Waters

Utilizing XBridge™ HPLC Columns for Method Development at pH Extremes

Kevin Jenkins, Diane Diehl, Damian Morrison, and Jeff Mazzeo
Waters Corporation

XBridge™ C_{18} columns provide outstanding phosphate buffer stability at pH 2, 7, and 12.

For the best peak shape, retention, and loading of basic analytes in reversed-phase HPLC, the pH of the mobile phase should be at least 2 units higher than the pKa of the molecule. For many bases, this means the pH of the mobile phase should be 10 or higher. Volatile buffers and additives such as ammonium bicarbonate and ammonium hydroxide are commonly used in high pH mobile phases, but their upper buffering capacity is pH 11. Phosphate buffer, a favorite of chromatographers due to its three pKa's of 2.15, 7.2, and 12.3 and transparency at low UV wavelengths, is rather aggressive at pH 12. Standard silica-based column packings are unusable at pH 12 with phosphate buffer, and many columns designed to work at high pH suffer from reduced lifetimes. With the introduction of the new XBridge™ family of HPLC columns, phosphate buffer at all three buffering ranges can now be routinely used.

Chromatographic Conditions

Stability Testing

Column: XBridge™ C_{18} 5 µm 4.6 × 150 mm
Mobile Phase A: 200 mM K_3PO_4 (pH 12):MeCN (80:20)
Mobile Phase B: Water
Mobile Phase C: Acetonitrile
Isocratic Mobile Phase Composition:
10% A; 37% B; 53% C
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL of 440 µg/mL (total concentration)
Detection: UV @ 254 nm

Selectivity Study

Column: XBridge™ C_{18} 3.5 µm 4.6 × 100 mm
Mobile Phase A1: 30 mM Potassium Phosphate, pH 2
Mobile Phase A2: 30 mM Potassium Phosphate, pH 7
Mobile Phase A2: 30 mM Potassium Phosphate, pH 12
Mobile Phase B: Acetonitrile
Flow Rate: 1.4 mL/min
Column Temperature: 30 °C
Injection Volume: 20 µL of 5 µg/mL (each) standard
Detection: UV @ 210 nm (pH 2, 7); 220 nm (pH 12)
Instrument (both studies): Waters Alliance® 2695 with 2996 PDA

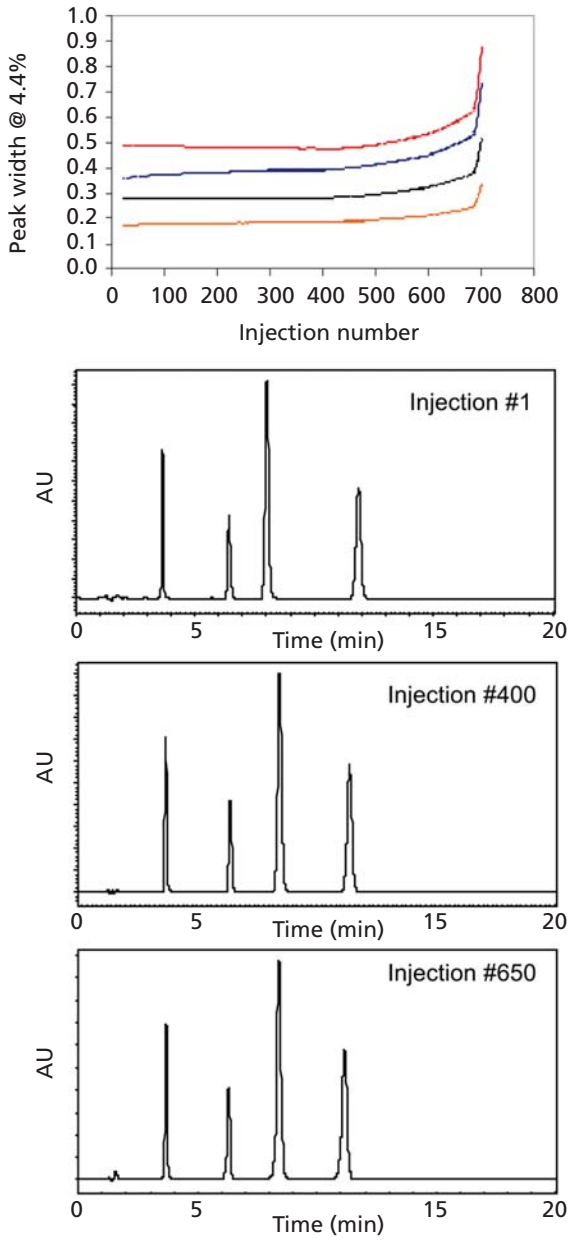

Figure 1: Column lifetime of XBridge™ C_{18} columns using pH 12 phosphate buffer at 30 °C. Elution: 1. Propranolol (orange), 2. Diphenhydramine (black), 3. Nortriptyline (blue), 4. Acenaphthene (red). Top plot is the peak width at 4.4% over the course of 700 injections.

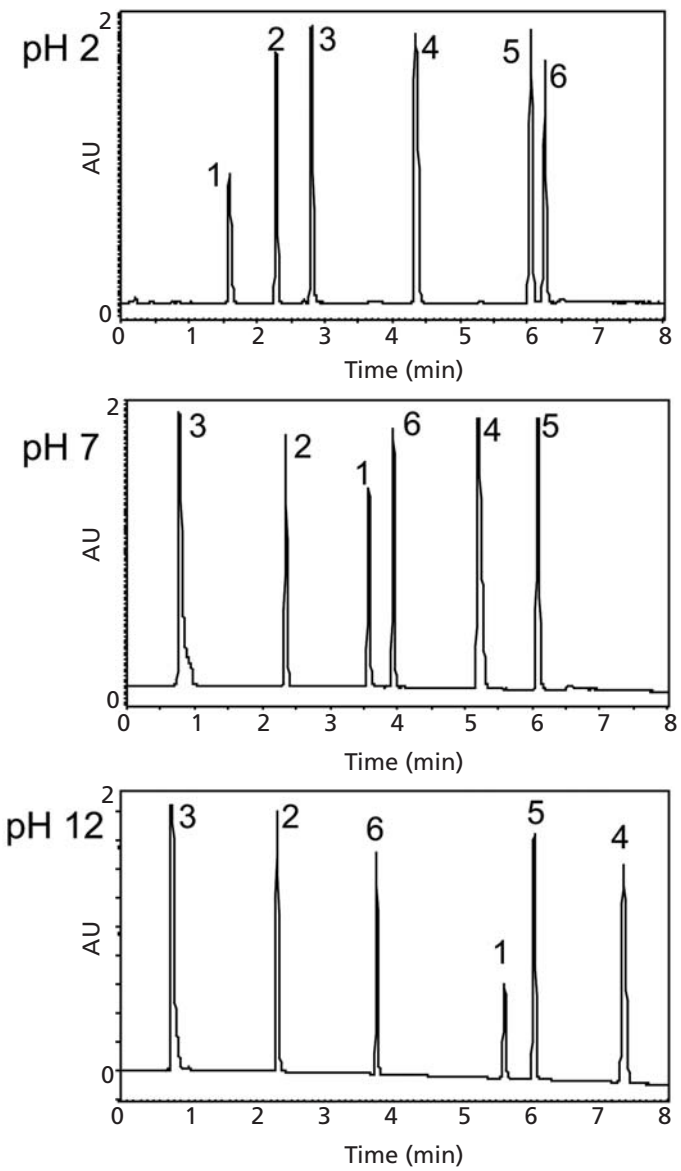

Figure 2: Selectivity differences at pH 2, 7, and 12. Analytes: 1. Doxylamine (B), 2. Benzamide (N), 3. Hydroxyisophthalic Acid (A), 4. Doxepin (B), 5. Flavone (N), 6. Fenoprofen (A). B: Base, N: Neutral, A:Acid.

Table I. Gradient

Time (min)	Profile %A	Profile %B
0.0	90	10
7.0	20	80
8.0	20	80

Results and Discussion

As shown in Figure 1, 650 continuous injections under pH 12 phosphate buffer conditions were successfully made on the XBridge™ C_{18} column. This is at least a 10-fold improvement over other commercially available HPLC columns. This result indicates that pH 12 phosphate buffer can now be routinely used in HPLC methods. Of course, the use of guard columns and careful sample preparation can also help to prolong the lifetime of the columns.

Mobile phase pH can have a significant contribution to chromatographic selectivity if the analytes of interest are ionizable. Figure 2 shows that for neutral analytes (flavone, benzamide) the mobile phase pH has no effect on chromatographic performance or retention time. For ionizable analytes, reversed-phase retention depends heavily on the pH of the mobile phase. At pH 2, which is below the pKa for most organic acids, we see maximum retention and the best peak shape for hydroxyisophthalic acid and fenoprofen. At pH 7 and 12 there is a significant decrease in retention for these acids; hydroxyisophthalic acid is nonretained and elutes in the void. The opposite is true for bases (doxepin, doxylamine), where maximum retention and best peak shape are observed at pH 12. At this pH, both analytes are pH neutral, allowing the reversed-phase retention mechanism to govern the separation.

Conclusions

XBridge™ C_{18} columns are stable in phosphate buffer pH 12 mobile phase conditions, allowing for full use of phosphate buffer across the pH 2–12 range. This now provides the method developer to fully utilize pH as a selectivity tool.

Waters Corporation
34 Maple Street, Milford, MA 01757
tel. (508) 478-2000, fax (508) 478-1990
www.waters.com