Fast HPLC Using Fused-Core Particle Technology for Rapid Screening of Pharmaceutical Compounds

By: **Hillel Brandes**, *Reporter EU Volume 30*

Hillel Brandes, Craig Aurand and Wayne K. Way

Introduction

HPLC is critical to the discovery, development and eventual commercialization of pharmaceutical products. HPLC is the benchmark analytical method in the pharmaceutical industry due to its ability to score such high marks in analytical validation characteristics; including accuracy, precision, limit of detection, specificity, linearity and range, and ruggedness. No other analytical techniques can consistently score high in all characteristics on compounds and matrices that are of interest to the pharmaceutical industry.

A major benefit of the Fused-Core particle is the small diffusion path. Furthermore, it has been generally accepted that a typical HPLC analysis takes 15 – 30 minutes, with some taking as long as an hour. When multiplied by the number of samples to be analysed either in discovery or product release, the total instrument time required is staggering. This overwhelming amount of instrument time has resulted in a growing number of instruments, around-the-clock analysis, and a push for faster methods.

Fast HPLC, using short columns (3 –10 cm) packed with small particles (<3 µm) and high fl ow rates has recently become an effective means to reduce analysis time. This is primarily due to the improved quality of sub-3 µm particle columns and the introduction of new instrumentation to meet the requirements of higher column backpressure and low instrument dispersion. The reasons for using sub-3 µm particle columns in fast HPLC are evident by examining Van Deemter plots for various particle sizes. The smaller particles yield lower HETP or higher effi ciency per unit length. Furthermore, the optimum fl ow rate is higher for smaller particles. Smaller particle columns have less effi ciency loss at high fl ow rates because mass transfer is less sensitive to velocity changes as illustrated by “fl atter” Van Deemter plots.

Unfortunately, column backpressure increases at a greater rate than column effi ciency as you decrease particle size. This increase in backpressure is so great for sub-2 µm particle columns that they are practically unusable using standard HPLC systems, such as Agilent® 1100. For this reason, a particle with high effi ciency plus low backpressure would be a more suitable candidate for Fast HPLC.

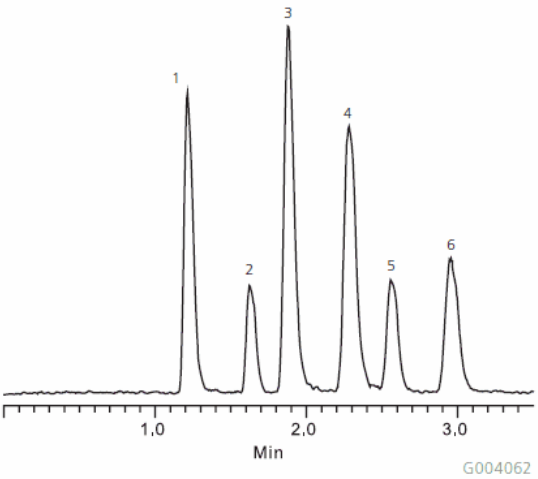
Fused-Core Particle Technology Delivers High Efficiency at Low Backpressure

Ascentis Express columns provide a breakthrough in fast HPLC performance. Based on Fused-Core particle technology, Ascentis Express provides the high-efficiency-based benefits of sub-2 µm particles, but at much lower backpressure. Due to the high efficiencies at low back pressures, Ascentis Express can provide Fast HPLC chromatography, which was previously unattainable on commercial LC systems.

The Fused-Core particle consists of a 1.7 µm solid core and a 0.5 µm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening. In fact, Ascentis Express columns are able to achieve efficiencies similar to that obtained with sub-2 µm particle columns, even though the backpressures are only 50% of that achieved under similar conditions with sub-2 µm particles. This means that Ascentis Express can turn almost any LC system into a fast HPLC workhorse for your lab.

Fast HPLC of Pharmaceutical Compounds

Shown in **Figures 1 – 3** on page 4 are the chromatograms for the separation of three sets of closely related pharmaceutical compounds. These examples include both basic and neutral, as well as, polar and nonpolar compounds. While each example utilizes 2.1 mm I.D. columns, three different flow rates and three unique mobile phase conditions are presented to demonstrate the versatility of fast HPLC with Fused-Core particle columns.



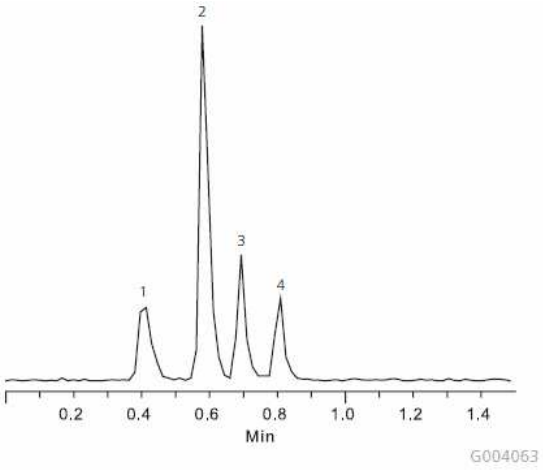
Column:	Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
Instrument:	Jasco X-LC
Mobile phase A:	100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide)
Mobile phase B:	water
Mobile phase C:	methanol
Online mixing:	A:B:C = 10:30:60
Flow rate:	0.3 mL/min

Temp.:	55 °C
Det.:	Thermo LCQ Advantage; ESI(+), m/z 250-320
Injection:	1 µL

1. Nordoxepin
2. Desipramine
3. Nortiptyline
4. Doxepin
5. Norclomipramine
6. Imipramine

Figure 1. TCAs sur Ascentis Express (product shown: **53823-U**)

Shown in **Figure 1** is the separation of six tricyclic antidepressants (TCAs). The separation of these closely related compounds was performed under isocratic mobile phase conditions with mass spectrometric (MS) detection. Baseline resolution was achieved with a total separation time of 3 minutes; demonstrating not only the potential speed of the Ascentis Express columns, but also the resolving power. Note the MS compatible mobile phase and flow rate. Furthermore, the use of 2.1 mm I.D. columns provides a reduction in solvent consumption compared to typical flow rates for 4.6 mm I.D. or monolithic columns.

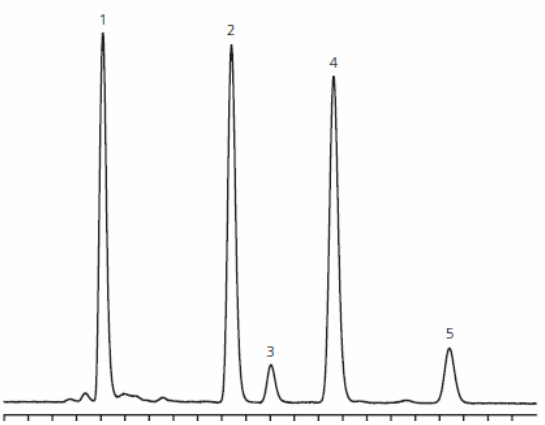


Column:	Ascentis Express C18, 5 cm x 2.1 mm ID (53822-U)
Instrument:	Agilent 1100
Mobile phase A:	0.1% acetic acid in water
Mobile phase B:	0.1% acetic acid in acetonitrile
Online mixing:	A:B = 74:26
Flow rate:	0.2 mL/min
Temp.:	35 °C
Det.:	ABI 3200 QT; ESI(+), MS/MS
Injection:	1 µL

1. Atenolol
2. Pindolol
3. Timolol
4. Metoprolol

Figure 2. β-Bloquants sur Ascentis Express (product shown: **53822-U**)

Data in **Figure 2** further illustrates the speed in which closely related compounds can be resolved using the Fused-Core particle. In this example, four β-blockers are resolved in less than one minute under isocratic conditions utilizing MS detection. While a 10 cm column was utilized for the TCAs separation, a 5 cm column was used for the β-blockers example.



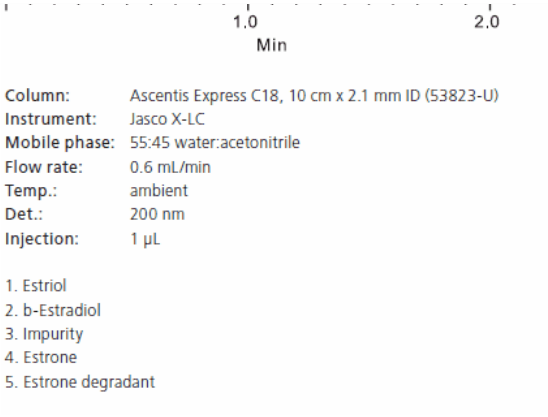


Figure 3. Steroides sur Ascentis Express (product shown: 53823-U)

The separation of three steroids, as well as a related impurity and degradant, is shown in **Figure 3**. A high mobile phase flow rate of 0.6 mL/min was utilized and is suitable for Ascentis Express columns due to the Van Deemter curve associated with these columns. Isocratic mobile phase conditions were utilized, as well as UV detection at 200 nm, a common detection wavelength for impurity profiling. Again, baseline resolution was achieved for all compounds with a total run time of less than two minutes. It should be noted that the isocratic conditions used in these examples further enhances sample throughput versus gradient conditions due to no need for column re-equilibration. With a backpressure of just 4500 psi, this analysis could be performed on almost any HPLC system. A similar separation was attempted using a sub-2 µm particle column, but was not possible given the same instrument constraints put on the Ascentis Express column.

Conclusions

Fast HPLC is increasingly becoming a reality for all phases of pharmaceutical product development, from discovery to production. Ascentis Express HPLC columns provide the high efficiency and low backpressure that make it a suitable candidate for fast HPLC using conventional instruments, such as an Agilent 1100. These columns also provide proportionate performance improvements on new, ultra-high pressure systems compared to sub-2 µm particles because the higher efficiency per unit backpressure characteristic of the innovative Fused-Core particle is a fundamental technology advance.

Materials

Product #	Description	Particle Size	Length × I.D.	Add to Cart
53823-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	10 cm × 2.1 mm	pricing
53814-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	10 cm × 3 mm	pricing
53827-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	10 cm × 4.6 mm	pricing
53793-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	15 cm × 10 mm	pricing
53825-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	15 cm × 2.1 mm	pricing
53816-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	15 cm × 3 mm	pricing
53829-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	15 cm × 4.6 mm	pricing
53799-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	2 cm × 2.1 mm	pricing
53802-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	3 cm × 2.1 mm	pricing
53805-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	3 cm × 3 mm	pricing
53818-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	3 cm × 4.6 mm	pricing
582711-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	5 cm × 1 mm	pricing
53822-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	5 cm × 2.1 mm	pricing
53811-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	5 cm × 3 mm	pricing
53826-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	5 cm × 4.6 mm	pricing
53804-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	7.5 cm × 2.1 mm	pricing
53812-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	7.5 cm × 3 mm	pricing
53819-U	Ascentis® Express C18, 2.7 Micron HPLC Column	2.7 µm	7.5 cm × 4.6 mm	pricing