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PESTICIDES DEVELOPMENT PROGRAMME IN INDIA

DP/IND/80/037

INDIA

Technical report: Quality control of pesticides: chromatographic methods of analysis

Prepared for the Government of India
by the United Nations Industrial Development Organization,
acting as the executing agency for the United Nations Development Programme

Based on the work of T.A. Antazo,
consultant in quality control of pesticides:
chromatographic methods of analysis

United Nations Industrial Development Organization
Vienna

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Explanatory notes

The following abbreviations have been used:

a.i.	active ingredient
AOAC	Association of Official Analytical Chemists
CIPAC	Collaborative International Pesticides Analytical Council Limited
D	dichlorophenoxyacetic acid
EC	emulsifiable concentrates
FAO	Food and Agriculture Organization of the United Nations
GIFAP	International Group of National Associations of Manufacturers of Agrochemical Products
g/l	grams per litre
GLC	gas liquid chromatography
HIL	Hindustan Insecticides Ltd.
HPLC	high pressure liquid chromatography
IR	infra-red
ISO	International Standards Organization
nm	nanometre
PDPI	Pesticide Development Programme in India
ppm	parts per million
t	tonnes
T	trichlorophenoxyacetic acid
UV	ultra-violet
WHO	World Health Organization
WP	wettable powders

Mention of firm names does not imply the endorsement of the United Nations Industrial Development Organization (UNIDO).

ABSTRACT

One of the objectives of the United Nations Development Programme (UNDP) project "Pesticides Development Programme in India" (DP/IND/80/037) is the improvement of the country's pesticide formulation capability through conducting training courses on various aspects of formulation technology.

A UNIDO consultant in the quality control of pesticides: chromatographic methods of analysis was in India from 25 March to 24 April 1985 to give lectures and demonstrations on the applications of gas chromatography and high-pressure liquid chromatography to the analysis of pesticides. Three papers presented during the Training Programme on Quality Control of Pesticide Formulations are annexed.

This report includes the consultant's comments and suggestions on the improvement of existing facilities for quality control testing available at the Pesticide Development Programme in India (PDPI) Centre.

CONTENTS

INTRODUCTION

A. Scope of the mission

The project "Pesticides Development Programme in India" (DP/IND/80/037) is a multipronged activity geared towards the strengthening and improvement of the pesticide formulation industry in the country. Established with UNDP/UNIDO assistance, this project began in July 1981 and is being implemented by the Hindustan Insecticides Ltd. (HIL) on behalf of the Government of India. Its centre is based at Udyog Vihar, Gurgaon, Haryana, some 25 km from Delhi, and is equipped with research and technology development facilities in the many aspects of pesticide formulation.

One of its objectives is the training of manpower for the pesticide industry with special emphasis on the small-scale sector. To this end, the Pesticides Development Programme in India (PDPI) conducted two training programmes especially designed to benefit the small-scale formulators: Pesticide Formulation Development (18-22 February 1985) and Pesticide Formulation Manufacture (18-22 March 1985). Since it was found that the quality control testing facilities of small-scale industrial units were in need of improvement, and since quality control was important in pesticide development, a third training programme was planned for quality control personnel.

For this reason an expert in quality control of pesticides: chromatographic methods of analysis was sent to India for the period of 25 March to 24 April 1985. The expert's duties were:

(a) To assist the scientists/engineers of the PDPI Centre in organizing a practically oriented training programme on quality control of pesticide formulations for technical personnel from the Indian pesticide industry;

(b) To give lectures and demonstrations on the application of gas chromatography and high pressure liquid chromatography for the analysis of pesticides;

(c) To train available HIL/PDPI staff in the operation and maintenance of the gas liquid and high pressure liquid chromatographs;

(d) To comment and suggest improvements on existing facilities for quality control testing available in the Centre.

B. Background

The production of pesticides in India is largely in the hands of the private sector. It is built around 32 large industrial units engaged in the manufacture of about 50 technical grade pesticides and over 400 small-scale formulators. An estimated total annual formulation capacity is of the order of 1.6 million t of formulated products. The small-scale sector accounts for 85 to 90 per cent of the total formulation capacity.

The major producer of pesticides in the public sector is Hindustan Insecticides Limited. It is under the administration of the Ministry of Chemicals and Fertilizers and has manufacturing units in Delhi, Cochin and Rasayani. These units are well-equipped research and development facilities for technical and formulated pesticides.

Dusts constitute the largest volume of pesticides used in the country, followed by emulsifiable concentrates (ECs), wettable powders (WPs) and granules. WP formulations are almost exclusively used in public health programmes.

Quality control facilities are fairly adequate in the case of manufacturers, especially of the large industrial firms, which maintain their own laboratories and have developed quality control systems. The quality control facilities of the small-scale formulators, however, need improvement, if not modernizing, in some cases.

The PDPI has established the necessary infrastructure and facilities for the quality control of pesticides and has formulated training capsules to cater to different needs of the pesticide formulation industry. It is with the training of personnel and with making available the services of sensitive sophisticated analytical instrumentation that PDPI can assist the small-scale formulators.

Whereas other testing laboratories, such as the Central Insecticides Laboratory, the Sri Ram Test House and the Industrial Testing and Analytical Laboratories Pvt. Ltd. are in existence, they are more or less confined to regulatory and/or routine commercial testing and are, therefore, product oriented. The goals of the PDPI are more in depth and ambitious, and while individual aspects of pesticide formulation are given importance as seen from the training courses, the approach, from the point of view of industry, is realistic. This should not, however, deter from interactions with technical personnel of these institutions for exchange of information on methodology, advances in the field of instrumentation, and other similar aspects.

I. RECOMMENDATIONS

1. In future training programmes, homogeneity of participants' qualifications should be strived at, where possible.

2. If the PDPI Centre is to function as a training base for pesticide analysis, an analytical development group should be constituted, with qualified, experienced pesticide analysts.

3. Since accurate and reliable analytical data depend on the proper operation and maintenance of analytical instruments, an intensive residency training of at least one month for a member of this analytical development group should be proposed and undertaken in a laboratory where such facilities exist.

4. A major shifting of instrument locations will have to be made, taking into account facility of use and safety requirements.

5. Additional equipment essential for the proper functioning of the analytical instruments has to be purchased.

6. A strong safety awareness programme should be implemented, with the usual laboratory safety equipment - safety goggles, gloves, protective clothing, first aid kits - provided and fume hoods and fire extinguishers installed.

7. As the non-availability of pure analytical standards is a major constraint in quality control, the generation of sufficiently pure analytical standards of pesticides from technical materials, as originally planned by PDPI, should be pursued and resultant purified standards made available to industrial laboratories.

8. All of the above-mentioned points are training aspects and priority training of centre personnel - present and to be hired - should first be accomplished before another programme for outsiders is considered.

9. If pesticide residue analysis is to be conducted in support of the Centre's bioefficacy trials, the purchase of gas chromatographs equipped with electron capture, flame photometric and nitrogen phosphorus detectors should be considered.

10. The library should be stocked with books, journals, periodicals and other reference literature pertaining to pesticide analysis, instrumentation and applications.

II. WORK CARRIED OUT

Training programme

A training programme on the quality control of pesticide formulations was conducted by PDPI for a group of 20 participants from the pesticide industry at the Management Development Institute, Gurgaon, Haryana from 15 to 19 April 1985. Practical demonstrations were held at the PDPI Centre at Udyog Vihar, Gurgaon. The programme capsule is given in annex I. During this course, the UNIDO expert delivered three lectures on international specifications, gas liquid chromatography (GLC) and high pressure liquid chromatography (HPLC) and was involved in practical demonstrations on GLC and HPLC techniques. These lectures are given in annexes II to IV.

For this training course, the analytical instruments mentioned had to be checked and operating conditions optimized. Minor adjustments in the operation of the gas chromatograph were made since the instrument was already operational. The unsuitability of the available stainless steel columns for routine analysis of pesticides was stressed. The recommended column material is glass since exposure of many pesticides to hot metal surfaces causes decomposition. This is especially true of the thiophosphates and most organochlorines because they are very labile in contact with hot metal surfaces resulting in breakdown and poor quantitation.

More time was spent in making the HPLC operational. Since the instrument had not been set to proper running after installation some time ago, problems ranging from column blockage to fluctuating flows and pressures were encountered. Leaks in the system caused by loose fitting connections in the different modules of the instrument had to be corrected. Availability of HPLC grade and sufficiently pure solvents proved yet another problem. In due time, however, test runs were made and conditions optimized for some applications.

During the conduct of the course, particularly the practicum sessions, the participants' background and experience were a limiting factor. While some were interested in the basics, the operation and manipulation of the instrument, a few with more experience wanted a discussion of problems encountered during an instrumental run of a particular pesticide. Considering the limited time allotted for these sessions, a more homogeneous grouping, by way of background and experience, would benefit the participants more.

Analytical development group

The PDPI Centre is equipped with top quality analytical instruments. It would, therefore, be a pity not to make use of such sophisticated equipment. If the PDPI Centre is to function as a training base for pesticide analysis, then the analytical development group, as presented in the PDPI organizational chart, should be constituted. This group can then make work plans for the utilization of the equipment in the instrument room. The present contingency of two in the instrument room could be absorbed by this group.

Since accurate and reliable analytical data are dependent on the proper operation and maintenance of analytical instruments, an intensive residency training of at least a month should be proposed for a member of this group to be undertaken in a laboratory where such facilities exist. Such aspects as instrumental methods of pesticide analysis, basic gas chromatography, basic high pressure liquid chromatography, trouble-shooting and maintenance, basic manipulations, and safety should be covered by such a course.

If the testing facilities of the PDPI Centre are to be made available to the pesticide industry and others, then the need for trained manpower is underscored. This is a priority area and should be given much attention before another training programme for outsiders is even considered.

Rearrangement of instruments

A major shifting of instrument locations will have to be made to ensure facility of use and safety. HPLC systems, because of the nature of solvents used, should not be operated in areas where open flames, sparks or excessive heat may be present. The GLC, which is equipped with a flame ionization detector, is situated facing the HPLC. As the GLC is ideally located by virtue of the easy access to the gas cylinders outside the window, it follows that the HPLC may have to be moved. The four electronic balances may be placed beside each other in a designated weighing corner. The two UV spectrophotometers and two IR spectrophotometers may be placed alongside each other.

Short simple operational instructions should be typed and taped on one side of the instrument as an aid to users of the instrument.

It should be stressed here that sensitive laboratory equipment needs regular service and maintenance. In many cases, this can be done by well-trained laboratory staff. A correct "user's attitude" could add mileage to the operating life of an instrument. It is as easy as reading the operating manual first.

It must be admitted that proper instrument service may be difficult to obtain since many local representatives of foreign companies are sales representatives who may have neither knowledge nor facilities to repair instruments sold by them. These foreign firms, however, have area representatives who visit the country at certain times during the year. It would be useful to get a schedule of these visits and have the area representative do a check and perhaps, recondition the instrument.

Additional equipment

Additional equipment should be purchased. Glass columns, as mentioned earlier, are a must in pesticide analysis. Solvent cleaners, filtering systems and sample clarification kits are needed to ensure quality solvents and trouble-free operation of the HPLC. Gas/liquid tight syringes must be purchased for HPLC. In addition, an explosion proof laboratory refrigerator with freezer is needed to store pure analytical standards and prepared standard solutions. A list of these equipments is given in annex V.

Safety awareness programme

Laboratory safety is the responsibility not only of the individual analyst but also of the management. PDPI management should focus on a safety awareness programme to keep the personnel properly informed and trained in safe laboratory practices. Not only should the usual laboratory safety equipment - safety glasses, gloves, gowns - be provided, but also, fume hoods and fire extinguishers should be installed. HPLC solvents must be prepared in a well-ventilated laboratory hood to minimize toxic vapours in the laboratory air. First aid kits are missing unfortunately.

Laboratory helpers should be made to realize the potential explosion hazards of filled gas cylinders and not allow them to dump these cylinders on the ground like bags of cement.

A clean uncluttered working area is not only healthy but will produce reliable results.

Pure analytical standards

The fact that pure analytical standards are not available is a major constraint in quality control. The PDPI should take the lead in the generation of sufficiently pure analytical standards from technical materials. These purified standards could then be made available to the many quality control laboratories of the small-scale formulators, which are otherwise forced to use technical materials as standards for quality checking.

These purified standards could be checked against pure analytical standards for quality and content. Several standards were brought over from the expert's laboratory: malathion, alpha-endosulfan, beta-endosulfan, mixed endosulfans, monocrotophos, butachlor and 2,4-D IBE.

Training of available staff

Two analysts were trained in the fundamentals of GLC operation and manipulation and one in HPLC. The training was, however, by no means extensive since the expert was busy making the HPLC operational in time for the training course.

Pesticide residue analysis

If pesticide residue analysis is to be conducted to support the Centre's bioefficacy trials, gas chromatographs equipped with electron capture, flame photometric and nitrogen phosphorus detectors should be purchased. These gas chromatographs should be for the exclusive use of residue analysts and under no circumstances should formulation analysis be allowed therein.

However, before any residue activity has been started, the analyst(s) should have undergone adequate training since the rudiments and basic considerations of residue analysis is an entirely different field from formulation analysis. Further, the need for a dust-free, immaculately clean working environment free of dust is essential. Under the present circumstances, this may be difficult to achieve.

Reference material

The library should be stocked with books on the latest developments in pesticide analysis, instrumentation, application, journals of chromatography, periodicals and other literature that would serve as ready reference for the analyst. Suggested literature references are given in annex VI.

Interactions

A list of contacts made during the mission visit is found in annex VII.

Annex I

TRAINING PROGRAMME

15 April 1985

Factors responsible for quality of pesticide formulations

Quality of raw materials
S. K. Khetan, N. K. Pillai

Production process and equipment
A. N. Dutta

Packaging, storage and transportation
V. C. Bhargava

Training of the production and quality control personnel
R. L. Bakshi

Welcome by S. P. Dhua, CMD
Hindustan Insecticides Ltd.

Inaugural address
by P. V. Shenoy, Addl. Secy.,
Min. of Agriculture

Key note address
by S. K. Mukherjee,
IARI

16 April 1985

Pesticide formulation specifications

Indian standard specifications
E. N. Sunder

International specifications
Thelma Antazo

Sampling and methods of testing for physical characteristics of formulations
D. R. Sharma

Shelf life of pesticide formulations and statutory requirements
K. D. Paharia

Practical demonstrations

17 April 1985

Methods of analysis

Chemical methods
D. Sengupta

Chromatographic methods

Gas liquid chromatography
D. Sengupta, Thelma Antazo

Course faculty-participants interaction

Demonstration

18 April 1985

High performance liquid chromatography
S. Mohan, Thelma Antazo

Spectroscopic methods
A. S. N. Murthy, P. K. Ramdas
Practical demonstrations

19 April 1985

Biological methods of quality control of pesticides
B. P. Shrivastava

Requirement of quality control laboratory for pesticide formulations
M. L. Kumar, Thelma Antazo
S. Mosinski

Annex II

INTERNATIONAL SPECIFICATIONS 1/

by

T. A. Antazo

Introduction

Specifications are standards against which products are measured. Specifically, they are allowable limits within which certain characteristics of a product may vary and still maintain acceptability of quality and performance.

In the field of pesticides, specifications have been set up as guideposts not only for manufacture, formulation and transport but also as guarantees when these products are sold, distributed and used in international commerce.

Specifications for insecticides and for spraying and dusting apparatus were first published by the World Health Organization (WHO) in 1953 to cover all the principal compounds used in controlling insects of public health importance. In 1956 the first edition of Specifications for Pesticides was published.

Extensive revisions, due in part to updated methods for quality control and considerable increase in the use of pesticides in public health, have led to the publication of the latest edition, the fifth, now titled Specifications for Pesticides Used in Public Health.

A decade later, the Food and Agriculture Organization of the United Nations (FAO) Working Party on the Official Control of Pesticides was created. One of its terms of reference was to produce specifications for pesticides used in agriculture analogous to those prepared by WHO for public health purposes. Today, this group is called the FAO Panel of Experts on Pesticide Specifications, Registration Requirements and Application Standards.

This Group of Experts has established liaison with scientists of the pesticide industry and with such organizations as WHO, the Collaborative International Pesticides Analytical Council Limited (CIPAC), the Association of Official Analytical Chemists (AOAC), and the International Standards Organization (ISO).

Methods of analysis that have been critically examined by interlaboratory collaborative trials are preferred for adoption and incorporation in the specifications. CIPAC and AOAC have both freely made available methods of analysis they have adopted after collaborative testing. The ISO standards for apparatus and common names for pesticides have been adopted.

Although the WHO specifications were designed specifically to meet the requirements of public health programmes for the control of vectors of human disease, they are in certain aspects similar to those required for plant

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1/ Presented during the training course on Quality Control of Pesticides conducted by Pesticide Development Programme in India at the Management Development Institute, Gurgaon, Haryana, India, 15-19 April 1985.

protection. So, where appropriate, FAO has adopted suitable WHO clauses in FAO specifications for those pesticides of mutual interest.

Largely, however, WHO specifications are not necessarily suitable for plant protection, even where the ingredients are identical. In many respects, these differ from the requirements for pesticides used in agriculture, particularly in the performance requirements and in the content of active ingredient in the various formulations.

In a collaborative effort in 1979 the FAO and WHO published specifications for rodenticides entitled Rodenticides: Analyses, Specifications, Formulations.

Requirements for specifications

Specifications are designed with the objective of ensuring that pesticides complying with them are satisfactory for the purposes for which they are intended. An emulsifiable concentrate should form a stable emulsion under the conditions of the method of testing and re-emulsify easily after long standing; water dispersible powders should pass realistic suspensibility tests; dusts should have particle size requirements. Acidity, alkalinity, presence of water, impurities, compaction, stability at elevated temperatures and other variables need to be considered. Physical appearance, colour, flowability need to be examined. Needless to say, active ingredient content should be given prime consideration.

The FAO specifications include the following physical and chemical properties:

Description:	colour, physical state
Active ingredient:	content, incl. tolerances identity
Impurities:	acidity, alkalinity, insolubles, water, other critical impurities
Physical properties:	(as applicable)
for dry products:	particle size and range, suspensibility, foaming, wettability, dustiness
for liquid products:	emulsion stability, re-emulsification, miscibility
Storage stability:	low temperature stability, heat stability
Containers:	stability, performance requirements

A summary of the above properties and a list of available CIPAC methods are given in appendix I.

Expression of content of active ingredient

The content of active ingredient of agricultural pesticides and plant protection products is expressed on a per cent weight/weight or g/l at 20°C basis. For solids, liquid technical active ingredients, volatile liquids (maximum boiling point 50°C) and viscous liquids (lower limit 1,000 centipoises at 20°C), the active ingredient content is expressed in per cent weight/weight. For other liquids, the active ingredient content is expressed in g/l at 20°C.

Tolerances for content

These tolerances take into account the difficulties likely to be encountered in manufacture and analysis. For consignments up to 5,000 kg, tolerance guidelines are presented in appendix II. For larger consignments, the tolerances in appendix II apply to individual samples, but where there are a number of samples, the negative tolerances should be halved.

For example, for a 50 per cent product, the average content of active ingredient of all samples taken shall be between $50 - \frac{2.5\% \text{ units}}{2}$ and 50 + 2.5% units (i.e., 48.75 to 52.5%).

Tests for product stability

Heat stability. Generally, products should be capable of being stored for at least two years in unopened containers, notwithstanding the conditions of this storage. Products shipped to the tropics may have to withstand temperatures of 50°C or more for considerable periods of time. Sometimes, a slow turnover of stocks may compound this problem.

A short heat test (54 ± 2°C for 14 days) has proved most useful in eliminating many products that do not meet an acceptable level of storage stability.

In certain instances, after a heat stability test, there may be an inevitable loss of active ingredient, a reduction in suspensibility and perhaps a change in acidity or another variable. A deterioration in the properties of a formulation is allowed by $\underline{x}$ per cent where $\underline{x}$ is normally not more than 10. This loss is based on the content of active ingredient found.

In the case of a newly developed product, the "heat stability clause" is regarded as "for information" for the first three years of the original patented life of the product. This allows information gained in the heat stability test to be supplemented with practical experience in field storage and other data on the stability of the formulation.

Where, owing to possible decomposition, products are tested for 14 days at less than 54 ± 2°C, a statement should be made on the label that the product shall not be stored above a specified temperature (often 20° or 30°C).

Low-temperature stability. Similarly, products intended for use in the tropics may have to withstand low temperatures since they are often shipped from cold climates. Thus, products undergo storage tests at 0 ± 1°C for seven days.

Emulsion stability. The stability of the diluted emulsion is determined in CIPAC Standard Waters A (20 ppm hardness) and C (500 ppm hardness) after the heat storage test, unless other Standard Waters are specified. This test is conducted at 30°C, but 10° or 20°C may be used in certain countries.

Foaming. This test is a check to avoid excessive foaming when filling the spray tank. A limit of 25 ml foam was adopted.

Impurities

The production of technical grade pesticides may result in the introduction of small quantities of other compounds such as: (a) basic starting materials, including their impurities, used in the synthesis of the active ingre-

dient; (b) side-reaction products formed during synthesis, e.g., isomers; (c) partial decomposition products of the a.i. arising from the working up of the final product; or (d) traces of solvent left from synthesis or the purification stages in the manufacture of the product.

These impurities are undesirable since they may influence the quality of the formulation by inducing the chemical decomposition of the active ingredient or the deterioration of packaging during storage. They may give rise to phytotoxicity in treated plants, lead to toxic residues in food crops or present an undue risk due to a change in the toxicological property of the product.

The nature of such impurities should be stated and maximum acceptable levels should be fixed in the specifications of the active technical material and its corresponding formulations. Where a minimum and maximum are used, they are absolute and no tolerance is permitted.

Examples of impurities for which limits have been established in relevant specifications:

TCDD, very toxic and teratogenic, limited to ppm 0.1 in 2,4,5-T formulations

Water in some dithiocarbamates, leads to decomposition of a.i., limits imposed

Free phenols in phenoxyacetic acid herbicides, causes taint in foodstuffs, limited in MCPA and 2,4,5-T to 1.5 per cent and in 2,4-D to 1 per cent

Packing of pesticides

A pesticide product consists of the pesticide and the container. The manufacturer of the product is responsible for the quality and performance of both. The importance of the container cannot be overemphasized. A good pesticide may be ruined by an unsuitable container.

Working on the philosophy that detailed requirements might hinder the development of novel packing materials, the FAO Group of Experts proposed only general advice in the specifications on packing. Where there are known precautions to take in packing a particular product (linings, moisture barriers, tight seal, polyethylene inserts etc.), these are incorporated in the specification requirement. Otherwise, only a general clause is given.

Labelling

The label is another important element of every pesticide product. Labels must be of such design and quality and must be affixed to the container in such a way that they do not deteriorate, become illegible or separate from the container, even under the rigours of international transport and storage, and hot and humid climatic conditions. Further, a physically satisfactory label may still be useless unless it is written in the language of the user.

Labelling recommendations have been issued by the FAO Group of Experts on Registration Requirements. As many pesticides are highly toxic to man and animal, it is essential that labels on the containers carry a clear warning of the hazards, with instructions for safe handling, and an indication of measures to be taken in case of accidental intoxication. Minimum statements of caution are included in the specifications.

Biological properties

In addition to the already mentioned requirements, certain biological properties are also very important to satisfactory product performance.

Phytotoxicity. A standard test to evaluate phytotoxicity is not yet available to the Group of Experts. Therefore, this clause is at present included in the specifications "for information" purposes.

Wetting of crop. The optimum wetting of the crop for maximum biological efficacy will vary with the crop, the pesticide, the volume and the mode of application. Information on suitable tests for evaluating wetting of leaf surfaces is given in an "information" clause, where applicable.

Mechanism for developing/adopting specifications

FAO issues three types of specifications:

(a) Draft specifications: those submitted for consideration;

(b) FAO provisional specifications: those that may require further work;

(c) FAO specifications: those that are fully acceptable on the basis of evidence presented.

Draft specifications are submitted by any organization or person for consideration by the Group of Experts, who assign a priority to it. The draft is circulated among the Group and GIFAP (pesticide industry) if it is a commodity product or to the concerned manufacturer if it is a patented product. At its annual meeting, the Group of Experts considers comments on the draft specifications and takes appropriate action, such as promotion to provisional or FAO specification.

FAO specifications are intended to:

(a) Provide basic standards of quality for the buying and selling of pesticides;

(b) Provide an international basis to assist and simplify the official approval and acceptance of pesticides;

(c) Provide standards of quality on which residue limits and safety measures may be based;

(d) Give an international official acknowledgment of the acceptability of a pesticide;

(e) Help protect the responsible vendors against inferior products.

Specifications for each pesticide are published as small booklets, while the corresponding methods of analysis are published in the CIPAC Handbook.

Appendix I

AIM OF THE PHYSICAL PROPERTIES AND AVAILABILITY OF CIPAC METHODS

Physical property	Aim of property	Applicable to formulations a/	Availability of CIPAC methods	Normal limits
1. Material soluble/ insoluble in a solvent	To determine the purity or impurity of the product	TC,SL	Soluble: MT.4, 5, 6, 7, 9, 71, 76, 87, 90 Unsoluble: MT.8, 10, 11, 16, 27, 35	Depending on the active ingredients
2. Acidity-alkalinity-pH	To ascertain decomposition of the active ingredients, deterioration of physical properties, danger of corrosion	TC, DP, WP, GR, EC, SL, UL, SC	MT. 31, 66, 75	Depending on the active ingredients
3. Water content	id.	TC, DP, WP, GR, EC, SL, UL	MT. 17, 30, 40	Depending on the formulation
4. Dry sieve test	To limit particles of unwanted size	DP, GR	MT. 59	Max.: 2% on 150 um 5% on 75 µm
5. Flowability	To ascertain the free flowing nature of the product	DP, GR	MT. 44 revised (for DP) no method for GR	Max.: 12
6. Dustability	To ascertain the ability of dust to be dispersed	DP	No CIPAC method but WHO/EQP/4R2	
7. Wet sieve test	To avoid the blockage of spray nozzles	WP, SC	MT. 59.3	Max.: 2% on 75 µm
8. Suspensibility	To determine that a sufficient amount of of a.i. is still in suspension to give a satisfactory, homogenous and effective spraying	WP, SC	MT. 15 (for WP)	Min. 50% but mainly depending on the a.i.
9. Wettability	To ascertain the product is rapidly wetted when added to water	WP	MT. 53	Max.: 1 min without swirling

continued

Appendix I (continued)

Physical property	Aim of property	Applicable to formulations a/	Availability of CIPAC methods	Normal limits
10. Persistent foam	To avoid excessive foam when filing the spray tack	WP, EC, SC	MT. 47.1 MT. 47.2 (For SC)	Max.: 25 ml after 1 min.
11. Flash point	To evaluate the danger of flammability	EC, SL, UL	MT. 1[illegible]	Depending on nat. or intern regulations
12. Viscosity	To evaluate the flow properties of liquid	UL, SC	MT. 22	Depending on the formulations
13. Emulsion stability	To evaluate the stability of the emulsion on standing	EC	MT. 36, 20	Cream: max. 4 ml after 2 hours free oil: nil
14. Re-emulsification	To evaluate the ability to be re-emulsified after standing	EC	MT. 36	Cream: max. 4 ml oil: max. 0.5 ml after 30 min.
15. Pour-tap bulk density	To provide information for packaging and application requirements	DP, WP, GR	MT. 33	Depending on the formulations
16. Dispersability	To assure that the product is adequately dispersed throughout the spray tank	WP, SC	MT. 160 (for SC)	Not yet fixed
17. Cold test	To evaluate the danger of crystallization or separation of ingredients in cold climate	EC, SL, UL, SC	MT. 39	Separation less than 0.3 ml at 0°C for 7 days
18. Heat stability	To evaluate the influence of temperature and time on the chemical and physical stability	DP, WP, EC, UL SC, GR, SL	MT. 46	Normal conditions 54°C for 14 days

a/ Code of formulations:

TC	Technical	SL	Soluble concentrate
DP	Dusting powder	UL	ULV formulations
WP	Wettable (water-dispersable) powder	GR	Granules
EC	Emulsifiable concentrate	SC	suspension concentrate

Annex II

CONSIGNMENTS UP TO 5,000 KG

Tolerance guidelines

Declared % of a.i. in formulation		Limits	Limits on % of declared a.i. content
in % w/w	in g/l		
50 and above	n.a.	± 2.5 percentage units	n.a.
n.a.	500 or higher	± 25 g/l	n.a.
25-50	250-500	n.a.	± 5
10-25	100-250	n.a.	± 6
2.5-10	25-100	n.a.	± 10
0-2,5	0-25	n.a.	± 15

Note: In each range, the upper value is not included (e.g., 25-50 means from 25 up to, but not including, 50%).

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Annex III

GAS LIQUID CHROMATOGRAPHY IN PESTICIDE FORMULATION ANALYSIS 1/

by

T. A. Antazo

Introduction

James and Martin introduced gas liquid chromatography in 1952 and since then it has become the most extensively used technique in analytical chemistry. Gas chromatography has made a marvellous development and expansion as a reliable separation method in various fields, such as chemical and petroleum industries, biochemistry, medical sciences, pharmacy and pollution monitoring.

In the field of pesticide residue chemistry, the gas chromatograph is the instrument of choice for the separation, identification and measurement of pesticidal compounds. Sensitive and specific detectors, such as the electron capture, thermionic, flame photometric and microcoulometric units, have allowed determination in the submicrogram range.

In contrast, the quantitative determination of pesticides in technical materials and formulations by gas chromatography is a macromethod and necessarily, accuracy and precision become fundamental factors. Accuracy expresses the correctness of the measurement. Precision expresses the reproducibility of the measurement. The same gas chromatographic principles used for residue methodology apply to formulation analysis but the demand and need for greater accuracy and precision are more acute.

The purpose of this paper is to present the mechanics, instrumental aspects and techniques that are considered essential and practical for the analysis of pesticide formulations. This paper does not go into the details of the theory and other aspects of gas chromatography.

Instrumental aspects

Detectors

The two types of detectors most commonly used in formulation analysis are the thermal conductivity, which detects changes in the thermal conductivity of the gas stream as solutes are eluted, and the flame ionization detector, in which the eluting solutes are burned in a hydrogen flame, producing a small electrical current, which after amplification, is measured by means of a suitable electrometer.

The thermal conductivity detector, although relatively simple and inexpensive, has several disadvantages: (a) it is non-specific; (b) it lacks sensitivity; and (c) it is sensitive to changes in operating parameters, such as temperature and carrier gas flow rate.

1/ Presented at the Pesticides Development Programme in India: Training Programme on Quality Control of Pesticide Formulations at the Management Development Institute, Gurgaon, Haryana, India from 15 to 19 April 1985.

Although most of the fixed gases (carbon dioxide, hydrogen sulfide, ammonia, air, water or carbon monoxide) produce no response with this detector, the flame ionization detector has become the most popular choice for the analysis of organic compounds. The detector is stable, relatively insensitive to flow and temperature changes, linear over a wide range and applicable to a wide range of solutes with high sensitivity.

Except for the flame photometric detector, the specific detectors used for residue analysis find little application in formulation analysis. The FPD is similar to the FID in operation and characteristics. It is based on the emission of light at 526 nm for P and at 394 nm for S when compounds containing these species are burned in a hydrogen rich flame. This light is collected with a mirror, filtered to a specific wavelength and measured by a photomultiplier tube. The response is dependent upon flame-fuel mixture, flow rate and temperature. The exact hydrogen-air-carrier gas ratios are critical in determining maximum sensitivity, selectivity and flame stability.

The sensitivity and selectivity of this detector makes it preferable to the FID for the analysis of formulations containing very low concentrations of organophosphorus or organosulphur compounds.

Columns

The column is the most important part of a gas chromatograph, the "heart", as chromatographers usually put it. The right choice of column material, liquid phase and support determines the efficiency of separation and quantitation that can be achieved with a gas chromatographic system.

Column requirements associated with resolution in pesticide residue analysis or resolution and efficiency in complex multicomponent mixtures are minimal in the gas chromatographic quantitation of formulations which are simpler mixtures.

When looking for a suitable column, the practical chromatographer must consider efficiency, resolution, separation, materials, and such factors as column dimensions and operating conditions that are necessary to give the shortest residence time possible of the compound on the column (many pesticides are known to be heatand metal-sensitive).

The recommended column material is glass since exposure of many pesticides to hot metal surfaces causes decomposition. This is especially true of the thiophosphates and most organochlorines, which are very labile in contact with hot metal surfaces resulting in breakdown and poor quantitation.

Pesticides are separated on primarily silicone liquid phases. Common nonpolar phases include DC-200, SE-30, OV-101, and OV-1. More polar phases, such as OV-17, QF-1, OV-210 and OV-225 or mixtures of these, are sometimes used to analyse for a multicomponent formulation. The more commonly used liquid phases for pesticide analysis are found in appendix I.

Recent findings recommend the use of liquid phases that can take high temperatures with low bleed rate, thus minimizing detector contamination. Instead of DC-200, it is advisable to use OV-101, which has a maximum temperature limit of 350°C as compared to 250°C for DC-200. QF-1 can be replaced by OV-210, which displays less bleed than the former. OV-17, a methyl silicone of moderate polarity with 50 per cent phenyl groups, has proven to be an excellent liquid phase for pesticides.

It is important to use a very inert silanized support to minimize absorption problems, since a liquid phase loading of 5 per cent or less cannot cover all the active sites on a non-inert or non-silanized support. Adsorptive and catalytic sites should be reactivated to avoid tailing and decomposition of the components to be separated. Commercially available supports that meet these requirements are chromosorb W HP, chromosorb G HP, gas chrom Q, diataport S or anakrom ABS. The mesh size of the support should be about 80 to 100, coated with a liquid phase of 3 to 5 per cent.

A liquid phase loading of 5 to 10 per cent on a chromosorb W support is commonly used in pesticide analysis. However, there are many advantages in using lower liquid phase loadings such as 1.5 to 3 per cent. Lower loadings mean lower column temperatures for equal retention times resulting in less column bleed and minimum detector contamination from the bleed.

Low coatings, when used with higher flow rates are very efficient and give well-resolved peaks. The result is very short residence of the compound on the column, minimizing decomposition. Decomposition, if instantaneous, will yield extraneous peaks. If it takes place slowly as the solute passes through the column, a low-topped curve either directly preceding or following the main peak will be obtained. In either case, the validity of the analysis is suspect.

Optimization of gas chromatographic conditions

Carrier gas flow rates are optimized at the selected operating column temperature so that peak broadening is minimal and the separation efficiency greatest possible. Analyses of pesticides are usually performed isothermally, i.e. with column maintained at constant temperatures. This mode of operation is preferable to programmed temperature gas chromatography because of baseline stability and the ability to control and more accurately reproduce column temperature conditions.

If quantitative results are to be obtained, the factors that influence the response of the detector, such as variations in sample size, flow rate, and column and detector temperatures, must be controlled.

Quantitative analysis

Internal standard technique

Formulations are considered simple mixtures whether they contain one or more pesticides. The use of the internal standard technique is ideal and applicable to such systems.

Using this technique, a known amount of a standard substance is added to a known concentration or volume of the formulation sample before it is chromatographed. The ratio of the peak height (or area) of the insecticide component to the peak height (or area) of the internal standard is measured. The concentration or percentage component present is then determined by comparison to a calibration curve or to the peak height (or area) ratio of a solution of the pure standard and the internal standard prepared to approximate the concentration in the formulation.

Choice of internal standard

An internal standard should be selected with care and should meet the following requirements: (a) it must not be present in the original sample; (b) it must elute close to the component of interest; (c) the ratio of the

peak height (or area) to that of the component should be close to unity; (d) it should be inert; and (e) it should be completely resolved from all other peaks.

A satisfactory quantitative procedure involves preliminary screening of possible internal standard candidates under the gas chromatographic conditions and column chosen for the pesticide to be analysed. A listing prepared by the United States Department of Agriculture Pesticides Regulation Division is given in appendix II.

Possible sources of error

The possible areas where errors can be introduced in the chromatographic technique are: (a) sampling technique; (b) sample adsorption or decomposition in the chromatograph; (c) detector performance; (d) recorder performance; (e) integration technique; and (f) calculations.

The gas chromatograph is a quantitative device with variable precision depending on the handling or interpretation of the results. In many cases, the physical measurement of the peak area is the limiting factor in the precision of the results.

The most common methods for peak area measurement are: (a) peak height; (b) height x width at half-height; (c) triangulation; (d) mechanical or electrical integrator and (e) computers.

Analysis of formulations

Sampling

For a valid analysis, a representative portion of the gross sample mixture must be obtained. It is as likely that there are as many incorrect determinations resulting from improper sampling as there are from the combined errors of manipulation, measurement, and calculation. An improper sample makes a subsequent analysis practically worthless. Valid and official methods for sampling formulations are described in Official Methods of Analysis of the Association of Official Analytical Chemists, 10th ed. and Scotts Standard Methods of Chemical Analysis, vol. II.

Sample preparation

Choice of solvent

The validity of any gas chromatographic method is dependent on the ability to quantitatively extract the pesticide from the sample matrix. No matter how selective and precise the gas chromatographic parameters and measurement conditions may be, when applied to standard pesticide solutions, if the pesticide is not completely extracted from the formulation matrix, then that procedure is worthless for that sample.

In choosing the proper solvent for the analysis, the following factors must be considered: (a) ability to extract the pesticide efficiently; (b) solvent must elute rapidly from the column; (c) solvent must be free of impurities that may have the same retention time as the pesticide or internal standard; and (d) solvent should not react with any of the formulation components in a manner that would affect the assay results.

Typical examples of sample preparation before gas chromatography

Some general procedures are given, which include separation of the pesticide from the formulation ingredients or merely a dilution of the sample with a suitable solvent.

Aerosols. The container and contents are weighed, then cooled in a freezing compartment for approximately 30 minutes. A very small hole is punched at the top of the container, which is allowed to stand in a fume hood while the propellant gas escapes. After the propellant has volatilized, the top of the container is carefully cut off. The container is then warmed on a steam bath to expel all the volatile solvents. After cooling to room temperature, the container with the nonvolatile material is weighed. This material is transferred to a suitable container and retained for analysis. The aerosol container is rinsed with ether, dried, and weighed. The difference between these weights represents the weight of nonvolatile material in the aerosol. The top of the container, which has previously been removed, is weighed. This weight is added to that of the empty dried container. The original gross weight of the aerosol minus the combined weight will give the net content. The percentage of nonvolatile residue in the aerosol is then calculated.

A weighed portion of the nonvolatile well mixed residual concentrate equivalent to 0.5 g of pesticide is transferred to a 50 ml flask (final concentration to be 10 mg/ml), and diluted to volume with solvent after addition of internal standard. The per cent pesticide in the concentrate is calculated:

$$\frac{\text{average peak height (area) sample}}{\text{average peak height (area) standard}} \times \frac{\text{weight (g) standard}}{\text{weight (g) sample}} \times \text{purity of standard} =$$

pesticide in concentrate (%)

The per cent pesticide in the aerosol is calculated:

$$\frac{\text{weight of concentrate} \times \text{pesticide in concentrate (\%)} \times 100}{\text{weight (g) of contents of container}} = \begin{matrix}\text{pesticide}\\ \text{in aerosol}\\ \text{formulation}\\ \text{(\%)}\end{matrix}$$

Solid samples (wettable powders, dust concentrates, baits and granules). A sample size equivalent to 2 g of pesticide is transferred to an empty 20 mm x 400 mm chromatographic column and the extraction solvent is percolated through the column at a drop rate of 1-2 drops per second until exactly 100 ml of the effluent is collected. A portion of the effluent to give a final concentration of 10 mg/ml is taken and mixed with a predetermined volume of internal standard.

Liquid formulations (emulsifiable concentrates, oil solutions, ultra low volume concentrates, water-miscible liquids, water soluble concentrates). A sample size equivalent to 0.5 g of the pesticide is transferred to a 50 ml volumetric flask (final concentration to be 10 mg/ml), the appropriate volume of internal standard is added to the flask and the sample diluted to volume with the proper solvent.

The advantages of gas liquid chromatography as the method of choice for formulation analysis are speed of analysis, simplicity of operation, high sensitivity, selective detectors, high separation efficiency, versatility and small sample size needed. Pesticide formulations that can be chromatographed directly need very little sample preparation. In most cases, one general procedure can be applied to all formulation types without prior clean-up.

Appendix I

LIQUID PHASES USED IN PESTICIDE ANALYSIS

Liquid phase	Chemical composition	Class of pesticide detected
QF-1	Tripropyl fluor-silicone	Organophosphates, chlorinated hydro-carbons
Silicone oil DC-200	Methyl silicone	Chlorinated hydro-carbons, organo-phosphates, chlorophenoxy acids and esters, thiocarbamates, dinitro herbicides
Silicone oil DC-550	Methyl phenyl silicone	Organophosphates
Silicone gum rubber, SE-30	Methyl silicone	Organophosphates, chlorinated hydro-carbons, chlorophenoxy acids and esters
OV-17	Methyl phenyl (25%) silicone	Organophosphates
OV-22	Methyl phenyl (65%) silicone	Organophosphates
OV-101	Methyl silicone	Organophosphates
OV-210	Methyl, trifluoro-propyl (50%) silicone	Organophosphates
QF-1 plus silicone oil DC-200 (1:1)	Fluorosilicone plus methyl silicone	Chlorinated hydro-carbons, chlorophenoxy acids and esters, triazine herbicides
DEGS	Diethylene glycol succinate polyester	Organophosphates
Reoplex 400	Polypropylene glycol adipate	Organophosphates
Carbowax 20M	Polyethylene glycol	Triazine herbicides

EGGS-X	Ethylene glycol succinate with 5% methyl silicone in chain	Organophosphates
EGGS-Y	Ethylene glycol succinate with 30% methyl silicone in chain	Organophosphates

Appendix II

INTERNAL STANDARDS EMPLOYED IN PESTICIDE ANALYSIS

Pesticide	Retention time (minutes)	Internal standard	Retention time (minutes)	Column conditions	
				Substrate	Temperature (°C)
Balan	4.01	Lindane	4.83	10% SE-30	186
Captan	4.4	Dieldrin	7.0	10% SE-30	210
Daconil	2.1	Aldrin	4.1	10% SE-30	190
Dacthal	6.0	Dieldrin	11.5	10% SE-30	210
Dasanit	9.4	Dieldrin	8.0	10% SE-30	210
o,p-DDT	6.5	Dieldrin	5.1	10% SE-30	208
p,p'-DDT	8.3	Dieldrin	5.1	10% SE-30	208
DDVP	4.2	Methyl nonyl ketone	5.6	10% SE-30	120
Dichlone	-	Aldrin	-	2% SE-30	198
Dieldrin	8.5	Methoxychlor	18.0	10% SE-30	210
Dimethoate	2.9	Dibutyl sebacate	5.9	5% DC-550	170
Disyston	3.4	Aldrin	5.8	10% SE-30	205
Dyrene	5.5	HEOD	8.5	10% SE-30	215
Famphur	3.0	Di-n-octyl phthalate	8.5	0.25% DC-550	200
Fenitrothion	2.8	Dibutyl sebacate	5.5	5% DC-550	165
Lanstan	2,8	α -Chlorotoluene	4.7	5% DC-550/0.2% Versamid 900	70
Lindane	6.2	Heptachlor	10.6	10% SE-30	195
Malathion	7.0	Dimethoate	3.4	5% OV-22	180
Malathion	2.5	Dibutyl sebacate	4.5	0.25% DC-200	160
Methyl parathion	1.9	Dieldrin	5.1	10% SE-30	208
Methyl parathion	2.17	Dibutyl sebacate	5.2	0.25% DC-550	160
Naphthylacetamide	8.3	Dieldrin	18.0	10% SE-30	215
Parathion	3.9	Dieldrin	7.0	10% DE-30	195
Parathion	3.17	Dibutyl sebacate	5.5	0.25% DC-550	160
PCNB (pentachloronitrobenzene)	8.5	Phorate	5.2	0.25% DC-550	130
Phorate	2.5	Benzyl benzoate	3.5	5% DC-550	165
Phorate	2.5	Lindane	3.5	5% DC-550	155
Tedion	10.3	Dieldrin	4.7	10% SE-30	235
Terrazole	2.3	Fluorene	5.4	0.25% DC-550	100
Trithion	3.6	Heptachlor	1.0	10% SE-30	170

Source: U.S. Department of Agriculture, Pesticide Regulation Division (now Environmental Protection Agency) and Pasarela (1970).

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Annex IV

A PRACTICAL APPROACH TO HIGH PRESSURE LIQUID CHROMATOGRAPHY 1/

by

T. A. Antazo

Introduction

Many of the disadvantages of column chromatography, such as low efficiency, long analysis time, non-reusable columns and poor quantitative reproducibility, have been resolved by innovations in column and instrument technology that have given rise to the technique of high pressure liquid chromatography (HPLC).

The fundamental theory behind HPLC is not new but it was not until 1969 that HPLC, as we know it today, was developed. In its simplest form, it operates on the same principle as traditional column chromatography. In this type of analysis, the mobile phase is a liquid that is pumped at relatively high pressures through a narrow bore column. The stationary phase consists of solid particles of very small size and large surface area. The use of microparticulate packings and narrow columns gives separation efficiencies much greater than those of any other chromatographic technique.

The decrease in column packing diameters created proportional increases in solvent flow resistance. As a result, constant flow and constant pressure liquid pumping systems were developed. Sample introduction systems were improved with valva injectors and liquid syringes.

The basic equipment necessary for HPLC are a pump to force the solvent over the stationary phase, an injection system to introduce the sample to the column, a column where the separation takes place, a detector and a recording device.

The acronym HPLC should be explained. High pressure liquid chromatography is also known as high performance liquid chromatography, high speed liquid chromatography (HSLC), high efficiency liquid chromatography (HELC), or simply, liquid chromatography (LC). A new definition has now been put forward, that of HPLC being high price liquid chromatography.

Considering this aspect, this paper will focus on critical points of the HPLC system - which, if ignored may create problems - because it is important to get good returns from this expensive investment.

Mobile phase

All chromatographers know that the quality and proper handling of the carrier (gas, mobile phase, developing solvent) are as important as any other part of the chromatographic system. Yet it is all too common that they take

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1/ Presented at the Pesticide Development Programme in India Training Programme on Quality Control of Pesticide Formulations at the Management Development Institute, Gurgaon, Haryana, India from 15-19 April 1985.

the carrier for granted. It is very easy to take a bottle of reagent grade solvent, pour it into the reservoir and start the chromatograph. However, when a problem develops with the mobile phase, it may not be easily or immediately identified, and once it has been, the entire system is already affected, causing a considerable waste of time and effort.

The most frequently encountered problems may be avoided or reduced by careful handling of the choice of solvents and mobile phase preparation.

Choice of solvent

The choice of solvent is crucial. Purity is a very important factor. Since large volumes of solvent are pumped through the column, trace solvent impurities can easily concentrate in the column and produce detrimental results. Spectrograde or HPLC-grade solvents are highly recommended.

Preparation of the mobile phase

Filtering. The most important consideration for the preparation of the mobile phase is for all solvents to be free of particulates before they are introduced into the chromatographic system. To remove these particulates the solvents should be filtered using an inert porous material having a 0.2 μ pore size.

Degassing. All solvents should be degassed before use. The basic reasons for this is to prevent gases dissolved in the liquid from coming out of solution in the system and disturbing the chromatographic performance. While under high pressure, gases cause fewer problems since they remain dissolved. Upon exiting the column, the pressure drops dramatically, causing the dissolved gases to leave the solvent and form bubbles. When these bubbles pass through the detector cell, signals to the recorder can become erratic, giving inaccurate results.

Solvent degassing can be done in three ways: heating, vacuum degassing and ultrasonification. Heating the solvent reduces gas solubility and the dissolved gases simply bubble out. Vacuum degassing does not extract all of the dissolved gases and for this reason, it should be repeated during the day to insure that gases do not redissolve during operation. Ultrasonification gives more rapid degassing. With this method, the container of solvent is placed in an ultrasonic bath for about 5 min.

Sample preparation

Solid samples have to be dissolved before introducing into the HPLC system. The choice of solvent is important. Ideally, the sample should be dissolved in the mobile phase, primarily to avoid precipitation in the column. If precipitation takes place before or on the column, unknown and randomly eluting peaks will be observed. These peaks may cover the peak of interest. Precipitation can also occur at the head of the column, clogging the inlet, increasing the column back pressure and restricting the flow.

Liquid samples should be dissolved in the mobile phase. If not, the sample may be evaporated to dryness and reconstituted with the mobile phase.

Filtration

It is just as important, if not more so, to filter the sample prior to injection as it is to filter the mobile phase. As against the mobile phase which may have a solvent reservoir filter and an inline pump filter before the sample inlet and column, the only filter of the sample is the column itself. Insoluble matter building at the head of the column can lead to restriction of the mobile phase flow, increasing the column back pressure, decreasing the column efficiency and producing split peaks.

Degassing

For quantitative analysis, the sample should not be degassed. Degassing causes solvent evaporation, which changes the sample concentration. However, the solvent must be degassed before preparing the sample.

Column

As in gas chromatography, the column is also the heart of the HPLC. Column selection is not always straightforward and no established set of rules exists that would apply for every separation problem. The selection of a column for a chromatographic separation requires consideration of the functional groups on the molecules to be separated and a knowledge of the characteristics of the various column stationary phases. It is important to know the molecular weight and the range of solubility of the sample. Then a mode of separation based on the molecular structure of the sample is chosen.

"Like associates with like" is a useful rule. Once the match has been made, a trial separation is attempted and followed by optimization of the chromatographic conditions.

Interactive chromatography involves four distinct modes of separation: partition, adsorption, ion exchange and exclusion. In HPLC the applicability of partition chromatography covers a wider range of samples because of the development of packing materials that have the liquid phase permanently bonded to a solid support, preventing inactivation of the column due to stripping of the liquid phase. Reverse phase partition chromatography, in which the bonded material is a long chain of nonpolar substance (e.g. octadecylsilyl), is used extensively because its selectivity for solutes can be adjusted over a wide range by varying the polarity of the mobile phase.

A column selection guide is given in appendix I.

Choosing separation conditions

Isocratic conditions of operation are those where the composition of the mobile phase is maintained during the entire analysis. Isocratic analysis is often the most suitable condition for quantitative analysis by HPLC.

Gradient elution, also called solvent programming, is used when the retention range is such that components cannot conveniently elute via isocratic conditions. It is employed in order to reduce analysis time.

Control of the mobile phase flow rate allows the operator to adjust the column efficiency. Flow rates primarily dictate analysis time with slower flow rates giving rise to longer analysis times. But increases in flow rates cause increases in column inlet pressure so this should be done with the other operating controls in mind. A typical flow rate for analytical separations is 0.5 to 2.0 ml/min for a 4.6 mm i.d. microparticulate column.

A mobile phase normally consists of two components: the weak component and the strong component. An increase in the strong component causes peaks to elute earlier.

The effect of changing mobile phase composition on capacity factor values in reversed phase chromatography is seen in appendix II. Methanol is the strong component, water the weak component. At 70 per cent methanol, there is little resolution between the peaks. The chromatographic system requires adjustment. With 50 per cent methanol in the mobile phase, resolution is achieved between the five components. A further decrease in strong component yields no advantage. At 30 per cent methanol, the last peak has become excessively broad. These effects are typical and apply to all modes of interactive chromatography.

These same materials separated at different temperatures with mobile phase flow rate and composition remain constant. Peaks become sharper as temperature is increased. This observation is fairly general.

Applications

Most classes of pesticides are amenable to HPLC analysis. With certain pesticides or groups of pesticides, HPLC may be the preferred method for quantification because it overcomes the limitations of gas liquid chromatography of thermal breakdown and lack of volatility of compounds.

Lawrence and Turton have reported HPLC data for 166 pesticides including 37 carbamates, giving the conditions, i.e. packing materials, column dimensions, mobile phase compositions, injection volumes, types of samples, detectors used for analysis.

The latest volume of the Analytical Methods for Pesticides and Plant Growth Regulators is devoted to applications of HPLC both for formulation and residue analysis.

A major potential advantage of HPLC is the feasibility of direct carbamate analysis without derivatization. HPLC procedures have been reported for a wide range of carbamates both in the combined form by UV detection and after derivatization by fluorometric detection.

HPLC is finding increasing application in the analysis of substituted ureas since degradation, which occurs at normal gc conditions, is practically eliminated.

In contrast to the well-developed GLC methodology for the determination of OCL and OP pesticides, the HPLC analysis of these classes has not been extensively explored. This may reflect, in part, the lack of available selective detection methods for these compounds. The utility of HPLC would be further extended if element-selective detectors were available, comparable to those in current use in GLC. As it is, only the UV and fluorescence detectors find wide applications in pesticide analysis.

It should be recognized that not all pesticides may be resolved and analysed by HPLC and that GLC or TLC or even spectrophotometry may even be more time efficient and suitable for the solution of particular problems in pesticide analysis. However, it should also be recognized that many pesticides and their metabolites could not have been easily analysed were it not for high pressure liquid chromatography.

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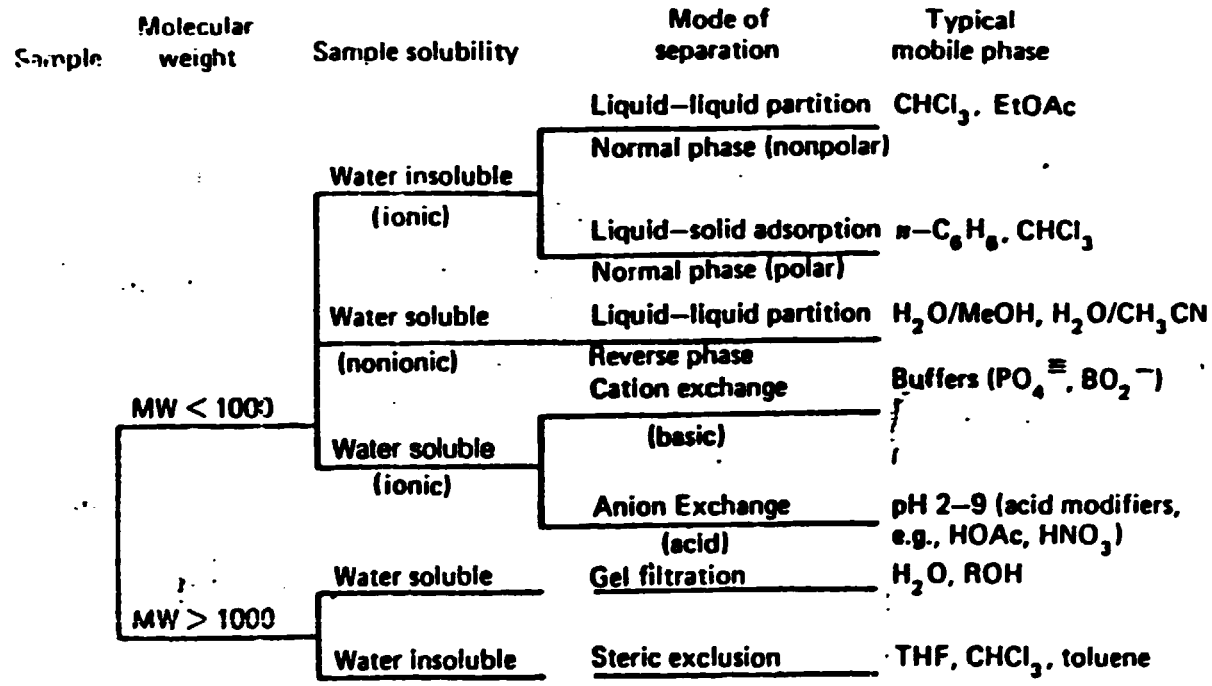
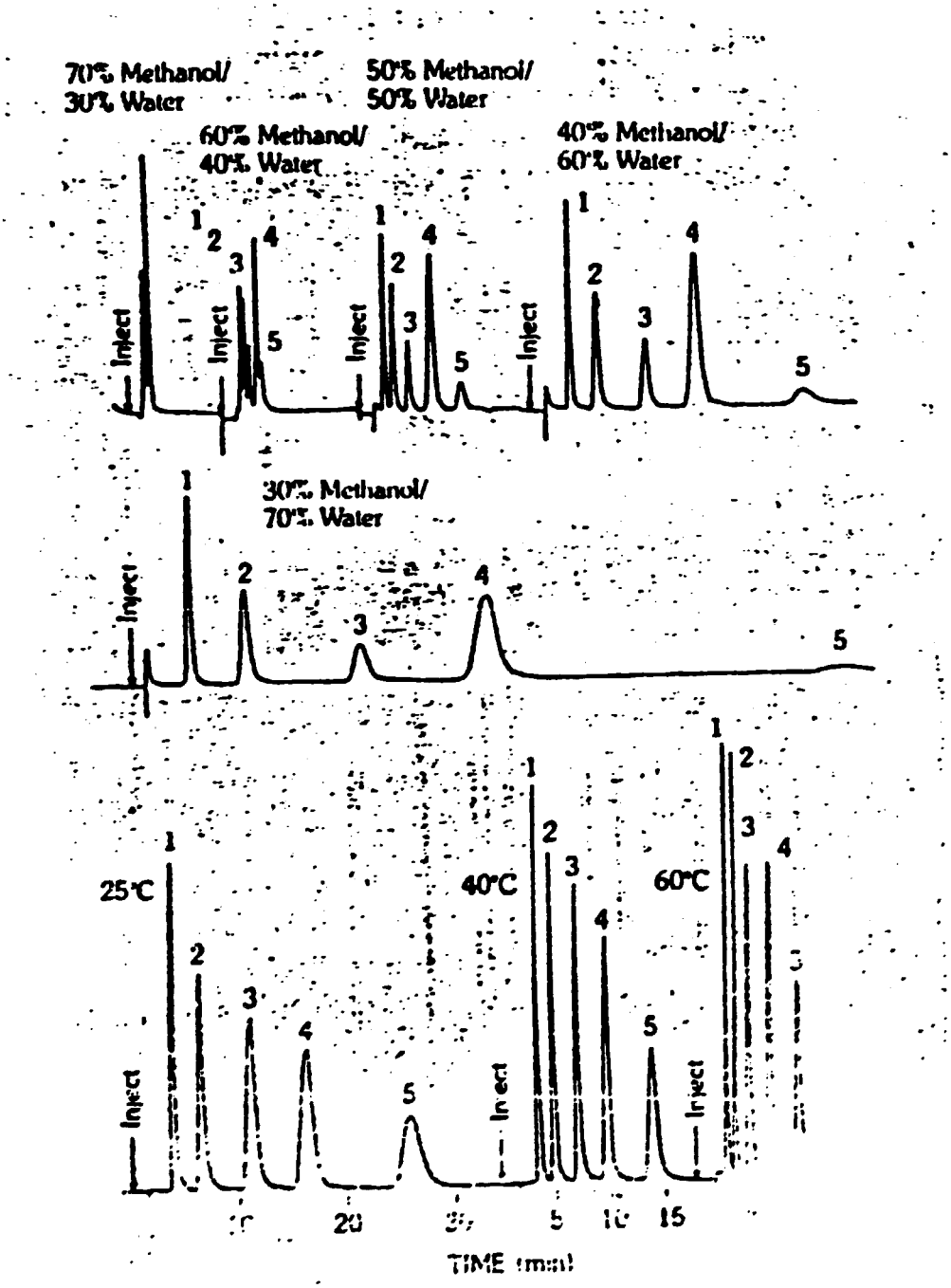
Appendix I

TYPICAL COLUMN SELECTION GUIDE



Appendix II

EFFECTS OF MOBILE PHASE AND TEMPERATURE MANIPULATIONS



Annex V

LIST OF EQUIPMENT

A. Supplementary equipment

1 Millipore's Milli-Q water purification system

1 Millipore filter purification set

1 Millipore sample clarification kit

1 Ultrasonic bath, for solvent degassing

1 Laboratory refrigerator with freezing compartment, explosion proof, self-defrosting (Ref: -10 to 7C; Freezer: -10 to -1C)

2 Gas/liquid tight syringes, 50 ul capacity

6 Glass columns, empty, 3 mm i.d., 1.0 m., for Perkin Elmer Sigma 2 B gas chromatograph

6 Glass columns, empty, 3 mm i.d., 2.0 m., for same equipment
Packing materials:

20 g 1.5% OV 17 on Chrom G HP 100/120 mesh

20 g 3% OV 101 on Chrom W 100/120 mesh

20 g 1.5% OV 17 + 1.95% QF 1

20 g 3% SE 30
Silanized glass wool
Assorted lab glassware

B. Safety equipment

1 First aid cabinet

1 Fume hood, with sliding doors, exhaust fans, with water and air outlets

2 Fire extinguishers, chemical type

Annex VI

RECOMMENDED LITERATURE

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Annex VII

LIST OF CONTACTS MADE

UNIDO	M. Kamal Hussein, SIDFA Sat Pal, assistant programme officer S. Mosinski, UNIDO consultant in pesticide formulation
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