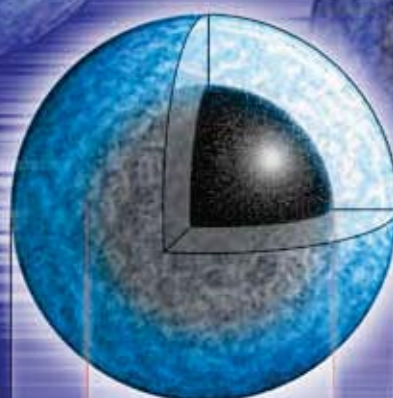
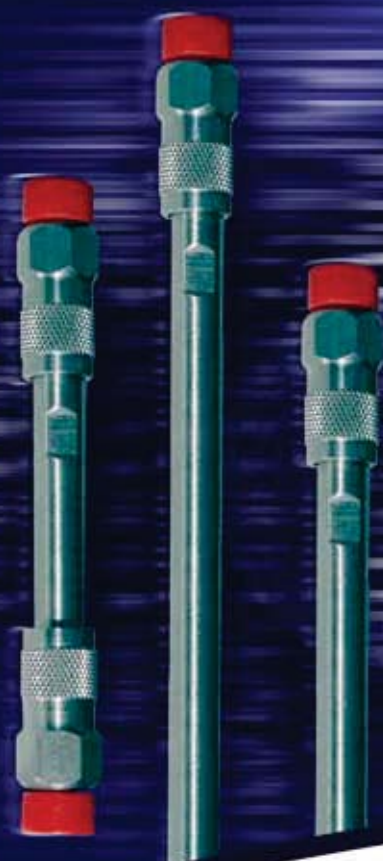


# Ascentis Express HPLC Columns with Fused-Core Technology

Extreme Performance on **Any** LC System



- Hyper-Fast Separations
- High Definition Resolution
- Super-Sensitive
- Super-Rugged



# A Breakthrough in HPLC Performance

## The Fused-Core Advantage

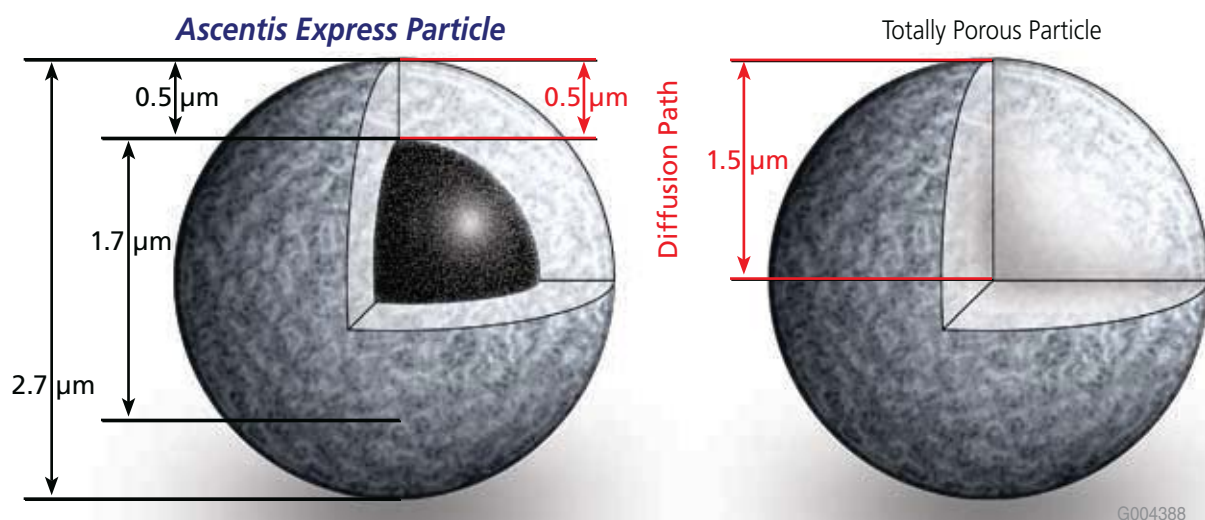
Ascentis Express provides the high speed and high efficiency of sub-2  $\mu\text{m}$  particles, but at approximately half the backpressure for the same column length. This lower pressure means that Ascentis Express can be run on conventional HPLC and LC-MS systems, as well as mid-pressure, UPLC™ and other ultra-high pressure systems. Lower pressure also means longer columns can be used for additional resolving power. Ascentis Express offers these benefits over sub-2  $\mu\text{m}$  particles, along with excellent column lifetime.

At the heart of Ascentis Express is the 2.7  $\mu\text{m}$  Fused-Core™ particle which comprises a 1.7  $\mu\text{m}$  solid core and a 0.5  $\mu\text{m}$  porous shell (Figure 1). Compared to totally porous particles, the Fused-Core particles have a much

shorter diffusion path because of the solid core. This partial porosity reduces axial dispersion of solutes and minimizes peak broadening. Other features, such as a very tight particle size distribution and high packing density, result in Ascentis Express columns that are capable of 240,000 N/m. This is comparable to the efficiency of sub-2  $\mu\text{m}$  particle columns and nearly twice the efficiency possible with 3  $\mu\text{m}$  particles.

While the Ascentis Express efficiency is as high as sub-2  $\mu\text{m}$  columns, the larger particle size delivers approximately half the backpressure for the same column dimensions and conditions. This allows Ascentis Express to turn any HPLC system into an extreme performance workhorse for your lab

Figure 1. Fused-Core Structure of Ascentis Express Compared to Totally Porous Particles



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# Ascentis Express FAQs

## What is unique about Ascentis Express?

Ascentis Express columns provide a breakthrough in HPLC performance. Based on Fused-Core particle technology, Ascentis Express provides the benefits of sub-2  $\mu\text{m}$  particles but at much lower backpressure. These benefits include the capability of providing fast HPLC and higher resolution chromatography. The Fused-Core particle consists of a 1.7  $\mu\text{m}$  solid core and a 0.5  $\mu\text{m}$  porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5  $\mu\text{m}$ ) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

## What phases are available in Ascentis Express?

Currently, C18, C8, RP-Amide, and HILIC (bare silica) phases are available for Ascentis Express.

## When are additional phases expected?

Additional phases are being developed. The best way to track new products is to visit [sigma-aldrich.com/express](http://sigma-aldrich.com/express) for the latest updates.

## Can I use Ascentis Express on any type of HPLC system?

Ascentis Express HPLC columns are capable of use on standard HPLC systems as well as UHPLC systems. Columns are packed in high pressure hardware capable of withstanding the pressures used in UHPLC systems.

## Is there anything I need to do to my HPLC system to use Ascentis Express?

Nothing special is required to use Ascentis Express HPLC columns. To obtain the full benefits of Ascentis Express, one should minimize dispersion or instrument bandwidth in the HPLC system (tubing, detector flow cell) as well as confirm the detector response system is set at a fast level. For more information, request Guidelines for Optimizing Systems for Ascentis Express Columns (T407102) or visit [sigma-aldrich.com/express](http://sigma-aldrich.com/express) and download.

## How can I measure my instrument bandwidth (IBW) and determine what columns can be used with minimal efficiency loss created by too much internal instrument volume?

For simple instructions on how to measure IBW, request *Guide to Dispersion Measurement* (T408143) or visit our website [sigma-aldrich.com/express](http://sigma-aldrich.com/express) and download.

## Do I need special fittings and tubing to connect Ascentis Express columns?

While operating pressures may not exceed the 400 bar (6,000 psi) capability of your traditional instruments, sustained pressures of about 200 bar (3,000 psi) will exceed the recommended pressure for conventional PEEK tubing and fittings at the column inlet. We recommend changing to stainless steel fittings in all high pressure locations and have designed special low-dispersion connectors (pg. 14) that will stay tight at pressures of 1,000 bar (15,000 psi) or greater, even when elevated column temperatures are employed.

## Can I use Ascentis Express on a UHPLC system?

Yes. Ascentis Express columns are packed in a way making them suitable for these ultra high pressure instruments. In fact, Ascentis Express outperforms sub-2  $\mu\text{m}$  columns on many applications since Ascentis Express provides the benefits of sub-2  $\mu\text{m}$  particles but at much lower backpressure.

## Can Ascentis Express columns be used for LC-MS?

Ascentis Express Fused-Core particles were designed with LC-MS in mind. Even extremely short column lengths exhibit sufficient plate counts to show high resolving power. The flat van Deemter plots permit resolution to be maintained at very high flow rates to maximize sample throughput. All Ascentis stationary phases have been evaluated for MS compatibility during their development, and the Express phases are no exception. A bonus of Ascentis Express columns for high throughput UHPLC and LC-MS is that they are extremely rugged and highly resistant to plugging, a very common failure mode for competitor columns.

## What flow rate should I use with Ascentis Express columns?

Based on the minimum in the van Deemter curves, higher flows than 5  $\mu\text{m}$  particle columns are required in order to maximize Ascentis Express column efficiency.

Ascentis Express HPLC Column ID	Suggested Starting Point for Flow Rate
4.6 mm I.D.	1.6 mL/min
3.0 mm I.D.	0.8 mL/min
2.1 mm I.D.	0.4 mL/min

## Are guard columns available?

Guard columns packed with Ascentis Express are currently not available. Ascentis Express columns are rugged and almost all users prefer operation of Ascentis Express columns without a guard column. If you would like to use a guard column, we recommend the Ascentis guard columns.



# Hyper-Fast Separations

## Double the Speed

- Designed for high flow rates
- Half the backpressure of sub-2  $\mu\text{m}$  particles

Compared to sub-2  $\mu\text{m}$  particles, the 2.7  $\mu\text{m}$  Ascentis Express particles generate approximately half the backpressure. This permits both longer columns and faster flow rates.

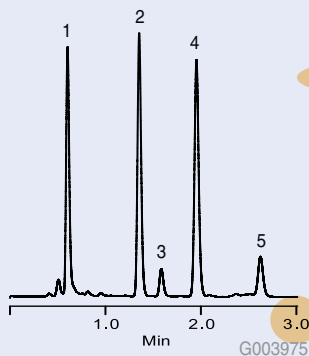
Figure 2 shows the separation of a steroid mixture on Ascentis Express (top) and a conventional sub-2  $\mu\text{m}$  column (lower) of the same dimensions. Because higher flow rates on Ascentis Express – even doubled in this

**Figure 2. Hyper-Fast Separations on Ascentis Express: Twice the Speed at Equivalent Pressure**

columns: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7  $\mu\text{m}$  particles (53823-U) and sub-2  $\mu\text{m}$  particle column (same dimensions)  
 mobile phase: 49:51 or 55:45, water:acetonitrile  
 flow rate: 0.4 or 0.2 mL/min.  
 temp.: ambient  
 det.: UV at 200 nm  
 injection: 1  $\mu\text{L}$

### Ascentis Express C18

0.4 mL/min flow rate

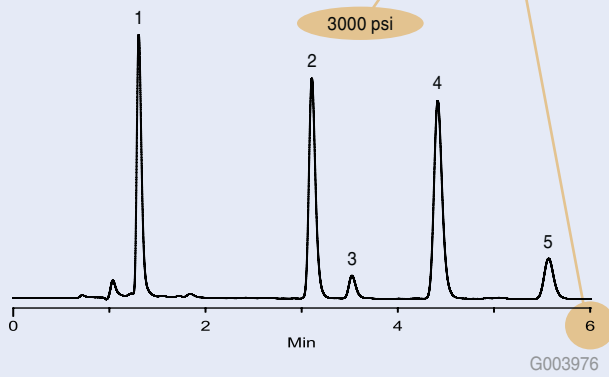


1. Estradiol
2.  $\beta$ -Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

**TWICE THE SPEED AT EQUAL PRESSURES**

### C18 Sub-2 $\mu\text{m}$

0.2 mL/min flow rate



example – generate similar backpressure, hyper fast separations are possible that have efficiency and resolution equal to the sub-2  $\mu\text{m}$  particle column.

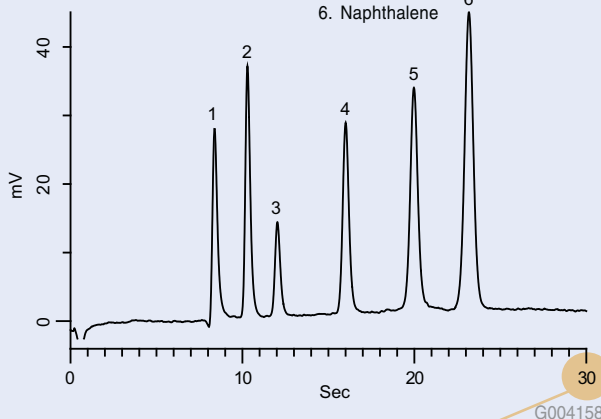
Shown in Figure 3 is a comparison against a traditional 15 cm, 5  $\mu\text{m}$  column and a 5 cm, Ascentis Express. The chromatograms further illustrate the high-speed capabilities of Ascentis Express at backpressures manageable by all HPLC systems. High flow rates are quite amenable to Ascentis Express HPLC columns due to the Fused-Core particle.

**Figure 3. Hyper-Fast Separations on Ascentis Express: Eight Times the Speed of Traditional Columns**

column: Ascentis Express C18, 5 cm x 3.0 mm I.D. and 5  $\mu\text{m}$  particle column, 15 cm x 3.0 mm I.D.  
 mobile phase: 31:69, water:acetonitrile  
 flow rate: 1.2 mL/min or 0.4 mL/min  
 temp.: 35° C  
 inj.: 0.5  $\mu\text{L}$

### Ascentis Express C18

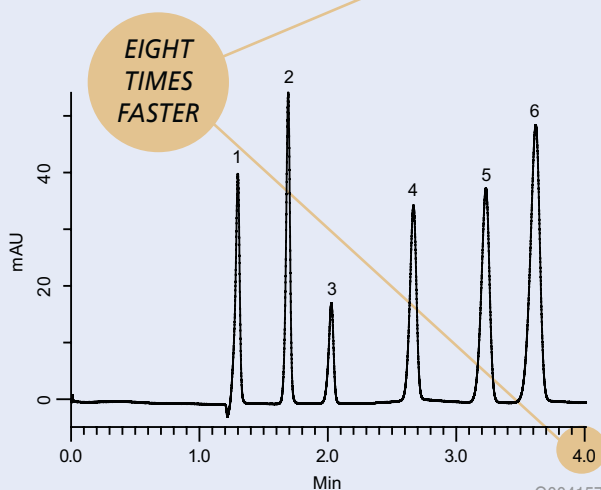
1.2 mL/min flow rate



1. Uracil
2. Phenol
3. Acetophenone
4. Benzene
5. Toluene
6. Naphthalene

### C18 5 $\mu\text{m}$

0.4 mL/min flow rate



**EIGHT TIMES FASTER**

# High Definition "HD"-Resolution

## Double the Efficiency

- Short analyte diffusion path
- Twice the efficiency of 3  $\mu\text{m}$  particles
- Longer columns permit doubling the plates over sub-2  $\mu\text{m}$  particles

Compared to conventional 3  $\mu\text{m}$  and 5  $\mu\text{m}$  particles, Ascentis Express HPLC columns provide sharper peaks under the same conditions. By simply swapping in an Ascentis Express HPLC column of the equivalent dimensions to your current 3  $\mu\text{m}$  and 5  $\mu\text{m}$  particle HPLC columns, an improvement in resolution can be achieved. This improvement is shown in Figure 4. Note: Remember Ascentis Express HPLC column recommended flow rates are higher than that for conventional 3  $\mu\text{m}$  and 5  $\mu\text{m}$  particles.

Ascentis Express and sub-2  $\mu\text{m}$  columns of the same dimensions give approximately the same number of

theoretical plates (efficiency). However, because Ascentis Express columns are more permeable and exhibit half the backpressure, you can use longer columns for even more resolving power. The high backpressure generated by the sub-2  $\mu\text{m}$  particles precludes the use of longer columns, even on ultra-high pressure systems under ambient conditions.

An example of the HD-Resolution is shown in Figure 5 where the additional theoretical plates on the 10 cm Ascentis Express column provided significantly better resolution of  $\beta$ -estradiol and the impurity compared to the 5 cm sub-2  $\mu\text{m}$  column at comparable backpressures.

Figure 4. HD-Resolution on Ascentis Express: Sharper Peaks than Traditional Columns

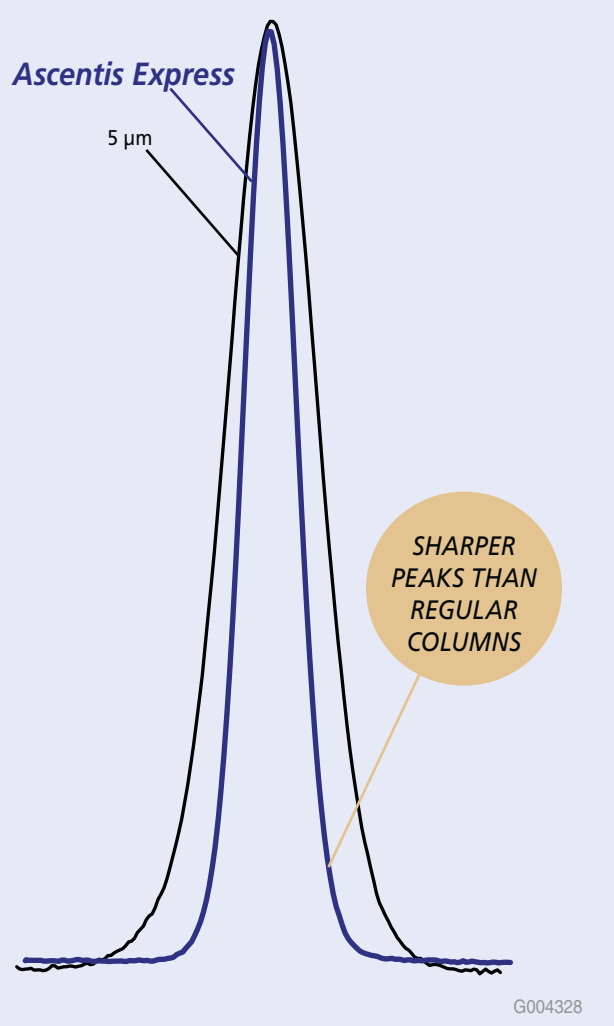


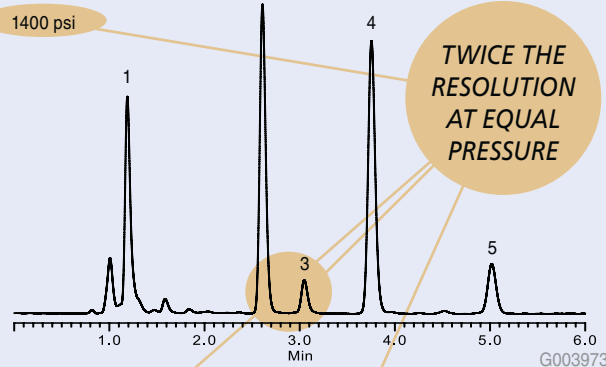
Figure 5. HD-Resolution on Ascentis Express Compared to Sub-2  $\mu\text{m}$  Columns

columns: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7  $\mu\text{m}$  particles (53823-U) and sub-2  $\mu\text{m}$  particle column, 5 cm x 2.1 mm I.D.  
mobile phase: 55:45 or 54:46, water:acetonitrile  
flow rate: 0.2 mL/min.  
temp.: ambient  
det.: UV at 200 nm  
injection: 1  $\mu\text{L}$

1. Estradiol
2.  $\beta$ -Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

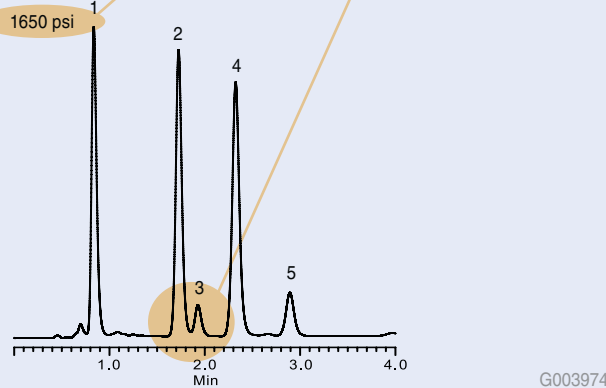
### Ascentis Express C18

10 cm length



### C18 Sub-2 $\mu\text{m}$

5 cm length





# Super-Sensitive

## High Sample Loading Capacity and Signal/Noise for Trace Analysis

- High column efficiency for high S/N
- High sample loading from thick, porous shell layer

Trace analysis benefits from high column efficiency. Efficient peaks are taller and provide higher S/N ratios. As discussed in earlier sections of this brochure, the Ascentis Express columns can provide higher efficiency than any traditional particle. The added sensitivity of the higher efficiency Ascentis Express particles is visualized as the "sensitivity gap" in Figure 6.

Figure 6. Higher Efficiency of Ascentis Express Provides Better Sensitivity than Traditional Columns

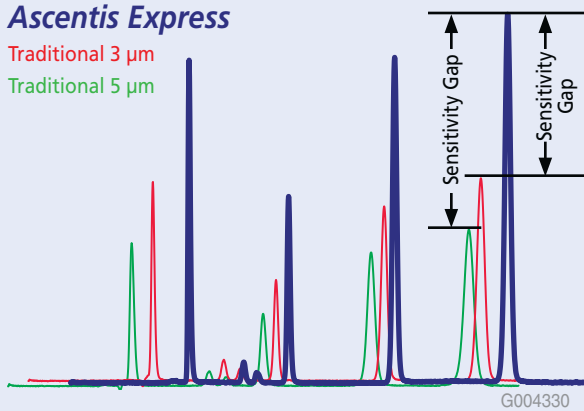
columns: as indicated; 10 cm x 4.6 mm I.D.  
 mobile phase : acetonitrile:water  
 flow rate: 1.8 mL/min  
 temp.: 35° C  
 det.: 254 nm  
 injection: 5 µL

1. Uracil
2. Acetophenone
3. Benzene
4. Toluene

**BETTER SENSITIVITY THAN REGULAR COLUMNS**

### Ascentis Express

Traditional 3 µm  
 Traditional 5 µm



Although they have a solid core, the 0.5 µm-thick "shell" of the Fused-Core particles provides roughly 75% of the surface area as a totally porous particle of the same diameter. Only the pores with very long diffusion paths are fused in the Ascentis Express HPLC columns. The resulting particles have effective surface areas of ~225 m<sup>2</sup>/g; comparable to totally porous particles. The higher surface area gives higher sample loading capacity compared to sub-2 µm particles, as evidenced by the symmetry vs. concentration relationship in Figure 7. Above 5 ppm, the sub-2 µm experiences sample overload and subsequent loss of peak shape.

Figure 7. Higher Loading Capacity of Ascentis Express Compared to Sub-2 µm Particles

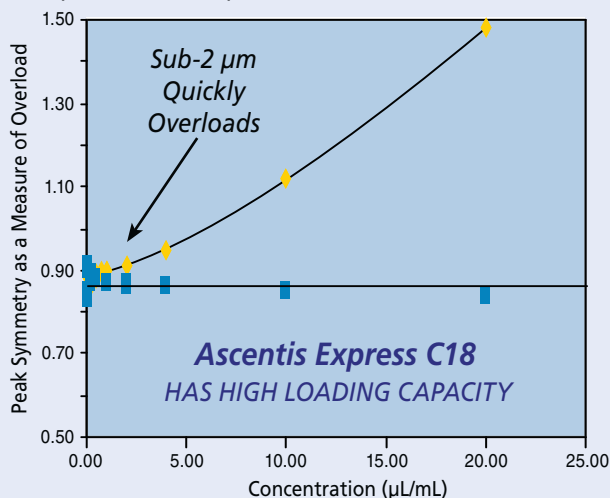
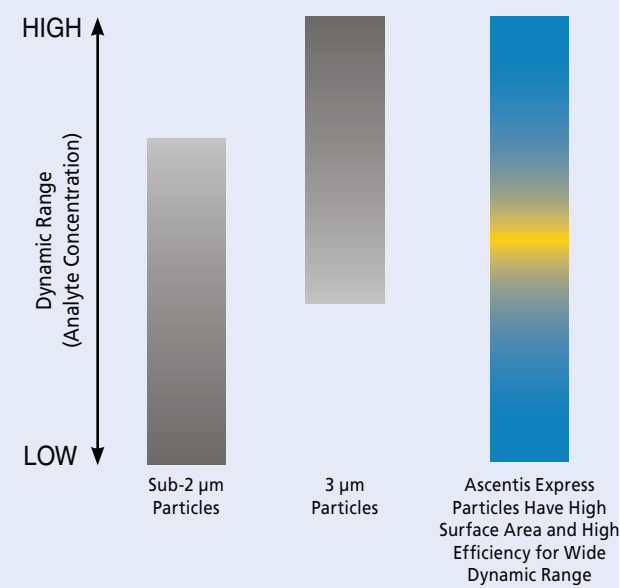


Figure 8 shows how Ascentis Express extends the dynamic range. It has the high efficiency of sub-2 µm particles needed for trace analysis, and the high surface area of totally porous particles needed for high sample capacity.

Figure 8. Extended Dynamic Range of Ascentis Express



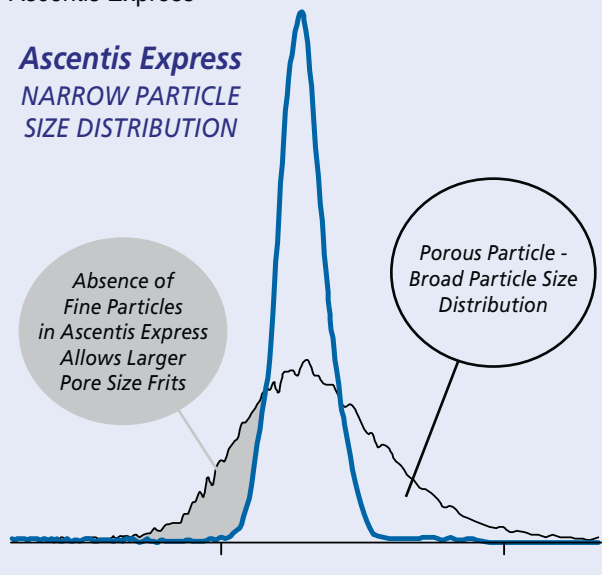
# Super-Rugged Columns

## Extended Column Lifetime Compared to Both 3 $\mu\text{m}$ and sub-2 $\mu\text{m}$ Columns

- Narrow particle size distribution allows use of 2  $\mu\text{m}$  frits
- Dense particles for more stable bed

Fused-Core particles are produced in a way that yields an extremely narrow particle size distribution (Figure 9). This narrow particle size distribution permits the use of frits with nominal 2  $\mu\text{m}$  pores, the same as used on most columns packed with 5  $\mu\text{m}$  particles. In comparison, sub-2  $\mu\text{m}$  particles require frits with much smaller pore size – 0.5  $\mu\text{m}$  or smaller – that are prone to fouling, lead to peak-splitting and high backpressure, and ultimately shorten the column lifetime. Another feature of the Fused-Core particles that contributes to their ruggedness is that they are denser than totally porous particles and form highly stable beds in the packed column.

Figure 9. Narrow Particle Size Distribution of Ascentis Express



## Sample Prep Simplicity

Combines the simplicity of protein precipitation and the selectivity of SPE for the targeted removal of phospholipids and proteins from biological samples

### **NEW!** HybridSPE™ – Precipitation Technology

- Reduce ion-suppression
- 100% removal of phospholipids & precipitated proteins
- Minimal to no method development
- Available in 96-well and 1 mL cartridge dimensions

To learn more, visit our website: [sigma-aldrich.com/hybrid-spe-ppt](http://sigma-aldrich.com/hybrid-spe-ppt) or contact Supelco Technical Service at 800-359-3041/814-359-3041 and [techservice@sial.com](mailto:techservice@sial.com)

# Alternative Selectivity with Ascentis Express RP-Amide

## Ascentis Express RP-Amide can solve

- Co-eluting peaks
- Unresolved components
- Poor retention of polar compounds
- Peak tailing of basic compounds
- Silanol interactions causing poor reproducibility

While the Ascentis Express C18 provides classic reversed-phase selectivity, the Ascentis Express RP-Amide provides increased selectivity for polar compounds, especially those that can act as a hydrogen-bond donor. Other attributes of the RP-Amide include improved peak shape for bases, 100% aqueous compatibility, and low bleed for LC-MS applications.

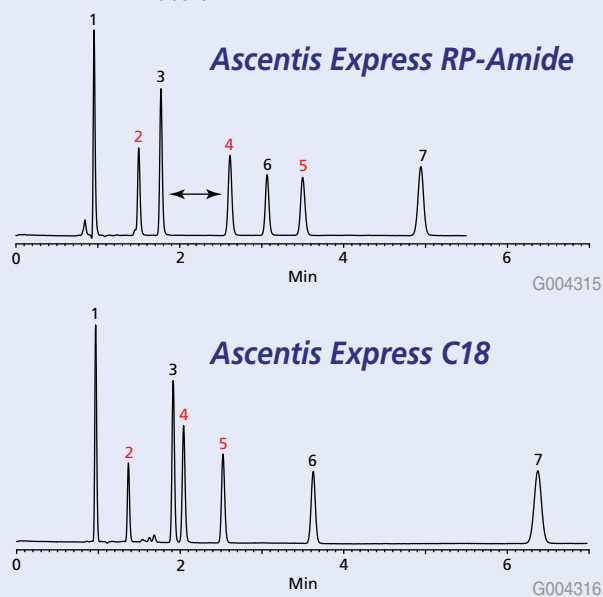
The amide group provides enhanced selectivity with analytes that have hydrogen bonded to a heteroatom. Phenols, carboxylic acids, amines and, to a lesser extent, alcohols show enhanced retention on the RP-Amide phase when compared to neutral non-polar analytes. An example of the power of the hydrogen bonding mechanism is shown in Figure 10. The Ascentis Express C18 and RP-Amide columns are compared. The analyte mixture contains neutral, non-polar analytes (benzene and toluene) and protic analytes (p-methoxyphenol, p-nitrobenzoic acid, and p-chlorophenol). As observed from the chromatograms in Figure 1, the neutral molecules show slightly reduced retention on the RP-Amide, but the protic molecules show greatly enhanced retention yielding a chromatogram with very different selectivities and even a change in elution order. The potential for solving separations difficulties is tremendous.

Two other points should be noted. The Ascentis Express RP-Amide has the same high efficiency as the Ascentis Express C18 with the same low back-pressure. Secondly, both separations were carried out in the same mobile phase. This is important since it simplifies method development. If a separation is not adequate on an Express C18, there is no need to change mobile phase to optimize the separation, simply switch to the Ascentis Express RP Amide and if protic moieties are present, a change in selectivity will be achieved.

Figure 10. Alternative Selectivity Provided by Ascentis Express RP-Amide Compared to C18

columns: Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D.  
Ascentis Express C18, 10 cm x 4.6 mm I.D.  
mobile phase: 40:60, 0.1% formic acid in water:methanol  
flow rate: 1.0 mL/min  
temp.: 25° C  
det.: 254 nm  
injection: 5 µL

1. Uracil
2. p-Methoxyphenol
3. Acetophenone
4. p-Nitrobenzoic acid
5. p-Chlorophenol
6. Benzene
7. Toluene



## Ascentis Express RP-Amide Applications

- Natural products
- Phenolics
- Bases
- Metabolites
- Polar Compounds

# Polar Compound Retention with Ascentis Express HILIC

## Benefits of HILIC Separation

- Retention of highly polar analytes like metabolites
- Complimentary selectivity to reversed-phase chromatography
- Increased MS sensitivity
- Quick transfer from final steps of sample prep (SPE, protein precipitation, etc.)

HILIC chromatography is gaining popularity due to increased retention of polar compounds. Many classes of polar compounds can be retained in HILIC. These include polar neutrals, polar acids, and polar and non-polar basic amines. Both polar and ionic interactions can contribute to retention and selectivity in this mode of chromatography.

## How HILIC works

HILIC separates compounds by using a mostly organic mobile phase across a polar stationary phase, causing solutes to elute in order of increasing polarity—the opposite of Reversed-Phase. Retention in HILIC is likely to be a combination of hydrophilic interaction, ion-exchange, and some reversed-phase retention. A typical mobile phase consists of 60-95% acetonitrile and an aqueous buffer. 10-20 mM ammonium acetate or ammonium formate are useful due to volatility and solubility. The sample solvent should be similar in type and strength as the mobile phase. The sample solvent can contain a higher amount of organic than the mobile phase, but should not contain more water than the mobile phase.

Shown in Figure 11 is a comparison of the analysis of highly polar molecules on Ascentis Express HILIC and Ascentis Express C18.

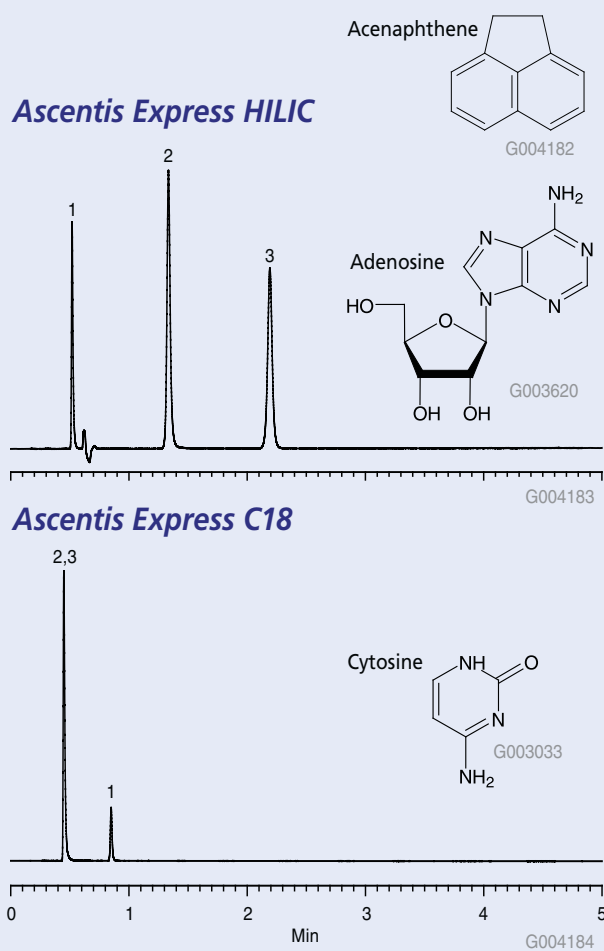
## Ascentis Express HILIC Applications

- Amino acids
- Small, polar acids – (metabolomics)
- Biogenic amines – (neurotransmitters, contaminants in food & beverage)
- Phosphates – (pesticides, herbicides)
- Sugars
- Drug metabolites and conjugates

Figure 11. Comparison of the Analysis of Polar Molecules on Ascentis Express HILIC and Ascentis Express C18

columns: Ascentis Express HILIC, 10 cm x 2.1 mm ID, 2.7  $\mu$ m particles (53939-U)  
Ascentis Express C18, 10 cm x 2.1 mm ID, 2.7  $\mu$ m particles (53823-U)  
mobile phase: 10:90; 100 mM ammonium formate, pH 3.0 with concentrated formic acid:acetonitrile  
flow rate: 0.4 mL/min  
temp.: 35  $^{\circ}$ C  
det.: UV at 254 nm  
injection: 1  $\mu$ L

1. Acenaphthene, 80  $\mu$ g/mL in mobile phase
2. Adenosine, 35  $\mu$ g/mL in mobile phase
3. Cytosine, 75  $\mu$ g/mL in mobile phase





# Improving HPLC Sample Throughput

## Do More Work in Less Time Without Changing Your Method

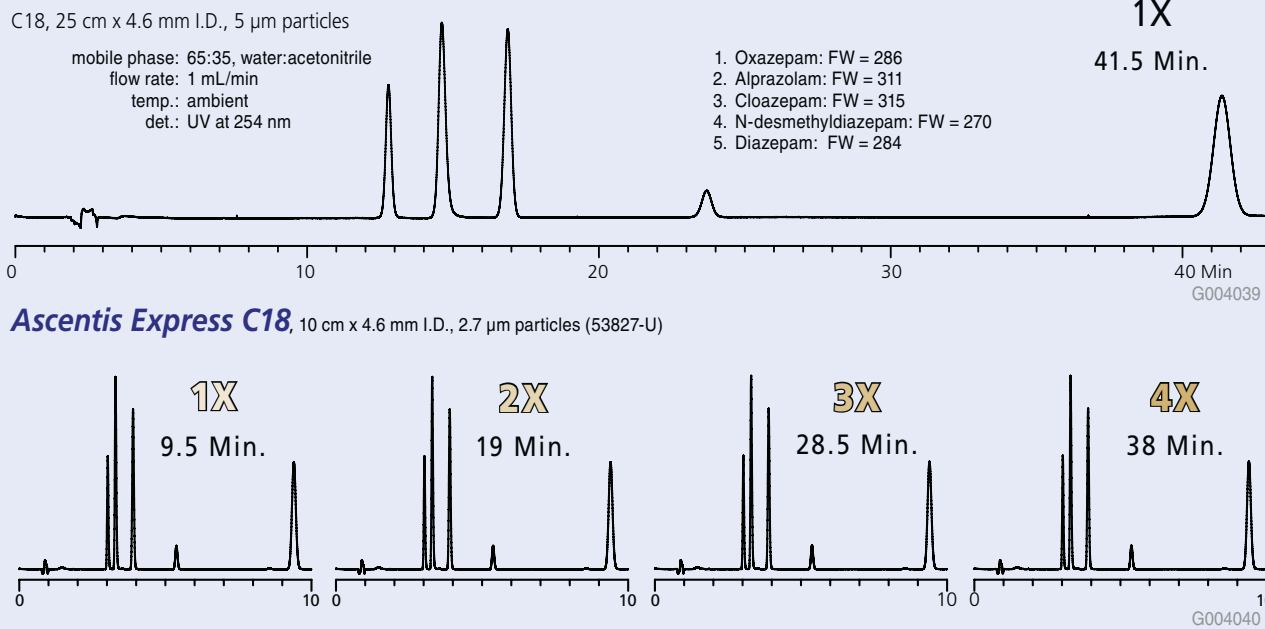
The demand for increased sample throughput and speed of results has driven HPLC users to search for breakthroughs in HPLC instruments and column technology. Although improvements have been realized, setbacks have been encountered. Reduction in column ruggedness, costly replacements of existing instrumentation, and difficulties in transferring methods to new systems have often made these past improvements unappealing to analysts.

The Fused-Core HPLC particle technology behind Ascentis Express permits 4- to 6-fold reduction in analysis time, with a subsequent increase in sample throughput compared to conventional HPLC columns, without sacrificing resolution or column ruggedness and without the need to change systems or sample prep procedures. The current high resolution column for traditional HPLC methods is a 25 cm column packed with 5  $\mu\text{m}$  particles.

Until now, this dimension provided the most efficiency within the pressure limit of a conventional HPLC system. With the high efficiency Ascentis Express, one can now achieve the same number of plates as a 25 cm column packed with 5  $\mu\text{m}$  particles with a 10 cm column or even more efficiency and resolution with a 15 cm Ascentis Express column. Therefore, by simply changing columns and keeping all other conditions the same, you can reduce the runtime and increase the resolution of your method.

Figure 12 compares the resolution of a five-component sample on 25 cm, 5  $\mu\text{m}$  C18 and 10 cm Ascentis Express C18 columns. Each column has approximately the same number of theoretical plates and hence the same resolving power. However the shorter Ascentis Express column delivers this separation in a much shorter time, in this case less than one-fourth the time as the 25 cm column.

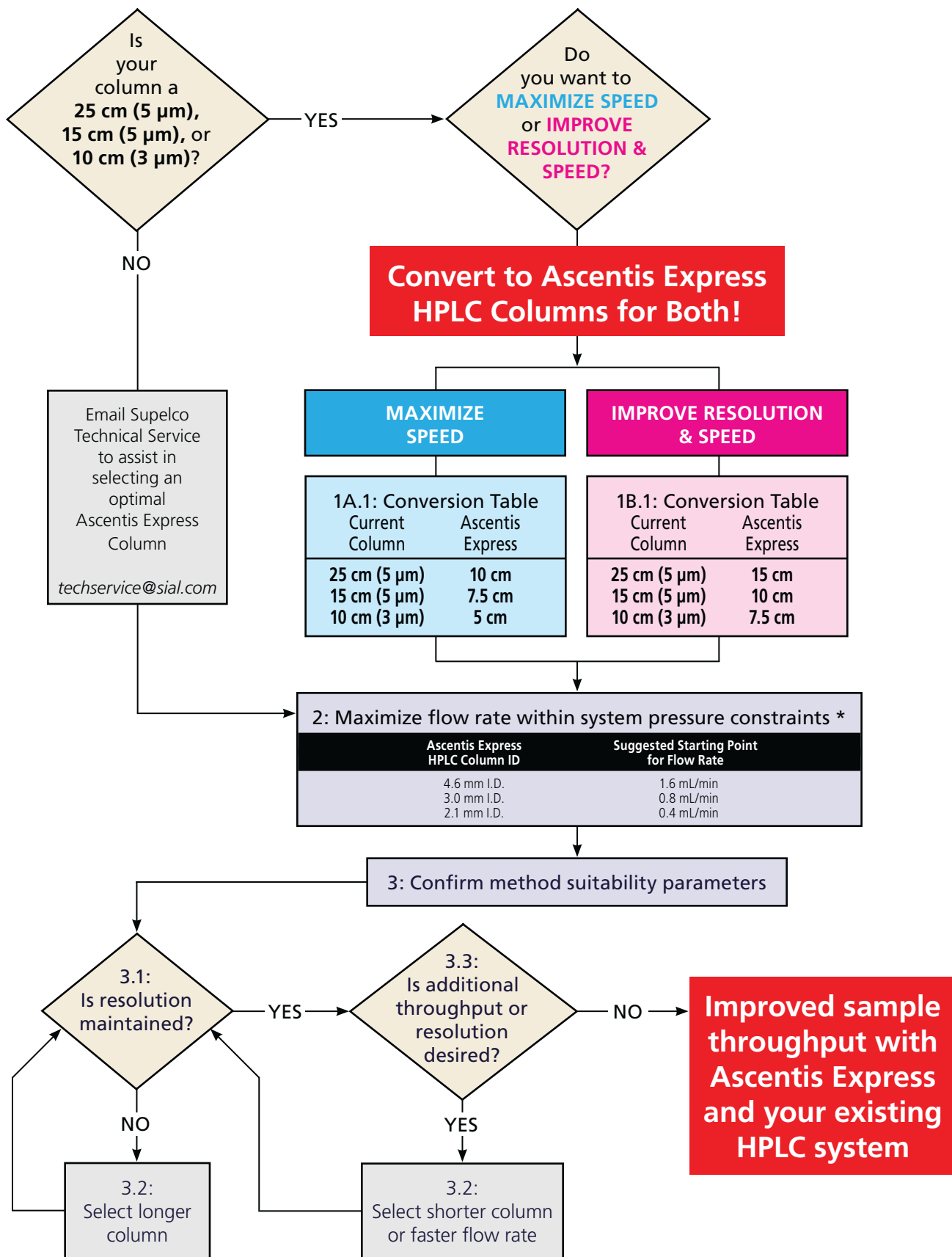
Figure 12. Increase Sample Throughput by Using Ascentis Express



Ready to do more work in less time?  
See the flow chart on the next page.



# Selecting the Optimal Ascentis Express Column



\*Read *Guidelines for Optimizing Systems for Ascentis Express Columns (T407102)* and *Guide to Dispersion Measurement (T408143)*.



# Fast HPLC for Rapid Screening of Pharmaceutical Compounds

## Ideal for Walk-up LC-MS Systems

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.

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  $\mu\text{m}$ ) 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  $\mu\text{m}$  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  $\mu\text{m}$  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.

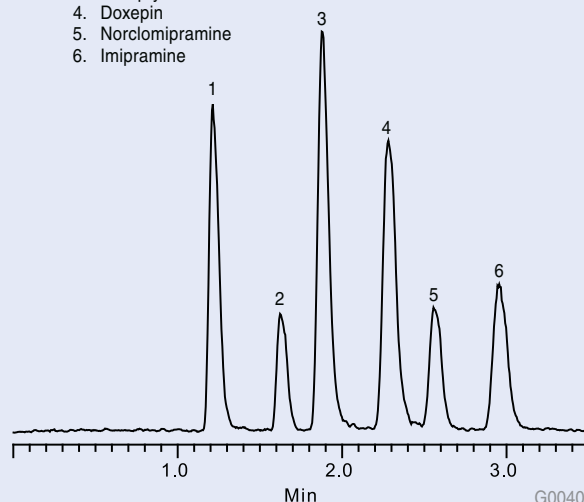
Unfortunately, column backpressure increases at a greater rate than column efficiency as you decrease particle size. This increase in backpressure is so great for sub-2  $\mu\text{m}$  particle columns that they are practically unusable using standard HPLC systems.

Shown in Figures 13-15 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 13. 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  
mobile phase ratios: 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  $\mu\text{L}$

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



G004062



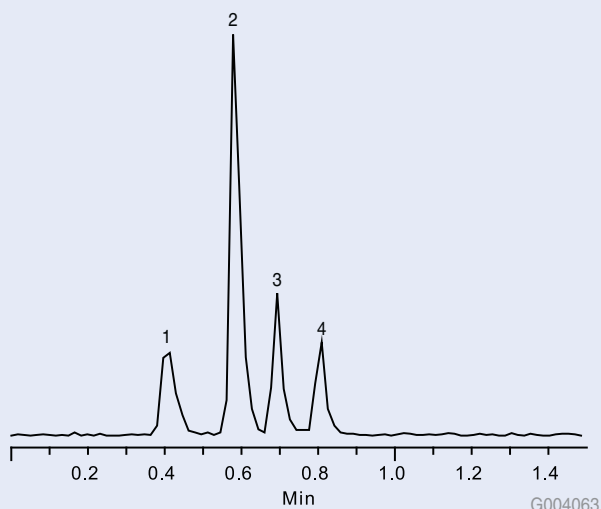
Shown in Figure 13 is the separation of six tricyclic antidepressants (TCAs). The separation of these closely related compounds was performed under isocratic mobile phase conditions with mass spectrometric (MS) detection. Baseline resolution was achieved with a total separation time of 3 minutes demonstrating 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 14 further illustrates the speed in which closely related compounds can be resolved using the Fused-Core particle. In this example, four  $\beta$ -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  $\beta$ -blockers example.

Figure 14.  $\beta$ -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  
mobile phase ratios: A:B = 74:26  
flow rate: 0.2 mL/min  
temp.: 35 °C  
det.: ABI 3200 QT; ESI(+), MS/MS  
injection: 1  $\mu$ L

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

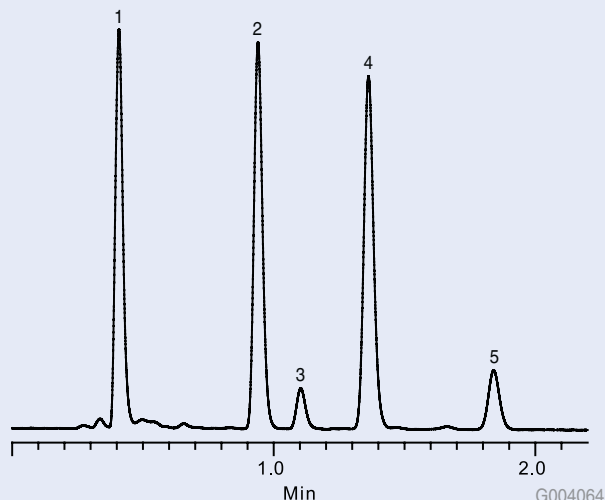


The separation of three steroids as well as a related impurity and degradant is shown in Figure 15. A high mobile phase flow rate of 0.6 mL/min was utilized and is suitable for Ascentis Express columns due to the Van Deemter curve associated with these columns. Isocratic mobile phase conditions were 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  $\mu$ m particle column but was not possible given the same instrument constraints put on the Ascentis Express column.

Figure 15. 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  
flow rate: 0.6 mL/min  
temp.: ambient  
det.: 200 nm  
injection: 1  $\mu$ L

1. Estriol
2.  $\beta$ -Estradiol
3. Impurity
4. Estrone
5. Estrone degradant





# Ultra-High Resolution HPLC: Column Coupling

## Maximize the Resolution of UHPLC systems

Column coupling in HPLC is gaining interest since LC systems are being designed to withstand column back pressures of up to 15,000 psi. Column coupling is a simple and practical way to increase resolution by simply increasing column length. Because Ascentis Express HPLC columns provide higher efficiencies at any pressure compared to 3  $\mu$ m and sub-2  $\mu$ m particles, the coupling of Ascentis Express columns enables significantly higher resolution than any other column on any commercial HPLC system.

Efficiencies greater than 150,000 plates/column are possible and demonstrated in the isocratic separation of benzene and toluene with various deuterium substitutions.

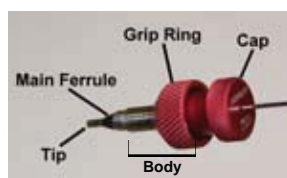
Figure 16 shows the efficiency obtained by coupling 5 Ascentis Express 15 cm columns together.

### Column Coupling Applications

- Natural product chemistry
- Tryptic digests
- Synthetic peptide mapping
- Stress studies of APIs
- LC-NMR

### High Performance HPLC Fittings/Interconnects

Improve HPLC performance with these fittings only from Supelco.



Use No Tools!

#### Key Benefits

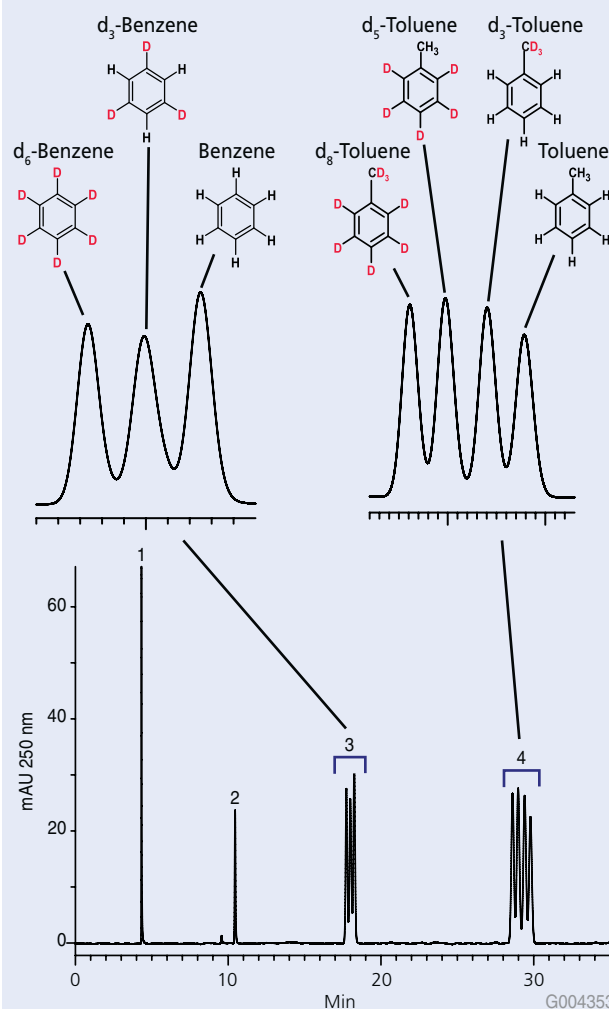
- Eliminate dead volume that contributes to peak broadening and decreased resolution
- Sliding ferrule design allows for use in any port
- Fingertight fittings, no tools required
- Rated to 15,000 psi

For a complete list, visit [sigma-aldrich.com](http://sigma-aldrich.com) and enter the keywords: **High Performance Fitting**

Figure 16. Column Coupling of Ascentis Express Provides over 100,000 Plates per Separation

column: Ascentis Express C18, 15 cm x 3 mm I.D., x5  
mobile phase: 56:44, water:acetonitrile  
flow rate: 0.6 mL/min  
temp.: 60° C  
pressure: 13500 psi (930 bar)

1. Uracil
2. Acetophenone
3. Benzene
4. Toluene



#### TRADEMARKS:

Ascentis, HybridSPE – Sigma-Aldrich Biotechnology LP  
Fused-Core – Advanced Materials Technology  
UPLC – Waters Associates, Inc.

# Ordering Information

## Analytical

ID (mm)	Length (cm)	Ascentis Express C18	Ascentis Express C8	Ascentis Express RP-Amide	Ascentis Express HILIC
2.1	3	53802-U	53839-U	53910-U	53933-U
2.1	5	53822-U	53831-U	53911-U	53934-U
2.1	7.5	53804-U	53843-U	53912-U	53938-U
2.1	10	53823-U	53832-U	53913-U	53939-U
2.1	15	53825-U	53834-U	53914-U	53946-U
3.0	3	53805-U	53844-U	53915-U	53964-U
3.0	5	53811-U	53848-U	53916-U	53967-U
3.0	7.5	53812-U	53849-U	53917-U	53969-U
3.0	10	53814-U	53852-U	53918-U	53970-U
3.0	15	53816-U	53853-U	53919-U	53972-U
4.6	3	53818-U	53857-U	53921-U	53974-U
4.6	5	53826-U	53836-U	53922-U	53975-U
4.6	7.5	53819-U	53858-U	53923-U	53977-U
4.6	10	53827-U	53837-U	53929-U	53979-U
4.6	15	53829-U	53838-U	53931-U	53981-U

## Capillary

	Ascentis Express C18 Length		Ascentis Express C8 Length	
	5 cm	15 cm	5 cm	15 cm
75 µm I.D.	53982-U	54219-U	53983-U	54229-U
100 µm I.D.	53985-U	54256-U	53987-U	54260-U
200 µm I.D.	53989-U	54261-U	53991-U	54262-U
300 µm I.D.	53992-U	54271-U	53997-U	54272-U
500 µm I.D.	53998-U	54273-U	53999-U	54275-U

## Ascentis Express Properties

### Stationary Phase Support

- Ultra-pure, Type B silica
- 1.7 µm solid core particle with 0.5 µm porous silica shell (effective 2.7 µm)
- 150 m<sup>2</sup>/gram surface area (comparable to ~225 m<sup>2</sup>/g porous particle)
- 90 Å pore size

### Bonded Phase

	Coverage µmoles/m <sup>2</sup>	pH Range	Endcapping
<b>C18</b>	3.5	2-9	Yes
<b>C8</b>	3.7	2-9	Yes
<b>RP-Amide</b>	3.0	2-9	Yes
<b>HILIC</b>	n/a	2-8	No

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