OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

21984-1

FINAL REPORT

TRAINING COURSE ON PRODUCTION OF PHYTOMEDICINES

UNIDO PROJECT TF/GLO/96/105

UNIDO CONTRACT No. 97/311

Report of Workshop sponsored by the International Center For Science and High Technology (ICS) / United Nations Industrial Development Organization (UNIDO), Trieste, Italy and Iberoamerican Program for Science and Technology for Development, CYTED, held at Panama, 24 November - 5 December 1997

Mahabir P. Gupta
International Coordinator, Subprogram X. Fine Pharmaceutical Chemistry, CYTED, University of Panama
Panama, Rep. of Panama

31 December 1997

Proj. no TF/GLO/96/105

UNIDO proj. (Final report) on training programme on the production of phytomedicines (reference (medicinal plants) ~~and flavourable plants~~) - (addition) covers (1) manufacturing of phytomedicines (medicinal products containing as active ingredients exclusively plant material) as registered pharmaceuticals, situation in Europe, (EC) registration attempts, the situation in Latin America, ~~and~~ (North America) and in the (Far East) (2) research and development, quality control, toxicological safety, evaluation methods (3) standardization of medicinal plants (4) medicinal and aromatic plants (farming) (5) field production, manufacturing regard (6) preparation of extracts, (packaging) (7) advanced technology (management) (training) needs (UNIDO) services (8) analytical and validation methodology e.a. ~~(training) (lectures) (list of~~ participants) (documentation) additional reference (chemical analysis) (chemicals) (information system) (information exchange) (NHC)

3110

4.6.98 mi

ABSTRACT

This report is rendered under the UNIDO Contract No. 97/311 to organize and execute an "*International Training Course on Production of Phytomedicines*" for Latinamerican scientists in collaboration with the CYTED Program. This Report describes briefly the objectives of the course, its detailed programs of lectures and practical exercises, list of expenses as per the assigned budget. In addition, it appends all the handouts and lecture outlines compiled by the visiting Faculty and resource persons.

TABLE OF CONTENTS

INTRODUCTION

This report describes all activities carried out under the UNIDO Contract No. 97/311, based on the UNIDO Project No. TF/GLO/96/105 to organize and executive an *International Training Course on Production of Phytomedicines*. The Terms of Reference of this Subcontract are described in Appendix 1.

This course shows how international cooperation between two organizations like the International Center for Science and High Technology (ICS/UNIDO, Trieste), and the Iberoamerican Program of Science and Technology for Development, CYTED can be beneficial.

This course took effect in Panama during the period 24 November -5 December 1997 . About a couple of months before the beginning of this course, the announcements along with the applications blank for a fellowship were sent to over 50 centers in Iberoamerica, mainly though the CYTED, Subprogram X. Fine Pharmaceutical Chemistry's focal points in 21 countries, Coordinators of 4 networks, and the Directors of 4 on-going research projects. Special effort was made to assure that the phytopharmaceutical industries of the region were informed of the course.

The Inaugural Ceremony held on 24 November 1997 was attended by Dr. Gustavo García de Paredes, Rector of the University of Panama, Dr. Ceferino Sánchez, National Secretary for Science, Technology and Innovation (SENACYT), Office of the President of Republic of Panama, Dr. Angela B. Aguilar, Dean of the College of Pharmacy, Prof. Enrico Feoli, Area Coordinator, ICS/UNIDO, Mr. Juan Palacios, Director of the Specialized Analysis Institute and Prof. Mahabir P. Gupta, International Coordinator of the Subprogram X. of Fine Pharmaceutical Chemistry, CYTED. The Rector of the University of Panama officially inaugurated the course. The Illustration 1 gives a view of the Inaugural Ceremony. Ms. Ligia Elizondo, Resident Representative of UNDP was invited, but because of her travel away from Panama, she could not attend. The Embassy of Italy was represented by the Commercial Attacheé, Ms. Rossana Rrico.

The University of Panama provided, through the Pharmacognostic Research Center on Panamanian Flora (CIFLORPAN) of the College of Pharmacy and the Specialized Analysis Laboratory (IEA) provided with all necessary facilities for the course. SENACYT also supported the course.

CONCLUSIONS

1. A total of 19 applications were received. Final selection was made in consultation with Ms. Elisa Sarti de Roa of ICS/UNIDO, Trieste.

2. Financial Support was provided to 14 foreign participants, marked with one (*) asterisk in the list of participants, representing 12 countries. The participation of the candidate from Portugal was made possible through the financial support of the CYTED program (Appendix 2)(**). In addition, two CYTED staff also attended.

3. A total of 16 Panamanian scientists from academic institutions, industry and Government sector also participated.

4. The total number of participants was 30.

   The participants from each sector were as follows:

Academia	17(56.6%)[1]
Industry	8 (26.%)
Government Sector	5 (16.7)

[1] Because of high number local participants from the university, this number is high. If we only consider foreign participants, the balance is in favor of the Industry.

5. Illustration 2 shows the participants. A newspaper cutting (Illustration 3) about the opening of the course is also included. Appendix 3 shows the detailed program of the course. Prof. Arnold Vlietinck of the University of Antwerp, served as the overall Coordinator of the technical program.

6. A field trip to a farm of the Spanish Agency for International Cooperation in Chorrera was organized to observe *in situ* the cultivation of medicinal plants.

7. The course was very intensive and covered 80 hours of theoretical and practical sessions. The students were grounded in the different aspects of production of phytomedicines. All the topics of the project Document were amply covered.

8. The course was evaluated at the end. The Technology Management Module was evaluated separately. The evaluation was also made according to the CYTED questionnaires. Appendices 4, 5 and 6, show the results of the course evaluation. In general, all the objectives were accomplished and the participants were extremely pleased with the organization, efficiency and the high academic level of the course.

9. Appendix 7 compiles all the handouts and literature given to the participants.

10. During the course, the participants were also informed about the activities of CYTED. Through the presentations of Dr. Armando Cáceres, International Coordinator of Network X.C: RIPROFITO, Dr. Roberto Pinzón, Director of Project X.3 Search for Immunomolulators and Chemoterapeutic agents from Tropical Biodiversity, and Dr. Mahabir P. Gupta, International Coordinator, Subprogram X. CYTED. The participants showed keen interest in its activities.

11. Prof. Enrico Feoli's presentations about the ICS and the Databases were highly praised by the participants.

12. Resource Persons and visiting Faculty who participated in the course are marked with the superscript 1 in the list of Participants (Appendix 2). Brief *Curriculum Vitae* of the Resource Persons are provided in Appendix 8.

RECOMMENDATIONS

1. The ICS/UNIDO should continue to hold further workshops in Latinamerica in the field of industrial utilization of medicinal and aromatic plants. Some topics for 1998 could be **Biodiversity and Newer Screening Technology to discover bioactive principles and bioprospecting and strategies for industrial utilization of medicinal and aromatic plants**. The approach should be proactive capacity building and technology transfer.

2. The Latinamerican countries must expedite enactment of appropriate and adequate legislation for registrations of herbal medicinal products. This is a bottleneck for the region, at the present time.

3. Latinamerican countries must take urgent action to ensure adequate capability and capacity building of human resources. Special effort should be made towards increasing public awareness on the importance of medicinal plants and their conservation and training seminars should be organized on the Intellectual Property Rights (IPR) issues within the local populations. Courses on Phytotherapy should also be organized.

4. Models of various aspects of bioprospecting including benefit sharing and commercial utilization should be studied during the process of developing national policies on conservation and sustainable utilization of biodiversity.

5. The workshop made it explicit the concern for lack of facilities for carrying out standardization and toxicological evaluation of medicinal and aromatic plants in Latinamerica. The workshop recommends the U.N. and other multilateral agencies, specially the W.H.O. and the UNIDO to offer technical assistance to this region.

6. The workshop notes with great concern the lack of facilities and capacity in the region to undertake cultivation of medicinal plants. Efforts should be made, in cooperation with FAO and other international bodies to offer state of the art technology in this field and stimulate participation of private enterpreneurs.

7. The workshop clearly showed the need for further international cooperation among other programs such as the CYTED, UNESCO, FAO and the IFS to maximize the efficiency and use of available resources.

8. Latinamerican countries are urged to take appropriate actions to inventory and study their biodiversity of medicinal and aromatic plants, as soon as possible.

ACKNOWLEDGEMENTS

Thanks are due to the CIFLORPAN staff, Angela Calderón, Dionisio Olmedo, Rosaura Jiménez, Carlos Guerra and Alex Espinosa for their tremendous support in the organization of the Course. Special thanks to the Director of the Institute of Specialized Analysis Laboratory, Lic. Juan Palacios and to the Dean of College of Pharmacy of University of Panama, Angela B. de Aguilar for their generous support. Financial support of ICS/UNIDO, and CYTED is gratefullly acknowledged. Special thanks are given to Dr. Ceferino Sánchez, SENACYT for official patronage of the course.

Appendix 1

TERMS OF REFERENCE OF THE SUBCONTRACT
for the
ICS TRAINING COURSE ON

"Production of Phytomedicines"

Panama, Panama, 24 November - 5 December 1997

1. Purpose of the Subcontract

The subcontract is requested for the organization of a Training Course on the Production of Phytomedicines.

The contents of the course will deal with several topics related to the theoretical and practical aspects of production of phytomedicines, with emphasis on problems and constraints in the production of phytomedicines, cultivation of medicinal plants, agrotechnology, pharmaceutical technology, safety and clinical evaluation and quality control, etc. A descriptive framework of the main topics to be considered during the training course is given in the Aide-Mémoire.

The implementation of this activity will be subcontracted to a local counterpart who will bear the hereunder stated responsibilities.

2. Duties and Responsibility for the Subcontractor

- Finalize, in cooperation with ICS Coordinator, the Project Document, the Aide-Mémoire, the announcement and the programme/agenda of the Training Course.
- Ensure that the resource persons provide written copies (approximately 10 pages each) of their contributions in order to prepare a Final Report of the event.
- Identify suitable candidates to participate in the Training Course and prepare a list (bearing in mind that at least 50% of the participants should be coming from the industrial sector) to be submitted to ICS Coordinator for the final selection.
- Prepare and organize all travel and logistic arrangements for both resource persons and participants in the Training Course (air tickets, board, lodging, local transportation, etc.).
- Prepare, for the duration of the Training Course, suitable meeting rooms, lecture halls and laboratories with the required scientific equipment.
- In cooperation and consultation with ICS Coordinator, he will be responsible for the carrying out of the programme according to the approved programme/agenda.
- Evaluate, under the responsibility of ICS Coordinator, the activities of the Training Course and the profile of the invited participants.
- Prepare, within one month after the completion of the Training Course, a comprehensive report of the event.

- Prepare a comprehensive package of all written contributions and overheads presented at the Training Course, including case studies, examples (possibly in soft format).

- Provide recommendations and suggestions on how to improve the quality and cost-effectiveness of the events that ICS-UNIDO intends to carry out in its future programme.

- Provide all administrative and secretarial support for the organization and execution of the event.

3. Number of Participants

25 (15 from Latin America and 10 from Panama).

4. Dates of the Subcontract

From 27 October 1997 to 15 January 1998.

5. Miscellaneous

For any additional detail or information on the training course please refer to the Aide-Mémoire.

Appendix 2

CYTED SENACYT

Training Course on "Production of Phytomedicines"
Panama, 24 November-5 December 1997

Training Course on "Production of Phytomedicines"
Panama, 24 November - 5 December 1997

BELGIUM

[1]De Beer, Jacques
IHE, Brussels
Juliette Wytsmanstraat 14
B-1050 Brussels
Belgium
Tel: 02-6425136
Fax: 02-6425327
e. mail: jacques.debeer@ihe.be

[1]Vlietinck, Arnold
University of Antwerp UIA
Deparment of Pharmaceutical Sciences
13 - 2610 Antwerp, Belgium
Tel: 03 - 8202733
Fax: 03 - 8202709
e. mail: vlietink@via.ac.be

BOLIVIA

[*]Flores Quisbert, Esther Ninoska
Instituto de Investigaciones
Fármaco Bioquímica (IIFB)
Facultad de Ciencias Farmacéuticas y Bioquímica
Universidad Mayor de San Andrés
Av. Saavedra Nro. 2224, 2do piso Miraflores
La Paz, Bolivia
Tel. +591 2 229021
Fax. +591 2 358404
E. mail: iifb@megalink.com

BRAZIL

[*]Lopes de Almeida, José Maria
Faculty of Pharmacy
Federal Fluminense University
Rua Mario Viana 523
2424100 Niteroi, Brazil
Tel. 55 21 7111012
Fax. 55 21 6106654
E. mail: farm@cruiser.com.br

[1]Parise, Edison Roberto
Universidades Federal de Sao Paulo
Rua Botucatu 740
2do Andar 04023-900
Sao Paulo, Brazil
Tel: 011 - 5491711
Fax: 011 - 5764050
e. mail: parise@gastro.epm.br

[1]Sharapin, Nikolai
Universidades Federal Fluminense
Rua Mario Viana 523
24241-000 Niterol, Rio de Janeiro, Brazil
Tel: 55-21-7111012
Fax: 55-21-5373725
E. mail: farm@cruiser.com.br

CHILE

[1]Lorenz, Matthias
Agroindustrial Department
FUNDACION CHILE
Avenida Paque Antonio
Rabat sur 6165
Vitacura, Santiago de Chile
Tel: 56-2-2185211 anexo 264

Fax: 56-2-2186720
e. mail: mlorenz@fundch.cl

COLOMBIA

***Luengas-Caicedo, Pilar Ester**
Departamento de Farmacia
Facultad de Ciencias
Universidad Nacional de Colombia
Apartado Aéreo 14490
Santafé de Bogotá, Colombia

Tel.571 3165000/3165080/3165045 Ext. 14607
Fax. 571 3165060
E. mail: peluengas@ciencias.ciencias.unal.edu.co

****Pinzón, Roberto**
Director
Proyecto de Productos Naturales
Universidad Nacional de Colombia
Apartado 14490
Santa Fe de Bogotá
Colombia
Tel./Fax. Res. (571) 250 7884
Tel. Res. 631-1030
Tel. Ofic. (571) 316-5080 ó 316-5045 (Directo)
Fax. Ofic. (571) 316-5060

COSTA RICA

***Hess Araya, Ileana**
Ministerio de Salud
Departamento de Drogas
San José, Costa Rica
Fax: (506) 222-1420

***Ruzicka Tarragó, Juan José**
Manager
Laboratory Ruta S. A.
Apartado 1634050
Alajuela, Costa Rica
Tel. +506 4410672
Fax. +506 4438757
E. mail: federico-ruzicka@bciecr.bcic.

CUBA

***Paéz, Barbara Natividad**
Biomundi Consulting
Calle 200, 1921 E/19 and 21
Reparto Atabey Playa
16015 La Habana, Cuba
Tel. +537 217711/217708
Fax. +537 336529
E. mail: barbara@biomundi.inf.cu

ECUADOR

***Dehesa González, Marco Antonio**
Laboratorio Renase
Alemania 102 (Pasaje) y Eloy Alfaro
Quito, Ecuador
Fax. +593 2 508256
E. mail: cpmujer@uio.satnet.net
ECUADOR

EL SALVADOR

***Campos, Ricardo Alberto**
Calle Arce No. 827
Ministerio de Salud Pública y Asistencia Social
San Salvador, El Salvador
Tel. 503 - 221 1001
Fax. 503 - 221 1001
E. mail:hcordova@sal.gbm.net

GERMANY

[1]Harnischfeger, Götz
Schaber and Brummer Gmbh

Bahnhofstrasse 35
D - 38259, Salzgitter (Ringelheim)
Tel: 05341-307-110
Fax: 05341-307-111
E. mail:

GUATEMALA

***Baldizon, Byron**
Universidad de San Carlos de Guatemala
Ciudad Universitaria
Zona 12, Edificio T 5, Guatemala
Tel. 4763992
E. mail: inge.cen-invest@usac.edu.gt.

****Cáceres, Armando**
Coordinador Internacional
Red Iberoamericana de Productos
Fitofarmacéuticos (RIPROFITO)
Laboratorio Farmaya, S. A.
Ave. Centroamérica 18-92
Apartado 1160
Zona 1, Guatemala
Guatemala

Tel. Ofic. (502) 4769889 (Univ.)
Fax. (502) 4769808 (Univ.)
Fax/Tel. (502) 2305006 (Farmaya)
E. mail: farmaya@uvalle.edu.gt

HONDURAS

***Abarca, José Francisco**
Extractos Vegetales de Centroamerica
carreters al Picacho
desvio al Hatillo, Kilómetro 6
Tegucigalpa, Honduras
Tel. +504 219476
Fax. +504 219068
E. mail: jabarca@volta.unah.hondunet.net

ITALY

Feoli, Enrico
ICS - UNIDO
Trieste, Italia
Tel: 3940-224572
Fax: 3940-224575
e. mail: feoli@ics.trieste.it

NICARAGUA

***Klein, Wilhelm**
Centro Nacional de la Medicina
Popular Tradicional
Apartado 77, Esteli,Nicaragua
Tel. +505 7134841
Fax. +505 7132240
E. mail: cnmpt@ibw.com.ni

NIGERIA

[1]Iwu, Maurice
Walter Reed Army Institute of Research
Division of Experimental Therapeutics
Department of Therapeutics
Department of Medicinal Plants
20307 - 5100, Washington, U.S.A.
United State of America
Tel: +1-301-2957793
Fax: +1-301-2957878
e. mail: iwum@igc.apc.org

PANAMA

Araúz, Yira
Instituto de Medicina Legal
Ministerio Público
Avenida Perú y calle 33 este
Apartado 6-9382, El Dorado
Panamá, Rep. de Panamá
Tel:(507) 227-7924
Fax:(507) 227-0272

Buitrago de Tello, Rosa
Depto. de Farmacología
Facultad de Medicina

Estafeta Universitaria
Universidad de Panamá
Tel: 507 - 223-8530
Fax: 507 - 223-8530
e.mail:buitrago@ancon.up.ac.pa

Calderón, Angela
CIFLORPAN
Facultad de Farmacia
Universidad de Panamá
Apartado 813 - 0164, Zona 13
Tel: 507 - 269-7655
Fax: 507 - 264-0789
E. mail: ciflorp2@ancon.up.ac.pa

Chávez, Marisol
Facultad de Farmacia
Departamento de Química Medicinal y Farmacognosia
Universidad de Panamá
Tel: 263-6133 ext 489
Fax: 223-7633

De Gracia, Esperanza de
Laboratorio Bionatura
Apartado 6-4821
El Dorado, Panamá
Tel./Fax. 221-9486

De León, Elvira
Facultad de Farmacia
Departamento de Química Medicinal y Farmacognosia
Universidad de Panamá
Tel: 263-6133 ext 489

Delvalle, Denise
Instituto Especializado de Análisis
Universidad de Panamá
Apartado 6-4240
El Dorado, Panamá
Tel: 263-6133 ext 305

Espósito Avella, Mario
Depto. de Farmacología
Facultad de Medicina
Universidad de Panamá
Tel: 507 - 223-8530
Fax: 507 - 269-0710
e.mail:marbella@up.ac.pa

Gupta, Mahabir P.
CYTED
Coordinador Internacional
Subprograma X Química Fina Farmacéutica
Facultad de Farmacia
Centro de Investigaciones Farmacognósticas de la Flora Panameña(CIFLORPAN)
Estafeta Universitaria
Apartado 10767
Universidad de Panamá
Tel: 507 - 223 - 6787
Fax: 507 - 264 - 0789
e. mail: gupta@pananet.com
ciflorp2@ancon.up.ac.pa

Martínez, Yariela
Instituto Especializado de Análisis
Universidad de Panamá
Tel: 263-6133 ext 306
Fax: 269-8880
e. mail: iea2@ancon.up.ac.pa

Monteza, César
Policia Técnica Judicial
ANCON
Apartado 1299, Zona 1
Panamá, Rep. de Panamá
Tel. 262-4501
Fax. 262-4416

Núñez, Gonzalo
Facultad de Farmacia
Departamento de Ciencia y Tecnologia Farmacéutica
Universidad de Panamá

Olmedo, Dionisio A.
CIFLORPAN
Facultad de Farmacia
Universidad de Panamá
Apartado 10676
Tel: 507 - 269-7655
Fax: 507 - 264-0789
E. mail: ciflorp2@ancon.up.ac.pa

Sánchez, Ceferino
Secretario Nacional de
Ciencia, Tecnología e Innovación
Presidencia de la República
República de Panamá
Apartado 7250
Zona 5, Panamá
Tel. 265-5204/ 265-5208
Tel/Fax: 265-0852 / 265-5205
E. mail:senacyt@ancon.up.ac.pa

Santana T., Ana Isabel
Instituto Especilizado de Análisis
Facultad de Farmacia
Universidad de Panamá
Apartado 10767
Tel: (507) 269-7655
Fax: (507) 264-0789
E. mail: ciflorp2@ancon.up.ac.pa

Solís, Nilka Guerrero de
Instituto Especializado de Análisis
Universidad de Panamá
Tel: 263-6133 ext 297
Fax: 2640789 or 269
e. mail: iea2@ancon.up.ac.pa
ciflorp1@ancon.up.ac.pa
psolis@ancon.up.ac.pa

Soto, Antonio Alberto
Facultad de Farmacia
Departamento de Química Medicinal y Farmacognosia
Universidad de Panamá
Tel: 263-6133 ext 489

PARAGUAY

***de García, Evelyn Báez**
CHEMINTER
LABORATORIES SAECA
Isaac Kostianovsky c/RI1 2 de mayo,
Bulnes 630 , Lillo, Villa Morra
Asunción, Paraguay
Tel. 595-21-200887 /595 21 610610
Fax. 595-21-610491 /595 21 607150
E. mail: chemi@pla.net.py
E. mail: garcía@infonet.com.py

PERU

Muñoz Benítez, Rosana
Laboratory S. J. Roxfarma, S. A.
Av. Alfredo Mendiola 5648
Urb. Industrial Infantas
Lima 39, Perú
Tel. +51 1 5281522 /51 1 3376627
Fax. +51 1 5280851
E. mail: rosanamunoz@hotmail.com
E. mail: a8912273@pucp.edu.pe

Plaza Parra, Alberto
Quimalfa, S. A.
701 Carretera Central Km. 2.5
Santa Anita
Lima 43,
Lima, Perú
Tel. +511- 3540606/ 511-3440419 / 511-4941010
Fax. +511- 3540633/ +511-494-2296
E. mail: aplaza@pucp.edu.pe

PORTUGAL

****Costa, Maria do Céu**
INETI - DTIQ
Estrada do Paço do Lumiar, 22

1699 Lisboa Codex
Portugal
Tel. +351 1 7164211
Fax. +351 1 7168100
E. mail: ceu.costa@ibqta.ineti.pt
E. mail: ceu.costa@mail.ineti.pt

* ICS/UNIDO Fellowship.
** CYTED Fellowship.
[1] Resource Persons.

Appendix 3

CYTED

SENACYT

Training Course on "Production of Phytomedicines"

Panama, 24 November-5 December 1997

Monday, 24 November

09h00-10h00	Inauguration
10h00-12h30	Lectures 1.1, 1.2.and 1.3 (Götz Harnischfeger)
14h30-18h00	TLC of plant preparations (1) (Jacques De Beer)

Tuesday, 25 November

09h00-12h30	Lecture 1.3, 1.5 (G.Harnischfeger) and 1.4 (G.Harnischfeger & Matthias Lorenz)
14h30-18h00	Field trip

Wednesday, 26 November

09h00-12h30	Lectures 2.1 and 2.2 (M.Lorenz)
14h30-18h00	TLC of plant preparations (2) (J.De Beer)

Thursday, 27 November

09h00-12h30	Lecture 3.1 (G.Harnischfeger) Lectures 3.2 and 3.3 (Nikolai Sharapin)
14h30-18h00	GC of plant preparations (1) (J.De Beer)

Friday, 28 November

09h00-12h30	Lecture 3.4 (G.Harnischfeger) Round Table Discussion
14h30-18h00	HPLC of plant preparations (1) (J.De Beer)

Monday, 1 December

09h00-12h30	Lectures 4 (UNIDO) (Piero Atella, Maurice Iwu & N. Sharapin)
14h30-18h00	Lectures 4 Contd. (UNIDO) (P. Atella, M. Iwu & N. Sharapin)

Tuesday, 2 December

09h00-12h300	Lectures 5 (Edison R. Parise)
14h30-18h00	Lectures 5 (E.R. Parise)

Wednesday, 3 December

09h00-12h30	Lecture 6.1, 6.2 and 6.3 (Anold J.Vlietinck & J.De Beer)
14h30-18h00	HPLC of plant preparations (2) (J.De Beer)

Thursday, 4 December

09h00-12h30	Lecture 2.3, 6.4, 6.5 and 6.6 (A.J.Vlietinck)
14h30-18h00	Free afternoon

Friday, 5 December

09h00-12h30	Lecture 7 (A.J.Vlietinck)
14h30-18h00	Evaluation of practical courses (J.De Beer)

CYTED SENACYT

UNIDO

Training Course on "Production of Phytomedicines"
Panama, 24 November-5 December 1997

UNIVERSIDAD DE PANAMA

Lecture 1.1 Present status of phytomedicines as registered drugs

Definitions : Phytomedicine in the rational system of medicine, in alternative medicinal systems
Legal status, registration
Alternates, e.g. nutraceuticals etc.
Economic importance
The situation in Europe: EEC registration attempts, hamnonization efforts, present status, notice to applicants
- the situation in various European states and their approaches to the problem

The situation in other countries: The Americas, Far-East, others
The WHO approach and efforts for world wide harmonization

Lecture 1.2 Ethnobotanical and ethnomedical evaluation, principles and applications

The ethnobotanical approach for selecting suitable plants:
- written tradition from alternative medicinal systems, e,g. TCM, Ayurveda;
- evaluation of traditional folk remedies in developed cultures;
- evaluation of medicines from indigenous people, the WHO approach.

Procedural outlay for the investigation of selected plants:
- bioassay guided physico-chemical and chemical research methods;
- principles of pharmacological screening methods;
- therapeutical and toxicological evaluation tools.

Lecture 1.3 Multidisciplinary research, Aspects of quality, Safety and efficacy

Guidelines of WHO for the assessment of herbal medicines
Outline of procedures for establishing qualify, safety and efficacy:
- information gathering techniques;
- definition of intended indication of the medicine
- application, formulation, dosage.

Detailled planning activities:
- manufacturing rationale;
- avaliability of raw materials and excipients
- consequences for quality assurance and control methods.

Assessment of efficacy:
- constraints and differences with regard to synthetic/chemically defined agents;
- overview of assessment methods for therapeutic efficacy;
- evaluation methods for toxicology problems.

Registraton and labeling, documentation procedures

Lecture 1.4 Industrialization of medicinal plants

Plant material collected from the environment, special problems:
- proper identification ;
- adulteration and admixtures
- quality assurance.

Plant material from cultured species, special problems:
- microbial contamination;
- pesticides;
- agrochemicals
- conformity with established specifications.

Breeding of optimized varieties, inculturing of wild plants
Processing of plant material:
- drying methods;
- decontamination
- cutting and milling.

Storage and shipping conditions
GAP procedural requirements, documentation.

Lecture 1.5 Problems and constraints in the production of medicines in developing countries

Legal uncertainties: status of phytomedicines: drugs, food additives or superstition
Registration requirements
Manufacturing problems: personnel, equipment, starting materials
Economic constraints:
- market volume;
- affordability for large parts of the population
- distribution problems
- investment and interest problems.

Lecture 2.1 Testing Medicinal and Aromatic Plant Production under Field Conditions

A. Introduction of known species to unknown areas
- causes of natural variability of active principles
- analyzing agroclimatic conditions
- prognosting agronomic yield and yield of active principles
- estimating production cost and quality
- mechanization
- post harvest technology

B. Domestication of unknown species
- domestication versus wild collecting
- finding the right germplasm
- multiplication techniques

Lecture 2.2 Field Production of Medicinal and Aromatic Plants

- multiplication of germplasm
- production of seedlings
- planting
- field production
- harvest
- post harvest
- transport and shipment
- production of medicinal and aromatic plants as contribution to development activities
- quality control
- GAP

Lecturo 2.3 Use of tissue cultures and fermentation cultures for the improvement of medicinal plants

Plant cell cultures: principles and methodologies, cell suspension cultures, protoplast cultivation, genetic manipulation of plant cells, transgenic medicinal plants, application of plant cell cultures such as *de novo* biosynthesis, biotransformation of precursors and enzymatic catalysis, industrial development

Lecture 3.1 Manufacturing process of medicinal plants, including control and validation of methods of preparation

Guideline for developing a manufacturing pathway
Review of technology and methods for:
- intermediates: extraction, concentration, drying;
- formulation;
- packaging.

Selected WHO-GMP requirements
Guidelines for process- and equipment validation
Quality assurance systems and quality control methods for phytomedicines
Development of quality control systems

Lecture 3.2 Methods of preparation of extracts and tinctures from medicinal plants including also control and validation of the used methods

Maceration, digestion and percolation techniques of extracts, production of essential oils, balsams, resins, waxes, gums, apparatus used for the production of plant preparations: control and validation of the used techniques.

Lecture 3.3 Methods of preparation and packaging of finished products from plant preparations including the validation of the used techniques

General aspects of drug packaging, drug packaging materials such as glass, metal, plastics, the fabrication and fitting of pharmaceutical containers, closures for pharmaceutical packages, labels and labeling, package used for pharmaceuticals such as solids, liquids and semisolids, issues in modern packaging.

Lecture 3.4 Overview of GMP for the manufacturing of plant preparations

WHO-GMP guidelines for the manufacture of herbal medicinal products General GMP requirements to be met:
- personnel;
- plant buildings;
- equipment;
- procedures.

Quality assurance and validation
Documentation.

Lecture 4 Technology managemant training: UNIDO

Introduction to technology management
- Basic technology management concepts
- Challenges for managers of Technology
- Creating technological competencies

Managing the technology innovation process
- Strategic innovation for new business
- Identifying sources of technology
- Assessment and selection of technology

Analysis of new investment opportunities
- The business plan approach
- Criteria for the selection of investments

Market opportunities in the phytomedicines industry in Latin America

Business alliances as a strategy to enter international markets
- Definition and types of business alliances
- Identification of potential partners
- Management of business alliances
- Strategic issues in SBAs
- Case studies analysis of SBAs in the phytomedicine and pharmaceutical industry.

Lecture 5 Clinical evaluation of phytomedicine

Good clinical practice, phamarcokinetic studies in men, dose-response information, clinical investigation for long term use, biostatistical methodology in clinical tals, fixed combination products, clinical twing of prolonged action, clinical requirements for locally acting plant preparations, clinical safety data management, investigation of bioavailability and bioequivalence
Abridged clinical testing dossier of phytomedicines.

Lecture 6.1 Quality control norms of phytopharmaceuticals: WHO, IMEA, ISO

WHO, IMEA and ISO norms for the control of starting materials *viz.* starting plant preparations, excipients and packaging material (immediate packaging) WHO- and E.P. Pharmacopoeia norms.
WHO, IMEA and ISO norms for the control of intermediate plant preparations *viz.* extracts and tinctures, and other plant preparations.
WHO, IMEA and ISO norms for the control of finished products viz. gelules, tablets, syrups, liquids, creams etc.

Lecture 6.2 Analytical methods used for the quality control of medicinal plants, plant preparations and finished plant drugs: overview

Spectroscopic methods: colorimetry and UV-spectroscopy
Chromatographic methods: TLC-densitometry, gaschromatography, high pressure liquid chromatography, size exclusion chromatography, supecritical fluid chromatography
Titrimetric methods and gravimetric methods
Capillary electrophoresis

Lecture 6.3 Validation methods: overview

Validation methods required for identity, tests and assays of E.P. plant preparations

Analytical performance parameters such as linearity, precision, accuracy, limit of detection, limit of quantitation, selectivity, range and ruggedness.

Lecture 6.4. Control of starting materials including plants, excipients and primary packaging material and/of intermediate plant preparations such as extracts and tinctures and others

Specification and routine tests including characteristics, identification tests such as macroscopic and microscopic description, qualitative chemical profile, chemical identity tests, detection of toxic adulterants, detection of potential contamination by microorganisms, products of microorganisms, pesticides, toxic metals, radioactivity, fumigants and assay of the active ingredients or markers

Scientific data including manufacture, quality control during manufacture, development of extracts and tinctures and other plant preparations.
Batch analyses.

Lecture 6.5 Control of finished products

Specification and routine testing including
- product specifications;
- control methods including identification, assay and other tests;
- pharmaceutical tests;
- identification and determination of excipients;
- scientific data including analytical validation and batch analysis.

Lecture 6.6 Stability testing of finished products

Batches tests specifying the packaging
Study methods: normal test conditions, accelerated test conditions
Characteristics studied including physical, chemical, microbiological, chromatographic characteristics and characteristics of the packaging
Evaluation of test procedures
Results of tests
Discussion, interpretations and conclussions: shelf-life and storage conditions.

Lecture 7 Toxicological and pharmacological evaluation of phytomedicines

Single dose and repeated dose toxicity: repeated dose tissue distribution studies, reproduction studies, testing for mutagenic and carcinogenic potential, specific aspects of regulatory genotoxicity tests
Pharmacokinetics and metabolic studies in the safety evaluation in animals, non-clinical local tolerance testing and preclinical biological safety testing
Abridged toxicological and pharmacological testing dossier of phytomedicines.

Notes:

[1]The lectures will be given in Room #A-11, Auditorium Bernardo Lombardo of the Faculty of Natural Sciences.

[2]The experimental sessions will be held in the Specialized Institute of Analysis and Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN), College of Pharmacy.

APPENDIX 4

RESULTS OF COURSE EVALUATION
ICS WORKSHOP EVALUATION QUESTIONNAIRE[1,2,3]

A	**ORGANIZATION**					
		CYTED	**ICS**	**NA**		
1	How did you obtain information about this workshop/course?	85	5	10		
		Excellent	**Very Good**	**Good**	**Fair**	**NA**
2	The information process was	33	57	10		
3	The announcement and pre-course material was:	19	33	19		29
Describe the content of the workshop/course Multidisciplinary research, aspects of quality, safety, efficacy, manufacturing process, validation, etc, of Phytomedicines; Legislation and Registration; Market trend; Clinical assays; cultivation; Industrialization of Medicinal Plants (Please see the questionnaires)						
		Excellent	**Very Good**	**Good**	**Fair**	**NA**
4	I found the scientific programme	61	29	10		
4.1	Applied Lecture/Workshop	38	52	10		
4.2	Use of small working groups	14	29	14	5	38
4.3	Case studies	10	48	5		38
4.4	The time spent by lectures in class and after class on specific questions/examples	33	52	10		5
		Balanced	**Unbalanced**			**NA**
4.5	Students scientific knowledge was	90	5			5
B	**Duration of Programme**	**Just Right**	**Too Long**	**Too Short**		
1	Number of days	76	14	10		
2	Length of working	95		5		
C	**Training facilities & Hotel**	**Excellent**	**Very Good**	**Good**	**Fair**	**NA**
1	Lecture/Training Rooms	48	33	5		14
2	Break/refreshments	33	43	5	5	14

		Excellent	Very Good	Good	Fair	NA
3	Hotel accomodation	48	23			29
4	Meals at the hotel	23	29	19		29
D	**Organizer's response to participants needs**	**Excellent**	**Very Good**	**Good**	**Fair**	**NA**
		52	48			
E	**Overal programme organization**	43	57			
F	**Would you recommend to others from your institution/country to attend a similar activity in the future?**	**Yes**	**Maybe**	**No**		
		95	5			

1	**Which part of the Activity did you find most useful?** R: All; Quality control, norms, methods, safety, control methods, market trends; Vlietnick, Harnishfeger, Parisse, Iwu speakers; manufacturing, industrialization, regulations & quality control; Sharapin's and De Beer's lectures and practices; registration, production and legislation.
2	**Which part of this activity do you think should be expanded?** R: Manufacturing process & stability assays; tissue and fermentation culture; marketing; specific case studies; Farmacological & toxicological studies; experimental; laboratories; More labs for clinical trials, examples and extraction, formulation of medicinal plants; plant extraction; cultivation and production.
3	**Which part of the activity do you think should be dropped?** R: 90% answer none; 10% answer Technology management training UNIDO.
4	**Any other suggestions for future** R: Interdisciplinary; more practical; none; more practice; practical part shoul be reorganized and improved, more field trips; invite industry people; Toxicological studies, include case/studies; increase formulation and extraction. Less european regulations.
5	**Do you think that the topics/tools you studied during the course could be used by industries in you country? If so, how? If not, why not?** R: Yes, for better products and competitive; legislation and registration; Very important; Development, analysis, presentation and registration of phytomedicines; manufacturing and quality control; Validation of medicinal plants; Dr. Lorenz's, Harnischfeger's lectures for cultivation and quality control; Industrial sector; highly relevant; Formulation and extraction.

6	**Can You suggest any programme and future activities which ICS could pursue in order to help with the technological and scientific advancement of your country?** R: Bioprospecting and newer screening methodology; Manufacturing, preformulation and formulations of phytopharmaceuticals; Tissues cultures and stability of phytopharmaceuticals; equipment acquisition; Similar workshop; develop phytomedicines without pesticides; Discussion on Latinoamerica about High quality on industry; Practical courses, Technical preparation; More seminars; Keep this programme running; Repeat the course for different enterprises; Formulations; Chromatography.
7	**Do you think you have benefited from participation in this course/workshop? If so, how? and your Institution?** R: Yes; Completely ; Evaluation, I will be able to help now my company in Quality control; Updated and registration; increased my knowledge about this issue.
8	**How do you intend to disseminate the information you have acquired during the activity once back in your own country?** R: Teaching and exchanging information; Reproducing the course; Conferences, meetings and seminars; National course; aplying this knowledge; potential herbal medicines; information practices; with seminars; lectures; speeches.

G	Evaluation of Lectures and Speakers	Excellent	Very Good	Good	Fair	NA
1	Course material	48	48	4		
2	Resident Lecture presentation	43	29			29
3	International Lecture presentation	67	33			
4	Ability of lecturers to answer specific questions	52	43	5		

Any Comments

R: This kind of training is very useful and gives us a complete overview of the topics of phytomedicines. The participants wish to emphasize that all the experts gave excellent lectures and labs sessions. This course gave a multidisciplinary approach to phytomedicines.

As the lecturers were the most of them Europeans, they presented the Europe-USA points of view, some thought the Asian view could also be included.

They acknowledged ICS/UNIDO for the unique opportunity in participating in such an ambicious multidisciplinary training course, whose goals were completely achieved, The Course overall was excellent, and should be repeated.

1 The number of questionnaires answered was 21.

2 The figures in the Table of Appendix 4 represent the answers in percentage.

3 NA No Answer

APPENDIX 5

RESULTS OF THE EVALUATION OF ICS TECHNOLOGY MANAGEMENT MODULE EVALUATION QUESTIONNAIRE[1,2,3]

A	Organization	CYTED	ICS	PC	NA	
1	How did you obtain information about this workshop/course?	85	4	7	4	
		Excellent	**Very Good**	**Good**	**Fair**	**NA**
2	The information process was	15	63	18		4
3	The announcement and pre-course material was	4	52	22	7	15
	Describe the content of the workshop/course R: Please see the questionnaires					
		Excellent	**Very Good**	**Good**	**Fair**	**NA**
4	I found the scientific programme	33	48	15		4
4.1	Applied Lecture/Workshop	15	48	22		15
4.2	Use of small working groups	7	11	11		70
4.3	Case studies	18	30	18		33
4.4	The time spent by lecturers in class and after class on specific knowledge was	33	44	15		7
4.5	Students scientific knowledge was	**Balanced**	**Unbalanced**		**NA**	
		85	4		11	
B	**Duration of programme**	**Just Right**	**Too Long**	**Too Short**		**NA**
1	Number of days	85	4	7		4
2	Lenght of working days	85	4	7		4

C	Training facilities & Hotel	Excellent	Very Good	Good	Fair	NA
1	Lecture/Training Rooms	59	22	7		11
2	Breaks/refreshmentns	44	22	18		15
		Excellent	**Very Good**	**Good**	**Fair**	**NA**
3	Hotel accommodation	52	11	11		26
4	Meals at the hotel	37	15	22		26
D	**Organizer's response to participants needs**	44	41	7		7
E	**Overall programme organization**	48	44	4		4
F	**Would you recommend to others from your institution/country to attend a similar activity in the future?**	**MAYBE**	**YES**	**NO**	**NA**	
		7	93			

1	**Which part of the Activity did you find most useful?** R: Please see the questionnaries.
2	**Which part of the activity do you think should be expanded?** R: Please see the questionnaires.
3	**Which part of the activity do you think should be dropped?** R: None; Anyone; None of it; No one; Economic aspect and the concept of cultivation.
4	**Any other suggestions for future improvements to the programme?** R: Small workshop on management; Give before hand literature; It would be better, if the students received the material for the course before the lectures; Courses like this should be given regularly. For more details please see the questionnaires.
5	**Do you think that the topics/tools you studied during the course could be used by industries in you country? If so, how? If not, why not?** R: Yes investing in growth, domestication and preservation of plants to be commercialized locally or for exportation; Yes it is important when considering the establishment of an industry, especially a small one, so as not to incurr in unnecessary expenses; We're trying to introduce our natural products in Europe and Japan, so we're making joint ventures with some foreign enterprises; Yes; Could be used in my country at least to stimulate cooperative agreementrs/contracts and the planing for adding value to my country's natural resource; These tools will be tremendously useful to help develop an industrial growth on this sector and more scientifical development, research and investigation. Please see the questionnaires for more details.

6	**Can you suggest any programme and future activities which ICS could pursue in order to help with the technological and scientific advancement of your country?** R: Please see the questionnaires.					
7	**Do you think you have benefited from participation in this course/workshop? If so, how? and your institution?** R: The majority of students opined positively.					
8	**How do you intend to disseminate the information you have acquired during the activity once back in your own country?** R: Organizing a national seminar and course about the phytomedicine with industry;Through publication in the local Journals; Planning a short course at the Universities. (Please see the questionnaries for more details).					
G	**Evaluation of Lecturers and Speakers**	**Excellent**	**Very Good**	**Good**	**Fair**	**NA**
1	Course material	11	52	15		22
2	Resident Lecture presentation	30	26	4		41
3	International Lecture presentation	48	30	11	4	7
4	Ability of lecturers to answer specific questions	56	33	4		7
Any Comments: R: It Would be helpful if interested people could be trained in well-equipped, high quality phytomedicine company; Was interesting, should have more course about practical parts; Simultaneous translation; More frequent courses like this; All Participants acknowledged sincerely the support of ICS/UNIDO, for which they are grateful.						

1 The number of questionnaires answered was 27.
2 The figures in the Table of Appendix 5 represent the answers in percentage.
3 NA No Answer
PC Personnal communications
ICS ICS/UNIDO
R Reply

APPENDIX 6

OVERALL COURSE EVALUATION[1,2,3]

Describe briefly about the follows aspects of the Training Course:

		Excellent	**Very Good**	**No Answer**	**Comments**		
1	**Organization**	43	43	14	See questionnaries		
2	**Content**	**Excellent**	**Good**	**Comments**			
		95	5	Updated; integral; fulfilled; high standard			
3	**Academic Level**	**Excellent**	**Very Good**	**Good**	**Comments**		
		72	14	14	Excellent; Very High; High; Updated		
4	**Adequate Documentation**	**Excellent**	**Very Good**	**Comments**			
		43	57	Adequate; Wide; Complete; Excellent; Updated; Absolutely; Useful;			
5	**Course Objectives Achieved**	**100%**	**95%**	**Comments**			
		86	14	Completely; Were fullfilled; Goals Met; Totally			
6	**Future Suggestions**	**Do the same**	**More Labs**	**No Answer**	**others**		
		19	19	38	22.9		
7	**Difficulties**	**No Answer**	**More Labs**	**Spanish Language**	**None**	**Comments**	
		24	9	5	62	Needs More Time & Practice	
8	**Additional Comments**	**Excellent**	**Film it**	**No Answer**	**Comments**		
		62	5	33	Group size adequate for laboratories; Congratulations; Well Organized!!		

1 The number of questionnaires answered was 21.

2 The figures in the Table of Appendix 6 represent the answers in percentage.

3 NA No Answer

Appendix 7

APPENDIX 8

BRIEF CURRICULUM VITAE OF RESOURCE PERSONS

1. Prof. Arnold Vlietnick

Professor and Head of the Department of Pharmacognosy, University of Antwerp, Belgium.
Ph.D. Pharmaceutical chemistry; Postdoctoral work at the University of Wisconsin. Chairman Gr XIII Expert Group, European Pharmacopoeia; Member, Commission of Belgian Pharmacopeia. WHO Collaborating Center on Tropical Medicine. Research on isolation and characterization of natural bioactive principles. Over 200 original publications and book chapters.

2. Prof. Götz Harnischfeger

Born in 1939. Pharmacy study in Frankfurt, Germany; Diploma 1964; License 1965; study of Chemistry, Florida University State 1966 - 1970; Ph. D. 1970; University of Göttingen from 1971 - 1979; Dr. of Science in Botany 1976; 1979 into the Phytomedicine Industry to Schaper & Brümmer, Germany: Head of Production, Head of Technical and Pharmaceutical Services, Members of the Board; Since 1982 Professor of Botany in Göttingen; Since 1996 members of the German and European Pharmacopeial Expert Group for Plant Drugs.

3 Prof. Jacques De Beer

Ph.D. Pharmacy; Head of the Belgian Goverment Analytical Lab in Brussels for Quality Control and Medicines. Member Expert Group XIII European Pharmacopoeaia.

4. Dr. Matthias Lorenz

Ph.D. University of Munich. Expert in Cultivation of medicinal plants with the G.T.Z. (Germany). Presently working in Chile with Fundación Chile. Hands on experience on cultivation projects in Nicaragua, Ecuador and Chile.

5. Nikolai Sharapin

Professor of Pharmaceutical Technology, Federal Fluminense University, Brazil. Expertise in Technology and Chemistry of natural products and phytopharmaceutical, Member of the Brazilian Pharmacopoeial commission. Ministry of Health, since 1982; over 100 research publications. Previously at University of Campinas, CODETECH, with vast experience in pharmaceutical technology.

6 Dr. Edison Roberto Parise

Associate Professor of Gastroenterology of the Federal University of Sao Paulo since 1983, Master and Ph. D. in Medicine and Gastroenterology. Research Fellow at the Royal Free Hospital, University of London & at Research and Advanced Studies, Polythecnical of National Institute, Mexico; Visiting Scientist at the University Pittsburg. Expertise in Clinical Evaluation of Phytomedicine and previous experience of Clinical trials in Brazil.

7. Maurice Iwu

Maurice Iwu is the Executive Director of Bioresources Development and Conservation Programme (BDCP) and a Senior Research Associate at Walter Reed Army Institute of Research, Washington, D.C. He is a founding, member of the strategy team and scientific adviser of Shaman Pharmaceuticals Inc. at San Francisco, California. He was the Vice-President for Research and Development at Toms of Maria a manufacturing company based on natural products. He is a consultant to the United Nations Industrial Development Organization (UNIDO) in Technology Management. Professor was educated at the University of Bradford, England where he trained as a pharmacist and obtained a master of Pharmacy degree and a Ph. D. in Pharmacognosy in 1978. He was a professor of Pharmacognosy at the University of Nigeria, Nsikica. He was a Fulbright scholar at the Columbia University and a W.H.O. visiting scholar at Dyron Perrins Laboratory, Oxford University, England. Professor Iwu has published over a hundred research articles in referenced journals and is the author three books. He is the president of the International Society for Ethnobiology. His current reserach interest is the development of novel therapeutic agents for treatment of tropic diseases and the creation of in countries trrough drug development for the conservation of biological diversity.

ILLUSTRATIONS

Illustration 1*

Opening Ceremony

Illustration 2*

Group Photographs of the Participants

* Photos by C. Guerra

Illustration 3
Newspaper Cutting

Panamá foco de atención sobre producción de fitomedicamentos

Con el propósito de capacitar a los científicos iberoamericanos en todos los aspectos de producción de fitomedicamentos se realiza en nuestro país el curso internacional sobre "Producción de Fitomedicamentos" coordinado por el Centro de Investigaciones Farmacognósticas de la Facultad de Farmacia de la Universidad de Panamá.

Entre los temas que abordará el Curso tenemos entre otros, los aspectos agrotecnológicos, control de calidad, registro, aspectos de tecnología farmacéutica y gestión tecnológica.

El Dr. Mahabir P. Gupta, Coordinador Internacional del Subprograma X. Química Fina Farmacéutica del Programa Iberoamericano de Desarrollo Científico y Tecnológico (CYTED), al dar la bienvenida a los participantes en el Curso señaló que "El mercado mundial de los fitomedicamentos se estima en unos 16,000 millones de dólares. La región latinoamericana consume solo un 8% del mercado mundial de medicamentos."

De igual forma indicó que "en el Curso se espera que los participantes estén en capacidad al finalizar el mismo de conocer y aplicar los conocimientos sobre estrategias de cultivos de plantas medicinales, diseñar y evaluar la seguridad y estudios clínicos de fitomedicamentos; introducir mejores y modernos métodos de tecnología en la producción y formulación de fitomedicamentos, entre otros".

Al Curso sobre "Producción de Fitomedicamentos" asisten científicos de Europa y América Latina y se desarrolla dentro del marco del convenio vigente entre la Organización de las Naciones Unidas para el Desarrollo Industrial (ONUDI), el Programa Iberoamericano de Desarrollo Científico y Tecnológico (CYTED), el Centro Internacional de Ciencia y Alta Tecnología (ICS/UNIDO) con sede en Trieste, Italia, el Subprograma X. Química Fina Farmacéutica, el Instituto Especializado de Análisis y la Facultad de Farmacia de la Universidad de Panamá, y la Secretaría Nacional de Ciencias, Tecnología e Innovación (SENACYT).

Appendix 9

SUMMARY OF EXPENSES

				US $
Travel				17,779.51
Annex 1	International air travel	16,355.00		
Annex 2	Local transportation	1,424.51		
Lodging				
Annex 3				6,652.65
Boarding				10,670.98
Annex 4	Per diems	7,130.00		
Annex 5	Meals & Reception	3,540.98		
Miscellaneus				4,934.82
Annex 6				
			TOTAL	**40,037.96**

21984-2

Appendix 7

Schaper & Brümmer
Naturstoff-Forschung für die Therapie

Schaper & Brümmer Postfach 61 11 60 38251 Salzgitter

Technisches Controlling
Apotheker Prof. Dr. G. Hamischfeger

Iberoamerican Program of Science and High Technology for Development (CYTED)

Training course on production of phytomedicines

College of Pharmacy, University of Panama
Panama / Panama, 24.11. - 5.12. 1997

Outline of lectures

AG Salzgitter HRA 836
pers. haft. Gesellschafterin
Schaper Verwaltungs-GmbH
AG Salzgitter HRB 310
Geschäftsführer
Hannelore Kracke, Arne Schaper

Schaper & Brummer GmbH & Co. KG
Hausadresse: Ringelheim
Bahnhofstraße 35
38259 Salzgitter
Telefon (0 53 41) 3 07-400/401
Telefax (0 53 41) 3 07-405
Telex 9 54 448

Present status of phytomedicines as registered drugs

Götz Harnischfeger

Before anything can be said about the „status" there has to be an understanding of the term phytomedicine. In the EC the name „Herbal medicinal product" is used more frequently and it is defined as follows: (figure 1)
In this definition the term „medicinal" implies, that the product is used primarily for health reasons, either to heal or to alleviate a disease or affliction, and that the medicine is labelled accordingly giving active ingredient, dosage and means of use. It furthermore implies, that the medicinal product is used in the context of rational, scientifically based conventional medical practice.
Of course, this European general view of what should be encompassed by the term „phytomedicine, herbal medicinal product or phytopharmakon" is not always found in practice. Especially the medical profession, at least the rigorous pharmacologist fraction, tends to subsumize all kind of products into the category „phytomedicine", which do not, or only marginally, fit the above definition. This is reflected in the various categories of legal recognition shown in figure 2.
The figure gives a compilation of terms into which these plant products have been divided and which can be used to classify the registration effort and the chances of its success in various states of the European community.

The situation in Europe

Although there is general agreement, that phytomedicines should comply with the requirements of regular, rational medicines, there is a still ongoing discussion about the extent of this compliance. Many of the conditions stated can not be fulfilled either by definition, e.g. active ingredient and its purity, or by economic reasons, e.g. clinical multicenter studies. The search for acceptable alternatives still differs from country to country and a great effort is in progress to harmonize in order to arrive on an acceptable, practical level of recognition. The present view of phytomedicines in the various European states, together with the resulting requirements for authorization as medicinal product, is given in the next figures
(figures 3, 4).
Phytomedicines are an important economic factor in Europe, not just a lingering remnant of 19 th century german romanticism. An inquiry of 1991 showed, that 1400 herbal drugs were used as raw material in the member states of the EEC. If only those drugs were considered, which are found in at least 5 of the 10 member states, still 145 herbal drugs remain (figure 5). The sales of phytomedicines in 1996 totaled around 7.2 billion U$. In the OTC sector, Germany holds the biggest share with 2.5 billion U$ followed by France with 1.6 billion U$ and Italy with 600 million Dollars.
Even if herbal medicines are widely used for self medication, there is no automatic link of phytomedicine and OTC status. In Germany and France, a relevant proportion of medical prescriptions is made up of phytomedicines. Some of them, e.g. those containing Belladonnae folium, are by prescription only.
Because legally all phytomedicines in finished, saleable form are considered medicinal products, they are required to be authorized (CD65/65 EEC). Applicants must document quality, safety and efficacy. The ongoing European assessment process should result in a harmonized „Summary of Product Characteristics" (SPC) for each drug and concomitant preparation. The process of harmonization, however, in spite of general guidelines (figure 6) is a tedious and sometimes discouraging process.
The point of conflict in the assessment of quality, safety and efficacy is the criterium which should be applied especially in the case of safety and efficacy.

Herbal medicinal products (phytomedicines) are medicinal products containing as active ingredients exclusively plant material and preparations thereof

A.1.1. Figure 1

Legal Definition of Phytomedicines

- regular medicines according to the standard legal requirements

- regular medicines but with special status

- non-conventional medicines

- medicines of alternative therapeutical systems

- nutraceuticals, food additives

- therapeuticals of more than dubious value

Austria

regular medicines, special status for about 500 active ingredients (generics) including some drugs and extracts

Belgium

regular medicines with simplified registration status for those drugs listed in a special list.

Denmark

special status for certain phytomedicines. Simplified registration, if traditional use, OTC status and indications suitable for self medication are claimed.

Finland

regular medicines. In registration, literature data are allowed for proof of efficacy

France

regular medicines with special status, at least for those drugs and preparations listed in the „ avis au fabricants“

Germany

regular medicines with special status, semi-alternative therapeutic products

Greece

regular medicines

Ireland

mixed status of either regular medicines or food additives, if consisting only of dried and cut drug and no health claim is put forward

Italy

both options, regular medicine or food additive are possible. Definition according to official lists

Spain

regular medicines with exceptions in registration

Sweden

same as Finland

United Kingdom

regular medicines but with special status

11. Figure 4

Table 1: Most relevant herbal drugs

Achillea millefolium	Herba
Acorus calamus	Rhizoma
Aesculus hippocastanum	Semen
Agar	
Agrimonia eupatoria	Herba
Agrimonia procera	Herba
Agropyron repens	Rhizoma
Alchemilla vulgaris	Herba
Allium cepa	Bulbus
Allium sativum	Bulbus
Aloe species (barbadensis, capensis, ferox)	Succus (sicc.)
Alpinia officinarum	Rhizoma
Althaea officinalis	Flores
Althaea officinalis	Folium
Althaea officinalis	Radix
Anethum graveolens	Fructus
Angelica archangelica	Radix
Arctium lappa	Radix
Arctostaphylos uva-ursi	Folium
Armoracia rusticana	Radix
Arnica montana	Flores
Artemisia absinthium	Herba
Atropa bella-donna	Folium
Barosma betulina	Folium
Betula pendula	Folium
Calendula officinalis	Flores
Capsella bursa-pastoris	Herba
Capsicum annuum	Fructus
Carum carvi	Fructus
Cassia angustifolia	Folium
Cassia senna	Folium, Fructus
Centaurium erythraea	Herba
Cephaelis ipecacuanha	Radix
Chamomilla recutica	Flores
Chondrus crispus	Thallus
Cimicifuga racemosa	Rhizoma
Cinnamomum aromaticum	Cortex
Citrus limon	Aetheroleum
Cnicus benedictus	Herba
Cola nitida	Semen
Commiphora molmol	Gum-Resin
Coriandrum sativum	Fructus
Crataegus laevigata	Folium
Crocus sativus	Stigma
Curcuma longa	Rhizoma
Cynara scolymus	Folium
Drosera rotundifolia	Herba
Equisetum arvense	Herba
Eucalyptus species	Aetheroleum
Ferula asa-foetida	Gum-Resin
Ficus carica	Fructus
Filipendula ulmaria	Flores, Herba
Foeniculum vulgare var. vulgare	Aetheroleum
Foeniculum vulgare var. vulgare	Fructus
Fraxinus excelsior	Cortex
Fraxinus excelsior	Folium
Fucus vesiculosus	Thallus
Fumaria officinalis	Herba
Geranium robertianum	Herba
Glycyrrhiza glabra	Radix
Hamamelis virginiana	Folium
Harpagophytum procumbens	Radix
Hedera helix	Folium
Humulus lupulus	Glandula
Humulus lupulus	Strobuli
Hydrastis canadensis	Rhizoma
Hypericum perforatum	Herba

1.1 Figure 5a

Hyssopus officinalis	Herba
Illicium verum	Fructus
Inula helenium	Rhizoma
Juniperus communis	Fructus
Krameria triandra	Radix
Lamium album	Flores
Laurus nobilis	Folium
Lavandula angustifolia	Flores
Levisticum officinale	Radix
Linum usitatissimum	Semen
Lobelia inflata	Herba
Malva sylvestris	Flores
Malva sylvestris	Folium
Marrubium vulgare	Flores
Marrubium vulgare	Herba
Melaleuca species	Atheroleum
Melissa officinalis	Folium
Mentha piperita	Aetheroleum
Mentha piperita	Folium
Menyanthes trifoliata	Folium
Myristica fragrans	Semen, Arillus
Myroxylon balsamum var. pereirae	Balsamum
Olea europaea	Folium
Olea europaea	Oleum
Origanum vulgare	Herba
Panax ginseng	Radix
Papaver rhoeas	Flores
Passiflora incarnata	Planta tota
Peumus boldus	Folium
Pimpinella anisum	Fructus
Pimpinella anisum	Fructus, Aetheroleum
Pinus species	Aetheroleum (Terpentin)
Plantago ovata	Semen
Podophyllum peltatum	Rhizoma, Resina
Polygonum aviculare	Herba
Potentilla erecta	Rhizoma
Primula veris	Radix
Prunus cerasus ssp. acida	Stipites
Prunus spinosa	Flores
Quercus robur	Cortex
Quillaja saponaria	Cortex
Rhamnus frangula	Cortex
Rhamnus purshianus	Cortex
Rheum officinale	Radix
Rosa canina	Fructus
Rosa centifolia	Flores
Rosmarinus officinalis	Folium
Rubus fruticosus	Folium
Rubus idaeus	Folium
Salvia officinalis	Folium
Sambucus nigra	Flores
Silybum marianum	Fructus
Silybum marianum	Herba
Solidago virgaurea	Herba
Tamarindus indica	Fructus
Taraxacum officinale	Radix
Thymus serpyllum	Herba
Thymus vulgaris	Herba
Tilia cordata	Flores
Trigonella foenum-graecum	Semen
Urtica dioica	Radix
Vaccinium myrtillus	Folium
Valeriana officinalis	Radix
Verbascum phlomoides	Flores
Verbascum thapsus	Flores
Verbena officinalis	Herba
Viburnum prunifolium	Cortex
Viola odorata	Flores
Viola tricolor	Flores
Viola tricolor	Herba
Vitis vinifera	Folium
Zea mays	Stipites
Zingiber officinale	Rhizoma

1.1 Figure 5B

Harmonization EEC

Quality: Directive 75/318/EEC amendment, Pharmacopoeia Europaea

Safety: Directives 91/507/EEC, 87/19/EEC, 88/320/EEC

Efficacy: Directives 91/507/EEC, 75/318/EEC

Reasons for divergent opinion are:

- different traditions in administrative practice,
- problems with the acceptance of bibliographic data,
- difficulties in assessment of typical OTC products with minor indications,
- reservations toward everything which comes from traditional, non- conventional, non-mainstream academic medicine.

There are concrete problems as well, e.g. the different use and indication of herbal drugs in the various states. An example is given in the figure (figure 7).

Nevertheless, there have been efforts from academic and industry organisations, working jointly in ESCOP i.e. European Scientific Cooperative for Phytotherapy, to draft standard, core SPC's for a sizeable number of plant drugs in form of monographs on safety and efficacy. These are evaluated by the CPMP (Committee for Proprietary Medicinal Products) at the EMEA (European Medical Evaluation Agency) and, if approved, legally binding for generic phytomedicines. For these products, then, safety and efficacy is considered proven fact. The next figure gives an overview (figure 8).

The CPMP has, however, already published a list of those drugs, which could pose a serious risk and should, therefore, be withdrawn for safety reasons, especially, since they possess no accepted benefit. (figure 9).

The situation in selected other states

a) The United States

Products of herbal origin (phytomedicines) are generally considered non prescription, OTC drugs with only a limited requirement for authorization by the FDA.

All OTC preparations are evaluated in groups according to medical indication by review panels. The findings are published in monograph form. All substances, including herbal products, are classified in 3 categories (figure 10).

In the review process, only a few herbals were found to be suited for category I, most landed in category III. The problem lies in the acceptance of bibliographic data or results of studies conducted outside the US.

In addition, the US definition of OTC considers the sale of products only without proper counceling by a specialist, be it a member of the medical profession or a registered pharmacist. If a product is sold in Europe under the category: pharmacies only, it will not be elegible for the US market.

The result of this policy is an almost uncontrollable market of phytomedicines in disguise as nutraceuticals, food additives, herbal foods etc. without proper supervision of the authorities, many with lack of proper quality and adorned with outlandish claims.

The FDA has awakened lately and established monographs on about 20 herbs of commercial importance, but they are unavailable to the public as yet. The USP contains quality monographs on 8 drugs, 8 more will be included in the next edition. In comparison the Pharm. Eur. contains 73 plant-drug monographs, the DAB an additional 67.

The American Botanical Council, an organization of scientists and commercial traders and manufacturers, tries to establish monographs for guidance in the trade. Although they are well researched and documented reviews, they are not officially accepted or recognized by the FDA (figure 11).

b) Japan

Japan has a long tradition in the use of phytomedicines, both, in the traditional Kampo medicinal system and the scientific, conventional, western medicine. Thus, the view of phytomedicines as drugs requiring licensing is dominant. Quality, safety and efficacy have to

Annex 1

Examples for different indications for the same phytomedicine in different EU-member states:

1. Hypericum

Comm. E/BfArM:	Psychovegetative disorders, depressive moods, anxiety and/or nervous restlessness.
ESCOP:	Mild to moderate depressive states (ICD-10-category F32, F32.1), somatoformic disturbances including symptoms such as restlessness, anxiety and irritability.
Swedish MPA:	Traditionally used for occasional sleeping disturbances and against mild restlessness.

2. Cimicifuga

Comm. E/BfArM:	Premenstrual symptoms and dysmenorrhoea as well as neurovegetative symptoms associated with menopause.
Swedish MPA:	Traditionally used against mild climateric symptoms such as hot flushes, sweatings, sleep disturbances and nervousness.

3. Vitex Agnus Castus

Germany:	Regeltempoanomalien. Prämenstruelle Beschwerden, Mastodynie (E-Monographie: BAnz Nr. 226 v. 02.12.1992)
Belgium:	This drug based on plant(s) is used as an adjuvant treatment of functional premenstrual disorders and premenopausal disorders, after each severe pathology has been excluded.
UK:	A traditional herbal remedy for the relief of occasional feeling of bloatedness.
France:	Traditionellement utilisé dans les règles douloureuses (avis aux Fabricants, planned)
[Switzerland:*	... wirkt bei monatlich wiederkehrenden Beschwerden vor Eintritt der Regelblutung (prämenstruelles Syndrom), **Rhythmusstörungen der Regelblutung** (Regeltempoanomalien) und bei Spannungs- und Schwellungsgefühl in den Brüsten (Mastodynie).]

* not a EU-member state

Status of Work in the ESCOP Scientific Committee

Published SPCs 15 March 1996 (20):

Althaeae radix
Betulae folium
Boldo folium
Calendulae flos
Foeniculi fructus
Harpagophyti radix
Hyperici herba
Lini semen
Melissae folium
Orthosiphonis folium
Plantaginis ovatae semen
Plantaginis ovatae testa
Salviae folium
Solidaginis herba
Tanaceti parthenii herba
Taraxaci folium
Taraxaci radix
Thymi herba
Urticae radix
Zingiberis rhizoma

Next publication (30):

Absinthii herba
Allii sativi bulbus
Aloes
Anisi fructus
Arnicae flos
Carvi fructus
Frangulae cortex
Gentianae radix
Hamamelidis folium
Humuli lupuli strobilus
Juniperi fructus
Lichen islandicus
Meliloti herba
Menthae pip. aetheroleum
Menthae pip. folium
Ononidis radix
Passiflorae herba
Polygalae radix
Primulae radix
Psyllii semen
Rhamni purshianae cortex
Ribis nigri folium
Rosmarini folium
Salicis cortex
Sennae folium
Sennae fructus acutifoliae
Sennae fructus angustifoliae
Urticae folium
Uvae-ursi folium
Valerianae radix

Under discussion:

Cardui mariae fructus	B
Centaurii herba	B
Crataegi folium cum flore	E
Echinaceae pallidae radix	E
Echinaceae purpureae herba	E
Echinaceae purpureae radix	B
Hamamelidis aqua	E
Hamamelidis cortex	E
Hippocastani semen	B
Matricariae flos	E
Myrrha	B
Rhei radix	B
Trigonella foenum-graecum	B
Vaccinium myrtillus	E

Herbal drugs with serious risks without any accepted benefit (Not acceptable for revision)

Aconitum all species
parts: all parts
reason: contains aconitine and other toxic alkaloids, benefit not proven.

Angelica archangelica L.
parts: fruit, herb
reason: contains phototoxic furanocumarins, benefit not proven

Aristolochia all species
parts: all parts
reason: contains aristolochic acids, strong carcinogen, genotoxicity, benefit not proven

Artemisia cina (BERG.) WILLKOMM.
parts: Flower-bud
reason: contains the toxic lactone santonin benefit/risk negative

Berberis vulgaris L.
parts: bark, root bark, root
reason: contains the alkaloid berberine

Borago officinalis
parts: herb, flowers
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties

Bryonia all species
parts: root
reason: cytotoxic cucurbitacines, drastic laxative and emetic

Chenopodium ambrosioides L. var. anthelminthicum (L.) A.GRAY
parts: essential oil
reason: contains the toxic principle ascaridole, benefit/risk negative

Chrysanthemum vulgare (L.) BERNH.
parts: flower, herb
reason: may contain essential oil with the neurotoxic thujone

Citrullus colocynthis (L.) SCHRAD.
parts: fruit
reason: contains cytotoxic cucurbitacines drastic laxative

Claviceps purpurea (FR.) TULASNE
parts: Secale cornutum (Sclerotium)
reason: contains toxic ergot-alkaloids. Benefit/risk negative.

Convolvulus scammonia L.
parts: Resin
reason: drastic laxative with irritant properties

1.1. Figure 9a

Croton tiglium L.
parts: seed, fatty oil from seed
reason: drastic laxative,
contains tumor-promoting phorbol diesters

Cynoglossum officinale L.
parts: herb
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties

Dryopteris filix mas (L.) SCHOTT
parts: rhizome
reason: the constituents drug are highly toxic, severe intoxications may occur when absorption is increased, benefit/risk is negative

Exogonium purga (WEND) BENTH.
parts: root, resin
reason: drastic laxative with irritant action

Juglans regia L.
parts: Fruit-shell
reason: may contain the naphtoquinone juglone which is mutagenic and possibly carcinogenic.
No benefit proven.

Juniperus sabina L.
parts: herb
reason: toxic herb, no benefit proven

Ledum palstre L.
parts: herb
reason: contains essential oil which is a potent irritant of the GI-tract, kidneys and urinary tract.
No benefit proven

Mallotus phillipinensis (LAM.)MÜLLER-ARG.
parts: gland and trichomes (Kamala)
reason: drastic laxative which may cause severe gastroenteritis, diarrhoea and vomiting when taken in higher dosages; benefit/risk negative

Ocimum basilicum L.
parts: essential oil
reason: contains high amounts of estragole which is genotoxic and a carcinogen in rodents. No benefit proven

Petasites hybridus (L.) GAERT. MEYER et SCHREB.
parts: leaf
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties

Petroselinum crispum (MILL.) Nym. ex A.W.HILL
parts: fruit
reason: contains significant amounts of essential oil with toxic apiole. Apiole and the fruits are used for self-induced abortions.

1.1 Figure 9G

Pulsatilla vulgaris MILLER
parts: herb
reason: higher doses may irritate the kidneys and urinary tract and pregnancy is an absolute contraindication. No benefit proven.

Ruta graveolens L.
parts: herb, leafs
reason: causes phototoxic reactions, genotoxic, the use for self induced abortions resulted in fatal intoxications. No benefit proven.

Rubia tinctorum L.
parts: root
reason: contains lucidin with genotoxic and probably carcinogenic activity. No benefit proven.

Sassafras albidum (NUTT.) NEES
parts: wood, root
reason: contains essential oil with carcinogenic and genotoxic safrole. No benefit proven.

Senecio all species
parts: herb, root
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties

Strychnos nux-vomica L.
parts: seed
reason: contains alkaloids, especially strychnine. Benefit / risk negative.

Symphytum all species, internal use
parts: herb, leaf, root
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties. No benefit proven.

Teucrium chamaedris L.
parts: herb
reason: Hepatotoxicity

Tussilago farfara L.
parts: flower, root
reasons: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties. No benefit proven.

Vinca minor L.
parts: herb, leaf
reason: hematological changes (leucocytopenia, lymphocytopenia, reduced globuline levels) have been observed in rabbits. No benefit proven.

1.1. Figure 9c

Drugs with toxic principles, where a more detailed discussion concerning the benefit/risk ratio is necessary:

1. Drugs with pyrrolizidine-alkaloids where a use is accepted under special precautions/labelling:

Symphytum officinale L., external use
parts: leafs, herb, root
restrictions: use only on unbroken, intact skin, use during pregnancy requires medical advice, use not longer than 6 weeks per year, temporarily tolerable dose (TTD) 100 µg PA/day

Tussilago farfara L.
parts: leaf
restriction: contraindicated during pregnancy and lactation, use not longer than 6 weeks per year, temporarily tolerable dose (TTD) 1 µg (herbal tea 10µg) PA/day

Petasites hybridus (L.) GAERT. MEYER et SCHREB.
parts: rhizome
restriction: contraindicated during pregnancy and lactation, use not longer than 6 weeks per year, temporarily tolerable dose (TTD) 1 µg (herbal tea 10µg) PA/day

For these drugs a limitation of the toxic principle and a strict definition of the conditions of use is necessary.

A similar approach is necessary for herbal drugs with small amounts of toxic constituents and accepted uses, for example estragole in (sweet) fennel.

2. Drugs with cardiac glycosides

for example:
Adonis vernalis L.
Convallaria majalis L.
Digitalis species
Nerium oleander L.
Urginea maritima (L.) BAKER
Strophanthus species

For these drugs a benefit/risk assessment must be done during revision.

3. Drugs with alkaloids

for example:
Atropa belladonna L.
Cephaelis ipecacuanha KARSTEN
Datura stramonium L.
Ephedra sinica STAPF
Hyoscymaus niger
Pausinystalia yohimbé (K.SCHUM.) PIERRE
Rauwolfia serpentina (L.) BENTHAM ex KURZ

For these drugs a benefit/risk assessment must be done during revision.

1.1 Figure 9a

US-FDA categories:

category I :
active substances which are considered effective and safe under the indication and labeling given in the monograph

category II :
active substances or indications whose safety and effectiveness is not generally recognized in medical science. These substances will not be listed in a monograph.

category III :
active substances or indications for which the material presented for safety and efficacy is insufficient for classification. Further studies might be considered necessary. If they are presented an inclusion into category I might be considered.

American Herbal Pharmacopoeia™ Monographs

AHP Monographs Completed	
Hawthorn	*Crataegus laevigata*
St. John's Wort	*Hypericum perforatum*
Valerian	*Valeriana officinalis*
Willow bark	*Salix* spp.
AHP Monographs near completion	
Ashwagandha	*Withania somnifera*
Astragalus root	*Astragalus membranaceus*
Garlic	*Allium sativum*
Reishi mushroom	*Ganoderma lucidum*
Schizandra	*Schisandra chinensis*
AHP Monographs in Process	
Billberry	*Vaccinium myrtillis*
Black Haw	*Viburnum prunifolium*
Chamomile	*Matricaria chamomilla*
Chaste berry	*Vitex agnus castus*
Cramp bark	*Viburnum opulus*
Dandelion Lf & Rt	*Taraxacum officinalis*
Dong Qui	*Angelica sinensis*
Echinacea	*Echinacea angustifolia, E. pallida, E. purpurea*
Ginger	*Zingiber officinale*
Ginkgo	*Ginkgo biloba*
Ginseng	*Panax ginseng, P. quinquefolius*
Goldenseal	*Hydrastis canadensis*
Lemon balm	*Melissa officinalis*
Licorice	*Glycyrrhiza uralensis, G. glabra*
Milk thistle	*Silybum marianum*
Momordica	*Momordica charantia*
Nettles Lf & Rt	*Urtica dioica*
Peppermint	*Mentha piperita*
Saw palmetto	*Serenoa repens*
Uva ursi	*Arctostaphylos uva-ursi*

be documented, either by studies or, to a lesser degree, by suitable biographical data pertaining to the medicinal system used.

c) China

The system in China, distinguishing between rational, western and TCM, works similar as in Japan. The level of requirements is in both countries relatively high, but, different from the US, leaves room for pragmatic solutions.

The world Health Organization

The aims of the WHO in the pharmaceutical sector are, in a nutshell, the availability of affordable, safe and effective medicines to every patient on earth. One step in this direction was the establishing of a list of basic substances for treatment of fundamental diseases around the globe, the edition of an international pharmacopoia to set standards and analytical methods for these substances, and also the publishing of GMP and GLP/GCP guidelines. The entire policy is laid down in the WHO drug strategy of 1991 (EB89/Inf. Doc./4).

Already early in its existence the WHO recognized, that phytomedicines are a welcome addition to the basic substances list, since they are readily available and traditionally used in many developing countries. Guidelines were published on „GMP for herbal medicinal products" (Pharm./92/178), on „Quality control methods for medicinal plant materials" (Pharm./90.152./TRM 90.3/rev.) and also „Guidelines for the assessment of herbal medicines" (WHO1991).

In 1994 WHO started a major effort to compile a list of herbs that are widely used in primary health care in various countries around the world. In a parallel step, monographs on each botanical are supposed to be developed, a task being given to Norman Farnsworth of the Univ. of Illinois in Chicago as spiritus rector of a group of experts. Presently 25 monographs encompassing 28 plant species on WHO's list of „Widely used medicinal plants" are being published. 30 more are scheduled for publication in a second volume (Figure 12).

These monographs vary from the standard pharmacopoial ones in such way, as they encompass both the quality aspect and the SPC aspect(figure 13). The acceptance of the WHO approach by national authorities of 2nd and 3rd world countries is presently unknown.

Some response is encouraging. In 1996, the International Conference of Drug Registering Agencies (ICDRA) accepted these monographs as helpful tools for decision. They constitute , accordingly, a recommendation to those states, which have no regulations as yet of their own to evaluate registration applications of phytomedicines. In this context, it is worthwhile to note, that ICDRA proposed in 1991 a list of activities to WHO in order to bring acceptable, safe and effective phytomedicines to market (Figure 14).

The situation in the southern hemisphere of America

This situation I cannot assess. A lot of research effort is known to me which occurs into the field of ethnobotanical use of medicinal plants. To us Europeans, the work in Mexico on native, indian medicine is well known e.g. However, nothing is known about the regulatory status of common phytomedicines and I would welcome Your comments on that to further my knowledge. Thank You.

WHO Monographs

Latin Name/Monograph Title	Common Name
Allium cepa	Onion
Allium sativum	Garlic
Aloe vera gel	Aloe vera
Aloe vera juice	Aloe vera
Astragalus membranaceus	Astragalus
Brucea javanica	Java brucea
Bupleurum falcatum	Bupleurum
Bupleurum falcatum p var. scorzonerifolium	Bupleurum
Centella asiatica	Gotu kola
Chamomilla recutita	Chamomile
Cinnamomum verum	Cinnamon
Cinnamomum cassia	Cassia
Coptis chinensis	Goldthread
Coptis deltoides	Goldthread
Coptis japonica	Goldthread
Curcuma longa	Turmeric
Echinacea angustifolia var. angustifolia	Echinacea
Echinacea angustifolia var. strigosa	Echinacea
Echinacea pallida	Echinacea
Echinacea purpurea	Echinacea, purple coneflower
Ephedra sinica	Ephedra, ma huang
Ginkgo biloba	Ginkgo
Panax ginseng	Ginseng, Asian
Glycyrrhiza glabra	Licorice
Glycyrrhiza uralensis	Licorice
Paeonia lactiflora	Peony
Plantago afra	Psyllium
Plantago indica	Psyllium
Plantago ovata	Psyllium
Plantago asiatica	Psyllium
Platycodon grandiflorum	Platycodon
Rauvolfia serpentina	Indian snakeroot
Rheum officinale	Rhubarb
Rheum palmatum	Rhubarb
Cassia senna (leaf)	Senna leaf
Cassia senna (fruit)	Senna pod
Thymus vulgaris	Thyme
Thymus zygis	Thyme
Valeriana officinalis	Valerian
Zingiber officinale	Ginger

Partial Outline of WHO Medicinal Plant Monographs

developed by Prof. Norman R. Farnsworth, Harry H.S. Fong, and Gail Mahady, PCRPS, University of Illinois, Chicago

TITLE (Latin)

Definition
Synonyms
    Vernacular names
Plant description
Description of plant material
    General appearance
    Organoleptic properties
    Microscopic characteristics
        Powdered drug
Geographical distribution
General identity tests
Purity tests
    Microbiological
        External use
        Internal use
    Chemical
    Foreign organic matter
    Total ash
    Acid-insoluble ash
    Alcohol-soluble extractive
    Pesticide residues
    Radioactivity residues
Assays
    Chemical assay
    Biological assay
Major chemical consituents
Dosage forms
    Storage
Clinical use
Pharmacology
    Experimental pharmacology
    Clinical pharmacology
Contraindications
Warnings
Precautions
    General
    Drug interactions
    Drug/laboratory test interactions
    Carcinogenesis, mutagenesis, impairment of fertility
    Pregnancy: teratogenic effects
    Pregnancy: non-teratogenic effects
    Nursing mothers
    Pediatric use
Side effects
Posology (dosage)
Additional comments
References

1.1. Figure 13

Recommendations of ICDRA to WHO concerning phytomedicines

-modification of the guidelines to local requirements of WHO member states in order to obtain a uniform standard

-continuing elaboration of monographs and methods for the assessment of safety and efficacy by WHO

-development of model guidelines for clinical assessment of phytomedicines by WHO

-exchange of information about the status of phytomedicines and experience concerning the application of the guidelines in the various countries

-listing of those medicinal plants, which are most frequently used to treat afflictions in the particular member state

-establishing of a monitoring system to assess risks originating from use of phytomedicines

INTERNATIONAL TRAINING

COURSE ON PRODUCTION OF PHYTOMEDICINES

Panama, 24 November - 5 December 1997

MARKET TRENDS FOR PHYTOPHARMACEUTICALS AND NATURAL PRODUCTS IN LATIN AMERICA

Prof. Nikolai Sharapin

Natural Products Technology Laboratory. Faculty of Pharmacy. Federal Fluminense University. Niteroi, RJ, Brazil

MARKET TRENDS FOR PHYTOPHARMACEUTICALS AND NATURAL PRODUCTS IN LATIN AMERICA

INTRODUCTION

Plants have always played major role in the treatment of human diseases. medicinal plants still account for approximately 25 % of all medical prescriptions in developed countries and for approximately 80 % in developing countries.

In the industrialized countries medicinal plants are used as raw materials for extraction of active compounds or for isolation of abundant but inactive compounds which can be converted in active substances by partial synthesis. In developing countries drugs are used as such or as extracts or as traditional preparations.

Latin America consumes only a small percentage (5%) of the total world consumption of drugs, although it has about 10 % of world's population. Due to the limited coverage by health services and the lack of access of a significant part of the population to the pharmaceuticals, plant based traditional medicine still play a role in the health care of the majority of the people in Latin America. It is important to point that while the consumption of medicament in the industrialized countries raised from 0,65 % to 0,9 % of G.I.R. during the period 1975 - 1990, the consumption of medicaments in the developing countries lowered from 0,79 to 0,67 % of the G.I.R. In Latin America the consumption of medicaments per-capita / year is of US 21.00, while it reaches US $ 256,00 in Japan and 182,00 in the United States. There are differences between Latin American countries (Argentina 65 dollars, Brazil 17, Bolivia 6) and between the regions of the same country (Brazil: Northeast - less than 5 dollars, south - 70 dollars, Sao Paulo - 90 dollars).(1)

Due to the lack of acquisitive power and the lack of public resources for purchase and distribution of medicaments, significative part - about 50 % - of Latin American population has no access to the industrialized medicaments and depend, on some extend, on medicinal plants for its health care.

The pharmaceutical market in Latin America is controlled by international laboratories. The participation of national companies is small. It varies from 50 % (Argentina) to 20 % (Brazil, Colombia) to 10 % (Costa Rica, Ecuador). The procession of raw materials for pharmaceutic industry is scarce, about 75 % of pharmaceutical raw materials being imported. Pharmaceutical end-products also are imported to some extend. Argentina, Brazil and Mexico import less than 10 % of their needs, while Central American countries import as much as 80 %.

The impossibility of access to the industrialized medicaments by a significative part of Latin America population, the increasing control of the pharmaceutical industry by the international laboratories and the decreasing participation of the national governments in the purchase and distribution of medicaments should stimulate the use of medicinal plants in order to improve the health care and to improve the deficit in the commercial balance.

Other reasons that should stimulate the national laboratories to produce plant based medicines should be:

- the green consumerism and the growing demand for "naturals" in developed countries

- the search for new pharmaceuticals from plant kingdom to combat chronic and life-threatening diseases
- the free market economy creating demand for new materials and products.

THE PRODUCTION OF PHYTOPHARMACEUTICALS IN LATIN AMERICA

The plant based pharmaceutical companies are usually small enterprises which formulate powdered plants or plant extracts into dosage forms. Some of them are concerned with the production of vegetable raw materials such as diosgenin, pilocarpine, rutin, essential oils and vegetable dyes. Few laboratories produce modern dosage forms based on vegetable extracts. Pharmaceutical end-products are consumed within the country, while essential oils and pure natural products are directed to exportations.

The industrial infrastructure of these industries is, in general, poor, the qualified human resources are scarce and, frequently, the quality of the products is poor.

The problems which influentiate the poor development of plant based industry are, in greater or smaller extend, related to:

- poor knowledge of economic, social and medical benefits of this type of industry,
- the non-prescription of phytomedicines by medical practitioners,
- the lack of technological knowledge for adequate fabrication of phytomedicines,
- inexistence of lack of knowledge of quality control and standardization methods,
- difficulties with a supply of medicinal plats in the amount and quality suitable for industrial use,
- the lack of investments in R & D in agrotechnology, phytochemistry, pharmaceutical technology, validation and therapeutic,
- regulation for registration of phytomedicines and other legal problems,
- few incentives from national governments to this type of industry.

The pharmaceutical industry does not seem interested in increase the production of phytopharmaceuticals end-products as they are considered as low-profitable. The production technology is poor and the products are not well accepted at the ethical market. The lack of investigation on native plants difficults the knowledge about commercial and medical possibilities of these products. On the other side, the government authorities are not sure that the industrialization of medicinal plats will really benefit the primary health care. The common point of view of the health authorities on referring to the medicinal plants is that they represent a cheap alternative for those populations that have no access to the industrialized medicaments.

The problems of quality control constitute serious limitation, as well as the problem of standardization of medicinal plants. Some countries count with monographs on medicinal plants, but the great majority of native medicinal plants

have no specifications to determine the authenticity, the purity and the quality of vegetable raw material as well of the pharmaceutical end-products.

PRODUCTION AND INTERNATIONAL TRADE ON VEGETABLE RAW MATERIALS

It is difficult to estimate Latin American production of vegetable raw materials. Statistics on international trade are incomplete and difficult to access. More informations are provided on aromatic plants, mainly from Argentina, Brazil and Chile.

ARGENTINA

The pharmaceutic industry at Argentina imports approximately 500 tons/year of vegetable extracts for medicinal uses, esteemed in 8 million dollars and some 20 tons / year of vegetable heterosides (15 million dollars). The main medicinal plant exported from Argentina is German chamomile (Matricaria recutita), the export being estimated at 20 million dollars.(2)

Concerning aromatic plants and their derivatives, the lemon oil is the main export item. The Argentinean production of lemon oil was of 1780 tons in the year of 1996, 1620 tons being exported, mainly to United States and United Kingdom. The import of essential oils was of 2 million dollars and comprised mainly orange essential oil.[3]

The commercial balance on essential oils is very favorable to Argentina, with an annual income of approximately 35 million dollars.

BRAZIL

Brazilian import of medicinal plants, plant extracts, glycosides, alkaloids, essential oils and steroid hormones (which are not natural products but are obtained by semi-synthesis from natural raw materials) reaches 40 - 45 million dollars/year. The summary of brazilian import / export can be seen below(7).

Brazilian trade on medicinal plants and related products

	IMPORT		EXPORT	
	tons	**US $ 1000**	**tons**	**US $ 1000**
Medicinal plants	1.500	1.600	800	3.500
Plant extracts	600	2.500	200	1.200
Heterosides	20	1.300	300	6.000
Alkaloids	25	15.000	20	30.000
Essential oils	12.000	15.000	?	2.500
Steroid hormones	20	12.000	?	7.000

Medicinal plants - The main items imported are liquorice (Glycyrrhiza glabra), Origanum (Origanum majorana) cascara (Rhamnus purshiana) and chamomile (Matricaria recutita). The exported plants are guarana (Paulinia cupana), tonka beans (Coumaruna odorata) and arruda, name which designs the specie Ruta graveolens, but is frequently used for the Pilocarpus microphyllus. This is a source of pilocarpine and its export is forbidden by law.

The extracts imported are mainly those of hops and liquorice and the export concerns mainly with the extracts of liquorice, Arnica montana and catuaba (Erythroxylon vaccinifolium), this one directed to Germany.

The main imported heterosides are digoxin, diosmin and glycyrrhizin. The balance of heterosides trade is very favorable to Brazil due to rutin which is one of natural products fabricated in the country. Caffeine is the principal alkaloid imported (both natural and synthetic). Other alkaloids are those of Claviceps purpurea, scopolamine and Cinchona alkaloids. This balance is also favorable to Brazil due to the export of pilocarpine salts produced in the country in the amount of 10 - 14 tons / year.

Among the essential oils the principal import item is that of Mentha arvensis, at the value of more than 8 million dollars. Brazil was, for many years, the main producer of Mentha arvensis oil but have lost its position due to the cultivation problems. Nowadays, the mentha oil is imported from Paraguay.

Steroid hormones are not natural products but they are obtained by partial synthesis from natural raw materials. Brazil does not produce steroid hormones so the export figures are referred to the products which were submitted to one or two synthetic steps, such as esterification.

CHILE

Few commercial enterprises are involved in the international trade of medicinal plants. Chile exports over than 20 million dollars of medicinal plants, of which Quillaja saponaria amounts top 800.000 dollars. Other export items are Peumus boldus, Origanum majorana, Rosa perruna (rosa mosqueta) and Smilax medica. Quillaja, rosa mosqueta and boldo are collected in the wild. Rosa mosqueta and boldo are exported mainly to Argentina and Brazil. Origanum and Quillaja are exported mainly to Germany. (6)

During the period 1992 - 1994 Chile imported 117 - 118 tons of medicinal and aromatic plats per year, which corresponds to about 320.000 dollars. The main items imported were ginseng roots (from Korea and Popular Republic of China), origanim (from Peru) and, in a minor scale, belladonna, cascara, valeriana, hamamelis and ipecac. The commercial balance on medicinal plants was positive during the above mentioned period.

Chile exports lemon and Mentha piperita essential oils. In 1994 the chilean export of essential oils reached 517 tons which corresponds to about 0,5 million dollars. The import of essential oils during the same period reached the volume of 100 tons/year corresponding to approximately 1,3 million dollars. The main products imported were citric oils, however the lavender essential oil was also imported during the above mentioned period. Cultivation trials in order to substitute the imported products have been established.

SPICES

The analysis of spice market in Japan and in European Union shows that the market grew 5,3 % in Japan an 3,45 % in the European Union. The Japanese market of spices is of about 100.000 tons/year corresponding to 140 million dollars. The main products for Japanese market are ginger, black pepper, capsicum,

curcuma and coriander. The main products at the EU market are black pepper, capsicum, ginger and coriander. The world market of spices showed a 2 % grow during the years 1992 - 1996. Mexico and Guatemala were the only Latin American countries which increased their export of spices in 14 % and 8 % respectively. (4, 5)

INCREASED DEMAND FOR HERBAL MEDICINES

At the same time that demand for herbal medicines is growing in the developing countries, research in the industrialized countries shows that a considerable part of population in those countries may be using some form of complementary medicine. This increasing demand for phytopharmaceuticals in both industrialized and developing countries is creating new patterns of medicinal plant harvesting. There are evidences that these patterns are exceeding the capacity of supply.

In 1997 the World Bank has issued reports on medicinal plants stating that with appropriate policies for conservation, cultivation, processing and marketing the medicinal plants may constitute a possible bridge between sustainable development, health care and conservation of the biodiversity. (8,9)

The demands of the majority of the people in developing countries for medicinal plants have been met by indiscriminate harvesting of spontaneous flora including those in the forest. The tropical rain forest regions in South America suffers processes such as deforestation, desertification and space occupation by agricultural areas, endangering several species of medicinal and economic value. The rational commercial exploitation of natural products from the forest is the only way to avoid felling and destruction by local populations and external economic interests in search of short term gain. Rational exploitation can be achieved with no permanent damage to the eco-system and scientific management of already damaged areas can ac accelerate recovery.

THE TROPICAL RAIN FOREST NATURAL PRODUCTS - AN INDUSTRIAL EXPLOITATION

While the exploitation of a small area with the object of industrializing a small number of known products is an activity that could be rapidly implemented, a program which aims at resolving the problem of forest preservation over a large area requires a number of diverse activities beyond the scope of single project and requires the collaboration of all organizations which are able to contribute technically.

The central rain forest region comprises parts of Bolivia, Brazil, Colombia, Ecuador, the Guyana's, Peru and Venezuela. It would be rational from the technical and economical points of view that the problems related to the industrial exploitation of the tropical rain forest should be held by the organizations in these countries working in cooperation.

As the problems of economic forest development are also relevant to Central America it would be important that these countries also participate in the program.

The destruction of the Amazonian forest and of the neighboring forest areas lead to the disappearance of diverse products characteristic of the habitat. The

commercialization of these products, of which several enjoy an expanding market, will create a strong break on the destructive process. Also a number of plants exists which could be cultivated in areas already devastated, contributing to the regional economic product and leading to the restoration of the forest cover.

The natural product market is undergoing rapid growth in the industrialized world. This is the market where Latin America countries possess an advantage as producer and, in some cases, a virtual monopoly.

The classification of commercializable or potentially commercializable natural products from the tropical rain forest can be made in three ways:

1. According to the origin

- Extractive products, that is products whose exploitation requires neither clearing nor plantation;
- Products of forest management, whose production requires some kind of agro-forestry operation, when continuing supply is envisaged;
- Products derived from the plantation of devastated marginal areas.

This classification determines the nature of primary operation.

2. According to the nature of the product:

- Vegetable oils, usually obtained by the expression or extracting fruits or seeds;
- Essential oils, obtained by steam destillation, extraction or direct destillation of plant material or by a combination of such processes;
- Crude extracts, obtained by the extraction with water, ethanol or other medium of vegetable material followed or not by concentration to a paste or dry powder;
- Pure or crude products, usually solid, normally obtained from the extracts by physical procedures;
- Powders obtained mechanically from the plant material or without extraction.

The three last subdivisions include the majority of medicinal, colors, aromas and pesticides. This classification determines the nature of industrial installation.

3. According to use:

- Food and drink additives
    - -colors
    - aromas and flavors
    - sweeteners and bitter principles
- Cosmetic and perfume materials
    - pigments
    - oils and fats
    - fragrances
- -Medicinals
- Insecticides or products for agricultural use or human and veterinary disease control
- Raw materials for industrial chemical transformations.

This classification is directly associated with marketing.

The objectives should be to preserve the tropical rain forest by means of the commercialization of regional natural products within international concepts of

quality and continuity of supply, aiming principally at the external market for medicinal products, aromas, food and soft drinks additives, cosmetics and pesticides. The formation of local cooperatives for the collection and primary processing of the regional products should be stimulated so that these cooperatives themselves take a direct interest in the preservation of forest. The commercial exploitation should be implemented envolving such cooperatives , private companies or land owners with the support of technical and scientific organizations, the range of commercializable natural products extended by the way of multidisciplinary research projects which cover not only chemical identification but also evaluation of their use.

Examples of some products for which a market exists or existed before synthetic products displaced them are listed below. There are many other potentially economic plant species that may be considered.(10)

PRODUCTS EXPLOITABLE BY FOREST MANAGEMENT				
PRODUCTS	**PLANT SOURCE**	**USE**	**TYPE**	**PROCESS**
Annatto	*Bixa orelana*	colour	poweder or concentrate	Extr. seeds or mechan separation
Candlewood (1)	*Vanillosmopsis erythrocarpa*	herb tea	ess. oil	Steam dist. wood
Capsaicin (1)	*Capsicum spp.*	pharmaceutical	concentrate	Extr. seeds org. solv.
Cedrelone	*Cedrela odorata* and other species	insecticide	cryst. solid	Ethanol extr. leaves fruits, etc.
Guaraná	*Paullinia cupana*	flavour (2)	concentrate	Aq. extr. fruit
Ipeca	*Cephaelis ipecacuanha (Uragoga ipecacuanha)*	medicinal (3)	concentrate or root	Ethanol extr. root, concentration
Neem (or azadirachtin) (1)	*Azadirachta indica*	insecticide	concentrate or solid	Aq. extr. fruit, concentration
Quassia	*Quassia amara*	insecticide	concentrate or powder	Aq. extr. wood, concentration
Rotenone (or Derris)	*Lonchocarpus nicou*	insecticide	solid/dust	Ethanol extr. root, concentration

(1) These products are derived from plants not native to the region, and the possibility of their adaptation requires study.

(2) Guaraná is classified as a medicine by the FDA.

(3) A large number of medicinal plants are reported for Amazonas. Few of these are commercialed outside the area but they represent an important economic and social potential of the region.

PRODUCTS DERIVABLE FROM NATURAL SOURCES WITHOUT PLANTING

PRODUCT (1)	PLANT SOURCE	USE	TYPE	(%) OIL IN SEED	PROCESS
Agaí (assai)	*Euterpe oleracea*	flavour	concentrate		Extr. fruit pulp. conc.
Andiroba	*Carapa guianensis*	medicinal	oil	63	Expr./extr. seed
BabaÇu (Babassu)	*Orbignya martiana*	food, cosmetic	oil	65	Expr./extr. seed
Bacuri	*Platonia insignis*	flavour	concentrate		Extr. fruit pulp. conc.
Balsamo-do-peru (Peru balsam)	*Myroxylon balsamum or M. peruiferum*	medicinal	resin		Tap trunk
Breu branco	*Burseraceae*	medicinal	resin		trunk exudate
Buriti (Miriti)	*Mauritia flexuosa*	nutritional	oil	pulp 8; kemel 48	Extr. fruit puilp; Expr./extr. seed
Carajurona chica red)	*Arrabidaea chica*	colour	powder or concentrate		Aq. extr. leaves ferment
Castanha-do-Pará (Brazil nut) (2)	*Bertholletia excelsa*	food	*in natura*	67	Note 2
Copaíba (Brazil copal)	*Copaifera spp.*	medicinal	resin		Tap trunk
CupuaÇu	*Theobroma bicolor and T.grandiflorum*	flavour	concentrate and fat		Extr. fruit pulp. or extr. seed
Patauá	*Jessenia bataua*	food	oil	21	Expr./extr. fruit pulp. and seed
Pequiá	*Caryocar villosum*	formulation, cosmetic	fat fat	nd 70	Extr. fruit pulp. Expr./extr. seed
Pupunha (pejibaye)	*Bactris gasipaes*	food	oil	21	Expr./extr. fruit pulp. and seed
Ryania (or ryanodine)	*Ryania acuminata Ryania speciosa*	insecticide	powder		Powered whole plant (or aq. extract, conc.)
Sapoti	*Achras sapota*	Chewing gum, balata	latex		Tap trunk
Sorva (3)	*Couma utilis (3)*	chewing gum, latex	latex		Tap trunk
Ucuuba	*Virola surianensis*	cosmetic	fat		Expr./extr. seed

(1) Some of the listed products are already commercialized on a large scale, but preliminary information indicates that new markets exist, specially in the cosmetic and foods areas. Other potential economic plants which have never been exploited, as for is known, are not listed here, but are described in publications cited in section 5.

(2) The nuts are commercilized as such, since the oil, once extracted, rancifies rapidly.

(3) These are many other batata producing species in tropical American both Sapotaceae such as Mimusops batata and Mnilkara bidentata and Apocynaceae such Couma rigida and Eschokkea lactescens.

In each case the industrial development procedure will consist of the following steps:

- setting up the local production scheme which should include primary industrialization to achieve maximum practical added value.
- Establishment of trading centers where the primary product is acquired by the industrial processes at previously agreeded price.

- Processing at the industrial level to an internationally accepted quality.
- Marketing.
- Support activities, including diverse social actions including health and educational services essential to the maintenance of production in a rain-forest environment.

In principle, the organization should be set in such a way as to concentrate the maximum added value in the region since it is this that will induce the conservation of the forest. However, it may be that in the initial stages some of the secondary upgrading of products may occur outside the region.

It should be established however from the start that crude products should be not exported for upgrading outside the country of origin, since this will conduce to predatory exploitation of the type that in the past brought about a virtual extinction of economic plants.

It is proposed that implementation should be encouraged in the form of individual agile projects, involving a company, a landowner and a rural cooperative at one side and one or two technical - scientific organizations with appropriate specialist capabilities at the other and, between the two, an interface company specialized in the industrialization of economic products.

To industrialize extractive products the local organization will normally be a cooperative and it would be responsible for collection and primary processing, such as oil-pressing, steam destilation, water extraction etc... The products will be packed and send for sale to previously agreed industrial organization at previously agreeded prices (experience shows that free trading of crude products leads to export of these by unqualified enterprises which do not maintain quality standards). The final producer will be a company able to place on the market a product with standard quality and to maintain adequate stock to meet the demand without delay. It would possess a chemical analytical laboratory for quality control and the equipment to attain the standard specification for each product.

It is foreseeable that this producer would also market the product, but in some cases this will better conducted by firms specializing who by the virtue of their market knowledge are able to obtain better prices or effect larger sales.

Laboratories with scientific and technological capacity will give support to the cooperatives and to the final producer in order to optimize production methods. This technical support will necessary include ecological and botanical informations as well as chemical and engineering know-how.

Concerning the agro-forestry products, the local organization will normally be a company possessing the land to be developed. This organization will modify the forest by the introduction of economic species which are adapted to permanent forest cover and will harvest and process the product where possible to specified quality.

Concerning the products derived from the cultivation of marginal or devastated areas, the local organization may be a cooperative or a company, the object being either the reflorestation of devastated areas or land uses compatible with the soil and climatic conditions. Thus not only trees but herbaceous cultures

could be contemplated in areas in which a reasonably quick economic return is essential to commercial success. However it is anticipated that species capable of restoring forest cover should be included in a mixed culture, when practical. Agronomic and biotechnological support, in these cases, will be fundamental.

In view of the fact that the market is a growing one and that, in the case of some products, is just beginning to appear or is still potential it is proposed therefore to further the market research in the industrialized countries not only with the object of discovering the demand for known products but also to introduce into the market new products which are potentially acceptable.

GENERAL GUIDELINES FOR THE PRODUCTION OF PLANT BASED PRODUCTS

Considering the production of medicinal and economic plants in areas other than tropical rain forest area, the first step to improve the supply and the quality of vegetable raw materials is to start agronomic trials on cultivation of medicinal plants. The introduction and acclimatization of foreign plants must also be considered. In Brazil there were successfully introduced plants such as Digitalis lanata (for cardiac glycosides), Duboisia spp. (for scopolamine) and Artemisia annua (for artemisinine and derivatives). After 8 years of agronomic research artemisinine content in cultivated plants was improved from 0,1 % to 1,0 %. /The agronomic research should be concerned with development of fast growing and disease resistant varieties, safe use of fertilizers and pesticides and the determination of time of harvest, as well as port-harvest procedures.

Considering that is lack of trained personnel in the fields of technology and engineering human resources in these areas have to be developed. The development of human resources will create direct impact on technological processes with the development of extraction procedures in laboratory, pilot and industrial scales. Also quality control protocols will be elaborated in order to assure the international quality standards.

Marketability is a determining factor for a success or failure of plant based industries. developing countries need better knowledge on demand and supply situation, price trends and qualities of products that could be marketed. They need identify marketing arrangements and trading companies and elaborate strategies for export promotion.

CONCLUSIONS

Developing countries which are producing plant based products have to overcome several problems to be competitive in the world market. Some of the problems associated with these industries are:

- the lack of technological knowledge in agrotechnology, pharmaceutical technology, extraction processes and quality control

- the lack of research and development on high yielding varieties of medicinal plants and on domestication of native species

- difficulties on marketing

- the lack of research and development on product and process development

- the lack of qualified man power. (11)

In order to overcome these constraints developing countries need to develop the technological and scientific capabilities and improve the production of plant derived [products to the internationally accepted standards. More emphasis should be put on the applied research strengthening the links between the university and the industry. Expert advice and assistment should be provided on market data. The assistance of international organisms in this field will be of great value.

Novel and non-conventional approach should be applied to conservation of tropical rain forest areas. The sustainable use of renewable resources will not only contribute to industrial development and improvement of living standards or rural populations but also to biodiversity and forest conservation.

REFERENCES

1. J. C. GEREZ. Industria Farmaceutica: histórico, mercado e competicao. Ciencia hoje 15(89); 21-33, 1993

2. A. L. BANDONI. Situación de las plantas medicinales y medicamentos herbolarios en Argentina. Seminario RIPROFITO. Resumenes. Guatemala, 1993

3. M.M. JUAREZ, G. FERNANDEZ y M. A. ELECHOSA. Produción, exportación y importación argentina de aceites esenciales citricos en el quinquenio 1992 - 1996. II WOCMAP. Resumenes. P-484. Argentina, 1997

4. O. ARIZIO. Caracterización del mercado mundial de especias de los primeros anos de la decada de 90. II WOCMAP. Resumenes. P-486. Argentina, 1997

5. O. ARIZIO. Analisis de la demanda de especias del mercado europeo durante lor primer anos de la decada de 90. II WOCMAP. Resumenes. P-487. Argentina, 1997.

6. G. PORTILLA. Comercio Internacional Chileno de Plantas Medicinales y/o aromaticas y de aceites esenciales. Actas. 2 Congreso de Plantas Medicinales - Chile 95. pg. 168-179. Chile, 1995.

7. L.C. PEREZ DIAS. Comercio Exterior de Materias-primas Farmaceuticas de Origem Vegetal - Panorama da Situacao Brasileira. XIII Simposio de Plantas Medicinais do Brasil, Resumos. Brazil, 1994.

8. J. SRIVASTAVA et alii. Medicinal Planyts: An expanding Role indevelopment. World Bank Technical Paper N. 320. The World Bank, Washington D.C., 1997.

9. J.LAMBERTm et alii. Medidcinal Plants: Rescuing a global heritage. World Bank Technical Paper N. 355. The World Bank, Washington D.C., 1997.

10. B. GILBERT. The industrial Exploitation of Tropical Rain Forest Natural Products. Unpublished. 1994.

11. T. de Silva. Production of Herbal Medicines in Developing Countries. International Symnposium on Herbal Medicine. Proceedings. Honolulu, Hawaii, USA. 1997.

Work Programme for the European Agency for the Evaluation of Medicinal Products 1997 - 1998

1. Introduction and priority tasks for the EMEA

This work programme for 1997 and 1998, presented by the Executive Director in accordance with Article 55(3) of Council Regulation (EEC) No 2309/93, was adopted by the Management Board on 5 February 1997.

Previous activities of the European Agency for the Evaluation of Medicinal Products (EMEA) are described in the 1995 and 1996 Annual Reports (see Reference Documents, p.37).

1.1 EMEA objectives

Council Regulation (EEC) No 2309/93 sets out the main objectives for the EMEA as follows:

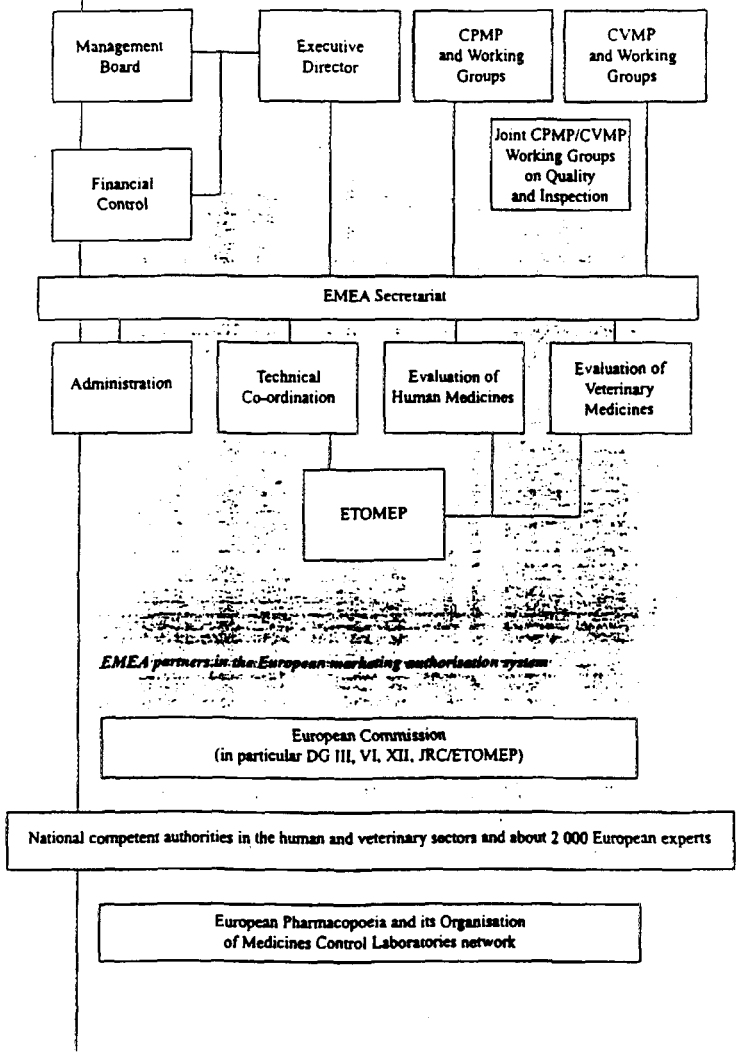
- to protect public health by mobilising the best scientific resources existing within the European Union (see Articles 49 and 51(a))
- to promote health care through the effective regulation of new pharmaceuticals and better information for users and health professionals (see Article 51(i))
- to facilitate the free circulation of pharmaceuticals within the European single market (see Article 51, first paragraph)
- to support the European pharmaceutical research and development industry by developing efficient, effective and responsive operating procedures (see Article 51, first paragraph)
- to support efforts in international co-operation (see Articles 51(f))

1.2 EMEA overall priorities

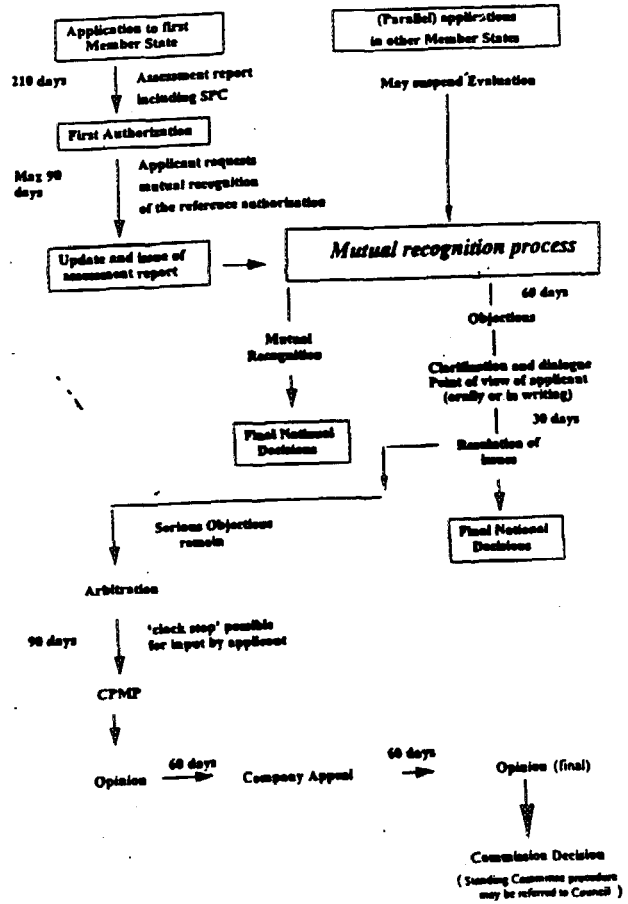
The Management Board has determined the following overall priorities for 1997-1998:

1. centralised applications for marketing authorisations for medicinal products (Council Regulation (EEC) No 2309/93, Article 4)
2. maintenance and pharmacovigilance activities (Council Regulation (EEC) No 2309/93, Articles 15-25, Articles 37-47)
3. establishment of maximum residue limits for substances in veterinary medicinal products (Council Regulation (EEC) No 2309/93, Article 51)
4. arbitrations and other Community referral procedures (Council Directive 75/319/EEC as amended, Articles 10, 11 & 12, and Council Directive 81/851/EEC as amended, Articles 18, 19 & 20)
5. scientific advice to future applicants and the EU institutions (Council Regulation (EEC) No 2309/93, Article 51)
6. information to health care professionals and public (Council Regulation (EEC) No 2309/93, Article 51)
7. technical support to international harmonisation initiatives (ICH, VICH, etc.) (Council Regulation (EEC) No 2309/93, Article 51)
8. support for the mutual recognition national authorisations, as requested
9. support for certain European policies at the request of the Commission or European Parliament

Organigram of the European Agency for the Evaluation of Medicinal Products



Mutual recognition procedure(s)



EUROPEAN COMMISSION
DIRECTORATE-GENERAL III
INDUSTRY
Industrial affairs III: Consumer goods industries
Pharmaceuticals and Cosmetics

Suggestion to include a paragraph on scientific monographs into the NTA:
In Chapter I - 'Marketing Authorisations'
Section 4 - 'Stand Alone Applications for a Marketing Authorisation';
it is suggested to insert the following new paragraph under Heading 4.2 :

4.2 BIBLIOGRAPHICAL APPLICATIONS

(1) Where the constituent or constituents of the medicinal product have a well established medicinal use, with recognised efficacy and an acceptable level of safety, demonstrated by detailed references to published literature presented in accordance with second paragraph of Article 1 of Directive 75/318/EEC, an application (so called 'bibliographical') for marketing authorisation may be submitted in accordance with Directive 65/65/EEC, article 4.8.(a)ii.

(2) An applicant wishing to use Article 4.8 (a)ii) of Directive 65/65/EEC must fully satisfy all the requirements of Article 1 of Directive 75/318/EEC as well as those of Directives 65/65/EEC and 75/319/EEC as amended, in effect, submit a 'complete' application.

(3) Directive 75/318/EEC Article 1 states that "where pursuant to point 8(a) of Article 4, second paragraph, of Directive 65/65/EEC, references to published data are submitted, the provisions of this Directive [i.e. Directive 75/318/EEC] shall apply in like manner." In such cases, the full article or reference should be supplied, with necessary translations. Moreover, the Expert Reports must clearly state the grounds for using published references under the conditions set out in Directive 75/318/EEC. This would include the completion of all of the tabular formats provided in 'The rules governing medicinal products in the European Union, Volume 2B Notice to Applicants: Presentation and content of applications' where relevant, unless there is a justification that the study is not relevant for the medicinal product. The impurity/related substances profile and the decomposition products arising during storage must be clearly indicated in order to allow assessment of appropriate efficacy and safety.

(4) In the event that neither detailed reference to published literature, nor appropriate justification is available to cover all the requirements, the applicant must supplement the missing data with appropriate additional studies.

NEW: *"Scientific monographs on certain - herbal - substances (e.g. those drafted by the European Scientific Co-operative on Phytotherapy (ESCOP) and the World Health Organisation (WHO)) offer a valuable and updated overview on published scientific literature, which may be used to demonstrate the safety and efficacy of a medicinal product in a bibliographical application in accordance with Article 4.8 (a)ii. These monographs may help to avoid duplication of work and bring about gradual harmonisation in the evaluation of : herbal - medicinal products. Therefore the Commission and Member States recommend that both applicants and competent authorities should make systematic use of these monographs."*

(5) It should be noted that summary assessment reports such as the EPAR for Community marketing authorisations or evaluation reports on Maximum Residue Limits which are made publicly available by competent authorities for reasons of transparency would generally not be considered to supply sufficient information to meet the requirements of Directive 75/318/EEC.

7. MANUFACTURE OF HERBAL MEDICINAL PRODUCTS

Principle

Because of their often complex and variable nature, and the number and small quantity of defined active ingredients, control of starting materials, storage and processing assume particular importance in the manufacture of herbal medicinal products.

Premises

Storage areas

1. Crude (i.e. unprocessed) plants should be stored in separate areas. The storage area should be well ventilated and be equipped in such a way as to give protection against the entry of insects or other animals, especially rodents. Effective measures should be taken to prevent the spread of any such animals and microorganisms brought in with the crude plant and to prevent cross-contamination. Containers should be located in such a way as to allow free air circulation.

2. Special attention should be paid to the cleanliness and good maintenance of the storage areas particularly when dust is generated.

3. Storage of plants, extracts, tinctures and other preparations may require special conditions of humidity, temperature or light protection; these conditions should be provided and monitored.

Production area

4. Specific provisions should be taken during sampling, weighing, mixing and processing operations of crude plants whenever dust is generated, to facilitate cleaning and to avoid cross-contamination, as for example, dust extraction, dedicated premises, etc.

Documentation

Specifications for starting materials

5. Apart from the data described in General Guide (chapter 4, point 4.11), specifications for medicinal crude plants should include, as far as possible:

- the botanical name (with, if appropriate, the name of the originator of the classification, e.g. Linnaeus);
- the details of the source of the plant (country or region of origin, and where applicable, cultivation, time of harvesting, collection procedures, possible pesticides used, etc.);
- whether the whole plant or only a part is used;
- when a dried plant is purchased, the drying system should be specified;
- the description of the plant and its macro and microscopical examination;
- the suitable identification tests including, where appropriate, identification tests for known active ingredients, or markers. A reference authentic specimen should be available for identification purposes;
- the assay, where appropriate, of constituents of known therapeutic activity or of markers;
- the methods suitable to determine possible pesticide contamination and limits accepted;
- the tests to determine fungal and/or microbial contamination, including aflatoxins and pest-infestations, and limits accepted;
- the tests for toxic metals and for likely contaminants and adulterants;
- the tests for foreign materials.

Any treatment used to reduce fungal/microbial contamination or other infestation should be documented. Specifications for such proce-ss should be available and should include details of process, tests a limits for residues.

Processing instructions

6. The processing instructions should describe the different operations carried out upon the crude plant such as drying, crushing and sifting, and include drying time and temperatures, and methods used to control fragment or particle size. It should also describe security sieving or other methods of removing foreign materials.

For the production of a vegetable drug preparation, instructions should include details of base or solvent, time and temperatures of extraction, details of any concentration stages and methods used (see also the note for guidance "Quality of herbal remedies", Volume III of "The rules governing medicinal products in the European Community").

Sampling

7. Due to the fact that crude drugs are an aggregate of individual plants and contain an element of heterogeneity, their sampling has to be carried out with special care by personnel with particular expertise. Each batch should be identified by its own documentation.

Quality Control

8. Quality Control personnel should have particular expertise in herbal medicinal products in order to be able to carry out identification tests and recognize adulteration, the presence of fungal growth, infestations, non-uniformity within a delivery of crude plants, etc.

9. The identity and quality of vegetable drug preparations and of finished product should be tested as described in the note for guidance "Quality of herbal remedies".

FIXED COMBINATION MEDICINAL PRODUCTS

Note for Guidance

[EMEA status as of 17 April 1996]

Note for guidance concerning the application of section C.6 Part 4 of the Annex to Directive 91/507/EEC as amended, with a view to the submission of an application for a marketing authorization for a new medicinal product. This guideline should be read in conjunction with current EC guidelines (e.g clinical investigation of oral contraceptives, 1987; Investigation of Chiral Active Substances, 1991; Biostatistical methodology in clinical trials, 1992; Dose-Response information to Support Product Authorisation, 1993).

1. JUSTIFICATION

1.1 Applicants will be required to justify the particular combination of active substances proposed. Fixed combination products will only be considered acceptable if the proposed combination is based on valid therapeutic principles.

1.2 For any individual fixed combination it is necessary to assess the potential advantages in the clinical situation against possible disadvantages, in order to determine whether the product meets the requirements of the standards and protocols with respect to efficacy and safety.

Potential advantages of fixed combinations include one of the following:

a) an improvement of the benefit/risk assessment due to :

i. addition or potentiation of therapeutic activities of their substances, which results in:

- a level of efficacy similar to the one achievable by each active substance used alone at higher doses than in combination, but associated with a better safety profile

or

- a level of efficacy above the one achievable by a single substance with an acceptable safety profile.

ii. the counteracting by one substance of an adverse reaction produced by another one.

b) a simplification of therapy

which improves patient compliance. When it is the only claim, it would be restricted to particular situations (e.g. non-prescription products).

Disadvantages of fixed combinations include :

i. the fact that even a combination which meets the needs of the average patient is unlikely to be ideally adjusted for the needs of each individual patient;

ii. the addition of the different adverse reactions specific to each substance.

1.3 General rules

Combinations, in principle, may not be considered rational if the duration of action of the substances differ significantly. This may not necessarily apply where it can be shown that the combination is clinically valid despite differences in this respect, e.g. if one substance is intended to enhance absorption of the other or where the substances are intended to exert their effects successively.

Each substance of the fixed combination must have documented contribution within the combination.

The inclusion of a substance to counteract an adverse reaction of an other substance may be considered justified, but only if the adverse reaction is a serious or a commonly ocurring one.

The inclusion of a substance intended to produce unpleasant adverse effects as a means of preventing abuse in undesirable.

Substances having a critical dosage range or a narrow therapeutic index are unlikely to be suitable for inclusion in fixed combinations.

2. INDICATIONS

The indications claimed for a fixed-combination medicinal product should be such that the presence of each active substance makes a contribution to the claimed effect. The product should be formulated so that the dose and proportion of each substance present is appropriate for the intended use.

An indication must be a well-recognised disease state, modification of a physiological state, dysfunctional state, syndrome or pathological entity. The individual substances of a fixed combination may be intended to relieve simultaneously different symptoms of such a disease state. In this case, it should be a prerequisite that these symptoms regularly occur simultaneously in a clinically relevant intensity and for a relevant period of time. It will not be proper to regard each individual symptom as an indication for the fixed combination, since it may also occur in other diseases and for treating this symptom alone the other substances may be irrelevant.

Fixed combination medicinal products may be indicated in different situations:

- in first line therapy, for patients receiving previously neither of the substances
- in second line therapy, when monotherapy has not demonstrated a satisfactory benefit/risk ratio.

The applicant should clearly state if the claimed indication is first line, second line therapy or other uses and the clinical development should be performed accordingly.

3. PHARMACODYNAMIC AND PHARMACOKINETIC STUDIES

The possibility of interactions between the substances should always be considered. The applicant should submit data either to establish that such interactions do not occur or that they are clearly recognized and defined.

3.1 Pharmacodynamic studies

Frequently, the addition or the potentiation of the pharmacodynamic effects of the various substances may constitute the rationale of the fixed combination.

In this case several dose combinations for each substance might have to be tested and the concentration-response information can help to select the fixed combination leading to a satisfactory response.

3.2 Pharmacokinetic studies

In general, the applicant must demonstrate that the various substances do not affect each others respective pharmacokinetic patterns.

In some cases, however, a pharmacokinetic interaction (i.e. combination with a metabolism inhibitor) constitutes the rationale of the fixed combination.

These interactions should be studied in healthy volunteers but also in patients if the disease modifies the pharmacokinetics of one substance and in high risk subgroups (elderly, patients with renal failure or hepatic impairment).

4. EFFICACY AND SAFETY

It is permissible to distinguish between the extent of the studies required in the case of those fixed combinations which correspond closely to combinations which are already in widespread use provided these are thoroughly and reliably documented, and those studies required in the case of those combinations which are essentially new :

a) When the fixed combination corresponds closely to combinations that are already in widespread use, a well founded bibliographical data analysis should be submitted. Provided that the respective data are thoroughly and reliably documented, this analysis may be helpful in reducing the amount of clinical trials to be performed and could facilitate the selection of doses for each substance and the proposed dose range of the fixed combination.

b) When the fixed combination is essentially new (active substances not usually combined, unusual quantitative composition of usually combined substances or one substance entirely new), the data needed are similar to a new chemical entity in the situation where the fixed combination is to be proposed (first line or second line therapy). Existing experience with the substances should be taken into account.

4.1 Composition and dosage regimen

The proposed dosage regimen must be justified.

The dosage of each substance within the fixed combination must be such as the combination is safe and effective for a significant population subgroup and the benefit/risk assessment of the fixed combination is equal or exceeds the one of each of its substances taken alone.

The multilevel factorial design may be used but other confirmatory strategies exist to prove that the combination is superior to its substances. Descriptive tools such as response-surface methods may be useful (see Dose-Response information to Support Product Authorisation).

In some cases, studies have to be specifically designed to determine the minimal effective dose and usual effective dose of the fixed combination. Multiple dose-effect studies may be required.

Where substances are intended to relieve simultaneously different symptoms or to prevent different diseases, selected doses of each substance are often those commonly used for the treatment of each symptom or the prevention of each disease.

4.2 Therapeutic trials

Confirmatory clinical trials are necessary to prove efficacy, preferably by parallel group comparisons in which the fixed combination is compared to its individual substances. Inclusion of a placebo group is recommended when feasible.

Comparative clinical studies of the fixed combination versus reference treatment might be necessary.

4.3 Safety aspects

Safety studies in animal should, as a general rule, have been performed with the active substances of the fixed combination in the proportion present in the product. Such studies will not be required where all the substances have been extensively and safely used in humans in identical or very similar combinations for a long period and the safety of such combinations is well documented.

In the case of combinations for long term use (see guidelines on the extent of population exposure to assess clinical safety for medicines intended for long term treatment of non-life-threatening conditions), safety data on 300-600 patients for six months or longer will be required. The absence of such data should be justified by the applicant.

Where there are grounds to expect that a fixed-combination product may be substantially more harmful or give rise to much more frequent adverse effects than any individual substances given alone, the applicant should provide evidence that this does not occur in therapeutic use, or that the advantages of the combination e.g. increased efficacy, outweigh such disadvantages.

5. COMBINATION PACKS

The principles applicable to fixed-combination products will also be applied in the assessment of preparations consisting of different medicinal products in combination packs where the products are intended for simultaneous or sequential administration.

6. CHEMICAL COMBINATIONS AND COMPLEXES

This guideline is also applicable to a new chemical substance which dissociates in vivo into two well known active substances. A rationale should be given.

Guidelines for the Testing of Drugs
(Verordung nach § 26 Arzneimittelgesetz über die Arzneimittel-Prüfrichtlinien)

Section 5
Divergent Requirements for Documents

Deviating from Sections 4 and 5, the following shall apply:

1. Document requirements for drugs with known active substances
In respect of drugs which contain a known active substance, the scientific documents submitted pursuant to § 22 (3) of the Medicines Act shall facilitate an evaluation of the therapeutic efficacy and safety of a drug when applied in the dosage indicated considering the proposed conditions for application. Tests on the bioavailability of new drugs containing known substances shall be required if they have been published in the list of the Federal Health Office pursuant to § 26 (3) of the Medicines Act.

Scientific documents shall include toxicological, pharmacological and clinical documents in the form of

- controlled studies,
- non-controlled studies,
- observational (non-interventional) studies,
- collections of case reports which enable scientific analyses.

- Documents on empirical medical findings prepared in accordance with scientific methods, e.g. in the form of scientific literature and expertises of professional societies, shall also be accepted as documents on scientific findings.

If the scientific documents provide sufficient information on the desirable and undesirable effects of the drug on human beings, new tests may not be required; in particular, it shall not be necessary in this case to submit documents on pharmacological and toxicological tests. However, any existing test results shall be submitted.
Where method and methodology have been further developed since the tests were conducted, this shall be duly considered when evaluating the results of the latter.
The general requirements for the particulars governing every study, as described in Sections 3 and 4, part A of these Guidelines, shall apply accordingly.

[redacted]

Commission E (1984)

The quality and extend of bibliographic data is required to correlate with the severity of indications claimed for the product and the risks of the active constituents. If there are no new controlled clinical trials, evidence of safety and efficacy is accepted as plausible

- if a herbal drug is mentioned in the standard literature or well documented review articles, or
- if there are clinical trials which are not conclusive alone but are supported by supplementary experimental data or
- if there is well documented knowledge on traditional use that is supported by significant experimental studies.

Traditional use without supplementary data or experimental data alone cannot be accepted as sufficient evidence of efficacy.

Federal Institute for Drugs and Medical Devices

Draft, May 1997

Recommendations for the preparing and carrying out of Observational (non-interventional)Studies

The following comments aim to give a precise description of the term „Observational Studies" taking into account both national and international documents and to make recommendations for the *planning, carrying out and evaluation of studies of this nature.*

1. Definition

An Observational Study is a study to collect findings on the application of approved, registered or fictitiously approved medicinal products. The special feature of these studies is that they seek as far as possible not to influence the individual doctor-patient relationship in respect of indication and selection and carrying out of the treatment. An Observational Study may be conducted without a comparator group, i.e. with specific references to a medicinal product or with two or more comparator groups, i.e. with a special emphasis on indications. Since an Observational Study is designed to generate findings on the use of medicinal products under routine conditions, it is normally conducted with commercial-grade products.

2. General requirements to be met by Observational Studies

Observational Studies must be planned, conducted, assessed and evaluated in line with the level of scientific knowledge of the disciplines involved. They must pursue a medical-scientific goal (Section 4) which must be formulated in advance as a detailed question. The design selected (based on a comparison, the time and scale of examination for each individual patient, number of patients) and the envisaged methods (data recording and evaluation) must be suited to providing answers to this question. An Observational Study is normally conducted in a prospective manner with a backdated starting point. The design and the manner in which it is conducted are similar to a cohort study. It may also be based on pharmaco-epidemiological data.

3. Methodological classification of Observational Studies

Observational Studies are one of several methodological instruments used to obtain information about medicinal products already on the market. Other important tools are clinical trials in Phase IV and case-control studies, longitudinal studies, correlation studies with aggregate data, evaluations of registers and spontaneous notifications. Aside from clinical trials, recommendations on the design of these instruments for drug research after marketing authorisation has been granted are not yet available.

The selection of the appropriate instrument is determined by the goal in terms of the desired results. For each specific question, the reasons must by given why the instrument selected is the right method and able to answer the question in a reliable and efficient (number of patients) manner.

4. Goals of Observational Studies

Possible goals of Observational Studies are:

a) To obtain knowledge about the use of medicinal products (prescription behaviour and habits, compliance with instructions on use and information for professional circles, acceptance and compliance, practicability, compliance with authorisation provisions etc.), the procurement of information about direct, indirect and intangible costs which are incurred through the routine use of treatment or in connection with it.

b) To deepen understanding of known adverse drug reactions (ADRs) under routine use (examination of the expected ADRs, frequency estimates), the procurement of information about so far unknown, particularly rare ADRs and interaction.

d) To extend knowledge on efficacy (e.g. under routine administration conditions: in groups not included in clinical trials, in sub-groups, to characterise non-responders etc.) Statements about efficacy from Observational Studies are only reliable when linked with findings from proof of efficacy from clinical trials conducted in line with recognised methodological criteria. Aside from substantiated exceptional cases, proof of efficacy by means of an Observational Study is not possible.

5. Non-intervention

Non-intervention within the framework of Observational Studies mainly involves not giving the attending physician any study-specific instructions about

a) whether treatment should be given at all or, if so, with what medicinal products;

b) the *details of* treatment (dose, *route of* administration);

d) under what circumstances treatment should be stopped or changed. A medicinal product may not be prescribed in order to include a patient in an Observational Study. The prescribing of a medicinal product and the inclusion of a patient in an Observational Study are two aspects which must be viewed separately.

In respect of post-authorisation monitoring non-intervention also requires that the diagnostic steps in order to record ADRs or to assess success correspond to the routine procedure. The systematic observation required in order to obtain information does, however, call in some

cases for additional data in order to record information about the type and scale of documentation and its control. There must be a certain degree of intervention vis à vis the physician in order to achieve uniformity of observation and a sufficiently high level in respect of quality and completeness of the data recorded.

6. Different forms of Observational Studies

Different goals (4a - 4c) call for different designs and forms of Observational Studies. In the case of 4c, and in part for 4b, comparative Observational Studies produce more reliable results than the drug-specific Observational Studies.

Depending on the question, the degree of intervention in post-authorisation monitoring will differ. In the case of the goals presented under 4a, efforts should be made to avoid any intervention. Here, thought should be given to retroselective data recording. In the case of the goals given under 4b and 4c, measures for the standardised recording of parameters are required. Recommendations should be made for the carrying out of diagnostic measures or reference should be made to published recommendations (e.g. guidelines).

7. Study Plan

Prior to the commencement of an Observational Study, a study plan must be established which corresponds to the current level of medical and biometric knowledge. It will mainly consist of an observation and evaluation plan. The observation plan should be oriented towards a routine approach but, particularly in the case of the goals under 4b and 4c, it should contain instructions to facilitate systematic observation and support the goal of uniform observation.

The study plan should at least contain the following details:

- Formulation of one (or more) detailed question(s) and the reasons why the Observational Study is the suitable tool for answering it (them);
- Description of the patient inclusion and, where appropriate, the procedure used to select the physicians involved (centres);
- Definition of the patients to be included and, where appropriate, a description of the procedure for the inclusion of patients;
- Description of the measures to guarantee that the study is representative (for both physicians and patients);
- Stipulation of the aspects to be recorded, a description of their relevance and their importance for answering the question (target parameter, influencing factor, disruptive factor);
- Discussion of possible disruptive factors and description of measures to control them;

- Timeframe for observation;
- Description of the recording instruments used in observation incl. the reasons why the data collected are suitable to answer the question posed;
- Reasons for the number of patients included;
- Description of the type and scale of documentation;
- Specification of reporting on ADRs;
- Description of quality assurance measures;
- Description of statistical evaluation;
- Provisions concerning responsible parties (sponsors, study coordinators, responsible biometrician etc.);
- Rules on reporting including biometric and medical evaluation.

8. Quality Assurance

The traditional quality requirements for epidemiological studies also apply to Observational Studies. The goal of quality assurance is to guarantee the completeness and validity of the data and to recognise and overcome any shortcomings early on.

9. Representative character

Since Observational Studies are designed to provide findings as a supplement to clinical trials, which are more closely related to day-to-day medical practice, measures are to be taken to ensure that patients included in the Observational Study are as representative as possible in respect of the situation under review, e.g. by including all the patients of each physician, the log book of the available patients etc.

10. Statistical evaluation

The evaluation of the data from an Observational Study is done on the basis of biometric methods appropriate to the problem in hand. The procedure is to be laid down in advance in the study plan.

11. Information to and consent from patients

Since there is no intervention in a decision about treatment, there is no need to inform patients beyond the normal obligation by a physician to inform his patient. However, there may be a

need to give the patient more information in conjunction with the use of patient data and additional measures. It is recommended that the patient's consent is obtained.

12. Ethics Commission

There are not likely to be any specific ethical problems arising from the treatment of patients in line with the latest level of medical knowledge. We refer here, more particularly, to the relevant ordinances (professional codes of practice) and laws and to different regulations in medical laws in the individual German states. Consideration should also be given to data protection aspects.

13. Duty of notification

In accordance with § 67 para 6 German Drugs Act, there is a duty of immediate notification for Observational Studies.

The duty of notification which applies to clinical trials in connection with ADRs (§ 29 para 1 German Drugs Act) also applies in full to Observational Studies.

14. Report, publication, archiving

A final report is to be prepared on the carrying out and results of an Observational Study. It must contain a biometric evaluation and a medical evaluation. The results of the Observational Report are to be published in accordance with scientific criteria.

All documents about an Observational Study are to be archived for at least ten years for later access and evaluation.

15. Marketing interests

An Observational Study is used primarily for scientific purposes. It may not be conducted solely for marketing reasons.

16. Reimbursement and fees

Reimbursement aspects may not impair a scientific, target-oriented approach to an Observational Study. Measures which go beyond the norm may be required to answer certain questions. Reimbursement of the costs of measures of this kind must be clarified separately.

The payment of fees to the physicians involved should correspond to the additional effort involved.

The need for data bases and regional networking for industrial exploitation of medicinal and aromatic plants.

Enrico Feoli
ICS-UNIDO Area Coordinator of "Earth, Environmental and Marine Sciences and Technologies"

A Summary for the ICS- CYTED training course on "Production of Phytomedicines", Panama (November 24-December 5 1997)

Introductory points

- Man is using plants from millennia not only for getting food, drinks, spices, wood and fibers but also for medical, aesthetic-cosmetic and flavouring aims. Trade of plants influenced the man history all over the world in these last 5 centuries both as far as legal and illegal market was and is concerned. Only in this last century man become aware that the plant kingdom is not an unlimited resource.

- The demographic growth and the depletion of the natural environment has reduced and is reducing the capability of plant kingdom of being renewable.
- Irreversible processes such as desertification and loss of biodiversity following the deforestation, space occupation by agricultural, urban and industrial areas and pollution are undermining the self-sustainability of plant kingdom.

- The consequences of the loss of biodiversity may not be easily foreseeable at global scale, however it is tangible that many species that are or can be useful for man in different ways are now endangered.
- This forces man to produce regulatory policies and to adopt measures of protection. Such measures can certainly help in preserving biodiversity at global scale, however they are not solving the major problem of improving life in rural areas.

- If there is an actual interest to develop the economy of the rural areas conservation policies have to be paralleled by exploitation policies of natural renewable resources of which medicinal and aromatic plants constitute an important economic component.

- Since people are dramatically leaving such areas to move to urban industrialized areas, the so called local knowledge on the environment and human traditions (the cultural heritage) is endangered.

- It follows that the traditional medicine based on indigenous plants is also endangered. It is remarkable that in many countries the ability of practitioners to identify plant species properly has decreased.

- In order to plan a sustainable rural development that may also include the industrial exploitation of indigenous medicinal and aromatic plants, tools have to be given to policy makers at different levels.

- Entrepreneurs on the other side need information on which to base decisions for investments.

- Furthermore farmers have to be convinced that breeding medicinal and aromatic plants would be profitable.

- Particularly in this respect a policy of suitable incentives would be needed to be activated.

- For supporting all the actions needed for the industrial exploitation of medicinal and aromatic plants it is necessary to have tools for getting the available information.

- Agencies such as FAO, UNEP, UNIDO, ESCAP, etc. of the UN should promote in a coordinated way the production of such tools. These tools are data bases and network management systems that allow to get access to the available information.

The necessary information

- The information necessary to support decisions for industrial exploitation of indigenous medicinal and aromatic plants system has to come out from the two systems that are involved, namely: the resource system and the exploitation system (Fig.1). Both systems have equal importance to offer the immense information that has to be organized in order to help the sustainable industrial exploitation of indigenous medicinal and aromatic plants.

- The resource sytem is the result of the long interaction between the plant genetic pool and the environment. Throughout history this interaction has produced the vegetation system (vegetation) that makes green our planet. Vegetation is organized in plant communities, this allows to distinguish vegetation types and to map them. Vegetation maps are useful tools to know plant distribution and to define the environment where plants are living. Medicinal and aromatic plants are not living alone they are living in plant communities and therefore vegetation maps are indispensable to know their distribution and environment. The knowledge about the ecology and the physiological requirements of the plants is the essential basis for planning their cultivation in the most suitable environment. This will prevent failures and vasting time and money.

- The exploitation system is based on the local knowledge that is conditioning and defining all the uses of the plants. There are many expoitation systems at different levels, from the village level, to national, regional until the world level. The use of medicinal and aromatic plants produces market at different levels. Markets at high level require standards and therefore taxonomy for plant identification, chemistry for chemical analysis and clinical tests are automatically involved in the picture. As a consequence of market requirements to improve cultivation, propagation, post harvesting and chemical techniques for industrial extraction of useful compounds is becoming an urgent matter.

The information system and network

- There are many datā bases developed for phytomedicines, they are mainly related to the different pharmacopeas. Many of them are listed in the book of T. da Silva "A manual on the essential oil industry" by UNIDO 1995. Of this list only few are specific for medicinal and aromatic plants. Many of them are commercial and deal only with bibliographic data (references). There are data bases on medicinal plants that are mainly reproducing books with wonderful pictures and recipies for preparing the medication and with the chemical formulas of the active principles.

- What is actually missing is a data base on industrial exploitation of medicinal and aromatic plants (inventories of uses, of pilot projects, of technologies for breeding and extraction, of exploitation projects, of clinical tests, etc.) that can be used as a decision support system. This can be developed and has to be developed on the basis of the already existing data bases: by integrating them in a system able to networking them. Since this data base is actually a bank of data bases it gives more than the description of plants, techniques for breedings, techniques for extraction and treatments, etc. but it will give access to data bases of the resource systems and the exploitation systems of different regions with particular emphasis to the international market system.

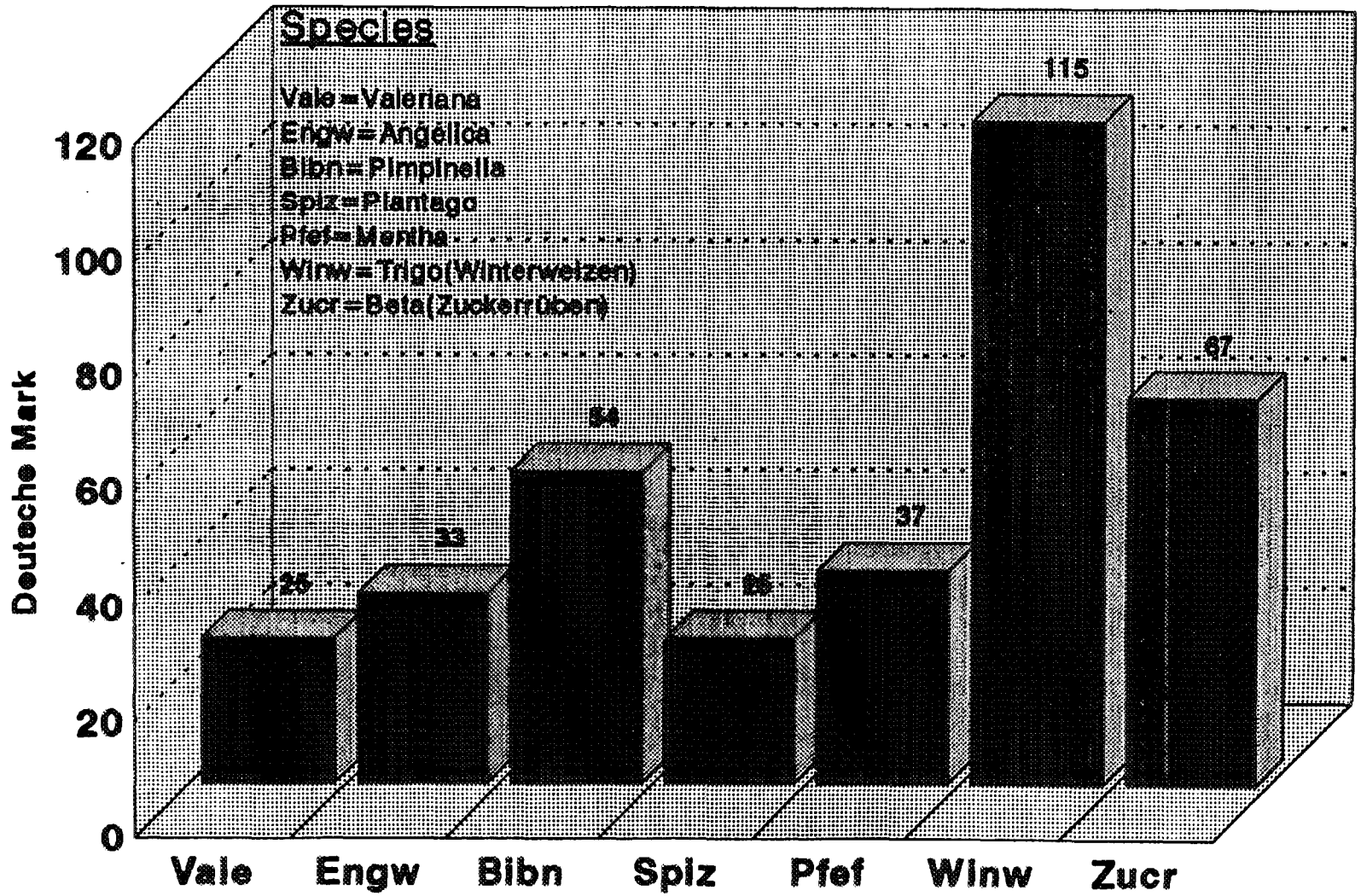
- To develop such an information system it would need the cooperation of many institutions and spontaneous networking in different regions.

- ICS-UNIDO will provide assistance in developing such a system by networking selected regional focal points and by action oriented research troughout fellowships, study tours, workshops and training courses.

Margen bruto de algunas especies medicinales y convencionales

por Hora de Mano de Obra (1US$=1,64DM)

Alemania 1988

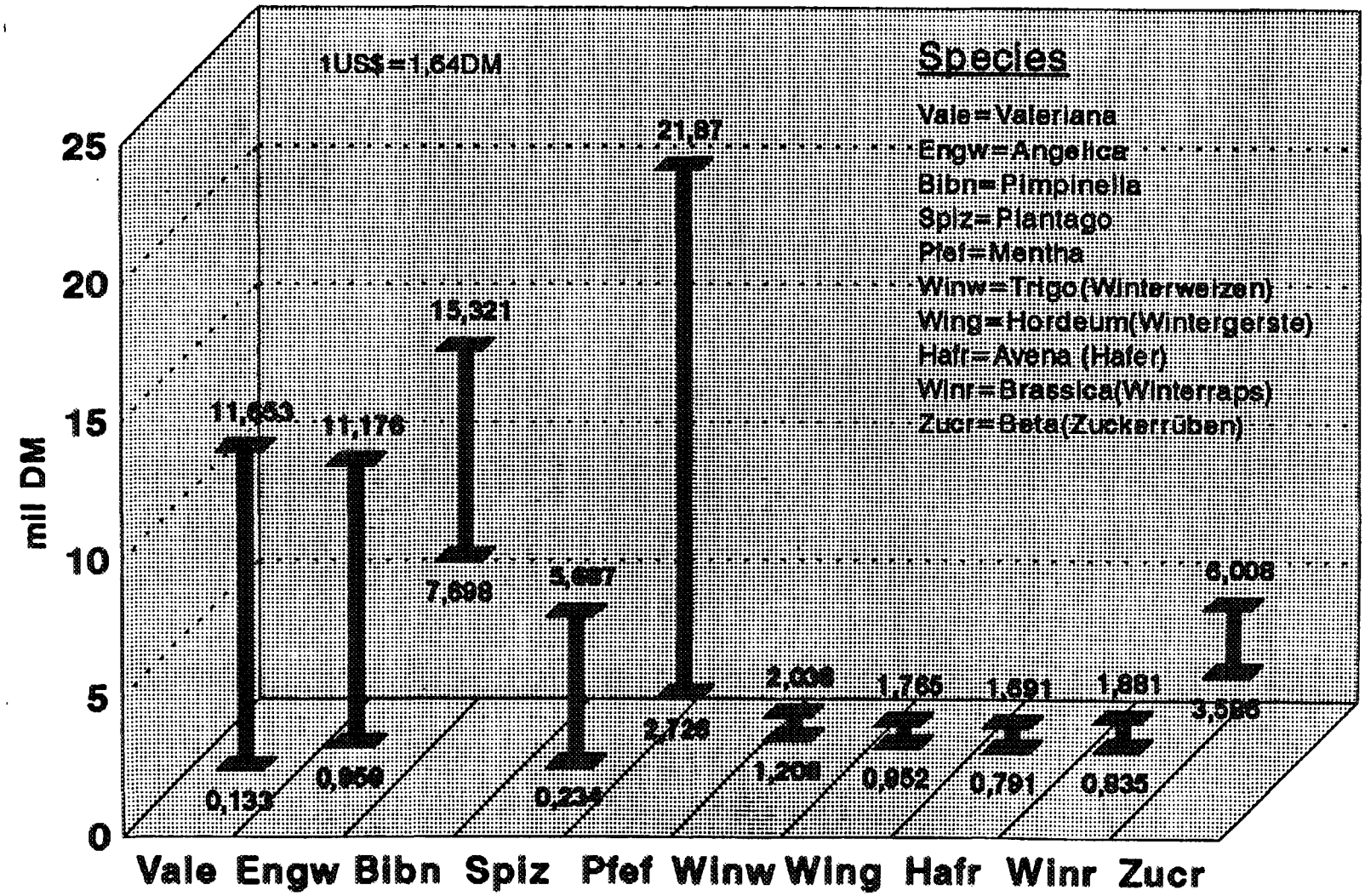


Santiago 1996

Margen bruto de algunas especies medicinales y convencionales

por Hectaria

Alemania 1988



Santiago 1996

Análisis/control de calidad en cultivos de plantas medicinales y aromáticas

Suelo	Nemátodos	
	Metales Pesados	
Aguas de Riego	Metales Pesados	
	Microbiano	
Producto final	Identidad	
	% Impurezas	
	Cenizas	
	Humedad	
	Compuestos activos	
	Microbiológico	Bacterias aeróbicas
		Hongos y Levaduras
		E. coli
		Enterobacterias
		Salmonella sp.
	Aflatoxina	Aflatoxina B1 y otras
	Residuo de Pesticidas	General
		Piretroides
	Metales Pesados	

Sobre la diferencia entre Plantas medicinales y Plantas aromaticas:

Plantas medicinales dan años a tu vida

Plantas aromaticas dan vida a tus años

(dicho chino)

Si quieres estar feliz para

3 horas	*- toma cerveza*
3 dias	*- mata a un chancho*
3 meses	*- casate*
todo tu vida	*- cultiva hierbas medicinales*

(dicho chino)

Testing Medicinal and Aromatic Plant Production under Field Conditions

Panama, Nov. 1997

M. Lorenz

Testing Medicinal and Aromatic Plant Production under Field Conditions

A. Introduction of known species to unknown areas

- causes of natural variability of active principles
- analyzing agroclimatic conditions
- estimating producton costs and quality
- mechanization
- post harvest technology

B. Domestication of unknown species

- domestication versus wild colletion
- finding the right germplasm
- multiplication techniques

Testing Medicinal and Aromatic Plant Production under Field Conditions

A.

Introduction of known species to unknown areas

Natural Variability of active ingrediances in medicinal and aromatic plants

I. Intraindividual Variability

II. Genetic Variability

III. Genome Variability

IV. Variability by Post Harvest Treatment

I. Intraindividual Variability

Morphological Variability

- e.g. Valeriana edulis, Chamomilla recutita

Ontogenetic Variability

- e.g.Mentha piperita, Carum carvi, Gentiana lutea, but: Hypericum perforatum

Agroclimatic Variability

- e.g. Temp.,Soil,Nutr.,Ligth,

Diurnale Variability

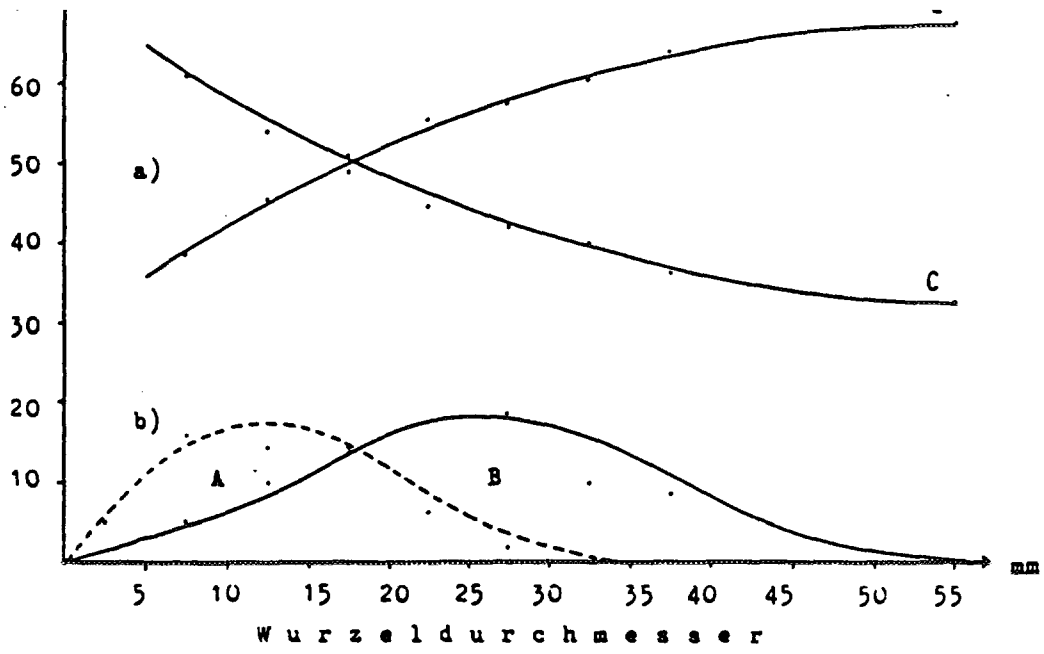


Abb. 36a: Massenanteile von Rinde (C) und Holz (L) bei zunehmendem Wurzeldurchmesser bei Valeriana edulis
b: Mengenanteile der Durchmesser-Fraktionen bei unterschiedlicher Kulturdauer
A 1 1/2 Jahre, B 3 Jahre

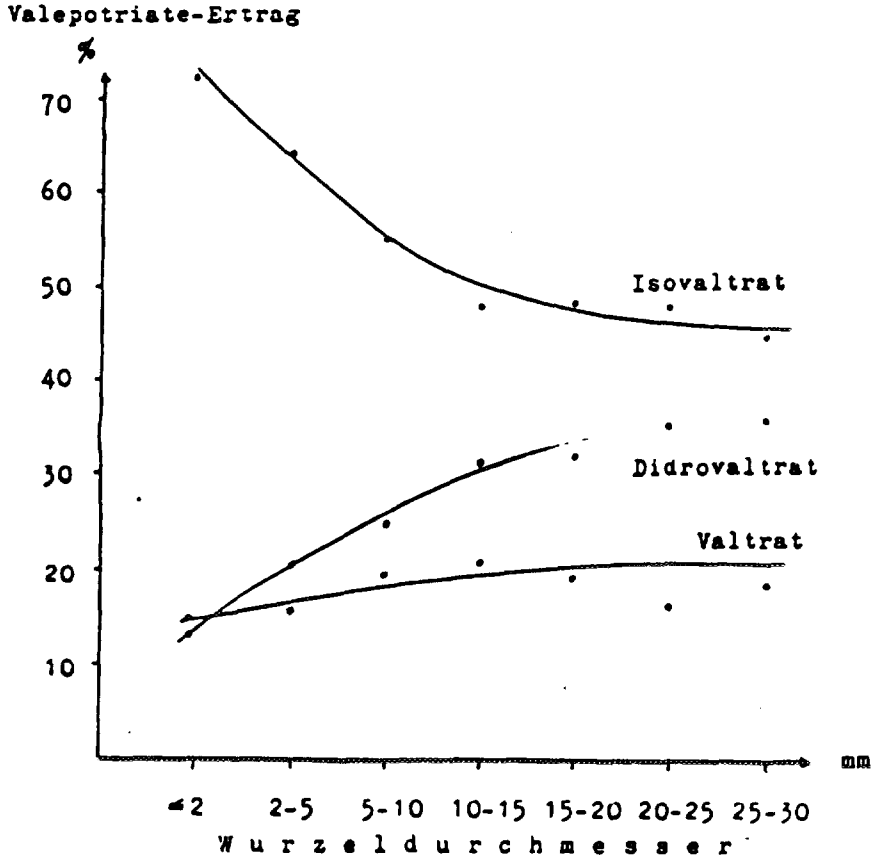
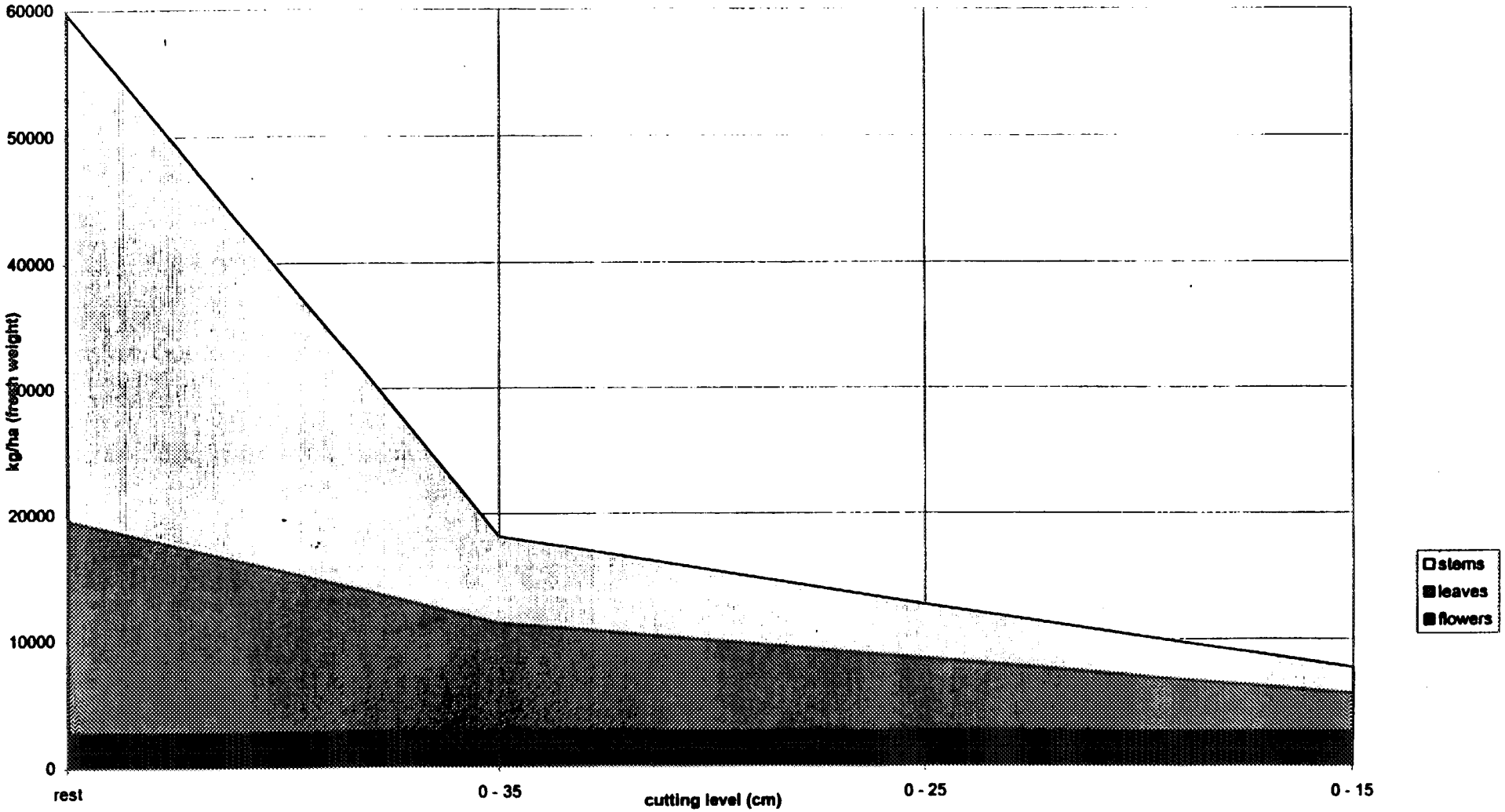
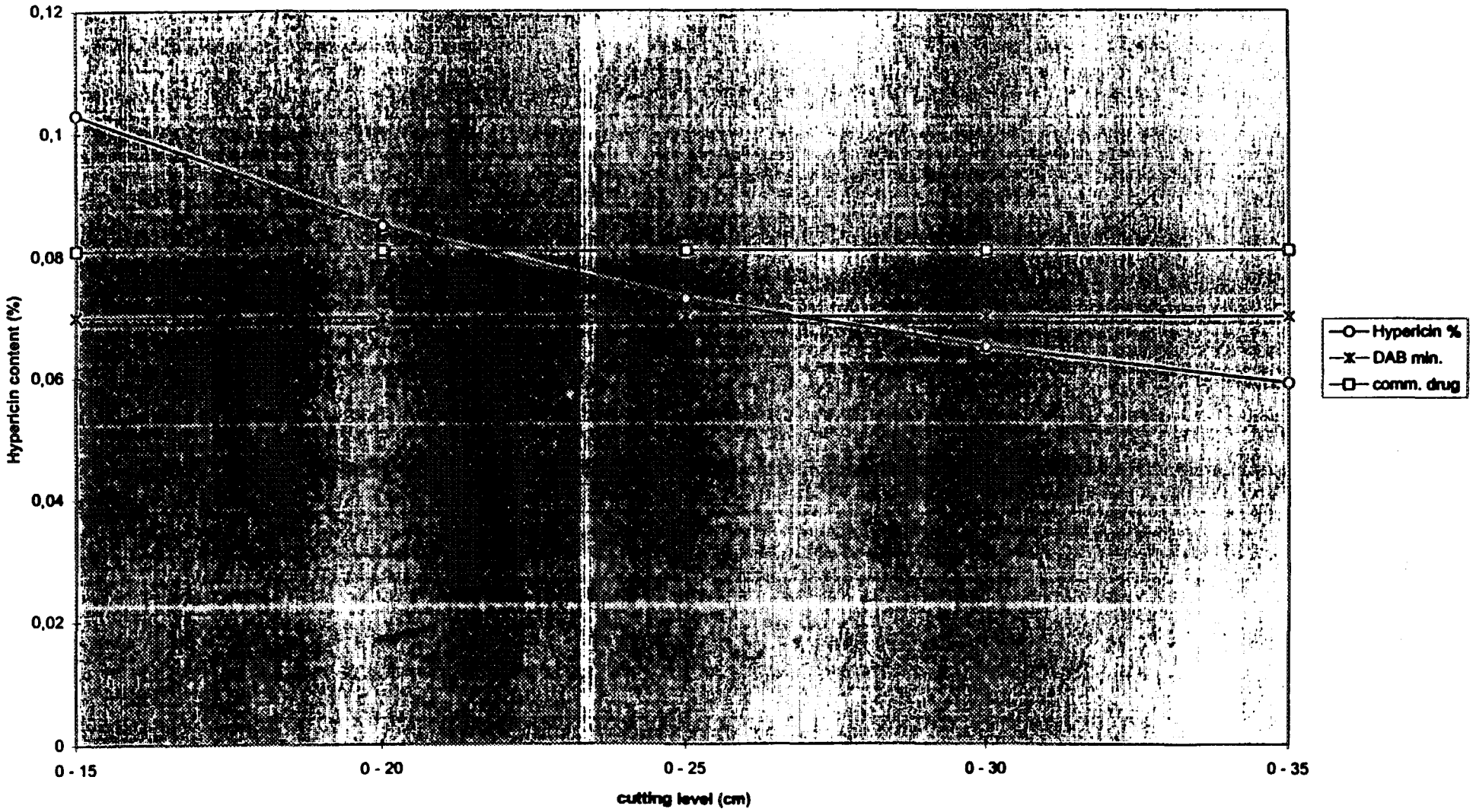


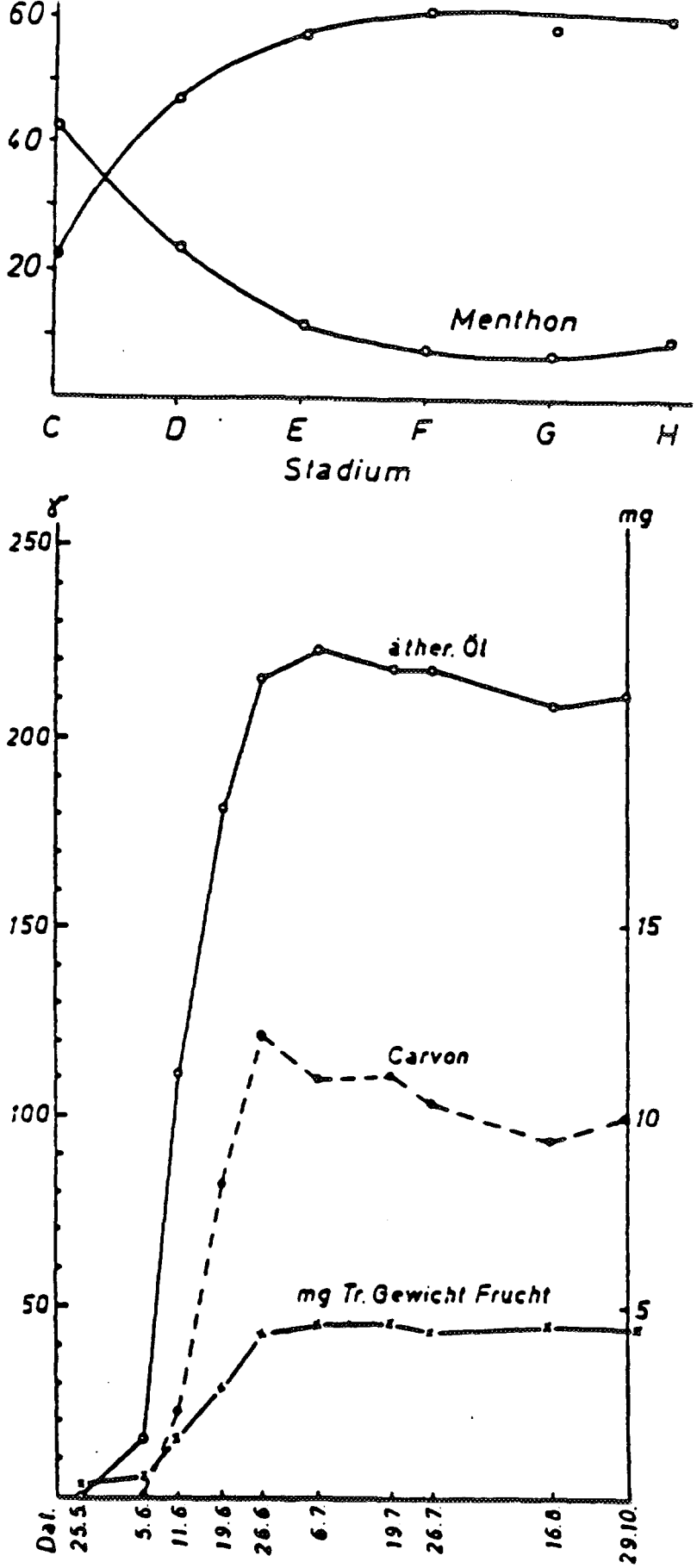
Abb. 37: Qualitative Veränderung des Valepotriate - Ertrags bei steigendem Wurzelumfang

Manual harvest of cultivated Hypericum perforatum
Linares 26.12.1996
II. fully flowering stems and stems in the stage of pre - flower



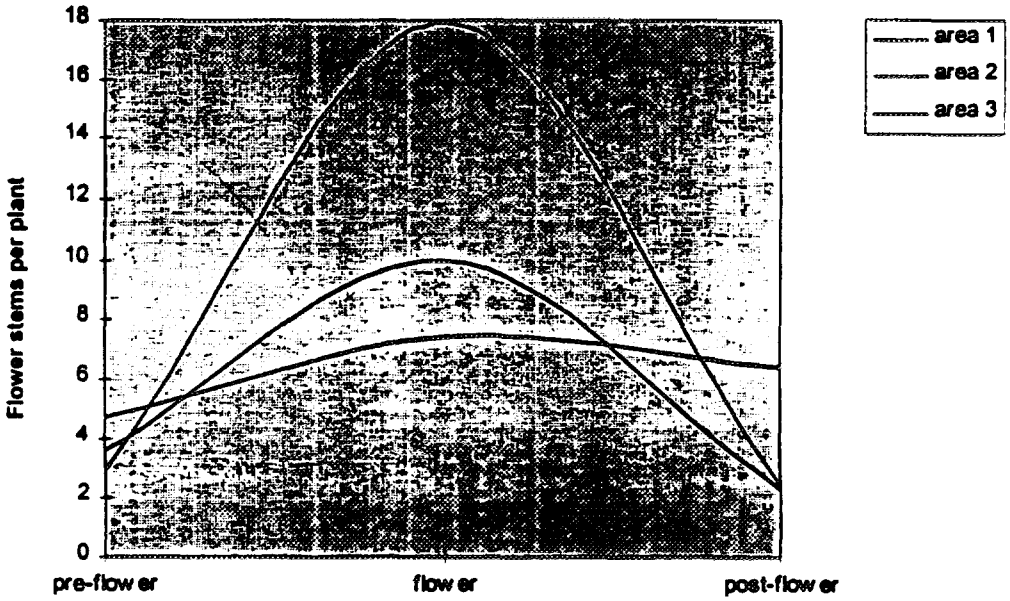
Hypericin - based quality of Hypericum perforatum as a result of different cutting levels





Absolute Menge an ätherischem Öl und Carvon in den Früchten verschiedenen Entwicklungsalters von *Carum carvi*

Maduration status within a Hypericum perforatum field
Linares 10.1.1997



II. Genetic Variability

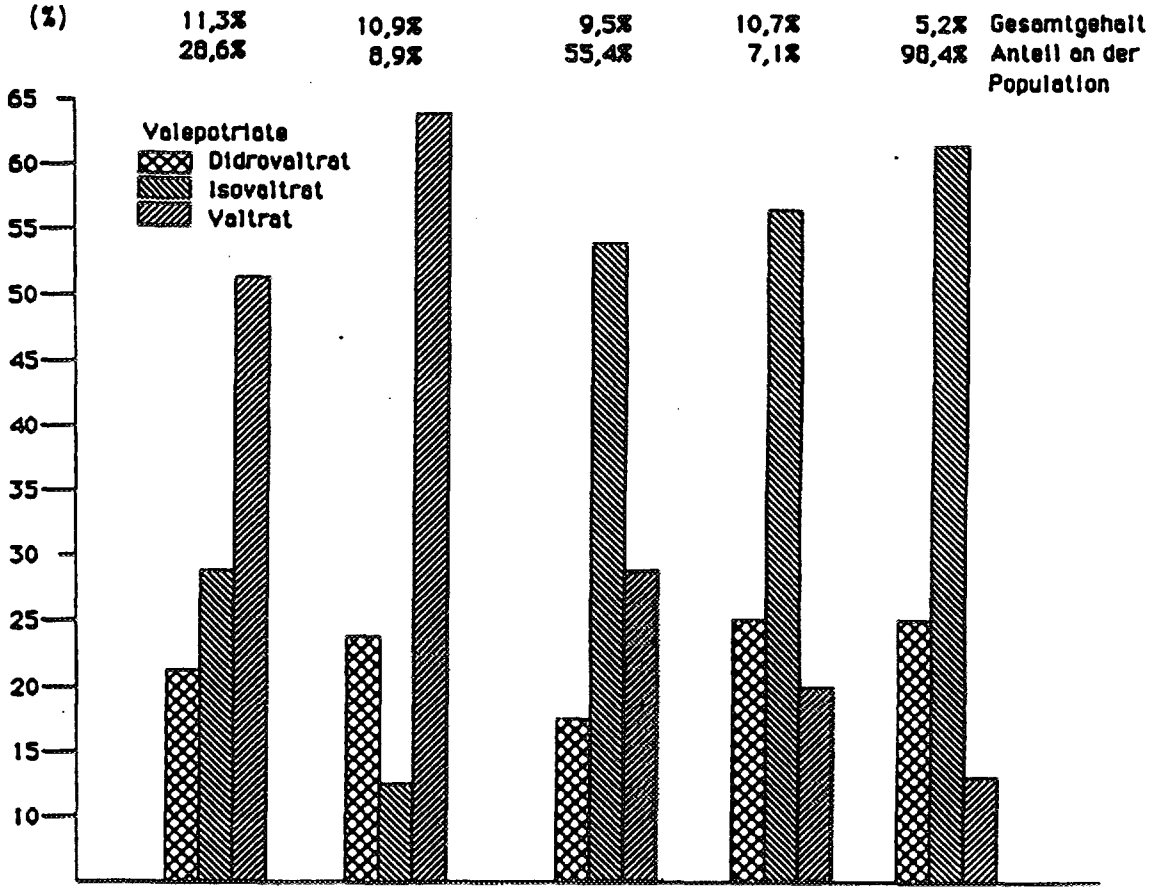
Wild Species

- Inter - population (geografic areas)
- Intra - population

Cultivated Species

- Generative propagation
  - » Varieties
- Vegetative propagation
  - » Clones

Anteil von Didrovaltrat, Isovaltrat u. Valtrat
am Gesamt-Valepotriate-Gehalt



I. Ökotypen

Meseta Neovolcanica	Durango

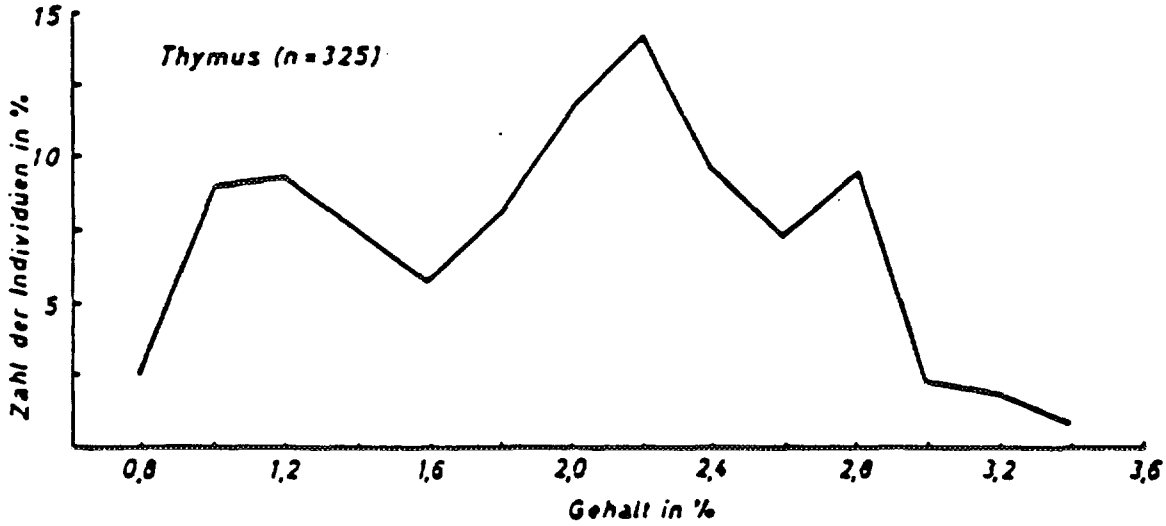
II. Chemovarietäten

'Váltratum'	'Isovaltratum'

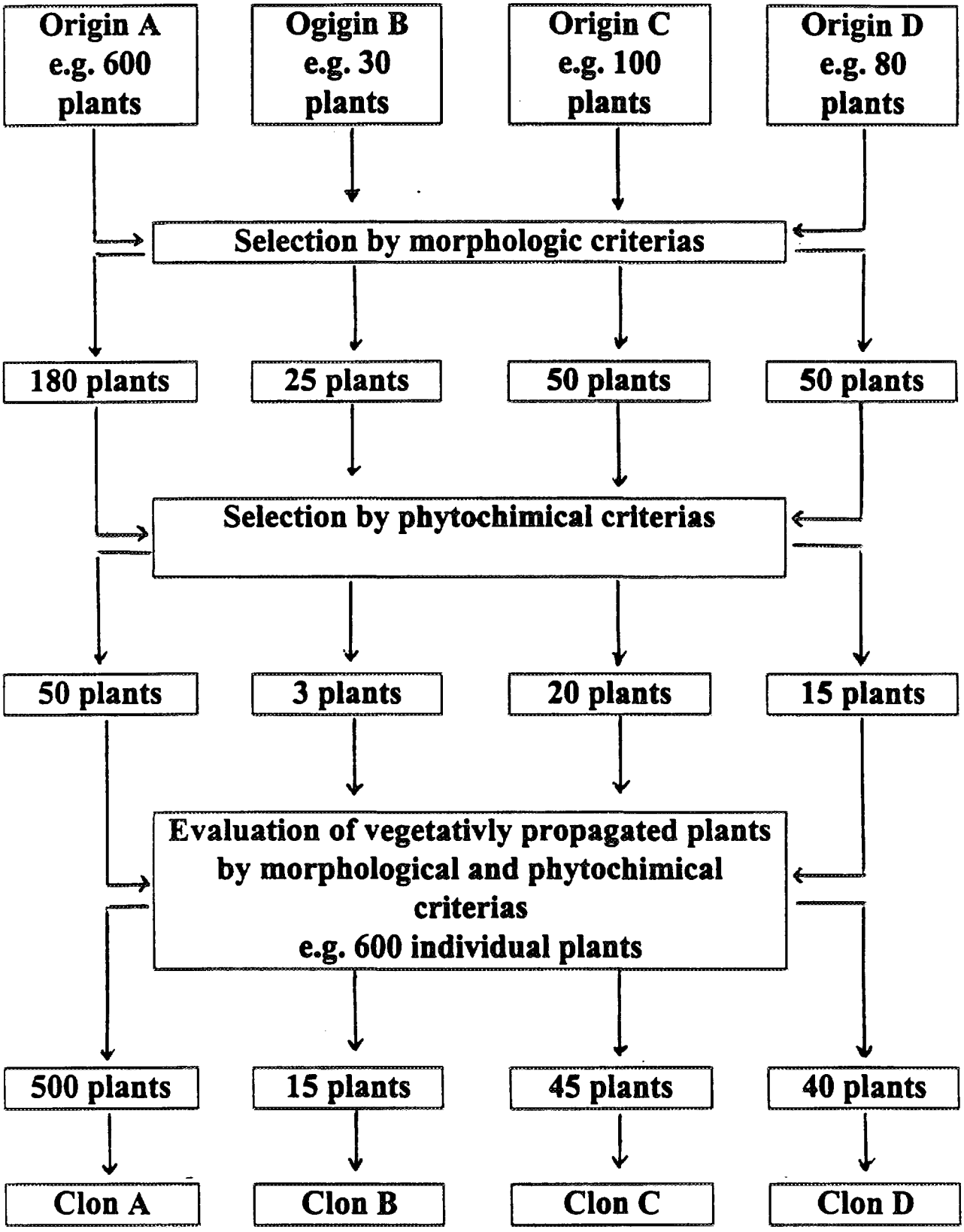
III. Chemoformen

VID	VDI	IVD	IDV	IDV

Abb. 64: Chemische Strukturen innerhalb der Ökotypen
'Meseta Neovolcanica' und 'Durango'


Thymus (n = 325)
Zahl der Individuen in %
15
10
5
0,8
1,2
1,6
2,0
2,4
2,8
3,2
3,6
Gehalt in %

Scheme of selection in medicinal and aromatic plant species



Dr. Matthias Lorenz Panama, 1997

III. Genome Variability

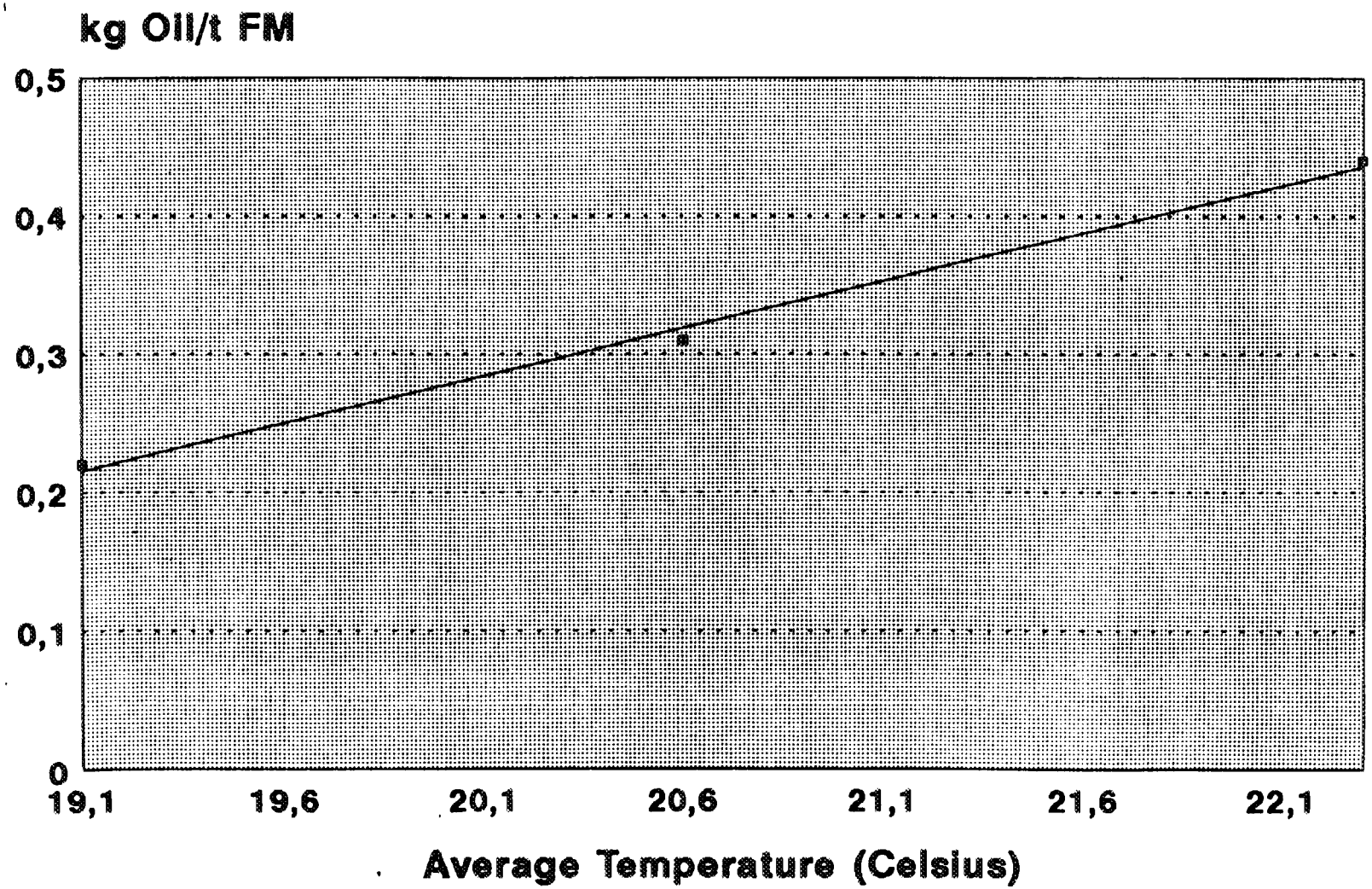
Diploide plants	**Tetraploide plants**
1. agronomic aspects	1. agronomic aspects
many but small leaves	prolonged vegetat. phase
	few but big leaves
	few flowers
2. quimical aspects	2. quimical aspects
	bigger but fewer oil cells
	alcaloide cont. may rise

IV. Variability by Post Harvest Treatment

Harvest	- hand/maschine (selective)
Cutting	- celldistruction, fermentation
Drying	- temperature, light
Packaging	- plastic/paper
Storage	- temp., humidity, time,infection
Transport	- temp., humidity

Essential Oil from Melissa officinalis

Temperature and Oil Content



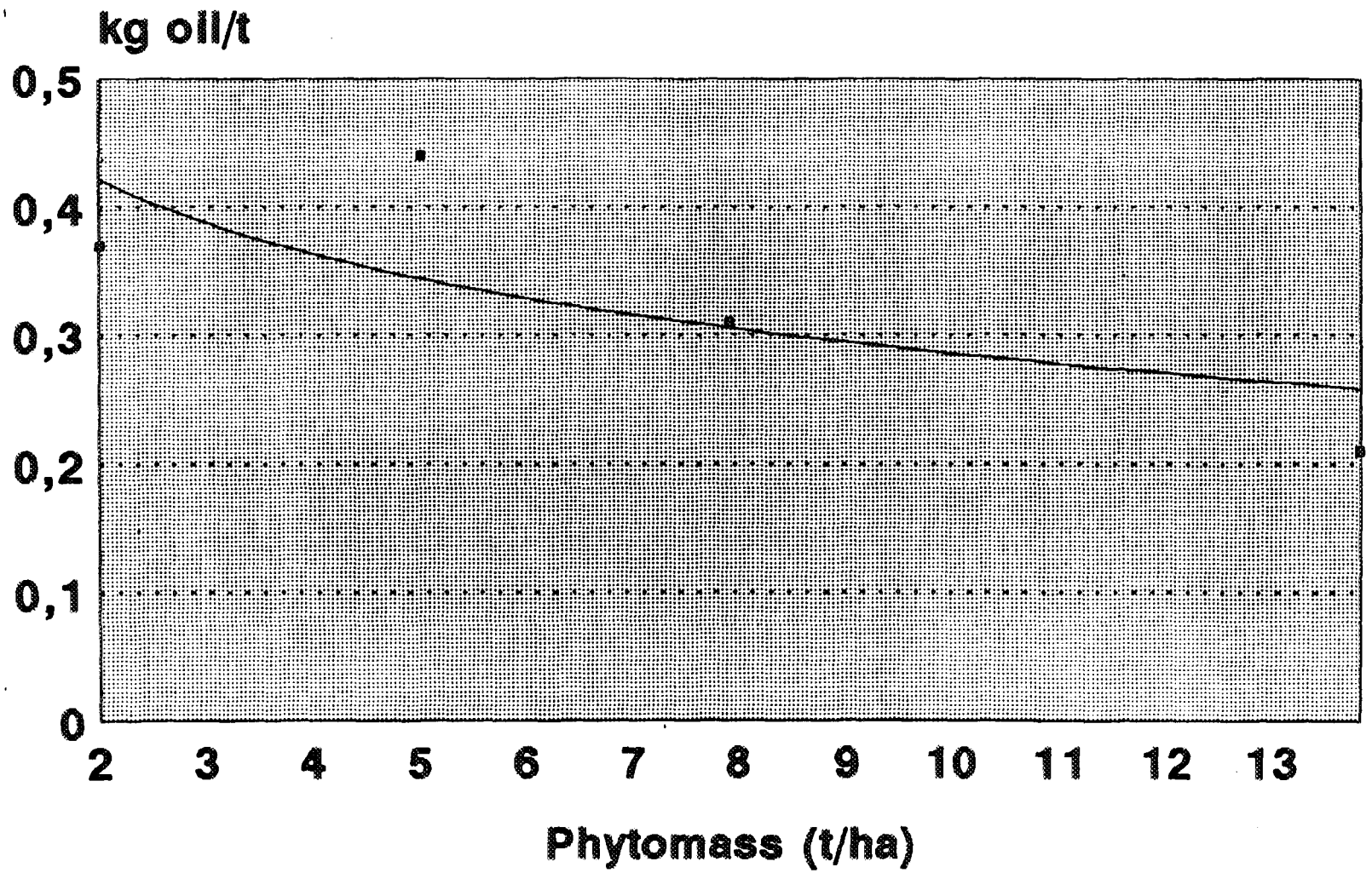
Fundacion Chile, 1996

Agroclimatic Conditions

Soil and Water	**Clima**
nutrients	temperature
physical properties (O2)	h sun/a and radiaton
pH	sealevel
havy metals	air humidity
salt	rainfall
microbial pollution	windexposion
	day length

Essential Oil from Melissa officinalis

Plant Development and Oil Content



Fundacion Chile, 1996

Prognosting agronomic yield and yield of active principles

- Field trials in different agroclimatic areas

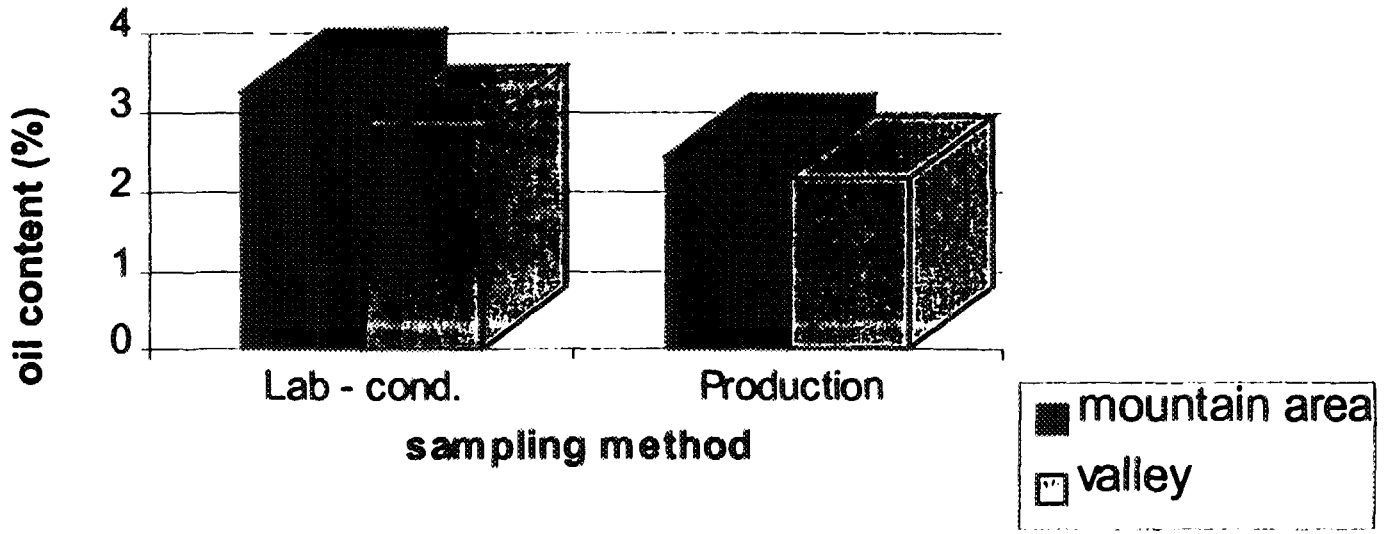
seedling production in one zone

distribution to different zones

- Agronomic, quimical and organoleptic evaluation of the obtained results

Estimation of production costs and quality

Quality of thyme as a result of production area and sampling method



Mechanization

seed (pneumatic seed, direct seed)

planting

weeding (organic acceptance?)

harvest (contamination, rapidness, quality)

postharvest (drying, cutting, sorting, packaging)

Post Harvest Technology

- Drying Facilities
  - natural drying
  - box dryer
  - band dryer
- Homogenization (charging)

Influence of drying parameters on the quality of Anethum graveolens

Temperature of drying (°C)	Time of drying process (h)	Essential oil content (mg/1000 g DM)
-	-	326
25	26	49
40	8	29
50	4	37
- 25	59	188

Testing Medicinal and Aromatic Plant Production under Field Conditions

B.

Domestication of unknown species

GENERAL METHODS, APPLIED IN PHARMACOPOEIAL HERBAL MONOGRAPHS
(Ph. Eur. 1997 : 69 monographs)

CHEMICAL REFERENCE STANDARDS (CRS)

- Digitalis leaf : ***digitoxin CRS*** : ASSAY : cardenolic glycosides, expressed as digitoxin
- Hamamelis leaf : ***hide powder CRS*** : ASSAY : tannins
- Ipeca root : ***emetine.2HCl CRS + cephaëline.2HCl CRS*** : IDENTIFICATION : TLC
- Liquorice root : ***glycyrrhizinic acid CRS*** : ASSAY : TLC + absorbance at 250 nm
- Rhatany root : ***hide powder CRS*** : ASSAY : tannins
- Senna leaf + pots : ***Senna extract CRS*** : IDENTIFICATION : TLC

REFRACTIVE INDEX *(2.2.6.)*

- Anise oil : 1.552 to 1.561
- Clove oil : 1.528 to 1.537
- Eucalyptus oil : 1.458 to 1.470
- Lemon oil : 1.474 to 1.476 + TEST for foreign essential oils (destillate max. 0.003 less)
- Peppermint oil : 1.457 to 1.467
- Sesame oil : 1.472 to 1.476

OPTICAL ROTATION *(2.2.7.)*

Angle of optical rotation

- Clove oil : 0 to -2°
- Eucalyptus oil : 0 to +10°
- Lemon oil : +57° to 70° + TEST for foreign essential oils (destillate max. 6° less)
- Peppermint oil : -10° to -30°

VISCOSITY *(2.2.8. , 2.2.9. and 2.2.10.)*

- Guar gallactomannan : ***with rotating viscosimeter*** : > 75 % and < 140 % of labeled value

DROP POINT *(2.2.17.)*

- Beeswax, white : 61 to 65 °C
- Beeswaw, yellow : 61 to 65 °C
- Wool fat : 38 to 44 °C
- Wool fat, hydrous : 38 to 44° C

FREEZING POINT *(2.2.18.)*

- Anise oil : 15 to 19 °C

ABSORPTION SPECTROPHOTOMETRY, ULTRAVIOLET AND VISIBLE *(2.2.25.)*

ASSAY *(with reference to dried drug)*

- **Aloes dry extract, dry extract standardised** : ≥ 19.0 % and ≤ 21.0 % *Hydroxyanthracene derivatives*, as barbaloin **: absorbance at 512 nm**

1. 0.400 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ether
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Aloes, barbados** : ≥ 28.0 % *Hydroxyanthracene derivatives*, as barbaloin **: absorbance at 512 nm**

1. 0.300 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ether
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Aloes, cape** : ≥ 18.0 % *Hydroxyanthracene derivatives*, as barbaloin **: absorbance at 512 nm**

1. 0.400 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ether
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Bearberry leaf** : ≥ 8.0 % *Hydroquinones* as anhydrous arbutin **: absorbance at 455 nm**

1. 0.400 g powder (m gram) + 50 ml water; reflux for 30 min.; cool; dilute to 250.0 ml
2. 5.0 ml solution in separating funnel + 45 ml water + 1.0 ml 2 % aminopyrazolone + 0.5 ml dilute NH_3 + 1.0 ml 8 % potassium ferricyanide solution; stand for 5 min.
3. shake with 25 ml methylene chloride; filter; repeat extraction with 3 x 25 methylene chloride
4. dilute to 100.0 ml with methylene chloride
5. measure absorbance; calculate : **A x 7.716/ m (specific absorbance = 648)**

- **Cascara** : ≥ 8.0 % *Hydroxyanthracene glycosides of which ≤ 40 % other than cascarosides* as cascaroside A **: absorbance at 515 nm ; absorbance ratio at 515 nm to 440 nm > 2.4**

1. 1.00 g powder in 100 ml boiling water + stirring for 5 min.
2. cool, dilute to 100.0 ml with water, shake, filter, discard first 20 ml
3. 10.0 ml filtrate to separating funnel + 0.1 ml 1 M HCl; shake with 2 x 20 ml ether-hexane (1/3 vol)
4. wash organic layer with 5 ml water; discard organic layer; add rinsings to aqueous layer
5. shake aqueous layers with 4 x 30 ml ethylacetate; combine ethylacetate extracts
6. aqueous layer ⇒ assay of cascarosides
7. evaporate organic layer to dryness
8. dissolve residue in 0.3-0.5 ml MeOH; transfer to volumetric flask; rinse with warm water
9. cool; dilute to 50.0 ml with water; transfer 20.0 ml to round-bottomed flask with 2 g ferric chloride and 12 ml HCl.
10. reflux for 4 h; cool, transfer to separating funnel and rinse with 3-4 ml NaOH 1 M and 3-4 ml water; add rinsings to separating funnel
11. shake with 3 x 30 ml ether-hexane (1/3 vol); wash organic layers with 2 x 10 ml water; discard water
12. dilute organic phase to 100.0 ml ether-hexane (1/3 vol); evaporate 20.0 ml
13. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
14. measure absorbance; calculate : **A x 6.95/ m (specific absorbance = 180)**

- **Cascara** : ≥ 8.0 % *Hydroxyanthracene glycosides of which ≥ 60.0 % Cascarosides,* as cascaroside A **: absorbance at 515 nm ; absorbance ratio at 515 nm to 440 nm > 2.7**

1. aqueous layer ⇒ assay of cascarosides : dilute to 50.0 ml with water
2. transfer 20.0 ml to round-bottomed flask with 2 g ferric chloride and 12 ml HCl.
3. reflux for 4 h; cool, transfer to separating funnel and rinse with 3-4 ml NaOH 1 M and 3-4 ml water; add rinsings to separating funnel
4. shake with 3 x 30 ml ether-hexane (1/3 vol); wash organic layers with 2 x 10 ml water; discard water
5. dilute organic phase to 100.0 ml ether-hexane (1/3 vol); evaporate 20.0 ml
6. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
7. measure absorbance; calculate : **A x 6.95/ m (specific absorbance = 180)**

- **Cinchona bark** : ≥ 6.5 % *total alkaloids of which ≥ 30 % and ≤ 60 % as quinine-type alkaloids; relative content of Quinine-type alkaloids* **: absorbance at 316 and 348 nm**

1. 1.000 g powder (m gram) + 10 ml water + 7 ml dilute HCl; heat for 30 min.; cool; add 25 ml chloroform, 50 ml ether, 5 ml 20 % NaOH
2. shake for 30 min.; add 3 g tragacanth powder; shake until solution becomes clear
3. filter, rinse flask with 5 x 20 ml chloroform-ether (1/2 vol); combine filtrate and washings
4. evaporate to dryness; dissolve residue in 10.0 ml ethanol; evaporate 5.0 ml to dryness
5. dissolve residue in 0.1 M HCl and dilute to 1000.0 ml
6. prepare 2 reference solutions (30.0 mg quinine and 30.0 mg cinchonine) in 0.1 M HCl and dilute to 1000.0 ml
7. measure absorbances of the 3 solutions at 316 nm and 348 nm; cfr formulas

- **Digitalis leaf** : ≥ 0.3 % *Cardenolic glycosides* as digitoxin : **absorbance at 540 nm**

1. shake 0.250 g powder with 50.0 ml water for 1 h.; add 5.0 ml 150 g/l Pb-acetate solution; shake; add after a few minutes 7.5 ml 4 % Na_2HPO_4 solution
2. filter; reflux 50.0 ml filtrate with 5 ml HCl (15 %) for 1 h.
3. transfer to separating funnel, rinse, shake with 3 x 25 ml chloroform
4. dry combined chloroform layers with anh. Na_2SO_4; dilute to 100.0 ml with chloroform
5. evaporate 40.0 ml to dryness; dissolve residue in 7 ml alcohol (50 %); add 2 ml dinitrobenzoic acid solution + 1 ml NaOH 1 M
6. prepare reference solution : 50.0 mg digitoxin CRS in 50.0 ml alcohol; dilute 5.0 ml to 50.0 ml; 5.0 ml dilution + 25 ml water + 3 ml HCl (15 %); reflux for 1 h.
7. transfer to separating funnel, rinse, shake with 3 x 25 ml chloroform
8. dry combined chloroform layers with anh. Na_2SO_4; dilute to 100.0 ml with chloroform
9. evaporate 40.0 ml to dryness; dissolve residue in 7 ml alcohol (50 %); add 2 ml dinitrobenzoic acid solution + 1 ml NaOH 1 M
10. measure absorbance of the 2 solutions during 12 min. until maximum
11. calculate content of cardenolic glycosides

- **Frangula bark** : ≥ 7.0 % *Glucofrangulins* as glucofrangulin A : **absorbance at 515 nm**;

1. weigh 0.250 g powder (m gram) + 25.0 ml 70 % methanol; mix; weigh again
2. reflux for 15 min; cool; weigh and adjust to first mass with 70 % methanol
3. filter; transfer 5.0 ml filtrate to separating funnel; + 50 ml water + 0.1 ml HCl;
4. shake with 5 x 20 ml light petroleum; transfer aqueous layer to volumetric flask
5. wash organic layers with 2 x 15 ml water; add water to aqueous layer in 100 ml volumetric flask
6. add 5 ml 5 % sodium carbonate; dilute to 100.0 ml with water; discard light petroleum;
7. 40.0 ml aqueous solution + 20 ml 20 % ferric chloride solution; reflux for 20 min.; add 2 ml HCl; reflux further for 20 min.; shake to dissolve precipitate; cool
8. transfer to separating funnel; shake with 3 x 25 ml ether; combine ether extracts; wash with 2 x 15 ml water
9. dilute ether layer to 100.0 ml; evaporate 20.0 ml to dryness;
10. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
11. measure absorbance : **A x 3.06/ m (specific absorbance = 204)**

- **Hamamelis leaf** : *Total polyphenols, Polyphenols not adsorbed by hide powder* as tannins with pyrogallol as Standard : **absorbance at 715 nm** (cfr. Phenols and polyphenols)

- **Liquorice root** : ≥ 4.0 % glycyrrhizinic acid : **absorbance at 250 nm after preparative TLC**

1. test solution: 1.00 g powder (m1) + 25 ml 1 M HCl + 2.5 ml dioxan in 100 ml flask; reflux for 2 h.; cool; filter; discard filtrate
2. rinse flask and filter with 5 x 20 ml water; discard rinsings
3. dry flask and filter at 105 °C for 20 min
4. transfer filter to the flask; add 50 ml chloroform; reflux for 5 min.; filter warm chloroform solution; repeat extraction with 3 x 25 ml chloroform; filter warm chloroform solutions;
5. evaporate combined chloroformic extracts to dryness; transfer residue quantitavely with chloroform-methanol (1/1 vol.) to 10.0 ml flask; rinse beaker with chloroform, evaporate to 2 ml; add to 10.0 ml flask; dilute to 10.0 ml with chloroform-methanol (1/1 vol.)
6. reference solution: 50.0 mg glycyrrhizinic acid CRS (m2) of C % declared content + 25 ml HCl + 2.5 ml dioxan in 100 ml flask; reflux for 2 h.; cool; filter; discard filtrate

7. cfr. 2-5
8. apply to TLC-plates as bands 20 mm x 3 mm 2 x 60 µl of test solution and 2 x 60 µl of reference solution; develop plate 2 x over 15 cm; dry; examine under 254 nm
9. mark zones, corresponding to ***β-glycyrrhetic acid*** in the chromatograms.
10. remove coatings; transfer to 25 ml flasks; add 5.0 ml ethanol; shake for 15 min.
11. filter each solution into 10 ml volumetric flask; rinse filter; dilute to 10.0 ml with EtOH.
12. prepare blank from plate
13. measure absorbance; calculate % glycyrrhizinic acid : **A1 x m2 x C/A2 x m1**

- **Rhatany root** : *Total polyphenols, Polyphenols not adsorbed by hide powder* as tannins with pyrogallol as Standard : **absorbance at 715 nm**

- **Rhubarb** : ≥ 2.2 % *Hydroxyanthracene derivatives* as rhein : **absorbance at 515 nm**

1. 0.100 g powder (m gram) + 30.0 ml water; mix and weigh; reflux for 15 min.
2. cool; add 50 mg $NaHCO_3$; weigh; adjust to original mass with water
3. centrifuge and transfer 10.0 ml liquid to reflux flask; add 20 ml ferric chloride solution; mix
4. reflux for 20 min., add 1 ml HCl, heat for further 20 min. shaking frequently; cool; transfer to separating funnel
5. shake with 3 x 25 ml ether, used to rinse the flask; combine ether extracts; wash with 2 x 15 ml water
6. filter ether extracts and dilute to 100.0 ml with ether
7. evaporate 10.0 ml to dryness; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
8. measure absorption; calculate % rhein: **A x 0.64/ m (specific absorbance = 468)**

- **Senna leaf** : ≥ 2.5 % *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. 0.150 g powder (m gram) + 30.0 ml water; mix and weigh; reflux for 15 min.
2. cool; weigh; adjust to original mass with water
3. centrifuge and transfer 20.0 ml supernatant liquid to separating funnel; add 0.1 ml dilute HCl; shake with 3 x 15 ml chloroform; discard chloroform layer;
4. add 0.10 g $NaHCO_3$; shake 3 min.;centrifuge; transfer 10.0 ml supernatant for reflux
5. add 20 ml ferric chloride solution; mix; reflux for 20 min.; add 1 ml HCl; heat for further 20 min.; shake to dissolve the precipitate
6. cool, transfer to separating funnel; shake with 3 x 25 ml ether, used to rinse the flask
7. combine ether layers; wash with 2 x 15 ml water; transfer ether layers and dilute to 100.0 ml with ether.
8. evaporate 10.0 ml ether to dryness; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
9. measure absorbance; calculate % sennoside B : **A x 1.25/ m (specific absorbance = 240)**

- **Senna pods, Alexandrian** : *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. cfr. Senna leaf

- **Senna pods, Tinnevelly** : *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. cfr. Senna leaf

- **Thyme** : *Phenols* as Thymol : **absorbance at 450 nm** (cfr. Phenols and polyphenols)

TESTS

- Almond oil : 0.100 g in 10.0 ml cyclohexane : **absorbance between 264 nm and 276 nm ≤ 0.20**
- Lemon oil : **absorbance between 260 nm and 400 nm : 0.20 to 0.96**
- Olive oil : 1.00 g in 100.0 ml cyclohexane : **absorbance at 270 nm ≤ 0.20 ; ratio absorbance at 232 nm/270 nm > 8**

PHENOLS AND POLYPHENOLS

ASSAY

- **Hamamelis leaf** : (protected from light)

1. 0.750 g powder (m in gram) + 150 ml water. Heat to boiling; cool; transfer and dilute to 250.0 ml
2. filter; discard first 50 ml of the filtrate

total polyphenols:

1. 5.0 ml filtrate + 25.0 ml water; mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
2. measure (after 3 min) absorbance (A1) at 715 nm

polyphenols not absorbed by hide powder:

1. 20.0 ml filtrate + 0.20 g hide powder CRS; shake for 60 min; filter
2. dilute 5.0 ml filtrate to 25.0 ml with water
3. mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
4. measure (after 3 min) absorbance (A2) at 715 nm

standard:

1. dissolve 50.0 mg pyrogallol R in water; dilute to 100.0 ml
2. dilute 5.0 ml solution to 100.0 ml with water
3. mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
4. measure (after 3 min) absorbance (A3) at 715 nm

% tannins: **13.12 x (A1 - A2)/ A3 x m**

- **Rhatany root :**

cfr. Hamamelis leaf

- **Thyme :**

1. dilute essential oil from assay to 50.0 ml alcohol (90.0 %)
2. 5.0 ml solution + 40 ml alcohol (90 %) + dilute with water to 100.0 ml
3. 5.0 ml in separating funnel + 45 ml water + 0.5 ml dilute ammonia + 1 ml 2 % aminopyrazolone; mix ; + 4 ml 2 % potassium ferrcyanide; mix;
4. + 25 ml methylene chloride; shake; separate methylene chloride layer
5. shake aqueous layer with 2 x 25 ml and 10 ml methylene chloride and filter
6. dilute to 100.0 ml with methylene chloride; measure absorbance at 450 nm
7. calculate % phenols, expressed as thymol; **(specific absorbance = 805)**

TESTS : Gelatin : phenolic preservatives + pentachlorophenol by TLC

THIN-LAYER CHROMATOGRAPHY *(2.2.27.)*

DETECTION IN ULTRAVIOLET LIGHT AT 254 nm :

- Anise oil : UV : anisaldehyde , anethol ; ***spray : vanillin reagent + heat*** : linalol, anethol, monoterpene hydrocarbons
- Aniseed : UV : anethol ; ***spray : phosphomolybdic acid + heat*** : anethol + triglycerides
- Cinnamon : 254 nm : cinnamaldehyde + eugenol ; 365 nm : o-methoxy-cinnamaldehyde ; ***spray : phloroglucinol solution*** : cinnamaldehyde +o-methoxy-cinnamaldehyde
- Clove : UV : eugenol + acetyleugenol; ***spray : anisaldehyde solution + heat*** : eugenol + acetyleugenol + caryophyllene
- Clove oil : UV : eugenol + acetyleugenol; ***spray : anisaldehyde solution + heat*** : eugenol + acetyleugenol + caryophyllene
- Devil's claw root : UV : harpagoside; ***spray : phloroglucinol solution + hydrochloric acid + heat*** : harpagoside
- Fennel bitter : UV : anethole; ***spray : sulphuric acid + heat*** : fenchone + anethole + terpenes
- Fennel sweet : UV : anethole; ***spray : sulphuric acid + heat*** : anethole + terpenes
- Gelatine : TEST : amino acid derivatives : derivatisation on the plate with ***dimethylaminonaphthalene-sulphonyl chloride + disodium tetraborate + dry at 60°C***; develop : 365 nm ; phenolic preservatives (ethyl-, methyl-, propyl parahydroxybenzoate, pentachlorophenol) : 254 nm
- Gentian root : UV : amarogentine; ***spray : fast red B salt; 10 min.*** : amarogentine; + ammonia vapour : amarogentine; TEST : other Gentiana species
- Lemon oil ***(no spray)***: UV 254 nm: citral + citropten + bergamotin + 5-genaryloxy-7-methoxycoumarin + psoralen derivative + biakangelicin; UV 365 nm : psoralen derivative, citropten, 5-genaryloxy-7-methoxycoumarin, bergamotin. TEST : adulterants : UV 254 nm : methyl anthranilate + methyl salicylate + chalcones ; UV 365 nm ; ***HCl-vapour + daylight***: chalcones + other adulterants
- Liquorice root : TESTS : UV : β-glycyrrhetic acid ; ***spray : anisaldehyde solution***; daylight: zones with Rf of 0.6 + β-glycyrrhetic acid; ASSAY: isolation by TLC of β-glycyrrhetic acid + absorbance at 250 nm
- Matricaria flower : UV : en-yne-dicycloether + matricin; ***spray : anisaldehyde solution + heat*** : bornyl acetate + matricin + bisabolol + en-yne-dicycloether + terpenes
- Peppermint leaf : UV : carvone + pulegone; ***spray : anisaldehyde solution + heat; daylight*** : menthol + cineole + carvone + pulegone + isomenthone + menthyl acetate + menthone + hydrocarbons
- Peppermint oil : UV : carvone + pulegone; ***spray : anisaldehyde solution + heat; daylight*** : menthol + cineole + carvone + pulegone + isomenthone + menthyl acetate + menthone + hydrocarbons + menthofuran
- Peru balsam : UV : benzyl benzoate + benzyl cinnamate; ***spray : phosphomolybdic acid + heat***; nerolidol ; no colophony
- Sterols in fatty oils : Separation of the sterol fraction from unsaponifiable matter *(2.4.23.)*: ***spray : dichlorofluorescein solution in ethanol + UV*** (before further GC analysis of isolated strerols)
- Shellac : TEST : colophony : UV ; ***spray : anisaldehyde solution + heat***
- Thyme : UV : thymol + quenching zones ; ***spray : anisaldehyde solution + heat*** : thymol + carvacrol + cineole + linalol + borneol

DETECTION IN ULTRAVIOLET LIGHT AT 365 nm :

- Aloes, barbados : ***spray : 10 % KOH in methanol + UV*** : barbaloin + aloesine ; ***heat*** : violet fluorescence zone
- Aloes, cape : ***spray : 10 % KOH in methanol, heat, UV*** : barbaloin + aloinosides A and B + aloesine; TEST : barbados aloes : no zone of violet fluorescence
- Aloes, dry extra extract standardised : ***spray : 10 % KOH in methanol,*** UV : barbaloin + aloesine + aloinosides A and B + violet fluorescence zone
- Cascara : ***spray : 5 % KOH in alcohol 50% + heat + UV*** : cascarosides (several zones with same fluorescence); TEST : other species of Rhamnus; anthrones: ***spray : 5 % KOH in alcohol 50% + heat + UV***: no zones of blue or orange-brown fluorescence; ***spray : 0.5 % nitrotetrazolium blue solution in methanol*** : no violet or greyish-blue zones
- Chamomille flower, roman : ***spray : solution of diphenylboric acid aminoethyl ester + macrogol 400 solution + stand for 30 min. + UV*** : apigenin + apigenin-7-glucoside + luteolin + apiin
- Cinchona bark : ***spray : anhydrous formic acid R + UV*** : quinine + quinidine ; ***spray : iodoplatinate reagent*** : quinine + quinidine + cinchonine + cinchonidine
- Cinnamon : 254 nm : cinnamaldehyde + eugenol ; 365 nm : o-methoxy-cinnamaldehyde ; ***spray : phloroglucinol solution*** : cinnamaldehyde +o-methoxy-cinnamaldehyde
- Digitalis leaf : ***spray : mixture of solution of chloramine + solution of trichloroacetic acid in alcohol + heat + UV*** : purpureaglycoside B + A + gitoxin + digitoxin
- Eucalyptus oil : ***spray : anisaldehyde solution + heat + UV*** : 1,8-cineole ; no citronellal
- Frangula bark : ***spray : 5 % KOH in alcohol 50% + heat + daylight*** : glucofrangulins + frangulins (several brownish-red zones); TEST : other species of Rhamnus; anthrones: ***spray : 5 % KOH in alcohol 50% + heat + UV*** : no yellow or blue fluorescence zones; ***spray : nitrotetrazolium blue solution in methanol*** : no violet or greyish-blue zones
- Gelatine : TEST : <u>amino acid derivatives</u> : derivatisation on the plate with dimethylaminonaphthalene-sulphonyl chloride + disodium tetraborate + dry at 60°C; develop ; 365 nm ; <u>phenolic preservatives</u> (ethyl-, methyl-, propyl parahydroxybenzoate, pentachlorophenol) : 254 nm
- Ipecacuanha root : ***spray : solution of iodine in alcohol + heat + daylight*** : emetine + cephaëline ; UV : intense yellow fluorescence : emetine +cephaëline
- Lemon oil *(no spray)* : UV 254 nm: citral + citropten + bergamotin + 5-genaryloxy-7-methoxycoumarin + psoralen derivative + biakangelicin; UV 365 nm : psoralen derivative, citropten, 5-genaryloxy-7-methoxycoumarin, bergamotin. TEST : adulterants : UV 254 nm : methyl anthranilate + methyl salicylate + chalcones ; UV 365 nm ; HCl-vapour : daylight: chalcones + other adulterants
- Lime flower : ***spray : solution of diphenylboric acid aminoethyl ester + macrogol 400 solution + stand for 30 min. + UV*** : rutine + hyperoside + different zones of fluorescence (pattern description)
- Rhubarb : UV : emodin + physcione + chrysophanol + rhein + aloe-emodin ; ***spray : 10 % KOH in methanol*** : zones become red to violet

DETECTION IN DAYLIGHT AFTER SPRAYING

- Acacia : 2 runs ; ***spray : aminohippuric acid reagent + heat*** : galactose + arabinose + rhamnose (no glucose)
- Bearberry leaf : ***spray : 1 % solution dichloroquinonechlorimide in methanol + 2 % solution of anhydrous sodium carbonate*** : arbutin + gallic acid + hydroquinone
- Belladonna leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Carnauba wax : ***spray : phosphomolybdic acid solution in alcohol*** : triacontanol + blue zones
- Fatty oils : identification *(2.3.2.)* : on octadecylsilyl silica gel : ***spray : 10 % phosphomolybdic acid solution in alcohol + heat***
- Guar galactomannan : 1 run ; ***spray : aminohippuric acid reagent + heat*** : galactose + mannose
- Hamamelis leaf : ***spray : ferric chloride solution*** : tannic acid + gallic acid + phenolic compounds
- Hyoscyamus leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Opium, raw : ***spray : potassium iodobismuthate solution + 0.4 % sulfuric acid*** : morphine + codeine + papaverine + noscapine + thebaine
- Rhubarb : TEST : Rheum rhaponticum : ***spray : phosphomolybdic acid solution*** : no rhaponticin
- Senega root : ***spray : anisaldehyde solution + heat*** : saponosides (red zones); ***spray : 20 % phosphomolybdic acid in ethanol + heat*** : saponosides (blue zones)
- Senna leaf ***: spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Senna pods, Alexandrian***: spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Senna pods, Tinnevelly: ***spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Star anise : ***spray : 20 % phosphomolybdic acid in alcohol + heat*** : anethole + triglycerides; TEST : no myristicine (Illicium anisatum)
- Sterols in Olive oil : separation of sterol fraction : ***spray : potassium permanganate solution***
- Stramonium leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Tragacanth : ***spray : aminohippuric acid reagent + heat*** : galactose + arabinose + xylose + fucose
- Valerian root : TEST : ***spray : anisaldehyde solution + heat*** : valereinc acid + valtrate + isovaltrate + acetoxyvalerenic acid

GAS CHROMATOGRAPHY *(2.2.28.)*

- Almond oil : Foreign fatty oils *(2.4.22.)* (fatty-acid fraction in %); Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Almond oil, refined : Foreign fatty oils *(2.4.22.)* (fatty-acid fraction in %); Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Anise oil : Chromatographic profile
- Arachis oil : Foreign fatty oils *(2.4.22.)*
- Clove oil : Chromatographic profile
- Fennel, bitter : Estragole (TESTS) and Anethole and Fenchone (ASSAY)
- Fennel, sweet : Estragole and Fenchone (TESTS) and Anethole (ASSAY)
- Olive oil : Foreign fatty oils *(2.4.22.)*; Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Peppermint oil : Chromatographic profile
- Pesticide residues *(2.8.13.)*: Organo phosphorus insecticides, Organochlorine and Pyrethroid insecticides : no monographs
- Sesame oil : Foreign fatty oils *(2.4.22.)*
- TEST for methanol and 2-propanol *(2.9.11.)* : extracts and tinctures
- Wool fat : Butylhydroxytoluene (< 200 ppm)
- Wool fat, hydrous : Butylhydroxytoluene (< 150 ppm)

LIQUID CHROMATOGRAPHY *(2.2.29.)*

- Devil's claw root : ASSAY : Harpagoside (1.2 %) : detection at 278 nm ; C18-column
- Opium, raw : ASSAY : Morphine (> 10.0 %), Codeine (> 2.0 %), Thebaine (< 3.0 %) : detection at 280 nm ; C8-column
- Sesame oil : TESTS : Composition of triglycerides : refractometer detection; 2 C18-columns

(Ph.Eur. - Supplem. 1988)

SIZE-EXCLUSION CHROMATOGRAPHY *(2.2.30.)*

- Purification of Organochlorine, Organophosphorus and Pyrethroid insecticides in **PESTICIDE RESIDUES** *(2.8.13.)*

LOSS ON DRYING *(2.2.32.)*

- Acacia - 100-105 °C: max. 15.0 %
- Acacia, spray dried - 100-105 °C : max. 10.0 %
- Agar - 100-105 °C : max. 20.0 %
- Aloes, barbados - 100-105 °C : max. 12.0 %
- Aloes, cape - 100-105 °C : max. 10.0 %
- Aloes, dry extract, standardised - 100-105 °C / 3 h : max. 4.0 %
- Bearberry leaf - 100-105 °C / 2 h : max. 10.0 %
- Belladona leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Cascara - 100-105 °C / 2 h : max. 10.0 %
- Devil's claw root - 100-105 °C : max. 12.0 %
- Digitalis leaf - 100-105 °C : max. 6.0 %
- Extracts : **Dry residue** : 100-105 °C / 3 h
- Frangula bark - 100-105 °C/ 2 h : max. 10.0 %
- Gelatine - 100-105 °C : max. 15 %
- Guar galactomannan - 100-105 °C / 5 h : max. 15.0 %
- Hamamelis leaf - 100-105°C / 4 h : max. 10.0 %
- Hyoscyamus leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Ipecacuanha, prepared - 100-105 °C : max. 5.0 %
- Ipecacuanha, root - 100-105 °C : max. 10.0 %
- Lime flower - 100-105 °C / 2 h : max. 12.0 %
- Maize starch - 100-105 °C : max. 15.0 %
- Marshmallow root - 100-105°C / 2 h : max.12.0 %
- Opium, raw -100-105 °C / 4 h : max. 15.0 %
- Potato starch - 100-105 °C : max. 20.0 %
- Psyllium seed - 100-105 °C / 2 h : max. 14.0 %
- Rhubarb - 100-105 °C : max. 12.0 %
- Rice starch - 100-105 °C : max. 15.0 %
- Senna leaf - 100-105 °C / 2 h : max. 12.0 %
- Senna pods, Alexandrian - 100-105 °C / 2 h : max. 12.0 %
- Senna pods, Tinnevelly - 100-105 °C / 2 h : max. 12.0 %
- Shellac - 40-45 °C / 24 h : max. 2.0 %
- Stramonium leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Wheat starch - 100-105 °C : max. 15.0 %
- Wool fat - 100-105 °C / 1 h : max. 0.5 %

SULPHATED ASH *(2.4.14.)*

- Ichtammol : < 0.3 %
- Linseed : < 6.0 %
- Liquorice root : < 10.0 %
- Maize starch : < 0.6 %
- Potato starch : < 0.6 %
- Rice starch : < 1.0 %
- Valerian root : < 15.0 %
- Wheat starch : < 0.6 %
- Wool fat : < 0.15 %
- Wool fat, hydrous : < 0.1 %

TITRATIONS

- ASSAYS

Ipecacuanha root : ≥ 2.0 % total alkaloids (emetine + cephaëline), calculated as emetine

1. 7.5 g powder + NH_3 and ether extraction
2. residue : dissolve in neutralised alcohol (90 %)
3. + 15.0 ml HCl 0.1 M
4. titrate excess acid with NaOH 0.1 M on methyl red mixed solution
5. 1 ml HCl 0.1 M = 24.03 mg total alkaloids, as emetine

Belladonna leaf : ≥ 0.30 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 10.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer
7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Hyoscyamus leaf : ≥ 0.05 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 40.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer
7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Stramonium leaf : ≥ 0.25 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 10.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer

7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Lemon oil : ≥ 2.2 % and ≤ 4.5 % carbonyl compounds, calculated as citral

1. 9.000 g + 20 ml ethanol
2. add 10.0 ml hydroxylamine.HCl + bromophenol blue solution
3. titrate with 0.5 M alcoholic KOH (from yellow to olive-green)
4. allow to stand for 5 min.; titrate again if necessary
5. 1 ml 0.5 M alcoholic KOH = 76.1 mg carbonyl compounds, as citral

Ichthammol : ≥ 4.5 % and ≤ 7.0 % total ammonia

1. dissolve 2.50 g in warm water
2. rinse solution into a 250 ml volumetric flask + add 200 ml NaCl-solution + dilute to 250 ml
3. filter (discard first 20 ml)
4. 100.0 ml clear filtrate + 25 ml formaldehyde solution (neutralised to phenolphthalein)
5. titrate with 0.1 M NaOH until faint pink colour is obtained
6. 1 ml 0.1 M NaOH = 1.703 mg NH_3

- TESTS

Gelatin : Sulphur dioxide: ≤ 200 ppm

1. cfr. Apparatus for determination of SO_2
2. boil gelatin with dilute HCl for 1 h.
3. collect SO_2 in test tube with 10 ml (neutralised) dilute hydrogen peroxide solution
4. heat contents of test tube for 15 min.
5. titrate with 0.1 M NaOH on bromophenol blue R

Eucalyptus oil : aldehydes

1. 10 ml oil + 5 ml toluene and 4 ml alcoholic $NH_2OH.HCl$ solution (contains methylorange)
2. shake
3. titrate with 0.5 M KOH in alcohol (60 %) until red colour changes to yellow
4. continue titration with shaking
5. end-point reached : permanent pure yellow colour of indicator in lower layer
6. repeat titration on further 10 ml with first determination liquid as reference
7. maximum 2.0 ml 0.5 M KOH in alcohol (60 %)

Olive oil : unsaponifiable matter : ≤ 1.5 %

1. 5.0 g oil + 50 ml 2 M alcoholic KOH : reflux for 1 h (+ shaking)
2. + 50 ml water; shake; cool ; transfer to separating funnel
3. + 50 ml light petroleum ; shake; transfer aqueous layer to second separating funnel

4. shake aqueous layer with 2 x 50 ml light petroleum; combine light petroleum layers
5. wash with 3 x 50 ml alcohol (50 %)
6. evaporate light petroleum and dry residue at 100-105 °C (15 min) : a gram
7. dissolve residue in 20 ml alcohol (neutralised to bromophenol blue solution)
8. if necessary, titrate with 0.1 M HCl (b ml)
9. % of unsaponifiable matter: 100 (a - 0.032 b)/m

Carnauba wax : Acid value : 2-7 ; Saponification value : 78-95

1. 2.000 g (m gram) + 40 ml xylene : reflux until completely dissolved
2. + 20 ml alcohol + 1 ml phenolphthalein solution
3. titrate hot solution with 0.5 M alcoholic KOH (n1 ml); carry out a blank test (n2 ml)
4. calculate acid value : 28.05 (n1- n2)/m

1. add to titrated solution (acid value) 20.0 ml 0.5 M alcoholic KOH; reflux for 3 h
2. add 1 ml phenolphthalein solution; titrate hot solution with 0.5 M HCl (n3 ml)
3. carry out blank (n4 ml)
4. calculate saponification value : 28.05 (n4 - n3)/m + acid value

- Acid value *(2.5.1.)*

Almond oil : < 2.0
Almond oil, refined : < 0.5
Anise oil : < 1.0
Arachis oil : < 0.6
Carnauba wax : 2 to 7 (own method)
Olive oil : < 2.0 ; if intended for use in manufacture of parenteral dosage forms : < 0.5
Peppermint oil : < 1.4
Sesame oil : < 0.6 ; if intended for use in manufacture of parenteral dosage forms : < 0.3
Wool fat : < 1.0
Wool fat, hydrous : < 0.8

- Ester value *(2.5.2.)*

Beeswax, white : 17 to 24 (own method)
Beeswax, yellow : 17 to 22 (own method)

- Hydroxyl value *(2.5.3.)*

Castor oil *(Ricini oleum)* : method A : > 150

- Iodine value *(2.5.4.)*

Castor oil : 82 to 90

- Peroxide value *(2.5.5.)*

Almond oil : < 10.0
Almond oil, refined : < 5.0

Arachis oil : < 5.0
Olive oil : < 5.0 (also for parenteral dosage forms)
Sesame oil : < 5.0
Wool fat : < 20
Wool fat, hydrous : < 15

- Saponification value *(2.5.6.)*

Carnauba wax (own method used) : 78 to 95
Castor oil : 176 to 187
Peru Balsam : 230 to 255
Wool fat : 90 to 105
Wool fat, hydrous : 67 to 79

- Unsaponifiable matter *(2.5.7.)*

Almond oil : < 0.7 %
Almond oil (refined) : < 0.7 %
Arachis oil : < 1.0 %
Castor oil : < 0.8 %
Olive oil (own method) : < 1.5 %
Sesame oil : < 2.0 % (Ph.Eur. Suppl. 1998)
Soya-bean oil : < 1.5 %

DETERMINATION OF WATER *(2.5.12.)*

If intended for use in the manufacture of parentral dosage forms

Almond oil, refined : 0.3 %
Arachis oil : 0.3 %
Castor oil : 0.3 %
Olive oil : 0.1 %
Sesame oil : 0.05 %
Soya-bean oil : 0.3 %

RESIDUE ON EVAPORATION OF ESSENTIAL OILS *(2.8.9.)*

- Lemon oil

SOLUBILITY OF ALCOHOL OF ESSENTIAL OILS *(2.8.10.)*

- Eucalyptus oil
- Clove oil

ASSAY OF 1,8- CINEOLE IN ESSENTIAL OILS *(2.8.11.)*

- Eucalyptus oil

DETERMINATIO OF ESSENTIAL OILS IN VEGETABLE DRUGS *(2.8.12.)*

- Aniseed : ≥ 20 ml/kg
- Caraway fruit : ≥ 30 ml/kg
- Chamomile flower, roman : > 7 ml/kg
- Cinnamon: > 12 ml/kg
- Clove: > 150 ml/kg
- Fennel, bitter : > 40 ml/kg
- Fennel, sweet : > 20 ml/kg
- Matricaria flower: > 4 ml/kg
- Peppermint leaf : > 12 ml/kg
- Star anise : > 70 ml/kg
- Thyme : > 12 ml/kg
- Valerian Root : > 5 ml/kg

MICROBIAL CONTAMINATION

- Acacia : total viable aerobic count *(2.6.12.)* < 10^4 /gram bacteria; TEST *E.coli (2.6.13.)*
- Agar : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Gelatin : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Maize starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Potato starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Wheat starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Rice starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Tragacanth : total viable aerobic count *(2.6.12.)* < 10^4 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Guar gallactomannan : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*

PART I

SUMMARY OF THE DOSSIER

A. Administrative data

The medicinal product which is the subject of the application shall be identified by name and name of the active ingredient(s), together with the *pharmaceutical form, the method of administration, the strength* and the final presentation, including packaging.

The name and address of the applicant shall be given, together with the name and address of the manufacturers and the sites involved in the different stages of the manufacture (including the manufacturer of the finished product and the *manufacturer(s) of the active ingredient(s)*), and where relevant the name and address of the importer.

The applicant shall identify the number of volumes of documentation submitted in support of the application and indicate what samples, if any, are also provided.

Annexed to the administrative data shall be copies of the manufacturing authorization as defined in Article 16 of Council Directive 75/319/EEC ([1]), together with a list of countries in which authorization has been granted, copies of all the summaries of product characteristics in accordance with Article 4a of Directive 65/65/EEC as approved by Member States and a list of countries in which an application has been submitted.

B. Summary of product characteristics

The applicant shall propose a summary of the product characteristics, in accordance with Article 4a of Directive 65/65/EEC.

In addition the applicant shall *provide samples or mock-ups of the packaging, labels and package leaflets* for the medicinal product concerned.

C. Expert reports

In accordance with Article 2 of Directive 75/319/EEC, expert reports must be provided on the chemical, pharmaceutical and biological documentation, the pharmacotoxicological documentation and the clinical documentation respectively.

The *expert report shall consist of a critical evaluation* of the quality of the product and the investigations carried out on animals and human beings and bring out all the data relevant for evaluation. It shall be worded so as to enable the reader to obtain a good understanding of the properties, quality, the proposed *specifications and control* methods, the safety, the efficacy, the advantages and disadvantages of the product.

All important data shall be summarized in an appendix to the expert report, whenever possible including report formats in tabular or in graphic form. The expert report and the summaries shall contain precise cross references to the information contained in the main documentation.

Each expert report shall be prepared by a suitably qualified and experienced person. It shall be signed and dated by the expert, and attached to the report shall be brief information about the educational background, training and occupational experience of the expert. The professional relationship of the expert to the applicant shall be declared.

([1]) OJ No L 147, 9. 6. 1975, p. 13.

PART 2

CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL TESTING OF MEDICINAL PRODUCTS

All the test procedures shall correspond to the state of scientific progress at the time and shall be validated procedures; results of the validation studies shall be provided.

All the test procedure(s) shall be described in sufficiently precise detail so as to be reproducible in control tests, carried out at the request of the competent authority; any special apparatus and *equipment which may* be used shall be described in adequate detail, possibly accompanied by a diagram. The formulae of the laboratory reagents shall be supplemented, if necessary, by the method of preparation. In the case of test procedures included in the *European Pharmacopoeia* or the pharmacopoeia of a Member State, this description may be replaced by a detailed reference to the pharmacopoeia in question.

A. Qualitative and quantitative particulars of the constituents

The particulars and documents which must accompany applications for marketing authorization, pursuant to point 3 of Article 4 (2) of Directive 65/65/EEC shall be submitted *in accordance with the following requirements.*

1. *Qualitative particulars*

1.1 *Qualitative particulars* of all the constituents of the *medicinal product shall mean* the designation or description of:

— the active ingredient(s),

— the constituent(s) of the excipients, whatever their nature or the quantity used, including colouring matter, preservatives, adjuvants, *stabilizers, thickeners*, emulsifiers, flavouring and aromatic substances, etc.,

— the constituents, intended to be ingested or otherwise administered to the patient, of the outer covering of the medicinal products – capsules, gelatine capsules, rectal capsules, etc.

These particulars shall be supplemented by any relevant data concerning the container and, where appropriate, its manner of closure, together with details of devices with which the medicinal product will be used or administered and which will be delivered with the product.

1.2 In the context of a radiopharmaceutical kit, which is to be *radiolabelled after* supply by the manufacturer, the active ingredient is considered to be that part of the formulation which is intended to carry or bind the radionuclide. Details of the source of the radionuclide shall be stated. In addition, any compounds essential for the radiolabelling shall be stated.

In a generator, both mother and daughter radionuclides are to be considered as active ingredients.

2. The *usual terminology*, to be used in describing the constituents of medicinal products, shall mean, notwithstanding the application of the other provisions of point 3 of Article 4 (2) of Directive 65/65/EEC:

— in respect of substances which appear in the *European Pharmacopoeia* or, failing this, in the national pharmacopoeia of one of the Member States, the main title at the head of the monograph in question, with reference to the *pharmacopoeia* concerned;

— in respect of other substances, the international non-proprietary name recommended by the World Health Organization, which may be accompanied by another non-proprietary name, or, failing these, the exact scientific designation; substances not having an international non-proprietary name or an exact scientific designation shall be described by a statement of how and from what they were prepared, supplemented, where appropriate, by any other relevant details,

— in respect of colouring matter, designation by the 'E' code assigned to them in Council Directive 78/25/EEC [1] of 12 December 1977 on the approximation of the rules of the Member States concerning the colouring matters authorized for use in medicinal products.

3. *Quantitative particulars*

3.1 In order to give *quantitative particulars* of the active ingredients of the medicinal products, it is necessary, depending on the pharmaceutical form concerned, to specify the mass, or the number of units of biological activity, either per dosage-unit or per unit of mass or volume, of each active ingredient.

Units of biological activity shall be used for substances which cannot be defined chemically. Where an International Unit of biological activity has been defined by the World Health Organization, this shall be used. Where no International Unit has been defined, the units of biological activity shall be expressed in such a way as to provide unambiguous information on the activity of the substances.

Whenever possible, biological activity per units of mass shall be indicated.

This information shall be supplemented:

— in respect of injectable preparations, by the mass or units of biological activity of each active ingredient in the unit container, taking into account the usable volume of the product, after reconstitution, where appropriate;

— in respect of medicinal products to be administered by drops, by the mass or units of biological activity of each active ingredient contained in the number of drops corresponding to 1 ml or 1 g of the preparation;

— in respect of syrups, emulsions, granular preparations and other pharmaceutical forms to be administered in measured quantities, by the mass or units of biological activity of each active ingredient per measured quantity.

3.2 Active ingredients present in the form of compounds or derivatives shall be described quantitatively by their total mass, and if necessary or relevant, by the mass of the active entity or entities of the molecule.

3.3 For medicinal products containing an active ingredient which is the subject of an application for marketing authorization in any Member State for the first time, the quantitative statement of an active ingredient which is a salt or hydrate shall be systematically expressed in terms of the mass of the active entity or entities in the molecule. All subsequently authorized medicinal products in the Member States shall have their quantitative composition stated in the same way for the same active ingredient.

3.4 For allergen products, the quantitative particulars shall be expressed by units of biological activity, except for well defined allergen products for which the concentration may be expressed by mass/unit of volume.

[1] OJ No L 11, 14. 1. 1978, p. 18.

3.5 The requirement to express the content of active ingredients in terms of the mass of active entities, as in point 3.3 above, may not apply to radiopharmaceuticals. For radionuclides, radioactivity shall be expressed in becquerels at a given date and, if necessary, time with reference to time zone. The type of radiation shall be indicated.

4. *Development pharmaceutics*

4.1 An explanation should be provided with regard to the choice of composition, constituents and container and the intended function of the excipients in the finished product. This explanation shall be supported by scientific data on development pharmaceutics. The overage, with justification thereof, should be stated.

4.2 For radiopharmaceuticals, this should include a consideration of chemical/radiochemical purity and its relationship to biodistribution.

B. Description of method of preparation

1. The description of the method of preparation accompanying the application for marketing authorization pursuant to point 4 of Article 4 (2) of Directive 65/65/EEC, shall be drafted in such a way as to give an adequate synopsis of the nature of the operations employed.

For this purpose it shall include at least:

— mention of the various stages of manufacture, so that an assessment can be made of whether the processes employed in producing the pharmaceutical form might have produced an adverse change in the constituents;

— in the case of continuous manufacture, full details concerning precautions taken to ensure the homogeneity of the finished product;

— the actual manufacturing formula, with the quantitative particulars of all the substances used, the quantities of excipients, however, being given in approximate terms in so far as the pharmaceutical form makes this necessary; mention shall be made of any substances that may disappear in the course of manufacture; any overage shall be indicated and justified;

— a statement of the stages of manufacture at which sampling is carried out for in-process control tests, where other data in the documents supporting the application show such tests to be necessary for the quality control of the finished product;

— experimental studies validating the manufacturing process, where a non-standard method of manufacture is used or where it is critical for the product;

— for sterile products, details of the sterilization processes and/or aseptic procedures used.

2. For radiopharmaceutical kits, the description of the method of preparation shall also include details of the manufacture of the kit and details of its recommended final processing to produce the radioactive medicinal product.

For radionuclides, the nuclear reactions involved shall be discussed.

Controls of starting materials

For the purposes of this paragraph, 'starting materials' shall mean all the constituents of the medicinal duct and, if necessary, of its container, as referred to in paragraph A, point 1, above.

the case of:

an active ingredient not described in the *European Pharmacopoeia* or in the pharmacopoeia of a Member State, or

an active ingredient described in the *European Pharmacopoeia* or in the pharmacopoeia of a Member when prepared by a method liable to leave impurities not mentioned in the pharmacopoeial monograph and for which the monograph is inappropriate to adequately control its quality,

hich is manufactured by a person different from the applicant, the latter may arrange for the detailed description the manufacturing method, quality control during manufacture and process validation to be supplied directly to e competent authorities by the manufacturer of the active ingredient. In this case, the manufacturer shall owever provide the applicant with all the data which may be necessary for the latter to take responsibility for e medicinal product. The manufacturer shall confirm in writing to the applicant that he shall ensure batch to atch consistency and not modify the manufacturing process or specifications without informing the applicant. ocuments and particulars supporting the application for such a change shall be supplied to the competent uthorities.

The particulars and documents accompanying the application for marketing authorization pursuant to points 7 and of Article 4 (2) of Directive 65/65/EEC shall include the results of the tests, including batch analyses articularly for active ingredients, relating to quality control of all the constituents used. These shall be submitted n accordance with the following provisions.

1.1 *Starting materials listed in pharmacopoeias*

The monographs of the *European Pharmacopoeia* shall be applicable to all substances appearing in it.

In respect of other substances, each Member State may require observance of its own national pharmacopoeia with regard to products manufactured in its territory.

Constituents fulfilling the requirements of the *European Pharmacopoeia* or the pharmacopoeia of one of the Member States shall be deemed to comply sufficiently with point 7 of Article 4 (2) of Directive 65/65/EEC. In this case the description of the analytical methods may be replaced by a detailed reference to the pharmacopoeia in question.

However, where a starting material in the *European Pharmacopoeia* or in the pharmacopoeia of a Member State has been prepared by a method liable to leave impurities not controlled in the pharmacopoeia monograph, these impurities and their maximum tolerance limits must be declared and a suitable test procedure must be described.

Colouring matter shall, in all cases, satisfy the requirements of Directive 78/25/EEC.

The routine tests carried out on each batch of starting materials must be as stated in the application for marketing authorization. If tests other than those mentioned in the pharmacopoeia are used, proof must be supplied that the starting materials meet the quality requirements of that pharmacopoeia.

In cases where a specification contained in a monograph of the *European Pharmacopoeia* or in the national pharmacopoeia of a Member State might be insufficient to ensure the quality of the substance, the competent authorities may request more appropriate specifications from the person responsible for placing the product on the market.

The competent authorities shall inform the authorities responsible for the pharmacopoeia in question. The person responsible for placing the product on the market shall provide the authorities of that pharmacopoeia with the details of the alleged insufficiency and the additional specifications applied.

In cases where a starting material is described neither in the *European Pharmacopoeia* nor in the pharmacopoeia of a Member State, compliance with the monograph of a third country pharmacopoeia can be accepted; in such cases, the applicant shall submit a copy of the monograph accompanied where necessary by the validation of the test procedures contained in the monograph and by a translation where appropriate.

1.2 *Starting materials not in a pharmacopoeia*

Constituents which are not given in any pharmacopoeia shall be described in the form of a monograph under the following headings:

a) The name of the substance, meeting the requirements of paragraph A, point 2, shall be supplemented by any trade or scientific synonyms;

b) the definition of the substance, set down in a form similar to that used in the *European Pharmacopoeia*, shall be accompanied by any necessary explanatory evidence, especially concerning the molecular structure where appropriate; it must be accompanied by an appropriate description of the method of synthesis. Where substances can only be described by their method of preparation, the description should be sufficiently detailed to characterize a substance which is constant both in its composition and in its effects;

c) methods of identification may be described in the form of complete techniques as used for production of the substance, and in the form of tests which ought to be carried out as a routine matter;

d) purity tests shall be described in relation to the sum total of predictable impurities, especially those which may have a harmful effect, and, if necessary, those which, having regard to the combination of substances to which the application refers, might adversely affect the stability of the medicinal product or distort analytical results;

e) with regard to complex substances of plant or animal/human origin, a distinction must be made between the case where multiple pharmacological effects render chemical, physical or biological control of the principal constituents necessary, and the case of substances containing one or more groups of principles having similar activity, in respect of which an overall method of assay may be accepted;

f) when materials of animal/human origin are used, measures to ensure freedom from potentially pathogenic agents shall be described;

g) for radionuclides, the nature of the radionuclide, the identity of the isotope, likely impurities, the carrier, the use and the specific activity shall be given;

h) any special precautions that may be necessary during storage of the starting material and, if necessary, the maximum period of storage before retesting shall be given.

Physic[illegible] l char[illegible] liable bio-ava[illegible]

The following items of information concerning active ingredients, whether or [illegible] in the [illegible]poeias, shall be provided as part of the general description of the active ingredients if the bio-availability of the medicinal product depends on them:

— crystalline form and solubility coefficients,

— particle size, where appropriate after pulverization,

— state of solvation,

— oil/water coefficient of partition (1).

The first three indents are not applicable to substances used solely in solution.

2. For biological medicinal products, such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma, the requirements of this paragraph shall apply.

For the purposes of this paragraph, starting materials shall mean any substance used in the manufacture of the medicinal product; this includes the constituents of the medicinal product, and, if necessary, of its container, as referred to in paragraph A, point 1 above, as well as source materials such as microorganisms, tissues of either plant or animal origin, cells or fluids (including blood) of human or animal origin, and biotechnological cell constructs. The origin and history of starting materials shall be described and documented.

The description of the starting material shall include the manufacturing strategy, purification/inactivation procedures with their validation and all in-process control procedures designed to ensure the quality, safety and batch to batch consistency of the finished product.

2.1 When cell banks are used, the cell characteristics shall be shown to have remained unchanged at the passage level used for the production and beyond.

2.2 Seed materials, cell banks, pools of serum or plasma and other materials of biological origin and, whenever possible, the source materials from which they are derived shall be tested for adventitious agents.

If the presence of potentially pathogenic adventitious agents is inevitable, the material shall be used only when further processing ensures their elimination and/or inactivation, and this shall be validated.

2.3 Whenever possible, vaccine production shall be based on a seed lot system and on established cell banks; for serums, defined pools of starting materials shall be used.

For bacterial and viral vaccines, the characteristics of the infectious agent shall be demonstrated on the seed. In addition, for live vaccines, the stability of the attenuation characteristics shall be demonstrated on the seed; if this proof is not sufficient, the attenuation characteristics shall also be demonstrated at the production stage.

(1) The competent authorities may also request the pK and pH values if they think this information is essential.

2.4 For allergen products, the specifications and control methods for the source materials shall be described. The description shall include particulars concerning collection, pretreatment and storage.

2.5 For medicinal products derived from human blood or plasma, the origin and the criteria and procedures for collection, transportation and storage of the source material shall be described and documented.

Defined pools of source material shall be used.

3. For radiopharmaceuticals, starting materials include irradiation target materials.

D. Control tests carried out at intermediate stages of the manufacturing process

1. The particulars and documents accompanying an application for marketing authorization, pursuant to points 7 and 8 of Article 4 (2) of Directive 65/65/EEC, shall include particulars relating to the product control tests that may be carried out at an intermediate stage of the manufacturing process, with a view to ensuring the consistency of the technical characteristics and the production process.

These tests are essential for checking the conformity of the medicinal product with the formula when, exceptionally, an applicant proposes an analytical method for testing the finished product which does not include the assay of all the active ingredients (or of all the excipient constituents subject to the same requirements as the active ingredients).

The same applies where the quality control of the finished product depends on in-process control tests, particularly if the substance is essentially defined by its method of preparation.

2. For biological medicinal products, such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma, the procedures and the criteria of acceptability published as recommendations of the WHO (*Requirements for Biological Substances*) shall serve as guidelines for all controls of production stages which are not specified in the *European Pharmacopoeia*, or failing this, in the national pharmacopoeia of a Member State.

For inactivated or detoxified vaccines, effective inactivation or detoxification shall be verified during each production run, unless this control is dependent upon a test for which the availability of susceptible animals is limited. In this case, the test shall be carried out until consistency of production and correlation with appropriate in-process controls have been established and thereafter compensated by appropriate in-process controls.

3. For modified or absorbed allergens, the allergen products shall be qualitatively and quantitatively characterized at an intermediate stage, as late as possible in the manufacturing process.

E. Control tests on the finished product

1. For the control of the finished product, a batch of a finished product comprises all the units of a pharmaceutical form which are made from the same initial quantity of material and have undergone the same series of manufacturing and/or sterilization operations or, in the case of a continuous production process, all the units manufactured in a given period of time.

The application for marketing authorization shall list those tests which are carried out routinely on each batch of finished product. The frequency of the tests which are not carried out routinely shall be stated. Release limits shall be indicated.

The particulars and documents accompanying the application for marketing authorization pursuant to points 7 and 8 of Article 4 (2) of Directive 65/65/EEC, shall include particulars relating to control tests on the finished product at release. They shall be submitted in accordance with the following requirements.

The provisions of the monographs for pharmaceutical forms, immunosera, vaccines and radiopharmaceutical preparations of the *European Pharmacopoeia* or failing that, of a Member State, shall be applicable to all products defined therein. For all controls of biological medicinal products such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma which are not specified in the *European Pharmacopoeia* or failing this, in the pharmacopoeia of a Member State, the procedures and the criteria of acceptability published as recommendations of the WHO (*Requirements for Biological Substances*) shall serve as guidelines.

If test procedures and limits other than those mentioned in the monographs of the *European Pharmacopoeia*, or failing this, in the national pharmacopoeia of a Member State, are used, proof shall be supplied that the finished product would, if tested in accordance with those monographs, meet the quality requirements of that pharmacopoeia for the pharmaceutical form concerned.

1.1 *General characteristics of the finished product*

Certain tests of the general characteristics of a product shall always be included among the tests on the finished product. These tests shall, wherever applicable, relate to the control of average masses and maximum deviations, to mechanical, physical or microbiological tests, organoleptic characteristics, physical characteristics such as density, pH, refractive index, etc. For each of these characteristics, standards and tolerance limits shall be specified by the applicant in each particular case.

The conditions of the tests, where appropriate, the equipment/apparatus employed and the standards shall be described in precise details whenever they are not given in the *European Pharmacopoeia* or the pharmacopoeia of the Member States; the same shall apply in cases where the methods prescribed by such pharmacopoeias are not applicable.

Furthermore, solid pharmaceutical forms having to be administered orally shall be subjected to in vitro studies on the liberation and dissolution rate of the active ingredient or ingredients; these studies shall also be carried out where administration is by another means if the competent authorities of the Member State concerned consider this necessary.

1.2 *Identification and assay of active ingredient(s)*

Identification and assay of the active ingredient(s) shall be carried out either in a representative sample from the production batch or in a number of dosage-units analysed individually.

Unless there is appropriate justification, the maximum acceptable deviation in the active-ingredient content of the finished product shall not exceed ± 5 % at the time of manufacture.

On the basis of the stability tests, the manufacturer must propose and justify maximum acceptable tolerance limits in the active-ingredient content of the finished product up to the end of the proposed shelf-life.

In certain exceptional cases of particularly complex mixtures, where assay of active ingredients which are very numerous or present in very low amounts would necessitate an intricate investigation difficult to carry out in respect of each production batch, the assay of one or more active ingredients in the finished product may be omitted, on the express condition that such assays are made at intermediate stages in the production process. This relaxation may not be extended to the characterization of the substances concerned. This simplified technique shall be supplemented by a method of quantitative evaluation, enabling the competent authority to have the conformity of the medicinal product with its specification verified after it has been placed on the market.

An *in vivo* or *in vitro* biological assay shall be obligatory when physico-chemical methods cannot provide adequate information on the quality of the product. Such an assay shall, whenever possible, include reference materials and statistical analysis allowing calculation of confidence limits. Where these tests cannot be carried out on the finished product, they may be performed at an intermediate stage, as late as possible in the manufacturing process.

Where the particulars given in section B show that a significant coverage of an active ingredient is employed in the manufacture of the medicinal product, the description of the control tests on the finished product shall include, where appropriate, the chemical and, if necessary, the toxico-pharmacological investigation of the changes that this substance has undergone, and possibly the characterization and/or assay of the degradation products.

1.3 *Identification and assay of excipient constituents*

In so far as is necessary, the excipient(s) shall be subject at least to identification tests.

The test procedure proposed for identifying colouring matters must enable a verification to be made that such matters appear in the list annexed to Directive 78/25/EEC.

An upper and lower limit test shall be obligatory in respect of preserving agents and an upper limit test for any other excipient constituent liable to affect adversely physiological functions; an upper and lower limit test shall be obligatory in respect of the excipient if it is liable to affect the bio-availability of an active substance, unless bio-availability is guaranteed by other appropriate tests.

1.4 *Safety tests*

1. Apart from the toxico-pharmacological tests submitted with the application for marketing authorization, particulars of safety tests, such as sterility, bacterial endotoxin, pyrogenicity and local tolerance in animals shall be included in the analytical particulars wherever such tests must be undertaken as a matter of routine in order to verify the quality of the product.

2. For all controls of biological medicinal products, such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma, which are not specified in the *European Pharmacopoeia*, or failing this, in the national pharmacopoeia of a Member State, the procedures and the criteria of acceptability published as recommendations in the WHO (*Requirements for Biological Substances*) shall serve as guidelines.

3. For radiopharmaceuticals, radionuclidic purity, radiochemical purity and specific activity shall be described. For content of radioactivity, the deviation from that stated on the label should not exceed ± 10 %.

...enerator, ... on th... ...or mot... ...aughter... ...lides a... ...d. For ...eluates tests for mother radionuclides and for other com... ...of the g... ...ystem s... ...ovided.

For kits, the specifications of the finished product shall include tests on performance of products after radiolabelling. Appropriate controls on radiochemical and radionuclidic purity of the radiolabelled compound shall be included. Any material essential for radiolabelling shall be identified and assayed.

F. Stability tests

1. The particulars and documents accompanying the application for marketing authorization pursuant to points 6 and 7 of Article 4 (2) of Directive 65/65/EEC shall be submitted in accordance with the following requirements.

A description shall be given of the investigations by which the shelf life, the recommended storage conditions and the specifications at the end of the shelf-life proposed by the applicant have been determined.

Where a finished product is liable to give rise to degradation products, the applicant must declare these and indicate characterization methods and test procedures.

The conclusions shall contain the results of analyses, justifying the proposed shelf life under the recommended storage conditions and the specifications of the finished product at the end of the shelf-life under these recommended storage conditions.

The maximum acceptable level of degradation products at the end of shelf-life shall be indicated.

A study of the interaction between product and container shall be submitted wherever the risk of such interaction is regarded as possible, especially where injectable preparations or aerosols for internal use are concerned.

2. Where for biological medicinal products, such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma, stability tests cannot be carried out on the finished products, it is acceptable to carry out stability indicating tests at an intermediate stage of production as late as possible in the manufacturing process. In addition, there should be an evaluation of the stability of the finished product using other secondary tests.

3. For radiopharmaceuticals, information on stability shall be given for generators, kits and radiolabelled products. The stability during use of radiopharmaceuticals in multi-dose vials shall be documented.

PART ...

TOXICOLOGICAL AND PHARMACOLOGICAL TESTS

I. Introduction

1. The particulars and documents accompanying the application for marketing authorization pursuant to point 8 of Article 4, second paragraph, Directive 65/65/EEC shall be given in accordance with the requirements below.

Member States shall ensure that the safety tests are carried out in conformity with the provisions relating to good laboratory practice laid down by Directives 87/18/EEC ([1]) and 88/320/EEC ([2]).

The toxicological and pharmacological tests must show:

a) the potential toxicity of the product and any dangerous or undesirable toxic effects that may occur under the proposed conditions of use in human beings; these should be evaluated in relation to the pathological condition concerned;

b) the pharmacological properties of the product, in both qualitative and quantitative relationship to the proposed use in human beings. All results must be reliable and of general applicability. Whenever appropriate, mathematical and statistical procedures shall be used in designing the experimental methods and in evaluating the results.

Additionally, it is necessary for clinicians to be given information about the therapeutic potential of the product.

2. Where a medicinal product is intended for topical use, systemic absorption must be investigated, due account also being taken of the possible use of the product on broken skin and absorption through other relevant surfaces. Only if it is proved that systemic absorption under these conditions is negligible may repeated dose systemic toxicity tests, foetal toxicity tests and studies of reproductive function be omitted.

If, however, systemic absorption is demonstrated during therapeutic experimentation, toxicity tests shall be carried out on animals, including where necessary, foetal toxicity tests.

In all cases, tests of local tolerance after repeated application shall be carried out with particular care and include histological examinations; the possibility of sensitization shall be investigated and any carcinogenic potential investigated in the cases referred to in paragraph II E of this Part.

3. For biological medicinal products such as vaccines, serums, toxins, allergen products and medicinal products derived from human blood or plasma, the requirements of this Part may have to be adapted for individual products; therefore the testing programme carried out shall be justified by the applicant.

In establishing the testing programme, the following shall be taken into consideration:

— all tests requiring repeated administration of the product shall be designed to take account of the possible induction of, and interference by, antibodies;

([1]) OJ No L 15, 17. 1. 1987, p. 29.
([2]) OJ No L 145, 11. 6. 1988, p. 35.

— examination of reproductive function, of embryo/foetal and perinatal toxicity, of mutagenic potential and of carcinogenic potential shall be considered. Where components other than the active ingredient(s) are incriminated, validation of their removal may replace the study.

4. For radiopharmaceuticals, it is appreciated that toxicity may be associated with a radiation dose. In diagnosis, this is a consequence of the use of radiopharmaceuticals, in therapy, it is the wanted property. The evaluation of safety and efficacy of radiopharmaceuticals shall, therefore, address requirements for medicinal products and radiation dosimetry aspects. Organ/tissue exposure to radiation shall be documented. Absorbed radiation dose estimates shall be calculated according to a specified, internationally recognized system by a particular route of administration.

5. The toxicology and pharmacokinetics of an excipient used for the first time in the pharmaceutical field shall be investigated.

6. Where there is a possibility of significant degradation during storage of the medicinal product, the toxicology of degradation products must be considered.

II. Performance of tests

A. Toxicity

1. *Single dose toxicity*

An acute test is a qualitative and quantitative study of the toxic reactions which may result from a single administration of the active substance or substances contained in the medicinal product, in the proportions and physico-chemical state in which they are present in the actual product.

The acute toxicity test must be carried out in two or more mammalian species of known strain unless a single species can be justified. At least two different routes of administration shall normally be used, one being identical with or similar to that proposed for use in human beings and the other ensuring systemic exposure to the substance.

This study will cover the signs observed, including local reactions. The period during which the test animals are observed shall be fixed by the investigator as being adequate to reveal tissue or organ damage or recovery, usually for a period of 14 days but not less than seven days, but without exposing the animals to prolonged suffering. Animals dying during the observation period should be subject to autopsy as also should all animals surviving to the end of the observation period. Histopathological examinations should be considered on any organ showing macroscopic changes at autopsy. The maximum amount of information should be obtained from the animals used in the study.

The single dose toxicity tests should be conducted in such a way that signs of acute toxicity are revealed and the mode of death assessed as far as reasonably possible. In suitable species a quantitative evaluation of the approximate lethal dose and information on the dose effect relationship should be obtained, but a high level of precision is not required.

These studies may give some indication of the likely effects of acute overdosage in man and may be useful for the design of toxicity studies requiring repeated dosing on the suitable animal species.

In the case of active substances in combination, the study must be carried out in such a way as to check whether or not there is enhancement of toxicity or if novel toxic effects occur.

2. *Repeated dose toxicity (sub-acute or chronic toxicity)*

Repeated dose toxicity tests are intended to reveal any physiological and/or pathological changes induced by repeated administration of the active substance or combination of active substances under examination, and to determine how these changes are related to dosage.

Generally, it is desirable that two tests be performed: one short-term, lasting two to four weeks, the other long-term. The duration of the latter shall depend on the conditions of clinical use. Its purpose shall be to determine by experiment the non-toxic dose range of the product and normally it shall last three to six months.

In respect of medicinal products to be administered once only to humans, a single test lasting two to four weeks shall be performed.

If, however, having regard to the proposed duration of use in human beings, the investigator sees fit to carry out experiments of greater or lesser duration than indicated above, he must give adequate reasons for doing so.

Reasons should also be given for the dosages chosen.

Repeated dose toxicity tests shall be carried out on two species of mammals one of which must be a non-rodent. The choice of route(s) of administration employed shall depend on the intended therapeutic use and the possibilities of systemic absorption. The method and frequency of dosage shall be clearly stated.

The maximum dose should be chosen so as to bring harmful effects to light. The lower doses will then enable the animal's tolerance of the product to be determined.

Wherever possible, and always in experiments on small rodents, the design of the experiment and the control procedures must be suited to the scale of the problem being tackled and enable fiducial limits to be determined.

The evaluation of the toxic effects shall be based on observation of behaviour, growth, haematological and biochemical tests, especially those relating to the excretory mechanism, and also on autopsy reports and accompanying histological data. The choice and range of each group of tests will depend on the species of animal used and the state of scientific knowledge at the time.

In the case of new combinations of known substances that have been investigated in accordance with the provisions of this Directive, the long-term tests may, except where acute and sub-acute toxicity tests have demonstrated potentiation or novel toxic effects, be suitably modified by the investigator who shall submit his reasons for such modification.

B. Examination of reproductive function

If the results of other tests reveal anything suggesting harmful effects on progeny or impairment of male or female reproductive function, this shall be investigated by appropriate tests.

C. Embryo/foetal and perinatal toxicity

This investigation comprises a demonstration of the toxic and especially the teratogenic effects observed in the issue of conception when the substance under investigation has been administered to the female during pregnancy.

Although up to the present these tests have had only a limited predictive value in regard to the application of the results to human beings, they are thought to provide important information where the results show effects such as resorptions and other anomalies.

Omission of these tests, either because the medicinal product will not normally be used by women capable of child-bearing or for other reasons, must be adequately justified.

Embryo/foetal toxicity studies shall normally be conducted on two mammalian species, one of which should be other than a rodent. Peri- and postnatal studies shall be conducted in at least one species. Where metabolism of a medicinal product in a particular species is known to be similar to that in man, it is desirable to include this species. Also, it is desirable that one of the species is the same as in the repeated dose toxicity studies.

The details of the test (number of animals, amounts administered, timing of administration and criteria for evaluation of results) shall depend on the state of scientific knowledge at the time when the application is lodged, and the level of statistical significance that the results must attain.

D. Mutagenic potential

The purpose of the study of mutagenic potential is to reveal the changes which a substance may cause in the genetic material of individuals or cells and which have the effect of making successors permanently and hereditarily different from their predecessors. This study is obligatory for any new substance.

The number and types of results and the criteria for their evaluation shall depend on the state of scientific knowledge at the time when the application is lodged.

E. Carcinogenic potential

Tests to reveal carcinogenic effects shall normally be required:

a) in respect of substances having a close chemical analogy with known carcinogenic or cocarcinogenic compounds;

b) in respect of substances which have given rise to suspicious changes during the long-term toxicological tests;

c) in respect of substances which have given rise to suspicious results in the mutagenic-potential tests or in other short-term carcinogenicity tests.

Such tests may also be required in respect of substances to be included in medicinal products likely to be administered regularly over a prolonged period of a patient's life.

The state of scientific knowledge at the time when the application is lodged shall be taken into account when determining the details of the tests.

F. Pharmacodynamics

This heading covers the variations caused by the substance in the functions of the physiological systems, whether these functions are normal or experimentally modified.

This study shall follow two distinct lines of approach.

Firstly, the actions on which the recommended application in therapeutic practice is based shall be adequately described. The results shall be expressed in quantitative terms using, for example, dose-effect curves, time-effect curves etc., and wherever possible, compared with data relating to a substance whose activity is known. Where a higher therapeutic potency is being claimed for a substance, the difference shall be demonstrated and shown to be statistically significant.

Secondly, the investigator shall provide a general pharmacological characterization of the substance, with special reference to collateral effects. In general, the main functions of the physiological systems should be investigated. The depth of this investigation must be increased as the doses liable to produce side-effects approach those producing the main effect for which the substance is being proposed.

The experimental techniques, unless they are standard procedures, must be described in such detail as to allow them to be reproduced, and the investigator must establish their validity. The experimental results shall be set out clearly and, when relevant to the test, their statistical significance quoted.

Unless good reasons are given to the contrary, any quantitative modification of responses resulting from repeated administration of the substance shall be investigated.

Tests on combinations of active substances may be prompted either by pharmacological premisses or by indications of therapeutic effect.

In the first case, the pharmacodynamic study shall demonstrate those interactions which might make the combination of value in therapeutic use.

In the second case, where scientific justification for the combination is sought through therapeutic experimentation, the investigation shall determine whether the effects expected from the combination can be demonstrated in animals, and the importance of any collateral effects shall at least be investigated.

If a combination includes a novel active substance, the latter must previously have been studied in depth.

G. Pharmacokinetics

Pharmacokinetics means the study of the fate of the active substance within the organism, and covers the study of the absorption, distribution, biotransformation and excretion of the substance.

The study of these different phases may be carried out both by means of physical, chemical or biological methods, and by observation of the actual pharmacodynamic activity of the substance itself.

Information on distribution and elimination (i.e. biotransformation and excretion) is necessary in cases where such data are indispensable to determine the dosage for humans, and in respect of chemotherapeutic substances (antibiotics, etc.) and substances whose use depends on their non-pharmacodynamic effects (e.g. numerous diagnostic agents, etc.).

Pharmacokinetic investigation of pharmacologically active substances is necessary.

In the case of new combinations of known substances which have been investigated in accordance with the provisions of this Directive pharmacokinetic studies may not be required, if the toxicity tests and therapeutic experimentation justify their omission.

II. Local tolerance

The purpose of local tolerance studies is to ascertain whether medicinal products (both active ingredients and excipients) are tolerated at sites in the body which may come into contact with the product as a result of its administration in clinical use. The testing strategy shall be such that any mechanical effects of administration or purely physico-chemical actions of the product can be distinguished from toxicological or pharmacodynamic ones.

PART 4

CLINICAL DOCUMENTATION

The particulars and documents accompanying applications for marketing authorizations pursuant to point 8 of Article 4 (2) of Directive 65/65/EEC shall be submitted in accordance with the provisions below.

A clinical trial is any systematic study of medicinal products in human subjects whether in patients or non-patient volunteers in order to discover or verify the effects of and/or identify any adverse reaction to investigational products, and/or study their absorption, distribution, metabolism and excretion in order to ascertain the efficacy and safety of the products.

Evaluation of the application for marketing authorization shall be based on clinical trials including clinical pharmacological trials designed to determine the efficacy and safety of the product under normal conditions of use, having regard to the therapeutic indications for use in human beings. Therapeutic advantages must outweigh potential risks.

A. General requirements

The clinical particulars to be provided pursuant to point 8 of Article 4 (2) of Directive 65/65/EEC must enable a sufficiently well-founded and scientifically valid opinion to be formed as to whether the medicinal product satisfies the criteria governing the granting of a marketing authorization. Consequently, an essential requirement is that the results of all clinical trials should be communicated, both favourable and unfavourable.

Clinical trials must always be preceded by adequate pharmacological and toxicological tests, carried out on animals in accordance with the requirements of Part 3 of this Annex. The investigator must acquaint himself with the conclusions drawn from the pharmacological and toxicological studies and hence the applicant must provide him at least with the investigator's brochure, consisting of all the relevant information known prior to the onset of a clinical trial including chemical, pharmaceutical and biological data, toxicological, pharmacokinetic and pharmacodynamic data in animals and the results of earlier clinical trials, with adequate data to justify the nature, scale and duration of the proposed trial; the complete pharmacological and toxicological reports shall be provided on request. For materials of human or animal origin, all available means shall be employed to ensure safety from transmission of infectious agents prior to the commencement of the trial.

B. Conduct of trials

1. *Good clinical practice*

1.1 All phases of clinical investigation, including bioavailability and bioequivalence studies, shall be designed, implemented and reported in accordance with good clinical practice.

1.2 All clinical trials shall be carried out in accordance with the ethical principles laid down in the current revision of the Declaration of Helsinki. In principle, the freely given informed consent of each trial subject shall be obtained and documented.

The trial protocol, procedures (including statistical design) and documentation shall be submitted by the sponsor and/or investigator for an opinion to the relevant ethics committee. The trials shall not begin before the opinion of this committee has been received in writing.

1.3 Pre-established, systematic written procedures for the organization, conduct, data collection, documentation and verification of clinical trials shall be required.

1.4 In the case of radiopharmaceuticals, clinical trials shall be carried out under the responsibility of a medical doctor authorized to use radionuclides for medical purposes.

2. *Archiving*

The person responsible for placing the medicinal product on the market shall make arrangements for archiving of documentation.

a) The investigator shall arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.

b) Patient files and other source data shall be kept for the maximum period of time permitted by the hospital, institution or private practice.

c) The sponsor or other owner of the data shall retain all other documentation pertaining to the trial as long as the product is authorized. These procedures shall include:

— the protocol including the rationale, objectives and statistical design and methodology of the trial, with conditions under which it is performed and managed, and details of the investigational product, the reference medicinal product and/or the placebo used,

— standard operating procedures,

— all written opinions on the protocol and procedures,

— the investigator's brochure,

— case report forms on each trial subject,

— final report,

— audit certificate(s), if available.

d) The final report shall be retained by the sponsor or subsequent owner, for five years after the product is no longer authorized.

Any change of ownership of the data shall be documented. All data and documents shall be made available if requested by relevant authorities.

C. **Presentation of results**

1. The particulars of each clinical trial must contain sufficient detail to allow an objective judgement to be made:

— the protocol, including the rationale, objectives and statistical design and methodology of the trial, with conditions under which it is performed and managed, and details of the investigational product used;

— audit certificate(s), if available;

— the list of investigator(s), and each investigator shall give his name, address, appointments, qualifications and clinical duties, state where the trial was carried out and assemble the information in respect of each patient individually, including case report forms on each trial subject;

— final report signed by the investigator and for multicentre trials, by all the investigators or the coordinating (principal) investigator.

2. The particulars of clinical trials referred to above shall be forwarded to the competent authorities. However, in agreement with the competent authorities, the applicant may omit part of this information. Complete documentation shall be provided forthwith upon request.

3. The clinical observations shall be summarized for each trial indicating:

a) the number and sex of patients treated;

b) the selection and age-distribution of the groups of patients being investigated and the control groups;

c) the number of patients withdrawn prematurely from the trials and the reasons for such withdrawal;

d) where controlled trials were carried out under the above conditions, whether the control group:

— received no treatment,

— received a placebo,

— received another medicinal product of known effect,

— received treatment other than therapy using medicinal products;

e) the frequency of observed side-effects;

f) details concerning patients who may be at increased risk, e.g. elderly people, children, women during pregnancy or menstruation, or whose physiological or pathological condition requires special consideration;

g) parameters or evaluation criteria of efficacy and the results in terms of these parameters;

h) a statistical evaluation of the results when this is called for by the design of the trials and the variable factors involved.

4. The investigator shall, in his conclusions on the experimental evidence, express an opinion on the safety of the product under normal conditions of use, its compatibility, its efficacy and any useful information relating to indications and contra-indications, dosage and average duration of treatment as well as any special precautions to be taken during treatment and the clinical symptoms of overdosage. In reporting the results of a multi-centre study, the principal investigator shall, in his conclusions, express an opinion on the safety and efficacy of the investigational product on behalf of all centres.

5. In addition, the investigator shall always indicate his observations on:

a) any signs of habituation, addiction or difficulty in weaning patients from the medicinal product;

b) any interactions that have been observed with other medicinal products administered concomitantly;

c) the criteria determining exclusion of certain patients from the trials;

d) any deaths which occurred during the trial or within the follow-up period.

6. Particulars concerning a new combination of medicinal substances must be identical to those required for new medicinal products and must substantiate the safety and efficacy of the combination.

7. Total or partial omission of data must be explained. Should unexpected results occur during the course of the trials, further preclinical, toxicological and pharmacological tests must be undertaken and reviewed.

If the medicinal product is intended for long-term administration, particulars shall be given of any modification of the pharmacological action following repeated administration, as well as the establishment of long-term dosage.

D. **Clinical pharmacology**

1. *Pharmacodynamics*

The pharmacodynamic action correlated to the efficacy shall be demonstrated including:

— the dose-response relationship and its time course,

— justification for the dosage and conditions of administration,

— the mode of action, if possible.

The pharmacodynamic action not related to efficacy shall be described.

The demonstration of pharmacodynamic effects in human beings shall not in itself be sufficient to justify conclusions regarding any particular potential therapeutic effect.

2. *Pharmacokinetics*

The following pharmacokinetic characteristics shall be described:

— absorption (rate and extent),

— distribution,

— metabolism,

— excretion.

Clinically significant features including the implication of the kinetic data for the dosage *regimen* especially for patients at risk, and *differences between man* and animal species used in the preclinical studies, shall be described.

3. *Interactions*

If the product is normally to be administered concomitantly with other medicinal products, particulars shall be given of joint administration tests performed to demonstrate possible modification of the pharmacological action.

If pharmacodynamic/pharmacokinetic *interactions exist between* the substance and other medical products or substances like alcohol, cafeine, tobacco or nicotine, likely to be taken simultaneously, or if such interactions are likely, they should be described and discussed; particularly from the point of view of clinical relevance and the relationship to the statement concerning *interactions in the* summary of product characteristics presented in accordance with Article 4a, point 5.6 of Directive 65/65/EEC.

E. Bioavailability/bioequivalence

The assessment of bioavailability must be *undertaken in all* cases where it is necessary, e.g. where the *therapeutic* dose is near the toxic dose or where the previous tests have revealed anomalies which may be related to pharmacokinetic properties, such as variable absorption.

In addition, an assessment of bioavailability shall be undertaken where necessary to demonstrate bioequivalence for the medicinal products referred to in Article 4 (2) point 8 (i) (ii) and (iii) of Directive 65/65/EEC.

F. Clinical efficacy and safety

1. In general, clinical trials shall be done as 'controlled clinical trials' and if possible, randomized; any other design shall be justified. The control treatment of the trials will vary from case to case and also will depend on ethical considerations; thus it may, in some instances, be *more pertinent* to compare the efficacy of a new medicinal product with that of an established medicinal product of proven therapeutic value rather than with the effect of a placebo.

As far as possible, and particularly in trials where the effect of the product cannot be objectively measured, steps shall be taken to avoid bias, including methods of randomization and blinding.

2. T ol of th ust inc rough ... t of the ... l metho ... employe ... number and reasons for inclusion of patients (including calculations of the power of the trial, the le... of significance to be used and a *description of the statistical unit*. Measures taken to avoid bias, particularly methods of randomization, shall be documented. Inclusion of a large number of subjects in a trial must not be regarded as an adequate substitute for a properly controlled trial.

3. Clinical statements concerning the efficacy or safety of a medicinal product under normal conditions of use which are not scientifically substantiated cannot be accepted as valid evidence.

4. The value of data on the efficacy and safety of a medicinal product under normal conditions of use will be very greatly enhanced if such data come from several competent investigators working independently.

5. *For vaccines* and serums, the immunological status and age of the trial population and the local epidemiology are of critical importance and shall be monitored during the trial and fully described.

For live *attenuated* vaccines, clinical trials shall be so designed as to reveal potential transmission of the immunizing agent from vaccinated to non-vaccinated subjects. If transmission is possible, the genotypic and phenotypic stability of the immunizing agent shall be studied.

For vaccines and allergen products, follow-up studies shall include appropriate immunological tests, and where applicable, antibody assays.

6. The pertinence of the different trials to the assessment of safety and the validity of methods of evaluation shall be discussed in the expert report.

7. All adverse events including abnormal laboratory values shall be presented individually and discussed, especially:

— in terms of overall adverse experience

and

— as a function of the nature, seriousness and causality of effects.

8. A *critical assessment* of relative safety, taking into account adverse reactions, shall be made in relation to:

— *the disease* to be treated,

— *other therapeutic approaches*,

— *particular characteristics in sub-groups* of patients,

— *preclinical data* on toxicology and pharmacology.

9. Recommendations shall be made for the conditions of use, with the intention of reducing the incidence of adverse reations.

G. ientatio plicatio :eption istance

When, in respect of particular therapeutic indications, the applicant can show that he is unable to provide comprehensive data on the quality, efficacy and safety under normal conditions of use, because:

— the indications for which the product in question is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence,

or

— in the present state of scientific knowledge comprehensive information cannot be provided,

or

— it would be contrary to generally accepted principles of medical ethics to collect such information,

marketing authorization may be granted on the following conditions:

a) the applicant completes an identified programme of studies within a time period specified by the competent authority, the results of which shall form the basis of a reassessment of the benefit/risk profile;

b) the medicinal product in question may be supplied on medical prescription only and may in certain cases be administered only under strict medical supervision, possibly in a hospital and for a radiopharmaceutical, by an authorized person;

c) the package leaflet and any medical information shall draw the attention of the medical practitioner to the fact that the particulars available concerning the medicinal product in question are as yet inadequate in certain specified respects.

H. Post-marketing experience

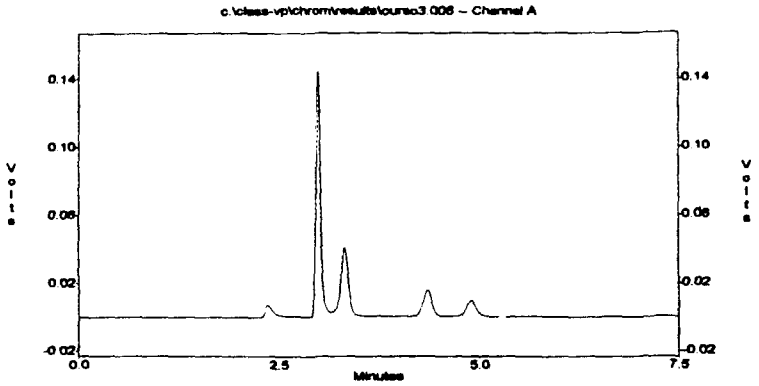
1. If the medicinal product is already authorized in other countries, information shall be given in respect of adverse drug reactions of the medicinal product concerned and medicinal products containing the same active ingredient(s), in relation to the usage rates if possible. Information from worldwide studies relevant to the safety of the medicinal product shall be included.

For this purpose, an adverse drug reaction is a reaction which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease or for the modification of physiological function.

2. In the case of vaccines already authorized in other countries, information on the monitoring of vaccinated subjects to evaluate the prevalence of the disease in question as compared to non-vaccinated subjects shall be submitted, when available.

3. For allergen products, response in periods of increased antigen exposure shall be identified.

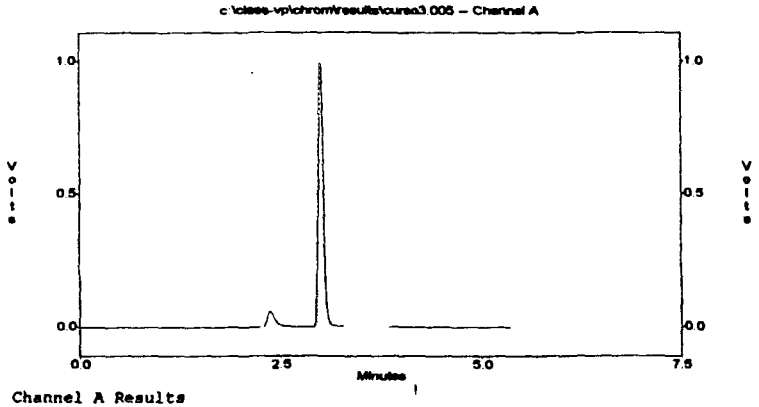
corrida 6
Muestra de corteza
Acquired : Dec 02, 1997 16:44:23
Printed : Dec 02, 1997 17:01:12
File Desc. : corrida 6
muestra de corteza
Fase móvil: AcN:ac. fosfórico 0,5% en MeOH al 5%
(20:80)
1mL/min 340nm 182Kgf/cm2
c-18 (col. de CIFLORPAN)



Channel A Results

Time	Area
2.37	65874.0
3.00	594176.0
4.35	128025.0
4.89	95160.0

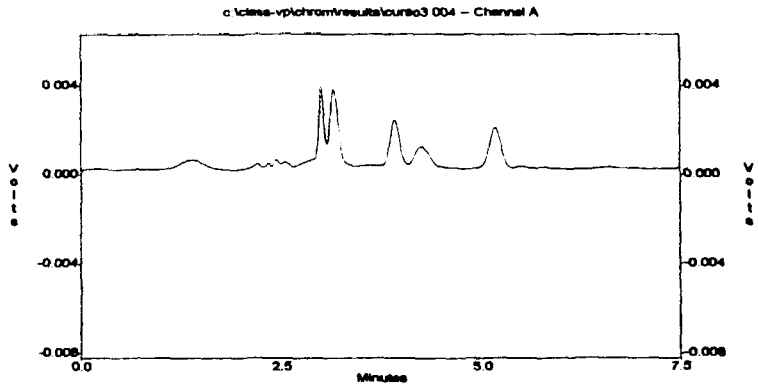
corrida 5
patrón de esculina
Acquired : Dec 02, 1997 16:35:02
Printed : Dec 02, 1997 16:58:52
File Desc. : Patrón estandarizado
misma condiciones de corrida 4



Channel A Results

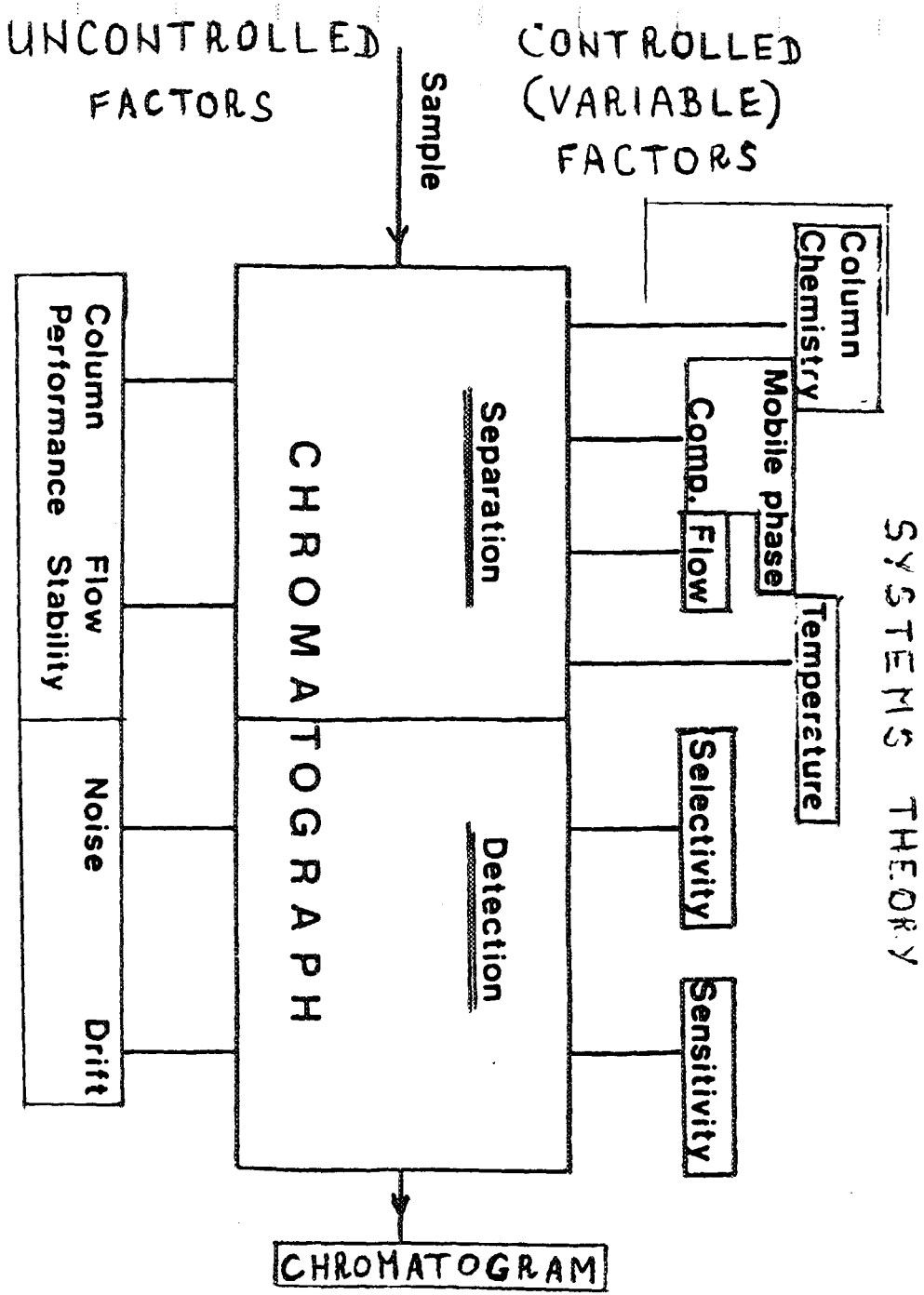
Time	Area
2.38	409097.0
3.00	4459207.0

corrida 4
Estándar estandarizado
Acquired : Dec 02, 1997 16:20:13
Printed : Dec 02, 1997 17:03:12
File Desc. : corrida 4
Extracto estandarizado
Fase móvil: AcN:ac. fosfórico 0,5% en MeOH al 5%
(20:80)
1mL/min 340nm 182Kgf/cm2
c-18a



Channel A Results

Time	Area
3.00	22677.0
3.16	29500.0
3.92	20029.0
4.24	15305.0
5.17	20822.0



OPTIMISATION METHODS

- ***Sequential***: Aim to approach optimum (LOCAL) conditions in a stepwise manner

e.g.:
- Simplex method → CRF, COF } BERRIDG
- Method of 'Iterative regression'
- (Phase selection diagrams)

- ***Simultaneous***: Proceed according to a fixed experimental design, established prior to any experimentation.

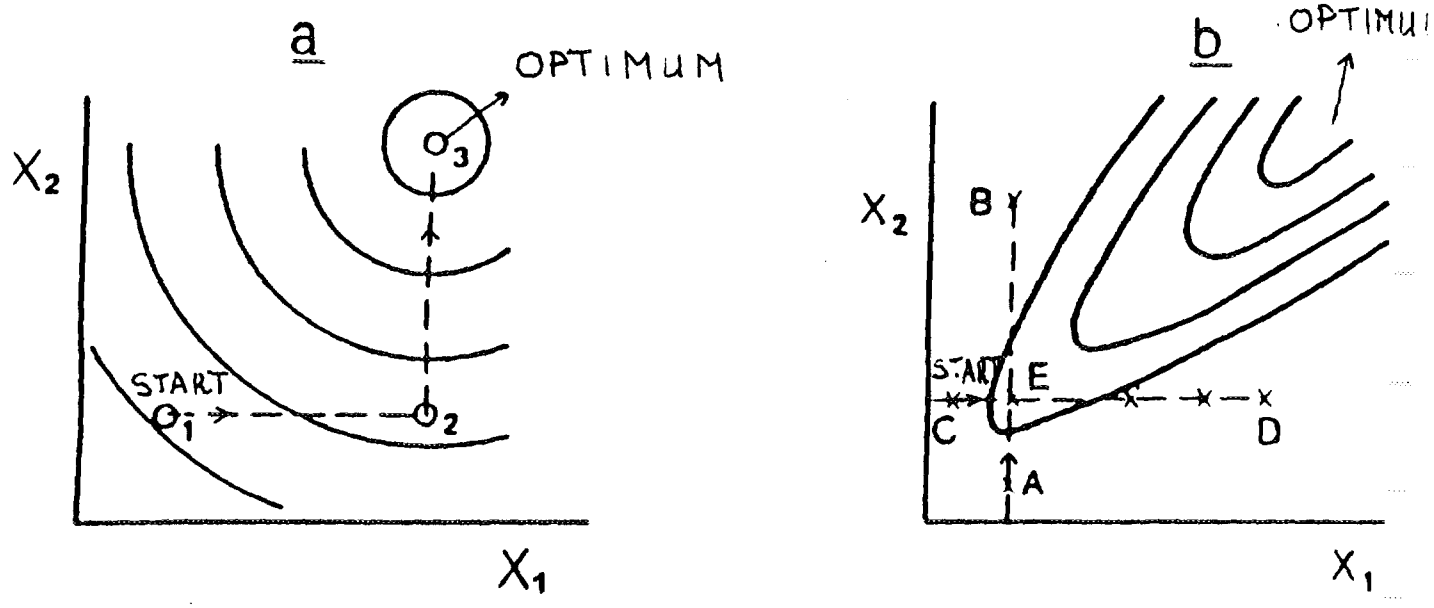
Objective:

to model the response surface

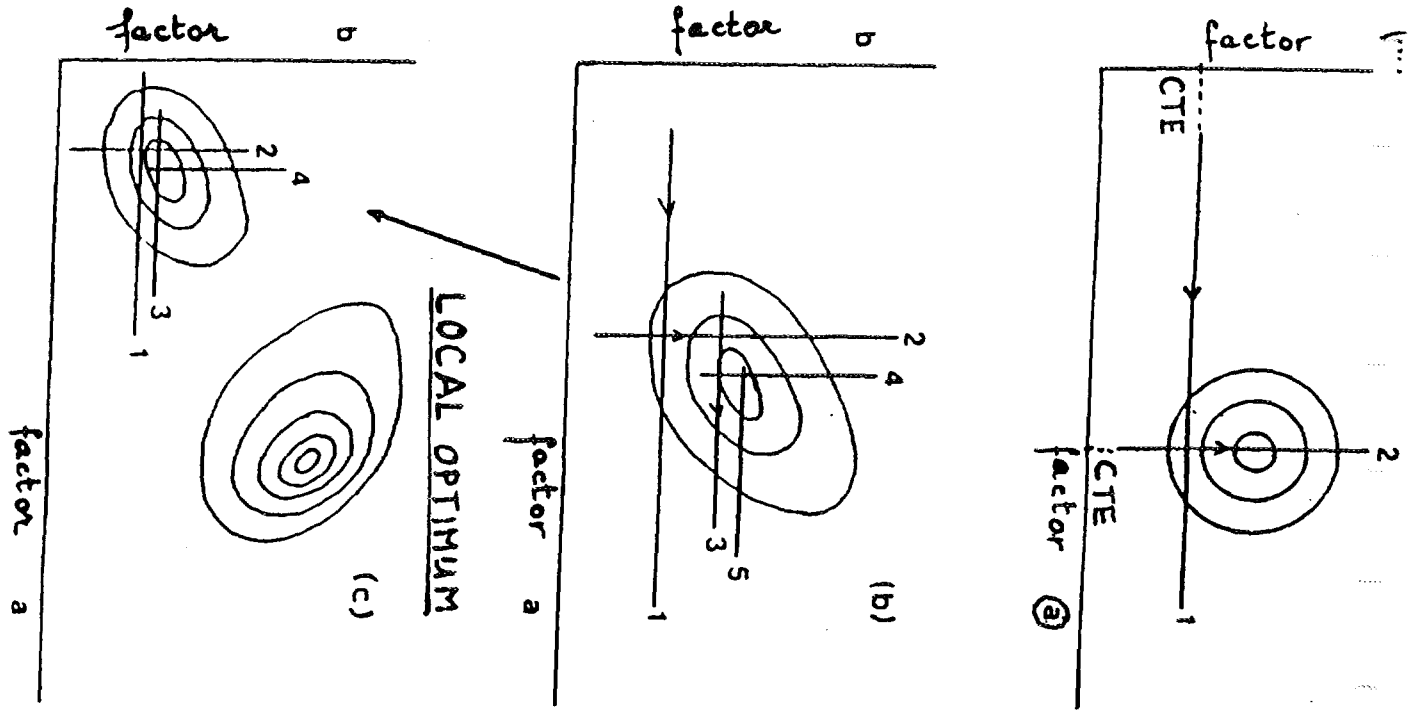
to predict the location of the optimum

to investigate the proposed parameters to establish their contribution to selectivity (Parameter space restricted)

e.g.:
- Factorial designs ←
- Simplex lattice designs → Σ variables = 1
- (Mixture designs)



One factor at a time optimisation on a three-dimensional response surface; a. successful; b. stuck on ridge



FACTORIAL DESIGNS

* Half fraction (*)
* Full
* Central composite: orthogonal
  face-centred (**)
  -> Full + Star rotable
* Box-Behnken
* Doehlert
* Plackett-Burman (7 Factors)

Experimental design on liquid chromatographic parameters in the analysis of tetracycline on poly(styrene-divinylbenzene) → K.U.Leuven

Expedition by experimental design of methyl and propyl parahydroxybenzoate, phenylephrine hydrochloride and chlorphenamine maleate by ion-pair liquid chromatography. → V.U.B.

FACTORIAL DESIGNS

PARAMETER SPACE

1) Which chromatographic parameters (variables) influence the response variable?
Response variable ? → t_R, k', N, A_s, $b_{0.5}$

2) Determination of the parameter boundaries and central values.

3) Which factorial design ? Practical performance of the chosen design.
Measurements of response variable.

SIGNIFICANCE

4) Estimation of the individual parameter and interaction effects. Significance ?

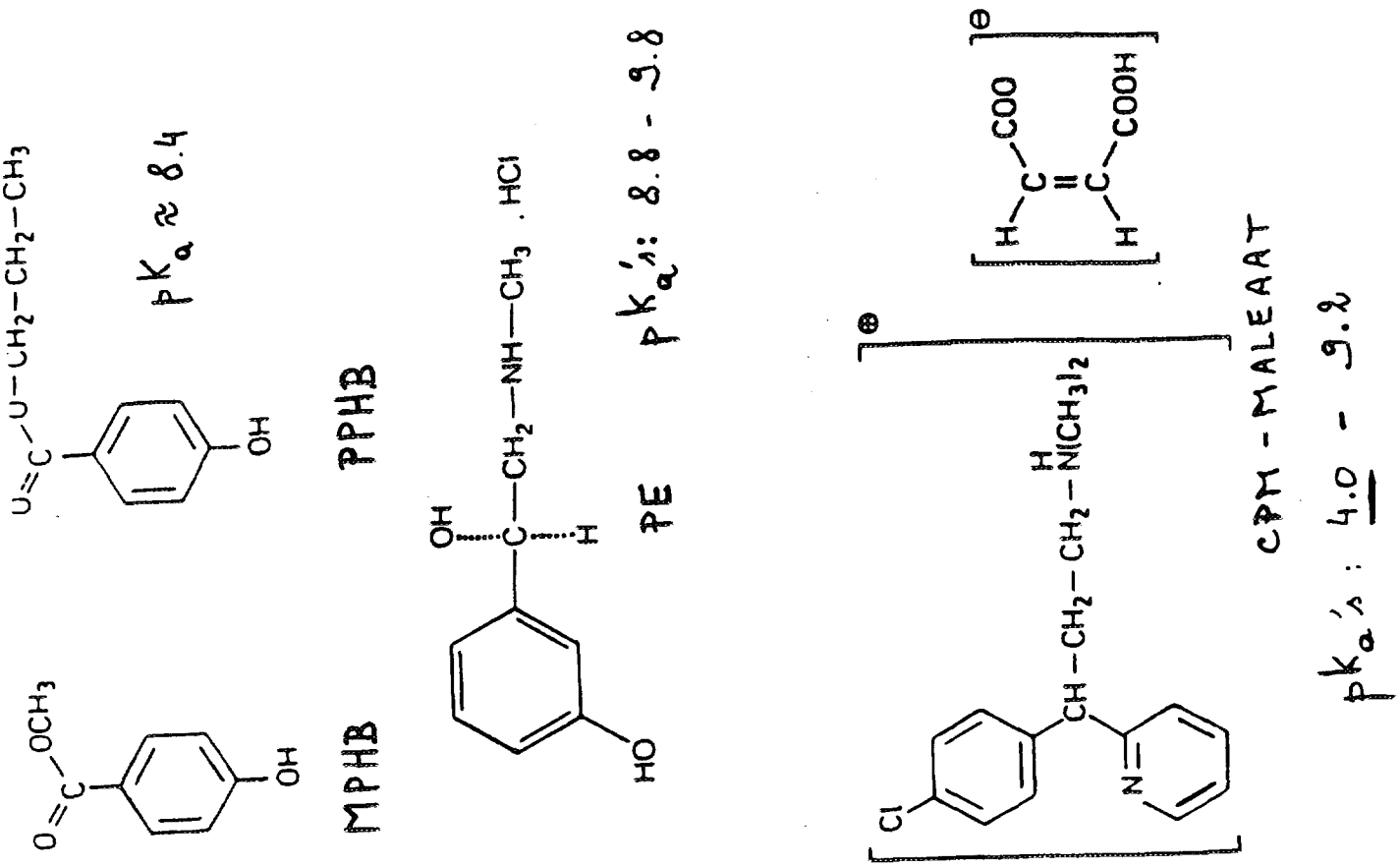
5) ANOVA tables.

6) Standardized Pareto charts.

7) Regression modelling: first/second order

8) Response surface plots.

9) Choice of optimal conditions. Which Optimization criterion ?
→ RESOLUTION
→ DURATION OF ANALYSIS



HPLC SYSTEM

Reservoir A. This contained a mixture of 80 % (v/v) of MeOH and 20 % (v/v) of a 0.05 molar solution of potassium dihydrogen phosphate, the pH of which was previously adjusted to the required pH-level (3.0, 4.0 or 5.0) with phosphoric acid or a 1 molar sodium hydroxide solution.

Reservoir B. This contained a 50 mmol/l solution of SDSS in a mixture of 80 % (v/v) of MeOH and 20 % (v/v) of water, the pH of which was previously adjusted to the required pH-level (3.0, 4.0 or 5.0) with phosphoric acid or a 1 molar sodium hydroxide solution.

Reservoir C. This contained a 50 mmol/l solution of DMOA in a mixture of 80 % (v/v) of MeOH and 20 % (v/v) of water, the pH of which was previously adjusted to the required pH-level (3.0, 4.0 or 5.0) with phosphoric acid or a 1 molar sodium hydroxide solution.

Reservoir D. This contained a 0.05 molar solution of potassium dihydrogen phosphate, the pH of which was previously adjusted to the required pH-level (3.0, 4.0 or 5.0) with phosphoric acid or a 1 molar sodium hydroxide solution.

The amounts (%, v/v) taken from reservoirs A, B and C were as to fulfil the different mobile phase combinations in the design by pH-level, with the restriction that reservoir D was only used to adjust the total volume to 100 % (v/v).

Apparatus: Waters Model 600 Multisolvent Delivery Syste

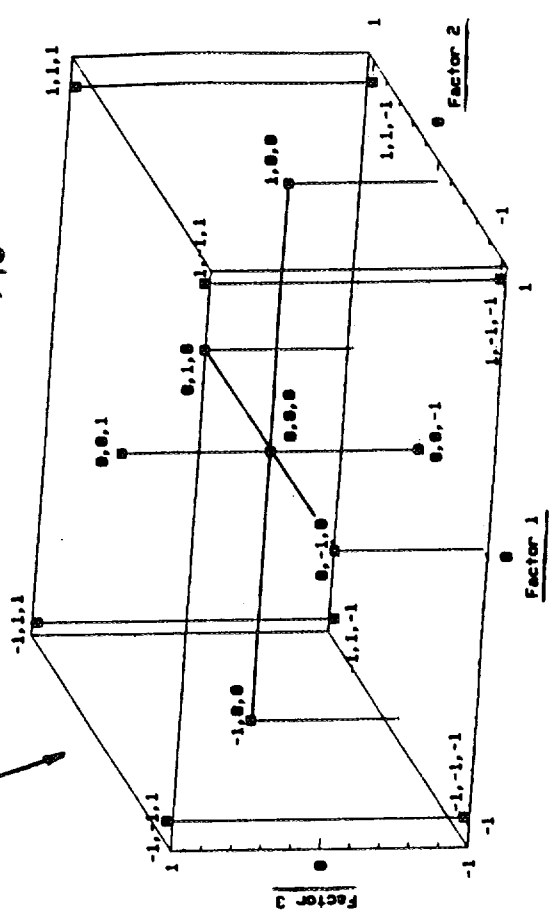
Column: 15 x 0.39 cm 4µ spheric C18 NOVAPAK

FACE-CENTERED CENTRAL COMPOSITE DESIGN

→ pure second order regression

$k=4 \Rightarrow (2^k + 2k + 1) = 16 + 8 + 1 = 25$ EXPERIM

$k=3 \Rightarrow (2^k + 2k + 1) = \underbrace{8+6+1}_{15}$



{ FULL FACTORIAL
CTDP

Practical performance of the applied central composite design

The central levels of the mobile phase parameters in the applied design were fixed to 70 % (v/v) for MeOH, 9.0 mmol/l for SDSS, 9.0 mmol/l for DMOA and 4.0 for the pH. To overcome solubility problems during solvent mixing, SDSS in reservoir B and DMOA in reservoir C had to be dissolved in 80 % (v/v) of MeOH solutions. This had to be taken into account when each examined mobile phase combination was composed with the Multisolvent Delivery System. For instance, to prepare the central level combination, 52 volumes of MeOH solution in reservoir A were mixed with 18 volumes of SDSS solution in reservoir B, 18 volumes of DMOA solution in reservoir C and 12 volumes of buffer solution (pH 4.0) in reservoir D.

The final mobile phase parameter values in the design were as follows:

PARAMETER SPACE

Chromatographic parameter	low value (-1)	central value (0)	high value (+1)
MeOH vol(reservoir A + B + C)	60	70	80
SDSS mmol/l (reservoir B)	3.0	9.0	15.0
DMOA mmol/l (reservoir C)	3.0	9.0	15.0
pH	3.0	4.0	5.0

The worksheet of the design, with the coded values -1, 0 and +1, is reproduced in Table 1.

Table 1.
Applied "face-centered central composite design" (coded units).

RUN	MeOH (Vol. %)	SDSS (mmol/l)	DMOA (mmol/l)	pH
1	0	0	0	0
2	-1	-1	-1	-1
3	+1	-1	-1	-1
4	-1	+1	-1	-1
5	+1	+1	-1	-1
6	-1	-1	+1	-1
7	+1	-1	+1	-1
8	-1	+1	+1	-1
9	+1	+1	+1	-1
10	-1	-1	-1	+1
11	+1	-1	-1	+1
12	-1	+1	-1	+1
13	+1	+1	-1	+1
14	-1	-1	+1	+1
15	+1	-1	+1	+1
16	-1	+1	+1	+1
17	+1	+1	+1	+1
18	-1	0	0	0
19	+1	0	0	0
20	0	-1	0	0
21	0	+1	0	0
22	0	0	-1	0
23	0	0	+1	0
24	0	0	0	-1
25	0	0	0	+1
26	0	0	0	0

EFFECT = $\frac{\Sigma(+1) - \Sigma(-1)}{9}$

PARAMETER

Table 2.
Measured response variables: retention times in minutes

	RUN	MPHB	PPHB	PE.HCl	CPM	
	1	1.45 (n=3)	1.94 (n=3)	1.89 (n=4)	6.67 (n=4)	
	2	1.69 (n=2)	2.90 (n=2)	2.42 (n=1)	38.05* (n=1)	H
	3	1.38 (n=2)	1.64 (n=2)	1.44 (n=2)	2.82 (n=2)	
H	× 4	1.55 (n=3)	2.42 (n=3)	2.86 (n=2)	75.50* (n=1)	
	5	1.36 (n=3)	1.62 (n=3)	1.83 (n=3)	6.80 (n=2)	
H	× 6	1.73 (n=2)	3.03 (n=2)	1.70 (n=3)	8.99 (n=3)	
	7	1.38 (n=3)	1.67 (n=3)	1.30 (n=3)	1.93 (n=3)	
	8	1.64 (n=2)	2.67 (n=2)	1.93 (n=2)	17.12 (n=2)	
	9	1.36 (n=2)	1.60 (n=2)	1.59 (n=4)	3.96 (n=4)	
	10	1.66 (n=3)	2.81 (n=3)	2.37 (n=3)	13.06 (n=3)	
	11	1.34 (n=1)	1.55 (n=3)	1.41 (n=1)	2.42 (n=3)	
	12	1.51 (n=2)	2.32 (n=2)	2.79 (n=2)	15.50 (n=2)	
L	× 13	1.33 (n=3)	1.54 (n=3)	1.75 (n=3)	3.63 (n=3)	
	14	1.63 (n=3)	2.63 (n=3)	1.65 (n=3)	5.27 (n=3)	
L	× 15	1.35 (n=2)	1.59 (n=2)	1.78 (n=3)	4.89 (n=2)	L
	16	1.53 (n=2)	2.31 (n=2)	1.93 (n=2)	6.61 (n=2)	
	17	1.36 (n=3)	1.59 (n=1)	1.57 (n=1)	2.88 (n=3)	
	18	1.63 (n=2)	2.66 (n=2)	2.24 (n=2)	16.15 (n=2)	
	19	1.34 (n=3)	1.58 (n=3)	1.51 (n=3)	2.90 (n=3)	
	20	1.50 (n=2)	2.10 (n=2)	1.62 (n=3)	4.60 (n=3)	
	21	1.42 (n=3)	1.89 (n=3)	2.00 (n=3)	7.66 (n=3)	
	22	1.46 (n=2)	2.00 (n=2)	2.28 (n=2)	11.30 (n=2)	
	23	1.43 (n=3)	1.91 (n=3)	1.68 (n=3)	4.91 (n=3)	
	24	1.44 (n=2)	1.99 (n=2)	1.74 (n=2)	8.41 (n=2)	
	25	1.40 (n=4)	1.84 (n=1)	1.79 (n=1)	4.68 (n=3)	
	26	1.45 (n=1)	1.99 (n=1)	1.94 (n=1)	6.68 (n=1)	

(*) Measured values not used; n = number of consecutive measurements.

Table 3.
Estimated effects with their standard errors on the retention times of MPHB and PPHB.

PARAMETER	MPHB		PPHB	
A: MeOH	-0.263333	+/- 9.48295E-3	-1.04111	+/-0.0296346
B: SDSS	-0.0666667	+/- 9.48295E-3	-0.217778	+/-0.0296346
C: DMOA *	0.0144444	+/- 9.48295E-3	0.0222222	+/-0.0296346
D: pH	-0.0466667	+/- 9.48295E-3	-0.151111	+/-0.0296346
AB	0.055	+/- 0.0100582	0.19375	+/-0.0314322
AC *	-0.01	+/- 0.0100582	-0.01125	+/-0.0314322
AD	0.0225	+/- 0.0100582	0.08625	+/-0.0314322
BC *	0.015	+/- 0.0100582	0.03125	+/-0.0314322
BD *	2.5E-3	+/- 0.0100582	0.01375	+/-0.0314322
CD *	-0.0125	+/- 0.0100582	-0.06125	+/-0.0314322
AA	0.0867619	+/- 0.0251407	0.327143	+/-0.0785655
BB *	0.0367619	+/- 0.0251407	0.0771429	+/-0.0785655
CC *	6.7619E-3	+/- 0.0251407	-2.85714E-3	+/-0.0785655
DD *	-0.0432381	+/- 0.0251407	-0.0828571	+/-0.0785655

"Standard error" estimated from "total error" with 11 d.f. (t = 2.20156)

Estimated effect * < 2 x Std. error : NOT SIGNIFICANT

↓

cfr. ANOVA

STD. PARETO

$2\sqrt{s^2 \times \text{diagonal in } (X'.X)^{-1} \text{ matrix}}$ → std. error

s^2 ↓ mean square / total error

- first order
- mixed sec. order
- pure sec. order

p. 1 Draper & Morgan

Table 4.
Estimated effects and their standard errors on the retention times of PE.HCl and CPM.

PARAMETER	PE.HCl(*)		CPM(**)	
A: MeOH	-0.69	+/- 0.0204602	-11.2191	+/-0.59956
B: SDSS	0.34	+/- 0.0204602	3.32162	+/-0.512898
C: DMOA	-0.502222	+/- 0.0204602	-5.4969	+/-0.59956
D: pH	-0.03 *	+/- 0.0204602	-4.62135	+/-0.59956
AB	-7.5E-3 *	+/- 0.0217013	-1.39682	+/-0.550021
AC	0.3175	+/- 0.0217013	4.13277	+/-0.651568
AD	2.5E-3 *	+/-0.0217013	3.56827	+/-0.651568
BC	-0.0625	+/- 0.0217013	-0.231824 *	+/-0.550021
BD	-2.5E-3 *	+/-0.0217013	-1.85932	+/-0.550021
CD	0.0175 *	+/-0.0217013	0.895266 *	+/-0.651568
AA	0.0289524 *	+/- 0.0542428	4.78199	+/-1.23979
BB	-0.101048 *	+/- 0.0542428	-2.00801 *	+/-1.23979
CC	0.238952	+/- 0.0542428	1.94199 *	+/-1.23979
DD	-0.191048	+/- 0.0542428	-1.17801 *	+/-1.23979

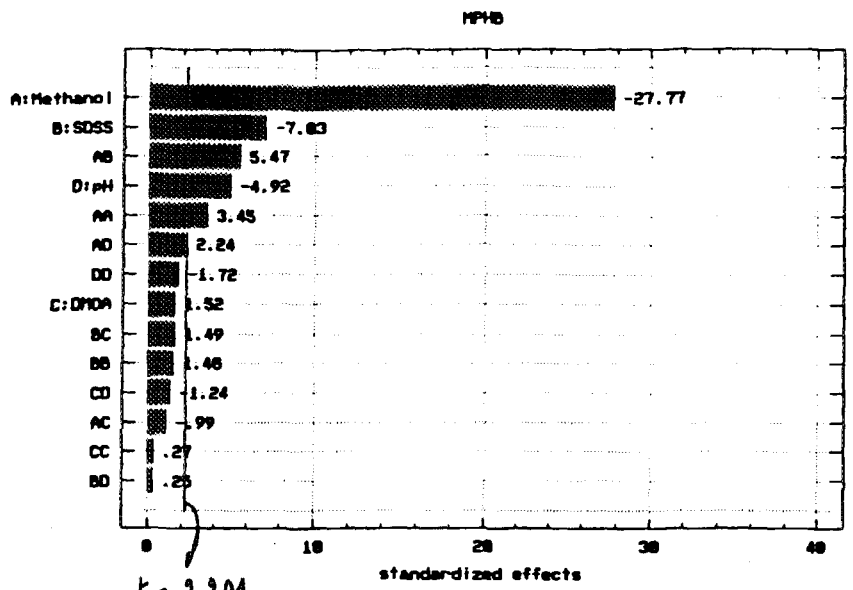
(*) "Standard error" estimated from "total error" with 11 d.f. (t = 2.20156)
(**) "Standard error" estimated from "total error" with 9 df (t = 2.26277)

* < 2 x Std. error

↓

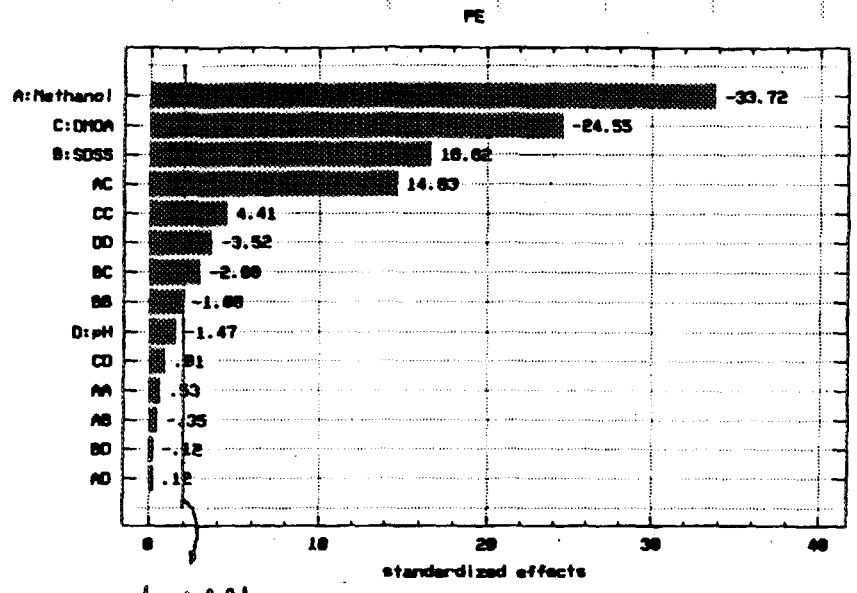
cfr. ANOVA

STD. PARETO



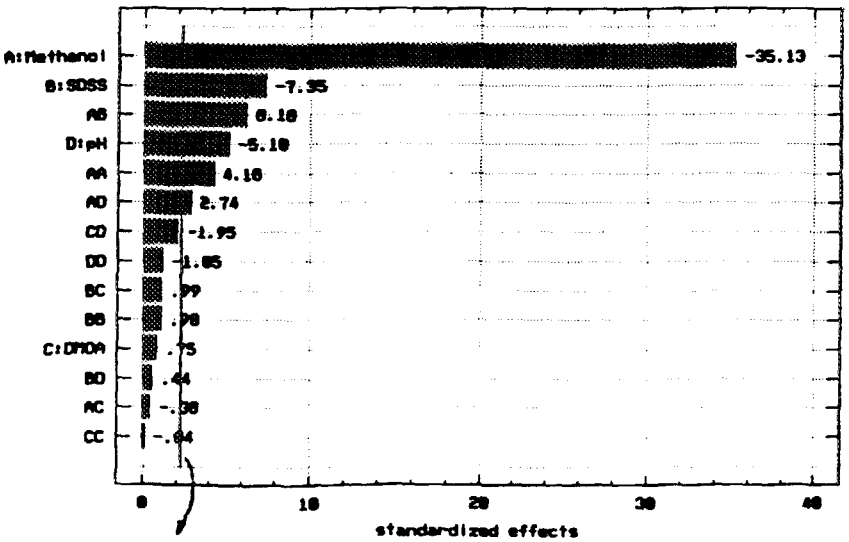
$t = 2.201$ (α = 0.05)

11 d.f.



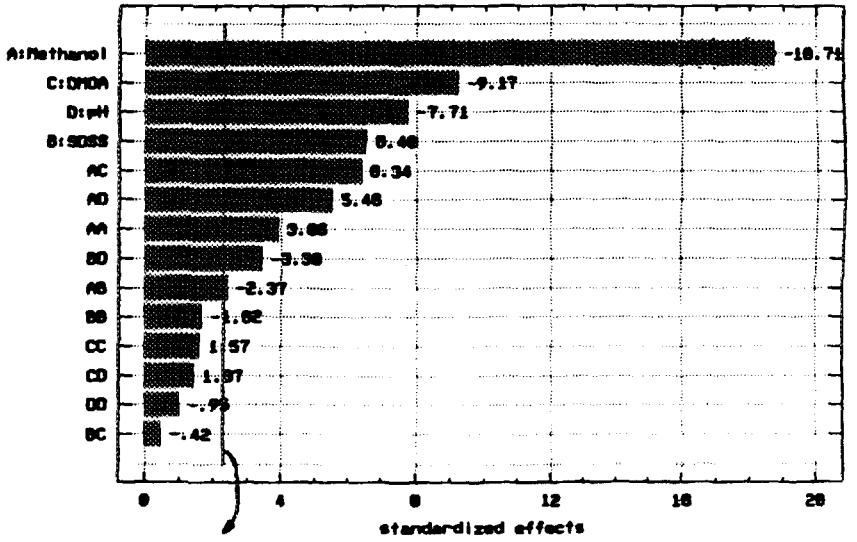
$t = 2.201$

11 df



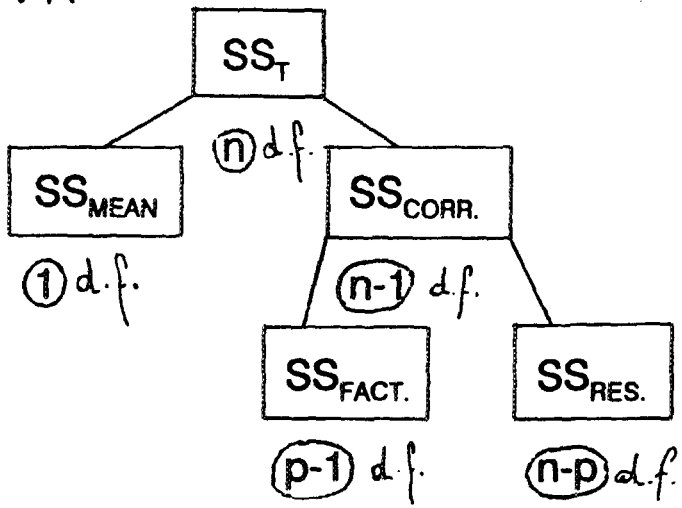
$t = 2.201$

11 d.f.



$t = 2.26$

9 df.



$$SS_T = Y'*Y = \sum y_{1i}^2$$

$$SS_{MEAN} = \bar{Y}'*\bar{Y} = \sum \bar{y}_1^2$$

$$SS_{CORR.} = C'*C = (Y-\bar{Y})'*(Y-\bar{Y}) = \sum (y_{1i}-\bar{y}_1)^2$$

$$SS_{FACT.} = F'*F = (\hat{Y}-\bar{Y})'*(\hat{Y}-\bar{Y}) = \sum (\hat{y}_{1i}-\bar{y}_1)^2$$

$$SS_{RES.} = R'*R = (Y-\hat{Y})'*(Y-\hat{Y}) = \sum (y_{1,r}-\hat{y}_{1i})^2$$

Table 5.
ANOVA-table for retention times of MPHB

Effect	Sum of squares	d.f.	Mean square	F-ratio	P-Value
A:MeOH	0.312050	1	0.312050	771.13	0.0000
B:SDS	0.020000	1	0.020000	49.42	0.0000
C:DMOA *	0.000939	1	0.000939	2.32	0.1559
D:pH	0.009800	1	0.009800	24.22	0.0005
AB	0.012100	1	0.012100	29.90	0.0002
AC *	0.000400	1	0.000400	0.99	0.3519
AD	0.002025	1	0.002025	5.00	0.0469
BC *	0.000900	1	0.000900	2.22	0.1640
BD *	0.000025	1	0.000025	0.06	0.8109
CD *	0.000625	1	0.000625	1.54	0.2398
AA	0.004819	1	0.004819	11.91	0.0054
BB *	0.000865	1	0.000865	2.14	0.1716
CC *	0.000029	1	0.000029	0.07	0.7957
DD *	0.001196	1	0.001197	2.96	0.1134
Total error	0.004513	11	0.000404		
Total(corr.)	0.375413	25			

* Not significant ← ok. estimated effects

Table 8.
ANOVA-table for retention times of CPM

Effect	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
A:MeOH	340.812530	1	340.81253	350.15	0.0000
B:SDSS	40.822730	1	40.82273	41.94	0.0001
C:DMOA	81.815147	1	81.81515	84.06	0.0000
D:pH	57.827553	1	57.82755	59.41	0.0000
AB	5.452663	1	5.45266	5.60	0.0421
AC	39.158461	1	39.15846	40.23	0.0001
AD	29.060888	1	29.06089	29.86	0.0004
BC *	0.172911	1	0.17291	0.18	0.6877
BD	11.122801	1	11.12280	11.43	0.0081
CD *	1.837588	1	1.83759	1.89	0.2027
AA	14.480397	1	14.48040	14.88	0.0039
BB *	2.553274	1	2.55327	2.62	0.1398
CC *	2.388119	1	2.38812	2.45	0.1517
DD *	0.878749	1	0.87875	0.90	0.3767
Total error	8.760029	9	0.97334		

Total(corr.) 483.483733 23 d.f.

* Not Significant

Table 7.
ANOVA-table for retention times of PE.HCl

Effect	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
A:MeOH	2.14245000	1	2.1424500	1137.31	0.0000
B:SDSS	0.52020000	1	0.5202000	276.15	0.0000
C:DMOA	1.13502222	1	1.1350222	602.52	0.0000
D:pH *	0.00405000	1	0.0040500	2.15	0.1706
AB *	0.00022500	1	0.0002250	0.12	0.7398
AC	0.40322500	1	0.4032250	214.05	0.0000
AD *	0.00002500	1	0.0000250	0.01	0.9116
BC	0.01562500	1	0.0156250	8.29	0.0150
BD *	0.00002500	1	0.0000250	0.01	0.9116
CD *	0.00122500	1	0.0012250	0.65	0.4455
AA *	0.00053668	1	0.0005367	0.28	0.6097
BB *	0.00653729	1	0.0065373	3.47	0.0894
CC	0.03655680	1	0.0365568	19.41	0.0011
DD	0.02336839	1	0.0233684	12.41	0.0048
Total error	0.02072159	11	0.0018838		

Total(corr.) 4.29563462 25 d.f.

* Not Significant

Table 6.
ANOVA-table for retention times of PPHB

Effect	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
A:MeOH	4.87760556	1	4.8776056	1234.23	0.0000
B:SDSS	0.21342222	1	0.2134222	54.00	0.0000
C:DMOA*	0.00222222	1	0.0022222	0.56	0.4769
D:pH	0.10275556	1	0.1027556	26.00	0.0003
AB	0.15015625	1	0.1501563	38.00	0.0001
AC *	0.00050625	1	0.0005063	0.13	0.7309
AD	0.02975625	1	0.0297563	7.53	0.0191
BC *	0.00390625	1	0.0039062	0.99	0.3519
BD *	0.00075625	1	0.0007563	0.19	0.6748
CD *	0.01500625	1	0.0150062	3.80	0.0773
AA	0.06852047	1	0.0685205	17.34	0.0016
BB *	0.00381010	1	0.0038101	0.96	0.3575
CC *	0.00000523	1	0.0000052	0.00	0.9720
DD *	0.00439547	1	0.0043955	1.11	0.3142
Total error	0.04347123	11	0.0039519		

Total(corr.) 5.58926538 25 d.f.

* Not Significant

MEASURED RESPONSE VARIABLE

INTERCEPT

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + \text{FIRST ORDER}$$

$$B_{12}X_1X_2 + B_{13}X_1X_3 + B_{14}X_1X_4 + B_{23}X_2X_3 + \text{MIXED SECOND ORDER}$$

$$B_{24}X_2X_4 + B_{34}X_3X_4 +$$

$$B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{44}X_4^2 + \text{error}$$

PURE SECOND ORDER

$$B = INV(X'.X).(X'.Y)$$

Central Composite Design | Design Matrix
(Met "Coded Units")

d =

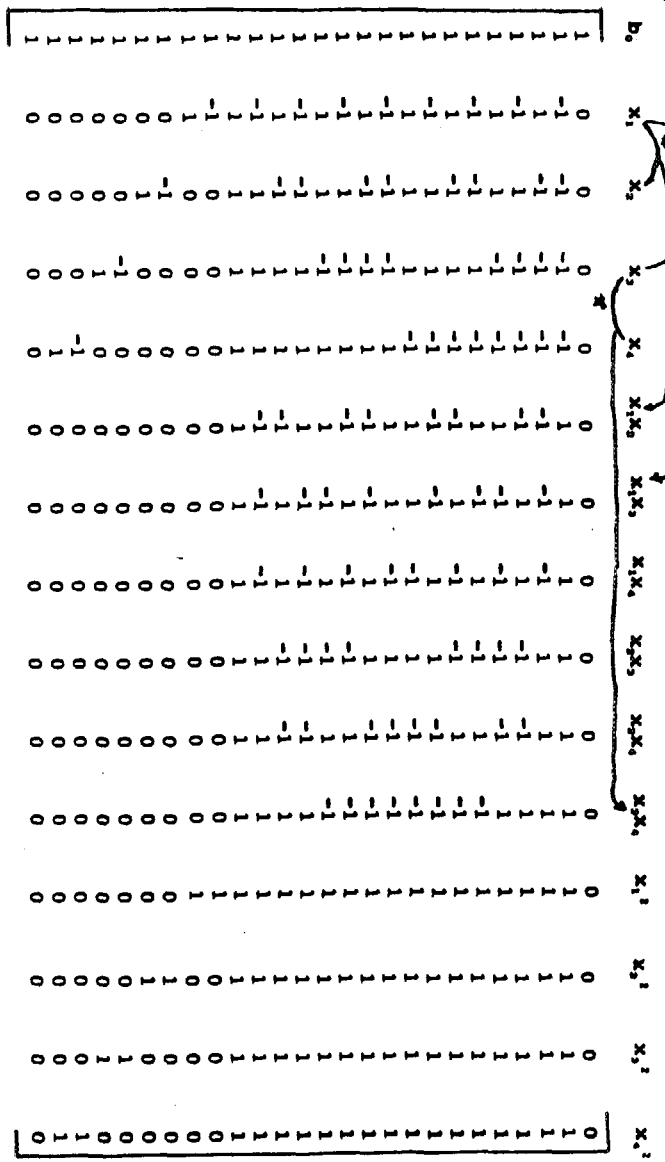
0	0	0	0
-1	-1	-1	-1
1	-1	-1	-1
-1	1	-1	-1
1	1	-1	-1
-1	-1	1	-1
1	-1	1	-1
-1	1	1	-1
1	1	1	-1
-1	-1	-1	1
1	-1	-1	1
-1	1	-1	1
1	1	-1	1
-1	-1	1	1
1	-1	1	1
-1	1	1	1
1	1	1	1
-1	0	0	0
1	0	0	0
0	-1	0	0
0	1	0	0
0	0	-1	0
0	0	1	0
0	0	0	-1
0	0	0	1
0	0	0	0

126

$Y_1 =$ [ ] Y_2 Y_3 Y_4

x_1 ($MeOH$) x_2 (SDS) x_3 ($DMOA$) x_4 (pH)

(... "Coded ...")



VARIANCE - COVARIANCE MATRIX

$\text{inv}(x'*x)$

	b_0	x_1	x_2	x_3	x_4	x_1x_2	x_1x_3	x_1x_4
b_0	0.1571	0	0	0	0	0	0	0
x_1	0	0.0556	0	0	0	0	0	0
x_2	0	0	0.0556	0	0	0	0	0
x_3	0	0	0	0.0556	0	0	0	0
x_4	0	0	0	0	0.0625	0	0	0
x_1^2	0	0	0	0	0	0.0625	0	0
x_2^2	0	0	0	0	0	0	0.0625	0
x_3^2	0	0	0	0	0	0	0	0.0625
x_4^2	-0.0429	0	0	0	0	0	0	0
x_1x_2	-0.0429	0	0	0	0	0	0	0
x_1x_3	-0.0429	0	0	0	0	0	0	0
x_1x_4	-0.0429	0	0	0	0	0	0	0

COVARIANCE-MATRIX

	b_0	x_1	x_2	x_3	x_4	x_1^2	x_2^2	x_3^2	x_4^2
b_0	0.3905	-0.1095	-0.1095	-0.1095	-0.1095	0	0	0	0
x_1	-0.1095	0.3905	-0.1095	-0.1095	-0.1095	0	0	0	0
x_2	-0.1095	-0.1095	0.3905	-0.1095	-0.1095	0	0	0	0
x_3	-0.1095	-0.1095	-0.1095	0.3905	-0.1095	0	0	0	0
x_4	-0.1095	-0.1095	-0.1095	-0.1095	0.3905	0	0	0	0
x_1^2	0	0	0	0	0	0.0625	0	0	0
x_2^2	0	0	0	0	0	0	0.0625	0	0
x_3^2	0	0	0	0	0	0	0	0.0625	0
x_4^2	0	0	0	0	0	0	0	0	0.0625
x_1x_2	0	0	0	0	0	0	0	0	0
x_1x_3	0	0	0	0	0	0	0	0	0
x_1x_4	-0.0429	-0.0429	-0.0429	-0.0429	0	0	0	0	0

$\neq 0$ ← NON ORTHOGONALITY

x'*y (y = de "response" vector voor methylparaben)

38.3200
-2.3700
-0.6000
0.1300
-0.4200
0.4400
-0.0800
0.1800
0.1200
0.0200
-0.1000
26.7700
26.7200
26.6900
26.6400

inv(x'*x)*(x'*y) = b

1.4437
-0.1317
-0.0333
0.0072
-0.0233
0.0275
-0.0050
0.0113
0.0075
0.0012
-0.0062
0.0434
0.0184
0.0034
-0.0216

Deze regressiecoëfficiënten zijn de helft van de berekende effecten !

"Residuals" : y - x*b =

0.0063 -0.0146 0.0062 -0.0154 0.0154 0.0035 0.0043 0.0226 -
0.0165 0.0146 -0.0196
-0.0013 -0.0054 -0.0124 0.0135 -0.0082 0.0176 0.0112 -0.0154
0.0046 -0.0088 0.0201
-0.0243 -0.0054 0.0012 0.0063

lparat reken ssidua

REGRESSION COEFFICIENTS FROM CODED VALUES				
	MPHB	PPHB	PE	CPM
A: MeOH	-0.131667	-0.520556	-0.345	-5.60956
B: SDSS	-0.0333333	-0.108889	0.17	1.66081
C: DMOA	7.22222E-3	0.0111111	-0.251111	-2.74845
D: pH	-0.0233333	-0.0755556	-0.015	-2.31067
A*B	0.0275	0.096875	-3.75E-3	-0.650912
A*C	-5E-3	-5.625E-3	0.15875	2.06638
A*D	0.01125	0.043125	1.25E-3	1.78013
B*C	7.5E-3	0.015625	-0.03125	-0.115912
B*D	1.25E-3	6.875E-3	-1.25E-3	-0.929662
C*D	-6.25E-3	-0.030625	8.75E-3	0.447633
A*A	0.043381	0.163571	0.0144762	2.39099
B*B	0.018381	0.0385714	-0.0505238	-1.00401
C*C	3.38095E-3	-1.4286E-3	0.119476	0.970993
D*D	-0.021619	-0.0414286	-0.0955238	-0.589007

1/2 Estimated effects

Table 9.
Regression equation characteristics

MPHB

Mobile phase parameter	Regression coeff.	Standard error	t-value	P-value
Intercept	5.242639	0.476636	10.9993	0.0000
A:MeOH	-0.082653	0.013145	-6.2878	0.0000
B:SDSS	-0.037639	0.006369	-5.8849	0.0000
D:pH	-0.102083	0.038375	-2.6601	0.0155
AB	0.000458	0.000091	5.0616	0.0001
AD	0.001125	0.000543	2.0706	0.0523
A^2	0.000435	0.000092	4.7076	0.0002

PPHB

Mobile phase parameter	Regression coeff.	Standard error	t-value	P-value
Intercept	16.172465	1.352958	11.9534	0.0000
A:MeOH	-0.309003	0.037313	-8.2815	0.0000
B:SDSS	-0.131169	0.018155	-7.2259	0.0000
D:pH	-0.377431	0.10893	-3.4649	0.0026
AB	0.001615	0.000257	6.2816	0.0000
AD	0.004312	0.001542	2.7963	0.0115
A^2	0.001608	0.000262	6.1357	0.0000

PE.HCl

Mobile phase parameter	Regression coeff.	Standard error	t-value	P-value
Intercept	5.998998	0.201041	29.8397	0.0000
A:MeOH	-0.058312	0.002786	-20.9328	0.0000
B:SDSS	0.036146	0.004643	7.7853	0.0000
C:DMOA	-0.219248	0.018922	-11.5867	0.0000
AC	0.002646	0.000262	10.0964	0.0000
BC	-0.000868	0.000437	-1.9875	0.0607

Table 9.(continued)

CPM

Mobile phase parameter	Regression coeff.	Standard error	*t*-value	*P*-value
Intercept	199.796647	28.866805	6.9213	0.0000
A:MeOH	-3.988126	0.730211	-5.4616	0.0001
B:SDSS	1.611037	0.434717	3.7059	0.0023
C:DMOA	-2.665258	0.390155	-6.8313	0.0000
D:pH	-12.189543	2.382353	-5.1166	0.0002
AB	-0.010471	0.004915	-2.1304	0.0514
AC	0.031859	0.005335	5.9712	0.0000
AD	0.162528	0.032013	5.0770	0.0002
BD	-0.151164	0.049148	-3.0757	0.0082
A^2	0.018559	0.004833	3.8399	0.0018

Table 10.
Compilation of regression results.(*) "Residuals" more than 3 sigma

MPHB

Run	Fitted retention time	Residuals	Standardized residuals
1	1.44375	0.00625	0.2999
2	1.71431	-0.02431	-1.42089
3	1.37347	0.00653	0.36318
4	1.59264	-0.04264	-2.84621
5	1.36181	-0.00181	-0.10012
6	1.71431	0.01569	0.88888
7	1.37347	0.00653	0.36318
(8*)	1.59264	0.04736	3.34212
9	1.36181	-0.00181	-0.10012
10	1.64514	0.01486	0.83978
11	1.34931	-0.00931	-0.51969
12	1.52347	-0.01347	-0.75865
13	1.33764	-0.00764	-0.42557
14	1.64514	-0.01514	-0.85611
15	1.34931	0.00069	0.03850
16	1.52347	0.00653	0.36318
17	1.33764	0.02236	1.29610
18	1.61889	0.01111	0.53195
19	1.35556	-0.01556	-0.75042
20	1.47708	0.02292	1.17662
21	1.41042	0.00958	0.47713
22	1.44375	0.01625	0.79146
23	1.44375	-0.01375	-0.66641
24	1.46708	-0.02708	-1.41226
25	1.42042	-0.02042	-1.04004
26	1.44375	0.00625	0.29999

Average Relative Deviation = 0.98%

(ARD) → $\frac{\Sigma \%\ \text{DEVIAT}}{9\,c}$

Table 10 (continued).
PPHB

RUN	Fitted retention time	Residuals	Standardized residuals
1	1.95750	-0.01750	-0.29590
2	2.96333	-0.06333	-1.29297
3	1.64222	-0.00222	-0.04340
4*	2.55181	-0.13181	-3.23798
5	1.61819	0.00181	0.03526
6	2.96333	0.06667	1.36791
7	1.64222	0.02778	0.54695
8	2.55181	0.11819	2.75091
9	1.61819	-0.01819	-0.35657
10	2.72597	0.08403	1.77943
11	1.57736	-0.02736	-0.53861
12	2.31444	0.00556	0.10853
13	1.55333	-0.01333	-0.26087
14	2.72597	-0.09597	-2.08910
15	1.57736	0.01264	0.24724
16	2.31444	-0.00444	-0.08681
17	1.55333	0.03667	0.72647
18	2.63889	0.02111	0.35453
19	1.59778	-0.01778	-0.29825
20	2.06639	0.03361	0.59150
21	1.84861	0.04139	0.73206
22	1.95750	0.04250	0.72733
23	1.95750	-0.04750	-0.81589
24	2.03306	-0.04306	-0.76248
25	1.88194	-0.04194	-0.74219
26	1.95750	0.03250	0.55283

ARD = 1.82 %

Table 10 (continued).
PE.HCl

Run	Fitted retention time	Residuals	Standardized residuals
1	1.86577	0.02423	0.38443
2	2.41938	0.00062	0.01173
3	1.41188	0.02812	0.53645
4	2.82188	0.03812	0.73189
5	1.81438	0.01562	0.29644
6	1.66216	0.03784	0.72641
7	1.28966	0.01034	0.19602
8	1.93966	-0.00966	-0.18303
9	1.56716	0.02284	0.43465
10	2.41938	-0.04938	-0.95729
11	1.41188	-0.00188	-0.03560
12	2.82188	-0.03188	-0.60952
13	1.81438	-0.06438	-1.26969
14	1.66216	-0.01216	-0.23053
15	1.28966	-0.00966	-0.18383
16	1.93966	-0.00966	-0.18383
17	1.56716	0.00284	0.05381
18	2.21077	0.02923	0.47878
19	1.52077	-0.01077	-0.17548
20	1.69577	-0.07577	-1.28622
21	2.03577	-0.03577	-0.58765
22*	2.11688	0.16312	3.34932
23	1.61466	0.06534	1.09704
24	1.86577	-0.12577	-2.23340
25	1.86577	-0.07577	-1.24538
26	1.86577	0.07423	1.21810

> 3.

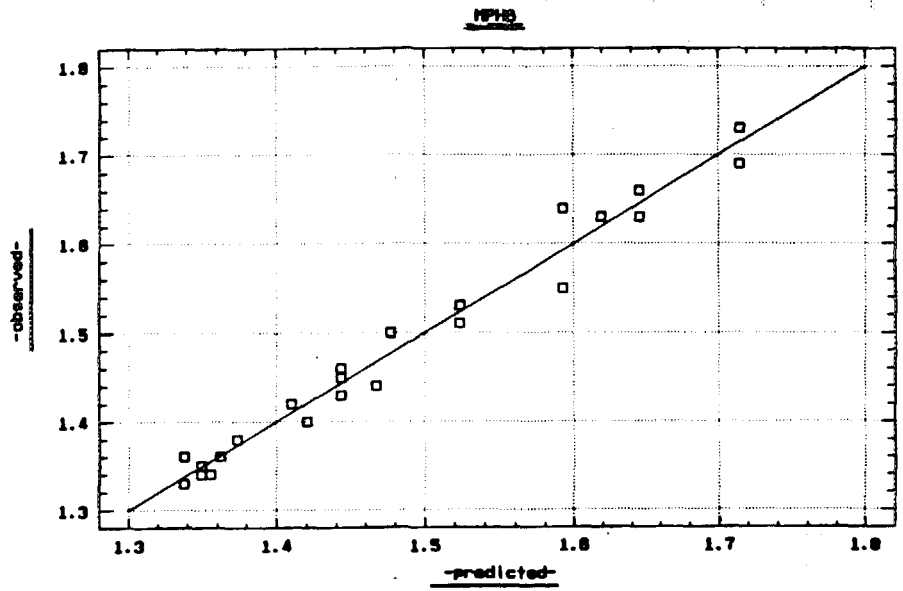
ARD = 2.09 %

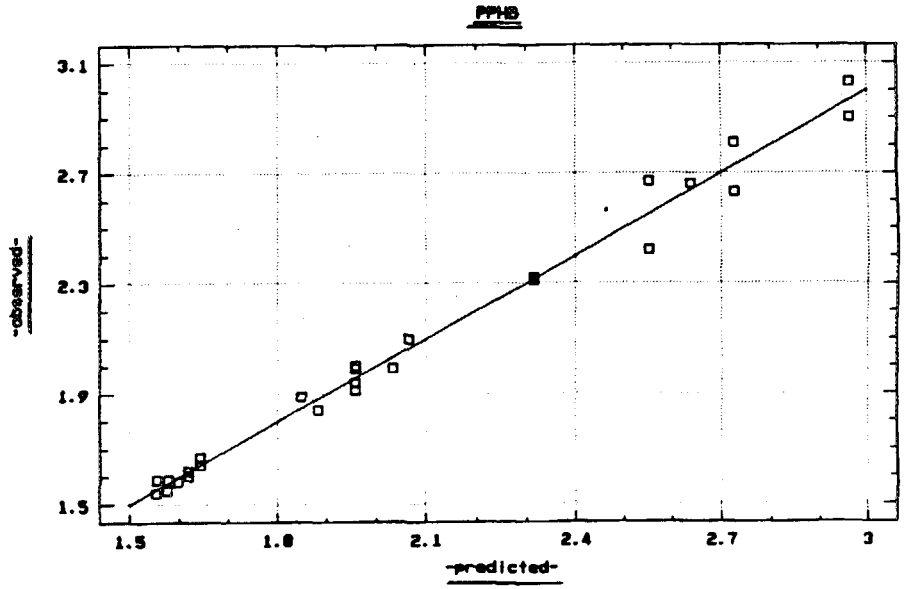
Table 10 (continued).

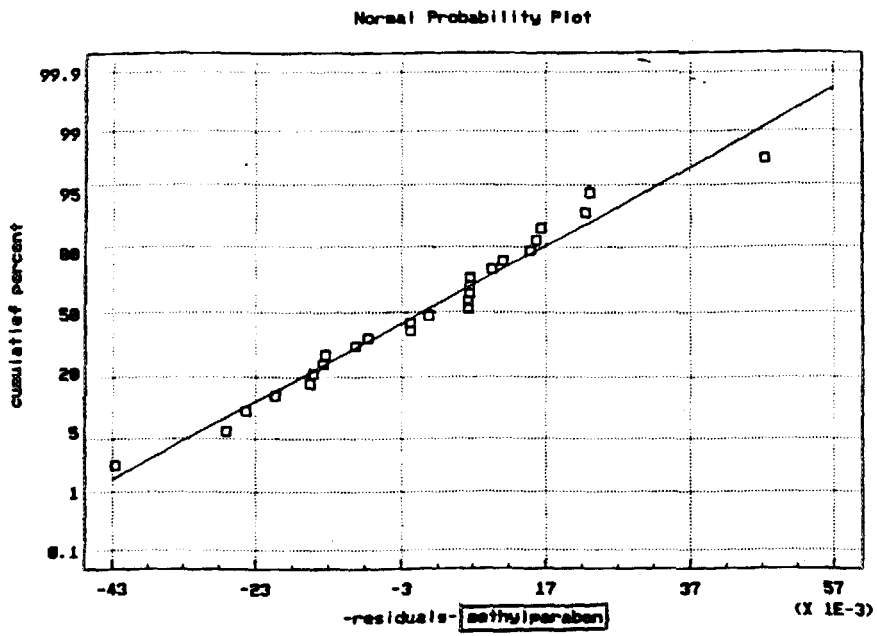
CPM

Run	Fitted retention time	Residuals	Standardized residuals
1	6.86375	-0.19375	-0.18527
2	-	-	-
3	2.57540	0.24460	0.32300
4	-	-	-
5	6.41420	0.38580	0.51252
6	10.2917	-1.30172	-2.70096
7	1.17685	0.75315	1.02870
8	16.6435	0.47654	0.81115
9	5.01565	-1.05565	-1.50205
10	13.5537	-0.49374	-0.72826
11	3.29387	-0.87387	-1.21876
12	16.2775	-0.77755	-1.18315
13	3.50474	0.12526	0.16567
14	4.50906	0.76094	1.19283
15	1.89532	-0.00532	-0.00704
16	7.23286	-0.62286	-0.95921
17	2.10619	0.77381	1.06774
18	14.1916	1.95838	2.31178
19	3.24778	-0.34778	-0.33090
20	5.22310	-0.62310	-0.62785
21	8.50440	-0.84440	-0.86184
22	9.47456	1.82544	2.11454
23	4.25294	0.65706	0.66770
24	9.03678	-0.62678	-0.63595
25	4.69072	-0.01072	-0.01071
26	6.86375	-0.18375	-0.17569

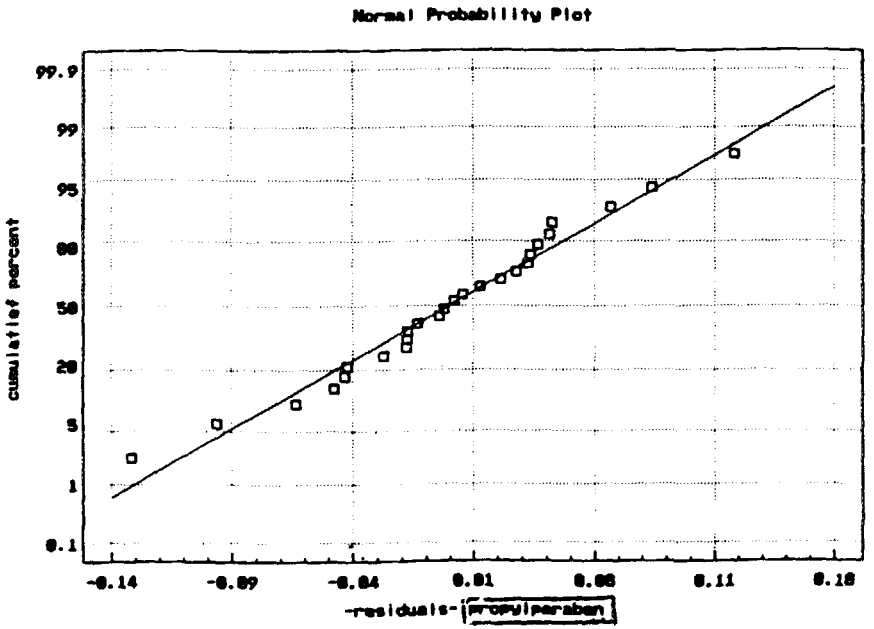
ARD = 12.95%

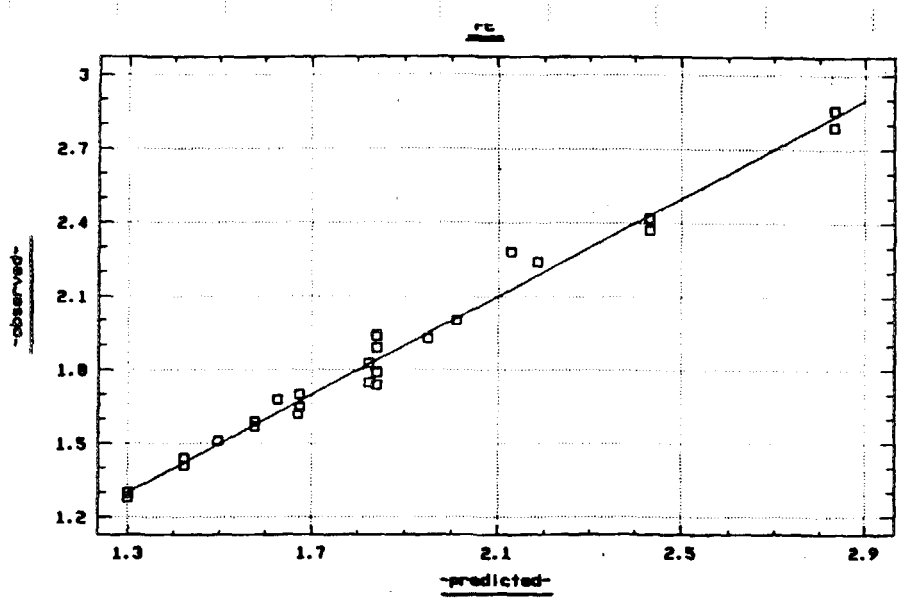


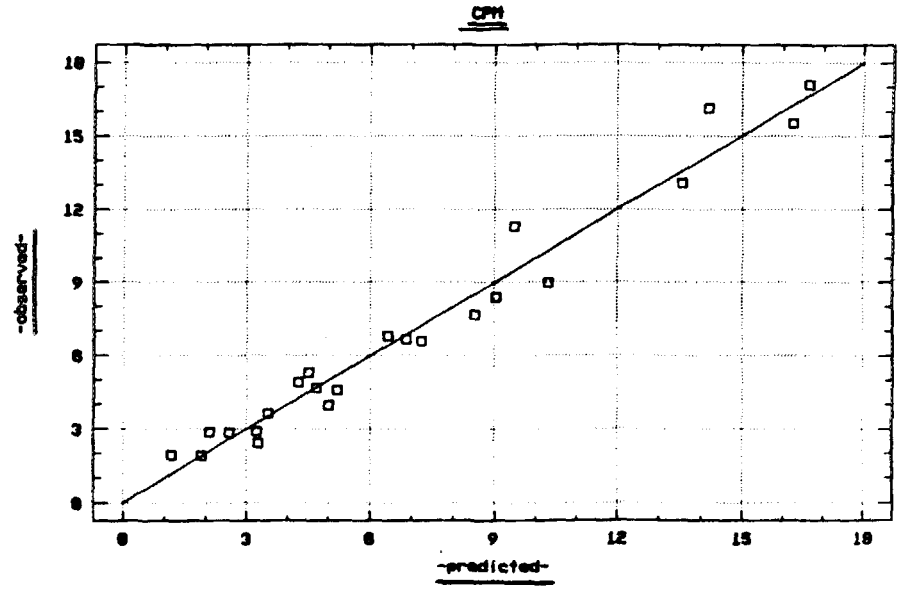




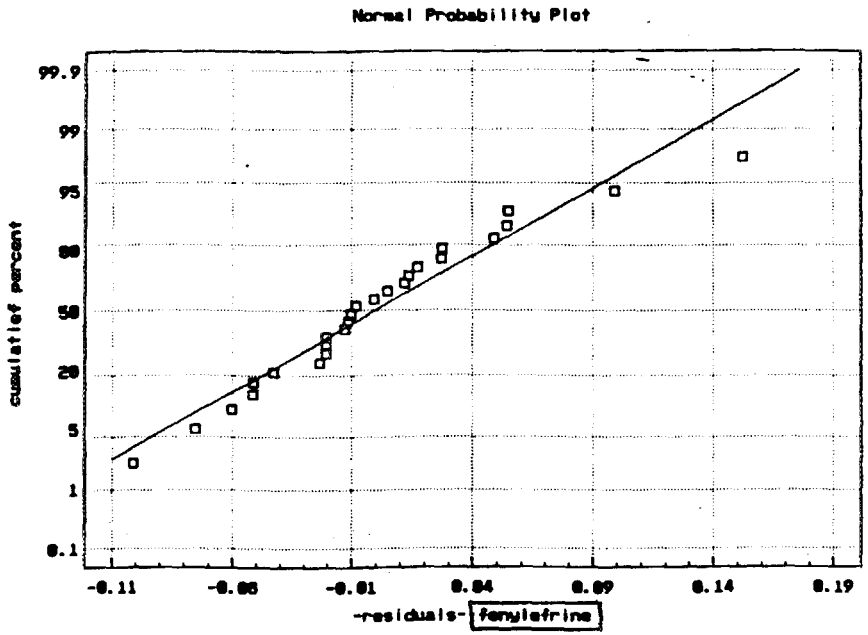
"DIAGNOSTIC PLOT": "RESIDUALS"



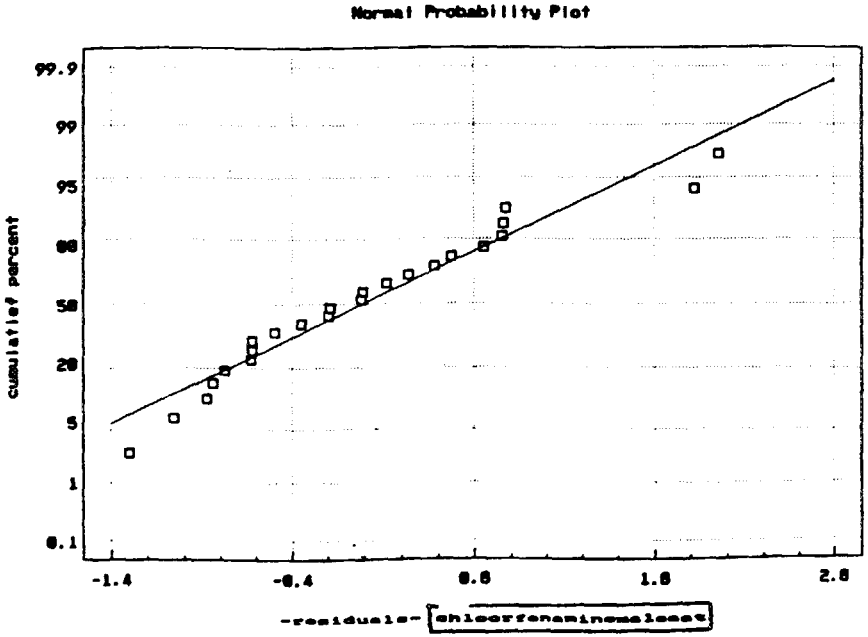




DIAGNOSTIC PLOT ... SIDU

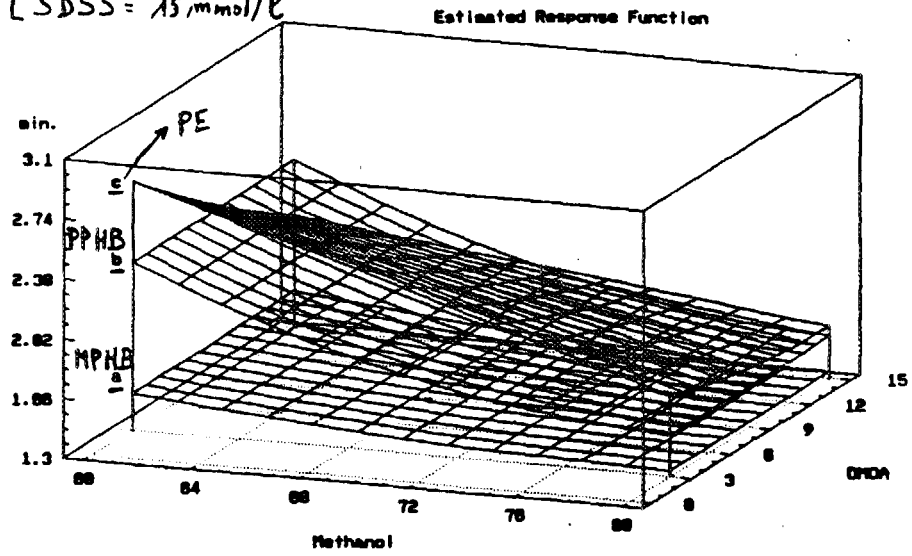


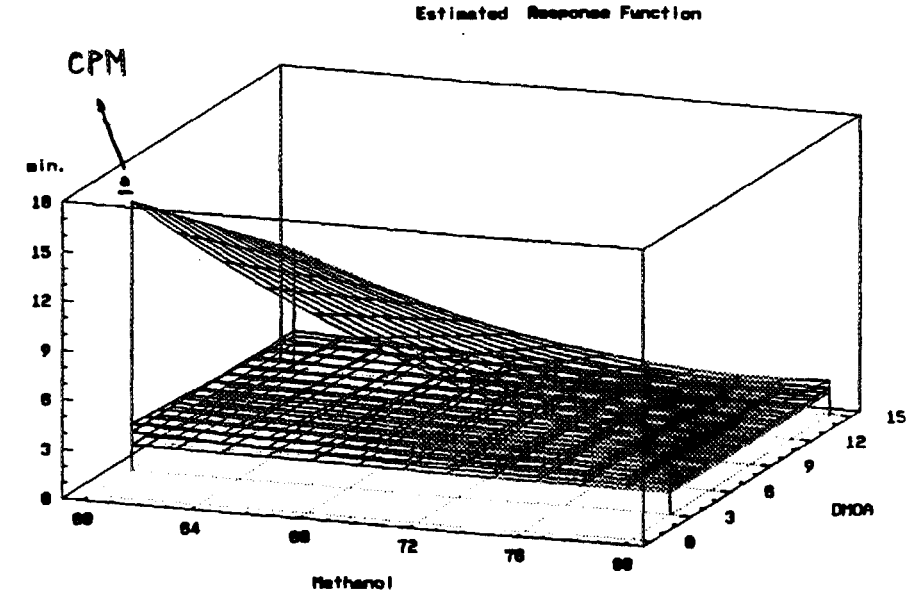
"DIAGNOSTIC PLOT": "RESIDUALS"

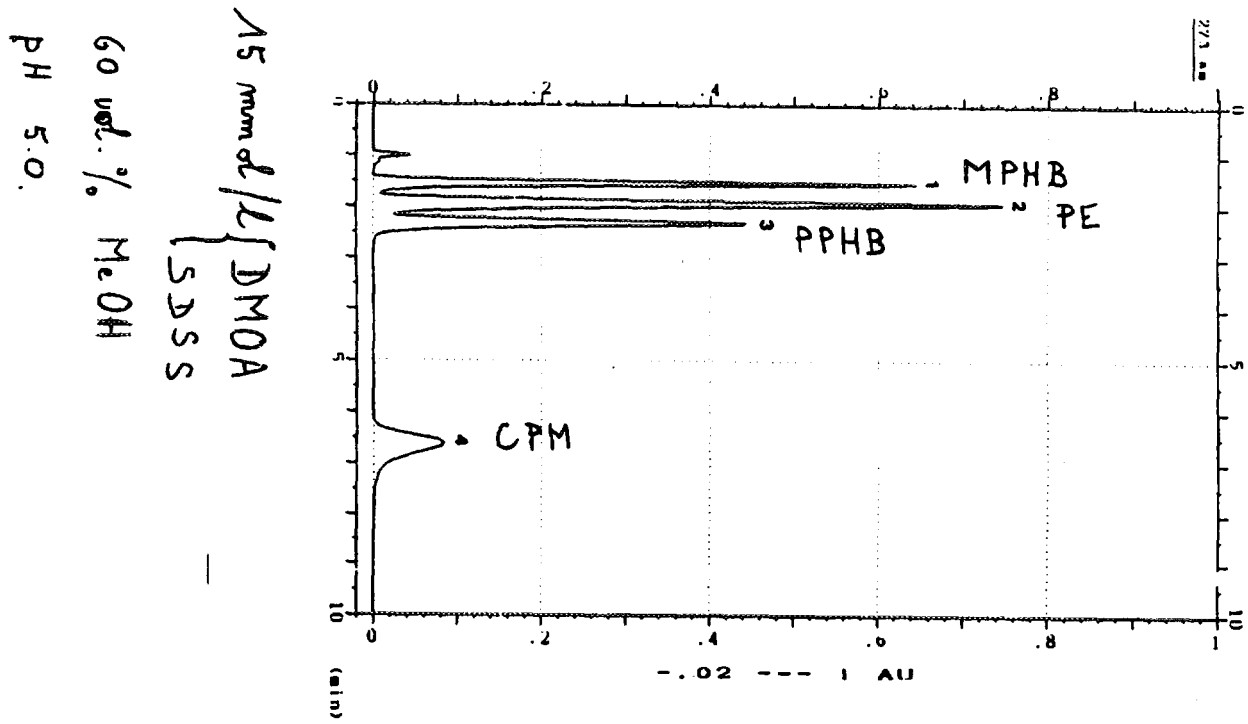


pH 10

LSDSS = 15 mmol/l







Conclusions

1) It is revealed that MeOH as organic modifier is the most influential parameter, within its examined concentration interval. Its estimated effect on the retention times is the most important for each compound.

2) The effect of the pH of the mobile phase is highly determining for the retention of CPM. On the contrary, the chromatographic behaviour of MPHB, PPHB and PE is almost insensitive to fluctuations between pH 3.0 and 5.0.

3) The retention times of PE and CPM also are clearly influenced by the DMOA and SDSS concentrations in the mobile phase. The effects of both parameters, however, are opposite.

4) Some important interactions between mobile phase parameters are discovered. Concerning the chromatographic behaviour of CPM, a remarkable interaction seems to exist between the SDSS concentration and the pH of the mobile phase. The effect of SDSS on the retention time of CPM is stronger at pH 3.0 than at pH 5.0.

5) Regression models with the significant chromatographic parameters and parameter interactions and the retention times as response variables, enable retention time calculation of the four compounds with good statistical reliability. From these regression models, three dimensional response surface plots can be constructed, which can help to select those parameter combinations, that ensure optimized chromatographic separations.

ANALYTICAL VALIDATION

The present document constitutes a note for guidance on the presentation of data validating test procedures used in the physico-chemical, biological or microbiological tests provided for in Directive 75/318/EEC as amended, with a view to marketing authorization in respect of a medicinal product.

1. INTRODUCTION

The objective of validation of a test procedure is to demonstrate that it is adequate for its intended use.

The analytical validation of test procedures used in pharmacokinetic, metabolic and bioavailability studies is the subject of a separate section of this note for guidance.

2. PARTS OF THE DOSSIER WHERE THIS NOTE FOR GUIDANCE IS APPLICABLE

This note for guidance is applicable to each test procedure used in the following sections of the chemical, pharmaceutical and biological documentation.

2-A Development pharmaceutics

2-B In-process control during manufacturing process

2-C Control of the starting materials (active substances - other components if necessary)

2-D Control tests on intermediate products

2-E Control tests of the finished product

2-F Stability

Revalidation of the test procedure may be necessary in certain circumstances e.g. transfer of the test procedure from the development stage to quality control (routine tests; in this case data will not normally be required) or when significant changes in the manufacturing procedure of the starting material or in the composition of the finished product have occurred. The degree of revalidation required depends on the nature of the changes.

3. ASPECTS TO BE VALIDATED AND CRITERIA FOR VALIDATION OF TEST PROCEDURES

The different criteria enumerated do not necessarily all apply for each test procedure. This depends very much on the circumstances.

Furthermore, the different aspects should not be considered separately, but may be linked (e.g. control of the purity and assay: see also annex).

Note: The Glossary and translation of some important terms can be found on pages 207-212.

a)	IDENTIFICATION:	Specificity
b)	TESTS: (Impurity content)	Specificity Limit of detection (LOD) or Limit of quantitation (LOQ)
c)	ASSAY: (Content or Potency)	Specificity Repeatability Precision Reproducibility Accuracy Linearity/Range/Sensitivity

4. GENERAL RECOMMENDATIONS

4.1 A short description of the main principle of the test procedure should be indicated.

4.2 The procedures must be described in such a way that they can be assessed and repeated by experts (e.g. authority experts or experts from a state laboratory) if they consider it necessary.

This includes:

- the exact description of the test conditions including precautions, reagents, reference substances and preparations;
- the verification of the test procedure under the defined operating conditions for example: verification of the separating power of a chromatographic system (system suitability test);
- the detailed formulae for the calculation of results including statistical evaluation as appropriate;
- the precise and detailed description of any equipment that is not commercially available;
- in the case where the analytical instrumentation is automated or not commercially available, it is recommended that (if available) details of a procedure as similar as possible to the background method and allowing the use of a standard equipment are provided.

4.3 For analytical methods (for instance disintegration or dissolution apparatus) or test procedures described in official and recognized publications (for instance pharmacopoeias), reference to the literature is sufficient. The test procedures in monographs of the starting materials described in pharmacopoeias are considered to be validated.

4.4 Reference substances and preparations (in house standards) should be characterized and evaluated for their intended purpose by additional methods other than those used in routine testing, unless reference substances or preparations of a pharmacopoeia or other official institutions are used. If a working standard is used, it must be characterized by comparison with the authentic reference standard.

4.5 In all cases, the complete data which demonstrate validity of test procedures should be indicated.

4.6 In all cases, the methods or procedures of analysis proposed must take account of technical and scientific progress and enable the starting material, intermediate and finished product to be checked by means of generally accepted methods (Article 9. of Directive 65/65/EEC as amended).

VALIDATION OF ANALYTICAL PROCEDURES DEFINITION AND TERMINOLOGY *)

1. INTRODUCTION

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text serves as a collection of terms, and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

2. TYPES OF ANALYTICAL PROCEDURES TO BE VALIDATED

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests.
- Quantitative tests for impurities' content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of substance substance or substance product or other selected component(s) in the substance product.

Although there are many other analytical procedures, such as dissolution testing for substance products or particle size determination for substance substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below.

- Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard.
- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.
- Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the substance substance. For the substance product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore revalidation may be necessary in the following circumstances:

- changes in the synthesis of the substance substance;
- changes in the composition of the finished product;
- changes in the analytical procedure;

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

GLOSSARY

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

This is sometimes termed trueness.

4. PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

4.1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision .

4.2. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

4.3. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

5. DETECTION LIMIT

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

6. QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

7. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

8. RANGE

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

9. ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Type of analytical procedure / characteristics	Identification	Testing for impurities: quantitat.	Testing for impurities: limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+(1)	-	+(1)
Specificity (2)	+	+	+	+
Detection Limit	-	-(3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

\- signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

8. SAMPLING OF STARTING AND PACKAGING MATERIALS

Principle

Sampling is an important operation in which only a small fraction of a batch is taken. Valid conclusions on the whole cannot be based on tests which have been carried out on non-representative samples. Correct sampling is thus an essential part of a system of Quality Assurance.

Note: Sampling is dealt with in Chapter 6 of the Guide, items 6.11. to 6.14. This annex gives additional guidance on the sampling of starting and packaging materials.

Personnel

1. Personnel who take samples should receive initial and on-going regular training in the disciplines relevant to correct sampling. This training should include:

   - sampling plans,
   - written sampling procedures,
   - the techniques and equipment for sampling,
   - the risks of cross-contamination,
   - the precautions to be taken with regard to unstable and/or sterile substances,
   - the importance of considering the visual appearance of materials, containers and labels,
   - the importance of recording any unexpected or unusual circumstances.

Starting materials

2. The identity of a complete batch of starting materials can normally only be ensured if individual samples are taken from all the containers and an identity test performed on each sample. It is permissible to sample only a proportion of the containers where a validated procedure has been established to ensure that no single container of starting material has been incorrectly labelled.

3. This validation should take account of at least the following aspects:

   - the nature and status of the manufacturer and of the supplier and their understanding of the GMP requirements of the Pharmaceutical Industry;
   - the Quality Assurance system of the manufacturer of the starting material;
   - the manufacturing conditions under which the starting material is produced and controlled;
   - the nature of the starting material and the medicinal products in which it will be used.

   Under such a system, it is possible that a validated procedure exempting identity testing of each incoming container of starting material could be accepted for:

   - starting materials coming from a single product manufacturer or plant;
   - starting materials coming directly from a manufacturer or in the manufacturer's sealed container where there is a history of reliability and regular audits of the manufacturer's Quality Assurance system are conducted by the purchaser (the manufacturer of the medicinal product) or by an officially accredited body.

   It is improbable that a procedure could be satisfactorily validated for:

   - starting materials supplied by intermediaries such as brokers where the source of manufacture is unknown or not audited;
   - starting materials for use in parenteral products.

4. The quality of a batch of starting materials may be assessed by taking and testing a representative sample. The samples taken for identity testing could be used for this purpose. The number of samples taken for the preparation of a representative sample should be determined statistically and specified in a sampling plan. The number of individual samples which may be blended to form a composite sample should also be defined, taking into account the nature of the material, knowledge of the supplier and the homogeneity of the composite sample.

Packaging Material

5. The sampling plan for packaging materials should take account of at least the following: the quantity received, the quality required, the nature of the material (e.g. primary packaging materials and/or printed packaging materials), the production methods, and what is known of the Quality Assurance system of the packaging materials manufacturer based on audits. The number of samples taken should be determined statistically and specified in a sampling plan.

POW___ FINL___3 AN_ _EVE ___

Powders

The coarseness or fineness of a powder is classed according to the nominal aperture size expressed in µm of the mesh of the sieve through which the powder is able to pass.

Terms used to describe particle size

• **Coarse powder (2000/355).**	A powder where all the particles pass through a No. 2000 sieve, and not more than 40% through a No. 355 sieve.
• **Moderately coarse powder (710/250).**	A powder where all the particles pass through a No. 710 sieve, and not more than 40% through a No. 250 sieve.
• **Moderately fine powder (355/180).**	A powder where all the particles pass through a No. 355 sieve, and not more than 40% through a No. 180 sieve.
• **Fine powder (180).**	A powder where all the particles pass through a No. 180 sieve.
• **Very fine powder (125).**	A powder where all the particles pass through a No. 125 sieve.

Sieves

The wire sieves used to sift powdered medicinal plant materials are classified by numbers as mentioned above which indicate the nominal aperture size expressed in µm.

Sieves are made of wire of uniform circular cross-section. They have the following specifications:

Number of sieve [µm]	*Nominal size of aperture* [mm]	*Nominal diameter of wire* [mm]	*Approximate screening area* [%]
2000	2.00	0.90	48
710	0.710	0.450	37
500	0.500	0.315	38
355	0.355	0.224	38
250	0.250	0.160	37
212	0.212	0.140	36
180	0.180	0.125	35
150	0.150	0.100	36
125	0.125	0.090	34
90	0.090	0.063	35
75	0.075	0.050	36
45	0.045	0.032	34

The nominal size of aperture of wire mesh sieves has been selected principally from among those recommended by ISO standard 565–1972.

GEI L AL ... ON ....PLIN(-

The reliability of any conclusions drawn from the analysis of a sample will depend upon how truly that sample represents the whole batch. General recommendations for sampling of pharmaceutical materials in connection with quality control are provided in the thirty-first report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations *(5)*.

Due to the specific characteristics of medicinal plant materials, in particular their inhomogeneity, special handling procedures are required in relation to sampling. The following procedures should be observed when selecting and preparing an average sample from a batch of material.

Recommended procedures

Sampling of material in bulk

Inspect each container/packaging unit (pack etc.) for conformity with pharmacopoeial monographs or other requirements regarding packaging and labelling. Check the integrity of the outer package and note any defects which may influence the quality or stability of the contents (physical damage, moisture etc.).

Damaged containers are sampled individually.

If initial inspection indicates that the batch is uniform, take the following samples:

When a batch consists of 5 containers/units, take a sample from all of them; and from a batch of 6–50 units, a sample from 5 packages. In the case of a batch of over 50 containers – sample 10% of the units – rounding up the number of units to the next highest figure of ten. For example, 51 units would be sampled as for 60.

After opening, inspect containers selected for sampling for:

- organoleptic characteristics (colour, texture and odour);
- presentation of the material (raw, cut, crushed, compressed);
- the presence of admixtures, foreign matter (sand, glass particles, dirt), mould, or signs of decay;
- the presence of insects;
- the presence of packaging material originating from poor or degraded containers.

From each container/package selected, take 3 original samples, taking care to avoid fragmentation. Samples should be taken from the upper, middle and lower parts. In the case of seeds and packages, 3 individual samples are taken by hand from a depth of not less than 10 cm from the top, and after cutting into the side of the package from the middle and lower parts. Samples of seeds are withdrawn with a grain probe. Material in boxes is first sampled from the upper layer; then approximately half of the contents is removed and samples are taken again. Finally after further removal of material, another sample is taken from the bottom. Samples should be as uniform as possible in mass. Individual samples are combined into a pooled sample which should be mixed carefully.

The average sample is obtained by *quartering*. The process of quartering consists of placing the sample, adequately mixed, formed as an even and square-shaped heap and dividing it diagonally into four equal parts. The two opposite parts are then taken diagonally, and carefully mixed. The process is repeated as necessary until the required quantity is obtained.

Pooled samples are quartered until the required amount remains which should be within ±10% (100–200 g for flowers and up to 10 kg for certain roots). Any remaining material should be returned to the batch.

The average sample is then *quartered* again and final samples are assembled and tested for the following characteristics:

- degree of fragmentation (sieve test);
- identity and level of impurities;
- determination of moisture and ash content;
- assay of active ingredients, where possible.

A portion of the final sample should be retained to serve as reference material, which may also be used for the purpose of checking quality control tests, if necessary.

Sampling of material in retail packages

From each wholesale container (boxes, cartons, etc.) selected for sampling, take at random two consumer packages. From small batches (1–5 boxes), take 10 consumer packages. Prepare the pooled sample by mixing the content of selected consumer packages and proceed as described above for the final sample.

2. DETERMINATION OF FOREIGN MATTER

Medicinal plant materials should be entirely free from visible signs of contamination by moulds, insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration should be detected.

It is seldom possible to obtain marketed plant materials that are entirely free of some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed.

During storage, products should be kept in a clean and hygienic place, so that no contamination occurs. Special care should be taken to avoid formation of moulds, since they may produce aflatoxins.

A macroscopical method of examination can conveniently be employed for whole or cut plant materials, however microscopy is indispensable for powdered plant materials.

Before the medicinal plant materials are cut, ground or powdered, soil, stones, sand, dust and other foreign inorganic matter must be removed.

Definition

Foreign matter is material consisting of any or all of the following:

(*a*) Parts of the medicinal plant material or materials other than those named with specified limits;

(*b*) Any organism, part or product of an organism, other than that named in the definition and description;

(*c*) Mineral admixtures not adhering to the medicinal plant materials, such as soil, stones, sand, and dust.

Sample size to be taken

It is difficult to prepare a pooled sample since most foreign matter adheres to the medicinal plant materials which is intrinsically nonuniform. Special procedures requiring considerable practice are therefore necessary. The problem is especially difficult when samples of unbroken crude medicinal plant materials are selected which are too small. Thus, it is crucial that the size of the sample should be sufficiently large to be representative.

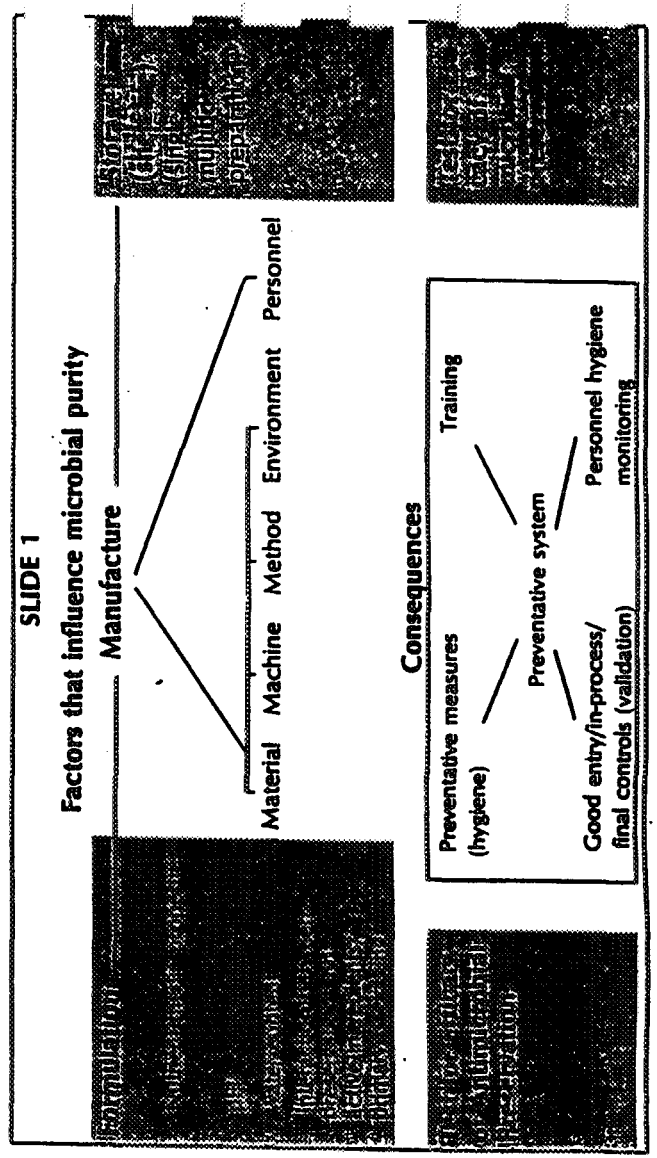
Recommended procedures

Foreign matter in whole or cut medicinal plant materials

Weigh the quantity of material as given below unless otherwise given in the instructions. Spread it in a thin layer and sort into groups of foreign matter either by visual inspection, using a magnifying lens (6× or 10×) or with the help of a suitable sieve, according to the requirements for the specific plant material.

The factors that may influence the microbial purity of drugs are:

- the formulation
- the manufacturing procedure
- the storage period
- the mode of use



SLIDE 2

Ph. Eur. Fascicule 19, May 1995
VIII. 15. MICROBIAL QUALITY OF PHARMACEUTICAL PREPARATIONS

In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable means must be taken to ensure their microbial quality. The pharmaceutical preparations should comply with the criteria given below.

CATEGORY 1

Preparations required to be sterile by the relevant monograph on the dosage form and other preparations labelled sterile

- Test for sterility (V.2.1.1).

CATEGORY 2

Preparations for topical use and for use in the respiratory tract except where required to be sterile

- Total viable aerobic count (V.2.1.8.1). Not more than a total of 10^2 aerobic bacteria and fungi per gram or per millilitre.
- Not more than 10^1 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (V.2.1.8.2).
- Absence of *Pseudomonas aeruginosa* (1.0 g or 1.0 ml) (V.2.1.8.2).
- Absence of *Staphylococcus aureus* (1.0 g or 1.0 ml) (V.2.1.8.2).

SLIDE 3

Ph. Eur. Fascicule 19, May 1995
VIII. 15. MICROBIAL QUALITY OF PHARMACEUTICAL PREPARATIONS

CATEGORY 3

A: *Preparations for oral and rectal administration*

- Total viable aerobic count (V.2.1.8.1). Not more than 10^3 aerobic bacteria and not more than 10^2 fungi per gram or per millilitre.
- Absence of *Escherichia coli* (1.0 g or 1.0 ml).

B. *Preparations for oral administration containing raw materials of natural origin (for which antimicrobial pretreatment is not feasible and for which the relevant authority accepts a microbial contamination of the raw material exceeding 10^3 viable micro-organisms per gram or per millilitre). Herbal remedies described in category 4 are excluded.*

- Total viable aerobic count (V.2.1.8.1). Not more than 10^4 aerobic bacteria and not more than 10^3 fungi per gram or per millilitre.
- Not more than 10^2 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (V.2.1.8.2).
- Absence of *Salmonella* (10.0 g or 10.0 ml) (V.2.1.8.2).
- Absence of *Escherichia coli* (1.0 g or 1.0 ml) (V.2.1.8.2).
- Absence of *Staphylococcus aureus* (1.0 g) (V.2.1.8.2).

SLIDE 4

Ph. Eur. Fascicule 19, May 1995

VIII. 15. MICROBIAL QUALITY OF PHARMACEUTICAL PREPARATIONS

CATEGORY 4

Herbal remedies

A. *Herbal remedies to which boiling water is added before use*

- Total viable aerobic count (V.2.1.8.1). Not more than 10^7 aerobic bacteria and not more than 10^5 fungi per gram or per millilitre.
- Not more than 10^2 *Escherichia coli* per gram or per millilitre (V.2.1.8.2, using suitable dilutions).

B. *Other herbal remedies*

- Total viable aerobic count (V.2.1.8.1). Not more than 10^5 aerobic bacteria and not more than 10^4 fungi per gram or per millilitre.
- Not more than 10^3 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (V.2.1.8.2).
- Absence of *Escherichia coli* (1.0 g) (V.2.1.8.2).
- Absence of *Salmonella* (10.0 g) (V.2.1.8.2).

Figure 2.8.12-2

Introduce into the flask the prescribed quantity of the drug and continue the distillation as described above for the time and at the rate prescribed. Stop the heating and after 10 min read the volume of liquid collected in the graduated tube and subtract the volume of xylene previously added. The difference represents the quantity of essential oil in the mass of the drug taken. Calculate the result as millilitres per 100 g of drug.

When the essential oil is to be used for other analytical purposes, the water-free mixture of xylene and essential oil may be recovered as follows: remove the stopper *K* and introduce 0.1 ml of a 1 g/l solution of sodium fluorescinate *R* and 0.5 ml of water *R*. Lower the mixture of xylene and essential oil into the bulb-shaped swelling *L* by means of the three-way tap, allow to stand for 5 min and lower the mixture slowly until it just reaches the level of the tap *M*. Open the tap sufficiently so that the water flows out of the connecting tube *BN*. Wash the tube with acetone *R* and with a little mixture *R* introduced through the filling funnel *N*. Turn the tap sufficiently in order to recover the mixture of xylene and essential oil in an appropriate flask.

2.8.13. PESTICIDE RESIDUES

Definition. For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of vegetable drugs. The term includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Limits. Unless otherwise indicated in the monograph, the drug to be examined at least complies with the limits indicated in Table 2.8.13.-1. The limits applying to pesticides that are not listed in the table and whose presence is suspected for any reason comply with the limits set by European Community directive 76/895 and 90/642, including their annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 nor in EC directive are calculated using the following expression:

$$\frac{ADI \times M}{MDD \times 100}$$

ADI - acceptable daily intake, as published by FAO-WHO in milligrams per kilogram of body mass,

M - body mass in kilograms (60 kg),

MDD - daily dose of the drug in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

E - extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

Table 2.8.13.-1

Substance	Limit (mg/kg)
Aldrin	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of cis-, trans- and Oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of *o,p'*-DDT, *o,p'*-DDE and *p,p'*-TDE)	1.0
Deltamethrin	0.5
Dicofol	0.5
Dieldrin	1.0
Dithiocarbamates (as CS_2)	2.0
Endosulfan (sum of Isomers and Endosulfan sulphate)	3.0
Endrin	0.05
Fenitrothion	0.5
Fenvalerate	1.5
Fluvalin	0.05
Heptachlor (sum of Heptachlor and Heptachlor epoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than γ)	0.3
Lindane (γ-hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.3
Permethrin	1.0
Pirimiphos-methyl	0.1
Procymidone	3.0
Propargite	0.9
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulfide)	1.0

Sampling

Method. For containers up to 1 kg, take one sample from the *total content,* thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount *sufficient to carry out* the tests.

Size of sampling. If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number *of containers* is more than three, take samples as indicated under Method from $\sqrt{n} + 1$ containers, rounding up to the nearest unit if necessary.

The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in airtight containers suitable for food contact, at a temperature below 0 °C, protected from light.

Reagents. All reagents and solvents are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been redistilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus. Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 h in a solution of phosphate-free detergent, rinse with large quantities of *distilled water R* and wash with acetone and hexane or heptane.

Qualitative and quantitative analysis of pesticide residues. The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed, and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination to be analysed,
- between 70 per cent to 110 per cent of each pesticide is recovered,
- the repeatability of the method is not less than the values indicated in Table 2.8.13.-2,
- the reproducibility of the method is not less than the values indicated in Table 2.8.13.-2,
- the concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

Table 2.8.13.-2

Concentration of the pesticide (mg/kg)	Repeatability (difference, ± mg/kg)	Reproducibility (difference, ± mg/kg)
0.010	0.005	0.01
0.100	0.025	0.05
1.000	0.125	0.25

The following section is given for information and guidance; it does not form a mandatory part of the General method.

TEST FOR PESTICIDES

Organochlorine, Organophosphorus and pyrethroid insecticides

The following methods may be used, in connection with the general method above. Depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry...) or a different method (immunochemical methods...) to confirm the results obtained.

This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

1. Extraction. To 10 g of the substance being examined, coarsely powdered, add 100 ml of *acetone R* and allow to stand for 30 min. Add 1 ml of a solution containing 1.8 µg per millilitre of *carbophenothion R* in *toluene R*. Homogenise using a high-speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml, of *acetone R*. Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40 °C until the solvent has almost completely evaporated. To the residue add a few millilitres of *toluene R* and heat again until the acetone is completely removed. Dissolve the residue in 5 ml of *toluene R*. Filter through a membrane filter (45 µm), rinse the flask and the filter with *toluene R* and dilute to 10.0 ml with the same solvent (solution A).

2. Purification

2.1. Organochlorine, organophosphorus and pyrethroid insecticides. Examine by size-exclusion chromatography (2.2.30).

The chromatographic procedure may be carried out using:

- a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with *styrene-divinylbenzene copolymer R* (5 µm),
- as mobile phase *toluene R* at a flow rate of 1 ml per minute.

Performance of the column. Inject 100 µl of a solution containing 0.5 g/l of *methyl red R* and 0.5 g/l of *orcein blue 2R R* in *toluene R* and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing, in *toluene R*, at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

Purification of the test solution. Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

2.2. Organochlorine and pyrethroid insecticides. In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat *silica gel for chromatography R* in an oven at 150 °C for at least 4 h. Allow to cool and add dropwise a quantity of *water R* corresponding to 1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 h using a mechanical shaker. Condition the column using 1.5 ml of *hexane R*. Prepacked columns containing about 0.50 g of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of *helium for chromatography R* or *oxygen-free nitrogen R* almost to dryness and dilute to a suitable volume with *toluene R* (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of *toluene R* as the mobile phase. Collect the eluate (solution C).

3. Quantitative analysis

3.1. Organophosphorus insecticides. Examine by gas chromatography (2.2.28), using *carbophenothion R* as internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution. Concentrate solution B in a current of *helium for chromatography R* almost to dryness and dilute to 100 µl with *toluene R*.

Reference solution. Prepare at least three solutions in *toluene R* containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using:

- a fused-silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of *poly(dimethyl)siloxane R*,
- *hydrogen for chromatography R* as the carrier gas. Other gases such as *helium for chromatography R* or *nitrogen for chromatography R* may also be used provided the chromatography is suitably validated,
- a phosphorus-nitrogen flame-ionisation detector or a atomic emission spectrometry detector,

maintaining the temperature of the column at 80 °C for 1 min, then raising it at a rate of 30 °C per minute to 150 °C, maintaining at 150 °C for 3 min, then raising the temperature at a rate of 4 °C per minute to 280 °C and maintaining at this temperature for 1 min, and maintaining the temperature of the injector port at 250 °C and that of the detector at 275 °C. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 2.8.13.-3. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

TABLE 2.8.13.-3

Substance	Relative retention times
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17
Phosalon	1.18

3.2. Organochlorine and pyrethroid insecticides. Examine by gas chromatography (2.2.28), using carbophenothion as the internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution. Concentrate solution C in a current of *helium for chromatography R* or *oxygen-free nitrogen R* almost to dryness and dilute to 500 µl with *toluene R*.

Reference solution. Prepare at least three solutions in *toluene R* containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using:

- a fused silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of *poly(dimethyl)(diphenyl)siloxane R*,
- *hydrogen for chromatography R* as the carrier gas. Other gases such as *helium for chromatography R* or *nitrogen for chromatography R* may also be used, provided the chromatography is suitably validated,
- an electron-capture detector,
- a device allowing direct cold on-column injection,

maintaining the temperature of the column at 80 °C for 1 min, then raising it at a rate of 30 °C per minute to 150 °C, maintaining at 150 °C for 3 min, then raising the temperature at a rate of 4 °C per minute to 280 °C and maintaining at this temperature for 1 min, and maintaining the temperature of the injector port at 250 °C and that of the detector at 275 °C. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 2.8.13.-4. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 2.8.13.-4

Substance	Relative retention times
α-Hexachlorocyclohexane	0.44
Hexachlorobenzene	0.45
β-Hexachlorocyclohexane	0.49
Lindane	0.49
δ-Hexachlorocyclohexane	0.54
ε-Hexachlorocyclohexane	0.56
Heptachlor	0.61
Aldrin	0.68
cis-Heptachlor-epoxide	0.76
p,p'-DDE	0.81
α-Endosulfan	0.82
Dieldrin	0.87
p,p'-DDE	0.87
o,p'-DDD	0.89
Endrin	0.91
β-Endosulfan	0.92
o,p'-DDT	0.95
Carbophenothion	1.00
p,p'-DDT	1.02
cis-Permethrin	1.29
trans-Permethrin	1.31
Cypermethrin*	1.40
Fenvalerate*	1.47
	1.49
Deltamethrin	1.54

* The substance shows several peaks.

LECTURES OF MR. MAURICE IWU

THE APPROACH -1

BDCP is essentially a cooperative of independent scientists, policy analysts, industrialists and institutions concerned with the deteriorating condition of life in the tropical parts of the world.

website: http://bioresources.org

KEY ISSUES THAT SHOULD BE ADDRESSED IN A MODEL CONTRACT FOR BIODIVERSITY PROSPECTING -2

- Future Supplies of Raw Materials: sustainable collection; collaborating institution and country as first source; fair price
- Provisions for Conservation
- Technology transfer
- Rights of indigenous People: reciprocity and equity considerations

TRADITIONAL MEDICINE AND MODERN SCIENCE: BRIDGING THE GAP

(Shaman Pharmaceutical Inc.)

Essential features of International Research collaborations

- Must yield tangible benefits for the partners or realistic hope of such benefits
- Should offer collaborative advantage
- Generate new shared values and not just exchange of skills
- Should offer organizational flexibility, not be rigidly directed by formal systems and contracts should involve interpersonal contacts
- Multilayer integration: strategic, tactical, cultural or interpersonal

RESEARCH COLLABORATIONS

Establish a Relationship - Not just a Deal

Fear of Being Engulfed

- Participation in the Process is the key - not Share of the Royalties
- Unrealistic Expectations May Hurt Biodiversity Conservation

BDCP - ROYALTY SHARING SCHEME THROUGH THE TRUST FUND MECHANISM

- 20 %: BDCP - International, to spend according to the Trust Fund General Principles on Conservation and development activities throughout Africa.
- 10 % : Universities in Nigeria
- 10 % : Universities in Cameroon
  Explicitly for the purposes of training graduate students.
- 10 % : National Botanical Gardens and Herbaria (split between Nigeria and Cameroon - not to replace existing goverment contributions or support.
- 50 % : Traditional healers organizations: community development funds, etc.

PARTNERSHIP ARRANGEMENTS

- The Relationship must yield tangible benefits for the partners- or realistic hope of such
- benefits
- Should offer collaborative advantage
- Generate new shared values and not mere exchange of skills
- Flexibility in organization: not rigidly directed by formal systems and contracts
- interpersonal connections
- Integration: strategic, tactical, cultural and interpersonal

Resources Development and Conservation Programme

TEN PRINCIPLES OF SUCCESSFUL PARTNERSHIPS

Finding a partner

- Step by step approach
- Develop a profile of your preferred partner
- Contact a multiple candidates

Creating a contact

- Focus on mutual benefits
- Start simple, set objectives
- involve lawyer - later

Manage the partnership

- Emphasize the partnership mentality
- Develop a team of champions
- Communicate frequently
- Think long-term but deliver short-term successes

Resources Development and Conservation Programme

SIX BRIDGING ELEMENTS

- **Education**
- **Health**
- **Access to information**
- **Basic Infrastructure**
- **Participation**
- **Small Scale Economic Activities**

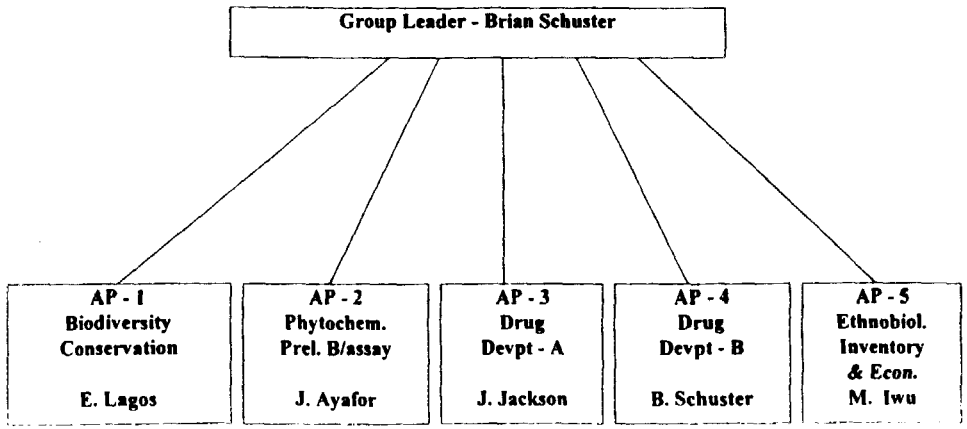
THE APPROACH - 2

Brings Innovative management and technical support to grass-root development, capacity building and self reliance are the underlying tenets in all BDCP projects.

THE RIO IMPERATIVE

1. INFORMED CONSENT
2. LEVEL PLAYING FIELD IN BIOLOGICAL RESOURCES EXCHANGE
3. RECOGNITION OF RIGHTS OF INDIGENOUS PEOPLE
4. RECOGNITION OF SOVEREIGNTY OF NATION SATES OVER GENETIC MATERIALS

ICBG STRUCTURE



ASSOCIATE PROGRAM - 1
Biodiversity Conservation, etc.

TRAINING:

Sponsored 4 participants to 1996 Smithsonian / Man and Biosphere Training course on Biodiversity Monitoring

Conducted two similar courses in Cameroon and Nigeria (involving participants from neighboring African countries)

FOREST DYNAMICS PLOT:

Establishing a large 50 hectare plot at Chimpanzee Camp in the Korup Forest of Cameroon

ASSOCIATE PROGRAM - 2
Phytochemistry & Prelim. Biossays - contd.

SCIENTIFIC OUT - PUT:

Processed over 150 plant extracts & fractions. Screened samples for activity against bacteria and fungi. System established for screening of random collected plants.

Isolated and characterized 52 compounds from bio-active plant extracts.

Identified and characterized 5 novel compounds.

ASSOCIATE PROGRAM - 4
Drug Development: Antiviral, etc

TRAINING:

Providing post-doctoral experience in drug development.

SCIENTIFIC OUT - PUTS:

HIV: Screened 25 extracts - 2 active substances identified: Most active constituent of one extract characterized, with high "therapeutic index".

EBOLA VIRUS: One of the plant isolates showed *in vitro* activity against ebola virus.

CYTOTOXICITY: 20 plant extracts tested against human colon tumor cell line - 16 active at 50 µg/ml level, 5 active below 5 µg/ml.

ANTIFUNGAL: 35 plant extracts with significant activity identified.

ASSOCIATE PROGRAM - 5
Ethnobiology, Inventory, Plant Collection

SCIENTIFIC OUT- PUT:

- Conducted 10 ethnobotanical field trips in Nigeria and Cameroon.
- Updates the database on African Medicinal Plants.
- Collected over 200 plants for the treatment of various target diseases.
- Prepared herbarium specimens for all the plants collected.
- Established a multidisciplinary team of experts to identify and selected plants species with greatest potential for biological activity.
- Maintains an inventory of plants used in the region for healing.

CAPACITY BUILDING:

- Establishing of the Center for Medicinal and Aromatic Plants at Nsukka, incorporating a medicinal plant herbarium, plant processing unit and data processing unit.
- Refurbishing of the Enugu Reference Herbarium.
- Establishing of a Trust Fund for Rural Development and Traditional Medicine.
- Purchase of a 4 wheel-drive vehicle for field work and plant collection.

BENEFIT SHARING PLAN:
Short Term & Immediate Compensation

- **Collection fees to individual & communities**
  - cash payment to informant/collector
  - Assist Community development projects
  - Medical members of ICBG assist local healers in treatment of diseases
- **Training & Capacity Building**

BENEFIT SHARING PLAN
Long Term Compensation

20 % - To the investigators and all the persons that contributed intellectually to the discovery and development efforts.

30 % - To be donated for the tropical diseases drug development program based at Walter Reed Army Institute of Research.

50 % - To BDCP for conservation and economic development projects to be disbursed through Trust Fund.

COMPUTERIZED INFORMATION SYSTEM ON AFRICAN MEDICINAL AND AROMATIC PLANTS

AFRICMED

- **Medicinal:** uses, constituents, pharmacology, literature
- **Floristic:** nomenclature, morphology, distribution, literature
- **Horticultural:** propagation, cultivation, literature

COMPUTERIZED INFORMATION SYSTEM ON AFRICAN MEDICINAL AND AROMATIC PLANTS

CISAMAP

- **Link interactively**: West and Central Africa
- **Network with Pretoria (Southern Africa) and Nairobi (East Africa)**

CONSERVATION PROJECTS

- Permanent biodiversity plots
- Inventory of flora and fauna of the region
- Economic value assessment studies
- Domestication and propagation of rare species
- Bioprospecting projects that add value at the grass root level
- Regional biodiversity network
- CISMAP database of African Medicinal Plants

TECHNOLOGY MANAGEMENT

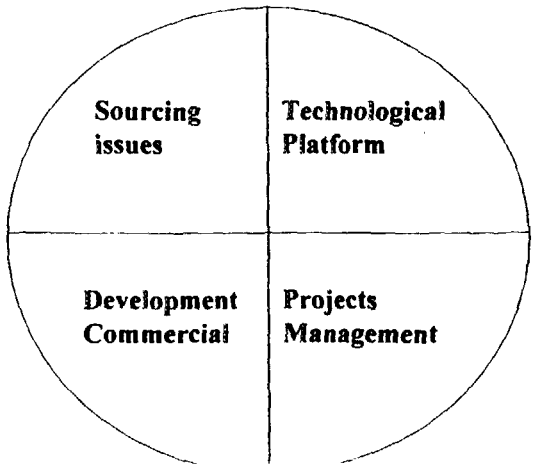
BDCP

Capability
Infrastructure
Technology Platform

Resources Development and Conservation Programme

Biodiversity
Microbial
culture collection
Ethnobiology

Marketing
Concerns, IPR



Extraction production and storage facility

Sole Effort
Network
Alliances &
Sub - contracts

BASIC ELEMENTS OF A STRATEGY

WHAT?
Products, goods, services
Extracts, phytomedicines, formulation

HOW?
Technology / management

WHO?
Client & beneficiaries

REVENUE AND ROYALTY RATES FOR MEDICINAL PLANTS

- **PLANT MATERIAL /EXTRACT** **0.5 - 2 %**
- **LEAD COMPOUND** **5 - 10 %**
- **DRUG CANDIDATE** **10 - 15 %**
- **LICENSED PRODUCT** **> 20 %**

SUSTAINABLE USE OF BIODIVERSITY

- **In Country Research and Development**
- **Product Development Through Cooperative Agreements**
  - Research collaborations
  - Strategic business Alliances
- **Bioprospecting**

BIOSPROSPECTING: REQUIREMENTS

- **IN COUNTRY**
  - Availability of material
  - Capability assessment
  - Capacity assessment
  - National environment
- **EXTERNAL**
  - Core areas
  - Market analysis
  - CBD compliance
  - Product development
  - Partnership arrangement

EXTRACT MARKET

- **License extract for specific period - not sell extract library.**
- **Add value by processing.**
- **Protect IPR.**
- **Be realistic in negotiating revenues - go far a share in the royalties.**
- **Keep good catalogue of the extracts for resupply request.**

ACCESS TO TECHNOLOGY & INTERNATIONAL MKT

- **NETWORKING**
- **COOPERATIVE AGREEMENTS**
- **STRATEGIC ALLIANCES**

STRATEGIC BUSINESS ALLIANCE

- Close collaboration between partners for shared objectives and agreed strategic goal
- Creates new comparative advantage
- May lead to loss of some degree of "sovreignty"
- A stronger form of cooperation than networking

CAPABILITY

1. Determine number and quality of essential personnel.
2. Level of training or retraining required.
3. Availability of identified personnel.

TECHNOLOGY PLATFORM

- *DEVELOPMENT:* **Phytomedicines, Antimalarial, Antifungal, Antiviral**
- *COMMERCIALIZATION:* **Extract Library, Novel Leads, Plant Materials**
- *PARTNERSHIP & ALLIANCES:* **Pharmaceuticals**

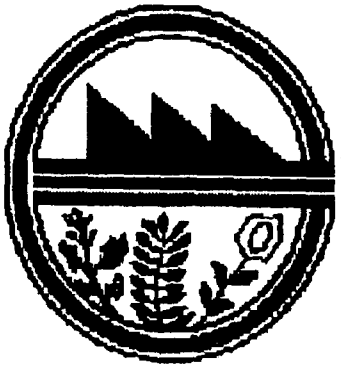
INFRASTRUCTURE

- Check for utilities and municipal services - may require lag phase to stablish a working system.
- Determine core requirements and secondary needs.
- Equip to reflect technological platform.

BUSINESS DEVELOPMENT OPTIONS

- *NETWORK:* Loose Alliance, Shared Information, Limited Commercial exchanges.
- *COOPERATIVE R & D AGREEMENTS:* Project based contracts, Close working relationships.
- *PARTNERSHIP & ALLIANCES:* Closer integration of resources, shared values.

LINKING BIODIVERSITY CONSERVATION AND ECONOMIC DEVELOPMENT



BIORESOURCES DEVELOPMENT AND CONSERVATION PROGRAMME
PROGRAMME DE DEVELOPMENT ET PRESERVATION DES RESOURCES BIOLOGIGUES

11303 AMHERST Ave. Silver Spring, MD 20906. Phone: 301-962-6201; Fax: 301-962-6205

SUPPLY CONTRACTS OR STRATEGIC ALLIANCES

PHYTOMEDICINE - *FOCUS*

- **Anti-oxidants**
- **Immune Stimulations**
- **Agents for Metabolic Disorders**
- **Cholesterol Reducing Agents**
- **Anti-infectives: Antifungal, Antiviral**
- **Antitumor**
- **Digestive Stimulants**
- **Adaptogenes**

PHYTOMEDICINES - FORMS 1

- **Aromatic teas**: essential oils.
- **Nonaromatic teas**: alkaloids, flavonoids, etc.
- **Infusions & Decoctions**: single or multiple plants, water or alcohol.
- **Baths**: solutions or steam, skin absorption or inhalation.
- **Powdered herbs**: whole plant / part.

PHYTOMEDICINES - FORMS 2

- **Saps:** usually unstable, freeze dry
- **Syrups**: drugs for respiratory diseases
- **Exudates**: processing affects chemical composition
- **Fresh Herbs**: activity may be lost if dried; bioassay each batch

WALTER REED ARMY INSTITUTE OF RESEARCH - AFRICA ICBG
BUILT ON THE FOUNDATION OF A SUCCESSFUL DRUG DEVELOPMENT PROGRAM

A. Virtual Drug Company - Multidisciplinary Staff

B. Rich in Tropical Diseases Expertise

C. Not Profit Driven

D. Interactive with W.H.O., Academia, Industry

- Walter Reed / Nigeria / Cameroon Project
  - Purpose: To develop drugs for parasitic diseases from tropical rainforest medicinal plants from Nigeria and Cameroon
    - Malaria
    - Trypanosomiasis
    - Leishmaniasis

ICBG: A NEW STANDARD OF COLLABORATION WITH INDIGENOUS PEOPLE.

INTERNATIONAL COOPERATIVE BIODIVERSITY GROUP (ICBG): DRUG DEVELOPMENT AND CONSERVATION OF BIODIVERSITY IN WEST AND CENTRAL AFRICA

JUNE 24-25, 1996
LAGOS, NIGERIA
INTERNATIONAL WORKSHOP ON
COMMERCIAL PRODUCTS OF INDIGENOUS PLANTS AS PHYTOMEDICINES AND COSMETICS

STRATEGIC BUSINESS ALLIANCES

- The BDCP Experience
  - Program Design
  - Program Structure
  - Program Staff
- Major Accomplishments
  - Problems

SOME PRODUCTS HAS BEEN PRODUCED AS NATURAL PRODUCTS LIKE *NATURAL* ***"TOOTHPASTE*** "BY "***TOM'S OF MAINE"*** WITH SPEARMINT FLAVOR AND IT IS ACCEPTED BY THE "AMERICAN DENTAL ASSOCIATION " (ADA)

NATURAL PERSONAL CARE PRODUCTS - SAFETY ISSUES

Possibility of irritation
indicate potential irritant.
limited "in use" history may not be sensitive enough to

Combination of Ingredients may produce entires different toxicity profile than observed in individual ingredients

Minor contaminants and impurities from handling or sourcing may create higher levels of toxicity

Poor or lack literature on the toxicity of the plant material

Chemical modification during production process may lead to "creation" of new toxicant

INGREDIENTS MUST SATISFY BOTH TECHNICAL SPECS. & MARKETING CONCERNS

MAJOR CLASSES OF NATURAL PRODUCTS FOR COSMETICS

Bio-saponins: *steroidal and triterpenoid*
Flavonoids: *bioflavonoids & biflavonoids*
Aminoacids: *non-protein, biocomp.*
Proteins & Phytoamines
Anti - oxidants
Alpha Hydroxy Acids
Formulation Aides

NATURAL PERSONAL CARE PRODUCTS

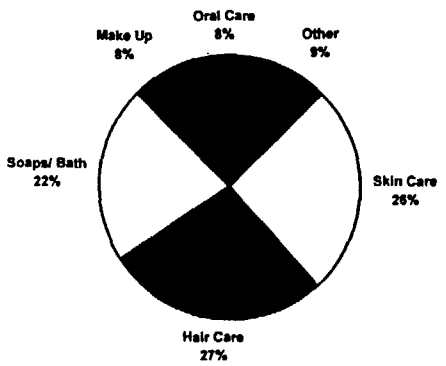
CONCERNS FOR NATURAL INGREDIENTS IN FORMULATIONS

Safety
Quality of Raw Materials
Reliable Source of Material
Regulatory Requirements
Claims Development

COSMETIC PRODUCTS (GENERAL DEFINITION)

- *Cleansers*
- *Perfumes*
- *Masking, i.e. changes appearance*
- *Prevent body odor*
- *Protection against environment effects*
- *Decorate*

% of Natural Personal Care Product Industry



Bioresources Development and Conservation Programme

NIGERIAN MEDICINAL PLANTS WITH POTENTIAL APPLICATIONS IN PRIMARY HEALTH CARE

Table # 1

Plant	constituent(s)	Activity/ Indications
1. ***Aframomum melegueta***	Essential oils Shagoal, gingerol	Antimicrobial, rubefacient
2. ***Ageratum conyzoides***	Ageratochromone	Would healing
3. ***Azadirachta indica***	Nortriterpenoids	Antimalaria antipyretic, seed insecticidal
4.***Balanites aegyptica***	Steroidal glycosides furanocoumarines	Laxative, Antiinflamtory, molluscicidal
5.***Bridelia ferruginea***	Coumestans flavonoids	Antifungal Mouth infections
6.***Butyrospermum paradoxum***	Fatty acids	Emmolient, antiinflamatory
7.***Cajanus cajan***	Amino glycosides, phenyl alanine	Management of Sickle-cell anemia
8.***Carica papaya***	Proteolytic enzymes (vol. oils in leaves)	Leaves for fevers, diabetes
9.***Cassia spp.***	Anthraquinone glycosides	Laxative
10.**Cola nitida**	Caffeine, aromatic acids	Tonic
11.***Cymbopogon citratus***	Volatile oils	Diuretic, tonic
12.***Dorstenia multiradiata***	Leucoanthocyanidins	Antifungal, antiviral
13.***Dracaena mannii***	Saponins	Local Antifungal, antiprotozoan
14.***Eucalyptus globulus***	Essential oils	Local antiseptic, colds rubefacient
15.***Garcinia kola***	Biflavonoids	Antihepatotoxic antiviral, adaptogen plaque inhibition
16.***Morinda lucida***	Anthraquinones	Malaria, jaundice
17.***Ocimum gratissimum***	Terpenes, xanthones	Antiseptic, cough, fevers
18.***Picramnia nitida***	Indole alkaloids	Antimalaria broad spectrum antiprotozoan
19.***Piper guineense***	Lignans, alkaloids	Antimicrobial, insecticidal tonic, antiinflamatory
20.***Psidium gujava***	Essential oils, vitamins	Carminative
21.***Sabiaceae calycina***	Alkaloids, flavonoids	Wound dressing, laxative
22.***Schwenkia guineensis***	Steroidal glycosides	Oral hygiene

23.*Sclerocarya birrea*	Catechins, flavonoids amino acids	Diabetes, tonic
24.*Tamarindus indica*	Ascorbic acid, citrates	Laxative, nausea
25.*Tetrapleura tretraptera*	Saponins, coumarins	Antiinfective, tonic
26.*Uvaria chamae*	Chalcones, terpenes	Antimicrobial
27.*Vernonia amygdalina*	Sesquiterpenes, saponins	Tonic, antidiabetic
28.*Xylopia aethiopica*	Diterpenes	Tonic, carminative, Antiviral
29.*Zanthoxylum xanthoxyloides*	Aromatic acids	Mangement of Sickle-cell anemia
30.*Zingiber officinale*	Terpenes	Hypertension, antihistamine

PHYTONUTRACEUTICALS & *HEALTH FOODS*

- Provitamins
- Adatogenes
- Cholesterol lowering agents
- Bioflavonoids
- Immune-stimulants
- Antioxidants
- Appetite suppressant

Bioresources Development and Conservation Programme

NOTICE TO APPLICANTS

VOLUME II B

SEPTEMBER 19

TABLE OF CONTENTS

PART I B I SUMMARY OF PRODUCT CHARACTERISTICS

In accordance with Article 4a of Directive 65/65/EEC[11], as amended by Directive 83/570/EEC[12], a proposal for a summary of product characteristics (SPC) must be included in the application. Part I B consists of the proposal for the SPC. Further, Article 4b of Directive 65/65/EEC[11] requires that the content must be approved by the competent authority. Thus the SPC forms an intrinsic and integral part of the marketing authorization.

The purpose and scope of the SPC is set out in Directive 83/570/EEC [12]:

"it is necessary, from the point of view of public health and the free movement of medicinal products, for the competent authorities to have at their disposal all useful information on authorized medicinal products, based in particular on summaries, adopted in other Member states, of the characteristics of products".

Therefore, the SPC sets out the agreed position of the medicinal product, as distilled during the course of the assessment process. It is the definitive statement between the competent authority and the marketing authorization holder, and it is the common basis of communication between the competent authorities of all the Member States. As such the content cannot be changed except with the approval of the originating competent authority.

In some Member States, a « data sheet » is prepared as a means of ,communication with prescribers/suppliers. This is based on the SPC. In order to avoid this duplication of effort, the value of using the SPC as a basis of information for the prescriber/supplier has been appreciated. Therefore, the sequence of topics in the SPC has been structured to highlight the clinical orientation and the section on "Clinical particulars" comes immediately following the sections which identify the medicinal product. In addition, sections on the marketing authorization number and the date of approval/revision of the SPC have been introduced.

In the light of harmonization activities and especially the inclusion of the SPC as part of the CPMP opinion, it was considered useful to have an agreed sequence for the presentation of information within the SPC. The competent authorities have all agreed to accept the sequence given in the guideline (III/9163/89)[13] which is reproduced on the following pages.

[11] see the annex, reference 7, complete article page 8 of the annex
[12] see the annex, reference 22
[13] see the annex, reference 128

SUMMARY OF THE PRODUCT CHARACTERISTICS :LIST OF HEADINGS

1. TRADE NAME OF THE MEDICINAL PRODUCT

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

3. PHARMACEUTICAL FORM

4. CLINICAL PARTICULARS
   - 4.1 Therapeutic indications
   - 4.2 Posology and method of administration
   - 4.3 Contra-indications
   - 4.4 Special warnings and special precautions for use
   - 4.5 Interaction with other medicaments and other forms of interaction
   - 4.6 Pregnancy and lactation
   - 4.7 Effects on ability to drive and use machines
   - 4.8 Undesirable effects
   - 4.9 Overdose

5. PHARMACOLOGICAL PROPERTIES
   - 5.1 Pharmacodynamic properties
   - 5.2 Pharmacokinetic properties
   - 5.3 Preclinical safety data

6. PHARMACEUTICAL PARTICULARS
   - 6.1 List of excipient(s)
   - 6.2 Incompatibilities
   - 6.3 Shelf-life
   - 6.4 Special precautions for storage
   - 6.5 Nature and contents of container
   - 6.6 Instructions for use/handling

7. MARKETING AUTHORIZATION HOLDER

8. MARKETING AUTHORIZATION NUMBER

9. DATE OF FIRST AUTHORIZATION/RENEWAL OF AUTHORIZATION

10. DATE OF (PARTIAL) REVISION OF THE TEXT

SUMMARY OF THE PRODUCT CHARACTERISTICS : NOTES ON HEADINGS

1. TRADE NAME OF THE MEDICINAL PRODUCT

To be completed by the applicant.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

In terms of the active substance(s) (Ph. Eur., National Pharmacopoeia, INN, trivial name and chemical description).

In the case of a radiopharmaceutical kit is to be considered to be that part of the formulation which carries or binds the radionuclide after the addition of a radioactive component. For a radionuclide generator both mother and daughter radionuclides are to be considered as active substance(s).

3. PHARMACEUTICAL FORM (WITH REFERENCE TO THE STANDARDIZED TERMINOLOGY)[14]

4. CLINICAL PARTICULARS

4.1. Therapeutic indications

- Avoid a global description. The indication(s) should relate as precisely as possible to the results of clinical trials.
- Indicate : treatment and/or prevention and/or diagnosis.

4.2 Posology and method of administration

e.g. adults, neonates, children[15] and the elderly and mention of the posology for each age category

- dosage (dose and interval) and duration
- dosage adjustment in renal or liver insufficiency, dialysis, concomitant disease
- maximum tolerated daily dose and the maximum dose for an entire course of therapy
- monitoring advice

Dosage for radioactive products should be expressed in Becquerels.

4.3 Contra-indications

Situations where patients should NEVER or GENERALLY NOT be treated. In rare cases where the medicinal product should NEVER be given, this must be specifically outlined.

[14] see the annex, reference 43

[15] see the annex, reference 102 ,103

4.4 ...ecial wa... ... d specif... ...tions for:

These are intended to :

- WARN prescribers or suppliers of the possibility of class- or drug-related adverse reactions (ADR) occurring under normal conditions of use in particular situations such as renal, hepatic or cardiac failure, elderly, young... [with the exception of pregnancy and lactation, ability to drive and use machines, interactions which are respectively dealt with in 4.5, 4.6, 4.7].

and

- Describe the conditions under which the medicinal product may be recommended for use in sub-groups of patients at risk, provided that the special conditions of use are fulfilled. Inform prescribers in the tentative ways to prevent the occurrence or the worsening of these ADR, by monitoring patients and/or reduction of doses, discontinuation of the treatment.

Emphasis can be given to a serious risk, by underlining the seriousness (i.e. possibility of death) and presenting the labelling at the top of the paragraph, in bold type, within a box.

4.5 Interaction with other medicaments and other forms of interaction

Show only interactions which are observed and/or for which there is potential on the basis of experience with drugs of the same pharmacotherapeutic group which are or may be clinically meaningful.

- medicinal products used for the same indication
- medicinal products used for other indications
- daily activities, e.g. meals

The following information should be given for each interaction :

a) mechanism of action (if known)

b) consequences on plasma levels of drugs and/or on laboratory and clinical parameters

c) recommendations :

- contra-indication (cross-referral with 4.3)
- not recommended association
- precautions for use (i.e. dose adjustment)

or

- to be taken into account.

-

4.6 Pregnancy and lactation

The following should be mentioned:

a) conclusions from the animal reproduction/fertility study and the human experience
b) the risk in humans at different times of pregnancy, as assessed from a)
c) information on the possibility of using the medicinal product in fertile and pregnant women.

Use during lactation

When the active substance(s) or its metabolites are excreted in the milk, a recommendation as to whether to stop or continue breast feeding, and the likelihood and degree of adverse reactions in the infant should be given.

Effe... ility to ... and use machines

On the basis of:

- the pharmacodynamic profile
- reported ADR

and/or

- impairment of driving performance or performance related to driving,

the medicine is :

a. presumed to be safe or unlikely to produce an effect

b. likely to produce minor or moderate adverse effects

c. likely to produce severe adverse effects or presumed to be potentially dangerous.

For situations b and c, special precautions for use/warnings should be mentioned.

4.8 Undesirable effects

- quantify these effects (frequency in general terms and seriousness)
- significant adverse reactions observed or the most predictable on the basis of :
  - toxicology, especially finding from repeated dose toxicity studies ;
  - previous clinical experience with products of the same class.

4.9 Overdose

- acute experience in animals
- human experience
- management of overdose in man.

5. PHARMACOLOGICAL PROPERTIES

(so far as this information is relevant for therapeutic purposes)
Statements should be brief and precise.

5.1. Pharmacodynamic properties

- pharmacotherapeutic group (ATC code)
- mechanism of action (if known)
- pharmacodynamic effects :

Relevant for prescription [effects for which there is a demonstration or at least some evidence of a relationship with the therapeutic effect or which may induce ADR] They should be concisely described.

5.2 ...rmacok... ...operties

Relevant information should be given on:

a) general characteristics of the active substance(s)

- absorption, with the bioavailability of the dosage form and, for the oral route, whether it is due to liver first pass effect ; incomplete absorption ; the influence of food;
- distribution, with reference to plasma protein binding, volume of distribution, tissue and/or plasma concentrations, pronounced multi-compartment behaviour;
- biotransformation, to active metabolites, inactive metabolites and in the case of prodrugs, to the active substance(s);
- elimination with reference to :
  - the elimination half-lives, the total clearance
  - excretion (with partial clearances)
  - the unchanged substance and metabolites (and their activities)
  - linear or non-linear kinetics.

b) characteristics in patients

- any known relationship between plasma/blood concentrations and the therapeutic activity or adverse drug reactions
- variations with respect to confounding factors, age, polymorphic metabolism and concomitant pathological situations (renal failure, hepatic insufficiency).

5.3 Preclinical Safety Data

Information should be given on any findings in the preclinical testing which could be of relevance for the prescriber, in recognising the safety and safety profile of the medicinal product used for the authorised indications(s), and which is not already included in other relevant sections of the SPC.

The information should be presented in a way that enables the prescribing physician to apply the benefit/risk of use of the product for the individual patient.

Note : During the development of a new medicinal product, a variety of pre-clinical studies will be performed. These are assessed by the competent authority when evaluating the application; if the results of the studies do not add to the information needed by the prescriber, then the results (either positive or negative) need not be repeated in the SPC.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipient(s); a full statement of the excipient(s) expressed qualitatively.

6.2 Incompatibilities

Information on physical and chemical incompatibilities of the medicinal product with others with which it is likely to be mixed or co-administered. This will be particularly important for medicinal products to be diluted before parenteral administration. Significant problems of sorption of product to syringes, large volume parenteral containers etc. should be stated.

medicinal products to be diluted before administration, Significant loss of
surpass 2 to 5yr volume content should be

6.3 Shelf life[16]

- shelf-life in the product as packaged for sale
- shelf-life after dilution or reconstitution according to directions
- shelf-life after first opening the container.

6.4 Special precautions for storage

The maximum (or minimum) storage temperature should be stated in Celsius to fully reflect conditions found in any EC Member State in which the medicinal product is likely to be sold or supplied, unless the stored medicinal product is stable at temperatures up to 30°C when the product need bear no special storage instructions.

Special precautions in relation to humidity and light should also be stated.

6.5 Nature and contents of container

Reference to standardized terminology with a description (see the list of standard terms European Pharmacopoeia [17])

6.6 Instructions for use/handling

Instructions for use/handling are needed where :

- the medicinal product as such is not intended for immediate use and has for instance to be suspended or diluted before administration. Claims on compatibilities can be given here provided these have been proven in the dossier.
- due to the nature of the medicinal product or the packaging/closure the way of using/handling the medicinal product is not obvious without instructions.
- a special dosing device to administer the product has to be used
- additional requirements for radiopharmaceuticals (Article 6 of Directive 89/343/EEC[18])

7. MARKETING AUTHORIZATION HOLDER

Name or style and permanent address or registered place of business of the holder of the marketing authorization.

8. MARKETING AUTHORIZATION NUMBER(S)

9. DATE OF FIRST AUTHORIZATION/RENEWAL OF THE AUTHORIZATION

[16] see the annex, reference 54
[17] see the annex, reference 43
[18] see the annex, reference 11,complete article page 11 of the annex

PA EXPER ITS

A. INTRODUCTION

Directive 75/319/EEC[19] as amended requires that the particulars and documents submitted in the application dossier are drawn up and signed by experts, with the necessary technical or professional qualifications. The chemical/ pharmaceutical/ biological, pharmaco-toxicological and clinical parts of the dossier should each include an Expert Report. The Expert Reports, their tabular formats and written summaries are placed in Part IC of the dossier.

It is important to emphasize that well prepared Expert Reports greatly facilitate the task of the competent authority in evaluating the dossier and contribute towards the speedy processing of applications. For these reasons particular care should be taken in the preparation of Expert Reports, following the guidance on the preparation of Expert Reports given in the current edition of the Notice to Applicants.

Where relevant Community guidelines on the conduct of tests, studies and trials on a medicinal product exist, these should be taken into consideration when Expert Reports are prepared. Any deviation from guidelines should be discussed and justified. In particular, the experts should give a justification for the statements in the proposed SPC_s taking into account the submitted data and the SPC guideline III/9163/89 [20]and also considering the need for bioavailability studies with reference to the guideline on bioavailability and bioequivalence in Volume III.

B. PRESENTATION OF THE EXPERT REPORT

Each Expert Report should be introduced by a "product profile" (1-2 pages) which is a brief extract of the summary of product characteristics and which repeats the following key points :

a) Type of application
- a product essentially similar to one already on the market, or
- a new active substance(s), or
- a new combination of previously known active substance(s), or
- a new pharmaceutical form, or
- a new strength, or
- an extension of indications

b) Chemical and pharmacokinetic properties
- the chemical structure of the active substance(s)
- the physico-chemical properties of the active substance(s) and the characteristics of the pharmaceutical form which could have an effect on the pharmacokinetic parameters and clinical efficacy

c) Indications
- the therapeutic indications proposed as a function of the posology and their justification
- the pharmacological and therapeutic classification of the active substance(s), defining the mode of action

[19] see the annex, reference 9
[20] see the annex, reference 128

- significant precautions and warnings derived from the principal results of the preclinical studies, both toxicology and animal pharmacology

e) Marketing/post-marketing

- a list of any post-marketing surveillance;
- a list of marketing authorizations already issued in other countries, and those applied for.

This product profile, as an extract of the summary of product characteristics, does not have to be signed by the expert.

The Expert Reports and the summaries of data should contain precise volume and page references to the specific studies or other relevant information contained in the study report tables and in the full dossier. It is recommended that these references be indicated in the right margin of the text and in a separate column or at the top of tables.

An Expert Report should bear the signature of the expert(s) and the place and date of its issue. Attached to the report, there should be brief (1 page) information on the expert(s): their name(s), educational background, training and occupation. The professional relationship of the expert to the applicant should be declared.

Each Expert Report should contain a critical discussion of the properties of the product (i.e. 3 Expert Reports, one each covering quality, safety and efficacy). The expert is expected to take and defend a clear position on the product in the light of current scientific knowledge. Applicants are reminded of the strict size limitation of the Expert Reports:

- Quality (normally less than 10 pages)
- Safety (normally less than 25 pages)
- Efficacy (normally less than 25 pages)

Each Expert Report should be followed by annexes, as set out in this Notice to Applicants.

- The tabular formats accompanying the Expert Report, in accordance to those set out in this Notice to Applicants, provide a standardized approach to the presentation of the documentation in tabular form. Equally, the formats are in many cases designed to facilitate the assessment which in turn benefits the applicant.
- The written summary should be factual, complete (i.e. covering all studies) and concise. It should contain cross-references to the documentation in the relevant part of the dossier as well as including tables, graphs, etc.

The written summary has proven useful, particularly for large complex dossiers and applicants are therefore encouraged to include (systematically) a written summary in the following applications:

- new active substance(s);
- abridged applications where the demonstration of well established medicinal use, with recognised efficacy and an acceptable level of safety relies on detailed references to published scientific literature;
- other abridged applications where, in the opinion of the applicant, the volume and complexity of the documentation would be such that a written summary would be helpful.

[illegible] dossier, [illegible] considered [illegible] be [illegible] written [illegible] except in [illegible] of biot[illegible] medici[illegible]nts and [illegible] product [illegible]ntain or consist of genetically modified organisms where a written summary of not more than 50 pages would be helpful).

For the toxicological section of Part III of the dossier, the tabular formats are considered to fulfil the function of a written summary.

For the pharmacological section of Part III of the dossier, a written summary could be useful. Normally, the written summary would not be more than 10 pages.

It is considered helpful to have an overview table which would precede a written summary.

For the efficacy part of the dossier, a written summary can be helpful for large, complex clinical documentation. In order to aid clarity, an overview table of clinical studies should precede the written summary.

The written summary should be factual, complete (i.e. covering all studies) and concise. Normally, it would not be longer than 30 pages. However, in cases of complex dossiers, with multiple indications and/or large numbers of patients evaluable for safety and efficacy, a larger summary (up to 100 pages) could be necessary.

C. EXPERT REPORTS FOR ABRIDGED APPLICATIONS

Consent from the marketing authorization holder

For applications based upon Article 4.8 (a)(i) of Directive 65/65/EEC[25] the Experts' Reports of the original marketing authorization holder may be used.

Bibliographical applications

For applications based upon Article 4.8 (a)(ii) of Directive 65/65/EEC[25], the experts' Reports should particularly focus on the following elements:

- the grounds for using published references and the relevance of the references selected;
- an update of published literature relevant to the substance and the present application. The expert may annotate review articles published in "peer review" journals which may be acceptable in this respect;
- a summary of impurities present in batches of the active substance(s) (and, where relevant, decomposition products arising during storage) as proposed for use in the product to be marketed;
- the issue of bioavailability, and bioequivalence where appropriate, related to the proposed formula for marketing should be addressed taking into account the relevant pharmacokinetic parameters of the formulation used in the literature;
- comparison of pharmacokinetic parameters (C_{max}, T_{max}, AUC etc.) of the formulations used in the literature and the formulation proposed for marketing;
- an evaluation of the results of additional studies to provide for missing data in the file. These data should be discussed in the perspective of what is known from published literature.

[25] see the annex, reference 7, complete article page 8 of the annex

Additional studies should also be submitted in tabular formats provided in this Notice to Applicants:

- every claim in the Summary of Product Characteristics (SPC) not known from or inferred from the properties of the medicinal product and/or its therapeutic group should be discussed in the Expert Reports and substantiated by published literature and/or additional studies.

Product essentially similar to a product authorised for 6 or 10 years

For applications based upon Article 4.8 (a)(iii) of Directive 65/65/EEC[33] the Expert Reports should particularly focus on the following elements:

- the grounds for claiming essential similarity;
- a summary of impurities present in batches of the active substance(s) (and where relevant decomposition products arising during storage) as proposed for use in the product to be marketed;
- an evaluation of the bioequivalence studies or a justification why studies were not performed with respect to the note for guidance on 'Investigation of Bioavailability and Bioequivalence
- an update of published literature relevant to the substance and the present application. It may be acceptable for articles in "peer review" journals to be annotated for this purpose.
- every claim in the Summary of Product Characteristics (SPC) not known from or inferred from the properties of the medicinal product and/or its therapeutic group should be discussed in the Expert Report and substantiated by published literature and/or additional studies.

Extensions

For applications based upon the second annex of the regulations on variations[29], an application for a new marketing authorization must be made.

The Expert Report should particularly focus on the following elements:

- an evaluation of the results of the additional studies. The results should be discussed in the perspective of what is known from published literature and previous submissions. Additional studies should also be submitted in tabular formats provided in this Notice to Applicants ;
- an update of published literature relevant to the substance and the present application. The expert may annotate articles published in "peer review" journals, which may be acceptable for this purpose;
- every claim in the Summary of Product Characteristics (SPC) not known from or inferred from the properties of the medicinal product and/or its therapeutic group should be discussed in the Expert Report and substantiated by published literature and/or additional studies.

[33] see the annex, reference 4

PART II CONCERNING CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION FOR ~~VEGETABLE~~ MEDICINAL PRODUCTS[55]

HERBAL

The principle of GMP and the detailed guidelines are applicable to all operations which require the authorization referred to in Article 16 of Directive 75/319/EEC[60] as modified. They are also relevant for all other large scale pharmaceutical manufacturing processes, such as that undertaken in hospitals, for the preparation of products for use in clinical trials, and for wholesaling, were applicable.

All analytical test procedures described in the various sections of the Part II chemical, pharmaceutical and biological documentation must be described in sufficient detail to enable the procedures to be repeated if necessary (e.g. by an official laboratory). All procedures need to be validated and the results of the validation studies must be provided.

PART II A: COMPOSITION

1 COMPOSITION OF THE MEDICINAL PRODUCT

NAMES OF INGREDIENTS	UNIT AND/OR PERCENTAGE	FUNCTION	REFERENCE TO STANDARDS
Active substance(s)			
Excipient(s)			

2 CONTAINER (BRIEF DESCRIPTION)

Nature of container materials; qualitative composition; method of closure; method of opening.

3 CLINICAL TRIAL FORMULA(E)

4 DEVELOPMENT PHARMACEUTICS

Explanation with regard to the choice of formulation, composition, ingredients and container, supported, if necessary, by data on development pharmaceutics .The overage, with justification thereof, should be stated .Tests carried out during pharmaceutical development must be described in detail, e. g. in vitro dissolution studies for solid pharmaceutical forms.

[55] see the annex, reference 61

[60] see the annex, reference 9,complete article page 11 of the annex

PART II B: METHOD OF PREPARATION

1 MANUFACTURING FORMULA (INCLUDING DETAILS OF BATCH SIZE)

2 MANUFACTURING PROCESS (INCLUDING IN-PROCESS CONTROL AND THE PHARMACEUTICAL ASSEMBLY PROCESS)

If vegetable active substance preparations are the starting material, the description of their manufacturing process and their control belong to section C.

A flow-sheet of the manufacturing process should be given.

3 VALIDATION OF THE PROCESS,

Validation of the process should be carried out when a non-standard method of manufacture is used or for steps of the manufacturing process which are critical for the product described in the finished product specifications (experimental data showing that the manufacturing process, using materials of the stated quality and the types of manufacturing equipment specified, is a suitable one and will consistently yield a product of the desired quality).

PART II C: CONTROL OF STARTING MATERIALS

1 ACTIVE SUBSTANCE(S)

1.1. Specifications and routine tests 1.1.2. Definition of a production batch

1.1.1 Active substance(s) described in a pharmacopoeia

For herbal drug preparation additional specifications as under 1.1.2 may be required.

1.1.2 Active substance(s) not described in a pharmacopoeia

- Characteristics
- Identification tests
- Purity tests (including limits for named, total, other single, unidentified single and unidentified total impurities)
  - Physical
  - Chemical
  - Potential contamination by micro-organisms, products of micro-organisms, pesticides, toxic metals, radioactivity, fumigants, etc.
- Other tests markers or of constituents
- Assay(s) of ~~excipients of vegetable active substances or vegetable active substance preparations~~ with known therapeutic activity
- In the case of ~~vegetable active substance preparations, a monograph on the vegetable active substance~~ a herbal drug preparation, a monograph on the herbal drug has to be presented

1.2. Scientific Data

1.2.1 Nomenclature

- International non-proprietary name (INN)
- Chemical name
- Other name
- Laboratory code
- In the case of vegetable active substance(s)
  - Scientific name of plant, with the name of the authority, variety and chemotype
  - Parts employed of the herb
  - Name of the preparation

1.2.2 Description

- Physical form
- Structural formula (including conformational data for macromolecules)
- Molecular formula
- Relative molecular mass
- Chirality Markers or substances with known therapeutic activity
- ~~Main excipients of vegetable active substances based on recent scientific data~~

1.2.3 Manufacture

- Name(s) and address(es) of the manufacturing source(s)
- Geographic source of vegetable active substance.
- Synthetic or manufacturing route
- Description of process
- Solvents, reagents; excipients .
- Catalysts
- Purification stages

1.2.4 Quality control during manufacture

- Starting materials
- Control tests on intermediate products (where appropriate)

1.2.5 Development (for active substance(s) of herbal vegetable origin)

1.2.5.1 Vegetable active substance the herbal drug or the herbal drug preparation

- Description of the ~~vegetable active substance(s)~~
  - macroscopic
  - microscopic constituents
- Composition and analytical research for ~~excipients~~ and physical characteristics
- Investigation for adulterants of known toxic excipients
- Analytical development and validation, commentary on the choice of routine tests and specifications

1.2.5.2 Vegetable ~~active substance preparation~~ (e.g. powder extract) Herbal drug preparations such as extracts

- Analytical chemical profile (qualitative and quantitative) constituents
- Detection of toxic ~~excipients~~/adulterants
- Analytical development and validation, commentary on the choice of routine tests and specifications.

1.2.6 Impurities

- Potential impurities originating from the route-of-synthesis
- Potential impurities arising during the production and purification
- Methods detecting potential contamination of the vegetable active substance(s) by micro-organisms and products of micro-organisms, pesticides, fumigation agents, toxic metals, radioactivity etc.
- Potential falsification and adulteration of the vegetable active substance(s)

1.2.7 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size, and use of batches including batches used in preclinical and clinical testing)
- Results of tests
- Reference material (analytical results), primary and others

2 EXCIPIENTS

2.1 Specifications and routine tests

2.1.1 Excipients described in a pharmacopoeia

2.2.2 Excipients not described in a pharmacopoeia

- Characteristics
- Identification tests
- Purity tests (including limits for named, total, other single, unidentified single and unidentified total impurities)
  - physical
  - chemical
- Other tests
- Assay(s) and/or evaluations (where necessary)

2.2 Scientific data

Data, where necessary, for example on excipient(s) used for the first time in medicinal products (see II C.1.2).

3 PACKAGING MATERIAL (IMMEDIATE PACKAGING)

3.1. Specifications and routine tests

- Type of material
- Construction
- Quality specifications (routine tests) and test procedures

3.2. Scientific data

- Development studies on packaging materials

- Batch analysis, analytical results

PART II D: CONTROL TESTS ON INTERMEDIATE PRODUCTS (IF NECESSARY)

A distinction should be made between in-process controls (Part II B) and control tests on intermediate products.

PART II E: CONTROL TESTS ON THE FINISHED PRODUCT

1 SPECIFICATIONS AND ROUTINE TESTS

1.1 Product specifications and tests for release at time of manufacture (general characteristics, specific standards)

1.2 Control Methods

1.2.1 Test procedures for identification and quantitative determination for the active substance(s)

It must be described in detail (including biological and micro-biological methods where relevant), together with other tests which include those in the appropriate general monograph for the type of dosage form in the European Pharmacopoeia:

- Identification tests
- Quantitative determination of active substance(s); and additionally for vegetable active substances and vegetable active substances preparation, quantitative determination of excipients with known therapeutic activity
- Purity tests
- Pharmaceutical tests (e.g. dissolution)

1.2.2 Identification and determination of excipient(s)

- Identification tests for approved colouring materials
- Determination of antimicrobial or chemical preservatives (with limits)

2. SCIENTIFIC DATA

2.1 Analytical validation of methods and comments on the choice of routine tests and standards (e.g. working standards)

2.2 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size and use of batches)
- Results obtained
- Reference material (analytical results), primary and others

PART II F: STABILITY

1 STABILITY TESTS ON ACTIVE SUBSTANCE(S)

- Batches tested
- General test methodology
  - accelerated test conditions
  - normal test conditions
- Analytical test procedures
  - assay
  - determination of degradation products
- Validation of all test procedures including limits of detection (including initial results)
- Results of tests
- Conclusions

2 STABILITY TESTS ON THE FINISHED PRODUCT

- Quality specification for the proposed shelf-life
- For radiopharmaceutical kits, data should be provided on the stability (including radiolabelling and biodistribution performance) of the unopened container (for shelf-life estimation) ; of the reconstituted radiolabelled product using maxima and minima of radionuclide content and volume of reconstituting medium (to establish the maximum radiolabelled shelf-life) ; and following simulated use of the product (to establish any limitation on the number of doses that may be removed from a prepared vial).
- Batches tested and packaging
- Study methods
  - real time studies
  - studies under other conditions
- Characteristics studied
  - physical characteristics
  - microbiological characteristics
  - chemical characteristics
  - chromatographic characteristics
  - characteristics of the packaging (container/closure interaction with the product)
- Evaluation test procedures
  - description of test procedures
  - validation of test procedures
- Results of test (including initials and reference to degradation products)
- Conclusions
  - shelf-life and storage conditions
  - shelf-life after reconstitution and/or first opening of the product
- Ongoing stability studies

Part II H : DATA RELATED TO THE ENVIRONMENTAL RISK ASSESSMENT FOR PRODUCTS CONTAINING, OR CONSISTING OF GENETICALLY MODIFIED ORGANISMS (GMOs) (see part II of biological medicinal products)

QUALITY OF HERBAL MEDICINAL PRODUCTS

Note for guidance concerning the application of Part 2 of the Annex to Directive 75/318/EEC, as amended. The special problems of herbal *medicinal products* and the differences between medicinal products containing chemically defined active *substances* are described in this note for guidance (*).

Consistent quality for products of *herbal* origin can only be assured if the starting materials are defined in a rigorous and detailed manner including especially the specific botanical identification of the plant material used. It is also important to know the geographical source and the conditions under which the *herbal* drug is obtained to ensure material of consistent quality.

This note for guidance should be read in conjunction with the Annex 7 "Manufacture of Herbal Medicinal Products" of Good Manufacturing Practice (GMP) for medicinal products; GMP recommendations should be respected.

Reference substances used in the control of all stages of the manufacturing process should be clearly defined.

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

1) In the case of a *herbal* drug

either (i) the quantity of *herbal* drug must be stated *if there are no constituents with known therapeutic activity*

or (ii) the quantity of a *herbal* drug may be given as a range corresponding to a defined quantity of constituents with known therapeutic activity

EXAMPLE

i) Active substance

Name	Quantity (**)
Valerianae radix	900 mg

or

ii) Active substance

Name	Quantity
Sennae folium	830-1000 mg, corresponding to 25 mg of hydroxyanthracene glycosides, calculated as Sennoside B.

Other substance

Name

...

(*) *In this note for guidance, the sequence used is designed to relate directly to Part 2 of the Annex to Directive 75/318/EEC, as amended.*

(**) *The quantity indicated refers to the specifications provided in the documentation.*

2) In the case of a *herbal* drug preparation,

either (i) ***in the case of constituents without established therapeutic activity***, the equivalent quantity *x - y* (*), or the ratio *a - b: 1* (*) of the ***herbal*** drug to the ***herbal*** drug preparation must be stated (this does not apply to fatty or essential oils).

or (ii) ***if the constituents are known***, the quantity of the ***herbal*** drug preparation may be given as a range corresponding to a defined quantity of constituents with known therapeutic activity (see example).

The composition of any solvent or solvent mixture and the physical state of the extract must be indicated.

If any other substance is added during the manufacture of the ***herbal*** drug preparation to adjust the ***herbal*** drug preparation to a certain level of constituents with known therapeutic activity, or for any other purpose, the added substance must be mentioned as an "other ***substance***" and the genuine extract as the "active ***substance***".

EXAMPLE

i) Active ***substance***

Name	Quantity
Valerianae radix dry extract ethanolic 60% *(V/V)* ***(a - b : 1)***	125 mg
or	
Valerianae radix dry extract ethanolic 60% *(V/V)*	125 mg ***equivalent to x - y mg Valeriane radix***

or

ii) Active ***substance***

Name	Quantity
Sennae folium dry extract ethanolic 60% *(V/V)* ***(a - b: 1)***	100-150 mg, corresponding to 25 mg of hydroxyanthracene glycosides, calculated as Sennoside B

Other ***substance***

Name

...

B. DESCRIPTION OF THE METHOD OF PREPARATION

The manufacturing process within the meaning of this section is the preparation of the finished product from the ***herbal drug or herbal drug preparation***. The description should include details of any comminution or size reduction step, and details of any process such as fumigation etc. used to reduce the levels of microbial contamination together with the controls exercised over the process. ***This section should be in accordance with the "Note for Guidance on Manufacture of the finished dosage form" (CPMP/QWP/486/95).*** If ***herbal*** drug preparations are the starting material, the manufacture of the ***herbal*** drug preparations and their controls do not belong under this section but under section C.

(*) ***'a' and 'b' or 'x' and 'y' have to be justified by the applicant***

C. CONTROL OF STARTING MATERIAL

1) Control of the ***herbal*** drug ***and of herbal drug preparations***

- Control of the ***herbal*** drug

A ***comprehensive specification*** for each ***herbal*** drug must be submitted, even if the starting material is a ***herbal*** drug preparation. This also applies if the applicant is not the manufacturer of the preparation. In the case of fatty or essential oils a ***comprehensive specification*** for the ***herbal*** drug is ***required unless fully justified***. The scientific name of the parent plant and its part(s) have to be stated.

If no monograph for the ***herbal*** drug is given in a Pharmacopoeia referred to in Directive 75/318/EEC, Annex I, a ***comprehensive specification*** on the ***herbal*** drug must be supplied and should be set out in the same way where practicable, as the monographs on ***herbal*** drugs in the European Pharmacopoeia. This should include the botanical name and authority and the common name if used for labelling purposes. Information on the site of collection, the time of harvesting and stage of growth, treatment during growth with pesticides etc., and drying and storage conditions should be included if possible. The ***comprehensive specification*** should be established on the basis of recent scientific data. In the case of ***herbal*** drugs with constituents of known therapeutic activity, assays of their content (with test procedure) are required. The content must be included as a range, so as to ensure reproducibility of the quality of the finished product. ***In the case of herbal drugs without constituents of known therapeutic activity, assays of marker substances (with test procedure) are required. The use of markers should be justified.***

As a general rule, ***herbal*** drugs must be tested for microbiological quality and for residues of pesticides and fumigation agents, radioactivity, toxic metals, likely contaminants and adulterants, etc., unless otherwise justified. Specifications and descriptions of the analytical procedures must be submitted, together with the limits applied. ***Analytical procedures not given in a Pharmacopoeia should be validated in accordance with the ICH guideline "Validation of analytical procedures: methodology" (CPMP/ICH/281/95).***

Reference samples of the ***herbal*** drugs must be available for use in comparative tests e.g. macro and microscopic examination, chromatography etc.

- Control of ***herbal*** drug preparations

If the ***herbal*** medicinal product contains not the ***herbal*** drug itself but a preparation, the ***comprehensive specification*** on the ***herbal*** drug must be followed by a description and validation of the manufacturing process for the ***herbal*** drug preparation. ***The information may be supplied either as part of the marketing authorisation application or using the European Drug Master File procedure. If the latter is chosen the documentation should be submitted in accordance with the note for guidance "European Drug Master File Procedure for Active Substances" (Eudra/Q/93/014).***

For each ***herbal*** drug preparation, a ***comprehensive specification*** must be submitted. This must be established on the basis of recent scientific data and must give particulars of the characteristics, identification tests and purity tests. This has to be done e.g. by different appropriate chromatographic methods. If deemed necessary by the results of the analysis of the starting material, tests on microbiological quality, residues of pesticides, fumigation agents, radioactivity, solvents and toxic metals have to be carried out. Quantitative determination (assay) of ***markers or of substances with known therapeutic activity*** is required. The content must be indicated with the lowest possible tolerance. The test methods must be described in detail.

If preparations from ***herbal*** drugs with constituents with known therapeutic activity are standardised (i.e. adjusted to a certain level of constituents with known therapeutic activity) it must be stated how such standardisation is achieved. If another ***substance*** is used for this purpose, it is necessary to specify as a range the quantity that can be added.

2) *Control of excipients*

Excipients including those added during the manufacture of the herbal drug preparations should be described according to the "Note for Guidance on Excipients in the dossier for application for marketing authorisation of a medicinal product" (Eudra/Q/91/015).

D. CONTROL TESTS CARRIED OUT AT AN INTERMEDIATE STAGE OF THE MANUFACTURING PROCESS OF THE FINISHED PRODUCT

Details of all control tests with details of test procedures and limits applied at any intermediate stages of the manufacturing processes are required, especially if these tests cannot be done in the finished product.

E. CONTROL TESTS ON THE FINISHED PRODUCT -

This section should be in accordance with the "Note for Guidance on Specifications and control tests on the finished product" (Eudra/Q/91/020) and the analytical procedures should be validated according to the ICH guideline "Validation of analytical procedures: methodology" (CPMP/ICH/281/95).
The control tests on the finished product must be such as to allow the qualitative and quantitative determination of the composition of the active ***substances*** and a specification has to be given which may be done by using markers if constituents with known therapeutic activity are unknown. In the case of ***herbal*** drugs or ***herbal*** drug preparations with constituents of known therapeutic activity, these constituents must also be specified and quantitatively determined.

If a ***herbal*** medicinal product contains several ***herbal*** drugs or preparations of several ***herbal*** drugs and it is not possible to perform a quantitative determination of each active ***substance***, the determination may be carried out jointly for several active ***substances***. The need for this procedure must be justified.

The criteria given by the European Pharmacopoeia to ensure the microbiological quality have to be respected.

F. STABILITY TESTS

This section should be in accordance with the "Note for Guidance on Stability testing of new active substances and medicinal products" (Eudra/Q/92/021).
Since the ***herbal*** drug or ***herbal*** drug preparation in its entirety is regarded as the active ***substance***, a mere determination of the stability of the constituents with known therapeutic activity will not suffice. It must also be shown, as far as possible e.g. by means of appropriate fingerprint chromatograms, that other substances present in the ***herbal*** drug or in the ***herbal*** drug preparation are likewise stable and that their proportional content remains constant.

If a herbal ***medicinal product*** contains several ***herbal*** drugs or preparations of several ***herbal*** drugs and if it is not possible to determine the stability of each active ***substance***, the stability of the medicinal product should be determined by appropriate fingerprint chromatograms, appropriate overall methods of assay and physical and sensory tests or other appropriate tests. ***The appropriateness of the tests should be justified by the applicant.***

In the case of herbal drug preparations containing constituents with known therapeutic activity, the limit should be ± 5% unless justified. In case of constituents without known therapeutic activity, a limit of ±10% can be accepted if justified by the applicant.

ANNEX

GLOSSARY

Herbal ***medicinal products*** are medicinal products containing as active ***substances*** exclusively plant material and/or ***herbal*** drug preparations.

Herbal drugs are plant material used for a medicinal purpose. A ***herbal*** drug or a preparation thereof is regarded as one active ***substance*** in its entirety whether or not the constituents with therapeutic activity are known.

Herbal drug preparations are comminuted or powdered ***herbal*** drugs, extracts, tinctures, fatty or essential oils, expressed juices, ***processed resins or gums***, etc...prepared from ***herbal*** drugs, and preparations whose production involves a fractionation, purification or concentration process. However, chemically defined isolated constituents or their mixture are not ***herbal*** drug preparations. Other ***constituents*** such as solvents, diluents, preservatives may form part of ***herbal*** drug preparations. These substances must be ***declared***.

Constituents with known therapeutic activity are chemically defined substances or groups of substances which are ***generally accepted to contribute substantially*** to the therapeutic activity of a ***herbal*** drug or of a preparation.

Markers are chemically defined constituents of a ***herbal*** drug which are of interest for control purposes. Markers may serve to calculate the quantity of ***herbal*** drug or preparation in the finished product if that marker has been quantitatively determined in the ***herbal*** drug or preparation when the starting materials were tested.

Annex II

AD HOC WORKING GROUP ON HERBAL MEDICINAL PRODUCTS MEETING

9-10 JUNE 1997

EMEA, 7 Westferry Circus, Canary Wharf, London E14 4HB

TRANSLATION OF THE TERM:

'HERBAL MEDICINAL PRODUCT'

Language	Translation
Danish	**Naturlægemidler** (term used today) **Plantelægemidler** (herbal medicinal products)
Dutch	**Kruidengeneesmiddel** (NL) **Plantaardige medicinale producten** (BE)
Finish	**Rohdosvalmiste/Naturmedel** (term used today) **Käsvirohdoslääke/Växtbaserade Läkemedel** (herbal medicinal products)
French	**Médicament à base de plantes**
German	**Pflanzliche Arzneimittel**
Greek	**Φυτικά φαρμακευτικά προϊόντα**
Italian	**Fitoterapici** or **Medicinali a base di piante**
Portuguese	**Produto medicinal a base de plantas** or **Medicamento a base de plantas**
Spanish	**Medicamento a base de plantas**
Swedish	**Naturläkemedel** (term used today) **Växtbaserade Läkemedel** (plant based medicinal products)

PART I C 1 EXPERT REPORT ON THE CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION

A. INTRODUCTION

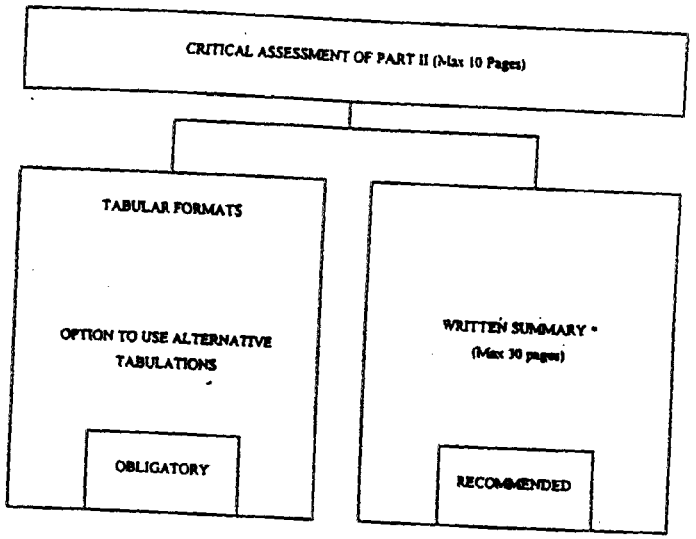
The pharmaceutical information should be presented in the following sequence :

- **Product profile**

- **Expert report**
  1. **Composition**
  2. **Method of preparation**
  3. **Control of starting materials**
     - a. **Active substance(s)**
     - b. **Excipients**
     - c. **Packaging material (immediate packaging)**
  4. **Control tests on intermediate products**
  5. **Control tests on the finished product**
  6. **Stability**
     - a. **Stability tests on active substance(s)**
     - b. **Stability tests on the finished product**
  7. **Other information**
  8. **Conclusions**
  9. **Reference list**
  10. **Information on the pharmaceutical expert**

- **Appendices to the expert report**
  1. **Tabular formats**
  2. **Written summary**

B. EXPERT REPORT ON THE CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION



* For the Quality part of the dossier, the tabular formats are considered to fulfil the function of written summary (except in case of biotechnology medicinal products and medicinal product which contain or consist of genetically modified organisms where a written summary would be helpful).

C. GENERAL ASPECTS

The pharmaceutical expert report should consist of a critical assessment of the methodology, results and conclusions.

For radiopharmaceuticals, appropriate discussions should be included on radiochemical, radiopharmaceutical and biodistribution studies aspects of the application.

Report formats which may be used by the pharmaceutical expert for compiling the factual tabular formats are given.

For the quality part of the dossier, the tabular formats are considered to fulfil the function of written summary (except in case of biotechnology medicinal products and medicinal product which contain or consist of genetically modified organisms where a written summary of not more than 30 pages would be helpful).

Use of these tabular formats facilitates a clear and well-ordered tabular presentation of the data. The format can however be adapted as necessary for an individual marketing authorization application by expanding or contracting sections, adding sections, and omitting sections where not relevant. Alternative tabular presentations may be used, however, the heading of such tables must be of the same structure i.e.

Name of Company: Name of Finished Medicinal Product: Name of Active substance(s):	Tabular format Referring to Part of the Dossier	(For National Authority use only)

Page references should be made to the appropriate volume and page of the Part II documentation or other relevant Parts of the full dossier. The "comments" space is intended for use by the assessor in the competent authority of the Member State concerned, and should therefore be left blank by the applicant.

"Drug Master Files" (confidential information)[24]

It is the responsibility of the applicant for a marketing authorization for a medicinal product to ensure that complete information is supplied to the authorities. The applicant must therefore consult and work together with the person submitting a separate Master File to ensure that all relevant information required is supplied as part of the Chemical, Pharmaceutical and biological Documentation (Part II) and in the Pharmaceutical Expert Report (Part IC).

Confidential data on the manufacture of the active substance(s) may be submitted in separate confidential documentation. However where is supplied separately, an Expert Report must be provided on any aspects not covered in the application for the marketing authorization for the product.

[24] see the annex, reference 47

D. CRITICAL ASSESSMENT

It is assumed, since the pharmaceutical expert has written and signed his Expert Report, that he is fully convinced that the product as developed is of the appropriate quality and that the proposed control tests and limits are those appropriate to ensure that the routinely manufactured batches continue to meet this quality requirement. The pharmaceutical expert should therefore not state this as his conclusion but instead critically review and discuss the elements of the dossier and tabular format which led him to this view. Some elements which might be included here:

1. Composition of the product[34]:

A discussion of the differences between the clinical trial formula(e) and the finally chosen composition and the significance of such differences (particularly in relation to product bioavailability).

2. Development pharmaceutics[35]:

A discussion of the choice of dosage form in relation to the intended indications. In relation to products where the bioavailability is critical, the data on bioavailability and the proposed routine control tests to ensure batch to batch consistency of bioavailability should be discussed (with a justification for the in vitro test limits). Where the in vivo absorption of the active substance(s) in man is low, the expert should discuss the evidence and conclude whether this relates to the intrinsic properties of the drug or is related to the particular dosage form.

The choice and concentration of additives (preservatives, antioxidants and others) should be discussed and shown to be optimised for their intended purpose in the product. In particular the results of preservative efficacy testing in relation to product storage, reconstitution, dilution and use should be discussed.

For special requirements for active substance(s) with one or more chiral centres see the following statement on Stereoisomerism. This gives the requirements both for data to be included in the Chemical and Pharmaceutical documentation (Part II), the Toxicological and Pharmacological documentation (Part III) and the Human Pharmacology and Clinical documentation (Part IV), and for discussion in the three Expert Reports on this documentation (Part IC).

In case of radiopharmaceuticals, particular attention should be paid to aspects affecting radiochemical properties of the product and biodistribution. Any radiolabelling procedure should be adequately discussed.

3. Stereoisomerism[37]:

When a new active substance(s) contains one or more chiral centres, it must be specified whether specific stereoisomers or a mixture of stereoisomers have been used in the animal and human studies, and information given as to the form of the active substance(s) to be used in the final product intended for marketing. Details should be provided on the chemical separation of different chiral forms used in the various tests reported in the application for marketing authorization.

Possible problems relating to stereoisomerism, which should be discussed in the appropriate expert report and cross referenced, should include:

- the batch to batch consistency of the ratio of stereoisomers in the various batches used
- the toxicological issues
- the pharmacological aspects (including evidence on which stereoisomers have the desired pharmacological properties)
- pharmacokinetics (including information on the relative metabolism of the stereoisomers)
- extrapolation of the preclinical data (paying particular attention to possible problems relating to species differences in handling of the stereoisomers)
- the significant clinical issues.

Where a mixture of stereoisomers has previously been marketed, and it is now proposed to market a product containing only one isomer, full data on this isomer should be provided.

[33] see the annex, reference 44
[34] see the annex, reference 46
[37] see the annex, reference 122

4. Method of preparation[38]:

A discussion as to how the particular manufacturing method and in-process control tests will consistently guarantee batches of product of the desired quality and that all individual dosage units within the batch are also acceptable.

5. Process validation[39]:

A discussion as to how the data gives the required assurance of suitable product quality (e.g. that a non-standard sterilization condition provides an acceptable level of assurance of product sterility).

6. Control of pharmacopoeial active substance(s)[40] :

A discussion as to the impurities in the starting material (particularly if it has been prepared by a method liable to leave impurities not mentioned in the pharmacopoeial monograph). Also in relation to possible impurities which might not be controlled by the pharmacopoeial monograph a cross-reference to the discussion of the possible toxicity of these impurities in the Toxicological Expert Report, levels found in typical batches, and the proposed test limits.

7. Control of non-pharmacopoeial active substance(s):

A discussion on the suitability of the manufacturing method and its controls to routinely and consistently produce material of suitable quality; an interpretation of the evidence of structure, isomerism and comment on the physico-chemical characteristics in relation to the specification (e.g. need for a particle size test in relation to a sparingly soluble active substance).

The expert should carefully review data on actual and potential impurities arising from the synthesis and together with the data from the analytical validation studies, show how the overall limits on individual and total impurities are set. The expert should also discuss the comparative analysis of the impurity levels in batches of the drug substances used in the toxicology studies, clinical trials and in typical batches as to be used in the marketed product to see whether the impurity levels have changed, and how the specified impurity limits relate to the levels found.

For active substance(s) (both pharmacopoeial and non-pharmacopoeial), the relevant impurities present in the active substance(s) from the specified manufacturing source must be known to the applicant for a product marketing authorization, and the toxico-pharmacological Expert Report in the application should, where necessary, consider the relevant impurities present in the active substance(s) and give a critical evaluation of what is known of their potential pharmacological and toxicological effects. The expert will need to consider the proposed impurity limits in relation to the toxicology of the impurity and the active substance(s) itself, the route of administration, daily dose, target population (e.g. children or the elderly), the duration of therapy and the proposed indications for the medicinal product.

For vegetable active substance, the test for potential contaminants (micro-organisms, pesticides, fumigants, radioactivity, toxic metals etc.) should be summarized. In the case of vegetable active substances the possibility of accumulation of pesticides or elimination of micro-organisms in comparison with the vegetable active substance and potential residues of fumigants should be discussed; levels found in typical batches; proposed test limits.

8. Excipients[41] :

A discussion of the suitability of the specification proposed. For new excipient(s) full data is needed and there should be a cross-reference to the data in the Toxicology Expert Report.

[38] see the annex, reference 58
[39] see the annex, reference 46
[40] see the annex, reference 45,53
[41] see the annex, reference 48

9. Packaging material (immediate packaging)[32]:

A discussion of the results of the studies on suitability of the packaging material in relation to proposed storage conditions and use of the product (e.g. moisture protection). Also a discussion of the specification and batch results.

10. Control tests on intermediate products[33]:

Where some tests on the finished product are not proposed to be carried out routinely because intermediate products are controlled, this should be discussed and justified.

11. Control tests on the finished product:

A discussion of the suitability of the proposed specification and control methods. The tests and limits (particularly for the quantitative determination of active substance(s) and purity tests) should be justified in relation to the results of the analytical validation studies, the batch analyses, and any information on production variability (incl. results of process validation studies). The results of production batch analyses should be compared to demonstrate reproducibility of the manufacturing process for the product. If necessary this may need to be provided on an ongoing basis.

12. Stability of the active substance(s)[34]:

A discussion of the conclusions as to the variability of batches of drug substance in stability, the most appropriate storage conditions, and the duration of storage before retesting to check compliance with specification. The expert should also discuss the significance of the degradation products and cross-refer to the information on their toxicity in the toxico-pharmacological Expert Report.

13. Stability of the finished product:

A discussion of the results of the stability trials and analysis of the data (including information on the active substance(s) content and content of significant degradation products, with comment on any discrepancies between these data), and a discussion of the variability between batches of the dosage form in the final packaging. The method of calculation or estimation of the shelf-life should be explained together with a justification for the recommended storage conditions. The basis for the recommendations on storage during marketing and use should be given.

For radiopharmaceuticals, any factors which might limit the number of doses of radiolabelled material that may be removed from a vial without affecting its safety or clinical efficacy should be discussed.

14. Other information:

A discussion of the results of other tests particularly on the validation of metabolic and pharmacokinetic assay methods with regard to the suitability of these methods.

15. Reference list:

A list of references used, in addition to those contained in the dossier, should be given and stated in accordance with internationally accepted standards of the 1979 Vancouver Declaration[35] on "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" or the system used in "Chemical Abstracts".

16 Information on the qualifications and experience of the pharmaceutical expert:

The qualifications and experience of the expert should be briefly summarized. Although only one expert may assume responsibility for the report, other experts may contribute to its preparation, according to their expertise.

32 see the annex, reference 49
33 see the annex, reference 50,51,53
34 see the annex, reference 54
35 see the annex, reference 42

E. TABULAR FORMATS

E. TABULAR FORMATS FOR RADIOPHARMACEUTICAL PRODUCTS

PART III TOXICO-PHARMACOLOGICAL DOCUMENTATION

Directive 91/507/EEC[37] states that Member States shall ensure that the safety tests are carried out in conformity with the provisions relating to good laboratory practice (GLP) laid down by Directives 87/18/EEC[38] and 88/320/EEC[39] , and the guideline III/3824/92 .In this context, the following are considered as safety tests which must conform to GLP:

If use is made of a list of published references pursuant to point 5 of the second paragraph of Article 4 of Council Directive 65/65/EEC[40] as modified by Directive 87/21/EEC[41] , the expert must show that this is justified.

The following must be provided in respect of each test:

1. Animals used (species, strain, sex, age, weight, etc.)
2. Product used (number of the batch, quality, etc.)
3. Experimental conditions including diet and husbandry
4. Results

PART III A: TOXICITY

1. single dose toxicity studies[42]
2. repeated dose toxicity studies[43]

PART III B: REPRODUCTIVE FUNCTION (FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE)[44]

PART III C: EMBRYO-FOETAL AND PERINATAL TOXICITY

PART III D: MUTAGENIC POTENTIAL[45]

1. In vitro
2. In vivo

[37] see the annex, reference 27
[38] see the annex, reference 37
[39] see the annex, reference 38
[40] see the annex, reference 7, complete article page 8 of the annex
[41] see the annex, reference 24
[42] see the annex, reference 77
[43] see the annex, reference 78,79
[44] see the annex, reference 80,81,82
[45] see the annex, reference 83,84

PART III E: CARCINOGENIC POTENTIAL[66]

PART III F: PHARMACODYNAMICS

1. Pharmacodynamics effects relating to the proposed indications
2. General pharmacodynamics
3. Drug interactions

PART III G[67]**: PHARMACOKINETICS**

1. Pharmacokinetics after a single dose
2. Pharmacokinetics after repeated administration
3. Distribution in normal and pregnant animals (e.g. autoradiography)
4. Biotransformation

PART III H[68]**: LOCAL TOLERANCE (WHERE APPROPRIATE)**

PART III Q [69]**: OTHER INFORMATION**

This section is intended for possible information not covered by any of the previous sections.

PART III R: ENVIRONMENTAL RISK ASSESSMENT / ECOTOXICITY

NOTE: Environmental risk for non GMO containing medicinal products
From 1.1.95, applications for marketing authorisations should include in Part IIIR an indication of any potential risks presented by the medicinal product for the environment. *This requirement is particularly applicable to new active substances and live vaccines.*
Applications for new active substances may include in the documentation provided, an indication of relevant environmental hazards, ranking reference to standard physicochemical tests and any appropriate testing they have conducted on biodegradability, including ecotox testing in sensitive species etc.
Applications for live vaccines should consider issues such as shedding, survival and capacity to disseminate.
The pre-clinical Expert Report should include an evaluation of possible risks to the environment from the point of view of use and/or disposal and make proposals for labelling provisions which would reduce this risk. (see annex p.xix)

[66] see the annex, reference 85,86,87
[67] see the annex, reference 88,89
[68] see the annex, reference 90
[69] see the annex, reference 91

PART I C 2 EXPERT REPORT ON THE TOXICO-PHARMACOLOGICAL (PRE-CLINICAL) DOCUMENTATION

A. INTRODUCTION

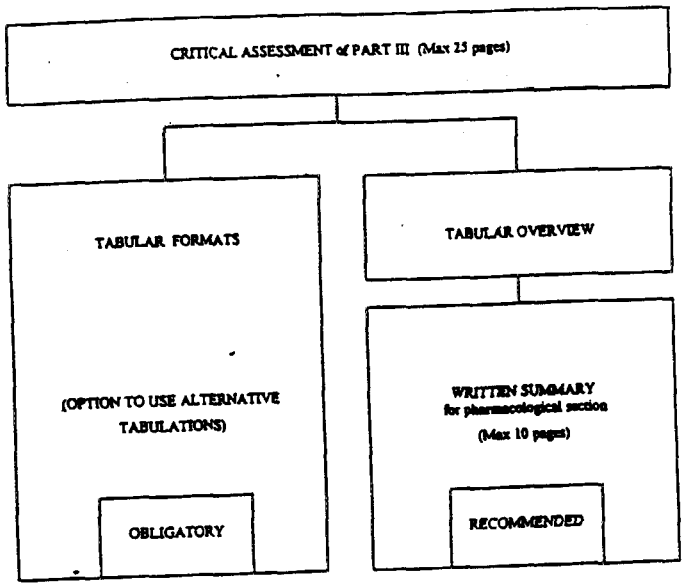
The toxico-pharmacological (pre-clinical) information should be presented in the following sequence:

- Product profile

- Expert report
  1. Pharmacodynamics
  2. Pharmacokinetics
  3. Toxicity
  4. Conclusions
  5. Reference list
  6. Information on the pre-clinical expert

- Appendices to the expert report
  1. Tabular formats
  2. Written summaries

Detailed instructions on the preparation of the above mentioned documents are given below.



Part III - Pharmacology/Toxicology

Toxicological studies should be summarised so as to enable a general check and overview.
Each study should be entered in the Table as a separate line

Type of Study	Species	Route	Duration of Treat-ment	Doses (as mg/kg bwt/day) administered as substance/ salt (Specify)	Conform to GLP yes/no
Pharmacodynamics (IIIF) Pharmacokinetics/ Toxicokinetics (IIIG) Toxicity; - single dose - repeated dose (IIIA) Reproduction function (IIIB) Embryo-foetal & perinatal toxicity (IIIC) Mutagenic potential (IIID) Carcinogenic potential (IIIE) Local tolerance (IIIH) Other (IIIQ)					
Environmental Risk Assessment/ Ecotoxicity (IIIR)	Include a short description of the studies in the dossier				

C. GENERAL ASPECTS

The expert should present a critical evaluation of the experimental studies and an interpretation of the pharmacodynamic, pharmacokinetic and toxicological results of an active substance(s).

The expert should comment on the GLP status of the studies submitted.

Relevant scientific literature should be taken into account for the evaluation. If detailed references to published scientific literature are to be used, all the requirements set out below for study report(s) have to be met. Information on the quality of batches of drug substance used in the pre-clinical studies must be provided.

Any association between findings and the quality of the medicinal product, the results of the clinical trials and effects seen with known medicinal products, should be indicated.

The expert should, when necessary, present a critical evaluation of the impurities present in the active substance(s) and give information on what is known of their potential pharmacological and toxicological effects. The Expert Report should form part of the justification for proposed impurity limits in the active substance(s) and be appropriately cross-referenced in the Pharmaceutical Expert Report. The expert will need to consider the proposed impurity limits in relation to the toxicology of the impurity and the active substance(s) itself, the route of administration, daily dose, target population (e.g. children or the elderly), the duration of therapy and the proposed indications for the medicinal product. The implications of any differences of the chirality, chemical form and impurity profile between the compound used in the pre-clinical studies and for the form to be marketed should be discussed. Attention should be drawn to the possible deficiencies in the design and conduct of the studies and their documentation.

For radiopharmaceuticals, the expert should address any safety issues of radionuclide or radiochemical purity which relates to preclinical studies.

Justification for the omission of particular studies (e.g. mutagenicity/carcinogenicity studies, reproduction toxicity studies) should be given. Requirements for additional studies should be discussed.

The Expert Report must contain page references for each relevant Tabular format (and the written summaries where prepared) appendixed to the Expert Report. The Tabular formats (and the written summaries where prepared) in turn indicate the page references in Part III of the documentation.

For the toxicological section of the dossier, the tabular formats are considered to fulfil the function of a written summary.

For the pharmacological section of Part III of the dossier, a written summary could be useful. Normally, the written summary would not be more than 10 pages.

It is considered helpful to have an overview table which would precede a written summary.

Tabular formats which may be used for such factual study reports and over-view tables are given. Use of these formats facilitates a clear and well ordered tabular presentation of the data. The format can however be adapted as necessary for an individual marketing authorisation application by expanding or contracting sections, adding sections and omitting sections where not relevant. Alternative tabular presentations may be used, however, the heading of such tables must be of the same structure, i.e.

Name of Company:	Tabular format Referring to Part of the Dossier	(For National Authority use only)
Name of Finished Medicinal Product:		
Name of Active substance(s):		

D. CRITICAL ASSESSMENT

The expert should clearly define the beneficial and advantageous aspects of the new medicinal product as demonstrated by toxico-pharmacological (pre-clinical) studies. Any necessary conditions for its use should be specified including, for radiopharmaceuticals, any precautions necessary because of the radioactive nature of the product. Taking the pharmacodynamic, pharmacokinetic and toxicological results and the group specific properties of known medicinal products into account, recommendations should be made for the "Summary of Product Characteristics" (Part IB) and suggestions made as to how these recommendations can be implemented.

The expert should especially and in each case refer to the following:

- the effects of an active substance(s) observed in toxico-pharmacological (pre-clinical) studies in relation to those expected or observed in man,
- the consequences of the use of the medicinal product before and during pregnancy and during lactation,
- mutagenic effects,[16]
- the tumorigenic risk to man - if epidemiological data are available they should be taken into account,[17]
- possible irreversible toxic effects,
- the consequence of the product being a radiopharmaceutical.

1. Pharmacodynamics

Studies conducted to establish the pharmacodynamic effects and the mode of action should be evaluated in the following order:

- studies demonstrating desired therapeutic effects (special pharmacodynamics),
- studies demonstrating effects in addition to desired effects (general pharmacodynamics),
- studies to detect drug interactions.

2. Pharmacokinetics[18]

For radiopharmaceuticals, distinction should be drawn between the physical and biological half-lives, with an indication of which is more relevant to the dosimetry.

The data of absorption, distribution, biotransformation, excretion, protein binding and the occurrence of metabolites necessary for extrapolation to humans should be assessed considering the relevance of:

- the methods used (including sensitivity and validation of assays),
- the pharmacokinetic models,
- the pharmacokinetic parameters.

3. Toxicity[19]

For radiopharmaceuticals, the chemical toxicology and effects or radiation dose should be considered both separately and interdependently.

[16] see the annex, reference 83
[17] see the annex, reference 84,86,87
[18] see the annex, reference 79,88,89
[19] see the annex, reference 77,78,81,82

The appearance and duration of the toxic effects, the dose-dependency and the reversibility or irreversibility, and all species- or sex-related differences should be reviewed and important features discussed particularly with regard to:

- toxic symptoms,
- causes of death,
- clinico-chemical and haematological findings,
- interactions between excipients of the medicinal product (fixed combinations),
- fertility, embryotoxicity (particularly teratogenicity), and peri-/postnatal toxicity,
- mutagenic activity, for which the chemical structure of the compound, its mode of action and the relationship to known mutagens should be taken into account,
- oncogenic/carcinogenic potential in the context of the chemical structure, the relationship of known carcinogens, the mutagenic potential, and the pharmacokinetics,
- local toxicity,
- immunotoxicity,
- studies conducted to clarify special problems.

The evaluation of toxicological studies should be arranged in a logical order so that all relevant data elucidating a certain effect/phenomenon are brought together. Extrapolation of the data from one animal species to another and from animals to man should be discussed considering the relevance of:

- animal species used,
- number(s) of animals used,
- route(s) of administration employed,[40]
- dosage(s) used (for radiopharmaceuticals, this should include the administered dose in Becquerels and the specific activity of the product).
- duration of treatment or of the study.

If alternatives to whole-animal experiments are employed, their validity should be proved.

If the dose-response relationship changes, e.g. with increasing doses or during repeated/long-term dosing, an explanation should be proposed.

4. Conclusions

Conclusions should be drawn as outlined above.

For radiopharmaceuticals, any use of animal studies to determine radiation exposure for extrapolation to man should be performed and evaluated as a separate exercise.

5. Reference list

A list of references used, in addition to those contained in the dossier, should be given and stated in accordance with internationally accepted standards of the 1979 Vancouver Declaration[41] on "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" or the system used in "Chemical Abstracts".

6. Information on the toxico-pharmacological (pre-clinical) experts

The qualifications and experience of the toxico-pharmacological (pre-clinical) expert should be briefly summarized. Although only one expert may assume responsibility for the Expert Report, other experts may contribute to its preparation, according to their expertise.

[40] see the annex, reference 90

[41] see the annex, reference 42

E. TABULAR FORMATS

(1) Name of the company

(2) Trade name or intended trade name; in case of change give the previous name in brackets

(3) Proposed or recommended I.N.N. or chemical name

(4) For use by registrating authority; all subsequent addenda to this application (cf (6)) must bear this N°.

(5) Date of submission.

(6) Insert « 0 » or « - » in case of application, otherwise the serial number of the addendum

PART IV CLINICAL DOCUMENTATION

The Commission Directive 91/507/EEC[70] requiring all phases of clinical investigation to be designed, implemented and reported in accordance with good clinical practice came into force the 1st January 1992. The CPMP guideline on Good Clinical Practice recommended that all studies commencing after the 1st July 1991 should be undertaken in accordance with GCP. The Clinical Expert as defined in this Notice to Applicants is therefore asked to ensure that all studies commencing after this date have been undertaken in accordance with GCP and to clearly state it in the introduction to the Clinical Expert Report in a additional section headed Compliance with GCP. The expert should comment on any studies not complying with GCP and give a clear statement as to why the guidelines have not been applied. In this section the expert should also comment on studies commencing before the 1st July 1991, noting whether these where undertaken according to GCP. The Expert should comment on any deficiencies in this studies.

If use is made of published references pursuant to point 8 of the second paragraph of Article 4 of Council Directive 65/65/EEC[71] as modified by Directive 87/21/EEC[72] the report must show that this is justified.

PART IV A: CLINICAL PHARMACOLOGY

1. PHARMACODYNAMICS

The following must be submitted for each of the studies:

1.1 A summary

1.2 The detailed research design (protocol)

1.3 The results including:

- The characteristics of the population studied
- The results in terms of efficacy
- The clinical and biological results relevant to safety (tables showing these results are useful).The safety and efficacy of radiopharmaceuticals (diagnostic or therapeutic) in comparison with alternative procedures or techniques should be discussed.
- The analysis of results

1.4 Conclusions

1.5 A bibliography if necessary

Tables recapitulating all the studies in a logical order may be necessary

2. PHARMACOKINETICS

The results of the investigations should be presented according to the populations tested

- Healthy volunteers
- Patients

[70] see the annex, reference 27

[71] see the annex, reference 7,complete article page 8 of the annex

[72] see the annex, reference 24

The following must be submitted for each of the studies:

2.1 A summary

2.2 The detailed research design (protocol)

2.3 The results

2.4 The conclusions

2.5 A bibliography if necessary

Tables recapitulating all the studies in a logical order may be necessary.

PART IV B: CLINICAL EXPERIENCE

The documentation must contain a description of all the tests carried out, including unfinished studies.

1. CLINICAL TRIALS

The following must be provided for each of the trials:

1.1 A summary

1.2 A detailed description of the main items in the research design (protocol) and the analytical methods (or the protocol itself)

1.3 The final (or intermediate) results including:

- characteristics of the population studied
- the results in terms of efficacy
  - clinical and biological monitoring
  - main criterion of efficacy
  - other criteria
- clinical and biological results concerning safety
- statistical evaluation of the results
- tabulated patient data, including clinical and laboratory monitoring results, presented in such a way as to enable a relation to individual patients.

1.4 Possible discussion

1.5 Conclusion

1.6 The following must be given in an annex:

- the research design (if not included in 1.2)
- the observation sheets or notes
- all individual data (if not included in 1.3)
- any bibliography

2. POST-MARKETING EXPERIENCE (IF AVAILABLE)

2.1 Adverse reaction and monitoring event and reports

2.2 Number of patients exposed

3. PUBLISHED AND UNPUBLISHED EXPERIENCE (OTHER THAN 1.)

3.1 Brief information on on-going trials and uncompleted trials

including the reason why the trials were not completed with full details on any safety issues raised in these studies.

3.2 Any other information

PART IV C: OTHER INFORMATION

160

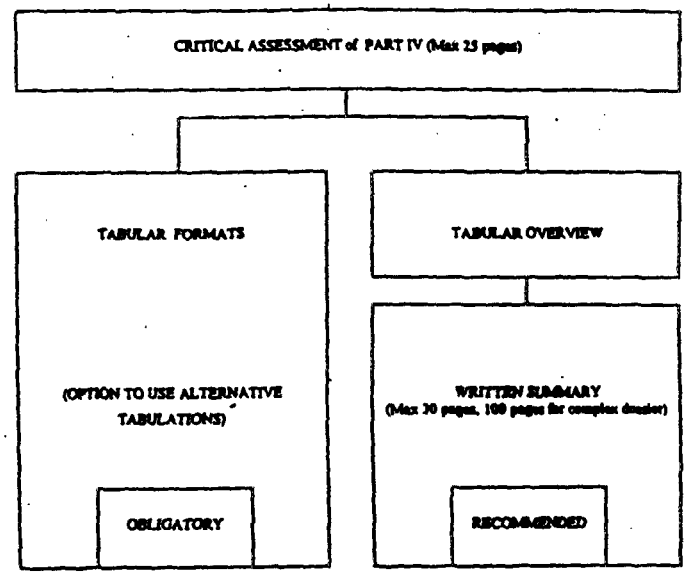
A. INTRODUCTION

The clinical information should be presented in the following sequence

- Product profile

- Expert report
  1. Problem statement
  2. Clinical pharmacology
  3. Clinical trials
     - efficacy
     - safety
  4. Post marketing experience
  5. Other information
  6. Conclusions
  7. Reference list
  8. Information on the clinical expert

- Appendice to the expert report
  1. Tabular formats
  2. Written summaries



Tabular Overview Part IV - Clinical Studies

Study Ref. No.	Type of Study	Doses	Duration of Treatment	No. of Patients	Study Design
	Dose evaluation				
	Efficacy studies: Placebo-controlled Efficacy Studies Active Control Efficacy Studies Non-controlled (open studies) Miscellaneous or aborted studies				

Total number of patients on product evaluable for efficacy =
Total number of patients on product evaluable for safety =

DURATION OF OBSERVATIONS***

	> 65 years*	< 12 years*	Special at risk groups**
Number of subjects evaluable for efficacy			
Number of subjects evaluable for safety			

Weeks	4	8	12	16	26	40	52
Numbers for Safety							
Numbers for Efficacy							

* If applicable
** If applicable and defined (e.g. renal failure, hepatic failure, diabetic patients)
*** The format of this table may be modified to accommodate different data sets

ANÁLISIS DE LAS TENDENCIAS DE I+D Y MERCADO DE LOS FITOMEDICAMENTOS

Ing. Bárbara Páez
Lic. Gabino Garrido*
Lic. Niurka Cruz

Consultoría BIOMUNDI. CITMA.Cuba
***C.Q.Farmacéutica.MINSAP.Cuba**

DEFINICIÓN DE FITOMEDICAMENTOS SEGÚN LA OMS

"Productos medicinales acabados y etiqueteados cuyos ingredientes activos están formados por partes aéreas o subterráneas de plantas u otro material vegetal, o combinaciones de éste, en estado bruto o en forma de preparaciones vegetales..."

PRINCIPALES LÍNEAS DE I+D EN LA RAMA FITOMÉDICA

- **Búsqueda de la explicación científica del conocimiento ancestral sobre el uso de las plantas medicinales**
- **Investigación etnobotánica de las especies por región**
- **Estudio de sus principios activos.**
- **Búsqueda de nuevas formulaciones de extractos vegetales**

OBJETIVOS A LOGRAR EN LAS INVESTIGACIONES, SEGÚN LA OMS

- **Obtener inventarios y clasificación terapéutica de las plantas.**
- **Hallar criterios científicos que aseguren la calidad de las preparaciones y su eficacia para el tratamiento de algunas enfermedades.**
- **Desarrollar normas internacionales que regulen el desarrollo de los**

CATEGORÍAS TERAPÉUTICAS MÁS INVESTIGADAS.

- Antiinfecciosos
- Cáncer
- S.N.C.
- Antihipertensivos
- Respiratorios
- Antioxidantes
- S. Inmunológico
- Hipoglicemiantes
- Analgésicos
- Dermatológicos

ALGUNAS DE LAS ESPECIES DE MAYOR UTILIZACIÓN EN LA ACTUALIDAD

- **Hypericum perforatum**
- **Salvia miltiorhiza**
- **Aloe spp.**
- **Zingiberis rhizoma**
- **Allium sativum**
- **Centella asiatica**
- **Hemidemus indicus**
- **Cortex cinnamoni**
- **Artemisia annua**
- **Momordica charantia**

ORIENTACIÓN DE LAS INVESTIGACIONES.
Bd Medline. 96-97

- **Farmacología**
- **Uso terapéutico**
- **Química**
- **Metabolismo**
- **Aislamiento y purificación**
- **Efectos adversos**
- **Administración y dosis**
- **Farmacocinética**
- **Contraindicaciones**

PRODUCTOS EN DESARROLLO Bd Pharmaproject.1996

Beta arteether	**Malaria**	**Artecef**
Zemaphyte	**Eczemas**	**Phytopharm**
MAP-30	**VIH,Cáncer**	**NYUMedic.**
PS 34WO	**Inmunoest.**	**Madaus**
Celastrol	**Cáncer**	**Schering P.**
PN-355	**VIH, Cáncer**	**Paracelsian**
Perthon	**VIH**	**Advanced P.**
PP-5217	**Inmunoest**	**Origene Tech.**
Androgapholide	**Cáncer**	**Paracelsian**

PAÍSES QUE MÁS INVESTIGAN Y PUBLICAN

Japón
China
Estados Unidos
India
Reino Unido
Brasil
Alemania
España
Tailandia
Corea del Sur

FACTORES ASOCIADOS CON EL DESARROLLO CIENTÍFICO DE LA FITOMEDICINA EN AMERICA LATINA

- **Necesidad de diversificar la tecnología moderna**
- **Desarrollo de la atención primaria al hombre**
- **Surgimiento de una actitud más cuidadosa al medio ambiente**
- **Revalorización del acervo cultural autóctono**

PAÍSES DE A. LATINA CON MAYOR DESARROLLO EN LAS INVESTIGACIONES FITOMÉDICAS

Argentina
Chile
México
Costa Rica
Cuba
Guatemala
Brasil

CAUSAS QUE MOTIVARON EL RESURGIMIENTO DEL MERCADO DE LOS FITOMEDICAMENTOS

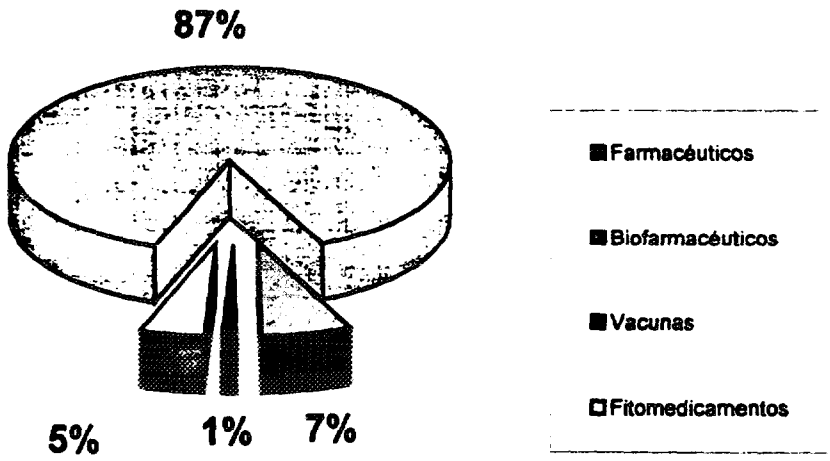
- **Permanente vigencia de su uso en determinadas culturas y medios sociales.**

- **El desarrollo del mercado mundial de estos medicamentos, como consecuencia de un mejor conocimiento de sus propiedades y la necesidad de la industria farmacéutica de desarrollar nuevos productos al menor costo posible.**

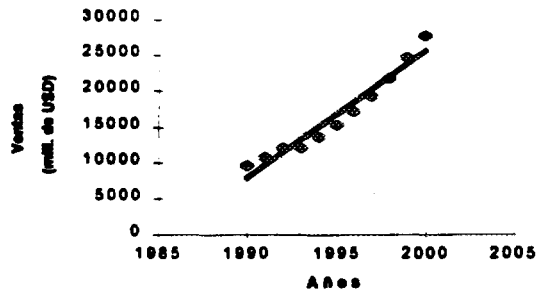
- **Una adecuación de las legislaciones sanitarias de muchos países,que han sabido rescatar este valor cultural**

- **El reclamo de la sociedad de medicamentos que generen menos efectos colaterales**

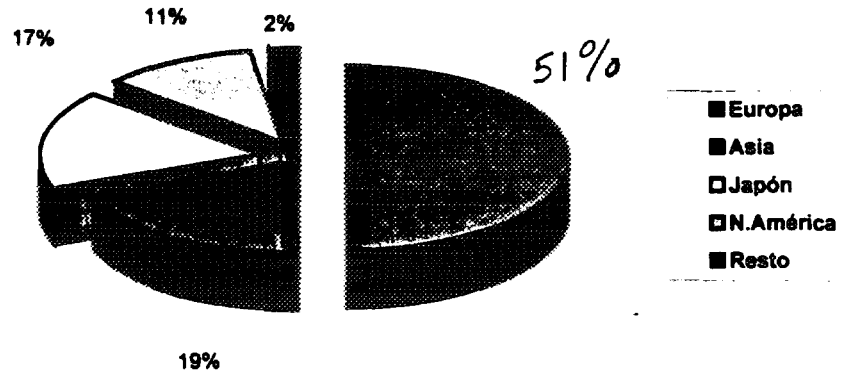
DISTRIBUCIÓN DEL MERCADO FARMACÉUTICO MUNDIAL.1996



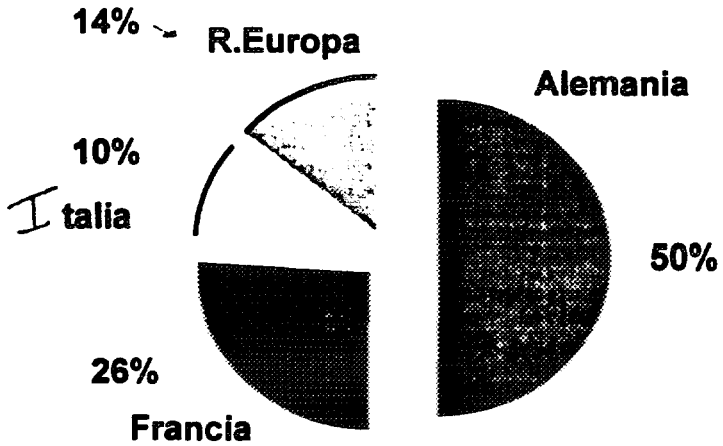
TENDENCIA DE LAS VENTAS DE LOS FITOMEDICAMENTOS



DISTRIBUCIÓN DE LAS VENTAS MUNDIALES DE LOS FITOMEDICAMENTOS.1996



DISTRIBUCIÓN DEL MERCADO DE LOS FITOMEDICAMENTOS EN EUROPA. 1996.



CATEGORÍAS TERAPÉUTICAS MÁS VENDIDAS EN EUROPA.1996

Cardiovasculares
Respiratorios
Tónicos
Digestivos
Antidepresivos
Sedantes
Hipnóticos

PRINCIPALES COMPAÑÍAS DEL MERCADO DE LOS FITOMEDICAMENTOS.1996

Bayer Ag.	**Alemania**
Madaus	**Alemania**
Bio-Ingemar	**Chile**
Shaman Pharmaceutical	**E.U.A.**
Pharmagenesis	**E.U.A.**
Nature Sunshine Product	**E.U.A.**
Indian Herbs Ltd.	**India**
Kanebo Ltd.	**Japón**
Ono Pharmaceutical	**Japón**
China Medicine Co.	**China**
XingYa Pharmaceutical	**China**
Sandoz LTD.	**Suiza**
Hileshong AB	**Suecia**

FACTORES QUE INFLUYEN EN EL DESARROLLO DE LOS FITOMEDICAMENTOS

Estado de los recursos naturales.
Patentes
Ambiente regulatorio
Disponibilidad de la información.

CONCLUSIONES

- **Las tendencias de I+D en la medicina verde están en la actualidad encaminadas a desarrollar amplias posibilidades futuras en la rama Fitomédica.**

- **Los Fitomedicamentos constituyen un segmento de la industria farmacéutica mundial con una tasa de crecimiento notable. Para el año 2000 se estiman ventas entre 23-25 mil millones de USD.**

La disponibilidad de información, patentes, ambiente regulatorio y estado de los recursos naturales, son los factores que influyen en el desarrollo futuro del mercado de los fitofármacos.

C. GENERAL ASPECTS

The Expert Report should contain a critical assessment of the methodology, results and conclusions of all studies.

In the body of the text, use of graphs and concise tables can facilitate understanding.

The Expert Report may be supplemented by written or tabulated summaries of data. Report formats which may be used for such factual study reports and over-view tables are given. Use of these formats facilitates a clear and well ordered tabular presentation of the data. The format can however be adapted as necessary for an individual marketing authorization application by expanding or contracting sections, adding sections and omitting sections where not relevant. Alternative tabular presentations may be used, however, the heading of such tables must be of the same structure, i.e.

Name of Company: Name of Finished Medicinal Product: Name of Active substance(s):	Tabular format Referring to Part of the Dossier	(For National Authority use only)

The format of the tables given as examples might not be suitable for human pharmacology studies. Applicants should therefore adapt the concept appropriately. It should also be noted that a tabular format for presentation of safety data has not been proposed. Applicants should provide appropriate tables that clearly present this data.

Precise volume and page references should be made to specific studies or other relevant information contained in the study report tables and in the Part IV documentation.

D. CRITICAL ASSESSMENT

The problem statement should be particularly centered upon clinical practice and should give all the useful information on the different treatments which could be envisaged in the pathology in question and the contribution which the medicinal product could represent, with recall of the therapeutic indications claimed, and the dosage.

CLINICAL PHARMACOLOGY (Part IV A)

PHARMACODYNAMICS

a) Data

All important data should be summarized and/or presented in tabular form. The documentation should particularly include the following:

- characteristics of the population studied;
- description and validation of the experimental methods;
- clinical and laboratory results as a function of dose and/or concentration; which are pertinent to the therapeutic efficacy and safety.

The use of graphs may also facilitate clear presentation of data.

b) Comments

The discussion, including the relevance of the experimental methods should cover:

- the pharmacodynamic action correlated to the therapeutic effect, including
- the dose-response relationship (intensity, duration),[48]
- the optimal dose and conditions of administration,
- the mode of action,
- the pharmacodynamic actions not correlated with the therapeutic effect.

The actions on different organs or physiological functions should be discussed; unwanted effects seen (including those observed in the course of the first administration to man in Phase I) should be evaluated as a function of dose as well as what might have been anticipated on the basis of the demonstrated pharmacodynamic properties.

PHARMACOKINETICS[49]

Pharmacokinetics of the active substance(s):

The report should provide the pharmacokinetic profile and parameters, dealing with the active substance(s) and as appropriate with active metabolite(s).

a) the results should be presented in relation to the populations studied:

- healthy volunteers,
- patients relevant to the therapeutic indications (particularly with the blood/plasma concentrations achieved at steady state during the study),
- patients at increased risk, for physiological reasons (children, elderly...) or for additional pathological reasons such as renal failure, liver insufficiency...

[48] see the annex, reference 98
[49] see the annex, reference 97

b) the report should include the pharmacokinetic characteristics:

- Absorption rate and extent, and if appropriate the influence of food
- Distribution including binding with plasma proteins and the distribution volumes
- Metabolism, including results concerning possible genetic polymorphism, the formation of active and inactive metabolites.
- Excretion of the unchanged substance and/or metabolites.

Parameters relevant to the rate and route of elimination should be assessed (elimination half-life, partial and total clearances).

c) comments should highlight:

- clinically significant features such as: the range of inter/intra-individual variations, non-linearity, deep compartment, diffusion into the fluids and target tissues for the indication (C.S.F., synovial fluid etc...), accumulation, role of metabolites in the clinical effect, liver enzyme induction etc....
- the implication of the kinetic data for the dosage regimen in normal conditions of use and in high risk patients,
- the possible interactions between the excipients of a fixed combination,
- differences between man and the animal species used in the preclinical documentation.

In vivo performance of pharmaceutical forms:

Bioavailability/bioequivalence[*]:

The pharmacokinetic results (C max, T max, A.U.C...) relevant to the comparison of formulations used in clinical development and particularly those proposed for marketing, should be assessed, and pertinent data presented in tables and/or graphs. Conclusions drawn should be based on appropriate statistical analyses, and interpretation should take into account results of dissolution rate studies.

Systemic absorption from pharmaceutical forms intended to have a non-systemic effect:

Studies and results with blood/plasma, urine or faeces levels should be summarized and processed as described above.

The clinical significance of systemic absorption, with respect to possible adverse effects, should be discussed.

Interactions:

If a pharmacodynamic and/or pharmacokinetic interaction exists between the substance and other medicinal products or substances like alcohol, caffeine, nicotine, likely to be taken simultaneously, or if such interactions are likely, they should be described and discussed; particularly from the point of view of clinical relevance, and the relationship to the statement concerning medicinal interactions in the summary of product characteristics.

Consideration should be given to results of the observations made in clinical pharmacological studies as well as clinical trials.

[*] see the annex, reference 109

The summary of results and the critical evaluation should give a clear picture of the therapeutic efficacy, the safety and other therapeutic characteristics.

Overall tabular presentation[*] of all of the studies:

A tabular presentation of all clinical trials and studies should be given. This should contain the principal characteristics of the trials, such as the title of the study and the country in which it took place, the design, the number of patients, the dose regimen and route of administration, the duration of treatment, the diagnosis and the reference medicinal product, if any, criteria and results for evaluation.

For a better understanding, it is recommended to successively present information relating to:

- controlled trials (divided between placebo and reference therapy),
- non-controlled trials.

Assessment of individual studies

The most important and significant studies should be summarized individually in tabular format.

When discussing these studies the expert should give special emphasis to the assessment of trials which give unequivocal evidence of the efficacy (phase II therapeutic studies) and provide a justification for the dosage regimens.

The compilation of the narrative and tabular information should facilitate clear understanding of each of the following aspects:

- the protocol (objective design, study population characteristics, type and duration of treatment, criteria for evaluation of efficacy and safety, statistical evaluation). Protocol deviation should be highlighted.
- data concerning

a) the patients:

- number(s) participating in the trial according to diagnosis,
- comparability of groups,
- number(s) of protocol deviance and drop-outs with reasons and treatments,
- number of observations available from efficacy and safety analysis.

b) therapeutic efficacy:

The results of each parameter of efficacy should be presented as a function of dose administered, with a statistical evaluation. The possibility of bias should be discussed and a judgement should be made on the clinical significance of the results.

and/or

c) safety:

Pertinent clinical and/or laboratory results should be summarized appropriately with statistical evaluation, where relevant. A clinical judgement should be made on the relationship to treatment, frequency and the seriousness of the observed adverse events.

d) quality of the trial:

Comments on quality control and on product formulation and on conformity with the principles of good clinical practices should be made.(Part IV)

[*] see the annex, reference 101

CONCLUSIONS SHOULD BE DRAWN ON THE BENEFIT/RISK RATIO FOR THE PRODUCT IN THE INDICATIONS UNDER THE CONDITIONS OF INVESTIGATION.

Global analysis of efficacy

a) Summary of available data should be presented as:

- the total number of patients with their characteristics,
- the number for each indication, age group, dosage regimen and duration of treatment.

b) Efficacy and dosage regimen(s)

The validity of the criteria for efficacy, methods of measurement, statistical methods, as well as the pertinence of the studies should be discussed.

The number of trials showing a positive and negative result should be indicated, accompanied with appropriate explanations. The number and percentage of drop-outs due to insufficient efficacy should be given.

The relationship between efficacy and dosage regimen should be justified and defined for each indication, in the different sub-groups of patients, with mention of the percentages of successes and failures.

For medicinal products intended for long term use, maintenance of long term efficacy and the establishment of long term dosage should especially be discussed.

If the treatment could be improved through plasma concentration monitoring, documentation for an optimal therapeutic plasma range should be included.

In so far as interactions or physico-chemical incompatibilities are considered as clinically significant, an examination of these problems should be included in the report.

c) Therapeutic value

The therapeutic efficacy of the tested medicinal product should be assessed by comparison with other reference therapies.

For fixed combinations, the therapeutic value should equally be considered by comparison to each of the individual components used separately. The doses and proportions of the components should be justified. A full account of the therapeutic advantages of such an association should be given.

Global analysis of safety

A full assessment should be made of adverse events. This should take into consideration the abnormal clinical and laboratory measurements from the trials.

For radiopharmaceuticals the evaluation of safety should include radiation dosimetry calculations.

Recommendations should be made on the conditions of use designed to reduce the impact of adverse events (e.g. dose adjustment, contra-indications, precautions for use etc...).

a) Review of patient population[4]

The total patient population studied must be defined. The number of patients for which there is adequate documentation to enable an assessment of safety should be stated. The overall figures should be analysed appropriately in sub-groups according to pertinent factors such as age, sex, race, diagnosis, dosage used etc. Patients with a particular risk factor should be high-lighted. The numbers of patients treated for

[4] see the annex, reference 99

specific durations of time, graded from short-term to, where relevant, long-term, should be indicated, i.e. number of patients treated for at least one year.

b) Assessment of adverse events

The pertinence of the different trials to the assessment of safety and the validity of methods of evaluation should be discussed.

Adverse events including abnormal laboratory values should be examined

- in terms of overall adverse experience: absolute number and frequency observed in relation to the total patient population and to the different sub-groups should be presented, highlighting number of deaths, serious effects and drop-outs (giving reasons)
- and as a function of
  - nature: effects of the same type should be grouped and numerically analysed by "body system". The summary should include poisoning reactions (overdose), the potential for dependence, rebound phenomena.
  - seriousness: one should distinguish between trivial adverse events and the more important ones which have lead to changes in the dosage regimens, stopping of the treatment and/or which were judged to be serious or potentially serious. All deaths should be reported and discussed whatever their cause.
  - causality: relationship of adverse events to treatment should be assessed, any deaths and other serious effects which are related to the drug should be appropriately discussed.

The circumstances of appearance of adverse reactions should be discussed as a function of treatment (dosage regimen, duration), concomitant therapy, characteristics of patients (age, sex, treated diseases, additional pathologies etc...).

When the substance is considered to be the cause of the adverse event, where possible, the relationship to the dosage used and the duration of treatment should be discussed.

c) Critical assessment

A critical assessment should be made on relative safety, taking into account adverse reactions recorded in relation to:

- diseases treated,
- product also studied, and more generally other therapeutic approaches,
- particular characteristics in sub-groups of patients,
- preclinical data on toxicology and pharmacology.

Recommendations should be made for the conditions of use with the intention of reducing the incidence of adverse reactions (e.g. modified dosage regimens, monitoring of blood/plasma levels, contra-indications, warnings, precautions for use, etc...).

POST MARKETING EXPERIENCE

If the product is already on the market in some countries, reported adverse reactions should be given, in relation to the consumption rates in these countries.

The discussion should deal with:

- the methods of detection and assessment of these adverse reactions;
- the results; in particular, it should be clearly shown how the post-marketing data complement and/or modify the safety profile and the conditions for use.

OTHER INFORMATION

This section is reserved for information not covered in the above parts.

Unfinished trials at the date of application should be mentioned, giving their nature, size, objectives and projected completion dates with results.

Studies discontinued prematurely should be mentioned and reasons given.

CONCLUSION

This section should cover the following points:

a) therapeutic justification for the product (especially for fixed combinations and new pharmaceutical forms);

b) efficacy;

c) safety,

taking account of preclinical pharmacology and toxicology studies. Adverse reactions, contra-indications, interactions, warnings and precautions for use should be defined. Treatment of overdosage should be described.

- The possible utilization during pregnancy and breast-feeding and the possible effect on driving ability should be taken into account.
- Regarding interactions, mention should be made of clinically significant interactions and of possible recommendations for use which would be appropriate.
- The concomitant use of other medicinal products studied during the clinical development, without clinical significance or not having caused any interaction should also be highlighted.

d) the dossier reviewer proposes (range, age, sex, duration of treatment and starting dose, etc...),

e) risk/benefit ratio: the product should be judged with regard to clinical practice and the different treatments which are available.

REFERENCE LIST

A list of references used, in addition to those contained in the dossier, should be given and stated in accordance with internationally accepted standards of the 1979 Vancouver Declaration [41] on "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" or the system used in "Chemical Abstracts".

References should be numbered and each reference should be easily found in the application file in question.

INFORMATION ON THE CLINICAL EXPERT

The qualifications and experience of the expert should be briefly summarized. Although only one expert may assume responsibility for the report, other experts may contribute to its preparation, according to their expertise.

[41] see the annex, reference 41

E TABULAR FORMATS

NON-CLINICAL TESTING OF HERBAL DRUG PREPARATIONS WITH LONG-TERM MARKETING EXPERIENCE ~~(OLD SUBSTANCES)~~

Guidance to facilitate mutual recognition and use of bibliographic data

Draft 22 November 1997

INTRODUCTION

~~Article 4, Nr. 8, a) ii) of Council directive 65/65 makes~~ It is clear that the applicant shall not be required to provide the results of pharmacological and toxicological tests if he can demonstrate by detailed reference to published scientific literature presented in accordance with the second paragraph of Article 1 of Directive 75/318/EEC that the constituent or constituents of the medicinal product have a well-established medicinal use, with recognised efficacy and an acceptable level of safety. This regulation in no way relieves the requirement of proof of safety set out by the Annex to CD 75/318. All aspects must be covered by bibliographic data and the expert report.

Published Non-clinical tests for ~~well established herbal drug preparations~~ old substances are often incomplete or not in accordance with today's state of the art. ~~In order to obtain a better understanding of the inherent risks with such products and avoid procedures that delay the mutual recognition of old substances in the EU, it is necessary to asses the minimum requirements of non-clinical testing. Well-presented~~ results of clinical trials as well as post-marketing experience gained by wide spread clinical use in humans contribute to the avoidance of unnecessary tests in animals. Protection of animals should be taken into consideration when requesting non-clinical testing of ~~old substances~~ well-established herbal drug preparations (86/609/EEC). Studies that do not agree with the current state of the art (e.g. GLP-conformity), should be judged for credibility; subsequent demands that could lead to a "blind" repetition of animal experiments should be avoided.

In cases of reasonable suspicion, additional appropriate non-clinical tests can be requested.

NON-CLINICAL TESTING

Where there is sufficient clinical experience available in humans, single dose and repeated dose toxicity, ~~immunotoxicity~~ as well as local tolerance testing of ~~well-established herbal drug preparations old substances~~ is not necessary. Likewise, pharmacological tests including safety pharmacology and ~~pharmacokinetics (preferably in animals)~~ are not necessary. The expert report must address these aspects and give the grounds why the documented medical experience justifies a safe use of the herbal drug preparation.

Non-clinical testing of ~~well-established herbal drug preparations old substances~~ should be directed towards the study of effects that are difficult, even impossible to detect clinically. These effects would include toxicity to reproduction, genotoxicity and carcinogenicity.

Reproductive toxicological investigations regarding fertility are generally not necessary, insofar as there are no grounds for suspicion that would necessitate testing.

The reproductive toxicological potential with regard to embryo-foetal and peri-post-natal development is to be clarified. Reproductive toxicity data are available for many old substances, however, these data are often not reliable. A repetition of the tests is only justified in cases in which the significance of the results is not clear and there are grounds for suspicion. Reproductive toxicological tests in animals are not necessary if one of the following criteria is fulfilled:

- Results from investigations in pregnant women and neonates are present.
- The medicinal product is not intended to be used in women of child-bearing age or during pregnancy.

The clinical Expert Report should justify the distinction made between women of child-bearing age and pregnancy.

The genotoxic potential of ~~herbal drug preparations old substances~~ should be clarified.

~~Genotoxicity data are available for many old substances, however, these data are often not reliable.~~ A repetition of the studies is only required in cases in which the significance of the results is unclear or they yield grounds for suspicion. Positive findings for ~~one herbal drug preparation or for~~ substances from one chemical class can frequently be extrapolated to another ~~herbal drug preparation substance in the class~~ without necessitating further testing (e.g. cytostatic agents).

It is recommended to first perform *in vitro* tests for substances in which the genotoxicity tests are insufficient. Substances with negative results *in vitro* also exhibit negative results *in vivo* in the majority of cases. In cases in which positive results in vitro are present, these are to be clarified by way of appropriate investigations, mainly *in vivo*. (CPMP/ICH 174/95, CPMP/ICH 141/95 and OECD 1995)

~~It is appropriate to assess genotoxicity initially in a bacterial reverse mutation test using a test battery of different bacterial strains and metabolic activation (e. CPMP/ICH and OECD Guidelines). This test has been shown to detect relevant genetic changes and the majority of genotoxic rodent carcinogens. If positive results can not be clearly attributed to specific constituents with a well-established safety-profile (e.g. Quercetin) additional in vitro and, if necessary, *in vivo* studies should be performed. A co-operative approach is encouraged to investigate herbal drug preparations with the same specification.~~

Carcinogenicity studies are not needed in cases where there is no suspicion for a carcinogenic potential (75/318/EEC, Part 3, IIE. Carcinogenic Effect, CPMP/ICH/140/95, CPMP/ICH/299/95, CPMP/ICH 166/96).

Even a positive suspicion of a carcinogenic effect of an well-established herbal drug preparation/old substance does not necessarily require a study to be performed. The following considerations should be included in the assessment:

- ~~Does a positive result alter the benefit-risk assessment?~~

—

- ~~Is tumour formation predictable because of some class effect, from the already available scientific data (i.e. tumour formation in well known target organs)?~~

—

- ~~If no carcinogenicity is expected from knowledge on other compounds of the same chemical class the outcome is usually known (i.e. no tumour formation), carcinogenicity testing can be normally avoided.~~

—

- Is the suspicion based on positive results of genotoxicity studies and can it be clarified in further genotoxicity studies, *mainly in vivo*?

- ~~Is the suspicion based on epidemiologically proven positive findings in humans (e.g. oestrogens cause mammary tumours in humans)?~~

- Is there sufficient epidemiological experience in humans that could refute the suspicion?

~~Toxicokinetic data are only required in connection with tests in animals.~~

EXPERT REPORT

The expert is obliged to point out the necessity or not of non-clinical testing for the herbal drug preparation/old substances. Plausible presentation of the facts contributes to the acceptance of the application for marketing authorisation and facilitates the evaluation performed by the authorities.

The expert should discuss available published toxicological data on closely related herbal drug preparations, different parts of the plant, data on related species of the same genus or plant family. If there are toxicological data on well-defined constituents of a herbal drugs preparation, the expert should discuss the relevance of these data for the safety assessment of the herbal drug preparation.

FUNDAMENTALS IN CLINICAL TRIAL

Edison R. Parise

I. ETHICAL PRINCIPLES

1. Research involving human subjects must

   - be according to scientific principles and based on previous laboratory and animal experimentation.
   - only be performed when knowledge can not be reached by other way
   - be formulated in experimental protocol approved by an independent ethical committee
   - conducted and supervised by scientifically qualified person
   - weight the potential advantage over the best current diagnostic and therapeutic method.
   - safeguard subject integrity respect. Precaution to resect privacy and minimize impact on personality

2. The investigator must follow four main principles:

   - *Respect for person.* Obtain free informed consent protecting vulnerable or incapable individuals (proxi consent).
   - *Beneficence.* Maximize benefits and minimize harms or discomfort
   - *Non-maleficence.* Assure that predictable harms will be avoided.
   - *Distributive justive.* Investigation should have social importance for the subject and for groups with equitable distribution of burden and benefits of participation of the research.

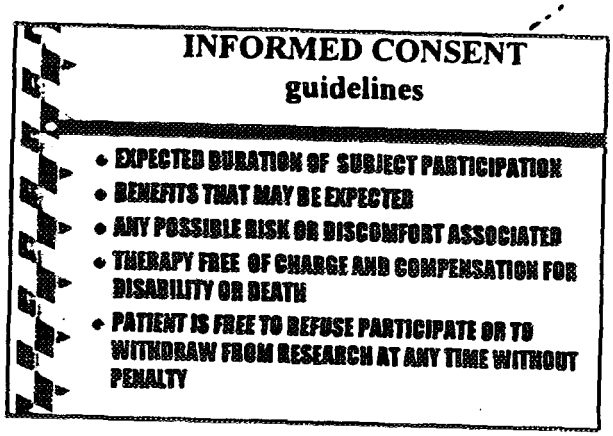
3. Induciment to participate:

- Subjects may be paid for inconveniences and time spent, be reimbursed for expenses incurred and receive free medical service. However payment or medical attention should not induce subjects to participate in the research against their better judgment. *Undue Inducement*

4. The investigator has the duty to:

- Communicate all the informations necessary for adequately informed consent
- Give the subject full opportunity and encouragement to ask questions
- Exclude possibility of unjustified deception, undue influence and intimidation
- Seek consent only if subjecthas adequate knowledge of relevant facts and has had opportunity of reflexion to consider wheter to participate

5. Informed consent - Guidelines



II. NON CLINICAL AND TOXICITY STUDIES ON HERBAL MEDICINE

1. The primary objectives in non-clinical studies are:

   - give support for clinical use or investigation
   - characterize pharmacological actions and toxicity
   - define chemical characteristics and mechanisms of action

2. Toxicological studies in animals:

   - The pharmacological assay should be performed in three diferents dosis according to the herbal pharmacological activity and toxicity
   - Maximum dose: dose that produce clinical, biochemical, haematological and anatomical disturbances, but the majority of the animals survive
   - Minimum dose: close efficacy dose, without side effects

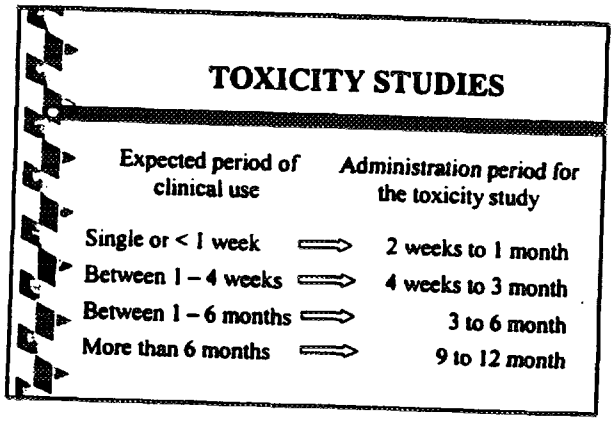
3. Phase I - toxicological studies:

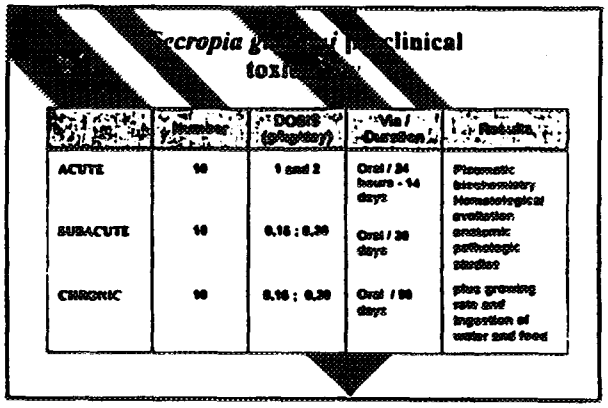
   - Acute toxicity 24 hours
   - Subacute toxicity 30 days
   - Chronic toxicity 90 days

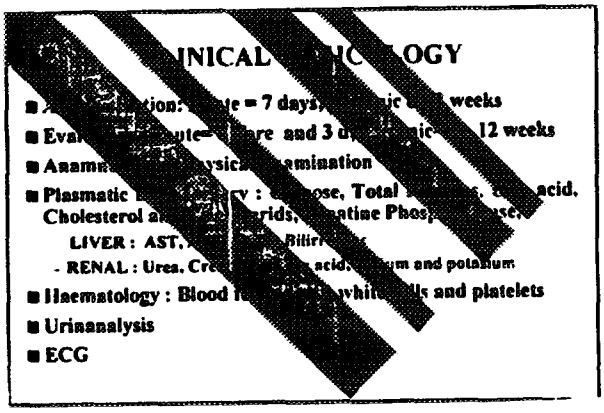
   Phase II - Special studies

   - Reproduction
   - Mutagenesis
   - Carcinogenesis

4. Toxicity Studies
5. Example of a preclinical toxicological experiment
6. Clinical toxicology







III. TYPES OF CLINICAL TRIAL

A. WHY TO PERFORM CLINICAL TRIALS ON HERBAL MEDICINE ?

Reazons for not relying only on conventional prescriptions

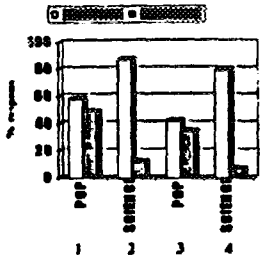
TRADITIONAL x POPULAR CONCEPTS IN LIVER DISEASES

1. Assessment symptomatology scientifically and popularly related to liver disease in patients with acute and chronic hepatitis and a control group with others acute and chronic viral infections or patients with liver cirrhosis compared with others chronic systemic disease

2. Inquery about scientific related symptoms in liver diseases with patients and medical students from the 1st and 5th year of the medical school

3. Inquery about popularly related symptoms in liver diseases with patients and medical students from the 1st and 5th year of the medical school

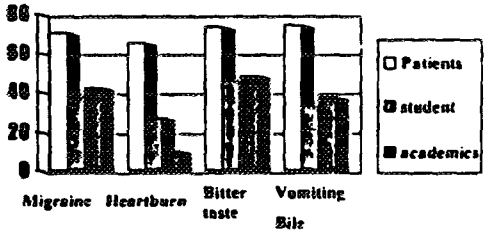
CLINICAL SYMPTOMATOLOGY IN PATIENTS WITH LIVER DISEASE



- 1 AND 2 PATIENTS WITH ACUTE AND CHRONIC HEPATITIS ; PATIENTS WITH ACUTE VIRAL INFECTIONS OTHERS THAN HEPATITIS (CONTROL)
- 3 AND 4 PATIENTS WITH LIVER CIRRHOSIS ; PATIENTS WITH CHRONIC SYSTEMIC DISEASES (CONTROL)

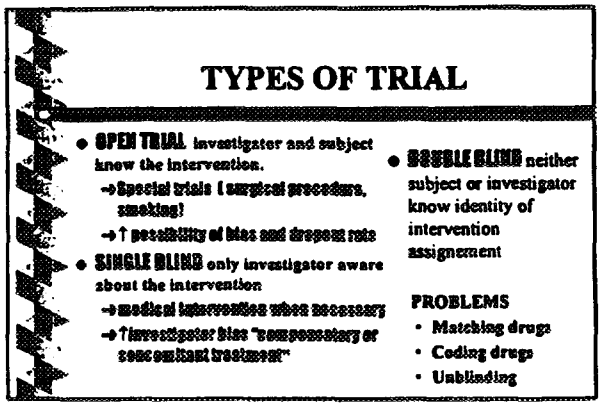
CLINICAL SYMPTOMS IN LIVER DISEASE
SCIENTIFIC RELATED SIGNALS AND SYMPTONS

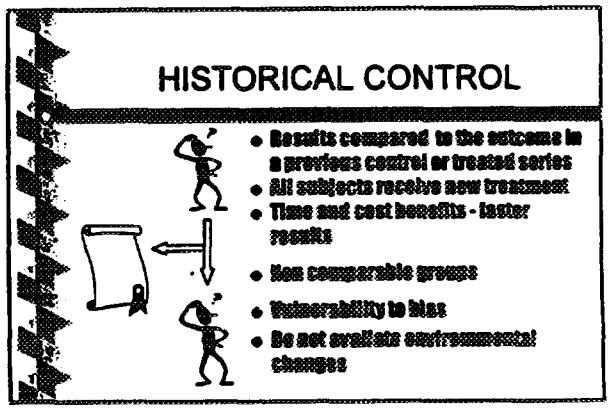
CLINICAL SYMPTOMS IN LIVER DISEASE
POPULARY RELATED SYMPTOMS AND SIGNALS

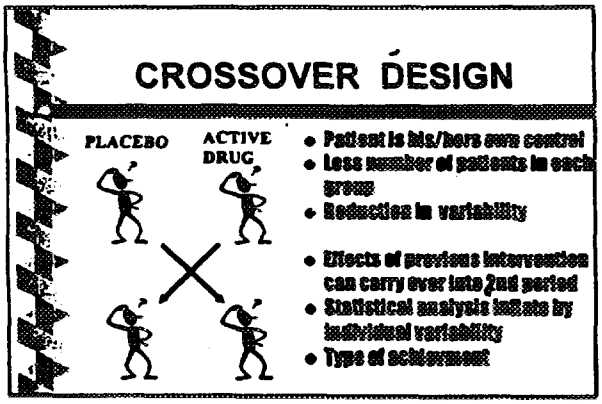


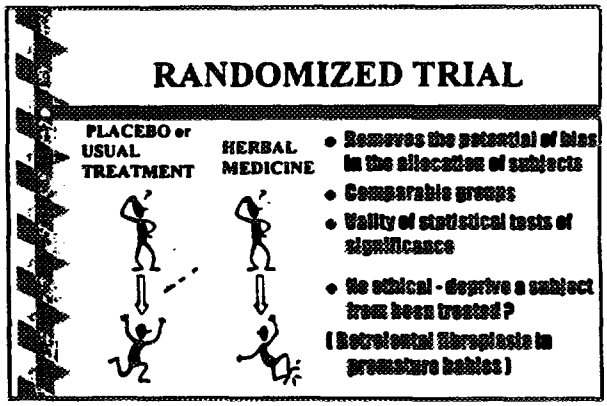
B. TYPES OF CLINICAL TRIALS

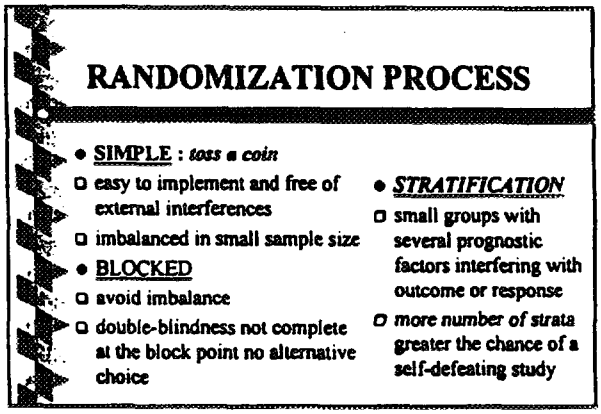
1. Open Trial, Single and Double Blind
2. Historical Control
3. Crossover Design
4. Randomized Trial
5. Randomization Process









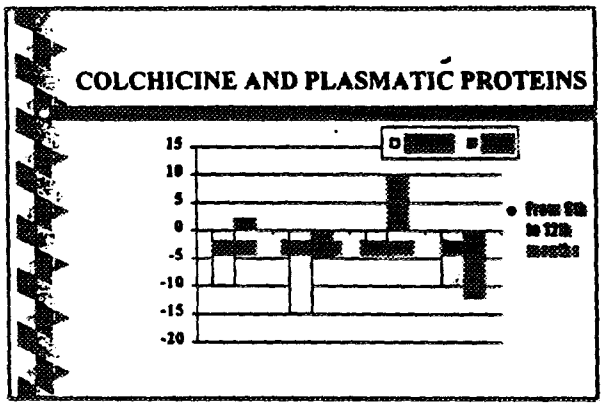


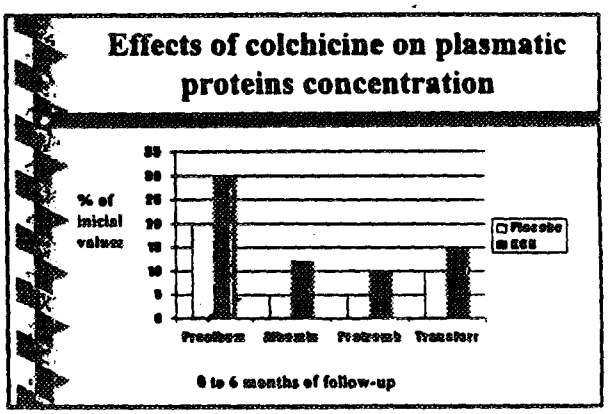
C. CLINICAL EXAMPLE FOR THE NECESSITY OF DOUBLE-BLIND, RANDOMIZED TRIALS

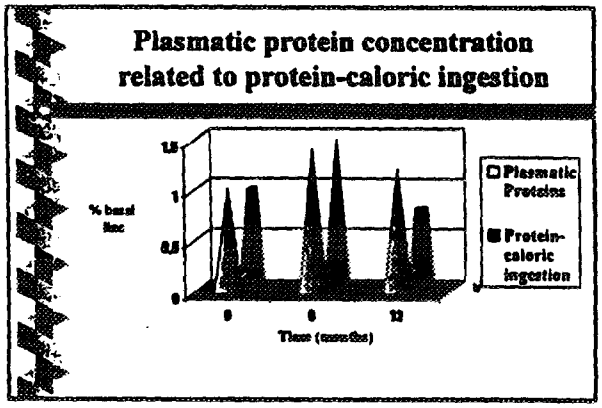
1. Effects of colchicine and placebo on plasmatic protein concentration (0 to 6th months, when patients were seen by doctors every months)

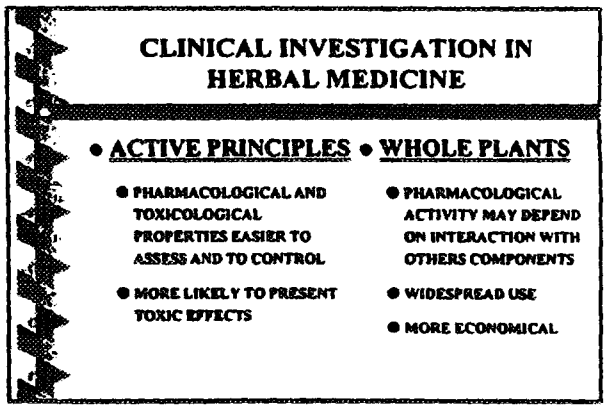
2. Effects of colchicine and placebo on plasmatic protein concentration (6th to 12th months. Clinical controls only every three months)

3. Correlation between plasmatic protein concentration and protein-caloric ingestion in both groups









IV - SAMPLE SIZE

1. Primary Response Variables
2. Sample size concepts
3. Sample size, formula for continous variables
4. Sample size, using the formula
5. Example of simple size just calculating Type I error
6. Simple size using Type I and Type II error for patients with peptic ulcer

PRIMARY RESPONSE VARIABLE

- Dichotomous - event rates *pi* and *pc*
- Continous - mean level μi and μc

Null hypothesis (Ho) : pc-pi = 0

Error type I - events rates are different by chance only and Ho is reject incorrectly

probability = α "p"value = difference when *Ho* is true

Error type II - *Ho* is not reject when it could be

probability = β depend on δ β-1= power of study

SAMPLE SIZE

- Statistical power to detect differences between groups should considered
- What differences are clinically significants
- The disease natural history

SAMPLE SIZE

- $2N=2\{Z\alpha\sqrt{2\,p\,(1-p)} + Z\beta\sqrt{pc\,(1-pc) + pi\,(1-pi)}\}^2 \;/\; (pc - pi)^2$

where: 2N total number of subjects

$Z\alpha$ = critical value correspond significant level

$Z\beta$ = power (1- β); "p"estimates of the event rates in *c* (control) and *i* (intervention) group

$p = pc+pi / 2$

SAMPLE SIZE

- Anual rate of one event = 20%
- the intervention - reduce to 15% in two years

(pc = 0,40 - pi = 0,30) two-sided with 5% significance and 90% power

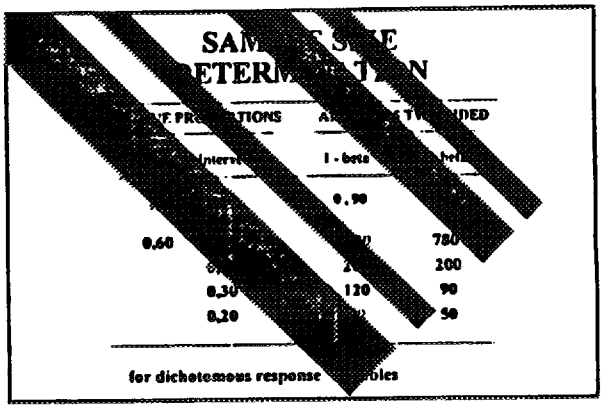
- $2N = \{1,96\sqrt{2(0,35)(0,65)} + 1,28\sqrt{0,4(0,6)+0,3(0,7)}\}^2 \;/\; (0,4)-(0,3)^2$

2N = 952

Number of patients required for peptic ulcer treatment

	Control	Expectation of healing		
Number	40%	50%	70%	90%
10	4	5	7	9*
20	8	10	14*	18**
50	20	25	35**	45**
100	40	50*	70**	90**
200	80	100**	140**	180**

(*)= p<0,05; (**)= p<0,01



V- PHASES ON CLINICAL TRIAL

CLINICAL TRIAL

- **PHASE I** - First trial of a new compound. Small number of healthy volunteers or patients
  Tolerance and Dose
- **PHASE II** - Limited number of patients Historical or randomized trial. **Clinical efficacy and Dose**
- **PHASE III** - Larger patient group. Double-blind randomized design. **Efficacy**
- **PHASE IV** - Studies after dosage form is available for general use. Unusual toxicity

Harnessing Technology to Achieve Corporate Objective

Mission: Purpose or constraint Focus

Business plan

Assessment of Environment

- proximate
- distant
- levels of uncertainty
    - * Clear enough future
    - * Alternate futures
    - * A range of futures
    - * True ambiguity
- Human resources capability
- Regulatory Environment
- Virtual organizations
- Joint ventures

STRATEGIC POSTURES

1. Leadership role: shape the future
   or
2. Adapt to the future
   or
3. Reserve the right to play

SUCCESSFUL STRATEGIC MANAGEMENT

* Analyze the industry
* Identify the organization's strength and weaknesses
* Exploit opportunities, take risks
* Develop core competencies with values
* Link external factors xxx
* Integrate long and short-term planning
* Leverage the linkage between the organization and the market place.

MARCHING LEVELS OF INNOVATION WITH ENVIRONMENTAL UNCERTAINTY AND HOSTILITY

1. Incremental expansion - low hostility
2. Comprehensive change - middle level of hostility
3. Discrete change - fairly hostile settings
4. Progressive innovation - highly hostile environment.

LABORATORIO DE TECNOLOGIA DE PRODUTOS NATURAIS
FACULDADE DE FARMACIA
UNIVERSIDADE FEDERAL FLUMINENSE
Niteroi - Brasil

TRAINING COURSE ON PRODUCTION OF PHYTOMEDICINES
PANAMA, 24/11 - 05/12, 1997

Prof. Nikolai Sharapin

TINCTURES - Liquid preparations. Extracts from drugs with ethanol of varying concentrations. Extraction ratio 1 : 10

FLUID EXTRACTS - Liquid preparations. Extracts from drugs with ethanol of varying concentrations. Extraction ratio 1 : 1

THICK EXTRACTS - Moisture content aprox. 45 -60 % No longer fluid at room temperature; thickly liquid or viscous when warm. Prepared by careful concentration of liquid extracts. May be added of calculated quantities of inert substances (dextrin, lactose, starch).

DRY EXTRACTS - Solid preparations. Obtained by careful concentration and drying of fluid extracts. May be added of inert substances.

PARAMETERS WHICH INFLUENTIATE EXTRACTION

CHOICE OF SOLVENT
SIZE OF PARTICLES
SWELLING OF THE DRUG
TEMPERATURE
pH
TIME OF EXTRACTION

ALCOHOL CONTENT X EXTRACTION TIME X TEMPERATURE

1. Constant alcohol content and extraction time			
Alcohol content	**50 %**	**50 %**	**50 %**
Extraction time	**1 h**	**1 h**	**1 h**
Temperature	**40°C**	**60 °C**	**80 °C**
Yield	**2 %**	**2,2 %**	**1,4 %**
2. Constant extraction time and temperature			
Alcohol content	**30 %**	**50 %**	**70 %**
Extraction time	**1 h**	**1 h**	**1 h**
Temperature	**60°C**	**60 °C**	**60 °C**
Yield	**1,7 %**	**2,2 %**	**2,9 %**
3. Constant alcohol content and temperature			
Alcohol content	**70 %**	**70 %**	**70 %**
Extraction time	**0,5 h**	**1 h**	**2 h**
Temperature	**60°C**	**60 °C**	**60 °C**
Yield	**1,8 %**	**2,9 %**	**1,2.9 %**

EXTRACTION OF DRUGS

1.DISSOLUTION OF EXTRACTIVE SUBSTANCES OUT OF DESINTEGRATED CELLS

2. DISSOLUTION OF EXTRACTIVE SUBSTANCES OUT OF INTACT PLANT CELLS BY DIFFUSION

IN ORDER TO INCREASE THE PERMEABILITY OF CELL WALLS REQUIRES STEEPING AND SWELLING OF THE DRUG PLANT MATERIAL

- **penetration of solvent into the plant cells and swelling the cells**
- **dissolution of extractive substances**
- **Diffusion of the dissolved extractive substances out of the plant cell**

EXTRACTION OF DRUGS

1. PROCESSES WHICH RESULT IN ESTABLISHMENT OF A CONCENTRATION EQUILIBRIUM BETWEEN SOLUTION AND SOLID RESIDUE

2. PROCESSES IN WHICH THE DRUG IS EXTRACTED EXHAUSTIVELY

EXTRACTION PROCESSES

MACERATION

KINETIC MACERATION

REMACERATION

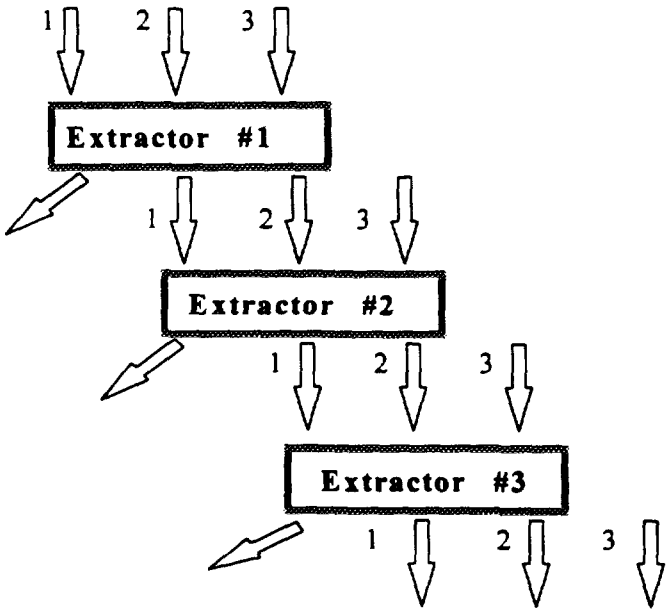
DIGESTION

EXHAUSTIVE EXTRACTION

PERCOLATION

REPERCOLATION

COUNTERCURRENT EXTRACTION



EXTRACTION OF ALKALOIDS 1

1.EXTRACTION WITH WATER IMMISCIBLE SOLVENTS

2. EXTRACTION WITH SOLVENTS MISCIBLE WITH WATER

3. EXTRACTION WITH ACIDULATED WATER

3.1 Extraction with aqueous solutions of inorganic acids

3.2 Extraction with aqueous solutions of inorganic salts

EXTRACTION OF ALKALOIDS 2

1. MOISTENING OF THE DRUG WITH Na_2CO_3 SOLUTION

2. EXTRACTION WITH WATER IMMISCIBLE SOLVENT

3. LIQUID - LIQUID EXTRACTION WITH AQUEOUS SOLUTION OF INORGANIC ACID

4. EXTRACTION OF AQUEOUS LAYER WITH ORGANIC SOLVENT (pH 9,0 - 9,5)

5. EVAPORATION OF THE SOLVENT AND SALT FORMATION

6. RECRYSTALLIZATION FROM ORGANIC SOLVENT

EXTRACTION OF ALKALOIDS 3

1. EXTRACTION WITH SOLVENT MISCIBLE WITH WATER
2. ADDITION OF WATER AND VACUUM CONCENTRATION UNTIL ELIMINATION OF ORGANIC SOLVENT
3. pH ADJUSTMENT TO 4.0 - 4.5
4. EXTRACTION WITH ORGANIC SOLVENT
5. pH ADJUSTMENT TO 9,0 - 9,5
6. EXTRACTION WITH ORGANIC SOLVENT
7. SOLVENT EVAPORATION AND SALT FORMATION
8. RECRYSTALLIZATION FROM ORGANIC SOLVENTS

EXTRACTION OF ALKALOIDS 4

1. EXTRACTION WITH AQUEOUS SULPHURIC ACID (2 % V/V)

2. $Al_2(SO_4)_3$ + NH_4OH until pH 5,5

3. FILTRATION

4. pH ADJUSTMENT TO 9.0 - 9.5

5. EXTRACTION WITH ORGANIC SOLVENT

6. EVAPORATION OF ORGANIC SOLVENT AND
   SALT FORMATION

EXTRACTION OF ALKALOIDS 5

1. EXTRACTION WITH 4 % (w/v) AQUEOUS SOLUTION
   OF ALUMINIUM SULPHATE

2. pH ADJUSTMENT TO 5,5

3. FILTRATION

4. pH ADJUSTMENT TO 9.0 - 9.5

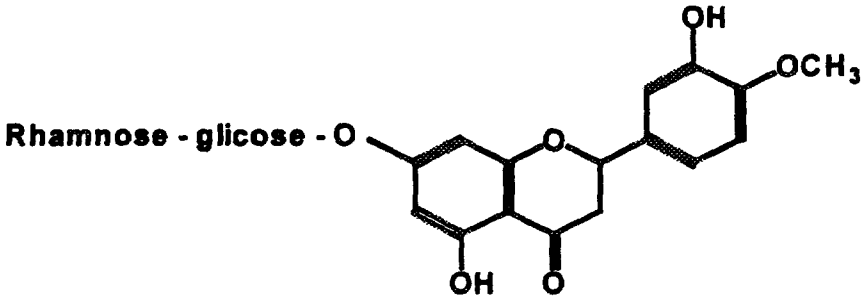
5. EXTRACTION WITH ORGANIC SOLVENT

6. SOLVENT EVAPORATION AND SALT FORMATION

ALKALOID EXTRACTION 6

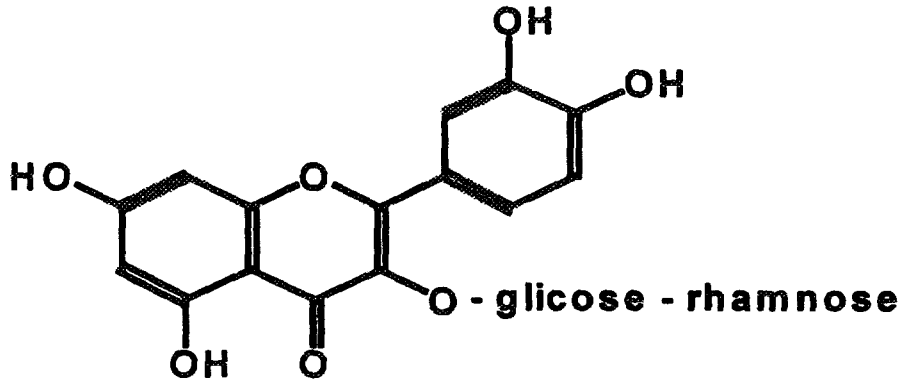
SALT FORMATION
- HYDROCHLORIDES
  - HCl gas.
  - Solvent: Acetone / metanol 95 - 5
- NITRATES
  - concetratred HNO_3
  - Solvent: EtOH; MeOH
- SULPHATES
  - H_2SO_4
  - Solvent: H_2O

EXTRACTION OF FLAVONOIDS



SOURCE: SWEET ORANGES; LEMONS
EXTRACTION: METHANOLIC SOLUTION OF NAOH

EXTRACTION OF FLAVONOIDS



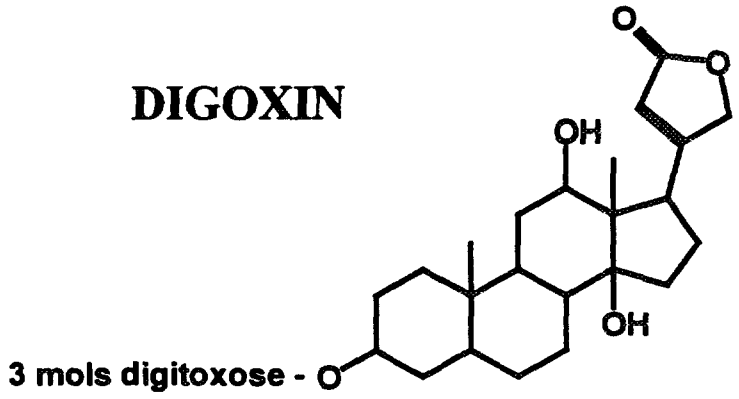
SOURCE: ***Dimorphandra gardneriana***

EXTRACTION: MeOH

LANATOSIDE C

β-D-glucose - acetyl-D-digitoxose-D-digitoxose -D-digitoxose -R
R = digitoxigenin

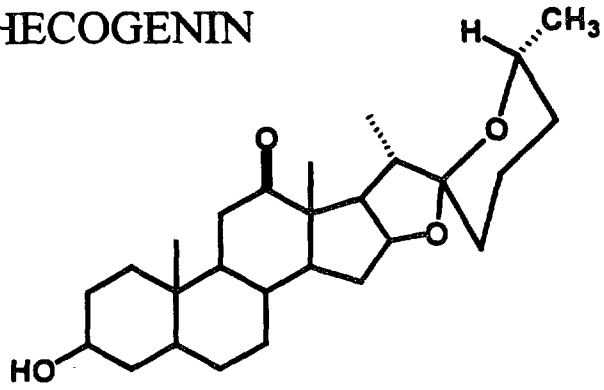
DIGOXIN



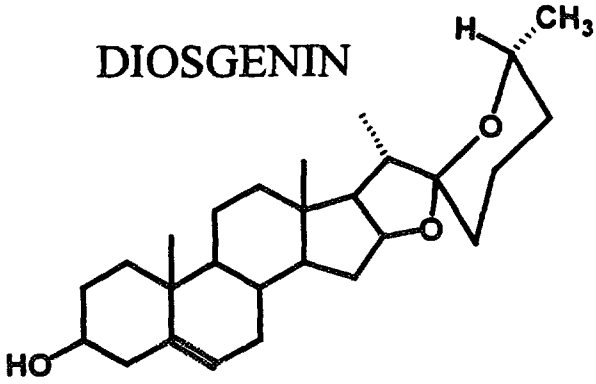
SOURCE: ***Digitalis lanata*** Ehrh

1. MOIST WITH WATER AND ALLOW TO FERMENTATE
2. EXTRACTION WITH MeOH
3. EVAPORATION OF MeOH. ADDITION OF WATER
4. EXTRACTION WITH CHLOROFORM
5. EVAPORATION OF CHLOROFORM AND DISOLUITION IN MeOH. ADDITION OF WATER.
6. DEFATTING WITH HEXANE
7. TREATMENT WITH NaOH IN CONTROLLED CONDITIO FOLLOWED BY NEUTRALIZATION WITH HCl
8. EXTRACTION WITH CHLOROFORM
9. EVAPORATION OF SOLVENT
10 PURIFICATION STEPS

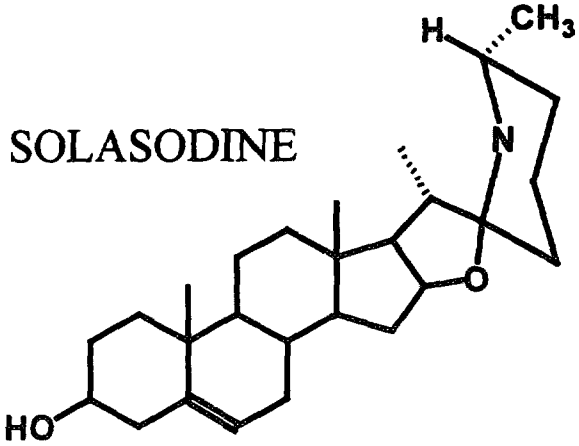
HECOGENIN



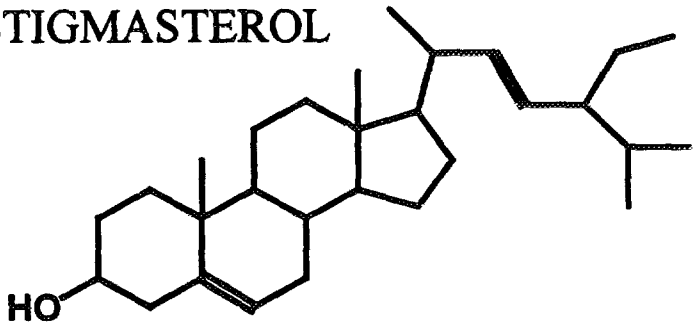
DIOSGENIN



SOLASODINE



STIGMASTEROL



ISOLATION OF HECOGENIN

SISAL JUICE —Fermentation→ FERMENTED JUICE

FERMENTED JUICE —separation of lower layer→ "SLURRY"

"SLURRY" —Hydrolysis→ "ACID SLURRY"

"ACID SLURRY" —Hydrolysis→ "ACID CAKE"

"ACID CAKE" —Solvent extraction→ CRUDE HECOGENIN

TLC of { standardized Aesculus hippocastanum extract / β-aescine

- stationary phase : RP-C18 plates
  - activate plates during 5 minutes in an oven of 100°C

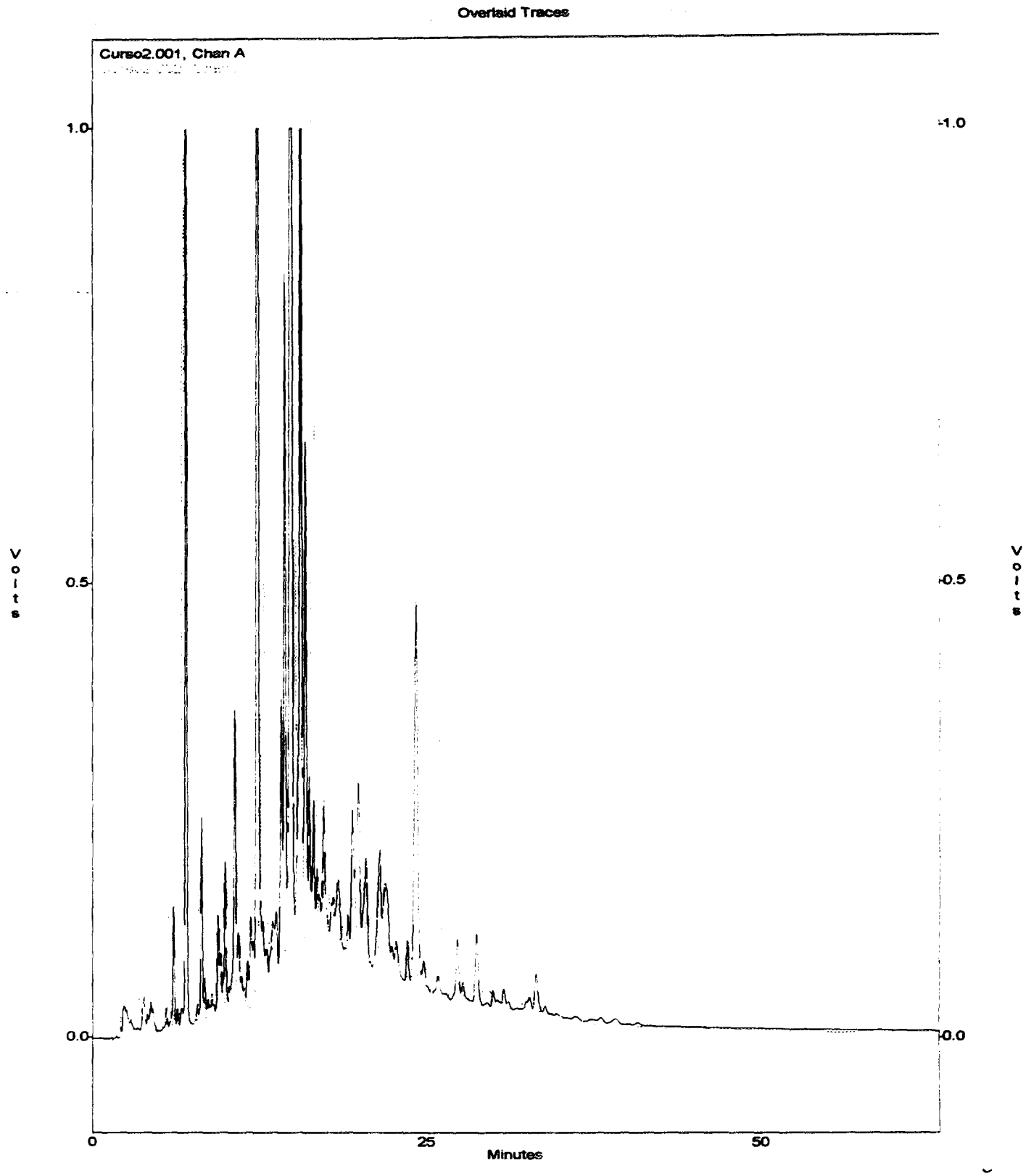
- mobile phase : acetonitrile - water (50:50) + 0.05 % trifluoroacetic acid

- apply the standard and the extract to the plate as line spots (5 - 10 - 15 µl)

- spray reagents : anisaldehyde - sulphuric acid

- β-aescine solution : 10 mg in 1 ml methanol 70 %.

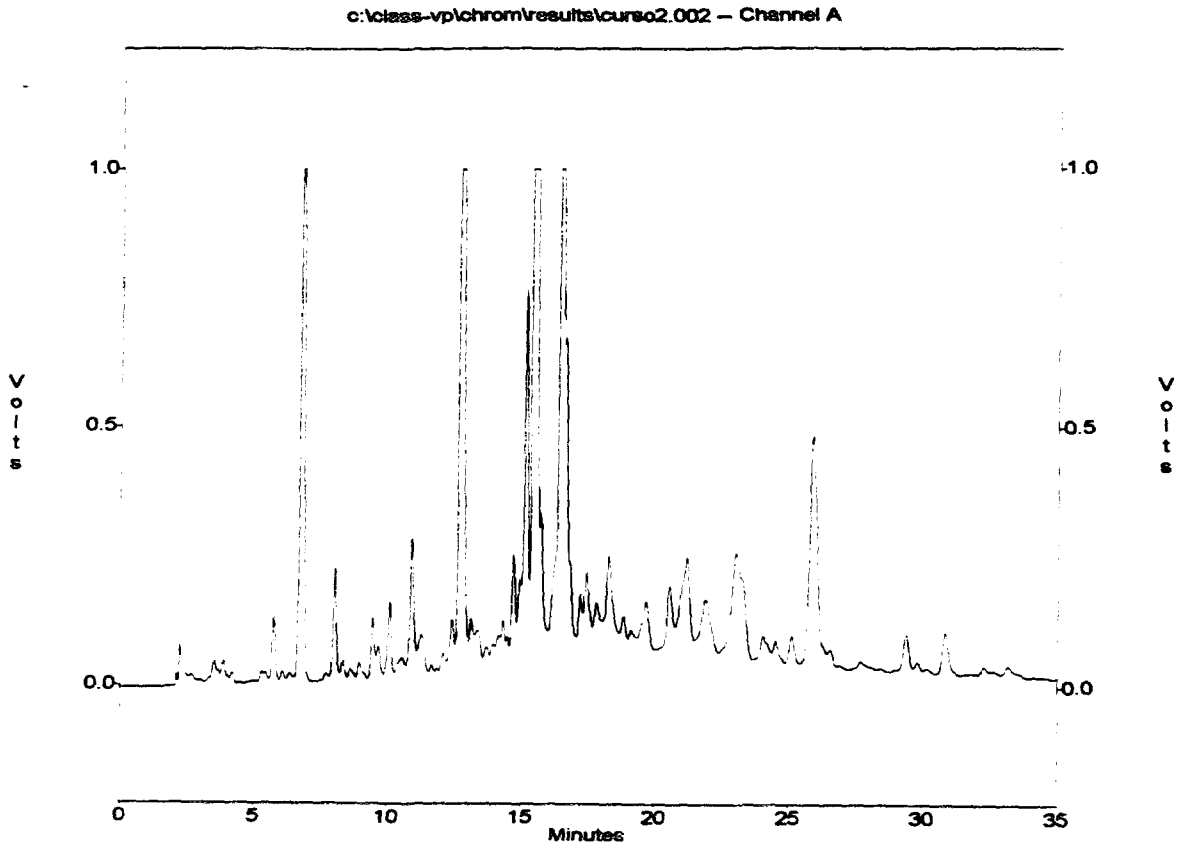
ND : 0.5 ml anisaldehyde
+ 10 ml acetic acid
+ 85 ml methanol
+ 5 ml sulphuric acid.


Overlaid Traces
Curso2.001, Chan A
1.0
0.5
0.0
Volts
0
25
50
Minutes

Muestra Tabebuia
1,5mL/min 254nm

t	A	B
0	10	90
30	55	45
60	55	45
62	10	90

Acquired : Nov 28, 1997 16:04:14
Printed : Nov 28, 1997 17:12:25
File Desc. : Muestra 2



Channel A Results

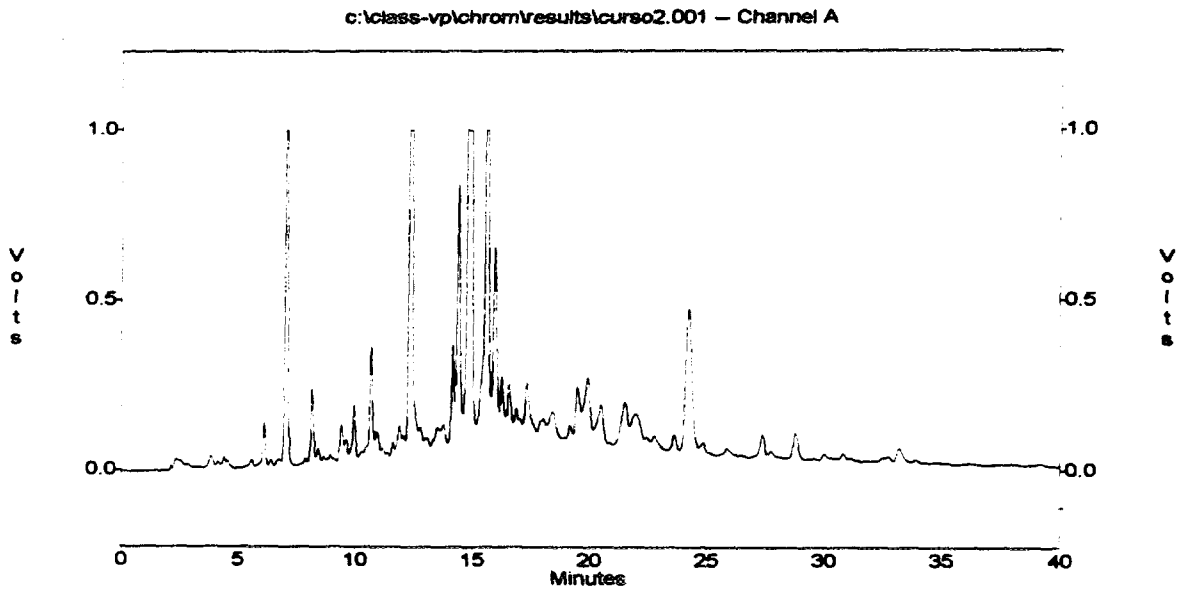
Time	Area
6.82	11405991.0
8.03	2219394.0
10.90	3382522.0
12.81	15274907.0
14.71	2694173.0
15.17	7842775.0
15.58	17492850.0
15.71	3666275.0
16.53	16858994.0
16.64	6032815.0
16.84	2459691.0
17.44	3551028.0
17.81	3030819.0
18.28	5900931.0
18.82	2194196.0
19.67	4532385.0
20.55	4834003.0

Continued...

Acquired : Nov 28, 1997 16:04:14
Printed : Nov 28, 1997 17:12:27
File Desc. : Muestra 2
Channel A Results

Time	Area
21.19	7151535.0
21.90	6075042.0
23.03	10328224.0
24.05	2809631.0
25.91	10901303.0
27.72	2005519.0
29.40	2284043.0
30.86	2165169.0

0 10 90
30 60 40
60 60 40
62 10 90
1,5mL/min 254nm
Acquired : Nov 28, 1997 14:56:50
Printed : Nov 28, 1997 16:07:53
File Desc. : MUESTRA DE Tabebuia (corteza)
Fase móvil: H20:H3PO4 1N en ACN
- 0 90 : 10
30 40 : 60
60 40 : 60
62 90 : 10
254nm 1,5mL/min 235Kgf/cm2
c-18



Channel A Results

Time	Area
7.09	10464989.0
8.17	2112276.0
9.96	2136180.0
10.68	4175393.0
12.43	15624030.0
13.51	2713222.0
14.17	4028907.0
14.42	8286408.0
14.98	19478964.0
15.68	16521214.0
15.96	8902105.0
16.27	3316638.0
16.58	3540294.0
16.87	2398079.0
17.34	5824854.0
18.42	5236204.0
19.50	3906749.0
19.95	6003460.0
20.52	4897263.0
21.54	5490040.0
21.99	4983450.0
22.77	3277599.0
24.27	10054810.0

Continued...

Acquired : Nov 28, 1997 14:56:50
Printed : Nov 28, 1997 16:07:55
File Desc. : MUESTRA DE Tabebuia (corteza)
Channel A Results

Time	Area
25.91	2325057.0
27.40	2287646.0
28.82	2123534.0

BLANCA GALVEZ ACN:H_3PO_4 IN Gradient 15:20 To [illegible]

Run : 02 Type : Sample

Path : C:\CRHOM Inst : 1022 LC Plus

Collection : 15:33:22 No. 28 1997 Method : [illegible] (15:20:04 No. 28 1997)

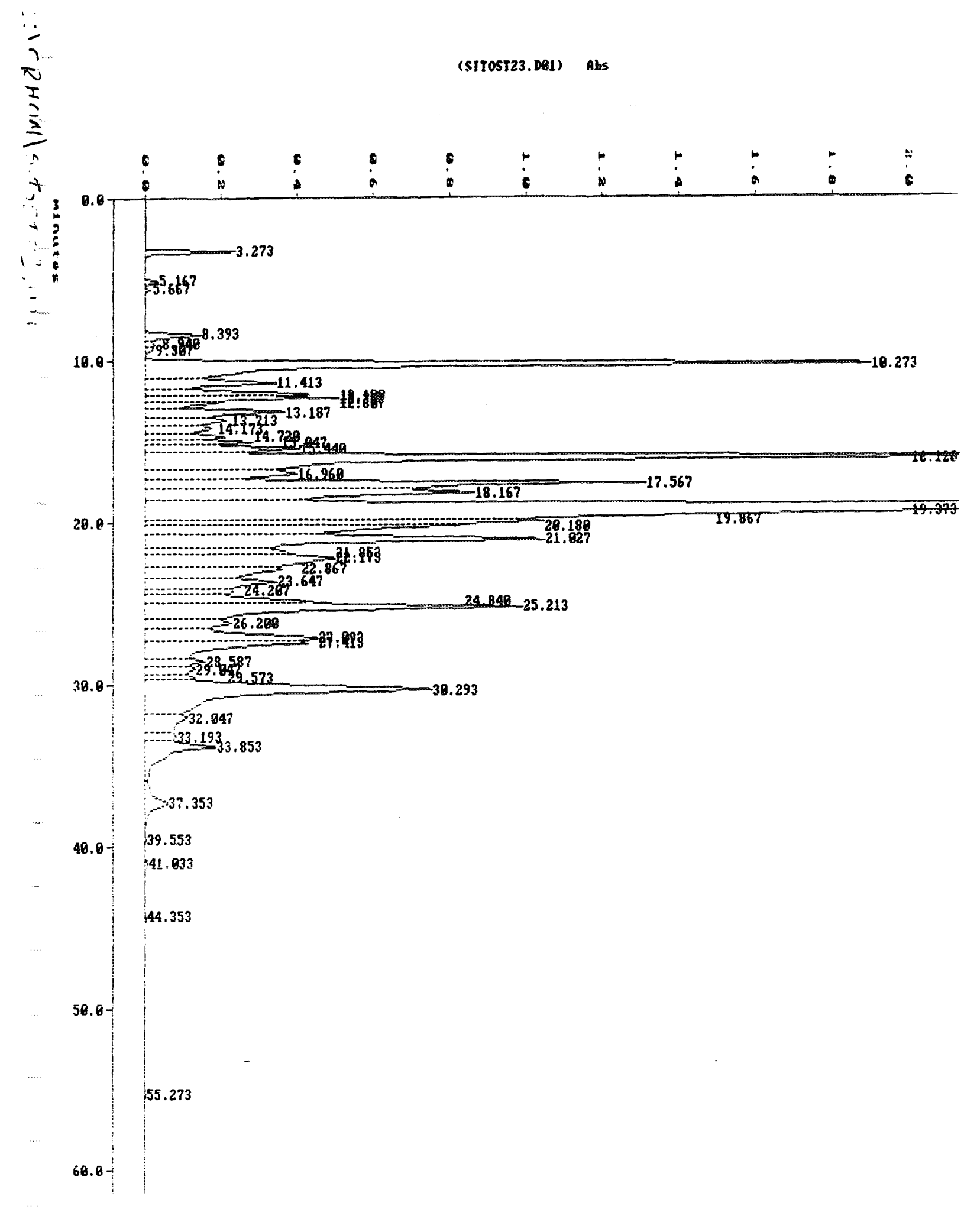
Integration: 15:33:22 No. 28 1997 Method : [illegible] (15:20:04 No. 28 1997)

Report : 16:35:26 No. 28 1997 Method : [illegible] (16:32:43 No. 28 1997)

Sample Amt : 1.00000e+0 Dilution: 1.00000e+0

EXTERNAL STANDARD (AREA)

RT	Area	BC	ExpRT	RF	Concn	Name
3.277	8759672			1.00000e+0	8759672.0000	Unknown
5.167	2324575	V		1.00000e+0	2324575.0000	Unknown
5.667	413086			1.00000e+0	413086.0000	Unknown
6.393	11650602	I		1.00000e+0	11650602.0000	Unknown
8.940	2343774	T		1.00000e+0	2343774.0000	Unknown
9.307	1731964	V		1.00000e+0	1731964.0000	Unknown
10.273	208288320	T		1.00000e+0	2.08288e+8	Unknown
11.413	41684920	T		1.00000e+0	41684920.0000	Unknown
12.100	34073204	T		1.00000e+0	34073204.0000	Unknown
12.313	35788064	I		1.00000e+0	35788064.0000	Unknown
12.607	16063985	T		1.00000e+0	16063985.0000	Unknown
13.187	42174460	I		1.00000e+0	42174460.0000	Unknown
13.713	27128077	I		1.00000e+0	27128077.0000	Unknown
14.173	21293694	I		1.00000e+0	21293694.0000	Unknown
14.723	20904600	I		1.00000e+0	20904600.0000	Unknown
15.047	21117488	I		1.00000e+0	21117488.0000	Unknown
15.447	45804920	I		1.00000e+0	45804920.0000	Unknown
16.120	235769216	I		1.00000e+0	2.35769e+8	Unknown
16.463	37543433	T		1.00000e+0	37543433.0000	Unknown
17.56	141202144	I		1.00000e+0	1.41202e+8	Unknown
18.167	113379165	T		1.00000e+0	1.13379e+8	Unknown
19.203	572783168	T		1.00000e+0	5.72783e+8	Unknown
19.867	99104912	T		1.00000e+0	99104912.0000	Unknown
20.180	99031744	I		1.00000e+0	99031744.0000	Unknown
21.027	164005696	T		1.00000e+0	1.64006e+8	Unknown
21.853	75909456	I		1.00000e+0	75909456.0000	Unknown
22.173	102591544	T		1.00000e+0	1.02592e+8	Unknown
22.86	59515856	I		1.00000e+0	59515856.0000	Unknown
23.347	38709812	T		1.00000e+0	38709812.0000	Unknown
24.207	20445384	I		1.00000e+0	20445384.0000	Unknown
24.941	47962080	I		1.00000e+0	47962080.0000	Unknown
25.323	148691008	I		1.00000e+0	1.48691e+8	Unknown
26.203	29844404	I		1.00000e+0	29844404.0000	Unknown
2[illegible].093	71008176	I		1.00000e+0	71008176.0000	Unknown
27.413	78716640	T		1.00000e+0	78716640.0000	Unknown
28.56	20346104	I		1.00000e+0	20346104.0000	Unknown
2[illegible].047	17677280	I		1.00000e+0	17677280.0000	Unknown
29.[illegible]	1141647[illegible]	I		1.00000e+0	1141647[illegible].0000	Unknown
[illegible].293	174684592	T		1.00000e+0	1.74685e+8	Unknown
32.041	29819714	I		1.00000e+0	29819714.0000	Unknown
33.193	11101013	T		1.00000e+0	11101013.0000	Unknown
[illegible].853	4096[illegible]796	I		1.00000e+0	4096[illegible]796.0000	Unknown
33.333	15906016			1.00000e+0	15906016.0000	Unknown
39.253	1[illegible]858	I		1.00000e+0	1[illegible]858.0000	Unknown
41.[illegible]	91[illegible]993			1.00000e+0	91[illegible]993.0000	Unknown
44.353	161352			1.00000e+0	161352.0000	Unknown
55.273	186509			1.00000e+0	186509.0000	Unknown


(SITOST23.D01) Abs
Minutes
0.0
10.0
20.0
30.0
40.0
50.0
60.0
3.273
5.167
5.667
8.393
9.307
10.273
11.413
13.187
16.960
17.567
18.167
19.867
20.180
21.027
22.867
23.647
24.840
25.213
30.293
32.047
33.193
33.853
37.353
39.553
41.033
44.353
55.273

MODEL 1022 RUNLOG for run: SITOS125

Pump Fault. Run Terminated Automatically.

ACN H_3PO_4 1N : H_2O Gradient 5 to 45 %

File : SITOS125.Dat tabebuia.cor BRANCA VALVES
Run : 04 Type : Sample
Path : C: CHROM Inst : 1022 LC Plus
Collection : 16:48:06 Nov 26 1997 Method : TABEBUIA [16:32:43 No. 26 1997]
Integration: 16:48:06 Nov 26 1997 Method : TABEBUIA [16:32:43 Nov 26 1997]
Report : 17:44:02 Nov 28 1997 Method : TABEBUIA [16:32:43 Nov 28 1997]

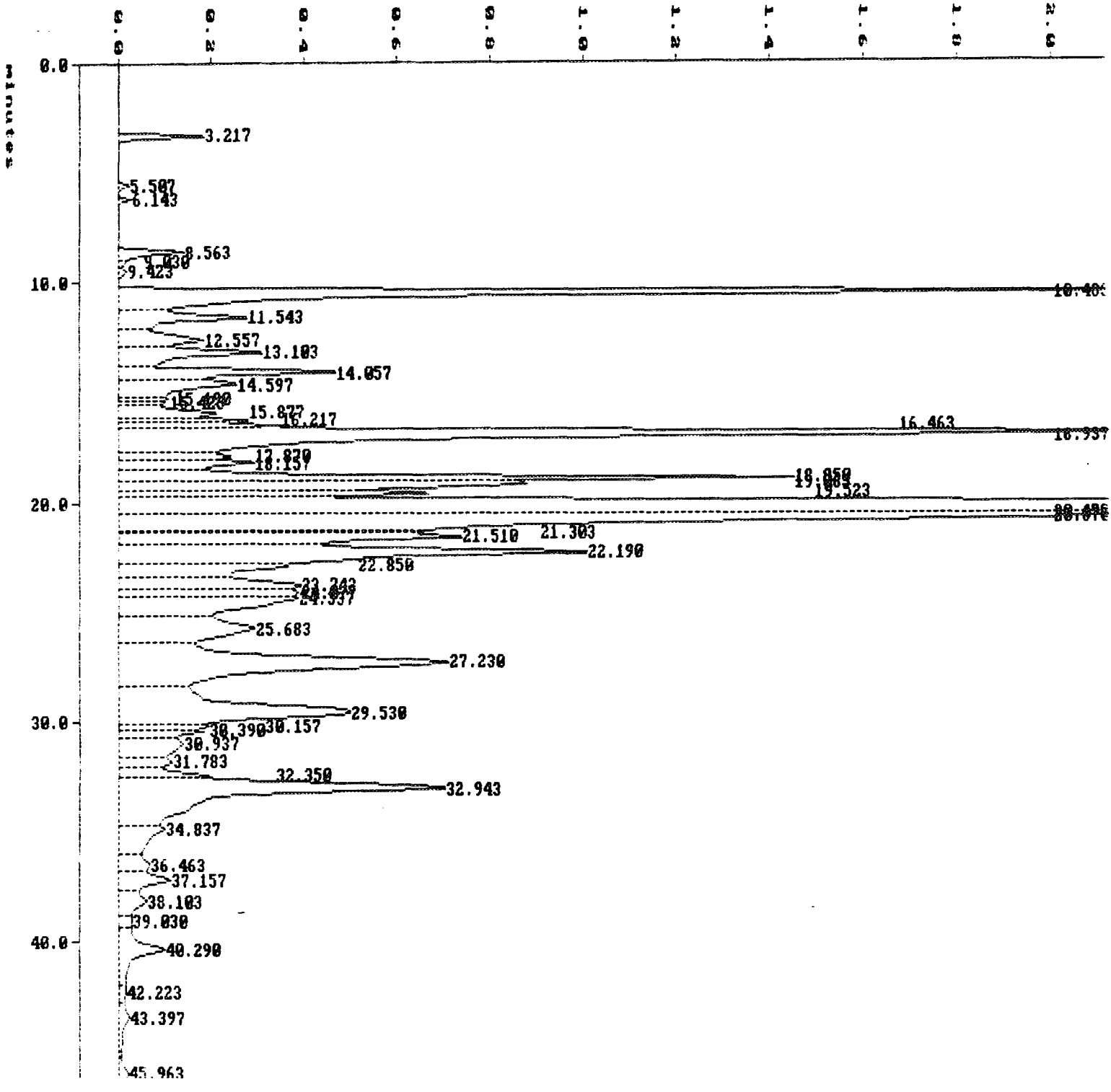
Sample Amt : 1.00000e+0 Dilution: 1.00000e+0

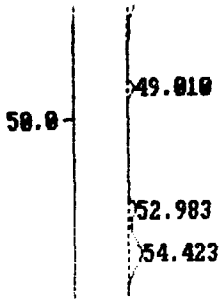
EXTERNAL STANDARD (AREA)

RT	Area	BC	ExpRT	Rf	Conc	Name
3.217	7a98b75			1.000000e+0	7a98b75.0000	Unknown
5.507	1297e90			1.000000e+0	1297e90.0000	Unknown
6.147	1200414			1.000000e+0	1200414.0000	Unknown
8.5a3	1149500			1.000000e+0	1149500.0000	Unknown
9.020	10e0999			1.000000e+0	10e0999.0000	Unknown
9.423	1494779			1.000000e+0	1494779.0000	Unknown
10.483	20812fd24			1.000000e+0	2.0812fd2e+8	Unknown
11.543	3e121f3e			1.000000e+0	3e121f3e.0000	Unknown
12.557	27957b20			1.000000e+0	27957b20.0000	Unknown
13.103	41552le0			1.000000e+0	41552le0.0000	Unknown
14.057	47282e9e			1.000000e+0	47282e9e.0000	Unknown
14.597	40ee51e8			1.000000e+0	40ee51e8.0000	Unknown
15.143	6187f4e			1.000000e+0	6187f4e.0000	Unknown
15.423	6e51307			1.000000e+0	6e51307.0000	Unknown
15.677	25287824			1.000000e+0	25287824.0000	Unknown
16.217	1588f304			1.000000e+0	1588f304.0000	Unknown
16.463	2170f04			1.000000e+0	2170f04.0000	Unknown
16.837	2857328e4			1.000000e+0	2.857328e+8	Unknown
17.870	19187f04			1.000000e+0	19187f04.0000	Unknown
18.157	298eee4e			1.000000e+0	298eee4e.0000	Unknown
18.350	99e0331e			1.000000e+0	99e0331e.0000	Unknown
19.0a3	10e522182			1.000000e+0	1.0e522e+8	Unknown
19.527	4859e7e8			1.000000e+0	4859e7e8.0000	Unknown
20.470	42998b1f			1.000000e+0	4.2998b1e+8	Unknown
20.677	3e2104e2			1.000000e+0	3.e2104e+8	Unknown
21.303	312e0f24			1.000000e+0	312e0f24.0000	Unknown
21.510	81e24224			1.000000e+0	81e24224.0000	Unknown
22.190	16e29785e			1.000000e+0	1.6e2978e+8	Unknown
22.650	51544e7			1.000000e+0	51544e7.0000	Unknown
23.743	5147e5e8			1.000000e+0	5147e5e8.0000	Unknown
24.077	145e2e2e			1.000000e+0	145e2e2e.0000	Unknown
24.337	7910f060			1.000000e+0	7910f060.0000	Unknown
25.e87	8857488			1.000000e+0	8857488.0000	Unknown
27.220	16e522e1f			1.000000e+0	1.6e522e+8	Unknown
29.510	147758192			1.000000e+0	1.4775de+8	Unknown
30.153	14e13899			1.000000e+0	14e13899.0000	Unknown
31.793	1e44e7e1			1.000000e+0	1e44e7e1.0000	Unknown
32.497	2046ee80			1.000000e+0	2046ee80.0000	Unknown
33.187	1424537e			1.000000e+0	1424537e.0000	Unknown
33.350	2e401508			1.000000e+0	2e401508.0000	Unknown
33.447	1e7140e8			1.000000e+0	1.e714e+8	Unknown
34.837	285e1380			1.000000e+0	285e1380.0000	Unknown
35.4e1	17451521			1.000000e+0	17451521.0000	Unknown
36.15	2e61e0e			1.000000e+0	2e61e0e.0000	Unknown

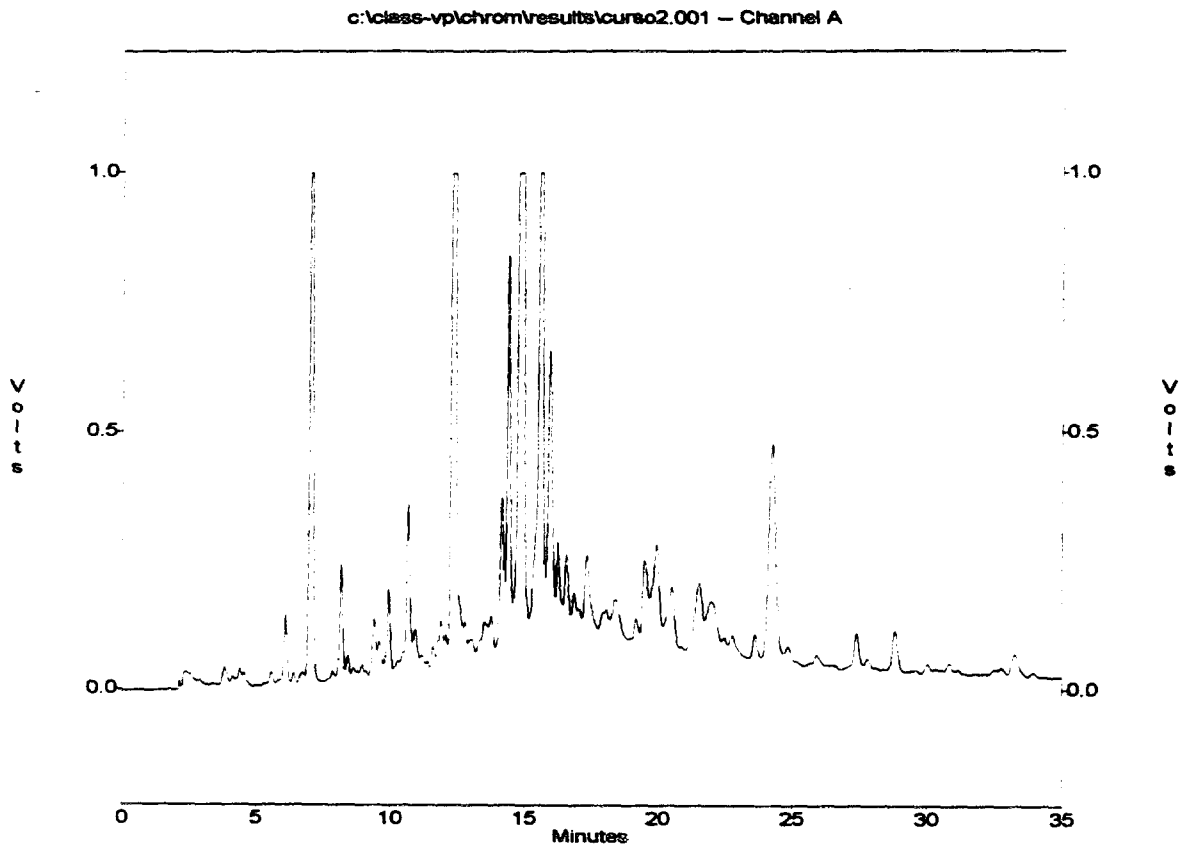
38.103	14685666 T	1.00000e+0	14685666.0000	Unknown
39.030	4289375 T	1.00000e+0	4289375.0000	Unknown
40.290	2785519 T	1.00000e+0	2785519.0000	Unknown
42.223	3164905 T	1.00000e+0	3164905.0000	Unknown
43.397	8065773 T	1.00000e+0	8065773.0000	Unknown
45.963	5431363	1.00000e+0	5431363.0000	Unknown
49.010	1127960	1.00000e+0	1127960.0000	Unknown
52.983	1629736 T	1.00000e+0	1629736.1250	Unknown
54.423	5933080	1.00000e+0	5933080.0000	Unknown

(SITOST25.D01) Abs




49.010
50.0
52.983
54.423

Muestra Tabebuia
1,5mL/min 254nm
Acquired : Nov 28, 1997 14:56:50
Printed : Nov 28, 1997 16:11:25
File Desc. : MUESTRA DE Tabebuia (corteza)
Fase móvil: H20:H3PO4 1N en ACN
- 0 90 : 10
30 40 : 60
60 40 : 60
62 90 : 10
254nm 1,5mL/min 235Kgf/cm2
c-18



Channel A Results

Time	Area
7.09	10464989.0
8.17	2112276.0
9.96	2136180.0
10.68	4175393.0
12.43	15624030.0
13.51	2713222.0
14.17	4028907.0
14.42	8286408.0
14.98	19478964.0
15.68	16521214.0
15.96	8902105.0
16.27	3316638.0
16.58	3540294.0
16.87	2398079.0
17.34	5824854.0
18.42	5236204.0
19.50	3906749.0

Continued...

Acquired : Nov 28, 1997 14:56:50
Printed : Nov 28, 1997 16:11:27
File Desc. : MUESTRA DE Tabebuia (corteza)
Channel A Results

Time	Area
19.95	6003460.0
20.52	4897263.0
21.54	5490040.0
21.99	4983450.0
22.77	3277599.0
24.27	10054810.0
25.91	2325057.0
27.40	2287646.0
28.82	2123534.0

CONFIDENTIEEL

Standardized Aesculus hipposcastanus extract:

1. Introduction:

The standardized extract is a mixture of Aesculus hippocastanus semen and cortex.

The 2 main compounds in this extract are:

- esculin: compound of Aesculus hippocastanus cortex
- escin: compound of Aesculus hippocastanus semen

Esculin is determined by HPLC and escin by spectrofotometry.

2. Quantitation of esculin by HPLC:

* Instrument:	Gilson HPLC - System with UV - VIS detector
* Stationary Phase:	Lichrospher RP 18e (5 µm) column: lenght = 244.00 mm Internal diameter = 4.00 mm
* Mobile Phase:	A: 0.5% Phosphoric acid in 5% methanol B: Acetonitril
* Detection:	λ = 340 nm
* Flow:	1.0 ml/min
* Injection volume:	20 µl test- or reference solution; the injection is done by a Gilson 234 automatic injector
* Reference solution:	+/- 20 µg/ml esculin in methanol 40%
* Test solution:	Bring in a measuring flask of 50 ml +/- 50.0 mg extract. Dissolve in 30 ml methanol 40% and add methanol 40% up to 50.0 ml.

CONFIDENTIEL

Aesculus hippocastanus cortex

I. Methodology:

* Quantitation of esculin by HPLC:

* Instrument:	Gilson HPLC - System with UV - VIS detector
* Stationary Phase:	Lichrospher RP 18e (5 µm) column: lenght = 244.00 mm Internal diameter = 4.00 mm
* Mobile Phase:	A: 0.5% Phosphoric acid in 5% methanol B: Acetonitril
* Detection:	λ = 340 nm
* Flow:	1.0 ml/min
* Injection volume:	20 µl test- or reference solution; the injection is done by a Gilson 234 automatic injector
* Reference solution:	+/- 20 µg/ml esculin in methanol 40%
* Test solution:	Add to 1.0 g cortex, 100 ml methanol 70% and reflux for 30 minutes. Decant the solution over a glass filter. Add 100 ml of methanol 70% to the sediment, reflux and decant again. These actions are done 2 more times. Evaporate the solvent till +/- 100 ml and bring quantitativily over in a measuring flask of 250 ml. Add methanol 40% up to 250 ml. Dilute 5.0 ml of this solution to 20.0 ml with methanol 40%.

The World Health Organization is a specialized agency of the United Nations with primary responsibility for international health matters and public health. Through this organization, which was created in 1948, the health professions of some 180 countries exchange their knowledge and experience with the aim of making possible the attainment by all citizens of the world by the year 2000 of a level of health that will permit them to lead a socially and economically productive life.

By means of direct technical cooperation with its Member States, and by stimulating such cooperation among them, WHO promotes the development of comprehensive health services, the prevention and control of diseases, the improvement of environmental conditions, the development of human resources for health, the coordination and development of biomedical and health services research, and the planning and implementation of health programmes.

These broad fields of endeavour encompass a wide variety of activities, such as developing systems of primary health care that reach the whole population of Member countries; promoting the health of mothers and children; combating malnutrition; controlling malaria and other communicable diseases including tuberculosis and leprosy; coordinating the global strategy for the prevention and control of AIDS; having achieved the eradication of smallpox, promoting mass immunization against a number of other preventable diseases; improving mental health; providing safe water supplies; and training health personnel of all categories.

Progress towards better health throughout the world also demands international cooperation in such matters as establishing standards for biological substances, pesticides and pharmaceuticals; formulating environmental health criteria; recommending international nonproprietary names for drugs; administering the International Health Regulations; revising the International Statistical Classification of Diseases and Related Health Problems; and collecting and disseminating health statistical information.

Reflecting the concerns and priorities of the Organization and its Member States, WHO publications provide authoritative information and guidance aimed at promoting and protecting health and preventing and controlling disease.

RESEARCH GUIDELINES FOR EVALUATING THE SAFETY AND EFFICACY OF HERBAL MEDICINES

World Health Organization
Regional Office for the Western Pacific
Manila
1993

1. INTRODUCTION

Background

Herbal medicines, as the major remedy in traditional medical systems, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. The use of these medicines has a particularly rich tradition among the peoples of the Western Pacific Region. In recent years, this has extended far beyond its original ethnic setting. The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas.

The World Health Organization is fully aware of the importance of herbal medicines to the health of many people throughout the world, as stated in a number of resolutions adopted by the World Health Assembly and the Regional Committee for the Western Pacific. Thus herbal medicines have been recognized as a valuable and readily available resource for primary health care, and WHO has endorsed their safe and effective use. A comprehensive programme for the identification, cultivation, preparation, evaluation, utilization and conservation of herbal medicines has been developed. Meanwhile, it has been realized that medicinal plants are a valuable resource for new pharmaceutical products and thus a potential source of new drugs as well as for economic development.

Definition of terms

Herbal medicine	A plant-derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. In some traditions, materials of inorganic or animal origin may also be present.
Characterizing compound	A natural constituent of a plant part that may be used to assure the identity or quality of a plant preparation, but is not necessarily responsible for the plant's biological or therapeutic activity.
Biological activity	A change in the base-line function of an animal or part of an animal brought about by the administration of a test substance.
Therapeutic activity	An intervention that results in the amelioration of the manifestations of human disease.
Processed plant materials	Plant materials treated according to traditional procedures to improve their safety and/or efficacy, to facilitate their clinical use, or to make medicinal preparations.
Medicinal preparations of plant materials	Medicinal preparations that contain one or more of the following: powdered plant materials, extracts, purified extracts, or partially purified active substances isolated from plant materials. In certain cases, materials of animal or mineral origin may also be included in such preparations.

2. GENERAL CONSIDERATIONS IN HERBAL MEDICINE RESEARCH

Legal considerations

Governments should actively promote the rational use of herbal medicines that have been scientifically validated. To do so, they need a national policy for approving those that are safe and effective for specified clinical indications. The adoption of such policy will help to overcome some of the legal barriers against the use of herbal medicines which in some countries may still be inadequately standardized.

Legislation concerning procedures for the registration of herbal medicine can play a very important role in ensuring that medicinal plant preparations are of acceptable quality, safety and efficacy. Research on herbal medicines, which is necessary to ensure their improved utilization by the public, would benefit from strong governmental endorsement.

Ethical considerations

Research on herbal medicines must be carried out in accordance with all relevant ethical guidelines.

The requirement of evidence as to the safety and efficacy of herbal medicines and the method of research chosen should be adjusted to the original purpose of the research.

Selection of research projects

Research projects should be selected with due consideration for several factors in addition to scientific interest. Three of these are:

(1) potential value of the research results for improving the health of the community with due regard to the prevalence of disease and the feasibility of using alternative treatments;

(2) the medical value of indigenous plants;

(3) technical and financial considerations.

Research approaches

Research on herbal medicines in the past has generally been carried out by individual researchers working independently. One researcher may find an active principle whose pharmacological and toxicological properties are then further studied elsewhere. Finally, yet another group may decide to go directly to human studies.

A single multidisciplinary group may enable more rapid progress. In such a group the first step might be to collect information on folkloric experience whose scientific validity is then investigated. If appropriate pharmacodynamic studies seem to verify the traditional use, the group can begin to conduct more general pharmacological and toxicological tests to assure the safety of the medicinal product, which can then be tested in an initial clinical trial. Additional confirmatory clinical trials may be conducted if warranted.

In certain instances, the isolation of an active substance may be useful in order to provide an exact dosage. In many cases, however, the plant preparation as a whole is therapeutically effective even though the active principle is not known. The clinical investigation of the therapeutic activity of such crude preparations may be useful, because that activity may depend not only on a single substance but may be influenced by a large number of other components in the herbal medicine.

Assuring access to relevant databases

Databases devoted to herbal medicines and natural products have been established in several countries and areas including China, Hong Kong, Japan and the United States. Easy access to such databases greatly facilitates the efforts of those interested in herbal medicines. Since the maintenance of such databases and access to them are costly, a government financial subsidy may be necessary in order to assure access of researchers and health planners to the information needed to hasten the rational use of herbal medicines in their countries.

Education

Dissemination of knowledge about herbal medicines in the form both of courses for professional health workers and of information for the public can greatly aid the overall effort to promote the rational use of herbal medicines.

Quality specifications of plant materials and preparation

All research on herbal medicines must specify the quality of the plant material or the preparation being investigated, in order that studies conducted by one investigator may be corroborated by other investigators (see Guidelines A, page 27).

Non-clinical studies

The primary objectives of non-clinical studies are:

- to determine whether such studies support the clinical use of a herbal medicine;
- to characterize the range of pharmacological actions of herbal medicines; and
- to define the chemical characteristics of pharmacologically active natural products and to elucidate their mechanisms or actions.

Pharmacodynamic investigations

Pharmacodynamic investigations are conducted in the light of the expected therapeutic effect of a herbal medicine using appropriate non-human systems.

General pharmacological investigations

General pharmacological investigations are conducted to elucidate various pharmacological activities other than the main pharmacodynamic action. Such investigations usually cover the tests on nervous, cardiovascular and respiratory systems, and if necessary others, and should be performed on conscious or

anaesthetized animals using adequate doses and proper routes of administration.

Toxicological investigations

Toxicological investigations are required to supplement human experience in defining possible toxicity from short-term use, but are particularly important in detecting toxicity that may occur either after prolonged exposure or years after the exposure has been discontinued. Generally, the longer the anticipated human use, the longer the test substance is administered to test animals.

Methods

In the conduct of non-clinical research on herbal medicines, standard methods are usually employed. However, the use of novel technologies and methods resulting from scientific progress should be encouraged.

1. Pharmacodynamic and general pharmacological methods should utilize animal models or bioassays that closely relate to human disease as described by either traditional or modern medicine (see Guidelines B, page 31).

2. Toxicological methods

   Animal and other toxicity studies are conducted according to generally accepted principles, referred to collectively as Good Laboratory Practice (GLP), which should be consulted in order to design appropriate studies (see Guidelines C, page 35).

Clinical trial protocol development

The development of a protocol should be the joint effort of representatives from several disciplines such as clinical pharmacologists, pharmacists, biostatisticians, physicians and other relevant health care workers, as well as experts in traditional medicine. Ordinarily, the protocol group is chaired by the chief investigator, who is a physician. The protocol should include the following:

1. The title of the trial.
2. A clear statement on the objectives of the study.
3. The justification of the proposed trial based on the available information on safety and efficacy, including a consideration of the non-clinical data as well as the drug utilization pattern and the disease spectrum for the country concerned.
4. The rationale for the composition of the formula being studied and its relation to the principles of both herbal medicine and pharmacodynamic data.
5. The type of trial (such as controlled, open) and trial design (parallel groups, cross-over techniques), blind technique (double blind, single blind), randomization (methods and procedures).
6. Entry and exclusion criteria for study subjects (which may be based on diagnostic criteria of either modern or traditional medicine).
7. Number of trial subjects needed to achieve the trial objective, based on statistical considerations.
8. The therapeutic or clinical end points that are to be analysed at the conclusion of the trial (the unique nature of traditional medicine, which can relate to subjective wellness or quality of life, should also be considered when selecting the end points of the trial).
9. Control groups to be used (whether a therapeutic control group or a placebo group is used will depend on the disease being studied and the availability of alternative modern drugs or herbal medicines of proven efficacy).
10. The subjective and objective clinical observations and laboratory tests which will be recorded during the course of the trial.
11. The treatment schedule for the duration of the trial, including dosage form and route of administration and the details of the product being used as a therapeutic control.
12. Criteria for other treatments that may or may not be given to subjects during the trial.
13. Procedures for the maintenance of subject identification code lists, treatment record, randomization list and/or Case Report Form (CRF).
14. Information on establishment of the trial code, where it will be kept and when, how and by whom it can be broken in the event of an emergency.
15. The qualifications and experience of the investigators.
16. The facilities and the sites where studies will be undertaken.
17. Methodology for the evaluation of results (such as statistical methods and reports on patients or participants who withdrew from the trial).
18. Information to be given to trial subjects.

patient protection, and issues of informed consent of patients. The work of the board should be guided by the World Medical Association's Declaration of Helsinki (Annex 2).

The board will work under standard operating procedures which will be developed by each institution taking into consideration all necessary requirements of local regulatory authorities and related governmental agencies including such rules as those for Good Clinical Practice (GCP).

Responsibilities of investigators

The investigators who participate in the design of the protocol will also be responsible for preparing all necessary material for review by the ethics review board.

The investigators must be aware of such responsibilities as the following:

- the appropriate medical care of patients in the study;
- the ethical requirements for the trial (such as selection of patients, advice to patients);
- a knowledge of the product used in the trial;
- an appreciation of research methodology and the conduct of clinical trials (such as the recording and evaluation of results);
- an appreciation of the importance of careful monitoring of the trial and the need to take necessary action, to alter or terminate the trial if patients appear to be harmed by some aspect of the trial.

Responsibilities of the sponsor

If the product under investigation is supplied by a manufacturer, or if the trial is undertaken at the request of a manufacturer, the manufacturer (sponsor) has obligations to maintain the integrity of the investigators, the protocol group and the ethics review board, and to prevent harm to a patient. The sponsor of a study can be an institution or an individual investigator as well as a manufacturer.

The material supplied for the trial will be prepared according to Good Manufacturing Practices (GMP) to ensure the quality of the material used in the investigation. All data on the product will be made available to the investigator before the trial design is completed.

The sponsor must meet all of the local requirements set by regulatory authorities and government agencies and should be aware of standards of good clinical practice.

Data management

The aim of record keeping and the handling of data is to gather information from the trial without error in a form that can later be analysed and reported. A Case Report Form (CRF) for each patient in the trial must be completed and signed by the investigator and the patient's files, CRFs and other sources of primary data must be kept for future reference. Patient data must be handled in a way that maintains confidentiality and yet ensures accuracy. All efforts should be made to maintain error-free records.

When subjects are randomized to different groups, the randomization procedure used must be documented. In the case of a blinded trial, a code for the medicine actually administered must be kept under appropriate conditions.

4. USING THE GUIDELINES

These research guidelines for evaluating the safety and efficacy of herbal medicines are intended to facilitate the work of research scientists and clinicians in this field and to furnish some reference points for the governmental, industrial and non-profit organizations that provide financial support for their work. It is hoped that these guidelines will be found general enough to enable each Member State to modify them to meet its own specific needs. It must be emphasized that these guidelines are offered as a summary of scientific standards governing various aspects of the study of herbal medicines. As such, they may be useful to the regulatory authorities who control the sale of these products and the governmental agencies and medical authorities who supervise their use in the health care system.

- characterizing compounds of the plant materials, which may also be the biologically or therapeutically active principle, should be quantified and described with their structural formulae, particularly if they are uncommon. For processed plant material, changes in the quantities of these characterizing compounds should be described.

Quality specifications

Authenticity. A description of the macroscopic, microscopic and sensory characteristics of the plant should be provided, including drawings or photographs if possible. A description should be provided of the physical or chemical tests done to identify the plant substances and chromatogram of the active fraction or characterizing compound should be provided. If this is not possible, it should be sufficient to identify a characteristic mixture of substances ("finger print") of the plant material.

Purity. Limits of foreign organic matter (such as stem and rachis fragments in the leaves or leaflets, leaf fragments in the flowers, etc.) and foreign mineral matter (such as sand and soil adhering to the plant material) should be specified; ash determinations should be provided.

Assay. A physical, chemical or biological assay of any known or active fractions should be described and the biological activity of the plant materials expressed in terms of this assay along with an acceptable range for the assay results.

Packaging, labelling and storage

The conditions for packaging, labelling and storage should all be recorded.

Information for medicinal preparations of plant materials

Among the medicinal preparations now widely used are powders, granules, pills, extracts, tablets and injections. Traditional powders and pills are made of powdered plant materials; tablets, granules, ointments and newer types of pills are mostly made of extracts; injections are made of purified extracts or pure active constituents isolated from the plant material. There are also certain medicinal preparations made of both powdered plant materials and extracts.

Name and formula of the product

- Name in Latin, English and native languages.
- Formula including the name of each ingredient and the quantities used for 1000 g or 1000 ml of the product. A quantity may be given as a range corresponding to a definite quantity of assayed active constituents. Any excipient used should be specified.
- Method of preparation to make 1000 g or 1000 ml of the product. The description of the method should include details of any process, such as solvent used, time and temperature of an extraction and concentration, as well as the process used to reduce the level of microbial contamination.
- The active constituents, as far as they are known, should be stated and their structural formulae given. Any chemical or pharmacological incompatibility should be mentioned.

Quality specifications

Authenticity. A description of macroscopic and sensory characteristics should be given and, if powdered plant materials are used as ingredients, their microscopic characteristics should be described

Animals with genetic defects can also be useful: for example the autoimmune mouse (NZB W/F1, MRL/1) and the hypertensive rat (SHR), etc. For study of those herbal medicines which are used under the principles of traditional medicine, animal models may need to be established according to those principles.

Test assays can use

- whole animals;
- isolated organs and tissues;
- blood and its components;
- *ex vivo* and tissue culture cells; and
- subcellular constituents.

Careful attention must be given to the selection of the test system since *in vitro* assays, although less expensive, may not provide such factors as metabolic activation which may be necessary for the biological activity of a herbal medicine. On the other hand, body fluids from test animals may contain such biologically active metabolites and be used successfully in less complex test systems.

Special attention should be given to the sensitivity, reproducibility and general acceptance of the test animals or test systems selected.

An examination of the literature may help to select the species and test systems considered to be most predictive of clinical results and therefore provide the most useful information.

Administration

Route of administration

Since oral dosage forms of herbal medicines are usually used clinically, the oral route of administration is ordinarily the most suitable for use with test animals. Additional routes may be used to approximate the intended route of administration in man.

Frequency of administration

Ordinarily, doses selected for a study should be established by means of a dose-response relationship but since such relationships often cannot be demonstrated with herbal medicines in whole animals, it may be sufficient to select one or more doses that provide a desired effect.

Selection of doses for animal studies should be in accordance with customary clinical doses.

Control group

It is essential that all studies include a negative (vehicle only) control group of animals and, if possible, a positive control group, that is, a group of animals in which the effect of a drug known to be positive is examined.

In cases where it is proposed to administer the herbal preparation to a human subject by the parenteral route, it may be sufficient to use this route alone for animal testing.

Dose levels

A sufficient number of dose levels should be used in rodents to determine the approximate lethal dose. In non-rodents, sufficient dose levels should be used for the observation of overt toxic signs.

Frequency of administration

The test substance should be administered in one or more doses during a 24-hour period.

Observation

Toxic signs and the severity, onset, progression and reversibility of the signs should be observed and recorded in relation to dose and time. As a general rule, the animals should be observed for at least seven to fourteen days.

Animals dying during the observation period, as well as rodents surviving to the end of the observation period should be autopsied.

If necessary, a histopathological examination should be conducted on any organ or tissue showing macroscopic changes at autopsy.

Long-term toxicity test

Animal species

Many regulatory agencies require that at least two species be used, one a rodent and the other a non-rodent.

Sex

Normally, the same number of male and female animals should be used.

Number of animals

In the case of rodents, each group should consist of at least ten males and ten females. In the case of non-rodents, each group should consist of at least three males and three females.

When interim examinations are scheduled, the number of animals should be increased accordingly.

Route of administration

Normally, the expected clinical route of administration should be used.

Administration period

The period of administration of the test substance to animals will depend on the expected period of clinical use. The period of administration of the toxicity study may vary from country to country, according to its individual regulations.

The following table reflects commonly used ranges of administration periods:

Expected period of clinical use	*Administration period for the toxicity study*
Single administration or repeated administration for less than one week	2 weeks to 1 month
Repeated administration, between one week to four weeks	4 weeks to 3 months
Repeated administration, between one to six months	3 to 6 months
Long-term repeated administration for more than six months	9 to 12 months

6. In order to maximize the amount of useful information that can be obtained during the administration period, all moribund animals should be sacrificed rather than allowed to die. Prior to sacrifice, clinical observations should be recorded and blood samples collected for haematological and blood chemical analysis. At autopsy, a macroscopic examination of organs and tissues and measurement of organ weights should be recorded. A full histopathological examination should be performed in an attempt to characterize the nature (severity or degree) of all toxic changes.

All survivors should be autopsied at the end of the administration period or of the recovery period after taking blood samples for haematological (including blood chemistry) examinations; organs and tissues should be examined macroscopically and organ weights measured. Histopathological examination of the organs and tissues of animals receiving lower dosage should also be performed, if changes are found on gross or macroscopic examination of their organs and tissues of these animals, or if the highest dose group reveal significant changes. On the other hand, histopathological examination of all rodents will further improve the chances of detecting toxicity.

Recovery from toxicity

In order to investigate the recovery from toxic changes, animals that are allowed to live for varying lengths of time after cessation of the period of administration of the test substance, should be examined.

Local toxicity test

Skin sensitization test

Dermatological preparations to be tested

- solid preparations:

  To be prepared by wetting the preparation with water or a suitable solvent to provide a uniform application.

- semi-solid preparations:

  To be tested as undiluted preparations.

- liquid preparations:

  To be tested as undiluted preparations. However, an aerosol agent can be diluted if necessary.

Experimental animals

Use a species with high susceptibility. Guinea-pigs are considered the most suitable experimental animals.

Test methods (in alphabetical order)

1. Adjuvant and patch test
2. Buehler test
3. Draize test
4. Freund's complete adjuvant test
5. Maximization test
6. Open epicutaneous test

4. Metabolic activation:

   Tests should also be performed with a suitable method of metabolic activation (such as, S9 mix)

5. Experimental procedure:

   a. Chromosomal preparations should be made at an appropriate time after treatment.

   b. At least two plates should be used for each dose level. Examination should be made for chromosomal structural aberrations and polyploid cells on 100 metaphase cells per plate.

6. Presentation of results:

   The relative frequency of cells with chromosomal aberrations and the frequency of chromosomal aberrations per cell should be presented in tables.

III. Micronucleus test with rodents

1. Animals:

   Male mice should normally be used.

2. Number of animals:

   Each group should consist of at least five animals.

3. Route of administration:

   Administration should be intraperitoneal or via the expected clinical route.

4. Dose levels:

   At least three dose groups should be employed.

5. Control groups:

   As a general rule, a solvent group should serve as a negative control. A positive control group should receive a substance known to induce micronuclei.

6. Frequency of administration:

   Single or repeated administration may be employed.

7. Experimental procedure:

   a. Animals should be sacrificed at an appropriate time after administration of the test substance, and bone marrow smears prepared.

   b. Normally, observation should be made of the incidence of micronuclei in 1000 polychromatic erythrocytes per animal. The relative frequency of polychromatic erythrocytes and total erythrocytes should also be calculated.

8. Presentation of results:

   The incidence of polychromatic erythrocytes with micronuclei and the frequency of polychromatic erythrocytes per total erythrocytes should be presented in tables.

Carcinogenicity test

Experimental animals

1. Species and strains of the animals should be selected in consideration of such factors as resistance against infectious disease, life span, spontaneous tumour incidence, and sensitivity to known carcinogens.

2. Animals of the same species and strain should be used for preliminary and full-scale carcinogenicity studies with the same test substance.

2. It is desirable that the highest dose should be set for each species and sex.

2. Full-scale carcinogenicity study

a. Animals:

At least two species of animals of both sexes should be employed. It is desirable to use animals with normal growth of the same age, up to the age of six weeks.

b. Number of animals:

Each group should comprise at least 50 males and 50 females. Allocation of the animals to each group should be made with the proper random sampling method based on body weight, etc.

c. Route of administration:

The expected route of clinical application should be used, if possible.

d. Dose levels:

At least three dose groups and a control group should be employed for each sex.

e. Control group:

i. A negative control group should be included.

ii. If various vehicles or emulsifiers are required to administer the test substance, the negative control group should receive such vehicles or emulsifiers alone. It is also desirable to establish an untreated control group.

f. Administration period:

The administration period should last from 24 to 30 months for rats and from 18 to 24 months for mice and hamsters, with administration normally performed seven days a week.

g. Experimental period:

Studies should be terminated from one to three months after the administration of the test substance has been terminated. However, the maximum experimental period should be 30 months for rats and 24 months for mice and hamsters. When cumulative mortality reaches 75% in either the lowest dose group or in the control group of either sex, the survivors of that sex should be sacrificed and the study terminated.

h. Experimental procedure:

i. All animals of each group should be observed daily for general signs, and body weight should be measured at least once a week during the first three months of administration of the test substance and at least once every four weeks thereafter.

ii. Animals that died during the experimental period should be autopsied immediately and macroscopic and histopathological examinations should be made of organs and tissues.

iii. Animals that appear to be moribund during the experimental period should be isolated or sacrificed and autopsied immediately and organs and tissues should be examined macroscopically and histopathologically. At the time of sacrifice, blood samples should be taken to

e. Control group:

i. A negative control group should be employed. A positive or a comparative control group is desirable.

ii. When vehicles or emulsifiers are required for the administration of the test substance, a negative control group should normally receive such vehicles or emulsifiers alone. A positive control group should receive a substance known to have potent reproductive and developmental toxicity, and a comparative control group should receive a drug with a similar chemical structure or pharmacological effects as the tested drug.

f. Administration period:

When rats or mice are used, males at least 40 days of age should be dosed daily for 60 days or more before mating, and administration should be continued until successful copulation. Sexually mature females should be dosed daily for at least 14 days before mating, during mating and after successful copulation until the beginning of organogenesis.

g. Experimental procedure:

i. During the experimental period, mortality should be recorded, general signs noted and body weights and food intake should be measured.

ii. A treated male and a treated female should be housed together and observed daily for confirmation of successful copulation.

iii. The mating period between the male and female pairs should be about two weeks. If necessary, a treated male and a non-treated

female, or a treated female and a non-treated male should be housed together and observed daily for confirmation of successful copulation.

iv. After successful copulation, females should be autopsied at term, and examined for the number of corpora lutea, successful pregnancies and mortality of fetuses. Additionally, a gross examination of the organs and tissues for all dams should be made.

v. Males used for mating and females without successful copulation should be autopsied at an appropriate time, and gross observation on organs and tissues should be made.

2. Segment II. Study on administration of the test substance during the period of organogenesis.

a. Animals:

Females of at least one species of rodent and a non-rodent such as rabbits should be used.

b. Number of animals:

Each group should consist of at least 30 animals for rats or mice and at least 12 animals for rabbits.

c. Route of administration:

The route of administration should ordinarily be that expected clinically.

d. Dose levels:

At least three different dosage groups plus a control group should be employed.

d. Dose levels:

At least three dose groups plus a control group should be employed.

e. Control group:

i. A negative group should be employed. A positive or a comparative control group may be employed, if necessary.

ii. When vehicles or emulsifiers are required for administration of the test substance, a negative control group should normally receive such vehicles or emulsifiers alone. A positive control group should receive a substance known to have potent reproductive and developmental toxicity and a comparative control group should receive a drug with a similar chemical structure or pharmacological effects.

f. Administration period:

i. During the experimental period, all the dams in each group should be examined for mortality and general signs and body weights and food intake should be measured.

ii. All the dams in each group should be allowed to deliver and nurse their offspring. Dams should be examined for abnormality on delivery.

iii. Litter size, mortality, sex and external changes of neonates should be examined, and body weights should be measured.

iv. Offspring should be examined for growth and development, appearance of specific signs, reproductive performance, etc. For observation of growth and development,

morphological, functional and behavioural examinations should be made. Reproductive performance of offspring should be examined on the basis of establishment of pregnancy. If necessary, observation for a longer period should be made.

v. At an appropriate time, autopsy and gross observations on organs and tissues should be made on treated dams. If necessary, an examination of the second litters should be done.

Analysis of results

1. The results obtained should be presented in the form of tables and figures with discussion of the results. For presentation, summary tables which give an overview of the results of all groups should be prepared. In addition, appendix tables which provide data for individual animals in each group should be prepared for reference.

2. For statistical analysis of the data obtained before weaning, it is desirable that the litter, instead of the individual fetus or offspring, serve as the unit for analysis.

3. The discussion should address the no-effect dose level of the test substance concerned with the reproduction of the parent animals and development of the next generation. It is desirable to compare the reproductive and developmental toxicity with that of similar drugs.

EXTRACTION OF DRUGS

1. ***GRINDING***

Grinding or mincing of drug means mechanically breaking down a given vegetable material. This normally is the first stage in the preparation of any vegetable derivative, whether simple or complex. In the process of grinding particle size homogeneity is normally a basic parameter. This governs at the extraction stage the uniform exhaustion of drug, which depends on the rate of diffusion of a substance from the granule to a solvent, the correct time, the rate of passage of the solvent through the drug and other aspects. Theoretically, the finer the granule, the faster (within certain limits) the extraction should be processed.

2. ***Extraction***

The extraction of drug is the separation by physical or chemical methods of a solid or liquid material from a solid (drug). Normally when the operation is performed with solvent for extracting the vegetable material it is called solid/liquid extraction. In the course of extraction two processes take place in parallel: the release of extractive substances from destroyed cells and the release of extractive substances from intact plant cells by a process of diffusion. The latter process is usually enhanced when the plant cell is treated with aqueous solvents, which causes swelling with consequent increased permeability or rupture of the cell wall. The procedures of extraction of drugs may be classified in two main groups

A. *Procedures in which it is sufficient to chive within set limits the equilibrium of concentration between drug and solution (macerations)*

B. *Procedures in which the drug is extracted until exhaustion of the soluble substances in the chosen medium.*

In type A of decreasing industrial importance the simplest case of is that of maceration, which may be static or dynamic; it also forms part of all processes, excluding countercurrent extraction processes, in which the aim is to ensure exhaustion of drug. In maceration equilibrium depends on the characteristics of drug, on its content of moisture, on the solvent used and on the contact time. These parameters influence one another and the optimal parameters have to be sought for each drug.

Every extractive procedure that leads to a concentration equilibrium stops when the distribution of the extractable substances between solvent and residual drug is constant. It is essential to know the value of this constant before deciding on the duration and number of extractions needed to exhaust a drug. Industrially the maceration process is often related to percolation. Percolation may be considered as repeated maceration.

Preatreating of a drug outside of the extractor is as a rule indispensable. The main reasons for this are:

1. To avoid sudden swelling of drug in a closed container, because if the solvent is aqueous, the drug may swell to two or three times its

original volume and so burst the extractor or make percolation impossible.
2. To ensure uniform moistening of the material for extraction and so prevent the formation of preferential channels, increasing the contact and passage of the solvent.
3 To increase the porosity of cell wall, thus facilitating diffusion of the extractive substances from cell to solvent or penetration of the cell by the solvent.

CHOICE OF EXTRACTION SOLVENT

To obtain the complete extraction of a given active principle from the drug the ideal solvent is obviously one that presenting maximum selectivity, has the best capacity for extraction in terms of coefficient of saturation of the product in the medium and is compatible with the properties of the material to be extracted. These requisites must as a rule be determined experimentally for each drug since the choice often depends on the stability of the compounds to be extracted and on possible interactions with other substances present. In principle, considering the above points separately, it may be said that aliphatic alcohols with up to three carbons or mixtures of them with water are the solvents with the greatest extractive power for almost all natural substances of low molecular weight like alkaloids, saponins and flavonoids. Ethyl alcohol in particular is the solvent of choice according to the pharmacopoeias for obtaining classic extracts such as tinctures, soft, fluid and dry extracts still widely used in pharmaceutical preparations. As these solvents have great extractive power they are the least selective and can still be use not only in the preparation of the above established extracts but also for the extraction of plants whose active principles is not yet known and an extract as complete as possible is needed. Still regarding the use of this type of solvent, the ideal alcohol / water ration for the extraction of woody parts of plants or barks, roots and seeds is about 7 : 3 or 8 : 2, whereas it must be lower (compatibly with the stability of the active principles)the 1 : 1 for extracting leaves or aerial green parts. With an alcohol / water ratio of 1 : 1 it is possible to avoid the extraction of chlorophyll, of resinous or polymeric substances that are normally of no importance to the activity of the extract but greatly complicate the subsequent stages of concentration by giving rise to gummy precipitates that are hard to eliminate. Lower strength hydroalcoholic mixtures with an alcohol/water ratio of 2 : 8 or 3 : 7 may be used in special cases not only for extraction but also under suitable temperature conditions for accomplishing target enzyme conversions in the actual course of extraction.
A case in the point is the classic conversion of primary glucosides of ***D. lanata***. lanatosides A,B and C into digitoxin, gitoxin and digoxin, respectively. This takes place at room temperature during the moistening of drug with water only or with water containing up to 20 % of alcohol In the contrary case, for the extraction of primary glucosides it is essential to operate with hydroalcoholic mixtures containing more than 50 % alcohol to block hydrolase activity

STATIC AND DYNAMIC MACERATION

The simplest process consist of pouring solvent onto the drug and, after a set time for every drug, straining of the extract and washing

the drug with fresh solvent to a prescribed weight. This procedure is useful for preparing tinctures or particular extracts and sometimes is the only process used for drugs rich in mucilage. However it is wasteful because it never exhaust the vegetable material. The drug retains a considerable portion of a solute which has to be recovered by pressing or centrifugation. This final stage is necessary step in any type of maceration, static or dynamic. If the material being extracted is costly, it is normal to choose the method that leads to exhaustion of the residue.

SIMPLE AND CONTINUOS PERCOLATION

In simple percolation the drug is extracted to exhaustion with fresh solvent. This is a long and expensive process due to the large quantities of solvent used, depending on several parameters.

1. Time taken to reach solvent solute equilibrium
2. Quantity of solvent needed to effect the first extraction on a reasonable industrial scale

3 Quantity of solvent needed do dilute completely the quantity of solute retained by the residual drug after first extraction.

Percolation and repercolation

In percolation as in maceration the drug is finely ground to the appropriate particle size, but not too finely so that the powder does not impede filtration of the solvent through the drug. As the drug is placed in very thick layer in the percolator, it is first moistened with extraction solvent as a rule outside of a extractor and allowed to swell before it is loaded

Lecture 2.3

Plant Cell Biotechnology

Use of tissue cultures and fermentation cultures for the improvement of medicinal plants

A.J. Vlietinck and L.Pieters
Departement of Pharmaceutical Sciences
University of Antwerp
Universiteitsplein 1
B-2610 Antwerp, Belgium

Introduction

One of the striking chemical attributes of plants is the range of natural products (secondary metabolites) which is formed throughout the plant kingdom. The possibility of this chemical virtuosity being expressed within culture systems quickly attracted attention. Although several decades of experience have tempered the initial optimism concerning the potential for industrial exploitation of plant cell cultures, scientific opportunities still abound. Pharmacognosy is at the threshold of a major expansion. Cell and gene technologies have extended our capabilities from plant description to control of development and design of products. This new technology, commonly referred to as biotechnology, may enhance the formation of desirable plant products and improve our health industry.

1. Plant cell cultures

Culture of plant tissues on defined media under controlled aseptic conditions enables many facets of plant biology to be experimentally manipulated with relative ease. The notion of culturing plant cells *in vitro* goes back to the beginning of this century. However, successful experiments in the culturing of unorganized plant cells for prolonged periods were first reported in 1939.

It is convenient to consider two types of plant culture that can be grown *in vitro*:

- organ cultures, *i.e.* isolated roots, leaves, flowers, etc., which retain the organisation of the intact organ;
- callus cultures (usually referred to as tissue cultures), which normally consist of a mass of mostly undifferentiated cells.

Callus cultures are species-specific, both morphologically and biochemically, but callus of a particular species may be derived from different parts of the plant. A further property of callus cultures is their ability, in response to certain stimuli, to regenerate intact plantlets which, unlike the callus, usually resemble the parent plant in their secondary metabolism. The factors controlling organogenesis in callus cultures are not at all well understood.

Tissue culturing techniques call for rigorously aseptic conditions since plant tissue cultures readily succumb to infection by bacteria, fungi and viruses. Nutrient media can be sterilised by autoclaving and thermo-labile substrates by filtration through bacterial filters. The initiation and transfer of culture material requires a sterile area or

laboratory and all the precautions employed in handling bacterial cultures. A common procedure for initiating a callus culture is to germinate a sterilised seed and to dissect aseptically from the resulting seedling a portion of stem, root or leaf tissue. This is placed on the surface of a suitable nutrient medium solidified with agar, which contains growth hormones (auxins and cytokins) to promote the formation of undifferentiated callus tissue. On prolonged subculture, some callus tissues lose their requirement for exogenously supplied auxin and are then said to be 'habituated'. A mass of light-coloured spongy or friable tissue grows from the original inoculum, and a portion must be periodically transferred to fresh nutrient medium at intervals of 2-3 weeks. Such cell-lines have been maintained for years, even decades, but genetic changes have been noted to occur on continued subculturing. Cultures grown on agar are referred to as static cultures. Suspension cultures on the other hand are obtained by inoculating callus grown on agar into liquid medium (usually of the same composition but lacking agar) and arranging for continuous agitation of the resulting suspension which consists microscopically of single cells and small cell clusters. Both the administration and extraction of products are faciltated in suspension cultures. Temperatures of 23° - 28° C and a fairly narrow pH range of 5.3 - 6.5 are commonly found satisfactory. The effect of light intensity and wavelength on growth and metabolism requires careful attention in each particular case. Some chemically defined growth regulators are kinetin (6-N-furfurylamino-purine), α-naphtalene acetic acid (NAA), 3-indolyl-acetic acid (IAA), 2,4-dichlorophenoxy-acetic acid (2,4-D), gibberelins and abscisic acid. These substances have profound effects on culture growth, the production of metabolites and differentiation.

The cells of many plant tissue cultures are totipotent, that is to say they possess all the information necessary to the functioning and replication of the whole plant including its secondary metabolism. This is evident from the fact, already mentioned, that callus tissues are often capable of regenerating whole plants which are in many respects comparable with the parent plant. In view of the many difficulties of growing intact higer plants under controlled and and reproducible conditions, chemists have been attracted by the potential of tisue culture in two distinct areas:

- the production of medicinally active secondary plant metabolites like steroids or alkaloids, either by *de novo* synthesis by the culture, or through biotransformations of more advanced but accessible intermediates;
- the study of biosynthetic and biodegradative pathways.

In both areas the stimulus has undoubtedly come from the highly successful exploitation of micro-organisms in the industrial synthesis of medicinals and in the exploration of primary and secondary metabolic pathways.

Several types of bioreactors to grow plant cell cultures are shown in fig. 1. Technologically it is feasible to grow plant cells on a large scale in bioreactors. Scaling up has been studied since the first successful *in vitro* growth of plant cells and tissues was described. Most work on large-scale cultures concerned the use of various types of low-shear bioreactors (*e.g.*, airlift-bioreactors), because plant cells were thought to be very sensitive to shear forces occurring in stirred-tank type-bioreactors. Little attention was paid to the cause of the supposed shear-sensitivity of plant cells. Recent studies showed that shear-sensitivity is not a general problem. On the contrary, many plant cell cultures are shear-tolerant and can be grown without any problem in stirred tanks. Why some cell lines are shear-sensitive and others shear-tolerant is not yet known, although it was noted that 'healthy' good-growing cell cultures were more shear-resistant than apparently 'stressed' (*e.g.* rapidly browning) cell cultures.

2. Regeneration of whole plants from single cells

When a plant is wounded mechanically, a patch of soft cells (a callus) grows over the wound. If a piece of young callus is removed and placed in a culture medium containing the appropriate nutrients and plant growth hormones, the cells will continue to grow and divide as a suspension culture. These cells can be plated out and they will grow to form new calli. The callus will then redifferentiate into shoots and roots, and ultimately a whole flowering plant will be produced. Differentiation of the cells in a callus depends on the relative concentrations of the plant hormones (phytohormones), auxins and cytokins. If the ratio of auxins to cytokins is high, then roots develop; shoots develop when the ratio is low. These cells are not very useful for uptake of DNA (in the case of genetic enginering – see below) because like all plant cells they are surrounded by a cellulose wall. However, this cellulose wall can be removed by treating the cells with fungal cellulase enzymes (fig. 2). The resulting protoplast is enclosed only by a plasma membrane and is much more amenable to experimental manipulation. Protoplasts will take up macromolecules like DNA, and they are capable of regenerating whole plants via the formation of calli.

Leaf disk technique. Growing whole plants from protoplasts is not easy, even for the most amenable species of plants. A simple but very significant improvement came with the development of the leaf disk technique (fig. 3). The technique is so important because it can be used with the most effective system for transferring genes into plants, a system using the Ti plasmid carried by the bacterium *Agrobacterium tumefaciens* (see below). Plant cells must be wounded to be targets for Ti gene transfer, and pieces of roots and stems have been used as targets. Leaves are a good source of regenerating cells, the cells coming from small disks cut from a leaf. The cells at the edge of the disk begin to regenerate, and when these disks are cultuerd briefly in a medium containing agrobacteria, these cells are efficiently exposed to the transfecting agent. The disks are then transferred several days to nurse cultures containing medium that stimulates shoot development. Cells carrying the plasmid are selected by culturing in shoot-stimulating medium with an appropriate antibiotic, such as kanamycin, and an antibiotic like cefotaxime to kill the *Agrobacterium*. Shoots develop within a few weeks, and these shoots are transferred to medium that induces root formation. The whole process, from cutting out the leaf disk to having rooted plants, takes between four and seven weeks. This process is extraordinarily fast compaerd with protoplast cultures.

3. Genetic engineering of plants

Genetic manipulation of plants has been practiced for many hundreds of years with great success by plant breeders, and plant breeding has become a very sophisticated branch of applied genetics. Breeders have developed elegant schemes for crossing plants to introduce and maintain desirables traits in inbred lines, and the yields of crops like maize and wheat have steadily increased over the past 60 years. However, the methods of classical plant breeding are slow and uncertain. To introduce a desired gene or set of genes by conventional methods requires a sexual cross between two lines, and then repeated back-crossing between the hybrid offspring and one of the parents until a plant with the desired characteristics is obtained. This process, however, is restricted to plants that can sexually hybridize, and genes in addition to the desired gene will be transferred.

Recombinant DNA techniques promise to circumvent these limitations by enabling plant geneticists to identify and clone specific genes for desirable traits, such as resistance to an insect pest, and to introduce these genes into already useful varieties of plants. Sexual compatibility becomes irrelevant, and the process becomes faster because transgenic plants expressing the gene can be selected directly. Plants have a number of unique biological features that can be explored with recombinant DNA techniques. These features include their pattern of growth, the means plants have devised to cope with the challenges of a changing environment from which they cannot escape, and, of course, photosynthesis.

Plants present advantages and disadvantages for the genetic engineer. The long history of plant breeding means that plant genecists have a wealth of strains carrying genetically characterized mutations that can be exploited at the molecular level. Plants are particularly amenable to genetic manipulation because many can be self-fertilized or *selfed*. When a plant heterozygous for a mutation is selfed, the progeny include wild-type plants, plants homozygous for the mutation, and also heterozygotes, in which the mutation is maintained. In addition, because plants produce very large numbers of progeny, rare mutations and recombinations can be found. Genetic manipulation of some plants is particularly refined because of the many years scientists have spent analyzing plant transposable elements, which can be exploited as vectors and as insertion mutagens.

However, although plants are attractive subjects for genetic research, they do have some disadvantages. For the molecular geneticist, one disadvantage is that many plants have very large genomes, often because of *polyploidy*, the presence of many genomes in the cell. Many groups of plants have polyploid species ; for example, about two-thirds of the grasses are polyploid, and species in the group that includes the potato have chromosome numbers ranging from 24 to 144. Polyploidy may contribute to the phenomenon of *somaclonal variation* exhibited by plants cells in tissue culture. In other words, plants generated from single cells are not genetically homogeneous, for it appears that plant cells growing in tissue culture are genetically unstable. This is a potentially serious problem in gene transfer experiments. A final difficulty arises because of our preoccupation with plants like maize, rice, and wheat, which have great agricultural importance. These are *monocotyledonous* plants ("monocots"), whose seeds have a single cotyledon (meaning "seed leaf"). These monocots are proving to be very difficult to transform with *dicotyledonous* plants (those with two cotyledon). Some novel methods are being devised to overcome this limitation.

Ti Plasmid of *Agrobacterium* causes crown gall tumors

Crown galls are tumors of plants that arise at the site of infection by some species of the bacteria *Agrobacterium* (fig. 4). The cells of crown galls have acquired the properties of independent, unregulated growth (that is, they are transformed). In culture, these cells grow in the absence of the plant hormones that are necessary for the culture of normal plant cells, and the cells retain this phenotype even in the absence of the bacterium. The tumor-inducing agent in *Agrobacterium* is a plasmid that integrates some of its DNA into the chromosome of its host plant cells. Ti plasmids are large, circular double-stranded DNA molecules of about 200 kb, and like other bacterial plasmids, they exist in *Agrobacterium* cells as independently replicating genetic units.

Ti plasmids are maintained in *Agrobacterium* because a part of the plasmid DNA, called *T-DNA*, carries the genes coding for the synthesis of unusual amino acids called *opines*. The infected plant cell is induced to synthesize these amino acids, but the plant cannot utilize them. Instead, the Ti plasmid is believed to carry genes coding for enzymes that can degrade opines, so the opines may act as a nutrient for the *Agrobacterium*. A second set of genes in T-DNA causes the unregulated growth of the plant cell. Two of these genes, *iaaM* and *iaaH*, code for enzymes that lead to the production of an auxin. The third gene, *iptZ*, codes for an enzyme that causes production of a second

phytohormone. These two hormones cause the infected plant cell to divide ; they also affect the neighboring cells.

T-DNA, part of the Ti plasmid, is transferred to plant cells

There are three components involved in Ti plasmid tumor induction (fig. 5). One is T-DNA, which is transferred to the host cell and is a form of mobile element. In addition, genes called *vir* (for virulence), present elsewhere on the Ti plasmid, are needed for the production of trans-acting proteins that are essential for, or at least enhance, plant cell transformation. These genes are carried on the *Agrobacterium* chromosome and are responsible for binding the bacterial cells to the plant.

The virulence genes in *Agrobacterium* are switched on by chemicals produced by wounded plant cells. Following activation of the *vir* genes, the T-DNA element is excised from the plasmid DNA. The T-DNA is flanked by Ti plasmid sequences, each 25 bp long. These flanking sequences are called *borders*, and they are involved in excision of the T-DNA sequence. Excision is a two-stage process in which the right-hand border is nicked between the third and fourth bases of the 25-bp repeat. A second nick in the left-hand border releases the T-DNA as a single strand. The process of transfer from the bacterial cell to the plant cell is analogous to the process of bacterial conjugation ; it is as though the *Agrobacterium* is mating with a plant cell!. The functions of the *vir* proteins in the transfer process are sill being explored. Incorporating extra copies of one of the *vir* genes into *Agrobacterium* leads to increased production of T-DNA and enhanced transformation. Other *vir* genes are associated with the single-stranded T-DNA itself and may be involved in the transfer process. However, this is not the whole story, because once inside the plant cell, the T-DNA has to enter the nucleus and integrate the plant cell DNA. Usually, multiple copies of T-DNA integrate at a single random site in the plant chromsome, but little is known of the mechanism.

T-DNA has been modified to sact as a gene vector

A method called *cointegration* was first used for gene transfer with the T-DNA, Ti plasmid, and *Agrobacterium* system (fig. 6). This method was developed to avoid the problems associated with manipulating large pieces of DNA the size of the Ti plasmid. T-DNA was first cloned into a standard *E. coli* cloning vector, and the plant gene subsequently cloned into a second cloning site carried in the vector. This intermediate

vector was introduced into *Agrobacterium* containing intact Ti plasmids. Recombination occurs between the homologous regions of the intermediate vector and the wild-type Ti plasmid, and on infection of a plant with the *Agrobacterium*, the recombinant plasmid is transferred to the plant cells. The *E. coli* plasmid used in this process is called an *integrative plasmid* because it becomes part of the Ti plasmid.

The standard method for T-DNA transfer is now the *binary system*. This method was devised when investigators realized that the essential functions for transfer are supplied separately by the T-DNA itself and by the Ti plasmid, and that the components can be carried on separate vectors. The *binary vector* contains the 25-bp borders of the T-DNA that are needed for excision and integration. The phytohormone genes of the T-DNA can be removed to create room for the insertion of foreign DNA, which will be transferred to the plant cell. At the same time deleting the phytohormone genes prevents the uncontrolled growth of the recipient cells. The other essential genes are the *vir* genes of the Ti plasmid, and these can act in trans if they are supplied on a separate plasmid, called the *helper* plasmid. A very important factor in the development of T-DNA-based vectors is the availability of selectable markers such as neomycin phosphotransferase II (NPTII), and dihydrofolate reductase. These markers are included within the 25-bp repeats of the binary vector, so they too are transferred into the plant cell. The vectors carry a second selectable marker so that they can be minipulated easily in *E. coli*. Binary vectors differ from integrative vectors in that the binary plasmid containing the DNA to be transferred to the plant cell is maintained as a separate replicating vector in *Agrobacterium*.

4. Metabolic engineering

Three options to improve the production of a secondary metabolite by means of metabolic engineering can be considered.

Increase flux through a pathway. Several factors might control the carbon flux through a biosynthetic pathway for example : rate-limiting enzymes, feedback inhibition, and competitive pathways. In the case of rate-limiting enzymes a higher activity could be achieved by introduction of the encoding gene in combination with a strong promotor. Alternatively a heterologous gene, encoding an enzyme with a similar function, could be introduced in the plant cells. Also to overcome feedback inhibition one might consider the use of a gene encoding a similar enzyme, but not sensitive for feedback inhibition. Protein engineering may be an even more sophisticated approach. Competitive pathways could be blocked by means of antisense genes. Sense genes could be used to try to overcome competition by having higher levels of activity of the enzyme leading to the desired product, or to introduce an enzyme with a similar function but with a higher affinity for the substrate. Obviously metabolic engineering requires thorough knowledge of the secondary metabolite pathway involved. One needs to know all the intermediates, the enzymes involved and the encoding genes, as well as the regulation on all three levels. In fact only for a few pathways is such information available, e.g. the flavonoid-anthocyanidin pathway. Many pathways are just known on the level of the intermediates, but in recent years further studies on the level of enzymes have been started, e.g. for terpenoid indole alkaloids, isoquinoline alkaloids, tropane alkaloids and certain terpenoids.

Increase the number of producing cells. For *Catharanthus roseus* cell cultures it was found that the production of anthocyanidins is determined by the percentage of producing cells. If one were able to increase the percentage of producing cells by genetic modification, the total yield of the desired product would increase. Unfortunately, very little is known about the processes which make a cell to produce a secondary metabolite, i.e. differentiate in a certain direction.

Decrease metabolism. From several studies it is known that in cell cultures catabolism occurs of what were thought to be end products, e.g. ajmalicine in *C. roseus* cell cultures is catabolized at almost the same rate as the biosynthetic rate. To be able to block metabolism, as far as it is not due to chemical degradation, the enzymes involved in catabolism have to be identified.

Problems

From the above mentioned studies it is clear that it is feasible to clone genes from secondary pathways and express them in other plants, resulting in a functional protein. However, some problems arise.

Cloning genes. Cloning genes from secondary pathways is quite elaborate, one has to follow the long way from secondary metabolite, via enzyme to the gene. The low levels of most secondary metabolism enzymes are a complicating factor in this approach. Moreover, the occurrence of a series of closely related genes may be a complication, as for example in the case of cytochrome P-450 enzymes, where 16 closely related genes were picked up from *C. roseus* by means of PCR.

Stability. A yet unknown aspect is the stability of a transgenic trait. It has been reported that transgenes are gradually silinced through generations of plants.

Compartmentation. Different parts of a biosynthetic pathway may occur in different cellular compartments. Consequently for genetic engineering, one needs to express an enzyme in the appropriate cellular compartment. Compartmentation may also be on a cellular level. It is not clear whether in the case of cellular compartmentation it will be feasible to eventually express all steps in one single cell.

Transport. As different compartments are involved, transport of intermediates has also an important regulatory function. This might be a selective transport, thus requiring the identification of genes of carrier proteins, or transport driven by pH gradients.

4. Production of Heterologous Proteins in Plants

A number of different heterologous proteins and peptides are now produced in a variety of plants. Novel DNA sequences may be introduced into plant cells by several means. These include use of *Agrobacterium* as a carrier, and direct injection of the DNA into certain plant cells. Using such techniques, plants can be engineered to produce insecticides, which when expressed may play a protective role. Plants may also be altered genetically to produce heterologous proteins of industrial interest. Expression of some such foreign proteins in plants has been reported.

Attempts to produce antibodies in a variety of heterologous systems have not usually been successful. This is most probably due to the complex structural nature of the mature antibody. Antibodies consist of four polypeptide chains, two light chains (identical) and two heavy chains (identical). Correct intrachain folding and interchain association is required to form a functional antibody. Such interactions are complex and are both covalent and non-covalent in nature. Functional antibodies have been produced in plants with limited success. Plant expression systems have the ability to carry out a number of post-translational modifications and can successfully glycosylate a range of heterologous proteins. However, recombinant glycoproteins produced by transgenic plant cells normally contain a glycosylation pattern different to the pattern associated with the protein produced in its natural source. Certain oligosaccharide epitopes commonly found on plant glycoproteins are highly immunogenic in mammals. This suggests that mammalian proteins intended for therapeutic application, if expressed in plant cells, might be highly immunogenic.

Heterologous Peptide Production in Plant Seeds

It is now possible to produce a range of heterologous peptides of commercial interest in plant expression systems. In recent years, a wide range of peptides of considerable commercial value have been identified. Many such peptides are of therapeutic significance. These occur naturally in the body and perform a variety of biological functions. Examples include thyrotrophin releasing factor (TRF), a 3-amino acid peptide produced in the hypothalamus which stimulates synthesis and release of the hormone thyrotrophin from the anterior pituitary gland. Oxytocin is a 9 amino acid peptide hormone secreted by the posterior pituitary. It stimulates uterine muscle contraction. Luteinizing hormone releasing hormone (LHRH) is a decapeptide produced by the hypothalamus which stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH)

from the pituitary gland. Other examples of peptides of clinical significance include bradykinin, a 9 amino acid hormone which inhibits inflammation of tissue, and the endorphins - a group of neuropeptides often referred to as the body's own opiates. Endorphins are endogenous ligands of the opiate receptors and hence exhibit a biological activity similar to morphine. Several endophin peptides have been characterized, the most important of which are known as α, β, and γ endophines, in addition to met-enkephalin and leu-enkephalin.

Most such peptides are synthesized in minute quantities in the body. As a result, purification from their natural source is fraught with technical difficulties. Many such peptides may be synthesized chemically. The cost of such chemical synthesis increases enormously with increasing peptide length. Production of a peptide containing modified amino acid residues by chemical synthesis may also present technical difficulties. However, despite such potential drawbacks, a number of peptides available commercially are synthesized chemically. Many have also been produced as heterologous peptides in fermentation systems utilizing procaryotes or yeast expression systems. Certain peptides are also successfully produced in plant seeds. Leu-enkephalin, for example, has been produced in this manner.

The seeds of higher plants contain large quantities of storage proteins. Some such storage proteins may constitute in excess of 50% of total seed protein. Production of leu-enkephalin was achieved by inserting its DNA coding sequence into the gene coding for a seed storage protein termed 2S albumin. The family of 2S albumines are among the smalles seed storage proteins, having a molecular weight of the order of 12 kDa. This family of proteins is derived from a group of structurally related genes - all of which exhibit both conserved and variable sequences. The variable regions vary not only in sequence but also in length. The strategy employed to produce leu-enkephalin involved substituting part of this variable sequence with a DNA sequence coding for the 5 amino acid neurohormone. The DNA construct was flanked on both sides by nucleotides encoding amino acid sequences recognised by the proteolytic enzyme trypsin. Expression of the altered 2S albumin gene resulted in production of a hybrid storage protein containing the leu-enkephalin sequence. The enkephalin was subsequently released from the altered protein by tryptic cleavage and purified by high-performance liquid chromatography (HPLC). Because of the incorporation of the tryptic cleavage sites, the purified product contained an extra lysine residue which was subsequently removed by treatment with

carboxypeptidase C - a proteolytic enzyme which hydrolyses only the peptide bond at the carboxyl terminus of a peptide or polypeptide.

Although production of heterologous proteins and peptides in plant seeds has been shown to be technically feasible, it is still unclear to what extent such production methods will be adopted by industry. Incorporation of significantly larger peptides into storage proteins may have adverse effects on the synthesis and stability of such hybrid proteins and thus may not be feasible. Economic considerations will constitute the important deciding factor. As yet, it is not clear if such methods of production would be economically more attractive when compared to chemical synthesis or microbial fermentation.

Literature

Constabel F., Planta Medica 56, 421 – 425 (1990)

Ellis B.E., Nat. Prod. Reports, 581 – 609 (1988)

Overton K.H. et al., Progress in the Chemistry of Organic Natural Products, pp. 293 – 298 (1977)

Verpoorte et al., J. Nat. Prod. 56, 186 – 207 (1993)

Verpoorte et al., Phytotherapy Research 10, S12 – S14 (1996)

Watson J.D. et al., Recombinant DNA, 2nd edition, Scientific American Books, New York 1992, chapter 15

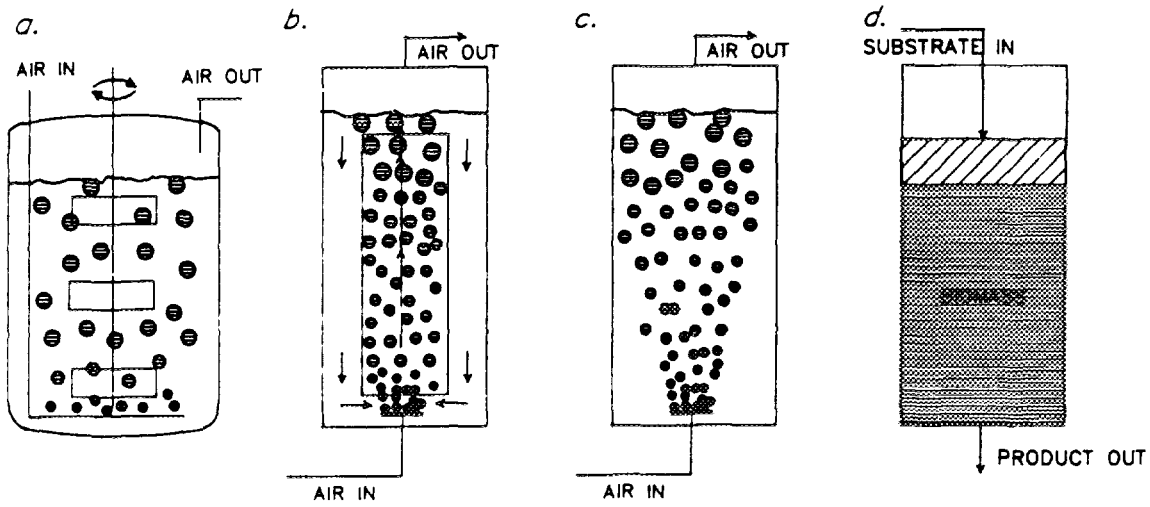


FIG. 1 BIOREACTORS

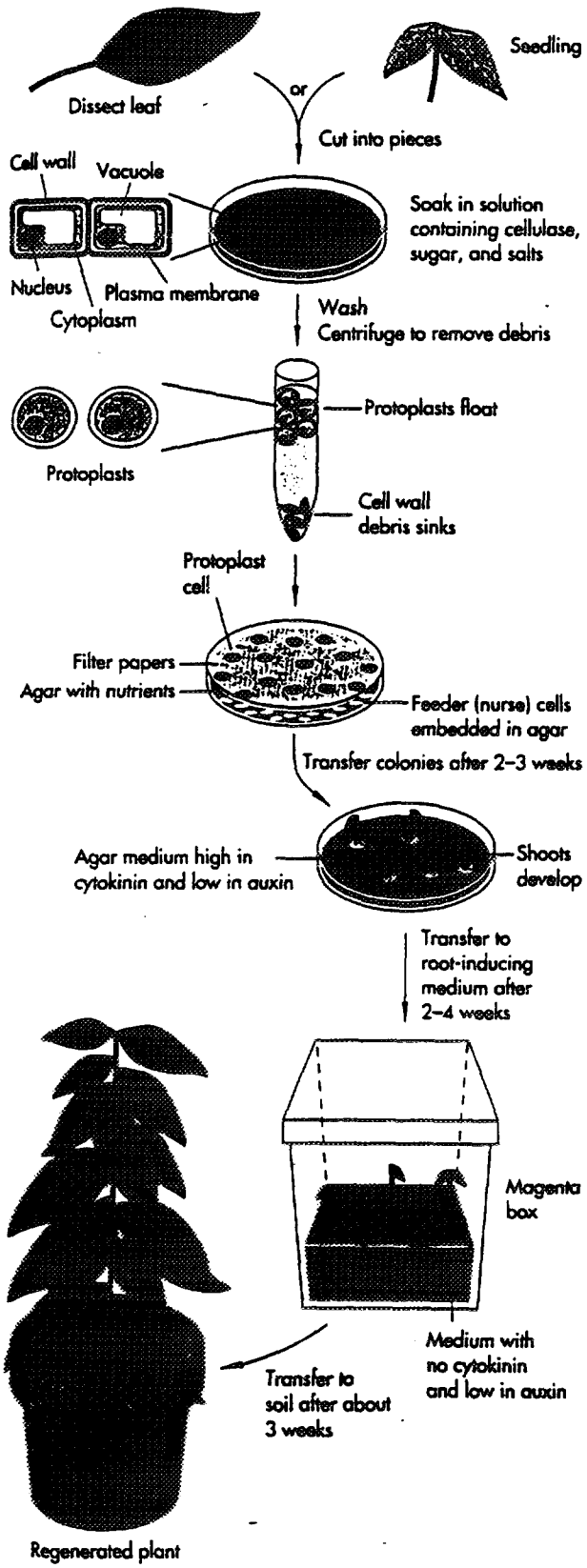


FIGURE 15-1
Regeneration of plants from protoplasts. Leaf cells are characterized by a cytoplasmic compartment containing numerous chloroplasts, a large vacuole, and a nucleus. The plasma membrane is surrounded by a tough cellulose cell wall that can be removed by incubating pieces of plant tissue in a solution containing cellulase. Sugars and salts are added to the solution to maintain osmotic balance and prevent the protoplasts from lysing. Once the cell debris is removed, the protoplasts are placed on filter paper covering a layer of nurse cells. The filter paper is impervious to the cells, but growth factors and other molecules produced by the nurse cells can diffuse into the protoplasts, which divide and grow to form microcolonies. For most plant cells nurse cell feeder layers are not needed. The microcolonies are carefully transferred to a medium high in cytokinin and low in auxin. Shoots appear in about two to four weeks. Then the cultured cells are transferred to a container called a Magenta box, which contains root-inducing medium lacking cytokinin and low in auxin. Once the roots appear, the plantlets can be placed in soil, where they develop into regenerated plants.

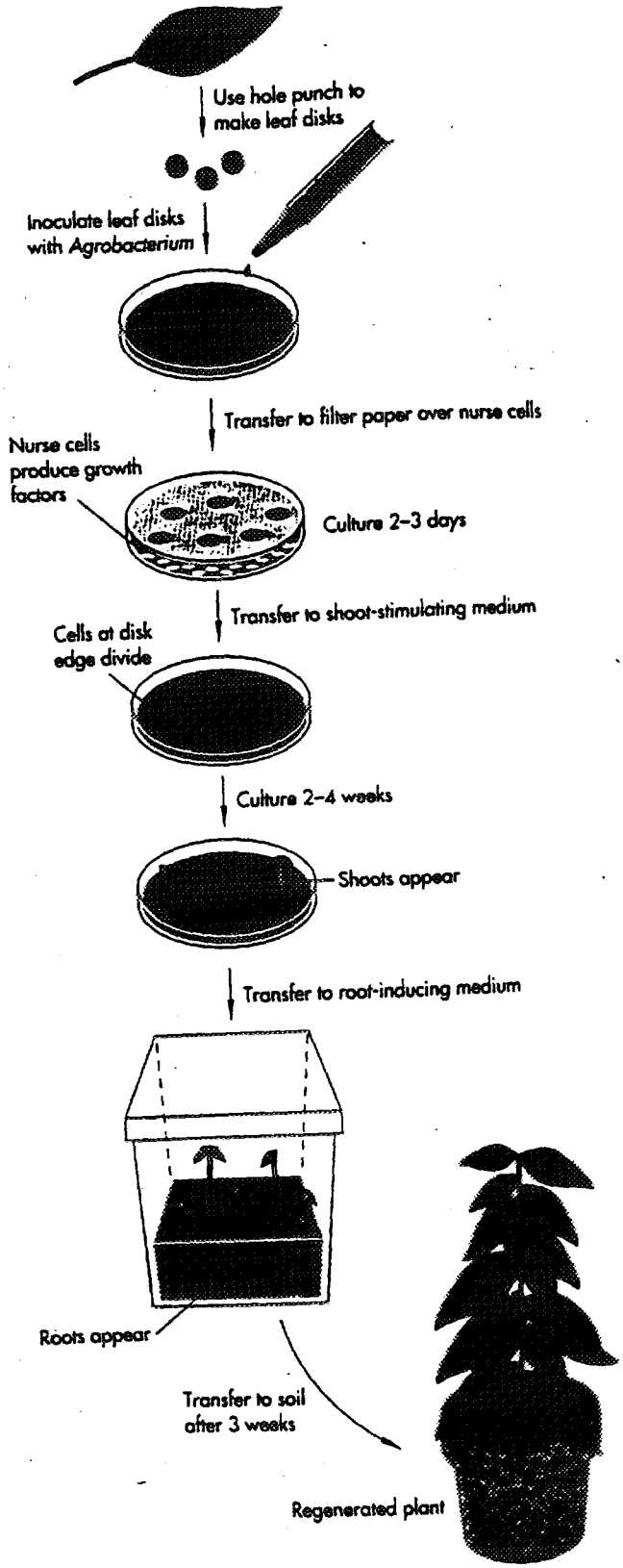
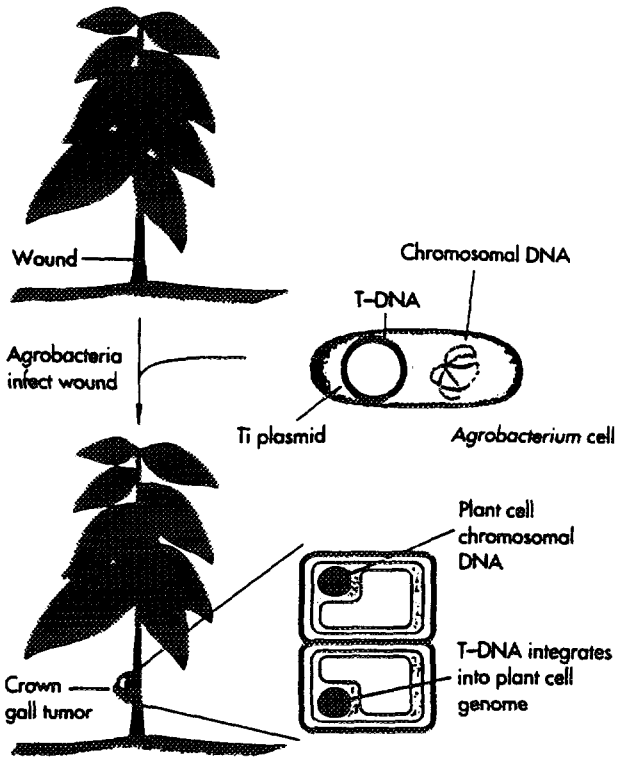


FIGURE 15-2
Regeneration of leaf disks infected by *Agrobacterium*. Leaf disks are cut out and placed in a shallow dish. A solution of agrobacteria is added, and after a few minutes, the leaf disks are transferred onto nurse cell medium. Wounded cells at the edge of the disk release factors that induce the agrobacteria to infect the cells. The plant disks are cultured in a fashion similar to that described for protoplasts in a medium containing an antibiotic such as cefotaxime that kills *Agrobacterium* but does not harm plant cells (Figure 15-1), to yield a regenerated plant.

FIGURE 15-3
Agrobacteria cause crown gall tumors in plants. When a wounded plant is infected by *Agrobacterium*, the agrobacteria cells do not enter the plant cell but transfer a DNA segment called the T-DNA from the circular extrachromosomal *tumor-inducing* (Ti) plasmid. The T-DNA becomes stably incorporated into the plant cell chromosomal DNA. Genes within T-DNA from natural Ti plasmids are expressed and their products stimulate the cells to divide uncontrollably. The structure formed by the rapidly dividing cells is called a *crown gall tumor.*



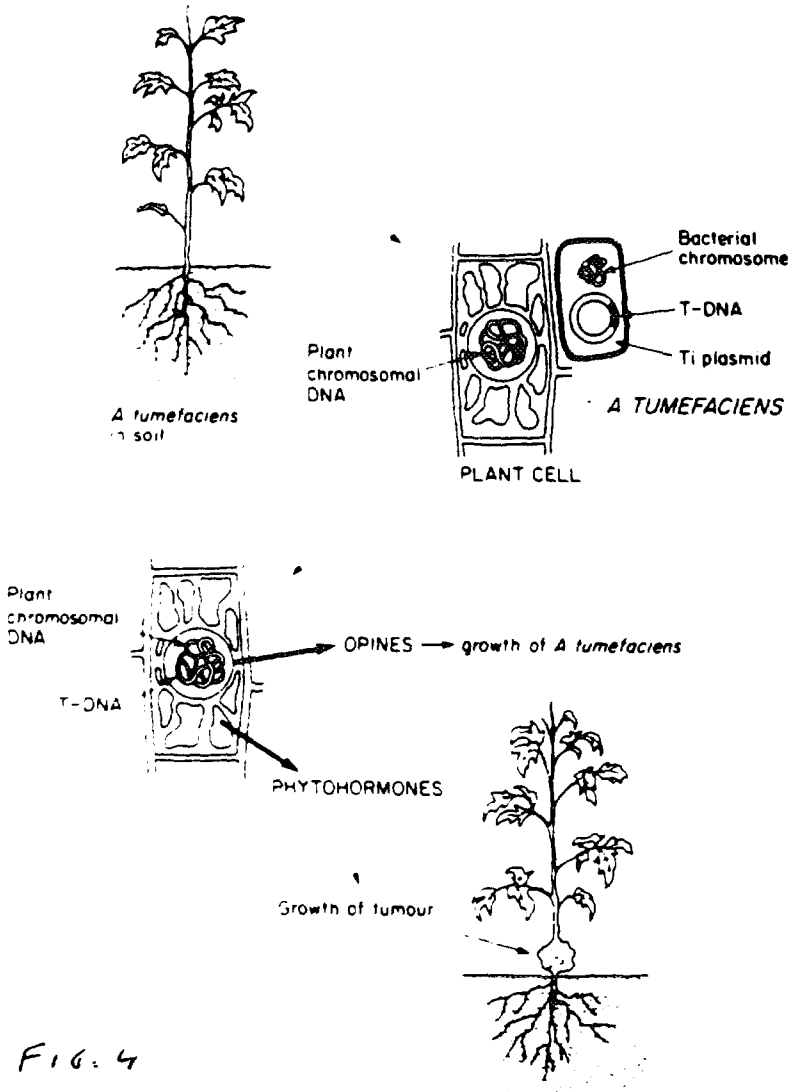


FIG. 4

The induction of plant tumours by strains of *Agrobacterium* carrying the Ti plasmid.

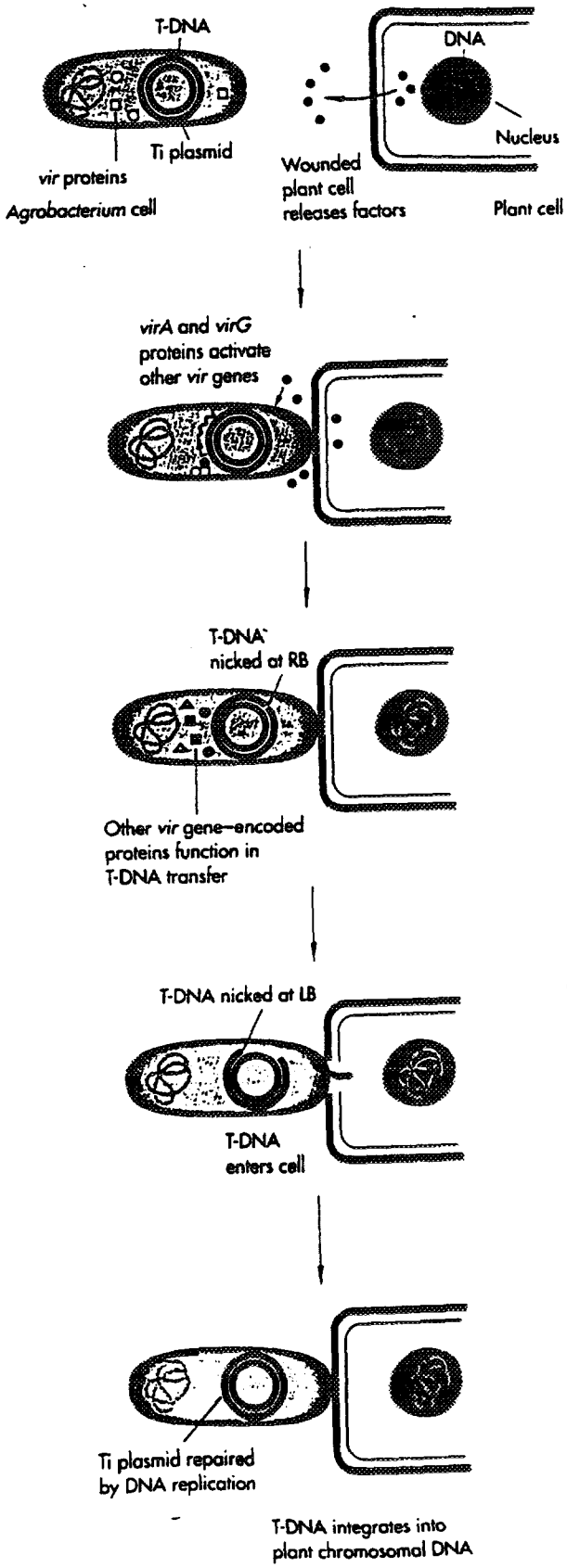


FIGURE 15-4
Transfer of T-DNA from *Agrobacterium* into a plant cell. When a plant cell is wounded, it releases factors that stimulate transcription of the *vir* genes on the Ti plasmid that function in the transfer of the T-DNA into the plant cell. Only the T-DNA region of the Ti plasmid is transferred to the plant cell. T-DNA is bounded by 25-bp imperfect repeats termed the *left border* (LB) and the *right border* (RB). Transfer begins with a nick in the DNA strand in the RB, then a nick occurs at the LB producing a single-stranded T-DNA molecule. By a mechanism that is still not completely worked out, the T-DNA molecule enters the plant cell, where it integrates randomly into the chromosomal DNA. The single-stranded T-DNA region of the Ti plasmid is repaired by DNA replication, so the *Agrobacterium* has not lost any information by transfering DNA to the plant cell.

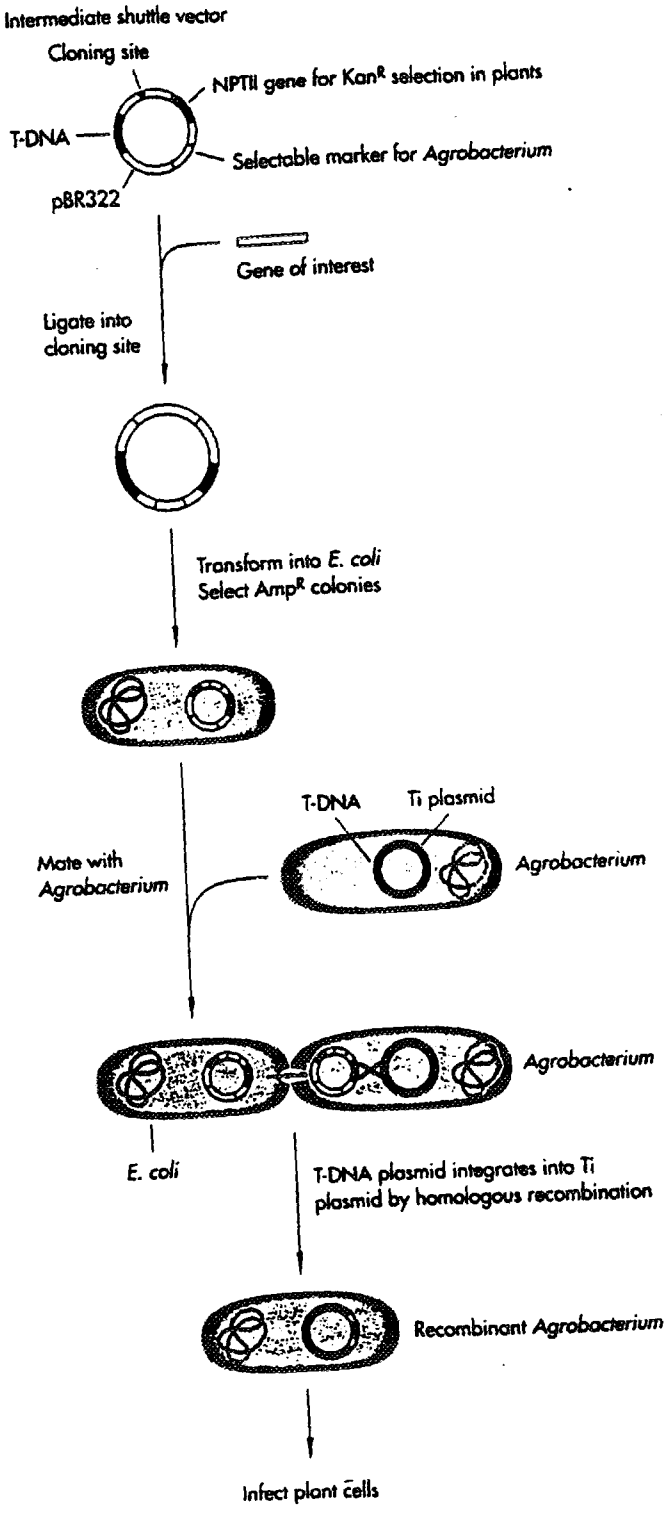


FIGURE 15-5
Transferring genes into plant cells by cointegration using T-DNA, Ti plasmids, and *Agrobacterium*. A cloned gene can be introduced into plant cells by first inserting it into the cloning site of a plasmid that can replicate in *E. coli* and contains a segment of T-DNA. The resulting intermediate shuttle vector is introduced into *E. coli* cells, and transformants are selected by resistance to ampicillin, encoded within the pBR322 sequences. Next, the plasmid is transferred from the *E. coli* cell to an *Agrobacterium* cell by mating. Once inside the *Agrobacterium*, the plasmid *integrates* into the Ti plasmid by means of homologous recombination of the T-DNA sequences on the two plasmids. This process places the entire integrative plasmid (the plasmid integrated into the Ti plasmid) between the left and right boundaries of the T-DNA. Plasmids that fail to integrate do not accumulate because they lack an origin of replication for *Agrobacterium*. Agrobacteria containing the recombinant Ti plasmid are selected and used to infect plant cells. Plant cells that have taken up the T-DNA are identified by the plant selectable marker NPTII, which confers resistance to kanamycin. These cells also contain the cloned gene of interest.

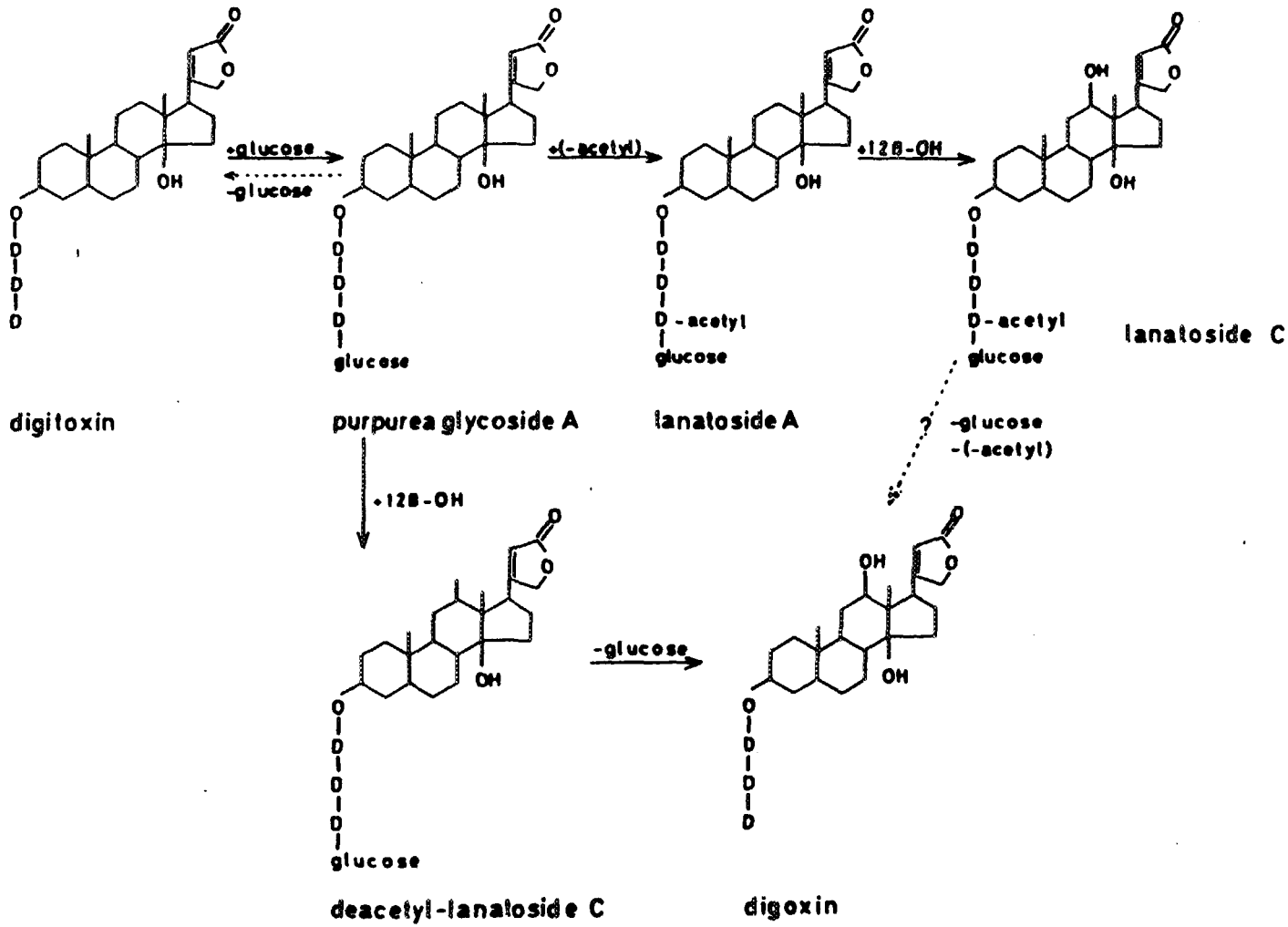


Fig. 2. Biotransformation of digitoxin by the cell strains 72L, 72D and C 3 of *Digitalis lanata*. Strains 72D and C3 are not able to produce lanatoside A and C

3. Digitoxigenin

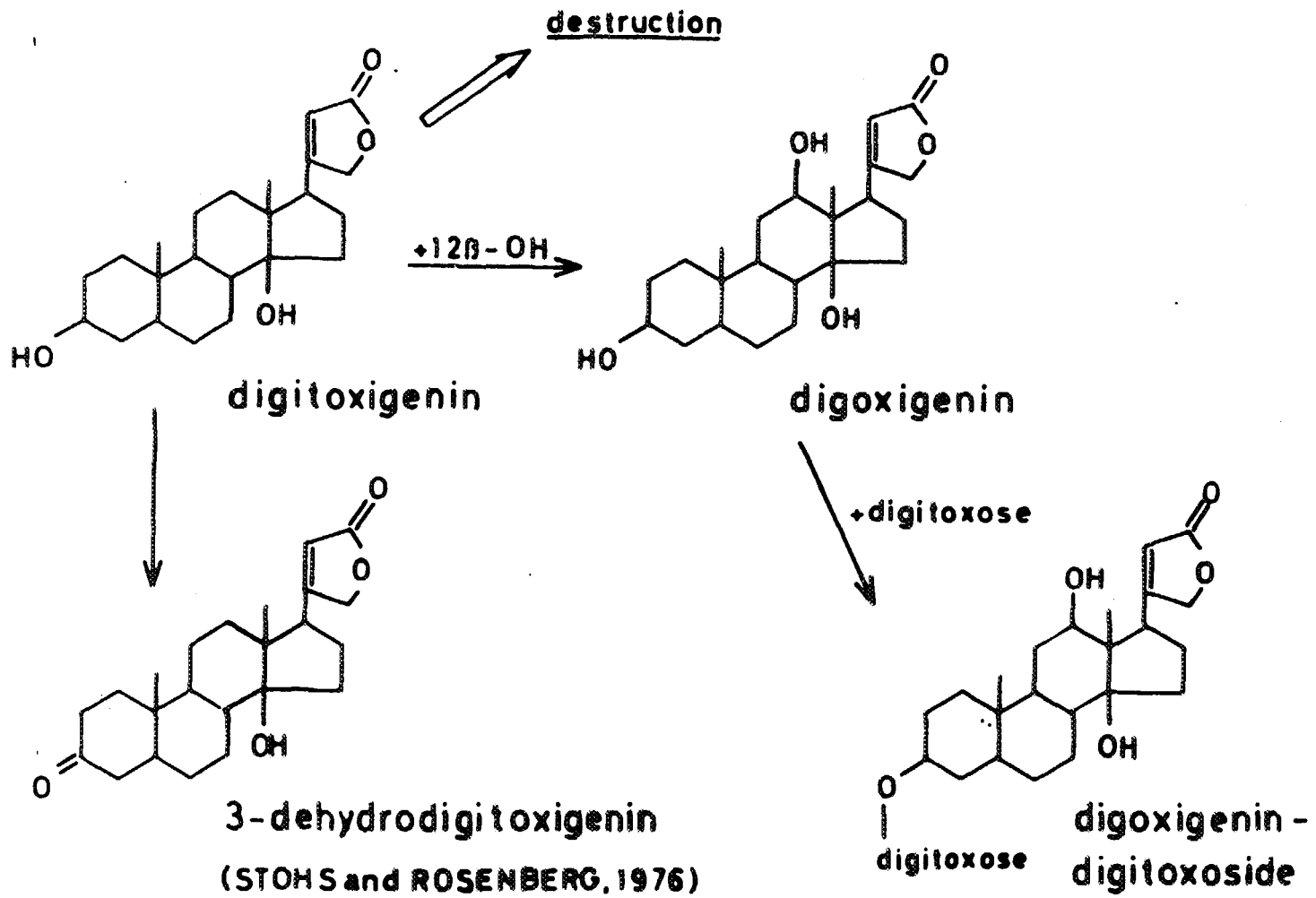
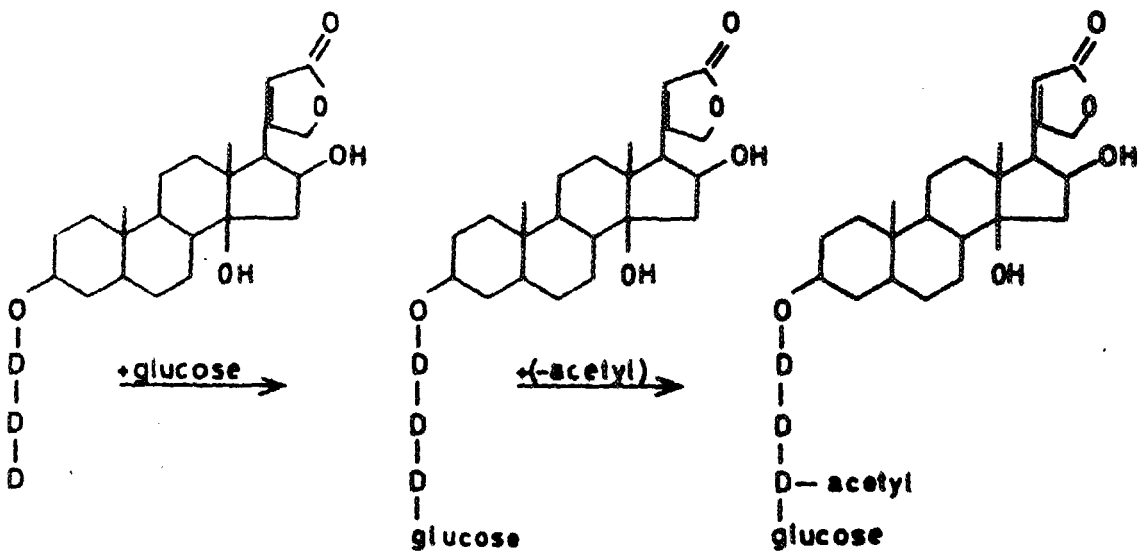


Fig. 3. Biotransformation of digitoxigenin and digitoxose by cell cul
D. lanata, strain 72L and according to Stohs and Rosenberg (1976)



gitoxin purpurea glycoside B lanatoside B

Fig. 6. Biotransformation gitoxin by cell cultures *D. lanata*, strain 72L

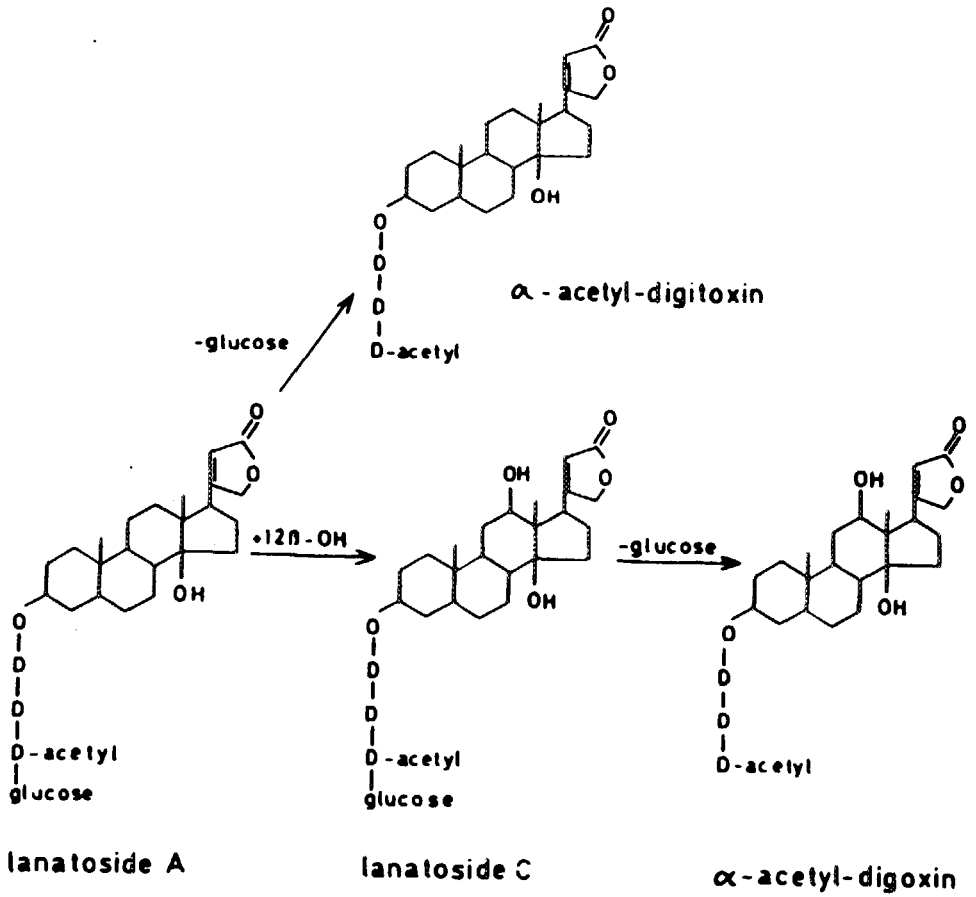


Fig. 7. Biotransformation of lanatoside A by cell cultures of *D. lutea ssp. lutea*, strain D.lu-1F

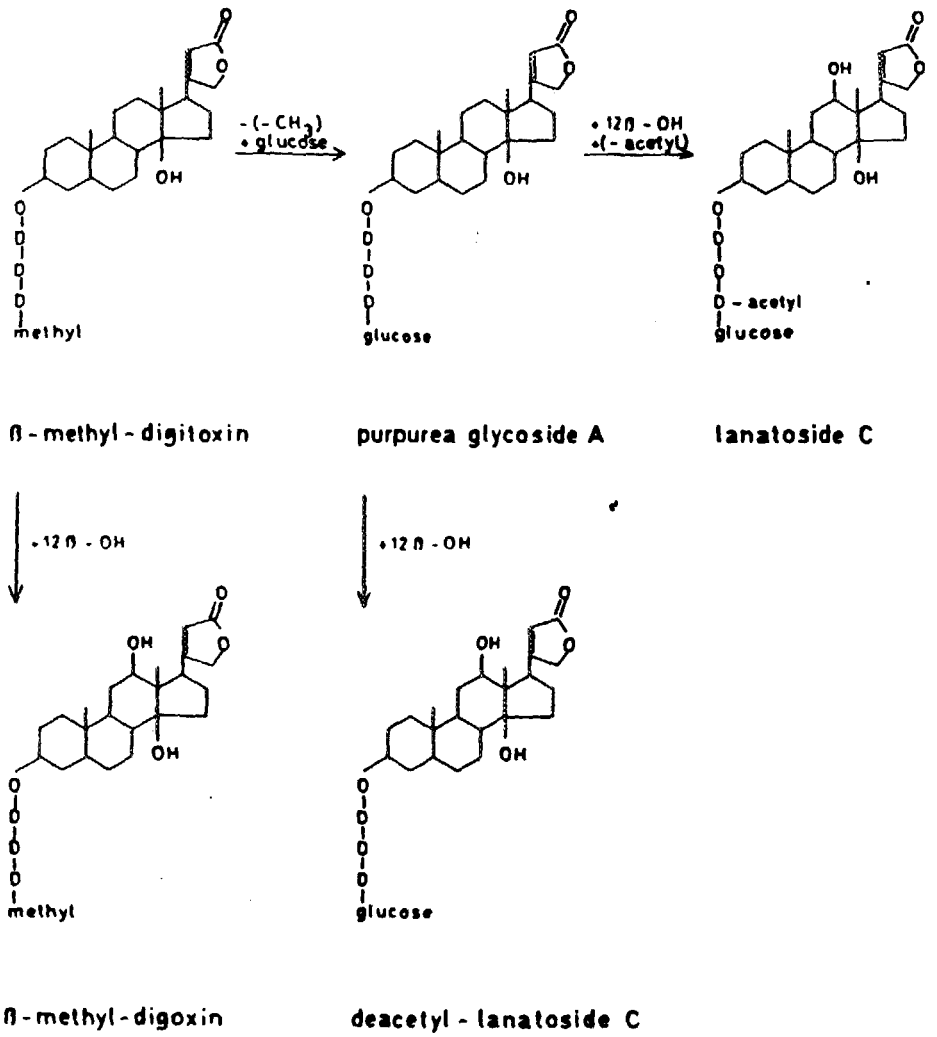
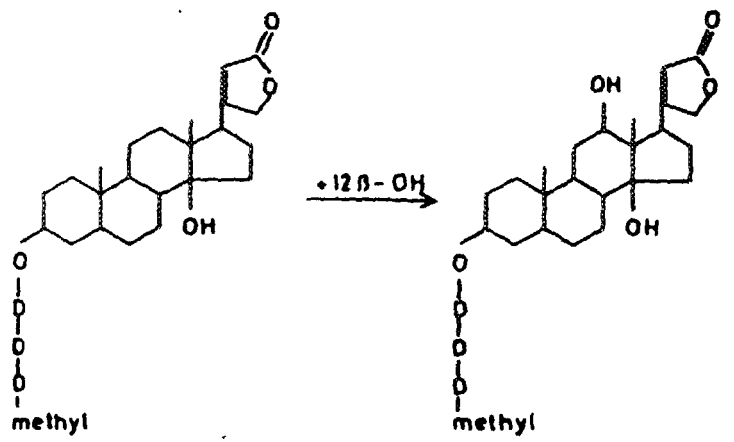


Fig. 10. Biotransformation of β-methyldigitoxin by cell cultures of *D. lanata*, strains 347, B2, 287, 293, 72L, 364 and E. Lanatoside C was only found in experiments with strain E, deacetyllanatoside C in experiments with strain 293



β-methyl-digitoxin

β-methyl-digoxin

Fig. 11. Biotransformation of β-methyldigitoxin by the cell strains 72D and 285 *(D. lanata)* and 30625-10-15S *(D. lanata ssp. leucophaea)*

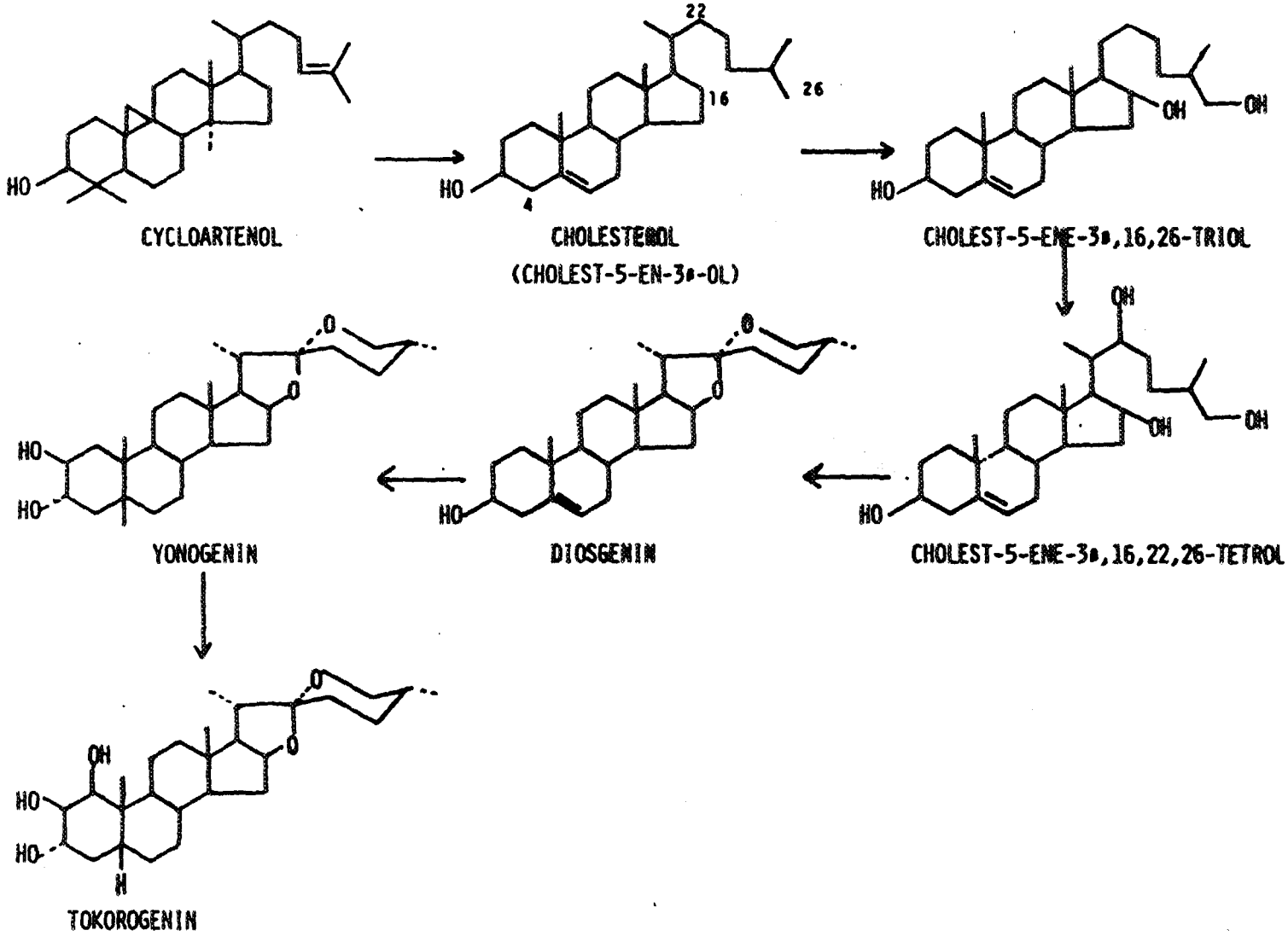


Fig. 4. Biotransformation of Steroidal Sapogenins

CELL SUSPENSION CULTURES of soybean and parsley:

Model systems for demonstrating
DIFFERENTIAL REGULATION OF INTERRELATED PATHWAYS

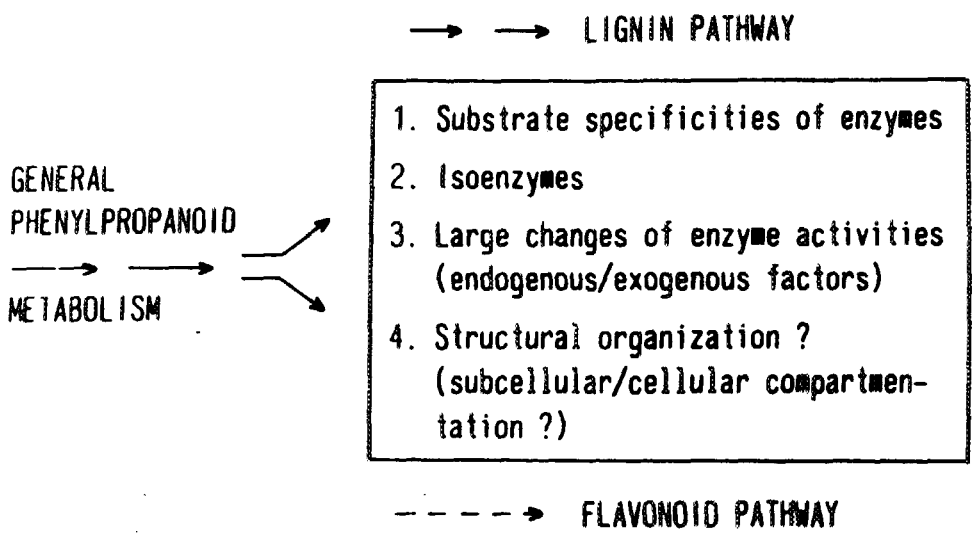


Fig. 7. Scheme summarizing modes of regulation of phenylpropanoid pathways

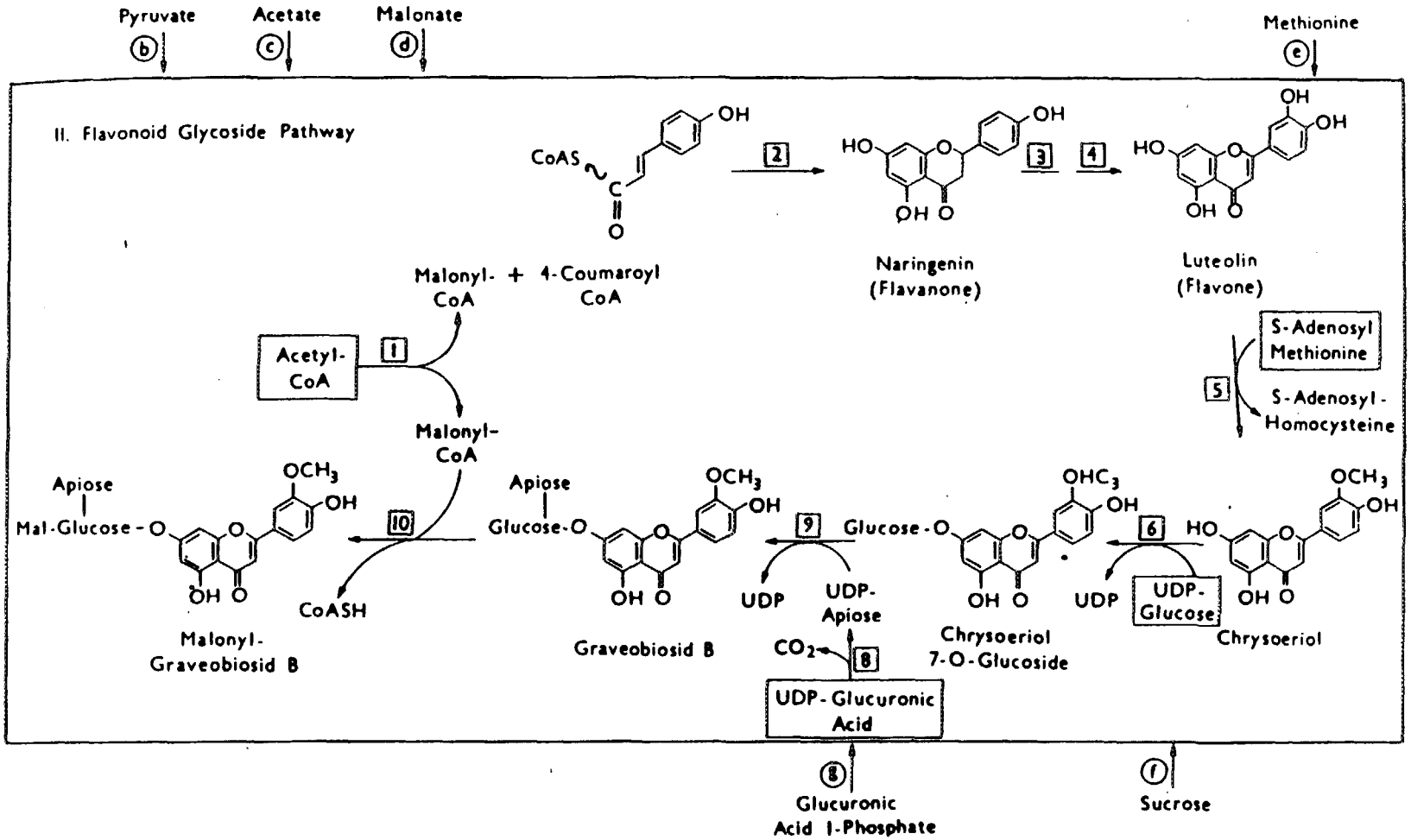


Fig. 4. Reactions catalyzed by the enzymes involved in the formation of the flavone glycoside, malonyl graveobioside B. *Arrows* within the frame: enzymes of the flavonoid glycoside pathway. *Arrows* outside the frame: enzymes of primary metabolism (for further explanation, see text)

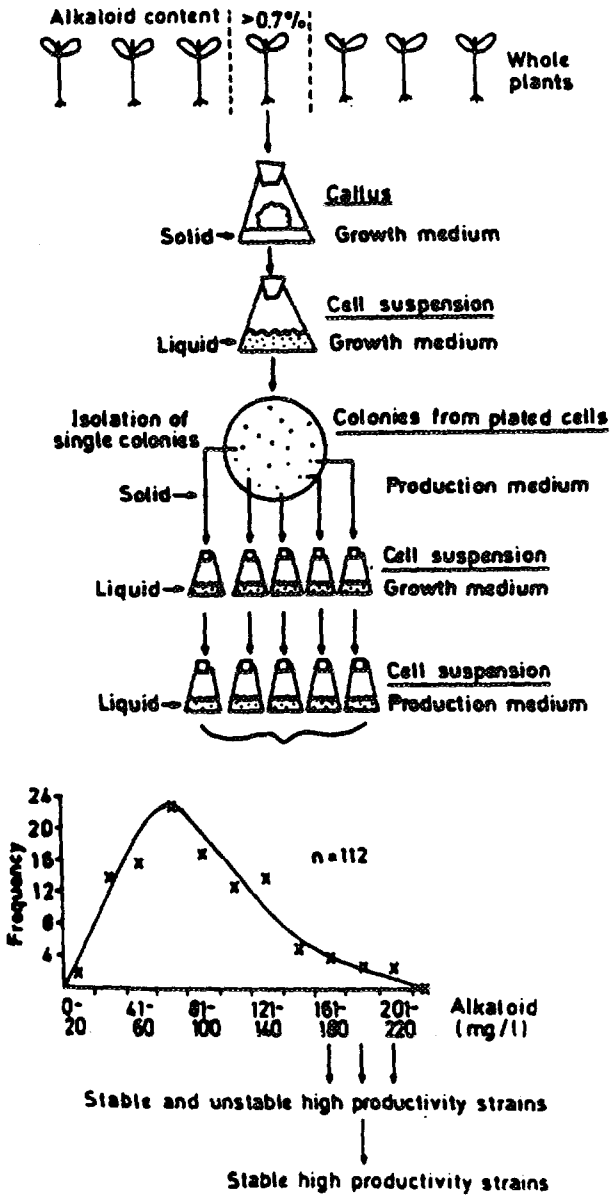


Fig. 8. Diagramatic outline showing procedure for selection of high alkaloid yielding cell strains

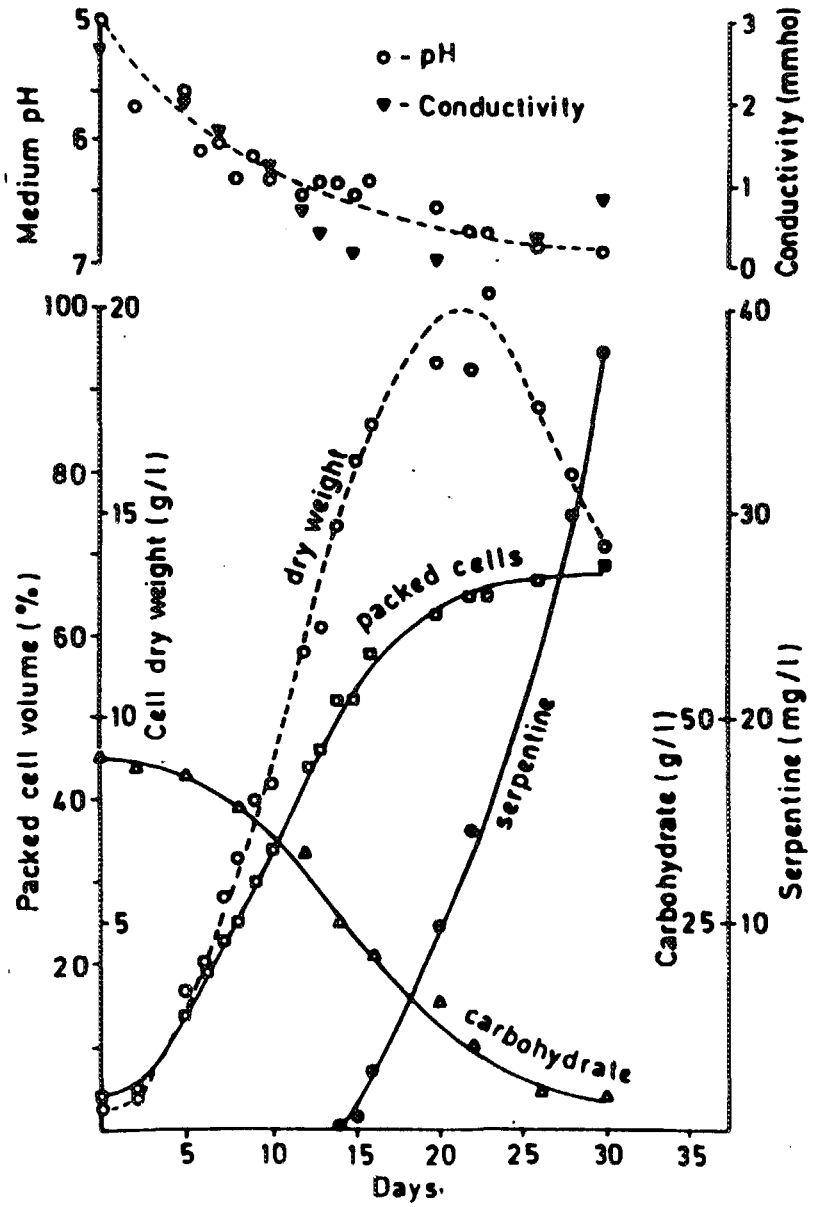


Fig. 10. Kinetics of growth characteristics and serpentine production observed when *C. roseus* cells were grown at 30°C in a 30 l airlift fermenter. The fermenter contained 22 l production medium and was aerated at 0.5 vvm

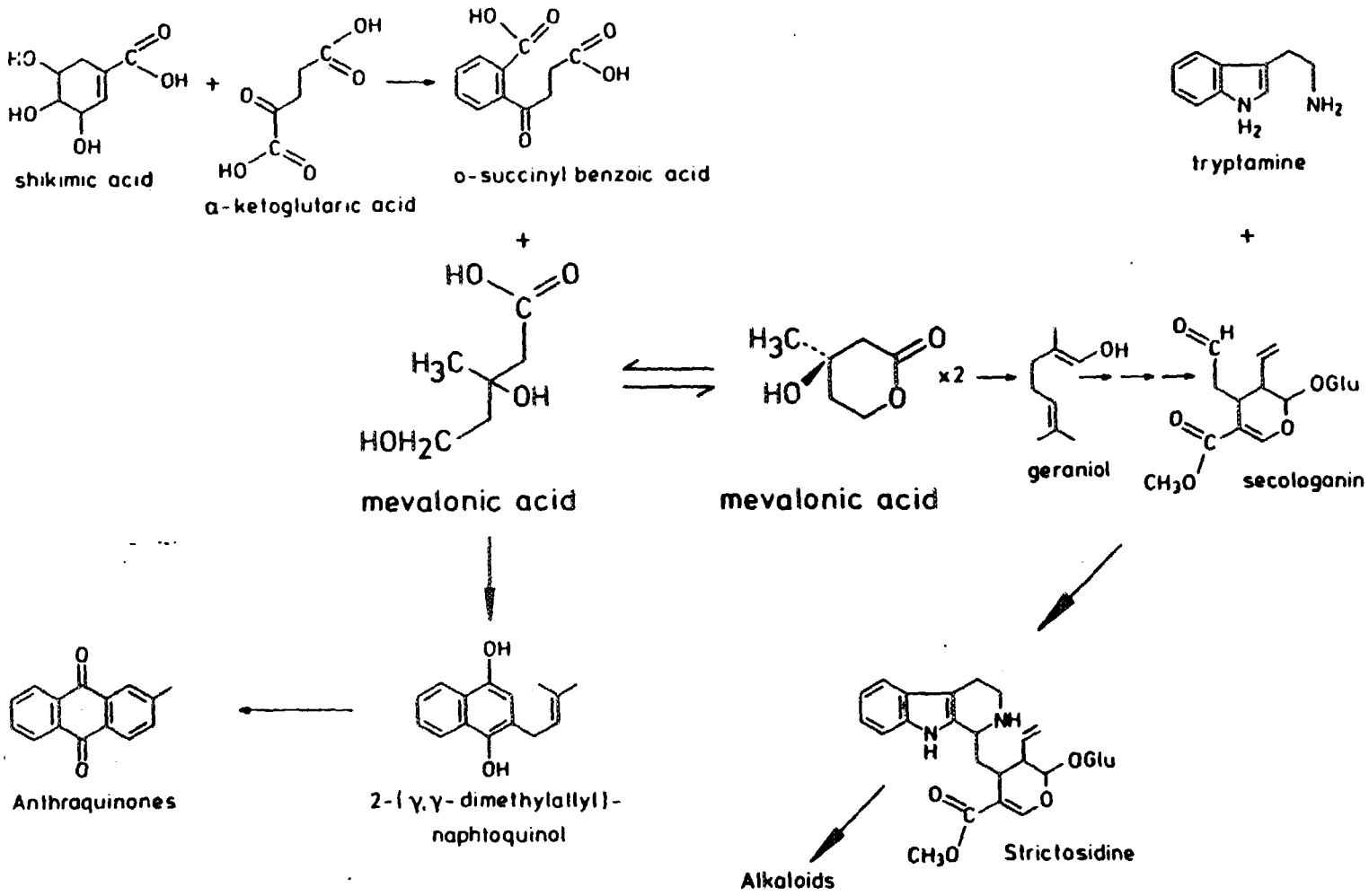
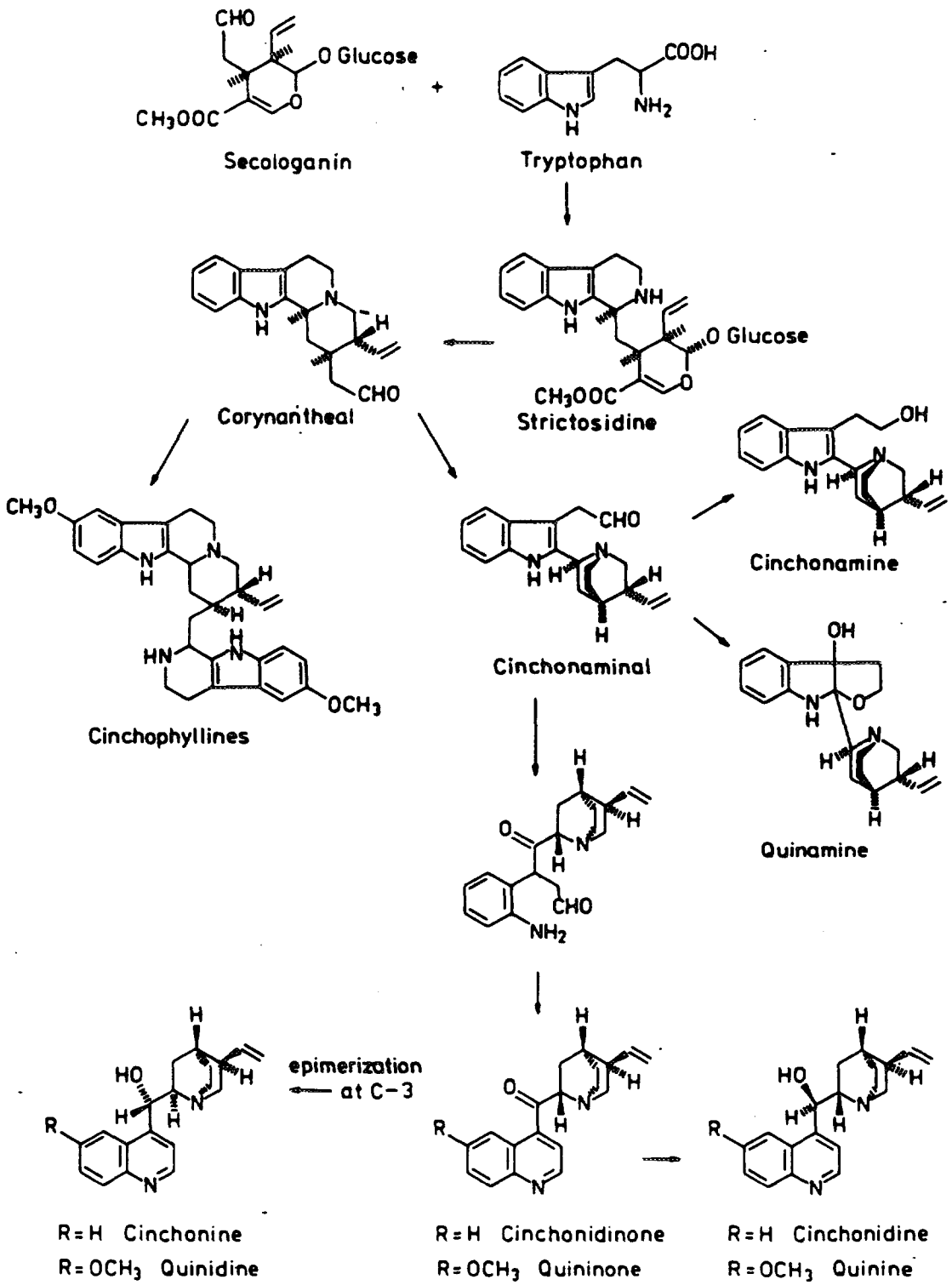


Fig. 3. The competition for mevalonic acid between anthraquinone- and alkaloid biosynthesis in tissue cultures of *Cinchona* sp.

Biosynthesis of Cinchona alkaloids



Scheme 1

Multidisciplinary research:

Aspects of quality, safety and efficacy

Götz Harnischfeger

The conditions to be met nowadays by every pharmaceutical product can be stated in the key principles of quality, safety and efficacy. These rigorous principles apply to phytomedicines as well, and as will be shown, require special techniques in the approach.

Definitions

Clear definitions and understanding of terms is a prerequisite. These are given as follows:

Phytomedicines, -synonym Herbal Medicines, Herbal Medicinal products-, should be regarded as

„Finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substances of this nature. Herbal medicines may contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be herbal medicines. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin. (WHO definition)

From this definition it can be clearly seen, since the entire plant or plant preparation is the active ingredient of a phytomedicine, that we are dealing with multicomponent systems in a chemical sense which might or might not been matrix bound. More generally formulated:

Plant drug preparations (active ingredients) are preparations ***used for the manufacture of herbal medicinal products***. They are comminuted or powdered vegetable substances, extracts, tinctures, fatty or essential oils, expressed juices etc prepared from vegetable substances, and preparations whose production involves a fractionation, purification or concentration process. If these processes are only preliminary stages of a further process, they are considered as processing stages. Chemically defined isolated constituents or their mixtures are not *plant drug* preparations. Other substances such as solvents, diluents, preservatives may form part of vegetable substance preparations. These substances must be indicated.

The multicomponent, in a chemical sense, **active ingredient** is composed basically of three types of substances, namely **active components, auxiliary components and neutral components.**

The active components encompass two functionally different subgroups, namely, the constituents with known therapeutic activity (active principles) and those which are pharmacologically relevant but do not account solely for the efficacy of the plant preparation.

Auxiliary components are those, which do not affect directly the physiology and thus, the efficacy, but facilitate the entry or crossover of active principles upon application of the medicine. According to the above, the plant drug itself is, therefore, normally the starting material and only in the case of direct application also the phytomedicine.

Planning the approach

The design of a proper evaluation and development routine for a given phytomedicine constitutes always an „interdepartemental" effort. The first step is the compilation and analysis of material pertaining to the questions outlined in figure 1 in order to establish a catalogue of necessary requirements.

The table shows three sections in order of priority. Without answering section 1 it is a little help to work on section 2, and without the conclusions of the latter it makes no sense to formulate requirements to be put down in section 3.

The approach can be used for both, the evaluation of phytomedicines already on the market and also the development of new products based on plant drugs.

1.3 Figure 1

1. Therapeutical information

- definition of medicinal indication
    - specified symptomatology of the affliction/disease
    - target organ
    - therapeutical aim
- reason for selecting this particular phytomedicine/plant drug
    - pharmacological data
    - therapeutical data
    - literature data, reports from experience, ethnomedicine
- intended rationale for using this particular phytomedicine
    - detailed proposal, where and why to establish this product in medicine

2. Technological requirements

- which compounds are liberated during processing of raw material ?
- which compounds undergo degradation or changes during processing ?
- which among those are active components in the intended indication ? Extent of alteration ?
- is the practiced or intended manufacturing procedure in line with the a. m. requirements ?
- can relevant compounds or groups already be selected, which are therapeutically relevant and present ?

3. Starting material

- average quality on the market
- necessary quality
- availability
- alternatives

Efficacy

The first section of the table constitutes conceptually the most difficult. As a rule, traditional use has to be translated into modern concepts of conventional „western" medicine, an even greater task, when alternative concepts of health and disease are involved, e.g. TCM or native philosophies. In addition, common ailments, like stomach or intestinal trouble, have a variety of causes including psychomatic ones and it is, therefore, mandatory to reduce them to the most plausible fitting of the symptomatology. The target organ for combating the disease should be defined with the overall strategy of maximum therapeutical effect.

It should be kept in mind, that the primary concern is not the proof of a better overall efficacy of phytomedicines compared to pure synthetic substances, but effectiveness at affordable costs.

Having established the therapeutical concept, the available data have to be screened and evaluated, not only for efficacy but also for safety.

The best way, in my experience, is a grading approach assigning various degrees of plausibility and relevance to the different sets of experimental and literature data. Such a system is outlined in figure 2.

The table lists the various items in decreasing order of importance for the documentation of proof. Textbooks e.g. are relatively unsuited. GCP studies, although expensive and tedious, are at the top of the ladder.

The figure 3 lists a personal approach to the problem of safety of a phytomedicine. It is only valid for a product on the market undergoing reassessment and mentioned as precaution in the product information. For newly developed phytomedicines the safety assessment has to follow the monitoring in phase III and IV of the general registration conditions.

Quality

Using the assessment derived from the section on therapeutical aims and efficacy of figure 1, it is possible to define the extraction and manufacturing process in more detail. Knowing, or at least having some good idea about the active principles involved, one can adjust the extraction parameters e.g. polarity, pH, temperature etc. accordingly to obtain the optimum content without disturbing the qualitative internal composition. Figure 4 gives an overview of the possible factors.

Technical experiments on a laboratory scale can, according to my experience , translated 1:1 into the commercial batch production of extracts.

For the purpose of a unified understanding of terms, especially when it comes to the declaration of contents for registration and labelling, a few definitions are added.

Extract means, as a matter of principle, the genuine extract, i.e. dried constituents extracted with a defined solvent.

Preparations of extracts are extracts containing added exipients (technical excipients, excipients for adjusting, or other excipients - e.g. solvents).

Drug-extract-ratio is the proportion of the genuine quantity of the starting drug used to the native extract obtained. Relevant information is provided within the natural range of fluctuation. More precise particulars can be given after an extended period of observation.

Parallel to the assessment or the development of an appropriate extract, intermediate or final product, a suitable quality control method has to be developed.

The finding of the right quality control parameters is an interdependent process shown schematically in figure 5.

For the **definition of analytic parameters** and specifications one has to work backwards, normally not necessary in work with medicines using synthetic active ingredients.

An outlay of the entire design procedure is given in figure 6. Please note, that in phytomedicines the botanical/pharmacognostic definition plays an important role and cannot be neglected. Microscopy is basic to the identification and determination of purity in the starting material and cannot be substituted for by physico-chemical methods. The latter are of primary importance for quantitative aspects of quality control.

Grading stages for efficacy and safety

Clinical, therapeutical

- complete agreement with an official therapeutical monograph, e.g. WHO, ESCOP, Kom. E, Avis

- partial agreement with an official monograph, supplemental evidence

- clinical/therapeutical study, double blind against placebo (GCP conform)

- clinical/therapeutical study, double blind against similar product (GCP conform)

- open (comparative) study

- studies involving therapeutical monitoring only

- structured reports of observations from medical practitioners; documented casuistics and observations involving at least 10 patients

- summaries of documented therapeutical experience incl. expert report

- evidence of mode of action in clinical matters from ethnomedicine incl. expert report

Literature documents

- results from publications in peer-reviewed scientific journals
- results from non peer-reviewed publications
- reviews and monographic summaries in modern handbooks and compendia (from 1985 onward)
- literature results from older medicinal handbooks (before 1985)
- extrapolation of pharmacological experiments from animal systems to human conditions

Safety evaluation

Adverse reaction: A response which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of the disease, or for the modification of physiological function (WHO)

Factorial evaluation of unexpected adverse reactions (UAR)

1. UARs concerning the phytomedicine in the marketed form

- no UARs known: 100 points

-serious UARs known: 1 point

a) - certain: factor 1
   - possible: factor 2

b) - occurrence very rare (less than 0.1 %) factor 10
   - occurrence rare (less than 1 %) factor 5
   - occurrence occasionally (1-10 %) factor 2
   - occurrence frequently (greater 10 %) factor 1

multiply: points X a X b

- non serious UAR known: 5 points
  - occurrence and corresponding factors as before

multiply as before

2. UARs concerning single active ingredients of the phytomedicine

- no UARs known: 100 points

- serious UARs known: 1 point
  factors as in 1
  additionally:
  c) same formulation and application form
  factor 1
  parenteral solution: factor 2
  d) reciprocal of percentage of content of UAR ingredient in the formulation
  factor: 100/percentage
  multiply: points X a X b X c X d

a) - certain: factor 1
   - possible: factor 2

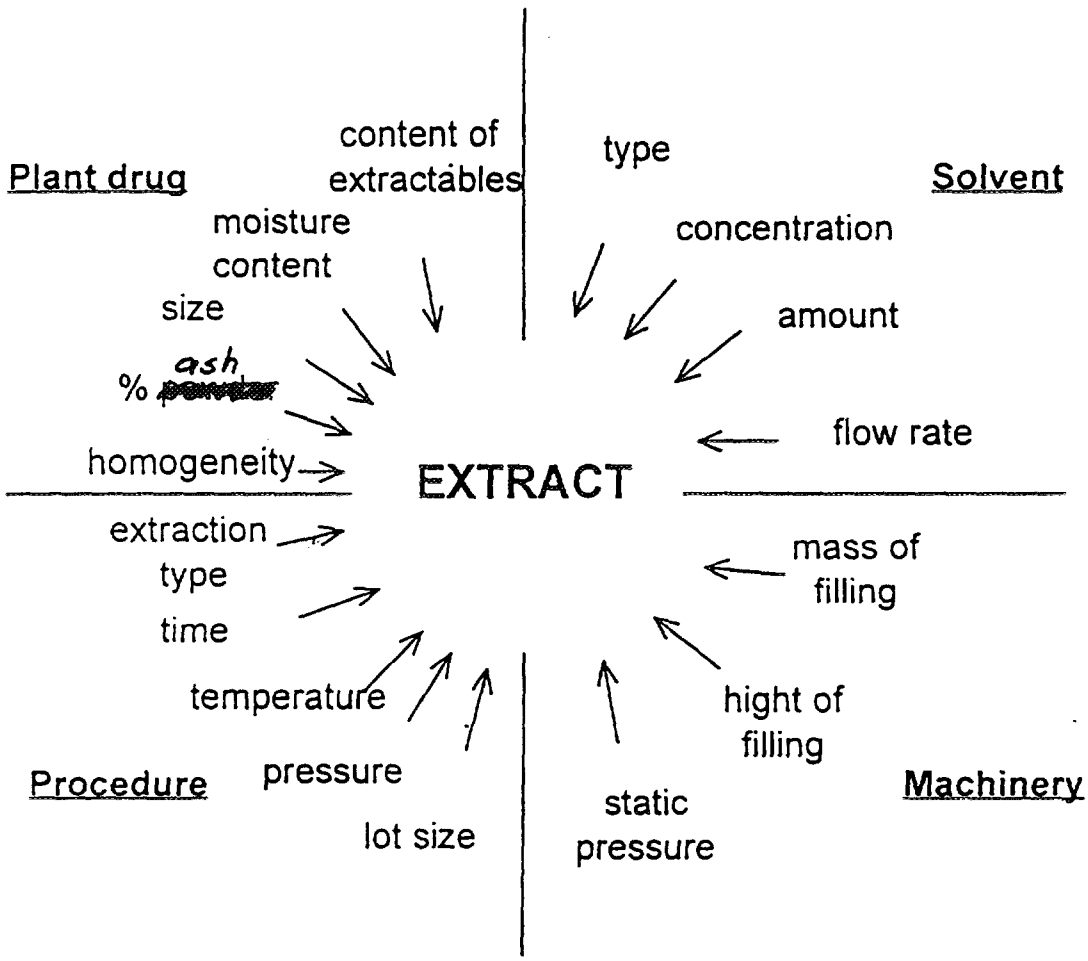
b) - occurrence very rare (less than 0.1 %)
   factor 10
   - occurrence rare (less than 1 %)
   factor 5
   - occurrence occasionally (1-10 %)
   factor 2
   - occurrence frequently (greater 10 %)
   factor 1

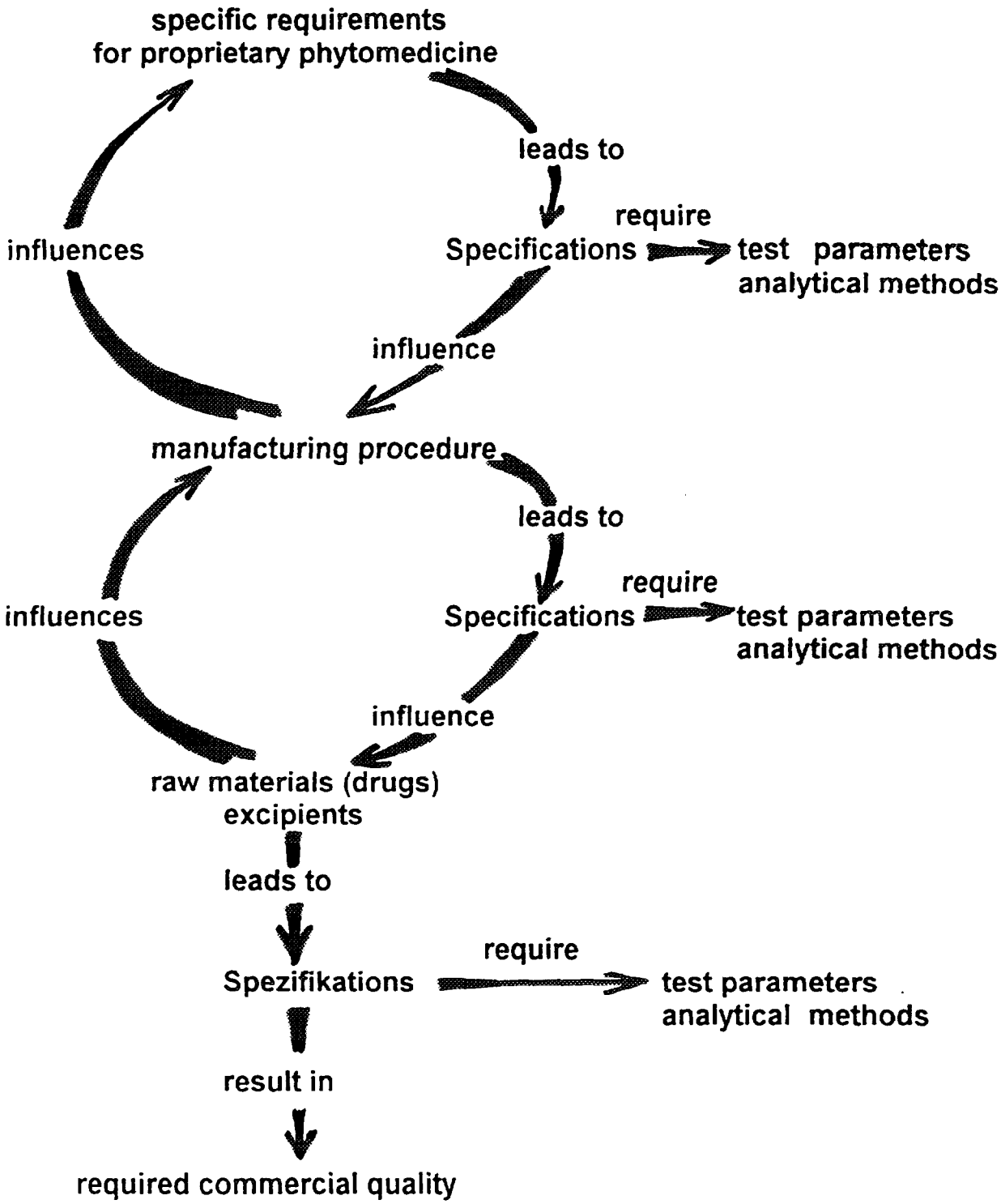
multiply: points X a X b

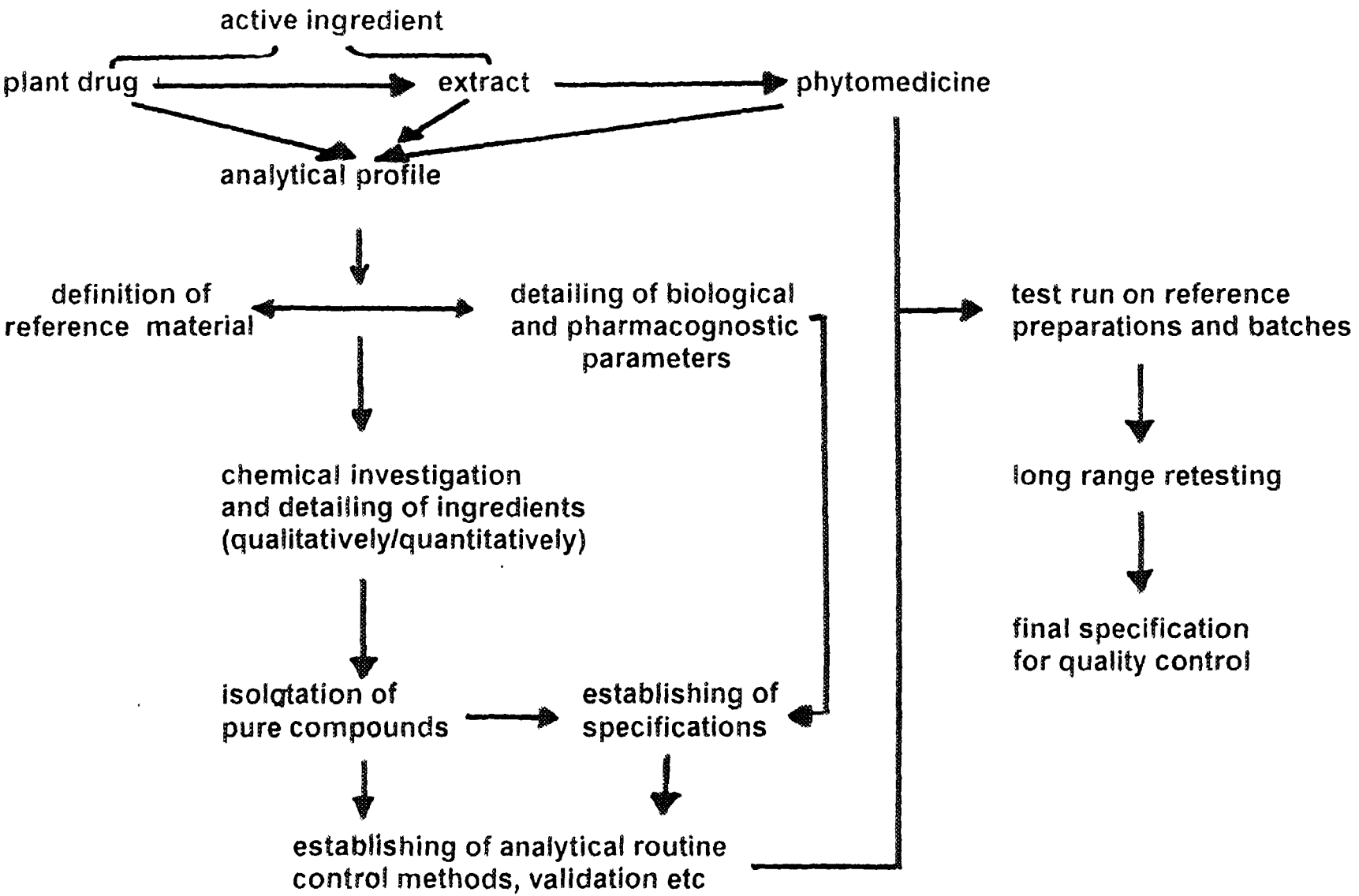
- non-serious UAR known: 5 points
  - occurrence and corresponding factors as before
  multiply as before

-no serious UARs known: 5 points
  factors as before
  multiply: points X a X b X c X d

A minimum value of 100 points should be achieved in order to consider the product safe.


Plant drug
content of extractables
moisture content
size
% ash
homogeneity
Solvent
type
concentration
amount
flow rate
EXTRACT
extraction type
time
temperature
pressure
lot size
Procedure
mass of filling
hight of filling
static pressure
Machinery


specific requirements
for proprietary phytomedicine
leads to
require
influences
Specifications
test parameters
analytical methods
influence
manufacturing procedure
leads to
require
influences
Specifications
test parameters
analytical methods
influence
raw materials (drugs)
excipients
leads to
require
Spezifikations
test parameters
analytical methods
result in
required commercial quality


active ingredient
plant drug
extract
phytomedicine
analytical profile
definition of
reference material
detailing of biological
and pharmacognostic
parameters
test run on reference
preparations and batches
chemical investigation
and detailing of ingredients
(qualitatively/quantitatively)
long range retesting
final specification
for quality control
isolqtation of
pure compounds
establishing of
specifications
establishing of analytical routine
control methods, validation etc

Starting materials should be specified along the line followed by the monographs in the pharmacopoiea, using the EP as guidance, since there most of the common plant drugs are described in a practical and useful manner (figure 7). When it comes to content however, the individual manufacturer has to go one step further. The pharmacopoeia gives only a minimum value, which has to be implemented by the manufacturer by a range in which quantitative parameters should lay in order to ensure reproducibility. It is also recommended that reference samples of the whole and cut drug be kept and used, since many plant drugs need proper botanical identification, especially, if closely related species are available under the same vernacular name.
With intermediates and final products physico-chemical methods like TLC and HPLC are in the foreground of analysis. Here it is important to select and characterize properly the reference substances necessary. Especially their purity is critical, since it can falsify quantitative results.
It should be mentioned, that many convention-methods used for determining ash, dry residue, water content, extractable matter etc. give important clues to the status of the raw material, intermediate or final phytomedicine. Sensory methods, chiefly used in the food industry, can also in times be helpful. Many of these analytical procedures have been described in the WHO document „Quality control methods for medicinal plant materials" (Pharm./90.152/rev.2, TRM/90.3).

When establishing the **specifications** it has been advantageous to assess the identity of the active ingredient, in phytomedicines defined as the sum of extractable components under the technological conditions used, using a chromatographic profile and its content by measuring the amount of one specific substance or group of substances of these extractables, thus being able to indicate the integrity of the internal composition and also, in proportion, its quantity.
At this point, the problem of uniformity of the phytomedicine from batch to batch has to be addressed. Since plants vary in their content of components due to environmental and climatic factors, a certain range of quantitative internal composition must be specified and maintained in the final product from batch to batch. The way to achieve this is by standardisation.
Standardisation is thus, the equalizing of an extract or tincture to a defined content of the compound used to assess the later activity of the product and is basically a problem of manufacturing. Here it suffices to state, that addition of the reference or measured substance to the extract is not the proper way, since the internal proportion of the extractables will be changed. Generally speaking, standardization is reached by way of mixing different batches of drugs or different batches of preparations and with the help of a validated manufacturing process. It includes all measures which lead to reproducible quality without using excipients to adjust a content. As measuring parameter the content of active principles or active markers, if known, is useful.
Control tests on the finished product must be such as to allow the qualitative and quantitative determination of the active ingredients, if known. Special methods must be used in the qualitative determination. The quantitative control is made in the form of
a) a batch-specific control using a given marker, usually with a range of ± 5 %; or
b) analogous to a) but determining pharmacologically relevant constituents (active markers) within the given specification, or
c) the control of the active principle, usually with a range of ± 5 %.

Because of the special situation of herbal medicinal products as mixtures consisting of numerous substances, deviations from the ± 5 % limit are justified under certain circumstances. Therefore, wider ranges can be specified if sound reasons are given.
If a *herbal medicinal product* contains several *plant drugs* or preparations thereof and it is not possible to perform a quantitative determination of each active ingredient, the determination may be carried out jointly for several active *ingredients (eg. flavonoids as a whole in Crataegi flos)*. The need for this procedure must be justified.

Nomenclature
engl./frz., latin

Definition
whole drug,reduced
drug,powder, fresh/dry
scientific name of plant
part of plant used
minimum content

Characters
organoleptic,odour,taste

Identification
macroscopic bot. charact.
microscopic bot. charact.
thin-layer-chromatography
chemical reactions

Tests
starch
ash
filth
foreign components
microbial contamination
foreign matter
swelling index
bitterness value
extractable matter
loss on drying/water

Assay
VIS./UV. spectrophotometry
volumetric titration
essential oil determination
liquid chromatography
quantitative TLC
gaschromatography

Storage and Labelling

For reasons of safety, parallel determination of residual solvents (for dry extracts), pesticides where expected, microbial contamination and heavy metal content have to be performed. The Pharm. Eur. gives for most of these aspects methods and limits of content.
One further topic to be considered is the evaluation of stability of a finished product, a factor important for shelf life and its economic value. The guidelines are specific on this.
Stability testing of the finished product comprises

a) for the batch-specific control of the preparation in the finished product, the determination of the marker within the limits of 90-105 % of the starting value;
b) for constituents with known therapeutic activity the determination of these constituents within the limits of 90 - 105 % of the declared values;
c) for active markers, the determination of these constituents within the specifications.

Furthermore, in any case it must be substantiated with the help of suitable fingerprint chromatograms that no essential changes in the pattern of constituents occur.
If a herbal medicinal product contains several plant drugs or preparations thereof and if it is not possible to determine the stability of each active ingredient, the stability of the medicinal product should be determined by appropriate fingerprint chromatograms or other suitable tests. The experimental design for such studies is outlined in the figure 8.
Generally, one tries to achieve a shelf life of 3 years at appropriate temperature and moisture conditions (figure 9). There are ICH guidelines for such testing which apply also for phytomedicines.

Starting material

The last segment of figure 1 concerns the starting material. The way of establishing its specifications has already been outlined before.
The standard for the material to be used is laid down in the proper Pharmacopoeia. The Ph. Eur. contains presently monographs on 69 drugs, 16 more are scheduled to appear 1998 in the supplement. In contrast, the USP monographs only 8 with 8 more appear in the future (figure 10).
It is requested for registration of phytomedicines, that for each *plant drug* preparation which is *used as active ingredient (starting material),* a monograph must be submitted *if no Pharmacopoeia monograph is available*. This monograph must be established on the basis of recent scientific data and must give particulars of the characteristics, identification tests and purity tests. This has to be done e.g. by different appropriate chromatographic methods. If deemed necessary by the results of the analysis of the starting material, tests on microbiological quality, residues of pesticides, fumigation agents, solvents and toxic metals have to be carried out.
Similar requirements, by the way, are also laid down for excipients.

Registration

In Europe, the rules and requirements of registration have been harmonized and are laid down in the „Notice of Applicants" mentioned before. What is asked for, is a structured version of the results of the applied research approach outlined above with a critical evaluation of the data laid down in an expert report. The various points are illuminated by the last figure showing the contents of such a dossier (figure11).

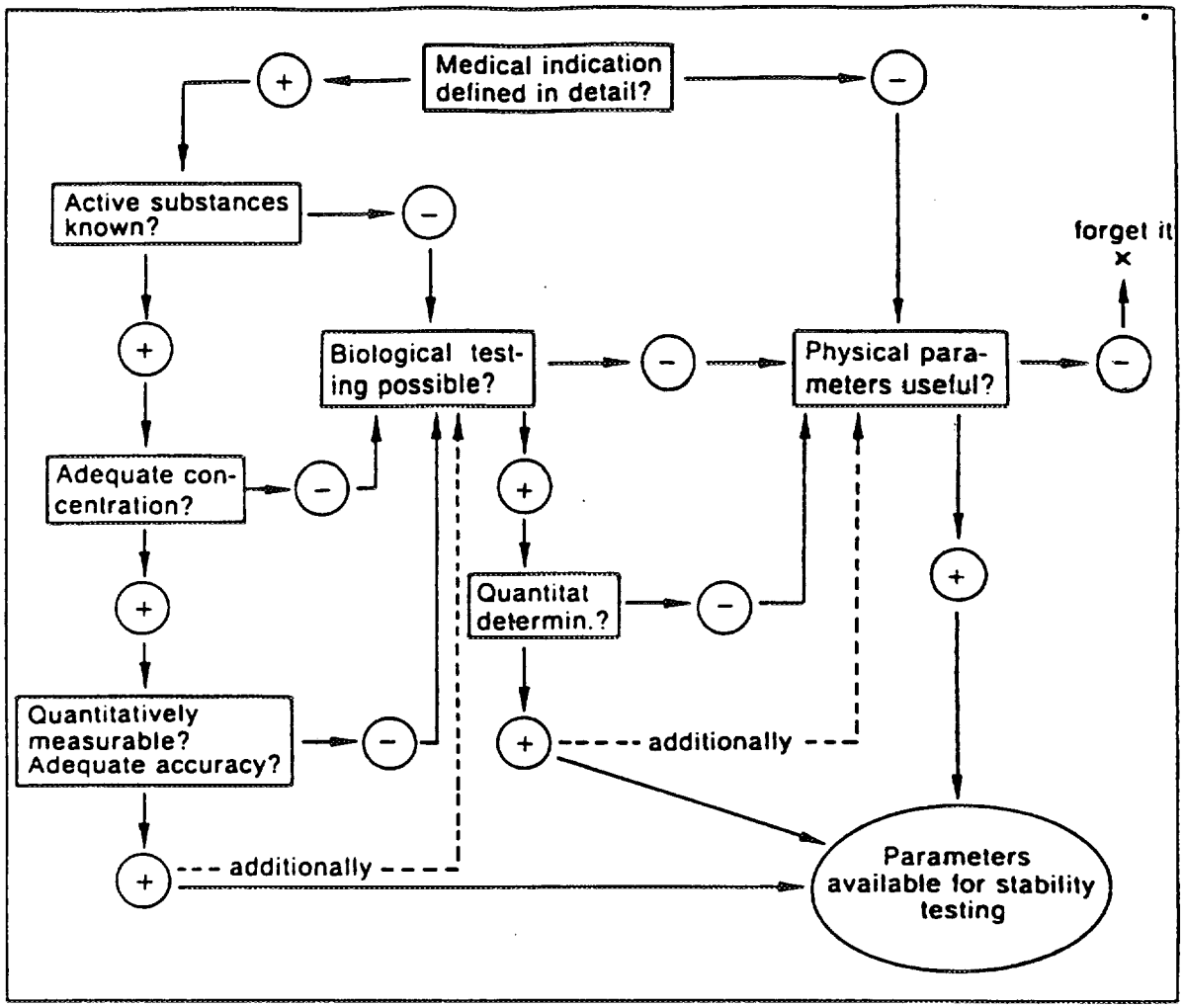


Fig. 1: Flow-chart for deciding the strategy in stability testing of phytopharmaka

1.3. Figure 8

Experimental stability testing parameters

temperature	rel. air humidity	information about
21°C	45%	temperate climate
25°C	60%	mediterranean cl.
30°C	35%	desert climate,trop.
30°C	70%	tropical climate

6 PHNC4T.DOC

9:1400

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (1)

- PRESENT STATUS

MONO-GRAPHS	EUR.PH. 1997	EUR.PH. 1998	DAB 10	PH.F. 10	IT.PH. 9	JAP.PH. 12
TOTAL NUMBER	1099	112	965	-	855	-
VEGETABLE DRUGS	69	16	127	141	103	47 (E.PH) ↑ 113 ↓ 66 (NON-E.PH)
PERCENTAGE VEGETABLE DRUGS	6.3%	14.2%	13	-	12	-

EUROPEAN PHARMACOPOEIA 1997 + 1998	MONOGRAPHS ALREADY PUBLISHED	MONOGRAPHS UNDER STUDY	TOTAL
VEGETABLE DRUGS	48	50	98
PLANT RAW MATERIALS OBTAINED AFTER TREATMENT	22	15	37
TINCTURES AND EXTRACTS	5	17	22
VEGETABLE OILS AND WAXES	10	10	20
TOTAL	85(7.0%)	92	177

- VOLATILE OILS, BALSAMS, RESINS, GUMS AND STARCHES

PART IA: SUMMARY OF THE DOSSIER

Administrative data

Fees, declaration and signature

Type of application

1. This application concerns:
2. The application is in accordance with the following legal base:
3. This is an application for:

Marketing authorisation particulars

Table of contents for remainder of the dossier

PART IB 1: SUMMARY OF PRODUCT CHARACTERISTICS

Summary of product characteristics: list of headings

Summary of product characteristics, notes on headings

1. Trade name of the medicinal product
2. Qualitative and quantitative composition
3. Pharmaceutical form
4. Clinical particulars
5. Pharmacological properties
6. Pharmaceutical particularls
7. Marketing authorisation holder
8. Marketing authorisation number
9. Date of first authorisation/renewal of authorisation
10. Date of (partial) revision of the text

PART IB 2: PROPOSAL FOR PACKAGING, LABELLING & PACKAGE LEAFLET

PART IB 3: SPCS ALREADY APPROVED IN THE MEMBER STATES

PART IC: EXPERT REPORTS

A. Introduction

B. Presentation of the Expert Reports

C. Expert Reports for abridged applications

PART IC 1: EXPERT REPORT ON CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION

A. Introduction
B. Expert Report
C. General aspects
D. Critical assessment
    1. Composition of the product:
    2. Development pharmaceutics:
    3. Stereoisomerism:
    4. Method of preparation:
    5. Process validation:
    6. Control of pharmacopoeial active substance(s) :
    7. Control of non-pharmacopoeial active substance(s):
    8. Excipients :
    9. Packaging material (immediate packaging):
    10. Control tests on intermediate products:
    11. Control tests on the finished product:
    12. Stability of the active substance(s):
    13. Stability of the finished product:
    14. Other information:
    15. Reference list:
    16 Information on the qualifications and experience of the pharmaceutical expert:
E. Tabular formats - chemical and biological products
F. Tabular formats - radiopharmaceutical products

PART IC 2: EXPERT REPORT ON THE TOXICO-PHARMACOLOGICAL (PRE-CLINICAL) DOCUMENTATION

A. Introduction
   - Product profile
   - Appendices to the expert report
B. Expert report
C. General aspects
D. Critical assessment
   1. Pharmacodynamics
   2. Pharmacokinetics
   3. Toxicity
   4. Conclusions
   5. Reference list
   6. Information on the toxico-pharmacological (pre-clinical) experts
E. Tabular formats

PART IC 3: EXPERT REPORT ON THE CLINICAL DOCUMENTATION

A. Introduction
   - Product profile
   - Appendice to the expert report
B. Expert report
C. General aspects
D. Critical assessment
   1. Clinical pharmacology (Part IV A)
   2. Pharmacodynamics
   3. Pharmacokinetics
   4. Clinical trials (PART IV B)
   5. Post marketing experience
   6. Other information
   7. Conclusion
   8. Reference list LIST
   9. Information on the clinical expert
E Tabular formats

PART II: CONCERNING CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION FOR VEGETABLE MEDICINAL PRODUCTS

PART II A: COMPOSITION

1 Composition of the medicinal product
2 Container (brief description)
3 Clinical trial formula(e)
4 Development pharmaceutics

PART II B: METHOD OF PREPARATION

1 Manufacturing formula (including details of batch size)
2 Manufacturing process
3 Validation of the process,

PART II C: CONTROL OF STARTING MATERIALS

1 Active substance(s)
2 Excipient(s)
3 Packaging material (immediate packaging)

PART II D: CONTROL TESTS ON INTERMEDIATE PRODUCTS (IF NECESSARY)

PART II E: CONTROL TESTS ON THE FINISHED PRODUCT

1 Specifications and routine tests
2. Scientific data

PART II F: STABILITY

1 Stability tests on active substance(s)
2 Stability tests on the finished product

PART IIG : Bioavailability/Bioequivalence

PART II H : DATA RELATED TO THE ENVIRONMENTAL RISK ASSESSMENT FOR PRODUCTS CONTAINING/CONSISTING OF GENETICALLY MODIFIED ORGANISMS (GMOS)

PART II Q: OTHER INFORMATION

PART III: TOXICO-PHARMACOLOGICAL DOCUMENTATION

PART III A: TOXICITY

PART III B: REPRODUCTIVE FUNCTION (FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE)

PART III C: EMBRYO-FOETAL AND PERINATAL TOXICITY

PART III D: MUTAGENIC POTENTIAL

PART III E: CARCINOGENIC POTENTIAL

PART III F: PHARMACODYNAMICS

PART III G: PHARMACOKINETICS

PART III H: LOCAL TOLERANCE (WHERE APPROPRIATE)

PART III Q : OTHER INFORMATION

PART III R: ENVIRONMENTAL RISK ASSESSMENT / ECOTOXICITY

PART IV: CLINICAL DOCUMENTATION

PART IV A: CLINICAL PHARMACOLOGY

1. PHARMACODYNAMICS
2. PHARMACOKINETICS

PART IV B: CLINICAL EXPERIENCE

1. CLINICAL TRIALS
2. POST-MARKETING EXPERIENCE (IF AVAILABLE)
3. PUBLISHED AND UNPUBLISHED EXPERIENCE (OTHER THAN 1.)

PART IV Q: OTHER INFORMATION

PART II
CONCERNING CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION FOR VEGETABLE MEDICINAL PRODUCTS[14]

The principle of GMP and the detailed guidelines are applicable to all operations which require the authorization referred to in Article 16 of Directive 75/319/EEC[15] as modified. They are also relevant for all other large scale pharmaceutical manufacturing processes, such as that undertaken in hospitals, for the preparation of products for use in clinical trials, and for wholesaling, were applicable.

All analytical test procedures described in the various sections of the Part II chemical, pharmaceutical and biological documentation must be described in sufficient detail to enable the procedures to be repeated if necessary (e.g. by an official laboratory). All procedures need to be validated and the results of the validation studies must be provided.

PART II A: COMPOSITION

1 COMPOSITION OF THE MEDICINAL PRODUCT

NAMES OF INGREDIENTS	UNIT AND/OR PERCENTAGE FORMULA	FUNCTION	REFERENCE TO STANDARDS
Active substance(s)			
Excipient(s)			

2 CONTAINER (BRIEF DESCRIPTION)

Nature of container materials; qualitative composition; method of closure; method of opening.

3 CLINICAL TRIAL FORMULA(E)

4 DEVELOPMENT PHARMACEUTICS

Explanation with regard to the choice of formulation, composition, ingredients and container, supported, if necessary, by data on development pharmaceutics .The overage, with justification thereof, should be stated .Tests carried out during pharmaceutical development must be described in detail, e. g. in vitro dissolution studies for solid pharmaceutical forms.

[14] see the annex, reference 61

[15] see the annex, reference 9

PART II B: METHOD OF PREPARATION

1 MANUFACTURING FORMULA (INCLUDING DETAILS OF BATCH SIZE)

2 MANUFACTURING PROCESS (INCLUDING IN-PROCESS CONTROL AND THE PHARMACEUTICAL ASSEMBLY PROCESS)

If vegetable active substance preparations are the starting material, the description of their manufacturing process and their control belong to section C.

3 VALIDATION OF THE PROCESS,

Validation of the process should be carried out when a non-standard method of manufacture is used or for steps of the manufacturing process which are critical for the product described in the finished product specifications (experimental data showing that the manufacturing process, using materials of the stated quality and the types of manufacturing equipment specified, is a suitable one and will consistently yield a product of the desired quality).

PART II C: CONTROL OF STARTING MATERIALS

1 ACTIVE SUBSTANCE(S)

1.1. Specifications and routine tests

1.1.1 Active substance(s) described in a pharmacopoeia

1.1.2 Active substance(s) not described in a pharmacopoeia

- Characteristics
- Identification tests
- Purity tests (including limits for named, total, other single, unidentified single and unidentified total impurities)
  - Physical
  - Chemical
  - Potential contamination by micro-organisms, products of micro-organisms, pesticides, toxic metals, radioactivity, fumigants, etc.
- Other tests
- Assay(s) of excipients of vegetable active substances or vegetable active substance preparations with known therapeutic activity
- In the case of vegetable active substance preparations, a monograph on the vegetable active substance

1.2. Scientific Data

1.2.1 Nomenclature

- International non-proprietary name (INN)
- Chemical name
- Other name
- Laboratory code
- In the case of vegetable active substance(s)
  - Scientific name of plant, with the name of the authority, variety and chemotype
  - Parts employed of the herb
  - Name of the preparation

1.2.2 Description

- Physical form
- Structural formula (including conformational data for macromolecules)
- Molecular formula
- Relative molecular mass
- Chirality
- Main excipients of vegetable active substances based on recent scientific data

1.2.3 Manufacture

- Name(s) and address(es) of the manufacturing source(s)
- Geographic source of vegetable active substance.
- Synthetic or manufacturing route
- Description of process
- Solvents, reagents; excipients .
- Catalysts
- Purification stages

1.2.4 Quality control during manufacture

- Starting materials
- Control tests on intermediate products (where appropriate)

1.2.5 Development (for active substance(s) of vegetable origin)

1.2.5.1 Vegetable active substance

- Description of the vegetable active substance(s)
  - macroscopic
  - microscopic
- Composition and analytical research for excipients and physical characteristics
- Investigation for adulterants of known toxic excipients
- Analytical development and validation, commentary on the choice of routine tests and specifications

1.2.5.2 Vegetable active substance preparation (e.g. powder extract)

- Analytical chemical profile (qualitative and quantitative)
- Detection of toxic excipients/adulterants
- Analytical development and validation, commentary on the choice of routine tests and specifications.

1.2.6 Impurities

- Potential impurities originating from the route of synthesis
- Potential impurities arising during the production and purification
- Methods detecting potential contamination of the vegetable active substance(s) by micro-organisms and products of micro-organisms, pesticides, fumigation agents, toxic metals, radioactivity etc.
- Potential falsification and adulterants of the vegetable active substance(s)

1.2.7 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size, and use of batches including batches used in preclinical and clinical testing)
- Results of tests
- Reference material (analytical results), primary and others

2 EXCIPIENTS

2.1 Specifications and routine tests

2.1.1 Excipients described in a pharmacopoeia

2.2.2 Excipients not described in a pharmacopoeia

- Characteristics
- Identification tests
- Purity tests (including limits for named, total, other single, unidentified single and unidentified total impurities)
  - physical
  - chemical
- Other tests
- Assay(s) and/or evaluations (where necessary)

2.2 Scientific data

Data, where necessary, for example on excipient(s) used for the first time in medicinal products (see II C.1.2).

3 PACKAGING MATERIAL (IMMEDIATE PACKAGING)

3.1. Specifications and routine tests

- Type of material
- Construction
- Quality specifications (routine tests) and test procedures

3.2. Scientific data

- Development studies on packaging materials
- Batch analysis, analytical results

PART II D: CONTROL TESTS ON INTERMEDIATE PRODUCTS (IF NECESSARY)

A distinction should be made between in-process controls (Part II B) and control tests on intermediate products.

PART II E: CONTROL TESTS ON THE FINISHED PRODUCT

1 SPECIFICATIONS AND ROUTINE TESTS

1.1 Product specifications and tests for release at time of manufacture (general characteristics, specific standards)

1.2 Control Methods

1.2.1 Test procedures for identification and quantitative determination for the active substance(s).

It must be described in detail (including biological and micro-biological methods where relevant), together with other tests which include those in the appropriate general monograph for the type of dosage form in the European Pharmacopoeia:

- Identification tests
- Quantitative determination of active substance(s); and additionally for vegetable active substances and vegetable active substances preparation, quantitative determination of excipients with known therapeutic activity
- Purity tests
- Pharmaceutical tests (e.g. dissolution)

1.2.2 Identification and determination of excipient(s)

- Identification tests for approved colouring materials
- Determination of antimicrobial or chemical preservatives (with limits)

2. SCIENTIFIC DATA

2..1 Analytical validation of methods and comments on the choice of routine tests and standards (e.g. working standards)

2..2 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size and use of batches)
- Results obtained
- Reference material (analytical results), primary and others

PART II F: STABILITY

1 STABILITY TESTS ON ACTIVE SUBSTANCE(S)

- Batches tested
- General test methodology
  - accelerated test conditions
  - normal test conditions
- Analytical test procedures
  - assay
  - determination of degradation products
- Validation of all test procedures including limits of detection (including initial results)
- Results of tests
- Conclusions

2 STABILITY TESTS ON THE FINISHED PRODUCT

- Quality specification for the proposed shelf-life
- Batches tested and packaging
- Study methods
  - real time studies
  - studies under other conditions
- Characteristics studied
  - physical characteristics
  - microbiological characteristics
  - chemical characteristics
  - chromatographic characteristics
  - characteristics of the packaging (container/closure interaction with the product)
- Evaluation test procedures
  - description of test procedures
  - validation of test procedures
- Results of test (including initials and reference to degradation products)
- Conclusions
  - shelf-life and storage conditions
  - shelf-life after reconstitution and/or first opening of the product
- Ongoing stability studies

PART II G: BIOAVAILABILITY/BIOEQUIVALANCE

Give reference to relevant sections in Part IV.

PART II H: DATA RELATED TO THE ENVIRONMENTAL RISK ASSESSMENT FOR PRODUCTS CONTAINING/CONSISTING OF GENETICALLY MODIFIED ORGANISMS (GMOS)

PART II Q: OTHER INFORMATION

This part is intended for information not covered by any of the previous parts, e.g. the analytical tests used for the pharmaceutical development of the product, studies concerning metabolism and bioavailability, etc.

Industrialization of medicinal plants

Götz Harnischfeger

The starting materials for all phytomedicines are plant drugs, mostly parts or plant organs of medicinally used species and usually in the dried form.

a) Plant material gathered by collection

According to WHO there are 21 000 plant species listed as being medicinally used as plant drugs. Between 70 - 90 % of these are commercially obtained by collecting the drugs in the natural habitat. About 50-100 species only are cultured as well.
The collecting practice is, interestingly, not always identical with the area of the species main occurrence. It is found mostly in regions with low wage levels, e.g. Eastern Europe, Africa, South America.
The reasons for the continuing practice of collection are manifold. Some of these arguments are given in figure 1.
The first argument encompasses all plant species which need more than 5 years to reach maturity or the stage of harvesting. Into this category belong trees like Aesculus hippocastanum, perennials like Arctostaphylos uvae ursi and bushes like Crataegus. These European species are not grown for commercial purposes but, if at all, as plants for alley-rims and natural hedges.
Many species are not amenable for agriculture for a variety of reasons, e.g. symbiotic relationships with other plants like in Viscum, Lichen islandicus etc. Inculturing might also prove difficult, especially with plants which developed the survival strategy of irregular flowering and seed formation, irregular germination parameters etc. Baptisia tinctoria is such a species where it took 15 years of agricultural research and high expenses to get a culture started.
This example illustrates the importance of the last two arguments of figure 1, namely, the total tonnage needed is uninteresting from a monetary point of view and collecting is a more economic alternative. In Baptisia, my company processes 95 % of the world demand, a great total of 4-5 tons per annum.
One has to consider also some dangers originating in the collection practice (figure 2). The figure lists the two main problems, namely extinction and elimination of genetic variety. Overharvesting of natural resources can lead to extinction of the plant in an entire region, e.g. Vinca rosea in Madagascar, or decrease, at minimum, the genetic variety of predominantly rare species. A third danger should not be omitted. It is the use of mostly uneducated collectors who destroy a whole plant to harvest just on plant organ, e.g. Tecoma bark.
In spite of these problems, collecting of plant material in the native habitat will represent for a long time to come the method of gathering starting material for phytomedicines. There are specific aspects of quality and concomitant analytics which are important in collected plant drugs. They are exemplified in figure 3.
Collected plant drugs, especially those used under their vernacular name, are very prone to be mislabelled, so that the aspect of analytical determination of identity becomes important. The best example is the well known drug Zarzaparilla, which is either Smilax species or at least in Peru, the root of Rumex obtusifolius (Roersch I, 201). Thus pharmacognostic analysis, coupled with knowledge about possible alternatives and synonym drugs, is the key operation in determining the exact identity of material.
One recent example, which happened in the US and luckily did not result in fatalities, may illustrate the importance of pharmacognostic analysis. Herbal tea of Plantago lanceolata leaves was containing leaves of Atropa belladonna, superficially not to distinguish in the cut stage. A simple microscopic analysis could have detected the difference, since these toxic Belladonna leaves show plenty of crystals of Ca-oxalate in the parenchymatic cells and also a specific, wavy cuticula on the epidermis.

Reasons for practice of drug collection from natural habitats

- the plant species grows slowly
- the plant species is not amenable to agriculture
- inculturing poses difficulties
- the tonnage needed for phytomedicines is unimportant
- collecting is more economic than inculturing

1.4 Fig 1.

Dangers from collecting practice

-overharvesting of endemic species

-reduction and/or elimination of local populations with the result of a decrease of genetic variety

-unnecessary destruction of plants during harvest

Specific analytical aspects of collected plant drugs

- identity

- admixtures

- foreign matter

1.4 Figure 3

Such mislabelling is mostly unintentional, since the collectors (and processors) are in many cases poorly educated people going by the native name in collecting.
A related problem is admixture, the above example would not have happened, if a proper identification protocol had been followed. But since it happened in the non medicinal OTC trade, none was required and most health food companies do not invest into such an enterprise. Admixture should not be confused with falsification, a criminal act.
A third aspect, which should be considered with emphasis, is foreign matter. Collected drugs tend to contain a higher percentage of sand, grass, non-drug parts of the species etc, than allowed by the general notices of the pharmacopoiea. Therefore, specific care should be taken in performing those tests described for this purpose. Generally, heavy metals, unusual microbial contamination and pesticide levels are of no or only minor importance. They are rather more frequent in crops from fertilized agricultural fields.

b) Plant material from cultured species

Only about 50 - 100 species are undergoing cultivation nowadays in Europe. Basically, all medicinal plants with a demand for more than 100 tons/a will eventually be agriculturally produced with exception of those meeting the criteria of figure 1. Besides this pure economic argument there are a variety of reasons given in figure 4.
The inculturing process itself should not be discussed at this point, the focus is rather on growing, harvesting and processing of a given, apt species. For the purpose of effective and constant quality a set of rules accompanying the crop from seed to storage is laid down in the GAP-guideline. It aims at minimization of undesirable quality by prevention.
Figure 5 shows the relevant passages for the cultivation part. Emphasis is placed on prevention of problems of microbiological nature, of pesticide residues and from agrochemical treatments.
In this context a sidetour will be added towards breeding of optimized varieties of medicinal plants.
The breeding of new varieties of known medicinal plants is almost exclusively motivated by economic forces. It requires a tight financial control and a continuous evaluation of the risk to benefit ratio in planning and implementing the individual project. In general it can be stated, that the goal pursued in plant breeding is either the improvement of quality or the lowering of costs due to cultivation, preferably both.
Quality, on the other hand, can only be assessed with analytical methods, which are, thus, a valuable tool in achieving the overall goal. The status of analytics is outlined in figure 6, which shows also the interdependence of the various requirements influencing the necessary assessments to be made.
The figure shows also, that the quality of the final product is the decisive factor in determining the requirements of the starting material for its manufacture. The starting material and its parameters are, on the other hand, the result of the efforts of the plant breeder. In consequence, when planning a breeding project, the requirements of the final use of the medicinal variety intended have to be set down and with it the analytical methods of assessing them.
For the medicinal plant in question, especially the control analytics, one has to develop a frame of basic requirements using the set of questions given in figure 7. While the left half of the figure gives relevant criteria applicable to all analytical method, the attributes highly desirable in plant breeding research are listed on the right side. Some consideration has to be given also to the processing of the drug to the final product, inasmuch the analytical variable chosen should not be influenced by this procedure.
This outline works only, however, if the pharmacopoiea set conditions are consistent for the next 10-15 years. But in our times methods and specifications change more quickly, so that breeding towards optimum conditions is a risky business.

(1) when too few of the plants grow wild;
(2) when the wild source is sparsely distributed;
(3) when the wild plants are inaccessible, e.g. mountainous plants or very tall trees from which leaves have to be collected;
(4) when there is need to improve the yield of active principles produced by the plants growing wild;
(5) when there is governmental control over the plants — for example, plants such as cannabis or plants yielding dangerous drugs (or addictive drugs) are best cultivated under licence;
(6) when only a desired species or variety of a particular plant is to be used for preparing galenicals because of its high yield of active constituents;
(7) cultivation can also produce more plant growth and hence a better yield, by introduction of good agricultural practices, good soil, pest control, etc.;
(8) cultivation can allow a better and quicker post-harvest treatment of the plant drug, such as drying and packaging before exportation. It is also useful where extraction of the active principle can be made on the spot, e.g. extraction of diosgenin on the plantation sites of *Dioscorea* yams in Mexico, North America.

Principles and Guidelines of Good Agricultural Practice (G.A.P.)

1. *Seeds and propagation material*

1.1. Seeding materials are to be identified botanically, possibly indicating plant variety and origin[1].

1.2. The occurrence of not species/variety-identical plants, plant-parts has to be controlled in the course of the entire production process (cultivation, harvest, drying, packaging) most strictly and such contaminants are to be eliminated promptly.

2. **Cultivation**

2.1. *Soil and Fertilization*

2.1.1. Medicinal and aromatic plants should not be grown in soils that are contaminated by sludge, heavy-metals, residues of plant protection and other chemicals, etc.

2.1.2. The manure applied should be void of human feces and prior to application it should be thoroughly composted. Application should take place exclusively in the period between harvest and the seeding of the new crop.

2.1.3. All other fertilizing agents should be applied sparingly and according to the necessary plant demand.

2.2. *Irrigation*

2.2.1. The soil must be well aerated. In case of necessity, irrigation should take place regularly and in uniform aliquots, in order to prevent water-logging, the build-up of high microclimatic humidity and as consequence rottenness and mould formation.

2.2.2. Irrigation-water should be of DIN 19650 quality ('hygienically safe water') and should be free of contaminants, such as feces, heavy-metals, pesticides, herbicides and toxicologically hazardous substances.

2.3. *Plant care and plant protection*

2.3.1. Plant density should be so adjusted as to reduce weed growth. Weeding should be carried out regularly, both died off weeds and other plant remnants should be eliminated and destroyed, in order to prevent mould and pest attacks, in the best possible way.

2.3.2. Pesticide and herbicide application should be avoided, as far as possible. In cases of necessity, they should be carried out according to national and international regulations.

The application should be carried out only by educated personal and should precede the harvest by a period either defined by the buyer or indicated by the producer of the plant protection product.

2.3.3. All measures regarding nutrient supply and chemical plant protection, should secure the marketability of the crude drug. It is obligatory that the buyer of the batch be informed of the brand, quantity and date of such chemicals in a written form.

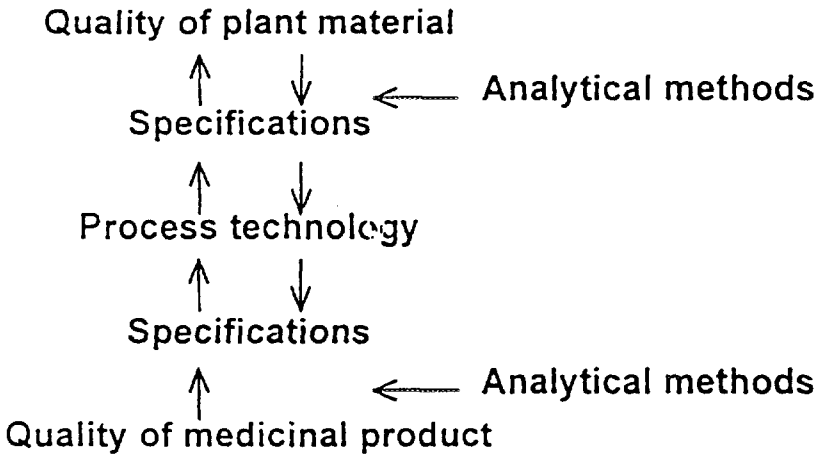

Quality of plant material
Analytical methods
Specifications
Process technology
Specifications
Analytical methods
Quality of medicinal product

Figure 6

Selection steps for analyte compound

- medicinal use of the drug (indication)
- pharmacologic/therapeutic range
- relevant components active at this indication
- priority
- guiding substance for assessing the uniformity of processed drugs (extracts)

Properties of a selected analytical method

- sensitive
- selective
- accurate/precise
- robust
- matrix independent
- simple
- high turnover
- acceptably priced

The a.m. prevention thought is also recognizable in the GAP section on harvesting (figure 8) and in the rules for drying (figure 9), packaging and storing (figure 10). The more general GAP requirements, being similar to those in GMP, are depicted in figure 11.

3. Harvest

3.1. The harvest should take place at a date when the plants - with regard to their aims of utilization- are of the best possible quality and contain their active principles in the highest quantities. Owing to the quality deteriorating effect of superfluously harvested plant matters, only such plants/plant organs should be harvested that will constitute the crude drug in the closest sense.

3.2. Harvest should take place under dry conditions (wet soils, dew, rain or exceptionally high air humidity are unfavorable).

3.3. Equipment should be in a both clean and technically possibly perfect working order. Those machine-parts incl. their housings that have a direct contact with the harvested crop, should be regularly cleaned and kept free of oil and other contamination (incl. plant left-overs).

3.4. Cutting devices of harvesters must be adjusted so that the collection of soil particles can be avoided.

3.5. In the course of harvest, care should be taken that possibly no quantities of contaminants (e.g. weeds) can mix with the harvested crop.

3.6. Damaged and perished plant parts must be promptly eliminated and destroyed.

3.7. All containers used in the harvest must be clean and must be kept free of the remnants of previous, inherent crops; containers out of use, must also be preserved in a dry condition, free of pests and inaccessible for pets as well as domestic animals.

3.8. The harvested crop must not establish direct contact with the soil. It must be promptly collected and under dry, clean conditions (e.g. sacks, baskets, trailers and hoppers, etc.) submitted to transport.

3.9. Mechanical damages, thickening of the crop that would result in undesirable quality changes must be avoided. In this respect, attention is to be paid that

- over filling of sacks is avoided,

- the stacking up of sacks should not result in thickening of the crop,

- plastic sacks are not used in the harvest.

3.10. The period between harvest and transport of the crop to the drying facility should be reduced to a minimum, in order to eliminate undesirable changes in both external appearance, active substances and microbial status.

3.11. The harvested crop must be protected from pests, pets and domestic animals. The extermination of pests should be carried out exclusively by licensed persons and registered chemicals.

4. Drying

4.1. Arriving at the drying facility the harvested crop is to be promptly unloaded and unpacked. It must not be exposed directly to the sun and it must be protected from rainfall.

4.2. Edifices used in the drying of harvested crops must be clean, as well as thoroughly aerated and must never be used for animal keeping.

4.3. Edifices must be built so that they provide protection for the harvested crop against birds, insects, rodents as well as pets and domestic animals.

4.4. Drying equipment and drying frames must be maintained clean and must be regularly serviced.

4.5. In case of air drying, the crop must be spread out in a thin layer. In order to secure unlimited air circulation, the drying frames must be located at a sufficient distance from the ground. It is to be attempted to achieve the uniform drying of the crop and as a consequence to prevent mould formation.

4.6. Provided not only the air drying method is applied, its conditions (e.g. temperature, duration, etc.) must be selected with utmost respect to the type (e.g. root, leaf or flower) and active substance content (e.g. essential oils and others) of the crude drug to be produced.

4.7. Drying directly on the ground or under direct exposure to the sun-light should be avoided.

4.8. The dried plant material (crop) should be screened and sieved in order to eliminate discolored, moulded or damaged substances, as well as soil, rock and other contaminants. Sieves must be maintained in a clean state and should be serviced regularly.

4.9 Clearly marked waste-bins should be kept ready, emptied daily and cleaned.

4.10. In order to protect it and to reduce the risk of pest attacks, the dried crop should be promptly packaged.

5. Packaging

5.1. After a repeated control and eventual elimination of low-quality materials and foreign matter, the well dried crude drug should be packaged in clean and dry, possibly new sacks, bags or cases.

5.2. Packaging materials should be stored in a clean and dry place that is free of pests and inaccessible for domestic animals.

5.3. Reusable packaging materials should be well cleaned and dried prior to their usage.

6. Storage and Transport

6.1. Packaged dried materials should be stored in a dry, well aerated edifice, in which the daily temperature fluctuations are limited and good aeration is given.

6.2. As a protection against pests, pets and domestic animals, the window and door openings are to be protected, e.g.: by wire netting.

6.3. It is to be recommended that the packaged dry crop will be stored:

- in edifices with concrete or similar easy to clean ground,

- on pallets,

- with a sufficient distance to the wall,

- thoroughly separated from other crops.

6.4. In the case of bulk transport, it is important to secure dry conditions and furthermore, in order to reduce the risk of pest attacks, it is extremely advisable to use aerated containers. As a substitute, the use of sufficiently aerated transport vehicles and other aerated facilities are recommended.

6.5. Fumigation against pest-attack should be carried out only in the case of necessity and it must be carried out exclusively by licensed personal. Only registered chemicals must be used.

6.6. Chemicals used either as pesticides or fumigants must be stored in separate rooms.

7. Equipment

7.1. Equipments used in plant cultivation and processing should be easy to clean, in order to eliminate contamination. It is recommended that possibly dry cleaning is implemented. Provided wet-cleaning became inevitable the equipments should be dried as soon as possible.

7.2. All machinery should be mounted in an easily accessible way. They must be well serviced and regularly cleaned.

7.3. In such processing procedures where the direct contact with the harvested crop is inevitable the use of wooden facilities is to be avoided.

7.4. Should wooden equipment (such as e.g. pallets, hopper, etc.) find application, it should not come into direct contact with chemicals and contaminated/infected materials, so that the infection of the plant material can be prevented.

8. Personal and Facilities

8.1. All processing procedures should be fully conform with both EU-Guidelines on Food Hygiene (1993) and the General Principles for Food-hygiene of Codex Alimentarius.

8.2. Personal entrusted to deal with the plant material should be

- required to have a high degree of personal hygiene,
- provided with dressing facilities as well as toilets incl. hand rinsing facilities.

8.3. The activity of persons suffering from known via food transmittable infectious diseases, including diarrhea, or transmitters of such diseases, must be suspended in areas where they are in contact with the plant material.

8.4. Persons with open wounds, inflammations and skin-infections should be suspended from the areas where plant processing takes place, until their complete recuperation.

9. Documentation

9.1. All starting materials and processing steps are to be documented.

9.2. All batches from coherent areas should be unambiguously and unmistakably labeled (e.g. by the application of a charge-number).

9.3. Batches from differing areas must be mixed only, provided it is guaranteed, that the mixture in itself will be homogenous. Such mixing procedures should also be documented.

9.4. It is essential to document the type, quantity and date of harvest of the crop, as well as the chemicals and other substances (e.g. fertilizers, pesticides and herbicides, growth regulators, etc.) used in the course of production.

9.5. The application of the fumigation agents methyl-bromide or Phosphin must be entered into charge-documentation.

9.6. All processes and procedures that could bear an impact on the quality of the product must be entered into the charge-documentation.

9.7. All agreements (production-guidelines, -contracts, etc.) between producer and buyer should be fixed in a written form.

9.8. The results of audits should be documented in an Audit-Report (copies of all documents, Schlagkartei, Audit-Reports, Analyse-Reports) are to be stored for a min. of 10 years.

10. Education

It is extremely advisable to educate all personal having to deal with the crop or those engaged in the direction of the production regarding productions techniques and the appropriate use of herbicides and pesticides.

11. Quality Assurance

11.1. Agreements between producers and buyers of medicinal and aromatic plants, with regard to quality questions, e.g. active principles and other characteristic ingredients, optical and sensoric properties, limit values of germ number, plant protection chemical residues and heavy metals, must be based on internationally recognized specifications and should be laid down in a written form.

Overview of GMP for the manufacturing of plant preparations

Götz Harnischfeger

GMP (good manufacturing procedures) is a set of guidelines universally applied in the production of pharmaceuticals. The definition of scope and contents is given in figure 1. The set of rules includes requirements and specifics on a variety of aspects, the most important ones are given in figure 2.
The general set of rules had to be annexed to take care of specific problems in the manufacture of phytomedicine, radio-chemicals, veterinary products, sterile medicinal products etc. National authorities have issued additional interpretations of individual rules in order to clarify them or to prevent misinterpretations. Unfortunately, the latter has formalized the use of GMP to such an extend, that the basic issue is hardly recognisable any more.
Phytomedicines have no special status in GMP. Most of the rules for the various aspects given in figure 2 apply to them as well. There are, however, some peculiarities in the complex of production and quality control. A general outline of these aspects is given in a supplemental guideline issued by the WHO.

Basic requirements

The basic requirements for GMP are outlined in figure 3. Some comments in the light of my experience with the manufacturing of phytomedicines might be allowed.

ad i) it is a necessity, that the manufacturing process is invariable, giving fixed target parameters for every step and relying on written SOP's.
Since the quality of every batch of raw material (plant drugs) varies, an equalizing step according to s specification should be applied at the earliest possible step.
It has an advantage, if the product batch is quarantained after every step in its manufacture and resumption of processing should only continue, if an el analysis at the control laboratory has resulted in „complies with the specification".

ad ii) a „critical" step is a matter of definition. It usually is reserved for process concerned with the safety of the finished product, e.g. pyrogenicity and purity in sterile products, where special sets of parameters have to be kept. In the normal manufacturing of phytomedicines, e.g. making of tinctures and extracts, the conditions can be classified as uncritical.
A critical variable, however, is the bioburden of starting material, final product and the environment. In this case, a validated method for reduction should exist, whose results are closely monitored at regular intervals. Especially water should be checked. The same should take place for the premises and equipment. The recommended values for upper limits of bioburden are given in figure 4.

ad iii) the expression „qualified and trained personnel" needs to be elaborated. In my experience, the senior level in overall supervisory functions should be filled with registered pharmacists. An additional qualification in pharmacognosy and technology as well as some industrial experience is an advantage. Pharmacists have by training a better understanding of the technical aspects of medicines, knowledge and a way of thinking which has to be acquired tediously by chemists or engineers.

ad IV) It is advisable to formulate the SOP's in a „step by step" way, with room for remarks and signatures of the operator. In this was, the SOP can be used as documented batch protocol.
The operators should undergo training every quarter year, especially in hygiene and safety. Faulty operating of machines should be discussed within the work-crew and remedies should be proposed. The supervisor present should write a report signed by everybody present.

The requirements for quality control are given in figure 5.

There are no special requirements in the area of training and personnel hygiene outside general GMP in the phytopharmaceutical industry.

GMP is that part of Quality Assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the Marketing Authorisation or product specification

aspects of GMP set of guidelines

- personnel

-premises and equipment

-production

-quality control

-contract manufacture

-complaints and product recall

-self inspection

-documentation

Basic requirements

i. all manufacturing processes are clearly defined, systematically reviewed in the light of experience and shown to be capable of consistently manufacturing medicinal products of the required quality and complying with their specifications;

ii. critical steps of manufacturing processes and significant changes to the process are validated;

iii. all necessary facilities for GMP are provided including:
   a. appropriately qualified and trained personnel;
   b. adequate premises and space;
   c. suitable equipment and services;
   d. correct materials, containers and labels;
   e. approved procedures and instructions;
   f. suitable storage and transport;

iv. instructions and procedures are written in an instructional form in clear and unambiguous language, specifically applicable to the facilities provided;

v. operators are trained to carry out procedures correctly;

vi. records are made, manually and/or by recording instruments, during manufacture which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the product was as expected. Any significant deviations are fully recorded and investigated;

vii. records of manufacture including distribution which enable the complete history of a batch to be traced, are retained in a comprehensible and accessible form;

viii. the distribution (wholesaling) of the products minimises any risk to their quality;

ix. a system is available to recall any batch of product, from sale or supply;

x. complaints about marketed products are examined, the causes of quality defects investigated and appropriate measures taken in respect of the defective products and to prevent reoccurrence.

Quality Control

Quality Control is that part of Good Manufacturing Practice which is concerned with sampling, specifications and testing, and with the organisation, documentation and release procedures which ensure that the necessary and relevant tests are actually carried out and that materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory.

The basic requirements of Quality Control are that:

i. adequate facilities, trained personnel and approved procedures are available for sampling, inspecting and testing starting materials, packaging materials, intermediate, bulk, and finished products, and where appropriate for monitoring environmental conditions for GMP purposes;

ii. samples of starting materials, packaging materials, intermediate products, bulk products and finished products are taken by personnel and by methods approved by Quality Control;

iii. test methods are validated;

iv. records are made, manually and/or by recording instruments, which demonstrate that all the required sampling, inspecting and testing procedures were actually carried out. Any deviations are fully recorded and investigated;

v. the finished products contain active ingredients complying with the qualitative and quantitative composition of the Marketing Authorisation, are of the purity required, and are enclosed within their proper containers and correctly labelled;

vi. records are made of the results of inspection and that testing of materials, intermediate, bulk, and finished products is formally assessed against specification. Product assessment includes a review and evaluation of relevant production documentation and an assessment of deviations from specified procedures;

vii. no batch of product is released for sale or supply prior to certification by a Qualified Person that it is in accordance with the requirements of the Marketing Authorization;

viii. sufficient reference samples of starting materials and products are retained to permit future examination of the product if necessary and that the product is retained in its final pack unless exceptionally large packs are produced.

Concerning the premises, the general GMP aspects are listed in figure 6. However, the WHO annex to GMP „Herbal medicinal products" has some special requirements. These are given in figure 7. One should, however, not be overly concerned with dust. Although dust carries a lot of microorganisms, most of them are no hazard to human health. If an ethanolic extraction is done (> 23 %) they will be decreased by several orders of magnitude. However, with water as the extraction solvent the microbes on the crude drug will certainly become a problem. In this case, dust free lots should be used and free dust on the premises has to be kept at a minimum. In general, it is advisable, to have dust eliminating ventilation at all those places installed, where it is generated.

There are also some special aspects in the specifications of phytomedicines and their plant starting materials mentioned in the annex to WHO-GMP. These are shown in figure 8 and have been treated in detail already before.

The precautionary measures and methods used for sampling, quality control and stability are addressed in figure 9.

In addition to the more or less official recommendations of GMP, there is a concomitant, rather large amount of accompanying rules and advices, most of them semi-official. There are regulations available for establishing a master qualification plan (DIN-ISO), quality assurance systems (DIN-ISO), PIC documents for inspection, validation etc., ICH documents on purity, stability testing etc. and a whole set of GLP and GCP guidelines of the EC. They will not be discussed in the context, but their existence and at least partial relevance to phytomedicines should be mentioned.

PREMISES AND EQUIPMENT

Principle

Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimize the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination, build up of dust or dirt and, in general, any adverse effect on the quality of products.

PREMISES

General

.1. Premises should be situated in an environment which, when considered together with measures to protect the manufacture, presents minimal risk of causing contamination of materials or products.

2. Premises should be carefully maintained, ensuring that repair and maintenance operations do not present any hazard to the quality of products. They should be cleaned and, where applicable, disinfected according to detailed written procedures.

3. Lighting, temperature, humidity and ventilation should be appropriate and such that they do not adversely affect, directly or indirectly, either the medicinal products during their manufacture and storage, or the accurate functioning of equipment.

4. Premises should be designed and equipped so as to afford maximum protection against the entry of insects or other animals.

5. Steps should be taken in order to prevent the entry of unauthorized people. Production, storage and quality control areas should not be used as a right of way by personnel who do not work in them.

Storage areas

WHO Annex

1. Crude (i.e. unprocessed) plants should be stored in separate areas. The storage area should be well ventilated and be equipped in such a way as to give protection against the entry of insects or other animals, especially rodents. Effective measures should be taken to prevent the spread of any such animals and microorganisms brought in with the crude plant and to prevent cross-contamination. Containers should be located in such a way as to allow free air circulation.

2. Special attention should be paid to the cleanliness and good maintenance of the storage areas particularly when dust is generated.

3. Storage of plants, extracts, tinctures and other preparations may require special conditions of humidity, temperature or light protection; these conditions should be provided and monitored.

Production area

4. Specific provisions should be taken during sampling, weighing, mixing and processing operations of crude plants whenever dust is generated, to facilitate cleaning and to avoid cross-contamination, as for example, dust extraction, dedicated premises, etc.

Apart from the data described in General Guide (chapter 4, point 4.11), specifications for medicinal crude plants should include, as far as possible:

- botanical name (with, if appropriate, the name of the originator of the classification, e.g. Linnaeus);
- details of the source of the plant (country or region of origin, and where applicable, cultivation, time of harvesting, collection procedures, possible pesticides used, etc.);
- whether the whole plant or only a part is used;
- when a dried plant is purchased, the drying system should be specified;
- plant description, macro and/or microscopical examination;
- suitable identification tests including, where appropriate, identification tests for known active ingredients, or markers. A reference authentic specimen should be available for identification purposes;
- assay, where appropriate, of constituents of known therapeutic activity or of markers;
- methods suitable to determine possible pesticide contamination and limits accepted;
- tests to determine fungal and/or microbial contamination, including aflatoxins and pest-infestations, and limits accepted;
- tests for toxic metals and for likely contaminants and adulterants;
- tests for foreign materials.

Any treatment used to reduce fungal/microbial contamination or other infestation should be documented. Specifications for such procedures should be available and should include details of process, tests and limits for residues.

Specifications for the finished product

The control tests on the finished product must be such as to allow the qualitative and quantitative determination of the composition of the active ingredients and a specification has to be given which may be done by using markers if constituents with known therapeutic activity are unknown. In the case of plant material preparations with constituents of known therapeutic activity, these constituents must also be specified and quantitatively determined.

If the final product contains several plant materials or preparations of several vegetable drugs and it is not possible to perform a quantitative determination of each active ingredient, the determination may be carried out jointly for several active ingredients. The need for this procedure must be justified.

QUALITY CONTROL

Personnel of Quality Control units should have particular expertise in herbal medicinal products in order to carry out identification tests, recognition of adulteration, presence of fungal growth, infestations, non-uniformity within a delivery of crude plants, etc.

Reference samples of the plant material must be available for use in comparative tests e.g. macro and microscopic examination, chromatography etc.

Sampling

Due to the fact that crude plant materials are an aggregate of individuals and present some heterogeneity, their sampling has to be carried out with special care by personnel with particular expertise. For additional advice see document "Quality Control Methods for Medicinal Plant Materials", Section 1, "General advice on sampling".

STABILITY TESTS

Since the plant materials or plant preparation in its entirety is regarded as the active ingredient, a mere determination of the stability of the constituents with known therapeutic activity will not suffice. It must also be shown, as far as possible e.g. by means of appropriate fingerprint chromatograms, that other substances present in the vegetable drug or in the vegetable drug preparation are likewise stable and that their proportional content remains constant.

If a herbal remedy contains several plant materials or preparations of several plant materials and if it is not possible to determine the stability of each active ingredient, the stability of the medicinal product should be determined by appropriate fingerprint chromatograms, appropriate overall methods of assay and physical and sensory tests or other appropriate tests.

Manufacturing process of medicinal plants, including also control and validation of methods of preparation

Götz Harnischfeger

The special feature of phytomedicines is the fact, that extracts from plants or part of plants constitute their active principle (figure 1).
These extracts are multicomponent systems containing

- pharmacologically active substances
- compounds which by themselves are not pharmacologically active but influence the biological effectiveness of active substances, i.e. supportive substances
- neutral, bulk material

From analytical data on these 3 groups of components information can be obtained concerning

- the relation of these groups of components with each other
- the technical options, to achieve a selective enrichment of defined substances for a particular phytomedicine

Standardization

This information leads automatically to considerations about standardization, the equalizing of batches of intermediates and final products with respect to one particular substance or group of substances, and technical methods to achieve this. For further discussion, the definitions of figure 2 will be used. It has to be emphasized, that the quantitative aspect of the extractable components in plant drugs, i.e. their ratio in the internal composition, is variable due to environmental influences (climate, soil, time of harvest etc.). It follows, that the bulk material has an influence on the quality parameters of the preparation, especially, since the amount of active ingredients is small compared to the inert bulk material.

Standardization in phytomedicines is possible and done by setting ranges, to which the relevant components comply. The range has to be declared on the label and should contain

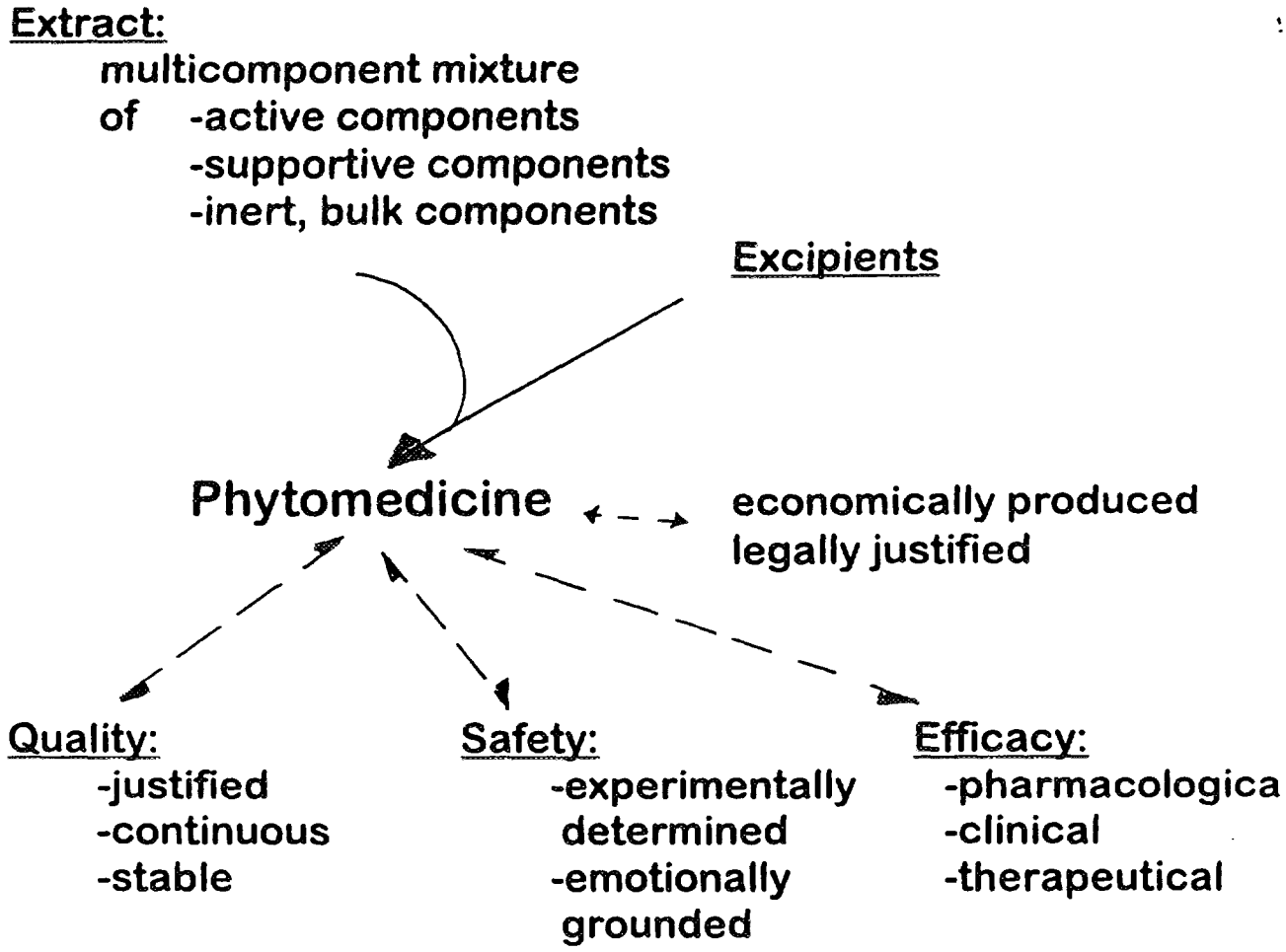
- the amount of starting material or the content of active ingredients per unit in the final product
- the mass of native extract (i.e. without technical excipient or solvent) and its range
- the range of the native plant drug/extract ratio
- information about type and concentration of solvent

An example is given in figure 3.
The use of ranges does not normally constitute a drawback or an inferiority compared to synthetic substances, because as a rule phytomedicines follow in their therapeutic activity a non linear dose/effect curve and have therapeutic ranges over various orders of magnitudes (figure 4).

Strategies for technical development

The basic scheme for determining the technical framework for the manufacture of a phytomedicine has been mentioned already and is shown in figure 5.
The second section of this scheme has, in a next step, to be modified in more detail, taking into consideration the standard to be used and the analytical methods available. Knowledge about the internal composition of the intended extracts should be obtained, either by trial experiments or from reliable literature sources. This concerns not only information about the active substances but also the neutral bulk material. The latter contains normally low molecular sugars, sugar-alcohols and similar substances which can influence a formulation. Stickiness and hygroscopy of extracts is, by the way, a constant problem in phytomedicines.
The composition of the extract and its physical behaviour is also the starting point for the choice of the excipients added in the formulation of the final product.

Another point, also mentioned in figure 5, is the anticipation of changes in the internal composition of the extract due to time and environmental conditions. The multicomponent


Extract:
multicomponent mixture
of -active components
-supportive components
-inert, bulk components
Excipients
Phytomedicine
economically produced
legally justified
Quality:
-justified
-continuous
-stable
Safety:
-experimentally determined
-emotionally grounded
Efficacy:
-pharmacologica
-clinical
-therapeutical

3.1 Figure 1

MEDICINAL PLANT

Plant, the whole or part of which, is used for medicinal purposes.

CRUDE PLANT MATERIAL

The fresh or dried medicinal plant or parts thereof, used for medicinal purposes.

PLANT PREPARATIONS

Comminuted or powdered plant material, extracts, tinctures, fatty or essential oils, expressed juices, etc. prepared from plant material, and preparations whose production involves a fractionation, purification or concentration process, excluding chemically defined isolated constituents.

FINISHED HERBAL MEDICINAL PRODUCT

Medicinal product containing, as active ingredients, exclusively plant material and/or preparations.

CONSTITUENTS WITH KNOWN THERAPEUTIC ACTIVITY

Substances or groups of substances which are chemically defined and known to contribute to the therapeutic activity of a plant material or of a preparation.

MARKERS

Constituents of a crude plant material which are chemically defined and of interest for control purposes. Markers may serve to calculate the quantity of plant material or preparation in the finished product if that marker has been quantitatively determined in the plant material or preparation when the starting materials were tested.

1 coated tablet contains:

Extractum Crataegi folii et floris (solvent ethanol 45 %, DER 4 - 7:1)

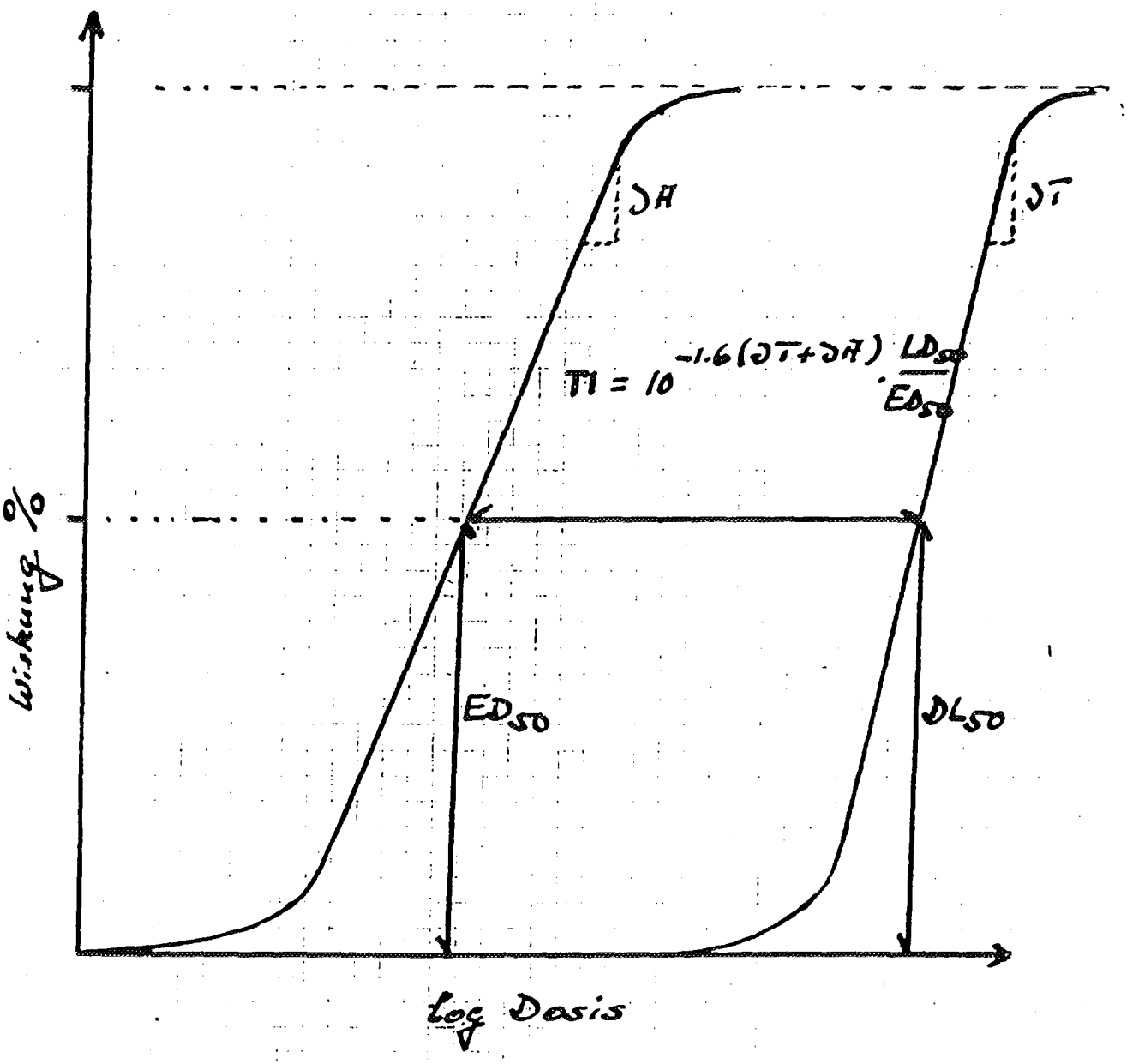
150 - 200 mg

Corresponding to 4 mg Flavonoids (DAB 10)

1 coated tablet contains:

Extractum Cimicifugae racemosae (extract from 20 mg drug, solvent ethanol 50 %, DER 4.5 - 8.5 :1)

Corresponding to 0.3 - 0.7 mg Triterpenglycosides



3.1 Figure 4

1. Therapeutical information

- definition of medicinal indication
    - specified symptomatology of the affliction/disease
    - target organ
    - therapeutical aim
- reason for selecting this particular phytomedicine/plant drug
    - pharmacological data
    - therapeutical data
    - literature data, reports from experience, ethnomedicine
- intended rationale for using this particular phytomedicine
    - detailed proposal, where and why to establish this product in medicine

2. Technological requirements

- which compounds are liberated during processing of raw material ?
- which compounds undergo degradation or changes during processing ?
- which among those are active components in the intended indication ? Extent of alteration ?
- is the practiced or intended manufacturing procedure in line with the a. m. requirements ?
- can relevant compounds or groups already be selected, which are therapeutically relevant and present ?

3. Starting material

- average quality on the market
- necessary quality
- availability
- alternatives

system „extract" is not static but, devoid of the cellular matrix, actually very labile and variable. Strategies have to be developed, to prevent such physical and chemical changes.

Extraction techniques

Generally there are two types of extraction procedures, leading either to exhaustive or to incomplete removal of extractives (figure 6).

In incomplete or discontinuous procedures, like maceration, an equilibrium will establish itself between extractibles in the solvent outside and inside the matrix. Since the solvent is not exchanged, some extractible substances will be left in the miscella. To obtain these, after finishing the extraction, the remaining miscella will be pressure treated using a hydraulic press. This step is critical, if large throughput is desired.

In exhaustive procedures, the equilibrium mentioned above is continuously disturbed through addition of fresh solvent. Larger throughput is possible but at the cost of larger amounts of less concentrated extracts and remaining solvent in the miscella. The method used to keep a loss of valuable solvent within limits is shown schematically in figure 7.

The above mentioned, classical extraction procedures can be regulated and controlled through (figure 8).

The extraction solvent influences with its polarity the type of components of the plant drug being dissolved. The temperature influences the solubility. The particle size of the drug modifies the accessibility and duration of establishing equilibrium. The latter is the guiding factor for optimum extraction time.

The accessibility of extractives in the matrix can be increased by a short steam treating of the drug before extraction. The cell walls become water logged and damaged. The yield of extractives increases.

Another method to achieve this is by cycles of pressure and expansion (up to 35-40 bar pressure and quick expansion).

Two technical extraction procedures, which gained some importance in the last decade, should illustrate some of the modern concepts..

1. The Extr-o-mat procedure
   Figure 9 depicts the scheme of this technique. A stainless steel basket with the drug is put into the closed container and solvent is pumped through continuously. Equilibrium is reached within 2 hrs, compared to the usual 10-12 hours necessary for 80 % extraction.
2. The fluid extraction procedure
   In this case gases, fluidized by high pressure, are applied as extraction solvent. Mostly CO_2 is used, which has a T_c of 31.04 °C and a P_c of 73,834 bar (figure 10).
   Criteria for the use of this procedure are given in figure 11. An example comparing traditional with fluid extraction is given in figure 12-14.

Concentration and Drying of Extracts

Concentration occurs chiefly in evaporators where the following conditions have to be met

- limited contact of the liquid phase with the heat delivering parts of the equipment
- high throughput to save time and costs

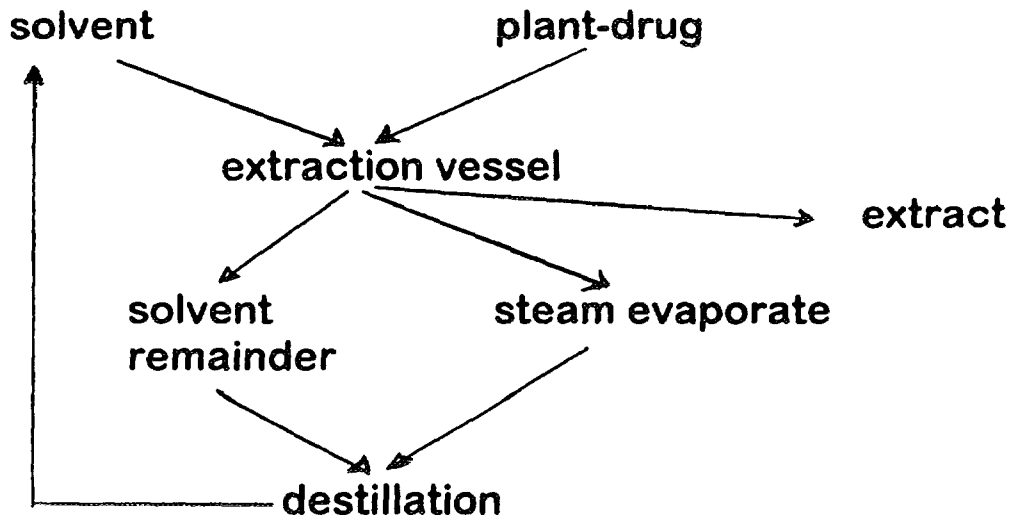
The basic design of a simple evaporator is shown in figure 15. The quality of the extract-concentrate is influenced by temperature (degradation of compounds or solvent) and duration of the process. Therefore, evacuated evaporators are used since a decrease by 10 ° C in temperature diminishes degradation by 50 %. Under proper conditions, even temperature-sensitive substances can be concentrated.

The following drying process transforms the concentrates, which have chiefly the character of a soft extract, unselectively into the solid form. Although the composition in a chemical sense

Extraction procedures

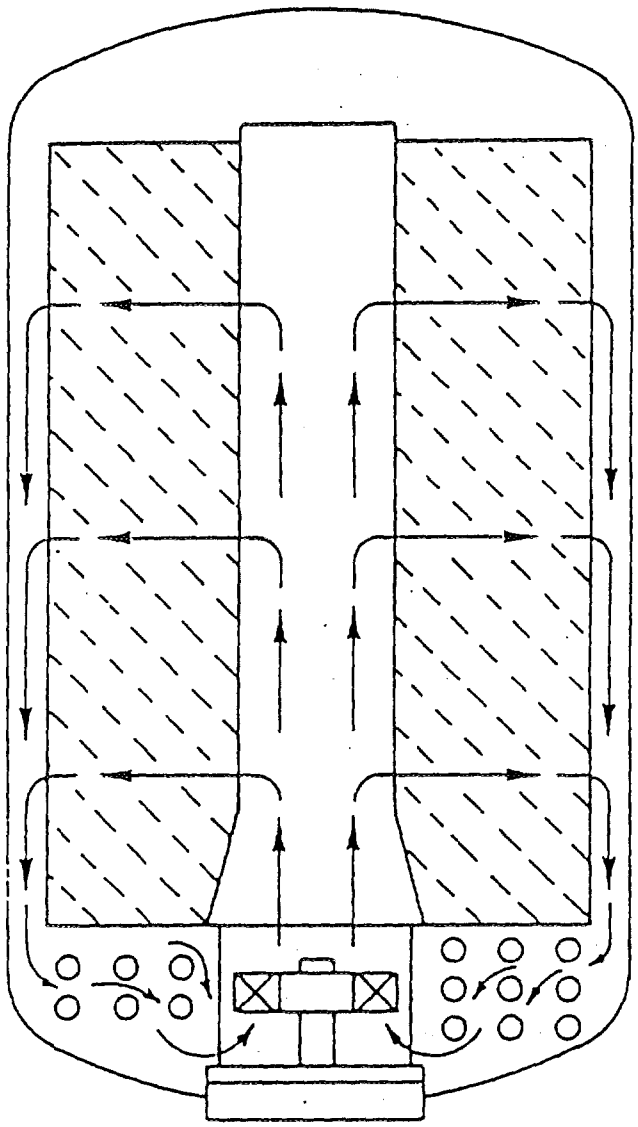
-incomplete:	immobile: maceration digestion agitated: ultrasound maceration stirring maceration
-exhaustive:	Soxleth percolation evacolation diacolation

3.1. Figure 6


solvent
plant-drug
extraction vessel
extract
solvent
remainder
steam evaporate
destillation

- choice of solvent polarity
- temperature
- particle size of the drug
- duration of extraction

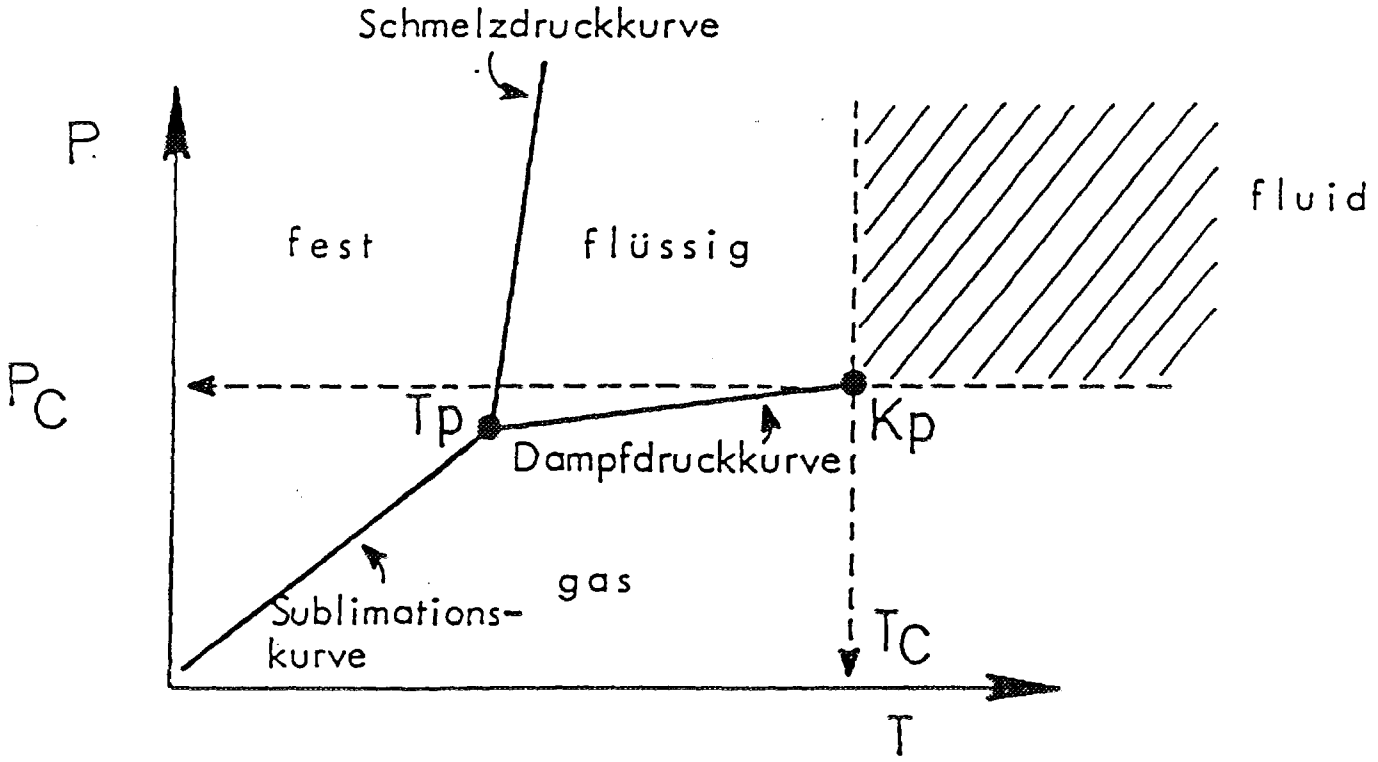
3.1 Figure 5



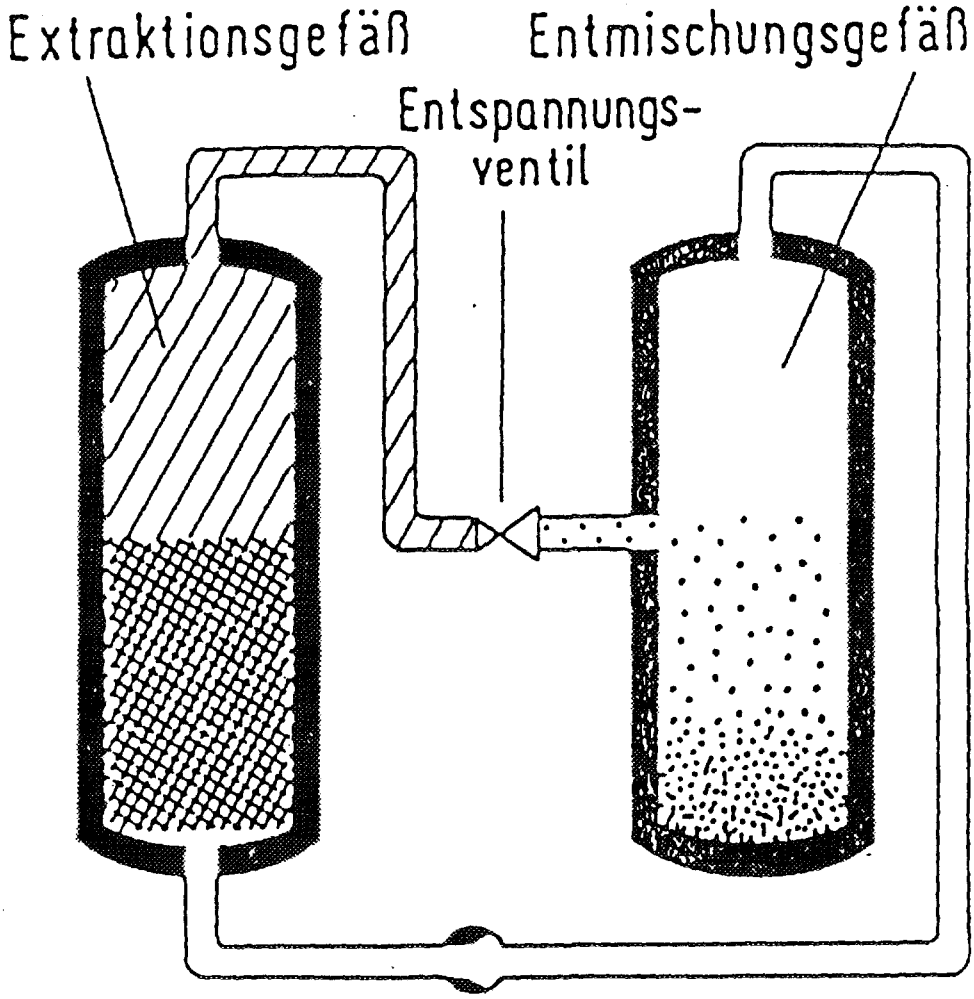
% gelöster N ($N_{gelöst}$) aus *Artemisia absinthium*, durchgeführt mit dem EXTR-O-MAT.

Extraktionszeiten (min)	% $N_{gelöst}$
15	28,06
30	29,75
60	30,64
120	32,82
180	32,74
240	32,52

3.1 Figure 9



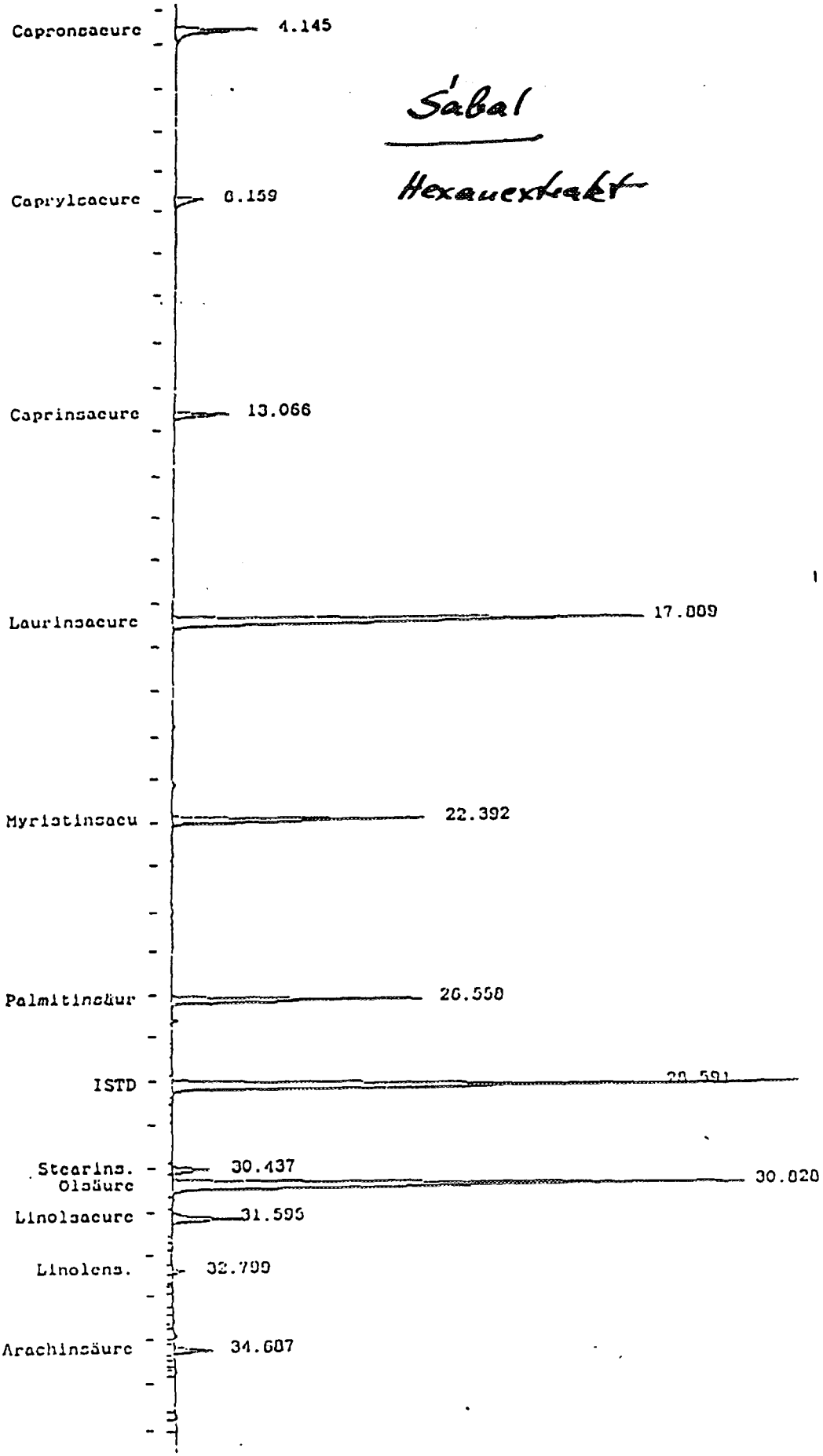
3.1 Figure 10a



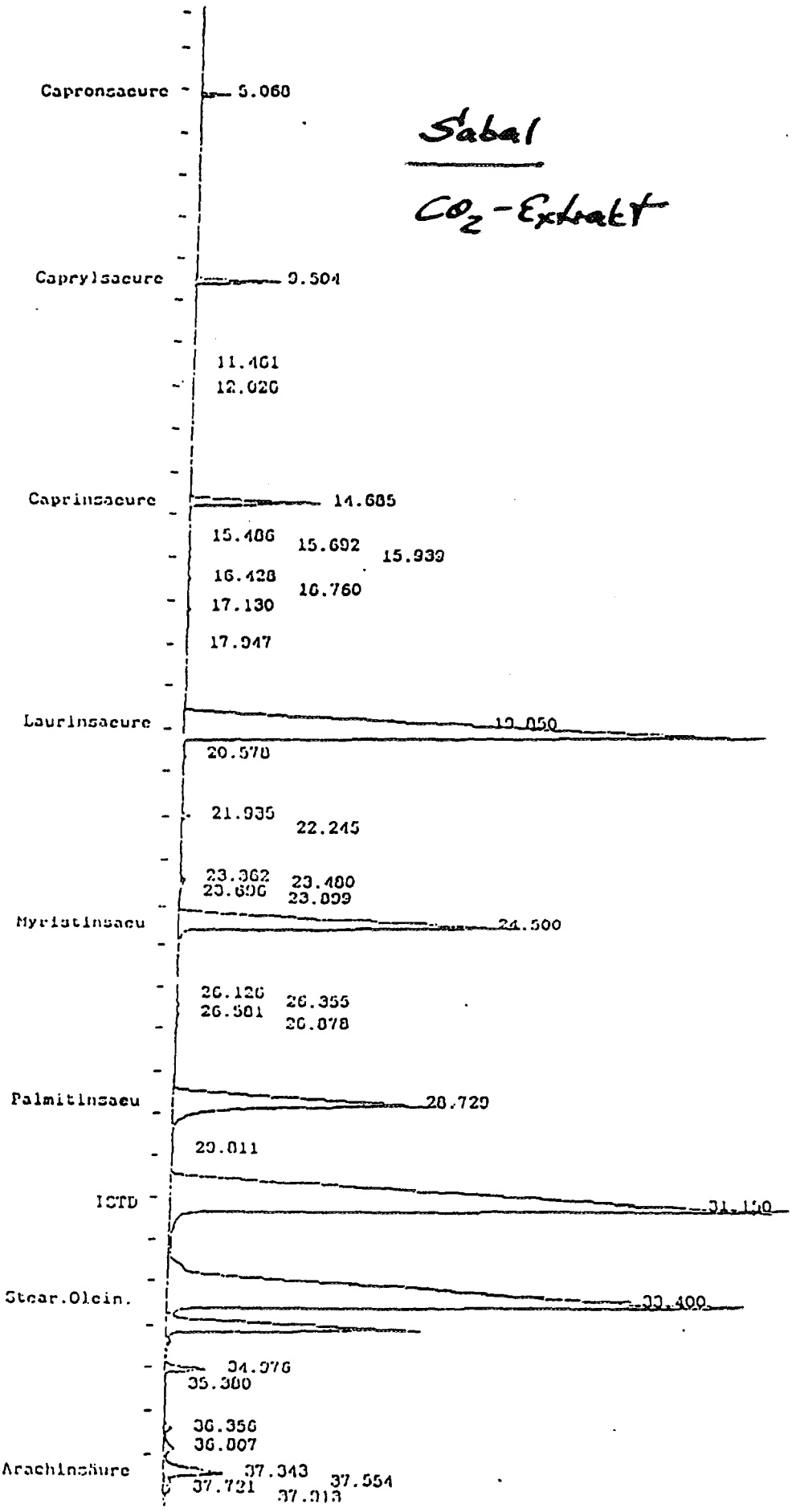
3.1 Figure 10B

- extraction of hydrocarbons with lipophilic character and a MW up to 300, e.g. esters, ethers, lactones
- strong polar groups decrease extractability; compounds with more than 3 hydroxyl- or with a carboxyl-group cannot be extracted
- the solubility of the extractive in the fluid should be more than 10 mg/L

3.1 Figure 11


Capronsaeure
4.145
Sabal
Hexanextrakt
Caprylsaeure
8.159
Caprinsaeure
13.066
Laurinsaeure
17.009
Myristinsaeu
22.392
Palmitinsäur
26.558
ISTD
28.591
Stearins.
Olsäure
30.437
30.820
Linolsaeure
31.595
Linolens.
32.799
Arachinsäure
34.687

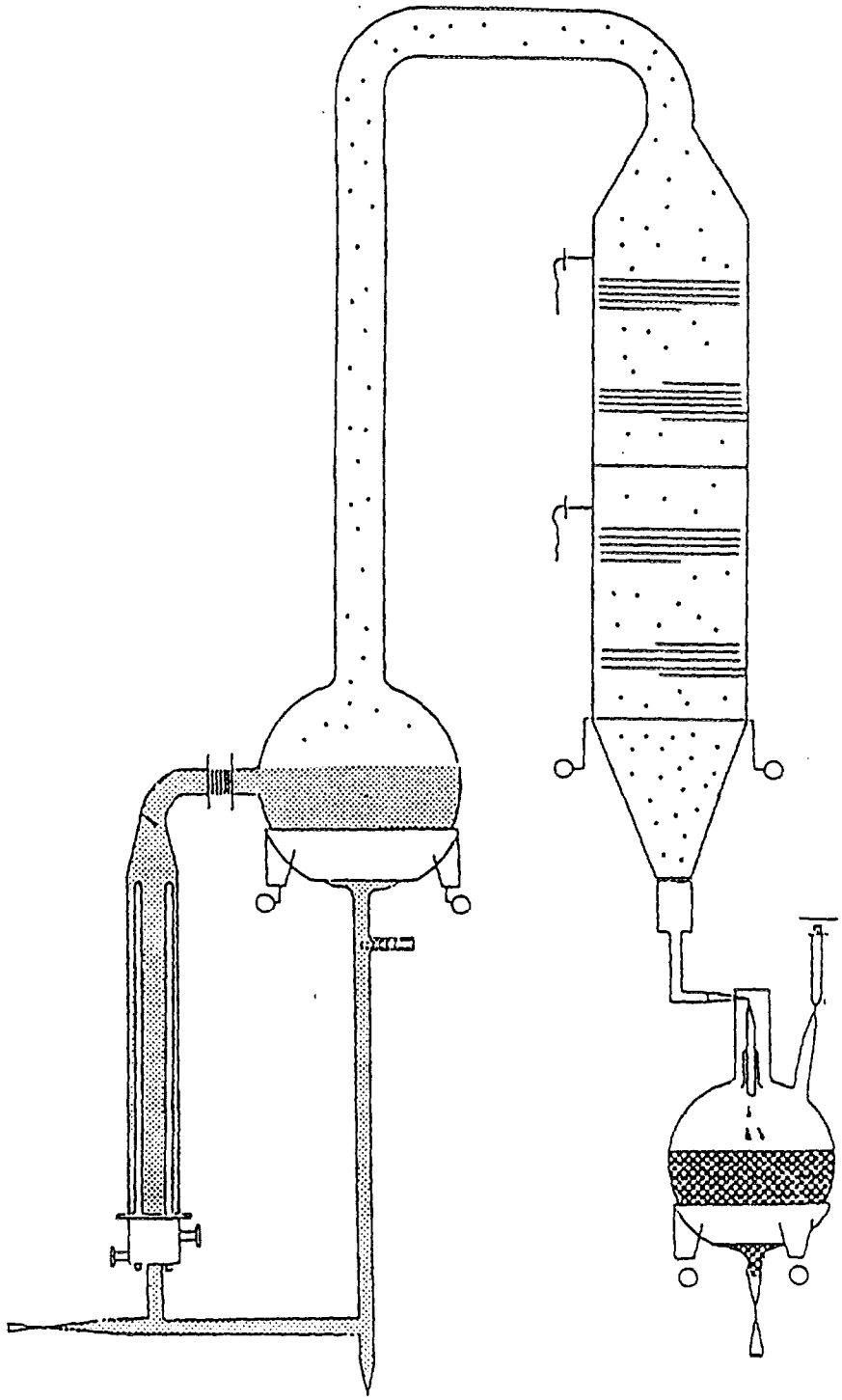
31 Figure 12


Sabal
CO_2-Extrakt
Capronsaeure
5.060
Caprylsaeure
9.504
11.461
12.020
Caprinsaeure
14.685
15.486
15.692
15.939
16.428
16.760
17.130
17.947
Laurinsaeure
19.050
20.570
21.935
22.245
23.362
23.480
23.606
23.899
Myristinsaeu
24.500
26.120
26.355
26.501
26.878
Palmitinsaeu
28.729
29.011
ISTD
31.130
Stear.Olein.
33.400
34.976
35.380
36.356
36.807
Arachinsäure
37.343
37.554
37.721
37.913

3.1 Figure 13

Prüfpunkt	Prüfergebnisse bisheriger Lieferungen des CO_2-Extraktes	Prüfergebnisse des Sabal-Spissum-Extraktes	Prüfergebnisse des Sabal-Hexan-Extraktes
Gehalt an höheren Fettsäuren (berechnet als Laurinsäure)	94,7 % 88,2 % 87,6 % - * 95,0 % 95,7 %	60,9 %	89,2 %
Gehalt an höheren Fettalkoholen	0,20 % 0,22 % 0,20 % 0,20 % 0,16 % 0,17 %	0,19 %	0,37 %
Gehalt an Gesamtsterolen (berechnet als ß-Sitosterol)	0,28 % 0,25 % 0,27 % 0,26 % 0,32 % 0,34 %	0,18 %	0,32 %
Gehalt an ß-Sitosterol	0,19 % 0,16 % 0,17 % 0,17 % 0,21 % 0,24 %	0,12 %	0,23 %
Unverseifbare Anteile	1,61 % 2,34 % 2,12 % 2,38 % 2,55 % 2,12 %	2,41 %	3,03 %

3.1. Figure 14



3 : Figure 5

is not changed, the physical parameters of the dry-extracts can be modified by the nature of the drying process. These parameters are: particle size, porosity, flowability and resolubility.
Drying always requires large amounts of heat energy, which can influence the composition of extracts with thermolabile compounds. The process however influences in a decisive manner the surface properties of the dry extract.

Formulation of Phytomedicines

a) Liquids

1.) chemical changes

Chemical processes which occur primarily in the liquid phase and influence the internal composition of the herbal medicine can be grouped as

- Redox reactions
- Interconversions
- Hydrolysis
- Condensations and polymerizations
- Isomerizations

The presence of heavy metals, enzymes and the influence of light and oxygen is inductive to such processes. It should be minimized wherever possible.

Redox reactions:

Redox reactions occur predominantly in those pharmaceutical specialities which contain plant extracts in aqueous or non-alcoholic form. Such reactions are normally catalyzed by enzymes, such as ubiquitous phenol oxidases and peroxidases co-extracted during manufacture. The risk of instability can be greatly reduced during manufacture by testing for latent peroxidase activity and subsequent steps for its inactivation.

Interconversions:

Especially flavane derivates can be altered in aqueous/alcoholic solution through acid-base catalysis. Plant extracts possess usually a pH between 2 and 4 due to co-extraction of organic plant acids, so that such interconversions are in the realm of possibility. An example is given by the conversion of isoliquiritigenine/liquiritigenine (figure 16).

Hydrolysis:

Acid catalyzed hydrolysis is one was for chemical change in many glycosides. A Well-known example is rutoside (figure 17) which is split into its various components by heat and acid pH (e.g. sterilization of an injectable solution).

Condensations and polymerisations:

These types of reactions play a special role of catechine and catechine-derivatives are present in the phytopharmakon (e.g. in Crateaegi flos.). Figure 17 depicts the mechanism given by WEINGES et al. (1969).

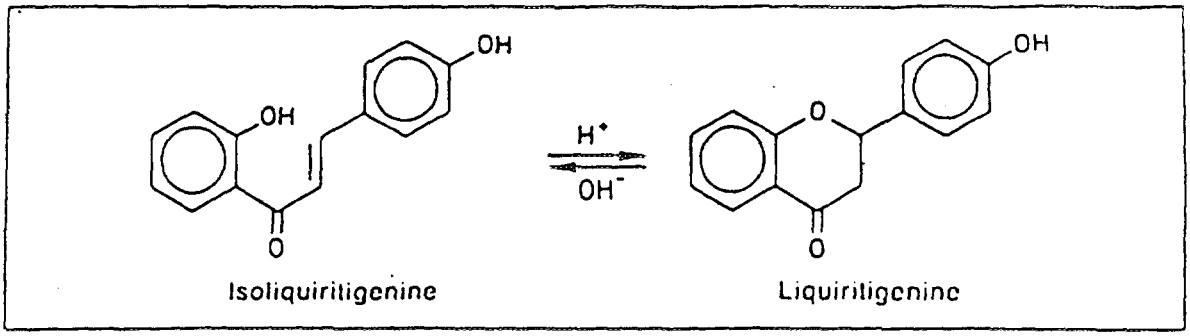
Isomerisations:

This type of alteration is depicted in figure 18 using griseofulvin as an example.

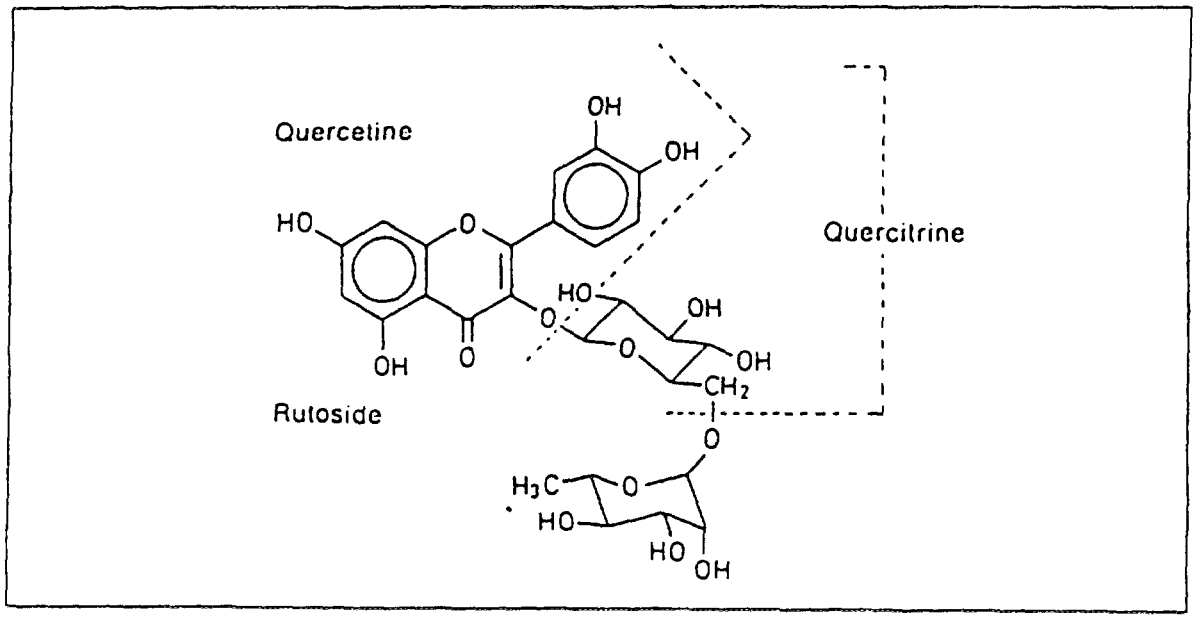
Photochemical processes:

Photochemically induced changes are quite frequent in pharmaceutical products and are found not only in the text-book example reserpine. For example, menthone in aqueous/ethanolic solution is changed by sunlight into menthocitronellal and saturated acid (figure 18). Similar processes, this time with free radicals as intermediates, are known for camphora (figure 19) which is an ingredient of about 250 pharmaceutical preparations, many of those phytopharmaka, in the Fed. Rep. of Germany.

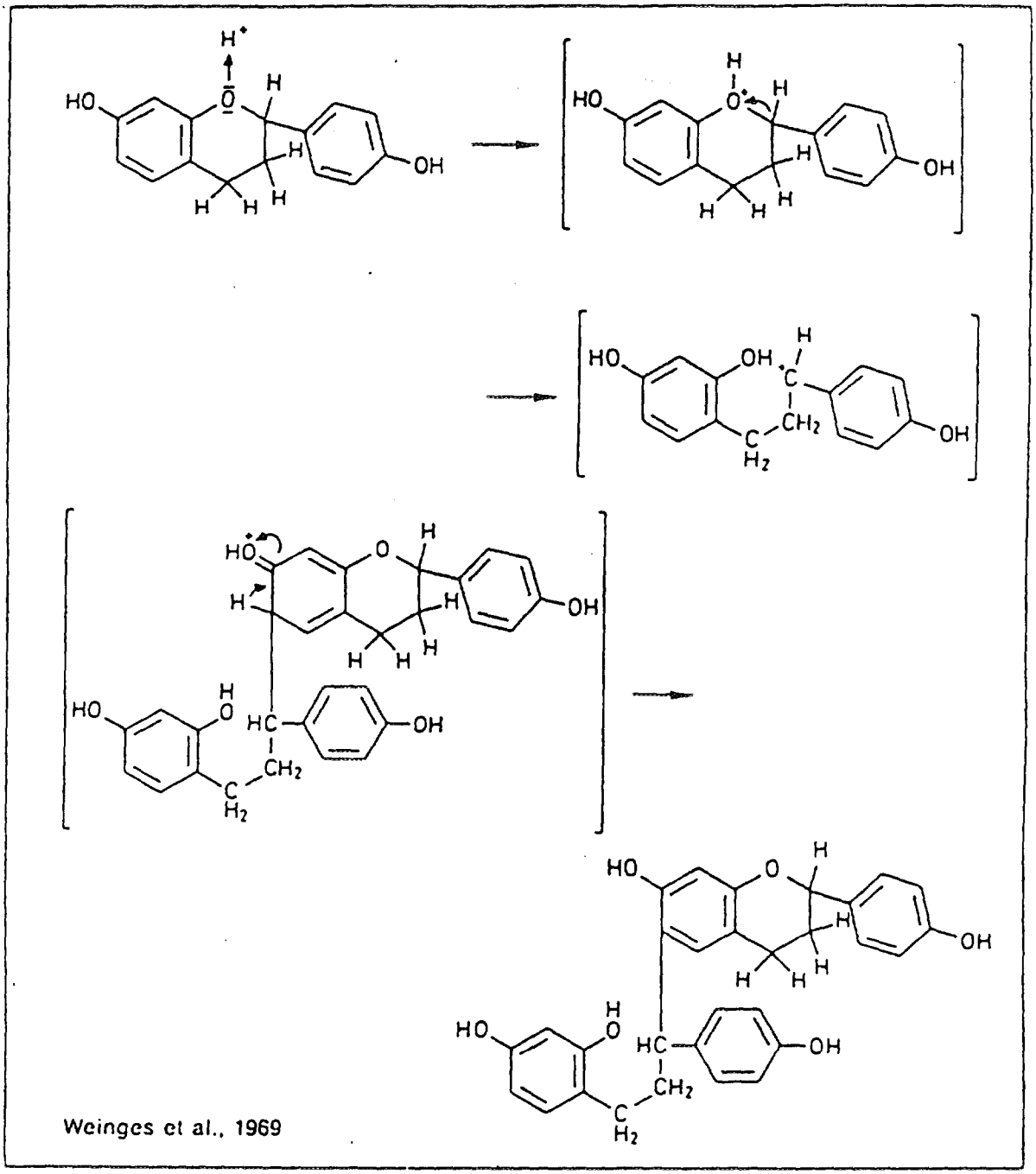
There is a number of other possibilities for chemical alterations which are not mentioned. One example is the time-dependent change of valepotriates into baldrinales where the underlying mechanism became known in the last few years with great repercussion on the stability data of preparations containing Valerianae radix.



Example of an acid-catalyzed conversion

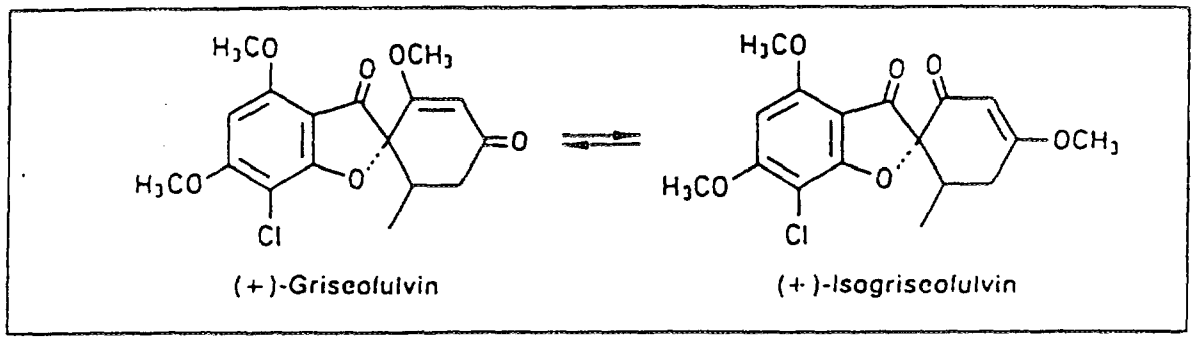


Rutoside and its partial components obtainable in glycoside hydrolysis

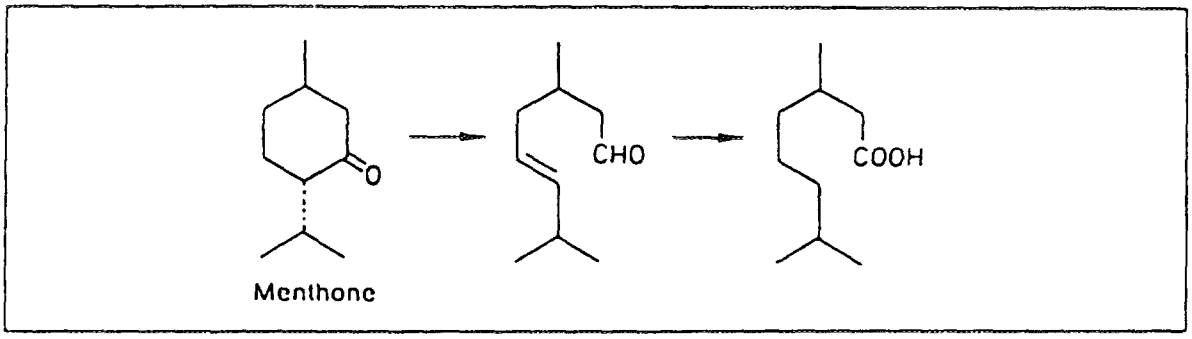


Mechanism of the acid-catalyzed self-condensation of catechines

3.1 Figure 17

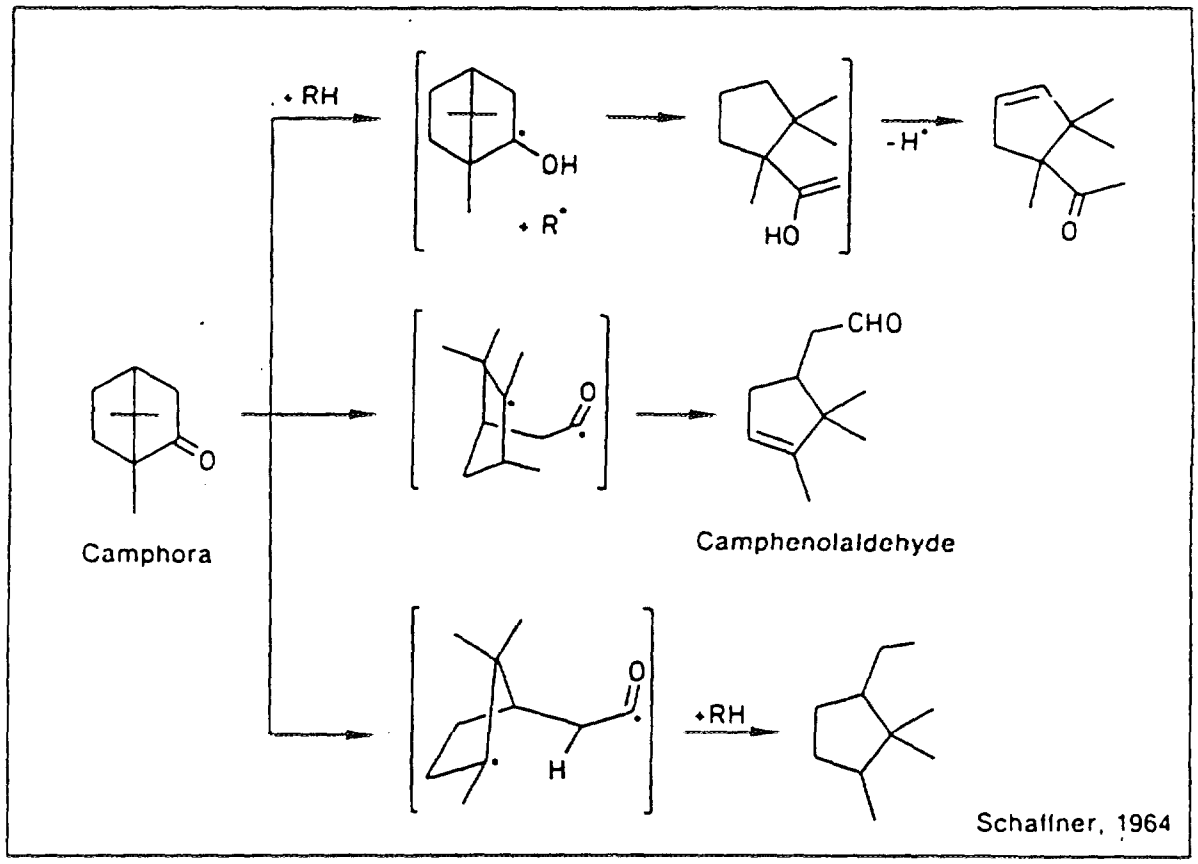


Example of an isomerisation



Photochemical conversion of menthone in aqueous ethanolic solution

R1 Figure 10



Photochemical conversion of camphora

3.1 Figure 19

The qualitative demonstration of the above-mentioned processes is in theory relatively simple. Since initially present components disappear and new ones are formed, main emphasis has to be placed onto chromatographic fingerprint analysis. In manufacture the framework conditions have to be rigidly observed, e.g. pH, ionic strength. Occurence of density, flocculation and opacity can give additional important hints. A small degree of turbidity is, by the way, common in liquids due to the interaction of acid tannines with the alkali of the bottle material „glass". This factor does not need to be considered in evaluating the stability of the preparation.

2. physical influences

Galenical incompatibilities are well known (figure 20). A combination of components incompatible with each other should be avoided. Attention should, in this respect, be focused on the excipients as well. Physical and chemical interactions with and among the bulk material of neutral coextractives are frequent causes for instabilities manifesting themselves as turbidity and flocculation (figure 21).

One aspect should be stated. Surose syrup, part of many liquid phytomedicines, cristallizes very easily. The cristallization process is dependent on the ration of sucrose to glucose plus fructose. Maximum ratios are 3:2 and below 1:4.

To prevent physical degradation, especially precipitation and flocculation, the following precautions should be introduced into the manufacturing process

1.) minimization of extraction of neutral bulk
2.) removal of oxygen and complexation of heavy metals
3.) treatment, if possible, through cooling for a prolonged time at 4 °C and addition of colloids, to neutralize surface charges of polymers and to flocculate them.

b) Solids

Although the same chemical changes, that have been mentioned for liquids, can take place in solids, they are of minor importance. A suitable solvent is normally absent.

The problem in the formulation of solid phytomedicines is the inhibition of phase transition form solid to liquid, i.e. prevention of water uptake from the surrounding atmosphere and separation of reactive reagents in the final formulation.

Overriding priority has the control of the hygroscopic nature of plant dry extracts.

An extract contains normally many, highly water soluble components, which adsorb humidity and get themselves dissolved. Water absorption of 5-10% changes most such dry extracts into syrupy, sticky masses (figure 22/23).

This feature has to be considered in planning the extraction parameters (use of selective extraction to minimize content of hygroscopic components) and when detailing the formulation process.

Analytical requirements

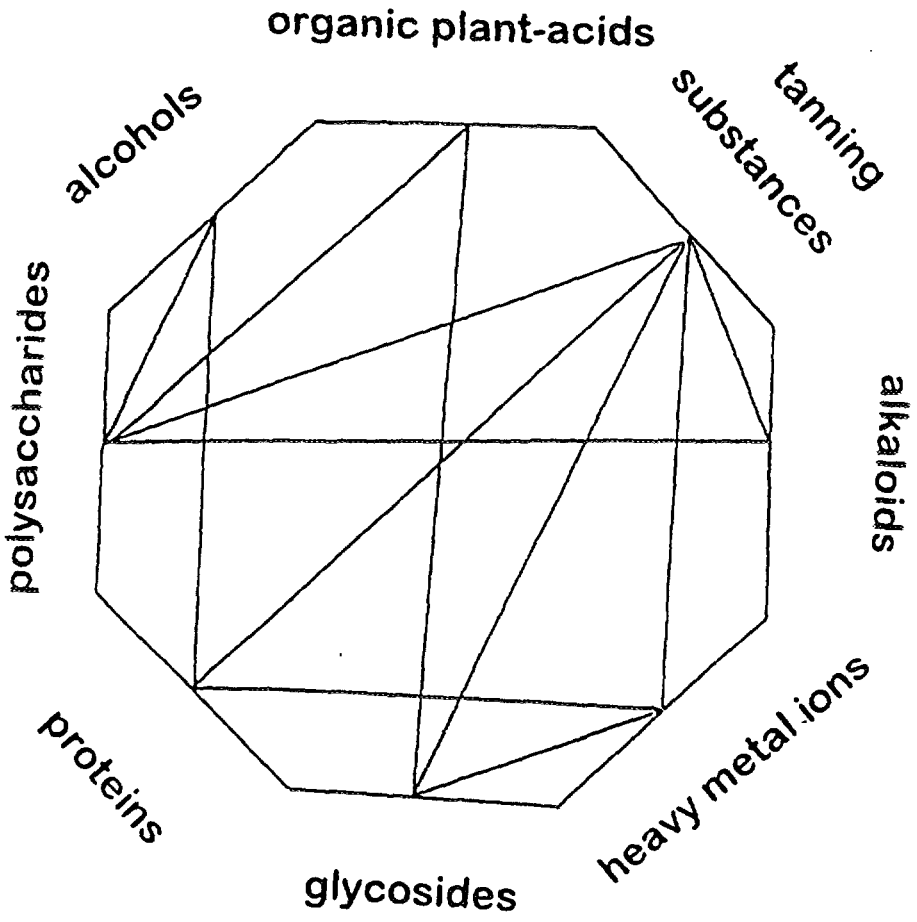
The planning of the analytical methods to be used has been described before (see lecture 1.3). Having established specifications for the analyte (starting material, intermediate, final product) one has to select the appropriate method. The method has to be

- appropriate
- sensitive in the specified range
- accurate
- robust and reliable
- economic in time and costs

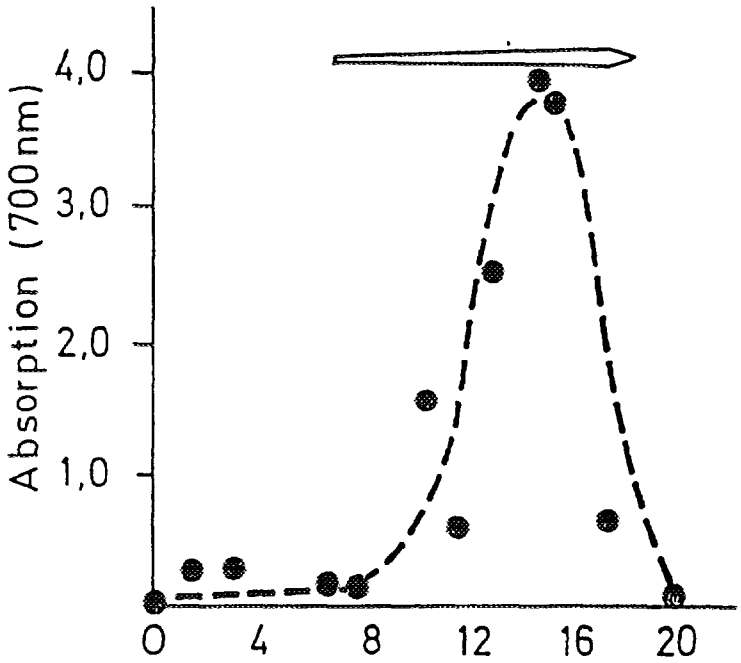
It is nowadays a necessary requirement to document the validation of the chosen method. This always includes the sample preparation as well. Figure 24 gives an overview.

Validation of the manufacturing process

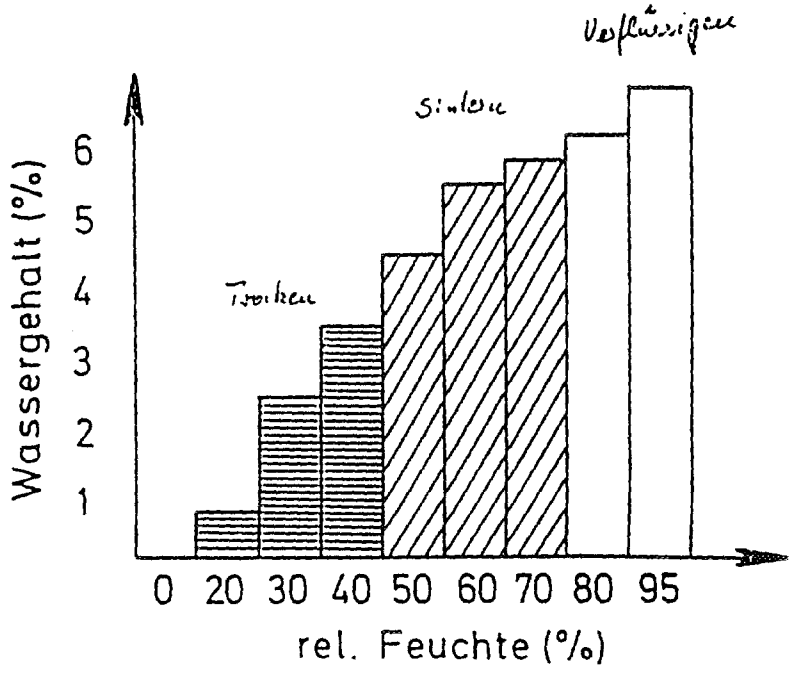
The basic processes in the manufacturing of phytomedicines are so simple and uncomplicated, that no large validation effort is necessary. If in the planning stage the specifications have been set, it has to be shown, that they can be kept continuously within the specified range. A simple design (figure 25) and reliable bookkeeping is normally sufficient to achieve certainty.


organic plant-acids
tanning substances
alkaloids
heavy metal ions
glycosides
proteins
polysaccharides
alcohols

3.1 Figure 20



3 / Figure 21



Konsistenz eines Baldriantrockenextrakts in Abhängigkeit von der rel. Feuchte

3.1 Figure 22

Einfluß von Feuchtigkeit auf das Verhalten von Trockenextrakten

Extractum	Wird pappig nach		Wird feucht nach	
	Einwirkungszeit von	Feuchtigkeitszunahme von	Einwirkungszeit von	Feuchtigkeitszunahme von
Belladonnae	sofort	0,47 % in 1 h	2 Tagen	6,87 %
Cocae	sofort	0,27 % in 1 h	2 Tagen	7,26 %
Colae	4 Std.	1,29 %	6 Tagen	9,61 %
Strychni	4 Std.	1,35 %	3 Tagen	9,02 %
Ipecacuanhae	8 Std.	2,39 %	2 Tagen	4,19 %
Cinchonae	1 Tag	3,22 %	3 Tagen	6,67 %
Hydrastis	2 Tagen	5,26 %	6 Tagen	9,62 %
Hyoscyami	2 Tagen	2,77 %	6 Tagen	8,90 %
Opii	2 Tagen	3,22 %	15 Tagen	6,02 %

Validation of analytical methods

accuracy : 6-10 fold repetition, starting with sample preparation, on the same starting material

linearity: spiking repeatedly with 75%, 125%, 150% of specified marker

appropriateness: spiking with known adulterations, test against reference sample

selectivity: test against a second, independent method

reliability, robustness: repetition by different analysts under slightly different environmental settings

economy: calculation of costs per test, including time, salary and equipment

Validation of manufacturing procedures

1. establishing a validation strategy

    -identification of critical steps (technical, microbiol.)
    -identification of suitable measuring parameters
    -selection of monitoring methods
    -selection of target criteria indicating validation

2. implementation of validation plan

    -collection of data
    -statistical evaluation
    -comparison with target criteria
    -review and critical assessment

3. documentation

    -validation plan
    -experimental design and methods
    -experimental results
    -statistical evaluation
    -assessment
    -proposals for improvement

Legal guidelines

The WHO has issued guidelines for GMP of phytomedicines and also for „Quality Control methods for Medicinal Plant Material". Although not legally binding, they constitute a help for further advancement.

The EEC has issued an entire codex of guidelines, legally binding for companies of the member states, which have been supplemented by PIC guidelines of equally binding nature. They include rules for plant design , equipment and working conditions, organization and safety, whose implementation is costly and sometimes self-defeating.

They will be discussed in a forthcoming lecture.

Problems and constraints in the production of medicines in developing countries

Götz Harnischfeger

The term developing country is usually applied to states, where the technical and intellectual capacity for achieving the standard of industrialization comparable to the EC or USA is present but political, or economic constraints and/or to few resources prevent a quicker pace towards this goal.
Usually these countries are burdened in addition with an uneven, limited educational system as well as an insufficient health care system, which requires large sums of money for improvement. The financial burden necessary is normally not in line with the economic status of the state finances.

The health care system itself, thus, is unable to provide high-tech conventional medicines to the entire population at affordable prices.
This led the WHO to issue a basic drug list, enumerating about 250 substances deemed absolute necessary to combat the most crippling and devastating illnesses especially in underdeveloped countries. The list constitutes a tool at the most basic level of medicinal care. Unfortunately, there are many countries in Africa, Asia and also some states in the Caribbean where even basic needs cannot be filled due to lack of funds.
For the majority of countries in the southern hemisphere it can be stated, that the basic level of medicinal care can be met. However, the population has relied in the past and still is relying to a great extend on herbal medicinal products not so much for the real „killers", like epidemics, but for the treatment of daily ailments and afflictions. This is not much different, by the way, to Europe or the US.
However, while the Europeans try to integrate the use of phytomedicines into conventional medicinal practices the US approach is diametrically opposite, i.e. complete neglect due to a dogma of „unproven" quality, safety and efficacy.

The problem which phytomedicines present in the developing countries is, in my opinion, primarily a mental one on the heads of the regulators trying to use the US-FDA as a guide. A rethinking and a legal framework is necessary, which defines phytomedicines as remedies on equal footing with synthetics and provides guidelines for assessing indigenous herbal drugs for their usefulness in conventional western medicine. The existing guidelines of the US-FDA, a model for many regulatory agencies, have to be modified in order to be applicable to the peculiarities of phytomedicines. Especially a directive for proof of efficacy through traditional experience is needed.
If phytomedicines are seen in this way, they provide one more tool to help developing countries to become self-reliant in their pharmaceutical services. The use of indigenous, locally available plants for the preparation of herbal medicinal products must be promoted, with the assistance of international organizations where necessary. For self reliance, the following steps are considered necessary (figure 1, Sofowora, 1979).
The need for effective phytomedicines is amply illustrated by the table on mortality in a not easily accessable region of Peru (figure 2).
The question which has to be solved by the various state authorities is the procedure of registration for use in conventional medicine. The intended guideline should be set pragmatically so that the registration can be achieved with a satisfactory level of quality, safety and efficacy, and no unnecessary demands. The level also results in grading of OTC and ethical form of marketing. In assessing efficacy, it should be demonstrated only, that the phytomedicine is active in the proposed indication, not, that it is superior to a synthetic substance. The decision, if OTC is suitable, can be made considering the criteria of (figure 3).
There are, in developing countries, a series of constraints which hamper modern manufacturing methods.
To strict GMP guidelines: They are constantly being improved and have reached a stage where overperfection is the rule rather than the exception. Here it is necessary to analyze what are

(1) developing countries must reduce unwarranted importation of drugs — only essential drugs (WHO, 1977) should be imported;
(2) they should attempt to produce some pharmaceuticals locally;
(3) they should utilize locally available medicinal plants as substitutes for

(4) they should encourage large-scale cultivation of medicinal plants such that any excess can be converted into drug products for exportation;
(5) they should direct research towards solving local problems in a collaborative manner.

1.5 Figure 1

Morbilidad en 4 comunidades campesinas durante junio 1987 - julio 1988

Infecciones Respiratorias Agudas*a	39%
Vias digestivas (diarrea, gastritis)	14%
Reumatismo	8%
Anemia	7%
Accidentes (inclusive, intoxicaciones)	5%
Piel	5%
Cefalea*c	4%
Diversos: (cólicos, hemorragia vaginal, conjuntivitis, parotiditis*b, epilepsia)	18%

*a) En un 10% de los casos se agravan a bronquitis o bronconeumonia.
*b) Durante la encuesta se presentó una epidemia de parotiditis.
*c) En muchas ocasiones, el viento, es la causa de la cefalea.

Fuente: de Paepe [illegible]

Mortalidad en el departamento de Cusco

Vias respiratorias	39.7%
Vias digestivas	13.7%
Sintomas mal o no definido	12.7%
Intoxicaciones, accidentes	5.6%
Diversos	28.3%

Fuente: Región de Salud.[illegible]

Selfmedication

Definition:

Selfmedication is the use of non prescription drugs by patients upon their own initiative and on their own responsibility with possibel guidance through a member of the health profession.

Criteria and safety precautions

1. Selfmedication is connected to symptoms not to medical diagnosis
2. Medicines for selfmedication must have approved quality, safety and efficacy
3. The time span for using such medicines should not exceed 3 - 7 days under normal circumstances

1.5 Liquid 3a.

4. Self medication is unsuited if

   - the symptoms continue
   - the physiological state deteriorates or recurres in worse form
   - strong pains persist
   - one or more apt appearing medicines have been used without success
   - Symptoms have been recognized as serious
   - parallel psychic symptoms are present, e.g. anxiety, depression, lethargy, overexcitement

5. Special caution should be applied during pregnancy and nursing, in babies and small children

basic , essential requirements, what are welcome and necessary additions and what are mere adornments.

The economic situation (cost/profit ratio): it does not allow the luxury of more than the bare minimum of personnel. Streamlining in acceptable (by the authorities) limits is of prime importance.

Trained man power: it is not always available. While this is easier to overcome at the manufacturing floor through in house training programs, key technical personnel at the supervising and laboratory level is more difficult to obtain. The system of higher education often neglects such special programs.

Equipment: It constitutes another important sector, which restricts development of pharmaceutical manufacturing enterprizes. Its cost is even for wealthy European companies outrageous, for countries in development prohibitive.

Ways of financing equipment must be found, if necessary, with the aid of multinational Organizations like WHO etc.

Logistics: they play a large role in running a pharmaceutical plant. The availability of water, electricity and infrastructure (road system, telecommunication) is elementary.

The long term supply of raw materials (plant drug): The question is, can the demand of the product either real or projected, be met by the natural resources? Supply can become a problem.

The biggest obstacle to a phytopharmaceutical industry with high quality products is, however, the market itself. It is mostly undefined, since medicines as such are in most cases only considered those available on prescription. The entire OTC, selfmedication, health food or nutraceutical market is uncontrolled in most countries. For a company to be profitable with high quality, state approved phytomedicines it is necessary, that it makes use of the chain of distribution installed for ethical drugs. That also means, that the distributors, e.g. pharmacists, nurses, hospitals, doctors, are educated in the use and limitations of the phytomedicines. Education has to start in the schools of pharmacy, nursing, medicine etc. with relevant courses. However, quality phytomedicines have a price. This price should be affordable in the general population, a requirement which in many instances cannot be fulfilled. Low prices can only be achieved by high volume output. This however needs a steady market, an effective and inexpensive distribution system and at least a minimum amount of financial resources either at the patient or, in socialized health systems, at the state level. The problem can be alleviated using phytomedicines instead of the usually high priced ethical drugs, but still requires attention at the economic and political level. This is aptly described in a paper given by Sofowora in 1982. Although now it is over 15 years later, the basic tenets are still the same.

I quote:

„when it is considered that the drug import bill (Ekwunife,1978) for Nigeria alone was about US$200 million in 1977 and that items such as laxatives costing about US$ 2.5 million were included in such imports it can be seen that there is a need for the production of laxatives from the many herbs that are used in traditional medicine as purges, in Nigeria.

A recent survey by UNCTAD has shown that 33 per cent of total drugs produced by the industialized nations are plant-derived and that if microbes are added, 60 per cent of medicinal products are of natural origin (UNCTAD, 1974). Indeed, higher plants have been described as the 'Sleeping Giant' of drug development

In a surveyin the US, 76 compounds obtained from plants commonly appeared in prescriptions. The survey showed that the statement often advanced, that plants may cease to be of importance to the drug industry, was a fallacy. Whereas many active agents derived from plants have been synthesized in the laboratory, commercial exploitation of such synthetic processes have proved impractical or uneconomic on an industrial scale (see also Chapter Six). Only seven drugs of natural origin used in the USA are known to be synthesized commercially; namely emetine, caffeine, theobomine, theophylline, psudoephedrine, ephedrine, and papaverine (Farnsworth and Morris, 1976). Therefore, developing countries should exploit

their medicinal plants to their own advantage by using them in their health care systems and producing drugs for export.

A look at the situation in Nigeria, for example, shows that there is need to promote the use of medicinal plants for drug manufacturing. A sample survey carried out in Nigeria showed that in the Lagos and Oyo States less than 1 per cent of total drugs dispensed in the health centres were of higher plant origin. Similarly in the retail pharmacies in Oyo State the proportion of drugs of plant origin stocked or dispensed was found to be less than 2 per cent. These figures indicate that these states (and probably the whole country) spend far less proportionately on drugs of plant origin than does the USA. The situation is probably similar for many other developing countries except India and China.

As a consequence, Sofowara proposes an approach to self reliance outlined in figure 4.

(1) developing countries must reduce unwarranted importation of drugs — only essential drugs (WHO, 1977) should be imported;
(2) they should attempt to produce some pharmaceuticals locally;
(3) they should utilize locally available medicinal plants as substitutes for

(4) they should encourage large-scale cultivation of medicinal plants such that any excess can be converted into drug products for exportation;
(5) they should direct research towards solving local problems in a collaborative manner.

1.5 Figure 4

class D (surface) : alert limit 60 cbu/plate,
action limit 100 cbu/plate
(air) : alert limit 500 cbu/m^3
action limit 1000 cbu/m^3

Classification

class A: laminar flow areas
filling areas for solutions to be sterilized in
in the final container
filling areas for aseptic filling of powders and
liquids

class B: standard sterile areas

class C: production areas for solutions to be sterilized
production areas for liquids to be filled aseptic.

class D: dermatics, nose and ear preparations, liquid
and solid oral forms

Microbiological limits

rooms, air

class (EG)	EG-GMP cbu/m^3	USP XXIII cbu/m^3
A	< 1	< 1
B	5	< 18
C	100	< 88
D	500	n. d.

surfaces

class (EG)	FIP recomm. cbu/25cm^3	USP XXIII cbu/30cm^3
A	5	3
B(eq.)	10	5
B(floor)	20	10
C(eq.)	n.d.	38.8
C(floor)	n.d.	58.8
D	n.d.	n.d.

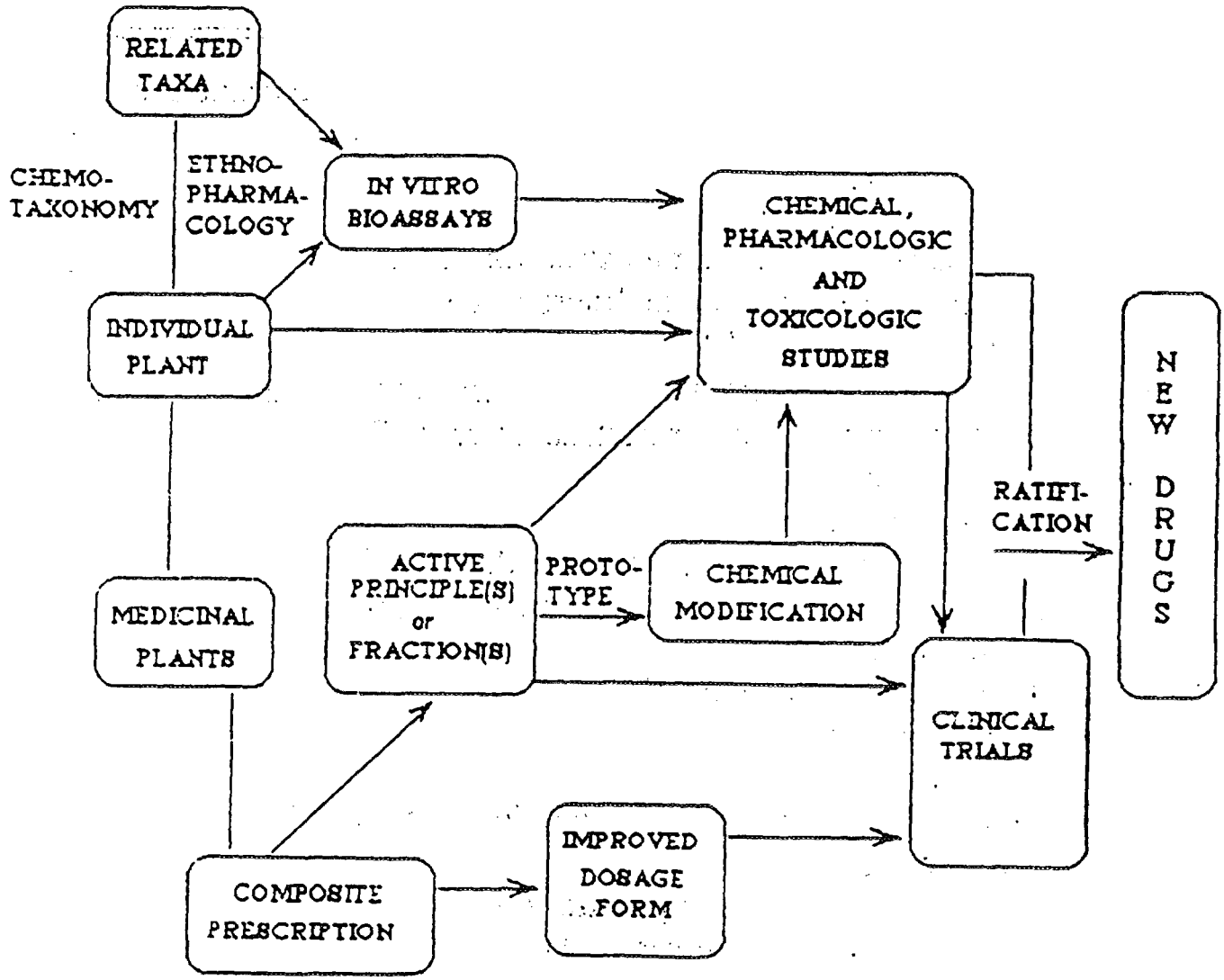
Ethonobotanical and Ethnomedical Evaluation:

Principles and Applications

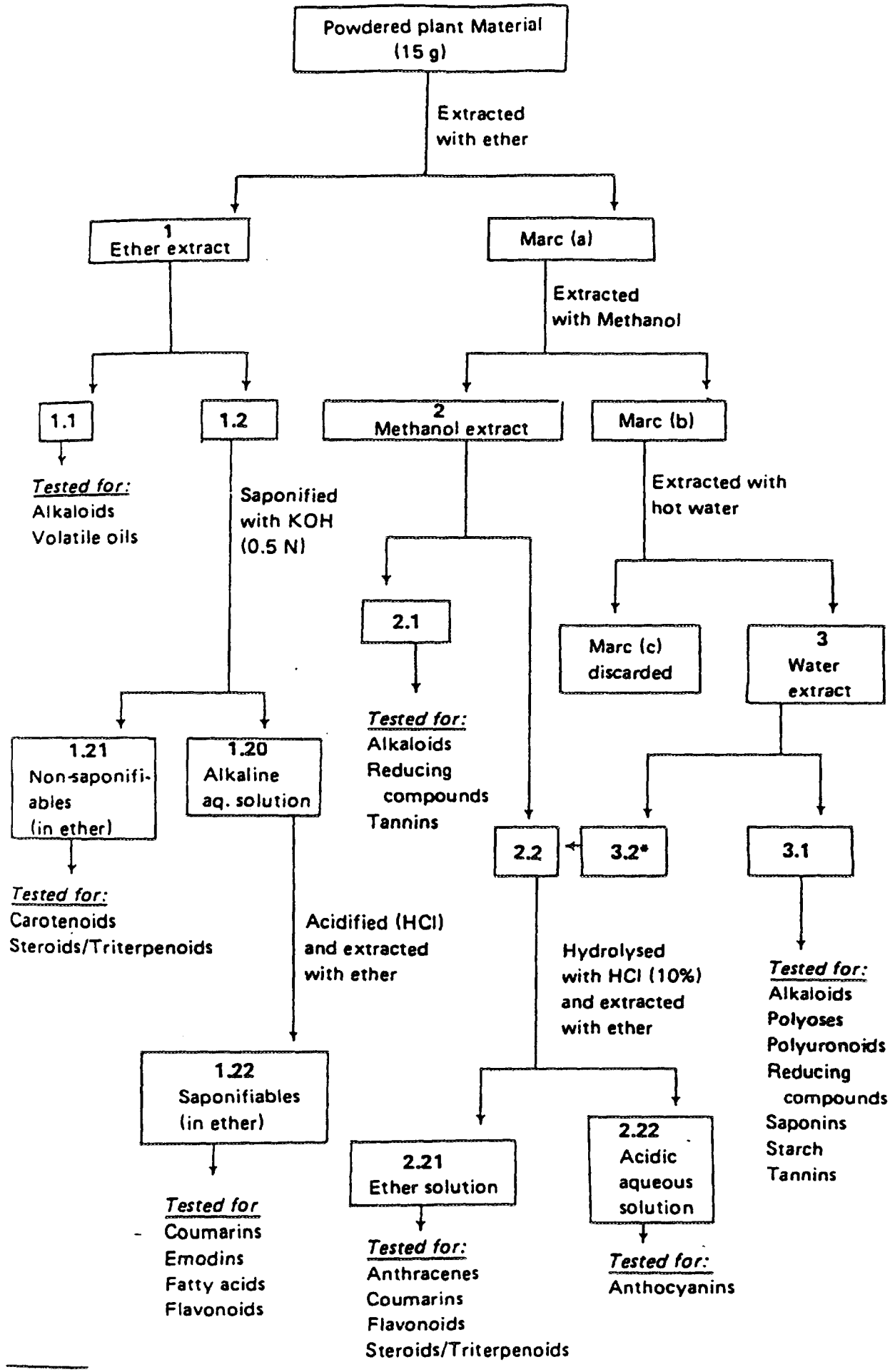
Götz Harnischfeger

The use of phytomedicines is in almost all instances the result of tradition carried over from times when synthetic substances for the treatment of diseases were unavailable. It can be safely stated, that up until the 1940s phytomedicines constituted the bulk of prescriptions made out by family doctors in Germany. The experiences gained with those formulations, many of them officially stated in the pharmacopoiea, were so sound and encouraging, that to the chagrin of purists in pharmacology phytomedicines still exist and are prescribed.
This shows, that there is a vast amount of empirical knowledge embedded in this type of pharmaceutical product. It just has to be transferred into modern scientific terms and the insufficient information about mode of action, pharmacokinetics etc. has to be filled by research. In many cases, e.g. valerian root (Valerianae radix), even the active principles are unknown.
The approach to fill in the missing knowledge is the same for the evaluation of traditional „western" medicine, the understanding for formulations in time proven alternative systems like Ayurveda and TCM with a long list of literature citations, or for the understanding of the practice of native healers, curandeiros, shamans etc., at least in respect to the scientific side of their trade. The approach is outlined in figure 1.
Starting with the plant drug or the formulation in question one fractionates its content by sequential extraction with solvents of decreasing or increasing polarity, and assays the resulting extracts not only chemically but also and primarily biologically. This bioguided method results, if successfully executed, in the isolation and characterization of single chemical compounds. An outlay (figure 2) and an example is given in figure 3.

Of utmost importance in this approach is the design of the bioassay. It has to be geared to the indication and its limitations in regard to reliability and projectability to the human condition have to be well known.
When dealing with relatively unexplored or unknown plant drugs, a general screening for pharmacological activity should precede any „specialized" bioassay.
The basic purpose of this sequence is, to let no true biological activity undetected. The initial screening procedure must unequivocally establish the activity, as well as its probable nature in order to indicate a course of further, more specific pharmacological evaluation. Therefore, in order to ensure an adequate scientific perspective, this very important initial screening must be designed to be unbiased, general in scope and, if possible, comprehensive, rather than being specifically directed to any particular type of activity or proposed use.
When one leaves out the general screening, there is a big chance to miss some of the most important effects. For instance, digitalis was originally classified by folklore as a diuretic, but when tested in a specific diuretic screen, this yielded negative results. Yet nobody can say that digitalis is not an active drug, and only when it is tested in a general screening, it can be established as a possible cardiotonic agent. By the way, in case of decompensatio cordis, it induces diuresis due to its cardiotonic action.
This initial screening must be carefully standardized to make it reliable and yield reproduceable results. Moreover, the methods must not be too elaborate or too expensive; the program must be designed so that it can be used for purified and for crude material as well, but does not require large quantities.
One widely used screening procedure was developed by Malone and Robichaud and improved by Irwin. This „hippocratic approach" integrates subjective impressions of the experimenter with objective measurements.
It requires only animals, a glass jar, a pair of forcepts, a hypodermic needle and a very well-trained observer. Three animals per dose are used and they are housed together in a plastic cage. The dosages are given in a logarithmic range. An example of the way drug effects are recorded is given in figure 4, where we can see that a rightening reflex which normally occurs every time an animal is laid on its back, can be abolished by drug treatment. Figure 5 gives an example of the way passivity is screened, ranging from 0 (a normal reaction of the animal) to 8


RELATED TAXA
CHEMO-TAXONOMY
ETHNO-PHARMA-COLOGY
IN VITRO BIOASSAYS
CHEMICAL, PHARMACOLOGIC AND TOXICOLOGIC STUDIES
INDIVIDUAL PLANT
NEW DRUGS
RATIFI-CATION
ACTIVE PRINCIPLE(S) or FRACTION(S)
PROTO-TYPE
CHEMICAL MODIFICATION
MEDICINAL PLANTS
CLINICAL TRIALS
IMPROVED DOSAGE FORM
COMPOSITE PRESCRIPTION

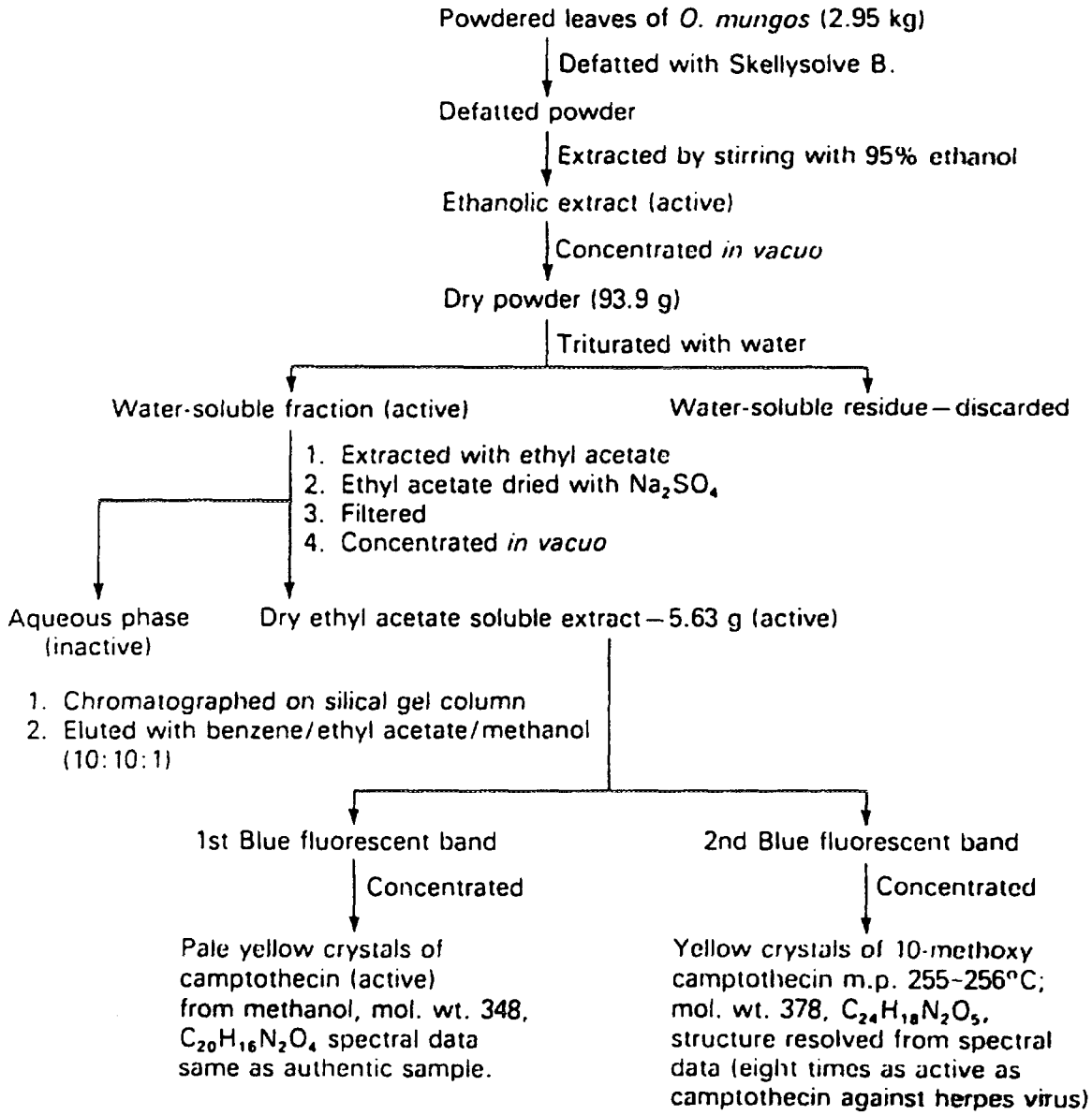
1.2 Figure 1



*3.2 is treated in the same manner as 2.2.

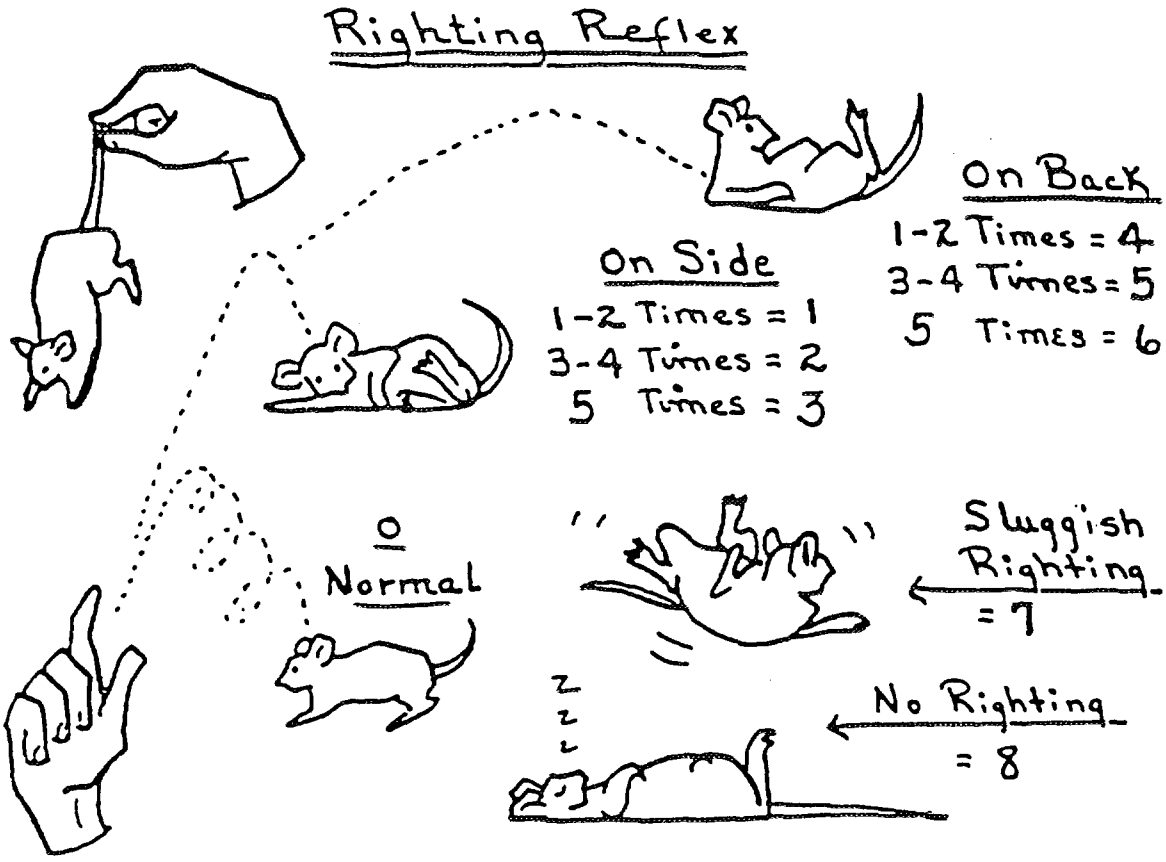
Isolation of antiviral component of *Ophiorrhiza mungos* (Rubiaceae)

After Susan Tafur, J. D. Nelson, D. C. Delong, and G. H. Svoboda (1976). *Lloydia*, **39** (4), 261.

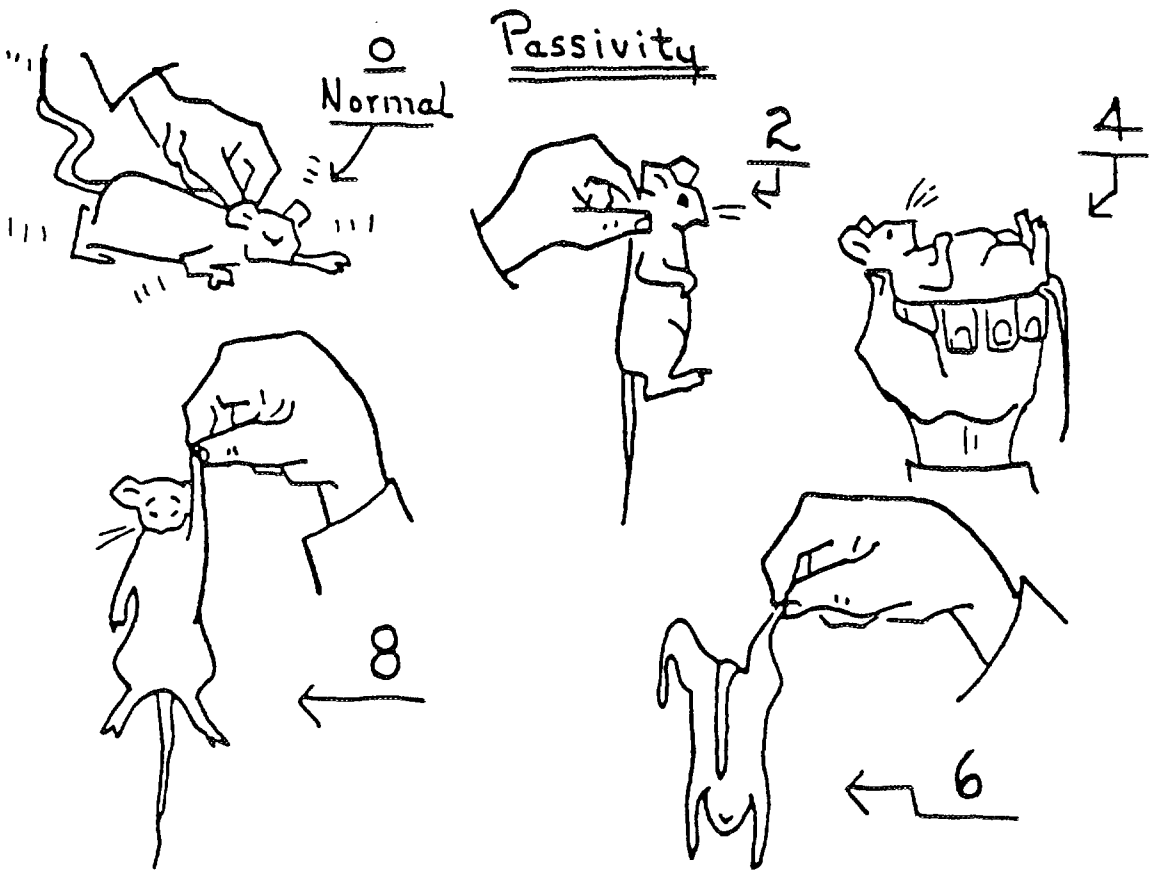


Purification was monitored by bioactivity using a plaque reduction of herpes virus as a direct quantitative assay to guide fractionation.

4.2 Figure 2



Impairment of righting reflex; illustrating of suring procedure to quantify on a 0-8 scale the intensity of drug effect.
After: S.Irwin, p. 47 (1964)



Struggle response (passivity) is scored from 0-8.
After: S.Irwin, p. 49 (1964)

1.2. Figure 5

(total passivity). Several symptoms an animal can show will give us information about the manner in which it is influenced (figure 6).
The points are recorded (figure 7/8) and a general picture about the profile of the tested substance emerges.
A well-trained observer with enough experience can indicate at first sight from such a profile to which class the drug probably belongs.
Another general screening method, going into more detail, thus circumventing the bias towards CNS activities, is given by the Swedish group of Sandberg and Samuelson. It consists of three levels of testing with increasing sophistication, outlined in figure 9.
Prerequisite for this test is a good solubility of the sample in aqueous medium.
Level 1 concerns the standard test of Malone and Irwin and is directed to the general pharmacological and toxicological profile.
Level 2 is geared towards more qualified information involving the basic mechanism of action, i.e.

- the brine shrimp test for toxicity
- antimicrobial tests
- the fertilized sea-urchin test gives information about cytostatic or cytotoxic activity and general effects on cell development.
- the Hexobarbital test gives hints towards neuronal interference
- opiate receptor studies involve analgetic, sedative antitussive, hypotensive and psychomimetic characters
- the cat model is used for the study of cardiovascular effects
- the guinea pig ileum is a model system for histamin- and spasmolytic effects
- Adenyl-cyclase and phosphordiesterase of human thrombocytes is a test system for an influence on the control of nucleotid metabolism, relevent in hypertension, asthma, diabetes

After a general profile of biological activity has been gained, investigations into detailed pharmacological effects can be made. The bioassays are standard procedures, often even given in the pharmacopoeia (e.g. tests for Mycobacterium tuberculosis, Mycoplasma, Histamin activity, blood pressure reducing activity, praekallikrein activation, complement activity, antibiotic activity tec. are standard laboratory procedures in pharmacology).
It has to be mentioned however, that many afflictions cannot be modelled in pharmacological systems. There is no exact evidence for a cause and neither exists an animal being similarly affected. An example is prostatic hyperplasia.
After this review on bioguided assays, the approach outlined before towards evaluation will be explained in more detail.

a) plants and phytopreparations used in native therapeutical systems with oral transmisssion or by „hands on" learning

   To clarify: the evaluation method described above is a scientific endeavour in the context of rational „western" medicine only, taking solely its tenets of illness and health into account. One has to be aware, that native reasoning about diseases and afflictions is based on entirely different concepts, mixing spiritual elements with observed causes.
   An outline of the investigational approach is given in figure 10.
   It has to be emphasized, that already a positive result in step 6 is sufficient to launch a new commercial phytomedicine.
   Very important is the information at the local level, its verification and substantiation by samples. The WHO, having used this approach extensively in the Ife and Tansania program, has developed a special questionnaire which is given in the next figures (figures 11/12).
   The drawback of this approach is the lack of information at the clinical level. There are normally no studies which have been made according to rigorous clinical standards (GCP) and they will be the exception even at an advanced stage of research (e.g. step 6). Everything depends on the interpretation of pharmacological and toxicological data, not

Concentration of solution
Rectal temperature before injection
Paw temperature before injection
Dose per kg
Weight in g
Dose level nr.
OBSERVATION: 15-30 min. after injection
IN CAGE
Dead within 30 minutes
Ptosis
Pilo erection
Straub tail
Hypoactivity
Hyperactivity
Urination
Defaecation
Shiver
Vocalization
Exophthalmos
Fighting
KEEPING THE ANIMAL IN THE HAND
Salivation
Lachrymation
Mydriasis
Myosis
Corneal reflex
Hypotonia
Reaction on touching
Agressive
Decreased respiration
Increased respiration
Pale ears
Red ears
Rectal temperature after injection
Temperature decrease
Temperature increase

ON SMOOTH SURFACE
Head drop
Righting reflex
Reaction to bumping the table
Reaction to sound
Abnormal gate
Abduced hin legs
Convulsions
Paralysed

Rotatin axle, 1 turn/2 seconds
Fatigueness
Analgesic activity "Hot plate" 55°C

Dead within 30 minutes
Dead within 1 hour
Dead within 24 hours
Dead within 48 hours

LD_{50}
Frightened jumping
Writhing

Date _____ Notebook No _____ pp ____

Hippocratic Qualitative/Semiquantitative Screen and Toxicity Report of _____

_____ Lot/Identification No _____

Dosage _____ mg/kg; Dosage Vehicle _____ Conc _____ mg/ml

PARAMETERS	Response Rating (0- +4) or Measurement														
	Control	+5 min	+10 min	+15 min	+30 min	+60 min	+2 hrs	+4 hrs	+6 hrs	+24 hrs	+48 hrs	+4 days	+7 days	*
Decr Motor Activity															
Decr. Resp. Rate															
Decr Resp. Depth															
Dyspnea															
Cheyne-Stokes Resp															
Analgesia															
Anesthesia															
Loss, Corneal Reflex															
Loss, Pinna Reflex															
Back Plasticity															
Ataxia															
Hind Leg Grip Loss															
Foreleg Grip Loss															
Hind Leg Paralysis															
Foreleg Paralysis															
Neck Paralysis															
Incr Motor Activity															
Incr Resp Rate															
Incr Resp Depth															
Fine Body Tremors															
Coarse Body Tremors															
Back Tonus															
Tonic Convulsions															
Clonic Convulsions															
Tail Erection															
Tail Grasping															
Tail Lashing															
Enophthalmos															
Exophthalmos															
Palpebral Ptosis															
Pupil Size, mm															
Pupil Size, (light)															
Nystagmus															

1-2 Figure 7

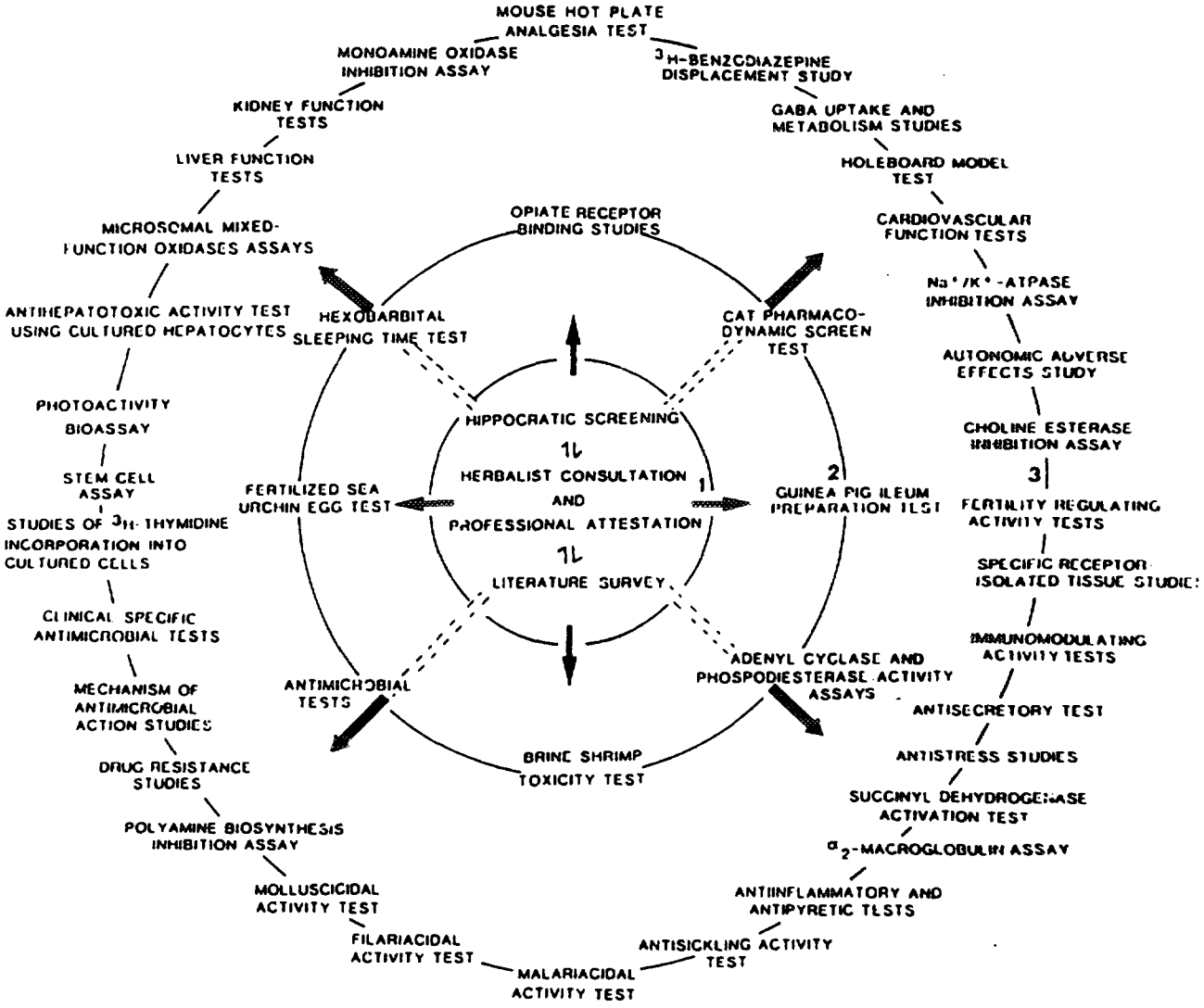
Project Code _____, Reader Signature _____

Test Animal _____, Fasted / Nonfasted _____, Sex _____; Weight _____ g

Mark _____; Dye Color _____, Cage No. _____; Special Treatment _____

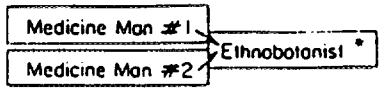
Injection Volume _____ ml; Injection Route _____; Clock Time _____

PARAMETERS	Response Rating (0 - +4) or Measurement														
	Control	+5 min	+10 min	+15 min	+30 min	+60 min	+2 hrs	+ hrs	+ hrs	+24 hrs	+48 hrs	+ days	+7 days	*
Chromodacryorrhea															
Ear Blanching															
Ear Hyperemia															
Ear Cyanosis															
Ear Metachrosis															
Fasciculation															
Thick Salivation															
Watery Salivation															
Pilomotor Erection															
Robichaud Positive															
Writhing Movements															
Rales															
Micturition															
Colored Micturition															
Diarrhea															
Stereotypy															
Circling Motions															
Disorientation															
Statue Positions															
Startle Sensitivity															
Head Tap: Aggressive															
Head Tap: Fearful															
Head Tap: Passive															
Body Grasp: Aggressive															
Body Grasp: Fearful															
Body Grasp: Passive															
Persistent Grooming															
Priapism/Colpectasia															
Rectal Temperature, °C		x	x	x											
Body Weight, g		x	x	x											

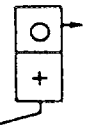

MOUSE HOT PLATE ANALGESIA TEST
MONOAMINE OXIDASE INHIBITION ASSAY
^{3}H-BENZODIAZEPINE DISPLACEMENT STUDY
KIDNEY FUNCTION TESTS
GABA UPTAKE AND METABOLISM STUDIES
LIVER FUNCTION TESTS
HOLEBOARD MODEL TEST
MICROSOMAL MIXED-FUNCTION OXIDASES ASSAYS
OPIATE RECEPTOR BINDING STUDIES
CARDIOVASCULAR FUNCTION TESTS
ANTIHEPATOTOXIC ACTIVITY TEST USING CULTURED HEPATOCYTES
HEXOBARBITAL SLEEPING TIME TEST
CAT PHARMACO-DYNAMIC SCREEN TEST
Na^+/K^+-ATPASE INHIBITION ASSAY
AUTONOMIC ADVERSE EFFECTS STUDY
PHOTOACTIVITY BIOASSAY
HIPPOCRATIC SCREENING
CHOLINE ESTERASE INHIBITION ASSAY
STEM CELL ASSAY
1L
HERBALIST CONSULTATION AND PROFESSIONAL ATTESTATION
1
2
3
FERTILIZED SEA URCHIN EGG TEST
GUINEA PIG ILEUM PREPARATION TEST
FERTILITY REGULATING ACTIVITY TESTS
STUDIES OF ^{3}H-THYMIDINE INCORPORATION INTO CULTURED CELLS
1L
LITERATURE SURVEY
SPECIFIC RECEPTOR ISOLATED TISSUE STUDIES
CLINICAL SPECIFIC ANTIMICROBIAL TESTS
IMMUNOMODULATING ACTIVITY TESTS
ANTIMICROBIAL TESTS
ADENYL CYCLASE AND PHOSPODIESTERASE ACTIVITY ASSAYS
MECHANISM OF ANTIMICROBIAL ACTION STUDIES
ANTISECRETORY TEST
BRINE SHRIMP TOXICITY TEST
ANTISTRESS STUDIES
DRUG RESISTANCE STUDIES
SUCCINYL DEHYDROGENASE ACTIVATION TEST
POLYAMINE BIOSYNTHESIS INHIBITION ASSAY
α_2-MACROGLOBULIN ASSAY
MOLLUSCICIDAL ACTIVITY TEST
ANTIINFLAMMATORY AND ANTIPYRETIC TESTS
FILARIACIDAL ACTIVITY TEST
ANTISICKLING ACTIVITY TEST
MALARIACIDAL ACTIVITY TEST

STAGE

1 ***Establish Folk History***

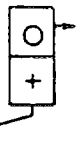


Decision # 1.
Do both medicine men use the same plant for the same disease?

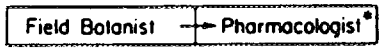


2 ***Establish Tentative Identification***

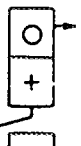
Decision # 2.
Genus? Species?



3 ***Blind Screen for Whole Material Activity***

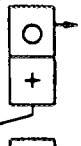


Decision # 3:
Dose-response activity at 1g/Kg or less?

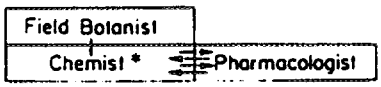


4 ***Absolute Plant Identification***

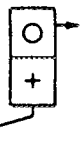
Decision # 4.
Agree on genus and species?



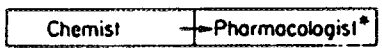
5 ***Quantitative Extractive / Marc Assays***



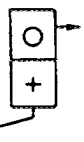
Decision # 5:
Using physical data, possible unique molecule?



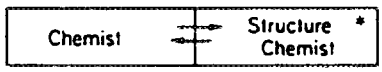
6 ***Classic Pharmacological Evaluation***



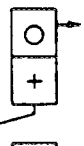
Decision # 6:
Unique activity?



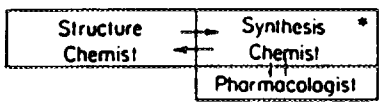
7 ***Classic Structure Determination***



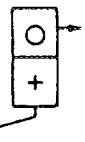
Decision # 7:
Unique molecule?



8 ***Synthesis and Structure Modification***



Decision # 8:
Patent synthesis?



Oviductus Ranae (哈蟆油 , Hamayou)

Dried oviduct fat of Chinese forest frog

This is the dried oviduct fat of female Chinese forest frog, *Rana temporaria chensinensis* David (Fam. Ranidae)., collected and dried.

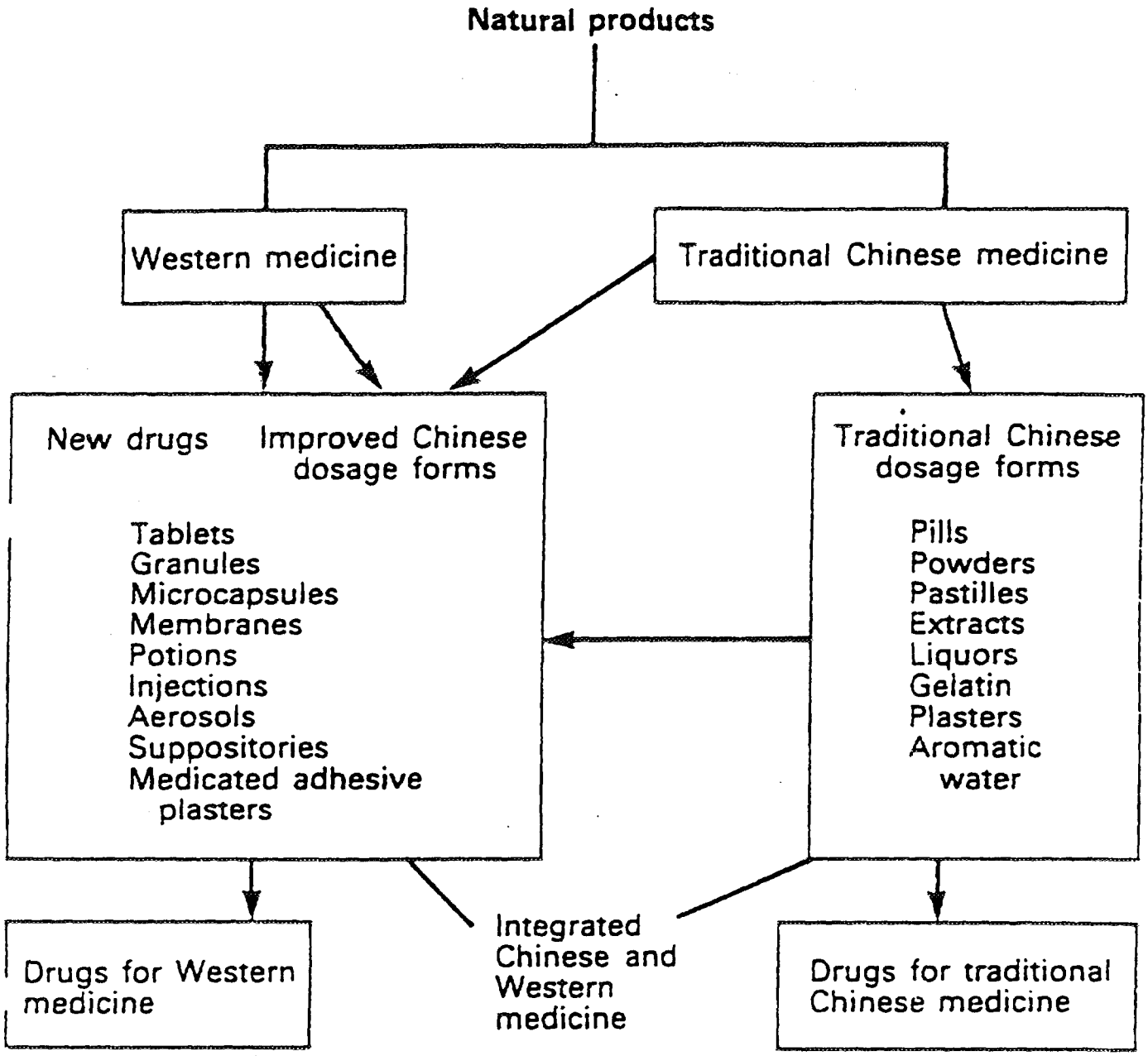
Description Irregular lump, crooked and overlaped, 1.5—2 cm long, 1.5—5 mm thick; surface yellowish-white, presenting fat like lustre, occasionally with greyish-white thin membranous dry skin, with satiny feeling. The volume can expand 10—15 times on soaking in lukewarm water. Odour, stinking; taste, slight sweet and slimy on chewing.

Action To replenish the vital *essence* of the *kidney*, and to nourish *yin* of the *lung*.

Indications General debility; listlessness, palpitation, insomnia and night sweating after an attack of disease; cough and hemoptysis in phthisis.

Usage and dosage 5—15 g, to be taken after soaked with water and stewed with sugar added, or to make pills.

Storage Preserve in a ventilated dry place, protected from moisture and moth.


Natural products
Western medicine
Traditional Chinese medicine
New drugs
Improved Chinese dosage forms
Tablets
Granules
Microcapsules
Membranes
Potions
Injections
Aerosols
Suppositories
Medicated adhesive plasters
Traditional Chinese dosage forms
Pills
Powders
Pastilles
Extracts
Liquors
Gelatin
Plasters
Aromatic water
Drugs for Western medicine
Integrated Chinese and Western medicine
Drugs for traditional Chinese medicine

1.2 Figure 12

always projectable onto humans. This seem to be the reason that development stops mostly with the isolation and characterization of active compounds. Clinical trials are to costly.

b) plants and phytopreparations used in alternative therapeutical systems with written tradition
This encompasses the research done on translating remedies of TCM, Ayurveda, islamic medicine, homoeopathy, spagyric aspect etc into modern scientific concepts of therapy. The outcome is many times an optimum of frustration, since definitions and circumstances of the various afflications are not apt for such translation.
Nevertheless, a sample of the power of the general approach is given in figure 11.
The effect of „oviductus ranae" is readily explainable by hormone, probably estrogen, substitution.
TCM has proven results within its system for about 3000 years. Thus, it is small wonder, that this medicinal system is practised side by side in China. More and more phytomedicines are available there encompassing „western" and TCM medicine (figure 12).

c) phytomedicines as traditional medicines in the context of conventional medicine
The general approach outlined before applies here as well, if a rational base for their efficacy is desired. It is just easier to obtain the starting materials, in good pharmacopoieal quality, and even official monographs on effectiveness based on the evaluation of reams of literature data.
The problem is less with a single plant or plant drug but with the combination-phytomedicines.

Figure 13 gives an **example** of a grandfather phytomedicine on the OTC market since the 1930ties with good acceptance and documented therapeutical efficacy. Quality control methods for its manufacture had to be updated to the current state of the art.
This cholagogue in the form of a tincture contained an extract of a mixture of Cardui benedicti herba, Cardui mariae fructus, Anserinae herba, Taraxaci radix cum herba, Chelidonii herba and Matricariae flos. The general indication for this phytomedicine was „Inflammation and affections of the gallbladder and the biliary tract". Broken up in todays medicinal terminology the indication encompasses: cholecystitis, cholangitis, irritation of the biliary tract, dyskinesia of the biliary tract, subsiding hepatitis epidemica and hepatogenic obstruction. Arguments for effectivity, as summarized in figure 13, came from the traditional use of the ingredients, from clinical and pharmacological studies in some instances and also from reports from practitioners. Test in animal experiments can be done using bile- secretion, pancreas-secretion and spasmolysis as measuring parameters. The figure allows, at least in theory, a first estimate of the effectiveness of the various partners in this particular combination.
Further detail is given in the next figure (figure 14). It shows the contents of each plant ingredient which are relevant to the intended use and which can be tested further in animal experiments for their suitability as quantitative markers. The figure argues for the elimination of Anserinae herba and Taraxaci radix cum herba, a decision which was confirmed through the outcome of the a.m. follow-up experiments.

Cholagogue

liquid formulation

mixture of extracts from: Cardui benedicti herba, Cardui mariae fructus, Anserinae herba, Taraxaci radix cum herba, Chelidonii herba, Matricariae flos

Indication:

common version: Disorders and affections of the gall-bladder, the biliary tract and colictype abdominal pains

medicinal version:

	shown by traditional use	pharmacol. clinical data	therap.exp.
-cholecystitis	+	+	
-cholangitis	+	+	
-affection of the biliary tract	+	+	
-dyskinesia of the b.t.	+		
-receding hepatitis	+	+	
-hepatogenic obstruction	+		+

medicinally relevant measuring parameters:

bile secretion, pancreatic juice secretion, spasmolysis (animal experimentation necessary)

Cnicus benedictus: bitter compounds of the germacrolide type(secretory stimulation by reflex mechanism) essential oil (mildly bacteriostatic)

Silybum marianum: flavolignanes (liverprotective), unknown (choleretic)

Potentilla anserina: unknown (antiinflammatory, mildly spasmolytic)

Taraxacum officinale: unknown (mild secretory stimulation)

Chelidonium majus: alkaloids of the chelidonintype (spasmolytic in smooth muscle, cholekinetic, slightly analgetic)

Matricaria recutita: azulenes and derivatives (antiinflammatory), flavonoidglycosides (spasmolytic)

PEPPERMINT OIL

Menthae piperitae aetheroleum

DEFINITION

Peppermint oil is obtained by steam distillation from the fresh overground parts of the flowering plant of *Mentha* × *piperita* L.

CHARACTERS

A colourless, pale yellow or pale greenish-yellow liquid with a characteristic odour and taste followed by a sensation of cold, miscible with alcohol, with ether and with methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 0.1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 10 mg of *thymol R*, 10 µl of *menthyl acetate R*, 20 µl of *cineole R* and 50 mg of *menthol R* in *toluene R* and dilute to 10 ml with the same solvent.

Apply separately to the plate as bands 10 µl of the reference solution and 20 µl of the test solution. Develop over a path of 15 cm using a mixture of 5 volumes of *ethyl acetate R* and 95 volumes of *toluene R*. Allow the plate to dry in air until the odour of the solvent is no longer perceptible and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution may show quenching zones (carvone, pulegone) situated just below the level of the zone (thymol) in the chromatogram obtained with the reference solution. Spray with *anisaldehyde solution R* and examine in daylight for 5 min to 10 min while heating at 100 °C to 105 °C. The chromatogram obtained with the reference solution shows, in order of increasing R_f value: an intense blue to violet zone (menthol) in the lower third; a violet-blue to brown zone (cineole); a pink zone (thymol); and a violet-blue zone (menthyl acetate). In the chromatogram obtained with the test solution: there is a zone due to menthol (the most intense) and a faint zone due to cineole; at R_f values between those of the cineole and thymol zones in the chromatogram obtained with the reference solution, there may be light pink or greyish-blue or greenish-grey zones (carvone, pulegone, isomenthone); in the middle of the chromatogram, there is a violet-blue zone (menthyl acetate) and just below it a greenish-blue zone (menthone); an intense violet-red zone (hydrocarbons) appears near the solvent front and below it a brownish-yellow zone (menthofuran); other less intensely coloured zones also appear.

B. Examine the chromatograms obtained in the test for chromatographic profile. The retention time of the principal peaks in the chromatogram obtained with the test solution is similar to that of the principal peaks in the chromatogram obtained with the reference solution. Carvone and pulegone may be absent from the chromatogram obtained with the test solution.

TESTS

Acid value (*2.5.1*). Not more than 1.4, determined on 5.0 g dissolved in 50 ml of the prescribed mixture of the solvents.

Relative density (*2.2.5*): 0.900 to 0.916.

Refractive index (*2.2.6*): 1.457 to 1.467.

Optical rotation (*2.2.7*). The angle of optical rotation is – 10° to – 30°.

Fatty oils and resinified essential oils (*2.8.7*). It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile. Examine by gas chromatography (*2.2.28*).

Test solution. The substance to be examined.

Reference solution. Dissolve 0.1 g of *limonene R*, 0.2 g of *cineole R*, 0.4 g of *menthone R*, 0.1 g of *menthofuran R*, 0.1 g of *isomenthone R*, 0.4 g of *menthyl acetate R*, 0.6 g of *menthol R*, 0.2 g of *pulegone R* and 0.1 g of *carvone R* in 1 ml of *hexane R*.

The chromatographic procedure may be carried out using:

— a fused-silica capillary column 60 m long and about 0.25 mm in internal diameter coated with *macrogol 20 000 R* as the bonded phase,

— *helium for chromatography R* as the carrier gas at a flow rate of 1.5 ml per minute,

— a flame-ionisation detector,

— a split ratio of 1/100,

maintaining the temperature of the column at 60 °C for 10 min, then raising the temperature at a rate of 2 °C per minute to 180 °C and maintaining at 180 °C for 5 min and maintaining the temperature of the injection port and of the detector at 220 °C.

Inject about 0.2 µl of the reference solution. When the chromatograms are recorded in the prescribed conditions, the components elute in the order indicated in the composition of the reference solution. Record the retention times of these substances.

The test is not valid unless: the number of theoretical plates calculated from the limonene peak at 110 °C is at least 30 000; the resolution between the peaks corresponding to limonene and cineole is at least 1.5.

Inject about 0.2 µl of the test solution. Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution (disregard the peak due to hexane).

Determine the percentage content of the components by the normalisation procedure.

The percentages are within the following ranges:

Limonene 1.0 to 5.0 per cent

Cineole 3.5 to 14.0 per cent

Menthone 14.0 to 32.0 per cent

Menthofuran 1.0 to 9.0 per cent

Isomenthone 1.5 to 10.0 per cent

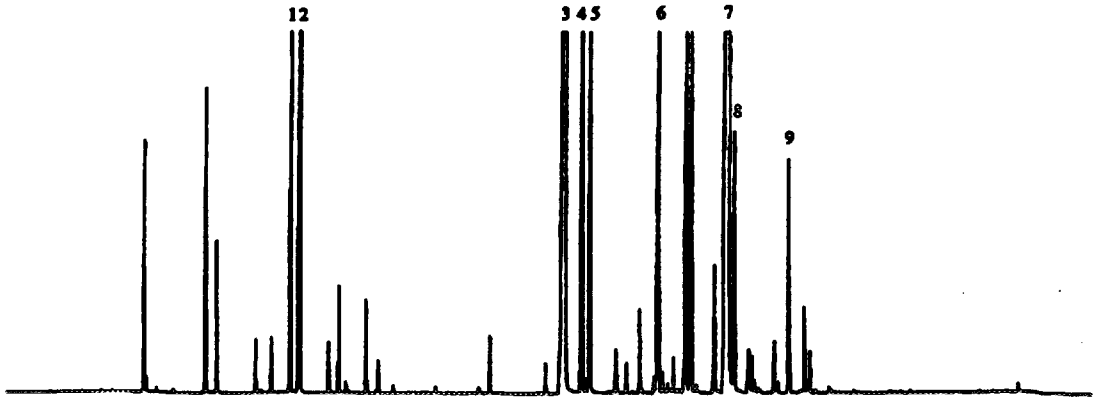
Menthyl acetate 2.8 to 10.0 per cent

Menthol 30.0 to 55.0 per cent

Pulegone not more than 4.0 per cent

Carvone not more than 1.0 per cent

The ratio of cineole content to limonene content is greater than two.



1. limonene
2. cineole
3. menthone
4. menthofuran
5. isomenthone
6. menthyl acetate
7. menthol
8. pulegone
9. carvone

1. limonene 4. menthofuran 7. menthol

2. cineole 5. isomenthone 8. pulegone

3. menthone 6. menthyl acetate 9. carvone

Figure 405-1.-*Type chromatogram for peppermint oil*

The type chromatogram is given for information and guidance in application of the analytical method. It is not part of the requirements of the monograph.

STORAGE

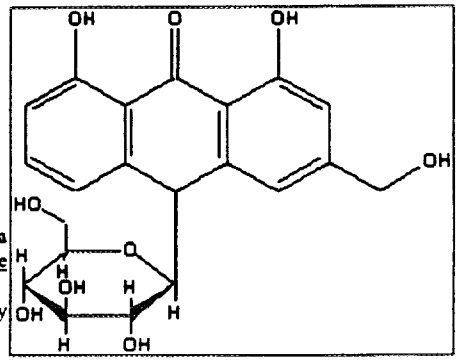
Aloin

[1415-73-2]

Synonyms: Barbaloin

$C_{21}H_{22}O_9$

418.40



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

ChemFinder (Macintosh) WebServer
Information about this particular compound
MSDS archive at the University of Utah
Information about this particular compound
Proposed list of medicines that may be taken by competeting sportsmen

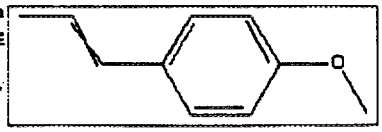
Return to searching

Anethole [104-46-1]

Synonyms: p-Propenylanisole; anise camphor; isoestragole; p-methoxy-beta-methylstyrene; 1-methoxy-4-propenylbenzene; nauli "gum"; oil of aniseed; 1-(p-methoxyphenyl)propene; p-1-propenylanisole; p-propenylphenyl methyl ether; 1-methoxy-4-(1-propenyl)benzene; Methoxy-4-propenylbenzene; Propenylanisole

$C_{10}H_{12}O$

148.20



Melting Point (°C)	21.35	Specific Gravity	0.9882
Boiling Point (°C)	234.5	Vapor Density	--
Vapor Press. Pa/kPa	--	Water Solubility	Slightly soluble
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	BZ8925000
Comments	White colorless crystals		

More Information about Anethole from Other Sources

Berkeley Carcinogenic Potency Database

BUA List of Existing Chemicals of Environment Relevance, incl. 1st and 2nd Priority Lists (Germany)

Information about this particular compound

CyberMol collection of molecules in VRML format

Information about this particular compound

Galactic Industries Corporation Spectral Database

FTIR SPECTRUM of ANETHOL

JICST Mass Spectral Database

Information about this particular compound

NIST Chemistry WebBook

Information about this particular compound

NTP Chemical Health and Safety Data

Information about this particular compound

Proton NMR Spectral Molecular Formula Index

Information about this particular compound

The Good Scents Company

Information about this particular compound

USEPA / OPP's Chemical Ingredients Database

Information about this particular compound

Web Molecules (in VRML)

Information about this particular compound

Return to searching

apigenin [520-36-5]

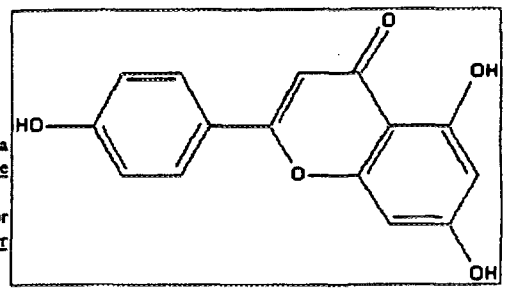
Synonyms: 4',5,7-Trihydroxyflavone; 5,7,4'-Trihydroxyflavone; Naringenin chalcone

$C_{15}H_{10}O_5$

270.24

This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures



Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
- Information about this particular compound

Ligand Chemical Database for Enzyme Reactions
- Information about this particular compound
- Information about this particular compound

Toronto Research Chemicals, Inc.
- apigenin

Web Molecules (in VRML)
- Information about this particular compound

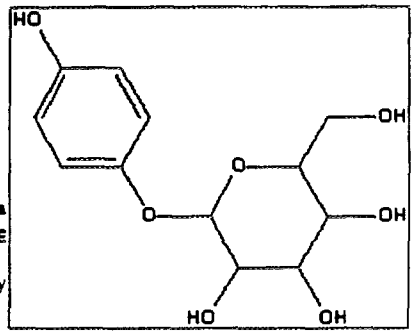
Return to searching

Arbutin [497-76-7]

Synonyms: Hydroquinone-beta-D-glucopyranoside

$C_{12}H_{16}O_7$

272.25



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound
Proton NMR Spectral Molecular Formula Index
Information about this particular compound
UMCP Partial list of teratogens

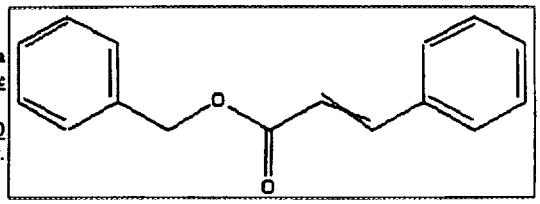
Return to searching

Benzylcinnamate [103-41-3]

Synonyms: 3-Phenyl-2-propenoic acid phenylmethyl ester; trans-Cinnamic acid benzyl ester; Cinnamein; benzyl 3-phenyl-2-propenoate; Benzyl 3-phenyl propenoate; Benzyl alcohol cinnamic ester; Cinnamic acid, benzyl ester; Benzyl beta-phenyl acrylate

$C_{16}H_{14}O_2$

238.29



Melting Point (°C)	37-39	Specific Gravity	--
Boiling Point (°C)	195-200 at 5 mm Hg	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	GD8400000
Comments	Pale yellow crystals		

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
Benzyl cinnamate, 99%
Contact Dermatitis Home Page
Information about this particular compound
JICST Mass Spectral Database
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
Proton NMR Spectral Molecular Formula Index
Information about this particular compound
The Good Scents Company
Information about this particular compound

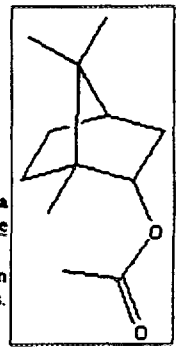
Return to searching

Bornyl acetate [76-49-3]

Synonyms: endo-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl acetate

$C_{12}H_{20}O_2$

196.29



This picture is a live chemical image

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Vaporization Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--

Comments: Colorless crystals

More information about this compound is available at:

Dielectric Constant Reference Guide
The Good Scents Company
- Information about this particular compound
- Information about this particular compound

USEPA / OPP's Chemical Ingredients Database
- Information about this particular compound

Return to searching

caryophyllene [87-44-5]

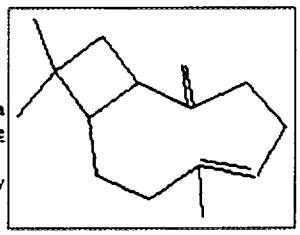
Synonyms: beta-caryophyllene; Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-; bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (E)-(1R,9S)-(-)-; bicyclo[7.2.0]undec-4-ene, 8-methylene-4,11,11-trimethyl-, (E)-(1R,9S)-(-)-; trans-caryophyllene; 1-caryophyllene; (-)-beta-caryophyllene; (-)-caryophyllene; (-)-trans-caryophyllene; 8-methylene-4,11,11-(trimethyl)bicyclo[7.2.0]undec-4-ene; 2-Methylene-6,10,10-trimethyl bicyclo[7.2.0]undec-5-ene

$C_{15}H_{24}$

204.35

This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?



Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	129-130	Vapor Density	--
Evaporation Rate	--	Water Solubility	
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	DT8400000
Comments	Oily liquid		

More information about this compound is available from

CyberMol collection of molecules in VRML format
- Information about this particular compound

Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
- Information about this particular compound

Flavornet
- Information about this particular compound

NTP Chemical Health and Safety Data
- Information about this particular compound

The Good Scents Company
- Information about this particular compound
- Information about this particular compound
- Information about this particular compound
- Information about this particular compound

UWI-Mona Chemistry Lectures
- This compound in PDB format
- Information about this particular compound
- Information about this particular compound

Return to searching

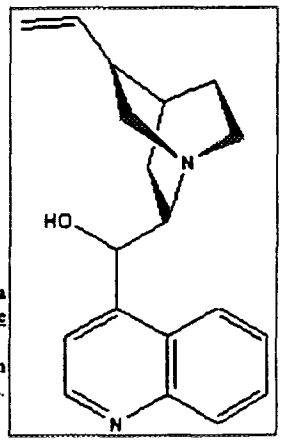
Cinchonine

[118-10-5]

Synonyms: Cinchonan-9-ol; (9S)-cinchonan-9-ol

$C_{19}H_{22}N_2O$

294.40



This picture is a live chemical image

This database is included in CS ChemOffice Ultra for Windows.

Why don't you get your own personal copy?

Melting Point (°C)	258-260	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	GD3500000
Comments	LIGHT SENSITIVE.		

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
Cinchonine, 99%(titr.)
ChemFinder (Macintosh) WebServer
Information about this particular compound
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound
MSDS archive at the University of Utah
Information about this particular compound

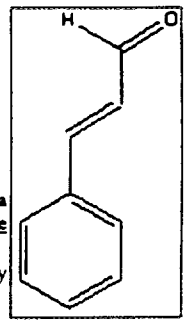
Return to searching

Cinnamaldehyde [104-55-2]

Synonyms: 3-Phenyl-2-propenal; Cinnamic aldehyde; 2-Propenal-3-phenyl; Cinnamal; Phenylacrolein; cassia aldehyde; 3-phenylpropenal; cinnamyl aldehyde; 3-phenylacrolein; benzylideneacetaldehyde; 3-phenyl-2-propenaldehyde; zimtaldehyde; 3-phenylacryaldehyde; Phenyl-2-propenal; Zimtaldehyde light; 3-Phenyl-2-propen-1-al

C_9H_8O

132.16



Melting Point (°C)	-7.5	Specific Gravity	1.05
Boiling Point (°C)	251	Vapor Density	4.5
Evaporation Rate	--	Water Solubility	Slightly soluble
Flash Point (°C)	71	EPA Code	--
DOT Number	--	RTECS	GD6475000

Comments: cinnamon tree bark, spice. Yellow oily liquid.

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
trans-Cinnamaldehyde, p.a.
Berkeley Smells Database
Information about this particular compound
Contact Dermatitis Home Page
Information about this particular compound
CyberMol collection of molecules in VRML format
Information about this particular compound
Dielectric Constant Reference Guide
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound
Galactic Industries Corporation Spectral Database
FTIR SPECTRUM of CINNAMALDEHYDE
JICST Mass Spectral Database
Information about this particular compound
Ligand Chemical Database for Enzyme Reactions
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
NTP Chemical Health and Safety Data
Information about this particular compound
OSHA Chemical Sampling and Methods
Information about this particular compound

Citral [5392-40-5]

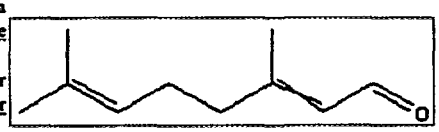
Synonyms: Neral; 3,7-Dimethyl-2,6-octadienal; Geranial; Citral A; geranal; geranialdehyde; trans-3,7-Dimethyl-2,6-octadienal; cis-Citral; cis-3,7-Dimethyl-2,6-octadienal; Dimethyl-2,6-octadienal; citral-b; Lemarome n; cis/trans-3,7-Dimethyl-2,6-octadienal

$C_{10}H_{16}O$

152.24

This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures



Melting Point (°C)	--	Specific Gravity	0.895
Boiling Point (°C)	229	Vapor Density	--
Evaporation Rate	--	Water Solubility	0.1-1 mg/mL at 18 C
Flash Point (°C)	101	EPA Code	--
DOT Number	--	RTECS	RG5075000
Comments	A component of lemon grass oil. Mobile, pale yellow liquid		

More information about this compound is available from

8(e) TRIAGE Chemical Studies Database
Acros Chemicals Catalog (with MSDSs)
Citral, 95%, mixture of cis and trans
Berkeley Smells Database
Information about this particular compound
Biodegradation and Bioaccumulation. Data of Existing Chemicals based on the CSCL, Japan (MITI)
Information about this particular compound
ChemFinder (Macintosh) WebServer
Information about this particular compound
Information about this particular compound
CyberMol collection of molecules in VRML format
Information about this particular compound
Information about this particular compound
Existing Chemicals: Literature Reviews and Evaluations
Information about this particular compound
Flavornet
Information about this particular compound
Information about this particular compound
Hyperreal Drugs Archive
Information about this particular compound
JICST Mass Spectral Database
Information about this particular compound
Ligand Chemical Database for Enzyme Reactions
Information about this particular compound
NFPA Chemical Hazard Labels
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
NTP Chemical Health and Safety Data

Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1alpha,2beta,5alpha)-

[89-78-1]

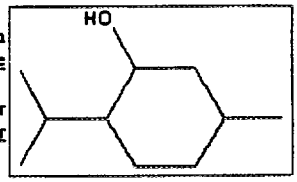
Synonyms: Menthol; 3-p-Menthanol

$C_{10}H_{20}O$

156.27

This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures



Melting Point (°C)	45	Specific Gravity	0.89
Boiling Point (°C)	212	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

BUA List of Existing Chemicals of Environment Relevance, incl. 1st and 2nd Priority Lists (Germany)
Information about this particular compound
California EPA List of Lists
ChemFinder (Macintosh) WebServer
Information about this particular compound
Critical Properties of Various Gases
CyberMol collection of molecules in VRML format
Information about this particular compound
Dielectric Constant Reference Guide
GESAMP List of Substances Carried by Ships
Information about this particular compound
National Toxicology Program (NTP) publications
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
Organic Compounds Database
Proposed list of medicines that may be taken by competeting sportsmen
Some molecular models in .pdb form
This compound in PDB format

Return to searching

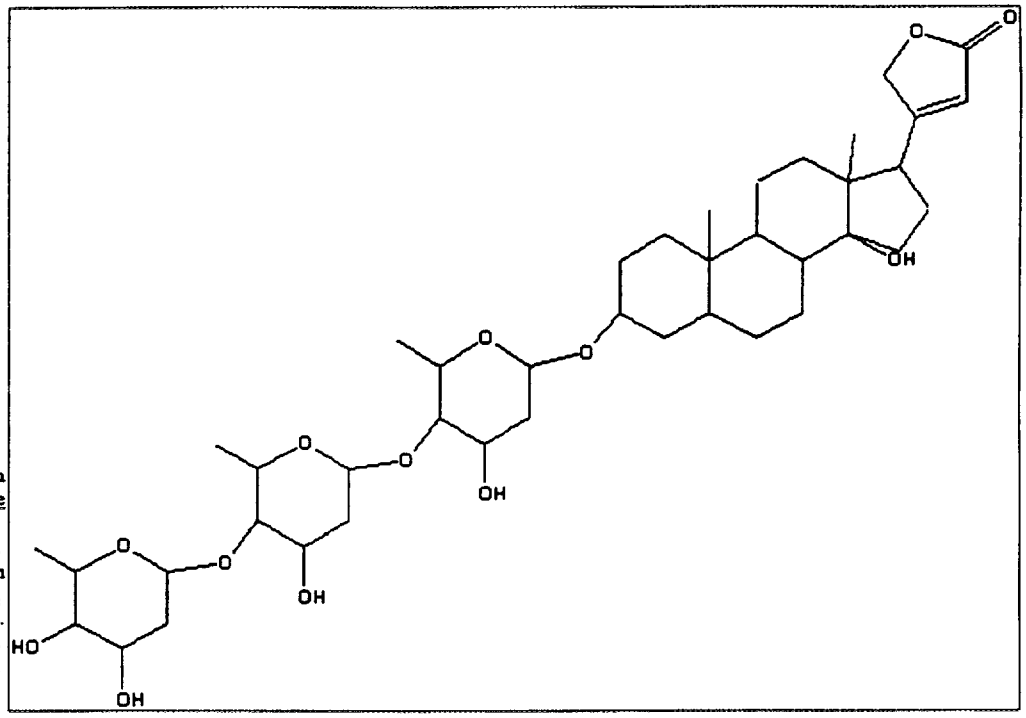
Digitoxin

Synonyms:

(3beta,5beta)-3-[(0-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1->4)-O-2,6-dideoxy-beta-D-ribo-hexo
Crystodigin; Digifortis; Card-20(22)-enolide,
3-[(O-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1.fwdarw.4)-O-2,6-dideoxy-beta-D-ribo-hexopyrano

$C_{41}H_{64}O_{13}$

764.95



Melting Point (°C)	228-230	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	IH2275000
Comments			

More information about this compound is available from

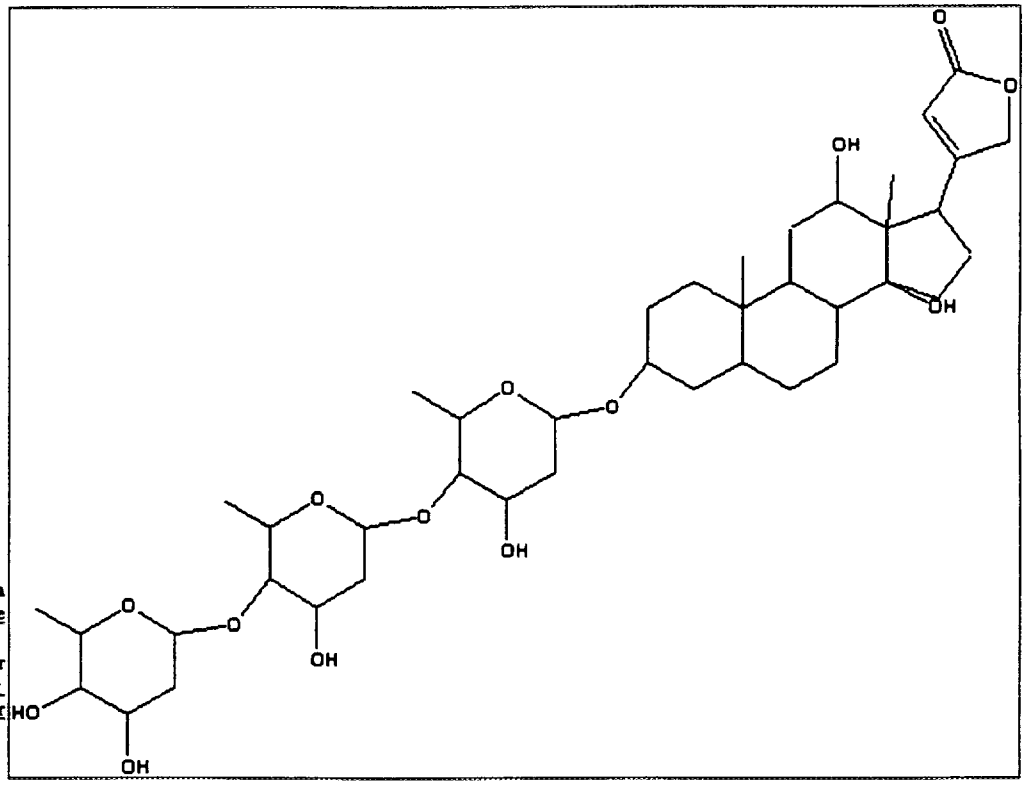
Acros Chemicals Catalog (with MSDSs)
Digitoxin, 99%
Australian Hazardous Substances Database
Information about this particular compound
California EPA List of Lists
Database for 3D Structures of drugs
Information about this particular compound
Drug brand name/generic name listing
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound
Florida Substance List
List of Dangerous Substances (EEC)
Information about this particular compound

Digoxin

Synonyms: Lanoxicaps; Lanoxin;
(3beta,5beta,12beta)-3-[(O-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1->4)-O-2,6-dideoxy-beta-D-rib
SK-Digoxin; Card-20(22)-enolide, 3-[(O-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1.fwdarw.4)-O-2,
(3beta,5beta,12beta)-;
3beta-((O-2,6-dideoxy-beta-D-Ribo-hexopyranosyl-(1rightarrow4)-O-2,6-dideoxy-beta-D-Ribo-hexo

$C_{41}H_{64}O_{14}$

780.95



This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures

Melting Point (°C)	248-250	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	IH6125000
Comments	CARDIAC GLYCOSIDE; INOTROPIC		

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
Digoxin, 95% (on dried substance)
California EPA List of Lists
CSMC On-Line Reference to Neonatal Medications
Information about this particular compound
Cutaneous Drug Reaction Database
Information about this particular compound
Database for 3D Structures of drugs
Information about this particular compound
Drug brand name/generic name listing
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion

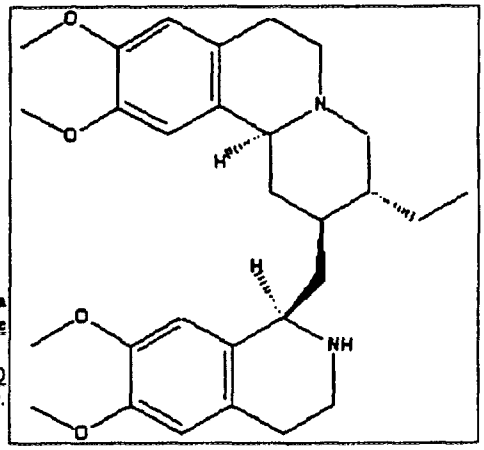
Emetine

[483-18-1]

Synonyms:

$C_{29}H_{40}N_2O_4$

480.65



This picture is a live chemical image

CS ChemOffice Pro 4.0 started shipping in Feburary. Are you using an older version?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--

Comments: Barash, Chem. Ind., 1958, 490.

More information about this compound is available from

ChemFinder (Macintosh) WebServer
Information about this particular compound
National Toxicology Program (NTP) publications
Information about this particular compound
Rain Forest Drugs
UMCP Partial list of mutagens

Return to searching

emodin [518-82-1]

Synonyms: 9,10-anthracenedione, 1,3,8-trihydroxy-6-methyl-; 6-methyl-1,3,8-trihydroxyanthraquinone; emodol; frangula emodin; persian berry lake; rheum emodium; schuttgelb; C.I. 75440; C.I. natural yellow 14; 1,3,8-Trihydroxy-6-methylanthraquinone

$C_{15}H_{10}O_5$

270.24

Melting Point (°C)	253	Specific Gravity	--
Boiling Point (°C)	(subl)	Vapor Density	--
Evaporation Rate	--	Water Solubility	
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	CB7920600
Comments	Orange needles		

More information about this compound is available from

CHEMICALS STUDIED through NIEHS's Reproductive Toxicology Group
NTP Chemical Health and Safety Data
Information about this particular compound
Web Molecules (in VRML)
Information about this particular compound

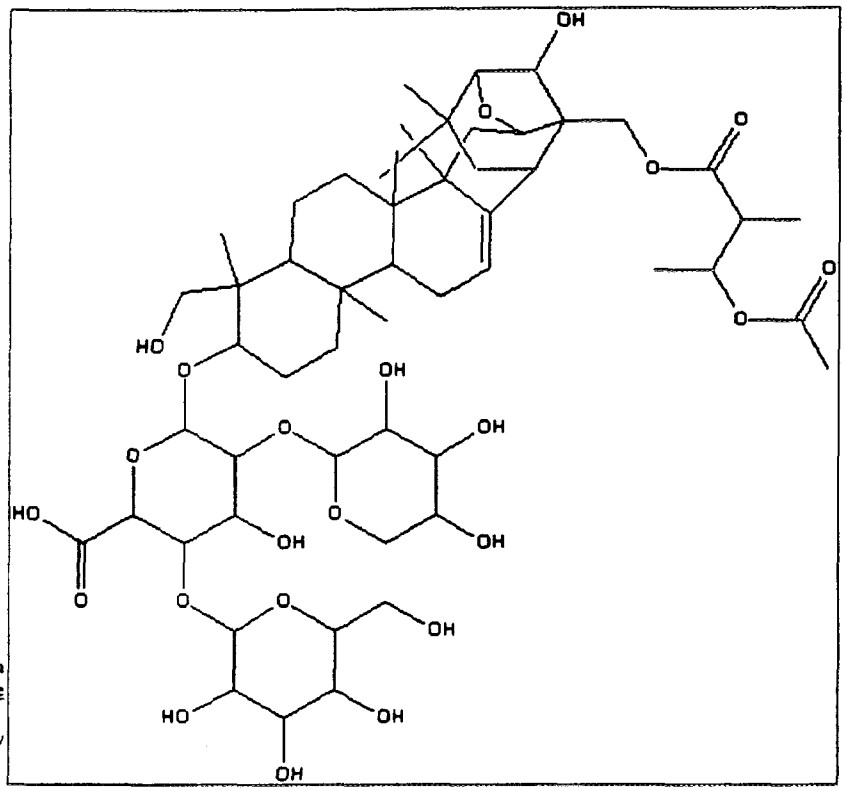
Return to searching

Escin

Synonyms:

$C_{54}H_{84}O_{23}$

1101.2



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

UMCP Partial list of teratogens

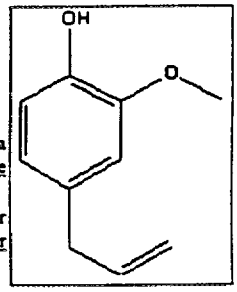
Return to searching

Eugenol [97-53-0]

Synonyms: 2-Methoxy-4-(2-propenyl)phenol; 1-Allyl-3-methoxy-4-hydroxybenzene; 2-Methoxy-4-allylphenol; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; Allylguaiacol; Caryophyllic acid; Eugenic acid; 4-allylcatechol-2-methyl ether; 4-allyl-1-hydroxy-2-methoxybenzene; 1-hydroxy-2-methoxy-4-prop-2-enylbenzene; 2-methoxy-4-prop-2-enylphenol; p-eugenol; 1,3,4-eugenol; 1-hydroxy-2-methoxy-4-allylbenzene; FA 100; fema no. 2467; 4-hydroxy-3-methoxyallylbenzene; 2-methoxy-1-hydroxy-4-allylbenzene; 1-allyl-4-hydroxy-3-methoxybenzene; 5-allylguaiacol; 1-hydroxy-4-allyl-2-methoxybenzene; 1-hydroxy-2-methoxy-4-propenylbenzene; 2-methoxy-4-(2-propen-1-yl)phenol; Allyl-2-methoxyphenol

$C_{10}H_{12}O_2$

164.20



This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures

Melting Point (°C)	15.44	Specific Gravity	1.066
Boiling Point (°C)	487 at 0 mm	Vapor Density	--
Evaporation Rate	--	Water Solubility	
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	SJ4375000

Comments: bay leaves, cloves. Clear, colorless or pale yellow liquid. AIR SENSITIVE.

More information about this compound is available from

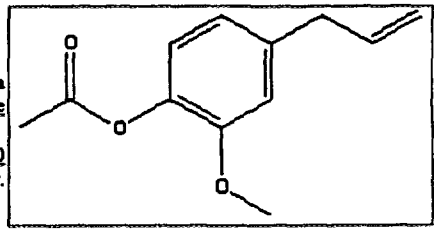
82 structural descriptors for NTP compounds
Acros Chemicals Catalog (with MSDSs)
  Eugenol, 99%
Berkeley Carcinogenic Potency Database
Berkeley Smells Database
  Information about this particular compound
California EPA List of Lists
ChemFinder (Macintosh) WebServer
  Information about this particular compound
Contact Dermatitis Home Page
  Information about this particular compound
CyberMol collection of molecules in VRML format
  Information about this particular compound
Database on Promoters of Chemical Carcinogesis
  Information about this particular compound
Dielectric Constant Reference Guide
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
  Information about this particular compound
Existing Chemicals: Literature Reviews and Evaluations

Eugenyl acetate [93-28-7]

Synonyms: acetyleugenol; 2-Methoxy-4,2-propen-1-yl phenyl acetate; Eugenol acetate; 4-Allyl-2-methoxyphenyl acetate

$C_{12}H_{14}O_3$

206.24



This picture is a live chemical image

CS ChemOffice Pro 4.0 started shipping in Feburary. Are you using an older version?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--

Comments: Colorless to pale yellow liquid

More information about this compound is available from

JICST Mass Spectral Database
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
Proton NMR Spectral Molecular Formula Index
Information about this particular compound
The Good Scents Company
Information about this particular compound
Information about this particular compound
Information about this particular compound
Information about this particular compound

Return to searching

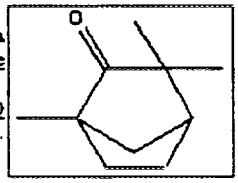
Fenchone [1195-79-5]

Synonyms: 1,3,3-trimethylbicyclo[2.2.1]heptan-2-one; L-1,3,3-Trimethyl-2-norbornanone

$C_{10}H_{16}O$

152.24

This picture is a live chemical image



Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

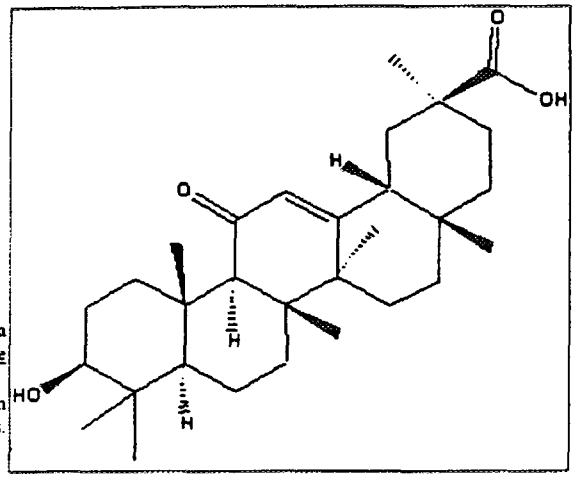
ATSDR Internet HazDat Site Contaminant Query
Information about this particular compound
Dielectric Constant Reference Guide
HLB numbers for surfactants and for emulsification of oils and waxes
JICST Mass Spectral Database
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
Proton NMR Spectral Molecular Formula Index
Information about this particular compound

Return to searching

Glycyrrhetinate [1449-05-4]

Synonyms:

$C_{30}H_{46}O_4$
470.69



This picture is a live chemical image

This database is included in CS ChemOffice Ultra for Windows.

Why don't you get your own personal copy?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from:

Ligand Chemical Database for Enzyme Reactions
Information about this particular compound

Return to searching

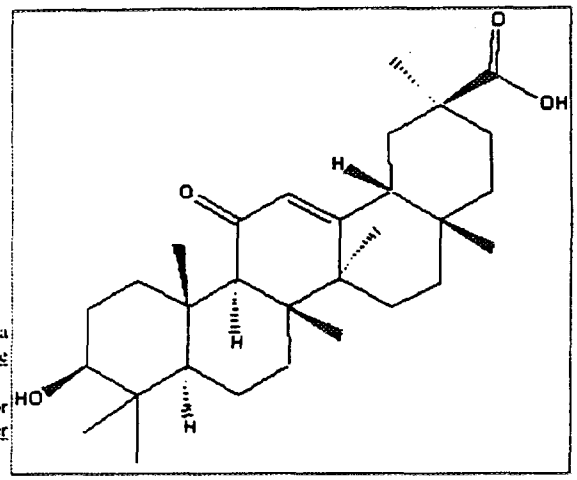
Glycyrrhetinic acid

[471-53-4]

Synonyms: 18-beta-Glycyrrhetinic acid; Enoxolone

$C_{30}H_{46}O_4$

470.69



This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures

Melting Point (°C)	292-295	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	RK0180000
Comments			

More information about this compound is available from:

Acros Chemicals Catalog (with MSDSs)
18-beta-Glycyrrhetinic acid, 99%
Berkeley Carcinogenic Potency Database
Database for 3D Structures of drugs
Information about this particular compound
Database on Promoters of Chemical Carcinogesis
Information about this particular compound
Ligand Chemical Database for Enzyme Reactions
Information about this particular compound

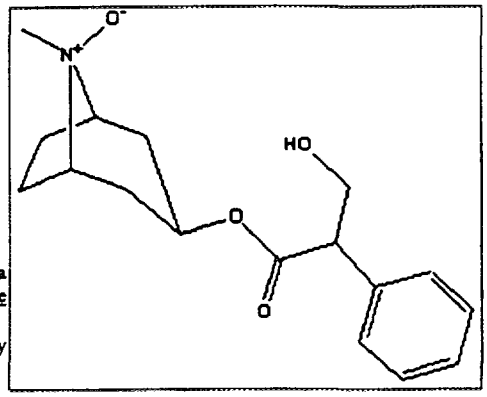
Return to searching

Hyoscine [51-34-3]

Synonyms: Scopolamine; transderm-SCOP; Murocoll; Plexonal; Scopoderm

$C_{17}H_{23}NO_4$

305.37



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--

Comments: Anticholintergic/antispasmodic. Sedative

More information about this compound is available from

Australian Hazardous Substances Database
Information about this particular compound
Cutaneous Drug Reaction Database
Information about this particular compound
Drug brand name/generic name listing
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound
Ligand Chemical Database for Enzyme Reactions
Information about this particular compound
List of Dangerous Substances (EEC)
Information about this particular compound
MedChem CLogP values for some drugs
Information about this particular compound
Nootropics bibliography
Proposed list of medicines that may be taken by competeting sportsmen
Rain Forest Drugs
UMCP Partial list of teratogens

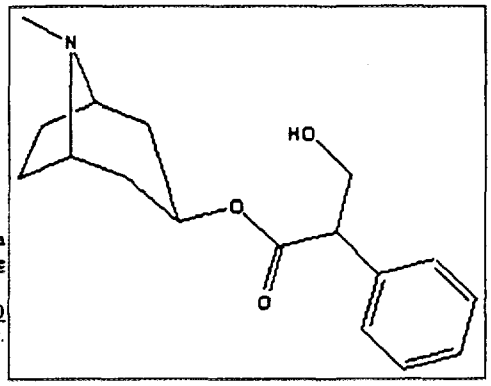
Return to searching

Hyoscyamine [101-31-5]

Synonyms: Benzeneacetic acid, alpha-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, [3(S)-endo]-; L-Hyoscyamine; L-Tropine tropate; Daturine; Duboisine

$C_{17}H_{23}NO_3$

289.37



This picture is a live chemical image

CS ChemOffice Pro 4.0 started shipping in Feburary. Are you using an older version?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--

Comments: Anticholinergic

More information about this compound is available from

Australian Hazardous Substances Database
Information about this particular compound
Ligand Chemical Database for Enzyme Reactions
Information about this particular compound
List of Dangerous Substances (EEC)
Information about this particular compound
Rain Forest Drugs
Web Molecules (in VRML)
Information about this particular compound

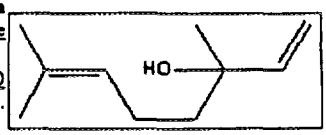
Return to searching

linalool [78-70-6]

Synonyms: 3,7-Dimethyl-1,6-octadien-3-ol; 3,7-Dimethylocta-1,6-dien-3-ol; Dimethyl-1,6-octadien-3-ol; 2,6-Dimethylocta-2,7-dien-6-ol; Linalool ex orange oil; Linalool ex bois de rose oil; Linalool ex ho oil; Linalol; (+/-)-Linalool

$C_{10}H_{18}O$

154.25



Melting Point (°C)	--	Specific Gravity	0.868
Boiling Point (°C)	199	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	75	EPA Code	--
DOT Number	--	RTECS	RG5775000

Comments: One of the principal components of bergamot or french lavender. Colorless liquid

---

More information about this compound is available from

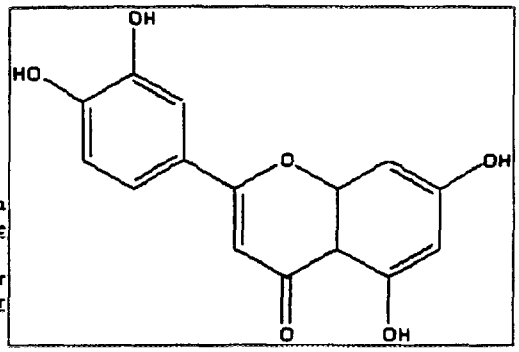
8(e) TRIAGE Chemical Studies Database
Acoustic Material Property Tables
  Information about this particular compound
Acoustic properties of liquids
  Information about this particular compound
Acros Chemicals Catalog (with MSDSs)
  Linalool, 97%
California EPA List of Lists
ChemFinder (Macintosh) WebServer
  Information about this particular compound
CyberMol collection of molecules in VRML format
  Information about this particular compound
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
  Information about this particular compound
Existing Chemicals: Literature Reviews and Evaluations
  Information about this particular compound
Flavornet
  Information about this particular compound
Galactic Industries Corporation Spectral Database
  FTIR SPECTRUM of LINALOOL
  FTIR SPECTRUM of (+/-)-LINALOOL, 97%
Ligand Chemical Database for Enzyme Reactions
  Information about this particular compound
NFPA Chemical Hazard Labels
  Information about this particular compound
NIST Chemistry WebBook
  Information about this particular compound
Proton NMR Spectral Molecular Formula Index

Luteolin [491-70-3]

Synonyms: 3',4',5,7-Tetrahydroxyflavone

$C_{15}H_{12}O_6$

288.26



This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
Information about this particular compound

Ligand Chemical Database for Enzyme Reactions
Information about this particular compound

Return to searching

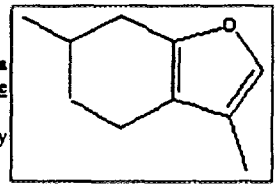
Menthofuran [494-90-6]

Synonyms:

$C_{10}H_{14}O$
150.22

This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?



Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	87 at 15 mm Hg	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
Menthofuran, 95% (GC)

Return to searching

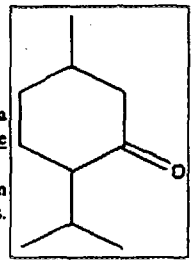
Menthone

[10458-14-7]

Synonyms: 5-Methyl-2-(1-methylethyl)cyclohexanone

$C_{10}H_{18}O$

154.25



This picture is a live chemical image

This database is included in CS ChemOffice Ultra for Windows.

Why don't you get your own personal copy?

Melting Point (°C)	-6	Specific Gravity	0.896
Boiling Point (°C)	207	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	69	EPA Codes	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from:

Acros Chemicals Catalog (with MSDSs)
Menthone, 90+ %, mixture of isomers
Flavornet
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound

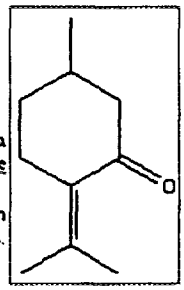
Return to searching

Pulegone [89-82-7]

Synonyms: Delta-4,8-p-menthen-3-one; 1-Isopropylidene-4-methyl-2-cyclohexanone; 1-Methyl-4-isopropylidene-3-cyclohexanone; (+)-4(8)-para-Menthen-3-one

$C_{10}H_{16}O$

152.24



This picture is a live chemical image

This database is included in CS ChemOffice Ultra for Windows.

Why don't you get your own personal copy?

Melting Point (°C)	--	Specific Gravity	0.93
Boiling Point (°C)	97 at 12 mm Hg	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	OT0261000

Comments: One of the chemicals that gives pennyroyal oil its odor. Colorless oily liquid

More information about this compound is available from:

Acros Chemicals Catalog (with MSDSs)
Pulegone, pract., 88% (GC)
ChemFinder (Macintosh) WebServer
Information about this particular compound
Dielectric Constant Reference Guide
JICST Mass Spectral Database
Information about this particular compound
NIST Chemistry WebBook
Information about this particular compound
Proton NMR Spectral Molecular Formula Index
Information about this particular compound
The Good Scents Company
Information about this particular compound
Information about this particular compound
Information about this particular compound
Information about this particular compound
Information about this particular compound
Web Molecules (in VRML)
Information about this particular compound

Return to searching

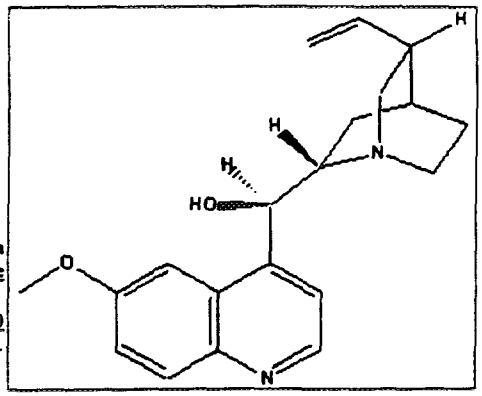
Quinine

[130-95-0]

Synonyms: Legatrin; Quin-260; Quin-amino; Quinamm; Quindan; Quiphile; Q-VEL; 6'-Methoxycinchonan-9-ol; 6'-Methoxychinchonan-9-ol sulfate (2:1) (salt); Novoquinine; Strema; 6-Methoxy-alpha-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol; Cinchonan-9-ol, 6'-methoxy-, (8alpha,9R)-

$C_{20}H_{24}N_2O_2$

324.42



This picture is a live chemical image

CS ChemOffice Pro 4.0 started shipping in Feburary.

Are you using an older version?

Melting Point (°C)	177 (dec)	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	VA6020000

Comments: Antipyretic. Antimalarial. Neuromuscular. LIGHT SENSITIVE.

More information about this compound is available from

ChemFinder (Macintosh) WebServer
- Information about this particular compound

Cutaneous Drug Reaction Database
- Information about this particular compound

Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
- Information about this particular compound

Hyperreal Drugs Archive
- Information about this particular compound

Introduction to Insecticides

MedChem CLogP values for some drugs
- Information about this particular compound

NLM AIDSDRUGS
- This compound in MDL Molfile format

Rain Forest Drugs

Some molecular models in .pdb form
- This compound in PDB format

The Dextromethorphan FAQ
- P450 Inhibiting Drugs

UMCP Partial list of mutagens

UMCP Partial list of teratogens

Web Molecules (in VRML)

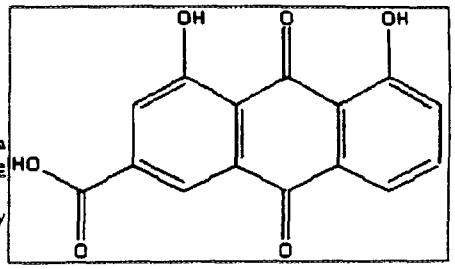
Rhein

[478-43-3]

Synonyms: 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthracenecarboxylic acid; 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthroic acid; cassic acid; chrysazin-3-carboxylic acid; 1,8-dihydroxyanthraquinone-3-carboxylic acid; monorhein; rheic acid; rhubarb yellow; 4,5-dihydroxy-2-anthraquinonecarboxylic acid; 1,8-dihydroxy-3-carboxyanthraquinone

$C_{15}H_8O_6$

284.22



Melting Point (°C)	321	Specific Gravity	--
Boiling Point (°C)	(subl)	Vapor Density	--
Evaporation Rate	--	Water Solubility	
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments	Yellow needles (in methanol)		

More information about this compound is available from

NTP Chemical Health and Safety Data
Information about this particular compound
Web Molecules (in VRML)
Information about this particular compound

Return to searching

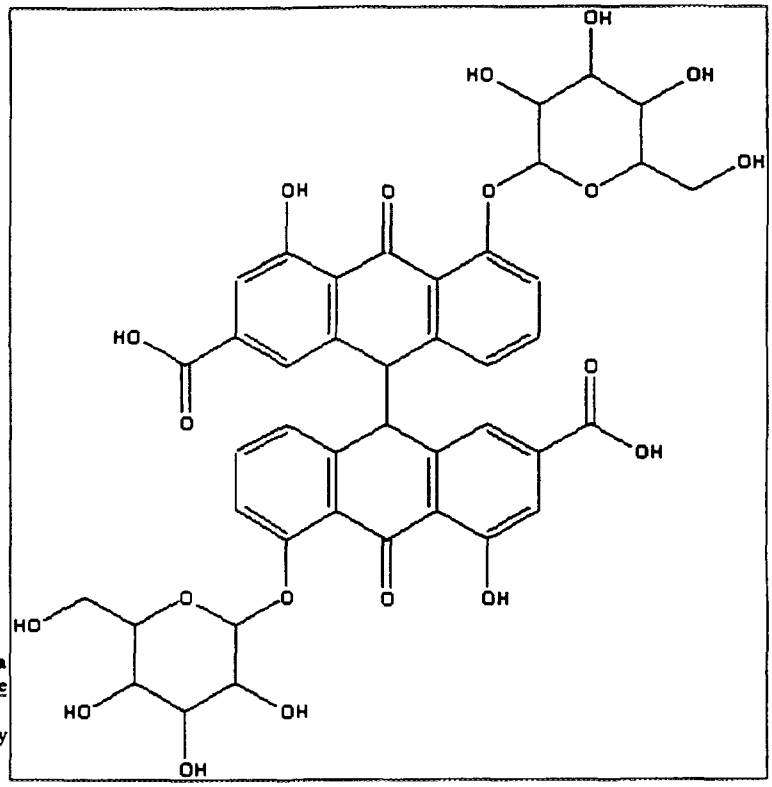
Sennoside A

[81-27-6]

Synonyms:

$C_{42}H_{38}O_{20}$

862.75



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Return to searching

Tannic acid [1401-55-4]

Synonyms: Gallotannic acid; Gallotannin; Tannin; Quebracho; Tannins; Galloylglucose.; Chinese tannin; Glycerite; Penta NM digalloyl glucose

Melting Point (°C)	210	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	198	EPA Code	--
DOT Number	--	NIOSH	WW5075000
Comments	LIGHT SENSITIVE; AIR SENSITIVE.		

More information about this compound is available from ...

Acros Chemicals Catalog (with MSDSs)
- Tannic acid, reagent ACS, powder
- Tannic acid, 95%

ATSDR Internet HazDat Site Contaminant Query
- Information about this particular compound

ChemFinder (Macintosh) WebServer
- Information about this particular compound

Database for 3D Structures of drugs
- Information about this particular compound

Database on Promoters of Chemical Carcinogesis
- Information about this particular compound
- Information about this particular compound
- Information about this particular compound
- Information about this particular compound

DuPont TYVEK® Protective Apparel Information Service
- Information about this particular compound

Existing Chemicals: Literature Reviews and Evaluations
- Information about this particular compound
- Information about this particular compound

Fisher Chemical Catalog (with MSDSs)
- Tannic Acid

Genium's Chemical Container Label Database
- Information about this particular compound

GESAMP List of Substances Carried by Ships
- Information about this particular compound
- Information about this particular compound
- Information about this particular compound

Gloves compatibility info

Guide to NIOSH/OSHA Air Sampling Methods
- Information about this particular compound

IARC (International Agency of Research on Cancer) Database
- Information about this particular compound

IARC Carcinogens
- Information about this particular compound
- Information about this particular compound

IARC Evaluations of Carcinogenicity to Humans

MSDS archive at the University of Utah

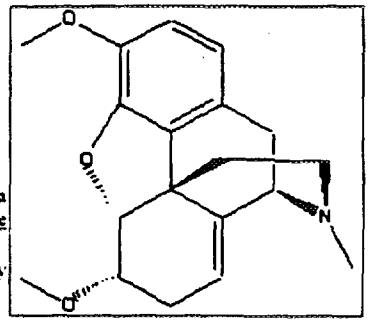
Thebaine

[115-37-7]

Synonyms: Paramorphine

$C_{19}H_{23}NO_3$

313.40



This picture is a live chemical image

Have you downloaded your copy of CS ChemOffice Net yet?

Melting Point (°C)	--	Specific Gravity	--
Boiling Point (°C)	--	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	--	EPA Code	--
DOT Number	--	RTECS	--
Comments			

More information about this compound is available from

Hyperreal Drugs Archive

- Information about this particular compound
- Information about this particular compound
- Information about this particular compound

Return to searching

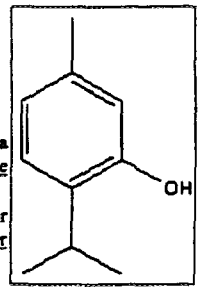
Thymol

[89-83-8]

Synonyms: 6-Isopropyl-m-cresol; 3-Hydroxy-p-cymene; Isopropyl cresol; 5-Methyl-2-(1-methylethyl)phenol; Methyl-2-(1-methylethyl)phenol; Methyl-2-isopropyl-1-phenol; 3-p-Cymenol; 2-Isopropyl-5-methyl phenol

$C_{10}H_{14}O$

150.22



This picture is a live chemical image

Instructions for Configuring your browser to view chemical structures

Melting Point (°C)	48-51	Specific Gravity	0.965
Boiling Point (°C)	233	Vapor Density	--
Evaporation Rate	--	Water Solubility	--
Flash Point (°C)	102	EPA Code	--
DOT Number	--	RTECS	XP2275000

Contents: plant oils,. White crystals

More information about this compound is available from

Acros Chemicals Catalog (with MSDSs)
  Thymol, 99%
Berkeley Smells Database
  Information about this particular compound
BUA List of Existing Chemicals of Environment Relevance, incl. 1st and 2nd Priority Lists (Germany)
  Information about this particular compound
California EPA List of Lists
ChemFinder (Macintosh) WebServer
  Information about this particular compound
Critical Properties of Various Gases
CyberMol collection of molecules in VRML format
  Information about this particular compound
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
  Information about this particular compound
Existing Chemicals: Literature Reviews and Evaluations
  Information about this particular compound
Fisher Chemical Catalog (with MSDSs)
  Thymol
JICST Mass Spectral Database
  Information about this particular compound
MSDS archive at the University of Utah
  Information about this particular compound
NIST Chemistry WebBook
  Information about this particular compound

Valeric Acid [109-52-4]

Synonyms: n-Pentanoic Acid; Butanecarboxylic Acid; pentanoic acid; n-Valeric Acid; 1-butanecarboxylic acid; propylacetic acid; valerianic acid

$C_5H_{10}O_2$

102.13

Melting Point (°C)	-20 to -18	Specific Gravity	0.939
Boiling Point (°C)	186	Vapor Density	--
Evaporation Rate	--	Water Solubility	soluble. 10-50 mg/mL at 22 C
Flash Point (°C)	86	EPA Code	--
DOT Number	NA 1760 Corrosive material	RTECS	YV6100000
Comments	Colorless liquid with unpleasant odor		

More information about this compound is available from

49 CFR Part 172: Hazardous materials shipping requirements
8(e) TRIAGE Chemical Studies Database
ATSDR Internet HazDat Site Contaminant Query
  Information about this particular compound
Australian Hazardous Substances Database
  Information about this particular compound
ChemFinder (Macintosh) WebServer
  Information about this particular compound
Critical Properties of Various Gases
CyberMol collection of molecules in VRML format
  Information about this particular compound
Dielectric Constant Reference Guide
Environmental Science Center database of Experimental Log P coefficients, with Ozone Depletion Potentials and Atmospheric Oxidation Rates
  Information about this particular compound
Existing Chemicals: Literature Reviews and Evaluations
  Information about this particular compound
Flavornet
  Information about this particular compound
Florida Substance List
Galactic Industries Corporation Spectral Database
  FTIR SPECTRUM of PENTANOIC ACID
GESAMP List of Substances Carried by Ships
  Information about this particular compound
  Information about this particular compound
Hazardous Chemicals Database at the University of Akron
  Information about this particular compound
IUPAC Assistant (in French)
  Information about this particular compound

Blühende Baumkrone von *Tabebuia impetiginosa*. Aufnahme von A. H. Gentry, Missouri Botanical Garden.

nen Konzentration isoliert: 0,005 % Dehydro-α-lapachon (= Xyloidon),[12] 0,001 % Deoxylapachol,[10] 0,014 % Lapachenol,[10] 0,002 % Lapacholmethylether,[10] 3,6 % Lapachol (= Tecomin),[11] 0,004 % α-Lapachon,[12] 0,001 % β-Lapachon,[12] Spuren von Lapachonon[11] und 0,001 % 2-Methyl-3-(dimethylallyl)-1,4-naphthochinon (= Menachinon-1).[10]

Verbreitung: *Tabebuia impetiginosa* ist in den tropischen Regenwäldern zwischen Nordmexiko und Argentinien sowie in Brasilien beheimatet.[5]

Anbaugebiete: Nur natürliches Vorkommen.

Drogen: Tabebuiae cortex.

Tabebuiae cortex (Tabebuia-Rinde)

Synonyme: Cortex Tabebuiae.

Sonstige Bezeichnungen: span./port. (lokale Namen): Ipé roxo, Lapacho, Pau d'arco, Taheebo.

Definition der Droge: Die getrocknete ganze oder geschnittene Rinde (vorwiegend der innere Teil der Rinde).

Stammpflanzen: *Tabebuia impetiginosa* (MARTIUS ex DC.) STANDLEY.

Herkunft: Aus tropischen Regenwäldern Südamerikas (Brasilien, Argentinien, Peru). Sammlung aus Wildvorkommen.

Gewinnung: Der Baum wird gefällt, die Rinde wird entfernt und luftgetrocknet.

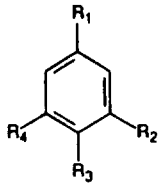
Ganzdroge: Dunkelbraune Rindenstücke, mit face

Schnittdroge: *Geruch.* Aromatisch, nach Vanillin. *Geschmack.* Adstringierend.

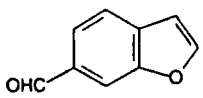
Inhaltsstoffe: Neben den nicht näher beschriebenen Cumarinen und Saponinen[13] sowie dem Flavonoid 4',7-Dihydroxyflavon-7-*O*-rutinosid[16] wurden kürzlich zahlreiche Verbindungen aus der Rinde von *Tabebuia impetiginosa* isoliert und mit Hilfe spektroskopischer Methoden strukturell definiert (Strukturformeln siehe S. 886):[17,18] 0,003 % 2-Acetylnaphtho[2,3-b]furan-4,9-dion, Benzo[b]furan-6-aldehyd (= 6-Formylbenzo[b]furan), 0,007 % (–)-6,8-Dihydroxy-3-methyl-3,4-dihydroisocumarin (= (–)-6-Hydroxymellein), 0,004 % (–)-2,3-Dihydro-2(1'-methylethenyl)naphtho[2,3-b]furan-4,9-dion (= (–)-Dehydro-*iso*-α-lapachon), 0,03 % 3,4-Dimethoxybenzaldehyd (= Veratrumaldehyd), 0,13 % 3,4-Dimethoxybenzoesäure (= Veratrumsäure), 0,003 % 2,2-Dimethylnaphtho[2,3-b]pyran-5,10-dion (= Dehydro-α-lapachon), < 0,001 % 8-Hydroxy-2-acetylnaphtho[2,3-b]furan-4,9-dion, < 0,001 % 5-Hydroxy-2-acetylnaphtho[2,3-b]furan-4,9-dion, 0,001 % 5-Hydroxy-2,3-dihydro-2-(1'-methylethenyl)naphtho[2,3-b]furan-4,9-dion (= 5-Hydroxydehydro-*iso*-α-lapachon), < 0,001 % 2-Hydroxy-3-(3',3'-dimethylallyl)naphtho-1,4-dion (= Lapachol), < 0,001 % (–)-5-Hydroxy-2-(1'-hydroxyethyl) naphtho[2,3-b]furan-4,9-dion, < 0,001 % (±)-8-Hydroxy-2-(1'-hydroxyethyl) naphtho[2,3-b] furan-4,9-dion, 0,006 % (+)-2-(1'-Hydroxyethyl) naphtho[2,3-b]furan-4,9-dion und 0,001 % 3,4,5-Trimethoxybenzoesäure (= Eudesminsäure). 0,02 % 4-Hydroxybenzoesäure, 0,02 % 4-Hydroxy-3-methoxybenzoesäure (= Vanillinsäure), 0,007 % 4-Hydroxy-3-methoxybenzaldehyd (= Vanillin), 0,004 % 4-Methoxybenzaldehyd (= Anisaldehyd) und 0,1 % 4-Methoxybenzoesäure (= Anissäure) wurden ebenfalls in der Rinde von *T. impetiginosa* nachgewiesen und mit Hilfe von Referenzsubstanzen identifiziert.[17,18]

Analytik: DC nach Lit.[17]:
- Stationäre Phase: Kieselgel-Fertigplatten 60 F_{254}, 0,25 mm Schichtdicke;
- Untersuchungslösung: 8 g fein pulverisierte Droge werden 24 h am Soxhlet mit Chloroform extrahiert. Der eingetrocknete Extrakt wird in 1 mL Chloroform aufgenommen und direkt zur dünnschichtchromatographischen Auftrennung verwendet (20 µL pro Spur).
- FM: Toluol-Chloroform-Ameisensäure (5 + 94 + 1);
- Detektion: Vis, UV 254 und 365 nm, Diethylamin (Vis);
- Auswertung: Das beschriebene Trennsystem ermöglicht eine gute Trennung aller Hauptinhaltsstoffe. Die Detektion mit Diethylamin zeigt selektiv die wichtigsten Chinone durch entsprechende Rotfärbung an.

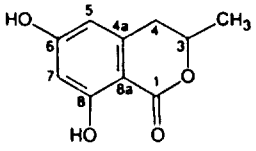
HPLC-Fingerprint-Analyse nach Lit.[17]:
- Stationäre Phase: LiChrosorb RP-18 (7 µm), 250-7;
- Mobile Phase: A: Wasser, B: Acetonitril + 0,1 N Phosphorsäure, Gradient 10 bis 60 % B in 0 bis 30 min



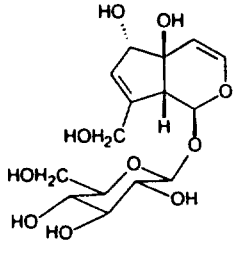
	R_1	R_2	R_3	R_4
p-Hydroxybenzoesäure	—COOH	—H	—OH	—H
Vanillinsäure	—COOH	—OCH_3	—OH	—H
Vanillin	—CHO	—OCH_3	—OH	—H
Veratrumsäure	—COOH	—OCH_3	—OCH_3	—H
Eudesminsäure	—COOH	—OCH_3	—OCH_3	—OCH_3
Veratrumaldehyd	—CHO	—OCH_3	—OCH_3	—H
Anissäure	—COOH	—H	—OCH_3	—H
Anisaldehyd	—CHO	—H	—OCH_3	—H



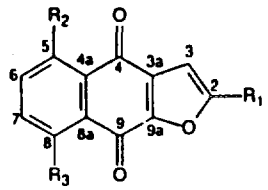
6-Formylbenzo[b]furan

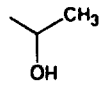
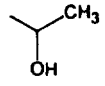
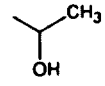
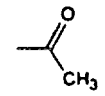
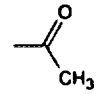
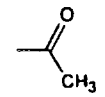


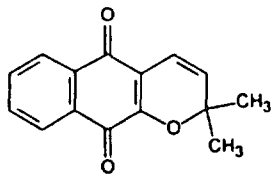
6-Hydroxymellein



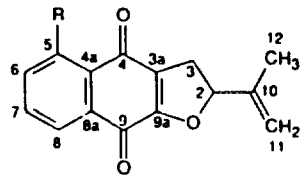
6-Epimonomelittosid



	R_1	R_2	R_3
2-(1'-Hydroxyethyl)-furanonaphthochinon		—H	—H
5-Hydroxy-2-(1'-hydroxyethyl)-furanonaphthochinon		—OH	—H
8-Hydroxy-2-(1'-hydroxyethyl)-furanonaphthochinon		—H	—OH
2-Acetylfuranonaphthochinon		—H	—H
8-Hydroxy-2-acetyl-furanonaphthochinon		—H	—OH
5-Hydroxy-2-acetyl-furanonaphthochinon		—OH	—H

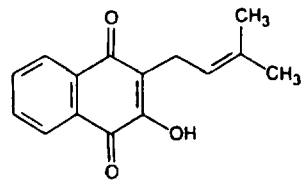


Dehydro-α-lapachon



Dehydro-*iso*-α-lapachon: R = —H

5-Hydroxydehydro-*iso*-α-lapachon: R = —OH

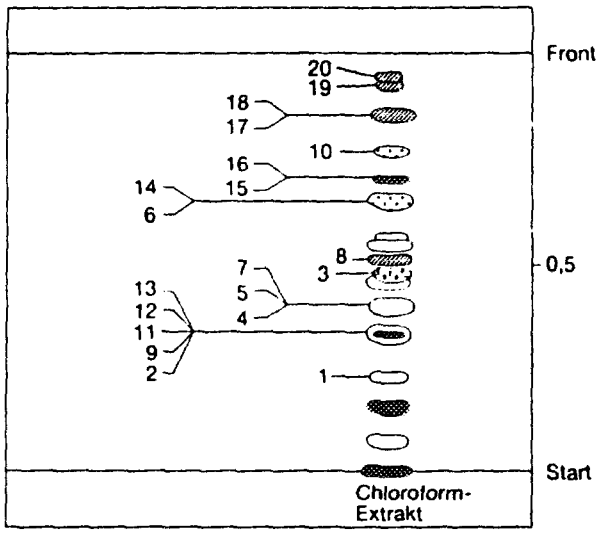


Lapachol

Darstell
nung de
1 *p*-Hy
4 Veratr
7 Anissä
lein, 10
thochin
thochin
hochino
droxy-2-
acetylfu
18 Dehy
lapachon

– Unte
von
Soxh
zur T
nom
den
– Ausv
ermi
inhal
rung
wert

Gehalt
– Stati
7;
– Mob
Phos
30 m
– Dete
– Unte
pulv
den
(Sox
Spitz



gelb (Vis), UV_{254nm} -Löschung

gelb (Vis), UV_{254nm} -Löschung, Rotfärbung mit Diethylamin

nur UV_{254nm} -Löschung

nur UV_{365nm} -Fluoreszenz blau

Darstellung der dünnschichtchromatographischen Trennung der Hauptinhaltsstoffe von Tabebuiae cortex:
1 *p*-Hydroxybenzoesäure, 2 Vanillinsäure, 3 Vanillin, 4 Veratrumsäure, 5 Eudesminsäure, 6 Veratrumaldehyd, 7 Anissäure, 8 6-Formyl-benzo[b]furan, 9 6-Hydroxymellein, 10 Anisaldehyd, 11 2-(1'-Hydroxyethyl)furanonaphthochinon, 12 5-Hydroxy-2-(1'-hydroxyethyl)furanonaphthochinon, 13 8-Hydroxy-2-(1'-hydroxyethyl)furanonaphthochinon, 14 2-Acetylfuranonaphthochinon, 15 8-Hydroxy-2-acetylfuranonaphthochinon, 16 5-Hydroxy-2-acetylfuranonaphthochinon, 17 Dehydro-α-lapachon, 18 Dehydro-*iso*-α-lapachon, 19 Hydroxydehydro-*iso*-α-lapachon, 20 Lapachol.

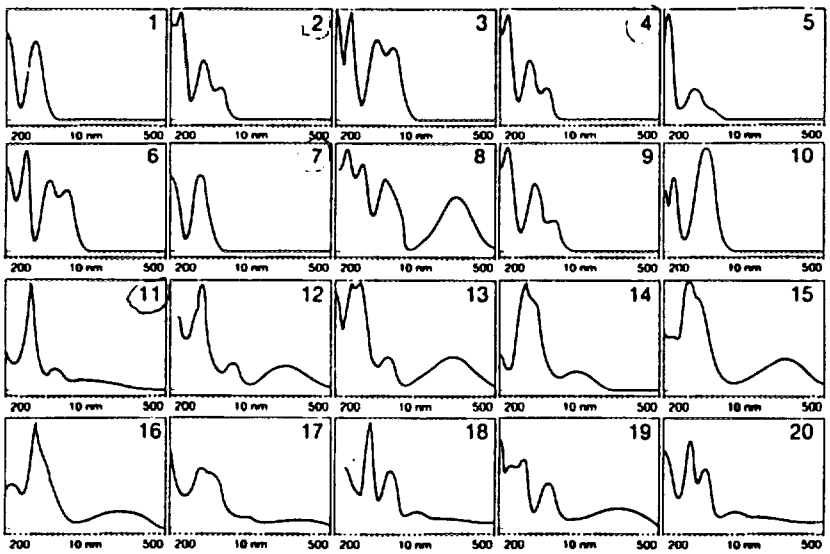
- Untersuchungslösung: 5 g pulverisierte Rinde von *Tabebuia impetiginosa* werden ca. 6 h am Soxhlet mit Chloroform extrahiert, anschließend zur Trockene eingeengt, in 5 mL Methanol aufgenommenen und filtriert. 5 µL dieser Lösung werden direkt zur HPLC-Analyse eingesetzt.
- Auswertung: Das beschriebene Trennverfahren ermöglicht eine sehr gute Trennung aller Hauptinhaltsstoffe sowie eine eindeutige Identifizierung durch Kombination mit On-line-UV-Auswertung.

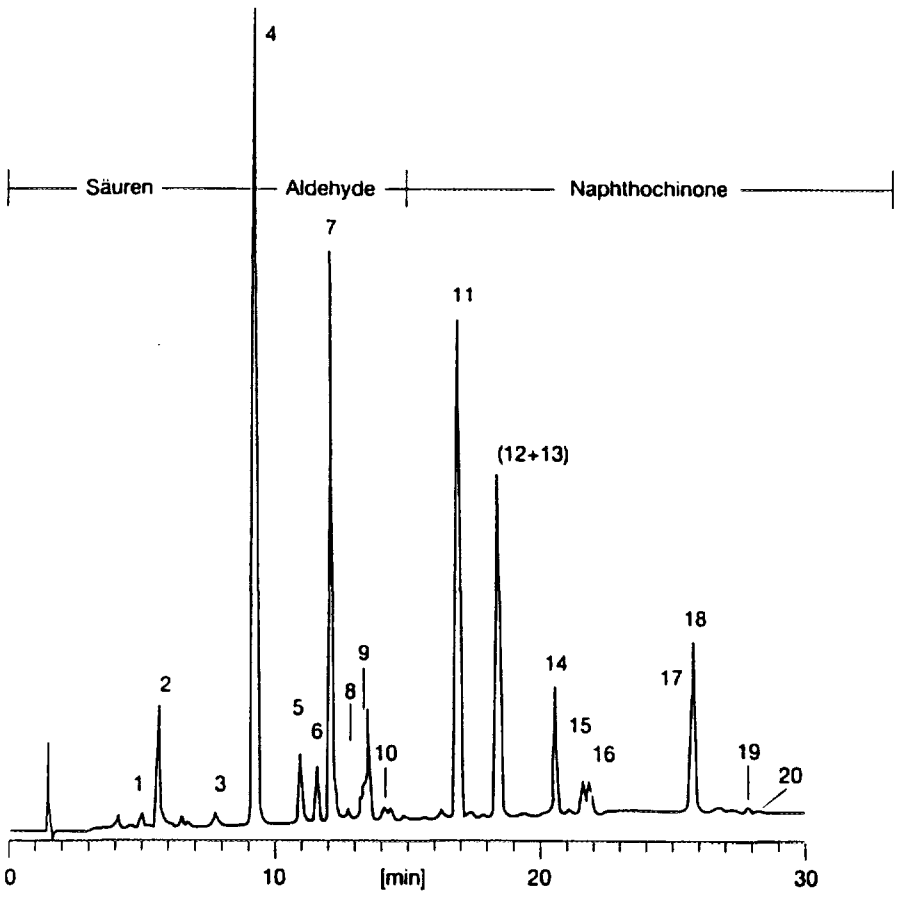
Gehalt: Quantitative HPLC-Analyse nach Lit.[17]:
- Stationäre Phase: LiChrosorb RP-18 (7 µm), 250-7;
- Mobile Phase: A: Wasser, B: Acetonitril + 0,1 N Phosphorsäure, Gradient 10 bis 60 % B in 0 bis 30 min;
- Detektion: UV 254 nm;
- Untersuchungslösung: 1,000 bis 3,000 g fein gepulverte Rinde von *Tabebuia impetiginosa* werden für 48 h mit ca. 50 mL Chloroform extrahiert (Soxhlet). Der erhaltene Extrakt wird in einem schließend 3mal unter leichtem Erwärmen in jeweils 1 mL Acetonitril aufgenommen, durch Watte filtriert und auf ca. 1 mL eingeengt. Diese Lösung wird auf eine mit Acetonitril durchfeuchtete Sep-Pak-Kartusche (C_{18}-Material) gegeben. Es wird mit 10 mL Acetonitril nacheluiert. Die erhaltene Lösung wird eingeengt und in 1 bis 2 mL Methanol aufgenommen (Analysenlösung I). Diese Analysenlösung eignet sich zur genauen Bestimmung der Benzoesäure- und Benzaldehyd-Derivate. Für die Chinonanreicherung engt man 50 bis 80 % der Analysenlösung I (genaue Volumenbestimmung) in einem Spitzkölbchen ein, nimmt anschließend den Trockenrückstand in ca. 1 mL Chloroform auf, filtriert über eine mit Chloroform durchfeuchtete Sep-Pak-Kartusche (Kieselgel) und spült mit 10 mL Chloroform nach. Die erhaltene Lösung wird wiederum in einem kleinen Spitzkölbchen (2 mL) eingeengt und in 300 bis 500 µL Methanol aufgenommen (Analysenlösung II). Zur quantitativen Erfassung der Naphthochinone wird die Analysenlösung II eingesetzt. Die quantitative HPLC-Analyse wird mit Hilfe der externen Standardmethode durchgeführt, wobei als externer Standard jeweils die isolierte Reinsubstanz in einer Konzentration von 1 mg/mL eingesetzt wird. Folgende Einspritzvolumina wurden untersucht:
- Externer Standard: 0,5 bis 5,0 µL * (insgesamt ca. 10 Meßpunkte) * = Lineares Verhalten der Eichgeraden nach Flächenintegration. Der Korrelationskoeffizient lag zwischen 0,998 und 0,98.
- Analysenlösung I: 1 bis 10 µL;
- Analysenlösung II: 5 bis 15 µL.

Um den methodischen Fehler (Anreicherungsverfahren über Sep-Pak-Kartuschen) so klein wie möglich zu halten, wird für jede Referenzsubstanz die Wiederfindungsrate aus der externen Standardlösung bestimmt und berücksichtigt.

Wirkungen: *Antitumorale Wirkung.* Peroral appliziert zeigt Lapachol bei einer Dosierung von 100 mg/kg KG im Yoshida-Sarcoma-Test eine 82 %ige und im Walker 256 Carcino-Sarcoma-Test eine 50 %ige Hemmung. α-Lapachon und Xyloidon (= Dehydro-α-lapachon) sind bis zu einer Dosis von 200 mg/kg KG in beiden Testsystemen unwirksam. β-Lapachon besitzt bei 7 mg/kg KG eine 16,2 %ige Hemmung beim Yoshida-Test und eine 33,5 %ige Hemmung beim Walker-Test. Der lipophile Hexan-Extrakt (Trockenrückstand, Droge:Extrakt-Verhältnis nicht angegeben) von Tabebuiae cortex ist in beiden Testsystemen bei peroraler Applikation von 150 mg/kg KG deutlich wirksamer (85 % bei Yoshida und 80 % bei Walker) als der wäßrige Extrakt bei einer Dosis von 500 mg/kg KG (32 % bei Walker).[19,20] Lapachol zeigt im Ascitic-Sarcoma-180-Test bei Mäusen eine ED_{50} von 141 mg/kg KG.[21] *Hemmung der Reverse Transkriptase.* 8 µmol β-Lapachon (2 µg/mL) hemmen die Reverse Transkriptase von Arian-Myeloblastose-Virus und Rauscher-Leukämie-Virus bei einer 60minütigen Inkubation um 50 %.[22]

Analgetische Wirkung. Im Hot-Plate-Test (50 bis [illegible] Lapachol eine deutlich





Darstellung der Trennung der Hauptinhaltsstoffe aus Tabebuiae cortex durch HPLC-Analyse. Die gezeigten on-line aufgenommenen UV-Spektren ermöglichen eine schnelle Identifizierung dieser Verbindungen.
1 *p*-Hydroxybenzoesäure, 2 Vanillinsäure, 3 Vanillin, 4 Veratrumsäure, 5 Eudesminsäure, 6 Veratrumaldehyd, 7 Anissäure, 8 6-Formyl-benzo[b]furan, 9 6-Hydroxymellein, 10 Anisaldehyd, 11 2-(1'-Hydroxyethyl)furanonaphthochinon, 12 5-Hydroxy-2-(1'-hydroxyethyl)furanonaphthochinon, 13 8-Hydroxy-2-(1'-hydroxyethyl)furanonaphthochinon, 14 2-Acetylfuranonaphthochinon, 15 8-Hydroxy-2-acetylfuranonaphthochinon, 16 5-Hydroxy-2-acetylfuranonaphthochinon, 17 Dehydro-α-lapachon, 18 Dehydro-*iso*-α-lapachon, 19 Hydroxydehydro-*iso*-α-lapachon, 20 Lapachol.

Instrument Method: tabebuiae_cortex

Millennium v2.15

Date Printed: 11:18:53 AM, November 12, 1997

Method Name: tabebuiae_cortex
Date Created: 06/11/97 01:24:22 PM

Solam · ODS Ultrasphere
4,6 x 250 mm
part no 235329
ser. no 5UE226

Channel Information

Channel: 996

Channel Type	3D PDA
Channel Name	996
Det. Units	AU
Description	

Instrument Information

Instrument Type PDA

Instrument Type	PDA
Instrument State	On
Start Wavelength	200.0
End Wavelength	500.0
Spec Resolution	2.4
Autoexposure	On
Exposure Time	15.0
Interpolate 656	Yes
Sample Rate	1.0
Lamp On	Yes
Spectral Filter	1
Use Analog One	Off
Use Analog Two	Off
Use Events	Off
Ch1 Output Mode	Off
Ch1 Offset	0.000
Ch1 Output WL	254.0
Ch1 Output BW	4.8
Ch1 Ratio WL	254.0
Ch1 Ratio TH	0.001
Ch1 Low Ratio	0.001
Ch1 High Ratio	100.000
Ch1 Filter Type	Hamming
Ch1 Filt Resp	none
Ch2 Output Mode	Off
Ch2 Offset	0.000
Ch2 Output WL	254.0
	4.8
Ch2 Output BW	4.8
Ch2 Ratio WL	254.0
Ch2 Ratio TH	0.001
Ch2 Low Ratio	0.001
Ch2 High Ratio	100.000
Ch2 Filter Type	Hamming
Ch2 Filt Resp	none

Table '996 Event Table' contains no data.

Instrument Type W600

Instrument Type	W600
Instrument State	On
Chan Name	600 PRESS
Description	
Use Chan	Off
Monitor	PRESS
Chart	%A
Pump Type	625
Pump Mode	Gradient
Flow	1.50
Percent A	90.0
Percent B	10.0

Percent C	0.0
Percent D	0.0
High Press Limit	4000.0
Low Press Limit	0.0
Sparge Rate	0
Sparge Rate A	Off
Sparge Rate B	Off
Sparge Rate C	Off
Sparge Rate D	Off
Temp Setpoint	0.0
High Temp Limit	25.0
Switch 1	Off
Switch 2	Off
Switch 3	Off
Switch 4	Off
Use Events	Off
Head Volume	50
MS Optimize % A	100.0
MS Optimize % B	0.0
MS Optimize % C	0.0
Optimizing Mass	194.0
%H2O in A	50.0
Silk	Off
Vacuum Degas	Off

Table 'W600 Event Table' contains no data.

W600 Gradient Table

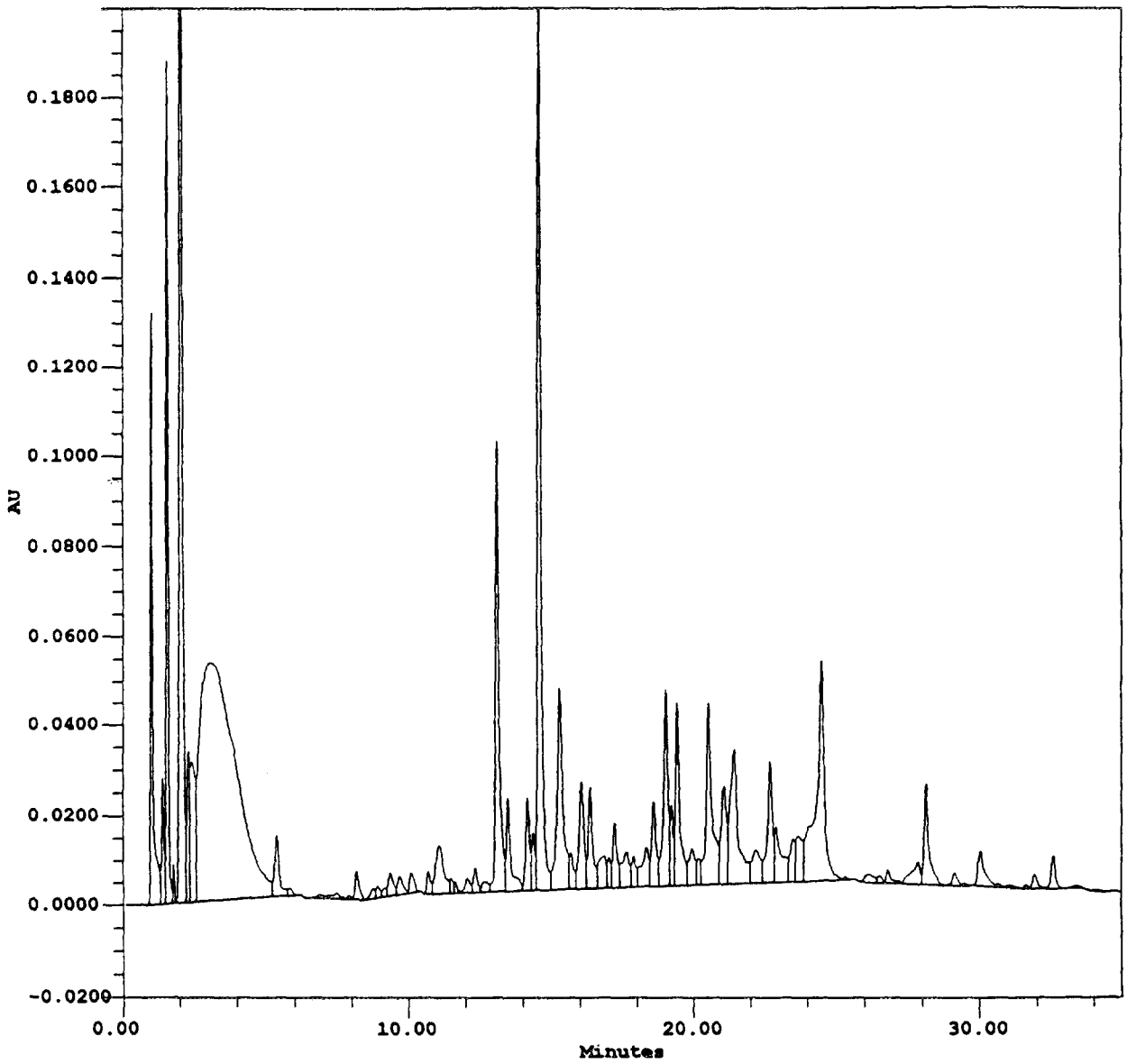
#	Time (min)	Flow (ml)	%A (%)	%B (%)	%C (%)	%D (%)	Curve
1	0.00	1.50	90.0	10.0	0.0	0.0	0
2	30.00	1.50	40.0	60.0	0.0	0.0	6
3	60.00	1.50	40.0	60.0	0.0	0.0	6
4	62.00	1.50	90.0	10.0	0.0	0.0	6

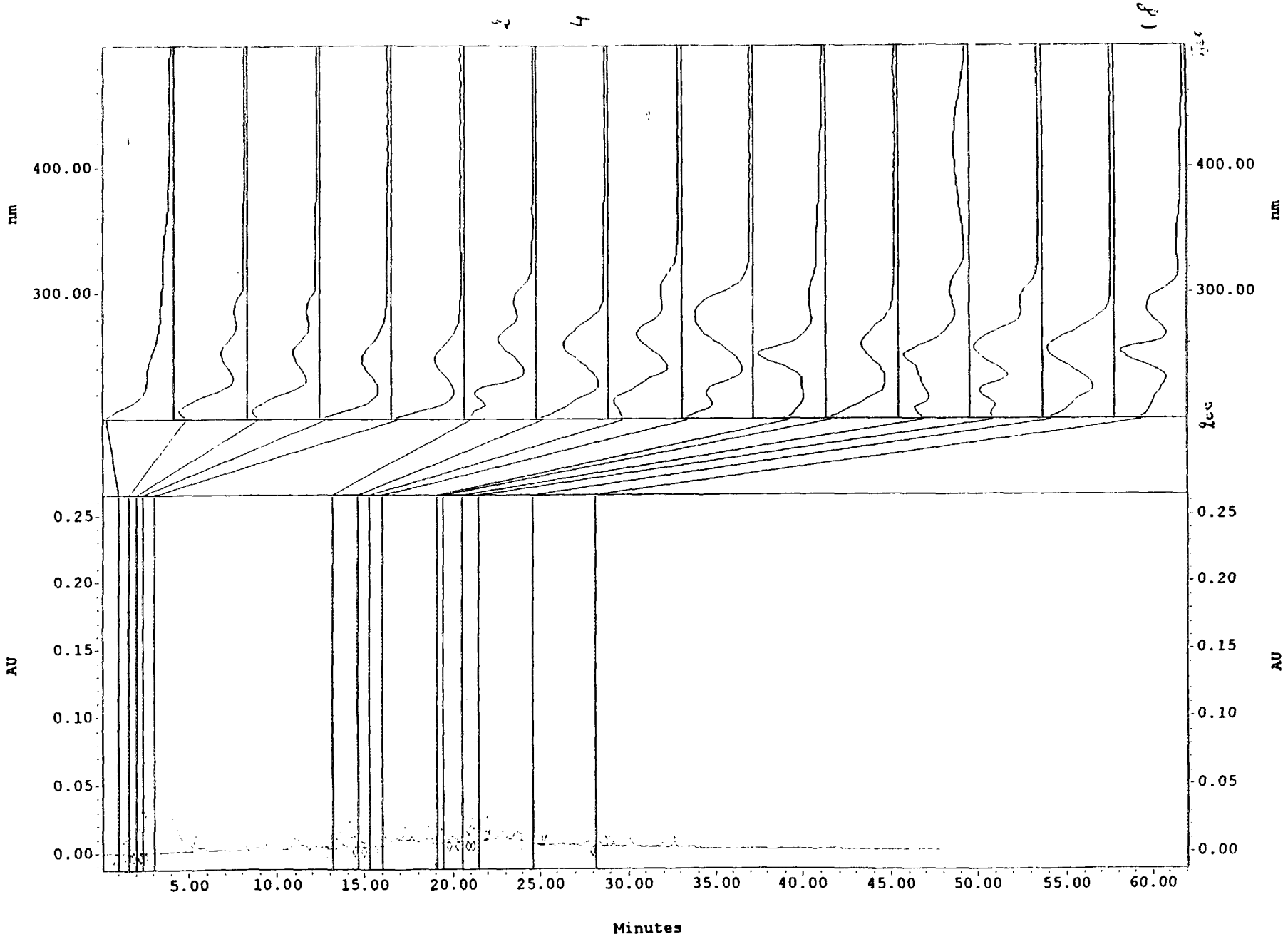
Instrument Type W717

Instrument Type	W717
Instrument State	On
Use Temp	No
Setpoint	25

Millennium Acquisition Report		Printed: November 6, 1997		Page: 1 of 1	
Report Method:	tabebuia_cortex		Version:	2.15	
Sample:	staal 1		Processed:	06/11/97 03:54:24 PM	
Vial:	1	Inj: 4	Channel:	996	

1,5 ml/min





Millennium PDA Spectrum Index Plot - SampleName staal 1, 254nm - PDA 254.0 nm