Waters

A New State-of-the-Art Silica C_{18} Column for Improved Peak Shape of Basic Analytes

SunFire™ C_{18} reversed-phase HPLC columns provide best-in-class peak shape, especially for basic analytes.

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Most of the leading HPLC column manufacturers have at least one, if not several, C_{18} columns based on a high-purity silica. These columns offer nearly symmetrical peak shapes for acids, bases, and neutrals, good column lifetimes, and excellent reproducibility at low pH. Many of these same columns also perform well in the neutral pH range for a variety of analytes. Chromatographers select a particular C_{18} column based on their own experiences and sets of experiments. With over 600 C_{18} columns on the market, is it worthwhile to develop yet another C_{18} silica-based column? We have sought to answer this question and have developed a new packing material that improves upon existing C_{18} chemistries. SunFire™ C_{18} offers the best peak shape for bases at pH 7, excellent efficiencies, and acid stability. Comparison of SunFire™ C_{18} with current leading C_{18} materials is discussed. Additionally, the use of this new material for stability-indicating assays is demonstrated.

Experimental Conditions

C_{18} Column Analysis Conditions

Columns: SunFire™ C_{18}, Luna® C_{18} (2) 100 Å, Ace® C_{18}, Inertsil® ODS-3, Zorbax® SB-C_{18}
Column dimensions: 4.6 × 150 mm, 5.0 µm
Mobile phase A: Water
Mobile phase B: MeOH
Mobile phase C: 30 mM sodium phosphate, pH 7.0
Isocratic conditions: 60% A, 10% B, 30% C
Flow rate: 1.0 mL/min
Temperature: 30 °C
Injection volume: 10.0 µL
Sample: 20 µg/mL
Detection: UV at 210 nm
Instrument: Waters Alliance® HT 2695 separations module with 2996 photodiode array detector

Stability-Indicating Assay Conditions

Column: SunFire™ C_{18}, 4.6 × 150 mm, 5.0 µm
Mobile phase A: 20 mM ammonium formate, pH 3.0
Mobile phase B: Acetonitrile
Isocratic conditions: 72% A, 28% B
Flow rate: 1.4 mL/min
Temperature: 30 °C
Injection volume: 7.0 µL
Sample: Pravastatin Sodium degraded by 3% hydrogen peroxide
Detection: UV at 254 nm
Instrument: Waters Alliance® HT 2795 separations module with 2996 photodiode array detector

Results

Several attempts have been made by manufacturers to develop new stationary phases that minimize peak tailing. High silanol activity at neutral pH generally promotes the tailing of basic analytes. As seen in Figure 1, superior peak shapes are observed for basic analytes under neutral pH operating conditions on SunFire™ C_{18} compared to other C_{18} chemistries with minimal peak tailing.

Figure 2 demonstrates the high efficiency and excellent peak shapes observed for degradant peaks present in low concentrations.With peaks below 0.1% peak area, the analyst is now able to detect and quantitate small degradant peaks. Spectral purity information concludes that all degradants are successfully resolved from pravastatin (peak angle [PA] is less than the peak threshold [PT]).

Conclusions

SunFire™ C_{18} columns yield narrow symmetrical peaks with good retention and low tailing factors for analytical analysis, therefore offering an improvement in sensitivity, resolution, and quantitation.

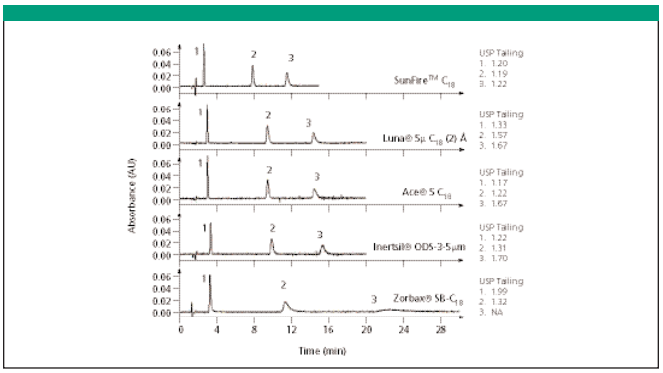

Figure 1: Basic analytes were run with 30 mM sodium phosphate pH 7 on SunFire™ C_{18} compared to other C_{18} chemistry. Peaks: 1 = phenylephrine; 2 = phenylpropanolamine; 3 = pseudoephedrine.

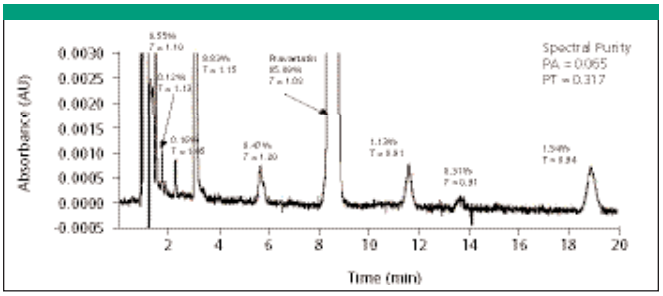

Figure 2: Degradation of pravastatin sodium by 3% hydrogen peroxide. Small degradant peaks yield high efficiency and low tailing factors, allowing for improved quantitation. USP tailing factors and % peak area are expressed.

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