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Separation of carboxylic acids on a weakly acidic cation-exchange resin by ion-exclusion chromatography

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Abstract

The separation of various carboxylic acids was performed on a polymethacrylate-based weakly acidic cation-exchange resin (TSKgel OApak-A) using ion-exclusion chromatography under the acidic elution conditions. When a diluted sulfuric acid solution was used as the eluent, highly sensitive conductimetric detection of carboxylic acids was achieved without increasing the background conductance of the eluent. This method was more sensitive than using benzoic acid eluent and enabled a good resolution of dicarboxylic as well as monocarboxylic acids. The addition of 5–20% methanol to the eluent considerably reduced the retention times of carboxylic acids with hydrophobic nature. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; Carboxylic acids

1. Introduction

Ion-exclusion chromatography is a useful technique for the separation of organic and inorganic weak acids. Especially, ion-exclusion chromatography is very useful for the determination of weak organic anions in inorganic salt solutions, because inorganic anions are excluded from the resin pores via the ionic repulsion and are completely separated from the weak organic anions which can partially or entirely permeate into the pores. So far, ion-exclusion chromatography has been discussed in many papers [1–12].

Typically, a polystyrene–divinylbenzene (PS–DVB)-based strongly acidic cation-exchange resin in the H⁺-form has been employed in ion-exclusion

chromatography [1–7]. When water was used as the eluent, a chromatogram with fronted peaks was obtained for carboxylic acids such as valeric acid with hydrophobic nature [1–3]. Accordingly, an aqueous solution of a strong acid such as sulfuric acid is generally used as the eluent for the separation of weak acids [2,4,5]. This is done to repress the ionization of the sample acids and to ensure that they are entirely in their molecular forms. However, when conductivity detection is used, the acidic eluent causes a rather high background conductance and reduces the ability to detect sample acids. In single-column ion-exclusion chromatography with conductimetric detection, weak organic acids (benzoic and succinic acids), whose solutions applied low conductivity, were used as eluents [1,6].

The purpose of this study was to understand the characteristics of a polymethacrylate-based weakly acidic cation-exchange resin (TSKgel OApak-A) in

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ion-exclusion chromatography under the acidic elution conditions. As the results described above, highly sensitive detection was achieved for carboxylic acids by the combination of this column, aqueous benzoic acid eluent and conductivity detector. However, when a sulfuric acid solution of such low concentration (0.05–0.5 mmol/l), which could not markedly raise the background conductivity, was used as the eluent, the separation of carboxylic acids was more sensitive than using the benzoic acid eluent. Furthermore, in this method, high-performance separation of dicarboxylic acids as well as monocarboxylic acids was attained. For reducing the time of analysis, organic solvents (methanol, ethanol, propanol and acetonitrile) have been added to the eluent [1,3,6,7]. In this study, the remarkable de-

crease in retention times of the hydrophobic carboxylic acids such as valeric and caproic acids by addition of organic modifier (methanol) to the eluent was observed.

2. Experimental

2.1. Apparatus

The ion chromatograph consisted of a Hitachi 7000 series, a Hitachi L-7100 pump at a flow-rate of 1 ml/min, a Hitachi L-7300 column oven at 35°C, a Hitachi L-7200 autosampler with a 100- μ l sample loop, a Hitachi L-7470 conductivity detector and a Hitachi D-7500 Integrator.

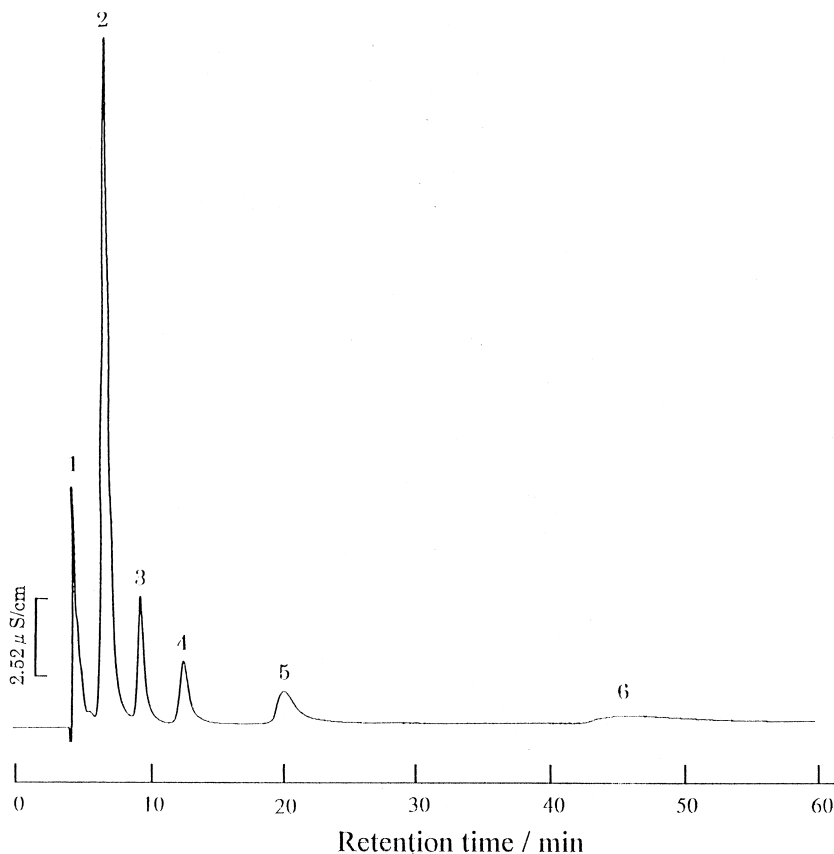


Fig. 1. Ion-exclusion chromatogram of hydrochloric and aliphatic acids by elution with 0.1 mmol/l H_2SO_4 . Column: TSKgel SCX PS-DVB-based strongly acidic cation-exchange resin, 300 mm \times 7.8 mm I.D.; column temperature: 35°C; flow-rate: 1.0 ml/min; detection: conductivity; injection volume: 100 μ l; sample concentration: 0.1 mmol/l for hydrochloric acid and 1.0 mmol/l for all aliphatic monocarboxylic acids. Peaks: 1=hydrochloric acid, 2=formic acid, 3=acetic acid, 4=propionic acid, 5=butyric acid, 6=valeric acid, 7=caproic acid.

2.2. Column

The separation column was a Tosoh glass column (300×7.8 mm I.D.) packed with a Tosoh TSKgel OApak-A polymethacrylate-based weakly acidic cation-exchange resin in the H⁺ form with a particle size of 5 μm. The exchange capacity was 0.1 mequiv./ml gel; but the information regarding the functional group and the cross-linkage was not provided by the manufacture. The column was equilibrated thoroughly with the eluent before the chromatographic run.

2.3. Reagents and solutions

Standard solutions of carboxylic acids and other inorganic acids were prepared from analytical-reagent grade chemicals without further purification.

Aqueous eluents were prepared as 0.05–1.0 mmol/l solutions by dissolving the acids (sulfuric

acid, benzoic acid) and methanol in distilled, deionized water. Aliphatic carboxylic acids were obtained from Kanto Chemicals (Tokyo, Japan) and the other acids and methanol from Wako Chemicals (Osaka, Japan).

3. Results and discussion

3.1. Effect of various eluents on ion-exclusion chromatographic separation of carboxylic acids

As reported in the previous papers [1–3], when water was used as the eluent, the resolution between carboxylic acids and the peak shape were not satisfactory. Turkelson and Richards [4] have reported that the use of acid eluents in ion-exclusion chromatography was very effective in the improvement of the peak shape. Therefore, in order to improve the peak shape, an aqueous strong acid such

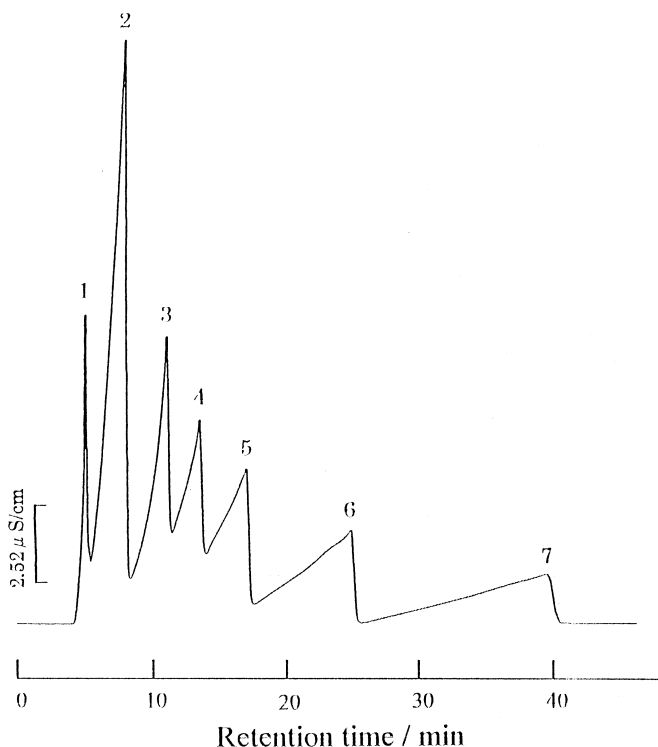


Fig. 2. Ion-exclusion chromatogram of hydrochloric and aliphatic acids by elution with distilled water. Column: TSKgel OApak-A polymethacrylate-based weakly acidic cation-exchange resin, 300 mm×7.8 mm I.D.; column temperature: 35°C; flow-rate: 1.0 ml/min; detection: conductivity; injection volume: 100 μl; sample concentration: 0.1 mmol/l for hydrochloric acid and 1.0 mmol/l for all aliphatic monocarboxylic acids. Peaks: 1=hydrochloric acid, 2=formic acid, 3=acetic acid, 4=propionic acid, 5=butyric acid, 6=varellic acid, 7=caproic acid.

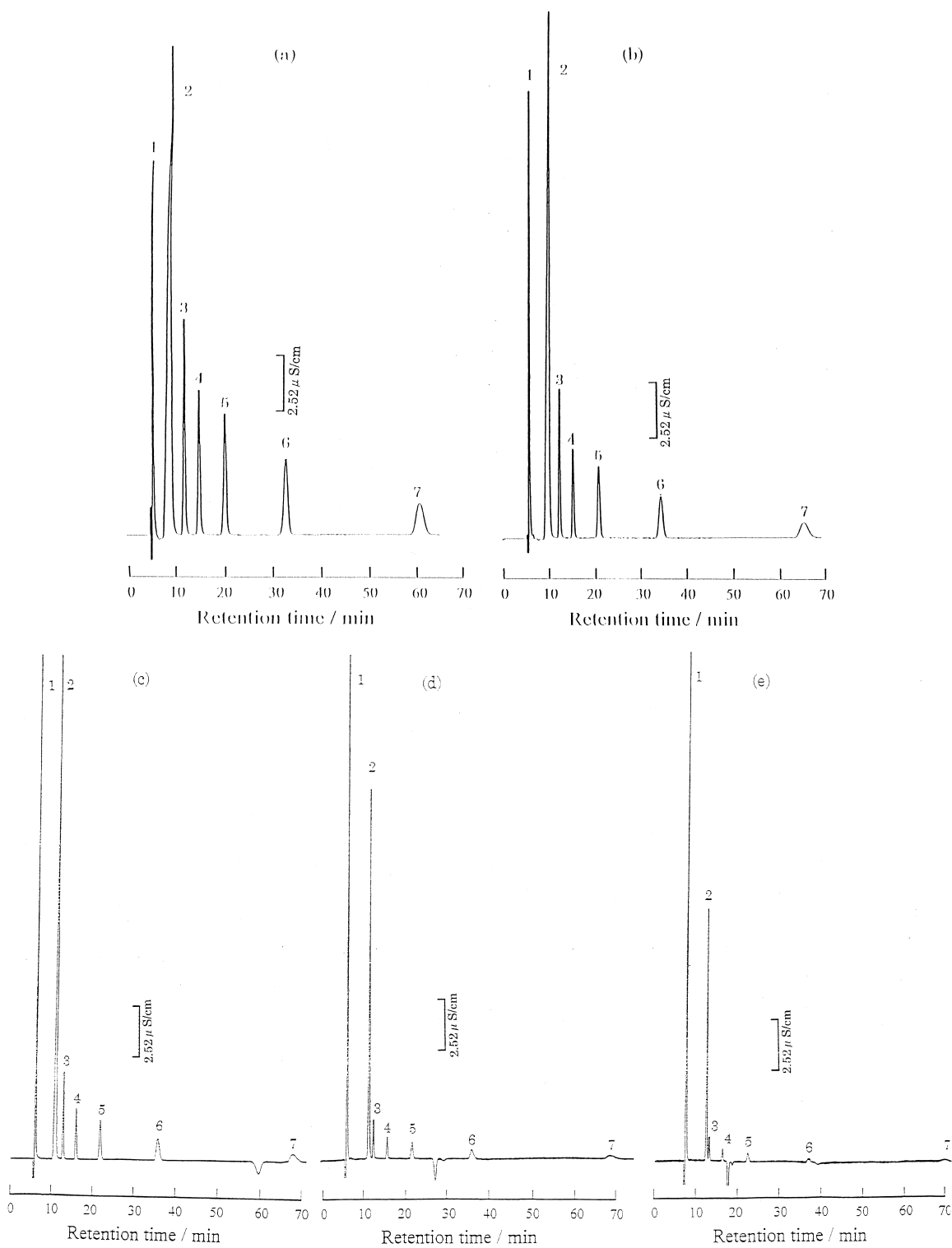


Fig. 3. Ion-exclusion chromatograms of hydrochloric and aliphatic acids by elution with (a) 0.05 mmol/l H₂SO₄, (b) 0.1 mmol/l H₂SO₄, (c) 0.2 mmol/l H₂SO₄, (d) 0.5 mmol/l H₂SO₄ and (e) 1.0 mmol/l H₂SO₄. Peak identification and other conditions as in Fig. 2.

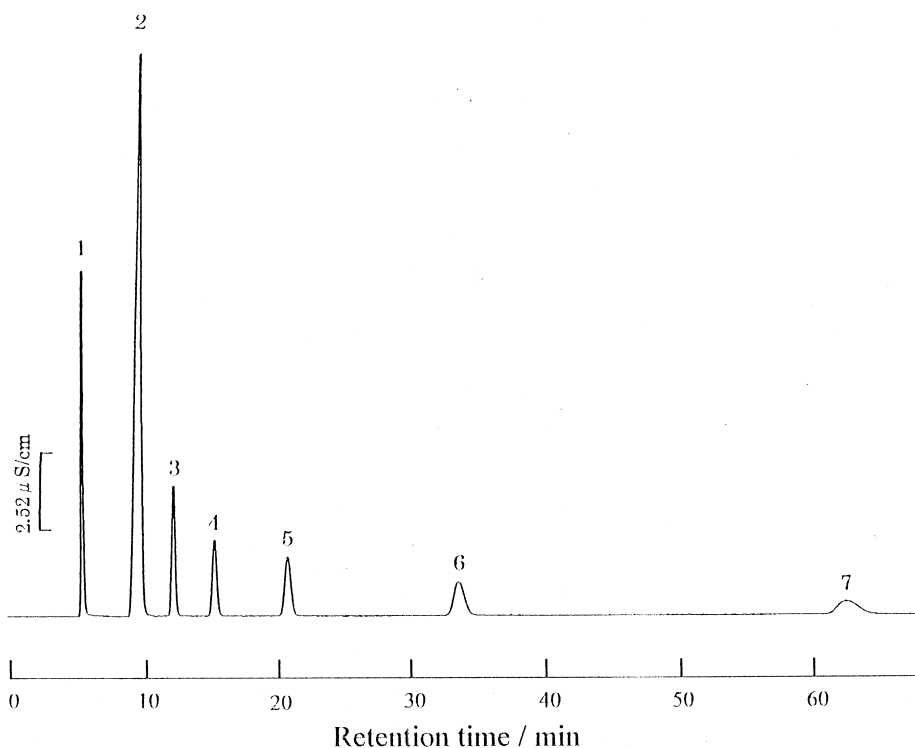


Fig. 4. Ion-exclusion chromatogram of hydrochloric and aliphatic acids by elution with 0.85 mmol/l benzoic acid. Peak identification and other conditions as in Fig. 2.

as sulfuric acid as an eluent has been investigated. When a PS–DVB-based strongly acidic cation-exchange resin generally employed in ion-exclusion chromatography was used, a good resolution of carboxylic acids by elution with sulfuric acid solutions was obtained, but the detection limits of carboxylic acids were decreased because of high background conductivity. When diluted sulfuric acid (0.05–0.5 mmol/l) with low background conductivity was used as the eluent, the resolution is very low and the peak is tailed due to the hydrophobic adsorption effects, as a side effect in ion-exclusion chromatography. For instance, Fig. 1 shows the separation of carboxylic acids with 0.1 mmol/l sulfuric acid as eluent by using a PS–DVB-based strongly cation-exchange resin (TSKgel SCX). However, when benzoic acid was used as the eluent, appropriate separation and detection of carboxylic acids was accomplished.

This section addresses some of the general properties of a polymethacrylate-based weakly acidic cation-exchange resin (TSKgel OApak-A) in ion-

exclusion chromatography. Then, the separation of aliphatic monocarboxylic acids by using this column was investigated with different eluents.

As shown in Fig. 2, the resolution of carboxylic acids by elution with water was very low and the peaks were fronted. In order to reduce peak fronting, sulfuric acid eluents with different concentrations (0.05–1.0 mmol/l) were studied, the elution performances for the separation of carboxylic acids were compared. From Fig. 3a–e, it can be seen that the ion-exclusion chromatographic separation of carboxylic acids was suitable even at the minimum concentration of sulfuric acid eluent. However, the resolution was improved with increasing the sulfuric acid concentration. That is to say that the H^+ from these eluents repressed the ionization of weak acid solutes and sharpened the chromatographic peak. Therefore, this means that the peak shapes are dependent on the proton concentration (pH value) of eluents. Subsequently, sulfuric, hydrochloric, nitric and benzoic acids at 0.2 mmol/l H^+ concentration were examined as eluents. The retention behavior of

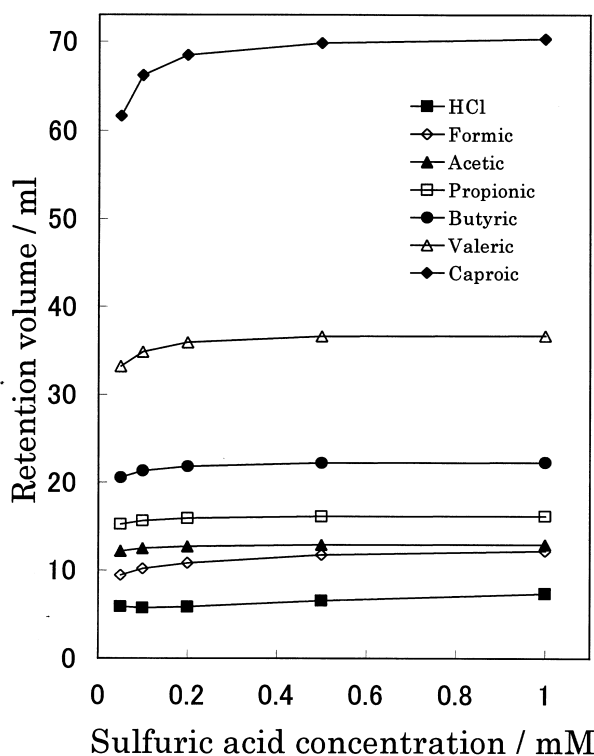


Fig. 5. Effect of sulfuric acid concentration in the eluent on retention volumes (V_R) of HCl and aliphatic monocarboxylic acids. Other chromatographic conditions as in Fig. 2.

the carboxylic acids was not affected by the anions in the eluents. For example, Fig. 4 shows the separation of carboxylic acids with 0.85 mmol/l

benzoic acid as the eluent. Table 1 lists the detection limits ($S/N=3$) of carboxylic acids by elution with water, 0.05–1.0 mmol/l sulfuric acid, 0.85 mmol/l

Table 1

Comparison of detection limits of aliphatic carboxylic acids by elution with (A) water, (B) 0.05–1.0 mmol/l sulfuric acid and (C) 0.85 mmol/l benzoic acid (other chromatographic conditions as in Fig. 2)

Eluent	A	B					C
		0.05 mM	0.1 mM	0.2 mM	0.5 mM	1.0 mM	
Background ^a	0.7	40	79	155	380	720	74
Noise ^b	0.0003	0.0006	0.001	0.0018	0.004	0.0075	0.0009
Detection limits ($S/N=3$) (μM)							
Formic	0.05	0.08	0.12	0.21	0.65	1.79	0.15
Acetic	0.10	0.18	0.44	1.23	6.28	19.20	0.64
Propionic	0.14	0.28	0.72	2.11	11.04	21.66	1.11
Butyric	0.18	0.33	0.90	2.74	18.30	53.25	1.42
Valeric	0.30	0.53	1.56	5.15	23.41	121.29	2.54
Caproic	0.57	1.91	5.13	16.64	95.47	197.89	8.84

^a Eluent background conductivity: $\mu S/cm$.

^b Eluent noise level: $\mu S/cm$.

benzoic acid. It can be seen that the separation by using 0.1 mmol/l sulfuric acid as the eluent was more sensitive than that by using 0.85 mmol/l benzoic acid corresponding to 0.2 mmol/l H^+ concentration.

In conclusion, a high resolution and sensitive detection in the ion-exclusion chromatography of carboxylic acids was achieved by combination of a TSKgel OApak-A column, diluted sulfuric acid eluents. Furthermore, the difference of elution behavior by the both columns probably depends on the difference of hydrophobicity of the matrix rather than that of the kinds of exchange group.

3.2. Effect of sulfuric acid concentration on retention volumes of carboxylic acids

In order to determine the optimum concentration of sulfuric acid in the eluent, the effect of sulfuric acid concentration on the ion-exclusion chromatographic separation of carboxylic acids was investi-

gated in the range of 0.05–1.0 mmol/l. The relationships between the sulfuric acid concentration and the retention volumes (V_R) of the carboxylic acids are shown in Fig. 5. As can be seen from Fig. 5, the V_R values of carboxylic acids were not entirely dependent on the sulfuric acid concentration.

3.3. Effect of methanol concentration

In order to accelerate the elution of carboxylic acids with hydrophobic nature, the effect of the addition of organic modifier (methanol) to a 0.1 mmol/l sulfuric acid eluent was investigated. The relationship between the methanol concentration and the V_R values of aliphatic carboxylic acids is shown in Fig. 6. With increasing methanol concentration, the V_R values of hydrochloric, formic, acetic and propionic acids were virtually unaltered, whereas those of the hydrophobic acids (C_4 – C_6) decreased drastically. Especially, the V_R values of caproic acid decreased from 66.2 ml with 0.1 mmol/l sulfuric

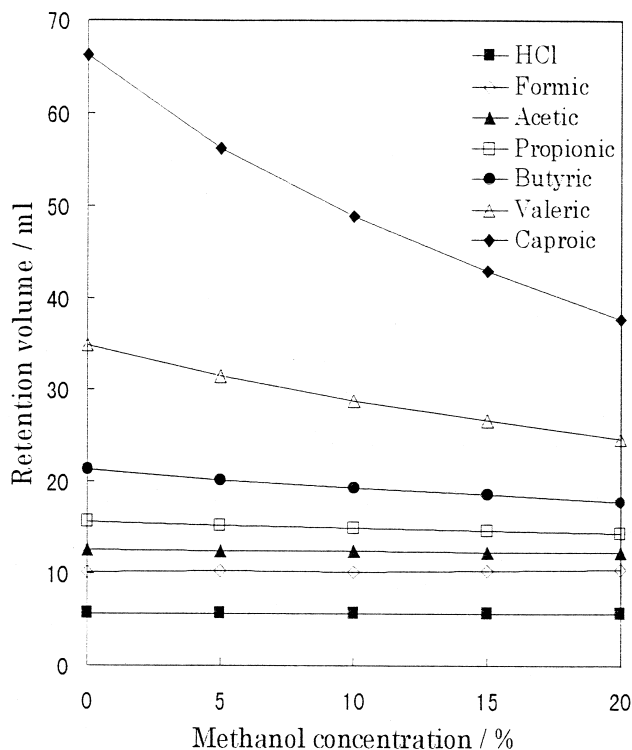


Fig. 6. Effect of methanol concentration in the eluent on retention volumes (V_R) of HCl and aliphatic monocarboxylic acids. Eluent: 0.1 mmol/l sulfuric acid containing 0–20% methanol. Other chromatographic conditions as in Fig. 2.

acid to 33.7 ml with 20% methanol–0.1 mmol/l sulfuric acid. This decrease might be due to the effect of the lipophilic property of the alkyl group in methanol rather than of the hydrophilic property of the alcoholic OH group in methanol. In conclusion, in order to reduce the analytical time, it is possible to enhance the methanol concentration to a limit of resolution of aliphatic carboxylic acids.

3.4. Separation of the other carboxylic acids

Using a TSKgel OApak-A column tested for the separation of the other carboxylic acids, dicarboxylic acids (oxalic, malonic, succinic, glutaric and adipic acids) and the other acids (maleric, malic, lactic, citric and tartaric acids). Dicarboxylic acids were completely separated from each solute, depending on

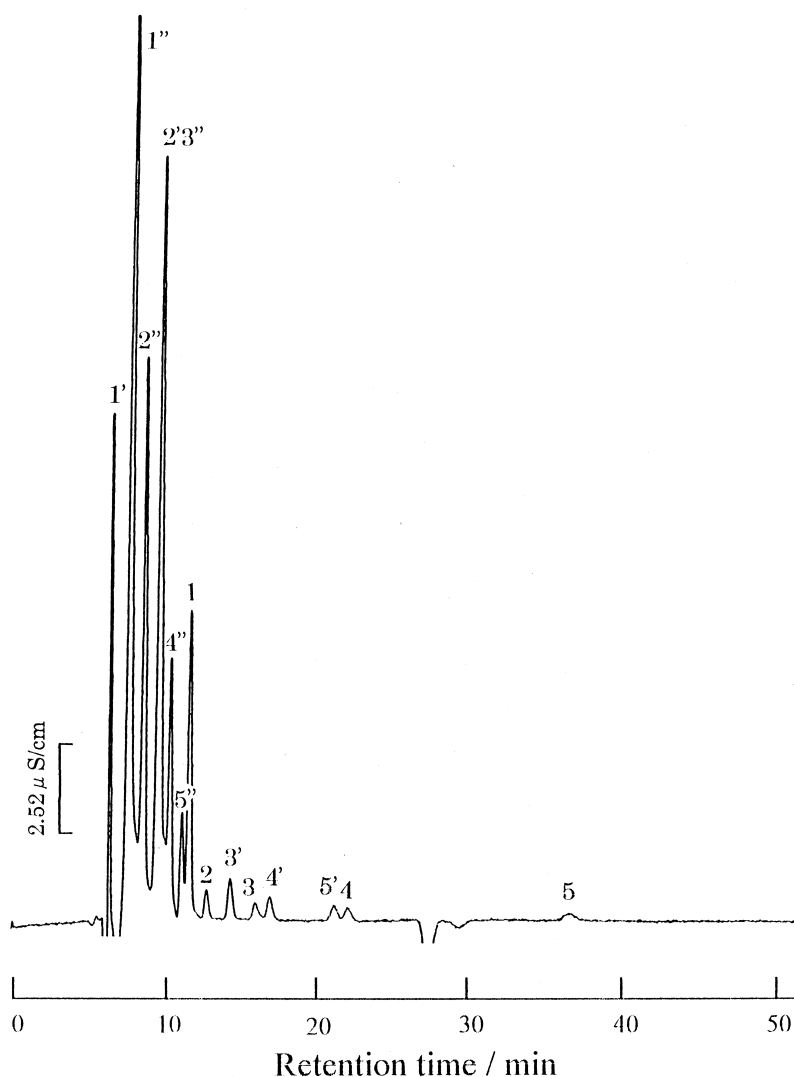


Fig. 7. Ion-exclusion chromatogram of aliphatic monocarboxylic, dicarboxylic and the other carboxylic acid by elution with 0.5 mmol/l H_2SO_4 . Peaks: monocarboxylic acids (1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid), dicarboxylic acids (1'=oxalic acid, 2'=malonic acid, 3'=succinic acid, 4'=glutaric acid, 5'=adipic acid) and the other carboxylic acids (1''=maleric acid, 2''=tartaric acid, 3''=citric acid, 4''=malic acid, 5''=lactic acid). Other chromatographic conditions as in Fig. 2.

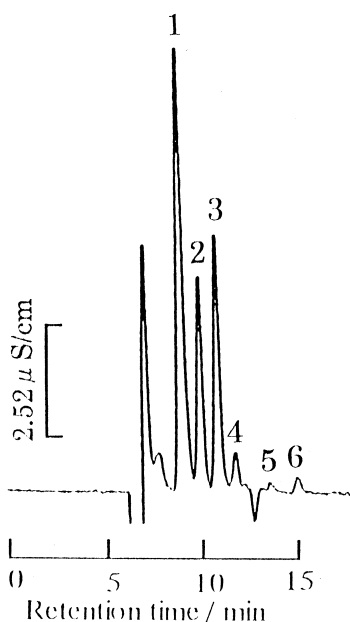


Fig. 8. Determination of organic acids in red wine using ion-exclusion chromatography. Eluent: 0.5 mmol/l H_2SO_4 ; other chromatographic conditions were as described in Fig. 2. Sample: red wine diluted 100-fold with the eluent. Peaks: 1=tartaric acid, 2=citric acid, 3=malic acid, 4=lactic acid, 5=acetic acid, 6=succinic acid; the negative peak corresponds to the ethanol.

the difference of hydrophobicity. However, it was not easy to completely separate the other carboxylic acids because each character was close. Therefore, sulfuric and benzoic acid eluents with various concentrations were tested for the separation of the mixture sample. As a result, the optimum resolution of carboxylic acids was achieved by elution with 0.5 mmol/l sulfuric acid. Fig. 7 shows the separation of the mixture of carboxylic acids described above and aliphatic monocarboxylic acids (formic, acetic, propionic and butyric acids) by elution with 0.5 mmol/l sulfuric acid. The method developed in this study is applicable for the detection of a number of carboxylic acids in a variety of real samples; Fig. 8 shows a typical result of the analysis of red wine.

3.5. Reproducibility, calibration graphs and detection limits

Under the optimum chromatographic conditions described above, the reproducibility of the peak areas

of the carboxylic acids except for caproic acid was less than 3.5% RSD, and calibration graphs obtained by plotting peak area of C_1 – C_6 carboxylic acids against the concentration were linear between 0.1 and 5.0 mmol/l. The detection limits ($S/N=3$) of C_1 – C_6 carboxylic acids by elution with 0.1 mmol/l sulfuric acid were in the range 0.12 μM (C_1) to 5.13 μM (C_6).

4. Conclusions

In this study, the ion-exclusion chromatography of carboxylic acids was investigated using a novel column (TSKgel OApak-A), with weakly acidic cation-exchange resin.

A good resolution and highly sensitive detection of carboxylic acids was accomplished by elution with diluted sulfuric acid solutions. It was possible to reduce the analytical time by the addition to organic modifier. This method made it possible to determine the complex mixture containing several kinds of dicarboxylic and hydroxycarboxylic acids.

In this method, it was difficult to determine aromatic carboxylic acids due mainly to more hydrophobic adsorption, so the development for the ion-exclusion chromatography separation of various aromatic acids on the TSKgel OApak-A will be the subject of future work.

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