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Three-Way Multivariate Calibration Procedures Applied To High-Performance Liquid Chromatography Coupled with Fast-Scanning Fluorescence Spectrometry Detection. Determination of Polycyclic Aromatic Hydrocarbons in Water Samples

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Three-way partial least-squares and *n* factor parallel factor analysis have been compared for the analysis of polycyclic aromatic hydrocarbons in water samples. Data were obtained with a chromatographic system set to record short-time chromatograms containing several unresolved peaks. The detection system consisted of a fast-scanning fluorescence spectra detector, which allows one to obtain three-dimensional data, where retention time, emission wavelengths, and fluorescence intensity are represented. The combined use of a multivariate calibration method and the three-dimensional data obtained from the HPLC−FSFS system allows resolution of closely eluting compounds, thus making a complete separation unnecessary. The procedure has been applied to tap water samples (spiked at 0.10 and 0.20 μg L^{-1} levels) with good results, similar to those obtained with a HPLC system with a conventional fluorescence detector.

The potential of separation techniques (such as liquid chromatography or capillary electrophoresis) has been vastly improved in the last years by the use of multichannel detectors [such as UV−visible diode-array (UV-DAD),[1−4] diode-array fluorescence,[5−10] charge-coupled devices (CCD),[11−12] or fast-scanning fluorescence spectrometry (FSFS)[13] detectors]. These detection systems allow the determination of the compounds to be analyzed not only by their retention times but also by their spectra (UV−visible or fluorescence). The additional information given by multichannel detectors offers possibilities that can be fully exploited if combined with multivariate calibration procedures.

Several multivariate methods have been applied to chromatographic data. Some of them, such as iterative target transformation factor analysis (ITTFA),[14] evolving factor analysis (EFA),[15,16] or window factor analysis (WFA),[17] do not provide direct quantitative information, because they only deal with one data matrix (one sample). The most useful are those that can deal with several samples simultaneously and that, therefore, can provide direct quantitative information. There are reports about the application of some of these methods, such as the adaptive Kalman filter[18] or generalizad rank annihilation matrix (GRAM),[19] to multichannel chromatographic data, but none about the use of three-way multivariate calibration procedures, such as *n* factor parallel factor analysis (PARAFAC)[20] or partial least squares (N-PLS),[21] although they have been applied to other trilinear data.[21−24]

PARAFAC and N-PLS are powerful techniques that, combined with the additional information provided by multichannel detection in chromatography, can, theoretically at least, resolve complex

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Table 1. Characteristics of the Method

working conditions for the chromatographic system	
column	15.0 × 4.6 mm Spherisorb S5 PAH
mobile phase	acetonitrile/water 90:10 (v/v)
detector conditions	
scan speed	50 nm s^{-1}
slit	16−16 nm

Spectrofluorometer Program

time (min)	λ_{ex} (nm)	λ_{em} (nm)
0.00−2.70	255	335−435
2.71−5.62	280	335−435
5.63−8.47	300	335−435
8.48−10.60	360	400−500

mixtures of compounds having similar chemical structures without the need of a complete chromatographic separation. As a consequence, the chromatographic system could be simplified and the time required for an analysis could also be shortened.

There are differences in the way that PARAFAC and three-way partial least squares (Tri-PLS) deal with the data. Tri-PLS sets the optimum calibration model by a reduction of the error in the concentrations, while PARAFAC reduces the error in the fit of the experimental data.

An important group of compounds usually determined by HPLC with fluorescence detection is that of polycyclic aromatic hydrocarbons (PAHs). They 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 16 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 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 an analysis.[25−27]

This paper describes the application of three-way PLS and PARAFAC to the analysis of multicomponent mixtures from three-dimensional, partially resolved, chromatograms obtained by HPLC−fast-scanning fluorescence spectrometry (FSFS). Both procedures have been compared, and the better models obtained have been used for the analysis of PAHs in spiked water samples, without a complete chromatographic separation, with good results.

Fast-scanning fluorescence spectrometry, which has been described previously,[13] has been chosen as the detection system because it shows higher sensitivity than conventional UV-DAD detectors and is similar to intensified-DAD (iDAD) or laser induced fluorescence-DAD (LIF-DAD). In addition, the use of a commercial spectrofluorometer for FSFS reduces the cost of the system in comparison with iDAD or LIF-DAD.

MODELING SYSTEMS

Three-Way PLS. The three-way partial least-squares algorithm decomposes the data cube (X) into three vectors: a score vector, t, and two weight vectors, w^J and w^K, in a model given by the equation

$$x_{ijk} = t_i w_j^J w_k^k$$

These vectors are calculated to have maximum covariance with the unexplained part of the dependent variable (concentrations), which satisfies

$$\max_{w^J w^K}[\mathrm{cov}(t,y)\ |\min(\sum_{j=1}^{J}\sum_{i=1}^{I}\sum_{k=1}^{K}(x_{ijk} - t_i w_j^J\ w_k^k)^2)]$$

Tri-PLS models the system by reduction of the error in the predictions (concentrations). It is thus more effective for quantitative than for qualitative purposes, as the qualitative information that can be obtained from the loading vectors is limited.

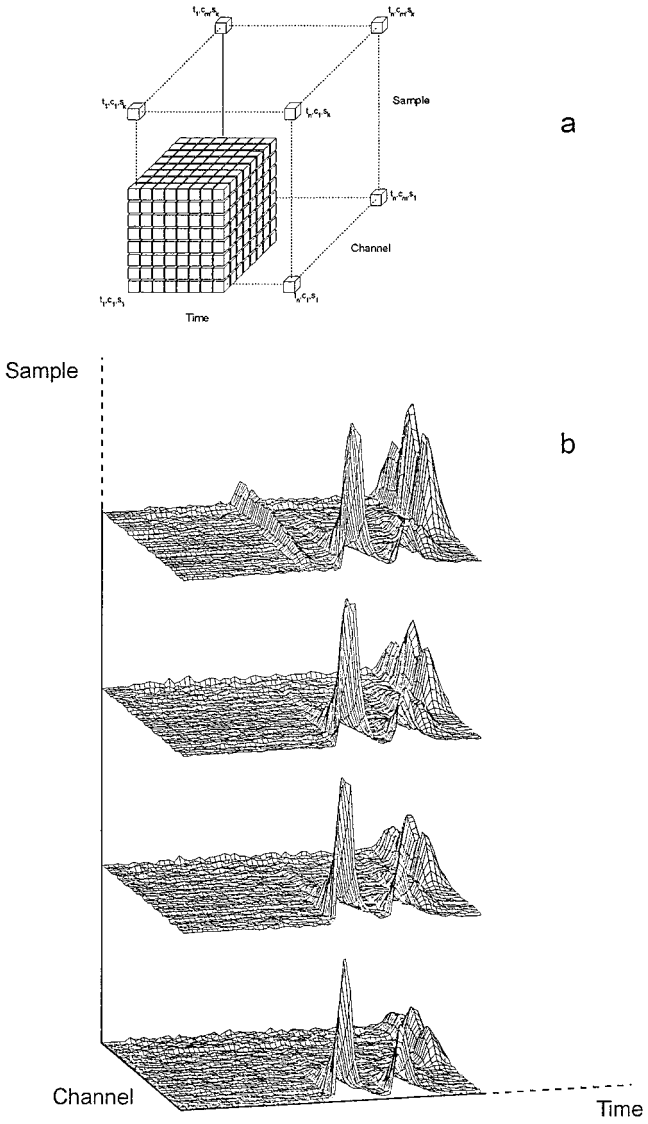

Figure 1. Disposition of data into the cube. (a) Dispositon of numerical data: time from t_1 to t_n, channels from c_1 to c_m, and samples from s_1 to s_k. (b) Three-dimensional representation of some chromatograms.

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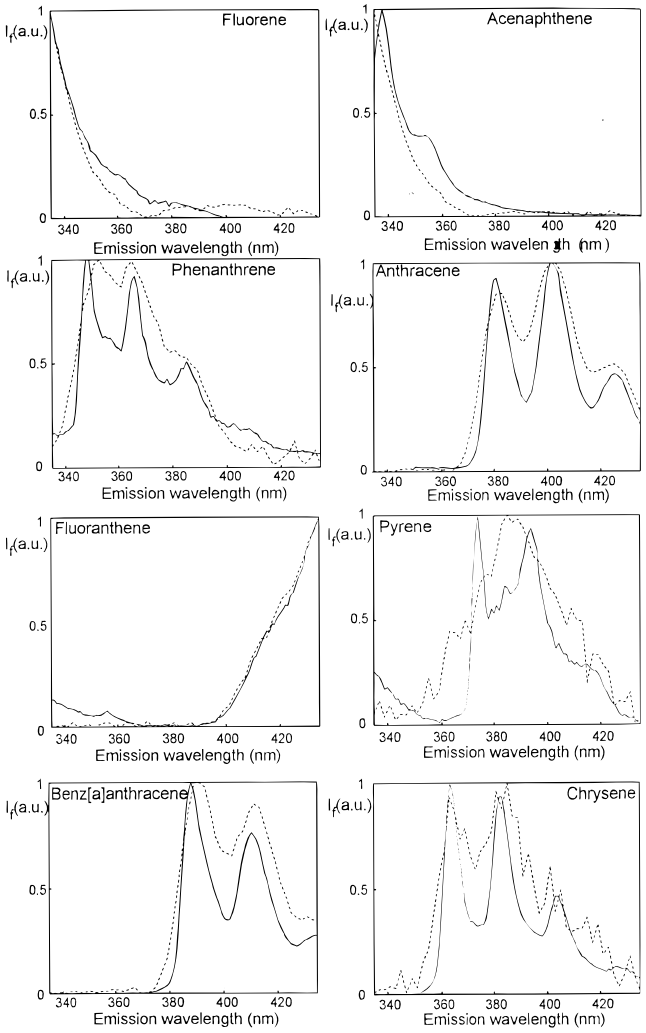

Figure 2. Emission spectra for the first eigth PAHs. Solid line, static emission spectra; dashed line, PARAFAC spectra.

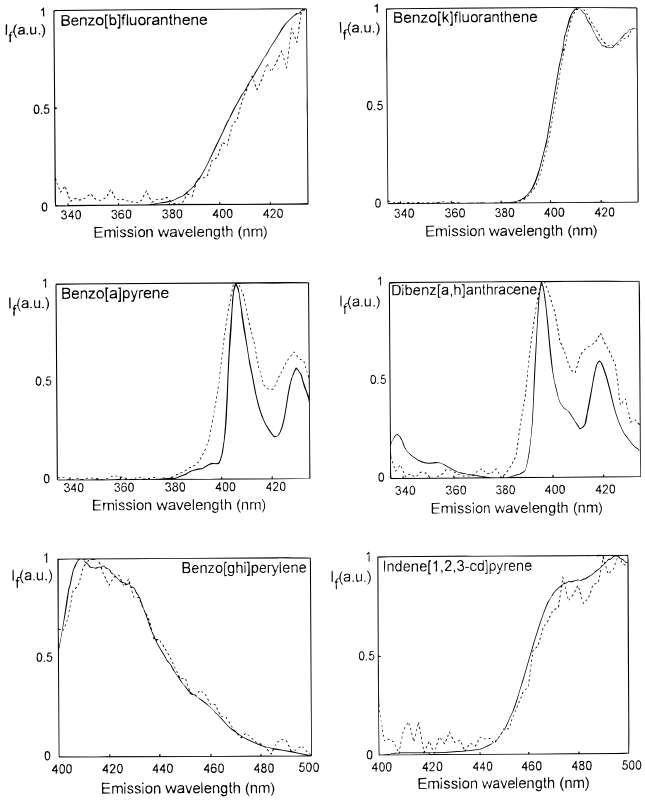

Figure 3. Emission spectra for the last six PAHs. Solid line, emission spectra; dashed line, PARAFAC spectra.

PARAFAC. The PARAFAC method allows the decomposition of bilinear data structures taking advantage of their uniqueness properties.[28] The simultaneous treatment of m samples allows one to obtain three matrices related to the fluorescence spectra (X), elution profiles (Y), and concentrations of the compounds (Z). For a sample (k), containing n compounds at different concentrations, the model R_k can be expressed as

$$R_k = \sum_{i=1}^{n} \hat{X}_i \hat{Y}_i' \hat{Z}_{k,i} + E_k$$

where Z is a $m \times n$ matrix containing numeric factors related to the concentration of each compound in each sample. The decomposition for all the m samples is done simultaneously, to obtain data related to the emission spectrum and the elution profile of each compound and also to its relative concentration in each sample. Two different approaches have been tested with PARAFAC decomposition. One of them had no constraints, meaning that the model found was a purely mathematical solution (that is, the coefficient matrices—or loadings—found for each factor could not be directly related to the chemical characteristics of the compounds, such as emission spectra or chromatographic peaks). The second model was obtained with nonnegative constraints, which means that the loadings found must be positive and that, theoretically, they should be directly related to chemical characteristics of the compounds and could be used to represent them, although in practice this is not always the case, at least for complex mixtures. Anyway, PARAFAC with nonnegative constraints provides useful qualitative information about the sample.

The solutions in PARAFAC models have been found by alternating least squares (ALS).[28] From the original data (R), initial estimates of X (X_0) and Y (Y_0) could be obtained, and the Z matrix was calculated from R, X_0, and Y_0, by a least-squares solution (Z_1). New values of Y (Y_1) were then obtained from X_0 and Z_1, and finally, X_1 was calculated from Y_1 and Z_1. This iterative procedure was repeated until convergence was achieved.

Number of Factors. For both modeling procedures, the minimum number of factors was calculated using a statistical test.[29] The optimum model was selected taking the minimum number of factors that yielded an error of prediction which did not have any significant differences with the minimum error of prediction.

For Tri-PLS the error was taken as the prediction error sum of squares (PRESS), calculated by a leave-one-out cross-validation procedure,[30] while in the case of PARAFAC, the error was obtained as the minimum error in the iterative process of the model fit.

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Table 2. Real and Predicted Values (ng mL^{-1}) for the Validation Set (PARAFAC Modeling)

compound	sample 1			sample 2			sample 3			global
	pred	real	*E* (%)	pred	real	*E* (%)	pred	real	*E* (%)	(all samples) *E* (%)
fluorene[a]	20.1	20.8	3.5	22.5	22.9	1.8	25.9	26.0	0.4	2.0
acenaphthene[a]	22.0	22.9	4.1	28.2	27.1	3.9	24.4	33.7	38.1	19.2
phenanthrene[a]	14.2	14.6	2.8	39.4	39.8	1.0	46.7	44.0	5.8	4.5
anthracene[a]	14.3	14.7	2.8	19.3	18.5	4.1	24.6	24.6	0.0	2.6
fluoranthene[a]	12.8	13.4	4.7	14.7	16.8	14.3	23.9	23.8	0.4	6.8
pyrene[a]	8.4	10.5	25.0	28.5	27.5	3.5	32.2	30.9	4.0	6.2
benz[*a*]anthracene[a]	11.6	10.9	6.0	18.6	17.9	3.8	32.6	33.0	1.2	2.7
chrysene[a]	13.3	10.7	19.5	24.2	25.3	4.5	24.0	29.1	21.3	14.6
benzo[*b*]fluoranthene[a]	8.5	8.7	2.4	12.0	11.6	3.3	20.1	20.0	0.5	1.9
benzo[*k*]fluoranthene[a]	6.0	7.4	23.3	13.8	15.3	10.9	3.9	3.1	20.5	12.7
benzo[*a*]pyrene[a]	34.4	36.7	6.7	17.9	18.7	4.5	31.2	32.5	4.2	5.3
dibenz[*a*,*h*]anthracene[a]	8.5	10.8	27.1	12.0	14.0	16.7	26.5	25.3	4.5	10.6
benzo[*ghi*]perylene[b]	10.5	9.7	7.6	27.9	25.8	7.5	15.9	16.1	1.3	7.1
indene[1,2,3-*cd*]pyrene[b]	29.8	31.5	5.7	23.2	23.3	0.4	11.3	14.6	29.2	8.9
global (all compounds)			8.1			5.3			11.5	9.2

[a] Six factors used to build the model. [b] Four factors used to build the model.

Table 3. Real and Predicted Values (ng ml^{-1}) for the Validation Set (Tri-PLS Modeling)

compound	sample 1			sample 2			sample 3			global
	pred	real	*E* (%)	pred	real	*E* (%)	pred	real	*E* (%)	(all samples) *E* (%)
fluorene[a]	22.7	20.8	8.4	23.5	22.9	2.6	16.0	26.0	62.5	25.2
acenaphthene[a]	23.9	22.9	4.2	26.3	27.1	3.0	34.2	33.7	1.5	2.8
phenanthrene[a]	14.2	14.6	2.8	38.3	39.8	3.9	43.8	44.0	0.5	2.6
anthracene[a]	16.3	14.7	9.8	18.2	18.5	1.6	25.7	24.6	4.3	5.8
fluoranthene[a]	13.3	13.4	0.8	15.4	16.8	9.1	23.6	23.8	0.8	4.4
pyrene[a]	11.4	10.5	7.9	28.0	27.5	1.8	28.5	30.9	8.4	5.1
benz[*a*]anthracene[a]	11.3	10.9	3.5	18.5	17.9	3.2	33.0	33.0	0.0	5.1
chrysene[a]	10.5	10.7	1.9	25.6	25.3	1.2	31.1	29.1	6.4	1.8
benzo[*b*]fluoranthene[a]	7.7	8.7	13.0	11.3	11.6	2.7	20.7	20.0	3.4	5.1
benzo[*k*]fluoranthene[a]	7.1	7.4	4.2	15.2	15.3	0.7	3.1	3.1	0.0	5.1
benzo[*a*]pyrene[a]	35.4	36.7	3.7	19.5	18.7	4.1	31.2	32.5	4.2	3.8
dibenz[*a*,*h*]anthracene[a]	10.0	10.8	8.0	14.1	14.0	0.7	25.6	25.3	1.2	2.8
benzo[*ghi*]perylene[b]	10.7	9.7	9.3	25.8	25.8	0.0	16.0	16.1	3.5	3.1
indene[1,2,3-*cd*]pyrene[b]	26.1	31.5	20.7	22.5	23.3	3.6	14.1	14.6	0.6	13.1
global (all compunds)			9.6			3.2			10.5	6.4

[a] Four factors used to build the model. [b] Five factors used to build the model.

EXPERIMENTAL SECTION

Apparatus. The chromatographic system consisted of a twin-piston Gynkotek 480 HPLC pump (Munich, Germany), a Gynkotek MSV6 automatic injector (Munich, Germany), with a 25-μL injection loop, and 12.5 cm × 4.6 mm Spherisorb S5-PAH column (Phase Separations Ltd.) with a 5-μm particle size.

An Aminco Bowman Series 2 spectrofluorometer (SLM-Aminco, Rochester, NY), equipped with a 25-μL flow cell (Hellma 176.752, Baden, Germany), was used for fast-scanning fluorescence spectra detection.[13]

Reagents. Stock standard solutions (~200 μg L^{-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) 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 ~200 μg L^{-1}), as well as a standard solution containing the 16 PAHs classified as primary pollutants by the EPA, were purchased from Supelco. Working standards were prepared by dilution of the stock solutions with acetonitrile.

Solid-phase extraction cartridges were C_{18} silica-based (Varian, San Fernando, CA).

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

Doubly distilled water (MilliQ+, Millipore, Bedford, MA) was used in the mobile phase. The mobile phase consisted of 90:10 acetonitrile/water, and before use, it was filtered through a 0.22-μm membrane filter and degassed with a stream of helium.

Safety Considerations. Several PAHs used in this study are suspected to be carcinogens and caution must be exercised with these compounds. All handling of standards should be performed in a ventilated hood, and latex gloves must be worn in order to avoid inhalation or skin contact. Waste solutions must be disposed properly.

Solid-Phase Extraction. Polycyclic aromatic hydrocarbons were extracted from the water sample (tap water spiked at 0.10 and 0.20 μg L^{-1}) by a solid-phase extraction (SPE) procedure.

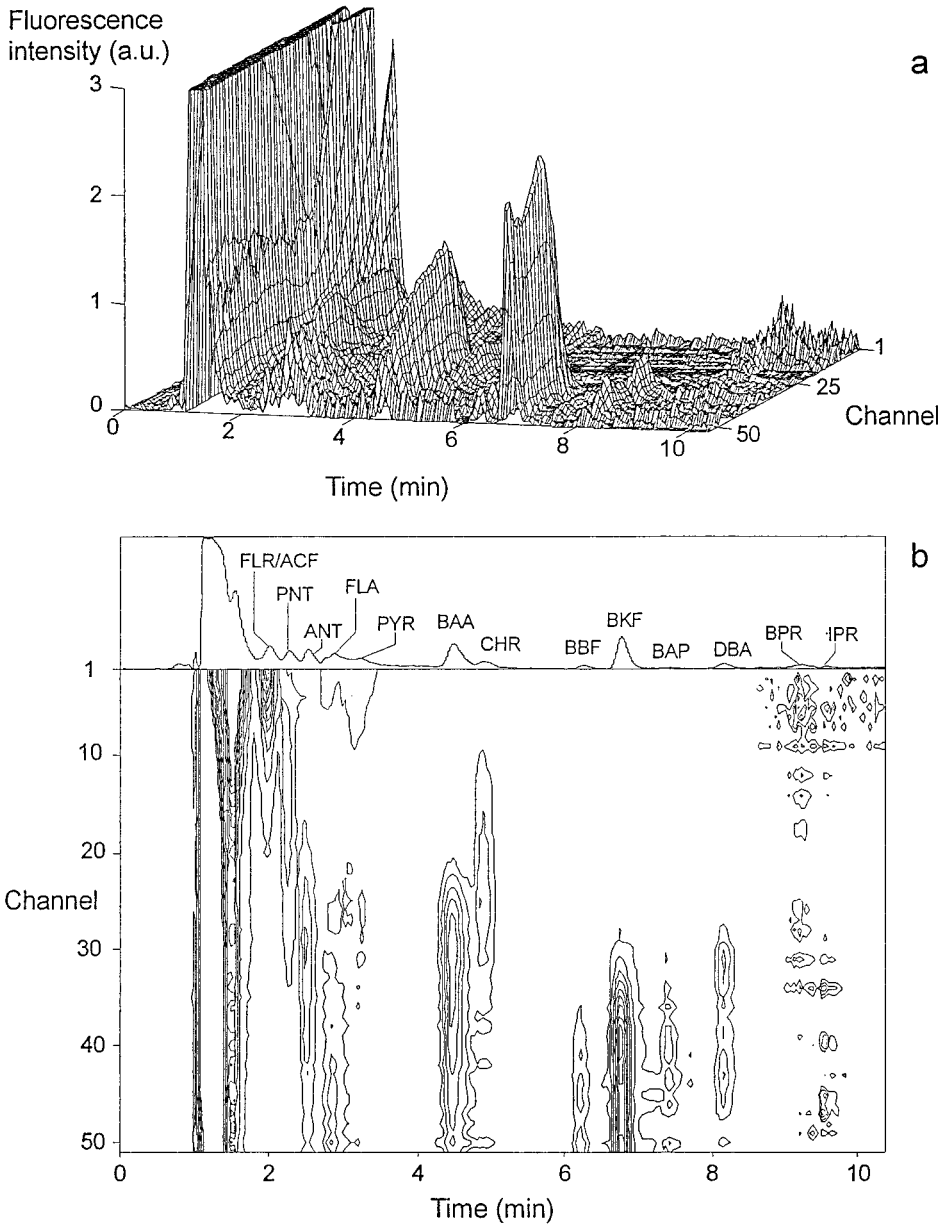

Figure 4. Tap water sample spiked at 0.1 μg L^{-1}. (a) Three-dimensional chromatogram; (b) profile and contour plot. For acronyms, see text.

The C_{18} cartridges were conditioned with 2.5 mL of methanol and 5 mL of a acetonitrile/water (5:95) mixture. The sample (100 mL of water, containing 5% v/v acetonitrile, to avoid adsorption of PAHs in the container) was introduced in the column at a flow rate of 2 mL min^{-1}, followed by 5 mL of the acetonitrile/water (5:95) mixture to remove any possible interferences. The cartridges were dried with a nitrogen stream, and the PAHs were eluted with three consecutive portions of 500 μL of acetone. This solution was evaporated to dryness with a nitrogen stream, and the PAHs were dissolved with 500 μL of acetonitrile.

HPLC−FSFS Detection. The chromatographic method has been modified with respect to that described in a previous paper,[13] where 20 min was required to obtain a full chromatogram. In this case, the flow gradient has been optimized in order to obtain a full chromatogram in a shorter time. The flow started at 1.5 mL min^{-1}, was constant for the first 4.5 min, rose to 4.0 mL min^{-1} from minute 4.5 to min 7.2, and remained constant at 4.0 mL min^{-1} from minute 7.2 until the end of the chromatogram. Under these conditions, the time required to obtain a full chromatogram was reduced to only 10 min, but this time still allowed the FSFS detector to record a number of spectra that was high enough to obtain good results by the multivariate calibration procedure. The change in chromatographic conditions led to a change in fluorometric conditions, as the time windows used to record the fast-scanning fluorescence spectra were shortened to fit the new situation (Table 1). Chromatograms contained four different sections, or blocks. In block 1, fluorene (FLR), acenaphthene (ACP), phenanthrene (PNT), and anthracene (ANT) were detected; in block 2, fluoranthene (FLA), pyrene (PYR), benz[*a*]-anthracene (BAA), and chrysene (CHR) were detected; in block 3, benzo[*b*]fluoranthene (BBF), benzo[*k*]fluoranthene (BKF), benzo[*a*]pyrene (BAP), and dibenz[*a*,*h*]anthracene (DBA) were detected; and finally, in block 4, benzo[*ghi*]perylene (BPR) and indene[1,2,3-*cd*]pyrene (IPR) were detected.

Fluorescence spectra were recorded along a range of 100 nm, with readings taken every 2 nm, at a scan speed of 50 nm s^{-1}.

Table 4. Spiked Tap Water Samples

	sample 1 (μg L^{-1})			sample 2 (μg L^{-1})	
compound	added	found[a] FSFS[b]	found[a] WP[c]	added	found[a] FSFS
fluorene	0.10	0.11 ± 0.01	0.08 ± 0.005	0.21	0.19 ± 0.01
acenaphthene	0.10	0.11 ± 0.01	0.14 ± 0.01	0.21	0.20 ± 0.01
phenanthrene	0.10	0.10 ± 0.01	0.11 ± 0.01	0.20	0.18 ± 0.01
anthracene	0.10	0.09 ± 0.005	0.06 ± 0.005	0.20	0.22 ± 0.01
fluoranthene	0.09	0.10 ± 0.01	0.10 ± 0.005	0.18	0.16 ± 0.01
pyrene	0.09	0.11 ± 0.01	0.07 ± 0.005	0.19	0.15 ± 0.02
benz[*a*]anthracene	0.10	0.09 ± 0.01	0.09 ± 0.01	0.19	0.21 ± 0.01
chrysene	0.10	0.09 ± 0.005	0.09 ± 0.005	0.20	0.21 ± 0.01
benzo[*b*]fluoranthene	0.10	0.10 ± 0.005	0.12 ± 0.005	0.21	0.22 ± 0.02
benzo[*k*]fluoranthene	0.10	0.10 ± 0.005	0.10 ± 0.005	0.21	0.22 ± 0.01
benzo[*a*]pyrene	0.10	0.08 ± 0.01	0.10 ± 0.01	0.20	0.23 ± 0.02
dibenz[*a*,*h*]anthracene	0.09	0.09 ± 0.005	0.11 ± 0.005	0.19	0.20 ± 0.01
benzo[*ghi*]perylene	0.10	0.08 ± 0.01	0.11 ± 0.01	0.20	0.24 ± 0.02
indene[1,2,3-*cd*]pyrene	0.10	0.11 ± 0.01	0.12 ± 0.01	0.21	0.19 ± 0.02

[a] Mean of three independent samples. [b] Fast-Scanning Fluorescence Spectra detector. [c] Wavelength Programming fluorescence detector.

Table 5. Comparison between Tri-PLS and PARAFAC for Different Concentration Ratio Samples

concn (ng mL^{-1})			Tri-PLS (3 factors)		PARAFAC (3 factors)	
C_{BAA}	C_{CHR}	ratio	C_{BAA}	C_{CHR}	C_{BAA}	C_{CHR}
20	1000	1:50	10.7 (46.5)	1034.2 (3.4)	6.8(66.0)	997.6(0.2)
25	100	1:4	21.4 (14.4)	88.1 (11.9)	29.2 (16.8)	123.9 (23.9)
30	250	1:8.3	31.0 (3.3)	270.1 (8.0)	29.5 (1.7)	259.1 (3.6)
35	500	1:14.3	34.0 (2.9)	496.7 (0.7)	30.7 (12.3)	492.7 (1.5)
15	600	1:40	19.4 (29.3)	564.1 (6.0)	9.73 (35.3)	552.2 (8.0)

concn (ng mL^{-1})				Tri-PLS (4 factors)			PARAFAC (4 factors)		
C_{BBF}	C_{BKF}	C_{BAP}	ratio	C_{BBF}	C_{BKF}	C_{BAP}	C_{BBF}	C_{BKF}	C_{BAP}
50	15	200	3.3:1:13.3	96.2 (92.4)	9.4 (37.3)	194.7 (2.6)	88.0 (76.0)	13.6 (9.3)	152.1 (23.9)
200	20	500	10:1:25	179.6 (10.2)	19.4 (3.0)	528.3 (5.7)	187.5 (6.2)	16.5 (17.5)	531.7 (6.3)
400	35	50	11.4:1:1.4	359.8 (10.0)	33.1 (5.4)	72.0 (44.0)	378.6 (5.3)	36.2 (3.4)	−0.14 (100.3)
650	50	100	13:1:2	631.1 (2.9)	53.3 (6.6)	59.3 (40.7)	667.7 (2.7)	51.0 (2.0)	72.0 (28.0)
500	20	1000	25:1:50	503.4 (0.7)	7.3 (63.5)	735.3 (26.5)	596.5 (19.3)	7.5 (62.5)	735.9 (26.4)

[a] BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluorantene; BKF, benzo[*k*]fluorantene; BAP, benzo[*a*]pyrene. Values in parentheses are the relative errors (%).

This allowed one to record a spectrum every 2.7 s (the time required by the monochromator to return to the initial position is included).

Data Processing. The data obtained from the spectrofluorometer (untreated data) must be corrected in order to avoid the displacement effect of the spectra in the chromatogram, provoked by the fact that each point in each spectrum corresponded to a different time and, in consequence, to a different portion of the effluent from the chromatogram. For this purpose, the untreated data were exported as ASCII files to be used with Matlab (MathWorks, Inc., Natick, MA) environment on a IBM Risc/6000 workstation. The value corresponding to the blank was subtracted and the effect of the flow was then corrected by a spline interpolation (raw data).

Six different kinds of data were used in the calculations, to decide which were most useful: raw data, scaled raw data, mean-centered raw data, smoothed data, smoothed scaled data, and smoothed mean-centered data.

Smoothing of raw data was done by a factor analysis procedure,[31−33] in order to reduce the instrumental noise. Mean-centered data were obtained by subtracting the mean of each column from all data in it. Scaled data were obtained by subtracting of the mean of each column from all data in it and dividing the result by the standard deviation of the column (thus obtaining a matrix where each column had zero mean and a variance equal to the unity).

RESULTS AND DISCUSSION

For the study and comparison of Tri-PLS and PARAFAC, the chromatograms of 10 standards were recorded. For each one of the four blocks forming a full chromatogram, data were first processed (as described in data processing) and then arranged into a cube so that the *x* axis corresponded to the retention time, the *y* axis corresponded to the channel (emission wavelengths), and, along the *z* axis (sample or concentration axis), the different chromatograms were placed. In Figure 1a, a graphical representation of the situation of the data points is shown, where each cube represents a data point. Figure 1b shows three-dimensional

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representations of some of the data in Figure 1a (each chromatogram corresponds to a horizontal plane in Figure 1a).

Qualitative Assessment. As could be expected, the best qualitative information was provided by PARAFAC when the decomposition was done with nonnegative constraints. This method gives good estimates of emission spectra and elution profiles of all of the components of a sample. Figures 2 and 3 show a comparison between the calculated emission spectra and the experimental spectra obtained in a static mode.

The loading vectors obtained by Tri-PLS also give some qualitative information, but this is of lower value.

Quantitative Assessment. For the quantitative assessment of Tri-PLS and PARAFAC, the 10 standards were divided into two groups, a calibration set composed of seven standards and a validation set formed of the three remaining standards.

The error of the model was expressed as a global error, calculated from the differences between the predicted and the real concentrations of the PAHs in the validation set, obtained by the equation

$$E\ (\%) = 100 \sqrt{\frac{\sum_{i=1}^{I}\sum_{j=1}^{J}(C_{\mathrm{pred}i,j} - C_{\mathrm{real}i,j})^2}{\sum_{i=1}^{I}\sum_{j=1}^{J}(C_{\mathrm{real}i,j})^2}}$$

where E is the percentage of error, C_{pred} is the concentration found by Tri-PLS or PARAFAC, I is the number of compounds, and J is the number of samples in the validation set.

In the case of Tri-PLS, smoothed scaled data gave poor results, while centered data gave good results only for the compounds of the second block (fluoranthene, pyrene, benz[*a*]anthracene, chrysene). Smoothed data were used for the other three blocks, although results obtained with raw data were similar. In the case of PARAFAC, the best results were obtained with smoothed data and without constraints.

The best estimates of the validation set obtained by both procedures are shown in Tables 2 and 3. Tri-PLS gave the lowest prediction error (6.4% against 9.2% for PARAFAC), which means a better global prediction, although results for two compounds (fluorene, benzo[*b*]fluoranthene) were worse than those obtained by PARAFAC.

Consequently, Tri-PLS has been selected for quantitative analysis and used for the determination of PAHs in spiked tap water samples. In Figure 4, a three-dimensional chromatogram, its profile, and its contour plot for a sample spiked at 0.10 μg l^{-1} are shown, and in Table 4, the concentrations calculated for each sample (corrected against the corresponding recoveries) are given. One of the samples was also analyzed by a conventional HPLC procedure, with a wavelength-programmed fluorescence detector,[34] and the results are also listed on Table 4, for comparison purposes.

Finally, several binary and ternary mixtures containing closely eluting compounds at very different concentration ratios were prepared and the chromatographic data analyzed by PARAFAC and Tri-PLS, to test the ability of these methods to solve such problems. As shown in Table 5, Tri-PLS offers better results than PARAFAC in all cases and can provide good results for closely eluting compounds at concentration ratios of even 25:1 in some cases.

CONCLUSIONS

The use of three-dimensional chromatograms combined with Tri-PLS or PARAFAC allows one to determine the concentration of PAHs in complex mixtures from partially resolved chromatograms. As a consequence, a total chromatographic separation is no longer necessary. This means than simpler chromatographic systems can be used and that the time required for an analysis is considerably shortened. The recently developed HPLC−FSFS detector has been used to record the chromatograms, because its sensitivity is better than that of UV-DAD detectors and similar to that of iDAD.

Both Tri-PLS and PARAFAC have good ability to predict the concentrations of the different compounds in a sample, although results obtained by Tri-PLS are slightly better than those given by PARAFAC, and for this reason, Tri-PLS was used for calibration and prediction. PARAFAC, however, gives more qualitative information, because estimates of the spectra and elution profiles for each compound can be calculated, if nonnegative restrictions are used, and this makes this procedure highly suitable for qualitative analysis.

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