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Separation of Polycyclic Aromatic Hydrocarbons by HPLC with Fluorescence Detection

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A method for the separation of polycyclic aromatic hydrocarbons by the application of Bakerbond PAH 16-Plus column under optimal operating conditions is described. The detection of compounds was made by the employment of fluorescence method with a wavelength programme in case of substance selection according to EPA and by operating with a constant wavelength pair in case of substance selection according to TVO.

Opisano sposób rozdziału policyklicznych węglowodorów aromatycznych przy użyciu kolumny Bakerbond PAH 16-Plus w optymalnych warunkach. Wykrywanie związków wykonywano przy zastosowaniu metody fluorescencyjnej ze zmianą długości fali w przypadku substancji według wzorca EPA i przy parze stałych długości fal w przypadku substancji według wzorca TVO.

The determination of polycyclic aromatic hydrocarbons (PAH) by HPLC requires separation columns of high selectivity and efficiency [1, 2]. The use of fluorescent detection with the attempt to attain optimal detection specificity and sensitivity for the individual PAH by altering the excitation and emission wavelength during separation (wavelength programming) also puts special requirements on the chromatographic separation. In order to be able to carry out wavelength switching it is necessary to achieve a chromatographic resolution (R) of at least 2.5 between adjacent signals to ensure that there is no interference with quantitative signal analysis by the normal variations in retention times. However, these severe requirements with regard to separation are frequently not fulfilled particularly for benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene where a wavelength switch would be particularly appropriate on account of the very different excitation and emission maxima [3]. Hence, these substances are usually detected at the same excitation and emission wavelengths. The compromise wavelength combination 360 nm/460 nm is often chosen here.

EXPERIMENTAL

Chemicals and equipment

The following polycyclic aromatic hydrocarbons were chosen for analysis: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*ah*]anthracene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene. The choice of these substances corresponds to the EPA standard apart from acenaphthylene and benzo[*e*]pyrene. The fluorescence detector does not respond to acenaphthylene.

The Bakerbond PAH 16-Plus column (Cat.No. 7504-00) was used. It is commercially available from J.T. Baker, through EUROCOLOR in Poland. The authors received their column from J.T. Baker, Deventer, Holland. The Bakerbond PAH 16-Plus column is 250 mm long with an i.d. of 3 mm. The particle size of the column filling is 5 μm.

Acetonitrile, methanol and water were HPLC grade from J.T. Baker.

EPA and TVO standards came from NDS and Dr. Ehrenstorfer, Augsburg (available through EUROCOLOR in Poland).

The liquid chromatograph used was equipped with fluorescence detector.

Procedure

The separation of polycyclic aromatic hydrocarbons using the Bakerbond PAH 16-Plus column is favored by low temperatures and should, therefore, be carried out below or at 30°C. The temperature was 25°C or 30°C during all separations.

For the separation of 16 EPA standards water and acetonitrile as a mobile phase were used and for 6 TVO standards only pure methanol was used. The optimal flow rate of the mobile phase was *ca.* 0.5 ml min^{-1}, so that there was a saving of about 50 % in solvent compared with the columns of 4 or 4.6 mm i.d. that are commonly used.

The samples of 10 μl containing PAHs in acetonitrile or methanol were introduced into a chromatographic column.

Sample origin

The following samples were used: sample 1: spiked water, sample 2: real soil sample, sample 3: spiked water, sample 4: real sediment sample from industrial harbour.

RESULTS AND DISCUSSION

A very good separation of 16 PAHs is presented in Fig. 1. It is so good that during fluorescence detection a wavelength switching is possible to obtain optimal excitation and emission maxima for individual PAHs.

The selectivity of the stationary phase and the separation efficiency of the column also find expression in the benzo[*a*]anthracene and chrysene, where a series of foreign substances are often detected in the case of real samples (Fig. 2).

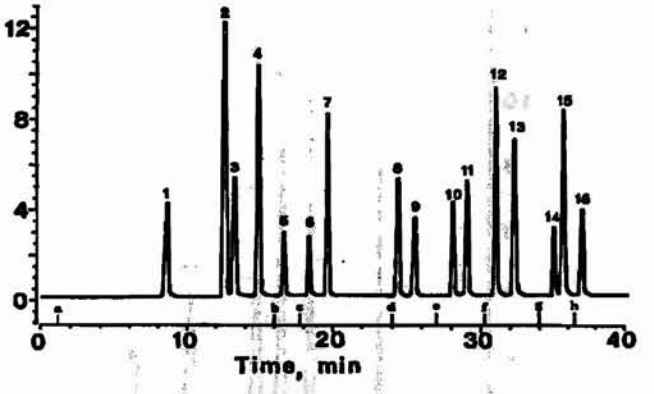

Figure 1. HPLC chromatogram of 10 μl PAHs standard (EPA) in CH_3CN, concentration of individual substances 90 pg μl^{-1}; Column – Bakerbond PAH 16-Plus; mobile phase H_2O–CH_3CN: 0–5 min isocratic (50 % CH_3CN), 5–35 min linear gradient (50–100 % CH_3CN), 35–45 min isocratic (100 % CH_3CN), flow rate 0.5 ml min^{-1}, equilibration – 15 min under initial conditions; column temperature 25°C; pressure 85 bar under initial conditions; fluorescence wavelength (nm) programme: a – ex. (excitation) 275, em. (emission) 350, b – ex. 375, em. 425, c – ex. 335, em. 440, d – ex. 315, em. 405, e – ex. 330, em. 420, f – ex. 375, em. 460, g – ex. 345, em. 420, h – e–1x. 300, em. 500; slit ex./em. = 25/50 nm; 1 – naphthalene, 2 – acenaphthene, 3 – fluorene, 4 – phenanthrene, 5 – anthracene, 6 – fluoranthene, 7 – pyrene, 8 – benzo[*a*]anthracene, 9 – chrysene, 10 – benzo[*e*]pyrene, 11 – benzo[*b*]fluoranthene, 12 – benzo[*k*]fluoranthene, 13 – benzo[*a*]pyrene, 14 – dibenzo[*ah*]anthracene, 15 – benzo[*ghi*]perylene, 16 – indeno[1,2,3-*cd*]pyrene

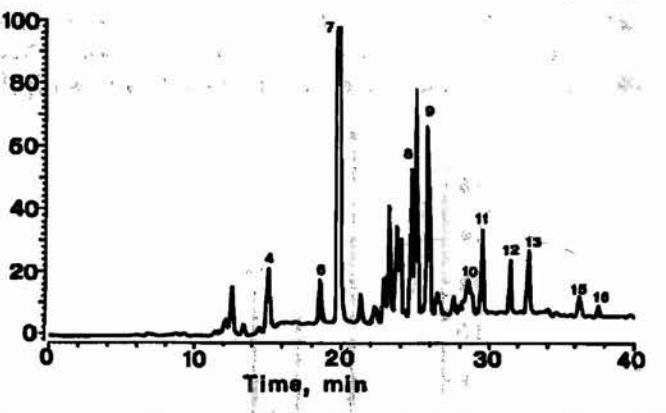

Figure 2. HPLC chromatogram of a contaminated soil sample. Chromatographic conditions (including a–h) and peak identification as in Fig. 1

For the examination of samples with respect to their contamination with PAH it is often sufficient to carry out a determination of 6 PAH according to TVO. The separation is very simple and on the column under discussion it can be carried out isocratically with pure methanol (Fig. 3).

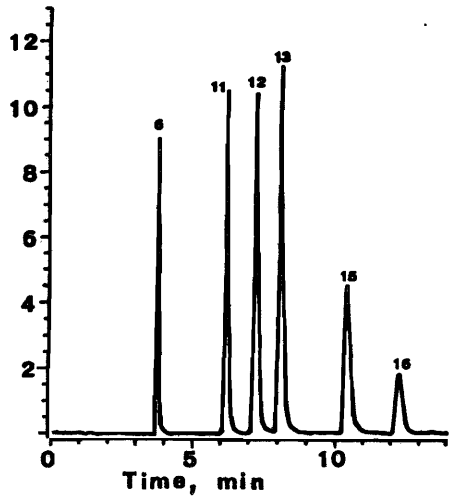

Figure 3. HPLC chromatogram of 10 µl PAHs standard (TVO) in CH_3OH, concentration of individual substances 100 pg μl^{-1}. Column – Bakerbond PAH 16-Plus; mobile phase CH_3OH, flow rate 0.5 ml min^{-1}; column temperature 30°C; pressure 55 bar; fluorescence detection – excitation 360 nm/emission 460 nm, slit ex./em. = 25/50 nm; peak identification as in Fig. 1

DIN draft 38407-F8 recommends that the detection of the 6 PAH be carried out by fluorescent detection at constant excitation and emission wavelengths [4]. The high resolution means that it would also be possible here to undertake wavelength switching in order to optimize detection. The isocratic procedure is preferred for the determination of the 6 PAH in drinking water and ground water, but it also yields reliable results in waste water and waste material investigations [5] (Fig. 4).

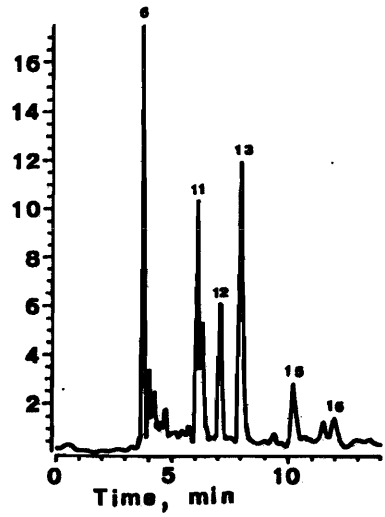

Figure 4. HPLC chromatogram of a sediment sample (industrial harbour). Chromatographic conditions as in Fig. 3; peak identification as in Fig. 1

Since the solubility of PAH in water is so low the samples should be injected in pure methanol or acetonitrile, whereby an injection volume of 10 µl has been found optimal for the 3 mm diameter column. When the gradient elution method is employed additional band broadening effects occur in the elution range naphthalene to fluorene but these have no appreciable effect on the separation of these substances (*cf.* Fig. 1). In contrast, however, the presence of apolar solvents, *e.g.* hexane or cyclohexane residues from sample preparation, has a great effect. Even a proportion of less than 1 %(V/V) of one of these solvents in the sample can so affect the reproducibilities of the retention times that it becomes necessary to make continual adjustment of the switching times for wavelength changes. Higher proportions lead to appreciable band broadening and even peak splitting. The effects of these solvents should be taken into account during sample preparation [5].

On account of relatively high amounts of PAHs in soil their determination is valuable for the assessment of the potential danger to ground water by waste material. Considering low solubility of PAHs in water very often selective enrichment of samples is necessary. It can be fulfilled by use of solid phase extraction on enrichment columns [6, 7].

The working lives of the Bakerbond PAH 16-Plus columns under routine conditions were found to be above average. If the stationary phase became coated with apolar fluorescent substances from strongly polluted samples the original separation performance could be restored by rinsing with acetonitrile–tetrahydrofuran 1+1 (V/V).

Conclusion

The Bakerbond PAH 16-Plus column has very good properties which enables to attain excellent separations of PAHs in different samples. The separations are so good that programming of fluorescence wavelength is possible.

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