

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

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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 as great as an hour. When multiplied by the number of samples to be analyzed 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 flow 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 efficiency per unit length. Furthermore, the optimum flow rate is higher for smaller particles. Smaller particle columns have less efficiency loss at high flow rates because mass transfer is less sensitive to velocity changes as illustrated by "flatter" Van Deemter plots.

Unfortunately, column backpressure increases at a greater rate than column efficiency 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 efficiency 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 that 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 non-polar 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.

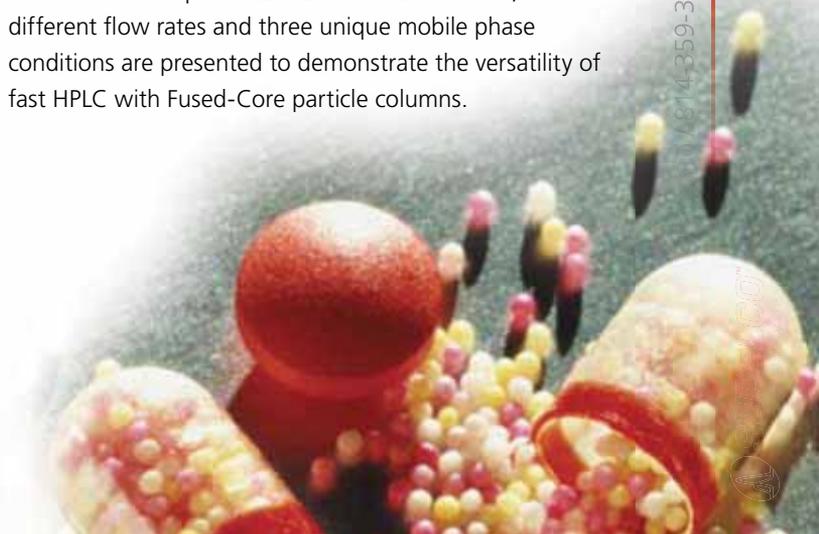


Figure 1. TCAs on Ascentis Express

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
 temperature: 55 °C
 flow rate: 0.3 mL/min
 detection: Thermo LCQ Advantage; ESI(+), m/z 250-320
 injection volume: 1 µL

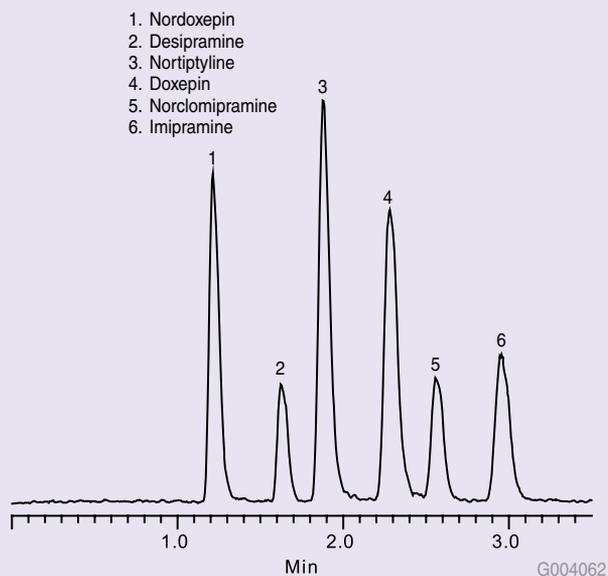


Figure 3. Steroids on Ascentis Express

column: Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
 instrument: Jasco X-LC
 mobile phase: 55:45 water:acetonitrile
 temperature: ambient
 flow rate: 0.6 mL/min
 detection: 200 nm
 injection volume: 1 µL

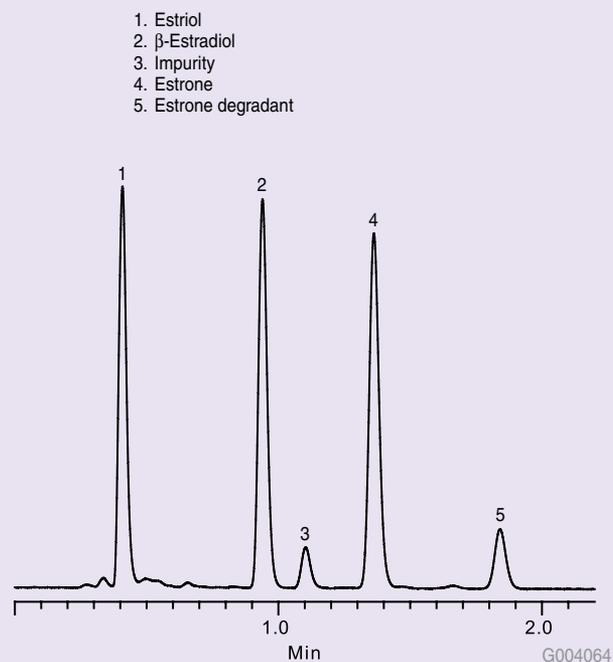
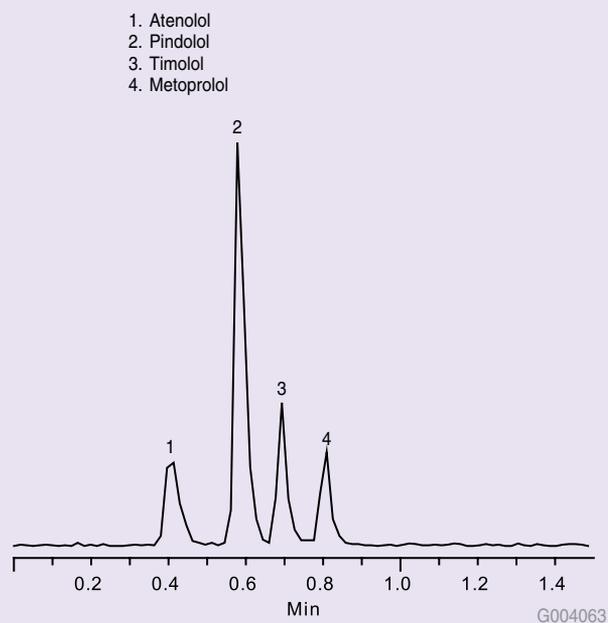


Figure 2. β-Blockers on Ascentis Express

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
 temperature: 35 °C
 flow rate: 0.2 mL/min
 detection: ABI 3200 QT; ESI(+), MS/MS
 injection volume: 1 µL



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.

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.

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 runtime 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.

+ Featured Products

ID (mm)	Length (cm)	Ascentis Express C18	Ascentis Express C8
Ascentis Express Columns			
2.1	3	53802-U	53839-U
2.1	5	53822-U	53831-U
2.1	7.5	53804-U	53843-U
2.1	10	53823-U	53832-U
2.1	15	53825-U	53834-U
3.0	3	53805-U	53844-U
3.0	5	53811-U	53848-U
3.0	7.5	53812-U	53849-U
3.0	10	53814-U	53852-U
3.0	15	53816-U	53853-U
4.6	3	53818-U	53857-U
4.6	5	53826-U	53836-U
4.6	7.5	53819-U	53858-U
4.6	10	53827-U	53837-U
4.6	15	53829-U	53838-U

! Related Information

For more information on Ascentis Express columns, request T407044 (JHD) or visit sigma-aldrich.com/express

Did you know...?

Selecting the Right Buffer

A partial list of common buffers and their corresponding useful pH range is supplied. Perhaps the most common buffer in HPLC is the phosphate ion. Although, with the growth of LC-MS, volatile buffers such as TFA, acetate, formate, and ammonia are becoming more frequently used. Remember, the purpose of a buffer in the mobile phase is to inhibit a pH change in the mobile phase after the introduction of a sample. When developing a method, it is important to select a mobile phase with a final pH at least one pH unit away from any analytes pK value. As a rule of thumb, one should work within a ± 1 pH unit of the buffer pKa. Typical buffer concentrations for HPLC tend to be 10-100 millimolar level.

Buffer	pKa @ 25 °C	Useful pH Range
Trifluoroacetic acid (TFA)	0.5	<1.5
Phosphate 1	2.1	1.1 - 3.1
Formate	3.8	2.8 - 4.8
Acetate	4.8	3.8 - 5.8
Phosphate 2	7.2	6.2 - 8.2
Ammonia	9.2	8.2 - 10.2
Phosphate 3	12.3	11.3 - 13.3

Guidelines for Preparing Mobile Phases

It should be understood that slight variations in pH and buffer concentration could have a dramatic affect on the chromatographic process; consistent and specific techniques should be a regular practice in the preparation of mobile phases. A common practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of buffer. The pH of the solution should be adjusted, if necessary, and then dilute to final volume of water prior to adding or blending of organic solvents. Then, add a volumetrically measured amount of organic solvent to obtain the final mobile phase. Thorough blending, degassing, and filtering prior to use is also recommended.

To view a listing of suitable HPLC and LC-MS additives and solvents, visit sigma-aldrich.com/lc-ms-solvents