Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

COMPARISON OF FULLY AND SUPERFICIALLY POROUS PARTICLE COLUMNS FOR THE ANALYSIS OF BASIC COMPOUNDS

Kenneth J. Fountain, Jane Xu, Zhe Yin, Pamela C. Iraneta, Diane M. Diehl

Waters Corporation

GOAL

Compare the performance of fully and superficially porous (fused-core™) particle columns.

INTRODUCTION

Since the introduction of high-performance liquid chromatography (HPLC) nearly 40 years ago, many improvements have been made to column stationary phases to achieve faster, more efficient separations. There are several ways of engineering a column in order to achieve these goals, including: increasing packed-bed homogeneity, decreasing intra-particle volume, narrowing particle-size distribution, and decreasing the diffusion distance—this can be achieved, in part, by using smaller particles. It is also thought that this may be achieved by using superficially porous particles that have a solid silica core and porous outer shell [1].

HPLC columns containing superficially porous (sometimes called fused-core) particles have recently gained increasing attention. Though this technology is not entirely new, it has been improved to the point where rapid, highly efficient separations can be achieved for some applications. For neutral compounds, 2.7-µm superficially porous particle columns were reported to have 70-80% of the efficiency of sub-2 µm, fully porous particles, and approximately 50-60% lower backpressure [2].

However a vast majority of the pharmaceutical drug compounds manufactured today are basic in chemical nature, so determining their chromatographic behavior on both fully and superficially porous particle columns has practical implications. Unlike neutral compounds, bases are ionized at low pH, and secondary interactions can occur with the stationary phase, causing asymmetric peak shape and efficiency loss. The goal of this work is to investigate the differences in separation performance between fully and superficially porous particle columns for routine analysis of basic pharmaceutical drug compounds. Major parameters of comparison are chemical stability, peak capacity (P_C), and column efficiency at different mass loads.

EXPERIMENTAL

For chemical stability and mass loading studies, experiments were performed on an ACQUITY UPLC® system equipped with an ACQUITY UPLC PDA detector. To determine chemical stability, a sample test mixture containing 50 µg/mL methyl paraben (prepared in H_2O) was injected every 20 minutes and retention time was monitored. The mobile phase was 0.5% TFA in H_2O. Flow rate was 1.4 mL/min. The column was heated to 60 °C. Injection volume was 2 µL. UV detection was performed at 254 nm using a sampling rate of 20 Hz (fast filter time constant).

The effect of mass load on column efficiency was determined by injecting solutions containing different concentrations of diphenhydramine and amitriptyline (both basic analytes). The isocratic mobile phase was either 32% ACN, 68% 10 mM ammonium formate, pH 3.17 or 57% ACN, 43% 10 mM ammonium bicarbonate, pH 10. Flow rate was 0.5 mL/min. Column temperature was 30 °C. Injection volume was 5 µL, and UV detection was performed at either 240 nm (amitriptyline) or 220 nm (diphenhydramine). Sampling rate was 40 Hz with no time constant. Samples were prepared in the corresponding mobile phase, and thiourea was used as the V_0 marker.

The peak capacity (P_C) of fully and superficially porous particle columns was compared with a standard mixture of basic compounds, and also with a forced degradation sample (glimepiride). These comparisons were all performed on an Alliance® 2695 Separations Module equipped with a 2998 PDA detector. Column dimensions used for these experiments were 4.6 x 75 mm. For separation of the standard mixture of bases, mobile phase A was 10 mM ammonium formate, pH 3. Mobile phase B was 100% ACN. The gradient was from 15–65% B in 13 min, hold at 65% B for 2 min, reset (24 min total run time). The flow rate was 1 mL/min and the column temperature was 30 °C. Injection volume was 10 µL, and UV detection was at 260 nm (5 Hz sampling rate, normal filter response). Peak capacity was measured using the peak width at 13.4% peak height (4σ).

For the forced degradation sample of glimepiride, mobile phase A was 0.1% TFA in H_2O. Mobile phase B was 0.1% TFA in ACN. The gradient was from 5-95% B in 11.36 min, hold at 95% B for 2.28 min, (17 min total run time). The flow rate was 1.9 mL/min and the column temperature was maintained at 40 °C. Injection volume was 18 µL, and UV detection was at 230 nm (20 Hz, normal filter time constant). The degradation sample was generated by first degrading two separate 0.25 mg/mL solutions of glimepiride (in MeOH) with 0.5N NaOH or 0.5N HCl at 60 °C for 90 min, respectively. The solutions were cooled, combined, and 5 µL of glacial acetic acid was added to neutralize the pH.

RESULTS

Chemical Stability

In order to improve the peak shape and retention of basic compounds in RP-HPLC, ion-pairing reagents such as TFA are used. However, these additives can potentially limit the lifetime of RP columns due to ligand hydrolysis. Therefore, the low pH stability of fully porous silica (SunFire™), fully porous ethylene-bridged hybrid (XBridge™), and superficially porous silica (HALO™) particle columns was measured (Fig. 1). Methyl paraben retention was monitored over the course of 20 hours in 0.5% TFA (pH ~ 1.3) at elevated temperature.

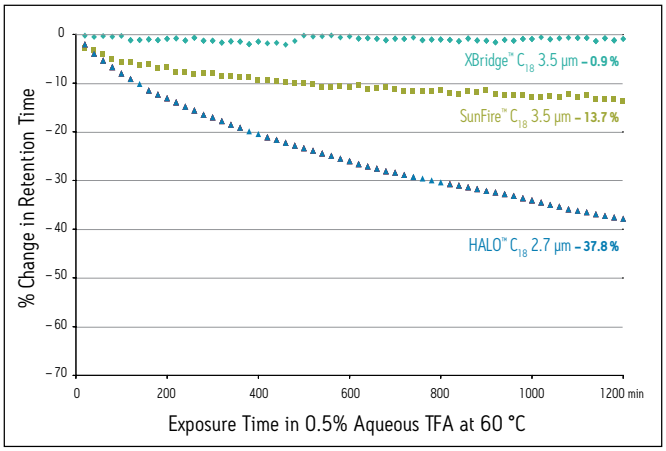

Figure 1. Low-pH chemical stability of XBridge C_{18}, SunFire C_{18}, and HALO C_{18}. Methyl paraben retention was corrected for system retention time. Column dimensions were 2.1 x 50 mm.

It is clear that the HALO column is the least stable at low pH. On this column, methyl paraben retention decreases by almost 40% over the course of the test, most likely due to gradual loss of the C_{18} ligand. Retention loss is also observed on the SunFire column, but to a much lesser extent. The SunFire column is almost three times more stable than the HALO column, due to difunctional bonding of the C_{18} ligand to the particle surface. Negligible retention loss was seen on the XBridge column, due to the fact that it contains hybrid particles with trifunctional bonding. These ethylene-bridged hybrid (BEH) particles have also been shown to have excellent high pH stability [3,4].

Peak Capacity

The ideal separation conditions for basic compounds involve high pH mobile phases. At high pH, bases are not ionized, and do not undergo secondary interactions with residual silanols on the stationary-phase surface. However, traditional silica-based stationary phases are not capable of routine operation at pH values >7 without severely compromising the column lifetime (<50-100 injections). As a result, chromatographers are limited to using silica-based RP columns at low pH conditions. Figure 2 shows the comparison between XBridge, SunFire, and HALO columns for the separation of a standard mixture of basic compounds in a low pH mobile phase.

The peak capacity for XBridge and HALO columns are nearly identical. The SunFire column has almost 60% higher peak capacity than the HALO column under the same conditions, indicating better peak shape for basic compounds at low pH. It is also interesting to note that the SunFire column gives slightly different selectivity (reversal in elution order of diltiazem and prednisone), that may be useful during HPLC method development.

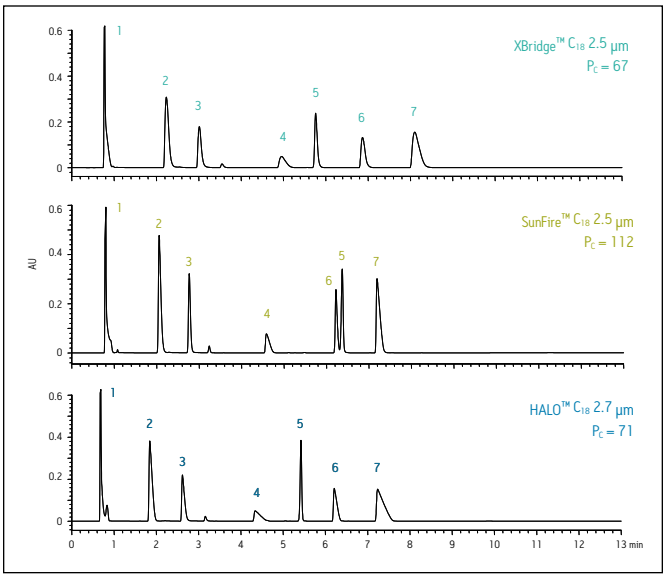

Figure 2. Peak capacity comparison for a standard mixture of basic compounds. Peaks: (1) uracil, (2) pindolol, (3) quinine, (4) labetalol, (5) prednisone, (6) diltiazem, (7) amitriptyline. For conditions [pH 3], see text on p. 2.

Another comparison of peak capacity between these three columns was performed using a forced degradation sample (Fig. 3). Again, the HALO column has the lowest peak capacity for separation of low-level impurities in the degraded sample. The XBridge column has the highest peak capacity (>10% higher than the HALO column), and the SunFire column is the most retentive. This example shows that there is no benefit to using the superficially porous Halo column instead of the fully porous, 2.5 µm XBridge or SunFire columns for "real-life" separations.

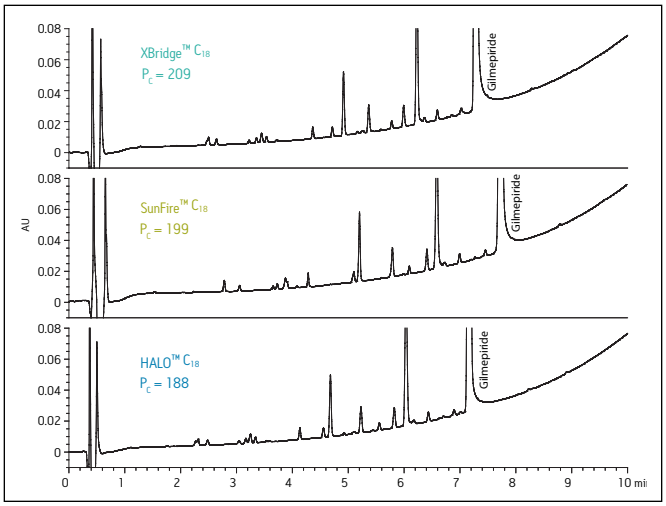

Figure 3. Separation of a glimepiride forced-degradation sample on XBridge, SunFire, and HALO columns. For conditions, see text on p. 3.

Effect of Mass Load on Column Efficiency

Maximizing the amount of sample that may be loaded onto an HPLC column is important for preparative purification and stability indicating methods, where a large amount of an active pharmaceutical ingredient (API) is injected in order to detect low-level impurities in the sample. As more material is loaded onto the column, the peak becomes wider, thereby decreasing column efficiency and resolution. The performance of fully and superficially porous particle columns was evaluated as a function of mass load for two basic compounds at low pH. Figure 4 shows the efficiency for amitriptyline on XBridge, SunFire, and HALO columns.

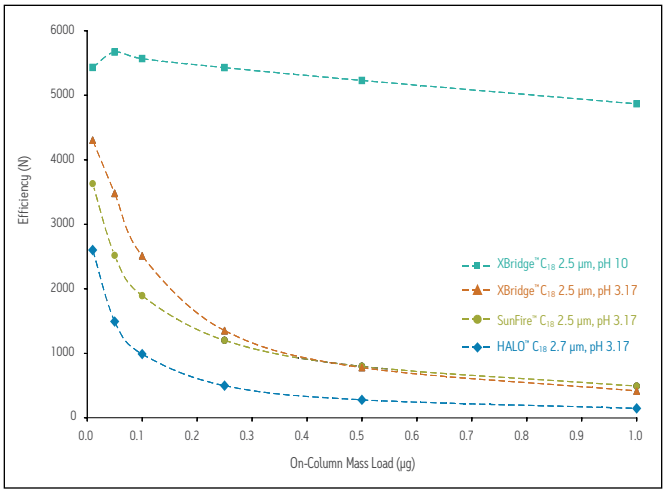

Figure 4. Efficiency of fully and superficially porous columns as a function of mass load. Amitriptyline was the test probe. Efficiency was calculated using the peak width at 4.4% peak height (5σ). Data were corrected for system band spreading.

At low pH, the HALO column has the lowest efficiency at all mass loads. This is expected, in large part because it is superficially porous and has the lowest surface area (150 m^2/g). At lower mass loads, the XBridge column has slightly higher efficiency than the SunFire column. As mass load increases, however, the XBridge and SunFire columns show similar performance. At the highest mass load tested at low pH, both XBridge and SunFire columns had ~ 3x higher efficiency than that of the HALO column.

As described earlier, the best conditions for analyzing basic compounds by HPLC are with high pH mobile phases. While it is not possible to use mobile phases with pH values above 7 with silica-based stationary phases (i.e., SunFire and HALO columns), it is possible to operate XBridge columns at pH values up to 12 with exceptional lifetime. Figure 5 shows the difference in peak shape and signal intensity for amitriptyline run at low and high pH on an XBridge C_{18} column.

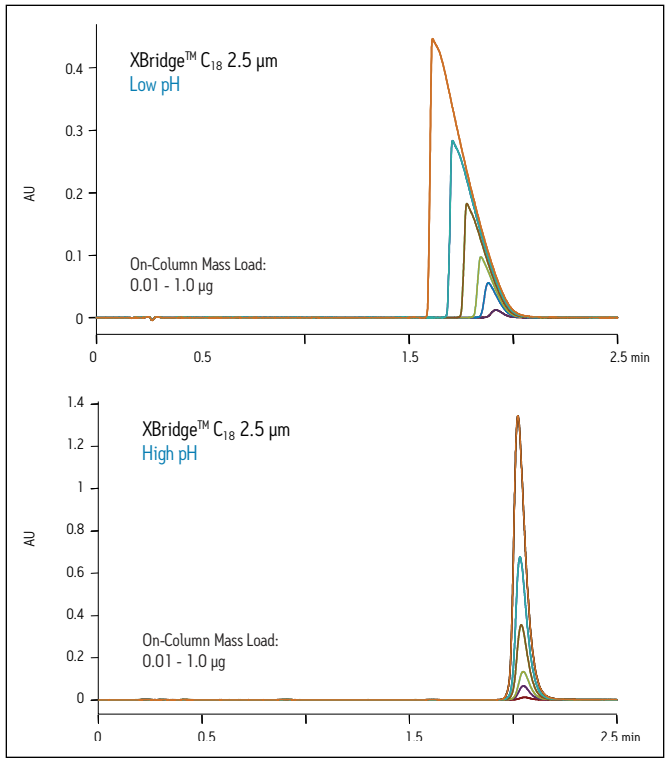

Figure 5. Analysis of amitriptyline in low and high pH mobile phases on an XBridge C_{18} column.

Peak shape is dramatically improved at high pH due to the lack of secondary interactions of the basic solute with the stationary phase under these conditions. As a result, signal intensity for amitriptyline is more than 3x higher than that at low pH mobile phases. Finally, no significant deterioration in column efficiency was observed at high pH, even for higher mass loads (Fig. 4, uppermost curve, black squares). Under these circumstances, the efficiency for the XBridge column was more than 10x higher than that at low pH. The trends shown in Figures 4 and 5 were similar for diphenhydramine (data not shown).

CONCLUSIONS

The benefits of using appropriate fully porous particle HPLC columns with comparable particle size clearly outweigh those of using current superficially porous (fused-core) particle columns for the analysis of basic compounds. Among the columns examined in this study, the fully porous particle columns have superior chemical stability at low pH when compared to that of the superficially porous particle column. Under gradient conditions, both XBridge and SunFire columns have higher peak capacities than the HALO column for a standard mixture of bases, as well as a forced degradation sample of glimepiride. Finally, XBridge and SunFire columns exhibit an approximately 3-fold higher efficiency than that of HALO columns at low pH. At high pH, XBridge columns demonstrated a 10-fold increase in column efficiency over that at low pH at the same mass loads. This dramatic advantage of hybrid particle technology is most important for increasing throughput in preparataive HPLC separations and as well as increasing capacity when developing superior stability indicating methods for impurity detection in APIs.

REFERENCES

1. J.J. DeStefano, T.J. Langlois, J.J. Kirkland, *J. Chromatogr. Sci.* **46** (2008) 254.
2. J.M. Cunliffe, T.D. Maloney, J. Sep. Sci. **30** (2007) 3104.
3. K.J. Fountain, D. Morrison, D.M. Diehl, J. Martin, ***LCGC:*** *The Application Notebook,* (June 200) 2.
4. K.D. Wyndham, J.E. O'Gara, T.H. Walter, K.H. Glose, N.L. Lawrence, B.A. Alden, G.S. Izzo, C.J. Hudalla, P.C. Iraneta, ***Anal. Chem.*** **75** (2003) 6781.

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 43 1 877 18 07, **Australia** 61 2 9933 1777, **Belgium** 32 2 726 1000, **Brazil** 55 11 5094 3788, **Canada** 1 800 252 4752 x2205, **China** 86 10 8586 8899, **CIS/Russia** 7 095 336 7000, **Czech Republic** 420 2 617 1 1384, **Denmark** 45 46 59 8080, **Finland** 358 9 5659 6288, **France** 33 1 30 48 72 00, **Germany** 49 6196 400600, **Hong Kong** 852 29 64 1800, **Hungary** 36 1 350 5086, **India and India Subcontinent** 91 80 2837 1900, **Ireland** 353 1 448 1500, **Italy** 02 265 0983, **Japan** 81 3 3471 7191, **Korea** 82 2 820 2700, **Mexico** 52 55 5200 1860, **The Netherlands** 31 76 508 7200, **Norway** 47 6 384 60 50, **Poland** 48 22 833 4400 **Puerto Rico** 1 787 747 8445, **Singapore** 65 6273 1221, **Spain** 34 93 600 9300, **Sweden** 41 56 676 7000, **Switzerland** 41 56 676 7000, **Taiwan** 886 2 2543 1898, **United Kingdom** 44 208 238 6100
All other countries: Waters Corporation U.S.A. 1 508 478 2000/1 800 252 4752

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

December 2008 720002825EN VW-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com