Phenylurea herbicides (urons), Dinocap, Dinoseb, Benomyl, Carbendazim and Metamitron in Waters 1994

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Methods for the Examination of Waters and Associated Materials

This booklet contains five basic methods:

A Phenylurea herbicides (urons) in waters (carbetamide may also be determined);

B Dinocap in waters by HPLC;

C Dinoseb in waters by HPLC;

D Carbendazim and benomyl (as carbendazim) in waters by HPLC; and

E Metamitron in waters by HPLC.

Only limited performance data is available for all the methods described in this booklet.

Chromatographic methods are very sensitive to minor physical and chemical variations in the quality of materials and apparatus used. These methods report the use of materials actually used in the evaluation tests but this in no way endorses these materials as superior to other similar materials. Equivalent materials are acceptable and it should be understood that the performance characteristics may differ with other materials used. It is left to users to evaluate these methods in their own laboratories.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, groundwater, river and seawater, waste water and effluents as well as sewage sludges, sediments and biota. In addition, short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests reported for most parameters. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results encompassing at least ten degrees of freedom from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors), systematic error (bias), total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available. An indication of the status of the method is shown at the front of this publication on whether or not the method has undergone full performance testing.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets in the series 'Methods for the Examination of Waters and Associated Materials' and their continuous revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is managed by the Drinking Water Inspectorate. At present there are nine working groups, each responsible for one section or aspect of water quality analysis. They are:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage works control methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members associated with these methods are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and the more important parameters and topics is available from HMSO (ISBN 0 11 752669 X).

Every effort is made to avoid errors appearing in the published text. If however, any are found, please notify the Secretary.

Dr D WESTWOOD
Secretary

18 February 1994

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with The Health and Safety at Work etc Act 1974 and any regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1988 SI 1988/1657. Where particular or exceptional hazards exist in carrying out the procedures described in this booklet then specific attention is noted. Numerous publications are available giving practical details on first aid and laboratory safety and these should be consulted and be readily accessible to all analysts. Amongst such publications are those produced by the Royal Society of Chemistry, namely 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', 5th edition, 1992; by Member Societies of the Microbiological Consultative Committee, 'Guidelines for Microbiological Safety', 1986, Portland Press, Colchester; and the Public Health Laboratory Service 'Safety Precautions, Notes for Guidance'. Another useful publication is produced by the Department of Health entitled 'Good Laboratory Practice'.

A Chlorotoluron, Diuron, Isoproturon, Linuron and other phenylurea herbicides (urons) in waters

Introduction

There are many phenylurea herbicides (urons) in use and the four most common are listed above. Provided there are no specific interferences, most of the phenylurea herbicides (urons) can be determined by the methods described in this booklet. With certain methods carbetamide may also be determined.

A selection of methods are given for the determination of phenylurea herbicides and the use of these methods will depend upon the type of equipment available to laboratories. This booklet contains two methods based on solid phase extraction and reverse or normal phase high performance liquid chromatographic analysis. Details of a liquid-liquid extraction procedure are also described. A confirmatory procedure, based on solid phase extraction, methylation and gas chromatographic analysis is presented. Brief details of a liquid chromatographic-mass spectrometric analysis are also described.

A1 The determination of phenylurea herbicides (urons) in waters by reverse phase HPLC

A1.1 Performance characteristics of the method

A1.1.1	Substances determined	Chlorotoluron, diuron, isoproturon and linuron. Other phenylurea herbicides of similar structure may also be determined.
A1.1.2	Type of sample	Drinking water.
A1.1.3	Basis of method	The determinands are extracted by solid phase extraction (SPE), eluted with dichloromethane (DCM) and evaporated to dryness. Methanol is added and the extracts are analyzed by reverse phase high performance liquid chromatography with ultra-violet detection (HPLC/UV).
A1.1.4	Range of application	Up to 2 μgL^{-1}. The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
A1.1.5	Calibration curve	The method is linear over the range of application.
A1.1.6	Standard deviation	See Table A1.
A1.1.7	Limit of detection	See Table A1.
A1.1.8	Sensitivity	Dependent on the instrument used. With a baseline fluctuation of 1.5% of full scale deflection, a diuron standard equivalent to an aqueous concentration of 0.05 μgL^{-1} gave approximately 15% full scale deflection.
A1.1.9	Bias	Extraction efficiencies are normally less than 100%. See Table A1.
A1.1.10	Interferences	Any co-extracted material which has a similar HPLC retention time to the compounds of interest and which gives a detector response at a wavelength of 245 nm will interfere.
A1.1.11	Time for analysis	Approximately 12 samples per day.

A1.2 Principle

The sample is extracted using a pre-conditioned C_{18} cartridge which is vacuum dried and then extracted with dichloromethane. Extracts are evaporated to incipient dryness and re-constituted in methanol. HPLC is carried out isocratically using reverse phase separation with UV detection at a wavelength of 245 nm.

Quantification is by an external standard procedure or by using an internal standard, added after extraction.

A1.3 Interferences

Substances adsorbed by the cartridge which are eluted by dichloromethane and soluble in aqueous methanol, and which absorb at 245 nm will interfere. Under certain conditions some triazine herbicides have been found to co-elute with certain phenylurea herbicides, for example atrazine with chlorotoluron.

A1.4 Hazards

Dichloromethane and methanol are toxic by skin absorption and inhalation. Methanol is flammable. Substituted phenylurea herbicides are toxic. Contact with skin or inhalation of solids or solutions should be avoided. Ensure adequate ventilation and work in a flame and spark proof area. Spark proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

A1.5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

A1.5.1 Dichloromethane (DCM). HPLC grade.

A1.5.2 Methanol. HPLC grade.

A1.5.3 Water. HPLC grade.

A1.5.4 Standard solutions.

A1.5.4.1 Stock solutions of phenylurea herbicides. Prepare individual stock solutions of pure or suitably certified phenylurea herbicides in methanol at a concentration of 1000 mgL^{-1}. For example in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of solvent.

A1.5.4.2 Stock solution of internal standard. Prepare a stock solution of suitably certified internal standard, for example caffeine at a concentration of 250 mgL^{-1} in methanol, using a similar procedure to that described in A1.5.4.1.

A1.5.4.3 Spiking solution of internal standard. By dilution of the stock solution (A1.5.4.2) prepare a solution of the internal standard at a concentration of 25 mgL^{-1} in methanol.

A1.5.4.4 Working standard solutions of phenylurea herbicides. By dilution of the stock solutions, prepare a series of mixed working standard solutions of phenylurea herbicides in methanol (A1.5.4.1), each containing 5.0 mgL^{-1} of internal standard. For example, a useful working range for each of the phenylurea herbicides is 0.1 to 0.5 mgL^{-1}.

If the external standard method is to be used, it is not necessary to prepare the internal standard solutions A1.5.4.2 and A1.5.4.3 and the internal standard can be omitted from the working standard solutions A1.5.4.4.

A1.6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then DCM and allowed to drain.

A1.6.1 Glass sample bottles, minimum 250 mL capacity, fitted with glass stoppers or PTFE-lined screw caps.

A1.6.2 Nitrogen. Oxygen-free, filtered and dry.

A1.6.3 C_{18} sorbent (SPE) cartridges (100 mg). The performance of the method can vary considerably with different batches of cartridge material and their supplier. It is important that different batches are performance tested before being used routinely.

A1.6.4 Cartridge manifold.

A1.6.5 High performance liquid chromatograph with a UV detector capable of working at a wavelength of 245 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic reverse phase.
Columns	: Precolumn Supelguard LC-18, 20 × 4.6 mm
	: Analytical Spherisorb C_{18}, 250 × 4.6 mm, 5 μm particle size.
Column temperature	: 35 °C.
Mobile phase	: Methanol:water (57:43 v/v).
Flow rate	: 1.5 $mLmin^{-1}$.
Injection volume	: 20 μL.
UV wavelength	: 245 nm.

A typical chromatogram of phenylurea herbicides obtained under conditions similar to these is given in Figure A1.1. Other columns and conditions (including gradient elution) may be used provided the performance is shown to be similar to or better than that reported here. Typical chromatograms of phenylurea herbicides and other compounds, obtained under alternative conditions, are given in figures A1.2 to A1.4.

A1.7 Sample collection and storage

Samples should be taken in glass bottles with glass or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should be stored in a refrigerator at about 4 °C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions. Extraction and analysis should be carried out as soon as possible after collection.

A1.8 Analytical Procedure

Step	Procedure	Notes
A1.8.1	Extraction	
A1.8.1.1	Condition a 100 mg C_{18} cartridge with 10 ± 0.5 mL of methanol followed by 10 ± 0.5 mL of water (A1.5.3).	(a) Do not let the sorbent bed dry out.
A1.8.1.2	Connect glass reservoirs to the cartridges by gently twisting, and then fit into a manifold.	
A1.8.1.3	Draw 250 ± 5 mL of sample through the cartridge at about 5–10 mL per minute.	
A1.8.1.4	Vacuum dry the cartridge for approximately 10 minutes.	

Step	Procedure	Notes
A1.8.1.5	Elute the cartridge with 1 ± 0.1 mL of DCM and collect the eluate in a tapered vial. Blow any remaining DCM in the solid phase cartridge into the vial.	
A1.8.1.6	Evaporate the eluate to incipient dryness with a stream of dry, clean nitrogen (A1.6.2).	
A1.8.1.7	Add 100 μL of methanol and 20 μL of spiking internal standard (A1.5.4.3), cap and mix thoroughly. This solution is now ready for HPLC analysis.	
A1.8.2	**HPLC/UV determination**	
A1.8.2.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section A1.6.5.	
A1.8.2.2	Inject aliquots of standards and extracts from blanks, samples and recoveries into the HPLC.	
A1.8.2.3	Measure the height or area of each of the phenylurea herbicide peaks corresponding to the compounds of interest and the peak height or area of the added internal standard.	
A1.8.2.4	Construct a calibration graph of peak ratios relative to the internal standard versus mass ratios relative to the internal standard for each phenylurea herbicide injected (see note b).	(b) If the external standard procedure is being used, construct a calibration graph of peak height or area for the standards versus concentration of each of the phenylurea herbicides (mgL^{-1}) injected.
A1.8.2.5	Read the mass ratio (or concentration, in the case of the external standard procedure) for each phenylurea herbicide in the extract from the calibration graph (note c) and calculate the concentration present in the original sample (see section A1.9).	(c) If the peak ratio for the sample exceeds the calibration range, dilute the extract appropriately and re-analyse. Account should be taken of the dilution of the internal standard.
A1.8.3	**Confirmation**	
A1.8.3.1	Change the HPLC conditions (note d). Re-analyse extracts following similar procedures to those described in sections A1.8.2.2 to A1.8.2.5.	(d) This can include mobile phase composition, column type or detector system or a combination of these. Other methods described in this part may also be used for confirmation.
A1.8.4	**Blanks and recoveries**	
A1.8.4.1	Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard material to separate samples of interference free water, for example HPLC grade, immediately before extraction (note e). Process these solutions under conditions identical with those to be used for the samples under analysis.	(e) Use up to 1 mL of an appropriate working standard solution in methanol.

Step	Procedure	Notes
A1.8.5	AQC	
A1.8.5.1	Carry out the entire procedure using distilled water (or water of a similar nature to the sample being anlaysed) spiked at approximately 0.1 μgL^{-1} with individual phenylurea herbicides. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

A1.9 Calculation

Internal standard procedure:

Concentration of each phenylurea herbicide is given by:

$$C = \frac{R \times A}{V} \mu gL^{-1}$$

External standard procedure:

Concentration of phenylurea is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}$$

where

C = concentration of phenylurea in original sample (μgL^{-1})

c = concentration determined in the extract from the calibration graph (μgmL^{-1})

v = volume of the extract for the determination (mL), normally 0.5 mL,

V = volume of sample extracted (L), normally 0.25 litre,

R = mass ratio of the determinand to internal standard from the calibration graph, and

A = amount of internal standard added to original sample (μg).

Alternative methods of calculation may be used provided they give equivalent results.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

When using the external standard procedure, the effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

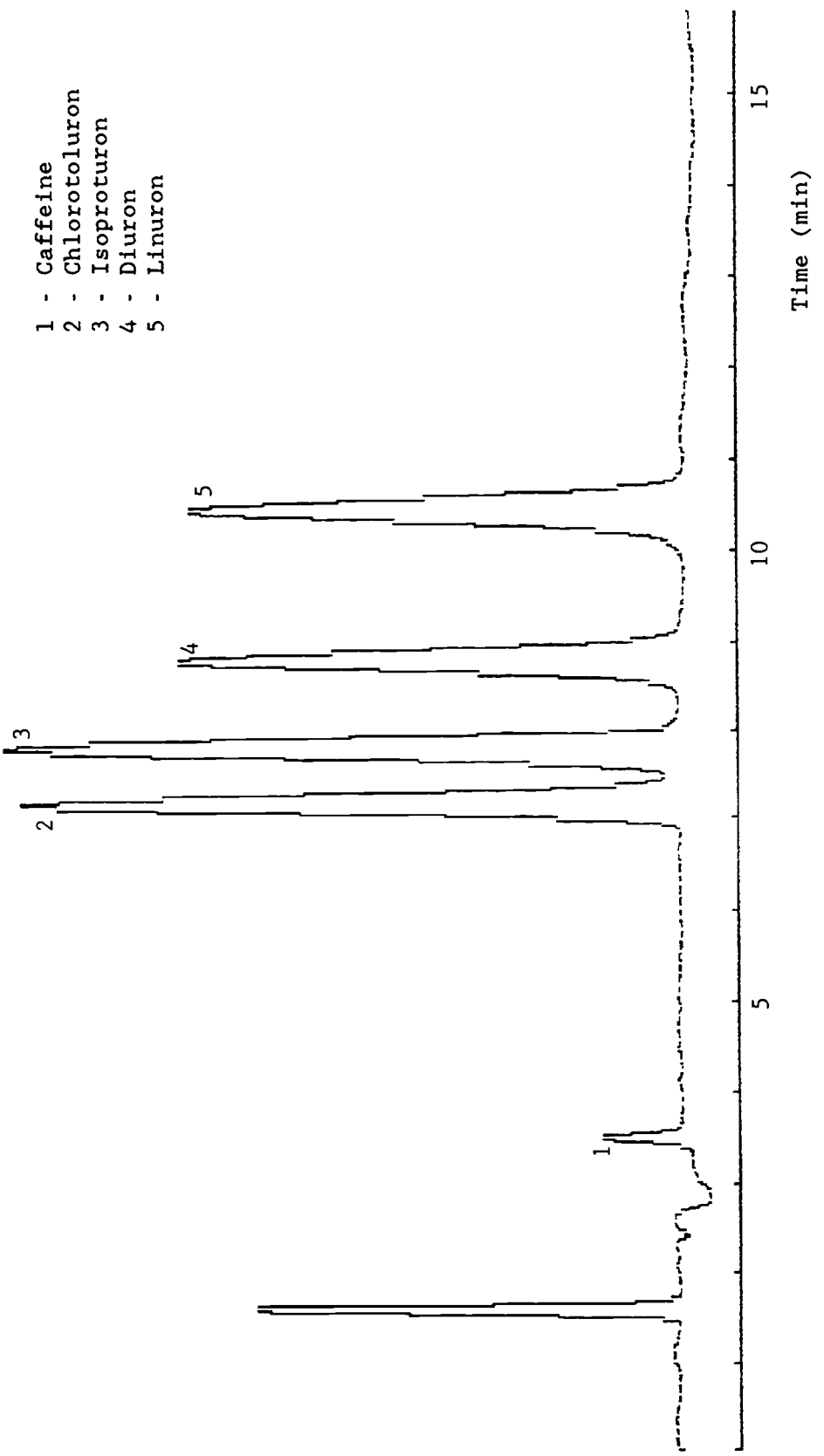
Table A1 Standard deviations, Recoveries and Limits of detection

	Spiked concentration	Standard deviation	Recovery %	Limit of detection
Chlorotoluron	0.1	0.0162	104	0.020
Diuron	0.1	0.0152	96	0.018
Isoproturon	0.1	0.0168	101	0.018
Linuron	0.1	0.0113	99	0.014

All units expressed in μgL^{-1} unless otherwise stated.
Good quality untreated borehole water was used in this analysis.
Data provided by Severn Trent Laboratories.

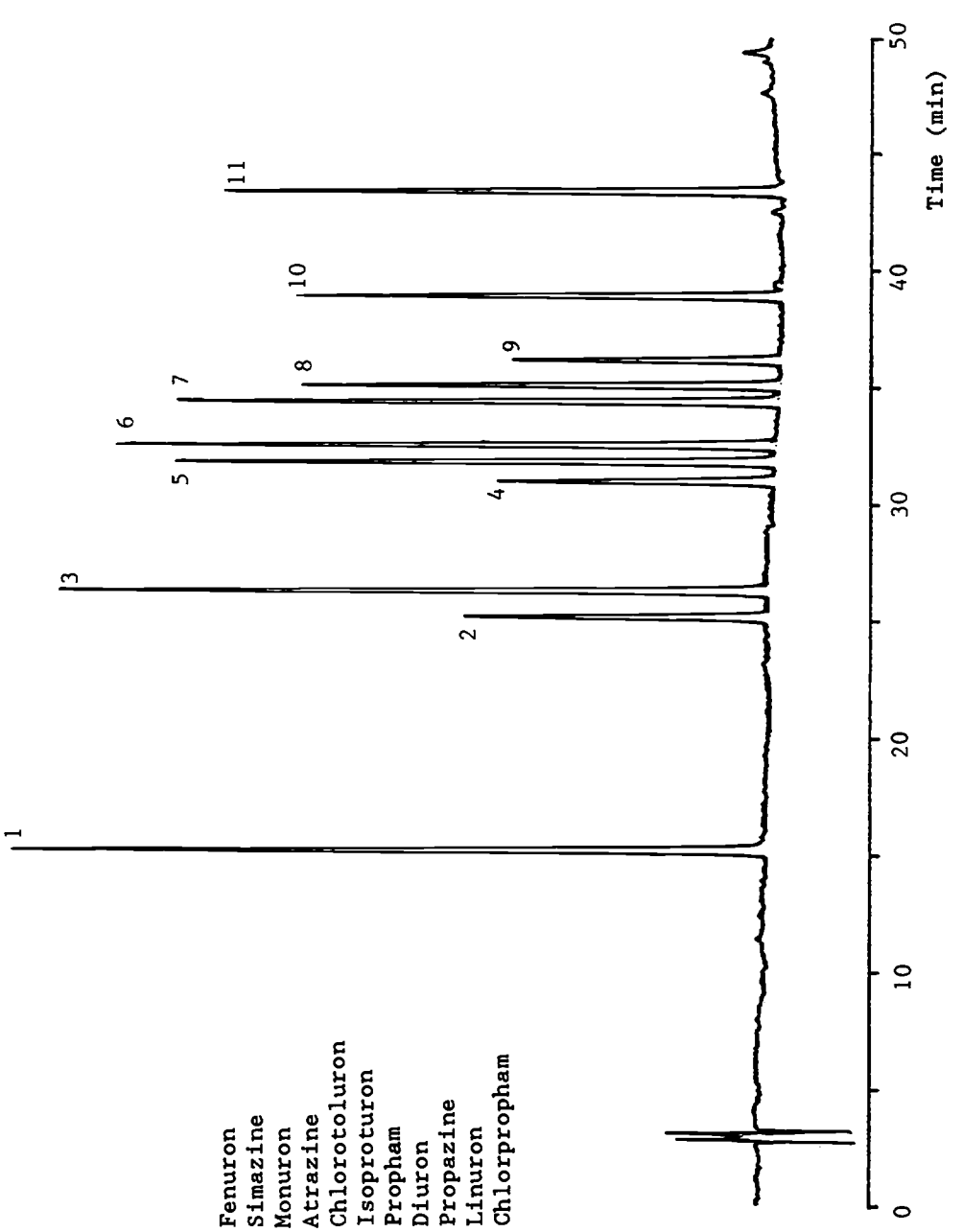
Figure A1.1 HPLC chromatogram of a phenylurea herbicides mixed standard (1 mgL^{-1} each phenylurea herbicide)

Figure A1.1 HPLC chromatogram of a phenylurea herbicides mixed standard (1 mgL^{-1} each phenylurea herbicide)



Chromatogram provided by Severn Trent Laboratories

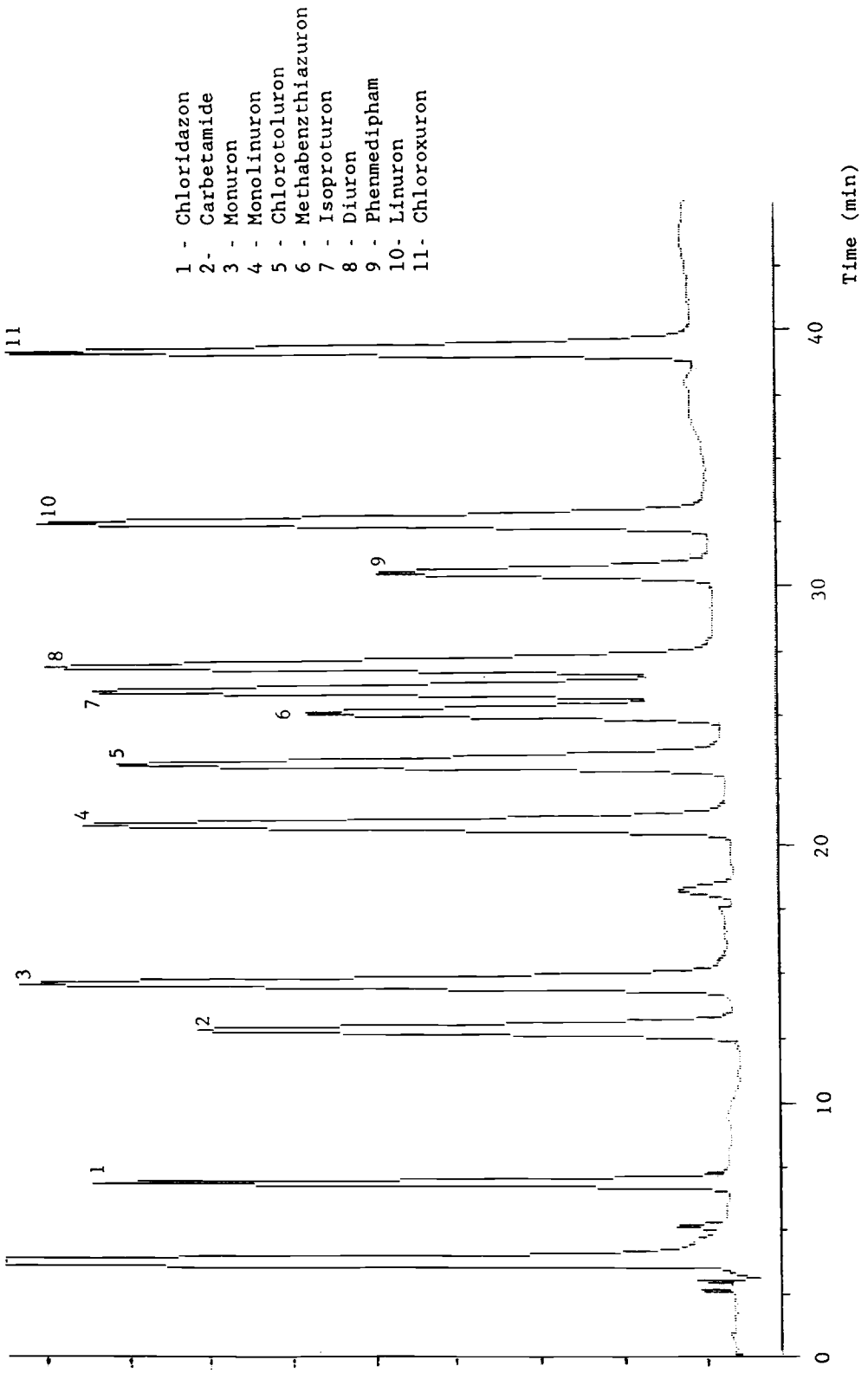
Figure A1.2 Gradient HPLC chromatogram of a herbicides mixed standard (10 mgL^{-1} each compound)



Notes: 1. HPLC conditions - Column: Supelcosil LC-ABZ (reverse phase) 250 x 4.6 mm, 5 µm particle size, linear mobile phase gradient: 100%[water + acetonitrile (9 + 1 v/v)]to 100% acetonitrile in 80 min and return to initial conditions over 10 min, flow rate 1.0 mLmin^{-1}, UV detection at a wavelength of 239 nm.

2. Chromatogram provided by NRA (Northumbria and Yorkshire Region).

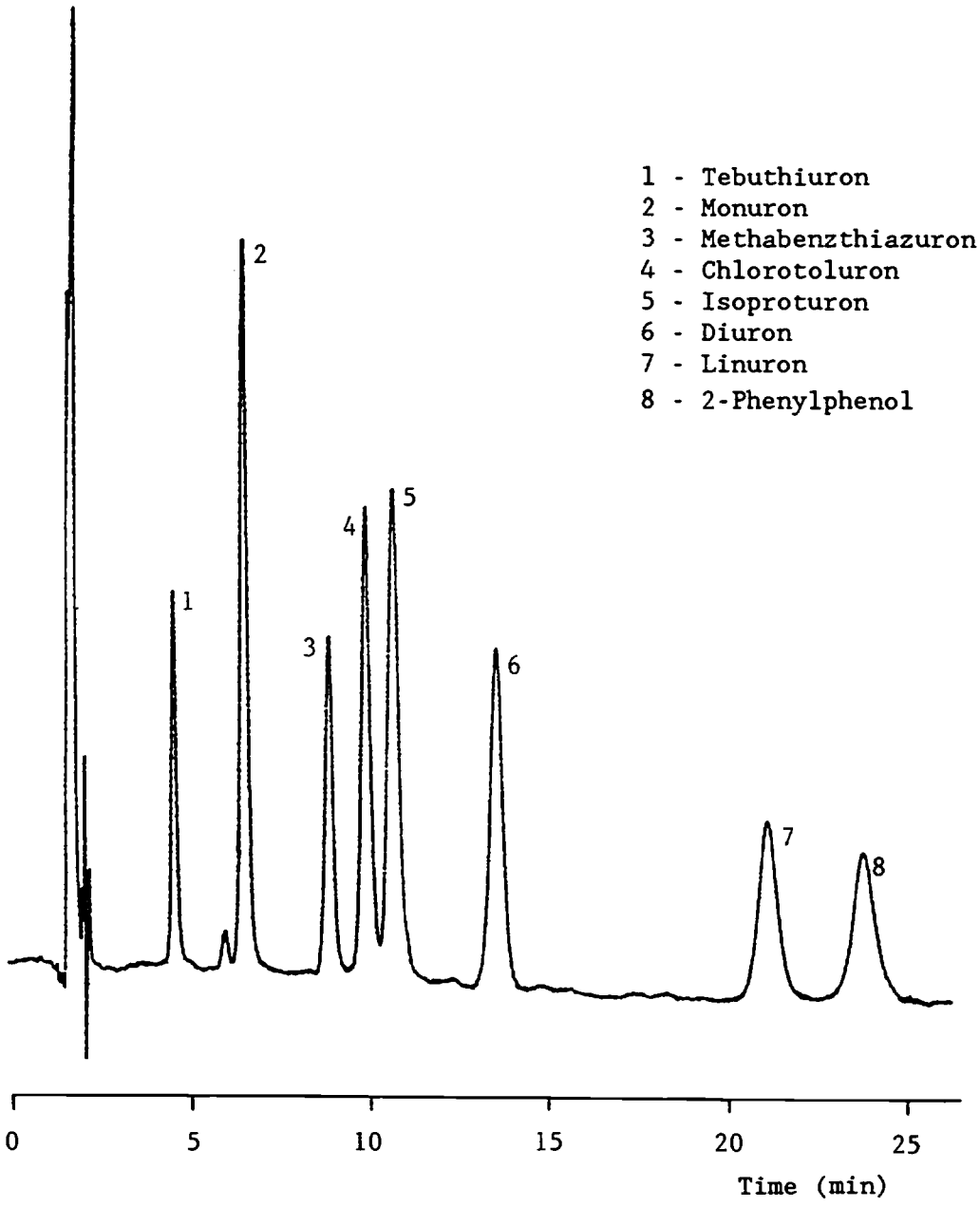
Figure A1.3 HPLC chromatogram of a herbicides mixed standard (0.2 mgL^{-1} each compound)



Notes: 1. HPLC conditions - Column: C_{18} (Nova-Pak) 100 x 8 mm, 4 μm particle size, mobile phase gradient: water + methanol (55 + 45 v/v) hold 4 min, linear to water + methanol (30 +70 v/v) at 35 min, hold to 38 min, return to initial conditions by 40 min and hold for a further 7 min, flow rate 1 mLmin^{-1}, UV detection at wavelengths 286 nm (0 - 10 min), 235 nm (10 - 17.5 min) and 245 nm (17.5 - 46.5 min).

2. Chromatogram provided by NRA (Anglia Region).

Figure A1.4 HPLC chromatogram of a phenylurea herbicides mixed standard (1 mgL^{-1} each compound)



Notes: 1. HPLC conditions - Column: Supelcosil LC-ABZ (reverse phase) 250 x 4.6 mm, 5 μm particle size, mobile phase: acetonitrile + water (32 + 68 v/v, isocratic), flow rate 1.5 $mLmin^{-1}$, temperature 60 °C, UV detection at a wavelength of 240 nm.

2. 2-Phenylphenol is the internal standard.

3. Chromatogram provided by Essex and Suffolk Water Company.

A2 The determination of phenylurea herbicides (urons) in waters by normal phase HPLC

A2.1 Performance characteristics of the method

A2.1.1	Substances determined	Chlorotoluron, diuron, isoproturon and linuron. Other phenylurea herbicides of similar structure may also be determined. Carbetamide is determined by this procedure.
A2.1.2	Types of sample	Drinking water and river waters with low suspended solids. The method has also been successfully applied to filtered waters from agricultural drainage ditches.
A2.1.3	Basis of method	The determinands are extracted by solid phase extraction (SPE), eluted with dichloromethane (DCM) and evaporated to dryness. 1,2-dichloroethane (1,2-DCE) is added and the extracts are analysed by normal phase high performance liquid chromatography with ultra-violet detection (HPLC/UV).
A2.1.4	Range of application	Typically up to 1 μgL^{-1}. The upper limit may be extended by dilution of the sample extracts or by taking a smaller sample volume.
A2.1.5	Calibration curve	The range of linearity depends on the equipment in use. The instrument used in the performance tests gave a linear response up to 2 μgL^{-1} for all the compounds listed in section A2.1.1.
A2.1.6	Standard deviation	See Table A2.
A2.1.7	Limit of detection	See Table A2.
A2.1.8	Sensitivity	Dependent on the instrument used. For the instrument used in the performance testing the baseline fluctuation was equivalent to 21 area counts and 0.1 mgL^{-1} standards gave responses of:— Chlorotoluron — 436 Diuron — 573 Isoproturon — 378 Linuron — 460 Carbetamide — 160
A2.1.9	Bias	The use of the internal standard minimises bias.
A2.1.10	Interferences	Any compound extracted by the procedure which gives a UV response at the wavelength used and with a retention time similar to any of the determinands will interfere.
A2.1.11	Time required for analysis	Approximately 12 samples per day.

A2.2 Principle

The sample is extracted using a pre-conditioned C_{18} sorbent (SPE) cartridge. After drying, the cartridge is eluted with dichloromethane. The eluate is evaporated to dryness and the residue dissolved in 1,2-dichloroethane. HPLC is carried out isocratically using normal phase separation with UV detection at a wavelength of 243 nm.

Quantification can be by an internal or external standard procedure.

A2.3 Interferences

The method will resolve most phenylurea herbicides likely to be present in water. Methabenzthiazuron emerges very close to isoproturon and the use of a more efficient column may be necessary to separate these compounds.

A2.4 Hazards

Isopropanol, 2,2,4-trimethylpentane, methanol and acetone are flammable. Dichloromethane and 1,2-dichloroethane are narcotic and toxic. The phenylurea herbicide standards and the internal standard, chloroxuron and their solutions are toxic. Contact with skin or inhalation with solids or solutions should be avoided. Ensure adequate ventilation and work in a flame and spark proof area. Spark proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

A2.5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

A2.5.1 Water. HPLC grade.

A2.5.2 Isopropanol. Analytical reagent grade.

A2.5.3 Methanol. Pesticide grade.

A2.5.4 Ammonium acetate. Analytical reagent grade.

A2.5.4.1 0.1M ammonium acetate solution, prepared in water (A2.5.1).

A2.5.5 Dichloromethane (DCM). Analytical reagent grade.

A2.5.6 Acetone. Analytical reagent grade.

A2.5.7 1,2-dichloroethane (1,2-DCE). HPLC grade.

A2.5.8 2,2,4-trimethylpentane. Analytical reagent grade.

A2.5.9 Standard solutions.

A2.5.9.1 Stock solutions of phenylurea herbicides. Prepare individual stock solutions of pure or suitably certified phenylurea herbicides in methanol at a concentration of 1000 mgL^{-1}. For example in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of solvent.

A2.5.9.2 Stock solution of internal standard. Prepare a stock solution of suitably certified internal standard, for example chloroxuron at a concentration of 500 mgL^{-1} in methanol, using a similar procedure to that described in A2.5.9.1.

A2.5.9.3 Spiking solution of internal standard. By dilution of the stock solution (A2.5.9.2) prepare a solution of the internal standard at a concentration of 5 mgL^{-1} in methanol.

A2.5.9.4 Working standard solutions of phenylurea herbicides. By dilution of the stock solutions (A2.5.9.1), prepare a series of mixed working standard solutions of phenylurea herbicides in 1,2-dichloroethane, each containing 1.0 mgL^{-1} of internal standard. For example, a useful working range for each of the phenylurea herbicides is 0.02 to 1.0 mgL^{-1}.

If the external standard method is to be used, it is not necessary to prepare the internal standard solutions A2.5.9.2 and A2.5.9.3 and the internal standard can be omitted from the working standard solutions A2.5.9.4.

A2.6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then DCM and allowed to drain.

A2.6.1 Sample bottles. Glass, 1.2 litre capacity, marked at 1.0 litre, fitted with glass stoppers or PTFE-lined screw caps.

A2.6.2 Cartridge manifold.

A2.6.3 C_{18} sorbent (SPE) cartridges (2g). The performance of the method can vary considerably with different batches of cartridge material and their supplier. It is important that different batches are performance tested before being used routinely.

A2.6.4 Centrifuge tubes. 10 mL, all glass, graduated.

A2.6.5 Microlitre syringes. 10–500 μL.

A2.6.6 Nitrogen. Oxygen-free, filtered and dry.

A2.6.7 High performance liquid chromatograph with a UV detector capable of working at 243 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic normal phase.
Columns	: Precolumn—Silica (Spherisorb S5) 50 × 4.6 mm, 5 μm particle size.
	: Analytical—Silica (Spherisorb S5) 250 × 4.6 mm, 5 μm particle size.
Column temperature	: Ambient.
Mobile phase	: Mixture of 9 mL methanol, 100 mL 2,2,4-trimethylpentane and 900 mL 1,2-dichloroethane.
Flow rate	: 1.5 $mLmin^{-1}$.
Injection volume	: 20 μL.
UV wavelength	: 243 nm.

A typical chromatogram of phenylurea herbicides and carbetamide obtained under these conditions is shown in Figure A2. Other columns and conditions may be used provided the performance is shown to be similar to or better than that reported here.

A2.7 Sample collection and storage

Samples should be taken in glass bottles with glass or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should be stored in a refrigerator at about 4 °C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions. Extraction and analysis should be carried out as soon as possible after collection.

If samples show any sign of turbidity, a cotton wool plug, pre-washed with DCM, is inserted into the end of the cartridge to prevent blocking. If the sample is very turbid, filtration will be necessary and a note of this should be reported.

A2.8 Analytical procedure

Step	Procedure	Notes
A2.8.1	Extraction	
A2.8.1.1	To 1000 ± 10 mL of sample in the sample bottle, add 5 mL isopropanol and shake. Add 100 ± 5 μL of the internal standard spiking solution (A2.5.9.3). (See note a).	(a) Blanks and spiked samples should also contain internal standard. If however, the external standard procedure is to be used, it is not necessary to add the internal standard.
A2.8.1.2	Set up the cartridge manifold with the 2 g C_{18} cartridges in place and condition them with 6 ± 0.5 mL isopropanol, followed by 6 ± 0.5 mL water (A2.5.1). (See note b).	(b) Do not allow the cartridges to dry out between addition of solutions.
A2.8.1.3	Pass the sample (containing internal standard) through the cartridge at a rate of approximately 15 $mLmin^{-1}$ (see note c).	(c) If smaller cartridges are used, the flow rate must be reduced to approximately 5 $mLmin^{-1}$.
A2.8.1.4	When all the sample has passed through the cartridge, wash with 5 ± 0.5 mL of 0.1M ammonium acetate: methanol (70:30 v/v).	
A2.8.1.5	Vacuum dry the cartridge (to eliminate water) for at least 40 minutes (see note d).	(d) It is important that all traces of water be removed.
A2.8.1.6	Elute the cartridge with 8 ± 0.5 mL DCM, collecting the eluate in a 10 mL centrifuge tube.	
A2.8.1.7	Evaporate the DCM to incipient dryness using a stream of purified nitrogen (see note e).	(e) If visible traces of water persist, add several drops of acetone and evaporate to incipient dryness.
A2.8.1.8	Dissolve the residue in 500 ± 5 μL 1,2-dichloroethane. The extract is now ready for HPLC analysis.	
A2.8.2	HPLC/UV determination	
A2.8.2.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section A2.6.7.	
A2.8.2.2	Inject aliquots of standards and extracts from blanks, samples and recoveries into the HPLC.	
A2.8.2.3	Measure the height or area of the peaks corresponding to each of the phenylurea herbicides and the internal standard.	
A2.8.2.4	Construct a calibration graph of peak ratios relative to the internal standard versus mass ratios relative to the internal standard for each phenylurea herbicide injected (see note f).	(f) If the external standard procedure is being used, construct a calibration graph of peak height or area for the standards versus concentration of each of the phenylurea herbicides (mgL^{-1}) injected.
A2.8.2.5	Read the mass ratio (or concentration, in the case of the external standard procedure) for each phenylurea herbicide in the extract from the calibration graph (note g) and calculate the concentration present in the original sample (see section A2.9).	(g) If the peak ratio for the sample exceeds the calibration range, dilute the extract appropriately and re-analyse. Account should be taken of the dilution of the internal standard.

Step	Procedure	Notes
A2.8.3	**Confirmation**	
A2.8.3.1	Change the HPLC conditions (note h). Re-analyse extracts following similar procedures to those described in sections A2.8.2.2 to A2.8.2.5.	(h) This can include mobile phase composition, column type or detector system or a combination of these.
A2.8.4	**Blanks and recoveries**	
A2.8.4.1	Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard material to separate samples of interference free water, for example HPLC grade, immediately before extraction (note i). Process these solutions under conditions identical with those to be used for the samples under analysis.	(i) Use an appropriate volume (up to 1 mL) of a mixed standard solution of phenylurea herbicides in methanol. When using the internal standard procedure, check that the recovery of the internal standard is acceptable, using external standardisation.
A2.8.5	**AQC**	
A2.8.5.1	Carry out the entire procedure using distilled water (or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with individual phenylurea herbicides. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous direct recovery tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

A2.9 Calculation

Internal standard procedure

Concentration of each phenylurea herbicide is given by:

$$C = \frac{R \times A}{V} \mu gL^{-1}$$

External standard procedure

Concentration of phenylurea is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}$$

where

C = concentration of phenylurea in original sample (μgL^{-1})

c = concentration determined in the extract from the calibration graph (μgmL^{-1})

v = volume of the extract for the determination (mL), normally 0.5 mL,

V = volume of sample extracted (L), normally 1 litre,

R = mass ratio of the determinand to internal standard from the calibration graph, and

A = amount of internal standard added to original sample (μg).

Alternative methods of calculation may be used provided they give equivalent results.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

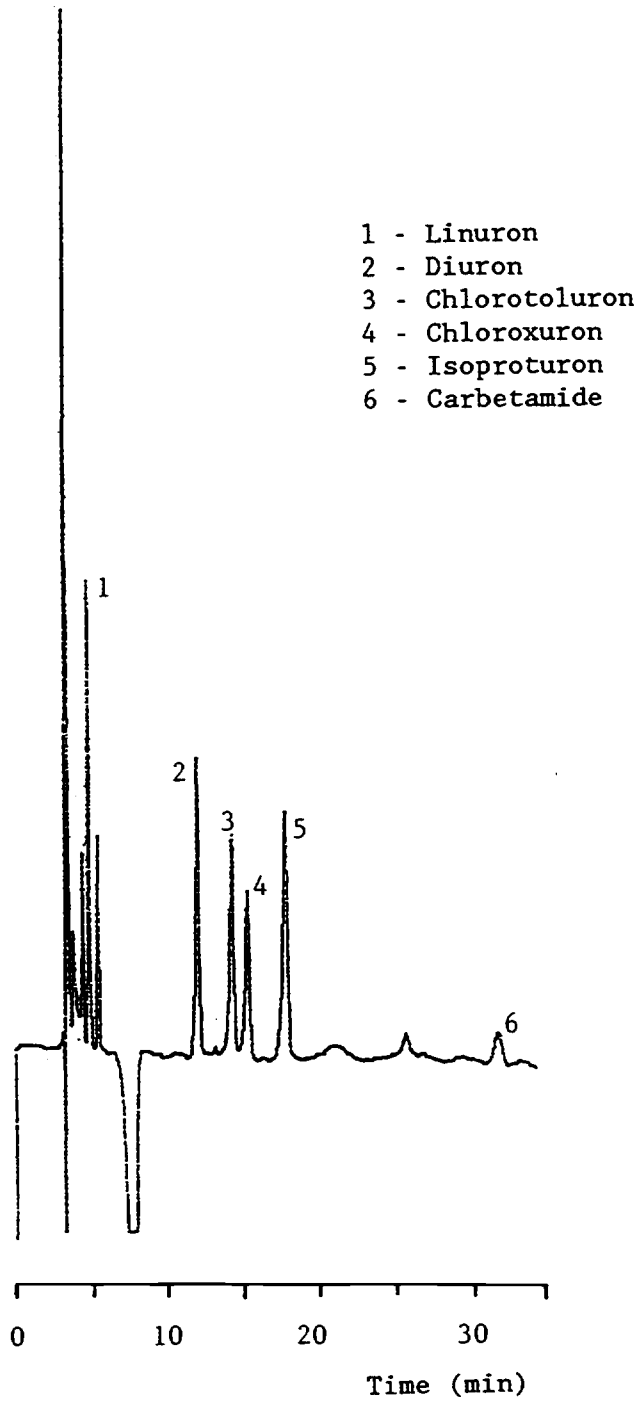
When using the external standard procedure, the effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

Table A2 Means, Standard deviations, Recoveries and Limits of detection

	Mean	S_t	Recovery %	LOD
Chlorotoluron				
Distilled water blank	0.002			0.004
0.1 spike	0.103	0.017(15)	103	
1.0 spike	0.925	0.055(11)	93	
Tap water blank	0.003			0.011
0.1 spike	0.095	0.021(14)	95	
1.0 spike	0.934	0.047(14)	93	
Diuron				
Distilled water blank	0.005			0.014
0.1 spike	0.103	0.028(11)	103	
1.0 spike	0.94	0.047(12)	94	
Tap water blank	0.004			0.014
0.1 spike	0.088	0.024(15)	88	
1.0 spike	0.95	0.051(15)	95	
Isoproturon				
Distilled water blank	0.005			0.009
0.1 spike	0.083	0.017(15)	83	
1.0 spike	0.72	0.033(13)	72	
Tap water blank	0.004			0.009
0.1 spike	0.083	0.022(15)	83	
1.0 spike	0.71	0.04(13)	71	
Linuron				
Distilled water blank	0.005			0.01
0.1 spike	0.115(8)	0.052(15)	115	
1.0 spike	0.90	0.063(12)	90	
Tap water blank	0.004			0.01
0.1 spike	0.124	0.029(14)	124	
1.0 spike	0.94	0.054(15)	94	
Carbetamide				
Distilled water blank	0.003			0.012
0.1 spike	0.079	0.015(15)	79	
1.0 spike	0.601	0.071(14)	60	
Tap water blank	0.003			0.008
0.1 spike	0.089	0.029(15)	89	
1.0 spike	0.62	0.081(14)	62	

All values expressed as μgL^{-1} unless otherwise stated.
LOD Limit of detection (calculated from $4.65 \times S_w$ ie standard deviation of the blank).
S_t Total standard deviation.
Figure in brackets represents degrees of freedom.
Data provided by SAC Scientific Ltd.

Figure A2 HPLC chromatogram of a phenylurea herbicides and carbetamide mixed standard (0.1 mgL^{-1} each compound)



Note: HPLC conditions are given in section A2.6.7

A3 The determination of phenylurea herbicides (urons) in waters by dichloromethane extraction (a note)

A3.1 Introduction

Dichloromethane will extract phenylurea herbicides (urons) from water. However, this solvent also extracts a large number of other compounds thus complicating the chromatographic interpretation considerably. This method gives details of a liquid-liquid extraction procedure to provide an extract which can be used in the chromatographic determinations of any of the other methods described in this part. In general, interference effects may be more noticeable and the analysis usually more time consuming. The limit of detection will be dependent on the sample size and the standard deviations may be similar to those obtained for the other methods.

Users are reminded to take notice of the details given in the sections in this part marked 'Hazards' and 'Sample preservation and storage'. Samples should be extracted as soon as possible after collection. If this is impracticable, the solvent should be added and the sample shaken. They should then be stored in a refrigerator at about 4 °C.

A3.2 Reagents

All reagents must be of sufficient purity that they do not give rise to signficant interfering peaks during the HPLC analysis. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed.

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

A3.2.1 Dichloromethane (DCM). HPLC grade.

A3.2.2 Methanol. HPLC grade.

A3.2.3 Anhydrous sodium sulphate. Granular, anhydrous. Heat to 500 ± 20 °C for 4 hr ± 30 min. Cool to about 200 °C in a muffle and then to ambient temperature in a desiccator. Store in a closed glass container.

A3.3 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then DCM and allowed to drain.

A3.3.1 Glass sample bottles of 1.2 L capacity, marked at 1.0 L, fitted with glass stoppers or PTFE-lined screw caps.

A3.3.2 Kuderna-Danish evaporators fitted with 10 mL graduated tubes. Equivalent evaporators may be used.

A3.3.3 Nitrogen. Oxygen-free, filtered and dry.

A3.3.4 Separating funnels, 2 L capacity, with a grease-free glass or PTFE tap, and stopper.

A3.4 Analytical procedure

Step	Procedure	Notes
A3.4.1	**Extraction**	
A3.4.1.1	To 1000 ± 10 mL of sample in the sample bottle, add 100 ± 5 mL of DCM (A3.2.1), stopper and shake on a shaking machine for 5 ± 0.5 min (see note a).	(a) A machine for shaking bottles in a horizontal plane may be used at 1–2 cycles per second.
A3.4.1.2	Transfer the contents of the bottle to a 2 L separating funnel and allow the phases to separate. Run the lower DCM layer into a flask containing approximately 10 g sodium sulphate (A3.2.3).	
A3.4.1.3	Rinse the bottle with a further 50 ± 1 mL of DCM. Transfer the washings to the separator, stopper and shake for 120 ± 10 sec. Allow the phases to separate and run the lower DCM layer into the flask containing the sodium sulphate.	
A3.4.1.4	Swirl the flask and leave to stand for at least 10 min, swirling occasionally. Transfer the extract to a Kuderna-Danish evaporator (note b). Wash the sodium sulphate with 10 ± 1 mL of DCM and decant the washings into the Kuderna.	(b) Alternative evaporating systems can be used.
A3.4.2	**Concentration**	
A3.4.2.1	Reduce the volume of the DCM extract in the evaporator to about 2–3 mL and gently evaporate the remaining solvent to incipient dryness with a stream of purified nitrogen (A3.3.3).	
A3.4.2.2	Depending on the procedure to be followed, redissolve the residue in the appropriate volume of solvent, see sections A1.8.1.7, A2.8.1.8, A4.8.2 and A5.8.2, or methylate in the case of method A4.	
A3.4.2.3	If the analysis cannot be carried out immediately store the extracts in small stoppered flasks at about 4°C.	
A3.4.2.4	Continue with the chromatographic determination as outlined in other part A methods. Calculate the amount of each phenylurea herbicide present in the original sample taking into account any volume changes used.	

A4 The determination of phenylurea herbicides (urons) in waters by GC/NPD

A4.1 Performance characteristics of the method

A4.1.1	Substances determined	Chlorotoluron, diuron, isoproturon, linuron and other phenylurea herbicides.
A4.1.2	Types of sample	Drinking water.
A4.1.3	Basis of method	The determinands are extracted by solid phase extraction (SPE), eluted with methanol and evaporated to dryness. The extract is methylated using iodomethane and sodium hydride. Water is added and the methylated herbicides partitioned into diethyl ether. The diethyl ether layer is separated and after evaporation is made up to known volume. The extract is analysed by gas chromatography with nitrogen-phosphorus detection (GC/NPD). Confirmation is by gas chromatography-mass spectrometry (GC-MS) operating in the electron impact (EI) mode.
A4.1.4	Range of application	Up to 8 μgL^{-1}. The upper limit may be extended by dilution of the sample extracts or by taking a smaller sample volume.
A4.1.5	Calibration curve	The method is linear over the range of application.
A4.1.6	Standard deviation	See Table A4.1.
A4.1.7	Limit of detection	See Table A4.1.
A4.1.8	Sensitivity	Dependent on the instrument being used. With a baseline fluctuation of less than 1%, a 0.1 mgL^{-1} standard gave approximately 60% fullscale deflection for chlorotoluron and isoproturon, and approximately 30% for diuron and linuron.
A4.1.9	Bias	Extraction efficiencies are normally less than 100%. See Table A4.1.
A4.1.10	Interferences	Any co-extracted material which after methylation has a similar GC retention time to any of the determinands and gives a response to the detector will interfere.
A4.1.11	Time required for analysis	Approximately 12 samples per day.

A4.2 Principle

The determinands are extracted from a 1 litre sample using a pre-conditioned C_{18} sorbent (SPE) cartridge which is vacuum dried and eluted with methanol. Extracts are then evaporated to incipient dryness and methylated with iodomethane in the presence of sodium hydride in dimethyl sulphoxide. After addition of water, the methylated extract is extracted with diethyl ether and the diethyl ether evaporated to incipient dryness. The extract is re-constituted in diethyl ether and analysed by GC/NPD.

A4.3 Interferences

Any substances present in extracts after extraction and methylation which have similar GC retention times to any of the determinands and respond to the NP detector will interfere.

A4.4 Hazards

Diethyl ether, iodomethane and methanol are toxic by skin absorption and inhalation. Diethyl ether, methanol and sodium hydride are flammable. Dimethyl sulphoxide is

harmful. Substituted phenylurea herbicides are toxic. Contact with skin or inhalation with solids or solutions should be avoided. Ensure adequate ventilation and work in a flame and spark proof area. Spark proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

A4.5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by light. They should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

A4.5.1 Water. Ultra high purity. Water prepared by double ion exchange followed by carbon column filtration and filtered (0.45 μm) before use is suitable.

A4.5.2 Diethyl ether. Pesticide residue grade.

A4.5.3 Dimethyl sulphoxide. HPLC grade.

A4.5.4 Methanol. HPLC grade.

A4.5.5 Iodomethane (99%) stabilised with copper.

A4.5.6 Sodium hydride in 80% dispersion with mineral oil.

A4.5.7 Standard solutions.

A4.5.7.1 Stock solutions: Prepare individual stock solutions of pure or suitably certified phenylurea herbicides in methanol at a concentration of 1000 mgL^{-1}. For example, in a volumetric flask dissolve 100 ± 0.1 mg of material in 100.0 ± 0.1 mL of methanol.

A4.5.7.2 Working standard solutions: Prepare a series of mixed working standard solutions of phenylurea herbicides in methanol by dilution of the stock solutions (A4.5.7.1). A useful working range is from 0.01 to 1.0 mgL^{-1}.

A4.6 Apparatus

Apparatus should be free from contamination before use. Glassware for sample collection should be rinsed with acetone then tap water, followed by water (A4.5.1). Clean glassware for use in analysis should be rinsed with approximately 10% (v/v) hydrochloric acid, then acetone and water followed by heating for 1 hour at 100°C.

A4.6.1 Glass sample bottles of 1.2 L capacity, marked at 1.0 L, fitted with glass stoppers or PTFE-lined screw caps.

A4.6.2 Nitrogen. Oxygen free, filtered and dry.

A4.6.3 Centrifuge tubes. Glass 10 mL with 0.1 mL graduations, glass stoppered, tapered and round bottomed.

A4.6.4 C_{18} sorbent (SPE) cartridges (1 g). The performance of the method may vary with different batches of cartridge material and their supplier. It is important that different batches are performance tested before routine use.

A4.6.5 Vacuum chamber and pump. Suitable for use with C_{18} sorbent cartridges.

A4.6.6 Gas chromatograph. A GC fitted with a suitable capillary column, injector and a NP detector. The instrument should be operated in accordance with the manufacturer's instructions. Operating conditions used to obtain the test data were as follows:

Column	:	Fused silica WCOT, 25 m × 0.22 mm ID, 0.25 μm film thickness coated with BP1
Carrier gas	:	Helium, 2 mLmin^{-1}
Injection mode	:	Split, 30:1 ratio
Column temperature	:	150°C (isothermal)
Injector temperature	:	250°C
Detector temperature	:	250°C

A typical chromatogram of a methylated phenylurea herbicide mixed standard obtained under these conditions is given in Figure A4.1. With slight temperature programming, seven methylated phenylurea herbicides can be resolved as shown in Figure A4.2. Other equivalent capillary columns can be used provided the performance obtained is shown to be similar to or better than that reported here.

A4.6.7 GC-MS equipment. A GC-MS capable of operating in the electron impact mode (EI) with selected ion monitoring (SIM). Major fragment ions for methylated phenylurea herbicides are given in Table A4.2.

Capillary conditions used for GC-MS are: Fused silica WCOT column, 60 m × 0.32 mm ID, 0.15 μm film thickness coated with DB 1701, with an oven temperature of 180°C and splitless injection.

A4.7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should be stored in a refrigerator at about 4°C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions.

A4.8 Analytical procedure

Step	Procedure	Notes
A4.8.1	Extraction	
A4.8.1.1	Condition a 1 g C_{18} cartridge with 6 ± 0.2 mL methanol followed by 6 ± 0.2 mL water (A4.5.1) (note a).	(a) Do not let the sorbent bed dry out.
A4.8.1.2	Connect a glass reservoir to each cartridge and then fit into a vacuum manifold.	
A4.8.1.3	Apply 3 ± 0.5 mL of water (A4.5.1) to the cartridge and then draw 1000 ± 10 mL of sample through the cartridge at not more than 15 ± 2 mLmin^{-1}.	
A4.8.1.4	Wash the cartridge with 3 ± 0.5 mL of water (A4.5.1) and dry under vacuum for approximately 5 minutes.	
A4.8.1.5	Elute the cartridge with 6 ± 0.2 mL of filtered methanol at a flow rate of about 9 mLmin^{-1}, into a tapered centrifuge tube.	
A4.8.1.6	Evaporate the eluate to incipient dryness with a stream of clean, dry nitrogen (notes b and c)	(b) The nitrogen supply should be adjusted such that the surface of the solvent is just indented and no splashing occurs. (c) Extracts from sections A1.8.1.6, A2.8.1.7 and A3.4.2.1 after evaporation to incipient dryness can be introduced at this stage into this method as a confirmatory procedure.

Step	Procedure	Notes
A4.8.2	**Methylation**	
A4.8.2.1	Place approximately 0.2 g of sodium hydride in a separate round bottomed centrifuge tube, followed by 1 ± 0.2 mL diethyl ether. Mix, pour off excess ether and evaporate to dryness under a stream of clean, dry nitrogen (note d). Add 1.5 ± 0.2 mL of dimethyl sulphoxide and mix to form a suspension (note e).	(d) Diethyl ether is added to remove traces of oil adhering to sodium hydride. (e) This amount of reagent is sufficient for at least three samples.
A4.8.2.2	Using a disposable glass pipette place approximately 300 ± 50 μL of the sodium hydride suspension in the sample tube (A4.8.1.6), and immediately add 50 ± 4 μL of iodomethane. Leave to stand for about 10 minutes (note f)	(f) These operations should be undertaken in a fume cupboard.
A4.8.2.3	Add 1 ± 0.2 mL of water (A4.5.1) to the mixture in the sample tube (notes f and g).	(g) Take care not to add too much water too quickly as the sample can effervesce vigorously.
A4.8.2.4	Add 8 ± 0.2 mL of diethyl ether to the sample, stopper the tube and shake vigorously. Allow the phases to separate. Transfer the organic phase to a tapered centrifuge tube using a disposable glass pipette.	
A4.8.2.5	Evaporate the organic phase to incipient dryness, accurately re-constitute in 1.00 ± 0.02 mL diethyl ether and stopper tightly.	
A4.8.3	**Preparation of standards**	
A4.8.3.1	Methanol solutions of the standards, 1.00 ± 0.02 mL, (A4.5.7.2) covering the range of concentrations expected to be found in the samples should be methylated by following steps A4.8.1.6 to A4.8.2.5. A concentration range of 0.01 to 1.0 mgL^{-1} will be adequate in most cases.	
A4.8.4	**GC/NPD determination**	
A4.8.4.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section A4.6.6.	
A4.8.4.2	Inject aliquots of the methylated extracts of standards, samples, blanks and recoveries into the GC.	
A4.8.4.3	Measure the height or area of each peak corresponding to the phenylurea compounds of interest.	
A4.8.4.4	Construct a graph of peak height or area versus concentration (mgL^{-1}) for each of the phenylurea herbicide standards injected.	
A4.8.4.5	Read the concentration of each phenylurea herbicide in the extract from the calibration graph (note h) and calculate the concentration in the original sample (see section A4.9)	(h) If the peak height or area for the sample exceeds the calibration range, dilute the extract and re-analyse.

Step	Procedure	Notes
	A4.8.5 Confirmation	
A4.8.5.1	Set up the mass spectrometer in the EI/SIM mode under conditions given in section A4.6.7 to select suitable ions (see Table A4.2). Re-analyse extracts following similar procedures to those described in section A4.8.4.	
	A4.8.6 Blanks and recoveries	
A4.8.6.1	Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure by adding suitable amounts of phenylurea herbicide standards to samples of interference free water (for example A4.5.1), immediately before extraction (note i). Process these solutions under identical conditions to those to be used for the samples under analysis.	(i) Use up to 100 μL of an appropriate working standard solution in methanol.
	A4.8.7 AQC	
A4.8.7.1	Carry out the entire procedure using water (A4.5.1 or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with individual phenylurea herbicides. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

A4.9 Calculation

Calculate the concentration of each phenylurea herbicide in the sample using the equation:

$$C = \frac{c \times v}{V} \ \mu gL^{-1}$$

Where

C = concentration of the phenylurea herbicide in the sample (μgL^{-1}),

c = concentration of the phenylurea herbicide in the extract (μgmL^{-1}),

V = volume of the sample taken (litres) and

v = volume of the extract for the determination (mL).

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

The effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

Table A4.1 Means, Standard deviations, Recoveries and Limits of detection

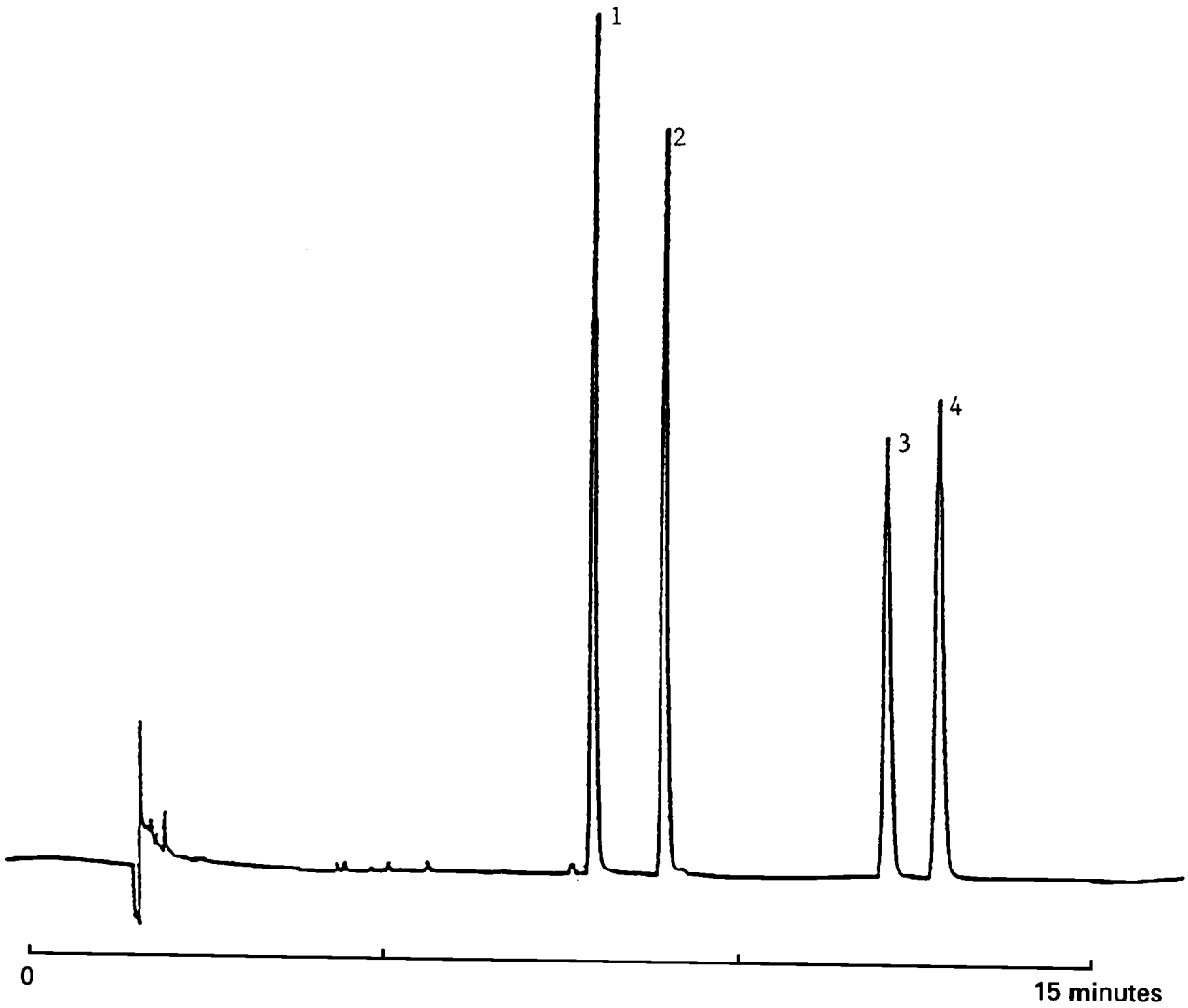
	Mean	S_t	Recovery %	DOF	LOD
Chlorotoluron					
Treated water blank					0.035
0.1 spike	0.083	0.0159	83.1	13.9	
1.0 spike	0.845	0.1618	84.5	14.6	
High purity water blank					0.039
0.1 spike	0.088	0.0156	87.5	15.4	
1.0 spike	0.886	0.1547	88.6	13.9	
Diuron					
Treated water blank					0.033
0.1 spike	0.082	0.0183	82.3	12.1	
1.0 spike	0.856	0.1637	85.6	15.6	
High purity water blank					0.036
0.1 spike	0.083	0.0164	82.9	13.8	
1.0 spike	0.889	0.1471	88.9	15.9	
Isoproturon					
Treated water blank					0.041
0.1 spike	0.089	0.0122	89.2	18.9	
1.0 spike	0.913	0.1625	91.3	18.9	
High purity water blank					0.035
0.1 spike	0.091	0.0132	90.9	16.1	
1.0 spike	0.936	0.1624	93.6	10.9	
Linuron					
Treated water blank					0.043
0.1 spike	0.087	0.0135	87.4	18.6	
1.0 spike	0.757	0.1982	75.7	18.2	
High purity water blank					0.041
0.1 spike	0.080	0.0177	79.8	14.5	
1.0 spike	0.792	0.1784	79.2	14.1	

All values expressed in μgL^{-1} unless otherwise stated.
S_t Total standard deviation.
DOF Degrees of freedom
LOD Limit of detection (calculated from $4.65 \times S_w$ ie within batch standard deviation of the blank)
Data provided by Essex and Suffolk Water company

Table A4.2 Major ions for GC-MS of methylated phenylurea herbicides

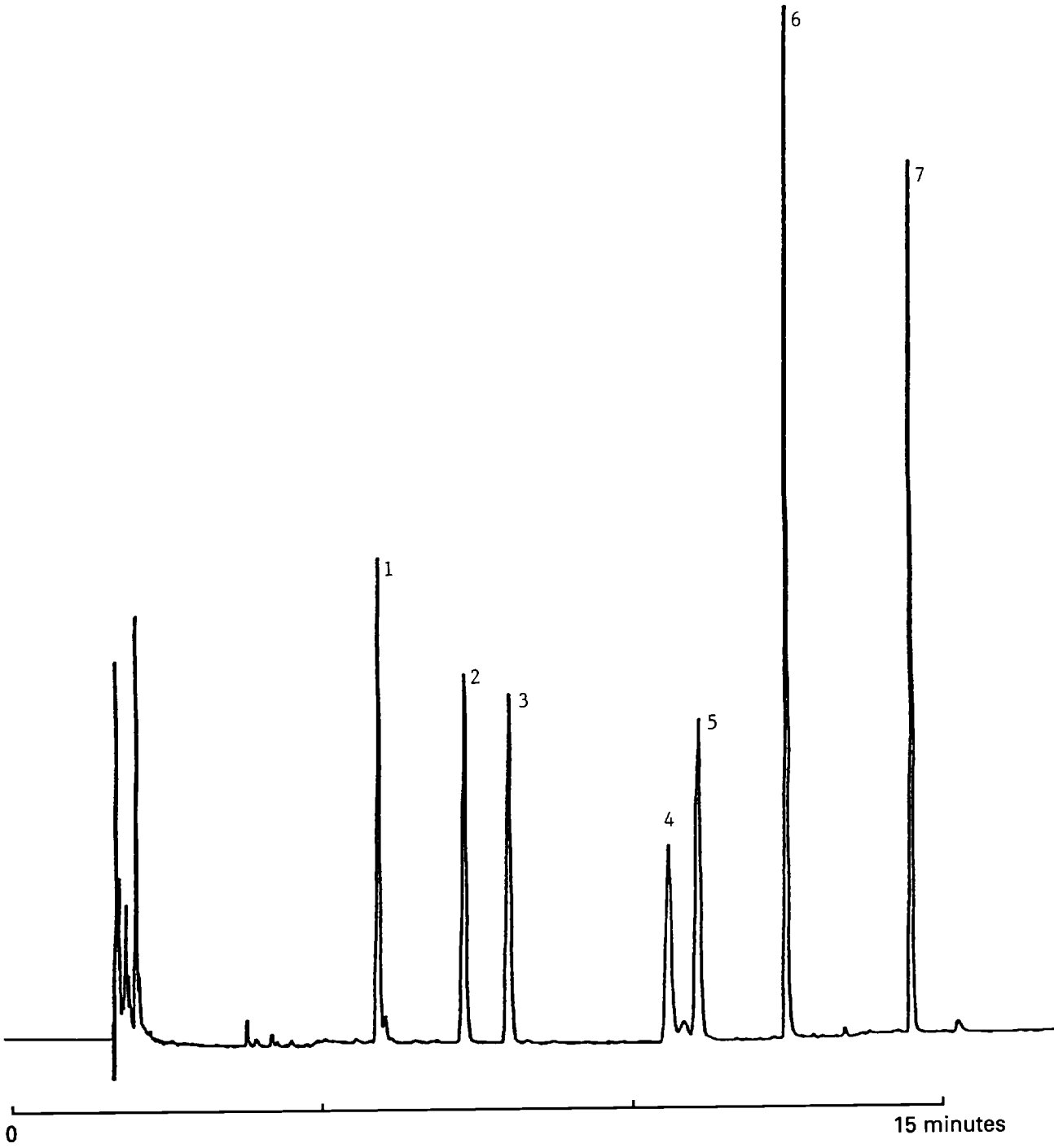
Phenylurea	Ions (m/z)
Chlorotoluron	72, 154, 226
Diuron	72, 174, 246
Isoproturon	72, 148, 220
Linuron	88, 174

Figure A4.1 **Isothermal GC/NPD chromatogram of a methylated phenylurea herbicides mixed standard (1.0 mgL^{-1} each compound)**



1 - Isoproturon
2 - Chlorotoluron
3 - Linuron
4 - Diuron

Figure A4.2 Temperature programmed GC/NPD chromatogram of a methylated phenylurea herbicides mixed standard (2.0 mgL^{-1} each compound)



Temperature programme: 140°C for 10 min, 140 to 190°C at 20°C min^{-1}, held at 190°C for 8 min.

1 - Monuron
2 - Isoproturon
3 - Chlorotoluron
4 - Linuron
5 - Diuron
6 - Methabenzthiazuron
7 - Tebuthiuron

A5 The determination of phenylurea herbicides (urons) in waters by thermospray LC-MS (a note)

A5.1 Performance characteristics of the method

A5.1.1	Substances determined	Chlorotoluron, diuron, isoproturon and linuron. Other phenlyurea herbicides may be determined, as well as carbendazim and carbetamide.
A5.1.2	Types of sample	River and drinking water.
A5.1.3	Range of application	Up to 0.125 μgL^{-1}. The upper limit may be extended by dilution of the sample extracts or by taking a smaller sample volume.
A5.1.4	Calibration curve	The method is linear over the range of application. The upper limit may be extended by dilution of the sample extracts or by taking a smaller sample volume.
A5.1.5	Standard deviation	See Table A5.1.
A5.1.6	Limit of detection	See Table A5.1.
A5.1.7	Sensitivity	Dependent on the instrument used. A signal to noise ratio better than 20:1 for 12.5 ng of each compound injected was observed.
A5.1.8	Bias	Extraction efficiencies are normally less than 100%. See Table A5.1.

A5.2 Principle

Any of the methods previously described in this part of the booklet may be used to obtain an extract of the compounds of interest. This extract is then analysed by thermospray liquid chromatography-mass spectrometry (LC-MS).

A5.3 Interferences

Interferences should be minimised by the multiple steps involved in this analysis. However, any compounds which pass through the extraction process, have similar LC characteristics, and give rise to similar ions in the thermospray source to any of the compounds of interest will interfere.

A5.4 Hazards

Methanol is toxic by skin absorption and inhalation. This solvent is also flammable. Substituted phenylurea herbicides are toxic. Contact with skin or inhalation with solids or solutions should be avoided. Ensure adequate ventilation and work in a flame and spark proof area. Spark proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

A5.5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

A5.5.1 Water. HPLC grade.

A5.5.2 Methanol. HPLC grade.

A5.5.3 Ammonium acetate. Analytical reagent grade.

A5.5.3.1 A 1% m/v methanolic solution of ammonium acetate.

A5.5.3.2 A 1% m/v aqueous solution of ammonium acetate.

A5.5.4 Standard solutions.

A5.5.4.1 Stock solutions of phenylurea herbicides. Prepare individual stock solutions of pure or suitably certified phenylurea herbicides in methanol at a concentration of 1000 mgL^{-1}. For example in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of solvent.

A5.5.4.2 Stock solution of internal standard. Prepare a stock solution of suitably certified internal standard, for example benzanilide at a concentration of 100 mgL^{-1} in methanol, using a similar procedure to that described in A5.5.4.1.

A5.5.4.3 Solution of internal standard for reconstituting extracts. By dilution of the stock solution (A5.5.4.2) prepare a solution of the internal standard at a concentration of 0.25 mgL^{-1} in 50/50 v/v aqueous methanol.

A5.5.4.4 Working standard solutions of phenylurea herbicides. By dilution of the stock solutions (A5.5.4.1), prepare a series of mixed working standard solutions of phenylurea herbicides in methanol, each containing 0.25 mgL^{-1} of internal standard. For example, a useful working range for each of the phenylurea herbicides is 0.05 to 0.625 mgL^{-1}.

A5.5.5 Helium. Suitable for chromatography.

A5.6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and allowed to drain.

A5.6.1 Sample bottles. All glass or with PTFE-lined screw caps, 1.2 litre capacity and marked at 1 litre.

A5.6.2 High performance liquid chromatography-mass spectrometry system (HPLC-MS) with thermospray interface. Operating conditions used to obtain the test data were as follows:

Mode:	Programmed reverse phase.
Column:	Analytical—C_{18} (Techsphere 5 ODS) 250 × 4.6 mm, 5 μm particle size
Column temperature:	30°C
Mobile phase:	Solution 1. Methanol:water (55:45 v/v) containing 1% m/v ammonium acetate.
	Solution 2. Methanol containing 1% m/v ammonium acetate (A5.5.3.1)
Programme:	0–10 minutes: 100% solution 1 10–15 minutes: 50/50 v/v mixture of solutions 1 and 2 15–20 minutes: 100% solution 2
Flow rate:	1 $mLmin^{-1}$.
Injection volume:	40 μL.
Detection:	Selected ion monitoring (SIM). See Table A5.2.

The post column reagent buffer required for thermospray ionisation is 1% m/v ammonium acetate (A5.5.3.2) used at a flow rate of 0.3 $mLmin^{-1}$. All solutions are filtered and de-gassed with helium prior to use.

Under these conditions approximate retention times of chlorotoluron, diuron, isoproturon and linuron were 12, 14, 13 and 17 minutes respectively. A typical selected ion mass chromatogram for the phenylurea herbicides, and for carbendazim and carbetamide, is given in Figure A5. Other suitable equipment may be used provided the performance obtained is shown to be similar to or better than that reported here.

A5.7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should be stored in a refrigerator at about 4°C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions.

A5.8 Analytical Procedure

Step	Procedure	Notes
A5.8.1	Extraction	
A5.8.1.1	1000 ± 10 mL of sample is extracted by any of the extraction techniques described in the previous methods. The extract obtained is evaporated to dryness as described (see note a)	(a) See sections A1.8.1.1–A1.8.1.6, A2.8.1.1–A2.8.1.7, A3.4.1.1–A3.4.2.1, A4.8.1.1–A4.8.1.6. In the case of method A1, only 250 mL of sample may be available.
A5.8.2	The residue is re-dissolved in 200 ± 5 μL reconstitution solution (A5.5.4.3). The extract is now ready for LC-MS analysis (note b).	(b) A concentration factor of 5000 is thus obtained.
A5.8.3	HPLC-MS determination	
A5.8.3.1	Set up the LC-MS and optimise the conditions according to the manufacturer's instructions. Exact conditions will vary depending on the instrument used. The analysis is carried out in SIM mode. The mass peaks monitored should normally be M + 1 and these correspond to those shown in Table A5.2. They can vary according to how the instrument is set up.	
A5.8.3.2	Inject aliquots of standards and extracts from blanks, samples and recoveries into the HPLC.	
A5.8.3.3	Continue as described in methods A1 or A2 using the internal standard procedures.	

Table A5.1 Standard deviations, Limits of detection and Recoveries

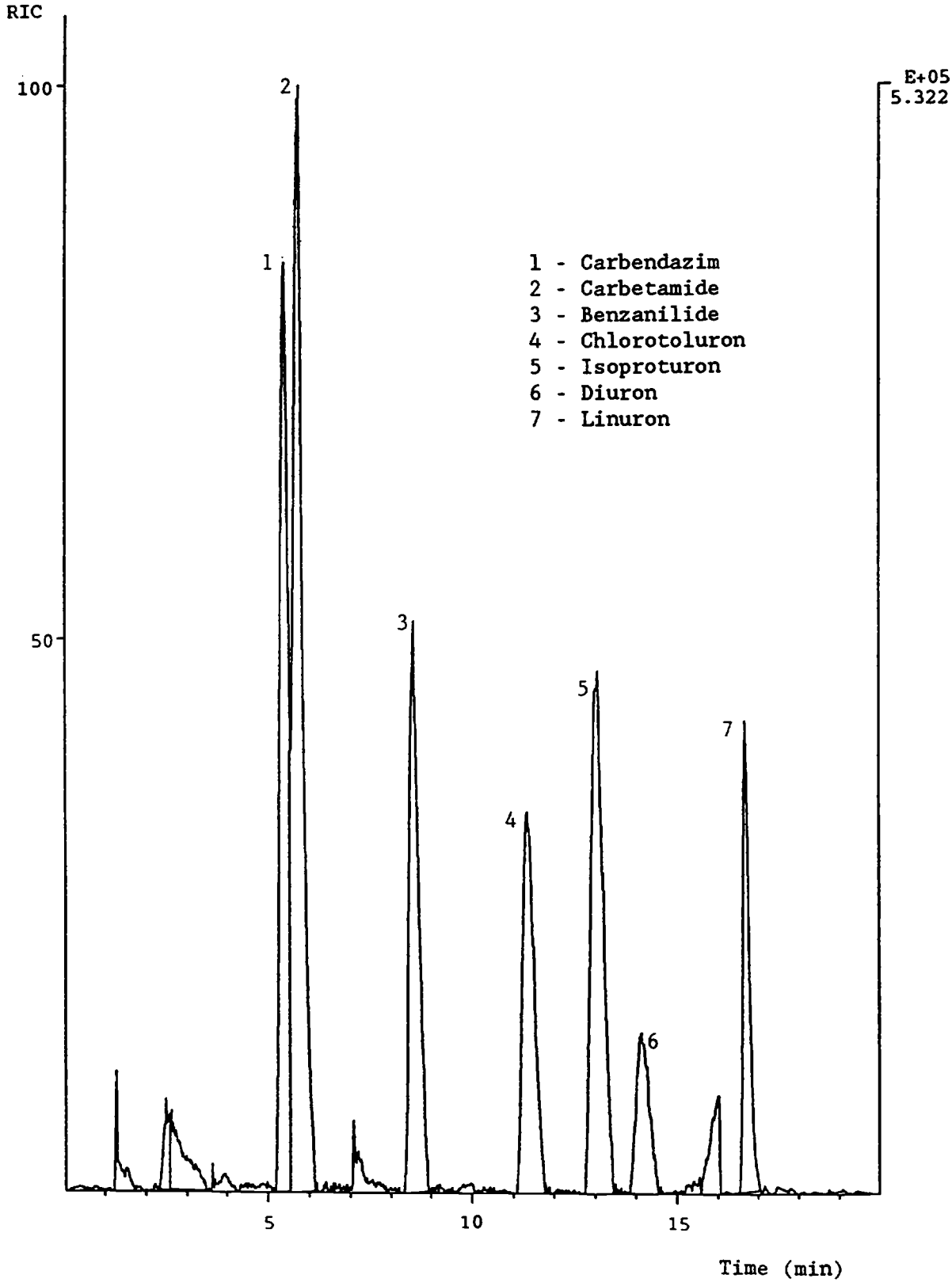
	Spike concentration	S_t	LOD	DOF	Recovery %
Chlorotoluron	0.1	0.0077	0.001	14	85.6
Diuron	0.1	0.0111	0.001	22	78.1
Isoproturon	0.1	0.0163	0.001	23	82.9
Linuron	0.1	0.0122	0.005	24	87.3
Carbendazim	0.1	0.0194	0.001	14	71.5
Carbetamide	0.1	0.0110	0.001	16	92.6

All units expressed as μgL^{-1} unless otherwise stated.
LOD Limit of detection (calculated from $4.65 \times S_w$ ie within batch standard deviation of blank).
DOF Degrees of freedom.
Data provided by North West Water Ltd.
Data represent complete sample preparation using procedures similar to those described in methods A1 and A2, and do not apply to MS analysis alone.

Table A5.2 M + 1 base peaks used to monitor compounds of interest

M + 1	Compound	Ion monitoring time (minutes)
192	carbendazim	0–10
237	carbetamide	0–10
198	benzanilide	0–10
213	chlorotoluron	10–16
207	isoproturon	10–16
233	diuron	10–16
249	linuron	16–20

Figure A5 Selected ion mass chromatogram for ground water spiked with phenylurea herbicides, carbendazim and carbetamide (0.1 μgL^{-1} each)



Chromatogram provided by North West Water Ltd.

B Dinocap in waters by HPLC

B1 Performance characteristics of the method

B1.1	Substances determined	Dinocap, isomeric mixtures of octyldinitrophenylcrotonate.
B1.2	Types of sample	Drinking and river waters.
B1.3	Basis of method	The determinand is extracted from a 1 litre sample of acidified water with dichloromethane (DCM) at a pH of approximately 3. The DCM extract is dried and evaporated to dryness. The residue is re-dissolved in methanol and dinocap is determined by high performance liquid chromatography with ultra-violet detection (HPLC/UV).
B1.4	Range of application	Up to 5 μgL^{-1}. The upper limit may be extended by dilution of the sample extracts or by taking a smaller sample volume.
B1.5	Calibration curve	The method is linear over the range of application.
B1.6	Standard deviation	0.008 μgL^{-1}, n = 5 (determined on blank samples).
B1.7	Limit of detection	0.04 μgL^{-1} for a 1 litre sample.
B1.8	Sensitivity	Dependent on the instrument used. With a baseline fluctuation of 0.5% a 1.05 mgL^{-1} standard gave approximately 20% full scale deflection.
B1.9	Bias	Extraction efficiencies are normally less than 100%. See Table B1.
B1.10	Interferences	Any co-extracted material which has a similar HPLC retention time to dinocap and which gives a detector response at a wavelength of 254 nm will interfere. The presence of the distinctive triplet of peaks for dinocap should aid in identification.
B1.11	Time required for analysis	Approximately 6 samples per day.

B2 Principle

Dinocap is extracted from 1 litre of acidified sample at pH 3 with dichloromethane. The extract is dried over anhydrous sodium sulphate and the volume reduced. The remaining dichloromethane is then removed under a stream of nitrogen, the residue re-dissolved in 0.5 mL of methanol and a portion of the extract analysed by isocratic reverse phase HPLC using a UV detector operating at a wavelength of 254 nm.

B3 Interferences

At low levels (<1 μgL^{-1}) there is usually interference present in extracts from river water samples.

B4 Hazards

Dinocap is toxic by skin absorption and ingestion. Caution must be exercised when preparing the stock solution. Skin contact, ingestion and inhalation must be avoided. Methanol is flammable. Dichloromethane and methanol are toxic by skin absorption and inhalation. Ensure adequate ventilation and work in a flame and spark free area. Spark

proof refrigerators should be used to store standard solutions and extracts. Sulphuric and phosphoric acids are corrosive. Appropriate safety procedures should be followed.

B5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

B5.1 Water. HPLC grade, filtered before use.

B5.2 Dichloromethane (DCM). Pesticide grade or equivalent.

B5.3 Methanol. HPLC grade.

B5.4 Sulphuric acid (5M). Measure about 700 ± 20 mL cold water into a 2 L beaker. With continuous stirring and cooling, slowly add 265 ± 5 mL sulphuric acid (d_{20} 1.84). When cool, make up to 1000 ± 10 mL with water.

B5.5 Orthophosphoric acid 85%. Analytical reagent grade.

B5.6 Sodium sulphate. Granular, anhydrous. Heat to 500 ± 20 °C for 4 hr ± 30 min. Cool to about 200 °C in a muffle and then to ambient temperature in a desiccator. Store in a closed glass container.

B5.7 Standards

B5.7.1 Stock solution: Prepare a stock solution of pure or suitably certified dinocap in methanol at a concentration of 1000 mgL^{-1}. For example, in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of methanol.

B5.7.2 Working standard solutions: Prepare a series of working standard solutions of dinocap in methanol by dilution of the stock solution (B5.7.1). A useful working range is from 0.05 to 10 mgL^{-1}.

B6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then dichloromethane and allowed to drain.

B6.1 Glass sample bottles of 1.2 L capacity, marked at 1.0 L, fitted with glass stoppers or PTFE-lined screw caps.

B6.2 Kuderna-Danish evaporators fitted with 10 mL graduated tubes. Equivalent evaporators may be used.

B6.3 Nitrogen. Oxygen-free, filtered and dry.

B6.4 Separating funnels, 2 L capacity, with a grease-free glass or PTFE tap, and stopper.

B6.5 High performance liquid chromatograph with a UV detector capable of working at 254 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic reverse phase.
Columns	: Precolumn—Co-pell C_{18}, 70 × 2 mm.
	: Analytical— Spherisorb C_{18} (ODS2) 250 × 4.6 mm, 5 μm particle size

Column temperature	: Ambient.
Mobile phase	: Methanol:water (90:10 v/v) adjusted to pH 3 with phosphoric acid.
Flow rate	: 1.0 $mLmin^{-1}$.
Injection volume	: 20 μL.
UV wavelength	: 254 nm.

Under these conditions retention times of the dinocap triplet peaks should be approximately 6.4, 7.0 and 7.5 minutes. Other suitable equipment may be used provided the performance obtained is shown to be similar to or better than that reported here.

B7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, the acid and extraction solvent should be added (see step B8.1.1) and the sample shaken. They should then be stored in a refrigerator at about 4 °C. The sample bottles should be protected from contamination and should not be placed in the close proximity of standard materials or their concentrated solutions.

B8 Analytical procedure

Step	Procedure	Notes
B8.1	**Extraction**	
B8.1.1	To 1000 ± 10 mL of sample in the sample bottle, add 0.5 ± 0.1 mL sulphuric acid (B5.4). Check the pH is approximately 3 and adjust if necessary (see note a). Add 100 ± 5 mL of DCM (B5.2) to the acidified sample, stopper and shake on a shaking machine for 5 ± 0.5 min (see notes b and c).	(a) pH paper may be used. (b) Dinoseb is also extracted under these conditions (see section C8.1) (c) A machine for shaking bottles in a horizontal plane may be used.
B8.1.2	Transfer the contents of the bottle to a 2 L separating funnel and allow the phases to separate. Run the lower DCM layer into a flask containing approximately 10 g sodium sulphate (B5.6)	
B8.1.3	Rinse the bottle with a further 50 ± 1 mL of DCM. Transfer the washings to the separator, stopper and shake for 120 ± 10 sec. Allow the phases to separate and run the lower DCM layer into the flask containing the sodium sulphate.	
B8.1.4	Swirl the flask and leave to stand for at least 10 min, swirling occasionally.	
B8.1.5	Transfer the extract to a Kuderna-Danish evaporator (note d). Wash the sodium sulphate with 10 ± 1 mL of DCM and decant the washings into the Kuderna.	(d) Alternative evaporation systems can be used.
B8.2	**Concentration**	
B8.2.1	Reduce the volume of the dichloromethane extract in the evaporator to about 0.5 mL and gently evaporate the remaining solvent to incipient dryness with a stream of purified nitrogen (B6.3).	
B8.2.2	Dissolve the residue in 0.50 ± 0.01 mL methanol. The solution is now ready for HPLC analysis.	

B8.3 HPLC/UV determination

B8.3.1 Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section B6.5.

B8.3.2 Inject aliquots of standards and extracts from blanks, samples and recoveries into the HPLC.

B8.3.3 Measure the total area of the three peaks in the triplet corresponding to dinocap (note e).

(e) The ratio of separated peaks from water samples may differ from that in the standards due to degradation or interference. In these circumstances, although initial quantification may be undertaken using those peaks which appear free from interference, results should be confirmed using alternative HPLC conditions (B8.4).

B8.3.4 Construct a calibration graph of peak area for the standards versus concentration of dinocap (mgL^{-1}) injected.

B8.3.5 Read the concentration of dinocap in the extract from the calibration graph (note f) and calculate the concentration present in the sample (see section B9).

(f) If the peak area for the sample exceeds the calibration range dilute the extract appropriately and re-analyse.

B8.4 Confirmation

B8.4.1 Change the HPLC conditions (notes g and h). Re-analyse extracts following similar procedures to those described in sections B8.3.2 to B8.3.5.

(g) This can include mobile phase composition, column type or detector system or a combination of these.

(h) See reference B10.

B8.5 Blanks and recoveries

B8.5.1 Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard material to separate samples of interference free water, for example HPLC grade, immediately before extraction (note i). Process these solutions under conditions identical with those to be used for the samples under analysis.

(i) Use up to 1 mL of an appropriate working standard solution in methanol.

B8.6 AQC

B8.6.1 Carry out the entire procedure using distilled water (or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with dinocap. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.

B9 Calculation

Concentration of dinocap is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}$$

where

C = concentration of dinocap in original sample (μgL^{-1})

c = concentration determined in the extract from the calibration graph (μgmL^{-1})

v = volume of the extract for the determination (mL), normally 0.5 mL, and

V = volume of sample extracted (L), normally 1 litre.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

The effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

B10 Reference

Method B in The Determination of Carbamates, Thiocarbamates, related compounds and Ureas in Water 1987 (ISBN 0117521515) in this series.

Table B1 Means and Recoveries

	Mean	Recovery %
Tap water blank	0.019	
Spike (0.26)	0.195	75
Spike (0.53)	0.43	81.1
Spike (1.05)	0.825	78.6
Spike (5.26)	5.07	96.3
River water blank	0.008	
Spike (0.53)	0.38	71.7

All values expressed as μgL^{-1} unless otherwise stated.
Data provided by Severn Trent Laboratories.

C Dinoseb in waters by HPLC

C1 Performance characteristics of the method

C1.1	Substance determined	Dinoseb (2-*sec*-butyl-4,6-dinitrophenol).
C1.2	Types of sample	Drinking and river waters.
C1.3	Basis of method	The determinand is extracted from a 1 litre sample of acidified water with dichloromethane (DCM). The DCM extract is dried and evaporated to dryness. The residue is redissolved in methanol and dinoseb is determined by high performance liquid chromatography with ultra-violet detection (HPLC/UV). Confirmation is by gas chromatography-mass spectrometry (GC-MS) operating in the electron impact (EI) mode.
C1.4	Range of application	Up to 5 μgL^{-1}. The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
C1.5	Calibration curve	The method is linear over the range of application.
C1.6	Standard deviation	0.003 μgL^{-1}, n = 5 (determined on blank samples).
C1.7	Limit of detection	0.014 μgL^{-1} for a 1 litre sample.
C1.8	Sensitivity	Dependent on the instrument used but with a baseline fluctuation of 0.5%, a 1.1 mgL^{-1} standard gave approximately 50% full scale deflection.
C1.9	Bias	Extraction efficiencies are normally less than 100%. See Table C1.
C1.10	Interferences	Any co-extracted material which has a similar HPLC retention time to dinoseb and which gives a detector response at a wavelength of 264 nm will interfere.
C1.11	Time required for analysis	Approximately 6 samples per day.

C2 Principle

Dinoseb is extracted from 1 litre of acidified sample with dichloromethane at a pH of approximately 3. The extract is dried over anhydrous sodium sulphate and the volume reduced. The remaining dichloromethane is then removed under a stream of nitrogen, the residue re-dissolved in 0.5 mL of methanol and a portion of the extract analysed by isocratic reverse phase HPLC using a UV detector operating at a wavelength of 264 nm.

C3 Interferences

None known, but any substance extracted under the same conditions and which elutes at the same retention time as dinoseb and absorbs at 264 nm will interfere.

C4 Hazards

Dinoseb is very toxic by skin absorption and ingestion. Caution must be exercised when preparing the stock solution. Skin contact, ingestion and inhalation must be avoided. Methanol is flammable. Dichloromethane and methanol are toxic by skin absorption and inhalation. Ensure adequate ventilation and work in a flame and spark free area. Spark

proof refrigerators should be used to store standard solutions and extracts. Sulphuric and phosphoric acids are corrosive. Appropriate safety procedures should be followed.

C5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark, if necessary.

C5.1 Water. HPLC grade, filtered before use.

C5.2 Dichloromethane (DCM). Pesticide grade or equivalent.

C5.3 Methanol. HPLC grade.

C5.4 Cyclohexane. Pesticide grade.

C5.5 Sulphuric acid (5M). Measure about 700 ± 20 mL cold water into a 2 L beaker. With continuous stirring and cooling, slowly add 265 ± 5 mL sulphuric acid (d_{20} 1.84). When cool, make up to 1000 ± 10 mL with water.

C5.6 Orthophosphoric acid 85%. Analytical reagent grade.

C5.7 Sodium sulphate. Granular, anhydrous. Heat to 500 ± 20 °C for 4 hr ± 30 min. Cool to about 200 °C in a muffle and then to ambient temperature in a desiccator. Store in a closed glass container.

C5.8 Standards

C5.8.1 Stock solution: Prepare a stock solution of pure or suitably certified dinoseb in methanol at a concentration of 1000 mgL^{-1}. For example, in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of methanol.

C5.8.2 Working standard solutions: Prepare a series of working standard solutions of dinoseb in methanol by dilution of the stock solution (C5.8.1). A useful working range is from 0.05 to 5 mgL^{-1}.

C6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then DCM and allowed to drain.

C6.1 Sample bottles : 1.2 L capacity, graduated at 1.0 L, made of glass and fitted with a glass stopper or PTFE-lined screw cap.

C6.2 Kuderna Danish evaporators fitted with 10 mL graduated tubes. Equivalent evaporating systems may also be used.

C6.3 Nitrogen. Oxygen-free, filtered and dry.

C6.4 Separating funnels, 2 L capacity, with a grease-free glass or PTFE tap, and stopper.

C6.5 High performance liquid chromatograph with a UV detector capable of working at 264 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic reverse phase
Columns	: Pre-column—Co-pell C_{18}, 70 × 2 mm : Analytical—Spherisorb C_{18}, (ODS2) 250 × 4.6 mm, 5 μm particle size
Mobile phase	: Methanol:water (80:20 v/v) adjusted to pH 3 with phosphoric acid.
Flow rate	: 1.0 mLmin^{-1}.
Injection volume	: 20 μL.
UV wavelength	: 264 nm

Under these conditions the retention time of dinoseb is approximately 7.5 minutes. Other suitable equipment may be used provided the performance obtained is shown to be similar to or better than that reported here.

C6.6 GC-MS equipment. A gas chromatograph—mass spectrometer capable of operating in the electron impact (EI) mode is required. The mass spectrum for dinoseb is shown in Figure C1.

Suitable gas chromatographic conditions are:

Column	:	Fused silica WCOT, 30 m × 0.25 mm id, 0.25 μm film thickness coated with DB-5, or equivalent.
Carrier gas	:	Helium, 1 mLmin^{-1}.
Injection mode	:	Splitless at 275 °C. Splitless time 0.5 min.
Temperature programme:		Two-stage ramp 60 °C for 1 min ramp to 175 °C at 35 °Cmin^{-1} ramp to 280 °C at 4 °Cmin^{-1} isothermal at 280 °C for 9 min. Total run time 39.5 min.

Under these conditions the retention time of dinoseb is approximately 11 minutes.

C7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, the acid and extraction solvent should be added (see step C8.1.1) and the sample shaken. They should then be stored in a refrigerator at about 4 °C. The sample bottles should be protected from contamination and should not be placed in the close proximity of standard materials or their concentrated solutions.

C8 Analytical procedure

Step	Procedure	Notes
C8.1	Extraction	
C8.1.1	To 1000 ± 10 mL of sample in the sample bottle, add 0.5 ± 0.1 mL sulphuric acid (C5.5). Check the pH is approximately 3 and adjust if necessary (see note a) Add 100 ± 5 mL of DCM (C5.2) to the acidified sample, stopper and shake vigorously for 120 ± 10 sec (notes b, c and d).	(a) pH paper may be used. (b) Dinocap is also extracted under these conditions (see section B8.1). (c) A machine for shaking bottles in a horizontal plane may be used. (d) The extraction can be undertaken in a separating funnel if required.
C8.1.2	Transfer the contents of the bottle to a 2 L separating funnel and allow the phases to separate. Run the lower DCM layer into a flask containing 5–10 g sodium sulphate (C5.7)	

Step	Procedure	Notes
C8.1.3	Rinse the bottle with a further 50 ± 1 mL of DCM. Transfer the washings to the separator, stopper and shake for 120 ± 10 sec. Allow the phases to separate and run the lower DCM layer into the flask containing the sodium sulphate.	
C8.1.4	Swirl the flask and leave to stand for at least 10 min, swirling occasionally.	
C8.1.5	Transfer the extract to a Kuderna-Danish evaporator (note e). Wash the sodium sulphate with 10 ± 1 mL of DCM and decant the washings into the Kuderna.	(e) Alternative evaporation systems can be used.
C8.2	**Concentration**	
C8.2.1	Reduce the volume of the dichloromethane in the evaporator to 0.5 mL and gently evaporate the remaining solvent to incipient dryness with a stream of purified nitrogen (C6.3).	
C8.2.2	Dissolve the residue in 0.50 ± 0.01 mL of methanol. This solution is now ready for HPLC analysis.	
C8.3	**HPLC/UV determination**	
C8.3.1	Set up the apparatus according to the manufacturer's instructions using the conditions described in section C6.5.	
C8.3.2	Inject aliquots of standards and extracts from blanks, samples and recoveries into the HPLC.	
C8.3.3	Measure the height or area of the peak corresponding to dinoseb.	
C8.3.4	Construct a calibration graph of peak height or area for the standards versus concentration of dinoseb (mgL^{-1}) injected.	
C8.3.5	Read the concentration of dinoseb in the extract from the calibration graph (note f) and calculate the concentration present in the original sample (see section C9).	(f) If the peak height or area for the sample exceeds the calibration range dilute the extract appropriately and re-analyse.
C8.4	**Confirmation**	
C8.4.1	Change the HPLC conditions (notes g and h). Re-analyse extracts following similar procedures to those described in sections C8.3.2 to C8.3.5.	(g) This can include mobile phase composition, column type or detector system or a combination of these. (h) See reference C10.
C8.4.2	Alternatively, a GC–MS procedure can be used. Set up the mass spectrometer in the EI/SIM mode under conditions given in section C6.6. Select suitable ions for monitoring. See Figure C1. Re-analyse extracts following similar procedures to those described in section C8.3.	

Step	Procedure	Notes
C8.5	Blanks and recoveries	
C8.5.1	Adequate blank values should be obtained using interference free water before analysing samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard material to separate samples of interference free water, for example HPLC grade, immediately before extraction (note i). Process these solutions under conditions identical with those to be used for the samples under analysis.	(i) Use up to 1 mL of an appropriate working standard solution in methanol.
C8.6	AQC	
C8.6.1	Carry out the entire procedure using distilled water (or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with dinoseb. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

C9 Calculation

Concentration of dinoseb is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}.$$

where

C = concentration of dinoseb in original sample (μgL^{-1}),

c = concentration determined in the extract from the calibration graph (μgmL^{-1}),

v = volume of the extract for the determination (mL), normally 0.5 mL, and

V = volume of sample extracted (L), normally 1 litre.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

The effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

C10 Reference

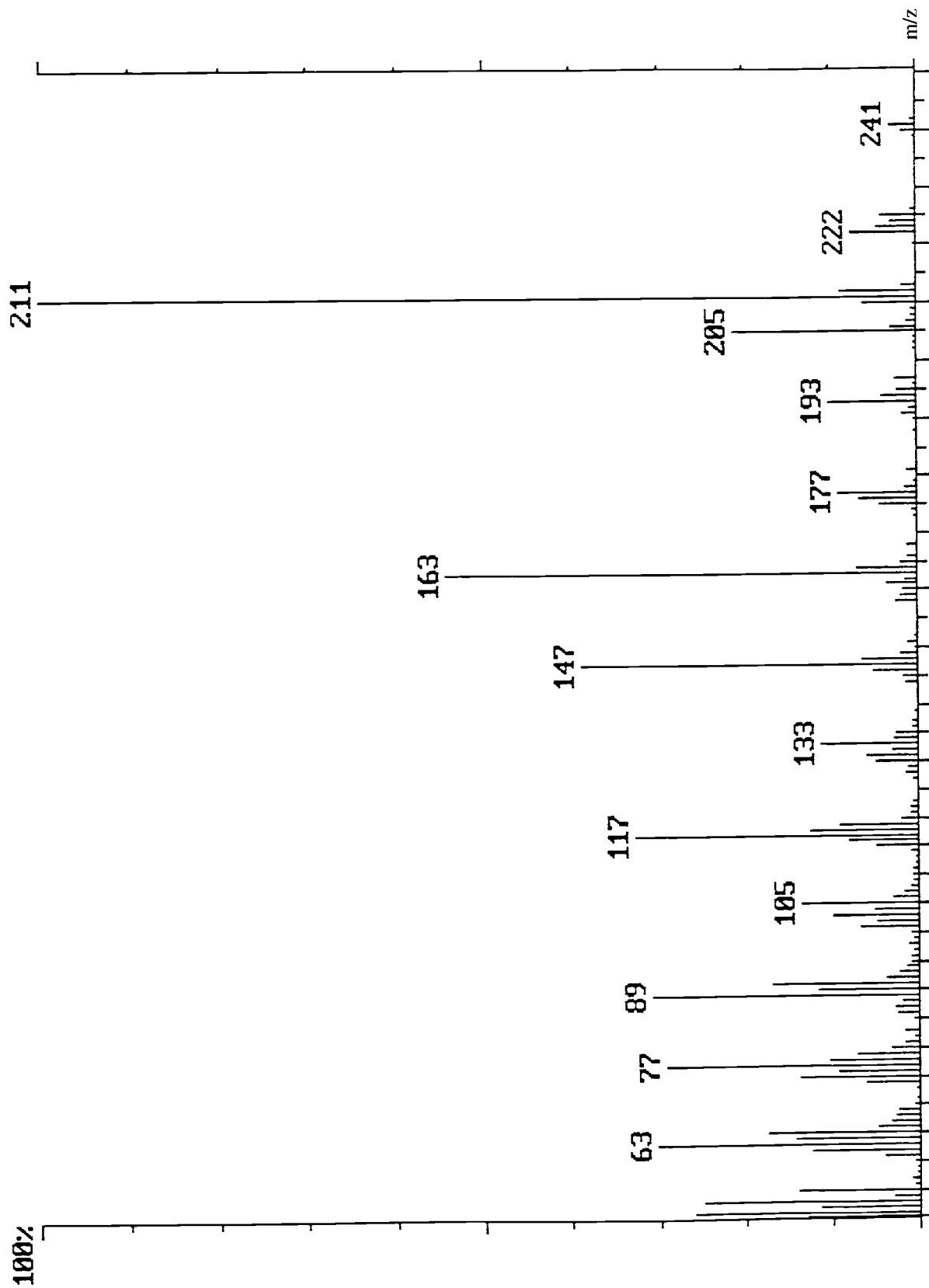
Method B in The Determination of Carbamates, Thiocarbamates, related compounds and Ureas in Water 1987 (ISBN 0117521515) in this series.

Table C1 Means and Recoveries

	Mean	Recovery %
Tap water blank	0.013	
Spike (0.22)	0.19	86.4
Spike (0.66)	0.61	92.4
Spike (1.1)	1.1	100.0
Spike (5.48)	4.67	85.2
River water blank	0.002	
Spike (0.66)	0.58	87.9

All values expressed as μgL^{-1} unless otherwise stated.
Data provided by Severn Trent Laboratories.

Figure C1 EI mass spectrum of dinoseb



Mass spectrum provided by Severn Trent Laboratories

Figure C1 EI mass spectrum of dinoseb

D Carbendazim and Benomyl (as carbendazim) in waters by HPLC

Benomyl converts to carbendazim during the analysis. No distinction is made between the two compounds using this method; the test data is the same for both substances.

D1 Performance characteristics of the method

D1.1	Substances determined	Carbendazim and benomyl, both expressed as carbendazim.
D1.2	Types of sample	Drinking and river waters.
D1.3	Basis of method	The determinands (as carbendazim) are extracted from a litre sample with dichloromethane (DCM) under basic conditions. The DCM extract is dried and evaporated to dryness. The residue is re-dissolved in methanol and carbendazim is determined by high performance liquid chromatography using ultra violet detection (HPLC/UV).
D1.4	Range of application	Up to 5 μgL^{-1} of each determinand. The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
D1.5	Calibration curve	The method is linear over the range of application.
D1.6	Standard deviation	0.003 μgL^{-1} for carbendazim.
D1.7	Limit of detection	0.014 μgL^{-1} (4.65 $\times$ standard deviation) for a 1 litre blank sample.
D1.8	Sensitivity	Dependent on the instrument used. With a baseline fluctuation of 30 units, 2 ng of carbendazim gave a response of 300 units.
D1.9	Bias	Extraction efficiencies are normally less than 100%. See Table D1.
D1.10	Interferences	Any co-extracted material which has a similar HPLC retention time to carbendazim and which gives a detector response at a wavelength of 288 nm will interfere.
D1.11	Time required for analysis	About 5 samples per day.

D2 Principle

A 1 litre sample is adjusted to a pH value of 2 with hydrochloric acid solution, and allowed to stand overnight. This converts any benomyl present in the sample to carbendazim. The pH is then raised to pH 9.5 with a solution of sodium hydrogen carbonate. The alkaline sample is extracted with dichloromethane and allowed to separate. The dichloromethane layer is dried with anhydrous sodium sulphate and transferred into a Kuderna-Danish evaporator fitted with a 10 mL graduated test tube. The sample is re-extracted with dichloromethane and treated as before. The combined dichloromethane extracts are evaporated to low volume and finally evaporated to dryness using a stream of nitrogen. The residue is dissolved in methanol and analysed by isocratic reverse phase HPLC using a UV detector operating at a wavelength of 288 nm.

D3 Interferences

Benomyl is not distinguished from carbendazim using this procedure due to its conversion to carbendazim.

D4 Hazards

Benomyl and carbendazim are toxic. Caution must be exercised when preparing the stock solution. Skin contact, ingestion and inhalation must be avoided. Methanol is flammable. Dichloromethane and methanol are toxic by skin absorption and inhalation. Ensure adequate ventilation and work in a flame and spark free area. Spark proof refrigerators should be used to store standard solutions and extracts. Hydrochloric acid and concentrated ammonia solutions are corrosive. Appropriate safety procedures should be followed.

D5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark, if necessary.

D5.1 Water. HPLC grade, filtered before use.

D5.2 Dichloromethane (DCM). HPLC grade.

D5.3 Acetone. Pesticide grade

D5.4 Methanol. HPLC grade

D5.5 Sodium sulphate granular, anhydrous. Heat to 500 ± 20 °C for 4 hr ± 30 min. Cool to about 200 °C in a muffle and then to ambient temperature in a desiccator. Store in a closed glass container.

D5.6 Hydrochloric acid (10% v/v).

D5.7 Sodium hydrogen carbonate solution (5% m/v). Weigh out 50 ± 0.5 g of sodium hydrogen carbonate, dissolve in about 800 mL of cold water, and make up to 1 L.

D5.8 HPLC mobile phase consisting of 60:40:0.6 v/v methanol: water: ammonia solution (d_{20} 0.880). Mix with stirring 600 ± 5 mL of methanol, 400 ± 5 mL of water and 6 ± 0.1 mL of ammonia solution (d_{20} 0.880).

D5.9 Carbendazim standards

D5.9.1 Stock solution: Prepare a stock solution of pure or suitably certified carbendazim in acetone at a concentration of 1000 mgL^{-1}. For example, in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of solvent.

D5.9.2 Working standard solutions: Prepare a series of working standard solutions of carbendazim in methanol by dilution of the stock solution (D5.9.1). A useful working range is from 0.05 to 5 mgL^{-1}.

D6 Apparatus

Apparatus should be clean and free from contamination before use.

D6.1 Glass sample bottles of 1.2 L capacity, marked at 1.0 L, fitted with glass stoppers or PTFE-lined screw caps.

D6.2 Kuderna-Danish evaporators fitted with 10 mL graduated tubes. Equivalent evaporators may be used.

D6.3 Nitrogen. Oxygen-free, filtered and dry.

D6.4 Separating funnels, 2 L capacity, with a grease-free glass or PTFE tap, and stopper.

D6.5 pH meter. Alternatively pH paper may be used.

D6.6 High performance liquid chromatograph with a UV detector capable of working at 288 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic reverse phase.
Columns	: Precolumn—Co-pell C_{18}, 70 × 2 mm.
	: Analytical—Spherisorb C_{18} (ODS2) 250 × 4.6 mm, 5 μm particle size
Column temperature	: Ambient.
Mobile phase	: See D5.8.
Flow rate	: 1.0 mLmin^{-1}.
Injection volume	: 20 μL.
UV wavelength	: 288 nm.

Under these conditions retention time of carbendazim is approximately 5 minutes. Other suitable equipment may be used provided the performance obtained is shown to be similar to or better than that reported here.

D7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should then be stored in a refrigerator at about 4°C. The sample bottles should be protected from contamination and should not be placed in the close proximity of standard materials or their concentrated solutions.

D8 Analytical procedure

Step	Procedure	Notes
D8.1	Extraction	
D8.1.1	To 1000 ± 10 mL of sample in the sample bottle, carefully add hydrochloric acid solution (D5.6) to adjust the pH to 2 and allow to stand overnight (note a). With the sodium hydrogen carbonate solution (D5.7) carefully adjust the pH to 9.5. Add 100 ± 5 mL of DCM (D5.2) to the resulting solution, stopper and shake on a shaking machine for 5 ± 0.5 min (see note b).	(a) pH paper may be used. This stage converts any benomyl present to carbendazim. (b) A machine for shaking bottles in a horizontal plane may be used at 1–2 cycles per second.
D8.1.2	Transfer the contents of the bottle to a 2 L separating funnel and allow the phases to separate. Run the lower DCM layer into a flask containing approximately 10 g sodium sulphate (D5.5).	
D8.1.3	Rinse the bottle with a further 50 ± 1 mL of DCM. Transfer the washings to the separator, stopper and shake for 120 ± 10 sec. Allow the phases to separate and run the lower DCM layer into the flask containing the sodium sulphate.	
D8.1.4	Swirl the flask and leave to stand for at least 10 min, swirling occasionally.	

Step	Procedure	Notes
D8.1.5	Transfer the extract to a Kuderna-Danish evaporator (note c). Wash the sodium sulphate with 10 ± 1 mL of DCM and decant the washings into the Kuderna.	(c) Alternative evaporation systems can be used.
D8.2	**Concentration**	
D8.2.1	Reduce the volume of the DCM extract in the evaporator to about 0.5 mL and gently evaporate the remaining solvent to incipient dryness with a stream of purified nitrogen (D6.3).	
D8.2.2	Dissolve the residue in 0.50 ± 0.01 mL methanol. The solution is now ready for HPLC analysis.	
D8.3	**HPLC/UV determination.**	
D8.3.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section D6.6.	
D8.3.2	Inject aliquots of standards and extracts of blanks, samples and recoveries into the HPLC.	
D8.3.3	Measure the height or area of the peaks corresponding to carbendazim.	
D8.3.4	Construct a calibration graph of peak height or area for the standards versus concentration of carbendazim (mgL^{-1}) injected.	
D8.3.5	Read the concentration of carbendazim in the extract from the calibration graph (note d) and calculate the concentration present in the original sample (see section D9).	(d) If the peak height or area for the sample exceeds the calibration range dilute the extract appropriately and re-analyse.
D8.4	**Confirmation**	
D8.4.1	Change the HPLC conditions (notes e and f). Re-analyse extracts following similar procedures to those described in sections D8.3.2 to D8.3.5.	(e) This can include mobile phase composition, column type or detector system or a combination of these. (f) For a normal phase method see reference D10.1.
D8.5	**Blanks and recoveries**	
D8.5.1	Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard carbendazim to separate samples of interference free water, for example HPLC grade, immediately before extraction (notes g and h). Process these solutions under conditions identical with those to be used for the samples under analysis.	(g) Use up to 1 mL of an appropriate working standard solution in methanol. (h) Benomyl degrades rapidly in organic solvents, particularly methanol. Carbendazim standards are used for quantification, (reference D10.2).

Step	Procedure	Notes
D8.6	AQC	
D8.6.1	Carry out the entire procedure using distilled water (or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with carbendazim. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

D9 Calculation

Concentration carbendazim and benomyl (expressed as carbendazim), is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}$$

where

C = concentration of carbendazim and benomyl (expressed as carbendazim) in the original sample (μgL^{-1}),

c = concentration determined in the extract from the calibration graph (μgmL^{-1}),

v = volume of the extract for the determination (mL), normally 0.5 mL, and

V = volume of sample extracted (L), normally 1 litre.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

The effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

D10 References

D10.1 Method B in The Determination of Carbamates, Thiocarbamates, related compounds and Ureas in Water 1987 (ISBN 0117521515) in this series.

D10.2 Chiba M, Cherniak E A, *J. Agric. Food Chem.*, Vol 26, No 3, 1978, p 573–576.

Table D1 Means and Recoveries

	Mean	Recovery %
Distilled water blank	0.012	
Spike (0.25)	0.17	68.0
Spike (0.50)	0.40	80.0
Spike (1.01)	0.71	70.3
Spike (5.04)	3.50	69.4
River water blank	0.035	
Spike (0.50)	0.28	56.0

The samples were spiked with carbendazim.
All values expressed as carbendazim in μgL^{-1} unless otherwise stated.
Data provided by SAC Scientific Ltd.
This performance data was obtained using alkaline conditions (pH 11) for the conversion of benomyl to carbendazim. Subsequent work showed that conversion is more effective under acid conditions (pH 2) and these conditions are now described in the method.

E Metamitron in waters by HPLC

E1 Performance characteristics of the method

E1.1	Substance determined	Metamitron.
E1.2	Types of sample	Drinking and river waters.
E1.3	Basis of method	The determinand is extracted from a 2 litre sample with dichloromethane (DCM) at pH approximately 8. Sodium chloride is added in order to improve the recovery. The extract is dried and evaporated to dryness. The residue is re-dissolved in aqueous methanol and metamitron is determined by high performance liquid chromatography with ultra-violet detection (HPLC/UV).
E1.4	Range of application	Up to 5 μgL^{-1}. The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
E1.5	Calibration curve	The range of linearity depends upon the system in use. For the instrument used in the performance tests the calibration curve was linear up to 5 μgL^{-1}.
E1.6	Standard deviation	See Table E1.
E1.7	Limit of detection	0.004 μgL^{-1} for a 2 litre sample volume.
E1.8	Sensitivity	Dependent on the instrument used. With a baseline fluctuation of 30 units, 2 ng of metamitron gave a response of 910 units.
E1.9	Bias	Extraction efficiencies are less than 100%. See Table E1.
E1.10	Interferences	Any co-extracted material which has a similar retention time to metamitron and which gives a detector response at a wavelength of 313 nm will interfere.
E1.11	Time required for analysis	A batch of 12 samples may be analysed in 3 days.

E2 Principle

A sample (2 L) is adjusted to approximately pH 8. Sodium chloride is added to the sample which is then extracted twice with dichloromethane (DCM). The extracts are combined, dried with anhydrous sodium sulphate and evaporated to dryness. The residue is re-dissolved in aqueous methanol and analysed by isocratic reverse phase HPLC using a UV detector operating at a wavelength of 313 nm.

E3 Interferences

There may be interferences present in extracts from river water. Plasticisers (phthalate and adipate esters) can cause interference problems.

E4 Hazards

Metamitron is toxic. Caution must be exercised when preparing the stock solutions. Skin contact, ingestion and inhalation must be avoided. Methanol is flammable. Dichloromethane and methanol are toxic by skin absorption and inhalation. Ensure adequate ventilation and work in a flame and spark free area. Spark proof refrigerators should be

used to store standard solutions and extracts. Concentrated ammonia solution is corrosive. Appropriate safety procedures should be followed.

E5 Reagents

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

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark if necessary.

E5.1 Water. HPLC grade, filtered before use.

E5.2 Dichloromethane (DCM). HPLC grade.

E5.3 Ammonia solution. d_{20} 0.880.

E5.4 Methanol. Pesticide grade.

E5.5 Mobile phase for HPLC. 50:50 v/v methanol:water.

E5.6 Sodium sulphate, granular, anhydrous. Heat to 500 ± 20 °C for 4 hr ± 30 min. Cool to about 200 °C in a muffle and then to ambient temperature in a desiccator. Store in a closed glass container.

E5.7 Sodium Chloride. Analytical reagent grade.

E5.8 Standards

E5.8.1 Stock solution: Prepare a stock solution of pure or suitably certified metamitron in methanol at a concentration of 1000 mgL^{-1}. For example, in a volumetric flask dissolve 100.0 ± 0.1 mg of material in 100.0 ± 0.1 mL of solvent.

E5.8.2 Working standard solutions: Dilute an aliquot of stock solution (E5.8.1) with water to produce a 50/50 v/v aqueous methanolic solution and use this to prepare a series of working standard solutions of metamitron in aqueous methanol (E5.5). A useful working range is from 0.05 to 1 mgL^{-1}.

E6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then dichloromethane and allowed to drain.

E6.1 Glass sample bottles of 2.4 L nominal capacity, marked at 2.0 L, fitted with glass stoppers or PTFE-lined screw caps.

E6.2 Kuderna-Danish evaporators fitted with 10 mL graduated tubes. Equivalent evaporators may be used.

E6.3 Nitrogen. Oxygen-free, filtered and dry.

E6.4 Separating funnels, 2 L capacity, with a grease-free glass or PTFE tap, and stopper.

E6.5 High performance liquid chromatograph with a UV detector capable of working at 313 nm, fitted with a C_{18} column. Operating conditions used to obtain the test data were as follows:

Mode	: Isocratic reverse phase.
Column	: Analytical—Spherisorb C_{18} (ODS2) 250 × 4.6 mm, 5 μm particle size

Column temperature : Ambient.

Mobile phase : See E5.5.

Flow rate : 1.0 $mLmin^{-1}$.

Injection volume : 20 μL.

UV wavelength : 313 nm.

Under these conditions the retention time of metamitron should be approximately 6 minutes. Other suitable equipment may be used provided the performance obtained is shown to be similar to or better than that reported here.

E7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted and analysed as soon as possible after sampling. If this is impractical, they should then be stored in a refrigerator at about 4 °C. The sample bottles should be protected from contamination and should not be placed in the close proximity of standard materials or their concentrated solutions. Metamitron degrades rapidly above pH 10.

E8 Analytical procedure

Step	Procedure	Notes
E8.1	**Extraction**	
E8.1.1	To 2000 ± 20 mL of sample in the sample bottle, add 0.880 ammonia solution (E5.3) to give a pH of 8.0 ± 0.5 (see note a). Add 100 ± 5 mL of DCM (E5.2) to the alkaline sample and 40 ± 1 g sodium chloride (E5.7), stopper and shake on a shaking machine for 5 ± 0.5 min (see note b).	(a) pH paper may be used. (b) A machine for shaking bottles in a horizontal plane may be used at 1–2 cycles per second.
E8.1.2	Transfer the contents of the bottle to a 2 L separating funnel and allow the phases to separate. Run the lower DCM layer into a flask containing approximately 10 g sodium sulphate (E5.6).	
E8.1.3	Rinse the bottle with a further 30 ± 1 mL of DCM. Transfer the washings to the separator, stopper and shake for 120 ± 10 sec. Allow the phases to separate and run the lower DCM layer into the flask containing the sodium sulphate.	
E8.1.4	Swirl the flask and leave to stand for at least 10 min, swirling occasionally.	
E8.1.5	Transfer the extract to a Kuderna-Danish evaporator (note c). Wash the sodium sulphate with 10 ± 1 mL of DCM and decant the washings into the Kuderna.	(c) Alternative evaporation systems can be used.
E8.2	**Concentration**	
E8.2.1	Reduce the volume of the DCM extract in the evaporator to about 2–5 mL and gently evaporate the remaining solvent to incipient dryness with a stream of purified nitrogen (E6.3).	

Step	Procedure	Notes
E8.2.2	Dissolve the residue in 1.0 ± 0.01 mL 50:50 v/v aqueous methanol. The solution is now ready for HPLC analysis.	
E8.3	**HPLC/UV determination.**	
E8.3.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section E6.5.	
E8.3.2	Inject aliquots of standards and extracts of blanks, samples and recoveries into the HPLC.	
E8.3.3	Measure the height or area of the peak corresponding to metamitron.	
E8.3.4	Construct a calibration graph of peak height or area for the standards versus concentration of metamitron (mgL^{-1}) injected.	
E8.3.5	Read the concentration of metamitron in the extract from the calibration graph (note d) and calculate the concentration present in the original sample (see section E9).	(d) If the peak height or area for the sample exceeds the calibration range dilute the extract appropriately and re-analyse.
E8.4	**Confirmation**	
E8.4.1	Change the HPLC conditions (note e). Re-analyse extracts following similar procedures to those described in sections E8.3.2 to E8.3.5.	(e) This can include mobile phase composition, column type or detector system or a combination of these. Gradient elution to 100% methanol may be used. Electrochemical detection is also suitable.
E8.5	**Blanks and recoveries**	
E8.5.1	Adequate blank values should be obtained using interference free water before analysing samples. At least one reagent blank should be analysed with each batch of samples. Check the efficiency of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard material to separate samples of interference free water, for example HPLC grade, immediately before extraction (note f). Process these solutions under conditions identical with those to be used for the samples under analysis.	(f) Use up to 1 mL of an appropriate working standard solution in methanol.
E8.6	**AQC**	
E8.6.1	Carry out the entire procedure using distilled water (or water of a similar nature to the sample being analysed) spiked at approximately 0.1 μgL^{-1} with metamitron. If the responses of extracted standards are used for comparison with those of the samples, an automatic correction is obtained. If not, the data from previous tests should be averaged and a mean correction factor determined to be used for correcting for recovery.	

E9 Calculation

Concentration of metamitron is given by:

$$C = \frac{c \times v}{V} \mu gL^{-1}$$

where

C = concentration of metamitron in original sample (μgL^{-1}),

c = concentration determined in the extract from the calibration graph (μgmL^{-1}),

v = volume of the extract for the determination (mL), normally 1 mL, and

V = volume of sample extracted (L), normally 2 litres.

The calculations are more easily performed using a laboratory data system to integrate the relevant peaks and to calculate the results automatically.

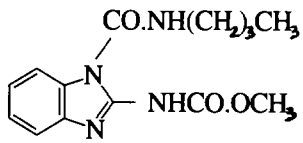
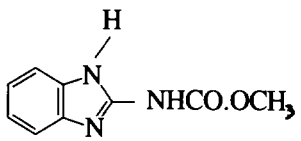
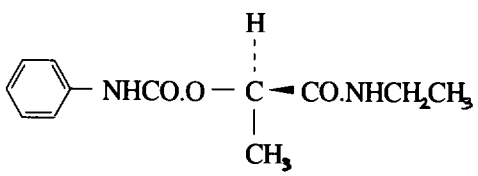
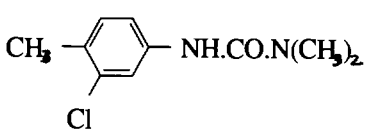
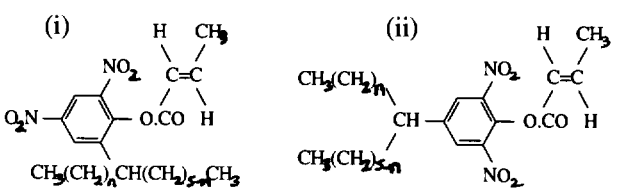
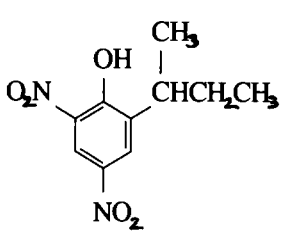
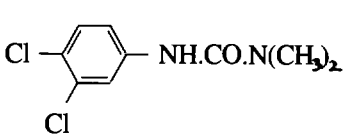
The effect of incomplete extraction and other systematic errors can be accounted for by running the standards through the complete procedure.

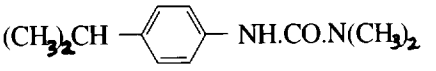
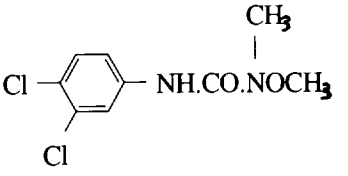
Table E1 Standard deviations and Recoveries

Sample	S_t	Recovery %
Distilled water (unspiked)	0.0001	
0.1 spike	0.003 (3)	67
1.0 spike	0.002 (4)	86
River water (unspiked)	0.004 (3)	
0.1 spike	0.003 (5)	48
1.0 spike	0.006 (5)	80

Figures in brackets represent degrees of freedom.
S_t = Estimate of total batch standard deviation.
Bessel's correction applied to all estimates of the standard deviations.
Blanks not subtracted.
All units expressed in μgL^{-1} unless otherwise stated.
Data provided by SAC Scientific Ltd.

Common names and chemical structures

Common Name	Structure
Benomyl	
Carbendazim	
Carbetamide	
Chlorotoluron	
Dinocap	
Dinoseb	
Diuron	

Common Name	Structure
Isoproturon	
Linuron	
Metamitron	

Analytical Quality Control

1 Routine control

Once a method has been selected for routine use, a system of analytical quality control should be adopted in order to validate the analysis. At least one control standard should be analysed with each batch of samples and the results plotted on a control chart. Corrective action should be taken if one value falls outside of the action limit (at ± 3s) or 2 consecutive values exceed the warning limit (at ± 2s). As more data are acquired, the standard deviation, s, should be updated and the control chart limits recalculated.

2 Estimation of the accuracy of analytical results using these methods

None of the methods given in this booklet have been thoroughly investigated and before general use, the accuracy achievable should be known. It would be of great value if any laboratory using or considering the use of any of these methods would estimate the accuracy of its own analytical results and report the findings to the Secretary of the Department of the Environment's Standing Committee of Analysts.

Address for correspondence

1. However well a method is tested, there is always the possibility of discovering a hitherto unknown problem. Users with information on these methods are requested to write to the address below.

2. At the present time, thorough test data are not available. Additional test data would be welcomed and results should be sent to the address below.

The Secretary
The Standing Committee of Analysts
The Department of the Environment
Drinking Water Inspectorate
43 Marsham Street
LONDON SW1P 3PY
England

Department of the Environment

Standing Committee of Analysts

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