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SEPARATION OF SODIUM SALTS OF PHENYLSULPHAMIC ACIDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING STRONG ELECTROLYTES

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SUMMARY

Sodium salts of phenylsulphamic acids have been separated on a μ Bondapak C₁₈ column using aqueous solutions of methanol and acetonitrile containing strong electrolytes. A study has been carried out on the effect of various salts on the separations. In addition, the effects of different amounts of salt and methanol concentration on the separations have been studied. The correlation of capacity factors with salt and methanol concentration is reported.

INTRODUCTION

The analysis of salts of sulphamic acids (RNHSO₃Na) is generally carried out by acid hydrolysis of the salt and determination of the liberated sulphate by titration¹ or by coupling of the liberated amines with 1,4-benzoquinone and subsequent determination by spectrophotometry^{2,3}. It was felt that high-performance liquid chromatography (HPLC) would be the method of choice for the separation and analysis of arylsulphamates, which would eliminate the use of an initial hydrolysis step. A report by Dubois and Stephenson⁴ described the use of HPLC to determine yields in the synthesis of sulphamic acids and their esters.

A survey of the literature showed that the separation of sulphonic acids by HPLC has been the subject of intense study. Ion-exchange chromatography has been used with some success⁵, but the major separations of sulphonic acids have been carried out using ion-pair chromatography, using normal-phase⁶⁻⁹ and reversed-phase techniques¹⁰⁻¹⁷. More recently, Jandera *et al.*¹⁸ showed that addition of a strong electrolyte to aqueous solutions without the use of ion-pairing reagents leads to very good separations of sulphonic acids. Accordingly, a systematic study was carried out to investigate the feasibility of separating phenylsulphamates using strong electrolytes in aqueous solutions by reversed-phase HPLC.

EXPERIMENTAL

Apparatus

All liquid chromatographic experiments were carried out on a Waters high-performance liquid chromatograph using a variable-wavelength UV detector (Waters Model 450). The chromatograph was equipped with a Waters Model U6K universal injector.

The separation column was a μ Bondapak C_{18} column (30 cm \times 3.9 mm I.D.) (Waters). Samples were injected into the system using a Hamilton microlitre syringe. The void volume (V_0) of the system was determined using uracil as substrate with 100% methanol as the mobile phase, $V_0 = 2.99 \text{ cm}^3$. All measurements were made at a wavelength of 254 nm.

Materials

The sodium salts of arylsulphamic acids were prepared and isolated from the corresponding amine and chlorosulphonic acid by the method of Audrieth and Sveda¹⁹. The compounds were recrystallized from 95% ethanol until they were free from occluded chloride and sulphate ions.

The mobile phases were prepared by mixing distilled water with methanol or acetonitrile in the required volume ratio. The electrolytes (analytical-reagent grade) were then dissolved in the mixed solvent. Methanol and acetonitrile were of HPLC grade (Fisons). All solutions were filtered through a Millipore 0.45- μm filter prior to analysis.

RESULTS AND DISCUSSION

Sulphonic and carboxylic acids are eluted unretained when chromatographed on reversed-phase columns using water or aqueous solutions of organic solvents¹⁴. In this work phenylsulphamates, when chromatographed using mobile phases containing no dissolved salt, were eluted with retention volumes slightly less than V_0 . Preliminary experiments showed, however, that when phenylsulphamates were chromatographed with mobile phases containing sodium sulphate and 20% methanol the retention volumes increased significantly.

In order to study the influence of the nature of the salt in the mobile phase upon separation, three salts (1, phenylsulphamate; 2, N-methylphenylsulphamate; and 3, *p*-bromophenylsulphamate) were chromatographed on a C_{18} column, using mobile phases containing different salts dissolved in 20% methanol at a concentration of 0.05 *M*. The retention volumes and relative retention values are listed in Table I. It can be seen that the nature of the salt does not affect to any great extent the separation and retention values of the three compounds. The compounds were separated satisfactorily for all salts used and typical separations are shown in Fig. 1. The retention volumes of the sulphamates increases slightly in the order nitrite > carbonate > nitrate > hydrogen phosphate > sulphate > formate > acetate. The nature of the cation of the salt does not affect the retention volume of the compounds to any great extent. When compared with Li^+ and K^+ ions, Na^+ ions in the mobile phase give slightly longer retention volumes for compounds 1, 2 and 3.

A brief study on the comparison of the effects of acetonitrile and methanol on

TABLE I

RETENTION VOLUMES, V_R , AND RELATIVE RETENTION VALUES OF (1) PHENYLSULPHAMATE, (2) N-METHYLPHENYLSULPHAMATE AND (3) *p*-BROMOPHENYLSULPHAMATE ON A C_{18} COLUMN (300 × 3.9 mm) IN DIFFERENT MOBILE PHASES

Mobile phase (20% v/v, methanol)	V_R			α	
	1	2	3	2/1	3/2
0.05 M Li_2SO_4	3.5	5.5	8.4	4.93	2.15
0.05 M Na_2SO_4	3.8	6.2	9.8	3.97	2.12
0.05 M K_2SO_4	3.6	5.6	8.5	4.27	2.11
0.05 M Na_2CO_3	3.5	5.5	8.3	4.02	2.11
0.05 M K_2CO_3	3.6	5.5	8.3	4.11	2.11
0.05 M KNO_3	3.5	5.3	7.8	4.52	2.07
0.05 M $NaNO_2$	3.2	4.7	6.9	8.15	2.28
0.05 M Na_2HPO_4	3.6	5.5	8.3	4.11	2.11
0.05 M $HCOONa$	3.8	6.5	10.8	4.34	2.22
0.05 M CH_3COONa	4.1	6.8	11.0	3.43	2.09

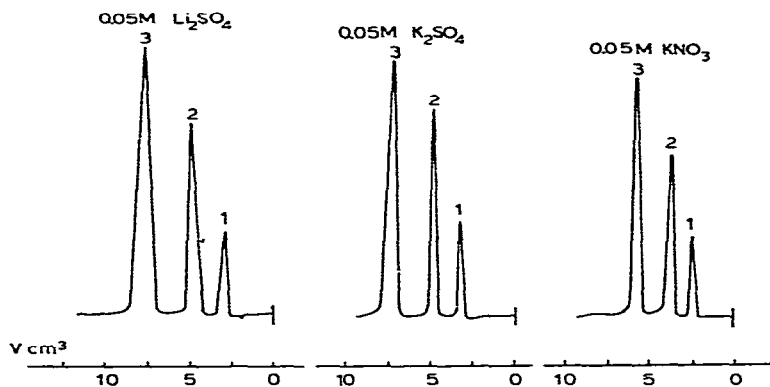


Fig. 1. Reversed-phase separations of a mixture of phenylsulphamates using mobile phases containing 20% (v/v) methanol with different salts on a μ Bondapak C_{18} column at a flow-rate of 2.5 ml/min: (1) phenylsulphamate; (2) N-methylphenylsulphamate; (3) *p*-bromophenylsulphamate.

TABLE II

COMPARISON OF RETENTION VOLUMES, V_R , AND CAPACITY FACTORS, k' , OF (1) PHENYLSULPHAMATE, (2) N-METHYLPHENYLSULPHAMATE AND (3) *p*-BROMOPHENYLSULPHAMATE FOR AQUEOUS SOLUTIONS OF ACETONITRILE AND METHANOL USING 0.05 M Na_2SO_4

Mobile phase	$V_R(1)$	$k'(1)$	$V_R(2)$	$k'(2)$	$V_R(3)$	$k'(3)$
10% Methanol	4.7	0.58	7.2	1.42	17.7	4.93
20% Methanol	5.3	0.75	11.9	2.96	18.3	5.15
10% Acetonitrile	4.5	0.50	7.2	1.42	12.2	3.09

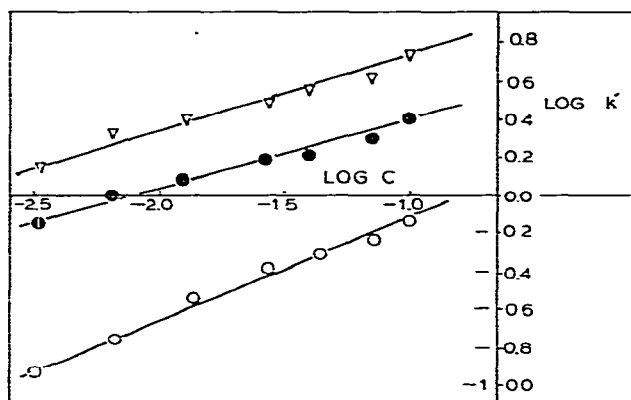


Fig. 2. Plot of $\log k'$ of phenylsulphamates as a function of the molarity of added electrolyte (Na_2SO_4). Column: $\mu\text{Bondapak C}_{18}$ with a mobile phase of 20% (v/v) methanol with varying Na_2SO_4 concentration. Flow-rate: 2.5 ml/min. ○ = Phenylsulphamate; ● = N-methylphenylsulphamate; ▽ = *p*-bromophenylsulphamate.

the separation of the three salts (1, 2 and 3) was carried out and the results are given in Table II. The results show that for salts 1 and 2 a lower acetonitrile content is required in order to achieve a separation similar to that obtained with methanol. However, a shorter retention volume overall for *p*-bromophenylsulphamate is observed when acetonitrile is used in the mobile phase. Methanol and sodium sulphate were used in the mobile phase for the remainder of the work for purposes of comparison where possible with the work on sulphonic acids.

It was felt that the influence of the concentration of the dissolved salt in the mobile phase on the separation of phenylsulphamates was important from the point of view of correct operating conditions. Accordingly, the retention volumes and capacity factors for the three compounds (1, 2 and 3) were measured in mobile phases containing different molarities of sodium sulphate in 15% (v/v) methanol. It was observed that the three compounds gave $V_R < V'_0$ when no sodium sulphate was incorporated into the mobile phase. In addition, each sulphamate gave asymmetric peaks. However, the retention volume and separation of the phenylsulphamates increased with increasing concentration of sodium sulphate in the mobile phase ($3 \cdot 10^{-3}$ to $1 \cdot 10^{-1}$ M). The data obtained can be plotted as $\log k'$ versus $\log C$, as shown in Fig. 2. It can be seen that each sulphamate gives a straight line, for the concentration range of salt used, with good linear regression coefficients. Horváth *et al.*¹⁵ have shown for ionized substances that the capacity ratios increase with increasing ionic strength and the following equation holds:

$$\ln k' = \ln k'_0 + AI + B(1)^{1/3} \quad (1)$$

where A and B are constants for a given solute solvent and column, respectively. Jandera *et al.*¹⁸ argue that for sulphonic acids where $k'_0 < 0$ eqn. 1 does not hold except perhaps at high salt concentrations. They found that the data for sulphonic acids (with $k' > 0$) with varying salt concentrations in the mobile phase can be correlated by the following empirical equation:

$$\ln k' = A + B \ln C \quad (2)$$

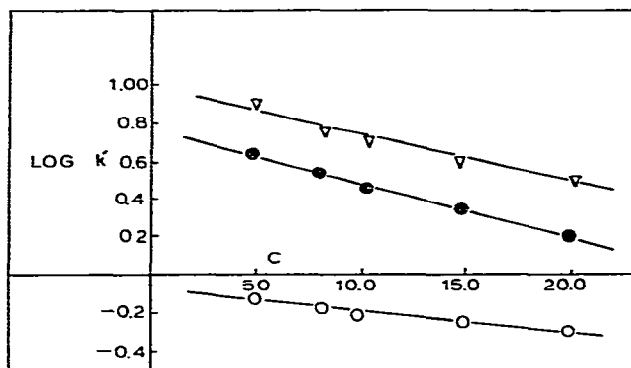


Fig. 3. Plots of $\log k'$ of phenylsulfamates as a function of methanol in the mobile phase. Column, μ Bondapak C_{18} ; mobile phase, 0.05 M Na_2SO_4 with methanol content varying from 5 to 20%; flow-rate, 2.5 ml/min. ○ = Phenylsulfamate; ● = N-methylphenylsulfamate; ▽ = *p*-bromophenylsulfamate.

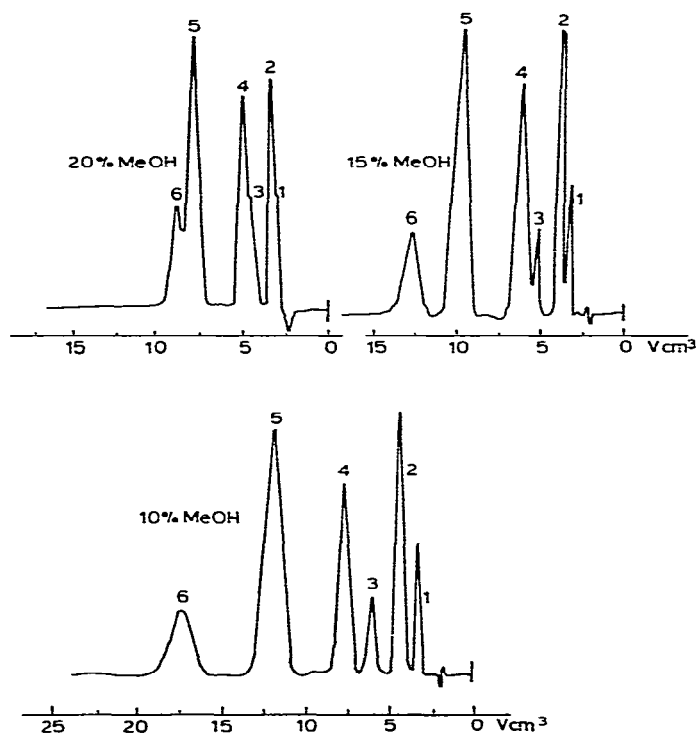


Fig. 4. Effect of methanol (MeOH) at constant salt concentration on the separation of six phenylsulfamates using 0.05 M Na_2SO_4 at a flow-rate of 2.5 ml/min. Column: μ Bondapak C_{18} . 1 = Phenylsulfamate; 2 = *p*-anisidinesulfamate; 3 = *p*-methylphenylsulfamate; 4 = N-methylphenylsulfamate; 5 = *p*-bromophenylsulfamate; 6 = N-ethylphenylsulfamate.

The results for the phenylsulphamates (Fig. 2) can be described by the same equation, and thus at least for the concentration range studied the influence of salt concentration on the capacity factors can be described (C = concentration of methanol).

A study was also carried out on the effect of varying the methanol concentration, while maintaining the salt concentration constant, on the separation of phenylsulphamates. Mobile phases with methanol varying from 5 to 20% (v/v) containing sodium sulphate at 0.05 M were used to separate the three acids used above. The data obtained were plotted as $\log k'$ versus C and the results are shown in Fig. 3. It can be seen that $\log k'$ decreases linearly with increasing concentration in the mobile phase. A similar behaviour is observed for sulphonic acids¹⁸ and in general it resembles the pattern for organic acids separated by ion-pair reversed-phase chromatography¹⁴. The effect of methanol concentration on the separation of six phenylsulphamates is shown in Fig. 4 using a constant salt concentration (0.05 M sodium sulphate). It can be seen that a decrease in the methanol concentration to 10% (v/v) results in the complete separation of all six compounds.

In conclusion, sodium salts of phenylsulphamic acids can be separated by reversed-phase HPLC using acetonitrile or methanol solutions containing strong electrolytes. The degree of separation is to a large extent independent of the nature of the salt used. The technique can be used to analyse arylsulphamates directly without any initial hydrolysis step.

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