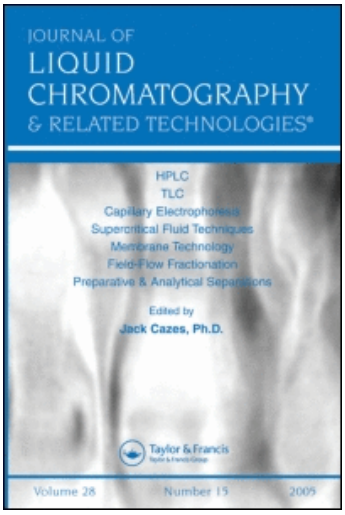

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RAPID DETERMINATION OF PAHs IN DRINKING WATER SAMPLES USING SOLID-PHASE EXTRACTION AND HPLC WITH PROGRAMMED FLUORESCENCE DETECTION

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ABSTRACT

A rapid, sensitive and selective method for determining 13 PAHs in drinking water samples using solid-phase extraction and HPLC with programmed fluorescence detection is developed. A solid-phase extraction method is described for preconcentrating the PAHs on Sep-Pak vac tC-18 cartridges. The volume of water analyzed was 1500 mL. The PAHs were eluted with ethyl ether, the eluates were evaporated to dryness and the residue was dissolved in methanol. The PAHs were analyzed on a Hypersil Green PAH column and a program of nine excitation and emission wavelength pairs were used. A mobile phase gradient of acetonitrile-water was used. It is possible to detect all the individual PAHs at very high sensitivity, at levels of ng/L. Recoveries were 60-96% for 12 PAHs at concentration levels of 2.33-48.7 ng/L with relative standard deviations in the range 0.4-10% (n=4). The method was applied to determine PAHs in tap-water and reservoir-water samples.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that represent the largest class of suspected chemical carcinogens. PAHs can be formed from both natural and anthropogenic sources. PAHs are produced by the incomplete combustion and pyrolysis of fossil fuels among other organic materials. The carcinogenic and toxic nature of PAHs, specifically benzo(a)pyrene and dibenzo(ah)anthracene, have increased the need to detect these compounds. The presence of PAHs in polluted air has been extensively studied but water has received much less attention. The distribution of PAHs in different bodies of water is dependent on their sources and the PAH solubilities in water.[1-3]

The analytical procedures most often used for PAH determination include: **a)** isolation of PAHs from water by solid phase extraction (SPE), such as reverse phase silica, polystyrene-divinyl benzene,[4-7] or **b)** liquid-liquid extraction (LLE) with organic solvents such as cyclohexane, n-hexane or methylene chloride.[6,8-11] The analytical techniques used to separate and determine them are gas chromatography (GC) with flame ionization (FID) or mass spectrometry (MS) detectors[7,9] and reverse phase high performance liquid chromatography (RP-HPLC) with spectrophotometric (UV-VIS) or fluorimetric (FL) detection.[11-13]

Because of the poor chromatographic resolution of some PAHs pairs, such as fluorene-acenaphthene and chrysene-benzo(a)anthracene, and the very low PAH concentrations in water samples, the combination of off-line RP-SPE with RP-HPLC-FL is attractive.

In a previous paper we reported a sensitive, selective method for fluorimetric detection which allows ng/L concentration levels to be determined using the Hypersil Green PAH column.[14] In this paper, we present a HPLC method for determining PAHs in drinking water using solid-phase extraction. To detect the PAHs, the excitation and emission wavelengths were time programmed over the chromatogram.

EXPERIMENTAL

Apparatus and Materials

The chromatographic system consisted of the following components: a Milton-Roy CM 4000 high-pressure-gradient pump (Rivera Beach, FL); a Rheodyne 7125 with 20 μL loop injector (Cotati, CA); a Perkin Elmer LS 30 luminiscence spectrometer (Norwalk, CT) and a Milton Roy CI 4100 integrator. The column

used was a Hypersil Green PAH (100 x 4.6 mm) (5 μm particulate size) by Shandon (England); a P-Selecta Precisterm bath was used to maintain the column temperature below 22 °C (Barcelona, Spain). A Sep-Pak vac tC-18 (500 mg, Waters; Milford, MA) and Extra-sep C-18 (1000 mg, Phenomenex) (Torrance, CA) cartridges were used to extract and preconcentrate the PAHs from water samples. A vacuum flask (1000 mL, Pobel; Madrid, Spain); separatory funnels (100, 1000 mL, Pobel); a Barna-vacio vacuum pump and a Visiprep vacuum manifold system (Supelco; Bellefonte, PA) were also used. All PAHs solutions were prepared using a P-Selecta ultrasonic bath. Solvents used to prepare the mobile phase and sample eluates were filtered through nylon Lida membrane filters (Kenosha, WI) with 0.45 μm pore size. A P-Selecta Meditronic centrifuge capable of 4200 rpm (3700 g) was also used.

Chemicals

Stock standard methanolic solutions of the PAHs with concentrations in the range $(10\text{-}1.0)\times10^{-4}$ M were prepared by dissolving the solids (Sigma; St. Louis, MO) in methanol. A working standard mixture was prepared by dilution of the stock standard solutions with methanol. HPLC purity acetonitrile, methanol and ethyl ether (Carlo Erba; Milan, Italy) were used. The other solvents and chemical reagents were also of HPLC purity. Water was purified with a Millipore Milli-Q system (Milford, MA).

Procedures

1. Water sample collection:

Tap-water samples were collected in the Faculty of Chemistry of the Universidad Complutense in Madrid city, and reservoir-water samples were collected from three reservoirs in Madrid (Picadas, La Pinilla and Valmayor). All samples were collected during the winter 1995, in amber bottles, previously cleaned with acetonitrile, in order to minimize photolytic decomposition, and were stored in a refrigerator at 4 °C from the time of collection until extraction. All samples were extracted immediately, within 24 hours of collection, and were then analyzed.

2. Sample preparation:

On receipt, the samples were filtered with nylon filters and extracted immediately with Sep-Pak vac tC-18 cartridges. The cartridges were previously conditioned with 6 mL of methanol, twice, and then with 6 mL of Milli-Q water, twice. 1500 mL of Milli-Q water containing the PAH mixture in the range 3.5-73

Table 1

Linear Gradient of the Mobile Phase

Time	Acetonitrile, %	Water, %
0.0	50	50
3.0	50	50
15.0	78	22
23.0	100	---
35.0	100	---
37.0	50	50

ng, or 1500 mL of water samples were preconcentrated with the cartridges at a flow rate of 50 mL/min. The cartridges were then dried in the vacuum system for 5 minutes, and then centrifugated at 1200 rpm for another 5 minutes. The adsorbed compounds were eluted from the Sep-Pak vac cartridges first with 3 mL, then with 1 mL of ethyl ether, at a flow rate of 1.5 mL/min.

The eluates were collected in a graduated glass tube and the solvent was evaporated by means of the vacuum manifold system; since solvent evaporation is an energy absorption process, in order to maintain the temperature constant at 20 ± 2 °C, the tube containing the eluate was inserted in a glass containing water at 20 ± 2° C. The residue was dissolved to 1 mL in methanol using the ultrasonic bath. This solution was filtered through nylon filters and analyzed by HPLC by injecting 20 μL into the HPLC system and applying the calibration procedure.

3. Calibration:

Standard solutions containing mixtures of the 13 PAHs were prepared at four concentrations levels in the range 0.4-150 μg/L. These solutions were analyzed by RP-HPLC with fluorimetric detection. In a previous paper, we have developed this chromatographic method.[14] The chromatographic parameters are the following: an acetonitrile-water mobile phase using the gradient is shown in Table 1, at a flow-rate of 1 mL/min at 22 °C.

Fluorescence detection was performed with time programming of excitation and emission wavelength pairs (the program is detailed in Table 2). The injection volume was 20 μL. The integrated peak areas were used to quantitate the PAHs. The mobile phase was degassed with helium. Figure 1 shows a chromatogram of a standard mixture of 13 PAHs.

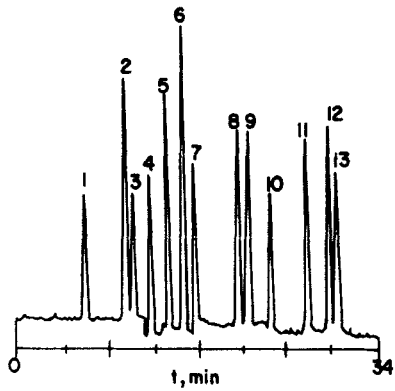

Figure 1: Chromatogram of a standard mixture of 13 PAHs. Conditions: Hypersil Green PAH (100 x 4.6 mm) column; Temperature, 22°C; Mobile phase, gradient of acetonitrile/water, see Table 1; Flow-rate, 1 mL/min; Fluorimetric detection, see Table 2; Injection volume, 20 µL. Peaks: 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, B(a)a; 9, chrysene; 10, B(e)p; 11, B(a)p; 12, Db(ah)a; 13, B(ghi)p.

Table 2

Program of Excitation and Emssion Wavelength Pairs

Detected Compound	Time, s	λ_{ex},nm	λ_{em},nm
Naphthalene, Acenaphthelene			
Fluorene	0	280	324
Phenanthrene	720	250	365
Anthracene	840	254	402
Fluoranthene	920	285	465
Pyrene	990	270	390
B(a)a, Chrysene	1230	270	384
B(e)p	1410	290	390
B(a)p	1590	295	405
Db(ah)a, B(ghi)p	1755	290	418

B(a)a, Benzo(a)anthracene; B(e)p, Benzo(e)pyrene; B(a)p, Benzo(a)pyrene; Db(ah)a, Dibenzo(ah)anthracene; B(ghi)p, Benzo(ghi)perylene

Table 3

Influence of Eluent Type on the Recoveries of PAHs

	Recoveries %*									
PAHs	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Naphthalene	19	42	--	--	14	5.4	--	--	--	--
Acenaphthene	33	24	--	--	10	6.3	--	--	--	--
Fluorene	26	18	--	--	16	8.7	1.3	--	--	--
Phenanthrene	43	24	45	56	33	18	54	47	11	13
Anthracene	43	19	44	53	21	30	49	84	11	12
Fluoranthene	41	20	52	40	44	22	50	66	46	44
Pyrene	44	25	67	44	42	30	36	80	98	28
B(a)a	35	18	67	86	31	25	42	57	61	54
Chrysene	30	15	65	81	30	23	36	56	58	60
B(e)p	19	12	67	74	29	27	36	85	97	63
B(a)p	19	13	68	78	28	31	41	53	72	55
Db(ah)a	33	27	91	74	34	51	47	55	57	59
B(ghi)p	17	13	80	71	25	32	35	55	80	63

* Mean of two determinations

1, Acetontrile; 2, Methanol; 3, Methylene chloride, 4, Ethyl ether; 5, n-Hexane; 6, n-Pentane; 7, n-Pentane/Ethyl ether (0.6/10, v/v); 8, Methylene chloride/Ethyl ether (1/1, v/v); 9 and 10, Methanol/Ethyl ether (1/1 and 1/2, v/v, respectively).

RESULTS AND DISCUSSION

Optimization Of Solid Phase Extraction (SPE) Experimental Conditions

1. Selection of the eluent:

Various solvents were tested for PAH elution on SPE: acetonitrile, methanol, methylene chloride, ethyl ether, n-hexane and n-pentane, and the following mixtures (v/v): n-pentane/ethyl ether(0.6/10), methylene chloride/ethyl ether (1/1) and methanol/ethyl ether (1/1 and 1/2). 1 mL of eluent was used. The results are shown in Table 3. The best results, for the majority of PAHs, were obtained with methylene chloride and ethyl ether; the mean recoveries were 50% and 51% for methylene chloride and ethyl ether, respectively.

Table 4

Influence of Water Volume on the Recoveries of PAHs

PAH	Recoveries, %* Water Volume, mL							
	50	**100**	**250**	**500**	**1000**	**1500**	**2000**	**1000****
Naphthalene	--	--	--	18	25	20	--	--
Acenaphthene	17	32	42	59	57	65	73	19
Fluorene	35	51	55	70	68	72	91	36
Phenanthrene	64	74	82	80	76	96	75	60
Anthracene	51	53	68	70	64	71	73	85
Fluoranthene	67	78	81	77	78	95	94	86
Pyrene	42	60	72	66	64	89	46	65
B(a)a	57	65	76	74	71	95	79	87
Chrysene	58	66	75	71	70	76	73	87
B(e)p	47	57	63	65	64	85	60	82
B(a)p	46	41	52	57	51	67	56	92
Db(ah)a	39	45	50	53	54	66	66	57
B(ghi)p	38	39	39	48	46	60	38	55
Mean Recoveries	43	51	58	63	63	74	63	62

* Mean of four determinations. Relative standard deviations are in the range of 0.4 to 10.

** Values obtained by applying the clean-up procedure recommended in the 525 EPA method.

2. Selection of the eluent volume:

The recoveries are a function of the number and eluent volume of the desorption steps. Volumes in the range of 1-4 mL of the two solvents were tested. Where 50%, 53%, 68% and 64% has been the mean recoveries of 1, 2, 3 and 4 mL of methylene chloride, respectively, and 51%, 74%, 79% and 76% has been the mean recoveries of 1, 2, 3 and 4 mL of ethyl ether, it was observed that best results were achieved with 3 mL of ethyl ether, with a mean recovery of 79% (n = 2). However, to improve reproducibility the elution was carried out in two steps. First eluting with 3 mL, then with 1 mL of ethyl ether, collecting the two eluates in a tube. For the selection study of nature and volume of eluent, 100 μL of standard mixture of PAHs in the range 35-730 μg/L were eluted through the tC-18 cartridge.

3. Selection of volume of water sample flow through Sep-Pak:

To improve recovery of the PAHs, several volumes between 50 to 2000 mL of Milli-Q water containing amounts of PAHs in the range 3.5-73 ng were tested. Table 4 shows the results obtained. Volumes above 250 mL gave mean recoveries of over 50 %. The best recoveries are achieved with 1500 mL of Milli-Q water, recoveries being in the range 60-96% for 12 PAHs. The low recovery obtained for napthalene (20%) is due to its volatility especially during the concentration of the eluates by evaporation of the solvents. These results are similar to those reported in the literature.[15-18]

The relative standard deviations were in the range 0.4-10% (n=4). The results were compared with those obtained by applying the procedure recommended in the EPA 525 Method,[7] using 1000 mL of water, which also are shown in Table 4. In general, higher recoveries were obtained by applying our method, except for the following PAHs: anthracene, chrysene and benzo(a)pyrene.

4. Effect of PAH concentration in water samples:

1500 mL of water were spiked with 200, 100, 50 and 25 μL of PAHs standard mixtures at concentration levels of 35-730 μg/L. The PAH concentrations and the PAH recovery results are shown in Table 5. The best mean recoveries were 74% and 73% corresponding to the PAH concentration levels of 2.33 - 48.7 ng/L and 1.16 -24.4 ng/L, respectively. The relative standard deviations were in the range 0.4 - 19% (n=4).

Determination of PAHs in Drinking Water

The proposed method was used to determine the 13 PAHs in two types of drinking water sample: tap-water and reservoir-water. Three tap-water samples were analyzed; in these samples no PAHs were detected. On the other hand, three samples from three reservoirs, indicated in experimental section, were also analyzed. A chromatogram corresponding to sample 1 (Picadas reservoir) is shown in Figure 2; in the chromatogram peaks were detected at the retention times of the PAHs, the first being naphthalene and the last, chrysene.

The results corresponding to three reservoir-water samples are summarized in Table 6, which also shows those obtained by applying the EPA clean-up procedure. In general the results of proposed method are higher than those obtained by applying the EPA Method 525, because the recoveries are also smaller for the latter method. Relative standard deviations were determined from four replicates on the same

Table 5

Influence of PAH Concentration in the Study of PAH Recoveries from Water Samples*

PAHs	C_1, ng/L	R_1, %	C_2, ng/L	R_2, %	C_3, ng/L	R_3, %	C_4, ng/L	R_4, %
Naphthalene	97.4	11	48.7	20	24.4	16	12.2	–
Acenaphthene	48.0	73	24.0	65	12.0	45	6.00	29
Fluorene	22.6	66	11.3	72	5.65	78	2.83	30
Phenanthrene	14.7	60	7.33	96	3.66	95	1.83	72
Anthracene	12.0	66	6.00	71	3.00	54	1.50	46
Fluroanthrene	5.34	86	2.67	95	1.34	82	0.670	82
Pyrene	6.66	68	3.33	89	1.66	93	0.830	48
B(a)a	9.34	68	4.67	95	2.34	81	1.17	73
Chrysene	17.3	83	8.67	76	4.34	91	2.17	78
B(e)p	14.7	80	7.33	85	3.66	84	1.83	38
B(a)p	4.66	70	2.33	67	1.16	56	0.580	43
Db(ah)a	10.7	71	5.33	66	2.66	79	1.33	64
B(ghi)p	9.34	73	4.67	60	2.34	90	1.17	–
Mean Recoveries		67		74		73		46

Water volume: 1500 mL; R, %: percentage recovery.

* Mean of four determinations. Relative standard deviations are in the range of 0.4-19%.

C_1, C_2, C_3 and C_4: PAHs concentration in spiked water samples.

sample; the values were between 3-15%. The amounts of PAHs in the samples are below the maximum permitted limits of 0.2 μg/L.[19] As can be seen in Table 6, the high molecular weight PAHs, such as benzo(e)pyrene, benzo(a)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene, which are the most toxic, were not detected. These results can be explained on the basis that these PAHs are very insoluble in water, there being a rough correlation between increasing molecular weight and decreasing solubility, and therefore it must be assumed that a large proportion of the PAH content of polluted water is adsorbed on suspended solids.[1,3]

CONCLUSIONS

The proposed procedure for the extraction and preconcentration of PAHs in water samples is faster than the EPA Method 525, due to the amount of packing material used in cartridges. Additionally, it affords a large reduction in the volume

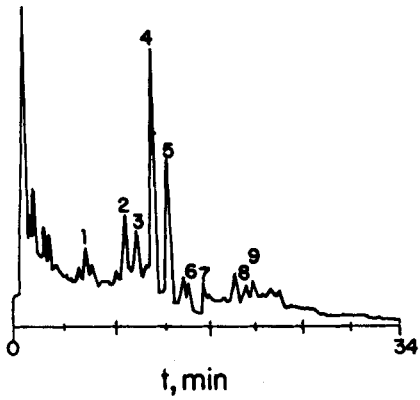

Figure 2: Chromatogram of a Picadas reservoir-water sample. Conditions: Hypersil Green PAH (100 x 4.6 mm) column; Temperature, 22°C; Mobile phase, gradient of acetonitrile/water, see Table 1; Flow-rate, 1 mL/min; Fluorimetric detection, see Table 2; Injection volume, 20 µL. Peaks: 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, B(a)a; 9, chrysene.

Table 6

Determination of PAHs in Reservoir-Water Samples. (ng/L)*

PAH	Samples** 1		2		3	
	A	B	A	B	A	B
Naphthalene	4.8	3.8	--	--	--	--
Acenaphthene	3.6	2.2	2.3	--	13	--
Fluorene	2.0	1.8	1.3	3.6	6.7	--
Phenanthrene	5.1	3.1	5.6	4.4	2.8	0.9
Anthracene	1.2	3.5	0.1	0.1	1.1	1.8
Fluoranthene	0.04	0.06	0.1	--	0.6	0.1
Pyrene	0.1	0.3	1.1	0.8	0.8	0.2
B(a)a	0.1	0.4	--	--	1.1	0.4
Chrysene	0.3	0.4	--	--	1.0	1.2

*Mean of four determinations. Relative standard deviations are in the range 3-15%.
**Samples collected from three reservoirs in Madrid during the winter 1995:
1) PICADAS; 2) LA PINILLA; 3) VALMAYOR. **A**: Proposed method;
B: Applying the clean-up procedure recommended in 525 EPA Method.

of solvent required and, consequently, is very economical. The chromatographic method allows sensitive, selective determination of PAHs in drinking water samples at ng/L levels, due to use of the Hypersil green PAH column and the excitation and emission wavelength pairs program.

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