

Linear Alkylbenzene Sulphonates (LAS) and Alkylphenol Ethoxylates (APE) in Waters, Wastewaters and Sludges by High Performance Liquid Chromatography 1993

This booklet contains two methods for the determination in waters, wastewaters and sludges of:

- A linear alkylbenzene sulphonates (LAS) by HPLC; and
- B alkylphenol ethoxylates (APE) by HPLC.

Method A is tentative.

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Contents

About this series	4				
Warning to users	5				
A	Determination of linear alkylbenzene sulphonates (LAS) in sewage, sewage effluents, river water, sewage sludge, river sediment and sludge-amended soil samples by HPLC with fluorescence detection	6	B	Determination of alkylphenol ethoxylates (APE) in sewage, sewage effluent and river water by HPLC	16
A1	Performance characteristics of the method	6	B1	Performance characteristics of the method	16
A2	Principle	7	B2	Principle	16
A3	Scope and limitations (including interferences)	7	B3	Scope and limitations (including interferences)	17
A4	Hazards	7	B4	Hazards	17
A5	Reagents	8	B5	Reagents	17
A6	Apparatus	8	B6	Apparatus	18
A7	Cleaning and preparation of apparatus	10	B7	Sample collection and preservation	19
A8	Sample collection and preservation	10	B8	Analytical procedure	19
A9	Analytical procedure	11	B9	Calculation of results	21
A10	Calculation of results	13	B10	References	22
A11	References	14		Address for correspondence	25
				Members assisting with these methods	26

About This Series

This booklet is part of a series intended to provide recommended methods for determining the quality of water and associated materials. In addition, short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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 is published as a series of booklets on single or related topics, thus allowing for the replacement or addition of methods as quickly as practicable without the need for waiting for the next edition. The rate of publication is also related to the urgency of the requirement for that particular method.

Although ideally all methods published should be fully tested, this is not often possible without delay in publication. Furthermore, the limit of detection, range, precision and interference effects applying to instrumental methods can depend on the actual instrument used, as well as on sample type, reagent purity and operator skill, etc. Even methods tested in many laboratories have been known to acquire problems, for example when new products appear (introducing new substances into effluents), when changes in production methods affect reagent quality, or when the method is used to analyse new types of sample (despite apparent similarity to samples already evaluated). As a guide, the following categories have been given to methods:

- (i) tested, usually in five or more laboratories
- no grade indicated;
- (ii) tested in one to three or four laboratories
- Tentative;
- (iii) evaluated, but not fully tested, but publication is urgently required
- Note;
- (iv) tested and found to be satisfactory by several laboratories, but in the opinion of experts requires a high degree of skill or has some other difficulty such that the method would be replaced if a better method were discovered.
- Provisional.

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

United 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 and senior technical staff to decide which method to use for the determination in hand. Whilst the attention of users is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

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

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

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

Publication of new or revised methods will be notified to the technical press. A current list of publications can be obtained from the Secretary.

Every effort is made to prevent errors from occurring in the published text. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically. However, should any errors be found, please notify the Secretary.

Dr D WESTWOOD
Secretary

5 August 1992

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 and COSHH 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 made available.

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

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

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', issued by the Royal Society of

Chemistry, London: 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Services Monograph 6, HMSO, London.

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

Safety while Sampling

Prior consideration must be given, especially when sampling in confined spaces or where access is difficult, to guard against suffocation, drowning, falls and poisoning or infection by ingestion, inhalation or skin contact.

Good Laboratory Practice

The Department of Health issues a booklet entitled: Good Laboratory Practice; the United Kingdom Compliance Programme, 1989. This can be obtained by writing to that Department in London. It deals chiefly with toxicity studies, but much can be applied to analytical chemistry.

A Determination of linear alkylbenzene sulphonates (LAS) in sewage, sewage effluent, river water, sewage sludge, river sediment and sludge-amended soil samples by high performance liquid chromatography (HPLC) with fluorescence detection (Tentative Method)

A1 Performance characteristics of the method

A1.1	Substance determined	Linear alkylbenzene sulphonates (LAS)						
A1.2	Type of sample	Sewage, sewage effluents, river waters, potable waters, sewage sludges, river sediments and sludge-amended soils.						
A1.3	Basis of the method	Concentration and clean-up of LAS in samples by a combination of solvent extraction, anion exchange and C ₁₈ column chromatography. Separation of individual LAS homologues by reverse-phase HPLC. Detection by fluorescence spectroscopy and quantification by peak area integration.						
A1.4	Range of application	0-0.5 µg of LAS expressed as some optimum suitable reference surfactant such as Marlon A.						
A1.5	Calibration curve	Linear.						
A1.6	Standard deviation	<p>A relative standard deviation of 4% for total LAS in aqueous samples and of 10% in samples with high solids content has been reported for the procedure outlined above with UV detection (1).</p> <p>With fluorescence detection (2) the following was achieved:</p> <table border="1"> <thead> <tr> <th>Soil µg/g LAS</th> <th>Total Standard Deviation µg/g</th> <th>Degrees of Freedom</th> </tr> </thead> <tbody> <tr> <td>2.4</td> <td>0.3</td> <td>4</td> </tr> </tbody> </table>	Soil µg/g LAS	Total Standard Deviation µg/g	Degrees of Freedom	2.4	0.3	4
Soil µg/g LAS	Total Standard Deviation µg/g	Degrees of Freedom						
2.4	0.3	4						
A1.7	Limit of detection: Aqueous samples (100 ml sample) Solid samples	<p>For liquor samples, 0.05 µg injected, equivalent to less than 2.5 µg/l.</p> <p>A detection limit of 0.2 µg LAS/g for sediment/soil and 2 µg/g for sewage sludge based on a typical sample size (see A9.2) and four times the standard deviation of the blank determination.</p>						
A1.8	Bias	Not known.						
A1.9	Interferences	Fluorescence active compounds not separated in the clean-up procedure which have similar retention times to the LAS components will interfere, for example, highly branched alkylbenzene sulphonates.						

- A1.10 Time required for analysis (i) Liquors. Set of 3–6 determinations total time 15–20 hours.
(ii) Solids. Set of 3–6 determinations total time 20–25 hours.
- The operator time will be approximately half the total time.
-

A2 Principle

The procedures outlined are essentially those described by Matthijs and De Henau (1) with the addition of improved recovery and detection techniques (2). Liquor samples are prepared for the HPLC analysis by extracting the LAS with methanol from the residue remaining after evaporation of a suitable portion of the unfiltered liquor. This extract is anion-exchanged to concentrate anionics, free of other surface active material. The LAS is eluted from the resin using methanolic/hydrochloric acid and then concentrated onto a C_{18} reverse-phase column from a neutralised aqueous solution. Any inorganic salts and highly polar organics are removed before LAS is selectively eluted from the column with methanol.

Solid samples are extracted using a Soxhlet reflux technique with a methanol extractant to recover the LAS material prior to the anion-exchange and C_{18} reverse-phase column clean-up steps described above for the liquor extracts. The resulting LAS residues are analysed using a reverse-phase HPLC system which separates the individual homologues by their carbon chain length. Detection is by fluorescence measurement and quantification by peak area assessment using external standards.

A3 Scope and limitations (including interferences)

The method can determine very low levels of LAS in the presence of other anionic surfactants not containing an aromatic ring, ie less than 0.05 μg LAS injected. It can assess the individual LAS homologues with carbon chain lengths of C_9 – C_{15} , although only those in the range of C_{10} – C_{14} will be relevant for LAS found in environmental samples. Depending upon the exact chromatographic conditions employed, the LAS homologues can show a varying degree of resolution for the individual phenyl positional isomers (see Figure 1). The response of the fluorescence detector to LAS has been shown to be linear up to at least 0.75 μg injected. The usual working range is approximately 0–0.5 μg LAS. The individual homologues have similar molar responses for fluorescence detection (2) (as also found for UV detection). Good recoveries of standard additions of LAS have been generally obtained for a range of environmental liquor and solid determinations, ie > 90%. (See Refs 1 and 2.)

A blank for solid determinations typically has a value of about 1 μg LAS even though pre-extracted Soxhlet equipment, high quality solvents (HPLC grade) and rigorous washing procedures for equipment are used. A blank determination should therefore be performed with each series of solid samples to correct the LAS concentrations found in these matrices. A detection limit of 0.2 μg LAS/g for sediment and soil and 2 μg /g for sludge determinations has been based on 10g and 1g sample weights respectively and four times the standard deviation of the blank determination.

For the majority of samples analysed a complete distribution of the LAS homologues by their chain length can be obtained without any serious matrix interferences because of the high specificity of the fluorescence detection for this material.

The application of the method to environmental samples in which the exact identities of LAS residues are unknown, necessitates the use of an appropriate reference LAS standard to express the level of LAS found (see section A5.10).

In addition to the method outlined above, an alternative reverse-phase HPLC separation system using UV detection (230 nm) can be used, if required, for analysing the LAS extracts prepared (see section A6.1 and Ref 1). The UV detection is less sensitive and less specific for determining LAS in environmental samples (2).

A4 Hazards

The following potentially hazardous reagents are used in this method: hexane, sodium perchlorate, hydrochloric acid and sodium hydroxide. Sodium perchlorate is a powerful oxidising agent. A high standard of hygiene should be maintained when working with sewage sludges (primary, secondary and digested).

A5 Reagents

Reagents should be analytical reagent quality except where otherwise specified.

A5.1 Water double deionised, free of surfactant residues.

A5.2 Water HPLC grade quality.

A5.3 Methanol HPLC grade, low in surfactant residues.

A5.4 Hexane fraction from petroleum.

A5.5 Sodium perchlorate.

A5.6 Methanol/Water (30 : 70 v/v) mixture.

Add 30 ± 1 ml of methanol (A5.3) to 60 ± 2 ml of double deionised water in a volumetric flask. Allow mixture to cool before making up to 100 ml with double deionised water.

A5.7 Methanol/Hydrochloric acid mixture (80 : 20 v/v).

Add 50 ± 1 ml of methanol (A5.3) to a 100 ml volumetric flask. Pipette cautiously 20 ± 0.5 ml of concentrated hydrochloric acid (d_{20} 1.18) into the methanol. Dilute to the mark with methanol. Allow to cool and make to the mark again. Mix well.

A5.8 Sodium Hydroxide, approximately 1.0M.

The use of a concentrated (commercially available) volumetric solution, provides a convenient means of preparing this solution, which is stable for up to 3 months. Alternatively, dissolve 4.0 ± 0.2 g of sodium hydroxide pellets in 100 ml of double deionised water.

It is convenient to prepare 0.1M and 0.01M solutions by dilution of the 1.0M solution.

A5.9 Hydrochloric acid, approximately 0.1M.

The use of a concentrated (commercially available) volumetric solution provides a convenient means of preparing this solution, which is stable for up to 1 month. Alternatively, add with stirring, 9.0 ± 0.5 ml of hydrochloric acid (d_{20} 1.18) to about 800 ml of double deionised water. Cool, make up to 1 litre with water and mix well.

A5.10 Standard linear alkylbenzene sulphonate (LAS) reference compound.

For general application with environmental samples, a commercial secondary LAS, for example Marlon A350, can be used for expressing the concentration of LAS found. Typically, Marlon A has a homologue distribution of 4% C_{10} ; 45.5–46% C_{11} ; 40–40.5% C_{12} ; 10% C_{13} and 0.1% C_{14} (with an average carbon chain length of 11.6).

A5.10.1 Standard LAS solutions.

A5.10.1.1 Stock solution 1000 mg/l in methanol.

Dissolve 0.1 ± 0.005 g of LAS in methanol (A5.3) and dilute to the mark in a 100 ml volumetric flask. This solution is stable for up to 3 months.

A5.10.1.2 Working solution 50 mg/l in methanol ($1 \mu\text{l} = 0.05 \mu\text{g}$ LAS).

Pipette 5.0 ± 0.05 ml of 1000 mg/l stock solution (A5.10.1.1) into a 100 ml volumetric flask and make up to the mark with methanol (A5.3). This solution is stable for up to 2 months.

A6 Apparatus

A6.1 Liquid chromatography, ancillary equipment and chromatographic conditions.

Any liquid chromatograph system capable of producing well separated peaks for the individual LAS homologues is suitable. This must be linked to a fluorescence detector which can operate at an excitation wavelength of 232 nm and an emission wavelength of 290 nm. An appropriate chromatographic data handling system may be used to quantify peak areas.

Instrument:	High performance liquid chromatograph capable of producing an isocratic solvent mixture.
Column:	Analytical column, for example μ -Bondapak C ₁₈ , 300 mm \times 3.9 mm, particle size 10 microns or equivalent (see note).
Guard column:	Column packed with C ₁₈ /Corasil Bondapak or equivalent.
Mobile phase:	16 : 84 v/v water/methanol mixture, 0.0875M with respect to sodium perchlorate. Prepare by dissolving 6.15 \pm 0.05 g of sodium perchlorate monohydrate in 80 \pm 1 ml of water and dilute to 500 ml with methanol. Filter before use (see note).
Solvent programme:	Isocratic.
Flow rate:	1 ml/min.
Detector:	Fluorescence detector. Excitation wavelength 232 nm Emission wavelength 290 nm Slit width 10 nm for both excitation and emission.

Note:

Alternative columns may be used and depending upon the efficiency of the analytical column the mobile phase may require slight modification to give the best separation for LAS. Generally, the C₁₀ – C₁₄ LAS homologues in the reference standard (Marlon A350) would be expected to elute in under 20 mins (see figure 1). The following linear gradient elution programme may be used with UV detection at 230 nm, if a fluorescence system is not available to analyse LAS in samples.

Mobile phase: Solvent A: 0.15M sodium perchlorate in water.
Solvent B: 0.15M sodium perchlorate in 70 : 30 v/v acetonitrile/water.

Time min	Flow ml/min	% A	% B
Initially	1	30	70
25	1	10	90
30	1	0	100
35	1	30	70

The solvent programme may also be used, if required, with fluorescence detection. The gradient programme will also elute the C₁₀ – C₁₄ LAS homologues in under 20 minutes but generally result in more separation of the individual phenyl positional isomers.

A6.2 Glassware.

A6.2.1 5 ml glass syringe with a Luer tip.

A6.2.2 Vials – used for reaction and storage – with solid top and PTFE-faced rubber liner (5 ml capacity), for example, Reacti-Vials or equivalent.

A6.2.3 Soxhlet extraction equipment – water cooled condensers and Soxhlet extractors designed to take extraction thimbles up to 30 \times 100 mm in size.

A6.3 Ancillary equipment

A6.3.1 Cellulose or glass fibre extraction thimbles up to a maximum size of 30 \times 100 mm.

A6.3.2 Solid phase extraction (SPE) vacuum manifold – its use would allow up to ten samples at a time to be concentrated and cleaned-up on disposable SPE columns (see section A9.3).

A6.3.3 SPE C₁₈ disposable columns – 3 ml capacity, 500 mg adsorbent.

A6.3.4 SPE Quaternary amine (N⁺) disposable columns – 3 ml capacity, 500 mg adsorbent.

A6.3.5 Plastic reservoirs for use with SPE system.

A6.3.6 pH meter.

A6.3.7 Heating block for use when the vials have to be heated.

A6.3.8 Solvent filtration equipment.

A6.3.9 Rotary sample mixer.

A6.3.10 HPLC injection syringe, capacity up to 25 μ l.

A7 Cleaning and preparation of apparatus

It is essential that all glassware be thoroughly cleaned to reduce to a minimum traces of linear alkylbenzene sulphonates.

Soxhlet equipment used for all solid determinations, including the thimbles, must be pre-extracted with methanol before use to minimise any LAS contamination. This can be done by refluxing with 200 ml methanol at a steady rate for 1 hour. The solvent is then discarded. A second reflux step may be necessary. Remove the thimble and dry completely before use. In addition, glass fibre thimbles, if used, must be conditioned to the solid matrices to ensure no significant losses of LAS from samples. These thimbles can be used repeatedly once conditioned.

The vials used for storing LAS extracts prior to HPLC analysis should be cleaned as follows:

(i) Place in an ultrasonic cleaning bath containing a 2% solution of a cleaning agent containing no LAS or alkylphenol ethoxylate and sonicate for 15-30 minutes.

(ii) Replace completely the cleaning solution with deionised water and sonicate for 15-30 minutes.

(iii) Wash 3 times with deionised water.

(iv) Rinse 3 times with small volumes of methanol and allow to air-dry.

Other glassware should be washed rigorously using water and methanol.

A8 Sample collection and preservation

A8.1 Liquor samples.

Because of their nature, surfactants will tend to become adsorbed onto any suspended solids, as well as on the walls of the containing vessel. The analyst must therefore ascertain the requirements of the analysis, ie whether the total or soluble surfactant concentration is required. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection, the samples should be stored in a refrigerator at 1-5°C and sterilised, for example, by addition of 1% v/v of a 40% formaldehyde solution. To obtain a representative sample, either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practicable, avoid the formation of foam during stirring or inversion, but in any case do not withdraw an aliquot while the bulk sample is foaming.

A8.2 Sludges (primary, secondary and digested sludge).

Sludge samples should be collected in pre-washed glass jars to which 1% v/v of a 40% formaldehyde solution has been added and the contents mixed well. The samples should be stored in a refrigerator at 1-5°C prior to use but not for extended periods.

A8.3 Soils.

Soil samples should be thinly spread over trays and allowed to air-dry to constant weight. The soil is periodically broken up with a trowel to facilitate the drying process. The dried soil should be finely ground using a mortar and pestle prior to being sieved through a laboratory sieve to obtain the soil with a particle size of less than 2 mm. The sieved samples can be stored in sealable plastic bags or glass bottles at room temperature prior to analysis.

A8.4 Sediments.

As described above for soils except sediments will be dried at 50°C to facilitate the removal of interstitial water.

A9 Analytical Procedure

Step	Procedure	Notes
A9.1	Recovery of LAS from liquor samples.	
A9.1.1	Take a suitable volume (V_s) of sample, up to 100 ml preferably containing 1 to 100 μg of LAS (note a) and evaporate to dryness in a suitable capacity beaker on a steam bath under a stream of nitrogen.	(a) Generally, take up to 10 ml of sewage, 50 ml of sewage effluent or 100 ml of river water.
A9.1.2	Add 25 ml of methanol and thoroughly dislodge all the sample residue from the sides and bottom of the beaker using a glass rod. Reduce the volume of the solvent to about 10 ml on a steam bath. Transfer the supernatant liquid into a clean beaker. Repeat the extraction and transfer procedures with two further 25 ml portions of methanol. Evaporate the combined supernatant to about 10 ml on a steam bath. Concentrate and clean-up the LAS in the resulting extract as outlined in section A9.3.	
A9.2	Recovery of LAS from solid samples.	
A9.2.1	Insure that the dried solids are well mixed before sampling. Weigh out accurately, about 0.5–1 g dried sludge (note b), 10 g air-dried sediment or 10 g air-dried soil into a pre-extracted, dry Soxhlet thimble (see section A7) for each determination.	(b) The procedure for preparing dried sludge has been detailed elsewhere (3). If necessary, coarse solids are removed by sieving (about 5 mm). The dry weight of the sludge must be determined. Dried sludge is ground and sieved before use.
A9.2.2	Set up the thimble in a Soxhlet extractor and add a few anti-bumping granules to the round-bottomed flask, along with 200 ± 5 ml of methanol (A5.3). Carry out a blank determination alongside the sample determination(s) to allow suitable correction for LAS contamination in reagents and equipment. Reflux the solvent at a steady rate for 4 hours (note c).	(c) After this period of time the extracts in the upper part of the Soxhlet apparatus should be completely colourless. If not, a longer reflux period may be needed.
A9.2.3	Use the total Soxhlet extract from soil and sediment determinations for the concentration/clean-up step (see section A9.3). Take the total Soxhlet extract from the sludge determinations, dilute to 200 ml with methanol in a volumetric flask and use 5 ml of this solution for the concentration/clean-up of the LAS recovered as outlined in section A9.3 (note d).	(d) The limit of detection may be reduced if a larger volume is taken for the concentration/clean-up procedure.
A9.3	Concentration and clean-up of LAS from liquor and solid extracts.	
A9.3.1	Pre-condition a quaternary amine (N^+) anion exchange SPE column (A6.3.4) by passing 3 ml of hexane through it (either using a vacuum manifold or a glass syringe fitted to a suitable column adaptor) followed by 10 ml of methanol at a flow rate of 1–2 ml/min. Do not let the column dry out at this stage.	
A9.3.2	Fit a plastic reservoir to the column and quantitatively transfer the extract in suitable portions (prepared in section A9.1 or A9.2). Pass through the column at a flow rate of 1–2 ml/min. Use a further 10 ml portion of methanol to rinse sample	

Step	Procedure	Notes
	<p>beaker or flask and also pass through the column at the same flow rate. Use a further 10 ml of methanol to wash the reservoir and column before drying the column by pulling air through it for several minutes.</p>	
A9.3.3	<p>Elute the column with 2 ml of methanol/hydrochloric acid (A5.7) at a rate of approximately 1-2 ml/min. Collect the eluate in a 100 ml beaker. Use a further 1 ml portion of methanol/hydrochloric acid (A5.7) to wash the column and combine the eluates. Dilute to approximately 50 ml with double-deionised water and adjust the solution pH to 7.0 ± 0.2 using sodium hydroxide and hydrochloric acid solutions, as required, and using a pH meter to measure the value.</p>	
A9.3.4	<p>Pre-condition a C_{18} SPE column (A6.3.3) by passing 10 ml of methanol through it, followed by 10 ml of double-deionised water at a flow rate of 1-2 ml/min. The column should not be allowed to dry out once solvated.</p>	
A9.3.5	<p>Fit a plastic reservoir to the column and transfer the whole of the neutralised solution to the reservoir, passing it through the column at a flow rate of 1-2 ml/min. Rinse the sample beaker with approximately 10 ml of double deionised water and use this to wash the reservoir and column. Remove the reservoir and wash the column with 2 ml of 30:70 methanol/water mixture (A5.6) at the same flow rate, before air-drying for several minutes.</p>	
A9.3.6	<p>Elute the column with 2 ml of methanol at a rate of approximately 2 ml/min. Collect the eluate in a reaction vial then place on a heating block module at $70 \pm 10^\circ\text{C}$ with a stream of nitrogen blowing over the surface of the solvent. When most of the solvent has been removed, re-elute the column with a further 1 ml portion of methanol and direct the end of the column so that the emerging solvent washes down the inside of the vial. Evaporate the solvent to dryness and cap the vial prior to the chromatographic analysis.</p>	
A9.4	<p>High Performance Liquid Chromatography (HPLC)</p>	
A9.4.1	<p>Filter and de-gas the HPLC mobile phase (A6.1) by passing through an appropriate membrane filter prior to use.</p>	
A9.4.2	<p>Set up the liquid chromatograph and any ancillary equipment according to the general conditions given in section A6.1 and the manufacturer's instructions. Ensure that stable, acceptable conditions are established for the subsequent analysis.</p>	

Step	Procedure	Notes
A9.4.3	Add accurately to the extract, a volume (V_1) of methanol, generally between 100 to 2000 μl , so that an appropriate injection aliquot (5–20 μl) can be taken, which should give a LAS response similar to that for the reference standards used, for example 0.125, 0.25, 0.375 and 0.5 μg Marlon A injected (note e). A degree of familiarity with the expected range of concentrations is required in order to ensure appropriate volumes are taken at this stage.	(e) For greater accuracy and in order to check the linearity of the response, several different standard determinations should be carried out simultaneously with the samples.
A9.4.4	Re-cap the vial and mix the contents using a rotary mixer. Allow any particulates in the sample to settle and inject a suitable sample volume (V_2) into the chromatograph.	
A9.5.	Calculation of Results	
A9.5.1	Determine the peak area under the chromatogram for the C_{10} - C_{14} homologues in the sample, blank and standards (note f).	(f) Correct these areas for any small LAS background associated with the injection of a methanol solvent blank run at the beginning of a set of analyses.
A9.5.2	Calculate the weight of LAS in the sample by directly comparing the area obtained with that for a known weight of LAS reference standard (Marlon A) run under identical chromatographic conditions using the expressions given in section A10.	

A10 Calculation of Results

If m = weight of LAS standard injected (μg)
 a = corrected area for LAS in sample (or blank determination)
 a_{std} = corrected area for LAS in standard
 V_1 = volume of methanol added to vial (μl)
 V_2 = volume of sample injected (μl)
 m_1 = weight of LAS in sample (μg)

then μg of LAS in sample (or blank determination), as Marlon A, is given by

$$m_1 = \frac{a \times V_1 \times m}{a_{\text{std}} \times V_2}$$

If m_2 = μg LAS in blank determination (solids)
 w = weight of dried solid sample taken for analysis, g
 V_s = volume of aqueous liquor taken for analysis, ml

then concentration in mg LAS/l in the aqueous liquor sample is given by

$$\frac{m_1}{V_s}$$

and the concentration in μg LAS/g of dried soil or sediment sample is given by

$$\frac{(m_1 - m_2)}{w}$$

and the concentration in g LAS/kg in a sludge sample is given by

$$\frac{(m_1 - m_2) \times 40}{w \times 1000}$$

where the factor 40 depends upon the volumes of sample taken and the volume made up to (see A9.2.3).

When required, the concentrations of the individual LAS homologue components can be determined and used to calculate the average carbon chain length for LAS in samples. The performance of the method must be checked by determining the recovery of appropriate standard additions of LAS from real samples.

A11 References

A11.1 Matthijs E and De Henau H (1987) Determination of LAS. *Tenside Detergents* 24(4) 193-199.

A11.2 Holt M S, Matthijs E and Waters J (1989). The Concentrations and Fate of Linear Alkylbenzene Sulphonate in Sludge-Amended Soils. *Water Research* 23(6), 749-759.

A11.3 The Sampling and Initial Preparation of Sewage and Waterworks Sludges, Soils, Sediments and Plant Materials and Contaminated Wildlife Prior to Analysis, 1986 (Second Edition) *Methods for the Examination of Waters and Associated Materials*, HMSO, London, in this series.

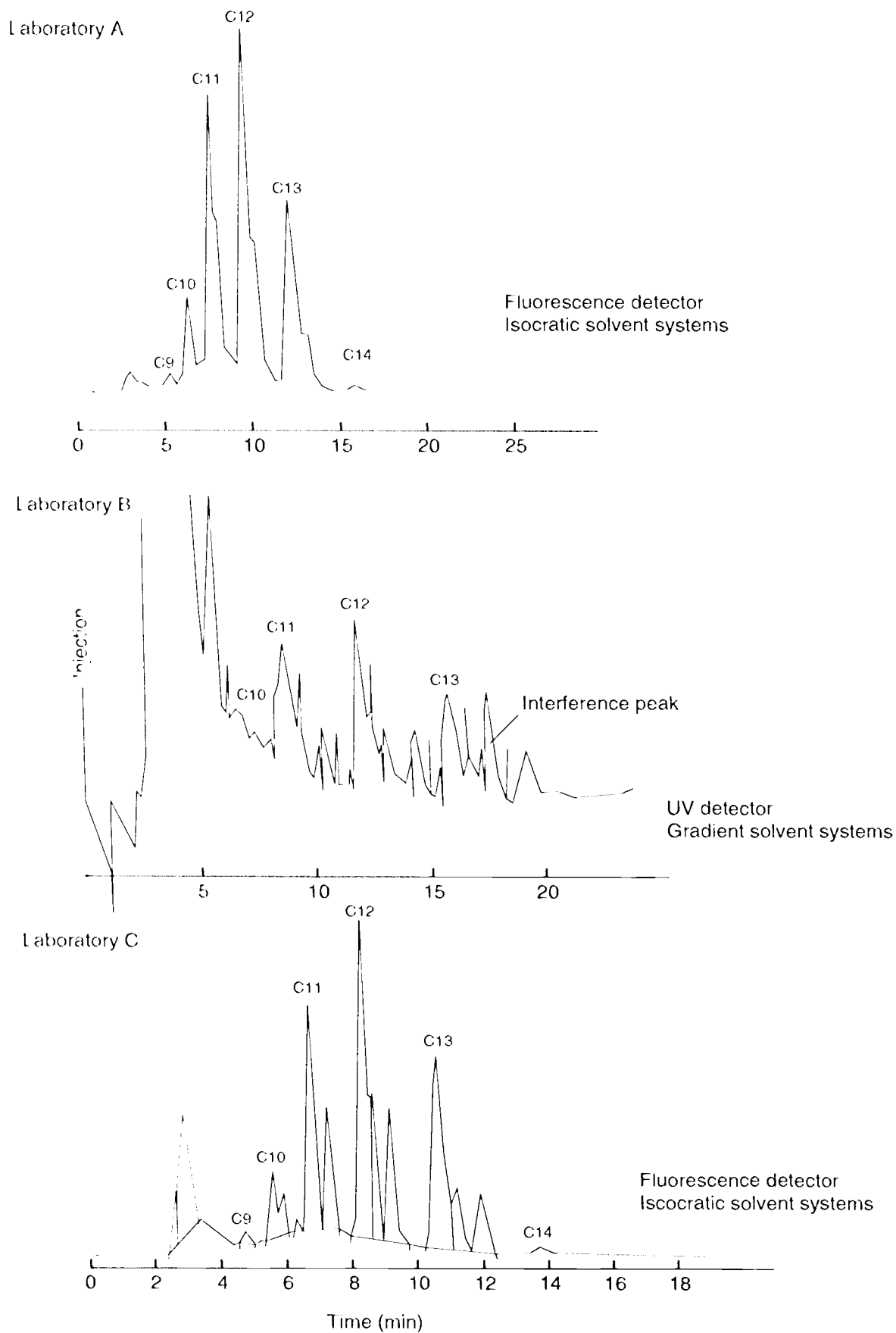


Figure 1 Reverse-phase high performance liquid chromatograms obtained by three participating laboratories

B Determination of alkylphenol ethoxylates (APE) in sewage, sewage effluent and river water by high performance liquid chromatography

B1 Performance characteristics of the method

B1.1	Substances determined	Alkylphenol ethoxylates (APE).			
B1.2	Type of sample	Sewage, sewage effluents, surface waters, biodegradation and toxicity test liquors.			
B1.3	Basis of the method	Concentration and clean-up of APE in samples by solvent sublation, ion exchange and alumina chromatography. Analysis of individual alkylphenol ethoxylates by normal phase HPLC. Detection by fluorescence spectroscopy* and quantification by peak area integration.			
B1.4	Range of application	0-5 µg of APE with fluorescence detection (expressed as some suitable reference material, for example, Marlophen 810).			
B1.5	Calibration curve	Linear over the range of application.			
B1.6	Standard deviation	Standard as Marlophen 810 mg/l	Sample	% Recovery	std dev
		1.0	deionised water	92	2.4
		1.0-5.0	settled sewage	91	4.6
		0.5-1.7	secondary sewage effluent	96	6.4
		4 degrees of freedom**			
B1.7	Limit of detection	0.5 µg of APE injected can be quantified giving the method a detection limit not higher than 0.005 mg/l for a 1 litre sample.			
		*UV detection at 280 nm may also be used			
		**Data supplied by Unilever Research, Port Sunlight Laboratory.			
B1.8	Bias	Not known.			
B1.9	Interferences	Compounds which fluoresce and have similar retention times to APE.			
B1.10	Time required for analysis	5-6 samples and necessary standards and blanks; operator time 22-30 hrs.			

B2 Principle

The procedure described determines the concentrations of alkylphenol ethoxylate nonionic surfactants extracted from aqueous media, for example, effluents and biodegradation test solutions, by solvent sublation (1).

The resulting sublation extracts are ion-exchanged under non-aqueous conditions, to remove any ionic surface active materials that may interfere in the subsequent HPLC determination, and transferred to a deactivated alumina column in hexane. Non-polar interferences are eluted whilst APE are retained on the column. The APE are recovered by elution with ethanol. The APE solution is then analysed using normal phase HPLC (which separates individual telomers) and fluorescence measurement. Quantification is carried out by peak area assessment. Other nonionic surfactants may be present but will not be detected by the fluorimeter, making the method specific for APE.

B3 Scope and limitations (including interferences)

The method can determine very low levels of individual alkylphenol ethoxylate telomers with 1 or more ethoxylate (EO) units in the presence of other nonionic surfactants such as alcohol ethoxylates. The response of the fluorescence detector to APE is linear up to at least 50 µg injected. The usual working range is approximately 0-0.5 µg. The individual APE telomers have similar molar response factors for fluorescence detection.

The application of this method to environmental samples (3,4) in which the exact identity of the APE residues are unknown, necessitates the use of an appropriate reference APE standard (for example, Marlophen 810) to express the concentration of APE determined. This material, one of several that can be used, is commonly used already in established wet chemical methods, including the BIAS (Wickbold) method (1).

The only predictable interferences arise from materials which actively fluoresce at the wavelengths used (excitation 230 nm, slit width 10 nm; emission 302 nm, slit width 5 nm) and which have similar retention times to APE. As an alternative, UV detection at 280 nm may be used to quantify APE extracts, although it is less sensitive (detection limit 0.1 mg/l) and less specific.

B4 Hazards

Chloroform, acetonitrile, methyl tertiary-butyl ether (MTBE), ethyl acetate, methanol and hexane are all potentially hazardous materials. A high standard of hygiene should be maintained when working with sewage samples.

B5 Reagents

Reagents should be analytical reagent grade quality unless otherwise stated.

B5.1 Aluminium oxide 90 active, neutral, Brockman grade 1 (70-230 mesh ASTM) for column chromatography. An equivalent alumina may also be used.

B5.2 Deactivated alumina (containing 5% m/m of water). The water content of the alumina must be controlled to ensure it has a reproducible retention capacity in column chromatography. Neutral alumina normally contains approximately 0.3-0.7% water. The water content is increased to 5% by the following procedure.

B5.2.1 Determine the water content of the alumina by heating approximately 5g (accurately weighed) in a silica crucible at 900°C for 1 hour. Cool the sample in a desiccator before reweighing. Calculate the weight loss as a percentage of water.

B5.2.2 Adjust the water content of the alumina by adding the amount of water necessary to make 100g of deactivated alumina, for example, if the alumina contains 0.3% water, use 4.7g of water and 95.3g of alumina. The water should be added to a clean, dry, amber glass or polypropylene bottle. Swirl and turn the bottle to wet the sides of the container. Add the appropriate amount of alumina to make 100g of deactivated material. Roll and tumble the bottle to mix the contents well. Allow the alumina to equilibrate overnight prior to use.

B5.3 Hexane fraction from petroleum, GLC grade.

B5.4 Ethanol.

B5.5 Methyl tertiary-butyl ether (MTBE).

B5.6 Acetonitrile. HPLC grade.

B5.7 Methanol. HPLC grade.

B5.8 Chloroform. HPLC grade.

B5.9 Glacial acetic acid.

B5.10 Sodium Hydroxide, 1.0 M.

The use of a concentrated (commercially available) volumetric solution provides a convenient means of preparing this solution. The solution is stable for at least three months.

B5.11 Reference APE standard. For general application with environmental samples, Marlophen 810, an octylphenol ethoxylate with an average of 10 ethoxylate units (EO) may be used to express the concentration of APE determined. For other applications alternative APE standards may be more appropriate.

B5.11.1 Marlophen 810 standard stock solution (0.5% m/v in chloroform). Weigh out 0.5000 ± 0.001 g of Marlophen 810 and make up to 100 ml with chloroform (B5.8) in a volumetric flask. This solution should be stable for up to six months if care is taken to avoid evaporation of the solvent.

B5.11.2 Marlophen 810 working solution (0.5 g/l). Dilute 10 ml of the stock solution (B5.11.1) to 100 ml in a volumetric flask with chloroform (B5.8).

B6 Apparatus

B6.1 Glassware.

B6.1.1 Chromatography column of conventional design having a ground glass joint to take a 250 ml cylindrical separating funnel as a solvent reservoir. (See Figure 2.)

B6.1.2 250 squat beakers.

B6.1.3 Glass rods.

B6.1.4 Pasteur pipettes.

B6.1.5 Vials— used for reaction and storage—with solid top and PTFE-faced rubber liner (5 ml capacity), for example, Reacti-Vials or equivalent.

B6.2 Ancillary equipment.

B6.2.1 Filtration equipment.

B6.2.2 Rotary sample mixer.

B6.2.3 100 and 500 μ l syringes.

B6.2.4 25 μ l HPLC syringe.

B6.2.5 Steam bath.

B6.3 Ion exchange resins.

Bio-Rad AG1-X2 anion exchange resin, hydroxide form (50–100 mesh) obtained by converting the chloride form of the resin (see below). Bio-Rad 50 W-X8 cation exchange resin, hydrogen form (50–100 mesh). Equivalent resins may also be used.

B6.3.1 Preparation of ion exchange resins.

Plug the lower constriction above the tap of the column with a small wad of glass wool and fill one-third full with water (Figure 2). Slurry about 7 ml of the wet anion exchange resin into the column with water. Remove any bubbles from the resin bed. Convert the resin from the chloride form to the hydroxide form by eluting with 20 bed volumes of 1M sodium hydroxide (approximately 140 ml) at a rate of 1–2 ml/min. Wash the column with 4 bed volumes of water (approximately 30 ml) at the same flow rate and ensure complete conversion by acidifying the final few millilitres of the aqueous eluate with dilute nitric acid and test for the presence of chloride using silver nitrate reagent. The absence of a white precipitate of silver chloride indicates that the column bed has been successfully converted. Wash the resin bed with 4 bed volumes of methanol (approximately 30 ml) at the same flow rate. Remove any bubbles from the column. Place a small wad of glass wool on top of the converted anion exchange resin. Slurry about 7 ml of wet cation exchange resin into the column with methanol, having

previously soaked the cation exchange resin in methanol for 30–60 min, to allow for any shrinkage before use. Wash the double-resin bed column with 50 ml of methanol at a rate of 2–3 ml/min. Remove any bubbles from the top resin bed and plug it with a small wad of glass wool. The column is now ready for use. Never allow resin beds to dry out.

B6.4 HPLC system and conditions.

Any high performance liquid chromatograph with a binary solvent programming capability will be suitable. This should be coupled to a fluorescence spectrometer which can operate at an excitation wavelength of 230 nm and an emission wavelength of 302 nm. A suitable chromatographic data handling system may be used to quantify peak areas.

Instrument:	HPLC with binary solvent programming capability.
Column:	250 mm × 4.6 mm I.D. stainless steel.
Packing:	Zorbax NH ₂ , particle size 5 µm. An equivalent packing material may be used. These columns are now commercially available.
Solvents:	A. 99.9:0.1 methyl tertiary-butyl ether (MTBE):acetic acid mixture. B. 95:5:0.1 acetonitrile:methanol:acetic acid mixture.
Flow:	2 ml/min.
Programme:	Linear gradient from 100% solvent A to 100% solvent B over 30 minutes. Hold at 100% solvent B for 15 minutes. Re-equilibrate column with 100% solvent A for 10 minutes before using again.
Detector:	Fluorescence spectrometer. Excitation wavelength 230 nm, slit width 10 nm Emission wavelength 302 nm, slit width 5 nm
Injector:	100 µl loop.
Temperature:	20°C

Note:

Alternative packings (for example, Partisil 5 PAC) may be used. Depending on the efficiency of the analytical column, both the solvent system and the gradient programme may require slight modifications to give the best separation of APE.

B7 Sample collection and preservation

Because of their nature, surfactants will be adsorbed onto any suspended solids as well as on the walls of the containing vessel. The analyst must therefore ascertain the requirements of the analysis, ie whether the total or soluble surfactant concentration is required.

Sampling bottles should be completely filled with sample, and if they are not to be analysed immediately, the samples should be sterilised to eliminate biodegradation by the addition of 10 ml of 40% v/v formaldehyde solution per litre of sample and stored in a refrigerator (0–5°C). To obtain a representative sample, the sample bottle should not be shaken but either slowly inverted, to avoid the formation of foam, or stirred, with a magnetic stirrer.

B8 Analytical procedure

Step	Procedure	Notes
B8.1	Sample concentration	
B8.1.1	Extract the surfactant from a known volume (V _s) of aqueous sample by the Wickbold sublation procedure (note a). Prepare at least one control and one APE standard (for example, 1 ml of 0.5 g/l	(a) See reference 1, Method B2. Four sublations will be required to give good recovery of APE from environmental samples.

Step	Procedure	Notes
	Marlophen 810 (B5.11.2) made up to volume in the sublation column with deionised water) for analysis with each batch of samples.	
B8.1.2	Evaporate the ethyl acetate extract to dryness on a steam bath in a fume-cupboard. Remove the sample from the steam bath when the solvent has completely evaporated (note b).	(b) The resulting dry sample residue will be stable for short periods (less than 7 days) prior to analysis.
B8.2	Ion exchange removal of ionic interferences	
B8.2.1	Dissolve any nonionic residue in 10 ml methanol, using a glass rod to break up the residue, and transfer the whole extract to the top of the prepared ion-exchange column (see section B6.3.1).	
B8.2.2	Pass the sample through the double resin at a rate of 1-2 ml/min (note c) and collect the eluate in a 400 ml beaker. Allow the whole extract to pass into the resin before using a further two 10 ml aliquots of methanol to ensure quantitative transfer of the sample to the column. Wash the sample through the column with 100 ml methanol at the same flow rate. Wash the columns with a further 150 ml of methanol after each separation. Never allow the resin bed to become dry (note d). Evaporate the column eluate to dryness carefully on a steam bath. Remove the sample from the bath as soon as the solvent has been evaporated.	(c) Inexperienced operators may find the use of a spare column, regulated by using a stop watch and measuring cylinder, helpful to establish the flow rates. (d) The capacities of the resin beds are such that a column may be used 5 times.
B8.3	Clean up by alumina chromatography	
B8.3.1	Insert a glass wool plug (note e) into the lower constriction of a chromatography column (above the tap). Slurry 10 g of deactivated alumina (B5.2) into the column with hexane. Wash the column with 50 ml hexane.	(e) Cotton wool can be used as an alternative. The plug must be tight enough to prevent significant amounts of alumina passing into the sample eluate but still allow sufficient flow through the column.
B8.3.2	Transfer the nonionic residue from B8.2.2 to the alumina column using several 10 ml aliquots of hexane. Elute at a rate of 1-2 drops per second (note f). Continue elution with hexane until a total of 100-125 ml of hexane has passed through the column. Discard the hexane.	(f) Allow the solvent level to reach the top of the alumina bed before adding each successive rinse.
B8.3.3	Rinse the sample beaker several times with 10 ml aliquots of ethanol (B5.4), add the ethanol rinses to the column and collect the eluent in a 250 ml beaker (note f). Continue elution with ethanol until 100 ml of the eluate has been collected (note g). Evaporate the eluate carefully just to dryness on a steam bath (note h).	(g) The elution with alcohol may be very slow and need to be left overnight. (h) An alternative method is to evaporate the alcohol under a stream of nitrogen.
B8.3.4	Dissolve the residue from step B8.3.3 using 4 × 1 ml portions of chloroform (B5.8) and quantitatively transfer these to a 5 ml vial using a Pasteur pipette. Evaporate the extract to dryness under a stream of nitrogen. Rinse the sides of the vial with a small volume of chloroform to concentrate the material	

Step	Procedure	Notes
	in the bottom of the vial and evaporate to dryness in the same way. Cap the vial and store until required for HPLC analysis.	
B8.4 HPLC analysis		
B8.4.1	Set up the liquid chromatograph and ancillary equipment in accordance with the manufacturer's instructions and the conditions laid out in step B6.4. Ensure that stable acceptable conditions are established for the subsequent analysis.	
B8.4.2	Immediately prior to the analysis, add a suitable volume (V_1) of chloroform to the vial (B8.3.4) containing the sample (note i). Recap the vial and mix the contents using a rota-mixer. Allow any particulate to settle and inject a suitable volume (V_2 , 10-20 μ l) of sample into the HPLC system. This volume of sample should have a response comparable to that for the APE standard. Generally 5 μ g of Marlophen 810 (10 μ l of solution B5.11.2) is a suitable amount of reference standard to inject into the HPLC (note j).	<p>(i) Avoid using 100 μl or less of chloroform since this may lead to significant evaporative losses from the sample and result in poor reproducibility of duplicate analyses.</p> <p>(j) One or more standard determinations should be run with each series of samples.</p>

B8.5 Calculation of results

- B8.5.1 Integrate the total area under the chromatogram for the known weight of Marlophen 810 standard injected.
- B8.5.2 Quantification of APE in a sample is made by integration of the total area under the chromatogram over the whole range of APE EO chainlengths being investigated. This area is then compared directly with that obtained for the known weight of APE reference standard (B5.11.2) (see Figure 3). When required the concentrations of the individual EO components can be determined and used to calculate the average EO chainlength for APE in the sample.

B9 Calculation of Results

- If m = weight of APE standard injected (μ g)
 a = area for APE in sample (or blank determination)
 a_{std} = area for APE in standard
 V_1 = volume of chloroform added to vial (μ l)
 V_2 = volume of sample injected (μ l)
 m_1 = weight of APE in sample (μ g)

then μ g of APE in sample (or blank determination), as Marlophen 810, is given by

$$m_1 = \frac{a \times V_1 \times m}{a_{std} \times V_2}$$

- If V_s = volume of aqueous liquor sample (litres)

then μ g APE/l in the aqueous liquor sample is given by

$$\frac{m_1}{V_s}$$

When required, the concentrations of the individual APE telomers can be readily calculated and used to determine the average ethoxylate chain length of the APE in the samples.

The performance of the method must be checked by determining the recovery of appropriate standard additions of APE from environmental samples.

B10 References

B10.1 Analysis of Surfactants in Waters, Wastewaters and Sludges, 1981. HMSO, London, in this series.

B10.2 Holt, M.S., McKerrel, E. H., Perry, J and Watkinson, R. J. (1986) Determination of alkylphenol ethoxylates in environmental samples by HPLC coupled to fluorescence detection. *J. Chromatogr.*, 362, 419—424.

B10.3 Brown, D., De Henau, H., Garrigan, J. T., Gerike, P., Holt, M., Keck, E., Kunkel, F., Matthijs, E., Waters, J. and Watkinson, R (1986) Removal of nonionics in a sewage treatment plant. *Tenside Detergents* 23, 190-195.

B10.4 Brown, D., De Henau, H., Garrigan, J. T., Gerike, P., Holt, M., Kunkel, E., Matthijs, E., Waters, J. and Watkinson, R (1987) Removal of nonionics in a sewage treatment plant. (II) *Tenside Detergents* 24, 14-19.

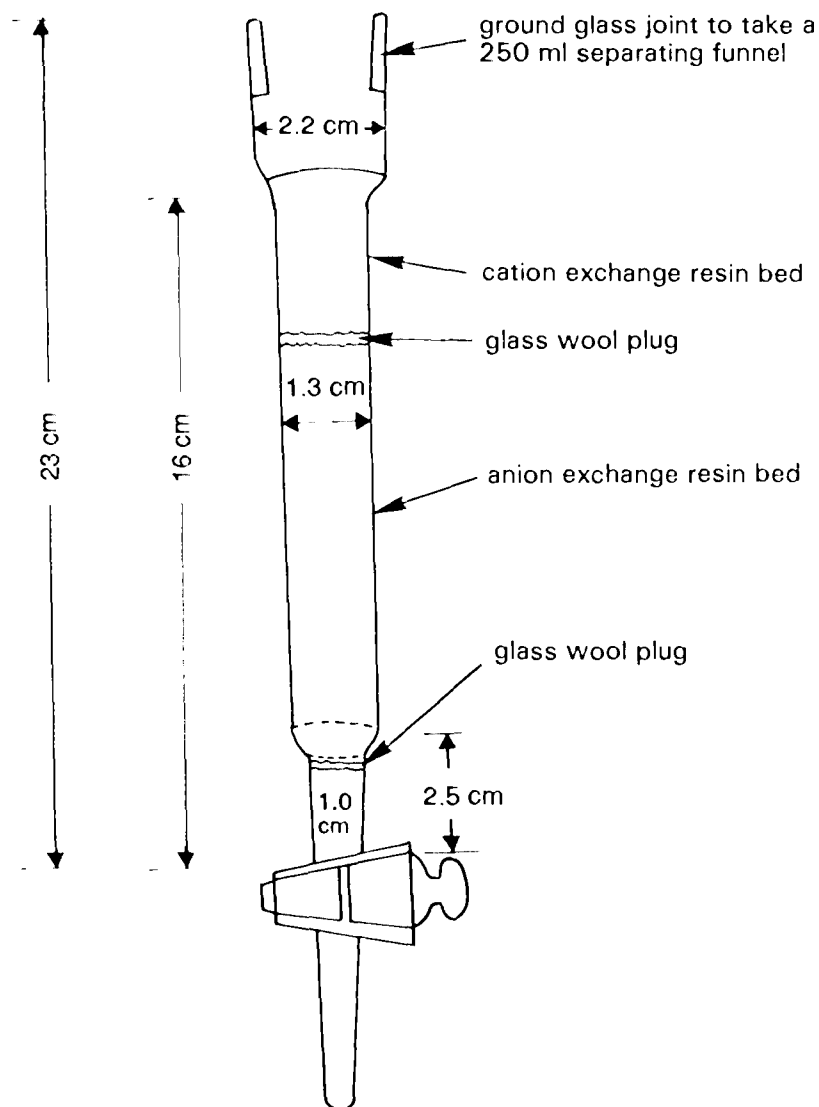


Figure 2

Chromatography/Ion exchange column

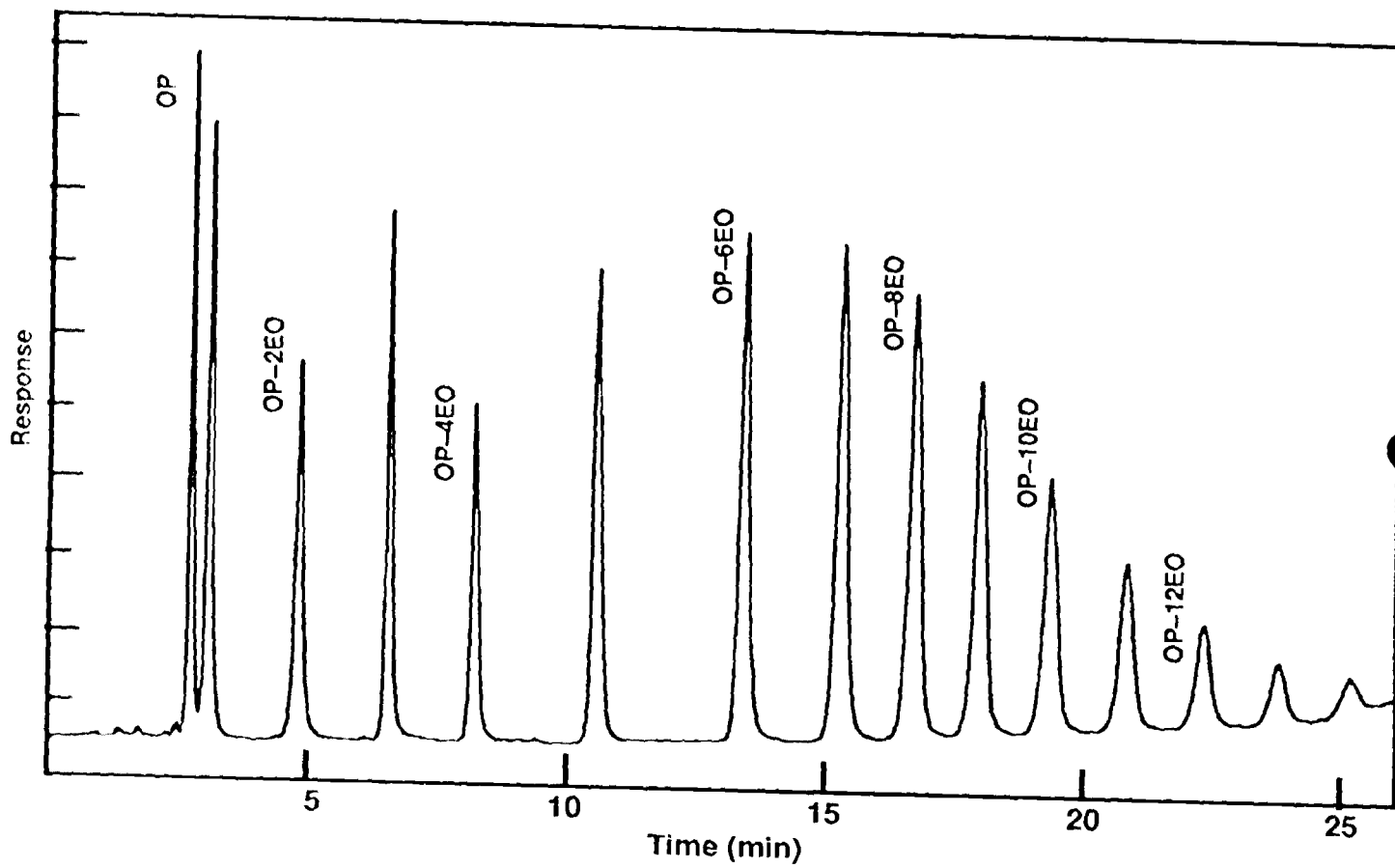


Figure 3 HPLC trace of octylphenol (OP) and ethoxylated octylphenols

Address for Correspondence

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

At the present time, thorough test data is not available. Additional test data would be welcomed and users are requested to send results to the Secretary at the address below.

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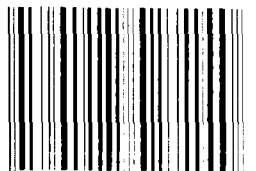
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