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SEPARATION OF ALIPHATIC CARBOXYLIC ACIDS BY ION-EXCLUSION CHROMATOGRAPHY USING A WEAK-ACID ELUENT

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SUMMARY

The chromatographic behavior of twelve aliphatic carboxylic acids was studied on a 5- μm cation-exchange resin column in the H^+ -form. A conductivity detector was used without any chemical suppression column. A 0.5 mM solution of benzoic acid or succinic acid was shown to be a suitable eluent. The background conductance of these eluents is much lower than the strongly acidic eluents commonly used and permits a more sensitive detection of the sample acids. Addition of 5% acetonitrile to the eluent greatly reduces the retention times of the later-eluting carboxylic acids and gives sharper peaks.

INTRODUCTION

Ion-exclusion chromatography is a technique for the separation of organic and inorganic weak acids, especially those of a hydrophilic nature. Earlier work in ion-exclusion chromatography has been attributed primarily to Wheaton and Bauman¹. The technique has been gaining in popularity, and a number of recent studies have been published by several workers²⁻⁵.

Typically, a high-capacity cation-exchange resin in the H^+ -form is used in ion-exclusion chromatography. A solution of sulfuric or hydrochloric acid is generally used as the eluent for the separation of weak acids. This is done to repress the ionization of the sample acids and to ensure that they are entirely in their molecular forms. However, when a conductivity detector is employed, the acidic eluent causes a rather high background signal and reduces the ability to detect sample acids. UV spectrophotometric detection is sometimes used⁶, but many aliphatic carboxylic acids absorb only weakly and their absorbance is often at the very low end of the UV spectral region.

A unique suppression system has been developed to reduce the background conductance when a hydrochloric acid eluent is used⁷. A cation-exchange column in the Ag^+ -form removes H^+ by exchange with Ag^+ and chloride by precipitation as silver chloride. However the precipitate tends to plug-up the column and greatly increase back pressure. Ito and Shinbori⁸ have proposed the use of a carbonic acid eluent for ion-exclusion chromatography with a conductivity detector.

In the present work a high-performance separation of various aliphatic carboxylic acids is achieved by using a strongly-acidic cation-exchange resin ($5\ \mu\text{m}$) in the H^+ -form with a conductivity detector. It is shown that excellent resolution and a very sensitive detection of aliphatic carboxylic acids is obtained using $0.5\ \text{mM}$ benzoic acid or $0.5\ \text{mM}$ succinic acid as the eluent. These eluents have a much lower background conductance than the strong-acid eluents previously used. It is also shown that incorporation of a small amount of acetonitrile or methanol in the eluent will reduce the adsorption of some carboxylic acids and speed up their elution.

EXPERIMENTAL

Apparatus

The liquid chromatograph used in this study was constructed in a modular fashion from individual components. The pump was a Milton-Roy Mini-Pump with single plunger (Laboratory Data Control, Riviera Beach, FL, U.S.A.). The eluent flow-rate for all work was $1.0\ \text{ml/min}$.

A Hitachi (Tokyo, Japan) 6-way valve injector with a $100\text{-}\mu\text{l}$ PTFE sample loop was used for all separations. A coil type pulse dampner Lichroma (Norrstown, PA, U.S.A.) Damp III, was placed between the pump and the sample injector to reduce the pump pulsation. The detector was a Wescan (Santa Clara, CA, U.S.A.) Model 213 conductivity detector. The recorder was a Fisher Scientific (Austin, TX, U.S.A.) Model 5000 strip chart recorder with 10-mV input. The separation columns were a TSK (Tokyo, Japan) glass column $20\ \text{cm} \times 8\ \text{mm}$ I.D.; "long column" and a plastic column $10\ \text{cm} \times 7.5\ \text{mm}$ I.D.; "short column".

The columns were packed using the slurry packing technique and equilibrated thoroughly with the eluent before chromatographic run. All fittings in contact with the eluent were either glass, PTFE, Kel-F or 316 stainless steel.

Column

A TSK polystyrene-divinylbenzene co-polymer based strongly acidic cation-exchange resin with $5\text{-}\mu\text{m}$ particle size and an exchange capacity of $4.2\ \text{mequiv./g}$ was used for all chromatographic runs.

Reagents and solutions

Standard solutions of twelve aliphatic carboxylic acids or salts were prepared from reagent-grade chemicals without further purification.

The eluents containing the acids with various $\text{p}K_{\text{a}1}$ values and some organic solvents were prepared by dissolving the acids in distilled, deionized water and filtering through a $0.45\text{-}\mu\text{m}$ membrane filter.

RESULTS AND DISCUSSION

Eluents and column efficiency

A major goal of this research was to find an eluent that is sufficiently acidic to repress the ionization of organic acids to give sharp chromatographic peaks without a high background conductance from the eluent itself. Accordingly, water alone and dilute aqueous solutions of sulfuric acid, phosphoric acid, salicylic acid, benzoic acid

and carbonic acid were compared as eluents for the separation of various aliphatic carboxylic acids by ion-exclusion chromatography.

Fig. 1 shows a complete separation of sulfuric acid and C_1 - C_5 aliphatic carboxylic acids with water as the eluent, but the separation is very slow and the peaks are unsymmetric.

The results were only slightly better with a dilute carbonic acid eluent. However, Fig. 2 shows that a faster separation is obtained with symmetric peaks using 5 mM benzoic acid as the eluent. Reasonable detection sensitivity can be obtained with this eluent by using a more sensitive setting for the conductivity detector. The detection sensitivity for C_1 - C_5 carboxylic acids with salicylic acid, phosphoric acid or sulfuric acid is very poor owing to the much higher background conductivity of these eluents.

Fig. 3 shows a separation of more polar carboxylic acids with 0.5 mM benzoic acid as the eluent. The mixture of seven acids is completely resolved except for lactic and formic acids, which have almost the same retention times.

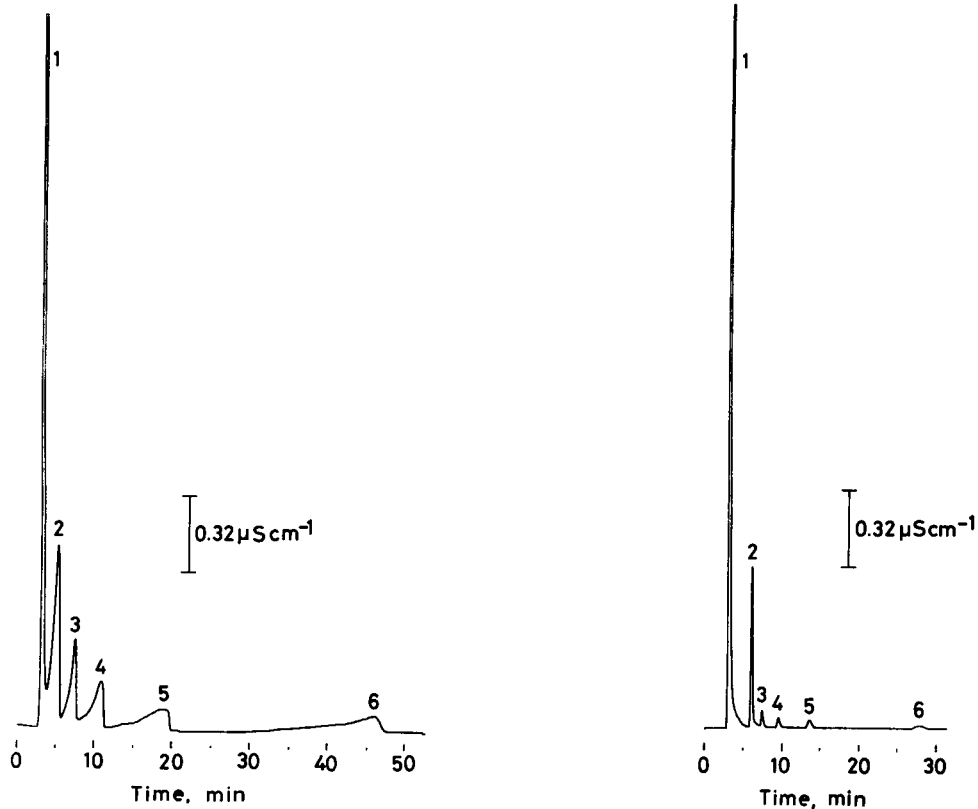


Fig. 1. Ion-exclusion chromatogram using water alone as the eluent. Peak identification: 1 = sulfuric acid; 2 = formic acid; 3 = acetic acid; 4 = propionic acid; 5 = butyric acid; 6 = valeric acid. Column was TSK gel, H^+ form, 20 cm \times 8 mm I.D. Sample acids were 1 mM each and a 100- μ l sample loop was used. Eluent flow-rate was 1.0 ml/min.

Fig. 2. Ion-exclusion chromatogram using 5.0 mM benzoic acid as the eluent. Peak identification and chromatographic conditions were the same as in Fig. 1.

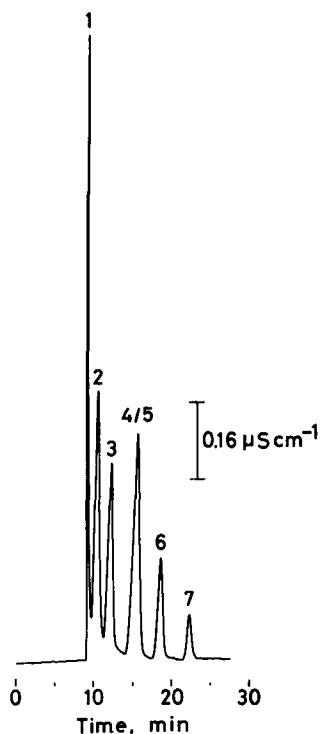


Fig. 3. Separation of (1) oxalic acid, (2) malonic acid, (3) malic acid, (4) lactic acid, (5) formic acid, (6) succinic acid and (7) acetic acid with 0.5 mM benzoic acid on the long and short column in series. Other chromatographic conditions were the same as in Fig. 1.

Retention volumes of sulfuric acid and C_1 – C_5 carboxylic acids are given in Table I for various eluents. Sulfuric acid is completely ionized and has the same retention volume for all of the eluents. Butyric acid and valeric acid have very long

TABLE I

COMPARISON OF RETENTION VOLUMES OF CARBOXYLIC ACIDS BY ELUTION WITH VARIOUS ACID ELUENTS

5 mM solutions of the eluent were used. N.D. = Not detected; neg. = negative peak.

Eluent	pK_{a1} of acid	Retention volume (ml)					
		Sulfuric acid	Formic acid	Acetic acid	Propionic acid	Butyric acid	Valeric acid
Water alone	15.7	3.2	5.1	7.5	10.8	19.2	46.2
Sulfuric acid–water	–2	3.2 (neg.)	7.8	11.0	13.3	N.D. (∞)	N.D. (∞)
Phosphoric acid–water	2.2	3.2	7.4	8.6	12.3	N.D. (∞)	N.D. (∞)
Salicylic acid–water	3.0	3.2	6.7	7.9	15.7	30.3	N.D. (∞)
Benzoic acid–water	4.2	3.2	6.4	7.9	10.2	25.0	27.4
Carbonic acid–water*	6.4	3.2	5.2	7.7	11.5	19.1	48.8

* Carbonic acid was produced continuously by passing sodium bicarbonate through a cation-exchange column.

TABLE II

COMPARISON OF COLUMN EFFICIENCIES OF FORMIC ACID USING VARIOUS ACID ELUENTS (5 mM ACID)

 N = Number of theoretical plates; HETP = height equivalent to a theoretical plate.

<i>Eluent</i>	<i>N</i>	<i>HETP (mm)</i>
Water alone	130	1.54
Sulfuric acid–water	2570	0.08
Phosphoric acid–water	2500	0.08
Salicylic acid–water	2330	0.09
Benzoic acid–water	2350	0.085
Carbonic acid–water	320	0.62

retention volumes with the strong and moderately strong acid eluents and could not be detected. The retention times of propionic acid, butyric acid and valeric acid were all shorter with benzoic acid than with any other eluent tested.

Column efficiency is compared in Table II. The number of theoretical plates, calculated from the formic acid peak, is almost identical for the acidic eluents, but is very low for water and carbonic acid eluents.

Detector response

The background conductances of water and 5.0 mM solutions of various acids are compared in Table III. It will be seen that the background conductance of benzoic acid is by far the lowest of the acidic eluents, except for carbonic acid. This table also shows a higher detector response for formic acid with benzoic acid as the eluent. Acetic acid is a weaker acid and has a lower detector response than formic acid, but the response is again higher with benzoic acid as the eluent.

Effect of benzoic acid concentration

Retention volumes were compared for C₁–C₅ alkylcarboxylic acids using 0.1, 0.5, 1.0, 5.0 and 10 mM benzoic acid eluents. There was almost no change in the

TABLE III

COMPARISON OF ELUENT BACKGROUND CONDUCTANCE AND RELATIVE DETECTOR RESPONSES OF FORMIC AND ACETIC ACID

<i>Eluent</i>	<i>Conductance</i> ($\mu\text{S cm}^{-1}$)	<i>Relative detector response (peak height)</i>	
		<i>Formic acid</i> ($pK_a = 3.8$)	<i>Acetic acid</i> ($pK_a = 4.8$)
Water	0.04	1.00	0.44
Sulfuric acid–water	81.6	0.15	0.02
Phosphoric acid–water	33.6	0.36	0.03
Salicylic acid–water	16.3	0.34	0.03
Benzoic acid–water	5.6	0.86	0.09
Carbonic acid–water	0.5	0.90	0.26

retention volumes of formic, acetic and propionic acids. The retention time of butyric acid decreased slightly and the retention time of valeric acid decreased significantly as the benzoic acid concentration was increased. A benzoic acid concentration of 0.5 mM was judged to be the optimum for separating these acids.

The background conductance of benzoic acid increased from about 0.41 μS at 0.1 mM to 6.8 μS at 10.0 mM benzoic acid; it was 1.2 μS at 0.5 mM benzoic acid. Detector responses for C_1 – C_5 carboxylic acids decrease somewhat as the benzoic acid concentration increases. Again, 0.5 mM benzoic acid seems to be a reasonable eluent concentration.

Retention volumes and distribution coefficients

The retention volumes, distribution coefficients (K_d), sensitivity ($\mu\text{S cm}^{-1}/\text{mM}$) and detection limits for elution with 0.5 mM benzoic acid are listed in Table IV. The distribution coefficients were calculated according to the following equation, which applies to pure ion-exclusion chromatography:

$$V_R = V_0 + K_d V_i$$

where V_R is the retention volume, V_0 the column void volume and V_i the volume of liquid inside the resin in the column.

From previous work³ it is evident that the retention volume of a strong acid (sulfuric acid) is ion-excluded completely from the resin and therefore has $K_d = 0.0$ and a retention volume equal to V_0 . A very weak acid (carbonic acid) penetrates completely into the resin phase and therefore has a K_d value of 1.0 and a retention volume equal to $V_0 + V_i$. Knowing V_0 and V_i , the K_d values of other acids can be calculated from their retention volumes.

TABLE IV

RETENTION VOLUMES, DISTRIBUTION COEFFICIENTS, AND DETECTOR RESPONSES OF CARBOXYLIC ACIDS BY ELUTION WITH 0.5 mM BENZOIC ACID

<i>Acid</i>	pK_{a1}	V_R (ml)	K_d	<i>Detector response</i> ($\mu\text{S cm}^{-1}/\text{mM}$)	<i>Detection limit</i> ($S/N = 2^*$) (μM)
Sulfuric acid	-2	3.2	0	8.56	0.16
Formic acid	3.8	5.4	0.36	1.09	1.28
Acetic acid	4.8	7.4	0.70	0.27	5.19
Propionic acid	4.9	10.1	1.15	0.12	11.7
Butyric acid	4.8	16.3	2.18	0.09	15.6
Valeric acid	4.8	34.3	5.18	0.03	46.7
Oxalic acid	1.3	3.3	0.02	4.60	0.30
Malic acid	1.9	4.2	0.17	1.97	0.71
Malonic acid	2.9	3.6	0.07	1.63	0.86
Tartaric acid	3.0	3.6	0.07	1.84	0.76
Citric acid	3.2	3.7	0.08	2.01	0.70
Lactic acid	3.9	5.2	0.33	0.61	2.30
Succinic acid	4.2	5.9	0.45	0.62	2.26
Carbonic acid	6.4	9.2	1.00	0.006	233

* Signal-to-noise level: 0.0007 $\mu\text{S cm}^{-1}$.

As can be seen from Table IV, the V_R values of carboxylic acids tend to increase with increasing pK_{a1} and most of the K_d values are between zero and one. However, the K_d values of propionic, butyric and valeric acid are greater than one. This means that adsorption by the resin is a side effect due to the greater hydrophobic nature of these acids.

Effect of organic solvents

Addition of a water-miscible organic solvent to the eluent might be expected to reduce the adsorption effects of propionic, butyric and valeric acids and thus decrease their retention volumes. This was proven experimentally, as is shown in Fig. 4, where methanol was added to the eluent, and in Fig. 5 where acetonitrile was added to the eluent. Acetonitrile is somewhat more effective than methanol.

The separation of sulfuric acid and C_1 - C_5 carboxylic acids on the short column with 0.5 mM benzoic acid is shown in Fig. 6. The same separation, under identical conditions except for 5% (v/v) of acetonitrile added to the eluent, is shown in Fig. 7. Thus only 5% acetonitrile greatly shortens the separation time.

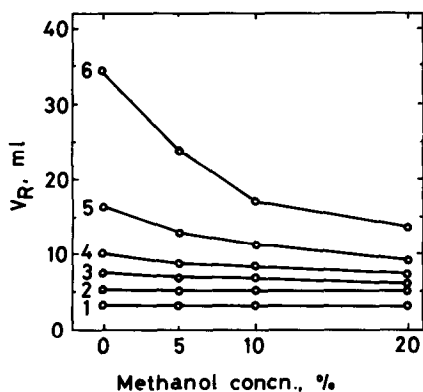


Fig. 4. Effect of methanol concentration of 0.5 mM benzoic acid on the retention volumes of various acids. Peak identification: 1 = sulfuric acid; 2 = formic acid; 3 = acetic acid; 4 = propionic acid; 5 = butyric acid; 6 = valeric acid.

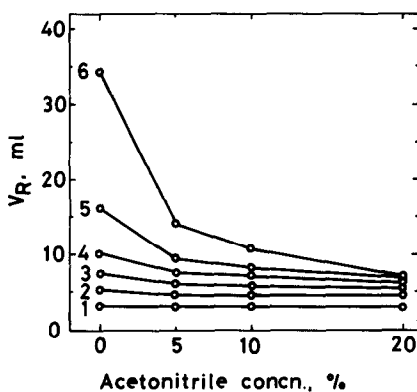


Fig. 5. Effect of acetonitrile concentration of 0.5 mM benzoic acid on retention volumes of various acids. See Fig. 4 for identification of the acids.

A negative "dip" was encountered between peaks 3 and 4 when 0.5 mM benzoic acid in 5% acetonitrile was used as the eluent and an aqueous sample was injected. However, this dip was eliminated by making the sample 0.5 mM in benzoic acid and 5% in acetonitrile.

Calibration curves

Samples containing varying concentrations of sulfuric acid and carboxylic acids were separated by elution with 0.5 mM benzoic acid in 5% acetonitrile using the short column. The various peak heights were measured and plotted against the acid concentration. As shown in Fig. 8, the plot for sulfuric acid was linear over the entire range covered, 0.1-5 mM. Formic acid curved off slightly above approximately

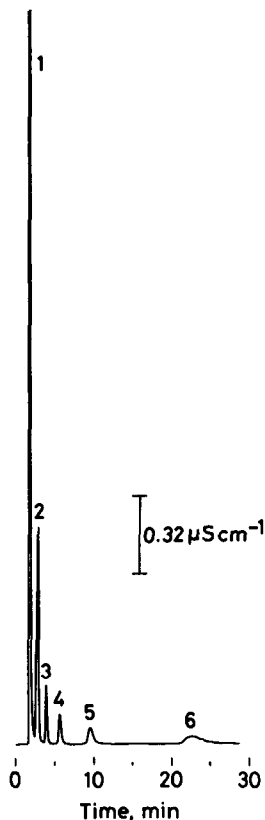


Fig. 6. Ion-exclusion chromatogram on the short column (10 cm \times 7.5 mm I.D.) using 0.5 mM benzoic acid eluent. Peak identification and other conditions were the same as in Fig. 1.

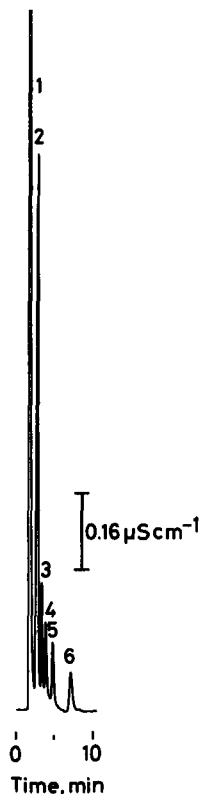


Fig. 7. Ion-exclusion chromatogram on the short column using 0.5 mM benzoic acid in 5% acetonitrile as the eluent. Peak identification and other conditions were the same as in Fig. 1.

1.0 mM. The plot for acetic acid was linear over almost the entire range. Propionic, butyric and valeric acid gave plots similar in linearity to that of acetic acid.

Succinic acid eluent

Although 0.5 mM benzoic acid, either in aqueous solution or in 5% aqueous acetonitrile, is an excellent eluent for ion-exclusion chromatography, 2 h or more is needed to condition a new column. A breakthrough curve showed that approximately 0.67 mg of benzoic acid is adsorbed per ml of wet resin by a cation-exchange column (20 cm \times 0.8 cm I.D.).

Fritz *et al.*⁹ noted that benzoic acid is adsorbed on the low-capacity resins used for cation-exchange chromatography, but that succinic acid and nicotinic acid (among others) are not appreciably adsorbed. Accordingly, 0.5 mM succinic acid was tried as an eluent for ion-exclusion chromatography. The breakthrough curve for this eluent indicated little if any adsorption, and a conditioning of the column used for ion-exclusion chromatography is complete within 5 min.

An excellent separation of sulfuric acid and C_1 - C_5 carboxylic acids was obtained on the short column using 0.5 mM succinic acid in 5% acetonitrile as the eluent (see Fig. 9). This chromatogram is very similar to that obtained with the benzoic eluent in 5% acetonitrile (see Fig. 7). When 0.5 mM succinic acid in entirely aqueous solution was used, the chromatogram was similar to that in Fig. 6 with aqueous benzoic acid.

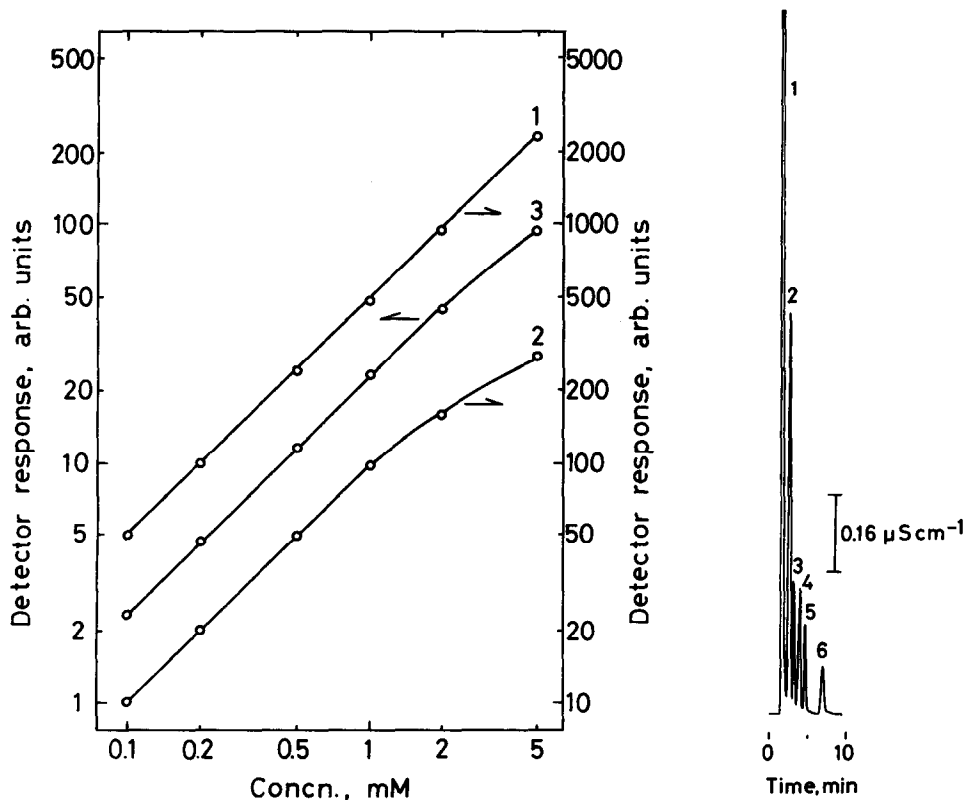


Fig. 8. Calibration curves of sulfuric and carboxylic acids by elution with 0.5 mM benzoic acid in 5% acetonitrile using a short column. 1 = Sulfuric acid; 2 = formic acid; 3 = acetic acid.

Fig. 9. Ion-exclusion chromatogram on the short column using 0.5 mM succinic acid in 5% acetonitrile as the eluent. Peak identification and other conditions were the same as in Fig. 1.

Comparison of separation columns

TSK SCX columns containing 5- μm resin particles were used for all of the work reported here, but two other types of columns were tested briefly. A Hitachi No. 2613 cation-exchange column containing 17- μm resin particles gave separations that were acceptable but not quite as good as the TSK column, which contained smaller particles. A Wescan No. 269-006 column was also satisfactory; it gave separations similar to those obtained with the TSK column. The Wescan column is longer (30 cm) and the retention times are correspondingly larger.

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