Journal of Chromatography, 370 (1986) 157–163
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 981

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN NATURAL WATERS BY THIN-LAYER CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

GIAMPAOLO CARTONI*, FRANCO COCCIOLI, MARINA RONCHETTI*, LORI SIMONETTI and LELIO ZOCCOLILLO
Dipartimento di Chimica, Università "La Sapienza", P. le Aldo Moro 5, 00185 Rome (Italy)
(Received July 27th, 1986)

SUMMARY

A procedure is reported for the determination in natural waters of the six polycyclic aromatic hydrocarbons (PAHs) listed by WHO as indicators of pollution. The method consists in continuous liquid–liquid extraction, separation of the PAH fraction by thin-layer chromatography and reversed-phase high-performance liquid chromatography with a spectrofluorimetric detector. The method has been applied to unfiltered samples of river and sea waters with PAH levels below 0.1 ng/l.

INTRODUCTION

Of the many pollutants in surface waters, polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogenic, are of great importance. The World Health Organization (WHO) has set a standard for the amount of PAHs in drinking water derived from surface waters, recommending a maximum level of 200 ng/l for "total PAHs"[1]. The term "total PAHs" refers to the sum of six specified PAHs: fluoranthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, benzo(*ghi*)perylene and indeno(1,2,3-*cd*)pyrene. A level of more than 200 ng/l indicates unacceptable contamination. This same limit has been established in Directives of the Commission of the European Economic Communities (EEC)[2]. The U.S. Environmental Protection Agency (EPA) has issued a list of organic compounds to be monitored in municipal and industrial discharges in the U.S.A.[3]; the list contains 129 compounds, 16 of which are PAHs. These 16 PAHs include the six listed by the WHO.

In recent years much work has been carried out to develop methods for the determination of PAHs in the aqueous environment. These methods differ in detail, but almost all include extraction, clean-up and analysis.

The extraction techniques used for concentrating PAHs from water include extraction with organic solvents[4–7] or adsorption on columns of macroreticular resins[8–10] and other materials [11–15]. Clean-up is normally carried out by thin-layer (TLC) or column chromatography[16–18]. For the determination of PAHs gas chromatography[19–21], gas chromatography–mass spectrometry[22,23], high-performance

* This research is part of the doctorate post lauream thesis of M.R.

0021-9673/86/$03.50

liquid chromatography (HPLC)[24–27] and fluorescence spectrometry[28–30] have been used.

At the present there is no official method for the determination of PAHs in natural waters; the WHO recommends the two-dimensional TLC method developed by Borneff[18] and Borneff and Kunte[31]. However, this method has the disadvantages that it is laborious and lacking in accuracy, so alternative methods are desirable[32].

In this paper we report a procedure for the determination in natural waters of the six PAHs listed by the WHO. The method, consisting in continuous liquid–liquid extraction, separation of the PAH fraction by TLC and reversed-phase HPLC with a spectrofluorimetric detector, is applicable to unfiltered samples. We do not use preventive filtration, consequently our results refer exactly to the total aqueous medium at the time of sampling.

The applicability of this technique was tested on real samples.

EXPERIMENTAL

Materials

All solvents were obtained from Carlo Erba (Milan, Italy). *n*-Hexane, benzene (analytical-reagent grade), methanol, acetonitrile and tetrahydrofuran (HPLC grade) were used as received. Cyclohexane (HPLC grade) was doubly distilled before use. The water used as the mobile phase in the HPLC system was doubly distilled and filtered through a Norganic Trace Organic Removal Cartridge (Millipore, Bedford, MA, U.S.A.). The purity of the solvents was checked before use.

Silica gel TLC plates (20 × 5 × 0.025 cm) were obtained from Merck (Darmstadt, F.R.G.).

Standard PAH samples were commercial products (Fluka, Buchs, Switzerland; Aldrich, Milwaukee, WI, U.S.A.: Eastman Kodak, Rochester, NY, U.S.A.) or were obtained from the Community Bureau of Reference, BCR (Commission of European Communities, Brussels, Belgium).

A standard solutions was prepared that contained the following amount of PAH per microlitre of methanol: 40 pg of fluoranthene, 20 pg of benzo(*b*)fluoranthene, 5 pg of benzo(*k*)fluoranthene, 20 pg of benzo(*a*)pyrene, 10 pg of benzo(*ghi*)perylene and 100 pg of indeno(1,2,3-*cd*)pyrene. This solution was obtained by appropriate dilution from individual solutions of the standards of concentration 0.1 μg/μl. The standard solution was stored at 4°C in the dark.

Apparatus

The HPLC analysis was performed using a Perkin-Elmer Series 2 liquid chromatograph equipped with a Series LS-5 spectrofluorimetric detector and a column (16.5 × 0.46 cm I.D.) packed with 5 μm silica, chemically modified with octadecylsilane (ODS). The packing materials were obtained from Carlo Erba and Phase Separations (Norwalk, CT, U.S.A.).

Sampling

Conventional sampling practices were followed. The water samples were collected in duplicate in glass containers. The volume drawn was 5 l.

Mercury(II) chloride (50 mg/l) was added to inhibit microbial activity and the samples were stored at 4°C.

Analytical procedure

The water samples (2 l) were subjected to continuous liquid–liquid extraction with cyclohexane for 50 h at a distillation rate of 5 ml/min. Approximately 150 ml of solvent were used; at the end of the extraction about 100 ml were stratified on the water and the remainder constituted the extract. This was concentrated to 1–2 ml by a rotary evaporator at room temperature and then reduced to dryness under a stream of nitrogen. The residue was dissolved in tetrahydrofuran (100 μl) and applied as a streak to a silica gel plate and developed with *n*-hexane–benzene (1:1) in the dark. After removal of the mobile phase, the plate was examined under ultraviolet light (254 nm) to establish the position of the PAH spot. The spot was scraped off, powdered and transferred to a filtration system consisting of a fritted glass Buchner funnel (porosity 16–40 μm) on a vacuum flask in which was placed a test-tube to collect the filtrate. The PAHs were eluted from the silica gel with 5 ml of tetrahydrofuran. Traces of silica gel, which could damage the column in the subsequent HPLC analysis, were removed from the tetrahydrofuran solution by passage through a Millipore filter (0.5 μm). The filtrate was evaporated to dryness using a stream of nitrogen and the residue dissolved in 50–100 μl of methanol for HPLC analysis (the methanol volume depends on the expected concentration of PAHs in the sample).

The HPLC analysis was performed on a column packed with Erbasil C_{18}. The PAHs were separated isocratically with methanol–water (85:15, v/v) as the mobile phase at a flow-rate of 1 ml/min. The compounds were detected under the optimum conditions by selecting the appropriate excitation and emission wavelengths. The excitation wavelength was fixed and the emission wavelength was varied during the analysis.

The PAH identification was accomplished by comparison of the retention times and the stop-flow excitation and emission spectra of the sample with those of standard compounds.

The determination of individual PAHs was carried out by comparison of the sample peak areas with those of standards. The samples were analysed in parallel with blanks to ensure that the glassware and reagents were interferences free.

RESULTS AND DISCUSSION

Most of the PAHs present in waters are found to be associated with suspended matter and this particulate fraction mostly conditions the choice of the extraction technique. Given the characteristics of the type of water to which we intend to apply the method (waters with relatively low levels of suspended matter), it is not necessary to apply preventive filtration, and consequently the determined PAH concentrations are relative to the total aqueous sample as such. Moreover, a filtration step would necessitate analyses of both the filtered material and the particulate matter, with a consequent probability of losses of the compounds of interest, expecially when working at the ng/l concentration level. Even if a suitable filter is chosen, some of the suspended matter could pass into the filtrate and make the sample non-homogeneous. As the adsorbed PAHs require a different extraction technique to the dissolved PAHs, in which the time of contact with the extraction solvent is longer, there is a risk of incomplete recovery of the PAHs. This has been observed by other workers[33] who have examined the filtration problem.

Preliminary studies were carried out to evaluate more suitable extraction techniques for use with unfiltered samples. Continuous and discontinuous liquid–liquid extraction and adsorption on a microcolumn packed with C_{18} reversed-phase material or with graphitized carbon black were examined. Continuous liquid–liquid extraction gave the highest PAH recovery. To obtain a good recovery, an extraction time of 50 h was sufficient.

The use of TLC to separate the PAHs from the total organic fraction was studied in our laboratory previously[34–37] at the μg/g concentration level in different matrices. Tests carried out with standard solutions showed that tetrahydrofuran is the most suitable solvent for elution from thin layers. Studies to evaluate the recovery of PAHs at concentrations comparable to those in the samples were carried out and some results are reported in Table I.

Coextracted interferents varied considerably with the type of water sample examined. In samples of tap water and sea water the TLC step is not necessary because these samples did not contain impurities at the retention times of the compounds to be determined.

The separation and detection of PAHs by HPLC was carried out using a fluorescence detector, which imparts both selectivity and sensitivity to the system. We compared the performances of some commercial C_{18} packing materials. These materials, although chemically similar, provided different separation efficiences and different retention characteristics for PAHs. We focused attention on pairs of PAH isomers that are difficult to separate, *viz.*, benzo(*b*)fluoranthene–benzo(*k*)fluoranthene (BbF–BkF) and benzo(*ghi*)perylene–indeno(1,2,3-*cd*)pyrene (BghiPe–IP). We found that Erbasil C_{18} gave the best resolution of these PAHs. Fig. 1 illustrates the HPLC separation obtained with columns packed with Erbasil C_{18} and Spherisorb ODS_2. Although the latter column has a higher number of theoretical plates it has a lower resolution for the PAH isomers examined. The resolutions for BbF–BkF were 1.6 and 2.0 and those for BghiPe–IP were 0.9 and 1.5, respectively.

The separation characteristics of the column also depend on the mobile phase. We examined the resolution obtained with methanol–water and acetonitrile–water, eluent systems generally used for this type of separation. Methanol–water is better because the BghiPe–IP isomers are unresolved using acetonitrile–water whatever the percentage of acetonitrile used.

The described procedure has been applied to the evaluation of the degree of PAH pollution of the River Tiber[38] (Fig. 2).

The PAH concentrations were corrected for a blank; samples of water, purified by distillation and passage through cartridges suitable for eliminating organic sub-

TABLE I

MEAN RECOVERY (%) OF PAHs FROM THIN LAYER

Compound	*0.25 ng*	*0.5 ng*	*1 ng*
Fluoranthene	93	100	90
Benzo(*b*)fluoranthene	76	89	90
Benzo(*k*)fluoranthene	61	85	86
Benzo(*a*)pyrene	68	82	83

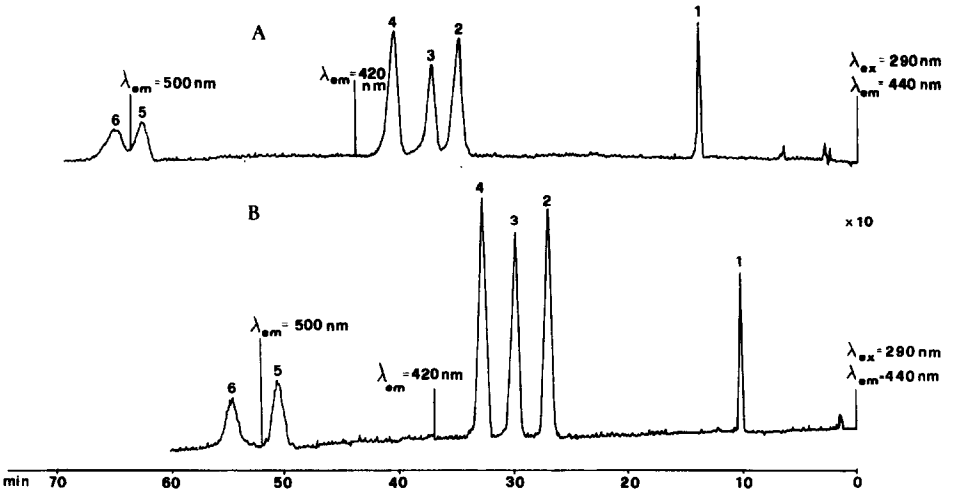

Fig. 1. HPLC traces of a standard mixture obtained on columns packed with different material: (A) 34 × 0.46 cm I.D. column of 5 μm Spherisorb ODS_2; (B) 16.5 × 0.46 cm I.D. column of 5 μm Erbasil C_{18}. Mobile phase: methanol–water (80:20). Flow-rate: 1 ml/min. Spectrofluorimetric detection. Peaks: 1 = fluoranthene; 2 = benzo(b)fluoranthene; 3 = benzo(*k*)fluoranthene; 4 = benzo(*a*)pyrene; 5 = benzo-(*ghi*)perylene; 6 = indeno(1,2,3-*cd*)pyrene.

stances, were prepared. These samples, when extracted and analysed under the same conditions as for the real samples, showed the presence of some PAHs. An accurate study to establish the origin of these compounds was therefore undertaken. We first verified the absence of PAHs from the materials and reagents used, then a water

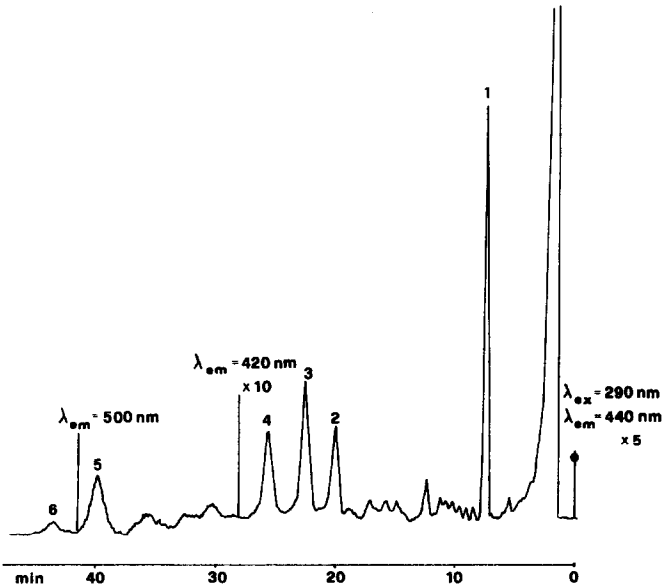

Fig. 2. HPLC trace of extract of River Tiber water. Column: 16.5 × 0.46 cm I.D. of 5 μm Erbasil C_{18}. Mobile phase: methanol–water (85:15). Flow-rate: 1 ml/min. Spectrofluorimetric detection. Peaks as in Fig. 1.

TABLE II

MEAN CONCENTRATIONS AND RELATIVE STANDARD DEVIATIONS FOR SOME PAHs DETERMINED IN WATER FROM THE RIVER TIBER

Compound	*Mean concentration* (ng/l)*	*Standard deviation* (ng/l)*
Fluoranthene	7.22	1.69
Benzo(*b*)fluoranthene	0.46	0.07
Benzo(*k*)fluoranthene	0.17	0.02
Benzo(*a*)pyrene	0.56	0.03
Benzo(*ghi*)perylene	0.11	0.02

* On 5 determinations.

sample of the described type was subjected to extraction and the extract was analysed at different times. After 50 h the concentration of the individual PAHs unexpectly remains almost constant. The probable explanation was that the small amount of PAHs present in the blank could have been formed during the process of boiling of cyclohexane in the extractor. In fact, PAH-free cyclohexane samples after boiling showed levels of some PAHs comparable to those of the blanks of distilled water.

The reproducibility of the method was determined by taking five sub-samples of the same sample of River Tiber water and executing three HPLC analyses on each sub-sample. Table II reports the mean values and the relative standard deviations obtained.

The recovery was determined by spiking samples of previously extracted distilled water with a solution of PAHs at a concentration of 5 ng/l each and values of $85 \pm 10\%$ were obtained.

REFERENCES

1 World Health Organization, *International Standard for Drinking Water*, WHO, Geneva, 3rd ed, 1971.
2 EEC, *Directive Relating of the Quality of Water for Human Consumption*, Official Gazette of the European Communities, L229, Brussels, 30-8-1980.
3 *Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants*, U.S. Environmental Protection Agency, Environment Monitoring and Support Laboratory, Cincinnati, OH, 1977.
4 M. A. Achenson, R. M. Harrison, R. Percy and R. A. Wellings, *Water Res.*, 10 (1976) 207.
5 D. Hoffman and E. L. Wynder, *Anal Chem.*, 32 (1960) 295.
6 S. F. Haenni and J. F. Smith, *Water Res.*, 11 (1977) 339.
7 W. H. Robbins, T. D. Searl, D. H. Wasserstrom and G. T. Boyer, in L. P. Jackson and C. C. Wright (Editors), *Analyses of Water Associated with Alternative Fuel Production*, ASTM STP T20, American Society for Testing and Materials, 1981, p. 149.
8 G. A. Junk, J. J. Richard, M. D. Grieser, D. Witiak, J. L. Witiak, M. D. Arguello, R. Vick, H. J. Svec, J. S. Fritz and G. V. Calder, *J. Chromatogr.*, 99 (1974) 745.
9 A. Tateda and J. S. Fritz, *J. Chromatogr.*, 152 (1978) 329.
10 P. E. Strup, J. E. Wilkinson and P. W. Jones, in P. W. Jones and R. I. Frendenthal (Editors), *Carcinogesis, Vol. 3: Polycyclic Aromatic Hydrogens*, Raven Press, New York, 1978, p. 241.
11 F. Bruner, F. Mangani and G. Furlani, *J. Chromatogr.*, 302 (1984) 167.
12 R. Kadar, K. Nagy and D. Fremstad, *Talanta*, 27 (1980) 227.
13 J. Saxena, J. Kozuchowski and D. K. Basu, *Environ. Sci. Technol.*, 11 (1977) 682.
14 D. K. Basu and J. Saxena, *Environ. Sci. Technol.*, 12 (1978) 791.
15 K. Ogan, E. Katz and W. Slavin, *J. Chromatogr. Sci.*, 16 (1978) 517.

16 E. P. Lankamayr and K. Müller, *J. Chromatogr.*, 170 (1979) 139.
17 G. Chatot, W. Jequier, M. Jay, R. Fontages and P. Obaton, *J. Chromatogr.*, 45 (1969) 415.
18 J. Borneff, in J. N. Pitts, R. C. Metcalf and A. C. Loyds (Editors), *Advances in Environmental Science and Technology*, Vol. 8, Wiley, New York 1977.
19 W. Giger and C. Schaffner, *Anal. Chem.*, 50 (1978) 243.
20 A. Biorseth, *Anal. Chim. Acta*, 94 (1977) 21.
21 A. Biorseth and G. Eklund, *Anal. Chim. Acta*, 105 (1979) 119.
22 F. M. Benoit, G. L. Label and D. T. Williams, *Int. J. Anal. Chem.*, 6 (1979) 277.
23 J. W. Elchelberger, E. H. Kerns, P. Olynyk and W. L. Budde, *Anal. Chem.*, 55 (1983) 1471.
24 B. S. Das and G. H. Thomas, *Anal. Chem.*, 50 (1978) 837.
25 K. Ogan, E. Katz and W. Slavin, *Anal. Chem.*, 51 (1979) 1315.
26 R. K. Sorrell and R. Reding, *J. Chromatogr.*, 185 (1979) 655.
27 N. T. Crosby, D. C. Hunt, L. A. Philp and I. Patel, *Analyst (London)*, 106 (1981) 135.
28 J. Borneff and H. Kunte, *Arch. Hyg. Bakt.*, 151 (1967) 202.
29 L. Scholz and H. Altmann, *Fresenius' Z. Anal. Chem.*, 240 (1968) 81.
30 S. Monarca, B. S. Causey and G. F. Kirkbright, *Water Res.*, 13 (1979) 503.
31 J. Borneff and H. Kunte, *Arch. Hyg. Bakt.*, 153 (1969) 220.
32 B. Crathorne and M. Fielding, *Proc. Anal. Div. Chem. Soc.*, 15 (1978) 155.
33 R. D. Smillie and D. T. Wang, in A. Biorseth and A. J. Dennis (Editors), *Polycyclic Aromatic Hydrocarbons, 4th International Symposium*, Battelle Press, Columbus, OH, 1981, p. 863.
34 L. Zoccolillo, A. Liberti and D. Brocco, *Atmos. Environ.*, 6 (1972) 715.
35 A. Candeli, G. Morozzi, G. Paolacci and L. Zoccolillo, *Atmos. Environ.*, 9 (1975) 843.
36 L. Zoccolillo, A. Liberti, F. Coccioli and M. Ronchetti, *J. Chromatogr.*, 288 (1984) 347.
37 M. Ronchetti, G. P. Cartoni and L. Zoccolillo, *J. Chromatogr.*, 348 (1985) 159.
38 G. P. Cartoni, F. Coccioli, T. La Noce, A. Liberatori, M. Ronchetti and L. Zoccolillo, *Proceedings of a Workshop Organized Within the Framework of the Concerned Action "Organic Micropollutants in the Aquatic Environment", Rome, March 1985*, Commission of the European Communities, Directorate-General for Science, Research and Development, Brussels, 1985, p. 43.