

# Hybrid Organic–Inorganic Particle Technology: Breaking Through Traditional Barriers of HPLC Separations



Hybrid organic–inorganic particles combine the best properties of silica — high efficiency and excellent mechanical strength — with the best properties of organic polymers — a wide pH stability range and reduced silanol effects. In this article, the authors describe reversed-phase HPLC packings based on hybrid particles and demonstrate the packing's retention and selectivity characteristics. They also show that these media have long lifetimes at elevated temperatures under conditions at which conventional bonded silica fails rapidly. The benefits of operating at elevated temperatures are increased efficiency and reduced back pressure. When combined with short columns containing 2.5- $\mu\text{m}$  hybrid particles, these conditions enable fast, high-resolution gradient separations, which are useful for high-throughput analyses.

**S**ilica-based reversed-phase materials are the most widely used packings for high performance liquid chromatography (HPLC) because they are mechanically strong and provide high separation efficiencies (1). However, they suffer from two disadvantages: First, they have a limited usable pH range, typically pH 2–8. Below pH 2, the bonded phase is susceptible to hydrolysis (2). Above pH 8, hydroxide ions ( $\text{OH}^-$ ) can attack and dissolve the silica, which causes the collapse of the packed bed and a catastrophic loss of efficiency (3). Second, basic analytes interact strongly with residual silanols and cause tailing peaks that are detrimental to resolution as well as to the accuracy and precision of quantitation (4).

Because both disadvantages are related to the use of silica supports, they can be avoided by using packings made from organic polymers. However, organic polymer packings yield significantly lower efficiencies than silica-based packings and are not as mechanically strong (5). Many organic polymer packings also shrink or swell when exposed to different solvents.

The ideal packing material would combine the best properties of bonded silicas — high efficiency and excellent mechanical

strength — with the best properties of organic polymers — wide pH stability range and absence of silanol effects. To create this type of packing we turned to a class of materials known as organic–inorganic hybrids. In hybrids, inorganic and organic components are combined to yield materials with properties intermediate between those of pure organic and pure inorganic compounds (6). The best characterized class of hybrid materials are those prepared by sol-gel synthesis using organosilanes (7,8).

We recently have introduced a family of reversed-phase columns that contain packings based on hybrid particles. We have shown that these columns deliver excellent mechanical strength, high efficiency, outstanding peak symmetry for bases, and stability at pH levels as high as pH 11.5 (9,10). These columns allow chromatographers to go beyond the traditional limitations of silica-based columns and retain those columns' best features. The extended pH and temperature stability enable fast separations with excellent peak shapes for a wide range of analytes. In this article, we demonstrate how these columns allowed us to use mobile-phase pH as a selectivity tool and to reduce analysis time significantly.

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## Experimental

We performed most of our experiments using an Alliance HPLC system, which comprised a model 2690 separation module, a column heater, a model 996 photodiode-array or a model 2487 UV absorbance detector, and a Millennium 32 Chromatography Manager data-management system (all from Waters Corp., Milford, Massachusetts). For the van Deemter experiments, we used a model 590 pump and a model 484 UV absorbance detector with a 2.6- $\mu\text{L}$  flow cell (both from Waters) and a model 7520 injector with a 0.5- $\mu\text{L}$  loop (Rheodyne, Rohnert Park, California).

We calculated efficiency using the half-height method and determined peak tailing factors at 5% of the peak height. We obtained Symmetry C18, XTerra MS C<sub>18</sub>, and XTerra RP<sub>18</sub> columns from Waters and Zorbax Eclipse XDB-C18 double-encapped dimethyl-C18 bonded sol-gel columns from Agilent Technologies (Wilmington, Delaware). All are reversed-phase columns.

The Symmetry C18 columns are based on a high-purity silica bonded with a monofunctional C<sub>18</sub> silane. The XTerra packings are based on the hybrid organic-inorganic carrier described below. The XTerra MS C<sub>18</sub> material is bonded with a trifunctional C<sub>18</sub> silane. The surface of the XTerra RP<sub>18</sub> packing is modified with a monofunctional silane containing an embedded polar group, specifically a carbamate group.

Unless otherwise indicated, the aqueous component of the mobile phase contained a 20 mM buffer solution. Potassium phosphate buffers were used for pH 2.5, 7.0, and 8.0 mobile phases. Ammonium acetate was used for pH 5.0 mobile phases, potassium borate for pH 9.5 mobile phases, and pyrrolidine hydrochloride for pH 10.6 mobile phases. All buffers were prepared in water purified with a Milli-Q water-purification system (Millipore, Bedford, Massachusetts). The organic modifier was either methanol or acetonitrile (both from J.T. Baker, Phillipsburg, New Jersey). The analytes were procured from Sigma Chemical Co. (St. Louis, Missouri) and Aldrich Chemical Co. (Milwaukee, Wisconsin).

For the column lifetime experiments at elevated temperature, the columns were maintained at 60 °C. We first passed a mobile phase containing 80:20 (v/v) 50 mM dibasic sodium phosphate (pH 7.00)-acetonitrile through the columns at 1 mL/min for 60 min. Next we washed the columns with water for 20 min, followed by 10:90 (v/v) water-acetonitrile for 10 min,

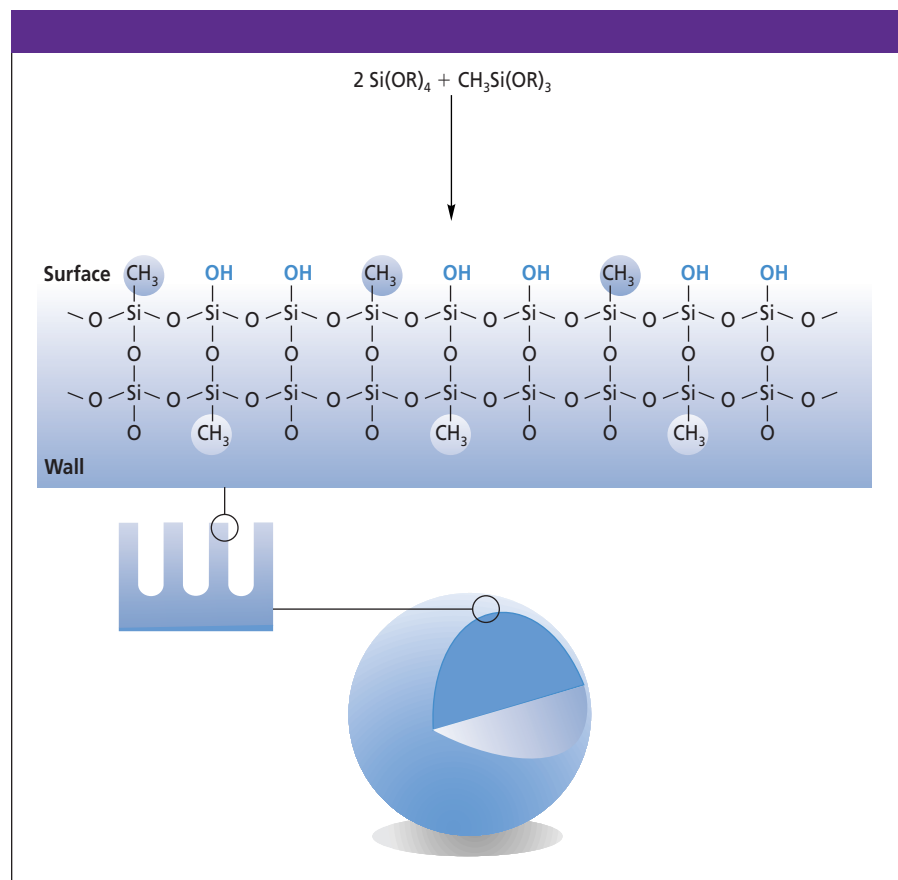
and 40:60 (v/v) water-acetonitrile for 60 min. We checked the condition of the column by injecting a solution of acenaphthene (uracil was the  $t_0$  marker) using a 40:60 (v/v) water-acetonitrile mobile phase at 1 mL/min for 15 min. Next we equilibrated the columns at 1.5 mL/min for 60 min with a 40:60 (v/v) 10 mM dibasic sodium phosphate (pH 7.00)-acetonitrile mobile phase and injected a mixture of four tricyclic antidepressants: doxepin, nortriptyline, amitriptyline, and trimipramine. After that step, we conditioned the columns with water for 20 min, acetonitrile for 10 min, and 80:20 (v/v) water-acetonitrile for 20 min. The entire sequence was repeated until we found that the columns lost more than 50% of their initial efficiency.

## Results and Discussion

**Column packings:** The porous spherical particles in the hybrid organic-inorganic columns are synthesized using a mixture of two organosilanes: one that forms silica units and another that forms methylsiloxane units (Figure 1). The resulting hybrid particles contain methylsiloxane groups throughout

their internal and external structure. We obtained optimum performance using a 2:1 molar ratio of silica to methylsiloxane units (10). These particles are additionally surface bonded to attach a variety of different reversed-phase groups. These bonded phases include two trifunctional materials (designated MS C<sub>8</sub> and MS C<sub>18</sub>) optimized for maximum stability and two monofunctional embedded carbamate materials (designated RP<sub>8</sub> and RP<sub>18</sub>) optimized for minimum peak tailing for basic analytes (11,12).

**Selectivity:** The most important features of any packing material are its retention and selectivity. To characterize these aspects of our bonded hybrid particles, we used a chromatographic test that has been described previously (13). The test involved the separation of a mixture of six analytes chosen for their sensitivity to different characteristics of reversed-phase packings. As Figure 2 shows, the separation appeared very similar on a C18-silica column (Figure 2a) and on a hybrid organic-inorganic reversed-phase C18 column (Figure 2b) with no change in elution order. Although all compounds were slightly less retained on the hybrid organic-



**Figure 1:** Schematic illustration of the synthesis and structure of the hybrid organic-inorganic particles in XTerra columns. Views of the particle structure are shown on three size scales: at the bottom an entire 5- $\mu\text{m}$  particle with a cutaway to indicate that the particle is solid, in the middle a segment showing idealized 125- $\text{\AA}$  pores, and on the top a schematic illustrating the idealized chemical structure.

inorganic column because of its lower surface area and carbon content (10), the retention of the hydrophobic marker acenaphthene was within the range observed with commercially available C18 columns (14). The selectivities for the polar analytes also were well within the range observed for commercially available C18 columns. However, the tailing factor for the base amitriptyline was significantly lower for the hybrid organic–inorganic reversed-phase C18 column (1.47 vs. 2.16), which indicates reduced silanol activity.

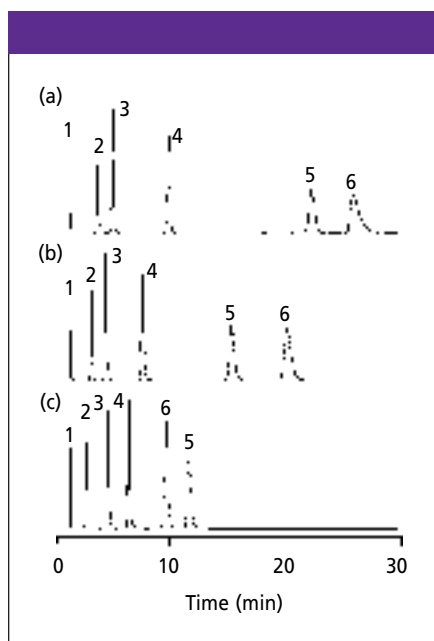
The chromatogram shown in Figure 2c was obtained using a hybrid organic–inorganic C18 column with an embedded polar group. This packing uses the same embedded-carbamate bonded phase that previously has been attached to another silica support (11,12). Consistent with previous observations for silica-based embedded-carbamate bonded phases, the hybrid organic–inorganic C18 column with an embedded polar group showed modified selectivity compared with the other two C18 columns. Most notable is a change in the elution order of the hydrophobic marker acenaphthene and the base amitriptyline. In addition, the hybrid organic–inorganic C18 column with an

embedded polar group also showed further improved peak symmetry for amitriptyline (U.S. Pharmacopeia [USP] tailing factor of 1.11). Reduced retention and tailing for basic analytes are general characteristics of embedded carbamate packings (11,12).

Thus the retention and selectivity characteristics of hybrid organic–inorganic C18 columns are well within the range observed for conventional C18–silica columns. These characteristics allow them to be used in previously developed methods without major changes in mobile-phase composition. In contrast, hybrid organic–inorganic C18 columns with embedded polar groups yield a significant change in selectivity, as previously demonstrated for silica-based, embedded-carbamate bonded phases. Chromatographers can use this selectivity difference advantageously to optimize separations.

**Peak shape:** Another important consideration for any packing is its ability to provide symmetrical peaks for a wide range of analytes. In addition to reducing resolution, tailing peaks are detrimental for the accuracy and precision of quantitation, and they result in inferior limits of detection and quantitation. Traditional silica-based packings often yield tailing peaks for basic analytes because of their interaction with residual silanols. With their unique particle composition, hybrid organic–inorganic packings have significantly reduced concentrations of residual silanols (10), so they deliver excellent peak shapes for a wide range of analytes, including acids, neutrals, and bases.

Table I lists the USP tailing factors obtained for a wide range of pharmaceutical compounds using a 150 mm × 3.9 mm,



**Figure 2:** HPLC separations of a selectivity mixture using 5- $\mu\text{m}$   $d_p$  (a) Symmetry C18, (b) XTerra MS C18, and (c) XTerra RP18 columns. Column dimensions: 150 mm × 3.9 mm; mobile phase: 65% (v/v) 20 mM  $\text{KH}_2\text{PO}_4$ – $\text{K}_2\text{HPO}_4$  (pH 7.00) with 35% methanol; flow rate: 1.0 mL/min; detection: absorbance at 254 nm; temperature: 23 °C. Peaks: 1 = uracil, 2 = propranolol, 3 = butyl paraben, 4 = naphthalene, 5 = acenaphthene, 6 = amitriptyline. The USP tailing factor for peak 6 was (a) 2.16, (b) 1.47, and (c) 1.11.

**Table I:** Summary of USP tailing factors obtained for pharmaceutical compounds using an XTerra RP18 column with various mobile phases\*

| Analyte                       | Buffer–Acetonitrile |        |        | Buffer–Methanol |        |        |
|-------------------------------|---------------------|--------|--------|-----------------|--------|--------|
|                               | pH 2.5              | pH 7.0 | pH 9.5 | pH 2.5          | pH 7.0 | pH 9.5 |
| Acetaminophen                 | 0.99                | 0.98   | 0.97   | 0.97            | 0.95   | 0.99   |
| Alprenolol                    | 1.03                | 1.03   | 1.09   | 1.01            | 1.03   | ND     |
| Atenolol                      | 1.03                | 1.03   | 1.03   | 0.96            | 1.02   | 1.05   |
| Benzamide                     | 1.01                | 0.98   | 0.98   | 1.28            | 1.15   | 1.20   |
| Brompheniramine               | 1.02                | 1.01   | 1.00   | 1.02            | 1.10   | 1.02   |
| Chlorpheniramine              | 1.02                | 1.04   | 1.00   | 1.09            | 1.04   | 1.02   |
| Chlorpromazine                | 1.05                | 1.02   | 0.98   | 1.04            | 0.98   | ND     |
| Dextromethorphan              | 1.04                | 1.18   | 1.08   | 1.04            | 1.22   | ND     |
| Dibucaine                     | 1.05                | 1.01   | 1.00   | 1.08            | 0.98   | 0.99   |
| Diphenhydramine               | 1.04                | 1.03   | 1.04   | ND              | 1.04   | 0.98   |
| Doxepin                       | 1.11                | 1.32   | 1.09   | 1.04            | 1.02   | 1.02   |
| Doxylamine                    | 1.05                | 1.02   | 1.03   | 1.15            | 1.04   | ND     |
| Ethyl <i>p</i> -aminobenzoate | 1.01                | 0.97   | 0.97   | 1.03            | 1.00   | 1.00   |
| Fenpropfen                    | 0.99                | 1.00   | 1.00   | 0.99            | 0.98   | 1.00   |
| Fluoxetine hydrochloride      | 1.04                | 1.08   | 1.04   | 1.04            | 1.05   | 1.02   |
| Hydroxyisophthalic acid       | 1.01                | 1.07   | 1.08   | 0.99            | 1.10   | 1.12   |
| Ibuprofen                     | 0.99                | 1.01   | 1.02   | 1.00            | 1.01   | 1.00   |
| Imipramine                    | 1.05                | 1.04   | 1.01   | 1.10            | 1.01   | 0.98   |
| Lidocaine                     | 1.00                | 0.95   | 0.97   | 1.03            | 1.02   | 0.99   |
| Methoxyverapamil              | 1.03                | 0.99   | 1.02   | 1.08            | 1.00   | 0.97   |
| Methylnicotinic acid          | 1.11                | 1.05   | 1.02   | 1.07            | 1.02   | 1.04   |
| ( $\pm$ )Metoprolol           | 1.02                | 1.02   | 1.04   | ND              | 1.04   | 1.03   |
| Nabumetone                    | 0.99                | 0.98   | 0.99   | 1.01            | 0.99   | 1.00   |
| Nicotinic acid                | 1.11                | 1.06   | 1.05   | 1.09            | 1.08   | 1.11   |
| Nordihydroguaiaretic acid     | 1.02                | 1.02   | 1.13   | 1.05            | 0.97   | 1.18   |
| Nortriptyline                 | 1.06                | 1.11   | 1.09   | 1.09            | 1.07   | 1.04   |
| Pheniramine                   | 1.02                | 1.03   | 0.98   | 1.03            | 1.17   | 1.06   |
| Procaine                      | 1.02                | 1.03   | 0.98   | 1.04            | 1.11   | 1.01   |
| Prednisolone                  | 1.04                | 0.98   | 1.00   | ND              | 0.95   | 0.99   |
| Pyrilamine                    | 1.02                | 1.01   | 0.97   | 1.13            | 1.01   | 1.01   |
| Risperidone                   | 1.00                | 1.01   | 1.00   | 1.06            | 1.17   | 0.98   |
| Suprofen                      | 1.03                | 1.01   | 1.00   | 1.03            | 1.00   | 1.00   |
| Trimipramine                  | 1.05                | 1.00   | 0.98   | ND              | 0.99   | 1.00   |
| Theophylline                  | 0.98                | 0.98   | 0.97   | 0.95            | 0.97   | 0.98   |
| <i>p</i> -Toluamide           | 0.98                | 0.98   | 0.99   | 0.98            | 1.01   | 1.02   |
| Uracil                        | 1.08                | 1.07   | 1.09   | 1.07            | 1.07   | 1.11   |
| Verapamil                     | 1.03                | 0.99   | 1.00   | 1.08            | 0.98   | 1.01   |

\*ND = not determined; experiment not performed.

5- $\mu\text{m}$   $d_p$  hybrid organic–inorganic C18 column with an embedded polar group with simple buffer compositions at three pH values (pH 2.5, pH 7.0, and pH 9.5). Both acetonitrile and methanol were used as organic modifiers. Retention factors were kept to 6 or less for all analytes by adjusting the strength of the mobile phase. At all three pH values, we obtained excellent peak shapes for all analytes, with USP tailing factors of 0.95–1.32.

**Effects of mobile-phase pH on selectivity:** Because most pharmaceutical analytes are ionic or ionizable, varying the mobile-phase pH is a powerful method development tool in reversed-phase chromatography. For ionizable analytes, pH changes induce larger shifts in selectivity than traditional solvent changes. The retention factor ( $k$ ) for a monoprotic acid can be predicted using the following equation:

$$k = k_{\text{HA}} + \frac{k_{\text{A}^-} - k_{\text{HA}}}{1 + 10^{(\text{pH} - \text{p}K_a)}} \quad [1]$$

where  $k_{\text{HA}}$  and  $k_{\text{A}^-}$  are the retention factors for the monoprotic acid and its conjugate

base, respectively, and  $K_a$  is the acid dissociation constant. Acids are most retained at pH values below their  $\text{p}K_a$ , at which they are predominantly in the uncharged protonated form. Conversely, bases are most retained at pH values above their  $\text{p}K_a$ , at which they are predominantly in the uncharged unprotonated form.

Equation 1 shows that retention is strongly dependent upon pH only within  $\pm 2$  pH units of  $\text{p}K_a$ . For a typical strong base with a  $\text{p}K_a$  of 9.5, retention shifts occur over the pH range of 7.5–11.5. To access this full range, it is essential to use a column that is stable over a wide pH range. Neue and co-workers (9) demonstrated that hybrid organic–inorganic packings can be used from pH 1.2 to pH 11.5. This broad pH range allows great flexibility in HPLC method development.

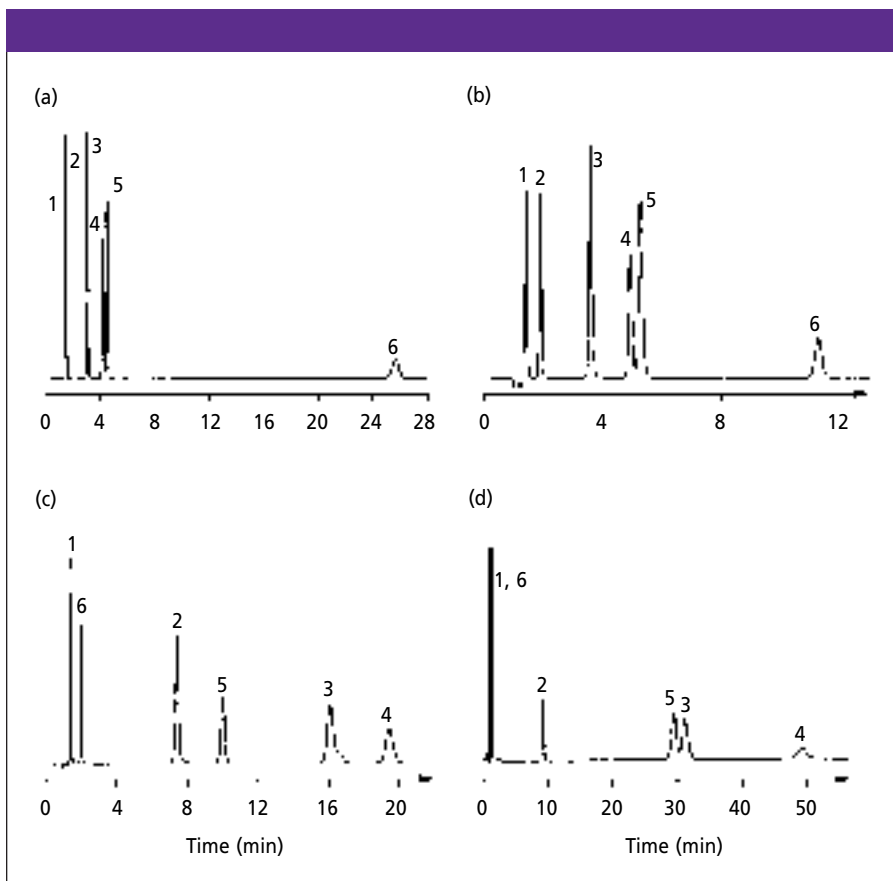
To illustrate the dramatic shifts in retention and selectivity possible by varying mobile-phase pH, we separated a mixture of acidic, neutral, and basic analytes on a 150 mm  $\times$  3.9 mm, 5- $\mu\text{m}$   $d_p$  hybrid organic–inorganic C18 column with an embedded polar group using four mobile-phase pH values (pH 2.5, 5.0, 8.0, and

10.6) (see Figure 3). At low pH values, the neutral analyte acetaminophen was eluted first, followed by the bases lidocaine, doxepin, imipramine, and nortriptyline — with  $\text{p}K_a$  values ranging from 7.9 to 9.7 — and the acid ibuprofen ( $\text{p}K_a$  4.4). At elevated pH values, the neutral and acidic analytes are eluted first, followed by the bases. These trends are as expected based on equation 1. The shoulder observed for peak 3 at pH 8.0 is caused by partial separation of the geometric isomers of doxepin.

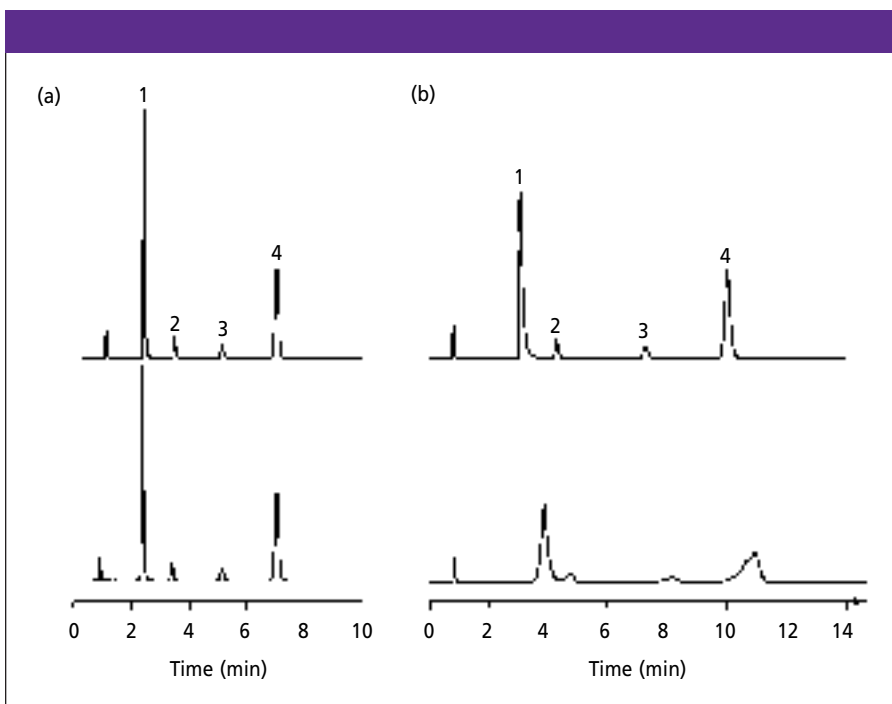
It is interesting to note that the retention factor of the neutral acetaminophen (peak 1) at pH 10.6 is only half as large as that observed at a lower pH. In fact, acetaminophen is a weak acid ( $\text{p}K_a$  9.5) that is ionized at pH 10.6. Clearly, these selectivity shifts can be advantageously applied in method development. For this particular mixture, we obtained the best separation at pH 8.0. It is also notable that excellent peak shapes were obtained for all analytes at all pH values.

**Temperature effects:** An additional benefit of the enhanced stability of the hybrid organic–inorganic columns is that they often exhibit longer lifetimes than C18–silica columns when used at elevated temperatures. Column deterioration at both low and high pH is strongly accelerated when the temperature is raised. Figure 4 shows an example. We compared the stability of a hybrid organic–inorganic C18 column with that of a double-encapped dimethyl–C18 bonded sol-gel silica column. The latter technology previously had been shown to provide superior high pH stability compared with other bonded silicas (15). The columns were purged with a 50 mM dibasic sodium phosphate (pH 7.00) at 60 °C and periodically evaluated by separating a mixture of tricyclic antidepressants. As previously shown (15), these conditions led to rapid failure of bonded silica columns because of dissolution of the silica particles and subsequent column voiding. In four days, the sol-gel C18–silica column had lost more than 75% of its initial efficiency. In contrast, a hybrid organic–inorganic C18 column with an embedded polar group showed no deterioration after 15 days.

Operation at elevated temperatures has several well-known benefits. The key benefit is that mobile-phase viscosity drops with increasing temperature. This reduced viscosity speeds up the rate of analyte diffusion in the mobile phase, reducing the dependence of efficiency on flow rate (16,17). Figure 5 demonstrates this effect by showing van Deemter curves (plots of plate



**Figure 3:** Separation of a mixture of acidic, basic, and neutral analytes at (a) pH 2.5, (b) pH 5.0, (c) pH 8.0, and (d) pH 10.6. Column: 150 mm  $\times$  3.9 mm, 5- $\mu\text{m}$   $d_p$  XTerra RP<sub>18</sub>; mobile phase: 65:35 (v/v) 20 mM buffer–acetonitrile; detection wavelength: 210 nm for pH 2.5, 5.0, and 8.0, and 230 nm for pH 10.6; flow rate: 1.0 mL/min; injection volume: 5  $\mu\text{L}$ . Peaks: 1 = acetaminophen, 2 = lidocaine, 3 = doxepin, 4 = imipramine, 5 = nortriptyline, 6 = ibuprofen.



**Figure 4:** Comparison of the stability of (a) XTerra RP<sub>18</sub> and (b) double-encapped C<sub>18</sub>-silica columns at 60 °C. Upper chromatograms: on day 1. Lower chromatograms: (a) on day 15, (b) on day 4. Column dimensions: 150 mm × 4.6 mm; mobile phase: 60:40 (v/v) acetonitrile–10 mM dibasic sodium phosphate (pH 7.0); flow rate: 1.5 mL/min; detection: absorbance at 254 nm. Peaks: 1 = doxepin, 2 = nortriptyline, 3 = amitriptyline, 4 = trimipramine.

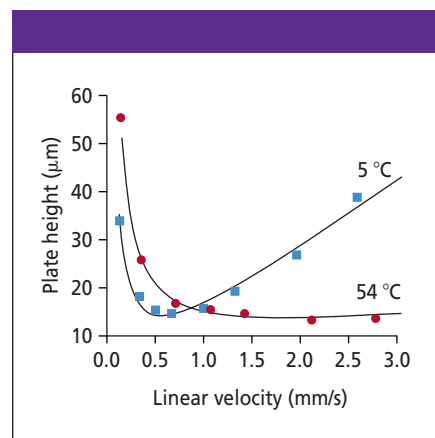
height [ $H$ ] versus linear velocity [ $u$ ] at two temperatures. At the higher temperature, the plate height shows a reduced dependence upon linear velocity at high velocities. Therefore, analyses may be accelerated by increasing the flow rate with little loss in efficiency.

Figure 6, which shows the separation of three basic analytes at four temperatures (30, 40, 50, and 60 °C), demonstrates the additional advantages of operating at elevated temperatures. We observed increases in efficiency and peak height as the temperature increased. In this experiment, we observed an improvement in plate count of as much as 34% by increasing the temperature from 30 to 60 °C (see Table II).

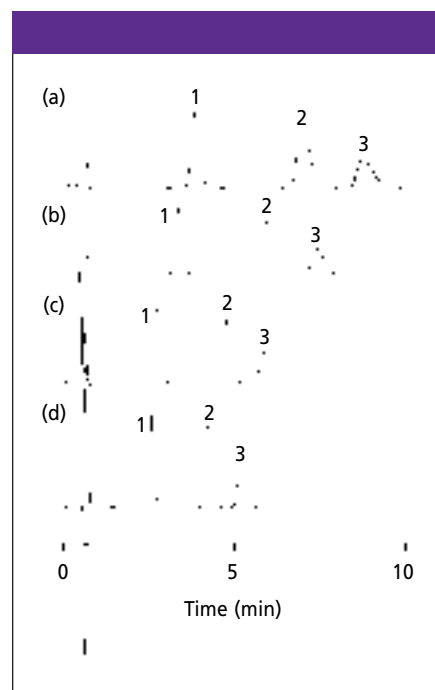
Another benefit of using elevated temperature is reduced column back pressure. Column back pressure is directly proportional to the viscosity of the mobile phase. A 1 °C increase in temperature decreases the mobile-phase viscosity by 1% (18). This decreased back pressure is particularly important as particle size is reduced to increase efficiency or speed. In the experiment described above, we observed that the column back pressure decreased steadily from 30 °C to 60 °C (see Table II). Thus, elevated temperature provides several benefits that are particularly important for fast separations.

**Fast gradient analysis:** Over the past few years, there has been a growing interest in fast, generic HPLC methods to support drug discovery and combinatorial synthesis. Through advances in HPLC instrumentation — low extracolumn bandspreading, low gradient delay volumes, and rapid injection cycles — and the use of 30–50 mm short columns packed with 3–5  $\mu\text{m}$  particles, researchers have been able to reduce analysis times to 3–5 min (19–21). These fast separations are accomplished using steep gradients and high flow rates. However, this approach has its limitations if analysts want to reduce the analysis time further; namely, the loss of separation efficiency and resolution.

To achieve a faster separation while maintaining high efficiency and resolution, chromatographers must use shorter columns with even smaller particle sizes. For this reason, we developed 2.5- $\mu\text{m}$   $d_p$  hybrid organic–inorganic particles that provide higher efficiency than 3–5  $\mu\text{m}$  particles. Columns containing 2.5- $\mu\text{m}$  particles also show a weaker dependence of plate height upon linear velocity (see Figure 7), so they can be used at high flow rates with little loss in efficiency. Because of the relatively high back pressures these columns generate, it is desirable to operate them at elevated temperatures to reduce the viscosity of the



**Figure 5:** Plots of plate height versus linear velocity at 5 °C and 54 °C. Column: 150 mm × 4.6 mm, 5- $\mu\text{m}$   $d_p$  XTerra MS C<sub>18</sub>; mobile phase: 50:50 (v/v) acetonitrile–water; analyte: acenaphthene. The symbols are the experimental results and the lines are the best fits to the van Deemter equation.



**Figure 6:** Separations performed at (a) 30, (b) 40, (c) 50, and (d) 60 °C. Column: 50 mm × 2.1 mm, 2.5- $\mu\text{m}$   $d_p$  XTerra MS C<sub>18</sub>; mobile phase: 25:75 (v/v) acetonitrile–10 mM ammonium acetate (pH 5.0); flow rate: 0.3 mL/min; injection volume: 3  $\mu\text{L}$ ; detection: 210 nm. Peaks: 1 = doxepin, 2 = imipramine, 3 = amitriptyline.

mobile phase. As discussed above, the stability of hybrid organic–inorganic particles enables long column lifetimes even at elevated temperatures.

To demonstrate the benefit of using columns containing 2.5- $\mu\text{m}$  particles, we separated a mixture of acidic, neutral, and basic drugs using three hybrid organic–

**Table II:** Dependence of column pressure (*P*), retention factor (*k*), efficiency (*N*), and USP tailing factor (*T*) on temperature\*

| Temperature (°C) | <i>P</i> (psi) | <i>k</i> |            |               | <i>N</i> |            |               | <i>T</i> |            |               |
|------------------|----------------|----------|------------|---------------|----------|------------|---------------|----------|------------|---------------|
|                  |                | Doxepin  | Imipramine | Amitriptyline | Doxepin  | Imipramine | Amitriptyline | Doxepin  | Imipramine | Amitriptyline |
| 30               | 1920           | 3.6      | 7.2        | 9.2           | 1430     | 1670       | 1680          | 1.25     | 1.42       | 1.34          |
| 40               | 1620           | 3.0      | 5.9        | 7.6           | 1550     | 1860       | 1940          | 1.24     | 1.33       | 1.24          |
| 50               | 1330           | 2.5      | 4.9        | 6.3           | 1650     | 2020       | 2100          | 1.22     | 1.31       | 1.20          |
| 60               | 1160           | 2.1      | 4.1        | 5.2           | 1680     | 2130       | 2250          | 1.21     | 1.26       | 1.22          |

\* Experimental conditions described in Figure 6.

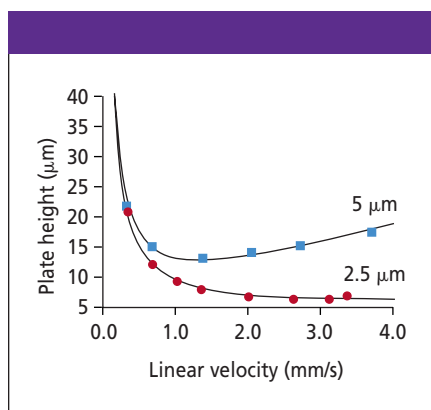
inorganic C18 columns: a 50 mm × 2.1 mm, 5- $\mu\text{m}$   $d_p$  column, a 30 mm × 2.1 mm, 3.5- $\mu\text{m}$   $d_p$  column, and a 20 mm × 2.1 mm, 2.5- $\mu\text{m}$   $d_p$  column. Note that the ratio of column length to particle size is roughly constant for the three columns, so they have similar efficiencies. The gradient duration was scaled in proportion to the length of the columns. We used a high flow rate of 1.5 mL/min and an elevated temperature (60 °C). The results of Figure 8 demonstrate that the column containing 2.5- $\mu\text{m}$  particles is able to cleanly separate the five analytes in only 1 min.

## Conclusion

We have shown that bonded hybrid organic-inorganic particles provide significant benefits over bonded silica. Because the surface chemistry of these packings is similar to that of bonded silicas, they exhibit similar selectivity and retention characteristics. However, the reduced residual silanol content of the bonded hybrid particles provides several advantages over bonded silica columns. One advantage is improved peak shape for basic compounds across a wide range of mobile-phase pH values. A second advantage is a dramatic improvement in stability to alkaline mobile phases. With a wide stability range, hybrid organic-inorganic columns allow the use of mobile-phase pH modification to optimize the separation of even strong bases. This enhanced stability also enables the use of elevated temperatures, which results in reduced back pressure and improved efficiency at high flow rates. We have demonstrated that 20-mm-long columns containing 2.5- $\mu\text{m}$  hybrid organic-inorganic particles enable high-speed gradient separations with analysis times as short as 1 min.

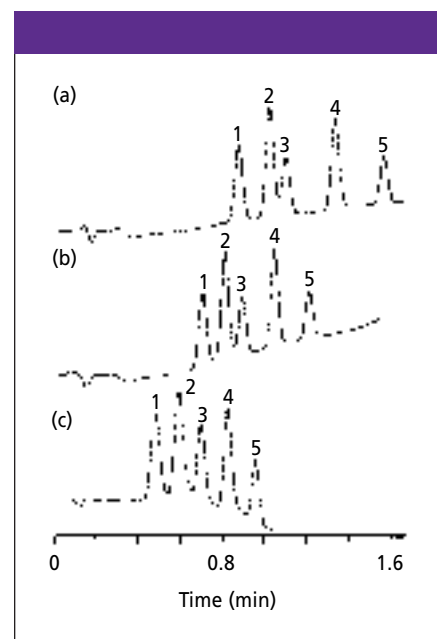
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**Figure 7:** Plots of plate height versus linear velocity for 5- and 2.5- $\mu\text{m}$   $d_p$  columns. Columns: 150 mm × 4.6 mm, 5- $\mu\text{m}$   $d_p$ , and 50 mm × 4.6 mm, 2.5- $\mu\text{m}$   $d_p$  XTerra MS C18; mobile phase: 50:50 (v/v) acetonitrile-water; analyte: acenaphthene; temperature: 25 °C. The symbols are the experimental results and the lines are the best fits to the van Deemter equation.

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**Figure 8:** Comparison of separation time at a constant ratio of column length to particle size using XTerra MS C18 columns. Shown are separations generated using (a) 50 mm × 2.1 mm, 5- $\mu\text{m}$   $d_p$ ; (b) 30 mm × 2.1 mm, 3.5- $\mu\text{m}$   $d_p$ ; and (c) 20 mm × 2.1 mm, 2.5- $\mu\text{m}$   $d_p$  columns. Mobile-phase A: 10 mM ammonium acetate (pH 5.0); mobile-phase B: acetonitrile with 10 mM ammonium acetate (pH 5.0); gradient: 20–95% B; gradient time: (a) 2.5 min, (b) 1.5 min, (c) 1 min; temperature: 60 °C; flow rate: 1.5 mL/min; injection volume: 1  $\mu\text{L}$ ; detection: absorbance at 210 nm. Peaks: 1 = prednisolone, 2 = diphenhydramine, 3 = naproxen, 4 = amitriptyline, 5 = ibuprofen.

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