

CHROM. 7317

ION-EXCLUSION CHROMATOGRAPHY OF SOME AROMATIC ACIDS

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SUMMARY

Mixtures of some aromatic monocarboxylic acids (benzoic, salicylic and *p*-hydroxybenzoic), dicarboxylic acids (phthalic, isophthalic and terephthalic) and tricarboxylic acids (trimellitic and trimesic) were separated by ion-exchange chromatography on Dowex 50W using the ion-exclusion technique.

The effect of cross-linking of the resin on the efficiency of the separation and also on the volume distribution coefficients of the acids was examined. It was found that the most efficient separation can be achieved by using Dowex 50W-X2, 200-400 mesh. The values found for the volume distribution coefficients of the acids decrease with increasing degree of cross-linking in most instances.

The ion-exclusion method was applied to the determination of benzoic acid as a preservative in mustard. The advantage of the method is that an aqueous extract of mustard can be analyzed directly, without the need for an additional work-up, in a relatively short time (65 min) with an accuracy of $\pm 6.5\%$.

INTRODUCTION

The use of ion-exclusion chromatography for the separation of mixtures of organic acids has been described by Harlow and Morman¹, who examined the effect of the cross-linking and mesh size of the resin and the length of the column on the efficiency of the separation of a standard mixture of formic, acetic and propionic acids. Having determined the conditions of separation, they applied the method to the separation of several mixtures of organic acids and determined the distribution coefficients of 58 acids.

The separation and the determination of some aliphatic acids using the ion-exclusion method was described by Czerwinski². The error in the determination of adipic acid in a mixture was 4.5%.

The method was developed for the determination of trace amounts of aliphatic organic acids in silage by Goodman *et al.*³. The accuracy of the method was $\pm 6\%$.

In the present work, the separation of mixtures of some aromatic acids by the ion-exclusion method has been studied. The aim of the work was to investigate the possibility of determining benzoic acid in complicated mixtures of substances.

The determination of benzoic acid, which is a widely used preservative in the food industry, has been described by a number of workers. Among the most up-to-

date methods applied are paper chromatography⁴, gas-liquid chromatography⁵ and gel filtration^{6,7}. The use of the ion-exclusion method for the determination of benzoic acid has the particular advantage that an aqueous extract can be analyzed directly without the need for an additional work-up.

EXPERIMENTAL

Columns (49.8 × 1.02 cm) of Dowex 50W-X2, -X4, -X8 and -X12 were packed in the usual manner and were maintained at 25° throughout the work. The closures of the columns were made as recommended by Thompson⁸. An aqueous solution of free aromatic acids was fed in using a piston-equipped screw-operated microburette. The individual acids in the eluate were detected by continuous conductivity measurements on the eluate, which was most conveniently accomplished by placing a 10-cm deionization column before the working column in order to keep the conductivity of the eluate at the lowest possible level. The flow conductivity micro-vessel consisted of two stainless-steel capillaries, which served as electrodes, joined together with a polyethylene tube. The distance between the electrodes was 1 mm, thus making the volume of the micro-vessel 0.3 μl. The electrical connection of the conductivity micro-vessel with another conductivity vessel of the same type, through which deionized water passed before entering the column, was made according to the Wickbold bridge arrangement⁹.

The change in the conductivity compared with the conductivity of deionized water was continuously registered using a recorder with a range of 2 mV per 25 cm.

Benzoic acid was determined in an aqueous (200 ml) extract of mustard (21.3083 g) manufactured by SKL (Liptovský Mikuláš, Czechoslovakia). The volume of the sample was 394.8 μl and the flow-rate of the deionized water was 1.65 ml/min. A typical separation of benzoic acid from other components in the analyzed mixture is shown in Fig. 1. Quantitation was carried out by standard additions of benzoic acid to the sample and the chromatograms were evaluated by using a planimeter.

RESULTS AND DISCUSSION

The separation of mixtures of aromatic acids can be seen in Fig. 2 (benzoic, salicylic and *p*-hydroxybenzoic), Fig. 3 (phthalic, isophthalic and terephthalic) and Fig. 4 (trimellitic and trimesic).

The effect of cross-linking of the Dowex 50W cation-exchange resins on the volume distribution coefficients was also investigated. The volume distribution coefficient (K_v) was calculated according to the equation

$$K_v = \frac{V_{\max.}}{X} - \varepsilon$$

where $V_{\max.}$ is the elution volume of the acid, X is the total bed volume and ε is the dead volume in 1 ml of the bed (determined according to the elution volume of hydrochloric acid). As the volume distribution coefficient also depends on the amount of the acid being analyzed, the values of K_v for different amounts of the acids are also given in Table I.

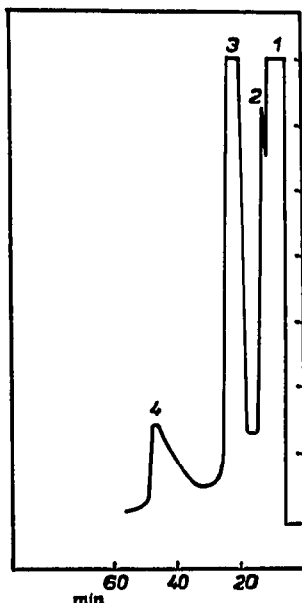


Fig. 1. Determination of benzoic acid in mustard on Dowex 50W-X2, 200-400 mesh. Column size, 49.8×1.02 cm; flow-rate, 1.65 ml/min; sample size, 394.8 μ l. 1, 2 and 3 = Unidentified acids; 4 = benzoic acid (the area under the elution curve = 2.860 cm^2).

The best separation of mixtures of aromatic acids was achieved using the cation-exchange resin Dowex 50W-X2, 200-400 mesh.

It can be seen from Table I that the K_p values of aromatic acids are higher than would be expected from their dissociation constants and similar values for aliphatic acids¹⁰. This can be explained by two different ways:

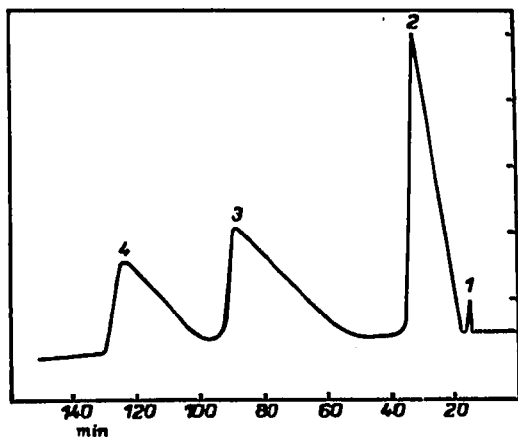


Fig. 2. Separation of some monocarboxylic aromatic acids on Dowex 50W-X2, 200-400 mesh, cation-exchange resin. Column size, 49.8×1.02 cm; flow-rate, 0.95 ml/min; amount of sample, 59.2 μ g per 180 μ l. 1 = Unidentified acid; 2 = salicylic acid; 3 = benzoic acid; 4 = *p*-hydroxybenzoic acid.

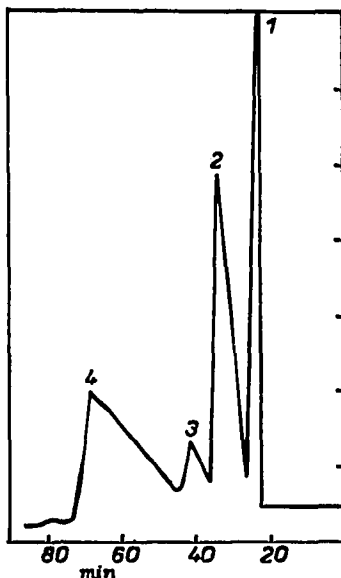


Fig. 3. Separation of some dicarboxylic aromatic acids on Dowex 50W-X2, 200-400 mesh, cation-exchange resin. Column size, 49.8×1.02 cm; flow-rate, 0.6 ml/min; sample size, 590 μ l. 1 = Unidentified acid; 2 = phthalic acid (19.7 μ g); 3 = terephthalic acid (9.9 μ g); 4 = isophthalic acid (19.7 μ g).

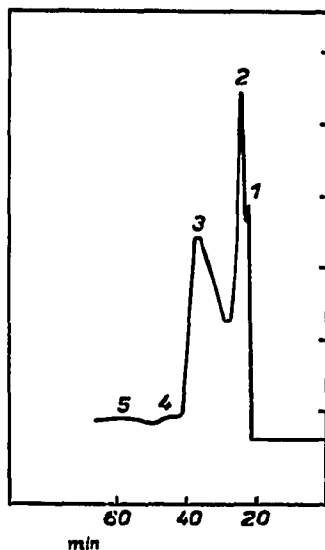


Fig. 4. Separation of some tricarboxylic aromatic acids on Dowex 50W-X2, 200-400 mesh, cation-exchange resin. Column size, 49.8×1.02 cm; flow-rate, 0.6 ml/min; sample size, 393 μ l. 1 = Unidentified acid; 2 = trimellitic acid (19.7 μ g); 3 = trimesic acid (19.7 μ g); 4 and 5 = unidentified acids.

TABLE I
VOLUME DISTRIBUTION COEFFICIENTS OF DIFFERENT AMOUNTS OF AROMATIC ACIDS

Acid	Amount of acid (μg)	Cross-linking			
		X2	X4	X8	X12
<i>p</i> -Hydroxybenzoic	19.7	2.26	—*	—	—
	29.8	2.31	—	—	—
	39.5	2.43	—	—	—
Benzoic	19.7	1.51	—	—	—
	29.8	1.57	—	—	—
	39.5	1.74	—	—	—
Terephthalic	19.7	0.31	0.05	0.03	0.03
	29.8	0.35	0.06	0.04	0.03
	39.5	0.39	0.06	0.04	0.03
Isophthalic	19.7	0.72	0.74	0.25	0.45
	29.8	0.82	0.77	0.34	0.46
	39.5	0.89	0.81	0.38	0.47
Trimesic	19.7	0.28	0.24	0.08	0.08
	29.8	0.34	0.30	0.09	0.09
	39.5	0.39	0.36	0.10	0.10
Salicylic	19.7	0.38	0.46	0.19	0.26
	29.8	0.43	0.48	0.21	0.31
	39.5	0.48	0.51	0.24	0.35
Phthalic	19.7	0.21	0.22	0.08	0.08
	29.8	0.24	0.24	0.09	0.09
	39.5	0.27	0.26	0.10	0.10
Trimellitic	19.7	0.09	0.09	0.03	0.03
	29.8	0.12	0.11	0.03	0.03
	39.5	0.12	0.12	0.04	0.04

* — denotes that, owing to considerable striking (unsharp peaks), the elution volumes could not be determined.

(1) by considering the absorption phenomena probably caused by the interaction of π -electrons of the benzene ring of the acid with those of the ion exchanger;

(2) by taking into account the solubility of the aromatic acids, which is, owing to the organic character of the resin¹¹, apparently greater in the inner phase of the ion exchanger than in the outer aqueous phase. This effect is more pronounced in the case of weaker aromatic acids, *e.g.* benzoic and *p*-hydroxybenzoic acids.

The volume distribution coefficient is not strictly dependent on the dissociation constants of the acids, unlike the case with aliphatic mono- and dicarboxylic acids¹⁰. Although some decrease in the volume distribution coefficients with a decrease in the dissociation constants occurs, there are exceptions (terephthalic and trimesic acids) to which this rule does not apply.

The effect of the degree of cross-linking of the resin on the K_v values for most aromatic acids is similar to that for aliphatic acids. Isophthalic and salicylic acids behave differently, their K_v values on Dowex 50W-X4 and -X12, for so far experimentally unexplained reasons, show a tendency to increase.

The results of the determination of benzoic acid in an aqueous extract of mustard show a relative accuracy of $\pm 6.3\%$ at the level of 0.12% of benzoic acid.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Hronec for preparing the aromatic acid samples.

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