ELSEVIER

Journal of Chromatography A, 779 (1997) 123–130

JOURNAL OF CHROMATOGRAPHY A

Development of fast-scanning fluorescence spectra as a detection system for high-performance liquid chromatography Determination of polycyclic aromatic hydrocarbons in water samples

R. Ferrer, J. Guiteras*, J.L. Beltrán

Departament de Química Analítica, Universitat de Barcelona, Avgda. Diagonal 647, E-08028 Barcelona, Spain

Received 24 December 1996; revised 11 March 1997; accepted 7 April 1997

Abstract

A new detection system based on full-spectrum fast scanning fluorescence has been developed for high-performance liquid chromatography (HPLC–FSFS). Emission spectra were obtained by scanning the effluent from a chromatographic column at 50 nm s^{-1}. The raw chromatograms were corrected by an interpolation method, because the fluorescence intensity measured for each consecutive wavelength corresponded to a different portion of the effluent. Quantification of samples was done by partial least squares (PLS). This procedure has been used for the determination of polycyclic aromatic hydrocarbons in water samples. © 1997 Elsevier Science B.V.

Keywords: Fast-scanning fluorescence detection; Polynuclear aromatic hydrocarbons

1. Introduction

Fluorescence spectroscopy is among the most sensitive and selective detection methods used in liquid chromatography. It is particularly useful for the determination of substances that have intrinsic fluorescence, such as some organic compounds that are of interest in biomedical and environmental analysis [1–8].

Polycyclic aromatic hydrocarbons (PAHs) are of natural and anthropogenic origin and, therefore, they are likely to be found in many kinds of environmental samples. Moreover, they are extremely hazardous, which has led sixteen of them to be included by the Environmental Protection Agency (EPA) in the list of priority pollutants. Most PAHs show strong intrinsic fluorescence and they are usually determined by high-performance liquid chromatography (HPLC) with fluorescence detectors. There is, however, a constant need to improve the sensitivity and selectivity of existing methods and to reduce the time required for analysis.

Fluorescence detectors used in HPLC are usually of first-order detectors. This means that the emission intensity, at a fixed pair of excitation and emission wavelengths, is recorded versus time. Many modern detectors can be programmed to set the optimum wavelengths for each compound, thus increasing the selectivity and the sensitivity [1,9–11].

The introduction of diode array detectors (DADs) led to an improvement in the selectivity, especially for compounds that have similar retention times

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00410-X

[5,12–14]. A DAD consists of an array of photosensitive semiconductor material (photo-diodes), which can take readings simultaneously. They are second-order detectors, because they can record a full spectrum of the effluent for each retention time. The chromatograms are three-dimensional, with axes for retention time, wavelength, and absorbance or emission intensity. DADs are mainly used as absorbance detectors, but some studies of intensified and non-intensified photodiode array fluorescence detectors have been published in recent years [15–20]. DADs, however, have the shortcoming of relatively low sensitivity. To improve the sensitivity, several procedures have been developed, such as the use of xenon flashlamps as the excitation source coupled to a DAD [15] or, most currently, detection with intensified DADs (IDAD), either with conventional Hg–Xe lamps [16–18] or with lasers as the excitation source (LIF: laser-induced fluorescence) [19,20]. The detection limits obtained for PAHs by these techniques ranged between 50–200 ng ml^{-1} for the flashlamp-DAD and conventional lamp-IDAD, although the use of LIF–IDAD improved the detection limits to about 2 ng ml^{-1}. Nevertheless, these are about 30 times higher than those obtained by LIF–photomultiplier tube (PMT) detection [19]. This shortcoming, and the fact that they are not commercially available as standard excitation–detection systems, seriously hampers their use and practical application.

Some studies have described the use of video-fluorimeters, based upon the silicon-intensified target (SIT) vidicon [21–23] or charge-coupled device (CCD) systems, which have been applied in the last years to capillary electrophoresis, using a laser as the excitation source [24,25], to obtain an emission spectrum for each retention time. This system provides high selectivity and sensitivity, but the cost of the instruments is very high.

Another method to improve the selectivity is fast-scanning fluorescence, where spectra of the effluent from the chromatographic column are recorded at a fixed excitation wavelength by scanning emission wavelengths at a high rate. Initial studies were carried out using the procedure of stopped-flow measurements [26], but some diffusion took place during the scan, with the consequent loss of chromatographic resolution. In 1973, a paper reporting the use of an early fast-scanning method was published, but the resolution was low, only three compounds were analyzed, and it was used for qualitative purposes [27]. Finally, another methodology, constant-energy synchronous fluorescence spectroscopy (CESFS), which can reach scan speeds of 200 nm s^{-1}, was also reported, but it had worse sensitivity than the other procedures [28].

In this paper, a new method for the determination of PAHs by fast-scanning fluorescence is described. This method has been developed to be used with a conventional spectrofluorimeter, so that no further instrumental modifications were needed. Moreover, the spectral data have been collected using the standard control programs provided with the spectrofluorimeter.

Under the conditions used, the effluent from a chromatographic column is scanned at 50 nm s^{-1}, so that a full spectrum can be obtained every 2.7 s. The proposed method has been applied to the analysis of PAHs in a spiked water sample (network supply) and to a water sample from a lagoon, with good results. The PLS-1 multivariate calibration algorithm (partial least squares for the determination of a single compound) [29–31] has been applied for the calibration and the quantification steps.

2. Experimental

2.1. Reagents

Stock standard solutions (about 200 μg ml^{-1}) of acenaphthene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, crysene, dibenzo[*a,h*]anthracene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene were prepared by dissolving the pure solid (Supelco, Bellefonte, PA, USA) in either methanol or acetonitrile, depending on its solubility. Solutions of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene in either acetonitrile or methylene chloride (all at about 200 μg ml^{-1}), and a standard solution containing the sixteen PAHs classified as primary pollutants by the EPA, were purchased from Supelco. Working standards were prepared by dilution of the stock solutions with acetonitrile.

Acetonitrile was of HPLC quality (J.T. Baker, Deventer, Netherlands). Cyclohexane and dichloromethane were of residue analysis quality (SDS, Peypin, France).

Doubly distilled water (Milli-Q+, Millipore, Bedford, MA, USA) was used in the mobile phase. The mobile phase consisted of acetonitrile–water (80:20, v/v) and, before use, it was filtered through a 0.22-μm membrane filter and degassed with a stream of helium.

2.2. Apparatus

The chromatographic system consisted of a twin-piston Gynkotek 480 HPLC pump (Munich, Germany), a Gynkotek MSV6 automatic injector, with a 25-μl injection loop, and a 15.0 cm×4.6 mm Spherisorb S5 PAH column (Phase Sep, UK) with a 5-μm particle size. An isocratic elution procedure with a flow gradient [10] was used throughout.

An Aminco Bowman Series 2 spectrofluorimeter (SLM-Aminco, Rochester, NY, USA), equipped with a 25-μl flow cell (Hellma 176.752, Baden, Germany) was used for detection.

2.3. Sample extraction

PAHs were extracted from the water samples by a cloud-point extraction procedure [10], using Triton X-114 (Aldrich, Milwaukee, WI, USA) as the non-ionic surfactant.

2.4. Data acquisition and pre-processing

In order to increase the sensitivity of the method, excitation wavelengths were programmed as a function of the PAHs eluted, so that four different excitation wavelengths were used: Acenaphthene, fluorene, phenanthrene and anthracene were excited at 255 nm, fluoranthene, pyrene, benz[*a*]anthracene and crysene at 280 nm, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and dibenz[*a,h*]anthracene at 300 nm, and benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene at 360 nm (Table 1). Two different ranges of emission wavelengths were scanned: 335 to 435 nm for fluorene to dibenz[*a,h*]anthracene, and 400 to 500 nm for benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene.

Table 1
Characteristics of the method

Chromatographic system[a]: Flow gradient program

Time (min)	Flow (ml min^{-1})
0.0	1.0
2.1	1.0
9.3	1.1
10.2	2.0
11.6	2.0
13.0	2.5

Detection system[b]: Spectrofluorimeter program

Block	Time (min)	λ_{ex} (nm)	λ_{em} range (nm)
1	0.00–5.75	255	335–435
2	5.76–10.75	280	335–435
3	10.76–17.50	300	335–435
4	17.51–25.00	360	400–500

[a] Column: Spherisorb S5 PAH (15.0 cm×4.6 mm).
Mobile phase: acetonitrile–water (80:20, v/v), isocratic elution.
[b] Scan speed: 50 nm s^{-1}. Excitation–emission slits: 16–16 nm.

The spectra were recorded at a rate of 50 nm s^{-1} and readings were taken every 2 nm. The total time needed to record a spectrum and to start the next was 2.7 s (this value includes the time to record the spectrum and the time needed by the emission monochromator to return to the start position). Under these conditions, the spectrofluorimeter took a reading every 0.04 s and, therefore, a distorted chromatogram was obtained, because each point in each spectrum corresponded to a different time and, in consequence, to a different portion of the effluent from the chromatographic column. This is an important difference from diode-array detectors, which can simultaneously take all the readings required to record a full spectrum.

The raw chromatograms must be processed to obtain valid data, and a computer program, written in MATLAB language (The MathWorks, Natick, MA, USA), has been developed for this purpose. The value of the blank, which corresponds to the mobile phase and to the flow cell, is subtracted from the raw chromatogram. The individual points of the fluorescence spectra, however, do not correspond to the same eluent composition, because of the flowing of the mobile phase and, therefore, they must be corrected to obtain the fluorescence intensities for

each wavelength at a fixed time. In our case, this correction is carried out by spline interpolation.

The last step of the process is to minimize the background noise generated by the spectrofluorimeter because of the fast-scanning of the emission wavelengths. This was achieved by a smoothing procedure, based on factor analysis of the fluorescence intensity data matrix [32–34], where the experimental matrix was decomposed in a pure data matrix and an error matrix.

The whole process is represented in Fig. 1: Fig. 1a shows the raw data; Fig. 1b is the raw data from which the blank has been subtracted; Fig. 1c shows the data from Fig. 1b converted by spline interpolation and Fig. 1d shows the final data after smoothing.

2.5. Quantification

To make full use of the advantages of multichannel detection, the determination of each PAH was done at its optimum emission wavelength, to obtain the maximum sensitivity for each compound. Moreover, the chromatogram was split in several sections according to the retention times of the different PAHs. In this way, the determination of each compound was performed using only data from the section of the chromatogram where its peak appears, and at the optimum wavelength.

After the instrumental conditions were established for each PAH, the PLS-1 multivariate calibration algorithm was used for the calibration and the quantification steps. In Table 2, the experimental conditions (range of retention times, and excitation and emission wavelengths) are shown for the different compounds.

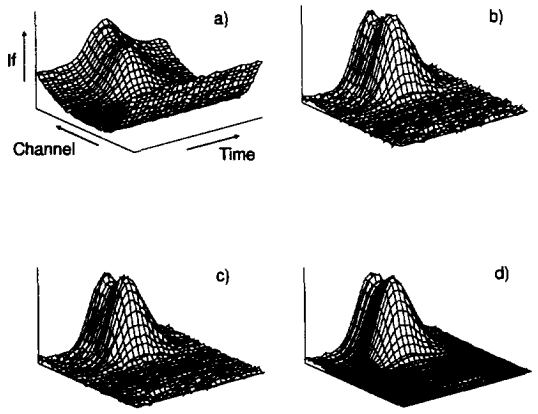

Fig. 1. Pre-processing of chromatographic data: (a) raw data; (b) raw data from which the blank has been subtracted; (c) data from (b) converted by spline interpolation; (d) final data after factor analysis smoothing.

Table 2
Parameters for the PLSR calibration

Compound	Retention time (s)	λ_{ex} (nm)	λ_{em} (nm)
Fluorene	205–265	255	337
Acenaphthene	205–265	255	337
Phenanthrene	245–295	255	403
Anthracene	285–320	255	361
Fluoranthene	330–375	280	433
Pyrene	395–455	280	391
Chrysene	530–575	280	353
Benz[*a*]anthracene	570–605	280	389
Benzo[*b*]fluoranthene	675–695	300	431
Benzo[*k*]fluoranthene	750–810	300	431
Benzo[*a*]pyrene	840–900	300	407
Dibenz[*a,h*]anthracene	950–1030	300	395
Benzo[*ghi*]perylene	1110–1175	360	412
Indene[1,2,3-*cd*]pyrene	1140–1210	360	484

The PLS calibration was carried out for seven different standards, which contained from 2 to 60 ng ml^{-1} of all of the compounds. The number of factors in the calibration model was figured out by cross-validation [35,36], leaving out one sample at a time. In all cases, two factors sufficed. From the calibration data set, and according to the PLS model, the different compounds in the samples were determined.

3. Results and discussion

All spectra were recorded at a scan speed of 50 nm s^{-1}. Higher scan speeds were tested, but this increased the risk of monochromator failure and did not improve the results, because of the increase in the spectral noise. A chromatogram of a standard mixture of several PAHs, in which the peaks corresponding to each PAH are labelled, is shown in Fig. 2. The fact that the emission intensity corresponding to each wavelength was measured only every 2.7 s did not significatively affect the resolution of the chromatograms, and the displacement of the spectra was easily corrected by the computer program developed for this purpose. In Fig. 3, some spectra

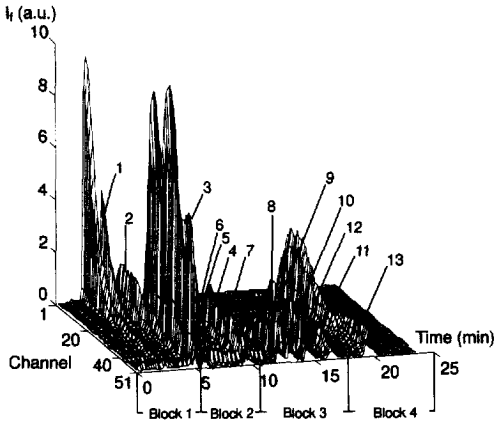

Fig. 2. Chromatogram of a standard solution of PAHs, 1= acenaphthene/fluorene, 2=phenanthrene, 3=anthracene, 4= fluoranthene, 5=pyrene, 6=chrysene, 7=benz[*a*]anthracene, 8=benzo[*b*]fluoranthene, 9=benzo[*k*]fluoranthene, 10=benzo[*a*]-pyrene, 11=dibenz[*a,h*]anthracene, 12=benzo[*ghi*]perylene and 13=indene[1,2,3-*cd*]pyrene. Detector conditions are as indicated in Table 1.

obtained by means of the FSFS method are compared with spectra obtained in a static method, showing a good agreement between both methods.

The use of wavelength programming to change excitation wavelengths and scan range led to a significant improvement in the selectivity of the method, because wavelengths used for each compound were as close as possible to their optimum values (Table 2).

Additionally, the possibility of recording the full-fluorescence spectrum allowed for a better determination of closely eluting PAHs. This is very clear for benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene (Fig. 4). From the chromatograms obtained by HPLC–FSFS (Fig. 4a), the appropriate emission wavelength to obtain the individual elution profiles for each independent compound (Fig. 4b) could be easily selected.

After the detection method was established, the quality parameters were determined (Table 3). Detection limits for the fast-scanning method, calculated as three times the standard deviation of the noise of a blank chromatogram, were compared with those obtained by first-order fluorescence detection with wavelength programming (HPLC–WP). As shown in Table 3, detection limits for HPLC–FSFS were usually higher than for HPLC–WP, although

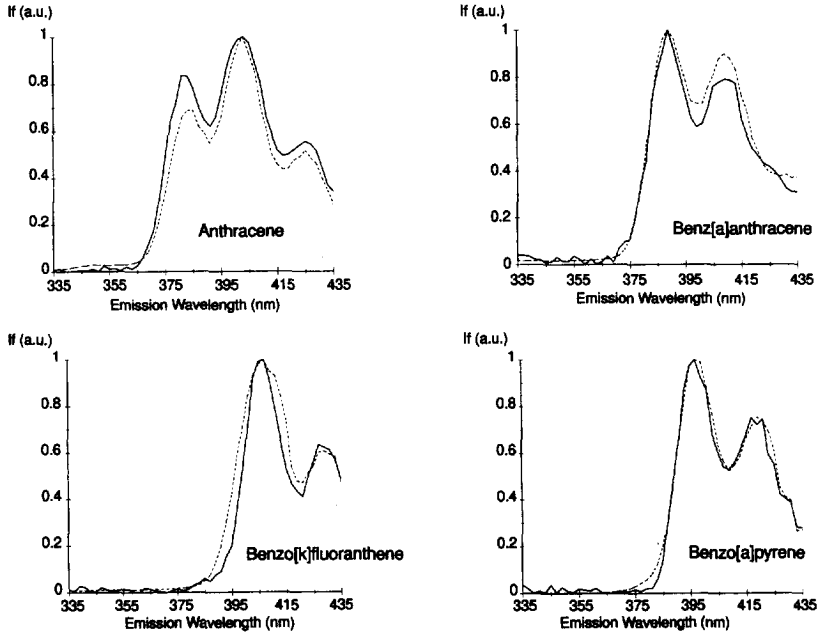

Fig. 3. Comparison between spectra obtained by fast scanning (solid line) and by scanning in a static mode (dotted line), both obtained at 50 nm s^{-1}.

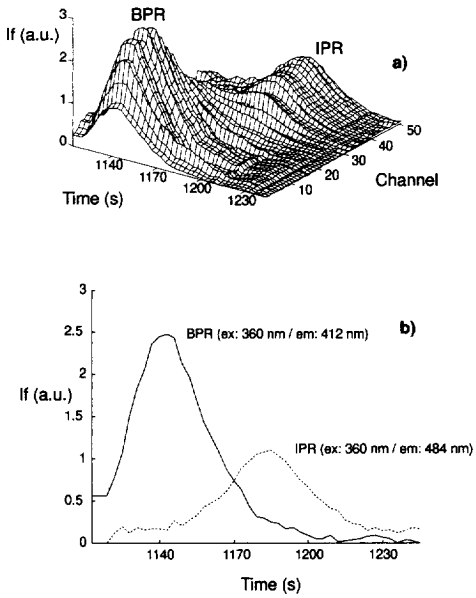

Fig. 4. (a) Chromatograms of benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene obtained by fast scanning fluorescence spectroscopy. (b) Elution profiles for each compound obtained from HPLC–FSFS data.

the differences were really important only for fluorene, acenaphthene, pyrene, dibenz[*a*,*h*]anthracene and benzo[*ghi*]perylene. Anyway, detection limits for any of these two methods were much better than those reported for some of these compounds when an intensified DAD was used in a modified spectrofluorimeter [15], and were similar to those obtained by LIF–IDAD [19]. Linear ranges are specified between the limit of quantification (calculated from ten times the standard deviation of the background noise of a blank) and the maximum amount of compound that still gives an emission intensity that can be read by the photomultiplier (i.e., before it becomes saturated). Relative standard deviation for the detection system has been determined by five injections of a standard solution of 10 ng ml^{-1} (this means 250 picograms of each compound injected into the chromatographic system).

The main advantage of second-order fluorescence detection lies in its ability to resolve co-eluting or closely eluting substances. With a first-order detector, closely eluting compounds must be measured together or, if there is some separation between the

Table 3
Quality parameters for the detection system

Compound	Limit of detection[a]		Linear range[a,d]	R.S.D. (%)[e]
	HPLC–WP[b]	HPLC–FSFS[c]		
Fluorene	4.2	26.5	90–2600	5
Acenaphthene	4.2	26.5	90–2600	5
Phenanthrene	4.7	6.2	21–2500	1.5
Anthracene	0.3	1.0	3–440	1.1
Fluoranthene	12.7	20.7	68–1800	5
Pyrene	6.5	56.5	188–5600	5
Chrysene	2.0	11.5	38–4100	8
Benz[*a*]anthracene	0.8	2.5	9–530	1.9
Benzo[*b*]fluoranthene	16.0	14.7	49–5600	7
Benzo[*k*]fluoranthene	0.5	1.2	4–450	3
Benzo[*a*]pyrene	1.0	3.2	11–1100	3
Dibenz[*a*,*h*]anthracene	1.8	10.0	33–2500	4
Benzo[*ghi*]perylene	13.5	49.0	163–3900	5
Indene[1,2,3-*cd*]pyrene	18.2	29.5	98–9800	14

[a] As picograms of PAH injected.
[b] HPLC–WP: HPLC with wavelength programmed fluorescence detection.
[c] HPLC–FSFS: HPLC with fast-scanning fluorescence spectra detection.
[d] Linear range is calculated between the limit of quantification and the value obtained for the saturation of the photomultiplier.
[e] Mean of five independent determinations.

peaks, an inclined baseline can be drawn from the baseline of the eluent to the intersection point of the peaks. With a second-order detector, totally independent signals can be obtained, provided that the co-eluting compounds have different spectra.

In order to test this new method of detection, the contents of PAHs in two water samples, a spiked water sample from a network supply, and a water sample from a lagoon, were determined.

Both samples were treated by a cloud-point extraction procedure. Fig. 5a shows the chromatogram of the spiked water sample. Some interfering compounds appear at short wavelengths (335–375 nm), but their influence decreases at longer wavelengths. Naphthalene, fluorene and acenaphthene, however, could not be determined in the samples; this is not a fault of the detection method (they can be determined in standards) but of the extraction method, because the peaks of these compounds are masked by the peaks of the surfactant. As shown in Table 4, the water was spiked at the levels designed by the directives of the European Community as the maximum admissible concentration of PAHs (0.2 μg l^{-1}), and the results obtained are in good agreement with the added values.

Table 4
Results for the spiked water samples (network supply)

Compound	Added (μg l^{-1})	Found (μg l^{-1}) [a]
Phenanthrene	0.19	0.16±0.02
Anthracene	0.20	0.31±0.03
Fluoranthene	0.18	0.18±0.03
Pyrene	0.18	0.16±0.01
Chrysene	0.19	0.14±0.01
Benz[*a*]anthracene	0.19	0.17±0.01
Benzo[*b*]fluoranthene	0.20	0.14±0.01
Benzo[*k*]fluoranthene	0.20	0.18±0.01
Benzo[*a*]pyrene	0.20	0.16±0.01
Dibenz[*a,h*]anthracene	0.18	0.18±0.02
Benzo[*ghi*]perylene	0.20	0.25±0.03
Indene[1,2,3-*cd*]pyrene	0.20	0.21±0.05

[a] Mean of four independent determinations.

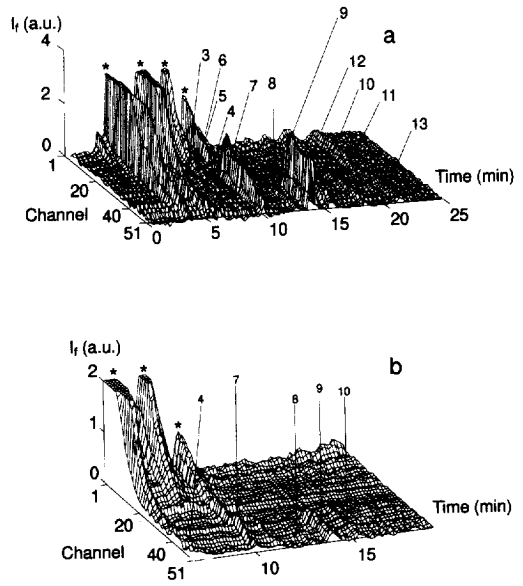

Fig. 5. (a) Chromatogram of a spiked water sample (network supply). (b) Chromatogram of a lagoon water sample. Only the sections where the detected compounds appear (blocks 2 and 3) are plotted. Peaks marked with an * correspond to the remains from the Triton X-114 used in the preconcentration step. Labelling of the peaks is as indicated in Fig. 2.

The second sample was analyzed for PAHs in two ways, first by HPLC–WP with fluorescence detection and then by the HPLC–FSFS method. The results obtained by the first method indicated that phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene and indene[1,2,3-*cd*]-pyrene were present, but not all of them could be quantified. Only benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene could be quantified when HPLC–FSFS was used, because the concentrations of the other compounds were below their limit of detection. In Table 5, the results obtained by both methods are summarized; when quantification was possible by HPLC–FSFS, the results were in good agreement with those obtained by HPLC–WP. The estimated limits of detection for these compounds in water samples are also given.

4. Conclusions

The use of a spectrofluorimeter as a second-order detector offers some important advantages. First, the possibility of recording the emission spectra of the compounds leads to a significant improvement of the selectivity of the method, because substances having

Table 5
Results for lagoon water samples (ng l^{-1})

Compound	HPLC–WP	HPLC–FSFS	
	Found[a]	Found[a]	LOD[b]
Phenanthrene	D	ND	10
Anthracene	D	ND	2
Fluoranthene	92±6	D	30
Pyrene	80±4	ND	90
Chrysene	6±2	ND	18
Benz[*a*]anthracene	112±8	119±6	4
Benzo[*b*]fluoranthene	162±12	151±12	24
Benzo[*k*]fluoranthene	102±9	89±5	2
Benzo[*a*]pyrene	139±11	138±7	5
Dibenz[*a,h*]anthracene	6±2	ND	16
Benzo[*ghi*]perylene	D	ND	78
Indene[1,2,3-*cd*]pyrene	D	ND	47

[a] Mean of three independent determinations.
ND: not detected.
D: detected, but below the quantification limit.
[b] Estimated detection limit for the cloud-point preconcentration procedure and determination by HPLC–FSFS.

very similar retention times can be easily identified and determined, provided that they have different spectra. Second, a spectrofluorimeter is much more sensitive than conventional diode-array detectors and, therefore, lower limits of detection can be reached; in fact, results are similar to those obtained by LIF–IDAD systems, but at a fraction of the cost, as the proposed method can be carried out using a standard spectrofluorimeter.

Acknowledgments

The authors thank the Comision Interministerial de Ciencia y Tecnología (CICYT) for supporting this study.

References

[1] H.G. Kicinski, S. Adamek, A. Kettrup, Chromatographia 28 (1989) 203.
[2] M.D. Nuñez, F. Centrich, Anal. Chim. Acta 234 (1990) 269.
[3] L. Hernández, S. Tucci, N. Guzman, X. Paez, J. Chromatogr. A 652 (1993) 393.
[4] C.G. Pinto, J.L.P. Pavón, B.M. Cordero, Anal. Chem. 66 (1994) 874.
[5] E.R. Brouwer, A.N.J. Hermans, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. A 669 (1994) 45.
[6] C. Escrivá, E. Viana, J.C. Moltó, Y. Picó, J. Mañes, J. Chromatogr. A 676 (1994) 375.
[7] Z. Deyl, F. Tagliaro, I. Miksik, J. Chromatogr. B. 656 (1994) 3.
[8] G. Gutnikov, J. Chromatogr. B 671 (1995) 71.
[9] J.L. Beltrán, R. Ferrer, J. Guiteras, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 477.
[10] R. Ferrer, J.L. Beltrán, J. Guiteras, Anal. Chim. Acta 330 (1996) 199.
[11] M. Mäkelä, L. Pyy, J. Chromatogr. A 699 (1995) 49.
[12] J.C. Fetzer, W.R. Biggs, J. Chromatogr. 642 (1993) 319.
[13] J.G. Dorsey, W.T. Cooper, J.F. Wheeler, H.G. Barth, F.P. Foley, Anal. Chem. 66 (1994) R500.
[14] O. Bruggemann, R. Freitag, J. Chromatogr. A 717 (1995) 309.
[15] T.L. Cecil, S.C. Rutan, J. Chromatogr. 556 (1991) 495.
[16] J. Wegrzyn, G. Patonay, M. Ford, I. Warner, Anal. Chem. 62 (1990) 1754.
[17] R.B. Poe, S.C. Rutan, Anal. Chim. Acta 283 (1993) 845.
[18] J.C. Gluckman, D.C. Shelly, M.V. Novotny, Anal. Chem. 57 (1985) 1546.
[19] R.J.v.d. Nesse, C. Gooijer, G.Ph. Hoornweg, U.A.Th. Brinkman, N.H. Velthorst, Anal. Lett. 23 (1990) 1235.
[20] S.J. Kok, R. Posthumus, I. Bakker, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, Anal. Chim. Acta 303 (1995) 3.
[21] I.M. Warner, M.P. Fogarty, D.C. Shelley, Anal. Chim. Acta. 109 (1979) 361.
[22] L.W. Hershberger, J.B. Callis, G.D. Christian, Anal. Chem. 53 (1981) 971.
[23] I.M. Warner, L.B. Mcgown, CRC Crit. Rev. Anal. Chem. 13 (1982) 155.
[24] G.A.M. Dalhoeven, N.V. Joshi, N. Rodriguez, L. Hernandez, J. Liq. Chromatogr. 18 (1995) 3729.
[25] J. Johanson, D.T. Witte, M. Larsson, S. Nilsson, Anal. Chem. 68 (1996) 2766.
[26] H. Harano, Y. Yamammoto, M. Saito, E. Mochida, S. Watanabe, J. Chromatogr. 83 (1973) 373.
[27] E.D. Pellizari, C.M. Sparcino, Anal. Chem. 45 (1973) 378.
[28] M.J. Kerkhoff, J.D. Winefordner, Anal. Chim. Acta 175 (1985) 257.
[29] H. Martens, Multivariate Calibration, John Wiley, New York, 1983.
[30] B.M. Wise, PLS Toolbox for use with MATLAB™, CPAC, Washington, 1992.
[31] A. Garrido Frenich, M. Martínez Galera, J.L. Martínez Vidal, M.D. Gil García, J. Chromatogr. A 727 (1996) 27.
[32] M. Meloun, J. Havel and E. Högfeldt, Computation of Solution Equilibria: A Guide to Methods in Potentiometry, Extraction and Spectrophotometry, Horwood, Chichester, 1988.
[33] E.R. Malinowski and D.G. Howery, Factor Analysis in Chemistry, Wiley, New York, 1980.
[34] J.J. Kankare, Anal. Chem. 42 (1970) 1322.
[35] S. Wold, Technometrics 20 (1978) 397.
[36] P.J. Gemperline, J. Chemometr. 3 (1989) 549.