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Extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) by solid phase micro-extraction/supercritical fluid chromatography (SPME/SFC)

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Abstract. Solid phase micro-extraction (SPME) is a clean and simple pre-concentration method. Previously used for trace analysis of volatil compounds, the use of SPME was extended to non volatil molecules with the development of an SPME /HPLC interface. This new interface allows the extraction and the analyses of high molecular weight compounds found in aqueous samples. Since supercritical fluid chromatography is particularly well suited for analysis of complex mixtures containing non volatil compounds, the faisability of coupling SPME and SFC has been investigated and applied to PAHs.
Several points have been studied: i/ behavior of interface and of fiber to supercritical fluid and high pressure required by the analytical method; ii/ kind of the compounds transfer from the fiber to the analytical column; iii/ relation between the nature of the fibers and the quantity of extracted compounds; iiii/ effect of salt addition.
The results show that the SPME/SFC technic may be used for extraction and analysis of PAHs, since the quantity of extracted compounds reachs 30%.

Key words. Polycyclic Aromatic Hydrocarbons (PAHs) – Solid Phase Micro Extraction (SPME) – Hyphenated SFC/SPME – fiber coating – salt addition.

The analysis of the Polycyclic Aromatic Hydrocarbons (PAH) diluted in water samples at very low concentration levels requires to concentrate them before the separation step.

The French AFNOR standard, applicable for six PAHs, recommends to quantify them at a level of 10 ng/L for three of them in the drinking water, whereas the US environmental protection agency has listed 16 PAHs, that have to be quantified in the waste water.

To extract these compounds from water based matrix, the liquid/liquid extraction (LLE) is mainly used, and HPLC is a suited separation method for these low volatile compounds. However, solid phase extraction (SPE) is became leading technique to replace liquid/liquid extraction [1,2], because trapping compounds in a solid adsorbant set in a cartridge, a disk (off-line), or in a pre-column (on-line), strongly reduces the use of solvents.

Solid phase micro-extraction (SPME) is another precontration method which can be used to replace the LLE. SPME is also based on the use of an adsorbant to concentrate compounds contained in liquid or solid samples. The sample is put into a hermetical closed vial [3-7], whose volume goes from 10 to 30 mL. Generally, a gazeous phase stays above a known volume of sample. The adsorbant is a fiber glued into a stainless steel plunger, inserted into a holder. When a vial is traited, the plunger is pulled back, drawing the fiber into the needle of the holder.

The fiber is either outside the needle during the adsorption or the desorption steps, or inside the needle when the needle is introduced or drawn out the vial piercing the septum or from the split/splitless injector.

The fiber can be placed into the gazeous phase above the samples, which corresponds to the head space SPME extraction [6-9], or into the liquid sample, which corresponds to direct SPME [3-5]. These two SPME methods are complementary one from another, the first one concentrating the volatile componds (the BTEX for instance), and the second one the less volatile (chloro-phenols, pesticides).

The stirring conditions [4,10], the thickness of the solid-phase coating of the silica fiber support [4] and the coated solid phase nature [11] have an effect on the speed required to reach the equilibria and determine the minimum adsorption time.

Other parameters can change the quantity of trapped compounds: sample pH [11,12], addition of salts [7,13], trapping temperature. Knowledge of the influence of these parameters is needed in order to decrease the detection limits of the compounds.

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Recently, an interface to hyphenate SPME with high performance liquid chromatography has been developped [14]. This interface consists of a regular six-port injection valve, in which the injection loop is replaced by a fiber desorption chamber. The volume of the desorption chamber is equal to 200 µL.

Before the introduction of the fiber, the desorption chamber may be either empty or fulled with an organic solvent. The needle is inserted into a teflon tubing until the outer needle comes to rest on a ferrule, in which the fiber slides. Closing the sealing clamp allows to constrict the ferrule around the inner needle, which seal the desorption chamber to wihstand the high pressure HPLC required.

When the valve is commuted from the load position to the inject one, the mobile phase is introduced from the top of the desorption chamber, either to push to extraction solvent in the case of static desorption, or to directely extract the compound and wash the fiber in the case of dynamic desorption. This interface enables SPME to be used couple before HPLC to concentrate and analysis compounds and opens the field of analysis of high molecular weight compounds such as surfactants [14].

Because the sub/supercritical fluid chromatography, is a technique particularly adapted to the analysis of these kind of compounds [15,16], the feasibility to coupled SPME/SFC, has been studied using as model a pure water solution spiked with PAHs.

Some parameters, such as the fiber chemical nature or the salt addition have been investigated.

Experimental

Supercritical fluid chromatography

Chromatographic separations were carried out using equipment manufactured by Jasco (Tokyo, Japan). The two pumps (Model 880-PU) were connected to a pulsation damper Séderé (Touzart et Matignon, Vitry-sur-Seine, France). The head of the pump used for carbon dioxide, was cooled to –2 °C by a cryostat (Julabo F 10c, Seelbach, Germany). The pulsation damper was connected to an injection valve Model 7125 fitted with a 20 µL loop (Rheodyne, Coati, CA, USA). The columns were placed in a thermostatically controlled oven (Crocosil, Cluzeau, Ste. Foy-la-Grande, France). One pressure regulator 880-81 Jasco controls the outlet pressure. Chromatograms were recorded using an electronic integrator Model CR 6A (Shilmadzu, Kyoto, Japan).

Detection was carried out on a Diode array UV/vis detector (Model 910, Jasco) equiped with a high-pressure resistant cell (20 MPa). The detection wavelenght is 210 nm.

Carbon dioxide (industrial quality) was purchased from Alphagaz (Bois d'Arcy, France).

Columns: Kromasil C18 (250 × 4.6 mm; 5 µm); Hypersil Green PAH (250 × 4.6 mm; 5 µm) (Hypersil, Runcorn, UK).

Solid Phase Micro-Extraction

Three commercially available coated fiber for autosampler were tested: Poly(dimethylsiloxane) (PDMS; 100 µm film thickness); Carbowax/TPR –100 (50 µm film thickness) and PDMS/Divinylbenzene (60 µm film thickness)(Supelco, Bellefonte, PA, USA).

The interface SPME/SFC with Valco Valve (Supelco, Bellefonte, PA, USA) is described more precisely elsewhere [14].

Before the extraction step, the PDMS coated fiber is desorbed under the analysis conditions (blank). This allows to extract the residual adsorbed compouds from the fiber that are detected by UV, and to guarantee cleanness of the fiber before extraction.

Static desorption was done by soaking 30 secondes the fiber in acetonitrile.

Reagents

EPA 610 PAH Mixture (Supelco, Bellefonte, PA, USA): the polycyclic aromatic hydrocarbons concentrations are indicated further, in methanol/methylene chloride 50:50 (v/v): naphtalene (n°1), acenaphthene (n°3): 1 mg/mL; acenaphthylene (n°2): 2 mg/mL; fluorene (n°4), fluoranthene (n°7), benzo(b)anthracene (n°11), dibenzo(a,h)anthracene (n°14), benzo(g,hi,)perylene (n°16): 0.2 mg/mL; phenanthrene (n°5), anthracene (n°6), pyrene (n°8), benzo(a)anthracene (n°9), chrysene (n°10), benzo(k)fluoranthene (n°12), benzo(a)pyrene (n°13), indeno(1,2,3-c,d)pyrene (n°15): 0.1 mg/mL.

Two test solutions (solution A and B) were prepared by dissolving the EPA 610 mixture in HPLC grade acetonitrile (Baker, Deventer, Holland). Different volume of the two test solutions were spiked in 10 mL of HPLC grade water (Baker, Deventer, Holland).

– Solution A: 5 µL of the EPA EPA 610 mixture previously diluted 5 times in acetonitrile were spiked to 10 mL of water (HPLC grade, Baker, Deventer, Holland). The final concentrations were: 1,3 = 0.1 ppm; 2 = 0.2 ppm; 4,7,11,14,16 = 20 ppb; 5,6,7,8,9,12,13,15 = 10 ppb. This first test solution has been used for the feasibility study.

– Solution B: 10 µL of the EPA 610 mixture previously diluted 100 times were spiked to 10 mL of water. The final concentrations were: 1,3 = 10 ppb; 2:= 20 ppb; 4,7,11,14,16 = 2 ppb; 5,6,8,9,12,13,15 = 1 ppb.

This second solution has been used for detection limit and for the salt addition effect studies (sodium chloride Rectapur, Prolabo, Fontenay sous bois, France).

After addition of a stirer bar, the vials (22 mL screw cap glass) were immediately capped with Teflon-faced silicone septum.

Results and discussions

Feasability study

The first part of the study has underlined the suitability of the sub/supercritical fluid chromatography for the analysis of PAH, due to the low viscosites and the high solvent strengh of these fluids [16].

However, many preliminary questions remain on the coupled SFC/SPME, related to the particular supercritical analytical conditions. Since the adsorbant phase is just coated on a solid support, it can be solubilised by the extraction solvent used in the static desorption, or by the mobile phase during the dynamic desorption. This explains why extraction of compounds with a PDMS fiber coupled with the HPLC can only be done using hydro-organic polar mobile phases, to not dissolve the fiber coating.

Thus, in our purpose, the coating solubility in neat carbon dioxyde or in modifiers added to carbon dioxyde has not been yet investigated.

In addition, when working with static or dynamic desorption, as the valve is switched from the load to the inject position, the pressure into the desorption chamber goes promply from the atmospheric pressure to a high pressure (15 to 20 MPa). Since the sub/super critical state of the fluid requires an outlet column pressure to be at least equal to 7.3 MPa, it then adds to the classical pressure drop in the column, and can increase the pressure at the head of the column. Subsequently, this supplementary pressure is applied to the desorption chamber when swiching the valve.

The mecanical resistance of the fiber to a so important pressure variation during the injection is not known either.

Finally, the seal at the interface to the high pressures applied in sub/supercritical chromatography need also be studied.

The seal of the device is due to the compression of a ferrule around the needle and upon a Teflon tube in which the needle is inserted.

The compression of the Slipfree connector should be strong enough to avoid leaks of the sub/supercritical fluid which can modify quantitative and qualitative results.

Moreover, if the compression is not strong enough, the needle is ejected through the ferrule, and as the fiber is keept outside the needle during the desorption step, the coating phase is pelt out the partialy constrited ferrule.

The preliminary experiments carried out to study the transfert conditions have shown that:

– the interface, and in particular the ferrule in which the needle is tighten into, resists well to high pressure imposed by the sub/supercritical fluid chromatography.

– both the 100 μm PDMS and the Carbowax/TPR fiber are not dissolved by supercritical fluids used for the PAH analysis, from neat carbon dioxide at the beginning of the analysis to the acetonitrile/carbon dioxide mixture (50:50; v/v) reached at the end of the gradient.

  On the other hand, the PDMS/DVB fiber is disolved in the mobile phase when the acetonitrile content increases. This fiber should be quickly pull out the desorption chamber after the extraction of the PAH by the neat carbon dioxyde.

– the dynamic desorption, used if the desorption chamber is empty before the introduction of the fiber, has been selected for the analyses. This desorption allows to obtain sharper chromatographic peaks, than with the static desorption. This seems to be due to a greater extraction capacity of the neat carbon dioxyde *vs.* acetonitrile. Moreover, with static desorption, since the desorption chamber is full of acetonitrile, the injection volume is equal to 200 μL. This volume is much larger than the one used in classic injection, and it reduces the chromatographic efficiency.

Recovery of extraction

Because of the apolar chemical nature of the PAHs, a PDMS fiber has been first chosen for this study. The PAHs have been trapped using the following SPME procedure:

– stirring of the test solution at maximum speed for 5 minutes, to homogenize and dissolve the spiked PAHs into water.

– total immertion of the fiber into the spiked water.

– exposition of the fiber for 45 minutes to the spiked water stirred (1000 turns/minute) at room temperature.

Figure 1 shows the chromatogram obtained by hyphenated SPME/SFC. In comparison to a direct injection of PAH in SFC (Fig. 6, 16), a slight loss of efficiency is observed when a dynamic desorption is used. This is due to a non instantaneous extraction of the fiber adsorbed compounds by the mobile supercritical phase.

Moreover, an important pressure drop occurs when shifting the Valco valve from the load to the inject position. As in classical injection, greater the injection volume is and worse the efficiency is. Thus, the pressure drop during the valve rotation leads to a fluid decompression, increasing the volume which contains the extracted compounds introduced in the analytical column.

However, as seen in the figure 1, all the peaks are correctly separated from a quantitative analysis point of view, especially the acenaphthylene, acenaphtene and fluoranthene.

To estimate the recovery of extraction, 5 microliters of the solution A have been injected, and the area obtained for each peak are used as reference. Three extractions were done to calculate the mean of the recovery.

Figure 2 reports the quantitative results obtained using the 100 μm PDMS fiber. These results are interesting on different aspects:

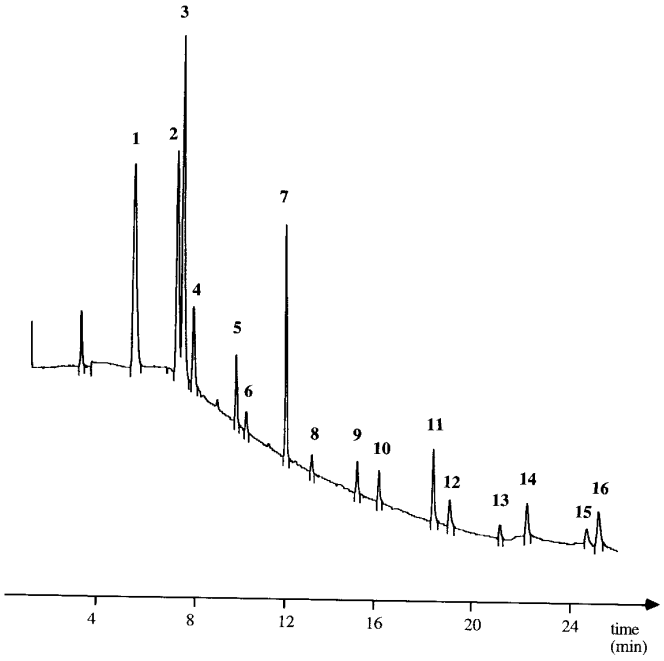

Fig. 1. Analysis of sixteen PAHs by coupling SPME/SFC. Extraction conditions are described in the text (solution A); fiber: 100 µm PDMS. Columns: Kromasil C18 (250 × 4.6 mm)- Hypersil Green PAH (250 × 4.6 mm). Mobile phase: elution gradient from CO_2/ACN (100:0; v/v) to CO_2/ACN (50:50; v/v) in 25 minutes, then CO_2/ACN (50:50; v/v) during 5 minutes. Total duration 30 minutes. *T* = 32 °C; Outlet pressure = 10 MPa; flow rate = 3 mL/min; UV detection: 210 nm, detector range: 0.32 AU, integrator attenuation = 2.

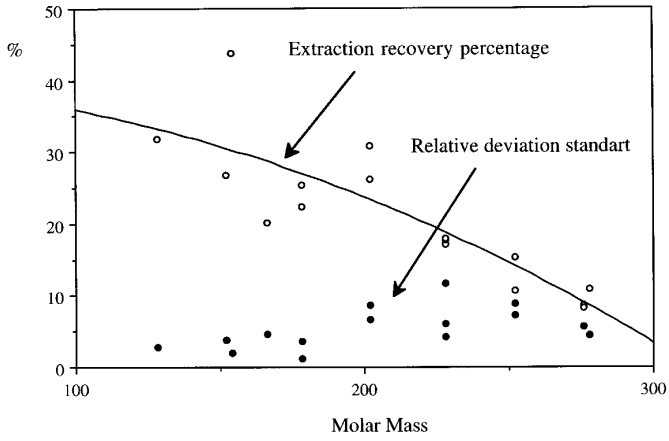

Fig. 2. Variation of recovery percentage and relative standart deviation *vs.* the molar mass of the PAHs using a 100 µm PDMS fiber.

– the recovery percentage of small PAHs (naphtalene, fluoranthene) is very high using this PDMS fiber (around 30%). This value is much higher than the ones more generally encounted for other types of compounds extracted by SPME (around 5%). This fact underlines the great molecular affinity between the PAHs and the Polydimethylsiloxane polymer coating the fiber.

– the extracted quantities decrease as the molecular weight of the PAHs increases. This result is surprising, since more the molecular weight increases, more the apolarity of the PAHs increases, as shown by the study of the octanol/water partage coefficient for instance. In this case, molecular interactions between the PAHs and the fiber should increase with their molecular weight. The opposite result is observed, which underline a low solubilisation of the PAHs in the water used as sample (the solubility of greater PAHs is lower than 1 ppb). Because this low solubility sharply reduces their equilibration rate with the fully immerged fiber [18], the sample time equal to 45 minutes is unadequate to concentrate these compounds.The adsorption of PAHs on the walls of the vessels can also reduce the available compound quantity into the solution.

– finally, the relative deviation standards (RSD) calculated using the three experiments range from 3 to 15%. These values are interesting, even if they are not homogeneous between smal and large PAHs. The non-homogeneity of the RSD does not seem related to the separation quality of the compounds, but rather to the partial solubility or the adsorption of the high molecular weight compounds.

Effect of salt addition

An identical extraction methodology than for the feasibility study of the SPME/SFC coupling has been used, exepted for the spiked solution. It is, the solution B which has been added to the water. Comparing to the solution one, this solution two is diluted 100 times, but 10 microliters are spiked. Thus, this aqueous sample is diluted by a factor 50.

Figure 3a shows that using low concentration solution (solution B), only a few compounds are detected. Among them, the three first PAH of 10 and 20 ppb concentration, and for the fluoranthene at the lowest concentration of 2 ppb.

The low detected concentration (2 ppb) of the fluoranthene is due to the absorbance maximum for this PAH, that is near the used detection wavelenght (210 nm).

Figure 4 shows the effect of the detection wavelenght on the respons of the compounds analysed using the SPME/SFC coupling (solution 1). After an complete study of PAHs spectra recorded using a diode array spectrophotometer, three other wavelenghts apart 210 nm have been selected.

These wavelenghts are 240, 260 and 280 nm. Six compounds exibit a maximum absorbance at 240 nm, three at 260 nm and two at 280 nm. As in HPLC analysis, figure 4 shows then the possibility of programing a selective change

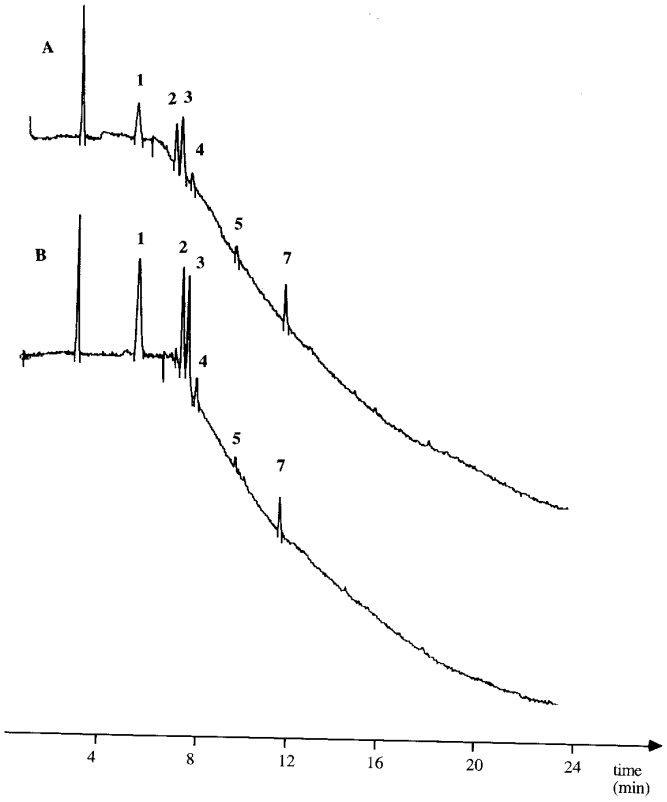

Fig. 3. Analysis of the sixteen PAHs of a low PAHs content aqueous sample. fiber: 100 µm PDMS; sampling conditions are described in the text (solution B), analysis conditions are the same as in figure 1, except for integrator attenuation = 0. A) without salt addition; B) with salt addition.

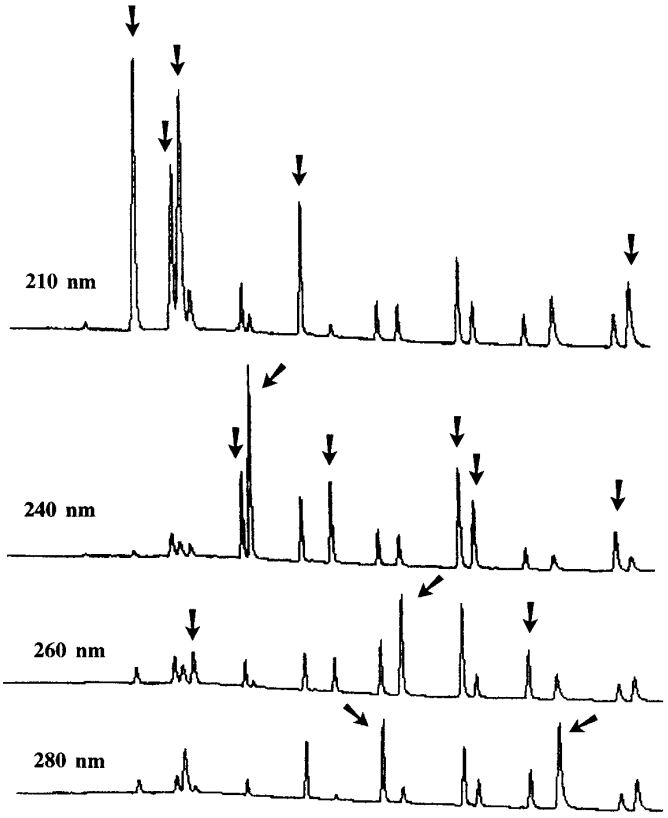

Fig. 4. Chromatogram of the sixteen PAHs by SFC recorded at different wavelength. Analytical conditions as in figure 1.

of the wavelenght during the analysis, in order to decrease the detection limit of the all PAHs to the value of 2 ppb.

Using the previous detection limit, the effect of salt addition has been studied.This effect is quite different depending on the compound (Fig. 3). For the first ones PAHs (peaks 2, 3, 4 and 5; Fig. 3b), sodium chloride addition increases around twice the extracted quantity, that is classically observed on the polar compounds. However, for the higher PAHs, the salt addition decreases their extraction yield, because their solubility in the aqueous sample is strongly reduced. Therefore since they are repulsed by the matrix/gazeous phase interface, they cannot interact with the fiber completly plunged into the aqueous matrix.

Effect of the fiber chemical nature

Two other fibers of different chemical nature have been studied, using the previously described extraction methodology. Solution A is used allowing the comparison with the PDMS fiber (Fig. 1).

The carbowax/TPR fiber exibits a lower recovery for all the PAHs than the others fibers, and the worst quality of fiber/analytical column transfert (Fig. 5A).

The increase of the peak width underlines a slower desorption rate of the compounds from the fiber into the supercritical mobile phase. This reduced transfer rate seems to be uncompatible with an efficient separation required for the quantification of PAHs.

The PDMS/DVB fiber increases the recovery of the smaller PAHs, but decreases that of the higher (Fig. 5B). An increase of dipole-dipole interactions between fiber and aromatic compounds passing from PDMS to PDMS-DVB fiber, when in the same time, the thickness of the fiber was reduced, may explain the result for smaller PAHs. The opposite effect noticed with higher PAHs could be due to reduce diffusion of compounds into the PDMS/DVB fiber.

However, as noticed previously, this fiber was dissolved by the supercritical fluids used for the separation, when the acetonitrile percentage reachs high level. Consequently, the

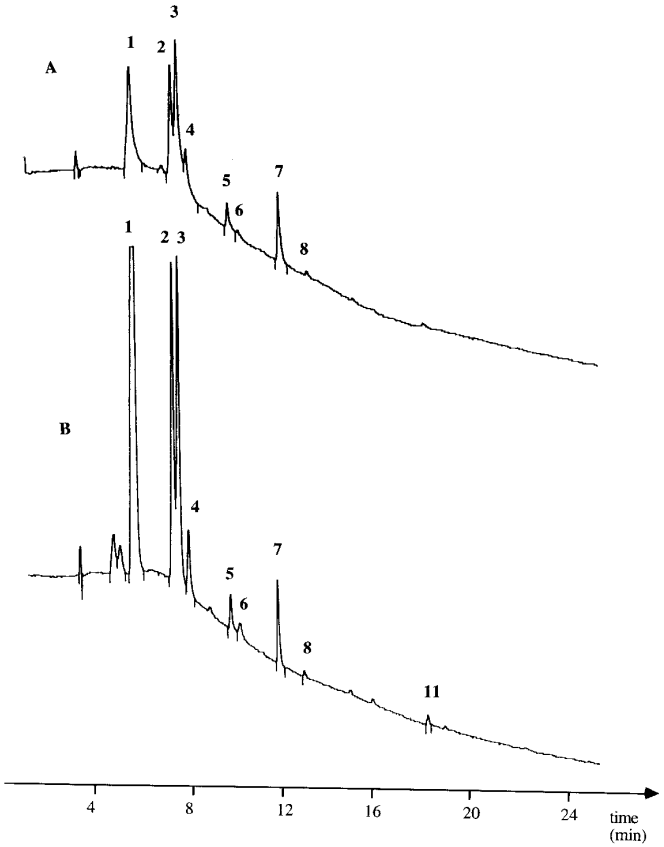

Fig. 5. Influence of the fiber chemical nature on the SPME/SFC results. Extraction and analysis conditions as in figure 1. A) Carbovax/TPR fiber; B) PDMS/DVB fiber.

use of this fiber requires to switch back the injection vanne one minute after the beginning of the analysis, to reduce the contact time between fiber and mobile phase. As prouved by the chromatogram, complete compound transfer from fiber to mobile phase (desorption step duration) is lower than one minute. This way of work enables the fiber reusability.

Conclusion

The hyphenated SPME/SFC requires more complex apparatus than classical liquid extraction and HPLC separation but enables both concentration of non volatil compounds and high separation level in a short analysis time.

The interface developped for HPLC, and SPME fibers can be used in the particular work conditions due to the supercritical fluid: high percentage of carbon dioxide in the mobile phase, high pressure in the chromatographic system and in the SPME/HPLC interface.

The discriminative recovery of PAHs by the PDMS fiber seems rather due to different diffusion rate than to interactions. However, because PDMS fiber enables extraction of the sixteen PAHs, this hyphenated technique should be successfully used for numerous applications.

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References

1. Loconto, P. R. *LC-GC int*. **1991** , *4*(9), 10-15.
2. Loconto, P. R. *LC-GC int*. **1991** , *4*(11), 10-16.
3. Arthur, C. L.; Pratt, K.; Motlagh, S.; Pawliszyn, J. *J. High. Res. Chromatogr*. **1992**, 741-744.
4. Louch, D.; Motlagh, S.; Pawliszyn, J. *Anal. Chem.* **1992,** *64*, 1187-1199.
5. Potter, D. W.; Pawliszyn, J. *J. Chromatogr.* **1992**, *625*, 247-255.
6. Zhang, Z.; Pawliszyn, J. *Anal. Chem.* **1993**, *65*, 1843-1852.
7. Zhang, Z.; Pawliszyn, J. *J. High. Res. Chromatogr.* **199**3, *16*, 689-692.
8. Page, B. D.; Lacroix, G. *J. Chromatogr.* **1993**, *648*, 199-211.
9. MacGillivray, B.; Pawliszyn, J. *J. Chromatogr. Sci*. **1994**, *32*, 317-322.
10. Arthur, C. L.; Killam, L. M.; Buchholz, K. D.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 1960-1996.
11. Buchholz, K. D.; Pawliszyn, J. *Anal. Chem.* **1994**, *66*, 160-167.
12. Krogh, M.; Johansen, K.; Tonnesen, F.; Rasmussen, K. E. *J. Chromatogr.* **1995**, *673*, 299-305.
13. Arthur, C. L.; Potter, D. W.; Buchholz, K. D.; Motlagh, S.; Pawliszyn, J. *LC-GC Int.* **1992**, *5*(10), 10-14.
14. Boyd-Boland, A. A.; Pawliszyn, J. *Anal. Chem*. **1996,** *68*, 1521-1529.
15. Lesellier, E.; Tchapla, A. In Packed Column supercritical fluid chromatography; Berger, C. and Anton, K. Ed.; Marcel Dekker inc, New York, 1997; pp 195-221.
16. Lesellier, E. accepted in *Analusis*.
17. Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. *Anal. Chem.* **1996**, *68*, 144-155.