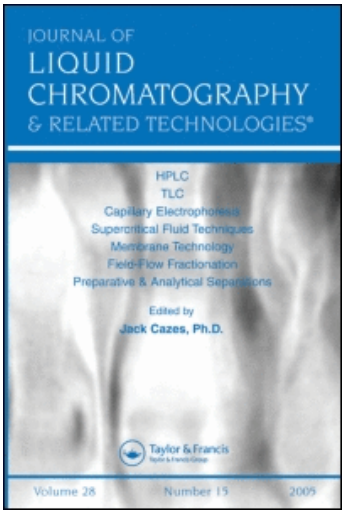

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Application of Iso-Selective Gradient Elution for the Separation of Selected Phthalates

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APPLICATION OF ISO-SELECTIVE GRADIENT ELUTION FOR THE SEPARATION OF SELECTED PHTHALATES

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ABSTRACT

In this paper, the overlapping resolution mapping scheme was applied to the gradient HPLC separation of selected phthalates. The procedure employs a minimum of seven experiments for the determination of the optimum mobile phase gradient for the desired separation. In the present work, solvent systems for iso-selective multisolvent gradient elution were used. The overlapping resolution mapping procedure was found to be a rapid and systematic approach for optimizing HPLC separations.

INTRODUCTION

Although liquid chromatography has been used for the analysis of phthalates [1-3], a systematic experimental design to optimize separation conditions was not used. In such

cases, laborious trial-and-error attempts to achieve a desired separation condition may be required. In this paper, a systematic experimental design was used to optimize the separation conditions of the phthalates using high performance liquid chromatography (HPLC). This was done by making use of mixture designs followed by overlapping resolution mapping (ORM)[4]. In this method, as few as seven experiments are required to optimize the separations of a set of compounds. This saves time and cost as fewer experiments means using lower quantities of solvent.

The ORM method can be used for isocratic separations as well as for gradient optimization. In a previous investigation, the use of the ORM procedure for the optimization of isocratic HPLC separations has been investigated[5]. In the present paper, we shall investigate the use of the ORM procedure for the optimization of gradient separation of six selected phthalates which were not satisfactorily separated using the isocratic procedure[5]. Although the biological effects of phthalates present in the environment have not been fully evaluated, the fact that these compounds are very widely used, and are therefore ubiquitous in the environment, and the potential that they may possess undesirable biological effects, justify the need to analyse for them. Indeed, five of the six phthalates, namely dimethyl phthalate, diethyl phthalate, dibutyl phthalate, benzyl-n-butyl phthalate and bis(2-ethylhexyl) phthalate, are in the priority pollutants list of the United States Environmental Protection Agency (USEPA).

EXPERIMENTAL

For the preparation of the mobile phases, HPLC grade methanol (J.T. Baker Chemical Co.), HPLC grade acetonitrile (Ajax Chemicals) and AR grade isoproponal (Ajax Chemicals) were used. Diethyl phthalate, dibutyl phthalate (purity both > 98%), bis(2-ethylhexyl) phthalate (purity > 97%) and dimethyl phathlate (purity > 99%) were obtained from Fluka Chemika. Diallyl phthalate and benzyl n-butyl phthalate were the purest grade obtained from Tokyo Kasei Kogyo Co..

Chromatographic work was performed using a Perkin Elmer Model Series 4 gradient pump connected to a Micro UVIS 20 (Carlo Erba, Italy) UV spectrophotometric detector. The wavelength was set at 224 nm. The chromatographic data were collected and analyzed on a Hewlett Packard 3390A integrator.

All chromatographic runs were duplicated with reproducibility between runs of $\pm$ 2% or better. The void volume / time was obtained by using methanol as the unretained component for all mobile phases. A Shimadzu Shimpack CLC-ODS column (5-micrometer particle size, 6 mm i.d. x 150 mm) was used for the exmperiments. The standards were prepared by dissolving known amount of phthalates in HPLC grade methanol. The concentration of each phthalate was 160 to 260 ppm in the standard mixture as well as in the individual standard solutions. All the samples were filtered and degassed before injecting into the column.

The mobile phases were prepared according to the procedure recommended by Runser[6]. The preparation was based

on the A + B quantum sufficit addition method where the correct volume of organic modifier was first added and this was followed by water, the inert carrier, to the mark. All the solvents were filtered by passing through Millipore membrane filter and degassed by helium sparging. Two chambers were used for the gradient elution. The organic modifiers were pumped through one chamber and water was pumped through another chamber.

The first practical position to be established is an estimate of the required solvent strength. The total solvent strength, ST, can be calculated using Eq(1):

$$ST = S_1X_1 + S_2X_2 + \ldots\ldots\ldots \quad (1)$$

where S_i are the individual solvent strength of the organic modifier[7] and X_i are the volume fraction of each component. In the present work, the isocratic multisolvent gradient elution (IMGE) scheme[8] was employed. The IMGE scheme involves changing the ratio of the carrier solent (e.g. water in reversed-phase systems) to organic modifiers during the separation so that the solvent strength changes during the run, although the separation selectivity does not. Thus, the isocratic solvent selectivity triangle actually represents a constant solvent strength cross-section or slice of a solvent selectivity "prism" in which solvent strength is continuously increased.

Based on the results of the isocratic runs performed independently[5], it was observed that one of the compounds

(bis (2-ethylhexyl) phthalate was eluted significantly later than the other five compounds. Therefore during the gradient runs, a linear gradient was employed to separate the first five compounds. Subsequently, the mobile phase was maintained at the final composition for a further 15 min to elute the last compound.

By using Eq (1) and tabulated values of S_i for the individual solvents, seven experimental runs are designed according to the IMGE scheme. The mobile phase compositions of the seven gradient elution experiments are listed in Table I.

In choosing the three binary mixtures for the solvent selectivity triangle, solvents with the widest potential differences in their interactions should be chosen as these are expected to be most successful in producing a separation of high selectivity.

The three major mobile phase effects which contribute to the selectivity result from proton donor, proton acceptor and dipole-dipole interactions with the compounds to be separated. The relative selectivities of chromatographic solvents have been conveniently grouped into sets by Snyder[7]. To effect changes in selectivity, solvents from different selectivity groups should be chosen. Since methanol and acetonitrile are classifed as Group II and Group IV solvents respectively and are the more common reversed-phase HPLC solvents, MeOH-water and ACN-water were used as two corners of the solvent selectivity triangle.

TABLE I
MOBILE PHASE COMPOSITIONS FOR THE SEVEN EXPERIMENTS

Mobile Phase 1

	MeOH	ACN	IPA	H_2O	Time
Initial	70.0	0.0	0.0	30.0	
Final (1)	100.0	0.0	0.0	0.0	15min
Final (2)	100.0	0.0	0.0	0.0	15min

Mobile Phase 2

	MeOH	ACN	IPA	H_2O	Time
Initial	0.0	67.7	0.0	32.3	
Final (1)	0.0	96.8	0.0	3.2	15min
Final (2)	0.0	96.8	0.0	3.2	15min

Mobile Phase 3

	MeOH	ACN	IPA	H_2O	Time
Initial	0.0	0.0	54.8	45.2	
Final (1)	0.0	0.0	76.2	23.8	15min
Final (2)	0.0	0.0	76.2	23.8	15min

Mobile Phase 4

	MeOH	ACN	IPA	H_2O	Time
Initial	35.0	33.9	0.0	31.1	
Final (1)	50.0	48.4	0.0	1.6	15min
Final (2)	50.0	48.4	0.0	1.6	15min

Mobile Phase 5

	MeOH	ACN	IPA	H_2O	Time
Initial	35.0	0.0	27.4	37.6	
Final (1)	50.0	0.0	38.1	11.9	15min
Final (2)	50.0	0.0	38.1	11.9	15min

Mobile Phase 6

	MeOH	ACN	IPA	H_2O	Time
Initial	0.0	33.9	27.4	38.7	
Final (1)	0.0	48.4	38.1	13.5	15min
Final (2)	0.0	48.4	38.1	13.5	15min

Mobile Phase 7

	MeOH	ACN	IPA	H_2O	Time
Initial	23.3	22.6	18.3	35.8	
Final (1)	33.3	32.3	25.4	9.0	15min
Final (2)	33.3	32.3	25.4	9.0	15min

Theoretically, the solvent used for the third corner of the triangle should come from a different selectivity group (i.e. Group III). Group III solvents include methoxyethanol, dimethylformamide (DMF) and tetrahydrofuran. Methoxyethanol was not chosen because of its toxicity,[10,11]; neither was dimethylformamide because it is an irritant [11]. Tetrahydrofuran was ruled out because it contains an

antioxidant which might cause interference in the chromatograms; removal of the antioxidant by distillation may be dangerous as peroxides may be formed which may be explosive. Thus although isopropanol is in the same selectivity group as methanol, IPA-water was chosen as the third corner of the triangle instead of methoxyethanol, dimethylformamide or tetrahydrofuran because IPA is a common HPLC solvent with good solvating power (solvent strength = 4.2) and also because of the disadvantages of the solvents from the solvent group III.

The computer software for the ORM method used to determine the optimum gradient elution solvent system is the same as that used in optimization of isocratic solvents[5]. This is because the routine in this program does not distinguish between retention times obtained on an isocratic or gradient elution basis. Consequently, selection of an optimum IMGE solvent for the best compromise resolution of all components in a mixture is carried out in the same manner for gradient elution as for an isocratic separation. However, in contrast to isocratic separations where peak widths increase with retention time, peaks from a linear solvent gradient elution run have approximately the same witdh (standard deviation) throughout the run. Therefore, in gradient elution, true plate count cannot be measured for a calculation of resolution of peak pairs in the mixtures. In this paper, apparent resolution based on the retention time differences of peaks is used as a measure of separation quality in gradient runs.

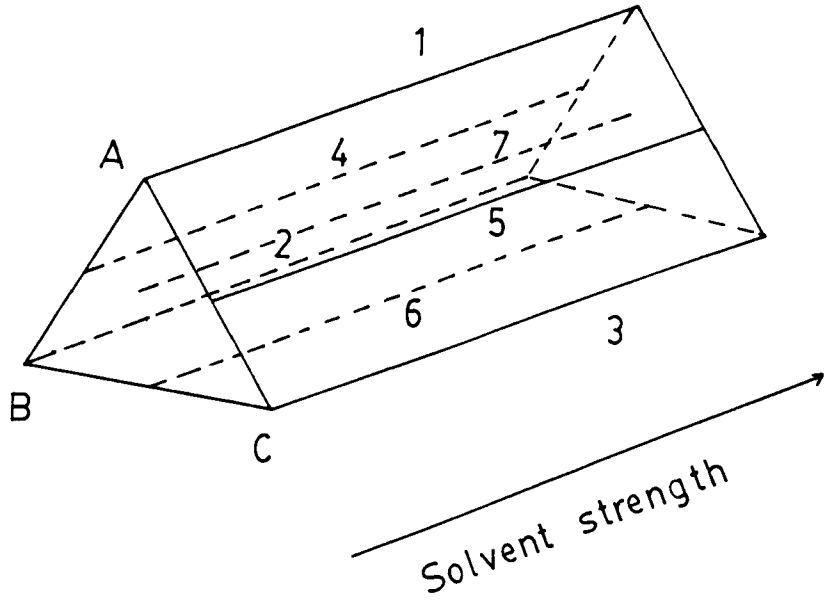

FIGURE 1 Experimental design for seven gradient elution runs to obtain basic data for optimization calculation. Solvent compositions given in Table I.

RESULTS AND DISCUSSION

The experimental approach used to determine the optimum mobile phase composition is very similar to that previously used for determining the optimum mobile phase composition in an isocratic system[5]. Seven experiments were conducted according to the design indicated in Figure 1.

Data from gradient elution chromatograms with these seven mobile phase systems are used to estimate the coefficients of equation (2) that describe the surface response plots for each of the peak pairs in the mixture within the solvent selectivity prism.

$$R = a_1x_1+a_2x_2+a_3x_3+a_{12}x_1x_2+a_{13}x_1x_3+a_{23}x_2x_3+a_{123}x_1x_2x_3 \quad (2)$$

The names of the phthalates are abbreviated as follows :

1) DMP = Dimethyl phthalate

2) DEP = Diethyl phthalate

3) DBP = Dibutyl phthalate

4) DAP = Diallyl phthalate

5) BBP = Benzyl n-butyl phthalate

6) DOP = Bis(2-ethylhexyl) phthalate

The chromatographic data obtained for the standard mixture of six phthalates using the seven eluent mixtures listed in Table I are shown in Table II. The apparent resolutions based on the retention time differences of peaks are tabulated in Table III.

TABLE II
RETENTION TIMES (MIN) OF THE SIX PHTHALATES IN EACH OF THE SEVEN ELUENT MIXTURES LISTED IN TABLE II

Compounds	DMP	DEP	DAP	BBP	DBP	DOP
Mobile Phase						
1	4.165	6.125	7.840	13.645	14.025	22.095
2	4.360	5.960	6.990	11.930	12.970	27.600
3	5.550	6.550	7.540	11.950	12.130	26.030
4	4.330	6.410	7.820	13.330	14.010	23.940
5	3.380	4.390	5.450	10.880	11.050	23.870
6	3.570	4.600	5.300	9.660	10.770	24.840
7	3.760	5.080	6.010	11.230	11.960	24.330

TABLE III
APPARENT RESOLUTION BASED ON RETENTION TIME DIFFERENCES OF PEAKS FOR THE SIX PHTHALATES IN THE MIXTURE

Peak Pairs	1,2	2,3	3,4	4,5	5,6
Mobile Phase					
1	1.960	1.715	5.805	0.380	8.070
2	1.600	1.030	4.940	1.040	14.630
3	1.000	0.990	4.410	0.180	13.900
4	2.080	1.410	5.510	0.680	9.930
5	1.010	1.060	5.430	0.170	12.820
6	1.030	0.700	4.360	1.110	14.070
7	1.320	0.930	5.220	0.730	12.370

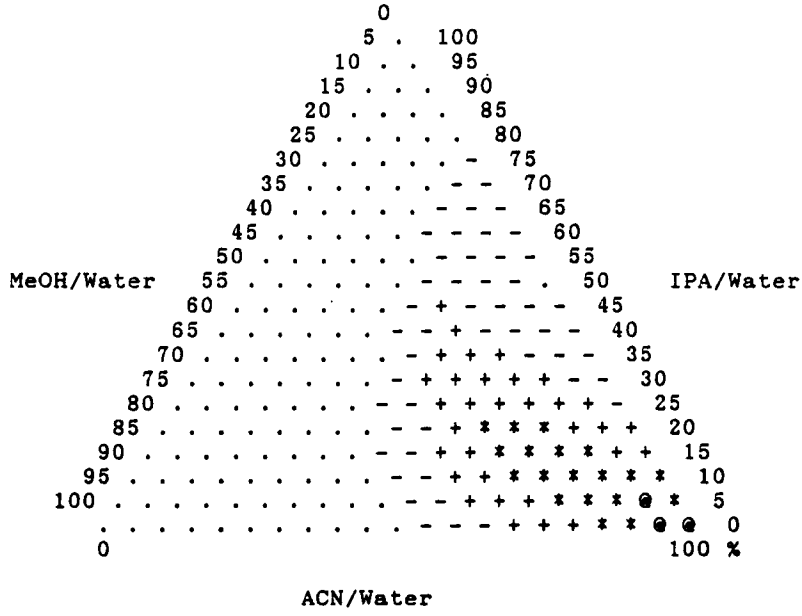

FIGURE 2 Overlapping Venn diagram for the five pair of peaks. Symbols representing range of resolution: (1) . : R < 0.7; (2) - : 0.7 <= R < 0.8; (3) + : 0.8 <= R < 0.9; (4) * : 0.9 <= R < 1.0; (5) @ : R >= 1.0

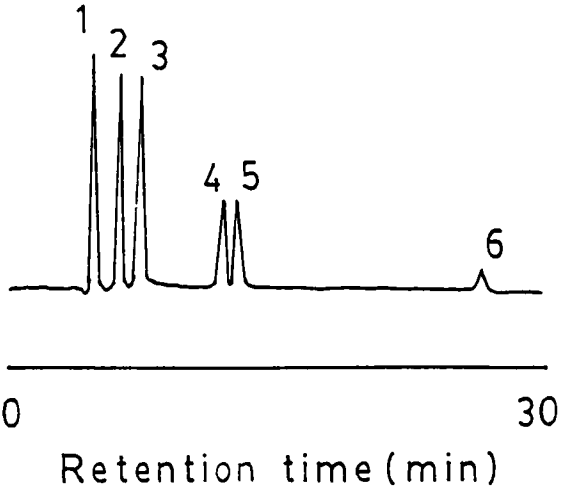

FIGURE 3 Optimum chromatogram of standard mixture of six phthalates using gradient mobile phase, methanol : acetonitrile : isopropanol : water (0.0 : 67.7 : 0.0 : 32.3 --(15 min)--> 0.0 : 96.8 : 0.0 : 3.2 --(15 min)--> 0.0 : 96.8 : 0.0 : 3.2). Peak numbers: 1 = DMP, 2 = DEP, 3 = DAP, 4 = BBP, 5 = DBP and 6 = DOP.

TABLE IV
OPTIMUM IMGE MOBILE PHASE COMPOSITION

	MeOH	ACN	IPA	H_2O	Time
Initial	0.0	67.7	0.0	32.3	
Final (1)	0.0	96.8	0.0	3.2	15min
Final (2)	0.0	96.8	0.0	3.2	15min

A minimum desired apparent resolution of 1.0 was specified. Venn diagram resolution plots for each peak pair and the overlapped resolution diagram were obtained using Eq (2) and the computer program. The five individual Venn diagrams for the five pair of peaks were then superimposed. The overlapped resolution diagram is shown in Figure 2. The chromatogram for one of the optimum mobile phase systems is shown in Figure 3. The optimum mobile phase used is tabulated in Table IV. Satisfactory separation was obtained for the six phthalates.

In conclusion, the overlapping mapping procedure was found to be a rapid and systematic approach for optimization of HPLC separations. In combination with the IMGE scheme, the ORM procedure can be extended to the optimization of gradient HPLC analysis. The application of the procedure to the separation of selected phthalates was successfully demonstrated.

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