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REVERSED-PHASE LIQUID CHROMATOGRAPHY OF AROMATIC SULPHONIC ACIDS AND OTHER STRONGLY POLAR COMPOUNDS WITHOUT ADDITION OF AN ION-PAIRING COUNTER-ION

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SUMMARY

Efficient separations of aromatic sulphonic acids, which are important as dye intermediates, can be achieved on chemically bonded reversed-phase columns using mobile phases containing strong inorganic electrolytes instead of organic ion-pair-forming substances. The system is highly selective and allows the rapid separation of a number of isomeric aromatic mono-, di- and trisulphonic acids. Other ionic or strongly polar substances, such as linear alkylbenzenesulphonates and water-soluble vitamins, can also be separated using this technique.

INTRODUCTION

Aromatic sulphonic acids, used as intermediates in the production of a large number of important dyes, are most often prepared using sulphonation of simple aromatic hydrocarbons or their derivatives. This operation usually yields mixtures of isomers and compounds with different numbers of sulphonic groups. Appropriate adjustment of reaction conditions, such as temperature and concentration of the sulphonation agent in the reaction mixture, can be used for control and optimization of the ratio of the individual products. For this purpose, as for the control of intermediates and technical products, a precise and rapid analytical method is necessary.

For the analysis of sulphonic acids, modern high-performance liquid chromatography is potentially superior to other methods, which are either time consuming and of insufficient efficiency and accuracy (paper and thin-layer chromatography) or require the preparation of volatile derivatives (gas chromatography).

Chromatography on ion-exchange columns has not been very successful for the analysis of aromatic sulphonic acids owing to the low selectivity and efficiency and especially the very strong retention on styrene–divinylbenzene ion-exchange resins[1].

Recently, ion-pair chromatography has been introduced for the separation of ionic and strongly polar organic substances[2–5]. This technique makes use of the

formation of ion pairs with appropriate counter-ions, either in the liquid stationary phase anchored on an appropriate support[6] (silica or a short alkyl-chain reversed phase) or in the aqueous–organic mobile phase in reversed-phase systems[4,7–10].

Good separations of some aromatic sulphonic acids have been achieved using reversed-phase ion-pair chromatography on chemically bonded packing materials[9,10], bleeding of the stationary phase being avoided. Retention of sample compounds and the selectivity, to a certain extent, can be controlled by adjusting the type and concentration of the ion-pair-forming counter-ion added to aqueous–organic mobile phase (usually a tetraalkylammonium salt, such as cetyltrimethylammonium bromide or tetrabutyl-, tetraethyl- and tetramethylammonium phosphate or sulphate) and by selection of the type and concentration of the organic solvent in the mobile phase[4,7–10]. Addition of inorganic electrolytes in minor concentrations to the mobile phase or adjustment of its pH can also be used to improve the separation[8,9,11–13]. An appropriate choice of the separation conditions in reversed-phase ion-pair chromatography is critical for the achievement of good separations. As the separation mechanism in ion-pair chromatography is complex and not fully understood yet, it is not always easy to find good separation conditions for each separation problem, and with certain ion-pair-forming counter-ions or certain organic solvents the compounds may be either strongly retained or, on the contrary, remain almost unretained over the whole composition range of the mobile phase used[7,9]. Peak splitting at certain compositions of the mobile phase was occasionally observed with some compounds[9]. Moreover, tetraalkylammonium salts and other ion-pair-forming substances added to the mobile phase in this method are expensive.

Most experiments with reversed-phase chromatography of ionic and strongly polar substances without the addition of ion-pair-forming counter-ions failed because pure water or aqueous–organic solutions were used, with subsequent addition of a buffer or other salt in relatively low concentrations. Then, the ionic and strongly polar compounds were eluted near or even prior to the column void volume, usually as strongly distorted or split peak with irreproducible shapes, owing to ionic-exclusion effects[10]. As we showed elsewhere[10,14], this effect can be overcome by addition of a strong electrolyte (inorganic or certain organic salts) in a relatively high concentration (usually 0.1 M or more) to the mobile phase. Then, most strongly polar and ionic compounds become retained, even strongly, and can be eluted as narrow, symmetrical peaks, like non-polar substances[14]. Retention (and the selectivity, to a certain extent) can be controlled conveniently and simply by adjusting the concentrations of the electrolyte and the organic solvent. An increase in the concentration of the electrolyte or a decrease in the concentration of the organic solvent promotes retention on reversed-phase columns[14]. No peak splitting was observed using this method[14]. Lastly, the mobile phases used in this system are much cheaper than those in ion-pair chromatography.

EXPERIMENTAL

The equipment consisted of an M6000 pump (Waters Assoc., Milford, MA, U.S.A.), an injection port which made possible direct syringe injection on the top of the column and an M440 UV detector (Waters Assoc.). A PPM 68005 gradient-forming device (Workshops of the Czechoslovak Academy of Sciences, Prague,

Czechoslovakia) connected to the inlet of the M6000 pump was used in gradient elution[15].

The reversed-phase column packing material was prepared from LiChrosorb SI 100 (10 μm) (Merck, Darmstadt, G.F.R.) by reaction with n-octadecyltrichlorosilane[16]. The chemically bonded reversed phase (C_{18}) prepared in this way contained 16.5% (w/w) of carbon and was slurry-packed[17] into a stainless-steel column (300 × × 4.2 mm I.D.). The void volume of the column was determined as the retention volume of ^2H_2O measured with aid of a differential refractometer (R-401, Waters Assoc.), and was 3.05 ml.

The solvents used as the components of the mobile phase were prepared by dissolving the calculated amount of sodium sulphate or lithium sulphate in water or in previously prepared aqueous methanol or by mixing water with methanol. Methanol and water were distilled in glass before use. The salts used were of analytical-reagent grade. The samples of acids were obtained from East-Bohemian Chemical Works Synthesia (Semtin, Czechoslovakia), and were dissolved in the mobile phase used as solvent A.

RESULTS AND DISCUSSION

Firstly, a separation of technically important sulphonic acids potentially present in reaction mixtures after the sulphonation of naphthalene was attempted. As the retention of sulphonic acids decreases with increasing number of sulphonic groups in the molecule of the acid, naphthalenetri- and -tetrasulphonic acids are only slightly retained on an octadecylsilica column; 0.4 M sodium sulphate in water without the addition of any organic solvent had to be used as the mobile phase in order to achieve their retention and separation. Under these conditions, naphthalenedi- and -monosulphonic acids are strongly retained and a decrease in concentration of sodium sulphate together with addition of an organic solvent (methanol) to the mobile phase was used for their elution.

Thus, the separation of a mixture containing one naphthalenetetrasulphonic, three naphthalenetrisulphonic, four naphthalenedisulphonic and naphthalene-1- and -2-sulphonic acids could be achieved using the elution of the tetra- and trisulphonic acids with 0.4 M sodium sulphate followed by linear gradient elution of di- and monosulphonic acids using 0.4 M sodium sulphate as solvent B. Fig. 1 shows the separation under these conditions, which took ca. 28 min.

Anthraquinonemono- and -disulphonic acids are more strongly retained than naphthalenesulphonic acids. A rapid separation of a five-component mixture of the acids produced in the sulphonation of anthraquinone could be achieved in 9 min using stepwise elution. Fig. 2 shows that anthraquinonedisulphonic acids were eluted with 0.133 M sodium sulphate in methanol–water (40:60) and the elution of anthraquinone-1- and -2-sulphonic acids followed using methanol–water (60:40) without the addition of a salt in the second step.

Fig. 3 shows the separation of five isomeric 1-naphthylaminesulphonic acids in 12 min. 1-Naphthylamine-5- and -4-sulphonic acids were eluted in an isocratic step using 0.32 M sodium sulphate in methanol–water (12:88) as the mobile phase, followed by linear gradient elution of the remaining three isomeric acids using methanol–water (60:40) as solvent B.
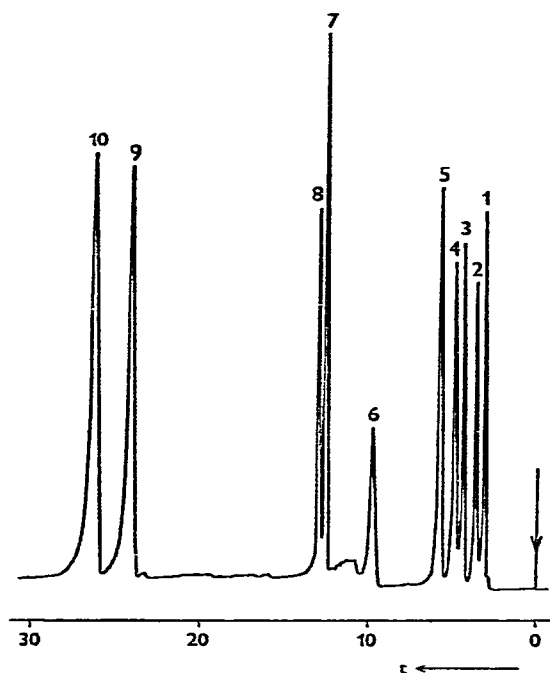
Fig. 1. Reversed-phase separation of naphthalenemono-, -di-, -tri- and -tetrasulphonic acids. Column: C_{18}, 10 μm, 300 × 4.2 mm. Mobile phase: step 1, isocratic elution with 5 ml of 0.4 M Na$_2$SO$_4$ in water; step 2, linear gradient elution, 0–90% B in 13.5 min. Solvent A, 0.4 M Na$_2$SO$_4$ in water; solvent B, methanol–water (40:60). Flow-rate: 1.0 ml/min. Detection: UV, 254 nm, 0.5 a.u.f.s. t = time elapsed (minutes). Compounds: 1 = naphthalene-1,3,5,7-tetrasulphonic acid; 2 = napthalene-1,3,6-trisulphonic acid; 3 = naphthalene-1,3,5-trisulphonic acid; 4 = naphthalene-1,3,7-trisulphonic acid; 5 = naphthalene-1,5-disulphonic acid; 6 = naphthalene-2,6-disulphonic acid; 7 = naphthalene-1,6-disulphonic acid; 8 = naphthalene-2,7-disulphonic acid; 9 = naphthalene-1-sulphonic acid; 10 = naphthalene-2-sulphonic acid.

The method described can be used for the separation of linear alkylbenzene-sulphonates, which are the major surfactants present in household detergents. These compounds can be separated using gas chromatography after desulphonation prior to the separation step[18], on organic gels[19–21] or using reversed-phase ion-pair chromatography with tetramethylammonium[7] or cetrimide[22] as the counter-ion added to the mobile phase[7]. Normal reversed-phase chromatography on chemically bonded phases using methanol–water as the mobile phase without further components was claimed to fail in the analysis of linear alkylbenzenesulphonates[7], but the separation can be achieved with the addition of an inorganic salt to the mobile phase, as demonstrated in Fig. 4, where chromatography of a dilute commercial household detergent is shown under gradient elution conditions.

Not only strong sulphonic (and carboxylic) acids can be separated using this technique, but also basic compounds or mixtures containing both basic and acidic compounds, as it demonstrated in Fig. 5, where the separation of five water-soluble vitamins was achieved in 13 min using as the mobile phase 0.5 M lithium sulphate in methanol–water (5:95) for isocratic elution.
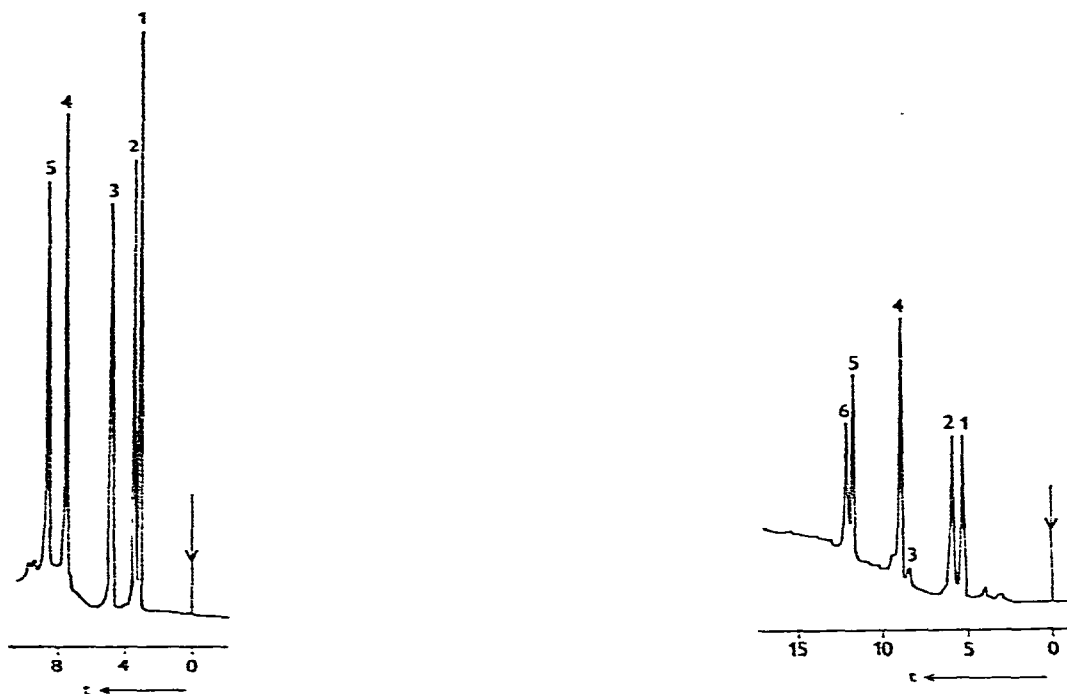
Fig. 2. Reversed-phase separation of anthraquinonemono- and -disulphonic acids. Column as in Fig. 1. Mobile phase: step 1, isocratic elution with 5 ml of 0.133 M Na₂SO₄ in methanol–water (40:60); step 2, isocratic elution with methanol–water (60:40). Flow-rate: 1.0 ml/min. Detection: UV, 254 nm, 0.1 a.u.f.s. t = time elapsed (minutes). Compounds: 1 = anthraquinone-1,5-disulphonic acid; 2 = anthraquinone-2,6-disulphonic acid; 3 = anthraquinone-1,8-disulphonic acid; 4 = anthraquinone-1-sulphonic acid; 5 = anthraquinone-2-sulphonic acid.

Fig. 3. Reversed-phase separation of isomeric 1-naphthylaminemonosulphonic acids. Column as in Fig. 1. Mobile phase: step 1, isocratic elution with 5 ml of 0.32 M Na₂SO₄ in methanol–water (12:88); step 2, linear gradient elution, 20–100% B in 12 min. Solvent A, 0.4 M Na₂SO₄ in water; solvent B, methanol–water (60:40). Flow-rate: 1.0 ml/min. Detection: UV, 254 nm, 0.5 a.u.f.s. t = time elapsed (minutes). Compounds: 1 = 1-aminonaphthalene-5-sulphonic acid; 2 = 1-aminonaphthalene-4-sulphonic acid; 3 = impurity; 4 = 1-aminonaphthalene-6-sulphonic acid; 5 = 1-aminonaphthalene 7-sulphonic acid; 6 = 1-aminonaphthalene-8-sulphonic acid.
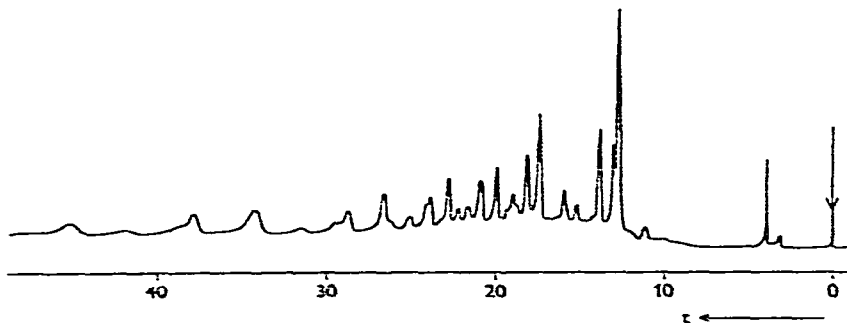

Fig. 4. Reversed-phase separation of alkylbenzenesulphonate surfactants in a commercial household detergent (10 μl of a sample diluted 1:10 with water). Column as in Fig. 1. Mobile phase: linear gradient elution. 0–100% B in 15 min. Solvent A, 0.4 M Na₂SO₄ in water; solvent B, methanol–water (60:40). After the end of the gradient, isocratic elution with pure solvent B followed. Flow-rate: 1.0 ml/min. Detection: UV, 254 nm, 1.0 a.u.f.s. t = time elapsed (minutes).
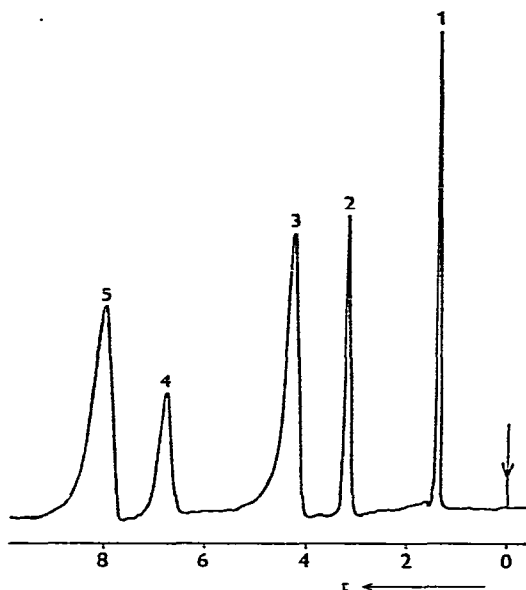
Fig. 5. Reversed-phase separation of a mixture of water-soluble vitamins. Column as in Fig. 1. Mobile phase: 0.5 M Li$_2$SO$_4$ in methanol–water (5:95) (isocratic elution). Flow-rate: 1.5 ml/min. Detection: UV, 254 nm, 0.05 a.u.f.s. t = time elapsed (minutes). Compounds: 1 = ascorbic acid; 2 = nicotinic acid; 3 = pyridoxine; 4 = nicotinamide; 5 = thiamine.

CONCLUSIONS

A few examples of the chromatographic separation of mixtures of aromatic sulphonic acids and other strongly polar compounds demonstrate the high selectivity and efficiency of reversed-phase chromatographic separations using high concentrations of salts in the mobile phase. Here, the ionic strength of the mobile phase is the most important factor controlling the separation. The retention and selectivity can, of course, be modified by selection of different salts used as the electrolytes in the mobile phase, but the type of salt seems to have only a minor effect on the separation[11]. A more detailed study would be necessary, however, for definite conclusions to be drawn.

The proposed method seems to be promising for the rapid separation of ionic and strongly polar compounds, and may compete successfully with reversed-phase ion-pair chromatography.

REFERENCES

1 P. Jandera and J. Churáček, J. Chromatogr., 86 (1973) 423.
2 S. Eksborg and G. Schill, Anal. Chem., 45 (1973) 2092.
3 S. Eksborg, P. O. Lagerström, R. Modin and G. Schill, J. Chromatogr., 83 (1973) 99.
4 D. P. Wittmer, N. O. Nuessle and W. G. Haney, Anal. Chem., 47 (1975) 1422.
5 J. H. Knox and G. R. Laird, J. Chromatogr., 122 (1976) 17.
6 J. C. Kraak and J. F. K. Huber, J. Chromatogr., 102 (1974) 333.
7 R. Gloor and E. L. Johnson, J. Chromatogr. Sci., 15 (1977) 413.
8 C. P. Terweij-Groen, S. Heemstra and J. C. Kraak, J. Chromatogr., 161 (1978) 69.

9 H. V. Ehmcke, H. Kelker, K. H. König and H. Ullner, *Z. Anal. Chem.*, 294 (1979) 251.
10 P. Jandera and H. Engelhardt, *Chromatographia*, 13 (1980) 18.
11 C. M. Riley, E. Tomlinson and T. M. Jefferies, *J. Chromatogr.*, 185 (1979) 197.
12 A. T. Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.*, 185 (1979) 225.
13 R. S. Deelder, H. A. J. Linssen, A. P. Konijnendijk and J. L. M. van de Venne, *J. Chromatogr.*, 185 (1979) 241.
14 P. Jandera, J. Churáček and J. Bartosová, *Chromatographia*, in press.
15 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 192 (1980) 37.
16 I. Halász and I. Sebestian, *Chromatographia*, 7 (1974) 371.
17 I. Halász and J. Asshauer, *J. Chromatogr. Sci.*, 12 (1974) 139.
18 H. Leidner, R. Gloor and K. Wuhrmann, *Tenside Deterg.*, 13 (1976) 122.
19 A. Nakae and K. Kunihiro, *J. Chromatogr.*, 152 (1978) 137.
20 S. Hashimoto, K. Sakurai and T. Nagai, *Bunseki Kagaku (Jap. Anal.)*, 25 (1976) 639.
21 K. Ikebe, Y. Tanaka, R. Tanaka and N. Kunita, *Shokuhin Eisei Hen*, 7 (1976) 31.
22 P. W. Taylor and G. Nickless, *J. Chromatogr.*, 178 (1979) 259.