J.Serb.Chem.Soc. 67(11)745–751(2002)
JSCS – 2997

UDC 66.012+543.344.5:547.56
Original scientific paper

Factorial design in isocratic high-performance liquid chromatography of phenolic compounds

ANTONIJE ONJIA[1#*], TATJANA VASILJEVIĆ[2], DJURO ČOKEŠA[1#] and MILA LAUŠEVIĆ[2#]

[1]*The Vinča Institute of Nuclear Sciences, P. O. Box 522, YU-11001 Belgrade and* [2]*Faculty of Technology and Metallurgy, P. O. Box 494, YU-11001 Beograde, Yugoslavia*

Abstract: A multifactor optimization strategy was utilized to predict the isocratic HPLC separation of nine phenols. The retention behavior was studied as a function of changing eluent (methanol – acetic acid) composition. The predicted and measured retentions are in rather good agreement. To locate the optimum in the factor space, the normalized resolution product criterion was applied. In virtually every case, the resolution is limited by the separation of the 2-chlorophenol and 2,4-dinitrophenol pair.

Keywords: factorial design, phenols, HPLC, resolution product, isocratic.

INTRODUCTION

High-performance liquid chromatography (HPLC) is a widespread analytical technique used predominantly for the separation of low- and non-volatile organic compounds. The capability of detecting down to ppb and sub-ppb levels, in addition to a simple sample preparation, makes HPLC as an important technique for trace pollutant analysis. Eleven phenolic compounds are classified by the U.S.EPA as priority pollutants that require monitoring in the environment. HPLC has already proved itself as a suitable technique for the analysis of phenols.[1–4]

Separation of phenols can be performed either by isocratic[2–7] or gradient elution.[1,3] The advantage of gradient elution is its ability to separate both weakly and strongly retained phenols in the same run. There are, however, several problems associated with changing the eluent during the run. The most severe problem is the baseline drift.[8] With isocratic elution a compromise must be made between resolution of the weakly retained phenols and the total length of the analysis. The rather complex task of performing a quality HPLC analysis of phenols requires an optimization of the separation.

The traditional approach to HPLC optimization is to perform an experiment by "trial and error" or by changing one control variable at time while holding the rest constant. Such methods can frequently require a very large number of experiments to identify the optimal

Serbian Chemical Society active member
* E-mail: onjia@vin.bg.ac.yu

conditions. Recently, computer-assisted HPLC separation has addressed this problem, using simplex,[9,10] neural network[11–13] or factorial design[14–16] strategies.

In this work a three-level two-factor design was applied to predict the retention behavior of nine phenols and to optimize their isocratic elution using a methanol – acetic acid mobile phase.

EXPERIMENTAL

Chemicals and reagents.

Individual stock solutions (1.0 mg/ml) of nine phenols, belonging to the U.S.EPA priority pollutant list of phenols, obtained from ChemService (West Chester, PA, USA), were used to prepare the working mixture. This mixture was diluted appropriately in mobile phase to prepare a solution containing 10 μg/ml of each phenol. HPLC-grade methanol and acetic acid (glac.) were purchased from E. Merck (Darmstadt, Germany). Milli-Q system (Millipore Co., Bedford, MA, USA) processed water was used for these experiments.

Chromatographic instrumentation and conditions.

The HPLC system consisted of a Model SP8810 pump, a Spectra200 variable-wavelength detector (both from Spectra-physics, San Jose, CA, USA), a Rheodyne (Cotati, CA, USA) 7125 injector fitted with a 10 μl sample loop. Detection outputs were computed with a Varian Star 4.5 Chromatography Workstation (Varian, Palo Alto, CA, USA). A Lichrochart ODS (25 cm × 4.0 mm × 10 μm) column (Merck, Darmstadt, Germany) was used at ambient temperature. Mobile phases comprising 30, 50 and 90 % (v/v) of methanol with 0.5, 1.0 and 1.5 % (v/v) acetic acid were applied to make a three-level two-factor experimental design. Separation and detection were performed at ambient temperature at a flow rate of 1.0 ml/min, and with UV detection at $\lambda = 280$ nm.

Software.

All calculations were performed using the Mathcad 2000 software package (MathSoft Inc. U.S.A.). Estimation of the retention model parameters was performed by applying the iterative Levenberg-Marquardt algorithm.[17] To make the Levenberg-Marquardt method more effective on actual calculations, the basic method was modified as described in Ref. 18. For the simulation of chromatograms, a laboratory-written programming routine taking into account the different experimental conditions, the resolution graph, and the mathematical functions given in details elsewhere[19,20] for the fitting of Gaussian and skewed peaks, was employed.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of the nine-component mixture of phenols at the central point (50 % methanol and 1.0 % acetic acid) of the applied experimental design. Two peak pairs, 2-chlorophenol and 2,4-dinitrophenol, as well as 4-chloro-3-methylphenol and 2-methyl-4,6-dinitrophenol, are fully overlapped. It is noteworthy that at the other experimental points, 4-chloro-3-methylphenol has a longer retention time than 2-methyl-4,6-dinitrophenol at low methanol percent, while the elution order is reversed at higher methanol percent. Without doubt, the worst separated pair of phenols is 2-chlorophenol and 2,4-dinitrophenol.

The retention behavior of phenols on an ODS reversed-phase column is related to their hydrophobicity (log P). While log P is in linear relation with the organic solvent in a reversed-phase system, this correlation is absent in the presence of ionic agents. Acetic acid can alter the retention of phenols according to the ion interaction mechanism.[21] Thus, the amount of acetic acid in the eluent affects the interaction of methanol with the stationary phase. A high degree of interaction between the two factors, concentration of methanol and acetic acid, can be described by an appropriate model as:

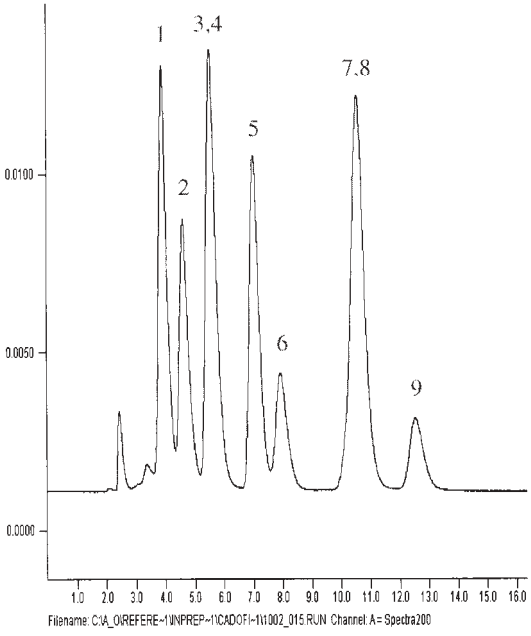

Fig. 1. Chromatogram of nine phenols at 50 % methanol and 1.0 % acetic acid. Peaks: (1) phenol, (2) 4-nitrophenol, (3) 2-chlorophenol, (4) 2,4-dinitrophenol, (5) 2-nitrophenol, (6) 2,4-dimethylphenol, (7) 2-methyl-4,6-dinitrophenol, (8) 4-chloro-3-methylphenol, (9) 2,4-dichlorphenol.

$$k = \beta_0 + \beta_1 \exp(-\beta_2 M) + \beta_1\beta_3 A^{1/\beta 4} \exp(-\beta_2 M) \quad (1)$$

where k is a capacity factor, β_0 is an offset term, β_1 is a measure of the capacity factor in the absence of methanol, β_2 and β_3 are measures of "effectiveness" of the added methanol and acetic acid, respectively. β_4 is a parameter of the Freundlich isotherm. M is the volume percent of methanol in the eluent, and A is the concentration of acetic acid in the eluent. The

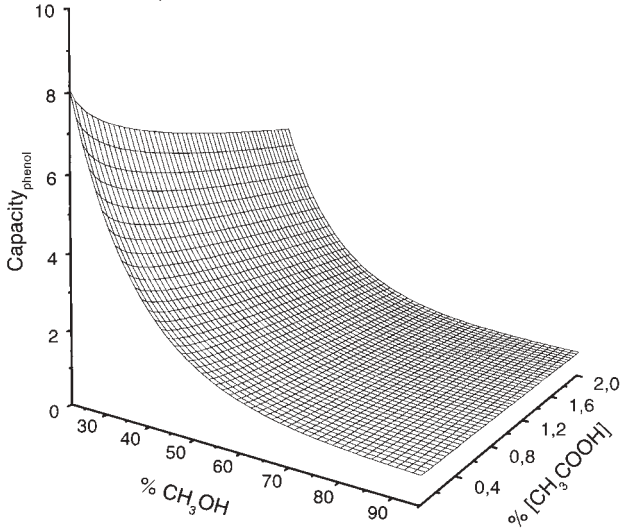

Fig. 2. Calculated retention surface for phenol eluted with methanol – acetic acid eluents.

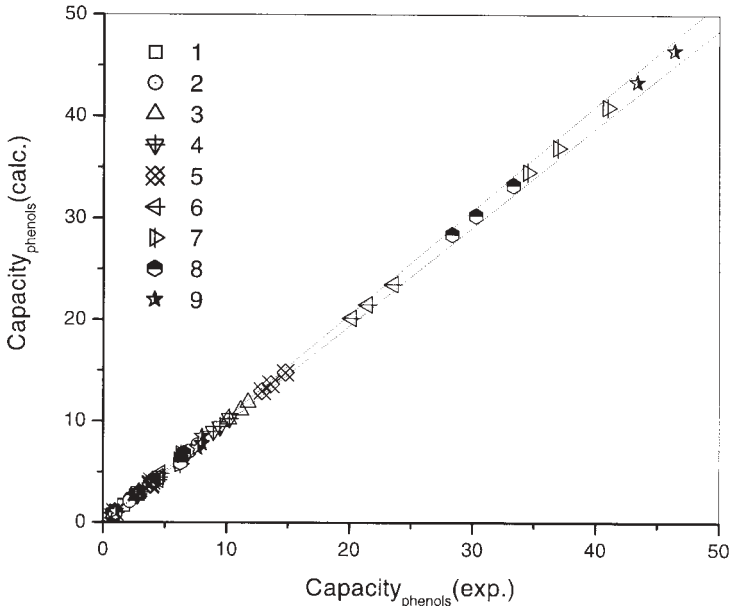

Fig. 3. Comparison between the measured and the calculated capacity factors for nine phenols. Symbols: see Fig. 1.

parameters in Eq. (1) were estimated by the non-linear least squares method described above. In all cases the sum of the squared relative residuals is less than 0.2.

A pseudo-three dimensional plot of the estimated capacity factor of phenol as a func-

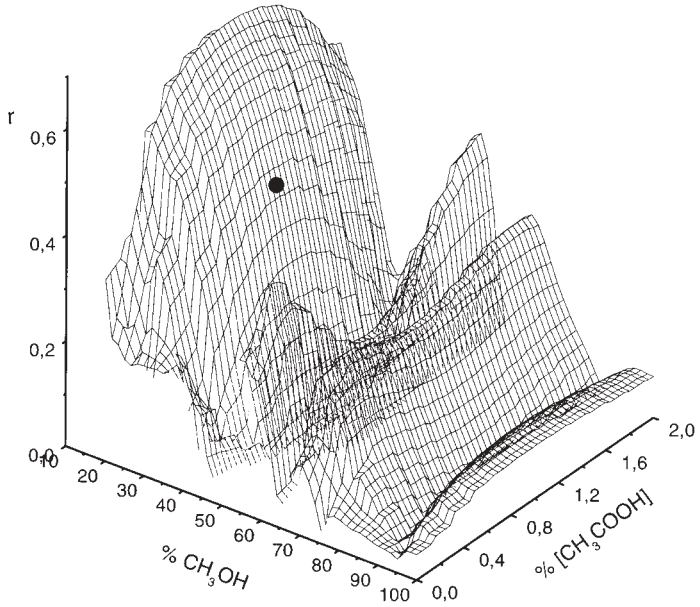

Fig. 4. Normalized resolution product as a function of the eluent composition.

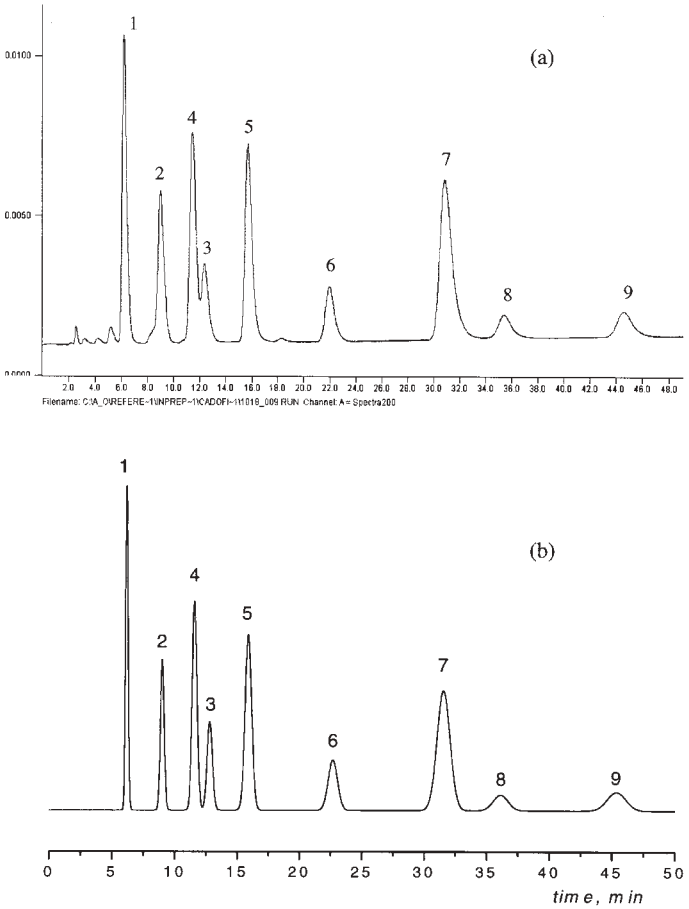

Fig. 5. Chromatograms of nine phenols at 36 % methanol and 0.9 % acetic acid: (a) obtained, (b) predicted. Peak identities as in Fig. 1.

tion of the methanol and acetic acid concentrations is shown in Fig. 2. The effect of acetic acid is more evident along the edge of the low methanol percent (20 %) whereas the effect is reduced at high methanol percent.

The comparison between the observed and the calculated capacity factors is summarized in Fig. 3. As can be seen, the average absolute magnitude of the difference between the calculated and observed values is generally within 5 %, which approaches the magnitude of the experimental precision.

A crucial step in a chromatographic optimization is the selection of an appropriate response function. Several such response functions exist and the choice of the appropriate function is dependent on the overall goal of the separation.[22] In this work, the normalized resolution product criterion[23] is employed to numerically quantify chromatograms. The normalized resolution product (r) may be estimated from the expression:

$$r = \prod_{i=1}^{n-1} \{R_{Si,i+1} / [(n-1)^{-1} \sum_{i=1}^{n-1} R_{Si,i+1}]\} \quad (2)$$

where n is the number of peaks and $R_{S\,i,i+1}$ is the resolution between peaks i and i+1. This criterion gives a value of zero to a chromatogram that has at least one peak fully overlapped, and a value of one for a chromatogram that has evenly spaced peaks. The predicted response surface of the normalized resolution product over the experimental space for the separation of the nine phenols is shown in Fig. 4. The point corresponding to 36 % methanol and 0.9 % acetic acid was selected as the optimum at which the retention times are still not too long and the resolution of 2-chlorophenol and 2,4-dinitrophenol acceptable.

On the basis of above results, the separation of the phenols was undertaken using the selected eluent composition (36 % methanol and 0.9 % acetic acid). The chromatograms obtained and predicted are shown in Fig. 5. It can be seen that a rather good agreement between the predicted and measured retention was obtained. This approach enables a simulated chromatogram for each point on the response surface. A detailed examination of the simulated chromatograms showed that the overlap region for 4-chloro-3-methylphenol and 2-methyl-4,6-dinitrophenol extends from approximately 45 to 65 % methanol whereas overlapping of the critical peaks 2-chlorophenol and 2,4-dinitrophenol occurs at all methanol-acetic acid combinations above 40 % methanol.

In this study, two phenols from the U.S.EPA priority pollutant list, pentachlorophenol and 2,4,6-trichlorophenol, were not considered. Preliminary experiments showed that these two phenols are fairly separated from the others. They are, however, strongly retained on the column, so that their retention times are excessive. In order to achieve shorter retention times for these phenols, a short column with small particle diameter is recommended.

CONCLUSION

A systematic optimization strategy using factorial design can accurately predict the separation for priority phenols when eluted from an isocratic HPLC system. This approach allows the determination of the combined effect of methanol and acetic acid in the eluent giving optimal separation. The normalized resolution product in the isocratic elution of nine phenols is limited by the separation of the 2-chlorophenol and 2,4-dinitrophenol pair.

ИЗВОД

ФАКТОРСКИ ДИЗАЈН У ИЗОКРАТСКОЈ ТЕЧНОЈ ХРОМАТОГРАФИЈИ (HPLC) ФЕНОЛА

АНТОНИЈЕ ОЊИА[1], ТАТЈАНА ВАСИЉЕВИЋ[2], ЂУРО ЧОКЕША[1] и МИЛА ЛАУШЕВИЋ[2]

[1]Институт за нуклеарне науке "Винча", п. пр. 522, 11001 Београд и [2]Технолошко-металуршки факултет, п. пр. 494, 11001 Београд

У раду је коришћена метода симултане мултифакторске оптимизације за теоријско предвиђање сепарације девет фенола методом HPLC у изократским условима. Ретенција фенола је испитивана у функцији састава мобилне фазе (сирћетне киселине и метанола).

Теоријски добијена ретенциона времена не одступају значајно од експерименталних. Као критеријум за оцену квалитета сепарације, у циљу лоцирања оптимума у факторском простору, коришћен је нормализовани резолуциони продукт. У свим експерименталним условима, сепарација 2-хлорофенола и 2,4-динитрофенола диктира тоталну резолуцију у систему.

(Примљено 2. априла 2002)

REFERENCES

1. D. A. Baldwin, J. K. Debowski, *Chromatographia* **26** (1988) 186
2. D. Puig, D. Barcelo, *Anal. Chim. Acta* **311** (1995) 63
3. E. Pocurrull, G. Sanchez, F. Borrull, R. M. Marce, *J. Chromatogr.* **696** (1995) 31
4. J. Frebortova, V. Tatarkovicova, *Analyst* **119** (1994) 1519
5. C. P. Ong, H. K. Lee, S. F. Li, *J. Chromatogr.* **464** (1989) 405
6. O. Busto, J. C. Olucha, F. Borrull, *Chromatographia* **32** (1991) 566
7. J. Torres-Lapasió, M. Roses, E. Bosch, M. Garcia-Alvarez-Coque, *J. Chromatogr.* **886** (2000) 31
8. L. Snyder, J. Glajch, J. Kirkland, *Practical HPLC Method Development*, 2nd Ed., Wiley, New York, 1997
9. J. Berridge, *Analyst* **109** (1984) 291
10. J. Berridge, *J. Chromatogr.* **485** (1989) 3
11. J. Smits, W. Melssen, G. Daalmans, G. Kateman, *Computers Chem.* **18** (1994) 157
12. S. Agatonović-Kuštrin, M. Zečević, Lj. Živanović, I. Tucker, *Anal. Chim. Acta* **364** (1998) 265
13. Y. Loukas, *J. Chromatogr.* **904** (2000) 119
14. J. Glajch, J. Kirkland, *J. Chromatogr.* **485** (1989) 51
15. R. Lopes Marques, P. Schoenmakers, C. Lucasius, L. Buyden, *Chromatographia* **36** (1993) 83
16. L. Snyder, J. Dolan, I. Molnar, N. Djordjević, *LC-GC* **15** (1997) 136
17. W. Press, W. Flannery, S. Teukolsky, B. Vetterling, *Numerical Recipes in C*, Cambridge Univ. Press, New York, 1992
18. J. More, B. Garbow, K. Hillstrom, *User's Guide to Minpack I*, Argonne National Lab. publ. ANL-80-74, 1980
19. J. R. Tores-Lapasió, J. J. Baeza-Baeza, M. C. Garcia-Alvarez-Coque, *Anal. Chem.* **69** (1997) 3822
20. V. B. Di Marco, G. G. Bombi, *J. Chromatogr.* **931** (2001) 1
21. B. A. Bidlingmeyer, S. N. Deming, W. P. Price, B. Sachok, M. Petrusek, *J. Chromatogr.* **186** (1979) 419
22. E. Klein, S. Rivera, *J. Liq. Chromatogr. Rel. Tech.* **23** (2000) 2097
23. P. Haddad, A. Drouen, H. Billiet, L. De Galan, *J. Chromatogr.* **282** (1983) 71.