On-line HPLC/SPME Interface using dynamic extraction

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Abstract. This study describes the determination of Polynuclear aromatic hydrocarbons in water using a new modification for the commercial SPME-HPLC interface which incorporates a dynamic SPME-HPLC extraction and vibration in the desorption chamber during the extraction and desorption steps. Extraction and desorption parameters were investigated using fourteen PAHs of different volatilities (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene) as test compounds and fluorimetric detection. Regression coefficients close to 0.99 with RSD < 8.1% and detection limits in the range 0.004–0.59 µg/L were found. A method was applied to determine the above PAHs in water samples. The results were compared with the 550.1 EPA method at the 0.05 significance level.

1. Introduction
Since its development, Solid Phase Microextraction (SPME) [1, 2] has been extensively used to separate a large number of organic compounds from different matrices such as environmental [3, 4, 5], biological [6, 7, 8] and food [9, 10, 11]. The advantage includes simplified sample preparation and solvent-free extraction. The extraction process may be carry out in two different ways: headspace (HS) in which the fiber is exposed in the vapor phase above a gaseous, liquid or solid sample and the direct immersion (DI) in which the fiber is immersed in the liquid sample, then a thermal desorption SPME in combination with gas chromatography (GC) using different detectors, especially mass spectrometry (MS), for volatile and semi-volatile organic compounds. [10, 12, 13]. On the other hand, liquid chromatography (LC) is essential for analysis of high molecular weight compounds, polar and/or thermally labile compounds not suitable for GC or GC/MS, as well as those with poor volatility; in this sense Supelco introduced a commercial version of the manual interface coupling the SPME with high performance liquid chromatography (HPLC); it consists of a standard six port HPLC injection valve with a special fiber desorption chamber, installed in place of the sample loop, because the fiber requires solvent desorption of analytes prior to HPLC or HPLC/MS analysis [14, 15, 16, 17].

Several authors have introducing changes in the SPME-HPLC interface with the aim to improve the method such as the study; these includes solvent direction of the flow in the interface [18, 19], reducing the internal volume of the interface [14, 19], the nature of the sealing mechanism [15], heating the interface to improve desorption efficiency [20, 21, 22] and modifying SPME/HPLC interface by using a 10-port valve and a C-8 refocusing unit [23]. However there are reasons why such methods have not been widely developed, which include a small number of available commercial fibers, in the automation...
of the method there is a lack and several limited lifetime due to the physical damage to the sorbent coating, particularly the due to the contact between with the solvent and the fiber coating during the desorption step [15, 18, 20, 24, 25, 26]; even so, the SPME-HPLC method has been a progress due to the benefits that offers as complement of the SPME-GC technique.

In this study the Supelco interface was modified to carry on an On-line dynamic extraction HPLC/SPME and improves the sensitivity, which includes a dynamic extraction with continuous flow of the sample on the fiber and the application of vibration in the desorption chamber during extraction and desorption; For this purpose, Polycyclic Aromatic Hydrocarbons (PAHs) have been chosen as test compounds to evaluate this method specifically the fourteen of them that the US Environmental Protection Agency (EPA) and European Union (EU) have been selected as priority contaminants [27]. HPLC-fluorescence detection was used. The method was applied successfully in water samples.

2. Experimental Section

2.1. Instrumental

2.1.1. Modified Supelco interface.
This interface was modified in several ways. The extraction step was made by putting the fiber in a continuous sample flow; a 100.0 mL syringe (Hamilton Bonaduz, Schwiz) which contain the sample, is inserted with the extraction chamber through six port valve (Reodyne model 9125) that is supply with vibration in load position; the syringe is pressurized by an infusion pump (Kd Scientific kds200) been able to continues flow rate.

Desorption step is made firstly in static mode by injecting into the chamber a volume of a suitable solvent to displace the sample and applying vibration in the chamber, then changing the valve to inject position; after a fixed time, the valve is returned to load and the displaced by the mobile phase. The figure 1a and 1b shows a schematic diagram of the SPME-HPLC interface.

2.1.2. HPLC conditions
PAHs analysis was carried out on a HPLC system which included an Agilent 1100 series HPLC-pump, a column oven with a temperature control module that allowed thermostatisation at 35°C (±0.5); the detection was made using an Agilent 1100 series programmable fluorescence detector. All the separations were carried out on a bonded phase specific for PAHs, Phenomenex Envirospe PP column (125x4.6 mm). The gradient started with 55:45 water:acetonitrile (v:v) during 2 minutes; next the acetonitrile content was increased to 90% in 25 minutes and returned to the initial composition after 15 minutes; the mobile phase flow rate was 1.0 mL/min. The excitation ($\lambda_{ex}$) and emission ($\lambda_{em}$) wavelength program used for detection is show in the table 1.

Manual SPME commercially available 65 $\mu$m film thickness polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fibers, from Supelco, were used; other fibers were also tested (PDMS-100 $\mu$m, PDMS-7 $\mu$m, PDMS/DVB-65 $\mu$m).

| PAHs          | excitation ($\lambda_{ex}$) | emission ($\lambda_{em}$) |
|---------------|----------------------------|---------------------------|
| Naphthalene   | 274                        | 335                       |
| Acenaphthene  | 266                        | 315                       |
| Fluorene      | 266                        | 315                       |
| Phenanthrene  | 249                        | 365                       |
2.1.3. Chemicals and reagents
Methanol and acetonitrile were of HPLC purity obtained from Scharlau, Barcelona, Spain. Water was purified with a Milli-Q system (Millipore; Milford, MA). Polynuclear aromatic Hydrocarbons Mix No. 49156 (naphthalene 500 µg/mL, acenaphthene 500 µg/mL, acenaphthylene 1000 µg/mL, fluorene 100 µg/mL, phenanthrene 40 µg/mL, anthracene 20 µg/mL, fluoranthene 50 µg/mL, pyrene 100 µg/mL, benzo(a)anthracene 50 µg/mL, chrysene 50 µg/mL, benzo(b)fluoranthene 20 µg/mL, benzo(k)fluoranthene 20 µg/mL, benzo(a)pyrene 50 µg/mL, dibenzo(a,h)anthracene 200 µg/mL, benzo(g,h,i)perylene 80 µg/mL and indene(1,2,3-c,d)pyrene 50 µg/mL) was obtained from Supelco their purity being higher than 99.999%. Stock standard solution was prepared taking 100 µL of the mixture of PAHs and they were diluted with methanol in a 10 mL volumetric flask; this standard solution was homogenized in the ultrasonic bath for five minutes. Standard dissolution was stored at 4°C in amber vial of 2.0 mL. The acenaphthylene and pyrene (1,2,3-c,d) indene were not included in this study because they show a low sensitive with fluorescence detector. Fresh standard solutions containing the stock standard solution PAHs mixture were prepared by diluting 100.0 µL into a 2.0 mL amber vial with 900.0 µL of methanol; these diluted standard solutions were homogenized in the ultrasonic bath for five minutes.

2.1.4. Real Samples.
Water samples were taken in the San Juan reservoir and the Guadalix River located in Madrid-Spain. Samples were collected in glass bottles Pyrex borosilicate amber and stored at 4°C. The water temperature at the time of sampling San Juan reservoir was 19°C, the sample was obtained in the GPS point. 40°22’16.1184” N 4°19’10.4016” W; the sample taken from the river Guadalix had a temperature of 16°C when was sampling and was collected in the GPS coordinates. 40°47’07.7” N 3°40’59.1” W.

2.2. Dynamic SPME/HPLC
2.2.1. Dynamic extraction process.
To carry out the extraction of the analytes are tested different fibers of Supelco CAR/PDMS 85 µm StableFlex, PDMS 100 µm, PDMS 7 µm and PDMS/DVB 65 µm.

The extraction of the PAHs was conducted in a dynamic mode; to optimize this methodology was prepared a sample by adding 50 µL of the diluted solution of PAHs in 100 mL of water Milli-Q with agitation in a platform (PSelecta Agimatic 243) 1200 rpm for ten minutes before the start of the process of SPME; then the 100 mL of the sample were transferred to a syringe of 100 mL (Hamilton, Bonaduz, Schwiz); right away, the needle of the syringe is introduced into the injector valve Rheodyne 7125 of 6 ports which is in position "load" to allow the passage of the sample through the chamber of desorption. On the other hand, the fiber of SPME stood at the house of desorption of the interface Supelco and exposed the fiber. The extraction process was performed by contact of the analytes with the fiber, passing the 100 mL of the sample by infusion from the syringe to a flow of 1.0 mL/min. During the
whole process of removal was applied vibration to the interface at room temperature (24-26°C). In figure 1 the experimental setup for the extraction process is shown.

Figure 1. a) Supelco interface modified: 1. SPME Holder, 2. SPME/HPLC Interface, 3. Vibration, 4. Syringe, 5. Infusion Pump, 6. Six Port Injector HPLC. b) SPME/ HPLC Flow path Sample.

2.2.2. Desorption process.
To desorb the analytes retained in the fiber sample syringe is removed. Then 90 µL of acetonitrile: methanol, 50:50, v:v, are injected into the desorption chamber; which is volume needed to completely fill the chamber and moving the remaining sample of the desorption chamber. Then is realized static desorption with vibration in the chamber by 3 min.; past this time the valve is turned to the “inject” position for the dynamic extraction for a 30 s. During the desorption process vibration is applied to the interface.

3. Results and discussion.
3.1. Dynamic extraction process.
In conventional SPME-HPLC the extraction is realized in an independent device introducing the coating fiber in the sample with stir until reaches the equilibrium, although the majority of the authors work in no-equilibrium conditions. In our method, when the dynamic extraction is used the partition coefficient between the sample and the fiber coating is reach pass the sample through of the internal diameter of the chamber extraction, in which the fiber is exposed to sample. Adsorption profiles were determined and optimized as function of the volume sample, the flow rate of sample and the vibration in the chamber extraction. The dynamic extraction was performed at room temperature (24-26°C). A 65 µm film thickness polydimethylsiloxane /divinylbenzene (PDMS/DVB) StableFlex fiber was selected because it
provided a best result regarding PDMS-100 µm, PDMS-7 µm, PDMS/DVB-65 µm fibers which were also tested.

The extraction volume profiles were studied (figure 2), shows that for 100 mL of sample the fiber reaches the equilibrium with the analytes obtain good extraction yields. The flow rate of sample was adjusted at 1 mL/min, rate optimal for maximum adsorb on the fiber, the slow dynamic extraction rate causes a better focusing result in a good adsorption on the fiber (see figure 3).

![Figure 2. Extraction volume profiles](image)
a) selected two rings to four rings PAHs; b) selected four rings to six ring PAHs.

The next step optimized was the vibration in the extraction chamber, the responses of the analytes were higher when the vibration was used, due to this increases the contact between the analytes and the fiber coating improves transport of analytes from the bulk sample phase to the vicinity of the fiber with the consequently increase of the sensibility and the reproducibility of the method as is show in the figure 4.

![Figure 3. Extraction rate profiles](image)
a) selected two rings to four rings PAHs; b) selected four rings to six rings PAHs.
3.2. Desorption process.
After the extraction step, with the fiber placed into desorption chamber of the SPME/HPLC interface was optimized the most efficient desorption solvent, a mixture of 50:50 methanol:acetonitrile, v:v, is a better solvent to desorbs the analytes from the fiber than the other solvents studied (ACN, MeOH and 30:70 MeOH:ACN). The time for static desorption was optimized in 3 min. The dynamic mode was optimized pass the mobile phase in inject position washing the fiber during 30 s. and immediately the valve was switched from inject to the load position. In both desorption modes was carried out applied vibration to the SPME/HPLC desorption chamber which has several effects on the SPME process due to enhance the diffusion and the mass transfer of the PAHs analytes since the fiber to the solvent.

3.3. Performance of SPME/HPLC method.
The optimized conditions established above were then used to prepare calibration curves for the 14 PAHs studied, using solutions of milli-Q water enriched 5 concentration levels of each of the PAHs margins between 0.1 and 500.0 µg/L; each calibrated concentration level was tested in triplicate; the results were related by the method of linear regression by the method of least squares. The linearly of the method was good with correlation coefficients ($r^2$) were between 0.925 and 0.995 for all standards and the relative standard deviations (RSD, %) were in range from 3.5 and 8.1 with reference to the midpoint of the concentration for each of the PAHs and triplicate analysis (n=3). The detection limits (LOD) were calculated as three times the standard deviation for a measurement value not than 10 times, these were in the range of 0.004 and 0.59 µg/L, the results are given in table 2. A typical SPME/HPLC chromatogram of PAHs in aqueous using the new methodology is shown in figure 5.

| PAHs          | Linear range, (µg/L) | $r^2$ | LOD, (µg/L) | RSD (n=3) |
|---------------|----------------------|-------|-------------|-----------|
| Naphthalene   | 2.5-250.0            | 0.995 | 0.28        | 6.3       |
| Acenaphthene  | 5.0-500.0            | 0.990 | 0.59        | 3.5       |
| Fluorene      | 0.5-500.0            | 0.928 | 0.01        | 7.7       |
| Phenanthrene  | 0.2-20.0             | 0.961 | 0.005       | 3.7       |

Table 2. Analytical characteristics of the method for standards

Figure 4. Peak areas obtained with and without vibration in the desorption chamber.
| Compound                  | Range    | Recovery | Detection Limit | LOD | LOQ |
|--------------------------|----------|----------|-----------------|-----|-----|
| Anthracene               | 0.1-10.0 | 0.989    | 0.007           | 3.5 |
| Fluoranthene             | 0.25-25.0| 0.940    | 0.04            | 5.7 |
| Pyrene                   | 0.5-50.0 | 0.992    | 0.03            | 8.1 |
| Benzo(a)anthracene       | 0.25-25.0| 0.987    | 0.01            | 6.5 |
| Chrysene                 | 0.25-25.0| 0.937    | 0.02            | 5.5 |
| Benzo(b)fluoranthene     | 0.1-10.0 | 0.968    | 0.008           | 5.3 |
| Benzo(k)fluoranthene     | 0.1-10.0 | 0.925    | 0.004           | 3.6 |
| Benzo(a)pyrene           | 0.25-25.0| 0.978    | 0.03            | 4.1 |
| Dibenzo(a,h)anthracene   | 1.0-100.0| 0.952    | 0.04            | 6.2 |
| Benzo(g,h,i)perylene     | 0.4-40.0 | 0.976    | 0.02            | 3.7 |

Figure 5. Chromatogram obtained from a standard mixture of PAHs in water using de the new methodology.

Accuracy was evaluated by recovery studies, R%, obtaining recoveries between 59 and 90% (Table 3), the results obtained with the proposed method were compared with the reference method EPA calculated the Student t, the results indicated no significant differences probability level 95% between the two methodologies [28].

The precision of the method the repeatability and reproducibility were studied, obtaining relative standard deviations for the method of dynamic extraction and desorption vibration (RSD) equal or below 8.1% for n = 3.
Table 3. Precision and accuracy obtained with dynamic extraction SPME-HPLC methodology and SPE (EPA method 550.1).

| PAHs                  | Dynamic Extraction SPME/HPLC | Method EPA 550.1 | t exp |
|-----------------------|------------------------------|------------------|-------|
|                       | µg/L RSD a RSD b % R c RSD d | µg/L % R c RSD d |
| Naphthalene           | 125.0 5.45 9.49 62.32 4.31 65.74 4.08 1.81 |
| Acenaphthene          | 250.0 6.20 10.18 65.07 5.53 61.02 5.85 1.61 |
| Fluorene              | 25.0 5.12 8.31 77.45 8.1 74.87 4.75 0.72 |
| Phenanthrene          | 10.0 8.90 10.09 76.80 7.7 69.58 7.93 1.79 |
| Anthracene            | 5.0 3.12 5.22 66.32 3.7 69.31 4.42 1.52 |
| Fluoranthene          | 12.5 5.55 4.48 81.12 8.1 81.27 7.49 0.03 |
| Pyrene                | 25.0 7.12 8.11 70.37 6.5 77.01 9.08 1.62 |
| Benzo(a)anthracene    | 12.5 3.66 4.51 59.78 9.4 75.81 5.01 1.75 |
| Chrysene              | 12.5 8.01 8.75 90.02 5.5 93.70 2.40 1.35 |
| Benzo(b)fluoranthene  | 5.0 6.42 8.94 89.19 10.6 81.14 7.26 1.44 |
| Benzo(k)fluoranthene  | 5.0 4.78 6.72 76.44 6.1 76.67 9.14 0.06 |
| Benzo(a)pyrene        | 12.5 3.15 6.33 72.44 11.2 64.74 7.26 1.66 |
| Dibenzo(a,h)anthracene| 50.0 7.65 7.02 81.33 7.4 81.64 2.25 0.15 |
| Benzo(g,h,i)perylene  | 20.0 7.36 8.21 76.79 5.7 80.91 3.54 1.57 |

a Repeatability (n = 3), b Reproducibility (n = 3), c R recovery (n = 6), d RSD % recovery (n = 6), Tabulated t value = 2.23 for P(0.05) [27].

3.4. Application to real samples
Analysis of water samples was carried out using the methodology EPA 550.1 and the method involves dynamic extraction; in both methods eight PAHs were found in samples of San Juan Reservoir (naphthalene, acenaphthene, fluorene, phenanthrene, fluorethene, benzo (a) anthracene, benzo (k) fluoranthene and benzo (a) pyrene) of which the phenanthrene was not quantified because it is outside the linear range; while the samples of the river Guadalix eight PAHs were detected by the method of dynamic extraction and seven by the EPA 550.1 (naphthalene, fluorene, phenanthrene, acenaphthene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene method and dibenzo (a, h) anthracene); Benzo (k) fluoranthene was detected but not quantified by the method of dynamic extraction and was not found by the EPA method; while fluoranthene was detected by both methods but was not quantified because they were outside the linear range. The results obtained in the quantization are in Table 4.

In order to validate the proposed SPME-HPLC method, its accuracy was evaluated by comparing the results with those found by the 550.1 EPA method [26], based essentially in SPE and HPLC with fluorimetric detection. The results obtained from the SPME method seemed to be slightly lower than those found from the EPA method; however, differences were not significant between both methods using the student t test at the significance level of 0.05 for all the PAHs detected (table 4).

Table 4. Determination of PAHs in real samples by dynamic extraction SPME-HPLC and SPE (EPA method 550.1).
### San Juan reservoir water samples

| PAH                | SPME Method (µg/L) | EPA 550.1 method (µg/L) | \( t_{\text{exp}} \) | SPME Method (µg/L) | EPA 550.1 method (µg/L) | \( t_{\text{exp}} \) |
|--------------------|--------------------|------------------------|-----------------|--------------------|------------------------|----------------|
| Naphthalene        | 27.90              | 6.86                   | 26.44           | 4.30               | 1.13                   | 14.34          | 2.87           | 9.82           | 3.09           | 0.98           |
| Acenaphthene       | 0.98               | 4.29                   | 1.21            | 1.50               | 2.71                   | n.d.           | -              | n.d.           | -              |
| Fluorene           | 1.18               | 8.36                   | 1.03            | 7.74               | 2.05                   | n.q.           | n.q.          | n.d.           | -              |
| Phenanthrene       | n.q.               | n.q.                   | -              | -                  | 7.89                   | 3.31           | 5.84           | 4.54           | 1.34           |
| Anthracene         | n.d.               | -                      | n.d.            | -                  | 1.53                   | 2.62           | 1.20           | 5.78           | 2.41           |
| Fluoranthene       | 0.55               | 7.23                   | 0.64            | 9.02               | 1.03                   | n.d.           | n.d.          | n.d.           | -              |
| Pyrene             | n.d.               | -                      | n.d.            | -                  | n.d.                   | n.d.           | -              | n.d.           | -              |
| Benzo(a)anthracene | 1.02               | 8.90                   | 0.95            | 7.06               | 1.50                   | n.d.           | n.d.          | n.d.           | -              |
| Chrysene           | n.d.               | -                      | n.d.            | -                  | 0.47                   | 4.70           | 0.55           | 3.01           | 1.21           |
| Benzo(b)fluoranthene | n.d.             | -                      | n.d.            | -                  | 0.31                   | 3.31           | 0.17           | 1.94           | 1.95           |
| Benzo(k)fluoranthene | 0.37              | 12.83                  | 0.43            | 10.23              | 0.86                   | n.q.           | n.d.          | n.d.           | -              |
| Benzo(a)pyrene     | 0.86               | 6.87                   | 0.72            | 6.25               | 1.97                   | n.d.           | n.d.          | n.d.           | -              |
| Dibenzo(a,h)anthracene | n.d.          | -                      | n.d.            | -                  | 0.91                   | 5.54           | 2.78           | 6.77           | 2.33           |
| Benzo(g,h,j)pyrene | n.d.               | -                      | n.d.            | -                  | n.d.                   | n.d.           | n.d.          | n.d.           | -              |

n.d., no detected, n.q. no quantified, \(^n=3\), Tabulated \( t \) value = 2.78 for \( P(0.05) \) [27]

### Guadalix River

| PAH                | SPME Method (µg/L) | EPA 550.1 method (µg/L) | \( t_{\text{exp}} \) | SPME Method (µg/L) | EPA 550.1 method (µg/L) | \( t_{\text{exp}} \) |
|--------------------|--------------------|------------------------|-----------------|--------------------|------------------------|----------------|
| Naphthalene        | 27.90              | 6.86                   | 26.44           | 4.30               | 1.13                   | 14.34          | 2.87           | 9.82           | 3.09           | 0.98           |
| Acenaphthene       | 0.98               | 4.29                   | 1.21            | 1.50               | 2.71                   | n.d.           | -              | n.d.           | -              |
| Fluorene           | 1.18               | 8.36                   | 1.03            | 7.74               | 2.05                   | n.q.           | n.q.          | n.d.           | -              |
| Phenanthrene       | n.q.               | n.q.                   | -              | -                  | 7.89                   | 3.31           | 5.84           | 4.54           | 1.34           |
| Anthracene         | n.d.               | -                      | n.d.            | -                  | 1.53                   | 2.62           | 1.20           | 5.78           | 2.41           |
| Fluoranthene       | 0.55               | 7.23                   | 0.64            | 9.02               | 1.03                   | n.d.           | n.d.          | n.d.           | -              |
| Pyrene             | n.d.               | -                      | n.d.            | -                  | n.d.                   | n.d.           | -              | n.d.           | -              |
| Benzo(a)anthracene | 1.02               | 8.90                   | 0.95            | 7.06               | 1.50                   | n.d.           | n.d.          | n.d.           | -              |
| Chrysene           | n.d.               | -                      | n.d.            | -                  | 0.47                   | 4.70           | 0.55           | 3.01           | 1.21           |
| Benzo(b)fluoranthene | n.d.             | -                      | n.d.            | -                  | 0.31                   | 3.31           | 0.17           | 1.94           | 1.95           |
| Benzo(k)fluoranthene | 0.37              | 12.83                  | 0.43            | 10.23              | 0.86                   | n.q.           | n.d.          | n.d.           | -              |
| Benzo(a)pyrene     | 0.86               | 6.87                   | 0.72            | 6.25               | 1.97                   | n.d.           | n.d.          | n.d.           | -              |
| Dibenzo(a,h)anthracene | n.d.          | -                      | n.d.            | -                  | 0.91                   | 5.54           | 2.78           | 6.77           | 2.33           |
| Benzo(g,h,j)pyrene | n.d.               | -                      | n.d.            | -                  | n.d.                   | n.d.           | n.d.          | n.d.           | -              |

n.d., no detected, n.q. no quantified, \(^n=3\), Tabulated \( t \) value = 2.78 for \( P(0.05) \) [27]

### 4. Conclusions
The results showed, that the modified interface has been successfully for the high recovery and good reproducibility, resulting in the fact that the On-line dynamic extraction methodology HPLC-SPME can be automated. The Validation of the new method for PAHs by comparing with the 550.1 EPA results indicate the potentiality of this modified interface to determine compounds of different volatilities.

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