Journal of Chromatography, 464 (1989) 405–410
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 133

Note

Optimization of mobile phase composition for high-performance liquid chromatographic analysis of eleven priority substituted phenols

C. P. ONG, H. K. LEE and S. F. Y. LI*

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511 (Singapore)

(First received September 20th 1988; revised manuscript received November 21st, 1988)

Substituted phenols are of great environmental concern particularly as water pollutants[1]. Eleven phenols, listed by the United States Environmental Protection Agency (U.S.E.P.A.) as priority pollutants[2], have been subjected to many previous investigations[3–10]. One of the more widely used techniques is high-performance liquid chromatography (HPLC) of which both reversed-phase isocratic and gradient elution analyses were reported. In this paper, the isocratic HPLC separation of these phenols is investigated. The separation of the eleven compounds is optimized by the use of the overlapping resolution mapping (ORM) scheme proposed by Glajch *et al.*[11]. The aim of the optimization scheme is to predict the best mobile phase composition consisting of mixtures of water with various proportions of three common organic modifiers, methanol, acetonitrile and tetrahydrofuran[12].

EXPERIMENTAL

Analyses were carried out using a Shimadzu LC-6A isocratic instrument, equipped with a Model SPD-6A variable-wavelength UV spectrophotometric detector set at 280 nm throughout this work. A reversed-phase Whatman Partisil-5 ODS-3 column (particle size 5-μm, 100 mm × 4.6 mm I.D.) was used. The chromatographic data were collected and analyzed on a Chromatopac C-R3A data processor. A flow-rate of 1 ml/min was used. The void volume, V_0, was determined using methanol as the unretained component.

The phenols mixture was supplied by Alltech Associates whereas the individual compounds used to identify peaks in the mixture chromatograms were obtained from Aldrich. These chemicals were the purest available. The solvents used were of HPLC grade supplied by J. T. Baker and the mobile phases were prepared according to the A + B (*quantum sufficit*) addition method[13]. All solvents were filtered through a Millipore membrane filter and then thoroughly degassed in an ultrasonic bath. The phenols were dissolved in the mobile phases, and filtered before injection with a Rheodyne 7125 injector. For each injection, 1.5 μl of the solution were used. This injection volume corresponds to 7.5 ng of each phenol.

0021-9673/89/$03.50

RESULTS AND DISCUSSION

The first step of the optimization scheme was to define the three vertices of the solvent selectivity triangle[14–16] which correspond to the compositions of three binary (organic modifier + water) solvents. The first vertex was established using a binary mixture of methanol–water as the mobile phase[14]. A total analysis time of 10 min was selected as a time constraint. Two preliminary experiments using different mixtures of methanol and water were performed. The results of these are given in Table I. The mobile phase composition of methanol–water (58.5:41.5, v/v) which achieved elution of all the peaks within the time constraint was selected as the first vertex[14]. The solvent strength of this mixture was then calculated using eqn. 1[14]

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

where ST represents the total solvent strength of the mixture, S_a, S_b are the individual solvent strengths and φ_a, φ_b are the volume fractions of components *a* and *b* respectively. Based on the total solvent strength found, the compositions of the other two binary mixtures which have equal solvent strength were then calculated using eqn. 1. The three binary mixtures were used as the vertices of the solvent selectivity triangle[14] and were denoted as A = methanol–water (58.5:41.5, v/v), B = acetonitrile–water (56.0:44.0, v/v) and C = tetrahydrofuran–water (40.0:60.0, v/v) respectively. Subsequently, a simplex design approach[11] was employed to select the mobile phases to be used in a set of seven HPLC experiments. The seven mobile phases are listed in Table II. Since the solvent compositions given in this table are based on the binary mixtures A, B and C, the corresponding compositions based on the individual solvents can be calculated easily from these values. The latter compositions are listed in Table III and the retention times of the phenols obtained when using them are listed in Table IV.

From the experiments using these seven different mobile phases, the resolutions, *R*, between every pair of peaks in the chromatograms obtained for each solvent composition were calculated using eqn. 2[17]

$$R = \frac{1}{4}(\alpha - 1)N^{1/2} \cdot \frac{K'}{(1 + K')} \quad (2)$$

TABLE I

RESULTS OF PRELIMINARY EXPERIMENTS USING BINARY MIXTURES OF METHANOL–WATER

Solvent composition (%, v/v)		*Capacity factor for last component eluted*	*Retention time (min) for last component eluted*
Methanol	*Water*		
50.0	50.0	23.65	12.96
58.5	41.5	19.68	10.78

TABLE II

SOLVENT COMPOSITION AS PERCENTAGE OF BINARY MIXTURES IN THE MOBILE PHASE

A = Methanol–water; B = acetonitrile–water; C = tetrahydrofuran–water.

Eluent mixture	*A*	*B*	*C*
1	100	0	0
2	0	100	0
3	0	0	100
4	50	50	0
5	0	50	50
6	50	0	50
7	33.3	33.3	33.3

TABLE III

SOLVENT COMPOSITIONS AS PERCENTAGE OF PURE SOLVENTS IN THE MOBILE PHASE

Eluent mixture	*Methanol*	*Acetonitrile*	*THF*	*Water*
1	58.50	0.00	0.00	41.50
2	0.00	56.00	0.00	44.00
3	0.00	0.00	40.00	60.00
4	29.25	28.00	0.00	42.75
5	0.00	28.00	20.00	52.00
6	29.25	0.00	20.00	50.75
7	19.50	18.65	13.30	48.55

TABLE IV

RETENTION TIMES (IN MIN) OF PHENOLS IN THE SEVEN ELUENT MIXTURES LISTED IN TABLE III

Compounds: 1 = 2,4-dinitrophenol; 2 = 2-methyl-4,6-dinitrophenol; 3 = phenol; 4 = *p*-nitrophenol; 5 = *o*-chlorophenol; 6 = *o*-nitrophenol; 7 = 2,4-dimethylpenol; 8 = 4-chloro-3-methylphenol; 9 = 2,4-dichlorophenol; 10 = pentachlorophenol; 11 = 2,4,6-trichlorophenol.

Compound	*Mobile phase*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
1	1.775	1.605	2.615	1.972	1.728	2.038	1.707
2	2.000	1.628	2.632	2.400	1.777	2.138	1.745
3	5.050	4.538	11.182	4.620	5.700	7.558	5.392
4	5.950	4.643	13.497	5.355	4.903	10.365	6.308
5	7.667	6.033	16.580	6.483	8.122	12.697	8.158
6	8.295	6.965	15.427	7.233	6.212	10.667	7.600
7	11.950	7.640	20.402	8.825	10.190	17.280	10.575
8	15.707	8.225	23.297	10.398	2.040	24.767	14.053
9	19.508	9.880	31.723	12.892	15.517	34.763	18.688
10[a]	15.658	3.192	4.442	15.398	9.295	7.528	4.892
11	28.937	8.073	24.142	21.033	8.217	31.383	19.142

[a] Standard phenol mixture was spiked with this component to improve detection.

where R is the resolution for a pair of adjacent peaks, α is the relative retention ratio, N is the number of theoretical plates and K' is the capacity factor for one of the peaks.

The calculated resolutions were then fitted by a second order polynomial equation

$$R = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \quad (3)$$

where a_i are coefficients and x_i are volume fractions of the binary mixtures A, B and C.

A minimum resolution of unity between each pair of peaks is specified. With the aid of a BASIC program[14] and the use of the minimum resolution criterion, a Venn diagram[11] was generated for each pair of peaks. Subsequently, by overlapping all the Venn diagrams, areas which satisfy the desired resolution for all the peaks were determined. The overlapping resolution diagram for the eleven phenols is illustrated in Fig. 1. The region that is shown with # represents the mobile phase compositions that give the best separation.

To confirm the success of the optimization procedure, a mobile phase composition from this region corresponding to 55.0% of A and 45% of B was chosen for a further experiment. Fig. 2 illustrates the chromatogram obtained using this mobile phase, *i.e.*, methanol–acetonitrile–tetrahydrofuran–water (32.2:25.2:0:42.6, v/v). The order of elution and retention times of the eleven phenols are listed in Table V. The chromatogram shows that all the eleven phenols are satisfactorily separated. Notably, the analysis time of 9 min is much shorter than the sequential procedure proposed by Buckman *et al.*[4], which involves actual times of 25 min for the first eluent mixture and 17 min for the second eluent mixture. The present method is also a significant improvement over the previous best isocratic separation of these

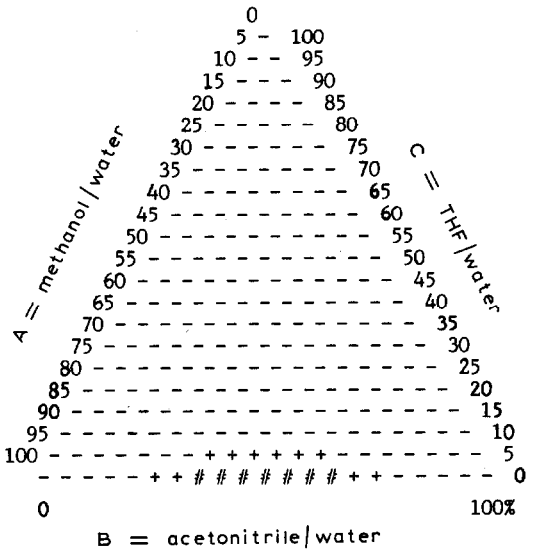

Fig. 1. Overlapping Venn diagram for the ten pairs of peaks: – –, $R < 0.5$; + +, $0.5 < R < 1.0$; # #, $R \geqslant 1.0$.

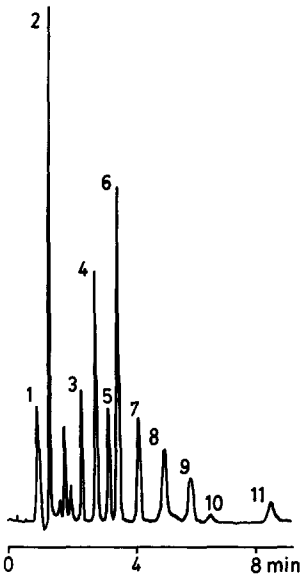

Fig. 2. Chromatogram of a mixture of eleven substituted phenols. Eluent: methanol–acetonitrile–THF–water (32.2:25.2:0:42.6, v/v). Peak numbers refer to Table IV. Chromatographic conditions as described in the text.

compounds obtained by Lee *et al.*[7] which requires an analysis time of 25 min, and is therefore suitable for adaptation to routine analysis of the phenols in environmental samples. Furthermore, the present work is the first application of a systematic approach to the optimization of mobile phase composition for the separation of these phenols. The method can readily be extended to the analysis of other compounds, which necessitates the use of ternary or more complicated eluent mixtures.

The results obtained in this work have successfully demonstrated the application

TABLE V

THE ELEVEN PRIORITY PHENOLS AND THEIR RETENTION TIMES FOR THE ELUENT MIXTURE METHANOL–ACETONITRILE–THF–WATER (32.2:25.2:0:42.6, v/v)

Peak No.	*Phenol*	*Retention time (min)*
1	2,4-Dinitrophenol	0.935
2	2-Methyl-4,6-dinitrophenol	1.313
3	Phenol	2.404
4	*p*-Nitrophenol	2.867
5	*o*-Chlorophenol	3.280
6	*o*-Nitrophenol	3.578
7	2,4-Dinitrophenol	4.258
8	4-Chloro-3-methylphenol	5.073
9	2,4-Dichlorophenol	5.948
10	Pentachlorophenol	6.595
11	2,4,6-Trichlorophenol	8.567

of a systematic approach to the optimization of mobile phase composition for HPLC. Optimization of the separation of the eleven phenols using an ORM scheme was achieved quite easily even though a quaternary mobile phase was considered.

REFERENCES

1 S. Hussain, *J. Chromatogr.*, 18 (1965) 419.
2 R. A. Hites, *Adv. Chromatogr. (N.Y.)*, 15 (1977) 69.
3 A. W. Wolkoff and R. H. Larose, *J. Chromatogr.*, 99 (1974) 731.
4 N. G. Buckman, J. O. Hill, R. J. Magee and M. J. McCormick, *J. Chromatogr.*, 284 (1984) 441.
5 F. P. Bigley and R. L. Grob, *J. Chromatogr.*, 350 (1985) 407.
6 S. Hussain and M. Kitayatulla, *J. Chromatogr.*, 168 (1979) 517.
7 H. K. Lee, S. F. Y. Li and Y. H. Tay, *J. Chromatogr.*, 438 (1988) 429.
8 S. F. Y. Li and H. K. Lee, *Chromatographia*, 25 (1988) 515.
9 K. Abrahamsson and T. M. Xie, *J. Chromatogr.*, 279 (1983) 199.
10 J. Folke and U. Lund, *J. Chromatogr.*, 279 (1983) 189.
11 J. L. Glach, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
12 E. Roggendorf and R. Spatz, *J. Chromatogr.*, 204 (1981) 263.
13 D. J. Runser, *Maintaining and Troubleshooting HPLC Systems, User's Guide*, Wiley, New York, 1981, p. 18.
14 J. C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley, Chichester, 1985, p. 70.
15 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
16 L. R. Snyder, *J. Chromatogr. Sci.*, 19 (1983) 223.
17 J. H. Purnell, *J. Chem, Soc.*, (1960) 1268.