Determination of polycyclic aromatic hydrocarbons and polycylic aromatic sulfur heterocycles by high-performance liquid chromatography with fluorescence and atmospheric pressure chemical ionization mass spectrometry detection in seawater and sediment samples

R.A. Gimeno, A.F.M. Altelaar, R.M. Marcé*, F. Borrull

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Imperial Tarraco 1, 43005 Tarragona, Spain

Abstract

Two methods for determining 10 polycyclic aromatic compounds were developed. Both methods were based on high-performance liquid chromatography (HPLC), but one method used fluorescence detection, while the other used atmospheric pressure chemical ionization mass spectrometry (APCI-MS). For water analysis, solid-phase extraction (SPE) was on-line coupled to the separation system. Using a styrene–divinylbenzene copolymer (PLRP-s) as sorbent in the SPE and adding 20% of acetonitrile to the water sample before its preconcentration, recoveries were above 70% for most of the compounds. For the fluorescence method, all compounds were detected and six of them could be quantified at concentrations higher than 0.02 μg l^{-1}. For the MS detection method, only seven of the compounds were detected and six were quantified at concentrations higher than 0.06 μg l^{-1}. To analyse sediment samples, an extraction with dichloromethane was used and, due to the complexity of the matrix, a standard addition calibration was carried out. Seawater and sediment samples taken from the Tarragona fishing port and marina on the coast of Catalonia (Spain) were analysed, and five compounds (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene) were quantified in the sediment samples. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Water analysis; Sediment; Polynuclear aromatic hydrocarbons; Polynuclear aromatic sulfur heterocycles

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are reported to have mutagenic and/or carcinogenic effects. The European Union (EU) has therefore developed a directive for controlling six PAHs in drinking water [1].

Some analogues of these compounds, such as polycyclic aromatic sulfur heterocycles (PASHs), are also potentially mutagenic and carcinogenic [2]. But, although they have a high bioaccumulation [3] and have been found in some water and sediment samples [4], they have not been studied as extensively as PAHs.

*Corresponding author. Tel.: +34-97-755-8137; fax: +34-97-755-9563.

E-mail address: marce@quimica.urv.es (R.M. Marcé).

These compounds occur in the environment as a result of natural or manmade incomplete combustion of organic materials, discharge from industrial processes, oil spill accidents, ballast operations, petroleum transport, traffic, etc. [5]. This is why it is very important to develop analytical methods for monitoring their presence in the environment.

The most common techniques used to separate PAHs are gas chromatography (GC) [6–10] and high-performance liquid chromatography (HPLC) [9–13]. With HPLC, the most common detection methods are UV–visible or diode-array detection (DAD) [10–12] and fluorescence detection [9–11,13]. Mass spectrometry (MS) has not been as extensively used because these compounds are difficult to ionize. Some studies have reported [14–18] using interfaces such as electrospray (ES), atmospheric pressure chemical ionization (APCI) or atmospheric pressure photoionization (APPI). The last one is a new interface that makes it possible to analyse compounds which are difficult to ionize due to their low polarity [18]. For ES, the addition of the tropylium cation to form the PAH–tropylium (π donor–π acceptor) complexes is proposed [17] in order to improve the ionization. APCI can also be used to determine PAHs, although for some compounds some authors found a decrease in sensitivity due to the presence of water in the mobile phase [14,15].

Because of the low concentration levels to be quantified in water samples, an enrichment step is needed before chromatographic analysis. This step has not been studied as much for PASH compounds. For PAHs, several preconcentration techniques have been used, but the most common preconcentration technique is solid-phase extraction (SPE) [19,20]. The sorbents that are most often used with SPE are C_{18} and styrene–divinylbenzene copolymers [5,11,19,20] like PLRP-s, although immunosorbents have also been used [10,21].

The main problem with these compounds is that they tend to become adsorbed onto the walls and surfaces they come into contact with. To avoid this, an organic solvent such as acetonitrile or 2-propanol or a surfactant must be added to the sample [11,19,22].

As mentioned above, the solubility of these compounds in water is very low and they tend to deposit in sediment samples. Thus, it is also important to determine them in such samples. One of the most common techniques for this extraction of the analytes from solid samples is using an organic solvent that is kept in contact with the sample [23,24].

The aim of this paper is to develop and compare two methods for determining polycyclic aromatic compounds, including the six PAHs regulated by the EU, and four PASHs, selected from bibliography. One methods involves HPLC separation with fluorescence detection and the other involves APCI-MS detection, as an alternative to the previous method. To analyse seawater, an on-line SPE step with a polymeric sorbent (PLRP-s) is used. For sediment sample analysis, the compounds are determined after extraction with an organic solvent. Finally, these methods are applied to determine these compounds in seawater and sediment samples from the marina and fishing port of Tarragona in Catalonia (Spain).

2. Experimental

2.1. Reagents and standard

Fig. 1 shows the structures of the studied compounds. All were obtained from Fluka (Buchs, Switzerland), Aldrich (Beere, Belgium), Sigma (Alcobendas, Madrid, Spain), Across (Geel, Belgium) or Supelco (Bellefonte, PA, USA), and all had a purity of more than 97%. Standard solutions of each compound at a concentration of 500 mg l^{-1} were prepared in acetonitrile (SDS, Peypen, France) and stored at 4 °C. All the working solutions were prepared by diluting these solutions.

For the mobile phase, HPLC gradient-grade acetonitrile and methanol (SDS) were used. To optimize the preconcentration step, HPLC gradient-grade 2-propanol (Merck, Germany) was also used. Ultra pure water was prepared by ultra filtration with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

A HP1100 system (Agilent Technologies, Barcelona, Spain) was used for HPLC separation. For detection, a HP1046A programmable fluorescence
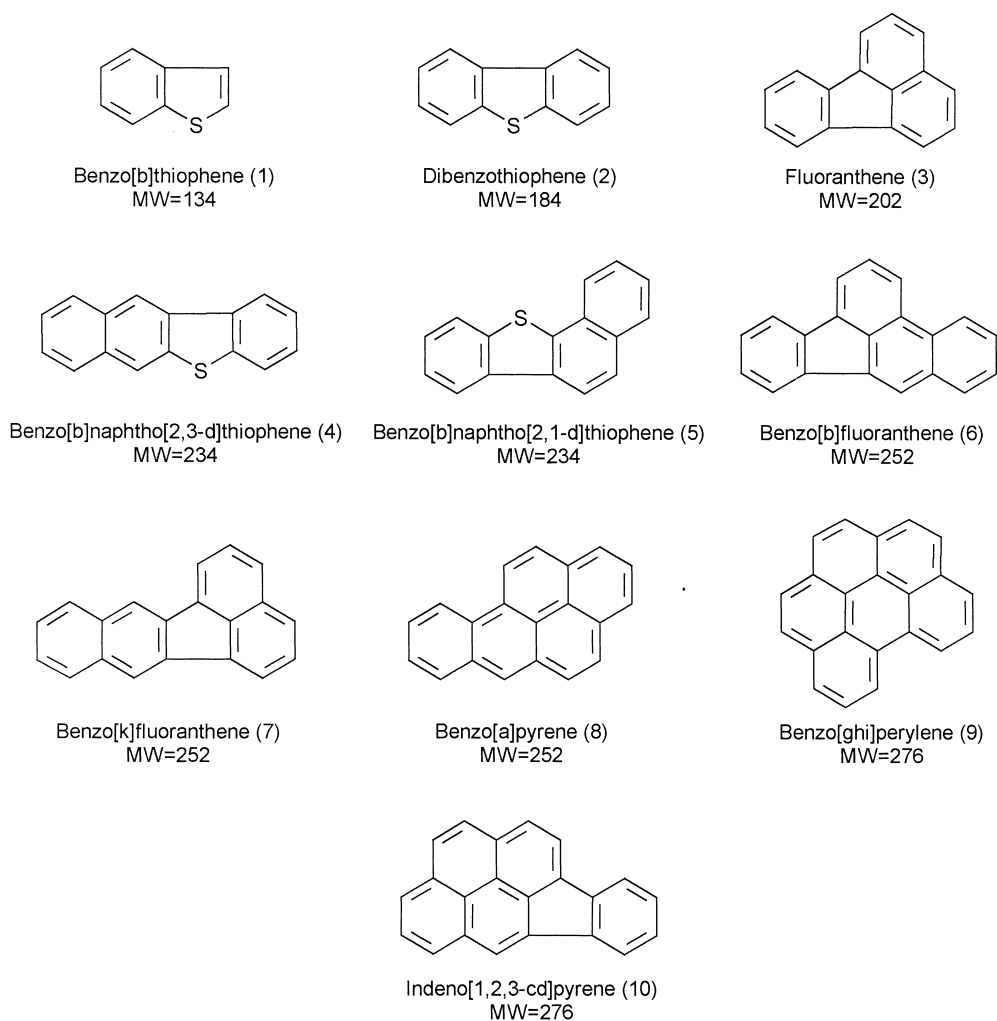
Benzo[b]thiophene (1)
MW=134

Dibenzothiophene (2)
MW=184

Fluoranthene (3)
MW=202

Benzo[b]naphtho[2,3-d]thiophene (4)
MW=234

Benzo[b]naphtho[2,1-d]thiophene (5)
MW=234

Benzo[b]fluoranthene (6)
MW=252

Benzo[k]fluoranthene (7)
MW=252

Benzo[a]pyrene (8)
MW=252

Benzo[ghi]perylene (9)
MW=276

Indeno[1,2,3-cd]pyrene (10)
MW=276

Fig. 1. Structures of the studied compounds. The identification numbers used in the figures and text are in parentheses. The molecular masses (M_r) of the compounds are also indicated.

detector (Hewlett-Packard, Barcelona, Spain) and an HP1100 mass-selective detection system with an APCI interface (Agilent Technologies) were used. The chromatographic column was a 15×0.46 cm Pinnacle PAH with a 5 μm particle size (Restek, Bellefonte, PA, USA). For direct injection experiments, 20 μl of sample volume was injected using an autosampler.

A HP1100 isocratic pump was used to add water into the mobile phase and improve the MS detection.

For the SPE step, a 10×3 mm stainless steel precolumn (Free University, Amsterdam, The Netherlands) laboratory-packed with 20 μm PLRP-s sorbent (Polymer Labs., Shropshire, UK) was used. The precolumn was on-line coupled to the chromatographic system with a Rheodyne 7000 valve, and an Applied Biosystems pump (Ramsey, NJ, USA) was used to preconcentrate the samples.

2.3. HPLC–fluorescence conditions

For HPLC separation with fluorescence detection, the mobile phase contained Milli-Q water and acetonitrile. The analysis started with 40% of acetoni-

trile, which was increased linearly up to 100% in 30 min. This percentage was maintained for 10 min to return to the initial conditions in 3 min. The column was equilibrated for 5 min. The column temperature was 30 °C and the mobile phase flow-rate was 1 ml min^{-1}.

For fluorescence detection, the following program of wavelengths was used: at 0 min, $\lambda_{ex}=233$ nm and $\lambda_{em}=312$ nm; at 15 min, $\lambda_{ex}=233$ nm and $\lambda_{em}=341$ nm; at 18 min, $\lambda_{ex}=233$ nm and $\lambda_{em}=420$ nm; at 23 min, $\lambda_{ex}=267$ nm and $\lambda_{em}=387$ nm; at 26 min, $\lambda_{ex}=271$ nm and $\lambda_{em}=363$ nm; at 27.5 min, $\lambda_{ex}=255$ nm and $\lambda_{em}=420$ nm; at 33 min, $\lambda_{ex}=230$ nm and $\lambda_{em}=450$ nm.

2.4. HPLC–APCI-MS conditions

For the MS detection, an isocratic separation with methanol as the mobile phase was used. The flow-rate was 0.75 ml min^{-1} and the column temperature was 45 °C. Water at a flow-rate of 0.5 ml min^{-1} was added between the separation and the MS systems to increase the response.

The positive ionization mode was selected and, after optimization, the vaporizer temperature was 500 °C, the nebulizer gas pressure was 60 p.s.i., the APCI drying gas was nitrogen at a 6 l min^{-1} at a temperature of 350 °C, capillary voltage was 4000 V and the corona current was 6 μA (1 p.s.i.=6894.76 Pa). The fragmentor voltage was set to 175 V and the gain was 16.

Chromatograms were recorded under selected-ion monitoring (SIM) acquisition. The molecular ion $[M]^{\cdot+}$ was selected for each compound. Under these conditions, only compounds 4–10 could be quantified because no signal was obtained for the rest.

2.5. Extraction methods

In the SPE process, the precolumn was first washed with 20 ml of acetonitrile and then with 20 ml of Milli-Q water at 4 ml min^{-1}. The tubes were then purged with the sample and finally 25 ml of this sample was preconcentrated at 2 ml min^{-1}. To inject the compounds into the chromatographic system, the retained analytes were eluted in back-flush mode by the mobile phase.

To analyse sediment sample, the enrichment step involved adding 2×30 ml of dichloromethane to 1 g of sample [25]. The mixture was shaken vigorously and kept for 30 min at each step. The supernatant was evaporated to dryness in a rotary evaporator. Finally, the residues were dissolved in 0.5 ml of acetonitrile. This solution was filtered through a 0.2-μm nylon syringe filter (Teknokroma, Barcelona, Spain) before being directly injected into the chromatographic system.

2.6. Sampling

Seawater samples were taken from the fishing port and marina in Tarragona, Catalonia (Spain). Seawater was also taken from the open sea as a blank sample. The samples were collected in 2.5-l pre-cleaned amber glass bottles. 20% of acetonitrile was added to stop the compounds from being adsorbed onto the wall of the bottles. The samples were then filtered through a 0.45-μm membrane filter (Whatman, Maidstone, UK) and kept at 4 °C until analysis.

Sediment samples were collected from the port of Tarragona and dried at room temperature.

3. Results and discussion

3.1. Optimization of HPLC with fluorescence detection

The chromatographic column specified in Section 2.2 was selected because it separates PAHs with high resolution. Moreover, mobile phase and wavelength program were optimized and the conditions are specified in Section 2.3. Under these conditions, all 10 compounds were separated in 35 min.

Calibration models were constructed and the highest concentration of the linear range was between 1 and 20 mg l^{-1} and the quantification limits ranged from 0.0005 to 0.075 mg l^{-1}. Linearity was good for all the compounds and correlation coefficients (R^2) were above 0.9997.

3.2. Optimization of HPLC–APCI-MS

The operational parameters of the APCI interface and MS detection were optimized for the positive ionization mode because for the negative mode there

was no response for most of the compounds. These parameters and their studied ranges were: drying gas flow (1–13 l min^{-1}) and temperature (150–400 °C), corona current (1–10 µA), nebulizer pressure (10–60 p.s.i.), capillary voltage (1000–6000 V), vaporizer temperature (200–500 °C), fragmentor voltage (25–350 V) and gain (4–16). The optimization was carried out by flow injection analysis (FIA) of several solutions containing the individual compounds at a concentration of 5 mg l^{-1}. An increase in the signal of most of compounds was observed when water was added to the carrier stream, although some authors [15,16] observed the opposite effect. So several percentages of water were checked. Acetonitrile and methanol were also checked as organic solvent in the carrier stream. Results were best with a water–methanol (40:60) solution, although compounds 1–3 were not detected, so this mobile phase was chosen for separating the compounds.

However, since at this mobile phase composition the analysis time was too long, the separation was established using methanol at a flow-rate of 0.75 ml min^{-1} as mobile phase and a post-column addition of water at a flow-rate of 0.5 ml min^{-1} before MS detection. Under these conditions, separation took only 20 min.

The optimum conditions outlined in Section 2.4 were chosen for the MS detection taking into account the results for the individual compounds. For all compounds, the molecular ion [M]$^{+}$ was obtained as base peak, and with a relative abundance (RA)

between 15 and 8%, the ion corresponding to the addition of a proton [M+H]$^{+}$.

Calibration models were constructed for the MS detection. The highest concentration of the linear range was 20 mg l^{-1} and the quantification limits were between 0.007 and 0.06 mg l^{-1}. Linearity was good for all compounds and correlation coefficients (R^2) were above 0.997.

3.3. Optimization of SPE process

The studied compounds tend to be adsorbed onto the walls and surfaces of the system and an organic solvent has to be added to the samples. This parameter has been studied for PAHs [19], but it had to be optimized for PASHs. For PAHs, the most common solvents are 2-propanol and acetonitrile [19], so both of these at different percentages were checked. In both cases some interferences from solvent coeluted with the first eluted compounds. Comparing optimum percentages of them, similar results were obtained, but less interferences were obtained for acetonitrile, so this solvent was chosen. In Table 1, some results are shown as an example. At low concentration of organic solvent, some of the last compounds were lost because they were adsorbed onto the system. Also, the more organic solvent added to samples, the lower the recoveries for the first eluted compounds. Finally, 20% of acetonitrile was chosen.

Recoveries were checked when a spiked seawater sample was preconcentrated and were similar to

Table 1
Recoveries obtained when 25 ml of Milli-Q water spiked at 0.8 µg l^{-1} containing different percentages of acetonitrile was preconcentrated ($n=3$)

Number	Compound	Acetonitrile		
		20%	25%	30%
1	Benzo[*b*]thiophene	108	42	17
2	Dibenzothiophene	104	102	104
3	Fluoranthene	100	95	96
4	Benzo[*b*]naphtho[2,3-*d*]thiophene	86	86	88
5	Benzo[*b*]naphtho[2,1-*d*]thiophene	86	86	89
6	Benzo[*b*]fluoranthene	88	89	91
7	Benzo[*k*]fluoranthene	78	79	83
8	Benzo[*a*]pyrene	71	69	72
9	Benzo[*ghi*]perylene	64	72	78
10	Indeno[1,2,3-*cd*]pyrene	40	51	58

The RSD was under 15% in all cases.

those obtained when Milli-Q water samples were preconcentrated.

3.4. Calibration

3.4.1. Seawater samples

Linearity for all the compounds was studied under optimum conditions for both methods by preconcentrating 25 ml of seawater sample spiked at different concentrations. Table 2 shows the linear ranges and limits of detection for a signal-to-noise ratio of 3. For both detectors, good linearity was obtained and correlation coefficients (R^2) were higher than 0.9996 for fluorescence and higher than 0.9967 for MS. For compounds 4, 8 and 9, the quantification and detection limits are similar when comparing both detection systems. For compounds 5–7, the results are lower with fluorescence detection but for the last compound, the quantification limit is lower with MS detection.

Repeatability was also evaluated by preconcentrating three seawater samples spiked at different concentrations of all the compounds. For fluorescence detection, the concentration was 0.3 μg l^{-1} for compounds 1, 2, 4, 5, 9 and 10, and 0.02 μg l^{-1} for compounds 3 and 6–8. The results, expressed as relative standard deviation (RSD), were between 2 and 15%, although most of the results were lower than 10%.

For MS detection, the concentration was 1 μg l^{-1} for compounds 5 and 6, 0.1 μg l^{-1} for compounds 4,7,9 and 10, and 0.01 μg l^{-1} for compound 8. In this case, the results were between 3 and 8%.

Fig. 2 show the chromatograms obtained at these conditions for both detection systems.

Reproducibility between days ($n=3$) was also evaluated for both methods at the same concentrations as for the repeatability analysis. Results were between 2 and 20% for fluorescence detection, being lower than 15% for most compounds, and between 6 and 13% for MS detection.

3.4.2. Sediment samples

From the different techniques available to analyse sediment samples, an extraction with an organic solvent was chosen because of the simplicity of this technique. As explained in Section 2.5, dichloromethane as organic solvent was selected from literature [25], and because of the complexity of the sample, a standard addition calibration was carried out.

3.5. Application to real samples

The developed methods were applied to analyse seawater and sediment samples from Tarragona fishing port and marina in Catalonia (Spain).

In seawater samples no compound was detected within the limits established in the methods, but when sediment samples were analysed, some of the studied compounds were detected. To quantify them, standard addition calibration was carried out. Sediment samples spiked at concentrations of between 75 and 600 μg kg^{-1} were treated as explained in

Table 2

Comparison of the linear ranges and limits of detection (LODs) when fluorescence or MS detection are used in the preconcentration of 25 ml of spiked seawater sample

| Number	Compound	Fluorescence		MS	
		Linear range (μg l^{-1})	LOD (μg l^{-1})	Linear range (μg l^{-1})	LOD (μg l^{-1})
1	Benzo[b]thiophene	0.06–10	0.03	–	–
2	Dibenzothiophene	0.009–10	0.003	–	–
3	Fluoranthene	0.008–10	0.004	–	–
4	Benzo[b]naphtho[2,3-d]thiophene	0.08–10	0.03	0.06–3	0.02
5	Benzo[b]naphtho[2,1-d]thiophene	0.07–10	0.02	0.7–100	0.3
6	Benzo[b]fluoranthene	0.02–5	0.008	0.6–100	0.2
7	Benzo[k]fluoranthene	0.007–5	0.003	0.07–10	0.03
8	Benzo[a]pyrene	0.004–1	0.001	0.006–0.3	0.003
9	Benzo[ghi]perylene	0.1–10	0.06	0.08–1.5	0.04
10	Indeno[1,2,3-cd]pyrene	0.3–10	0.1	0.08–3	0.04
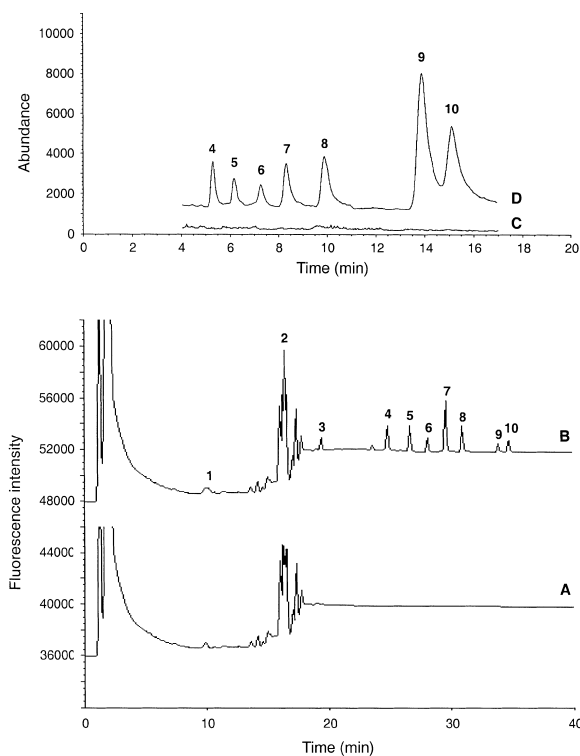
Fig. 2. Chromatograms obtained at the optimum conditions for SPE–HPLC–fluorescence and SPE–HPLC–APCI-MS when 25 ml of seawater sample was preconcentrated. Signal identification: (A) and (B), blank and spiked sample for fluorescence detection; (C) and (D), blank and spiked sample for MS detection. The concentration levels for the spiked samples are specified in the text. For peak identification, see Fig. 1.

Fig. 3. Chromatograms obtained for a sediment sample with fluorescence (A) and MS (B) detection.

Section 2.5 and analysed by both fluorescence and MS detection. Three unspiked sediment samples were also analysed for quantification. Fig. 3 shows the chromatogram for each method.

Because of the different quantification limits of the methods, not all the compounds could be quantified with the two methods. With fluorescence detection, compounds 6–8 could be identified and quantified at concentrations of 230, 86 and 174 μg kg^{-1}. With the APCI-MS system, compounds 7–10 were identified and quantified. The results were 526, 218, 281 and 123 μg kg^{-1}, respectively. For both detection systems, the RSDs were less than 15% in most cases. As can be seen, there are some difference between the concentration of compound 7 when calculating it using the different detection systems. These could be
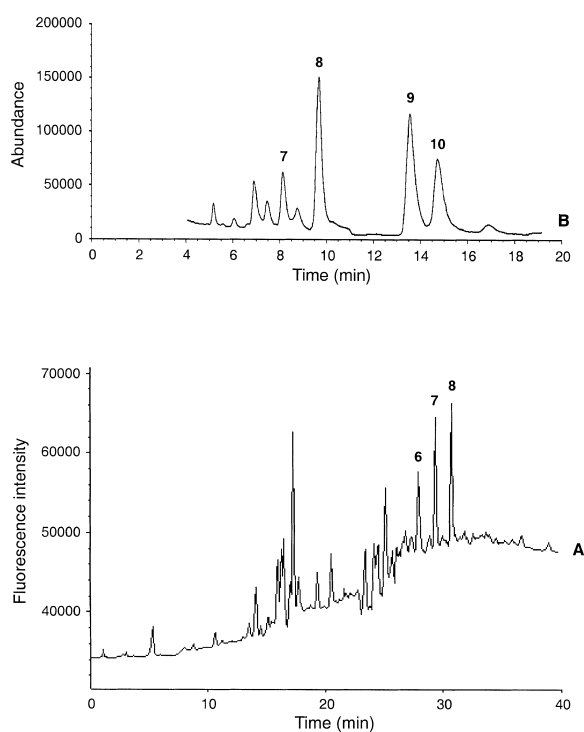
explained by the presence of interfering peak because in the MS chromatogram under the SIM acquisition mode there are some other peaks that appear at similar retention time to the studied PAHs. These interferences could not be identified under full-scan acquisition mode because of the low concentration levels.

Therefore, to detect whether there is any interference, we suggest on-line coupling between fluorescence and MS detection, since each detector provides complementary information.

4. Conclusions

Two methods have been optimized for determining a mixture of polycyclic aromatic compounds in seawater and sediment samples. Methods consist on fluorescence or APCI-MS detection after HPLC separation. APCI-MS detection was found to be not an alternative technique to the mostly used fluores-

cence but a good complementary technique for the detection of these compounds. To analyse seawater samples, 25 ml of sample was preconcentrated by on-line SPE with PLRP-s as sorbent and 20% of acetonitrile was added to the sample prior to preconcentration. For the fluorescence detection method, quantification limits ranged from 0.004 to 0.3 μg l^{-1} depending on the compound. Using the APCI-MS detection, only seven of the studied compounds could be detected and the quantification limits for these compounds ranged from 0.006 to 0.7 μg l^{-1}. To analyse sediment samples, an extraction with dichloromethane was used and a standard addition calibration was carried out. These methods were used to quantify these compounds in seawater and sediment samples from Tarragona fishing port and marina on the coast of Catalonia (Spain). Although no compound was detected in the seawater samples, five compounds were quantified in the sediment samples using both detection systems.

References

[1] Proposal for a Council Directive, Commission of the European Communities, Brussels, 1994.
[2] S.G. Mössner, S.A. Wise, Anal. Chem. 71 (1999) 58.
[3] J. Hellou, D. Mackay, B. Fowler, Environ. Sci. Technol. 29 (1995) 2555.
[4] R.M. Vilanova, P. Fernández, C. Martínez, J.O. Grimalt, Water Res. 35 (2001) 3916.
[5] E. Manoli, C. Samara, Trends Anal. Chem. 18 (1999) 417.
[6] S. Lacorte, I. Guiffard, D. Fraisse, D. Barceló, Anal. Chem. 72 (2000) 1430.
[7] L. Wolska, K. Galer, T. Górecki, J. Namiesnik, Talanta 50 (1999) 985.
[8] R. Doong, S. Chang, Y. Sun, J. Chromatogr. A 879 (2000) 177.
[9] J.D. Berset, M. Ejem, R. Holzer, P. Lischer, Anal. Chim. Acta 383 (1999) 263.
[10] C. Miège, M. Bouzige, S. Nicol, J. Dugay, V. Pichon, M.C. Hennion, J. Chromatogr. A 859 (1999) 29.
[11] R. El Harrak, M. Calull, R.M. Marcé, F. Borrull, Int. J. Environ. Anal. Chem. 64 (1996) 47.
[12] F. Sun, D. Littlejohn, M.D. Gibson, Anal. Chim. Acta 364 (1998) 1.
[13] I. Urbe, J. Ruana, J. Chromatogr. A 778 (1997) 337.
[14] C.H. Marvin, R.W. Smith, D.W. Bryant, B.E. McCarry, J. Chromatogr. A 863 (1999) 13.
[15] S. Pérez, D. Barceló, Chromatographia 53 (2001) 475.
[16] J.F. Anacleto, L. Ramaley, F.M. Benoit, R.K. Boyd, M.A. Quillian, Anal. Chem. 67 (1995) 4145.
[17] H. Moriwaki, A. Imaeda, R. Arakawa, Anal. Commun. 36 (1999) 53.
[18] D.B. Robb, T.R. Covey, A.P. Bruins, Anal. Chem. 72 (2000) 3653.
[19] R. El Harrak, M. Calull, R.M. Marcé, F. Borrull, J. High Resolut. Chromatogr. 21 (1998) 667.
[20] R.M. Marcé, F. Borrull, J. Chromatogr. A 885 (2000) 273.
[21] E.R. Brouwer, A.N.J. Hermans, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. A 669 (1994) 45.
[22] M. Bouzige, V. Pichon, M.C. Hennion, J. Chromatogr. A 823 (1998) 197.
[23] J. Slobodník, S. Ramalho, B.L.M. Van Baar, A.J.H. Louter, U.A.Th. Brinkman, Chemosphere 41 (2000) 1469.
[24] D. Thomas, S.M. Crain, P.G. Sim, F.M. Benoit, J. Mass Spectrom. 30 (1995) 1034.
[25] A. Jiries, H. Hussain, J. Lintelmann, Water Air Soil Pollut. 121 (2000) 217.