The Determination of Carbamates, Thiocarbamates, Related Compounds and Ureas in Waters 1987

Methods for the Examination of Waters and Associated Materials

For the determination of urea itself ($CO[NH_2]_2$), see Urea in Waters 1985, published in this series.

Method B also determines at least one acrylate.

Method D will also determine thiuram disulphides.

London Her Majesty's Stationery Office

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First published 1988

ISBN 0 11 752151 5

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The Determination of Carbamates, Thiocarbamates and Ureas in Waters 1987 (An Acrylate, and some Thiuram Disulphides are also included)

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About This Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being issued when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has 9 Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic 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, under the overall supervision of the appropriate working group and the main committee.

The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a seperate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary

1 July 1987

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

Lone working, whether in the laboratory or field, should be discouraged.

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

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

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

Introduction

This booklet contains four methods for the analysis of carbamate and urea herbicides, pesticides and fungicides. An acrylate is also included.

These methods are:

(1) A normal-phase HPLC method, in part A,
(2) A reverse-phase HPLC method, in part B,
(3) A TLC method, in part C;
(4) A method for alkyldithiocarbamates by conversion to CS_2, in part D.

The best and most versatile methods are the HPLC procedures. Most compounds are measured with good detection limits and precision. Small amendments to the method enable it to be used for almost all the compounds in the group.

The TLC procedure is adequate as a confirmatory technique and perhaps for identification purposes; but as with all TLC methods the results obtained are imprecise.

The method for alkyldithiocarbamates involving conversion to carbon disulphide and estimation by gas chromatography with flame-photometric detection is essentially a screening technique for a number of dithiocarbamates which are not detected by the HPLC or TLC procedures. It is based on a method for crop residues and soil and the procedure can be adapted to cope with these samples as well as water. It is unlikely that it will be necessary to analyse mixtures of these few compounds for individual pesticides. The chief difference between them is the metal of which they were a salt (usually manganese, or manganese plus zinc). Analysis for the various metals by the appropriate methods in this series would suffice to indicate the probable ratio of the various pesticides in the sample; but the natural levels present may make this impossible. At present most pesticides in this class are ethylenedithiocarbamates. If other dithiocarbamates need to be determined, as hydrolysis to carbon disulphide is a general reaction, they are determinable by this method; but some additional analysis would be needed to differentiate by amine residue if specific identification were necessary. Xanthates, if present, would give carbon oxysulphide, a poisonous gas soluble in water and octane (see D4). The method also determines thiuram disulphides reducible to dithiocarbamates.

A Estimation of Carbamate and Related Herbicides in River and Drinking Waters by Normal Phase High Performance Liquid Chromatography

(For modifications to improve the sensitivity for specific compound determination see Section A10).

A1 Performance Characteristics of the Methods

A1.1	Substances determined	Most carbamate herbicides. The compounds for which test data are given are: Pebulate, EPTC, Triallate, Propham, Chloropham, Chlorbufam, Methiocarb and Carbaryl. (The use of the reversed phase method (B) may also determine soluble dithiocarbamates and xanthates if concentration is by cartridge or precolumn and acidification is avoided).
A1.2	Types of sample	River and drinking waters.
A1.3	Basis of methods	
A1.3.1	Normal Phase HPLC	Extraction of the sample with dichloromethane, concentration, and evaporation, and evaporation to dryness. The residue is redissolved in a mixture of trimethylpentane/isopropanol and analysed by normal phase HPLC with UV detection at 220 nm.
A1.3.2	Reverse Phase HPLC (See Method B).	Samples are preconcentrated either on a cartridge or precolumn, or by solvent extraction. Dissolved in menthanol-water or acetonitrile-water, injected onto a reverse phase HPLC column and eluted. Detection is as for normal phase HPLC.
A1.4	Range of application	Typically up to 100 μg/l.
A1.5	Calibration curve	Linear up to 100 μg/l for all the compounds tested.
A1.6	Standard deviation	See Tables 1 to 7.
A1.7	Limit of detection	See Tables 1 to 7.
A1.8	Sensitivity	For the instruments used in the performance tests, set with a baseline fluctuation of 0.5% full scale deflection (FSD), the weights of herbicide required to give a peak height of

	50% FSD were:—	Pebulate	20 ng
		EPTC	40 ng
		Triallate	40 ng
		Propham	12 ng
		Chlorpropham	20 ng
		Chlorbufam	24 ng
		Methiocarb	45 ng
		Carbaryl	20 ng

A1.9	Bias	Extraction efficiences are less than 100% (See Section A9.2).
A1.10	Interferences	Any co-extracted material which has similar retention time to any of the determinands and which responds at the selected wavelength will interfere.
A1.11	Time required for analysis	Assuming the apparatus and equipment is prepared, eight samples may be analysed in two days.

A2 Principle

Normal Phase HPLC. The carbamates are extracted from an acidified sample into dichloromethane (DCM). The extract is concentrated to less than 5 ml using a Kuderna-Danish evaporator and then blown down to incipient dryness with a purified nitrogen line. 500 μl of trimethylpentane containing 2% propan-2-ol is added to redissolve the residue and the solution injected into an HPLC machine fitted with a silica column and a UV detector operated at 220 nm. Peak heights for the samples are compared to those of standards made up in the HPLC mobile phase. For Reverse Phase see Method B.

A3 Interferences

Most interferences are eliminated in the sample preparation or by the selective wavelength used. In the performance tests however, some peaks appeared in the river water samples which did not interfere with the compounds tested, but which might give rise to interference if the method were extended to cover a wider range of carbamate or urea herbicides. The reverse phase method, by almost completely reversing the order of elution, offers improved accuracy for those compounds eluting late with normal phase and allows the determination of some which barely elute at all with normal phase sampling and preconcentration without acidification may allow soluble dithiocarbamates and xanthates to be determined.

Under some conditions phenoxyacidic herbicides such as 24D and 245T may cause interference. These however absorb at 270–280 nm. Hence indentification and correction may be made. Analysts are advised to check separations and indentify peaks with known or spiked samples on their own columns.

A4 Hazards

Carbamate herbicides are toxic, Acetonitrile is toxic and flammable, avoid inhalation, ingestion and skin contact, clean up spillage. 2.2.4-trimethylpentane and propan-2-ol are flammable. Dichloromethane is narcotic. Ensure adequate ventilation and work in a flame and spark free area.

A5 Reagents

The reagents vary with procedure chosen.

A5.0 Water for use in the preparation of reagents, blanks, controls and standards should be distilled from water made slightly acid with sulphuric acid, the first and last 10% being discarded.

A5.1 Dilute sulphuric acid, approx 5M. Cautiously, with cooling and stirring, pour 265 ± 5 ml of sulphuric acid (d_{20}1.84) into about 500 ml of water and make up to 1 litre ±10 ml of water.

A5.2 Sodium sulphate, anhydrous, roasted at 500°C.

A5.3 Dichloromethane, HPLC grade.

A5.4 2,2,4-trimethylpentane (iso-octane) HPLC grade.

A5.5 Propan-2-ol HPLC grade.

A5.6 Standard Solutions.

A5.6 Stock solution of herbicides: Prepare a stock solution of each herbicide by disolving 25 mg of pure or certified material in 25 ml of the solvents used as the mobile phase (98% 2,2,4-trimethylpentane plus 2% propan-2-ol by volume). NOTE: Some carbamates will not dissolve at this concentration. A lower concentration may be prepared or an alternative solvent used.

A5.6 Working standards: Prepare working standard solutions in the same solvent mixture from the stock solution using microlite syringes.

Some useful standards are:—

0.05 mg/l	0.5 µl stock in 10ml solvent
0.1 mg/l	1.0 µl stock in 10ml solvent
0.5 mg/l	5.0 µl stock in 10ml solvent
1.0 mg/l	10.0 µl stock in 10ml solvent
5.0 mg/l	50.0 µl stock in 10ml solvent
10.0 mg/l	100.0 µl stock in 10ml solvent
25.0 mg/l	250.0 µl stock in 10ml solvent

A5.6.3 When the retention times for each herbicide has been determined multiple standards may be prepared.

A5.7 Blank Water. See Step C8.8 note n for a suggested purification.

A5.8 Acetonitrile, HPLC grade.

A6. Apparatus

Glassware should be clean and dry. Rinsing with acetone before use and then drying in a stream of clean air assists with this process.

A6.1 Sample bottles. Of all glass construction capable of holding 1.2 litres and calibrated at 1 litre

A6.2 Separating funnel 1 litre.

A6.3 Kuderna-Danish evaporator 250 ml.

A6.4 Centrifuge tubes, 10 ml graduated, tapered glass, glass-stoppered.

A6.5 Microlitre syringes: 500 µl, 100 µl, 10 µl, 1 µl and an HPLC syringe.

A6.6 HPLC machine fitted for normal phase operation with a silica column (Lichrosphere-Si 60II, 25 cm, or equivalent) and a UV detector capable of operation at 220 nm. Wavelengths of 206–300 nm are required for some carbamate herbicides. For reverse phase operation a C18 or similar column should be used, see Method B.

A6.6.1 HPLC mobile phase compostition for Normal Phase Operation.

Initial composition:	trimethylpentane	98%
	propan-2-ol	2%
After emergence of chlorbufam:	trimethylpentane	97%
(approx 15 min)	propan-2-ol	3%

Flow rate 1.3 ml/min

Other solvent ratios may be preferred for specific determinations, see Section 10.

A6.2 Sample loop size 20 µl. (See also A8.3.2 note g).

A6.8 A cartridge or precolumn (as an alternative method of sample concentration.

A7 Sample storage

Samples should be extracted as soon as possible after sampling. Sample extracts are stable for at least a week. Sample bottles should be protected from contamination by sealing each bottle in a polythene bag.

A8 Analytical Procedure for Normal Phase Operation

Step	Procedure		Notes
A8.1	Extraction		
A8.1.1	To a 1 litre (±10 ml) sample in the sample bottle add dilute sulphuric acid (A5.1) until the pH is 3 (note a).	(a)	Indicator paper is suitable although a pH meter may be used. Acidified samples of dithiocarbamates and xanthates are readily decomposed and oxidized (see Section D11). They must be kept cool with minimal exposure to air and processed as quickly as possible.
A8.1.2	Add 40.0±0.1 g anhydrous sodium sulphate (A.5.2) and shake the bottle until all the sodium sulphate has dissolved.		
A8.1.3	Add 50 ± 5 ml DCM (A5.3) and shake for 2 minutes. (note b).	(b)	A shaking machine may by used, but the extraction time must be increased to 10 minutes.
A8.1.4	Transfer the entire contents of the bottle into a 1 litre separating funnel (A6.2). Rinse the sample bottle with a further 10 ± 1 ml DCM and add the washings to the separator. Allow the layers to separate.		
A8.1.5	Run off the lower DCM layer into a Kuderna-Danish evaporator fitted with a graduated centrifuge tube (A6.4) (note c)	(c)	If an interfacial cuff is present, a sodium sulphate drying column may be used, or the extract swirled with sodium sulphate to remove residual water.
A8.1.6	Re-extract the sample in the separating funnel with a further 25±1 ml DCM by shaking for 2 min. (Note b). Allow the layers to separate and run off the lower DCM layer into the same Kuderna-Danish evaporator used in Step A8.1.5. Add one anti-bumping chip to the evaporator. (See also C8.1.8 note c)		
A8.2	Concentration		
A8.2.1	Evaporate the solvent to about 2–3 ml. After allowing the evaporator to drain, remove the Kuderna and blow down the extract to incipient dryness with a gentle stream of purified nitrogen (note d).	(d)	DCM boils rapidly. A gap of about 5 cm should be left between the steam collar and the evaporator to avoid violent boiling.
A8.2.2	Using a syringe (A6.5) add 500 µl of the mobile phase (note e) used for the HPLC analysis to the residue in the centrifuge tube, swirl to dissolve. Stopper the tube ready for HPLC analysis.	(e)	The usual composition is 98% trimethylpentane and 2% propan-2-ol but see also Section A10.
A8.3	HPLC Analysis (normal phase operation)		
A8.3.1	Set up the machine in accordance with the manufacturer's instructions using the conditions given in A6.5.1, using the mobile phase given in A6.6.1. For many carbamates a wavelength of 220 nm is suitable (see note f).	(f)	For alternative wavelengths see Section A10. (see also Section A3)

Step	Procedure		Notes
A8.3.2	Inject aliquots of the standards and samples into the HPLC using loop injection (20 μl) (note g)	(g)	The loop size may be varied depending on the concentration of the sample and the resolution required.
A8.3.3	Construct a calibration graph of peak heights for the standards versus weight (ng) of herbicide injected.		
A8.3.4	Read off the weight of herbicide in the samples from the calibration graph and calculate the concentration present in the original sample.		
A8.4	Blanks		
	Adequate blank values should be obtained prior to analysing samples.		

A9 Calculation

If a 1 litre sample is extracted and concentrate to 500 μl before injection then:—

$$C = \frac{W}{2V}$$

Where C = Concentration in the original sample (μl/1)

W = Weight (ng) of herbicide in the injected sample of extract read off from the calibration graph

V = Volume (μl) of extract injected

If a 20 μl sample loop is used then:

$$C = \frac{W}{40}$$

A9.1 For sample volumes other than 1 litre proportionate by dividing by the sample volume in litres.

A9.2 Note that for more accurate values, correction also needs to be made for extraction efficiency.

If the compound exists in the same molecular state in both the solvent and water, the extraction efficiency for a real sample can be computed by also analysing a spiked sample.

If C is the concentration of the sample and the extraction efficiency is E% then the value C_2 found will be $\frac{C_1E}{100}$

Then for a spiked sample of concentration $C_1 + S_1$ where S_1 is the increase in concentration, the increase S_2 in the found value of the spike will be

$$S_2 = \frac{E}{100}(C_1 + S_1) - C_2$$

Hence the extraction efficiency can be found.

If the compound is associated in either the solvent or water the calculation becomes more complicated (see ref 1). This can be ascertained by analysing a second spiked sample with a different value spike. Ideally, the values for the extraction efficiency obtained should be approximately the same.

Tables

Means and Estimates of Standard Deviation
(Degrees of Freedom are in brackets)

Table 1

Unspiked distilled water

Compound	Mean found (μg/l)	Sw (9) (μg/l)	L of D (μg/l)
Pebulate	0.056	0.033	0.15
EPTC	0.018	0.012	0.06
Triallate	0.024	0.015	0.07
Propham	0.029	0.030	0.14
Chlorpropham	0.047	0.063	0.29
Chlorbufam	0.016	0.005	0.02
Methiocarb	0.022	0.028	0.13
Carbaryl	0.014	0.005	0.02

Table 2

Unspiked river water

Compound	Mean found (μg/l)	Sw (9) (μg/l)	L of D (μg/l)
Pebulate	0.030	0.028	0.13
EPTC	0.020	0.014	0.07
Triallate	0.020	0.012	0.06
Propham	0.034	0.028	0.13
Chlorpropham	0.017	0.008	0.04
Chlorbufam	0.025	0.018	0.08
Methiocarb	0.025	0.027	0.13
Carbaryl	0.018	0.006	0.03

Table 3

Low spiked distilled water 1 μg/1

Compound	Mean found (μg/1)	Sw (μg/l)	Sb (μg/l)	St (μg/l)
Pebulate	0.977	0.105 (5)	n.s.	0.182 (6)
EPTC	0.722	0.133 (5)	n.s.	0.164 (7)
Triallate	0.780	0.072 (5)	n.s.	0.075 (9)
Propham	0.802	0.098 (5)	0.193 (4)	0.217 (5)
Chlorpropham	0.961	0.101 (5)	n.s.	0.146 (6)
Chlorbufam	0.910	0.155 (5)	n.s.	0.179 (8)
Methiocarb	0.922	0.157 (5)	n.s.	0.210 (7)
Carbaryl	0.974	0.087 (5)	n.s.	0.121 (7)

Table 4

High spiked distilled water (8μg/l)

Compound	Mean found (μg/l)	Sw (μg/l)	Sb (μg/l)	St (μg/l)
Pebulate	6.97	1.072 (5)	0 (4)	1.072 (9)
EPTC	6.26	0.659 (5)	n.s.	1.007 (6)
Triallate	5.6	0.400 (5)	0.902 (4)	0.986 (5)
Propham	6.31	0.891 (5)	n.s.	1.230 (7)
Chlorpropham	6.89	1.154 (5)	0 (4)	1.154 (9)
Chlorbufam	7.04	1.157 (5)	n.s.	1.177 (9)
Methiocarb	7.06	1.100 (5)	0 (4)	1.110 (8)
Carbaryl	7.24	1.077 (5)	0 (4)	1.077 (9)

Table 5

Low spiked river water (1 μg/l)

Compound	Mean found (μg/l)	Sw (μg/l)	Sb (μg/l)	St (μg/l)
Pebulate	0.832	0.126 (5)	n.s.	0.197 (6)
EPTC	0.910	0.243 (5)	n.s.	0.173 (8)
Triallate	0.862	0.086 (5)	0 (4)	0.086 (9)
Propham	0.844	0.153 (5)	n.s.	0.210 (7)
Chlorpropham	0.825	0.144 (5)	0 (4)	0.144 (9)
Chlorbufam	0.673	0.103 (5)	n.s.	0.123 (8)
Methiocarb	0.707	0.129 (5)	n.s.	0.162 (7)
Carbaryl	0.743	0.141 (5)	n.s.	0.163 (8)

Table 6

High spiked river water (8 μg/l)

Compound	Mean found (μg/l)	Sw (μg/l)	Sb (μg/l)	St (μg/l)
Pebulate	5.80	0.200 (5)	1.517 (4)	1.530 (4)
EPTC	5.91	0.809 (5)	n.s.	1.172 (6)
Triallate	5.50	0.736 (5)	1.117 (4)	1.338 (5)
Propham	5.74	0.755 (5)	n.s.	1.048 (7)
Chlorpropham	5.97	1.027 (5)	n.s.	1.199 (8)
Chlorbufam	5.95	1.029 (5)	n.s.	1.522 (6)
Methiocarb	5.92	0.467 (5)	1.759 (4)	1.820 (4)
Carbaryl	6.22	0.918 (5)	1.351 (4)	1.634 (5)

Table 7

Mean recoveries % (blank subtracted)

Compound	Distilled low spike	Distilled high spike	River low spike	River high spike
Pebulate	92.1	87.0	80.2	72.1
EPTC	75.4	78.2	89.0	72.6
Triallate	76.6	70.8	84.2	68.5
Propham	77.3	78.9	81.0	71.4
Chlorpropham	91.4	86.0	82.5	74.5
Chlorbufam	89.4	88.0	64.8	74.1
Methiocarb	90.0	88.2	68.2	73.7
Carbaryl	96.0	90.5	72.4	77.5

A10 Adaptation to Determine Specific Compounds

With minimal modification the method can be adapted to determine a wide range of carbamates and related compounds. The compromise wavelength selected for measurement of the compounds tested is satisfactory for many carbamates, but use of the optimum wavelengths for individual compounds will give better responses and should be used where appropriate. The method will determine aldicarb, but the retention time is long and the response poor. Reversed phase HPLC (Method B) or the use of a more polar mobile phase in normal phase improves the response and shortens the analysis time. In general, the less polar the compound the higher the ratio of 2.2.4 trimethylpentane to propan-2-ol, and conversely the more polar the lower the optimum elutant composition ratio is. Examples of specific wavelengths are given in Table 8.

Table 8 Alternative Detection Wavelengths

Compound	Suggested Wavelength nm
Propoxur	217
Aldicarb Methiocarb	213
Bendiocarb Carbaryl	221
Pebulate Propham Azulam Triallate	214
Diuron	215
Propham	233
Chlorpropham	235

For further information on optimum absorption wavelengths and alternative columns and eluents, see Ref. 3.

B Estimation of Carbamate and Urea Herbicides in River and Drinking Water by Reverse Phase High Performance Liquid Chromatography (An Acrylate is also included).

B1 Performance Characteristics of the Method

B1.1	Substances determined	Most carbamates and urea herbicides, also Dinocap.
B1.2	Types of sample	River and drinking waters.
B1.3	Basis of method	The sample is concentrated by extraction or adsorption, evaporated to incipient dryness and dissolved in the mobile phase and analysed, using polar solvents, by HPLC with UV or electrochemical detection. Cartridge or precolumn concentration may be used for clean samples (if such samples are not acidified soluble dithiocarbamates and xanthates may be determinable).
B1.4	Range of application	Depends upon the response of the individual determinands under the conditions used. Typically the range is 0–20 μg/l.
B1.5	Calibration curve	Linear up to at least 50 μg/l for all the determinands tested.
B1.6	Standard deviation	See table 9.
B1.7	Limit of detection	See table 9.
B1.8	Bias	Efficiency of the extraction is normally less than 100%. See table 9.
B1.9	Interferences	Any substance eluting with the same elution time as any of the determinands and giving a detector response at the wavelength used will interfere. At low levels of determinand in water (<0.1 μg/l) background peaks are significant and without additional quantitative data should be regarded as maxima. Phenoxy-herbicides may interfere. (See Table 10, note iii and Section A3).
B1.0	Time for analysis	2h per sample assuming a batch of six is analysed.

B2 Principle

The sample is pre-concentrated as in Method A Sections A8.1 and A8.2. An aliquot of the concentrate dissolved in the HPLC mobile phase is injected on to the HPLC column and the determinands eluted with polar solvents. Isocratic or gradient elution may be used with UV or electrochemical detection.

Clean samples may be concentrated on a suitably absorbing cartridge or precolumn from which they are recovered using the same mobile phase as is used for elution.

B3 Interferences

At low levels of determinand (<0.1 μg/l) in water there are significant background peaks and baseline excursions. The solvents used should be bubbled with helium to remove air and only HPLC grade solvents and reagents used. Air absorbs below 210 nm when UV detection is used. Phenoxyalkanoic acid herbicides may interfere with some determinands. It is essential to analyse blanks when the procedure is used. Interferences can usually be overcome by:—

(i) Changing the wavelength.
(ii) Changing the eluting solvent composition.
(iii) Changing the column.
(iv) Changing the gradient.

B4 Hazards

Dichloromethane is narcotic; acetonitrile is toxic; acetonitrile and methanol are flammable. The standard compounds and their solutions are toxic.

B5 Reagents

(For information on the HPLC mobile phase see B7.7)

B5.1 Dilute sulphuric acid, approx 5M.
Carefully pour 266±2 ml of sulphuric acid (d_{20} 1.84) into about 600 ml of cold water (B5.4), with continuous stirring and external cooling. Allow to cool. Transfer to a 1 litre measure and make up to the mark with water (B5.4). This solution should keep indefinitely.

B5.1.1 Alternatively phosphoric acid, approx 5M.
Carefully pour 267±2 ml of phosphoric acid (d_{20} 1.83) into about 600 ml of cold water (B5.4), with continuous stirring and external cooling. Allow to cool. Transfer to a 1 litre measure and make up to the mark with water (B5.4). This solution should keep indefinitely.

B5.2 Sodium sulphate, anhydrous, roasted at 500°C for 2h.

B5.3 Dichloromethane, HPLC grade.

B5.4 Water HPLC grade (see C8.8 note n).

B5.5 Methanol HPLC grade.

B5.6 Acetonitrile HPLC grade.

B5.7 Stock solutions of herbicides.
Prepare a stock solution of each herbicide in 25 ml of the solvent mixture constituting the HPLC mobile phase (eg water: methanol 1:1)

B5.8 Working solution of herbicides.
Prepare working solutions in the same solvent mixture from the stock solutions using microlitre syringes. Multicomponent standards may be used once the elution times under the conditions used have been established. Some useful standards are:—

0.05 mg/l	0.5 μl stock in 10 ml solvent
0.1 mg/l	1.0 μl stock in 10 ml solvent
0.5 mg/l	5.0 μl stock in 10 ml solvent
1.0 mg/l	10.0 μl stock in 10 ml solvent

5.0 mg/l	50.0 μl stock in 10 ml solvent
10.0 mg/l	100.0 μl stock in 10 ml solvent

For Benomyl determinations only

B5.9 Sodium hydroxide solution, approx 10M.
Dissolve 400±1 g of sodium hydroxide in about 600 ml of cold water, (B5.4), with continuous stirring and external cooling. Allow to cool. Transfer to a 1 litre measure and make up to the mark with water (B5.4). This solution should keep for at least six months.

B5.10 Sodium hydrogen carbonate solution 5% W/V.
Dissolve 50±1 g of sodium hydrogen carbonate in 1 litre±10 ml of water (B5.4). This solution should keep for at least one year.

B5.11 Ammonia Solution (d_{20} 0.880).

B6 Sample Storage

Samples should be analysed as soon as possible. Otherwise, the pH should be adjusted to 3.0±0.5 and the samples stored in a refrigerator. (But see B1.3)

B7 Apparatus

B7.1 Sample bottles. All glass capable of holding 2.2 litres and calibrated at 2.0 litres.

B7.2 Separating funnels 2 litre.

B7.3 Kuderna-Danish evaporator 250 ml.

B7.4 Centrifuge tubes, 10 ml, graduated, tapered glass, glass stoppered.

B7.5 Microlitre syringes 1 μl – 100 μl, and an HPLC syringe.

B7.6 High Performance Liquid Chromatograph fitted with an ODS or similar column and an UV detector. Wavelength optima are between 200–400 nm for individual herbicides but a wavelength of 220 nm is a reasonable compromise for a range of determinands (see section A10). Electrochemical detectors are also satisfactory. A 20 μl sample loop is normally used for injection; but larger sizes have been used successfully.

B7.7 HPLC mobile phase.
Any mixture of polar solvents which will effect the separations required is satisfactory. A common mixture for isocratic elution is a 50:50 water: methanol mixture. Other ratios, water: acetonitile mixtures and gradient elution have also been used. A mobile phase flow rate of 1 ml/min is suitable but may be varied depending on the compounds to be determined. See also Ref 3.

B8 Analytical Procedure

Step	Procedure	Notes
B8.1	Concentration	
B8.1.1	Except for the determination of Benomyl, concentrate the sample as described in Section A8.1 and A8.2 (notes a and b) (Phosphoric acid may be substituted for sulphuric acid if preferred). If dithiocarbamates or xanthates are suspected, see B8.1.2.	(a) A two litre sample is advantageous. Use 100 ml DCM for the first extraction (b) Pre-column or cartridge concentration may be used. For many river waters the maximum useable sample size is about 250 ml. This will result in a poor limit of detection. Cartridges must be precleaned before use by washing thoroughly with water and eluent until blank tests show that they are clean.
B8.1.2	Acidified samples of dithiocarbamates and xanthates are readily decomposed or oxidized (see Section D11). Such samples should be kept cool and processed as quickly as possible, or treated as in B8.1.3.	
B8.1.3	For Benomyl procede as in Sections A8.1 and A8.2 except that the pH value is adjusted to 11 with sodium hydroxide (ION) and re-adjusted to 9.5 with sodium hydrogen carbonate (5%) solution at step A8.1.1	
B8.2	Redissolve the extracted material in 500 μl of the mobile phase to be used for the HPLC analysis. (note d)	(c) A 20 μl loop injector is suitable.
B8.3	HPLC Analysis (reverse phase operation)	(d) Suggested mobile phases compositions and detector wave lengths are given in table 10.
B8.3.1	Set up the apparatus according to the manufacturer's instructions.	
B8.3.2	Inject suitable aliquots of the standards and extracts on to the HPLC column (note c). Allow the components to separate. (note d)	
B8.4	Construct a calibration graph of peak heights of the standards v.s. weights (ng) of herbicide injected.	
B8.5	Read off the weight of herbicide (W) in the sample from the calibration graph and calculate the concentration present in the original sample.	
B8.6	Blanks Adequate blank values should be obtained prior to analysing samples.	

B9 Calculation

If a 2 litre sample is extracted into 500 μl before injection then:—

$$C = \frac{W}{4V}$$

Where C = Concentration in original sample μl/1

W = Weight (ng) of herbicide in the injected sample extract read off from the calibration graph

V = Volume (μl) of extract injected.

B9.1 For more accurate values correction also needs to be made for extraction efficiences (See table 7). These should be checked for the system in use.

Table 9 Means, Standard Deviations, Limits of Detection and Recoveries

Determinand	**Spiking Level** µg/l*	**Mean** µg/l	**St** µg/l	**L of D** µg/l	**% Recovery**
Pebulate (a)	—	0.035	0.016	0.08	—
	0.1	0.162	0.063	—	127
	0.5	0.425	0.050	—	78
EPTC (a)	—	0.042	0.011	0.05	—
	0.1	0.108	0.069	—	66
	0.5	0.432	0.067	—	78
Tri-allate (a)	—	0.028	0.011	0.055	—
	0.1	0.090	0.016	—	62
	0.5	0.500	0.104	—	94
Propham (a)	—	0.017	0.005	0.025	—
	0.1	0.110	0.025	—	93
	0.5	0.420	0.045	—	81
Carbaryl (a)	—	0.017	0.008	0.04	—
	0.1	0.108	0.029	—	91
	0.5	0.418	0.064	—	80
Methiocarb (a)	—	0.020	0.004	0.04	—
	0.1	0.108	0.024	—	88
	0.5	0.407	0.020	—	77
Benomyl (b)	blank			0.016	
	1.0				72
	1.0				53
Dinocap (b)*	—	0.19	0.008	0.04	83

Notes: (a) Data from SAC Scientific

(a)* Samples were taken from a lowland surface reservoir and spiked, where appropriate, with a stock solution of the determinands. The means and estimates of standard deviation are not blank corrected. Recoveries are blank corrected.

(b) Data from Severn Trent Finham Laboratory

(b)* Benomyl samples were distilled and river water (spiked) respectively. Dinocap sample data were means of eight sets of river water samples with spikes ranging from 0.3 to 5.3 µg/l. River water samples were also analysed and gave similar data.

All with 5 degrees of freedom except the triallate data which has 4.

Table 10 UV Wavelengths and Mobile Phase Compositions

Compound	Wavelength (nm)	Solvent composition Methanol: Water % v/v
EPTC	220	65:35
Triallate	220	65:35
Pebulate	220 (254)	50:50
Propham	220 (254)	50:50
Chlorpropham	220 (254)	50:50
Chlorbufam	220 (254)	50:50
Methiocarb	220 (254)	50:50
Carbaryl	220 (254)	50:50
Carbetamide	220	50:50
Carbofuran	220	50:50
Benomyl	364	60:40 plus 6% v/v of ammonia solution (d_{20} 0.880) (total 106%)
Dinocap	364	90:10 but the water is acidified to pH3 with phosphoric acid prior to mixing

Notes: (i) The wavelengths suggested are not necessarily optimal, but represent pragmatic compromises for a range of determinands. Values in brackets are literature alternatives.

(ii) Other solvent mixtures may be used for particular determinands. Gradient elution techniques are also suitable. (Acrylonitrile-water mixtures between 40–60 and 60–40 are preferred by some analysts but need careful handling.

(iii) If phenoxyacidic herbicide contamination is suspected, also measure at 270–280 nm and see Section A3.

C Estimation of some Carbamate and Urea Herbicides in River Waters by Thin Layer Chromatography

C0 Introduction

The method is easy to use and requires simple and inexpensive apparatus. The required limit of detection (0.1 μg/l) is unattainable but spots at 5 μg/l are visible and this concentration is lower than any quoted toxic limit for this type of herbicide. The method is adequate for many carbamate and urea herbicides when reasonable levels are expected to be present.

C1 Performance Characteristics of the Method

C1.1	Substances determined	Most carbamate and urea herbicides. The compounds tested were Carbaryl, Diuron, Pirimicarb, Propham, and Triallate.
C1.2	Types of sample	River and drinking waters.
C1.3	Basis of method	Extraction with dichloromethane, concentration of the extract, thin layer chromatography, hydrolysis and visualization of spots by a chromogenic reaction using ninhydrin.
C1.4	Range of Application	Typically up to 100 μg/l.
C1.5	Calibration curve	All the compounds tested gave a graded response up to 100 μg applied.
C1.6	Standard deviation	Because of the nature of TLC determinations, a figure for standard deviation is of dubious value. The results of replicate tests however, are presented in Table 11.
C1.7	Limit of detection	For all the compounds tested 5 μg/l was discernible.
C1.8	Sensitivity	Not applicable.
C1.9	Bias	Extraction efficiencies are less than 100%.
C1.10	Interferences	Any co-extracted material which reacts with the chromogenic reagent or which obscures visualization will interfere. Some organo-phosphorus pesticides and some phenols may give coloured spots in this analysis.
C1.11	Time required for analysis	Assuming the plates and reagents are prepared, 24 samples per week can be analysed.

C2 Principle

The carbamates are extracted from an acidified sample into dichloromethane. The extract is evaporated to low volume and applied to a TLC plate and the compounds separated. After hydrolysis with hydriodic acid the spots are visualized by ninhydrin.

C3 Interferences

Direct interferences may arise if some organophosphorus compounds, some phenols or some amines are present. No interferences were observed in the performance testing but if interference should occur a different solvent system or a different visualization reagent may be used to isolate the spots. Indirect interferences may be encountered from naturally coloured materials whose presence masks the chromogenic reaction. In the performance testing, after TLC and visualization, a coloured streak appeared on the river water plates but all the spots had a greater Rf value and had moved beyond the end of the coloured streak.

C4 Hazards

Dichloromethane is narcotic. Hexane and methanol are flammable. Hydriodic acid is toxic by inhalation. The oven used for heating the treated plates should be mounted in a fume cupboard or under a fume hood. TLC plates sometimes crack in the oven during the heating stage.

C5 Reagents

All reagents must be of sufficient purity such that they do not give rise to significant interference on the TLC plates. This should be checked for each batch of material and verified by running procedural blanks. Reagents should be stored in all-glass containers.

C5.1 Indicator papers. To cover pH range 1–5.

C5.2 Sodium sulphate, anhydrous, granular; roasted at 500°C for 4h, allowed to cool to 200°C in the furnace and then to ambient in a desiccator.

C5.3 Sulphuric acid, d_{20} 1.84, AR.

C5.4 Diluted sulphuric acid, approximately 9M. Carefully add 50 ml of conc. sulphuric acid (C5.3) to 50 ml water, stirring the whole of the time and keeping the container cool in a sink of cold water (EXTREME CARE, HEAT WILL BE GENERATED).

C5.5 Dichloromethane AR (DCM).

C5.6 Hexane AR.

C5.7 Hydriodic acid d_{20}. 1.7 AR.

C5.8 Acetic acid, glacial, AR.

C5.9 Ninhydrin AR.

C5.10 Ethanol, absolute.

C5.11 Spray reagent 1. Mix 25±1 ml of hydriodic acid (C5.7), 25±1 ml of glacial acetic acid (C5.8) and 50±1 ml water. The solution is stable for at least a week.

C5.12 Spray reagent 2. Dissolve 3±0.3 g of ninhydrin (C5.9) in 95±5 ml ethanol (C5.10) and 5±0.5 ml glacial acetic acid (C5.8). The solution is stable for several days.

C5.13 Blank Water. See Step C8.8 note n for a suggested purification.

C5.14 Standard Samples. See Step C8.4.2 note e and for preparative procedure A5.6.

C6 Apparatus

Glassware should be clean and dry. Rinsing with acetone before use and then drying in a stream of clean dry air assists with this process.

C6.1 Sample bottles. Of all-glass construction capable of holding 1.2 litres and calibrated at 1 litre.

C6.2 Separating funnels. 1 litre.

C6.3 Drying tubes. Glass tubes approximately 130 mm long by 10 mm I.D. fitted with a reservoir at the top and a jet or tap at the bottom. The jet should be loosely plugged with dichloromethane-washed cotton wool and the tube half filled with sodium sulphate (C5.2). Wash with 20 ml DCM before use.

C6.4 Kuderna-Danish evaporator, 250 ml.

C6.5 Centrifuge tubes, 10 ml, graduated, tapered, glass stoppered.

C6.6 TLC plates, 20×20 cm with a 250 micron layer of Silica-gel (Merck or equivalent) Ready-made plates may be used or plates can be prepared n the laboratory:— Slurry 30 g silica gel G with 60 ml water for 2 min. Spread 5 plates using a manual or automatic plate spreader with a thickness of 250 microns. Allow the plates to dry, activate at 106°C for 60 min.

C6.7 TLC Development tank: of sufficient size to hold at least two TLC plates and lined with chromatography paper.

C6.8 TLC sprays (2), all glass.

C7 Sample storage

Samples should be extracted as soon as possible after sampling. The bottles should be protected from contamination by sealing each bottle in a polythene bag. If analysis has to be delayed, acidify the sample to pH3 with dilute sulphuric acid (C5.4) and omit step C8.1.1 of the experimental procedure.

C8 Analytical Procedure

Step	Procedure	Notes
C8.1	Extraction	
C8.1.1	To a 1 litre sample in the bottle add dilute sulphuric acid (C5.4) until the pH is 3 (note a)	(a) Indicator paper is suitable, although a pH meter may be used. See also A8.1.1 note (a)
C8.1.2	Add 40 g of anhydrous sodium sulphate (C5.2) and shake the bottle until all the sodium sulphate has dissolved	
C8.1.3	Add 50±5 ml of DCM and shake for 2 min (note b)	(b) A shaking machine may be used, but the extraction times must be increased to 10 min
C8.1.4	Transfer the entire contents of the sample bottle to a separating funnel. Rinse the sample bottle with about 10 ml DCM and add the washings to the separator. Allow the layers to separate.	
C8.1.5	Transfer the lower DCM layer to a drying column (C6.3). Collect the column eluate in a Kuderna-Danish evaporator (C6.4) fitted with a centrifuge tube (C6.5).	
C8.1.6	Re-extract the sample in the separating funnel by shaking for 2 min with 25±2 ml DCM. (Note b). Allow the layers to separate and add the lower DCM layer to the same drying tube. Allow the combined extracts to run into the Kuderna-Danish evaporator.	
C8.1.7	Wash the drying column with a further 10 ml DCM and collect the eluate in the same Kuderna.	

Step	Procedure	Notes
C8.1.8	Remove the drying tube, add *one* anti-bumping granule to the Kuderna. (Note c).	(c) The chip promotes even boiling, but may be omitted if difficulty is experienced in removing the extract from the tube for spotting. An alternative to the use of an antibumping granule is to modify the Kuderna Danish evaporator system with a ground glass jointed tee piece or side arm through which is inserted a tube drawn out into a fine capillary which dips down to the bottom of the sample tube. Purified nitrogen is very slowly bubbled through this tube during the evaporation. At the conclusion, the capillary is rinsed off with a drop of solvent. The nitrogen is not turned off until this has been done and the sample tube removed. The final evaporation is carried out using a slightly larger bore jet for the purified nitrogen which then just impinges above the liquid surface.
C8.2	Concentration	
C8.2.1	Evaporate the solvent to about 2–3 ml. After draining, remove the Kuderna and blow down the extract to 20–50 μl with a gentle stream of purified nitrogen. (note c)	
C8.3	Preparation of TLC tank	
C8.3.1	Line the walls of the tank with Whatman No. 1 filter paper or equivalent.	
C8.3.2	Pour sufficient solvent into the tank to give a depth of about 15 mm of solvent (See section C8.5.1 for solvent systems used).	
C8.3.3	Replace the tank lid and rock the tank gently to wet the walls and lining paper. Allow the system to equilibrate for at least 30 minutes.	
C8.4	Spotting of Plates	
C8.4.1	Apply all the sample from C8.2.1 to the plate, 2 cm from the lower edge, using a microlitre syringe (note d). Add a further 20 μl DCM to the centrifuge tube, swirl and add the washings to the same spot on the plate using the microlitre syringe. Repeat the process with a further 20 μl DCM and add the washings to the same spot.	(d) The spot applied should be as small as possible.
C8.4.2	On the same plate apply a series of standards of the compound or compounds to be determined employing the same spotting technique (note e).	(e) Convenient standards are 2, 5, 20, 25, 50 μg of each determinand. The volume of DCM in which these quantities are dissolved should match the volumes used for the samples (approx 80 μl). Up to 6 spots may be conveniently placed on one plate.
C8.4.3	Draw a line with an awl, 3 cm from the top edge of the plate.	
C8.5	Solvent systems	
C8.5.1	Hexane: acetone, 2:1 by volume was used in testing the method. Other solvent systems which have been used are: chloroform: acetone 9:1 hexane: acetone 5:1 (note f).	(f) Other solvent systems may be used if experience indicates a better separation is obtained for certain determinands.

Step	Procedure	Notes
C8.6	Thin Layer Chromatography	
C8.6.1	Carefully lower the plate into the tank, replace the lid and allow the solvent front to rise to the marked line (C8.4.3) (note g).	(g) Running time is normally about 25 minutes.
C8.6.2	Remove the plate from the tank and allow it to air dry (note h).	(h) All naked flames and sources of ignition must be absent.
C8.7	Visualisation	
C8.7.1	Wearing rubber gloves and in a fume hood, spray the plate with the hydriodic acid reagent (spray reagent 1, C5.11). Clip a clean glass plate over the sprayed plate and heat in an oven at 180°C±10°C for 20±5 min (note i). Cool the oven slowly by opening the door slightly and lowering the temperature control (note j).	(i) The plates should stand in a vertical plane to assist the heat flow. (j) Cracking of the plates may occur if cooling is too rapid.
C8.7.2	Transfer the warm plates to a fume cupboard and remove the cover plate (CAUTION: Iodine fumes are evolved) (note k).	(k) If the plates are cold before removal of the cover plate residual iodine will stain the silica and make interpretation of the spots more difficult.
C8.7.3	Interpretation of the spots may be made at this stage (see C8.7.5) and may be adequate for some purposes. Better visualization is obtained if the second spray reagent is also used. (Note m).	
C8.7.4	In a fumehood spray the plate with ninhydrin reagent (spray reagent 2, C5.12) and heat in an oven at 120°C±5°C for 20±5 min (notes l and m).	(l) Sufficient ninhydrin solution must be deposited on the plate to ensure full colour development. Care is necessary when spraying as it is all too easy to wash off the silica layer.
C8.7.5	Compare the Rf values and colour reactions of the sample extract spots with those of the standards. For quantitative results the sizes and intensities of the spots should be compared visually or a plate-reader may be used where available.	(m) Other colour development systems have been used and the use of more than one system improves the reliability of the determination.
C8.8	Blanks	
	An adequate number of blanks using pesticide free (note n) water as sample should be carried through the whole procedure.	(n) Use high quality distilled water, extracted with several portions of DCM and redistilled rejecting the first and last 20%.

Table 11 Means and estimates of total standard deviation

Distilled water

Herbicide	Mean blank µg/l	Low spike (5 µg/l)			High spike (50 µg/l)		
		Mean	Range	st	Mean	Range	st
Diuron	0	6	0–10	3.8	46	40–50	3.8
Carbaryl	0	6	5–10	2.0	42	40–50	4.1
Pyrimicarb	0	6	0–10	4.9	44	40–50	4.9
Propham	0	3	0–5	2.6	52	40–60	6.8
Triallate	0	3	0–5	2.7	41	40–45	2.0

River water

Herbicide	Mean blank µg/l	Low spike (5 µg/l)			High spike (50 µg/l)		
		Mean	Range	st	Mean	Range	st
Diuron	0	5	5	0	46	40–50	3.8
Carbaryl	0	3	0–10	4.1	42	40–45	2.6
Pyrimicarb	0	9	5–10	2.0	46	40–50	4.9
Propham	0	5	5	0	53	50–60	5.2
Triallate	0	4	0–10	3.8	44	40–50	4.9

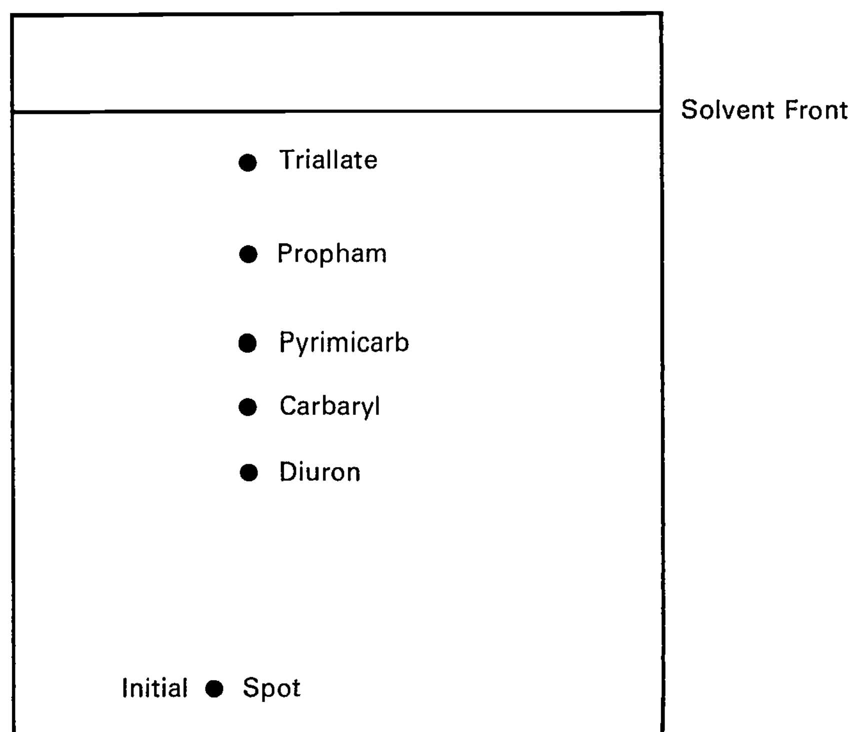
Notes: (i) Standard plates were prepared at 0, 5, 10, 15, 35, 40, 45, 50, 55 and 60 µg/l. The sample spots were equated to the nearest standard spot without any attempt at interpolation.

(ii) Means are rounded off to the nearest whole number.

(iii) None of the blanks gave discernible spots with appropriate Rf values.

(iv) 10 blanks were run on a single occasion. 6 spiked samples were run in pairs on 3 occasions.

FIG.1 SEPARATION OF THE HERBICIDES TESTED



D Estimation of Dithiocarbamates and Related Compounds as Carbon disulphide in River Water By Reduction and Gas Chromatography

D0 Introduction

The method which was found to be reasonably sensitive is in practice fairly simple to use; approximately 0.5 μg/l as CS_2 can be detected, but lower levels may be determined if suitable equipment is available. Rather variable results may be obtained however from replicate samples, hence it is recommended that several portions of each sample be analysed. Within its limits the technique is satisfactory. For the problem of identifying individual dithiocarbamates see the Introduction to this booklet.

D1 Performance characteristics of the Method

D1.1	Substances determined	Maneb, Mancozeb, Nabam, Zineb, Ferbam, other dialkyldithiocarbamates, Thiram*, and substances producing carbon disulphide (CS_2) on reduction and or hydrolysis. Soluble Metham Sodium can also be determined, as well as its oxidation product symdimethyl thiruram disulphide. (* Thiram the commercial fungicide and animal repellent, is actually tetramethyl thiuram disulphide)
D1.2	Types of sample	River and drinking waters.
D1.3	Basis of method	The sample is heated with acid in the presence of stannous chloride and 2,2,4-trimethylpentane (i-octane). The CS_2 formed dissolves in the i-octane and is determined by capillary column gas-chromatography using a flame photometric detector.
D1.4	Range of application	Up to 50 μ/l as CS_2. The range can be extended by dilution of the sample.
D1.5	Calibration curve	The plot of response versus concentration is not linear. A plot of the square root of the response versus concentration is approximately linear up to 50 μg/l CS_2. A set of standards bracketing the samples should be run with each set of analyses.
D1.6	Standard deviation	See Table 12.
D1.7	Limit of detection	0.48 μg/l as CS_2. 0.84 μg/l as Maneb. See Table 12. See also Section D10 for a way to lower the limit of detection, which has been tested.
D1.8	Sensitivity	For the instrument used in the performance tests, a solution containing 25 μg/l as CS_2 gave a deflection of 50% FSD when the baseline fluctuation was 0.5% FSD.

D1.9	Bias	Mean recoveries are less than 100%. Incomplete conversion of the fungicide to CS_2 will lead to bias. Loss of CS_2 via leaking bottle caps will also produce low results.
D1.10	Interferences	Carbon disulphide will interfere. Any compound producing CS_2 under the conditions of the analysis will also interfere.
D1.11	Time required for analysis	Assuming the apparatus and equipment are prepared, twelve samples may be analysed per day.

D2 Principle

The sample (200 ml) is heated to 80°C with an acidic solution of stannous chloride in a sealed bottle containing 2.2.4 trimethyl pentane. The fungicides are hydrolysed to carbon disulphide which dissolves in the trimethyl pentane. The concentration of CS_2 in the trimethyl pentane is measured using a gas-chromatograph fitted with a capillary column and a flame photometric detector used in the sulphur mode. The responses of the samples are compared with those of a series of standards of CS_2 treated in the same way as the samples. Thiuram disulphides are reduced to dithiocarbamates, which then hydrolyse.

D3 Interferences

As the determinands are measured as carbon disulphide, samples containing this substance will interfere. The method measures all the substances listed in 1.1; thus the result is the total of the compounds listed together with any others which undergo the same reaction. The method is not specific for any one determinand.

D4 Hazards

Dialkyldithiocarbamates, stannous chloride and carbon disulphide are toxic. Carbon disulphide and i-octane are flammable. Hydrochloric acid is corrosive. If xanthates are present, carbon oxysulphide will be formed which is volatile and toxic and precedes carbon disulphide in GC.

During the course of the analysis it is necessary to heat sealed bottles to 80°C in a water bath and, at intervals, to remove the bottles for shaking. As the pressure in the bottles is likely to be high, safety guards and or screens should be used, protective clothing, gloves and a face-mask should be worn during this operation. As an additional precaution bottles may be wrapped in cloth or wire gauze, but unless wrapped carefully this may be cumbersome and so also hazardous. If it is necessary to use large (c3 litre) bottles, they should be well inspected for flaws and tightness of cap seal. They should be enclosed in a strong wire mesh or gauze screen and handled using plastic bottle crates or baskets when hot and under pressure. Mechanical shaking should be used. If it can be carried out safely, end over end shaking is preferred as solvent and substance extracted float and sink in water respectively.

Similar non-manual operation is also suggested for the smaller 350 ml bottles.

Bottles should be securely fixed in both screen and crate if these are used.

Do not open bottles until they have cooled.

During the performance tests this procedure was carried out about 200 times without mishap using 350 ml bottles. The low concentration procedure given in Section D10 has also been evaluated using 3 litre Winchester bottles in plastic cases. No problems were encountered.

D5 Reagents

D5.1 Stannous chloride: weigh out 15 ± 0.5 g of stannous chloride dihydrate ($SnCl_2 .2H_2O$). Add 450 ± 5 ml conc hydrochloric acid (d_{20}1.18), mix and make up to 1 litre with distilled water.

D5.2 2,2,4-trimethylpentane (i-octane) A.R.

D5.3 Carbon disulphide A.R.

D5.4 Stock solution of carbon disulphide: Using a microlitre syringe inject 79.2 μl of carbon disulphide (D5.3) in to 100 ml acetone (A.R.). The concentration of this solution is 1000 mg/l. (Solution A).

D5.5 Stock solution of carbon disulphide: Using a microlitre syringe inject 100 μl of the stock solution of CS_2 (D5.4) into 10 ml acetone. The concentration of this solution is 10 mg/l. (Solution B).

D6 Apparatus

All glassware should be clean and dry.

D6.1 Bottles, Glass 350 ml with screw cap. Medical flat bottles with aluminium caps are suitable. Bottles with corroded caps should not be used. (c3 litre bottles may be needed if Section C10 is used).

D6.2 PTFE cap liners (Fi-seal or equivalent) To fit inside the screw caps of the bottles and ensure a tight seal and prevent corrosion of the cap if of aluminium (D6.1).

D6.3 Water bath With lid, capable of maintaining 80° ± 1°C.

D6.4 Gas chromatograph, capillary column instrument fitted with split, split less or on-column injection and a flame photometric detector operated in the sulphur mode. An oven temperature of 40°C isothermal is suitable for non-polar columns.

D6.5 G.C. columns. A 25 m wall coated open tubular column constructed of fused silica and coated with a 1 μm layer of OV1. Other capillary columns may also be used (eg Carbowax 20M OV17). Packed columns have been used for the analysis (eg 2m OV1) but the sensitivity and the detection limits are impaired.

D6.6 Selection of microlitre syringes. (1,2,5,10,20,100 μl).

D7 Sample storage

Samples should be analysed as soon as possible after sampling. Samples, distilled and river water, spiked with Maneb and stored at 4°C for three days showed no apparent deterioration.

As at least 200 ml samples are required, and it is recommended that several replicate analyses be made, the total volume of initial sample taken should, if possible, be at least one litre.

D8 Analytical Procedure

Step	Procedure	Notes
D8.1	Add 200 ± 1 ml of sample to a screw capped bottle (D6.1). Add 10.0 ± 0.1 ml 2,2,4-trimethylpentane (D5.2) and 50 ± 1 ml stannous chloride solution. (D5.1). **Immediately** fasten the PTFE lined screw cap on to the bottle. (note a).	(a) The bottle must seal completely when the cap is screwed tightly down.
D8.2	To eight other bottles add 10.0 ± 0.1 ml 2,2,4-trimethylpentane (D5.2) and 50 ml stannous chloride solution (D5.1). Fasten the cap on to one of the bottles labelled 'blank'. (note a).	

D8.3 To the remaining seven bottles add
(1) 2 µl CS_2 Stock Solution B (D5.5)
(2) 10 µl CS_2 Stock Solution B (D5.5)
(3) 20 µl CS_2 Stock Solution B (D5.5)
(4) 100 µl CS_2 Stock Solution B (D5.5)
(5) 2 µl CS_2 Stock Solution A (D5.4)
(6) 5 µl CS_2 Stock Solution A (D5.4)
(7) 10 µl CS_2 Stock Solution A (D5.4)
(note b and c)

All with a maximum tolerance of ± 1%

Immediately fasten the screw caps. (note a). Label the bottles appropriately and shake vigorously for 10 seconds.

(b) Experience may indicate that some of the standards may be omitted.

(c) These standards produce responses equivalent to 0.1, 0.5, 1.0, 5.0, 10.0, 25, and 50.0 µg/l CS_2 in the original sample.

D8.4 Place the bottles containing the blank, standards and samples in a water bath pre-heated to 80° ± 1°C (note d). Allow the bottles to come approximately to reaction temperature.

(d) See Hazards Section 4, Protective clothing gloves and a face mask must be worn to prevent injury if a bottle cracks.

D8.5 Then after 15 min, 30 min and 45 min at reaction temperature remove the bottles (note d) shake vigorously for 10 seconds and return to the water bath. After 60 min at temperature remove the bottles shake and allow to cool to room temperature (note e).

(e) Immersing the bottle in cold water accelerates the cooling of the bottles. Do not open until cooled to room temperature.

D8.6 Chromatography:

D8.6.1 Immediately before injection add water to each bottle so that the organic layer rises into the neck of the bottle. Inject 1 µl of this organic layer on to the column. (note f).

(f) If split injection is used a larger volume may be injected with an appropriate adjustment of the split ratio.

D8.6.2 The peak for CS_2 emerges before the solvent peak. After the CS_2 has emerged wait for the solvent peak before making the next injection. (note g).

(g) The CS_2 peak emerges very rapidly and the solvent peak after about 4 min.

D8.6.3 Inject all the samples, standards and blanks and measure the peak heights associated with the CS_2 peaks.

D8.6.4 Construct a calibration graph of the square root of the peak heights vs. concentration of CS_2 in the original sample. Read off the concentration of CS_2 in the original sample directly from the calibration graph. (note h).

(h) Use of the automatic linearizing circuits diminishes the response. Plots of response vs. concentration are not exactly linear and a range of standards bracketting the samples must be run.

D9 Calculation

The concentrations of CS_2 in the samples is read directly from the graph. If only one, dithicarbamate is thought to be present, the CS_2 figure can be converted arithmetically to express the results as the compound sought.

D10 Lowering the Limit of Detection

If a lower limit of detection is required, it will be necessary to use a larger sample—1 to 3 litre. Adequate safety precautions can be readily devised, see Hazards Section D4.

D10.1 Take a sound tightly closing screw capped bottle of 2.5–3 litre capacity. Add 2 litres (± 10 ml) of sample. Add 10.0 ± 0.1 ml of 2.2.4 trimethylpentane (D5.2) and 500 ± 20 ml of stannous chloride solution (D5.1). **Immediately** fasten the screw cap on the bottle (see D8.1 note a).

D10.2 Using 2 litres of pesticide free distilled water, repeat D10.1. Use this as a blank.

D10.3 Using 2 litres of pesticide free distilled water add 20 μl of CS_2 Stock Solution B (D5.5) and the other reagents from D10.1. Complete D10.1. Use this as an analytical quality control sample (0.1 $\mu g/l$ CS_2).

D10.4 Carry out D8.4 to D8.6.3. Observing the Hazards Section D4.

D10.5 Using a calibration curve prepared as in D8.2 to D8.6.4 and D9 determine the CS_2 content of the sample, using the standard prepared in D10.3 as a control. Proportionate for difference in sample size between D8.1 and D10.1.

D11 Note (on oxidation and decomposition)

It is not possible to concentrate samples by evaporation. Dithiocabamates (and also ureas in methods A, B and C) are subject to hydrolysis. Thiocarbamates are also subject to oxidation giving compounds of the type $R_2NCS.S_2.CS.NR_2$, or if a sulphur receptor such as cyanide (which goes to thiocyanate) is present, $R_2.N.CS.S.CS.NR_2$. Hence the addition of stannous chloride to ensure nonoxidizing conditions during the hydrolysis on which this method is based. Hydrolysis of dithiocarbamates, xanthates, and ureas give CS_2, COS, and CO_2 respectively. Some ureas also partially isomerize to ammonium and cyanate ions.

The fungicide Thiram is the oxidation product of dimethyldithiocarbamate $[(CH_3)_2N.CS.S]_2$. Metham sodium may form an insoluble coating of the related symmetrical dimethylated compound $[(CH_3)\ HN.CS.S]_2$. Tests show that these compounds are reduced back to dithiocarbamates by stannous chloride and so are determinable.

Should it be necessary to distinguish between Metham sodium, Serbam and the other dithiocarbamates, concentrate the residual acidic aqueous layer left at step D8.6.1. cool. Make alkaline, allow precipitated tin compounds to settle and examine the supernatant aqueous layer by cation chromatography. Metham sodium gives mono methylamine, Serbam gives dimethylamine, while the others give 1.2 diamino-ethane. For further information see the booklet in this series on Chromatography of Ions.

Table 12 Means and estimates of standard deviation

Water sample	Spiking level		Mean Conc found $\mu g/l$ CS	Standard deviation			Mean Recovery %
	As CS_2 $\mu g/l$	As Maneb $\mu g/l$		Sw $\mu g/l$	Sb $\mu g/l$	St $\mu g/l$	
Unspiked dist	—	—	−0.02	0.10	—	—	—
Unspiked river	—	—	0.09	0.10	—	—	—
Low spiked dist	2.3	4	2.07	0.24 (5)	n.s	0.35 (6)	91
High spiked dist	11.5	20	7.85	0.24 (5)	2.31 (4)	2.32 (4)	69
Low spiked river	2.3	4	2.10	0.33 (5)	n.s	0.49 (6)	91
High spiked river	11.5	20	8.16	0.88 (5)	2.19 (4)	2.36 (5)	71

Numbers in brackets indicate degrees of freedom. n.s. = not significant.

Using LD = 4.65 sw for the blanks, the limit of detection for the method is:
0.48 $\mu g/l$ as CS_2 (0.84 as Maneb) in distilled water.
0.47 $\mu g/l$ as CS_2 (0.82 as Maneb) in river water.

Analytical Quality Control

1 Introduction

All the methods given in this booklet have only been thoroughly investigated in one or two laboratories, and are therefore Tentative. Before firmly recommending the method for general use, it is desirable to know the accuracy achievable in other laboratories. It would, therefore, be of great value if any laboratory using or considering the use of this method could 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.

The precision achieved and the effects of any interfering substances that may be present in samples are of particular interest. Any information on these aspects would be useful, but the value of such information would be greatly enhanced if it were obtained to a common plan so that the information can be compared and valid conclusions drawn. Accordingly, suggestions for a suitable experimental design and analysis of results are given in the following sections and it is strongly urged that laboratories follow this design whenever possible. The design has been chosen to be as simple as possible; more complex designs are possible and would give more information.

2 Basis of suggested Tests

The limit of detection is governed by the within-batch variability of blank determinations. The precision of analytical results may depend on the concentration of determinand in the sample analysed and on the type of sample, eg worse precision may be obtained with samples than with standard solutions. For these reasons the basic design recommended is the analysis of one portion of each of the following solutions on each of n days, where n is at least 5 and preferably up to 10.

Solution No.	Description
1	Blank
2	Another blank
3	Standard solution low concentration
4	Standard solution high concentration
5	Typical sample
6	Same sample spiked a known amount of determinand

It is essential that these solutions be treated exactly as if they were samples and the appropriate specified procedure be rigidly followed. These solutions should be analysed in random order in each batch of analyses. Solutions 1 to 4 should be prepared each day exactly as described in the method and should contain the same amount of acid or alkali as is present in the samples. The same batch of water should be used on each day to prepare all four solutions. For solutions 5 and 6 a total of 5 litres of typical sample are required. Prepare solution 6 each day when required by spiking solution 5. The results of the analyses of solutions 5 and 6 will provide a check on the effect of sample type on precision. Any deviation of the recovery of spiked material from 100% may give an indication of the presence of interfering substances.

Note that the Blank water should be appropriate for the method being evaluated.

3 Evaluation of Results

The raw experimental results should be sent direct to the Department of the Environment for evaluation together with the results obtained from the standards used to establish the calibration curve in each batch of analyses. However, for those laboratories wishing to make the calculations themselves, the details are given below.

3.1 Convert all results to concentrations as described in the method. Deduct the first of the two blank values (solution 1) from each of the other solution values.

3.2 Calculate the mean concentration of the n results for each solution.

3.3 Calculate the standard deviation, s, of the n results for each solution from:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

Where x_i = the result from the ith batch
$\bar{x}$ = the mean value of x_i

3.4 Calculate the within-batch standard deviation, S_w, of the blank from:

$$S = \sqrt{\frac{\sum(x_{1i} - x_{2i})^2}{2n}}$$

Where x_{1i} = the 1st blank result (solution 1) from the ith batch
x_{2i} = the 2nd blank result (solution 2) from the ith batch.

3.5 Calculate the mean percentage recovery, R, of the spiked determinand in solution 6 from:

$$R = \frac{(\bar{x}_6 - \bar{x}_5) \times 100}{10}$$

Where $\bar{x}_5$ = the mean value of the results for solution 5
$\bar{x}_6$ = the mean value of the results for solution 6.

3.6 Summarize the results as in the following table:

Solution	No of results n	Mean Concentration μg/l	Standard Deviation μg/l	Mean Recovery %
2 Blank				—
3 Standard, low				—
4 Standard, high				—
5 Sample				—
6 Solution 5 + spike				—

The appropriate sample description should be entered in the space for solution 5. The standard deviation from step 3.4 is entered for the blank solution 2 and the standard deviations from step 3.3 are entered for solutions 3 to 6.

4 Evaluation of Interference Effects

If interference effects are suspected, analyse a series of standard samples with and without known amounts of interference plus also real samples, spiked real samples and spiked real samples with interferent added. If interference removal is contemplated, this should be evaluated in the same way.

5 Routine Analytical Quality Control

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of tests are possible and they should be used as appropriate. As a minimum, however, it is suggested that a standard solution of the determinand of suitable concentration should be analysed at the same time and in exactly the same way as normal samples. The results obtained should then be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be estimated.

Address for Correspondence

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

The Secretary
The Standing Committee of Analysts
The Department of the Environment
Romney House, 43 Marsham Street
LONDON SW1P 3PY
England

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Dd290818 10/88 C10 G443 10170

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