Pyrethrins and Permethrin in Potable Waters by Electron-Capture Gas Chromatography 1981

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £2.25 net

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Methods for the Examination of Waters and Associated Materials

Chromatographic methods are very sensitive to minor physical and chemical variations in the quality of the materials and apparatus used. Hence this method sometimes mentions the actual equipment and materials used for the evaluation tests. This in no way endorses these materials as superior to other similar materials.
Equivalent materials and instruments are acceptable though it must be understood that the performance characteristics may be different, and can vary with batch. It is left to the senior supervising analyst to evaluate and choose from the appropriate brands available.

Contents

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Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

First published 1982

ISBN 011 751628 7

About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Instrumentation and on-line analysis*
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis*
9.0 Radiochemical methods.

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No. 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9TB. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

* These two working groups are in process of being wound up. Their tasks are being redistributed among the other Working Groups.

T A DICK
Chairman

L R PITTWELL
Secretary

25 September 1981

Pyrethrins and Permethrin in Potable Waters by Electron-Capture Gas Chromatography 1981 Tentative Method

1. Performance Characteristics of the Method

1.1	Substances determined	Pyrethrins or Permethrin.
1.2	Type of sample	Potable Waters.
1.3	Basis of Method	Extraction into hexane, followed by gas chromatography using an electron-capture detector.
1.4	Range of application (a)	Up to 25 μg/l
1.5	Calibration Curve (a)	Range of linearity depends on the detector in use. The instrument used in the performance tests gave a straight line calibration graph on log-log ordinates over the following range of weights of compound injected. Pyrethrins 0 to 40 ng Permethrin 0 to 50 ng

1.6 Standard Deviation (a)

Pyrethrins Concentration (μg/l)	Total Standard Deviation (μg/l)	Degrees of Freedom
0.0	0.01*	4
1.0	0.05	4
10.0	0.54	5
25.0	0.92	5

* Within-batch standard deviation.

Permethrin Concentration (μg/l)	Total Standard Deviation (μg/l)	Degrees of Freedom
0.0 (b)		
1.0	0.07	6
10.0	0.72	5
25.0	0.90	5

1.7	Limit of detection (a)	Pyrethrins 0.06 μg/l (4 degrees of freedom) Permethrin 0.24 μg/l (b) (6 degrees of freedom)
1.8	Sensitivity (a)	At a peak to peak baseline noise of 0.5%, 50% recorder deflection was given by the following weights of determinand. Pyrethrins (pyrethrin I) 0.3 ng Permethrin 0.5 ng

1.9 Bias (a)

Only solvent extraction recoveries have been tested. These were as follows:–

Pyrethrins Concentration (μg/l)	Mean Recovery %	95% Confidence limits %
1.0	96.9	4.1
10.0	97.1	4.3
25.0	98.9	2.5

Permethrin Concentration (μg/l)	Mean Recovery %	95% Confidence limits %
1.0	101.9	8.0
10.0	103.6	6.4
25.0	99.2	4.6

See also Section 5.

1.10 Interferences

Potentially any electron-capturing materials which pass through the procedure and have similar gas chromatographic retention times to the component or components of the insecticide being measured for quantification.

In practice no interferences have yet been detected in waters in the United Kingdom. (c)

1.11 Time required for analysis (a)

(i) Assuming that all reagents and apparatus are prepared and the instrument calibrated the following times are typical for the determination of either Pyrethrins or Permethrin:–
For a single determination-extraction 12 minutes, gas chromatography and quantification a further 18 minutes. For multiple determinations 10 samples in approx 2.5 hrs.

(ii) Total analysis time including instrument calibration, and preparation of apparatus and reagents, approx 15 samples per man day.

(a) These results were obtained by the Anglian Water Authority using a constant current electron capture detector 2 μl injections and the gas chromatography conditions given in Sections 9.3.1(a) and 9.3.2(a). Overlapped injections were used for the Pyrethrins analysis. Although similar standard deviations, biases, interferences and times required for analysis should be achieved on other types of electron capture detector using suitable chromatographic conditions, ranges of application, calibration curves, limits of detection and sensitivities may vary markedly.

(b) All blanks for Permethrin were recorded as less than 0.02 μg/l, which was the minimum discrimination of the measuring system in use. No standard deviation could be calculated. The limit of detection quoted is based on the within-batch standard deviation of the 1 μg/l sample. This should be regarded as an upper estimate and it is likely that the true result is somewhat lower. It is unlikely, given 10 blank results less than the minimum discriminable interval of 0.02 μg/l, that the within-batch standard deviation of blank results exceeded 0.01 μg/l. If so, this suggests that the Limit of Detection could be approximately 0.06 μg/l or less.

(c) Information provided by Anglian Water Authority, Essex Water Company, North West Water Authority, Severn-Trent Water Authority and the Water Research Centre.

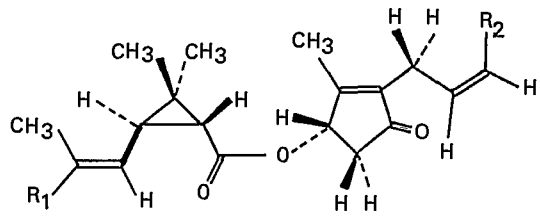
2. Introduction

Pyrethrins or Permethrin are added to potable water supplies for the control of infestation by species such as *Asellus aquaticus*. The material is usually supplied as a 2% m/v solution in an alcohol which is dosed into the supply, after appropriate dilution if necessary, to give nominal concentrations of 10 or 25 µg/l. As the compounds are relatively insoluble in water, precipitation and adsorption can occur reducing their concentration. It is thus necessary to check concentrations at source and in supply during disinfestation exercises. This method is suitable for these purposes.

3. Determinand Structure and Nomenclature

3.1 Pyrethrins

Pyrethrin is the name given to a mixture of naturally occurring insecticidal compounds extracted from the flowers of the pyrethrum plant (*Chrysanthemum cinerariaefolium*). Initially the insecticidal materials were isolated and then separated into two fractions which were named Pyrethrins I and II. Subsequently these fractions were further separated into six pure compounds as shown in Figure 1.



		R_1	R_2
Pyrethrin I	pyrethrin I	$-CH_3$	$-CH=CH_2$
	jasmolin I	$-CH_3$	$-CH_2-CH_3$
	cinerin I	$-CH_3$	$-CH_3$
Pyrethrin II	pyrethrin II	$-COOCH_3$	$-CH=CH_2$
	jasmolin II	$-COOCH_3$	$-CH_2-CH_3$
	cinerin II	$-COOCH_3$	$-CH_3$

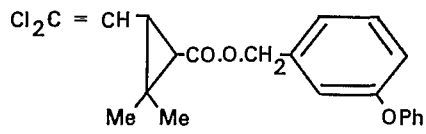
Fig 1 The Structure of Pyrethrins

Thus the word Pyrethrin is used to indicate both mixtures of compounds and single compounds. Throughout this method the mixtures will be named using an initial capital letter (Pyrethrins) and the single compounds using an initial letter in the lower case (pyrethrin).

3.2 Permethrin

Permethrin is the commercial name for a mixture of the cis and trans isomers of a synthetic pyrethroid whose structure is shown in figure 2 below.

Fig 2 The Structure of Permethrin



4 Principle

The Pyrethrins or Permethrin are extracted into hexane. The hexane is analysed by gas chromatography using an electron-capture detector. The Pyrethrins are separated into six gas chromatographic peaks as in figure 3. The instrument is calibrated and quantative results are obtained by measurement of one of the gas chromatographic peaks, normally pyrethrin I. Permethrin appears as either an unresolved doublet (figure 4) in which case the maximum height of this is measured or as a doublet, in which case one of the peaks is measured.

5 Bias

5.1 Adsorption

Pyrethrins and Permethrin are adsorbed onto surfaces, including glass sample containers, from aqueous solution. In order to overcome this source of bias the method includes solvent extraction of the sample vessel.

5.2 Pyrethrins

The composition of different batches of Pyrethrin extracts will vary and with it the ratio of the insecticidal materials. Calibration standards are therefore best prepared from the same batch of 2% alcoholic solution of Pyrethrins as is added to the potable water. The different insecticidal compounds may adsorb or degrade at different rates on passage through the water distribution systems, bias may therefore be introduced by using the method of calibration recommended. Such errors have normally been found to be sufficiently small to be acceptable during mains disinfestation operations.

The 2% Pyrethrins solutions are made up by the supplier from pyrethrum extracts which have been analysed for Pyrethrins, using the PMBK method in the United Kingdom (1). Because of the diffficulties of preparing the pure insecticidal materials primary standardization is restricted and is not normally made. Approximate composition may be checked against other batches of 2% Pyrethrins in an alcohol or standard extracts analysed by the PMBK method. The latter are available from:-

Wellcome Research Laboratories (Berkhamstead)
Berkhamstead Hill,
Berkhamstead,
Herts, HP4 2QE.

In practice the alcohol solutions have been found to be sufficiently reliable for use as standards during mains disinfestation operations.

5.3 Permethrin

The 2% alcoholic solutions supplied for mains disinfestation have been found to be sufficiently reliable for use as standards during the monitoring of such exercises. The ratio of isomers may change from that in the material dosed, in samples taken after several days residence in distribution systems. This will introduce calibration errors. The use of Endrin as an internal standard (2) reduces these errors. However, in most cases the results are adequate for operational purposes.

6 Interferences

No specific tests have yet been made on the interference effects of known concentrations of other substances, however, in principle any substance in the solvent extract capable of producing a response on the electron-capture detector at a gas chromatographic retention time similar to the peak being measured will interfere. In practice no such intefering materials have been found in potable waters analysed in the United Kingdom. Interferences have been found to arise from contact of samples and extracts with certain plastics and from contaminated reagents and apparatus. (See Sections 8 and 9).

7 Hazards

Pyrethrum extracts are toxic and may cause dermatitis of both allergic and contact types (3). Hexane and acetone are highly flammable and appropriate precautions should be taken, including the use of sparkproof refrigerators for the storage of solvents. Electron-capture detectors contain radioactive materials and must be used strictly in accordance with the manufacturer's instructions.

8 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks in the gas chromatographic analysis of the solvent extract. This must be checked for each batch of material and verified by the running of procedural blanks with each batch of samples analysed.

Reagents may become contaminated by contact with air and other materials, particularly plastics or by degradation due to the action of light. Storage should be in the dark in tightly sealed all glass containers or other vessels found to be suitable.

8.1 Pyrethrins or Permethrin

2% m/v solution in an alcohol. This should be the same concentrate as is being added to the potable water. When stored in a refrigerator (4°C) its composition has been sufficiently stable for the purposes of mains disinfestation analyses for at least one year.

8.2 **Hexane**

Pesticide analysis grades are suitable, some batches of other grades may be acceptable without redistillation. Unacceptable batches should be redistilled using a 300 mm Dufton spiral fractionation column or equivalent.

Note – Other volatile, mainly aliphatic hydrocarbon, solvents such as 60–80°C or 40–60°C boiling point petroleum ether have been found to be suitable substitutes for hexane (4) (5). Their suitability should be checked before use, as full performance characteristics are not available.

8.3 **Acetone**

as 8.2 (excepting note).

8.4 **Blank Water**

The water used for blank determinations and for the preparation of standards should be the water which is to be treated with Pyrethrins of Permethrin, immediately prior to the addition of the insecticide. This should be collected on the day of use and if necessary dechlorinated.

Pyrethins are degraded by free chlorine (6), this is therfore removed from the water being treated prior to Pyrethrins addition. In practice it may not always be possible to obtain a sample of dechlorinated, Pyrethrin-free water. If free chlorine is present in the blank water being used it must be removed by the addition of excess sodium thiosulphate (normally 1 ml of a 3% m/v solution of $Na_2 S_2 O_3$ per 500 ml sample will be suitable).

Permethrin isomers are degraded only slowly by free chlorine (7), approx. 25% loss of both cis and trans isomers occurring after 120 hours at both 0.30 and 0.64 mg Cl_2/1. It should not be necessary to dechlorinate blank water as the losses of Permethrin occurring in the short period between the preparation and extraction of aqueous standards (Section 12.3 and 14) will be negligible.

8.5 **Distilled Water**

Laboratory distilled water produced from all – glass or metal stills is normally satisfactory.
Note – Both Pyrethrins and Permethrin steam distil and will contaminate laboratory distilled water if the laboratory is in the dosed area.

9 Apparatus

Apparatus should be checked for contamination by shaking with hexane for at least two minutes and then analysing the hexane as in section 11.2. Some brands of detergent may give rise to interferences.

A suitable cleansing procedure for new or stored glassware is as follows:

Wash with a detergent which does not give rise to interferences, rinse with distilled water and dry in an oven (60°C to 250°C). Where apparatus is being used for successive analysis within a batch, rinsing with hexane between determinations has been found to avoid cross contamination.

Apparatus which has been in contact with high levels of Pyrethrins or Permethrin compared with those being analysed should be rejected unless shown to be contamination-free after cleansing. Heating glassware at 250°C overnight can be effective in removing such contamination. Plastics, except PTFE, should not be used where contact with samples, extracts or standards is expected unless shown to be contamination-free.

9.1 **Separating funnel**

1l capacity with a PTFE tap or glass tap (without grease) and a glass stopper.

9.2 **Tubes – glass stoppered**

15 to 25 ml capacity.

9.3 Gas Chromatography

A gas chromatograph with an elecron-capture detector is required. This should be operated in accordance with the manufacturer's instructions. On-column or glass lined injection systems should be used.

Electron-capture detectors give a linear response over a limited range. This range must be determined for each detector and set of conditions. Contamination of the detector will cause a loss of linearity and change in calibration. These effects will vary with the instrument and in unfavourable cases will necessitate full calibration daily and check standards being run between every four sample injections.

The weights of Pyrethrins in the standards and sample extracts injected must be chosen or adjusted such that the detector operates within its linear range, This is best accomplished by maintaining a fixed injection volume (normally between 1 and 5 μl) and diluting the sample extracts if necessary.

Optimum injector and detector temperatures will vary with the instrument, however these should not be lower than the column temperature.

Many different columns have been used to analyse Pyrethrins and Permethrins, these should have an efficiency of better than 600 theoretical plates. Two suitable columns and conditions are described below:-

9.3.1 Pyrethrins

(a) *Glass column*

0.9 m long, 4 mm internal diameter packed with 100 to 120 mesh Chromosorb W HP supporting 2.5% by weight of OV–25 and operated at 210°C and 40 ml/min N_2. Pyrethrin II should emerge completely within 20 minutes (See figure 3).

(b) *Glass column*

0.9m long, 4mm internal diameter packed with 80 to 100 mesh Volaspher A2 supporting 3% by weight of OV – 210 and operated at 220°C and 60 ml/min N_2. Pyrethrin II should emerge completely within 15 mins. The chromatogram is similar to Figure 3.

9.3.2 Permethrin

(a) *Column as in 9.3.1*

(a) but operated at 250°C and 40 ml/min N_2. The Permethrin isomers are unresolved and should emerge completely within 5 minutes. (See figure 4)

(b) *Column and conditions as 9.3.1 (b)*

The Permethrin isomers are resolved and should emerge completely within 7 mins (see Figure 5).

10 Sample Collection and Storage

Sample bottles should be all glass (including glass stoppers) of approx. 500ml capacity and cleaned in the manner described for glassware (Section 9). Other sample vessels may be appropriate but no information is available.

The sample bottle should not be rinsed with the water being sampled as this may lead to positively biased results due to insecticide being adsorbed from the rinse water onto the sample bottle walls. Samples should not be placed in close proximity to concentrated solutions of the determinand.

Water samples for Pyrethrins analysis should be extracted as soon as possible after collection in order to avoid decomposition of the determinand. Aqueous solutions of Permethrin are stable for at least 120 hours (7). Solvent extracts have been found to be stable for several months when stored in stoppered glass tubes in a refrigerator (4°C).

Evaporation of the solvent may occur even under refrigeration.

Extracts must not be allowed to evaporate to dryness and must be restored to their original volume before analysis.

CAUTION – See section 7.

11 Analytical Procedure

READ SECTION 7 ON HAZARDS BEFORE STARTING THIS PROCEDURE

Step	Procedure	Notes
	Extraction of Pyrethrins	
11.1	Transfer the contents of the sample bottle (approx. 500 ml) to a 1l separating funnel. Rinse the sample bottle with 5 to 10 ml hexane. Make the rinsings up to 10 ± 0.2 ml with hexane and add to the separating funnel. Stopper the funnel and shake vigorously for at least 2 mins (a) (b). Allow the phases to separate for at least 5 mins (c). When good separation has been achieved run off the lower aqueous phase and measure its volume (V ml) to ± 10 ml. Run the hexane layer into a glass stoppered tube.	(a) Alternatively extraction may be performed in the sample bottle if the stopper fits well enough or a PTFE sealing sleeve or 2 turns of PTFE jointing tape are used to seal the stopper. The bottle should be emptied to the shoulder if necessary and 10 ml ± 0.2 ml hexane added. After shaking for 2 mins the phases are added to a separating funnel for separation. Alternative methods are also suitable (8,9). The volume extracted should be known. (b) If different volumes of sample are used the volume of solvent should be adjusted proportionally. Lower ratios of water: solvent i.e. less than 50:1 should give good results and may avoid dilution of the extract for levels of Pyrethrins or Permethrin greater than 25 µg/l or for more sensitive elecron-capture detectors. (c) Many potable waters will give adequate separation in less than 5 min, very few will take longer. If emulsions do form, these may be broken by the addition of salts (usually NaCl, $MgSO_4$ or Na_2SO_4) or by centrifugation. Care is needed to avoid contamination. Provided that sufficient solvent is separated to enable the injections to be made into the gas chromatograph the interfacial emulsion may be discarded.
	Gas Chromatography	
11.2	Inject the hexane extract into the gas chromatograph (d) (e). (See also 11.5 below).	(d) Injection volumes of 1 to 5 µl have been used apparently satisfactorily. The performance characteristics in Section 1 were obtained using a 2µl injection volume.
	Blank	
11.3	Analyse a sample of Blank Water as in sections 11.1 and 11.2	(e) It should not be necessary to dry the extract, however, care must be taken not to draw any water droplets into the syringe.
	Calculation of concentration	
11.4	Measure the height (f) (g) of the same peak as used in plotting the calibration graph, read off the quantity (X ng), of Pyrethrins or Permethrin in the aliquot (Z µl) of sample injected, from the calibration graph. Similarly determine the quantity of Pyrethrins or Permethrin in the Blank (Y ng) (h). Calculate the concentration (C µg/l) of determinand in the sample as:- (j) $C = \frac{10\,(X - Y)}{ZV}\ \mu g/l$ where V = Volume (ml) of sample extracted.	(f) Peak areas may also be used. (g) If the response is above the linear portion of the calibration graph the extract may be diluted with hexane and the calculation of concentration adjusted accordingly. No performance characteristics are available.

Step	Procedure	Notes

(h) Blanks are normally sufficiently small to be regarded as zero.

(j) Extraction recoveries are normally sufficiently close to 100% for recovery correction factors to be ignored in the calculation. This must be checked (see Section 14). If the % recovery (R%) is sufficiently less than 100% to affect the practical usage of the results then the calculation should be amended to:-

$$C = \frac{10\,(X-Y)}{ZV} \quad \frac{100}{R}$$

where R = calculated percentage extraction recovery.

11.5 Note on Gas Chromatography

In the analysis of Pyrethrins only one peak of the Pyrethrin I group (usually the third, pyrethrin I) is used for quantification, thus the Pyrethrin II group is superfluous to this method. GC analysis times may be shortened to approx. one quarter that shown in Figure 3 by using back-flushing systems to remove the Pyrethrin II group (10). Such systems are not generally available and a useful reduction in GC analysis time may be achieved by overlapping injections as shown in Figure 6. This system can increase throughout by approximately 70%.

12 Standards

12.1 Standard A

Pipette 1 ± 0.01 ml of the Pyrethrins or Permethrin concentrate being used for the mains disinfestation (2% m/v insecticide in an alcohol) into a 100 ml volumetric flask, make up to the mark with acetone. This gives a standard of nominally 200 mg insecticide/1 in acetone.

12.2 Gas Chromatography Standards

Standard A should be diluted in hexane to cover the linear range of the detector in use in steps of 1,2,5,10 etc. Typically the following dilutions will cover the working range of the method (in steps of 1,2.5,5, 10) and follow conveniently from Standard A above.

Equivalent Units						
ng/5 µl of hexane	0.25	0.5	1	2.5	5.0	10.0
µg/l of hexane	50	100	200	500	1000	2000
µg/l of water (assuming 100% recovery)	1	2	4	10	20	40

Dilutions may be made using syringes, pipettes or burettes whichever are found to be suitable. The drainage errors for solvents, using pipettes calibrated for delivery of water, have been found to be acceptable for the method. Micro-syringe deliveries may not always be accurate, however, many workers find then suitable. Micro-pipettes which have an air space between the plunger and the solvent volume are NOT suitable due to the volatility of the solvent.

Fig 3 Pyrethnns (4 ng) on OV-25, column and conditions as 9.3.1. (a).

1. Pyrethrin I
2. Jasmolin I
3. Cinerin I
4. Pyrethrin II
5. Jasmolin II
6. Cinerin II

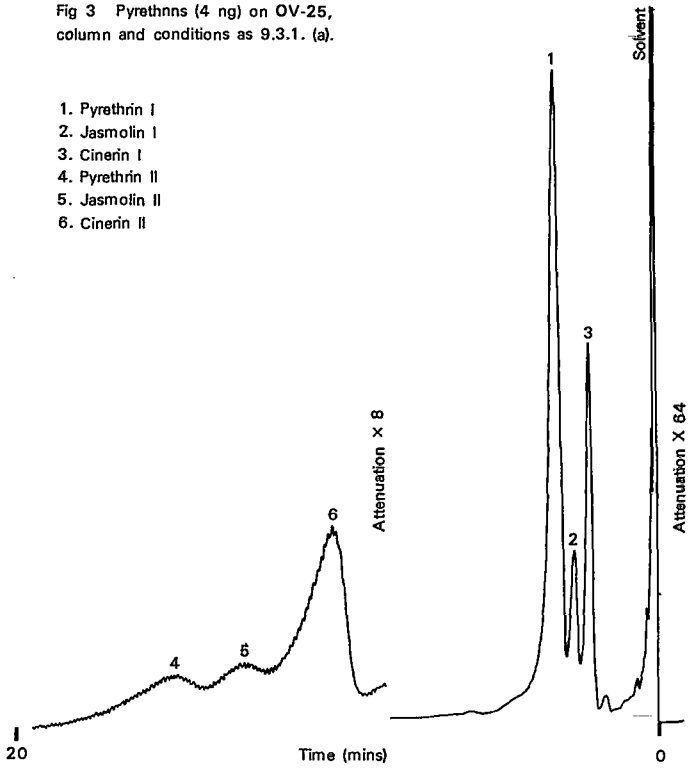


Fig 4 Permethrin (4ng) on OV-25, column and conditions as 9.3.2 (a).

1. Impurity
2. cis + trans Permethrin

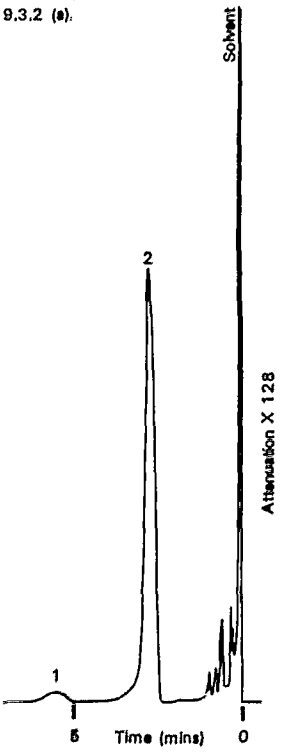


Fig 5 Permethrin on OV-210, column and conditions as 9.3.1 (b).

1. trans Permethrin
2. cis Permethrin
3. Impurity

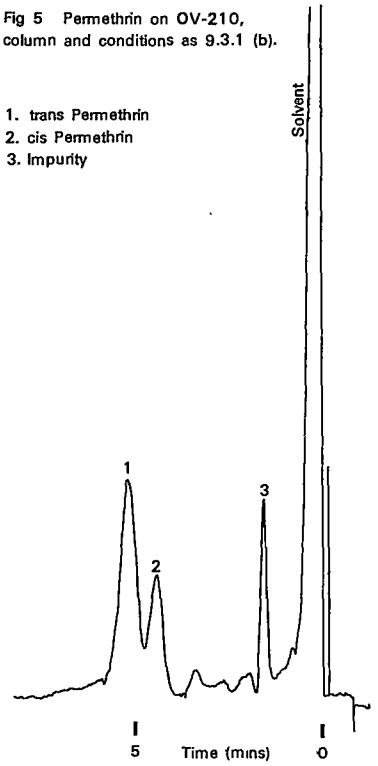
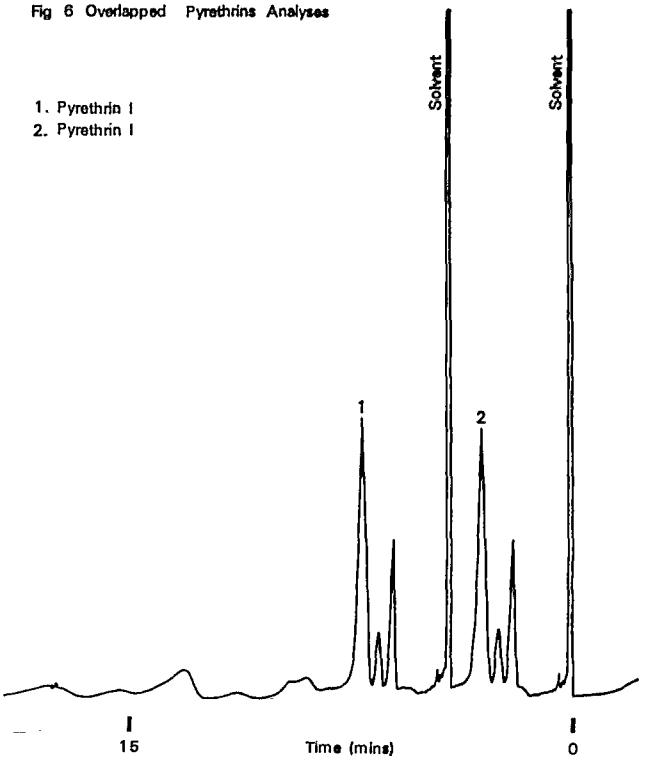


Fig 6 Overlapped Pyrethrins Analyses

1. Pyrethrin I
2. Pyrethrin I



12.3 **Aqueous standards for recovery checks –**

Using a microlitre syringe (100 µl) add a suitable quantity of Standard A to approx. 500 ml Blank Water in a sample bottle to approximate to the maximum level of Pyrethrins or Permethrin expected during the disinfestation operation.
Typically:
Std. (i) 25 µl Std. A for 10 µg/l water
Std. (ii) 62.5 µl Std. A for 25 µg/l water
as 62.5 µl will be difficult to judge using a 100 µl syringe this is better adjusted to the nearest calibration mark on the syringe, e.g. 62 µl. The syringe needle should be held just beneath the water surface and removed immediately after delivery. Other concentrations may be similarly prepared.

12.4 **Storage of Standards**

Standards are normally only required to be stored during the period of a mains disinfestation operation (i.e. maximum 4 weeks). Standards have been shown to be stable over this period when stored in the dark, using well fitting stoppers and preferably refrigerated to minimize evaporation. Working standards should be stored as above when not in use.

13 Calibration of the Gas Chromatograph

Inject the appropriate fixed aliquot of each of the standards prepared as in Section 12.2 at amplifier attenuations such that the peak height of the major peak is between one quarter and three quarters of full scale deflection (on less sensitive instruments the lowest standards may be less than one quarter f.s.d.). Measure the height of one determinand peak in each injection (usually the largest). Plot a calibration graph of peak height against quantity of insecticide injected (in ng). At the beginning of each day, check calibration by injection of a high and a low standard. If either has drifted outside the expected scatter of results re-run. If the result is confirmed re-calibrate. The standards given in Section 12.2 will be within the linear range of most electron-capture detectors. If outside, injection volumes, dilutions and calculations should be adjusted to bring analyses within the linear range.

In order to cover easily the linear range of the detector and achieve good discrimination at low concentrations, it is often convenient to plot calibration graphs using log-log ordinates. Some electron-capture detectors will give calibration graphs with a straight line section using log-log ordinates, but which would not give a linear section on linear ordinates. In these cases the straight line portion of the log-log curve may be regarded as the linear range of the detector.

14 Checking the recovery of the solvent extraction stage

Analyse the aqueous standard as prepared in Section 12.3 by the procedure as in sections 11.1 and 11.2. Measure the height (a mm) of the peak being used for calibration. Inject the same quantity of Standard A (12.1) as was used to prepare the aqueous standard above, using the same syringe and technique, into 10 ml ± 0.2 ml Hexane in a glass stoppered tube. Analyse by step 11.2 and measure the height (b mm) of the peak being used for calibration. The recovery of the solvent extraction stage is then 100 a/b %.

This procedure is adopted because, although microlitre syringe delivery volumes are highly reproducible, their absolute delivered volumes are sometimes questionable. Larger volumes of water-miscible solvents can adversely affect recoveries. In some cases it may be possible to calculate recoveries by the more usual procedure of analysing an aqueous standard by Sections 11.1 to 11.4 and comparing the result with the nominal concentration.

15 Checking the Accuracy of Analytical Results

Once the method has been put into routine use many factors may adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of test are possible and they should be used as appropriate. As a minimum it is suggested that an aqueous standard (Section 12.3) equivalent to the dosing level of Pyrethrins or Permethrin into the potable supply be analysed with each batch of samples (approx 8 samples). The concentration of the aqueous standard should be calculated as though it were a sample and the results plotted on a quality control chart. This will facilitate detection of inadequate accuracy and will also allow the standard deviation of analytical results to be estimated.

Should more frequent checks of detector calibration be required then repeated injection of the same solution may be made and the results plotted on a control chart. Greater than 5% drift would indicate that re-calibration was necessary.

Calibration, Blank and recovery checks should be made daily and the latter two also when new waters are analysed or new batches of reagents introduced.

16 References

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17 Request for Further Information

17.1 Further Method Testing

The method as written has been tested in only one laboratory and must therefore be regarded as tentative. Further information on performance characteristics from other Laboratories is required before recommended status can be achieved; your help in providing this information is requested.

All information will be useful, however, the following experiments are suggested:

1. Analysis of one, some or all of the following solutions in duplicate on each of at least five days: aqueous standards (Section 12.3) made up at 0.0, 1.0, 10.0 and 25.0 μg insecticide. Results to be returned in μg/l.

2. Recovery checks on the solutions analysed as in 1. above. Also on higher concentrations of insecticide.

3. Repeat analysis on real samples: This is likely to be limited to large bodies of dosed water such as service reservoirs and batches analysed on one day. It is suggested that at least five replicates be made on one day.

17.2 Analysis of Pyrethrins or Permethrin in waters other than potable water. This method may be suitable for the analysis of waters other than potable water. Information from laboratories attempting to use the method in this manner is requested. Work at The Water Research Centre, Stevenage, England indicates low recoveries (about 60%) of Permethrin from sewage effluent (W.R.C Laboratory Report No. 1093).

17.3 Analysis of pyrethroids other than Pyrethrins of Permethrin. The solubility data for other synthetic pyrethroids indicates that this analytical method may be suitable for their analysis. Information from laboratories attempting to use the method in this manner is requested. The retention times, relative to pyrethrin I, of some of these materials are given in Table 1.

Data from The Shell Toxicology Laboratory at Sittingbourne, Kent, show recoveries of 72 to 130% of Cypermethrin from water using a similar extraction system to that given in this method.

17.4 *Results* (17.1) or *information* (17.2 and 17.3), or *correspondence* on other matters concerning this method should be sent to:

either

Dr. B.T. Croll,
Chairman SCA Working Group 6,
Anglian Water Authority,
Ambury Road,
HUNTINGDON,
Cambridge, PE18 6NZ

or

The Secretary,
SCA Working Group 6,
Department of the Environment,
Romney House,
43 Marsham Street,
LONDON, SW1 P 3PY.

Table 1

Pyrethroids data relative to pyrethrin I

Column / Pyrethroid	3% OV–210 on Volaspher A2 220°C (py I r.t. = 2.8 mins)	2.5% 0V–25 on Chromosorb W – HP 250°C (py I r.t. = 0.7 mins)	
	Retention Time	Retention Time	Peak Height*
Allethrin	0.57	0.56	2.96
cinerin I	0.73	0.70	0.53
jasmolin I	0.87	0.86	0.28
pyrethrin I	1.00	1.00	1.00
cinerin II	2.89	2.33	0.13
jasmolin II	3.50	2.83	0.05
pyrethrin II	4.00	3.24	0.06
cis Permethrin	1.64	4.11	0.31
trans permethrin	1.86		
Resmethrin – ve	0.79	1.50	0.04
+ ve	2.46	4.14	0.01
Cypermethrin A	4.16	5.86	1.60
B	4.68		
C	5.05		
cis Decamethrin	6.96	12.0	1.56
trans Decamethrin	7.48		
Sumicidin A	2.80	8.14	0.37
B	5.64	8.86	0.31
C	6.32		

* 2 ng injection of each insecticide.

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Dd 718267 C13 9/82

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