Fused-Core Particles: A Practical Alternative to Sub-2 Micron Particles

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Abstract

The benefits of sub-2 micron particle size columns have been widely researched and published. The use of these columns on ultrahigh-pressure liquid chromatography (UHPLC) instrumentation may lead to increased efficiencies and higher throughput. However, these instruments may not be readily available to the pharmaceutical chemist. Within the past year, a practical alternative has been introduced which offers increased efficiencies, but at conventional HPLC pressure limitations. These particles are called fused-core particles and are comprised of a 1.7-micron solid core encompassed by a 0.5-micron porous silica layer (d_p = 2.7 micron). The goal for this research was to test these columns for efficiency and robustness utilizing a mixture of Torcetrapib and its relative impurities. Our results indicate that excellent theoretical plates (~14000) were achievable for run times less than 5 min. Compared to the Waters Acquity particles, the fused-core particles achieved approximately 80% of the efficiency but with half the observed backpressure. Our robustness results concluded that these separations were reproducible for at least 500 injections while the % RSD for retention time, theoretical plates, peak asymmetry, and resolution was found to be less than 1%.

Introduction

It is clear that ultrahigh-pressure liquid chromatography (UHPLC) has a role in pharmaceutical analysis and is an emerging technology (1,2,3). UHPLC can improve traditional pharmaceutical method development and optimization through the utilization of sub-2 micron column particle sizes at high linear velocities (4,5,6). While UHPLC has proven to be a viable technology, new instrumentation is required that is capable of handling pressures greater than 6000 psi. In Pfizer Analytical Research and Development located in Groton, CT, UHPLC instrumentation accounts for approximately 5% of all liquid chromatography instrumentation. This can be attributed to the increased cost (~25%) compared to a traditional HPLC and the fact that it is a relatively new technology so legacy instrumentation accounts for roughly 95% of the inventory. As the system lifecycle for legacy instruments sunset, UHPLC systems will replace these retired instruments. However, it may take several years or even a decade to reach complete replacement.

Since the availability of the UHPLC is limited, chromatographers are searching for alternatives. There are several column chemistries commercially available which allow increased efficiencies, but without the pressure cost of sub-2 micron columns. These alternatives include the silica monolithic column and the fused-core column particles [Advanced Materials Technology (AMT), Inc., Wilmington, DE]. Monolithic silica columns are made from a single substrate of porous silica gel and can be operated at higher flow rates without the backpressures associated with sub-2 micron particles. While there have been some recent advances in this field, monoliths are not the focus of this paper (7,8). Instead, this report will evaluate the fused-core column particles.

Unlike sub-2 micron column technology, which utilizes a completely porous particle (i.e., 1.7 µm), the particles developed by AMT are 2.7 µm in diameter consisting of a 0.5 µm porous shell fused to a solid 1.7 µm silica core particle. Recent work by Kirkland and co-authors suggests that these particles exhibit efficiencies that are comparable to sub-2 micron porous particles, but with modest backpressures. This may be due to the narrow particle size distribution and higher density of fused-core particles (9,10). Further, the small diffusion path for the analyte may reduce the resistance to mass transfer (C-term in van Deemter equation) thus allowing operation at higher flow rates with minimal losses in efficiency (11).

In this study, fused-core silica particles were evaluated on both conventional high-performance liquid chromatography (HPLC) and the Acquity UPLC using a sample mixture containing Torcetrapib and its relevant impurities. This report is intended to provide an overview of the applicability of fused-core particles to pharmaceutical separations. There are several recent publications that describe the theoretical aspects of fused-core particles in detail (12,13,14,15).

Experimental

Materials and reagents

All chemicals were ACS reagent grade or HPLC grade. Acetonitrile was purchased from JT Baker. MilliQ (Moshiem,

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France) purified water (> 10mΩ, < 99 ppb TOC) was used throughout the analyses. Chemical Research and Development, Pfizer Inc, (Groton, CT) provided samples of Torcetrapib (Figure I) drug substance and impurities. Impurities consisted of the diastereomer of the bulk material, impurities with strong polar differences, and impurities structurally similar to the bulk material.

System conditions and Van Deemter plots

A mobile phase of water–acetonitrile (30:70) was utilized throughout the experiments. For conventional HPLC, a 10-µL injection of a 0.6 mg/mL sample mixture of Torcetrapib and several impurities spiked in at 0.2% was injected using a variable flow rate of 1.0 mL/min to 3.0 mL/min on a Halo C18, 4.6 mm × 100 mm, 2.7 µm column (MAC-MOD, Chadds Ford, PA). Data was collected at 210 nm utilized at a rate of 2 Hz using a Waters 2695. Similar experiments were conducted utilizing an Acquity UHPLC but on a Halo C18 2.1 mm × 100 mm, 2.7 µm column.

For the Van Deemter analysis, peak efficiencies as a function of linear velocity were plotted. The ranges of linear velocities for the HPLC were 0.1–4.7 mm/s on a Halo C18, 4.6 mm × 100 mm, 2.7 µm column. The analyte was a 0.6 mg/mL solution of Torcetrapib drug substance (Pfizer Inc., Groton, CT) that eluted with a retention factor of approximately 8. Efficiencies were calculated based on the USP tangent plate calculations. The applicable equations for the Van Deemter analysis and the system conditions for Waters Acquity experiments were previously published (1).

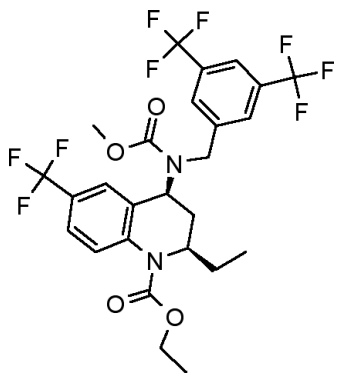

Figure 1. Torcetrapib drug substance (molecular weight: 600.47).

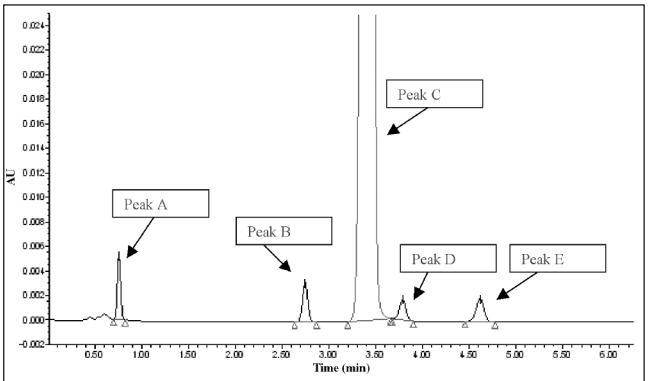

Figure 2. Initial separation of KPSS on a Halo C18, 4.6 mm × 100 mm, 2.7 µm column. (Isocratic 30:70 Water–Acetonitrile, 2.75 mL/min, 35°C, 210 nm, observed pressure = 4500 psi).

Results and Discussion

It is the opinion of this author that UHPLC should be the preferred approach to method development within the pharmaceutical industry. However, the limiting factor is the availability of these instruments to the pharmaceutical chemist. It may take several years or even decades before the conventional HPLC systems are replaced by high-pressure chromatographic systems. This can be attributed to the increased costs (~25%) compared to conventional HPLC instrumentation and the fact that it is still a relatively new technology. Further, conventional HPLC has been proven as a rugged and robust instrument, so chromatographers may be reluctant to make the transition. The goal of this study was to find a practical alternative to sub-2 micron columns, which would yield similar efficiencies but without the pressure cost. The utilization of fused-core column particles on conventional HPLC is a practical approach but requires an extensive evaluation to prove its applicability to the pharmaceutical industry.

Initial observations

For these column evaluations, a key predictive sample set (KPSS) was evaluated consisting of Torcetrapib drug substance and four key impurities: two structurally related impurities, a precursor, and a diastereomer that is difficult to separate. Figure 2 shows that the entire separation was completed within a 5 min run time using a flow rate of 2.75 mL/min. Torcetrapib (Peak C) typically tails on most historic C18 columns, but perfect symmetry (USP tailing = 1.0) was observed with excellent theoretical plates (~ 11000 plates). As a result, baseline resolution was achieved between the key pair of Peak C and Peak D, its diastereomer. The pressure observed was within the boundaries of conventional HPLC even at a flow rate of 2.75 mL/min (~ 4500 psi). Though the flow rate is excessive due to the solvent consumption, the columns are commercially available in 2.1 mm i.d. and 3.0 mm i.d. columns, which would allow the geometric scaling of flow rates to 1 mL/min or less. Table I summarizes some of the key results from this analysis.

In Figure 2, Torcetrapib maintains efficiency with adequate resolution of its impurities even at an excessive flow rate of 2.75 mL/min. DeStefano and co-authors suggest that the thin porous shell of these particles allows larger molecular weight solutes to interact with the stationary phase faster than smaller molecular weight solutes due to the shorter diffusion path. For fused-core

Table I. Theoretical Plates for Torcetrapib and Resolution Between Peaks for Several Flow Rates on a Halo C18, 4.6 mm x 100 mm, 2.7 µm Column

Flow rate	Theoretical plates for Torcetrapib (peak C)	Resolution of key pair (peaks C and D)
2.0	14293	3.33
2.25	13380	3.21
2.5	12428	3.02
2.75	11674	2.96
3.0	10722	2.73

particles, DeStefano found that there is a C-term improvement for compounds with approximate molecular weights of 600 (10). Torcetrapib, which has a molecular weight of 600.47, appears to support these conclusions. An electron micrograph of a fused-core particle is shown in Figure 3.

For the separation that was achieved using the 2.75 mL/min flow rate, a 2.1 mm i.d. column was substituted and the linear velocity was reconstructed by geometrically scaling the flow rate to the equivalent linear velocity. The initial separation was then reproduced utilizing an Acquity UPLC. We observed a ~15% increase in theoretical plates from 11674 to 13545. From these results, it is evident that the fused-core particles yield approximately 80% of the efficiency compared to the Waters Acquity BEH particles (13545 versus 17346), but with half the backpressure (4875 psi versus 10125 psi) (1). This research is consistent with previously published data from Maloney and Cunliffe (16). It is important to note that the Acquity UPLC was specifically engineered to reduce the extra-column effects associated with running separations at high pressures (> 6000 psi). The Acquity UPLC used for this analysis utilized a column switcher component and was found to have a system dwell close to 200 µL. This is approximately a five-fold reduction in system volume as compared to traditional HPLC. Because of the reduced system volumes, the observed theoretical plate increase on the Acquity was expected.

Van Deemter analysis

A van Deemter plot was constructed for the Halo C18, 4.6 mm × 100 mm, 2.7 µm column utilizing Torcetrapib as the analyte over the linear velocity range of 0.1–4.7 mm/s. The plot can be seen in Figure 4. Based on the van Deemter plot (VDP), a reduced plate height of 2.1 microns was observed. The VDP curve falls in between the plate heights observed for conventional HPLC columns (> 3 µm) and UHPLC columns (< 2 µm). It is important to note that the shape of the van Deemter curve for the fused-core particles is similar to that of totally porous particles. Though the column achieves approximately 80% compared to the Waters Acquity particle, it is clear that these columns offer the potential to run separations at higher flow rates without sacrificing resolution and within conventional HPLC pressure boundaries.

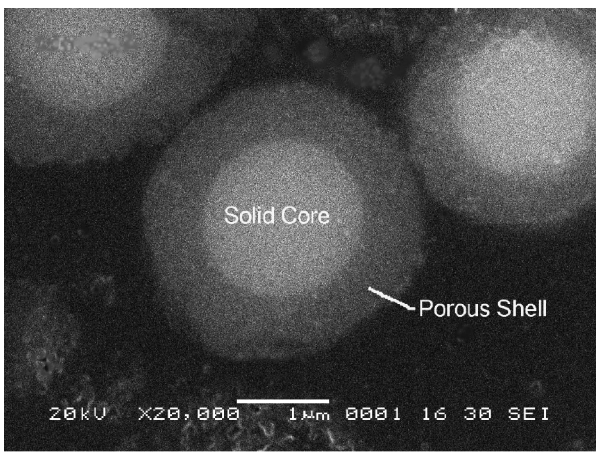

Figure 3. Electron micrograph cross-section of 2.7 µm fused-core particle *(Courtesy of Dr. J. Kirkland, Advanced Materials Technology, Inc.).*

Table II. Summary of Robustness Study on Halo C18, 4.6 mm × 100 mm, 2.7 µm column

Parameter tested	Result
% RSD (Retention time, *n* = 500) for Torcetrapib (peak C)	0.09%
% RSD (Theoretical plates, *n* = 500) for Torcetrapib (peak C)	0.70%
% RSD (Tailing, *n* = 500) for Torcetrapib (peak C)	0.27%
% RSD (Resolution, *n* = 500) for Key Pair (peaks C and D)	0.39%

Robustness studies

A particle stability test was performed on the Halo C18, 4.6 mm × 100 mm, 2.7 µm column by monitoring retention time, theoretical plates, and tailing of Torcetrapib (Peak C). The resolution with its diastereomer (Peak D) was additionally monitored. An overlay of the 1st injection vs. the 500th injection can be seen in Figure 5. The results are summarized in Table II. Based on these results, the Halo C18 would easily meet commercial needs in the pharmaceutical industry.

Though the Halo column particles were designed for use on HPLC, the column hardware and particles are capable of han-

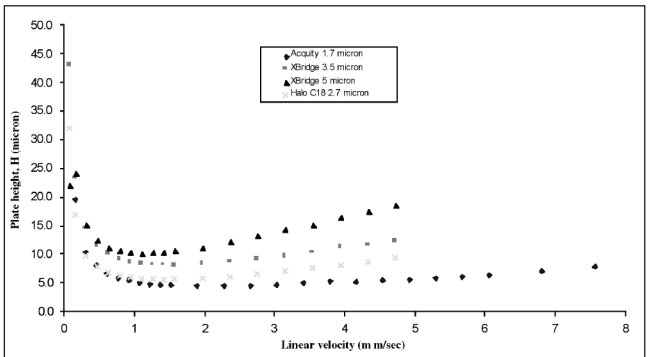

Figure 4. H–*u* plots obtained for Torcetrapib on Acquity, XBridge, and Halo columns. Columns: Acquity UPLC BEH C18, 1.7 µm, 100 mm × 2.1 mm i.d.; XBridge C18, 3.5 µm, 150 mm × 4.6 mm i.d.; XBridge C18, 5 µm, 150 mm × 4.6 mm i.d., Halo C18, 2.7 µm, 100 mm × 4.6 mm i.d. All results have been previously published with the exception of the Halo results (1).

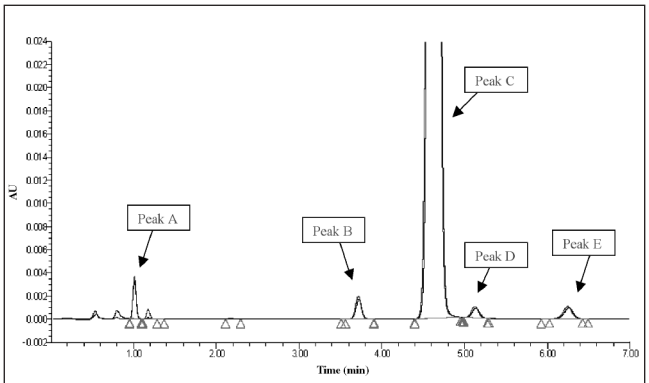

Figure 5. Overlay of 1st injection and 500th injection on a Halo C18, 4.6 mm × 100 mm, 2.7 µm column (isocratic: 30:70 water–acetonitrile, 2.0 mL/min, 35°C, 210 nm, observed pressure: 3270 psi). This figure was previously published (10).

Table III. Summary of Robustness Study on Halo C18, 2.1 mm × 100 mm, 2.7 µm column

Parameter Tested	Result
% RSD (Retention time, *n* = 250) for Torcetrapib (Peak C)	0.06%
% RSD (Theoretical plates, *n* = 250) for Torcetrapib (Peak C)	0.38%
% RSD (Tailing, *n* = 250) for Torcetrapib (Peak C)	0.53%
% RSD (Resolution, *n* = 250) for key pair (Peaks C and D)	2.7%

dling pressures greater than conventional limits (6000 psi). An experiment was performed at 1.6 mL/min on a 2.1 mm × 100 mm, 2.7 µm column for 250 injections utilizing a Waters Acquity system. The observed pressure for this experiment was 12525 psi yet the results were reproducible even at these high pressures. These results are summarized in Table III. The increase in % RSD for resolution between Peak C and D at these higher pressures compared to conventional pressure is due to peak shape irregularities for Peak D. In this experiment, the % RSD for theoretical plates for Peak D was found to be approximately 10%. This is slightly evident in an overlay of the 1st and 250th injection, which has been previously published (10). Nevertheless, the data found in Table II and Table III illustrates the excellent stability of the fused-core particles even beyond the recommended pressure limit of 9000 psi. This may be due to the ability to pack homogenous beds as a result of the narrow particle size distribution (10).

Conclusion

It is clear that the utilization of fused-core particles offers a practical alternative to sub-2 micron particles. Columns packed with these particles are capable of running fast and efficient separations at conventional pressure limits. Particle stability tests concluded that separations were reproducible for at least 500 injections while the % RSD for retention time, theoretical plates, peak asymmetry, and resolution was found to be less than 1%. As a result, these particles would easily meet the commercial needs of a pharmaceutical chemist. The fact that this column can be utilized at even higher pressures (~ 9000 psi) allows it to be an excellent choice for achieving high peak efficiencies while maintaining low backpressures (16).

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