The Determination of 6 Specific Polynuclear Aromatic Hydrocarbons in Waters (with notes on the determination of other PAH) 1985

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About this Series

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

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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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

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

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

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sewage Works Control Methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee.

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

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

L R PITTWELL
Secretary

1 July 1986

Warning to Users

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

Local Safety Regulations must be observed.

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

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

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

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

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

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

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

Six Specified Polynuclear Aromatic Hydrocarbons in Water

1 Introduction

Two methods are given.
A using High-Performance liquid chromatography (HPLC)
B using Thin-layer chromatography (TLC)

Method A would be the method of choice given that HPLC apparatus is available. Method B is subject to the large random errors which are typical of visual estimation from TLC plates. However, it is the method specified in the EC Directive relating to the quality of water intended for human consumption (80/778/EEC). It is also inexpensive and can be used for screening purposes when the number of samples to be analysed is so small that HPLC could not be cost justified. It is recommended that TLC results which approach the EC Directive MAC of 200 ng/l (sum of the 6 polynuclear Aromatic Hydrocarbons (PAH) in Table 1) be checked by HPLC.

2 General Information

Some PAH are known to cause cancer in experimental animals (5). They occur in nearly all waters either in solution or absorbed onto particulate material (suspended solids, sediment). The 6 PAH shown in Table 1 may be used as an indicator of a much larger group of PAH occurring in natural waters and sewage effluents (6). Based on this evidence the World Health Organisation set a maximum acceptable level of the sum of the 6 PAH in potable waters of 200 ng/l (7). The standard has also been adopted by the European Community (8, 9). Some samples of waters and effluents may contain PAH distributions for which the 6 PAH in this method are not a good indicator.

Waters uncontaminated with waste water generally contain very low levels of PAH—totals for the 6 PAH usually in the low ng/l range. Very contaminated waste waters may contain totals of several orders of magnitude higher. However, it must be realised that PAH show a strong tendency to absorb on to particulate matter and sediments. Consequently, samples of relatively uncontaminated waters containing appreciable levels of suspended solids, eg rivers in flood, may exhibit enhanced levels due to the absorbed PAH.

3 Hazards Warning and Safety Precautions

Certain PAH are highly carcinogenic and must be handled with **extreme** care. Contact of solid materials, solvent extracts and solutions of standard PAH with the body must not be allowed to occur. PAH may co-distil with solvents and become deposited on the outside of stoppered bottles, containers containing solutions of PAH in solvent must therefore always be handled using gloves which are solvent resistant and preferably disposable. PAH contamination of vessels may be detected by irradiation with 366 nm UV light. Besides being dangerous such deposits indicate a change in solution concentration. Care should be taken during this operation not to irradiate the bulk of the solution. Vessels containing PAH solutions should be stored standing in beakers to contain any spillage in the case of breakage.

Solid PAH are obviously the most dangerous and give rise to a dust hazard due to their crystals becoming electrostatically charged. These materials must only be handled where proper facilities are available (eg adequate fume cupboards, protective clothing, dust masks etc). When pure PAHs are weighed out, it is prudent to do this in only one step ie repeated adjustments of the amount of pure PAHs in order to achieve a specific quantity should not be carried out. This minimises exposure to dangerous PAHs. Any necessary adjustment is performed on a solution of the PAH, for example, as described in the method. It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or are purchased from suppliers specialising in their preparation.*

Solvent solutions containing PAH, used gloves etc, must be disposed of in a manner approved for disposal of toxic waters.

Before commencing PAH analysis in a laboratory it would be wise to discuss the facilities with the appropriate Health Authority. It is suggested that initial familiarisation with the techniques be carried out using a non-carcingenic PAH such as fluoranthene. General techniques for the handling of hazardous materials have been given elsewhere (1, 2, 3, 4), these publications or their equivalent should be studied before handling PAH.

NB As this section covers both methods, these chemicals have to be added:

Methanol and Acetonitrile are both flammable and toxic. Appropriate precautions must be observed when handling them. Cyclohexane, ethanol, toluene, diethylether, n-hexane and acetone are flammable. Spectrofluorimeters may emit ozone generated by Xenon lamps. This gas must be vented (using the hose provided with the instrument).

HPLC procedures involve the use of liquids at high pressure. The apparatus should be shielded in case of leaks. Care must be taken to ensure that the pump is switched off and the pressure has fallen before breaking connections on the high pressure parts.

4 Scope and Field of Application

The methods are applicable to the estimation of the 6 PAH in Table 1. Most potable, underground and relatively non-polluted surface waters may be analysed.

The methods may be applicable to unfiltered samples only. Filtration of samples gives problems due to absorption of PAH onto apparatus and no suitable filtration technique can be recommended at the present time.

A large percentage of the PAH in a sample may be absorbed onto the particuate matter. If samples contain particulate matter then it is important to ensure representive sampling.

---

* Standard solutions of PAH are available from a limited number of suppliers. As an aid to the analyst 2 suppliers are given below. Other suitable sources may be available and this footnote does not indicate any endorsement by SCA.

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

Ferak Berlin,
Nobelstrasse 36–44,
1000 Berlin 44,
West Germany

A

6 Specified Polynuclear Aromatic Hydrocarbons in Water Using High-Performance Liquid Chromatography

A1 Performance Characteristics of the Method (19)*

A1.1	Substances determined	Six Polynuclear Aromatic Hydrocarbons (PAH) are measured, these are: Fluoranthene (FLU) Benzo (ghi) perylene (Bghi Pe) Benzo (k) fluoranthene (BkF) Indeno (1, 2, 3-cd) pyrene (I 123cd P) Benzo (b) fluoranthene (BbF) Benzo (a) pyrene (BaP)
A1.2	Type of sample	Potable, underground, reservoir, lake and river waters.
A1.3	Basis of method	PAH are extracted from the sample by solvent extraction using cyclohexane. The concentrated extract is analysed using reversed-phase high-performance liquid chromatography with fluorimetric detection.
A1.4	Range of application	FLU up to approx 100 ng/l BbF up to approx 20 ng/l BkF up to approx 20 ng/l BaP up to approx 20 ng/l I 123cd P up to approx 20 ng/l B ghi Pe up to approx 20 ng/l
A1.5	Limit of dection	5 degrees of freedom FLU 1.2 ng/l BbF 1.4 ng/l BkF 1.0 ng/l BaP 1.3 ng/l I 123cd P 1.1 ng/l B ghi Pe 1.7 ng/l
A1.6	Calibration curve	Linear over the range of application.
A1.7	Standard deviation	See Table 2.
A1.8	Recovery	See Section A3.
A1.9	Sensitivity	Dependent on instrument. The instrument used for these tests gave a 70% FSD peak for 6.5 ng of fluoranthene injected with a baseline fluctuation of approximately 0.1%.
A1.10	Interference	See section A4.

---

* Performance characteristics were determined by Stevenage Analytical Consultants.

A1.11 Time required

(a) Assuming all apparatus to be prepared and calibrated, time to analyse a sample:

dependent on time taken to separate phases but minimum 70 minutes.

(b) Total operator time

1 sample 4 hours
(4 samples 8 hours)

A2 Principle of Method

The PAHs are extracted from the sample using cyclohexane. Other solvents (pentane, hexane, dichloromethane) have been used successfully by some workers. The solvent extract is concentrated to small volume and separated by reversed phase HPLC using isocratic elution. Gradient elution may be required in some applications. The PAH are detected and quantified by measuring their fluorescence under UV irradiation.

A3 Recovery

The presence of suspended matter in samples may give rise to poor recovery of the PAH.

Usually the efficiences of extraction of the 6 PAH from distilled water range from about 70–90%. This inability to extract all the determinand is not, however, a source of bias if the results obtained are corrected using a predetermined figure for mean recovery, checked in each batch of analyses.

The extraction efficiencies from river waters can be poorer and variable. For a lowland river, the following recoveries of the 6 PAH, *uncorrected for the extraction efficiencies from distilled water*, have been observed.

At the 10 ng/l spiking level:

FLU	67%	BaP	52%
BbF	58%	I 123 cd P	26%
BkF	54%	Bghi Pe	23%

At the 90 ng/l spiking level:

FLU	72%	BaP	68%
BbF	81%	I 123 cd P	58%
BkF	73%	Bghi Pe	61%

Higher recoveries have been reported using similar methodology applied to other river waters. Whenever the method is applied to river waters, or potable waters consequently containing unusually high levels of suspended solids, it is recommended that a spiking recovery test be performed and the recovery data obtained used to correct the analytical results.

A4 Interference

Any material which fluoresces or which quenches fluorescence, and has similar chromatographic properties to the specified PAH will interfere in the method. Other co-extracted materials may modify the properties of the chromatographic stationary phase or solvent. Such interferences will give distorted chromatograms which it will not be possible to interpret accurately if at all.

One aspect which appears impossible to evaluate experimentally is the exact magnitude of the interference due to other PAH's. The evidence suggests that when the 6 measured PAH's occur in natural waters, such as river water, they are invariably present as part of a complex mixture with other PAH's (10). Analytically, it is not possible to completely resolve such a mixture and therefore there may be some interference from the unspecified PAH's. In particular pyrene may interfere with fluoranthene and perylene with benzo(b) fluoranthene, benzo(k) fluoranthene or benzo(a) pyrene.

The presence of some types of interfering materials can be detected by standard additions of PAH to the solvent extracts of samples. It is recommended that such a procedure be used as a periodic check.

In practice, no significant general interferences have been encountered in the analysis of potable waters and rivers and reservoirs. In very specific circumstances interference may occur. For example, Optiblanc RG has interfered with the estimation of benzo(b) fluoranthene. The compound originated from an industrial effluent.

A5 Reagents

Due to the extremely low concentration being measured using this method extreme care must be taken to avoid contamination of apparatus and reagents. All materials must be checked by the running of procedural blanks. All reagents are analytical reagent grade except where stated.

A5.1 Cyclohexane—the purity of each batch must be checked by concentrating 200 ml and examining the concentrate for the presence of PAH's by the HPLC procedure (Steps A8.9 to A8.12). No PAH's will be detected in suitable batches of solvent.

Most batches of solvent will require purification. General purpose reagent grade has been found suitable after purification by one of the following procedures:

A5.1.1 Distillation—shake the cyclohexane with concentrated sulphuric acid, wash with water (PAH free), dry over anhydrous sodium sulphate and distil.

A5.1.2 Column chromatography—A 25 × 500 mm glass column with a PTFE stopcock (or glass used without grease) is prepared by first adding 50 g basic aluminium oxide, activity grade I (see A5.9), and placing 20 g dry silica gel (dried by heating in an oven at 250°C for 30 mins on top). Both absorbants are added dry. Cyclohexane is slowly added to the top of the column from a dropping funnel and approximately the first 50 ml of cyclohexane passing through the column are reintroduced to the top of the column. If the cyclohexane is of good quality, 2.5 l can be cleaned with the column. If a yellow area develops in the column, the flow should be stopped. The absorbent cannot be reused.

A5.2 Methanol—suitable for HPLC and with a sufficiently low blank.

A5.3 Acetonitrile—as A5.2.

A5.4 Ethanol—as A5.2.

A5.5 Acetone.

A5.6 Water

A5.6.1 Procedural Blanks

Distilled or de-ionised water (this is generally suitable as prepared in the laboratory, but should be checked by running procedural blanks).

A5.6.2 HPLC Mobile Phase

Ultra-pure water is required, double distilled membrane filtered (0.45 μm) water is usually suitable. Commercial systems incorporating ion-exchange, activated carbon filtration and 0.45 μm membrane filtration have also given good results.

A5.7 Sodium Sulphate anhydrous.

A5.8 Sodium Thiosulphate solution approximately 10%—dissolve 160 ± 10 g of sodium thiosulphate pentahydrate ($Na_2S_25H_2O$) in 1l of water.

A5.9 Aluminium Oxide—basic for column chromatography, particle size 50 to 200 μm, Brockmann Activity I.

The Brockmann Activity of the Aluminium Oxide should be checked (11). If activity is I it is suitable for solvent purification (A5.1.2). If not prepare Brockmann Activity I as described below:

Heat Aluminium Oxide* to 500°C. Finish the cooling in a desiccator using magnesium perchlorate or an equivalent desiccant. This gives Aluminium Oxide of Brockmann Activity I. It may be stored in the desiccator for up to 5 days.

*The low temperture or gamma isomer, not the high temperature alpha isomer.

A5.10 HPLC Mobile Phase

The solvent mixture used as the HPLC Mobile phase will vary accordingly to which stationary phase is used, and sometimes which batch of stationary phase, it should be chosen to give optimum separation of the 6 PAH of interest (see section A9). The solvent mixture should be membrane filtered (0.2 to 0.5 μm), to provide maximum protection for the pump and column and, if necessary, degassed.

A5.11 Standard solutions

CAUTION: PAH are carcinogenic and must be handled with care—see Section 3. Preparation of PAH standards from the solid materials should not be undertaken unless the correct safety equipment is available and the proper handling techniques are used.

Standard solutions should be stored in a spark-proof refrigerator in order to minimise PAH decomposition and solvent evaporation. Standard solutions should be stable for at least 6 months when stored under these conditions.

A5.11.1 Preparation from solid PAH

Stock Solutions (100 to 150 μg/ml)—Accurately weigh out 10 to 15 mg of each PAH, dissolve separately in 50 to 70 mls of cyclohexane and make each up to 100 ± 0.1 ml using cyclohexane. The stability and concentration of these solutions may be checked by Ultra-violet spectrophotometry.

Note. The above procedure is adopted to eliminate the hazard of multiple weighing. Dissolution of solid PAH in cyclohexane can be difficult and may take several days, the solvent may be warmed to speed the process or the PAH dissolved in a small quantity of a more polar solvent and then made up to the mark using cyclohexane.

Standard Solution

To a 100 ml graduated flask add a volume of each stock solution equivalent to 200 μg each PAH (1.3 to 2.0 ml ± 0.02 ml). Make up to 100 ± 0.1 ml using methanol, acetonitrile or HPLC solvent as appropriate to the HPLC procedure. The composition of this solution is as below.

PAH	ng/μl
Fluoranthene	2
Benzo (ghi) perylene	2
Benzo (k) fluoranthene	2
Indeno (1, 2, 3-cd) pyrene	2
Benzo (b) fluoranthene	2
Benzo (a) pyrene	2

A5.11.2 Commercially prepared standards

The stock solutions and the standard solutions in benzene or cyclohexane are available commercially. Whilst normally reliable, problems have been encountered due to poor storage etc, and in some cases cross checking with a standard of known reliability is advisable.

6 Apparatus

The following washing procedure has generally been found to give contamination-free apparatus when starting with new glassware or glassware which has contained low levels of PAH: Wash using detergent and rinse thoroughly in tapwater. Rinse using deionised water followed by acetone and cyclohexane.

Glassware which has been used for solutions containing high levels of PAH will not be completely cleaned by the above procedure and should be discarded for low-level PAH analysis. It may be reserved for high level PAH analysis only. Cleaned glassware should be protected from contamination (especially atmospheric dust in city and urban areas).

Plastics, except PTFE, should be avoided as they may give problems with contamination of solutions or absorption of PAH from solutions.

A6.1 **Sample bottles**—Glass bottles with glass stoppers nominal capacity 2 l calibrate before use at 1.8 l.

A6.2 **Micro-separator**—constructed in glass as Figure 1 (12) to fit sample bottles or

Separating funnel—50 ml capacity, with PTFE tap (or glass tap used without grease) and glass stopper.

A6.3 **Vacuum rotary evaporator**—with suitable water bath.

A6.4 **Concentration flask**—constructed in glass, as Figure 2 to fit the rotary evaporator.

A6.5 **Nitrogen Supply**—cleaned by passage through molecular sieve and silica gel, line terminating a fine jet of glass or metal and controllable such that the glas flow just indents the meniscus of the solvent being evaporated; typically 200 ml/min from a jet of internal diameter 0.5 um at a distance of 20 mm from the liquid).

A6.6 HPLC Apparatus

Injector system and high pressure pump suitable for use with the analytical columns and conditions required (See Section A6.8 and A9). Fluorimetric detector system suitable for use at excitation wavelengths of 300 nm and emission wavelengths of 460 nm. The wavelengths given are a compromise and the subject is discussed further in Section A10. Monochromator and filter fluorimeters have proved suitable for the analysis.

A6.7 HPLC Column and column operation

A reversed-phase system is used. This consists of a column packed with a silica stationary phase of particle size 5 to 10 μm (chemically modified by octadecylsilane) and an eluting solvent of methanol + water, acetonitrile + water or ethanol + acetonitrile + water. All 6 PAH should be resolved using isocratic elution. In addition, separation of perylene and pyrene (see section A4) from the 6 is a good test of resolution. However, nominally similar packing materials vary in their separating power not only between manufacturers, but also between batches. Solvent composition should therefore be varied to acheive optimum separation and for some applications it may be necessary to use gradient elution (see section A9). The following columns and conditions have proved suitable for the analysis but new columns should be checked. Suitable equivalents are available.

A6.7.1 Isocratic elution:

1. Column 450 mm long by 4 mm internal diameter LiChrosorb RP 18 (5 μm). Eluting solvent 9 parts Acetonitrile + 1 part water, flow rate 1 ml/min. (See figs 3 and 4).

2. Column 250 mm long by 2.6 mm internal diameter Zorbax 5 μm ODS. Eluting solvent 82 parts Acetonitrile + 18 parts water, flow rate 1.0 ml/min.

3. Column 250 mm long by 3.2 mm internal diameter packed with Vydac TP 5 μm. Eluting solvent 35 parts Acetonitrile + 30 parts Ethanol + 35 parts water, flow rate 1.4 ml/min.

A6.7.2 Gradient elution:

Various systems have been applied that use the above solvent system. Figure 5 was produced using: acetonitrile/water gradient: 75% acetonitrile—100% acetonitrile in 35 mins; 25 cm × 4.6 mm 6 ODS Zorbax.

A6.8 Microlitre syringes for HPLC 10 μl and 100 μl capacity.

A6.9 Microlitre syringes for Aqueous Standard Preparation 10 μl, 100 μl and 500 μl capacity.

A7 Sample Collection and Preservation

Sample bottles should be completely filled at the sampling site and the stoppers inserted, leaving no air space.

In the case of surface water samples the use of sampling devices through which the sample flows before entering the sample bottle should be avoided as these may give contamination or PAH absorption problems. It may be necessary to avoid including the surface layer in the sample as this may contain higher levels of PAH than the main body of the water. This is particularly so if an oil film is present. Owing to the strong absorption of PAH onto particulate matter, precuations must be taken to ensure the representative sampling of turbid samples.

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 potable waters, PAH samples must be taken before the tap is 'flamed' to sterilise for bacteriological analysis. Residual chlorine must be removed using sodium thiosulphate, add 0.5 ml of a 10% solution of sodium thiosulphate to the sample and mix. This quantity is sufficient to remove levels of chlorine found in normal waterworks practice.

The bottle tops should be wrapped in aluminium foil to protect from contamination during transport and storage, and sources of such contamination (eg dust, motor vehicle exhaust fumes, cigarette smoke) avoided. Samples should be stored in the dark and extracted as soon as possible after collection (within 24 hours).

A8 Procedure

Caution: Some PAH are carcinogenic Read Section 3—Hazards Warning and Safety Precautions. Organic solvents used in this procedure are flammable and in some cases toxic. HPLC procedures involve the use of liquids at high pressure, leaks can be dangerous. Ensure adequate venting and shielding.

Note: PAH are degraded by light and all stages of procedure should be protected from strong sunlight and sources of UV irradiation.

Step	Procedure	Notes
	Extraction	
A8.1	Empty water from the bottle until the level corresponds to the 1.8 l mark.	
A8.2	Weigh the bottle plus sample to ± 1 g Note the weight W_{1g}.	
A8.3	To the sample add 40 ml cyclohexane. Shake vigorously by hand for 5 mins ensuring good mixing and solvent dispersion. (Note a).	(a) 40 ml is the minimum volume known to be suitable. Larger volumes (eg 100 ml) may be convenient in some cases, eg when emulsions form. Mechanical shaking machines may also be used; recoveries should be checked.

Step	Procedure	Notes
A8.4	Stand for at least 5 minutes to allow the layers to separate. (Notes b, c).	(b) 5 minutes is adequate for most potable waters. Other waters may need several hours standing.
A8.4	**Either:** Replace the stopper with the micro-separator. Through the lateral tube A (Figure 1) add sufficient water to bring the cyclohexane layer into tube B. Transfer the extract to a conical flask using a pipette. Rinse the pipette with a small volume of cyclohexane and add the washings to the flask, **Or:** Siphon off most of the water from beneath the cyclohexane layer using a clean glass tube and then transfer the solvent plus the remaining water to a separating funnel. Rinse the sample bottle with 20 ± 2 ml cyclohexane and add the rinsing to the separating funnel. Discard the lower aqueous layer and run the cyclohexane layer into a conical flask. Rinse the separating funnel with 20 ± 5 ml cyclohexane and add the rinsings to the flask.	(c) Emulsions may form which will not separate on standing. If only a small proportion of the solvent is contained in the emulsion then an aliquot of the solvent may be used for the analysis and the final calculation adjusted accordingly. The procedure may also be used when only a small amount of solvent separates if PAH levels are sufficiently high. Alternatively the emulsion may be centrifuged to achieve separation. **Caution:** The centrifuge must be spark-proof due to the volatility and inflammability of the solvent.
A8.6	Weigh the empty sample bottle to ± 1 g. Note the weight W_2 g.	
	Concentration of Solvent Extract	(d) Some workers omit this stage but great care must be taken to avoid the carry-over of water droplets to the next stage of analysis.
A8.7	Dry the solvent for at least 30 mins using sodium sulphate (Note d).	
A8.8	Filter the dry extract through a small cotton wool plug into the concentration flask (Fig 2). Rinse the sodium sulphate and conical flask with 10 ± 0.5 ml cyclohexane and add to the concentration flask via the filter (Note e).	(e) If a concentration flask is not available other flasks may be used to achieve concentration to about 5 ml. The concentrated extract is then transferred to a 10 ml pointed bottom flask or centrifuge tube (calibrated) and progressed to step A8.9 or A8.10.
A8.9	Concentrate to 2 ml (± 0.5 ml) using the rotary evaporator. The water bath heating the flask should be at a maximum temperature of 40°C and the vacuum adjusted to achieve concentration in 5 to 15 minutes without bumping (Note f). Wash the walls of the flask with 1 ml (± 0.2 ml) of cyclohexane using a pipette.	(f) Other apparatus for the removal of solvent may be used if a rotary evaporator is not available. However, the extract should not be heated above 40°C to avoid losses. Recoveries of PAH should be checked.
A8.10	Using the nitrogen supply, with the flask placed in a water bath at ambient temperature, further concentrate just to dryness in a fume cupboard. Take up the residue in 200 ± 5 μl of methanol, acetonitrile or HPLC solvent (notes g, h, j).	(g) The nitrogen supply should be adjusted such that the surface of the solvent is just indented and no splashing occurs. Higher nitrogen flow rates may result in loss of PAH by evaporation or splashing. The nitrogen supply must be removed immediately the last trace of solvent has evaporated, otherwise the more volatile PAH may be lost. (h) With the 200 μl of solvent it is sufficient to rinse the 'lower walls' above the 'collection tube'. The

Step	Procedure	Notes
		optimum volume and composition of redissolution solvent will vary according to the HPLC apparatus (see Section A9) and the concentration of PAH in the sample.
		(j) Manipulation of small volumes of solvent may be conveniently achieved using glass syringes or Pasteur pipettes. Teats for the latter must be checked for interfering materials.
	HPLC Analysis	
A8.11	Set up the HPLC apparatus to give the desired separation and allow to equilibrate, until a stable baseline is achieved (note k).	(k) See Sections A9 and A10 for HPLC and fluorimetric detector conditions.
A8.12	Inject 50 µl of concentrated solvent extract (from step A8.10) and run the sample under the appropriate chromatographic conditions (note l).	(l) The volume injected may be reduced for samples containing high levels of PAH in order to stay within the linear range of the instrument. The calculation of PAH concentrations must be suitably modified.
	Blank Test	
	Pass an appropriate quantity of blank water through steps A8.2 to A8.12. The blank should not contain detectable PAH (note m).	(m) This procedure assumes that the blank water contains undetectable PAH levels. Should it be suspected that detectable PAH are present then the blank water should be further purified as for HPLC water. A modification of this procedure using purified water can be used to confirm that apparatus is PAH free as in note j.
	Calibration	
A8.13	Inject in turn 1, 2, 5, 8 and 10 µl of the standard solution (A5.11) into the HPLC apparatus.	
A8.14	Measure the height of the response for each PAH and plot peak height (in mm) against quantity of each PAH injected (in ng). The graph should be linear over the range given (note n).	(n) Peak areas may be measured instead of peak heights. The nature and quantity of solvent injected may affect the response given by some HPLC systems (see Section A9).
	Calculation	
A8.15	Using the chromatogram obtained in step A8.12, identify the PAH by comparison of the retention times of recorded peaks with those of the standard mixture (note o).	(o) Chromatographic retention times are not absolute proof of the identity of a peak but such identification is adequate for this method.
A8.16	Measure the height of each PAH peak (in mm) and read off the quantity of each PAH present ($W_p$ng) from the calibration graph.	

Step	Procedure	Notes
A8.17	The quantity (ng/l) of each PAH in the sample is then calculated as: $C = \frac{\text{total volume of extract (in l)}}{\text{volume of extract injected (in l)}} \times \frac{1000}{\text{weight of sample}}$ $= \frac{200}{50} \times \frac{1000}{W_1 - W_2} \times W_p$ where: C = Concentration of PAH in the sample in ng/l [NB in the calculation 1000 g = 1000 ml]. W_1 = wt of sample bottle + sample (g) W_2 = wt of sample bottle (g) W_p = Mass of PAH in the sample peak (in ng).	

A9 High-Performance Liquid Chromatography

The requirements for high performance liquid chromatography instruments have been reviewed (13). For the purposes of PAH analysis optimum performance is required to give good separation of a closely related group of compounds. Thus bandspreading must be minimised during injection and chromatography. It is difficult to prescribe exactly the column performance required but a good target would be at least rear baseline separation of the 6 PAHs with separation of pyrene and perylene from the 6. However it may well be that less resolution provides acceptable results for many purposes.

A large number of column-solvent combinations have been used for the separation of PAH (14, 15, 16). The most convenient, commercially available systems involve the use of reversed-phase HPLC with a hydrocarbon-bonded silica packing and a mixed solvent of methanol or acetonitrile and water. Commonly these bonded packings are produced by a chemical reaction between 5 or 10 μm particle size silica and octadecylchlorosilane. Although these materials are chemically similar, their separating properties vary when prepared by different manufacturers, even from batch to batch. This may significantly alter the degree of resolution of the 6 PAH of interest in this method (16). Gradient elution can usually give a separation of all 6 compounds with the majority of the materials but few have been reported as giving complete separation under isocratic conditions. Subsequent work suggests that no untested batch of packing material can be relied upon to give separation of all 6 under isocratic conditions.

Isocratic mobile phases have usually consisted of mixtures of methanol or acetonitrile and water in the ratio of 70 to 90 parts solvent plus 30 to 10 parts water. Ternary mixtures of ethanol, acetonitrile and water have also proved useful. Isocratic elution is faster and more reproducible than gradient elution. Gradient elutions have normally been performed with water and one of the above organic solvents. In general acetonitrile is preferred as the organic solvent due to its lower viscosity and therefore lower pumping pressures to achieve a given flow rate. Figure 5 shows separation of the 6 PAHs using gradient elution.

The column temperature has normally been 20 to 30°C, with ambient most frequently used.

The solvent in which the sample is injected into the HPLC apparatus and its quantity, can induce bandspreading. Ideally it will be the same as the eluting solvent, but in practice up to 100 μl injections of solutions in the organic phase of the eluting solvent mixture or a water miscible solvent of lower polarity than the eluting solvent have proved satisfactory. It has also been reported that up to 300 μl may be injected if the sample is dissolved in the same mixture of solvents as used for the HPLC separation, but with a lower percentage of organic solvent (eg 50:50).

A10 Fluorimetric Detection

The fluorimetric detector system must be connected to the HPLC column outlet and have a sufficiently small volume cell such that bandspreading is minimal. Both monochromator and filter instruments have been used successfully for this determination. For ease of working detector excitation and emision wavelengths are kept constant throughout the analysis and are thus a compromise. Many combinations have been used, but for monochromator instruments excitation at 300 nm and monitoring of emission at 460 nm has been found to be suitable. These conditions minimise interference from some other commonly occurring PAH such as perylene.

For optimisation of response to any given PAH more specific spectrophotofluorimeter conditions may be chosen, for guidance the following excitation and emission wavelengths are suitable:

Compound	Excitation Wavelength (nm)	Emission Wavelength (nm)
Fluoranthene	365	462
Benzo (b) fluoranthene	302	452
Benzo (k) fluoranthene	302	431
Benzo (a) pyrene	297	405 or 430
Benzo (ghi) perylene	302	419 or 407
Indeno (1, 2, 3-cd) pyrene	300	500

The performance characteristics quoted in this method were obtained using an excitation wavelength of 360 nm and an emission wavelength of 460 nm for FLU, BbF, BkF and BaP, and 300 nm excitation and 465 nm emission for I 123 cd P and B ghi Pe.

For filter instruments, the fixed conditions for monochromator instruments should be matched as closely as available filters allow.

Satisfactory results have been obtained using an excitation filter of 300 nm and an emission filter transmitting at wavelengths larger than 370 nm. The choice of lamp can be critical with filter instruments, as an unsuitable lamp will give low energy at the required wavelengths and therefore poor sensitivity.

A11 Checking the Accuracy of the Analytical Results

Once the method has been put into routine operation, many factors may subsequently affect the accuracy of the analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of test are possible and they should be used as appropriate (19).

The following minimum procedure is recommended. In each batch of analyses, a distilled water standard solution containing concentrations of each PAH near the upper end of the range, should be taken through the entire procedure. The results should be plotted on a control chart, which will facilitate assessment of the extraction efficiency from distilled water. Provided results remain in control, they may be pooled periodically with the predetermined distilled water recoveries data to provide a better correction factor.

A12 Concentration range of the method

When concentrations of PAH higher than the quoted range of application are encountered it is recommended that large aliquots of the samples be diluted quantitatively to bring the diluted sample into the correct range. Results must be modified appropriately by the dilution factor.

A13 Other PAH

The method can in principle be extended to other PAH. Some information on the separation of other PAH can be gained from figures 6, 7 and 8. Further information is available in references 17 and 18.

6 Specified Polynuclear Aromatic Hydrocarbons in Water Using Thin-Layer Chromatography

B1 Performance Characteristics of the Method (19)*

B1.1	Substances determined	6 Polynuclear Aromatic Hydrocarbons (PAH) are measured, these are: Fluoranthene (FLU) Benzo (ghi) perylene (B ghi Pe) Benzo (k) fluoranthene (BkF) Indeno (1, 2, 3-cd) pyrene (I 123cd P) Benzo (b) fluoranthene (BbF) Benzo (a) pyrene (BaP)
B1.2	Type of sample	Potable, underground, reservoir, lake and river waters.
B1.3	Basis of method	Extraction of sample (1.8 litres) with cyclohexane, concentration of the extract and analysis using 2-dimensional thin layer chromatography. The PAH are estimated from the intensity of their fluorescence under ultraviolet illumination.
B1.4	Range of application	Up to 90 ng/l of each PAH.
B1.5	Limit of detection	5 ng of each PAH, being the smallest amount present on the standard plates. This corresponds to 2 ng/l with a 1.8 litre sample.
B1.6	Calibration curve	By visual comparison of 6 standard TLC plates containing 5, 10, 20, 40, 80 and 160 ng of each PAH.
B1.7	Standard deviation	See Section B9, and Appendix C.
B1.8	Recovery	See Section B3, and Appendix B.
B1.9	Interference	See Section B4.
B1.10	Typical Operator time required	4 hours for a single analysis 7 hours for 4 analyses 10 hours for 8 analyses

* Performance Characteristics were obtained by Stevenage Analytical Consultants.

B2 Principle of Method

PAH are extracted from the sample (1.8 l) using cyclohexane.

The solvent extract is concentrated to a small volume. Most of the extract is then applied to one corner of a thin layer chromatography plate. The PAH are separated by development in 2 dimensions. The PAH are visualised by irradiation with 366 nm wavelength ultra-violet light. The spots caused by the 6 specified PAH may be identified by their position on the plate and their characteristic colours. Comparison with a standard chromatogram run under identical conditions to the sample is essential for adequate identification. A typical standard chromatogram is illustrated in Figure 9 and an example of a polluted river water chromatogram in Figure 10.

Quantitative analysis is made by visual comparison of the sample chromatogram with chromatograms of standard solutions containing various quantities of the 6 PAH. Although the precision of this method is relatively poor, the results are adequate for some applications. One tenth of the extract is reserved for further analysis if the concentration of PAH is above the ranges of application of the method.

B3 Recovery

The solvent extraction stage is not quantitative. Recoveries from distilled water and a river water are shown in table 5. Recoveries may be lower from waters of high turbidity. Further losses can occur during other stages of the procedure.

B4 Interferences

Any extracted material which fluoresces, or quenches fluorescene, and has similar chromatographic properties to the specified PAH will interfere in the method. Other co-extracted materials may modify the properties of the chromatographic stationary phase or solvent, or impede the movement of the solvent up the TLC plate. Such interferences will give distorted chromatograms which cannot be interpreted accurately if at all. As a guide to the analyst, concentrated extracts which are colourless to deep yellow in colour may generally be analysed. Extracts which are more intensely coloured (eg brown or black) may show unacceptable interference.

One aspect which appears impossible to evaluate experimentally is the exact magnitude of the interference due to other PAHs. The evidence suggests that when the 6 measured PAHs occur in natural waters, such as river water, they are invariably present as part of a complex mixture with other PAHs. Analytically, it is not possible to completely resolve such a mixture and therefore there may be some interference from the unspecified PAHs. In practice this effect does not appear to be important (10).

Spiking water samples with the 6, specified PAHs in order to estimate the magnitude of any interference is unrealistic in that with real samples such increases of the six PAHs would almost certainly be accompanied by proportionate increases in the interfering PAHs.

The exact nature of the potential interference is unknown so that addition of ‘synthesised’ interference cannot be evaluated.

B5 Reagents

Due to the extremely low concentration being measured using this method extreme care must be taken to avoid contamination of apparatus and reagents. All materials must be checked by the running of procedural blanks. All reagents are analytical reagent grade except where stated.

B5.1 Cyclohexane—Suitable grades are commercially available. The purity of each batch must be checked by concentrating 100 ml and examining the concentrate for the presence of PAHs by the TLC procedures (Steps B8.7 to B8.14). No PAHs will be detected in suitable batches of solvent.

If a suitable solvent grade is not available then general purpose reagent grade has been found suitable after purification as below. The procedure may also be used to purify poor batches of other solvent grades—

Shake the cyclohexane with concentrated sulphuric acid, wash with water (PAH-free), dry over anhydrous sodium sulphate and distill.

B5.2 Acetone

B5.3 Toluene—sulphur-free, free from fluorescent materials

B5.4 n-Hexane

B5.5 Methanol

B5.6 Diethyl Ether—peroxide free. (See method for Chlorophenoxy acid herbicides, Tricholorobenzoic acid, Chlorophenols, Triazines and Glyphosate in Water, 1985 in this series for details of testing for, and removing, peroxides).

B5.7 Ethanol

B5.8 Water—distilled or de-ionised (This is generally suitable as prepared in the laboratory, but should be checked by running procedural blanks).

B5.9 Sodium sulphate anhydrous

B5.10 Sodium Thiosulphate solution approximately 10%—dissolve 160±10 g of sodium thiosulphate pentahydrate ($Na_2S_2O_3\ 5H_2O$) in 1 l of water. Before use extract with 100 ml cyclohexane to remove any PAH contamination.

B5.11 Aluminium Oxide G—for thin layer chromatography (only required if not using pre-coated TLC plates).

B5.12 Cellulose 40% acetylated—for thin layer chromatography (only required if not using pre-coated TLC plates).

B5.13 TLC Solvent System 1—a mixture of 9 volumes of n-hexane and 1 volume of toluene, dry using self-indicating silica gel.

B5.14 TLC Solvent System 2—a mixture of 4 volumes of methanol, 4 volumes of diethyl ether and 1 volume of water.

Caution: Heat is generated on mixing and a build-up of pressure can occur. Allow the mixture to cool before stoppering the storage bottle.

B5.15 Standard solutions

Caution: PAH are carcinogenic and must be handled with care—see Hazards Warning and Safety Precautions (Section 3). Preparation of PAH standards from the solid materials should NOT be undertaken unless the correct safety equipment is available and the proper handling techniques are used—see Warning and Safety Precautions.

Standard solutions should be stored in a spark-proof refrigerator in order to minimise PAH decomposition and solvent evaporation. Standard solutions should be stable for at least 6 months when stored under these conditions.

B5.16 Preparation from solid PAH

Stock Solution

Weigh out accurately 10–15 mg of each PAH and dissolve each in 100 ml cyclohexane in a volumetric flask to give 6 stock solutions of known but differing concentrations. This procedure is to avoid the hazard due to multiple weighings. The concentrations will normally be 100–150 μg/ml. Benzo(ghi)perylene dissolves slower than the other PAH.

Standard Solution

Pipette aliquots of each solution (equivalent to 800 μg of each PAH) using a 10 ml pipette, into a 100 ml volumetric flask and make up to 100 ml with cyclohexane. Normally between 5 and 8 ml are required depending on the concentration of the stock solution. This stock mixture contains 8.0 ng of each PAH/μl.

Spotting Solution A

Prepare spotting solution A containing 1 ng each PAH/μl by pipetting 1.25 ml (±0.02 ml) of stock mixture (using a 2 ml calibrated pipette) into a 10 ml volumetric flask and make up to 10 ml with cyclohexane.

Spotting Solution B

Prepare spotting solution B containing 8.0 ng each PAH/μl by transferring 10 ml of stock mixture to a 10 ml volumetric flask. (This is necessary to facilitate spotting). Standard solutions must be stored in the dark in a sparkproof refrigerator or freezer and are stable for at least one year under these conditions.

B5.17 PAH Spiking Solution

Prepare the spiking solution by pipetting an aliquot of each stock solution equivalent to 100 μg of each PAH (between 0.5 and 1 ml) into a 100 ml volumetric flask using a 2 ml graduated pipette and make up to 100 ml with acetone.

B5.18 Commercially prepared standard solutions

Stock solutions and TLC standard solutions are available commercially. Whilst normally reliable, problems have been encountered due to poor storage etc and in some cases cross checking with a standard of known reliability is advisable. They will not always correspond with the standards given in this procedure.

B6 Apparatus

The following washing procedure has generally been found to give contamination-free apparatus when starting with new glassware or glassware which has contained low levels of PAH;—Wash using detergent and rinse thoroughly in tapwater. Rinse using de-ionised water followed by acetone and cyclohexane. Dry inside before use.

Glassware which has been used for solutions containing high levels of PAH will not be completely cleaned by the above procedure and should be discarded for low-level PAH analysis. It may be reserved for high level PAH analysis only.

Plastics, except PTFE, should be avoided as they may give problems with contamination of solutions or adsorption of PAH from solutions.

B6.1 Samples bottles—glass with glass stopper nominal capacity 2 l calibrate before use at 1.8 l.

B6.2 Micro-separator—constructed in glass as Figure 1 (12) to fit sample bottles or

Separating funnel—500 ml capacity, with PTFE tap (or glass tap used without grease) and glass stopper.

B6.3 Vacuum rotary evaporator—with suitable water bath.

B6.4 Nitrogen Supply—cleaned by passage through molecular sieve and silica gel, line terminating in fine jet of glass or metal and controllable such that the gas flow just indents the meniscus of the solvent being evaporated (typically 200 ml/min from a jet of internal diameter 0.5 mm at a distance of 20 mm from the liquid).

B6.5 Centrifuge tubes—15 ml nominal capacity, ground-glass stoppers, 2 required per sample with at least one graduated to 10 ml × 0.1 ml.

B6.6 Chromatography tanks for single 200 × 200 mm plates, lined inside with Whatman No 4 paper and wrapped on the outside with black paper.

B6.7 Desiccator—suitable for storage of prepared TLC plates.

B6.8 Micro-capillary pipettes—5, 10 and 50 μl–50 μl long enough to reach bottom of centrifuge tube.

B6.9 Ultra-Violet lamp—emitting at 366 nm.

B6.10 TLC plates—20 cm × 20 cm coated in a mixture of 7 parts of aluminium oxide G and 3 parts of cellulose 40% acetylated to a thickness of 0.25 mm. These are available commercially, but may need re-activating at 100°C±5°C for 30±5 mins before use. Cool and store in a desiccator.

Plates may be prepared as follows using conventional glass TLC plates and plate coating apparatus:

Thoroughly mix, by shaking, 28 g±0.2 g aluminium oxide G, 12 g±0.2 g cellulose 40% acetylated and 65 ml±0.5 ml ethanol. Coat onto clean glass plates (20 × 20 cm) at a thickness of 0.25 mm. (The quantities given are sufficient for coating 5 plates). Air dry at room temperature for 10 to 15 minutes, then activate at 100°C±5°C for 30 mins±5 minutes. Cool and store in a desiccator.

Note: Some workers find it advantageous to scrape 10 mm of adsorbent from the edges of the plates.

B6.11 Dark cabinet—to hold at least 7 plates to be viewed simultaneously under 366 nm illumination.

B6.12 TLC plate standards

To prepare the 6 reference plates, spot 6 activated TLC plates in the manner described above with the following solutions, using 5 or 10 μl microcapillary pipettes.

Plate 1	5 μl of spotting solution A
Plate 2	10 μl of spotting solution A
Plate 3	20 μl (2 × 10μl) of spotting solution A
Plate 4	5 μl of spotting solution B
Plate 5	10 μl of spotting solution B
Plate 6	20 μl (2 × 10μl) of spotting solution B

Six plates are thus prepared (using the TLC development procedure described in Section B8) with the following concentrations:

Plate 1	5 ng of each PAH
Plate 2	10 ng of each PAH
Plate 3	20 ng of each PAH
Plate 4	40 ng of each PAH
Plate 5	80 ng of each PAH
Plate 6	160 ng of each PAH

Store in a desiccator over silica gel in the dark. These plates deteriorate slowly on exposure to ultra-violet light, but with careful use should maintain adequate accuracy for at least 2 months. The stability of the plate standards should be checked periodically (every month) by comparison with a freshly prepared plate standard.

B7 Sample Collection and Preservation

Sample bottles should be completely filled at the sampling site and the stoppers inserted, leaving no air space.

In the case of surface water samples the use of sampling devices through which the sample flows before entering the sample bottle should be avoided as these may give contamination or PAH adsorption problems. It may be necessary to avoid including the surface layer in the sample as they may contain higher levels of PAH than the main body of the water. This is particularly so if an oil film is present. Owing to the strong adsorption of PAH onto particulate matter precautions must be taken to ensure the representative sampling of turbid samples.

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 potable waters PAH samples must be taken before the tap is 'flamed' to sterilise for bacteriological analysis. Residual chlorine must be removed using sodium thiosulphate; add 0.5 ml of a 10% solution of sodium thiosulphate to the sample and mix. This quantity is sufficient to remove levels of chlorine found in normal waterworks practice.

The bottle tops should be wrapped in aluminium foil to protect samples from contamination during transport and storage, and sources of such contamination (eg dust, motor vehicle exhaust fumes, cigarette smoke) avoided. Samples should be stored in the dark and extracted as soon as possible after collection (within 24 hours).

B8 Procedure

Caution: Some PAH are carcinogenic, see Section 3. Cyclohexane and other organic solvents used in this procedure are flammable.

Note: PAH are degraded by light and all stages of the procedure should be protected from strong sunlight and sources of UV irradiation.

Step	Procedure	Notes
	Extraction	
B8.1	Empty water from the bottle until the level corresponds to the 1.8 l mark.	
B8.2	Weigh the bottle plus sample to ±lg Note the weight W_1g.	
B8.3	Add 40 ml cyclohexane to the sample (Note a). Stopper.	(a) 40 ml is the minimum volume known to be suitable. Larger volumes (eg 100 ml) may be convenient in some cases, eg when emulsions form.
B8.4	Shake vigorously by hand for 5 mins ensuring good mixing and solvent dispersion then stand for at least 5 minutes to allow the layers to separate. (Notes b, c).	(b) A shaking machine may be used but recoveries should be checked. 5 minutes standing is adequate for most potable waters. Other waters may need several hours standing.
B8.5	**Either:**	
B8.5A	Replace the stopper with the micro-separator. Through the lateral tube A (Figure 1) add sufficient water to bring the cyclohexane layer into tube B. Transfer the extract to a conical flask using a pipette. Rinse the pipette with a small volume of cyclohexane and add the washings to the flask, **or:**	(c) Emulsions may form which will not separate on standing. If only a small proportion of the solvent is contained in the emulsion then an aliquot of the solvent may be used for the analysis and the final calculation adjusted accordingly. The procedure may also be used when only a small amount of solvent separates if PAH levels are sufficiently high. Alternatively the emulsion may be centrifuged to achieve separation. Caution: the centrifuge must be spark proof due to the volatility and inflammability of the solvent.
B8.5B	Siphon off most of the water from beneath the cyclohexane layer into a conical flask. Transfer the solvent and the residual water to a separating funnel. Rinse the sample bottle with 20±2 ml cyclohexane and add the rinsings to the separating funnel. Discard the lower aqueous layer and run the cyclohexane layer into a conical flask. Rinse the separating funnel with 20±5 ml cyclohexane and add the rinsings to the flask.	
B8.6	Weigh the empty sample bottle to ±1 g. Note the weight W_2 g.	
	Concentration of Solvent Extract	
B8.7	Dry the solvent for at least 30 mins using sodium sulphate (Note d).	(d) Some workers omit this stage but great care must be taken to avoid the carry-over of water droplets to the next stage of analysis.
B8.8	Filter the dry extract through a small cotton wool plug into a 250 ml round-bottomed flask. Rinse the sodium sulphate and conical flask with 10±0.5 ml cyclohexane and add to the round-bottomed flask via the filter.	

Step	Procedure	Notes
B8.9	Concentrate to 5±2 ml using the rotary evaporator. The water bath heating the flask should be at a maximum temperature of 40°C and the vacuum adjusted to achieve concentration in 15 to 20 minutes without bumping (Note e).	(e) Other apparatus for the removal of solvent may be used if a rotary evaporator is not available. However, the extract should not be heated above 40°C. Recoveries of PAH should be checked.
B8.10	Transfer the extract to a calibrated 10 ml centrifuge tube using a Pasteur pipette. Rinse the flask with 1.5 ml (±0.2 ml) cyclohexane and add the rinsings to the main extract.	(f) Retain 1/10 of the extract in case the linear range of the method is exceeded and re-analysis is necessary.
	Adjust the main extract to 10 ml by addition of cyclohexane. At this stage remove 1 ml (± 0.1 ml) from the calibrated tube and transfer to another calibrated tube (Note f). Stopper and store in a flameproof refrigerator or freezer. Concentrate the remaining 9 ml of extract to about 50 μl by means of a gentle stream of purified air delivered from a jet held about 1 cm above the surface. (Notes g, h).	(g) The nitrogen supply should be adjusted such that the surface of the solvent is just indented. Higher nitrogen flow rates may result in loss of PAH by evaporation or splashing. (h) Manipulation of small volumes of solvent may be conveniently achieved using glass syringes or Pasteur pipettes. Teats for the latter must be checked for interfering materials.
	Thin-Layer Chromatography	
B8.11	The concentrated extract (0.05 ml to 0.1 ml is spotted in the bottom left hand corner of a TLC plate 20 to 25 mm from each edge. The diameter of the spot must be controlled to 4 to 6 mm (Note j). Allow the spotting solvent to evaporate at room temperature (2 to 3 mins).	(j) Micro syringes or capillaries may be used to apply the solvent to the plate. Spotting may be aided by various commercially available frames and cold or warm blowers. Care must be taken not to unduly damage the surface of the plate. However a small hole in the plate made by the syringe generally does not affect chromatography and is practically unavoidable.
B8.12	Develop the plates until the solvent has moved 150±15 mm from the spotting position, in TLC solvent system 1 (notes k, l). Use a tank which has previously been allowed to equilibrate with the solvent for at least 15 minutes. Remove the plates from the tank and dry at ambient temperature in a fume cupboard for 10 to 15 minutes.	(k) Some workers find that better solvent fronts are obtained when the absorbent is scraped from the edges of the plate in a straight line to a distance of 10 mm from the edge of the plate.
B8.13	Rotate the plates through 90° anti-clockwise from the first direction of development and develop in TLC solvent system 2 under the same conditions as step B8.12 (Note l).	(l) The development times are approximately 30 mins for solvent system 1 and 90 mins for solvent system 2.
	Remove the plates from the tank and air dry in a fume cupboard at ambient temperature (10 to 20 mins).	
	Calculation	
B8.14	Irradiation of the plates with 366 nm light will visualise the PAH as a series of fluorescent spots. Typical chromatograms are shown in Figures 9 and 10.	
B8.15	Compare the plate with the 6 reference plates.	

Step Procedure Notes

Comparison of the plates should take place in a darkened room or cabinet. Irradiation is carried out with the uv lamp held approximately 30 cm above the plates. The individual PAHs are recognised from their position and characteristic colour of the fluorescence by comparison with standards, while the quantitative estimation is made by visually comparing their intensities. Estimate each PAH individually as being either nominally equal to the intensity of a corresponding spot on a reference plate or, nominally intermediate in intensity between 2 corresponding PAH concentrations on the reference plate. For example, with the specified reference plates the following values are possible for PAH estimated as nominally equal; ng on plates 5, 10, 20, 40, 80, 160 or greater than 160 and for PAH estimated as nominally intermediate; ng on plate less than 5, 7, 14, 28, 57, or 113. (Notes m, n).

NB Only grade A observers (see section B9) are capable of the latter assessment.

(m) Excessive exposure of the plate to uv light must be avoided.

(n) See fig 9 for a typical plate.

B8.16 Calculate the concentration of each PAH in the sample C (ng/l) as follows:

$$C = \frac{W_p \times 10^3}{W_1 - W_2} \times \frac{10}{9} \text{ ng/l}$$

Where:

G = concentration of the PAH in the sample in ng/l

W_1 = wt of sample bottle plus sample (g)

W_2 = wt of sample bottle (g)

W_p = estimated mass of PAH in the sample spot (in ng).

NB $\frac{10}{9}$ factor since 1/10 of the extract was removed (see step B8.10).

Blank

B8.17 A procedural blank using water should be processed through all the steps of the experimental procedure involved in the analysis of the relevant samples. This blank should be sufficiently small to be ignored, but if significant with respect to the PAH levels in the samples should be subtracted from the results (Note o).

(o) PAH present in the blank water must not be subtracted from the result. If blank water contamination is suspected, a procedural blank must be run which omits the water during the extraction stage, in order to ascertain the PAH blank due to the other sources of contamination.

Spiked water sample

B8.18 The inclusion of a spiked water sample with each batch of samples is recommended (see section B9) to check satisfactory recovery and estimation of PAH. Thus, 80 µl of spiking solution 1 is added to 2 litres of water using a 100 µl syringe. The water may be blank water or a real water sample but it must have negligible levels of PAHs (see section B9). The spiking solution is injected just below the surface of the sample. The sample bottle is stoppered and shaked for 2 mins. It is then treated as a normal sample.

B9 Checking the accuracy of the method

The reference scale used in this method is constructed on the basis that the fluorescence energy emitted by a chromatogram spot is directly proportional to the amount of PAH present and that the visual response of the analyst is a logarithmic function of the light intensity. The 6 reference plates employed constitute concentration steps of 5, 10, 20, 40, 80, 160 ng of each PAH. Visually the steps represent equivalent increases in fluorescence which should be readily discernible to most analysts. The minimum discrimination error attached to such a reference scale can be shown to be +41% to −29%. To this should be added the estimated error introduced in preparing the reference chromatograms by the technique, which is about ±5%. Thus most analysts would be within +46% to −34% discrimination error. A good observer is able to discriminate between the fluorescence intensities of spots of the reference concentrations and spots of concentrations intermediate between reference concentrations. Therefore nominal interpolated mid-points were incorporated in the reference scale, which gives, after considering the logarithmic nature of the scale and rounding off the figures, the following reference scale points;

5, 7, 10, 14, 20, 28, 40, 57, 80, 113, 160 ng each PAH.

(It would be feasible to construct 11 reference chromatograms corresponding to the above steps, but, bearing in mind the nature of the method this would be impractical in most circumstances).

The minimum discrimination error attached to such a reference scale can be shown to be +18.9% to −15.9%.

Once the method has been put into operation, regular checks are recommended. Precise checking of recovery from standard solutions and/or spiked samples is precluded by the large random errors associated with visual estimation, but such tests are nevertheless recommended as the only feasible control. A good observer must be used to check recoveries.

To aid assessment of incomplete recovery it is recommended that a spiked blank water and/or water sample is run with each batch of analyses, at a concentration (for each PAH) equivalent to 80 ng on the TLC plate. The unspiked sample should contain a negligible concentration of each PAH (5 ng on plate).

If the results obtained are consistently reported, by a good observer, at the 80 ng level the recovery is probably better than 80%. If, however, the results are consistently reported at the next lowest reference scale level (57 ng) the recovery could be as low as about 60%. If such a low recovery is obtained for a standard solution, the procedure should be carefully scrutinised and the cause identified and remedied. If it is obtained for a real sample, but greater than 80% recovery is obtained for a standard solution, adsorption of the PAH upon suspended matter may be the cause and greater than 20% bias should be allowed for in assessing the results (see Appendices B and C).

The ability of observers to evaluate the TLC plates with adequate accuracy should be regularly checked. This can be done as follows:

Step 1. Prepare a set of reference plates (see Section B6) and mark the back of each plate for recognition after the test.

Step 2. Instruct a colleague to set out the plates in random order then attempt to place the 6 plates in correct order, basing the decision entirely on the fluorescent intensity observed. This should be repeated at least 4 times. The analyst must be able to place the 6 plates in correct order for each PAH. After the plates have been placed in correct order for one PAH it may be necessary to re-scramble the plates before attempting to place in correct order for the next PAH. This can be carried out in 'one sitting' as long as the analyst is confident that each PAH can be ordered correctly. Should this not be the case, and problems are encountered for just one of the 6 PAH then the plates should be stored in the dark for 24 hr and the experiment repeated. When repeating the experiment those PAH incorrectly ordered in the first experiment, should be observed first. If the plates are still placed incorrectly then a fresh set of plates should be prepared and the experiment repeated.

Step 3. If Step 2 is successfully carried out prepare 2 further plates having levels of PAH intermediate (ie midpoints) to the levels on the reference plates. Levels of 14 ng of each PAH per plate and 57 ng of each PAH per plate are suitable for this purpose. Mark the back of these plates for recognition after the test and place with the set of reference plates prepared in Step 1.

Step 4. Instruct a colleague to set out the plates in random order then attempt to place the 8 plates in correct order. This is repeated at least 4 times. As described in Step 2 the analyst must confirm that each of the 6 PAH can be correctly placed in order.

If Step 4 is carried out successfully a discrimination error of ±25% (approximately the figures mentioned above) should be applied to the results obtained by the analyst (a grade A observer). If Step 2 can be successfully completed but the 2 intermediate points cannot be placed in correct position then a discrimination error of ±45% (approximate figure) should be applied (a grade B observer) (IMPORTANT: See Appendix C on assessment of results).

B10 Sources of error

B10.1 Contamination

PAHs are unbiquitious in the environment and therefore throughout the analysis great care must be taken to avoid contamination of the sample, extract or concentrate. Contact with grease, oils, plastics and organic material should be eliminated.

Contamination from the atmosphere is a problem especially relevant to PAH analysis. Particulate matter from smoke emissions (fires, chimneys, cigarettes, industry in general) could lead to intermittent contamination. It is advisable before setting up the method for routine analysis to analyse a sufficient number of blanks, for example 2 per day over a period of 2 weeks, to ensure that sporadic contamination is absent.

B10.2 Decomposition

Exposure to strong sunlight or fluorescent lighting must be minimised.

B10.3 Improper Sampling and Absorption

See Sections A7 and B7.

B11 Concentration range of the method

See Section A12.

Appendix A—Precision

The principal source of random error will normally be the visual estimation of the amounts of the PAH on the TLC plates, by comparison of fluorescent intensities. When the 'sample' plate has an amount of a PAH close to that of a reference scale point, an unbiased observer should record the amount as that of the scale point, so the error should be small. When the amount of PAH on the 'sample' plate lies at the geometric mean of the amounts on the adjacent reference scale points, an unbiased observer should record the amount as that of the higher reference scale point on 50% of occasions and as that of the lower reference scale point on 50% of occasions. It will, therefore, be seen that the error will be greatest at such geometric mean concentrations and will be as large as the difference between the true amount and the amount at the reference scale point above or below (whichever is judged to be of equivalent intensity to the sample plate for the PAH in question).

To check the visual acuity of Grade A and Grade B observers (see B9) plates were prepared having amounts of each PAH between those of the reference scale points, at levels of one-half, one-quarter and three-quarters of the reference scale point interval. This was done at both low and medium PAH levels (in relation to the range of the method), for 2 Grade A observers and 2 Grade B observers. In each case, the observers each made 20 estimations of each of the 3 'unknown' plates (for each PAH), assigning the lower or higher standard amount as they considered correct on each occasion. (All observations were randomised). The percentage of high scale point estimations was recorded in each case. The results are shown in Tables 3 and 4.

In general, the results approximated the expected pattern of 'high' estimations with concentration (ie roughly 25% at the one-quarter point, 50% at the one-half point and 75% at the three-quarter point) though there was evidence of both random and systematic observer errors. Errors tended to be greater in the case of Grade B observers, despite the fact that the differences between their test plates were larger (corresponding to the wider intervals between standard plate amounts) in their case. (It may be noted, however, that Grade B observers soon improved to Grade A status with increasing experience). It should be noted also that these tests were somewhat more stringent than those outlined in Section B9 which are more suitable for periodic routine checking of observer ability.

Appendix B—Bias

In addition to biased visual estimation (see Appendix A), biased results can arise from incomplete recovery of the PAH through the extraction step. Precise estimation of recoveries from spiked water samples is difficult because of the large random errors associated with visual estimation (see Appendix A), but recoveries of all 6 PAH of about 80–90% have been estimated, using distilled water spiked with about 90 ng/l of each PAH. Recoveries from river water, particularly at much smaller concentrations, may be considerably lower (see Table 4). Correction could in principle be made by using the observed recoveries of PAH from individual spiked water samples (see Section B9) but the random error of the visual estimation is so large that this will be less than satisfactory in practice. Given the screening nature of the method (see Appendix C), it is probably best to always allow for a negative bias of 20% in assessing results. Incomplete recovery may be exacerbated by the presence of suspended matter in the sample, and if recovery tests on typical samples reveal substantially lower recoveries than 80% (see Section B9) a correspondingly larger allowance should be made in assessing results (see Appendix C).

Table 5 summarises information on estimation of recovery of PAH. It must be noted that in this work attempts were made to achieve much finer discrimination than implied in Section B9 of the method. However, the information given gives a good indication of the overall recovery of PAH achieved.

See also Checking the Efficiency of Extraction.

Appendix C—Assessment of Results

It is common to consider the sum of the concentrations of all 6 PAH in relation to the standards for drinking water and related waters. For example, the EC Directive for drinking water specifies a limit for all 6 PAH of 200 ng/l. This process of summation, in combination with the complex pattern of errors associated with visual estimation of TLC plates (see Appendix A) and the problems of incomplete extraction (see Appendix B), makes assessment of analytical results far from straightforward. For simplicity and safety, the following approach is recommended:

(i) Record, for each PAH, the amount equivalent to the next highest plate above the recorded amount.

(ii) Convert the resulting values for each PAH to concentrations, and sum the 6 estimates so obtained.

If the sum approaches or exceeds 200 ng/l in any sample, or frequency exceeds 120 ng/l in samples from a given location, an alternative method (TLC) with instrumental evaluation of plates or HPLC should be used).

The above approach errs substantially on the side of safety[*], and more complex assessment procedures could be devised which do no incorporate the large safety margin involved in always using the 'next-highest-plate-value' approach of (ii) above. However, given the difficulties of error estimation and quality control with the method, it is recommended that an alternative (TLC with instrumental evaluation, or HPLC) can be used in preference to further elaboration of the assessment procedures applied to the results obtained using this method.[†]

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[*] With consequently increased risks of falsely reporting that the standard has been exceeded.

[†] For similar reasons, it is recommended that Grade A observers are always used unless total PAH concentrations are very low.

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Table 1 The 6 Specific PAH

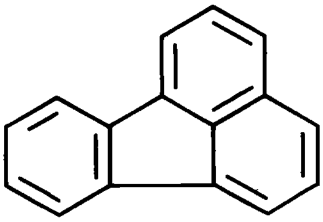
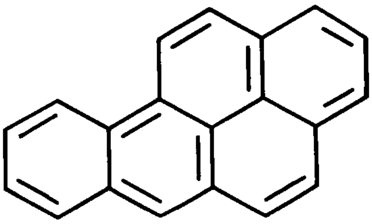
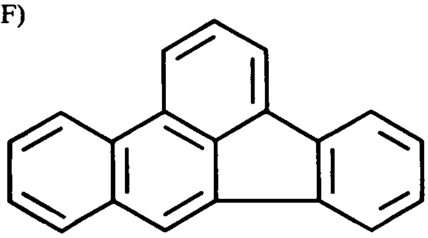
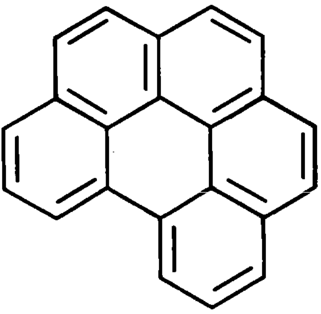
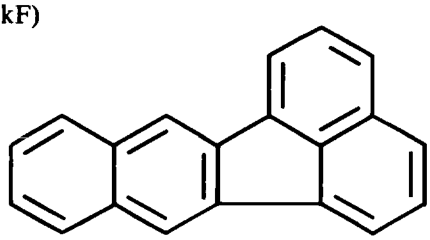
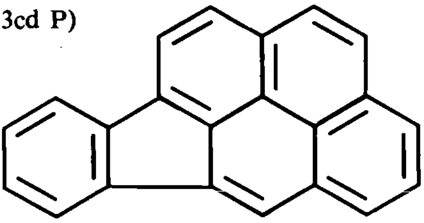
Name	Structure	Name	Structure
Fluoranthene (FLU)		Benzo (a) pyrene (Bap)	
Benzo (b) fluorathene (BbF)		Benzo (ghi) perylene (B ghi Pe)	
Benzo (k) fluoranthene (BkF)		Indeno (1,2,3-cd) pyrene (I 123cd P)	

Table 2 Standard Deviation of the HPLC Method

Sample	PAH	(ng/l)	S_w	S_b	S_t
Distilled water	Fl	7.34	1.44	ns	1.70(8)
	BbF	0.64	0.31	ns	0.45(6)
	BkF	0.48	0.21	0.39	0.45(5)
	BaP	0.30	0.28	ns	0.39(9)
	IP	0.35	0.24	0.48	0.54(5)
	BghiP	0.35	0.37	ns	0.52(7)
Doionised water	Fl(rpt)	0.83	0.25(11)		
Distilled water 10ng/l spike	Fl	14.28	1.59	0	1.59(8)
	BbF	8.30	1.36	ns	1.42(8)
	BkF	8.24	1.32	0	1.32(9)
	BaP	7.47	1.20	ns	1.54(9)
	IP	7.92	1.43	ns	1.72(8)
	BghiP	7.56	1.16	ns	1.44(7)
Distilled water 90ng/l spike	Fl	84.1	7.77	0	7.77(9)
	BbF	79.4	12.02	0	12.02(7)
	BkF	77.5	12.34	0	12.34(6)
	BaP	68.7	11.5	0	11.5(9)
	IP	72.4	14.19	0	14.19(8)
	BghiP	61.5	13.43	0	13.43(7)
River water (R Ouse)	Fl	8.75	1.14(5)		
	BbF	6.68	0.79(5)		
	BkF	5.00	0.89(5)		
	BaP	7.05	1.07(5)		
	IP	5.62	0.85(5)		
	BghiP	7.92	0.73(5)		
River water (R Ouse) 10ng/l spike	Fl	15.42	1.10(5)		
	BbF	12.43	0.73(5)		
	BkF	10.35	0.48(5)		
	BaP	12.28	0.65(5)		
	IP	18.25	0.72(5)		
	BghiP	10.2	1.31(5)		
River water (R Ouse) 90ng/l spike	Fl	73.5	7.04(5)		
	BbF	79.8	5.78(5)		
	BkF	70.8	5.19(5)		
	BaP	68.5	6.86(5)		
	IP	58.0	8.67(5)		
	BghiP	62.5	7.12(5)		
River water (R Wear tributary)	Fl	3.4	0.91(5)		
	BbF	8.47	1.87(5)		
	BkF	4.22	1.47(5)		
	BaP	5.83	1.49(5)		
	IP	5.93	1.35(5)		
	BghiP	6.37	1.40(5)		
River water (Stevenage Brook)	Fl	55	2.7(5)		
	BbF	156	7.2(5)		
	BkF	100	5.5(5)		
	BaP	157	8.8(5)		
	IP	123	7.7(5)		
	BghiP	168	3(5)		

Figures in brackets indicate degree of freedom
ns = not significant

Table 3 Precision of Observations (Grade A Observer) For detailed explanation see note after Table 5

Observer	PAH	Relative Number of High Scale Point Estimations (%)					
		Low Scale Point 10ng High Scale Point 14ng			Low Scale Point 56ng High Scale Point 80ng		
		11ng	12ng	13ng	62ng	68ng	74ng
A1	FL	20	55	65	29	60	55
	BbF	25	45	70	0	35	75
	BkF	50	30	80	25	40	80
	BaP	15	80	70	25	80	65
	IP	30	15	60	20	35	55
	BghiP	10	85	70	35	55	65
Mean for A1		25	52	69	21	51	66
A2	FL	30	50	100	25	55	80
	BbF	15	60	85	5	40	90
	BkF	35	60	75	15	85	90
	BaP	10	100	100	10	80	90
	IP	5	50	85	20	50	100
	BghiP	35	75	100	65	80	90
Mean for A2		22	66	91	23	65	90
Overall Mean for A1 and A2		23	59	80	22	58	78
Theoretical mean for observations		25	50	75	25	50	75

Table 4 Precision of Observations (Grade B Observer) For detailed explanation see note after Table 5

Observer	PAH	Relative Number of High Scale Point Estimations (%)					
		Low Scale Point 10ng High Scale Point 20ng			Low Scale Point 40ng High Scale Point 80ng		
		12ng	14ng	17ng	48ng	57ng	68ng
B1	FL	5	60	100	10	60	95
	BbF	10	85	100	60	70	100
	BkF	50	60	100	20	55	100
	BaP	30	15	100	40	95	85
	IP	15	75	100	10	60	100
	BghiP	45	70	100	40	60	100
Mean for B1		26	61	100	30	67	97
B2	FL	15	35	75	5	40	95
	BbF	25	55	75	20	55	90
	BkF	15	45	90	20	25	75
	BaP	60	35	100	20	45	90
	IP	10	20	35	10	30	85
	BghiP	60	75	90	50	80	100
Mean for B2		31	44	78	21	46	89
Overall Mean for B1 and B2		28	53	89	25	56	93
Theoretical mean for observations		25	50	75	25	50	75

Table 5 % Recovery of PAH from water

PAH	Distilled water + 10ng/l (each PAH)	Distilled water + 90ng/l (each PAH)
FL	45 ± 25	90 ± 5
BbF	56 ± 6	88 ± 5
BkF	54 ± 6	84 ± 6
BaP	52 ± 6	80 ± 4
IP	55 ± 9	84 ± 4
B(ghi)P	56 ± 5	81 ± 5
	River water + 10ng/l	River water + 90ng/l
FL	42 ± 44	82 ± 23
BbF	15 ± 14	75 ± 11
BkF	27 ± 25	67 ± 8
BaP	12 ± 15	72 ± 4
IP	17 ± 9	53 ± 9
B(ghi)P	8 ± 17	59 ± 10

Detailed Procedure for obtaining Tables 3 and 4

Two plates were prepared. The first had 10 ng spots of each PAH and the second plate at 14 ng spots of each PAH. These were regarded as the 'standard' plates. Three additional plates spotted with intervening amounts (11 ng, 12 ng and 13 ng) of each PAH were also prepared, representing 'sample' plates. The 'sample' plates were compared with the 'standard' plates and the observer was asked to decide whether the samples were nearer to the low (10 ng) standard or the high (14 ng) standard. The process was repeated on 20 occasions. The figures given in the table represent the percent of occasions when each 'sample' spot was considered to be nearer the high (14 ng) 'standard' spot. A similar exercise was performed for higher concentrations with plates spotted with 56 ng and 80 ng as 'standard' and 62, 68 and 74 ng as 'samples'. Two different, but experienced, analysts (observers A1 and A2) interpreted the spots. Results of all these tests are given in Table 3.

Less experienced observers were used for a less demanding test in which the 'standards' were 10 and 20 ng of each PAH, and the intervening 'sample' spots were at 12, 14 and 17 ng. Again the exercise was repeated 20 times at two levels of spotting and with two independent observers. The results of this series of tests is given in Table 4. In all the tests, the plates were randomized between observations.

Figure 1 Micro separator

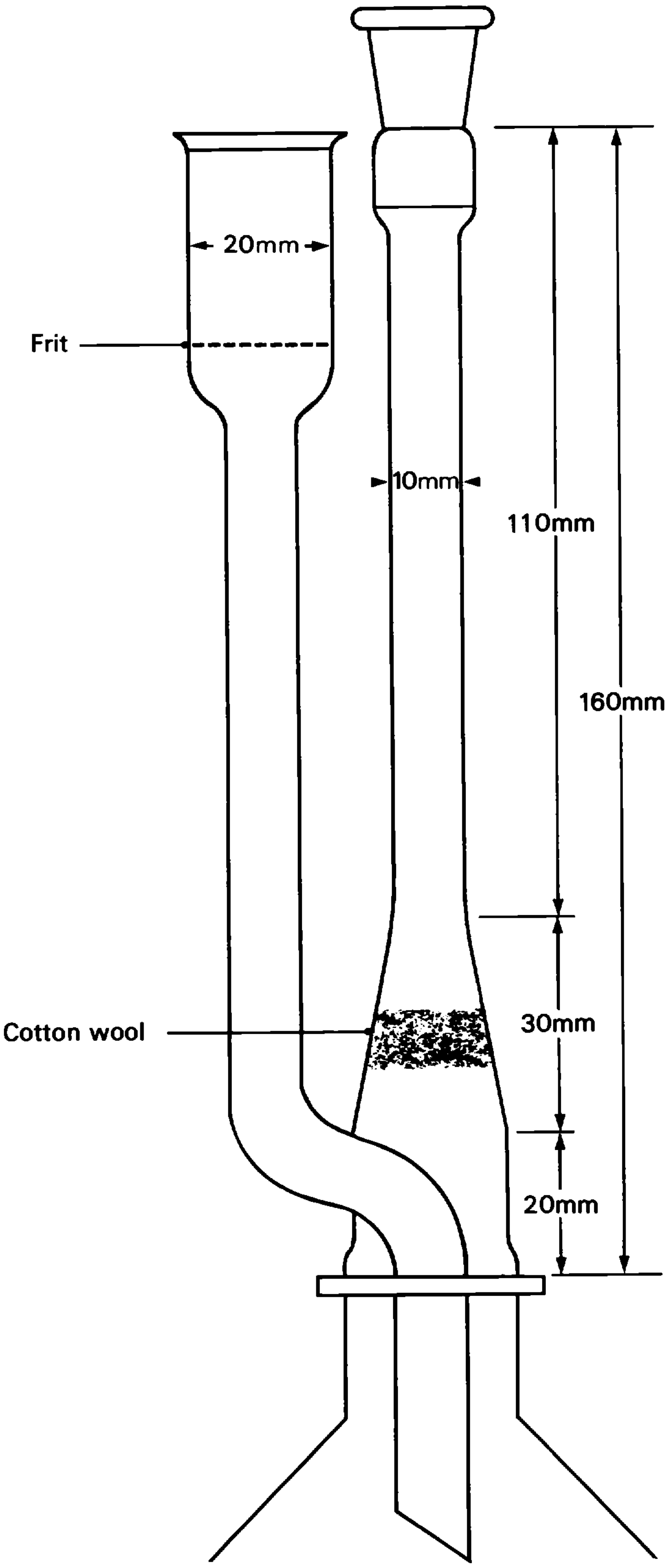


Figure 2 Concentration flask (capacity 100ml)

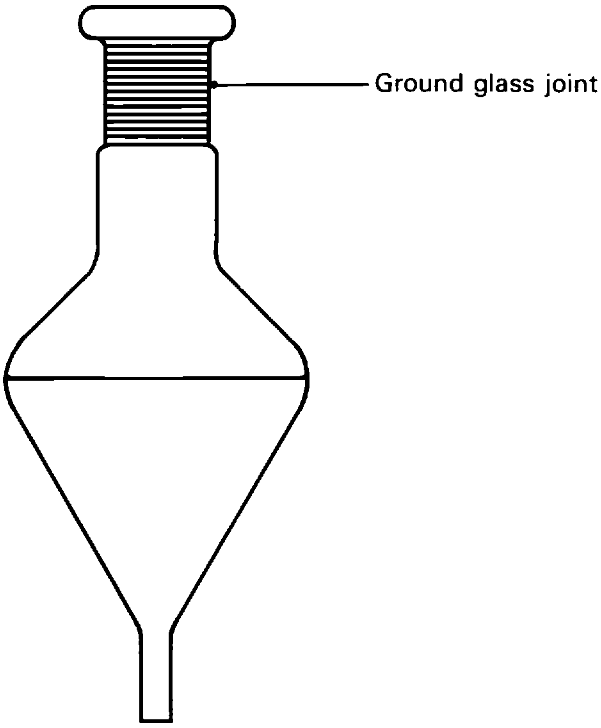


Figure 3 Typical chromatogram (0.0144 ng/ul std)
Columns and conditions as A6.7 (1)

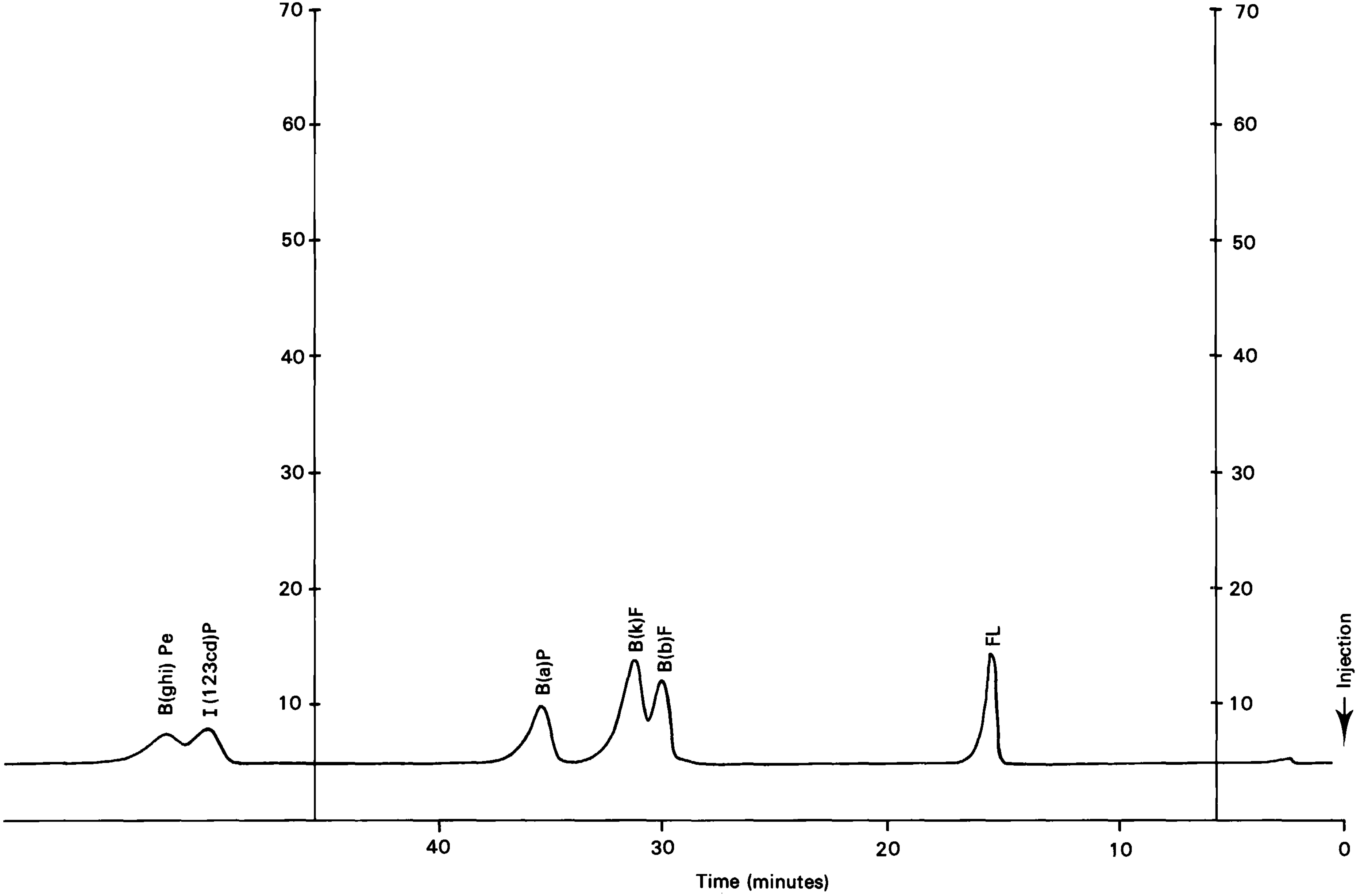


Figure 4 Typical chromatogram (river water)
Columns and conditions as A6.7 (1)

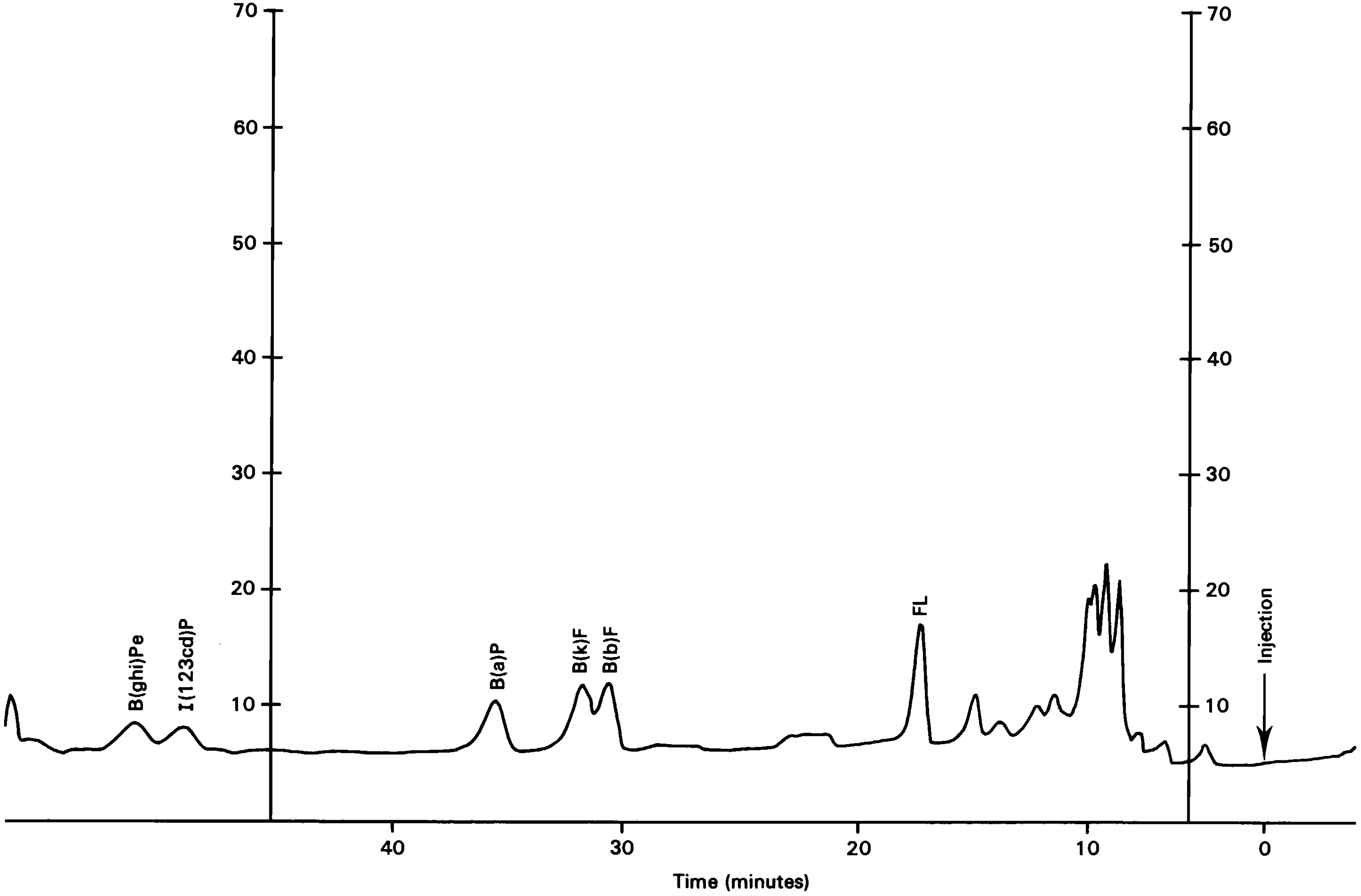


Figure 5 Separation of the six PAH using gradient elution

Conditions: 7.5% aqueous acetonitrite to 100% acetonitrite
25cm X 4.6mm i.d. 6μm ODS column

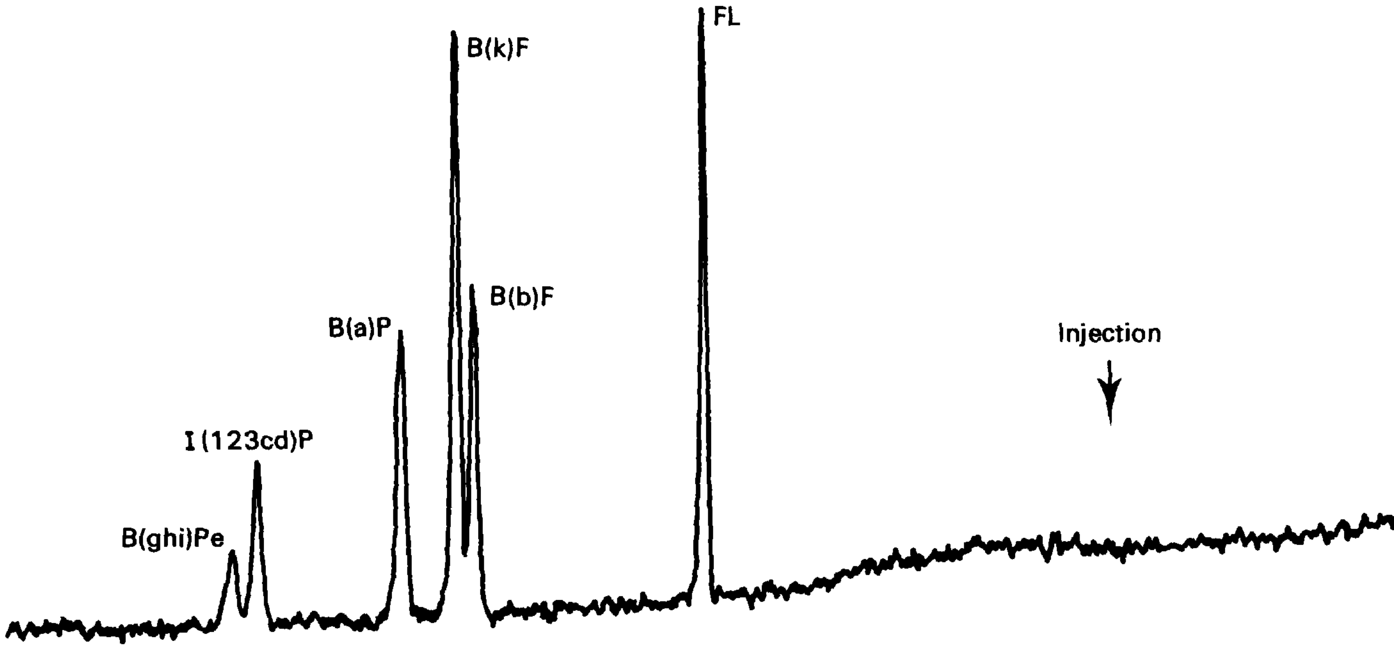
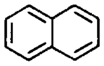


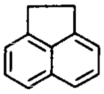
Figure 6 PAH separated isocratic and gradient elution HPLC shown in Figures 7 and 8
(based on data and chromatograms from reference 17)

Compound

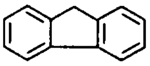
1) NAPHTHALENE



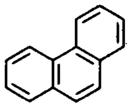
2) ACENAPHTHENE



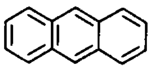
3) FLUORENE



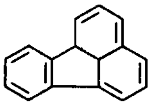
4) PHENANTHRENE



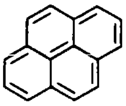
5) ANTHRACENE



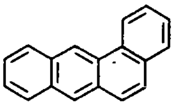
6) FLUORANTHENE



7) PYRENE

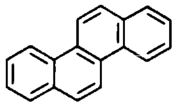


8) BENZ(a)ANTHRACENE

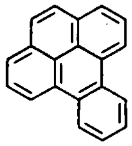


Compound

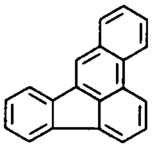
9) CHRYSENE



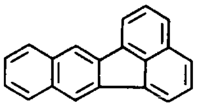
10) BENZO(e)PYRENE



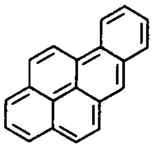
11) BENZO(b)FLUORANTHENE



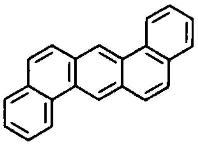
12) BENZO(k)FLUORANTHENE



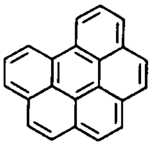
13) BENZO(a)PYRENE



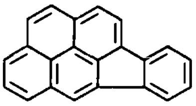
14) DIBENZ(a,h)ANTHRACENE



15) BENZO(ghi)PERYLENE



16) INDENO(123cd)PYRENE



17) PERYLENE

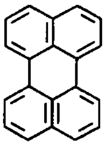


Figure 7 Chromatogram of 12 PAH standards using isocratic elution
(80% acetontrile in water at a flow rate of 0.5mL/min)

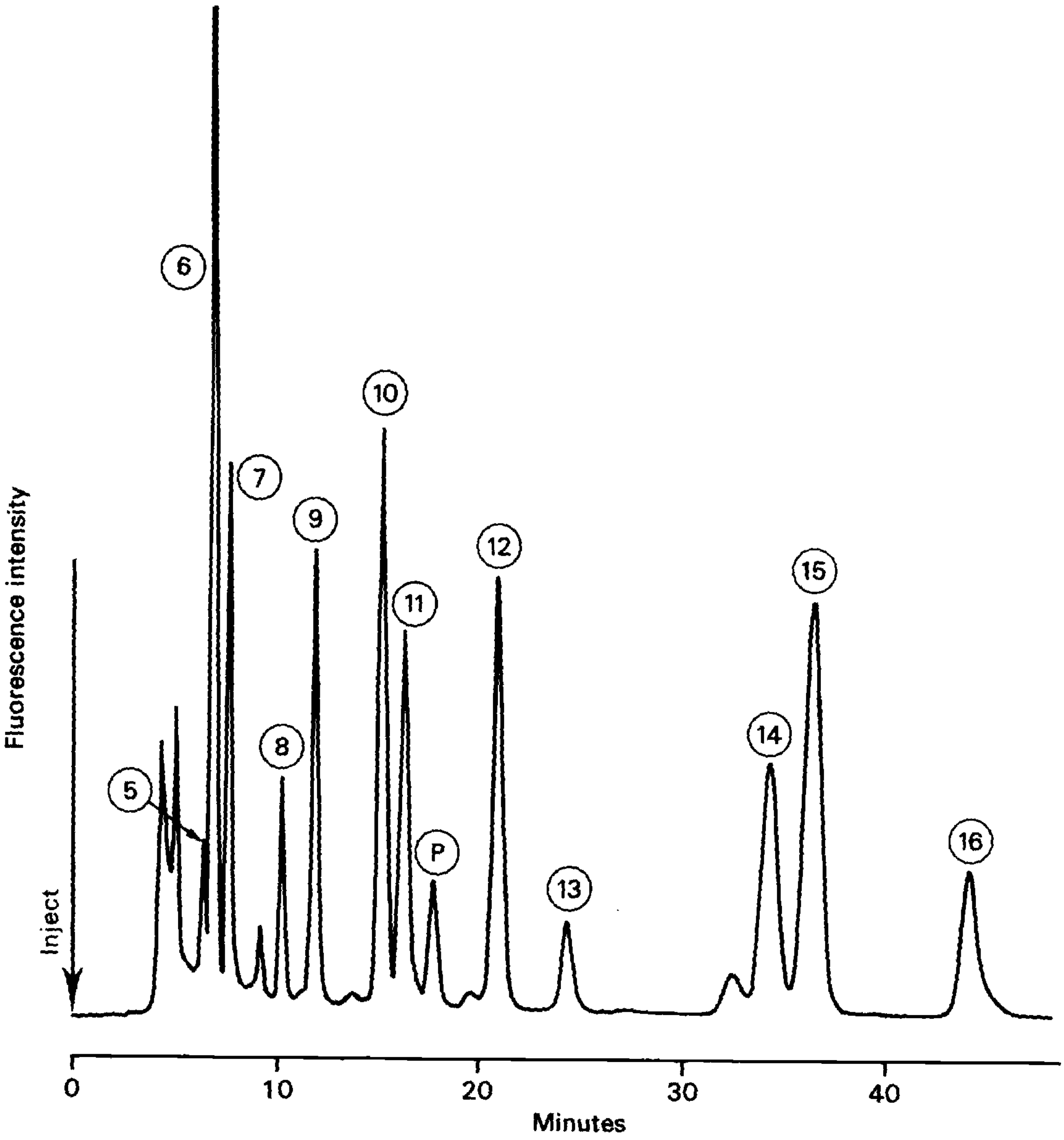


Figure 8 Chromatogram of 16 PAH standards using gradient elution
(50% aqueous acetonitrile to 100% acetonitrile)

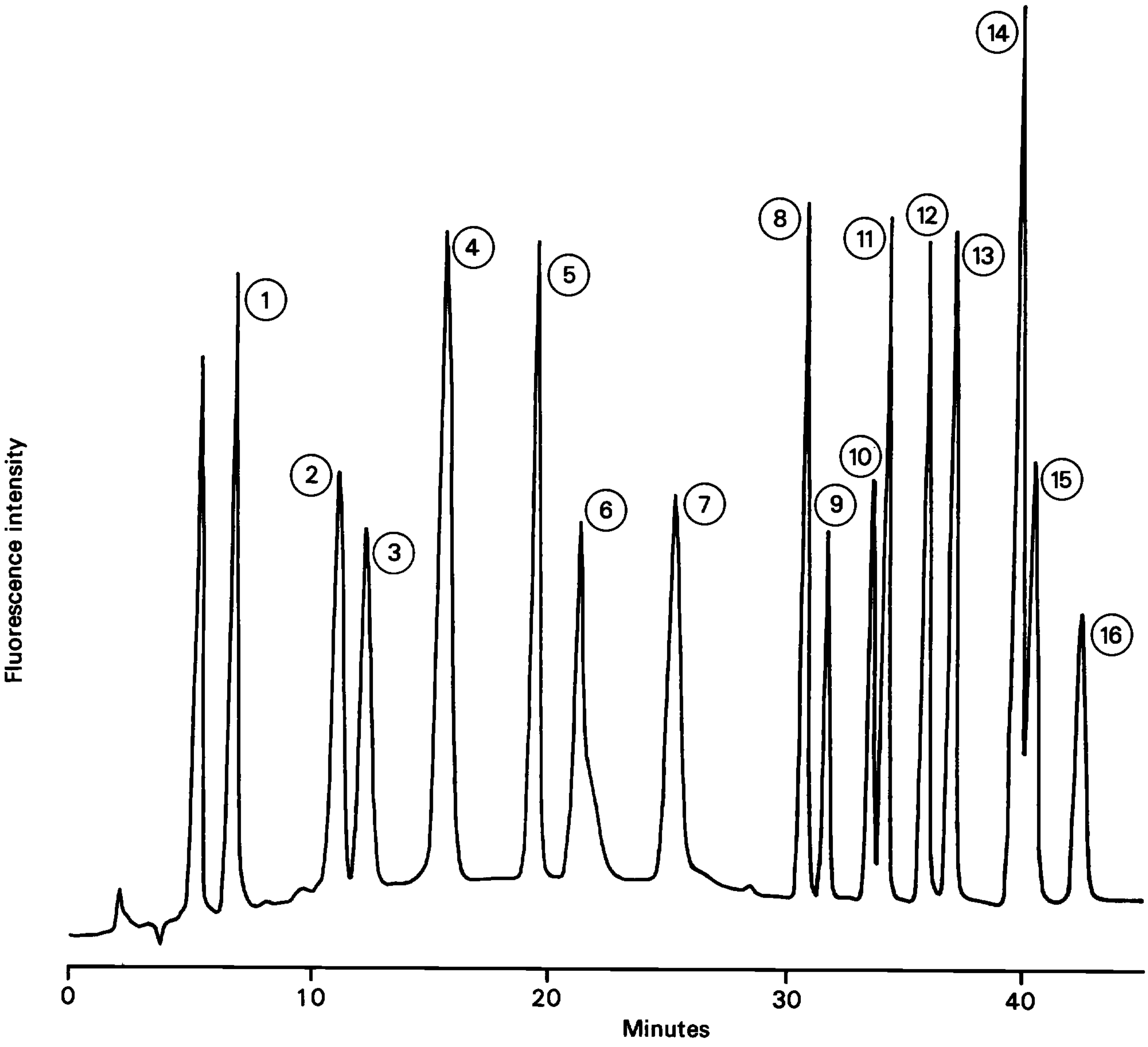
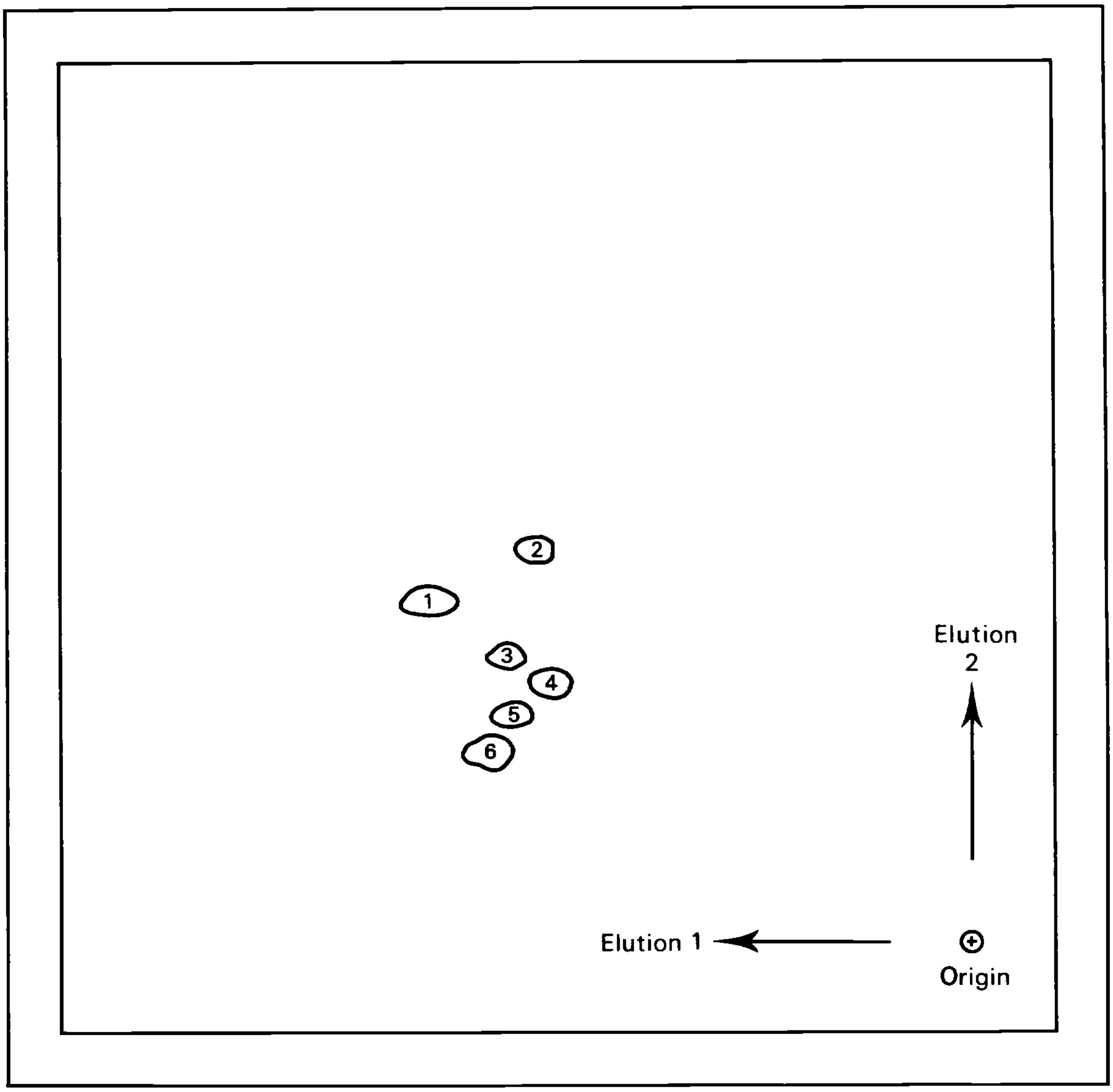


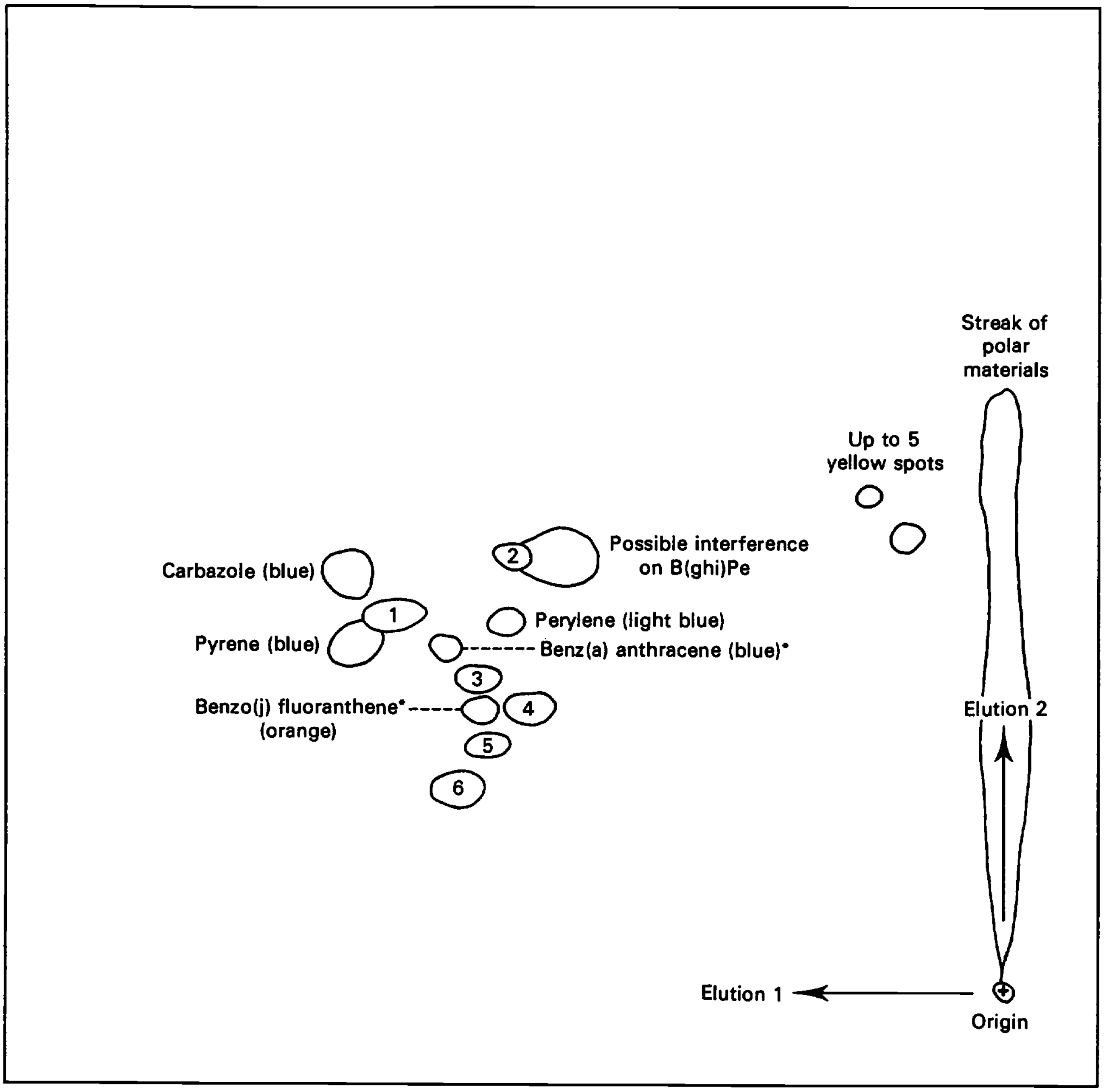
Figure 9 Standard PAH two-directional chromatogram as viewed under UV light



Spot	PAH	Colour
1	FLUORANTHENE	Turquoise
2	BENZO(ghi)PERYLENE	Violet
3	BENZO(k)FLUORANTHENE	Violet
4	*INDENO(123cd)PYRENE	Yellow
5	*BENZO(b)FLUORANTHENE	Turquoise
6	*BENZO(a)PYRENE	Violet

*Carcinogenic

Figure 10 Typical river water two-dimensional PAH chromatogram



Spot	PAH	Abbreviation	Colour
1	FLUORANTHENE	FL	Turquoise
2	BENZO(ghi)PERYLENE	B(ghi)Pe	Violet
3	BENZO(k)FLUORANTHENE	B(k)F	Violet
4	*INDENO(1 23cd)PYRENE	I(1 23cd)P	Yellow
5	*BENZO(b)FLUORANTHENE	B(b)F	Turquoise
6	*BENZO(a)PYRENE	B(a)P	Violet

*Carcinogenic

Checking the Efficiency of Extraction (Both Methods)

As mentioned in the methods, extraction efficiency is dependent on the amount and type of suspended solids present in the sample. If no suspended matter is present, extraction efficiency can be determined by analysis of a control spike sample to which known amounts of the PAH of interest have been added. Analytical results may be corrected accordingly, but this should be noted in the report.

If suspended matter is present, it might be filtered off and the filter extracted with solvent using a soxhlet apparatus; but the more usual procedure is to analyse a control spike sample. However, it is necessary to allow time for the added PAH to equilibrate between water and solid. For ordinary work, standing for 24 hours (but not less) is sufficient, but for more accurate work, on occasional check on a sample stood for 7 days or even longer is suggested.

Address for Correspondence

At the present time, though based on work in several laboratories, thorough test data is only available from one laboratory, hence the tentative status of the method. Additional test data would be welcomed. Results should be sent to the address below.

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

The Secretary
The DOE Standing Committee of Analysts
The Department of the Environment
2 Marsham Street
LONDON
SW1P 3EB
England.

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Department of the Environment

Standing Committee of Analysts

Members assisting with the production of this method.

Because of the hazardous nature of some of the determinands, evaluation and testing of these 2 methods has taken considerable time. Hence only Working Group and Main Committee members actually involved in this project are listed; but the Panel is given in full.

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