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Note

Optimization of the isocratic high-performance liquid chromatographic separation of selected phthalates using the overlapping resolution mapping technique

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The use of a systematic experimental design to optimize the separation conditions in high-performance liquid chromatography (HPLC) serves to minimize, if not eliminate, the laborious trial-and-error attempts to achieve desired separation conditions generally associated with the latter technique. One of these systematic approaches makes use of mixture designs followed by overlapping resolution mapping[1]. This method requires only seven preliminary experiments based on the solvent selectivity triangle[2–4] to predict the mobile phase compositions for optimum separation. The selection of the criteria desired for a particular analysis is made at this stage; these criteria include a reasonable analysis time (which forms the basis on which the solvent strength of the eluent mixture may be calculated) and a certain required resolution between adjacent component peaks in the chromatogram.

The overlapping resolution mapping technique has previously been applied with success to the analysis of eleven priority substituted phenols[5]. In this paper, the use of five plasticizers (dimethyl, diethyl, dibutyl, diallyl and benzyl-*n*-butyl phthalate) as model systems to test the isocratic overlapping resolution mapping scheme is described. Although the biological effects of plasticizers present in the environment have not been fully evaluated, the facts that these compounds are very widely used, and are therefore ubiquitous in the environment and that they may possess undesirable biological effects, justify the need to analyse for them. Indeed, four of the five phthalates mentioned above (the exception being diallyl phthalate), plus a sixth, bis(2-ethylhexyl) phthalate (not considered in this present work, for reasons given below), are on the priority pollutant list of the United States Environmental Protection Agency (USEPA).

EXPERIMENTAL

Chemicals and reagents

Dimethyl, diethyl, dibutyl, diallyl and benzyl-*n*-butyl phthalate (of at least 97% purity) were obtained from Fluka (Switzerland). Standard solutions of the individual

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phthalates and mixtures (in the concentration range 160–260 ppm for each component) were prepared using methanol. The methanol (J. T. Baker, U.S.A.) and acetonitrile (Ajax, Australia) used were of HPLC grade and the 2-propanol and dimethylformamide (Ajax) were of analytical-reagent grade. Mobile phases were prepared according to the A + B (*quantum sufficit*) addition procedure recommended by Runser[6]. In this method, the modifier amounts are measured, and then the volume is brought to the desired value with water, the final component in the mixture. All solutions and solvents were filtered and degassed by sonication before use.

Instrumentation

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump, a Shimadzu SPD-6A variable-wavelength UV spectrophotometric detector and a Chromatopac CR-3A data processor. The reversed-phase column used for the evaluation of the optimization scheme was a Shimadzu Shimpack CLC-ODS (15 cm × 6 mm I.D.; 5 μm particle size). Once the optimum mobile phase had been determined, a Whatman Partisil-5 ODS-3 column (10 cm × 4.6 mm I.D.; 5 μm particle size) was used to separate the phthalates. The detection wavelength was 224 nm. The flow-rate used was 1.0 ml min^{-1}, except for 2-propanol–water (0.6 ml min^{-1}); the lower flow-rate was used because of the excessively high column pressure obtained with 2-propanol–water as mobile phase.

A Rheodyne 7125 injection valve with a 20-μl loop was used for sample injections. All chromatographic runs were duplicated with a reproducibility between runs of 2% or better. The void volume was obtained for all mobile phases by using methanol as the unretained component.

RESULTS AND DISCUSSION

Preliminary experiments

In defining the first vertex of the solvent selectivity triangle using methanol–water[2] as the binary mixture, the first selection criterion to be specified was the desired analysis time. With three randomly selected methanol–water compositions (60:40, 70:30 and 72.4:27.6), it was determined that the shortest retention time for the last-eluting component in the standard phthalate mixture was a reasonable 22 min for the composition 72.4:27.6. The solvent strength (*ST*) of the eluent mixture of this composition was then calculated from the equation[2]

$$ST = s_a\varphi_a + s_b\varphi_b + \ldots \quad (1)$$

where s_i = the individual solvent strengths and φ_i = the volume fractions of each component *i*.

The solvent compositions of the other binary mixtures (acetonitrile–water and 2-propanol–water, which define the other two vertices of the triangle) having the same solvent strength were then determined using eqn. 1.

To effect changes in selectivity, solvents from different selectivity groups[4] should be chosen to establish the solvent selectivity triangle. As methanol and acetonitrile are the most common reversed-phase HPLC solvents, and they are in different solvent groups, they were picked (as binary mixtures with water) to be the two corners of the

TABLE I

ELUENT MIXTURES USED IN PRELIMINARY EXPERIMENTS

Solvent compositions are given as percetages of binary mixtures in the mobile phase.

Eluent mixture	*Methanol–water*	*Acetonitrile–water*	*2-Propanol–water*
1	100	0	0
2	0	100	0
3	0	0	100
4	50	50	0
5	50	0	50
6	0	50	50
7	33.3	33.3	33.3

triangle. For the third corner, 2-propanol (+ water) was selected instead of the customary tetrahydrofuran (THF) for work of this nature mainly because of the added expense of obtaining antioxidant-free THF. The other solvents in the same group as THF, methoxyethanol and dimethylformamide, were ruled out because of their toxic and irritant properties. Moreover, 2-propanol is in the same group as methanol which, in our view, is an added advantage because of its good solvating power.

The solvent selectivity triangle having been established, the next step was to conduct experiments using the other six eluent mixtures representing the acetonitrile–water and 2-propanol–water vertices, the three mid-points of the vertices and the centre of the triangle[1]. The seven eluent mixtures used for these preliminary experiments are shown in Table I. Table II shows each of these eluent mixtures as a percentage of the pure solvents in the mobile phase.

It is worth mentioning that during the setting up of the solvent compositions representing the second and third vertices of the solvent selectivity triangle, it is usual to make small adjustments to the amounts of the organic modifier in order to obtain equivalent k' values[2]. If this were done, however, the solvent strength would no longer be constant. A change in solvent strength is not useful for improving the resolution

TABLE II

ELUENT MIXTURES USED IN PRELIMINARY EXPERIMENTS

Solvent compositions are given as percentages of pure solvents in the mobile phase.

Eluent mixture	*Void time, t_0 (min)*	*Methanol*	*Acetonitrile*	*2-Propanol*	*Water*
1	2.662	72.40	0.00	0.00	27.60
2	2.683	0.00	70.00	0.00	30.00
3	4.037	0.00	0.00	51.70	48.30
4	2.760	36.20	35.00	0.00	28.80
5	2.532	36.20	0.00	25.85	37.95
6	2.325	0.00	35.00	25.85	39.15
7	2.600	24.13	23.33	17.23	35.31

TABLE III

CAPACITY FACTORS (k') OF PHTHALATES CHROMATOGRAPHED USING THE ELUENT MIXTURES LISTED IN TABLE II

DMP = Dimethyl phthalate; DEP = diethyl phthalate; DAP = diallyl phthalate; BBP = benzyl-*n*-butyl phthalate; DBP = dibutyl phthalate.

Eluent mixture	*DMP*	*DEP*	*DAP*	*BBP*	*DBP*
1	0.381	0.991	1.538	6.848	7.211
2	0.371	0.838	1.133	3.517	4.145
3	0.288	0.618	0.958	2.733	2.776
4	0.407	1.022	1.485	5.670	6.385
5	0.337	0.810	1.296	5.057	5.377
6	0.449	0.996	1.373	4.311	5.220
7	0.363	0.904	1.337	5.027	5.935

between peaks[7]. Therefore, in this work, no adjustments to the composition of the mobile phase were made.

From the results of the seven preliminary runs, the resolutions, R_s, between every pair of peaks in the chromatogram were obtained for each eluent mixture using the equation[8]

$$R_s = \frac{1}{4}(\alpha - 1)N^{0.5}\left(\frac{k'}{1 + k'}\right) \tag{2}$$

where k' is the average capacity factor for the two peaks, α the relative retention ratio and N the number of theoretical plates. The calculated R_s values were then fitted into a second-order polynomial equation[2]:

$$R_s = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \tag{3}$$

TABLE IV

RESOLUTION (R_s) BETWEEN ADJACENT PEAKS CALCULATED FOR PHTHALATES CHROMATOGRAPHED USING THE ELUENT MIXTURES LISTED IN TABLE II

Eluent mixture	R_s			
	Peak pair[a]			
	DMP–DEP	*DEP–DBP*	*DBP–DAP*	*DAP–BBP*
1	5.428	2.569	23.233	0.387
2	3.955	1.456	12.260	1.183
3	2.977	2.020	10.015	0.183
4	5.249	2.100	18.353	0.901
5	3.492	2.564	18.393	0.441
6	3.818	1.713	13.191	1.453
7	4.817	2.109	17.499	1.276

[a] Abbreviations of phthalates as in Table III.

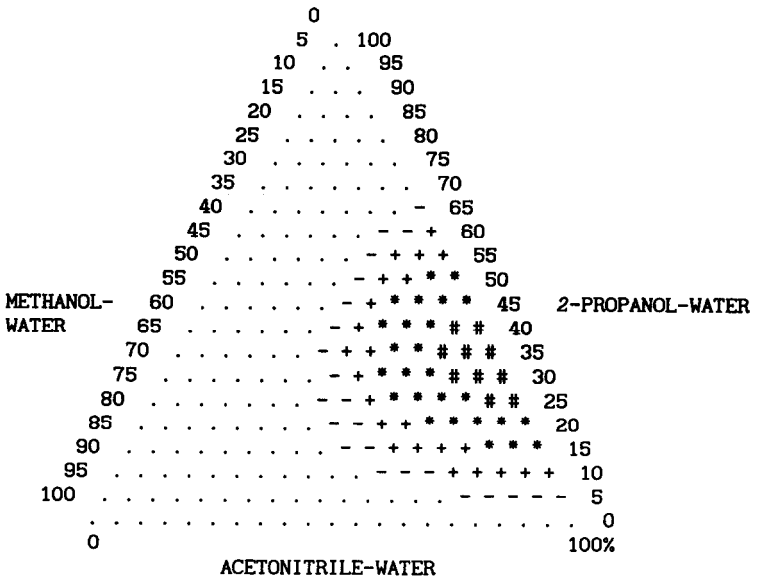

Fig. 1. Overlapping resolution mapping plot for all four phthalate peak pairs. · = $R_s \leqslant 1.2$; − = $1.2 \leqslant R_s \leqslant 1.3$; + = $1.3 \leqslant R_s \leqslant 1.4$; * = $1.4 \leqslant R_s \leqslant 1.5$; # = $R_s \geqslant 1.5$.

where a_i = coefficients and x_i = volume fractions of the three binary mixtures, methanol–water, acetonitrile–water and 2-propanol–water. The values of a_i for each pair of peaks were determined using a BASIC program[2] slightly modified for our purposes. Subsequently, using eqn. 3, R_s values within the solvent selectivity triangle were calculated. Table III shows the capacity factors of the five phthalates chromatographed using the seven eluent mixtures. The calculated R_s values for adjacent peaks in each of the eluent mixtures are given in Table IV [note that with eluent mixture 6, the minimum resolution is close to that specified (1.5) for our optimization scheme (see below)].

Overlapping resolution mapping: optimization of solvent composition

From the calculated R_s values, Venn diagrams[1] were generated[2] for each pair of components in the chromatogram at a specified resolution of 1.5 between each adjacent pair of peaks. By superimposing (overlapping) all seven Venn diagrams, areas corresponding to solvent compositions giving the desired resolution amongst all the peaks in a phthalate mixture were established. Such an overlapping resolution mapping diagram for the five phthalates considered in this work is depicted in Fig. 1. The area described by the symbol # represents all possible mobile phase compositions using which the optimum separation (*i.e.*, where $R_s \geqslant 1.5$) of all the phthalates may be achieved. The other symbols indicate various mobile phase compositions which are not optimized to achieve a complete separation between each peak pair for all the components in the mixture.

To evaluate the optimization scheme, a point within the region encompassed by the symbol # on the overlapping resolution mapping diagram was taken; this

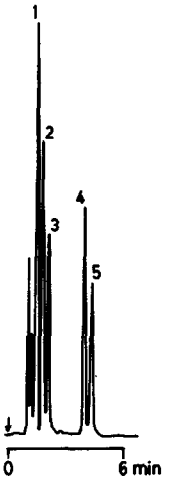

Fig. 2. Chromatogram of phthalates obtained using optimum eluent mixture (methanol–acetonitrile–2-propanol–water, 3.6:42.0:18.1:36.3) derived from overlapping resolution mapping diagram in Fig. 1. Peaks: 1 = dimethyl phthalate; 2 = diethyl phthalate; 3 = diallyl phthalate; 4 = benzyl-*n*-butyl phthalate, 5 = dibutyl phthalate. The Whatman Partisil 5 ODS-3 column was used to generate this chromatogram. Other conditions are as given in the text. The void time for this column was 1.1 min.

corresponds to a mobile phase composition of methanol–acetonitrile–2-propanol–water (3.6:42.0:18.1:36.3). Using this mobile phase, a different C_{18} column and under isocratic elution, a chromatogram of the five phthalates was generated, as shown in Fig. 2. As can be seen, satisfactory separation was achieved. Note that the analysis time was less than 6 min. The value of the overlapping resolution mapping technique has thus been clearly illustrated. In spite of the fact that not all of the adjacent peaks could be separated in each of the seven preliminary experiments, after the overlapping resolution mapping scheme had been established an optimized mobile phase composition could be identified by the simple expedient of selecting a point (and consequently a solvent composition) within the region of the overlapping resolution mapping diagram where $R_s \geqslant 1.5$ (or any other required resolution). Although, as mentioned above, with eluent mixture 6 it was possible to obtain a minimum resolution of 1.45 (for diallyl phthalate and benzyl-*n*-butyl phthalate), which is close to the specified R_s value, an even better resolution (*i.e.*, 1.5 or greater, symbolized by # in Fig. 1) could be achieved for this pair when the optimum mobile phase, derived from the optimization scheme, was used. This systematic approach therefore reduces to a minimum the time spent in method development and is also economical in terms of amounts of solvents used. Another point worth emphasizing is that the chromatogram in Fig. 2 was obtained using a different reversed-phase column from that used to establish the optimum mobile phase composition in the optimization exercise. This further illustrates the flexibility and versatility of the overlapping resolution mapping scheme; for a particular application, subsequent routine analyses may be carried out without resort to the original column used for method development.

Limitations of the present approach

As indicated by the results, the present isocratic technique has been found to be highly satisfactory for the separation of five priority phthalates. However, with the addition of a sixth plasticizer, bis(2-ethylhexyl) phthalate, possibly because its retention behaviour is different from that of the other five under the conditions employed here, it was not possible to generate an overlapping resolution map from which an optimized solvent composition (with specified $R_s \geqslant 1.5$) could be selected for the complete separation of all six components. Obviously, a different approach needs to be considered in this situation. Work is currently being conducted to overcome this difficulty.

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