Phenols in Waters and Effluents by Gas-Liquid Chromatography, 4-aminoantipyrine or 3-methyl-2-benzothiazolinone hydrazone, 1981

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £3.40 net

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Methods for tri-, tetra-, and pentachloro phenols will be included in the booklet containing methods for TBA and DICAMBA. The method for dichloro phenols will be included with the method for MCAA.

CONTENTS

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

*Also called 4-aminophenazone
†Also called 3-methyl-2-benzothiazolinone hydrazone

Warning to users

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

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

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

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

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About this series

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

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

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

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

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

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

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

TA DICK
Chairman

LR PITTWELL
Secretary

25 September 1981

General Introduction

The presence of phenols in a river or stream is objectionable because of their strong bactericidal action, their toxicity to aquatic life and the unpleasant odour and tastes produced when water containing even microgram quantities of phenols is chlorinated.

In addition to phenol itself, the term "phenols" covers a wide range of compounds – which includes monohydroxy (monohydric), dihydroxy (dihydric) and polyhydroxy (polyhydric) derivatives of benzene – and which can be present in effluents from many industrial processes. The monohydroxy derivatives include phenol, the three methylphenol isomers (cresols) and the six dimethylphenol isomers (xylenols) which, together with phenols containing halogen or larger alkyl groups, are the major sources of phenolic pollution. The dihydroxy derivatives, which include catechol, resorcinol and quinol together with their alkyl homologues, and polyhydroxy derivatives are normally only found in certain industrial effluents.

For the purposes of the test, the term "phenols" does not include naturally occurring compounds such as humic acids and vegetable tannins.

Because of the wide range of concentrations at which phenols can occur in effluents, rivers and potable water, it has been necessary to provide several procedures for their determination. The choice of method will depend on the sample type and the range of phenols expected as summarized below.

1. **For effluents and waters in which the phenols concentration is greater than 100 µg/l,** two methods are provided

   Method A, a gas chromatographic procedure

   This gives the higher accuracy and will permit the separation and determination of most monohydric and dihydric phenols and also of chlorinated phenols. It is the only method that will give the true phenols concentration of a sample.

   Method B, a colorimetric method

   Monohydric phenols only can be estimated colorimetrically, after distillation, by reaction with 4-aminoantipyrine in aqueous solution at pH 10. However, the result obtained by this method can only give an indication of the true phenols concentration since substituted phenols do not react with 4-aminoantipyrine to the same extent as phenol itself.

   It is, therefore, unlikely that the result obtained by method B will correspond exactly to that obtained by method A.

2. **For effluents and waters in which the phenols concentration is below 100 µg/l,** three methods are provided.

   Method C, a colorimetric method

   Monohydric phenols are distilled, reacted with 4-aminoantipyrine at pH 10 and the coloured product concentrated by extracting into chloroform.

   Method D, a colorimetric method

   This is based on the same principles as method C, but involves reaction at pH 7.9, at which pH greater sensitivity for chloro-substituted phenols is obtained.

   Method E, a colorimetric method

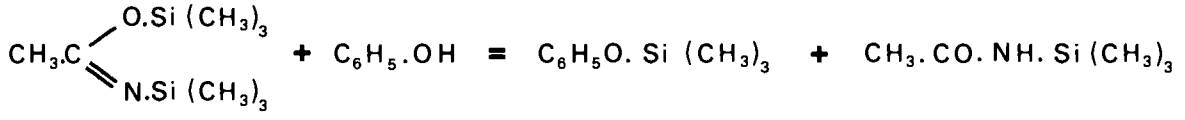
   This method uses a different colorimetric reagent from methods B, C and D. The reagent, 3-methyl-2-benzothiazolinone hydrazone (MBTH), reacts with more phenols – particularly phenols substituted in the para position – than does 4-aminoantipyrine. Consequently, method E may be advantageous for certain types of sample. It will not, however, give the true concentration of phenols in a sample, nor will it produce the same result as the 4-aminoantipyrine methods.

A table showing the relative responses of the 4-aminoantipyrine methods and the MBTH method to a range of phenols is given (Table 1).

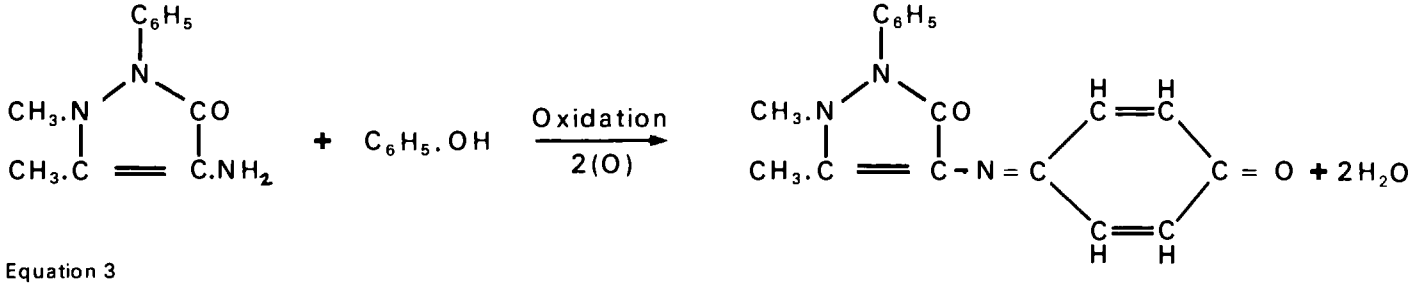
Note that none of the above methods is capable of reaching the very low levels of detection of phenols required for monitoring compliance with certain EEC directives. Research into a suitable method using gas chromatography with electron capture detection is being developed by the Standing Committee of Analysts and it is hoped that a suitable method will be published in a subsequent booklet.

Equations for the reactions used

Equation 1



Equation 2



Equation 3

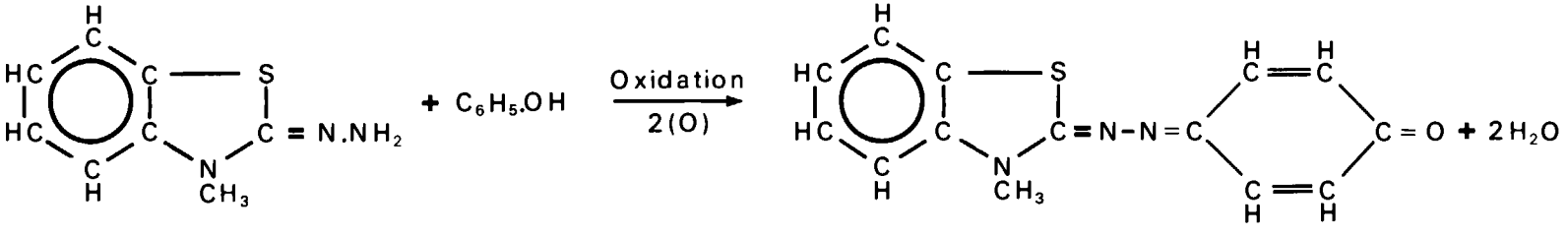


Table 1
Relative Responses of 4-AAP Methods and MBTH Method to a Range of Phenols

	Relative Sensitivity (Phenol = 1.0)										
	Phenol	2-Cresol	3-Cresol	4-Cresol	2-Chloro Phenol	Di-chloro Phenol	2,4,6-Tri-chloro Phenol	Guaia-col	2,4-Xylenol	2,5-Xylenol	2-Napthol
Method B Direct 4-APP (Ref. 1)	1.0	0.7	0.7	0.1	NDA	NDA	NDA	NDA	0.1	0.4	NDA
Method C 4-AAP pH 10 (Ref. 2)	1.0	0.8	NDA	0.0	0.9	0.3	0.1	0.8	NDA	0.5	0.1
Method D 4-AAP pH 7.9 (Ref. 2)	1.0	0.7	NDA	0.0	0.1	0.8	0.6	0.8	NDA	0.5	0.2
Method E MBTH (Ref. 5)	1.0	1.0	1.0	0.6	0.9	0.4	0.2	NDA	0.4	NDA	0.3

Notes
1. These data refer to the colorimetric stage only.
2. NDA – no data available.

Method A Determination of Phenols Gas Chromatographic Method

A1 Performance Characteristics of the Method

A1.1	Substances determined	Most simple monohydric and dihydric phenols and chlorophenols
A1.2	Types of Sample	Rivers, waters, sewage effluents and industrial effluents.
A1.3	Basis of method	Phenols are extracted into an organic solvent the trimethyl-silylethers prepared and separated by gas chromatography.
A1.4	Range of application	0–50 mg/l of phenols in total.
A1.5	Calibration curve	Not applicable.
A1.6	Standard deviation (a) (s_w) (with 5 degrees of freedom)	(i) Using Synthetic solutions

Phenolic compound	Standard deviation, mg/l	
	Synthetic solution, each phenol conc'n 1 mg/l	Synthetic solution, each phenol conc'n 1 mg/l
Phenol	0.02	1.07
4-Cresol	0.04	0.97
2,5-Xylenol	0.15	1.30
4-Chlorophenol	0.06	0.96
Catechol	0.22	1.38

(ii) Using drinking water 'spiked' with phenols

Phenolic compound	Concentration of spike mg/l	Standard deviation mg/l
Phenol	2.30	0.09
4-Cresol	2.87	0.08
2,5-Xylenol	4.84	0.09
4-Chlorophenol	6.22	0.23
Catechol	7.72	0.15

A1.7 Limits of detection (a) (with 5 degrees of freedom)

Phenolic compound	Limit of detection, mg/l
Phenol	0.1
4-Cresol	0.2
2,5-Xylenol	0.6
4-Chlorophenol	0.3
Catechol	0.9

A1.8	Sensitivity	Dependent on individual phenol and on instrument used.
A1.9	Bias	Low results may be obtained with samples which contain very water soluble phenols such as polyhydric phenols and which may not be fully extracted with the solvent used.

A1.10	Interferences	Co-extracted compounds could interfere. See Section 3.
A1.11	Time required for analysis (a)	Assuming all reagents prepared and the instrument already calibrated, for each sample the extraction stage takes 1–1½ h and the gas chromatography takes a further ½ h.

(a) These data were obtained at the Department of Industry, Laboratory of the Government Chemist using a Varian 3700 gas chromatograph fitted with a 12′ × ¼″ column packed with 10% OV–101 on 80–100 mesh Diatomite CLQ. The column oven temperature was 130°C and the injector and detector temperatures were 150°C and 250°C respectively.

A2 Scope

Phenols are extracted into 4-methylpentan-2-one and a known amount of an internal standard is added. The phenols are then converted into their trimethylsilyl ethers (TMSE) and the mixture is examined by gas chromatography. By reference to the quantity of internal standard added, the concentration of each phenol present is calculated.

A3 Field of Application

The method has been applied to the determinations of the following phenols in river waters, sewage effluents and some industrial effluents.

Phenol
Alkyl phenols
Aryl phenols
Chlorinated phenols
Dihydric phenols

Other phenols may well be determined by this method, but these have not been investigated. For example higher molecular weight phenols can be determined by employing higher column temperatures than those given in the examples in the Appendix.

Interferences may be experienced from some co-extracted co-chromatographed organic compounds, for example some neutral organic compounds. When large numbers of phenols are present in a sample, especially if a mixture of chlorinated and non-chlorinated phenols, it may not be possible to separate and identify each individual phenol even with the use of a capillary column.

A4 Principle

A phenol is identified by the retention time of its trimethylsilyl ether after gas chromatographic separation. Three stationary phases are recommended –

(i) a methylsilicone,
(ii) Carbowax 20M,
(iii) tri-2,4-xylenylphosphate.

Choice of stationary phase depends upon the separation required and the nature of the sample. For examples see the Appendix.

The use of two or more of the above stationary phases may help to confirm the identity of a peak.

A5 Hazards

A5.1 N,O-BIS (TRIMETHYLSILYL) ACETAMIDE (REAGENT A7.10) IS TOXIC AND MUST BE HANDLED WITH CARE. IT MUST NOT BE ALLOWED TO COME INTO CONTACT WITH THE SKIN AND ITS VAPOUR MUST NOT BE INHALED. MANUFACTURERS WARNING AND ADVICE, WHEN GIVEN, SHOULD BE STRICTLY FOLLOWED

A5.2 Sodium arsenite (Reagent A7.6) is poisonous.

A5.3 4-Methylpentan-2-one (Reagent A7.6) is flammable and has a harmful vapour. It must not be pipetted by mouth.

A5.4 Phenols (Reagent A7.12) are poisonous and corrosive. Some can be absorbed through the skin.

A5.5 Chromic Acid is corrosive. The dust of Chromium VI compounds must not be inhaled.

A5.6 Hydrochloric Acid (Reagent A7.5) can cause burns and gives off a harmful vapour.

If samples might contain cyanide or sulphide, acidification of samples should be carried out in a well-ventilated place.

A6 Reactions

The trimethylsilyl ethers are prepared by mixing the phenol with N,0-bis (trimethylsilyl) acetamide.

For the equation, for this reaction, see Equation 1.

A7 Reagents

Use only reagents of analytical grade where appropriate.

A7.1 **Water**
It is essential that water of negligible phenol content (compared with the smallest concentrations to be determined in the samples) be used for the preparation of reagent solutions, phenol standard solutions and blanks. The water should be stored in an all-glass aspirator.

A7.2 **Nitrogen,** oxygen-free, passed through a molecular sieve trap at an appropriate flow rate (usually in the range 20–50 ml/min).

A7.3 **Hydrogen** passed through a molecular sieve trap at a flow rate recommended by by the chromatograph manufacturer.

A7.4 **Air** passed through a molecular sieve trap at a flow rate recommended by the chromatograph manufacturer.

A7.5 **Hydrochloric acid solution** (50% V/V)
Add 500±5 ml hydrochloric acid (d_{20}1.18) cautiously to 400 ml of water in a 1-litre measuring cylinder stirring continuously. Cool and dilute with water to 1 litre. Store in a glass bottle with a ground glass stopper.

A7.6 **Sodium arsenite solution** (0.2% W/V)
Dissolve 2.00±0.01 g of sodium arsenite in water and dilute to 1 litre in a calibrated flask. Store the solution in a glass bottle with a ground glass stopper. Caution: Sodium arsenite is poisonous.

A7.7 **4-methylpentan-2-one,** redistilled
Check the purity of this reagent before use by evaporating 20±2 ml down to about 0.5 ml and injecting 2±0.1 µl directly into the gas chromatograph using the operating conditions used for the determination.

A7.8 **Calcium sulphate,** anhydrous granular.

A7.9 **pH indicator paper,** wide range.

A7.10 **N.O-bis (trimethylsilyl) acetamide (BSA)**
This can be obtained in small ampules or septum – sealed bottles. The reagent is rapidly hydrolysed and should be stored in a refrigerator and kept free from moisture. CAUTION: BSA IS TOXIC.

A7.11 **Internal standard solution – 0.5 mg/ml in 4-methylpentan-2-one.**
The choice of internal standard will depend on the type and number of phenols to be determined. Generally heptanol, nonanol or decanol will be suitable, but other compounds may be more appropriate for some specific applications.

A7.12 **A selection of pure phenols.** CAUTION: PHENOLS ARE POISONOUS AND CORROSIVE.

A8 Apparatus

A8.1 **Gas chromatograph** equipped with flame ionization detector, operated in accordance with manufacturers instructions.

A8.2 **Glass or stainless steel column** packed with 85–100 mesh diatomaceous earth (AW-DCMS) coated with the stationary phase. Suitable stationary phases are

(i) 3–10% methylsilicone eg SE-30, OV-1 and OV-101.
(ii) 5% Carbowax 20M.
(iii) 5% tri-2,4-xylenylphosphate.

The efficiency of the column should be at least 5000 total theoretical plates, measured using p-cresyl TMSE if complete separation of the three cresols is required; if this separation is not required, a column with a lower efficiency may be satisfactory. If improved separation of the majority of monohydric phenols is required, the use of a capillary column is recommended.
Operating conditions which have been found suitable are given in Section A13.

A8.3 **Pipettes,** Grade A, 1.00 ml, 2.00 ml and 5.00 ml provided with suction devices for use with A7.11.

A8.4 **Microlitre syringe,** 100 µl capacity, for reagent A7.10.

A8.5 **Microlitre syringe,** 5 µl capacity, for injection of sample into gas chromatograph.

A8.6 **Separating funnel,** 100 ml capacity.

A8.7 **Small glass reaction tube with cap.** The cap should be glass, PTFE or aluminium-lined.

A9 Sample Collection and Preservation

Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenols. Thus the addition of preserving agents is necessary.
The samples must be collected and stored in glass bottles with ground glass stoppers, previously cleaned by standing overnight in chromic acid or by employing an equivalent cleaning procedure, and then rinsed with water (Caution: Chromic acid is corrosive). Add 2 ml of sodium arsenite solution per litre of sample immediately after sampling. Then add 5 ml of 50% V/V hydrochloric acid per litre of sample. Check that the pH is 2 or below with wide range indicator papers, adjust with 50% hydrochloric acid if necessary.

CAUTION: If sulphide and/or cyanide is present, toxic fumes may be evolved.

Step	Procedure	Notes
	Analysis of samples	
A10.1	Add 100±5 ml of preserved sample (notes a, b and c) to the separating funnel and extract the solution twice with 10±1 ml portions of 4-methylpentan-2-one.	(a) Wide range indicator paper should be used to ensure that the pH of the sample is less than 2.
A10.2	Combine the extracts and dry them by adding 1.0±0.2 g of anhydrous calcium sulphate and swirl. Leave for at least 10 minutes.	(b) The sample may be filtered if necessary. (c) A larger or smaller aliquot may be taken if desired.
A10.3	Add by pipette to the dried extract a suitable amount (note d), depending on the expected phenols concentration, of internal standard solution and mix (note e).	(d) A useful guide is: Highest expected individual phenol conc'n, mg/l — Volume of internal standard solution, ml 0–5 — 1.00 5–10 — 2.00 10–25 — 5.00 25–50 — 10.00 (e) Do not pipette by mouth.

Step	Procedure	Notes
A10.4	Decant into an evaporting basin or small beaker and carefully evaporate the solution down to about 0.5 ml by blowing with a stream of nitrogen with gentle heating to about 60°C (note f). Decant into a small reaction tube.	(f) Alternatively, a rotary evaporator may be used.
A10.5	Dry the concentrated solution by adding a small amount of anhydrous calcium sulphate. Leave for at least 5 minutes.	
A10.6	Add 100±5 µl of BSA, replace the cap and mix.	
A10.7	Allow to react at room temperature for at least 20 minutes.	
A10.8	Inject 2.0±0.1 µl of the reaction mixture into the gas chromatograph, and adjust the attenuation settings such that the peak height of the internal standard is similar to the peak height of the major phenol component of the sample (preferably between one-third and two-thirds scale deflection).	
A10.9	Measure the height of each peak (note g) and calculate the concentration of each individual phenol by the internal standard technique.	(g) When an integrator is available the area may be used but the attenuation should not be adjusted.
	Blank determination	
A10.10	To 100±5 ml of water add 0.2 ml of sodium arsenite solution and 0.5 ml of 50% V/V hydrochloric acid. Repeat steps 1–9.	
	Internal standard calibration	
A10.11	Prepare a calibration mixture containing accurately about 100 mg of each of the phenols to be determined and the appropriate internal standard, and dissolve them in 4-methylpentan-2-one and make up to 100 ml in a volumetric flask and mix well.	
A10.12	Transfer about 0.5 ml of the calibration mixture into a small reaction tube and proceed with steps 6, 7 and 8 (note h).	(h) It is recommended that this step be carried out in duplicate.
A10.13	Measure the height of each peak (note g) and, after making any necessary corrections for attenuation changes made, calculate the response factor for each phenol as follows: $R_p = \frac{H_s \times W_p}{H_p \times W_s}$ where R_p = response factor for each phenol. H_s = height of internal standard peak corrected for blank if necessary. H_p = height of each phenol peak corrected for blank if necessary. W_p = weight of phenol used. W_s = weight of internal standard used.	

A11 Calculation of Results

The concentration of individual phenols in the sample is given by the formula

$$\frac{R_p \times H_p \times W_s}{H_s} \times \frac{1000 \text{ mgl}}{V}$$

where R_p = response factor for individual phenol (see Section 10, Steps 11–13).

H_p = height of individual phenol peak, corrected for blank if necessary.

H_s = height of internal standard peak, corrected for blank if necessary.

W_s = weight of internal standard used (mg).

V = volume of sample taken (ml).

If the total concentration of phenols in the sample is required, the individual phenol concentrations are summed.

A12 Checking the Accuracy of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests of the accuracy should be made regularly. As a minimum, it is recommended that with every batch of samples a known amount of phenol should be added to 100 ml of water used for the reagent blank and then analysed by stages 10 and 1–9 of Section A10. The results obtained should be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be calculated.

A13 Typical Chromatograms

The following series of eight chromatograms which demonstrates the wide range of phenolic compounds which may be determined using the operating conditions described in this method. These chromatograms are given to assist the analyst in the appropriate choice of column packing and operating conditions.

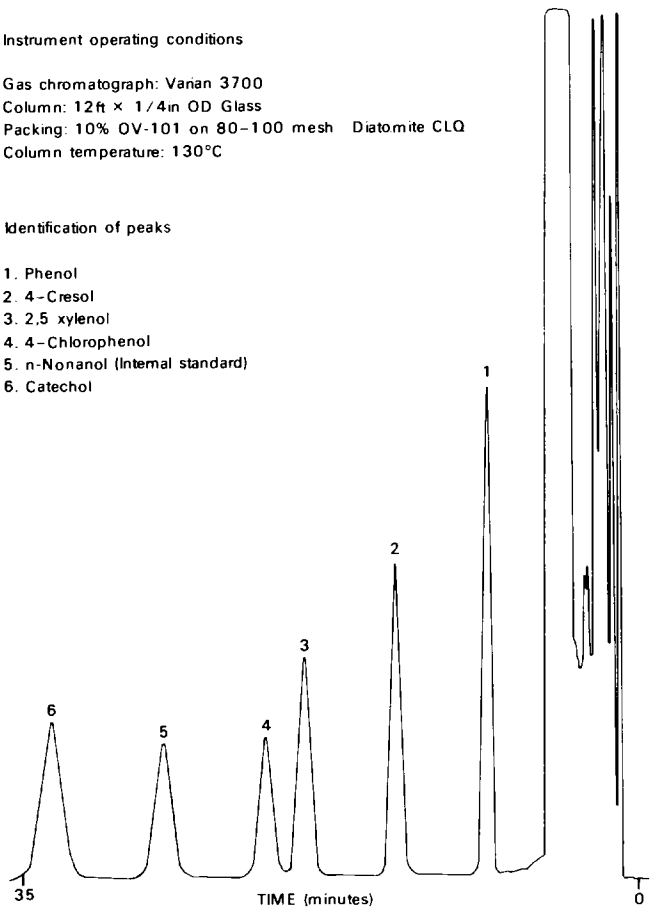
A13·1

Instrument operating conditions

Gas chromatograph: Varian 3700
Column: 12ft × 1/4in OD Glass
Packing: 10% OV-101 on 80–100 mesh Diatomite CLQ
Column temperature: 130°C

Identification of peaks

1. Phenol
2. 4-Cresol
3. 2,5 xylenol
4. 4-Chlorophenol
5. n-Nonanol (Internal standard)
6. Catechol



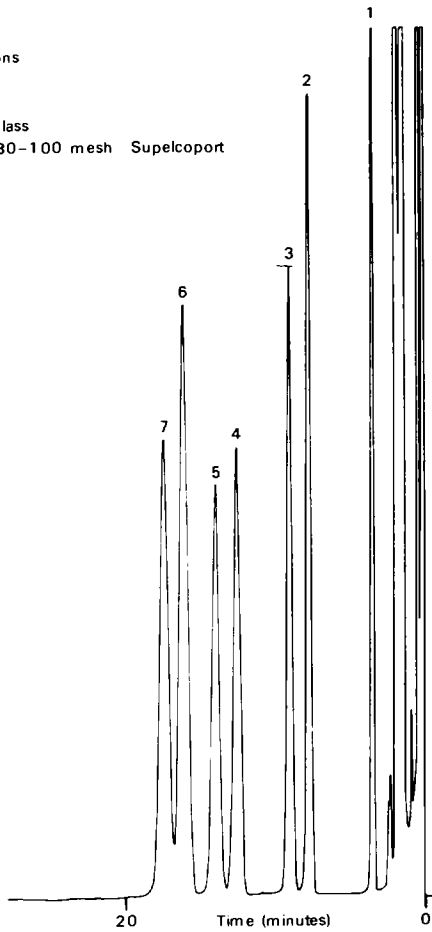
A13·2

Instrument operating conditions

Gas chromatograph: PE F11
Column: 6ft × 1/4in OD Glass
Packing: 10% OV-101 on 80–100 mesh Supelcoport
Column temperature: 140°C

Identification of peaks

1. Phenol
2. 2-Chlorophenol
3. 4-Chlorophenol
4. 2-Chloro-6-methyphenol
5. 4-Chloro-2-methyphenol
6. 2,6-Dichlorophenol
7. 2,4-Dichlorophenol



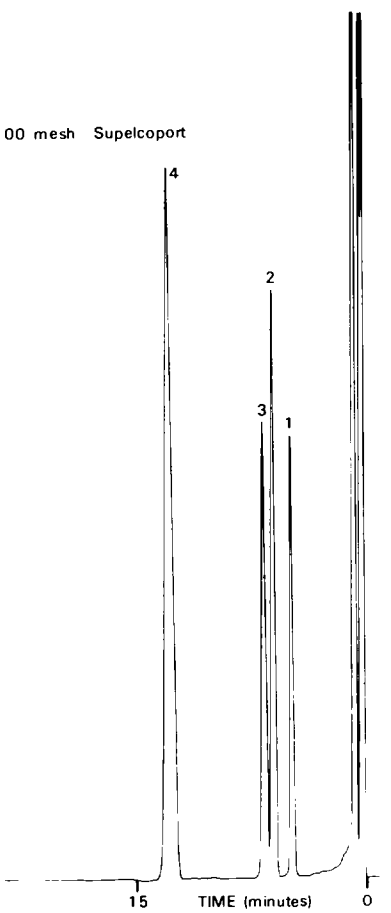
A13·3

Instrument operating conditions

Gas chromatograph: PE F11
Column: 6ft × 1/4in OD Glass
Packing: 10% OV-101 on 80–100 mesh Supelcoport
Column temperature: 180°C

Identification of peaks

1. Catechol
2. Resorcinol
3. Quinol
4. Pyrogallol



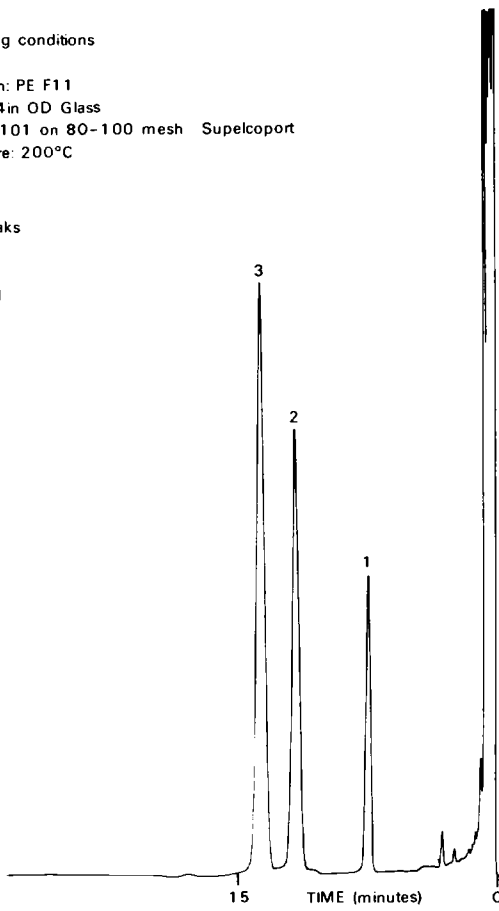
A13·4

Instrument operating conditions

Gas chromatograph: PE F11
Column: 6ft × 1/4in OD Glass
Packing: 10% OV-101 on 80–100 mesh Supelcoport
Column temperature: 200°C

Identification of peaks

1. 2-Phenylphenol
2. 3-Phenylphenol
3. 4-Phenylphenol



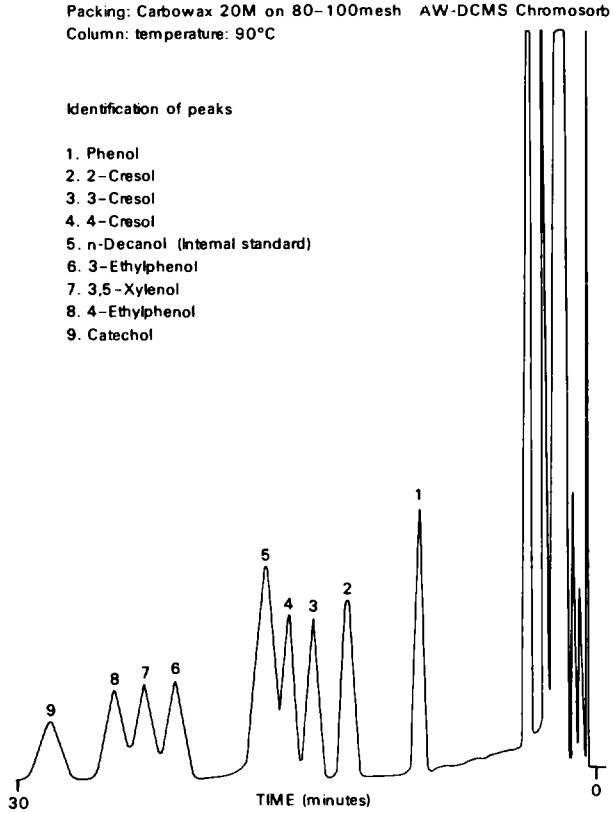
A13·5

Instrument operating conditions

Gas chromatograph: Varian 1700
Column: 12ft × 1/8in OD Stainless Steel
Packing: Carbowax 20M on 80–100mesh AW-DCMS Chromosorb W
Column: temperature: 90°C

Identification of peaks

1. Phenol
2. 2-Cresol
3. 3-Cresol
4. 4-Cresol
5. n-Decanol (Internal standard)
6. 3-Ethylphenol
7. 3,5-Xylenol
8. 4-Ethylphenol
9. Catechol



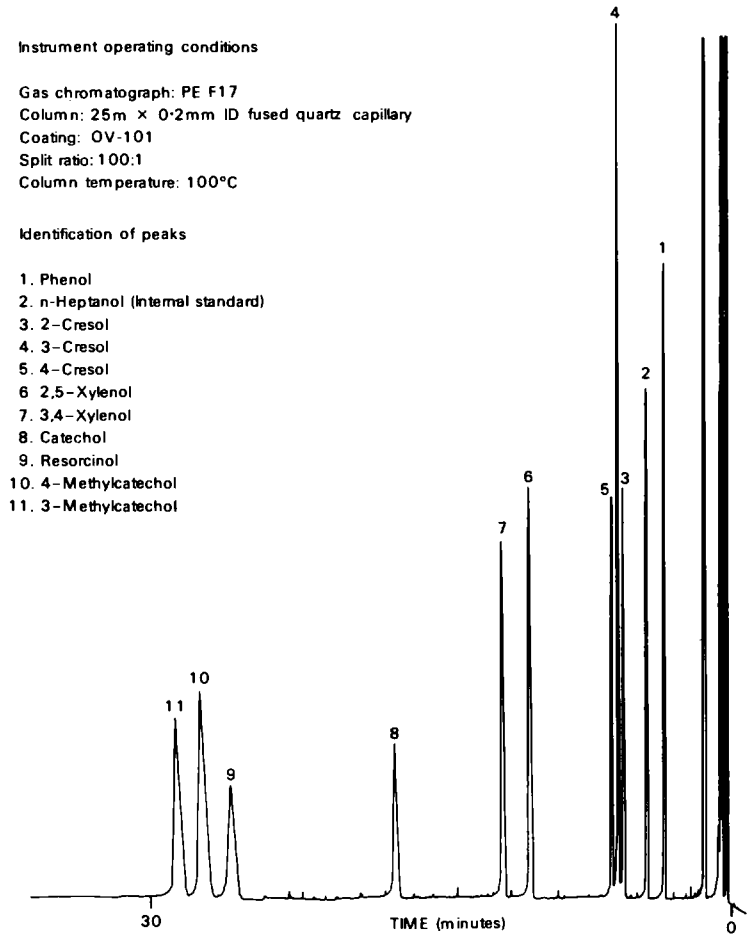
A13·6

Instrument operating conditions

Gas chromatograph: PE F17
Column: 25m × 0·2mm ID fused quartz capillary
Coating: OV-101
Split ratio: 100:1
Column temperature: 100°C

Identification of peaks

1. Phenol
2. n-Heptanol (Internal standard)
3. 2-Cresol
4. 3-Cresol
5. 4-Cresol
6. 2,5-Xylenol
7. 3,4-Xylenol
8. Catechol
9. Resorcinol
10. 4-Methylcatechol
11. 3-Methylcatechol



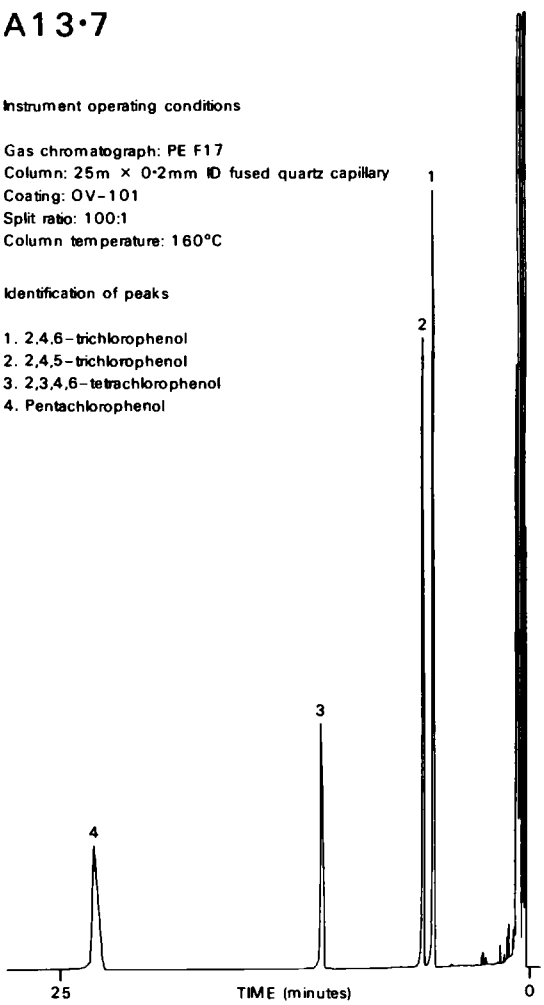
A13·7

Instrument operating conditions

Gas chromatograph: PE F17
Column: 25m × 0·2mm ID fused quartz capillary
Coating: OV-101
Split ratio: 100:1
Column temperature: 160°C

Identification of peaks

1. 2,4,6-trichlorophenol
2. 2,4,5-trichlorophenol
3. 2,3,4,6-tetrachlorophenol
4. Pentachlorophenol



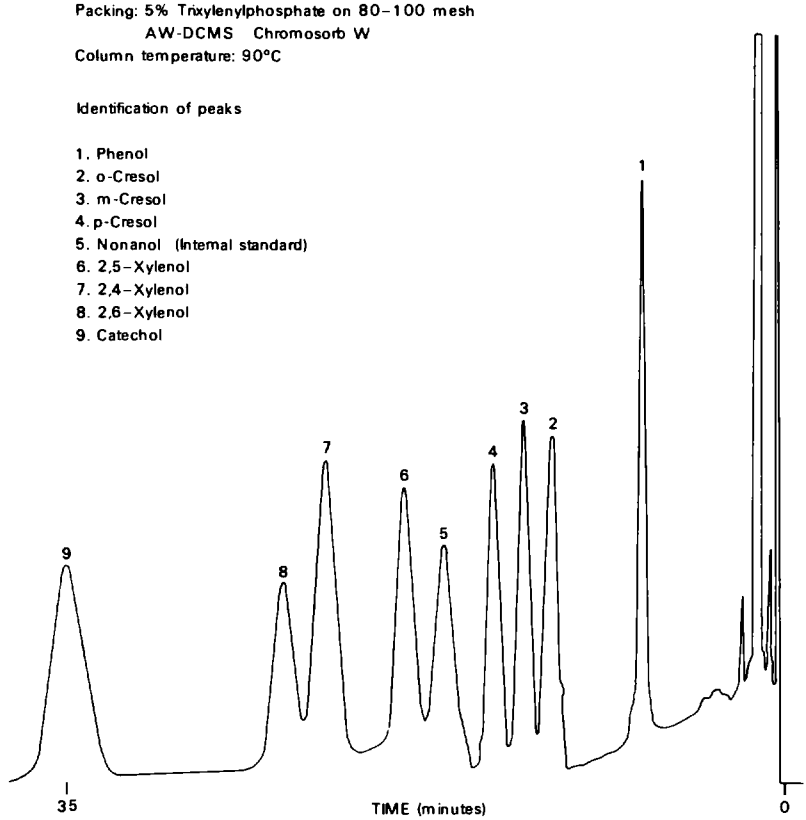
A13·8

Instrument operating conditions

Gas chromatograph: Pye Unicam 104
Column: 12ft × 1/8in OD Stainless Steel
Packing: 5% Trixylenylphosphate on 80–100 mesh
AW-DCMS Chromosorb W
Column temperature: 90°C

Identification of peaks

1. Phenol
2. o-Cresol
3. m-Cresol
4. p-Cresol
5. Nonanol (Internal standard)
6. 2,5-Xylenol
7. 2,4-Xylenol
8. 2,6-Xylenol
9. Catechol



Method B Determination of Monohydric Phenols in Effluents and Wastewaters, 4-Aminoantipyrine (pH 10.0) Spectrophotometric Method

B1 Performance Characteristics of the Method

B1.1	Substances determined	Those monohydric phenols reacting with 4-aminoantipyrine at pH 10.0 (see Section B2).
B1.2	Types of sample	Effluents and wastewaters (see Section B2).
B1.3	Basis of the method	After distillation to minimize interferences the sample is treated with reagents to form a 4-aminoantipyrine dye the concentration of which is determined spectrophotometrically.
B1.4	Range of application (a) (b)	0–1 mg/l phenol. The range can be extended by dilution with water after the distillation stage.
B1.5	Calibration curve (a) (b)	Linear to 1 mg/l phenol.
B1.6	Standard deviation (a) (b) (c)	

	Concentration mg/l phenol	Standard deviation mg/l phenol
Phenol solution	0.164	0.0062
Phenol solution	0.672	0.0122
Coke oven effluent	0.230	0.0085
Industrial effluent (d)	4.98	0.128

B1.7	Limit of detection (a) (b)	0.0092 mg/l (with 9 degrees of freedom).
B1.8	Sensitivity (a) (b)	1 mg/l phenol gives an absorbance of about 0.55.
B1.9	Bias	The concentration found will be less than the total concentration of monohydric phenols if phenols other than phenol are present (see Section B2).
B1.10	Interferences	None known. Tests using known concentrations of phenol with equal concentrations of aniline and with equal concentrations of phenol showed no interference (e).
B1.11	Time required for analysis	For six samples, a blank and a control sample the total analytical time is about 3.5 hours, and the operator time is about 1.5 hours.

(a) These data refer to the determination of phenol only.
(b) These data were obtained at the British Carbonization Research Association, using a spectrophotometer at 510 mm.
(c) Total standard deviations with 5 degrees of freedom.
(d) Sample diluted after distillation stage.
(e) Severn Trent Water Authority information.

B2 Scope and Field of Application

The method is applicable to sewage effluents but may not be applicable to all trade effluents. The analyst should make appropriate tests to ensure the method is satisfactory for these samples.

Monohydric phenols are separated from non-volatile impurities by distillation. Dihydric and polyhydric phenols are not recovered in the distillation procedure and therefore are not determined by the method.

Although none of the absorptiometric methods for the determination of phenols in waters are completely satisfactory, methods involving the use of 4-aminoantipyrine are at present recommended for the analysis of waters and effluents. The main limitations of 4-aminoantipyrine methods are:—

i) Many monohydric phenols respond to a lesser extent than phenol. The relative responses of some phenols to the 4-aminoantipyrine direct spectrophotometric method are given in Table 1.

ii) Substituents in the position para to the hydroxyl group prevent the reaction except as follows: halogen, carboxyl, sulphonic acid, hydroxyl and methoxyl.

iii) A nitro group in the ortho-position prevents reaction, a nitro group in the meta position inhibits the test but not completely.

iv) No colour reaction occurs when the ortho-positions are open and the para-position is blocked by aryl, alkyl, ester, nitro, benzoyl, nitroso or aldehyde groups.

v) Results are obtained as an apparent concentration of phenol and give no indication of which phenols are present.

B3 Principle

Biochemical oxidation of phenols in the sample is inhibited by the addition of hydrochloric acid which also eliminates any chemical changes resulting from alkaline conditions. Oxidizing agents are removed by the addition of sodium arsenite. The monohydric phenols are distilled from acid solution and separated from non-volatile impurities. The steam-distilled phenols react with 4-aminoantipyrine at a pH of 10.0 ($\pm$0.2) in the presence of potassium ferricyanide to form a coloured antipyrine dye, the absorbance of which is measured at 510 nm.

B4 Hazards

B4.1 Hydrochloric acid (reagent B6.2) can cause burns and gives off harmful vapour.

B4.2 Sodium arsenite (reagent B6.3) is poisonous.

B4.3 Ammonia (reagent B6.4) is poisonous and gives off harmful vapour.

B4.4 Phenol (reagent B6.7) is poisonous and corrosive and can be absorbed through the skin.

B4.5 Chromic acid (section B7.2) is corrosive.

B5 Reaction

The reaction scheme for the formation of the 4-aminoantipyrine complex is given in equation 2.

B6 Reagents

For the analysis, use only reagents of recognized analytical reagent grade unless otherwise stated.

B6.1 Water

It is essential that water of negligible phenol content (compared with the smallest concentrations to be determined in the samples) be used for rinsing apparatus and for the preparation of reagent solutions, phenol standard solutions and blanks. The water should be stored in an all glass aspirator.

B6.2 **Hydrochloric Acid Solution** (50% V/V)

Add 500 (±5) ml hydrochloric acid (d_{20} 1.18) cautiously to 400 ml of water in a 1-litre measuring cylinder stirring continuously. Cool and dilute with water to 1 litre. Store in a glass bottle with a ground glass stopper. Caution: Hydrochloric acid can cause burns and gives off harmful vapour.

B6.3 **Sodium Arsenite Solution** (0.2 W/V)

Dissolve 2.00 (±0.01) g of sodium arsenite in water and dilute to 1 litre in a calibrated flask. Store the solution in a glass bottle with a ground glass stopper. Caution: Sodium arsenite is poisonous.

B6.4 **Ammonia/Ammonium Chloride Solution**

Dissolve 21.4 (±0.1) g of ammonium chloride in 127 (±2) ml of ammonia solution (d_{20} 0.880) and dilute with water to 250 ml in a calibrated flask. Store this solution in a glass bottle with a ground glass stopper. For each batch of ammonia/ammonium chloride solution the pH should be checked as follows:—

Add 100 ml (±5 ml) of water to a 250 ml beaker and add 2 ml (±0.05 ml) of ammonia/ammonium chloride buffer solution. Mix the solution well and measure the pH which should be 10.0±0.2. Caution: Ammonia is poisonous and gives off harmful vapour.

B6.5 **4-Aminoantipyrine Solution** (2% W/V)

Dissolve 2.00 (±0.01) g of laboratory-reagent-grade 4-aminoantipyrine (4-aminophenazone) in water and dilute to 100 ml in a calibrated flask. Prepare freshly on the day of use and keep in a dark glass bottle with a ground glass stopper.

B6.6 **Potassium Ferricyanide Solution** (8% W/V)

Dissolve 8.0 (±0.1) g of potassium ferricyanide in water and dilute to 100 ml in a calibrated flask. Prepare freshly each week and store in the dark in a dark glass bottle with a ground glass stopper.

B6.7 **Standard Solutions of Phenol**

Solution A 1 ml=1 mg of phenol

Dissolve 1.000 (±0.001) g of phenol C_6H_5OH in water 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.

Solution B 1 ml=10 µg of phenol

Pipette 10.00 (±0.02) ml of the phenol *Solution A* into a 1-litre calibrated flask and dilute with water to 1-litre. Prepare on the day of use.

B7 Apparatus

B7.1 **Photometric Equipment**

A spectrophotometer of prism or grating type is required. The instrument should be capable of use at 510 nm (the exact wavelength of maximum absorption to be checked for each instrument) and should be equipped with 40 mm cells. Alternatively an instrument employing narrow band pass filters having maximum light transmission at or near 510 nm (eg. Ilford No. 604) and equipped with 40 mm cells may be used. Not all commercially available instruments have facilities for the use of 40 mm cells and where shorter path length cells are used the sensitivity of the method will be decreased but the precision of the analytical results is unlikely to be affected.

B7.2 **Glassware**

Glassware should have ground glass joints where appropriate; the use of grease is not recommended.

Distillation flask, 1 litre.
Splash head.
Water cooled condenser with receiver adaptor.
Calibrated flask, 500 ml.
Conical flask, 250 ml.

All glassware should be thoroughly cleaned before use by standing overnight in chromic acid or by employing an equivalent cleaning procedure and then rinsed with water. (Caution: Chromic acid is corrosive). After each determination the apparatus should be rinsed with water. Any yellow stains due to 4-aminoantipyrine complex should be removed with acetone before rinsing with water.

B8 Sample Collection and Preservation

Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenols. Thus the addition of preserving agents is necessary.

The samples must be collected and stored in glass bottles with ground glass stoppers previously cleaned by the procedure given in B7.2. 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.

B9 Procedure

Step	Procedure	Notes
	Analysis of samples†	
B9.1	Add 500 ml (±5 ml) of sample to a 1-litre distillation flask. (note a)	(a) It is recommended that if a sample is likely to contain more than 1 mg phenol/litre, an appropriately smaller aliquot of the sample be diluted with water after distillation.
B9.2	Distill off 450 ml (±5 ml) of the sample into a 500 ml calibrated flask (note b) and make up to 500 ml with water.	(b) It is useful to make a mark at 450 ml on the calibrated flask.
B9.3	Add 100 ml of the distilled sample (or an appropriate volume, V, of the distillate diluted to 100 ml with water) to a 250 ml conical flask.	
B9.4	Add in sequence to the flask swirling the contents after each addition; 2.00 ml (±0.05 ml) of ammonia/ ammonium chloride buffer solution (note c) 2.00 ml (±0.05 ml) of 2% W/V 4-aminoantipyrine solution and 2.00 ml (±0.05 ml) of 8% W/V potassium ferricyanide solution. Allow to stand for 10±5 minutes (note d).	(c) Do not pipette by mouth. (d) The 4-aminoantipyrine complex in aqueous solution has been found to be stable between 5 and 15 minutes.
B9.5	Meanwhile set up the spectrophotometer (Section 7.1) according to the manufacturers instructions. Adjust the zero of the instrument with water in the reference cell. Measure the absorbance (See Section B10) of the well mixed solution at 510 nm using 40-mm cells (note e). Recheck the instrument zero. Let the absorbance of the sample be S.	(e) Other sizes of cells may be used but the performance characteristics quoted in Section 1 would no longer apply.

*Ferrous sulphate should not be used to remove oxidizing agents in samples; only sodium arsenite should be used for this purpose.
†Interferences have been reported if the distillation stage is omitted.

Step	Procedure	Notes
B9.6	After measurement discard the contents of the sample cell, rinse it with water and allow to drain before the next solution to be measured is placed in the cell.	
	Blank determination	
	A blank determination must be run with each batch of determinations using exactly the same reagent solutions as those used for samples.	
B9.7	Add 500 ml (±5 ml) of water to a 1-litre distillation flask.	
B9.8	Add in sequence to the distillation flask carefully swirling the contents after each addition: 1.00 ml (± 0.05 ml) of 0.2% W/V sodium arsenite solution (note f) and 2.5 ml (±0.05 ml) of 50% V/V hydrochloric acid (note f).	(f) Do not pipette by mouth.
B9.9	Repeat stages 2–6 inclusive. Let the absorbance of the blank be B.	
	Calculation of results	
B9.10	Calculate the absorbance due to phenolic compounds, in the sample R, from $R=S-B$	
B9.11	Calculate the concentration of phenol C_A in the sample from $C_A=K.R.\frac{100}{V}$ where K is the calibration factor ie. the concentration of phenol in mg/l equivalent to an absorbance of 1 on the calibration curve. V the volume of distillate diluted to 100 ml for the spectrophotometric stage.	
	Preparation of Calibration Curve	
B9.12	Add 500, 495, 490, 480, 470, 460 and 450 ml of water (all ±5 ml) to a series of 1-litre distillation flasks. Add to these flasks 0.00, 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml of the phenol standard solution B.	
B9.13	Repeat stages 8 and 2–6 on these samples. These determinations should be repeated at least once on another day and then again as necessary until the calibration curve is defined with the required accuracy. Normally two batches of determinations will suffice. (note g).	(g) A new calibration curve should be prepared with each bottle of 4-aminoantipyrine used.
B9.14	Subtract the average absorbance of the blank from the absorbance average for each of the phenol concentrations and plot the corrected absorbances against the concentrations of phenol added to the water. The solutions in stage 12 are equivalent to 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg phenol/litre.	

B10 Notes on Procedure

Measurement of absorbance

The exact instrument setting for the wavelength of the absorption peak must be checked for each instrument and then used in all future work. The procedure used for measuring absorbance should be rigorously controlled to ensure satisfactory precision of measurements. The same cells should always be used for the reference and sample cells; and they should always be placed in the same position in the cell holder with the same face towards the light source.

It is difficult to ensure reproducible alignment of cells with chipped corners and they should be discarded. Similarly optical faces of the cells and the slide of the cell holder should be kept scrupulously clean. Before every set of measurements the optical density of the sample cell should be measured against the reference cell when both are filled with water. This will help to indicate when the cells need cleaning, and it will also enable the true absorbance of the blank to be determined.

B11 Checking the Accuracy of Analytical Results

Once the method had been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests of the accuracy should be made regularly. As a minimum, it is recommended that with every batch of samples a known amount of phenol should be added to 500 ml of water used for the reagent blank and then analysed by stages 8, 2–6, 10 and 11 of Section 9. The results obtained should be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be calculated.

Method C Determination of Monohydric Phenols in Unchlorinated Freshwaters and Effluents, 4-Aminoantipyrine, (pH 10.0) Chloroform Extraction Spectrophotometric Method

C1 Performance Characteristics of the Method

C1.1	Substances determined	Those monohydric phenols reacting with 4-aminoantipyrine at pH 10.0. (See Section 2).
C1.2	Types of sample	The method has been tested for river waters. For other types of samples see Section 2.
C1.3	Basis of the method	After distillation to minimize interferences the sample is treated with reagents to form a 4-aminoantipyrine dye. This is extracted into chloroform and its concentration determined spectrophotometrically.
C1.4	Range of application (a) (b)	0–100 µg phenol/litre. The range can be extended by dilution with water after the distillation stage.
C1.5	Calibration curve (1) (b)	Linear to 100 µg phenol/litre.
C1.6	Standard deviation (a) (b) (c)	
C1.7	Limit of detection (a) (b)	2.9 µg/l (with 10 degrees of freedom).
C1.8	Sensitivity (a) (b)	100 µg/l phenol gives an absorbance of about 1.75.
C1.9	Bias	The concentration found will be less than the total concentration of monohydric phenols if phenols other than phenol are present.
C1.10	Interferences	Not known.
C1.11	Time required for analysis	For six samples, a blank and a control sample the total analytical time is about 4 hours and the operator time about 2 hours.

	Concentration µg/l phenol	Standard deviation µg/l phenol
Phenol solution	10.0	0.83
Phenol solution	79.2	2.49
Spiked river water	10.3	1.20
Spiked river water	80.2	1.77

(a) These data refer to the determination of phenol only.
(b) These data were obtained at the Southern Water Authority, using a spectrophotometer at 460 nm.
(c) Total standard deviation with 5 degrees of freedom.

C2 Scope and Field of Application

The method has been tested for river waters and should be applicable to groundwaters and unchlorinated potable waters. If the method is to be applied to sewage effluents and trade effluents, the analyst should make appropriate tests to ensure that the method is satisfactory for these samples.

Monohydric phenols are separated from non-volatile impurities by distillation. Dihydric and polyhydric phenols are not recovered in the distillation procedure and therefore are not determined by the method.

Although none of the absorptiometric methods for the determination of phenols in waters are completely satisfactory, methods involving the use of 4-aminoantipyrine are at present recommended for the analysis of waters and effluents. The main limitations of 4-aminoantipyrine methods are:—

i) Many monohydric phenols respond to a lesser extent than phenol. The relative responses of some phenols to the 4-aminoantipyrine (pH 10.0) method are given in Table 1. Further information on this subject may be found in references 3 and 4.

ii) Substituents in the position para to the hydroxyl group prevent the reaction except as follows: halogen, carboxyl, sulphonic acid, hydroxyl and methoxyl.

iii) A nitro group in the ortho-position prevents reaction, a nitro group in the meta position inhibits the test but not completely.

iv) No colour reaction occurs when the ortho-positions are open and the para-position is blocked by aryl, alkyl, ester, nitro, benzoyl, nitroso or aldehyde groups.

v) Results are obtained as an apparent concentration of phenol and give no indication of which phenols are present.

C3 Principle

Biochemical oxidation of phenols in the sample is inhibited by the addition of hydrochloric acid which also eliminates any chemical changes resulting from alkaline conditions. Oxidizing agents are removed by the addition of sodium arsenite. The monohydric phenols are distilled from acid solution and separated from non-volatile impurities. The steam-distilled phenols react with 4-aminoantipyrine at a pH of 10.0 ($\pm$0.2) in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is extracted into chloroform and the absorbance of the extract is measured at 460 nm.

C4 Hazards

C4.1 Hydrochloric acid (reagent C6.2) can cause burns and gives off harmful vapour.

C4.2 Sodium arsenite (reagent C6.3) is poisonous.

C4.3 Ammonia (reagent C6.4) is poisonous and gives off harmful vapour.

C4.4 Chloroform (reagent C6.7) is poisonous and gives off harmful vapour.

C4.5 Phenol (reagent C6.8) is poisonous and corrosive and can be absorbed through the skin.

C4.6 Chromic acid (Section C7.2) is corrosive.

C5 Reaction

The reaction scheme for the formation of the 4-aminoantipyrine complex is given in equation 2.

C6 Reagents

For the analysis, use only reagents of recognized analytical reagent grade unless otherwise stated.

C6.1 Water

It is essential that water of negligible phenol content (compared with the smallest concentrations to be determined in the samples) be used for rinsing apparatus and for the preparation of reagent solutions, phenol standard solutions and blanks. The water should be stored in an all-glass aspirator.

C6.2 Hydrochloric Acid Solution (50% V/V)

Add 500 ($\pm$5) ml hydrochloric acid (d_{20} 1.18) cautiously to 400 ml of water in a 1-litre measuring cylinder stirring continuously. Cool and dilute with water to 1 litre. Store in

a glass bottle with a ground glass stopper. Caution: Hydrochloric acid can cause burns and gives off harmful vapour.

C6.3 **Sodium Arsenite Solution** (0.2% W/V)

Dissolve 2.00 (±0.01) g of sodium arsenite in water and dilute to 1 litre in a calibrated flask. Store the solution in a glass bottle with a ground glass stopper. Caution: Sodium arsenite is poisonous.

C6.4 **Ammonia/Ammonium Chloride Solution**

Dissolve 21.4 (±0.1) g of ammonium chloride in 127 (±2) ml of ammonia solution (d_{20} 0.880) and dilute with water to 250 ml in a calibrated flask. Store this solution in a glass bottle with a ground glass stopper. For each batch of ammonia/ammonium chloride solution the pH should be checked as follows:

Add 500 ml (±5 ml) of water to a 1-litre beaker and add 2 ml (±0.05 ml) of ammonia/ammonium chloride buffer solution. Mix the solution well and measure the pH which should be 10.0±0.2. Caution: Ammonia is poisonous and gives off harmful vapour.

C6.5 **4-Aminoantipyrine Solution** (2% W/V)

Dissolve 2.00 (±0.01) g of laboratory-reagent-grade 4-aminoantipyrine (4-aminophenazone) in water and dilute to 100 ml in a calibrated flask. Prepare freshly on the day of use and keep in a dark glass bottle with a ground glass stopper.

C6.6 **Potassium Ferricyanide Solution** (8% W/V)

Dissolve 8.0 (±0.1) g of potassium ferricyanide in water and dilute to 100 ml in a calibrated flask. Prepare freshly each week and store in the dark in a dark glass bottle with a ground glass stopper.

C6.7 **Chloroform**

Caution: Harmful vapour.

C6.8 **Standard Solutions of Phenol**

Solution A 1 ml=1 mg of phenol

Dissolve 1.00 (±0.001)g of phenol C_6H_5OH in water 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.

Solution B 1 ml=10 µg of phenol

Pipette 10.00 (±0.02) ml of the phenol *Solution A* into a 1-litre calibrated flask and dilute with water to 1 litre. Prepare on the day of use.

Solution C 1 ml=1 µg of phenol

Pipette 25.00 (±0.03) ml of the phenol *Solution B* into a 250 ml calibrated flask and dilute with water to 250 ml. Prepare immediately before use.

C7 Apparatus

C7.1 **Photometric Equipment**

A spectrophotometer of prism or grating type is required. The instrument should be capable of use at 460 nm (the exact wavelength of maximum absorption to be checked for each instrument) and should be equipped with 40 mm cells. Alternatively an instrument employing narrow band pass filters having maximum light transmission at or near 460 nm (eg. Ilford No. 602) and equipped with 40 mm cells may be used. Not all commercially available instruments have facilities for the use of 40 mm cells and where shorter path length cells are used the sensitivity of the method will be decreased but the precision of the analytical results is unlikely to be affected.

C7.2 **Glassware**

Glassware should have ground glass joints where appropriate, the use of grease is not recommended.

Distillation flask, 1 litre.
Splash head.
Water cooled condenser with receiver adaptor.
Calibrated flask, 500 ml.
Separating funnel, 1 litre.

It is preferable that the glassware should be reserved solely for phenol determinations. All glassware should be thoroughly cleaned before use by standing overnight in chromic acid or by employing an equivalent cleaning procedure and then rinsed with water (Caution: Chromic acid is corrosive). After each determination the apparatus should be rinsed with water. Any yellow stains due to 4-aminoantipyrine complex should be removed with acetone before rinsing with water.

C8 Sample Collection and Preservation

Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenols. Thus the addition of preserving agents is necessary.

The samples must be collected and stored in glass bottles with ground glass stoppers previously cleaned by the procedure given in 7.2. 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.

C9 Procedure

Step	Procedure	Notes
	Analysis of samples	
C9.1	Add 500 ml (±5 ml) of the preserved sample to a 1-litre distillation flask. (note a).	(a) It is recommended that if a sample is likely to contain more than 100 µg phenol/litre, an appropriately smaller aliquot of the sample be diluted with water after distillation.
C9.2	Distil off 450 ml (±5 ml) of the sample into a 500 ml calibrated flask (note b) and make up to 500 ml with water.	(b) It is useful to make a mark at 450 ml on the calibrated flask.
C9.3	Add the distilled sample (or an appropriate volume, V, of the distillate diluted to 500 ml with water) to a 1-litre separating funnel.	
C9.4	Add in sequence to the separating funnel swirling the contents after each addition, 2.00 ml (±0.05 ml) of ammonia/ammonium chloride buffer solution (note c), 3.00 ml (±0.1 ml) of 2% W/V 4-aminoantipyrine solution and 3.00 ml (±0.1 ml) of 8% W/V potassium ferricyanide solution.	(c) Do not pipette by mouth.
C9.5	Allow the solution to stand for 10±5 minutes (note d).	(d) The 4-aminoantipyrine complex in aqueous solution has been found to be stable between 5 and 15 minutes.

*Ferrous sulphate should not be used to remove oxidizing agents in samples; only sodium arsenite should be used for this purpose.

Step	Procedure	Notes
C9.6	Add 25.0 (±0.1 ml) of chloroform to the separating funnel (note c) shake the funnel gently for a few seconds and then release the excess pressure in the funnel (note e).	(e) Care should be taken that no chloroform is lost when the excess pressure is released.
C9.7	Shake the funnel vigorously for 1 minute (±10 second) and allow the two phases to separate for at least five minutes.	
C9.8	Pass the chloroform extract (note f) through a Whatman No. 4 filter paper into a clean dry 40 mm cell. Discard the filter paper.	(f) Dry the stem of the separating funnel with a strip of filter paper before running off the extract.
C9.9	Meanwhile set up the spectrophotometer (Section 7.1) according to the manufacturers instructions. Adjust the zero of the instrument with chloroform in the reference cell. Within 30 minutes of the extraction measure the absorbance (see Section 10) of the extract (note g) at 460 nm using 40-mm cells (note h). Recheck the instrument zero. Let the absorbance of the sample be S.	(g) Do not allow direct sunlight to fall on the extract. (h) Other sizes of cells may be used but the performance characteristics quoted in Section 1 would no longer apply.
C9.10	After measurement discard the contents of the sample cell, rinse it with chloroform and allow to dry before the next extract to be measured is placed in the cell.	
	Blank determination	
	A blank determination must be run with each batch of determinations using exactly the same reagent solutions as those used for samples.	
C9.11	Add 500 ml (±5 ml) of water to a 1-litre distillation flask.	
C9.12	Add in sequence to the distillation flask carefully swirling the contents after each addition: 1.00 ml (±0.05 ml) of 0.2% W/V sodium arsenite solution (note h), and 2.5 ml (±0.05 ml) of 50% V/V hydrochloric acid solution (note i).	(i) Do not pipette by mouth.
C9.13	Repeat stages 2–10 inclusive. Let the absorbance of the blank be B.	
	Calculation of results	
C9.14	Calculate the absorbance due to phenolic compounds in the sample, R, from $R = S - B$	
C9.15	Calculate the concentration of phenol C_A in the sample from $C_A = K.R.\frac{500}{V}$ where K is the calibration factor ie. the concentration of phenol in µg/l equivalent to an absorbance of 1 on the calibration curve. V the volume of distillate diluted to 500 ml for the spectrophotometric stage.	

Step	Procedure	Notes
	Preparation of Calibration Curve	
C9.16	Add 500, 495, 490, 480, 470, 460 and 450 ml of water (all ±5 ml) to a series of 1-litre distillation flasks. Add to these flasks 0.00, 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml, respectively, of phenol standard solution C.	
C9.17	Repeat stages 12 and 2–10 on these samples. These determinations should be repeated at least once on another day and then again as necessary until the calibration is defined with the required accuracy. Normally two batches of determinations will suffice (note j).	(j) A new calibration curve should be prepared with each bottle of 4-aminoantipyrine used.
C9.18	Subtract the average absorbance of the blank from the average absorbances for each of the phenol concentrations and plot the corrected absorbances against the concentrations of phenol added to the water. The solutions in stage 16 are equivalent to 0, 10, 20, 40, 60, 80 and 100 µg/litre.	

C10 Notes on Procedure

Measurement of absorbance

The exact instrument setting for the wavelength of the absorption peak must be checked for each instrument and then used in all future work. The procedure used for measuring absorbance should be rigorously controlled to ensure satisfactory precision of measurements. The same cells should always be used for the reference and sample cells, and they should always be placed in the same position in the cell holder with the same face towards the light source.

It is difficult to ensure reproducible alignment of cells with chipped corners and they should be discarded. Similarly optical faces of the cuvette and the slide of the cell holder should be kept scrupulously clean. Before every set of measurements the optical density of the sample cell should be measured against the reference cell when both are filled with chloroform. This will help to indicate when the cells need cleaning, and it will also enable the true absorbance of the blank to be determined.

C11 Checking the Accuracy of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests of the accuracy should be made regularly. As a minimum, it is recommended that with every batch of samples a known amount of phenol should be added to 500 ml of water used for the reagent blank and then analysed by stages 12, 2–10, 14 and 15 of Section 9. The results obtained should be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be calculated.

Method D Determination of Monohydric Phenols in Chlorinated Freshwaters, 4-Aminoantipyrine (pH 7.9) Chloroform Extraction Spectrophotometric Method

D1 Performance Characteristics of the Method

D1.1	Substances determined	Those monohydric phenols reacting with 4-aminoantipyrine at pH 7.9. (See Section D2).
D1.2	Types of sample	River waters and chlorinated potable waters.
D1.3	Basis of the method	After distillation to minimize interferences the sample is treated with reagents to form a 4-aminoantipyrine dye. This is extracted into chloroform and its concentration determined spectrophotometrically.
D1.4	Range of application (a) (b)	0–50 µg phenol/litre. The range can be extended by dilution with water after the distillation stage.
D1.5	Calibration curve (a) (b)	Linear to 50 µg phenol/litre.
D1.6	Standard deviation (a) (b) (c)	

	Concentration µg/l phenol	Standard deviation µg/l phenol
River water	3–13	2.1
Spiked river water	8–18	3.4
Spiked river water	56–63	2.1

D1.7	Limit of detection (a) (b)	4.7 µg/l (with 10 degrees of freedom).
D1.8	Sensitivity (a) (b)	50 µg/l phenol gives an absorbance of about 0.90.
D1.9	Bias	The concentration found will be less than the total concentration of monohydric phenols if phenols other than phenol are present.
D1.10	Interferences	Not known.
D1.11	Time required for analysis	For six samples, a blank and a control sample the total analytical time is about 4 hours and the operator time about 2 hours.

(a) These data refer to the determination of phenol only.
(b) These data were obtained at the Water Research Centre (Medmenham Laboratory) using a spectrophotometer at 460 nm.
(c) Within-batch standard deviations with 8 degrees of freedom.

D2 Scope and Field of Application

The method has been tested for river waters and chlorinated potable waters. The method has the advantage over the pH 10.0 method with chloroform extraction when applied to chlorinated freshwaters in that greater sensitivity is obtained for chlorosubstituted phenols. (See Table 1).

Monohydric phenols are separated from non-volatile impurities by distillation. Dihydric and polyhydric phenols are not recovered in the distillation procedure and therefore are not determined by the method.

Although none of the absorptiometric methods for the determination of phenols in waters are completely satisfactory, methods involving the use of 4-aminoantipyrine are at present recommended for the analysis of waters and effluents. The main limitations of 4-aminoantipyrine methods are:

(i) Many monohydric phenols respond to a lesser extent than phenol. The relative responses of some phenols to the 4-aminoantipyrine (pH 7.9) method are given in Table 1. Further information on this subject may be found in reference 3.

(ii) Substituents in the position para to the hydroxyl group prevent the reaction except as follows: halogen, carboxyl, sulphonic acid, hydroxyl and methoxyl.

(iii) A nitro group in the ortho-position prevents reaction, a nitro group in the meta position inhibits the test but not completely.

(iv) No colour reaction occurs when the ortho-positions are open and the para-position is blocked by aryl, alkyl, ester, nitro, benzoyl, nitroso or aldehyde groups.

(v) Results are obtained as an apparent concentration of phenol and give no indication of which phenols are present.

D3 Principle

Biochemical oxidation of phenols in the sample is inhibited by the addition of hydrochloric acid which also eliminates any chemical changes resulting from alkaline conditions. Oxidizing agents are removed by the addition of sodium arsenite. The monohydric phenols are distilled from acid solution and separated from non-volatile impurities. The steam-distilled phenols react with 4-aminoantipyrine at a pH of 7.9 ($\pm$0.1) in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is extracted into chloroform and the absorbance of the extract is measured at 460 nm.

D4 Hazards

D4.1 Hydrochloric acid (Reagent D6.2) can cause burns and gives off harmful vapour.

D4.2 Sodium arsenite (Reagent D6.3) is poisonous.

D4.3 Ammonia (Reagent D6.4) is poisonous and gives off harmful vapour.

D4.4 Chloroform (Reagent D6.8) is poisonous and gives off harmful vapour.

D4.5 Phenol (Reagent D6.9) is poisonous and corrosive, and can be absorbed through the skin.

D4.6 Chromic acid (Section D7.2) is corrosive.

D5 Reaction

The reaction scheme for the formation of the 4-aminoantipyrine complex is given in equation 2.

D6 Reagents

For the analysis, use only reagents of recognized analytical reagent grade unless otherwise stated.

D6.1 Water

It is essential that water of negligible phenol content (compared with the smallest concentrations to be determined in the samples) be used for rinsing apparatus and for the preparation of reagent solutions, phenol standard solutions and blanks. The water should be stored in an all-glass aspirator.

D6.2 Hydrochloric Acid Solution (50% v/v)

Add 500 ($\pm$5) ml hydrochloric acid (d_{20} 1.18) cautiously to 400 ml of water in a 1-litre measuring cylinder stirring continuously. Cool and dilute with water to 1 litre. Store in a glass bottle with a ground glass stopper. Caution: Hydrochloric acid can cause burns and gives off harmful vapour.

D6.3 **Sodium Arsenite Solution** (0.2% w/v)

Dissolve 2.0 (±0.01) g of sodium arsenite in water and dilute to 1-litre in a calibrated flask. Store the solution in a glass bottle with a ground glass stopper. Caution: Sodium arsenite is poisonous.

D6.4 **Ammonia Solution** (0.5N)

Dilute 27.5 (±1) ml of ammonia solution (d_{20} 0.880) with water to 1 litre in a calibrated flask. Store the solution in a glass bottle with a ground glass stopper. Caution: Ammonia is poisonous and gives off harmful vapour.

D6.5 **Phosphate Buffer Solution**

Dissolve 104.5 (±1) g of anhydrous di-potassium hydrogen phosphate K_2HPO_4 and 72.3 (±1) g of potassium dihydrogen orthophosphate KH_2PO_4 in water and dilute with water to 1 litre in a calibrated flask. The pH of this solution should be 6.8. Store the solution in a glass bottle with a ground glass stopper.

D6.6 **4-Aminoantipyrine Solution** (2% w/v)

Dissolve 2.00 (±0.01) g of laboratory-reagent-grade-4-aminoantipyrine (4-aminophenazone) in water and dilute to 100 ml in a calibrated flask. Prepare freshly on the day of use and keep in a dark glass bottle with a ground glass stopper.

D6.7 **Potassium Ferricyanide Solution** (8% w/v)

Dissolve 8.0 (±0.1) g of potassium ferricyanide in water and dilute to 100 ml in a calibrated flask. Prepare freshly each week and store in the dark in a dark glass bottle with a ground glass stopper.

D6.8 **Chloroform**

Caution: Poison, harmful vapour.

D6.9 **Standard Solutions of Phenol**

Solution A 1 ml = 1 mg of phenol

Dissolve 1.000 (±0.001) g of phenol C_6H_5OH in water 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.

Solution B 1 ml = 10 µg of phenol

Pipette 10.00 (±0.02) ml of the phenol *Solution A* into a 1-litre calibrated flask and dilute with water to 1 litre. Prepare on the day of use.

Solution C 1 ml = 0.5 µg phenol

Pipette 25.00 (±0.003) ml of the phenol *Solution B* into a 500 ml calibrated flask and dilute with water to 500 ml. Prepare immediately before use.

D7 Apparatus

D7.1 **Photometric Equipment**

A spectrophotometer of prism or grating type is required. The instrument should be capable of use at 460 nm (the exact wavelength of maximum absorption to be checked for each instrument) and should be equipped with 40 mm cells. Alternatively an instrument employing narrow band pass filters having maximum light transmission at or near 460 nm (eg Ilford No 602) and equipped with 40 mm cells may be used. Not all commercially available instruments have facilities for the use of 40 mm cells and where shorter path length cells are used the sensitivity of the method will be decreased but the precision of the analytical results is unlikely to be affected.

D7.2 **Glassware**

Glassware should have ground glass joints where appropriate; the use of grease is not recommended.

Distillation flask, 1 litre.
Splash head.
Water cooled condenser with receiver adaptor.
Calibrated flask, 500 ml.
Separating funnel, 1 litre.

It is preferable that the glassware should be reserved solely for phenol determinations. All glassware should be thoroughly cleaned before use by standing overnight in chromic acid or by employing an equivalent cleaning procedure and then rinsed with water. (Caution: Chromic acid is corrosive). After each determination the apparatus should be rinsed with water. Any yellow stains due to 4-aminoantipyrine complex should be removed with acetone before rinsing with water.

D8 Sample Collection and Preservation

Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenols. Thus the addition of preserving agents is necessary.

The samples must be collected and stored in glass bottles with ground glass stoppers previously cleaned by the procedure given in 7.2. 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 hydroxhloric acid per litre of sample. CAUTION: If sulphide and/or cyanide is present toxic fumes may be evolved.

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* Ferrous sulphate should not be used to remove oxidizing agents in samples; only sodium arsenite should be used for this purpose and check that the pH is 2 or less with wide range indicator paper and adjust if necessary with 50% v/v hydrochloric acid.

D9 Procedure

Step	Procedure	Notes
	Analysis of samples	
D9.1	Add 500 ml (±5 ml) of sample to a 1-litre distillation flask. (note a).	(a) It is recommended that if a sample is likely to contain more than 50 µg phenol/litre an appropriately smaller aliquot of the sample be diluted with water after distillation.
D9.2	Distil off 450 ml (±5 ml) of the sample into a 500 ml calibrated flask (note b) and make up to 500 ml with water.	(b) It is useful to make a mark at 450 ml on the calibrated flask.
D9.3	Add the distilled sample (or an appropriate volume, C, of the distillate diluted to 500 ml with water) to a 1-litre separating funnel.	
D9.4	Add in sequence to the separating funnel swirling the contents after each addition; 5.0 ml (±0.1 ml) of 0.5N ammonia solution (note c), the appropriate volume of phosphate buffer (±0.05 ml) (note d) 1.00 ml (±0.05 ml) of 2% w/v 4-aminoantipyrine solution and 3.00 ml (±0.1 ml) of 8% w/v potassium ferricyanide solution.	(c) Do not pipette by mouth (d) For each batch of 0.5N ammonia prepared, determine the appropriate volume of phosphate buffer as follows. Add 500 ml (±5ml) of distilled water and 5.0 ml (±0.1 ml) of 0.5N ammonia solution to a 1-litre beaker. Titrate the solution with the phosphate buffer solution, and note the volume required to give a pH of 7.9. Use that volume of buffer solution for all determinations using the same batch of 0.5N ammonia solution.

Step	Procedure	Notes
D9.5	Allow the solution to stand for 15±5 minutes (note e).	(e) The 4-aminoantipyrine complex in aqueous solution has been found to be stable between 10 and 20 minutes.
D9.6	Add 25.0 (±0.1 ml) of chloroform to the separating funnel (note c) shake the funnel gently for a few seconds and then release the excess pressure in the funnel (note f).	(f) Care should be taken that no chloroform is lost when the excess pressure is released.
D9.7	Shake the funnel vigorously for 1 minute (±10 seconds) and allow the two phases to seapate for at least five minutes.	
D9.8	Pass the chloroform extract (note g) through a Whatman No 4 filter paper into a clean dry 40 mm cell. Discard the filter paper.	(g) Dry the stem of the separating funnel with a strip of filter paper before running off the extract.
D9.9	Meanwhile set up the spectrophotometer (Section D7.1) according to the manufacturers instructions. Adjust the zero of the instrument with chloroform in the reference cell. Within 30 minutes of the extraction measure the absorbance (see Section 10) of the extract (note h) at 460 nm using 40 mm cells (note i). Recheck the instrument zero. Let the absorbance of the sample be S.	(h) Do not allow direct sunlight to fall on the extract. (i) Other sizes of cells may be used but the performance characteristics quoted in Section D1 would no longer apply.
D9.10	After measurement discard the contents of the sample cell, rinse it with chloroform and allow to dry before the next extract to be measured is placed in the cell.	
	Blank determination	
	A blank determination must be run with each batch of determinations using exactly the same reagent solutions as those used for samples.	
D9.11	Add 500 ml (±5 ml) of water to a 1-litre distillation flask.	
D9.12	Add in sequence to the distillation flask carefully swirling the contents after each addition: 1.00 ml (±0.05 ml) of 0.2% w/v sodium arsenite solution (note j), 2.5 ml (±0.05 ml) of 50% v/v hydrochloric acid solution (note j).	(j) Do not pipette by mouth.
D9.13	Repeat stages 2–10 inclusive. Let the absorbance of the blank be B.	
	Calculation of results	
D9.14	Calculate the absorbance due to phenolic compounds in the sample, R, from $R = S - B$	
D9.15	Calculate the concentration of phenol C_A in the sample from $C_A = K.R.\frac{500}{V}$ where K is the calibration factor ie. the concentration of phenol in µg/l equivalent to an absorbance of 1 on the calibration curve. V the volume of distillate diluted to 500 ml for the spectrophotometric stage.	

Step	Procedure	Notes
	Preparation of calibration Curve	
D9.16	Add 500, 495, 490, 480, 470, 460 and 450 ml of water (all ±5 ml) to a series of 1-litre distillation flasks. Add to these flasks 0.00, 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml of phenol standard solution C.	
D9.17	Repeat stages 12, and 2–10 on these samples. These determinations should be repeated at least once on another day and then again as necessary until the calibration curve is defined with the required accuracy. Normally two batches of determinations will suffice (note k).	(k) A new calibration curve should be prepared with each bottle of 4-aminoantipyrine used.
D9.18	Subtract the average absorbance of the blank from the average absorbances for each of the phenol concentrations and plot the corrected absorbances against the concentrations of phenol added to the water. The solutions in stage 16 are equivalent to 0, 5, 10, 20, 30, 40 and 50 µg phenol/litre.	

D10 Notes on Procedure

Measurement of absorbance

The exact instrument setting for the wavelength of the absorption peak must be checked for each instrument and then used in all future work. The procedure used for measuring absorbance should be rigorously controlled to ensure satisfactory precision of measurements. The same cells should always be used for the reference and sample cells, and they should always be placed in the same position in the cell holder with the same face towards the light source.

It is difficult to ensure reproducible alignment of cells with chipped corners and they should be discarded. Similarly optical faces of the cell and the slide of the cell holder should be kept scrupulously clean. Before every set of measurements the optical density of the sample cell should be measured against the reference cell when both are filled with chloroform. This will help to indicate when the cells need cleaning, and it will also enable the true absorbance of the blank to be determined.

D11 Checking the Accuracy of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests of the accuracy should be made regularly. As a minimum, it is recommended that with every batch of samples a known amount of phenol should be added to 500 ml of water used for the reagent blank and then analysed by stages 12, 2–10, 14 and 15 of Section D9. The results obtained should be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be calculated.

Method E Determination of Monohydric Phenols in Raw and Treated Waters MBTH Chloroform Extraction Spectrophotometric Method

E1 Performance Characteristics of the Method

E1.1	Substances determined	Phenolic substances which react with 3-methyl-2-benzothiazolinone hydrazone, (MBTH) (See Section E2).
E1.2	Types of sample	The method has been tested for raw and potable waters.
E1.3	Basis of the method	After distillation to minimize interferences the sample is treated with MBTH to form a pink coloured compound which is extracted into chloroform and measured spectrophotometrically.
E1.4	Range of application (a) (b)	0–100 µg phenol/litre. The range can be extended by dilution with water after the distillation stage.
E1.5	Calibration curve (a) (b)	Linear to 100 µg phenol/litre.
E1.6	Standard deviation (b)	

	Concentration µg'l phenol	Standard deviation µg/1 phenol
Phenol solution (c)	0	0.22
Phenol solution (c)	5	0.24
Phenol solution (c)	10	0.46
Phenol solution (c)	20	0.88
River water (d)	30.6	1.50
Spiked river water (d)	60.6	3.00

E1.7	Limit of detection (a) (b)	1.0 µg/l (with 10 degrees of freedom).
E1.8	Sensitivity (a) (b)	50 µg/l phenol gives an absorbance of about 1.50.
E1.9	Bias	The concentration found will be less than the total concentration of monohydric phenols if phenols other than phenol are present.
E1.10	Interferences	See Section E2 for details.
E1.11	Time required for analysis	For 20 samples (including distillation) total analytical time is about 6 hours, and operator time about 2 hours.

(a) These data refer to the determination of phenol only.
(b) These data were obtained at the Head Office Laboratory, Yorkshire Water Authority.
(c) Within-batch standard deviations with 10 degrees of freedom.
(d) Total standard deviations with 19 degrees of freedom.

E2 Scope and Field of Application

The method has been tested and used routinely for river waters, potable supplies (including chlorinated waters) and groundwaters in general. If the method is to be applied to sewage effluents and trade effluents, the analyst should make appropriate tests to ensure that the method is satisfactory for these samples.

Monohydric phenols are separated from non-volatile impurities by distillation. Dihydric and polyhydric phenols are not recovered in the distillation procedure and therefore are not determined by the method. MBTH is also used to determine aliphatic aldehydes and aromatic amines, which are therefore potential interferants. Distillation has been shown to remove aromatic amines (5), whereas aliphatic aldehydes remain in the distilled sample. The effect of these compounds was determined by analysing a sample containing n-butyraldehyde, glyoxal and formaldehyde each at a concentration of 100 µg/l. With the procedure a green dye was formed with MBTH, but this was destroyed when the buffer was added and gave no response at the wavelength used to measure phenols.

Gales (5) & Friestad (4) have shown that the MBTH method is more universal in reactivity to phenols than the 4-aminoantipyrine method, and also that it leads to higher molar absorptivities. As with that method, results are obtained as an apparent concentration of phenol and give no indication of which phenols are present.

E3 Principle

Biochemical oxidation of phenols in the sample is inhibited by the addition of hydrochloric acid which also eliminates any chemical changes resulting from alkaline conditions. Oxidizing agents are removed by the addition of sodium arsenite. The monohydric phenols are distilled from acid solution and separated from non-volatile impurities. The steam distilled phenols react with MBTH in an acidic medium using ammonium ceric sulphate as an oxidant. The coloured dye produced is extracted into chloroform and the absorbance of the extract is measured at 490 nm.

The coupling takes place in the para-position, if this is occupied the MBTH reagent will react at a free ortho position. Therefore, the reaction of MBTH with phenolic compounds is less dependent on the position of the substituent group than with 4-aminoantipyrine. A comparison of the two methods for various phenols is given in table 1.

E4 Hazards

4.1 Hydrochloric acid (reagent E6.2) can cause burns and gives off harmful vapour.

4.2 Sodium arsenite (reagent E6.3) is poisonous.

4.3 Chloroform (reagent E6.7) is poisonous and gives off harmful vapour.

4.4 Phenol (reagent E6.8) is poisonous and corrosive, and can be absorbed through the skin.

4.5 Chromic acid (section E7.2) is corrosive.

E5 Reaction

The reaction scheme for the formation of the 3-methyl-2-benzothiazolinone hydrazone complex is given in equation 3.

E6 Reagents

For the analysis, use only reagents of recognized analytical reagent grade unless otherwise stated.

E6.1 Water

It is essential that water of negligible phenol content (compared with the smallest concentrations to be determined in the samples) be used for rinsing apparatus and for the preparation of reagent solutions, phenol standard solutions and blanks. The water should be stored in an all glass aspirator.

E6.2 Hydrochloric Acid Solution (50 % v/v)

Add 500 (±5) ml hydrochloric acid (d_{20} 1.18) cautiously to 400 ml of water in a 1-litre measuring cylinder stirring continuously. Cool and dilute with water with 1 litre. Store in

a glass bottle with a ground glass stopper. Caution: Hydrochloric acid can cause burns and gives off harmful vapour.

E6.3 **Sodium Arsenite Solution** (0.2% w/v)

Dissolve 2.00 (±0.01) g of sodium arsenite in water and dilute to 1 litre in a calibrated flask. Store the solution in a glass bottle with a ground stopper. Caution: Sodium arsenite is poisonous.

E6.4 **MBTH solution** (0.04% w/v)

Dissolve 0.080±0.001 g of 3 methyl-2-benzothiazolinone hydrazone hydrochloride in approx. 100 ml of distilled water. Dilute to 200±2 ml with distilled water. Store in a glass bottle.

E6.5 **Ammonium cerium sulphate solution** (1.25% w/v)

Dissolve 2.5±0.1 g of ammonium cerium (iv) sulphate dihydrate in approx. 150 ml of distilled water. Add 1.5±0.1 ml of concentrated sulphuric acid. After the solid has dissolved (filter if necessary) dilute to 200±2 ml with distilled water. Store in a glass bottle. This reagent is stable for at least five days.

E6.6 **Buffer solution**

Dissolve in the following order, 11.2±0.1 g of sodium hydroxide, 2.8±0.1 g ethylene diamine tetra-acetic acid disodium salt, and 11.2±0.1 g boric acid in approx. 200 ml of distilled water. Dilute to 250±2 ml with distilled water. Store in a glass bottle. This reagent is stable for at least ten days.

E6.7 **Chloroform**

Caution: Harmful vapour.

E6.8 **Standard Solutions of Phenol**

Solution A 1 ml=1 mg of phenol

Dissolve 1.000 (±0.001) g of phenol C_6H_5OH in water 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.

Solution B 1 ml=10 µg of phenol

Pipette 10.00 (±0.02) ml of the phenol *Solution A* into one 1-litre calibrated flask and dilute with water to 1 litre. Prepare on the day of use.

Solution C 1 ml=1 µg of phenol

Pipette 25.00 (±0.03) ml of the phenol *Solution B* into a 250 ml calibrated flask and dilute with water to 250 ml. Prepare immediately before use.

E7 Apparatus

E7.1 **Photometric Equipment**

A spectrophotometer of prism or grating type is required. The instrument should be capable of use at 490 nm (the exact wavelength of maximum absorption to be checked for each instrument) and should be equipped with 40 mm cells. Alternatively an instrument employing narrow band pass filters having maximum light transmission at or near 490 nm (eg Ilford No 603) and equipped with 40 mm cells may be used. Not all commercially available instruments have facilities for the use of 40 mm cells and where shorter path length cells are used the sensitivity of the method will be decreased but the precision of the analytical results is unlikely to be affected.

E7.2 **Glassware**

Glassware should have ground glass joints where appropriate, the use of grease is not recommended.

Splash head.
Water cooled condenser with receiver adaptor.
Calibrated flask, 500 ml.
Separating funnel, 1 litre.

It is preferable that the glassware should be reserved solely for phenol determinations. All glassware should be thoroughly cleaned before use by standing overnight in chromic acid or by employing an equivalent cleaning procedure and then rinsed with water. (Caution: Chromic acid is corrosive). After each determination the apparatus should be rinsed with water.

E8 Sample Collection and Preservation

Chemical and biochemical processes in the sample may occur between sampling and analysis and affect the concentrations of phenol. Thus the addition of preserving agents is necessary.

The samples must be collected and stored in glass bottles with ground glass stoppers previously cleaned by the procedure given in 7.2. 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.

* Ferrous sulphate should not be used to remove oxidizing agents in samples; only sodium arsenite should be used for this purpose.

E9 Procedure

Step	Procedure	Notes
	Analysis of Samples	
E9.1	Add 500 ml (±5 ml) of the preserved sample to a 1-litre distillation flask (note a).	(a) It is recommended that if a sample is likely to contain more than 100 µg phenol/litre, an appropriately smaller aliquot of the sample be diluted with water after distillation.
E9.2	Distil off 450 ml (±5 ml) of the sample into a 500 ml calibrated flask (note b) and make up to 500 ml with water.	(b) It is useful to make a mark at 450 ml on the calibrated flask.
E9.3	Add the distilled sample (or an appropriate volume, V, of the distillate diluted to 500 ml with water) to a 1-litre separating funnel.	
E9.4	Add in sequence to the separating funnel swirling the contents and standing for 5.0±0.5 mins. after each addition, 5.00 ml (±0.10 ml) of 0.04% w/v MBTH solution, 5.00 ml (±0.10 ml) of 1.25% w/v ammonium ceric sulphate solution and 5.00 ml (±0.10 ml) of buffer solution.	(c) Do not pipette by mouth.
E9.5	Allow the solution to stand for 15±2 mins. (note d).	(d) The 3 MBTH complex in aqueous solution has been found to be stable between 12 and 60 mins.
E9.6	Add 25.0 (±0.1 ml) of chloroform to the separating funnel (note c) shake the funnel gently for a few seconds and then release the excess pressure in the funnel (note e).	(e) Care should be taken that no chloroform is lost when the excess pressure is released.

Step	Procedure	Notes
E9.7	Shake the funnel vigorously for 1 min. (±10 seconds) and allow the two phases to separate for at least five mins.	
E9.8	Pass the chloroform extract (note f) through a Whatman No. 4 filter paper into a clean dry 40 mm cell. Discard the filter paper.	(f) Dry the stem of the separating funnel with a strip of filter paper before running off the extract.
E9.9	Meanwhile set up the spectrophotometer (Section 7.1) according to the manufacturers instructions. Adjust the zero of the instrument with chloroform in the reference cell. Within 30 mins of the extraction measure the absorbance (see Section 10) of the extract at 490 nm using 40 mm cells (note h). Recheck the instrument zero. Let the absorbance of the sample be S.	(g) Other sizes of cells may be used but the performance characteristics quoted in Section 1 would no longer apply.
E9.10	After measurement discard the contents of the sample cell, rinse it with chloroform and allow to dry before the next extract to be measured is placed in the cell.	
	Blank determination	
	A blank determination must be run with each batch of determinations using exactly the same reagent solutions as those used for samples.	
E9.11	Add 500 ml (±5 ml) of water to a 1-litre distillation flask.	
E9.12	Add in sequence to the distillation flask carefully swirling the contents after each addition: 1.00 ml (±0.05 ml) of 0.2% w/v sodium arsenite solution (note g) and 2.5 ml (±0.05 ml) of 50% v/v hydrochloric acid solution (note h).	(h) Do not pipette by mouth.
E9.13	Repeat stages 2–10 inclusive. Let the absorbance of the blank be B.	
	Calculation of results	
E9.14	Calculate the absorbance due to phenolic compounds in the sample R, from $R=S-B$	
E9.15	Calculate the concentration of phenol C_A in the sample from $C_A=K.R.\frac{500}{V}$	
	Where K is the calibration factor ie, the concentration of phenol in µg/l equivalent to an absorbance of 1 on the calibration curve. V the volume of distillate diluted to 500 ml for the spectrophotometric stage.	
	Preparation of Calibration Curve	
E9.16	Add 500, 495, 490, 480, 470, 460 and 450 ml of water (all ±5 ml) to a series of 1-litre distillation flasks. Add to these flasks 0.00, 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml, respectively, of phenol standard solution C.	

Step	Procedure	Notes
E9.17	Repeat stages 12 and 2–10 on these samples. These determinations should be repeated at least once on another day and then again as necessary until the calibration is defined with the required accuracy. Normally two batches of determinations will suffice (note i).	(i) A new calibration curve should be prepared with each bottle of 3 MBTH used.
E9.18	Subtract the average absorbance of the blank from the average absorbances for each of the phenol concentrations and plot the corrected absorbances against the concentrations of phenol added to the water. The solutions in stage 16 are equivalent to 0, 10, 20, 40, 60, 80 and 100 µg/litre.	

E10 Notes on Procedure

Measurement of absorbance

The exact instrument setting for the wavelength of the absorption peak must be checked for each instrument and then used in all future work. The procedure used for measuring absorbance should be rigorously controlled to ensure satisfactory precision of measurements. The same cells should always be used for the reference and sample cells, and they should always be placed in the same position in the cell holder with the same face towards the light source.

It is difficult to ensure reproducible alignment of cells with chipped corners and they should be discarded. Similarly optical faces of the cell and the slide of the cell holder should be kept scrupulously clean. Before every set of measurements the optical density of the sample cell should be measured against the reference cell when both are filled with chloroform. This will help to indicate when the cells need cleaning, and it will also enable the true absorbance of the blank to be determined.

E11 Checking the Accuracy of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests of the accuracy should be made regularly. As a minimum, it is recommended that with every batch of samples a known amount of phenol should be added to 500 ml of water used for the reagent blank and then analysed by stages 12, 2–10, 14 and 15 of Section 9. The results obtained should be plotted on a quality control chart which will facilitate detection of inadequate accuracy, and will also allow the standard deviation of routine analytical results to be calculated.

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[2]Member of the Phenols Panel (from November 1973 unless stated. This panel started as a joint panel for Organic Carbon and Phenols, but separated in April 1974. In January 1976 it became subordinate to Working Group 6.

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