Chlorophenoxy Acidic Herbicides Trichlorobenzoic Acid, Chlorophenols Triazines and Glyphosate in Water 1985

Methods for the Examination of Waters and Associated Materials

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Chlorophenoxy Acidic Herbicides, Trichlorobenzoic Acid, Chlorophenols, Triazines and Glyphosate in Water 1985 (for a compound list see Substances Determined, below)

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Contents

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Figures

Substances Determined

This booklet contains methods for the following organochlorine acidic herbicides and related compounds:

2,4 dichloro- and 2,4,5 trichloro-phenoxyacetic acids,
2,3,6 trichloro- and 3,6 dichloro- 2 methoxy-benzoic acids,
4 (4 chloro- 2 methyl-phenoxy) butyric acid,
4 chloro- 2 methyl-phenoxyacetic acid,
2,2 dichloro- and 2 (4 chloro- 2 methyl-phenoxy) propionic acids,
their salts and esters, also called 24D, 245T, 236 TBA,
Dicamba, MCPB, MCPA, Dalapon and Mecoprop or MCPP respectively),
and di-, tri-, tetra- and penta-chlorophenols.

It also contains methods for Glyposate and Triazine Herbicides.

Methods for the Organo Chlorine Compounds

Methods Given

Five methods are given for the organochlorine compounds, each made up of a number of steps which are often interchangeable between methods, hydrolysis being omissible for salts and free acids. Dichlorophenols are best analysed without the derivative formation step. These methods are:

- **A Extraction, Hydrolysis, Butylation, Gas Chromatography**
- **B Extraction, Hydrolysis, Methylation, Gas Chromatography**
- **C Extraction, Perfluorobenzylation, Gas Chromatography**
- **D Extraction, Hydrolysis, Methylation, Gas Chromatography, Mass Spectroscopy**
- **E Extraction, Hydrolysis, Nitration, Methylation, Gas Chromatography.**

In general methyl derivatives come off the GC column sooner than the butyl, which may be of importance when analysing for substances such as pentachlorophenol with long retention times: However butyl derivatives are often (but not always) more clearly resolved than the methyl. Perfluorobenzyl derivatives are used for substances which are otherwise difficult to detect following separation by gas chromatography.

Two confirmatory procedures are given for the chloro-methylphenoxy alkanoic acids. Chlorinated phenols may also be analysed by the methods for phenols at low levels in waters, to be issued in this series, some will also be determinable by the trimethylsilyl ether gas chromatographic method given in Phenols in Waters and Effluents etc, 1981 in this series.

Preferred Methods

The determinands most suited to each method are:

Method	Preferred for	Also suitable for*
A (Butylation)	24D, 245T, Dalapon	
B (Methylation)	236 TBA, Dicamba, Polychlorophenols	24D, 245T, dichlorophenols
C (Pentafluorobenzylation)	MCPA, MCPB, MCPP	Dicamba, TBA
D (Gas Chromatography–Mass Spectrometry)	MCPA, MCPB, MCPP, 24D, 245T	
E (Nitration–Methylation)	MCPA, MCPB, MCPP	

Methods for Other Herbicides

F	Glyphosate
G	Triazines

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 emphasized 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. Known or suspected poisoning cases are usually sent to the nearest hospital having special equipment. To ensure admission to the correct hospital at once, always state whether poisoning is likely when calling an ambulance or arranging for an admission to hospital. Similar warning should be given if a biological or radio-chemical 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 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. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. 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.

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About this Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, 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 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 the 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 a committee of the Department of the Environment set up in 1972. Currently it has seven 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
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods.

The actual methods and reviews 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.

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 and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. 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 that booklet.

L R PITTWELL
Secretary

31 October 1983

CHROMATOGRAM OF 2,4-D AND 2,4,5-T BUTYL ESTERS

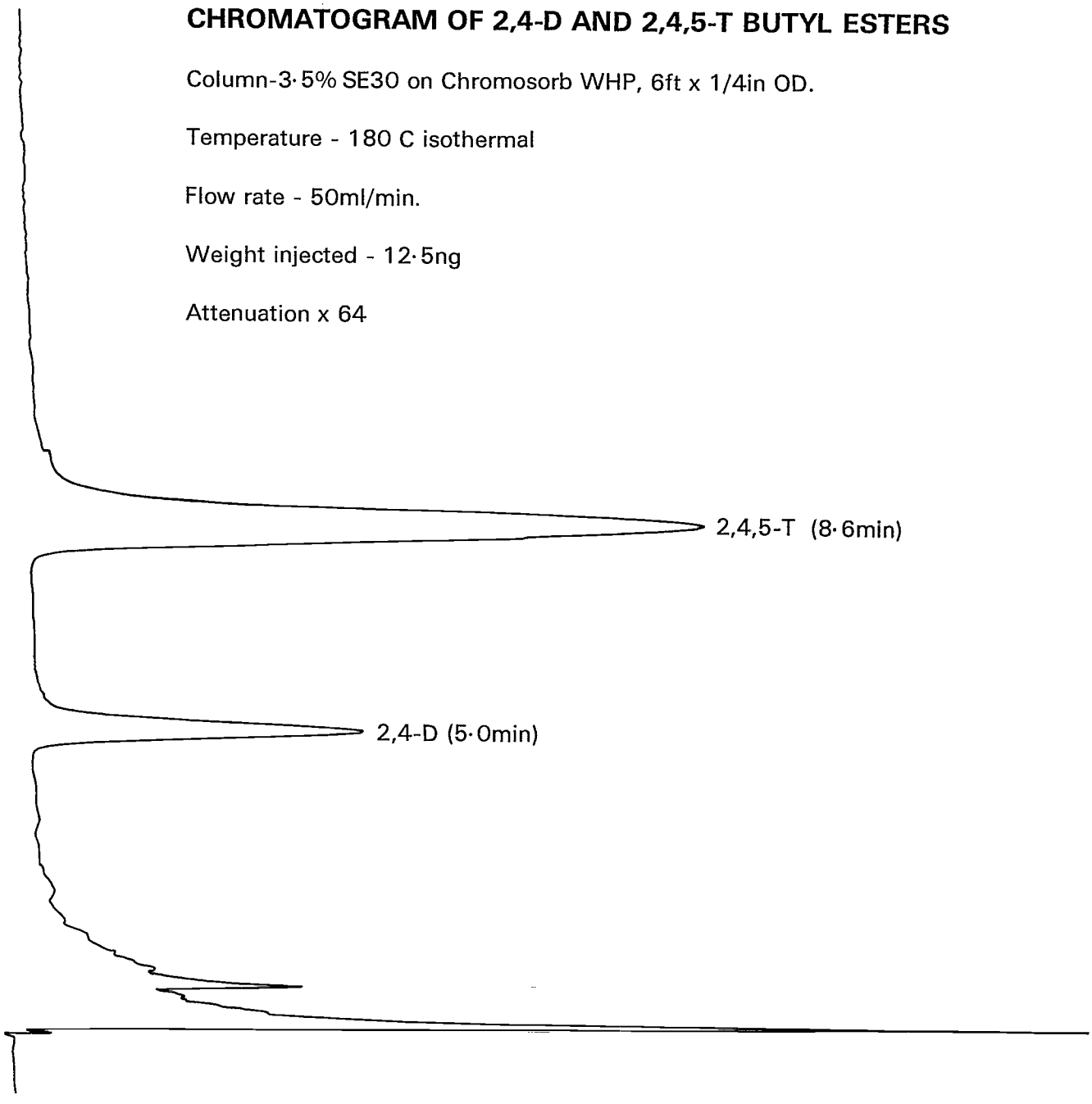
Column-3·5% SE30 on Chromosorb WHP, 6ft x 1/4in OD.

Temperature - 180 C isothermal

Flow rate - 50ml/min.

Weight injected - 12·5ng

Attenuation x 64



A The Determination of 2,4-D and 2,4,5-T Herbicides in Rivers and Drinking Waters

A1 Performance Characteristics of the Method

A1.1	Substances determined	2.4-dichlorophenoxy and 2.4.5-trichlorophenoxy acetic acids (2.4-D and 2,4,5-T), their salts and esters and Dalapon.
A1.2	Types of sample	River and drinking waters.
A1.3	Basis of method	Extraction into diethyl ether followed by alkaline hydrolysis. Derivatization and examination by gas–liquid chromatography using an electron capture detector (EC/GC).
A1.4	Range of application	Up to 10 µg/l.
A1.5	Calibration curve	Range of linearity depends upon the detector in use. For the instrument used in the performance tests (Varian 3700) the linear range for 2,4-D was up to 2.5 ng and for 2,4,5-T up to 20 ng.
A1.6	Standard deviation	See Table 1.
A1.7	Limit of detection (9 degrees of freedom)	

	Detection Limit (µg/l)	SW Within batch Standard deviation ng/l
2,4-D	0.024	0.0053
2,4,5-T	0.004	0.0009

A1.8	Sensitivity	Dependent on the determinand and the instrument in use. For the instrument used in the performance tests, using an attenuation of 16, 2,4-D gave 22% f.s.d. and 2,4,5-T gave 58% f.s.d. for 1 ng of herbicide injected.
A1.9	Bias	Extraction efficiencies are normally less than 100%. Bias will vary with the extraction efficiency of any particular determinand and its derivative (see Table 1) for samples relative to standards.*
A1.10	Interferences	Any coextracted material or its derivative which has a similar GC retention time and which gives a detector response will interfere.
A1.11	Time required for analysis	3 samples/day

* See advice at B1.9

A2 Principle

The herbicides are extracted from the acidified sample into diethyl ether. The extract is evaporated to low volume and hydrolysed with alkali. After acidification, the free acids are extracted into diethyl ether and evaporated to dryness before the formation of the butyl ester. The derivative is extracted into hexane and examined by electron capture gas chromatography.

A3 Interferences

The detector is sensitive to many compounds other than herbicides. Gas chromatography using two or more columns may assist in differentiating herbicide derivatives from interfering peaks on the chromatogram.

A4 Hazards

Diethyl ether is narcotic. Diethyl ether, hexane, butanol and acetone are flammable. All electrical equipment should be flame proof. Herbicides in the undiluted state are toxic. Caution must be exercised when preparing the stock solutions. Skin contact, ingestion and inhalation must be avoided. The acid and alkali solutions used are corrosive. Formation of peroxides in ether should be minimized using the procedure given in A5.1. Subsequent prolonged storage should be avoided to minimize explosion hazards. Always test for absence of peroxide if the ether is to be distilled, or if long storage prior to or since purchase is suspected.

A5 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 derivatives. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Reagents should be stored in all glass containers.

A5.1 Diethyl ether – freshly distilled through a fractionating column. Ether should be stored in dark bottles containing copper gauze.

A5.1.1 Tests for the presence of ether peroxides

i. Highly peroxidized ethers have a different smell from pure ethers, but this provides only a rough indication and samples should be examined as described in ii, iii, or iv.

ii. Test papers are available commercially. Otherwise, either

iii. Prepare ferrous thiocyanate from solutions of ferrous sulphate, potassium thiocyanate and dilute sulphuric acid, reduce any ferric thiocyanate by adding small pieces of iron wire; (for a suitable apparatus for preparation and storage see that described for chromous chloride in Ref A9.1). Shake a sample of the ether with a few drops of the colourless ferrous thiocyanate solution, being careful to avoid oxidation during transfer and ensuring that no air is present. The reddish ferric thiocyanate colour indicates the presence of ether peroxide (for more details see Ref A9.2);

or

iv. Prepare an acidified solution of potassium iodide using potassium iodide and dilute sulphuric acid. Remove any free iodine by shaking with a little carbon tetrachloride or chloroform just prior to use, allow to settle and decant off the colourless aqueous solution. Add the suspect ether to this aqueous solution. A brown coloration indicates the presence of ether peroxide. The exact amounts of reagents used in this test are not critical.

A5.1.2 Safe Destruction of ether peroxides

Do not attempt to distil an ether containing ether peroxide. Either dissolve some ferrous sulphate in dilute sulphuric acid and pour this slowly into the ether, mix thoroughly, allow to separate and retest the ether (do not use electrical appliances for the mixing);

or carry out the procedure given in Section B5.2. Finally, redistill the ether using a fractionating column.

A5.2 Potassium hydroxide solution – 5% w/v in distilled water, freshly prepared.

A5.3 Hexane – Fraction from petroleum. Boiling range not less than 95% between 67°C and 70°C.

A5.4 Sodium sulphate – Anhydrous, granular, neutral, roasted in a muffle furnace at 500°C ± 20°C for 4 h ± 30 min. Cool to about 200°C in the furnace and then to ambient temperature in a desiccator and store in a sealed jar. Some batches of sodium sulphate have been found to be alkaline. If this is so, wash with a solution containing 0.5 ml conc. hydrochloric acid per litre of methanol before roasting.

A5.5 Sulphuric acid – Analytical Reagent grade concentrated (d_{20}1.84).

A5.6 Dilute sulphuric acid – Approximately 5M. CAREFULLY add 250 ml of concentrated sulphuric acid to 600 ml distilled water with swirling and cooling. Cool to ambient temperature and dilute to 1 litre with distilled water.

A5.7 Dichloromethane – Analytical Reagent grade.

A5.8 Butan-1-ol – Analytical Reagent grade.

A5.9 Acetone – Analytical Reagent grade.

A5.10 Anti-bumping granules – washed with Dichloromethane (DCM) before use.

A5.11 Standard solutions of 2,4-D and 2,4,5-T.
A5.11.1 Stock Solutions – These may be prepared by dissolving pure or certified materials in acetone. A suitable concentration is 50 mg/100 ml

WARNING
CAUTION MUST BE EXERCISED WHEN PREPARING THE STOCK SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

A5.11.2 Working Standards – these may be prepared from stock solutions using microlitre syringes which are reserved solely for this purpose and using hexane as the dilutent. Some useful working standards are:

0.5 µg/ml	(100 µl A5.12.1 diluted to 100 ml)
1.0 µg/ml	(200 µl A5.12.1 diluted to 100 ml)
2.5 µg/ml	(500 µl A5.12.1 diluted to 100 ml)
5.0 µg/ml	(1.0 ml A5.12.1 diluted to 100 ml)
10 µg/ml	(2.0 ml A5.12.1 diluted to 100 ml)

A6 Apparatus

Glassware should be clean and dry. Rinsing with acetone just before use assists in freeing glassware from possible contaminants.

A6.1 Sample bottles – (Information on the supply of suitable bottles is available from Glass Manufacturers Federation, 19 Gt Portland Street, London W1 — 01-580 6952). These should be of all glass construction capable of holding 1.2 litres and calibrated at 1 litre. Each bottle should be checked for contamination by rinsing with a small volume of acetone and examining the rinsings by gas chromatography. Bottles showing evidence of contamination should be rejected.

A6.2 Drying tubes – Glass tubes approximately 130 mm long by 10 mm internal diameter fitted with a reservoir at the top and a jet at the bottom. The jet should be loosely plugged with acetone-washed cotton wool and the tube half filled with sodium sulphate (A5.4).

A6.3 Separating funnels – Glass 1 litre and 100 ml capacity, with ungreased glass or PTFE taps.

A6.4 Round bottom flask – 50 ml capacity with ground glass socket.

A6.5 Kuderna – Danish evaporator (Figure 1).

A6.6 3 bubble micro – Snyder column (Figure 2).

A6.7 Graduated centrifuge tubes, glass 10 ml–0.1 ml graduations, tapered, glass stoppered.

Fig 1 KUDERNA-DANISH EVAPORATOR SYSTEM

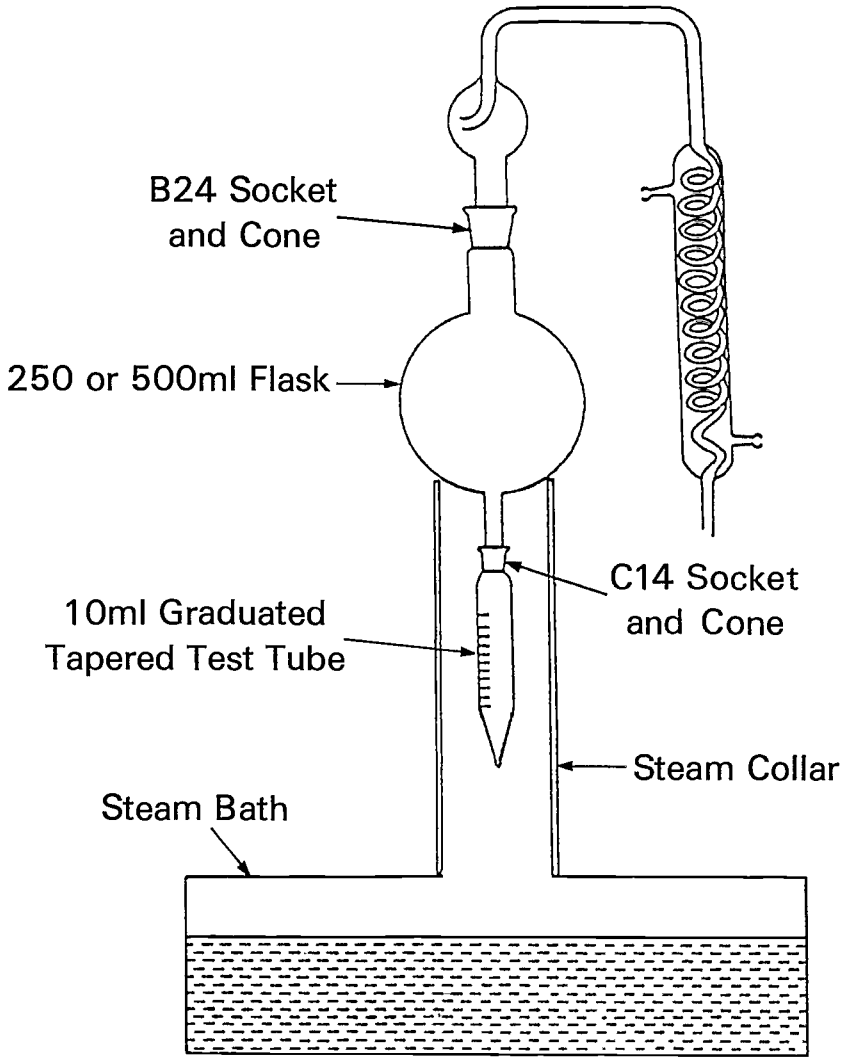
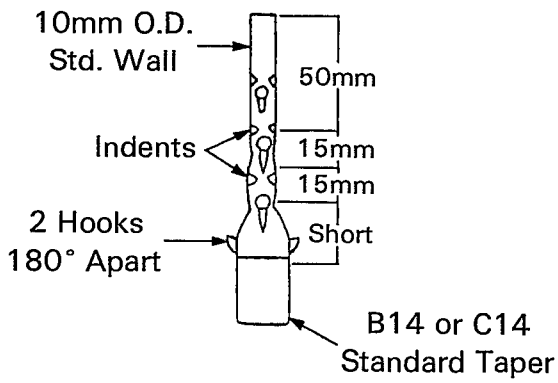


Fig 2 MICRO SNYDER COLUMN



A6.8 Gas–liquid chromatograph (GC).

A gas chromatograph with an electron capture detector is required, to be operated in accordance with the manufacturer's instructions. On-column or glass lined injection systems should be used. Many different columns have been used for herbicide analysis; some suitable columns are:

i. Glass column 1.5 m × 3 mm id packed with 80–100 mesh AW-DMCS Chromosorb W supporting 4% by weight of OV1 or SE 30 stationary phase operated at 180 °C.
ii. A similar column supporting 3% by weight of diethylene glycol succinate.
iii. Fused silica wall-coated open tubular column (25 m coated with a methyl silicone stationary phase).

A7 Sample Storage and Preservation

Samples should be extracted as soon as possible after sampling. If it is impractical to analyse the samples immediately, the acid and solvent should be added to the sample bottles at once (see A8.1.1). These are then shaken and stored in a spark-proof refrigerator. The sample bottles should be protected from contamination by covering the top and shoulders with polythene sheeting. Alternatively the whole bottle may be sealed in a polythene bag. Samples should not be placed in close proximity to concentrated solutions of herbicides.

A8 Analytical Procedure

Step	Procedure	Notes
A8.1	Extraction of Herbicides	
A8.1.1	Add 5 ml of dilute sulphuric acid (A5.6) to 1 litre of sample and check that the pH is between 1 and 2 (see Note a). Add more acid if required. Add 150±5 ml of diethyl ether and shake vigorously for 2 minutes (see Notes b and c). Transfer the contents into a 1 litre separating funnel and allow the phases to separate. Rinse the sample bottle with a few ml of ether and add the rinsings to the 1 litre separating funnel.	a. Narrow range pH paper or a pH meter may be used. b. Vent the bottle at intervals especially at the start, to prevent a build up of pressure.
A8.1.2	Run off the aqueous layer into a second 1 litre separating funnel and transfer the remaining ether layer to a drying tube (A6.2). Collect the eluate from the drying tube in a Kuderna Danish evaporator fitted with a 50 ml round bottomed flask (A6.4).	c. A shaking machine may be used once any risk of pressure build up is over, but the extraction time must be extended to at least ten minutes.
A8.1.3	Add a further 50±5 ml ether to the separating funnel containing the sample and again shake vigorously for 2 minutes. Allow the phases to separate then run off the extract into the same drying tube, combining the extracts in the Kuderna Danish evaporator. Wash the drying tube with 20±2 ml ether and combine the washings with the extracts.	
A8.1.4	Discard the aqueous layer. Care must be exercised because this layer will contain dissolved ether.	

Step	Procedure	Notes
A8.2	Hydrolysis	
A8.2.1	Add an anti-bumping granule (A5.10) to the extract from A8.1.3 and carefully concentrate in a fume cupboard to approximately 5 ml on a water bath maintained at 55°C (see Note d). Fit a micro-Snyder column (A6.6) and further reduce the volume to approximately 1 ml. Alternatively the volume may be reduced using a gentle stream of dry, oil-free air or nitrogen in a fume cupboard.	d. Initial violent ebullition may occur.
A8.2.2	Add 5 ± 1 ml of potassium hydroxide solution (A5.2) from a measuring cylinder to the concentrated extract and re-fit the column (see Note e). Place the flask on a steam bath in a fume cupboard for one hour.	e. Binding of the ground glass joint can be avoided by the use of a P.T.F.E. sleeve.
A8.2.3	Cool the flask to ambient temperature and transfer the contents quantitatively to a 100 ml separating funnel rinsing the flask with two aliquots of 5 ± 1 ml of distilled water. Add each rinsing to the separating funnel and extract twice with 20 ± 1 ml aliquots of hexane. Allow the phases to separate and reject the organic layer each time (see Note f).	f. The potassium salts of the herbicides remain in the aqueous phase.
A8.2.4	Add 2 ± 0.2 ml dilute sulphuric acid (A5.6) and swirl to mix. Extract the free acids of the herbicides with 20 ± 2 ml of dichloromethane (DCM) (A5.7).	
A8.2.5	Allow the phases to separate and then run the DCM through a second drying tube collecting the eluate in a Kuderna Danish evaporator fitted with a 10 ml graduated centrifuge tube (A6.7). Wash the drying tube with 10 ± 1 ml DCM and collect the washings in the same evaporator (see Note g).	g. Steps A8.2.1 to A8.2.5 may be omitted if only the free acids are to be determined.
A8.3	Concentration	
A8.3.1	Add an anti-bumping granule (A5.11) and carefully concentrate the extracts to 5 ± 1 ml on a steam bath (see Note d).	
A8.3.2	Further evaporate the extracts just to dryness using a micro-Snyder column or a gentle stream of dry oil-free nitrogen or air in a fume cupboard.	
A8.4	Derivatization	
A8.4.1	Add 0.5 ± 0.1 ml butan-1-ol (A5.8) and dissolve the residue, warming if necessary. Add 2 drops of concentrated sulphuric acid (A5.5), loosely stopper and immerse the bottom of the tube in boiling water for 45 minutes ± 5 minutes, then cool to ambient temperature (see Note h).	h. A heating block maintained at 100°C may be used.
A8.4.2	Add 10 ml distilled water followed by 1 ± 0.05 ml hexane (A5.3) (see Note i). Stopper and shake vigorously for 30 seconds. Allow the layers to separate.	i. A pipette calibrated for this purpose or a burette must be used.

Step	Procedure	Notes
A8.4.3	Evaporate 1 ± 0.05 ml of each of the working solutions (A5.12.2) and a blank to dryness as in step A8.3.2 and derivatize as in steps A8.4.1 and A8.4.2.	
A8.5	**Gas Chromatography**	
A8.5.1	Inject 5 µl of the hexane layer of each derivatized standard (A8.4.3) and sample extract (A8.4.2) into the GC. Compare the retention times of sample peaks with those of the standards (see Note j). (See Frontispiece)	j. If a capillary column is used the injected volume should be decreased.
A8.6	**Calculation of Concentration**	
A8.6.1	Construct a calibration chart of the weight of standard material in ng against peak height or area. Read off the weight of herbicide injected (see Note j). Calculate the concentration of herbicide in the original sample from: $C = \frac{1000.E.A.D}{I.V}$ µg/l where C = concentration in sample (µg/l) E = volume of final extract (ml) A = weight of herbicide (ng) in the injected volume of extract (blank corrected) I = volume injected in µl V = volume of original sample in ml D = concentration or dilution factor If exactly 1 litre of sample is used and the volumes stated in the method adhered to, the calculation is simplified to: $C = \frac{A}{5}$ µg/l	k. It may be necessary to dilute or concentrate the sample hexane extract to bring the instrument response into the same range as the standard. Note the dilution or concentration factor D.
A8.7	**Blanks and Standards** To check for contamination and interferences, at least one blank and one standard should be analysed by the entire procedure with each batch of analyses.	

A9 References

A9.1 *Analysis of Sludge Digester Gas 1979* (in this series) p15. Section B5.2.2. HMSO.

A9.2 *Analar Standards for Laboratory Chemicals* 8th edition p258 1984. BDH.

Table 1 Recoveries and Estimates of Standard Deviation*

	2,4-D			2,4,5-T		
Spiking level (µg/l)	% recovery	Mean (µg/l)	Total (9) Standard Deviation (µg/l)	% recovery	Mean (µg/l)	Total (9) Standard Deviation (µg/l)
9.0	94	8.5	0.503	98	8.8	0.144
0.1	85	0.08	0.005	66	0.07	0.010

Figures in brackets indicate the number of degrees of freedom used in the calculation of the estimate of standard deviation. Means and recoveries have been blank corrected.

*These data were obtained at The Polytechnic of North London, Holloway, London, using spiked river water containing 61 mg/l of suspended solids.

B The Determination of Substituted Benzoic Acid Herbicides and Chlorinated Phenols in River and Drinking Water

B1 Performance Characteristics of the Method

B1.1	Substances determined	2, 3, 6-trichlorobenzoic acid and its salts (TBA) 3, 6-dichloro-2-methoxybenzoic acid and its salts (Dicamba) 2, 4, 6-trichlorophenol (2,4,6 TCP) 2, 4, 5-trichlorophenol (2,4,5 TCP) 2, 3, 4, 6-tetrachlorophenol (2,3,4,6 TeCP) Pentachlorophenol (PCP) Other chlorinated phenols may be extracted by the method (see Section B8.4)
B1.2	Types of sample	River and drinking water.
B1.3	Basis of method	Acidification of the sample, extraction into diethyl-ether, methylation and, examination by gas chromatography using an electron-capture detector (EC-GC).
B1.4	Range of application	0–80 µg/l. The range can be extended upwards by dilution of the sample.
B1.5	Calibration curve	The range of linearity depends upon the detector in use.
B1.6	Standard deviation	See Table 2.
B1.7	Limit of detection	See Table 3.
B1.8	Sensitivity	Dependent upon determinand and instrument in use. See Table 4.
B1.9	Bias	Extraction efficiencies are normally less than 100%. This will lead to results consistently lower than the true value. See Table 5. (This error may be somewhat reduced if control standards or spiked samples are run through the whole procedure and correction made; but even this may not completely eliminate systematic errors with turbid or polluted samples due to the effects of other materials.)
B1.10	Interferences	Any co-extracted material or its derivative which has a similar GC retention time will interfere.
B1.11	Time required for analysis	Six samples per working day.

Table 2 Standard Deviations

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
(a) Standard Deviations of River Water					
2, 4, 6-TCP	0.009	0.002	0.007	0.007	10
2, 4, 5-TCP	0.001	0.010	0.011	0.015	14
2, 3, 4, 6-TeCP	0.011	0.002	0.025	0.025	9
PCP	0.017	0.002	0.007	0.007	9
2, 3, 6-TBA	0.0849	–	–	0.007	5
(b) Standard Deviations of River Water Plus Low Level Spikes of Each Compound (1 µg/l)					
2, 4, 6-TCP	1.098	0.036	0.047	0.059	13
2, 4, 5-TCP	1.046	0.041	0.079	0.089	11
2, 3, 4, 6-TeCP	1.039	0.042	0.058	0.072	13
PCP	1.028	0.036	N.S.	0.044	15
2, 3, 6-TBA	0.208	0.0053	0.0012	0.0054	9
(c) Standard Deviations of River Water Plus High Level Spikes of Each Compound					
2, 4, 6-TCP	10.49	0.20	0.87	0.89	10
2, 4, 5-TCP	10.38	0.16	0.96	0.97	9
2, 3, 4, 6-TeCP	10.02	0.20	N.S.	0.25	17
PCP	9.38	0.27	N.S.	0.31	15
2, 3, 6-TBA	18.45	–	–	0.76	5

Table 3 Limits of Detection
Each with 9 degrees of freedom except 2, 3, 6-TBA which had 5.

Compound	Limit of Detection µg/l
2, 4, 6-TCP	0.07
2, 4, 5-TCP	0.2
2, 3, 4, 6-TeCP	0.02
PCP	0.02
2, 3, 6-TBA	0.0005

Table 4 Sensitivity

Compound	Quantity ng	% Full Scale Deflection at a Base-line Noise Level of 0.5%
2, 4, 6-TCP	0.03	84
2, 4, 5-TCP	0.03	16
2, 3, 4, 6-TeCP	0.02	62
PCP	0.04	66
2, 3, 6-TBA	0.03	17

Table 5 Recoveries

Compound	Recovery %	Standard Deviation	Degrees of Freedom
(a) Recoveries 1 µg/l Level (2, 3, 6-TBA at 0.2 μgl^{-1} Level)			
2, 4, 6-TCP	115.8	9.3	11
2, 4, 5-TCP	114.6	4.8	18
2, 3, 4, 6-TeCP	109.1	10.1	11
PCP	105.9	7.3	12
2, 3, 6-TBA	100.8	3.2	8
(b) Recoveries 10 µg/l (2, 3, 6-TBA at 18.4 μgl^{-1} Level)			
2, 4, 6-TCP	107.8	5.9	10
2, 4, 5-TCP	104.1	2.8	13
2, 3, 4, 6-TeCP	103.9	4.7	11
PCP	101.7	3.5	15
2, 3, 6-TBA	98.2	3.6	5

B2 Principle

The herbicides and/or chlorinated phenols are extracted from the acidified sample into diethyl-ether, methylated with diazomethane and examined by EC-CG.

B3 Interferences

The detector is sensitive to many compounds other than herbicides and chlorinated phenols. Gas chromatography using two or more columns may assist in differentiating herbicide and chlorophenol derivatives from interfering peaks on the chromatogram.

B4 Hazards

Diethyl ether and methanol are toxic, narcotic and flammable. Iso-octane is flammable. All electrical equipment should be flameproof. Herbicides especially in the undiluted state, are toxic. Caution must be exercised when preparing the stock solutions. Diazomethane is toxic, carcinogenic and potentially explosive. The sulphonamide reagent has been shown to induce tumours in laboratory animals. Skin contact, ingestion and inhalation must be avoided. Ethers can form peroxides on storage or oxidation which are explosive. For detection see Section A5.1.1, for removal see Sections A5.1.2 or B5.2.

B5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks on the chromatograms ultimately obtained. This should be verified for each batch of material by running procedural blanks with each batch of samples analysed. Reagents should be stored in all-glass containers.

B5.1 Dilute sulphuric acid – Approximately 5 M. Very carefully add 250 ml of concentrated sulphuric acid (Analytical Reagent grade) (d_{20} 1.84) to 600 ml of distilled water with swirling and cooling. Cool to ambient temperature and dilute to 1 litre with distilled water.

B5.2 Diethyl ether – Freshly re-distilled from a mixture of 5 ± 1 g of potassium hydroxide and 1 ± 0.2 g of quinol per litre in all glass apparatus. (Alternatively see Section A5.1 and subsections therein.)

B5.3 Methanol – Analytical Reagent grade.

B5.4 N-methyl-N-nitrosotoluene-4-sulphonamide – Analytical Reagent grade.

B5.5 Potassium hydroxide solution – Dissolve 60±0.5 g potassium hydroxide in 100±5 ml distilled water.

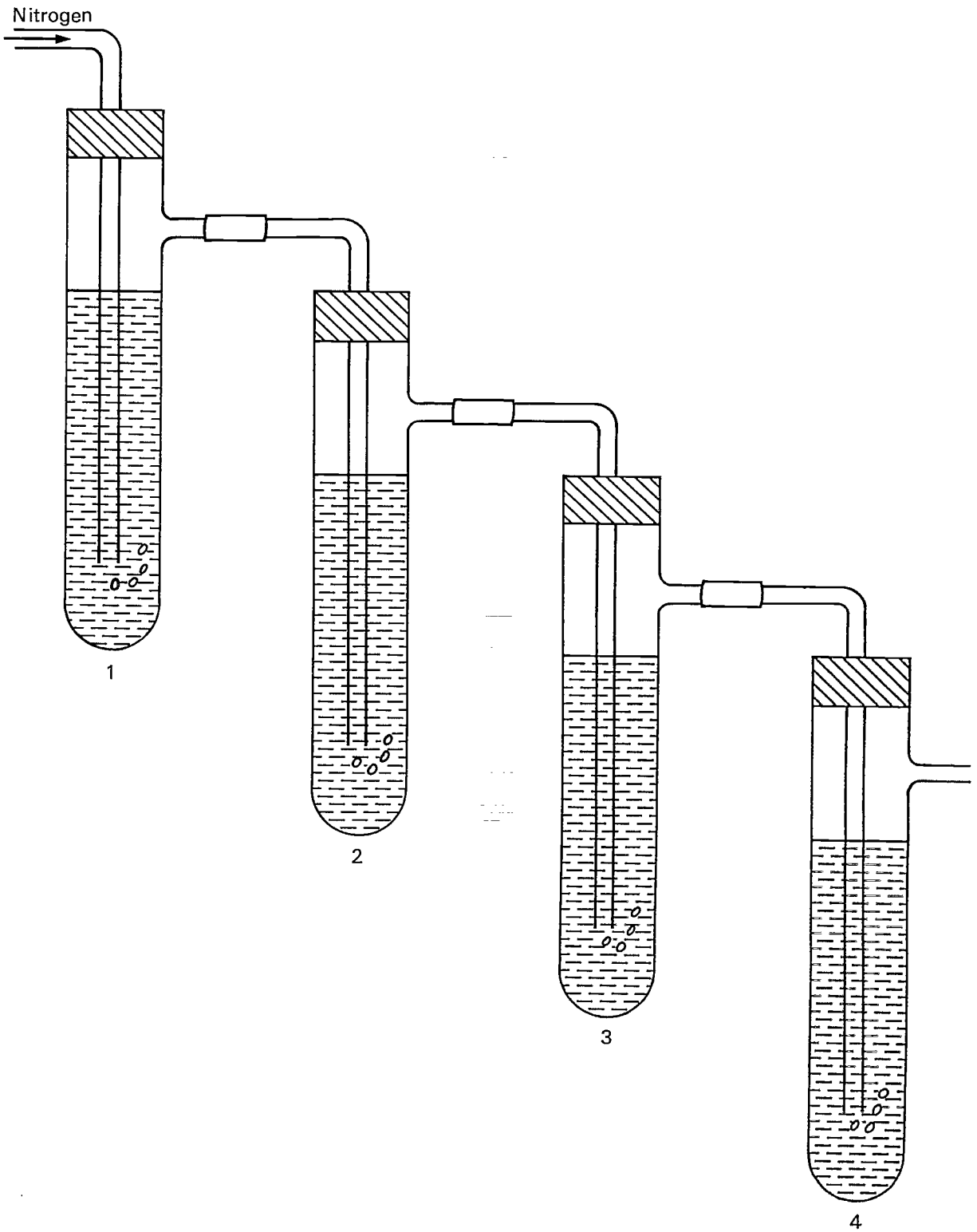


FIGURE 3 METHYLATION APPARATUS

B5.6 Standard solutions of the herbicides and chlorophenols.

B5.6.1 Stock solutions – these may be prepared by dissolving pure or certified materials in methanol. A suitable concentration is 50 mg/100 ml methanol.

B5.6.2 Working standards – these may be prepared by diluting aliquots of the stock solutions with water-saturated ether (B5.8) using microlitre syringes or calibrated pipettes which are reserved solely for this purpose. Some useful working standards are:

0.01 µg/ml	(2 µl B5.6.1 diluted to 100 ml)
0.025 µg/ml	(5 µl B5.6.1 diluted to 100 ml)
0.05 µg/ml	(10 µl B5.6.1 diluted to 100 ml)
0.1 µg/ml	(2 µl B5.6.1 diluted to 10 ml)
0.5 µg/ml	(10 µl B5.6.1 diluted to 10 ml)
1.0 µg/ml	(20 µl B5.6.1 diluted to 10 ml)

B5.7 2, 2, 4-Trimethylpentane (iso-octane) – Redistilled in all-glass apparatus.

B5.8 Water saturated ether – Vigorously shake 200 ml distilled water with 2l of distilled diethyl ether (B5.2) in a glass stoppered Winchester taking care to release any build up of pressure.

B5.9 Glacial acetic acid – Reagent grade.

B5.10 Roasted sodium sulphate – Heat at 500±20°C for 4 hours ± 30 minutes. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator containing magnesium pechlorate or equivalent alternative.

B5.11 Anti-bumping granules – For 2, 3, 6-TBA analysis only.

B5.12 Nitrogen – Clean, dry.

B6 Apparatus

Glassware should be clean and dry. Rinsing with diethyl ether (B5.2) just before use assists in freeing apparatus from possible contaminants.

B6.1 Sample bottles – (Information on the supply of suitable bottles is available from Glass Manufacturers Federation, 19 Great Portland Street, London, W1 — 01-580 6952).

These should be of all-glass construction, capable of holding 1.2 litres and calibrated at 1 litre. Each bottle should be checked for contamination by rinsing with a small volume of ether and examining the rinsings by gas chromatography. Bottles showing evidence of contamination should be rejected.

B6.2 Separating funnels – Glass 1 litre capacity with ungreased glass or PTFE taps.

B6.3 Methylation apparatus (Fig 3) – The whole apparatus should be mounted in a glass-fronted fume cupboard.

B6.4 Kuderna-Danish evaporator (Fig 1) – 2, 3, 6-TBA analysis only.

B6.5 Graduated centrifuge tubes – glass 10 ml, 0.1 ml graduations, tapered and glass stoppered.

B6.6 Volumetric flasks – 50 ml capacity.

B6.7 Gas-liquid chromatograph (GC). A GC fitted with an electron capture detector is required, to be operated in accordance with the manufacturer's instructions.

On column or glass-lined injection systems should be used. Many different columns have been used; some suitable columns are:

i. Glass column 2 m × 3 mm i.d. packed with Diatomite CLQ 100/120 mesh supporting 4% by weight of OV101 and 6% by weight of OV210 as the stationary phase.

ii. An identical column as in (i) but supporting 3% by weight of OV210.

iii. An identical column as in (i) but supporting 1.5% by weight of Apiezon L on 100/120 mesh Chromosorb W-HP.

iv. Fused silica wall-coated open tubular column (25 m) coated with a methyl silicone stationary phase.

B7 Sample Storage and Preservation

Samples should be extracted as soon as possible after sampling. If it is impractical to analyse the samples immediately, the acid and solvent should be added to the sample bottles at once (see B8.1.1). These are then shaken and stored in a spark-proof refrigerator. The sample bottles should be protected from contamination by covering the top and shoulders with aluminium foil. Samples should not be placed in the proximity of concentrated soultions of herbicides or phenols.

B8 Analytical Procedure

Step	Procedure	Notes
B8.1	Extraction of herbicides and chlorinated phenols	
B8.1.1	To 1 litre of sample in its glass bottle add 5±0.5 ml of dilute sulphuric acid (B5.1) and shake thoroughly to mix. Check that the pH is less than 2 (Note a).	(a) Narrow range pH paper may be used.
B8.1.2	Transfer the acidified sample to a 1 litre separating funnel.	
B8.1.3	Rinse the bottle with 100±5 ml of water-saturated diethyl ether (B5.8) and transfer the washings to the separating funnel. Repeat with a further 25 ml of the ether.	
B8.1.4	Shake the separating funnel vigorously for 2 minutes (see notes b and c). Allow the phases to separate.	(b) Vent the funnel to prevent pressure build up. (c) A shaking machine may be used but the extraction time must be extended to at least 10 mins.
B8.1.5	Run off the aqueous phase, measure its volume and discard (note d).	(d) If emulsification of interfacial cuff appears run off as much water as possible and add sodium sulphate, shake and separate.
B8.1.6	Run the ether layer into a 50 ml volumetric flask (note e).	(e) For 2, 3, 6-TBA analysis only, collect the ether extract and washings in a Kuderna-Danish evaporator (B6.4) fitted with a graduated centrifuge tube and proceeed to B8.1.9.
B8.1.7	Wash the separating funnel with 15±2 ml of ether followed by a further 10±2 ml of ether, collecting all the washings in the 50 ml volumetric flask containing the main extract.	

Step	Procedure	Notes
B8.1.8	Make the ether in the volumetric flask up to the mark with more ether. For determination of chlorophenols proceed to step B8.2 (note f).	(f) For dichlorophenols continue as for TBA (step B8.1.9). For suspected mixtures, multiple analyses by each variant may be necessary.
B8.1.9	Add an anti-bumping granule (B5.10) and concentrate the extract to about 5 ml in the Kuderna-Danish evaporator on a steam bath.	
B8.1.10	Reduce the volume of the extract from step B8.1.9 to 2±0.1 ml with a stream of nitrogen. Except for samples being analysed for dichlorophenols, proceed to step B8.2. For samples being analysed for dichlorophenols proceed to Step B8.4. (Note g.)	(g) For suspected mixtures run multiple samples by both procedures.
B8.2	Methylation	
B8.2.1	Set up a train of four dry test tubes each fitted with a gas bubbler in a fume cupboard as shown in Figure 3. Add the following materials to the tubes specified. Tube 1 — 20±2 ml of diethyl ether (B5.2) Tube 2 — 10±0.5 ml of diethyl ether plus 12±0.5 ml methanol plus 0.8±0.1 g of N-methyl-N-nitrosotoluene-4-Sulphonamide (B5.4). Tube 3 — 25 ml of diethyl ether — water saturated (B5.8) to be saturated with diazomethane. Tube 4 — 15±1 ml of a 1:1 mixture of glacial acetic acid (B5.9) and diethyl ether (B5.2).	
B8.2.2	Add 2±0.2 ml of 60% aqueous potassium hydroxide (B5.5) to Tube 2 and pass nitrogen through the train of tubes at a rate of 2–3 bubbles per second until the ether in Tube 3 turns deep yellow. Stop the nitrogen flow and remove Tube 3.	
B8.2.3	Carefully add a large volume of glacial acetic acid to neutralize the alkali in Tube 2 and discard the contents, washing away with a copious supply of water.	
B8.2.4	Transfer 2±0.1 ml of the ethereal extract (from B8.1.8) to a 10 ml graduated centrifuge tube (B6.5).	
B8.2.5	Add 2±0.1 ml of diazomethane saturated ether from Tube 3 (B8.2.2). Mix thoroughly and leave for 15 minutes.	
B8.2.6	Add 2±0.1 ml of iso-octane (B5.7) to each tube; mix well and then concentrate back down to the 2 ml graduation mark using a stream of nitrogen. The methylated extract is now ready for GC examination.	
B8.3	Calibration Standards	
	Repeat steps 8.2.4 to 8.2.6 using 2±0.1 ml of each of the prepared working standards (B5.6.2).	

Step	Procedure	Notes
B8.4	Gas Chromatography	
	Inject a suitable volume of the methylated extract into the GC. Inject the same volume of similarly derivatized standards into the GC and compare the retention times and peak heights of the standards and samples (notes h and i). For dichlorophenols (which have not been methylated) inject suitable volumes of standards and concentrated extracts from B8.1.10.	(h) It may be necessary to dilute or concentrate the iso-octane extract to bring the instrument response into the same range as the derivatized standards. Record the dilution or concentration factor D.
B8.5	Calculation of Concentration	
	Construct a calibration graph of the weight of standard material (ng) against peak height or area. Read off the weight of herbicide or chlorophenol presented in the injected sample. Calculate the concentration of herbicide in the original sample from: $C = \frac{1000\ E.A.D}{I.V}\ \mu g/l$ where C = concentration in sample (µg/l) E = volume of final extract (ml) A = weight of substance to be measures (ng) in the injected volume of extract I = volume injected (µl) V = volume of sample (ml) D = concentration or dilution factor (note h).	(i) If a capillary column is used the injected volume should be decreased.

C The Determination of MCPA, MCPB and MCPP Herbicides in River and Drinking Waters

C1 Performance Characteristics of the Method

C1.1	Substances determined	4-chloro-2 methylphenoxy acetic acid (MCPA) 4-(4-chloro-2 methylphenoxy) butyric acid (MCPB) 2-(4-chloro-2-methylphenoxy) propionic acid (Mecoprop or MCPP) The method will also determine other herbicides and related compounds but is primarily designed for those herbicides with poor electron-capturing qualities. 2,4-D, 2,4,5-T Dicamba and TBA have been analysed by this method. The salts of the acids are also determined.
C1.2	Types of sample	River and drinking water.
C1.3	Basis of method	Extraction with di-isopropyl ether, clean-up, formation of the pentafluorobenzyl ester and examination by electron capture gas chromatography (EC-GC).
C1.4	Range of application	Up to 2 µg/l.
C1.5	Calibration curve	The range of linearity depends upon the detector in use. The detector used in the testing of the method gave a non-linear but usable response in the range up to 2 µg/l.
C1.6	Standard deviation	See Tables 6–10.
C1.7	Limit of detection	See Table 11.
C1.8	Sensitivity	See Table 12.
C1.9	Bias	Bias will vary with the extraction efficiency and with the efficiency of derivatization (see Table 13).*
C1.10	Interferences	Any co-extracted material which is not removed by the clean-up of any derivative formed, which has a similar retention time to the herbicide derivatives and gives a detector response, will interfere.
C1.11	Time required for analysis	4 samples per day.

* See advice at B1.9

Table 6 Standard Deviations of River Water

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
Mecoprop	0.273	0.047	0.096	0.107	5
MCPA	0.029	0.038	0.031	0.049	7
2, 3, 6-TBA	0.033	0.021	0.032	0.038	5
2, 4, 5-T	0.511	0.195	0.235	0.306	6
MCPB	0.49	0.136	0.290	0.321	5

Table 7 Standard Deviations of Blank + 0.4 µg/l Each Herbicide

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
Mecoprop	0.370	0.008	0.019	0.021	2
Dicamba	0.308	0.004	0.010	0.011	2
MCPA	0.418	0.020	0.057	0.060	2
2, 3, 6-TBA	0.325	0.017	0.028	0.033	3
2, 4-D	0.383	0.018	0.046	0.049	2
2, 4, 5-T	0.413	0.033	0.074	0.081	2
MCPB	0.437	0.053	0.051	0.074	3

Table 8 Standard Deviations of Blank + 2.0 µg/l Each Herbicide

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
Mecoprop	1.89	0.052	0.068	0.086	4
Dicamba	1.77	0.035	0.085	0.091	3
MCPA	2.15	0.026	0.297	0.298	3
2, 3, 6-TBA	1.86	0.038	0.084	0.093	4
2, 4-D	1.94	0.133	0.111	0.173	5
2, 4, 5-T	2.05	0.110	0.132	0.172	5
MCPB	2.09	0.214	0.163	0.269	6

Table 9 Standard Deviations of River Water + 0.4 µg/l Each Herbicide

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
Mecoprop	0.390	0.031	0.075	0.081	3
Dicamba	0.370	0.013	0.039	0.041	3
MCPA	0.420	0.026	0.080	0.084	3
2, 3, 6-TBA	0.351	0.024	0.035	0.043	4
2, 4-D	0.548	0.013	0.117	0.117	3
2, 4, 5-T	0.474	0.081	0.041	0.190	6
MCPB	0.451	0.186	0.000	0.186	7

Table 10 Standard Deviations of River Water + 2.0 µg/l Each Herbicide

Compound	Mean Conc Found µg/l	Standard Deviations µg/l			Degrees of Freedom
		Sw	Sb	St	
Mecoprop	1.99	0.041	0.113	0.120	3
Dicamba	2.02	0.053	0.039	0.066	6
MCPA	2.45	0.102	0.365	0.379	3
2, 3, 6-TBA	2.03	0.043	0.000	0.043	6
2, 4-D	2.43	0.062	0.215	0.224	3
2, 4, 5-T	2.24	0.142	0.230	0.276	4
MCPB	2.06	0.0180	0.276	0.329	4

Table 11 Limits of Detection (8 degrees of freedom)

Compound	Mean conc µg/l	Limit of Detection µg/l
Mecoprop	0.11	0.05
Dicamba	0.10	0.02
MCPA	0.08	0.05
2, 3, 6-TBA	0.08	0.02
2, 4-D	0.14	0.07
2, 4, 5-T	0.11	0.06
MCPB	0.10	0.10

Table 12 Retention Times and Detector Sensitivity

Compound	Retention Time Mins	Quantity (ng) of each compound giving a reponse of 50% fsd at a baseline noise level of 0.5%
Mecoprop	5.13	0.05
Dicamba	5.78	0.03
MCPA	5.93	0.04
2, 3, 6-TBA	6.27	0.03
2, 4-D	6.93	0.07
2, 4, 5-T	8.39	0.07
MCPB	8.65	0.07
Dichloroprop	6.16	0.05

Table 13 Recoveries of Herbicides Spiked into Water Samples at 0.4 and 2.0 µg/l

	Recovery %			
	Distilled Water		River Water	
	Low spike	High spike	Low spike	High spike
Mecoprop	93	95	98	100
Dicamba	77	89	93	101
MCPA	105	108	105	123
2, 3, 6-TBA	81	93	88	102
2, 4-D	96	97	137	122
2, 4, 5-T	103	103	118	112
MCPB	109	105	113	103

C2 Principle

The herbicides are extracted with di-isopropyl ether and cleaned up by extraction into alkali, acidification and re-extraction into dichloromethane (DCM). The cleaned up extract is reacted with pentafluorobenzyl bromide to form the esters which are then determined by capillary column gas chromatography with electron-capture detection.

C3 Interferences

The detector is sensitive to many compounds other than herbicides and their derivatives. Because of the reactive nature of the reagent used and the strongly electron capturing properties of the derivatives formed this analysis is often subject to interference and poor baselines. The use of one or more capillary columns assists in differentiating the peak due to the determinands. Confirmation of the identity of the peaks is most desirable (see confirmation procedures).

C4 Hazards

Di-isopropyl ether can form a peroxide. See Section A5.1 and subsections therein. Di-isopropyl ether peroxidizes readily and is thus potentially explosive. The material normally supplied is stabilised with quinol, but fresh solvent should be purchased just sufficient for immediate use. Old stock should *never* be used. Di-isopropyl ether and iso-octane are flammable. DCM is narcotic. Pentafluorobenzyl bromide is lacrymatory. The herbicides are toxic; caution should be exercised when preparing the stock solutions. Skin contact, ingestion and inhalation must be avoided. The acid and alkali solutions used are corrosive.

C5 Reagents

C5.1 Di-isopropyl ether (IPE) – Purify a suitable volume before use. Shake twice with 1M Sodium Hydroxide (500 ml IPE with 2 × 40 ml NaOH). Discard the NaOH (see also Sections A5.1.1, A5.1.2 and B5.2 which are applicable).

C5.2 Dichloromethane (DCM) – Shake 200 ml DCM with 30 ml 1M NaOH. Discard the NaOH.

C5.3 Sodium Hydroxide (1M) – Dissolve 40 g NaOH in 1 litre distilled water.

C5.4 Hydrochloric acid – Concentrated (d_{20} 1.18).

C5.5 Sulphuric acid – (35% v/v) — CARE — slowly, with constant stirring and cooling pour 350±5 ml of concentrated sulphuric acid (d_{20} 1.84) into about 400 ml of water. Allow to cool to room temperature and make up to 1,000±10 ml in a measuring cylinder. Mix and store in a glass stoppered bottle.

C5.6 Sodium carbonate – Saturated in water (highly temperature dependent, approximately 2M at 20°C approximately 210 g/l).

C5.7 α-Bromo-2,3,4,5,6-pentafluorotoluene (Bromo-PFT) – Solution — 0.25% in acetone prepared immediately before use (65 µl Bromo-PFT in 25 ml of acetone).

C5.8 2,2,4 Trimethylpentane – (iso-octane) — Pure.

C5.9 Standard solutions of the herbicides.

C5.9.1 Stock solution – this may be prepared by dissolving pure or certified material in acetone.

A suitable concentration is 20 mg per litre.

C5.9.2 Working standards – these may be prepared from the stock solution using microlitre syringes reserved solely for this purpose or calibrated pipettes using hexane as the diluent. Some useful working standards are:

0.1 µg/l	(0.5 µl C5.9.1 diluted to 100 ml)
0.2 µg/l	(1.0 µl C5.9.1 diluted to 100 ml)
0.5 µg/l	(2.5 µl C5.9.1 diluted to 100 ml)
1.0 µg/l	(5 µl C5.9.1 diluted to 100 ml)
2.0 µg/l	(10 µl C5.9.1 diluted to 100 ml)
5.0 µg/l	(25 µl C5.9.1 diluted to 100 ml)
10.0 µg/l	(50 µl C5.9.1 diluted to 100 ml)
100 µg/l	(500 µl C5.9.1 diluted to 100 ml)
200 µg/l	(1000 µl C5.9.1 diluted to 100 ml)
500 µg/l	(5000 µl C5.9.1 diluted to 100 ml)

C6 Apparatus

Glassware should be clean and dry. Rinsing with acetone just before use assists in freeing glassware from possible contaminants.

C6.1 Sample bottles* – these should be of all glass construction capable of holding 1.2 litres and calibrated at 1 litre. Each bottle should be checked for contamination by rinsing with a small volume of acetone and examining the rinsing by gas chromatography. Bottles showing evidence of contamination should be rejected.

C6.2 Drying columns – Glass tubes approximately 130 mm long by 4–10 mm I.D fitted with a reservoir at the top and a jet at the bottom. The jet should be loosely plugged with acetone washed cotton wool and the tube half-filled with sodium sulphate.

C6.3 Separating funnels – glass 1 litre and 100 ml capacity with ungreased glass or PTFE taps.

C6.4 Round-bottom flask – 50 ml capacity with ground glass socket.

C6.5 Kuderna-Danish evaporator – (Fig 1).

C6.6 3-bubble micro-Snyder column – (Fig 2).

C6.7 Graduated centrifuge tubes – glass, 10 ml, 0.1 ml graduations, tapered, glass-stoppered.

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*Information on the supply of suitable bottles is available from the Glass Manufacturers' Federation, 19 Great Portland Street, London W1 Tel: 01-580 6952.

C6.8 Gas-liquid chromatograph (GC) – a gas chromatograph with an electron capture detector is required, to be operated in accordance with the manufacturer's instructions.

Capillary columns are to be preferred for this analysis, for example:
10–50 m fused-silica of glass wall-coated open tubular column coated with a methyl silicone stationary phase.

Alternatively a packed column may be used, for example:
Glass column 1.5 m × 3 mm ID packed with 80–100 mesh AW-DCMS Chromosorb W supporting 4% by weight of OV1 or SE30 stationary phase.

C7 Sample storage and preservation

Samples should be extracted as soon as possible after sampling. If it is impractical to analyse the samples immediately, the acid and solvent should be added to the sample bottles at once. These are then shaken and stored in a spark-proof refrigerator. The sample bottles should be protected from contamination by covering the top and shoulders with polythene sheeting. Alternatively the whole bottle may be sealed in a polythene bag. Samples should not be placed in close proximity to concentrated solutions of herbicides.

C8 Analytical Procedure

Step	Procedure	Notes
C8.1	Extraction of herbicides	
C8.1.1	Add 5 ml of dilute sulphuric acid (C5.3) to 1 litre of sample and check that the pH is between 1 and 2 (see note a). Add more acid if required. Add 100 ± 10 ml IPE and shake vigorously for 5 minutes (note b). (See Hazards section C4). Transfer the contents into a 1-litre separating funnel and allow the phases to separate.	(a) Narrow range pH paper or a pH meter may be used. (b) A shaking machine may be used but the extraction time must be extended to at least 20 minutes.
C8.1.2	Discard the aqueous phase and transfer the extract to a 150 ml separating funnel.	
C8.2	Clean up	
C8.2.1	Shake the extract with 25 ± 1 ml 1M NaOH for 30 ± 5 seconds and allow the phases to separate.	
C8.2.2	Run the aqueous layer into a second 150 ml separating funnel containing 15 ml dichloromethane. Add 35% sulphuric acid (2.5 ml) and shake for 30 ± 5 seconds and allow the phases to separate.	
C8.2.3	Pass the DCM layer through a drying column into a Kuderna-Danish evaporator. Wash the column with a further 15 ± 1 ml DCM and collect the washings in the same evaporator.	
C8.2.4	Using a steam bath reduce the extract in the evaporator to about 5 ml. When cool remove the graduated tube and reduce the extract to incipient dryness using a gentle stream of dry nitrogen.	

Step	Procedure	Notes
C8.3	Derivatization	
C8.3.1	To the tube add 1.0±0.1 ml freshly prepared PFT solution and 1 drop of saturated sodium carbonate solution. Mix stopper and place the tubes in a water bath at 60±10°C for 40 minutes (note c).	(c) Only the bottom 1 cm of the tube should be immersed.
C8.3.2	Remove the tube, cool, add 10±0.5 ml distilled water followed by 1.0±0.05 ml iso-octane. Shake for 30 seconds. The supernatant iso-octane layer is then taken for the GC analysis.	
C8.3.3	Using a series of standards equivalent to 0.1, 0.2, 0.4, 1.0 and 2.0 µg/l and higher if needed, blow to dryness and derivatize as in steps C8.3.1 and C8.3.2. A procedural blank should also be run with each batch of samples.	
C8.4	Chromatography	
C8.4.1	Inject a suitable volume of the iso-octane layer from C8.3.2 on to the gas-chromatographic column and compare the retention time of the sample peaks with those of the standards treated in the same way.	
C8.4.2	Plot a calibration curve of the weight (ng) of derivatized herbicide injected versus the peak height or area. The sample concentrations should always lie within the range of the standards.	
C8.5	Calculation of concentration	
C8.5.1	Calculate the weight (A) of the material in the injected volume (I) from: $A = \frac{P_1.S}{P_2}$ where P_1 = peak area or height of the sample injection P_2 = peak area or height of the standard injection S = weight in ng of standard (see note d) Calculate the concentration of herbicide in the original sample from: $C = \frac{1000.E.A(D)}{I.V}$ µg/l where C = concentration in sample (µg/l) E = volume of final extract (ml) A = weight of substance in the injected volume of extract (ng) I = volume injected (µl) V = volume of original sample (ml) D = concentration or dilution factor	(h) It may be necesary to dilute or concentrate the sample hexane extract to bring the instrument response into the same range as the standard. Note the dilution or concentration factor D.

D Confirmatory Procedure for Phenoxy Herbicides by Gas Chromatography Mass Spectrometry (GC/MS)

D1 Performance Characteristics of the Method

D1.1 Substances determined	MCPA, MCPB, Mecoprop, 2,4-D, 2,4,5-T
D1.2 Type of sample	River water
D1.3 Basis of method	The samples are extracted with ether and methylated with diazomethane. The derivatized extracts are examined by GC/MS using multiple ion detection
D1.4 Range of application	Up to 5 mg/l
D1.5 Calibration curve	Range of linearity depends upon the equipment used. For the instrument used in testing the method the linear range was up to 5 mg/l
D1.6 Standard deviation	To be determined
D1.7 Limit of detection	Typically 1 µg/l
D1.8 Bias	Bias will vary with the extraction efficiency of each determinand. See advice at B1.9.
D1.9 Interferences	None known at the m/e values selected
1.10 Time required for analysis	4 samples per day

D2 Principle

The methyl extracts from the GC procedure are injected into a gas chromatograph connected to a mass spectrometer operated in the electron impact mode with multiple ion detection.

D3 Interferences

It is unlikely, if the ions used are selected carefully and the GC column used is efficient, that any interference will occur. Isomeric compounds may produce similar MS peaks but their retention times are different from the herbicide peaks.

D4 Hazards

Herbicides and their solutions are toxic. Hexane and ether are flammable. Diazomethane is toxic, possibly carcinogenic and potentially explosive. The sulphonamide reagent has been shown to produce tumours in laboratory animals. Skin contact, ingestion and inhalation must be avoided. Ether occasionally forms an explosive peroxide on storage, see Sections A4 and A5.1 and parts therein.

D5 Reagents and Standards

Reference compound — Perfluorokerosene, heptacosafluorotributylamine or dibutyl phthalate have been used to produce suitable reference ions. The reference ion should have a mass/charge ratio as close as possible to that of the herbicide ions selected.

Standards — Those standards described in A5.12.2 or C5.9.2 are satisfactory. If other herbicides are being determined these should be made up in the same manner to produce a multiple standard.

D6 Apparatus

D6.1 GC/MS Equipment

D6.1.1 Column – 25 or 50 m glass or fused silica wall coated open tubular column coated with a methyl silicone stationary phase.

D6.1.2 Oven – maintained isothermally at about 180°C.

D6.1.3 Carrier gas – helium or hydrogen at a flow rate of 1–2 ml per minute.

D6.1.4 Interface – direct, jet or open split.

D6.1.5 Mass spectrometer – either a quadrupole or magnetic sector instrument is suitable, set up to discriminate between whole number values of m/e ratios (unit resolution). The instrument should be operated in the electron inpact mode and be fitted with a multiple ion detection unit and, if necessary, a multiple pen chart recorder.

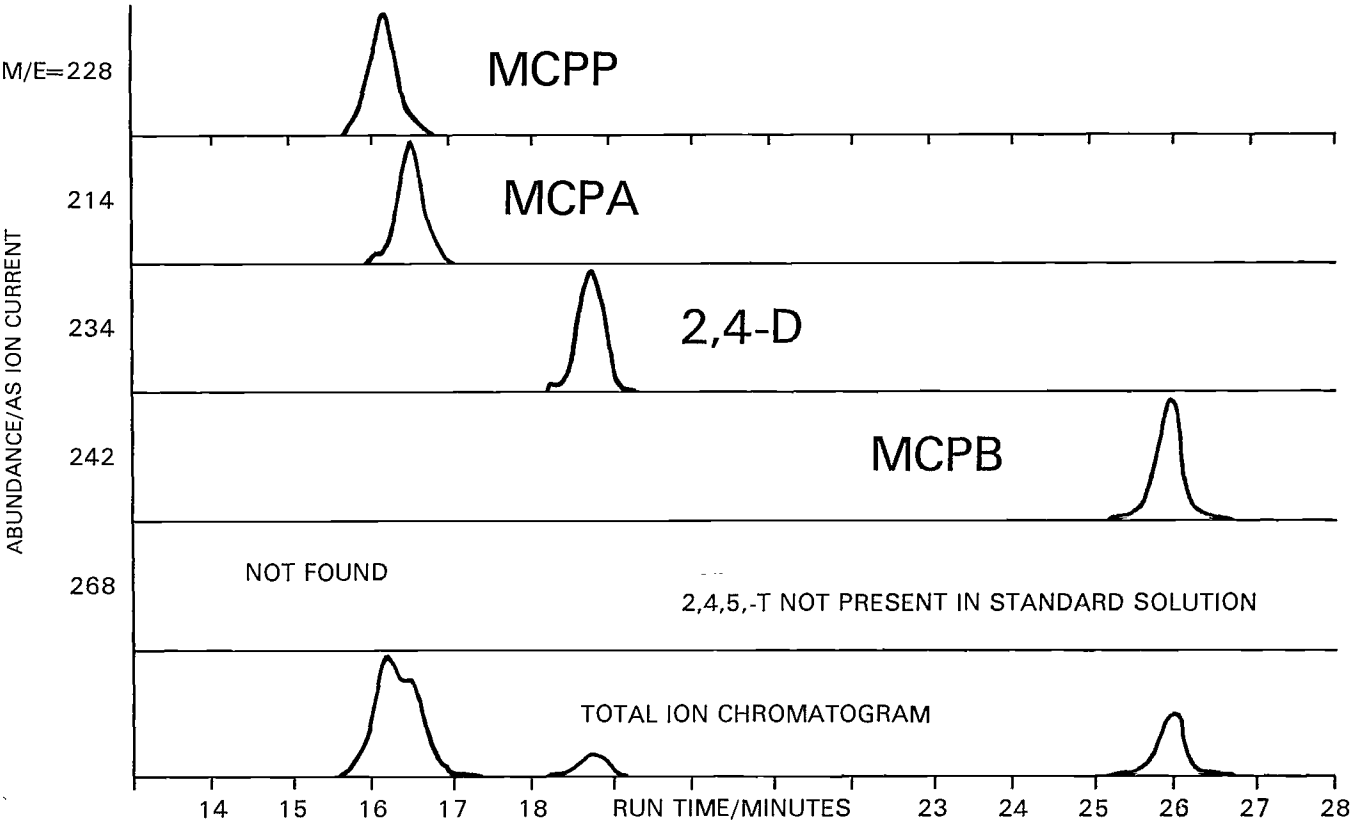
D7 Sample Preservation

The extracts should be stored in a spark proof refrigerator. PTFE tape wrapped around the joint of the extract tube minimizes evaporation. Samples should be analysed within one week of derivatization.

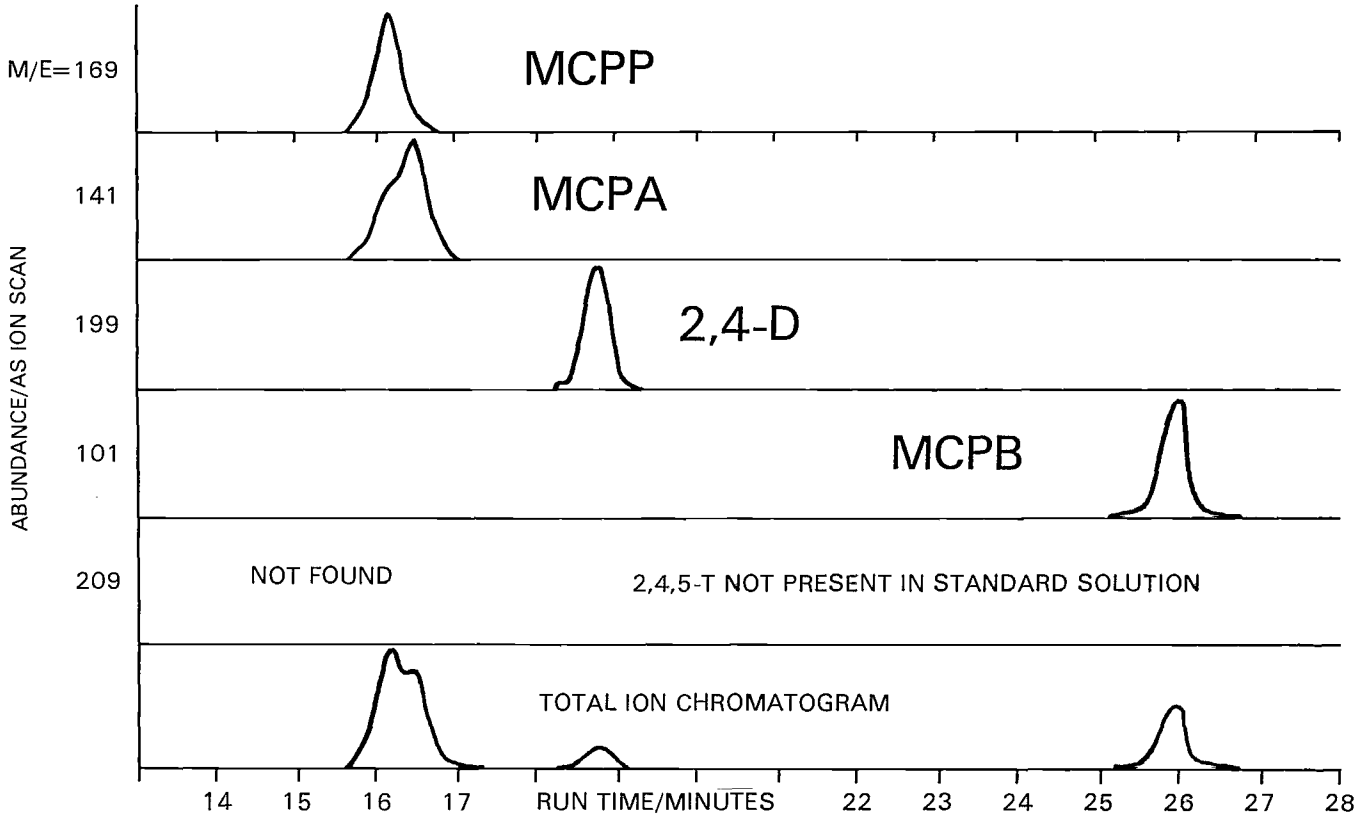
D8 Analytical Procedure

Step	Procedure	Notes
D8.1	Extraction and Methylation Using the procedure for TBA, steps B8.1 to B8.2.5, extract and methylate the sample. Adjust the volume of the extract to 2.0±0.05 ml with a gentle stream of dry nitrogen.	
D8.2	GC/MS 1	
D8.2.1	Set up the multiple ion detector to select the appropriate ions for the herbicides to be determined with a suitable lock mass (note a). Suitable ions are: m/e 2,4-D 199, 234 m/e 2,4,5-T 209, 268 m/e MCPA 141, 214 m/e MCPB 101, 242 m/e Mecoprop 169, 228	a) The 149 ion of dibutyl phthalate is usually suitable. If different herbicide ions are chosen the reference ion should have an m/e value as close as possible to the chosen ion. See also Fig 4.

Fig 4 Selected ion scan using molecular ions to identify individual methylated pesticides



Ion scan using selected ion masses to identify individual methylated pesticides



Data provided courtesy of Thames Water

Step	Procedure	Notes
D8.2.2	Inject a suitable aliquot in duplicate of each esterified standard to give a response within the linear range of the instrument into the chromatograph. Select suitable ions for the methyl esters of the herbicides from the spectra of the standards (notes b, c and d). If the duplicates agree closely, plot a calibration curve of the response of each ion measured (peak height) against weight of herbicide injected. (Note e.) Run procedural blanks in duplicate.	b) The molecular ions of the herbicide esters may be used although more interference is likely. Other major ions may also be used if interference occurs. c) More than one ion can be monitored for each herbicide. This increases the certainty of identification. d) Published spectra for some of these compounds can vary considerably. Major ions from the spectra given by the instrument in use should be selected. e) Peak areas may also be used if an integrator is fitted.
D8.2.3	Inject the same volume of sample extracts as those of the standards and compare the responses obtained. Read off the concentrations of the herbicides from the calibration graph.	
D8.3	Calculation of Concentration The calculation is the same as that given in A8.6.1. If a 1 µl injection is used and the volumes in the method adhered to then C = A mg/l where C = Concentration in the sample A = Weight of herbicide in the volume injected.	

E Confirmatory Procedure for MCPA, MCPB and MCPP by Nitration, Methylation and Gas Chromatography

Introduction

As the pentafluorobenzyl bromide reagent used in the primary procedure is very reactive, the method is prone to interference and the chromatogram baselines are often poor. A confirmation technique is therefore desirable and if GC-MS is not available the extracts may be nitrated and methylated followed by gas chromatographic analysis using an electron-capture detector.

E1 Performance Characteristics of the Method

E1.1 Substances determined	MCPA, MCPB, Mecoprop
E1.2 Type of sample	Drinking waters and many river waters
E1.3 Basis of method	Extraction into ether, evaporation, nitration, methylation followed by electron capture gas chromatographic (ECGC) analysis
E1.4 Range of application	Up to 500 µg/l
E1.5 Calibration curve	Range of linearity depends upon the equipment in use. The calibration line typically is linear over two orders of magnitude.
E1.6 Standard deviation	At a spiking level of 250 µg/l of MCPA the estimate of standard deviation was 25 µg/l
E1.7 Limit of detection	0.004 µg/l for MCPA
E1.8 Bias	Bias will vary with the extraction efficiency of each determinand. See advice at B1.9
E1.9 Interferences	Any compound which is co-extracted or formed during derivatization and which has a similar retention time to the derivatized herbicides will interfere. Problems have been encountered with some river waters high in sediment
E1.10 Time required for analysis	4 samples per day

E2 Principle

The sample is extracted with ether and some potentially interfering compounds removed by alkaline partition. The extract is evaporated to dryness and nitrated. An ethereal solution of the nitrated product is then methylated with diazomethane and the nitrated methyl esters analysed by EC-GC. (Ref E9).

E3 Interferences

The detector is sensitive to many compounds other than the derivatized herbicides. Some of these may occur in the sample or be formed in the derivatization procedures. The use of two or more columns may assist in differentiating the determinand peak from interfering peaks in the chromatogram. The greater efficiencies achieved by capillary columns improve the certainty of the identifications.

E4 Hazards

Herbicides and their solutions are toxic. Ether is flammable, phosphoric acid and acetic acid are corrosive. Diazomethane is toxic, possibly carcinogenic and potentially explosive. The sulphonamide reagent has been shown to produce tumours in laboratory animals. Skin contact, ingestion and inhalation must be avoided. Ethers may occasionally form explosive peroxides on storage — see Sections A4 and A5.1 and sections therein.

E5 Additional Reagents

Nitrating reagent: Add 1.0±0.1 g sodium nitrate to 100±0.5 ml o-phosphoric acid in a round bottomed flask. Solution is aided by heating the mixture on a steam bath. This must be prepared immediately before use.

E6 Apparatus

See Sections B6 and C6.

E7 Sample Storage and Preservation

As Section C7.

E8 Analytical Procedure

Step	Procedure	Notes
E8.1	Extraction	
E8.1.1	Extract the sample using the procedure for TBA, steps B8.1 to B8.1.9.	
E8.1.2	Transfer the ether extract to a 100 ml separating funnel. Extract the ether solution with 20 ml 1M NaOH by shaking. Allow the layers to separate. Pass the aqueous layer into a second 100ml separating funnel and discard the ether layer.	
E8.1.3	Acidify the alkaline solution with 5±0.5 ml 3M H_2SO_4 and extract by shaking with 15±1 ml diethyl ether. Allow the layers to separate and discard the aqueous layer.	
E8.1.4	Dry the ether extract by passage through a 10 cm column of anhydrous sodium sulphate, washing the column with an additional 10±1 ml of diethyl ether. Collect the ether extract and the washings in a 50 ml round bottomed flask.	
E8.1.5	Evaporate the ether just to dryness in a fume cupboard using a water bath and finally a gentle stream of purified nitrogen.	
E8.1.6	Prepare the nitrating reagent and add 2±0.2 ml of the hot fresh mixture to the residue in the round bottomed flask. Place the flask on a steam bath for 15±2 min swirling occasionally.	
E8.1.7	Allow the contents of the flask to cool to room temperature. Transfer the nitrated solution to a 100 ml separating funnel. Use 25±2 ml of 2% sodium sulphate to wash the flask contents into the funnel.	

Step	Procedure	Notes
E8.2	Derivatization	
E8.2.1	Extract the nitrated herbicides with 15 ± 1 ml diethyl ether by shaking. Discard the aqueous layer. Transfer the sample into a test-tube.	
E8.2.2	Methylate the sample following the procedure for TBA, steps B8.2.1–B8.2.5.	
E8.2.3	Using a purified nitrogen line evaporate the extract to about 5 ml. Transfer to a graduated centrifuge tube using ether to wash the test-tube and finally evaporate using the nitrogen line to 5 ± 0.05 ml.	
E8.3	Standardization	
	Repeat the procedure with a range of standards which bracket the expected concentration of herbicide. A reagent blank should be analysed with each batch of standards and samples.	
E8.4	Gas Chromatography	
	Inject 5 µl (or other suitable volume) of the nitrated, methylated extract into the chromatograph fitted with an electron capture detector. The same columns as are used for the TBA analysis are suitable. Inject the same volume of derivatized standards and compare the retention times and peak heights or areas of the standards and samples (note a). Plot a calibration graph of the peak response against the weights of herbicide originally used to prepare the derivatized standards (note b).	a) It may be necessary to dilute or concentrate the sample to bring the response into the range of the standards. b) Log — log paper may be necessary.
E8.5	Calculation	
	Using the suggested volumes the calculation is: $C = x$ µg/l where C = concentration of herbicide present in the original 1L water sample x = wt (ng) of herbicide present in the 5 µl injection read off from the calibration graph.	

E9 References

Wilson I, Croll B T, T.P. 105 WRA Medmenham 1973 *Determination of phenoxy-acetic acid herbicides in water.*

F The Determination of Glyphosate in River Water

F1 Performance Characteristics of the Method

F1.1	Substances determined	N-(phosphonomethyl) glycine (Glyphosate) (see also 10.1).
F1.2	Type of sample	River water.
F1.3	Basis of method	The aqueous sample is concentrated by evaporation and passed through an ion-exchange column. After further concentration, the Glyphosate is separated by reversed phase HPLC and fluorogenically labelled before fluorimetric detection.
F1.4	Range of application	0–100 µg/l. The range can be extended to 100 mg/l if the concentration step is omitted.
F1.5	Calibration curve	Linear up to 100 µg/l.
F1.6	Standard deviation	See Table 14.
F1.7	Limit of detection	0.08 µg/l (calculated from 4.65 Sw with 9 degrees of freedom).
F1.8	Sensitivity	With a baseline fluctuation of 0.5% FSD, 50% FSD is given by approximately 10 µg/l in the original sample.
F1.9	Bias	The recovery is less than quantitative. See Table 14.*
F1.10	Interference	Any compound which passes through the procedure and gives a detector response at the same retention volume as the determination will interfere.
F1.11	Time required for analysis	Assuming all the apparatus and reagents are prepared, 12 samples per man-week. At least a week should be allowed for setting-up and familiarization.

* See advice at B1.9

Table 14 Means, Standard deviations and Recoveries

Sample	Spike	Mean ug/l	% found ug/l	Estimates of standard deviation Recovery	Sw	Sb	St
Distilled unspiked	–	0.06	–	0.017 (9)	–	–	
Distilled low spike	1	0.74	74	0.063 (6)	0.124 (5)	0.139 (6)	
Distilled high spike	10	7.6	76	0.56 (6)	n.s	0.70 (9)	
River unspiked	–	0.06	–	0.007 (6)	n.s	0.11 (7)	
River low spike	1	0.72	72	0.083 (6)	0 (5)	0.083 (10)	
River high spike	10	7.5	75	0.49 (6)	0.87 (5)	0.99 (6)	

Figures in brackets indicate the number of degrees of freedom calculated from:

$$f = \frac{m\,(m-1)(M_1 + [n-1]\,M_0)^2}{m\,M_1^2 + (m-1)(n-1)\,M_0^2}$$

where M_0 = within batch mean square
M_1 = between batch mean square
m = no of batches
n = no of replicates per batch
f = degrees of freedom (rounded to nearest whole number)

n.s. = not significant

Recoveries have not been blank corrected as the blanks were attributable to baseline noise.

F2 Principle

The sample (1L) is concentrated by evaporation to 20 ml and the pH adjusted if necessary to <7. A cation-exchange column (Dowex 50W-X8) is prepared and washed through with 0.01M hydrochloric acid and then with water until the washings are neutral. The sample is placed on the column and eluted with water until 60 ml have been collected. The sample is further concentrated in a rotary evaporator to 5 ml. The HPLC apparatus is set up as shown in Fig. 1 and 20 µl injected via a loop on to a Partisil/Sax column using an acid mobile phase. The column eluate is reacted with a calcium hypochlorite cleavage reagent and then with an o-phthalaldehyde fluorogenic reagent and is then passed to a fluorimeter cell where the fluorescence is measured.

F3 Interferences

Most interferences are eliminated by the ion-exchange and the HPLC column as well as by the selectivity of the reagent and the wavelengths used for measurement. Amino acids which respond and are likely interferences are separated from the Glyphosate by the column. The column used should be checked to ensure that glycine can be satisfactorily separated from the Glyphosate peak.

F4 Hazards

o-phthalaldehyde and Glyphosate are toxic. Hydrochloric acid is corrosive.

F5 Apparatus and Reagents

F5.1 Ion exchange resin – Dowex 50W–X8. 20–50 mesh, Hydrogen form or equivalent.

F5.2 Hydrochloric acid – conc. AR grade.

F5.3 Hydrochloric acid – 0.01M (1 ml/l).

F5.4 Mobile phase: 0.09M o-phosphoric acid and 0.01M sulphuric acid – dissolve 26 ml H_3PO_4 and 2.7 ml H_2SO_4 in 5L distilled water.

F5.5 di-potassium hydrogen phosphate – K_2HPO_4: AR grade.

F5.6 Sodium chloride – AR grade.

F5.7 Calcium hypochlorite – (35% available chlorine).

F5.8 Boric acid – AR grade.

F5.9 Potassium hydroxide – AR grade.

F5.10 Mercaptoethanol – AR grade.

F5.11 o-phthalaldehyde – puriss. (phthaldialdehyde) (Fluka)

F.12 Methanol – HPLC grade.

F5.13 Cleavage reagent:

F5.13.1 Buffer solution – dissolve 34.8 g potassium hydrogen phosphate and 23.2 g sodium chloride in 2L distilled water.

F5.13.2 Working solution – weigh out 12 mg calcium hypochlorite and dissolve in 200 ml of the buffer solution. The hypochlorite is slow to dissolve. It is convenient to prepare the reagent on the day preceding analysis. The solution should be discarded after 48h.

F5.14 Fluorogenic reagent – Into a 1l standard flask in a fume cupboard add approximately 700 ml distilled water, 100 g boric acid, 72 g potassium hydroxide. Add 1 ml mercaptoethanol and 0.8 g o-phthalaldehyde dissolved in 10 ml methanol. Shake until dissolved and make up to the mark. (This solution is available commercially prepared.)

F5.15 Rotary evaporator – (reduced pressure).

F5.16 Ion exchange columns – 50 cm × 2 cm ID fitted with a frit and stopcock. A 250 ml reservoir fitting the neck of the column is useful.

F5.17 Graduated centrifuge tubes – 10 ml.

F5.18 HPLC – fitted with a 20 µl sampling loop and a fluorimeter. The HPLC column used in the performance testing was a 25 cm Partisil 10 u SAX column (HPLC Technology). Other similar columns may be used.

F5.19 Reagent pumps – several alternative systems can be used for adding the post-column reagents. Alternatives are given in F5.19.1–F5.19.3. The preferred system is that given in F5.19.2.

F5.19.1 Pressure pots (2) – maintained with sufficient head pressure to ensure delivery of the correct flow rate of reagent. With this system it is difficult to balance the flow rates of the cleavage and fluorogenic reagents resulting in drifting baselines.

F5.19.2 HPLC dosing pumps (2) – or double pump capable of delivering the low flows necessary (eg Orlita Dosiertechnik DMP 16 16 or equivalent).

F5.19.3 Peristaltic pumps – fitted with pump tubing of an appropriate size (eg LKB Perpex or Technicon pumps).

F5.20 Fluorimeter – equipped with a flow cell. Either a scanning or filter instrument can be used. The excitation wavelength is 360 nm and the emission wavelength 455 nm. If a filter instrument is used, suitable filters are: excitation — Balzers R-UV 362 23 (narrow band pass 362 nm emission — Corning CS-3-74 (3391) (cut off 408 nm).

F5.21 Teflon tubing – 0.5 mm ID: for delay coils.

F6 Standards

Standards are prepared by dissolving pure or certified material in water. A suitable stock solution is 100 mg/l (50 mg in 500 ml). Dilutions can be prepared to give 20, 10, 5, 1, 0.5 amd 0.1 mg/l. The stock solution is stable for at least one month when stored in a refrigerator.

F7 Sample Storage

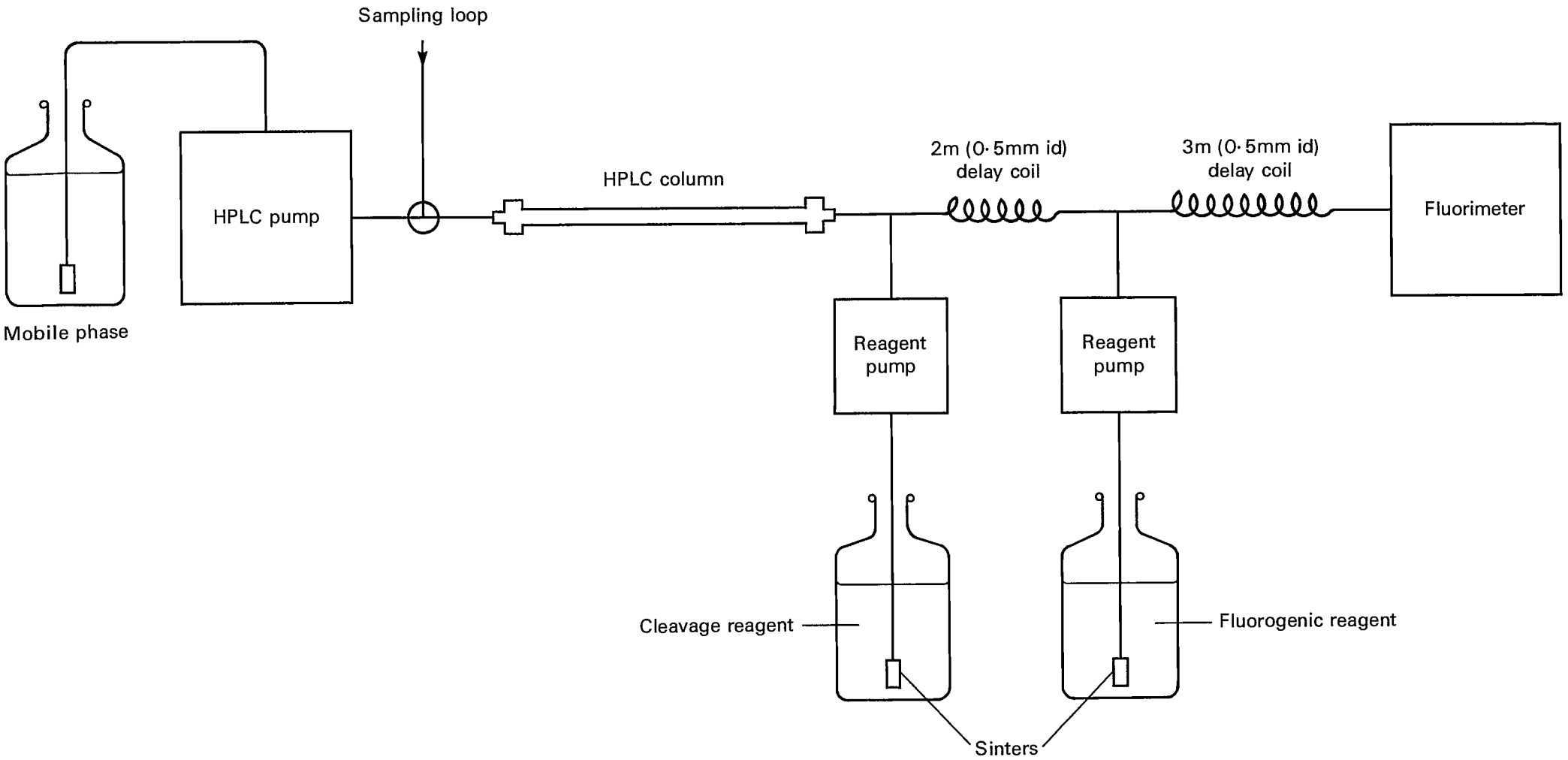
Glyphosate metabolises to (aminomethyl) phosphonic acid. Samples should therefore be analysed as soon as possible after receipt. Glass bottles fitted with glass stoppers or metal screw caps are suitable containers.

If analysis has to be delayed, samples should be stored in a refrigeraor (without freezing).

F8 Analytical Procedure

Step	Procedure	Notes
F8.1	Place 1L of sample into a rotary evaporator under reduced pressure and reduce the volume to 20±1 ml (note a). Adjust the pH to below 7 if necessary.	(a) If numbers of samples are to be analysed, hotplate evaporation is acceptable.
F8.2	Set up an ion exchange column (F5.16) and pack with 12±0.2 g of the resin (5.1) by slurrying with 250 ml 0.01M HCl (5.3). Allow all the acid to pass through the column. When the meniscus has reached the top of the resin wash with water until the washings are approximately neutral when tested with pH paper (note b).	(b) The resin may be regenerated with the acid followed by washing with water. If solids collect on the surface, these may be removed by flotation with water in a beaker. Do not allow the resin to dry. Replace the resin in the column and regenerate.
F8.3	Pour the concentrated sample carefully on to the column. Rinse with 5±0.5 ml water and add the washing to the column. Any dissolved solids which have precipitated in the concentration step should be washed onto the column.	
F8.4	Allow the sample to pass through the column at a flow rate of 2 ml/min. When the meniscus reaches the top of the resin add approximately 100 ml distilled water. Collect the first 60 ml of column eluate.	
F8.5	Concentrate the column eluate to < 5 ml on a rotary evaporator under reduced pressure, transfer to a graduated centrifuge tube and finally adjust the volume to 5.0±0.05 ml (note c).	(c) This concentration step may be omitted if the concentration of Glyphosate is expected to be greater than 20 µg/l
F8.6	Set up the HPLC system as shown in Fig 5. Fit a 20 µl sampling loop. Set the flow rate of the mobile phase to 1 ml/min. Connect the cleavage reagent junction to the fluorogenic reagent junction with 2 m of tubing and the fluorogenic reagent junction to the fluorimeter flow cell with 3 m of tubing (notes d & e). Set the flow rate of the cleavage reagent to 0.2 ml/min and that of the fluorogenic reagent to 0.4 ml/min. Changing the flow rate of one pump affects the other reagent flow and time must be allowed for the system to stabilize.	(d) The length of Teflon tubing from the outlet of the column to the cleavage reagent junction should be minimal. (e) The reactions are not instantaneous and the tubing must be long enough to give a reasonable reaction time. Use of excessive lengths of tubing causes peak spreading. The lengths used should be optimized by experiment for the system in use.
F8.7	When a stable baseline has been achieved inject the standards listed in Section F6. Plot a calibration graph of weight injected (ng) versus peak height.	

Fig 5 Schematic diagram of equipment for post-column fluorogenic labelling and HPLC for glyphosate analysis



F8.8 Inject the sample, measure the peak height at the appropriate retention time (approx 12 min) and read off the weight of Glyphosate present from the calibration graph (note f).

F8.9 Calculation

If the volumes suggested are used then C = 0.25 w

where C = Concentration in original sample (µg/l)
w = weight (ng) in injected sample.

(f) With river water samples a large peak often emerges shortly before the Glyphosate peak.

F9 Reference

Moye A H, Miles, C J and Scherer, C J. A Simplified High-Performance Liquid Chromatographic Residue Procedure for the Determination of Glyphosate Herbicide and (Aminomethyl) phosphonic Acid in Fruits and Vegetables Employing Post-column fluorgenic Labelling. *J Agric Good Chem.* **31** 69–72 1983.

F10 Notes

F10.1 Aminomethyl phosphonic acid, the major glyphosate metabolite, may also be determined by a modification of this method. See the reference above.

F10.2 Columns – An Aminex A27 column has also been used for this analysis, see the reference above. PRPI columns (F5.18) were found to give a better separation from interfering compounds although, with use, the performance of the column deteriorates.

G The Determination of Triazine Herbicides in River and Drinking Water

G1 Performance Characteristics of the Method

G1.1	Substance determined	Triazine herbicides
G1.2	Types of sample	River and drinking water.
G1.3	Basis of method	Extraction of an alkaline sample into dichloromethane. Concentration and estimation of the triazines by gas chromatography using a nitrogen selective detector.
G1.4	Range of application	Typical up to 50 ng injected.
G1.5	Calibration curve	Linear up to 40 µg/l
G1.6	Standard deviation	See Table 1.
G1.7	Limit of detection	For atrazine, using a packed column 0.15 µg/l. Similar detection limits would be expected for the other triazines such as Simazine, Prometryne, Propazine, and Terbutryne. Using a polar capillary column the main blank was 0.015 µg/l with a within batch (5) estimate of standard deviation of 0.005 and a detection limit of 0.025 µg/l.
G1.8	Sensitivity	Dependent upon the instrument in use. In these tests 50% F.S.D. with a baseline noise level of 0.5% was given by 6 ng of atrazine.
G1.9	Bias	Extraction efficiencies are normally less than 100%. Bias will vary with the extraction efficiency of any particular determinand. See advice at B1.9.
G1.10	Interferences	Any co-extracted material which has a similar GC retention time and to which the detector responds will interfere.
G1.11	Time required for analysis	2 h per sample, assuming that these are analysed in batches of six or more.

Table 1 Estimates of Standard Deviation (Packed Column)

Spiking level (µg/l)	Atrazine			Terbutryne		
	Mean	SW	Degrees of freedom	Mean	SW	Degrees of freedom
0	0.21	0.032	9	–	–	–
1.0	0.86	0.13	5	0.89	0.054	5
8.0	7.35	0.22	5	7.45	0.14	5
Unspiked tap water	0.45	0.026	5	–	–	–

G2 Principle

The herbicides are extracted from the alkaline sample with dichloromethane. The extract is evaporated to dryness and the residue redissolved in a known volume of methanol prior to gas chromatography.

G3 Interferences

Substances with similar retention times to those of the triazine herbicides will interfere but only if they produce a response from the nitrogen selective detector. The use of two or more different GC columns may assist in differentiating triazines from interfering peaks in the chromatograms.

G4 Hazards

Dichloromethane is narcotic; methanol is flammable and toxic by skin absorption and ingestion. Ammonia is toxic and lachrymatory.

G5 Reagents

All reagents must be of such purity that they do not give rise to significant peaks during chromatography. This should be verified by running procedural blanks. Reagents should be stored in glass containers.

G5.1 Dichloromethane – reagent grade redistilled using all glass apparatus.

G5.2 Ammonia solution – S.G. 0.880.

G5.3 Sodium sulphate – anhydrous granular, roasted in a muffle furnace at 500±20°C for 4 hr±30 min. Cooled and stored in a desiccator.

G5.4 Methanol – AR grade.

G5.5 Standard triazine solution – prepared by dissolving pure or certified materials in methanol. A suitable concentration is 25 mg/100 ml.

G5.5.1 Working standards – these may be prepared by diluting an aliquot of the stock solution using microlitre syringes, reserved solely for this purpose.

Methanol is used as the diluent.

Some useful working standards are:

0.25 µg/ml (1 µl — 10 ml)
0.1 µg/ml (4 µl — 10 ml)
0.5 µg/ml (20 µl — 10 ml)
1.0 µg/ml (40 µl — 10 ml)
5.0 µg/ml (200 µl — 10 ml)
10 µg/ml (400 µl — 10 ml)

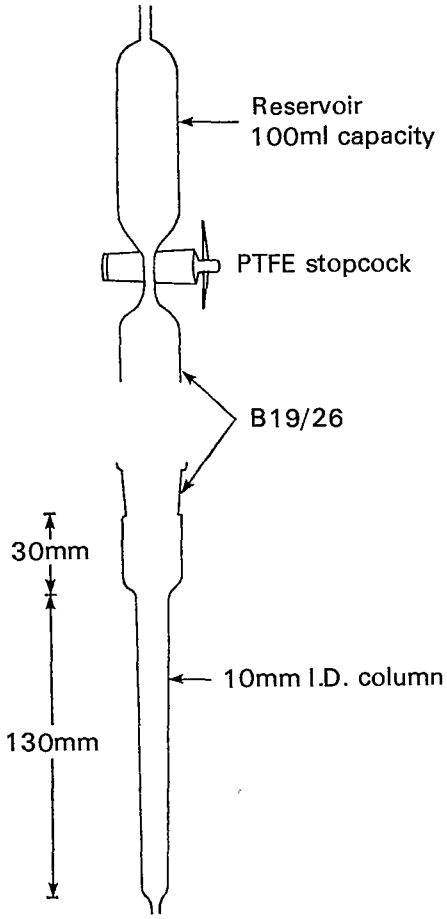
G6 Apparatus

G6.1 Sample bottles – these should be of all glass construction, capable of holding 1.2 l and calibrated at 1.0 l. Glass bottled with P.T.F.E lined caps may be used.

G6.2 Separating funnels – glass, 1 litre capacity with ungreased glass or PTFE taps.

G6.3 Drying tubes – glass tubes approximately 130 mm long by 10 mm internal diameter fitted with a reservoir at the top and a jet at the bottom. The jet should be loosely plugged with dichloromethane-washed cotton wool and the tube half filled with sodium sulphate. (Fig 6.)

Fig 6 Drying column



G6.4 Conical flasks – glass, 250 ml.

G6.5 Round bottomed flasks – 250 ml capacity, thick-walled glass, with ground necks fitted with B24 glass stoppers.

G6.6 Kuderna-Danish evaporator – (Fig 1)

G6.7 Graduated cylinders – 100 ml glass.

G6.8 Pipettes.

G6.9 Water bath – temperature controlled.

G6.10 Centrifuge tubes – tapered, graduated 10 ml capacity.

G6.11 Gas-liquid chromatograph (GC) – operated isothermally in accordance with the manufacturer's instructions. A GC fitted with a flame thermionic, cold flame thermionic, or conductivity detector or similar nitrogen specific detector is required.

Suitable columns are:

1. 50 m W.C.O.T coated with Carbowax 20M.
2. 2 m × 4 mm i.d. glass column packed with 3% stabilised diethylene glycol succinate on 80–100 mesh acid washed Gas Chrom P unsilanized. The use of the capillary column is to be preferred because of the improved separation of the peaks of interest.

G6.12 Air or Nitrogen Line – the supply of gas must be cleaned by passage through a column of 1/16 in pellets of type 13X molecular sieve and 15–40 mesh silica gel ending in a fine jet of glass or metal controlled such that the gas jet just indents the meniscus of the solvent being evaporated (typically 200 ml/min from a jet of internal diameter 0.5 mm at a distance of 20 mm from the liquid).

G7 Sample Storage

Samples should be extracted as soon as possible after sampling. If it is impractical to analyse the samples immediately, the ammonia and solvent should be added to the sample bottles at once (see G8.1.1/2). These are shaken and then stored in a refrigerator.

G8 Analytical Procedure

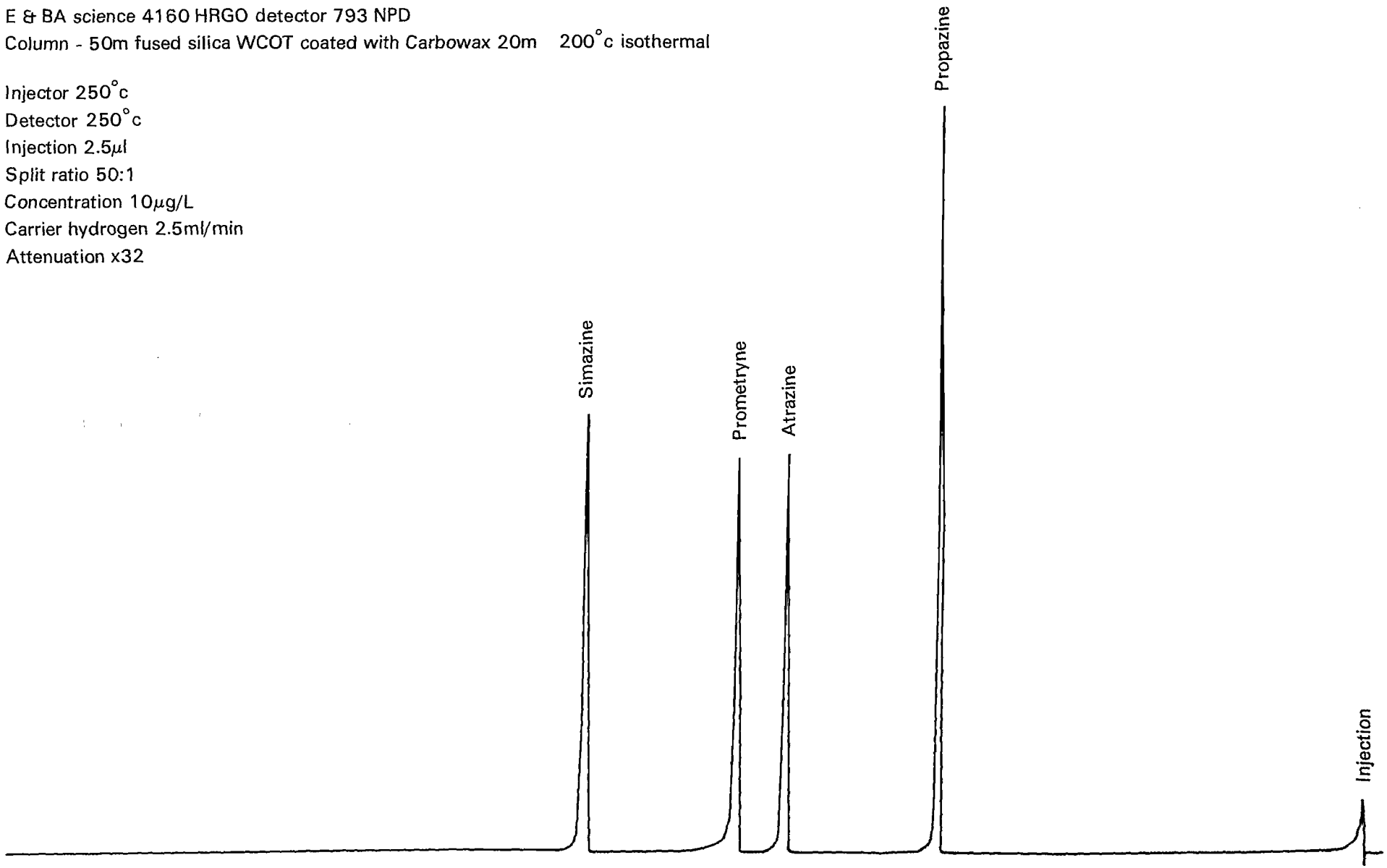
Step	Procedure	Notes
G8.1	Extraction of herbicides	
G8.1.1	Add 2±0.2 ml of 0.880 ammonia to 1.0 l of sample in sample bottle and check that the pH is in excess of 8.0 (note a).	(a) pH paper may be used.
G8.1.2	Add 100±5 ml dichloromethane and shake for 2 min (notes b and c). Transfer the contents to a 1 l separating funnel and allow the phases to separate.	(b) Ventilate the bottle to avoid pressure build up. (c) A shaking machine may be used but the extraction time must be extended to at least 10 min.
G8.1.3	Run off the organic layer into the drying tube and collect the elute in a Kuderna-Danish evaporator fitted with a 10 ml graduated centrifuge tube (note d).	(d) Emulsions may form. Transfer the bulk of the solvent to the drying tube as in 8.1.3. Transfer the interfacial cuff into a 250 ml conical flask and absorb the water with sodium sulphate. Decant the dried solvent into the drying tube. Wash the sodium sulphate in conical flash with a few ml of dichloromethane and transfer the washings to the drying tube.
G8.1.4	Repeat the extraction of the aqueous phase with a further 50±5 ml of dichloromethane. Transfer the solvent to the same drying tube.	
G8.1.5	Run off the aqueous layer into a measuring cylinder. Record the volume of the aqueous phase.	
G8.1.6	Wash the drying tube after the extracts have passed through with 20±2 ml of dichloromethane, collecting the solvent in the same Kuderna-Danish evaporator.	

Step	Procedure	Notes
G8.2	**Evaporation**	
G8.2.1	Add an anti bumping granule to the evaporator. Place the evaporator over a steam bath and reduce the volume of solvent to 3–5 ml (note e).	(e) Violent ebullition of the solvent may occur. Take care to avoid loss of sample.
G8.2.2	Remove the centrifuge tube and evaporate the solvent just to dryness with a gentle stream of dry, oil-free air or N_2 (G6.12).	
G8.2.3	Dissolve the residue in 2.0±0.05 ml methanol.	
G8.3	**Chromatography**	
G8.3.1	Inject 5 ul of the solution (G8.2.4) into the GC (note f). Inject 5 µl of the standard solutions and compare the peak heights or areas of the standards and samples.	(f) The solution may be diluted with methanol if necessary. Smaller volumes should be injected if a capillary column is employed. Figure 7 shows a typical GC trace.
G8.4	**Calculation of concentration**	
	Construct a calibration graph from the series of standard solutions injected (G8.3.1) using peak height or integrated peak area measurement.	
G8.4.1	Then:	
	$C = \frac{N.Vf \times 1{,}000}{Vi \,.\, Vs}$ µg/l	
	of herbicide in the sample, where C = concentration in original sample µg/l N = weight of triazine in the injected volume of extract (ng) from calibration chart Vi = volume injected (µl) Vs = volume of original sample (ml) Vf = volume of extract (ml)	
	If the volumes given in the method are adhered to, the calculation is	
	C = 0.4 N µg/l	
G8.5	**Blanks and Standards**	
	To check for contamination and interferences at least one blank and one standard should be analysed by the entire procedure with each batch of analyses.	

Fig 7 Chromatogram of triazine herbicides

E & BA science 4160 HRGO detector 793 NPD
Column - 50m fused silica WCOT coated with Carbowax 20m 200°c isothermal

Injector 250°c
Detector 250°c
Injection 2.5μl
Split ratio 50:1
Concentration 10μg/L
Carrier hydrogen 2.5ml/min
Attenuation x32



(Courtesy of SAC Chromatography Ltd.)

Checking the Accuracy of Analytical Results

Once the methods have been put into normal routine operation many factors may subsequently 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 tests are possible and they should be used as appropriate. As a minimum, however, it is suggested that at least one sample, of suitable concentration, in each batch of analyses be analysed in duplicate. The results obtained should then be plotted on a quality control chart which will facilitate detection of inadequate precision and allow the standard deviation of routine analytical results to be estimated.

The routine checking of the recovery of the procedure is also desirable.

For further information see Refs 1 and 2.

References

(1) British Standards BS 5700 to 5703 inclusive.

(2) Davey D J and Hunt D T E. The use of cumulative sum charts in Analytical Quality Control. WRC Technical Report TR 174. Water Research Centre Medmenham 1982.

Addresses for Correspondence

1. However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this method are requested to write to the address below.

2. At the present time, though based on work in several laboratories, thorough test data is only available from a few laboratories. Additional test data would be welcomed. Results should be sent to the address below.

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The Standing Committee of Analysts
The Department of the Environment
Romney House
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