Determination of Some Polycyclic Aromatic Hydrocarbons in the Caspian Seawater by HPLC Following Preconcentration with Solid-Phase Extraction

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ABSTRACT: *A solid-phase extraction (SPE) method for sample clean-up and preconcentration followed by reversed-phase high-performance liquid chromatography (RP-HPLC) with uv detection is reported for determination of polycyclic aromatic hydrocarbons (PAHs) in the caspian seawater. A good resolution was obtained using acetonitrile:water (40:60) as mobile-phase for separation of these compounds. The effect of experimental variables, such as breakthrough volume with different concentrations of PAHs, type and volume of solvents for elution step were studied in SPE using C_{18} cartridge as sorbent. Different solvents (methanol, hexane and chloroform) were used for elution step in SPE. The best solvent for elution was hexane with volume of 3 ml, recoveries over 82 % and relative standard deviations lower than 6 %. Detection limits ranging from 0.02 to 0.14 $\mu g ml^{-1}$ were obtained for different PAHs using HPLC method. Concentration of PAHs in the caspian seawater were 0.34 to 14.11 $ng ml^{-1}$.*

KEY WORDS: *Caspian seawater, Solid-phase extraction, Polycyclic aromatic Hydrocarbons, HPLC, Water analysis.*

INTRODUCTION

Since PAH compounds are carcinogenic, identi-ficatation and determination of these compounds in environment are very important [1].These substances can be produced in natural and anthropogenic processes and they can be found in many different kinds of samples, both biological and environmental. For this reason, their detection and monitoring has become an important problem and this has led to the development of new analytical methods with improved selectivity and sensitivity [2-5].

PAHs are typical non-polar compounds and have excellent retention on a reversed-phase adsorbent such as C_{18} bonded silica [6]. Although their solubility in water is very low, concentrations in the $\mu g l^{-1}$ level are commonly encountered in the environment. Since these compounds are considered toxic at this level, their presence needs to be monitored. Determination of PAHs in natural waters has to be carried out with great care to avoid serious losses occurring during the sampling and storage stage. This is due to the hydrophobicity of this compounds, and

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their tendency to be adsorbed to surfaces they are in contact with, including suspended particulate matter [7].

The most common techniques used to separate PAHs are gas chromatography (GC) [8-13] and high-performance liquid chromatography (HPLC) [12-19]. With HPLC, the most common detection methods are uv-visible or diode-array detection (DAD) [13-15, 20] and fluorescence detection [21-25].

Because of low concentration levels to be quantified in water samples, an enrichment step is needed before chromatographic analysis. For PAHs, several precon-centration techniques have been used, but the most preconcentration technique is solid-phase extraction [6,26-29].

SPE is a technique that is becoming increasingly popular, because unlike liquid-liquid extraction (LLE) it does not require large volumes of toxic organic solvents, analysis time can be decreased significantly and on-line and/or automated procedures are easily designed. Another advantage of SPE over LLE is the wide variety of extraction conditions which may be used to achieve the desired separation and concentration. The great variety of types of sorbents commerically available has increased the use of SPE[29].

In this work, a simple, suitable and rapid procedure by use of SPE with C_{18} sorbent is proposed for the extraction of some PAHs from the caspian seawater. After preconcentration by using SPE, separation with HPLC is carried out in isocratic mode by a mixture of water and acetonitrile as the mobile phase.

EXPERIMENTAL

Apparatus

The HPLC pump was the series 10 liquid chromatography with Model 7125 Manual Injector, Perkin Elmer (Norwalk, CT, USA). Recorder used was a AR-55 signal Penlinear (Pye Unicam, Holand). The column used was a 5 μm particle size, NOVA PAK C_{18} (150×3.9 mm) from waters company (Milford, MA, USA). Detection was performed using a UV-Visible detector Model LC-95, Perkin Elmer (Norwalk, CT, USA). For preconcentration of analytes in SPE step, the C_{18} Bond Elut cartridge (3 ml, 300 mg) used was from Varian (Harbor city, CA).

Reagents

Naphtalene, acenaphtene, hexane and chloroform were supplied from Merck (Darmstadt, Germany). Fluorene, phenanthrene, anthracene, fluoranthene and pyrene were obtained from Fluka (Buchs, Switzerland), all with a purity of more than 97 %. The stock solutions of these compounds at a concentration of 500 mgl^{-1} were prepared in methanol. These standards were kept in the dark at 4 °C. Working solutions used for direct injection were made up in methanol. Mobile phases used for HPLC were mixture of HPLC-grade acetonitrile and de-ionized doubly distilled water filtered by 0.45 μm filter.

Extraction Methods

Before sample preconcentration, the cartridge was cleaned with 5 ml n-hexane and subsequently conditioned with 5 ml methanol and 10 ml water. Flow rate of the sample through the cartridge was controlled at 5 ml/min under vacuum. After all the sample had percolated through the cartridge for the concentration step, the cartridge was dried for 10 min using a vacuum pump and the analytes were removed from the cartridge with a proper solvent. The SPE extracts were evaporated to dryness, redissolved in 300 μl methanol, filtered and injected into the HPLC system. This extraction method was used for both standards and real samples.

Sampling

Samples were collected from the Caspian sea in babolsar at distance almost 1000 meters away from the coast. These samples were collected into pre-cleaned amber glass bottles and immediately were transferred to laboratory for analysis. To avoid analyte adsorption problems on surface of bottle and vessels, some acetonitrile (10 %) was added to the samples, filtered through 0.45 μm filter and were transferred to cartridge for preconcentration.

Separation of PAHs with HPLC using UV Detection

Mobile-phases for HPLC separation of these compounds with UV detection were doubly distilled water and acetonitrile. The analysis performed with different percentages of acetonitrile and 40% of acetonitrile was selected. Factor for this selection was selectivity factor between the PAHs peaks (table 1). Separations were performed at room temperature with a constant flow rate of 1.5 ml/min. For detection of these compounds λ=254 nm was used. Fig. 1 shows typical chromatogram of PAHs standard mixture.

Table 1: Effect of % CH_3CN on Selectivity Factor.

% CH_3CN	$\alpha_{1,2}$	$\alpha_{2,3}$	$\alpha_{3,4}$	$\alpha_{4,5}$	$\alpha_{5,6}$	$\alpha_{6,7}$
40	2.300	1.087	1.260	1.175	1.405	1.096
50	1.945	1.000	1.179	1.152	1.263	1.167
60	1.500	1.000	1.667	1.143	1.250	1.120
70	1.909	1.000	1.238	1.000	1.154	1.000

Conditions: Column; C_{18} (150×3.9 mm) 5 µm, Flow rate: 1.5 ml/min, Inj.volume: 10 µl, λ=254 nm. (1- Naphtalene=50, 2- Acenaphtene.=100, 3- Fluorene.=10, 4- Phenantrene=5, 5- Antheracene =5, 6- Flourantene =20, 7- Pyrene =20 µgml^{-1}).

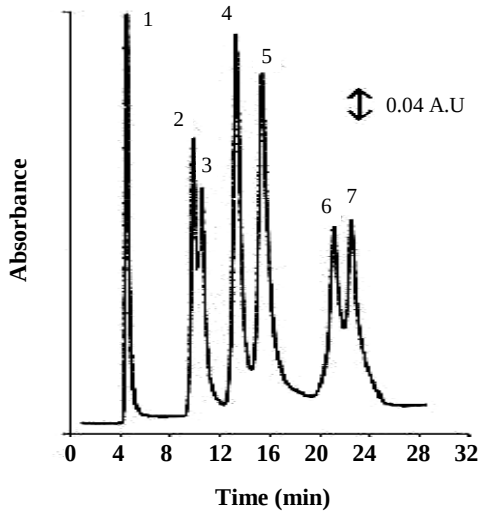

Fig. 1: Separation of Standard PAHs by HPLC. Conditions: Mobile Phase; acetonitrile:water (40:60 V/V). Column; C_{18} (150×3.9 mm) 5 µm; Flow Rate: 1.5 ml/min; Wavelegth Detection; 254 nm; Samples: [1) Naphtalene=50, 2) Acenaphtene= 100, 3) Fluorene=10, 4) Phenanthrene=5, 5) Anthracene=5, 6) Fluoranthene=20, 7) Pyrene=20 µgml^{-1}].

Solid-Phase Extraction (SPE) process

Cartridge (C_{18}) of SPE was activated with 5 ml methanol and 10 ml water. Then, the samples were loaded to it. When the analytes are retained, a sorbent drying step is needed to remove water before elution of analytes. Presence of water in the final extract causes some difficulties during solvent removing from analytes [21,29]. After 10 min of drying in vacuum system, water was completely removed from SPE Cartridge.

Several SPE variables (such as breakthrough volume of different PAHs, type and solvent volume for elution) had to be examined to establish the optimum conditions. The procedure is based on the preconcentration of 5 ml of mixture of PAHs in methanol, which contained different concentration of PAHs (5 to 100 µgml^{-1}), dilluted to 100 ml with doubly distilled water. The solvents tested for elution step were methanol, chloroform and hexane. Results showed that hexane had good recoveries (over 82 %) for these compounds (table 2). After selection of proper solvent for elution, its appropriate volume for extraction was evaluated using different volume of hexane (0.5-5 ml) from C_{18} cartridge. Results showed that good recoveries were obtained with 3 ml hexane (table 3).

Finally, breakthrough volumes of different PAHs were determined using different volumes of standard solutions (50-1000 ml) with the same absolute amount of these compounds. The maximum recoveries were observed with 500 ml of standard solutions (table 4). Thus, to extract PAH compounds from seawater, 500 ml solution of seawater and acetonitrile (10 % acetonitrile) was loaded into C_{18} cartridge. The cartridge was dried for 10 min to remove solvent. Then the analytes were eluted with 3 ml of hexane. To separate PAH compounds by HPLC, hexane was evaporated at room temperature and the residue was dissolved in 300 µl methanol, filtered and injected to HPLC system (10 µl). Fig. 2 shows chromatograms of PAHs at λ=254 nm. Detection limits, calculated at a signal-to-noise ratio of three, ranged from 0.02 to 0.14 µg/ml at 254 nm. Table 5 shows Limits of Detection and concentration of PAH compounds in seawater.

Conclusions

A simple, rapid and suitable procedure for determination of seven environmentally important PAHs by preconcentration of analytes by SPE using C_{18} cartridge as sorbent and HPLC with UV detection has been described. The limits of detection at λ=254 nm with acetonitrile:water (40:60) as mobile phase and flow rate of 1.5 ml/min were found to be between 0.02-0.14 µgml^{-1}. Separation for these compounds was about 24 min. Good recoveries and RSDs were achieved by hexane as eluent in SPE step. On the basis of these results, the method was applied to the PAHs analysis in seawater samples. A preconcentration step with octadecyl cartridge (C_{18}) was necessary because of very low concentration of PAHs in seawater before injection to HPLC system. Results of analysis of PAHs by HPLC showed a concentration range of 0.34-14.11 ngml^{-1} in caspian seawater.

Table 2: Effect of type of solvent on Recoveries (R %) of PAHs and RSD % (n=6) for elution step in SPE process.

Compound	t_R (min)	Hexane		Methanol		Chloroform	
		% R	% RSD	% R	% RSD	% R	% RSD
Naphtalene	5/15	82	2	103	2	56	3
Acenaphtene	10/20	100	4	101	4	77	3
Fluorene	11/00	99	3	102	4	59	4
Phnanterene	13/34	102	3	82	3	57	3
Antheracene	15/42	89	2	80	4	52	3
Flourantene	21/33	111	3	67	5	51	6
Pyrene	23/33	106	4	62	4	56	4

Table 3: Effect of volume of hexane on R % and RSD % (n=6) for elution step in SPE process.

Compoud	Hexane volume (ml)											
	0/5		1/0		2/0		3/0		4/0		5/0	
	% R	% RSD	% R	% RSD	% R	% RSD	% R	% RSD	% R	% RSD	% R	% RSD
Naphtalene	38	3	46	3	50	3	82	2	82	2	85	3
Acenaphtene	54	3	46	5	86	4	100	3	102	3	98	2
Fluorene	36	4	42	5	71	5	99	3	98	5	99	4
Phenantrene	24	6	56	2	77	2	102	2	98	4	102	3
Antheracene	24	6	48	4	76	3	89	4	88	3	91	4
flourantene	29	4	63	4	102	4	111	6	109	5	109	4
Pyrene	19	4	55	4	92	4	106	4	99	4	102	3

Table 4: Effect of breakthrough volume on R % and RSD % (n=5). Compounds were eluted using 3 ml of hexane.

Compound	breakthrough volume (ml)									
	50		100		250		500		1000	
	R %	RSD %	R %	RSD %	R %	RSD %	R %	RSD %	R %	RSD %
Naphtalene	52	4	82	3	78	3	78	4	77	3
Acenaphtene	82	3	100	3	98	3	102	4	100	3
Fluorene	76	5	99	4	98	4	92	5	91	4
Phenantrene	81	2	102	2	104	2	108	2	106	2
Antheracene	70	4	89	3	97	4	96	3	94	4
Flourantene	98	4	111	4	111	5	112	4	107	5
Pyrene	88	3	106	4	108	3	122	3	122	3

Table 5: Concentration of PAHs in the caspian seawater and their Detection Limits.

Compound	LOD (μgml^{-1})	Concentration in Caspian seawater ($ngml^{-1}$)
Naphtalene	0.14	0.63
Acenaphtene	0.12	1.24
Fluorene	0.06	0.34
Phenanthrene	0.02	0.57
Anthracene	0.02	2.06
Fluoranthene	0.04	10.71
Pyrene	0.05	14.11

*Conditions: Mobile Phase; acetonitrile:water (40:60 V/V); Flow rate; 1.5 ml/min, Column; C_{18} (150×3.9 mm) 5 µm; λ=254 nm; T= AmbientTemperature. Injection volume; 10 µl. *Concentration of PAHs were measured after preconcentration with HPLC and then converted to concentration in sea water.*

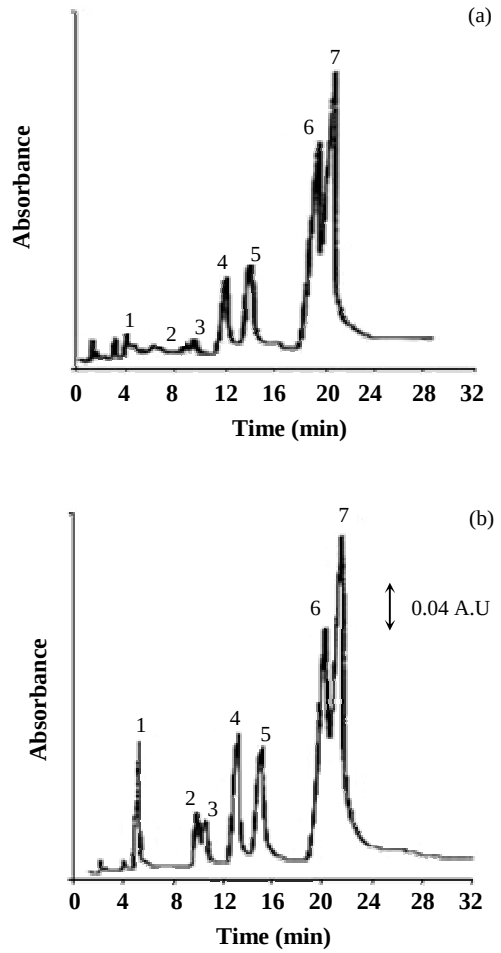

Fig. 2: Separation of PAHs in the Caspian Seawater at λ=254 nm (a) non spike (b) after being spiked with different concentration of PAHs (Naphtalene=5, Acenaphtene =10, Fluorene=1, Phenanthrene=0.5, Anthracene=0.5, Fluoranthene =2 and Pyrene=2 μgml^{-1}). Other conditions as figure 1.

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