The Determination of Microgram and Submicrogram Amounts of Individual Phenols in River and Potable Waters 1988

Methods for the Examination of Waters and Associated Materials

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Users should remember that the accuracy of an analysis for individual phenols is highly dependent on the other substances present including other substituted phenols and therefore they should evaluate the methods themselves for their own analytical problem.

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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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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 users 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 nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 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 separate 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 checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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 specialised hospital.

The Determination of Microgram and Submicrogram Amounts of Phenols in River and Potable Waters

Introduction

The EC Directive on the Quality of Water for Human Consumption (Ref 5) has a Maximum Allowable Concentration for the "Phenol Index" or Phenol itself of 0.5 μg/l, but some natural phenols not causing taste problems may be excepted. Some substituted phenols can be detected by taste at very low concentrations indeed, even at picograms per litre for the worst tasting ones.

A compendium of 5 methods (Ref 6) was issued in this series, and a subsequent booklet (Ref 7) gives methods for di, tri, tetra and pentachloro-phenols.

Very many substituted phenols are known to occur both naturally and industrially and the limits of detection vary between the phenols as well as with the method. This booklet gives 2 Gas Chromatographic methods and 2 HPLC methods which have proved useful. The following table summarizes typical limits of detection found with the various methods.

Method	Typical Limits of Detection
Ref 6 A	0.1–c 1 mgl^{-1} for different compounds (phenol and catechol quoted)
Ref 6 B	9 μgl^{-1} for phenol
Ref 6 C	3 μgl^{-1} for phenol
Ref 6 D	5 μgl^{-1} for phenol
Ref 6 E	1 μgl^{-1} for phenol
Ref 7 B with methylation	0.02–c 0.1 μgl^{-1} for various polychlorophenols (penta, tetra, and tri chloro)
Ref 7 B without methylation	c 0.1 μgl^{-1} for dichlorophenols
This booklet A	0.02–0.1 μgl^{-1} for many different substituted phenols. Some but not all xylenols are less sensitive (often used as a confirmation for methods C and D).
This booklet B	0.25 μgl^{-1} for phenol. Many other phenols may also be determined.
This booklet C	c 0.01 μgl^{-1} for many different substituted phenols with good separations (using electrochemical detection).
This booklet D	Mainly for investigations and confirmation 0.05–0.2 μgl^{-1} for many different substituted phenols with good separations using electrochemical detection; up to ca 1 to 10 μgl^{-1} using UV detection, up to 0.2 mgl^{-1} if direct injection is used, possibly higher with smaller samples.

As there are so many possible phenolic compounds which can occur naturally, in effluents, or be made unwittingly in water treatment, great care is needed to ensure correct identification. This is especially important as the instrumental response varies

between phenols and calibration is by comparison with a standard sample of the phenol. Hence correct identification also effects quantification.

If in doubt, analysis on two different columns or with different eluents or even different methods is suggested. Mass spectrometric detection may also be used to distinguish between some phenols and may be coupled with these methods.

Note that ethyl phenols have been reported in silage liquor, benzyl and phenyl phenols have commercial uses; and phenols with other substituents than halogens, nitro and amino groups are of common occurrence.

Although Method A has been fully tested to the normal SCA standard of at least 5 independent laboratories and much data is available for the other methods, the possible variation in samples is so numerous that analysts seeking to determine or identify a specific phenol should evaluate the various methods using synthetic and spiked samples containing the sought or suspected compounds prior to placing confidence in their results.

It must be remembered that some phenols are also used as their esters or ethers which require mild hydrolysis to liberate the free phenol. This may actually occur during sample storage or during the analysis. Analysts are warned to be alert and if necessary check whether the method they are using will hydrolyse such compounds or not. Common among such esters is pentachlorophenyl laurate; phenolic acetates are also encountered.

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

A Determination of Phenols by Electron-Capture Gas Chromatography of Pentafluorobenzoyl esters

A1 Performance Characteristics Of The Method*

A1.1	Substances determined	Phenols as shown in Table 1.
A1.2	Type of Sample	Rivers and potable waters.
A1.3	Basis of Method	Phenols are converted to their pentafluorobenzoyl esters. The derivatives are then extracted into hexane and determined using electron-capture gas chromatography.
A1.4	Range of Application	The method has been tested over the range 0 to 1 $\mu g l^{-1}$ for each phenol listed.
A1.5	Calibration Curve	Linear or slightly curved over the range of application.
A1.6	Standard Deviation	See Tables 2 to 7.
A1.7	Limits of Detection	See Table 1.
A1.8	Sensitivity	See Table 1.
A1.9	Bias	Recoveries from distilled, river and potable waters are shown in Table 8.
A1.10	Interferences	Any material which produces pentafluorobenzoyl derivatives of similar gas chromatographic characteristics to the determinands (see section 3).
A1.11	Time required for Analysis	Six hours to obtain a result from receipt of a sample assuming all reagents, calibration standards etc, are prepared. 12 man-hours to analyse a batch of 20 samples including all preparation time.

* Data obtained by Huntingdon Research Centre using a 10 m, BP5 fused silica capillary column.

Similar data, with slight variations in the method used to suit the sample, have also been obtained by Grampian RC., Severn Trent WA, (Finham Laboratory), SAC Scientific, Anglian WA and Thames WA.

A2 Principle

The sample is made alkaline and pre-extracted with hexane to reduce levels of interfering compounds. The sample is then buffered using sodium carbonate and reacted with a solution of pentafluorobenzoyl chloride in hexane, by shaking. The phenol pentafluorobenzoyl esters partition into the hexane layer, excess reagent is removed by rinsing with NaOH solution and the esters are determined using electron-capture gas chromatography (1).

A3 Interferences

Pentafluorobenzoyl chloride will react with several functional groups other than phenolic hydroxyl groups which may be present in water samples, principally amines. The extraction conditions eliminate most of these interfering compounds but in practice some interferences appear in the chromatograms. Even the purest available pentafluorobenzoyl chloride gives some interfering peaks. It is therefore essential to check the blank for each batch of reagent.

Many laboratory atmospheres contain phenols which can cause interference during the extraction stages. It is essential that the extraction and sample handling be performed away from such areas. The use of phenol for other analytical methods, particularly ammonia analysis, and the use of pheolic disinfectants have been found to cause problems.

A4 Hazards

Hexane is flammable. The toxicological properties of pentafluorobenzoyl chloride are not well defined, however, it is a very reactive chemical and lachrymatory and should be handled with caution, in a fume cupboard. 2, 4, 6-trichlorophenol is a suspect carcinogen and must be handled with great care. Electron capture detectors contain radioactive materials and must be used in accordance with the manufacturers instructions.

A5 Reagents and Standards

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

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

A5.1 Water

The water used for blank determinations, pre-extraction and preparation of standard solutions should have a phenol content that is negligible in comparison with the smallest concentrations to be determined. This should be checked by analysis as in Section 8. Unchlorinated borehole water is often suitable.

A5.1.1 Laboratory distilled or de-ionized water often contains interfering materials and is not suitable, however, contamination from this source can usually be eliminated either by filtration through activated carbon (the size, contact time and life of the column will depend upon the level of contamination encountered, the particular phenols and the grade of activated carbon used), or by distillation as below:

Measure water (1.8 litre) into a distillation flask (2 litre) add boiling chips and commence distillation. Reject the first 200 ± 20 ml then add solid NaOH (3.0 ± 0.2 g) and continue distillation. Reject the next 100 ± 10 ml. Collect and use the next 1 litre ± 50 ml. Reject the still residue.

A5.1.2 Non-chlorinated borehole water is often suitable but should be checked as in section A8.

A5.2 Blank Water

To 400. ± 10 ml water add 20.0 ml approx 1M sodium hydroxide solution (see A5.8).

A5.3 Hexane—pesticide analysis grade.

A5.4 Acetone.

A5.5 Sodium Hydroxide.

A5.6 Sodium Bicarbonate.

A5.7 Pentafluorobenzoyl chloride (store in a refrigerator at c 4°C).

A5.8 Sodium Hydroxide solution (approx 1M)—dissolve 16.0 ± 0.2 g sodium hydroxide in 400 ± 10 ml water. This reagent will keep for about 6 months.

A5.9 Sodium Bicarbonate solution (approx 1M)—dissolve 16.8 ± 0.2 g sodium bicarbonate in 200 ± 5 ml water. This reagent will keep indefinitely.

A5.10 Pentafluorobenzoyl chloride solution—dissolve 150 ± 5 µl pentafluorobenzoyl chloride in 120 ± 5 ml hexane. Prepare daily.

A5.11 Standard Solutions.

A5.11.1 Stock solutions 200 µgml^{-1}

Weigh out 20 ± 0.1 mg of each phenol shown in Table 2 and dissolve individually in acetone. Make up each in separate flasks to 100 ± 0.1 ml with acetone. Store refrigerated in the dark. These solutions should be stable for six months.

If necessary, standards containing other suspected phenols should also be prepared.

A5.11.2 Diluted Stock Solutions 10 µgl^{-1}

Transfer 5 ± 0.02 ml of each stock solution to a flask and make up to 100 ± 0.1 ml with acetone.

The above solution is suited to the gas chromatography column given in A6.3.i, other columns will require different compositions chosen to give good resolution of a maximum number of phenols.

A5.11.3 Working Standard Solutions

Prepare dilutions of the standard as follows:

STD A. 1.0 ± 0.01 ml 10.0 µgml^{-1} standard to 25.0 ± 0.02 ml acetone = 0.40 µgml^{-1}
STD B. 2.0 ± 0.01 ml 10.0 µgml^{-1} standard to 25.0 ± 0.02 ml acetone = 0.80 µgml^{-1}
STD C. 4.0 ± 0.01 ml 10.0 µgml^{-1} standard to 25.0 ± 0.02 ml acetone = 1.60 µgml^{-1}
STD D. 7.0 ± 0.01 ml 10.0 µgml^{-1} standard to 25.0 ± 0.02 ml acetone = 2.8 µgml^{-1}
STD E. 10.0 ± 0.01 ml 10.0 µgml^{-1} standard to 25.0 ± 0.02 ml acetone = 4.0 µgml^{-1}

A6 Apparatus

New glassware should be washed with concentrated HCl, rinsed well with distilled water and finally with acetone. Reserve the glassware for phenols analysis. Subsequent washing is as above but using approx 1 M HCl. Use gloves to avoid contamination. (see also B6).

A6.1 Test tubes—B24 stoppered approx 50 ml volume calibrated to 42 ± 0.5 ml.

A6.2 Mechanical shaker—flat bed 400 oscillations per minute. Suitable for shaking the horizontally held test tubes.

A6.3 Gas Chromatograph—A gas chromatograph fitted with an electron-capture detector and split-splitless injection. This should be operated according to the manufacturer's instructions. The following columns and conditions have been found to be satisfactory:

i. Column — 10M fused silica capillary—BP5 (SE54 equivalent) 0.33 mm id 0.5 µm coating thickness.

Temperature — Inj—230°C, Det: 250°C
130°C for 1 min then 3°C min^{-1} to 145°C then 15°C min^{-1} to 165°C then 25°C min^{-1} to 250°C. Hold for 3 mins.

Gases — He carrier (0.40 Bar)
N_2 make up (30 ml min^{-1} total) to detector.

Injection vol — 1.5 µl (0.15 min residence).

Typical retention times are shown in Table 9 and capillary chromatograms are shown in Fig 1a, b and c.

ii. Column — Glass column 1.5 m in length, internal diameter 4 mm. Packed with 80 to 100 mesh Supelcoport supporting 10% by weight of OV-101.

Temperature — Column 200°C, Injector 245°C, Detector 300°C.

Gases — Column 20 ml $N_2 min^{-1}$ plus Detector 20 ml $N_2 min^{-1}$.

Typical retention times are shown in Table 9.

Users should optimize conditions for their own equipment and analytical separation before routine use.

A7 Sample Collection And Preservation

A7.1 Samples should be collected in glass bottles. Plastic screw caps must be checked to ensure absence of interference. Bottles should be completely filled so as to eliminate any air space.

A7.2 If further reaction between free chlorine and phenols in the sample, (to produce chlorinated phenols), is to be eliminated; an excess of sodium thiosulphate must be added to the sampling bottle after rinsing out the bottle with samples, but prior to finally filling with sample. The quantity of sodium thiosulphate added to the sample is not critical but must be sufficient to react with all the chlorine present. Normally 0.1 to 0.2 ml of a 3% w/v solution will be appropriate. For samples taken in the field it is convenient to add 2 or 3 drops of the above solution or a few crystals of the solid (sufficient to cover a microspatula tip).

A7.3 Samples should be analysed as soon as possible after receipt at the laboratory. If this is not possible within one day, then the samples should be processed through step 8.1 of the analytical procedure, when they can be stored for up to 14 days.

A8 Analytical Procedure

CAUTION—Before proceeding with the analysis read the whole method carefully, particularly Section 4 HAZARDS and Section 3 INTERFERENCES.

Step	Procedure	Notes
	Sample Pre-treatment	
A8.1	Decant the water sample into a test tube up to the calibration mark (42 ± 0.5 ml). Add 2.0 ± 0.05 ml sodium hydroxide solution (approx 1M), and mix. Add 6.0 ± 0.5 ml hexane and shake to mix thoroughly for 2 ± 0.5 min. Allow layers to separate and using a pipette draw off the hexane layer plus any interfacial emulsion to the 42 ml calibration mark. (Notes a, b, c). Discard the hexane layer.	(a) 5 min settling is usually adequate. (b) 40.1 ml ± 0.5 of the original sample remains after this procedure. (c) A clean pasteur pipette attached to a vacuum line is convenient for removing the hexane.
	Derivatisation/Extraction	
A8.2	Add 6.0 ± 0.1 ml sodium bicarbonate solution (approx 1M) and 4.0 ± 0.01 ml pentafluoro-benzoyl chloride solution to each tube, stopper and shake for 30 ± 2 min on the mechnical shaker (note d).	(d) Vigorous hand shaking for 5 ± 0.5 min may also be used.

Step	Procedure	Notes
A8.3	Allow to settle for 5 ± 1 min or until the layers have separated. Transfer most of the hexane layer to a second test tube containing 10 ± 0.5 ml sodium hydroxide solution (approx 1M) using a pasteur pipette. Stopper the second tube and shake vigorously for 1.5 ± 0.2 min (note e, f).	(e) This step destroys excess reagent. (f) Care must be taken not to transfer any of the aqueous layer from step A8.2 into the second tube.
A8.4	Allow to settle for 5 ± 1 min or until the layers have separated. Retain the hexane layer for GC analysis.	
	Blank Determination	
A8.5	A sample of blank water must be analysed with each batch of samples. Carry out steps A8.2 to A8.4 above but substituting blank water for the sample (see Section A5.2).	
	Calibration Standards	
A8.6	Calibration must be performed with each batch of samples. To a test tube containing blank water (40 ml) add 10 µl of standard solution A. Stopper and shake to mix thoroughly. This standard contains 0.1 $\mu g l^{-1}$ each phenol.	
A8.7	Repeat step A8.6 using Standard Solutions B, C, D and E. The Standards contain 0.2, 0.4, 0.7 and 1.0 $\mu g l^{-1}$ each phenol.	
A8.8	Process each standard solution through steps A8.2 to A8.4 above.	
	Gas Chromatography	
A8.9	Inject 1.5 µl of each calibration standard extract into the gas chromatograph and measure the peak height (a) produced for each phenol.	(g) Peak heights may be measured either manually or using an integrator. Peak areas may also be used.
A8.10	Repeat A8.9 using the blank extract. Measure the peak height (b) of each peak corresponding to a phenol. Plot graphs of **a—b** against concentration for each phenol (note g).	
A8.11	Inject 1.5 µl of each sample extract into the gas chromatograph, run and measure the peak height (c) of each phenol present. Read off the concentration of each phenol from the calibration graph (note h, i) using **c—b**.	(h) Calibration graphs will vary in linearity but should be linear or only slightly curved over the calibration range given. For higher phenol concentrations the sample should be diluted with water. (i) This procedure relies on the quantity of each phenol in the blank water being very low.

Table 1 Detector Sensitivity at the 1.0 μgl^{-1} Level in Water (*0.5 μgl^{-1}) and Limits of Detection

Compound	% fsd at a baseline noise level of 0.5%	Limit of Detection† μgl^{-1}
Phenol	74	0.038
2-cresol	66	0.021
3-cresol	70	0.018
4-cresol	72	0.025
2-chlorophenol	51	0.030
3-chlorophenol	47	0.031
4-chlorophenol	42	0.036
2, 3-xylenol	54	0.025
3, 4-xylenol	42	0.014
3, 5-xylenol	54	0.015
2, 4-dichlorophenol	48	0.043
2, 4, 6-trichlorophenol	53	0.032
2, 4, 5-trichlorophenol	46	0.11
Pentachlorophenol	34	0.11
*2, 4-xylenol	22	
*2, 5-xylenol	26	
*2, 6-xylenol	6	

† (9 degrees of freedom except 2, 4, 5-TCP 7 degrees)
* This limit of Detection only applies to the compounds marked

Table 2 Standard Deviations of Blank + 0.2 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	0.177	0.011	0.007	0.013	7.4	6
2-cresol	0.178	0.008	0.010	0.013	7.4	4
3-cresol	0.190	0.008	0.009	0.012	6.3	5
4-cresol	0.194	0.008	0.010	0.013	6.6	4
2-chlorophenol	0.194	0.005	0.011	0.012	6.1	4
3-chlorophenol	0.193	0.006	0.016	0.017	8.9	3
4-chlorophenol	0.194	0.005	0.014	0.015	7.8	3
3, 5-xylenol	0.196	0.007	0.012	0.014	7.0	4
2, 3-xylenol	0.183	0.007	0.011	0.013	7.2	4
3, 4-xylenol	0.191	0.006	0.006	0.009	4.4	5
2, 4-dichlorophenol	0.206	0.009	0.019	0.021	10.0	4
2, 4, 6-trichlorophenol	0.201	0.014	0.010	0.018	8.8	6
2, 4, 5-trichlorophenol	0.181	0.024	0.014	0.027	15.1	6
Pentachlorophenol	0.199	0.005	0.034	0.035	17.4	3

Table 3 Standard Deviations of Tap Water + 0.2 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	0.163	0.009	0.003	0.009	5.8	7
2-cresol	0.175	0.006	0.006	0.008	4.5	5
3-cresol	0.186	0.006	0.007	0.009	4.9	5
4-cresol	0.192	0.007	0.009	0.012	6.2	4
2-chlorophenol	0.182	0.006	0.009	0.011	5.8	4
3-chlorophenol	0.185	0.006	0.017	0.018	9.7	3
4-chlorophenol	0.187	0.009	0.016	0.018	9.7	4
3, 5-xylenol	0.193	0.012	0.000	0.012	6.2	7
2, 3-xylenol	0.178	0.009	0.005	0.010	5.5	6
3, 4-xylenol	0.187	0.008	0.000	0.008	4.3	5
2, 4-dichlorophenol	0.176	0.015	0.003	0.015	8.6	7
2, 4, 6-trichlorophenol	0.214	0.012	0.011	0.016	7.5	5
2, 4, 5-trichlorophenol	0.181	0.024	0.014	0.027	15.1	6
Pentachlorophenol	0.186	0.026	0.017	0.031	16.9	6

Table 4 Standard Deviations of River Water + 0.2 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	0.145	0.014	0.015	0.020	14.0	5
2-cresol	0.165	0.007	0.009	0.012	7.0	4
3-cresol	0.193	0.006	0.012	0.013	6.9	4
4-cresol	0.195	0.010	0.017	0.020	10.0	4
2-chlorophenol	0.184	0.007	0.011	0.013	6.9	4
3-chlorophenol	0.179	0.007	0.016	0.017	9.5	3
4-chlorophenol	0.191	0.007	0.020	0.021	10.9	3
3, 5-xylenol	0.190	0.012	0.013	0.017	8.9	5
2, 3-xylenol	0.165	0.008	0.013	0.015	9.0	4
3, 4-xylenol	0.182	0.007	0.004	0.008	4.5	6
2, 4-dichlorophenol	0.180	0.006	0.009	0.011	6.1	4
2, 4, 6-trichlorophenol	0.180	0.009	0.004	0.010	5.7	6
2, 4, 5-trichlorophenol	0.176	0.018	0.000	0.018	10.1	7
Pentachlorophenol	0.131	0.019	0.004	0.020	15.0	7

Table 5 Standard Deviations of Blank + 1.0 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	0.99	0.043	0.077	0.089	9.0	4
2-cresol	0.99	0.036	0.014	0.038	3.9	7
3-cresol	1.02	0.027	0.000	0.027	2.6	7
4-cresol	0.99	0.017	0.040	0.043	4.4	3
2-chlorophenol	1.00	0.023	0.092	0.095	9.5	3
3-chlorophenol	0.99	0.041	0.072	0.083	8.4	4
4-chlorophenol	0.96	0.035	0.074	0.082	8.5	4
3, 5-xylenol	1.01	0.029	0.075	0.081	8.0	3
2, 3-xylenol	0.94	0.035	0.041	0.054	5.7	5
3, 4-xylenol	1.00	0.040	0.060	0.073	7.3	4
2, 4-dichlorophenol	0.97	0.023	0.078	0.081	8.4	3
2, 4, 6-trichlorophenol	1.03	0.050	0.056	0.075	7.2	5
2, 4, 5-trichlorophenol	0.95	0.041	0.034	0.053	5.6	5
Pentachlorophenol	0.98	0.062	0.100	0.118	12.1	4

Table 6 Standard Deviations of Tap Water + 1.0 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	1.00	0.054	0.064	0.084	8.4	5
2-cresol	0.98	0.031	0.000	0.031	3.1	7
3-cresol	1.03	0.027	0.020	0.034	3.3	6
4-cresol	1.02	0.032	0.033	0.046	4.5	5
2-chlorophenol	1.03	0.059	0.054	0.080	7.8	5
3-chlorophenol	1.03	0.059	0.080	0.100	9.7	4
4-chlorophenol	0.99	0.057	0.054	0.079	8.0	5
3, 5-xylenol	1.02	0.033	0.092	0.097	9.5	3
2, 3-xylenol	0.94	0.008	0.051	0.051	5.4	3
3, 4-xylenol	1.03	0.033	0.065	0.073	7.1	4
2, 4-dichlorophenol	0.99	0.068	0.066	0.095	9.6	5
2, 4, 6-trichlorophenol	1.10	0.018	0.049	0.053	4.8	3
2, 4, 5-trichlorophenol	1.01	0.059	0.060	0.084	8.4	5
Pentachlorophenol	0.94	0.058	0.037	0.069	7.3	6

Table 7 Standard Deviations of River Water + 1.0 μgl^{-1} each Phenol

Compound	Mean Conc Found μgl^{-1}	Standard Deviations μgl^{-1}			% S_c	Degrees of Freedom
		S_w	S_b	S_c		
Phenol	1.02	0.066	0.126	0.142	13.9	4
2-cresol	0.92	0.036	0.007	0.037	4.0	7
3-cresol	1.02	0.023	0.084	0.087	8.5	3
4-cresol	0.99	0.026	0.043	0.050	5.1	4
2-chlorophenol	1.03	0.024	0.132	0.134	13.0	3
3-chlorophenol	1.04	0.037	0.179	0.183	17.6	3
4-chlorophenol	1.01	0.032	0.142	0.146	14.4	3
3, 5-xylenol	0.99	0.020	0.093	0.095	9.6	3
2, 3-xylenol	0.87	0.035	0.046	0.058	6.7	4
3, 4-xylenol	0.96	0.034	0.057	0.066	6.9	4
2, 4-dichlorophenol	1.01	0.051	0.205	0.211	20.8	3
2, 4, 6-trichlorophenol	1.06	0.063	0.080	0.102	9.7	4
2, 4, 5-trichlorophenol	1.04	0.066	0.147	0.161	15.5	4
Pentachlorophenol	0.87	0.094	0.083	0.125	14.4	5

Table 8 % Recoveries for each Phenol

Compound/μgl^{-1}	Blank		Tapwater		River Water	
	0.2	1.0	0.2	1.0	0.2	1.0
Phenol	89	99	82	100	73	102
2-cresol	89	99	88	98	83	92
3-cresol	95	102	93	103	97	102
4-cresol	97	99	96	102	98	99
2-chlorophenol	97	100	91	103	92	103
3-chlorophenol	97	99	93	103	90	104
4-chlorophenol	97	96	94	99	96	101
3, 5-xylenol	98	101	97	102	95	99
2, 3-xylenol	92	94	89	94	83	87
3, 4-xylenol	96	100	94	103	91	96
2, 4-dichlorophenol	103	97	88	99	90	101
2, 4, 6-trichlorophenol	101	103	107	110	90	106
2, 4, 5-trichlorophenol	91	95	91	101	88	104
Pentachlorophenol	100	98	93	94	66	87

Table 9 Typical Retention Times for a Blank + 0.2 $\mu g l^{-1}$ each Phenol

Compound	10 m BP5 Capillary	10% OV-1
	mins	mins
Phenol	2.34	4.80
2-cresol	3.21	6.30
3-cresol	3.52	6.75
4-cresol	3.66	6.90
2-chlorophenol	4.48	8.20
3-chlorophenol	4.62	8.40
4-chlorophenol	4.77	8.80
2, 3-xylenol	5.34	.
3, 4-xylenol	5.88	.
3, 5-xylenol	5.11	.
2, 4-dichlorophenol	7.14	13.50
2, 4, 6-trichlorophenol	8.41	19.70
2, 4, 5-trichlorophenol	8.76	.
Pentachlorophenol	10.66	.
2, 4-xylenol	4.85	.
2, 5-xylenol	4.71	8.60
2, 6-xylenol	4.43	.

Fig. 1 Typical Chromatograms for Method A

a. A blank

b. Synthetic Standards (lugl^{-1})

c. A river sample

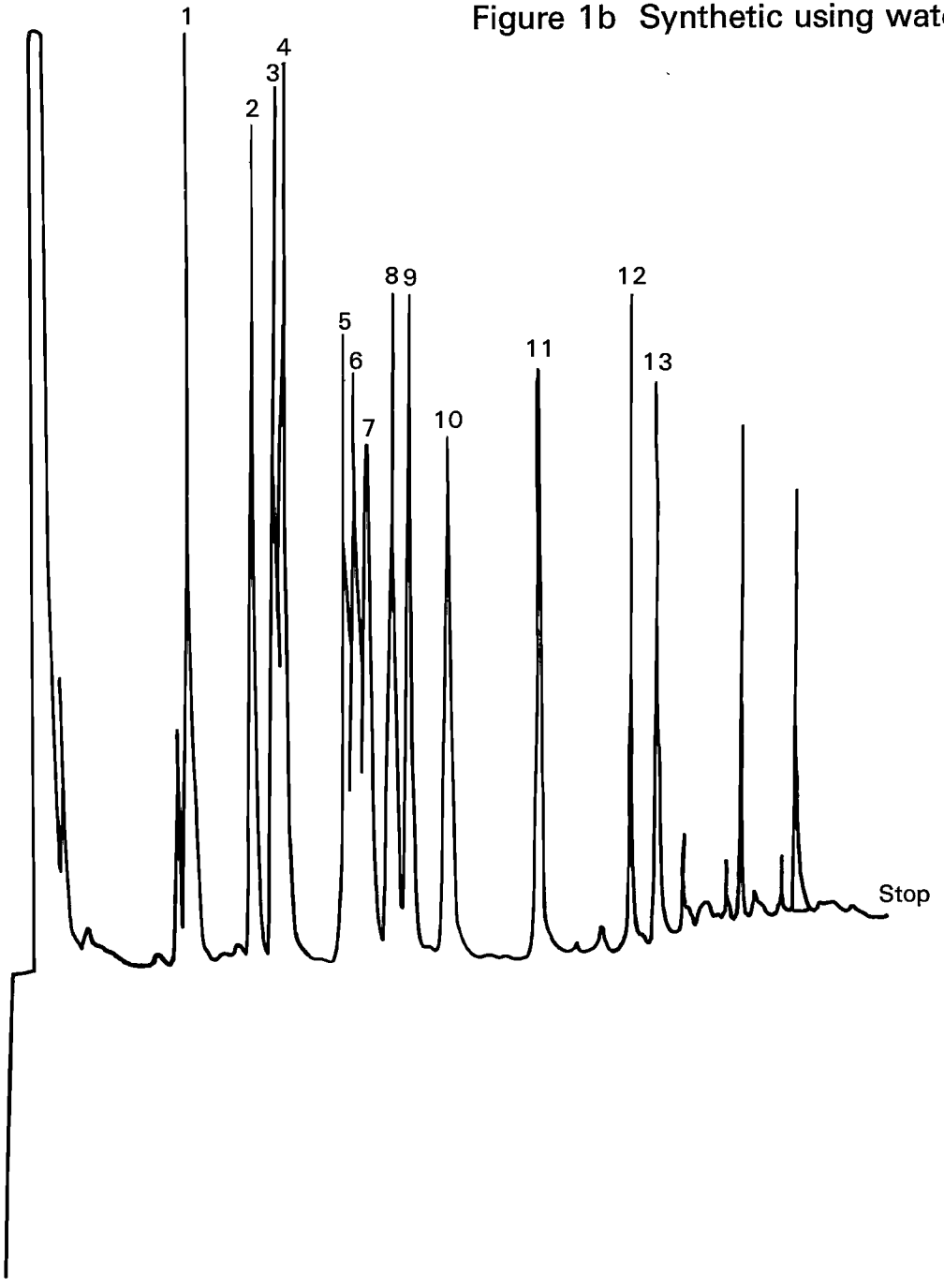
Fig. 1 Typical Chromatograms for Method A

a. A blank

b. Synthetic Standards (lugl^{-1})

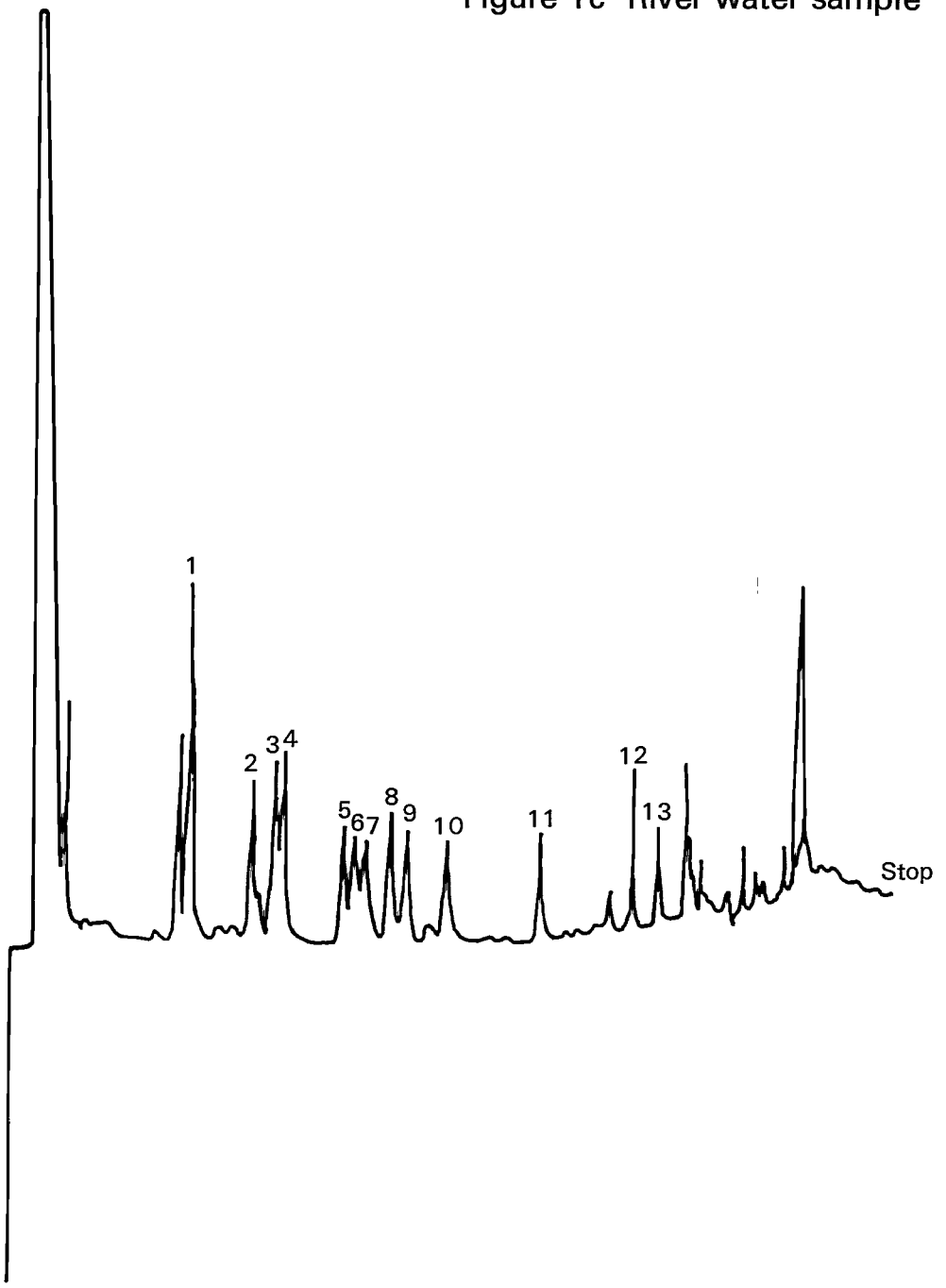
c. A river sample

Figure 1b Synthetic using water in Figure 1a



	Name	Time
1	Phenol	2.34
2	2-Cresol	3.21
3	3-Cresol	3.52
4	4-Cresol	3.66
5	2-Chlorophenol	4.48
6	3-Chlorophenol	4.62
7	4-Chlorophenol	4.77
8	3.5 Xylenol	5.11
9	2.3 Xylenol	5.34
10	3.4 Xylenol	5.88
11	2.4 Dichlorophenol	7.14
12	2.4.6 - Trichlorophenol	9.41
13	2.4.5 - Trichlorophenol	9.76

Figure 1c River water sample



	Name	Time
1	Phenol	2.34
2	2-Cresol	3.22
3	3-Cresol	3.53
4	4-Cresol	3.65
5	2-Chlorophenol	4.48
6	3-Chlorophenol	4.63
7	4-Chlorophenol	4.77
8	3.5 Xylenol	5.11
9	2.3 Xylenol	5.35
10	3.4 Xylenol	5.88
11	2.4 Dichlorophenol	7.13
12	2.4.6-Trichlorophenol	9.41
13	2.4.5-Trichlorophenol	9.76

B Determination of Phenol by Bromination and Electron Capture Gas Chromatography

B1 Performance Characteristics of the Method

B1.1	Substances determined	Phenol
B1.2	Type of Sample	Rivers and potable waters
B1.3	Basis of Method	Bromination of the Phenol in aqueous solution, extraction of the 2, 4, 6-tribromophenol into n-Hexane and analysis of the n-Hexane extract by electron-capture gas chromatography.
B1.4	Range of application	Up to at least 10 $\mu g\ l^{-1}$
B1.5	Calibration curve	Linear to at least 10 $\mu g\ l^{-1}$
B1.6	Standard deviation	Estimated for river water as about 0.1 $\mu g\ l^{-1}$ at concentrations of 0.6–5 $\mu g\ l^{-1}$ (ref 2)
B1.7	Limit of detection	0.25 $\mu g\ l^{-1}$ (ref 2)
B1.8	Sensitivity	Depends on detector used.
B1.9	Bias	Not known.
B1.10	Interference	Any organic material which passes through the procedure, has a strong electron affinity and has similar gas chromatographic characteristics to the determinand; or which reacts with bromine to form the same or similar compounds eg 2 and 4 mono and 2, 4-bromophenols.

Data from North West W A Consumer Protection Laboratory, Huntington, Cheshire

B2 Principle

The phenol is brominated in aqueous solution. The 2, 4, 6-tribromophenol thus formed is then extracted into n-hexane. The solution is then examined by gas chromatography with elelctron capture detection, such that the 2, 4, 6-tribromophenol is well separated on the column from the solvent as well as from other electron capturing substances resulting from the procedure or already present in the sample (references 2, 3 and 4). Some other phenols may be analysed using this technique.

B3 Interferences

Other phenols reacting to form 2.4.6 tribromophenol or eluting close to that phenol and activating the detector will interfere with the method when used specifically for phenol. If used to detect other phenols, analysts are advised to evaluate for themselves.

B4 Hazards

n-Hexane is flammable.

B5 Reagents and Standards

All organic reagents must be of sufficient purity such that they do not give rise to significant interfering peaks in the gas chromatographic analysis of the solvent extract. Analytical Reagent grade should be used for inorganic reagents. Reagents and standards should be stored in all-glass vessels with no contact with organic matter, such as plastics etc.

B5.1 Water

The water used for blank determinations and preparation of standard solution should have a phenol content that is negligible in comparison with the smallest concentration to be determined. Unchlorinated borehole water is often suitable.

B5.2 Bromide/Bromate solution

Dissolve 10±0.1 g potassium bromide and 2.8 g±0.1 g potassium bromate in 1000±1 ml of phenol-free water.

B5.3 Hydrochloric acid d_{20} 1.18 (AR grade)

B5.4 Sodium thiosulphate solution 0.1 N

Dissolve 24.8±0.2 g of Sodium thiosulphate pentahydrate in 1000±5 ml of water.

B5.5 n-Hexane

Pesticide grade.

B5.6 Standard Solutions

See C5.7–C5.11, A5.11 or D5.5–D5.7.

B6 Apparatus

All glassware should be washed with chromic acid followed by thorough rinsing with phenol-free water. Distillation equipment should be steam cleaned with water acidified with hydrochloric acid. Separating funnels should be rinsed with the extraction solvent before use. All syringes must be cleaned and checked by GC before use. A separate set of glassware should be used for each level of concentration of calibration standards and for samples. (See also A6).

B6.1 Glass stoppered graduated separating funnel 250 ml, glass tap (grease free) or with PTFE tap.

B6.2 Pipettes, various (1–10 ml).

B6.3 Erlenmeyer flasks, 250 ml Pyrex.

B6.4 Nessler Cylinders, 100 ml.

B6.5 Syringe 5 μl.

B7 Gas Chromatograph

A gas chromatograph with electron capture detector. This should be operated in accordance with the manufacturers' instructions. Various GC columns are potentially suitable for the analysis. However, the method is based on the following GC conditions;

Column:	Glass 1.5 m×4 mm ID 4.5% Carbowax 20 M on 80–100 Anakrom Q
Detector:	Electron capture
Detector oven temperature:	300°C
Column oven temperature:	170°C
Injector temperature:	250°C
Carrier gas:	N_2; 60 ml min^{-1} (oxygen-free)
Chart speed:	1 cm min^{-1}
Injection volume:	5 μl
Typical retention time (from injection):	7 min

B8 Sample Collection and Preservation

See sections C7.1 and C7.2. If storage is essential, acidification to a pH value of 2.5±0.1 and refrigeration at 4±1°C will prevent loss of phenol for at least 24 hours.

B9 Analytical Procedure

Step	Procedure	Notes
B9.1	**Distillation Stage**	
	Place 100±1 ml of sample in a 250 ml round bottomed flask containing a few anti-bumping granules. Connect flask to a suitable distillation apparatus and collect the distillate (90±1 ml). Add 10±0.5 ml of Phenol-free water to the distillation flask and continue distilling until the total distillate volume collected is 100±1 ml.	
B9.2	**Bromination Stage**	
	Transfer the distillate to a 250 ml Erlenmeyer flask. Add 10±0.2 ml of (B5.2) Bromide/Bromate solution using a pipette. Then add 1±0.05 ml of Hydrochloric acid (B5.3)	
	Shake and allow to react for 10±0.5 minutes (note a). Then add 1.5±0.05 ml, of 0.1 N Sodium Thiosulphate solution and shake. Transfer this solution to a 250 ml separating funnel and add 10±0.05 ml n-Hexane using a pipette (note b).	(a) The Solution should become straw coloured. (b) The solvent should not be pipetted by mouth.
	Shake the mixture vigorously for 2±0.1 min venting frequently. Allow the layers to separate (note c)	(c) Typically this should take about 1 minute.
	Run off the aqueous layer and discard. Collect the remaining solvent in a 10 ml stoppered test tube containing 1±0.1 g anhydrous sodium sulphate.	
B9.3	**Blank Determination**	
	A blank must be analysed with each batch of samples (note d).	(d) All blanks and standards should be processed through stages B9.1 and B9.2 prior to GC analysis.
B9.4	**Calibration Standards**	
	Calibration standards must be run with each batch of determinations. A control standard (2.5 $\mu g\, l^{-1}$) is also required (note d).	

Step	Procedure	Notes
B9.5	**Gas Chromatograph**	
	Set up the instrument according to the manufacturers' instructions and optimize operating conditions (see section B6).	
B9.5.1	Run blank and determine peak area (note e).	(e) Observe the general blank quality and then check specifically at the 2, 4, 6-tribromophenol retention time. Ideally this area should be zero.
B9.5.2	Run calibration standards and determine peak area for the 2, 4, 6-tribromophenol peak.	
B9.5.3	Run samples and determine peak area for the 2, 4, 6-tribromophenol peak.	
B9.5.4	Calculate results by comparison of peak areas.	

B10 Determination of Other Phenols

Some other phenols brominate to give derivatives soluble in hexane, but with different GC peaks. These may be determined in like manner using their own brominated derivative peaks.

B10.1 2 and 4 monobromo and 2, 4 dibromophenol will be determined as if they were phenol, as will 2, 4, 6-tribromophenol itself. Similar confusions may occur if other phenols are analysed. However the pre-existence of such bromophenols is rare in most samples.

2,4,6-tribromophenol may be determined by omitting the bromination steps in stage D7.2, and extracting directly into hexane

C Determination of Phenols in Water by Reverse Phase HPLC with Isocratic Elution and Electochemical Detection

C1 Performance Characteristics of the Method
(see also C9)

C1.1	Substances Determined	Phenol and any other organic compound undergoing oxidation under the electrochemical conditions specified.
C1.2	Type of Sample	Raw and potable waters.
C1.3	Basis of Method	Direct injection of sample via a sample loop onto a divinyl-benzene/polystyrene copolymer column followed by reverse phase HPLC using coulometrically efficient electrochemical detection.
C1.4	Range of Application	Up to at least 100 μl^{-1}
C1.5	Calibration Curve	Linear to at least 100 $\mu g\ l^{-1}$
C1.6	Standard Deviation (within batch) (5 degrees of freedom)	

	Phenol Conc ($\mu g\ l^{-1}$)	Std Dev ($\mu g\ l^{-1}$)
Standards	0.5	0.019
	1.0	0.034
Samples		
River Water	0.023	0.012
River spiked	1.048	0.063

C1.7	Limit of detection (5 degrees of freedom)	0.034 $\mu g\ l^{-1}$ phenol
C1.8	Bias	Less than 10% as indicated by recovery data from spiked samples. (Table 10).
C1.9	Interference	Any compound which has similar chromatographic characteristics and electrochemical qualities to the determinand.
C1.10	Time required for analysis.	6 samples per hour.

See also Sections C9 and C10.

Table 10 Recovery of phenol from 'spiked' samples

Sample	Initial conc as Phenol ($\mu g\ l^{-1}$)	Conc of Phenol added ($\mu g\ l^{-1}$)	Conc of Phenol found ($\mu g\ l^{-1}$)	% Recovery of added Phenol
Raw Water	0.023	1.00	1.048	102.4%

Test data from North West Water Authority. Consumer Protection Laboratory, Huntington, Cheshire.

C2 Principle

Separation of the phenols is achieved by direct aqueous injection of the sample via a sample loop onto a polystyrene/divinyl benzene copolymer column. The elution takes place isocratically using an acetonitrile/water mobile phase buffered to pH 9.2. Detection is via a coulometrically efficient dual electrode electrochemical detector.

C3 Interference

Any compound having similar LC characteristics to the determinand and undergoing electrochemical oxidation at the detector electrode at the potentials used.

C4 Hazards

C4.1 Phenol and acetonitrile are both toxic and should be handled with care.

C4.2 Acetonitrile is inflammable and should be handled with care, ensure that no naked flames are in the vicinity.

C5 Reagents and standards

C5.1 Water (for standard and blank preparation)

Water used for standard and blank preparation should have a phenol content which is negligible compared with the smallest concentration of phenol to be determined in the samples. Unchlorinated borehole water was found to be the most suitable.

C5.2 Water (Mobile phase)

HPLC grade water.

C5.3 Acetonitrile

HPLC grade Acetonitrile.

C5.4 Sodium Phosphate, dibasic

Gold Label grade (Aldrich Chemical Company Inc).

C5.5 10 mM Dibasic Sodium Phosphate Solution

Dissolve 1.420±0.005 g of sodium phosphate (C5.4) in 1,000±1 ml HPLC grade water C5.2).

C5.6 Acetonitrile/Dibasic sodium phosphate mobile phase

Add 1,000±1 ml 10 mM dibasic sodium phosphate solution (5.5) to 1,000±1 ml HPLC grade acetonitrile (C5.3) mix thoroughly and degas.

C5.7 Phenol Stock Solution, 1 ml ≡ 1 mg of phenol

Dissolve 1,000±0.001 g of phenol (C_6H_5OH) in water (C5.1) and dilute to 1 litre in a calibrated flask. Store in the dark in a dark glass bottle. Prepare monthly. CAUTION: PHENOL IS POISONOUS AND CORROSIVE.

C5.8 Phenol intermediate standard solution A

1 ml ≡ 50 μg phenol

Dilute 5.00±0.01 ml of phenol stock solution (5.7) to 100 ml with water (C5.1) in a calibrated flask and mix. This solution should be prepared freshly on the day of use.

C5.9 Phenol intermediate standard solution B

1 ml ≡ 500 ng phenol

Dilute 2.50±0.01 ml of the phenol intermediate standard solution A(C5.8) to 250 ml with water (5.1) in a calibrated flask and mix. This solution should be prepared freshly on the day of use.

C5.10 Phenol working standard solution C

1 ml ≡ 1.0 ng phenol

Dilute 0.200±0.005 ml of the phenol intermediate standard solution B(C5.9) to 100 ml with water (C5.1) in a calibrated flask and mix. This solution should be prepared freshly within one hour of use.

C5.11 Phenol working standard solution D

1 ml ≡ 0.5 ng phenol

Dilute 0.100±0.002 ml of the phenol intermediate standard solution B(C5.9) to 100 ml with water (C5.1) in a calibrated flask and mix. This solution should be prepared freshly within one hour of use.

Preservatives (see C7)

C5.12 Copper sulphate solution

Dissolve 50 g $CuSO_4$, $5H_2O$ in 400 ml of Water and dilute to 500±5 ml. Stored in a glass bottle this reagent is stable for at least 1 year.

C5.13 Phosphoric Acid

Phosphoric acid, d_{20} 1.6 at 20°C.

C6 Apparatus

C6.1 LC pump

A pulse free HPLC pump is required capable of delivering up to 1.0 ml min^{-1} with a flow rate accuracy of 1% and a flow rate precision of 0.3%. A dual reciprocating pump is preferable. To determine the test data above, a Kontron 420 HPLC pump fitted with a microbore head and flow-through Bourdon tube type pulse damper was used.

C6.2 Coulometric Detector

ESA Coulochem 5100A detector with a model 5011 Analytical Cell and model 5020 Guard Cell or equipment with equivalent performance.

C6.3 Injection Valve

Rheodyne 7125 injector or similar, fitted with a 100 μl sample loop.

C6.4 Sample syringe

250 μl Rheodyne or similar syringe (Hamilton 725 SNR).

C6.5 Flushing syringe

2 ml syringe with Luer tip fitted wit a 7125 needle port cleaner.

C6.6 LC Column

A 15 cm 5 μm PRP-1 reverse phase Styrene/Divinyl benzene copolymer (Hamilton Co) or similar should be used. Prior to this analytical column a 5 × 3.0 mm guard cartridge (PLRP-S 100 Polymer Laboratories Ltd) or similar must be fitted.

C6.7 Computing Integrator

C6.8 The apparatus if configured as shown in Fig 2:

C7 Sample Collection and Preservation

C7.1 The samples must be collected in glass bottles (Borosilicate 250 ml) with ground glass stoppers, previously cleaned by standing overnight in chromic acid (Caution: **Chromic acid is corrosive**). The stopper must be replaced immediately after sampling ensuring that no air bubbles are present.

C7.2 As no preservative is used, samples must be analysed as soon as possible and no later than 2 hrs after sampling as chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentration of the phenols.

C7.3 If analysis is not to be carried out shortly after sampling then the addition of preserving agents is essential. An additional distillation step is then required (C8.7) to remove these prior to injection (Caution: The injection of any sample containing preservative onto the analytical column may cause serious and irreversible damage to the detector). To preserve a sample, phosphoric acid (0.34±0.02 ml) (C5.13) and 2.5±0.1 ml of copper sulphate solution (C5.12) are added per 250 ml sample immediately after sampling and the stopper replaced, ensuring that no air bubbles are present. The sample is stored at 4°C and analysed as soon as possible and not later than 24 hours after sampling.

Step	Procedure	Notes
C8.1	**Coulometric Detector Set-up** (note a)	(a) Other instruments may require slightly different settings.

The following potentials and gain settings are used:

Detector 1 potential	=250 mV
Detector 2 potential	=650 mV
Guard Cell potential	=750 mV
Gain (Detector 2)	=150
Response time (Detector 2)	=10 seconds

Only the current from detector 2 is monitored.

C8.2 **Chromatographic Conditions** (note a)

Mobile phase flow is 0.50 ml min^{-1}.

Preparation of calibration curve

C8.3 Flush injector with 2×2 ml water (C5.1). Inject 2×250 µl aliquots of the blank water (C5.1) into the analytical loop with the injector in the 'load' position. Rotate injector to the 'inject' position and monitor the current from detector 2 with the computing integrator. When phenol peak has eluted rotate the injector to 'load' and integrate the data obtained.

C8.4 Repeat C8.3 using phenol working standard solution C(C5.10) followed by phenol working standard solution D(C5.11).

C8.5 Prepare a calibration curve of peak area against concentration of the standards (corrected for the blank).

Use of linear regression

C8.6 Instead of constructing a calibration graph, linear regression may be used to calculate coefficients and constants for a $y=mx+c$ type equation.

Removal of preservative (if necessary)

If the sample contains no preservative go directly to step C8.8.

C8.7 Place 100±1 ml of the sample in a 250 ml round bottom flask containing a few anti-bumping granules. Connect the flask to a suitable distillation apparatus and collect 90 ml of distillate. Add 10±0.02 ml water (C5.1) and continue distilling until 100 ml of distillate has been collected.

Proceed with step C8.8.

Analysis of samples

C8.8 Repeat step C8.3, injecting the sample.

C8.9 Using the calibration curve (C8.5) or linear regrssion (C8.6) convert the peak area of the phenol peak in the sample into concentration of phenol in the sample.

C9 Validation of Method

Statistical evaluation of the method was carried out according to the procedures given by Cheeseman and Wilson (8) (viz precision of standards and samples and spiking recovery).

The precision target for the determination of phenol in raw water had been set at a total standard deviation of an individual determination of 10% of concentration or 0.1 µg l^{-1} phenol, whichever was the greater. To test the performance of the method against this target duplicate analyses were carried out on each of 5 days, of borehole water

(blank), 0.5 $\mu g\ l^{-1}$ and 1.0 $\mu g\ l^{-1}$ standards, river water from the River Dee at Huntington and River Dee water spiked with a 1.0 $\mu g\ l^{-1}$ phenol addition. The results from the last 2 samples were used to calculate the spiking recovery.

The results were analysed to derive the corresponding within-batch (S_w), between-batch (S_b) and total (S_t) standard deviations. The results are summarised in Table 11; the number of degrees of freedom were derived from duplicate analyses on each of 5 days and are given in parentheses.

C9.1 Users should carry out a similar procedure for their own samples prior to putting the method into routine use or reporting a special investigation.

Table 11 Precision of Analytical Results

Solution	Standard deviation* $\mu g\ l^{-1}$ phenol			Mean concn found $\mu g\ l^{-1}$ phenol
	S_w	S_b	S_t	
Blank (Borehole water)	0.006(5)	—	—	0.022
0.50 $\mu g\ l^{-1}$ phenol	0.019(5)	N.S.(4)	0.023(6)	0.483
1.00 $\mu g\ l^{-1}$ phenol	0.034(5)	N.S.(4)	0.038(6)	1.000
R. Dee water	0.012(5)	0.021(4)	0.025(4)	0.023
Dee + 1.00 $\mu g\ l^{-1}$ phenol	0.063(5)	0.054(4)	0.083(7)	1.048

* Figures in parentheses are the degrees of freedom. N.S. indicates that the result is not statistically significant.

Analysis of variance showed that the 0.5 and 1.0 $\mu g\ l^{-1}$ phenol standards showed no significant between-batch variability.

A limit of detection of 0.034 $\mu g\ l^{-1}$ of phenol was calculated by using the formula $2t\sqrt{2}(S_w)$, where t is Student's single sided t for a probability of 0.10 and S_w is the within-batch standard deviation of the blanks. This satisfies a target of 0.05 $\mu g\ l^{-1}$ of phenol in raw and potable water determinations.

The spiking recovering of 102.4% was considered satisfactory.

C10 Scope of Method

Although validation is only given for monohydric unsubstituted phenol many other substituted phenols can be determined by this method as shown by the chromatogram in Fig 3 obtained under the stated conditions.

Figure 2

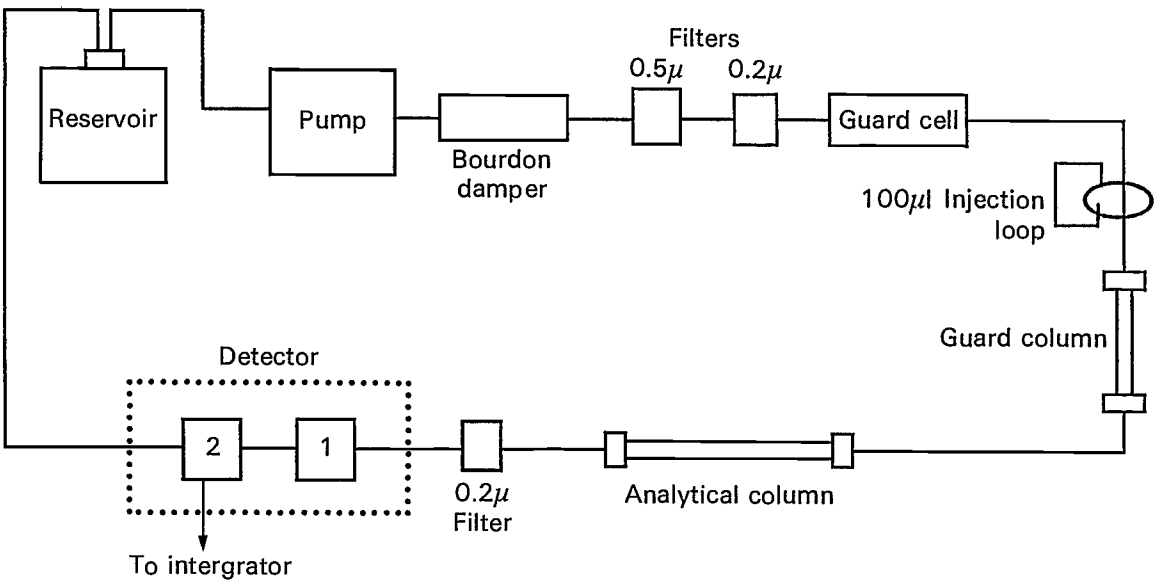
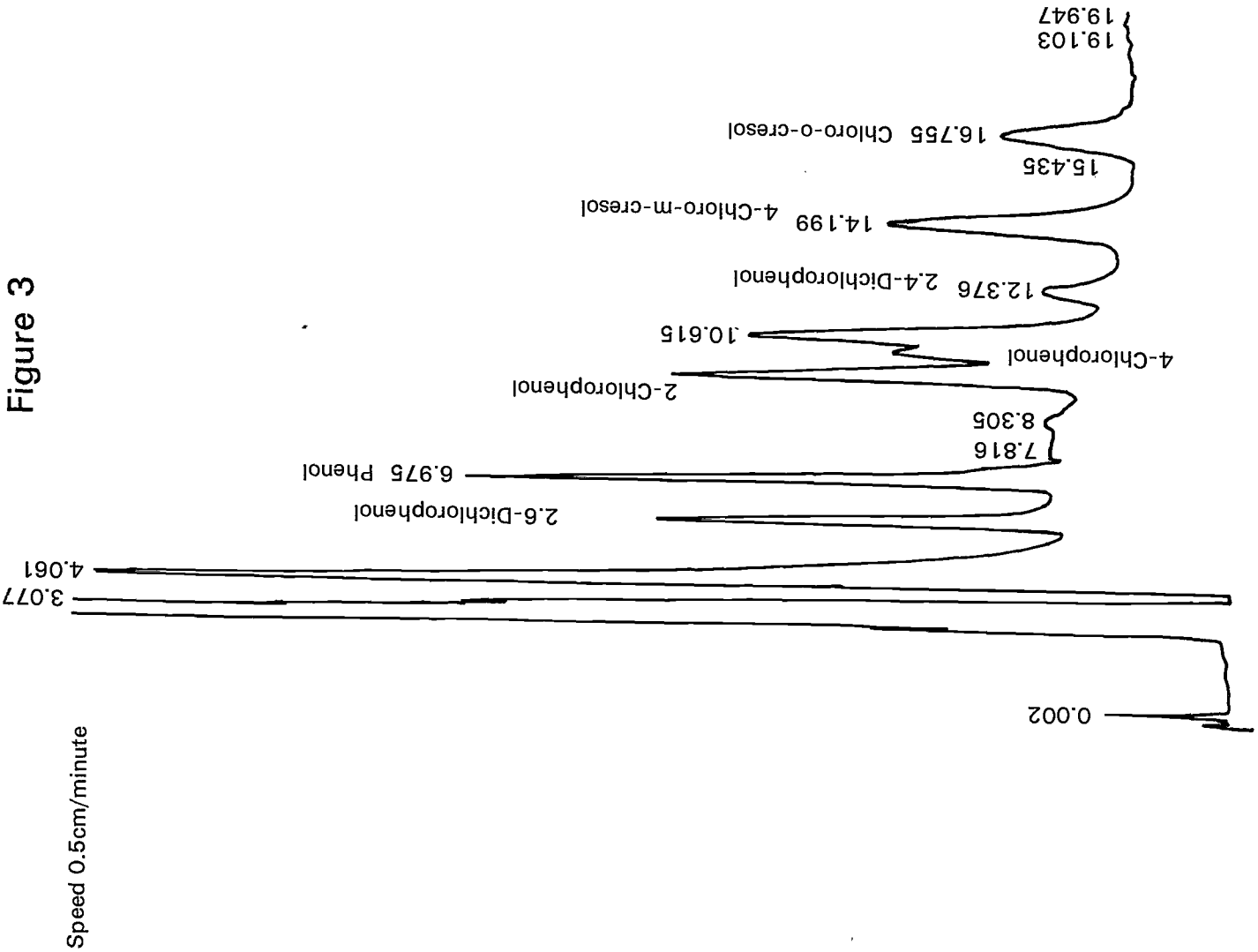

Reservoir
Pump
Bourdon damper
Filters
0.5μ
0.2μ
Guard cell
100μl Injection loop
Guard column
Detector
2
1
0.2μ Filter
Analytical column
To intergrator

Figure 3


0.002
3.077
4.061
2.6-Dichlorophenol
6.975 Phenol
7.816
8.305
2-Chlorophenol
4-Chlorophenol
10.615
12.376 2.4-Dichlorophenol
14.199 4-Chloro-m-cresol
15.435
16.755 Chloro-o-cresol
19.103
19.947
Speed 0.5cm/minute

D Determination of Phenols in Water by HPLC with Ultra-violet detection (electrochemical detection may also be used)

Mainly intended for investigations and confirmation

D0 Introduction

There are very many phenols known, and though the number causing problems for the water industry are only a fraction of these, unexpected phenols do sometimes occur. This 'method' or more strictly series of procedures is intended for the identification of phenols when the unexpected happens.

Three basic procedures are given, but each is capable of variation. It is suggested that an analyst without any prior information as to which is the best way to separate the phenols in the sample being studied should try a small aliquot with each of the main methods (using D8 extraction with D10) and then, if necessary, proceed to variants of these procedures. Often the analyst will have some prior knowledge of what is likely to be present and prior experience of these procedures which will help in making the initial examinations. Even so, be prepared for the unexpected.

This method relies on making final confirmation by comparison with known materials. If however the phenol proves to be one not known or available to an analyst, the procedure is capable of adaptation to give small quantities of material which could be used for conventional organic compound identification procedures such as Linear Temperature Programmed Retention Indexes, Mass Spectrometry, UV and IR absorption spectrometry or even micro-chemical testing.

D1 Performance Characteristics of the Method

D1.1	Substances determined	Phenols and substituted phenols.
D1.2	Type of sample	Potable waters; and rivers depending upon phenol concentration and background contamination. Two extraction procedures have been evaluated, along with three HPLC procedures. Test data is given for the combinations $a_1 + b_1$ (procedure D8), $a_2 + b_2$ (procedure D9), and $a_1 + b_3$ and $a_2 + b_3$ (procedure D10). These various stages are:—
D1.3	Basis of the method	(a) Concentration of phenols from water sample using:— a1. C18 extraction cartridge (note Cyclohexyl or graphitized carbon may be used with other performance characteristics). a2. Automated sample preconcentration on a reverse phase precolumn. (b) Separation of phenols by HPLC:— b1. Isocratically using a buffer of orthophosphoric acid: Methanol: Ethanol. Two different ratios of eluent composition may be used dependent on the phenols expected.

		b2. Extraction and gradient HPLC using a methanol/phosphate buffer mobile phase and ultra-violet detection. (Steps a_2 and b_2 are a continuous operation). b3. Gradient elution using 0.1% aqueous acetic acid and methanol.
D1.4	Range of application	Variable depending upon phenol and concentration volume and technique. The use of an electrochemical detector will extend the range downwards. The method has been tested up to 10 $\mu g\ l^{-1}$ but recovery may be substantially less than 100% at higher concentrations. Reduction of the volume sampled can extend the range upwards. Having identified or confirmed the phenols present, users should check recoveries and, if more accurate results are required, repeat the analysis using methods A, B or C.
D1.5	Calibration curve	Linear provided the breakthrough volumes at the concentration stage have not been exceeded. Breakthrough volumes are phenol and sample type dependent. Analysts should check this for themselves.
D1.6	Standard deviation	Depending upon concentration technique. See tables 15 and 18.
D1.7	Limit of detection	Dependant upon phenol and concentration technique. See table 19.
D1.8	Bias	The lower homologues may have poor recoveries due to their low breakthrough volumes.
D1.9	Interferences	(a) Any substance with similar elution time absorbing at the wavelength measured (214, 254 or 280 nm) will interfere. The use of dual wavelength detection may alleviate the problem. or (b) Any substance with similar elution time causing a response with the electrochemical detector if used. (c) Substances which elute just prior to the phenol peak and mask early peak detection.
D1.10	Time for analysis	For Procedure D8 With multiple sample extraction; typically 2 samples per hour. For procedure D9

	Dependent on the complexity of the sample analysed. For complete separation of components listed in Table 16, typical analysis time allowing for instrument re-equilibration is 37 minutes. For procedure D10 the time is dependent on the initial extraction procedure used and resembles that for D8 or D9 accordingly.
D1.11 Additional data	See tables 12–20 and Figures 4–6 and 8–10.

Test data was provided as follows

D8 (both eluents) Southern WA, Otterbourne
D9 Philips Scientific Cambridge
D10 South West WA, Countess Wear

D2 Principle

There are three possible ways of making this analysis

D2.1 The phenols are extracted from acidified water using a C_{18} extraction cartridge; followed by elution with methanol; water. The eluted sample is made *up to* volume and an aliquot is separated at pH 3.1 isocratically on a Chromsphere C_{18} Column at 45°C. The absorption is measured at 214 nm (alternatively or in conjunction with an electrochemical detector). The phenols are identified and quantified by comparison with known standard mixtures using a computing integrator.

D2.2 A liquid sample pump supplies acidified and filtered sample to a C8 reverse phase precolumn mounted across a pneumatically operated 6-port Rheodyne valve. Phenols are preconcentrated by absorption onto the reverse phase packing. When the required volume of sample has passed through the precolumn, the valve is operated, and mobile phase supplied by the primary liquid pump, elutes preconcentrated sample components to the head of a reverse phase C_{18} analytical column. Separation by gradient elution takes place on the analytical column at ambient temperature using methanol and phosphate buffer as the mobile phase. Ultra-violet absorbance of the column effluent is measured at 280 nm, and phenols are identified and quantified by comparison with known standard mixtures.

D2.3 Following extraction as in D2.1 the sample is separated using gradient elution with acetic acid methanol mixtures on a suitable C_{18} column at 30°C. The absorption is measured at 214 nm and 254 nm. The phenols are identified and quantified by comparison of the retention times, relative UV absorbance and peak area with known standard mixtures.

D3 Interferences

See D1.9 above

D4 Hazards

Phenols are toxic, can be absorbed through the skin on long exposure and may cause burns. 2.4.6 trichlorophenol has been suspected of being carcinogenic.

High pressure and vacuum are used, so adequate care and screening are necessary.

Methanol is highly flammable and toxic. Avoid breathing vapour and contact with skin and eyes. Orthophosphoric acid d_{20} 1.7 is corrosive and causes burns. Acetic acid is flammable and causes severe burns.

D5 Reagents and Standards

These are dependent upon procedure used

Reagents for all procedures

D5.1 Methanol (HPLC grade) Degassed.

D5.2 Water. Either bottled HPLC grade water, or deionized water purified to reagent grade. Filter through a 0.45 μm filter. Store in glass bottles free of all organic contamination. Degas and use as required.

D5.3 Orthophosphoric acid d_{20} 1.7 analytical reagent grade (15.7 Molar)

D5.4 Helium for degassing

Reagents for Procedure D8 and possibly D10.

D5.5 Ethanol absolute.

D5.6 1 Molar phosphoric acid.

Dilute with care 6.1 mls of orthophosphoric acid (D5.3) to 100 ml with HPLC water (D5.2).

D5.7 Buffer 45:40:15 (0.01M H_3PO_4:Methanol:Ethanol)

Mix together 450 ml of HPLC water (D5.2); 400 ml of Methanol (D5.1) and 150 ml of Ethanol (D5.5). Add 4.5 ml of 1 M phosphoric acid (D5.6).

Mix and filter through a 0.45 μm filter and degas.

This solution is prone to contamination and it is recommended that it be prepared just prior to use.

D5.7.1 Buffer 56:32:12 (see D8.2.1 note d).
Mix 560 ml of HPLC grade water, 320 ml of Methanol, 120 ml of Ethanol and add 5.6 ml of 1M phosphoric acid. Handle as D5.7.

D5.7.2 90:10 Methanol-Water

Measure out 90±1 ml of methanol (D5.1) and add 10± ml of water and mix well

D5.8 Stock Standards

Dissolve 1.000±0.001 g of each phenol in methanol (D5.1) and make up to 100 ml with methanol in volumetric flasks. Providing these solutions are stored at normal room temperatures and in the dark they will be stable for at least one month. Discard any solutions which change colour on keeping.

D5.9 Intermediate Stock Standard Solutions

Accurately transfer 1.00±0.01 ml of each stock standard (D5.8) to a 100 ml volumetric flask and make up to the mark with Methanol (D5.1). These solutions will normally be stable for several weeks when kept in the dark at room temperature.

D5.10 Working Standard Solutions

1 ml contains 1 μg of phenols.

Accurately transfer 1.00±0.01 ml of each Intermediate Stock (D5.9) to a 100 ml volumetric flask and make up to the mark with buffer used (D5.7 or D5.7.1), see D8.2.1 note d. It will be found useful to prepare one or more mixed standards depending upon the phenols of interest.

This solution will normally be stable for several days.

Reagents for Procedure D9

D5.11 5 mM Phosphate Buffer pH 2.5. Carefully and with cooling dissolve 31.8±0.1 ml of H_3PO_4 (D5.3) in HPLC grade water (D5.2) and make up to 100±0.1 ml (5 M H_3PO_4). Accurately transfer 1 ml of this solution to a 1000 ml volumetric flask and make up to the mark with water. Dissolve 0.78±0.01 g of analytical reagent grade NaH_2PO_4. $2H_2O$ (sodium dihydrogen orthophosphate

dihydrate) in HPLC grade water and make up to 1000±1 ml. Both these solutions are 5 mM with respect to phosphate ions.

In order to prepare 1 litre of working phosphate buffer (5 mM, pH 2.5), mix 223±1 ml of 5 mM H_3PO_4 solution with 777±1 ml of 5 mM NaH_2PO_4 solution, filter through a 0.45 μm filter.

Check that the pH of the buffer is 2.5±0.1 pH unit with a suitably calibrated pH meter. Adjust the pH with acid or phosphate solution as necessary. Degas and use as required. This solution deteriorates with time and ideally should not be kept for more than one week.

D5.12 Stock Standard Solution

The phenols selected for inclusion in the stock standard should reflect the expected complexity of the samples analysed. Dissolve 0.100±0.001 g of each phenol required in methanol (D5.1), combine the aliquots, and make the mixed phenol standard up to 200 ml in a volumetric flask with methanol. Tables 12, 13 and 16 indicate a possible range of phenols for inclusion in a stock standard solution; but other phenols such as ethyl phenol, benzyl phenol and polyhydric phenols may also occur which should then be included.

This stock standard solution contains 500 μg ml^{-1} of each phenol. Discard any solutions which change colour on keeping.

D5.13 Intermediate Standard Solution

Intermediate standard solution, 2.5 μg ml^{-1} of each phenol. Accurately transfer 1 ml of the stock standard solution to a 200 ml volumetric flask and make up to the mark with water (D5.2) acidified to pH 2.5 with H_3PO_4 (D5.6). This solution should be stable for at least a week if kept in the dark.

D5.14 Working Standard Solution

Working standard solution 10 ng/ml of each phenol. Accurately transfer 2 ml of the intermediate standard solution to a 500 ml volumetric flask and make up to the mark with water, acidified to pH 2.5 with H_3PO_4 (D5.6). This solution should be stable for at least 24 hours if kept in the dark.

Reagent for Procedure D10

D5.15 0.1% Aqueous Acetic Acid

Dissolve 1 ml of HPLC grade glacial acetic acid in HPLC grade water and make up to 1 litre in a volumetric flask.

Filter through a 0.45 μm filter and degas.

D6 Apparatus

Note, where specific manufacturers are mentioned these were the models used to obtain the test data. Other makes with comparable or better performance may be substituted.

For procedure D8

D6.1 Pump

A high performance pump capable of delivering up to 1.0 ml of pulse free solute. A solvent switching valve would be useful.

D6.2 Injector

Any suitable injector capable of accepting 100 μl of sample. Note Rheodyne 7125 with 100 μl sample loop was used to obtain the test data.

D6.3 Detector

A UV detector capable of operating at 214 nm and 0.01 AUFS.

D6.4 Column

Procedure (a) 2 × 100 mm Chromsphere C_{18} Columns ID 3 mm, particle size 5 μm, complete with guard column, housed in an oven at 45°C.

D6.5 Computing Integrator

A Spectra Physics SP4270 has been found suitable.

D6.6 Sample Syringe

Rheodyne 500 μl (Hamilton 750 SNR).

D6.7 Standards Syringe

25 μl Rheodyne syringe (Hamilton 702-SNR)

D6.8 Flushing Syringe

10 ml Luer-lock syringe and 7125 needle port cleaner.

D6.9 5 ml Volumetric Flasks

D6.10 Sep-pak C_{18} Cartridges
or Bond Elute C_{18}
or Bond Elute cyclohexyl
or Supelco graphitized carbon

For Procedure D9

D6.11 A high performance liquid chromatograph capable of solvent mixing and gradient operation (Philips Scientific PU4100).

D6.12 A manually operated injection valve with a 20 μl sample loop.

D6.13 For automated, unattended operation, a pneumatically actuated 6-port switching valve (Rheodyne 7010) mounted in the column compartment and operated by the external switching functions of a computing integrator* (a manually operated valve may also be used).

* Obtainable from Philips Scientific—Special Engineering Group (SEG)

D6.14 Precolumn—reverse phase Spheri-5C8, particle size 5 μm, 30 mm × 4.6 mm ID.

D6.15 Precolumn holder (suitable for use with D6.14).

D6.16 Analytical column—Ultrasphere IP reverse phase C_{18}, particle size 5 μm, 25 cm × 4.6 mm ID. (Altex, USA).

D6.17 Ultra-violet detector capable of monitoring column effluent absorbance at 280 nm. (Philips Scientific PU4110)

D6.18 A liquid pump to supply sample at flow-rates between 0.5 and 5.0 ml min^{-1} (Sample pump).

D6.19 A computing integrator capable of performing external switching functions, if automatic operation is required, or a chart recorder for manual operation.

D6.20 A pH meter.

D6.21 Vacuum filter and reservoir. Filter papers 0.45 μm pore size.

D6.22 Standards syringe 25 μl (Hamilton # 702)

D6.23 Pumps

A high performance gradient elution pump capable of delivering up to 1 ml min^{-1} of pulse-free eluent, or two pumps with a gradient controller and solvent mixing system.

D6.24 Injector

A manual or automatic loop injector capable of accepting up to 100 μl of sample.

D6.25 Column

A 25 cm column packed with a 5 micron C_{18} silica phase. A Merck Lichrocart HPLC cartridge was used.

D6.26 Detector

A dual wavelength UV absorbance detector set to 214 nm and 254 nm, or a diode array multiwavelength detector.

A Philips Scientific PU 4021 detector linked to a PU 4850 data system was used.

An electrochemical detector cannot be used with this procedure.

D7 Sample Collection and Preservation

Phenols are readily oxidized, many are volatile, hence there should be no airspace (ullage) in bottles and the oxidant used to clean bottles must be completely removed before use. Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenols. Addition of preserving agents is therefore necessary. Various preservatives have been recommended (see A7, C7 or the quotation from Ref 6 given below).

D7.1 Bottle Preparation

Samples should be taken in ground glass stoppered one litre glass bottles. Bottles are pre-cleaned with potassium permanganate-sulphuric acid solution and stood for 10 mins before rinsing well with tap water, then HPLC water. Alternatively chromic acid may be used and the bottles stood overnight and rinsed as before.

D7.2 Sampling

The bottle should be completely filled without rinsing and securely stoppered and returned to the laboratory for extraction as soon as possible.

D7.3 Sample Preservation

As soon as possible the pH of the sample should be reduced to pH 2.5 or less with orthophosphoric acid (D5.3) and stored at not more than 5°C with no air space.

Tests have shown that trace phenol samples may lose all detectable phenols within 2 days. Hence arrangements should be made to extract the sample as soon as possible after sampling.

Other preservatives have also been recommended. If used, the operator should ensure these have no adverse effects on the extraction and chromatography. One such method given in Ref 6 is:

Add 2 ml of 0.2% W/V sodium arsenite solution per litre of sample immediately after sampling. Then add 5 ml of 50% V/V hydrochloric acid per litre of sample. CAUTION: If sulphide and/or cyanide is present toxic fumes may be evolved. Check that the pH is 2 or less with wide range indicator paper and adjust if necessary with 50% v/v hydrochloric acid.

D8 Analytical Procedure
(alternative procedures are given in Sections D9 and D10)

Step	Procedure	Notes

D8.1 Sample Extraction

Samples should be acidified as in D7.3 and extracted as soon as possible after sampling.

D8.1.1 Prepare a Sep-Pak C_{18} cartridge for use by passing 2 ml of Methanol (D5.1) followed by 2 ml of HPLC water (D5.2). (note a)

(a) This is most easily achieved using a 10 ml luer-lock glass syringe.

D8.1.2 Extract the phenols by passing the sample through the cartridge at a flow rate of not more than 10 ml min^{-1}. (note b). Do not allow the cartridge to become dry.

(b) Higher flow rates result in reduced recovery of phenols.

D8.1.3 When the sample has passed through the cartridge, allow the vacuum to remove as much water as possible. Using the 10 ml glass luer-lock syringe, elute the phenols from the cartridge with 2.5 ml of 90:10 methanol: water (HPLC grade). (note c). The eluent must pass through the cartridge in the same direction as the sample. Collect the eluate in a clean 5 ml volumetric flask and make up to to the mark with HPLC water (D5.2).

(c) An elutant of 90:10 methanol-water (D5.7.2) is necessary to remove PCP and tetrachlorophenols from the C_{18} cartridge.

D8.2 HPLC

D8.2.1 **Operating Conditions**

Buffer:	45:40:15 0.01 M H_3PO_4: Methanol: Ethanol (note d)
Flowrate:	0.5 ml min^{-1}
Column:	2 × 100 mm Chromsphere C_{18} 3 mm ID 5μm particle size
Column Temp:	45°C
Detector:	UV 214 nm range 0.01 AUFS
Integrator Speed:	0.5 cm sec_{-1}

(d) A buffer of 56.32.12 (D5.7.1) may be used to improve the separation of lower phenols.

D8.2.2 Switch on and allow to warm up. Ensure solutions are degassed and the pump primed. Run methanol for 10 mins; then switch to buffer and allow to stabilize. (Approximately 1 hour)

D8.2.3 Calibrate using 20 μl portions of suitable mixed standards such as D5.10.

D8.2.4 Run samples using 100 μl portions of sample extract.

D8.2.5 After the analysis is complete, in order to clean the column, switch to methanol and run for a further 30 minutes before switching off.

D8.3 Identification of Phenols

Phenols are identified by their retention times on the column. See tables 12 and 13 and figs 4 and 5.

If necessary the analysis can be repeated with different buffers such as D5.7.1 to give better separation of early eluting phenols (see D8.2.1) and Fig 5.

Step	Procedure	Notes
D8.4	Quantification	
	This method is not intended for quantitative use; but approximate concentrations can be deduced by comparing peak areas of standards and samples and correcting for percentage recoveries (see Table 14):	

D9 Analytical Procedure
(alternative procedures are given in Sections D8 and D10)

Step	Procedure	Notes
D9.1	Sample Extraction and HPLC Analysis	
	Samples should be acidified as in D7.3 and extracted as soon as possible after sampling.	
D9.1.1	The 6 port Rheodyne switching valve, precolumn, analytical column, liquid pumps and detector should be configured as indicated in Figure 7.	
D9.1.2	Switch on the instrumentation and allow to warm up. Set the operating conditions as indicated in D9.2. Ensure that the solutions are degassed and that the pumps are primed. With the Rheodyne valve in the 'INJECT' position, pump mobile phase (30% Methanol: 70% Phosphate buffer, D5.11) through the precolumn and analytical column. Allow the system to stabilize and check that the baseline is steady. Simultaneously, use the sample pump to flush the sample delivery lines, firstly with methanol and then with HPLC water.	
D9.1.3	Prepare the precolumn and analytical column for use by running the gradient indicated in the operating instructions (D9.2). Check that the baseline does not drift unduly. After running the gradient, allow the mobile phase to re-equilibrate to the initial conditions. (note a)	(a) This need only be done prior to the first analysis after switch on.
D9.1.4	Still with the switching valve in the 'INJECT' position, replace the HPLC water reservoir (supplying the sample pump) with the sample to be analysed. Pump the sample down to waste for 5 minutes to ensure thorough flushing of the pump head and delivery lines.	
D9.1.5	Depending on the mode of operation used, either manually or automatically operate the switching valve ('LOAD' position) and extract phenols by pumping sample through the precolumn for 2.5 minutes at the flow-rate indicated in D9.2. Simultaneously start the gradient. (note b)	(b) If volumes greater than 5 ml are sampled phenol may breakthrough the precolumn.
D9.1.6	After extracting for 2.5 minutes, again operate the switching valve ('INJECT' position). Extracted phenols are backflushed by the mobile phase onto the head of the analytical column, and separation takes place by gradient elution.	

Step	Procedure	Notes

D9.2 Operating Conditions

Precolumn:	Reverse phase Spheri-5 C_8 30 mm × 4.6 mm ID. Particle size 5 μm
Analytical Column:	Reverse phase Ultrasphere IP C_{18} 25 cm × 4.6 mm ID. Particle size 5 μm
Column Temperature:	Ambient
Elution Gradient:	

Mobile Phase Composition Ratio 5 mM Phosphate Buffer: Methanol	Times in minutes
70:30	for first 2.5 min.
change to	
25:75	linearly over 22 min
change to	
0:100	linearly over 2 min
0:100	maintain for 5 min

Flow-rate:	1.5 ml min^{-1}.	
Detector:	UV 280 nm	Range 1.0 AUFS
Chart Speed:	0.5 cm min^{-1}.	
Sampling Pump Flow-rate:	2.0 ml min^{-1}.	

D9.3 System Clean-up

After analysis and before switching the instrument off, switch the mobile phase to 100% methanol and flush for 15 minutes. (note c)

(c) Traces of buffer should be removed from the system to prevent blockage due to precipitation.

D9.4 Identification of Phenols

Phenols are identified by their retention times on the column. See Table 16 and Figure 8. If necessary, additional standards with different phenols from those given in Table 16 should be used.

D9.5 Calibration and Quantification

The system can be calibrated by extracting the working standard solution (D5.14) in the same way as described in the analytical procedure (D9.1) and obtaining peak area data for the phenols of interest. Concentration of sample components can be calculated by comparison with the standard peak areas. (note d)

(d) Recovery of sample components from the pre-column should be checked periodically and the results recorded with a view to establishing pre-column life and monitoring its performance. Absolute recovery can be determined by pre-concentrating known standards; calculating the oncolumn loading for each phenol analysed, and comparing the peak areas obtained with those observed when loading the analytical column with the same weight of each phenol by direct syringe injection. (Dilution and injection of the intermediate standard solution (D5.14) has been found to be most suitable for this purpose.)

D10 Analytical Procedure
(for alternative procedures see Sections D8 and D9)

Step	Procedure

D10.1 Sample Extraction

Either sample extraction procedure given in Section D8 or Section D9 may be used.

D10.2 HPLC

D10.2.1 **Operating Conditions**

Solvent: The analysis is started with a mixture of 9:1 0.1% aqueous acetic acid: methanol. After sample injection, the following gradient is used:

Run Time (Mins)	0.1% Acetic Acid (D5.15)	Methanol (D5.1)	Timed Events
0	90	10	Sample Injection
2	90	10 (a)	
5	60	40 (a)	
12			Start Integration
35	0	100 (a)	

Flow Rate: 1 ml min^{-1}
Column Temperature: 30°C
Detector: UV 214 nm and 254 nm.

Note (a) Linear gradient ratio changes occur between 2 and 5 minutes and between 5 and 35 minutes.

D10.2.2 Switch on and allow to warm up. Filter and degas solvents and prime the pump(s). Purge the system and injector with pure methanol.

D10.2.3 Set up a gradient to change the solvent to 90:10 0.1% aqueous acetic acid: methanol over 10 minutes. Allow to stabilize (about 20 minutes) and check the baseline. Inject 50 μl of a suitably mixed standard (see D5.10 and D5.14) and run the gradient detailed in D10.2.1. (But see also D10.3).

D10.2.4 After elution of all components, repeat the above procedure for each of the sample extracts, using the same injection volume as the standard mixture.

D10.2.5 After all the analyses are completed, purge the injector, column and pumping system with pure methanol before switching off.

D10.3 Identification of Phenols

Phenols are identified by their retention times and the ratio of their absorbances at 214 nm and 254 nm.

The gradient may be varied to improve the resolution of the particular phenols being analysed.

D10.4 Quantification

The concentration of the individual components can be calculated using the relative peak areas of the sample and standard at 214 nm. The recovery from the particular sample matrix should be taken into consideration.

D11 Range of the Method

D11.1 0 to 1 μg phenol/litre for D8 or D8+D10.

D11.2 0 to 10 μg phenol/litre for D9 or D9+D10.

D11.3 For higher concentrations of phenols, smaller sample injection volumes can be used. Alternatively smaller sample volumes may be used at the extraction stage.

D11.4 Direct injection, avoiding sample concentration, might give a typical range of 0 to 0.2 mgl^{-1} phenol. In which case the sample should be filtered through a 0.45 μm filter prior to injection.

D11.5 Unless advised otherwise an electrochemical detector will be more sensitive to phenols and may be used to reduce the range; **BUT DO NOT USE Electrochemical detection with gradient elution procedures.**

D11.6 If procedure D9 is used with higher concentrations of phenols, either the sampling time or flow-rate may be reduced. However, under these circumstances, the operator should check recovery and linearity for the phenols determined.

D12 Confirmation of Identity and Quantification

D12.1 Confirmation of identity is made firstly by comparison with synthetic known samples, then by use of spiked real samples and finally by showing that the sample, spiked sample and standard behave similarly with two or preferably three procedures, columns or eluents.

D12.2 Approximate quantification can be obtained by standard additions and measurement of peak height or area (which ever is the most convenient to measure accurately); **but only after the peak has been truly identified.**

D13 Note of Peak Broadening and False Splitting

Peak Broadening and ultimately separation into multiple peaks has been reported with repeatedly used reverse-phase HPLC columns, due to channeling in the column (9). Various solutions have been suggested. Users of this method are warned to watch for the onset of channeling by use of AQC samples, watching for the onset of peak broadening and are advised to take remedial action should it occur.

Table 12 Typical Retention Times Using Buffer 45:40:15

Compound	RT (mins)	RRT
Phenol	1.70	1.0
2,Chlorophenol + Cresols	2.17	1.28
3,Chlorophenol + 4, Chlorophenol	2.50	1.47
Xylenols	2.77	1.63
2,6-Dichlorophenol	2.90	1.70
2,3-Dichlorophenol	3.31	1.95
2,5-Dichlorophenol	3.76	2.21
2,4-and 3,4-Dichlorophenol	4.08	2.40
2-and 4-Phenylphenol	4.35	2.56
3,5-Dichloro + 2,3,6-Trichlorophenol	5.87	3.45
2,3,4-Trichlorophenol	6.25	3.68
2,4,6-Trichlorophenol	7.06	4.15
2,4,5-Trichlorophenol	7.81	4.59
2,3,5-Trichlorophenol	8.39	4.93
3,4,5-Trichlorophenol	8.99	5.29
2,3,4,6-Tetrachlorophenol	12.44	7.32
2,3,5,6-Tetrachlorophenol	12.84	7.55
2,3,4,5-Tetrachlorophenol	14.92	8.78
Pentachlorophenol	26.57	15.6

Table 13 Typical Retention Times Using Buffer 56:32:12

Compound	RT (min)	RRT
Phenol	3.57	1.0
2,Chlorophenol	4.81	1.35
4,Chlorophenol	5.88	1.65
2,6-Dichlorophenol	6.85	1.92
2,3-Dichlorophenol	8.02	2.25
2,5-Dichlorophenol	8.79	2.46

Table 14 Recoveries of Phenols at 1 $\mu g l^{-1}$ level.
1 Litre of Water passed through cartridge. Phenol eluted with 90:10 Methanol: Water.

Compound	Sep-Pak C_{18}	Bond Elute Cyclohexyl
	%	%
2,6-Dichlorophenol		127
2,5-Dichlorophenol	17.4	114
3,5-Dichlorophenol	20.3	74
*2,4,6-Trichlorophenol	63.8	90
*2,4,5-Trichlorophenol	80.9	102
*2,3,4,6-Tetrachlorophenol	89.0	98
*2,3,4,5-Tetrachlorophenol	82.9	88
*Pentachlorophenol	88.3	68

* 100% MeOH will give quantitative recovery of phenols using Sep-Pak C_{18} but may give interferences coinciding with other phenols.

Table 15 Standard Deviation for Selected Phenols—Blank + 1.0 $\mu g l^{-1}$ of Each Phenol

Compound	Mean Conc Found $\mu g l^{-1}$	SD (Total for 5 degree of Freedom)
3,5-DCP	0.203	0.029
2,4,6-TCP	0.638	0.013
2,4,5-TCP	0.809	0.014
2,3,4,6-Tet CP	0.890	0.016
2,3,4,5-Tet CP	0.829	0.039
Pentachlorophenol	0.883	0.021

Table 16 Typical Retention Times and Relative Retention Times Using Gradient Elution

Component	RT(min)	RRT
Phenol	6.55	1.0
4-Nitrophenol	8.85	1.35
2,4-Dinitrophenol	10.17	1.55
2-Chlorophenol	10.90	1.66
2-Nitrophenol	11.43	1.75
2,4-Dimethylphenol	14.32	2.19
2-Methyl-4,6-Dinitrophenol	15.58	2.38
4-Chloro-3-Methylphenol	16.33	2.49
2,4-Dichlorophenol	17.61	2.69
2,4,6-Trichlorophenol	21.49	3.28

Note: Retention times are corrected for sampling time.
ie (Retention time reported in Figure 8) −2.5 minutes.

Table 17 Recovery of Phenols at 10 μgl^{-1} Level

River water found to contain no detectable phenols was spiked to 10 μgl^{-1} with each of the phenols indicated and analysed by the procedure described in D9.1 (50 μg theoretical on-column loading of each phenol.) Percentage recovery was determined as described in D9.5 and note d.

Component	Recovery C_8 Precolumn (%)
Phenol	35.7
4-Nitrophenol	90.9
2,4-Dinitrophenol	92.1
2-Chlorophenol	73.3
2-Nitrophenol	87.9
2,4-Dimethylphenol	85.8
2-Methyl-4,6-Dinitrophenol	96.5
4-Chloro-3-Methylphenol	86.5
2,4-Dichlorophenol	96.4
2,4,6-Trichlorophenol	84.6

Table 18 Statistical Evaluation

River water found to contain no detectable phenols was spiked to 10 μgl^{-1} (phenol only 5 μgl^{-1}) with each of the phenols indicated and analysed in triplicate by the procedure described in D9. Peak area data was collected for statistical evaluation.

Component	Mean Peak Area (n = 3)	Standard Deviation (n − 1)	%Rel. Std. Dev.
Phenol (5 μgl^{-1})	4,348	181	4.2
4-Nitrophenol	54,251	1,336	2.5
2,4-Dinitrophenol	79,934	1,766	2.2
2-Chlorophenol	24,137	1,744	7.2
2-Nitrophenol	78,378	1,215	1.6
2,4-Dimethylphenol	25,585	917	3.6
2-Methyl-4,6-Dinitrophenol	107,675	551	0.5
4-Chloro-3-Methylphenol	20,949	1,514	7.2
2,4-Dichlorophenol	20,172	97	0.5
2,4,6-Trichlorophenol	11,896	473	4.0

Table 19 Limit of Detection

The limits of detection for the 10 phenols listed in Table 16 were determined by spiking river water found to contain no detectable phenol, to 10 μgl^{-1} with each phenol and analysing by the preconcentration method described. Detector range was set to 0.2AUFS and the limits of detection were calculated on the basis of 2 × Baseline noise.

The percent relative standard deviations of peak areas at the 2.5 μgl^{-1} level are also listed as a guide to the expected errors at lower concentrations.

Component	Limit of Detection (μgl^{-1})	% Rel. Std. Dev (n − 1) 2.5 μgl^{-1})
Phenol	2.35	No data
4-Nitrophenol	0.44	2.44 (n = 4)
2,4-Dinitrophenol	0.34	5.51 (n = 4)
2-Chlorophenol	0.92	3.98 (n = 4)
2-Nitrophenol	0.32	3.30 (n = 5)
2,4-Dimethylphenol	0.71	5.28 (n = 4)
2-Methyl-4,6-Dinitrophenol	0.25	3.17 (n = 6)
4-Chloro-3-Methylphenol	1.03	4.44 (n = 3)
2,4-Dichlorophenol	1.13	10.35 (n = 4)
2,4,6-Trichlorophenol	1.43	10.52 (n = 3)

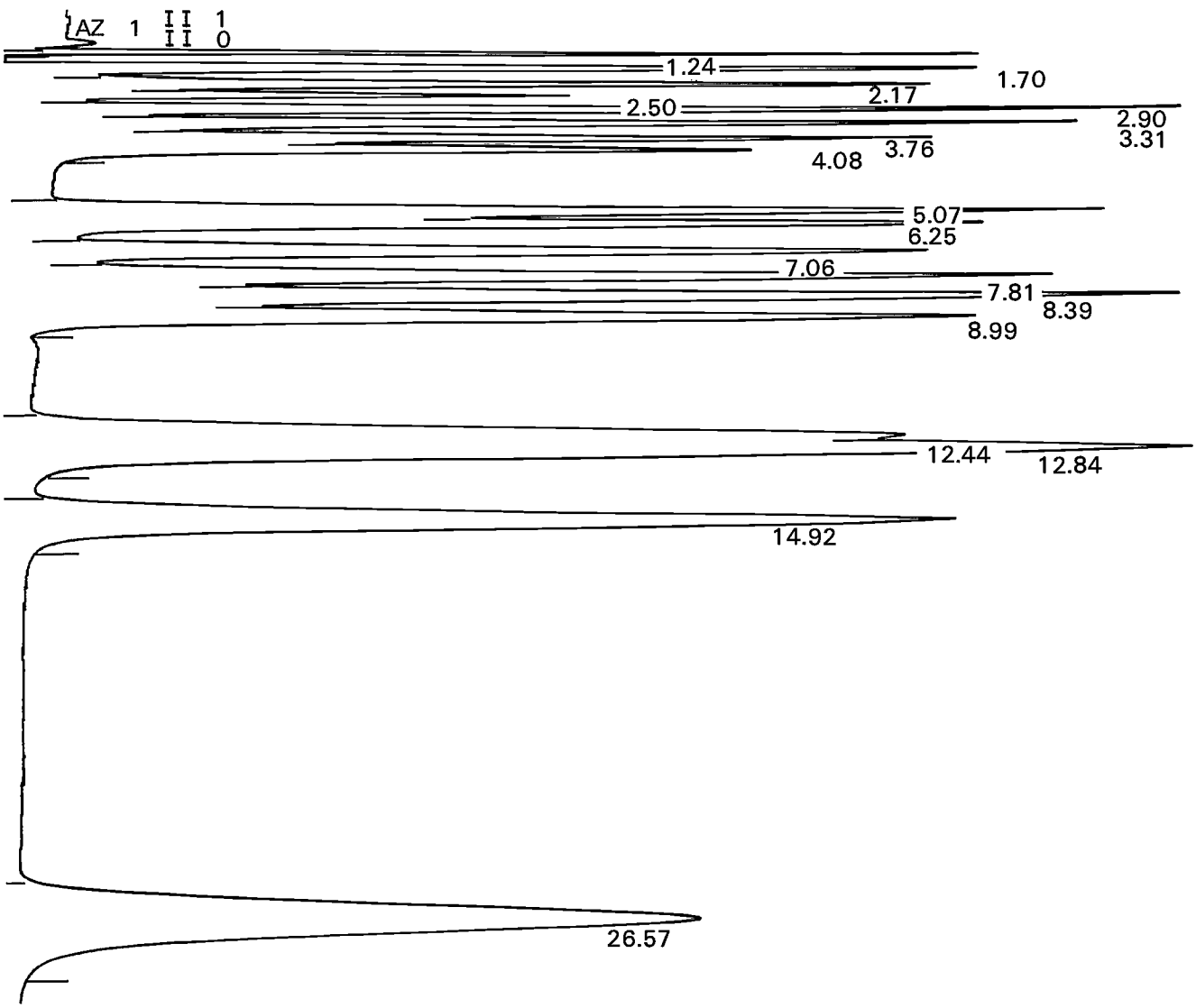
Table 20 Typical Retention Times and Wavelength Ratios Using Gradient Elution (Procedure D10)

Compound	RT (Mins)	RRT	Absorbance Ratio 214 nm: 254 mm
Phenol	13.9	1.0	12.6 : 1
2-Chlorophenol	18.4	1.32	20.3 : 1
4-Chlorophenol	20.0	1.44	19.9 : 1
3-Chlorophenol	20.4	1.47	22.6 : 1
2,6-Dichlorophenol	22.0	1.58	37.5 : 1
2,4-Dichlorophenol	24.8	1.78	28.7 : 1
2,4,6-Trichlorophenol	28.9	2.08	36.0 : 1
2,4,5-Trichlorophenol	29.3	2.11	24.1 : 1
2,3,4,6-Trichlorophenol	32.1	2.31	22.1 : 1
Pentachlorophenol	35.4	2.55	14.7 : 1

Figure 4

Phenol chromatogram with solvent 45:40:15 0.01M H_3PO_4: methanol: ethanol

Procedure D8 (D5.7)

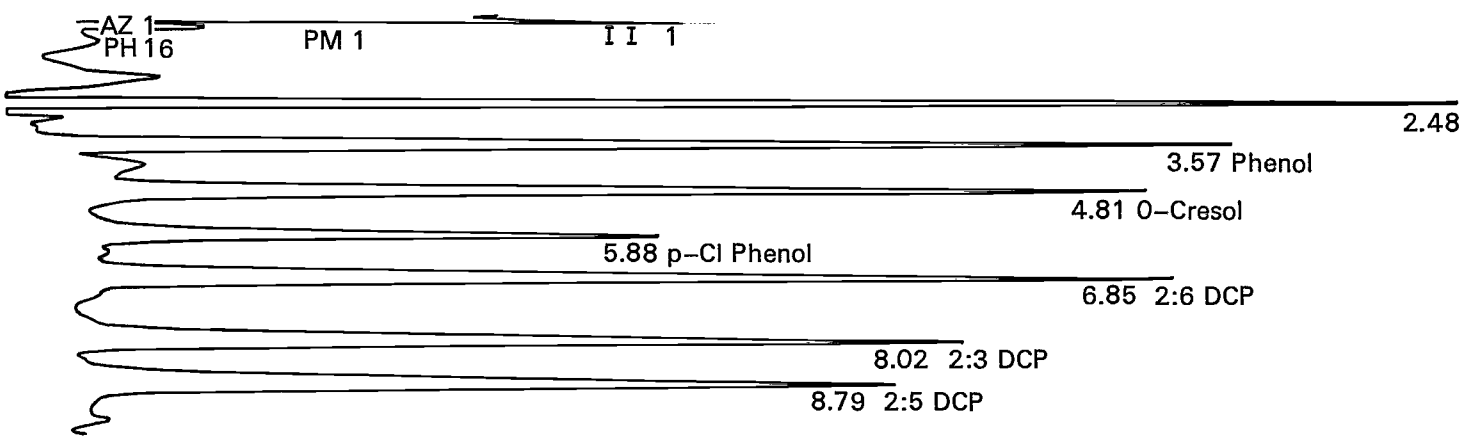


Peak #	Area %	RT	Area BC	
1	2.374	1.24	224355 01	
2	7.719	1.7	729517 02	Phenol 20ng each injected
3	6.586	2.17	622480 02	0–Chlorophenol
4	3.911	2.5	369619 02	m + p Chlorophenol
5	6.28	2.9	593555 02	2:6 DCP
6	5.234	3.31	494663 02	2:3
7	4.113	3.76	388740 02	2:5
8	3.167	4.08	299319 03	2:4 and 3:4
9	4.126	5.87	389952 02	3:5 DCP and 2:3:6 TCP
10	3.843	6.25	363251 03	2:3:4
11	3.943	7.06	372637 02	2:4:6
12	4.875	7.81	460698 02	2:4:5
13	5.949	8.39	562286 02	2:3:5
14	5.171	8.99	488684 03	3:4:5
15	5.264	12.44	497539 02	2:3:4:6 Tetra CP
16	9.107	12.84	860747 03	2:3:5:6
17	7.877	14.92	744508 01	2:3:4:5
18	10.46	26.57	988583 01	Penta CP

Figure 5

Selected phenols elution with solvent 56:32:12 0.01M H_3PO_4: methanol: ethanol

Procedure D8 (D5.7.1)



Figures 6a–j Linearity (Procedure D9)

River water found to contain no detectable phenols was spiked to 2.5, 5.0, 7.5 and 10.0 $\mu g l^{-1}$ with each of the 10 phenols indicated in Table 16 and analysed in replicate by the procedure described in D9. River water blanks were also treated in the same way. Figures 6a–j show linearity plots for all 10 phenols across this concentration range.

Figure 6a Phenol

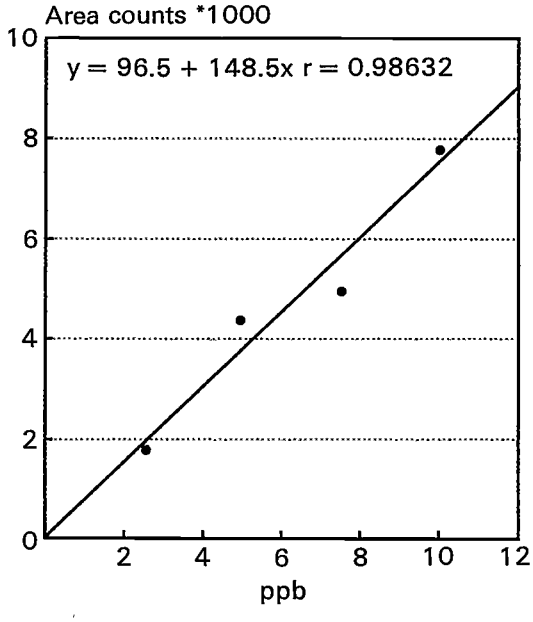


Figure 6b 4–Nitrophenol

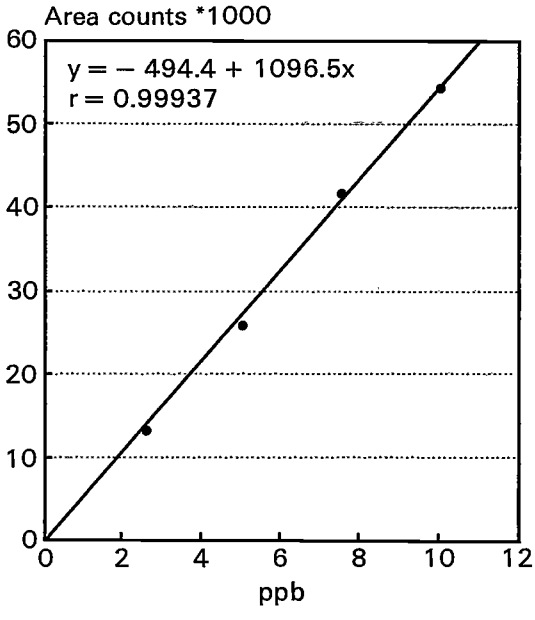


Figure 6c 2,4–Dinitrophenol

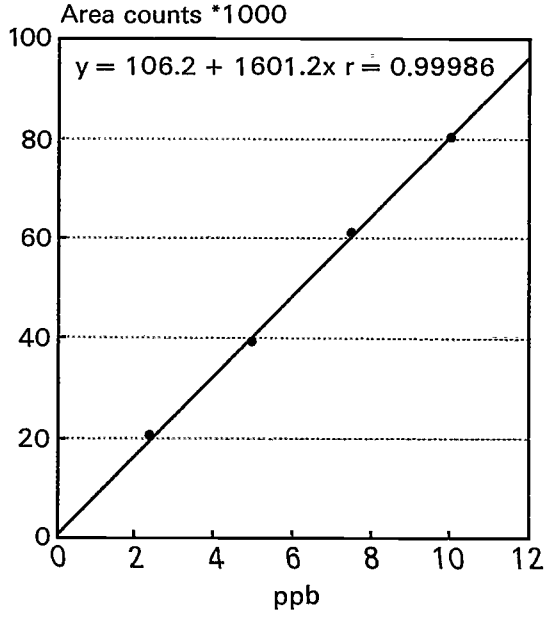


Figure 6d 2–Chlorophenol

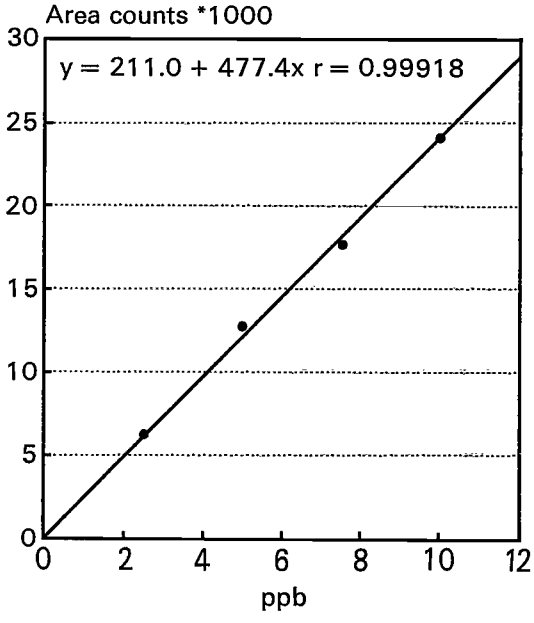


Figure 6e 2–Nitrophenol

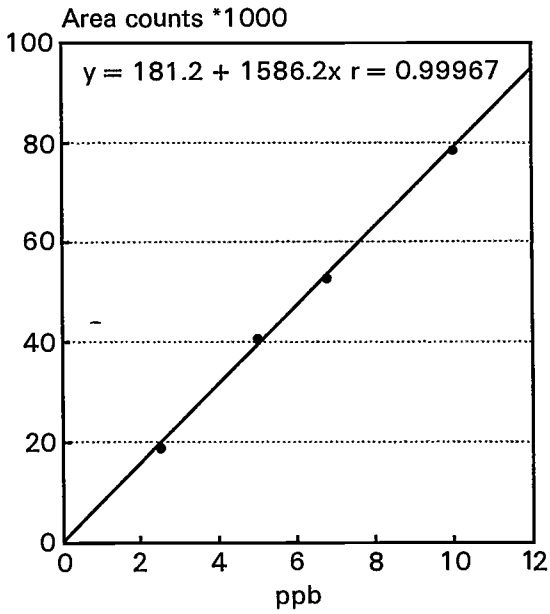


Figure 6f 2,4–Dimethylphenol

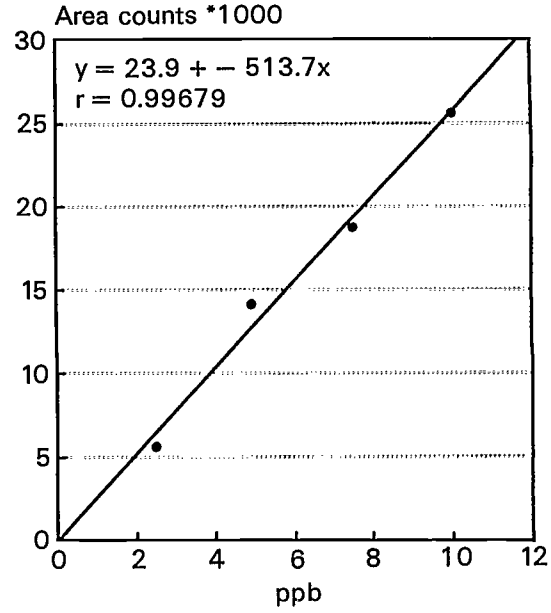


Figure 6g 2–Me–4,6–dinitrophenol

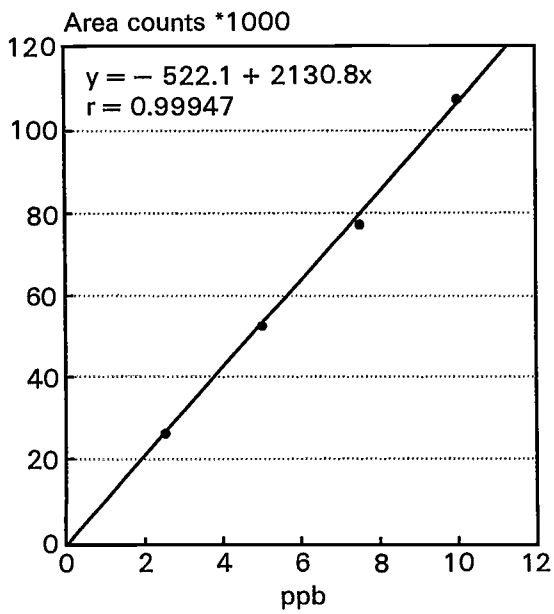


Figure 6h 4–Cl–3–methylphenol

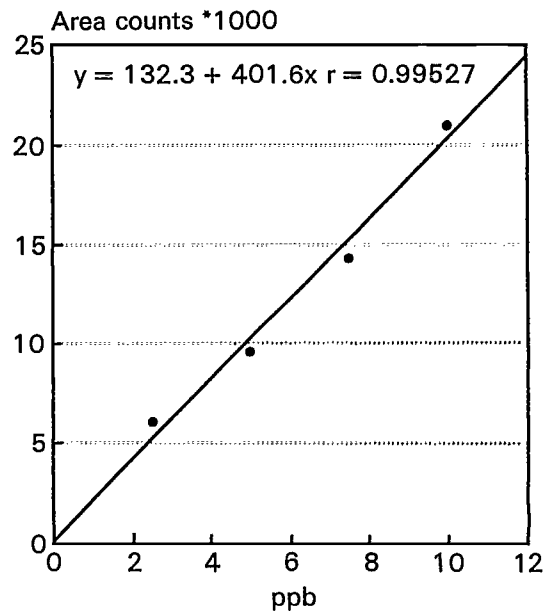


Figure 6i 2,4–Dichlorophenol

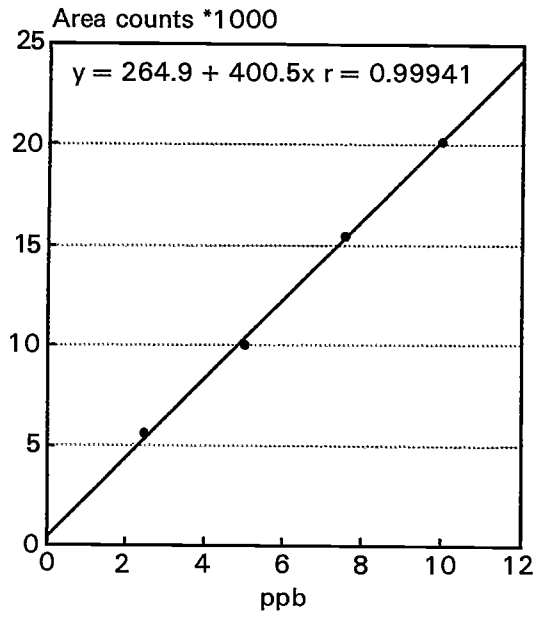


Figure 6j 2,4,6–Trichlorophenol

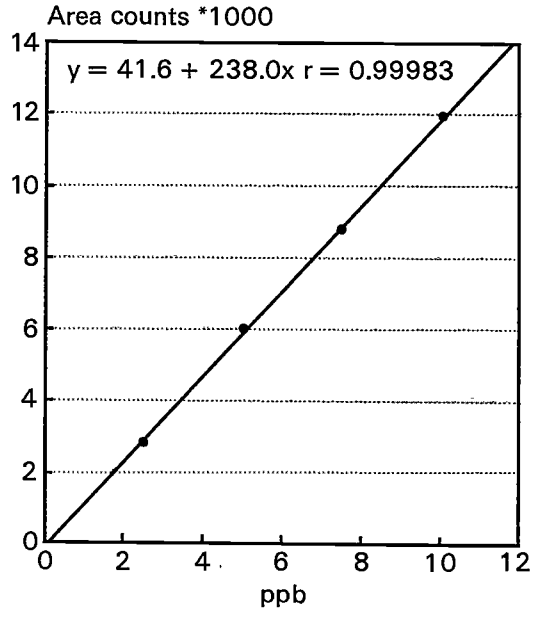


Figure 7 Valve configuration

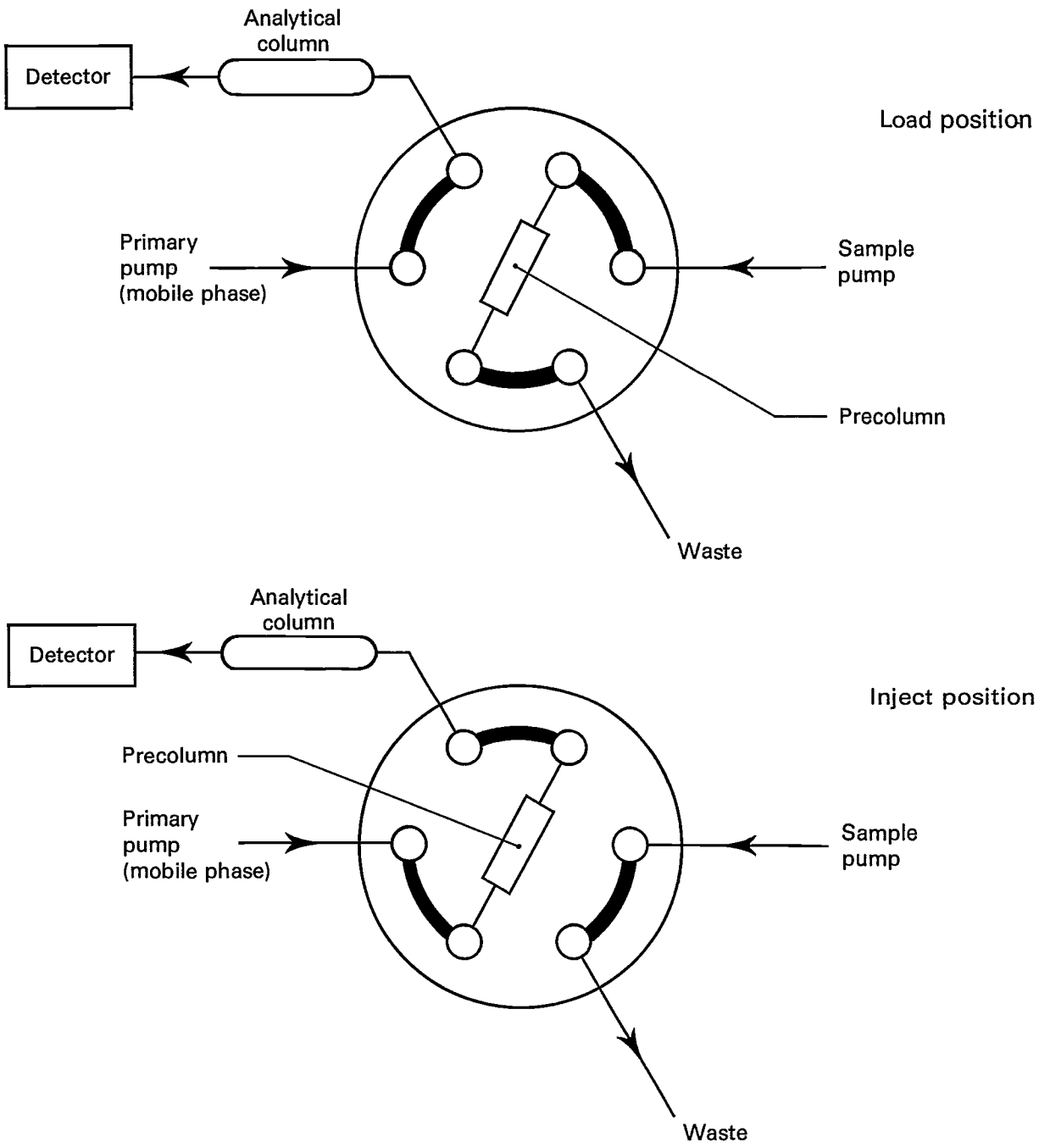
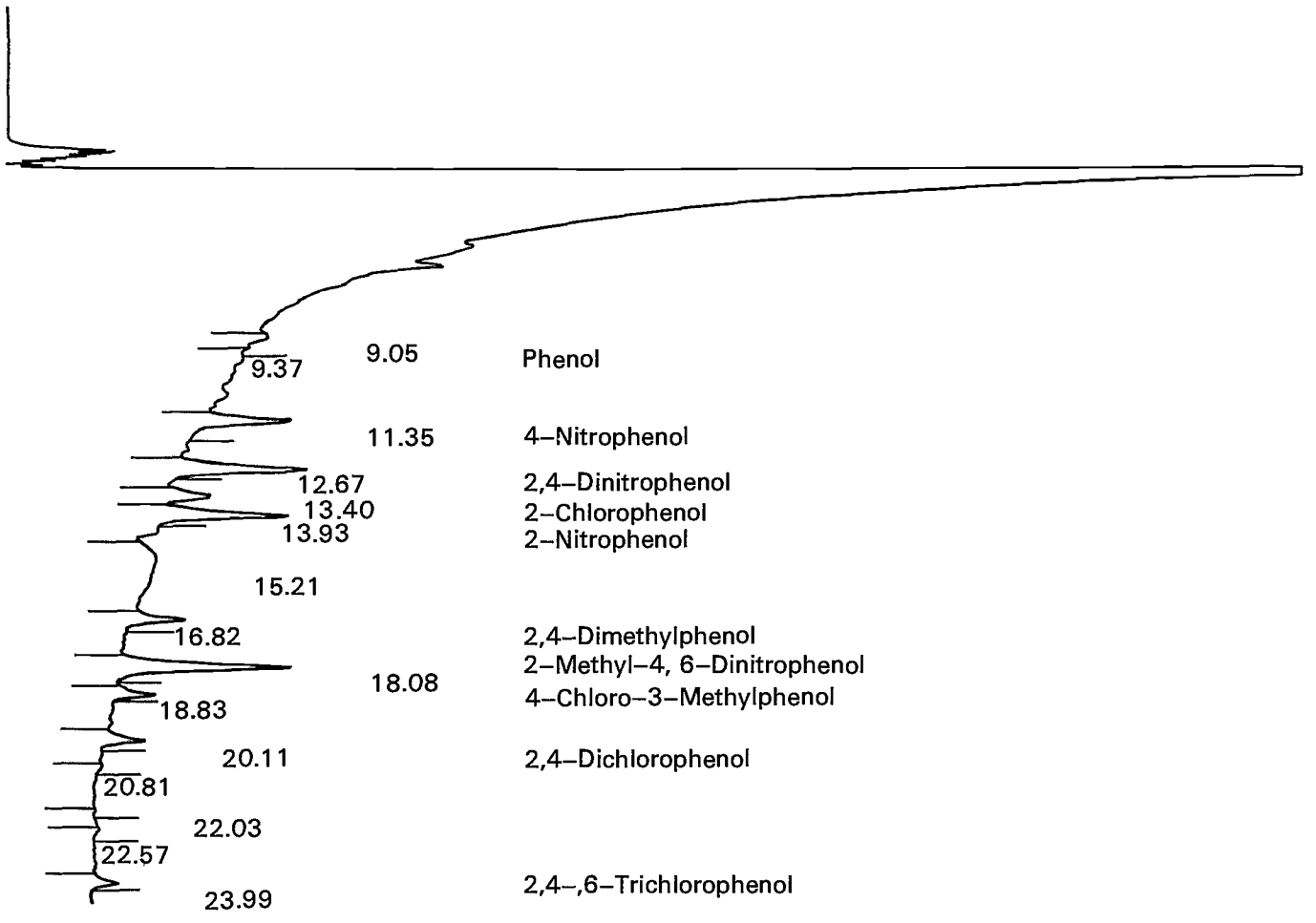


Figure 8 Analysis of phenols in river water (10μg /L each component)

Procedure D9



1	9.05
2	9.37
3	11.35
4	12.67
5	13.4
6	13.93
7	15.21
8	16.82
9	18.08
10	18.83
11	20.11
12	20.81
13	22.03
14	22.57
15	23.99

Figure 9 Standard chlorophenols at 214nm using gradient elution

(D10) (Integration started 12 minutes after injection)

Retn time	Rel ret	Peak HT	Peak area	Peak conc	%conc	Peak name
114.0	1.0000	299.759	4389.903	12.636	5.297	Phenol
386.0	3.2414	254.791	3942.889	20.337	8.525	2-C/Phenol
488.0	4.1043	212.369	2860.251	19.871	8.330	4-C/Phenol
510.0	4.2759	238.898	4106.761	22.615	9.480	3-C/Phenol
610.0	5.1034	283.924	4715.332	37.517	15.727	2,6-DCP
780.0	6.5517	203.808	3361.071	28.661	12.014	2,4-DCP
1024.0	8.6724	349.926	4829.220	36.019	15.099	2,4,6-TCP
1046.0	8.8793	378.448	6794.676	24.125	10.113	2,4,5-TCP
1214.0	10.3276	392.050	6932.337	22.118	9.272	2,3,4,6-TP
1408.0	12.0172	792.925	15067.776	14.654	6.143	PCP
				.....		
				238.555		

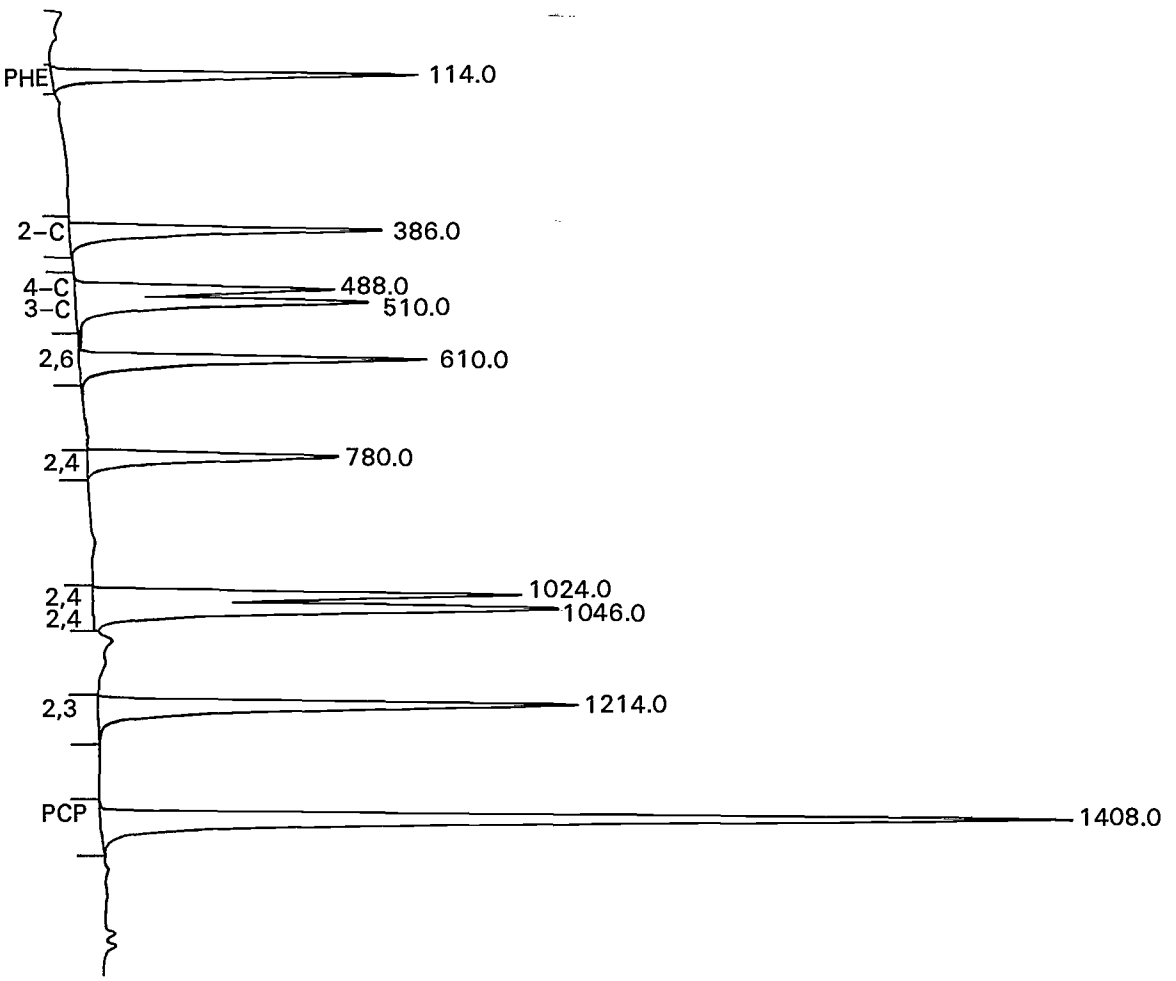
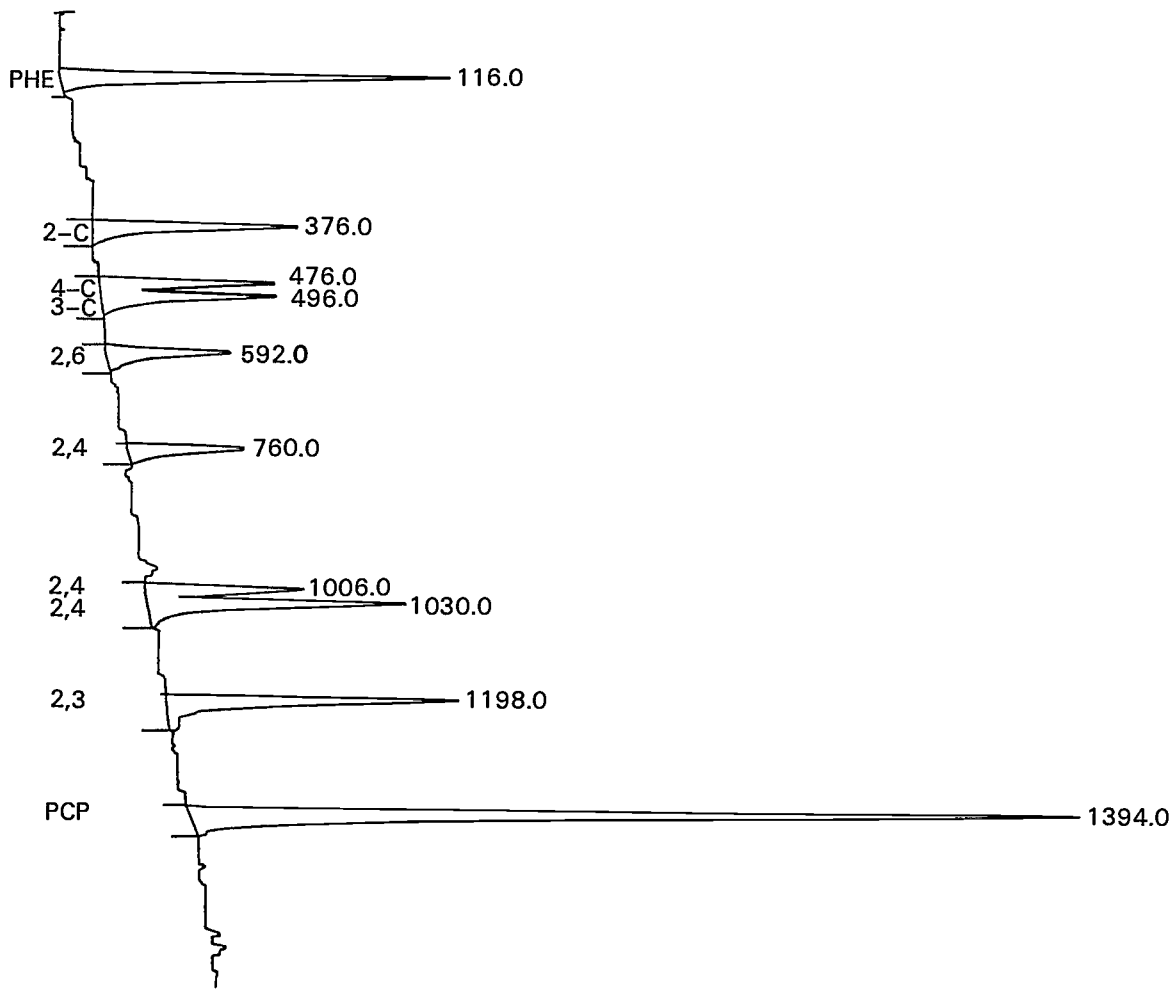


Figure 10 Standard chlorophenols at 254nm using gradient elution

(D10) (Integration started 12 minutes after injection)

Retn time	Rel ret	Peak HT	Peak area	Peak conc	% conc	Peak name
116.0	1.0000	23.723	330.630	1.000	10.000	Phenol
376.0	3.2414	12.528	189.931	1.000	10.000	2–C/Phenol
476.0	4.1034	10.687	140.144	1.000	10.000	4–C/Phenol
496.0	4.2759	10.564	176.077	1.000	10.000	3–C/Phenol
592.0	5.1034	7.568	129.998	1.000	10.000	2,6–DCP
760.0	6.5517	7.111	113.134	1.000	10.000	2,4–DCP
1006.0	8.6724	9.715	140.190	1.000	10.000	2,4,6–TCP
1030.0	8.8793	15.687	276.417	1.000	10.000	2,4,5–TCP
1198.0	10.3276	17.726	305.818	1.000	10.000	2,3,4,6–TP
1394.0	12.0172	54.110	882.534	1.000	10.000	PCP
				.....		
				10.000		



Analytical Quality Control

(see also sections D9.5 note d and D13)

1 It is desirable to carry out strict analytical quality control procedures. As phenol solutions have a poor shelf life, and as correct identification is critical for correct analysis, it is suggested that quality control charts be run for the standards used routinely.

2 Prepare aqueous solutions of the phenols of interest by proceeding as in C5.7 but using smaller accurately weighed amounts of the phenols and dissolving in 1 litre of water by warming. Consult solubility tables before preparing the master solutions. Quantitatively, successively dilute to give Control Standard Solutions of about the concentrations expected in the samples. Analyse these solutions by the full procedure used.

3 Although most of the data given in these methods is for phenols, cresols, and xylenols and their chloroderivatives tests have shown that nitro, amino and other substituted phenols and phenylphenols can be determined by these methods, and that polarity is the guide to the order in which phenols tend to appear. To avoid embarassing errors, when sure identification is required, analysts should at least check the identification by using spiked samples and also an alternative method with both real and spiked samples.

4 For additional information see Refs 10, 11 and 12.

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2 E Lindley and P J Rennie. 'Phenolic compounds in Potable Water Treatment Effects and Analytical Review', paper presented to the Scientific Section of Water Engineers and Scientists, Matlock, Derbyshire, September, 9th 1981.

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4 B T Croll. Personal Communication.

5 *O. J. European Communities* (30 August 1980) L229/18 and Department of the Environment *Circular 20/82* and related Welsh, Scottish and Northern Ireland Office Circulars.

6 *Phenols in Waters and Effluents by Gas-Liquid Chromatography, 4-aminoantipyrine or 3-methyl-2 benzothiazolinone hydrazone 1981,* HMSO, in this series.

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9 Irwin W J, Hempenstall J M, Li Wan Po A, *Laboratory Practice* 1984 **33**(2), 74–76

10 *General Principles of Sampling and Accuracy of Results 1980.* HMSO in this series.

11 Davy, D J, and Hunt D T E, WRC *Technical Report TR 174,* Medmenham 1982.

12 British Standards BS 5700 to 5703 inclusive and BS 5750.

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:

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The Standing Committee of Analysts
The Department of the Environment
Romney House
43 Marsham Street
London SW1P 3PY

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Membership responsible for these methods

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