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Optimization of mobile phase composition for high-performance liquid chromatographic separation by means of the overlapping resolution mapping scheme

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SUMMARY

The use of the overlapping resolution mapping (ORM) scheme to predict the optimum mobile phase composition for isocratic reversed-phase separation was examined. The application of the method to the separation of a mixture of ten polycyclic aromatic hydrocarbons was demonstrated. The ORM scheme was found to be a rapid and versatile method.

INTRODUCTION

In a previous paper[1], we reported the application of the overlapping resolution mapping (ORM) scheme to the optimization of mobile phase composition for the separation of eleven priority phenols. Although high-performance liquid chromatography (HPLC) has been used extensively for the analysis of polycyclic aromatic hydrocarbons (PAHs)[2–5], the use of the ORM scheme for the optimization of separation of these compounds has not been examined. The ORM scheme is an interpretative optimization method in which the extent of chromatographic separation is predicted indirectly from the retention behaviour of the individual solutes[6]. For the optimization of eluent mixtures with up to quaternary compositions, only seven experiments need to be carried out according to the Snyder selectivity triangle[7] before one can locate the optimum conditions. Computer programs have been written to assist in the computation steps and in the prediction of optimum conditions[8].

In this paper, a procedure is described that enables optimum solvent systems to be selected by simple experimentation. The application of the procedure to the separation of ten PAHs is demonstrated.

EXPERIMENTAL

The chromatographic work was performed using a Shimadzu LC-6A isocratic instrument equipped with a Model SPD-6A variable-wavelength UV spectrophoto-

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metric detector. Samples were injected with a Rheodyne 7125 injector with a 20-μl loop. The chromatographic data were collected and analysed on a Chromatopack C-R3A data processor.

All chromatographic runs were duplicated with a reproducibility between runs of $\pm 2\%$ or better. The void volume was obtained by using methanol as the unretained component for all mobile phases. The results obtained were within 1% of each other. The amount of sample injected did not affect the retention times at the concentrations used. Two reversed-phase columns were employed for the analyses: a Whatman Partisil-5 ODS-3 column of 5-μm particle size (100 mm $\times$ 4.6 mm I.D.) and a Perkin-Elmer 3 $\times$ 3 HS C_{18} column of 3-μm particle size (33 $\times$ length and 4.6 mm I.D.). The wavelength of the detector was set at 254 nm. The organic modifiers used were methanol, acetonitrile and isopropanol with water as the inert carrier. A flow-rate of 0.8 ml/min was used throughout. The Perkin-Elmer column was first used to determine the optimum mobile phase composition using the ORM scheme. Once the optimum mobile phase had been established, the Whatman column was then used to test the validity of the results of the optimization procedure.

Seven of the PAHs, fluoranthene, chrysene, fluorene, benzophenanthrene, dibenz[*a,h*]anthracene, benz[*a*]anthracene and coronene, were supplied by Fluka and were of the purest grade available. The remaining three PAHs, naphthalene, pyrene and phenanthrene, were obtained from Aldrich and their purities were better than 99%. HPLC-grade acetonitrile and methanol (J. T. Baker) and analytical-reagent grade isopropanol (Aldrich) were used for the preparation of the mobile phases. The A + B (*quantum sufficit* or sufficient quantity) addition method recommended by Runser[9] was used. According to this method, correct volumes of organic modifier were first added, followed by water which was used to make up to the required volume. All the solvents were filtered through Millipore membrane filters and degassed in an ultrasonic bath.

The PAH mixture was prepared by dissolving known amounts of individual PAHs in the minimum amount of dimethyl sulphoxide (DMSO) and then diluting with the mobile phase. This procedure was essential as many of the PAHs are not very soluble in water. However, as DMSO has a high UV cut-off (330 nm), which might interfere with the analyses, the amounts used were kept as small as possible. The concentrations of the PAHs in the standard mixture ranged between 0.33 and 27.50 ng/μl. Owing to the posibility of degradation or decomposition of the PAHs under light, the standard solution was protected from light and stored in a cool location. Individual PAHs were also prepared in the same manner to assist in the identification. All the samples were filtered and degassed before injection into the column.

RESULTS AND DISCUSSION

A schematic diagram of the ORM scheme is illustrated in Fig. 1. The first step in the optimization procedure was to establish the goals of the process. For HPLC separations, this task is not always straightforward as the separation is governed by many factors and parameters[10]. Nevertheless, two criteria were used in the investigation reported here. The first was that all the peaks in the final chromatogram (*i.e.*, the "optimized" chromatogram) should have a resolution, R_s, of at least unity between peaks. The second criterion was that all the peaks should have capacity

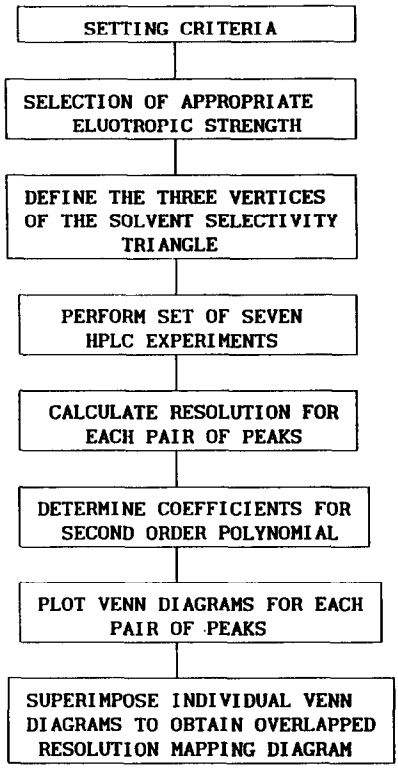


Fig. 1. Schematic diagram of the ORM scheme.

factors, k', in the range 0–20, to ensure that the total analysis time falls within a reasonable range.

The next step was to identify the three vertices of the Snyder selectivity triangle[7], which is illustrated in Fig. 2. The three points on the triangle (A, B and C) represent the three iso-eluotropic binary mixturs (*i.e.*, organic modifier + carrier, water) of equivalent solvent strength. Before the composition of these three binary mixtures could be determined, it was first necessary to define the appropriate eluotropic strength for the system. The eluotropic strength was chosen so that the second criterion

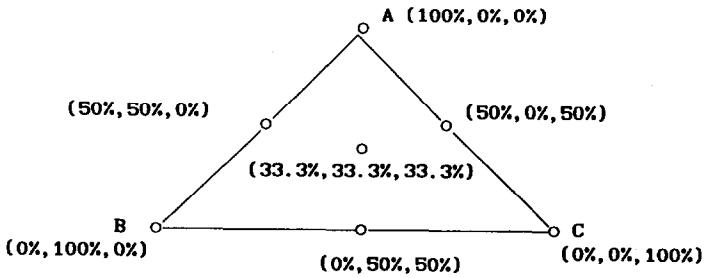


Fig. 2. Solvent selectivity triangle showing compositions (%, v/v) of mobile phase consisting of mixtures of binary solvents A (methanol–water), B (acetonitrile–water) and C (isopropanol–water).

TABLE I

RESULTS OF PRELIMINARY RUNS USING ELUENT MIXTURES CONSISTING OF METHANOL AND WATER

Methanol–water composition (v/v)	*k' for last-eluting peak*	*Solvent strength*
60:40	26.0	1.80
70:30	22.0	2.10
72:38	19.5	2.16

TABLE II

MOBILE PHASE COMPOSITIONS IN TERMS OF VOLUME PERCENTAGES OF THE BINARY MIXTURES A (METHANOL–WATER), B (ACETONITRILE–WATER) AND C (ISOPROPANOL–WATER)

Eluent mixture	*A*	*B*	*C*
1	100.0	0.0	0.0
2	0.0	100.0	0.0
3	0.0	0.0	100.0
4	50.0	50.0	0.0
5	0.0	50.0	50.0
6	50.0	0.0	50.0
7	33.3	33.3	33.3

defined earlier could be satisfied. The selection was based on the suggestion by De Galan *et al.*[11], who recommended the use of methanol-water binary mixtures for identifying the mobile phase composition that gives k' values for all the peaks within the desired range (0–20). Consequently, three preliminary runs were carried out using methanol–water mobile phases with the compositions given in Table I. The results of these preliminary runs are also shown in Table I. The mobile phase methanol–water (72:28, v/v) gave the smallest capacity factor for the last-eluting component and

TABLE III

MOBILE PHASE COMPOSITIONS AS PERCENTAGES OF PURE SOLVENTS IN MIXTURE

Eluent mixture	*Concentration (%, v/v)*			
	Methanol	*Acetonitrile*	*Isopropanol*	*Water*
1	72.0	0.0	0.0	28.0
2	0.0	70.0	0.0	30.0
3	0.0	0.0	51.6	48.4
4	36.0	35.0	0.0	29.0
5	0.0	35.0	25.8	39.2
6	36.0	0.0	25.8	38.2
7	24.0	23.3	17.2	35.5

TABLE IV

RETENTION TIMES (min) OF PAH COMPOUNDS IN EACH OF THE SEVEN ELUENT MIXTURES LISTED IN TABLE III

No.	*Compound*[a]	*Eluent mixture*						
		1	*2*	*3*	*4*	*5*	*6*	*7*
1	Cor	0.482	0.472	0.452	0.587	0.455	0.469	0.459
2	Nap[b]	2.050	0.940	1.210	1.408	1.056	1.517	1.186
3	Fl	2.697	1.260	2.041	5.782	1.517	2.685	1.828
4	Ph	2.952	1.405	2.157	2.518	1.652	2.836	1.995
5	Ft	4.280	1.783	2.679	3.484	2.080	3.820	2.649
6	Pyr	4.717	1.808	2.836	3.842	2.289	4.078	2.903
7	Benzo	6.408	2.285	3.580	4.963	4.573	5.443	3.623
8	BiB(a,h)[b]	2.230	4.332	3.582	13.822	3.115	5.518	3.766
9	Ch[b]	7.513	2.477	3.977	5.567	3.005	6.382	4.110
10	B(a)	7.798	2.542	4.196	5.783	3.135	6.724	4.268

[a] (1) Cor = coronene; (2) Nap = naphthalene; (3) Fl = fluorene; (4) Ph = phenanthrene; (5) Ft = fluoranthene; (6) Pyr = pyrene; (7) Benzo = benzophenanthrene; (8) DiB(a,h) = dibenz[*a,h*]anthracene; (9) Ch = chrysene; (10) B(a) = benz[*a*]anthracene.

[b] The standard mixture was spiked with this component to improve detection.

satisfied the second optimization criterion. This mobile phase was then selected as solvent A in the solvent selectivity triangle shown in Fig. 2. Subsequently, the solvent strength of this mobile phase was calculated using the following equation:

$$ST = S_a\varphi_a + S_b\varphi_b + \ldots \quad (1)$$

where ST represents the solvent strength of the mixture, φ_i are the volume fractions of each component and S_i are the individual solvent strengths of the organic modifiers[11]. After determining the solvent strength for mobile phase A, the other two iso-eluotropic

TABLE V

RESOLUTION BETWEEN ADJACENT PEAKS IN THE CHROMATOGRAMS OBTAINED USING THE SEVEN ELUENT MIXTURES LISTED IN TABLE III

Peak pair	*Eluent mixture*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
1	4.094	4.216	4.181	4.829	4.237	4.579	3.978
2	0.636	1.981	3.890	4.933	2.573	3.877	2.748
3	1.550	0.773	0.461	3.217	0.666	0.453	0.775
4	0.702	1.622	1.968	0.630	1.909	2.512	2.956
5	3.648	0.108	0.555	1.824	0.830	0.561	0.972
6	0.991	2.344	2.446	1.132	1.568	2.826	2.055
7	2.918	0.736	0.006	0.386	0.925	0.182	0.357
8	1.670	0.247	1.254	0.003	0.375	1.481	0.836
9	0.425	4.680	0.864	11.843	0.057	0.408	0.363

mixtures B (acetonitrile–water) and C (isopropanol–water) could be calculated using eqn. 1. Four additional mobile phase compositions were then selected from the solvent selectivity triangle. The seven eluent mixtures chosen are listed in Table II and are also illustrated in Fig. 2. As the compositons shown in Table II are in terms of the binary solvents A, B and C, the actual compositons in terms of the pure solvents were recalculated and are given in Table III.

Experiments using these seven mobile phases were then performed and the results are listed in Table IV. All chromatographic results were obtained in duplicate with reproducibilities better than $\pm 2\%$. The void time for the Perkin-Elmer column was 0.38 min.

In addition to the standard mixture, individual PAHs were also analysed to assist in the identification and calculation of the resolution. From the results of the seven experiments the resolution, R_s, could be calculated using either of the following two equations:

$$R_s = \frac{1}{4}(\alpha - 1)N^{\frac{1}{2}}\left(\frac{k'}{k'+1}\right) \tag{2}$$

$$R_s = \frac{2(t_2 - t_1)}{w_2 + w_1} \tag{3}$$

where R_s is the resolution for a pair of adjacent peaks, α is the relative retention ratio, N is the number of theoretical plates of the column, k' is the capacity factor for one of the peaks, t_i is the retention time of the ith peak and w_i is the width of the ith peak. The values of R_s calculated are listed in Table V.

Subsequently, the R_s values were fitted into a second-order polynomial:

$$R_s = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + a_{123}x_1x_2x_3 \tag{4}$$

TABLE VI

COEFFICIENTS OF EQN. 4 FOR THE NINE PAIRS OF PEAKS

Peak pair	*Coefficient*						
	a_1	a_2	a_3	a_{12}	a_{23}	a_{13}	a_{123}
1	4.094	4.216	4.181	2.696	0.398	1.522	− 18.861
2	0.636	1.981	3.890	14.498	1.240	3.766	− 42.879
3	1.550	0.773	0.461	8.222	−1.358	−0.656	− 22.755
4	0.702	1.622	1.968	−2.128	2.296	2.868	32.076
5	3.648	0.108	0.555	−0.216	−5.086	0.918	0.597
6	0.991	2.344	2.446	−2.142	−0.602	1.724	6.516
7	2.918	0.736	0.006	−5.764	−2.148	−0.756	2.703
8	1.670	0.247	1.254	−3.822	−4.348	2.922	9.777
9	0.425	4.680	0.864	37.162	−2.350	9.456	−119.988

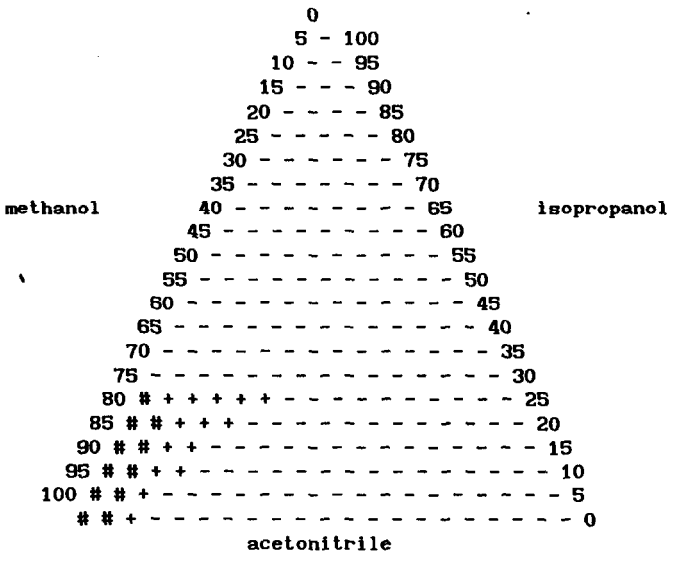
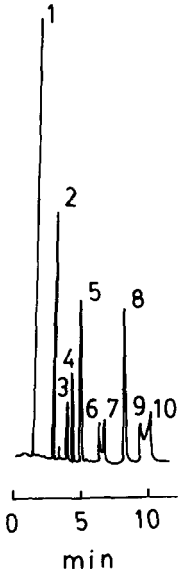


Fig. 3. Overlapping resolution diagram for the nine pairs of peaks. Symbols: −, R ⩽ 0.5; +, 0.5 < R < 1.0; # $R \geqslant 1.0$.



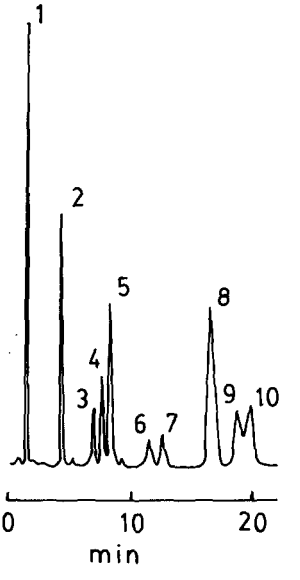


Fig. 4. Chromatogram of standard mixture of PAHs using a mobile phase consisting of methanol–acetonitrile–isopropanol–water (61.20:3.50:5.16:30.14, v/v). Column: Perkin-Elmer 3 × 3 HS C_{18}. Other chromatographic conditions as described in text. For peak identification, see Table IV.

Fig. 5. Chromatogram of standard mixture of PAHs using a mobile phase consisting of methanol–acetonitrile–isopropanol–water (61.20:3.50:5.16:30.14, v/v). Column: Whatman Partisil-5 ODS-3. Other chromatographic conditions as described in text. For peak identification, see Table IV.

where a_i are coefficients and x_i are the volume fractions of the mobile phases A, B and C. With the aid of a modified version of the Basic program given by Berridge[8], the coefficients for each peak pair were determined. These coefficients are listed in Table VI. Using eqn. 4, together with the coefficients listed in Table VI, the R_s values for other mobile phase compositions within the solvent triangle could be calculated. These values were used to construct Venn diagrams or resolution plots for all the peak pairs. The individual resolution plots were then superimposed to give an overlapped resolution diagram, which is shown in Fig. 3. The area marked with # represents the optimum region where the solvent compositions would result in satisfactory separation of all the peaks (*i.e.*, $R_s > 1$).

To confirm the results of the optimization procedure, an additional experiment using one of the mobile phase compositions in the optimum region was performed to verify that a satisfactory separation of all the peaks could be achieved. A mobile phase consisting of methanol–acetonitrile–isopropanol–water (61.20:3.50:5.16:30.14, v/v) was used for this purpose. A typical chromatogram obtained is illustrated in Fig. 4. Satisfactory separation was achieved for the ten PAHs. A further test was performed using a different column (the Whatman column). The same mobile phase was used and the chromatogram obtained is shown in Fig. 5. Complete separation was also observed. As the latter column is longer, longer retention times were observed.

The above results demonstrated that the ORM optimization scheme is a rapid and versatile method. Optimum separation can be achieved without much difficulty even when quaternary mobile phases are considered, because only seven different mobile phase systems need to be examined in order to obtain the necessary data for the resolution plots. Moreover, no re-optimization is required once the optimum mobile phases have been established. This would certainly be a useful advantage over many other optimization techniques, as a shorter column can be used first to determine the optimum mobile phase. Subsequently, a longer column can be used to improve the separation further if necessary. With such a scheme, the overall analysis time can be significantly reduced. Further, in any case of column failure during the course of the experiment, a new column with the same type of stationary phase can be used to replace the damaged column and there is no need to repeat the whole optimization procedure. This is, of course, a very useful feature of the ORM technique as it would mean that wastage of expensive solvents and analysis time can be minimized.

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