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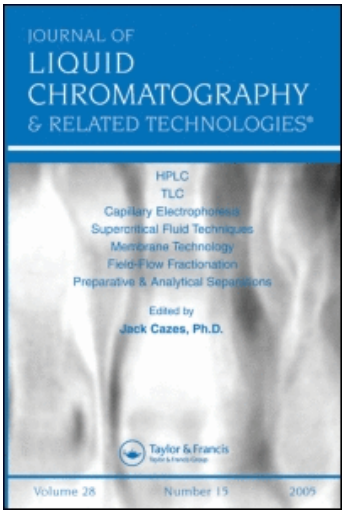

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Optimization of the Detection Wavelength Applied to the HPLC Analysis of Polycyclic Aromatic Hydrocarbons

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OPTIMIZATION OF THE DETECTION WAVELENGTH APPLIED TO THE HPLC ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS

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ABSTRACT

Presented paper discuss a mathematical procedure which utilises the individual spectra of fifteen polyaromatic hydrocarbons for the optimization of the detection wavelength. Procedure is based upon use of weighting factors appropriate for the components under consideration (toxicological data). Two criteria have been used in the optimization procedure. If suggested criterion was used and all the components were of equal importance, then the optimum was at 220 and 254 nm respectively. When the weighting factors based on the toxicological data were employed, the optimum was found at 287 nm.

INTRODUCTION

The sensitivity of chromatographic method (observed peak height in a chromatogram) is an important aspect of the chromatographic process. It can be increased by the injection of larger volumes of samples, preconcentration on a pre-column in HPLC, by using a good column operated at the optimum flow rate [1] and selection of optimal

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detection conditions. The use of programmable UV detectors which operate at wavelengths that are changed at pre-selected times [2-4] or the use of multiple wavelength detectors [5,7-9] represent an experimental way of increasing of the sensitivity of UV detection. The second way is mathematical optimization based on the optimization criteria [6,10,11].

The aim of this paper is to discuss the mathematical procedure which utilises the spectral data obtained by the UV photo diode array detector for the optimization of the detection wavelength from the toxicological point of view. The optimization has been applied to the chromatographic analysis of selected polycyclic aromatic hydrocarbons.

THEORY

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Before any optimization, the goal of the process should be defined unambiguously. The goal of the optimization of detection wavelength of a group of components with different spectra is to obtain the areas of all peaks in a chromatogram as maximal as possible.

The area of Gaussian peak P_i can be related to the peak height at the maximum $h_{max,i}$ by the following equation [1]:

$$P_i = h_{\max,i} \cdot \sigma_i \cdot \sqrt{2 \cdot \pi} \tag{1}$$

where σ_i is the standard deviation of a Gaussian peak i.

If the absorbance is the quantity measured by a detector, then $h_{max,i}$ can easily be expressed in terms of the absorptivity ε_i, concentration of the peak maximum $c_{max,i}$ and a width of a detection cell l.

$$h_{\max,i} = A_{\max,i} = \varepsilon_{\max,i} \cdot c_{\max,i} \cdot l \tag{2}$$

where $A_{max,i}$ is the absorbance at the peak maximum.

Thus, equation (1) can be transformed to the following expression:

$$P_i = A_{\max,i} \cdot \sigma_i \cdot \sqrt{2 \cdot \pi} = \varepsilon_{\max,i} \cdot c_{\max,i} \cdot l \cdot \sqrt{2 \cdot \pi} \tag{3}$$

As can be seen from equation (3), the area of the Gaussian peak depends linearly on the ε_i and therefore the area of a peak can be represented by the absorptivity ε or by the absorbance A.

Optimization criterion.

The elemental criterion *EC* can be defined by the following expression:

$$EC_i = \frac{\varepsilon_{i,\lambda_j}}{\varepsilon_{i,\lambda_{max}}} = \frac{A_{i,\lambda_j}}{A_{i,\lambda_{max}}} \quad (4)$$

where ε_i is the absorptivity of the component *i* at the wavelength λ_j which lies in the interval 200-400 nm and $\varepsilon_{i,\lambda max}$ is the absorptivity of component *i* at the wavelength of the main spectral maximum λ_{max} and $A_{i,\lambda j}$, $A_{i,\lambda max}$ are the absorbances in the spectrum.

The criterion *C* can be written as:

$$C = \prod_{i=1}^{N} (EC_i)^{\alpha_i} \quad (5)$$

where *N* is the number of components and α_i is the weighting factor. By the proper choice of α_i one can optimize the detection wavelength for the peaks of interest. For the choice of the weighting factors, we have used the toxicological data of 15 polyaromatic hydrocarbons.

EXPERIMENTAL

Chromatographic system

Reversed phase HPLC was performed on a Waters Assoc. model 501 pumps with a Vydac - 5μm, C-18 column (length = 250 mm, inner diameter = 4.6 mm) and a Waters Assoc. photo diode array detector model 990. The composition of the gradient mobile phase is listed in Tab. 1.

Chemicals

The standards of 15 polycyclic aromatic hydrocarbons naphtalene (1), acenaphtylene (2), acenaphtene (3), fluorene (4), phenantrene (5), anthracene (6), fluoranthene (7), pyrene (8), benzo(a)anthracene (9), chrysene (10), benzo(b)fluoranthene (11), benzo(k)fluoranthene (12), benzo(a)pyrene (13), dibenz(a,h)anthracene (14),

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Table 1. The Composition of the Gradient Mobile Phase

Time / min.	w(A) / %	w(B) / %
0	100	0
3	100	0
18	0	100
27	0	100
30	100	0

A = 50 % ^{V}/v acetonitrile in water, B = pure acetonitrile, flow rate = 1.4 ml / min..

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benzo(g,h,i)perylene (15) were purchased from Supelco USA. The acetonitrile for gradient was from Merck, Germany. Standard solution was prepared by dissolving of the analytes in acetonitrile for gradient (0.1 mg.cm^{-3}).

Optimization procedure

One obvious advantage of the product criteria is that the result will be mainly determined by the smallest value of the elemental criterion EC_i. The value of EC_i lies in the interval (0-1) and it is zero if $A_{i,\lambda j}$ is equal to zero. All product criterion will be zero if any term EC_i is zero, hence the value of the absorptivity ε_i for the component i at λ_j is zero. During optimization process the maximum of criterion C has been found.

The second way is to maximize the minimal value of EC for the given set of compounds over whole range of wavelengths. The optimization method is shown in the next algorithm.

RESULTS AND DISCUSSION

In the first optimization procedure all peaks were considered to be of equal importance. The results of optimization are shown in Fig. 2-5. As can be seen from

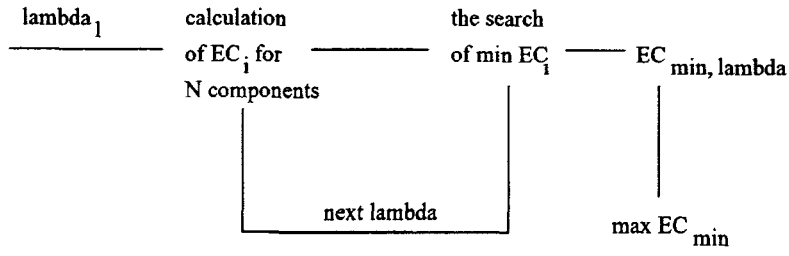

Figure 1. The algorithm of the optimization procedure.

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Fig. 2, 4 the main maximum of criterion C resp. $maxEC_{min}$ was around 220 nm, but at this low wavelength some coeluting interferences could cause a reduction of selectivity. Therefore, we have considered the range of wavelengths over 250 nm. In Fig. 3 can be seen the detail of Fig.2 and the maximum of C was detected around 254 nm. This result is in agreement with the commonly used wavelength in HPLC for detection of polyaromatic hydrocarbons [13].

The toxicological characteristics of selected PAHs show that the health risks of individual PAHs are not at the same level, therefore in the optimization procedure they shouldn't be of equal importance. Many PAHs are known to be carcinogenic or cocarcinogenic as a result of oxidative reactions in the body [14]. In Table 2 the carcinogenities of 15 PAHs are summarised [15-17].

The polyaromatic hydrocarbons listed in Table 2 can be classified into 3 groups according to their carcinogenity. Naphtalene, acenaphtylene, acenaphtene, fluorene (group A) are relatively weak carcinogenic resp. non-carcinogenic (and also their carcinogenities are inadequately documented). Phenantrene, anthracene, fluoranthene, pyrene, chrysene and benzo(g,h,i)perylene (group B) are moderately active and benzo(a)anthracene, benzo(k) resp. benzo(b)fluoranthene, benzo(a)pyrene and dibenz(a,h)anthracene (group C) are the most dangerous carcinogens. We have assigned the following three levels of weighting factor α_i for the groups: A -0.01; B- 0.10; C- 1.00.

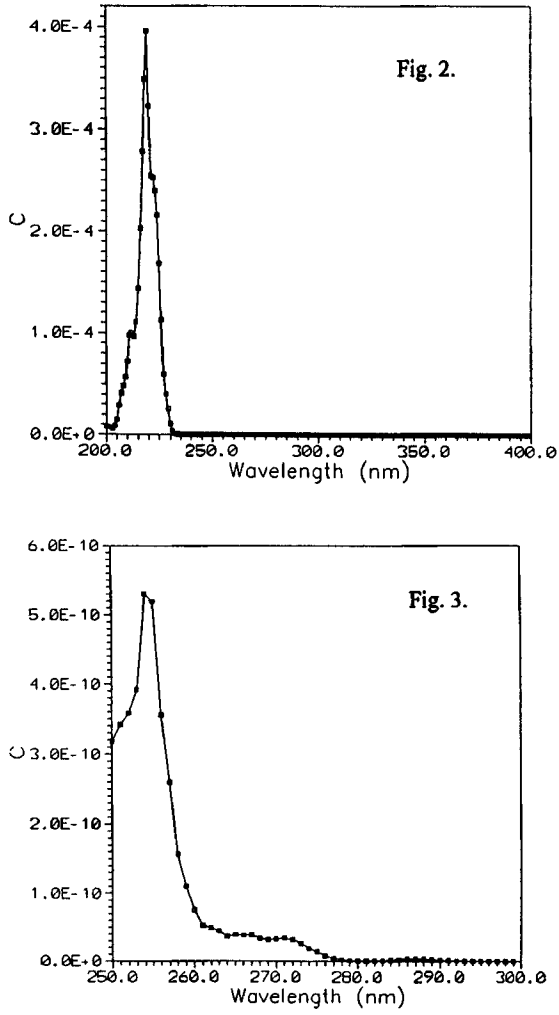

Fig. 2 - 5. The dependences of criterion C and EC_{min} on the wavelength.

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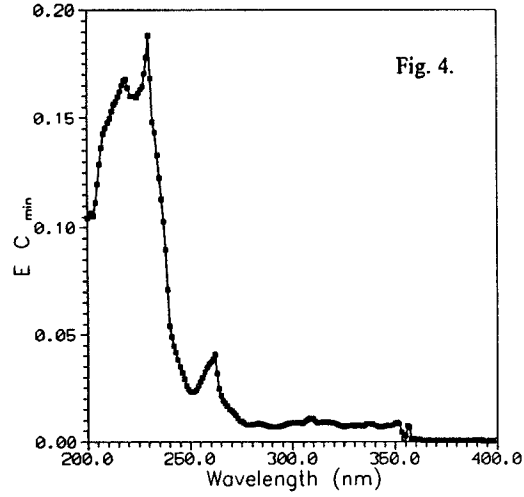

Fig. 4.

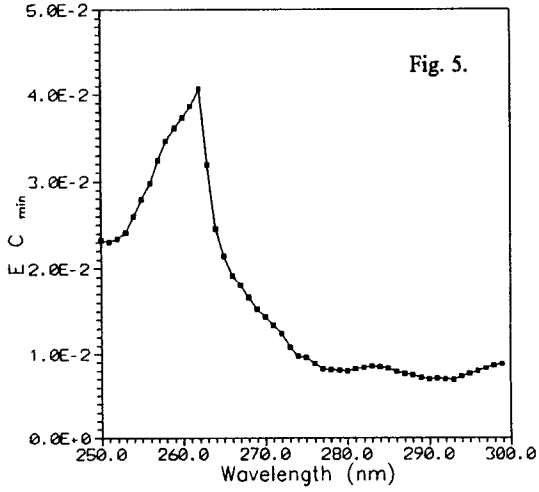

Fig. 5.

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TABLE 2.The Toxicological characteristics of 15 PAHs

compound	classification according to p system	
	acute toxicity	carcinogenity
naphtalene	!C	?C
acenaphtylene	?B	??C
acenaphtene	?B	??C
fluorene	?B	?B
phenantrene	:C	?D
anthracene	:C	?D
fluoranthene	?C	?D
pyrene	?C	:D
benzo(a)anthracene	?C	!E
chrysene	?C	:D
benzo(b)fluoranthene	?C	:E
benzo(k)fluoranthene	?C	:E
benzo(a)pyrene	?C	!F
dibenz(a,h)anthracene	?C	!E
benzo(g,h,i)perylene	?C	:D

The reliability;!-strong, :-moderate, ?-weak, ??-very weak.

The activity; B - very weakly active,
C - weakly active,
D - active,
E - very active,
F - extremely active.

The results of the optimization procedure is shown in Fig 6. The maximum of *C* was detected around 287 nm. The significance of individual maxima (220, 254, 287 nm) are illustrated by the three chromatograms shown in Fig.7-9. Table 3 lists the values of normalized spectra (*EC*) of individual components at this maxima.

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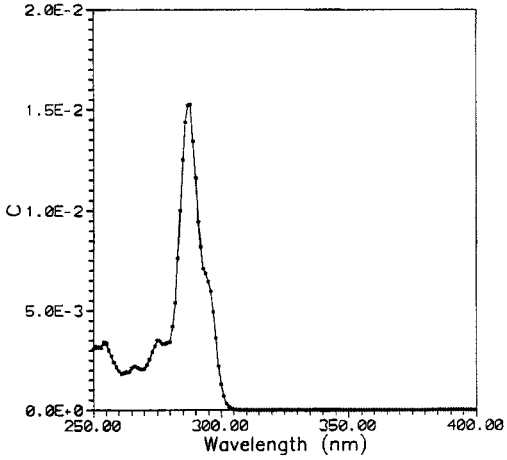

Fig. 6. The dependence of criterion C calculated with the toxicological weighting factors

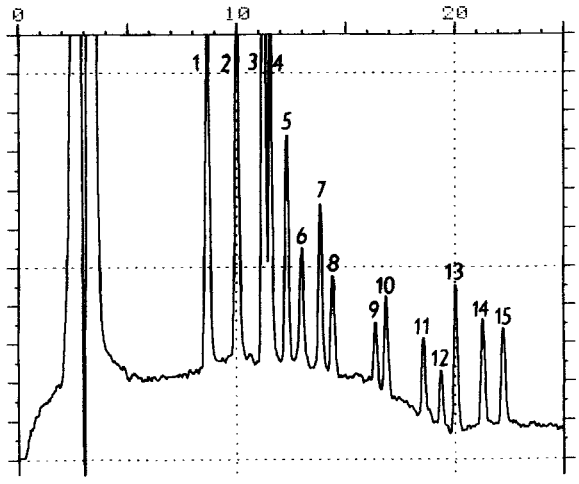

Fig. 7 The chromatogram of 15 PAHs corresponding to the maximum of C at 220 nm. (For the numbering of the peaks see experimental)

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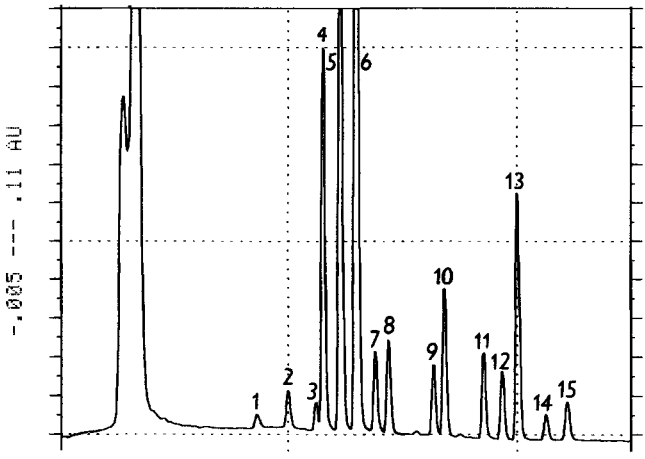

Fig. 8 The chromatogram of 15 PAHs recorded at 254 nm.

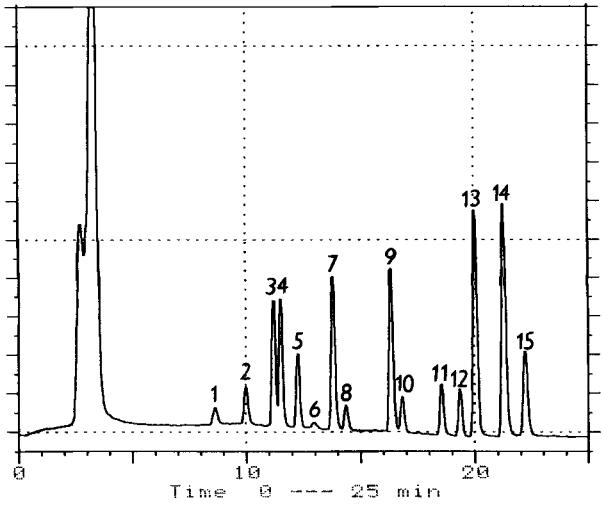

Fig. 9 The chromatogram of 15 PAHs recorded at 287 nm.

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Table 3. The Normalized Spectra at 220, 254, 287 nm

compound	EC_{220}	EC_{254}	EC_{287}
naphtalene	1.00	0.04	0.06
acenaphtylene	0.62	0.06	0.06
acenaphtene	0.68	0.02	0.10
fluorene	0.47	0.37	0.13
phenantrene	0.52	0.95	0.12
anthracene	0.17	0.95	0.01
fluoranthene	0.85	0.26	0.50
pyrene	0.37	0.18	0.05
benzo(a)anthracene	0.85	0.41	0.96
chrysene	0.49	0.38	0.10
benzo(b)fluoranthene	0.99	0.57	0.34
benzo(k)fluoranthene	0.80	0.55	0.34
benzo(a)pyrene	0.60	0.78	0.78
dibenz(a,h)anthracene	0.47	0.06	0.74
benzo(g,h,i)perylene	0.75	0.18	0.43

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CONCLUSION

We have used the two criteria for the optimization of the detection wavelength applied to the analysis of polyaromatic hydrocarbons. If EC_{min} is used as the function describing the sensitivity of the detection, then no attention is paid to all but one component in a chromatogram which is a disadvantage of this criterion. The criterion C evaluates the suitability of the detection wavelength more realistically. The obtained results confirmed the optimum commonly used wavelength used in HPLC of polyaromatic hydrocarbons (254 nm). If the weighting factors based on the toxicological characteristics of tested PAHs are used, then the optimum was found at 287 nm. The sensitivity of detection for selected polyaromatic hydrocarbons (group C) after optimization (287 nm) in compare with sensitivity at 254 nm was approximately the same for benzo(b),

benzo(k)fluoranthene and benzo(a)pyrene, two times higher for benzo(a)anthracene and ten times higher for dibenz(a,h)anthracene.

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