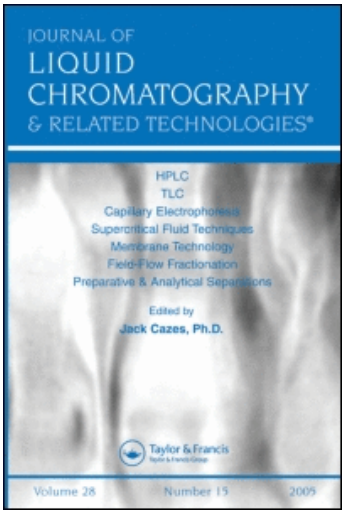

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SYNTHESIS AND EVALUATION OF A NOVEL C_8 ESTER-BONDED STATIONARY PHASE FOR REVERSED-PHASE HPLC

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ABSTRACT

A novel ester-bonded packing for reversed-phase HPLC (RP-HPLC) was synthesized by reacting octanoic acid with β-(3,4-epoxycyclohexyl) ethyltrimethoxy silane; then, the intermediate product was coupled onto porous silica. Characterization of the prepared packing was carried out with elemental analysis, solid-state ^{13}C NMR, and Fourier transform infrared (FT-IR) analysis.

The chromatographic properties of the novel ester-bonded phase have been investigated in RP-HPLC with acidic, basic, and neutral analytes as probes. The influence of organic modifier and pH of mobile phase and the hydrolytic stability of the packing were also studied. Results showed that the new stationary phase has excellent chromatographic properties in RP-HPLC and can resist hydrolysis between pH = 2.5 ~ 7.5.

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INTRODUCTION

Silica-based RP-HPLC has become the method of choice for a large proportion of liquid chromatographic separations. Many of the benefits of RP-HPLC can be attributed to the silica itself, which lends mechanical stability, narrow distribution of pore and particle sizes, and chemical reactivity for easy modification of the surface silanol groups; this provides a number of possibilities for stationary phases. Today, more than 600 kinds of RP columns are commercially available worldwide, and new and better RP columns are being brought onto the market (1).

A variety of stationary phases were bonded to siliceous supports through intermediate silane-containing active functional groups (e.g., amino-, epoxy-, etc.) for further modification. For example, a number of packings were prepared using glycidoxypropyltrimethoxysilane, which is a widely used and important coupling agent; intermediate silanes have been used as stationary phases in RP-LC (2,3) HIC (4–6) ion exchange (7), SEC (8,9), etc. Many packings were prepared through aminopropyl bonded phases which were acylated to form an embedded polar amide functional group in the ligand's alkyl chain (10–13). These and other embedded polar functional groups, such as ureas and carbamates were found to effectively reduce silanol interactions with basic analytes (14,15).

In this study, we first used a new silane coupler—β-(3,4-epoxycyclohexyl) ethyltrimethoxy silane (β-ECTS) which contains a highly reactive group, an oxirane ring, and a bulky group, cyclohexyl, to react with octanoic acid; then the intermediate was coupled onto silica to obtain a C_8 ester-bonded RP-LC packing (16). The characterization and chromatographic evaluation of the novel packing were studied.

EXPERIMENTAL

Chemicals and Solvents

The octanoic acid (97%) was purchased from Shanghai No. 2 Chemical Factory (Shanghai, China), β-(3,4-epoxycyclohexyl) ethyltrimethoxy silane (β-ECTS, 98%) was purchased from Aldrich. Kromasil silica (mean particle diameter, 5 μm; specific surface area, 340 m^2g^{-1}, mean pore diameter, 10 nm) was purchased from Akzo Nobel (Bohus, Sweden). HPLC grade methanol was from Yuwang Chemical Factory (Shandong, China). Toluene and other reagents utilized were all of analytical-reagent grade. Double distilled water was used.

Apparatus

HPLC separations were carried out at ambient temperature with a system consisting of an LC-10AD HPLC pump (Shimadzu, Japan), SPD-10A UV/visible spectrophotometer detector (Shimadzu, Japan); Rheodyne 7125 injection valve (Cotati. CA., USA). A JS-3000 chromatography workstation (Dalian, China) was used for chromatogram recording and data processing.

Carbon, hydrogen, and nitrogen percentages for the new stationary phase were determined with a Carlo Erba Model 1106 analyzer (Milan, Italy). The infrared spectra were acquired with an FT-IR 170-SX infrared spectrophotometer (Nicolet, USA). The solid state ^{13}C CP-MAS-NMR spectra of the bonded silica were obtained with a Bruker MSL-300 spectrometer (Billerica, MA, USA).

Synthesis of C_8 Ester-Bonded Phase

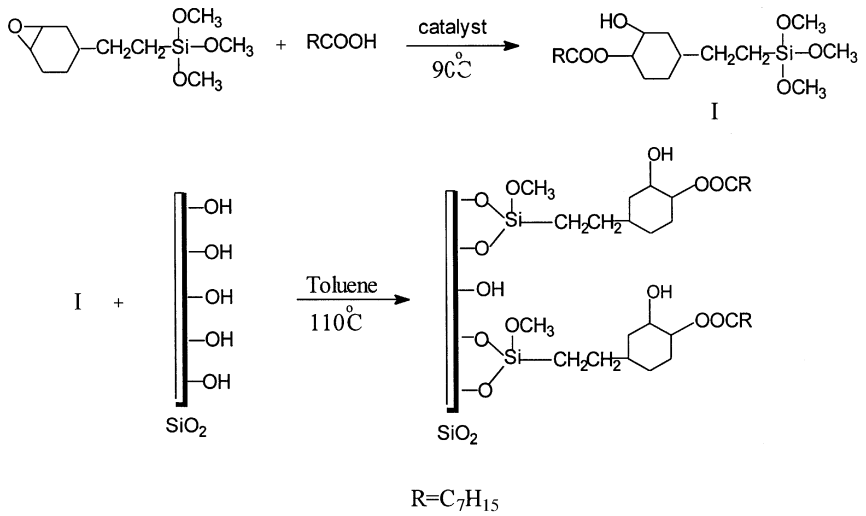
Pretreatment of Silica Gel

Twenty grams of silica gel was added to a round-bottom flask containing 200 mL of 20% hydrochloric acid and the mixture was refluxed for 4 hours. The treated silica gel was filtered, washed several times successively with water and acetone, then dried in a vacuum oven at 100°C for 12 h. The dried silica was kept in a vacuum desiccator.

Preparation of Bonded Phase

The synthesis route is shown in Scheme 1. β-ECTS (3.2 mL) and triethylamine (0.5 mL) were dissolved in a three-neck round-bottom flask with 30 mL of dried toluene; then, a mixture of 4.5 mL of octanoic acid in 20 mL of dried toluene was added dropwise to the flask while stirring. Then, the flask was placed in an oil bath at 90°C for 6 h to ensure complete reaction of the ring-opening of the epoxide (7). The intermediate product (I) was stored in a vacuum desiccator for later use.

Dried silica gel (5 g) was placed in a 250 mL three-neck round-bottom flask and heated up to 150°C for 6 h. Then, the intermediate product (I) was drawn into the flask by vacuum. After the slurry was agitated at reflux temperature in an oil bath for $8 \sim 12$ h, the bonded silica gel was filtered and washed with toluene and methanol several times, successively, then dried in vacuum at 100°C for 6 h to obtain the C_8 ester-bonded packing.



R=C_7H_{15}

Scheme 1. The synthetic route for C_8 ester-bonded phase.

Column Packing

Bonded silica was packed into a 150×4.6 mm stainless steel column using conventional high-pressure slurry techniques. Chloroform and ethanol were used as packing solvent and displacement solvent, respectively.

RESULTS AND DISCUSSION

Characterization of the Packing

The results of elemental analysis for three batches of the packing are listed in Table 1. Knowing that the specific surface of silica gel support is $340\,m^2g^{-1}$

Table 1. Elementary Analysis of C_8 Ester-Bonded Phase

Batch	C%	H%
1	14.18	2.36
2	14.50	2.01
3	14.10	2.37
Ave	14.26	2.47

(see Experimental), the surface coverage of C_8 ester-bonded phase was calculated as 2.67 μmol m^{-2}.

Figure 1 show the FT-IR spectra of the final product and starting silica for this packing. From Figure 1b, compared with the spectrum of the starting silica gel (Figure 1a), several new absorption bands are apparent in the spectrum of the final product. The peaks at 2931.5 cm^{-1} and 2857.5 cm^{-1} are the asymmetric stretching vibration and symmetric stretching vibration of $-CH_2-$, respectively; and the peak at 1455.3 cm^{-1} is assigned to the bending vibration of $-CH_2-$. They indicate the existence of a methylene group. The carbonyl peak appears at 1758.2 cm^{-1}.

The solid-state ^{13}C CP-MAS-NMR of the final product is shown in Figure 2. It can be seen from the spectrum that the peak near 173 ppm is assigned to the carbonyl carbon 9. Peaks at 51 and 55 ppm are assigned to the carbons 6 and 7, respectively, which indicates the success of the ring opening reaction. Peaks in the range of 7 to 31 ppm indicate the successful bonding of target long-chain-alkyl group onto the phase.

In short, the elemental analysis data, the FT-IR spectra, and the solid-state ^{13}C CP-MAS-NMR confirm the success of the synthetic procedure.

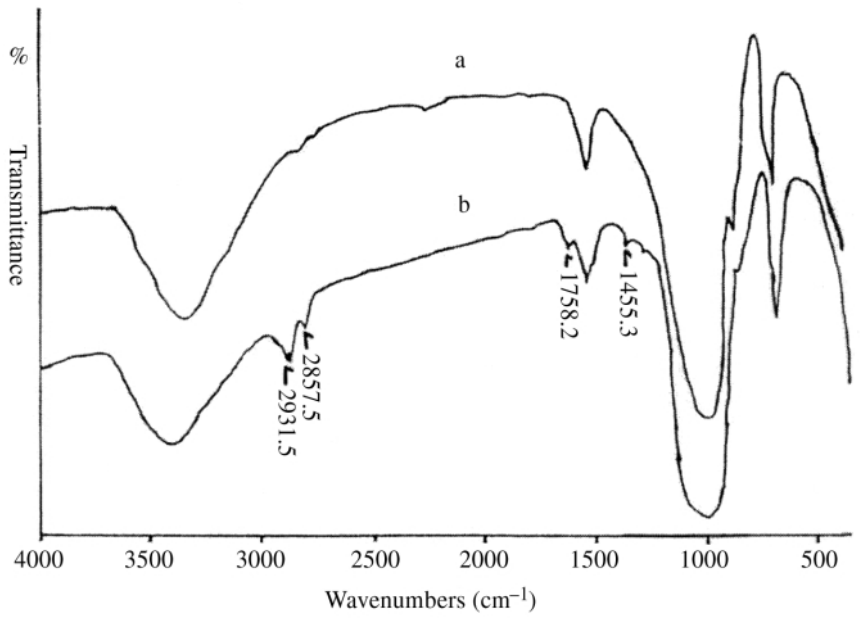


Figure 1. FT-IR spectra of silica treated with 20% hydrochloric acid (a), and silica bonded phase (b).

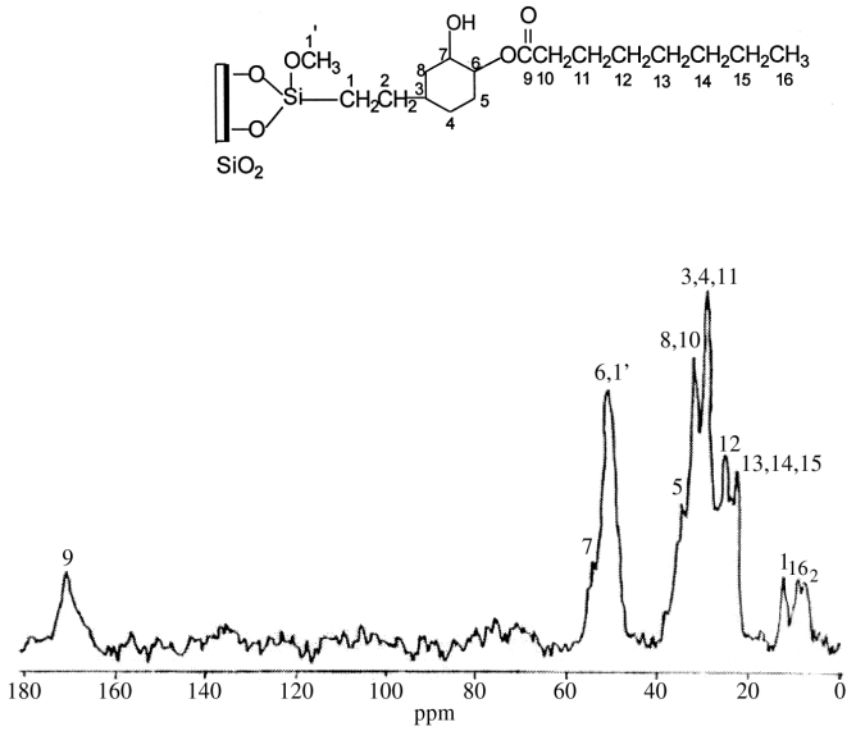


Figure 2. Solid state ^{13}C-CP-MAS NMR spectrum of the bonded phase.

Chromatographic Evaluation

General Chromatographic Character

Figure 3 is the result of separation of some aromatic compounds with the new stationary phase. It can be seen that the four solutes have high theoretical plate numbers and good peak shapes (e.g., naphthalene, $N = 61{,}000$, $A_s = 0.97$), which demonstrates the excellent hydrophobic properties of the C_8 ester-bonded phase.

Figure 4 is the result of separation of a test mixture proposed by Engelhardt and coworkers (11). From the figure, it can be seen that aniline was eluted before phenol and the asymmetry factor of the aniline peak divided by that of the phenol peak was 1.13 (Table 2), less than 1.3. Isomers of o- and p-toluidine with identical hydrophobicity, but different basicity, were co-eluted as a symmetrical peak and the retention factor for ethylbenzene was 5.02 in the range of $2 \sim 11$ for

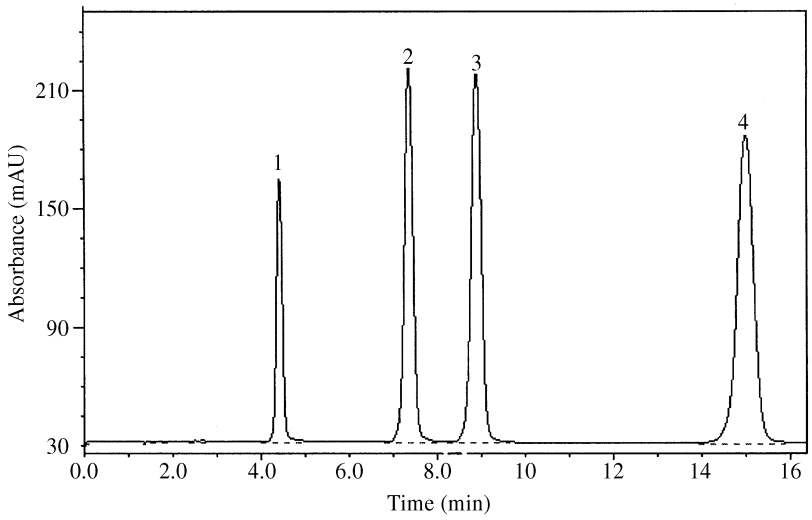


Figure 3. The chromatogram of some aromatic compounds. Conditions: mobile phase, 75 : 25 (v/v) methanol/water; flow rate, 0.8 mL/min; column temp., 25°C; detection wavelength, 254 nm; peaks, 1 = benzene, 2 = naphthalene, 3 = biphenyl, 4 = phenanthrene.

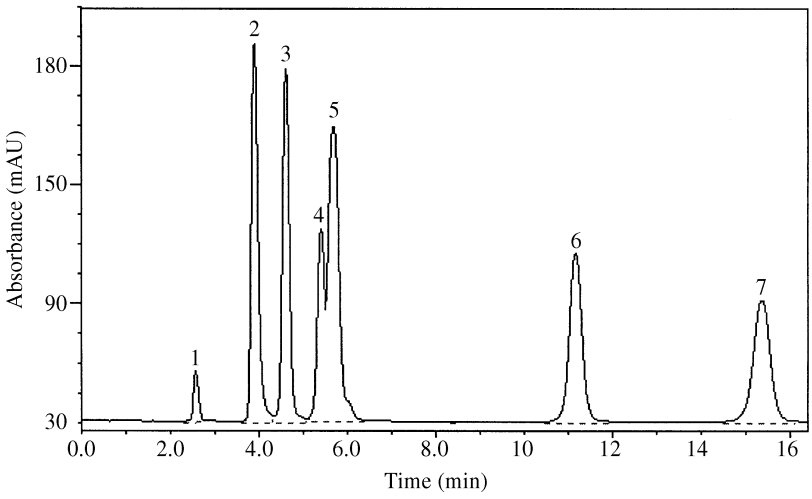


Figure 4. The chromatogram of some neutral, acidic, and basic compounds. Conditions: mobile phase, 55 : 45 (v/v) methanol/water; flow rate, 0.8 mL/min; column temp., 25°C; detection wave length, 254 nm; peaks, 1 = uracil, 2 = pyridine, 3 = aniline, 4 = o,p-toluidine, 5 = phenol, 6 = toluene, 7 = ethylbenzene.

Table 2. Chromatographic Data of Some Neutral, Acidic and Basic Compounds

Samples	Uracil	Pyridine	Aniline	Phenol	o,p-Toluidine	Toluene	Ethylbenzene
[a]k	0	0.52	0.80	1.11	1.22	3.37	5.02
[b]N(n/m)	31210	43740	71150	30320	61910	61770	
[c]As	1.14	1.16	1.13	0.96	1.02	1.01	0.95

[a]Retention factor.
[b]Theoretical plate number.
[c]Peak asymmetry factor.
The conditions are the same as in Figure 4.

C_8 column. In short, according to the standard of a "good" column, as proposed by Engelhard (17,18), the column packed with the new stationary phase is obviously a "good" column.

Figure 5 is the result of separation of some basic compounds, including aminophylline, nicotine, caffeine, and papaverine with methanol and water as mobile phase. From the figure, it can be seen that these basic solutes all have good peak shapes and high theoretical plate numbers (e.g., caffeine, $N = 44{,}190$, $A_s = 1.17$). The excellent chromatographic properties are attributed to the existence of a bulky group in the prepared stationary phase, which weakens the interaction between basic analytes and residual silanol groups on the silica surface.

Effect of Organic Modifier

Figure 6 depicts plots of the logarithms of the capacity factors of the solutes (acidic, basic, and neutral species) versus mobile phase methanol content. As is apparent from the figure, the logk' decreases linearly with increasing mobile phase methanol content, which demonstrates that the column operated by a reversed-phase mechanism, even with very basic compounds.

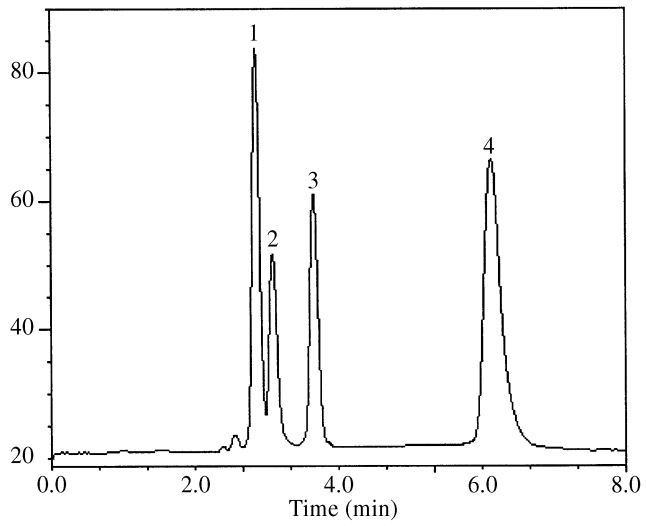


Figure 5. The chromatogram of some basic compounds. Conditions: mobile phase, 65 : 35 (v/v) methanol/water; other conditions are the same as in Figure 4. peaks, 1 = aminophylline, 2 = nicotine, 3 = caffeine, 4 = papaverine.

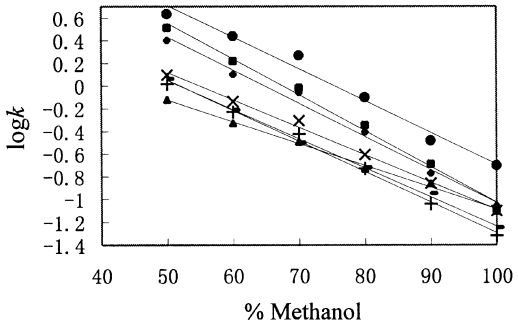


Figure 6. Effect of methanol concentration on log k' of some neutral, acidic, and basic compounds at a flow rate of 0.8 mL min^{-1} on the C_8 ester-bonded phase column. ● = N,N-diethylaniline, ■ = Toluene, ◆ = ethylbenzoate, **x** = p-Toluidine, − = Pyridine, + = Phenol, ▲ = Aniline.

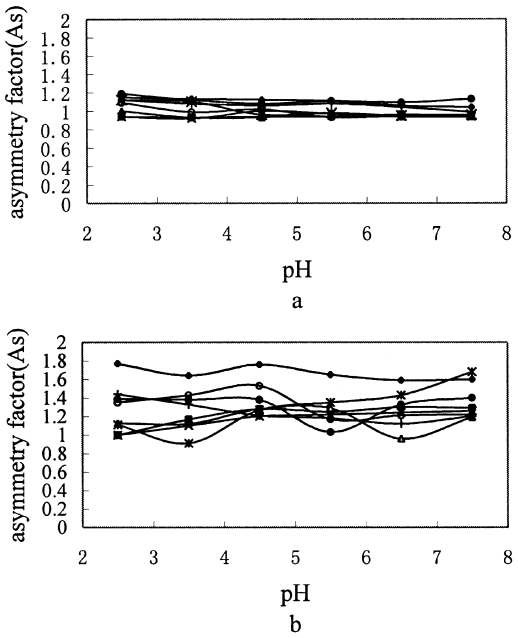


Figure 7. Effect of pH on peak asymmetry factors for some basic compounds with (a) C_8 ester-bonded phase, (b) Kromasil C_8. Conditions: The mobile phase was methanol-buffer (65 : 35, v/v); the other conditions are the same as in Figure 4. ◆ = pyridine, + = papaverine, ● = aminophylline, ○ = procaine, △ = p-toluidine, * = N,N-diethyl-aniline, ■ = aniline, ▼ = o-toluidine.

Effect of pH

Figure 7 shows the greatly reduced interaction of surface silanols when using the new packing for basic compounds. Asymmetry factors for a series of basic compounds remain essentially constant throughout the pH 2.5 ~ 7.5 range for a methanol-citric acid buffer mobile phase. In contrast, peak asymmetry factor of a traditional RP-HPLC packing, such as Kromasil C_8, had larger changes with increasing pH. These results suggest that the existence of a bulky group, cyclohexyl in the prepared stationary phase weakens the interaction between basic solutes and residual silanol groups on the silica surface.

Aging Investigation

Figure 8 depicts the hydrolytic stability of the C_8 ester-bonded phase with pH 2.5 and pH 7.5 buffered mobile-phase (65 : 35, methanol/buffer), respectively.

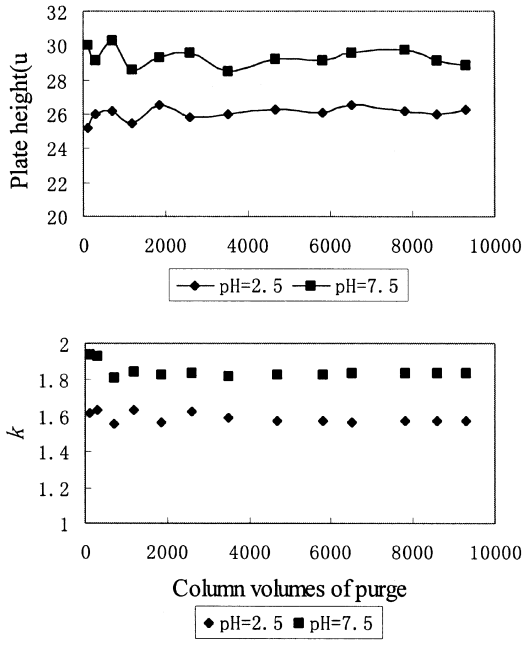


Figure 8. Aging of C_8 ester-bonded phase with ethyl benzoate. Mobile phase, 65 : 35 (v/v) methanol : 0.02 mol/L citric acid-disodium hydrogen phosphate buffer (pH = 2.5) and 65 : 35 (v/v) methanol : 0.02 mol/L disodium hydrogen phosphate-sodium dihydrogen phosphate buffer (pH = 7.5). Other conditions are the same as in Figure 4.

The results show that little change occurred in retention factor *k*' values and plate height (μm) during continuous purging with almost 10,000 column volumes of the mobile phase. The good hydrolytic stability may arise from the steric protection by the cyclohexyl in the packing.

CONCLUSION

A new C_8 ester-bonded phase for RP-HPLC was synthesized by using *β*-(3,4-epoxycyclohexyl) ethyltrimethoxy silane as coupling reagent. The effects of organic modifier composition and mobile phase pH has been investigated. Because there is a bulky-group, cyclohexyl, in the packing, compared with conventional reversed-phase materials, the new packing exhibits less "silanol effect" and can be useful in the separation of basic compounds. In addition, the new packing exhibits high hydrolytic stability and exhibits a long lifetime in the operating range of pH 2.5 ~ 7.5.

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