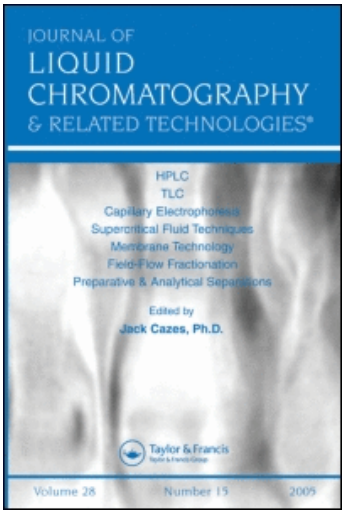


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Optimization of Mobile Phase Composition for HPLC Separations of Nitroaromatics Using Overlapping Resolution Mapping

Y. J. Yao[a]; H. K. Lee[a]; S. F. Y. Li[a]
[a] Department of Chemistry National, University of Singapore Kent Ridge, Republic of Singapore

To cite this Article Yao, Y. J. , Lee, H. K. and Li, S. F. Y.(1993) 'Optimization of Mobile Phase Composition for HPLC Separations of Nitroaromatics Using Overlapping Resolution Mapping', Journal of Liquid Chromatography & Related Technologies, 16: 11, 2223 — 2235
To link to this Article: DOI: 10.1080/10826079308020981
URL: http://dx.doi.org/10.1080/10826079308020981

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(11), 2223-2235 (1993)

OPTIMIZATION OF MOBILE PHASE COMPOSITION FOR HPLC SEPARATIONS OF NITROAROMATICS USING OVERLAPPING RESOLUTION MAPPING

Y. J. YAO, H. K. LEE, AND S. F. Y. LI*
Department of Chemistry
National University of Singapore
Kent Ridge, Republic of Singapore 0511

ABSTRACT

The use of the overlapping resolution mapping (ORM) procedure for the optimization of the HPLC separations of eight nitroaromatics employing reversed-phase isocratic elution is described. The ORM technique was used to establish the optimum mobile phase compositions for a given column. Further improvements in resolution were thereafter obtained on longer columns with similar packing materials, without any further optimization.

INTRODUCTION

The use of systematic strategies and computer-aided optimization procedures for separations has found widespread acceptance by HPLC practitioners in recent years. Indeed, there have been entire volumes devoted to the subject [1-3]. Some optimization procedures employed

for the selection of the optimal mobile phase compositions in HPLC includes sequential simplex method [4], "PRISMA" model method [5], window diagrams [6], overlapping resolution mapping (ORM) method [7], and optimization based on the function of mutual information (FUMI) [8]. These optimization schemes have generally proved to be efficient and quite often have provided satisfactory separations.

In this work, the ORM approach is empolyed. The ORM optimization approach has been applied to many forms and types of liquid chromatography. For instance, for reversed-phase HPLC, eleven substituted phenols were successfully separated using this scheme [9,10]. In normal-bonded phase, the separation of a steroid mixture using this method has been demonstrated [11]. The ORM method is not restricted to isocratic separations. Its application to gradient separations has also been described [12,13]. Besides optimization of the compositions of organic modifiers in the mobile phase, ORM has also been used to optimize other variables such as stationary phases [14] and the pH of buffer solutions [15].

Nitroaromatic compounds are commonly found in industrial waste effluent. They are toxic, even at low concentration levels. Dinitrotoluene isomers are currently an environmental problem, with 2,4-dinitrotoluene listed as a priority pollutant by the United States Environmental Protection Agency (U.S.E.P.A). Although HPLC separation of nitroaromatic compounds has been investigated [16,17], optimum experimental conditions were obtained by trial-and-error type of approaches. The application of systematic optimization schemes for these compounds has not been reported. In view of the potential environmental concern of these compounds, it is expected that there will be a growing

interest in methods which can be used to determine optimum conditions for their separation. In the present work, the use of the ORM scheme for the optimization of the separation of eight nitroaromatics is demonstrated.

EXPERIMENTAL

Equipment

The Shimadzu (Kyoto, Japan) liquid chromatograph system employed in this study consisted of a LC-9A pump, a SPD-6A variable-wavelength UV spectrophotometric detector set at 254 nm, and a C-R6A Chromatopac integrator for data collection and integration. Injections were made using a Rheodyne 7125 injector with a 20μl sample loop (Alltech Assoc., Carnforth, U.K.). The columns used included (1) a 10cm x 4.6mm i.d. Whatman Partisil-5 ODS-3 (New Jersey, U.S.A.) column; (2) a 11cm x 4.7mm i.d. Whatman Partisphere-5 C_{18} cartridge; (3) a 25cm x 4.6mm i.d. Whatman Partisil-5 ODS-3 column; and (4) a 15cm x 6.0mm i.d. Shimpack CLC-ODS column (Kyoto, Japan). The particle size of the packing material used in all the columns is 5 μm. A flow rate of 0.8 ml/min was used throughout, unless otherwise specified. The experiments were performed at ambient temperature.

Reagents and Materials

HPLC-grade acetonitrile and isopropanol were obtained from J.T.Baker (Phillipsburg, N.J., U.S.A.) and HPLC-grade methanol from Carlo Erba (Milan, Italy). The water used was purified on a Milli-Q system (Millipore,

TABLE 1

Nitroaromatics Investigated and Their Codes

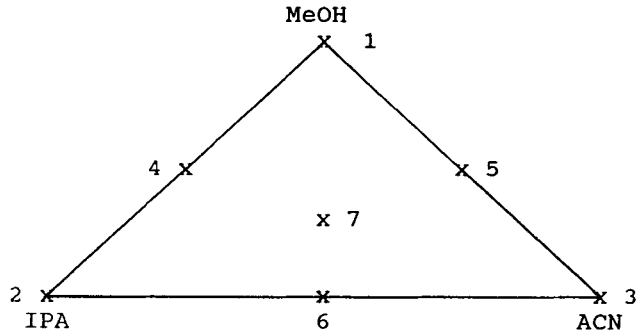
COMPOUNDS	CODES
3,4-dinitrotoluene	A
1-chloro-2,4-dinitrobenzene	B
2,4-dinitrotoluene	C
4-nitrotoluene	D
1-chloro-4-nitrobenzene	E
1-chloro-3-nitrobenzene	F
4-chloro-2-nitrotoluene	G
2-chloro-4-nitrotoluene	H

U.S.A.). Mobile phases mixtures were prepared according to the A + B addition method [18], in which exact volumes of all the solvents were measured and mixed.

The nitroaromatics were obtained from Fluka Chemika (Buchs, Switzerland). Standard solutions were prepared in methanol with solute concentrations of 100 ppm. Table 1 lists the eight compounds used in the present study.

RESULTS AND DISCUSSION

The first step of the optimization procedure was to chose a mobile phase system. Solvents that exhibit wide differences in chemical interactions with the compounds of interest are preferred to effect selectivity changes for better separations. In reversed-phase liquid chromatography, methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran (THF) are the usual solvents. As commercially available THF contains an antioxidant which might cause interference during detection, it was replaced by isopropanol (IPA) which has comparable



experiments	mobile phase composition %MeOH	%ACN	%IPA	%water
1	50.0	——	——	50.0
2	——	——	35.7	64.3
3	——	48.4	——	51.6
4	25.0	——	17.9	57.1
5	25.0	24.2	——	50.8
6	——	24.2	17.9	57.9
7	16.7	16.1	11.9	55.3

FIGURE 1
Experimental design and conditions for the seven preliminary runs.

solvent strength (4.3) to that of THF (4.0) [19]. Although MeOH and IPA belong to the same group under the solvent classification proposed by Snyder [20], they have been shown to possess sufficient selectivity differences for satisfactory separations to be attained [9,13].

Subsequently, seven preliminary runs designed as shown in Fig. 1 were used to determine the coefficients, a_i's, in equation (1) that describes the response

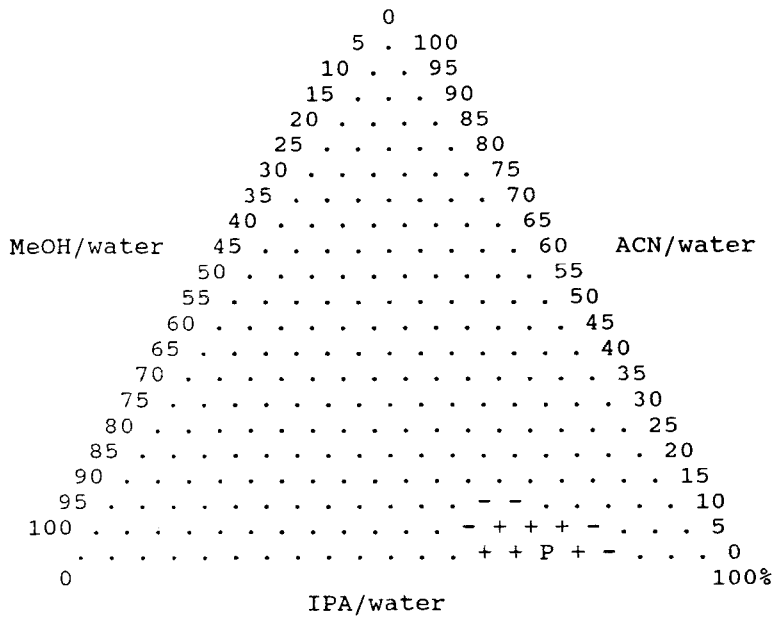


FIGURE 2
Overlapped resolution map for the seven peak pairs:
.. R > 0.1, -- 0.1 ≤ R < 0.15, ++ 0.15 ≤ R < 0.2.

surface generated by the resolution data,

$$R = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \quad (1)$$

where R is the resolution between adjacent peaks, and x_i's are volume fractions for each organic modifier. Resolutions were calculated using the equation

$$R = \frac{2\Delta t}{w_1 + w_2}$$

where Δt is the difference in retention times between two peaks and w_1, w_2 are peak widths at the baseline.

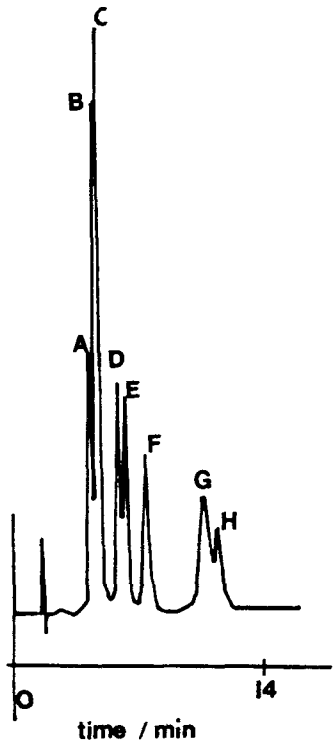


FIGURE 3
Chromatogram for isocratic separation of the eight nitroaromatics using the mobile phase MeOH/IPA/water (12.5 : 26.78 : 60.72); flow rate: 0.8 ml/min; detection at 254 nm; performed on a Whatman Partisil 10cm column. Compounds as listed in Table 1.

Resolution maps for every adjacent peak pair are generated with the aid of a modified BASIC program given by Berridge [1]. Subsequently, by superimposing all the resolution maps, an overlapped resolution map is obtained, as shown in Fig. 2.

In Fig. 2, the region denoted by "+" represents a minimum resolution of 0.15 between adjacent peaks. A

TABLE 2

Experimentally Measured and Predicted Resolutions for Adjacent Peaks at Optimum Conditions on Column 1.

peak pairs	Resolution measured	predicted*
A-B	0.66	0.42
B-C	0.08	0.15
C-D	1.50	1.49
D-E	0.52	0.42
E-F	1.24	1.30
F-G	2.15	2.45
G-H	0.52	0.48

* Predicted values were based on Equation (1)

point P was chosen from the region which would be expected to provide the highest resolution. Point P corresponded to a mobile phase consisting of 12.5% MeOH, 26.78% IPA and 60.72% water. The chromatogram obtained with this mobile phase is shown in Fig. 3. Resolutions of more than 0.5 could be achieved for all peak pairs except for the pair 2,4-dinitrotoluene and 1-chloro-2,4-dinitrobenzene, which are barely separated. The measured and predicted resolutions are listed in Table 2. In general, it is expected that baseline separation between a pair of peaks would be obtained if $R > 1.5$ [1]. However, the results of the optimization procedure showed that for the nitroaromatic compounds, baseline separation could not be obtained using column (1) and the mobile phase system consisting of MeOH, IPA and water. The failure in separating all the peaks could be partly attributed to the inadequacy of the solvent system to provide selectivity. Ideally, when a combination of three organic modifiers and a base solvent (water in the case for reversed-phase

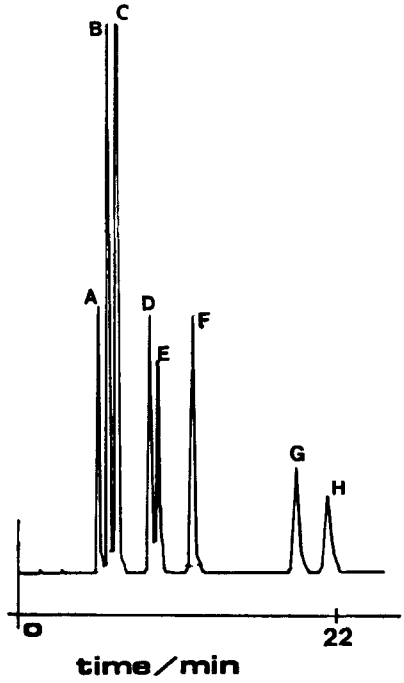


FIGURE 4
Chromatogram obtained on a Whatman Partisphere 11cm cartridge. Other conditions same as in Fig. 3.

separation) is used, the region of optimum mobile phase mixture should be found near the centre of the triangle. In the present case, points denoting better resolutions are localised near the base of the triangle, corresponding to mobile phase compositions having very low or zero percentage of ACN. The bias observed indicates that ACN is a poor choice for this separation. One of the approaches to further improve resolution is to use a different mobile phase system. However, this approach would require further optimization to be performed.

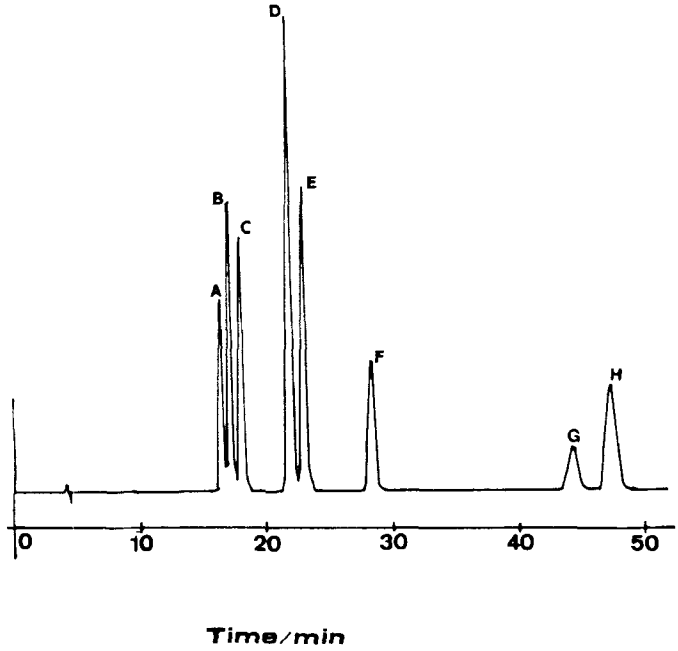


FIGURE 5
Chromatogram obtained on a Whatman Partisil 25cm column. Flow rate 0.75 ml/min. Other conditions same as in Fig. 3

Alternatively, resolution can be improved by using columns which provide higher efficiencies. The selectivity would not be expected to vary significantly for columns with similar packing materials. Thus instead of repeating the optimization procedure with different solvent systems, it was decided to test the optimal mobile phase composition established on other columns. Two longer columns from the same supplier (Whatman Partisphere, 11cm and Whatman Partisil, 25cm) and a column from a different supplier (Shimpack, 15cm) were investigated. The chromatograms obtained are shown in Fig. 4 to 6.

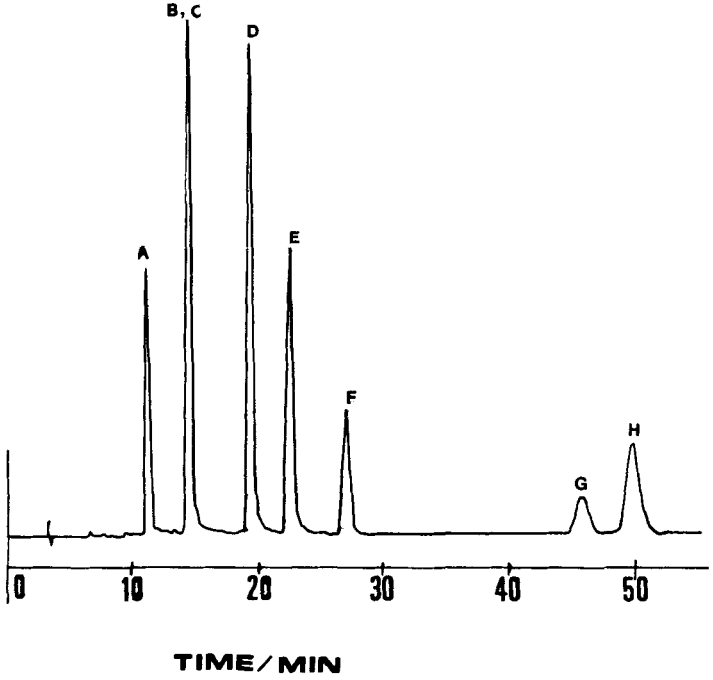


FIGURE 6
Chromatogram obtained on a Shimpack 15cm column. Other conditions same as in Fig. 3.

With the Whatman 11cm and 25cm columns, all peaks were satisfactorily resolved. Although these improved resolutions were obtained at the expense of longer analysis times, the elution order remained the same. In the case of the Whatman 25cm column, a lower flow rate (0.75 ml/min) was used to prevent excessively high pressure. Separations performed on a Shimpack column showed a slightly different solute selectivity. Better separation was observed between 3,4-dinitrotoluene and 1-chloro-2,4-dinitrobenzene, and between 1-chloro-4-nitrobenzene and 4-nitrotoluene. However, 2,4-dinitrotoluene and 1-chloro-2,4-dinitrobenzene coeluted. The

difference in selectivity from that of columns 1 to 3 is probably the consequence of variations in the packing materials used by the different manufacturers.

These results showed that satisfactory separations of the eight nitroaromatics could be obtained based on results of the ORM procedure. Although completely resolved peaks were not attainable on the original column, improvement in resolution could be obtained without having to go through the optimizing scheme again. This was achieved through the application of the established optimal condition on longer columns of similar packing material, which provided higher efficiencies.

ACKNOWLEDGEMENTS

The authors thank the National University of Singapore for financial support.

REFERENCES

1. J. C. Berridge, Techniques for the Automated Optimization of HPLC Separations, Wiley, Chichester,1985.

2. P. J. Schoenmakers, Optimization of chromatographic selectivity, Elsevier, Amsterdam, 1986.

3. S. Nyiredy ed, Special Issue on Optimization of Mobile Phase, J. Liq. Chromatogr., 14: (1991)

4. J. C. Berridge, Analyst (London), 109: 291 (1984)

5. Sz. Nyiredy, B. Meier, C. A. J. Erdelmeier, O. Sticher, J. High Resolut. Chromatogr., 8: 186 (1985)

6. H. J. Issaq, G. M. Muschik, G. M. Janini, J. Liq. Chromatogr., 6: 259 (1983)

7. J. L. Glajch, J. J. Kirkland, K. M. Squire, J. M. Minor, J. Chromatogr., 199: 51 (1980)

8. Y. Hayashi, R. Matsuda, Anal. Lett., 23: 1765 (1990)

9. C. P. Ong, H. K. Lee, S. F. Y. Li, J. Chromatogr., 464: 405 (1989)

10. O. Busto, J. C. Olucha, F. Borrull, Chromatographia 32: 566 (1991)

11. P. E. Antle, Chromatographia, 15: 277 (1982)

12. J. J. Kirkland, J. L. Glajch, J. Chromatogr., 255: 27 (1983)

13. Y. J. Yao, M. R. Khan, C. P. Ong, H. K. Lee, S. F. Y. Li, Environ. Monitor. & Assess., 19: 83 (1991)

14. J. L. Glajch, J. C. Gluckman, J. G. Charikofsky, J. M. Minor, J. J. Kirkland, J. Chromatogr., 318: 23 (1985)

15. A. P. Goldberg, E. L. Nowakowska, P. E. Antle, L. R. Snyder, J. Chromatogr., 316: 241 (1984)

16. T. Nielson, Anal. Chem., 55: 286 (1983)

17. Z. Jin, S. M. Rappaport, Anal. Chem., 55: 1778 (1983)

18. D. J. Runser, Maintaining and Trouble shooting HPLC Systems, User's Guide, Wiley, New York, 1981, Chap 1

19. L. R. Snyder, J. W. Dolan, J. R. Grant, J. Chromatogr., 165: 3 (1979)

20. L. R. Snyder, J. Chromatogr. Sci., 16: 223 (1978)

Received: October 20, 1992
Accepted: November 5, 1992