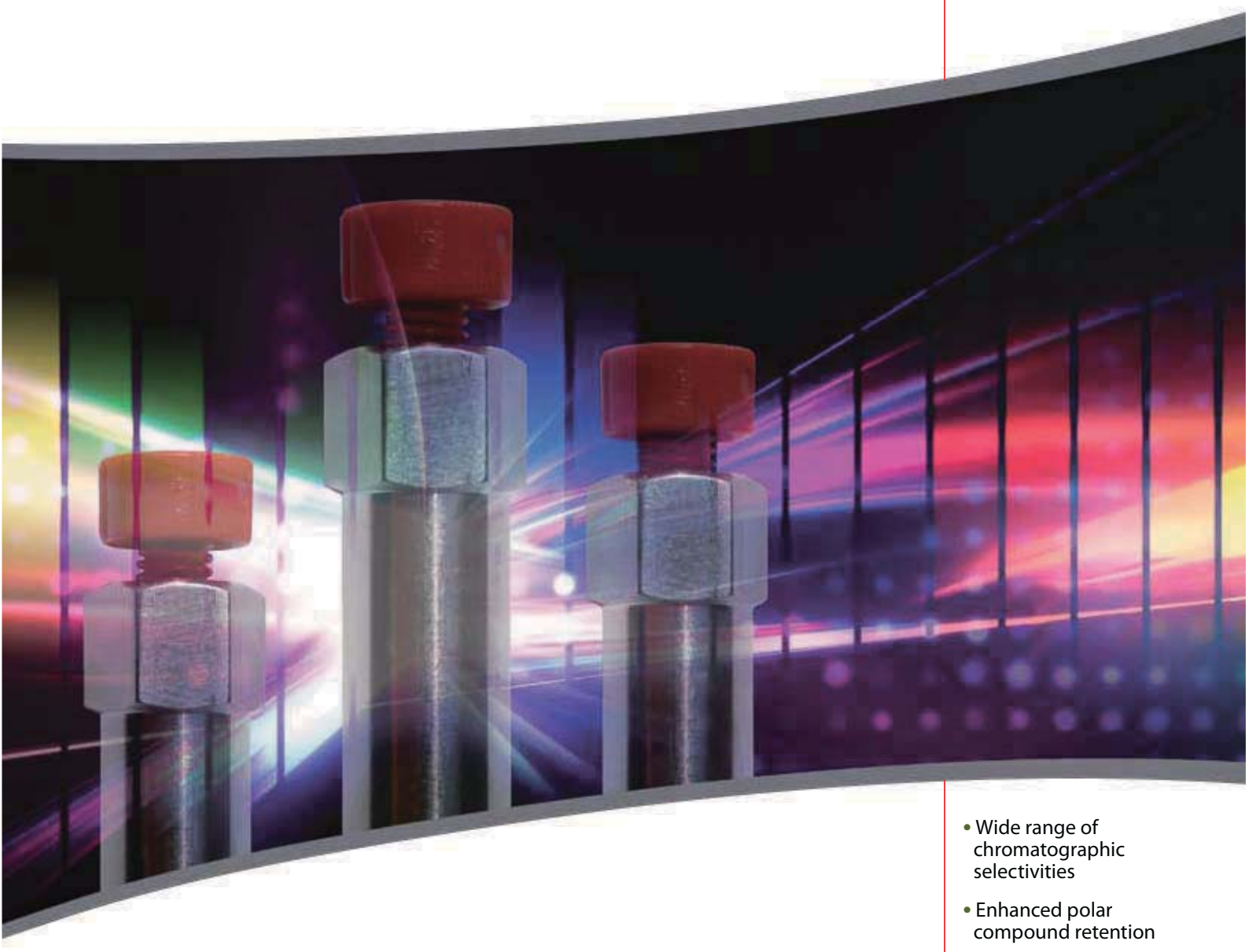
Ascentis® HPLC Columns

SUPELCO® Analytical

Elevated HPLC Performance from a Wide Range of Phases



- Wide range of chromatographic selectivities
- Enhanced polar compound retention
- Ideal for LC-MS and all modern applications

SIGMA-ALDRICH®

Ascentis: Elevated HPLC Performance

We have placed heavy emphasis on optimizing Ascentis phases with relation to the three terms of the resolution equation: efficiency, retention and selectivity. However, our strongest emphasis has been on the most powerful term, selectivity. Together, Ascentis bonded phases have a wide range of selectivities. It is likely that one or more Ascentis phase will accomplish any small molecule HPLC separation.

Packed in micro- to preparative hardware dimensions, Ascentis products cover all HPLC application areas, including the most sensitive trace-level analyses.

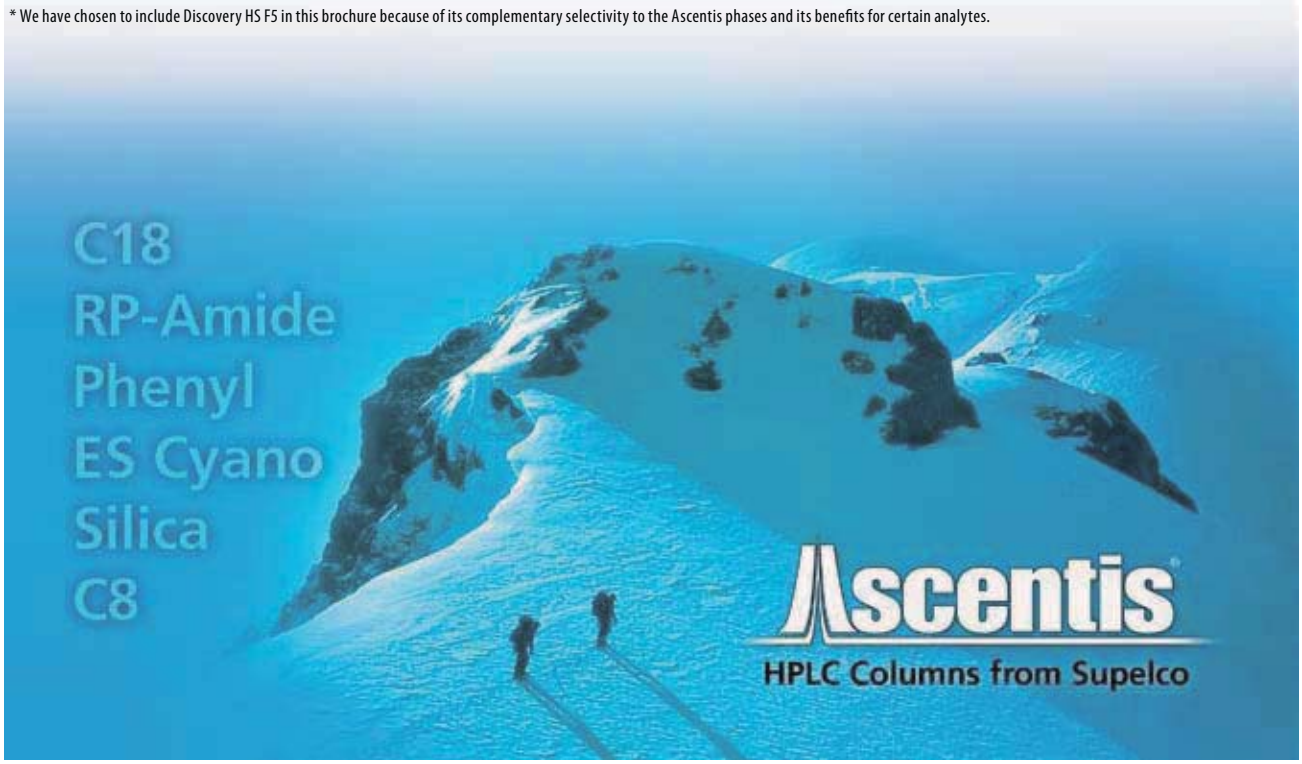
The General Features of the Ascentis Family Include:

- High purity, type B silica for inertness, reproducibility and stability
- Modern bonding processes that optimize bonded phase coverage and maximize stability, while minimizing bleed and unwanted secondary interactions
- Wide selection of bonded phase chemistries and bare silica
- Phases with enhanced polar compound retention
- Compatible with LC-MS and all of today's sensitive instruments and methods
- Scalable selectivity from analytical to preparative
- High surface area silica for high preparative loading capacity

Ascentis Characteristics

Phase	USP Code	Key Competitive Feature	Modes	Primary Uses	Page
Ascentis C18	**L1**	High surface area, inert surface	**Reversed-phase**	Small molecules and peptides	**9**
Ascentis RP-Amide	**L60**	Phase stability, low bleed	**Reversed-phase**	Excellent "first choice" alternative to C18 for routine RP method development. Polar molecules, especially phenolics and other H-bond donors, acids, bases (uncharged), anilines	**10**
Ascentis Phenyl	**L11**	Phase stability, low bleed	**Reversed-phase, HILIC**	Ring systems and strong dipoles, π-acids, π-electron acceptors, heteroaromatics, nitroaromatics	**12**
Ascentis ES Cyano	**L10**	Phase stability	**Reversed-phase, HILIC, 100% Aqueous**	Polar compounds, strong dipoles, tricyclic antidepressants	**14**
Ascentis Silica	**L3**	High loading capacity, controlled and uniform surface activity	**Normal phase (non-aqueous), HILIC**	Non-polar compounds (in NP mode), highly-polar compounds (in HILIC mode), nucleosides, amino acids	**16**
Ascentis C8	**L7**	High surface area, inert surface	**Reversed-phase**	Small molecules and peptides	**18**
Discovery® HS F5*	**L43**	Orthogonal selectivity to C18, ours is well-characterized	**Reversed-phase, HILIC, ion-exchange**	All electron and π-electron donors, bases (charged), positional isomers	**20**

* We have chosen to include Discovery HS F5 in this brochure because of its complementary selectivity to the Ascentis phases and its benefits for certain analytes.



Ascentis HPLC columns represent a continuum of improvement through innovations in HPLC technology.

Developing HPLC Methods on Ascentis

Selecting An Ascentis Column

Column Screening: The Ascentis & Discovery Method Development "Tool Kit"

We recommend every HPLC method developer have these five columns in their arsenal.

Ascentis C18 – Classic C18 selectivity will achieve most reversed-phase separations

Ascentis RP-Amide – For enhanced retention and performance of polar compounds, especially bases (uncharged) and compounds with H-bond potential

Ascentis Phenyl – For enhanced retention and performance of polar compounds, especially ring systems, dipoles, and nitroaromatics

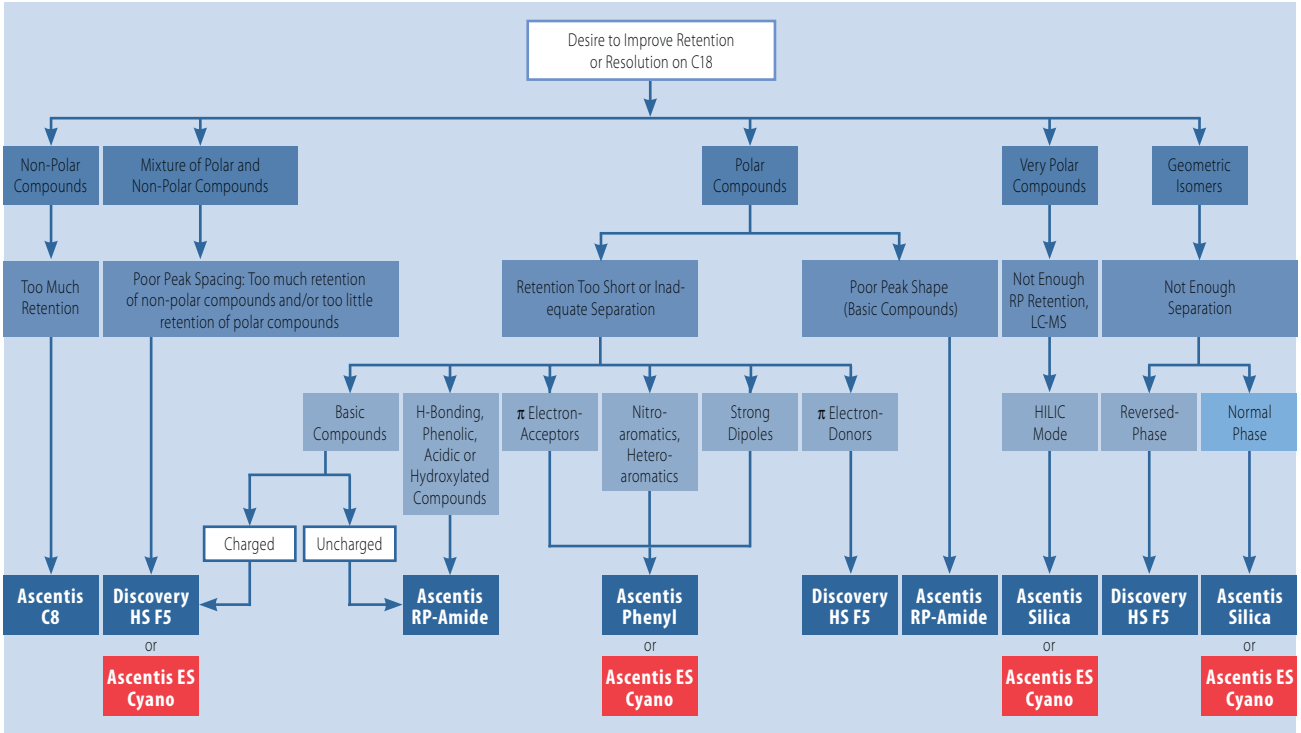
Ascentis ES Cyano – Extra stable for low pH mobile phases due to sterically protected phase. Useful for polar selectivity in reversed-phase mode. Also useful as an alternative to silica in HILIC mode

Discovery HS F5 – For enhanced retention and performance of polar compounds, especially bases (charged) and when the sample contains a mixture of non-polar and polar compounds

Simply screen these five columns in your desired mobile phase, using your preferred column.

Choosing an Ascentis or Discovery Phase Based on Compound Class and Separation Challenge or Objective

Typically, Ascentis C18 is the first choice for starting a new method. However, 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 the stationary phase. The range of selectivity provided by Ascentis and Discovery phases makes this easy. The flow chart below helps guide the selection of Ascentis or Discovery phase based on the particular compound type or separation challenge. For more information about each phase, and the other Ascentis phases, please refer to their dedicated pages in this brochure.



Column selection guidelines for Ascentis and Discovery phases based on compound class and separation challenge or objective.

Harnessing the Power of Chromatographic Selectivity

Chromatographic resolution is a function of column efficiency (***N***), retention (***k***) and selectivity (α). It is usually written in the form of the resolution equation:

$$R = \frac{\sqrt{N}}{4} \times \frac{k}{k+1} \times \frac{\alpha - 1}{\alpha}$$

When resolution is plotted vs. these three parameters in Figure 1, it becomes apparent that selectivity has the greatest affect on resolution.

The Power to Accomplish Difficult Separations

One of the most important reasons why selectivity is leveraged in HPLC is to resolve closely-eluting compounds. A good example of this is the need to quantify a compound that elutes in the tail of a more abundant compound, perhaps a low-level impurity in the presence of the parent compound, like shown in the Figure 2. By altering the stationary phase, in this case going from a C18 to an RP-Amide, the impurity can be eluted before the main peak, thereby allowing more sensitive and reliable quantification.

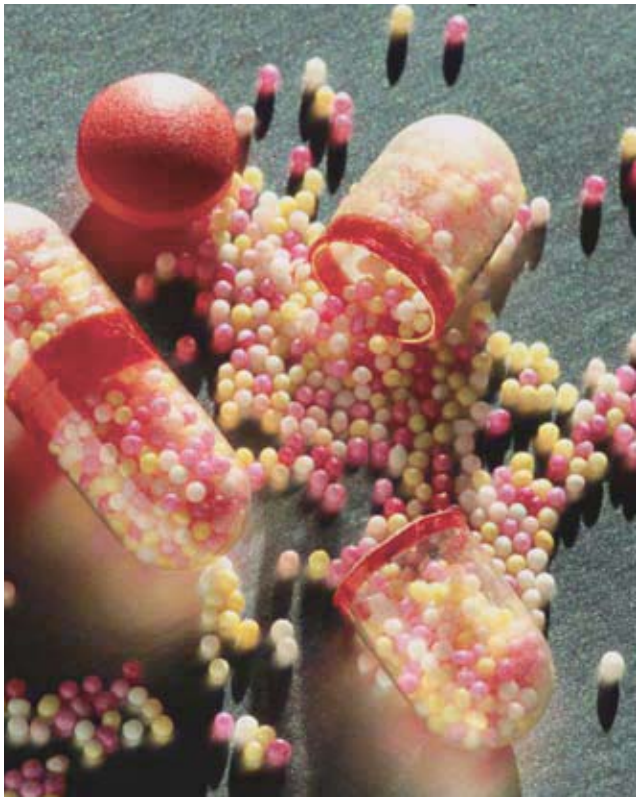


Figure 1. Affect of Selectivity on Improving Resolution

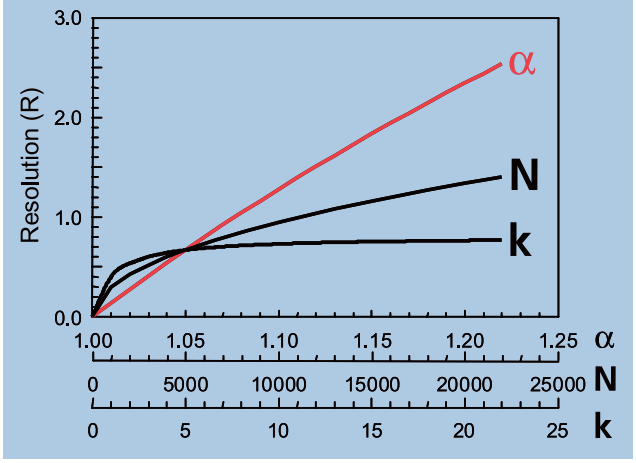
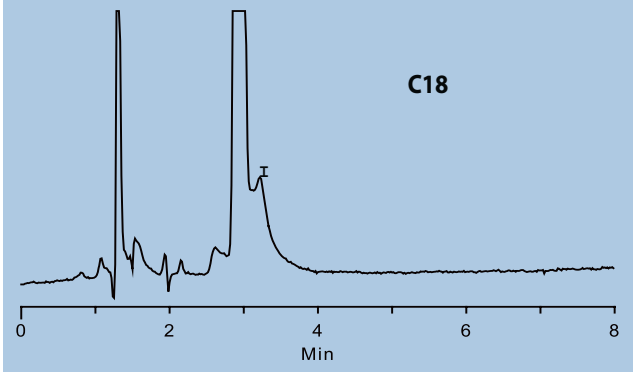
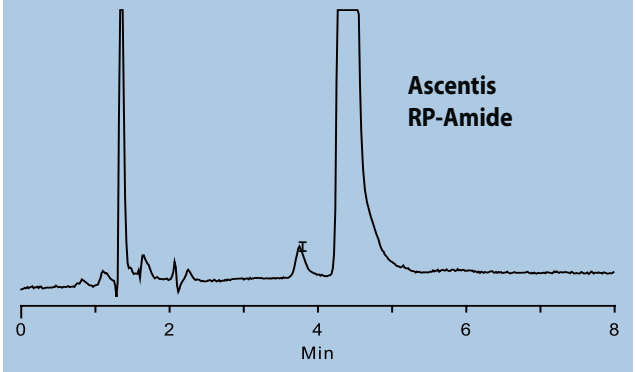


Figure 2. Impurity Eluted on the Downslope of the Main Peak on C18 — Difficult to Quantify



Impurity Eluted Before the Main Peak on Ascentis RP-Amide – Better Quantification



The power of chromatographic selectivity is demonstrated in this example. The C18 column elutes the impurity in the downslope of the major peak, limiting the ability to detect and quantify the impurity. By using a column with different selectivity, in this case an Ascentis RP-Amide, the impurity peak is eluted before the main peak.

Key Ascentis Application Areas

Polar Analytes: Enhanced Retention, Selectivity, and Compatibility with Highly-Aqueous Mobile Phases

Polar compounds are difficult to analyze by traditional reversed-phase because they lack the high proportion of hydrophobic character necessary for retention. Since most pharmaceutically- and biologically-active compounds are highly polar, this has presented a continual problem in HPLC. From the beginning of our commitment to HPLC innovation, we have focused on bonded phase to enhance polar compound retention.

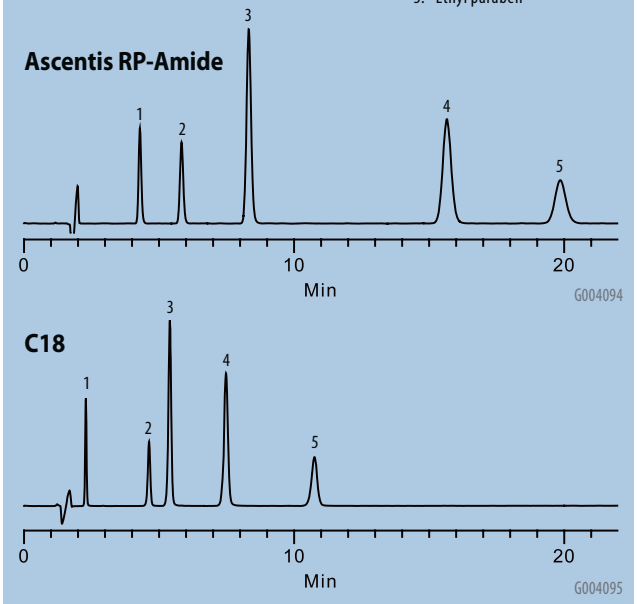
Stationary Phases with Enhanced Polar Compound Retention Compared to C18

Rather than relying solely on dispersive forces to achieve retention, our column portfolio contains bonded phases that lend additional retentive character toward analytes with specific polar functional groups.

Figure 3. H-bonding (Ascentis RP-Amide)

column: Ascentis RP-Amide & C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 70:30, water with 0.1% TFA:acetonitrile
flow rate: 1 mL/min
temp.: 35 °C
det.: UV at 220 nm
injection: 10 µL

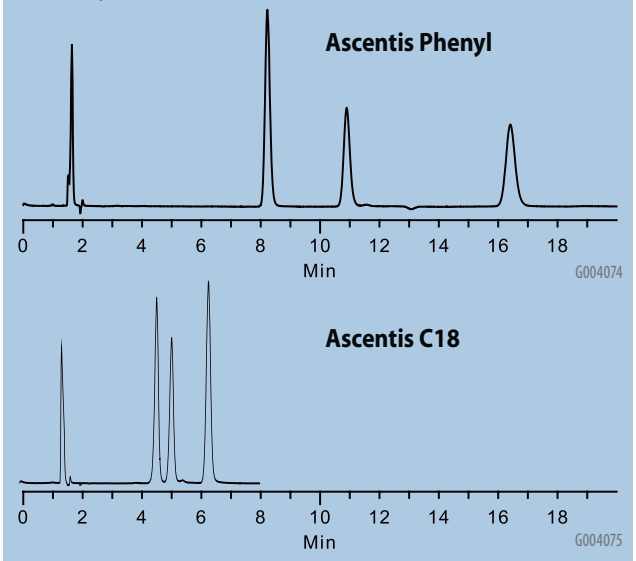
1. 4-Hydroxybenzoic Acid
2. Acetylsalicylic Acid
3. Benzoic Acid
4. 2-Hydroxybenzoic Acid
5. Ethyl paraben



Ascentis RP-Amide exhibits strong retention relative to C18 for phenols and organic acids.

Figure 4. π-π Interactions (Ascentis Phenyl)

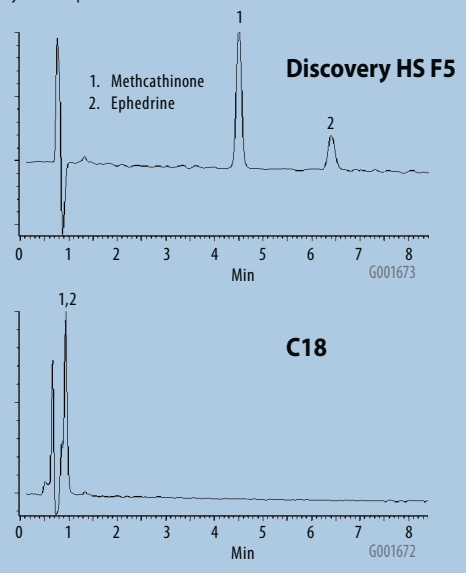
column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase: 40:60, acetonitrile:water
flow rate: 1.0 mL/min.
temp.: 30 °C
det.: UV at 210 nm
injection: 5 µL
sample: nitrobenzene, m-dinitrobenzene, 1,3,5-trinitrobenzene



Ascentis Phenyl exhibits strong π-π interactions with the π-acidic nitroaromatics. Addition of each nitro group increases retention.

Figure 5. Ion Exchange (Discovery HS F5)

column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
conventional C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 30:70, 10 mM ammonium acetate (pH 6.98): CH_3CN
flow rate: 2.0 mL/min.
temp.: 35 °C
det.: photodiode array
injection: 5 µL



Discovery HS F5 exhibits enhanced retention from ion exchange interactions with ionized analytes.

HILIC Mode: Enhanced Retention and High MS-Suitability

Highly-polar compounds, like underivatized amino acids, nucleosides and nucleotides, are not well-retained by reversed-phase HPLC. However, under HILIC (hydrophilic interaction chromatography) conditions, they can be retained. HILIC is a variation of normal phase HPLC where the mobile phase contains high percentages of organic modifier. It is also called "aqueous normal phase" or ANP. Under high organic conditions, polar interactions become prominent which can lead to increased retention. Ascentis Phenyl, Ascentis ES Cyano, Ascentis Silica and Discovery HS F5 exhibit HILIC character under highly-organic mobile phases. An added benefit of HILIC mobile phases is the high organic (often >90%) is amenable to MS detection.

Stability in Highly-Aqueous Mobile Phases

Unless working in HILIC mode, mobile phases for polar compounds are often highly-aqueous, with only small percentages of organic modifiers. Under such conditions, C18 phases are not wetted, which causes two problems. First, the bonded phase molecules coalesce resulting in phase collapse and subsequent loss of hydrophobic retention. Second, analytes have an unpredictable approach to the silica surface, resulting in irreproducible and unstable retention run-to-run and column-to-column. Ascentis Phenyl, Ascentis ES Cyano, Ascentis RP-Amide, and Discovery HS F5 are completely aqueous-compatible, and will not undergo phase collapse even in mobile phases that contain 100% water.

Figure 6. Separation of Nucleosides on Ascentis Phenyl

column: Ascentis Phenyl, 15 cm x 2.1 mm I.D., 5 µm particles (581613-U)
mobile phase: 10 mM ammonium formate (pH 3.0 with formic acid)
flow rate: 0.2 mL/min
temp.: 35 °C
det.: UV at 270 nm
injection: 1 µL

1. Cytidine (0.1 g/L)
2. Deoxycytidine (0.1 g/L)
3. Uridine (0.1 g/L)
4. Guanosine (0.1 g/L)
5. Adenosine (0.1 g/L)
6. Deoxyguanosine (0.1 g/L)
7. Deoxyadenosine (0.1 g/L)
8. Thymidine (0.1 g/L)

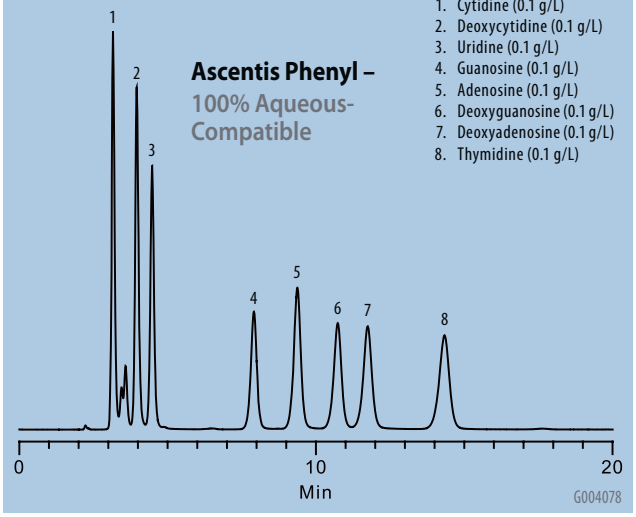
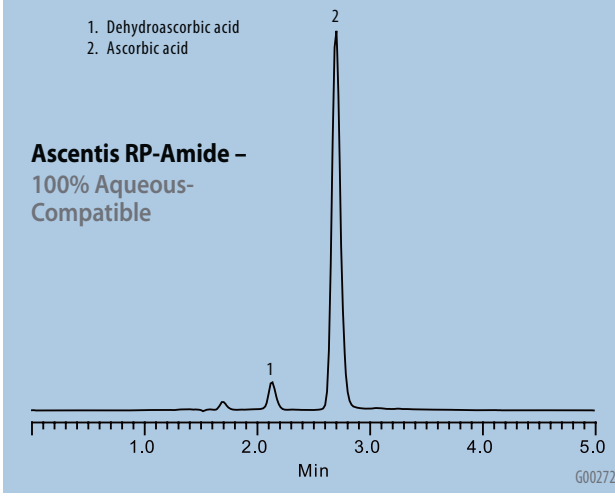


Figure 7. Separation of Organic Acids on Ascentis RP-Amide

column: Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase: 25 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid)
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV at 230 nm
injection: 10 µL

1. Dehydroascorbic acid
2. Ascorbic acid



Selecting the Right Buffer

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

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

LC-MS Compatibility Through Phase Stability, Retentivity and Inertness

In today's laboratory, HPLC columns and bonded phases must be compatible with mass spectrometric detection. Complete MS compatibility is an important design input for all Ascentis phases.

Negligible Phase Bleed

Loss of stationary phase can contribute to high background interference in all forms of detection, but it is most notable in MS detection where phase bleed can also lead to fouling of the instrument and subsequent downtime for cleaning and repair. Modern bonding procedures and an intelligent selection of the Ascentis phase chemistry combine to give all Ascentis phases low detectable bleed under MS and sensitive UV detection.

Amide Chemistry Avoids the Need for TEA and TFA Additives

Silanol-suppressing mobile phase additives, like TEA and TFA, are required for good peak shape on traditional HPLC phases. However, because they suppress the MS signal they should be avoided. Ascentis RP-Amide, by virtue of the embedded amide group, does not require silanol-suppressing additives for good peak shape. Formic acid is a suitable acidic modifier for use with Ascentis RP-Amide columns.

Ascentis pH Stability: Extending the Working pH Range

Occasionally, HPLC mobile phases outside the normal pH 2-7 range are desired to control sample stability, solubility or ionization state, or for compatibility with detection methods. A limitation of most silica-based HPLC phases is their instability outside this range where hydrolysis of the bonded phase and dissolution of the underlying silica can occur. Ascentis columns have excellent stability compared to competitive silica-based columns. The high bonded phase coverage and proprietary endcapping combine to increase resistance to hydrolysis and dissolution. As a result, Ascentis columns can be used successfully between pH 1.5-10 under certain conditions. Note, however, that it is important to avoid storing Ascentis columns, and any silica-based column, in harsh mobile phases.

Ascentis Provides Scalable Separations from Microbore to Preparative

Time and precious samples are wasted during scale-up if the analytical and preparative columns do not give the same elution pattern. The high surface area of the underlying Ascentis silica provides high loading capacity to purify larger quantities of material. Additionally, bonded phase and silica chemistry are uniform across 3,5, and 10 µm particle sizes. These features combine to ensure that analytical separations that are developed on Ascentis 3 or 5 µm particles are completely scalable to preparative separations on Ascentis 10 µm particles and larger columns. Additionally, separations developed on 5 or 10 µm particles can be scaled down for fast analysis on Ascentis 3 µm particles.

- Ascentis 10 µm particles in large column dimensions are ideal for isolating and purifying mg to gram amounts of compounds for further characterization.
- Ascentis 3 µm particles in short columns are ideal for rapid analysis and LC-MS applications.

Guidelines for Preparing Mobile Phases

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

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

Ascentis C18

The First Choice for Classic C18 Retention and Selectivity

Optimization of silica and bonded phases make Ascentis C18 a true workhorse for the vast majority of HPLC separations. High surface area and phase stability give it perfect character for demanding LC-MS and preparative separations.

Features:

- **Classic C18 selectivity**
- **High non-polar retentivity**
- **Symmetric peak shape**
- **Highly reproducible and stable**
- **Ideal for LC-MS**

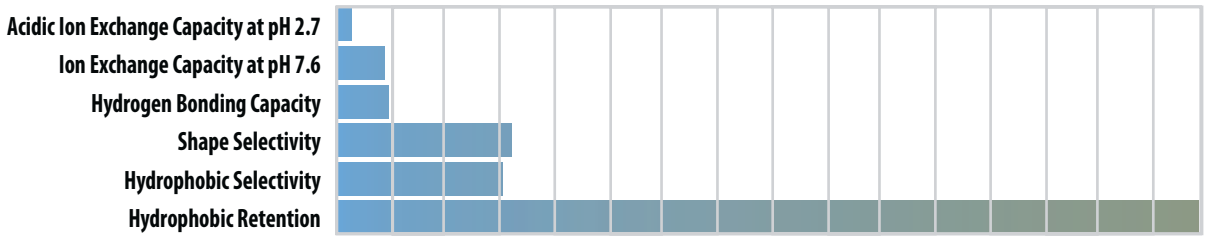
Key Applications:

General reversed phase, hydrophobic and polar compounds

Properties:

USP code:	L1
Bonded phase description:	Octadecyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	25%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis C18



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

The classic reversed-phase column, Ascentis C18 is suitable for any method that specifies a C18-type column. Its high surface area gives Ascentis C18 strong hydrophobic retention and high loading capacity for preparative applications.

LC-MS Implications

Ascentis C18 is low-bleed for clean ESI and APCI traces. The high retentivity means that the mobile phase can contain high levels of organic modifier that are more readily desolvated.

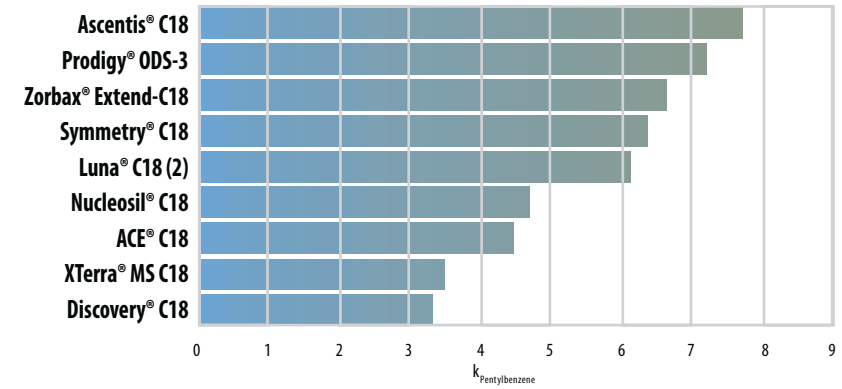
Notes

Modern C18 columns have very similar selectivity, even for basic compounds, because silica quality and bonding techniques have improved to the point that silanol effects are minimal. Unless quality with your current column is the issue, the main reasons for evaluating different brands of C18 columns are for improved peak shape and for slight changes in selectivity.

* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Ascentis C18 is one of the most retentive reversed-phase columns available. High retentivity extends the linear range of loading capacity, making Ascentis C18 ideal for separations that are or might be used for preparative applications. High retentivity also means Ascentis C18 can accommodate the highly-organic mobile phases encountered in LC-MS.

Ascentis C18: Top of Its Class in Hydrophobic Retentivity



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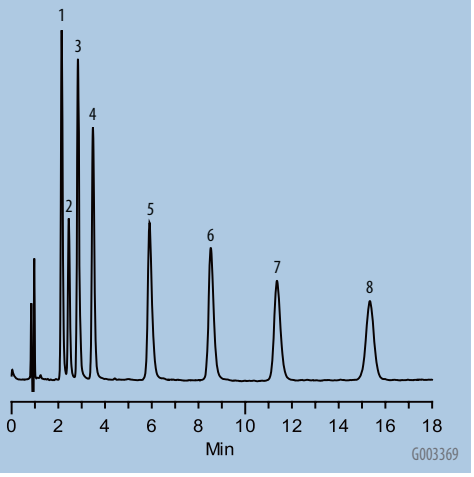
Analysis of basic compounds at neutral pH often gives longer retention compared to acidic mobile phases, but sometimes causes poor peak shape due to silanol interactions. The highly-inert surface of Ascentis C18, as with all Ascentis phases, permits analysis in neutral pH mobile phases. In this example, a mix of tricyclic antidepressants at pH 7 shows excellent peak shape on Ascentis C18.

Figure 8. Symmetrical Peaks for Basic Compounds Indicative of High Surface Deactivation of Ascentis Phases

column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase: 30:70; 25 mM ammonium phosphate, pH 7.0: methanol
flow rate: 1.5 mL/min.
temp.: 35 °C
det.: UV at 254 nm
inj.: 20 µL

1. Desmethyl doxepin
2. Protriptyline
3. Desimpramine
4. Nortriptyline
5. Doxepin
6. Impramine
7. Amitriptyline
8. Trimpramine

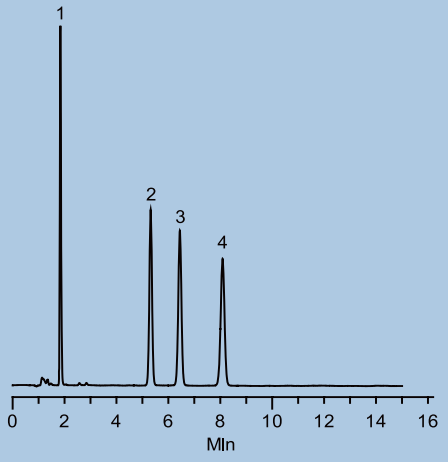


Ascentis C18 provides the selectivity and retention for a range of compounds including steroids. Ascentis C18 is a reliable first choice HPLC column that gives symmetric peak shape and excellent retention for even difficult compounds.

Figure 9. Estrogenic Steroids on Ascentis C18

column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase: 55:45, water:acetonitrile
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV at 220 nm
inj.: 10 µL

1. Estriol
2. α-Estriol
3. β-Estriol
4. Estrone



Ascentis RP-Amide

Ultra-Low Bleed Alkyl Amide Phase that Rivals C18 as a Generic Scouting Column. Excellent Peak Shape and Resolution, Especially for Polar Compounds or Mixtures of Compound Polarity

As pioneers in embedded polar group (EPG) phases for HPLC, Supelco is pleased to offer Ascentis RP-Amide, which has all the benefits of enhanced polar compound retention and selectivity, without any of the disadvantages of competitive EPG phases.

Features

- **Improved peak shape for bases compared to C18**
- **Different selectivity than C18 or C8 for wide range of polar compounds (especially acids)**
- **Lower bleed than competitive EPG phases**
- **100% aqueous compatible**

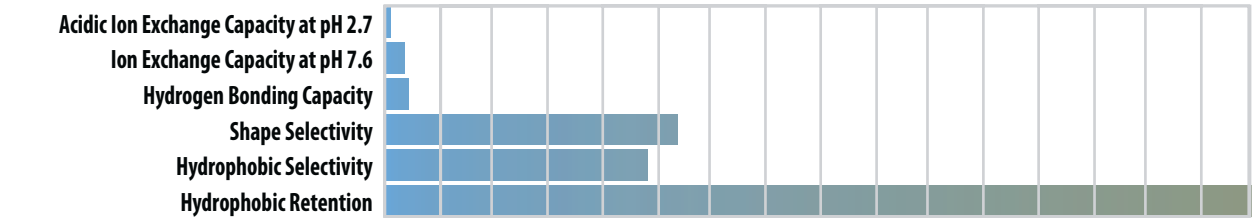
Key Applications

Small, water soluble molecules and peptides, H-bond donors, acids, phenols, basic compounds, polar compounds

Properties

USP code:	L60
Bonded phase description:	Stable amide group embedded in an 18-carbon chain
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	19.5%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis RP-Amide



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Ascentis RP-Amide can be used for many of the same separations as a C18 while avoiding some of the disadvantages of C18 such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl-only phases, Ascentis RP-Amide has enhanced retention and selectivity for phenols, organic acids and other polar solutes due to strong H-bonding between the amide carbonyl (H-bond acceptor) and H-bond donors, like phenols and acids. Compared to other EPG phases, like carbamates, ureas, sulfonamides and ethers, Ascentis RP-Amide gives retention comparable to C18 and C8 for easy column comparison without the need to change mobile phase conditions.

LC-MS Considerations

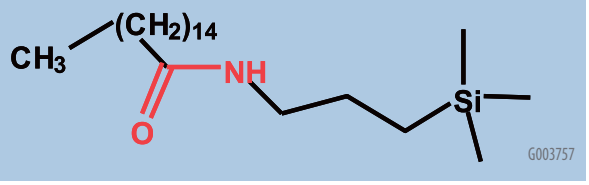
Unlike other amide-based phases, Ascentis RP-Amide uses an amidosilane reagent and a one-step bonding method similar to C18. Polymeric reagents are also employed to achieve maximum stability and low bleed with all modern HPLC detectors.

Notes

- Generally, acids are retained more and bases retained less on RP-Amide compared to C18 and C8 columns.
- Methanol can be comparable in elution strength to acetonitrile when compounds are retained by H-bonding mechanism on the RP-Amide phase.

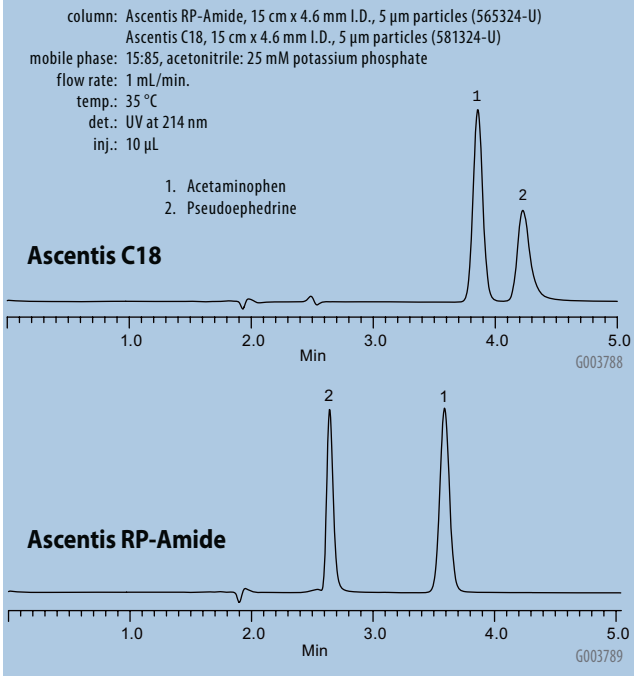
* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Figure 10. Structure of Ascentis RP-Amide



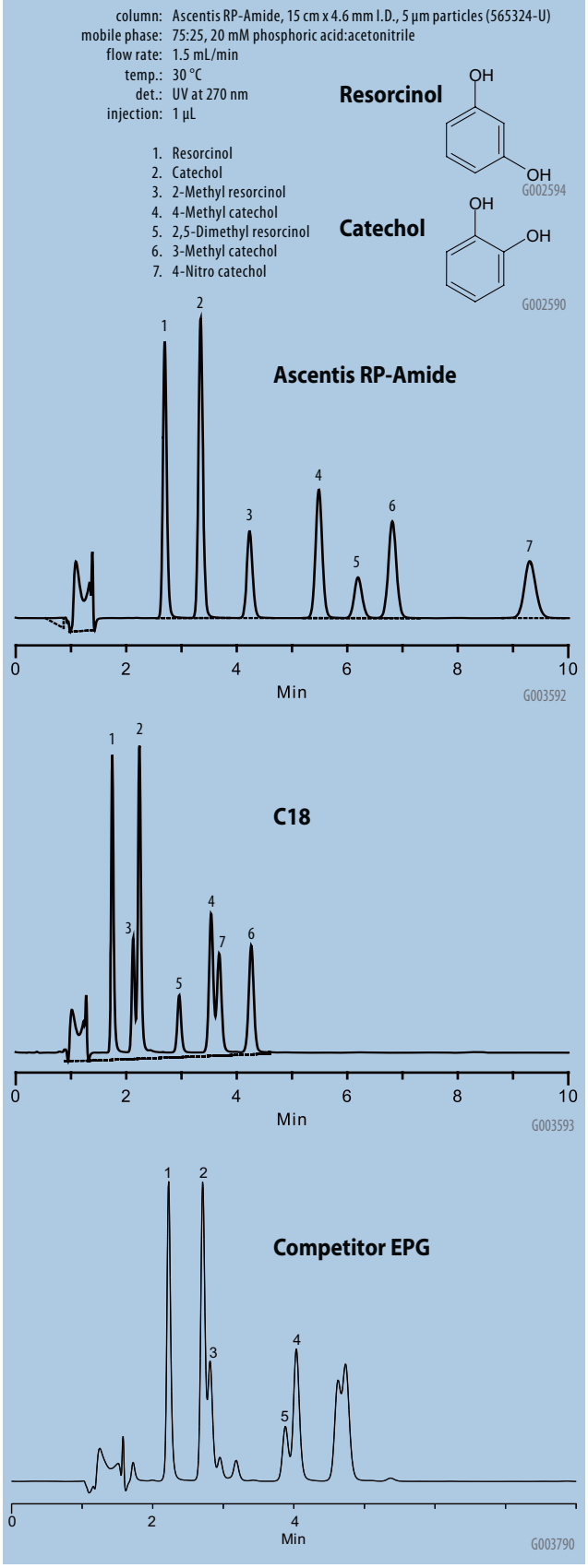
Because the amide group lies near the silica surface, it is believed to suppress tailing of basic solutes by electrostatic shielding (repulsion) or by interacting preferentially with the silanols (H-bonding between the phase and the substrate). The absence of unwanted silanol and other secondary interactions gives the Ascentis RP-Amide excellent peak shape for both acids and bases. This example of internal deactivation with acetaminophen and pseudoephedrine (Figure 11) shows the dramatic effect of changing stationary phase: not only are the peaks more symmetrical, but elution order is reversed.

Figure 11. Improved Efficiency, Selectivity and Resolution of Ascentis RP-Amide vs. C18



An Ascentis RP-Amide column is more retentive and selective for phenolic compounds, like catechols and resorcinols, compared to a C18 and an ether-type polar embedded phase (Figure 12). The ether phase does not have the ability to H-bond with phenolic groups like the amide group does. Although comparing an ether phase to a C18 phase may be useful if only slightly different selectivity is needed, the most dramatic results for acids such as phenols and carboxylic acids will be obtained with amide-based phases, like Ascentis RP-Amide.

Figure 12. Enhanced Phenolic Compound Retention via H-bonding on Ascentis RP-Amide



Ascentis Phenyl

Ultra-low Bleed Phenyl Phase with Enhanced Phenyl Selectivity

Phenyl-based reversed-phases were one of the first alternatives to C18 selectivity. Our Ascentis Phenyl has been improved to offer exceptional phase stability and enhanced phenyl retention. Ascentis Phenyl offers versatility by also operating in the HILIC mode.

Features

- **Low-bleed for MS or UV gradient applications due to the use of trifunctional bonding reagent**
- **Outstanding phenyl selectivity due to high phase loading and short butyl spacer**
- **100% aqueous-compatible for highly-polar compounds**

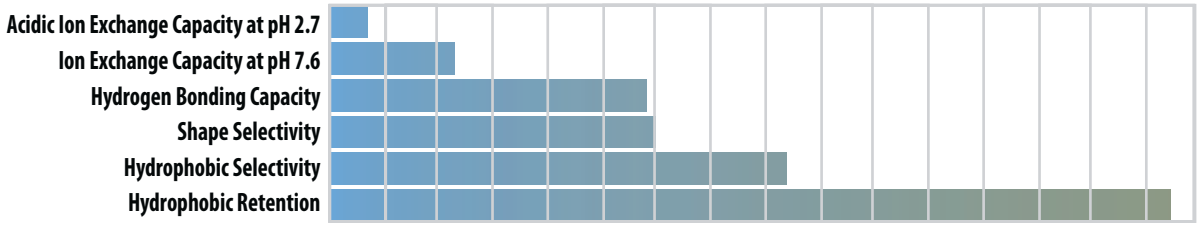
Key Applications

Small, water soluble molecules and peptides, π-acceptors, nitroaromatics, polar compounds, dipoles, heterocyclics, HILIC mode

Properties

USP code:	L11
Bonded phase description:	Phenyl ring with short butyl spacer
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	19.5%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis Phenyl



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Phenyl phases are π-basic (electron donating) and are similar in overall retention to alkyl and EPG phases for easy column screening. The alternate selectivity of phenyl phases is often explained by the π-π interactions available through the phenyl ring. Compounds that appear to exhibit differential selectivity on the phenyl phase include:

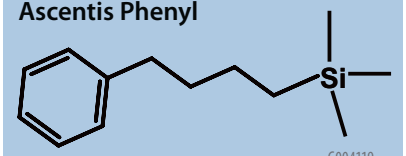
- hydrophobic bases (TCAs, tetracyclines)
- moderate bases (anesthetics and narcotic analgesics)
- benzodiazepines
- some acidic compounds such as ACE inhibitors and quinoline antibiotics
- nucleosides (e.g. cytidine)
- nitro, azide and sulfonyl compounds

Notes

- Methanol can be a more selective mobile phase component than acetonitrile.
- Activate HILIC mode by using highly-aqueous (>90%) mobile phases.

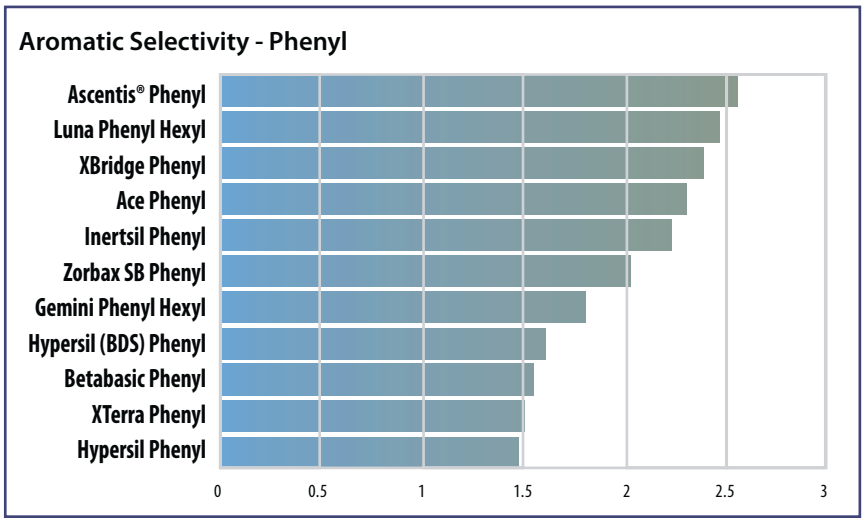
* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Figure 13. Structure of Ascentis Phenyl



Most commercially-available phenyl phases actually show a great deal of C18-like selectivity, negating their impact on improving the separation. With the highest level of true phenyl character among all phases tested, Ascentis Phenyl has excellent aromatic selectivity, making it a true alternative to traditional C18.

The short, butyl spacer of Ascentis Phenyl does not dilute the phenyl character as conventional hexyl spacers do. Figure 14 shows the stronger contribution of π-electron interactions from the phenyl ring on Ascentis Phenyl compared to the phenylhexyl phase allowing it to resolve buspirone and trazadone. The phenyl and alkyl selectivity tend to cancel each other out on the competitive phenylhexyl phase in this case.

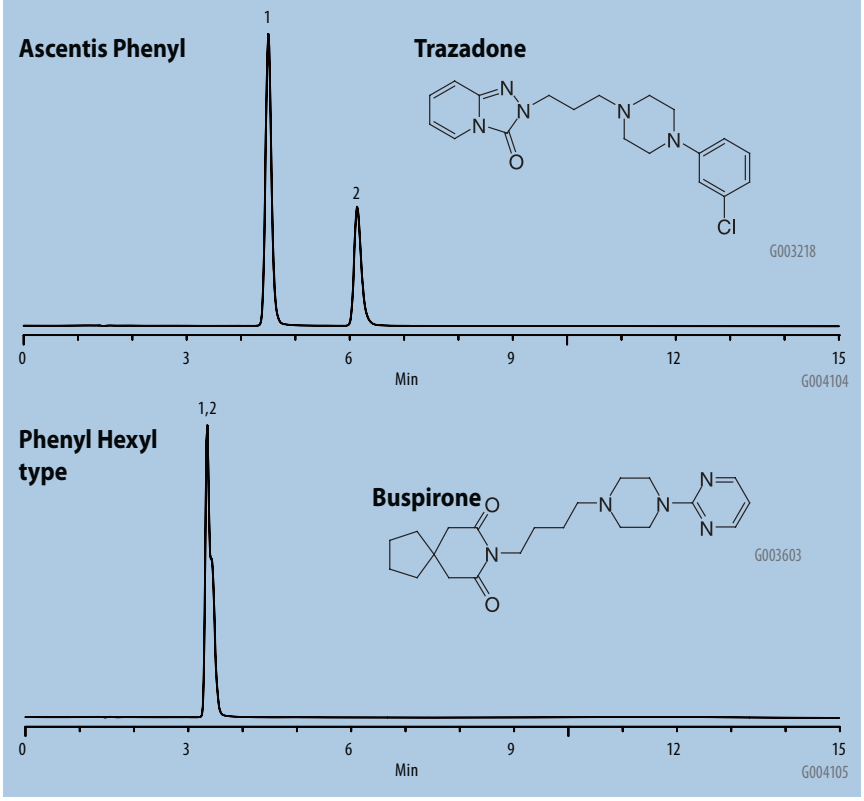


All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Figure 14. Better Phenyl Selectivity on Ascentis Phenyl Compared to Competitive Phases

column: Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase: 40:60, 10 mM ammonium acetate (pH 5.5 with acetic acid):acetonitrile
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV at 254 nm
injection: 1 µL

1. Trazadone
2. Buspirone



Ascentis ES Cyano

Extra stable for low pH mobile phases due to sterically protected phase

Useful for polar selectivity in the reversed-phase mode, including π-π and dipole-dipole interacting compounds. Can also be used in HILIC mode and normal phase chromatography.

Features:

- **Enhanced stability at low pH**
- **Operates in reversed-phase, HILIC and normal phase modes of chromatography**
- **Low MS bleed**
- **100% aqueous compatible**
- **Available as 3 µm and 5 µm particles**

Key applications:

polar compounds, nitroaromatics, tricyclic antidepressants, steroids

Properties:

USP Code:	L10
Bonded phase description:	diisopropyl cyano propyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	< 5 ppm metals
Particle shape:	Spherical
Particle size:	3 and 5 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	10 %
pH range recommended:	1-8

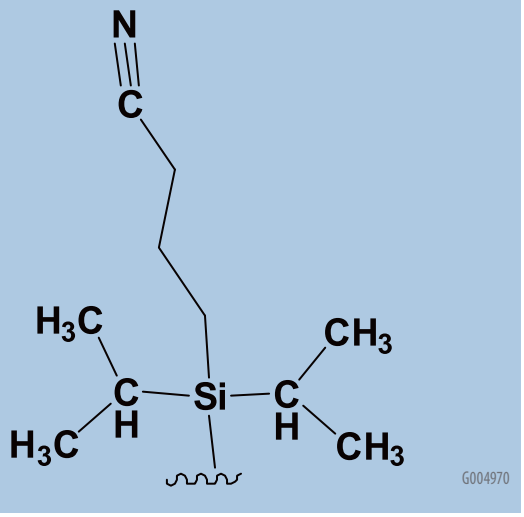
Use

Cyano phases have become very popular because of their unique selectivity for polar groups and double bonds. Their potential for dipole/dipole and dipole/induced-dipole interaction made them one of the earliest stationary phase functional groups when alternate selectivity was needed. In the past, stability of the cyano phase under reversed-phase and HILIC conditions has been poor. Exposure to aqueous organic mobile phases, acidic pH and elevated temperatures can create gradual retention and selectivity loss that is reportedly due to stationary phase hydrolysis. A new Ascentis ES Cyano column, based on 3 and 5 µm porous silica substrate, has been developed. This new phase compares very favorably in stability to C18, C8, Amide and Phenyl.

Notes

- Can be used in reversed, HILIC and normal phase modes
- It is best to dedicate a specific column to one mode of chromatography mentioned above.
- Methanol gives more selectivity than acetonitrile in the reversed phase mode.
- Cyano phases are used in EPA Method 8330 (1) for the analysis of explosives and nitroaromatics.

Figure 15. Structure of Ascentis ES Cyano



1. US EPA Method 8330A, "Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)" Revision 1 (February 2007), obtained from the *www.epa.gov web site.*

Cyano columns have been used for the analysis of tricyclic antidepressants for some time. A comparison of the most popular competitor column and the Ascentis ES Cyano phase is shown to the right. Better resolution is seen using the ES Cyano than the competitor column for three critical peak pairs. In addition, note the selectivity change of the trimipramine and doxepin (peaks 1 and 2) between these two phases under these conditions.

Figure 16. Comparison of Tricyclic Antidepressants on Ascentis ES Cyano and a Competitor Cyano Phase

column: as indicated; 15 cm x 4.6 mm, 5 µm particle
mobile phase A: 20 mM potassium phosphate, dibasic (pH 7.0 with phosphoric acid)
mobile phase B: acetonitrile
mobile phase C: methanol
mixing proportions: A:B:C, 25:60:15
flow rate: 2.0 mL/min
temp.: 25 °C
det.: 215 nm
injection: 5 µL
sample: 100 µg /mL in 70:30, water: methanol

1. Trimipramine
2. Doxepin
3. Amitriptyline
4. Imipramine
5. Nordoxepin
6. Nortriptyline
7. Desipramine
8. Protriptyline

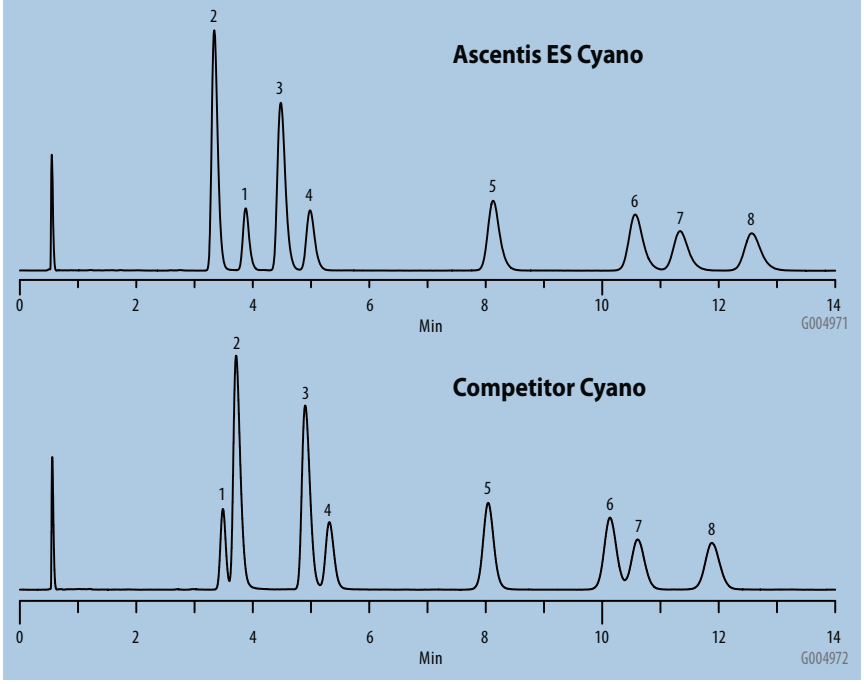
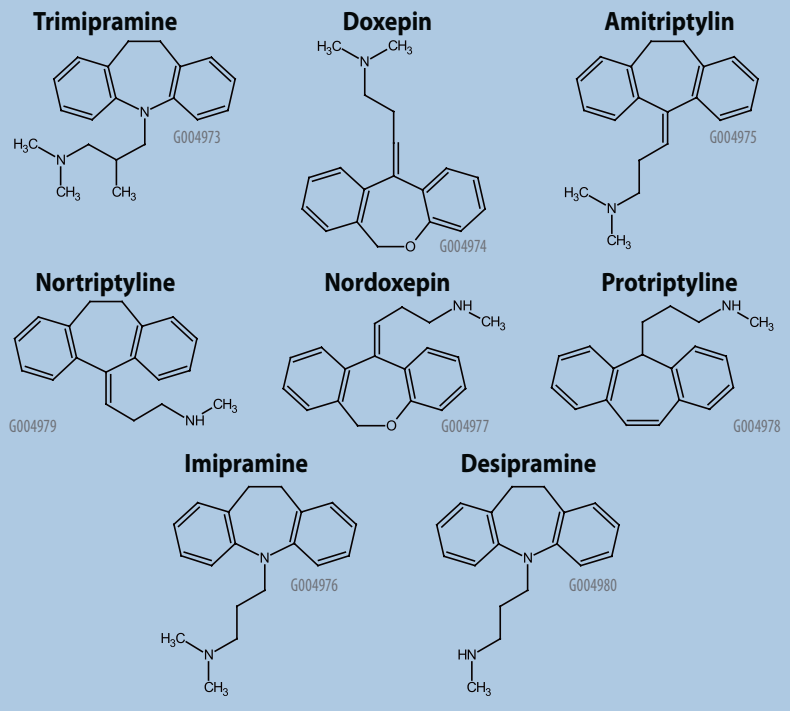


Figure 17. Structures of Tricyclic Antidepressants



Ascentis Silica

High Surface Area and High Surface Deactivation Combine to Give Ascentis Silica Exceptional Performance as a Normal Phase, HILIC and Preparative HPLC Material

Besides being the underlying support for all Ascentis phases, Ascentis Silica has applications in its own right. Silica is widely used to separate positional isomers in normal phase mode, and polar compounds in HILIC (aqueous normal phase modes). Silica is also used in organic synthesis to purify reaction mixtures. In each case, a high purity, controlled and uniform surface is necessary to impart the desirable chromatographic performance.

Features

- **High-loading capacity**
- **Operates in both normal-phase and HILIC modes**
- **Tested in both modes and shipped in ethanol, Ascentis Silica is ready to use in either mode**
- **Ultra-pure, spherical silica**
- **Available in 3, 5, and 10 µm**

Key Applications

Small molecular weight positional (geometric) isomers, non-polar compounds (in NP mode), vitamins, steroids, polar compounds (in HILIC mode)

Properties

USP code:	L3
Bonded phase description:	None (surface comprises silanol, -Si-OH, and siloxane, -Si-O-Si-, groups)
Endcapped:	No
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	0%
pH range (recommended):	2-6

Use

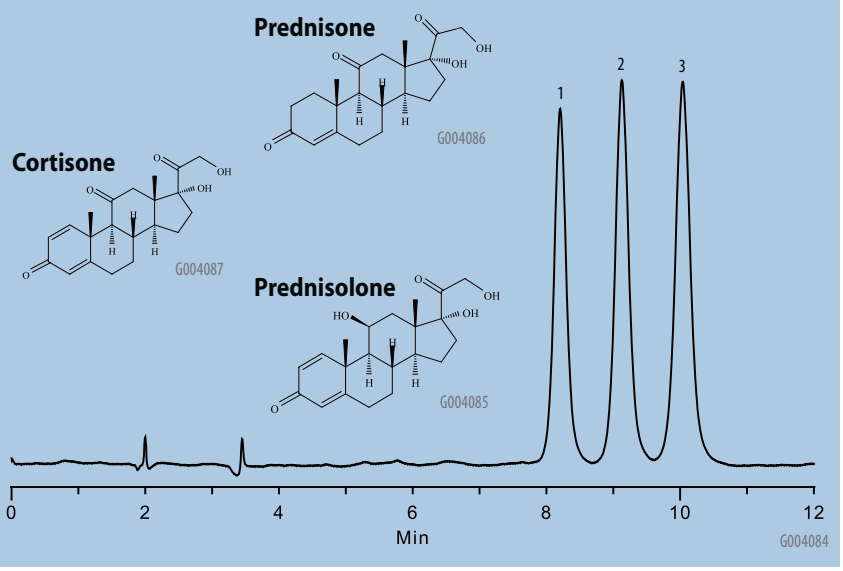
- Normal phase and HILIC HPLC modes
- Preparative chromatography
- Purification (organic synthesis)
- LC-MS

The classic use of silica columns is for normal phase HPLC. The rigid structure of the silica surface, as opposed to the flexible nature of bonded phases, allows it to distinguish between molecules with different footprints that may have the same hydrophobicity. Geometric isomers and closely-related substances, like the steroids shown in Figure 15, can be separated on Ascentis Silica under normal phase conditions. Normal phase is also widely used in preparative chromatography because the mobile phase is more easily removed by evaporation than the water-containing reversed-phase mobile phases.

Figure 18. Ascentis Silica: Normal Phase Separation of Geometric Isomers and Closely-Related Compounds

column: Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase: 88:12, hexane:ethanol
flow rate: 1.0 mL/min.
temp.: 35 °C
det.: UV at 245 nm
injection: 10 µL
sample: 50 µg/mL in 85:15, hexane: 2-propanol

1. Cortisone
2. Prednisone
3. Prednisolone

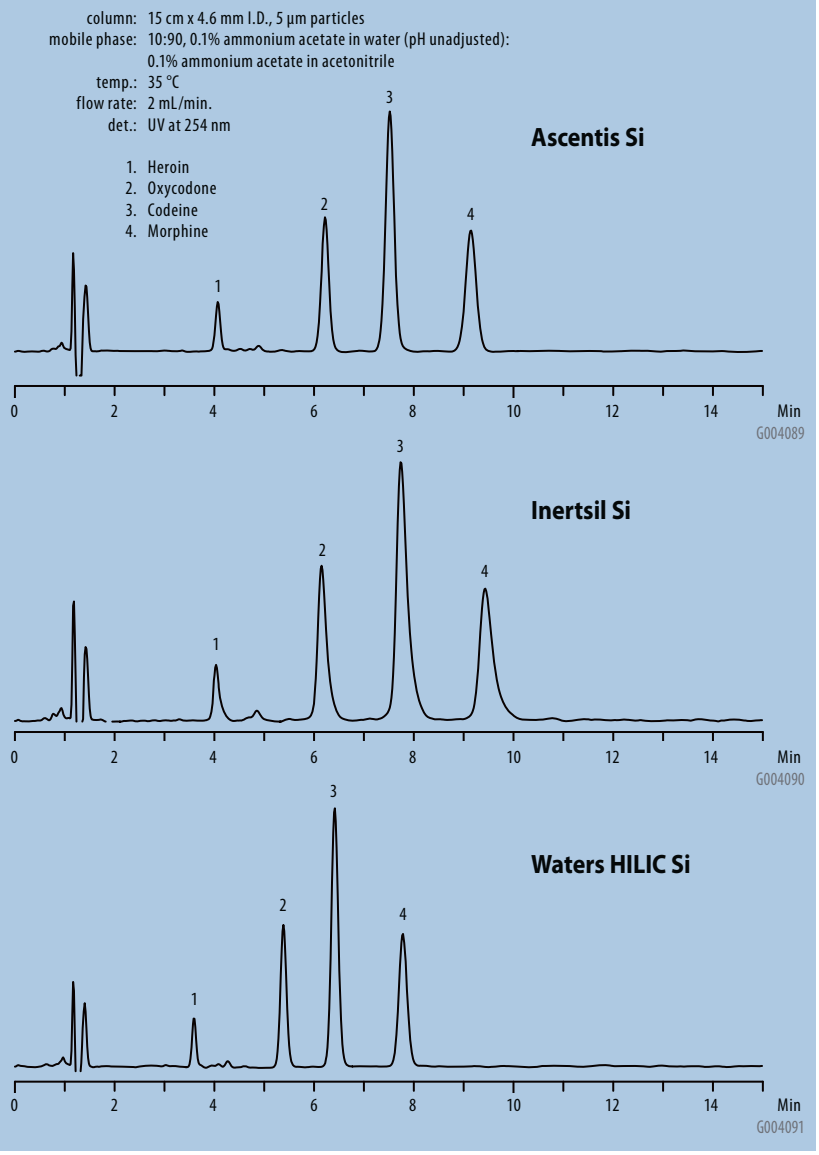


Ascentis Silica is used successfully in the aqueous normal-phase or HILIC mode. In this mode, water is the strong modifier and the organic is the weak modifier of the mobile phase. Like reversed-phase, HILIC offers the flexibility of using pH and ionic strength to control retention.

HILIC is ideal for very polar compounds and is highly compatible with LC-MS.

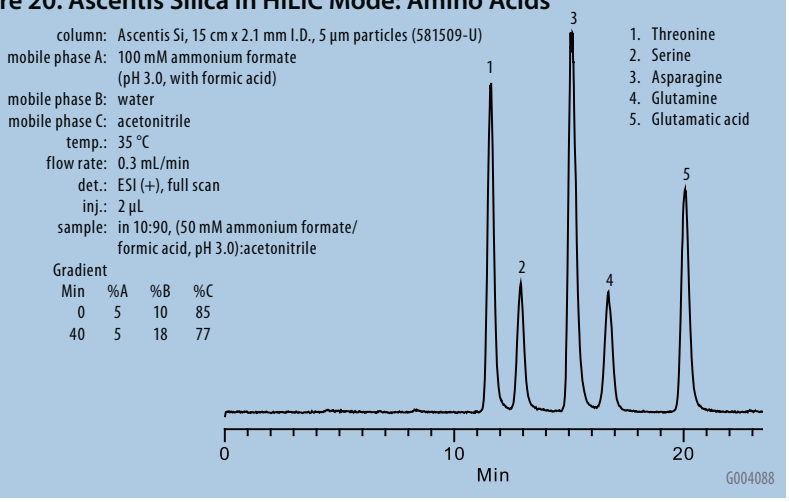
Elution order is generally opposite to that obtained under reversed-phase conditions. Figure 16 compares Ascentis Silica with two competing silicas, demonstrating better resolution by virtue of excellent peak shape and high retentivity.

Figure 19. Ascentis Silica in HILIC Mode Gives Better Peak Shape and Retention of Basic Drugs than Competitive Silicas



Polar biomolecules, like amino acids, nucleotides and nucleosides, typically require derivatization for their analysis by reversed phase HPLC. The HILIC mode offered by Ascentis Silica permits the retention and resolution of these compounds without derivatization, eliminating a time-consuming sample preparation step (Figure 17).

Figure 20. Ascentis Silica in HILIC Mode: Amino Acids



Ascentis C8

One of The Most Hydrophobic C8 Phases Available

Leveraging the improvements to silica and bonded phase properties that made Ascentis C18 so useful, its shorter alkyl chain cousin, Ascentis C8, is also suitable for routine HPLC and LC-MS.

Features:

- **Selectivity similar to C18 for non-polar compounds**
- **Different selectivity for polar compounds**
- **Less hydrophobic than C18, more hydrophobic than other C8 phase**
- **Symmetric peak shape**
- **Highly reproducible and stable**
- **Ideal for LC-MS**

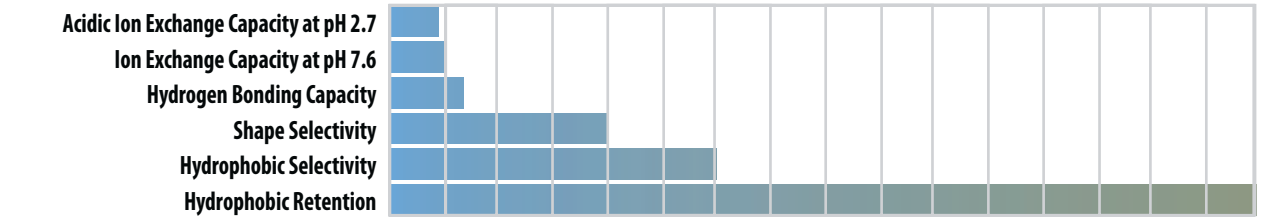
Key Applications:

Small, water soluble molecules and peptides, less hydrophobic retention than C18 but comparable selectivity, LC-MS

Properties:

USP code:	L7
Bonded phase description:	Octyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3 and 5 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	15%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis C8

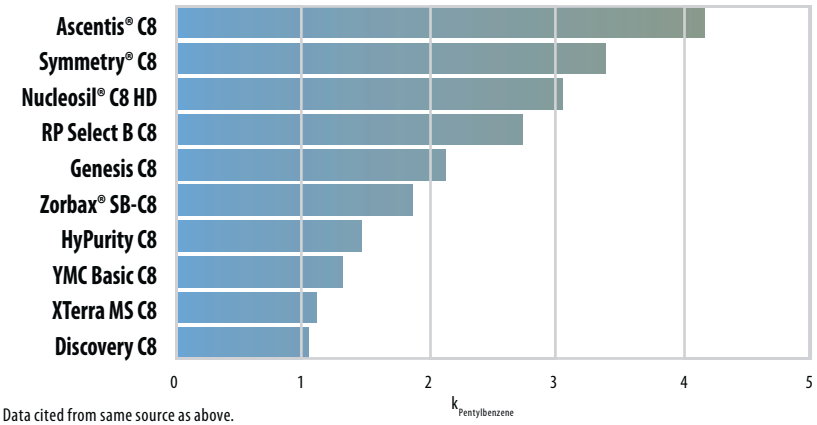


All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Ascentis C8 is suitable for any method that specifies a C8-type column. Although C8 columns often show similar selectivity to C18 columns, shorter alkyl chains sometimes show different selectivity toward polar compounds because they can solvate differently with the mobile phase and interact differently due to the size and shape of certain molecules. Also, C8 reagents are smaller than C18 reagents and have improved primary phase coverage, thereby requiring less end-capping. Ascentis C8 has excellent peak shape and very high phase stability.

Ascentis C8: Top of Its Class in Hydrophobic Retentivity



Data cited from same source as above.

Among commercially available C8 columns, Ascentis C8 has the highest degree of hydrophobic retention. This permits the use of higher percentages of organic modifier, a benefit to LC-MS users.

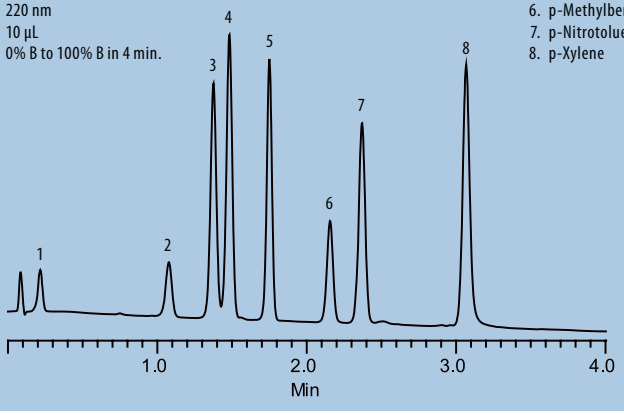
* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Ascentis C8 is an excellent choice for fast gradient analysis. Ascentis C8 is typically more retentive at low organic composition that C18 and less retentive at high organic composition. Furthermore, Ascentis C8 has better aqueous compatibility for gradients that start at 100% aqueous composition.

Figure 21. Fast Gradient Analysis on Ascentis Columns

column: Ascentis C8, 3 cm x 3.0 mm I.D., 5 µm particles (581440-U)
mobile phase A: 95:5, 10 mM ammonium phosphate:acetonitrile, pH 2.5
mobile phase B: 40:60, 10 mM ammonium phosphate:acetonitrile, pH 2.5
flow rate: 3.5 mL/min
temp.: 35 °C
det.: 220 nm
inj.: 10 µL
gradient: 0% B to 100% B in 4 min.

1. p-Toluidine
2. p-Nitroaniline
3. p-Nitrophenol
4. p-Cresol
5. p-Nitrobenzoic acid
6. p-Methylbenzoic acid
7. p-Nitrotoluene
8. p-Xylene



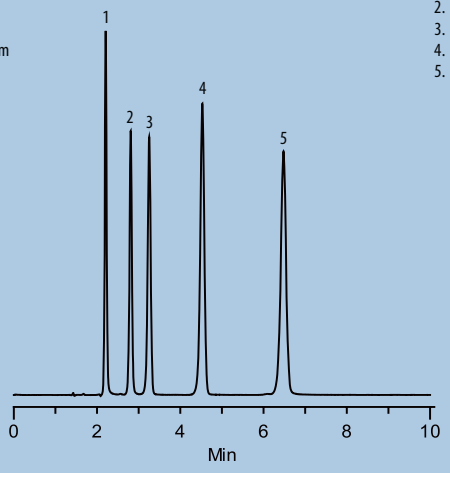
G003377

Ascentis C8 often yields enhanced retention than Ascentis C18 for small polar molecules under highly aqueous conditions. Greater retention for Ascentis C8 may be related to greater wettability of Ascentis C8 as compared to Ascentis C18.

Figure 22. Polar Molecules Under Highly Aqueous Conditions

column: Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase: 3:97, methanol:water with 0.1% TFA
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV at 220 nm
inj.: 10 µL

1. L-tartaric
2. Lactic Acid
3. Citric Acid
4. Fumaric Acid
5. Acrylic Acid



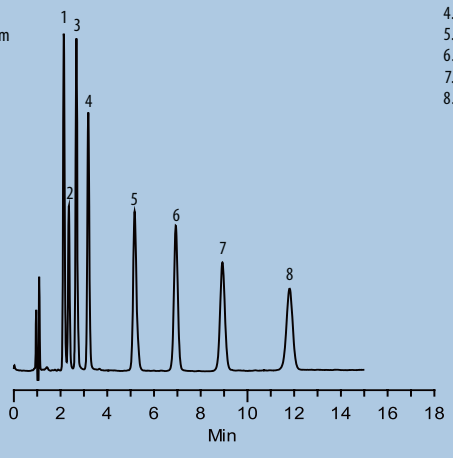
G003368

Analysis of bases at neutral pH often yields enhanced retention over acidic mobile phase but sometimes causes poor peak shape with C18 and C8 columns due to silanol interaction. In this example, a mix of tricyclic antidepressants at pH 7 shows excellent peak shape on Ascentis C8.

Figure 23. Pharmaceutical Bases at pH 7

column: Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase: 30:70; 25 mM ammonium phosphate, pH 7.0: methanol
flow rate: 1.5 mL/min.
temp.: 35 °C
det.: UV at 254 nm
inj.: 20 µL

1. Desmethyl Doxepin
2. Protriptyline
3. Desimpramine
4. Nortriptyline
5. Doxepin
6. Impramine
7. Amitriptyline
8. Trimpramine



G003370

Discovery HS F5

Unique Reversed-phase Selectivity Compared to C18 and C8

Discovery HS F5 provides reversed-phase separations that are distinctly different from C18 columns. However, compounds will generally elute within the same retention time window, making most C18 methods easily transferable.

Features

- **Unique (orthogonal) selectivity compared to C18 and C8**
- **Stable, low-bleed LC-MS separations**
- **Both reversed-phase and HILIC modes**
- **Possesses multiple types of interactions: dispersive, dipole-dipole, π-π, charge-transfer**

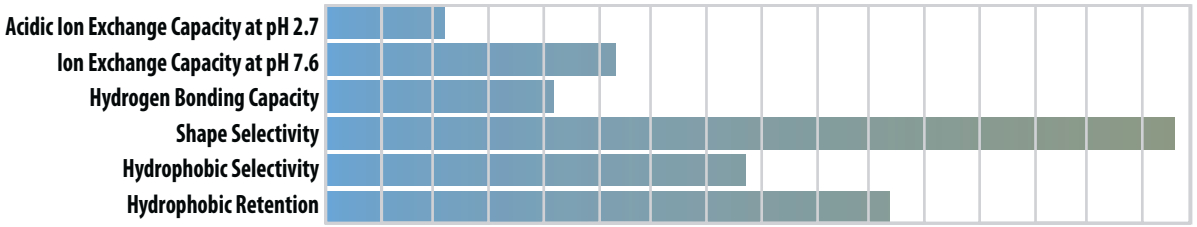
Key Applications

Small, water soluble molecules and peptides, polar compounds, basic compounds, positional isomers

Properties

USP code:	L43
Bonded phase description:	Pentafluorophenylpropyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<10 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	120 Å
Surface area:	300 m^2/g
Carbon load:	12%
pH range (recommended):	2-8

Characterization of Chromatographic Performance on Discovery HS F5



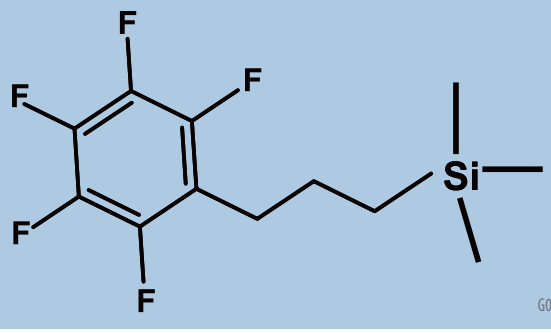
All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Notes

Compared to a C18: Generally, bases are retained longer on the HS F5 than on a C18, hydrophobic compounds are retained less. Increasing the organic content of a C18 separation 5 to 10 percent will generally provide similar retention on a HS F5. Also, as a general rule, solutes with log $P_{o/w}$ values less than 2.5 will be retained longer on HS F5 compared to a C18.

Compared to a phenyl phase: Although aromatic in nature, the pentafluorophenylpropyl (F5) phase does not resemble a phenyl phase in retention or selectivity. The F5 is a strong Lewis acid due to the electron withdrawing effects of five fluorine groups; the F5 ring is electron deficient whereas the phenyl ring is electron rich.

Figure 24. Structure of Discovery HS F5



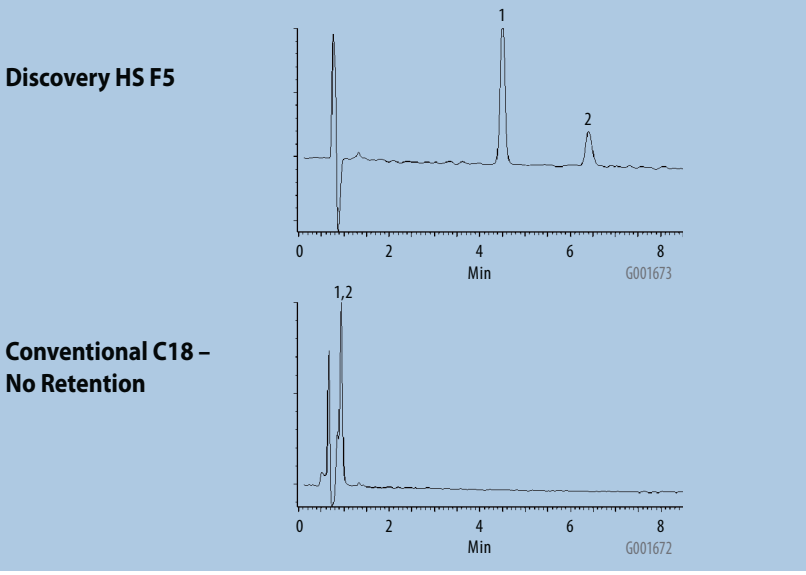
Guidelines for Transferring a C18 Method to Discovery HS F5

Generally, bases are longer retained on the HS F5 than on a C18. Increasing the organic content of a C18 separation 5 to 10 percent will generally provide similar retention on a HS F5. Results with other compounds are highly variable. However, it is generally true that solutes with $logP_{o/w}$ values less than 2.5 will be retained longer on HS F5 compared to a C18. The degree of difference is highly solute dependent.

Figure 25. HS F5 Provides Excellent Separation - Solutes Are Not Retained on C18

column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
Conventional C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 30:70, 10 mM Ammonium Acetate (pH 6.98): CH_3CN
flow rate: 2.0 mL/min
temp.: 35 °C
det.: Photodiode Array
inj.: 5 µL

1. Methcathinone (100 µg/mL)
2. (+/-) Ephedrine (200 µg/mL)

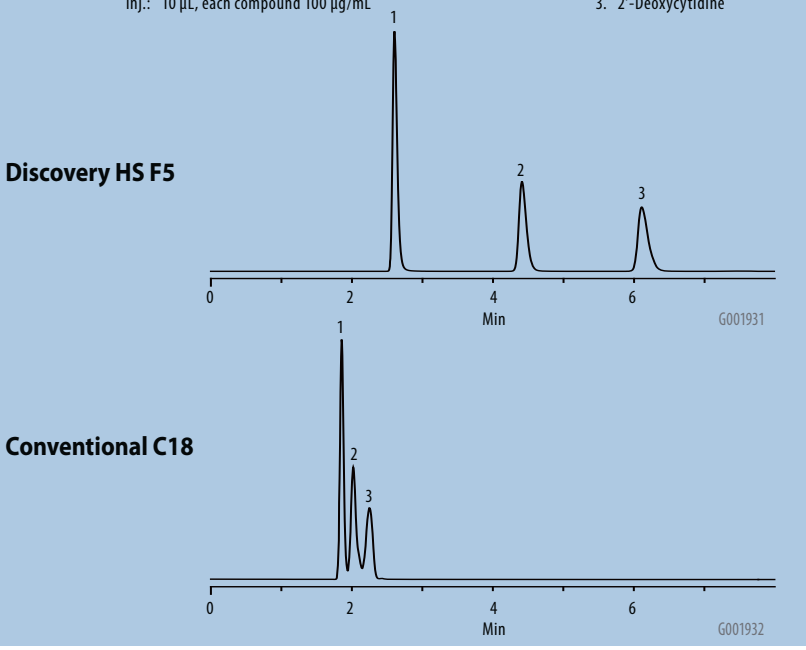


In Figure 25, cytidine and related compounds provide another example of the power of HS F5 to provide unique and valuable separations compared to a C18. An added benefit of the HS F5 is its resistance to phase collapse under 100% aqueous conditions.

Figure 26. Unique Selectivity of HS F5 Resolves Compounds Better than C18

column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
Conventional C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 10 mM KH_2PO_4, pH 3.0 with H_3PO_4, (C18 separation has 5% CH_3CN)
flow rate: 1 mL/min
temp.: 30 °C
det.: UV at 280 nm
inj.: 10 µL, each compound 100 µg/mL

1. Cytidine
2. Cytosine
3. 2′-Deoxycytidine



Ascentis® Express

Extreme Performance on Any LC System

The demand for increased sample throughput and speed of results has driven HPLC users to search for breakthroughs in HPLC instrument and column technology. Although improvements have been realized, setbacks have been encountered. Reductions 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.

Ascentis Express has changed all of that.

Ascentis Express with Fused-Core™ Particle Technology provides the ultimate solution for today's separation demands - high speed and high efficiency with low backpressure.

By simply changing to Ascentis Express Columns, sample throughput can be improved by 400%!

No longer will you have to make changes to:

- **sample prep**
- **flow rate**
- **system pressure**

And no new instrumentation is required!

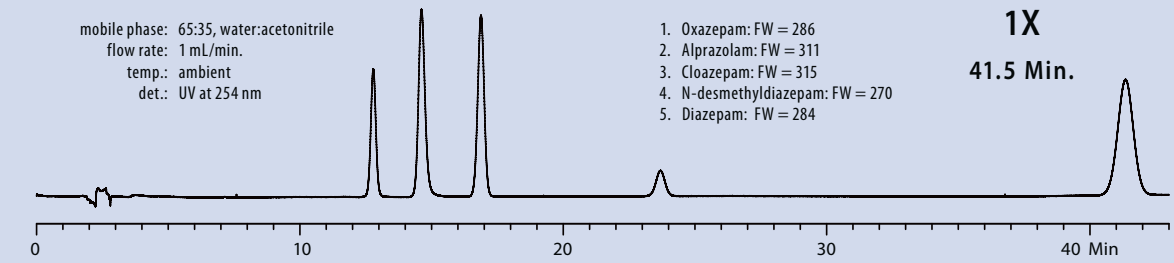
For more information on this exciting new technology, visit our website: ***sigma-aldrich.com/express***

Do More Work in Less Time without Changing your Method

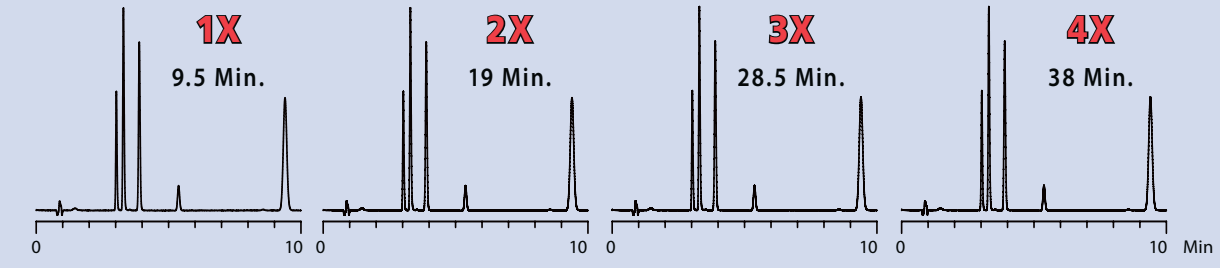
With identical conditions, the Ascentis Express C18 column performs **4X** as many separations as a standard C18 column in less time.

CONDITIONS				Theoretical Plates	Standard C18 Throughput	**Ascentis C18 Throughput**
Sample Prep	Flow Rate	System Pressure	HPLC System			
SAME	SAME	SAME	SAME	SAME	1X	4X

C18, 25 cm x 4.6 mm I.D., 5 µm particles



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



Ordering Information

Particle Size	ID (mm)	Length (cm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis ES Cyano	Ascentis Silica	Ascentis C8	Discovery HS F5
3 µm									
	1	5	581311-U	565309-U	inquire	inquire	inquire	581412-U	inquire
	1	10	581364-U	565389-U	581600-U	inquire	581520-U	581435-U	inquire
	1	15	581365-U	65566-U	581601-U	inquire	581521-U	581436-U	inquire
	2.1	2	581312-U	565313-U	inquire	inquire	inquire	581413-U	inquire
	2.1	3	581313-U	565314-U	581602-U	inquire	581522-U	581414-U	567501-U
	2.1	5	581300-U	565300-U	581603-U	577308-U	581500-U	581400-U	567500-U
	2.1	10	581301-U	565301-U	581604-U	577309-U	581501-U	581401-U	567502-U
	2.1	15	581302-U	565302-U	581605-U	577310-U	581502-U	581402-U	567503-U
	3	2	581314-U	565315-U	inquire	inquire	inquire	581415-U	inquire
	3	3	581302-U	565310-U	581606-U	inquire	581523-U	581403-U	567505-U
	3	5	581307-U	565311-U	inquire	inquire	inquire	581404-U	inquire
	3	10	581308-U	565312-U	581607-U	inquire	581503-U	581405-U	567581-U
	4.6	2	581315-U	565316-U	inquire	inquire	inquire	581416-U	inquire
	4.6	3	581316-U	565317-U	inquire	inquire	inquire	581417-U	567509-U
	4.6	5	581320-U	565320-U	581608-U	577311-U	581504-U	581406-U	567504-U
	4.6	10	581321-U	565321-U	581609-U	577312-U	581505-U	581407-U	567505-U
	4.6	15	581322-U	565322-U	581610-U	inquire	581506-U	581408-U	567507-U
5 µm									
	2.1	2	581368-U	565391-U	inquire	inquire	inquire	581439-U	inquire
	2.1	3	581327-U	565331-U	inquire	inquire	inquire	581430-U	inquire
	2.1	5	581303-U	565303-U	581611-U	577300-U	581507-U	581420-U	567508-U
	2.1	10	581326-U	565304-U	581612-U	577301-U	581508-U	581419-U	567510-U
	2.1	15	581304-U	565305-U	581613-U	577303-U	581509-U	581421-U	567511-U
	2.1	25	581305-U	565306-U	581614-U	inquire	581510-U	581422-U	567512-U
	3	2	581328-U	565332-U	inquire	inquire	inquire	581431-U	inquire
	3	3	581369-U	565392-U	inquire	inquire	inquire	581440-U	inquire
	3	5	581329-U	565333-U	inquire	inquire	inquire	581432-U	inquire
	4.6	2	581330-U	565335-U	inquire	inquire	inquire	581433-U	inquire
	4.6	3	581331-U	565336-U	inquire	inquire	inquire	581434-U	inquire
	4.6	5	581323-U	565323-U	581615-U	577304-U	581511-U	581423-U	567513-U
	4.6	10	inquire	565328-U	inquire	577305-U	inquire	inquire	567515-U
	4.6	15	581324-U	565324-U	581616-U	577306-U	581512-U	581424-U	567516-U
	4.6	25	581325-U	565325-U	581617-U	577307-U	581513-U	581425-U	567517-U
	10	5	581340-U	565340-U	inquire	inquire	inquire	inquire	567518-U
	10	10	581341-U	565341-U	inquire	inquire	inquire	inquire	567537-U
	10	15	581342-U	565343-U	inquire	inquire	inquire	inquire	567519-U
	10	25	581343-U	565344-U	581618-U	inquire	581514-U	inquire	567520-U
	21.2	5	581344-U	565345-U	inquire	inquire	inquire	inquire	inquire
	21.2	25	581347-U	565348-U	581619-U	inquire	581515-U	inquire	567523-U
10 µm									
	4.6	15	581350-U	565352-U	inquire	inquire	inquire	inquire	inquire
	4.6	25	581351-U	565353-U	inquire	inquire	581524-U	inquire	inquire
	10	5	581352-U	565354-U	inquire	inquire	inquire	inquire	inquire
	10	10	581353-U	565355-U	inquire	inquire	inquire	inquire	inquire
	10	15	581354-U	565356-U	inquire	inquire	inquire	inquire	inquire
	10	25	581355-U	565357-U	inquire	inquire	581516-U	inquire	inquire
	21.2	5	581356-U	565358-U	inquire	inquire	inquire	inquire	inquire
	21.2	10	581357-U	565359-U	inquire	inquire	inquire	inquire	inquire
	21.2	15	581358-U	565360-U	inquire	inquire	inquire	inquire	567528-U
	21.2	25	581359-U	565361-U	inquire	inquire	581517-U	inquire	567529-U

	ID (mm)	Length (cm)	Particle Size (µm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis Silica	Ascentis C8	Discovery HS F5
Ascentis Supelguard Cartridges									
Kit	2.1	2	3	581376-U	inquire	inquire	inquire	inquire	567571-U
Pack of 2	2.1	2	3	581377-U	inquire	inquire	inquire	inquire	567570-U
Pack of 2	2.1	2	5	581370-U	565372-U	inquire	inquire	inquire	567574-U
Kit	2.1	2	5	581371-U	565373-U	inquire	inquire	inquire	567575-U
Pack of 2	3	2	5	581374-U	565374-U	inquire	inquire	inquire	inquire
Kit	3	2	5	581375-U	565375-U	inquire	inquire	inquire	inquire
Kit	4	2	3	581378-U	inquire	inquire	inquire	inquire	567573-U
Pack of 2	4	2	3	581379-U	inquire	inquire	inquire	inquire	567572-U
Pack of 2	4	2	5	581372-U	565370-U	581620-U	581518-U	581426-U	567576-U
Kit	4	2	5	581373-U	565371-U	581621-U	581519-U	581427-U	567577-U

Kits include one cartridge, stand alone holder, a piece of tubing, 2 nuts and 2 ferrules.

Ascentis Validation Packs

4.6	15	5	581390-U	565394-U	581695-U	inquire	inquire	inquire
4.6	25	5	581391-U	565395-U	581696-U	inquire	inquire	inquire

Validation Packs include 3 columns, each from a different bond lot.

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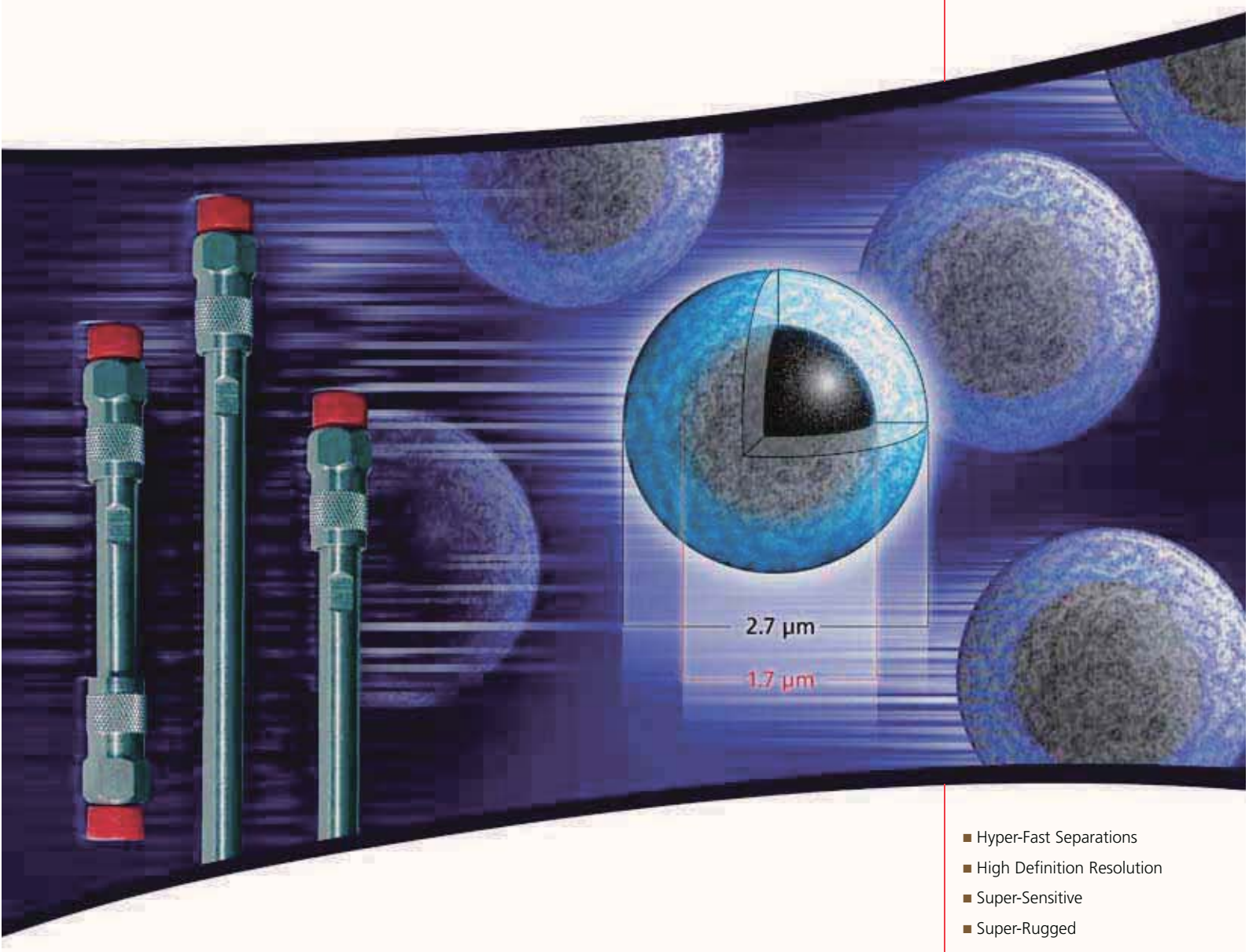

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- High Definition Resolution
- Super-Sensitive
- Super-Rugged

SIGMA-ALDRICH®

A Breakthrough in HPLC Performance

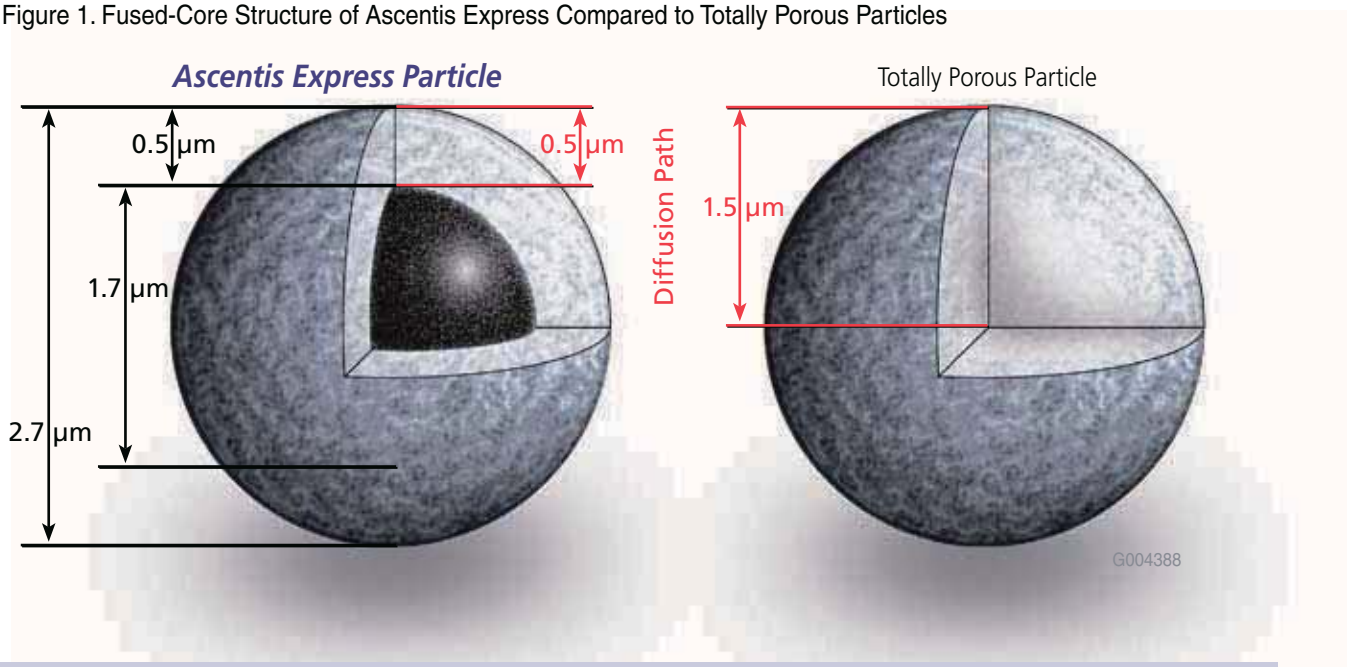
The Fused-Core Advantage

Ascentis Express provides the high speed and high efficiency of sub-2 µm 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 µm particles, along with excellent column lifetime.

At the heart of Ascentis Express is the 2.7 µm Fused-Core™ particle which comprises a 1.7 µm solid core and a 0.5 µm 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 µm particle columns and nearly twice the efficiency possible with 3 µm particles.

While the Ascentis Express efficiency is as high as sub-2 µm 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 µm 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 µm solid core and a 0.5 µm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

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* 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* 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* 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 µm columns on many applications since Ascentis Express provides the benefits of sub-2 µm 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 µm 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 µm particles

Compared to sub-2 µm particles, the 2.7 µm Ascentis Express particles generate approximately half the back-pressure. 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 µm column (lower) of the same dimensions. Because higher flow rates on Ascentis Express – even doubled in this example – generate similar backpressure, hyper fast separations are possible that have efficiency and resolution equal to the sub-2 µm particle column.

Shown in Figure 3 is a comparison against a traditional 15 cm, 5 µm 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 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 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
mobile phase: 49:51 or 55:45, water:acetontrile
flow rate: 0.4 or 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

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

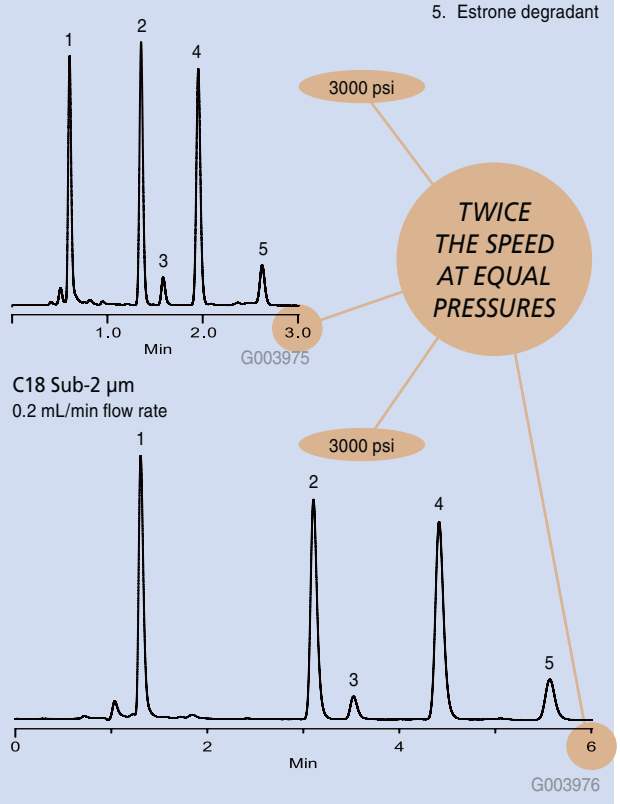
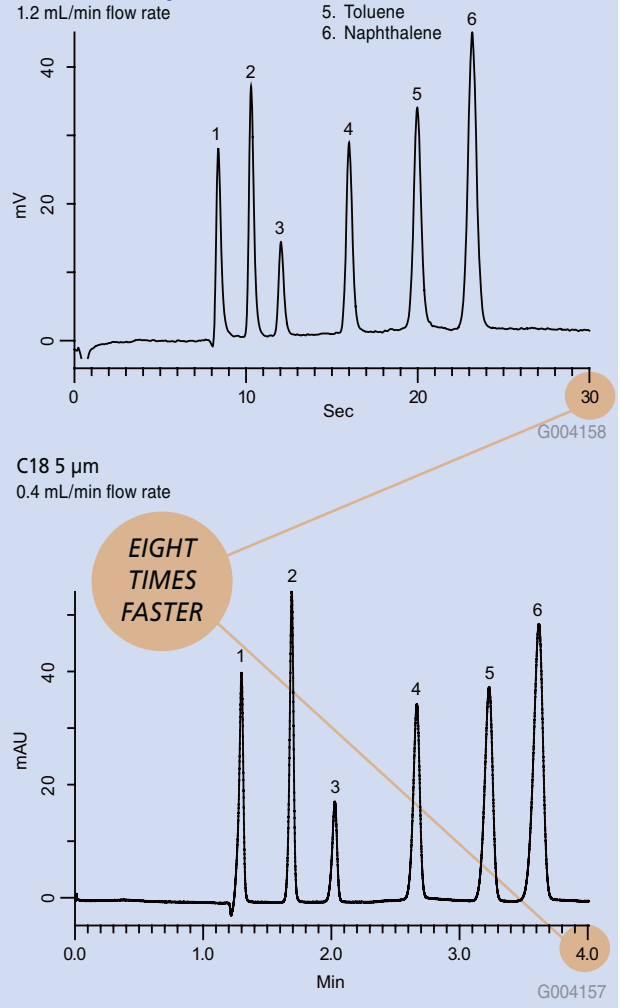


Figure 3. Hyper-Fast Separations on Ascentis Express: Eight Times the Speed of Traditional Columns

column: Ascentis Express C18, 5 cm x 3.0 mm I.D. and 5 µm 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 µL

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



High Definition "HD"-Resolution

Double the Efficiency

- Short analyte diffusion path
- Twice the efficiency of 3 µm particles
- Longer columns permit doubling the plates over sub-2 µm particles

Compared to conventional 3 µm and 5 µm 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 µm and 5 µm 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 µm and 5 µm particles.

Ascentis Express and sub-2 µm 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 µm 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 β-estradiol and the impurity compared to the 5 cm sub-2 µm column at comparable backpressures.

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

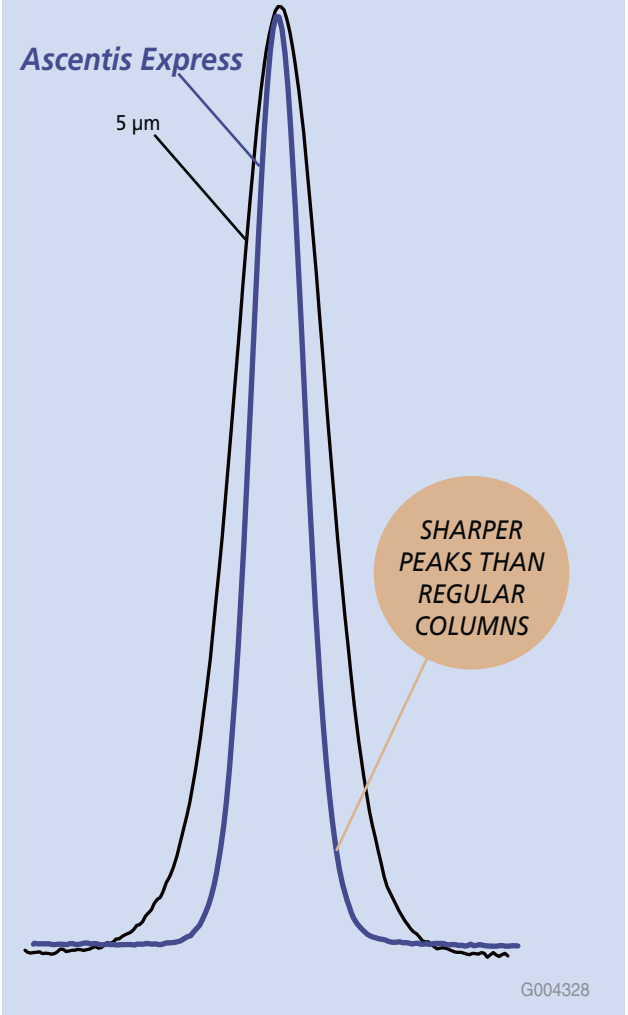
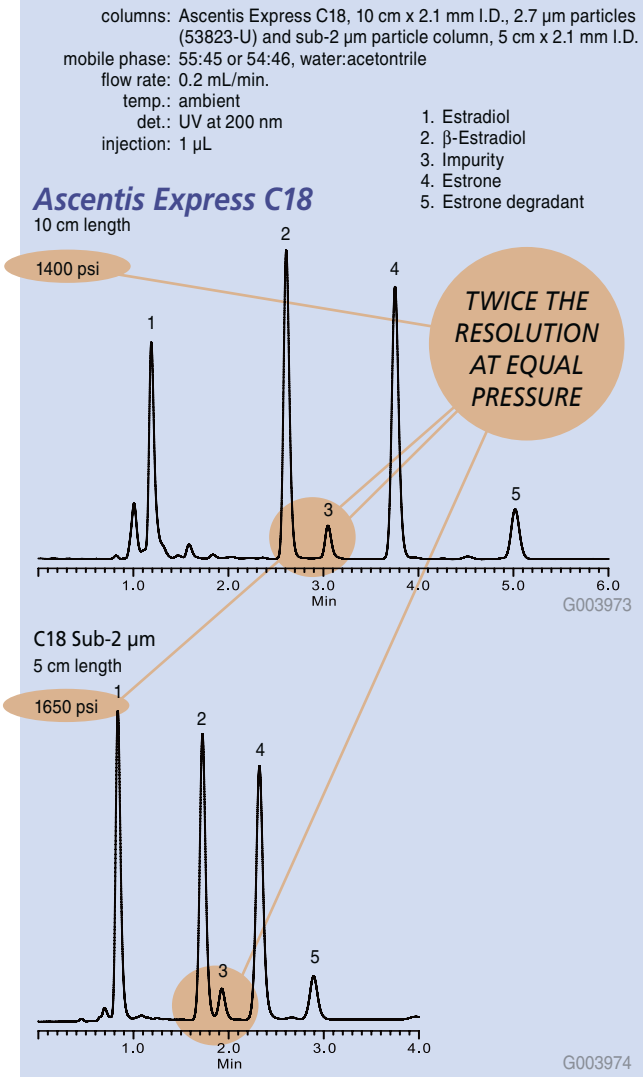


Figure 5. HD-Resolution on Ascentis Express Compared to Sub-2 µm Columns



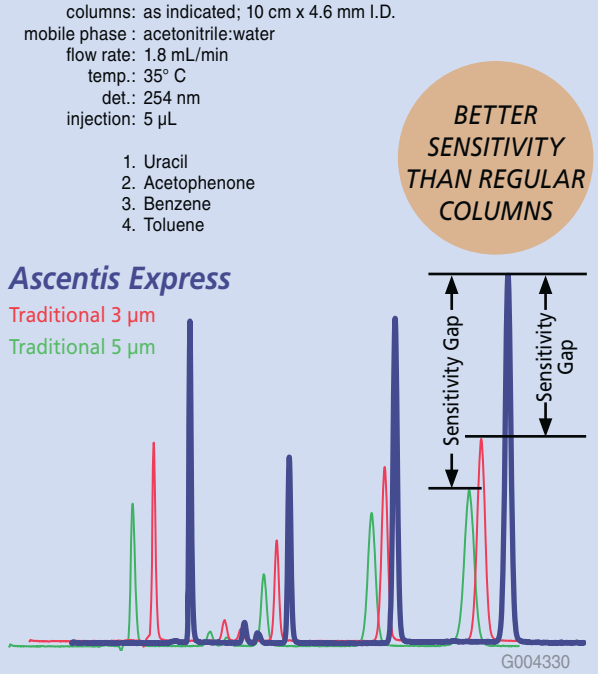
Super-Sensitive

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



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^2/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

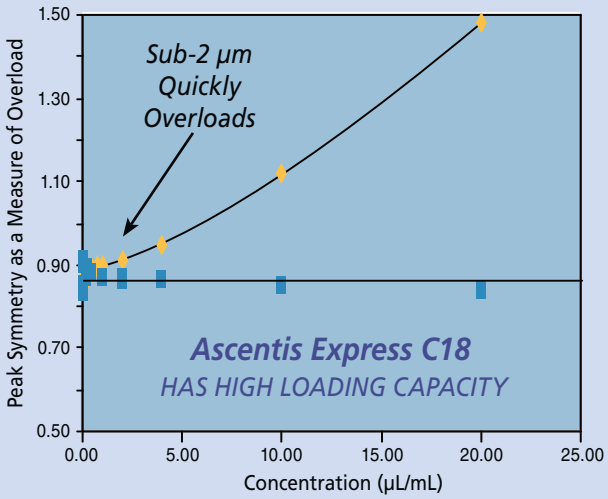
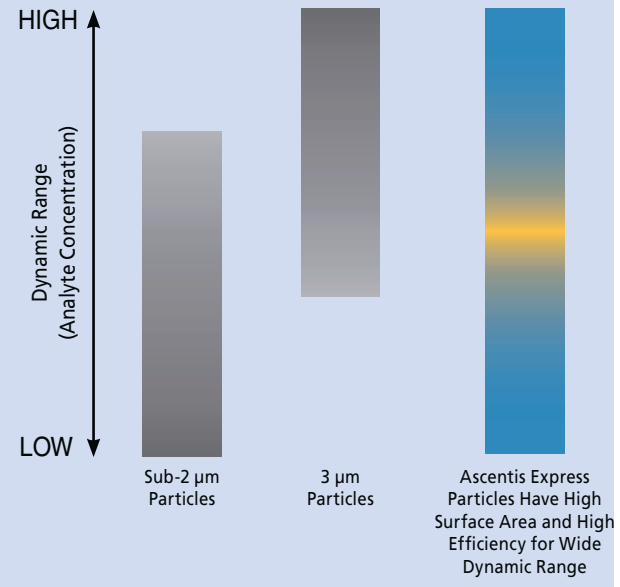


Figure 8 shows how Ascentis Express extends the dynamic range. It has the high efficiency of sub-2 µm particles needed for trace analysis, and the high surface area of totally porous particles needed for high sample capacity.

Figure 8. Extended Dynamic Range of Ascentis Express



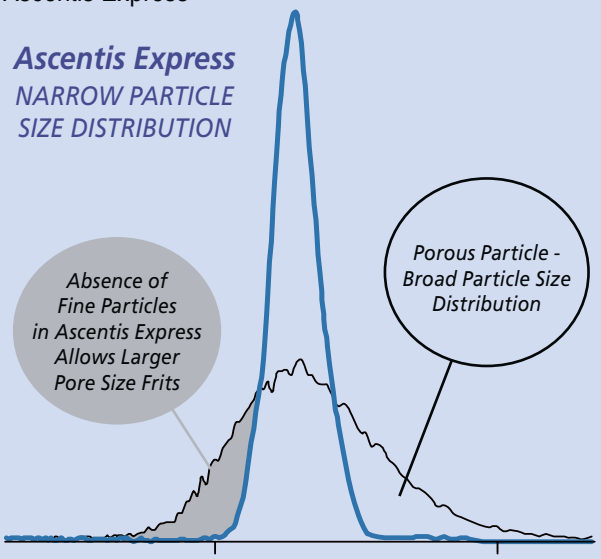
Super-Rugged Columns

Extended Column Lifetime Compared to Both 3 µm and sub-2 µm Columns

- Narrow particle size distribution allows use of 2 µm 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 µm pores, the same as used on most columns packed with 5 µm particles. In comparison, sub-2 µm particles require frits with much smaller pore size – 0.5 µm 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



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.

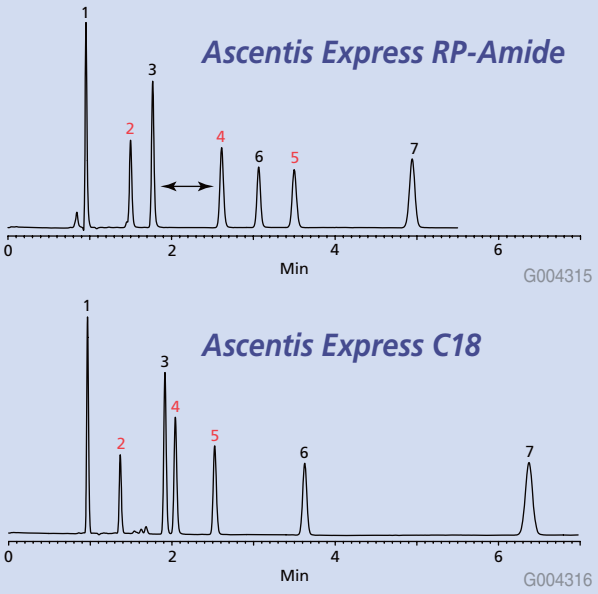
The amide group provides enhanced selectivity with analytes that have hydrogen bonded to a heteroatom. Phenols, carboxylic acids, amines and, to a lesser extent, alcohols show enhanced retention on the RP-Amide phase when compared to neutral non-polar analytes. An example of the power of the hydrogen 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.

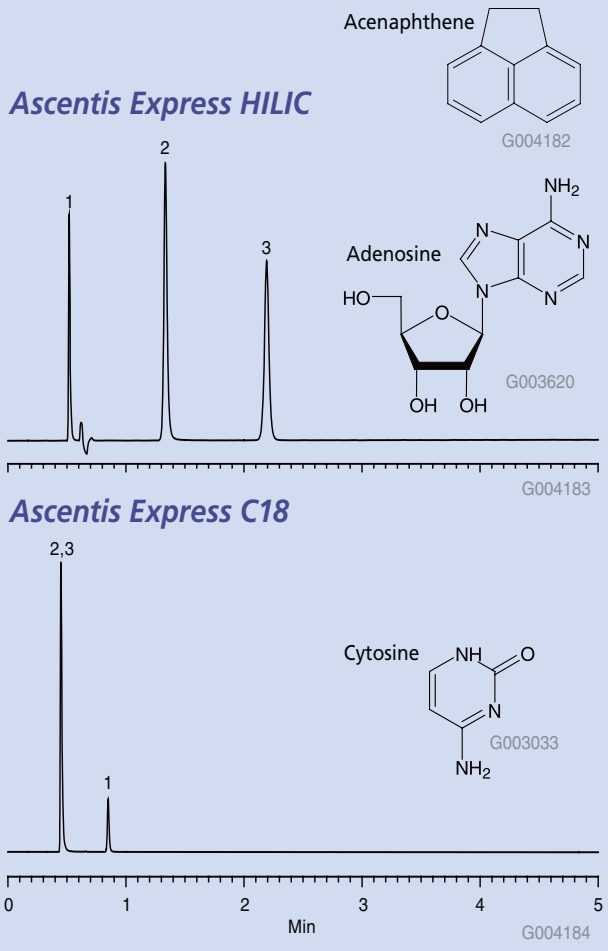
Ascentis Express HILIC Applications

- Amino acids
- Small, polar acids – (metabolomics)
- Biogenic amines – (neurotrans-mitters, 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 µm particles (53939-U)
Ascentis Express C18, 10 cm x 2.1 mm ID, 2.7 µm 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 °C
det.: UV at 254 nm
injection: 1 µL

1. Acenaphthene, 80 µg/mL in mobile phase
2. Adenosine, 35 µg/mL in mobile phase
3. Cytosine, 75 µg/mL in mobile phase



Improving HPLC Sample Throughput

Do More Work in Less Time Without Changing Your Method

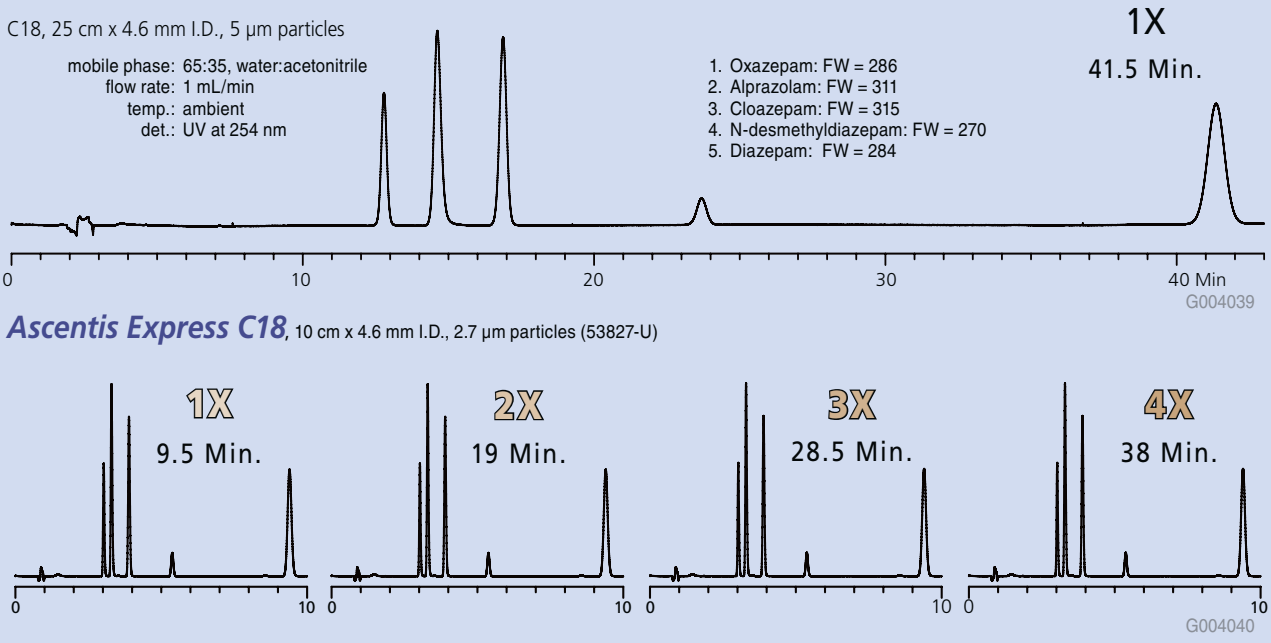
The demand for increased sample throughput and speed of results has driven HPLC users to search for breakthroughs in HPLC instruments and column technology. Although improvements have been realized, setbacks have been encountered. Reduction in column ruggedness, costly replacements of existing instrumentation, 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 µm 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 µm 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.

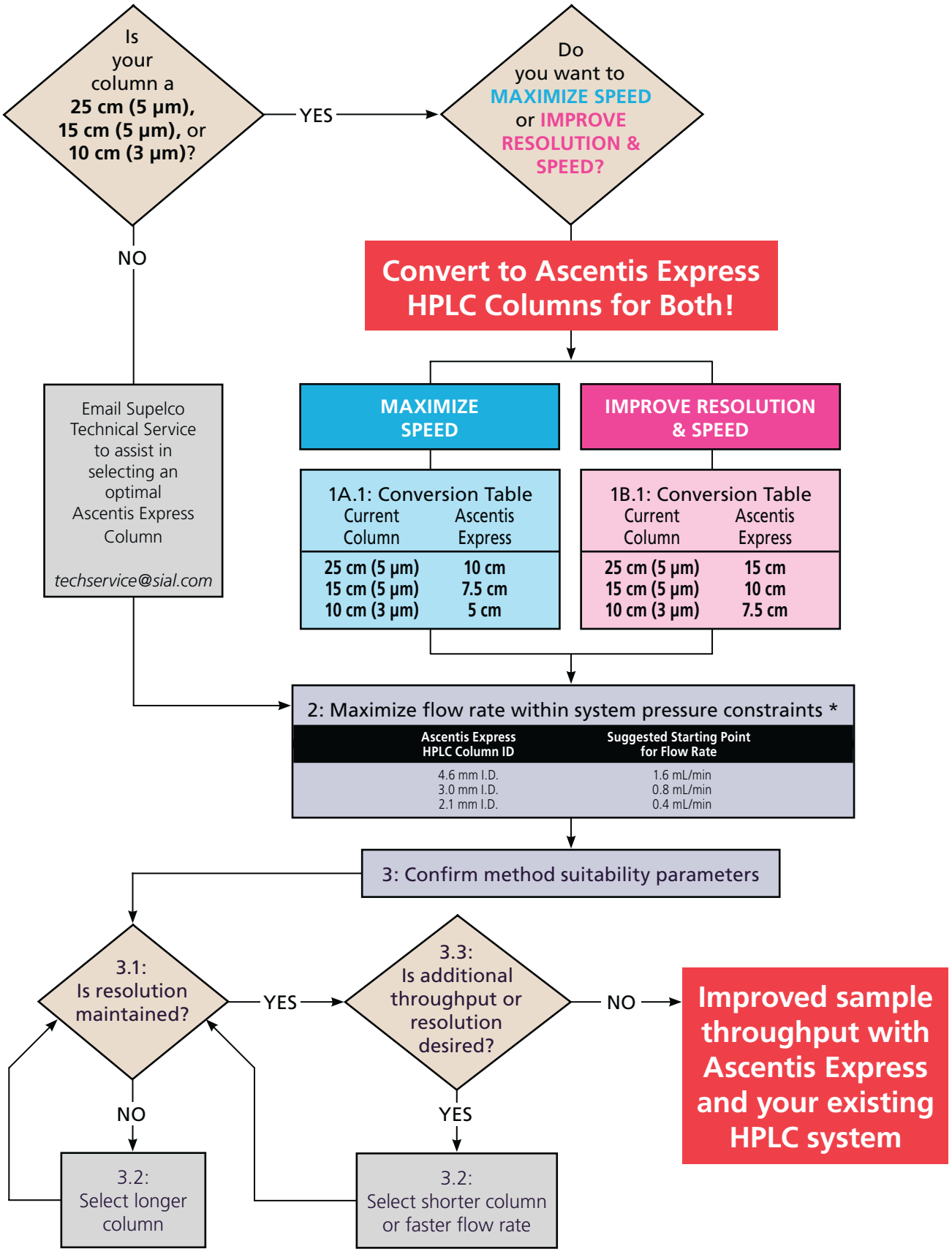
Figure 12 compares the resolution of a five-component sample on 25 cm, 5 µm C18 and 10 cm Ascentis Express C18 columns. Each column has approximately the same number of theoretical plates and hence the same resolving power. However the shorter Ascentis Express column delivers this separation in a much 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).

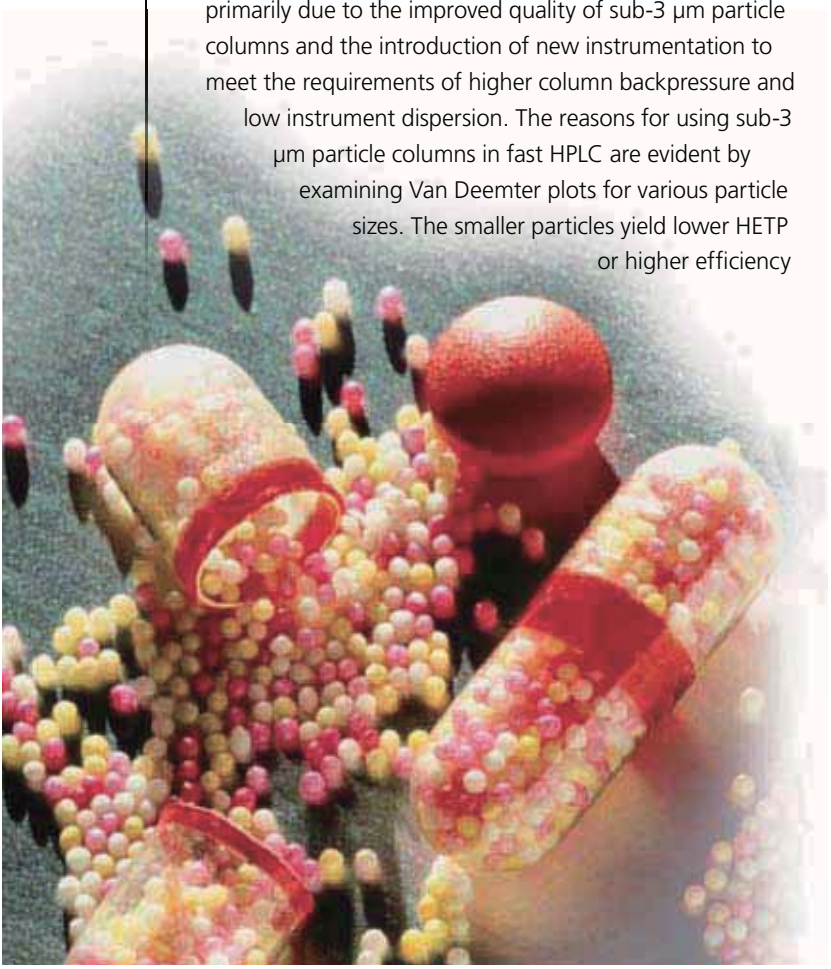
Fast HPLC for Rapid Screening of Pharmaceutical Compounds

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 µm) and high flow rates has recently become an effective means to reduce analysis time. This is primarily due to the improved quality of sub-3 µm particle columns and the introduction of new instrumentation to meet the requirements of higher column backpressure and low instrument dispersion. The reasons for using sub-3 µm particle columns in fast HPLC are evident by examining Van Deemter plots for various particle sizes. The smaller particles yield lower HETP or higher efficiency per unit length. Furthermore, the optimum flow rate is higher for smaller particles. Smaller particle columns have less efficiency loss at high flow rates because mass transfer is less sensitive to velocity changes as illustrated by "flatter" Van Deemter plots.



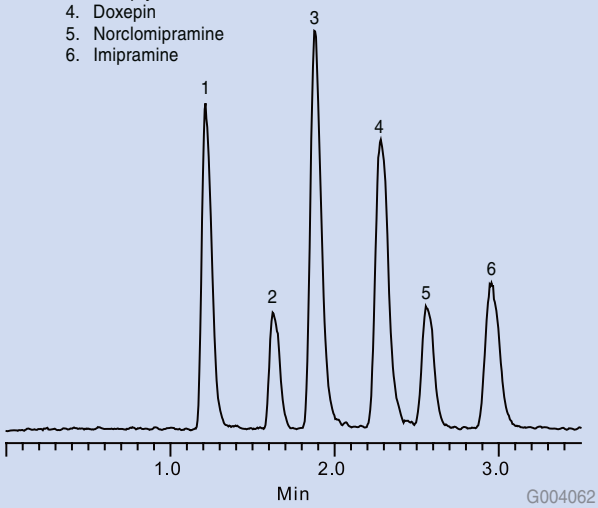
Unfortunately, column backpressure increases at a greater rate than column efficiency as you decrease particle size. This increase in backpressure is so great for sub-2 µm particle columns that they are practically unusable using standard HPLC systems.

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 µL

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



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 β-blockers are resolved in less than one minute under isocratic conditions utilizing MS detection. While a 10 cm column was utilized for the TCAs separation, a 5 cm column was used for the β-blockers example.

The separation of three steroids as well as a related impurity and degradant is shown in Figure 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 µm particle column but was not possible given the same instrument constraints put on the Ascentis Express column.

Figure 14. β-Blockers on Ascentis Express

column: Ascentis Express C18, 5 cm x 2.1 mm ID (53822-U)
instrument: Agilent 1100
mobile phase A: 0.1% acetic acid in water
mobile phase B: 0.1% acetic acid in acetonitrile
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 µL

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

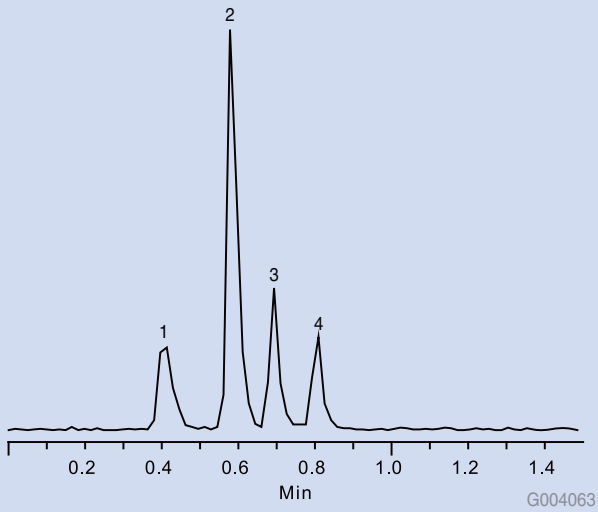
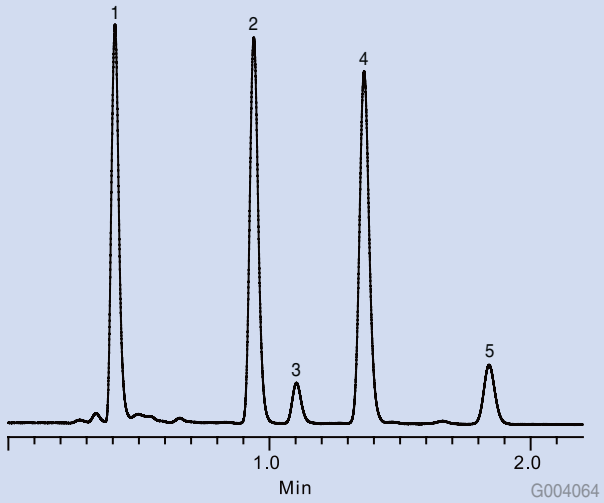


Figure 15. Steroids on Ascentis Express

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 µL

1. Estriol
2. β-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 µm and sub-2 µm 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.

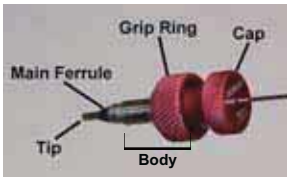
Figure 16 shows the efficiency obtained by coupling 5 Ascentis Express 15 cm columns together.

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.



P001255

Use No Tools!

Key Benefits

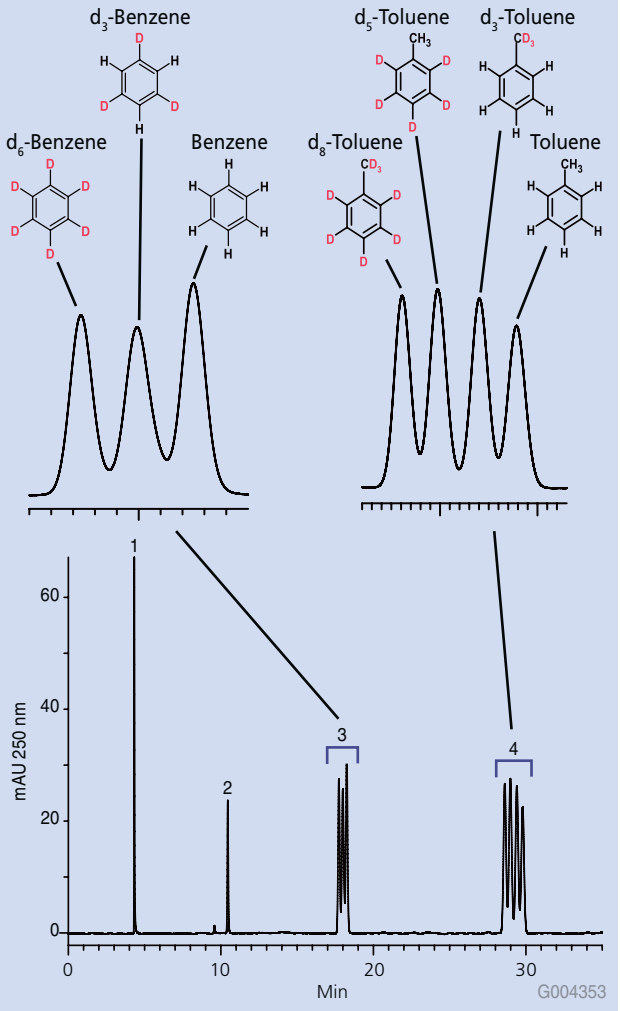
- Eliminate dead volume that contributes to peak broadening and decreased resolution
- Sliding ferrule design allows for use in any port
- Fingertight fittings, no tools required
- Rated to 15,000 psi

For a complete list, visit *sigma-aldrich.com* 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



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^2/gram surface area (comparable to ~225 m^2/g porous particle)
- 90 Å pore size

Bonded Phase

	Coverage µmoles/m^2	pH Range	Endcapping
C18	*3.5*	*2-9*	*Yes*
C8	*3.7*	*2-9*	*Yes*
RP-Amide	*3.0*	*2-9*	*Yes*
HILIC	*n/a*	*2-8*	*No*

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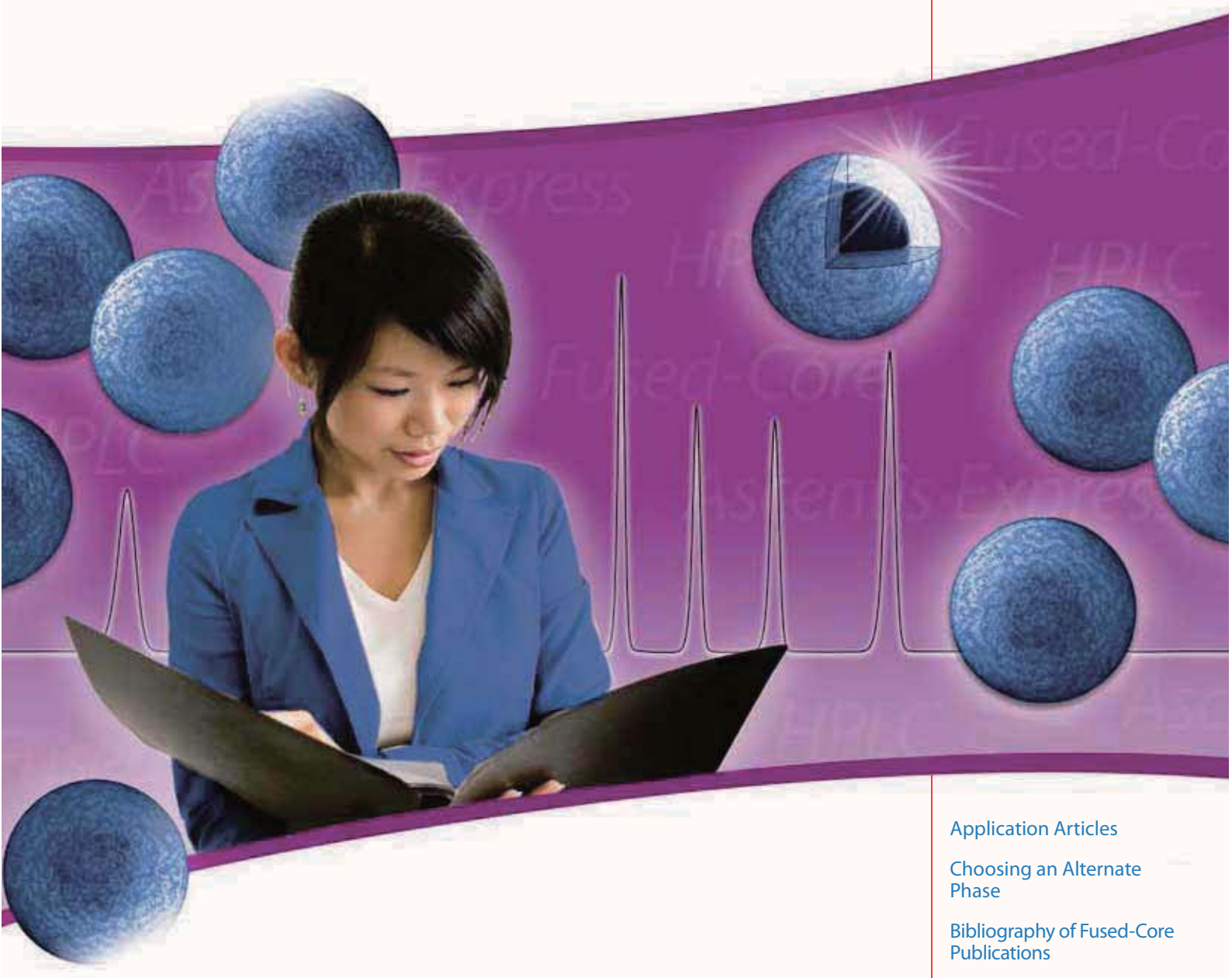
Ascentis® Express HPLC Resource Guide

SUPELCO® Analytical



- Application Articles
- Choosing an Alternate Phase
- Bibliography of Fused-Core Publications
- Practical Recommendations for Success
- Listing of Available Technical Literature

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Available Resources

US Technical Service

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EU Technical Services

email: EurTechServ@sial.com

Internet: sigma-aldrich.com/express

Webinars: sigma-aldrich.com/videos (available 24 hours/day)

Email updates: Fused-Core Report (register at sigma-aldrich.com/express)

Twitter: twitter.com/HPLCSessions

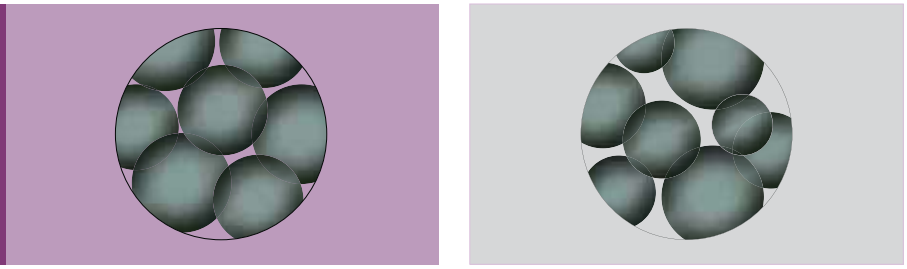
The Fused-Core™ Advantage

Extreme performance on any LC system

Half the backpressure of sub-2 µm columns
Twice the performance of 5 µm columns

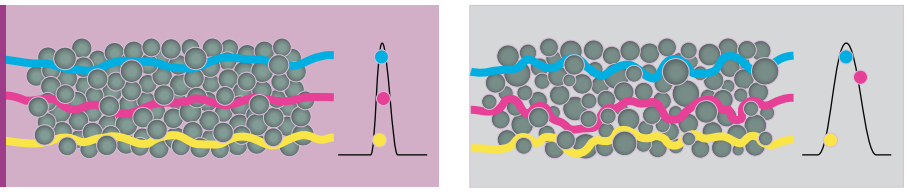
Fused-Core Particles | **Traditional Porous Particles**

Narrow Particle Size Distribution



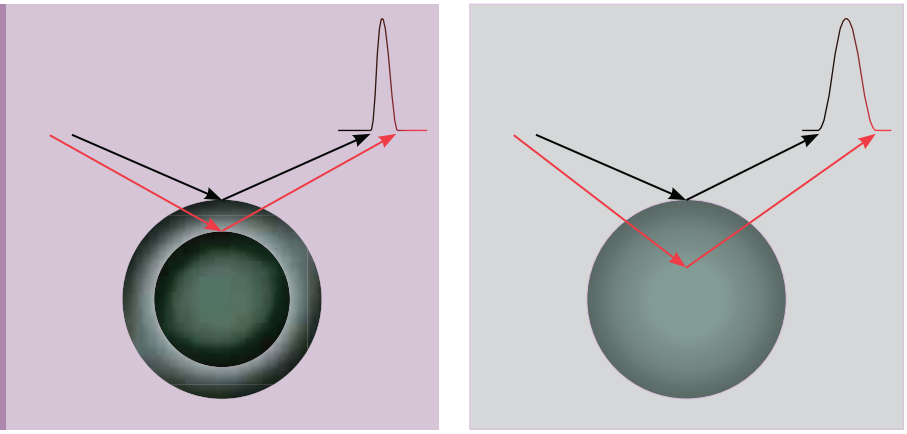
The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured in a way to yield extremely narrow particle size distributions.

More Consistent Bed



The "A" term in the van Deemter equation accounts for the effects of inhomogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistent packed bed and a consistent path length, **minimizing analyte diffusion** through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path



The short diffusion path of the Fused-Core particle **yields sharper peaks** than traditional 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 peak broadening.

Introducing Ascentis Express…

Now, High Speed and High Efficiency HPLC Separations are Possible on Any LC System

Increasing speed and resolution of HPLC analyses are drivers for innovation in both HPLC column and hardware design. While columns packed with 5 µm particles have been the standard, reducing particle size has been the strategy of many column manufacturers and users alike. Smaller particles result in faster chromatography. The cost for the improved speed is higher column backpressures. To obtain the benefit of the small particles, instrumentation beyond conventional HPLC is required.

Ascentis Express columns provide a breakthrough in HPLC column performance. Based on Fused-Core particle technology, Ascentis Express provides the benefits of high speed of much smaller particles but at a backpressure suitable to conventional HPLC systems. Due to this fundamental performance advantage, Ascentis Express can benefit both conventional HPLC users as well as UPLC™ or other ultra pressure system users.

Exceeding the Performance of Other "Fast" HPLC Particles

Designed to deliver speed and resolution on all LC systems, Ascentis Express meets and exceeds the benefits of competitive particles, including 3 µm and sub-2 µm particles. Under the same conditions, Ascentis Express columns deliver the same efficiencies at half the backpressure of sub-2 µm particles and nearly twice the efficiency of 3 µm particles.

Compared to Sub-2 µm Particles:

Advantage: *Ascentis Express columns can be run successfully on conventional, mid-pressure and ultra high pressure HPLC and LC-MS instruments.*

Advantage: *Double the flow rate. Run Ascentis Express columns at higher flow rates for faster analyses.*

Advantage: *Double the column length. Longer Ascentis Express columns can be used, giving additional resolving power.*

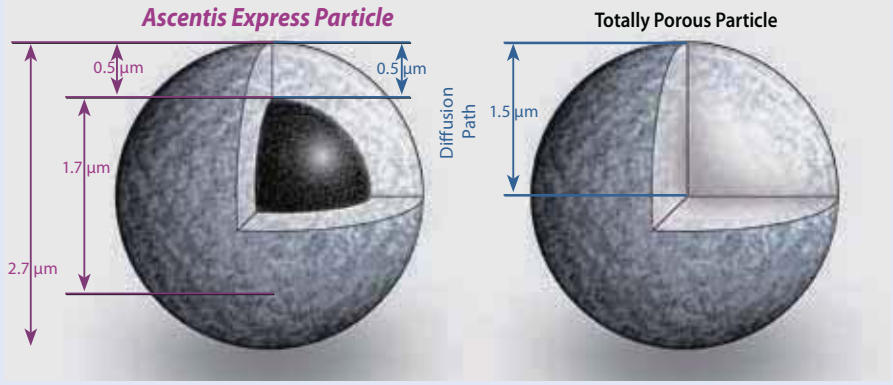
Compared to 3 µm Particles:

Advantage: *Double the efficiency. Ascentis Express columns have nearly twice the column efficiency of 3 µm particles.*

Practical Recommendations for Success

- Use 0.005 in. I.D. inlet and outlet tubes. Broadening is much less sensitive to the tube length than to the I.D. Minimize lengths of the inlet and outlet tubes for best performance, but do not worry about having a few extra centimeters of length if it makes maintenance or column installation easier.
- If high pressure becomes a problem, then use acetonitrile as modifier and elevate the column temperature whenever possible. If methanol, THF, or another more viscous modifier is required, then elevating the temperature becomes even more beneficial. Even a modest temperature increase will greatly reduce the mobile phase viscosity and the required pressure while improving mass transfer.
- Use only 4.6 mm diameter Fused-Core columns on conventional HPLC systems to minimize broadening problems from the remaining system components. Extra column broadening worsens as the column diameter is decreased.
- Keep the sample volumes small — 5 µL or less if the peaks of interest elute early ($k = 1$). Up to 20 µL is acceptable if k exceeds 10.
- Avoid sample solvents that are stronger than the mobile phase.
- Use data rates of 10 Hz or greater, and watch out for bunching factors.

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



The Particle Platform Innovations Behind Ascentis Express

Like most modern HPLC particles, Ascentis Express particles are high surface area spheres made from high purity silica gel. The total particle diameter is 2.7 µm. However, here the comparison ends. What sets apart Ascentis Express from conventional HPLC particles is the patent pending Fused-Core technology. Ascentis Express particles comprise a solid 1.7 µm diameter silica core that is encapsulated in a 0.5 µm thick layer of porous silica gel.

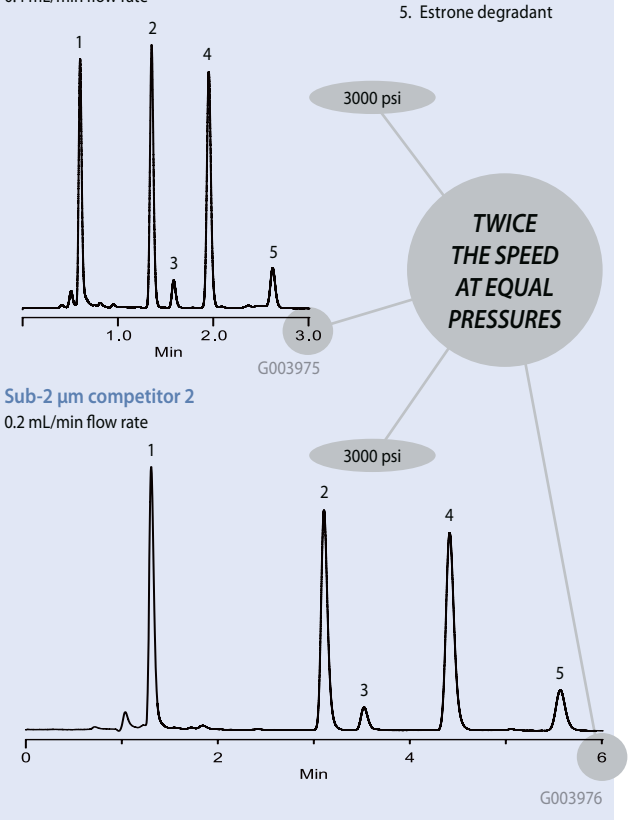
There are five distinct properties of Ascentis Express particles that account for their high performance and are worth emphasizing:

1. **The solid core**
   Because of the solid core, analytes cannot diffuse as deeply into the particle, resulting in less band broadening, and hence higher efficiency and sensitivity, compared to totally porous particles of the same diameter.
2. **The 0.5 µm porous shell surrounding the solid core**
   The porous shell gives the particles a surface area comparable to totally porous particles for excellent phase loading and sample capacity.
3. **The total particle diameter (2.7 µm)**
   Compared to sub-2 µm porous particles, Ascentis Express yields half the column backpressure, allowing longer columns and faster flow rates (Figures 2 and 3).
   Compared to 3 µm porous particles, Ascentis Express yields nearly twice the efficiency (Figure 4).
4. **The narrow particle size distribution.**
   Compared to both sub-2 µm and 3 µm particles, Ascentis Express provides longer column lifetime because the narrow particle size distribution allows us to use larger pore size frits (2 µm vs. 0.5 µm) that are less susceptible to fouling.
5. **The high particle density**
   By virtue of the solid core, Ascentis Express particles yield a more densely packed bed for added stability and long column lifetime.

Figure 2. Hyper-Fast Separations on Ascentis Express

column: 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)
mobile phase: 49:51 or 55:45, water:acetontrile
flow rate: 0.4 or 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

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



Ascentis Express: High Speed, High Efficiency Separations Adaptable Equally to R&D and Routine Analysis Settings

The recent introduction of UPLC™ and other ultra high pressure LC systems addressed the need for high throughput separations. However, speed is not the only important criteria: the need for more sensitivity, more resolution and improved ruggedness of the technique has lead to a continual stream of new LC and LC-MS instruments. Coupled with the large installed base of conventional HPLC instruments, the result is that most laboratories have a mixture of instruments, old and new. Whereas columns packed with sub-2 µm particles require ultra high pressure instruments, Ascentis Express columns can be run on any LC system. Methods developed on Ascentis Express can be readily and reliably validated and transferred from R&D to routine analysis labs, whether across the building or across the world.

We hope this article has sparked an interest in Ascentis Express and the benefits it can bring to your laboratory. Subsequent articles will develop the Ascentis Express message by focusing on specific features and application areas.

Figure 3. HD-Resolution on Ascentis Express Compared to Sub-2 µm Columns

column: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column, 5 cm x 2.1 mm I.D.
mobile phase: 55:45 or 54:46, water:acetontrile
flow rate: 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

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

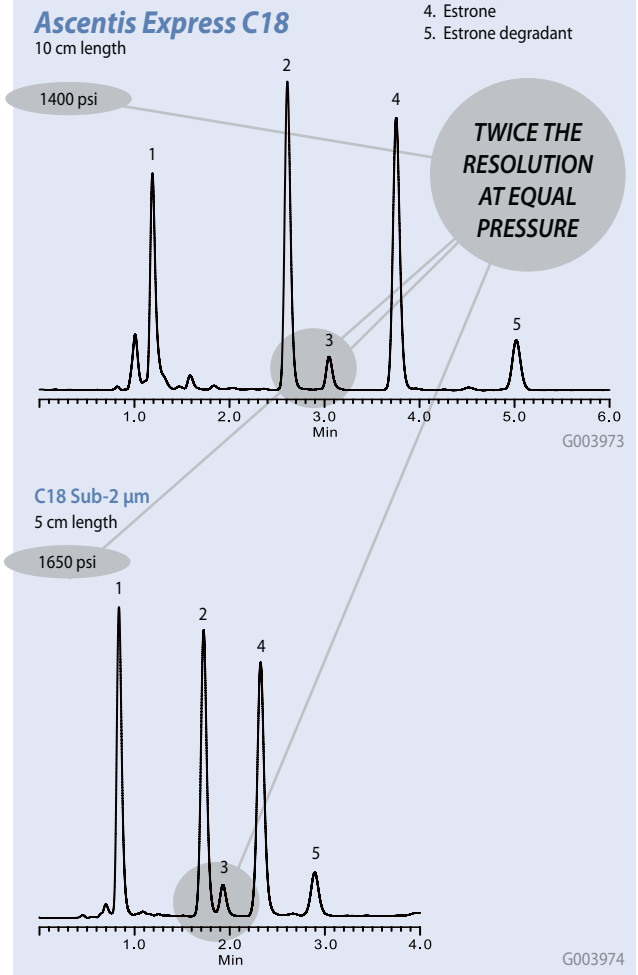
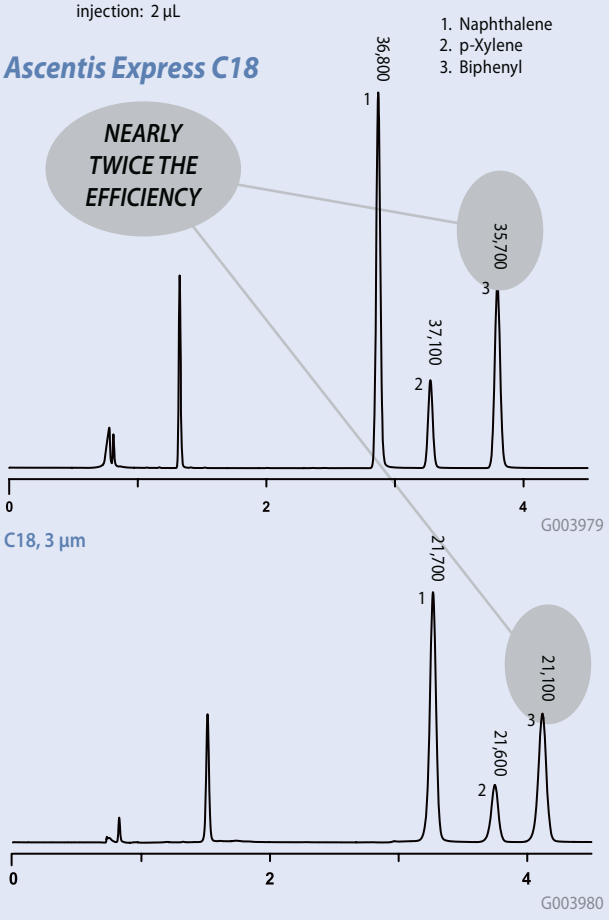


Figure 4. HD-Resolution on Ascentis Express Compared to 3 µm Particles

column: Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U) and C18, 15 cm x 4.6 mm I.D., 3 µm particles
mobile phase: 35:65 or 27.5:72.5, water:acetontrile
flow rate: 1.5 mL/min.
temp.: ambient
det.: UV at 220 nm
injection: 2 µL

1. Naphthalene
2. p-Xylene
3. Biphenyl



Rapid, Sensitive, General-Purpose Cleaning Validation Using Ascentis Express HPLC Columns

Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

S. Bannister, M. Talbott, F. Hanciles
Xcelience LLC, Tampa, FL

Verification of the removal of drug residue from multi-product manufacturing equipment is required by GMP regulations and the suitability of applied analytical methods is judged with a combination of sensitivity, selectivity, and because the release of equipment is dependent - speed. The FDA does not set quantitative acceptance specifications, but the commonly used limit is based on not more than 0.1% of a dose carried over into a single dose of the next product. Translation of this into an analytical limit combines the total product contact area, the mass (or volume) of product contacting the surface, the mass (or volume) of each dose unit, the sampled area, the rinse volume and the fraction of the rinse sample used for analysis. The requisite limits are commonly measured in ng/mL of injected sample.

The ubiquity of HPLC in drug analysis makes it an attractive choice for cleaning validation. Methods qualified for cleaning validation are often adaptations of drug-substance methods. The original methods are capable of determining the drug and its related impurities, but the ability to simultaneously measure multiple closely related analytes comes at the expense of run time and is not needed in cleaning validation.

This work was undertaken to investigate the use of rapid gradients using recently introduced FCP columns on conventional instrumentation in the development of general-purpose methods for cleaning validation. The benefits include high sensitivity and reductions in the time needed to set up and run the method.

Resolution, limits of detection and quantitation, and run time in HPLC analyses are improved by reducing the width of eluted bands. Contributions to bandwidth include both column (particle size, packing structure and resistance to mass transfer in the stationary and mobile phases) and extracolumn volumes (injection, unswept and tubing). Columns packed with 5 µm fully porous particles have been the standard for conventional HPLC for twenty-five years. Smaller-particle packings (3 µm) have been available almost as long and offer higher efficiency (lower band dispersion)

Figure1. Acidic and Neutral Drug Panel

column: Ascentis Express C18, 10 cm x 4.6 mm I.D. (53827-U)
mobile phase A: water with 0.1% phosphoric acid
mobile phase B: acetonitrile with 0.1% phosphoric acid
temp.: ambient
flow rate: 1.76 mL/min
det.: UV at 215 nm
inj.: 100 µL
gradient:

Min	%A	%B
0	70	30
2	60	40
4	5	95

1. Hydrochlorothiazide (9)
2. Chlorthalidone (2)
3. Prednisolone (2)
4. Pravastatin (4)
5. Carbamazepine (2)
6. Diclofenac (14)
7. Ibuprofen (15)
8. Progesterone (2)

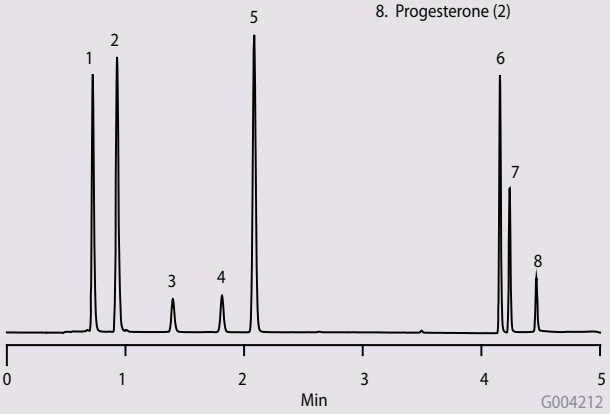
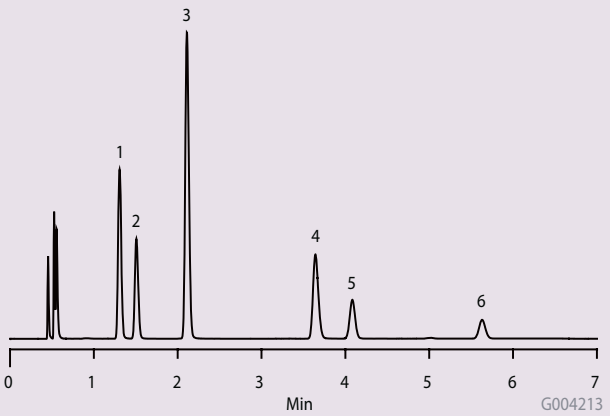


Figure 2. Basic Drug Panel

column: Ascentis Express C18, 10 cm x 4.6 mm I.D. (53827-U)
mobile phase A: water with 0.05M potassium phosphate and 0.1% TEA and 0.6% OSA-Na at pH = 2.9
mobile phase B: acetonitrile
temp.: ambient
flow rate: 1.76 mL/min
det.: UV at 215 nm
inj.: 100 µL
gradient:

Min	%A	%B
0	63	37
5	59	41

1. Quinidine (5)
2. Dipyridamole (29)
3. Propranolol (12)
4. Haloperidol (8)
5. Amlodipine (29)
6. Fluoxetine (3)



dispersion) on conventional instrumentation, but require higher pumping pressures due to lower bed permeability. Efficiency can be further increased by the use of particles smaller than 3 µm but only with the use of instrumentation optimized with respect to both pressure and extra-column effects.

Supelco has recently introduced reversed-phase packings based on 2.7 µm silica particles in which a 0.5 µm layer of 90-Å porous silica has been deposited onto a 1.7 µm solid spherical core. Advantages of columns packed with these particles include high efficiency, lower backpressure due to a very narrow particle size distribution, and smaller efficiency losses with increasing velocity due to improved mass-transfer kinetics in the shallow porous layer. The narrow particle size distribution allows the use of larger pore column frits, which combined with the greater stability of the packed bed should produce longer column lifetimes in routine use.

The high resolving power of gradient elution in the analysis of closely related substances is the result of the reduction of peak width as a band moves through the column. The back of the band is accelerated by the stronger solvent. A broad gradient will elute a wide range of substances and a steep gradient will elute them quickly.

Versatile Separations

To judge the utility of Ascentis Express columns in cleaning validation, an Agilent 1100 component system with standard components (including a 10 mm/13 µL flow cell) was used to develop a short gradient separation using Ascentis Express C18, 10 cm x 4.6 mm for each of two panels: eight acidic or neutral drugs (AN) and six basic drugs (B). For each separation, the flow rate was 1.76 mL/min, detection was at 215 nm, and 100 µL injections were made of aqueous solutions representing the final equipment rinse. The separations are shown in Figures 1 & 2. Limits of detection (ng/mL) are listed next to each analyte in Figures 1 and 2.

These separations demonstrate the capabilities of Ascentis Express columns on conventional, robust, instrumentation in rapid analyses of multiple drugs at low ppb levels suitable for development as methods for cleaning validations in multiproduct manufacturing facilities.

Selecting the Right Buffer

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

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

Guidelines for Preparing Mobile Phases

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

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

Ascentis Express Peptide ES-C18 Expands the Fused-Core Particle Platform into Bioseparations

Introduction

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 bigger pores (160 Å versus 90 Å in standard Ascentis Express), which greatly expands the application range for Ascentis Express columns.

Key Applications for Ascentis Express Peptide ES-C18:

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

Key Advantages:

- Higher peak capacity providing greater resolution
- Amenable to higher flow rates for faster analysis
- Exceptional ruggedness providing long column lifetime

Ascentis Express Peptide ES-C18 columns utilize 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 combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.01-0.1% concentration, most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion-pairing properties. Additional opportunities for low pH operation include the normal short chain carboxylic acids, formic acid and acetic acid, as well as mineral acids, such as phosphoric acid (0.001-0.02 M).

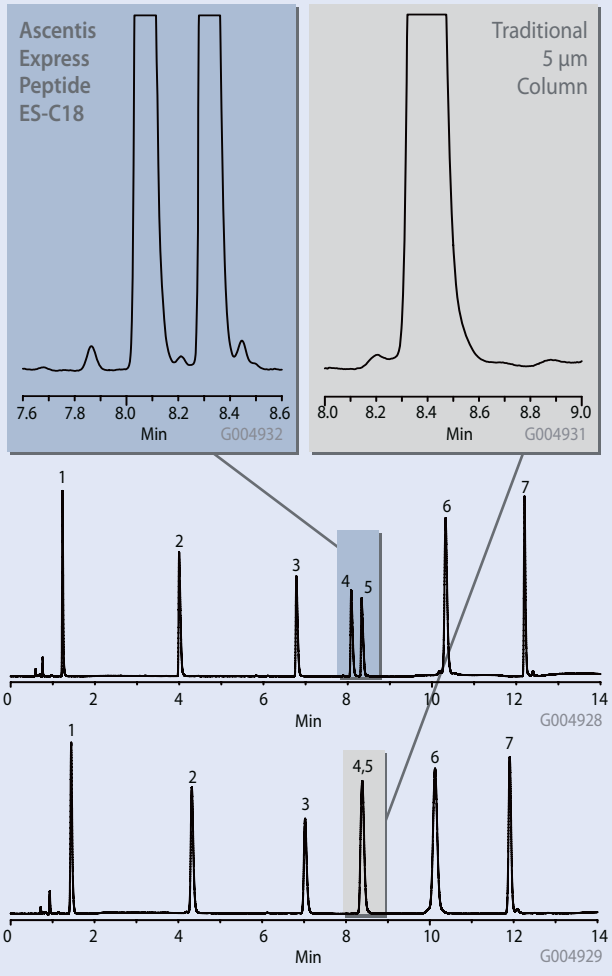
Shown in Figure 1 is the chromatographic separation of a peptide mix. The peptide mix contains a range of peptides in terms of molecular weight, basicity, and hydrophobicity. Excellent peak shape and peak width are achieved with a standard acetonitrile gradient and 0.1% TFA modifier. The resolution of 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 column.

Figure 1. Comparison of Peptide Test Mix with Ascentis Express Peptide ES-C18 and Traditional Column

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 10% acetonitrile / 90% water / 0.1% trifluoroacetic acid
mobile phase B: 75% acetonitrile / 25% water / 0.1% trifluoroacetic acid
gradient: 0% to 50% B in 15 min
flow rate: 1.5 mL/min.
det: UV at 220 nm
temp: 30 °C
injection: 5 µL

Peptide Test Mix

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



For more information on the Ascentis Express Peptide ES-C18, request brochure T410043 (MII).

Improving the Current USP Method for the Analysis of Lansoprazole Drug Substance Using HPLC Columns Based on Fused-Core Particle Technology

Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

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Introduction

Compendial methods from the USP (United States Pharmacopeia) are widely used in pharmaceutical drug product and raw materials testing. However, not all methods in the USP use modern technologies. In chromatographic methods, it is not uncommon that older brands of columns are specified. Therefore, the USP methods are under continuous revision to improve existing procedures or to allow the user to obtain better results.

Due to the improved resolving power of Fused-Core particles, the method was optimized with a shorter runtime without sacrificing resolution.

In an effort to improve the compliance of drug product, drug substance, and excipient monographs with current scientific/regulatory standards, USP is seeking the submission of proposals for improved methods. The intent is to replace the current procedures that may be deficient, flawed, or unsafe (e.g. *http://www.usp.org/USPNF/submitMonograph/improveMon.html)*. Requests for revision of an existing monograph are encouraged by USP in light of advances in analytical technologies. Furthermore, ease of operation, suitability for automation, and potential for high-throughput analysis can be considered in a revision. To develop the best possible analytical test method for its intended use, a fully integrated method development process such as the selection of column, mobile phase, detection technology, and LC hardware by utilizing the most advanced technologies viable should be considered to ensure the methods are robust, consistent, and easy to use.

In this study, the USP method for lansoprazole was considered for improvement. Several drawbacks in the current USP monograph for lansoprazole prompted the investigation. These drawbacks include sample solution instability, use of different columns and samples preparations for the evaluation of assay and impurity, the requirement of using internal standard for assay and a long HPLC runtime (60 min). A new HPLC column, Ascentis Express C18, based on Fused-Core particle technology was investigated for this study. The Ascentis Express HPLC column claims high efficiencies as a result of a 0.5 µm layer of porous silica on a 1.7 µm solid silica core. An additional advantage to the column is that standard HPLC instrumentation can be used as opposed to UHPLC that is required for sub-2 µm columns.

Results and Discussion

Initially, a traditional 5 µm C18 column as specified in the USP monograph was compared to the 2.7 µm Ascentis Express C18 using the standard USP conditions for chromatographic purity for lansoprazole (1, 2). Improved resolution and sensitivity were obtained using the Fused-Core column that allowed us to make several significant improvements to the method. Due to the improved resolving power of Fused-Core particles, the method was optimized with a shorter runtime without sacrificing resolution. The total run time was reduced from 60 min to 40 min (Table 1). Moreover, the improved sensitivity allowed for the reduction in concentration of the test sample for chromatographic purity from 250 mg/mL to 100 mg/mL, the level required for assay in the USP monograph. Therefore, simultaneous evaluation of assay and chromatographic purity is achieved. Finally, a change of diluent pH, was implemented to improve sample solution stability removing the requirement of injecting sample within 10 minutes after preparation.

Table 1. Method Parameters for Improved Lansoprazole Method

column:	Ascentis Express C18, 15 cm x 4.6 mm, 2.7 µm (53829-U)
mobile phase A:	Water
mobile phase B:	Acetonitrile: 0.5% Triethylamine in Water, pH=7.0 [80:20]
flow rate:	0.8 mL/minute
column temp.:	Ambient
autosampler Temp.:	5 °C
injector volume:	15 µL
detector wavelength:	285 nm
run time:	40 min
gradient:	

Time (Min)	%A	%B
0.0	90	10
30.0	20	80
35.0	20	80
35.1	90	10
40.0	90	10

Figure 1. Linearity Curve from 50 to 150% Nominal Concentration

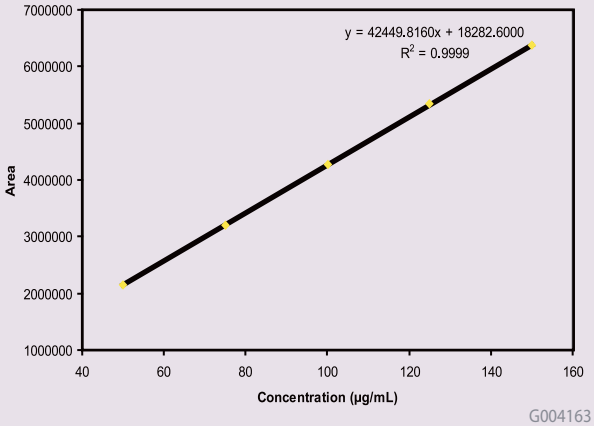

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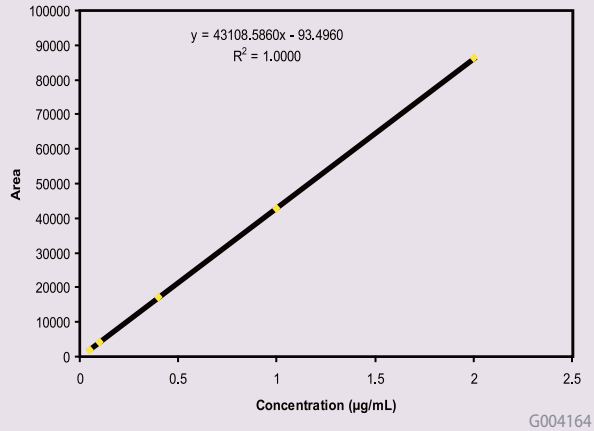
Figure 2. Linearity Curve from 0.05 to 2.0% Nominal Concentration



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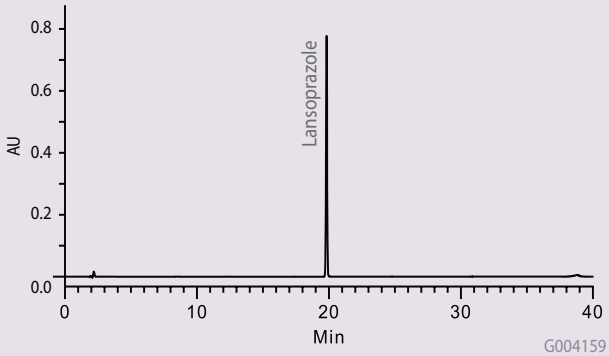
The new method was shown to be linear from 0.05% to 150% of nominal concentration of 100 mg/mL, with quantitation limit less than 0.05%. The broad range of linearity allows for simultaneous impurity and assay analysis. The linearity data are shown in Figures 1 and 2. The RSD of 5 replicate injections of standard solution was 0.11%. In additional experiments, the method was evaluated by analysis of degraded lansoprazole drug substance. Lansoprazole was stressed under four separate conditions by exposure to acid, base, heat and hydrogen peroxide. The chromatograms of the acid and peroxide exposed drug substance along with the unstressed drug substance are shown for reference. The resolving power of the Ascentis Express HPLC column makes it very suitable for these types of studies.

Figure 3. Analysis of Lansoprazole Using Improved Method with Ascentis Express C18 HPLC Column

Unstressed Lansoprazole



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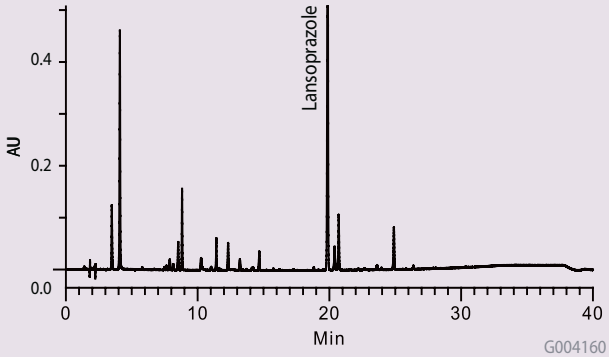
Aged Resolution Solution



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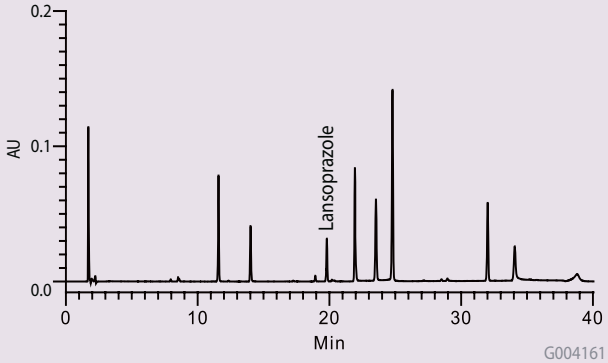
Degraded Lansoprazole Exposed to 0.1 N HCl



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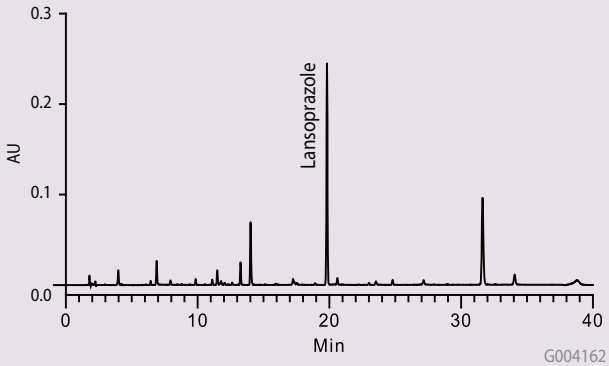
Degraded Lansoprazole Exposed to 0.05% H_2O_2



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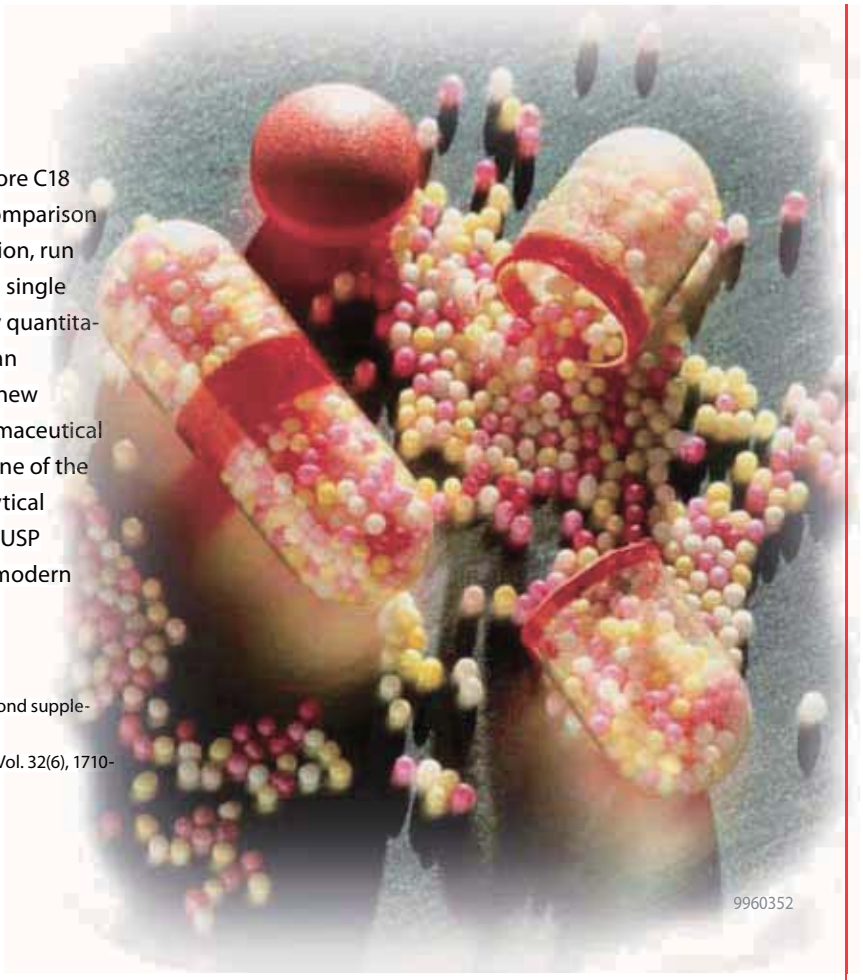
Conclusion

The method developed using 2.7 µm Fused-Core C18 column provided significant improvements in comparison with the original USP method in terms of resolution, run time and sensitivity. As a result, the consolidated single method can be used for both assay and impurity quantitation. The advantages of Fused-Core columns as an alternative for sub-2 µm columns without using new UHPLC instruments could be appealing for pharmaceutical testing. Furthermore, this paper has presented one of the ways (a road map) that could be utilized by analytical scientists in the pharmaceutical field to improve USP monographs for their intended purposes using modern analytical technologies.



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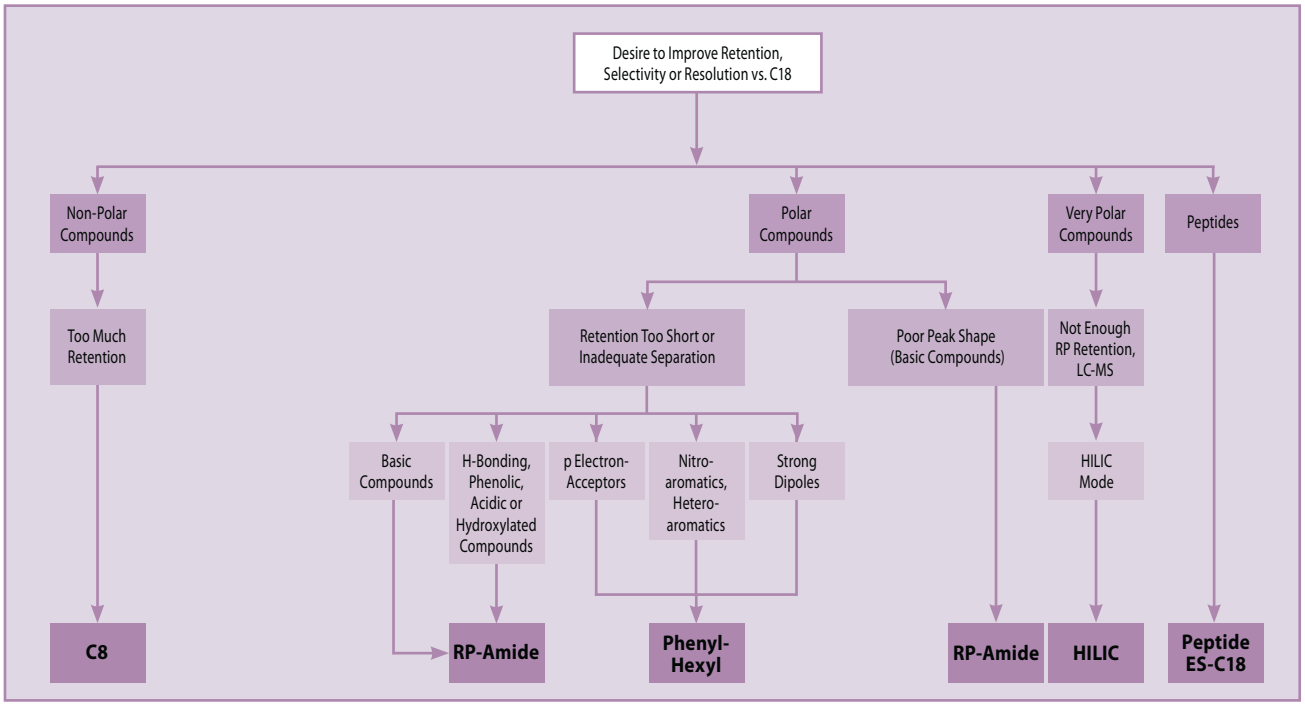
2. Pharmacopeial Forum, In-Process Revision, Lansoprazole, Vol. 32(6), 1710-1712 (2006).

Selecting an Ascentis Express Phase

Ascentis Express C18 is the first choice for starting a new method. However, 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 stationary phases. The range of selectivity provided by Ascentis Express makes this easy. The flow chart below helps guide in the selection of an Ascentis Express phase, based on the particular compound type or separation challenge.



Profiling of Stevia rebaudiana Extract by Accurate Mass Using HILIC and Reversed-Phase Chromatography

Introduction

There is growing public interest in low-calorie alternatives to carbohydrate-based sweeteners. Synthetic sweeteners are often regarded as having an undesirable aftertaste. Recent publications have shown a dramatic increase in attention toward natural extracts including the *Stevia rebaudiana* plant, not only for its sweetening effect but also for additional health benefits attributed to the plant. The major sweetening components are stevioside, rebaudioside A, rebaudioside C, and dulcoside A, each of which is over 300 times sweeter than sucrose-based sweeteners. The concern with the human consumption of the stevia leaf had been attributed to the possible mutagenic properties of steviol, but more recent studies conducted by the World Health Organization have established the safety for steviol and its glycosides.

In this study, an evaluation of the *Stevia rebaudiana* plant extract was conducted using modern chromatographic and mass spectrometry techniques for the determination of extracted components. The purpose was to evaluate the utility of performing two different modes of chromatographic separation for component identification. An accurate mass time of flight (TOF) mass spectrometer was used in the detection and identification of components. A novel software package was then utilized for the determination of common components between the two chromatographic modes and to depict the impact of chromatographic selectivity.

The concept behind the study was to utilize both reversed-phase chromatography and HILIC chromatography for the determination of extract components. By using two different modes of selectivity, components that co-retain, do not retain, or do not elute under one chromatographic mode may be resolved under a separate mode. By resolving a component chromatographically, a more accurate assessment of the component can be made without relying specifically on accurate mass data.

With traditional reversed-phase chromatography, analytes are primarily retained on an alkyl based stationary phase by partitioning interaction between the non polar stationary phase and the analyte. Though this mode of chromatography is widely accepted for separation of moderately polar to non-polar compounds, highly polar analytes often have minimal or no retention on these phases. More popular polar embedded stationary phases address this issue with the addition of a polar functional group within the alkyl chain. Polar embedded phases can enhance retention of polar compounds, but it is not a solution for all applications. Often highly polar analytes require alternative modes of chromatographic retention. In particular, HILIC chromatography allows for alternative selectivity by utilizing a highly polar stationary phase with a relatively non polar mobile phase. Under HILIC conditions, the partitioning of analytes is achieved through a preferential solvation of an aqueous environment on the polar surface. More polar analytes will partition more into the surface solvent and thus be retained longer than a less polar analyte. In addition to the partitioning, the polar surface of the stationary phase allows for adsorptive interactions via hydrogen bonding, dipole, etc. When ionic samples are separated, the potential for ion-exchange interactions also exists and in many cases becomes the dominant retention mechanism. Using silica-based stationary phases, ionized surface silanol groups may interact via ion-exchange with positively charged analytes.

Experimental

In this study, both reversed-phase and HILIC separations were conducted using the Ascentis® Express RP-Amide and Ascentis Express HILIC. The polar embedded group of the Amide was chosen over traditional C18 phases to increase the retention of the polar analytes in the stevia


Stevia rebaudiana

extract. The Ascentis HILIC allowed for alternative selectivities for polar analytes. Because of the large amount of unknown components in the stevia extract, using both reversed-phase and HILIC modes enabled orthogonal selectivity to resolve co-retained components and enable better determination of components in the extract with confirmation between the two modes.

Stevia leaves were obtained from Sigma Aldrich (S5381). Sample extraction of the stevia leaves was performed by weighing 400 mg of crushed stevia leaves into a 7 mL amber vial. A total of 4 mL of 50:50 acetonitrile:water was added and the sample was vortexed and sonicated for 3 minutes. The sample was then centrifuged for 2 minutes at 15000 rpm. The supernatant was then collected and analyzed directly.

The sample extract was analyzed using a gradient elution profile for both HILIC and reversed-phase chromatographic modes. Analysis was conducted using an Agilent® 1200SL Rapid Resolution system in sequence with an Agilent 6210 TOF mass spectrometer. The TOF enabled the use of accurate mass for determination of components. The acquired data was processed using the Mass Hunter software package. The data was pushed to the Mass Profiler package for statistical comparison of the two chromatographic modes. This software package enabled the identification of common components between the two chromatographic separations of the stevia extract. By performing this type of statistical comparison, the components attributed to the stevia extract were differentiated from components attributed to chromatographic anomalies. From this comparison the major components of the stevia extract were determined. Available standards were then used to confirm the identification of several of the components.

Results and Discussion

Figure 1 and Figure 2 represent the total ion chromatogram for the stevia extract under both HILIC and reversed-phase conditions. Both of these chromatographic separations demonstrate the complexity of the stevia extract. Table 1 depicts the major components that were common in both the reversed-phase and HILIC separations of the stevia extract. More than 250 components were identified with this comparison, but only the major components were targeted in this study. The highlighted components in Table 1 depict co-retention of analytes under reversed-phase conditions. A good example of using this orthogonal approach is observed in the case of steviobioside and ducloside A. Under the reversed-phase separation, these components were co-retained. By performing the separation under HILIC conditions, steviobioside and ducloside A were well separated. Other unidentified major components that were unresolved under the reversed-phase conditions were also separated under the HILIC conditions. The data in
Table 1 also depicts the selectivity difference between the two chromatographic modes. Polar components that were poorly retained in the reversed-phase conditions were

(continued on page 16)

Figure 1. Component Chromatogram of Stevia Extract on Ascentis Express HILIC

column: Ascentis Express HILIC, 15 cm x 2.1 mm I.D., 2.7 µm with upchurch inline filter
flow: 0.2 mL/min.
mobile phase A: 2 mM ammonium formate (98:2 acetonitrile:water)
mobile phase B: 2 mM ammonium formate (80:20 acetonitrile:water)
temp.: 35 °C
inj. vol.: 1 µL
system: Agilent 1200SL 6210 TOF, ESI(+)
datafile: 1228082.d

gradient:

Time	%A	%B	Flow
0.00	100	0	0.2
10.00	100	0	0.2
40.00	0	100	0.2
45.00	0	100	0.2
45.01	100	0	0.4
57.00	100	0	0.2
60.00	100	0	0.2

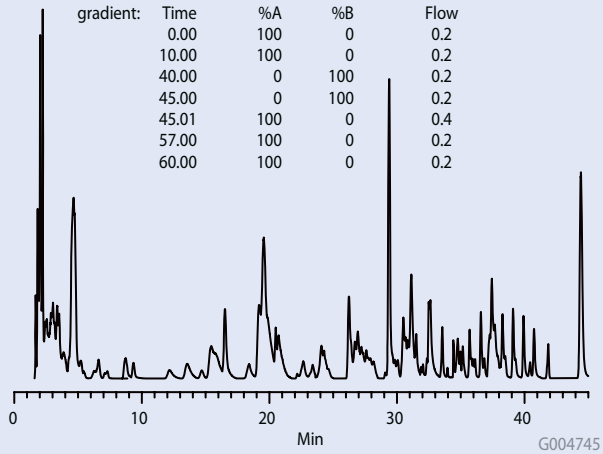
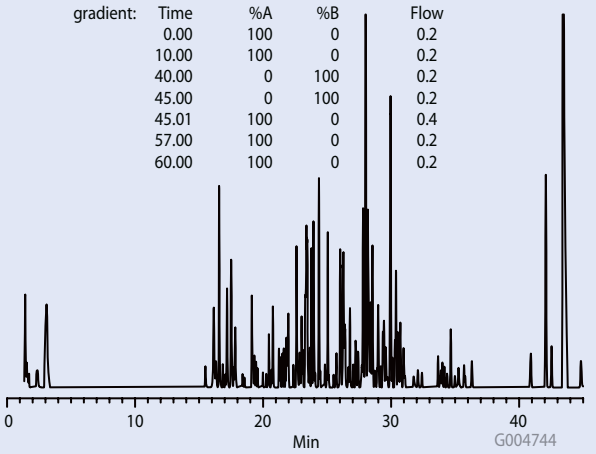


Figure 2. Component Chromatogram of Stevia Extract on Ascentis Express RP-Amide

column: Ascentis Express RP-Amide, 15 cm x 2.1 mm I.D., 2.7 µm with Upchurch inline filter
flow: 0.2 mL/min.
mobile phase A: 10 mM ammonium formate water
mobile phase B: 10 mM ammonium formate (95:5 acetonitrile:water)
temp.: 35 °C
inj. vol.: 1 µL
system: Agilent 1200SL 6210 TOF, ESI(+)
datafile: 012009002.d

gradient:

Time	%A	%B	Flow
0.00	100	0	0.2
10.00	100	0	0.2
40.00	0	100	0.2
45.00	0	100	0.2
45.01	100	0	0.4
57.00	100	0	0.2
60.00	100	0	0.2



Bibliography of Fused-Core Publications

Title: Comprehensive two-dimensional liquid chromatography to quantify polyphenols in red wines.
Authors: Paola Dugo, Francesco Cacciola, Paola Donato, Diego Airado-Rodríguez, Miguel Herrero, Luigi Mondello
Affiliation: Dept of Food & Environment, University of Messina, Messina, Italy; Dept of Pharmaceutical Chemistry, University of Messina, Messina, Italy; Dept of Analytical Chemistry, University of Extremadura Avda, Badajoz, Spain; Campus-Biomedico, Roma, Italy
Journal: Journal of Chromatography A, 2009

Title: Serial coupled columns reversed-phase separations in high-performance liquid chromatography – Tool for analysis of complex real samples
Authors: Luigi Mondello, Miguel Herrero, Francesco Cacciola, Paola Donata, Daniele Giuffrida, Giacomo, Dugo, Paola Dugo
Affliation: Dept of Pharmaceutical Chemistry; University of Messina, Messina,Italy; Campus-Biomedico, Roma, Italy; Dept of Food & Environment, University of Messina, Messina, Italy
Journal: Journal of Chromatography A, 2008, 208-215

Title: Characterization of new types of stationary phases for fast liquid chromatographic applications
Authors: Szabolcs Fekete, Jenö Fekete, Katalin Ganzler
Affliation: Formulation Development, Gedeon Richter Plc, Budapest X, Hungary; Dept of Inorganic & Analytical Chemistry; Budapest University of Technology & Economics
Journal: Journal of Pharmaceutical and Biomedical Analysis, 2009

Title: The use of partially porous particle columns for the routine, generic analysis of biological samples for pharmacokinetic studies in drug discovery by reversed-phase ultra-high performance liquid chromatography-tandem mass spectrometry
Authors: David N. Mallett, César Ramírez-Molina
Affliation: GlaxoSmithKline Medicines Research Centre; Respiratory CEDD DMPK & Immuno-Inflammation CEDD DMPK; Stevenage, Herts, United Kingdom
Journal: Journal of Pharmacuetical and Biomedical Analysis, 2009, 100-107

Title: Shell and small particles; Evaluation of new column technology
Authors: Szabolcs Fekete, Jenö Fekete, Katalin Ganzler
Affiliation: Formulation Development, Gedeion Richter Plc, Budapest X, Hungary; Dept. of Inorganic & Analytical Chemistry; Budapest University of Technology & Economics, Budapest, Hungary
Journal: Journal of Pharmaceutical and Biomedical Analysis, 2008

Title: Modeling of the Mass-Transfer Kinetics in Chromatographic Columns Packed with Shell and Pellicular Particles
Authors: Krzysztof Kaczmarski, Georges Guiochon
Affiliation: Dept of Chemical & Process Engineering; Rzesnow University of Technology, Rzeszow, Poland; Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Analytical Chemistry 2007, 4648-4656

Title: Mass-Transfer Kinetics in a Shell Packing Material for Chromatography
Authors: Georges Guiochon, Alberto Cavazzini, Fabrice Gritti, Krysztof Kaczmarski, Nicola Marchetti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee; Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland
Journal: Analytical Chemistry, 2007, 5972-5979

Title: Comprehensive Off-Line, Two-Dimensional Liquid Chromatography. Application to the Separation of Peptide Digests
Authors: Georges Guiochon, Nicola Marchetti, Jacob N Fairchild
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee, Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Analytical Chemistry 2008, 2756-2767

Title: Comparison between the loading capacities of columns packed with partially and totally porous fine particles. What is the effective surface area available for adsorption?
Authors: Georges Guiochon, Fabrice Gritti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Journal of Chromatography A, 2007, 107-122

Title: Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials
Authors: Georges Guiochon, Fabrice Gritti, Alberto Cavazzini, Nicola Marchetti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee; Dept of Chemistry, University of Ferrara, Ferrara, Italy
Journal: Journal of Chromatography A, 2007, 289-303

Title: Gradient elution separation and peak capacity of columns packed with porous shell particles
Authors: Georges Guiochon, Nicola Marchetti, Alberto Cavazzini, Fabrice Gritti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee, Div of Chemical & Analytical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee, Dept of Chemistry, University of Ferrara, Ferrara, Italy
Journal: Journal of Chromatography A 2007, 203-211

Title: Comparative study of the performance of columns packed with several new fine silica particles. Would the external roughness of the particles affect column properties?
Authors: Georges Guiochon, Fabrice Gritti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Journal of Chromatography A, 2007, 30-46

Title: High peak capacity separations of peptides in reversed-phase gradient elution liquid chromatography on columns packed with porous shell particles
Authors: Georges Guiochon, Nicola Marchetti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Journal of Chromatography A, 2007, 206-216

Title: Evaluation of the properties of a superficially porous silica stationary phase in hydrophilic interaction chromatography
Authors: David V McCalley
Affiliation: Center for Research in Biomedicine, University of the West of England, Frenchay, Bristol, United Kingdom
Journal: Journal of Chromatography A, 2008, 85-91

Title: Optimization of two-dimensional gradient liquid chromatography separations
Authors: Pavel Jandera, Petr Cesla, Tomás Hájek
Affiliation: Dept of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic
Journal: Journal of Chromatography A, 2008

Title: Comprehensive two-dimensional liquid chromatography separations of pharmaceutical samples using dual Fused-Core columns in the 2nd dimension
Authors: Anthony J Alexander, Lianjia Ma
Affiliation: Analytical R & D, Bristol Myers Squibb Co, New Brunswick NJ
Journal: Journal of Chromatography A, 2009, 1338-1345

Title: Determination of fatty alcohol ethoxylates with diphenic anhydride derivatization and liquid chromatography with spectrophotometric detection. A comparative study with other anhydrides.
Authors: G. Ramis-Ramos, A. Micó-Tormos, F. Bianchi, E. F. Simó-Alfonso
Affiliation: Dept de Química Analitica, Universitat de València, Valencia, Spain
Journal: Journal of Chromatography A, 2009, 3023-3030

Title: Determination of fatty alcohol ethoxylates by derivatisation with maleic anhydride followed by liquid chromatography with UV-vis detection
Authors: G. Ramis-Ramos, A. Micó-Tormos, C. Collado-Soriano, J. R, Torres-Lapasió, E. Simó-Alfonso
Affiliation: Dept de Química Analitica, Universitat de València, Valencia, Spain
Journal: Journal of Chromatography A, 2008, 32-41

Title: On-column epimerization of dihydroartemisinin: An effective analytical approach to overcome the shortcomings of the International Pharmacopoeia monograph
Authors: Francesco Gasparrini, Walter Cabri, Alessia Ciogli, Ilaria D'Acquarica, Michela Di Mattia
Affiliation: Analytical Development, R & D Dept.,Pomezia, Italy; Dept di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Rome, Italy
Journal: Journal of Chromatography B, 2008, 180-191

Title: Use of partially porous column as second dimension in comprehensive two-dimensional system for analysis of polyphenolic antioxidants
Authors: Paola Dugo, Francesco Cacciola, Miguel Herrero, Paola Donato, Luigi Mondello
Affiliation: Dipartimento de Scienze degli Alimenti e dell'Ambiente, Facoltà di Scienze, Messina, Italy; Dipartimento Farmacia-chimco, Facoltà di Farmacia, Università di Messina, Messina, Italy; Campus-Biomedico, Roma, Italy
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Authors: Paola Donato, Paola Dugo, Francesco Cacciola, Giovanni Dugo, Luigi Mondello
Affiliation: Dipartimento Farmacia-chimco, Facoltà di Farmacia, Università di Messina, Messina, Italy; Dipartimento de Scienze degli Alimenti e dell'Ambiente, Facoltà di Scienze, Messina, Italy; Campus-Biomedico, Roma, Italy
Journal: Journal of Separation Science, 2009, 1129-1136

Title: Maximizing peak capacity and separation speed in liquid chromatography
Authors: Patrik Petersson, Andre Frank, James Heaton, Melvin R. Euerby
Affiliation: AstraZeneca R&D Lund, Lund, Sweden; AstraZeneca, R&D Charnwood, Loughborough, Leicestershire, England, UK
Journal: Journal of Separation Science, 2008, 2346-2357

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Authors: Isabelle François, Deirdre Cabooter, Koen Sandra, Frédéric Lynen, Gert Desmet, Pat Sandra
Affiliation: Separation Sciences Group & Pfizer Analytical Research Centre, Ghent University, Ghent, Belgium; Dept. of Chemical Engineering, Vrije Universiteit Brussel, Brussels, Belgium; Research Institute for Chromatography, Kortrijk, Belgium
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Authors: Jennifer M. Cunliffe, Todd D. Maloney
Affiliation: Pfizer Global Research & Development, Michigan Laboratories, Ann Arbor
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Title: High specific activity tritium-labeled N-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): A high-affinity 5-HT2A receptor-selective agonist radioligand
Authors: David E. Nichols, Stewart P. Frescas, Benjamin R. Chemel, Kenneth S. Rehder, Desong Zhong, Anita H. Lewin
Affiliation: Dept. of Medicinal Chemistry & Molecular Pharmacology, School of Pharmacy & Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana; Center for Organic & Medicinal Chemistry, Research Triangle Instutite, Durham, NC
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Authors: Joseph J. Kirkland, Timothy J. Langlois, Joseph J. DeStefano
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Journal: American Laboratory, 2007

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Authors: Thomas L. Chester
Affiliation: Dept. of Chemistry, University of Cincinnati, Cincinnati, OH
Journal: American Laboratory, 2009

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Authors: Yunsheng Hsieh, Christine J. G. Duncan, Jean-Marc Brisson
Affiliation: Drug Metabolism & Pharmacokinetics Dept., Schering-Plough Research Institute, Kenilworth, NJ
Journal: Anal. Chem., 2007, 79 (15), pp 5668–5673

Title: Discovery of Pyridine-Containing Imidazolines as Potent and Selective Inhibitors of Neuropeptide Y Y5 Receptor
Authors: Makoto Ando, Nagaaki Sato, Tsuyoshi Nagase, Keita Nagai, Shiho Ishikawa, Hirobumi Takahashi
Affiliation: Tsukuba Research Institute, Merck Research Laboratories, Banyu Pharmaceutical Co, Ltd, Tsukuba, Japan
Journal: Bioorganic & Medicinal Chemistry, 2009

Title: Discovery of potent and orally active 3-alkoxy-5-phenoxy-N-thiazolyl benzamides as novel allosteric glucokinase activators
Authors: Tomoharu Iino, Daisuke Tsukahara, Kenji Kamata, Kaori Sasaki, Sumika Ohyama, Hideka Hosaka
Affiliation: Banyu Tsukuba Research Institute, Banyu Pharmaceutical Co Ltd, Tsukuba, Japan
Journal: Bioorg Med Chem. 2009 Apr 1;17(7):2733-43

Title: Achiral Liquid Chromatography with Circular Dichroism Detection for the Determination of Carnitine Enantiomers in Dietary Supplements and Pharmaceutical Formulations
Authors: Ángel Ríos, Fernando de Andrés, Gregorio Castañeda
Affiliation: Dept. of Analytical Chemistry & Food Technology, University of Castilla-La Mancha, Ciudad Real, Spain
Journal: Journal of Pharmaceutical and Biomedical Analysis, 2009

Title: Characterization of new types of stationary phases for fast liquid chromatographic applications
Authors: Szabolcs Fekete, Jenö Fekete, Katalin Ganzler
Affiliation: Formulation Development, Gedeon Richter Plc, Budapest, Hungary; Budapest University of Technology & Econmics, Dept. of Inorganic & Analytical Chemistry, Budapest, Hungary
Journal: Journal of Pharmaceutical and Biomedical Analysis, 2009

Title: Comprehensive Two-Dimensional Liquid Chromatography Separations of Pharmaceutical Samples using Dual Fused-Core Columns in the Second Dimension
Authors: Anthony J. Alexander, Lianjia Ma
Affiliation: Analytical Research & Development, Bristol Myers Squibb Co, New Brunswick, NJ
Journal: Journal of Chromatography A, 2009, Pages 1338-1345

Title: Curcumin Structure-Function, Bioavailability, and Efficacy in Models of Neuroinflammation and Alzheimer's Disease
Authors: Aynun N. Begum, Mychica R. Jones, Giselle P. Lim, Takashi Morihara, Peter Kim, Dennis D. Heath
Affiliation: Dept. of Medicine & Neurology & Psychiatry & Biobehavioral Sciences and the Semel Institute, UCLA, Los Angeles, CA; Greater LA Healthcare System, Geriatric Research Education Clinical Center, Sepulveda, CA; Cancer Prevention & Control Program, Moores UCSD Cancer Center, UCSD, San Diego, CA; Dept. of Post-Genomics & Diseases, Division of Psychiatry & Behavioral Proteomics, Osaka University Graduate School or Medicine, Osaka, Japan
Journal: J Pharmacol Exp Ther. , 2008, 196-208

Title: Liposome-Encapsulated Curcumin Suppresses Growth of Head and Neck Squamous Cell Carcinoma In vitro and in Xenografts through the Inhibition of Nuclear Factor kB by an AKT-Independent Pathway
Authors: Dorothy Wang, Mysore S. Veena, Kerry Stevenson, Christopher Tang, Baran Ho, Jeffrey D, Suh
Affiliation: Dept. of Surgery & Pathology, VA Greater LA Healthcare System; David Geffen School of Medicine, UCLA, Los Angeles, CA; Dept of Psychiatry & Biobehavioral Sciende, Semel Institute, UCLA, Los Angeles, CA; Dept. of Experimental Therapeutics, MD Anderson Cancer Center, University of Texas, Houston, TX
Journal: Clinical Cancer Research, 2008

Title: Analysis of Impurities in Streptomycin and Dihydrostrepomycin by Hydrophilic Interaction Chromatography/Electrospray Ionization Quadrupole Ion Trap/Time-of-Flight Mass Spectrometry
Authors: Shin-ichi Kawano
Affiliation: Analytical Applications Dept., Shimadzu Corp., Kyoto, Japan
Journal: Rapid Communications in Mass. Spectrometry, 2009, 907-914

Title: Determination of Fatty Alcohol Ethoxylates by Derivatization with Phthalic Anhydride followed by Liquid Chromatography with UV-vis Detection
Authors: G. Ramis-Ramos, A. Micó-Tormos, E. F. Simó-Alfonso
Affiliation: Dept de Química Analitica, Universitat de València, Valencia, Spain
Journal: Journal of Chromatography A, 2009, 47-53

Title: Highly Efficient Peptide Separations in Proteomics: Part 1. Unidimensional High Performance Liquid Chromatography
Authors: Koen Sandra, Mahan Moshir, Filip D'hondt, Katleen Verleysen, Koen Kas, Pat Sandra
Affiliation: Dept. of Chemical Engineering, Vrije Universiteit Brussel, Brussels, Belgium
Journal: Journal of Chromatography B, 2008, 48-63

Title: Multidimensional LCxLX analysis of Phenolic and Flavone Natural Antioxidants with UV-electrochemical coulometric and MS Detection
Authors: Tomás Hájek , Veronika Skerikova, Petr Cesla, Katerina Vynuchalova, Pavel Jandera
Affiliation: Dept of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic
Journal: Journal of Separation Science, 2008, 3309-3328

Title: Separation of Natural Product using columns packed with Fused-Core Particles
Authors: P. Yang, G.R. Litwinski, M. Pursch, T. McCabe, K. Kuppannan
Affiliation: The Dow Chemical Company, Analytical Sciences, Midland, MI
Journal: J. Sep Sci., 2009, 1816-1822

(continued from page 13

Table 1. Major Component Retention Comparison Between HILIC and Reversed-Phase Modes

Component	Accurate Mass	Ascentis Express HILIC	Ascentis Express RP-Amide
		RT	RT
	137.0476	44.397	1.429
	120.0574	34.695	3.075
	102.0473	29.394	3.077
	368.1698	18.414	15.502
	120.0574	31.082	17.166
	402.1518	15.471	17.196
	162.1405	8.727	19.128
	378.2242	8.736	19.13
Rebaudioside A/E	966.4281	34.439	21.755
	516.1254	20.784	23.016
	498.1154	20.714	23.016
Stevioside	804.3743	26.52	23.63
Steviolbioside	642.3234	19.213	24.356
Dulcoside A	788.3817	26.247	24.475
	338.2448	2.248	25.737
	176.1555	2.049	26.026
	284.2134	3.372	26.028
	246.1977	3.359	26.06
	380.255	2.002	26.672
	284.2131	2.811	27.881
	360.083	1.867	28.187
	444.2002	1.825	30.391
Steviol	318.2186	1.91	32.19
	592.2655	2.239	42.539

Figure 3. Stevia Extract on Ascentis Express RP-Amide, Extracted Ion Chromatogram for Steviol

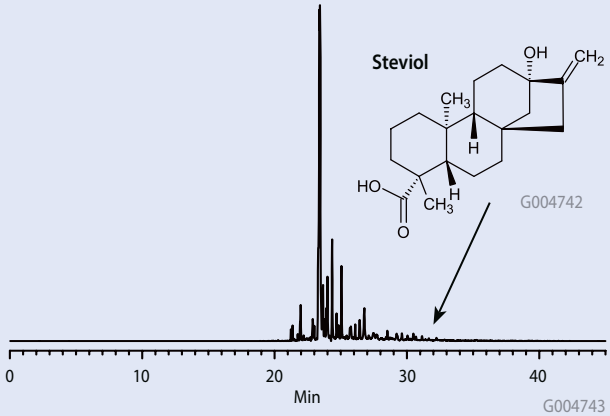
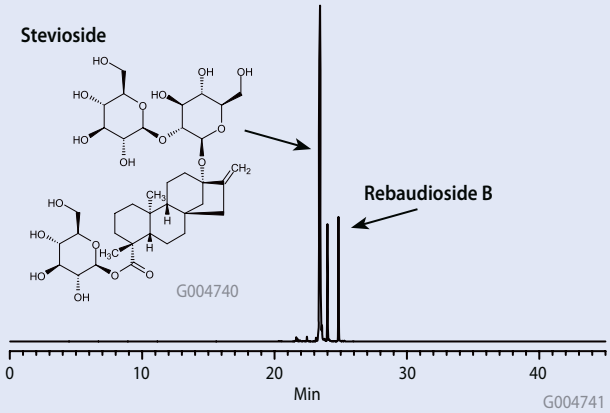


Figure 4. Stevia Extract on Ascentis Express RP-Amide, Extracted Ion Chromatogram for Stevioside



strongly retained under HILIC conditions. In two cases, where known components were identified, it was necessary to use standards to confirm their retention. Figures 3 and 4 depict the extracted ion chromatogram for the accurate mass of steviol and stevioside. As can be seen in both chromatograms, multiple peaks are observed for each of the accurate masses. In the case of stevioside, it is isobaric with rebaudioside B making identification difficult. A stevioside standard (Sigma Aldrich) was used for positive identification. In addition, the reversed-phase separation of the extract resulted in multiple peaks observed for the accurate mass of steviol. This was due to fragments from additional glycosides that resulted in a steviol fragment ion, again it was necessary to confirm the steviol retention with a standard.

Conclusions

The profiling of the *Stevia rebaudiana* extract demonstrates the utility of performing orthogonal chromatographic modes when handling complex samples. The two modes of chromatography were complimentary for the determination of major components from the stevia extract. In most cases where coelution occurred in one chromatographic mode, the components were separated under the orthogonal mode. Though component identification was made easier through the accurate mass of the TOF, it was still necessary to have good chromatographic resolution to confirm component identity. In both cases, the Fused-Core™ particle demonstrated the ability to perform complex matrix analysis in both HILIC and reversed-phase separations.

Colors of the World: Fast Separation of Dyes with Ascentis Express

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 unusual places like 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 (1, 2).

Further, a sensitive HPLC method is needed for quality control testing of dyes and the identification of byproducts. Supelco's Ascentis Express HPLC columns provide outstanding sensitivity and resolution for such applications.

Method Development for Dyes

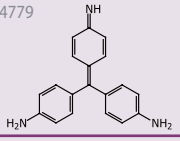
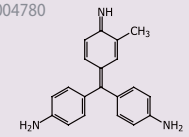
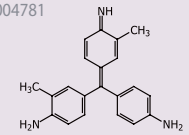
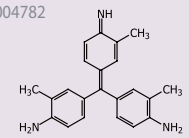
Table 1 contains a list of dyes added to one sample and dissolved in a mixture of methanol and acetonitrile. The sample was injected on an Ascentis Express C8 HPLC column under varying mobile phase conditions to determine the best separation parameters. Temperature, injection volume, detector settings, and flow rate were kept constant.

The chemical and physical properties of the dyes differ strongly, so the first step in developing a suitable HPLC method was the use of a gradient run ranging from 25% acetonitrile to 100% acetonitrile (B) and 0.1% formic acid in water as an aqueous counterpart (A). The UV chromatogram of the combined wavelengths 360, 550, and 620 nm showed good chromatography of all compounds except for the poor peak shape of Sudan 410 at 17.35 minutes (Figure 1A).

To optimize that peak shape, methanol was added to the organic mobile phase (acetonitrile:methanol, 90:10); the gradient run was repeated, resulting in better peak shape for Sudan 410 (Figure 1B). In a final experiment, the gradient profile was changed and optimum conditions were attained (see Table 2). Figure 1C shows the step-by-step improvements in the chromatography.

Only three runs were needed to get the final method, showing how easy and fast it is to develop methods with Ascentis Express columns. Further, Ascentis Express columns contain Fused-Core particles that allow for faster run times; even separations performed on standard HPLC systems can be sped up by up to 30% with Ascentis Express.

Table 1. Structure and Mass of the Dyes in the Sample Mixture. Most of the Compounds are Detected as $[M+H]^+$ Ions except (5), which gives $[M]^+$ Ions

Peak No.	Structure	Name / Exact Mass
1	G004779 	Parafuchsin $C_{19}H_{17}N_3$ 287.142247
2	G004780 	Basic Fuchsin $C_{20}H_{19}N_3$ 301.157897
3	G004781 	Methylfuchsin $C_{21}H_{21}N_3$ 315.173547
4	G004782 	Newfuchsin $C_{22}H_{23}N_3$ 329.189197

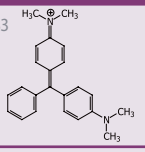
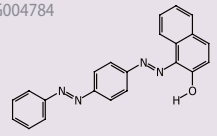
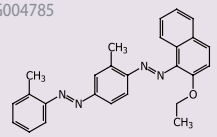
Peak No.	Structure	Name / Exact Mass
5	G004783 	Malachite Green $C_{23}H_{25}N_2$ 329.201773
6	G004784 	Sudan III $C_{22}H_{16}N_{4O}$ 352.132411
7	G004785 	Sudan 410 $C_{26}H_{24}N_{4O}$ 408.195011

Table 2. Initial and Final HPLC Method Settings for Separation of the Seven Dyes Listed in Table 1, After Optimization

Fixed Parameters

column:	Ascentis Express C8, 10 cm × 4.6 mm I.D., 2.7 µm particles
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)

Variable Parameters

Initial Conditions

solvents: (A) water with 0.1% formic acid
(B) acetonitrile

gradients:

Time	%A	%B
0.0	75	25%
1.5	75	25%
15.0	0	100%
22.0	0	100%
25.0	75	25%

Final Conditions

(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%

Figure 1. UV Chromatograms of Sudan III and Sudan 410. (A) Initial Conditions, (B) Addition of Methanol to Mobile Phase, (C) Final Conditions after Adjusting Gradient

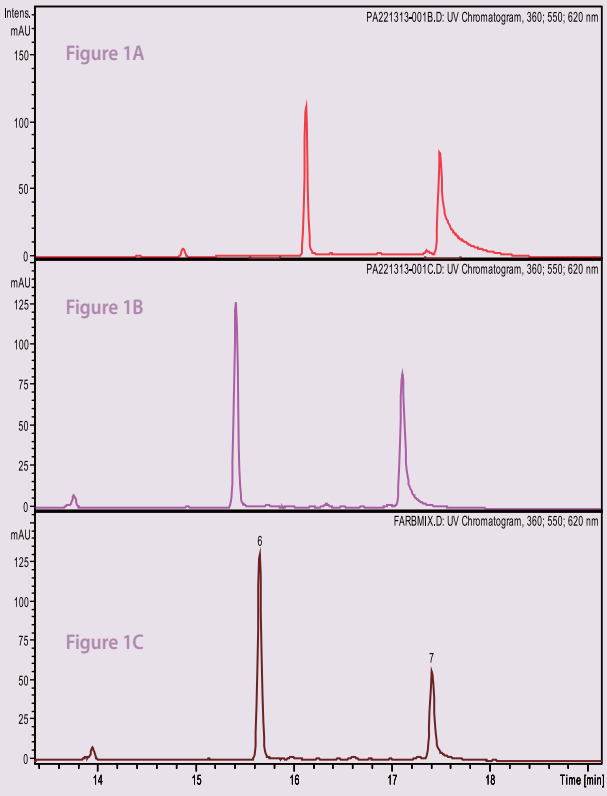
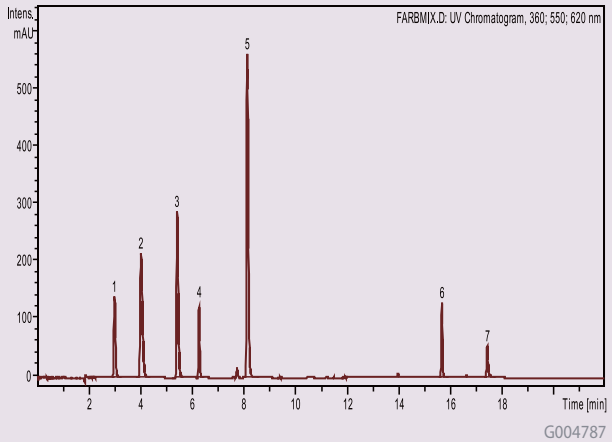


Figure 1 shows UV chromatograms of Sudan III (Peak 6) and Sudan 410 (Peak 7) after three optimization steps. An organic phase mixture of methanol:acetonitrile (10:90) and a final gradient composition of 98% organic mobile phase resulted in the best overall peak shapes with a minimum of tailing of compounds (Peak 7). Figure 3 shows the final chromatogram with very good separation of all analytes.

Figure 2 is a UV chromatogram of the final HPLC method. Resolution, sensitivity, and peak symmetry were optimal for all analytes. The total run time on a standard HPLC instrument (Agilent 1100) was 25 minutes, but the separation could easily be performed faster on ultra-performance instruments.

Figure 2. UV Chromatogram of the Final HPLC Method

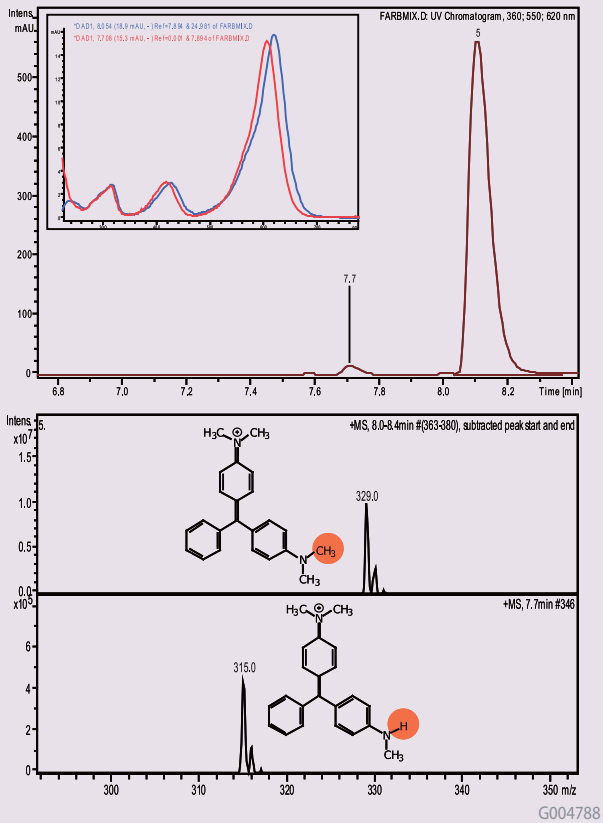


Ascentis Express HPLC Columns: High Efficiency and LC-MS Compatibility

Using Ascentis Express columns on standard HPLC, fast LC, and ultra-performance instruments can yield heightened sensitivity (Figure 2). Mass detectors in LC-MS systems are very sensitive to contaminants in solvents and column bleed, both of which are very low with Ascentis Express columns combined with the right set of Fluka® LC-MS solvents and additives. Both aspects, high efficiency and low column bleed, are basic requirements in trace analysis of small target analyte concentrations or in identification of byproducts which may influence dramatically the quality and application of dyes. Figure 3 shows an example of the identification of low concentrations of byproducts even in very complex mixtures. The unknown substance at the retention time of 7.68 minutes shows a nearly identical UV spectrum to Malachite Green (Peak 5 at 8.10 minutes), but the mass is 14 Da lower. This may correspond to the exchange of a methyl residue with a proton at a position in the Malachite Green molecule that has no influence on the chromophore. Only the displayed molecular structures fit the UV and mass spectroscopic results.

Figure 3. Expanded View of UV Chromatogram Showing Unknown Impurity at 7.7 min. and Malachite Green (5)

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



To get optimal results from your LC-MS system and accurate UV and mass spectra of impurities with a high signal-to-noise level, it is best to use high purity LC-MS solvents from Fluka and high performance HPLC columns such as Ascentis Express from Supelco.

References

1. Commission Decision. Official Journal of the European Union. L154/114. June 10, 2003.
2. Rapid Alert System for Food and Feed (RASFF). 2004. Annual Report. European Commission of Health & Consumer Protection Directorate General.

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^2/gram surface area (comparable to ~225 m^2/g porous particle)
- 90 Å pore size, 160 Å for Peptide ES C-18

Bonded Phase

	Coverage µmoles/m^2	pH Range	Endcapping
C18	*3.5*	*2-9*	*Yes*
C8	*3.7*	*2-9*	*Yes*
RP-Amide	*3.0*	*2-9*	*Yes*
HILIC	*n/a*	*2-8*	*No*
Phenyl-Hexyl	*3.4*	*2-9*	*Yes*
Peptide ES-C18	*3.5*	*1-9*	*No*

Ascentis Express RP-Amide:

Combining an Embedded Polar Group Stationary Phase and Fused-Core Particles

Ascentis Express RP-Amide HPLC columns are the most recent product additions to the Supelco HPLC product line. Combining an embedded polar group (EPG) stationary phase with the Fused-Core particles, Ascentis Express RP-Amide provides a host of useful benefits to the HPLC chromatographer. The benefits come from both the phase technology and the particle technology and can be summarized as:

Fused-Core Benefits

- Higher efficiency than traditional HPLC columns (3 and 5 µm)
- Half of the backpressure of sub 2 micron columns
- Capable of UHPLC performance on traditional HPLC systems

RP-Amide Benefits

- Alternative reversed-phase selectivity to C18
- Improved peak shape for bases
- 100% aqueous compatible reversed-phase column

At the heart of the Ascentis Express RP-Amide is the 2.7 µm Fused-Core particle that comprises a 1.7 µm solid core and a 0.5 µm porous shell. Compared to totally porous particles, the Fused-Core particles have a much shorter diffusion path because of the solid core. This partial porosity reduces 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 capable of delivering extreme performance to any HPLC system. In fact, there have been many reports on the vast improvements in efficiency and speed provided by Ascentis Express HPLC columns versus traditional HPLC columns. The improvements provide UHPLC performance on traditional HPLC systems.

While the Ascentis Express C8 and C18 provide classic reversed-phase selectivity, the RP-Amide phase offers an alternative selectivity. Supelco first commercially introduced the EPG phase in 1988. At that time, large tailing factors for basic analytes continued to plague conventional C18 and C8 bonded phases. The EPG phase was found to improve peak shape of basic analytes. The early generation EPG phases were based on a two-step bonding process (Figure 1). The first step was the bonding of an aminopropylsilane to the bare silica surface creating a surface with amine functionality. In step two, palmitoyl chloride was reacted with the amine to create a long chain amide. Not all amines would be converted in the process, leaving a mixed system. These early generation EPG phases suffer from poor reproducibility.

Next generation phases, including Ascentis Express RP-Amide, are produced using a one-step process (Figure 2). In the single step process, no free amino ligands occur since the amide is introduced as a whole unit. This one-step bonding process yields excellent batch-to-batch reproducibility. Interestingly, not all EPG phases on the market use the modern, one-step bonding approach.

Improved Peak Shape for Basic Compounds

As previously mentioned, Ascentis Express RP-Amide phase reduces silanol interactions with basic analytes improving peak shape. A good test to demonstrate this effect is highly basic compounds using a mobile phase pH of 7. At this pH, many of the residual silanols are in the ionized form and the basic compounds are protonated.
The protonated (charged) bases interact with the charged silanols via ion exchange and result in a tailing peak. A test

Figure1. Early Generation Two-step Bonding Process for EPG Amide Phases

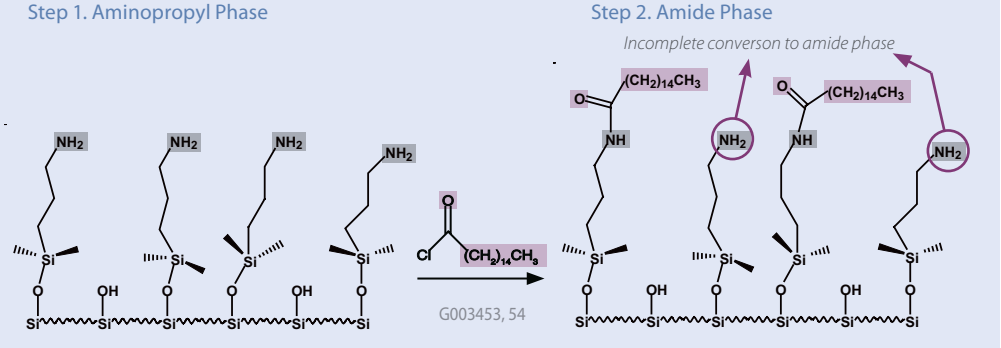
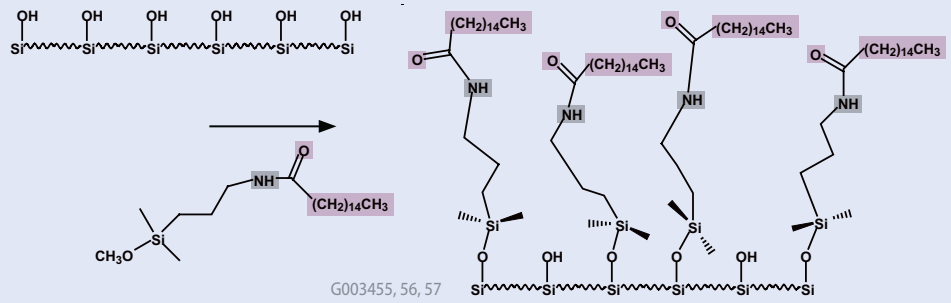


Figure 2. Next Generation One-step Bonding Process for EPG Amide Phases



mix of tricyclic antidepressants was analyzed on Ascentis Express RP-Amide and a C18 column with a mobile phase pH of 7 (Figure 3). As shown in Figure 3, the RP-Amide produces more symmetrical peaks than the C18 for these difficult test probes. Asymmetry data is summarized in Figure 4 for doxepin, imipramine, and amitriptyline.

Alternative Selectivity

Ascentis 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 Figure 5. 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. In comparing the Ascentis Express RP-Amide to the Waters™ BEH Shield RP18,
a competitive EPG phase, the selectivity is very similar. The difference in this example is the Ascentis Express RP-Amide yields a backpressure half of the 1.7 µm column. This difference in backpressure means the Ascentis Express column is suitable for traditional HPLC systems while the 1.7 µm column is not.

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.

Figure 3. Separation of Tricyclic Antidepressants on Ascentis Express RP-Amide and Conventional C18

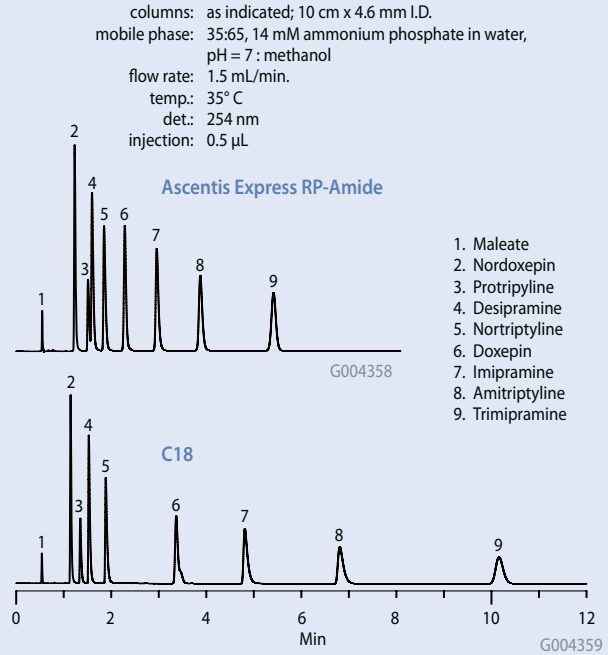


Figure 4. Asymmetry Factors for Difficult Bases on Ascentis Express RP-Amide and Conventional C18

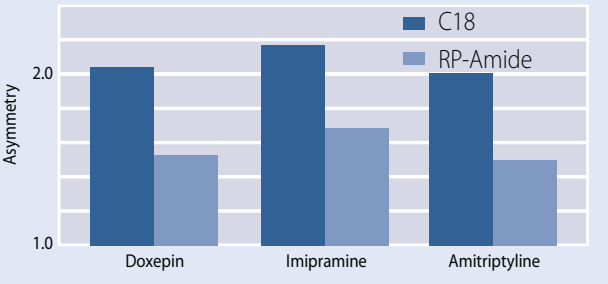
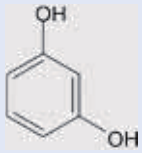


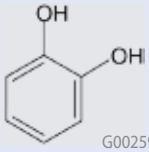
Figure 5. Separation of Phenolics on Ascentis Express RP-Amide & C18, & Waters BEH Shield RP18, 1.7 µm

columns: as indicated; 10 cm x 2.1 mm I.D.
mobile phase A: 20 mM phosphoric acid, pH 2 (unadjusted)
mobile phase B: water
mobile phase C: acetonitrile
mobile phase ratios: A:B:C = 75:5:20
flow rate: 0.3 mL/min.
temp.: 35° C
det.: 270 nm
injection: 1 µL
sample: 50 mg/L ea. in 20 mM phosphoric acid

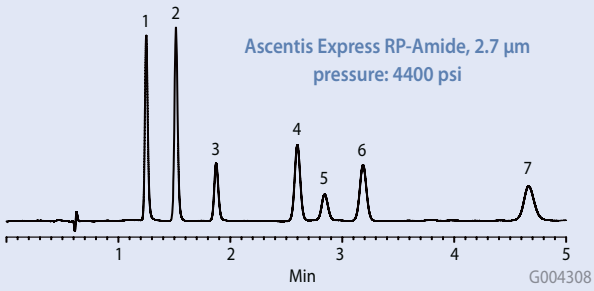
1. Resorcinol
2. Catechol
3. 2-Methylresorcinol
4. 4-Methylcatechol
5. 2,5-Dimethylresorcinol
6. 3-Methylcatechol
7. 4-Nitrocatechol

Resorcinol 

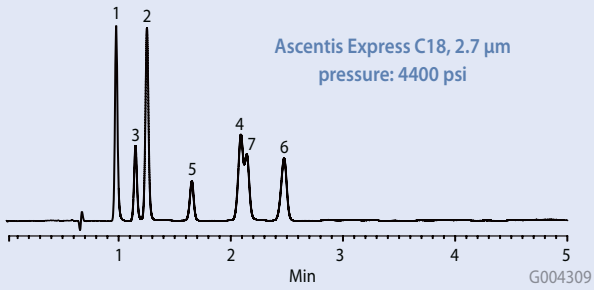
G002594

Catechol 

G002590



G004308



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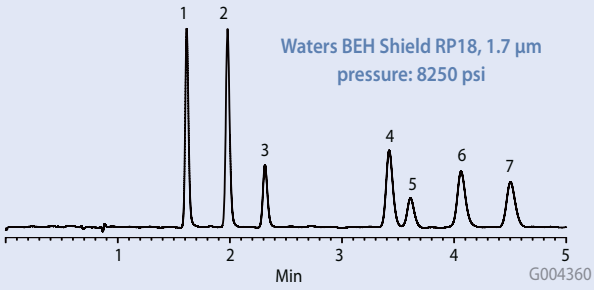
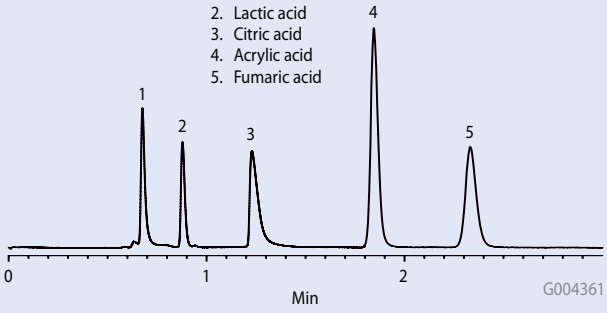


G004360

Figure 6. Separation of Small Organic Acids Under 100% Aqueous Conditions

column: Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D.
mobile phase: 0.1% TFA (v/v) in water
flow rate: 0.3 mL/min.
temp.: 35° C
det.: 210 nm
injection: 1 µL
sample: 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

1. Tartaric acid
2. Lactic acid
3. Citric acid
4. Acrylic acid
5. Fumaric acid



G004361

Aqueous Compatible Reversed-Phase Column

Ascentis Express RP-Amide provides stable and reproducible analyte retention in 100% aqueous mobile phases. Many C18 phases are known to suffer from phase collapse under highly aqueous mobile phase conditions causing loss of retention. Shown in Figure 6 is 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.

Conclusion

Ascentis Express RP-Amide is a blend of modern phase technology and innovative particle technology. The Fused-Core particle provides benefits in terms of speed, resolution, sensitivity, and ruggedness. The one-step RP-Amide bonding chemistry provides benefits in terms of selectivity, aqueous stability, and improved peak shape for bases.

Ascentis Express Phenyl-Hexyl:

Combining the popular Phenyl-Hexyl Stationary Phase and Fused-Core Particles

Even though new column technologies have more than doubled the plates per meter possible with traditional 5 µm columns, resolution still cannot be routinely achieved in every case without the ability to adjust retention and selectivity by proper selection of column stationary and mobile phases. This article features Ascentis Express Phenyl-Hexyl phase, a new addition to the Fused-Core column family, and describes how column selectivity and higher efficiency can be coupled to achieve much faster separations than have previously been possible.

The vast majority of UHPLC separations have been carried out with C18 columns in the classic reversed-phase (RP) mode; however, suppliers now offer many different phases. Although no one would dispute the fact that UHPLC columns with different phases are needed, very little has been published yet on the performance that can be expected from UHPLC columns having different, complementary selectivity to C18 and C8. Two of the most popular polar-RP phases are RP-Amide, which is often categorized as an embedded polar-group phase, and Phenyl, which can interact with solutes by π-π mechanisms. A brief retention and selectivity comparison for the Ascentis Express column family is given in Table 1.

C18 and C8 phases are highly popular because they are stable, reproducible, and easy-to-use. Retention correlates closely with log P values, which have been established for many solutes. Solute ionization causes retention to decrease in a predictable manner and is relatively easy to control by adding dilute acids, bases, and buffers to the mobile phase. Changing the organic component of the mobile phase between acetonitrile and methanol (or other solvents) allows the user to tweak resolution because solvation affects phase structure and selectivity. Temperature is also a useful variable for optimizing phase selectivity. Columns with C18 and C8 phases will frequently give optimum resolution when solutes are nonpolar or slightly polar; however, columns with polar-RP phases such as RP-Amide or Phenyl-Hexyl will often show improved retention and selectivity for more polar solutes. It should be emphasized that even polar-RP phases have a significant alkyl phase character in addition to their polar character. The same mobile phase solvents and techniques may be employed with polar-RP phases, with comparable phase stability to C18.

Table 1. Brief Overview of Ascentis Express Column Retention and Selectivity

Ascentis Express Fused-Core Phase	Principle Retention Mode	Principle Solute Interaction
C18	Reversed-Phase (RP)	Hydrophobic (dispersive)
C8	Reversed-Phase (RP)	Hydrophobic (dispersive)
RP-Amide	RP with embedded polarity	Hydrophobic and H-bonding
Phenyl-Hexyl	RP with pendant aromaticity	Hydrophobic and π-π
HILIC (Silica)	HILIC (or normal phase)	Hydrophilic (dipole, H-bonding, ion exchange)

The RP-Amide phase is complementary to C18 because the amide group has several unique features: 1) strong interaction by H-bonding when solutes can donate or accept protons, 2) effective shielding of silanols by internal H-bonding between amide group and silica surface, and 3) the ability to wet and operate well, even in 100% aqueous solvents. H-bonding allows solutes with carboxyl and phenol groups to be retained much longer and separate much better on RP-Amide than on C18 or C8. Shielding prevents solutes with amino groups from interacting with silanols and can result in shorter retention and sharper peaks on amide phases. Another interesting feature of amide phases is that methanol and other alcohols become much stronger solvents when H-bonding between phase and solute occurs. Except for the special situations listed above, an RP-Amide phase often performs similar to C18 due to the long alkyl chain extending away from the surface.

The Phenyl phase has unique selectivity arising from solute interaction with the aromatic ring and its delocalized electrons. It is complementary (orthogonal) to both C18 and RP-Amide phases because of this unique aromaticity. An unsubstituted phenyl ring is a π-donor or Lewis base, which interacts strongly with π-acceptors and any electron-deficient Lewis acid. Phenyl phases also tend to exhibit good shape selectivity, which may originate from solute multipoint interaction with the planar ring system. More retention and selectivity will often be observed for solutes with aromatic electron-withdrawing groups (fluorine, nitro, etc.) or with a delocalized heterocyclic ring system such as the benzodiazepine compounds.

UHPLC Results with Ascentis Express Phenyl-Hexyl

Low-pressure drop with high efficiency and a flat van Deemter curve have been confirmed for Phenyl-Hexyl, as shown in Figure 1. In general, more than twice the column efficiency of 5 µm particles can be expected for all Ascentis Express Fused-Core columns at pressures that are easily managed with all HPLC instruments. Note that 20,000 plates have been achieved for a 10 cm x 3 mm I.D. column operating at optimum flow. A Jasco X-LC HPLC instrument was used for the study. Figure 2 illustrates the benzodiazepine chemical structures used in this study. As shown in Figure 3, the selectivity of Ascentis Express Phenyl-Hexyl is very similar to that of other commercial Phenyl columns, so methods can be readily transferred between columns.
The difference in efficiency and pressure drop for the two porous 3 µm columns can be explained by different particle size distributions.

Figures 4-5 show comparisons of five benzodiazepines separated on the four Ascentis Express RP phases in water:acetonitrile and water:methanol mobile phases. No additives were employed in order to observe the interaction between these polar solutes and the different phases; however, a dilute buffer will normally be used for development of a validated method. The addition of 10-20 mM buffer at neutral pH typically has little or no effect upon the separation with these highly deactivated column phases.

Figure 1. Flow Performance of Ascentis Express Phenyl-Hexyl Column with Neutral Probes

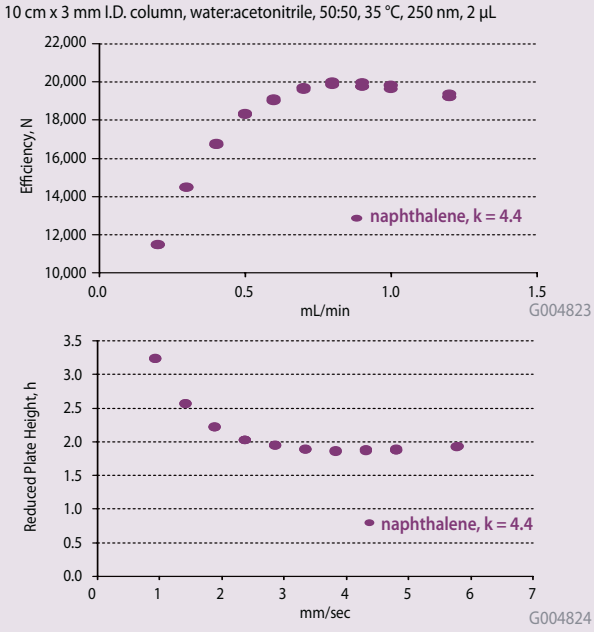


Figure 2. Benzodiazepine Structures

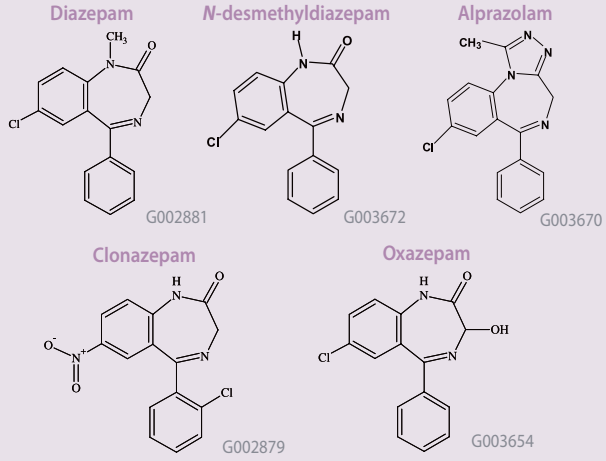
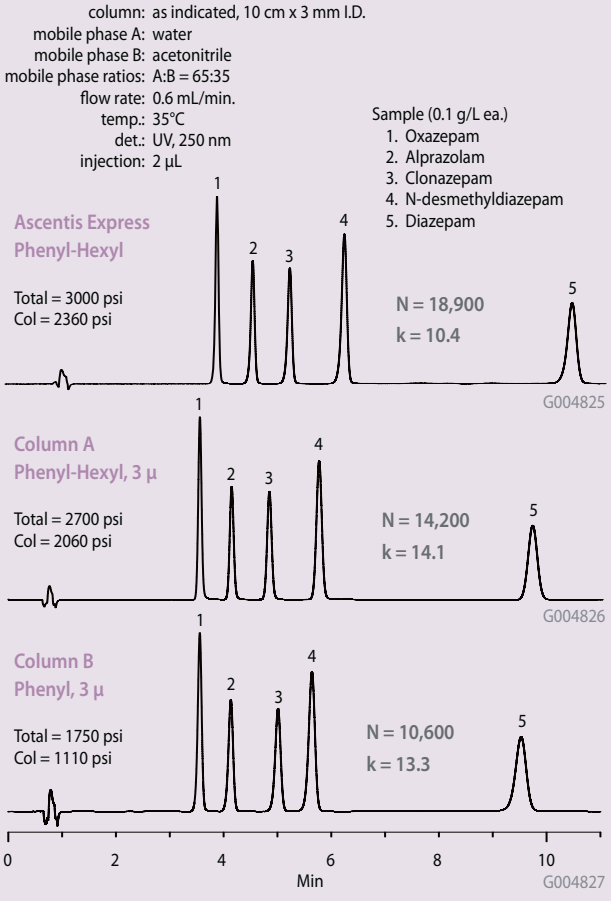


Figure 3. Comparison of Phenyl Column Selectivity for Benzodiazepines



Note that overall retention in acetonitrile is similar for the four bonded phases, but elution order is different. The two less polar compounds, diazepam and desmethyldiazepam, elute late and show the same order for all columns due to predominance of hydrophobic interactions. The more polar solutes, however, elute earlier and interact differently with Phenyl-Hexyl and the other phases.

Reference

1. Kazakevitch, Y. V., et al. J. Chromatogr., A. 2005, 1082, 158–165.

With this test sample and operating conditions, three of the four Ascentis Express RP columns provide good resolution with different selectivity, however, Phenyl-Hexyl shows the best retention and selectivity.

A switch to water:methanol in Figure 5 shows a dramatic change in retention for Ascentis Express Phenyl-Hexyl. In water:methanol mobile phase, the phenyl group interacts much more strongly than the other phases with the solute heterocyclic ring system, presumably by a π-π mechanism. Kazakevitch (1) has published evidence that methanol forms only monolayer coverage on aromatic phases (and also thinly solvates other phases), which allows the aromatic selectivity to shine through more strongly. Elution order for the polar compounds also changes from that of water:acetonitrile conditions. For this test sample, Ascentis Express Phenyl-Hexyl selectivity is clearly superior in water:methanol to the other phases.

Figure 4. Benzodiazepines in 35% Acetonitrile Mobile Phase with no Additive

column: Ascentis Express, as indicated, 10 cm x 3 mm I.D., 2.7 µm particles
mobile phase A: water
mobile phase B: acetonitrile
mobile phase ratios: A:B = 65:35
flow rate: 0.6 mL/min.
pressure: 3000 psi total, 2360 psi column
temp.: 35 °C
det.: UV, 250 nm
injection: 2 µL

Sample (0.1 g/L ea.)
1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-desmethyldiazepam
5. Diazepam

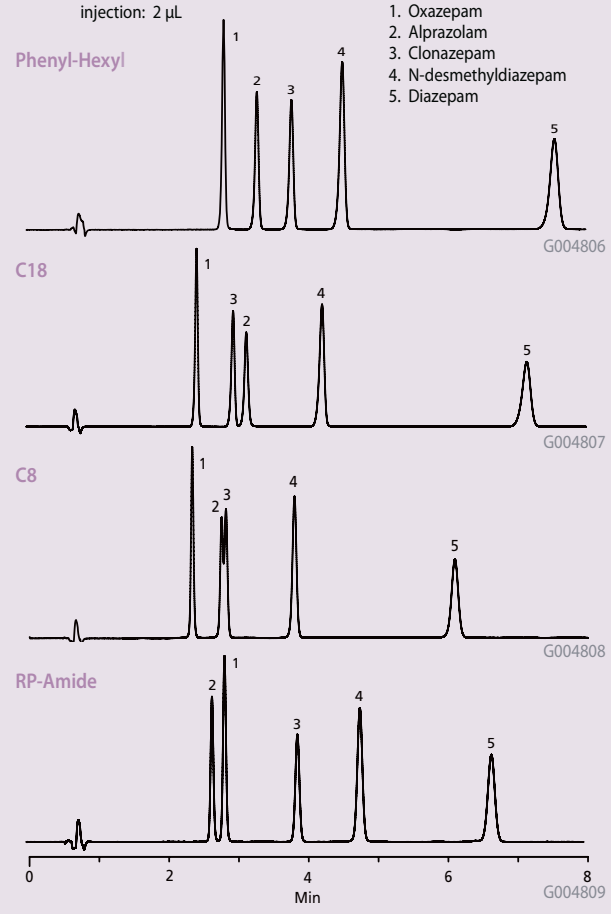
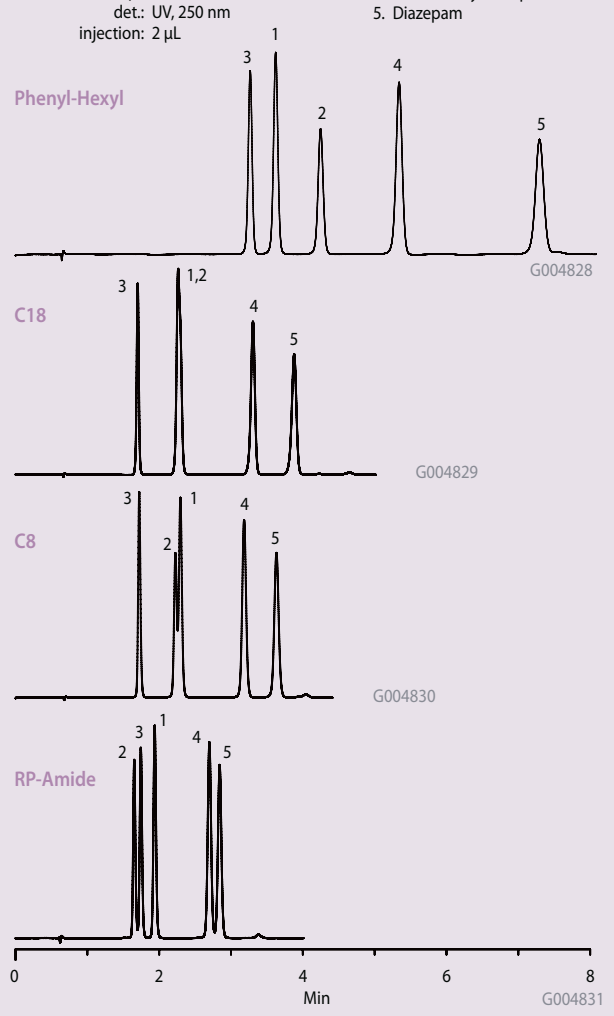


Figure 5. Benzodiazepines in 60% Methanol Mobile Phase with no Additive

column: Ascentis Express, as indicated10 cm x 3 mm I.D.
mobile phase A: water
mobile phase B: methanol
mobile phase ratios: A:B = 40:60
flow rate: 0.6 mL/min
pressure: 4200 psi total; 3420 psi column
temp.: 35 °C
det.: UV, 250 nm
injection: 2 µL

Sample (0.1 g/L ea.)
1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-desmethyldiazepam
5. Diazepam



Conclusion

A new Phenyl-Hexyl phase has been paired with Fused-Core particles to complete the primary Ascentis Express column family. High performance with lower pressure drop than other UHPLC columns has been confirmed for all Fused-Core particle phases. Ascentis Express Phenyl-Hexyl correlates well to other Phenyl phases for easy method development or method transfer. Selectivity for benzodiazepine compounds has been compared to the other Ascentis Express RP phases in water:acetonitrile and water:methanol. The extra retention possible with Phenyl phases in water:methanol has been demonstrated for these heterocyclic aromatic compounds. The potential for faster, more sensitive assays using Ascentis Express Phenyl-Hexyl and all Ascentis Express phases has been shown.

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 µm 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 µm solid core and a 0.5 µm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

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* and download.

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 µm columns on many applications since Ascentis Express provides the benefits of sub-2 µm 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 µm 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

Key Technical Literature (available by request through technical service)

Code	Publication Title
T409113	Method Optimization using Alternative Selectivities in Fused-Core Particle HPLC Column Technology
T409110	Increased Bioanalytical Throughput Using Fused-Core HPLC with Selective Phospholipid Depletion
T409041	Extended Performance of LC Instruments with Fused-Core Particle Columns
T408141	Utilizing Fused-Core Technology for LC-MS Applications
T408088	Transfer and Speedup of Methods to Fused-Core Particle Columns
T408087	Optimization of HPLC Instrumentation for High Efficiency Separations
T408077	Achieving Sub-2 µm LC-MS Performance at Moderate Pressures using Fused-Core Particle Technology
T408035	High-Resolution HPLC Through Coupling Columns
T408034	High Resolution HPLC Performance Under Both Isocratic and Gradient Conditions
T408033	Achieving Optimum UHPLC Column Performance by Measuring and Reducing Overall System Dispersion
T408031	Achieving Ultra-HPLC Column Performance with Older Instruments
T407127	Achieving Efficient Bioanalytical Separations at Moderate Pressures using Fused-Core Particle Technology
T407078	Optimizing HPLC Particles and Column Dimensions for Fast, Efficient Separations
T407102	Guidelines for Optimizing Performance with Ascentis Express HPLC Columns
T408143	Guide to Dispersion Measurement

Ordering Information

Analytical Ascentis Express Columns

ID (mm)	Length (cm)	C18	C8	Phenyl-Hexyl	HILIC	RP-Amide	Peptide ES-C18
2.1	2	**53799-U**	—	—	—	—	—
2.1	3	**53802-U**	**53839-U**	**53332-U**	**53933-U**	**53910-U**	**53299-U**
2.1	5	**53822-U**	**53831-U**	**53334-U**	**53934-U**	**53911-U**	**53301-U**
2.1	7.5	**53804-U**	**53843-U**	**53335-U**	**53938-U**	**53912-U**	**53304-U**
2.1	10	**53823-U**	**53832-U**	**53336-U**	**53939-U**	**53913-U**	**53306-U**
2.1	15	**53825-U**	**53834-U**	**53338-U**	**53946-U**	**53914-U**	**53307-U**
3.0	3	**53805-U**	**53844-U**	**53341-U**	**53964-U**	**53915-U**	**53308-U**
3.0	5	**53811-U**	**53848-U**	**53342-U**	**53967-U**	**53916-U**	**53311-U**
3.0	7.5	**53812-U**	**53849-U**	**53343-U**	**53969-U**	**53917-U**	**53312-U**
3.0	10	**53814-U**	**53852-U**	**53345-U**	**53970-U**	**53918-U**	**53313-U**
3.0	15	**53816-U**	**53853-U**	**53346-U**	**53972-U**	**53919-U**	**53314-U**
4.6	3	**53818-U**	**53857-U**	**53347-U**	**53974-U**	**53921-U**	**53316-U**
4.6	5	**53826-U**	**53836-U**	**53348-U**	**53975-U**	**53922-U**	**53318-U**
4.6	7.5	**53819-U**	**53858-U**	**53351-U**	**53977-U**	**53923-U**	**53323-U**
4.6	10	**53827-U**	**53837-U**	**53352-U**	**53979-U**	**53929-U**	**53324-U**
4.6	15	**53829-U**	**53838-U**	**53353-U**	**53981-U**	**53931-U**	**53328-U**

Capillary Ascentis Express Columns

Length (cm)	I.D. (µm)	Cat. No.
C18		
5	75	**53982-U**
15	75	**54219-U**
5	100	**53985-U**
15	100	**54256-U**
5	200	**53989-U**
15	200	**54261-U**
5	300	**53992-U**
15	300	**54271-U**
5	500	**53998-U**
15	500	**54273-U**
C8		
5	75	**53983-U**
15	75	**54229-U**
5	100	**53987-U**
15	100	**54260-U**
5	200	**53991-U**
15	200	**54262-U**
5	300	**53997-U**
15	300	**54272-U**
5	500	**53999-U**
15	500	**54275-U**

Ascentis Express Guard Columns


E001103

Universal Guard Holder

Description	Cat. No.
Holder w/EXP Titanium Hybrid Ferrule -- cartridge not included with holder	**53500-U**

Ascentis Express Guard Cartridges

Description	I.D. (mm)	Pkg. Size	Cat. No.
C18	2.1	3	**53501-U**
C18	3.0	3	**53504-U**
C18	4.6	3	**53508-U**
C8	2.1	3	**53509-U**
C8	3.0	3	**53511-U**
C8	4.6	3	**53512-U**
RP-Amide	2.1	3	**53514-U**
RP-Amide	3.0	3	**53516-U**
RP-Amide	4.6	3	**53519-U**
HILIC	2.1	3	**53520-U**
HILIC	3.0	3	**53521-U**
HILIC	4.6	3	**53523-U**
Phenyl-Hexyl	2.1	3	**53524-U**
Phenyl-Hexyl	3.0	3	**53526-U**
Phenyl-Hexyl	4.6	3	**53531-U**
Peptide ES-C18	2.1	3	**53536-U**
Peptide ES-C18	3.0	3	**53537-U**
Peptide ES-C18	4.6	3	**53542-U**

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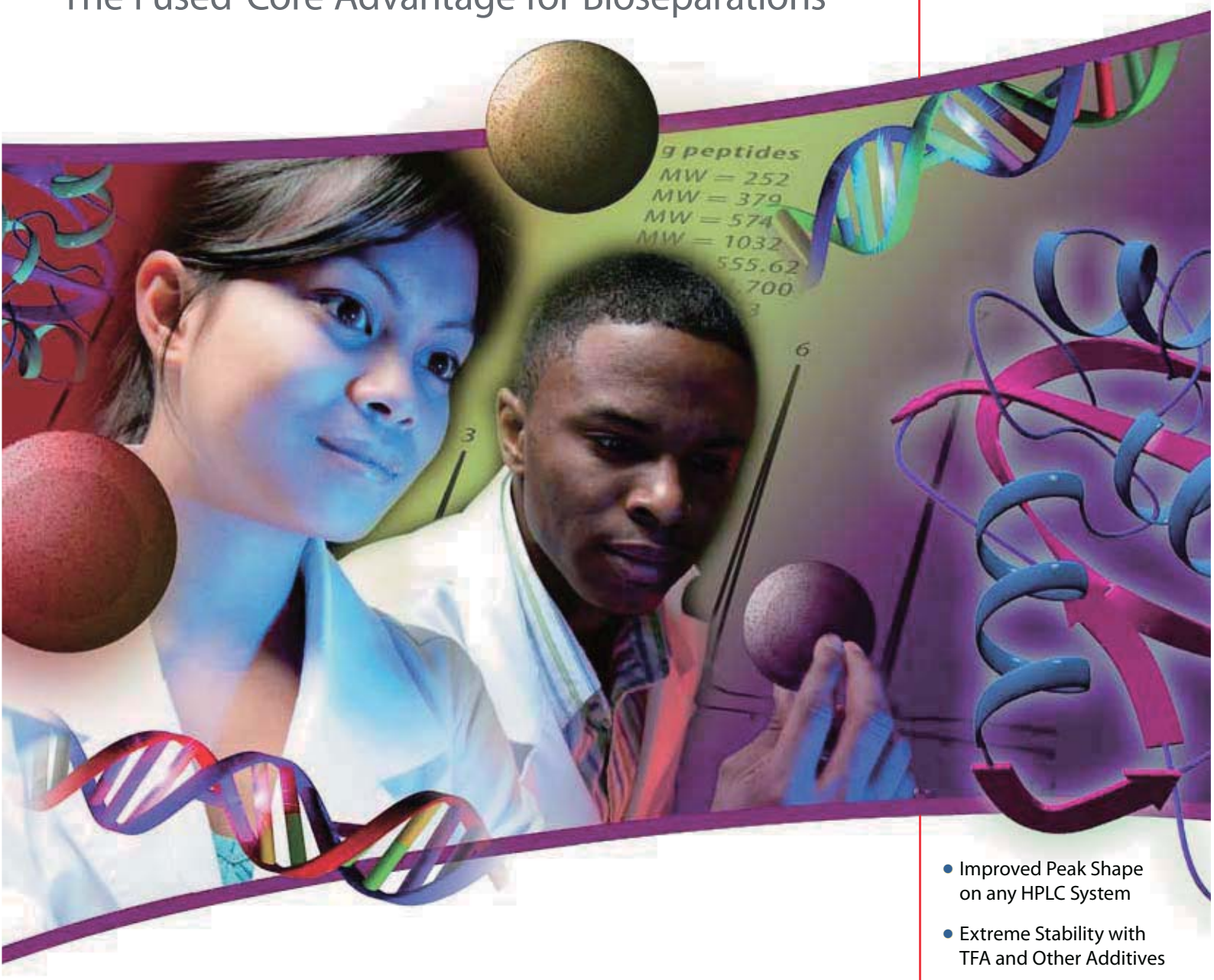
The Fused-Core Advantage for Bioseparations

SUPELCO Analytical



- Improved Peak Shape on any HPLC System
- Extreme Stability with TFA and Other Additives
- Rugged HPLC Column Design

A Breakthrough in Bioseparations Performance

Ascentis Express Peptide ES-C18

Ascentis® Express Peptide ES-C18 is a high-speed, high-performance liquid chromatography column based on a new 160Å Fused-Core™ particle design. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency for high MW solutes (up to 20 kDa) due to the shallow diffusion paths in the 0.5-micron thick porous shell and the small overall particle size of 2.7-microns. The non-end-capped, sterically protected C18 bonded phase of Ascentis Express Peptide ES-C18 provides a stable, reversed phase packing with a pore structure and pore size that is optimized for reversed-phase HPLC separations of peptides and polypeptides, using typical acidic mobile phases favored for protein structure-function and proteomic applications.

Table 1. Specifications for Ascentis Express Peptide ES-C18

Silica	High Purity Type B
Phase	Sterically protected C18
pH range	1 – 9
Temperature	100 °C
Average pore diameter	160 Å
Surface area, nitrogen	80 sq.m/g
Pore volume	0.30 mL/g
Particle density	1.3 g/cc

Ordering Information

Ascentis Express Peptide ES-C18 Columns

I.D. (mm)	Length (cm)				
	3	5	7.5	10	15
2.1	53299-U	53301-U	53304-U	53306-U	53307-U
3.0	53308-U	53311-U	53312-U	53313-U	53314-U
4.6	53316-U	53318-U	53323-U	53324-U	53328-U

Applications

The Ascentis Express Peptide ES-C18 columns are best utilized with mobile phases that are mixtures of acetonitrile and water or methanol and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Using elevated temperatures (e.g., 40 – 100 °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. Gradient-elution techniques using 5 -10% organic component as the initial mobile phase and increasing to 100% organic component as the final mobile phase often can affect separations of complex sample mixtures in minimal time.

Ionizable compounds, such as acids and bases, are generally best separated with mobile phases buffered at pH of 2 to 3. The use of 10-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds.

Ascentis Express Peptide ES-C18 columns utilize 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 combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.1% concentration. Most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion pairing properties. Additional opportunities for UV detection at low pH operation is with mineral acids such as phosphoric acid (1-20 mM). For MS detection 0.1% formic acid is most commonly employed (sometimes acetic acid), but significant benefit to peak shape can be realized with 0.1% formic acid adjusted to pH 3.5 (with ammonium hydroxide, for instance), especially with basic peptides. This is likely due to greater availability of formate anion for ion pairing.

Pharmaceutical Peptides

Many peptides have been investigated as therapeutic pharmaceutical drugs and are active vasodilators, vasoconstrictors, hormones, and neuropeptides. Using reversed-phase HPLC, it is possible to solve the tasks of identification, purity monitoring, and quantitative analysis in many cases, including those where the application of other methods is impossible.

Synthetic Peptides

The difficulty with synthetic peptides involves the production of many "deletion variants". A deletion may occur at any point in the peptides synthesis and so several versions of the peptide are produced which are absent one, two or three amino acids from the desired product. This makes for an interesting chromatographic problem because the resultant peptide mix contains peptides that are very similar in structure.

Peptide Mapping

Protein analysis and characterization has become more crucial due to many biopharmaceutical advances. Peptide mapping via LC-MS is one such technique that is commonly used today. A typical procedure involves the preparation of a tryptic digest from the protein, with subsequent characterization using reversed-phase, gradient HPLC separation followed by mass spectral analysis and database search.

Difficult Separations on Ascentis Express Peptide ES-C18

Figure 1. Basic Peptides

column: Ascentis Express Peptide ES-C18, 10 cm x 2.1 mm I.D. (53306-U)
mobile phase A: 0.1% additive in water
mobile phase B: 25:75, (0.4 % additive):acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

Peptide probes (listed in order of elution):

1.	ac-GGGLGGAGGLKG	monoisotopic mass: 941.5
2.	ac-KYGLGGAGGLKG	monoisotopic mass: 1118.6
3.	ac-GGAVKALKGLKG	monoisotopic mass: 1139.7
4.	ac-KYALKALKGLKG	monoisotopic mass: 1330.8

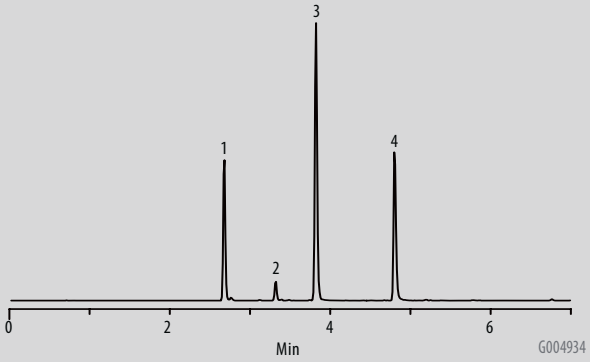


Figure 2. Peptide Test Mix

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 90:10, (0.1 % TFA):acetonitrile
mobile phase B: 25:75, (0.1% TFA):acetonitrile
gradient: initial = 0% B to 50% B in 15 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 5 µL

The test mix employed contains the following peptides

1.	Gly-Tyr	MW = 252
2.	Val-Tyr-Val	MW = 379
3.	Met Enkephalin	MW = 574
4.	Angiotensin II	MW = 1032
5.	Leu-Enkephalin	MW = 555
6.	Ribonuclease	MW = 13,700
7.	Bovine Insulin	MW = 5733

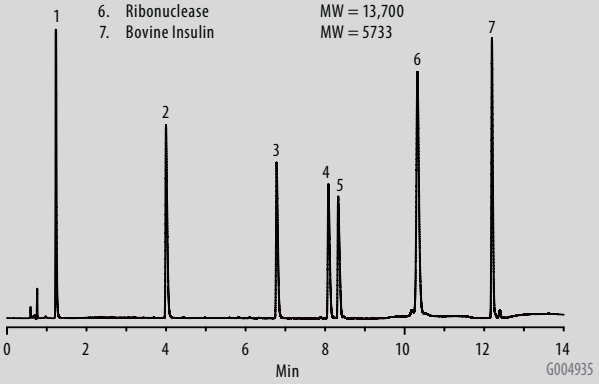


Figure 3. Carbonic Anhydrase Tryptic Digest

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 0.1 % TFA in water
mobile phase B: 40:60, (0.1% TFA):acetonitrile
gradient: initial = 3% B to 100% B in 53 min.
flow rate: 1.0 mL/min
temp.: 30 °C
det.: UV at 215 nm
injection: 20 µL

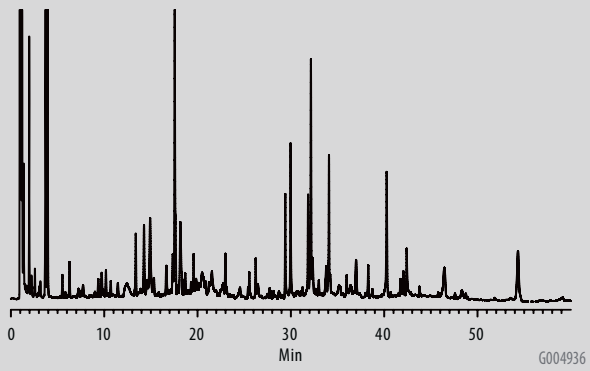
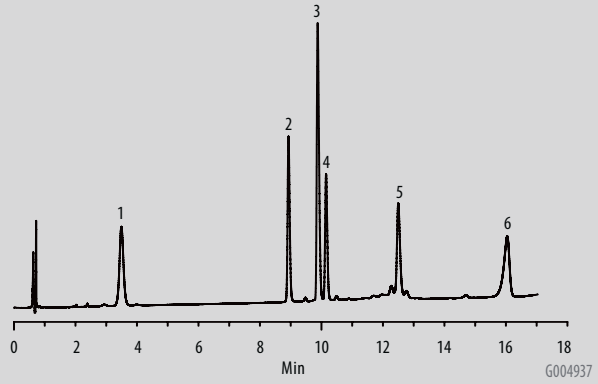


Figure 4. Small Proteins

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 90:10, (0.1% TFA):acetonitrile
mobile phase B: 25:75, (0.1% TFA):acetonitrile
gradient: initial = 25% B to 40% B in 15 min.; then to 60% B at 20 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 4 µL

1.	Ribonuclease	MW = 13,700
2.	Porcine Insulin	MW = 5,780
3.	Bovine Insulin	MW = 5,730
4.	Human Insulin	MW = 5,800
5.	Cytochrom C	MW = 12,327
6.	Lysozyme	MW = 14,700



Competitor Comparison

Basic Peptides

Columns run under equivalent conditions of gradient slope (Δ % MeCN per column volume).

column: C18, 10 cm x 2.1 mm I.D.
mobile phase A: 0.1% additive in water
mobile phase B: 25:75, (0.4 % additive):acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

Peptide probes (listed in order of elution):

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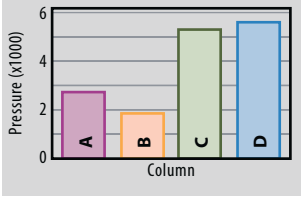
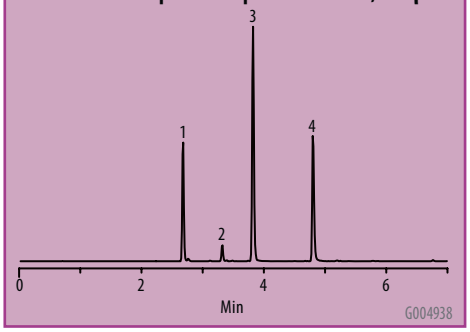
3. ac-GGAVKALKGLKG Monoisotopic mass: 1139.7
4. ac-KYALKALKGLKG Monoisotopic mass: 1330.8

Peptide probes increase in basicity and hydrophobicity.

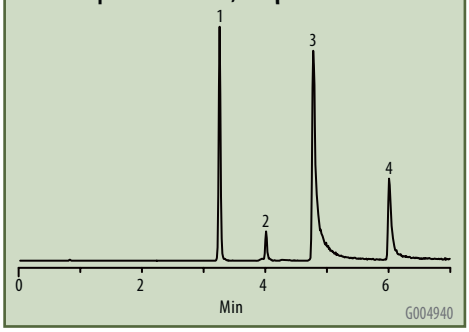
Column Backpressure



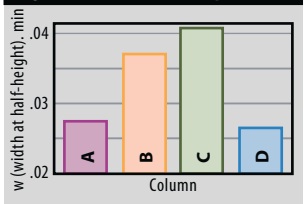
A. Ascentis Express Peptide ES-C18, 2.7µm



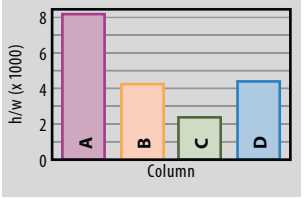
C. Competitor V C18, 1.5µm



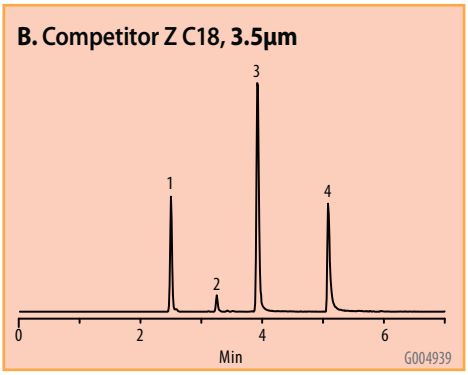
Avg Peak Width of Basic Peptides



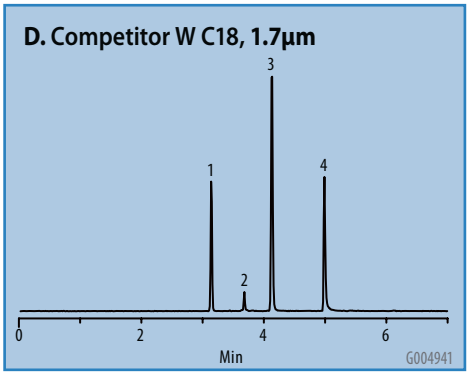
Avg h/w of Basic Peptides



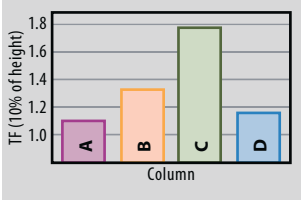
B. Competitor Z C18, 3.5µm



D. Competitor W C18, 1.7µm



Avg Tailing Factor of Basic Peptides



Experimental Setup: Columns and Elution Gradients

Column	µm	Pore (Å)	ID (mm)	L (cm)	CV (mL)*	start %B	Grad slope [Δ % MeCN / CV]
A. Ascentis Express Peptide ES-C18	2.7	160	2.1	10	0.190	15	2
B. Competitor Z C18	3.5	300	2.1	10	0.216	15	2
C. Competitor V C18	1.5	120	2.0	10	0.205	15	2
D. Competitor W C18	1.7	130	2.1	10	0.186	15	2

* CV determined as follows: t_0 = r.t. of unretained component. d_0 = r.t. of unretained component with ZDV union in place of column. **CV** = (t_0 - d_0) x mL/min

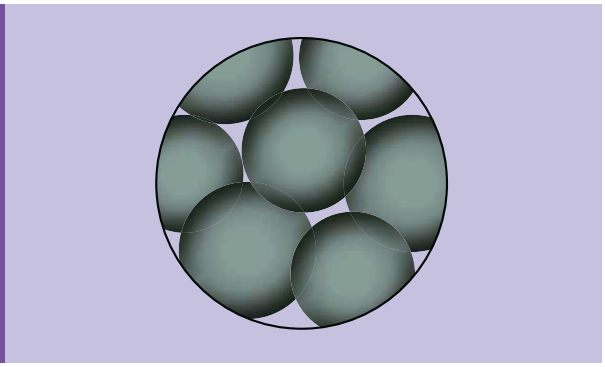
Some Quantitative Comparisons

Column	Peak 1			Peak 2			Peak 3			Peak 4		
	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$
A. Ascentis Express Peptide ES-C18, 2.7 µm	0.0258	9725	1.20	0.0224	1520	1.00	0.0344	11321	1.00	0.0267	9706	1.30
B. Competitor Z C18, 3.5 µm	0.0344	4082	1.14	0.0344	572	1.25	0.0387	8267	1.08	0.0387	3871	1.74
C. Competitor V C18, 1.5 µm	0.0344	4418	1.03	0.0344	548	1.06	0.0516	3044	2.69	0.0430	1281	2.38
D. Competitor W C18, 1.7 µm	0.0258	7568	1.10	0.0241	1203	1.20	0.0241	1942	1.00	0.0301	6684	1.30

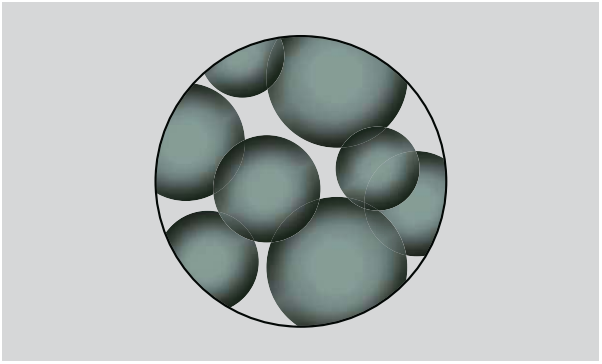
The Fused-Core Advantage

Narrow Particle Size Distribution

Fused-Core Particles

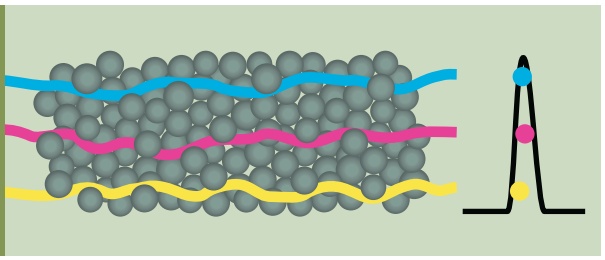


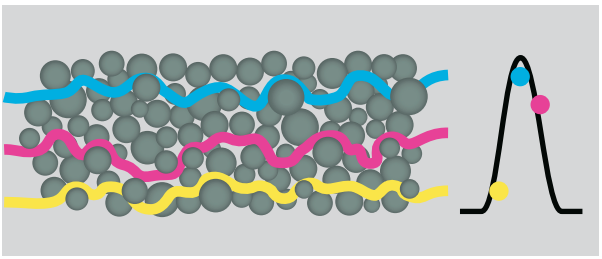
Traditional Porous Particles



The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured in a way to yield extremely narrow particle size distributions.

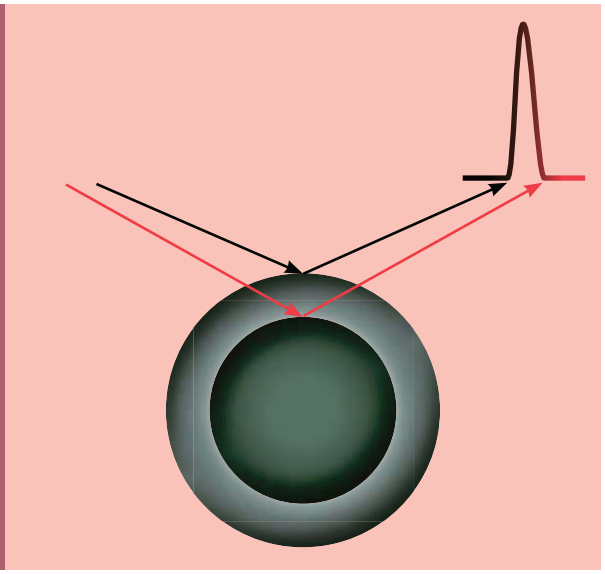
More Consistent Bed

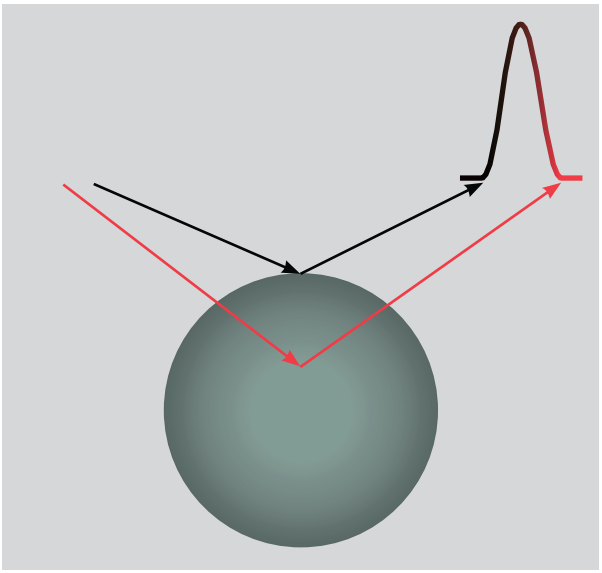




The "A" term in the van Deemter equation accounts for the effects of inhomogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistent packed bed and a consistent path length, **minimizing analyte diffusion** through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path





The short diffusion path of the Fused-Core particle **yields sharper peaks** than traditional 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 peak broadening.

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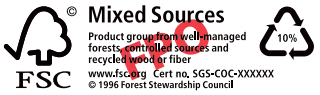

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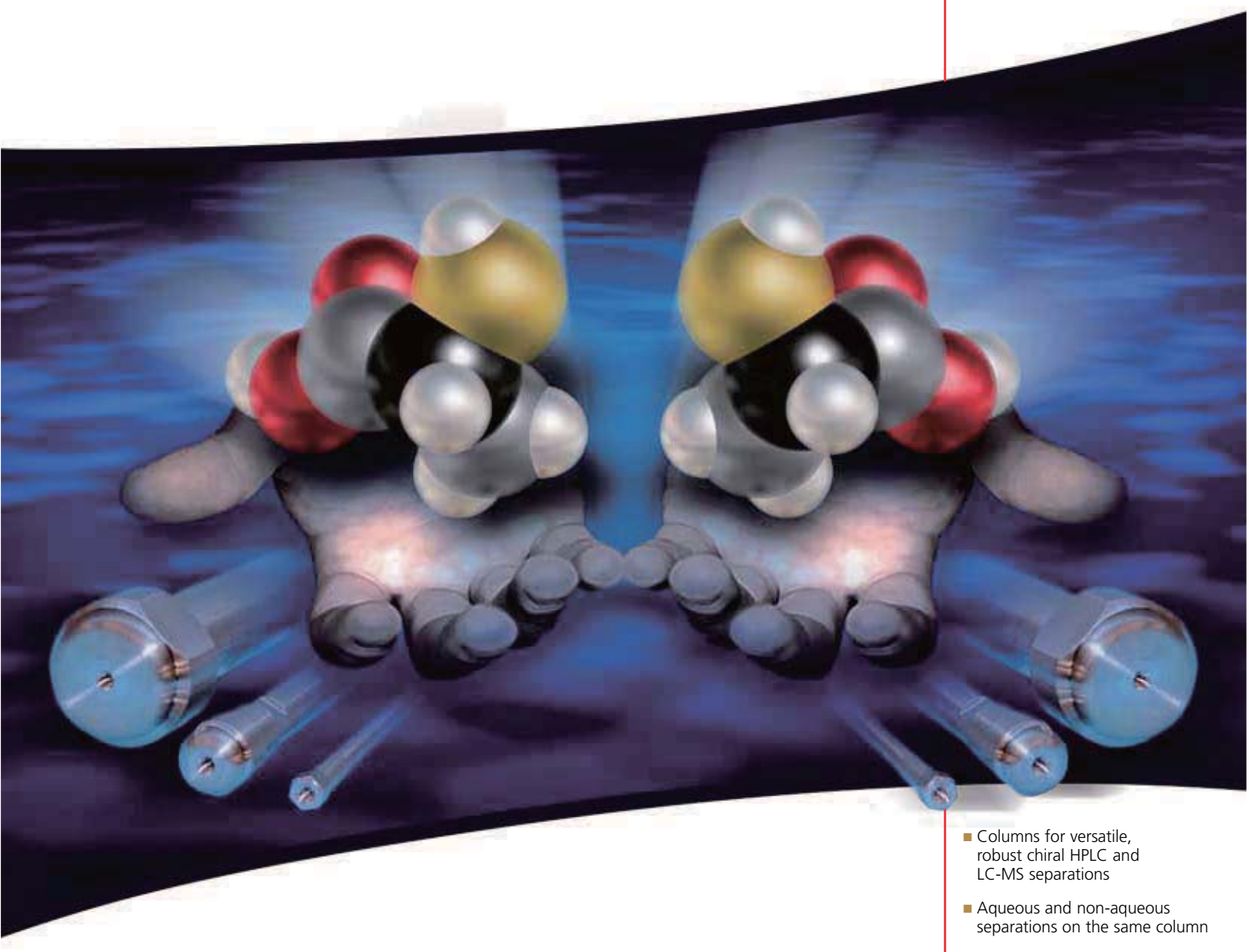


- Columns for versatile, robust chiral HPLC and LC-MS separations
- Aqueous and non-aqueous separations on the same column
- No solvent or additive memory effects
- Wide applicability, especially suited to polar and ionizable compounds
- Predictable scale-up from analytical to prep

CHIROBIOTIC

Versatile Chiral HPLC and LC-MS Separations of Polar, Ionizable and Neutral Compounds

CHIROBIOTIC™ CSPs (chiral stationary phases) interact with polar, ionizable and neutral analytes via multiple molecular interactions. This versatility means that the same CHIROBIOTIC column can be successfully used in a variety of mobile phases, a significant benefit over CSPs that operate only in a single mode, normal or reversed-phase, for example, and must be dedicated to those mobile phase systems. However, the most interesting feature of CHIROBIOTIC CSPs is the **presence of ionic interactions**, which allows them to be used in polar ionic and reversed-phase modes for sensitive LC-MS operation.

Key application areas

- **Drug Discovery** – High enantioselectivity, fast screening protocols, scalability to prep, reproducibility for reliable methods, polar and non-polar analytes
- **Organic Synthesis** – Compatible with all HPLC solvents to optimize sample solubility, fully scalable to prep
- **Bioanalytical, Drug Metabolism** – High throughput, MS-compatibility, aqueous samples, short run times, rugged columns
- **Amino Acid and Peptide Analysis** – Resolves underivatized natural and synthetic chiral amino acids and peptides, different selectivity and higher preparative capacity than C18 for achiral amino acids

What is the CHIROBIOTIC family?

Developed originally by Advanced Separations Technologies (Astec), the CHIROBIOTIC family comprises highly enantioselective CSPs based on macrocyclic glycopeptides that have been bonded through multiple covalent linkages to high purity silica particles. CHIROBIOTIC CSPs offer flexibility in choice of mobile phase conditions, both aqueous and non-aqueous, and are ideal for analytical and preparative separations of neutral, polar and ionic compounds.

How do CHIROBIOTIC CSPs separate enantiomers?

CHIROBIOTIC CSPs offer six different types of molecular interactions: ionic, H-bond, π-π, dipole, hydrophobic and steric. They also possess multiple inclusion sites that influence selectivity based on the molecular shape of the analyte. The optimization of enantiomer resolution is achieved by changing the mobile phase to leverage the types and relative strengths of the various interactions.

What makes CHIROBIOTIC CSPs unique?

The bonded macrocyclic glycopeptide itself (Figure 1), in terms of its morphology, molecular composition and multiple covalent linkages to the silica surface, is what makes CHIROBIOTIC CSPs unique and gives them significant and valuable benefits over other CSPs. The truly differentiating feature of CHIROBIOTIC CSPs is the presence of ionic interactions. These interactions are unique to CHIROBIOTIC CSPs and are responsible in large part for their desirable retention characteristics toward polar and ionizable analytes in aqueous and non-aqueous solvents.

How do the CHIROBIOTIC CSPs differ?

The various CHIROBIOTIC phases share the benefits of robustness, flexibility in mobile phase options, ionic interactions, compatibility with polar compounds and LC-MS and preparative scalability. However, CHIROBIOTIC CSPs differ in selectivity, primarily because of their differing number and types of interaction sites, and the number, type and accessibility of ionic sites in the bonded macrocyclic glycopeptide.

The CHIROBIOTIC CSP Family

CHIROBIOTIC CSPs are based on 5, 10 or 16 µm, high purity, porous silica gel. They differ in the nature of the bonded macrocyclic glycopeptide and resulting enantioselectivity.

- **CHIROBIOTIC V and V2*** – Vancomycin
- **CHIROBIOTIC T and T2*** – Teicoplanin
- **CHIROBIOTIC R** – Ristocetin
- **CHIROBIOTIC TAG** – Teicoplanin Aglycone

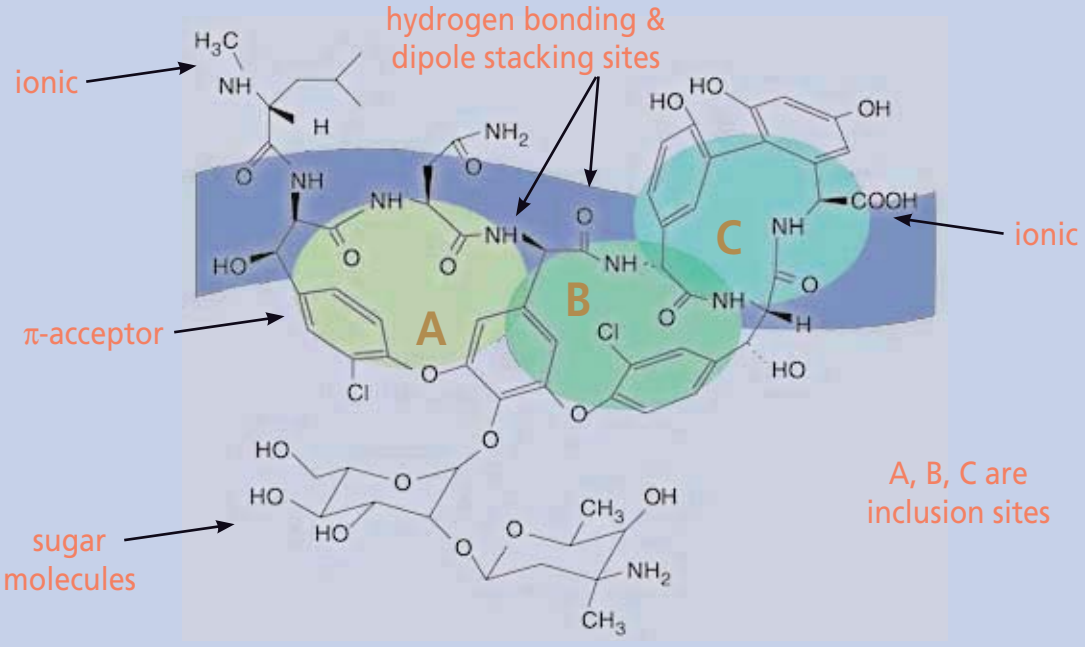
*CHIROBIOTIC V and T differ from V2 and T2, respectively, in their bonding chemistry that gives them different selectivity and preparative capacity for certain classes of analytes.

Key features of CHIROBIOTIC CSPs:

- **Aqueous and non-aqueous separations on the same column** – CHIROBIOTIC CSPs have H-bond, ionic, dispersive, π-π, dipole stacking, steric and inclusion mechanisms, usually multiple types of interactions per analyte.
- **Wide applicability** – Applications cover a very broad range of compound classes, with the different CHIROBIOTIC CSPs showing complementary selectivity.
- **LC-MS compatibility** – The wide choice of mobile phases makes CHIROBIOTIC CSPs ideal for LC-MS, where analyte ionization and detection sensitivity are of critical concern.
- **No solvent or additive memory effect** – The same CHIROBIOTIC column can be used alternately in polar, reversed-phase and normal phase solvents without damage, unlike cellulosic and amylosic phases that require dedicated operation.
- **Robust columns with long lifetimes** – Each macrocyclic glycopeptide molecule is linked to the silica surface via four or five covalent bonds for exceptional stability and long column life. They are designed to withstand high pressure and flow rates, as well as rapid changes in mobile phase conditions.
- **Solvent choices maximize sample solubility** – CHIROBIOTIC CSPs operate in highly aqueous and non-aqueous polar mobile phases for polar compound solubility. They also operate in normal phase mobile phases to maximize solubility of non-polar compounds. CHIROBIOTIC CSPs are compatible with all organic solvents.
- **Excellent preparative scalability and capacity** – From narrowbore to prep, separations on CHIROBIOTIC are fully scalable, even with polar analytes. By relying on primarily aqueous eluents, the use and disposal of toxic organic solvents are eliminated. Additionally, preparative methods in the non-aqueous polar ionic mode are just as easy to process as normal phase solvents.
- **Fast kinetics for speed and efficiency** – The kinetics of the molecular interactions between the analyte and the CHIROBIOTIC CSP are fast, providing efficient separations and relatively short retention times.
- **Orthogonal selectivity to other CSPs** – The six CHIROBIOTIC CSPs are different from each other, and from other types of CSPs to offer choices in enantioselectivity, like reversal of elution order.

Figure 1. Proposed Structure of Vancomycin-based CHIROBIOTIC V and V2

CHIROBIOTIC chiral HPLC stationary phases are macrocyclic glycopeptides attached to the surface of high purity, porous silica by four or more covalent bonds. Vancomycin is shown here, but all four CHIROBIOTIC CSPs possess multiple functional groups that can undergo different types of interactions. The CHIROBIOTIC surface is essentially ionic, allowing the use of polar ionic and aqueous mobile phases. Additionally, the glycopeptide structure has several inclusion sites that provide selectivity based on the molecular structure of the analyte.



Incorporating CHIROBIOTIC CSPs into Your Chiral Column Screening Protocol

We recommend that you incorporate CHIROBIOTIC into your routine screening protocol. Experience has shown that one or more of the CHIROBIOTIC CSPs, particularly V2, T and TAG, will perform the majority of chiral separations. Even if other CSPs give adequate resolution, a CHIROBIOTIC CSP may allow use of mobile phases that are better suited to your sample and detection method, or the CHIROBIOTIC method may be faster, more efficient or more robust. A CHIROBIOTIC method may also have advantages from a preparative standpoint in terms of solvent selection and sample capacity.

For developing a new chiral HPLC method, we have created and use routinely in our laboratories a simple and rapid chiral column screening protocol (Table 1). It is important to keep in mind that a single CHIROBIOTIC column possesses multiple types of molecular interactions that are different in each of the four distinct modes. The same column can be exposed to all of the conditions outlined in the screening protocol shown in Table 1 without any change or loss of performance. This versatility is just one advantage that CHIROBIOTIC CSPs have over other CSPs.

The four CHIROBIOTIC CSPs we recommend in the screening protocol are available in 25 cm or 10 cm column kits. Also, you can further expand the screening field by incorporating the CYCLOBOND™ bonded cyclodextrin and P-CAP™ chiral polymer CSPs into your screening protocol to accommodate other types of compounds not covered by the routine screen.

Table 1. The CHIROBIOTIC Screening Protocol

columns: CHIROBIOTIC V2, T, R and TAG
procedure: Method development follows a simple strategy that tests polar ionic, polar organic, reversed-phase and normal phase modes.

Separation Mode	Description	Types of Compound	Screening Mobile Phase	Parameters to Optimize
Polar Ionic	Polar organic solvents (CH_3OH or CH_3CN) containing small amounts of acid and base or salt	Acids, Bases, Zwitterions	(100:0.1:0.1, v:v:v) CH_3OH:Acetic Acid: Triethylamine	Change acid-base ratio, change the type of acid or base, add a volatile salt (test different ammonium salts)
Reversed-Phase	Typical RP eluents, water or buffers with CH_3OH or CH_3CN	Acids, Bases, Zwitterions, Neutrals	(30:70) CH_3CN:20mM Ammonium Acetate, pH 4.0	Change the % and type of organic modifier, adjust pH, buffer type and ionic strength
Polar Organic	Polar organic solvents without ionic additives	Neutrals	100% Ethanol	Use other polar organic solvents or blends
Normal Phase	Non-polar organic solvents with polar solvent modifiers	Neutrals	(30:70) Ethanol:Heptane	Increase % of polar modifier, change both solvents

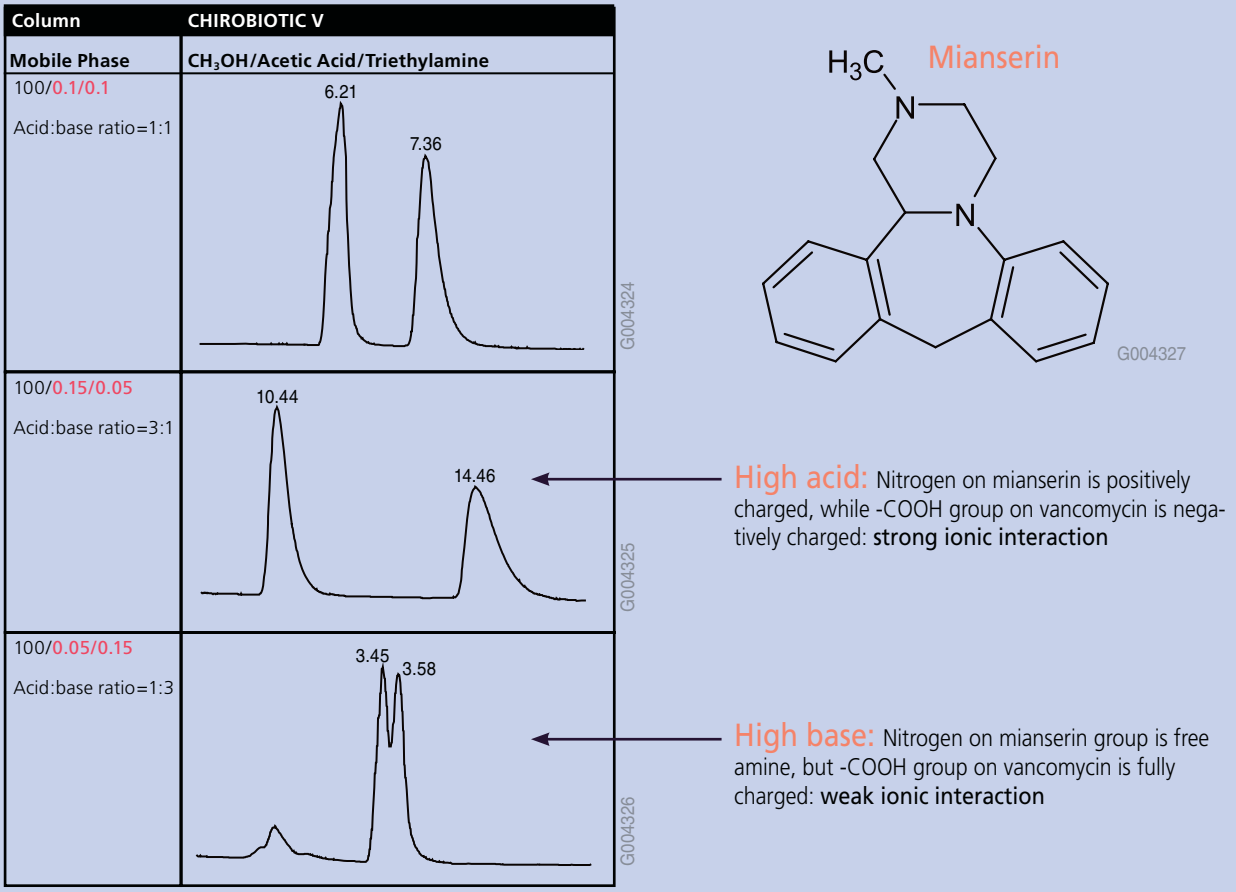
Method Optimization: Acid-Base Ratio, Temperature and Flow Rate in Polar Ionic Mode

Using CHIROBIOTIC CSPs in the polar ionic mode has the highest probability of success. Optimizing resolution usually involves changing the contribution to retention of ionic interactions between the analytes and functional groups in the macrocyclic glycopeptide structure by:

- Changing the ratio of acid to base (Figure 2)
- Adding a soluble, volatile salt (instead of the acid and base) directly to the methanol

The acid, base or salt that is ultimately selected is based on its compatibility with the detection method (e.g. LC-MS), sample solubility and whether the separation will be scaled up to preparative.

Figure 2. Demonstration of Polar Ionic Mode Mechanism: Effect of Acid:Base Ratio



CHIROBIOTIC: Ideally Suited for LC-MS of Polar, Ionizable and Neutral Compounds

Each of the various ionization sources has an optimal set of mobile phase conditions. Outside this set, ionization may be suppressed with resulting loss in sensitivity. CHIROBIOTIC phases are uniquely able to operate across all mobile phase systems. CSPs that are limited to normal phase operation, like many cellulosic and amylosic CSPs, reduce the options in detection methods.

ESI – Operate CHIROBIOTIC CSPs in reversed-phase and unique polar ionic modes.

APCI – Operate CHIROBIOTIC CSPs in polar ionic mode.

APPI – Operate CHIROBIOTIC CSPs in normal phase mode.

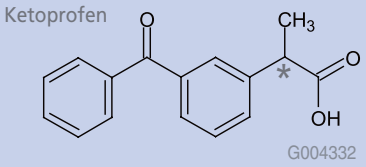
Typical polar ionic mobile phases are methanol with low concentrations (0.1 – 0.001%) of volatile salts like ammonium acetate or ammonium formate. Figures 3 and 4 show examples of CHIROBIOTIC CSPs for LC-MS in reversed-phase and polar ionic mode mobile phases, respectively.

In addition to mobile phase compatibility, the allowable high flow rates and short columns make them ideally suited to fast MS applications.

CHIROBIOTIC columns can be used in conjunction with HybridSPE™-PPT plates to enhance sensitivity by completely removing endogenous proteins and phospholipids. This approach was used to resolve the enantiomers of clenbuterol on a CHIROBIOTIC T column in Figure 4.

Figure 3. ESI-MS of Ketoprofen on CHIROBIOTIC R in Reversed-phase Mode

column: CHIROBIOTIC R, 15 cm x 2.1 mm, 5 µm particles (13019AST)
mobile phase: (30:70) CH_3OH:20 mM ammonium acetate, pH 5.6
flow rate: 0.2 mL/min.
det.: ESI(-)
temp.: 35 °C
analyte: Ketoprofen



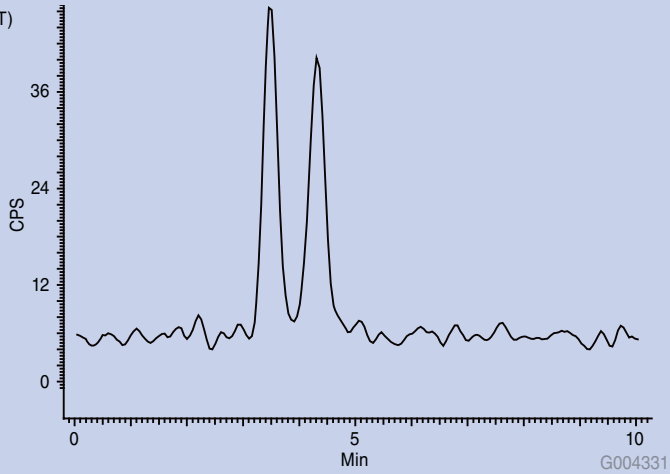
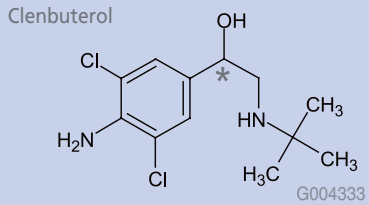
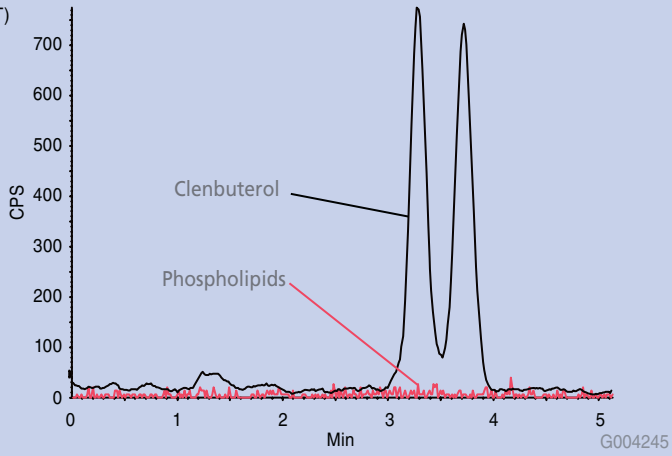


Figure 4. ESI-MS of Clenbuterol Extracted from Plasma on CHIROBIOTIC T in Polar Ionic Mode

column: CHIROBIOTIC T, 10 cm x 2.1 mm, 5 µm particles (12018AST)
mobile phase: 10 mM ammonium formate in CH_3OH
flow rate: 0.3 mL/min.
det.: ESI(+)
temp.: 30 °C
analyte: Clenbuterol in rat plasma (10 ng/mL)



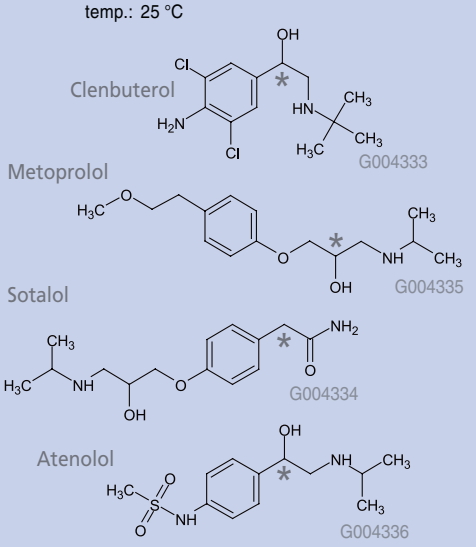


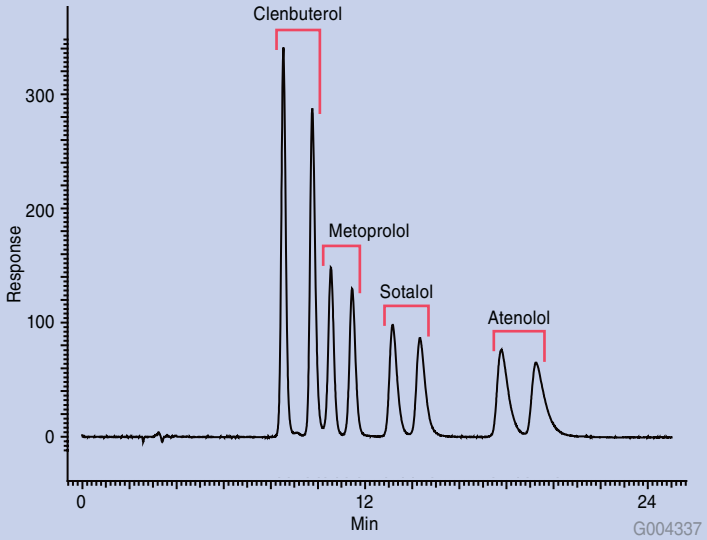
Unique Polar Ionic Mode

A valuable feature of CHIROBIOTIC CSPs, the novel and very versatile polar ionic mode is popular because its mobile phases are polar organic solvents containing volatile additives that are ideally suited for preparative and LC-MS applications. An example is shown in Figure 5. Additionally, compared to normal phase separations, the polar ionic mode has speed, efficiency and sensitivity advantages, all valuable assets for LC-MS.

Figure 5. Beta-Receptors on CHIROBIOTIC T in Polar Ionic Mode

column: CHIROBIOTIC T, 25 cm x 4.6 mm, 5 µm particles (12024AST)
mobile phase: 15 mM ammonium formate in CH_3OH
flow rate: 1 mL/min.
det.: UV at 220 nm
temp.: 25 °C





Multi-modal Interactions Permit Use in Aqueous and Non-aqueous Solvents

All CHIROBIOTIC CSPs possess multiple interaction sites on the same column. Changing the mobile phase affects the relative strength of specific types of interactions. The power and flexibility of multi-modal CHIROBIOTIC CSPs are demonstrated in Figures 6 through 10. The vancomycin-based CHIROBIOTIC CSPs were used successfully in four different modes.

Polar Ionic Mode

A valuable feature of CHIROBIOTIC, the novel and very versatile polar ionic mode mobile phase system is desirable because of its high volatility and beneficial ionization effect for LC-MS (Figure 6).

Reversed-phase Mode

Also highly suitable for LC-MS and polar analytes, reversed-phase (RP) is a mode familiar to all chromatographers. CHIROBIOTIC CSPs have RP character and can be used in a wide range of buffers and solvents (Figure 7).

Polar Organic Mode

Enantiomers of polar neutral analytes have been successfully separated on CHIROBIOTIC in the polar organic mode where the mobile phase is typically a polar organic solvent or solvent blend. Reaction mixtures, even in pyridine, can be run on CHIROBIOTIC in this mode (Figure 8).

Normal Phase Mode

Normal phase chiral separations are desirable to maintain solubility of hydrophobic compounds and when analyzing reaction mixtures in non-polar organic solvents. CHIROBIOTIC CSPs have the flexibility to operate in normal phase mode. The same column can be used with normal phase and polar/aqueous solvents and additives without memory effects (Figure 9).

Figure 6. Polar Ionic Mode

column: CHIROBIOTIC V2, 25 cm x 4.6 mm, 5 µm particles (15024AST)
mobile phase: 15 mM ammonium formate in CH_3OH
flow rate: 1 mL/min.
det.: UV at 230 nm
temp.: 25 °C
analyte: Fluoxetine

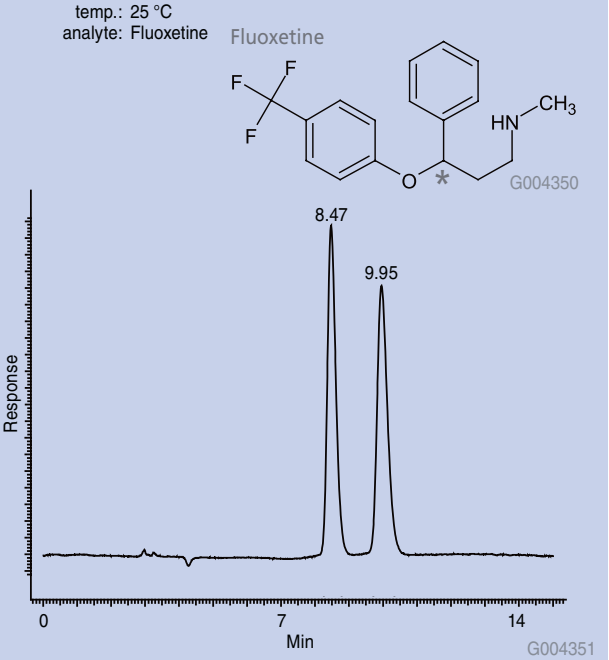


Figure 7. Reversed-phase Mode

column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
mobile phase: (30:70) CH_3CN:5 mM ammonium acetate, pH 4.1
flow rate: 1 mL/min.
det.: UV at 254 nm
temp.: 25 °C
analyte: Warfarin

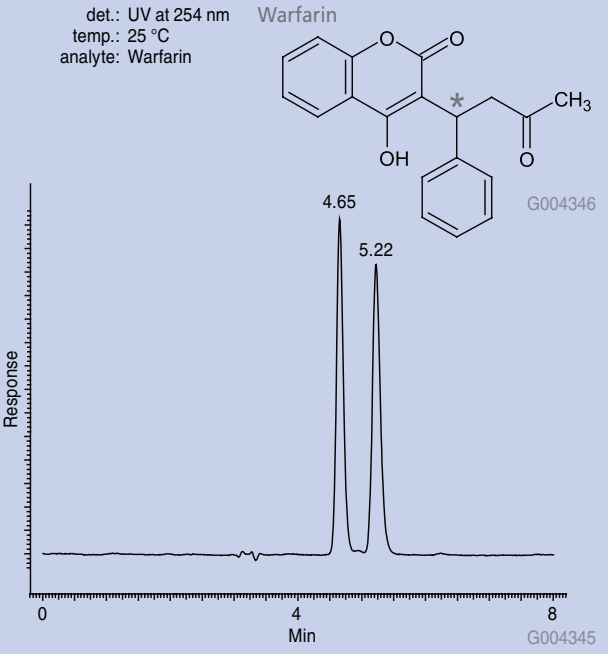


Figure 8. Polar Organic Mode

column: CHIROBIOTIC V2, 25 cm x 4.6 mm, 5 µm particles (15024AST)
mobile phase: CH_3OH
flow rate: 1 mL/min.
det.: UV at 230 nm
temp.: 25 °C
analyte: Thalidomide

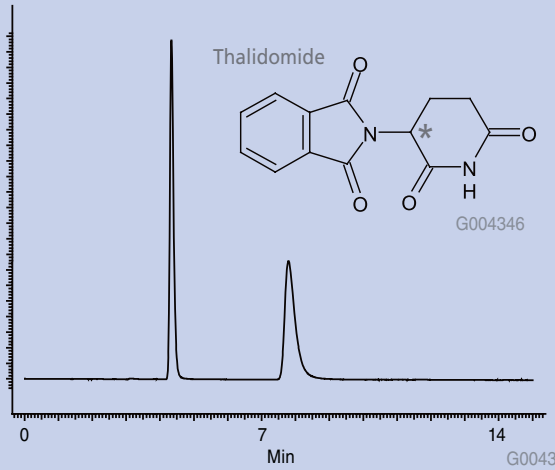
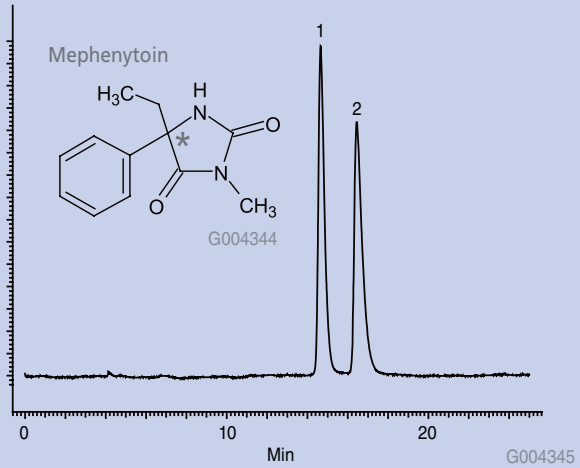


Figure 9. Normal Phase Mode

column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
mobile phase: (95:5) hexane:ethanol
flow rate: 1 mL/min.
det.: UV at 205 nm
temp.: 25 °C
analyte: Mephenytoin



Preparative Applications Using CHIROBIOTIC CSPs

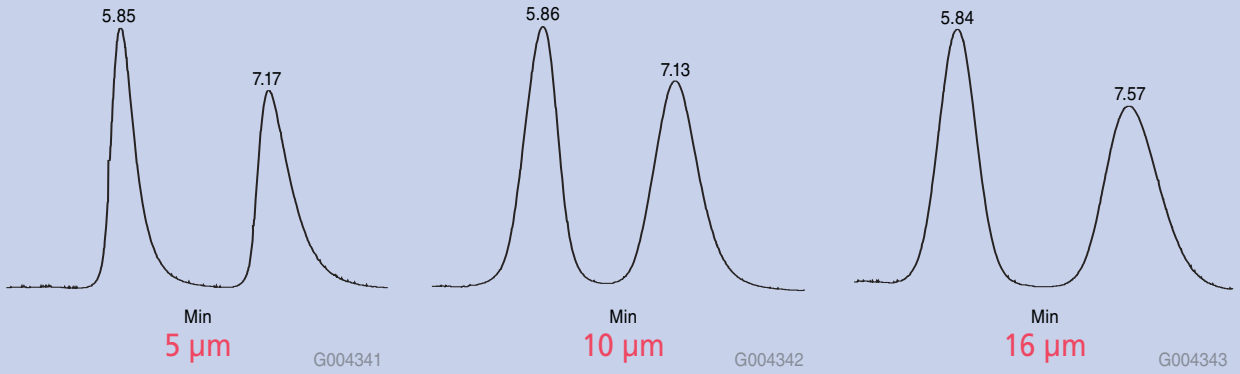
- Scalability across all CHIROBIOTIC particle sizes
- Low retention times give high throughput

CHIROBIOTIC columns can be used in all preparative HPLC techniques, including elution and recycle chromatography, mass-directed prep, SFC and simulated moving bed (SMB). Scale-up is highly predictable because the same bonded phase chemistry is employed across all particle sizes. Multiple covalent bonds attach the CHIROBIOTIC macrocyclic glycopeptides to the silica surface, meaning no CSP ligand will contaminate the product. Preparative separations on CHIROBIOTIC columns often have speed and efficiency benefits over other CSPs. In terms of loading capacity, a 25 cm x 21.2 mm column has medium to high loadings, from a few mg to over 300 mg per injection.

Prep separations on CHIROBIOTIC are reproducible and scalable. Figure 10 shows the separation of phenylalanine isomers in reversed-phase mode on columns packed with 5, 10, and 16 µm particles of CHIROBIOTIC T.

Figure 10. Scalability Across CHIROBIOTIC CSP Particle Sizes

column: CHIROBIOTIC T, 25 cm x 4.6 mm
mobile phase: (50:50) ethanol:water
flow rate: 0.9 mL/min.
det.: UV at 220 nm



Preparative Applications (contd.)

A significant advantage of CHIROBIOTIC for preparative applications is the fact that the mobile phase can be chosen to optimize sample solubility – a critical preparative consideration. The examples here show preparative CHIROBIOTIC separations in three different mobile phase systems.

Preparative Reversed-phase and Polar Ionic Modes

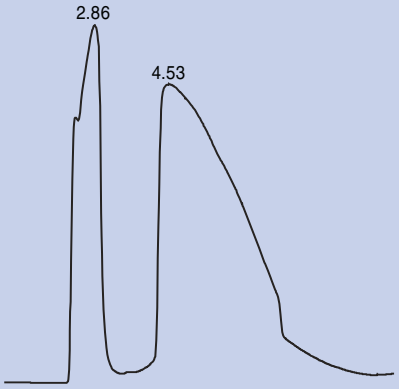
Preparative separations in reversed-phase and polar ionic mode solvents have benefits over normal phase preparative separations in terms of solvent safety and waste disposal costs. Figure 11 shows the use of CHIROBIOTIC TAG in a preparative separation in polar ionic mode.

Preparative Polar Organic Mode

Figure 12 shows the analytical and preparative separations of thalidomide enantiomers on CHIROBIOTIC T. The analytical scale gave an α value of 3.35 in 100% methanol and a retention time under 10 minutes. However, since thalidomide is fairly insoluble in pure methanol, it was possible to add 20% dioxane to the mobile phase to increase solubility 3.5-fold while still achieving the necessary separation.

Figure 11. Preparative Separation on CHIROBIOTIC TAG in Polar Ionic Mode

column: CHIROBIOTIC TAG, 25 cm x 21.2 mm, 5 µm particles (14044AST)
mobile phase: 0.1% ammonium acetate in CH_3OH
flow rate: 35 mL/min.
det.: UV at 300 nm
throughput: 20 mg/g CSP/hr.
load: 200 mg in 6 mL
analyte: N-Acetyl Tryptophan

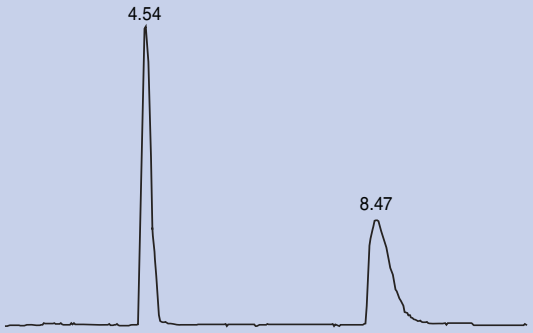


G004340

Figure 12. Sample Solubility Considerations in Preparative

Analytical Scale

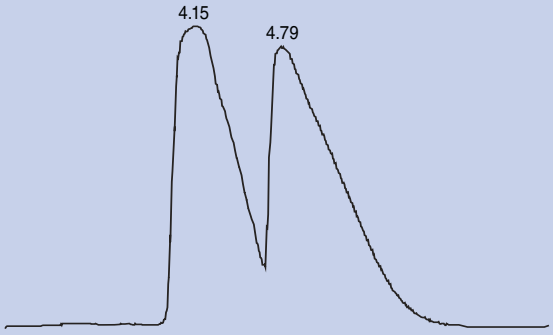
column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
mobile phase: CH_3OH
det.: UV at 293 nm
flow rate: 1 mL/min.
analyte: Thalidomide



G004338

Prep Scale

column: CHIROBIOTIC V, 25 cm x 21.2 mm, 5 µm particles (11044AST)
mobile phase: (80:20) CH_3OH:dioxane
det.: UV at 313 nm
flow rate: 20 mL/min.
load: 70 mg in 12 mL
analyte: Thalidomide



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CHIROBIOTIC V and V2

Bonded Macrocylic Glycopeptide:	Vancomycin
Chiral Centers:	18
Sugar Moieties:	2
Inclusion Cavities:	3

Separates a wide variety of secondary and tertiary amines in the polar ionic mode. Have many of the separation characteristics of protein-based stationary phases but with exceptional stability and much higher sample capacity. CHIROBIOTIC V2 and V differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

CHIROBIOTIC T and T2

Bonded Macrocylic Glycopeptide:	Teicoplanin
Chiral Centers:	23
Sugar Moieties:	3
Inclusion Cavities:	4

These CSPs have resolved all of the known beta-blockers and dihydrocoumarins and many other compound classes. Generally reproduces chiral crown ether or ligand-exchange for amino acid separations. CHIROBIOTIC T2 and T differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

CHIROBIOTIC TAG

Bonded Macrocylic Glycopeptide:	Teicoplanin Aglycone
Chiral Centers:	8
Sugar Moieties:	0
Inclusion Cavities:	4

The removal of the three sugar moieties enhances resolution of many of the amino acids (alpha, beta, gamma and cyclic). CHIROBIOTIC TAG has shown remarkable selectivity for sulfur-containing molecules, such as sulfoxides and the amino acids methionine, histidine and cysteine. Neutral molecules, like oxazolidinones, hydantoins and diazepines, have shown enhanced resolution and, more remarkably, in single-solvent systems like methanol, ethanol and acetonitrile. Some acidic molecules have also shown increased selectivity.

CHIROBIOTIC R

Bonded Macrocylic Glycopeptide:	Ristocetin A
Chiral Centers:	38
Sugar Moieties:	6
Inclusion Cavities:	4

The presence of amines in the ristocetin structure makes it a good choice when screening acidic compounds.

CHIROBIOTIC Product Listing

For more information and to review our complete offering of CHIROBIOTIC columns, please visit *sigma-aldrich.com/chiral*

Method Development Kits

ID (mm)	Length (cm)	Cat. No.	
4.6	10	**10300AST**	- One each of CHIROBIOTIC V2, T, TAG and R
4.6	25	**10305AST**	- One each of CHIROBIOTIC V2, T, TAG and R

CHIROBIOTIC Columns*		V	V2	T	T2	TAG	R
ID (mm)	Length (cm)	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.
5 µm							
2.1	15	11019AST	15019AST	12019AST	16019AST	14019AST	13019AST
2.1	25	11020AST	15020AST	12020AST	16020AST	14020AST	13020AST
4.6	5	11021AST	15021AST	12021AST	16021AST	14021AST	13021AST
4.6	10	11022AST	15022AST	12022AST	16022AST	14022AST	13022AST
4.6	15	11023AST	15023AST	12023AST	16023AST	14023AST	13023AST
4.6	25	11024AST	15024AST	12024AST	16024AST	14024AST	13024AST
10	25	11034AST	15034AST	12034AST	16034AST	14034AST	13034AST
10	50	11036AST	15036AST	12036AST	16036AST	14036AST	13036AST
21.2	25	11044AST	15044AST	12044AST	16044AST	14044AST	13044AST
10 µm							
4.6	25	11124AST	15124AST	12124AST	16124AST	14124AST	13124AST

*Other column dimensions, including guard columns and preparative dimensions are available on our website or by inquiring to ***techservice@sial.com.***

Chiral Services: Column Screening and Small-Scale Purification

Consult Supelco to obtain a quotation for our expert services for chiral column screening (HPLC and GC), method development and optimization, as well as isolation of up to 10 grams of purified enantiomer.

The complete listing of our chiral HPLC and GC columns can be found at *sigma-aldrich.com/chiral*, our corporate chiral web portal, where you can view our other products for chiral chemistry, like chiral catalysts, building blocks, mobile phase additives, derivatization reagents and more.

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Astec P-CAP™ and P-CAP™-DP

Polycyclic amine polymer stationary phases for chiral HPLC

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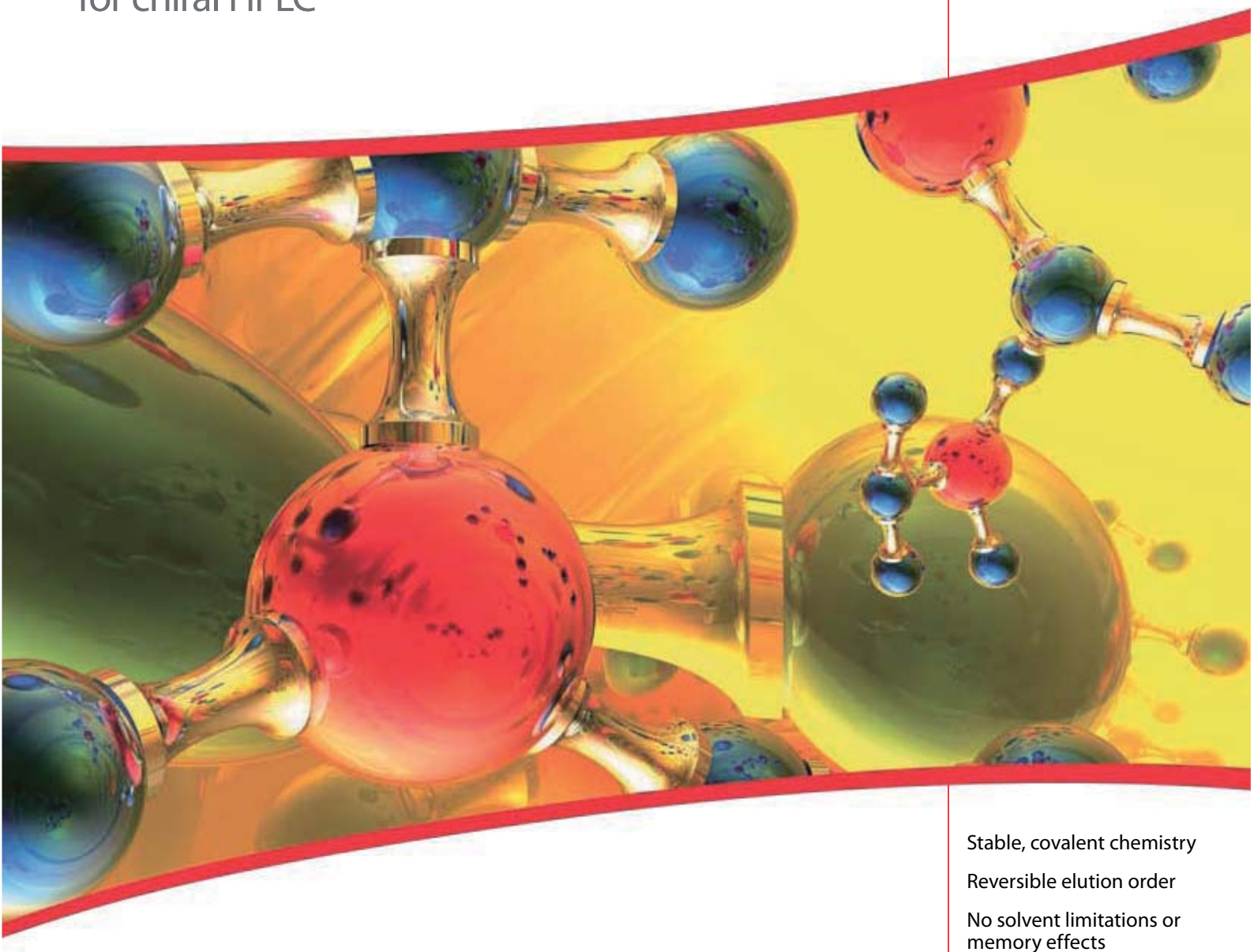


- Stable, covalent chemistry
- Reversible elution order
- No solvent limitations or memory effects
- High capacity for preparative applications
- MS and SFC-compatible

Chiral HPLC for Chemists: Ultimate Solvent Choice with High Capacity Using Astec's P-CAP and P-CAP-DP

Useful for chiral HPLC and SFC separations, Astec P-CAP and P-CAP-DP polymeric chiral stationary phases (CSPs) have a thin, ordered layer of chiral polymer covalently bonded to the silica surface. They offer high stability, high sample loadability, easy scale-up, and no memory effect.

Today's chiral HPLC columns too often give excellent enantioselectivity at the expense of solvent choice. Sample solubility and its link to preparative separations can mean that a compromise has to be reached between selectivity and solvent choice. Astec P-CAP and P-CAP-DP chiral HPLC columns have no solvent restrictions, so the user can select a solvent that provides optimum enantioselectivity and analyte solubility.

Astec P-CAP

– **Bonded phase: Poly(trans-1,2-cyclohexanediyl-bis-acrylamide)**

– **Invented by Prof. Francesco Gasparrini (1), P-CAP is made from a diacryloyl -trans-1,2-diphenylethylenediamine polymerization, and utilizes hydrogen bonding and steric effects as enantiomer separation mechanisms.**

Astec P-CAP-DP

– **Bonded phase: Poly(diphenylethylenediamine-bis-acryloyl) or Poly-DPEDA**

– **Invented by Prof. Daniel Armstrong (2), Astec P-CAP-DP introduces phenyl rings to add π-π interactions, giving it one additional type of interaction compared to P-CAP. P-CAP-DP is less polar than P-CAP.**

Solvent Choice and Reversal of Elution Order

The P-CAP and P-CAP-DP polyamide CSPs feature a thin, ordered polymer layer chemically bonded to 5 µm or 3.5 µm spherical silica using a patented radical polymerization. This gives the phases high permeability across the surface and, because they are synthetic, they can be identically manufactured in both R,R and S,S forms. This provides a predictable reversal of elution order in the same mobile phase (Figure 1).

The P-CAP phases have no solvent or additive memory effects, so the same column can be used in a number of different mobile phases without any detrimental effects. These phases form a new generation bridge between the traditional 'brush' type CSPs and the conventional polymeric phases.

Chiral method development is typically carried out either in normal phase (heptane/IPA or hexane/ethanol) or polar organic (acetonitrile/methanol) modes. For method optimization, a wide range of organic solvents can be used, from acetone to dichloromethane to dioxane, and many others. For acids and bases, the addition of 0.1% TFA often

Figure 1. Predictable Elution Order Reversal

columns: 25 cm x 4.6 mm I.D., 5 µm
mobile phase: 95:5, methylene chloride:methanol
flow rate: 1 mL/min.

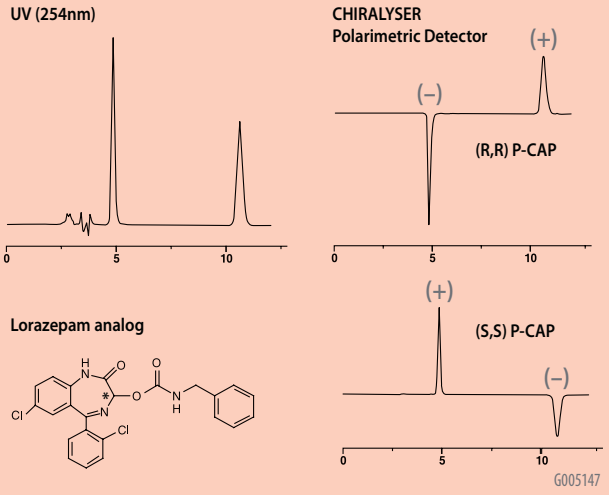
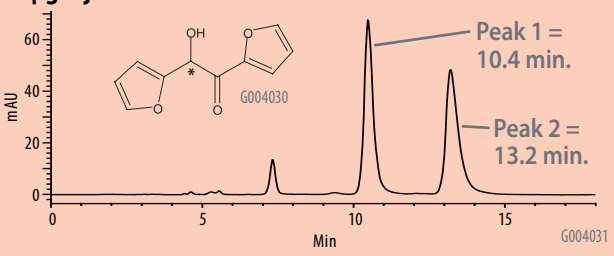


Figure 2. High-capacity Separations

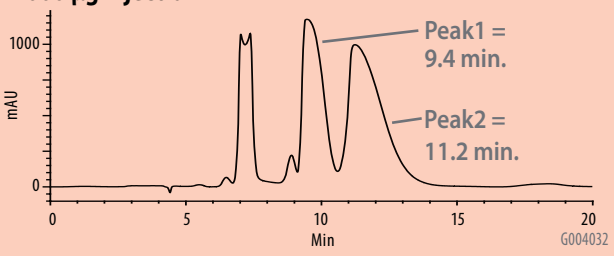
Analyte: furoin

column: (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase: 80:20, hexane:ethanol
flow rate: 1.0 mL/min.
temp.: 23 °C
det.: UV at 235 nm
injection: (a) 2 µL at 4 mg/mL (8 µg total)
(b) 50 µL at 20 mg/mL (1000 µg total)

8 µg injection



1000 µg injection



increases resolution and efficiency and decreases retention times. There are no known limitations on the kind of solvents that can be used with these phases. For MS detection, volatile acids and buffers such as ammonium acetate can be added to enhance peak efficiency or to enhance ionization when needed.

High Capacity for Preparative HPLC

Solvent flexibility and high loading capacity make P-CAP and P-CAP-DP CSPs ideal for analytical, preparative and process scale separations. The separation of furoin enantiomers is shown in Figure 2 using an analytical 25 cm x 4.6 mm Astec (R,R) P-CAP-DP column and mobile phase of 80:20 hexane:ethanol. Excellent separation is demonstrated with an injection of 8 µg of the furoin racemate. Increasing the load to 1 mg on this analytical column demonstrates the phase's high loading capacity.

MS-Compatible Operation

Astec P-CAP and P-CAP-DP operate in mobile phases that are amenable to MS-detection. Salt and/or acetic acid can be added to improve efficiency or enhance ionization and detection (Figure 3).

Applications

Astec P-CAP CSPs have been used for a wide variety of molecular types and are ideal for medium to high polarity compounds. The mechanism of separation is either through hydrogen bonding for P-CAP, or through both hydrogen bonding (donor and acceptor) with additional π-π interaction for the P-CAP-DP. Both also use dipole-dipole and steric interactions. Examples of the separations completed to date are shown in Table 1.

Table 1. Examples of P-CAP and P-CAP-DP Application Areas

Hydroxycarboxylic acids	**Benzene sulfonamides**
Alcohols	**Binaphthols**
Sulfoxides	**Benzodiazepines**
Esters	**Phosphonic acids**
Amides	**Bis-Sulfones**
N-blocked amino acids	**Chromemones**

Complementary to Other Astec CSPs

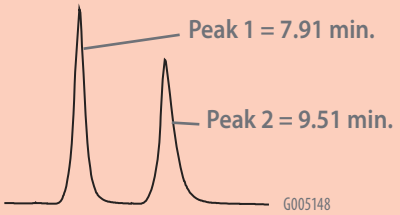
Astec P-CAP and P-CAP-DP are complementary to Astec CHIROBIOTIC, CYCLOBOND and the polysaccharide-based CSPs. We suggest you incorporate them into your chiral column screening protocol.

Figure 3. Improved Analytical Separation with MS-Compatible Mobile Phases

column: (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
flow rate: 1.0 mL/min.
temp.: 25 °C
det.: UV @ 254 nm

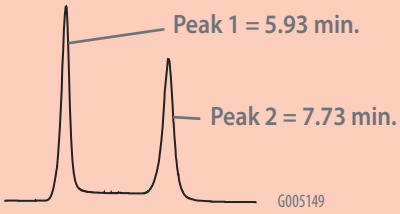
Separation of 1,1'-Bi-2-naphthol

mobile phase: 95:5:10 mM ammonium acetate:methanol:acetate



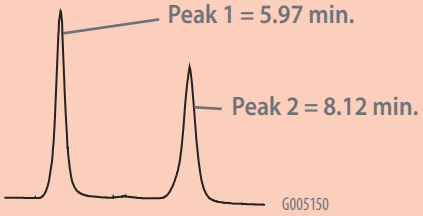
Oxazepam

mobile phase: 70:30:20 mM ammonium acetate:methanol:acetate



Lorazepam

mobile phase: 70:30:20 mM ammonium acetate:methanol:acetate



Summary

Astec P-CAP and P-CAP-DP are rugged chiral HPLC phases, experience no memory effects, and can be run in a wide variety of solvents with speed and high efficiencies. For preparative applications, a combination of wide solvent choice and high capacity make them ideal for large-scale purification.

References

1) New hybrid polymeric liquid chromatography chiral stationary phase prepared by surface initiated polymerization. Gasparrini, F.; Misiti, D.; Rompietti, R.; Villani, C. J Chromatogr, A. (2005), 1064(1), 25-38.

2) Chromatographic evaluation of poly(trans-1,2-cyclohexanediyl-bisacrylamide) as a chiral stationary phase for HPLC. Zhong, Qiqing; Han, Xinxin; He, Lingfeng; Beesley, Thomas E.; Trahanovsky, Walter S.; Armstrong, Daniel W. Department of Chemistry, Iowa State University, Ames, IA, USA. Journal of Chromatography, A (2005), 1066(1-2), 55-70.

Ordering Information

Particle Size (µm)	Length (cm)	I.D. (mm)	Cat. No.
Astec (R,R) P-CAP			
3.5	5	4.6	30021AST
3.5	10	4.6	30022AST
3.5	15	4.6	30023AST
5	5	4.6	31021AST
5	10	2.1	31018AST
5	10	4.6	31022AST
5	15	2.1	31019AST
5	15	4.6	31023AST
5	25	2.1	31020AST
5	25	4.6	31024AST
5	25	10	31034AST
5	25	21.2	31044AST
10	25	4.6	31124AST
Guards*			
5	2	1	31101AST
5	2	4	31100AST
Astec (S,S) P-CAP			
3.5	5	4.6	32021AST
3.5	10	4.6	32022AST
3.5	15	4.6	32023AST
5	5	4.6	33021AST
5	10	2.1	33018AST
5	10	4.6	33022AST
5	15	2.1	33019AST
5	15	4.6	33023AST
5	25	2.1	33020AST
5	25	4.6	33024AST
5	25	10	33034AST
5	25	21.2	33044AST
10	25	4.6	33124AST
Guards*			
5	2	1	33101AST
5	2	4	33100AST

Particle Size (µm)	Length (cm)	I.D. (mm)	Cat. No.
Astec (R,R) P-CAP-DP			
3.5	15	4.6	34023AST
5	15	2.1	35019AST
5	15	4.6	35023AST
5	25	4.6	35024AST
5	25	10	35034AST
5	25	21.2	35044AST
Guards*			
5	2	4	35100AST
Astec (S,S) P-CAP-DP			
3.5	15	4.6	36023AST
5	15	2.1	37019AST
5	15	4.6	37023AST
5	25	4.6	37024AST
5	25	10	37034AST
5	25	21.2	37044AST
Guards*			
5	2	4	37100AST

*Guard Column Holders	
Guard Holders for 4 mm I.D. cartridges (holder not required for 1 mm I.D. guards)	21150AST

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