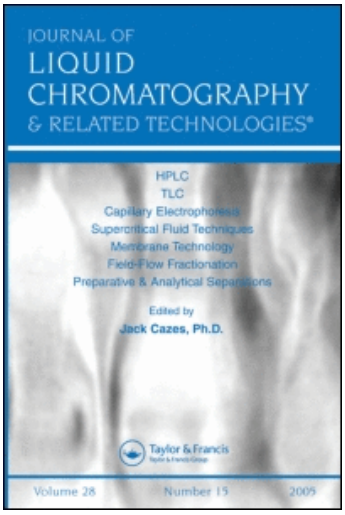

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Comparison Between Different Extraction (LLE and SPE) and Determination (HPLC and Capillary-LC) Techniques in the Analysis of Selected PAHs in Water Samples

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Comparison Between Different Extraction (LLE and SPE) and Determination (HPLC and Capillary-LC) Techniques in the Analysis of Selected PAHs in Water Samples

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Abstract: In this work, the extraction of 9 out of 16 PAHs pollutants according to US Environmental Protection Agency (EPA) procedures, was studied through liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The analysis of PAHs was made by high performance liquid chromatography (HPLC), using both a Supelcosil LC 18 (25 cm × 4.6 mm, 5 μm) column operating in the conventional HPLC mode and a capillary column (20 cm × 0.25 mm, 5 μm), packed in house with Spherisorb ODS-2 particles and operating in the capillary liquid chromatography (c-LC) mode.

Of the extraction techniques used, LLE revealed itself to be efficient in the extraction of the higher-molecular-weight PAHs, while SPE was adequate for the extraction of all PAHs. HPLC revealed to be more sensitive than c-LC in the detection of PAHs in the sample concentration. However, since in c-LC the dilution of the compounds in the mobile phase is less, the mass sensitivity was significantly higher than that obtained with conventional HPLC (that is important when a limited sample amount is available). In the real water samples analyzed no PAH was found under the analytical conditions used.

Keywords: Liquid-liquid extraction, Solid-phase extraction, HPLC, Capillary liquid chromatography, Polycyclic aromatic hydrocarbons

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants resulting from emissions of a variety of sources, including industrial combustion and discharge of fossil fuels and residential heating (both fossil fuels and wood burning). Because of their mutagenic and carcinogenic properties, the study of PAHs in environmental matrices including air, water, and soils is of great importance. PAHs are usually present in environmental samples as extremely complex mixtures; these mixtures contain many isomeric structures and alkylated isomers. These compounds can be introduced in aqueous medium by several ways, amongst them the sewer waters produced from industries, and particulate materials carried by the wind and by rainwater.[1]

Since its inception in the early 1970's, high performance liquid chromatography (HPLC) has been used for the separation of PAHs. Since Schmit's report, reversed-phase on chemically bonded C-18 phases has become the most popular HPLC mode for the separation of PAHs.[2–5]

The miniaturization of chromatography started in 1957 when Golay[6] introduced capillary columns into the gas chromatography. Since then, capillary GC proved its usefulness; however, at present only a little further development of GC is observed. On the contrary, liquid chromatography is still being developed, new packing materials are being prepared and the chromatographic systems are miniaturized.[7]

The introduction of capillary liquid chromatography (c-LC) is usually attributed to Horváth et al., who in 1967[8,9] used 0.5–1.0 mm inner diameter (ID) stainless steel columns packed with pellicular particles for the separation of ribonucleotides. c-LC has established itself as a complementary technique to conventional sized LC columns, which are nowadays routinely used in high performance liquid chromatography.

It is very important to discuss the terminology used in the literature regarding the nomenclature of columns of smaller ID.[7] Ishii[10] divided columns into groups accordingly to the ID:

- 4.6 mm-conventional HPLC;
- 1.5 mm-semimicro HPLC;
- 0.46 mm-micro HPLC;
- 0.15 mm-ultramicro HPLC;
- 0.05–0.2 mm-packed micro-capillary column;
- 0.01–0.06 mm-open tubular capillary column.

More recently, Vissers et al. and Chervet[11,12] classified the LC techniques according to the flow rate range. These trends may be combined to generate a more general classification, as in Table 1.

The most important advantages of c-LC are the ability to work with minute sample sizes, small volumetric flow rates, and enhanced detection

Table 1. Terminology used in this work for LC techniques

Column ID	Flow rate	Name
3.2–4.6 mm	0.5–2.0 mL min^{-1}	Conventional HPLC
1.5–3.2 mm	100–500 μL min^{-1}	Microbore HPLC
0.5–1.5 mm	10–100 μL min^{-1}	Micro-LC
150–500 μm	1–10 μL min^{-1}	Capillary-LC
10–150 μm	10–1000 nL min^{-1}	Nano-LC

performance with the use of concentration sensitivity detection devices due to reduced chromatographic dilution.[13–16] Reducing the column ID from 4.6 mm to 0.32 mm will increase a UV or fluorescence signal by a factor of 200, and from 4.6 mm to 0.046 mm by a factor of 10000. Naturally, the loading capacity of the conventional columns is correspondingly higher. Furthermore, it is important to be aware of the statement, "if conditions are otherwise equal", since equal conditions may not always be easily obtained. Examples are: lowered S/N compared to theoretical values as a result of shorter light path, light scattering through the curved wall of fused silica with on-column detection, increased noise in cells with Z- or U-configuration, and dead volumes and adsorptive surfaces in detectors.[17]

The potential of capillary liquid chromatography for routine analysis of trace environmental pollutants was demonstrated by Lee[18] in the determination of PAHs at sub-ppb levels in natural water. Focus of that work was placed on enhancing concentration sensitivity by the combined use of off-line solid-phase extraction (SPE), on-column sample focusing, and a U-shaped capillary flow cell. Under optimal conditions, the detection limit was the range of 0.04–0.2 μg L^{-1} and recoveries for PAHs were higher than 84%.

The recent trend in the LC column miniaturization can be verified by the number of published articles during the last ten years. Through a search on the Science Direct homepage (http://www.sciencedirect.com), using capillary liquid chromatography as key word, an increase of more than 1000% was discovered on the number of published papers in the period of 1994 to 2004.

In this study, two extraction methods and two LC modes are investigated and compared for the determination of selected PAHs in water samples.

EXPERIMENTAL

Chemicals

Different manufacturers supplied the PAHs analytical standards used in this work. SPE cartridges (C-18, 300 mg) were obtained from Supelco (Bellefonte, New Jersey, USA). Methanol, acetonitrile (HPLC grade) and methylene

chloride, used in LLE, were obtained from Mallinckrodt (Paris, Kentucky, USA). HPLC grade water was obtained in a Milli-Q system (Millipore, São Paulo, Brazil).

The stock solutions of the PAHs were prepared in acetonitrile and the working solutions were prepared by dilution of the stock solution, also in acetonitrile, in order to get PAHs mixtures containing each compound in the concentrations of 100, 50, 10, 7, 5, 2, 1, 0.1, 0.05, 0.01, and 0.001 $\mu g\,mL^{-1}$.

Extraction Methods

Liquid-Liquid Extraction (LLE)

One hundred milliliters of milli-Q grade water spiked with a standard mixture containing selected PAHs (naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and dibenz(a,h) anthracene), at concentrations of 10 $\mu g\,mL^{-1}$ of each compound, were shaken with 3 portions of 30 mL of methylene chloride. After, the organic phase containing the PAHs was evaporated to dryness by a controlled and gentle flow of nitrogen, and the extracted PAHs were redissolved in acetonitrile in order to get a PAHs mixture in the final concentration of 5 $\mu g\,mL^{-1}$ of each compound.

Solid-Phase Extraction (SPE)

Initially, SPE cartridges containing 300 mg of C_{18} phase were conditioned with 5 mL of methanol followed by 5 mL of milli-Q grade water. After this step, one hundred milliliters of milli-Q grade water spiked with a standard solution containing selected PAHs, in the concentration of 10 $\mu g\,mL^{-1}$ of each compound, were introduced into the cartridge for the concentration step. Each cartridge was dried for 10 min using a vacuum pump, and the analytes were removed from the cartridge with acetonitrile, in order to get a PAHs mixture in the final concentration of 5 $\mu g\,mL^{-1}$ of each compound.

Determination Methods

High Performance Liquid Chromatography (HPLC)

HPLC analyses were performed in a Shimadzu SPD M10A liquid chromatograph. The analysis was performed using a Supelcosil LC 18 (25 cm × 4.6 mm, 5 μm) column in the following chromatographic conditions: acetonitrile/water (70 : 30 v/v) at a flow-rate of 0.8 mL min^{-1} as mobile phase; oven temperature of 30°C; injected volume of 20 μL, isocratic elution

mode. Detection was performed at 220 *nm* for naphthalene, acenaphthylene, acenaphthene, and dibenz(a,h)anthracene and at 254 *nm* for phenanthrene, anthracene, fluoranthene, pyrene, and chrysene.

Capillary Liquid Chromatography (c-LC)

The c-LC analyses were made using a setup containing several modules, all from Fisons (Rodano, Italy). The system included a Phoenix 20 micro pump, a 60 nL Valco injection valve, a UV VIS 20 micro detector equipped with a 8 mm Z-shaped micro flow cell, and a data acquisition module. The micro column used (20 cm × 0.25 mm, 5 μm) was slurry packed in house using a Spherisorb ODS-2 (particle diameters of 5 μm) phase. The chromatographic conditions used in c-LC included: acetonitrile/water (75 : 25 v/v) at a flow rate of 4 $\mu L\,min^{-1}$ as mobile phase (isocratic elution mode) and a column at room temperature. Detection was performed at 220 and 254 nm (such as in the HPLC method).

RESULTS AND DISCUSSION

Capillary Liquid Chromatography: General Aspects

To verify the better mass sensitivity of the c-LC compared to HPLC as predicted in the literature,[9] the same amount, in mass, of anthracene and fluoranthene was injected in the two techniques. As can be observed in Figure 1, good agreement between the theory and experimental results was obtained. An increase of up to 50 units in the absorbance scale for the anthracene shows the higher sensitivity of the c-LC (please note the difference in the full scale in Figure 1).

Another interesting aspect observed in c-LC, refers to the difficulty of the syringe pump in maintaining constant flow rate of mobile phase, as it becomes empty. This was verified through the variation of the retention times of the analytes when the pump presented a capacity of ca. 50% or less, as illustrated in Figure 2.

Determination of Selected PAHs in Spiked Water Samples

Figure 3 shows the chromatograms obtained through the injection of a standard mixture of selected PAHs (each compound in the concentration of $10\,\mu g\,mL^{-1}$) in the HPLC system, while in Figure 4 are illustrated the chromatograms obtained by c-LC (of the same standard mixture).

Analyzing the chromatograms obtained by the two techniques, a satisfactory resolution is verified with a very close run time. The plate numbers obtained for the PAHs investigated were higher in c-LC than HPLC

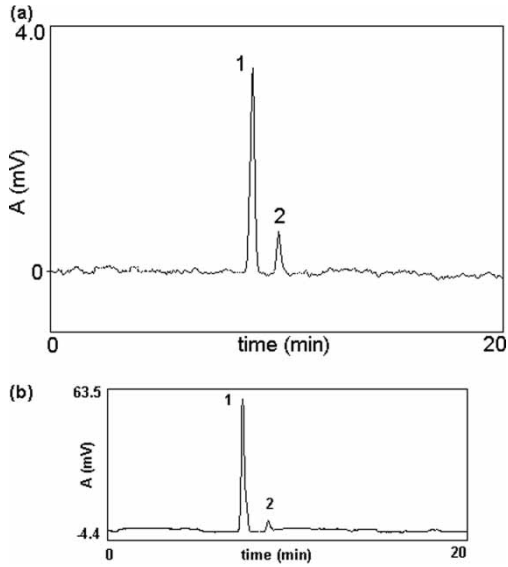

Figure 1. Comparative chromatograms indicating the sensitivity difference between (a) HPLC and (b) c-LC. Peaks identity: (1) anthracene; (2) fluoranthene. It is important to note the difference in the full scale of the chromatograms.

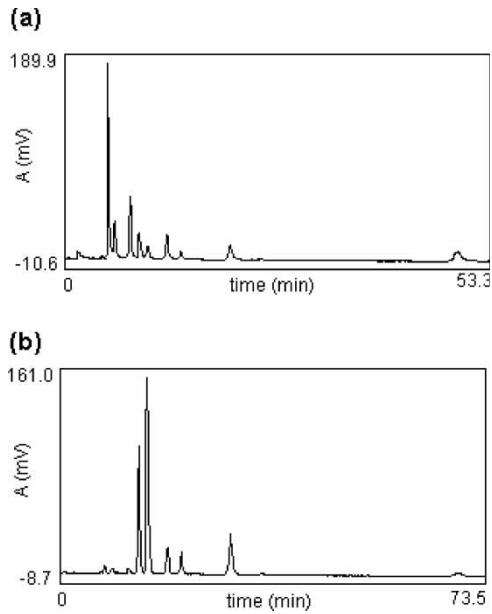

Figure 2. c-LC chromatograms of a standard mixture containing selected PAHs ($10\,\mu g\,mL^{-1}$) with: (a) pump filled with 47.6% and (b) pump filled with 38.8% of its capacity. All other nominal conditions are the same.

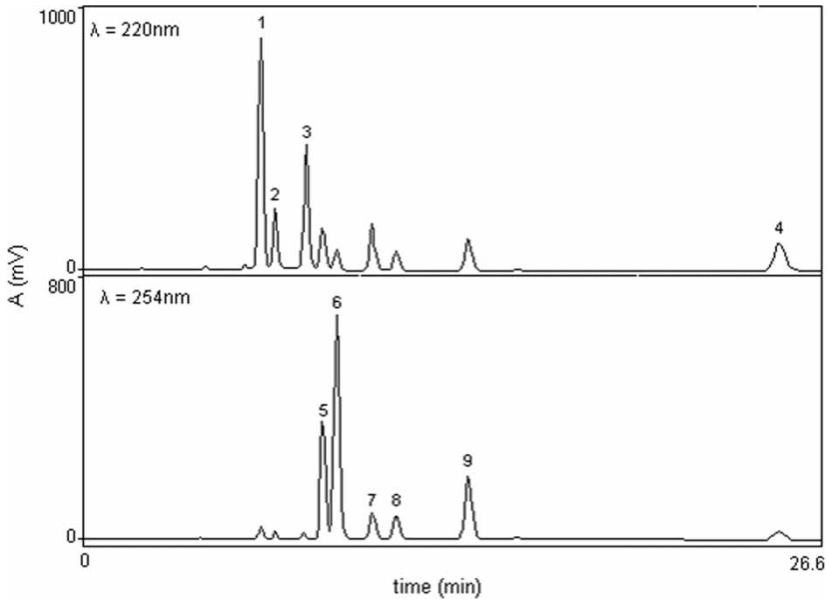

Figure 3. HPLC chromatogram of a standard mixture containing selected PAHs ($10\,\mu g\,mL^{-1}$). Peaks identity: (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) dibenzo(a,h)anthracene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) chrysene.

(Table 2), probably due to lesser dispersion of the analytes in the mobile phase produced by the low flow rate characteristic of the c-LC. The identification of the analytes was done through the retention times obtained by individual injection, in triplicate, of the PAHs analytical standard solutions and through the UV spectrum obtained by HPLC.

The detection and quantification limits for the PAHs determined through HPLC and c-LC are shown in Table 3. Analyzing the results obtained, it can be observed that the detection limits for PAHs are lower for HPLC than c-LC, in relation to the concentration of the injected compounds. This occurs because of the different injection volume of these compounds in the two techniques. While in HPLC the injected volume is $20\,\mu L$, in c-LC the injected volume is only 60 nL. However, the concentration sensitivity in the detection cell (mass of compounds that reach the detector) is higher in c-LC, since the flow rate used in this technique is very low and the dilution of the PAHs in the mobile phase becomes much less. This feature of lesser dilution of the analytes in c-LC compensates the higher injected volume in HPLC (in relation to the mass sensitivity), making the c-LC technique more attractive when small size samples are available.

The linearity ranges studied were: 0.1 to $10\,\mu g\,mL^{-1}$ for HPLC and 1 to $10\,\mu g\,mL^{-1}$ for c-LC (for all investigated PAHs). Between the extraction

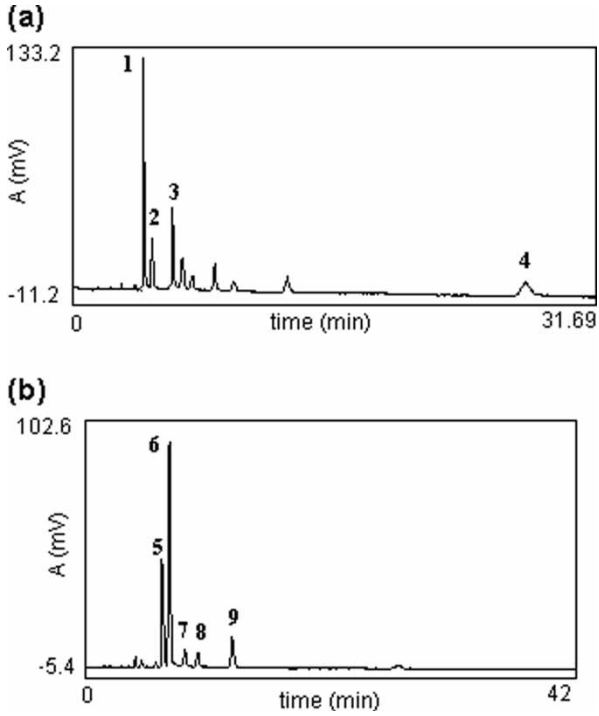

Figure 4. c-LC chromatograms of a standard mixture containing selected PAHs ($10\,\mu g\,mL^{-1}$); (a) $\lambda = 220$ nm, peaks identity: (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) dibenzo(a,h)anthracene; (b) $\lambda = 254$ nm, peaks identify: (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) chrysene.

techniques used, LLE gave good recoveries for the high-molecular-weight PAHs and not as good for the extraction of volatile PAHs (Table 4). Another inconvenience of this technique was the high amount of solvent required for the extraction. Comparing the two chromatographic techniques (HPLC and c-LC), similar recovery results were obtained (Table 4), with c-LC presenting a better mass sensitivity in all cases (due to smaller dilution factor).

Good recoveries for all compounds, were achieved through SPE (Table 5), including the high-molecular-weight PAHs showing to be more adequate than LLE on the PAHs extraction. As in LLE, similar recoveries were obtained through HPLC and c-LC.

Determination of Selected PAHs in Real Water Samples

After the optimization of all extraction and determination conditions with the spiked water samples, analysis of the Araraquara city (São Paulo state, Brazil)

Table 2. Retention times (t_R) and plate numbers (N) obtained for the investigated PAHs by HPLC and c-LC

	HPLC		c-LC	
Compound	t_R (min)	N/m	t_R (min)	N/m
Naphthalene	6.35	21445	4.20	35996
Acenaphthylene	6.87	25086	4.73	49579
Acenaphthene	7.99	30334	5.98	54152
Phenanthrene	8.58	31658	6.47	60683
Anthracene	9.09	27595	7.04	60001
Fluoranthene	10.37	35923	8.38	56412
Pyrene	11.23	38871	9.48	54342
Chrysene	13.84	45382	12.46	63024
Dibenz(a,h)anthracene	25.08	62915	27.43	67451

river water samples, aiming at the identification and quantification of the investigated PAHs, were performed. The extraction method employed was SPE. As the LOD values obtained by HPLC were lower than that obtained by c-LC, and there was no limit of sample amount, HPLC was chosen and applied in the real samples due to the better sensitivity of this method. Water collections were made in several basins that supply this city. Typical chromatograms obtained by HPLC for one-basin river water samples analyzed are shown in Figure 5. As a result, in all water samples analyzed, no PAH was found under the analytical condition used.

Table 3. Detection and quantification limits (LOD and LOQ) for selected PAHs obtained by HPLC and c-LC

	HPLC ($\mu g\,L^{-1}$)		c-LC ($\mu g\,L^{-1}$)	
Compound	LOD	LOQ	LOD	LOQ
Naphthalene	1.0	3.3	10.0	33.3
Acenaphthylene	10.0	33.3	500.0	1650.0
Acenaphthene	1.0	3.3	300.0	990.0
Phenanthrene	1.0	3.3	100.0	330.0
Anthracene	0.8	2.6	10.0	33.0
Fluoranthene	10.0	33.3	1000.0	3300.0
Pyrene	10.0	33.3	1000.0	3300.0
Chrysene	5.0	16.6	800.0	2640.0
Dibenz(a,h)anthracene	30.0	99.9	1000.0	3300.0

Table 4. Recovery values for selected PAHs (LLE) obtained by HPLC and c-LC

	HPLC			c-LC		
Compound	Recov. (%)	Mass (ng)	RSD[a] (%)	Recov. (%)	Mass (ng)	RSD[a] (%)
Naphthalene[b]	—	—	—	—	—	—
Acenaphthylene[b]	—	—	—	—	—	—
Acenaphthene[b]	—	—	—	—	—	—
Phenanthrene	84.7	84.7	8.2	85.6	0.25	1.5
Anthracene	91.0	91.0	7.7	93.3	0.28	1.5
Fluoranthene	95.1	95.1	8.5	93.1	0.28	2.8
Pyrene	92.2	92.2	8.3	94.3	0.28	1.9
Chrysene	98.2	98.2	8.0	99.6	0.30	4.0
Dibenz(a,h)anthracene	94.1	94.1	7.7	103.4	0.31	1.7

[a]Analysis in triplicate (n = 3).
[b]Compound not extracted.

CONCLUSIONS

In this work, c-LC presented a better mass sensitivity when compared to HPLC, although HPLC showed to be more concentration sensitive; c-LC revealed to be a good technique in the determination of PAHs (in relation to the mass sensitivity) when a limited sample amount is available.

Table 5. Recovery values for selected PAHs (SPE/C18 phase) obtained by HPLC and c-LC

	HPLC			c-LC		
Compound	Recov. (%)	Mass (ng)	RSD[a] (%)	Recov. (%)	Mass (ng)	RSD[a] (%)
Naphthalene	86.0	86.0	5.0	78.5	0.23	7.5
Acenaphthylene	91.4	91.4	4.8	88.9	0.26	2.3
Acenaphthene	87.1	87.1	5.0	77.1	0.23	3.5
Phenanthrene	90.7	90.7	5.0	85.4	0.25	1.9
Anthracene	66.0	66.0	4.9	59.8	0.18	3.2
Fluoranthene	91.1	91.1	4.8	90.6	0.27	1.9
Pyrene	90.0	90.0	4.9	84.6	0.25	3.3
Chrysene	80.5	80.5	5.6	78.3	0.23	1.5
Dibenz(a,h)anthracene	75.6	75.6	5.5	74.1	0.21	2.3

[a]n = 3.

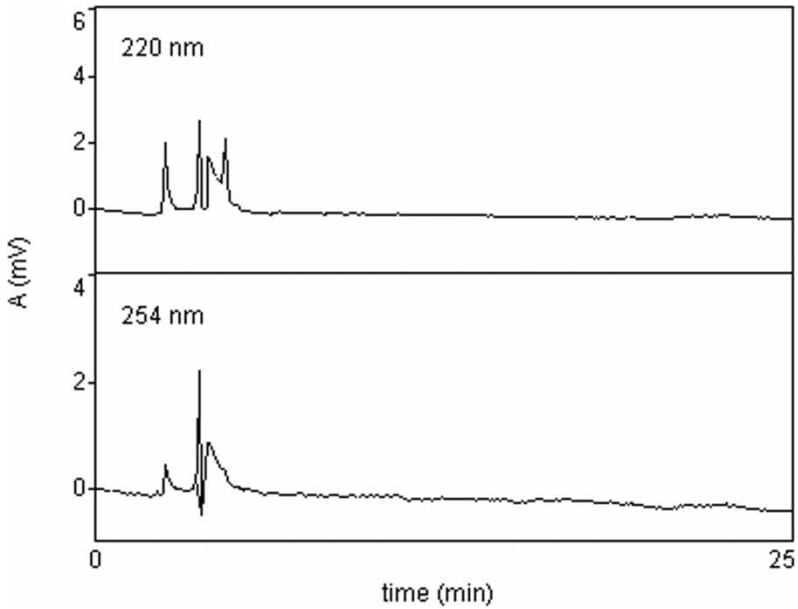

Figure 5. HPLC chromatogram obtained from Córrego do Paiol extracted water sample (using SPE method). Extraction conditions: 100 mL of river water passed through the cartridge (filled with 300 mg of C18 phase); elution with acetonitrile.

However, if there is no limitation of the sample volume, HPLC must be preferred, due to their higher loading capacity and, consequently, higher sensitivity than c-LC. The better technique for extraction of selected PAHs was SPE; LLE was only efficient in the extraction of the high-molecular-weight compounds.

It was also verified in c-LC, that the retention profiles of the analytes are dependent on the percentage of the filled pump. Using values lower than 50% of the pump capacity, a variation of the retention times between the chromatographic run was observed; with the pump reservoir presenting more than 50% of its capacity filled, the retention times were reproducible.

Analyses by SPE and HPLC from river water samples were made and no studied PAH was found under the analytical conditions used.

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