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Optimization of high-performance liquid chromatography and solvent parameters for the separation of polycyclic aromatic hydrocarbons compared with supercritical fluid chromatography with UV detection

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ABSTRACT

Ten packed standard columns were evaluated for the separation of the sixteen US Environmental Protection Agency polycyclic aromatic hydrocarbons (PAHs) by gradient HPLC. This was done in a systematic way by the use of chemometric parameters such as window diagrams and chromatographic response factors and a computer program to calculate k', α, N and R_s. New approaches to a more economical and ecological use of HPLC solvents were evaluated. Good results were achieved by recycling acetonitrile–water waste by distillation as the azeotrope and using it again with methanol in an HPLC system. Supercritical fluid chromatography with carbon dioxide as supercritical fluid and acetonitrile as modifier was compared with HPLC, using the same column in both methods, for the determination of PAHs.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants because of their carcinogenic, mutagenic and toxic potential [1] and they are ubiquitous in different concentrations in air, water and soils. Their identification and determination are a great challenge for the analytical chemist.

PAHs can be determined by various chromatographic techniques. High-performance liquid chromatography (HPLC) in combination with a fluorescence detector is the most powerful method [2]. It is a common procedure to use acetonitrile–water gradients in HPLC [3,4]. Supercritical fluid chromatography (SFC) is also possible, but at the moment only with UV detection.

The selection of a suitable stationary phase for PAH separation depends on the particle size, which should be small (<5 μm), and on the pore size. Commercially available RP-18 columns show great differences with respect to PAH separation, depending on the separation conditions and stationary phases used [5–7]. A comprehensive comparison of fused-silica capillary columns for GC–electron-capture detection for chlorinated hydrocarbons was published by Lopez-Avila *et al.* [8].

Nevertheless, often various PAHs cannot be separated from each other or only with poor quality. The reason for this is the very slight differences in their properties *e.g.*, polarity and polarizability. The quality of a column can be judged from the chromatographic resolution R_s

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of pairs of compounds, which can be calculated as follows:

$$R_s = \frac{2\Delta t}{w_{b1} + w_{b2}} \quad (1)$$

where Δt is the difference of retention times of peaks 1 and 2 and w_b is the peak width at base. The peak separation function (f/g) (where f is the depth of valley below a straight line connecting the two adjacent peak maxima and g is the height of the straight line above the baseline at the valley) was developed by Kaiser [9] and generalized by Morgan and Deming [10], who developed the chromatographic response function (CRF):

$$CRF = \sum_{i=1}^{k} \ln (P_i), \quad P_i = \frac{f}{g} \quad (2)$$

where P_i is the peak separation for n peak pairs. This CRF value allows the separation of pairs of peaks to be judged in an easy manner.

The window diagram technique is a chemometric procedure for solvent optimization in HPLC [11]. The selectivity factor α is plotted against the solvent composition and the maxima of the curves show the most suitable separation conditions. This allows proper conditions to be selected and also permits other factors to be considered [12].

In this work we tested ten different reversed-phase columns for the determination of PAHs.

EXPERIMENTAL

An Applied Biosystems HPLC system was used. It consists of two pumps (Model 400 solvent-delivery system), an injector (Rheodyne Model 7125 with a 20-μl loop), a mixer (Model 491 dynamic mixer–injector), a fluorescence detector (ABI Model 980 programmable fluorescence detector) and a UV detector (ABI Model 783 A programmable absorbance detector). For data storage and evaluation, a PE Nelson interface (900 Series interface) with PE Nelson 2600 SI software and a Vector 80386 SX personal computer was employed. The column temperature was adjusted with a Colora column oven.

The Abimed–Gilson SFC system consists of two pumps (Model 308 Pump A Master 10 SC for carbon dioxide, Model 306 Pump B Slave 5 SC for acetonitrile as modifier), a mixer, an autosampler (Model 401 diluter with Model 231 ASI injection port), an oven (Model 831 temperature regulator), a UV detector (Model 117) and a back-pressure regulation valve as restrictor (regulation valve in Model 821 pressure regulator).

Acetonitrile (LiChrosolv; Merck, Darmstadt, Germany), doubly distilled water, methanol (b.p. 64°C) and acetonitrile–water azeotrope (b.p. 77°C) distilled through a 1.5-m column were used as solvents.

National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA) PAH standard containing sixteen US Environmental Protection Agency (EPA) priority PAHs was obtained from Promochem (Wesel, Germany).

The following columns were used: Eurosphere 80 C_{18} (250 × 2 mm I.D.; 5 μm) (column 1) and Eurosphere 80 C_{18} (250 × 4 mm I.D.; 5μm) from Knauer (Berlin, Germany); Nucleosil 5 C_{18} PAH, (150 × 4 mm I.D.; 5 μm) (column 2) from Macherey–Nagel (Düren, Germany); Aluspher 100 RP SelectB (250 × 4 mm I.D.; 5 μm) (column 4), Superspher 100 RP 18 (250 × 4 mm I.D.; 5 μm) (column 5) and special column KU 48 (250 × 4 mm I.D.; 5 μm) (column 10) from Merck; and Hypersil phenyl (250 × 4.6 mm I.D.; 5 μm) (column 6), Hypersil-MOS (250 × 4 mm I.D., 5 μm) (column 7), Hypersil-PAH C_{18} (100 × 4.6 mm I.D.; 5 μm) (column 8), Nucleosil C_{18} (120 × 4.6 mm I.D.; 3 μm) (column 9) and Nucleosil 120 C_{18} (250 × 4 mm I.D.; 5 μm) (column 3) from Muder & Wochele (Berlin, Germany).

A computer program was written to calculate the chromatographic parameters k', α, N and R_s from storage data files of a PE 2600 data system [13] using equations from the literature [14].

RESULTS AND DISCUSSION

In order to evaluate the differences between standard and special stationary phases, the chromatographic parameters (gradient, temperature, flow-rate) and the detector parameters (excita-

tion and emission wavelengths for fluorescence detection) were individually optimized for each column.

The resolution R_s for certain compounds was calculated. In Fig. 1, the resolution of all columns is plotted for four pairs of PAHs that are difficult to separate. Only three columns (2, 6 and 8) gave a sufficient separation ($R_s \geq 1$) for these pairs. The experiments showed that special materials as the stationary phase gave better results.

Another aim of this study was to find new ways for the more economical and ecological use of HPLC solvents. Acetonitrile–water solvent waste was distilled [azeotrope of acetonitrile–water (84:16), b.p. 77°C] and reused after adjusting it to acetonitrile–water (70:30) as solvent A with methanol as solvent B in an HPLC system.

With the help of the above-mentioned chemometric parameters, the time-consuming trial-and-error method should be replaced through systematic work. The following data were obtained from experiments with a Knauer Eurosphere 80 C_{18} column (250 × 4 mm I.S., 5 μm).

The window diagram can help to select the conditions for a good separation of mixtures, which are at the maxima of the curve. Here 0 or 40% methanol represented the best or second best conditions. For isocratic separation a ternary mixture with 40% methanol was chosen. Isocratic separation conditions offer many advantages, *e.g.*, no column equilibration is neces-

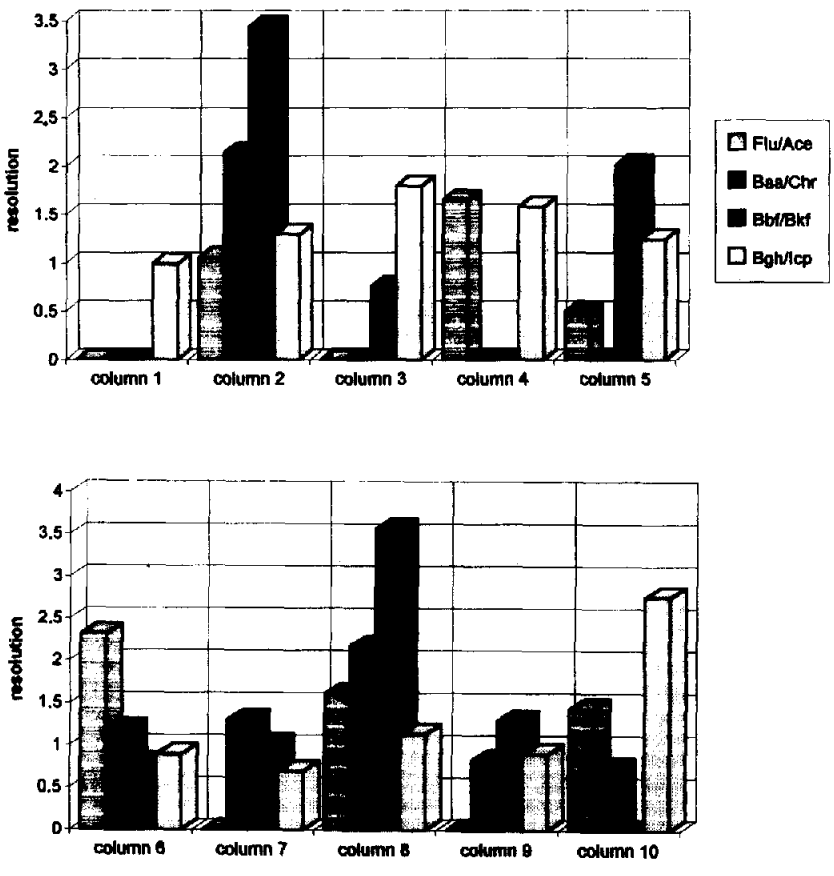

Fig. 1. Resolution of the four poorly resolved pairs acenaphthene–fluorene, benz[*a*]anthracene–chrysene, Benzo[*b*]fluoranthene–Benzo[*k*]fluoranthene and benzo[*ghi*]perylene–indeno[1,2,3-*cd*]pyrene with ten reversed-phase columns. For identification of columns see Experimental section.

sary, thus the analysis time can be decreased, or a straighter baseline, and/or less solvent noise under certain detection conditions.

A plot of the Van Deemter curve yielded an optimum flow-rate of 0.8 ml/min.

To judge the quality of the separation at different temperatures, the chromatographic response factor (CRF) was calculated. The CRF became less negative for all four pairs with increasing temperature (25°C, −2.600; 30°C, −2.125; 35°C, −1.954; 40°C, −1.598).

According to the retention times, the chromatographic parameters for the excitation and emission wavelengths of the fluorescence detector had to be adjusted so as to give optimum sensitivity.

Fig. 2 shows two chromatograms for an acetonitrile–water binary gradient and another binary gradient with a ternary mixture of methanol with acetonitrile–water (70:30) obtained with the Nucleosil 5 C_{18} PAH column.

The quality of the separation (α, k', N) is comparable for both methods. The α values are given in Table I.

A Hypersil-PAH C_{18} column was selected for a comparison of the HPLC and SFC methods. The SFC trace obtained with carbon dioxide as supercritical fluid and acetonitrile as modifier is shown in Fig. 3. The major advantage of SFC is the shorter analysis time, and further the

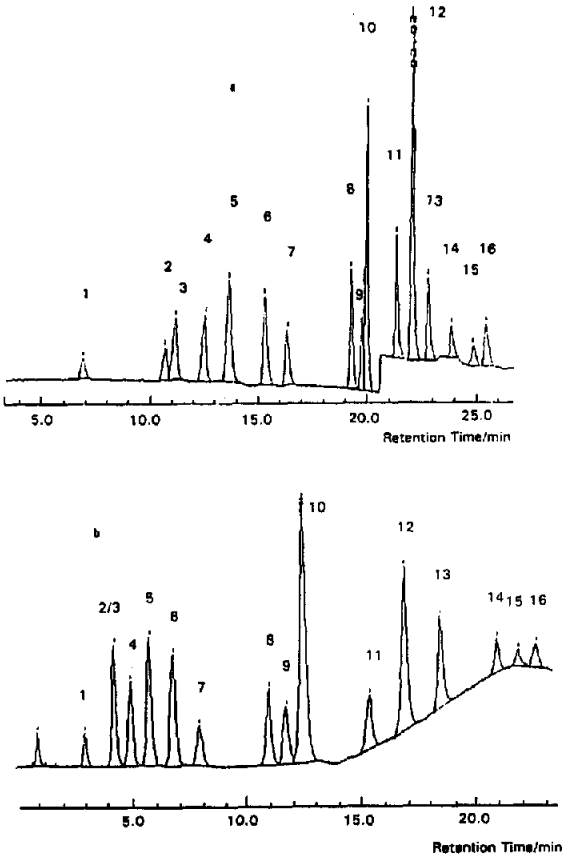

Fig. 2. HPLC of a PAH standard with (a) a binary acetonitrile (ACN)–water gradient at 30°C and a flow-rate of 1 ml/min and (b) a binary gradient of a ternary mixture of methanol with acetonitrile–water at 40°C and a flow gradient (0–8.5 min, 0.7 ml/min; 8.5–10.5 min, gradient to 1.1 ml/min; 10.5–26 min, 1.1 ml/min). Solvent gradient (a): $t = 0$, ACN–water (55:45); $t = 6$ min, gradient in 10 min to ACN–water (80:20); $t = 16$ min, gradient in 3 min to ACN; $t = 19$ min, ACN; $t = 32$ min, gradient in 2 min to ACN–water (55:45). Solvent gradient (b) [with B = MeOH and A = ACN–water (70:30)]: $t = 0$, 100% A; $t = 10$ min, gradient in 8 min to 100% B; $t = 18$ min, 10 min 100% B; $t = 28$ min, gradient in 2 min to 100% A. Wavelengths for excitation and emission were set to optimum sensitivity, similar to those explained in the literature [16]. For peaks 1–16, see Table I.

TABLE I

RESOLUTION α ACCORDING TO FIG. 2a AND b

No.	Compound	α	
		Gradient a	Gradient b[c]
1	Naphthalene		
	Acenaphthylene[a]	1.27	1.18
2	Acenapthene	1.37	1.38
3	Fluorene	1.04	1.00
4	Phenanthrene	1.14	1.22
5	Anthracene	1.12	1.20
6	Fluoranthene	1.11	1.21
7	Pyrene	1.07	1.20
8	Benz[*a*]anthracene	1.19	1.43
9	Chrysene	1.03	1.07
10	2,2'-Binaphthyl[b]	1.01	1.07
11	Benzo[*b*]fluoranthene	1.07	1.25
12	Benzo[*k*]fluoranthene	1.04	1.11
13	Benzo[*a*]pyrene	1.04	1.10
14	Dibenz[*a*,*h*]anthracene	1.05	1.14
15	Benzo[*ghi*]perylene	1.04	1.05
16	Indeno[1,2,3-*cd*]pyrene	1.03	1.04

[a] Acenaphthylene: UV detection in HPLC.
[b] As internal standard.
[c] According to Fig. 2.

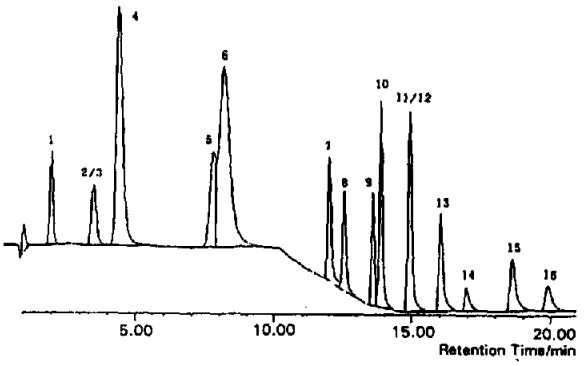

Fig. 3. SFC of a PAH standard obtained at 90°C with UV detection at 254 nm. Pressure gradient: $t = 0$, 150 bar; $t = 4$ min, gradient in 6 min to 160 bar; $t = 10$ min, gradient in 2 min to 400 bar. Flow gradient: $t = 0$, 1 ml/min; $t = 10$ min, gradient in 2 min to 2 ml/min. Modifier gradient: $t = 0$, 0% ACN; $t = 10$ min, gradient in 2 min to 2% ACN. For peaks 1 and 11–16 see Table I; peaks 2–10 correspond with acenaphthylene to chrysene, same order as in Table I.

equilibration time is lower. The separation of the sixteen EPA PAHs is comparable with both methods, but slightly better using HPLC. A different column material will improve the separation but is not yet commercially available [15].

CONCLUSIONS

The quality of the separation showed great differences for the ten columns tested. The special PAH columns gave better results than the standard columns, owing to the larger pore size is the former instance.

Each column requires an individual programme for the gradient, temperature, flow-rate and excitation and emission wavelengths for fluorescence detection. By using chemometric parameters such as the window diagram and CRF value and a computer program for the calculation of important chromatographic values, the solvent optimization and the judgement of peak separation can be achieved in an easy and systematic manner.

Recycling of the acetonitrile–water eluate and reusing it in a ternary mixture with methanol using a binary gradient showed new possibilities for the use of HPLC. The acetonitrile consumption can be reduced to 56% and the costs to nearly 23% when costs of solvents, disposal and additional costs for distillation, etc., are taken into account.

A comparison between HPLC and SFC with UV detection showed the great advantages of SFC, *i.e.*, shorter analysis time, lower operating costs and the use of carbon dioxide, which is non-toxic, non-flammable, inert and does not have to be discharged. The detection limits are similar with the HPLC and SFC methods, between 1.2 ng for phenanthrene and 17.7 ng for benzo[*ghi*]perylene. The importance of SFC will increase further in the future when it becomes possible to use fluorescence detection and columns specially made for SFC.

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