

# Ion-exclusion chromatographic separations of C<sub>1</sub>–C<sub>6</sub> aliphatic carboxylic acids on a sulfonated styrene–divinylbenzene co-polymer resin column with 5-methylhexanoic acid as eluent

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## Abstract

The application of C<sub>7</sub> aliphatic carboxylic acids (heptanoic, 2-methylhexanoic, 5-methylhexanoic and 2,2-dimethyl-*n*-valeric acids) as eluents in ion-exclusion chromatography with conductimetric detection for C<sub>1</sub>–C<sub>6</sub> aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids) was carried out using a highly sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) in the H<sup>+</sup> form as a stationary phase. When using 0.05 mM sulfuric acid at pH 4.0 as the eluent, peak shapes of hydrophobic carboxylic acids (isovaleric, valeric, isocaproic and caproic acids) were tailed strongly. In contrast, when using 1 mM these C<sub>7</sub> carboxylic acids at pH ca. 4 as the eluents, although system peaks (vacant peaks) corresponding to these C<sub>7</sub> carboxylic acids appeared, peak shapes of these hydrophobic acids were improved drastically. Excellent simultaneous separation and relatively high sensitive conductimetric detection for these C<sub>1</sub>–C<sub>6</sub> aliphatic carboxylic acids were achieved in 25 min on the TSKgel SCX column (150×6 mm I.D.) using 1 mM 5-methylhexanoic acid at pH 4.0 as the eluent.

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**Keywords:** Mobile phase composition; Carboxylic acids; Methylhexanoic acid

## 1. Introduction

Ion-exclusion chromatography developed by Wheaton and Baumann [1] is recognized as a simple and convenient analytical technique for the determination of various carboxylic acids, especially hydrophilic carboxylic acids [2]. Separation of carboxylic acid is commonly performed on highly sulfonated styrene–divinylbenzene (PS–DVB) co-polymer resin in the H<sup>+</sup> form with large hydrophobicity as the stationary phases. In the earliest works [3–5], water

was mainly used as the eluent. Both strongly fronted and broadened peaks for carboxylic acids were obtained in ion-exclusion chromatography, because carboxylic acids were well dissociated and, as a consequence, these acids were strongly excluded from the sulfonated PS–DVB as the stationary phase by electrostatic repulsion.

Richards [6] and Turkelson and Richards [7] applied dilute strong acids, such as HCl and H<sub>2</sub>SO<sub>4</sub>, as the eluents in ion-exclusion chromatography for the improvement of peak shapes of carboxylic acids by the suppression of the dissociation of carboxylic acids. Peak shapes of hydrophilic carboxylic acids were improved drastically, whereas peaks of hydrophobic carboxylic acids were tailed strongly. This is

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due mainly to an increase in hydrophobic interaction between carboxylic acids and the sulfonated PS–DVB as the stationary phase. Furthermore, the ion-exclusion chromatographic conditions resulted in low conductimetric detection sensitivities for carboxylic acids, because of both an increase in eluent conductivity and a decrease in the degree of the dissociation of carboxylic acids. Therefore, a further investigation was required for the development of advanced ion-exclusion chromatography for hydrophobic carboxylic acids.

Tanaka and co-workers [8,9] applied dilute benzoic acid as the eluent in the ion-exclusion chromatography of carboxylic acids, in order to eliminate these drawbacks. Benzoic acid is believed to coat the surface of the cation-exchange stationary phase and to reduce the hydrophobicity of the resin. Although a system peak corresponding to benzoic acid in the eluent appeared, symmetrical peaks and excellent separation of several aliphatic carboxylic acids (formic, acetic, propionic, butyric and valeric acids) were achieved on a highly sulfonated PS–DVB column. The authors applied  $C_6$  aliphatic carboxylic acids as the eluents in ion-exclusion chromatography using a highly sulfonated PS–DVB column (TSKgel SCX column,  $150 \times 4.6$  mm I.D.) for  $C_1$ – $C_5$  aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) [10]. Symmetrical peaks and excellent simultaneous separation of these aliphatic carboxylic acids were achieved in 15 min using 1 mM isocaproic acid at pH 4.0 (eluent conductivity:  $44 \mu\text{S cm}^{-1}$ ) as the eluent. Isocaproic acid was also adsorbed strongly on the TSKgel SCX column and acted as an advanced elution modifier for hydrophobic carboxylic acids. Relatively high sensitive conductimetric detection was also achieved. This is due mainly to the pH (pH 4.0) and low conductivity of the eluent.

The aim of this study was to extend the utility of TSKgel SCX as the stationary phase in ion-exclusion chromatography of carboxylic acids. Then, the application of the TSKgel SCX column ( $150 \times 6$  mm I.D.) in the ion-exclusion chromatographic separation of  $C_1$ – $C_6$  aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids) was carried out using various  $C_7$  aliphatic carboxylic acids (heptanoic, 2-methylhexanoic, 5-methylhexanoic and 2,2-di-

methyl-*n*-valeric acids) as the eluents. These  $C_7$  carboxylic acids also functioned as advanced elution modifiers for these  $C_1$ – $C_6$  carboxylic acids. When using 1 mM 5-methylhexanoic acid at pH 4.0 (eluent conductivity:  $44 \mu\text{S cm}^{-1}$ ) as the eluent, symmetrical peaks, excellent simultaneous separation and relatively high sensitive conductimetric detection for these  $C_1$ – $C_6$  carboxylic acids were achieved on the TSKgel SCX column in 25 min.

## 2. Experimental

### 2.1. Instrumentation

The ion-exclusion chromatograph consisted of a Tosoh (Tokyo, Japan) LC-8020 chromatographic data processor, a Tosoh CCPM-II solvent delivery pump operated at a flow-rate of  $1 \text{ ml min}^{-1}$ , a Tosoh CM-8020 conductimetric detector, a Tosoh CO-8020 column oven operated at  $35^\circ\text{C}$ , a Tosoh DS-8023 on-line degasser and a Rheodyne (Cotati, CA, USA) Model 9125 injector equipped with a  $100\text{-}\mu\text{l}$  sample loop.

A Toa Denpa (Tokyo, Japan) IM-40S ion meter with a glass electrode was used for the measurement of pH of eluents. A Toa Denpa CM-20 conductimetric detector was also employed for the measurement of conductivities of the eluents.

### 2.2. Separation column

A separation column ( $150 \times 6$  mm I.D.) packed with a Tosoh TSKgel SCX highly sulfonated styrene–divinylbenzene co-polymer resin in the  $\text{H}^+$  form (cation-exchange capacity of ca. 1.5 mequiv.  $\text{ml}^{-1}$  and particle size of ca.  $5 \mu\text{m}$ ) was used in this study.

### 2.3. Chemicals

All chemicals were of analytical-reagent grade and were purchased from Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan). Distilled, deionized water was used for the preparation of eluents and standard solutions.

### 3. Results and discussion

#### 3.1. Effect of concentration of sulfuric acid in eluent on chromatographic behavior of $C_1$ – $C_6$ aliphatic carboxylic acids on the TSKgel SCX column

In ion-exclusion chromatography, when using water as the eluent, fronted and broadened peaks for carboxylic acids are obtained. Turkelson and Richards [7] have demonstrated the effectiveness of dilute strong acids, such as hydrochloric acids and sulfuric acid, as the eluents in ion-exclusion chromatography for the improvement of peak shapes of carboxylic acids. Then, the effect of the concentration of sulfuric acid in the eluent on the chromatographic behavior of  $C_1$ – $C_6$  aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids) on the TSKgel SCX column (150×6 mm I.D.) was investigated for the simultaneous separation of these carboxylic acids. Fig. 1 shows the relationship between the concentration of sulfuric acid and the retention volumes of these  $C_1$ – $C_6$  carboxylic acids. Fig. 2A–C show chromatograms of these  $C_1$ – $C_6$  carboxylic acids using 0.005 mM sulfuric acid at pH 5.0 (eluent conductivity:  $5.5 \mu\text{S cm}^{-1}$ ), 0.05 mM sulfuric acid at pH 4.0 ( $41 \mu\text{S cm}^{-1}$ ) and 0.5 mM sulfuric acid at pH 3.0 ( $391 \mu\text{S cm}^{-1}$ ) as the eluents, respectively.

As shown in Fig. 1, with increasing the concentration of sulfuric acid, the retention volumes of these  $C_1$ – $C_6$  carboxylic acids increased. The retention volumes of higher carboxylic acids increased largely in comparison with those of lower carboxylic acids. This is due main to both (a) a decrease in the electrostatic repulsion and (b) an increase in hydrophobic interaction between these  $C_1$ – $C_6$  carboxylic acids and the surface of the TSKgel SCX, caused by suppressing the dissociation of these carboxylic acids. As shown in Fig. 2A, when using 0.005 mM sulfuric acid as the eluent, both largely fronted peaks of  $C_1$ – $C_5$  carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) and slightly fronted peaks of  $C_6$  carboxylic acids (isocaproic and caproic acids) were obtained. This is because the  $C_1$ – $C_5$  carboxylic acids were well dissociated and, as a consequence, these acids were mainly separated by the ion-exclusion chromatog-

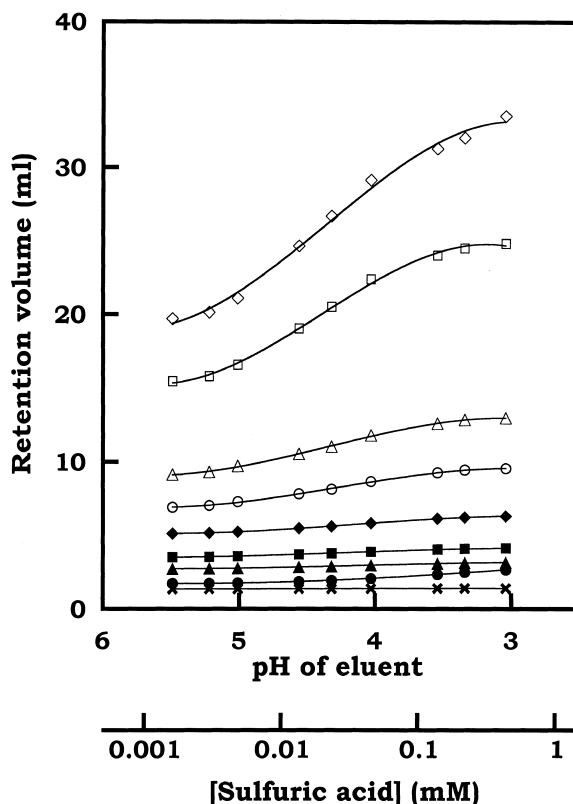


Fig. 1. Effect of concentration of sulfuric acid in eluent on retention volumes of  $C_1$ – $C_6$  aliphatic carboxylic acids on TSKgel SCX column. Conditions: column: TSKgel SCX, column size: 150×6 mm I.D., column temperature: 35 °C, eluent: 0.0015–0.5 mM sulfuric acid, flow-rate: 1 ml min<sup>-1</sup>, detection: conductivity, injection volume: 100  $\mu\text{l}$ , sample concentration: 0.1 mM for formic acid and 0.2 mM for other carboxylic acids, symbols:  $\times$ =sulfuric acid,  $\bullet$ =formic acid,  $\blacktriangle$ =acetic acid,  $\blacksquare$ =propionic acid,  $\blacklozenge$ =butyric acid,  $\circ$ =isovaleric acid,  $\triangle$ =valeric acid,  $\square$ =isocaproic acid,  $\diamond$ =caproic acid.

raphy process. In contrast, due to large hydrophobicity, the  $C_6$  carboxylic acids were separated by both ion-exclusion chromatography and the hydrophobic adsorption process. As shown in Fig. 2B, when using 0.05 mM sulfuric acid as the eluent, both symmetrical peaks of  $C_1$ – $C_4$  carboxylic acids (formic, acetic, propionic, isobutyric and butyric acids) and tailed peaks of  $C_5$ – $C_6$  (isovaleric and valeric, isocaproic and caproic acids) were obtained. Peak resolution between the  $C_1$ – $C_4$  carboxylic acids was improved. Since the dissociation of these  $C_1$ – $C_6$  carboxylic acids was largely suppressed under the

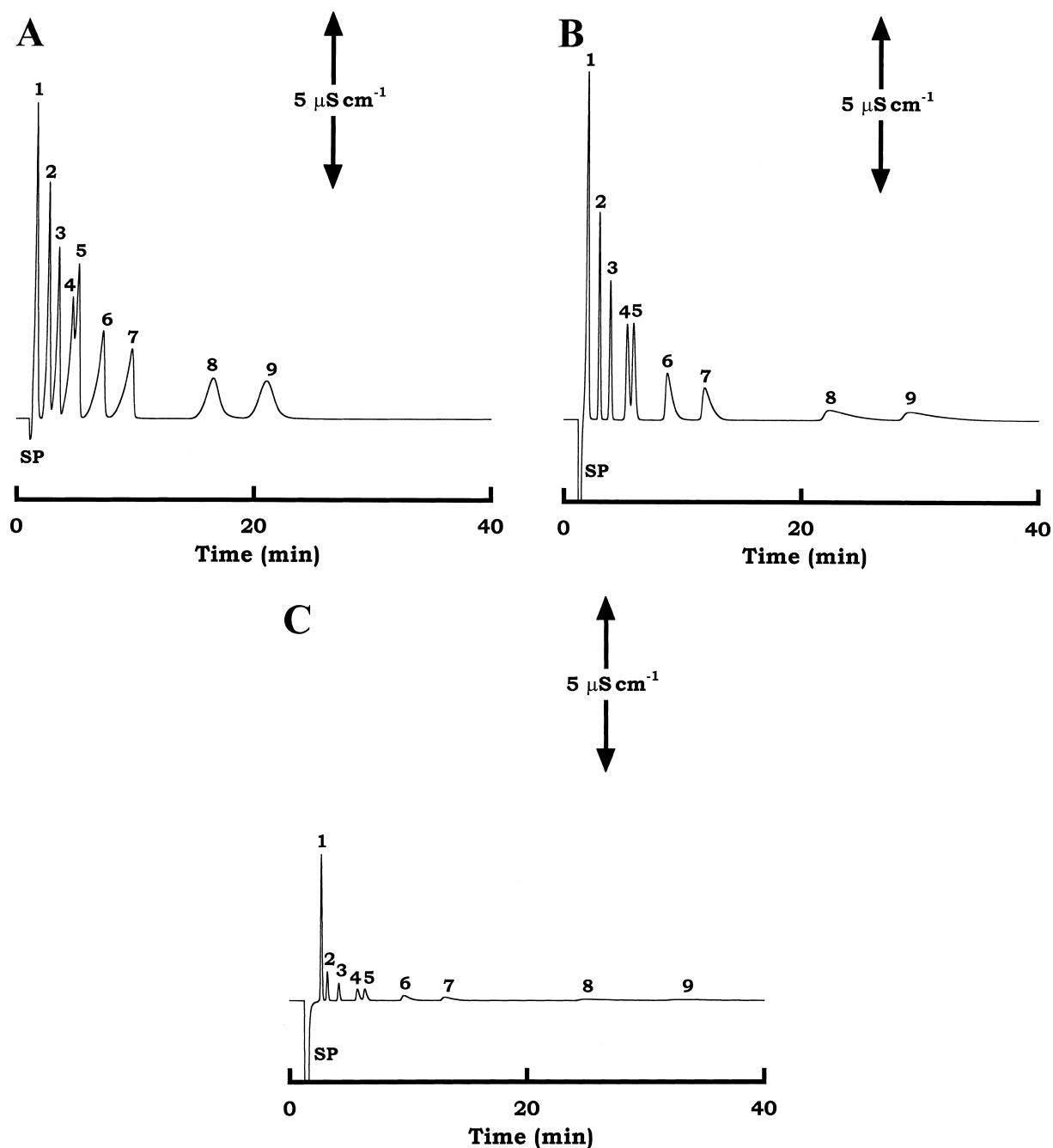


Fig. 2. Chromatograms of  $C_1$ – $C_6$  aliphatic carboxylic acids on TSKgel SCX column using various concentrations of sulfuric acid as eluent. Conditions: eluent: (A) 0.005 mM sulfuric acid at pH 5.0 (eluent conductivity:  $5.5 \mu\text{S cm}^{-1}$ ), (B) 0.05 mM sulfuric acid at pH 4.0 (eluent conductivity:  $41 \mu\text{S cm}^{-1}$ ), (C) 0.5 mM sulfuric acid at pH 3.0 (eluent conductivity:  $391 \mu\text{S cm}^{-1}$ ). Peaks: SP=system peak (vacant peak corresponding to sulfuric acid in eluent), 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutyric acid, 5=butyric acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid, 9=caproic acid.

ion-exclusion chromatography conditions, it appeared that these carboxylic acids were mainly separated by hydrophobic adsorption process. Unfortunately, as shown in Fig. 2A–C, with increasing the concentration of sulfuric acid, the conductimetric detection sensitivities decreased drastically. This is due to both (a) an increase in eluent conductivity and (b) a decrease in the conductimetric detector response, caused by suppressing the dissociation of these carboxylic acids.

Considering peak shape, peak resolution and conductimetric detection sensitivity, it was decided that the optimum concentration of sulfuric acid in the eluent was 0.05 mM (pH 4). However, a further investigation was required for the improvement of peak shapes for hydrophobic carboxylic acids ( $C_5$ – $C_6$  carboxylic acids).

### 3.2. Effect of benzoic, $C_7$ aliphatic carboxylic acids as eluents on chromatographic behavior of $C_1$ – $C_6$ aliphatic carboxylic acids on the TSKgel SCX column

Tanaka and Fritz [8] applied dilute benzoic acid as the eluent in ion-exclusion chromatography with conductimetric detection (CD) using a highly sulfonated styrene–divinylbenzene co-polymer resin column for both the improvement of peak shape and the decrease in retention volume for hydrophobic aliphatic carboxylic acid (valeric acid). Benzoic acid in the eluent is believed to adsorb onto the surface of the stationary phase and to decrease hydrophobicity of the stationary phase. Then, the application of diluted benzoic acid as the eluent in ion-exclusion chromatography–CD was carried out for the improvement of peak shapes and the decrease in the retention volumes for the  $C_5$ – $C_6$  carboxylic acids. Fig. 3 shows the chromatogram of these  $C_1$ – $C_6$  carboxylic acids using 0.25 mM benzoic acid at pH 4.0 as the eluent.

As shown in Fig. 3, although symmetrical peaks of the  $C_1$ – $C_5$  carboxylic acids were obtained, peak shapes of the  $C_6$  carboxylic acid (isocaproic acid) remained tailed. The decrease in the retention volumes of hydrophobic carboxylic acids was small. A system peak (vacant peak) corresponding to benzoic acid in the eluent appeared and interfered seriously with the determination of caproic acid. When using

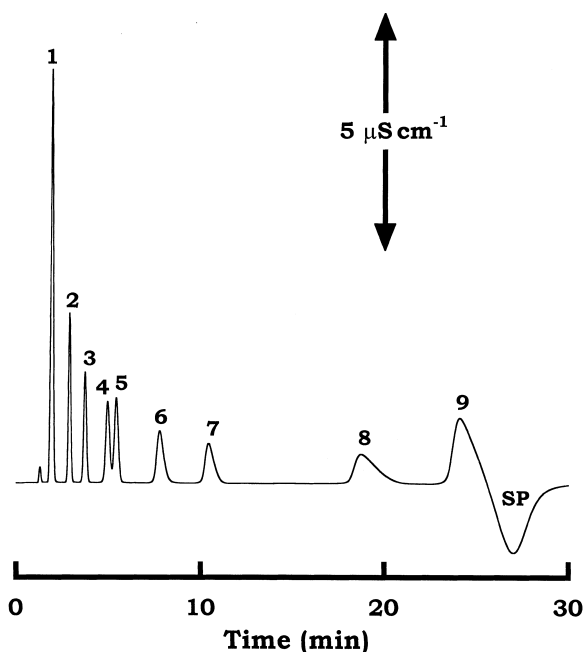


Fig. 3. Chromatogram of  $C_1$ – $C_6$  aliphatic carboxylic acids on TSKgel SCX column using benzoic acid as eluent. Conditions: eluent: 0.25 mM benzoic acids at pH 4.0 (eluent conductivity:  $43 \mu\text{S cm}^{-1}$ ). Peaks: SP=system peak (vacant peak corresponding to benzoic acid in eluent), 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutyric acid, 5=butyric acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid, 9=caproic acid. Other chromatographic conditions as in Fig. 2.

0.5 and 1.0 mM benzoic acid as the eluents, system peak corresponding to benzoic acid also interfered seriously with the determination of caproic acid. The main reason for the drawback was due mainly to a relatively small affinity of benzoic acid for the TSKgel SCX stationary phase. Unfortunately, it was concluded that benzoic acid eluent was not an appropriate eluent in ion-exclusion chromatography–CD for the simultaneous separation of these  $C_1$ – $C_6$  carboxylic acids.

Table 1 lists the retention volumes of benzoic acid and several  $C_7$  aliphatic carboxylic acids (2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) on the TSKgel SCX column using 0.05 mM sulfuric acid as the eluent. The elution order was 2,2-dimethyl-*n*-valeric acid < benzoic acid < 2-methylhexanoic acid < 5-methylhexanoic acid < heptanoic acid. The order strongly suggested that the 5-methylhexanoic and heptanoic acids

Table 1  
Retention volumes of C<sub>1</sub>–C<sub>7</sub> aliphatic carboxylic acids and benzoic acids on the TSKgel SCX column using 0.05 mM sulfuric acid as eluent

Carboxylic acid	Retention volume (ml)
Formic acid	2.1
Acetic acid	3.0
Propionic acid	3.9
Isobutyric acid	5.3
Butyric acid	5.8
Isovaleric acid	8.7
Valeric acid	11.8
Isocaproic acid	22.4
Caproic acid	29.1
2,2-Dimethyl- <i>n</i> -valeric acid	36.1
2-Methylhexanoic acid	49.2
5-Methylhexanoic acid	62.0
Heptanoic acid	89.1
Benzoic acid	41.7

would be successful applied as the eluents in ion-exclusion chromatography–CD for the simultaneous separation of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids. Secondary, the application of these C<sub>7</sub> aliphatic carboxylic acids as the eluents was carried out. Fig. 4A–D show chromatograms of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids using 1 mM 2,2-dimethyl-*n*-valeric acid at pH 4.1, 1 mM 2-methylhexanoic acid at pH 4.0, 1 mM 5-methylhexanoic acid at pH 4.0 and 1 mM heptanoic acid at pH 4.0 as the eluents, respectively.

As shown in Fig. 4A, when using 1 mM 2,2-dimethyl-*n*-valeric acid as the eluent, although symmetrical peaks of the C<sub>1</sub>–C<sub>5</sub> carboxylic acids were obtained, peaks of the C<sub>6</sub> carboxylic acids remained tailed. A system peak corresponding to 2,2-dimethyl-*n*-valeric acid interfered seriously with the determination of isocaproic acid. As shown in Fig. 4B, when using 1 mM 2-methylhexanoic acid as the eluent, although symmetrical peaks of the C<sub>1</sub>–C<sub>5</sub> carboxylic acids and isocaproic acid were obtained, a system peak also interfered with the determination of caproic acid. In contrast, as shown in Fig. 4C and D, when using 1 mM 5-methylhexanoic acid or 1 mM heptanoic acid as the eluents, symmetrical peaks of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids were obtained with no interference. This is because 5-methylhexanoic and heptanoic acids were strongly retained on the TSKgel SCX column and, consequently, complete

separation of caproic acid and their system peaks could be achieved easily. Furthermore, the retention volumes of caproic acid varied depending on the isomeric structure of heptanoic acid used as an eluent; 19.5 min using 1 mM 2,2-dimethyl-*n*-valeric acid, 16.9 min using 1 mM 2-methylhexanoic acid, 16.2 min using 1 mM 5-methylhexanoic acid and 15.1 min using 1 mM heptanoic acid. This result indicated that 5-methylhexanoic and heptanoic acid also acted as very effective elution modifiers for the decrease in the retention volumes of hydrophobic carboxylic acids.

Considering peak shape, interference of system peak and chromatographic time, it was concluded that the most suitable eluent in ion-exclusion chromatography–CD was 5-methylhexanoic acid for the simultaneous separation of these C<sub>1</sub>–C<sub>6</sub> aliphatic carboxylic acids.

### 3.3. Effect of concentration of 5-methylhexanoic acid in eluent on chromatographic behavior of C<sub>1</sub>–C<sub>6</sub> aliphatic carboxylic acids on the TSKgel SCX column

The effect of the concentration of 5-methylhexanoic acid in the eluent on the retention behavior of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids was investigated. Fig. 5 shows the relationship between the concentration of 5-methylhexanoic acid and the retention volumes of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids. Fig. 6A–C show chromatograms of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids using 0.01 mM 5-methylhexanoic acid at pH 5.2 (eluent conductivity: 3.4 μS cm<sup>-1</sup>), 0.1 mM 5-methylhexanoic acid at pH 4.5 (12 μS cm<sup>-1</sup>) and 10 mM 5-methylhexanoic acid at pH 3.5 (135 μS cm<sup>-1</sup>) as the eluents, respectively. A chromatogram of these acids, using 1 mM 5-methylhexanoic acid at pH 4.0 (42 μS cm<sup>-1</sup>) as the eluent, has been already shown in Fig. 4C.

As shown in Fig. 5, with increasing the concentration of 5-methylhexanoic acid in the eluent, although the dissociation of these C<sub>1</sub>–C<sub>6</sub> carboxylic acids was suppressed, the retention volumes of these carboxylic acids decreased. The degree of the decrease in the retention volumes of higher carboxylic acid was considerably larger than that of lower carboxylic acids. This result strongly indicated that 5-methylhexanoic acid was adsorbed onto the

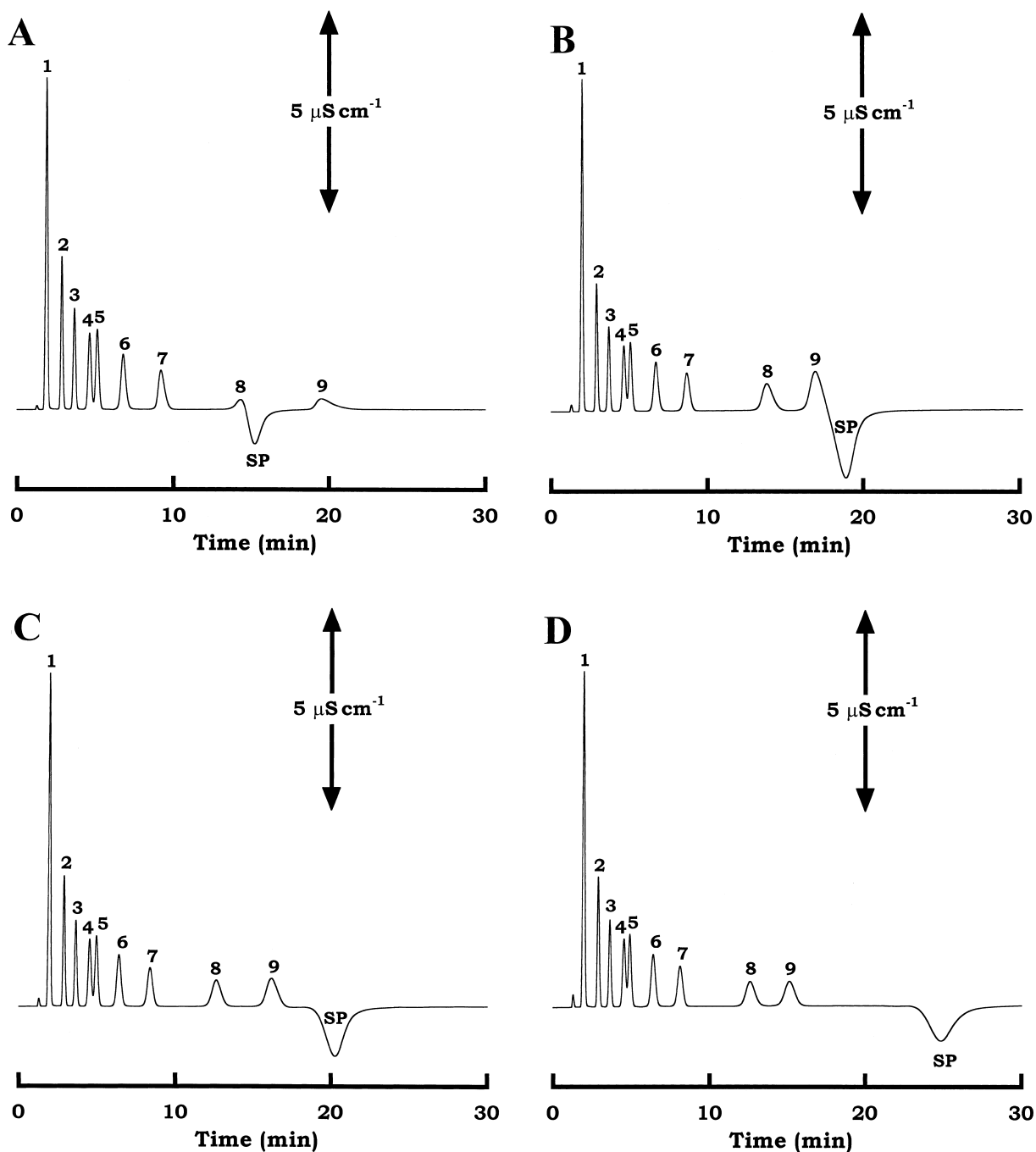


Fig. 4. Chromatograms of  $C_1$ - $C_6$  aliphatic carboxylic acids on TSKgel SCX column using various  $C_7$  aliphatic carboxylic acids as eluent. Conditions: eluent: (A) 1 mM 2,2-dimethyl-*n*-valeric acid at pH 4.1 (eluent conductivity:  $33 \mu\text{S cm}^{-1}$ ), (B) 1 mM 2-methylhexanoic acid at pH 4.0 ( $44 \mu\text{S cm}^{-1}$ ), (C) 1 mM 5-methylhexanoic acid at pH 4.0 ( $42 \mu\text{S cm}^{-1}$ ), (D) 1 mM heptanoic acid at pH 4.0 ( $41 \mu\text{S cm}^{-1}$ ). Peaks: SP=system peak (vacant peak corresponding to  $C_7$  aliphatic carboxylic acids in eluent), 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutyric acid, 5=butyric acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid, 9=caproic acid. Other chromatographic conditions as in Fig. 3.

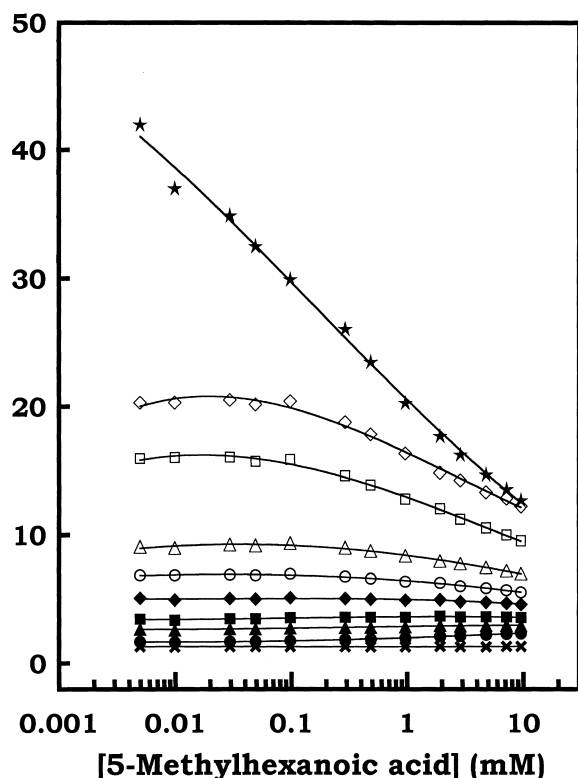


Fig. 5. Effect of concentration of 5-methylhexanoic acid in eluent on retention volumes of  $C_1$ – $C_6$  aliphatic carboxylic acids on TSKgel SCX column. Conditions: eluent: 0.003–10 mM 5-methylhexanoic acid, symbols:  $\times$ =sulfuric acid,  $\bullet$ =formic acid,  $\blacktriangle$ =acetic acid,  $\blacksquare$ =propionic acid,  $\blacklozenge$ =butyric acid,  $\circ$ =isovaleric acid,  $\triangle$ =valeric acid,  $\square$ =isocaproic acid,  $\diamond$ =caproic acid,  $\star$ =system peak (vacant peak corresponding to 5-methylhexanoic acid in eluent). Other chromatographic conditions as in Fig. 4.

TSKgel SCX and acted as an advanced elution modifier for hydrophobic carboxylic acids. The retention volume of system peak decreased greatly. This is because 5-methylheptanoic acid in the eluent itself acted as a retention modifier for 5-methylhexanoic acid adsorbed onto the TSKgel SCX. As shown in Fig. 6A, when using 0.01 mM 5-methylhexanoic acid as the eluent, both fronted peaks of these  $C_1$ – $C_6$  carboxylic acids and poor peak resolution between the  $C_1$ – $C_5$  carboxylic acids were achieved. This is because these  $C_1$ – $C_6$  carboxylic acids were well dissociated and, consequently, these acids were mainly separated by the ion-exclusion chromatography process. As shown in Fig. 6A and B and Fig. 4C, with increasing concentration

of 5-methylhexanoic acid in the eluent, peak shapes of these  $C_1$ – $C_6$  carboxylic acids improved drastically. As shown in Fig. 4C, when using 1 mM 5-methylhexanoic acid as the eluent, both symmetrical peaks and excellent simultaneous separation for these  $C_1$ – $C_6$  carboxylic acid were achieved in 25 min. In contrast, as shown in Fig. 6C, when using 10 mM 5-methylhexanoic acid as the eluent, a system peak interfered seriously with the determination of caproic acid. As shown in Fig. 6B and C and Fig. 4C, with increasing the concentration of 5-methylhexanoic acid, the conductimetric detection sensitivities for these  $C_1$ – $C_6$  carboxylic acid decreased drastically. This is because of (a) an increase in eluent conductivity and (b) a decrease in the conductimetric detector response of these carboxylic acids caused by suppressing the dissociation of these carboxylic acids.

Considering peak shape, peak resolution, conductimetric detection sensitivity and chromatographic time, it was concluded that the optimum concentration of 5-methylhexanoic acid in the eluent was 1 mM (Fig. 4C).

### 3.4. Analytical performance parameters

Table 2 shows the detection limits (signal-to-noise ratio of 3, injection volume of 100  $\mu$ l) of these  $C_1$ – $C_6$  aliphatic carboxylic acids. Highly sensitive conductimetric detection was achieved. The main reasons are that (a) the eluent conductivity of 1 mM 5-methylhexanoic acid as the eluent was relatively low ( $42 \mu\text{S cm}^{-1}$ , noise:  $1.3 \times 10^{-3} \mu\text{S cm}^{-1}$ ) and (b) these carboxylic acids were partly dissociated under the ion-exclusion chromatography–CD conditions.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these carboxylic acids. Linear calibration graphs ( $r^2 \geq 0.99$ ) were obtained in the concentration range between 0.005 and 2.0 mM for these carboxylic acids.

The relative standard deviations of the chromatographic peak areas of these carboxylic acids, whose concentrations were 0.1 mM for formic acid and 0.2 mM for the other acids, were less than 0.7% ( $n=10$ ). Reproducible chromatograms were obtained during repeated chromatographic runs.



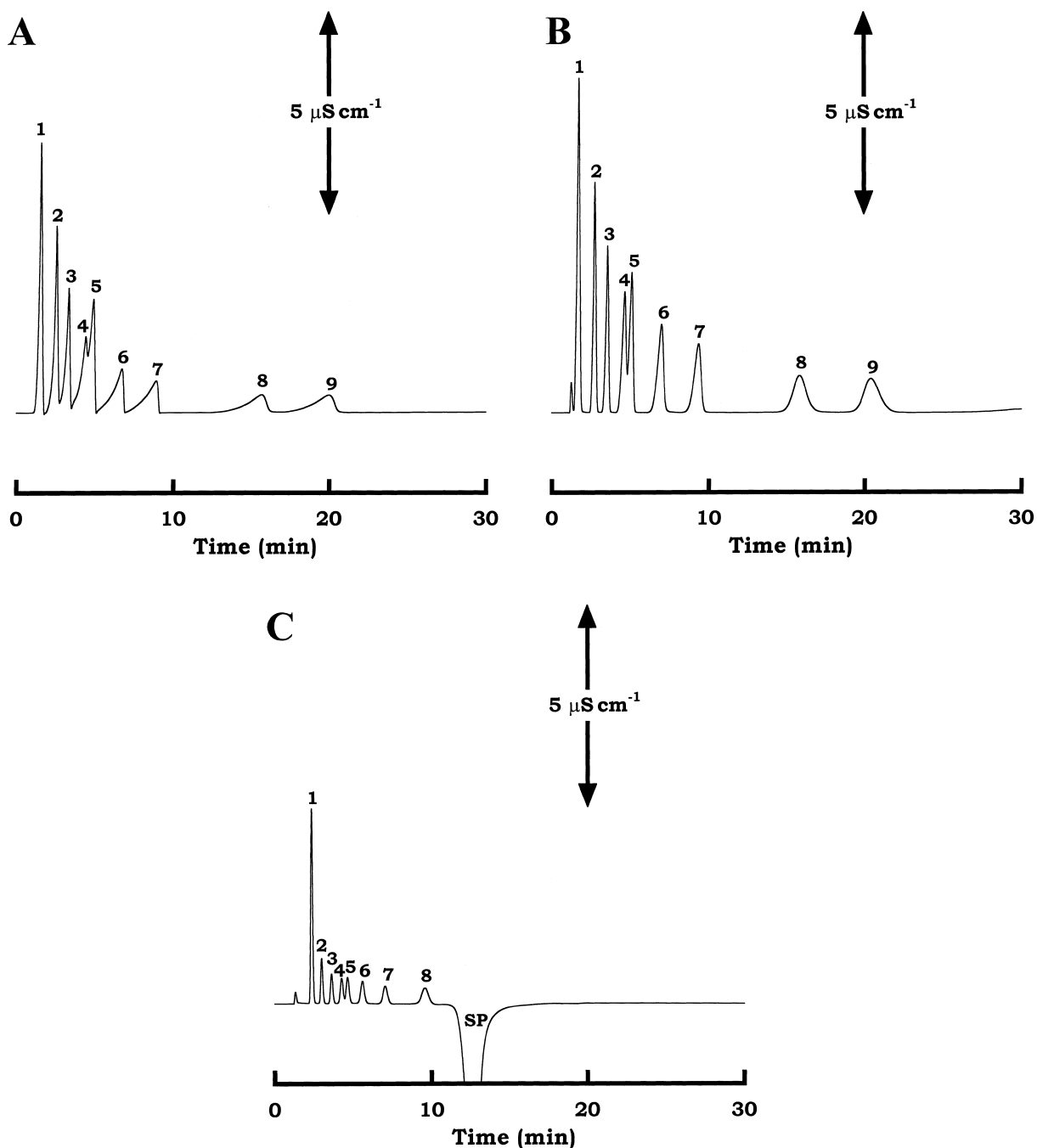


Fig. 6. Chromatograms of C<sub>1</sub>-C<sub>6</sub> aliphatic carboxylic acids on TSKgel SCX column using various concentration of 5-methylhexanoic acid as eluent. Conditions: eluent: (A) 0.01 mM 5-methylhexanoic acid at pH 5.2 (3.4 μS cm<sup>-1</sup>), (B) 0.1 mM 5-methylhexanoic acid at pH 4.5 (12 μS cm<sup>-1</sup>), (C) 10 mM 5-methylhexanoic acid at pH 3.5 (135 μS cm<sup>-1</sup>). Peaks: SP=system peak (vacant peak corresponding to 5-methylhexanoic acid in eluent), 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutyric acid, 5=butyric acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid, 9=caproic acid. Other chromatographic conditions as in Fig. 5.

Table 2

Detection limits (signal-to-noise ratio of 3, injection volume of 100  $\mu\text{l}$ ) of  $\text{C}_1$ – $\text{C}_6$  aliphatic carboxylic acids

Carboxylic acid	Detection limit	
	$\mu\text{M}$	$\text{ng ml}^{-1}$
Formic acid	0.047	2.2
Acetic acid	0.23	14
Propionic acid	0.39	26
Isobutyric acid	0.45	40
Butyric acid	0.43	38
Isovaleric acid	0.59	60
Valeric acid	0.79	81
Isocaproic acid	1.1	$1.3 \times 10^2$
Caproic acid	0.98	$1.2 \times 10^2$

Eluent conductivity of 1 mM 5-methylhexanoic acid: 42  $\mu\text{S cm}^{-1}$ . Noise:  $1.3 \times 10^{-3}$   $\mu\text{S cm}^{-1}$ .

#### 4. Conclusion

In order to extend the utility of a highly sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) in the  $\text{H}^+$  form as a stationary phase in ion-exclusion chromatography for carboxylic acids, the application of the TSKgel SCX column (150 $\times$ 6 mm I.D.) in ion-exclusion chromatography–CD was carried out for the simultaneous separation of  $\text{C}_1$ – $\text{C}_6$  aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids) using  $\text{C}_7$  aliphatic carboxylic acids (heptanoic, 2-methylhexanoic, 5-methylhexanoic and 2,2-dimethyl-*n*-valeric acids) as eluents.

As a result, it was found that 5-methylhexanoic acid, which was strongly adsorbed onto the TSKgel SCX in comparison with these  $\text{C}_1$ – $\text{C}_6$  carboxylic

acids, was the most suitable eluent in ion-exclusion chromatography–CD for these carboxylic acids. 5-Methylhexanoic acid in the eluent was strongly adsorbed onto the surface of the TSKgel SCX at first and then decreased the hydrophobicity of the TSKgel SCX. When using 1 mM 5-methylhexanoic acid at pH 4.0 as the eluent, symmetrical peaks, excellent simultaneous separation and relatively high sensitive conductimetric detection for these carboxylic acids were achieved on the TSKgel SCX column in 25 min.

The use of higher aliphatic carboxylic acid, such as 5-methylhexanoic acid, as the eluent strongly expands the utility of the TSKgel SCX column in the ion-exclusion chromatography–CD of aliphatic carboxylic acids.

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