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PILOT PRODUCTION OF MEDICINES USING INDIGENOUS RAW MATERIALS

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Technical Report :

Strengthening the Present Capabilities of the Institute of Materia Medica, Hanoi, to Conduct Research and Develop Technology for Processing Pharmaceuticals from Indigenous Plants*

Preapred for the Government of Vietnam
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

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* This document has not been edited.

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INTRODUCTION

Modern drugs as well as drugs produced from indigenous plants are used in the health care of the people in Vietnam. As the availability of modern drugs is limited due to the high costs involved in importation, the majority of the drugs used presently are those produced in Vietnam from indigenous plant resources. In order to improve the quality of these drugs the Ministry of Health has initiated action to develop factory processing of these herbal medicines by including this project as a national priority.

The Institute of Materia Medica (I.M.M.) in Hanoi has been entrusted with the responsibility of conducting research and development to improve and generate suitable technologies for the systematic production of drugs based on new improved formulations.

The Institute of Materia Medica has a multidisciplinary set up consisting of all divisions needed for the development of new drugs and dosage forms. It has a strong pharmacology division engaged in evaluating the claims of therapeutic efficacy of individual compounds as well as compound formulations in the pharmacopoeia including toxicity studies on them. Another major function of the Institute is to develop routine quality control methods for the standardization of raw materials, intermediates, processes, finished products and packaging. The Institute also has divisions entrusted with the responsibilities of improving agrotechnology and post-harvest technology to generate the raw materials of the required quality. As such there cannot be a better choice than the Institute of Materia Medica to be the implementing agency for this project.

The Institute of Materia Medica has already conducted extensive research on the phytochemistry and the pharmacological effectiveness of certain plant extracts and compound formulations. Certain phytochemicals such as Tetrahydropalmatine and Tetrahydroberberine have been tested and developed as new single drugs. These new

drugs, formulations and dosage forms developed by the Institute have not been transferred to production factories due to the lack of proper pilot plant facilities to scale up the laboratory findings.

It is envisaged that the quality control protocols and pilot plant technologies developed at the Institute would be passed on to the State - run factories resulting in the vast resources of the flora in Vietnam being exploited to deliver health care to the people. Thus the realization of the objectives of the project would lead to the development of a Vietnamese Pharmaceutical Industry based on local Plant resources.

1. PROJECT OBJECTIVES RELEVANT TO THE CONSULTANT

The primary objective of the project is the development of suitable technologies at the pilot plant scale for the production of new and improved dosage forms based on indigenous plant raw materials. Hence the inputs from UNDP covers pilot plant equipment, other equipment to strengthen the infrastructure of the associated multidisciplinary laboratories, training of personnel in new methods and techniques overseas and the training of personnel locally by international experts. Within the framework of the project document, the work expected from an Industrial Pharmacist Consultant consists of strengthening the good manufacturing practices by introducing where necessary the procedures for

(a) quality control at raw material, intermediate, processing and finished product stages.
(b) improving, monitoring, standardization and optimization of process technology to ensure purity, reproducibility of therapeutic efficacy and stability of finished products.
(c) presentation of finished products in modern dosage forms based on new and developed formulations.
(d) developing protocols for the transference of pilot scale production procedures to industrial factories for large scale production.

The list of drugs to be initially produced in Phase I of the project utilizing the pilot plant facilities consists of the following two categories:

Category I : Purely traditional multicomponent or. single plant crude extracts to be converted into modern dosage in liquid or tablet form.

Category II (Pure active principles) : Pure phytochemicals and their simple modified forms derived from indigenous raw materials which can be made into modern allopathic preparations in tablet or injection form.

The outputs envisaged within the project include :

1. The development of nine new standardized drug formulations from among commonly used drugs (listed in Page 9 of Project Decument) which were at first produced in crude, poorly presented unstandardized form.
2. Technology for manufacture of two phytochemicals originally discovered by the Institute of Materia Medica and production in sufficient quantity for elaborated pharmacological, toxicity and clinical trials - tetrahydropalmatine and tetrahydroberberine.
3. Technology for manufacture of two phytochemicals already in use in other countries and production on pilot plant scale - berberine chloride and D - strophanthin.

In order to achieve the above outputs, the relevant activities expected of the Industrial Pharmacist Consultant are detailed on page 14 of the Project Document under 13, 14, 16, 17 and 18.

13. Production of large quantities of medicaments needed for sustained clinical trials and to monitor patient - doctor acceptability of the drugs produced.
14. Refinement and improvement of the formulatien of dosage forms.
16. Studies on process monitoring, process optimization, process control and standardization of drugs to improve the quality of traditional drug forms.
18. Demonstrate through pilot scale production the soundness of the new drug and their manufacturing technology and feasibility for commercial production.

The time period allocated to the consultant is two split missions of three months each.

2. TERMS OF REFERENCE OF THE CONSULTANT

The duties of the Industrial Pharmacist Consultant as included in the job description are as follows:

The expert will function under the direction of the UNIDO Chief Technical Adviser (Project Co-ordinator) and work in collaboration with national counterparts and other international experts in accomplishing the following:

a) Organize the pilot scale production of modern dosage forms - such as tablets, syrups etc., suitably standardised and stabilised from indigenous plant extracts in accordance with recognised good manufacturing practices.
b) Establish a research unit for formulation studies to provide continuous improvement of finished products.
c) Train local counterpart staff within the Institute of Materia Medica in modern manufacturing practices with lectures as well as practical demonstrations.
d) In collaboration with the process engineering expert develop techniques to obtain extracts specifically suited for modern dosage forms.
e) In collaboration with the international expert in Organic/Analytical Chemistry develop quality standards for both products and raw materials as well as methods for process monitoring.

3. WORK ASSIGNED TO THE CONSULTANT

The consultant was briefed by the Substantive Officer of the UNIDO Headquarters before arrival in Hanoi. The performance of the duties (a) and (d)given above was not expected during this mission of the consultant as the pilot plant machinery were not operative. Hence the terms of reference were limited to (b), (c) and (e) for this mission. The major duties (a) and (d) are expected to be performed by the consultant along with the next visit of the process engineering expert at the time of commissioning the pilot plant and the tablet machine.

The consultant arrived in Hanoi on 9th December 1987 and reported to the Chief Technical Advisor and met the Director of the Institute of Materia Medica and the National Project Director. The Chief Technical Advisor briefed him further on the present status of the project and explained the unavoidable and unforseen circumstances which might even further restrict the activities of the consultant.

The management of the Institute and the National Project Director had a discussion with the C.T.A. and the consultant on the specific tasks to be performed by the consultant. The management briefed the consultant as to the limitations of the availability of infrastructural facilities which might restrict the activities of the consultant as his main function would have been to develop and organise pilot plant production in accordance with good manufacturing practices. The management of the Institute and the National Project Director in consultation with the C.T.A. indicated the following activities within the framework of the duties (b), (c) and (e) detailed in the job description.

1. Research and development to improve the formultions of Achyranthes syrup and tablets, Adenosma tablets and APD (Anti Periodontitis) liquid.
2. Development of new tablet formulations for l-Tetrahydropalmatine hydro chloride, Tetrahydroberberine hydrochloride and Berberine chloride.
3. Improvement of sugar coating procedure and demonstration of film coating of tablets.
4. Development of quality control protocols for the raw materials; Achyranthes oidentata (roots), Adenosma indianum (aerial parts).
5. Development of pharmacopoeial specifications for Tetrahydropalmatine hydrochloride, Tetrahydroberberine hydrochloride and Berberine chloride.
6. Drawing up of quality specifications for the finished products; Achyranthes syrup and tablets, Adenosma tablets, APD liquid, Tetrahydropal matine HCl tablets, Tetrahydroberberine HCl tablets and Berberine chloride tablets.
7. Studies on stability and shelf-life of above products.

The consultant spent the first few days discussing with the group-leaders of the standarization, formulation and pilot plant divisions and getting himself apprised of the facilities available and the problems at hand in order to draw up his programme of work. He was also shown around the Institute and the pilot plant machinery.

4. ACTIVITIES OF THE CONSULTANT

The activities of the consultant were confined to the formulation, pilot plant standardisation and analytical divisions. A programmed time schedule of the work to be carried out in the formulation and pilot plant divisions was drawn up by the consultant in consultation with the heads of the two divisions and the vice director. But it was not possible to strickly keep to the plan, due to various factors including the non-receipt of chemicals and equipment that have been ordered and even humid weather conditions. Nevertheless a substantial part of the programme was executed with a lot of improvisations. The staff of the standarisation and analytical divisions participated in the preparation of monographs and the development of assay procedures.

The consultant conducted the following activities:

1. Discussions and progress review meetings with the C.T.A., Management of the Institute, National Project Director and the relevant group leaders (Annexure I)
2. Lectures on production of tablets and syrups, standardisation and quality control, stability and storage of medicaments and good manufacturing practices.
3. Development of tablet formulations for l-tetrahydropalmatine hydrochloride (THP, HCl), tetrahydroberberine chloride (THB,HCl) and berberine chloride.
4. Laboratory scale production of tetrahydropalmatine hydrochloride, tetrahydroberberine hydrochloride and berberine chloride tablets.
5. Improvement of the method of extraction and formulation of Achyranthes (Syrup and tablets).
6. Laboratory scale production of Achyranthes syrup and ampoules (oral use).
7. Formulation trials on Achyranthes tablets.
8. Sugar coating (laboratory scale) of tetrahydropalmatine tablets.
9. Film coating (laboratory scale) of berberine chloride tablets..
10. Accelerated stability studies on the above tablets.
11. Demonstration of direct compression and double compresssion in tablet production.
12. Development of quality control protocols (Pharmocopoeial specifi cations) for Achyranthes roots and Adenosma aerial parts.

13. Development of pharmacopoeial specifications for tetrahydro palmatine HCl, tetrahydroberberine HCl and berberine chloride (as raw material).
14. Development of assay precedures for tetrahydropalmatine, HCl and tetrahydroberberine HCl in tablets.
15. Work out the parameters for the measurement of haemolytic index to determine the saponin content in Achyranthes roots and syrup.
16. Development of specifications for Achyracthes syrup.
17. Draw up in-process control procedures for the uncoated tablets of tetrahydropalmatine HCl, tetrahydorberberine HCl and berberine chloride.
18. Preliminary studies on the extraction of Adenosma indianum and recommendations on improved methods of extraction.
19. Recommend improved formulation for Adenosma tablets.
20. Recommend protocols for stability testing of pharmaceuticals in Vietnam.
21. Recommend a new solid dosage form for APD liquid preparation and improvements in the formulation of the liquid itself.
22. Formulation and demonstration of the preparation of effervescent granules.

The tablets were made on a laboratory scale single punch machine using worn out punches and dies without much heed to the shape and size of tablets. The non availability of a heavy duty slugging machine and concave punches larger than 6 mm were limitations to the development of formulations of tablets from plant extracts. The in-process controls during tablet manufacture as required by good manufacturing practices could not be demonstrated due to non-receipt of the necessary instruments up to now.

Pending the arrival of orders, some new formulations were made using the chemicals brought by the consultant (Annexure 2) and the C.T.A.

The high humid (90%) weather condition was a continuous problem for tablet making and coating. The plant extracts tended to absorb moisture and some experiments had to be delayed or even abandoned as the humid weather continued up to the time of departure of the consultant.

The consultant received the fullest cooperation of the CTA, NPD, Director and the Management of the Institute and all scientists who worked with him. The scientists who worked with the consultant were guided and trained to be competent in handling new plant extracts, phytochemicals, development of formulations including stability testing and problem solving but as bench scale operations.

5. OUTPUT AND DETAILS OF WORK

A. Lectures

The consultant conducted the following series of lectures (12 hours) in order to update and enhance the knowledge of scientists and pharmacists on the necessary theoretical backup and the latest developments in pharmaceutical product development.

i. Formulation, production, quality assessment and stability of syrups
ii. Tablets as a dosage form. Excipients, Size reduction, mixing, granulation and granule characteristics, compression, handling of tablet defects.
iii. Tablet formulation and characteristics, inprocess controls and manufacture.
iv. Coating of pharmaceuticals including sustained-release forms.
v. Good manufacturing practices, sampling, stability testing and packaging.

The extent to which the latest developments could be used in a developing country and the processes that could be effected in a pilot plant were discussed. The procedures required by good manufacturing practices that could not be demonstrated due to lack of facilities, were explained and the need to adopt them was emphasized.

This enrichment of know-how was very useful in that the pharmacists participated actively in the discussions about the work they were performing under the guidance of the consultant. As a result they became aware of the problems and attempted to seek solutions with a good understanding of the theory. This enabled the consultant to guide and train them with ease and to get an increased work output.

B. Tetrahydropalmatine Hydrochloride (THP , HCl)

After extensive pharmacological research, the Institute of Materia Medica has drveloped l-tetrahydropalmatine as a single drug to be used as a sedative in the treatment of some mental disorders. The drug has been extracted from the tubers of various species of Stephania growing in Vietnam. The drug has not yet been included in the Vietnamese Pharmacopoeia. The hydrochloride form has been used in the production of tablets of 50 mg.

1. Monograph specifications :

The specifications available were the melting point, specific rotation and the λ max. The consultant guided the personnel in the standardisation division to develop pharmacopoeial specifications for THP, HCl (Annexure 3). As the pharmacopoeias available did not list this drug, specifications for identification, purity and assay had to be developed.

The TLC system used at the Institute could not distinguish THP, HCl from tetrahydroberberine hydrochloride. Hence a system suitable for the identification of the two compounds was developed. In addition a number of other identification tests were included, so that the factories that do not have facilities could have a choice. The purity criteria required for pharmacopoeial specifications were included.

2. Assay :

The assay procedure hitherto used in the Institute was found to be defective as it was based on a UV spectrophotometric measurement of λ max at 282 nm. The consultant demonstrated that the oxidised products of THP including (Annex 3 A) palmatine absorb at this wavelength and the extinction coefficient of palmatine is even much larger than that of THP at this wavelength. Hence spurious results would be obtained. Further the formula used in the calculation included an instrument factor although reproducible readings could not be obtained due to voltage fluctuations. Both the scientist who is in charge of the instrument and the head of the standardisation division were unable to obtain reproducible readings even during the same day. The instrument was in a room which had variations of temperature and weather and it was switched on just before taking readings. Hence the Cecil

instrument which is a non - recording manual type could therefore be used with a reference standard if meaningful results are to be obtained.

The scientists were trained in how to use it for an assay procedure taking tetrahydropalmatine hydrochloride as the example (Annexure 4). However they were warned that this method could not be used to test the purity of THP,HCl as the impurities in it would interfere with the determination.

The assay developed by the consultant (Annexure 5) was based on HPLC and had the following advantages:

(i) The THP, HCl could be directly extracted from the tablets instead of extracting the base as previously.

(ii) The impurities were separated and appeared as a separate peak at the same wavelength (282 nm).

Attempts to separate the peaks of THP, HCl and tetrahydroberberine hydrochloride at this wavelength were not completely successful due to the limited number of HPLC solvents available (Methanol, Ethanol and water).

However, use of methanol and monitoring at 282 nm partially resolved the two peaks. This could be used to detect any contamination with THB, HCl (in addition to the TLC method). As peaks were symmetrical quantitative estimations could even be made.

The consultant also stressed the importance of measuring the specific rotation of the drug as the l-form is reported to be a sedative while the d-form is reported to sometimes show the opposite effect. The [α] will thus ensure that palmatine isolated from other plant sources or oxidised products have not been reduced to get the racemic tetrahydroform.

3. Accelerated stability studies

Tetrahydropalmatine hydrochloride tablets produced in the Institute developed a yellow colouration during normal handling. The consultant conducted some accelerated stability tests in order to ascertain the cause of instability. He took this opportunity to train the scientists in the formulation division on how to solve such problems and the procedure for conducting accelerated stability studies. Exposure

of the tablets to high temperature (60°C), high humidity (80%) and direct sunlight revealed that only the direct sunlight had a dele terious effect and that too only on the surfaces that were exposed. The change of colour during normal handling was oobserved mostly at the edges of the tablets. Exposure of the raw material and half tablets indicated that the discolouration rate was much less in the crystalline material and at the cut end of the tablet. Hence the problem was identified as a photoxidation reaction catalysed by the excipients used and contact with metal surfaces.

A testing model for conducting accelerated statibility studies (Annexure 6) for the prediction of the shelf life of new drugs was handed over to the head of formulation along with a protocol recommending a stability sampling progamme (Annexure 7) for actual shelf life determinations. These recommendations have been based on the changing weather conditions and the facilities available in most of the pharmacies and houses.

4. Trial tablet formulations :

The scrutiny of the formula used for the tablets revealed that talc had been used as the diluent and magnesium stearate as the lubricant. As the THP base was more prone to oxidation than the salt, the alkaline magnesium stearate was replaced by stearic acid. Talc was replaced by starch as metallic ions even in trace amounts would catalyse the photo-oxidation reactions. In any case talc is hardly used as a diluent. A small amount of EDTA was incorporated as a chelating agent to reduce any effects due to punches, die and sieves used. The consultant was restricted in his choice of excipients as where ever possible he had to select from among the locally availa-ble material eg. lactose was not available.

The consultant conducted some trial formulation experiments taking this opportunity to demonstrate direct compresssion and double compression formulae (using chemicals brought by him) as a means of eliminating an exposure to moisture and heat. But good flow properties and compressibility could only be obtained by the wet granu-lation experiments. The tablets produced had good characteristics and were stable during normal handling. They were still effected by direct sunlight while those kept inside the laboratory were white. Hence THP, HCl tablets should be packed in well closed containers protected from light. If necessary the cores could be protected by sugar coating.

5. Laboratory scale production :

The best formula (Annexure 8) in terms of stability, flow properties, compressibility and friability was chosen for the laboratory scale production. Only 300 g of THP, HCl of required quality was available. 25 g were used in the trial formulation experiments and 275 g were granulated to be tabletted using 6 mm concave punches to produce tablets of 100 mg weight. Unfortunately a mishap occured during the drying of granules in the oven overnight. The temperature of the oven had shot up to over 90°C, that the granules were brownish in colour in the morning. The oven used had no controls and the temperature has shot up due to high voltage fluctuations during the night. On keeping the good trial tablets in the same oven overnight the same discolouration occurred.

The consultant prefered to re-work the whole sample by extracting the THP,HCl from the granules but time constraints (two weeks prior to the departure of the consultant and the New Year Holidays (Tet) intervening) and the non availability of further raw material (THP, HCl) made him to find means of salvaging the process. Quantitative estimation of the granules indicated a 97.2% purity and a very small amount of palmatine. 20 g of the granules were handed over to the phytochemistry division to work out the procedure to recover the pure drug and the balance quantity was regranulated after adding more starch. The tablet size therefore had to be increased. As concave punches larger than 6 mm were not available, 7 mm flat punches were used eventhough the tablets were to be used for demonstrating the sugar coating procedure.

Details of general quality control parameters for the evaluation of tablets were given (Annexure 9). A protocol for the in-process control of THP,HCL plain tablets was worked out (Annexure 10).

A monograph on Tetrahydropalmatine tablets was also drawn up (Annuxure 11).

6. Sugar coating :

The flat 7 mm tablets produced using the old 4-punch machine showed noticeable differences in tablet thickness and hardness. About 25 - 30% of the tablets were thicker than the rest indicating a non-uniformity in the punch lengths (At least one punch should have been slightly shorter than others).

Due to time constraints and the need to demonstrate the somewhat different sugar coating procedure recommended by the consultant (Annexure 12) it was decided to coat the tablets. The consultant noticed that there was no practice of seal coating of tablet cores even when they were moisture sensitive. As such tablets prepared from plant extracts developed cracks after some time. The need to seal coat certain types of tablets was stressed. The THP,HCl tablet cores had to be protected from moisture as starch had been used as the diluant, binder and the disintegrating agent.

As coating of tablets had to be done without a proper air handling system and under very humid (90%) conditions, a lot of improvisations had to be done. A laboratory scale coating pan was used heating it directly by keeping a hot plate underneath. The area was heated using a room heater and the drying air was applied using a hair drier. Still for all the conditions as required by good manufacturing practices could not be obtained.

The drying of tablets overnight after the seal coat, had not been done as recommended as the oven had been switched off exposing the tablet cores to the high humid conditions in the laboratory. At the sub coating stage the differences in the hardness showed up by some tablets tending to laminate.

The defective tablets were removed and the sub coating was continued. Another problem that surfaced at this stage was the uneven lumpy appearance of the tablet cores particularly at the edges. The acacia powder in the dusting powder had absorbed moisture and become gummy in contact with the sub coating solution. The unevenness at the faces could be corrected but that at the edges was difficult to correct resulting in some tablets not being quite circular even after the grossing and smoothing stages. The formula of the sub coat dusting powder was thus modified by removing the

acacia powder and increasing the percentage of powdered sugar although in the experience of the consultant acacia powder had given good results under different atmospheric conditions.

Colour coating was continued with the defective tablets being present as the load was just sufficient for the proper use of the coating pan. Although the consultant wanted to demonstrate the use of a lake dye suspension it was not possible due to unavailability of a lake dye.

The polishing procedure practised in the Institute was to warm the tablets to 60 - 70°C, add a small quantity of a melt of paraffin wax in liquid paraffin, put them in a bag and roll the bag in the pan for 3 - 5 hours. The consultant demonstrated the use of a polishing pan and a solution of wax for polishing. The coating pan was converted to a polishing pan by lining it with a layer of paraffin wax and a solution of beewax in chloroform was used as the polishing solution.

The whole coating and colouring procedure was not up to the standard expected by the consultant. It is a difficult task to change the practices of an experienced coater by a single run. But it is hoped that at least the new stages (sealing and polishing) be introduced in coating of tablets in the future. It is hoped that one would appreciate the difficulties of a consultant who has to do makeshift arrangements and improvisations. The consultant would like to emphasize that production of good quality coated tablets would not be possible unless an air conditioned room with a dehumidified air circulation and a coating pan with the necessary air handling system and spray instruments are made available.

C. Berberine chloride

Berberine chloride is used as a drug in a number of countries. Japanese and Indian pharmacopoeias contain monographs on it. It is used in the treatment of non-specific diarrhoeas. The raw material for the extraction of Berberine is available in Vietnam and already it is being extracted on a large scale at the Ho Chi Minh City Drug Factory. The Institute has produced tablets of Berberine Chloride containing 100 and 50 mg.

1. Pharmacopoeial Monograph (Annexure 13)

As Berberine Chloride has not yet been included in the Vietnamese Pharmacopoeia, monograph specifications for the raw material was worked out. Most of the details in the J.P. and I.P. were similar and could be directly adopted. The sample handed over to the consultant had no moisture although Berberine Chloride crystallises out with up to three molecules of water of crystallisation. On inquiry it was revealed that the sample supplied had been crystallised from methanol. So the drug had to be recrystallised from hot water before the specifications were checked.

As the identification tests for Berberine and Palmatine closely resemble each other a system was developed to distinguish between them so that any contamination with palmatine could be detected. This was an addition to the specifications found in the other two pharmacopoeias. The assay procedure given in the J.P. and I.P. was retained. But an additional assay proceduring using HPLC was included as it can be conveniently used for the routine assay of tablets (Annexure 14).

2. Tablet formulation trials

Although the Institute has previously made Berberine Chloride tablets, the formula has been misplaced and was not available to the consultant. Hence he had to try a few formulae taking into account the properties of the compound and the decision to film coat the tablets. Magnesium stearate and talc were excluded from the formulation and stearic acid was used as the lubricant (Annexure 15). Although 6 mm tablets were to be made 7 mm flat tablets had to be made because of the small amount of material supplied.

The inprocess controls for the tablets were also prepared (Annexure 16).

A monograph on Berberine tablets was also drawn up (Annexure 17).

3. Laboratory scale production

The consultant was supplied with 160 g of berberine chloride which had been crystallised from methanol. 20 g were used in formulation trials and the balance was used in producing tablets to be film coated. The formula which gave good tablets (Annexure 15) was used with some modifications to increase the weight so as to produce a sufficient load for film coating. Drying of granules was again affected by the high voltage during the night although a different oven was used. Even good trial tablets when kept in that oven showed a darkening of colour overnight. This was brought to the notice of the management so that remedial measures could be taken to stabilise the voltage at night. The tablets were prepared using these granules so that film coating could be demonstrated. The tabletting was done on a 4 punch machine using 7 mm punches.

4. Film Coating

Although the load was not sufficient for the laboratory coating pan, the process was demonstrated just before the departure of the consultant. The prevalent weather conditions necessitated a lot of improvisations such as heating the pan using a hot plate, heating the room but the humidity was too high. There was no exhaust system. The drying air was supplied using a hair drier. The procedure (Annexure 18) had been given by the consultant and the formula II (without colourant) was used in this instance.

D. Tetrahydroberberine Hydrochloride

This is a new drug developed by the scientists of the Institute after extensive chemical and pharmacological research. It is a safe tranquilizer with no addictive properties and with a high therapeutic index. The racemic form is used as the drug and there are no reports of investigations on the two individual isomers. It is produced as a semisynthetic drug by the reduction of the naturally occurring berberine.

1. Pharmacopoeial specfications

As there were no previous reports on the specifications for the drug, the consultant guided the staff in drawing up pharmacopoeial specifications. The whole monograph was worked out including an assay based on HPLC (Annexures 19 & 20). UV spectrophotometric method could not be used (λmax 284 - 286 nm) as berberine too absorbs at this wavelength. The product supplied to the consultant was pale yellow in colour and contained same berberine chloride as an impurity (shown by the HPLC and TLC). Hence a permissible limit for berberine chloride was included in the monograph. This could be deleted once pure crystalline THB,HCl is made available. In contrast to THP, HCl this compound was much less stable in solution. Solutions became yellow in colour and showed the presence of berberine and other oxidation products.

2. Trial Formulations

Several formulations were tried varying some of the excipients (limited choice) and a good formula (Annexure 21) was selected after subjecting the tablets to accelerated stability testing. The drug was becoming yellow when exposed to light and temperature in the presence of moisture in the raw material state. Hence care has to be taken during the production stage to control these conditions. The humid conditions delayed the laboratory scale production.

3. Laboratory scale production

The consultant was supplied with 200 g of THB, HCl as a pale yellow crystalline powder. 30 g were used in the formulation trials and the balance was tabletted using the selected formula and 6 mm concave punches. Good tablets with the required characteristics were obtained. The specifications (Annexure 22) and inprocess controls (Annexure 23) were drawn up using these tablets.

E. Achyranthes bidentata Blume (Amaranthaceae)

The roots of Achyranthes bidentata had been used in traditional medicine for a long time. It has been indicated for a number of ailments including inflammatory conditions, painful micturition, amenorrhoea, congestion due to trauma, lumbago and haematuria. The pharmacological research carried out at the Institute has established that the extract has antiinflammatory, hypotensive and hypocholesteremic effects on animal models. In combination with other plants, it has been shown to be active against periodontitis, lumbago and polyarthritis. Clinical research has supported the hypocholesteremic, hypotensive and antiinflammatory effects.

Chemical investigations have indicated the presence of triterpenic saponins and steroids in the roots. The genin of the saponins has been identified as oleanolic acid. Pharmacological studies conducted at the Institute indicate that saponin fraction is active against chronic and acute phases of experimental inflammatory action and inactive in lowering the cholesterol level in animal models. The fraction that is responsible for the hypocholesteremic action has yet to be determined.

1. Pharmacopoeial Monograph

The current Vietnamese Pharmacopoeia contains a monograph on the roots of Achyranthes. It describes the morphology, microscopy, powder microscopy of the roots and froth tests as a means of identification. Purity tests only include the moisture content and foreign matter. The monograph also describes the method of processing the roots. Fumigation with sulphur is done a number of times to reduce fungal growth as the roots contain a large proportion of moisture and polysaccharides.

The consultant was supplied with the raw material from the market. The samples supplied had different organoleptic characteristics. They could be broadly classified as light brown, dark brown and brownish black samples. The sweetness of the three categories also varied; the brownish black being the sweetest. The differences in colour were explained as being due to the age of the sample. The degree of sweetness indicates that a process of fermentation

or maturation is taking place during the storage. The three samples were studied separately for the moisture content, extractives, ash content, reducing sugar and the saponin content. The results were interesting in that the oldest sample had less saponin than the other two as shown by haemolytic index and more water soluble and ethanol soluble extractives than the other two. The content of reducing sugar was similar. Saponins seems to undergo hydrolysis on storage giving more nonreducing sugars. These variations made the drawing up of specifications difficult. Wide ranges were allowed to accomodate the different types. The current pharmacopoeial specifications were improved to include identification tests, purity criteria and quantitative estimation of reducing sugars and saponins (Annexure 24). More work is needed if the types are to be officially recognised. Procedures for the determination of monograph specifications are given in Annexure 24 A.

2. Haemolytic Index

The estimation of the saponins was carried out using the haemolytic index. As a standard saponin was not available only the apparent haemolytic index was determined. The quantities of extract and the blood suspension to be used were determined after a few experiments (Annexure 25).

3. Improvement of Achyranthes syrup formulation

Achyranthes syrup prepared in the Institute had stability and clarity problems. The sample handed over to the consultant in December 1987 was a cloudly brown suspension although prepared in August 1987. The single dose ampoules (oral use) were the same.

After scrutinising the method of preparation, the consultant conducted a series of extractions to determine the extractibility into 96% alcohol and n-butanol. Alcohol (96%) extracted half as much and n-butanol a quarter as much as the weight extracted by the aqueous alcohol (40%) used in preparing the syrup. The concentration of the aqueous alcohol to $\frac{1}{8}$ th the volume would lead to the precipitation of some water as well as alcohol solubles giving a saturated concen trate with might further precipitate material once the syrup is added. Hence the process had to be modified taking into account

the costs on alcohol and the pharmacological finding that some water soluble extractive is necessary to lower the cholesterol level.

Percolation of cut roots was found to be inefficient as the marc retained a lot of alcohol which could not be expressed with the available equipment. The moisture content of the roots is as high as 25% and unless the cell structure is damaged, it is difficult to get a good extraction. As this could only be done using the facilities of the pilot plant, the consultant could only improve on the percolation procedure and that too on a very small scale (2 kg) (Annexure 26).

The volume of alcohol used was the same as that used in the Institute's method but without dilution. The recovery of alcohol from the marc was more efficient as water was used in continuing the percolation. The extract was not subjected to drastic heat treatment as only the alcohol was recovered by distillation. The bulkiness of the extractive was less but should contain the saponin. The recovery of alcohol from the alcoholic (96%) percolate and the aqueous alcoholic percolate were done separately and the aqueous extracts were mixed. Some precipitation occurred at this state and the precipitate should be further investigated chemically and pharmacologically. Filtration gave a clear extract to which a preservative was added. The solution was sweet and could have been developed as the finished product after flavouring.

This extract showed a slight cloudiness on storage particularly at lower temperature. The solution cleared on warming. Investigations revealed that the cloudiness was due to the precipitation of an organic acid. The pH of the solution was 3.5. Adjustment of the pH to 6 - 6.5 with sodium bicarbonate resulted in the evolution of carbon dioxide and a very clear solution. This extract was given for pharmacological evaluation.

The syrup was then made by incorporating sugar directly into the extract. A small quantity of glycerin was added to aid any solubility problems.

The syrup was flavoured with anise oil as it gave a better flavour. Besides the use of synthetic isoamyl acetate could be avoided as anise oil is obtained from locally available star anise.

The consultant recommeds that the extract be marketed as the finished product in order to avoid the use of sugar which is contraindicated for diabetics thereby even saving on the costs. Ampoules or multidose containers could be packed.

4. Standardisation of the extract

The standardisation of the extract was to have been done using the haemolytic index after pharmacological evaluation. The concentrated percolate could have been assayed for saponins and then diluted to the required strength in making up the finished product. But the pharmocological findings (discussed below) forcussed a problem that whether it was correct to standardise on the saponin content. This matter remains to be settled.

5. Pharmacological Evaluation

The result of the pharmacological evaluation revealed that the new syrup was much more active than the old formulation in lowering the cholesterol levels in rabbits. The results were significant and a further study is awaited. The determination of saponin content using the haemolytic index showed that the new formulation had substancially less saponins than the old syrup. This supports the earlier finding that saponins themselves were not active in lowering the cholesterol levels. It will be interesting to test the new formulation against acute and chronic inflammation where saponins have been shown to be responsible for the activity. It is also possible that the old syrup has undergone some change (as seen by the brownish suspension), which is interfering with the determination of haemolytic index.

6. Specifications for the syrup

The specifications for the quality assessment of the syrup were developed (Annexure 27). Some accelerated stability studies were done under high and low temperatures, exposure to light and the atmospheric humidity conditions.

7. Achyranthes Tablets

The tablets produced in the Institute had the following problems

(1) The desired disintegration time could not be achieved
(2) Sugar coated tablets tended to crack on storage
(3) The colour of the tablets faded with time

The extract had been subjected almost to caramelization that they formed hard tablets, which would not disintegrate within 30 minutes. No disintegrating agent had been added. In order to solve this problem for the procedure presently used, starch was used as part of the filler and dry starch was included after granulation as a disintegrant.

The cracking of the sugar coat can be due to many reasons including storage conditions. The fact that the final granules were absorbing some moisture during tabletting would lead to hydrolysis and oxidative processes which show up with time. Hence the incorporation of a stabilizer is indicated. Further the sugar coating had been done without a seal coat exposing the tablet to the syrup solution. A seal coat or a better and a more stable extract may solve this problem.

The colourant and the method of colour coating could not be examined but a new colour coating formulation was suggested.

As mentioned previously, the raw material and the extraction parameters were so variable from batch to batch, standardisation on the weight of the extract would not give uniformity of dosages. Scrutiny of the extraction procedure and the tablet formulation showed that the procedure could be improved so as not to subject the active principles to drastic heat treatment. The bulkiness of the extract could also be reduced such that the dose of five tablets at one time could be cut down. The concentrated extract contained a lot of sugary and polar material making drying difficult. The consultant carried out two separate percolations, one with 96% alcohol and the other with 40% alcohol in order to study the procedure used in the Institute. Comparison of the first 1.4 l of percolates showed that the weight of material extracted using 40% alcohol was almost double the weight extracted with 96% alcohol. Thus a lot of water soluble gummy material was being extracted with 40% alcohol which brought about some of the

problems mentioned. n-Butanol extraction of portions of the two extracts gave nearly the same weight of extractives. Hence if the butanol extractive is pharmacologically evaluated, the bulk can be reduced to almost a quarter and a realistic dose regimen can then be produced.

As the alcoholic (96%) extracts were found to be active in lowering the cholesterol levels, the formulation of tablets using the two extracts was continued. After the recovery of the alcohol, the extracts were evapourated to obtain a liquid concentrate which could not be dried further. Diluents were incorporated (to get 0.25 g of extract per tablet) and the sticky mass could not be dried even at 80 - 90°C. The practice had been to get a dry mass after adding the filler and subject it to dry granu lation in a oscillating granulator. This was not possible with these extracts, may be, because of the high humid conditions. Part of the 96% alcoholic extract was formulated as a.125 g tablet (Annexure 28). Hence more diluent could be added to carry out a wet granulation. Good granules were obtained but the tabletting could not be done due to high humid conditions. The granules tended to pick up moisture during tabletting. The strength was reduced to half as the extractive was half that produced by the 40% alcoholic extraction. These tablets once made should be sent for pharmacological evaluation.

When dry extracts are obtained using the pilot plant facilities, slugging may be a better method of preparing the granules. At present the extract is subjected to drastic heat treatment in the preparation of the concentrate and again to 80 - 90°C for long periods to get a dry mass.

The consultant suggested 3 alternative procedures (Annexure 29) for the extraction of roots for the preparation of tablets. The choice amongst them would have to await pharmocological evaluation.

8. Standardisation of Dry Extracts

The variability of the quality of raw material and the extraction precedure parameters would result in batchwise variations of the extractive. Hence standardisation of the extract is essential.

Although it was expected to have been standardised on the saponin content, the latest pharmocological results have created some doubts. Hence till these are cleared, the consultant recommends that two preparations be marketed, one as an antiinflammatory drug and other as a hypocholesteremic drug. The antiinflammatory drug can be standardised using the haemolytic index. As for the hypocholesteremic effect further experimentation is necessary. Activity directed fractionation of the extract has to be carried out in order to define the fraction that is responsible for the cholesterol lowering effect. Once this is done, a procedure for its standardisation could be developed.

F. ADENOSMA INDIANUM (LOUR.) MERR - SCROPHULARIACEAE

Three of the nine species of Adenosma found in Vietnam are used in traditional medicine in the treatment of fever, jaundice, vertigo and as a digestive and a diuretic. Only Adenosma caeruleum has been included in the current Vietnamese Pharmacopoeia although all three species are widely used in the treatment of liver and bile diseases. Adenosma indianum is the most abundant, widely distributed raw material. The Institute of Materia Medica has conducted very detailed and elaborate pharmacological research on Adenosma indianum to demonstrate a number of activities among which its cholagogue action in the treatment of hepatitis constitute an original contribution of Vietnamese traditional medicine to health care. The pharmacological findings on liver disease has been supplemented by clinical trial results. Some chemical constituents have been identified. Activity studies have only been done using aqueous extracts, alcoholic extract and the essential oils. Further investigations on activity directed fractionations are necessary to isolate the active principles or determine the most active fraction.

1. Monograph specifications

The institute has been preparing a monograph on Adenosma indianum to be included in the second edition of the National Phamacopoeia. The consultant recommended that more details should be included in the mongraph and guided the staff in doing so. Identification tests using TLC of essential oils and flavonoids so as to distin guish indianum from the other two species were worked out.

Other criteria such as the ash content, water and alcohol soluble extractives were also included (Annexure 30). Further work is necessary to determine the differences in the flavonoid polyphenol and saponin constituents among the three species. GLC analysis of essential oils of the three species was done to use the chromatograms as finger-prints to differenciate the three species. The work on this plant could not be completed as the scientist responsible for this work had to go on official leave.

2. Adenosma Tablets

The protocol used presently for the extraction of Adenosma needs quantitative details as the extraction is carried out by immersing a sackful of cut pieces of the aerial parts in water and boiling for 2 hours. Two such extracts are made. There is no attempt to retain the volatile oil components which will get steam distilled during the process. There is also drastic heat treatment in evaporating the combined extracts to get a concentrate and again heating with the excipients to get a dry mass. No attempt has been made to standardise the extract.

The consultant conducted some preliminary work using organic solvents for extraction but could not complete the work as the scientist detailed to do this work had to go abroad on a training fellowship. However the consultant recommended procedures for the improvement of the method of extraction (Annexure 31) and trial formulations for the tablets (Annexure 32).

A method for the standardisation of the drug extract has to be worked out. It is suggested that the flavonoid or polyphenol content be used for this purpose.

G. APD Liquid

APD is a liquid compound preparation used in the treatment of periodontitis, gingivitis and acute inflammation of the buccal mucosa. Considerable amount of research had been done in the Institute of Materia Medica to determine the pharmacological activities of the three individual plant extracts. The compound liquid preparation has been subjected to clinical trials with encouraging results.

The preparation contain the extractives of the following :

Aerial parts of Solanum hainanensis	- 6 parts	by weight
Roots of Achyranthes bidentata	- 2 parts	"
Bulbs of Eleutherina subaphylla	- 2 parts	"

The final preparation contains 13.5% of alcohol and is menthol flavoured. The mode of administration is to keep 10 ml of liquid in the mouth for one hour or longer. The consultant had discussions about the dosage form, as firstly an alcoholic solution is being used to be in contact with the sensitive buccal mucosa for a long time and secondly that the mode of treatment is very inconvenient to the patient. He suggested that a slow releasing lozenge or pastille form would be a more convenient form or if a liquid is still essential, then an aqueous preparation to be formulated. /The purpose of using alcohol was not evident as alcohol has not been used in the extraction and as benzoic acid has been added as a preservative/. The sample examined by the consultant was a cloudy suspension and seemed to have stability problems.

The method of preparation lacked a great deal of efficiency and reproducibility as large pieces were being extracted, the quantity of water used being not measured and the time of extraction being conveniently fixed. The final dilution was made to get the extractive of 1 g of the compound drugs per ml.

The consultant after considering the chemical constituents of the three plants, recommended improved procedures for the extraction (Annexure 33). The use of alcohol for extraction although is expensive at present due to difficulties of recovery will be a saving when pilot plant facilities are used to recover nearly the total quantity of alcohol used. Besides a better product will result as lower temperatures are used for the extraction and removal of solvent, thus retaining active principles which are thermolabile. Extracting with a large volume of water and then concentrating would be wasteful as much material will be precipitated some times together with the active principles (for a liquid preparation).

The consultant requested the prepared extract concentrate so that he could do some trials on a solid formulation but due to time constraints it was not supplied. Hence he recommended two formulations (Annexure 34) which could be tried and clinically tested. A new liquid formulation was also recommended (Annexure 33) in the case of a preference for a liquid dosage form on grounds of efficacy.

6. UTILIZATION OF RESULTS

The specifications developed for both plant and phytochemical raw materials can be included as monographs in the National Pharma copoeia. They can immediately be used for the quality essessment of raw materials before purchasing or before being used in manu facture of the products. The assays developed can be used in the determination of the potency and content uniformity of the finished products. The specifications for the finished products may have to be amended slightly after pilot scale production as they were drawn up on lab scale production.

The tablet formulation developed for the phytochemicals can be used for the production of the tablets but some modifications would be necessary to upscale them to pilot scale level production.

The recommended inprocess controls and the evaluation criteria for tablets can be introduced once the instruments are received.

Achyranthes syrup could be produced using the new formulation and its quality assessed but tablets have to await the evaluation of the proposed methods of extraction.

The methods of extraction and formulations recommended for Adenosma and APD preparation are to be evaluated both chemically and pharmacologically to see whether further modifications would be required.

Standardisation of Achyranthes extract could now be done for the preparations to be used as antiinflammatory drugs using the haemo lytic index for saponins.

Systematic stability testing and shelf life determination of the finished products could be commenced for every product made in the Institute. Tablets with a better stability and finish could be produced by following the sugar coating procedure recommended. It is hoped that the seal coat and the polishing procedures will be introduced. Film coating on a large scale will have to await proper equipment and facilities.

The good manufacturing practices demonstrated and recommended to be introduced should be given high priority in implementation.

7. CONCLUSIONS

The consultant within the constraints of facilities and the allocated time could only accomplish the tasks that have been described in chapter five. When one scrutinizes the job description leaving items (a) and (d) the tasks have been adequately covered.

The counterpart staff were given a good training in research methods on how to improve formulations, develop new formulations, solve problems, conduct accelerated stability studies and determine the shelf life of medicaments. As the facilities in the lab were very scanty and inadequate, make shift arrangements and improvisations had to be done. For example clarification problems had to be settled with filtration through filter papers. Though methodology has been imparted, actual processes are still to be demonstrated using pilot plant facilities. The product and formulation development procedures were restricted only to the facilities and chemicals available in the laboratory.

Some granulations are done by drying the wet mass and granulating the dried mass using a dry granulator. This is an improvisation done at the Institute which has many disadvantages. The necessary range of stainless sieves are also not available for large scale manual procedure.

The quality of tablets cannot be higher than the quality of granules from which they are made. Thus the design of the granulation system is a crucial one and the procurement of a drying cabinet should be urgently considered.

Achyrathes syrup was produced using a new formulation but could still be very much improved using the facilities available in the pilot plant. The development of tablet formulations of plant extracts will have to wait the commissioning of the pilot plant as the facili ties available presently are not efficient. Drying during sugar coating on a large scale is done by using a hot plate system to heat the pan. There is no exhaust system except a room fan. This method should be discontinued if the products of a modern pilot plant facility are to be improved to the desired quality. Hot air inlet system and an efficient exhaust system are essential. If film coating is to be introduced spray equipment is also necessary. A couple

of steam jacketted kettles would be a worthwhile addition to the facility. Another important factor which affects the quality of coating and the process in general is the relative humidity of the hot air and the environment. Good results could be obtained using air with a relative humidity between 35 - 50%. The RH of the laboratory was near 90% when the coating was done. Hence taking the weather conditions of Hanoi into account, an air conditioned room with a dehumidified air flow should be considered.

The counterpart staff were trained in the modern manufacturing practices by conducting a series of lectures. Wherever possible demonstrations were done to train the persons who were on the job. The practical training was restricted to the equipment and chemicals available but the necessary theory was imparted with the hope that the theory could be applied when required.

The facilities in the standardisation laboratory were limited being a newly established laboratory. But most of the tasks expected from that division were accomplished in that quality standards for most of the raw materials indicated by the management were completed. Those of finished products were done in a draft form pending the products of large scale manufacture. The counterpart staff were trained in use of modern spectroscopic and analytical methods and on the development of assay procedures and identification tests. Standardisation of plant extracts was demonstrated using the haemolytic index for saponins as an example.

Process monitoring methods were explained and protocols was recommended but could not be demonstrated. The production of dosage forms, stability tests, labelling and packaging, purchase and storage and quality assessment of pharmaceuticals in accordance with good manufacturing practices were stressed and demonstrated wherever possible.

The main tasks of pilot scale production of modern dosage forms in a standardised and a stabilised form and the development of techniques to obtain extracts of the required quality using the pilot plant facilities can only be accomplished after the commissioning of the pilot plant including the tablet machine.

The consultant has to conduct well controlled scale up experiments on the tablet formulations developed in the laboratory in order to accurately define the variables such as drying time of granules,

residual moisture, granule size reduction, blending times etc. within specified limits. When a new formulation is to be transferred to production, the research pharmacist should be responsible for the initial scale up and the production runs.

The high speed tablet production also requires the proper granule flow from the hopper to the die cavity. The flow has to be monitored so as to make the necessary modifications to the formula.

Another important aspect of pilot plant production is to ensure that the tablet characteristics developed by the consultant are efficiently reproduced on the production scale.

The consultant feels that unless he completes the above tasks, his job only would have been half done and the benefits of his mission would not be fully realised. Hence he recommends a re-visit during the time of the presence of the process engineering expert so that the full utilisation of the pilot plant facilities in the production of drugs made by the Institute could be demonstrated and the pilot scale production of dosage forms organised.

8. RECOMMENDATIONS

1. As the quality of a medicament has to be built into the product and not assayed in, it is recommended that all operations connected with the production of drugs from raw materials to the packaged product should be carried out in accordance with good manufacturing practices detailed and developed by the consultant.

2. An important requirement for a tablet making area is a controlled environment. The temperature, air flow, humidity and light have to be maintained at optimal levels throughout the year in spite of the changes in the weather. The consultant therefore recommends that the Institute considers the suitable air conditioning of the tabletting areas including a dehumidified air flow system as an urgent need for the production of good quality tablets throughout the year.

3. The facilities afforded by the pilot plant are modern, efficient and time saving. But some equipment are required to ensure the production of efficacious and accurate dosage forms of reproducible quality.

The consultant therefore recommends for the kind consideration of the UNDP the procurement of the following items to realize the objectives as envisaged.

(1) A heavy dury slugging machine. (2) Drying cabinet with trays.

(3) An air handling system and spray equipment for the coating pan. It would be preferable to have a deduster. A strip packing machine would ensure a suitable packing system for the modern dosage forms.

4. As the attainment of the major objectives of the project is the pilot scale production of quality dosage forms for sustained clinical trials in the first instance, the consultant recommends that he be recalled after the commissioning of the pilot plant to upscale laboratory processes, to conduct studies on process monitoring and process optimization, to standardise the plant extracts, and to demonstrate through the pilot plant facilities the stages where the facilities of unit operations could be properly utilized in the improvement of the preparation of extracts and finished products so that the pilot plant would be maximally used. He could also develop the protocols necessary for the transference of pilot scale production procedures to industrial factories for large scale production.

5. Proper drying of wet granules is an essential requirement to get the required flow properties for the achievement of weight and content uniformity in tablets. The consultant recommends that the Institute get the ovens repaired to include the necessary variable controls.

6. The development and improvement of products, stability testing and quality assessment are continuous operations of a pilot plant set up. As such the consultant recommends that the Institute considers the upgrading of facilities in the formulation and standardisation laboratories on a priority basis. Improvement of the facilities in these labs even stagewise will add to the quality performance and efficiency of operations.

9. ACKNOWLEDGEMENTS

The consultant gratefully acknowledges with thanks

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2. The kind cooperation and assistance received from Prof.Dr. Nguyen Van Dan, the Hon'ble Vice Minister of Health, Prof. Nguyen Gia Chan, the Director of the Institute, Mme Prof. Dean Thi Nhu, the National Project Director, Mr. Le Tung Chau and Mr. Truong Canh, the Vice - Directors of the Institute and Mrs. Khuong Bang Tuyet, the Project Officer.

3. The valuable work and cooperation received from the counterpart scientists who worked with the consultant without whose efforts the work described here would not have been possible. Special mention should be made of the Heads of Divisions Mr. Nguyen Thuong Thuc in particular, Mrs. Le Thi Thuyet, Mr. Nguyen Que, Prof. Do Viet Trang and Mrs. Nga in particular Mrs. Thu, Mrs. Hoa and Mr. Voi who worked hard to achieve these results.

4. The valuable cooperation and assistance received from the hard working Project Secretary, Mr. Nguyen Tuong Dung who helped the consultant throughout the mission in accomplishing many a task that would have otherwise been delayed.

5. The friendship and cooperation received from all the staff of the Institute.

ANNEXURE 1

PARTICIPANTS AT THE MEETINGS

1. Porf.Dr. Nguyen Van Dan, Vice Minister of Health
2. Prof. Nguyen Gia Chan, Director of I.M.M.
3. Dr. C.K. Atal, C.T.A.
4. Prof. Doan Thi Nhu, N.P.D.
5. Mr.Truong Canh, Vice Director of I.M.M.
6. Mr. Le Tung Chau, Vice Director of I.M.M.
7. Mrs. Khuong Bang Tuyet, Project Officer
8. Mr. Nguyen Thoung Thuc, Head of Formulation Division
9. Mr. Nguyen Que, Head of Pilot Plant
10. Mrs. Nguyen Thi Thuyet, Head of Standardisation Division
11. Prof. Do Viet Trang, Head of Analytical Division
12. Mr. Pham Kim Man, Head of Planning Division
13. Dr. Do Trung Dam, Head of Pharmacological Division
14. Mr. Nguyen Tuong Dung, Project Secretary

ANNEXURE 2

CHEMICALS BROUGHT BY THE CONSULTANT

1. Sodium benzoate
2. Benzoic acid
3. Sodium metabisulphite
4. Sodium bisulphite
5. Methyl, p-hydroxybenzoate (Methylparaben)
6. Propyl, p-hydroxybenzoate (Propylparaben)
7. Butylated Hydroxyanisole (BHA)
8. Butylated Hydroxytoluene (BHT)
9. Talc (Asbestos free)
10. Stearic acid
11. Magnesium stearate
12. Sorbic acid
13. Polyethylene glycol 2000 (PEG)
14. Methyl cellulose 400
15. Methyl cellulose 4000
16. Microcrystalline cellulose
17. Carboxymethyl cellulose (CMC)
18. Hydroxypropylmethyl cellulose (HPMC)

ANNEXURE 3

MONOGRAPH ON 1 - TETRAHYDROPALMATINE HYDROCHLORIDE

Tetrahydropalmatine Hydrochloride contains not less than 98.0% and not more than 102.0% of $C_{21}H_{25}O_4N.HCl$ calculated with reference to the dried substance.

Description . White or creamy white crystalline powder; odourless. Turns yellow when exposed to direct sun light.

Solubility . Soluble in 500 parts of water, 100 parts of chloroform, 100 parts of methanol, 150 parts of alcohol. It is fairly soluble in hot water.

Identification

A. Dissolve .01 g of the drug in 2.0 ml of conc. sulphuric acid and warm. Gives a colourless solution which turns orange red upon the addition of 2.0 ml of conc. nitric acid. Dilution with water results in an orange red precipitate.

B. Dissolve 0.10 g in 20 ml of water by warming, add 0.5 ml of dilute nitric acid. Cool and filter after 10 minutes. Add to 3.0 ml of the filtrate, 1.0 ml of silver nitrate solution (5%); white precipitate is formed.

C. Carry out thin layer chromatography on a 0.05% solution of the drug in methanol using the following systems

(a). Silica GF_{254} - solvent system; Toluene : Ethyl acetate :Dimethyl amine (14 : 2 : 1)

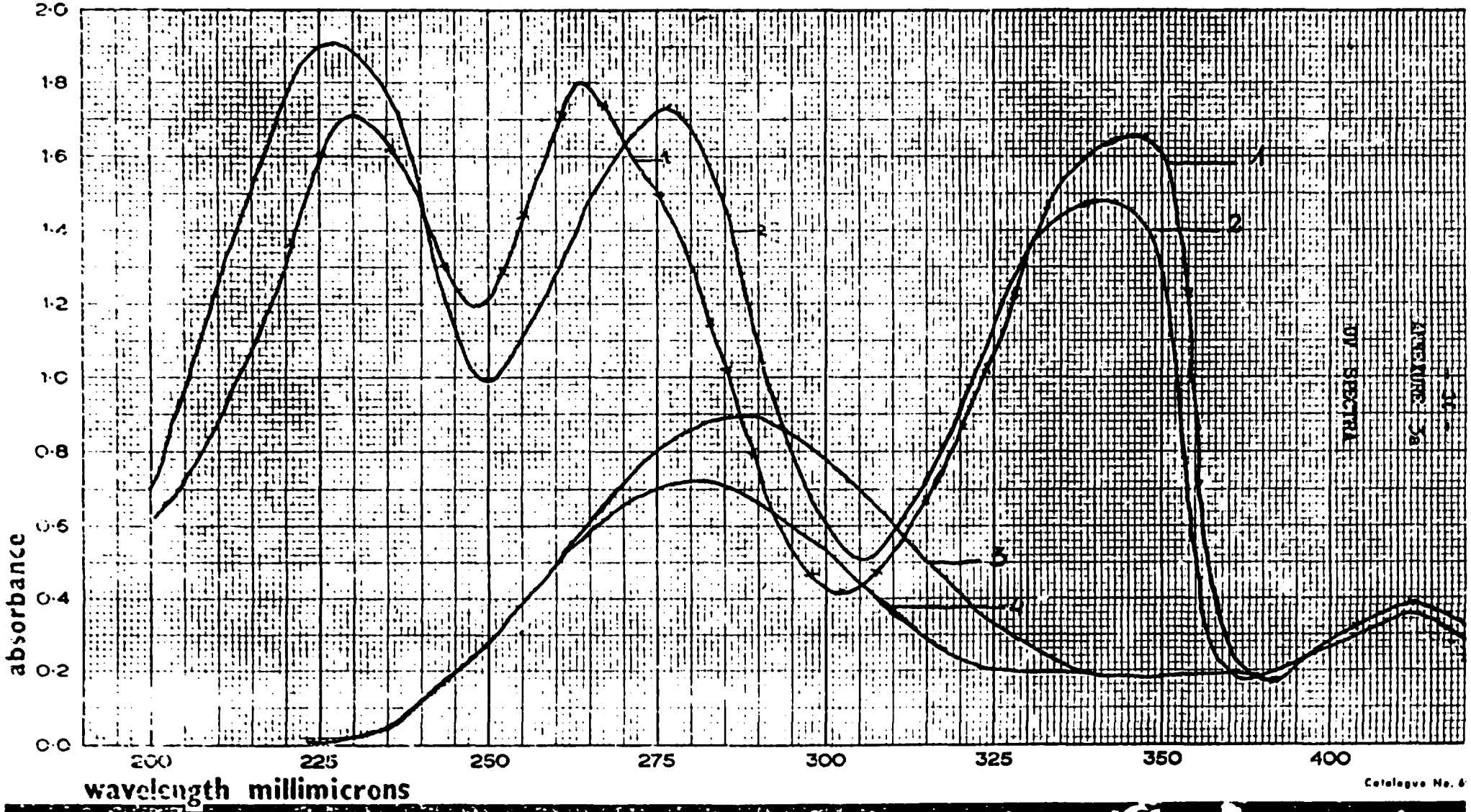
Flourescent spot when examined at 365 nm. Rf 0.76

(cf THB, HCl Rf 0.84)

ULTRAVIOLET SPECTROPHOTOMETER

UNICAM SP.800

UNICAM INSTRUMENTS LIMITED, CAMBRIDGE



Catalogue No. 6

ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA UV Spectra (of equivalent dilutions)

CONCENTRATION
REFERENCE
PATH LENGTH

1. Berberine Chloride
2. Palmatine Chloride
3. THB. HCl
4. TMP. HCl

SPEED FAST ☐ SLOW ☐

DATE

OPERATOR

(Contd. Annex 3)

(b) Alumina F_{254} - Solvent system; n-hexane : Chloroform :GL actic acid (45 : 45 : 10)
Flourescent spot when examined at 365 nm; Rf 0.46
(cf Rf .49 for THB, HCl)
Dragendorf's reagent gives orange coloured spots.

N.B.

Rf values depend on the plates as they are hand made.

Purity
1. A solution of 0.10 g in hot water should be clear.
2. Specific rotation $[\alpha]_D^{20}$ = - 291 (0.9% in Ethanol)
3. Melting range 213 - 216°C.

Related substances . In the HPLC assay, any peaks other than the main peak should be less than 2% of the area of the main peak.

Acidity . Shake 0.10 g with 30 ml of water, filter. Add 2 drops of phenolphthalein to the filtrate and then 0.10 ml of .1M sodium hydroxide; colourless solution should change to pink colour.

Loss on drying to constant weight at 105°C. Not more than 5.0%

Ash content . Not more than 0.2%

Assay . Carry out the assay described in Annexure 5. The amount of drug should be within the limits specified in the monograph.

Storage . Tetrahydropalmatine hydrochloride should be kept in well closed containers protected from light.

ANNEXURE 4

USE OF CECIL CE 202 UV SPECTROPHOTOMETER FOR ASSAY PURPOSES

Example - Tetrahydropalmatine Hydrochloride should be in the pure form.

(This method is not applicable for the assay of THP, HCl in finished products or to test for purity as the wavelength selected (λ max) is interfered by the oxidised products of THP, HCl).

As the instrument does not give reproducible readings, the use of a reference solution is recommended. For this assay potassium dichromate (heated at 110°C for 4 hours and cooled) was used as the standard reference. Pure THP, HCl was also available.

After studying the absorbance of the two compounds at 282 nm a suitable concentration was selected in order to determine the specific absorbance ($E^{1\%}_{1cm}$) of each compound.

0.004% w/v of the two solutions were made accurately by diluting concentrated solutions. Potassium dichromate solution was made with 0.01 N sulphuric acid while THP, HCl solution was made in absolute alcohol. The absorbances of the two solutions were measured several times to get the average value.

The absorbance (A_R) of dichromate = 0.40

The absorbance (A_T) of THP, HCl = 0.56

$A_R = E_R \times .004$ $\quad E_R = E^{1\%}_{1cm}$ of dichromate

$A_T = E_T \times .004$ $\quad E_T = E^{1\%}_{1cm}$ of THP, HCl

$$\frac{E_R}{E_T} = \frac{A_R}{A_T} = \frac{.40}{.56} = \frac{1}{1.4}$$

Assay

1. When the concentration of THP, HCl test sample is unknown, a solution is made weighing accurately the equivalent of 20 mg of THP, HCl and making up to 100 ml with alcohol. 10 ml of this solution is diluted to 50 ml to give the test solution (C_T)

(Contd. Annex 4)

2. A solution of potassium dichromate is made by dissolving 20 mg in 500 ml of 0.01 N H_2SO_4.
   Measure the absorbances with reference to the respective solvents.
   Let the absorbance of test solution be $A_{T'}$
   Let the absorbance of dichromate be A_R

$$A_{T'} = E_T \times C_T \quad , \quad A_R = E_R \times .004$$

$$\frac{A_{T'}}{A_R} = \frac{E_T}{E_R} \times \frac{C_T}{.004}$$

$$\therefore C_T = \frac{E_R}{E_T} \times \frac{A_{T'}}{A_R} \times .004$$

$$= \frac{1}{1.4} \times \frac{A_{T'}}{A_R} \times .004$$

$\therefore C_T$ can be calculated as $A_{T'}$ + A_R are known.

This is the method of assay of Berberine Chloride given in the Japanese and Indian Pharmacopoeias.

ANNEXURE 5

HPLC ASSAY PROCEDURE FOR TETRAHYDROPALMATINE HYDROCHLORIDE (THP,HCl)

1. Prepare a standard solution

   Weigh accurately about 50 mg of anhydrous pure THP, HCl transfer to a 10 ml volumetric flask, dissolve in methanol and make up to volume.

2. Prepare the sample solution to be tested in the same way.
   (a) Weigh accurately the equivalent of about 50 mg of anhydrous THP,HCl and make up the solution to 10 ml with methanol.
   (b) If tablets are being tested, powder 20 tablets (weighed) and weigh accurately the powder equivalent to about 50 mg of THP,HCl. Transfer the powder into a 10 ml volumetric flask, add 5 ml of methanol warm to get THP,HCl into solution, cool and make up to volume.
   (c) Each 50 mg tablet could be extracted and made up to 10 ml with methanol if content uniformity of the tablets is being determined.

3. Carry out HPLC analysis using the following operating conditions.

Machine	HITACHI 365 A Liquid Chromatograph
Column	2.1 x 500 mm
Stationary phase	Hitachi gel 3010 (reverse phase)
Mobile phase	Methanol
Detection	UV absorbance at 282 nm
Flow rate	1 ml per minute
Chart speed	2.5 mm / min.

Inject between 5 and 10 µl of the solutions separately. Symmetrical peaks with an elution time between 10 and 20 minutes will be obtained. Hence peak heights could be used to determine the amount of drug in the test solution. (Calibration curve or matching peaks could be used.

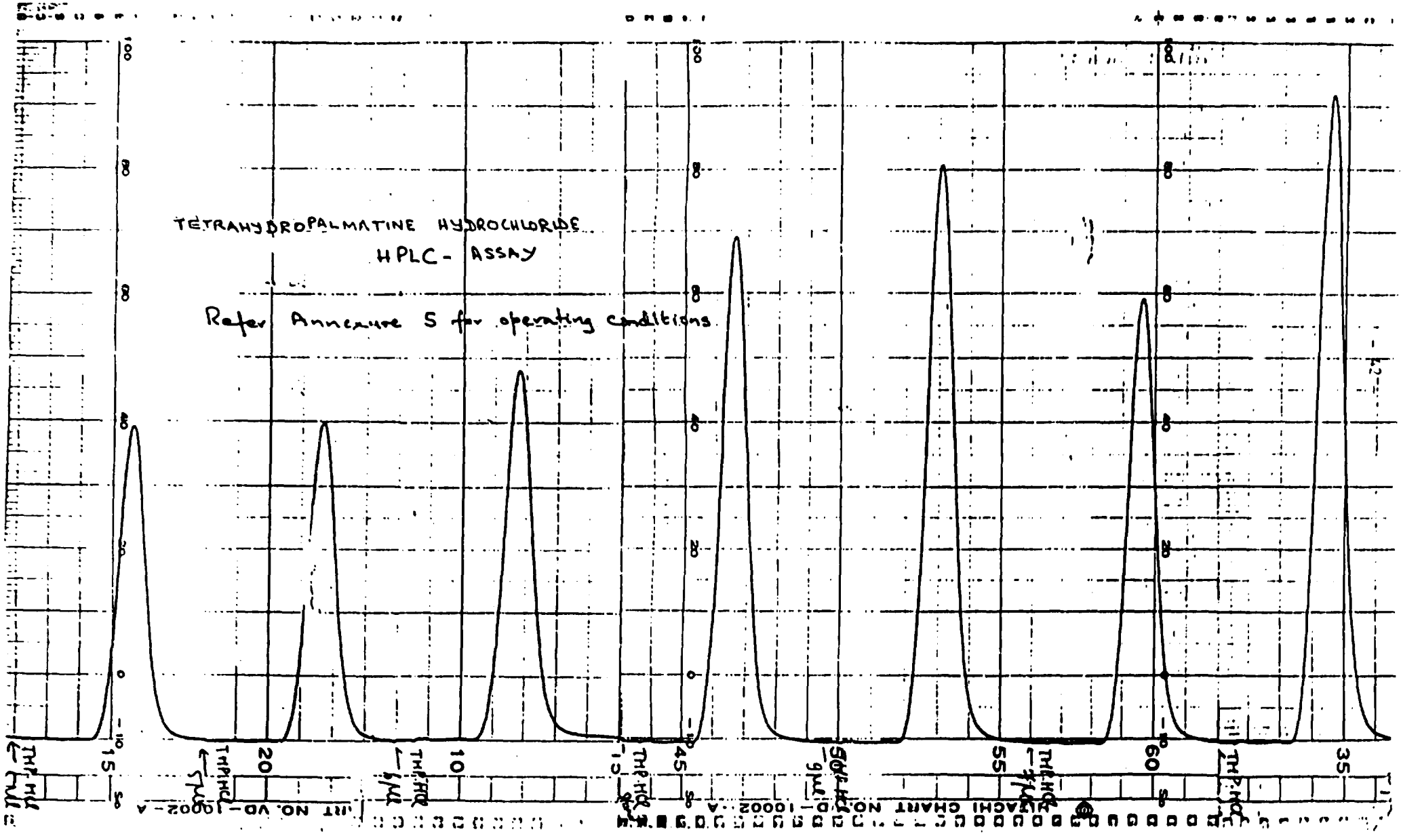
If any peaks other than the main peak appear, contamination is indicated. Peak appearing between 3 and 6 minutes could be due

(Contd. Annex 5)

to palmatine chloride in the sample. Check the area to determine whether the related substance is within permissable limits.

N.B. 1. It is strongly recommended that the standard and the test be run under the same instrumental conditions each time.

2. Repeat determinations are necessary to ensure reproducibility of the responses.


TETRAHYDROPALMATINE HYDROCHLORIDE
HPLC - ASSAY
Refer Annexure 5 for operating conditions

ANNEXURE 6

ACCELERATED STABILITY TESTING MODEL

The Table I given below is based on a model which indicates the maximum and minimum times at which the potency of a product must be at least 90% of that claimed in the label when stored at indicated temperatures, if it is to have a shelf life of 2 years at room temperature (25°C).

Temperature (°C)	Maximum time	Minimum time
37	12 months	6.4 months
50	6.8 "	1.7 "
60	4.1 "	3 weeks
70	2.6 "	9 days

Table I

In order to test whether the product will have a shelf life of 2 years at room temperature, one has to determine the potency (assay) of the product after storing at various temperatures for the periods indicated. The potency should not be less than 90% of the label claim for both the maximum and minimum times indicated. At least two temperatures must be studied.

The effect of light and moisture could be combined with these temperatures to determine their effects on stability.

These results could be used to fix a tentative expiration date until the actual stability tests are performed.

The assay schedule required for the above model will be as follows:

Temperature (°C)	Times at which samples are to be assayed
37	6, 8, 12 months
50	1, 2, 5, 7 months
60	20 days, 2, 4 months
70	9 days 2, 3 months

N.B. 1. Sample size examined should ensure statistical validity.

2. Storage when testing, should be done in the same containers as that in which the drug is to be marketed.

3. Storage conditions should also related to those to be stated in the label.

ANNEXURE 7

STABILITY PROTOCOL

The main variables to be considered are temperature, light and moisture. In addition colour, hardness and other physical charac teristics, preservative stability and container properties are also included.

The effect of moisture is usually studied by varying the relative humidity, the effect of temperature in constant temperature ovens and the effect of light in a light cabinet using clear and amber bottles.

Storage conditions can be combinations of these variables eg. RH 60% at 15°C, RH 80% at 15°C, RH 60% and 80% at 25°C.
The stability sampling programme recommending when samples are to be assayed is given below :

Temp.(°C)	Time at which a sample has to be assayed (months)												
	0	1	2	3	4	6	9	12	18	24	36	48	60
4					x			x		x			
15	x		x	x	x	x	x	x		x		x	x
25	x	x		x		x	x	x	x	x	x	x	x
37		x	x		x	x		x		x			
50		x	x		x	x		x					

N.B.

1. Sample size examined should ensure statistical validity
2. Storage when testing should be in the same containers as those in which the drug is marketed.
3. At each sampling stage, the characteristics of the dosage form have to be measured eg. colour, hardness, friability, weight variation, disintegration time and drug content for tablets
4. Preservative stability and container characteristics when applicable will have to be studied.

ANNEXURE 8

FORMULATION AND MANUFACTURING INSTRUCTIONS

Product and Potency : Tetrahydropalmatine Hydrochloride Tablets - 50 mg

Product Code No.

Batch size 5000

Formula	Grams per tablet	Grams per batch
1. Tetrahydropalmatine Hydrochloride	.050	250.0
2. Starch	.040	200.0
3. EDTA	.002	10.0
4. Starch (binder)	.003	15.0
5. Dry starch	.004	20.0
6. Stearic acid	.001	5.0
Total :	.100	500.0

Steps

1. Mill items 1, 2, and 3 and screen through a 0.1 mm sieve
2. Blend the milled powder thoroughly
3. Prepare 10% w/w starch paste binder
4. Mix the binder with the powder blend to form wet mass
5. Granulate the wet mass through a 1.0 mm sieve
6. Dry the granules in paper lined trays after spreading them only ½" thick at 45°C overnight
7. Screen the drug granules through 1.0 mm sieve
8. Screen item 5 and 6 through a fine mesh sieve (nylon cloth)
9. Mix the screened granules with items 5 and 6
10. Submit representative samples to standardisation division to determine if the blend is homogeneous
11. Upon receipt of clearance compress using 6 mm concave punches
12. Submit representative samples of the finished product to quality control before release for coating

N.B. In-process controls should be carried out during tabletting. The tablets should be stored protected from moisture and light.

ANNEXURE 9

QUALITY CONTROL PARAMETERS FOR THE EVALUATION OF TABLETS

1. Appearance, colour, taste
2. Hardness, between 5 - 7 kg (uncoated tablets)
3. Friability, not more than 1% (uncoated tablets)
4. Thickness uniformity, ±5% (uncoated tablets)
5. Weigh uniformity of uncoated tablets

   Weigh 20 tablets individually and collectively, calculate the average weight. The tablets are acceptable only if no more than two tablets differ from the average weight by no more than the percentage indicated below and no tablet differs by more than double the percentage.

Average weight	Variation allowed	Only up to 2 tablets can be between	No tablet shall be above
130 mg or less	10 %	10 - 20%	20%
131 - 325 mg	7.5%	7.5- 15%	15%
More than 325 mg	5 %	5 - 10%	10%

6. Content uniformity (for tablets of 50 mg or less of drug) Select 30 tablet samples. Assay 10 of these individually. The tablets are acceptable if the content of each of the 10 tablets are within the limits of 85 and 115% of the average of the tolerences specified in the potency definition of the monograph of the drug.

   If the content of more than one tablet falls outside these limits and none of tablets falls outside the limits of 75 and 125%, the remaining 20 tablets are assayed individually. All 20 tablets should fall within the limits 85 - 115% of the average potency if the batch is to be acceptable.

7. Disintegration time.

   Disintegration apparatus could be used.

(Contd.Annex 9)

Uncoated tablets

Place six tablets in the six tubes and add a disc to each tube. Immerse the basket in the beaker containing the required volume of water at 37°C (±2°C) and start the apparatus. The time taken for the six tablets to disintegrate and pass through the mesh is taken as the time of disintegration. In order to fix this parameter a representative number of samples should be tested. Once the time is included in the monograph all subsequent batches of tablets should conform to this specification.

At this stage if one of the six fails to disintegrate during the specified time, the test is repeated with 12 additional tablets. Not less than 16 of the total 18 tablets must disintegrate within the time specified.

Plain coated tablets (sugar and film coated other than enteric coated)

The six tablets are placed in the tubes and the basket is immersed in water at room temperature for 5 minutes. Then a disc is added to each tube and the basket is immersed in water at 37°C (±2°C). The apparatus is operated for 60 minutes. If the tablets do not disintegrate within this time they are immersed in a IN HCl solution at 37°C (± 2°C) and the test continued for another 30 minutes. If still one or two fail to disintegrate, the test is repeated with 12 additional tablets and not less than 16 of the total 18 tablets must diintegrate within the time specified.

Dissolution Time - May be required for certain tablets.

ANNEXURE 10

IN-PROCESS CONTROLS FOR TETRAHYDROPALMATINE HYDROCHLORIDE (50 mg)

uncoated tablets

Weight of tablet	0.100 g
Weight range	0.090 - 0.110 g
Hardness range	6 - 8 kg
Thickness range	2.5 mm ± 5%
Friability	less than 1.0%
Disintegration time	not more than 20 minutes
Diameter	6 mm
Colour	white or creamy white

ANNEXURE 11

TETRAHYDROPALMATINE TABLETS (Gindarin tablets)

Tetrahydropalmatine Tablets contain l-tetrahydropalmatine hydrochlo ride. They may be sugar coated.

Content of l-tetrahydropalmatine hydrochloride should be 92.5 to 107.5 per cent of the stated amount.

Identification

A. Extract 0.05 g of powdered tablets with 5 ml of methanol. Filter, evaporate off the methanol. Dissolve the residue in 2.0 ml of conc. sulphuric acid and warm the solution. Colourless solution turns orange - red on adding 2.0 ml of conc. nitric acid.

B. Exctract 0.02 g of powdered tablets with 20 ml of water by warming, filter and add 0.5 ml of dilute nitric acid. Cool and filter again after 10 minutes. Add to 3.0 ml of filtrate, 1.0 ml of silver nitrate solution (5%); white preci pitate is obtained.

C. Extract .01 g of powdered tablets with 5 ml of methanol and carry out the TLC analysis given in Annexure 3 for THP. HCl.

Disintegration time — Not more than 20 minites. Sugar coated tablets up to a maximum of 60 minutes.

Optical rotation — Extract a quantity of powdered tablets equiva lent to 250 mg of THP, HCl with absolute alcohol (10 ml), filter into a 25 ml volumetric flask and make up to volume with washings from the residue on the filter paper. Measure the optical rotation of the solution at 20°C. It should be between - 2.4 and -2.6.

Related substance . Any peak if present in the HPLC chromatogram used for the assay, the area of it should not be more than 2% of the area of the main peak.

Assay . Perform the assay for tablets as decribed in Annexure 5. The results should be within the limits specified in this monograph.

Storage. Tetrahydropalmatine hydrochloride tablets should be kept in well closed containers protected from light.

ANNEXURE 12

PROCEDURE FOR SUGAR COATING
(LADLE PROCESS)

Pilot Plant Scale

The drying times indicated will be different in the absence of a proper air handling system.

I. Sealing :

1. Prepare the dusting powder, pass the powder through fine sieve, weigh the required quantities, mix thoroughly and sieve again.

Calcium carbonate 65%
Talc 35%

2. Prepare the sealing solution

40% w/v solution of shellac in alcohol or
3-7% w/w of cellulose Acetate Phthalate or
Hydroxyprophyl-methylcellulose
or
Hydroxypropylcellulose
in Acetone or methanol or alcohol.

3. Heat the tablets to 30°C.
4. Start the pan and immediately apply the sealing solution in sufficient quantity to wet all the tablets (Mix and distribute). In 2-3 minutes the tablet mass will become tacky.
5. Before excess tack develops, add sufficient quantity (q.s) of dusting powder evenly throughout the tablet bed to prevent tablets from sticking together and to the pan. After 2 - 3 minutes the powder will be absorbed into the seal coat.
6. Apply full volume drying air at 25°C for 20 - 30 minutes.
7. If there is excess dusting powder in the pan, remove the powder before applying the next coat.

(Contd. Annex 12)

8. Repeat procedures 4 - 7 using somewhat less sealing solution and dusting powder for a second and if required for a third coat.
9. Remove the tablets from the pan and place in a tray and dry at 25 - 30°C overnight to ensure that all residual solvent is removed.

N.B. Care must be taken not to apply more coating than is required for sealing purposes.

II Sub coating :

1. Prepare the subcoating dusting powder mix fine powder and sieve

Calcium carbonate	40%	w/w
Titanium dioxide	6%	w/w
Talc	25%	"
Powdered sugar	29%	"

2. Prepare the subcoating solution -

Gelatin	6%	w/w
Acacia powder	6%	"
Sugar	47%	"
Water	41	"

3. Preheat the sealed tablets to 30°C using warm dry air (40 - 50°C). The pan should be rotated every 2 - 3 minutes during the heating process. Alternatively if the tablets were main tained at 30°C overnight they can be added to the pan just before coating.
4. Start the pan rotating and apply the first subcoat with the drying air off. Exhaust must be on.
5. Mix the tablets to get all the tablets wet and within a few minutes the tablets will become tacky and ride up in the pan.
6. Add dusting powder (q.s.) immediately to cover all the tablets sufficiently (may be within 2 - 4 revolutions of the pan).
7. Once the dusting powder is evenly distributed, allow the tablets to roll with the exhaust on for 3 - 5 minutes.

(Contd. Annex 12)

8. If too much powder has been added, it will collect under the bottom of the tablets. This powder must be removed by taking out the tablets in this area first, then removing the powder. Replace the tablets in the pan.
9. Direct the drying air (40 - 50°C) on to the tablet bed to dry the tablets. 25 - 30 minutes may be required depending on the efficiency of the air flow system. Tablets should be hand mixed to ensure even drying.
10. Check the degree of dryness by scraping a tablet with your thumb nail and also with the end of a spatula. Dried tablet will be impervious to the thumb nail and slightly penetrable by the edge of the spatula.

    N.B. Do not make the next application before the present application is thoroughly dry.
11. Repeat the procedures 4 to 10 for the second coat. Allow 30 minutes between each application.
12. As much as seven subcoats (or even more until the tablets are rounded) can be applied. Increase slightly the volume of coating solution and dusting powder from the forth coat onwards.
13. Remove tablets from the pan and if they are still spongy or damp dry overnight to ensure thorough drying. If dry the smoothing procedure can start once the pan is cleaned.

III. Smoothing :

1. Prepare the smoothing suspension -

Calcium carbonate or		
Tricalcium phosphate	13 %	w/w
Titanium dioxide	4 %	"
Starch	3 %	"
Sugar	50 %	"
Water	30 %	"

   N.B. If the subcoated tablets are very smooth, simple sugar syrup (70% w/w) can be used.
2. Preheat the tablets to 30°C.
3 Apply the first coat with the exhaust on and the drying air off.

(Contd. Annex 12)

4. Mix the tablets to ensure that all the tablets are wet, paying particular attention to see that the edges of the tablets are wet.
5. In 2 - 3 minutes the tablets will start to dry losing their glossy wet appearance. Then direct the drying air (40 - 50°C) on to the lower one third of the tablet bed till the tablets are completely dry (Mix the tablets occassionally)
   N.B. Check drying by placing the back of the hand against the moving tablets when dust will be evident on it.
6. Repeat procedures 3 to 5 for the successive coats until the desired weight and the smoothness are reached.
7 The drying time of the last two coats must be increased to further smooth the tablets for colouring.

   This is done by turning on the drying air about 5 - 6 minutes later instead of the earlier 2 - 3 minutes and having the drying air at room temperature.

IV. Colouring :

1. Prepare the colour syrup.
   First prepare simple syrup (70% w/w) preferably in a steam jacketted kettle. Maintain the temperature around 45°C (25 - 60)

   Either Prepare a stock solution of colour syrup 01 - 06%
   Dilute to .005% for the first few coats and gradually increase the concentration of colourant.

   or Use a colour concentrate (Lake colour dispersion)
   Mix one volume of concentrate with two volumes of syrup at 45° C.
   Add another six volumes of syrup and agitate until a uniform suspension results.

   Prepare the solution needed for 2 - 4 hours.

(Contd. Annex 12)

2. Preheat tablets to 30°C. It is important that all the tablets are at the same temperature.

3. Add colour solution/suspension to the rotating tablet bed. Heated air (40°C) is directed on the tablet bed and the exhaust is fully opened. In about one minute the tablets should be completely wet. Check whether the edges are wet and mix.

4. Check drying by placing the back of the hand against the moving tablet bed (dust)

   N.B. Do not allow the tablets to tumble after they are dry and dusting freely.

5. Repeat the procedures 3 and 4 until the desired colour is obtained. In about eight application, the full colour should develop and then another seven applications will serve as smoothing coats. The conditions recommended are as follows:

	Drying Time	Drying Air	Exhaust
Coats 1 to 8	5 minutes	40° C	Full
" 9 to 12	10 minutes	25° C	Full
" 13 to 14	15 minutes	None	Full
" 15	15 minutes	None	None

6. Finally three finishing applications of clear syrup (70% w/w) are done.

7. Wet the tablets with clear syurp, dry for 10 - 12 minutes until the tablets from front and back of the pan show the same dusty appearance.

8. Do another application similar to 7.

9. In the third application, mix the tablets and in 2 - 3 minutes the tablets will lose their gloss and begin to ride higher in the pan. Then stop the pan and rotate it at intervals as

(Contd. Annex 12)

indicated below :

First minute	-	Rotate ½ turn every 10 seconds
Next 5 minutes	-	Rotate ½ turn every 30 seconds
Next 54 minutes	-	Rotate ½ turn increasing the interval from one minute to 10 minutes

10. Remove the tablets from the pan and place in racks (25 - 30° C) for at least three hours or overnight prior to polishing.

V. Polishing :

1. Prepare the polishing pan (wax lined or convas lined pan)
2. Prepare the polishing solution or suspension - White Bees wax 5 - 7% w/w in chloroform or acetone.

   or

   Carnauba wax 6% w/w Bees wax 4% w/w in isopropanol
   (Disperse the powders in isopropanol)

3. Add the polishing wax suspension or solution to the tablet bed. As the tablets tumble in the pan the solvent evaporates giving a shine to the tablets. After 10 - 15 minutes, check a tablet by polishing with a piece of cloth. If it takes high polish, do not add any more wax but allow the pan to rotate until the expected shine is obtained.

   N.B. More wax may be added if the tablets do not give a good polish.
   Do not allow the tablets to tumble once the desired polish is obtained.

ANNEXURE 13

MONOGRAPH ON BERBERINE CHLORIDE

$C_{10}H_{18}CINO_4 . xH_2O$

Berberine chloride contains not less than 97.0 % and not more than 102.0 % of $C_{20}H_{18}CINO_4$ (M.W. 371.82) calculated with reference to the anhydrous substance. It crystallises with up to three molecules of water of crystallization.

Description . Yellow crystalline powder; odourless with a very bitter taste.

Solubility . Sparingly soluble in cold water but freely soluble in hot water. Soluble in 500 parts of methanol, sparingly in alcohol and insoluble in chloroform. It is soluble in hot ethanol.

Identification

A. Dissolve 0.01 g in 20 ml of water by warming. Cool and add 1.0 ml of potassium iodide solution (10%), yellow precipitate is formed.

B. Dissolve 0.10 g in 20 ml of water by warming, add 0.5 ml of dilute nitric acid. Cool and filter after 10 minutes. Add 1.0 ml of silver nitrate solution (5%) to 3.0 ml of the filtrate; white precipitate is formed.

C. Carry out thin layer chromatography on a 0.05% solution of the drug in 50% alcohol using the following system

Alumina F_{254}. Solvent system, n-hexane : chloroform ; Glacial acetic acid (45 : 45 : 10)
Yellow fluorescent spot when examined at 365 nm.
Rf = 0.19
(cf Palmatine Rf .21)
Rf values vary as hand prepared plates are used.

(Contd. Annex 13)

Purity 1. 0.10 g of drug when dissolved in water by warming should give a clear solution.

Acidity Shake 0.10 g with 10 ml of water, filter. To the filtrate add 2 drops of phenolphthalein and 0.1 ml of .1M sodium hydroxide; yellow colour should change to orange red colour.

Loss on drying (0.5 g) Not more than 16.5%

Ash content (1.0 g) Not more than 0.5 %

Assay Carry out the assay using HPLC as described in Annexure 14 or the following method.

1. Dissolve 20 mg of potassium dichromate (dried at 110°C for 4 hours and cooled) in 0.01N sulphuric acid and make up to 100 ml in a volumetric flask. Determine the absorbance of this solution at 421 nm (A_R).

2. Dissolve 20 mg of the drug in 20 ml of water by warming in a 100 ml volumetric flask and make up to volume. Measure 10 ml of this solution accurately into another 100 ml volumetric flask and dilute with water to 100 ml. Determine the absorbance of the second solution at 421 (A_B).

   The weight of berberine chloride in mg =

$$\text{mg of } K_2Cr_2O_7 \times \frac{A_B}{A_R} \times \frac{1}{1.006}$$

   The amount of berberine chloride should be within the limits specified in this monograph.

Storage Berberine Chloride should be kept in well closed containers protected from light and stored at a temperature not exceeding 40° C.

ANNEXURE 14

HPLC ASSAY OF BERBERINE CHLORIDE

1. Prepare a standard solution of berberine chloride
   Weigh accurately about 10 mg of berberine chloride crystallized from methanol, transfer into a 10 ml volumetric flask, add 5 ml of distilled water and warm to dissolve the drug. Cool and make up to volume with methanol.

2. Prepare the test solution in the same way
   (a) Weigh accurately the equivalent of 10 mg of anhydrous berberine chloride, dissolve in 5 ml of water by warming and make up to 10 ml with methanol.

   (b) To assay tablets (50 mg), powder 10 tablets after weighing, and weigh accurately the powder equivalent to 10 mg of berberine chloride. Transfer to a 10 ml volumetric flask, add 5 ml of water, warm to extract the drug, cool and make up to volume methanol.

   (c) 50 mg tablets could be assayed by extracting each tablet with 25 ml of hot water and making up to 50 ml with methanol to check the uniformity of content.

3. Carry out the HPLC analysis using the following running conditions

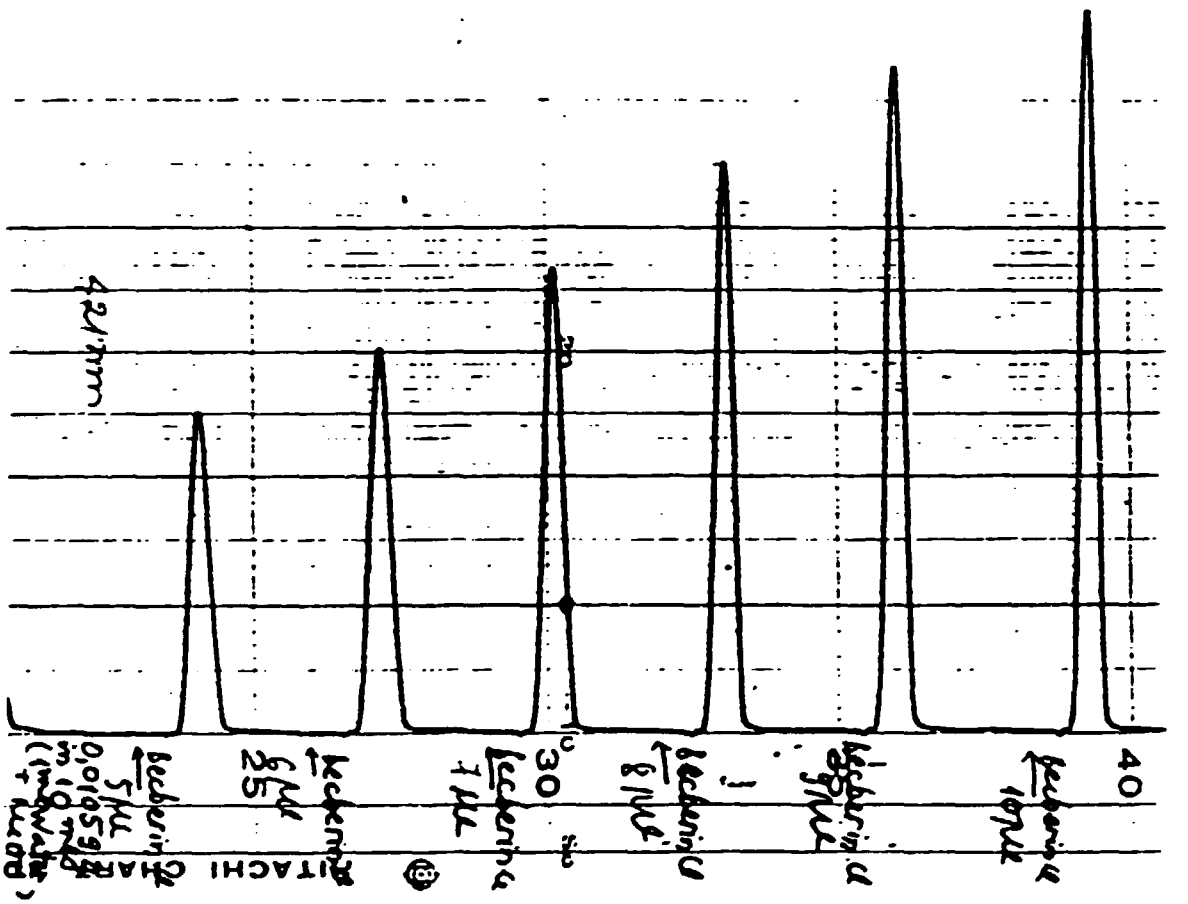
Machine	Hitachi 635 A Liquid Chromatograph
Column	2.1 mm x 500 mm
Stationary phase	Hitachi gel 3010 (reverse phase)
Mobile phase	Methanol
Detection	UV absorbance at 421 nm
Flow rate	1.0 ml per minute
Chart speed	2.5 mm/ minute

Inject between 5 and 10 µl of each solution separately. Symmetrical peaks with an elution time between 3 and 6 minutes will be obtained. Hence peak heights could be used to calculate the content of the drug in the test sample (If the peaks are not symmetrical peak areas will have to be used).

N.B. 1. Repeat determinations to ensure reproducibility of response
2. It is strongly recommended that both the standard and test solutions be run under the same instrumental conditions.

BERBERINE CHLORIDE - HPLC ASSAY

See Annexure 14 for operating conditions



ANNEXURE 15

FORMULATION AND MANUFACTURING INSTRUCTIONS

Product and Potency - Berberine Chloride, 50 mg (crystallized from water)

Product Code Number

Batch Size 3000

Formula	Grams/Tablet	Grams/Batch
1. Berberine Chloride	.050	150.0
2. Starch	.040	120.0
3. Binder	.003	9.0
4. Dry starch	.005	15.0
5. Stearic acid	.002	6.0
Total :	.100	300.0

Steps

1. Mill items 1 and 2 and screen through a 0.1 mm sieve
2. Blend the two powders thoroughly
3. Prepare 10 % w/w starch paste
4. Mix the binder with the powder blend to get a wet mass
5. Granulate the wet mass through a 1.0 mm sieve
6. Dry the granules at 40°C in paper lined trays after spreading them ½" thick
7. Screen the dry granules through a 1.0 mm sieve
8. Screen items 4 and 5 through a fine mesh
9. Mix the screened granules with items 4 and 5
10. Submit representative samples to standardisation division to determine if the blend is homogeneous
11. On clearance, compress using 6 mm concave punches
12. Submit representative samples of finished product to quality control before final release

N.B. 1. In process controls should be carried out during tabletting of large batches.

2. Tablets should be stored in closed containers at a temperature not exceeding 40°C.

ANNEXURE 16

IN-PROCESS CONTROLS FOR BERBERINE CHLORIDE TABLETS (50 mg)

Weight of tablet	0.100 g
Weight range	0.090 - 0.110 g
Hardness range	6 - 8 kg
Thickness range	2.5 mm ± 5%
Friability	less than 0.5 %
Disintegration time	not more than 15 minutes
Colour	yellow

ANNEXURE 17

BERBERINE TABLETS

Berberine tablets contain Berberine Chloride. They may be film coated. Content of berberine chloride should be 92.5 to 107.5% of the stated amount.

Identification

A. Extract 0.20 g of powdered tablets with 20 ml of water by warming, filter and add 0.5 ml of dilute nitric acid. Cool and filter again after 10 minutes. Add 1.0 ml of silver nitrate solution (5%) to 3 ml of the filtrate; White precipitate results.

B. Extract 0.02 g of the powdered tablets by warming with 50 ml of water. Cool, filter and add 1 ml of potassium iodide solution (10 %); yellow precipitate is formed.

C. Carry out thin layer chromatography as follows :
Extract 0.01 g of powdered tablets into 5 ml of methanol. Run a chromatogram. Alumina F_{254} - Solvent system; n-hexane : chloroform : Glacial acetic acid (45 : 45 : 10).
Yellow fluorescent spot when examined at 365 nm Rf = 0.19 (cf with Rf = 0.21 for palmatine)

Orange spot with Dragendorf's reagent.

Disintegration time : Not more than 15 minutes

Related substances : The above thin layer chromatogram should not contain other yellow fluorescent spots.

Assay : Perform the assay as described in Annexure 14 for tablets or Annexure 13 using a quantity of powdered tablets equivalent to 20 mg of the drug to prepare the test solution. The results should be within those specified in this monograph.

Storage : Berberine Chloride Tablets should be protected from light and stored at a temperature not exeeding 40 ° C.

ANNEXURE 18

PROCEDURE FOR FILM COATING
(Lad le Technique)

Essential Requirements :

(a) An efficient and an adequate air handling system for drying and removal of solvent. The exhaust fan should have much larger capacity of the drying air to ensure that no solvent escapes into the room.
eg. for a 36 inch pan with a charge of 20 kg of tablets, the capacity of drying air should be 300 c.f.m. with an exhaust of 500 c.f.m. for an application interval of thirty minutes.

(b) If conventional pans are used, they should preferably be equipped with baffles to ensure adequate mixing of the tablets and to prevent sliding.

(c) A system for spraying the solution.

Prepare the film coating solution :

The solution should consist of the following constituents :

Essential (a) Resin (e.g. Hydroxypropylmethyl cellulose (HPMC), Ethyl cellulose, Cellulose acetate phthalate (AP), Methyl cellulose, Shellac, Methacrylic polymer)

N.B. If a non water dispersable resin is used, a small quantity of water dispersable resin such as polyethylene glycol (PEG), Glyceryl monostearate should also be used.

(b) Plasticizer (e.g. Propylene glycol, Glycerin, castor oil, Diethylphthalate, Acetylated monoglycerides)

N.B. One part per 10 parts of polymer

(c) Solvent (e.g. Methylene chloride, Ethanol, Methanol, Isopropanol, Acetone or preferably solvent blends.)

Optional (a) Colourant Insoluble aluminium lake pigment

(b) Opacifier (e.g. Titanium dioxide, Magnesium carbonate, Magnesium oxide, Aluminium hydroxide when colouring or protection from sun light are required.)

(Contd. Annex 18)

(c) Wetting agent (e.g. Tween 80, when the polymer solution does not wet the tablet easily).

A suitable coating solution formulation can be tried out on a trial scale.

The following two formulae could be tried.

A. Formula I

For a transparent coat (Base solution)

H.P.M.C.	4.4 %	w/w
Ethylcellulose	1.0 %	"
Acetylated monoglycoside	0.6 %	"
Methyl alcohol	34.0 %	"
Methylene chloride	60.0 %	"

Colour solution

Colour dispersion (concentrate)	6.0 %	w/w
Base solution	94.0 %	"

or 0.1 % w/w of colour in base solution

N.B. An opacifier should have been added to the base solution before coating.

Gloss solution

Glycerin	2.0 %	w/w
Base solution	33.0 %	"
Methyl alcohol	22.0 %	"
Methylene chloride	43.0 %	"

B. Formular II

1. Cellulose acetate phthalate	5.0 g
2. Propylene glycol	2.0 ml
3. Tween 80	0.6 ml
4. Colourant	0.1 g
5. Castor oil	0.3 ml
6. Stearic acid	1.0 g
7. Polyethylene glycol 6000	14.0 g
8. Alcohol	20.0 g
9. Acetone to	100 ml

Dissolve 1,2,3 and 8 in about 20 ml of acetone. Warm about 30 ml of acetone and add 4,5,6 and 7. Mix the two solutions and make up to volume with warm acetone.

(Contd. Annex 18)

1. Prewarm the tablets to 25° - 30° C.
2. Apply the coating solution with the drying air off and the exhaust on.
3. Mix to ensure that all the tablets are wet. It is safer to under-wet than overwet.
4. After about a minute apply the drying air when the tablets will begin to dry and start to ride in the pan.
5. If there is sticking apply small quantities of talc.
6. Stop the pan when the tablets have lost most of the tack and are free.
7. Rotate the pan as follows :

   (a) ½ a turn every 10 seconds for the first minute.
   (b) ½ a turn every 30 seconds for four minutes.
   (c) ½ a turn every two minutes for 25 minutes.

   N.B. The pan can be rotated at 2 r.p.m. for this whole period.
8. Onece the tablets are dry repeat the procedures 2 to 7 for the subsequent coats until the colour has developed.
9. Two further applications without the colourant can then be applied after dilution with an equal volume of solvent.
10. Thirty minutes after completion of coating (drying) the tablets can be tumbled freely to enhance the lustre.
11. Remove the tablets from the pan, place in racks and dry at 25 - 30° C for 24 hours.

ANNEXURE 19

MONOGRAPH ON TETRAHYDROBERBERINE HYDROCHLORIDE

Tetrahydroberberine hydrochloride contains not less than 98.0% and not more than 102.0 % of $C_{20}H_{21}O_4N$. HCl calculated with reference to the dried substance.

Description White to pale yellow crystalline powder; odourless. Yellow colour intensifies when exposed to air and light.

Solubility Soluble in 150 parts of hot water, 300 parts of chloroform, 150 parts of methanol and 250 parts of ethanol. It is very slightly soluble in cold water.

Identification

A. Dissolve 0.01 g in 2 ml of conc. sulphuric acid; gives a yellow coloured solution which turns red on warming.

B. Dissolve 0.01 g in 20 ml of water by warming, add 0.5 ml of dilute nitric acid. Cool and filter after 10 minutes. Add to 3.0 ml of filtrate, 1.0 ml of silver nitrate solution (5 %); White precipitate is formed.

C. Carry out thin layer chromatography on a 0.05 % solution of drug in methanol using the following systems.

(a) Silica GF_{254} - Solvent system; Toluene : Ethylacetate: Dimethylamine (14 : 2 : 1).
Flourescent spot when examined at 365 nm, Rf 0.84 (as compared with THP, HCl Rf 76)

(b) Alumina F_{254} - Solvent system ; Benzene : Chloroform : Glacial acetic acid (45 : 45 : 10)
Flourescent spot when examined at 365 nm Rf 0.49 (as compared with Rf 0.46 for THP, HCl)
Spray reagent : Dragendorf's reagent gives orange spots
Rf values may vary as hand made plates are used.

(Contd. Annex 19)

Purity

1. 0.10 g when dissolved in water by warming should give a clear solution.

2. Melting point range 170 - 172° C.

Limit for Berberine Chloride

Assay for Berberine Chloride using the method given in Annexure 13 or 14

The amount of berberine chloride should not be more than 2.0% calculated with reference to dried substance.

Acidity

Shake 0.10 g with 30 ml of water, filter. Add 2 drops of phenolphthalein to the filtrate and then 0.1 ml of 0.1 M sodium hydroxide; colourless or pale yellow solution should change to pink colour.

Loss on drying to constant weight at 105° C Not more than 1.0%

Ash content Not more than 0.5 %

Assay Conduct the assay described in Annexure 20
The amount of drug should be within the limits specified in this monograph.

Storage Tetrahydroberberine Hydrochloride should be kept in well closed containers protected from light and stored at a temperature not exceeding 30° C.

ANNEXURE 20

HPLC ASSAY OF TETRAHYDROBERBERINE HYDROCHLORIDE (THB,HCl)

1. Prepare a standard solution
   Weigh accurately about 50 mg of anhydrous pure THB, HCl; transfer to a 10 ml volumetric flask, dissolve in methanol and make up to volume.

2. Prepare the sample solution to be tested in the same way
   (a) Weigh accurately the equivalent of about 50 mg of anhydrous THB, HCl and make up the solution to 10 ml with methanol
   (b) If tablets are being tested, powder 20 tablets (weighed) and weigh accurately the powder equivalent to about 50 mg of THB, HCl. transfer the powder into a 10 ml volumetric flask, add 5 ml of methanol, warm to get the THB, HCl into solution, cool and make up to volume.
   (c) Each 50 mg tablet could be extracted and made up to 10 ml with methanol if the content uniformity of the tablets is being determined.

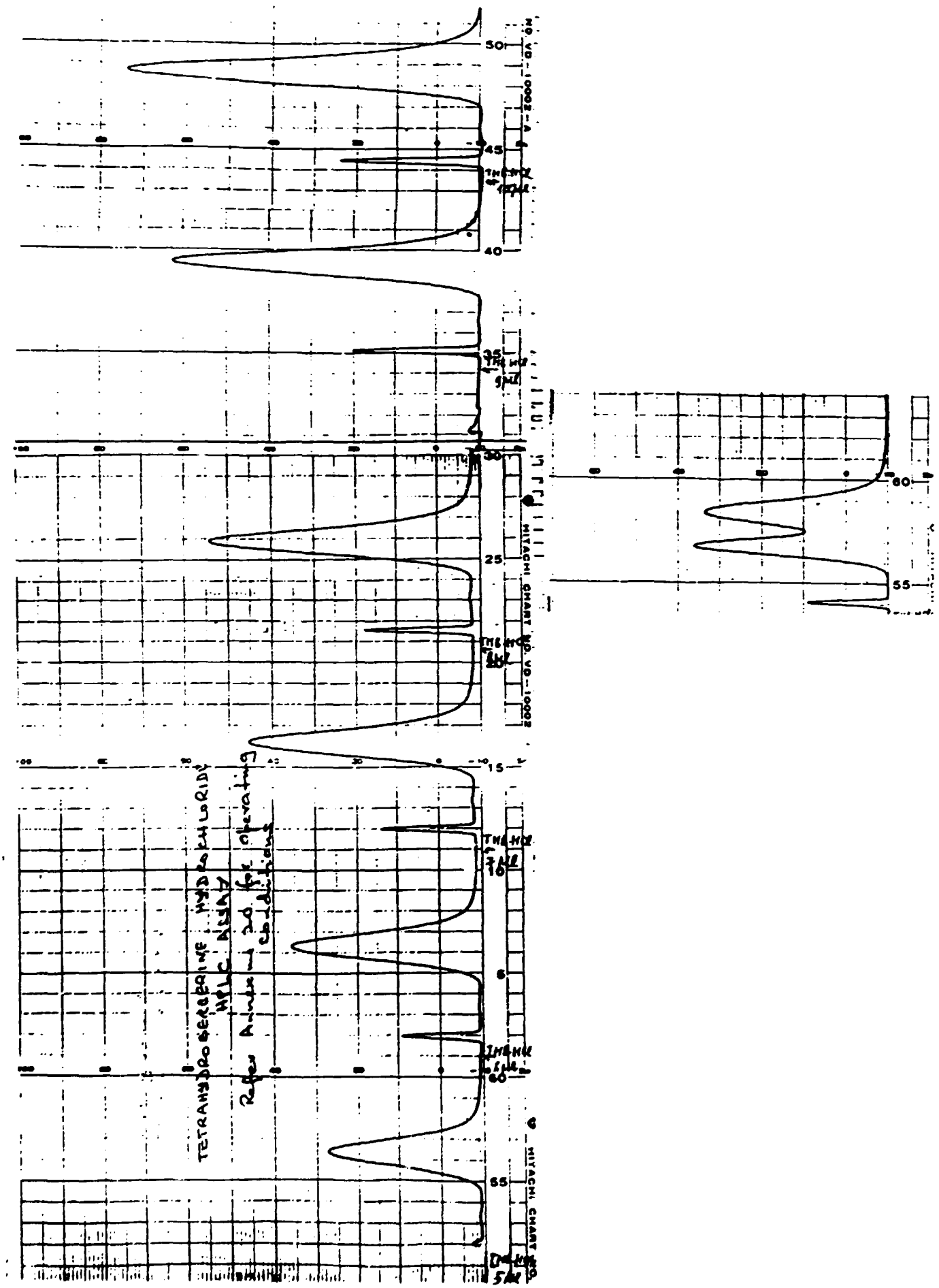
3. Carry out HPLC analysis using the following running conditions.

Machine	Hitachi 365 A Liquid Chromatograph
Column	2.1 mm x 500 mm
Stationaly phase	Hitachi gel 3010 (reverse phase)
Mobile phase	Methanol
Detection	UV absorbance at 286 nm
Flow rate	1 ml per minute
Chart speed	2.5 mm/minute

Inject between 5 and 10 µl of the solution separately. Symmetrical peaks with an elution time between 14 and 30 minutes will be obtained. Hence peak heights could be used to calculate the amount of drug in the test solution.

If any peaks appear before this time (or after), contamination is indicated. If the peak has an elution time between 3 - 6 minutes the presence of berberine chloride in the sample is indicated. In this case the sample solution should be run with berberine chloride as standard at 421 nm to determine the amount of berberine chloride and check whether it is within permissible limits.

N.B. :It is strongly recommended that fresh solution of both standard and test be made as solutions of THB, HCl undergoes oxidation readily.


TETRAHYDROSERPENTINE HYDROCHLORIDE
HPLC ASSAY
Refer Analytical Conditions for operating
HITACHI CHART NO. VD-10002

ANNEXURE 21

FORMULATION AND MANUFACTURING INSTRUCTIONS

Product and Potency - Tetrahydroberberine Hydrochloride Tablets, 50 mg

Product Code Number

Batch size 4000

Formula	Grams/Tablet	Grams/Batch
1. Tetrahydroberberine Hydrochloride	.050	200.0
2. Starch	.038	152.0
3. EDTA	.002	8.0
4. Starch (binder)	.004	16.0
5. Dry starch	.004	16.0
6. Stearic acid	.002	8.0
Total :	.100	400.0

Steps

1. Mill items 1, 2 and 3 and screen through a 0.1 mm sieve
2. Blend the sieved powder well
3. Prepare a 10% w/w starch paste binder
4. Mix the starch paste with powder blend to get a wet mass
5. Screen through a 1.0 mm sieve
6. Dry the wet granules in paper lined trays ½" thick at 45°C
7. Screen dry granules through 1.0 mm sieve
8. Screen items 5 and 6 through a fine mesh sieve
9. Mix the screened granules with items 5 and 6
10. Submit representative samples to standardisation division to check if blend is homogeneous
11. Upon clearance compress using 6 mm concave punches
12. Submit representative samples of finished product to quality control for clearance

N.B. In process controls should be carried out during tabletting Storage Tablets should be stored protected from moisture and light.

ANNEXURE 22

TETRAHYDROBERBERINE TABLETS

Tetrahydroberberine tablets contain tetrahydroberberine hydrochloride. They may be sugar coated.

Content of tetrahydroberberine hydrochloride should be 90 to 110% of the stated amount.

Identification

A. Extract 0.05 g of the powdered tablets with 5 ml of methanol. Filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of conc. sulphuric acid and warm the solution; a red coloured solution is formed.

B. Extract 0.20 g of the powdered tablets with 20 ml of water by warming, filter and add 0.5 ml of dilute nitric acid to the filtrate. Cool and filter after 10 minutes. Add 1 ml of silver nitrate solution (5%) to 3 ml of the filtrate; white precipitate is formed.

C. Carry out thin layer chromatography as follows :
Extract 0.01 g of powdered tablets with 5 ml of methanol and run a chromatogram using the systems described in annexure 19. the spot should correspond to that of authentic sample.

Disintegration time : Not more than 20 minutes. Sugar coated tablets up to a maximum of 60 minutes.

Berberine Chloride : Determine the amount of berberine chloride in the powdered tablets using the procedure given in annexure 14 for tablets. It should not be more than 2% of the stated amount of drug.

Assay : Carry out the HPLC assay for tablets given in Annexure 20. The results should be within those specified in this monograph.

Storage : Tetrahydroberberine Hydrochloride tablets should be kept in well closed containers protected from light and moisture.

ANNEXURE 23

IN-PROCESS CONTROLS FOR TETRAHYDROBERBERINE HYDROCHLORIDE (50 mg)

uncoated tablets

Weight of tablet	0.100 g
Weight range	0.090 - 0.110 g
Hardness range	5 - 7 kg
Thickness range	25 mm ± 5 %
Friability	less than 1 %
Disintegration time	Not more than 20 minutes
Colour	pale yellow

ANNEXURE 24

MONOGRAPH ON ACHYRANTHES ROOTS

Achyranthes roots consists of partially dried roots of Achyranthes bidentata Blume.

Description : The drug contains long slender cylindrical main root with some lateral roots. Sometimes parts of the stem are found at the crown. The roots are coloured light brown to brownish black depending on the period of storage. The taste also varies from slightly sweet newly dried roots to sweeter old roots. The roots are soft with longitudinal wrinkles. Fracture is smooth with no fibrous material.

Macroscopy :

Microscopy :

Identification :

A. An aqueous extract (0.5 %)gives a lasting foam on shaking.
B. An aqueous extract (5 %) gives a positive test for reducing sugars.
C. Carry out the following thin layer chromatographic analysis:

(a) Extract 5 g of finely cut roots with 20 ml of water by heating on a water bath. Cool and filter. Extract the filtrate with 2 x 15 ml of n-butanol. Run a chromatogram of the butanol extract using silica GF 254.
Solvent system; n-butanol: Ethanol: Ammonia (7:2:5).
Spray with vanillin/H_2SO_4 reagent. Compare the TLC profile with the authentic finger print (3 spots).

(b) Evaporate the above butanol extract to dryness, add 10 ml of 5N hydrochloric acid to the residue and reflux for 15 minutes. Cool and extract the contents with 2 x 10 ml of ether. Wash the combined ether extracts with water, dry with Na_2SO_4 (anhydrous) and evaporate to dryness. Run a chromatogram on the residue using silica GF 254.
Solvent system; n-butanol : Gl. Acetic Acid : water (5:1:4).
Use oleanolic acid as a reference.
The spot obtained should correspond to oleanolic acid.
Spray reagent - Vanillin/H_2SO_4.

(Contd. Annex 24)

Foreign matter : Not more than 10 % stem parts. Good quality drug should contain less than 5 % of stem material. Other foreign matter not more than 1 %.

Loss of drying (6 hours) : Not more than 20 %.

Ethanol soluble extractive : Not less than 50 %.

Water soluble extractive : Not less than 60 %.

Reducing sugars : Not more than 15 %.

Ash content : Not more than 5 %.

Acid insoluble ash : Not more than 1.5%

Assay : Apparent haemolytic index as determined by the procedure given in Annexure 25 should be above 130.

Storage : Achyranthes roots should be stored in a dry place at a temperature not exceeding 25° C.

ANNEXURE 24A

PROCEDURE FOR THE DETERMINATION OF
MONOGRAPH SPECIFICATIONS

1. Foreign matter : Take coarsely powdered 100 g of drug. Spread thin and pick the parts not included in the monograph. Then pick the other foreign matter which are not plant parts.

   (1) Determine the % of plant parts not specified.
   (2) Determine the % of foreign matter

2. Loss on drying : Weigh 1 - 5 g of powdered material to a weighed dish. Dry to constant weight at 105° C. Certain drugs will require prolong drying periods to get to constant weight. Then a time limit can be specified in the monograph.

3. Ash content : Heat 1 - 3 g of powdered drug in a weighed platinum or silica dish to a temperature not exceeding 450° C until free from carbon. Cool and weigh. (If it still contains carbon wash the mass with hot water, filter through an ashless filter paper collecting the filtrate. Ignite again to 450°C the filtrate and evaporate to dryness at 450° C).

4. Acid insoluble ash : Boil ash obtained from 3 for five minutes with 25 ml of 2M HCl, filter through a weighed gooch crucible, wash with hot water and ignite to 450° C. Cool and weigh.

5. Water soluble ash : Boil ash from 3 for five minutes with 25 ml of water. Collect insoluble matter in a weighed gooch crucible wash with hot water, ignite for 15 minutes at 450° C. The difference in weights will give the water soluble ash.

6. Water extractive : Take 5 g of powdered air dried drug, add 100 ml of water into a flask. Shake frequently for six hours and keep closed for another 16 hours. Filter, evaporate 20 ml of filtrate in a weighed vessel to dryness and dry to constant weight at 105° C.

7. Ethanol soluble extractive : Add 5 g of powdered air dried drug into 100 ml of 40% alcohol in a closed vessel. Shake frequently during the first six hours and keep closed for additional 16 hours. Filter rapidly, evaporate 20 ml of filtrate to dryness in a weighed vessel and dry the residue to constant weight at 105° C.

(Contd. Annexure 24A)

8. Chloroform/Hexane soluble extractive : Extract 5 g of powdered with 100 ml of solvent for 24 hours shaking and releasing the pressure during the first six hours. Filter through a Buchner funnel containing a weighed filter paper taking care to transfer all the residue. Wash residue with the solvent, dry it in air to constant weight. Difference in weight will give the solvent soluble extractive.

ANNEXURE 25

DETERMINATION OF HAEMOLYTIC INDEX OF ACHYRANTHES

1. Prepare the test solution as follows :

   A. Roots : Extract about 4 g (accurately weighed) of finely cut roots of Achyranthes with 100 ml of buffer solution in 250 ml flask by heating on a hot water bath for 30 minutes. (keep the flask covered with a glass funnel). Agitate the contents at least four times. Filter hot into a 100 ml volumetric flask.

   B. Extract from percolation : Dilute 15 ml of extract buffer solution to 100 ml.

   C. Syrup : Dilute 25 ml of syrup with buffer solution to 100 ml.

2. Buffer solution (pH 7.4)

KH_2PO_4	1.743 g
Na_2HPO_4	9.596 g
NaCl	9.00 g
Distilled water to	1000 ml

3. Blood suspension : Remove fibrin in bovine blood by agitating with glass beads or a rough stick. Take 10 ml and dilute to 500 ml with buffer solution.

4. Standard Saponin Solution (0.02 %) : Dissolve 0.02 g of saponin (HI = 25000) in 100 ml of buffer solution.

TEST Take 10 tubes and add the solutions (ml) as indicated below:

Solution No;	1	2	3	4	5	6	7	8	9	10
1. Test solution	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2
2. Buffer solution	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8
3. Blood suspension	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Note the first tube which is transparent as the limit dilution after 2 hours.

eg. Let the tube with limit dilution be 5

Repeat test using this limit dilution as the twelfth tube as follows:

Solution No.	1	2	3	4	5	6	7	8	9	10	11	12
1. Test soln	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10	2.15	2.20
2. Buffer soln.	3.35	3.30	3.25	3.20	3.15	3.10	3.05	3.00	2.95	2.90	2.85	2.80
3. Blood	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Determine the limit dilution (first transparent tube) after 6 hours

eg. Let it be tube 9. Then the Apparent Haemolytic Index (hi) $= \frac{10}{W}$

W = weight of the drug contained in the test solution filled into the test tube with limit dilution (9) expressed in grams (related to dry drug at 100° C).

Haemolytic Index = hi x F

F = Blood Factor

Determination of F : Conduct the test with seven test tubes using the standard saponin solution

Solution No.	1	2	3	4	5	6	7
1. Standard saponin soln.	1.0	1.5	2.0	2.5	3.0	3.5	4.0
2. Buffer solution	4.0	3.5	30	2.5	2.0	1.5	1.0
3. Blood suspension	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Note the limit dilution after 6 hours.

Perform dilution test using the limit dilution as tube No. 12 as before.

Keep for 12 hours and note the limit dilution.

Calculate hi(s) for the standard saponin

$$F = \frac{25000}{hi(s)}$$

ANNEXURE 26

PREPARATION OF ACHYRANTHES SYRUP

1. Cut the roots (2 kg) into very small pieces
2. Mix with alcohol (96 %) and keep covered for 4 hours
3. Pack the percolator, add alcohol to completely soak and cover the material
4. Macerate for 48 hours
5. Percolate slowly with alcohol until 2 l are collected (Percolate I)
6. Continue percolation with water to collect 3 litres (Percolate II)
7. Remove the alcohol saparately from the two percolates
8. Mix the two aqueous concentrates
9. Dilute with water up to 2.2 l and add sodium benzoate (3 g)
10. Adjust the pH to 6 - 6.5
11. Keep closed for 24 hours.
12. Filter to get the extract
13. Add glycerin (can be ampouled at this stage)
14. Get the extract evaluated
15. Prepare the syrup if necessary using the following formula

Liquid extract	2.2 l
Glycerin	0.3 l
Sugar	1.25 kg
Sod. metabisulphite	.002 kg
Purified water to	3.0 l

Dissolve the sugar in the liquid extract by warming and and boil for 2 - 3 minutes. Cool, add sodium metabisulphite and flavour. Make up to volume with water. Keep closed for 24 hours - 48 hours. Filter.

N.B. Check the pH and adjust to 6 - 6.5

Storage : Achyranthes syrup should be stored in well closed containers at a temperature not exceeding 30° C.

ANNI 'E 27

SPECIFICATIONS FOR ACHYRANTHES SYRUP

Description	A clear golden brown syrupy liquid with a sweet taste and an aroma of anise
Solubility	Miscible with water forming a clear solution
Acidity	pH 6 - 6.5
Weight per ml	1.25 - 1.27 g
Identification	A. A solution of 0.5 ml of syrup in 5 ml of water when shaken should give a lasting foam B. Extract 5.0 ml of syrup with 3 x 10 ml portions of n-butanol. Combine the butanol extracts, wash with water and evaporate to dryness to get residue A. Carry out TLC analysis on res idue A using the system given in Annexure 24. Compare with the finger print from authentic sample. C. Add 15 ml of 5N Hydrochloric acid to residue A and reflux for 15 minutes. Cool and extract the contents with 3 x 15 ml portions of ether. Combine the ether extracts, wash with water and dry with anhydrous sodium sulphate. Evaporate off the ether and carry out the TLC analysis on the residue using the system given in Annexure 24. The only spot should correspond to oleanolic acid.
Reducing sugars	6.0 - 8.0 %
Ash content	Not more than 2.0 %
Assay	Assay for the saponin content using the method described in Annexure 25. The apparent haemolytic index should be between 20 - 30.
Storage	Achyranthes syrup should be kept in well closed containers and stored at a temperature not exceeding 30° C. If a turbidity develops it must be cleared by warming in hot water before use.

ANNEXURE 28

PREPARATION OF ACHYRANTHES TABLETS (LABORATORY SCALE)

1. Percolate 2 kg of roots using the procedure in Annexure 26
2. Remove the solvent from the two fractions separately
3. Evaporate the aqueous concentrates to dryness under reduced pressure at a temperature not exceeding 60°C.
4. Combine the dry extracts or the viscous pastes
5. Determine the water content if it is a paste
6. Assay this extract for saponin content to standardise the extract
7. Add the sufficient quantity of excipients and granulate using 1.5 mm sieve.
8. Dry the granules at 50°C
9. Screen the dried granules through 1 mm sieve
10. Mix with lubricant and disintegrating agent
11. Compress using 6 mm concave punches

Formula

Extract from 2 kg

Blend of dry starch and $MgCO_3$ (Equal weights) q.s.

Dry starch 3%

Magnesium stearate 1 %

ANNEXURE 29

TRIAL EXTRACTION PROCEDURES

FOR ACHYRANTHES ROOTS

I. Percolate 2 kg of finely cut roots of Achyranthes with 70 % alcohol. Collect 5 l of percolate. Remove the alcohol (reduced pressure) to obtain an aqueous concentrate A.

(a) Evaporate the concentrate A (from one percolation) to dryness under reduced pressure at a temperature not exceeding 60° C. Assay for saponin content to standardise the extract. Get the extract pharmacologically evaluated.

(b) Extract the aqueous concentrate A (from another percolation) with n-butanol. Remove n-butanol under reduced pressure to obtain a less bulky dry extract. Assay for saponin content and get its activity tested.

II. Macerate 2 kg of cut roots in 70 % alcohol (5 l) using a waring blender. Reflux the contents. Cool and filter. Remove the alcohol and process the aqueous concentrate as in I (a) and I (b).

III. Macerate 2 kg of cut roots in water (5 l) using a waring blender. Heat contents up to 70° C. Cool and filter. Extract the filtrate with n-butanol. Remove n-butanol under reduced pressure and get the dry extract evaluated.

The dry extract could be formulated into tablets by using Dry or Wet granulation. Dosage regimen could be reduced if n-butanol extract is found to be active.

The syrup too can be formulated using the best extraction procedure.

ANNEXURE 30

MONOGRAPH ON ADENOSMA INDIANUM

Adenosma consists of the dried aerial parts of Adenosma indianum (Lour.) Merr. harvested during the time of flowering.

Description : Dark brown material with an aromatic odour and a strong taste.

Macroscopy :

Microscopy :

Identification :

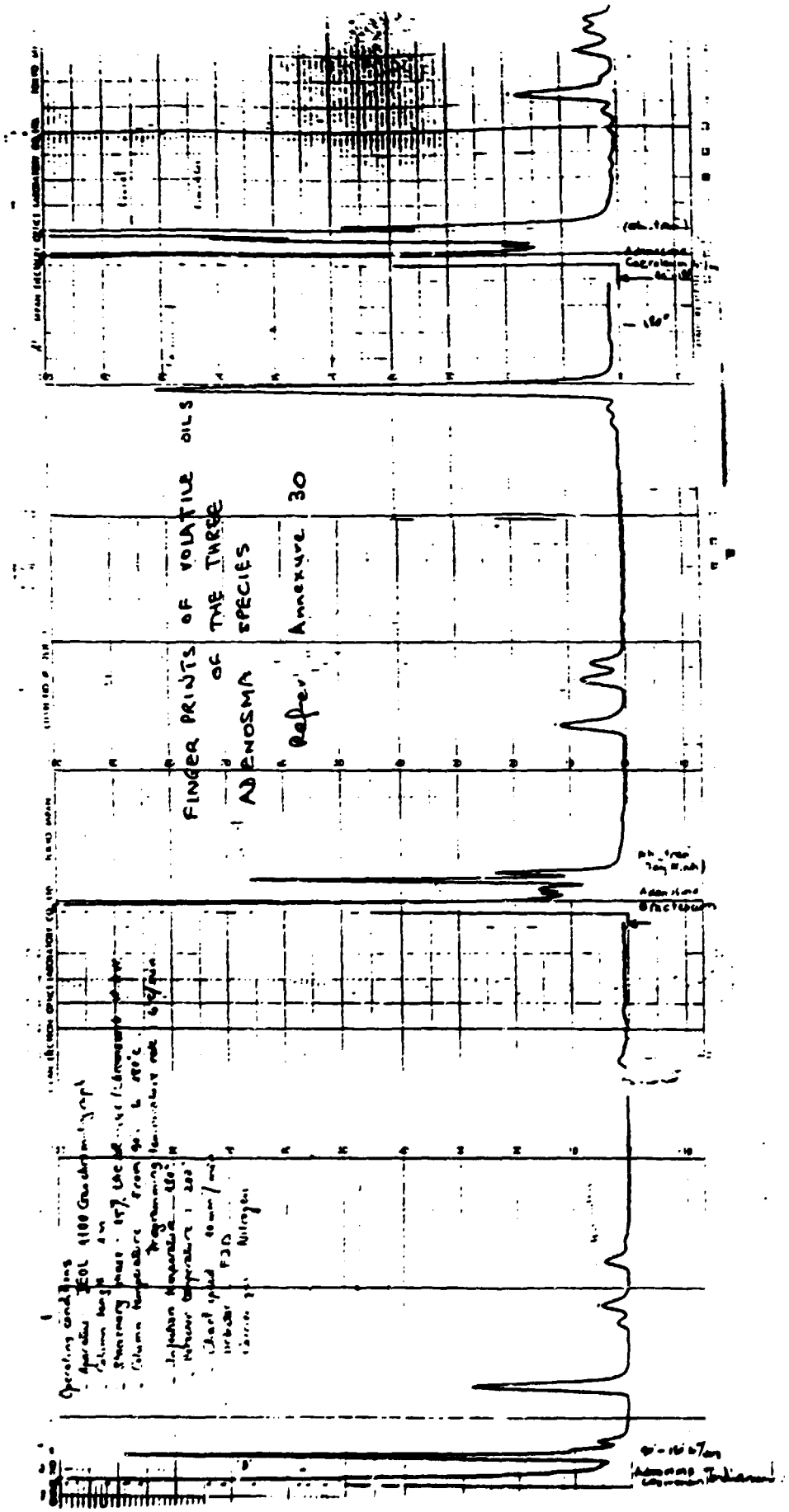
A. Extract the volatile oils and subject to GLC analysis using the following running conditions :

Machine	JEOL 1100 Gas Chromatograph
Column	1 m
Stationary phase	15 % LAC 2R - 446/ Chromasorb WAW
Column temperature	90° - 180°C at 4 /min.
Injection temperature	180° C
Detector temperature	200° C
Detector	FID
Carrier gas	Nitrogen
Chart speed	10 mm / min.

The chromatogram obtained should match the finger print of the authentic sample (differences from the other two species should be noticed). Chromatogram attached.

B. The volatile oil can also be run on a TLC system. Silica GF_{254} - Toluene : Ethylacetate (93:7). The chromatogram after spraying with vanillin/H_2SO_4 reagent should match the finger print of the authentic sample. An additional yellow spot is evident in indianum (cf caeruleum and bracteosum).

C. Extract 0.5 g of the powdered drug with 10 ml of alcohol by heating on a water bath. Subject the extract to TLC. Silica GF_{254} -Ethylacetate: formic acid: Acetate acid: water(100:11:11:27). After spraying with conc. H_2SO_4 reagent, the chromatogram


FINGER PRINTS OF VOLATILE OILS
OF THE THREE
SPECIES
ADENOSMA
Ref:
Annexure 30

(Contd. Annex 30)

should match the finger print of the authentic sample.

Stems :	Not more than 60 %
Foreign matter	Not more than 2 %
Volatile oils	Not less than 0.3 %
Moisture content	Not more than 2.0 %
Ash content	Not more than 3.0 %
Acid insoluble ash	Not more than 2.0 %
Alcohol soluble extractive	Not less than 5 %

Assay :

A method has to be developed once the active fraction has been defined.

Storage :

Adenosma should be stored in a dry place at a temperature not exceeding 25° C.

N.B. It is recommended that the specifications be re-checked using a new sample.

ANNEXURE 31

PROPOSED PROCEDURES FOR THE EXTRACTION OF ADENOSMA

I. Steam distil 2 kg of coarsely powdered aerial parts of Adenosma until the condensate is free from volatile oils. Separate the volatile oils from the distillate.
Filter the water extract in the steam distillation vessel.

(a) In one extraction evaporate the filtrate to dryness under reduced pressure at a temperature not exceeding 60° C.
Mix the volatile oils with the dry extract and get it pharmacologically evaluated.

(b) Extract the filtrate from another steam distillation with normal butanol. Remove the n-butanol under reduced pressure to obtain a less bulky dry extract. Incorporate the volatile oils and send for pharmacological testing.

II. Method I may further be improved by refluxing the residue and the extract left after steam distillation after adding dilute alcohol (20 %). The filtrate from this can be extracted into n-butanol to continue with procedure I (b).

III. Percolate 2 kg of coarsely powdered drug with 70 % alcohol. Collect 6 litres of percolate and distil the alcohol collecting the condensate. Recover the volatile oils. Evaporate the aqueous concentrate under reduced pressure at a temperature not exceeding 60° C. Incorporate the volatile oils into the dry extract and get its activity evaluated.

ANNEXURE 32

TRIAL FORMULATIONS FOR ADENOSMA TABLETS

I. Dry granulation

Dry extract powdered
(without the volatile oils)

Blend of equal quantities of dry starch and $MgO/MgCO_3$
Magnesium stearate

Compress into large hard tablets

Granulate using the dry granulator

Incorporate the volatile oils by either spraying or with an absorbant.

Mix with the lubricant and compress into tablets.

II. Wet granulation

Dry extract powdered

Blend of equal parts of dry starch and $MgO/MgCO_3$
Starch paste q.s.

Magnesium stearate 1.0 %

Dry starch 4.0 %

Prepare wet granules, dry and screen through sieve. Incorporate the volatile oils by blending with dry starch, add the lubricant and compress.

ANNEXURE 33

APD LIQUID - PROPOSED EXTRACTION PROCEDURES & FORMULATIONS

Each extract should be clinically evaluated.

Solanum procumbens/S. hainanensis (aerial parts)	1200 g
Achyranthes bidentata (roots)	400 g
Eleutherine subaphylla (Bulbs)	400 g

I. Extract the mix of cut pieces of the drugs with hot water (4 1). Cool and filter. Extract the filtrate with n-butanol. Remove n-butanol under reduced pressure to get a dry extract. This could be formulated into a liquid or solid dosage form.

II. Extract the mix with 70% alcohol (4 1) under reflux. Filter and remove alcohol by distillation. The aqueous extract so obtained can be formulated into a liquid dosage form. In order to prepare a solid dosage form, the aqueous concentrate should be evaporated to dryness under reduced pressure at a temperature not exceeding 60°C.

Liquid Formulation

Dry or liquid extract q.s.	
Glycerin	5 %
Menthol or Peppermint Emulsion	0.2 %
Sodium benzoate	0.1 %
Water to	100 %

Dilute the extract with some water and add glycerin. Dissolve sodium benzoate and peppermint emulsion in water, add to the extract and make up to volume. If menthol is used it has to be dissolved in a little alcohol and then added.

Allow to stand for 24 hours and filter.
If a precipitate forms, check whether it is soluble in alcohol and if it is soluble incorporate up to 3 % of alcohol in the formulation.

ANNEXURE 34

FORMULATIONS FOR SOLID DOSAGE FORMS OF APD

I. Tablets (Lozenge)

Dry extract q.s.
Lactose
Starch paste
Polyethylene glycol 2 %
Compress into hard tablets
May be formulated as a chewable tablet if necessary.

II. Pastilles

Concentrated liquid extract or	
Dry extract q.s.	
Gelatin	200 g
Glycerin	400 g
Sodium benzoate	2 g
Citric acid	20 g
Sweetener	q.s.
Menthol	2 g
Water to	1000 g

Soak gelatin in about 200 ml of water until softened, add glycerin and heat on a water bath until the gelatin has dissolved. Dissolve the other ingredients separately (except menthol) in about 60 ml water and add to the gelatin mix. Dissolve the menthol in a little alcohol add to the extract and add the extract and sufficient water to make 1000 g. Pour into moulds or trays and allow to cool.