Calibration

E5.3 Switch the system to the configuration shown in Figure E1A. With the aid of the sample pump, transfer 25 mL of acetonitrile through the enrichment column and allow to drain to waste.

E5.4 Start the analytical pump.

E5.5 Change the configuration to that of Figure E1B and pump mobile phase through the enrichment column until a stable baseline is obtained. Continue pumping throughout the procedure.

E5.6 Pump 50 mL of one of the standard solutions (E3.5.4) from the sample pump to waste.

E5.7 While maintaining flow, simultaneously change the configuration to that shown in Figure E1A and for raw waters place a dry 20 mL measuring cylinder at the waste outlet. (For potable waters use a 100 mL cylinder). Allow 20 ± 0.5 mL (or 100 ± 1 mL) to collect and switch off the sample pump. Simultaneously, switch to the configuration shown in Figure E1B and start the data collection sequence.

E5.8 While data is being collected, prepare the system for the next standard.

Pump to waste, from the sample pump, 25 mL of acetonitrile followed by 50 mL of the next standard. When the chromatographic run has ended, repeat the procedure at E5.7.

E5.9 When the three calibration standards have been run use the data obtained to calibrate the integrator.

Sample quantification

E5.10 Add to each sample bottle the appropriate volume of internal standard solution, (note a). Mix thoroughly.

(a) This is calculated from the following equation: (100xV/500) µL where V is the volume of sample bottle (mL).

E5.11 Pump 50 mL of sample from the sample pump to waste.

E5.12 While maintaining flow, simultaneously change to the configuration shown in Figure E1A and for raw waters place a dry 20 mL measuring cylinder at the waste outlet, note b.

Allow 20.0 ± 0.5 mL (or 100 ± 1 mL) to collect and switch off the sample pump. Simultaneously, switch to configuration shown in Figure E1B and start the data collection sequence.

(b) For potable water use a 100 mL cylinder.

E5.13 While data are being collected prepare the system for the next sample.

Pump to waste, from the sample pump, 25 mL of acetonitrile followed by 50 mL of the next sample. When the chromatographic run has ended, repeat the procedure at E5.12.

E6 Calculation

The internal standard method of calculation is used. The response factor for the analyte is given by the equation:-

$$RF = [A_s][C_{is}]/[A_{is}][C_s]$$

Where

$[A_s]$ = Response of parameter to be measured

$[A_{is}]$= Response for the internal standard

$[C_s]$ = Concentration of parameter to be measured

$[C_{is}]$= Concentration of internal standard

Calculate the response factors for each calibration level. An average value may be used if the response values over the working range are reasonably constant (say for example 20% rsd or less).

If an average response value is used, unknown values for [Cs] may be determined by substituting the corresponding value for [As] in the rearranged equation:

$$[C_s] = [A_s][C_{is}]/[A_{is}].RF$$

For graphical methods of calibration, plot a calibration curve of the response ratio $[A_s]/[A_{is}]$ versus $[C_s]$.

For samples containing PAH concentrations above the range of calibration standards, dilutions must be made using 17.5% v/v propan-2-ol in water containing internal standards which has been shown by the blank procedure to contain negligible concentrations of PAH or interferents. Samples taken through the procedure can be corrected for blanks also taken through the procedure.

A typical chromatogram is shown in Figure E2.

Table E1 Range of application and Limits of detection

PAH	Concentration (ngL^{-1})	LOD (ngL^{-1}) #	*
Fluoranthene	up to 100	5	10
Benzo[3,4]fluoranthene	up to 100	2	10
Benzo[11,12]fluoranthene	up to 100	1	5
Benzo[3,4]pyrene	up to 100	2	5
Benzo[1,12]perylene	up to 200	10	20
Indeno[1,2,3-*cd*]pyrene	up to 500	10	50

LOD Limit of detection:
based on 100 mL of potable water:
* based on 20 mL of river water:

Table E2 Standard deviations

PAH	Mean (ngL^{-1})	Standard deviation (ngL^{-1})	Degrees of freedom
Fluoranthene	40.5	1.03	5
	83.2	2.41	5
Benzo[3,4]fluoranthene	61.6	1.93	5
	114.1	5.95	5
Benzo[11,12]fluoranthene	63.8	1.35	5
	115.7	5.3	5
Benzo[3,4]pyrene	63.8	1.29	5
	116.9	5.08	5
Benzo[1,12]perylene	136.8	4.5	5
	248	14.46	5
Indeno[1,2,3-cd]pyrene	364.5	4.07	5
	630.6	38.7	4

Figure E1 Configuration of switching valves

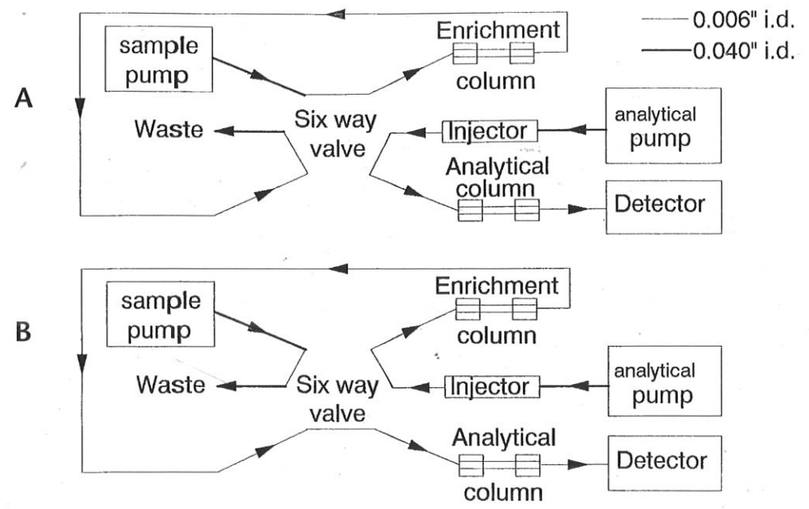
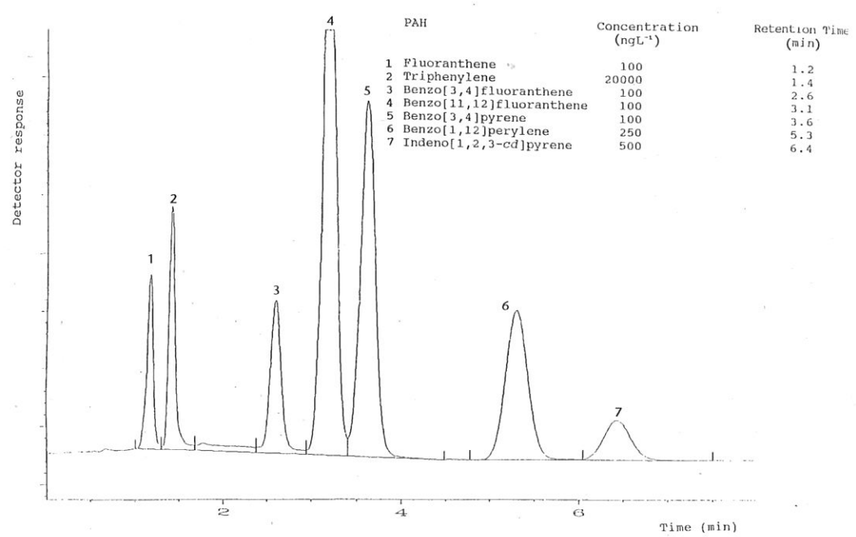


Figure E2 Chromatogram of on line HPLC



Appendix A: Confirmation of results

The methods in this booklet are based on HPLC with fluorescence detection. Whilst this technique is a very sensitive and reasonably specific method for the determination of PAH in extracts of water samples it can be subject to interferences. In potable waters, PAH concentrations are generally very low and when present are often due to the presence of coal tar pitch lined water mains in the distribution system. Where the methods for six PAH are used fluoranthene will usually be the sole, or major PAH determined. In some samples, problems have been observed with the determination of fluoranthene either as a result of the presence of one or more chemicals eluting and being detected at the same retention time or by the presence of other chemicals quenching the fluorescence of fluoranthene. Where fifteen PAH are analysed, these problems will be apparent since PAH mixtures derived from coal tar pitch may also have phenanthrene and anthracene present in significant concentrations. Thus, if there is an unusual ratio of the fluoranthene:phenanthrene, or fluoranthene:anthracene concentrations it may be necessary to confirm the presence and concentration of fluoranthene. There are several potential methods of confirmation and some of these are discussed below.

a) HPLC with fluorescence detection

A range of fluorescence excitation and emission wavelengths have been reported in the scientific literature (see Table 1) and are used in the methods described in this booklet. A change of fluorescence conditions and re-analysis by HPLC may be sufficient to overcome interferences and provide confirmation of results.

b) Thin layer chromatography

The thin layer chromatographic (TLC) method published previously in this series (1) can be used to provide confirmation of results obtained by HPLC.

c) Gas chromatography-mass spectrometry (GC-MS)

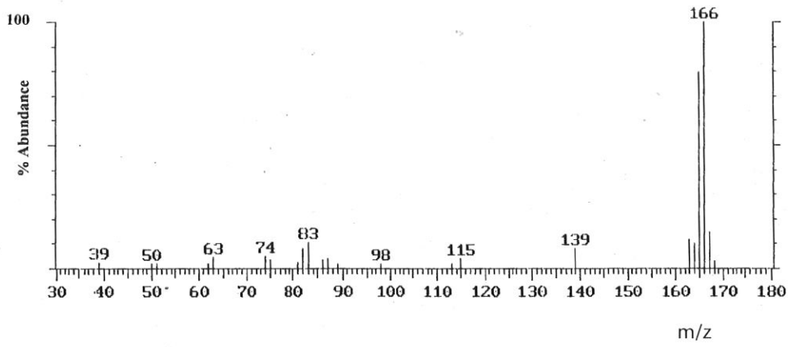
This technique provides greater specificity than the HPLC fluorescence method, but may not be as sensitive. However, in instances where problems have been observed with the HPLC fluorescence determination of fluoranthene, GC-MS has usually been able to confirm the presence or absence of fluoranthene. Confirmation should be carried out by first exchanging the HPLC mobile phase for a solvent more compatible with gas chromatography, for example dichloromethane. The extract is then analysed by GC-MS in specific ion monitoring mode (with electron impact ionisation) with the mass spectrometer set to detect the nominal (or accurate, if a high resolution instrument is available) molecular masses of the fifteen PAH (see Figure 1). PAH show relatively simple mass spectra, for example see Figure 2, with the molecular ion often the most intense ion. Standard solutions of the fifteen PAH should be analysed by GC-MS before any samples are determined in order to assign the retention times of the PAH, to calculate their relative response factors and, if accurate quantification is required, to construct calibration curves.

Table 1 HPLC fluorescence detection conditions

PAH	Reference 8		Reference 9		Reference 10		Reference 11	
	(ex)	(em)	(ex)	(em)	(ex)	(em)	(ex)	(em)
Naphthalene	280	340	280	334	-	-	-	-
Acenaphthene	280	340	292	324	245	350	-	-
Fluorene	280	340	268	308	245	350	-	-
Phenanthrene	280	340	292	366	260	420	-	-
Anthracene	305	430	253	402	260	420	260	430
Fluoranthene	305	430	360	460	260	420	260	430
Pyrene	305	430	336	376	265	380	260	430
Benz [1,2]anthracene	305	430	288	390	265	380	285	385
Chrysene	305	430	268	383	265	380	285	385
Benzo [3,4] fluoranthene	305	430	300	436	290	430	285	385
Benzo [11,12] fluoranthene	305	430	308	414	290	430	305	405
Benzo [3,4] pyrene	305	430	296	408	290	430	305	405
Dibenz[1,2:5,6]anthracene	305	430	297	398	290	430	-	-
Benzo [1,12] perylene	305	430	300	410	290	430	300	455
Indeno [1,2,3 - *cd*] pyrene	305	500	302	506	300	500	300	455

ex (excitation wavelength, nm)
em (emission wavelength, nm)

Figure 2 Mass spectrum of fluorene



Appendix B: Quantification

The recovery of the 15 PAH covered by these methods is generally high; recovery being measured by comparison of the concentration of an extracted standard with that of an un-extracted standard. In order to determine performance data it is important that any of the methods in this booklet used by a laboratory is validated in that laboratory by examining samples of the matrix of interest. Validation is carried out by examining a number of batches of replicate samples containing known levels of PAH. Hence, information will be obtained on the recoveries of individual PAH which may be required to correct for incomplete recovery. If a recovery correction is to be used, it is important to have an indication that a consistent recovery is obtained for each batch of analyses carried out. This indication may be achieved over a period of time using a standard mixture of PAH in a suitable matrix and is referred to as a `recovery control'.

A second approach, which is commonly used, is to add standard compounds to each sample prior to analysis. These standards are taken through the entire procedure, including extraction, concentration and chromatographic determination, as carried out for the sample. They are used to provide an indication of the recovery of each PAH in the sample or to provide an automatic correction for recovery of the PAH in the sample. For automatic correction, the standards should be included in the method validation and the assumption is made that the ratio of recovery of the PAH to the standard is a constant factor and is independent of sample matrix.

A further approach is to use a set of PAH calibration standards added to appropriate blank or matrix samples. These calibration solutions are taken through the entire procedure, including extraction, concentration and chromatographic determination, and are used to construct calibration graphs for individual PAH. Hence, the concentrations determined for the PAH in the samples are automatically corrected for recovery.

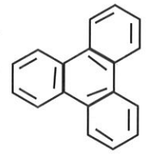
A supplementary use of standards for quantification purposes is the addition of standards to the sample extract prior to the concentration and chromatographic steps. These additions are used to correct for volumetric changes during concentration and chromatographic determination.

Standard compounds can also be added to samples as a check on the performance of the analytical method but are not used in the calibration procedure.

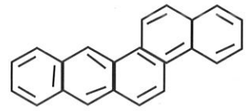
Standards should be of similar structure and possess similar properties to the PAH being determined. They should also possess chromatographic and fluorescence properties similar to the PAH being determined and should be readily discernible from the PAH of interest. They should not be present in the samples being analysed. Typically, aromatic compounds may be suitable and Figure 3 shows some that have been used for this purpose, of which triphenylene has been the most commonly used. Elution of triphenylene occurs between pyrene and chrysene on reverse phase HPLC. Benzo-*b*-chrysene is also suitable in some cases. If samples contain high levels of PAH, then other PAH often occur which may cause problems due to co-elution.

Figure 3 Structures of possible internal standards

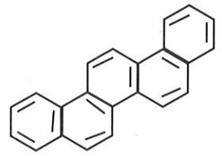
Triphenylene
$C_{18}H_{12}$ = 228



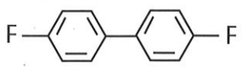
Benzo-*b*-chrysene
$C_{22}H_{14}$ = 278



Picene
$C_{22}H_{14}$ = 278



4,4' - Difluorobiphenyl
$C_{12}H_8F_2$ = 190



References

1. Methods for the Examination of Waters and Associated Materials (1986). The determination of 6 Specific Polynuclear Aromatic Hydrocarbons in Waters (with notes on the determination of other PAH), 1985. Her Majesty's Stationery Office, London.

2. IUPAC (1979). Nomenclature of Organic Chemistry. Rules 19-24 to A-28, Fused Polycyclic Hydrocarbons. Editors Rigaudy, J. and Klesney, S.P. Pergamon Press, Oxford, pp 20-31.

3. Crane, R.I., Fielding, M., Gibson, T.M. and Steel, C.P. (1981) A Survey of Polycyclic Aromatic Hydrocarbon Levels in British Waters. WRc Technical Report TR158.

4. Hazards in the Chemical Laboratory (1986) Bretherick, L. (Ed) The Royal Society of Chemistry, London, 4th Edition.

5. Working with Carcinogens (1977) United States National Institute for Occupational Safety and Health publication No 77-206.

6. Guidelines for work with chemical carcinogens in MRC establishments. Sub-committee of the Medical Research Council, Cell Biology and Disorders Board, October 1979.

7. Code of Practice for handling carcinogens in the occupational medicine and hygiene laboratory (1979). Health and Safety Executive, Occupational Medicine and Hygiene Laboratories. Her Majesty's Stationery Office, London.

8. Ogan, K., Katz, E. and Slavin, W. (1979). Determination of Polycyclic Aromatic Hydrocarbons in Aqueous Samples by Reversed-Phase Chromatography. Analytical Chemistry, **51**, 8, 1315-1320.

9. ISO TC 147/SC2/WG19 Draft Method for High Performance Thin Layer Chromatographic Determination of Six Polynuclear Aromatic Hydrocarbons (PAH).

10. Kicinski, H.G., Adamek, S. and Kettrup, A. (1989). Trace Enrichment and HPLC Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples using Solid Phase Extraction in Connection with UV/VIS Diode-Array and Fluorescence Detection. Chromatographia, **28**, 34, 203-208.

11. Kirk, P.W.W. and Lester, J.N. (1990) The Fate of Polycyclic Aromatic Hydrocarbons during Sewage Sludge Digestion. Environmental Technology, **12**, 13-20.

Address for correspondence

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

The Secretary
The Standing Committee of Analysts
Environment Agency
Steel House
11 Tothill Street
LONDON SW1H 9NF
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Standing Committee of Analysts

Members assisting with these methods

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D Britnell
P Chamberlain
G Mills
S Nash
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A Parry
M Rolph
C Watts

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Methods for the Examination of Waters and Associated Materials