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# Optimization of separation of some polycyclic aromatic compounds by thin-layer chromatography

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## Abstract

A new modelling of the chromatographic separation process of some polar and nonpolar polycyclic aromatic compounds in the form of 3rd degree functions is reported. For the selection of the optimum composition of the benzene–acetone–water mobile phase used in the separation of eight polycyclic aromatic compounds (benzo[*a*]pyrene-7-hydroxy; benzo[*a*]pyrene-8-hydroxy; benzo[*a*]pyrene-*trans*-7,8-dihydrodiol; benzo[*a*]pyrene-*trans*-9,10-dihydrodiol; benzo[*a*]pyrene-*trans*-7,8-diol-9,10-epoxy(*syn*); benzo[*a*]pyrene; dibenz[*a,h*]anthracene; chrysene) on *n*-octyl and *n*-octadecyl silica gel R thin layers, some computer programs were performed. These programs are written in GW-BASIC language and can be examined on any adequate IBM PC (386, 486, 586) computer. The best mixtures of the mobile phase as given by the computer programs have been experimentally tested. These programs allow the choice of a mobile phase which ensures the best spreading of  $R_f$  values on a chromatographic plate and satisfactory differences between them ( $\Delta R_{f,\min}$  takes maximum values). Information concerning the general aspect of the chromatographic separation process can be obtained by curve analysis from diagrams ( $R_f \times 100$  versus mobile phase composition). © 1997 Elsevier Science B.V.

**Keywords:** Computer simulation; Optimization; Mobile phase composition; Polynuclear aromatic hydrocarbons

## 1. Introduction

The carcinogenic and/or mutagenic potential and the great stability in natural biological systems of some polycyclic aromatic hydrocarbons (PAHs) and of their biologically reactive metabolites are well known [1]. Because of their toxicity, PAHs are widely studied [2–13]. Studies concerning PAH separation on silica gel layers with and without lipophilic modifications and with various eluent systems have been presented in the literature [5–9]. Baranowska et al. [8] have analyzed PAHs in soil extracts from a coke plant by adsorption and re-

versed-phase TLC on plates coated with silica gel, a 1:1 mixture of silica gel and Kieselguhr, aluminium oxide, RP-18 or RP-8 developed with various solvent systems. Kosera and Śliwiok [9] have performed a chromatographic and physicochemical investigation by RP-TLC and RP-HPTLC on the hydrophobic properties of izomeric PAHs. Determination of benzo[*a*]pyrene in food was performed by high-performance liquid chromatography (column RP-18) with fluorimetric detection for quantification [10]. PAHs in marine sediment were separated by means of on-line coupling of liquid-chromatography to thin-layer chromatography and identification by fluorescence excitation and emission spectroscopy [11]. In a lot of cases, PAH separation was carried out by

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HPTLC RP-18 [5–10]. A simple, efficient and very precise system for the rapid separation of PAHs is charge transfer chromatography on caffeine-impregnated HPTLC silica gel layers. Determination and quantification are performed by fluorimetry [12]. Chiral stationary phases were used for enantiomeric separation of *trans*-dihydrodiol PAHs by  $\pi$ -donor–acceptor interactions [13].

Thin-layer chromatography is a simple analytical technique; however, the selection of the mobile phase that gives the best resolution of all solutes in a mixture is more difficult. Investigations on the optimization of the mobile phase in planar chromatography have been published [14–16]. Nurok [14] summarized the strategies on optimization of TLC systems. The methods used in one-dimensional planar chromatography can be simple or more sophisticated (window diagrams, sequential simplex method, mixture design statistical approach, prisma model) [14,17]. A graphic presentation of binary mobile phase optimization in TLC has been given [18]. A new quaternary mobile phase system for optimization of TLC separations of basic drugs and alkaloids using mixture designs and response surface modelling has been presented. The selection was based on the classification of solvents according to selectivity and solubility parameters [19]. A simple mathematical approach to the optimization of the separation process in TLC consists in the possibility of calculating optimum values of the differences in chromatographic parameters for the components of separated mixtures performed by means of the retention model based on the law of mass action. The method has been used to calculate the extreme separation values of mixtures of phenols and naphthalene derivatives [20]. A comparative study of the three different optimization methods based on the relationships between retention and binary mobile phase composition proposed by Snyder, Schoenmakers and Kowalska has been presented [21]. Computer-aided optimization of liquid–solid systems in TLC was presented in a cycle of papers and it is based on retention versus eluent composition relationships [22–25]. A Pascal computer program for selecting the optimum eluent composition for a given set of solutes from a database has been developed. The database program uses the Snyder–Soczewiński model of competitive adsorption [24]. Informational

theory based on mathematical structure has been elaborated for general chromatographic optimization [26–29].

In the present paper we report a new modelling of the chromatographic separation process of some polar and nonpolar polycyclic aromatic compounds (PACs) (Fig. 1) in the form of 3rd degree continuous functions. This way of experimental data processing is different from the models found in the literature which use logarithmical, exponential or 2nd degree functions [17,19–25].

The selection of optimum mobile phase composition for the separation of this mixture has been obtained by means of some new computer programs written in GW-BASIC language on the basis of experimental data. The study was performed for the separation of eight PACs on *n*-octyl and *n*-octadecyl chemically bonded silica gel R thin layers [30,31] using benzene–acetone–water as mobile phase. For the selection of optimum mobile phase composition on a certain stationary phase four chromatographic runs are necessary.

## 2. Experimental

### 2.1. Thin-layer chromatography

Chromatographic tests were performed on 10×20 cm glass plates coated with *n*-octyl and *n*-octadecyl silica gel R thin layers. The silica gel R and the chemically bonded *n*-octyl and *n*-octadecyl silica gel R used in these experiments were produced at the Institute of Chemistry ‘Raluca Ripan’ Cluj-Napoca, Romania [30,31]. The plates were coated with 0.30 mm thin layers by application of an ethanolic slurry of a mixture of the stationary phase, organic binder and fluorescent indicator  $F_{254}$  for TLC (Merck). Standard solutions (0.05%) of the eight PACs (Fig. 1), both single and mixture, were prepared in benzene. 0.2  $\mu$ l/spot volumes were applied with Brand micropipets on chromatographic plates. The chromatographic plates were developed over a distance of 10 cm in a saturated chamber, by ascending technique at room temperature. Several ternary mixtures of benzene–acetone–water (85:15:1; 70:30:1; 30:70:1; 10:90:1, v/v/v) were used as mobile

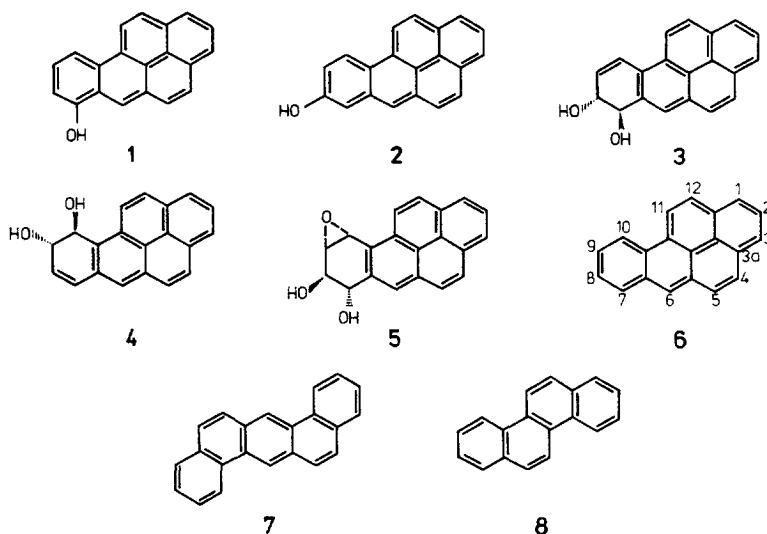


Fig. 1. Structures of the investigated polycyclic aromatic compounds: (1) benzo[a]pyrene-7-hydroxy; (2) benzo[a]pyrene-8-hydroxy; (3) benzo[a]pyrene-*trans*-7,8-dihydrodiol; (4) Benzo[a]pyrene-*trans*-9,10-dihydrodiol; (5) benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxy(*syn*); (6) benzo[a]pyrene; (7) dibenz[a,h]anthracene; (8) chrysene.

phases. PACs were examined in UV light at 254 and 366 nm (Camag UV).

## 2.2. Optimization studies

The analysis of the experimental data shows that for various compounds the  $R_F$  values versus mobile phase composition curves differ substantially in shape. For this reason, a choice was made for the representation of  $R_F$  values versus mobile phase composition for one certain compound as being a 3rd degree continuous function. Thus, for each compound, the  $R_F$  values in a range of 1–100 of the mobile phase composition were calculated as a 3rd degree continuous function according to the equation:

$$R_{F(i,j)} = a_i k_j^3 + b_i k_j^2 + c_i k_j + d_i$$

where  $i$  = number of PAC compound;  $k_j$  = mobile phase composition for experiment  $j$ .

This way of experimental data processing permits the analysis of different compounds with a reduced number of experiments. The model does not require tables because it is built on the basis of experimental data.

By means of the experimental  $R_F$  values at four different mobile phase compositions, for the compound  $i$  the system can be written:

$$R_{F(i,1)} = a_i k_1^3 + b_i k_1^2 + c_i k_1 + d_i$$

$$R_{F(i,2)} = a_i k_2^3 + b_i k_2^2 + c_i k_2 + d_i$$

$$R_{F(i,3)} = a_i k_3^3 + b_i k_3^2 + c_i k_3 + d_i$$

$$R_{F(i,4)} = a_i k_4^3 + b_i k_4^2 + c_i k_4 + d_i$$

where  $i$  = number of PAC compound ( $i=1-8$ );  $k_j$  = mobile phase composition for experiment  $j$  ( $j=1-4$ ).

Solving this system, the  $a_i$ ,  $b_i$ ,  $c_i$  and  $d_i$  parameters of the curve for compound  $i$  are determined.

In agreement with each mobile phase composition  $\Delta R_{F,\min}$  is calculated. By means of experimental  $R_F$  values,  $C_m^2$  combinations (combinations of  $m$  into groups of 2) are calculated at different mobile phase compositions, where  $m$  is the number of studied substances ( $m=8$ ). These combinations represent differences between the 3rd degree previously defined continuous functions. Thus,  $\Delta R_F$  values are calculated for each combination. We chose the  $\Delta R_F$

with the smallest value, i.e.  $\Delta R_{F,\min}$ . For a certain composition of mobile phase, two curves, namely the closest curves, correspond to  $\Delta R_{F,\min}$ . The mobile phase composition is calculated according to the polarity of the mixture. Data from tables and diagrams are represented in the sense of increasing polarity. The variation of  $\Delta R_{F,\min}$  versus mobile phase composition presents, as a rule, more maximum points and minimum ones. The maximum of  $\Delta R_{F,\min}$  value (max.  $\Delta R_{F,\min}$ ) is represented by the greatest  $\Delta R_{F,\min}$  value and corresponds to a certain mobile phase composition. The best separation is to be found at that mobile phase composition for which  $\Delta R_{F,\min}$  has a maximum.

For the selection of the optimum mobile phase composition some computer programs were performed. These computer programs are written in the GW-BASIC language and can be examined on an adequate IBM PC (386, 486, 586) computer (the computer programs are available upon request). The PACs1, PACs2 and PACs3 programs performed the data introduction of the experimental measurements, the  $a_i$ ,  $b_i$ ,  $c_i$  and  $d_i$  parameter calculus of curves and saved them in a card index. The PACs4 and PACs5 programs calculated  $R_F$  values (for each compound at each mobile phase composition) and the  $\Delta R_{F,\min}$  value, displayed the results (mobile phase composition,  $R_{F(i,j)}$ ,  $\Delta R_{F,\min}$ ) and produced the graphic representations.

The PAC separations were performed on chromatographic plates coated with *n*-octyl and *n*-octa-

decyl silica gel R thin layers. These plates were eluted with benzene–acetone–water as mobile phase at four different compositions in the range 1–100, where it was important that two of the determinations were close to the extreme range compositions.

### 3. Results and discussion

#### 3.1. Visualization

The colour and the intensity of PAC spots in UV light before and after development are presented in Table 1. The data show that all the studied compounds can be visualized in UV light. The different structures of PACs influence the spot intensity. The detection limits are in the range 10–100 ng/spot.

#### 3.2. Separation and optimization

Experimental data (PAC  $R_F$  values, mobile phase compositions) were processed by means of computer programs. Thus, the optimum mobile phase composition was found, for which the best separation of the eight PACs is obtained. For this composition, each compound has a calculated  $R_F$ . The optimum compositions of the mobile phase found by means of computer programs for the separation of the eight PACs were experimentally verified. The experimental  $R_F$  values correspond to the calculated ones.

Table 1  
Spot colour of investigated polycyclic aromatic compounds in UV light at 366 and 254 nm before and after development

Compound	Visualization		
	Before development	After development	
		336 nm	254 nm
1	light blue + + + +	lilac + + + +	lilac + + +
2	blue + + + +	blue + + + +	blue + + +
3	violet + + +	violet + + +	violet + +
4	violet + + +	violet + + +	violet + +
5	blue–violet + + +	violet + + +	violet + +
6	lemon–green + + + +	blue–green + + + +	green–violet + + +
7	violet + +	brownish–violet + +	brownish–violet +
8	violet + +	brown	brown + +

$\lambda = 366$  nm, dark plate background;  $\lambda = 254$  nm, greenish plate background.

+ = spot intensity.

Table 2

$R_f \times 100$  values of the investigated polycyclic aromatic compounds on *n*-octyl silica gel R thin layers

Compound	$R_f \times 100$			
	Mobile phase: benzene–acetone–water (v/v/v)			
	85:15:1	70:30:1	30:70:1	10:90:1
1	16	38	44	39
2	12	31	35	31
3	9	22	41	40
4	8	18	34	33
5	0	0	11	12
6	74	75	70	65
7	75	77	72	68
8	78	79	75	71

The results obtained from the separation of PACs on *n*-octyl silica gel R layers and by computer programs are given in Table 2, Table 3 and Fig. 2.

The data from Table 3 indicate  $R_f \times 100$  values (calculated and experimentally obtained) for some max.  $\Delta R_{F,\min}$  of the studied PACs and the corresponding mobile phase compositions for the optimum separation. The calculated values of max.  $\Delta R_{F,\min}$  for the range extremes of the mobile phase composition were not considered. The obtained results during the experimental verification are in agreement with the calculated data. For a good separation of the studied PACs on *n*-octyl silica gel

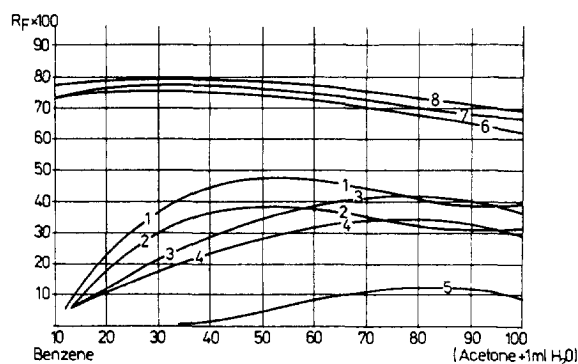


Fig. 2. Diagram obtained ( $R_f \times 100$  values versus mobile phase composition) after computer processing of the experimental data for the investigated polycyclic aromatic compounds on *n*-octyl silica gel R thin layers.

R thin layers can be considered the optimum, with 52:48:1 (max.  $\Delta R_{F,\min} \times 100 = 2.12$ ) or 70:30:1 (max.  $\Delta R_{F,\min} \times 100 = 1.99$ ) (v/v/v) being the composition of the benzene–acetone–water mobile phase. The analysis of curves from Fig. 2 shows that the eight PACs can be separated by a max.  $\Delta R_{F,\min} \times 100 \approx 2$  in the range 70:30:1–50:50:1 (v/v/v) of benzene–acetone–water mobile phase composition. In this range, 1–6 PACs are well separated, the  $\Delta R_{F,\min} \times 100 \approx 2$  values refer only to 6–8 PACs. The composition range of 38:62:1–33:67:1 (v/v/v) of ben-

Table 3

$R_f \times 100$  values for some max.  $\Delta R_{F,\min}$  of investigated polycyclic aromatic compounds and optimum mobile phase composition (benzene–acetone–water) on *n*-octyl silica gel R thin layers

Compound	$R_f \times 100$							
	$\Delta R_{F,\min} \times 100$							
	1.99		2.12		1.98		1.98	
	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>
1	37.6	38	47.2	47	46.1	46	44.8	45
2	30.7	31	38.4	38	37.0	37	35.7	36
3	21.7	22	33.6	34	39.4	39	40.6	40
4	17.7	18	27.6	28	32.5	32	33.6	34
5	0.0	0	4.5	5	9.0	9	10.4	10
6	75.0	75	73.9	74	71.6	72	70.6	71
7	77.0	77	76.0	76	73.6	74	72.6	73
8	79.0	79	78.2	78	76.3	77	75.5	76
Mobile phase	70:30:1		52:48:1		38:62:1		33:67:1	

<sup>a</sup> Calc. = Calculated.

<sup>b</sup> Exp. = Experimentally obtained.

Table 4

$R_F \times 100$  values of the investigated polycyclic aromatic compounds on *n*-octadecyl silica gel R thin layers

Compound	$R_F \times 100$			
	Mobile phase: benzene–acetone–water (v/v/v)			
	85:15:1	70:30:1	30:70:1	10:90:1
1	13	32	19	15
2	11	27	17	12
3	10	21	28	25
4	4	15	21	18
5	0	6	6	0
6	79	71	67	60
7	80	75	70	65
8	81	80	76	70

zene–acetone–water mobile phase indicates a separation of the eight PACs by a  $\Delta R_{F,\min} \times 100 = 1.98$ , but the separations are no longer good for Compounds 1–4.

The results obtained on *n*-octadecyl silica gel R layers with the separation of the eight PACs are given in Table 4, Table 5 and Fig. 3. The best separation, max.  $\Delta R_{F,\min} \times 100 = 4.37$ , is performed with a 61:39:1 (v/v/v) benzene–acetone–water mobile phase composition. Analysing the curves from Fig. 3, a good separation of the eight PACs (max.  $\Delta R_{F,\min} \times 100 \approx 4$ ) can be observed in the benzene–

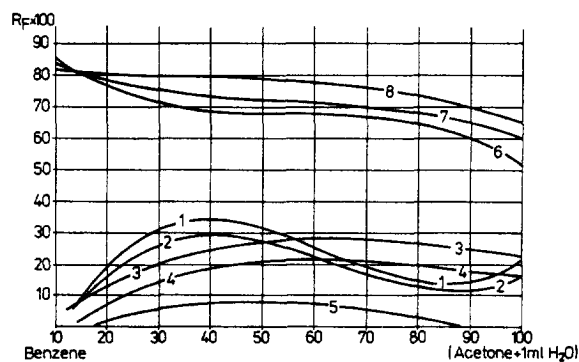


Fig. 3. Diagram obtained ( $R_F \times 100$  values versus mobile phase composition) after computer processing of the experimental data for the investigated polycyclic aromatic compounds on *n*-octadecyl silica gel R thin layers.

acetone–water mobile phase composition range 70:30:1–60:40:1 (v/v/v). Around the 10:90:1 (v/v/v) mobile phase composition PAC separations can be obtained by a max.  $\Delta R_{F,\min} \times 100$  close to a value of 3.

Analysing Figs. 2 and 3, we can note that the curve shape is similar for the compounds with similar structures (1–2, 3–4, 6–8) and, also, that all unsubstituted PACs (6–8) have  $R_F \times 100$  values higher than 60, while the hydroxy PACs (1–5) have  $R_F \times 100$  values lower than 50. In all the cases, the

Table 5

$R_F \times 100$  values for some max.  $\Delta R_{F,\min}$  of investigated polycyclic aromatic compounds and optimum mobile phase composition (benzene–acetone–water) on *n*-octadecyl silica gel R thin layers

Compound	$R_F \times 100$							
	$\Delta R_{F,\min} \times 100$							
	3.61		4.05		4.37		2.95	
	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>	Calc. <sup>a</sup>	Exp. <sup>b</sup>
1	29.5	30	32.3	33	34.4	35	14.9	15
2	24.8	25	27.3	28	29.3	29	12.0	12
3	19.0	19	21.3	21	24.9	25	25.0	25
4	13.0	13	15.3	15	18.7	19	18.0	18
5	5.0	5	6.2	6	7.6	8	0.0	0
6	72.3	72	70.8	71	69.0	69	60.1	60
7	75.9	76	74.9	75	73.4	73	65.1	65
8	80.2	80	80.0	80	79.5	80	70.1	70
Mobile phase	73:27:1		69:31:1		61:39:1		10:90:1	

<sup>a</sup> Calc. = Calculated.

<sup>b</sup> Exp. = Experimentally obtained.

curve of Compound 5 is placed in the lowest part of the diagrams. This compound contains one epoxy group, besides the two hydroxy groups. This distribution of curves on the diagrams is in accordance with the polarity of PACs used in this study.

Analysis of the diagrams permits us a rapid choice of the mobile phase composition which offers the best separation of the mixture substances and presents a good general aspect of the chromatographic separation process. Comparison of max.  $\Delta R_{F,\min}$  values and the diagrams of the two stationary phases shows that the best separations are obtained on *n*-octadecyl silica gel R thin layers.

#### 4. Conclusions

The utilisation of the achieved computer programs permits the determination of optimum benzene–acetone–water mobile phase composition for the separation of the eight investigated PACs on *n*-octyl and *n*-octadecyl silica gel R thin layers. These computer programs for the separation of some different substances from a mixture are written in the GW-BASIC language and model the chromatographic separation process in the form of 3rd degree equations. This modelling does not require tables, for it is built on the basis of experimental data. These programs allow the choice of mobile phases which ensure the best spread of  $R_F$  values on the chromatographic plates and satisfactory differences between them ( $\Delta R_{F,\min}$  takes maximum values).

For the *n*-octyl stationary phase, the optimum composition of the benzene–acetone–water mobile phase is in the range 70:30:1–50:50:1 (v/v/v) and for *n*-octadecyl silica gel R stationary phase it is in the range 70:30:1–60:40:1 (v/v/v). These theoretical data have been experimentally verified and are similar.

The elaborated computer programs process the experimental data of four chromatographic runs at different mobile phase compositions and ensure that we can find the optimum conditions for TLC separation of any substance mixture, where it is important that two of the determinations are close to the extreme range compositions. The graph representations ( $R_F \times 100$  values vs. mobile phase composition)

give information concerning the general aspect of the chromatographic separation process.

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