

Ion-exclusion chromatography of aliphatic carboxylic acids on an unmodified silica gel column

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Abstract

In order to demonstrate the effectiveness of a silica gel column in ion-exclusion chromatography, the elution behavior of aliphatic carboxylic acids was investigated on an unmodified silica gel column having cation-exchange characteristics. When water was used as an eluent, the elution order of the carboxylic acids was dependent on the pK_a and the hydrophobicity, but the resolution between strong acids, e.g. HNO_3 , and carboxylic acids was not satisfactory. Therefore, the composition of eluents containing sulfuric acid and organic modifiers was investigated for the separation of HNO_3 and C_1 – C_{10} aliphatic carboxylic acids. When 0.5 mM sulfuric acid containing 0.05% heptanol was used as an eluent, an excellent ion-exclusion chromatographic separation was achieved in 30 min using UV-detection at 220 nm.

Keywords: Mobile phase composition; Organic modifiers; Carboxylic acids; Sulfuric acid

1. Introduction

Ion-exclusion chromatography developed by Wheaton et al. [1] has been recognized as a useful technique for the separation of inorganic and organic weak acids. The weak acids are separated on a cation-exchange resin depending on their first dissociation constant (pK_{a1}) and the hydrophobicity [2].

In ion-exclusion chromatography, the combination of a column packed with a high-capacity sulfonated styrene–divinylbenzene (PS–DVB) co-polymer resin in the H^+ form and an acidic eluent has been commonly employed for the separation of the weak acids. However, when using such acidic eluents, hydrophobic weak acids such as higher aliphatic carboxylic acids and aromatic carboxylic acids are retained strongly on the PS–DVB column due to hydrophobic interaction and thus the peak obtained is

usually tailed. In order to improve the peak shape of the hydrophobic weak acids by reducing the hydrophobic interaction on the PS–DVB resin, various organic modifiers, such as alcohols [3], acetonitrile [4] and sugar [5], have been added to the aqueous eluent. However, the use of higher concentrations of the organic modifiers is limited because shrinking of the PS–DVB resin occurs. Therefore, it has been difficult to obtain a satisfactory ion-exclusion chromatogram for the higher hydrophobic carboxylic acids. On the other hand, silica gel is recognized as a useful column packing material for HPLC [6,7] because of its physical and chemical stability and inertness to organic solvents. The silanol group on the surface of silica is known to act as a weakly acidic cation exchanger at $pH > 2$ because the isoelectric point is approximately 2. Considering the above characteristics of silica, it can be expected that a silica gel column should be applicable to the ion-exclusion chromatographic separation of hydro-

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phobic carboxylic acids by elution with neutral or moderately acidic eluents.

The purpose of this study is to demonstrate the effectiveness of an unmodified silica gel column in the ion-exclusion chromatographic separation of hydrophobic aliphatic carboxylic acids. Using a 0.5 mM sulfuric acid eluent containing 0.05% heptanol (v/v), a good ion-exclusion chromatographic separation of the C₁–C₁₀ aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic, caprylic, pelargonic and capric acids) was achieved in 30 min using UV detection at 220 nm.

2. Experimental

2.1. Ion chromatograph

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) SC-8010 chromatographic data processor, a Tosoh CCPM delivery pump at a flow-rate of 1 ml/min, a Tosoh CO-8000 column oven at 35°C, a Tosoh SD-8012 on-line eluent degasser, a Shimadzu (Kyoto, Japan) SPD-10AV UV-Vis spectrophotometric detector at 220 nm and a Reodyne (Conti, CA, USA) 7125 injector equipped with a 100 µl sample loop.

2.2. Columns

The stainless-steel separation column (300 mm × 7.8 mm I.D.) was packed with Nomura Chemical (Seto, Japan) Develosil 30-5 spherical porous silica gel and equilibrated thoroughly with eluent before each chromatographic run.

The TSKgel SCX column was used as an example of a conventional ion-exclusion chromatography separation column to compare elution behavior of various kinds of inorganic and organic acids with that obtained using the silica gel column.

The details of these columns are shown in Table 1.

2.3. Chemicals

All chemicals were of analytical-reagent grade or HPLC grade and were purchased from Wako (Osaka, Japan).

The eluents were prepared by diluting 0.5 M

Table 1
Properties of ion-exclusion chromatography columns used in this study

Column	Develosil 30-5	TSKgel SCX
Matrix	Silica	PS-DVB
Functionality	-SiOH	-SO ₃ H
Capacity (mequiv. g ⁻¹)	nd	>4.2
Particle size (µm)	5	5
Pore size (Å)	ca. 27	uk
Surface area (m ² g ⁻¹)	ca. 770	uk
Column size (mm)	300 × 7.8	300 × 7.8
Column void volume (ml)	5.2	3.4
Volume of liquid inside the resin in the column (ml)	5.3	6.9

nd = not determined; uk = unknown.

sulfuric acid with distilled deionized water or organic solvent-water.

3. Results and discussion

3.1. Elution behavior of various acids on the silica gel column by elution with water

In order to characterize the silica gel column in the ion-exclusion mode, the elution behavior of various strong and weak acids, including inorganic and organic acids, was investigated using water as an eluent, and the results were compared to those from the TSKgel SCX ion-exclusion column.

Table 2 lists the retention volumes (V_R) and the distribution coefficients (K_d) for these acids together with the pK_{a1} values. The K_d value was calculated according to the following equation [8]:

$$V_R = V_0 + V_i K_d$$

where V_R is the retention volume, V_0 is the column void volume and V_i is the volume of liquid inside the resin in the column. The V_0 value was taken as the V_R of H₂SO₄, which was completely excluded from the resin phase. The value of $V_0 + V_i$ was calculated from the mass difference between the wet silica gel and the silica gel after drying at 130°C for 24 h [8]. The V_0 value of the silica gel column was 5.2 ml and the V_i value was 5.3 ml.

From Table 2, it can be seen that strong acids

Table 2
Retention volume (V_R) and distribution coefficient (K_d) of common inorganic and organic acids on the ion-exclusion chromatography columns by elution with water

Solute	pK_{a1}	Develosil 30-5		TSKgel SCX	
		V_R (ml)	K_d	V_R (ml)	K_d
HCl	-7.0	5.4	0.03	3.4	0.00
H ₂ SO ₄	-4.0	5.2	0.00	3.4	0.00
HNO ₃	-1.5	5.3	0.01	3.4	0.00
H ₃ PO ₄	2.15	6.4	0.16	3.6	0.03
HF	3.17	31.8	5.05	4.2	0.11
H ₂ CO ₃	6.35	17.7	2.38	12.5	1.31
H ₃ BO ₃	9.23	17.2	2.27	12.9	1.37
Formic acid	3.75	7.7	0.47	6.3	0.42
Acetic acid	4.76	10.2	0.95	9.0	0.81
Propionic acid	4.87	11.8	1.26	11.9	1.22
Butyric acid	4.82	14.0	1.66	17.4	2.03
Valeric acid	4.86	17.4	2.31	32.6	3.10
Caproic acid	4.86	21.1	3.01	70.2	9.67
Heptanoic acid	4.87	24.9	3.73	154.4	21.8
Caprylic acid	4.89	29.1	4.54	S.R.	V.L.
Pelargonic acid	5.00	35.3	5.72	S.R.	V.L.
Capric acid	~5	46.4	7.87	S.R.	V.L.
Lactic acid	3.89	8.3	0.59	6.3	0.42
Oxalic acid	1.27	8.1	0.55	3.7	0.03
Malonic acid	2.84	6.5	0.25	4.5	0.15
Tartaric acid	3.04	6.4	0.23	4.4	0.14
Malic acid	3.46	6.9	0.32	5.0	0.23
Succinic acid	4.21	8.1	0.55	6.7	0.48
Citric acid	3.13	6.6	0.25	4.2	0.11
Benzoic acid	4.20	7.8	0.48	36.2	4.74
Phthalic acid	2.95	5.5	0.05	4.4	0.14
Trimellitic acid	2.52	5.3	0.01	3.8	0.06
Pyromellitic acid	1.92	5.3	0.01	3.5	0.01
Methanol	15.5	12.9	1.47	11.6	1.18
Ethanol	16.6	15.0	1.86	13.6	1.48
Propanol	~17	17.2	2.28	19.0	2.25
Butanol	~17	20.5	2.91	30.2	3.87
Phenol	9.70	12.8	1.45	S.R.	V.L.

S.R. = strong retention; V.L. = very large value.

(HCl, H₂SO₄ and HNO₃) were eluted simultaneously, close to the V_0 value, and weak acids were eluted later than the strong acids, indicating that an ion-exclusion process occurred on the silica gel column. Accordingly, this result suggested that these acids were excluded from the silica gel surface by electrostatic repulsion from the dissociated silanol group.

The K_d values of most of the acids listed in Table 2 were between 0 (complete ion exclusion) and 1 (complete ion permeation), but those of the higher aliphatic monocarboxylic acids with hydrophobic characteristics were greater than 1 due to adsorption

effects on the surface of the silica gel. The K_d values of these higher aliphatic carboxylic acids on the silica gel column were much smaller than those on the TSKgel SCX column, which indicated that the hydrophobicity on the surface of the silica gel was much smaller than that on PS-DVB resin. The retention order of the aliphatic carboxylic acids on the silica gel column was the same as that on the TSKgel SCX column.

The retention mechanism of aromatic carboxylic acids (benzoic, phthalic, trimellitic and pyromellitic acids) on the silica gel column was based mainly on

ion-exclusion effects because the K_d values for these species were between 0 and 1, depending on pK_{a1} . The K_d value of HF was extremely large on the silica gel column, perhaps because of the strong chemical adsorption of HF on the surface of the silica gel. On the other hand, the K_d values of H_2CO_3 and H_3BO_3 were considerably larger than 1. This might have been due to hydrophilic adsorption on the silica gel. Tanaka et al. [9] have reported the separation of non-ionic organic compounds by ion-exclusion chro-

matography on the TSKgel SCX column. In the present method, the separation of these compounds was again achieved, based on the adsorption effect on the silica gel, as listed in Table 1.

A typical chromatogram of aliphatic monocarboxylic acids using water as an eluent is shown in Fig. 1A. Fronted peaks were obtained for formic acid (C_1) to valeric acid (C_5), while tailed peaks were obtained for caproic acid (C_6) to caprylic acid (C_8). This result indicated that the separation mechanism

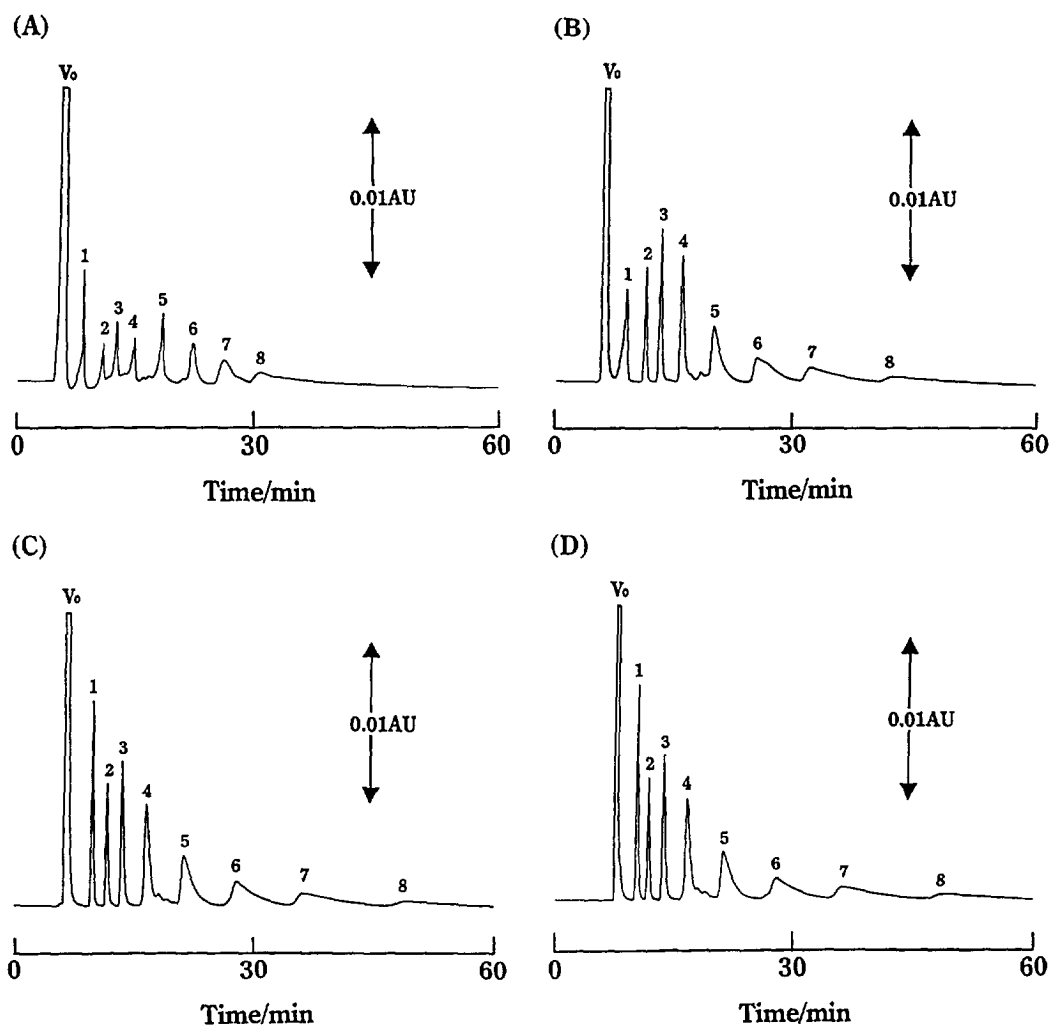


Fig. 1. Chromatograms of nitric acid and aliphatic carboxylic acids under the various concentrations of sulfuric acid in the eluent. Column: Develosil 30-5, 300 mm \times 7.8 mm I.D.; column temperature: 35°C; eluent: (A) distilled water, (B) 0.05 mM sulfuric acid, (C) 0.5 mM sulfuric acid, (D) 5 mM sulfuric acid; flow-rate: 1 ml/min; detection: UV at 220 nm; injection volume: 100 μ l; sample concentration: 0.1 mM for nitric acid and 1 mM for all aliphatic monocarboxylic acids. Peaks: V_0 = nitric acid, 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid, 6 = caproic acid, 7 = heptanoic acid, 8 = caprylic acid.

of the acids was based mainly on ion-exclusion effects for low hydrophilic acids and on adsorption effects for hydrophobic acids. From the above results, it was concluded that the silica gel was a useful column packing material for the ion-exclusion chromatographic separation of aliphatic mono-carboxylic acids having either hydrophilic and hydrophobic characteristics.

3.2. Effect of sulfuric acid concentration

Using water as an eluent, the higher aliphatic carboxylic acids were eluted easily, but the resolution between these acids and the peak shape were not satisfactory. Turkelson et al. [10] have reported that the use of acidic eluents in ion-exclusion chromatography were very effective in the improvement of the peak shape. Therefore, in order to improve the peak shape, the effect of the concentration of sulfuric acid in the eluent was investigated between 0–5 mM. Typical chromatograms of the aliphatic carboxylic acids obtained with 0.05, 0.5 and 5 mM sulfuric acid eluent are shown in Fig. 1B, C and D, respectively. The peak shapes for formic, acetic and propionic acids were improved with increasing concentrations of sulfuric acid in the eluent, whereas the peak shapes for butyric and valeric acids remained tailed. Furthermore, the peak shapes for higher carboxylic acids exhibited extensive tailing. The V_R values of the hydrophobic carboxylic acids increased with increasing concentration of sulfuric acid in the eluent.

These results indicated that suppression of the degree of dissociation of both the silanol groups and the solutes themselves caused an increase in the extent of tailing and the V_R of these acids, especially the more hydrophobic species. As can be seen from Fig. 1, the resolution between nitric acid and formic acid improved with increasing concentration of sulfuric acid in the eluent. A reasonable chromatogram was obtained using a 0.5 mM sulfuric acid eluent at pH 3 (Fig. 1C).

Fig. 1D indicates that the ion-exclusion effects occur even in a 5 mM sulfuric acid eluent (pH 2) because the V_R value of nitric acid was smaller than that of a weak acid. Additionally, the V_R value of nitric acid, which corresponds to V_0 , increased with increasing concentration of sulfuric acid in the eluent

because suppression of the dissociation of the silanol groups causes a decrease in electrostatic repulsion. Considering the resolution and V_R values of higher carboxylic acids, the optimum concentration of sulfuric acid in the eluent was concluded to be 0.5 mM.

3.3. Effect of organic modifier

In order to improve the peak shape and to accelerate the elution of higher aliphatic carboxylic acids, the effect of addition of organic modifiers (methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, ethyleneglycol, glycerol and acetonitrile) to a 0.5 mM sulfuric acid eluent was investigated.

First, the effect of methanol was investigated in detail. The relationship between the methanol concentration and the V_R values of aliphatic carboxylic acids is shown in Fig. 2. With increasing methanol concentration, the V_R values of nitric, formic and acetic acids were virtually unaltered, whereas those of the hydrophobic acids (C_3 – C_{10}) decreased drastically. Using a 0.5 mM sulfuric acid eluent containing

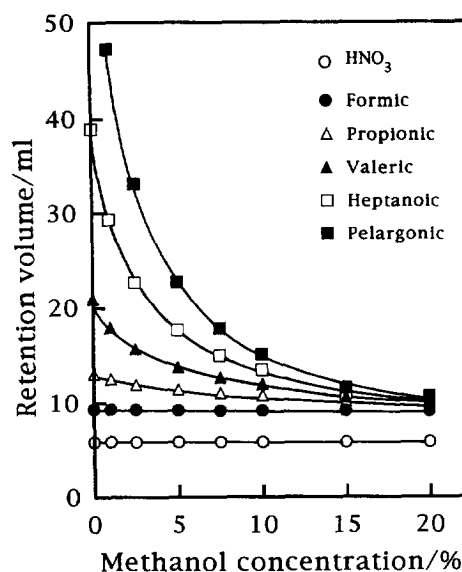


Fig. 2. Effect of methanol concentration in the eluent on retention volumes (V_R) of HNO₃ and aliphatic monocarboxylic acids. Eluent: 0.5 mM sulfuric acid containing 0–20% methanol; sample concentration: 0.025 mM for nitric acid and 0.25 mM for all aliphatic monocarboxylic acids. Other chromatographic conditions as for Fig. 1.

20% methanol, the V_R values of propionic and higher carboxylic acids were almost the same and were close to the $V_0 + V_i$ value for the column. The result indicated that the hydrophobic carboxylic acids were separated depending on their hydrophobicity in the ion-exclusion mode on the silica gel column. Using a 0.5 mM sulfuric acid eluent containing 2.5% methanol, reasonable resolution and V_R values for these acids were obtained (Fig. 3A).

As described above, although reasonable resolution and V_R values for aliphatic carboxylic acids were achieved when using the eluent containing methanol, the peak shape and V_R values for the hydrophobic carboxylic acids were not satisfactory. Morris et al. [11] have reported that the use of an eluent containing higher alcohols, such as butanol, in ion-exclusion chromatography was very effective in improving peak shape. Therefore, the effect of various alcohols in the eluent was investigated. The elution behavior obtained for all of the eluents containing alcohols was similar to that for the methanol eluent described above.

The addition of the alcohols, especially higher alcohols, such as hexanol and heptanol, to the eluent was very effective both in improving the peak shape and in reducing the V_R values of the hydrophobic

carboxylic acids. The result indicated that heptanol was the most effective organic modifier in the ion-exclusion chromatography. As shown in Fig. 3B, an excellent chromatogram of nitric acid and C_1 – C_{10} carboxylic acids was obtained in 30 min using a 0.5 mM sulfuric acid eluent containing 0.05% heptanol. Other organic modifiers (acetonitrile, ethyleneglycol and glycerol) were also tested, but the results obtained were inferior to those for the higher alcohols (>propanol).

3.4. Reproducibility, calibration graphs and detection limits

Under the optimum chromatographic conditions described above, a mixture of aliphatic carboxylic acids was chromatographed. Fig. 4 shows multi-chromatograms of the aliphatic carboxylic acids in the concentration range 0.1–1.0 mM. The reproducibility of the V_R values of the carboxylic acids was less than 0.5% R.S.D., and calibration graphs obtained by plotting peak area of C_1 – C_8 carboxylic acids against the concentration were linear between 0.1 and 1.0 mM. The detection limits ($S/N=3$) of C_1 – C_{10} carboxylic acids were in the range 3 μM (C_1) to 10 μM (C_{10}).

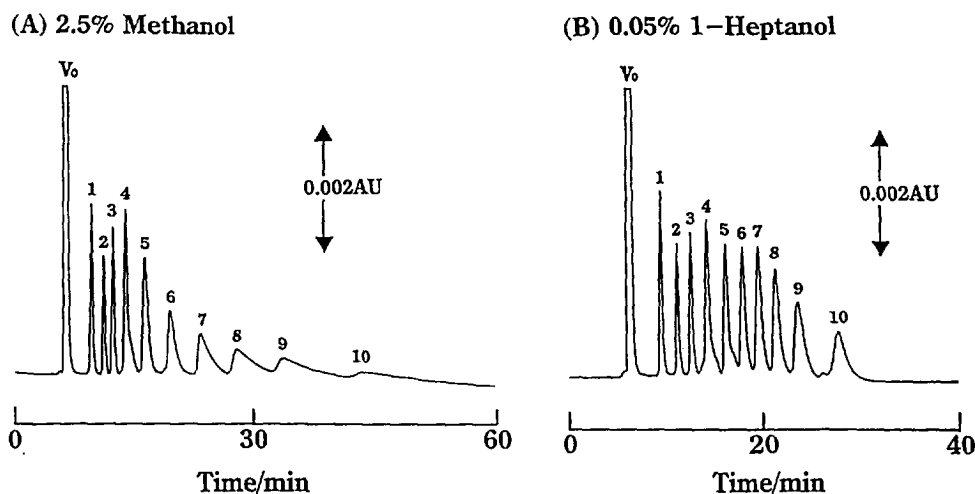


Fig. 3. Chromatograms of HNO_3 and aliphatic carboxylic acids using a 0.5 mM H_2SO_4 eluent containing (A) methanol and (B) 1-heptanol. Peaks: V_0 = nitric acid, 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid, 6 = caproic acid, 7 = heptanoic acid, 8 = caprylic acid, 9 = pelargonic acid, 10 = capric acid. Other chromatographic conditions as for Fig. 2.

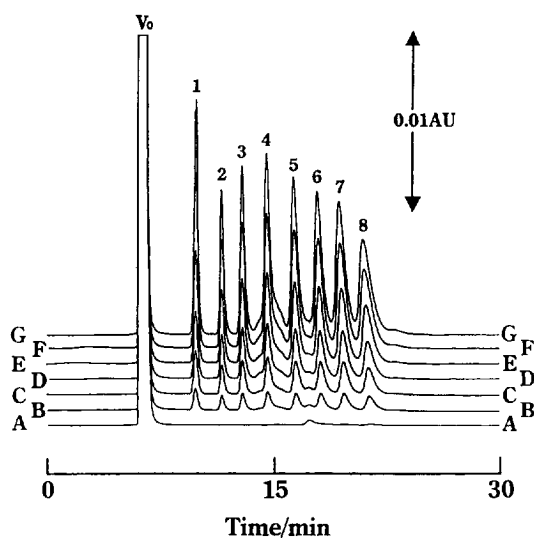


Fig. 4. Multi-chromatogram of HNO_3 and aliphatic carboxylic acids under the optimum ion-exclusion chromatography conditions. Eluent: 0.5 mM sulfuric acid containing 0.05% 1-heptanol. Concentration of aliphatic carboxylic acids: A=0 mM, B=0.1 mM, C=0.2 mM, D=0.3 mM, E=0.5 mM, F=0.75 mM, G=1.0 mM. Peaks: V_0 =nitric acid, 1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid, 5=valeric acid, 6=caproic acid, 7=heptanoic acid, 8=caprylic acid. Other chromatographic conditions as for Fig. 3.

4. Conclusion

A new ion-exclusion chromatographic method has been developed using an unmodified silica gel column and a sulfuric acid–heptanol eluent. The proposed method is useful for the separation of aliphatic carboxylic acids having either hydrophilic or hydrophobic characteristics. A high resolution separation of C_1 – C_{10} carboxylic acids was achieved in 30 min.

Since the detection sensitivity of the aliphatic

carboxylic acids by the proposed method with UV-detection was only moderate, the development of highly sensitive detection methods such as conductivity and spectrophotometry after postcolumn derivatization is required and will be the subject of future work.

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