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Application of solid-phase extraction discs with a glass fiber matrix to fast determination of polycyclic aromatic hydrocarbons in water

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Abstract

The extraction of polycyclic aromatic hydrocarbons (PAHs) in water with solid-phase extraction (SPE) discs on a glass fiber matrix has been less studied than other systems such as SPE column extraction or the carbofluor matrix discs. In this paper we have studied SPE discs with a glass fiber matrix (SPE disc GFM) to extract PAHs from aqueous samples, which have then been separated and detected with high-performance liquid chromatography–fluorescence detection. We have found that the proposed method of analysis allows us to obtain detection limits of 0.1 ng/l for benzo[*a*]pyrene and a variation of 6% in the recovery of said compound at the level of 1 ng/l, and it complies with the required specifications for PAHs in the EU Directive draft on drinking water. The use of GFM discs allows us to shorten the extraction times of PAHs by between 3 and 12 times in comparison with other SPE systems. They can concentrate volume samples of up to 1 l, with PAH recoveries at the level of 1–2 ng/l higher than 80±10% and detection limits of between 0.1–2 ng/l, depending on the compound studied. © 1997 Elsevier Science B.V.

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1. Introduction

Different laws on water for public consumption passed by the European Union (EU), the United States (US) and the World Health Organisation (WHO) include control over polycyclic aromatic hydrocarbons (PAHs). The recent EU Directive draft [1] on drinking water proposes the determination of six PAHs (fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]-perylene and indeno[1,2,3-*cd*]pyrene), and limits the maximum concentration to 200 ng/l for all of them, and to 10 ng/l for the particular case of benzo[*a*]pyrene. Furthermore, it specifies that the analytical method used in the determination of these compounds must comply with the requirement of 25% precision (measured as variation rate at the analyzed level of concentration), a detection limit equal to 25% of maximum admissible concentration (MAC) (2.5 ng/l for benzo[*a*]pyrene and 50 ng/l for the rest), and also an accuracy of 25%.

US legislation [2] includes benzo[*a*]pyrene and limits it to a concentration of 200 ng/l, but does not specify the characteristics of the method to determine PAHs, though the most widespread methods in the US are those of the US Environmental Protection Agency (EPA) [3] or those of the standard methods (SM) [4], which are similar. In these two methods sixteen compounds are checked, whereas in the EU only six PAHs are checked. Furthermore, they propose gas chromatography (GC) or high-performance liquid chromatography (HPLC) after a previous liquid–liquid extraction of the PAHs. HPLC yields better detection limits (from 280 to 18 ng/l) than

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GC. The WHO, in its latest "Guidelines for drinking water quality" [5], considers only benzo[*a*]pyrene with a reference level of 700 ng/l, and in Volume 2 on "Health criteria and other supporting information" [6] proposes GC–MS or HPLC with fluorescence detection (FD) as reference techniques. It also mentions that with the latter method the detection limits obtained are 0.1 ng/l. HPLC–FD has been generally reported to provide better detection limits [4,6].

SPE columns off-line and on-line [7–10] and C_{18} extraction discs of different matrices have been used as alternative techniques for the isolation and concentration of PAHs with liquid–liquid extraction. The best known SPE discs are the ones that support the reversed-phase C_{18} on a PTFE matrix. Recently SPE discs with a glass fiber matrix have appeared on the market. For such discs the only analytical procedure to analyze PAHs is proposed by the manufacturer [12].

In this paper we compared the three SPE systems of extraction (columns, PTFE discs, GFM discs), taking into account the filtering time that each case requires, the analysed sample volume and the recoveries of the PAHs at the ng/l level. Then, we studied the GFM discs more closely after having proved that they had fewer clogging problems than the other systems when we analysed real water samples that were already filtered per 0.45 μm. Thus we studied the PAH recoveries at the level of ng/l set by the proposed Directive. We also improved these recoveries by adding isopropanol to the standards and to the samples, and we determined the optimal % of isopropanol for 1 l samples with the established equipment and conditions. We followed the supplier's advice for the conditioning of the GFM discs. The elution of the analytes was carried out with different fractions of *n*-hexane.

Furthermore, we evaluated the detection limits of the method from calibrated curves of five levels, where each level of the curve included the whole analytical process (extraction with GFM disc of standards 1 l HPLC–FD). Therefore, it was not necessary to take into account the recoveries to calculate the final concentrations. We studied the detection limits for two concentration intervals, one ranging from 0 to 8 ng/l and the other ranging from 0 to 2 ng/l, noticing a great improvement in the detection limits when we used low range curves.

The determination of PAHs by means of HPLC–FD has been sufficiently verified [3,4,13,14]. In previous studies we fixed the conditions of separation and detection of the six analysed PAHs and we optimized the programming criterium of the excitation and emission wavelengths for benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene.

The method of analysis proposed in this paper has been applied to determine PAHs in real water samples from different origins (sea water, river water and drinking water), in order to study the possible interferences in the various matrices, especially in the case of benzo[*a*]pyrene, the best studied analyte.

Though this technique complies with the specifications on precision and detection limits required in the Directive draft, we have not been able to verify the accuracy because we do not have reference waters with certified PAHs.

The use of SPE discs with a glass fiber matrix allows us to considerably reduce the extraction times of PAHs compared with other existing systems. We have obtained detection limits of 0.1 ng/l (less than 2.5 ng/l) for benzo[*a*]pyrene, recoveries of 85% and variation rates of 6% (less than the 25% value of the Directive) for 1 ng/l standards.

2. Experimental

2.1. Sample treatment and preparation of standards

The water samples were collected in an amber glass bottle and filtered with glass fiber membranes of 0.45 μm (AP 1504700) from Millipore (Bedford, MA, USA), and they were processed in the 24 h following their collection.

The PAH standards were prepared from individual solutions in acetonitrile of 10 ng/μl of fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]-pyrene from Dr. Ehrenstorfer (Augsburg, Germany), supplied by Scharlau, (Spain). The intermediate standard of 100 μg/l was prepared from the originals by means of dilution with acetonitrile, HPLC grade, from Merck (Darmstadt, Germany). These standards were kept in the dark at −20°C. The calibration standards of 1–50 ng/l were prepared from the standard of 100 μg/l in Milli-Q water (RX system+

Milli-Q system) of Millipore, and they were prepared just prior to use. The intermediate standards of 1–10 μg/l were prepared in a mixture of acetonitrile–Milli-Q water (50:50, v/v).

10% of isopropanol HPLC grade, from Merck, was added as a surfactant to the blanks, standards and already filtered samples before the extraction of the organic compounds.

2.2. Cleaning and conditioning of columns and SPE discs

Three different SPE systems were studied: (1) SPE columns from J.T. Baker (Denventer, Netherlands) with a glass body and 500 mg of C_{18} filling; (2) C_{18} discs with a PTFE matrix with a diameter of 47 mm (Empore Discs) from Varian (Harbor City, CA, USA); (3) C_{18} discs with a glass fiber matrix with a diameter of 47 mm (Envi-Disc) from Supelco (Bellefonte, PA, USA). The Envi-Discs have a glass fiber matrix and a C_{18} modified surface and were studied more closely. Filtration equipment from J.T. Baker was used to carry out the extraction of PAHs with SPE columns, while Supelco equipment (Envi-Disc TM Holder Manifold) was used in the extraction of discs under the conditions advised by the manufacturer.

The conditioning of the columns was carried out by passing at low flow 6 ml of *n*-hexane, followed by 3 ml of methanol (both from Merck) and 6 ml of Milli-Q water (from Millipore). Both kinds of discs studied were conditioned in the same way: 5 ml of cyclohexane (from Merck) followed by 5 ml of hexane, 5 ml of methanol and 5 ml of Milli-Q water. The extraction systems were conditioned just before filtering the sample.

2.3. Elution and reconcentration of analytes

The elution of PAHs was carried out with four fractions of 0.5 ml of *n*-hexane in the case of the SPE columns and three fractions of 5 ml of *n*-hexane. The extracts obtained were dehydrated by passing them through anhydric sodium sulfate from Panreac (Montcada i Rexac, Barcelona, Spain). The extracts were evaporated at room temperature under a current of nitrogen 5.0, and 200 μl of acetonitrile was added to the residue. This final solution was passed to a microvial (HP5181-1270) from Hewlett-Packard (Palo Alto, CA, USA) for later analysis by HPLC.

2.4. Separation with HPLC–FD

The HPLC system consising of an automatic injector-sampler, degassing system, pumping system and gradient formation was an HP-1050 system from Hewlett-Packard, controlled by a Vectra VL2 4/50 computer, which handles the equipment control programs, the analysis sequence and the integration (Chemstation HP rev. A3-01).

For the separation of the compounds we used a Vydac 201 TP 54 column (Hesperia, CA, USA), 150 mm×4.0 mm, filled with reversed-phase of 5 μm. We injected 25 μl of sample and we worked at 27°C with a constant flow-rate of 1.8 ml/min. The elution of the compounds was carried out with the following rates of acetonitrile–water: initial conditions acetonitrile–water (60:40, v/v); at 2.5 min acetonitrile–water (60:40, v/v); at 10 min acetonitrile–water (90:10, v/v); at 20 min acetonitrile–water (0:100, v/v). The programmable fluorescence detector used to detect the PAHs was an HP-1046A from Hewlett-Packard and the excitation (λ_{exc}) and emission (λ_{ems}) wavelengths were optimized, setting the following program: time 0 min λ_{exc} 250 nm, λ_{ems} 40 nm; time 9 min λ_{exc} 255 nm, λ_{ems} 420 nm; time 14.5 min λ_{exc} 230 nm, λ_{ems} 400 nm; time 15.8 min λ_{exc} 250 nm, λ_{ems} 495 nm; time 20 min λ_{exc} 250 nm, λ_{ems} 400 nm.

3. Results and discussion

In our laboratory we normally use the screening technique proposed by Tomingas and Grover [15] to determine PAHs, which includes liquid–liquid extraction, separation with high-performance thin-layer chromatogarphy (HPTLC) and detection with fluorescence photodensitometry, with a detection limit for benzo[*a*]pyrene of 10 ng/l. We also carried out the separation and detection of PAHs with HPLC–FD.

Due to the large amount of solvent (dichloromethane) used in the process of liquid–liquid extraction and to the formation of emulsions in the case of some real samples, we began to work with C_{18} SPE columns to concentrate PAHs. We noticed that many

samples from river water and drinking water caused clogging of the columns, and so it was usual for the processing of 500 ml of sample to take about 3.5–4 h. Therefore, we decided to use SPE discs instead of columns and we tried discs with PTFE matrix, with which the processing of 1 l samples of water previously filtered per 0.45 μm took 1 h. When the discs with a glass fiber matrix (GFM) were used, the same samples needed between 10–20 min to complete the process, depending on the particular case. Due to the additional advantage obtained from the improvement of the filtering time, we thought we should study the GFM discs in more detail to test whether they would satisfy the analytical requirements in the Directive draft on drinking water.

Before studying the GFM discs, we determined the optimal chromatographic conditions of separation and detection for the studied compounds. In Fig. 1 we can see the separation obtained for a mixture of 10 μg/l of the six PAHs mentioned in the Directive draft. Generally, the separation technique with HPLC–FD for the PAHs in aqueous matrices has not given any problems. However, the most delicate part of the chromatogram, from the point of view of detection, is between benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene, because its λ_{exc} and λ_{ems} are different, and both compounds, though they can be correctly separated, elute close together. This means that, before analysing the samples, the change time of indeno[1,2,3-*cd*]pyrene wavelengths is proved to be correct. In order to do that we use a standard of PAHs. We calculate the correct change time by adding 0.05 min to the time when the previous compound (benzo[*ghi*]perylene) has just eluted.

Table 1 shows the results of the recoveries of PAHs using the three SPE extraction systems mentioned above. The same table shows the analysed sample volumes, the concentration of PAHs used and the number of repetitions made in each case. The results are given as %recovery±%relative standard deviation (R.S.D.). We should note that these studies were carried out at very low concentrations of analyte, especially in the case of GFM discs where standards of 1 ng/l have been used. In all three cases, the concentration of fluoranthene and indeno[1,2,3-*cd*]pyrene are ten and five times superior to the concentration of benzo[*a*]pyrene, due to their lower sensitivity. The recoveries of PAHs with the three systems of extraction are very similar but we can see a slightly higher variability in the case of SPE columns and PTFE discs. The most important difference is the time of filtration required in each case: 3.5–4 h for a sample of 500 ml with SPE columns, 1 h for a sample of 1000 ml with PTFE discs and 10–15 min for 1000 ml with GFM discs.

We studied the effect of isopropanol on the recovery of PAHs at low concentration and determined the optimal %. Table 2 shows the results obtained, given as %recovery. We observed that the addition of 5% and 10% of isopropanol to the sample increased the compound recoveries, whereas no improvement was achieved when 15% of isopropanol was used. Taking these facts into account, we chose to use isopropanol at 10%, because we observed better recovery of benzo[*a*]pyrene.

Table 3 shows the results of the sensitivity of the PAHs relative to fluoranthene, the least sensitive compound in the studied series. The most sensitive of all is benzo[*k*]fluoranthene, the response being of the same order as for benzo[*a*]pyrene. Table 3 also shows the detection limits of the method, obtained by using two different criteria. The first criterion is based on the determination of the detection limits from the standard deviation of the blanks ($3S_b$), and the other is based on the standard deviation of the residuals of the measured calibrated curve (for $n=4$ repetitions)(statistical $S_{y/x}$). If we compare the results obtained with both criteria we can observe that the criterion for the residuals is much stricter than that of the blanks. We determined the detection limits from calibrated curves with different concentration intervals. We can observe that when we use low interval curves (between 0–2 ng/l) the limits improve by between two and ten times, depending on the compound. The calibrated curves were obtained from five points (0, 0.5, 1, 1.5 and 2 ng/l for the low interval; 0, 2, 4, 6 and 8 ng/l for the high interval), and the complete analytical procedure was applied to each point. The recoveries obtained for the different levels are of 80–90% and the curves show correlations (R^2) of 0.999, which allows us to quantify the compounds by means of the external standard method. If we compare the detection limits with those required in the Directive draft, of 2.5 ng/l for the benzo[*a*]pyrene, we have in all cases limits of 0.56 and of 0.09 ng/l, even by the strictest criterion. The

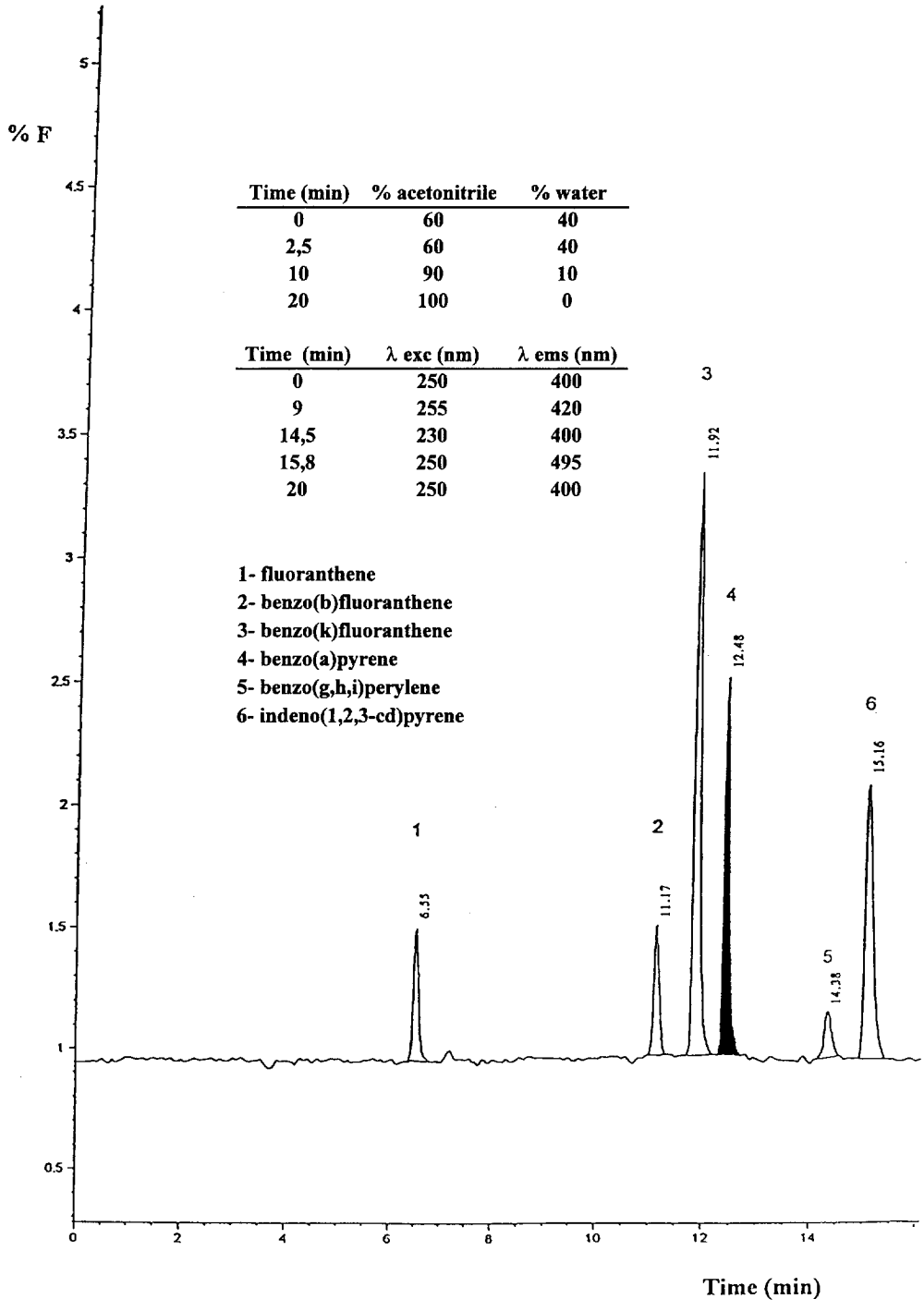

Fig. 1. Separation of PAHs by HPLC–FD. The standard was 10 μg/l for each compound, except fluoranthene (100 μg/l) and indeno[1,2,3-*cd*]pyrene (50 μg/l). The chromatographic conditions were: flow-rate 1.8 ml/min, injection volume 25 μl, temperature 27°C, column Vydac 201 (150×4 mm), eluent acetonitrile–water. The gradient and wavelength programme are indicated at the top of the figure.

Table 1
PAHs recoveries with SPE columns, SPE disk PTFE matrix and SPE disk glass fibre matrix (GFM)

Compound	SPE column V=0.5 l 5–50 ng/l, n=7	PTFE disk V=1 l 1–5 ng/l, n=6	GFM disk V=1 l 1–2 ng/l, n=9
Fluoranthene	105±12	85±12	78±9
Benzo[*b*]fluoranthene	89±12	92±15	85±9
Benzo[*k*]fluoranthene	77±9	86±13	85±9
Benzo[*a*]pyrene	80±6	86±13	85±6
Benzo[*ghi*]perylene	82±9	83±11	93±5
Indeno[1,2,3-*cd*]pyrene	58±14	75±15	78±10

Results are shown as %recovery±%relative standard deviation (R.S.D). Volume of sample, concentrations and number of repetitions are also indicated.

Table 2
Effect of isopropanol on the PAH recoveries

Compound	Isopropanol			
	0%	5%	10%	15%
Fluoranthene	66	80	77	65
Benzo[*b*]fluoranthene	78	79	87	72
Benzo[*k*]fluoranthene	77	86	88	78
Benzo[*a*]pyrene	77	81	88	73
Benzo[*ghi*]perylene	78	92	96	89
Indeno[1,2,3-*cd*]pyrene	70	73	82	70

Extractions made from 1 l of water spiked with 1 ng/l for each compound, except for fluoranthene (10 ng/l) and indeno[1,2,3-*cd*]pyrene (5 ng/l).

most unfavorable case is that of the fluoranthene, with limits of 1.63 ng/l and of 7.90 ng/l, much lower than the 50 ng/l value required by the Directive for this compound.

The limiting factor to the precision of an analytical method in which extractions are made at the level of ng/l is the variability in the recoveries of the analysed compound. Table 1 shows that we obtained an R.S.D. inferior to the 25% of the Directive.

The proposed method of analysis has been used to determine PAHs in samples of sea water, river water and drinking water. In sea water and river water no matrix problems were detected when additions of 1–2 ng/l were made. But in the samples of drinking water (Fig. 2a) we could see that the area of the benzo[*a*]pyrene peak decreased significantly, obtaining recoveries of only 10% (Fig. 2b). Therefore, we carried out tests adding sodium sulphite to the samples of water to remove the free residual chlorine and thus obtained recoveries of about 80% (Fig. 2c). Fig. 3a shows a chromatogram of the extract of 1 l of river Ebro water fortified with a mixed standard of PAHs of 1 ng/l for every compound, except for fluoranthene (10 ng/l) and indeno[1,2,3-*cd*]pyrene (5 ng/l). Quantification is more complex in the zone of fluoranthene, because in this initial zone a larger number of unidentified compounds elute. However, no problems have been detected in the zone of

Table 3
Sensitivity, relative to fluoranthene, and detection limits (DL, ng/l) for PAHs according to the criteria explained in the paper (blanks S_b and calibration curves $S_{y/x}$)

	Sensitivity relative	Range 2–8 ng/l		Range 0.5–2 ng/l	
		DL (ng/l) Criterion ($3S_b$)	DL (ng/l) Criterion ($3S_{y/x}$)	DL (ng/l) Criterion ($3S_b$)	DL (ng/l) Criterion ($3S_{y/x}$)
Fluoranthene	1	2.57	9.68	0.17	1.63
Benzo[*b*]fluoranthene	9	0.17	0.84	0.05	0.07
Benzo[*k*]fluoranthene	34	0.08	0.81	0.04	0.11
Benzo[a]pyrene	*33*	*0.12*	*0.68*	*0.05*	*0.09*
Benzo[*ghi*]perylene	10	0.09	0.78	0.04	0.23
Indeno[1,2,3-*cd*]pyrene	4	0.58	5.58	0.02	0.80

DL=Detection limit.

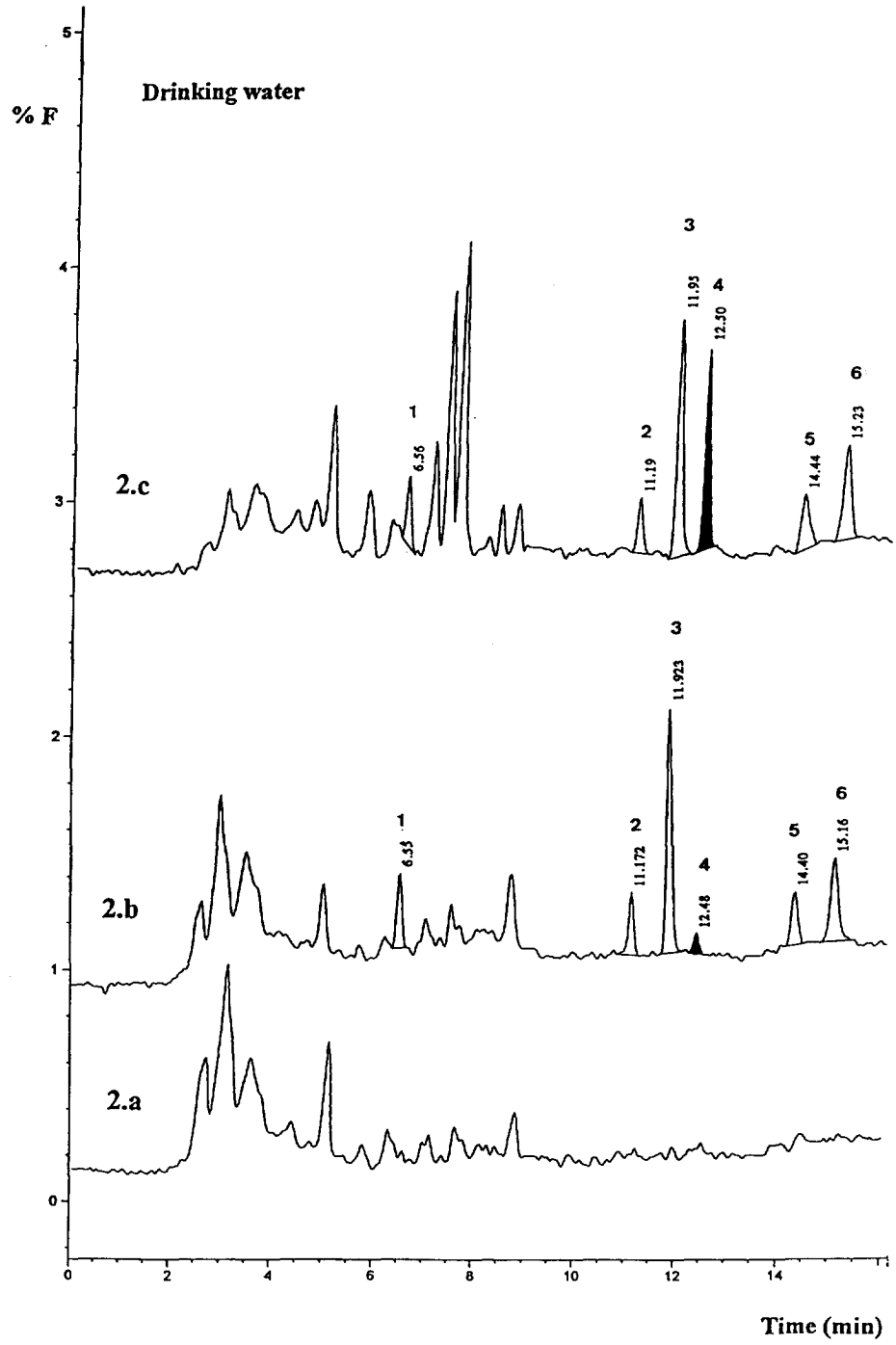

Fig. 2. Effect of sodium sulfite on the benzo[*a*]pyrene recovery in chlorinated water. (a) 1 l of drinking water; (b) 1 l of drinking water spiked with 1 ng/l for each compound except fluoranthene (10 ng/l) and indeno[1,2,3-*cd*]pyrene (5 ng/l); (c) the same as (a), but the sample was treated previously spiked with sodium sulfite.

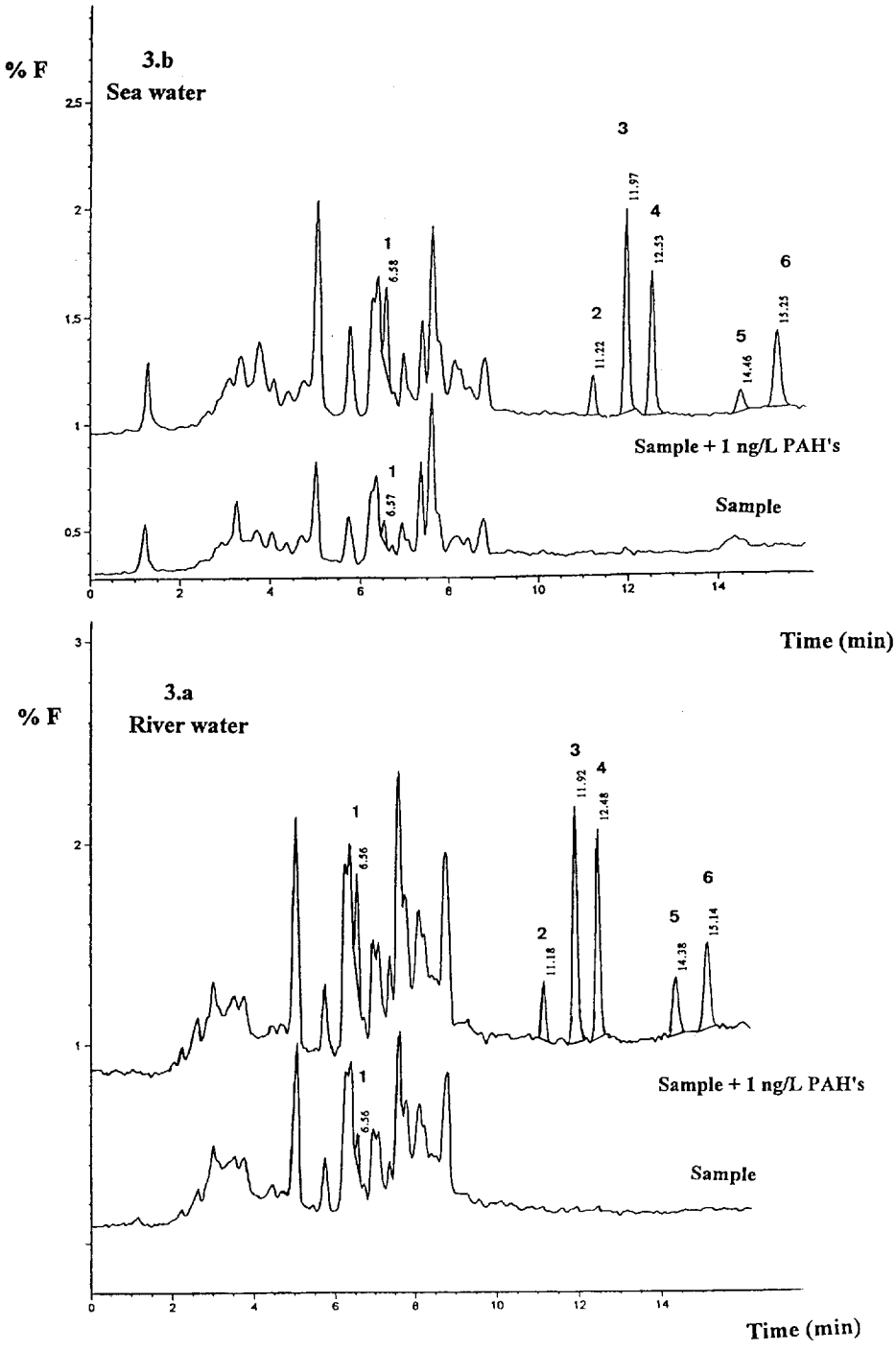

Fig. 3. Separation and recovery of PAHs in sea water and river water, after being spiked with 1 ng/l for each compound, except fluoranthene (10 ng/l) and indeno[1,2,3-*cd*]pyrene (5 ng/l) to 1 l of sample. (a) River water; (b) sea water.

benzo[*a*]pyrene, the compound that interests us most because it is the PAH that receives the strictest treatment in all the consulted legislation. Fig. 3b shows the chromatogram obtained for sea water (1 l sample; 1 l sample spiked with standard of 1 ng/l). In this case, the initial zone of the chromatogram is less complex. The recoveries of PAHs are in both cases about 80%.

After testing the validity of the method according to the criteria of the Directive draft, we applied this technique over a period of five months to determine PAHs in the river Ebro water and in drinking water coming from said river. During the study period we could only quantify fluoranthene and benzo[*k*-]fluoranthene at the level of 0.8–15 ng/l and 0.2–0.7 ng/l, respectively. The rest of the PAHs were below the detection limit.

We could not verify the accuracy of the method because we could not find natural samples of water with concentrations of certified PAHs. The inter-laboratory tests in which our laboratory takes part do not include these kinds of tests. In the future these compounds should be included in inter-laboratory tests, so that accuracy can be studied.

4. Conclusions

The use of SPE discs with a GFM as a preparation procedure, combined with the analysis HPLC–FD, is an ideal method to determine PAHs in aqueous samples, regardless of the special characteristics of the matrix. In this way, the method allows the concentration of a large volume of sample (1 l) in less than 20 min, thus achieving the detection and precision criteria required by the Directive (method with 25% of precision and detection limit of 2.5 ng/l for the benzo[*a*]pyrene).

Furthermore, the SPE discs with GFM present additional advantages compared with other existing extraction techniques. The amount of solvent required in the process is lower in the case of the SPE discs and it is normally possible to considerably reduce the sample processing time. The major surface area of discs in relation to C_{18} cartridges permits the filtering of a large volume of sample without clogging problems (up to 1 l of real samples), improving the detection limit (DL) of the method. Changing the C_{18} support on the discs (PTFE matrix by GF matrix) the time of filtration improves by five, probably because the GF matrix is more compatible with aqueous samples than the PTFE matrix. Recoveries and precision for both SPE discs are comparable in this report, but other authors [11] have obtained better results for PTFE discs than us. Our results may be explained by the fact that the range of concentrations used varied considerably, 80 ng/l compared with the 1 ng/l used here. Nevertheless, in both cases the standard deviations are lower than 10%.

More studies are needed to determine the accuracy of the method. One possibility would be to include the study of these compounds in inter-laboratory analysis comparisons in the future.

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