Stability of Silica-Based, Monofunctional C_{18} Bonded-Phase Column Packing for HPLC at High pH

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Abstract

Previous studies have shown that nonendcapped, monofunctional bonded-phase column packings made from silica supports prepared by the sol-gel method have surprising stability at up to pH 10 in certain mobile phases. Even when purged with NaOH at pH 12, these columns demonstrate properties that suggest that useful chromatographic separations might be feasible under such conditions. Separations at high pH (pH 10 or greater) are attractive for separating certain basic compounds. Such solutes would be in the free-base form, which would minimize potential deleterious interaction with unreacted silanol groups on the silica support. Results from this study indicate that use of lithium-based buffers may reduce the dissolution of silica supports and thus enhance column stability at high pH. Experiments also suggest that pH 12 separations with sol-gel silica supports are feasible, although such high pH operation limits column lifetime.

Introduction

The reversed-phase separation of basic compounds at low pH (less than or equal to 3) often is recommended because the best peak shape and highest column efficiency usually result (1,2). However, chromatography at low pH sometimes is not feasible because of solute instability or band-spacing problems. In such cases, intermediate (pH 4–8) or higher pH (9 or greater) separating conditions are indicated. Although intermediate pH operation can produce useful band spacings for ionizable compounds, problems with band shapes and retention reproducibility can arise as a result of the partial ionization of the basic solutes or the unreacted silanols or a combination of both on the silica support surface (3). Operation at higher pH (9 or greater) is potentially attractive because basic compounds can be separated as free bases, which minimizes interaction with the silica support. Here, silanol groups on the support surface are ionized so that electrostatic interaction with free-base solutes cannot take place.

Separations with silica-based column packings at higher pH traditionally have been discouraged because of potential problems with silica support dissolution and rapid column failure. However, studies have shown that certain column packings can be routinely used at a pH of 9 or more under certain operating conditions (4–8). Specifically, bonded-phase column packings made with silicas that were produced by aggregating silica sols (i.e., sol-gel silicas) have demonstrated superior resistance to dissolution at high pH; this results in higher stability for more rugged and reproducible separation methods (7,8).

This study indicates that high pH separations with certain silica-based bonded-phase column packings can be accomplished under some operating conditions. Basic pharmaceuticals are eluted with good peak shape and column efficiency at a pH of 12. Column stability is sufficient for some applications that require high pH conditions.

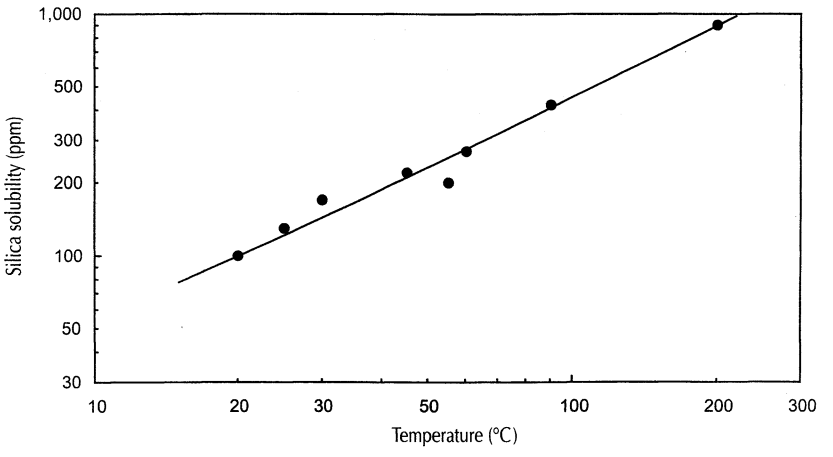

Figure 1. Aqueous solubility (ppm) of amorphous silica as a function of temperature. (Figure adapted from R.K. Iler, *The Chemistry of Silica*, John Wiley & Sons, New York, NY, 1979, p 31.)

Experimental

Apparatus and reagents

Column purging ("aging") studies were performed with a Model 860 pump (DuPont Instruments; Wilmington, DE). Column tests were undertaken with a Model 1050 pump/detector system (Hewlett-Packard; Wilmington, DE). Column temperatures were controlled with a DuPont column compartment. Samples were injected with a Model 7125 sampling valve (Rheodyne; Cotati, CA). Chromatographic data were processed with a personal computer equipped with ChromPerfect software (Justice Innovations; Palo Alto, CA). Solvents and reagents were obtained from EM Science (Gibbstown, NJ). The Zorbax Rx-C18 columns (15 × 0.46 cm) were prepared by Rockland Technologies and are available from Mac-Mod Analytical (Chadds Ford, PA). The packing for these columns was made from ultrapure, low acidity ("Type B") sol-gel silica support (9–11) with 8-nm pores and a nominal surface area of 180 m^2/g. The silica support was densely bonded with dimethyl-C_{18} groups (3.3 µmol/m^2) but was not endcapped. Test samples were obtained from Aldrich Chemicals (St. Louis, MO).

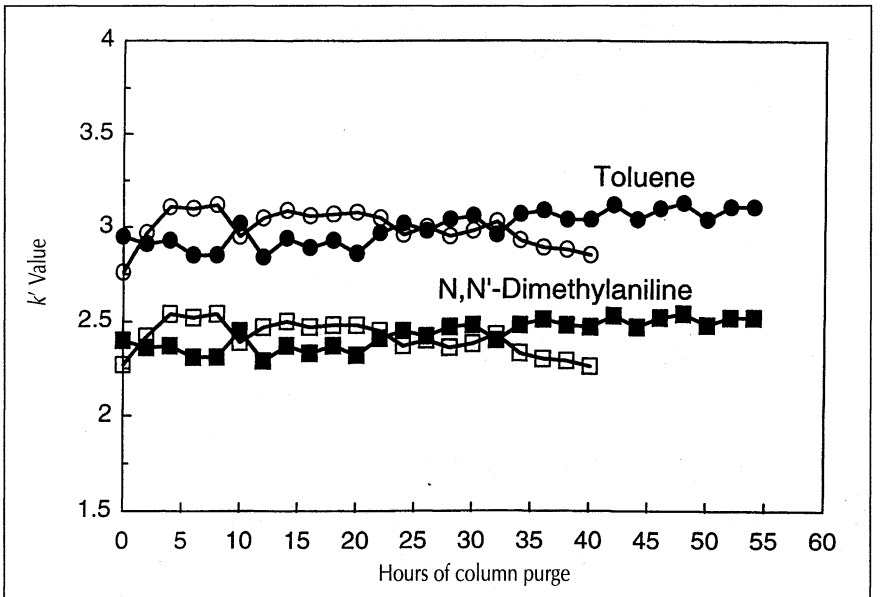

Figure 2. The effect of the cation on k' values for columns purged at a pH of 12.3. Conditions: column, Zorbax Rx-C18 (15 × 0.46 cm); mobile phase, 20% acetonitrile–80% 0.02M NaOH or LiOH; purge and chromatographic test, 1.0 mL/min at 22°C; solutes, 1.0 and 0.01 mg/mL toluene and *N,N'*-dimethylaniline, respectively; sample, 5 µL. Key: toluene with NaOH, —○—; *N,N'*-dimethylaniline with NaOH, —□—; toluene with LiOH, —●—; *N,N'*-dimethylaniline with LiOH, —■—.

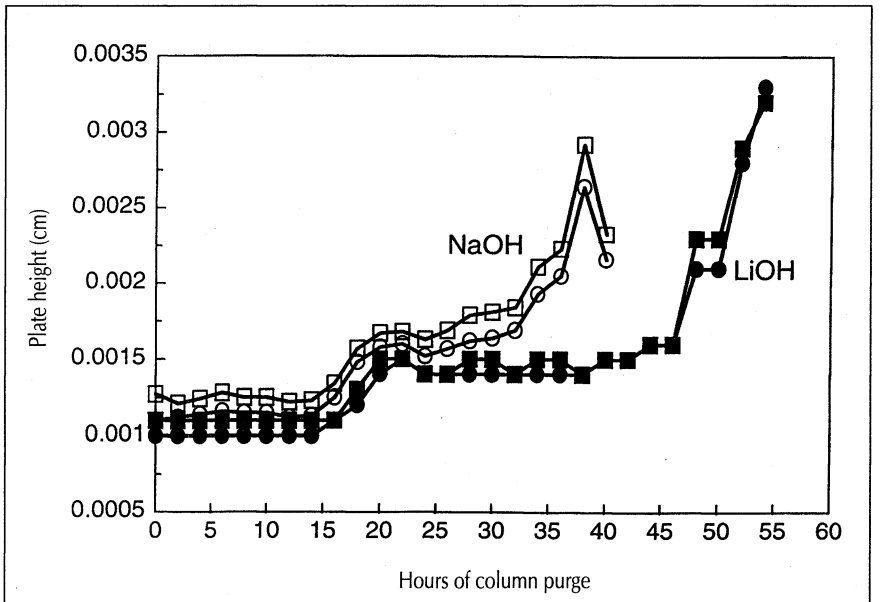

Figure 3. The effect of the cation on plate height for columns purged at a pH of 12.3. Conditions: column, Zorbax Rx-C18 (15 × 0.46 cm); mobile phase, 20% acetonitrile–80% 0.02M NaOH or LiOH; purge and chromatographic test, 1.0 mL/min at 22°C; solutes, 1.0 and 0.01 mg/mL toluene and *N,N'*-dimethylaniline, respectively; sample, 5 µL. Key: toluene with NaOH (k' = 3.0), —○—; *N,N'*-dimethylaniline with NaOH (k' = 2.4), —□—; toluene with LiOH (k' = 3.0), —●—; *N,N'*-dimethylaniline with LiOH (k' = 2.4), —■—.

Procedures

For stability testing, the high pH mobile phase was continuously pumped through each column at a flow rate of 1.0 mL/min at ambient temperature (approximately 22°C). To closely simulate actual chromatographic usage, I did not recycle this mobile phase. Periodically during this purging, columns were flushed with at least 20 volumes of mobile phase, and 5 µL of the test mixture was chromatographed. The test mixture consisted of 0.05, 0.01, and 0.25 mg/mL uracil (void volume marker), *N,N'*-dimethylaniline, and toluene, respectively. Conditions for other chromatographic data are shown in the appropriate figure captions.

Results and Discussion

Previous studies have shown that the failure of bonded-phase, silica-based columns in the pH region of 6–10 primarily is due to dissolution of the silica support rather than hydrolysis and subsequent loss of the silane stationary phase (7,8). The solubility of the silica support is especially affected by the column temperature (8). The influence of temperature on the solubility of amorphous silica in water is illustrated in Figure 1 (12). The strong increase in silica dissolution with an increase in temperature significantly affects column stability; this effect has been demonstrated in chromatographic studies (8). The important conclusion is that lower

column temperatures should be used for maximum column stability, especially for intermediate and high pH separations.

Studies also have shown that column packings made with silica supports prepared by aggregating silica sols (sol-gel silicas) are more stable in the pH 6–10 region than columns prepared with silicas made by gelling soluble silicates (xerogels) (7,8). Presumably, this dissimilarity largely is a function of differences in porosity and pore structure of the silica support. The end result is that columns with sol-gel silica supports are more stable at intermediate and high pH and appear to be preferred for rugged, reproducible methods (3,7,8).

Both the anions and cations used in the mobile phase buffer also affect column stability by influencing the rate of silica support dissolution at intermediate and high pH (8). Phosphate and carbonate buffers enhance silica support dissolution in the pH 6–10 region, whereas borate, citrate, and other organic buffers (e.g., TRIS) show a much smaller effect (8). Thus, the use of certain buffers greatly enhances the stability of silica-based columns and substantially increases method reliability in the intermediate and high pH region.

The type of cation in the buffer also can affect silica-based column performance at high pH. Figure 2 shows the results of purging a nonendcapped dimethyl-C_{18} column with a mobile phase of 20% acetonitrile–80% 0.02M NaOH or LiOH at 1.0 mL/min. The k' values for toluene (neutral) and *N,N'*-dimethylaniline (basic, but neutral at this pH) are essentially constant after more than 40 h of purging at ambient temperature for both NaOH and LiOH. However, after this period, k' values for both solutes begin to decrease for the NaOH purge, but they do not decrease for the comparable LiOH test.

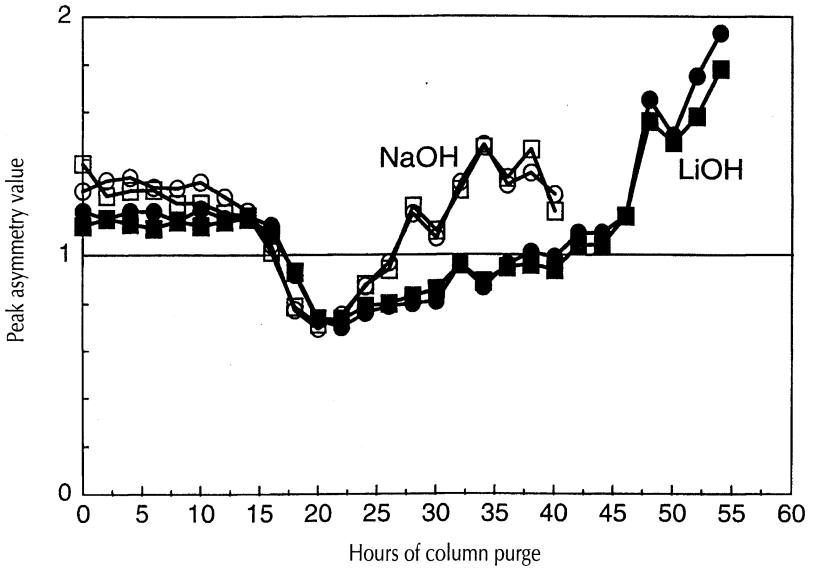

Figure 4. The effect of the cation on band asymmetry for columns purged at a pH of 12.3. Conditions: column, Zorbax Rx-C18 (15 x 0.46 cm); mobile phase, 20% acetonitrile–80% 0.02M NaOH or LiOH; purge and chromatographic test, 1.0 mL/min at 22°C; solutes, 1.0 and 0.01 mg/mL toluene and *N,N'*-dimethylaniline, respectively; sample, 5 µL. Key: toluene with NaOH (k' = 3.0), —○—; *N,N'*-dimethylaniline with NaOH (k' = 2.4), —□—; toluene with LiOH (k' = 3.0), —●—; *N,N'*-dimethylaniline with LiOH (k' = 2.4), —■—.

The effect of the cation on column performance is better illustrated in Figure 3 with plate height data obtained at the same time as the k' data of Figure 2. Both the NaOH and the LiOH purges slightly increased plate heights for toluene and *N,N'*-dimethylaniline after approximately 17 h of purging. The origin of this increase is obscure but may involve a minor shift in column packing bed structure that degraded column efficiency. After approximately 35 h, the column purged with NaOH showed significant degradation, as evidenced by the sudden and significant increase in plate heights. Similar evidence for degradation of the LiOH-purged column did not appear until approximately 50 h of purging. Peak symmetries gave similar information, as shown in Figure 4. The results of this experiment suggest that silica-based column stability at pH 12 may be enhanced by using lithium-based rather than sodium-based buffers. This result may be due to the fact that the solubility of lithium silicate (formed by the dissolution of silica support) is less than that of sodium silicate.

There is a question of whether highly basic compounds can interact with the ionized silanol groups on the silica support surface, even though at pH 12 solutes might be in the free amine form. If such an interaction is possible (e.g., by dipolar interaction), then retention and peak shape would be affected by the concentration of buffer (i.e., the ionic strength) used in the mobile phase. To test this possibility, we chromatographed 2-amino-4,6-dimethylpyridine (pK_a = 9.5) with a mobile phase of 50% methanol–50% LiCl/LiOH buffer, pH 12.0, at ambient temperature. Under these conditions, this solute should be a free base, and

Table I. Band Characteristics for a Highly Basic Compound at pH 12.0*

LiCl (M)	LiOH (M)	Comments†	k' Value	Plate height (cm)	Asymmetry factor
0.025	0.011	pH 12.02	2.31	0.0108	3.65
0.075	0.033	pH 12.07	1.94	0.0031	1.51
0.225	0.099	pH 12.10	1.80	0.0027	1.33
0.225	0.099	1/10 sample mass	1.85	0.0029	1.18
0.225	0.099	10mM dimethyloctylamine	0.88	0.0031	1.28
0.225	0.099	1/10 sample; 10mM dimethyloctylamine	0.80	0.0034	1.25

* Conditions: column, Zorbax Rx-C18 (15 x 0.46 cm); mobile phase, 50% MeOH–50% LiCl/LiOH buffer; flow rate, 1.0 mL/min; solute, 2-amino-4,6-dimethylpyridine; approximate pK_a, 9.5; temperature, ambient.
† Five microliters was injected; the initial sample mass was 5 µg.

the unreacted silanol groups on the silica support should be fully ionized.

Table I shows the effect of changing the buffer concentration while maintaining the mobile phase buffer at an essentially constant pH of 12. Increasing the buffer concentration initially decreased the solute k' value, the plate height, and peak asymmetry values. These solute band characteristics then leveled off with approximately 0.075M LiCl–0.033M LiOH buffer and changed little with further increase in buffer concentration. Decreasing the solute mass by a factor of 10 produced only a small change in band characteristics, which suggested that the initial test conditions did not overload the column with solute. Adding 10mM dimethyloctylamine (pK_a = 11.5) had no significant effect on plate height or peak asymmetry but did decrease the solute k' value, presumably because of the increase in mobile phase strength. Decreasing the sample mass 10-fold with dimethyloctylamine modifier had no additional effect.

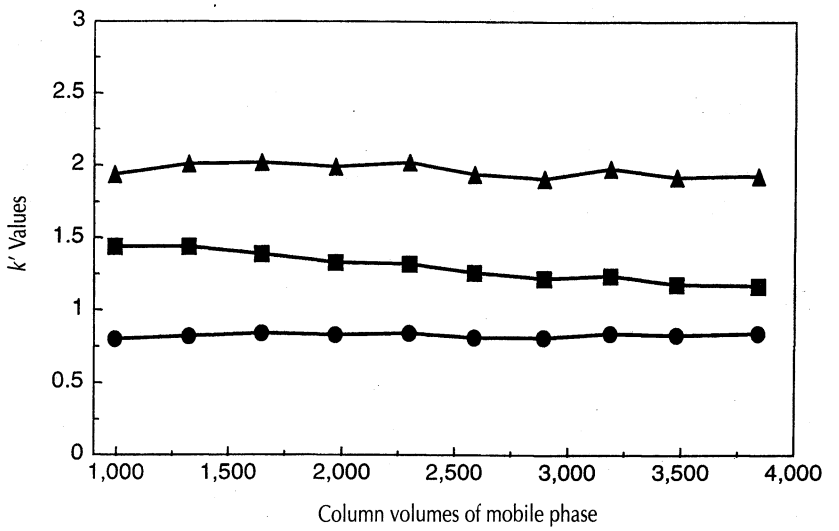

Figure 5. Column aging at a pH of 12.6: k' values for highly basic compounds. Conditions: column, Zorbax Rx-C18 (15 x 0.46 cm); mobile phase, 40% methanol–60% 0.025M LiCl–0.05M LiOH buffer, pH 12.6; purge and chromatographic test, 1.0 mL/min at 22°C; sample, 5 µL. Solutes: —●—, 0.05 mg/mL caffeine (pK_a = 10.4); —■—, 0.19 mg/mL tetracaine (pK_a = 8.5); —▲—, 0.14 mg/mL butacaine (pK_a = 9.0).

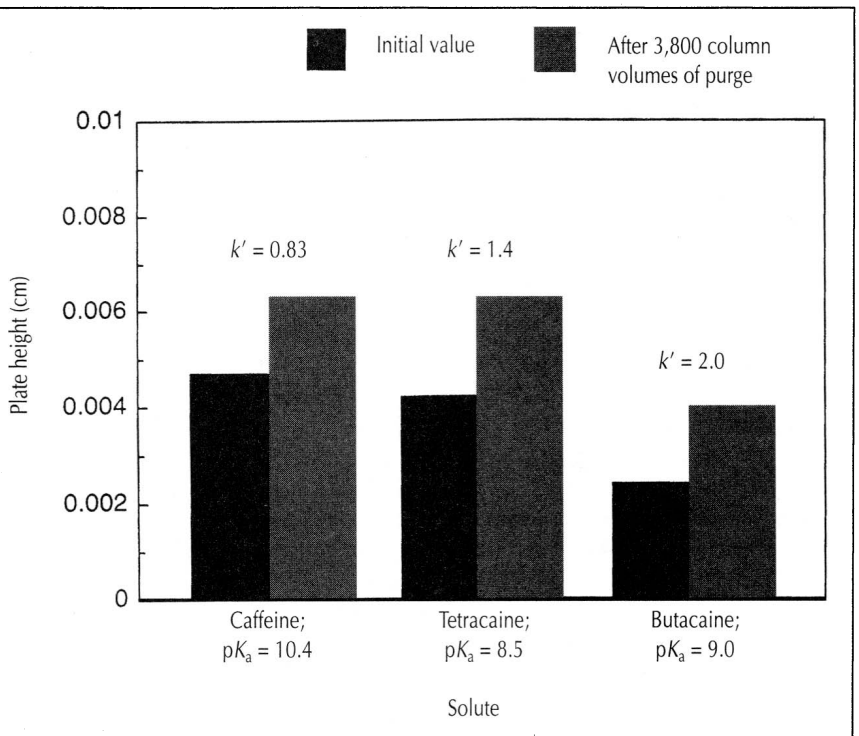

Figure 6. Column aging at a pH of 12.6: plate heights for highly basic compounds. Conditions: column, Zorbax Rx-C18 (15 x 0.46 cm); mobile phase, 40% methanol–60% 0.025M LiCl–0.05M LiOH buffer, pH 12.6; purge and chromatographic test, 1.0 mL/min at 22°C; solutes, 0.05 mg/mL caffeine, 0.19 mg/mL tetracaine, and 0.14 mg/mL butacaine; sample, 5 µL.

The results in Table I suggest that this solute engages in some interaction with the silica support at a low pH 12 buffer concentration. However, with increased buffer concentration, this interaction is minimized or eliminated and presumably leaves hydrophobic interactions as the main retention mechanism. The retention effects on silica-based packings at high pH likely are strongly affected by solute, silica support, and bonding type, as well as buffer type and mobile phase composition. Additional studies are needed to better define these effects.

Column aging studies also were carried out with the basic compounds, caffeine (pK_a = 10.4), tetracaine (pK_a = 8.5), and butacaine (pK_a = 9.0). Figure 5 shows the plots of the k' values for these solutes when a nonendcapped dimethyl-C_{18} column was continuously purged with 40% methanol–60% LiCl/LiOH buffer (pH 12.6) at a rate of 1.0 mL/min and a temperature of 22°C. A slight decrease in the k' value for tetracaine only was observed after approximately 3,800 column volumes (approximately 5.7 L) of purge. The change in plate heights during this experiment is illustrated in Figure 6; the solutes show a 13–33% increase after 3,800 column volumes of purge. Figure 7 shows the initial chromatogram of this mixture and a chromatogram (different sample mixture) with the same column after 3,800 column volumes of purge with the pH 12.6 buffered mobile phase (approximately 2 weeks of 8-h working days). The slight fronting of the peaks for the purged column may signify a change in the packed bed; it may be a small void beginning to form in the column inlet. Minor selectivity changes occurred as a result of the pH 12.6 treatment, but the resulting separation still could be used for precise analysis.

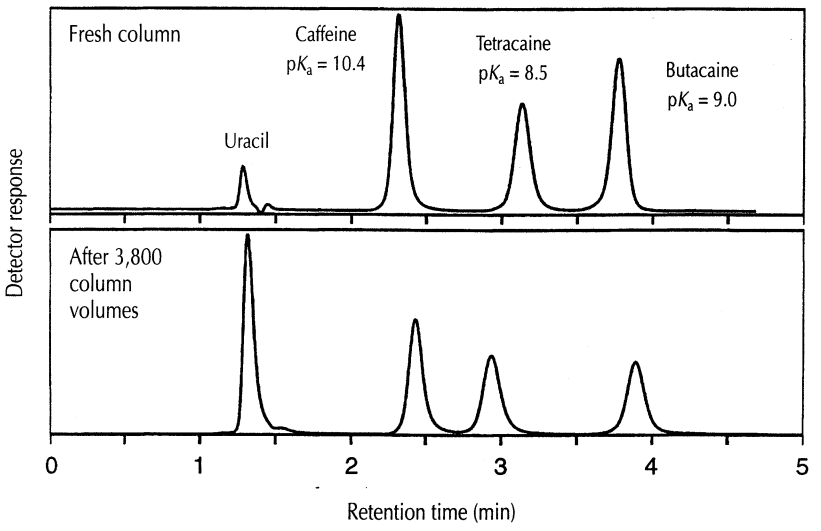

Figure 7. Column aging at a pH of 12.6: chromatograms with initial and aged columns. Conditions: column, Zorbax Rx-C18 (15 x 0.46 cm); mobile phase, 40% methanol–60% 0.025M LiCl–0.05M LiOH buffer, pH 12.6; purge and chromatographic test, 1.0 mL/min at 22°C; solutes, 0.05 mg/mL caffeine, 0.19 mg/mL tetracaine, and 0.14 mg/mL butacaine; sample, 5 µL.

Conclusions

This study confirms that certain densely bonded, nonendcapped dimethyl-C_{18} bonded-phase packings prepared with sol-gel porous silica microspheres are surprisingly resistant to degradation in the pH 10–12 range. The data suggest that such columns can be safely purged periodically with 0.02M NaOH solution to clean unwanted, highly retained materials (e.g., endotoxins) from the column bed. Buffers made with lithium salts appear to be less aggressive at high pH than those prepared with sodium salts. Strongly basic compounds can be successfully separated with good column efficiency and band shapes at high pH in instances in which solutes are free bases. It can be anticipated, however, that operation of silica-based columns at high pH will shorten column life compared with much longer operation at a lower pH. We are currently studying the stability of endcapped dimethyl-C_{18} columns at high pH operating conditions to determine if the lifetime of silica-based columns can be extended with this approach.

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