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Application of polymethacrylate resin as stationary phase in liquid chromatography with UV detection for C_1–C_7 aliphatic monocarboxylic acids and C_1–C_7 aliphatic monoamines

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Abstract

The application of unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as a stationary phase in liquid chromatography with UV detection for C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) was carried out. Using dilute sulfuric acid as the eluent, the TSKgel G3000PW$_{XL}$ resin acted as an advanced stationary phase for these C_1–C_7 carboxylic acids. Excellent simultaneous separation and symmetrical peaks for these C_1–C_7 carboxylic acids were achieved on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) in 60 min with 0.25 mM sulfuric acid containing 1 mM 2-methylheptanoic acid at pH 3.3 as the eluent. Using dilute sodium hydroxide as the eluent, the TSKgel G3000PW$_{XL}$ resin also behaved as an advanced stationary phase for these C_1–C_7 amines. Excellent simultaneous separation and good peaks for these C_1–C_7 amines were achieved on the TSKgel G3000PW$_{XL}$ column in 60 min with 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine at pH 11.9 as the eluent.

Keywords: Stationary phases, LC; Carboxylic acids; Methylheptanoic acid; Methylheptylamine; Amines; Alkylamines; Polymethacrylate

1. Introduction

Ion-exclusion chromatography developed by Wheaton and Baumann [1] is a simply and convenient analytical technique for the determination of low molecular weak acids (carboxylic acids) [2,3] and weak bases (amines) [3,4]. The separation of carboxylic acids mainly proceeds on a cation-exchange resin [low cross-linked styrene–divinylbenzene copolymer (PS–DVB)-based strongly acidic cation-exchange resin] in the H^+ form as the stationary phase with acidic solution as the eluent [5,6]. The separation of amines proceeds on anion-exchange resin (low cross-linked PS–DVB-based strongly basic anion-exchange resin) in the OH^- form as the stationary phase with basic solution as the eluent [7,8]. In these conditions, the dissociation of carboxylic acids and amines are largely suppressed and, consequently, these acids and amines are mainly separated by both ion-exclusion chromatographic process and hydrophobic interaction process. As a result, strongly tailed peaks and extremely long retention times for higher carboxylic acids and amines are obtained. The addition of organic solvent is one of the most useful ways for elimination these drawbacks [9–12]. However, the concentration of organic solvent in the eluent is strongly limited, because shrinkage of these low cross-linked PS–DVB resins occurs. Therefore, the separations of higher carboxylic acids and amine are considerably difficult applications in ion-exclusion chromatography.

Recently, Li and Fritz [13] applied unfunctionized high cross-linked PS–DVB resin was as the stationary phase in liquid chromatography for various carboxylic acids and amines. Good separations of several carboxylic acids and amines were achieved on the PS–DVB resin column. This result supposed strongly that carboxylic acids and amines

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were separated by only hydrophobic interaction between the PS–DVB resin and carboxylic acids and that between the PS–DVB resin and amines. Unfortunately, due to large hydrophobicity, aquatic solution containing large amount of organic solvent was required as the eluent for utilizing this type PS–DVB resin. Hydrophilic unfunctionized resins, such as silica gel and polymethacrylate resin, could be applied as stationary phases in LC with aquatic solution as the eluent for both carboxylic acids and amines. However, when using silica gel as the stationary phase, pH range of eluent is strongly limited. This is because the solubility of silica gel in aquatic solution as the eluent increases drastically with increasing pH of the eluent [14]. Therefore, it was expected that polymethacrylate would be one of the most suitable stationary phases in the LC for both carboxylic acids and amines. However, the application has not been carried out yet.

The aim of this study was to demonstrate the effectiveness of unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as the stationary phase in liquid chromatography with UV detection for carboxylic acids and amines. Then, the chromatographic behavior of C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) was investigated with sulfuric acid and sodium hydroxide as the eluents. The TSKgel G3000PW$_{XL}$ resin behaved as advanced stationary phase for these carboxylic acids and amines. Excellent simultaneous separation and symmetrical peaks for these C_1–C_7 carboxylic acids were achieved on the TSKgel G3000PW$_{XL}$ column in 60 min with 0.25 mM sulfuric acid containing 1 mM 2-methylheptanoic acid (C_8 carboxylic acid) at pH 3.3 as the eluent. Excellent simultaneous separation and good peaks for these C_1–C_7 amines were also achieved on the TSKgel G3000PW$_{XL}$ column in 60 min with 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine (C_8 amine) as the eluent.

2. Experimental

2.1. Instruments

Liquid 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 ml min^{-1}, a Tosoh CO-8020 column oven operated at 35 °C, a Tosoh UV-8020 UV-Vis spectrophotometric detector operated at 210 nm, a Tosoh SD-8023 on-line degasser and a Rheodyne (Cotati, CA, USA) Model 9125 injector equipped with a 100 μl sample loop. A Tosoh CM-8020 conductimetric detector was also used for the measurement of cation-exchange capacity of an unfunctionized polymethacrylate resin (Tosoh TSKgel G3000PW$_{XL}$).

A Toa Denpa (Tokyo, Japan) IM-40S ion meter equipped with a glass electrode was used for the measurement of pH of eluents.

2.2. Separation column

Separation column (150 mm × 6 mm i.d., stainless steel) packed with a Tosoh TSKgel G3000PW$_{XL}$ unfunctionized polymethacrylate resin (particle size of ca. 7 μm) by using slurry-packing method was used in this work.

The TSKgel G3000PW$_{XL}$ resin shows small cation-exchange behavior under basic condition, due to small amount of carboxylic group on the resin, originated from starting materials [15]. The determination of cation-exchange capacity of the resin was carried out according to following cation-exchange reaction;

$$\text{resin} - \text{COO}^-\text{Na}^+ + \text{KOH} \rightarrow \text{resin} - \text{COO}^-\text{K}^+ + \text{NaOH}$$

A cation-exchange capacity (A, meq. ml^{-1}) of the resin was calculated from the equations:

$$A = (V_R - V_o)\frac{C}{1000\ \text{V}}$$

where V_R is the breakthrough volume of the column (ml), V_o the total dead volume (column void volume+connected tube volume, ml), C the concentration of potassium hydroxide solution (mM) and V is column volume (4.24 ml).

First, the column was equilibrated with 1 mM sodium hydroxide solution. Sample of 1 mM sodium hydroxide solution containing 1% methanol was injected. Elution volume of peak corresponding to methanol was considered as V_o (3.34 ml). Next, 1 mM potassium hydroxide solution was passed through the column and the conductimetric detector response (breakthrough curve) was monitored. Volume corresponding to breakthrough point in the detector response curve was considered as V_R (49.0 ml). The amount of cation-exchange capacity of the TSKgel G3000PW$_{XL}$ resin was ca. 0.011 meq. ml^{-1}.

2.3. Chemicals

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

3. Results and discussion

3.1. Separation of C_1–C_7 aliphatic monocarboxylic acids on TSKgel G3000PW$_{XL}$ column

3.1.1. Effect of concentration of sulfuric acid in eluent on chromatographic behavior of C_1–C_7 aliphatic monocarboxylic acids

The application of an unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as a stationary phase in liquid chromatography with UV detection (210 nm) for C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) was attempted with dilute sulfuric acid as eluent. Fig. 1 shows the relationship between the concentration of sulfuric acid in the eluent and the retention volumes of these C_1–C_7 carboxylic acids on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.). Fig. 2A–D show chromatograms of these C_1–C_7 carboxylic acids with: (A) 0.005 mM sulfuric acid at pH 5.1; (B) 0.05 mM sulfuric acid at pH 4.1; (C) 0.25 mM sulfuric acid at pH 3.3; and (D) 0.5 mM sulfuric acid at pH 3.1 as the eluents.

As shown in Fig. 1, with increasing the concentration of sulfuric acid in the eluent, the retention volumes of these C_1–C_7 carboxylic acids increased. The degree of the increase in the retention volumes was formic acid (C_1) < acetic acid (C_2) < propionic acid (C_3) < butyric acid (C_4) < valeric acid (C_5) < hexanoic acid (C_6) < heptanoic acid (C_7). These results indicated that (a) the increase in the retention volume of these C_1–C_7 carboxylic acids was due mainly to an increase in hydrophobicity of these carboxylic acids, caused by suppressing their dissociation and (b) these carboxylic acids were mainly separated by hydrophobic interaction process. As shown in Fig. 2A–D, with increasing the concentration of sulfuric acid, peak shapes of these C_1–C_7 carboxylic acids changed drastically. As shown in Fig. 2A, when using 0.005 mM sulfuric acid as the eluent, strongly fronted peaks of these C_1–C_7 carboxylic acids were observed. This is because these carboxylic acids were well dissociated under the eluent condition. With increasing the concentration of sulfuric acid, peak shapes of C_1–C_5 carboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid and valeric acid) were improved. Symmetrical peaks of the C_1–C_5 carboxylic acids were obtained at the concentration of sulfuric acid in the eluent ≥ 0.25 mM. In contrast, peaks of C_6–C_7 carboxylic acids (3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) were

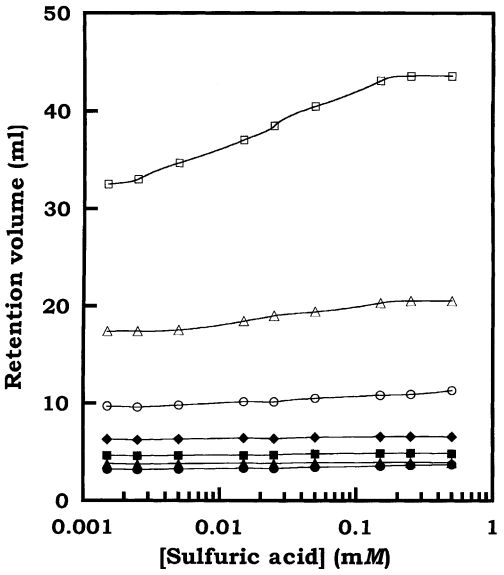

Fig. 1. Effect of concentration of sulfuric acid in eluent on retention volumes of C_1–C_7 aliphatic monocarboxylic acids. Conditions—column: TSKgel G3000PW$_{XL}$; column size: 150 mm × 6 mm i.d.; column temperature: 35 °C; eluent: 0.0015–0.5 mM sulfuric acid; flow rate: 1 ml min^{-1}; detection: UV at 210 nm; injection volume: 100 μl; sample: 1 mM C_1–C_7 carboxylic acids in eluent. Symbols: (●) formic acid; (▲) acetic acid; (■) propionic acid; (◆) butyric acid; (○) valeric acid; (△) hexanoic acid; and (□) heptanoic acid.

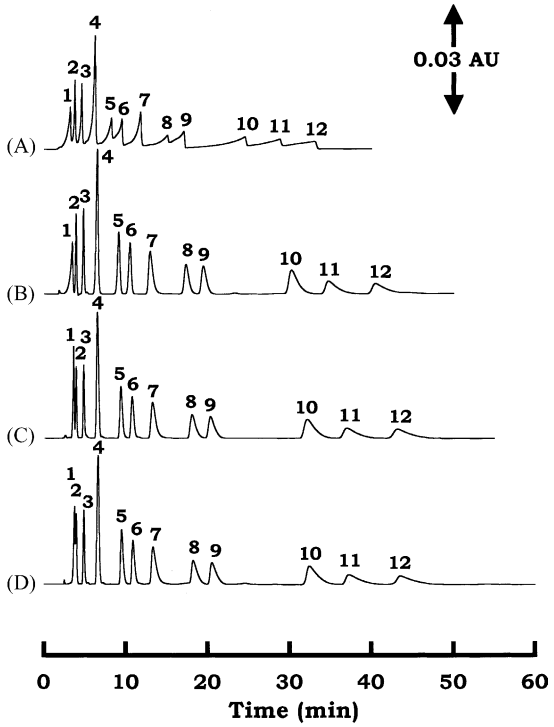

Fig. 2. Chromatograms of C_1–C_7 carboxylic acids with various concentrations of sulfuric acid as eluents. Conditions—eluents: (A) 0.005 mM sulfuric acid at pH 5.1; (B) 0.05 mM sulfuric acid at pH 4.1; (C) 0.25 mM sulfuric acid at pH 3.3; (D) 0.5 mM sulfuric acid at pH 3.1. Other chromatographic conditions as in Fig. 1. Peaks: (1) formic acid; (2) acetic acid; (3) propionic acid; (4) isobutyric and butyric acids; (5) isovaleric acid; (6) valeric acid; (7) 3,3-dimethylbutyric acid; (8) 4-methylvaleric acid; (9) hexanoic acid; (10) 2-methylhexanoic acid; (11) 5-methylhexanoic acid and; (12) heptanoic acid.

tailed largely. This is due mainly to strongly hydrophobic interaction between the resin and the hydrophobic C_6–C_7 carboxylic acids. Peak resolution between these C_1–C_7 carboxylic acids was improved at the concentration range of sulfuric acid in the eluent between 0.0015 and 0.25 mM. Good separation of these C_1–C_7 carboxylic acids was achieved in 50 min with 0.25 mM sulfuric acid as the eluent.

Considering peak shape and peak resolution, it was concluded that a reasonable concentration of sulfuric acid in the eluent was 0.25 mM for these C_1–C_7 carboxylic acids (Fig. 2C). However, a further investigation was required for improving peak shapes of the C_6–C_7 carboxylic acids.

3.1.2. *Effect of C_8 aliphatic monocarboxylic acids added to sulfuric acid as eluent on chromatographic behavior of C_1–C_7 aliphatic monocarboxylic acids*

In previous study, we demonstrated that C_7 aliphatic monocarboxylic acid (5-methylhexanoic acid) was very effective eluent in ion-exclusion chromatography with a sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) column for C_1–C_6 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 4-methylvaleric acid and hexanoic acid) [16]. 5-Methyhexanoic acid in the eluent was adsorbed on the resin at first and then reduced hydrophobic interaction between the resin and these C_1–C_6 carboxylic acids. Hence, the addition of C_8 aliphatic monocarboxylic acids (2-propylvaleric acid, 2-ethylhexanoic acid, 2-methylheptanoic acid and octanoic acid) to 0.25 mM sulfuric acid as the eluent was carried out, for improving peak shapes of the C_6–C_7 carboxylic acids. Fig. 3A–D show chromatograms of these C_1–C_7 carboxylic acids with 0.25 mM sulfuric acid containing: (A) 1 mM 2-propylvaleric acid; (B) 1 mM 2-ethylhexanoic acid; (C) 1 mM 2-methylheptanoic acid; and (D) 1 mM octanoic acid as the eluents.

As shown in Fig. 3A–D, when using sulfuric acid containing the C_8 carboxylic acids as the eluents, peak shapes of the C_6–C_7 carboxylic acids were improved drastically and their retention times decreased largely. These results indicated that the C_8 carboxylic acids acted as an advanced elution modifier for these hydrophobic carboxylic acids. Unfortunately, system peaks corresponding to the C_8 carboxylic acids in the eluent appeared. As shown in Fig. 3A and B, when using 0.25 mM sulfuric acid containing 1 mM 2-propylvaleric acid or 1 mM 2-ethylhexanoic acid as the eluents, system peaks corresponding to the C_8 acids (2-propylvaleric acid or 2-ethylhexanoic acid) interfered seriously for the determination of heptanoic acid. In contrast, as shown in Fig. 3C and D, when using 0.25 mM sulfuric acid containing 1 mM 2-methylheptanoic acid or 1 mM octanoic acid as the eluents, no interferences of system peaks corresponding to the C_8 carboxylic acid (2-methylheptanoic acid or octanoic acid) for the determination of these C_1–C_7 carboxylic acids were observed. However, when using 0.25 mM sulfuric acid containing 1 mM octanoic acid as the eluent, since the retention time of the system peak was

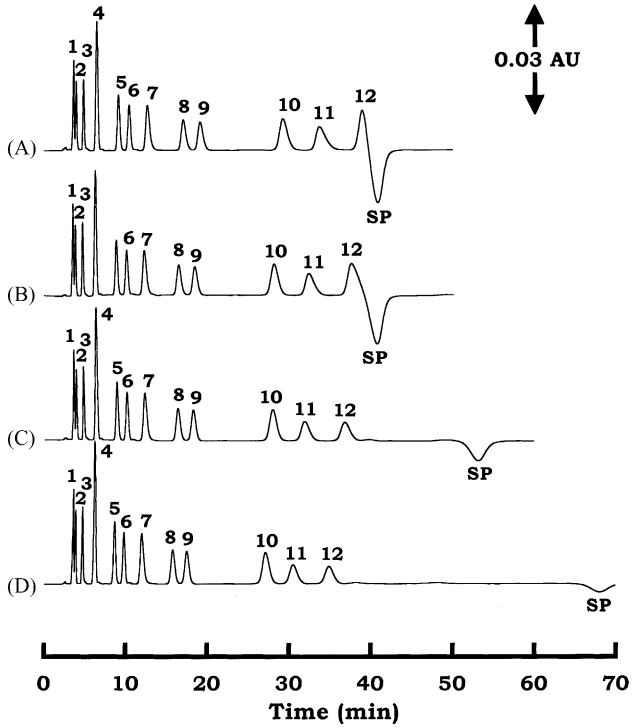

Fig. 3. Chromatograms of C_1–C_7 carboxylic acids with 0.25 mM sulfuric acid containing various C_8 carboxylic acids as eluents. Conditions—eluents: (A) 1 mM 2-propylvaleric acid in 0.25 mM sulfuric acid at pH 3.3; (B) 1 mM 2-ethylhexanoic acid in 0.25 mM sulfuric acid at pH 3.3; (C) 1 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid at pH 3.3; (D) 1 mM octanoic acid in 0.25 mM sulfuric acid at pH 3.3. Sample: 1 mM C_1–C_7 carboxylic acids in 0.25 mM sulfuric acid. Other chromatographic conditions as in Fig. 2. Peaks: (SP), system peak corresponding to C_8 acid in eluent, other peaks identification is as in Fig. 2.

ca. 67 min, it took ca. 70 min for each chromatographic run.

Considering peak shape, interference of system peak and chromatographic time, it was concluded that 2-methylheptanoic acid was the most suitable C_8 carboxylic acid added to 0.25 mM sulfuric acid as the eluent for these C_1–C_7 carboxylic acids (Fig. 3C).

Next, the effect of the concentration of 2-methylheptanoic acid in 0.25 mM sulfuric acid as the eluent on the chromatographic behavior of these C_1–C_7 carboxylic acids was investigated in detail. Fig. 4 shows the relationship between the concentration of 2-methylheptanoic acid in the eluent and the retention volumes of these C_1–C_7 carboxylic acids. Fig. 5A–D show chromatograms of these C_1–C_7 carboxylic acids with 0.25 mM sulfuric acid containing: (A) 0.125 mM; (B) 0.25 mM; (C) 0.5 mM; and (D) 2 mM 2-methyheptanoic acid as the eluents. A chromatogram with 0.25 mM sulfuric acid containing 1 mM 2-methyheptanoic acid as the eluent has been already shown in Fig. 3C.

As shown in Fig. 4, with increasing the concentration of 2-methylheptanoic acid in the eluent, the retention volumes of these C_1–C_7 carboxylic acids decreased. The order of the decreased in the retention volumes was formic acid

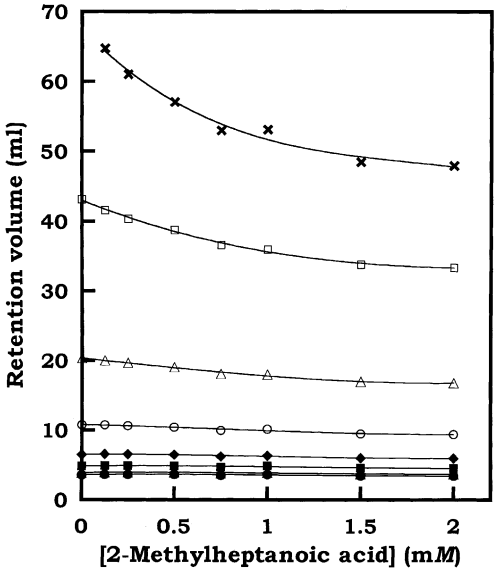

Fig. 4. Effect of concentration of 2-methylheptanoic acid in 0.25 mM sulfuric acid as eluent on retention volumes of C_1–C_7 carboxylic acids. Conditions—eluents: 0–2 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid. Other chromatographic conditions are as in Fig. 3. Symbols: (✖) system peak corresponding to 2-methylheptanoic acid in eluent, other identification symbols as in Fig. 1.

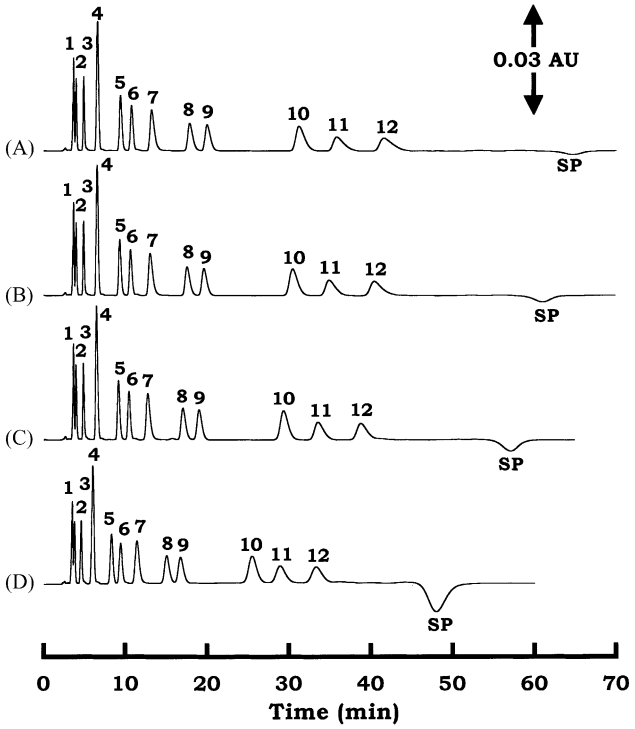

Fig. 5. Chromatograms of C_1–C_7 carboxylic acids with 0.25 mM sulfuric acid containing various concentrations of 2-methylheptanoic acid as eluents. Conditions—eluents: (A) 0.125 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid at pH 3.3; (B) 0.25 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid at pH 3.3; (C) 0.5 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid at pH 3.3; (D) 2 mM 2-methylheptanoic acid in 0.25 mM sulfuric acid at pH 3.3. Other chromatographic conditions as in Fig. 4. Peaks: (SP), system peak corresponding to 2-methylheptanoic acid in eluent, other peaks identification as in Fig. 2.

(C_1) < acetic acid (C_2) < propionic acid (C_3) < butyric acid (C_4) < valeric acid (C_5) < hexanoic acid (C_6) < heptanoic acid (C_7) < system peak corresponding to 2-methylheptanoic acid (C_8). The order indicated clearly that 2-methylheptanoic acid was strongly adsorbed on the resin and acted as an effective elution modifier for higher carboxylic acids including 2-methylheptanoic acid itself. As shown in Figs. 3C and 5A–D, with increasing the concentration of 2-methylheptanoic acid, peak shapes of the C_6–C_7 carboxylic acids were improved. Symmetrical peaks of these C_1–C_7 carboxylic acids were obtained at the concentration of 2-methyheptanoic acid in the eluent ≥ 1 mM. Excellent simultaneous separation and symmetrical peaks for these C_1–C_7 carboxylic acids were achieved in 60 min with 0.25 mM sulfuric acid containing 1 mM 2-methylheptanoic acid as the eluent. In contrast, with increasing the concentration of 2-methyheptanoic acid, UV detector responses of these C_1–C_7 carboxylic acids somewhat decreased at 210 nm.

Considering peak shape, UV detection sensitivity and chromatographic time, it was concluded that the optimum concentration of 2-methylheptanoic acid in 0.25 mM sulfuric acid as the eluent was 1 mM for these C_1–C_7 carboxylic acids (Fig. 3C).

3.1.3. Analytical performance parameters

Table 1 shows the detection limits (UV detection at 210 nm, signal-to-noise ratio of 3 and injection volume of 100 μl) of these C_1–C_7 carboxylic acids. Unfortunately, due to low molar extinction coefficients of these carboxylic acids at 210 nm, detection sensitivities were moderate.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these C_1–C_7 carboxylic acids. Linear calibration graphs (r^2 > 0.99) were obtained in the concentration range between 0.03 and 2 mM for these carboxylic acids.

The relative standard deviations of the chromatographic peak area of these C_1–C_7 carboxylic acids, whose concen-

Table 1
Detection limits (UV detection at 210 nm, signal-to-noise ratio of 3 and injection volume of 100 μl) of C_1–C_7 carboxylic acids

Carboxylic acid	Detection limit	
	μM	μg ml^{-1}
Formic acid	2.3	0.11
Acetic acid	3.0	0.18
Propionic acid	2.9	0.21
Butyric acid	3.2	0.28
Isovaleric acid	3.6	0.37
Valeric acid	4.3	0.44
3,3-Dimethylbutyric acid	4.4	0.51
4-Methylvaleric acid	6.5	0.76
Hexanoic acid	6.8	0.79
2-Methylhexanoic acid	6.7	0.87
5-Methylhexanoic acid	11	1.4
Heptanoic acid	11	1.5

trations were 1 mM, were less than 0.9% ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

3.2. Separation of C_1–C_7 aliphatic monoamines on TSKgel G3000PW$_{XL}$ column

3.2.1. Effect of concentration of sodium hydroxide in eluent on chromatographic behavior of C_1–C_7 aliphatic monoamines

The application of the TSKgel G3000PW$_{XL}$ column in liquid chromatography with UV detection at 210 nm for C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) was attempted with dilute sodium hydroxide as the eluent. Fig. 6 shows the relationship between the concentration of sodium hydroxide in the eluent and the retention volumes of these C_1–C_7 amines on the TSKgel G3000PW$_{XL}$ column. Fig. 7A–C show chromatograms of these C_1–C_7 amines with: (A) 2 mM sodium hydroxide at pH 11.3; (B) 5 mM sodium hydroxide at pH 11.6; and (C) 10 mM sodium hydroxide at pH 11.9 as the eluents.

As shown in Fig. 6, with increasing the concentration of sodium hydroxide in the eluent, the retention volumes of these C_1–C_7 amines decreased drastically. The retention behavior of these C_1–C_7 amines on the column with sodium hydroxide as the eluent was completely different from that of these C_1–C_7 carboxylic acids on the column with sulfuric acid as the eluent (Fig. 1). Since the TSKgel G3000PW$_{XL}$ resin showed small cation-exchange behavior under basic condition (cation-exchange capacity: ca. 0.011 meq. ml^{-1} at pH 11.0) [15], it was expected that these C_1–C_7 amines were separated by not only hydrophobic interaction process but also cation-exchange process. As shown in Fig. 7A–D, these chromatograms were quite different. As shown in Fig. 7A, when using 2 mM sodium hydroxide as the eluent, C_1–C_2 amines (methylamine and ethylamine) were detected indirect-photometrically and C_3–C_7 amines (propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) were detected direct-photometrically at 210 nm. The C_1–C_2 amines were detected direct-photometrically at the concentration of sodium hydroxide in the eluent $\geq$ 3 mM. With increasing the concentration of sodium hydroxide, peak resolution between these C_1–C_7 amines was also improved. Good separation of these C_1–C_7 amines was achieved in 60 min with 10 mM sodium hydroxide as the eluent. Unfortunately, peaks of C_6–C_7 amines (1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) were largely tailed, due to strongly hydrophobic interaction between these hydrophobic amines and the resin.

Considering peak shape, peak resolution and chromatographic time, it was concluded that a reasonable concentration of sodium hydroxide in the eluent was 10 mM for these C_1–C_7 amines (Fig. 7C). However, a further investigation was also required for improving peak shapes of the C_6–C_7 amines.

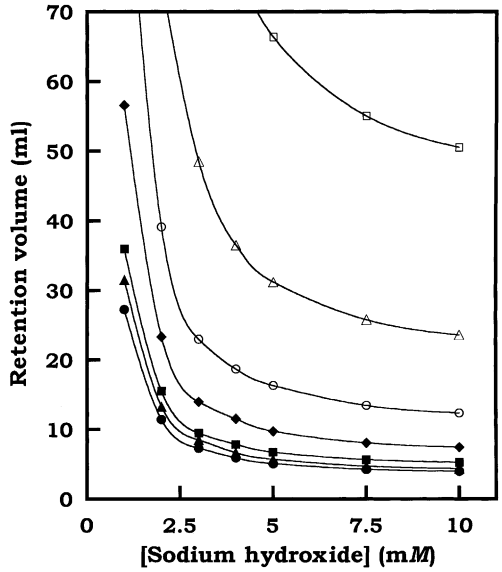

Fig. 6. Effect of concentration of sodium hydroxide in eluent on retention volumes of C_1–C_7 aliphatic monoamines. Conditions—eluents: 1–10 mM sodium hydroxide, sample: 1 mM C_1–C_7 aliphatic monoamines in eluent. Other chromatographic conditions as in Fig. 5. Symbols: (●) methylamine; (▲) ethylamine; (■) propylamine; (◆) butylamine; (○) amylamine; (△) hexylamine; and (□) heptylamine.

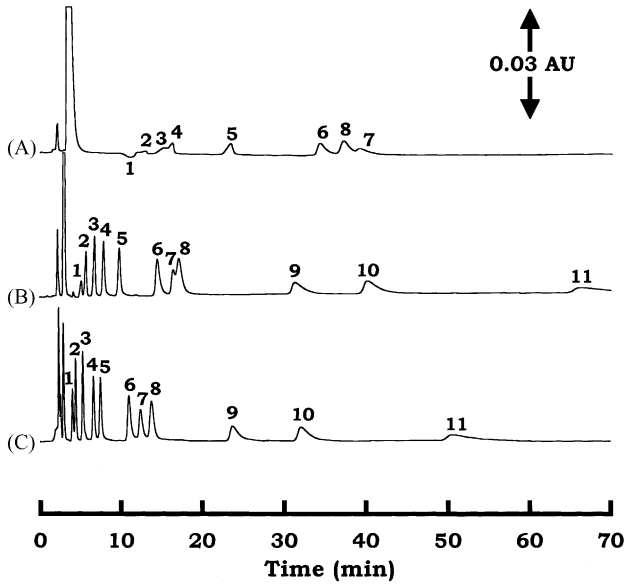

Fig. 7. Chromatograms of C_1–C_7 amines with various concentrations of sodium hydroxide as eluents. Conditions—eluents: (A) 2 mM sodium hydroxide at pH 11.3; (B) 5 mM sodium hydroxide at pH 11.6; (C) 10 mM sodium hydroxide at pH 11.9. Other chromatographic conditions as in Fig. 6. Peaks: (1) methylamine; (2) ethylamine; (3) propylamine; (4) isobutylamine; (5) butylamine; (6) isoamylamine; (7) amylamine; (8) 1,3-dimethylbutylamine; (9) hexylamine; (10) 2-heptylamine and; (11) heptylamine.

3.2.2. Effect of C_8 aliphatic monoamines added to sodium hydroxide as eluent on chromatographic behavior of C_1–C_7 aliphatic monoamines

The addition of C_8 amines (1,5-dimethylhexylamine, 2-ethylhexylamine, 1-methylheptylamine and octylamine) to 10 mM sodium hydroxide as the eluent was carried out for improving peak shapes of the C_6–C_7 amines. Fig. 8A–D show chromatograms of these C_1–C_7 amines with 10 mM sodium hydroxide containing: (A) 0.5 mM 1,5-dimethylhexylamine; (B) 0.5 mM 2-ethylhexylamine; (C) 0.5 mM 1-methylheptylamine; and (D) 0.5 mM octylamine as the eluents.

As shown in Fig. 8A–D, when using 10 mM sodium hydroxide containing the C_8 amines as the eluent, peak shapes of the C_6–C_7 amines were improved and their retention times decreased largely. This is because the C_8 amines in the eluent were strongly adsorbed on the resin at first and then acted as an effective elution modifier for the C_6–C_7 amines. Unfortunately, system peaks corresponding to the C_8 amines in the eluents also appeared. As shown in Fig. 8A, when using 10 mM sodium hydroxide containing 0.5 mM 1,5-dimethylhexylamine as the eluent, a system peak corresponding to 1,5-dimethylhexylamine interfered seriously for the determination of heptanoic acid. As shown in Fig. 8B, when using 10 mM sodium hydroxide containing 0.5 mM 2-ethylhexylamine as the eluent, a system peak corresponding to 2-ethylhexylamine also interfered for the determination of heptanoic acid. As shown in Fig. 8C, when using 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine as the eluent, excellent simultaneous separation and good peak shapes for these C_1–C_7 amines were achieved in 60 min with no interferences of a system peak corresponding to 1-methylheptylamine. As shown in Fig. 8D, when using 10 mM sodium hydroxide containing 0.5 mM octylamine as the eluent, since the retention time of a system peak corresponding to octylamine was ca. 90 min, it took very long time (ca.100 min) for each chromatographic run. Furthermore, peak of methylamine disappeared at 210 nm.

Considering peak shape, interference of system peak and chromatographic time, it was concluded that 1-methylheptylamine was the most suitable C_8 amine added to 10 mM sodium hydroxide as the eluent for these C_1–C_7 amines (Fig. 8C).

Next, the effect of the concentration of 1-methylheptylamine in 10 mM sodium hydroxide as the eluent on the chromatographic behavior of these C_1–C_7 amines was investigated in detail. Fig. 9 shows the relationship between the concentration of 1-methylheptylamine in the eluent and the retention volumes of these C_1–C_7 amines. Fig. 10A–C show chromatograms of these C_1–C_7 amines with 10 mM sodium hydroxide containing: (A) 0.1 mM; (B) 0.3 mM; and (C) 1 mM 1-methylheptylamine as the eluents. A

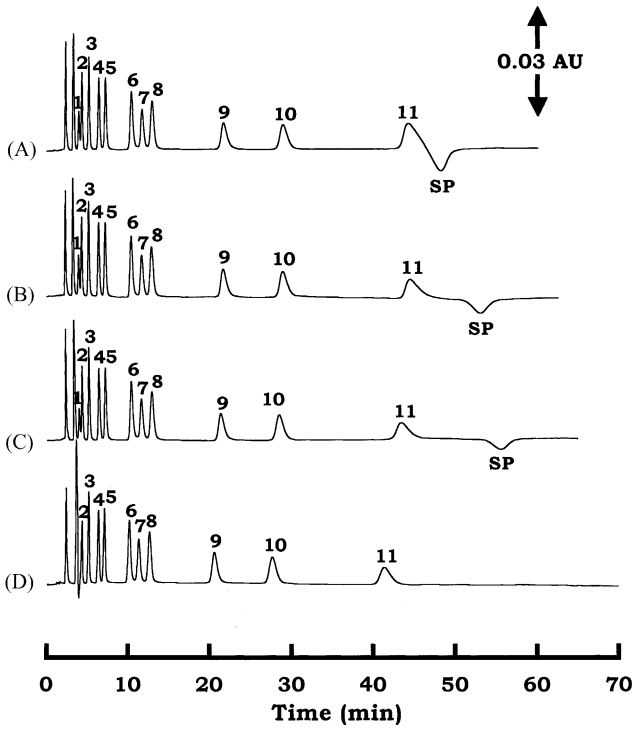

Fig. 8. Chromatograms of C_1–C_7 amines with 10 mM sodium hydroxide containing various C_8 amines as eluents. Conditions—eluents: (A) 0.5 mM 1,5-dimethylhexylamine in 10 mM sodium hydroxide at pH 11.9; (B) 0.5 mM 2-ethylhexylamine in 10 mM sodium hydroxide at pH 11.9; (C) 0.5 mM 1-methylheptylamine in 10 mM sodium hydroxide at pH 11.9; (D) 0.5 mM octylamine in 10 mM sodium hydroxide at pH 11.9, sample: 1 mM C_1–C_7 amines in 10 mM sodium hydroxide. Other chromatographic conditions as in Fig. 7. Peaks: (SP), system peak corresponding to C_8 amine in eluent, other peaks identifications as in Fig. 7.

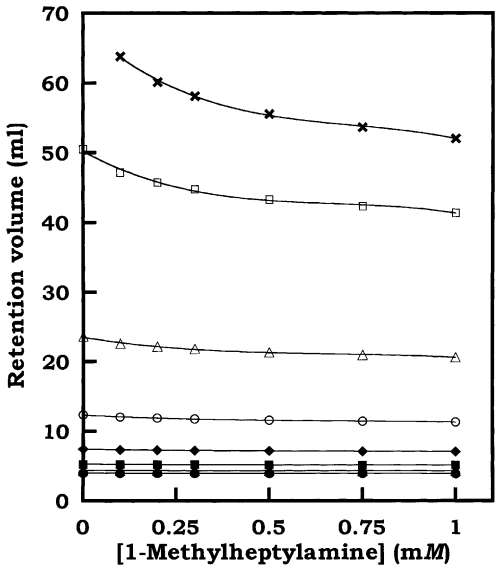

Fig. 9. Effect of concentration of 1-methylheptylamine in 10 mM sodium hydroxide as eluent on retention volumes of C_1–C_7 amines. Conditions—eluents: 0–1 mM 1-methylheptylamine in 10 mM sodium hydroxide. Other chromatographic conditions as in Fig. 8. Symbols: (✖) system peak corresponding to 1-methylheptylamine in eluent, other symbols identification as in Fig. 6.

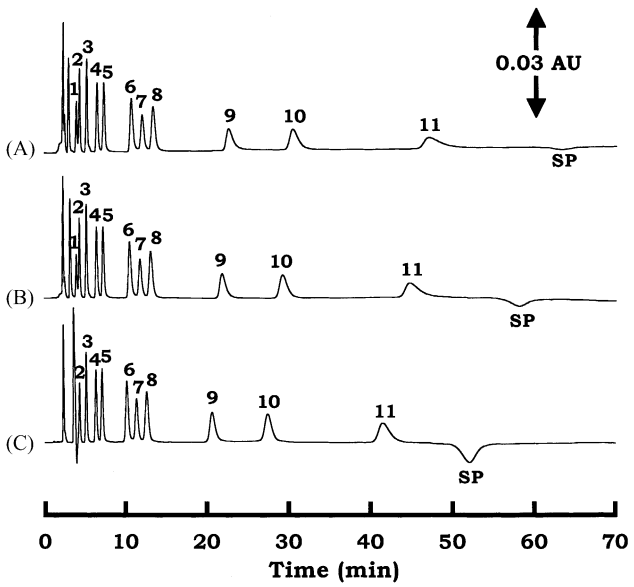

Fig. 10. Chromatograms of C_1–C_7 amines with 10 mM sodium hydroxide containing various concentrations of 1-methylheptylamine as eluents. Conditions—eluents: (A) 0.1 mM 1-methylheptylamine in 10 mM sodium hydroxide at pH 11.9; (B) 0.3 mM 1-methylheptylamine in 10 mM sodium hydroxide at pH 11.9; (C) 1 mM 1-methylheptylamine in 10 mM sodium hydroxide at pH 11.9. Other chromatographic conditions as in Fig. 9. Peaks: (SP), system peak corresponding to 1-methylheptanoic acid in eluent, other peak identification as in Fig. 7.

chromatogram with 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine as the eluent has been already shown in Fig. 8C.

As shown in Fig. 9, with increasing the concentration of 1-methylheptylamine in the eluent, the retention volumes of these C_1–C_7 amines decreased. The order of the decreased in the retention volumes was methylamine (C_1) < ethylamine (C_2) < propylamine (C_3) < butylamine (C_4) < amylamine (C_5) < hexylamine (C_6) < heptylamine (C_7) < system peak corresponding to 1-methylheptylamine (C_8). This order indicated that 1-methylheptylamine functioned as an effective elution modifier for higher amines including 1-methylheptylamine itself. As shown in Figs. 8C and 10A–C, with increasing the concentration of 1-methylheptylamine, peak shapes of the C_6–C_7 amines were improved largely. Good peak shapes of these C_1–C_7 amines were obtained at the concentration of 1-methyheptyamine $\geq$ 0.5 mM. Excellent simultaneous separation and good peak for these C_1–C_7 amines were achieved in 60 min with 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine as the eluent. In contrast, with increasing the concentration of 1-methylheptylamine in the eluent, the UV detection sensitivities of these C_1–C_7 amines slightly decreased at 210 nm. Furthermore, as shown in Fig. 10C, when using 10 mM sodium hydroxide containing 1 mM 1-methylheptylamine as the eluent, peak of methylamine disappeared at 210 nm.

Table 2
Detection limits (UV detection at 210 nm, signal-to-noise ratio of 3 and injection volume of 100 μl) of C_1–C_7 amines

Amine	Detection limit	
	μM	μg ml^{-1}
Methylamine	11	0.33
Ethylamine	4.5	0.21
Propylamine	3.6	0.22
Isobutylamine	4.7	0.34
Butylamine	4.6	0.34
Isoamylamine	5.7	0.50
Amylamine	8.3	0.72
1,3-Dimethylbutylamine	6.9	0.70
Hexylamine	12	1.3
2-Heptylamine	13	1.5
Heptylamine	20	2.3

Considering peak shape, peak resolution, UV detection sensitivity at 210 nm and chromatographic time, it was concluded that the optimum concentration of 1-methylheptylamine in 10 mM sodium hydroxide as the eluent was 0.5 mM for these C_1–C_7 amines (Fig. 8C).

3.2.3. *Analytical performance parameters*

Table 2 shows the detection limits (UV detection at 210 nm, signal-to-noise ratio of 3 and injection volume of 100 μl) of these C_1–C_7 amines. Unfortunately, due to both (a) high UV adsorption of the 10 mM sodium hydroxide containing 0.5 mM 1-methylheptylamine as the eluent at 210 nm (ca. 0.8 AU, noise 0.031 mAU) and (b) low molar extinction coefficients of these amines at 210 nm, detection sensitivities for these amines were moderate.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these C_1–C_7 amines. Linear calibration graphs ($r^2 > 0.99$) were obtained in the concentration range between 0.05 and 2 mM for these amines.

The relative standard deviations of the chromatographic peak area of these C_1–C_7 amines, whose concentrations were 1 mM, were less than 1.1 % ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

4. Conclusions

The application of unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as a stationary phase in liquid chromatography with UV detection (210 nm) for both C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine,

hexylamine, 2-heptylamine and heptylamine) was carried out, for demonstrating the effectiveness of this type resin as stationary phase in liquid chromatography for various inorganic and organic compounds. The TSKgel G3000PW$_{XL}$ resin acted as an advanced stationary phase for these C_1–C_7 carboxylic acids and these C_1–C_7 amines. Excellent simultaneous separation and symmetrical peaks for these C_1–C_7 carboxylic acids were achieved on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) in 60 min with 0.25 mM sulfuric acid containing 1 mM C_8 carboxylic acid (2-methylheptanoic acid) at pH 3.3 as the eluent. Excellent simultaneous separation and good peak shapes for these C_1–C_7 amines were also achieved on the TSKgel G3000PW$_{XL}$ column with 10 mM sodium hydroxide containing 0.5 mM C_8 amine (1-methylheptylamine) at pH 11.9 as the eluent. The UV detection sensitivities of these C_1–C_7 carboxylic acids and these C_1–C_7 amines were moderate. A study on the development of highly sensitive detection methods will be the subject of future work.

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