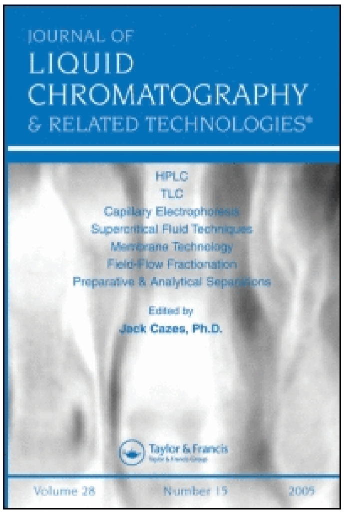

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SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Utilizing experimentally estimated capacity factors and calculated activity coefficients of the solutes in the mobile phase, as well as octadecylsilica stationary phases of varying coverage, the selectivity of separation in a reversed-phase liquid chromatographic system is studied. The retention times were measured for n-alkanes (C_5-C_7), n-alcohols (C_5-C_7) and benzene derivatives in methanol - water mobile phases. A strictly linear relationship between the experimentally determined selectivity factor (α) and the calculated selectivity factor in the mobile phase (α^m) is observed. The relative retention in the stationary phase (α^s) is independent of the mobile phase strength and the silica surface coverage, i.e., α^s is merely a function of the molecular structure of related solutes. Therefore, a change in surface coverage does not lead to a change in α.

INTRODUCTION

The prime intention of method development in liquid chromatography (LC) is to achieve satisfactory resolution of the sample components. The important factor for the calculation of the chromatographic resolution is the selectivity of a separation system. The selectivity factor (α) in LC with a certain support and mobile phase composition is defined as the ratio of the capacity factors (k) of two solutes. As the capacity factor of a solute in LC has been expressed in terms of activity coefficients (1-4), the selectivity can be characterized in the following way (5):

$$\alpha = \frac{k_2}{k_1} = \frac{\gamma_2^m \gamma_1^s}{\gamma_1^m \gamma_2^s} = \frac{\alpha^m}{\alpha^s} \qquad (1)$$

where γ is the activity coefficient of a solute in the mobile (m) or the stationary (s) phase and α is the selectivity factor. By definition, solute 2 is more retained.

In reversed-phase liquid chromatography (RPLC) the selectivity factor in the mobile phase (α^m) will be dependent on the hydrophilic interactions between the aqueous mobile phase and the polar moieties of the two solutes. On the other hand, the relative retention in the stationary phase (α^s) will be determined mainly by the hydrophobic interasction between the bonded alkyl ligands and the non-polar moieties of the two solutes. Both, changes in the mobile phase composition and changes in the concentration of bonded n-alkyl ligands (6,7) affect chromatographic retention. In RPLC, retention can usually be described by the approximate relationship:

$$\ln k_i = \ln k_{iw} - m_i\varphi \qquad (2)$$

where φ is the volume fraction of organic solvent in the mobile phase, k_{iw} is the value of k_i with water as the mobile phase ($\varphi = 0$), and m_i is a constant characteristic for the organic solvent and the solute **i**. Formerly, it has been also shown (3) that:

$$\ln \gamma_i^m = \ln \gamma_i^w - m_i'\varphi \qquad (3)$$

where γ_i^w is the activity coefficient of a solute in water and m_i' is a constant. When the m_i in eqn. 2 is approximately equal to m_i' in eqn. 3 then:

$$\Delta m_i = \Delta m_i' = \frac{d(\ln \alpha)}{d\varphi} = \frac{d(\ln \alpha^m)}{d\varphi} \qquad (4)$$

where Δm_i and $\Delta m_i'$ are equal to $m_2 - m_1$ and $m_2' - m_1'$, respectively. For the accurate evaluation of α^m reliable γ_i^m values are required. The activity coefficients of solutes in the aqueous mobile phase can be determined from experimental gas-liquid (1,8-10) or combined gas-liquid and liquid-liquid (11,12) chromatographic data. Alternatively, it can be estimated by the method of Pierotti et al. (13), "a priori" calculated (3) utilizing the UNIFAC group model of activity coefficients (14-16) or estimated from the aqueous solubilities of solutes (17).

Several studies (e.g. 6,7) have shown an increase in the capacity factor with increasing concentration of bonded n-alkyl ligands on the surface of the stationary phase. In one of these studies (6) an increase of the selectivity factor (α) with increasing bonded-phase carbon content is demonstrated.

To compare the selectivity factor (α) of a pair of solutes with the selectivity factor in the mobile phase (α^m), the capacity factor of various solutes were experimentally determined using HPLC, methanol-water mobile phases and a range of well characterized monomeric ODS stationary phases. The activity coefficients of solutes in the mobile phase were "a priori" calculated or were estimated from aqueous solubilities.

EXPERIMENTAL

A Model ALC/GPC 244 liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model 440UV detector and an R401 differential refractometer was employed. The chromatograms were obtained with a Model M 730 data module.

The eluent was methanol (E. Merck, Darmstadt, F.R.G.), mixed volumetrically with doubly distilled water in various proportions. The range of capacity factors was kept between 0.2-50 whenever it was possible; the range of methanol concentrations was from 0 to 100%. The flow-rate was 1 ml/min and the column temperature was maintained at

22±0.5°C. The mobile phase was filtered through a 0.5-μm Millipore filter (Millipore, Bedford, MA, U.S.A.).

LiChrosorb Si 60, 10 μm (E. Merck) was used for preparing ODS packings. Appropriate quantities of dimethyloctadecylchlorosilane (Fluka, Bucks, Switzerland) were refluxed with dry silica (10 g) for 15 h in freshly distilled xylene, with 1 ml pyridine. Subsequently the bonded silica was extensively washed with toluene, tetrahydrofuran, methanol and water.

The bonded phases were characterised by elemental microanalysis and packed into 300 x 3.9 mm I.D. stainless-steel columns (Waters Assoc.) using a high-pressure slurry-packing technique. The solutes were obtained from various sources and are listed in Table 1. Deuterated water (E. Merck) was used as a non-retained species for determining the column dead volume.

The calculation of activity coefficients was described previously (3).

RESULTS AND DISCUSSION

For the solutes listed in Table 1 the retention data obtained on columns of varying ODS coverage are presented as slopes (m_i) and intercepts ($\ln k_{iw}$) of eqn. 2, calculated from experimentally determined capacity factors by linear regression analysis. Correlation coefficients of the linear regression of experimental capacity factors varied from 0.9986 - 0.9999. The activity coefficients

TABLE 1. Linear regression constants of eqns. 2 and 3 for the test compounds and for varying silica coverage.

	Coverage (mmol/g)									
	0.255		0.335		0.499		0.690			
	m_i	$\ln k_{iw}$	m_i	$\ln k_{iw}$	m_i	$\ln k_{iw}$	m_i	$\ln k_{iw}$	m_i'	$\ln \gamma_i^w$
Benzene	6.14	3.41	6.07	3.78	6.26	4.33	6.30	4.72	6.00	7.85
Toluene	7.44	4.94	7.48	5.29	7.44	5.84	7.55	6.23	7.37	9.47
Ethylbenzene	9.01	6.60	8.98	6.95	9.02	7.48	8.89	7.87	8.92	11.24
Acetophenone	6.05	3.30	6.12	3.64	6.37	4.12	6.29	4.52	6.21*	7.02**
o-Cresol	6.16	3.22	6.40	3.55	6.46	4.05	6.27	4.44	6.32*	5.50**
Benzyl alcohol	5.22	2.19	5.44	2.51	5.31	2.97	5.37	3.35	5.34*	5.07**
Phenol	5.27	1.97	5.32	2.22	5.17	2.69	5.29	3.07	5.26*	4.15**
Aniline	4.83	1.84	4.92	2.19	4.86	2.70	5.01	3.12	4.91*	5.00**
Pentane	8.52	6.26	8.74	6.74	8.46	7.06	8.61	7.40	8.52	10.98
Hexane	9.90	7.84	10.03	8.24	9.85	8.62	9.81	8.95	9.95	12.65
Heptane	11.28	9.40	11.33	9.76	11.09	10.07	11.10	10.49	11.38	14.30
Pentanol-1	5.38	2.74	5.70	3.07	5.68	3.54	5.56	3.93	5.58*	5.47**
Hexanol-1	6.72	4.15	6.80	4.47	6.81	4.93	6.75	5.31	6.77*	6.89**
Heptanol-1	7.82	5.56	8.07	5.88	8.04	6.34	7.95	6.71	7.97*	8.31**

* The average value of experimentally determined m_i values of various silica coverage.
** Calculated from the solute aqueous solubility.

of n-alkanes and n-alkylbenzenes, used for the evaluation of α^m, were calculated using the UNIFAC mathod (14-16) as described previously (3). The γ_i^m values calculated in this way are presented in Table 1 as slopes (m_i') and intercepts ($\ln \gamma_i^w$) of eqn. 3. Correlation coefficients of the linear regression of "a priori" calculated γ_i^m values varied from 0.9988 - 1.0000. For alcohols and other benzene derivatives from Table 1 it has been argued (3) that the UNIFAC parameters produce unreliable γ_i^m values. Therefore, for the calculation of γ_i^m values of these solutes, their aqueous solubilities (3,17-19) and the following equation (3) were used:

$$\ln \gamma_i^m = \ln \gamma_{i,exp}^w - \overline{m}_i \varphi \qquad (5)$$

Here, $\overline{m}_i$ is the average value (from various silica coverages) of experimentally determined m_i values of a solute from Table 1; $\gamma_{i,exp}^m$ is the activity coefficient of the same solute in water, calculated from its aqueous solubility. In the previous paper (3) it was argued that on the basis of experimentally determined aqueous solubility of a compound and the slope of eqn. 2, it is possible to calculate fairly reliable activity coefficients in the mobile phase for different φ values. Both $\overline{m}_i$ and $\gamma_{i,exp}^m$ are also presented in Table 1.

The selectivity factors α and α^m are calculated as k_2/k_1 and γ_2^m/γ_1^m, respectively. The most retained (reference) compound 2 for the benzene derivatives was ethylbenzene and for the aliphatic compounds heptane. Both $\ln \alpha$ and $\ln \alpha^m$ were strictly linear (correlation coefficients varied from 0.9982 - 1.0000) with the variation of the volume fraction of methanol in the aqueous mobile phase (φ), according to the equations:

$$\ln \alpha = \ln \alpha_w - \Delta m_i \varphi \tag{6}$$

and

$$\ln \alpha^m = \ln \alpha_w^m - \Delta m_i' \varphi \tag{7}$$

where $\ln \alpha_w$ and $\ln \alpha_w^m$ are equal to $\ln(k_{2w}/k_{1w})$ and $\ln(\gamma_2^w/\gamma_1^w)$, respectively.

The selectivity factors α and α^m of the various solute pairs are shown in Table 2 as the slopes and intercepts of eqns. 6 and 7.

The values of the slopes (Δm_i values) of eqn. 6 for a given solute pair are very similar for stationary phases of different coverages, indicating that the change in selectivity (or retention) that results from a change in φ is independent of the concentration of bonded ligands. The same conclusion can be drawn from the data of Kaliszan et al. (7). The mean Δm_i value of a solute pair is also very close to the corresponding calculated $\Delta m_i'$ value, suggesting the possibility of using eqn. 4 for the prediction of changes in relative retention on the basis of accurate mobile-phase activity coefficients. However, for mobile-phase optimization in RPLC, the use of "a priori" calculated activity coefficients based on data from other non-chromatographic sources can lead to large errors, predominantly due to unreliable group interaction parameters (3). The experimental determination of activity coef-

TABLE 2. Linear regression constants of eqns. 6 and 7 of solute pairs and varying silica coverage.

	Coverage (mmol/g)									
	0.255		0.335		0.499		0.690			
	Δm_i	$\ln \alpha_w$	Δm_i	$\ln \alpha_w$	Δm_i	$\ln \alpha_w$	Δm_i	$\ln \alpha_w$	$\Delta m_i'$	$\ln \alpha_w^m$
Ethylbenzene/										
Toluene	1.55	1.65	1.50	1.66	1.58	1.64	1.36	1.65	1.51	1.74
Benzene	2.90	3.21	2.90	3.16	2.78	3.16	2.60	3.16	2.87	3.35
Acetophenone	2.97	3.31	2.85	3.31	2.66	3.36	2.60	3.35	2.68	4.20
o-Cresol	2.87	3.39	2.58	3.40	2.57	3.44	2.62	3.43	2.58	5.72
Benzyl alcohol	3.85	4.44	3.55	4.45	3.71	4.51	3.52	4.52	3.56	6.15
Phenol	3.84	4.68	3.66	4.73	3.87	4.80	3.56	4.76	3.64	7.08
Aniline	4.21	4.78	4.02	4.74	4.19	4.80	3.88	4.75	3.98	6.22
Heptane/										
Hexane	1.37	1.55	1.30	1.52	1.24	1.45	1.30	1.55	1.43	1.65
Pentane	2.74	3.13	2.57	3.01	2.65	3.03	2.51	3.11	2.85	3.31
Heptanol-1	3.44	3.83	3.26	3.88	3.03	3.72	3.14	3.77	3.40	5.98
Hexanol-1	4.56	5.25	4.53	5.29	4.28	5.14	4.53	5.18	4.61	7.41
Pentanol-1	5.90	6.66	5.63	6.69	5.41	6.53	5.54	6.56	5.80	8.83

ficients in the aqueous liquid phase using GLC or GLC/LLC methods have at least two imperfections: the number of solutes studied is limited and the accuracy of the estimated activity coefficients is insufficient. For example, Djerki and Laub (12) determined for the first time the activity coefficients of C_3-C_7 aliphatic aldehyde, ketone, ester and alcohol solutes at infinite dilution in methanol, water and mixtures of the two by appropriate combination of GLC and LLC retention data. The slopes (m_i') of eqn. 3, calculated for n-alcohols are 1.8-2.4 times lower than those presented in Table 1 for the same alcohols. According to the data of Janini et al. (11), also determined by the GLC/LLC method, the activity coefficients of phenol in water exceeds that of o-cresol, indicating a higher aqueous solubility of o-cresol. Comparing the different methods, the activity coefficients based on the aqueous solubility of solutes and HPLC data seem to be more reliable because the GLC technique is limited primarily to the study of volatile solutes with solvents of low vapor pressure at the column temperature. Also, calculations in GLC are very sensitive to the saturation vapor pressure of a solute. In the LLC technique, the mobile and stationary phases must obviously be immiscible; each must exibit sufficient solvency for the solutes such that the retention volumes are neither too small nor too large to compromise the accuracy of their measurement. However, the slopes of eqn. 7 calculated for some solutes of Table 1 cn the basis of experimentally determined m_i

values (eqn. 2) are also disputable, mainly due to the fact that m_i values obtained on the same LC system may diverge significantly from laboratory to laboratory. The experimental m_i values of Hafkenscheid et al. (20) for some solutes are about 50% higher than the values of Opperhuizen et al. (21), although both experiments were carried out on Hypersil ODS columns (5 µm particles). On the other hand, the experimental data of this study are in close agreement with, for example , corresponding data in refs. (7),(20),(22) and (23), obtained with octyl-silica or ODS stationary phases from various manufacturers.

The second factor in eqn. 6 affecting the relative retention is the intercept (ln α_w), representing the extrapolated value for the selectivity factor in water. The data of Table 2 demonstrate an independence of α_w on the concentration of alkyl ligates on the stationary phase. That is, a change in the silica surface coverage does not lead to a change in the separation factor α. Data of ref. (7) also support this statement. Since there is a general trend in RPLC of increasing retention with increasing surface coverage, it may be concluded for the present set of solutes and with metanol-water mixtures as the mobile phase that a change in the surface coverage causes only a change in the phase ratio of a system.

Retention data obtained with a standard gradient by Antle et al. (24), estimated on commercial Zorbax ODS and two Zorbax C-8 columns (differing in surface areas) demonstrate very small differences in α between octyl-silica and ODS stationary phases (±1.9% with aqueous methanol and ±3.7% with aqueous acetonitrile) for a variety of solutes with simple and more complex molecular structures. For many of the solutes tested, it was formerly had already been concluded that selectivity is independent of bonded alkyl chain length (25). Thus, it can be generally stated that the optimization of a RPLC system can best be achieved by changing the mobile phase composition or polarity of column. Nevertheless, it should be noted here that for some solutes and non-monomeric stationary phases (6) an increase in α was demonstrated with both increasing surface coverage and increasing alkyl chain length.

The substraction of the ln α_w value for a solute pair of Table 2 from the corresponding ln α_w^m value gives ln α^s value, independent on both φ and surface coverage. Values of α^s obtained in this way are presented in Table 3, together with α_w and α_w^m values for solute pairs of Table 2. According to eqn. 1, a linear relationship is obtained between α^m and α with slope equal to α^s with correlation coefficients varying from 0.9997 - 0.9999. Values of α^s calculated in this way are also listed in Table 3.

It has been pointed out previously (26-28) that in RPLC the interaction of the solute with stationary phases of low surface energy, such as n-alkyl chains attached to a silica surface, is weak and non-selective. Thus, the driving force for retention is the unfavourable interaction of a solute

TABLE 3. Selectivity factors α_w, α_w^m and α^s for various solute pairs of Table 2.

	α_w^*	α_w^m	α^s		
			α_w^m/α_w	Eqn. 1	Mean
Ethylbenzene/					
Toluene	5.21	5.70	1.09	1.01	1.05
Benzene	23.80	28.50	1.20	1.12	1.16
Acetophenone	27.94	66.69	2.39	2.38	2.39
o-Cresol	30.57	304.90	9.97	10.10	10.04
Benzyl alcohol	88.23	468.72	5.43	5.31	5.37
Phenol	115.58	1188.00	10.28	10.42	10.35
Aniline	117.92	502.70	4.26	4.33	4.30
Heptane/					
Hexane	4.57	5.21	1.14	1.12	1.13
Pentane	21.54	27.66	1.27	1.15	1.21
Heptanol-1	44.70	395.44	8.85	8.13	8.49
Hexanol-1	184.93	1652.43	8.94	8.20	8.57
Pentanol-1	742.48	6838.29	9.21	8.26	8.74

* Mean value of varying silica coverage in Table 2.

with the surrounding water molecules of the mobile phase. It can be generally concluded from the data in Table 3 (see also ref. 3) that more polar solutes demonstrate a higher affinity toward both the mobile and the stationary phase in comparison with less polar solutes. The relative retention in both phases increases from the less polar solutes such as toluene and hexane to the more polar solutes such as phenol and butanol. The increase in α^s is significantly lower than that in α^m. However, it can not be neglected, especially for polar solutes. An increasing difference in polarity between two solutes leads to an increase in α^s. A comparison of the data presented in Table 3 shows that all three selectivity factors increase when the polarity difference between, for example two benzene derivatives is increased, but the order of the increase is not the same. For example, α^m for the solute pairs ethylbenzene/benzene derivative is changed in the following order: phenol > aniline > benzyl alcohol > o-cresol > acetophenone > benzene > toluene. However, the increasing order of α^s is different: phenol > o-cresol > benzyl alcohol > aniline > acetophenone > benzene > toluene. The reason for such different order is the dual behaviour of n-alkyl bonded silica, i.e. reversed phases possess both hydrophobic and the hydrophilic properties, even though untreated silica also shows reversed phase behaviour in contact with aqueous mobile phases (24,29,30). Phenol and o-cresol have approximately the same retention in the stationary phase, because the hydrophobic moiety of the bonded silica (alkyl chains) is only slightly selective to the methyl group. The same phenomenon has also noticed for alkanes and alkylbenzenes. The selectivity difference in the stationary phase per methylene group for these solutes is small. Thus, these solutes are separated in RPLC only on the basis of their significantly different interaction with the mobile phase. On the other hand, the hydrophilic moiety of the stationary phase is significantly selective to the polar groups. The solubility of benzyl alcohol and aniline in water is similar, but their interaction with the stationary phase is different enough to achieve separation (α_w^m = 1.07 , α_w = 1.34). For the aniline/phenol pair α_w^m and α^s values are approximately equal, so their separation is poor. The situation is similar in the case of acetophenone and o-cresol. Generally, it may be concluded that n-alkyl bonded stationary phases are slightly selective to the non-polar moiety of a solute and are noticably selective to polar solute groups.

CONCLUSIONS

"A priori" calculated activity coefficients can successfully be used for the prediction of the selectivity of the mobile phase. For RPLC systems varying in the silica surface coverage the selectivity factor α depends only on the strength of the mobile phase. Therefore, the selectivity factors α and α^m are linearly related to each other with the proportionality constant equal to α^s. The relative retention in the stationary phase (α^s) is an approximately constant value, only slightly dependent on the mobile phase strength, the silica surface coverage and/or the length of n-alkyl bonded shains. Thus, the selectivity in the stationary phase is only a function of the molecular structure of the solutes. The stationary phases studied show a slight selectivity to the hydrophobic moiety of solutes (e.g. methyl or methylene groups), while the selectivity to the polar substituents is significant and can not be neglected.

REFERENCES

1. Slaats, E.H., Kraak, J.C., Brugman, W.J.T., Poppe, H., J. Chromatogr., 149, 255 (1978).
2. Rizzi, A., J. Chromatogr., 348, 1 (1985).

3. Petrović, S.M., Lomić, S., Šefer, I., J. Chromatogr., 348, 49 (1985).

4. Petrović, S.M., Lomić, S., Šefer, I., Chromatographia, 23, 915 (1987).

5. Petrović, S.M., Lomić, S., Šefer, I., Chromatographia, 24, 800 (1987).

6. Hennion, M.C., Picard, C., Caude, M., J. Chromatogr., 166, 21 (1978).

7. Kaliszan, R., Osmialowski, K., Tomellini, S.A., Hsu, S.-H., Fazio, S.D., Hartwick, R.A., J. Chromatogr., 352, 141 (1986).

8. Slaats, E.H., Thesis, Amsterdam, 1980.

9. Pescar, R.E., Martin, J.J., Anal. Chem., 38, 1661 (1966).

10. Shafter, D.L., Daubert, T.E., Anal. Chem., 41, 1585 (1969).

11. Janini, G.M., Qaddora, L.A., J. Liq. Chromatogr., 9, 39 (1986).

12. Djerki, R.A., Laub, R.J., 11th International Symposium on Column Liquid Chromatography, Amsterdam, 1987.

13. Pierotti, G.J., Deal, C.H., Derr, E.L., Ind. Eng. Chem., 51, 95 (1959).

14. Fredenslund, A., Jones, R.L., Prausnitz, J.M., AIChE J., 21, 1086 (1975).

15. Fredenslund, A., Gmehling, J., Michelsen, M.L., Rasmussen, P., Prausnitz, J.M., Ind. Eng. Chem., Process Des. Dev., 16, 450 (1977).

16. Gmehling, J., Rasmussen, P., Fredenslund, A., Ind. Eng. Chem., Process Des. Dev., 21, 118 (1982).

17. Wasik, S.P., Tewari, Y.B., Miller, M.M., Martire, D.E., NBS Technical Report NBSIR-81-2406, National Bureau of Standards, Washington, D.C., 1981.

18. Stephen, H., Stephen, T., Solubilities of Inorganic and Organic Compounds, Vol. I, Pergamon press, New York, 1963, Table 1.

19. Funasaki, N., Hada, S., Neya, S., J. Chronatogr., 361, 33 (1986).

20. Hafkenscheid, T.L., Tomlinson, E., J.Chromatogr., 218, 409 (1981).

21. Opperhuizen, A., Sinnige, T.L., van der Steen, J.M.D., Hutzinger, O., J. Chromatogr., 388, 51 (1987).

22. Schoenmakers, P.J., Billiet, H.A.H., de Galan, L., J. Chromatogr., 218, 261 (1981).

23. Snyder, L.R., Quarry, M.A., Glajch, J.L., Chromatographia, **24**, 33 (1987).

24. Antle, P.E., Goldberg, A.P., Snyder, L.R., J. Chromatogr., **321**, 1 (1985).

25. Karch, K., Sebastian, I., Halàsz, I., J. Chromatogr., **122**, 3 (1976).

26. Horvàth, Cs., Melander, W., Molnàr, I., J. Chromatogr., **125**, 129 (1976).

27. Locke, D.C., J. Chromatogr. Sci., **12**,425 (1974).

28. Karger, B.L., Gant, J.R., Hartkopf, A., Weiner, P.H., J. Chromatogr., **128**, 65 (1976).

29. Bij, K.E., Horváth, Cs., Melander, W.R., Nahum, A., J. Chromatogr., **203**, 65 (1981).

30. Petrović, S.M., Lončar, E., Kolarov, Lj., Chromatographia, **18**, 683 (1984).