The Determination of Polynuclear Aromatic Hydrocarbons in Waters (additional methods) 1997

Methods for the Examination of Waters and Associated Materials

This booklet contains five methods for determining polynuclear aromatic hydrocarbons. All methods are based on fluoresence detection and only limited performance data are available.

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

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About this series

Introduction

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

Performance of methods

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

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

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

Standing committee of analysts

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

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

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

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

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

Dr D Westwood
Secretary
5th August 1996

Warning to users

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

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

1 Introduction

Methods are given for determining polynuclear aromatic hydrocarbons (PAH) in waters; these methods are additional to those published in an earlier booklet in this series (1).

These additional methods, using high performance liquid chromatography (HPLC) are for the determination of:

A six PAH using solid phase extraction;

B six or fifteen PAH using liquid-liquid extraction:

C fifteen PAH using liquid-liquid extraction;

D dissolved and particulate phase speciation of six PAH using filtration and solvent extraction; and

E six PAH using on-line enrichment.

Methods using HPLC are the methods of choice for PAH analysis and methods A and E provide alternative procedures to the liquid-liquid extraction procedure published previously (1). This reflects developments in the technology of extraction methods for liquid samples and the desire to increase automation of sample extraction. Due to the possibility of incomplete and non-reproducible extraction of particulate-associated PAH, caution is required in the use of methods A and E for samples containing significant amounts of particulate matter. Methods B and C are extensions of the previously reported method for six PAH to allow determination of fifteen PAH. The United Kingdom (UK) drinking water regulations require the determination of six PAH, however, there may be instances where the determination of other PAH is considered necessary in order to check on the results obtained when only 6 PAH are first determined. Method D allows separate determinations of the "dissolved" and "particulate-associated" fractions of PAH, as differentiated by membrane filtration. This may be useful in surveys carried out on samples from coal tar-lined mains where particulate matter may be high and where the majority of the PAH are associated with these particulates.

Some problems have been observed with the analysis of PAH as a result of interfering compounds present in the extracts causing either false positive or false negative results. This has been a particular problem for fluoranthene. In addition, a variable fluorescence response has been observed for benzo[1,12]perylene over short periods of time which affect the quantification of this compound. The problem appears not to be instrument-related, but its cause is at present unknown. Appendix A has therefore been included which describes methods on the confirmation of results. The precision and accuracy of trace organic analysis can be improved by the use of internal standards to estimate recovery and/or aid quantification. Appendix B gives a short overview of the use and choice of internal standards for analysis of PAH.

2 General information

2.1 Origin of PAH

The PAH covered by these methods are shown in Figure 1, together with their empirical formulae, nominal molecular weights and IUPAC (2) and alternative common names. PAH occur ubiquitously in the environment and are present in nearly all natural waters either in solution or adsorbed onto particulate material. PAH enter the environment primarily from combustion of organic materials, particularly fossil fuels, although substituted versions of PAH are produced biogenically.

Some PAH are reported to be carcinogenic and limits for drinking water have been incorporated into UK legislation. A maximum value (0.2 μgL^{-1}) is based on the summation of the concentrations of six individual PAH, marked * in Figure 1. These PAH are often used as indicators of the wider range of PAH which occur in natural waters and effluents. There is also a UK individual maximum concentration for benzo[3,4]pyrene of 10 ngL^{-1}.

The methods in this booklet are suitable for the determination of PAH in clean river waters, but are principally intended for potable water analysis. Levels of PAH in uncontaminated river

Figure 1 **PAH** (in order of elution on reverse phase HPLC)

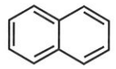
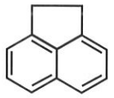
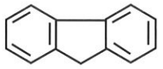
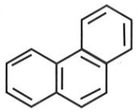
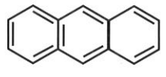
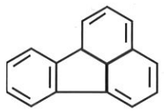
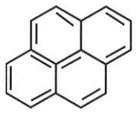
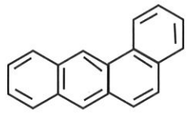
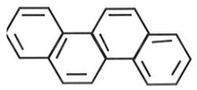
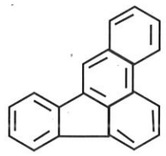
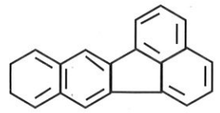
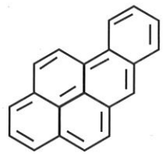
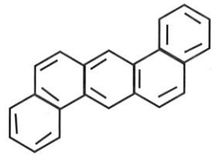
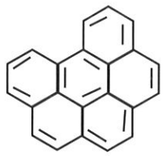
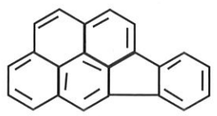
	IUPAC Name	Common Names
 $C_{10}H_8 = 128$	Naphthalene	
 $C_{12}H_{10} = 154$	Acenaphthene	1,2 Dihydroacenaphthylene
 $C_{13}H_{10} = 166$	Fluorene	
 $C_{14}H_{10} = 178$	Phenanthrene	
 $C_{14}H_{10} = 178$	Anthracene	
 $C_{16}H_{10} = 202$	Fluoranthene*	1,2 Benzacenaphthene
 $C_{16}H_{10} = 202$	Pyrene	Benzo [*def*] phenanthrene
 $C_{18}H_{12} = 228$	Benz [*a*] anthracene	Benz [1,2] anthracene
 $C_{18}H_{12} = 228$	Chrysene	

Figure 1 (cont) **PAH** (in order of elution on reverse phase HPLC)

	IUPAC Name	Common Names
 $C_{20}H_{12} = 252$	Benz [*e*] acephenanthrylene	Benzo [*b*] fluoranthene Benzo [3,4] fluoranthene*
 $C_{20}H_{12} = 252$	Benzo [*k*] fluoranthene	Benzo [11,12] fluoranthene*
 $C_{20}H_{12} = 252$	Benzo [*def*] chrysene	Benzo [*a*] pyrene Benzo [3,4] pyrene*
 $C_{22}H_{14} = 278$	Dibenz [*a,h*] anthracene	Dibenz [1,2:5,6] anthracene
 $C_{22}H_{12} = 276$	Benzo [*ghi*] perylene	Benzo [1,12] perylene*
 $C_{22}H_{12} = 276$	Indeno [1,2,3 -*cd*] pyrene*	

* PAH for which legislative limits are prescribed in the UK

waters are generally very low, with total PAH typically in the 5-20 ngL^{-1} region. However, waters contaminated with effluents may contain levels of PAH several orders of magnitude higher. In addition, because of the tendency of PAH to adsorb strongly to particulate matter and sediments, apparently clean river waters that contain significant quantities of suspended particulate matter, for example rivers in spate, may contain high levels of PAH due to particulate-associated PAH.

High levels of PAH may also occur in potable water where pipes in the distribution systems are lined with coal tar pitch. This is because coal tar pitch contains large amounts (up to several % by weight) of PAH, which over long periods of time may leach out of the lining into the water. Alternatively, particles of coal tar pitch lining may be deposited into the water (3). Leaching of PAH from linings is particularly likely where hard groundwater is the source of potable water. In this case, dissolved levels of PAH may be high (up to several hundred ngL^{-1}). High PAH levels, associated with coal tar pitch particulates, may occur in dead-ends of mains distribution systems, where particulates can accumulate, and concentrations up to 10000 ngL^{-1} have been measured. The major components of coal tar pitch lining from the 15 PAH determined are often fluoranthene, pyrene, phenanthrene and anthracene.

2.2 Analytical

The methods in this booklet are based on reverse-phase HPLC separation. It is important to note that the HPLC column temperature must be maintained at a constant temperature in order to ensure that the resolution and retention times required to separate (and identify) the PAH are routinely obtained. This can be achieved by the use of a column jacket with a temperature controlled liquid flowing through it. The ambient temperature should also be maintained at a reasonably constant value (±2 °C). The chromatography column and conditions used in the determination should achieve adequate efficiencies to allow separation, preferably to baseline, of all components. In addition, special care should be taken with methods based on solid phase extraction due to the wide variability of the extraction properties between batches of solid phase cartridges and cartridges from different manufacturers.

As mentioned earlier, PAH in water samples may be strongly associated with particulate matter present in natural waters and in some drinking water mains distribution systems. This has two important consequences in respect of the determination of PAH concentrations in samples containing particulate matter. Firstly, it may prove difficult to obtain a representative sample, since relatively small differences in the amount of particulate matter sampled with the water may lead to large differences in the measured concentration of PAH. Secondly, the analytical procedures may be unsuitable for the extraction of PAH from the particulate matter present in the water, and result in poor and variable recovery. Since a larger portion of the PAH present in the sample may be associated with the particulate matter, rather than with the dissolved phase, large differences can result in the measured concentration of PAH for apparently identical samples.

3 Hazards warning and safety precautions

Certain PAH are highly carcinogenic and should be handled with extreme care. Contact with solid materials, solvent extracts and solutions of standard PAH should not be allowed to occur. PAH may become deposited on the outside surface of glassware containing solvent solutions of PAH. Glassware should therefore always be handled using gloves which are solvent resistant and preferably disposable. PAH contamination of the outside surface of vessels may be detected by irradiation with 366 nm wavelength ultra violet (UV) light. Care should be taken during this operation not to irradiate the bulk of the solution. As well as being hazardous, such deposits may indicate a change in solution concentration. Vessels containing PAH solutions should be stored upright in beakers, so as to contain any spillage in the case of breakage, and under refrigeration.

Solid PAH may give rise to a dust hazard due to crystals becoming electrostatically charged. These materials should be handled only where proper facilities are available (for example adequate fume cupboards, protective clothing, dust masks etc). When pure PAH are weighed, it is prudent to do so in a single step, ie repeated adjustments of the amount of pure PAH in order to achieve a specific quantity should be avoided. This minimises exposure to hazardous PAH. Any necessary adjustment can be performed on a solution of the PAH. It is strongly advised that standard solutions are prepared centrally in suitably equipped

laboratories or are purchased from suppliers specialising in their preparation.

Standard solutions of PAH are available from a number of suppliers. As an aid to the analyst, a list of suppliers are given below. Other suitable sources may also be available and reference to the listed suppliers does not constitute any endorsement.

Community Bureau of Reference - BCR
Directorate General XII
Commission of the European Communities
200 rue de la Loi
B-1049 Brussels, Belgium

Supelco (Sigma-Aldrich)
Fancy Road
Poole
Dorset
BH17 7NH

Promochem Ltd
6 S Mundells
Welwyn Garden City
AL7 1EP

Greyhound Chromatography
and Allied Chemicals
88 Grange Road West
Birkenhead, Merseyside L43 4XF

Qm_X Labs Ltd
Qm_X House,62 High St,
Saffron Walden
Essex CB10 1EE

Concentrated solutions of PAH, used gloves etc, should be disposed of in a manner approved for disposal of toxic waste.

General techniques for the handling of hazardous materials have been given elsewhere (4, 5, 6, 7). These, or similar publications should be consulted before handling PAH.

Methanol, toluene and acetonitrile are both flammable and toxic and appropriate precautions should be observed when handling these solvents. Cyclohexane, ethanol, diethyl ether, petroleum ether (40:60), acetic acid, n-hexane, propan-2-ol and acetone are flammable. Dichloromethane is a presumptive carcinogen and should be handled with appropriate precautions. Sulphuric acid, nitric acid and acetic acid are corrosive and cause severe burns.

4 Scope and field of application

Methods A, B, D and E are applicable to the measurement of six PAH, marked * in Figure 1, in most potable waters, groundwaters and relatively un-polluted surface waters. Methods A and E are applicable to unfiltered samples with low levels of particulate matter. Incomplete and non-reproducible recovery may be obtained if they are used for water samples containing PAH adsorbed on suspended particulate matter.

Methods B and C are applicable to the measurement of fifteen PAH shown in Figure 1 in most potable waters, groundwaters and probably relatively un-polluted surface waters. The methods have not been validated for un-polluted surface waters. Method D can be used for water samples containing high levels of particulate matter by analysing separately the particulate-associated and dissolved PAH.

5 Interferences

Any material which is co-extracted under the conditions used for a particular method and which has similar chromatographic behaviour to any of the PAH determined and which fluoresces or quenches fluorescence will interfere. Molecular oxygen present in any solvent used in the determination can cause fluorescence quenching of PAH. Hence, HPLC solvents should be thoroughly degassed before use. Sample extracts which show a positive fluorescence at any point on the chromatogram where a wavelength change occurs will produce a false baseline, which may interfere with peak detection and quantification.

6 Sample collection and storage

Samples should be collected in clean (amber) glass bottles with screw caps and polytetrafluorethylene (PTFE) liners and should be filled to leave no headspace. Any residual chlorine in the sample should be neutralised by the addition of sodium thiosulphate or sodium sulphite. When sampling from taps, the tap should be allowed to flush for at least 2 minutes, unless `first flush' samples are specifically required. When sampling drinking waters, the tap should not be `flamed' before a sample is taken for PAH analysis. Extra protection from contamination during transport and storage by, for example, dust, vehicle exhaust fumes and cigarette smoke can be provided by wrapping the bottle tops in aluminium foil. Samples should be stored in the dark, under refrigeration and analysed as soon as possible after collection.

Sample bottles should be carefully cleaned before use and contamination with dirtier, water samples should be avoided. Bottles can be rinsed out and hand washed in hot water with detergents or, preferably, in an automatic glass washer. After an optional dilute acetic acid wash, bottles should be thoroughly rinsed in deionised water. After draining, they can be heated slowly in an oven to 500 °C, held at that temperature for 1 hour and carefully cooled. Alternatively, bottles can be rinsed with dichloromethane. Bottle tops and any PTFE liners should be considered disposable and new ones fitted.

A Determination of six polynuclear aromatic hydrocarbons in waters using solid phase extraction and high performance liquid chromatography

A1 Performance characteristics of the method

A1.1	**Substances determined**	PAH marked * in Figure 1.
A1.2	**Type of sample**	Drinking water.
A1.3	**Basis of method**	Samples are extracted using C_{18} solid phase cartridges which are then eluted with dichloromethane. After evaporating the eluent to a small volume, acetonitrile is added and this solution is analysed by HPLC using fluorescence detection. **This method is not suitable for any sample containing significant visible solids.**
A1.4	**Range of application**	Individual compounds vary; typically up to a maximum of 40ngL^{-1}.
A1.5	**Calibration curve**	All calibrations are linear up to the top of their working ranges, after which detector saturation effects are evident.
A1.6	**Standard deviation**	See Table A1.
A1.7	**Limit of detection**	See Table A1.
A1.8	**Sensitivity**	Instrument dependent.
A1.9	**Bias**	Recovery efficiencies are less than 100%. See Table A1.
A1.10	**Time required for analysis**	Batches of 40 or more samples can be extracted in 1 day. Typical run time for 12 samples with calibration standards and analytical quality control solutions is six hours.

Data provided by Severn Trent Laboratories Ltd

A2 Principle

Samples are extracted by sorption onto C_{18} solid phase cartridges which are eluted with dichloromethane. After evaporating the eluent to a small volume, acetonitrile is added and this solution is analysed by HPLC using fluorescence detection.

A3 Interferences

See section 5.

A4 Hazards

See section 3.

A5 Reagents

All chemicals used to prepare reagent and standard solutions should be of sufficient purity that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemical by analysing procedural blanks with each batch of samples analysed.

Standards should be stored at 4 °C in tightly sealed all-glass containers, wrapped in metal foil.

A5.1 **Propan-2-ol.** HPLC grade.

A5.2 **Dichloromethane.** HPLC grade.

A5.3 **Acetonitrile.** HPLC grade.

A5.4 **Water.** HPLC grade.

A5.5 **Triphenylene.** Analytical grade reagent. (Used as internal standard).

A5.6 **Sodium thiosulphate solution** (3 % m/v). Dissolve 46.0 ± 0.2 g sodium thiosulphate pentahydrate in water in a 1 litre volumetric flask, and make up to volume with water.

A5.7 **PAH standard solutions.** 100mgL^{-1} of each of the six compounds.

A5.8 **Preparation and storage of standard solutions.** Standard solutions should be stored in a cool environment (to avoid excessive evaporation of solvent), but all solutions and solvents should be adjusted to room temperature before volumetric measurements are made. When a standard is required for use, the container and contents can be weighed, a portion of the solution sub-sampled into a clean, dry beaker of low, but sufficient volume, and the container and its (reduced) contents weighed again. Any significant difference in the weight of the flask since its last use would indicate loss of solvent and appropriate action should be taken.

A5.8.1 **Stock internal standard solution.** (150 mgL^{-1}). Weigh accurately, approximately 15 mg of triphenylene and note the exact weight. Transfer the solid quantitatively to a 100 mL volumetric flask and add sufficient acetonitrile to dissolve the material. The volume is then made up to the mark with acetonitrile. This solution is stable for up to six months.

A5.8.2 **Working internal standard.** (15 mgL^{-1}). Add10.0 ± 0.1 mL of the stock internal standard (A5.8.1) to a l00 mL volumetric flask and make to the mark with acetonitrile. This solution is stable for up to one month.

A5.8.3 **Individual stock PAH standards.** (5000 µgL^{-1}). A volume of 1.00 ± 0.02 mL of the standard solution (A5.6) is transferred to a 20 mL volumetric flask, and the volume made up to the mark with acetonitrile. This solution is stable for up to six months.

A5.8.4 **Mixed intermediate standard.** (Benzo[11,12]fluoranthene concentration = 50 µgL^{-1}, other PAH concentrations = 100 µgL^{-1}).

Transfer 0.50 ± 0.01 mL of the benzo[11,12] fluoranthene stock solution (A5.8.3), and 1.00 ± 0.02 mL of the other five stock solutions, to a 50 mL volumetric flask, and make up to volume using acetonitrile. This solution is stable for up to one month.

A5.8.5 **Working calibration standard.** Transfer 500 ± 10 µL of the mixed intermediate standard (A5.8.4) to a 2 mL glass vial, the vial is sealed with a crimp cap, and stored in a refrigerator, until required. When required (for example with every batch of samples), a vial should be de-capped, 100 ± 2 µL of working internal standard (A5.8.2) added, and the vial re-capped. This solution is stable for up to one week. The 600 µL volume now contains 25 ng benzo[11,12]fluoranthene, 50 ng of other PAH and 1.5 µg of internal standard.

A5.8.6 **Low level check standard (sensitivity check).** A low level check standard can be prepared by transferring volumes of stock PAH standard solutions (A5.8.3) tabulated below, to a 50 mL volumetric flask, and making to volume with acetonitrile.

PAH	Volume of A5.8.3 solution (µL)	Concentration in standard solution (µgL^{-1})
Fluoranthene	50	5.0
Benzo[3,4]fluoranthene	50	5.0
Benzo[11,12]fluoranthene	25	2.5
Benzo[3,4]pyrene	20	2.0
Benzo[1,12]perylene	200	20.0
Indeno[1,2,3-*cd*]pyrene	200	20.0

For a working low level check standard, 500 ± 10 µL of this solution is transferred to a 2 mL vial and 100 ± 10 µL of internal standard (A5.8.2) added. This solution is stable for up to one week. The 600 µL volume now contains 1.25 ng benzo[11,12]fluoranthene, 2.5 ng of fluoranthene and benzo[3,4]fluoranthene, 1.0 ng of benzo [3,4]pyrene, 10 ng of benzo[1,12]perylene and indeno[1,2,3-cd]pyrene, and 1.5 µg of internal standard.

A6 Apparatus

A6.1 **A top pan balance,** capable of weighing up to 2000 g in increments of 1 g.

A6.2 **Vacuum manifold** fitted with on/off valves and a pressure gauge.

A6.3 **Suitable stand** to hold cartridges.

A6.4 **Nitrogen blow-down apparatus** with a supply of nitrogen (99.9% v/v).

A6.5 **Glass micro-vials** together with crimp top and crimper tool.

A6.6 **Variable volume pipettes** (0-50 µL, 100-250 µL, 200-1000 µL).

A6.7 **Pasteur pipettes**, glass, disposable.

A6.8 **100 mg syringe C_{18} cartridges** together with 250 mL nominal volume glass reservoirs, manufactured to fit on the top of the cartridges.

A6.9 **Volumetric flasks,** grade B or better.

A6.10 **Solvent resistant dispensers** (capable of dispensing 30 mL of propan-2-ol).

A6.11 **Analytical balance**, capable of weighing to the fourth decimal place, in grams.

A6.12 **Suitable syringes**, for preparation of standard solutions (250 µL, 500 µL, 1000 µL can be used).

A6.13 **Guide needles** with a luer fitting for cartridge elution, and sample blowdown.

A6.14 **1.1 mL tapered chromatography vials**.

A6.15 **10 mL glass pipette**, grade B or better.

A6.16 **Bottle shaker** capable of taking one litre glass bottles.

A6.17 **High performance liquid chromatograph**.

A6.18 **Fluorescence detector** with programmable wavelength selection.

A6.19 **Strip chart recorder** or data station for processing detector output.

A6.20 **Amber glass sample bottles**. Nominal 1 litre capacity with PTFE-lined screw plastic cap.

A7 Sample collection and storage

See section 6.

A8 Analytical procedure

Step	Procedure	Notes
A8.1	Connect the glass reservoirs to the sample numbered cartridges by gently twisting and fit onto the vacuum manifold, (note a).	(a) Check the waste reservoir to ensure it is empty.
A8.2	Turn on the vacuum pump, adjust the vacuum to 13 mm of Hg, open the stop valves and condition the cartridges with 10.0 ± 0.5 mL of propan-2-ol (A5.1), followed by 10.0 ± 0.5 mL of distilled water (A5.4). When the meniscus of the water just reaches the cartridge packing close the stop valves so as to ensure the cartridges do not dry out.	
A8.3	Thoroughly shake the sample bottle to homogenise the sample and discard approximately 50 mL of sample. Add 30 ± 3 mL of propan-2-ol to the 1 litre sample bottle and shake on a bottle shaker for 10 ± 1 minute. Weigh the bottle (W_1). Pour approximately 200 mL of sample into a reservoir, and reweigh the bottle, (W_2). Volume of water extracted = (W_1 - W_2) / 1.03.	
A8.4	Set vacuum pump to 38-51 mm Hg and open the stop valve. Allow the sample to be drawn through the cartridge, ensuring that it does not run dry, note b. Close the stop valve, note c.	(b) This should take no more than 40-45 minutes. (c) If the sample takes more than 40-45 minutes to pass through the cartridge it may contain an unacceptable concentration of solids and this method should not then be used.
A8.5	When all the samples in a batch have passed through the cartridges remove the reservoirs and vacuum dry the cartridges for ten minutes.	
A8.6	Transfer the dry cartridges to a suitable stand and fit a guide needle to each. Number a 1.1 mL tapered vial for each sample and add 20 ± 2 µL of acetonitrile (A5.3) to each, and place under the appropriate cartridge ensuring the guide needle is in the mouth of the vial. Add 1.0 ± 0.1 mL of dichloromethane (A5.2) to the cartridge and elute into the vial.	

A8.7 Blow down the samples under a gentle stream of nitrogen until a small volume (approximately 10-20 µL) is left in the narrow taper.

A8.8 Add 100 ± 2 µL of working triphenylene internal standard (A5.8.2). Mix the solution in the vial using a rotary mixer and transfer the solution to a glass micro-vial. Seal tightly with a crimp top.

A8.9 The extracts are now ready for HPLC determination.

A9 HPLC conditions

The following HPLC conditions were used to generate the data.

Mode	:	Isocratic reverse phase.
Column	:	Shandon Hypersil (reverse phase) C_{18} (ODS) 10 cm x 4.6mm.
Column temperature	:	Ambient (23-24 °C).
Mobile phase	:	Acetonitrile:water (92:8 v/v).
Flow rate	:	1.2 $mLmin^{-1}$.
Injection volume	:	20 µL
Fluorescence detector program	:	Wavelength changes should be made between the peaks of the PAH noted and times of changes optimised on actual samples).

Excitation Wavelength (nm)	Emission Wavelength (nm)	Wavelength Changes	
		After	Before
270	420	Initial conditions	
290	410	Triphenylene	Benzo[3,4]fluoranthene
295	460	Benzo[3,4]pyrene	Benzo[1,12]perylene

At stop time, excitation and emission wavelengths are returned to initial conditions. A typical chromatogram is shown in Figure A1.

A10 Calculation

A calibration graph of ratio of determinand to internal standard peak height or area against mass of determinand in sample injected is constructed either manually or via the instrument data station. The original sample concentration is calculated from the graph taking into account sample volume extracted, sample volume injected and any dilutions that may have been used. Since an internal standard is added after extraction, correction for incomplete recoveries should be made where necessary.

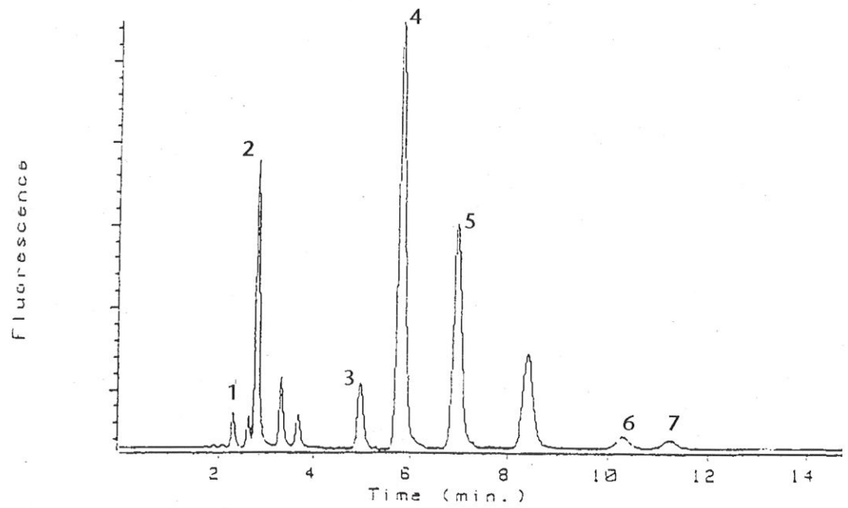
Table A1 Standard deviations, Recoveries and Limits of detection

Compound	Concentration (ngL^{-1})	Standard deviation (ngL^{-1})	Recovery (%)	Limit of detection (ngL^{-1})
Fluoranthene	40	1.0	99.6	4.4 (12)
Benzo[3,4]fluoranthene	40	0.7	97	1.6 (17)
Benzo[11,12]fluoranthene	20	0.5	92.7	0.7 (14)
Benzo[3,4]pyrene	20	0.5	74.3	0.5 (14)
Benzo[1,12]perylene	40	0.9	82.3	3.0 (11)
Indeno[1,2,3-*cd*]pyrene	20	0.9	86.7	2.4 (14)

Figures in brackets represent degrees of freedom.

Figure A1 A typical chromatogram of six PAH

1 Fluoranthene
2 Triphenylene (internal standard)
3 Benzo[3,4]fluoranthene
4 Benzo[11,12]fluoranthene
5 Benzo[3,4]pyrene
6 Benzo[1,12]perylene
7 Indeno[1,2,3-*cd*]pyrene



B Determination of six or fifteen polynuclear aromatic hydrocarbons in waters using liquid-liquid extraction and high performance liquid chromatography

B1 Performance characteristics of the method

B1.1	**Substances determined**	PAH (see Figure 1). The method describes two sets of instrumental conditions for the separation of groups of 6 and 15 PAH respectively. (Acenaphthylene fluoresces insufficiently to be quantified).
B1.2	**Type of sample**	Drinking water.
B1.3	**Basis of method**	Solvent extraction of the sample followed by concentration and high performance liquid chromatography (HPLC) with fluorescence detection.
B1.4	**Range of application**	Working ranges vary depending on individual compounds, but can be up to 200 ngL^{-1}.
B1.5	**Calibration curve**	All calibrations are linear up to the top of the working ranges after which detector saturation effects are evident.
B1.6	**Standard deviation**	See Tables B1 and B2.
B1.7	**Limit of detection**	See Tables B1 and B2.
B1.8	**Sensitivity**	Instrument dependent.
B1.9	**Bias**	Recovery efficiencies are less than 100%. See Tables B1 and B2.
B1.10	**Time required for analysis**	For a batch of 24 samples, preparation time is typically 1 day for 1 person. Typical run time for a batch of 12 samples by HPLC is 6 hours for 6 PAH and 12 hours for the 15 PAH.

Data provided by Severn Trent Laboratories Ltd

B2 Principle

Samples are extracted using dichloromethane and the extract evaporated to a small volume. Acetonitrile is added to form the major portion of the solvent and the solution is analysed by HPLC using fluorescence detection.

B3 Interferences

See section 5.

B4 Hazards

See section 3.

B5 Reagents

All chemicals used to prepare reagent and standard solutions should be of sufficient purity that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemical by analysing procedural blanks with each batch of samples analysed.

Standards should be stored at 4 °C in tightly sealed all-glass containers, wrapped in metal foil.

B5.1 **Dichloromethane.** HPLC grade.

B5.2 **Acetonitrile.** HPLC grade.

B5.3 **Water.** HPLC grade.

B5.4 **Triphenylene.** Analytical grade reagent. (Used as internal standard).

B5.5 **Sodium sulphate.** Anhydrous analytical grade. Heat to 500 ± 20 °C for 4 hours ± 30 minutes.

B5.6 **Sodium thiosulphate solution (3 % m/v).** Dissolve 46.0 ± 0.2 g sodium thiosulphate pentahydrate in water in a 1 litre volumetric flask, and make up to volume with water.

B5.7 **PAH standard solution.** For determination of the 15 PAH it is convenient to use a commercial mixture. The dilutions used in this method are shown in Table B3 but any suitable commercially available solution can be used.

B5.8 **Preparation and storage of standard solutions.** Standard solutions should be stored in a cool environment (to avoid excessive evaporation of solvent), but all solutions and solvents should be brought to room temperature before volumetric measurements are made. When a standard is required for use, the container and contents can be weighed, a portion of the solution sub-sampled in a clean, dry beaker of low, but sufficient volume, and the sealed container and its (reduced) contents weighed again. Any significant difference in weight of the container and contents since its last use would indicate loss of solvent and appropriate action should be taken.

B5.8.1 **Stock internal standard solution.** (150 mgL^{-1}). Weigh about 15 ± 1 mg of triphenylene and note the exact weight. Transfer the solid quantitatively to a 100 mL volumetric flask and add sufficient acetonitrile (B5.2) to dissolve the material. The volume is then made up to the mark with acetonitrile. This solution is stable for up to six months.

B5.8.2 **Working internal standard solution.** (15 mgL^{-1}). A volume of 10.0 ± 0.1 mL of the stock internal standard (B5.8.1) is made up to 100 ml with acetonitrile. This solution is stable for up to one month.

B5.8.3 **Stock PAH standard solution.** A volume of 500 ± 5 µL of PAH standard mixture (B5.7) is diluted to 25 mL in dichloromethane (B5.1) in a volumetric flask. This solution is stable for up to six months.

B5.8.4 **Calibration standard solution.** A volume of 1.00 ± 0.01 mL of stock solution (B5.8.3) is diluted with acetonitrile in a 50 mL volumetric flask.

A volume of 0.50 + 0.01 mL of this solution is transferred to a 2 mL glass vial, see Table B3 for concentrations, and 100 ± 2 µL of internal standard (B5.8.2) is added and the vial sealed with a crimp top. This solution is stable for up to one week.

B5.8.5 **High check standard solution.** A volume of 2.00 ± 0.01 mL of stock solution (B5.8.3) is diluted with acetonitrile in a 25 mL volumetric flask.

A volume of 0.50 + 0.01 mL of this solution is transferred to a 2 mL glass vial, and 100 ± 2 µL of internal standard (B5.8.2) is added and the vial sealed with a crimp top. See Table B3 for concentrations. This solution is stable for up to one week and can be used to check the fluoresence response.

An appropriate low level check standard, to confirm performance at concentrations close to the limits of detection, can be included if necessary.

B6 Apparatus

B6.1 **Amber glass sample bottles.** Nominal 1 litre capacity with PTFE-lined screw plastic cap.

B6.2 **Solvent resistant dispenser** (preferably to screw directly onto Winchester or other container) capable of delivering 10-50 mL.

B6.3 **A top pan balance** with a capacity of at least 2000 g in increments of 1 g.

B6.4 **Bottle shaker** capable of taking 1 litre bottles.

B6.5 **Glass separating funnels** (one litre capacity).

B6.6 **Glass chromatography column** (approximately 30 cm long x 2 cm diameter) fitted with a coarse glass sinter and quickfit connections suited to the micro Kuderna-Danish evaporators and Snyder columns.

B6.7 **Micro Kuderna-Danish evaporators** (approximately 100 mL volume).

B6.8 **Snyder columns**, two or three ball, fitting into the evaporators.

B6.9 **Water bath** set at 60 ± 5 °C, together with a support for the Kuderna/Snyder apparatus.

B6.10 **Glass boiling chips** (washed with dichloromethane and dried).

B6.11 **Nitrogen evaporation apparatus** (for reducing solvent extracts to low volume).

B6.12 **Glass chromatography vials**, together with crimp top and crimper tool.

B6.13 **Variable volume syringe dispensers** (100-250 µL and 10-20 µL).

B6.14 **Analytical balance**, capable of weighing to the fourth decimal place in grams.

B6.15 **Pasteur pipettes**, glass, disposable.

B6.16 **Graduated test tubes** to fit a micro Kuderna-Danish apparatus.

B6.17 **High performance liquid chromatograph.**

B6.18 **Fluorescence detector** with programmable wavelength selection.

B6.19 **Strip chart recorder** or data station for processing detector output.

B7 Sample collection and storage

See section 6.

B8 Analytical procedure

Step	Procedure	Notes
B8.1	Shake the bottle to homogenise the sample. Discard a portion (50-60 mL) and reseal. Weigh the bottle and contents (W1 g).	
B8.2	Add 50 ± 5 mL of dichloromethane (B5.1) directly to the sample bottle. Reseal, ensuring the PTFE insert is correctly placed in the cap. Shake the bottle on the mechanical shaker for 10 ± 1 minutes.	

B8.3 Pour the contents of the bottle into a 1 litre separating funnel and allow the phases to separate. Run the lower dichloromethane phase through a glass chromatography column, containing about 25 g of sodium sulphate (B5.5) which has previously been washed and wetted with dichloromethane, into a labelled micro Kuderna-Danish evaporator fitted with a graduated test tube.

B8.4 Pour the aqueous sample back into the sample bottle. Repeat steps B8.2 to B8.3 using 40 ± 1 mL of dichloromethane to obtain a second extract.

The sample bottle should be rinsed with approximately 10 mL of dichloromethane and the washings added to the separating funnel before running off the second extract through the sodium sulphate and into the micro-Kuderna.
Weigh the empty bottle, (W2 g).

Remove the micro-Kuderna, add two boiling chips and a Snyder column with a connecting clip.

B8.5 Immerse the micro Kuderna-Synder apparatus in the water bath (60 ± 5 °C), (note a). Reduce the volume of the extract to about 2 mL, (note b). Remove the apparatus allow to cool. Remove the Snyder column.

(a) The water level should not cover more than the bottom of the micro and Kuderna bulb.

(b) The solution should not "bump" or boil dry.

B8.6 When the combined extracts have been reduced to 1-2 mL and allowed to cool, the volume is further reduced to approximately 0.1 mL by using a stream of clean, dry nitrogen blown onto the surface of the extract (the extract in the micro Kuderna is warmed in a water bath at not more than 40 ± 1°C).

B8.7 Add acetonitrile so as to bring the volume of the extract up to approximately 0.5 mL. Add 100 µL working of internal standard solution (B5.8.2) using a variable volume syringe dispenser and mix on a rotary mixer. Transfer the extract to a 2 mL autosampler vial and seal with a crimp top.

B8.8 The extract is now ready for HPLC determination.

B9 HPLC conditions

The following HPLC conditions were used to generate the data.

a) 6 PAH:-

Oven temperature	:	Ambient (23-24 °C)
Flow rate	:	1.2 mLmin^{-1}
Maximum pressure	:	400 BAR
Injection volume	:	20 µL
Isocratic mixture	:	acetonitrile:water (92:8 v/v)
Column	:	Hypersil Green PAH (reverse phase) 10 cm x 4.6 mm id.
Pre-column	:	Pelliguard (reverse phase) LC-18 2 cm
Fluorescence detector program	:	Wavelength changes should be made between the peaks of the PAH noted and times of changes optimised on actual samples; similarly for solvent changes.

Excitation Wavelength (nm)	Emission Wavelength (nm)	Wavelength changes After	Before
270	420	Initial conditions	
290	410	Triphenylene	Benzo[3,4]fluoranthene
295	460	Benzo[3,4]pyrene	Benzo[1,12]perylene

At stop time, excitation and emission wavelengths are returned to initial conditions.

b) 15 PAH:-

Oven temperature	:	Ambient (23-24 °C)
Flow rate	:	1.3 mLmin^{-1}
Maximum pressure	:	400 BAR
Injection volume	:	20 µL
Solvents	:	Water (A), acetonitrile (B)
Column and pre-column	:	Same as for 6 PAH

Solvent timetable

Time (min)	A (%)	B (%)	
0.00	55	45	
10.00	50	50	These are gradient changes in composition.
27.50	0	100	
33.00	0	100	
35.00	55	45	
36.00	55	45	

Fluorescence detector program : Wavelength changes should be made between the peaks of the PAH noted and times of changes optimised on actual samples; similarly for solvent changes.

Excitation Wavelength (nm)	Emission Wavelength (nm)	Wavelength changes Before	After
270	340	Initial conditions	
240	380	Phenanthrene	Fluorene
270	420	Fluoranthrene	Anthracene
290	380	Triphenylene	Pyrene
290	410	Benzo[3,4]fluoranthene	Chrysene
295	460	Dibenz[1,2:5,6]anthracene	Benzo[3,4]pyrene

At stop time, excitation and emission wavelengths are returned to initial conditions.

B10 Calculation

A typical chromatogram is shown in Figure B1.

A calibration graph of ratio of determinand to internal standard peak height or area against mass of determinand in sample injected is constructed either manually or via the instrument data station. The original sample concentration is calculated from the graph taking into account sample volume extracted, sample volume injected and any dilutions that may have been used. Since an internal standard is added after extraction, correction for incomplete recoveries should be made where necessary.

Table B1 Standard deviations, Recoveries and Limits of detection - 6 PAH

Compound	Concentration (ngL^{-1})	Standard deviation (ngL^{-1})	Recovery (%)	Limit of detection (ngL^{-1})
Fluoranthene	40	1.6	89.0	4 (8)
Benzo[3,4]fluoranthene	40	1.6	96.8	3 (8)
Benzo[11,12]fluoranthene	20	1.0	96.5	2 (8)
Benzo[3,4]pyrene	20	1.0	96.1	1 (9)
Benzo[1,12]perylene	40	1.6	92.1	3 (9)
Indeno[1,2,3-*cd*]pyrene	20	1.3	88.0	5 (9)

Figures in brackets represent degrees of freedom.

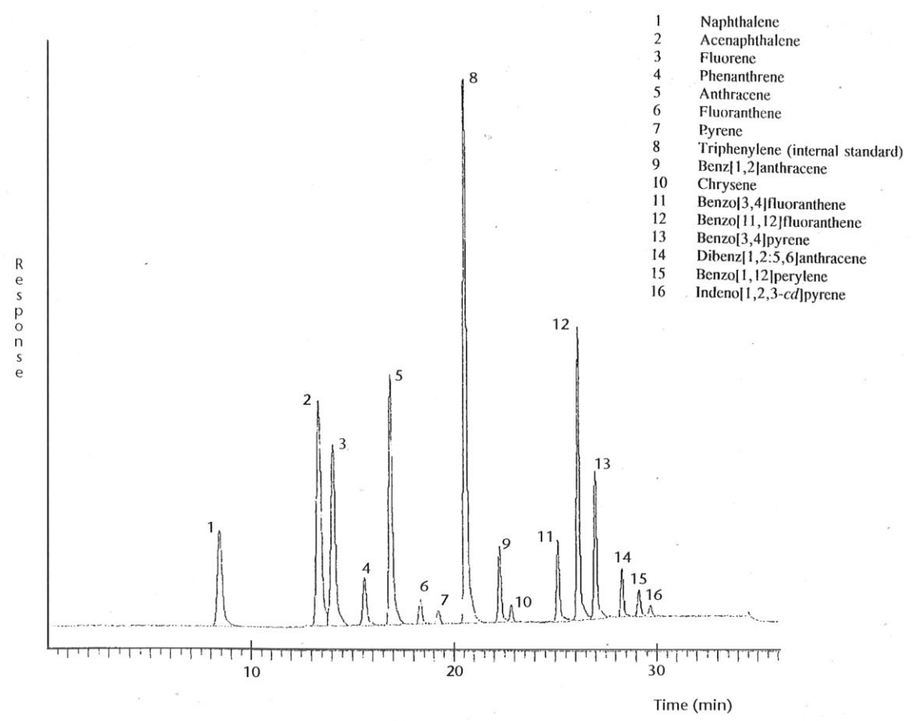
Table B2 Standard deviations, Recoveries and Limits of detection -15 PAH

Compound	Concentration (ngL^{-1})	Standard deviation (ngL^{-1})	Recovery (%)	Limit of detection (ngL^{-1})
Naphthalene	40	4.0	50	9
Acenaphthene	40	3.6	55	8
Fluorene	10	1.2	62	2
Phenanthrene	5	0.4	93	1
Anthracene	5	0.6	65	1
Fluoranthene	10	0.9	105	3
Pyrene	5	0.5	99	2
Benz[1,2]anthracene	5	0.5	91	1
Chrysene	5	0.6	88	2
Benzo[3,4]fluoranthene	10	1.4	98	2
Benzo[11,12]fluoranthene	5	0.9	99	1
Benzo[3,4]pyrene	5	0.7	90	1
Dibenz[1,2:5,6]anthracene	10	1.5	106	4
Benzo[1,12]perylene	10	1.5	102	4
Indeno[1,2,3-*cd*]pyrene	5	1.6	96	7

Table B3 Concentrations of PAH standard solutions

Compound	Commercial PAH solution (B5.7) (μgmL^{-1})	Stock standard (B5.8.3) (μgmL^{-1})	Calibration standard (B5.8.4) ($ngmL^{-1}$)	High check standard (B5.8.5) ($ngmL^{-1}$)
Naphthalene	1000	20	400	1600
Acenaphthene	1000	20	400	1600
Fluorene	200	4	80	320
Phenanthrene	100	2	40	160
Anthracene	100	2	40	160
Fluoranthene	200	4	80	320
Pyrene	100	2	40	160
Benzo[1,2]anthracene	100	2	40	160
Chrysene	100	2	40	160
Benzo[3,4]fluoranthene	200	4	80	320
Benzo[11,12]fluoranthene	100	2	40	160
Benzo[3,4]pyrene	100	2	40	160
Dibenz[1,2:5,6]anthracene	200	4	80	320
Benzo[1,12]perylene	200	4	80	320
Indeno[1,2,3-*cd*]pyrene	100	2	40	160

Figure B1 A typical chromatogram of fifteen PAH



C Determination of fifteen polynuclear aromatic hydrocarbons in waters using liquid-liquid extraction and high performance liquid chromatography

C1 Performance characteristics of the method

C1.1	**Substances determined**	The fifteen PAH shown in Figure 1.
C1.2	**Type of sample**	Potable water and groundwater.
C1.3	**Basis of method**	PAH are extracted from the aqueous sample by solvent extraction using dichloromethane (DCM). The extract is concentrated into acetonitrile. Separation and quantification is achieved using reverse-phase liquid chromatography with fluorescence detection.
C1.4	**Range of application**	Up to 15 ngL^{-1} of each PAH.
C1.5	**Calibration curve**	Linear for each PAH over the range of application.
C1.6	**Standard deviation**	See Table C1.
C1.7	**Bias**	See Table C1.
C1.8	**Limit of detection**	See Table C1.
C1.9	**Time required for analysis**	A batch of 8 samples and associated control solutions (2 blanks and 2 spiked groundwater samples) takes approximately 2 days.

Data provided by Water Research centre.

C2 Principle

PAH are extracted from the sample using dichloromethane and the solvent extract is concentrated to a small volume (1 mL) in acetonitrile. The PAH are separated by reverse-phase HPLC using gradient elution, and quantified by fluorescence detection.

C3 Apparatus

C3.1 **General**

C3.1.1 **2.5 litre, amber coloured clean glass bottles,** with polytetrafluoroethylene (PTFE) lined screw-caps.

C3.1.2 **2 litre separating funnels,** with ground glass stoppers.

C3.1.3 **Syringes** for standard preparation. 500 µL, 1000 µL.

C3.1.4 **Syringe for filtration.** 50 mL luer tipped, glass.

C3.1.5 **250 mL flat bottomed flasks,** with ground glass stoppers.

C3.1.6 **Membrane filters,** cellulose nominal pore size 0.2 µm, 47 mm diameter.

C3.1.7 **Syringe top filters,** 0.2 µm, 10 mm diameter.

C3.1.8 **Shaker.**

C3.1.9 **All glass filtration unit.**

C3.1.10 **Ultrasonic bath.**

C3.1.11 **Solvent concentration apparatus** (for example, Zymark TurboVap, Kuderna-Danish apparatus, rotary evaporator or equivalent).

C3.1.12 **2 mL amber coloured sample vials** with caps and seals.

C3.1.13 **Microlitre syringes** for HPLC. (The HPLC instrument used for this method employed an auto-injector).

C3.2 **Instrumental**

A high performance liquid chromatograph equipped with a variable excitation and emission wavelength fluorescence detector and a suitable reverse phase PAH column (250 x 4.6 mm; 5 µm diameter particles).

The following elution conditions were used.

Column Temperature: 17.0 ± 0.5 °C

Solvent gradient

Time (min)	Acetonitrile (% v/v)	Water (% v/v)	Flow ($mLmin^{-1}$)
0	48	52	1.0
3	48	52	1.0
26	93	7	1.0
29	99	1	1.0
50	99	1	1.0
51	48	52	1.0

End time : 65 min

NOTE: The water (C4.1) and acetonitrile (C4.4) should be continually degassed by purging with helium (C4.6). This purging will also apply to the acetonitrile used for the autosampler system wash.

The performance characteristics shown for this method were obtained using specific auto-injector and fluorescence detector parameters. Since these parameters will be dependent on the instrument, column and exact elution conditions used, a typical chromatogram for the separation of the 15 PAH is shown in Figure C1.

C4 Reagents

All chemicals used to prepare reagent and standard solutions should be of sufficient purity that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemical by analysing procedural blanks with each batch of samples analysed.

Standards should be stored at 4 °C in tightly sealed all-glass containers, wrapped in metal foil.

C4.1 **Water.** The water used for the HPLC mobile phase is glass double distilled grade which has previously been filtered through a 0.2 µm membrane filter.

C4.2 **Dichloromethane.** (DCM). HPLC analysis grade.

C4.3 **Anhydrous sodium sulphate.** Heat in a furnace at 500 °C for at least 2 hours and cool before use.

C4.4 **Acetonitrile.** HPLC analysis grade.

C4.5 **Nitrogen.** Oxygen-free grade.

C4.6 **Helium.**

C4.7 **Stock standard solution of 15 PAH.** (2000 μgmL^{-1} of each PAH). A standard solution is commercially available .

C4.8 **Intermediate standard solution of 15 PAH.** (20 μgmL^{-1} of each PAH). Add 1.00 ± 0.01 mL of stock PAH solution (C4.7) to about 90 mL of acetonitrile (C4.4) contained in a 100 mL grade A calibrated flask. Make up to the mark with acetonitrile, stopper and mix well. This solution is stable for at least one year when stored at 4 °C in the dark.

C4.9 **Working standard solution of 15 PAH.** (1 μgmL^{-1} of each PAH). Add 1.00 ± 0.01 mL of intermediate PAH solution (C4.8) to about 15 mL of acetonitrile (C4.4) contained in a 20 mL grade A calibrated flask. Make up to the mark with acetonitrile, stopper and mix well. This solution is stable for at least six months when stored at 4 °C in the dark.

C4.10 **Calibration standard solutions of 15 PAH.** To a series of 20 mL grade A calibrated flasks add 0.1, 0.2, 0.4, 0.5 and 0.6 mL respectively of working PAH solution (C4.9). Make up to the mark with acetonitrile, stopper and mix well. These solutions contain 10, 15, 20, 25 and 30 $ngmL^{-1}$ of each PAH respectively. These solutions are stable for at least three months when stored at 4 °C in the dark.

NOTE: All of these standard solutions are protected from degradation by light by wrapping the containers in metal foil and storing in the dark at 4 °C when not in use.

C4.11 **Spiking standard solution of 15 PAH.** (0.02 μgmL^{-1}). Add 2.00 ± 0.02 mL of working PAH solution (C4.9) to a 100 mL grade A calibrated flask. Make up to the mark with acetone, stopper and mix well. This solution is stable for at least three months when stored at 4 °C in the dark.

C4.12 **Spiked groundwater.** (10 ngL^{-1}). Add 1.00 ± 0.01 mL of spiking PAH solution (C4.11) to about 1 litre of groundwater contained in a 2 litre calibrated flask. Make up to the mark with groundwater, stopper and mix well. This solution is the control solution used for recovery purposes and is prepared on the day of sample preparation.

C4.13 **Blank water.** An unspiked groundwater is also subjected on the same day to the analytical procedure to act as a blank solution.

C5 Analytical procedure

The procedure is applicable to the determination of "total" PAH in (unfiltered) samples. The additional sample preparation requirements prior to analysis for dissolved and particulate-associated PAH are stated in section D.

Step	Procedure	Notes
	Extraction	
C5.1	Mix the sample in the bottle thoroughly and discard a small amount so that the remaining volume is approximately 2 litres (note a).	(a) This volume approximates to just below the level of the shoulder of the bottle.
C5.2	Add 50 ± 1 mL of DCM (C4.2) to the bottle and replace the cap tightly to prevent leakage at the next step (note b).	(b) Ensure that there is a new PTFE liner in the cap before replacing it on the bottle.
C5.3	Place the bottle on its side on a shaker and shake the bottle contents for 10 ± 2 min (note c).	(c) About 125 revolutions per minute is sufficient.
C5.4	Quantitatively transfer the contents of the bottle to a 2.5 litre separating funnel, rinse the bottle with approximately 5 mL of DCM (C4.2) and transfer to the separating funnel.	
C5.5	Stopper the separating funnel and shake manually for at least 1 minute. Allow the aqueous and DCM layers to separate (note d). Transfer the lower DCM layer into a 250 mL flask.	(d) Separation may take up to 5 minutes. Periodically release the pressure during the shaking.
C5.6	Add 30 ± 1 mL of DCM (C4.2) to the retained sample bottle. Stopper and shake manually for 1 minute and transfer the DCM to the separating funnel.	
C5.7	Repeat steps C5.5 to C5.6 (note e) and add the DCM to the 250 mL flask containing the first transferred DCM volume (note f).	(e) Rinsing with approximately 2 mL of DCM is sufficient. (f) The DCM extract is approximately 87 mL at this stage.
C5.8	*Carefully transfer the aqueous layer into a 2-litre measuring cylinder (note g) and measure the volume, V1 mL.	(g) On those infrequent occasions where the volume of aqueous layer is greater than 2 litres, use a second smaller measuring cylinder, eg 100 mL to measure the additional volume, V2 mL. * Alternatively, the volume can be determined using pre-calibrated bottles marked at the 2 L volume or by weighing bottle plus sample and subtracting the weight of the empty bottle after extraction.
C5.9	Add 20 ± 1 mL of DCM (C4.2) to the separating funnel and shake manually for at least 0.5 min. Add this DCM volume to the 250 mL flask used previously (note h).	(h) Periodically release the pressure during shaking. The total volume of DCM extract is now approximately 107 mL.

C5.10 Add a quantity of anhydrous sodium sulphate (C4.3) (note i) to the volume of the DCM extract and allow it to remain in contact for at least 0.5 hour. Cover the flask with metal foil to protect from light.

(i) The quantity should be sufficient to completely dry the solvent. A dried DCM extract at this stage promotes a smooth evaporation at step C5.13.

C5.11 Repeat steps C5.1 to C5.10 for all samples and control solutions (note j) to be processed as one batch.

(j) See sections C4.12. and C4.13.

Concentration of DCM Extract

Concentration of the DCM extract can be achieved either by a suitable evaporative concentration method or as described here by use of a Zymark TurboVap.

C5.12 Quantitatively transfer the dried DCM extract (C5.10) to a TurboVap tube by rinsing the 250 mL flask with approximately 10 mL of DCM (C4.2) (note k).

(k) Ensure that the sodium sulphate bulk remains in the 250 mL flask.

C5.13 Add 0.50 ± 0.01 mL of acetonitrile (C4.4) to the tube and place the tube into the TurboVap unit (note l) and reduce the volume of extract to 0.5 mL (note m).

(l) Switch on the Turbovap unit at least 30 min before use to reach the 38 ± 2°C operating temperature.

(m) The TurboVap unit should automatically stop when this volume has been reached and simultaneously issue an audible signal to alert the operator.

C5.14 Remove the sample extract from the tube using a 500µL syringe and transfer to a vial (C3.12).

C5.15 Adjust to a final volume (V4) of 1.00 ± 0.01 mL with acetonitrile (C4.4) (note n). Place a septum on the top of the vial and screw the cap on tightly (note o).

(n) This can be achieved by rinsing the TurboVap tube with (1.00 - V3) ± 0.01 mL acetonitrile and adding this volume by syringe to the vial. V3 is the exact measured volume of the sample extract from C5.14.

(o) If the sample extract is not to be analysed immediately it can be stored frozen for at least four weeks.

Instrument performance evaluation and calibration

C5.16 Set up the HPLC equipment for use (notes p and q).

(p) See section C3.2 instrument conditions.

(q) Refer to manufacturer's operating instructions.

C5.17 Equilibrate the HPLC by pumping eluent (mobile phase) at the starting composition through the column, (note r).

(r) Use a flow rate of 1 $mLmin^{-1}$.

C5.18 Inject the 30 $ngmL^{-1}$ calibration standard (C4.10) (note s) either manually or using an autosampler. A suitable volume may be 10 µL. If the PAH concentrations are to be calculated manually, measure the accepted peak heights.

(s) Repeat this injection at least three times to establish and confirm the consistency and acceptability of the retention times, peak shapes and heights of the PAH in the calibration standard.

Analysis of Extract

C5.19 Inject the full set of calibration standards, samples, procedural control and blank solutions in the desired order.

Manual calculation of the PAH concentration of the sample

The procedure described below is given only as an example of a manual calculation.

C5.20 Identify each peak on the chromatogram of the calibration standards.

C5.21 Measure the peak height of each PAH in each calibration standard.

C5.22 Produce a calibration curve for each PAH by plotting the peak height versus the concentration of the PAH in the calibration standard.

C5.23 Use the calibration curves to convert the peak height for the extracts of the samples and procedural control and blank solutions into the concentration of each PAH in the extract (C μgL^{-1}).

C6 Calculation

Calculate the concentration (S ngL^{-1}) of each PAH in the original aqueous sample as follows. See steps C5.8 and C5.15.

$S = C \times [V4/(V1 + V2)]\ ngL^{-1}$

where

V1 + V2 is the sample volume in mL,
V4 is the final extract volume in mL, and
C is the concentration of PAH in the extract in μgL^{-1}.

The concentration of each PAH calculated is then corrected for blank and recovery if applicable. These two values are calculated from the HPLC response of the procedural blank and control solution (C4.12 and C4.13).

C7 Extension of the concentration range of the method

The method exhibits a linear relationship between the HPLC system response and concentration of each PAH up to a concentration of 15 ngL^{-1} for the aqueous sample.

Higher concentrations of PAH in aqueous solutions may be determined by:

(i) using a smaller volume of sample, diluted to 2 litres with glass double distilled water; or

(ii) analysing a dilution of the solvent extract.

In either case, the appropriate correction factor will need to be applied when calculating the analytical result. The limits of detection stated in the method may not apply for the new conditions used to extend the range.

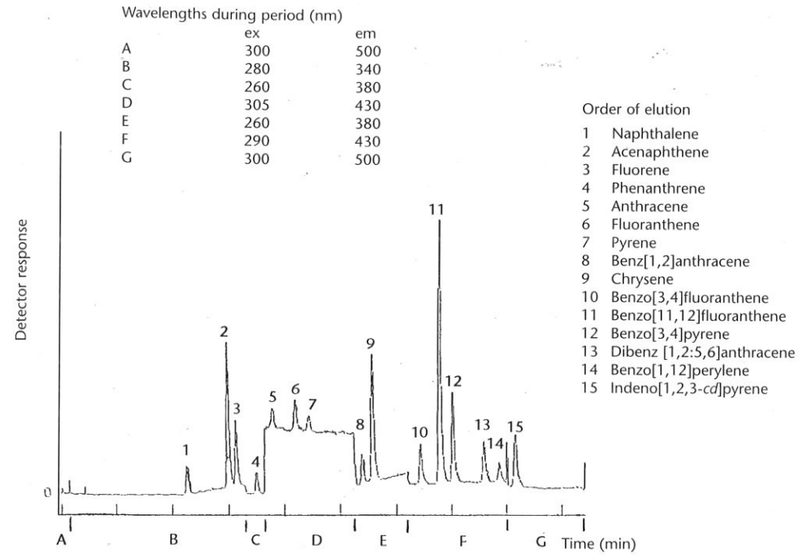
Table C1 Precision of method for replicate injections of standard solutions of PAH (with 7 degrees of freedom; within batch)

	Relative standard deviation (%)			Recovery (%)	LOD (ngL^{-1})
Concentration (μgL^{-1})	**10**	**20**	**30**		
Naphthalene	4	7	4	55	4
Acenaphthene	5	4	3	55	3
Fluorene	7	5	3	57	4
Phenanthrene	8	6	5	67	3
Anthracene	18	8	7	55	4
Fluoranthene	11	8	4	86	2
Pyrene	14	17	12	81	3
Benz[1,2]anthracene	9	6	4	80	1
Chrysene	6	4	4	82	1
Benzo[3,4]fluoranthene	4	3	3	83	1
Benzo[11,12]fluoranthene	3	2	2	85	1
Benzo[3,4]pyrene	6	4	4	72	4
Dibenz[1,2:5,6]anthracene	4	4	3	79	1
Benzo[1,12]perylene	9	7	6	81	1
Indeno[1,2,3-*cd*]pyrene	2	2	2	82	6

Recoveries based on each PAH from spiked groundwater (at a concentration of 10 ngL^{-1} with 6 degrees of freedom).

LOD Limit of detection

Figure C1 Chromatogram of HPLC calibration standard containing 30 μgL^{-1} of each compound



D Determination of dissolved and particulate phase speciation of six PAH in waters using filtration, solvent extraction and high performance liquid chromatography

D1 Sample preparation for dissolved and particulate-associated PAH

Step	Procedure	Notes
	Separation of dissolved (D) PAH from particulate-associated (P) PAH	
D1.1	Shake thoroughly and remove some of the sample from the full bottle so that the remaining volume in the bottle is approximately 2 L, (notes a and b).	(a) See note(a) of step C5.1. (b) If "total" PAH and "D and P" PAH are to be determined, a 4 litre volume of sample will be required.
D1.2	Place a 0.2 µm, 47mm membrane filter in the filtration unit (note c) and filter 200-300 mL of glass double distilled water into the 1 litre receiving vessel. Rinse the receiving vessel with this water which is then discarded.	(c) See section C3.1.9.
D1.3	Shake the capped sample bottle vigorously for at least 1 min and then carefully filter the whole of the sample through the membrane filter (note d).	(d) The filtration is carried out in approximately 250 mL aliquots of sample. Periodically the volume of filtrate in the 1 litre receiving vessel will need to be carefully transferred into a second clean, dry 2.5 litre glass bottle.
D1.4	Retain the total volume of filtrate in the second 2.5 litre bottle for extraction of the dissolved PAH.	
	Extraction, concentration and analysis of dissolved PAH	
D1.5	Add 25 ± 0.5 mL of DCM (C4.2) to the receiving vessel, swirl the vessel and then transfer the volume of DCM to the 2.5 litre bottle.	
D1.6	Add a further 25 ± 0.5 ml of DCM to the bottle, replace the cap tightly to prevent leakages at the next step (note e).	(e) Ensure that there is a new PTFE liner in the cap.
D1.7	Follow steps C5.3 to C6. (note f).	(f) The PAH calculated is "dissolved".

Step	Procedure	Notes
	Extraction of particulate-associated PAH	
D1.8	Dismantle the filtration unit and carefully transfer the filter membrane and the particulates to a 200 mL glass beaker. Rinse the reservoir section of the unit with 50 ± 1 mL of DCM, collecting the rinsings in the same beaker (note g).	(g) If the extraction is not to take place immediately, cover the reservoir completely with metal foil to protect the particulates from dust and light.
D1.9	Place the beaker in a sonicating bath and sonicate the beaker contents for at least 2 minutes.	
D1.10	Remove the membrane filter from the beaker using tweezers. Wash the filter and the tips of the tweezers separately with a collective total of 30 mL of DCM. The washings are collected in the beaker.	
D1.11	Attach a syringe top filter to a 50 mL luer tipped glass syringe with its plunger removed. Securely place a TurboVap tube beneath the syringe.	
D1.12	Quantitatively transfer the DCM extract (D1.8 to D1.10) into the syringe (note h) and replace the syringe plunger. Pass the DCM through the syringe filter at a flow rate of about 5 $mLmin^{-1}$.	(h) The transfer is made in two stages.
D1.13	Rinse the beaker with about 20 mL of DCM (C4.2) into the syringe and pass this volume through the syringe filter at the same flow rate as before.	
D1.14	Add 0.50 ± 0.01 mL of acetonitrile (C4.4) to the tube and place the tube into the TurboVap unit (note i) and reduce the volume according to the instructions at step C5.13.	(i) Switch on the TurboVap unit at least 30 min before use.
	Concentration and analysis of particulate associated PAH	
D1.15	Follow steps C5.14 to C6 (note j).	(j) Note that the PAH calculated is "Particulate-associated".

D2 Accuracy of dissolved and particulate-associated PAH sample preparation

Spiking a quantity of particulate matter with PAH presents many problems. A 10 ngL^{-1} solution containing six PAH was subjected to the D and P procedure and a summary of the analytical results expressed as recovery (%) is given in Table D1.

Table D1 Collective recovery of dissolved and particulate-associated PAH

PAH	Recovery (%)				
	Dissolved# (D)	Particulate# (P)	(D)+(P) = C	Total* T	[C/T].100
Fluoranthene	78	2.4	80.4	86	93.5
Benzo[3,4]fluoranthene	71.4	8.2	79.6	83	96
Benzo[11,12]fluoranthene	72.7	7.5	80.2	85	94.3
Benzo[3,4]pyrene	60.4	8.1	68.5	72	95
Benzo[1,12]perylene	51	10.0	61	81	75.3
Indeno[1,2,3-*cd*]pyrene	55	6.0	61	82	74.3

Degrees of freedom (* n=6, # n=7)

Column 3 shows the total recoveries obtained using the dissolved and particulate-associated method and column 4 shows the recoveries using the "total" solvent extraction method described in method C. The final column shows the percentage of the recoveries obtained by the D and P method compared to the "total" method.

E Determination of six polynuclear aromatic hydrocarbons in waters by on-line trace enrichment and high performance liquid chromatography

E1 Performance characteristics of the method

E1.1	Substances determined	The six PAH marked * in Figure 1.
E1.2	Sample type	Raw and potable waters.
E1.3	Basis of method	PAH are extracted from the sample by trace enrichment onto a short pellicular C_{18} column. The concentrated samples are analysed by reverse phase liquid chromatography with fluorescence detection.
E1.4	Range of application	See Table E1.
E1.5	Limit of detection	See Table E1.
E1.6	Calibration curves	Linear over the range of application.
E1.7	Standard deviation	See Table E2.
E1.8	Bias	The efficiency of the enrichment stage has not been determined. However, no significant difference has been detected between the recoveries from standards and potable waters or clean natural waters. The efficiency of the trace enrichment cartridge will decrease with use and should be renewed when the internal standard height falls significantly compared to the value obtained when the cartridge was new. After replacement of the cartridge the method must be re-calibrated.
E1.9	Time required for analysis	Approximately 20 minutes per sample or standard.

Data provided by National Rivers Authority

E2 Principle

PAH are concentrated by passing a measured volume of aqueous sample through a short (10 mm) column packed with pellicular C_{18} material. The sample contains 17.5% v/v propan-2-ol to minimise adsorption onto glass containers and pump components. The trace enrichment column is switched into the system upstream of the analytical column and PAH are eluted by the mobile phase. After separation, components are detected fluorimetrically. The PAH are quantified by comparison with standard solutions of PAH containing 17.5% propan-2-ol run under the same conditions. The internal standard method of calculation is used.

E3 Reagents

Due to the low concentrations being measured by this method, extreme care must be taken to avoid contamination of apparatus and reagents. All materials should be checked by running procedural blanks.

E3.1 **Water.** Water used for this analysis should be of high quality. Water produced by purification equipment incorporating activated carbon has proved satisfactory. Water should be blank tested as part of the analytical procedure.

E3.2 **Propan-2-ol.** HPLC grade reagent is generally suitable. Each batch should be blank tested before use. The blank obtained should be negligible.

E3.3 **Acetonitrile.** HPLC grade reagent is suitable.

E3.4 **Mobile phase.** Add 300 mL of water to a clean 2 L volumetric flask and make to the mark with acetonitrile. Allow the mixture to return to room temperature and make to the mark with acetonitrile. Filter through a 0.45 µm membrane. Transfer the mobile phase to the container from which it will be used, place under mild vacuum and sonicate for 5 minutes with periodic vacuum adjustment to prevent boiling.

Variations in mobile phase composition will affect retention times therefore the mobile phase should not be changed within a batch of determinations.

E3.5 **Standard solutions.**

E3.5.1 **Preparation from solid PAH.** Stock solutions (100 μgmL^{-1}). Accurately weigh out a sufficient quantity of each PAH and dissolve separately in methanol to produce a 100 μgmL^{-1} concentration.

E3.5.2 **Intermediate standard solutions.** Dilute the individual standard solutions (E.3.5.1) to produce a mixed intermediate standard solution in a suitable solvent of composition:

PAH	Concentration (μgmL^{-1})
Fluoranthene	0.1
Benzo[3,4]fluoranthene	0.1
Benzo[11,12]fluoranthene	0.1
Benzo[3,4]pyrene	0.1
Benzo[1,12]perylene	0.2
Indeno[1,2,3-*cd*]pyrene	0.5

E3.5.3 **Internal standard solution.** Triphenylene (100 mgL^{-1}) Weigh sufficient triphenylene into a volumetric flask. Add methanol and make to volume to produce a 100 mgL^{-1} standard solution.

E3.5.4 **Working standard solution.**

Use the mixed intermediate solution (E3.5.2) to prepare working standard solutions in water 1, 2 and 3 of the following concentrations;

PAH	Concentration (ngL^{-1})		
	1	2	3
Fluoranthene	20	50	100
Benzo[3,4]fluoranthene	20	50	100
Benzo[11,12]fluoranthene	20	50	100
Benzo[3,4]pyrene	20	50	100
Benzo[1,12]perylene	40	100	200
Indeno[1,2,3-*cd*]pyrene	100	250	500

Add 87.5 ± 0.5 mL of propan-2-ol to each of three 500 mL volumetric flasks. Make each flask to the mark with standard solution 1, 2 or 3 above and number appropriately. Add 100 µL of internal standard solution (E3.5.3) to each flask and mix.

E3.5.5 **Blank solution.** Add 87.5 ± 0.5 mL of propan-2-ol to a 500 mL volumetric flask and make to the mark with water.

Add 100 µL of internal standard solution (E3.5.3) to the flask and mix.

E4 Apparatus

E4.1 **Sample bottles.** Bottles are nominally 500 mL amber glass bottles fitted with ground glass stoppers. The volume of each individual bottle should be calibrated by weighing empty and then filled (leaving no head space) with water at 20 ± 1 °C and the volume scored into the glass with a diamond pencil. To each bottle is added an amount of (blank tested) propan-2-ol equivalent to 17.5% of the bottle volume.

Bottles should be labelled "Fill only. Do not rinse or drain. Flammable".

E4.2 **HPLC Equipment.**

E4.2.1 **Analytical pump.** Any good quality pump capable of a pulse free flow rate of at least 4 $mLmin^{-1}$.

E4.2.2 **Sample pump.** This can be of a lower specification and should be capable of operating at a flow rate of 10 $mLmin^{-1}$.

E4.2.3 **Switching valve.** A six-port, three-way, two-position liquid chromatography valve suitable for high pressure operation.

E4.2.4 **Injector.** This is an optional component but it does allow trace enrichment efficiency and the band-broadening effect of the enrichment column to be assessed.

E4.2.5 **Trace enrichment column.** A 10 mm column packed with a pellicular C_{18} packing.

E4.2.6 **Analytical column.** A proprietary column capable of resolving the PAH of interest.

E4.2.7 **Fluorescence detector.** The minimum instrument requirement is a fluorimeter fitted with a 365 nm excitation filter and a wide band pass emission filter centred at 425 nm.

If wavelength programming is available, the following wavelengths can be used.

PAH	Excitation (nm)	Emission (nm)
Fluoranthene	280	452
Benzo[3,4]fluoranthene	290	430
Benzo[11,12]fluoranthene	290	430
Benzo[3,4]pyrene	290	430
Benzo[1,12]perylene	300	410
Indeno[1,2,3-*cd*]pyrene	370	490

E5 Analytical procedure

Step	Procedure	Notes
E5.1	Set up and prime the LC pumps according to the manufacturer's instructions.	
E5.2	Set up the conditions for the fluorimeter according to the manufacturer's instructions.	