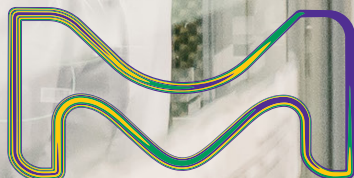


MERCK

# Ascentis<sup>®</sup> Express HPLC and UHPLC Columns

Maximum Resolution on any System



The life science business  
of Merck operates as  
MilliporeSigma in the  
U.S. and Canada.

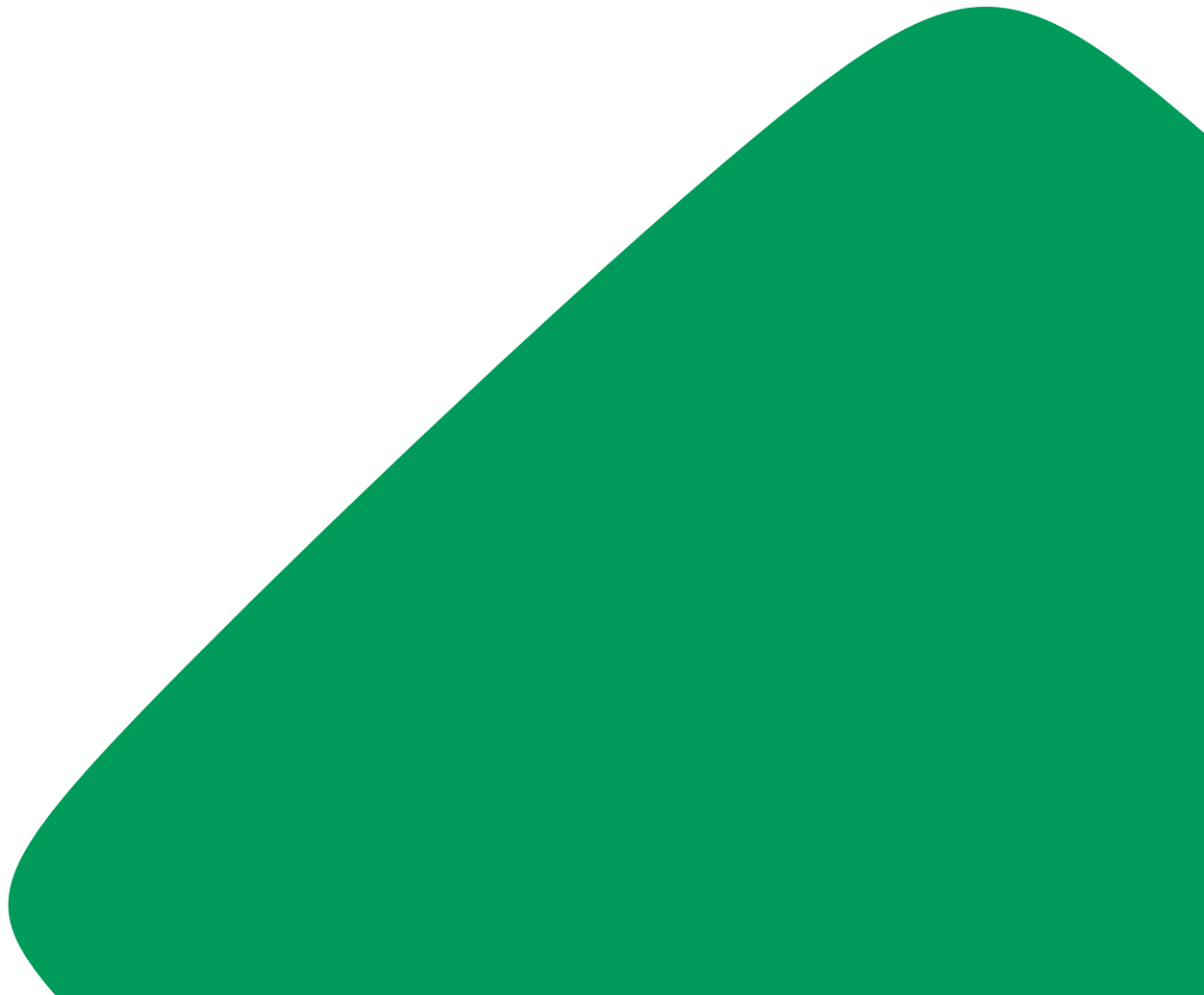
Supelco<sup>®</sup>  
Analytical Products

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## Experience the Benefits of Ascentis® Express HPLC and UHPLC Columns

Based on Fused-Core® particle technology, Ascentis® Express columns provide a exceptional advancements in HPLC column performance and the benefits of high sample throughput at maximum resolution.

- Fused-Core® technology (Superficially Porous Particles; SPP)
  - Maximum speed and efficiency on both UHPLC and HPLC systems (particle sizes: 2 µm, 2.7 µm and 5 µm)
  - 40% more efficiency in comparison to Fully Porous Particles (FPP) of same particle size
  - UHPLC columns with 2 µm particles (pressure stable 1000 bar)
  - Column dimensions from 0.075 mm ID (capillary columns) to 4.6 mm ID (analytical HPLC columns)
  - Broadest range of phases/selectivities for optimal method development
- 

# Increase Resolution and Speed

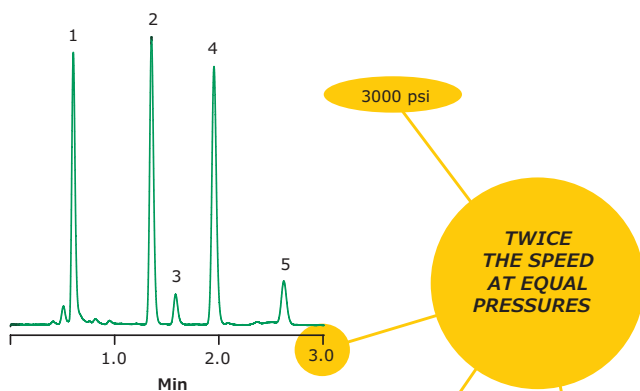
Ascentis® Express HPLC and UHPLC columns are based on Fused-Core® particle technology enabling fast results with highest resolution. Ascentis® Express columns provide about 40% more efficiency in comparison to columns with fully porous particles (FPP) of the same size. This performance enhancement is applicable to all HPLC instruments (in addition to UHPLC systems).

## Twice the Speed at Equivalent Pressure vs. sub-2 µm

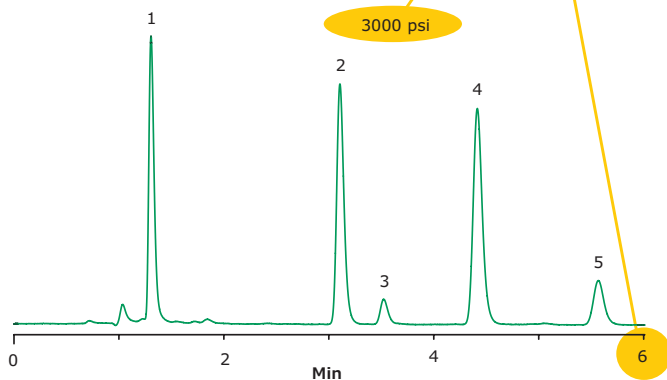
Compared to fully porous sub-2 µm particles typically used in UHPLC, Ascentis® Express Fused-Core® 2.7 µm particles generate approximately half the backpressure while providing the same high resolution. This permits both longer columns, for more resolving power, and faster flow rates, for higher throughput.

Demonstrating this point, the separation below shows a steroid mixture on Ascentis® Express (top) and a sub-2 µm UHPLC column (bottom) of the same dimensions. Due to the lower backpressure of the Ascentis® Express an increased flow rate (double in this case) can be applied providing the same back pressure, separation efficiency and resolution as on a sub-2 µm UHPLC, just with a 50% shorter runtime, increasing sample throughput.

Ascentis® Express C18  
0.4 mL/min flow rate



Sub-2 µm competitor 2  
0.2 mL/min flow rate

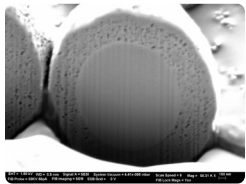


### Chromatographic conditions:

<b>Columns:</b>	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
<b>Mobile phase:</b>	water/acetonitrile 49:51 (for Ascentis® Express); water/acetonitrile 55:45 (for sub-2 µm)
<b>Column temp:</b>	ambient
<b>Detector:</b>	UV, 200 nm
<b>Injection:</b>	1 µL

1. Estradiol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

# Benefit from the exceptional advancements of Fused-Core® technology



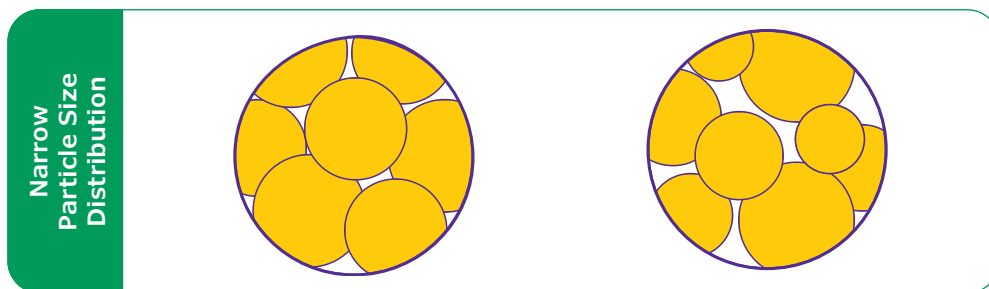
Fused-Core® particles consists of a solid silica core and a porous silica shell allowing a shorter diffusion path compared to conventional fully porous particles.

## Features of Fused-Core® particles over Fully Porous Particles

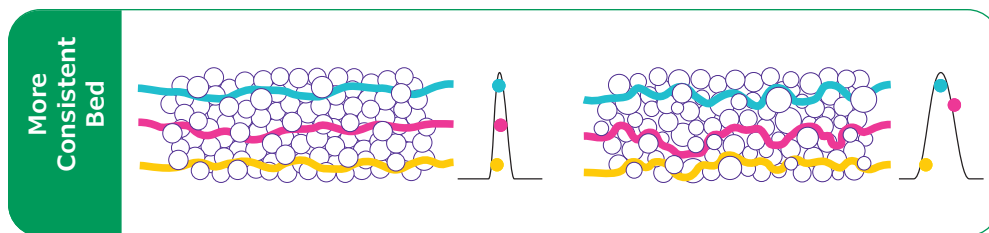
- Narrower particle size distribution
- More consistently packed bed
- Shorter diffusion path

### Fused-Core® (Superficially Porous Particles, SPP)

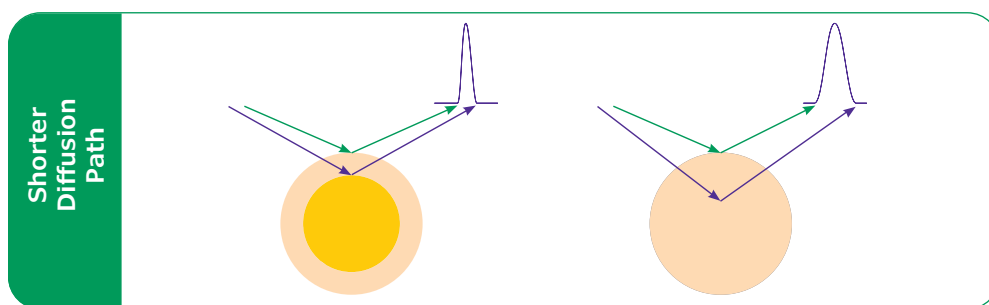
### Fully Porous Particles (FPP)



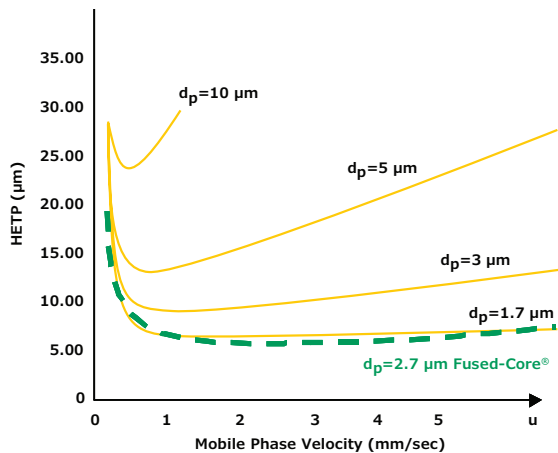
The innovative manufacturing process for Fused-Core® particles produces a very narrow particle size distribution. This allows for the use of larger porosity frits that clog less, resulting in a more rugged column. Traditional fully porous particles provide a larger particle size distribution, requiring smaller pore frits, that clog more easily.



The "A" term in the van Deemter equation accounts for the effects of heterogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistently packed bed and a more consistent path lengths, minimizing analyte dispersion (peak broadening) through the column. This eddy diffusion is effectively independent of mobile phase velocity.



The short diffusion path of the Fused-Core® particle yields sharper peaks than on traditional fully porous particle columns. The minimized resistance to mass transfer, the "C" term in the van Deemter equation, of the Fused-Core® particle provides sharper peaks than traditional porous particles. The short diffusion path also permits the use of higher flow rates without significant peak broadening / loss in efficiency.



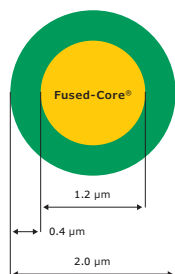
The factors that affect chromatographic efficiency are eddy diffusion, longitudinal diffusion, and resistance to mass transfer, the A, B and C terms respectively from the van Deemter equation.

$$H = A + \frac{B}{u} + Cu$$

- H Height equivalent to theoretical plate (column length/efficiency)
- A Eddy diffusion
- B Longitudinal diffusion
- C Resistance to Mass Transfer
- u Mobile phase linear velocity

# Best fit for HPLC and UHPLC

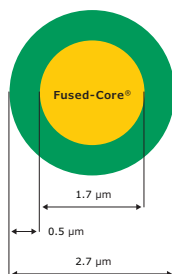
## Best Fused-Core® UHPLC column



An optimized solution for high throughput small molecule analysis

Pressure stability: 2 µm: 1000 bar

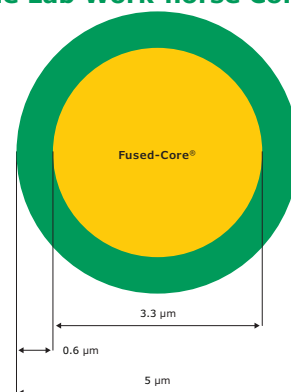
## Fast on any System



A practical solution that delivers UHPLC performance from any HPLC

2.7 µm: 600 bar

## The Lab Work-horse Column



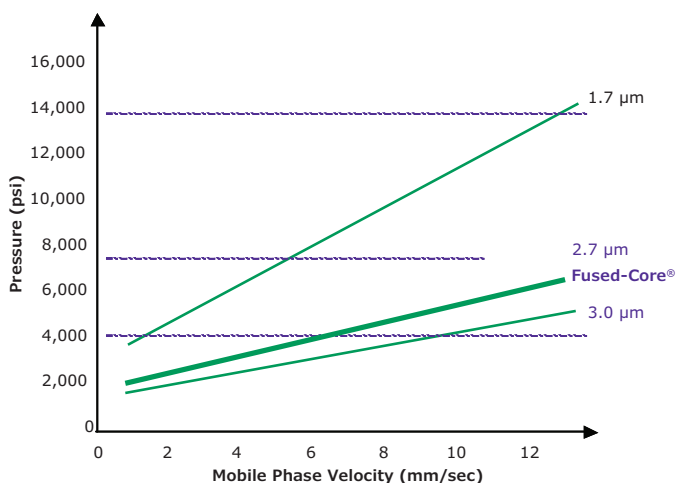
True plug and play solution for improving existing 3 or 5 µm porous particle HPLC columns

5 µm: 600 bar

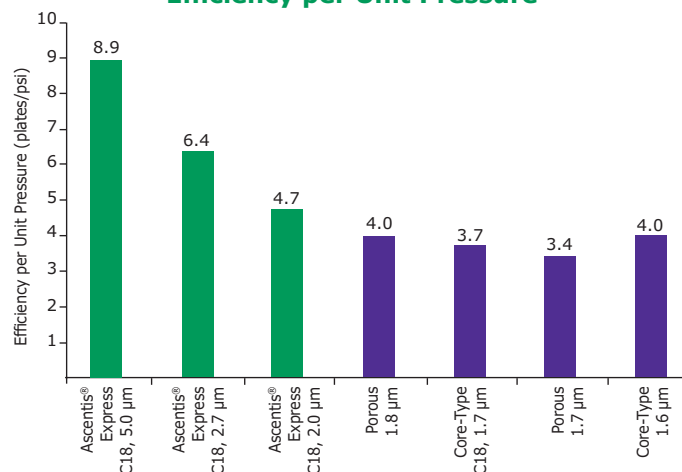
## More separation power per unit pressure

Designed to deliver speed and resolution on all UHPLC and HPLC systems, Ascentis® Express columns with Fused-Core® technology exceed the benefits of sub-2, 3 and 5 µm particles. Ascentis® Express 2.7 µm delivers more resolving power per unit pressure than even sub-2 µm particles on any HPLC system (including UHPLC). Ascentis® Express 5 µm columns are able to achieve greater speed and efficiency than any other

5 µm particle-based column. This means that Ascentis® Express 5 µm can be the standard column for all fully porous 5 µm-based methods. With the addition of 2.0 µm Ascentis® Express UHPLC columns, we now offer three U/HPLC Fused-Core® particle size, making the Ascentis® Express column line truly scalable from HPLC to UHPLC.

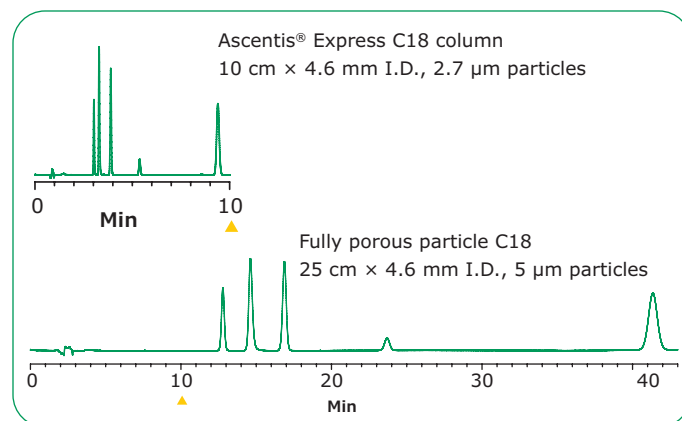


## Efficiency per Unit Pressure



## Higher Sample Throughput Without Compromises

The outstanding separating power of Ascentis® Express HPLC columns allows the use of shorter column dimensions while maintaining good resolution. This results in higher sample throughput and reduction in costs.



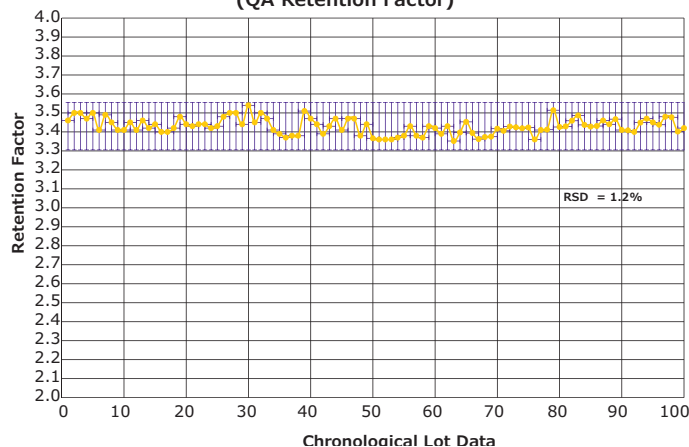
# We ensure consistent results

## Excellent Lot-to-Lot Reproducibility

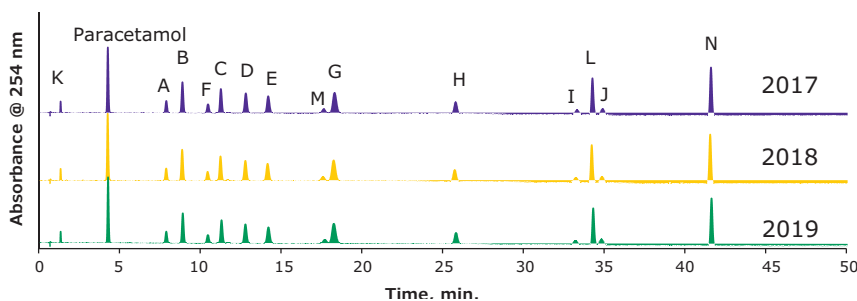
The consistency of chromatographic results depends on many factors. One major contributor to consistent and reliable results is the HPLC column. Therefore, the Lot-to-Lot and column-to-column reproducibility is a major concern.

Ascentis® Express HPLC and UHPLC columns show an excellent reproducibility. Over the last 10 years the relative standard deviation (RSD) of the QA retention factor was 1.2%. The high Lot-to-Lot reproducibility is also demonstrated by the selectivity test below.

Ascentis® Express Reproducibility Data (2010-2020)  
(QA Retention Factor)



Lot-to-Lot Ascentis® Express C18, 2.7 µm, 10 cm x 2.1 mm



### Chromatographic Conditions:

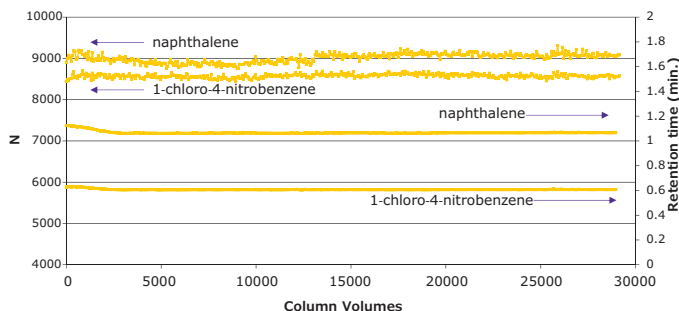
#### Gradient elution

<b>Mobile Phase:</b>	[A] pH 7 phosphate buffer [B] methanol
<b>Wavelength:</b>	254 nm
<b>Injection:</b>	1.0 µL
<b>Temperature:</b>	30 °C
<b>Flow Rate:</b>	0.3 mL/min.

## Temperature Stability

In addition to high reproducibility, the stability of HPLC column materials is important. The test-set below demonstrates the stability of Ascentis® Express, C18 column 5 µm at 60 °C pH 2.

Stability of Ascentis® Express (5µm) at pH 2 and 60 °C



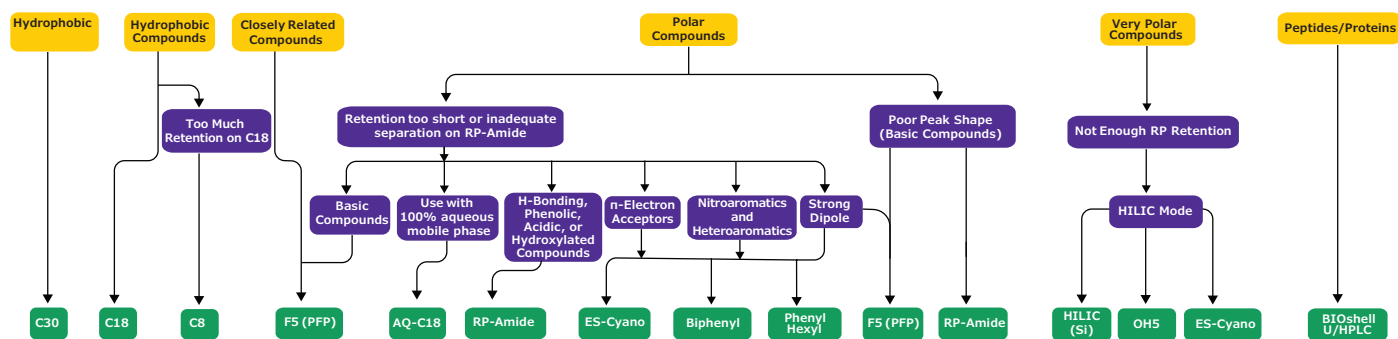
### Chromatographic Conditions:

<b>Column:</b>	Ascentis® Express C18, 5 cm x 4.6 mm I:D, 5 µm Particles (50530-U)
<b>Temperature:</b>	60 °C;
<b>Mobile phase:</b>	50% acetonitrile/50% aqueous 0.1% trifluoroacetic acid;
<b>Flow rate:</b>	1.8 mL/min
<b>Injection:</b>	1.0 µL
<b>Solutes:</b>	1-chloro-4-nitrobenzene, naphthalene, k = 1.7 and 3.8, respectively.

# A broad range of column selectivities for all compound classes

Column selectivity has the highest influence on resolution in chromatography. Selection of the best suitable column chemistry for your target analytes is therefore an important selection parameter. C18 column chemistries are typically the first choice. Nevertheless, when a C18 doesn't give the desired separation or the sample contains compounds that

are known to be difficult to retain or resolve on a C18, consider changing stationary phase early in method development for more optimal applications. The range of selectivity provided by Ascentis® Express makes this easy. The flowchart below helps to guide users in the selection of an Ascentis® Express phase, based on the particular compound type or separation challenge.



Bonded Phase	Chemistry	USP Designation	Chromatographic Properties / Use	Particle Size (s) (µm)	Pore Size (Å)
C30	Triacontyldimethyl	L62	Excellent selectivity for very hydrophobic compounds, long-chain and structurally related isomers	2.7	160
C18	Dimethyloctadecyl	L1	Outstanding performance for a broad range of analytes	2, 2.7, 5	90
Peptide ES-C18	Diisobutyloctadecyl	L1	Fast separation of peptides and polypeptides with high peak capacity	2.7	160
AQ-C18	Polar modified Octadecyl	L1	Resistant to dewetting; compatible to 100% aqueous mobile phase	2, 2.7, 5	90
C8	Dimethyloctyl	L7	Enhanced retention for less hydrophobic compounds or faster separation if retention on C18 is too long	2, 2.7, 5	90
RP-Amide	C16-Amide	L60	Complementary selectivity to alkyl phases	2, 2.7, 5	90
Phenyl-Hexyl	Dimethylphenyl-hexyl	L11	Enhanced selectivity for aromatic compounds; strong pi-pi donor	2, 2.7, 5	90 and 160
Biphenyl	Dimethylbiphenyl	L11	Enhanced selectivity for aromatic compounds	2, 2.7, 5	90
F5 (PFP)	Pentafluorophenylpropyl	L43	Outstanding selectivity for stereoisomers, strong pi-pi acceptor	2, 2.7, 5	90
ES-Cyano	Diisopropylcyanopropyl	L10	Enhanced retention for polar compounds and much less retention for hydrophobic compounds	2, 2.7, 5	90
OH5	Penta-hydroxy	L95	Ideal for the HILIC separation of very polar compounds with a LogP value close to 0 or less than 0	2, 2.7, 5	90
HILIC	Bare silica	L3	Enhanced separation of polar compounds; can be used in HILIC and normal-phase mode.	2, 2.7, 5	90



# Ascentis® Express C18

## U/HPLC columns for fast and high resolution applications

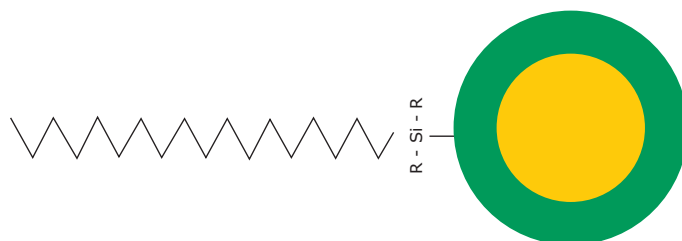
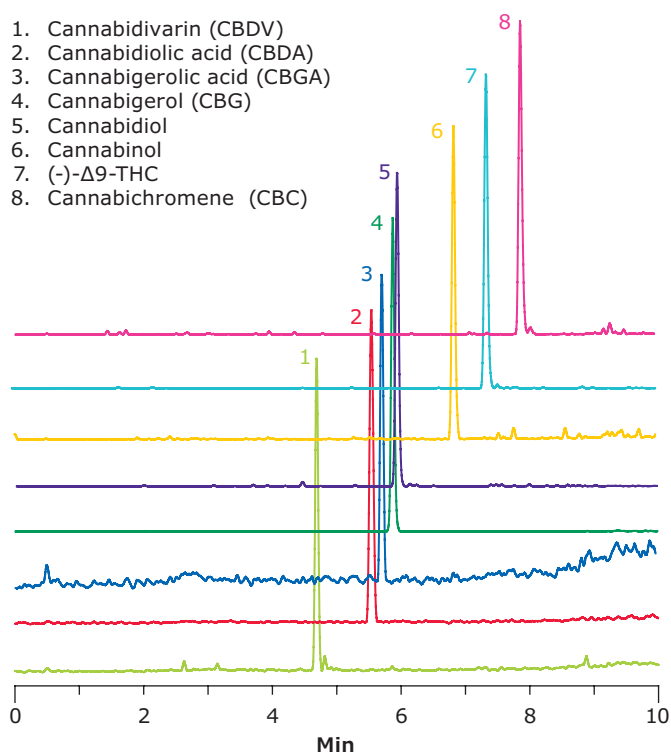
Ascentis® Express C18 is the first choice for starting a new method. The Fused-Core® particle technology of the Ascentis® Express C18 column enables high speed and high resolution separations on HPLC and UHPLC instruments. The column efficiency performs typically 40% higher in comparison to fully porous particulate columns with same particle size.

Ascentis® Express C18 bonded phase is nonpolar in nature. It is best used with mobile phases that are mixtures of methanol and water or acetonitrile and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Using elevated temperatures (e.g., 40–60 °C) will reduce the viscosity of the mobile phase and allow the use of faster flow rates and lower column pressure for high sample throughput.

### Application: UHPLC-MS Analysis of Cannabinoids on Ascentis® Express C18

Cannabis compounds reportedly have therapeutic efficacy in the treatment of pain, mood disorders, and inflammatory diseases. Testing in relation to Cannabis is typically done in GC/MS, LC-MS, or HPLC for applications in clinical toxicology, testing of cannabis potency or impurity profiling by growers, pharmaceutical research, forensic analysis, and urine drug testing. Shown here is the separation of cannabis compounds on an Ascentis® Express C18 column.

1. Cannabidivarin (CBDV)
2. Cannabidiolic acid (CBDA)
3. Cannabigerolic acid (CBGA)
4. Cannabigerol (CBG)
5. Cannabidiol
6. Cannabinol
7. (-)- $\Delta^9$ -THC
8. Cannabichromene (CBC)



#### Ascentis® Express C18 Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Octadecyl
<b>Particle Size:</b>	2.0 $\mu\text{m}$ / 2.7 $\mu\text{m}$ / 5.0 $\mu\text{m}$
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	6.5% / 6.7% / 5.6%
<b>Surface Area:</b>	120 $\text{m}^2/\text{g}$ / 135 $\text{m}^2/\text{g}$ / 90 $\text{m}^2/\text{g}$
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

Also Available as Ascentis® Express C18 PCP: PCP in the name means the bonded phase has been pre-conditioned with phosphoric acid.

#### Chromatographic properties\*

##### Hydrophobicity

$k$  (pentylbenzene)/ $k$  (butylbenzene)

1.51

##### Shape Selectivity

$k$  (triphenylene)/ $k$  (o-terphenyl)

1.37

##### H-Bonding Capacity

$k$  (caffeine)/ $k$  (phenol)

0.39

##### Ion-Exchange Capacity (acidic)

$k$  (benzylamine)/ $k$  (phenol)

0.11

\*Tanaka test modified by Euerby (ref. Journal of Chromatography A, 994 (2003) 13–36)

#### Chromatographic conditions

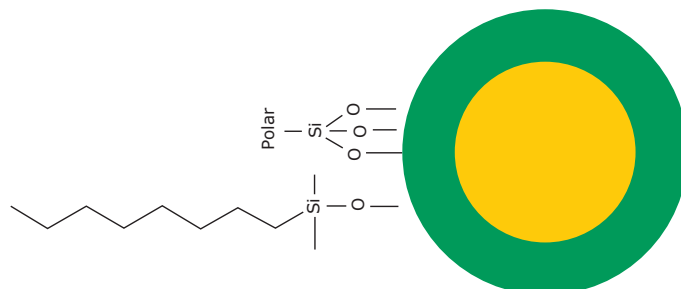
<b>Column:</b>	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.0 $\mu\text{m}$ particles (50813-U)
<b>Sample:</b>	100 ng/mL each in methanol
<b>Mobile phase:</b>	[A] 0.1% formic acid; [B] 0.1% formic acid in acetonitrile
<b>Gradient:</b>	60% B to 100% B in 10 min
<b>Flow rate:</b>	0.4 mL/min
<b>Pressure:</b>	7200 psi (496 bar)
<b>Injection:</b>	1 $\mu\text{L}$
<b>Column temp.:</b>	35 °C
<b>Detector:</b>	MS, ESI(+), ESI(-), MRM m/z (see figure for transitions)

# Ascentis® Express AQ-C18

## U/HPLC columns resistant to de-wetting; compatible with 100% aqueous mobile phase

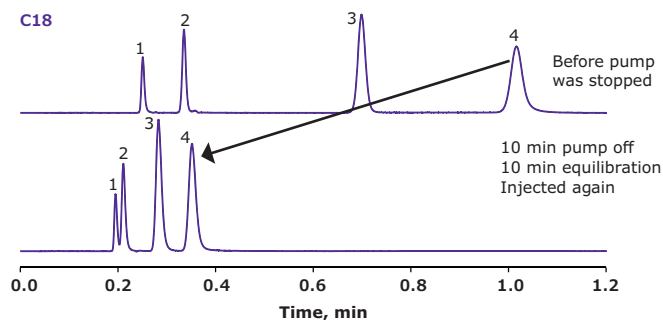
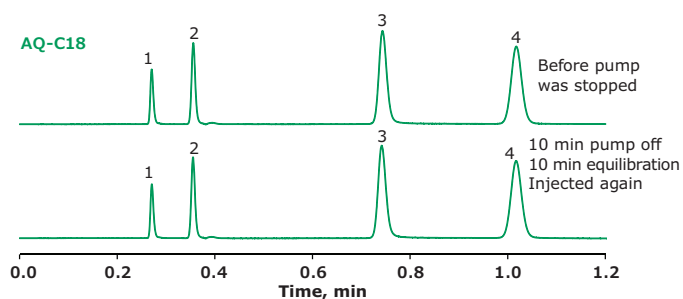
The Ascentis® Express AQ-C18 column, based on Fused-Core® particles, is a C18 bonded phase prepared using a proprietary procedure that incorporates a small amount of polar silane which makes the phase resistant to de-wetting. This allows the users of the AQ-C18 phase to run highly-aqueous (up to 100%) mobile phases without the risk of phase collapse. The modified C18 phase exhibits enhanced retention of polar compounds under 100% aqueous conditions.

The Ascentis® Express AQ-C18 reversed-phase packing can be used for basic, acidic, and neutral compounds.



### Stability test under 100% aqueous conditions

The separation example demonstrates the stability of Ascentis® Express AQ-C18 stationary phase under 100% aqueous conditions in contrast to a common C18 phase. After the pump was stopped and restarted, the retention and resolution were maintained with the Ascentis® Express AQ-C18 column, where the C18 phase showed a dramatic change in retention.



### Ascentis® Express AQ-C18 Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Polar modified Octadecyl
<b>Particle Size:</b>	2.7 µm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	6.5% / 6.7% / 5.6%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k (butylbenzene)

1.45

#### Shape Selectivity

k (triphenylene)/k (o-terphenyl)

1.82

#### H-Bonding Capacity

k (caffeine)/k (phenol)

0.47

#### Ion-Exchange Capacity (acidic)

k (benzylamine)/k (phenol)

0.03

\*Tanaka test modified by Euerby (ref. Journal of Chromatography A, 994 (2003) 13–36)

### Chromatographic conditions:

<b>Columns:</b>	Ascentis® Express AQ-C18, 5 cm x 4.6 mm I.D., 2.7 µm Ascentis® Express 90 Å C18, 5 cm x 4.6 mm I.D., 2.7 µm
<b>Mobile phase:</b>	Water/0.1% TFA
<b>Temperature:</b>	30 °C
<b>Injection Volume:</b>	0.5 µL
<b>Detection:</b>	UV 254 nm, PDA 1. Thiourea 2. 5-Fluorocytosine 3. Adenine 4. Thymine

# Ascentis® Express Peptide ES-C18

## HPLC columns enabling highest efficiency, capacity and robustness for peptide separations

Ascentis® Express Peptide ES-C18 columns were specifically engineered to separate higher molecular weight compounds such as peptides and small proteins. These columns contain advanced Fused-Core® particles that have larger pores (160 Å versus 90 Å in standard Ascentis® Express columns), which greatly expands the application range for Ascentis® Express columns.

Key applications for Ascentis® Express Peptide ES-C18 columns:

- Pharmaceutical/therapeutic peptide separation
- Peptide mapping
- Natural and synthetic peptide analysis
- Oligonucleotide analysis

Key Advantages:

- Higher peak capacity providing greater resolution
- Exceptional ruggedness providing long column lifetime

Ascentis® Express Peptide ES-C18 HPLC columns use a steric-protected C18 bonded-phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the column tolerates the combination of low pH and elevated temperature well.

### Comparison of Peptide Test Mix with Ascentis® Express Peptide ES-C18 HPLC column and Traditional Column

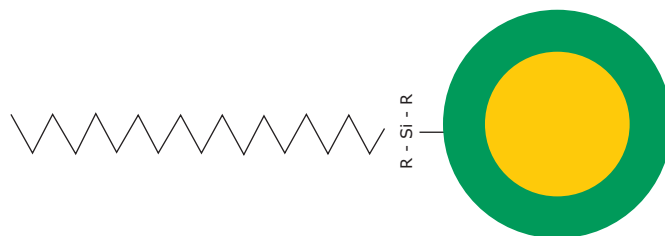
The peptide mix used for the separation below contains a range of peptides in terms of molecular weight, basicity, and hydrophobicity. Excellent peak shape and peak width are achieved with a standard acetonitrile gradient and 0.1% TFA modifier. The resolution of small baseline impurities are shown in the inset, demonstrating the resolving power of the Ascentis® Express Peptide ES-C18 column versus a traditional 5 µm fully porous particle column.

#### Chromatographic conditions

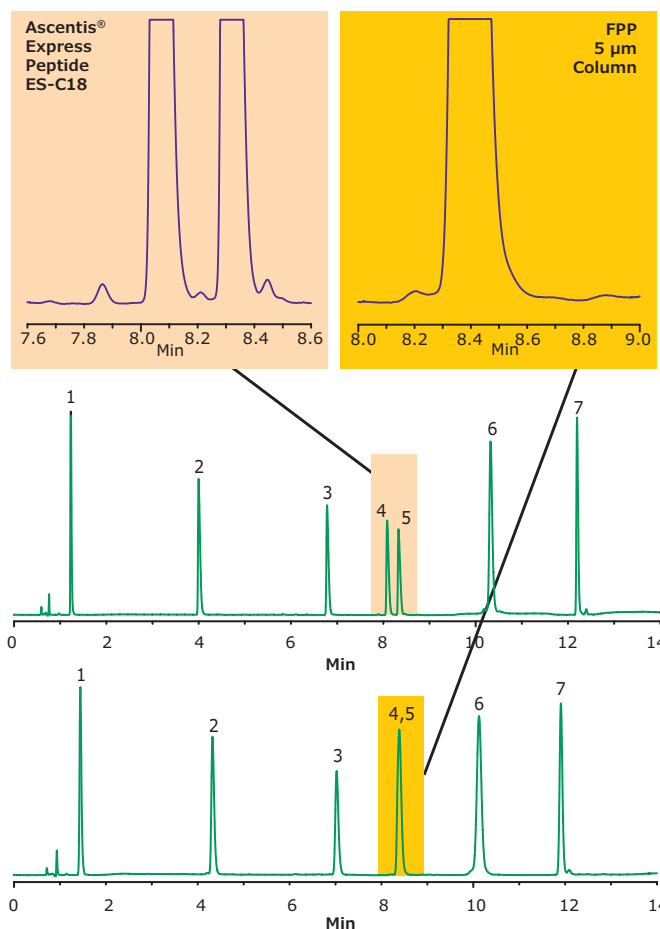
<b>Column:</b>	Ascentis® Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
<b>Mobile phase A:</b>	[A] 10% acetonitrile / 90% water / 0.1% trifluoroacetic acid
<b>Mobile phase B:</b>	[B] 75% acetonitrile / 25% water / 0.1% trifluoroacetic acid
<b>Gradient:</b>	0% to 50% B in 15 min
<b>Flow rate:</b>	1.5 mL/min.
<b>Detection</b>	UV at 220 nm
<b>Temp:</b>	30 °C
<b>injection:</b>	5 µL

#### Peptide Test Mix

- |                             |                               |
|-----------------------------|-------------------------------|
| 1. Gly-Tyr MW = 252         | 5. Leu-Enkephalin MW = 555.62 |
| 2. Val-Tyr-Val MW = 379     | 6. Ribonuclease MW = 13,700   |
| 3. Met Enkephalin MW = 574  | 7. Bovine Insulin MW = 5733   |
| 4. Angiotensin II MW = 1032 |                               |



Ascentis® Express Peptide ES-C18 Specifications	
<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Diisobutyloctadecyl
<b>Particle Size:</b>	2.7 µm
<b>Pore Size:</b>	160 Å
<b>Carbon Load:</b>	4.6%
<b>Surface Area:</b>	90 m <sup>2</sup> /g
<b>pH Range:</b>	1 - 8
<b>Max Temperature at Low pH:</b>	90 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	No



# Ascentis<sup>®</sup> Express C8

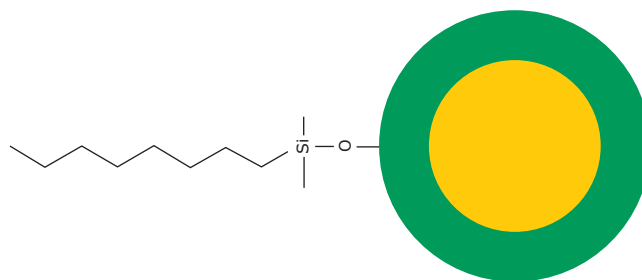
## Enhanced retention for less hydrophobic compounds or faster separation if retention on C18 is too long

When a C18 doesn't give the desired separation, or your sample contains compounds that are known to be difficult to retain or resolve on a C18, consider changing to an Ascentis<sup>®</sup> Express C8 column. The Ascentis<sup>®</sup> Express C8 phase can be ideal for non-polar compounds that are retained too strongly by a traditional C18 column.

### Fast Separation of Dyes with an Ascentis<sup>®</sup> Express C8 U/HPLC Column

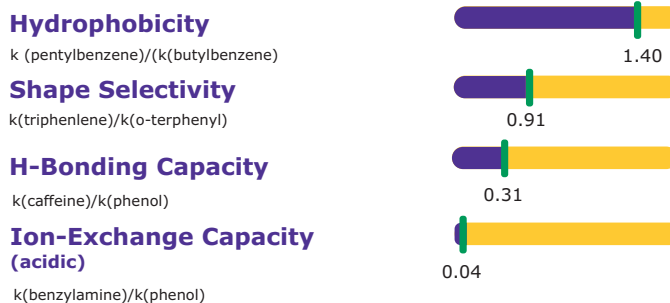
Dyes surround us everywhere every day. They can be found in common places like the printing ink in magazines or books and in plastics, textiles, and leather, but also in diesel fuel and tattoo color. Most of these synthetic colors are based on aromatic ring structures containing heteroatoms and tend to have a high potential for causing cancer; as a result, they are not intended for use in food coloring. But since 2003, there have been several incidents of Sudan I contamination in chili powder. This situation necessitates the analysis of spice mixtures to determine if they have been adulterated.

Table 1 contains a list of dyes (in elution order) added to one sample and dissolved in a mixture of methanol and acetonitrile. The sample was injected on an Ascentis<sup>®</sup> Express C8 HPLC column.\*

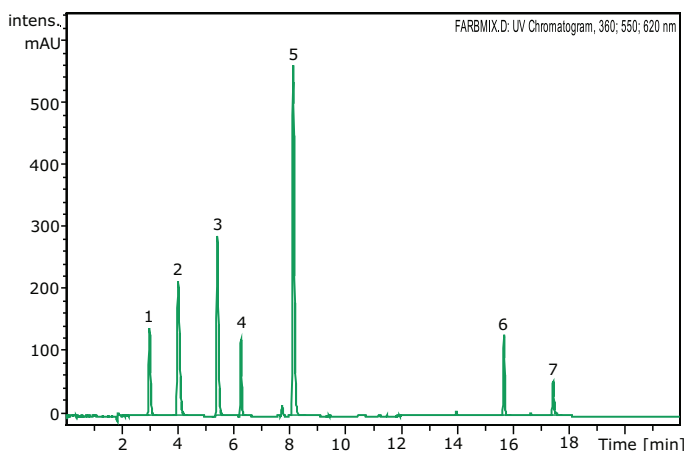


Ascentis <sup>®</sup> Express C8 Specifications	
Silica:	Type B (High purity silica)
Particle Platform:	Superficially porous particles (SPP)
Phase Chemistry:	Dimethyloctyl
Particle Size:	2.0 μm / 2.7 μm / 5.0 μm
Pore Size:	90 Å
Carbon Load:	4.8% / 5.4% / 3.7%
Surface Area:	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
pH Range:	2 - 9
Max Temperature at Low pH:	60 °C
Max Temperature at High pH:	40 °C
Endcapped:	Yes

### Chromatographic properties\*

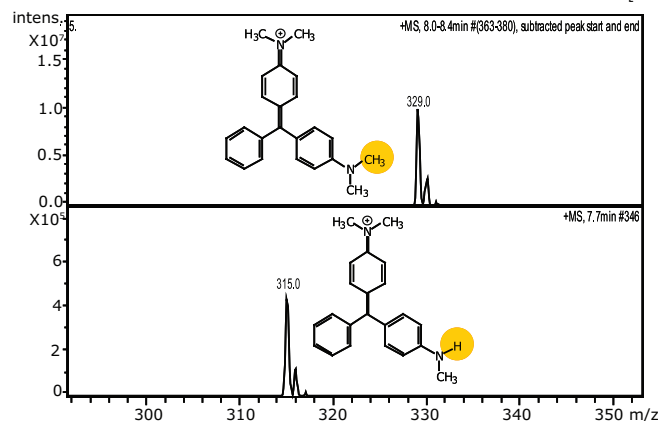
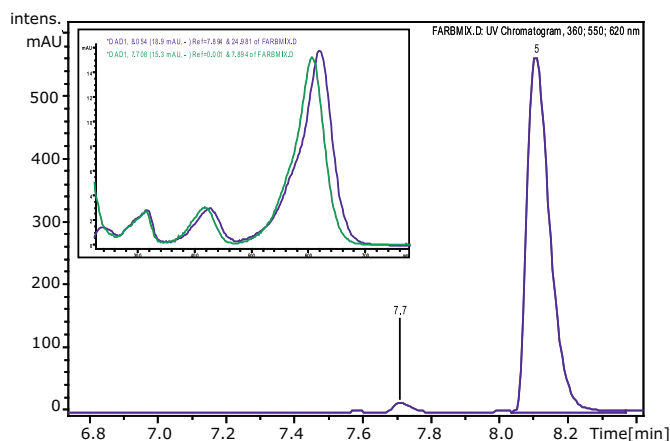


\*Tanaka test modified by Euerby (ref. Journal of Chromatography A, 994 (2003) 13-36)



## Expanded View of UV Chromatogram Showing Unknown Impurity at 7.7 min and Malachite Green

The inset shows the UV spectra of malachite green (purple) and the unknown impurity (green). The mass spectra are of malachite green (top) and unknown impurity (bottom).



Peak No.	Structure	Name / Exact Mass
1		Parafuchsin C <sub>19</sub> H <sub>17</sub> N <sub>3</sub> 287.142247
2		Basic Fuchsin C <sub>20</sub> H <sub>19</sub> N <sub>3</sub> 301.157897
3		Methylfuchsin C <sub>21</sub> H <sub>21</sub> N <sub>3</sub> 315.173547
4		Newfuchsin C <sub>22</sub> H <sub>23</sub> N <sub>3</sub> 329.189197
5		Malachite Green C <sub>23</sub> H <sub>25</sub> N <sub>2</sub> 329.201773
6		Sudan III C <sub>22</sub> H <sub>16</sub> N <sub>4</sub> O 352.132411
7		Sudan 410 C <sub>26</sub> H <sub>24</sub> N <sub>4</sub> O 408.195011

### Chromatographic conditions

**Column:** Ascentis® Express C8, 10 cm × 4.6 mm I.D., 2.7 μm (53837-U)

**Mobile phase** [A] water with 0.1% formic acid  
[B] acetonitrile:methanol (90:10)

Time	%A	%B
0.0	75	25
1.5	75	25
15.0	2	98
22.0	2	98
25.0	75	25

**Flow rate:** 0.8 mL/min

**Temp:** 55 °C

**UV DAD:** 200–950 nm

**MS:** ESI(+), SPS target 500 m/z, stability 100%, trap lvl. 100%, optimize normal, range 100–1500 m/z, nebulizer 50 psi, dry gas 12 L/min, dry temp. 365 °C

**Injection volume:** 3 μL

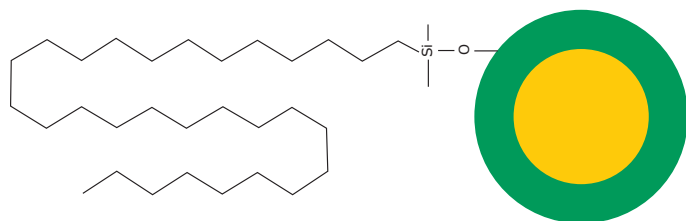
**Run time:** 25 min (5 min posttime)

**Sample:** Mix of dyes in methanol and acetonitrile

# Ascentis® Express C30

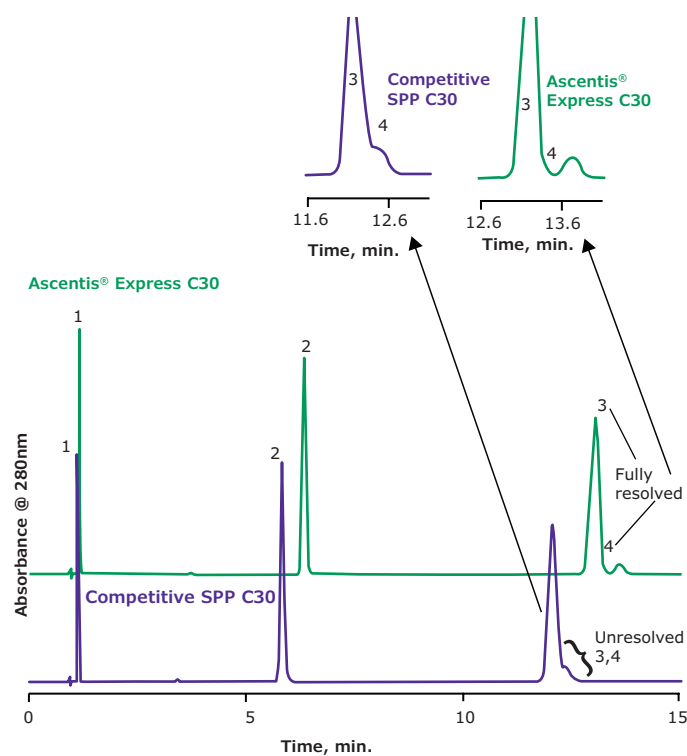
## Excellent selectivity for very hydrophobic compounds, long-chain and structurally related isomers

Ascentis® Express 160 Å C30 HPLC columns are highly suited for the reversed-phase separation of geometric and positional isomers. Ionizable compounds, such as acids and bases, are generally best separated with mobile phases buffered at pH of 2 to 3. The use of 20-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds.



### Separation of hydrophobic vitamins

These columns show increased retention and baseline resolution of vitamin K1 trans and cis isomers compared to a competitive SPP C30 column. Since the cis isomer of K1 is biologically inactive, it is important to know how much of each isomer is present in vitamin enriched products.



Ascentis® Express C30 Specifications	
<b>Silica:</b>	Type B(High purity silica)
<b>Particle Platform:</b>	Superficially porousparticles (SPP)
<b>Phase Chemistry:</b>	Triacetyldimethyl
<b>Particle Size:</b>	2.7 µm
<b>Pore Size:</b>	160 Å
<b>Carbon Load:</b>	4.5%
<b>Surface Area:</b>	90 m <sup>2</sup> /g
<b>pH Range:</b>	1-9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k(butylbenzene)

1.47

#### Shape Selectivity

k(triphenylene)/k(o-terphenyl)

1.48

#### H-Bonding Capacity

k(caffeine)/k(phenol)

0.60

#### Ion-Exchange Capacity (acidic)

k(benzylamine)/k(phenol)

0.14

\*Tanaka test modified by Euerby

### Chromatographic conditions:

<b>Columns:</b>	Ascentis® Express 160 Å C30, 15 cm x 4.6 mm I.D., 2.7 µm Competitive SPP C30, 15 cm x 4.6 mm I.D., 2.7 µm (577136-U);
<b>Mobile Phase:</b>	[A] Water; [B] Methanol
<b>Isocratic:</b>	95% B
<b>Flow Rate:</b>	1.5 mL/min
<b>Temperature</b>	25 °C
<b>Injection Volume</b>	1 µL
<b>Detection:</b>	PDA at 280 nm
<b>Vitamins</b>	1. Menadione (K3) 2. Menaquinone 4 (K2) 3. 2,3-trans-phyllquinone (K1) 4. cis-phyllquinone (K1)

# Ascentis<sup>®</sup> Express RP-Amide

## U/HPLC columns providing alternative selectivity to C18 and improved separation of basic compounds

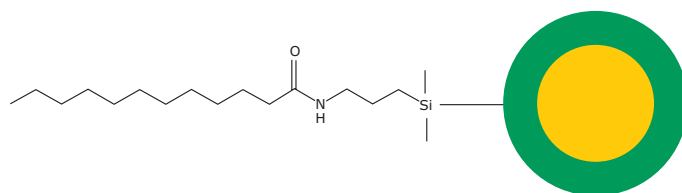
While the Ascentis<sup>®</sup> Express C8 and C18 provide classic reversed-phase selectivity, the RP-Amide phase provides besides an alternative reversed-phase selectivity:

- Improved peak shape for basic compounds
- 100% aqueous compatibility
- LC-MS compatibility
- Fast separation with Fused-Core<sup>®</sup> technology

The one-step RP-Amide bonding chemistry provides benefits in terms of selectivity, aqueous stability, and improved peak shape for bases.

### Alternative Selectivity

Ascentis<sup>®</sup> Express RP-Amide provides increased selectivity for polar compounds, especially those that can act as a hydrogen bond donor. 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 the figure below. The phenolic nature of catechols and resorcinols provides a good test for demonstrating enhanced selectivity of the RP-Amide phase. The RP-Amide phase shows complete baseline resolution of these related compounds while the C18 phase shows reduced retention, resolution, and selectivity for the phenolics. The selectivity differences between the RP-Amide and the C18 can be a useful tool in method development. In many cases, when peaks co-elute on a C18 phase, the RP-Amide can be substituted to achieve separation without a change in mobile phase.



### Ascentis<sup>®</sup> Express RP-Amide Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Amide-C16
<b>Particle Size:</b>	2.0 μm / 2.7 μm / 5.0 μm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	7.3% / 8.3% / 5.1%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k (butylbenzene)

1.43

#### Shape Selectivity

k (triphenylene)/k (o-terphenyl)

1.75

#### H-Bonding Capacity

k (caffeine)/k (phenol)

0.47

#### Ion-Exchange Capacity (acidic)

k (benzylamine)/k (phenol)

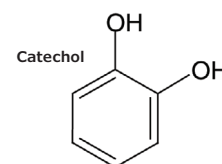
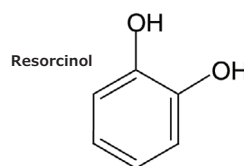
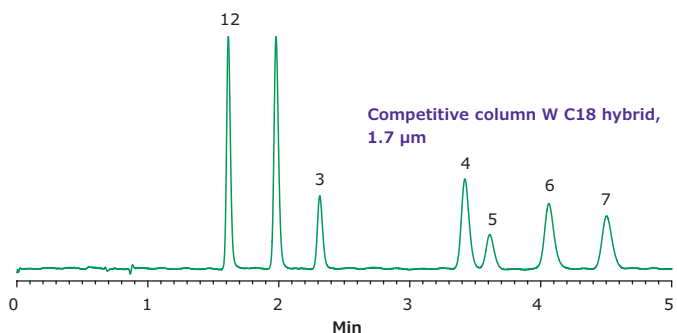
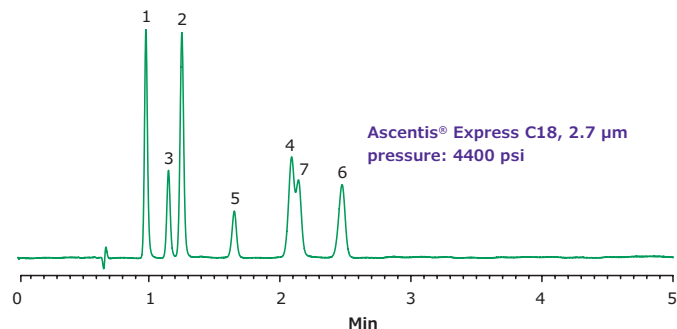
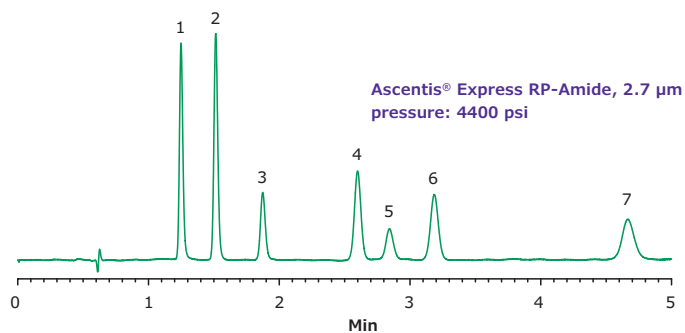
0.10

\*Tanaka test modified by Euerby

## Separation of phenolic compounds resorcinol and var. catechols on RP-Amide and C18

### Chromatographic conditions

<b>Columns:</b>	as indicated; 10 cm x 2.1 mm I.D.
<b>Mobile Phase:</b>	[A] 20 mM phosphoric acid, pH 2 (unadjusted) [B] water [C] acetonitrile
<b>Mobile Phase Ratios:</b>	A:B:C = 75:5:20
<b>Flow Rate:</b>	0.3 mL/min.
<b>Temp.:</b>	35° C
<b>Det.:</b>	270 nm
<b>Injection:</b>	1 µL
<b>Sample:</b>	50 mg/L ea. in 20 mM phosphoric acid



1. Resorcinol
2. Catechol
3. 2-Methylresorcinol
4. 4-Methylcatechol
5. 2,5-Dimethylresorcinol
6. 3-Methylcatechol
7. 4-Nitrocatechol

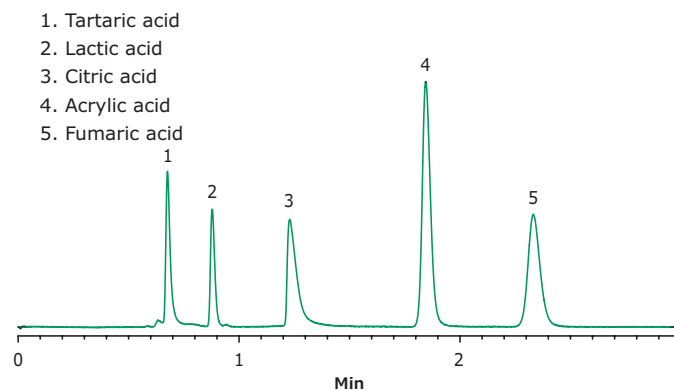
## Aqueous Compatible Reversed-Phase Column

Ascentis® Express RP-Amide U/HPLC column provides stable and reproducible analyte retention in 100% aqueous mobile phases. Below the separation of a mix of organic acids analyzed under 100% aqueous

conditions. Excellent selectivity and peak shape is noted for all the test probes, even citric acid, which is a notoriously difficult analyte.

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express RP-Amide, 10 cm x 2.1 mm I.D. 2.7 µm
<b>Mobile Phase:</b>	0.1% TFA (v/v) in water
<b>Flow Rate:</b>	0.3 mL/min.
<b>Temp.:</b>	35 °C
<b>Det.:</b>	210 nm
<b>Injection:</b>	1 µL
<b>Sample:</b>	in mobile phase; tartaric acid, 2 g/L; lactic acid, citric acid, 4 g/L; acrylic acid, 0.5 g/L; fumaric acid, 0.2 g/L

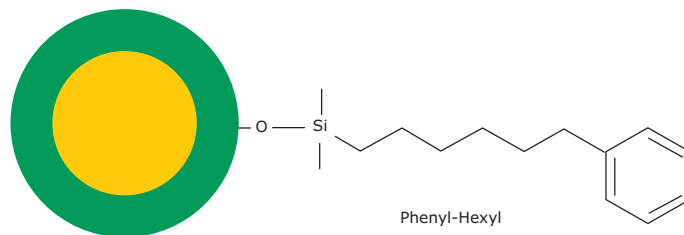




# Ascentis<sup>®</sup> Express Phenyl-Hexyl

## Improved reversed phase selectivity for polar aromatics and heterocyclic compounds

The phenyl phase of the Ascentis<sup>®</sup> Express Phenyl-Hexyl U/HPLC column has unique reversed phase selectivity, particularly for polar aromatics and heterocyclic compounds, which arises from solute interaction with the aromatic ring and its delocalized electrons. The Ascentis<sup>®</sup> Express Phenyl-Hexyl column is complementary (orthogonal) to both the C18 and RP-Amide Ascentis<sup>®</sup> Express phases because of this unique aromaticity. Phenyl phases also tend to exhibit good shape selectivity, which may originate from solute multipoint interaction with the planar rigid phenolic ring system. More retention and selectivity will often be observed for analytes with an aromatic ring with electron-withdrawing groups attached (e.g. fluorine, nitro, etc.) or with a delocalized heterocyclic ring system such as the benzodiazepine compounds.



### Ascentis<sup>®</sup> Express Phenyl-Hexyl Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Dimethylphenyl-hexyl
<b>Particle Size:</b>	2.0 $\mu\text{m}$ / 2.7 $\mu\text{m}$ / 5.0 $\mu\text{m}$
<b>Pore Size:</b>	90 $\text{\AA}$ and 160 $\text{\AA}$
<b>Carbon Load:</b>	6.3% / 7.1% / 5.2%
<b>Surface Area:</b>	120 $\text{m}^2/\text{g}$ / 135 $\text{m}^2/\text{g}$ / 90 $\text{m}^2/\text{g}$
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 $^{\circ}\text{C}$
<b>Max Temperature at High pH:</b>	40 $^{\circ}\text{C}$
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k (butylbenzene)



#### Shape Selectivity

k (triphenylene)/k (o-terphenyl)



#### H-Bonding Capacity

k (caffeine)/k (phenol)



#### Ion-Exchange Capacity (acidic)

k (benzylamine)/k (phenol)

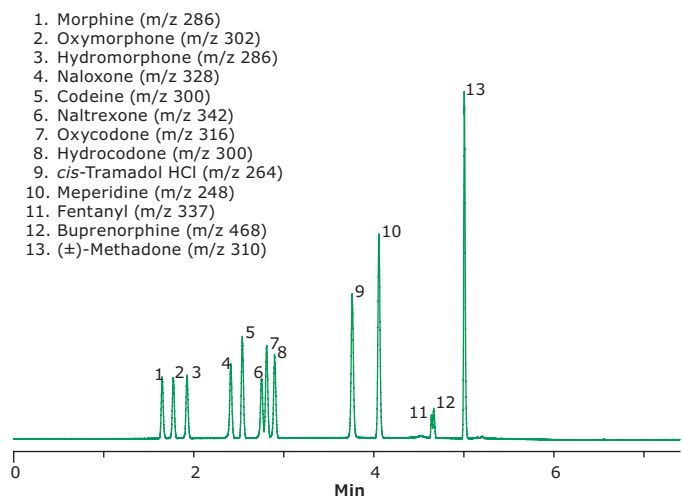


\*Tanaka test modified by Euerby

## Rapid LC-MS Analysis of Pain Management Opioids on Ascentis<sup>®</sup> Express Phenyl-Hexyl

### Chromatographic conditions

<b>Column:</b>	Ascentis <sup>®</sup> Express Phenyl-Hexyl 10 cm x 2.1 mm, 2.7 $\mu\text{m}$ particles (53336-U)
<b>Column Temp.:</b>	30 $^{\circ}\text{C}$
<b>Mobile Phase:</b>	[A] water with 0.1% formic acid; [B] methanol with 0.1% formic acid
<b>Gradient:</b>	10 to 45% B in 3 min; to 100% B in 2 min; held for 2.4 min
<b>Flow Rate:</b>	0.3 mL/min
<b>Pressure:</b>	6940 psi (478 bar)
<b>Sample:</b>	Pain Management $\mu\text{Lti}$ -Component Opiate Mixture-13 (P-071) dilute to 30-300 ng/mL in 99:1, water:methanol
<b>Injection:</b>	2 $\mu\text{L}$
<b>Detector:</b>	MS, ESI(+), SIR

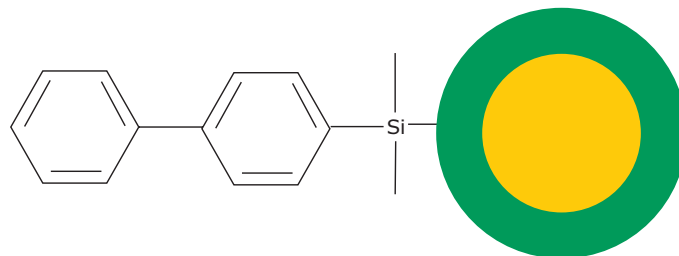


# Ascentis® Express Biphenyl

## U/HPLC columns providing Enhanced selectivity for aromatic compounds

The Ascentis® Express Biphenyl U/HPLC column offers extra separation power for the optimization of compounds that are challenging to resolve or elute early on C18 and other phenyl phases.

The bonded, densely endcapped, dimethyl-biphenyl stationary phase, provides a stable, reversed phase packing with enhanced pi-pi and mild steric interactions due to the two sequential phenyl groups bonded to the base silica. Ascentis® Express Biphenyl column can be used for basic, acidic, or neutral compounds.



### Chromatographic properties\*

#### Hydrophobicity

$k(\text{pentylbenzene})/k(\text{butylbenzene})$



#### Shape Selectivity

$k(\text{triphenylene})/k(\text{o-terphenyl})$



#### H-Bonding Capacity

$k(\text{caffeine})/k(\text{phenol})$



#### Ion-Exchange Capacity (acidic)

$k(\text{benzylamine})/k(\text{phenol})$



\*Tanaka test modified by Euerby

### Ascentis® Express Biphenyl Specifications

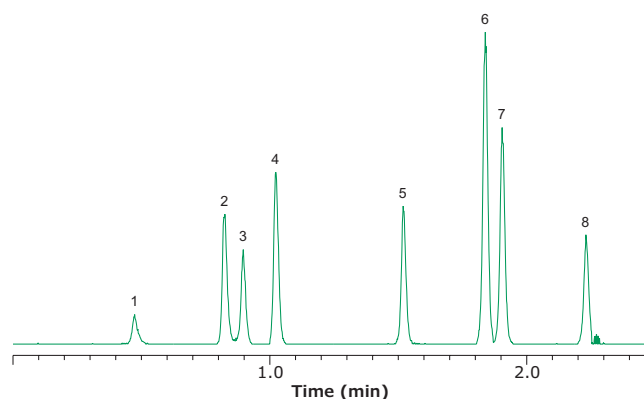
<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Dimethylbiphenyl
<b>Particle Size:</b>	2.0 µm / 2.7 µm / 5.0 µm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	6.7% / 7.0% / 5.5%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

## LC-MS Analysis of a Pain Panel using an Ascentis® Express Biphenyl Column

The retention and selectivity offered by the biphenyl column are ideal for rapid, efficient drug and metabolite analysis using conditions that are compatible with MS detection

### Chromatographic conditions

<b>column:</b>	Ascentis® Express Biphenyl, 5 cm x 2.1 mm I.D., 2.7 µm (64057-U)
<b>mobile phase:</b>	[A] water with 0.1% formic acid; [B] acetonitrile with 0.1% formic acid
<b>gradient:</b>	5% B to 100% B in 5 min
<b>flow rate:</b>	0.5 mL/min
<b>pressure:</b>	1320 psi (91 bar)
<b>column temp.:</b>	60 °C
<b>detector:</b>	MS-TOF, ESI+, XIC
<b>injection:</b>	0.5 µL
<b>sample:</b>	500 ng/mL in 99:1, water:methanol



Peak	rt	Analyte	m/z
1	0.474	Normorphone	288.0898
2	0.825	6a-Naloxol	330.1339
3	0.897	Naloxone	328.1178
4	1.023	6b-Naltrexol	344.1495
5	1.519	Norbuprenorphine	414.2246
6	1.839	Fentanyl	337.1915
7	1.904	Buprenorphine	468.2691
8	2.230	(-)-11-nor-9-Carboxy-Δ9-THC	345.1692

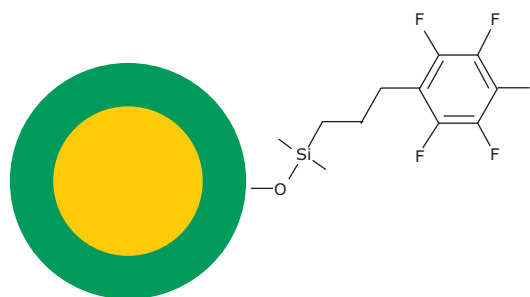
# Ascentis<sup>®</sup> Express F5 (pentafluorophenyl, PFP)

## Enhanced selectivity for structural isomers and closely related compounds

The pentafluorophenylpropyl stationary phase of the Ascentis<sup>®</sup> Express F5 U/HPLC column provides a stable, reversed-phase packing with electron-deficient phenyl rings due to the presence of electronegative fluorines. In addition to forming pi-pi and mildly steric interactions, F5 phases also retain compounds by polar interactions. As a result of having both polar and non-polar character, F5 phases can show dual-mode retention behavior, sometimes producing a “U-shaped” retention as a function of acetonitrile content of the mobile phase, with retention increasing at both low and high concentrations of ACN (reversed-phase and HILIC retention modes). The Ascentis<sup>®</sup> Express F5 phase can be used for basic, acidic, or neutral compounds and provides and orthogonal selectivity to C18.

- In comparison to the C18 phases, the F5 phase shows longer retention time of basic analytes and less retention of hydrophobic analytes
- Suitable for-phase, HILIC, and 100% aqueous applications
- Stable, low bleed for LC-MS and LC-UV

Chromatographers are often faced with the challenge of separating compounds that are very similar in solubility. Separation on non-polar phases such as C18 is driven by differential partitioning of analytes, therefore, the alkyl phases are often ineffective in meeting this challenge.



### Ascentis<sup>®</sup> Express F5 Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	pentafluorophenylpropylsilane
<b>Particle Size:</b>	2.0 μm / 2.7 μm / 5.0 μm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	5.3% / 5.5% / 3.9%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	2 - 9
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k(butylbenzene)

1.26

#### Shape Selectivity

k(triphenylene)/k(o-terphenyl)

2.51

#### H-Bonding Capacity

k(caffeine)/k(phenol)

0.56

#### Ion-Exchange Capacity (acidic)

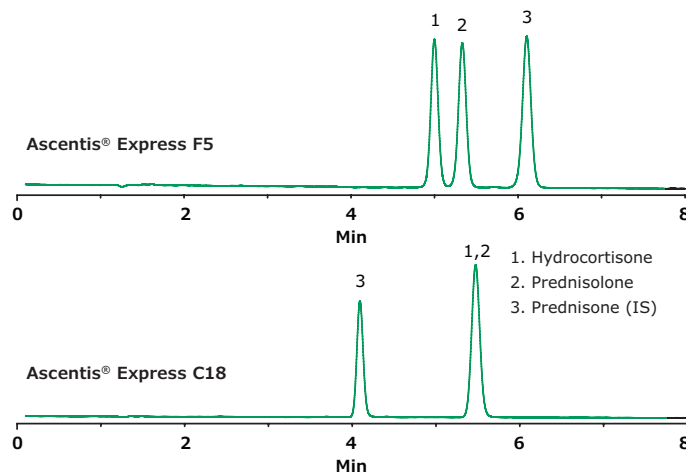
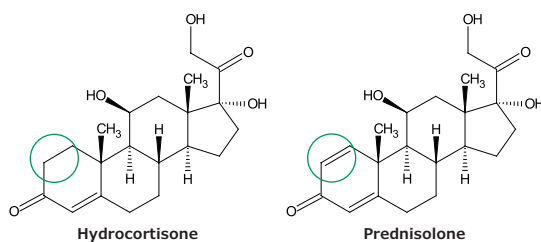
k(benzylamine)/k(phenol)

0.25

\*Tanaka test modified by Euerby

## Separation of Closely Related Compounds the F5 Phase

Hydrocortisone and prednisolone differ by a single double bond. Their solubilities are very similar; however, their shapes differ significantly. The application example shows a comparison of their separation (along with prednisone internal standard (IS) using both a C18 and an F5 stationary phase. The fluorinated phase, apparently due to the enhanced shape selectivity, is shown to provide the separation of these closely related compounds.



### Chromatographic conditions

**Column(s):** Ascentis® Express F5, 10 cm x 4.6 mm, I.D., 2.7 µm particle size (53590-U)

Ascentis® Express C18, 10 cm x 4.6 mm, I.D., 2.7 µm (53827-U)

**Mobile Phase:** [A] water  
[B] methanol

**Mobile Phase Ratio:** A:B 50:50, v/v

**Flow Rate:** 0.8 mL/min

**Temp.:** 35 °C

**Pressure:** ~2400 psi

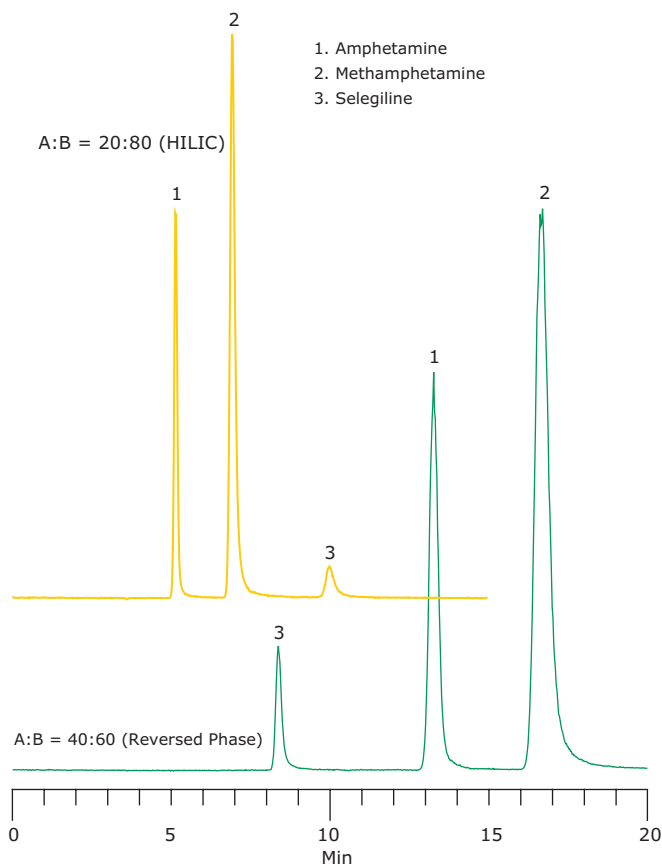
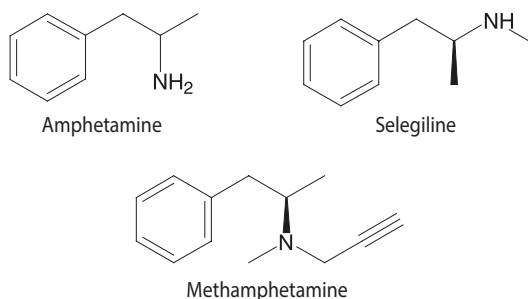
**Det.:** UV at 240 nm

**Injection:** 5 µL

**sample:** 10 µg/mL each in 90:10 water:methanol

## Separation of selegiline and amphetamines using an Ascentis® Express F5 column in Reversed Phase and HILIC mode

The multi-modal retention mechanisms in HILIC, which offers orthogonal selectivity to reversed-phase, is evident in the following separation of selegiline and amphetamines on an Ascentis® Express F5 column. Selegiline under HILIC conditions elutes last, whereas under reversed phase conditions it elutes first. Selegiline therefore is retaining primarily based in RP partitioning, whereas the amphetamines are retaining primarily by IEX.



### Chromatographic conditions

**Column:** Ascentis® Express F5, 10 cm x 4.6 mm I.D., 2.7 µm particles (53590-U)

**Mobile Phase:** [A] 10 mM ammonium acetate, pH 4.0 with acetic acid; [B] acetonitrile; (20:80, A:B - HILIC), (40:60, A:B - reversed phase)

**Flow Rate:** 0.6 mL/min

**Pressure:** 260 bar (2300 psi)

**Temp.:** 35 °C

**Det.:** MS ESI (+), SIR m/z 136, 150, 188

**Injection:** 2 µL

**Sample:** 10 µg/mL in methanol

# Ascentis® Express columns for HILIC mode

The separation of polar analytes continues to be an exceptional challenge to scientists. Reversed phase (RP) chromatography, though most commonly applied, is not well-suited for analytes that are hydrophilic, due to poor retention. HILIC (hydrophilic interaction liquid chromatography) is a technique that has been adopted for analysis of these hydrophilic analytes by researchers over the last 20 years, owing to its complimentary nature to reversed phase (RP) and normal phase (NP) chromatography. HILIC often provides retention and selectivity that RP and NP techniques lack and can successfully be used to improve separation and resolution of very polar analytes by improving their retention. HILIC can also provide a more suitable separation for mixtures of polar, hydrophilic and ionizable compounds. In addition, It may also provide increased LC-MS response, due to the high organic mobile phase applied. These benefits have made HILIC a potential solution for separation of polar analytes and an alternate technique to RP for challenging separations.

Four different stationary phases of Ascentis® Express columns can be used to separate of basic, acidic, or neutral polar compounds in HILIC (Hydrophilic Interaction Liquid Chromatography) mode:

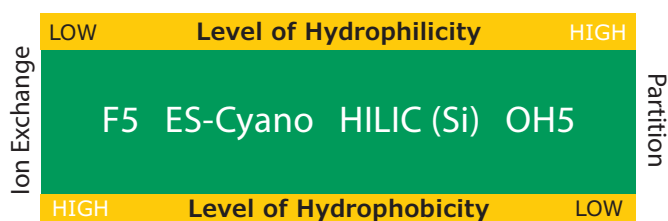
**OH5:** Pentahydroxy phase

**F5:** Pentafluorophenylpropyl stationary phase

**ES-Cyano:** Cyano linked by a propyl chain

**HILIC (Si):** Bare silica

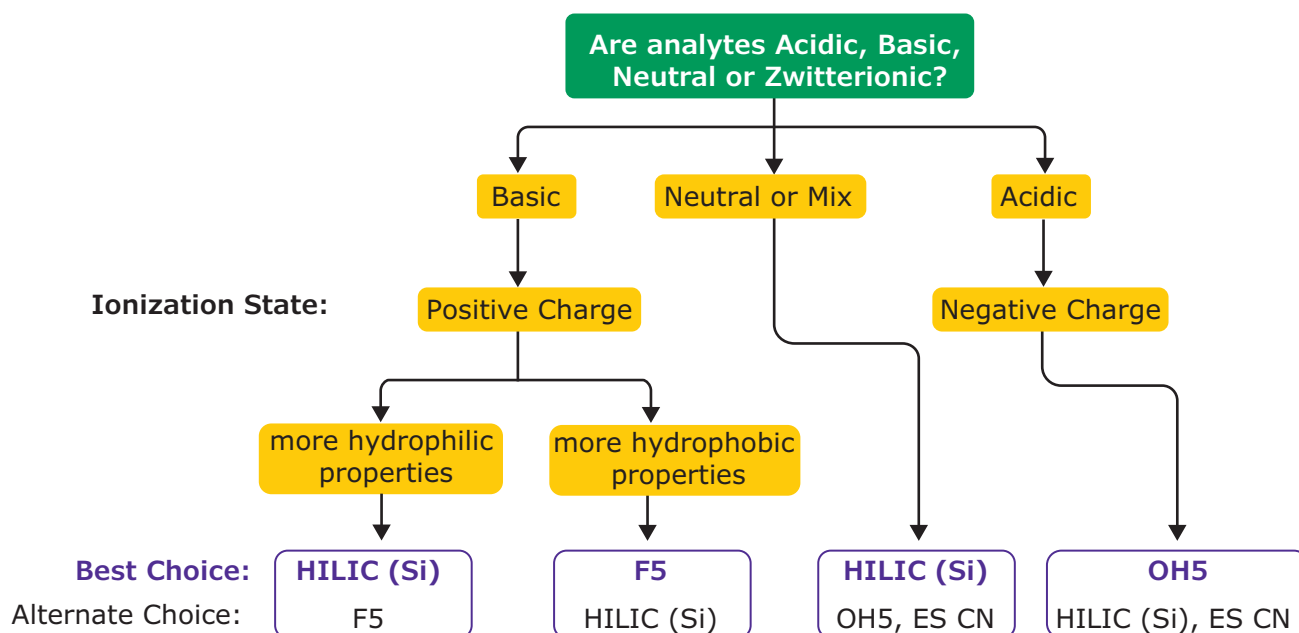
## Relative Hydrophilicity of Ascentis® Express HILIC Phases



## Reference

1. Grushka, E., Behhaim, D., *J. Chromatogr., A.* 2010, 1217, 65-74.

## HILIC Phase Selection Guide

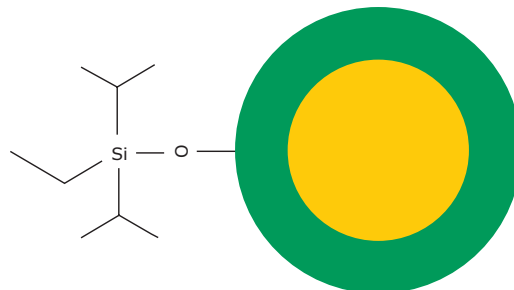
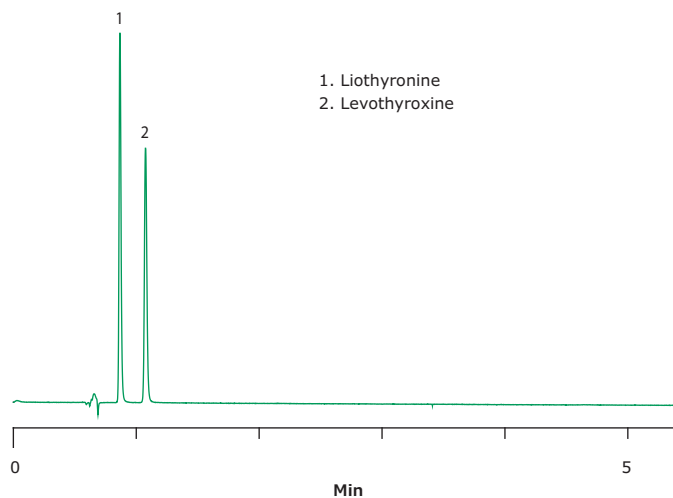
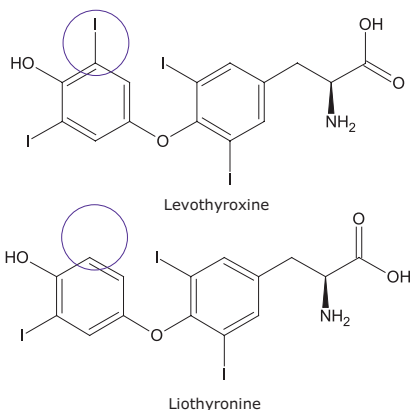


# Ascentis® Express ES-Cyano

## Stable, reversed phase packing for basic, acidic or neutral compounds

Ascentis® Express ES-Cyano phase is moderately polar in nature and highly suited for the separation of acids, bases, and neutral analytes. Ascentis® Express ES-Cyano U/HPLC columns use a steric-protected cyano bonded-phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the cyanopropyl chain to the surface. The combination of low pH and elevated temperature operation of the column is therefore well tolerated. Ascentis® Express ES-Cyano columns offers the following key advantages in HILIC mode:

- Ion-exchange mechanism in a HILIC mode
- Stable at low pH and high temperature
- Ideal for non-polar bases in HILIC mode



### Ascentis® Express ES-Cyano Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Diisopropylcyanopropyl
<b>Particle Size:</b>	2.0 µm / 2.7 µm / 5.0 µm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	3.4% / 3.5% / 2.5%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	1 - 8
<b>Max Temperature at Low pH:</b>	80 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

### Chromatographic properties\*

#### Hydrophobicity

k (pentylbenzene)/k (butylbenzene)

1.22

#### Shape Selectivity

k (triphenylene)/k (o-terphenyl)

2.56

#### H-Bonding Capacity

k (caffeine)/k (phenol)

0.47

#### Ion-Exchange Capacity (acidic)

k (benzylamine)/k (phenol)

0.19

\*Tanaka test modified by Euerby

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express ES-Cyano, 10 cm x 2.1 mm I.D., 2.7 µm (53473-U)
<b>Mobile Phase:</b>	[A] water with 0.05% phosphoric acid; [B] acetonitrile with 0.05% phosphoric acid; (60:40, A:B)
<b>Flow Rate:</b>	1.5 mL/min
<b>Pressure:</b>	3270 psi
<b>Temp.:</b>	30 °C
<b>Detector:</b>	UV, 225 nm
<b>Injection:</b>	5 µL
<b>sample:</b>	20 µg/mL in mobile phase

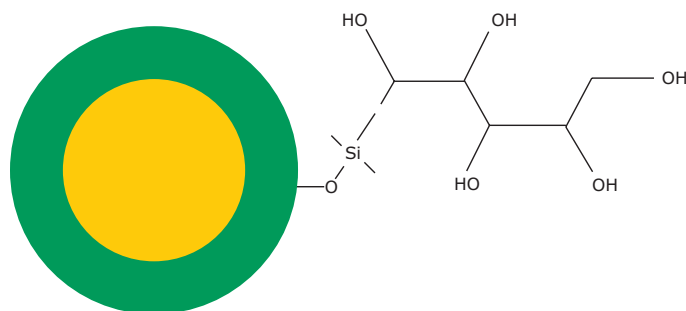
# Ascentis® Express OH5

## Pentahydroxy phase for fast analysis of polar compounds

The Ascentis® Express OH5 phase is a highly polar ligand that possesses 5 hydroxyl groups tethered to the silica via novel proprietary linkage phase chemistry. The unique phase being bonded to the Fused-Core® particle exhibits enhanced retention and performance, delivering strong HILIC partitioning interaction and only limited ion exchange retention.

- Exhibits predominantly HILIC partitioning retention, limited silanol anionic character, and is relatively insensitive to ionic strength
- High column stability

The impact of partitioning and ion exchange in HILIC separations is demonstrated by a mixture of acidic, neutral, and basic polar compounds. All three analytes have similar log D values, both acid and base are ionized at mobile phase pH.



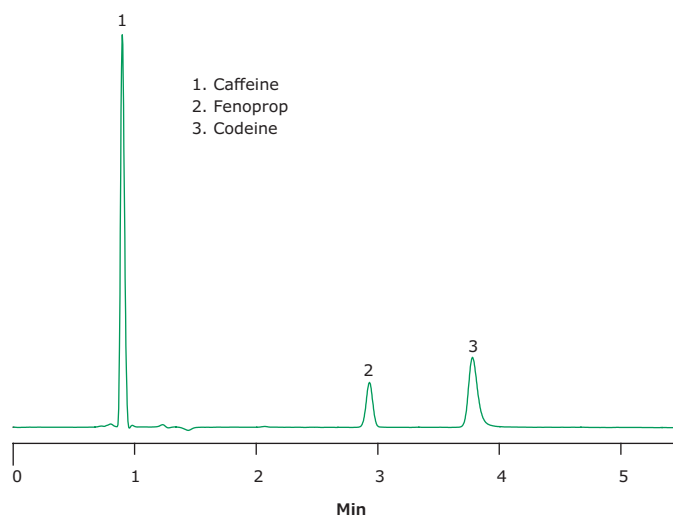
### Ascentis® Express OH5 Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	Pentahydroxy
<b>Particle Size:</b>	2.0 µm / 2.7 µm / 5.0 µm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	2.8% / 3.2% / 2.1%
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	1 - 8
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	Yes

## Mixed Polar Compounds on Ascentis® Express OH5 U/HPLC column

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express OH5, 10 cm x 3.0 mm I.D., 2.7 µm particles (53769-U)
<b>Mobile Phase:</b>	5 mM ammonium formate (95:5 acetonitrile:water) pH 6.8
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	965 psi
<b>Temp.:</b>	30 °C
<b>Det.:</b>	254 nm
<b>Injection:</b>	1 µL
<b>Sample:</b>	100 µg/mL



# Ascentis® Express HILIC (Si)

## U/HPLC columns for Separation of polar compounds

Ascentis® Express HILIC (Si) offers mainly high surface area and high surface deactivation, which combine to give Ascentis® Express Silica an exceptional performance as a HILIC phase. Besides being the underlying support for all Ascentis® Express phases, Ascentis® Express HILIC (Si) has applications in its own right. Silica is widely used to separate positional isomers and polar compounds in normal phase mode. In each case, a high purity, controlled and uniform surface is necessary to impart the desirable chromatographic performance.

- High-loading capacity
- Offers both ion-exchange and partition mechanisms of separation in HILIC mode
- Ultra-pure silica
- Ideal for the separation of polar compounds

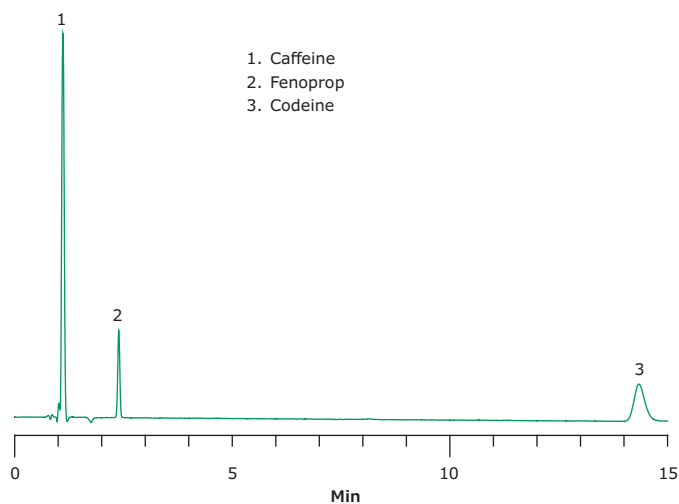


### Ascentis® Express HILIC (Si) Specifications

<b>Silica:</b>	Type B (High purity silica)
<b>Particle Platform:</b>	Superficially porous particles (SPP)
<b>Phase Chemistry:</b>	n. a.
<b>Particle Size:</b>	2.0 µm / 2.7 µm / 5.0 µm
<b>Pore Size:</b>	90 Å
<b>Carbon Load:</b>	n. a.
<b>Surface Area:</b>	120 m <sup>2</sup> /g / 135 m <sup>2</sup> /g / 90 m <sup>2</sup> /g
<b>pH Range:</b>	1 - 8
<b>Max Temperature at Low pH:</b>	60 °C
<b>Max Temperature at High pH:</b>	40 °C
<b>Endcapped:</b>	

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express HILIC (Si), 10 cm x 3.0 mm I.D., 2.7 µm (53970-U)
<b>Mobile Phase:</b>	5 mM ammonium formate (95:5 acetonitrile:water) pH 6.8
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	855 psi
<b>Temp.:</b>	30 °C
<b>Det.:</b>	254 nm
<b>Injection:</b>	1 µL
<b>Sample:</b>	100 µg/mL





# Application Examples

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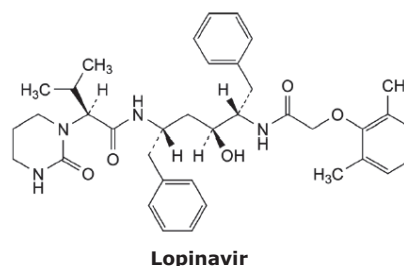
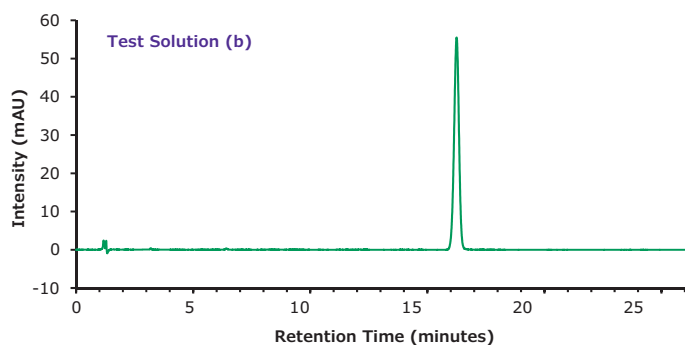
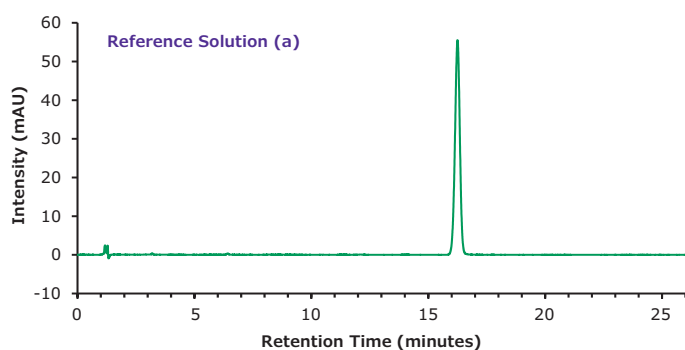
# High resolution and speed in pharmaceutical applications

## Lopinavir – Assay Method Ph. Eur.

### Introduction

This paper illustrates how it is possible to set-up the assay method for Lopinavir testing following the current European pharmacopeia guidelines (10.2). The monograph assay method calls for a column with  $l = 0.25$  m,  $\varnothing = 4.6$  mm end-capped octadecylsilyl silica gel for chromatography with  $4 \mu\text{m}$  particle size. No particular HPLC column is referenced in the Ph. Eur. knowledge database for assay method, and the method is of isocratic nature. This gives a chance

to replace the monograph column geometry/particle size with a shorter and faster alternative column (up to 70% reduction in length) packed with smaller particles (up to 50% reduction). This can save valuable time, and at the same time you can benefit from improved separation efficiency, which typically translates into better method performance and sensitivity. In this study, the limit of detection (LOD) is better than 1 ppm using HPLC-UV detection.

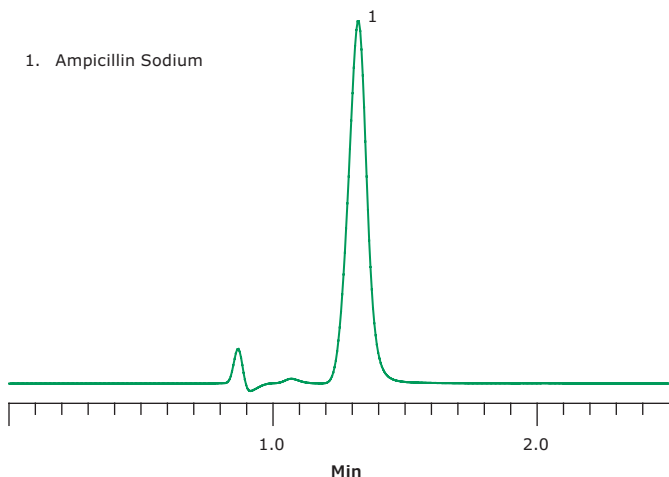


No.	Compound	Retention Time (min)	Tailing Factor
1	$t^0$ void volume	1.1	
2	Lopinavir CRS	16.2	0.97

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 15 cm x 4.6 mm ID, 2.7 $\mu\text{m}$
<b>Detection:</b>	UV = 215 nm (micro flow cell; 1.4 $\mu\text{L}/7\text{mm}$ )
<b>Mobile phase A:</b>	Acetonitrile/phosphate buffer solution 45/55 (v/v)
<b>Buffer preparation:</b>	Dissolve 0.9 g of dipotassium hydrogen phosphate and 2.7 g of potassium dihydrogen phosphate in 900 mL of water and mix well. Adjust to pH 6.0 with phosphoric acid, dilute to 1000 mL with water and filter.
<b>Injection:</b>	12 $\mu\text{L}$
<b>Flow Rate:</b>	1.0 mL/min
<b>Temperature:</b>	50 °C
<b>Pressure Drop:</b>	153 bar (2219 psi)
<b>Solvent mixture:</b>	Acetonitrile/water 50/50 (v/v)
<b>Test solution (a):</b>	Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100 mL with the solvent mixture.
<b>Test solution (b):</b>	Dilute 5.0 mL of the test solution (a) to 100 mL with the solvent mixture.
<b>Reference solution (a):</b>	Dissolve 50.0 mg of Lopinavir CRS in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 5 mL of this solution to 100 mL with the solvent mixture.

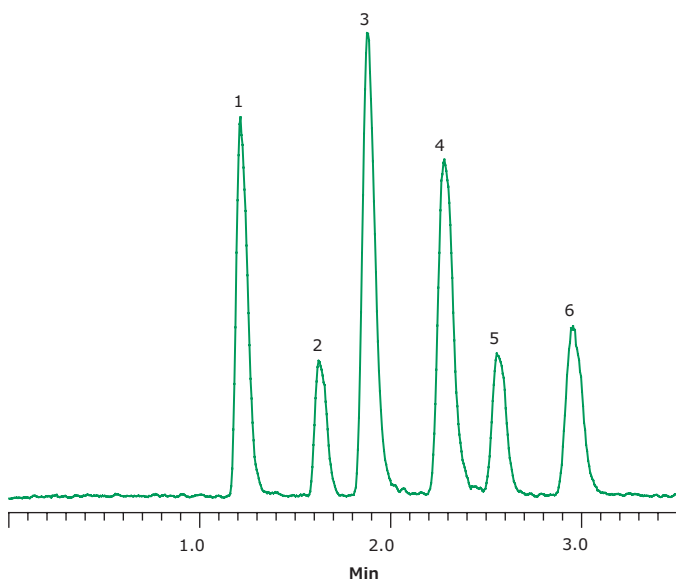
## USP HPLC Analysis of Ampicillin Sodium using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 4.6 mm I.D., 5 µm
<b>Column Temp.:</b>	30 °C
<b>Mobile Phase</b>	[A:B:C:D] [A] acetonitrile, [B] water, [C] 1M Potassium phosphate monobasic in water, [D] 1N acetic acid in water (80:9:10:1)
<b>Flow Rate:</b>	1.0 mL/min
<b>Sample:</b>	1 mg/mL in mobile phase
<b>Injection:</b>	10 µL
<b>Detector:</b>	UV, 254 nm

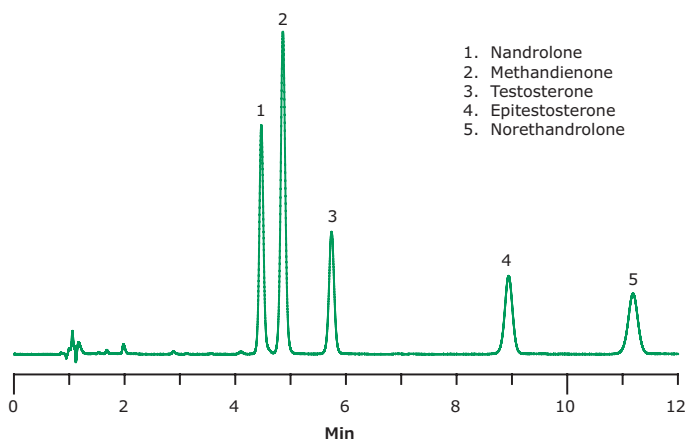
## Rapid Separation of Tricyclic Antidepressants using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
<b>Column Temp.:</b>	55 °C
<b>Mobile Phase:</b>	[A] 100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide), [B] water, [C] methanol (10:30:60, A:B:C)
<b>Flow Rate:</b>	0.3 mL/min
<b>Injection:</b>	1 µL
<b>Detector:</b>	ESI(+), m/z 250-320

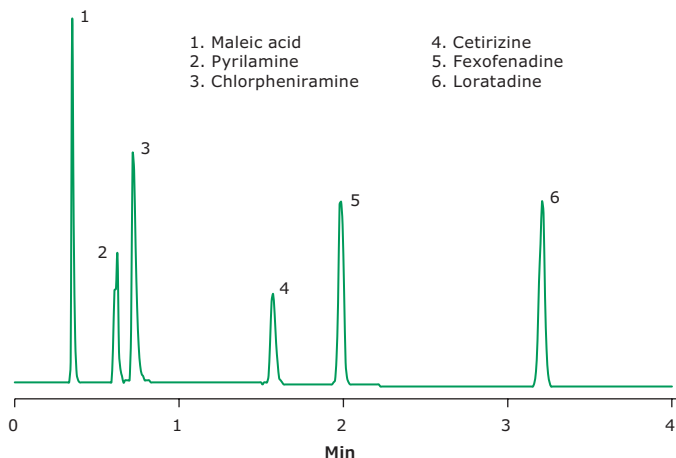
## HPLC Analysis of Steroids using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] water; [B] acetonitrile; (60:40, A:B)
<b>Flow Rate:</b>	0.2 mL/min
<b>Pressure:</b>	2944 psi (203 bar)
<b>Sample:</b>	50 mg/L in 75:25, water:methanol
<b>Injection:</b>	2 µL
<b>Detector:</b>	UV, 254 nm

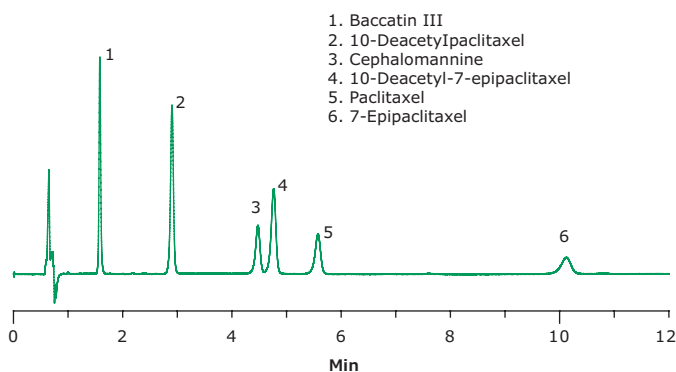
## Fast Separation of Antihistamines using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 3.0 mm I.D., 5 µm
<b>Column Temp.:</b>	40 °C
<b>Mobile Phase:</b>	[A] 0.02M sodium phosphate, pH 2.6; [B] methanol
<b>Gradient:</b>	50% B for 0.5 min; to 75% B in 2 min; held for 2 minutes
<b>Flow Rate:</b>	1.0 mL/min
<b>Pressure:</b>	2770 psi (191 bar)
<b>Sample:</b>	Dissolved in 20:80 water:methanol
<b>Injection:</b>	1.0 µL
<b>Detector:</b>	UV, 230 nm

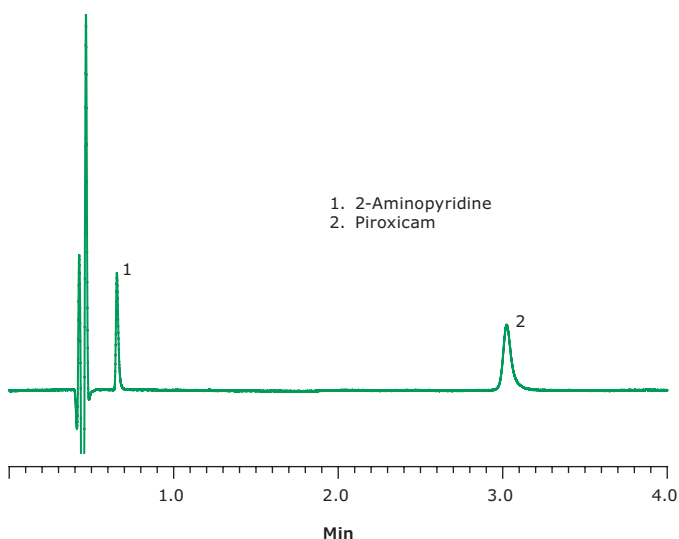
## HPLC Analysis of Taxols using an Ascentis® Express F5 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express F5, 10 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	30 °C
<b>Mobile Phase:</b>	[A] water; [B] acetonitrile; (60:40, A:B)
<b>Flow Rate:</b>	0.3 mL/min
<b>Sample:</b>	25 mg/L in 70:30, water:methanol
<b>Injection:</b>	2 µL
<b>Detector:</b>	UV, 227 nm

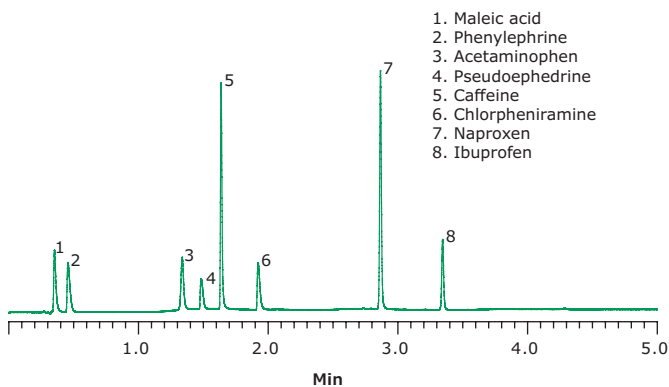
## UHPLC Analysis of Piroxicam and 2-Aminopyridine Impurity using an Ascentis® Express F5 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express 10 cm x 2.1 mm I.D., 2.0 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 10 mM ammonium formate pH 3.0 with formic acid; [B] acetonitrile; (75:25, A:B)
<b>Flow Rate:</b>	0.5 mL/min
<b>Pressure:</b>	7570 psi (522 bar)
<b>Sample:</b>	5 - 100 µg/mL in 90:10, water:methanol
<b>Injection:</b>	0.5 µL
<b>Detector:</b>	UV, 250 nm

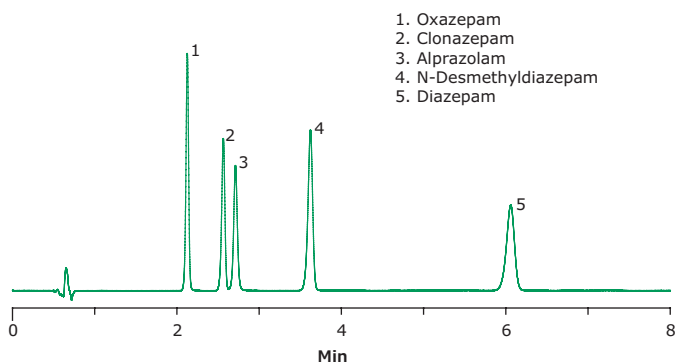
## HPLC Analysis of Acetaminophen, Caffeine, Chlorpheniramine, Ibuprofen, Naproxen, Phenylephrine, and Pseudoephedrine using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 5 mM ammonium phosphate monobasic, pH 2.0 with phosphoric acid: acetonitrile (98:2); [B] acetonitrile;
<b>Gradient:</b>	0 to 68% B in 3 min; held at 68% B for 2 min
<b>Flow Rate:</b>	0.4 mL/min
<b>Sample:</b>	100 µg/mL in 95:5, water: methanol
<b>Injection:</b>	1 µL
<b>Detector:</b>	UV, 210 nm

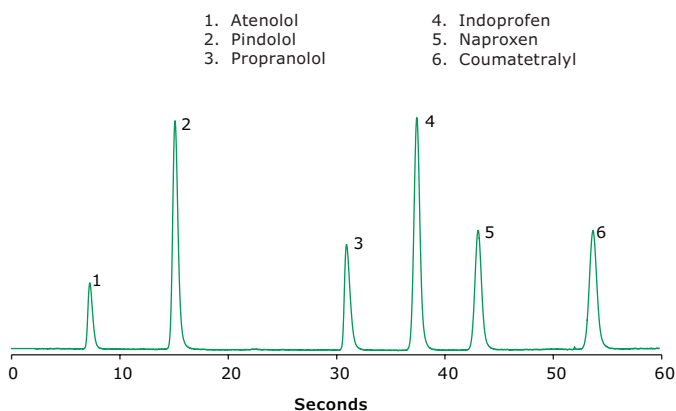
## HPLC Analysis of Benzodiazepines using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 3 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] water; [B] acetonitrile; (66:34, A:B)
<b>Flow Rate:</b>	0.6 mL/min
<b>Sample:</b>	0.1 g/L each in 20% methanol
<b>Injection:</b>	2 µL
<b>Detector:</b>	UV, 250 nm

## HPLC Analysis of Beta Blockers using an Ascentis® Express C18 Column with a 60 Second Gradient



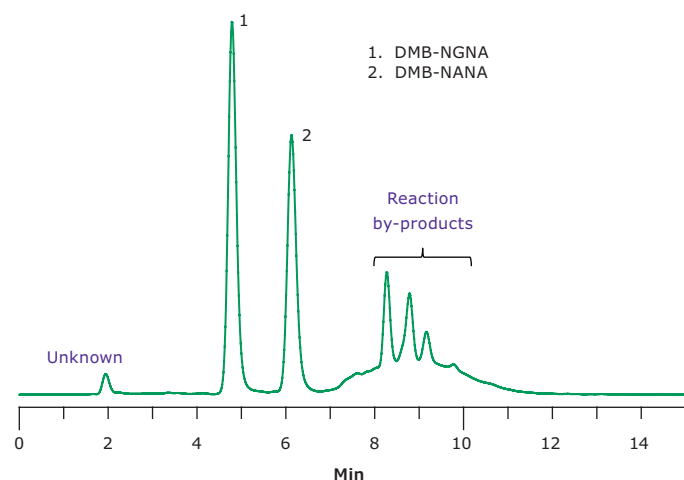
### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 2 cm x 2.1 mm I.D., 5 µm
<b>Column Temp.:</b>	40 °C
<b>Mobile Phase:</b>	[A] water/0.1% TFA; [B] acetonitrile/0.1% TFA
<b>Gradient:</b>	5-50% B in 60 seconds
<b>Flow Rate:</b>	2 mL/min
<b>Sample:</b>	0.17-0.33 mg/mL in 71% water/29% acetonitrile
<b>Injection:</b>	0.5 µL
<b>Detector:</b>	UV, 254 nm

## HPLC Analysis of DMB-Labeled Sialic Acids using an Ascentis® Express RP-Amide Column: Comparison of Biosimilars to Reference Materials

Sialic acids affect the bioavailability, function, stability, and metabolism of glycoproteins. Two forms of sialic acid are commonly present in therapeutic glycoproteins: N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA). One of the most common quantification methods involves releasing sialic acids from the glycoprotein, derivatizing NANA and NGNA with 1,2-diamino-4, 5-methylenedioxybenzene (DMB), and analyzing by C18-HPLC with fluorescence

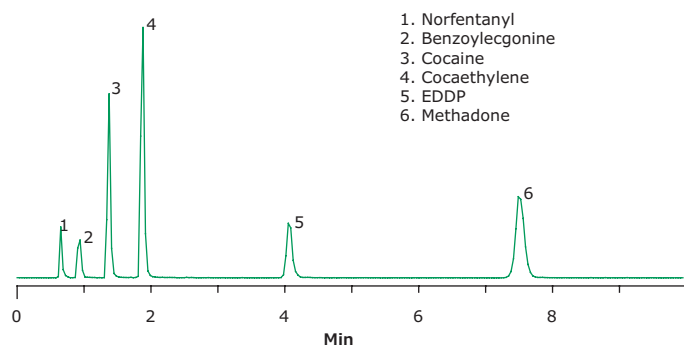
detection. This procedure is subject to interference from peaks originating from excess reagent and other derivatized impurities, limiting sensitivity and reproducibility. The objectives of this study were to develop a significantly improved HPLC-fluorescence method for DMB-NANA and DMB-NGNA, and to apply this method to compare two candidate biosimilar therapeutic proteins to their respective reference materials.



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	30 °C
<b>Mobile Phase:</b>	[A] Water, 0.1% formic acid, [B] acetonitrile, 0.1% formic acid
<b>Gradient:</b>	0-1 min 6% B; 1.01-4 min 20% B; 4.01-12 min 6% B, total run time 15 min
<b>Flow Rate:</b>	0.2 mL/min
<b>Pressure:</b>	1300 psi (89.6 bar)
<b>Sample:</b>	Mix of DMB-labeled NGNA and NANAsialic acid, 5 mg/mL each
<b>Injection:</b>	0.5 µL
<b>Detector:</b>	fluorescence, lambda excitation = 373 nm, lambda emission = 448 nm

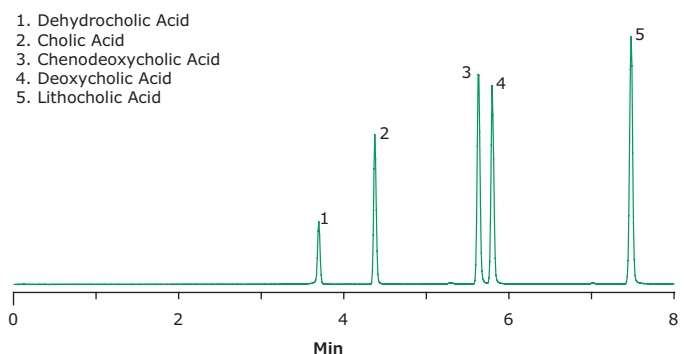
## LC-MS Analysis of Drugs of Abuse using an Ascentis® Express RP-Amide Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express RP Amide, 10 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 10 mM ammonium formate in water; [B] 10mM ammonium formate in acetonitrile; (75:25, A:B)
<b>Flow Rate:</b>	0.2 mL/min
<b>Sample:</b>	500 ng/mL in water
<b>Injection:</b>	2 µL
<b>Detector:</b>	ESI(+)

## HPLC Analysis of Bile Acids using an Ascentis® Express C18 Column

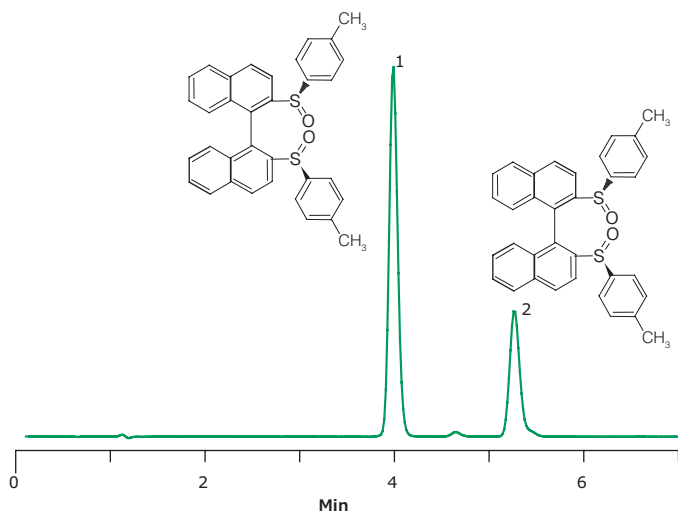


### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 5 cm x 4.6 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 10 mM ammonium acetate, pH 4 (adjusted with acetic acid); [B] acetonitrile; (80:20, A:B)
<b>Gradient:</b>	20 to 100% B in 10 min
<b>Flow Rate:</b>	1.0 mL/min
<b>Sample:</b>	10 µg/mL
<b>Injection:</b>	5 µL
<b>Detector:</b>	ESI(-) in SIR mode

## (1R)-2,2'-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1'-binaphthalene Diastereomer Separation using an Ascentis® Express C18 Column

1. (1R)-2,2'-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1'-binaphthalene
2. (1S)-2,2'-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1'-binaphthalene

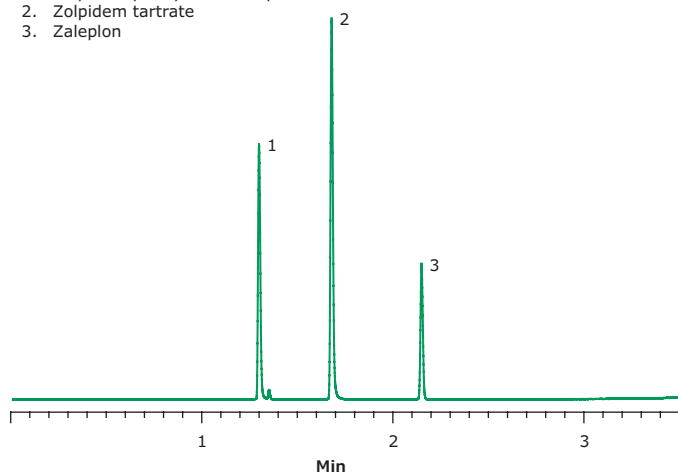


### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 4.6 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] water; [B] methanol; (25:75, A:B)
<b>Flow Rate:</b>	0.8 mL/min
<b>Pressure:</b>	2450 psi (169 bar)
<b>Sample:</b>	(1R)-2,2'-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1'-binaphthalene, 25 µg/mL in methanol; (1S)-2,2'-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1'-binaphthalene, 50 µg/mL in methanol
<b>Injection:</b>	5 µL
<b>Detector:</b>	UV, 240 nm

## Rapid HPLC Analysis of Z-Drugs using an Ascentis® Express C18 Column

1. Zolpidem phenyl-4-carboxylic acid
2. Zolpidem tartrate
3. Zaleplon

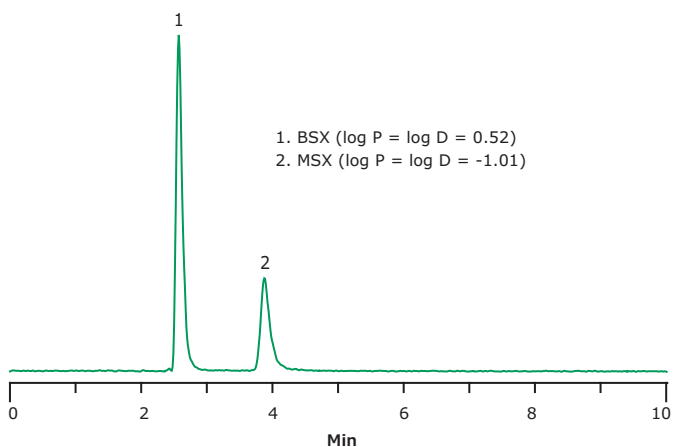
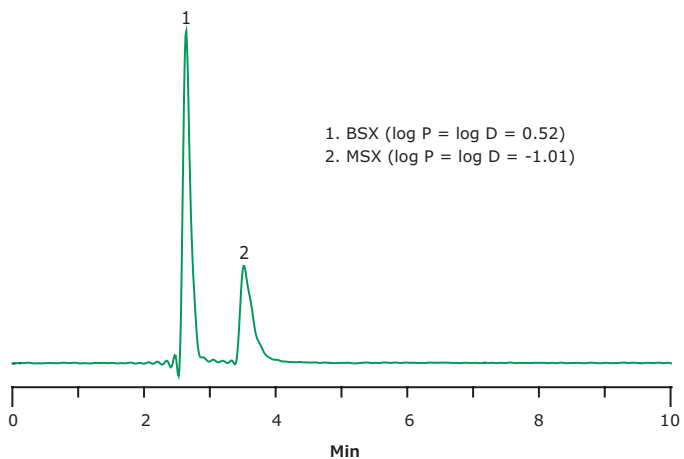


### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 3.0 mm I.D., 2.0 µm
<b>Column Temp.:</b>	30 °C
<b>Mobile Phase:</b>	[A] 10 mM ammonium formate pH 3.0 with formic acid in 90:10, water:acetonitrile; [B] 10 mM ammonium formate pH 3.0 with formic acid in 10:90, water:acetonitrile;
<b>Gradient:</b>	0 to 100% B in 3.0 min; held at 100% B for 0.5 min
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	5405 psi (373 bar)
<b>Sample:</b>	10 µg/mL in 96:4, water:methanol
<b>Injection:</b>	0.5 µL
<b>detector:</b>	MS, ESI+, combined SIR

## Extracted Ion Chromatogram of Methionine Sulfoximine (MSX) and Buthionine Sulfoximine (BSX) using an Ascentis® Express HILIC Column and an Ascentis® Express OH5 Column

The separation of methionine sulfoximine and buthionine sulfoximine on the Ascentis® Express HILIC and OH5 phases provide adequate separation and peak shape, the OH5 exhibits improved selectivity as well as peak efficiency for the pair of analytes.



The objective was to obtain good chromatographic separation of MSX and related compounds. Both the Ascentis® Express HILIC (Si) and OH5 are shown to be good candidates for MSX analysis.

### Chromatographic conditions

<b>Column:</b>	Ascentis® Express HILIC (Si), 10 cm x 3.0 mm I.D., 2.7 µm
<b>Mobile Phase:</b>	0.1% formic acid, pH to 3.5 w/ammonium hydroxide:acetonitrile, 25:75, v/v
<b>Flow Rate:</b>	0.4 mL/min
<b>Pressure:</b>	1350 psi
<b>Temp:</b>	ambient
<b>Det:</b>	ESI (+), scan m/z 150-300
<b>Injection:</b>	5 µL
<b>Sample:</b>	10 µg/mL in 90% methanol

### Chromatographic conditions

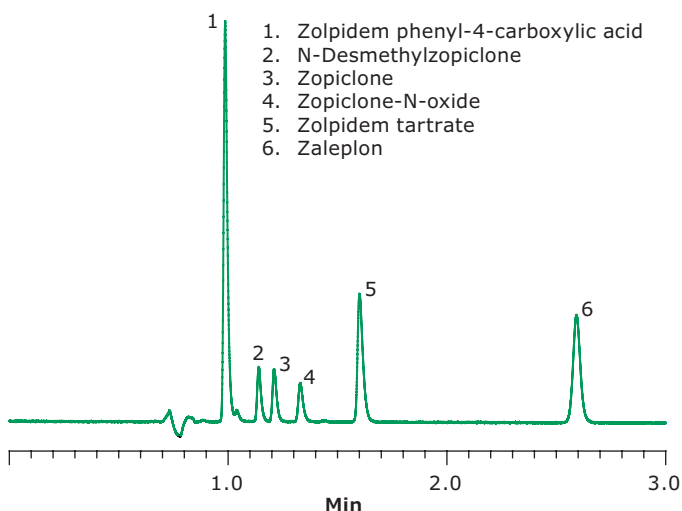
<b>Column:</b>	Ascentis® Express OH5, 10 cm x 3.0 mm I.D., 2.7 µm
<b>Mobile Phase:</b>	0.1% formic acid, pH to 3.5 w/ammonium formate:acetonitrile, 25:75, v/v
<b>Flow Rate:</b>	0.4 mL/min
<b>Pressure:</b>	1350 psi
<b>Temp:</b>	ambient
<b>Det:</b>	ESI (+), scan m/z 150-300
<b>Injection:</b>	5 µL
<b>Sample:</b>	10 µg/mL in 90% methanol (CHROMASOLV LC-MS grade, 34966)



## HPLC Analysis of Z-Drugs using an Ascentis® Express ES-Cyano Column

A rapid method for the simultaneous determination of the Z-drugs or sleep aids: zopiclone, zolpidem, and zaleplon is presented here. The need for greater analytical capacity and throughput for the analysis of

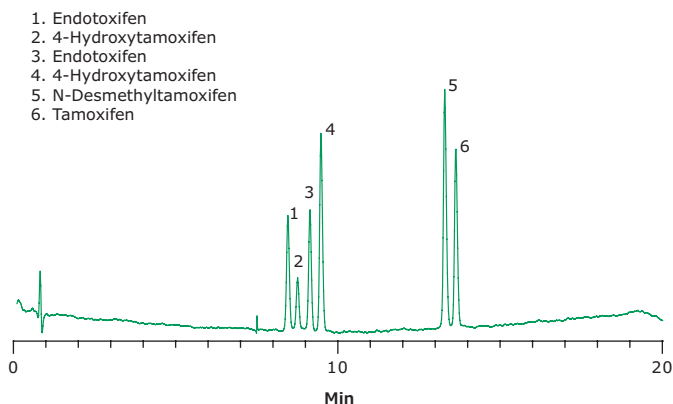
sleep aid medicines (Z-drugs) in forensic toxicology laboratories can be met by the use of fast Ascentis® Express 2.0 µm Fused Core UHPLC Columns.



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express ES-Cyano, 10 cm x 3.0 mm I.D., 2.0 µm
<b>Column Temp.:</b>	30 °C
<b>Mobile Phase:</b>	[A] 10 mM ammonium formate pH 3.0 with formic acid; [B] acetonitrile; (70:30, A:B)
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	5405 psi (373 bar)
<b>Sample:</b>	10 µg/mL in 75:25, water:methanol
<b>Injection:</b>	2 µL
<b>Detector:</b>	UV, 254 nm

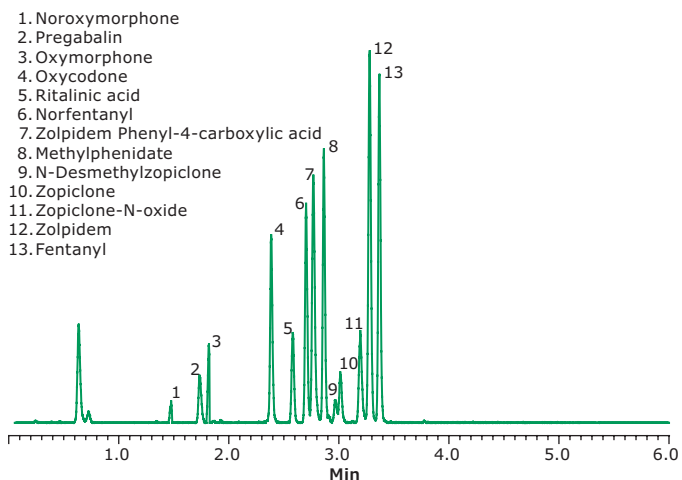
## HPLC Analysis of Tamoxifen E/Z isomers and Related Compounds on an Ascentis® Express RP-Amide Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 0.1% formic acid in water; [B] 0.1% formic acid in acetonitrile
<b>Gradient:</b>	held at 25% B for 2 min; 25 to 40% B in 15 min; 40 to 25% B in 0.1 min; held at 25% B for 2.9 min
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	2292 psi (158 bar)
<b>Sample:</b>	10 mg/L in 10:90, water: methanol
<b>Injection:</b>	2 µL
<b>Detector:</b>	UV, 260 nm

## LC-MS Analysis of a Combination Drug Panel in Urine using an Ascentis® Express Biphenyl Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express Biphenyl, 5 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	25 °C
<b>Mobile Phase:</b>	[A] 5 mM ammonium formate, 0.1% formic acid in water; [B] 5 mM ammonium formate, 0.1% formic acid in water:methanol (5:95)
<b>Gradient:</b>	5% B for 1 min; to 100% B in 5 min; held at 100% B for 1 min
<b>Flow Rate:</b>	0.5 mL/min
<b>Pressure:</b>	431 psi (29.8 bar)
<b>Sample:</b>	300 ng/mL in urine after dilution 9:1 in water
<b>Injection:</b>	1 µL
<b>Detector:</b>	MS, ESI+, EIC

# High resolution and speed in Food & Beverage

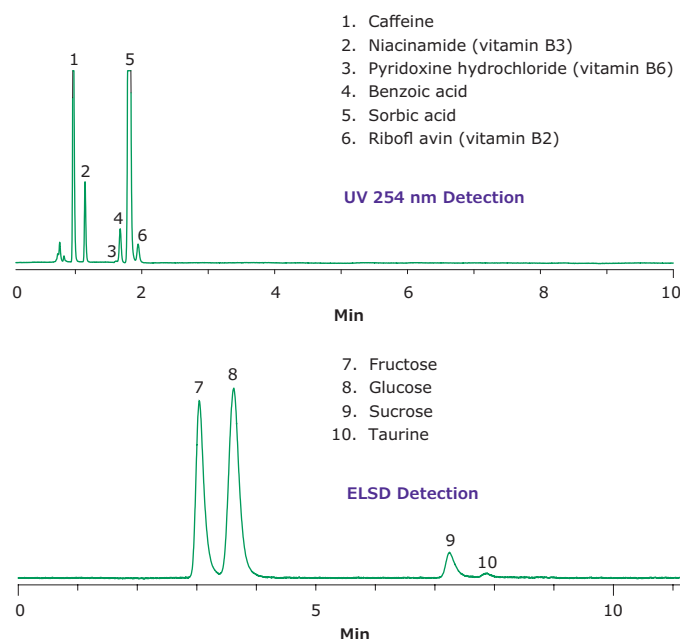
## Analysis of the Rock Star Energy Drink using an Ascentis® Express HILIC (Si) with UV and ELSD Detection in Series

Caffeinated energy drinks contain a variety of ingredients that usually include a sweetener (sugars, synthetic sugar substitutes, zero-calorie natural sweeteners), vitamin B supplements, and, of course, caffeine. They may also include amino acids, organic acids, and various plant extracts. The sample complexity makes it important to use highly-efficient, highly-selective phases and columns that are compatible with different detection systems to maximize the information from HPLC experiments. Ascentis® Express Fused-Core® columns meet these requirements.

The results on the Ascentis® Express HILIC column are found in. Here, UV and ELSD detection was used to detect different types of compounds; ELSD allowed us to see the non-UV absorbing sugars. The HILIC conditions yielded extremely rapid analysis (under 2 minutes) and MS-friendly mobile phase. The low backpressure of HILIC mobile phases also permits high flow rates for fast analysis.

### Chromatographic conditions

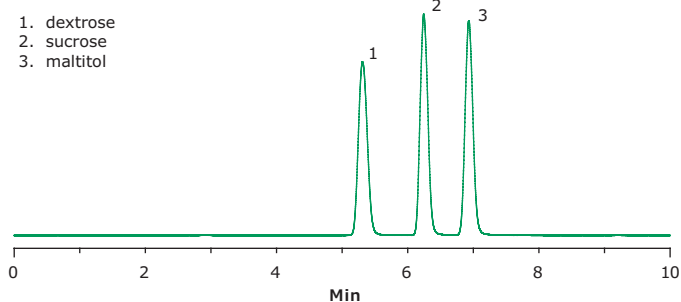
<b>Column:</b>	Ascentis® Express HILIC (Si), 10 cm x 3.0 mm I.D., 2.7 µm
<b>Mobile Phase:</b>	(A) 100 mM ammonium acetate, pH 5.0 with acetic acid; (B) water; (C) acetonitrile; (9:1:90, A:B:C) mixing proportions: A:B:C = 9:1:90
<b>Flow Rate:</b>	0.6 mL/min
<b>Pressure:</b>	815 psi
<b>Temp.:</b>	35 °C
<b>Det.:</b>	UV at 254 nm; ELSD, 55 °C, 3.5 bar nitrogen
<b>Injection:</b>	2 µL
<b>Sample:</b>	dilute 1:9 in acetonitrile



## HPLC Analysis of Sugars by HILIC Chromatography using Ascentis® Express OH5 Column with ELSD Detection

This application shows the analysis of sugars by HILIC with ELSD detection. The purpose of the work shown here was to develop a HILIC method that could quickly separate mono- and di-saccharides with good

sensitivity and reproducibility using an Ascentis® Express OH5 column and an Evaporative Light Scattering Detector (ELSD) detector.



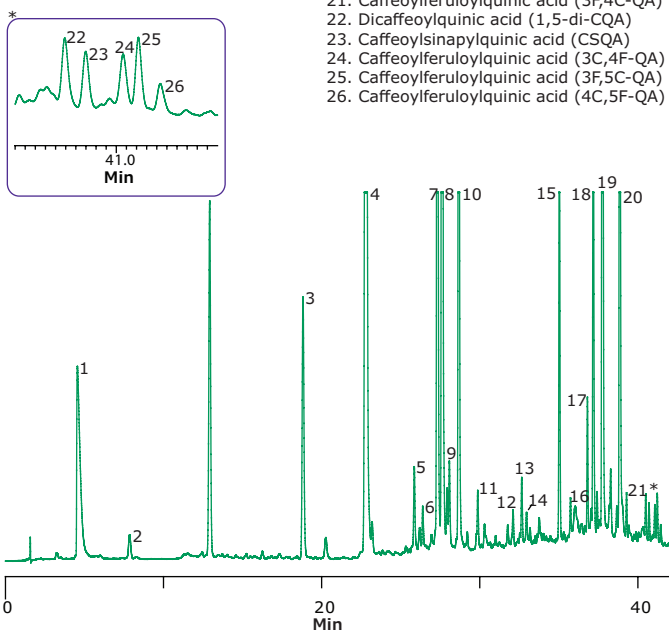
### Chromatographic conditions

<b>Column:</b>	Ascentis® Express OH5, 15 cm x 4.6 mm, I.D., 2.7 µm
<b>Column Temp.:</b>	35 °C
<b>Mobile Phase:</b>	[A] 0.005 M ammonium formate, pH 3.15 with concentrated TFA [B] acetonitrile (30:70, A:B)
<b>Flow Rate:</b>	0.5 mL/min
<b>Pressure:</b>	841 psi (58 bar)
<b>Sample:</b>	0.5 mg/mL each prepared in 50:50 Water:Acetonitrile
<b>Injection:</b>	10 µL
<b>Detector:</b>	ELSD (detector other: Nebulizer 60° C, Evaporator 80° C, Gas flow 1.60 SLM (Standard L/Min.), LED 100%, Smoothing 5.0 S, PMT (Photomultiplier) Gain 1.0

## HPLC Analysis of Maté Leaves using an Ascentis® Express C18 Column

1. Gentisic acid
2. 3,4-Dihydroxy-phenylacetic acid
3. Theobromine
4. Neo-chlorogenic acid (3-CQA)
5. Dicafeic acid
6. Coumaroylquinic acid (CQA)
7. Chlorogenic acid (5-CQA)
8. Caffeine
9. Dicafeic acid
10. Crypto-chlorogenic acid (4-CQA)

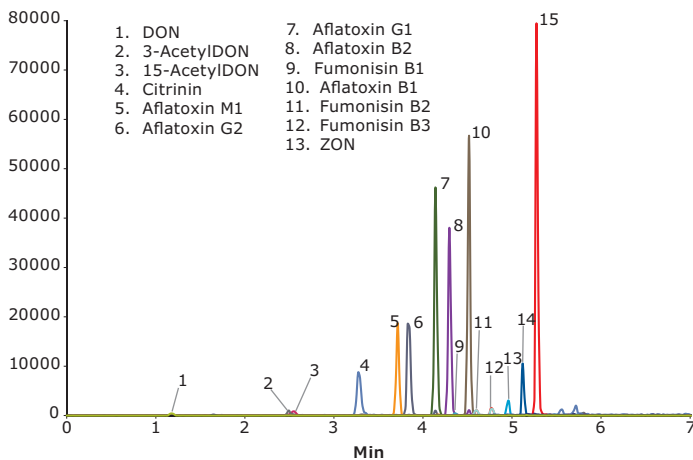
11. Caffeoylquinic acid (1-CQA)
12. Feruloylquinic acid (3-FQA)
13. Feruloylquinic acid (5-FQA)
14. Feruloylquinic acid (4-FQA)
15. Rutin
16. Quercetin-3-O-glycoside
17. Kaempferol-3-O-glycoside
18. Dicafeoylquinic acid (3,4-di-CQA)
19. Dicafeoylquinic acid (3,5-di-CQA)
20. Dicafeoylquinic acid (4,5-di-CQA)
21. Caffeoylferuloylquinic acid (3F,4C-QA)
22. Dicafeoylquinic acid (1,5-di-CQA)
23. Caffeoylsinapylquinic acid (CSQA)
24. Caffeoylferuloylquinic acid (3C,4F-QA)
25. Caffeoylferuloylquinic acid (3F,5C-QA)
26. Caffeoylferuloylquinic acid (4C,5F-QA)



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 150 x 4.6 mm I.D., 2.7 µm
<b>Column Temp.:</b>	25 °C
<b>Mobile Phase:</b>	[A] water, pH 3 with formic acid; [B] acetonitrile, pH 3 with formic acid
<b>Gradient:</b>	0 to 5% B in 20 min; 5 to 40% B in 30 min
<b>Flow Rate:</b>	1 mL/min
<b>Injection:</b>	10 µL
<b>Detector:</b>	PDA: range 215- 420 nm (8 µL detector cell volume, cell temp. 40 °C, sampling rate 12.5 Hz, time constant 0.025 s)
<b>Sample/ Matrix:</b>	UAE (Ultrasonic Extraction) Mate extract (dried and minced leaves (7.5 g) from maté were extracted with 225 mL of methanol in an ultrasonic bath, thermostat at 75 ±0.5 °C (potency: 90 W; frequency: 40 kHz))

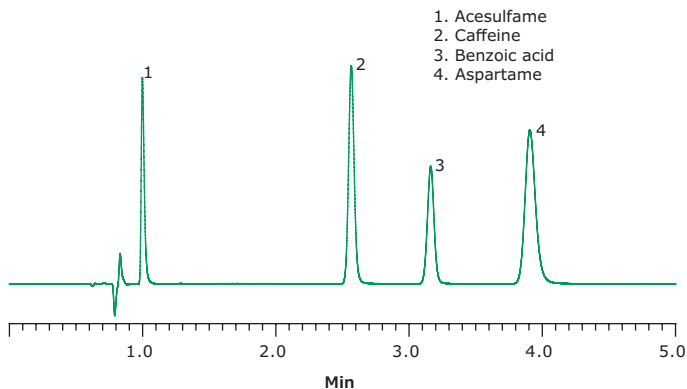
## LC-MS/MS Analysis of Multiple Mycotoxins using an Ascentis® Express Phenyl-Hexyl Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm
<b>Column Temp.:</b>	40 °C
<b>Mobile Phase:</b>	[A] 5mM ammonium formate with 0.1% formic acid in water ; [B] 5mM ammonium formate with 0.1% formic acid in acetonitrile
<b>Gradient:</b>	30 to 60% B in 3 min; to 100% B in 2 min; hold at 100% B for 2 min; 100 to 30% B in 0.5 min; hold at 30% for 2 min
<b>Flow Rate:</b>	0.4 mL/min
<b>Pressure:</b>	5511 psi (380 bar)
<b>Injection:</b>	2 µL
<b>Detector:</b>	MS/MS, ESI(+), MRM (See Figure for transitions)

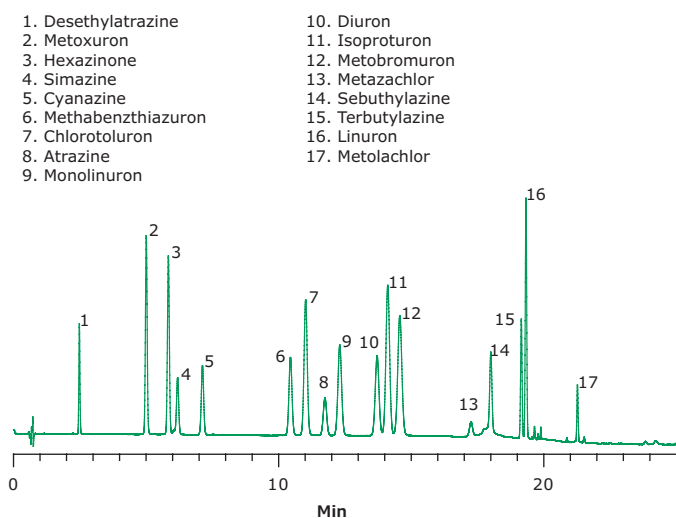
## HPLC Analysis of Beverage Additives in Diet Cola using an Ascentis® Express RP-Amide Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express RP-Amide, 10 cm x 3.0 mm I.D., 2.7 µm
<b>Column Temp.:</b>	40 °C
<b>Mobile Phase:</b>	[A] 20 mM ammonium acetate, pH 4.7 with acetic acid; [B] acetonitrile; (92:8, A:B)
<b>Flow Rate:</b>	0.6 mL/min
<b>Sample:</b>	500 - 1000 µg/mL in 95:5, buffer: acetonitrile
<b>Injection:</b>	1 µL
<b>Detector:</b>	UV, 214 nm

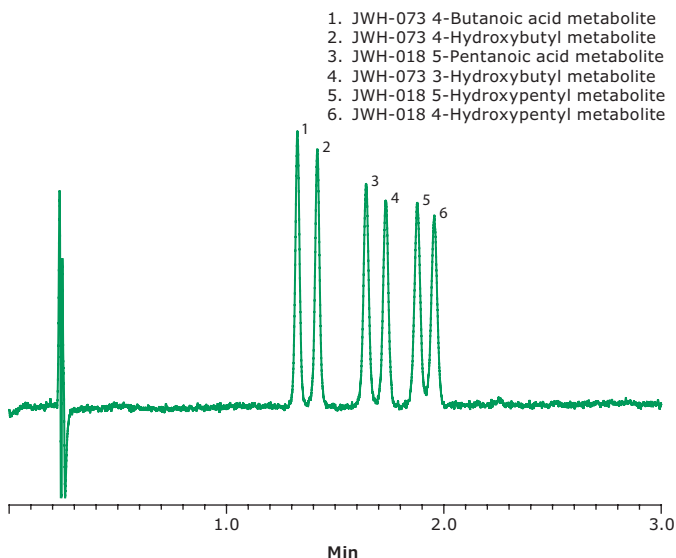
## HPLC Analysis of Atrazine Herbicides using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 3.0 mm I.D., 2.7 µm
<b>Column Temp.:</b>	46 °C
<b>Mobile Phase:</b>	[A] 20 mM ammonium acetate, pH 6.4 unadjusted; [B] acetonitrile
<b>Gradient:</b>	20 to 28% B in 11 min; 28 to 65% B in 5 min; held at 65% B for 4 min
<b>Flow Rate:</b>	0.6 mL/min
<b>Sample:</b>	10-25 µg/mL in 10:90, water: acetonitrile
<b>Injection:</b>	5 µL
<b>Detector:</b>	UV, 240 nm

## Rapid UHPLC Analysis of Synthetic Cannabinoid Metabolites using an Ascentis® Express C18 Column



### Chromatographic conditions

<b>Column:</b>	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.0 µm
<b>Column Temp.:</b>	45 °C
<b>Mobile Phase:</b>	[A] 0.1% formic acid; [B] 0.1% formic acid in acetonitrile; (55:45, A:B)
<b>Flow Rate:</b>	0.8 mL/min
<b>Pressure:</b>	10800 psi (744 bar)
<b>Sample:</b>	50 µg/mL in 70:30, water:methanol
<b>Injection:</b>	0.5 µL
<b>Detector:</b>	UV, 250 nm

# Ordering information

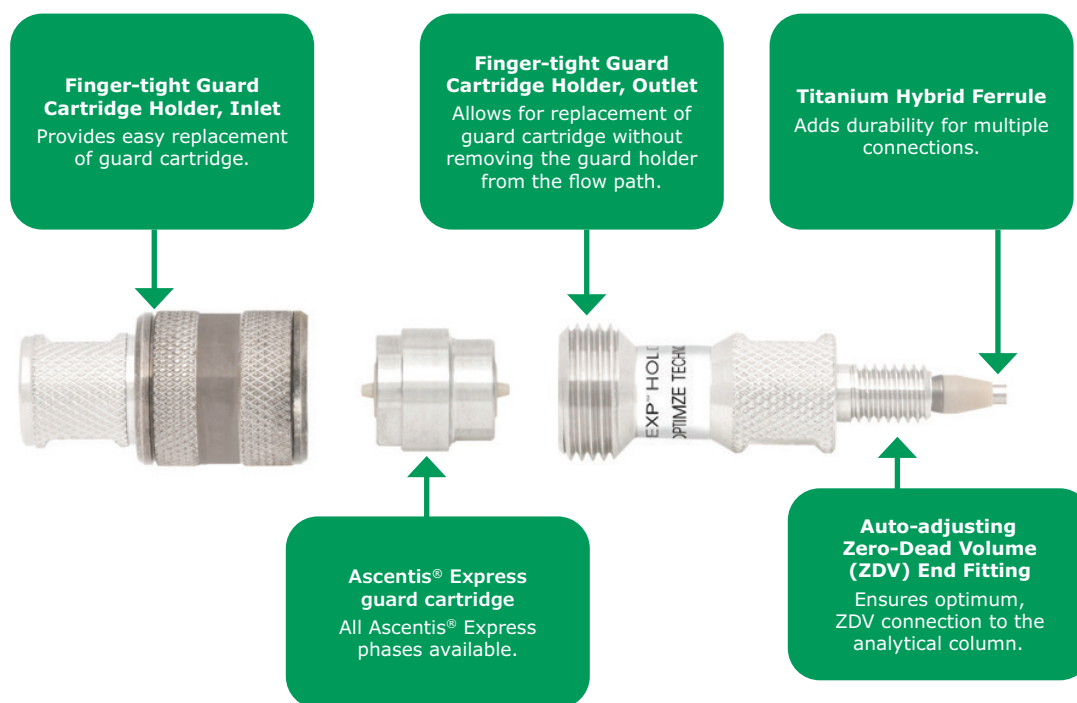
Ascentis® Express (5 µm)										
Length	ID	C18	C8	RP-Amide	Phenyl-Hexyl	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)
2 cm	2.1 mm	50507-U	50362-U	50732-U	50442-U		50603-U	50557-U	50313-U	50255-U
3 cm	2.1 mm	50508-U	50363-U	50733-U	50443-U		50604-U	50558-U	50314-U	50256-U
5 cm	2.1 mm	50509-U	50364-U	50734-U	50446-U	584585-U	50605-U	50559-U	50317-U	50257-U
7.5 cm	2.1 mm	50511-U	50367-U	50735-U	50451-U		50607-U	50562-U	50321-U	50258-U
10 cm	2.1 mm	50517-U	50368-U	50737-U	50454-U	584586-U	50612-U	50563-U	50322-U	50260-U
15 cm	2.1 mm	50518-U	50372-U	50739-U	50455-U	584587-U	50613-U	50564-U	50327-U	50261-U
25 cm	2.1 mm	50521-U	50373-U	50747-U	50456-U	584588-U	50614-U	50566-U	50328-U	50262-U
3 cm	3.0 mm	50522-U	50376-U	50749-U	50459-U	584589-U	50615-U	50567-U	50329-U	50264-U
5 cm	3.0 mm	50523-U	50377-U	50751-U	50464-U	584590-U	50616-U	50568-U	50335-U	50265-U
7.5 cm	3.0 mm	50525-U	50378-U	50752-U	50466-U		50619-U	50569-U	50336-U	50268-U
10 cm	3.0 mm	50526-U	50381-U	50753-U	50469-U	584591-U	50622-U	50570-U	50338-U	50269-U
15 cm	3.0 mm	50527-U	50382-U	50758-U	50470-U	584592-U	50623-U	50574-U	50339-U	50270-U
25 cm	3.0 mm	50528-U	50385-U	50759-U	50472-U		50624-U	50575-U	50341-U	50276-U
3 cm	4.6 mm	50529-U	50386-U	50767-U	50474-U	584593-U	50625-U	50577-U	50343-U	50278-U
5 cm	4.6 mm	50530-U	50389-U	50768-U	50477-U	584594-U	50626-U	50581-U	50344-U	50284-U
7.5 cm	4.6 mm	50533-U	50390-U		50479-U		50627-U	50583-U	50345-U	50286-U
10 cm	4.6 mm	50536-U	50391-U	50773-U	50482-U	584595-U	50628-U	50585-U	50346-U	50288-U
15 cm	4.6 mm	50537-U	50392-U	50774-U	50483-U	584596-U	50631-U	50588-U	50347-U	50289-U
25 cm	4.6 mm	50538-U	50394-U	50775-U	50487-U		50632-U	50591-U	50348-U	50294-U
Guard 5mm	2.1 mm	50539-U	50395-U	50776-U	50496-U	584597-U	50633-U	50592-U	50349-U	50295-U
Guard 5mm	3 mm	50541-U	50396-U	50777-U	50497-U	584598-U	50634-U	50593-U	50350-U	50297-U
Guard 5mm	4.6 mm	50542-U	50399-U	50779-U	50498-U	584599-U	50635-U	50597-U	50355-U	50298-U

Ascentis® Express (2.7 µm)														
Length	ID	C30	C18	AQ-C18	Peptide ES-C18	C18*, PCP	C8	RP-Amide	Phenyl-Hexyl	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)
2 cm	2.1 mm		53799-U	577320-U			53795-U			64043-U	53592-U	53494-U	53779-U	
3 cm	2.1 mm		53802-U	577321-U	53299-U		53839-U	53910-U	53332-U	64054-U	53566-U	53468-U	53748-U	53933-U
5 cm	2.1 mm	577100-U	53822-U	577322-U	53301-U		53831-U	53911-U	53334-U	64057-U	53567-U	53470-U	53749-U	53934-U
7.5 cm	2.1 mm		53804-U	577323-U	53304-U		53843-U	53912-U	53335-U	64061-U	53568-U	53472-U	53755-U	53938-U
10 cm	2.1 mm	577101-U	53823-U	577324-U	53306-U		53832-U	53913-U	53336-U	64065-U	53569-U	53473-U	53757-U	53939-U
15 cm	2.1 mm	577102-U	53825-U	577325-U	53307-U		53834-U	53914-U	53338-U	64068-U	53571-U	53475-U	53764-U	53946-U
25 cm	2.1 mm	577103-U												
2 cm	3.0 mm			577326-U						64047-U				
3 cm	3.0 mm	577104-U	53805-U	577327-U	53308-U		53844-U	53915-U	53341-U	64055-U	53574-U	53476-U	53766-U	53964-U
5 cm	3.0 mm	577105-U	53811-U	577328-U	53311-U		53848-U	53916-U	53342-U	64058-U	53576-U	53478-U	53767-U	53967-U
7.5 cm	3.0 mm		53812-U	577329-U	53312-U		53849-U	53917-U	53343-U	64062-U	53577-U	53479-U	53768-U	53969-U
10 cm	3.0 mm	577106-U	53814-U	577330-U	53313-U		53852-U	53918-U	53345-U	64066-U	53578-U	53481-U	53769-U	53970-U
15 cm	3.0 mm	577107-U	53816-U	577331-U	53314-U		53853-U	53919-U	53346-U	64069-U	53579-U	53483-U	53771-U	53972-U
2 cm	4.6 mm			577332-U						64051-U				
3 cm	4.6 mm	577108-U	53818-U	577333-U	53316-U		53857-U	53921-U	53347-U	64056-U	53581-U	53484-U	53772-U	53974-U
5 cm	4.6 mm	577134-U	53826-U	577334-U	53318-U		53836-U	53922-U	53348-U	64059-U	53583-U	53486-U	53774-U	53975-U
7.5 cm	4.6 mm		53819-U	577335-U	53323-U		53858-U	53923-U	53351-U	64064-U	53584-U	53489-U	53775-U	53977-U
10 cm	4.6 mm	577135-U	53827-U	577336-U	53324-U	50461-U	53837-U	53929-U	53352-U	64067-U	53590-U	53491-U	53776-U	53979-U
15 cm	4.6 mm	577136-U	53829-U	577337-U	53328-U	50462-U	53838-U	53931-U	53353-U	64071-U	53591-U	53492-U	53778-U	53981-U
Guard 5mm	2.1 mm	577137-U	53501-U	577338-U	53536-U		53509-U	53514-U	53524-U	64074-U	53594-U	53495-U	53780-U	53520-U
Guard 5mm	3 mm	577138-U	53504-U	577339-U	53537-U		53511-U	53516-U	53526-U	64076-U	53597-U	53496-U	53781-U	53521-U
Guard 5mm	4.6 mm	577139-U	53508-U	577340-U	53542-U		53512-U	53519-U	53531-U	64078-U	53599-U	53497-U		53523-U

\*Ascentis® Express C18 PCP: pre-conditioned with phosphoric acid.

Ascentis® Express (2 µm)										
Length	ID	C18	C8	RP-Amide	Phenyl-Hexyl	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)
2 cm	2.1 mm	50805-U	51652-U	51567-U	51600-U		50857-U	51709-U	50951-U	51403-U
3 cm	2.1 mm	50809-U	51654-U	51568-U	51601-U		50858-U	51712-U	50952-U	51404-U
5 cm	2.1 mm	50811-U	51656-U	51569-U	51603-U	584600-U	50859-U	51717-U	50957-U	51406-U
7.5 cm	2.1 mm	50812-U	51657-U	51571-U	51605-U		50861-U	51721-U	50958-U	51408-U
10 cm	2.1 mm	50813-U	51658-U	51576-U	51608-U	584601-U	50863-U	51724-U	50959-U	51409-U
15 cm	2.1 mm	50814-U	51661-U	51577-U	51609-U	584602-U	50867-U	51725-U	50962-U	51418-U
25 cm	2.1 mm					584603-U				
3 cm	3 mm	50815-U	51663-U	51582-U	51611-U	584604-U	50869-U	51727-U	50963-U	51419-U
5 cm	3 mm	50816-U	51664-U	51583-U	51614-U	584605-U	50871-U	51728-U	50964-U	51421-U
7.5 cm	3 mm	50817-U	51672-U	51587-U	51616-U		50876-U	51729-U	50965-U	51424-U
10 cm	3 mm	50819-U	51673-U	51588-U	51617-U		50879-U	51732-U	50967-U	51428-U
15 cm	3 mm	50821-U	51674-U	51589-U	51618-U	584606-U	50881-U	51734-U	50968-U	51429-U
Guard 5mm	2.1 mm	50822-U	51676-U	51594-U	51619-U	584607-U	50884-U	51736-U	50969-U	51430-U
Guard 5mm	3 mm		51679-U	51595-U	51623-U	584608-U	50886-U	51739-U	50973-U	51433-U

### Ascentis® Express Guard Cartridge Holder 53500-U



Ascentis® Express Capillary columns (2.7µm)				
Length	ID	C18	Peptide ES-C18	C8
5 cm	0.075 mm	53982-U	53543-U	53983-U
5 cm	0.100 mm	53985-U	53544-U	53987-U
5 cm	0.200 mm	53989-U	53545-U	53991-U
5 cm	0.300 mm	53992-U	53546-U	53997-U
5 cm	0.500 mm	53998-U	53547-U	53999-U
5 cm	1 mm		53548-U	
15 cm	0.075 mm	54219-U	53549-U	54229-U
15 cm	0.100 mm	54256-U	53552-U	54260-U
15 cm	0.200 mm	54261-U	53553-U	54262-U
15 cm	0.300 mm	54271-U	53554-U	54272-U
15 cm	0.500 mm	54273-U	53558-U	54275-U
15 cm	1 mm		53561-U	

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