

1 Short Communications

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3 **Poly(L-lactic acid)-modified silica stationary phase for reversed-phase and**
4 **hydrophilic interaction liquid chromatography**

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6 Kaname Ohyama, Shizuka Takasago, Naoya Kishikawa and Naotaka Kuroda*

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8 Course of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki
9 University

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11 * Corresponding author. Course of Pharmaceutical Sciences, Graduate School of Biomedical
12 Sciences, Nagasaki University, Nagasaki, Japan. Fax: +81-95-819-2444

13 E-mail address: n-kuro@nagasaki-u.ac.jp

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15 Keywords: poly(L-lactic acid); mixed-mode; hydrophilic interaction; reversed-phase

16

1 **Abstract**

2 Poly(L-lactic acid) is a linear aliphatic thermoplastic polyester that can be produced
3 from renewable resources. Poly(L-lactic acid)-modified silica stationary phase was
4 newly prepared by amide bond reaction between amino groups on aminopropyl silica
5 and carboxylic acid groups at the end of poly(L-lactic acid) chain. Poly(L-lactic
6 acid)-silica column was characterized in reversed-phase liquid chromatography and
7 hydrophilic liquid chromatography with the use of different mobile phase composition.
8 Poly(L-lactic acid)-silica column was found to work in both modes, and the retention of
9 test compounds depending on acetonitrile content exhibited “U-shaped” curves, which
10 was an indicator of reversed-phase liquid chromatography/hydrophilic liquid
11 chromatography mixed-mode retention behavior. In addition, carbonyl groups
12 included into poly(L-lactic acid) backbone work as an electro-accepting group toward a
13 polycyclic aromatic hydrocarbon and provide π - π interaction.

14

1 **1 Introduction**

2 Reversed-phase liquid chromatography (RPLC) is widely used to retain and separate
3 hydrophobic and moderate hydrophobic compounds; however, it often does not work in
4 the separation of polar compounds due to lack of retention. Normal phase liquid
5 chromatography is another choice in such case, but non-polar mobile phases are not a
6 good solvent for the polar compounds. For this case, hydrophilic interaction
7 chromatography (HILIC), where bare silica or polar groups (amine, amide, cyano,
8 diol)-bonded silica stationary phase and hydro-organic mobile phase are used, has
9 become a valuable alternative.

10 Mixed-mode chromatographic separation based on more than one retention
11 mechanism is a better concept than a single mode one. This concept is gathering
12 attention and several mixed-mode separation materials have been reported. Most
13 mixed-mode separation methods combine RP and anion- or cation-exchange, but the
14 materials for mixed-mode of RPLC and HILIC are limited although the combination
15 may expand the applicability most widely [1-7]. Some researchers have utilized the
16 materials possessing long alkyl chain (hydrophobic moiety) and ionizable group
17 (hydrophilic moiety) for RPLC/HILIC mixed-mode separation [2]. However, ionized
18 group can electrostatically interact with ionized analytes, resulting in a severe peak
19 tailing. Contrastingly, Wu *et al.* synthesized nonionic polar stationary phases with
20 hydroxyl and sulfoxide groups and reported that some of them exhibited RPLC and
21 HILIC mode separations [3].

22 Poly(L-lactic acid) (PLLA) is a linear aliphatic thermoplastic polyester that can be

1 produced from renewable resources (e.g. corn, wheat, or rice). PLLA has been
2 extensively explored for biodegradable drug delivery carriers and tissue engineering
3 scaffolds due to their superior biocompatibility and versatile processabilities [8].
4 PLLA is relatively hydrophobic with a static water contact angle of approximately 80°
5 [9]. In addition, a number of carbonyl groups can contribute to hydrogen bond with
6 amino group and π - π interaction with aromatic ring. These characteristics were
7 expected to realize RPLC/HILIC mixed-mode chromatography.

8 In the present study, PLLA-modified silica (PLLA-silica, Fig. 1) stationary phase
9 was newly prepared by amide bond reaction between amino groups on aminopropyl
10 silica (APS) and carboxylic acid groups at the end of PLLA chain. PLLA-silica
11 column was characterized in RPLC and HILIC modes with the use of different mobile
12 phase composition.

13

14 **2 Materials and methods**

15 *2.1 Chemicals*

16 APS (particle size, 5 μm ; pore size, 120 \AA) was a kind gift from Daiso Chemical
17 (Osaka, Japan). HPLC grade of acetonitrile (ACN), benzene, naphthalene, anthracene,
18 toluene, uracil, thymidine, cytosine, uridine, adenosine, thymine, cytidine, theobromine
19 and caffeine were obtained from Wako Pure Chemicals (Osaka, Japan). PLLA
20 (average molecular weight: 5,000) and dioxane was from Nacalai Tesque (Kyoto, Japan).
21 Hypoxanthine was purchased from Merck KGaA (Darmstadt, Germany).
22 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM),

1 inosine, phenanthrene and pyridoxine were from Tokyo Chemical Industry (Tokyo,
2 Japan). Ethylbenzene and adenine were from Kishida Chemicals (Osaka, Japan).
3 Paraxanthine was obtained from Sigma (St. Louis, MO, USA).

4

5 2.2 Preparation of PLLA-silica stationary phase

6 PLLA-silica was obtained by a single-step reaction as follows: PLLA (10 g) and
7 DMT-MM (0.83 g) were added to a suspension of APS (0.77 mmol/g, 2.6 g) in dioxane
8 (25 mL) and the mixture was shook at 40 °C for 24 h. After the reaction, PLLA-silica
9 was filtered and washed with dioxane, 0.1% hydrochloric acid and water. Elemental
10 analysis: C 3.47%; H 0.87%; N 1.23% for APS; C 7.19%, H 1.41%, N 1.53% for
11 PLLA-silica. The modification ratio (mmol/g) of PLLA on APS was 0.015 estimated
12 from the value of nitrogen and the average molecular weight by elemental analysis.
13 Infrared spectra indicated the presence of carbonyl group on PLLA-silica obtained by
14 the above reaction (1750 cm^{-1} , data not shown).

15

16 2.3 Chromatography

17 PLLA-silica was packed into HPLC columns (150 mm x 1.5 mm I.D.) by a slurry
18 packing method with methanol [10].

19 The HPLC system included a Shimadzu LC-20AT pump, SPD-6A UV detector and
20 CR-8A recorder (Kyoto, Japan). Flow rate was set at 1.0 mL/min with UV detection at
21 260 nm. All aqueous solutions were made with the water that was deionized and
22 distilled using WG 203 (Yamato Scientific, Tokyo, Japan) and then passed through a

1 water purification system (Puric-Z, Organo, Tokyo, Japan).

3 **Results and discussion**

4 *3.1. Retention properties in RPLC mode*

5 The influence of ACN content in the mobile phase on the retention of hydrophobic
6 compounds (benzene, toluene, ethylbenzene, butylbenzene, naphthalene, anthracene and
7 phenanthrene) was studied with varying the percentage of ACN from 10 to 20%. The
8 retention of test compounds decreased with the increase in ACN content and a linear
9 relationship between $\log k$ and ACN content was obtained. It is a typical characteristic
10 of the RPLC mode and the retention of hydrophobic compound was dominated by
11 hydrophobic interaction. It can also be found that the retention of test compounds
12 increased with their hydrophobicity.

13 Plots of $\log k$ against $\log P$ (octanol-water partition coefficient) values for
14 alkylbenzenes, benzene, naphthalene, anthracene and phenanthrene are drawn (Fig. 2).
15 It was clearly found that PLLA-silica more highly recognized aromatic hydrocarbons,
16 compared with alkylbenzenes. A carbonyl group is polarized to be δ^+ (carbon) and δ^-
17 (oxygen), respectively, and these polarized atoms enable to work as an electrostatic
18 source for a π - π interaction [11]. It has been reported that a carbonyl π -benzene π
19 interaction is comparatively larger than a benzene π -benzene π interaction [11]. The
20 hydrophobic interaction was less than we expected; however, considering that a number
21 of carbonyl groups are included into the PLLA backbone, PLLA-silica may be useful
22 for high recognition to aromatic hydrocarbons.

1

2 3.2. Retention properties in HILIC mode

3 HILIC separation commonly employs hydro-organic mobile phase with a higher
4 organic content than 60%. The effect of ACN content in the mobile phase on the
5 retention of polar compounds (nucleic acids: uracil, thymine, cytosine; nucleosides:
6 adenosine, uridine, cytidine, thymidine, inosine; vitamins: riboflavin, pyridoxine;
7 xanthines: 1,7-dimethylxanthine, 3,7-dimethylxanthine, 1,3,7-trimethylxanthine) was
8 investigated. The retention factors decreased drastically or slightly when the ACN
9 content decreased from 95% to 90%. This observation exhibited a typical HILIC
10 retention behavior. Hence, PLLA-silica stationary phase acts as a HILIC phase at high
11 ACN content. The representative chromatogram is shown in Fig. 3.

12

13 3.3. Comparison between PLLA and APS

14 In order to illustrate the impact of PLLA modification to APS, the retention factors
15 and the elution orders of test compounds on APS-packed column, which was prepared
16 as the aforementioned procedure, were studied in both RPLC and HILIC modes. In
17 RPLC mode, hydrophobic compounds (benzene, naphthalene and alkylbenzenes) were
18 hardly retained on APS column even though mobile phase with high water content
19 (80%) was used. In HILIC mode, nucleic acids and nucleosides (thymine, uracil,
20 cytosine, thymidine, uridine, adenosine) were tested on APS column. The elution
21 orders of thymine, uracil, cytosine, thymidine and adenosine were same as PLLA-silica.
22 Cytosine was most retained on PLLA-silica, while uridine was most retained on APS

1 silica. This tendency was consistent with surfactin-modified APS column which were
2 very recently reported and works in mixed-mode of HILIC/RPLC [12].

3 4 3.4. RPLC/HILIC mixed-mode

5 The RPLC/HILIC mixed-mode retention behaviors were investigated with a set of
6 moderate polar and polar compounds. As shown in Fig. 4, PLLA-silica stationary
7 phase showed a “U-curve” retention profile [1, 4], which is an indicator of
8 RPLC/HILIC mixed-mode retention behavior. The retention time of test compounds
9 decreased with an increase in ACN content at low and middle contents, according to the
10 RPLC mode. Then, the retention times increased with increasing ACN content from
11 80% to 95%, indicating that the retention was governed by hydrophilic interaction
12 between the stationary phase and compounds. The ACN content is about 50% for the
13 weakest retention and that is the boundary between the two different retention modes.
14 This feature potentially provides a greater flexibility in real sample analyses, compared
15 to a single use of conventional RPLC or HILIC columns.

16 1,3,7-Trimethylxanthine (caffeine), 1,7-dimethylxanthine and hypoxanthine are all
17 purine derivatives with different numbers of methyl groups. Despite their methylated
18 sites, they still retain a certain degree of polarity. This makes them suitable for a
19 HILIC separation although their separation is commonly performed by RPLC [3, 13].
20 The separation in RPLC mode (1% ACN) caused these analytes to elute due to
21 hydrophobicity; thus, caffeine showed the strongest retention (Fig. 5a). On the other
22 hand, the stationary phase could reverse the elution order, and a good separation was

1 obtained in HILIC mode (95% ACN) (Fig. 5b). This suggests that PLLA-silica
2 column can be used for both RPLC and HILIC separation modes only by changing the
3 mobile phase composition.

4

5 **Conclusion**

6 Newly synthesized PLLA-silica stationary phase was characterized in RPLC or
7 HILIC mode and was found to work in both modes. The retention of test compounds
8 depending on ACN content exhibited “U-shaped” curves, which was an indicator of
9 RPLC/HILIC mixed-mode retention behavior. In addition, carbonyl groups included
10 into PLLA backbone work as an electro-accepting group toward a polycyclic aromatic
11 hydrocarbon and provide π - π interaction.

1 **6 References**

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1 **Figure captions**

2

3 **Fig. 1** PLLA- silica stationary phase.

4

5 **Fig. 2** Relationship between $\log k$ and $\log P$ with PLLA-silica for alkylbenzenes and
6 polyaromatic hydrocarbons. Conditions: mobile phase, H₂O/ACN=90/10 (v/v %);
7 flow rate, 1mL/min; UV detection, 260 nm.

8

9 **Fig. 3** Separation of test compounds. Conditions: mobile phase, H₂O/ACN=5/95
10 (v/v %); Other conditions are the same as those given in Fig. 2. Peaks: 1, uracil; 2,
11 thymine; 3, thymidine; 4, uridine; 5, adenosine; 6, cytosine; 7, inosine; 8, cytidine.

12

13 **Fig. 4** Effect of ACN content in the mobile phase on the retention times. Conditions:
14 mobile phase, H₂O -ACN; Other conditions are the same as Fig. 2.

15

16 **Fig. 5** Separation of xanthines in RPLC mode and HILIC mode. Conditions: mobile
17 phase, H₂O/ACN=99/1 (v/v %) (RPLC), H₂O/ACN=5/95 (v/v %) (HILIC) ; Other
18 conditions are the same as Fig. 2. Peaks: 1, hypoxanthine; 2, 1,7-dimethylxanthine; 3,
19 1,3,7-trimethylxanthine (caffeine)

Fig. 1

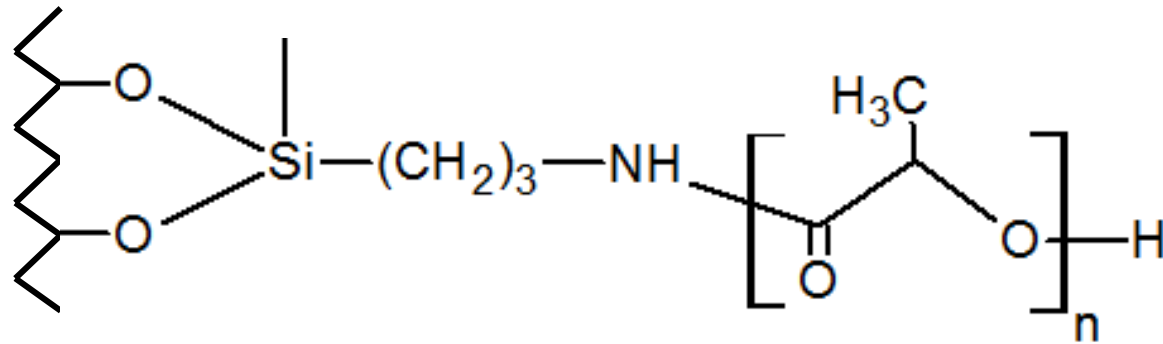


Fig. 2

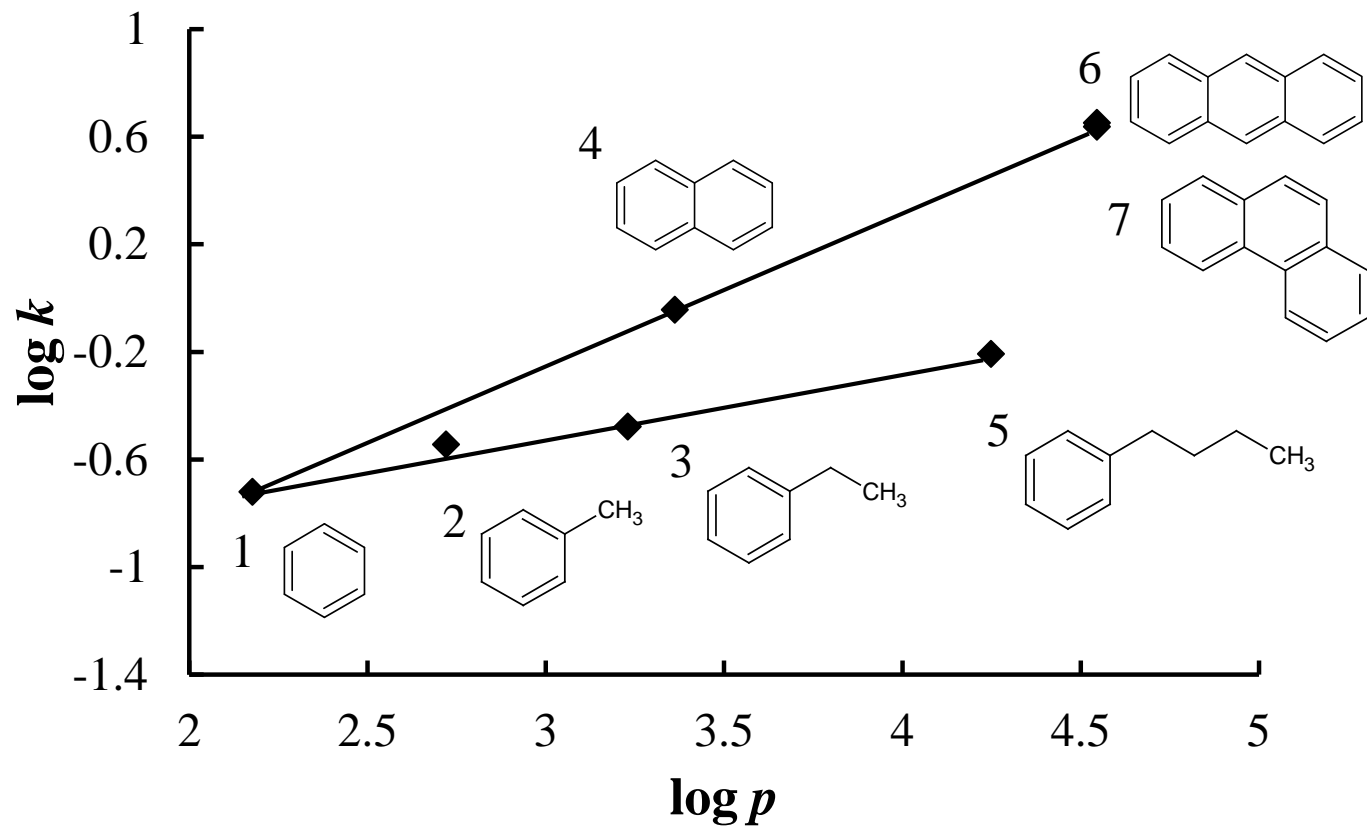


Fig. 3

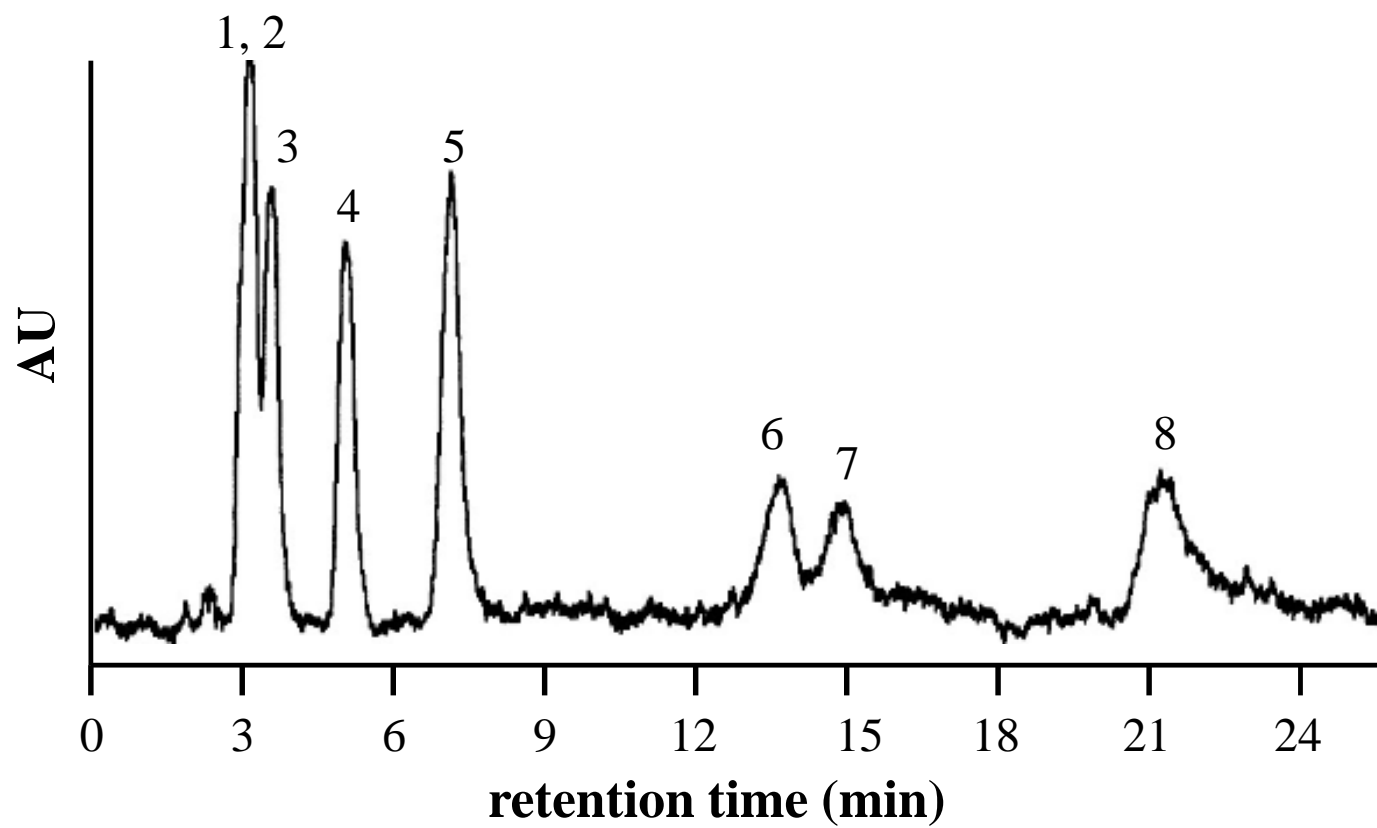


Fig. 4

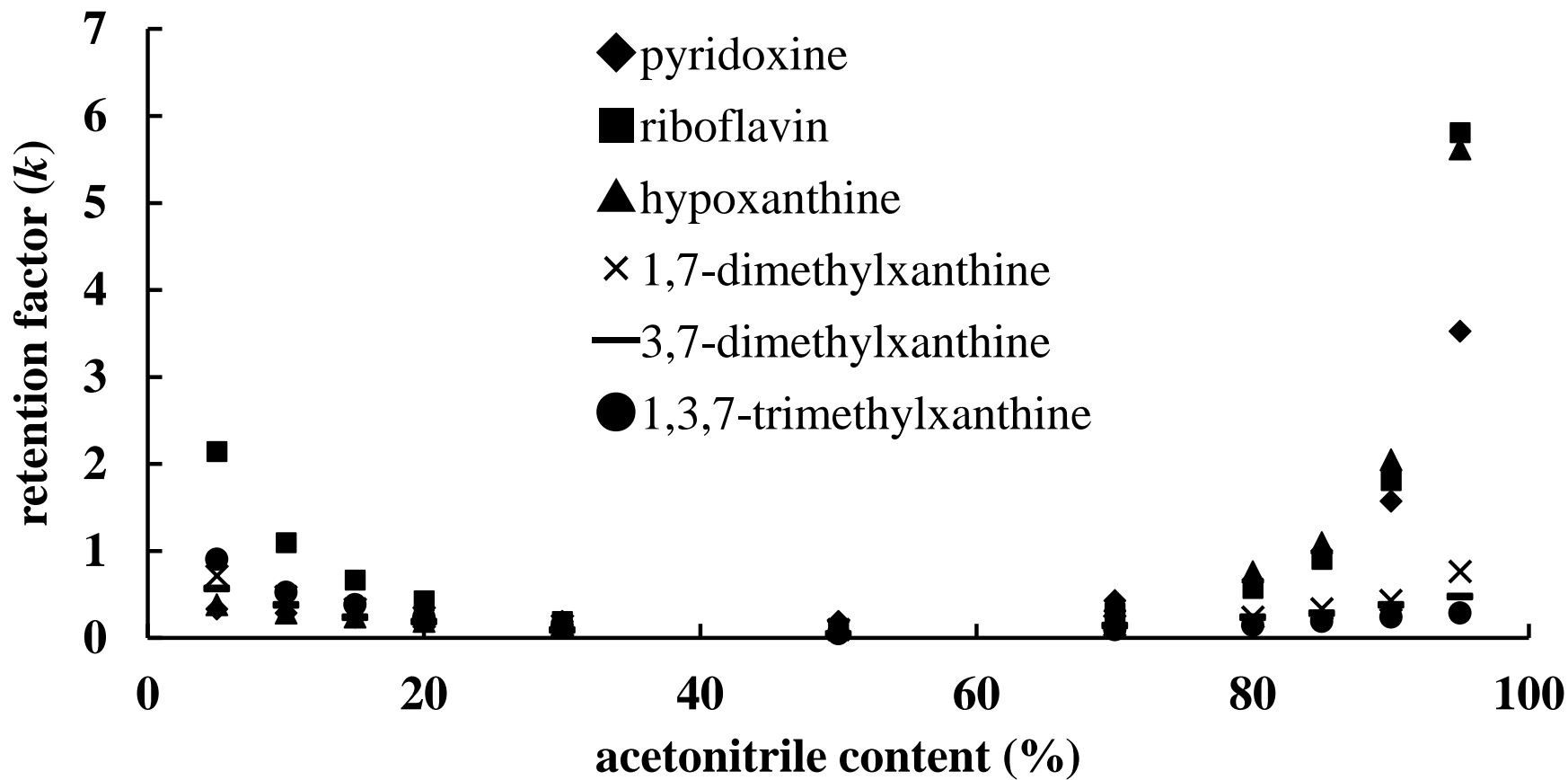


Fig. 5

