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Preparative Purification of Challenging Basic Compounds: What are the Options?

Fang Xia, Jie Y. Cavanaugh, and Diane M. Diehl
Waters Corporation

The new reversed-phased SunFire™ C_{18} column possesses high preparative mass loading and excellent efficiency for challenge separations under low pH conditions.

Structurally-related analytes are challenging to purify efficiently and are difficult to separate due to selectivity and loadability limitations. The use of pH range to impact the selectivity of ionizable compounds is a very powerful approach. As has been shown previously, purification of compounds in the non-ionized form can achieve 20–50 folds or even higher loadability compared to the ionic form (1). However, due to procedure or equipment limitations, it is often the case that it is only practical to work in a specific pH. The SunFire™ C_{18} preparative OBD™ columns are designed to have reduced peak tailing, high loadability, and extraordinary low pH stability. Therefore, in this study, we compare the preparative loading of basic compounds on SunFire™ Prep columns at low pH and XTerra® Prep columns at high pH conditions.

Experimental Conditions

Low pH Conditions:
Column: SunFire™ Prep C_{18}, 19 × 100 mm, 5 µm
Mobile phase A: 0.1% trifluoroacetic acid in water
Mobile phase B: 0.1% trifluoroacetic acid in acetonitrile
Flow rate: 18.0 mL/min (Figure 1), 23.9 mL/min (Figure 2)
Gradient: 10 min linear from 10% to 90% B and hold at 90%B for 5 min (Figure 1); 10 min linear from 45% to 90% B (Figure 2)
Injection volume: 3000 µL (Figure 1) and 848 µL (Figure 2)
Sample in Figure 1: econazole and miconazole at 10 mg/mL each in DMSO
Sample in Figure 2: nitrendipine and nimodipine at 25 mg/mL each in DMSO
Detection: UV at 270 nm (Figure 1); UV at 290 nm (Figure 2)
Instrument: Waters®, AutoPurification™ system

High pH Conditions:
Column: XTerra® Prep MS C_{18}, 19 × 100 mm, 5 µm
Mobile phase A: 100 mM NH_4HCO_3/water (10/90), pH 10
Mobile phase B: 100 mM NH_4HCO_3/acetonitrile (10/90), pH 10
Flow rate: 18.0 mL/min (Figure 1), 23.9 mL/min (Figure 2)
Gradient: 10 min linear from 0% to 100% B, and hold at 100% B for 5 min (Figure 1); 10 min linear from 50% to 100% B (Figure 2)
Injection volume: 4000 µL (Figure 1) and 1020 µL (Figure 2)

Results

The separations of two structurally related antifungal drugs under low- and high-pH conditions are shown in Figure 1. A total load of 60 mg is achieved on a SunFire™ Prep column at pH 2.3 (as indicated in 1a), while 80 mg is achieved on a XTerra® Prep column at pH 10 (Figure 1b). Similarly, the separations of two calcium blockers on the SunFire™ Prep and XTerra® Prep columns are shown in Figure 2. A comparable mass loading is achieved on SunFire™ Prep column at low pH and the XTerra® Prep column at high pH.

Depending on the conditions required, you have the option of using SunFire™ for low pH, or XTerra® for high pH conditions to separate and purify basic analytes.

Conclusions

New SunFire™ Prep columns provide comparable high mass loading for basic compounds under low pH to XTerra® Prep columns under high pH.

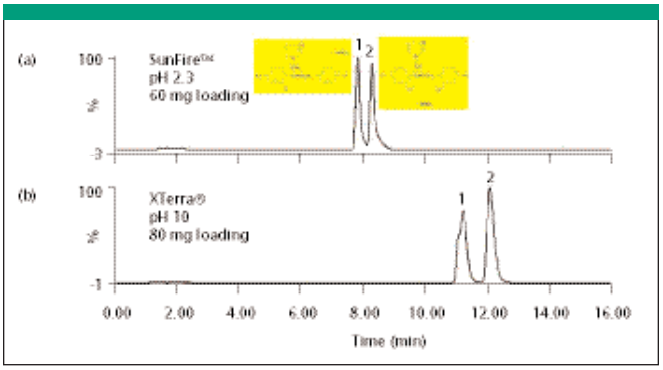

Figure 1: Separation of antifungal drugs under various pHs. (a) SunFire™ Prep C_{18}, 19 × 100 mm, 5 µm at pH 2.3 and (b) XTerra® Prep MS C_{18}, 19 × 100 mm, 5 µm at pH 10. Analytes: 1 = econazole, 2 = miconazole.

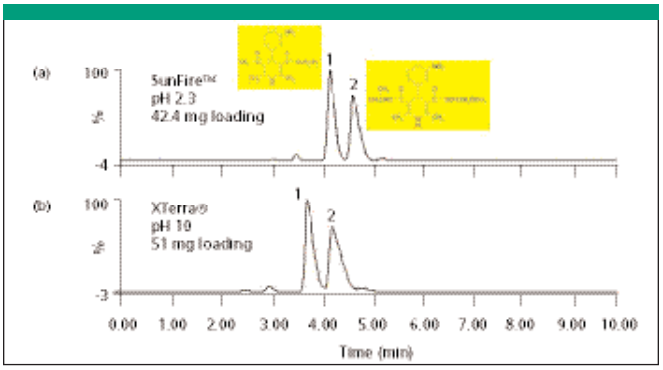

Figure 2: Separation of calcium blockers under various pHs. (a) SunFire™ Prep C_{18}, 19 × 100 mm, 5 µm at pH 2.3 and (b) XTerra® Prep MS C_{18}, 19 × 100 mm, 5 µm at pH 10. Analytes: 1 = nitrendipine, 2 = nimodipine.

References

(1) U.D. Neue, et al. "Differences in preparative loadability between the charged and uncharged forms of ionizable compounds," *J. Chromatogr.*, **1030**, 123-134 (2004).

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