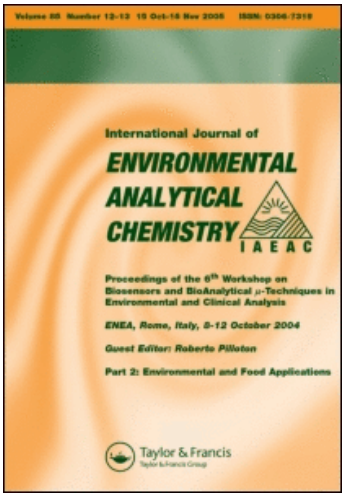

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Simultaneous Determination of Polycyclic Aromatic Hydrocarbons by Variable Angle Spectrofluorimetry

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SIMULTANEOUS DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY VARIABLE ANGLE SPECTROFLUORIMETRY

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The use of variable-angle fluorescence spectra together with multilinear curve-fitting techniques for the resolution of mixtures of polycyclic aromatic hydrocarbons (PAHs), is described. Only one spectrum is necessary to determine four compounds in a mixture even when a severe overlap occurs. The PAHs are quantified at ng/ml levels.

INTRODUCTION

The determination of polycyclic aromatic hydrocarbons (PAHs) is the subject of many papers published in recent years, because they are widespread environmental contaminants and many of them are known to be carcinogenic. Fluorescence spectroscopy has been shown to be selective and sensitive towards PAHs.[1] The main problem of the analysis of PAHs is that spectra of complex mixtures often cannot be well resolved because of overlap.[2] High-performance liquid chromatography (HPLC) with fluorimetric detection has been successfully used,[3–6] although previous clean-up and separation steps are then necessary to avoid interferences of related compounds. In order to avoid these time-consuming steps, synchronous fluorescence spectroscopy is frequently used to resolve PAH mixtures.[7] The suitable selection of $\Delta\lambda$ is the main handicap in its application. New software from the authors for instrumentation control and data acquisition allows a simple optimization of this parameter. The use of this technique for quantitative analysis of mixtures of anthracene, benz[a]anthracene, pyrene and phenanthrene is discussed.

We have also attempted the simultaneous determination of PAHs with highly overlapping spectra by combining the use of variable-angle fluorescence spectroscopy and multilinear fitting algorithms. Variable-angle fluorescence spectroscopy is a variant of synchronous fluorescence spectroscopy, in which excitation and emission wavelengths are varied simultaneously but at different rates. The

wavelength difference between both monochromators varies continuously. This allows one to select the best path through the excitation-emission matrix (EEM) to the particular mixture to be resolved. Thus, the quantitative analysis of several components is possible from only one spectrum.

This technique has had limited use due mainly to the difficulties in obtaining this type of spectrum. In this paper, variable-angle fluorescence spectroscopy is applied to resolve PAH mixtures. The spectra are obtained with a simple fluorimeter under computer control. No hardware modification is required. The procedure is fast and simple and can be used for routine determination of PAH in samples when all the constituents are known.

EXPERIMENTAL

Reagents

The PAHs used were obtained from the US Environmental Protection Agency (EPA Quality Assurance Branch, Cincinnati, USA) and used without further purification. Stock solution of these PAHs were prepared by diluting them in methanol at μg/ml levels. The methanol used in this study was Pestipur quality for residue analysis from SDS (Peypin, France). The water was deionized and distilled.

Apparatus

All the fluorimetric experiments were carried out on a Perkin–Elmer LS-5 luminescence spectrometer, provided with a Xenon discharge lamp (9.9 W) pulsed at line frequency, F/3 Monk–Guillieron monochromators and 1 × 1 cm quartz cells. The spectrometer was connected via interface RS232C to an IBM Personal System/2 model 50 equipped with hard disk and 3.5″ floppy disk.

Software

Instrument control, spectral data acquisition and representation were performed by using the fluorescence program package FLUOROPACK,* previously described by the authors.[8,9] This package has been designed to perform the most useful basic functions in fluorimetry and it also includes a powerful subroutine which allows the experimental obtention of variable angle fluorescence spectra in few minutes with no hardware modification.

Multilinear regression analysis was performed by using the program MULTIC.*[10] This program provides simultaneous determination of the PAH

*FLUOROPACK and MULTIC may be requested from SCIWARE Bank of Programs, Association of Environmental Sciences and Techniques (AEST), Department of Chemistry, Universitat de les Illes Balears, 07071-Palma de Mallorca, Spain.

Table 1 Characteristic analytical parameters of PAH

PAH	*If = Ax + B*[a]		*Linear range* (*ng/ml*)	*Exc-ems* (*nm*)	*LOQ*[b] (*ng/ml*)	*R.S.D.* (%)
	A	*B*				
B[a]Anthracene	2.81	8.2	5–200	285–389	3.0	0.42
Anthracene	15.54	3.9	0.4–40	248–400	0.25	1.20
Phenanthrene	1.023	7.7	40–200	248–366	7.0	1.07
Pyrene	0.394	4.8	32–400	269–390	12	1.03

[a]x is the concentration in ng/ml.
[b]LOQ, limit of quantitation.

concentrations, with their confidence limits (99% confidence level) as well as the variance analysis.

Procedure

Linear relationships between fluorescence intensity and the concentrations of each PAH were established within the range of 5–200 ng/ml of benz[a]anthracene, 40–200 ng/ml of phenanthrene, 0.4–40 ng/ml of anthracene and 32–400 ng/ml of pyrene. Analytical parameters are given in Table 1.

A number of standard isoluminescent solutions of each PAH were prepared (benz[a]anthracene, 40 ng/ml; anthracene, 8 ng/ml; phenanthrene, 100 ng/ml and pyrene, 160 ng/ml), as well as several synthetic mixtures of two, three and four components.

The excitation and emission spectra of each component were recorded under the following conditions: 480 nm/min scan speed, scale factor 1, response factor 1. Both excitation and emission width slits were 5 nm. These conditions were used for all subsequent experimental data collection. The scattered light spectra was similarly recorded using a methanol blank.

The tridimensional spectra were obtained by successive recording of 50 emission spectra (from 340 to 470 nm). On starting the measurements, the excitation wavelength was set at the lowest limit (230 nm). When a fluorescence scan was completed, the excitation monochromator automatically advanced an increment of 2 nm, and the emission spectrum was recorded again. The process was continued until 50 spectra were recorded within the excitation range of 230 to 328 nm with no hardware modification. Each tridimensional plot is formed by 50×131, that is 6550, experimental points in an excitation-emission matrix (EEM).

The contour plots, in which each line represents the isoluminescent points of the tridimensional excitation-emission representations, were obtained with the program SURFER from Golden Software (Colorado, USA).

The tridimensional synchronous fluorescence spectra were recorded by successive registration of 50 synchronous spectra at an excitation range of between 230 and 350 nm, with $\Delta\lambda$ varying from 30 to 177 nm (increment of $\Delta\lambda$ from one spectrum to another was 3 nm).

Different concentrations in the linear range of each standard were prepared in such a way that the solutions were isoluminescent. The variable-angle synchronous

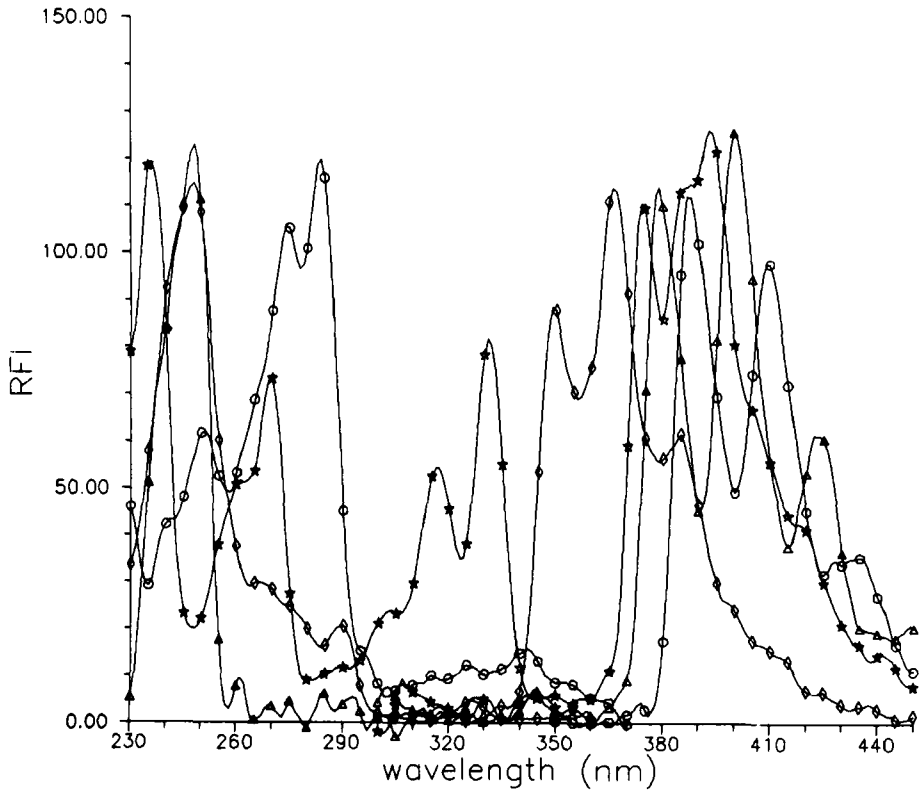


Figure 1 Excitation and emission fluorescence spectra of PAHs. ○, Benz[a]anthracene (40 ng/ml). △, Anthracene (8 ng/ml), ◇, Phenanthrene (100 ng/ml), ☆, Pyrene (160 ng/ml).

scanning was executed in triplicate for each concentration, and a standard spectrum was obtained for each component by averaging the three spectra. The variable-angle fluorescence spectra of samples were also obtained in triplicate and then averaged.

RESULTS AND DISCUSSION

Figure 1 shows the excitation and emission spectra of the four polycyclic aromatic hydrocarbon standards, phenanthrene, anthracene, benz[a]anthracene and pyrene. This figure illustrates the considerable overlap of these spectra, which hinders the multicomponent analysis of their mixtures by conventional fluorimetry.

Table 2 gives the composition of the mixtures of two, three and four components used in this study. These mixtures were prepared according to the following criteria: (i) to keep the concentration of one component constant while varying the others; (ii) to modify all the concentrations proportionally (constant relative proportions); (iii) to modify the concentrations without any defined

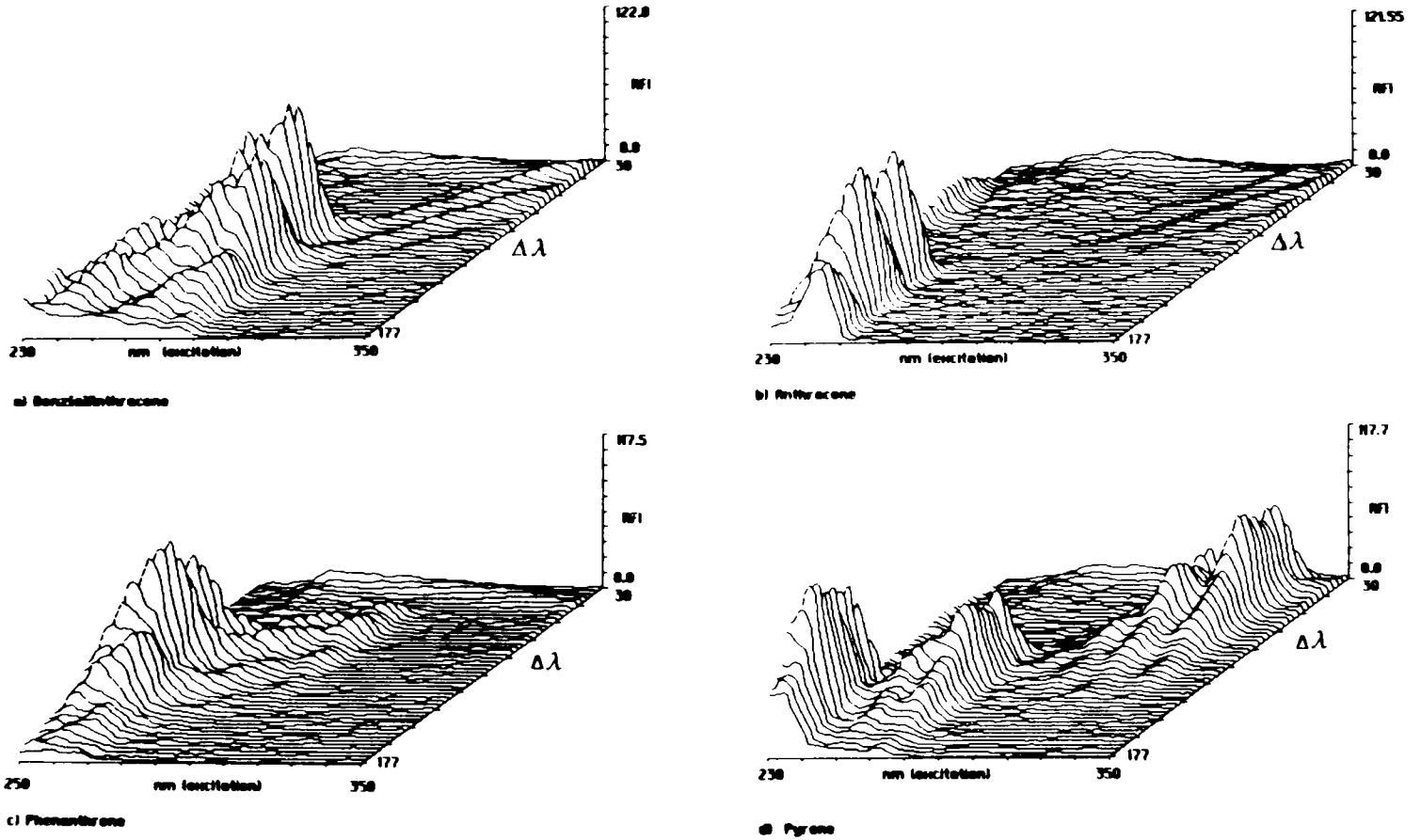


Figure 2 Tridimensional synchronous fluorescence spectra of PAHs. Concentrations as in Figure 1.

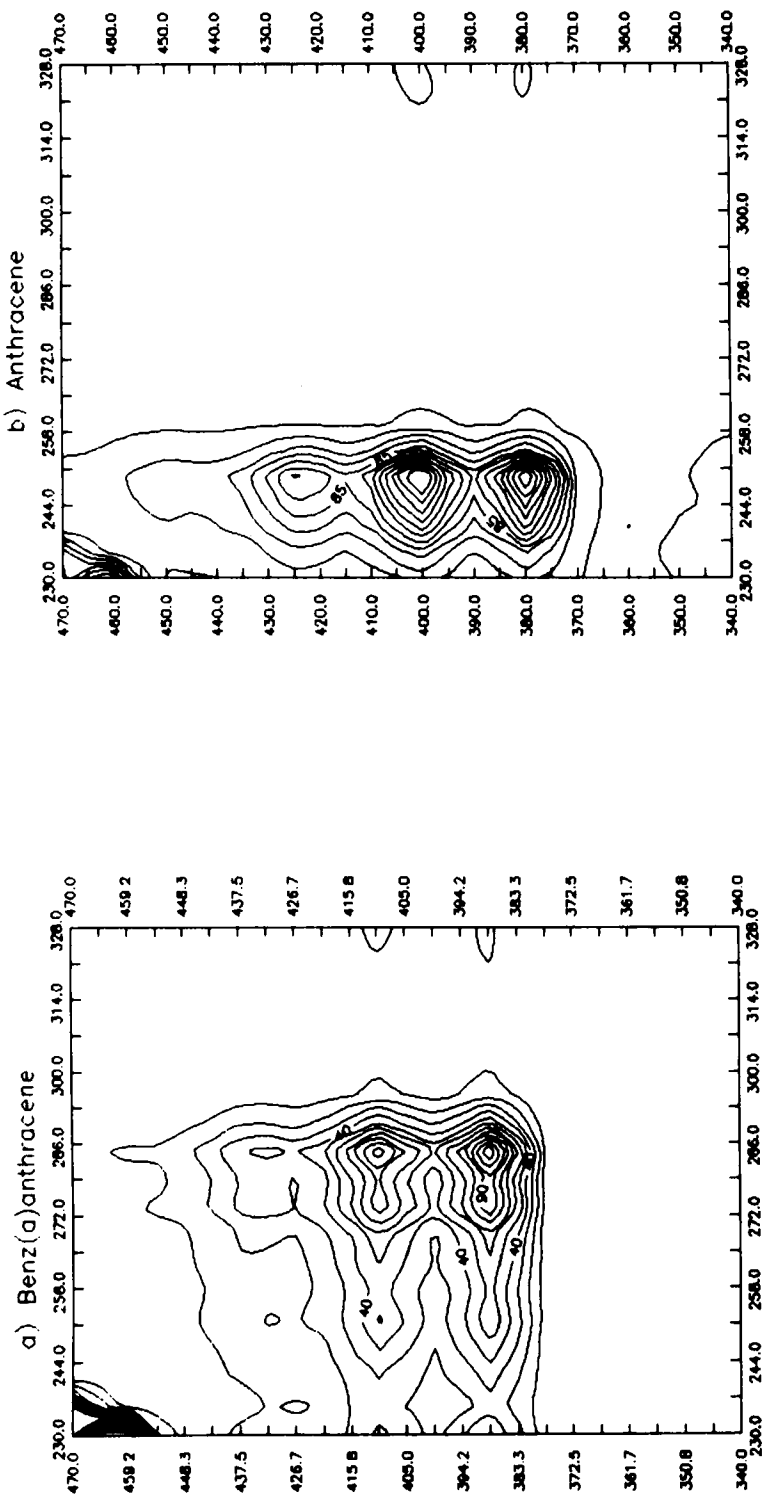
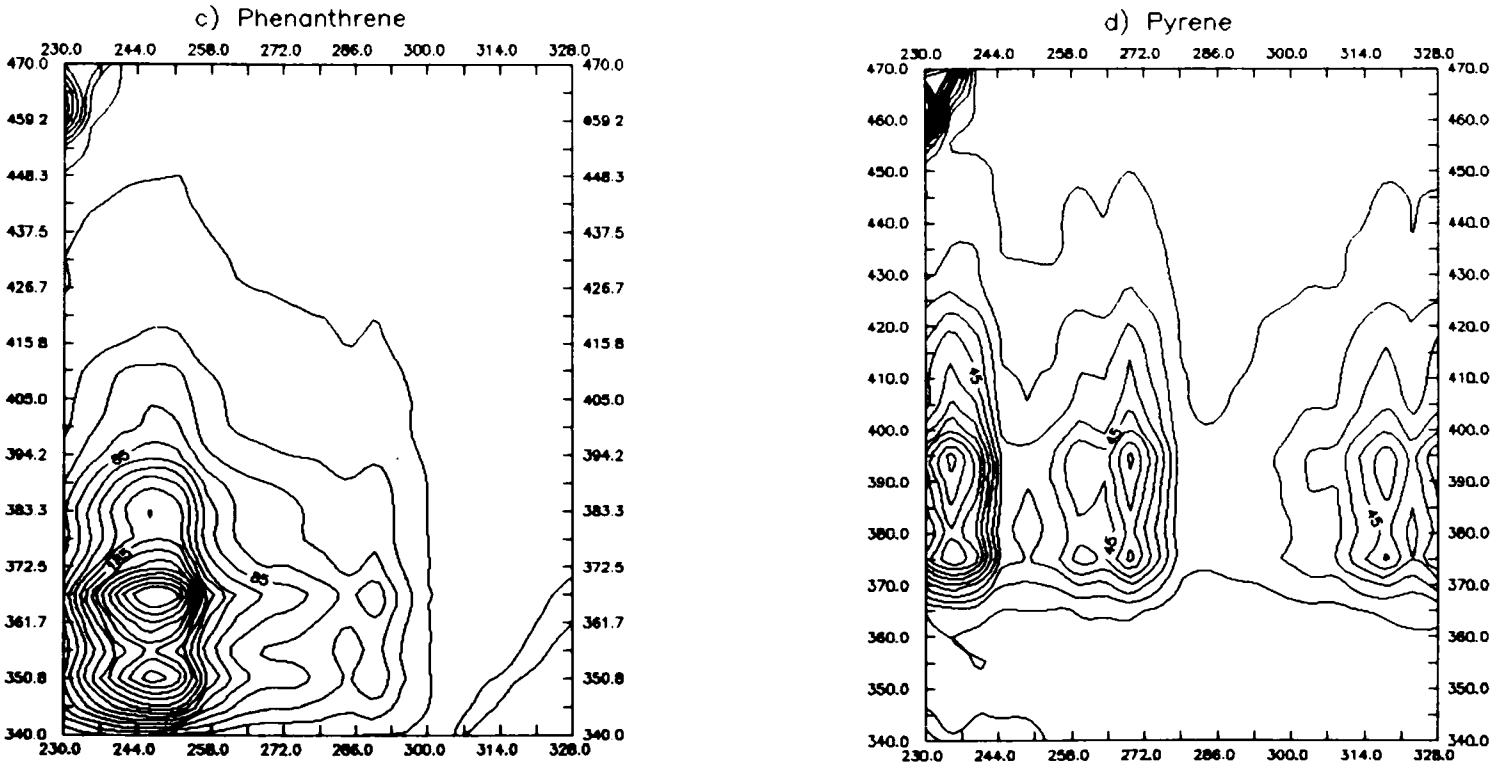


Figure 3 Contour plots of the PAHs. Concentrations as in Figure 1.



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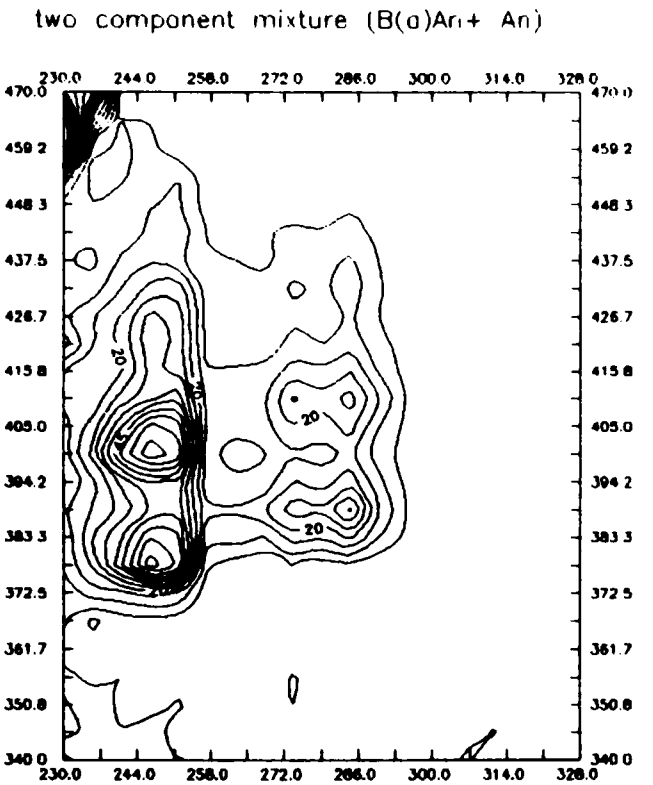

Figure 4 Contour plots of three synthetic mixtures of PAHs. (a) Benz[a]anthracene + anthracene; Sample 6 in Table 2. (b) Benz[a]anthracene + anthracene + phenanthrene; Sample 16 in Table 2. (c) Benz[a]anthracene + anthracene + phenanthrene + pyrene; Sample 19 in Table 2. The selected route for variable-angle synchronous scanning has been marked on map c.

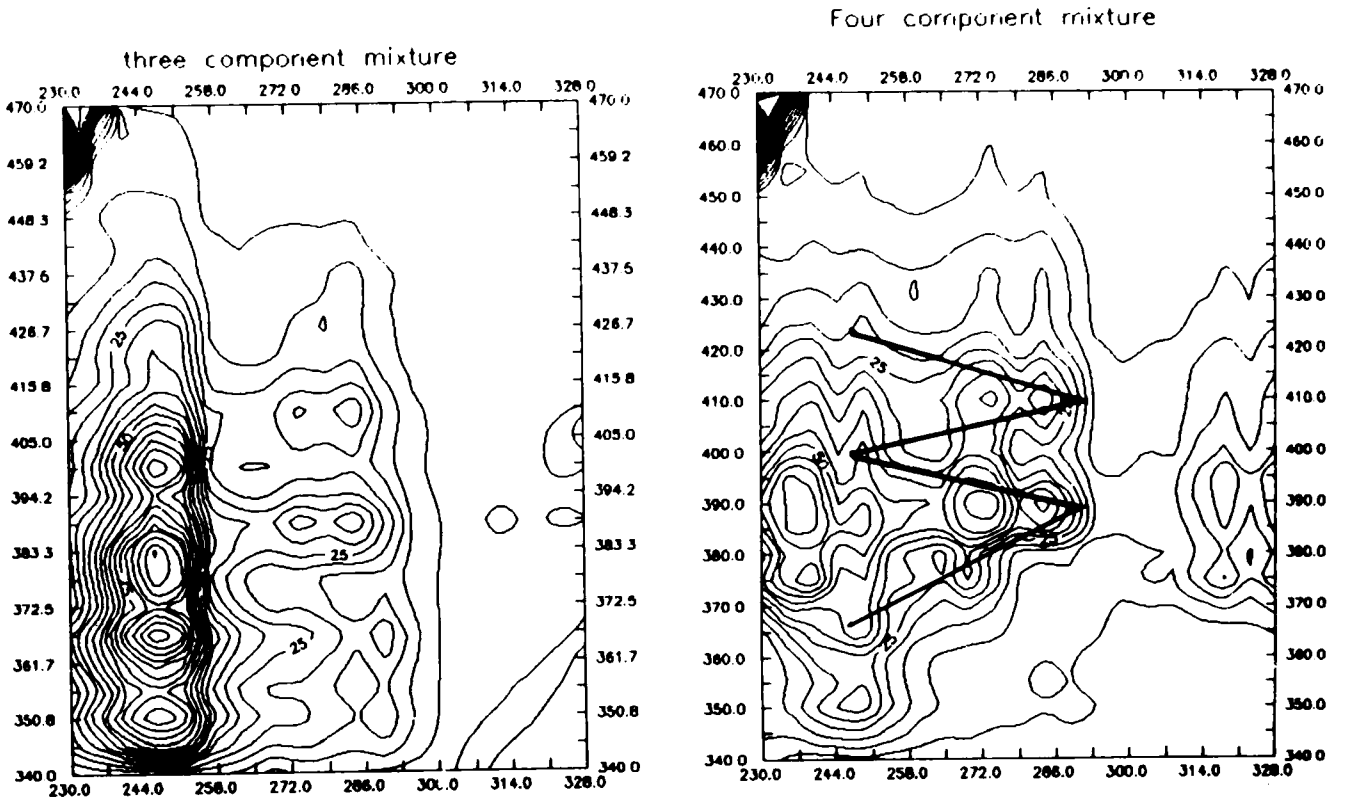

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Table 2 Composition of prepared samples in ng/ml

No.	*B[a]Ant*	*Ant*	*Phe*	*Pyr*
1	20	4	—	—
2	40	4	—	—
3	80	4	—	—
4	20	2	—	—
5	16	4	—	—
6	16	8	—	—
7	16	4	—	—
8	16	1.6	—	—
9	5	5	—	—
10	—	4	40	—
11	40	—	40	—
12	40	4	200	—
13	80	4	80	—
14	16	4	40	—
15	40	1.6	80	—
16	20	2	80	—
17	50	10	50	—
18	40	4	80	—
19	16	1.6	40	80
20	40	4	80	80
21	80	8	80	32
22	16	3.2	200	160
23	80	3.2	120	112
24	120	1.6	40	80
25	50	2	100	140

proportion (non-constant relative concentrations). The same numbers are used for the samples in Tables 2 and 3.

Selection of Method

According to the general tendency in PAH fluorimetric analysis, the simultaneous determination of the PAH was firstly attempted by using synchronous fluorimetry. In order to determine the best $\Delta\lambda$ values, the three-dimensional synchronous spectra were recorded (Figure 2). They showed clearly that, although it is possible to analyze selectively pyrene in the presence of the other compounds by selecting $\Delta\lambda = 43$ nm, there is no $\Delta\lambda$ fully suitable to allow the quantitative estimation of each PAH from a single spectrum.

Other techniques were investigated as an alternative means of providing multicomponent analysis of PAH mixtures. With this purpose, the total luminescent spectra of PAHs and their contour maps were examined. The contour plots of the four PAH standards are shown in Figure 3. As can be seen from these figures, there are no selective excitation-emission pairs to resolve the mixture by using conventional fluorimetry. The spectra of their mixtures are even more complicated, as shown in Figure 4 in which the contour maps of two-, three- and

four-component PAH mixtures (sample numbers 6, 16 and 19 in Table 2) are plotted.

Fortunately, the information contained in the EEM is quite excessive for each component and not all of it is necessary in quantitative analysis. For these reasons, we considered the application of variable-angle synchronous fluorimetry (VASF) to the analysis of PAHs.

The VASF is a useful tool in multicomponent analysis because it allows one to explore the portions of tridimensional spectra where the main information is contained, thus providing the proper route. It has had only limited application due to the difficulties in obtaining the variable-angle synchronous spectra. Until now, two methods to change the $\Delta\lambda$ separation between both monochromators have been described. The first one is mechanical and involves hardware modification, while the second one is a digital processing of tridimensional maps. Several contour maps are then necessary to obtain the calibration graphs which make this approach time-consuming and limits its potential application.

A new software developed by the authors offers a simpler alternative to produce VASF spectra directly from the fluorimeter Perkin Elmer LS-5 device, following a previously selected route. The VASF spectra obtained are experimental, in contrast with the theoretical ones obtained from data processing of tridimensional spectra. This is possible by giving to the fluorimeter the excitation and emission wavelengths, which have been previously stored in a file. The spectra of standards and samples were obtained and then mathematically treated by a least-squares method for quantitative analysis of the multicomponent mixtures.

The route for simultaneous analysis of anthracene, benz[a]anthracene, phenanthrene and pyrene was chosen with emphasis on maximum sensitivity criteria. Thus, we have followed a scan route through the maximum and minimum emission intensities of individual spectra. Optimization of the route was done by a trial-and-error method. The selected route for variable-angle scanning is marked out in Figure 4. In Figure 5 the planar projection of the variable-angle spectra of standards and a mixture are shown (sample number 22 in Table 2).

Quantitative Analysis

As illustrated in the quoted figures, the quantification of PAHs in mixtures is prevented by spectral overlap. Mathematical algorithms must be used, even if the variable-angle synchronous fluorescence spectra are recorded. The least-squares fitting technique has proved to be satisfactory and easy to use for analysis of mixtures of known components. Summarizing the methods described in ref. 11, the following equation may be deduced:

$$I_{fij} = \sum k_{ij} c_j \tag{1}$$

where i represents each excitation-emission pair and j represents the compound in the mixture. The k_{ij} values are derived from variable-angle spectra of standards (it is assumed that there is no error). The concentration of each component in a

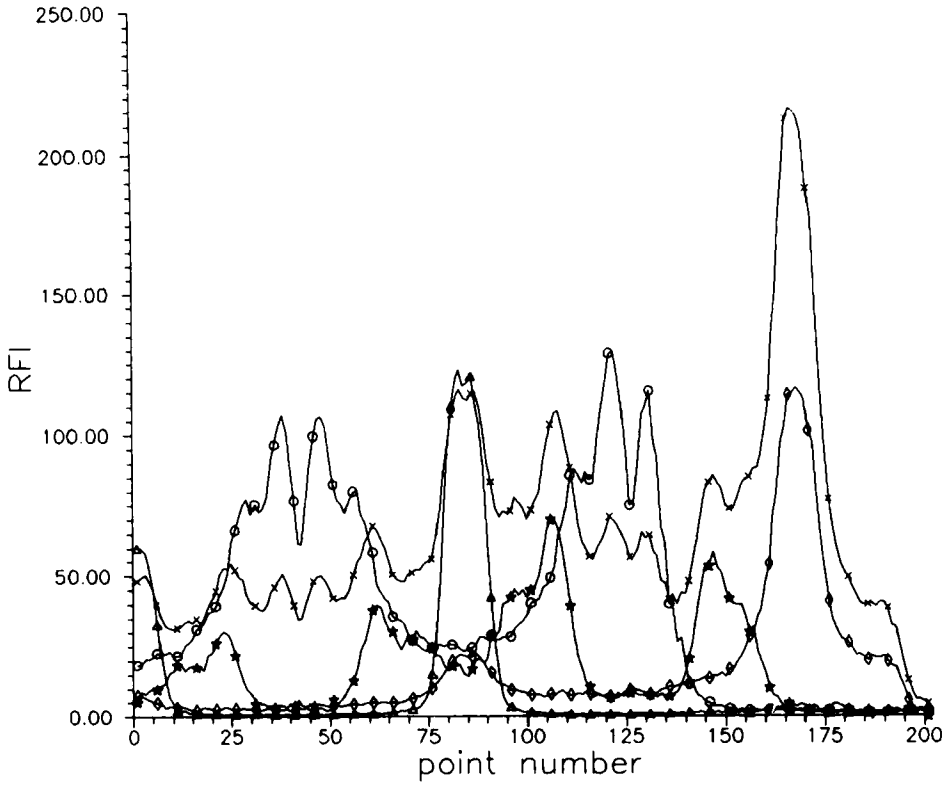

Figure 5 Planar projection of the VASF spectra of the PAHs and a mixture of them. ○, Benz[a]anthracené (40 ng/ml), △, Anthracene (8 ng/ml), ◇, Phenanthrene (100 ng/ml), ☆, Pyrene (160 ng/ml), ×, Four-component mixture (sample 22 in Table 2).

mixture is determined by using a least-squares regression method. The analysis of variance provides an estimation of the goodness of the fit.

Based on this model the resolution of mixtures of two, three and four components was accomplished. Calculated concentrations and confidence intervals for a probability of 99% are shown in Table 3.

CONCLUSIONS

The use of variable-angle fluorescence spectroscopy has been shown to be a powerful tool in quantitative multicomponent analysis, as it provides in the same measurement the specific information for each compound. There is no loss of information compared with the complete tridimensional spectrum, that is to say, no negative effect on the analysis is observed on discarding the points outside the areas of interest, thus allowing time saving in data acquisition as well as in computational calculations.

The strategy of applying a personal computer in both stages of the technique—instrumentation control and data acquisition on the one hand and

Table 3 Calculated concentrations of PAHs in ng/ml (for a probability of 99%)

No.[a]	*B[a]Ant*	*Ant*	*Phe*	*Pyr*
1	22.1±0.2	4.34±0.05	—	—
2	42.7±0.2	3.93±0.05	—	—
3	81.0±0.4	4.17±0.10	—	—
4	21.8±0.1	1.97±0.04	—	—
5	14.6±0.2	3.63±0.05	—	—
6	18.0±0.0	8.38±0.09	—	—
7	17.3±0.2	4.24±0.05	—	—
8	16.9±0.2	1.81±0.04	—	—
9	5.7±0.2	5.45±0.04	—	—
10	—	3.78±0.04	39.9±0.6	—
11	38.1±0.2	—	40.6±0.8	—
12	35.6±0.4	3.31±0.09	173.9±1.5	—
13	75.8±0.4	3.76±0.08	77.6±1.4	—
14	16.0±0.2	3.92±0.03	40.1±0.6	—
15	39.6±0.2	1.50±0.05	78.9±0.8	—
16	19.4±0.2	1.96±0.05	76.0±0.8	—
17	47.6±0.4	9.40±0.08	48.2±1.4	—
18	37.8±0.3	3.69±0.07	83.1±1.1	—
19	14.8±0.2	1.41±0.05	38.0±0.7	81.8±1.5
20	38.0±0.4	3.43±0.09	85.1±1.46	80.9±3.0
21	74.3±0.6	6.97±0.16	75.8±2.5	33.7±5.1
22	15.8±0.4	3.24±0.09	181.4±1.5	163.0±3.1
23	75.5±0.6	2.63±0.14	112.6±2.3	111.1±4.6
24	109.7±0.8	1.53±0.19	38.6±3.1	85.1±6.4
25	48.5±0.3	1.62±0.08	96.9±1.3	139.1±2.7

[a]Sample numbers refer to Table 2.

mathematical treatment of these data on the other hand—to analyze the multicomponent mixtures has had very satisfactory results.

The present method is simple and easy to apply and can be used for the resolution of mixtures of known components, thus avoiding previous time-consuming separation steps. Because of its great selectivity, VASF can be applied to relatively complex matrices.

A VASF-related application to resolution of pesticide mixtures[12] yielded good results and encouraged us to accomplish the resolution of PAH mixtures, as PAH are potent carcinogens in animals and man. A very interesting application is the determination of PAH in foods. Certain PAHs have been found in foods as a result of either external contamination or by generation during food processing (mainly smoking and high temperature cooking). Although recent analytical techniques such as HPLC are successfully used, the necessary previous stages of separation and purification make the complete method often tedious and time-consuming. Losses occur at each step and final recoveries are only 50–80%. Variable angle synchronous fluorimetry may be a valuable alternative for these situations as the separation step is eliminated and the individual components are quantified in a single spectrum.

Acknowledgments

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