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Solvent-free method for the determination of polynuclear aromatic hydrocarbons in waste water by solid-phase microextraction–high-performance liquid chromatography with photodiode-array detection

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Abstract

Solid-phase microextraction (SPME) has rapidly been established among the practical alternatives for sample preparation for gas chromatography. Nevertheless polynuclear aromatic hydrocarbons (PAHs) are more effectively monitored by high-performance liquid chromatography (HPLC), but initially, there was no simple way to introduce analytes extracted by SPME into an HPLC system. A SPME–HPLC interface was developed by Supelco, which enables one to take advantages of the time and solvent savings offered by SPME. In the present work six PAHs from the European Union directives: fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[ghi]perylene, indeno[1,2,3-*cd*]pyrene were evaluated after optimization of a sample preparation method with a 100-μm poly(dimethylsiloxane) fiber. Repeatability, reproducibility, correlation coefficients, linearity, recoveries and limits of detection where determined and are indicated.
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1. Introduction

Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, which present a potential health concern because their toxicity, mutagenicity and carcinogenicity in animals [1,16]. In 1976 the European Union (EU) listed 132 dangerous substances of target analytes that should be monitored as dangerous substances unloaded into the aquatic environmental [2]. In this context it is fundamental to develop analytical methods and improve sample preparation techniques, so that this directive could be respected and that human health can be protected. Today classical extraction techniques are useless and solid-phase extraction (SPE) has become the sample preparation technique of choice [10,14,15]. More recently a new extraction technique appeared – solid-phase microextraction (SPME) – which integrates sampling, extraction, concentration and sample introduction, in a single step [3]. This new technique constitutes a new environmental chemistry philosophy as this phasing out of the solvent is expected to induce a major change in analytical methodology [4] and also allows for the scientific community formulate new and practical alternatives to the known sample preparation methods. SPME consists of two processes: first, the partitioning of the analytes between the coating

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of the fiber and the sample matrix and secondly, the desorption of the concentrated analytes into an analytical instrument, followed by separation and quantification. SPME applications have focused on extracting organic compounds from various matrixes such as air, water and soils. SPME presents all the advantages of SPE such as simplicity, low cost, easy automation and on-site sampling as well as eliminates disadvantages of SPE such as plugging and the use of solvents [5]. The automation and optimization of SPME has also been studied by some authors [6,17] and its importance is justified for application in other different situations. The study of more complex samples will implicate the study of a higher number of factors that will affect the process, so a deep concern will be needed to ensure consistent results. Also it is expected that improvements will be made on the fibers by the manufacturer in response to new environmental problems. As far as we know, waste water samples collected from the effluent of the treatment plant of a petrochemical refinery have never been studied until now. Some approaches are scarcely shown [7] when some aromatic compounds, mainly from the benzene/toluene/ethylbenzene/xylenes (BTEX) fraction were extracted by this technique and analysed by ultraviolet absorption spectroscopy [8]. The objectives of the present work were: (i) to develop an analytical method based on the use of poly(dimethylsiloxane) (PDMS) fibers for the determination of PAHs in contaminated effluents; (ii) to apply the knowledge to the study of real samples.

2. Experimental

2.1. Chemicals and reagents

The acetonitrile was HPLC-grade obtained from Riedel-de Haen (Germany). Fluoranthene (FLT), benzo[*b*]fluoranthene (B[*b*]FLT), benzo[*k*]fluoranthene (B[*k*]FLT), benzo[*a*]pyrene (B[*a*]P), benzo-[ghi]perylene (B[*ghi*]PER), indeno[1,2,3-*cd*]pyrene (I[1,2,3-*cd*]P), were purchased from Supelco, Bellefonte, PA, USA. They were mostly of >99% purity. The water was of HPLC-grade. Each PAH was first diluted in acetonitrile and then a PAH mixture of 20 mg/l in acetonitrile was prepared.

2.2. Equipment

The chromatographic determinations were carried out on a Waters high-performance liquid chromatography (HPLC) system which includes an automated gradient controller, two HPLC-pumps W.510, a column oven with a temperature control module, a six-port Rheodyne 7725i manual injector and a photodiode array detector W.996 all from Waters, Milford, MA, USA. Data were collected by a personal computer using Waters Millenium 2010 software. All separations were carried out on a C_{18} HPLC column, a Vydac 201 TP (250×4.6 mm, 5 μm) (Sigma–Aldrich, Hesperia, USA) purchased from Supelco. It used a SPME–HPLC interface, that consisted of a desorption chamber and a mounting bracket adapted to the six-port injection valve referred to above. The desorption chamber replaces the injection loop in the HPLC system. This chamber is made of stainless steel, to which the mobile phase line from the pump can be connected. All surfaces that come in contact with the SPME fiber or the mobile phase are stainless steel or Vespel, for inertness and solvent resistance. The SPME fiber is introduced into the desorption chamber under ambient pressure when the injection valve is in the "load" position. The fiber is sealed in place with a double-tapered Vespel ferrule, and locked with a convenient pressure clamp. Within the interface, mobile phase contacts the SPME fiber, removes the adsorbed analytes and delivers them to the column for separation. Commercialized 7- and 100-μm PDMS fibers were used to extract compounds from water. A 100-μm PDMS fiber, without epoxy glue produced by Supelco especially for this work was also used. The fused-silica fiber is connected to a stainless steel tubing in order to increase the mechanical strength of the fiber assembly for repeated analysis. The stainless steel tubing is then contained in a specially designed syringe. A SPME fiber holder, for manual use in HPLC, was used with the reusable, SPME fibers assemblies. All these materials were produced by Supelco. To ensure controlled temperature and agitation during extraction was used an heated agitation platform (Variomag). The ultrasonic apparatus was a Sonorex, from Bandelin Electronic, Berlin, Germany. Borosilicate 4-ml amber glass vials and a SPME sampling hand were

used for sample extraction all from Supelco. The magnetic stir bars were purchased from Sipaco.

2.3. Sample collection

Treated plant effluent was collected at a discharge pipe of a petrochemical plant in Porto on October 1997. Sample was maintained in a pyrex borosilicate amber glass container. Each bottle was conveniently rinsed prior to sample addition. Sample preservation was accomplished by storing the bottles at 4°C, protected from light, immediately after sampling.

2.4. Sample preparation

The water samples used for study were prepared by spiking the PAH mixture standard solution into HPLC-grade water, at different concentrations. The waste water sample was also prepared by spiking a sample volume with the same standard solution. All spiked samples were allowed to stand in the ultrasonic apparatus for some minutes, to assure perfect homogeneity.

2.5. Chromatographic conditions

All samples introduced by the SPME unit were performed manually. The column temperature was set to 30°C. Chromatograms were extracted at 254 nm. The solvents used as mobile phase were acetonitrile and HPLC-grade water. The solvent program was: 87% acetonitrile, isocratic for 5 min, then increasing linearly to 100% acetonitrile in 20 min, staying there for 5 min and finally back to initial conditions in 5 min, always with a flow-rate of 0.8 ml/min.

2.6. Solid-phase microextraction procedure

The fibers were conditioned as recommended by Supelco, directly in the HPLC system. So, the fiber was placed in the interface, the injection valve was switched from the ''load'' to the ''inject'' position, allowing the mobile phase to pass through the interface during the chromatographic run. Each day, prior to sample analysis, several blank desorptions were made to ensure that fiber and needle were free from contaminants. All the vials used for sample extraction were washed with tap water, high-purity water and solvent in use, prior to heating in a muffle. The magnetic stir bars had the same treatment as the vials except the heating. The first step in SPME is to allow the fiber to dry for some minutes (2–3 min) if it has been desorbed in organic solvents, because if they are present they may affect the extraction of the next sample. Then the fiber was exposed to the water sample for a period of time. The fiber was withdrawn from the water sample and introduced in the interface into the HPLC system. Within the interface the mobile phase contacts the SPME fiber, removes the adsorbed analytes and delivers them to the column for separation. Analytes were removed in a moving stream of mobile phase-dynamic desorption. The SPME procedure was optimized. For that purpose HPLC spiked water at 200 μg/l each compound was used. A 4-ml extraction sample was used to study the effect of temperature (room temperature and 45°C) and agitation during extraction. The desorption time was optimized to avoid memory effects. For compound extractions, 7- and 100-μm PDMS fibers were used. The relation between the time of extraction and maximum adsorption obtained for each compound was also studied for a 100-μm PDMS fiber: several samples of 200 μg/l spiked HPLC-water were submitted to SPME under optimized conditions for different extraction times (30, 60, 90 and 120 min). The behaviour of two different 100-μm PDMS fibers for the extraction of 4 ml of spiked water with 200 μg/l of each PAH, at room temperature, with agitation, during 30 min is described. The calibration curve was done with seven points whose concentrations were: 300, 200, 100, 50, 10, 5 and 1 μg/l. For each point three extractions of HPLC spiked water were made. The matrix effects were studied with 100 μg/l of spiked waste-water. A 100-μm PDMS fiber was used to extract 4 ml of this sample, at room temperature, with agitation, during 30 min.

3. Results and discussion

The direct contact of cross-linked polymers with organic solvents often results in swelling of polymers. So a 7-μm PDMS coating has been recommended to avoid blockage of flow inside the desorption chamber due to swelling of PDMS [5].

Table 1
SPME of 4 ml of spiked water at 200 μg/l each PAH, at room temperature (20°C), with and without agitation, during 30 and 60 min

Compounds	Peak area (μV s)[a]			
Temperature/agitation Time of extraction	20°C/with agitation 30 min	20°C/with agitation 60 min	20°C/without agitation 30 min	20°C/without agitation 60 min
FLT	171 278	389 824	58 393	109 397
B[*b*]FLT	62 622	198 830	15 517	24 705
B[*k*]FLT	70 363	235 133	12 345	13 644
B[*a*]P	69 948	242 254	14 346	10 729
B[*ghi*]PER	21 221	74 359	3154	7261
I[1,2,3-*cd*]P	81 670	282 935	22 933	11 957

[a] Mean area of four extractions.

Nevertheless 100-μm PDMS provided highest extraction levels (greatest sensitivity) [9], as we could verify. This was very important for the objective of the work and this latter fiber was chosen, although the equilibration was faster with 7-μm coating. The extraction volume of 4 ml was recommended by Supelco. The sensitivity of SPME analysis is not significantly enhanced by larger sample volumes [11] but mainly by the film thickness of the fiber. Better results for extraction of PAHs with 100-μm SPME were obtained at room temperature (maximum R.S.D. was 28.1%). At 45°C the mean areas obtained can be better for some compounds, but there are much more variations in the results (maximum R.S.D. was 47.0%). Due to the temperature, little bubbles are formed around the fiber preventing the analytes from reaching it. Better results were also obtained with agitation. A thin static aqueous layer, adjacent to the fiber is formed in SPME without agitation [3] and analytes have to diffuse through this layer of water so that they can be adsorbed by the fiber coating, which is more difficult. All these results are very well expressed in Tables 1 and 2. SPME is an equilibrium process, in which analytes partition between the sample matrix and a polymeric stationary phase, exposed to the sample for a predetermined period of time. Extraction occurs according to analyte's partition coefficient, K, between the two phases. At equilibrium, the amount extracted by the stationary phase can be calculated [12]. Fig. 1 shows the time of extraction necessary to reach the equilibrium for the studied compounds, which is about 90 min. Nevertheless a 30-min extraction time was chosen, because for routine analysis it is not necessary to reach the equilibrium since the time of the fiber exposure is maintained [13] for the sample extractions and for the calibration procedure. The adsorption time of 30 min is a reasonable compromise between a good peak area and a acceptable time analysis. During a chromatographic run we can

Table 2
SPME of 4 ml of spiked water at 200 μg/l each PAH, at 45°C, with and without agitation, during 30 and 60 min

Compounds	Peak area (μV s)[a]			
Temperature/agitation Time of extraction	45°C/with agitation 30 min	45°C/with agitation 60 min	45°C/without agitation 30 min	45°C/without agitation 60 min
FLT	303 402	429 873	152 049	252 246
B[*b*]FLT	120 940	205 225	61 177	80 743
B[*k*]FLT	78 192	108 707	44 522	42 641
B[*a*]P	88 554	143 014	43 434	64 269
B[*ghi*]PER	20 331	26 487	12 621	11 240
I[1,2,3-*cd*]P	93 517	137 983	59 846	63 541

[a] Mean area of four extractions.

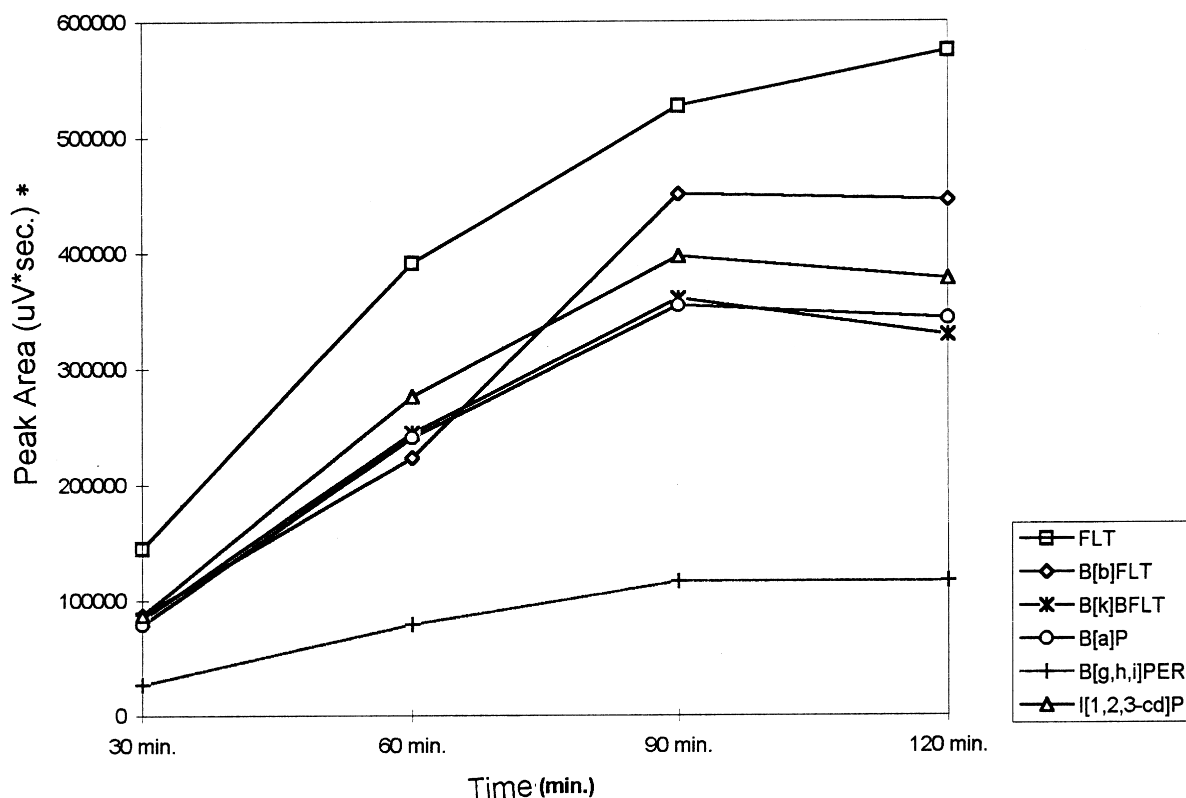

Fig. 1. Equilibration time for each PAH for the SPME of 4 ml of a spiked water at 200 μg/l, at room temperature, with agitation, with the 100-μm PDMS fiber.

be extracting the next sample. The desorption process will be 90% complete at time $t=L^2/2D$, where L is the coating thickness and D is the diffusion coefficient of an analyte. In this case the thickness of the PDMS coating is 100 μm, and the diffusion coefficient of analytes in a liquid layer is about 10–10 m^2/s. The desorption time required is thus less than 1 s, assuming continuously flowing mobile phase and good solubility of analyte in the mobile phase [5]. The chromatograms obtained for direct injection throw the loop and SPME fiber injection, showed that retention times are almost identical which is consistent with a very fast desorption process of the analytes from the fiber coating and with a very little contribution of desorption chamber geometry to the dead volume of the whole system as it was reported [5]. The desorption procedure is very important in HPLC and needs to be optimized for each application as well as for different solvent compositions. If the desorption process is not complete, analytes left in the coated phase may be subsequently desorbed, giving rise to false signals in blank analyses. The carry-over could also influence analysis of samples containing the target analytes if the extraction time is significantly less than the equilibrium time [12]. So, a desorption time of 2 min was used to ensure that almost all compounds were desorbed from the fiber, and that no significant carry-over was found, which was happening with shorter desorption times, for the highest concentrations. With this desorption time no peaks appeared in the chromatogram of the second blank. The repeatability of the process was evaluated by the study of peak retention time and peak area for three extractions of 200 μg/l spiked HPLC-water sample at selected extraction conditions in the same day. R.S.D. values indicate good quantitative accuracy of the method. Reproducibility was studied with the results of five

Table 3
Repeatability[a] of migration time and peak area of SPME of 4 ml 200 μg/l spiked water with a 100-μm PDMS fiber, at room temperature and with agitation, during 30 min

Compound	t_R (min)	R.S.D. (%)	Peak area (μV s)	R.S.D. (%)
FLT	6.079±0.08	1.2	$15.40\cdot10^4\pm0.95\cdot10^4$	5.9
B[*b*]FLT	11.257±0.45	3.9	$7.91\cdot10^4\pm0.46\cdot10^4$	12.2
B[*k*]FLT	12.645±0.61	4.6	$8.34\cdot10^4\pm0.18\cdot10^4$	2.0
B[*a*]P	14.323±0.72	4.9	$8.53\cdot10^4\pm0.73\cdot10^4$	8.0
B[*ghi*]PER	18.962±1.10	5.9	$2.59\cdot10^4\pm0.24\cdot10^4$	8.5
I[1,2,3-*cd*]P	20.140±1.30	6.6	$9.48\cdot10^4\pm0.77\cdot10^4$	7.8

[a] Average of three extractions in the same day.

extractions under the same conditions with different 100-μm PDMS fibers on three different days. The R.S.D. values exhibit that peak retention times and areas are quite reproducible with the SPME–HPLC interface. Results are listed in Tables 3 and 4. The results obtained for the same extraction conditions, using two different 100-μm PDMS fibers, are shown in Table 5. The results are quite different from each other, which of course is a big problem for routine analysis. So, as we already indicated, there is a need of concern that in future the manufacture will attempt this problem and find a solution for it. Calibration was performed by extraction of spiked samples. The linear regression equations with slopes and correlation coefficients are summarised in Table 6. The minimum detectable amounts (LODs) at a signal-to-noise ratio of 3 for the six PAHs are also given in Table 6 and range between 1 and 5 μg/l. Industrial waste waters contain a complex matrix characterized by the presence of different interferences and pollutants. Therefore, to evaluate the matrix effect and its influence on SPME, waste water sample was spiked at 100 μg/l and submitted to SPME, under the selected conditions. Table 7 shows the recoveries obtained for the target compounds, varying from 73 to 104%. A chromatogram obtained for the injection of a 100-μm PDMS fiber after SPME of 4 ml spiked waste water 100 μg/l each PAH, at room temperature, with agitation, during 30 min can be seen in Fig. 2.

Table 5
Comparation of the results obtained for the SPME of 4 ml of a spiked water at 200 μg/l, at room temperature, with agitation, during 30 min, with two different 100-μm PDMS fibers

Compound	Peak area (μV s)[a]	
	Fiber 1	Fiber 2
FLT	291 485	163 020
B[*b*]FLT	183 399	81 570
B[*k*]FLT	162 422	85 133
B[*a*]P	141 355	92 610
B[*ghi*]PER	43 371	27 766
I[1,2,3-*cd*]P	174 372	101 880

[a] Mean area of two extractions.

4. Conclusions

The extraction of PAHs from water by SPME is a very fast and simple method that requires no organic

Table 4
Reproducibility[a] of migration time and peak area of SPME of 4 ml 200 μg/l spiked water with a 100-μm PDMS fiber, at room temperature and with agitation, during 30 min

Compound	t_R (min)	R.S.D. (%)	Peak area (μV s)	R.S.D. (%)
FLT	6.065±0.07	0.95	$15.75\cdot10^4\pm1.2\cdot10^4$	5.0
B[*b*]FLT	11.249±0.46	2.84	$7.49\cdot10^4\pm1.6\cdot10^4$	15.5
B[*k*]FLT	12.639±0.62	3.40	$8.19\cdot10^4\pm2.5\cdot10^4$	19.8
B[*a*]P	14.332±0.71	3.50	$8.75\cdot10^4\pm2.7\cdot10^4$	20.8
B[*ghi*]PER	19.009±1.21	4.20	$2.55\cdot10^4\pm0.6\cdot10^4$	17.4
I[1,2,3-*cd*]P	20.215±1.24	4.70	$9.72\cdot10^4\pm3.0\cdot10^4$	20.8

[a] Average of five extractions on three different days.

Table 6
Calibration data obtained for SPME of 4 ml spiked water with a 100-μm PDMS fiber, at room temperature and with agitation, during 30 min

Compounds	Calibration equation	R	R^2	Linear range (μg/l)	LOD (μg/l)
FLT	$y=1.05 \cdot 10^3 x$	0.991	0.982	1–300	1
B[*b*]FLT	$y=9.40 \cdot 10^2 x$	0.990	0.976	1–200	1
B[*k*]FLT	$y=8.00 \cdot 10^2 x$	0.988	0.977	5–200	5
B[*a*]P	$y=6.78 \cdot 10^2 x$	0.997	0.995	1–300	1
B[*ghi*]PER	$y=2.13 \cdot 10^2 x$	0.990	0.981	5–300	5
I[1,2,3-*cd*]P	$y=8.25 \cdot 10^2 x$	0.997	0.994	1–300	1

Table 7
Recoveries for the six PAHs after SPME in 4 ml waste water spiked with 100 μg/l, at room temperature, with agitation, during 30 min ($n=3$)

Compound	Recovery (%)	R.S.D. (%)
FLT	104	4.6
B[*b*]FLT	94	11.7
B[*k*]FLT	90	13.5
B[*a*]P	97	21.5
B[*ghi*]PER	73	29.4
I[1,2,3-*cd*]P	86	20.4

solvents, others than the mobile phase, and does not need sophisticated equipment. The SPME technique is an inexpensive alternative to other extraction techniques as each fiber can be used repeatedly, for about 50 extractions. Another advantage of SPME is that only a very small sample volume is necessary and on-site field sampling study is facilitated. The agitation during SPME is very important as results can be substantially improved. On the other hand the effect of the heating seems to be prejudicial for the PAHs extraction. No significant carry-over was found after the optimization of the method. The method is relatively insensitive to matrix effects if standard parameters are controlled, as good recoveries and good detection limits obtained for the waste water proved. SPME seems to be a powerful approach for analysis of PAHs in waste water. The main problem emerges from the fact that the performance of the 100-μm PDMS fibers is not linear. This is a problem to be solved by the manufacturer.

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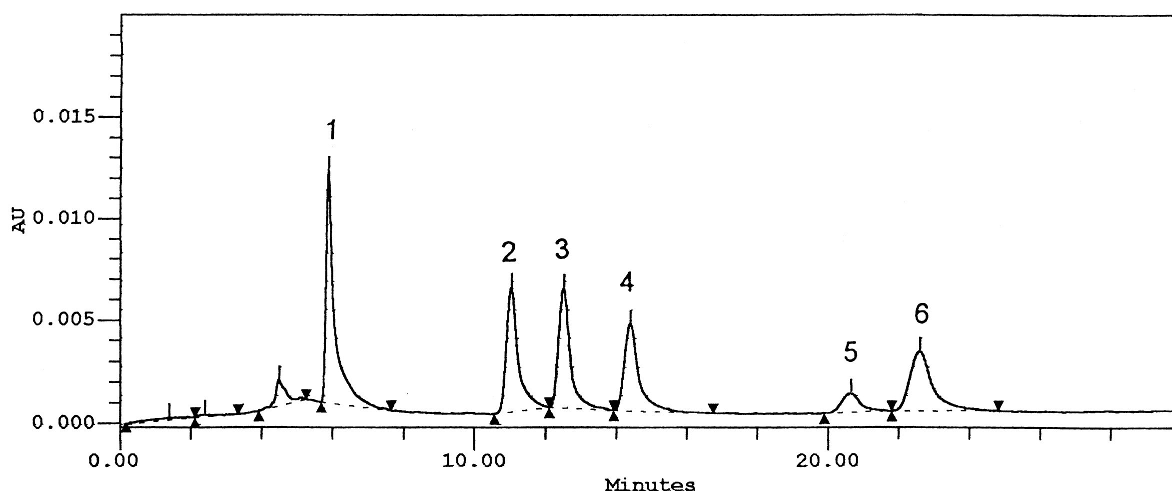

Fig. 2. Chromatogram obtained for the injection of a 100-μm PDMS fiber after SPME of 4 ml spiked waste water 100 μg/l each PAH, at room temperature, with agitation, during 30 min. 1=FLT; 2=B[*b*]FLT; 3=B[*k*]FLT; 4=B[*a*]P; 5=B[*ghi*]PER; 6=I[1,2,3-*cd*]P.

given to the resolution of some problems that appeared during the work.

References

[1] A. Byorseth, T. Ramdahl (Eds.), Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 2, Emission, Sources and Recent Progress in Analytical Chemistry, Marcel Dekker, New York, Basel, 1985, pp. 1–21.
[2] Directive 76/464/CEE, Commission of the European Community, Brussels, 4 May 1976.
[3] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A–853A.
[4] D. Noble, Anal. Chem. 65 (1993) 693A.
[5] J. Chen, J.B. Pawliszyn, Anal. Chem. 67 (1995) 2530–2533.
[6] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, Anal. Chem. 64 (1992) 196.
[7] B.L. Wittkamp, S.B. Hawthorne, D.C. Tilotta, Anal. Chem. 68 (1997) 1204.
[8] B. Macgillivray, J. Pawliszyn, P. Fowlie, C. Sagare, J. Chromatogr. Sci. 32 (1994) 317–322.
[9] Application Note 99, Supelco, Bellefonte, PA, 1995.
[10] D. Barceló, M.F. Alpendurada, Chromatographia 42 (1996) 704–711.
[11] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, Anal. Chem. 68 (1996) 144–155.
[12] A. Boyd-Boland, J. Pawliszyn, Anal. Chem. 68 (1996) 1521–1529.
[13] M.T. Almeida, P.M.A.R. Conceição, M.F. Alpendurada, Analusis 25 (1997) 51–54.
[14] A. Tanabe, H. Mitobe, K. Kawata, M. Sakai, J. Chromatogr. A 754 (1996) 159.
[15] I. Ferrer, D. Barceló, J. Chromatogr. A 737 (1996) 93–99.
[16] K.J. Hageman, L. Mazeas, C.B. Grabanski, D.J. Miller, S.B. Hawthorne, Anal. Chem. 68 (1996) 3892–3898.
[17] C. Rivasseau, M. Caude, Chromatographia 41 (1995) 462–470.