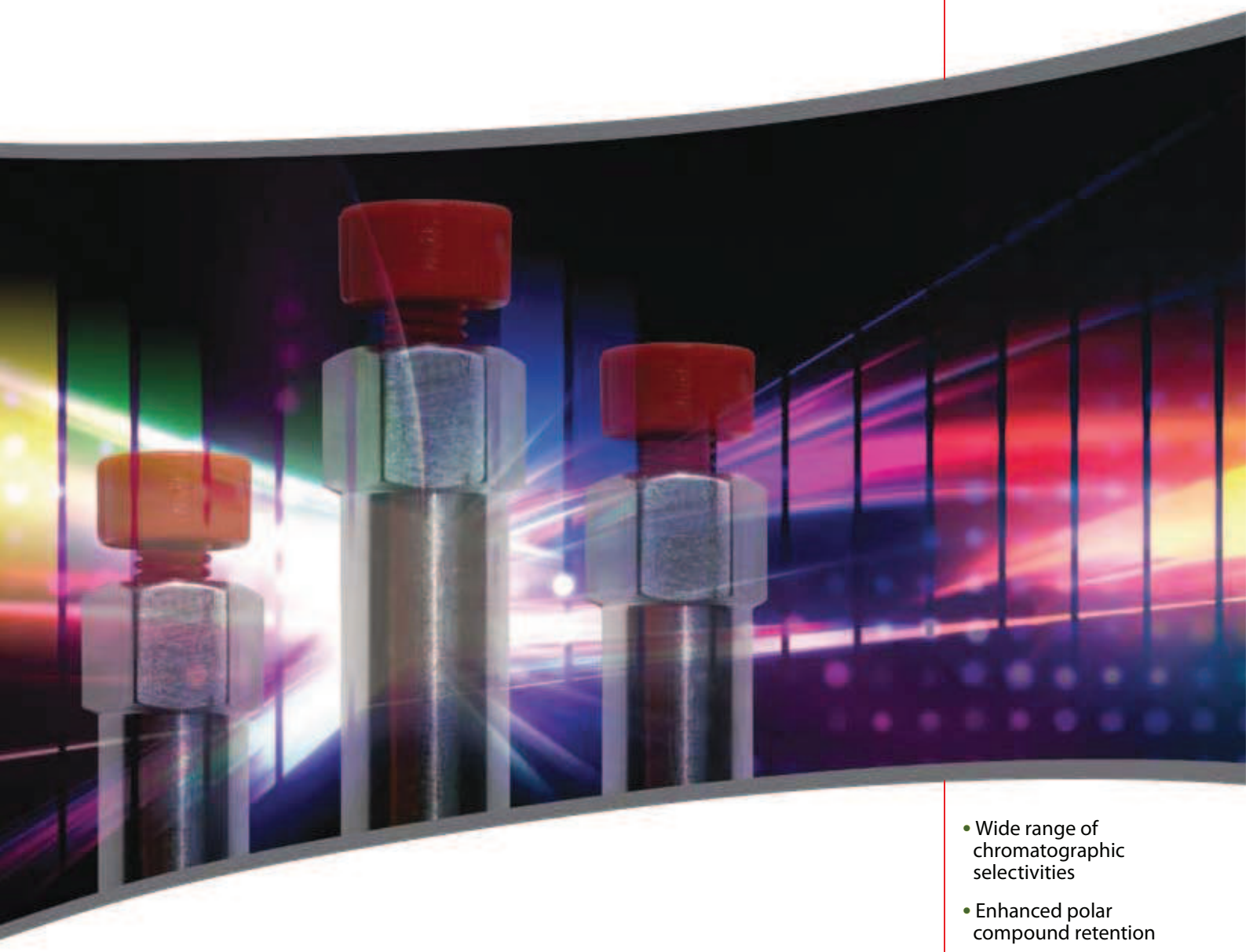
Ascentis® HPLC Columns

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.

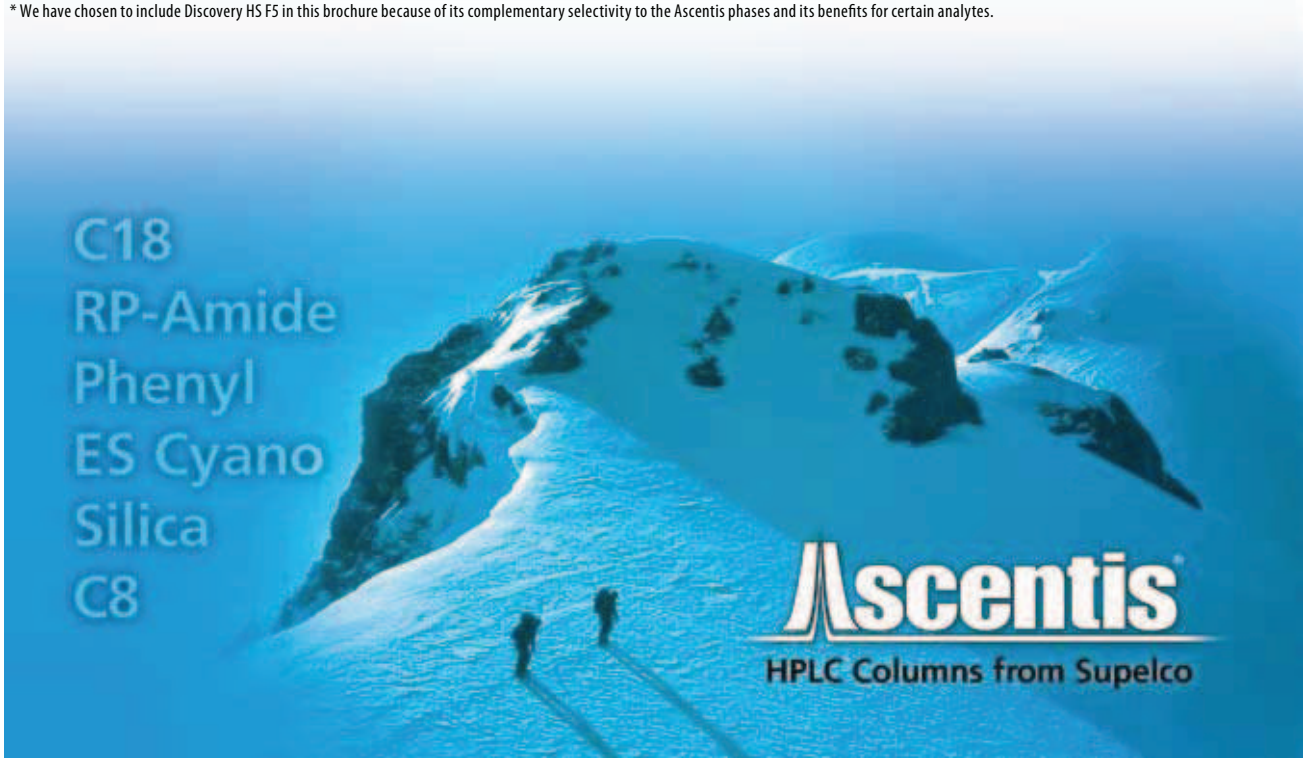
The General Features of the Ascentis Family Include:

- 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

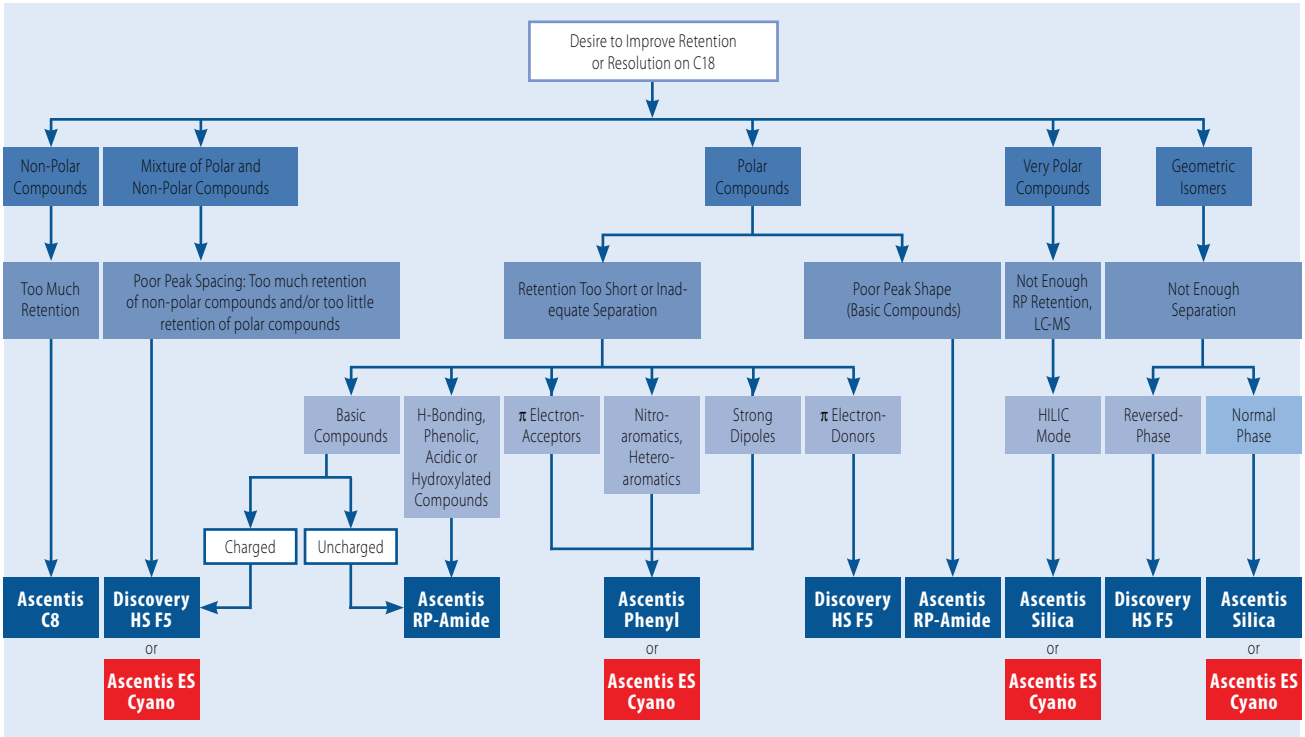
Ascentis ES Cyano – Extra stable for low pH mobile phases due to sterically protected phase. Useful for polar selectivity in reversed-phase mode. Also useful as an alternative to silica in HILIC mode

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

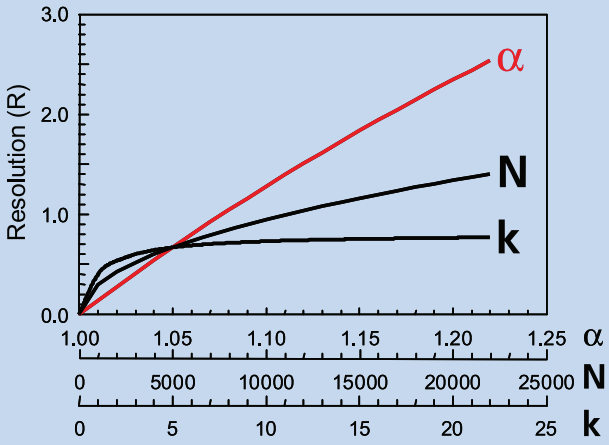
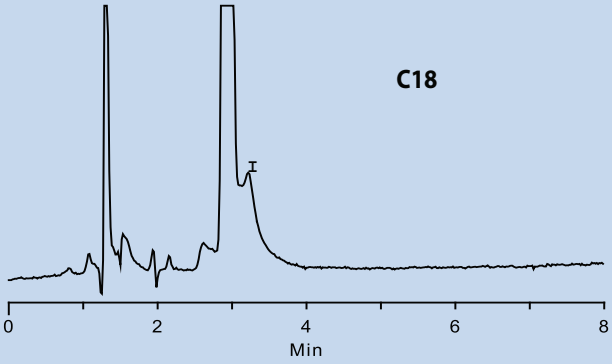
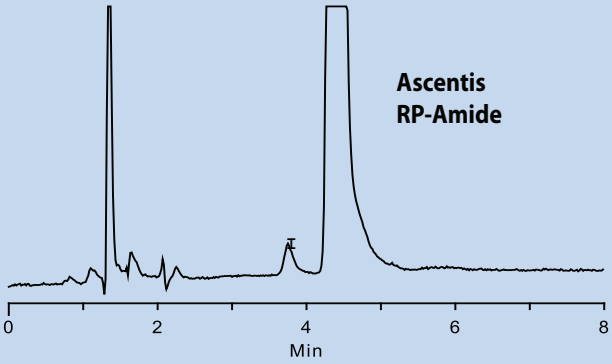


Figure 2. Impurity Eluted on the Downslope of the Main Peak on C18 — Difficult to Quantify



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.

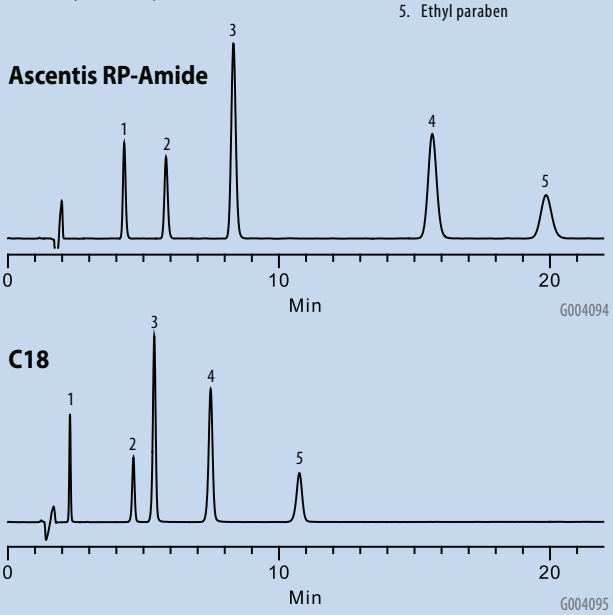
Stationary Phases with Enhanced Polar Compound Retention Compared to C18

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

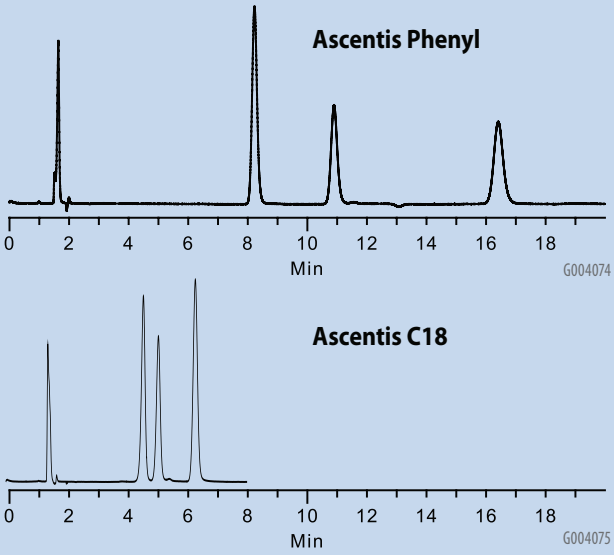
1. 4-Hydroxybenzoic Acid
2. Acetylsalicylic Acid
3. Benzoic Acid
4. 2-Hydroxybenzoic Acid
5. Ethyl paraben



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

Figure 4. π-π Interactions (Ascentis Phenyl)

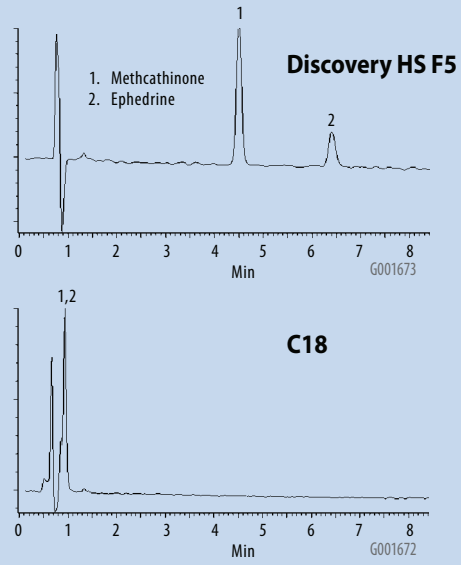
column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase: 40:60, acetonitrile:water
flow rate: 1.0 mL/min.
temp.: 30 °C
det.: UV at 210 nm
injection: 5 µL
sample: nitrobenzene, m-dinitrobenzene, 1,3,5-trinitrobenzene



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

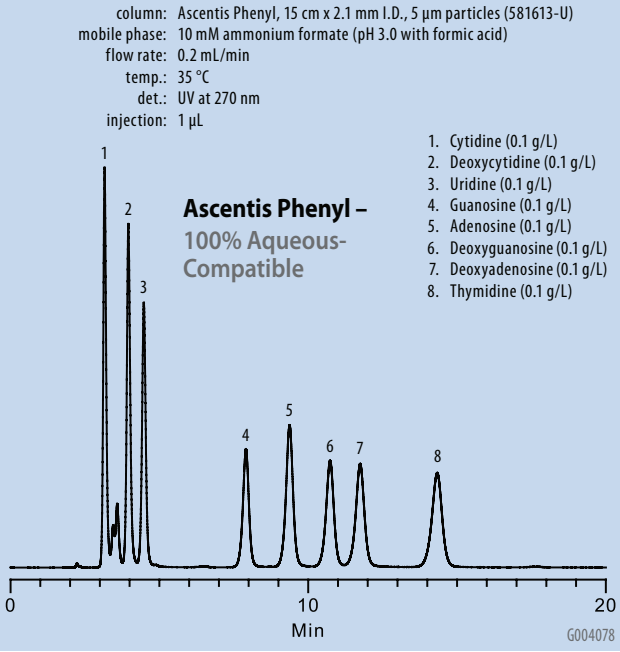
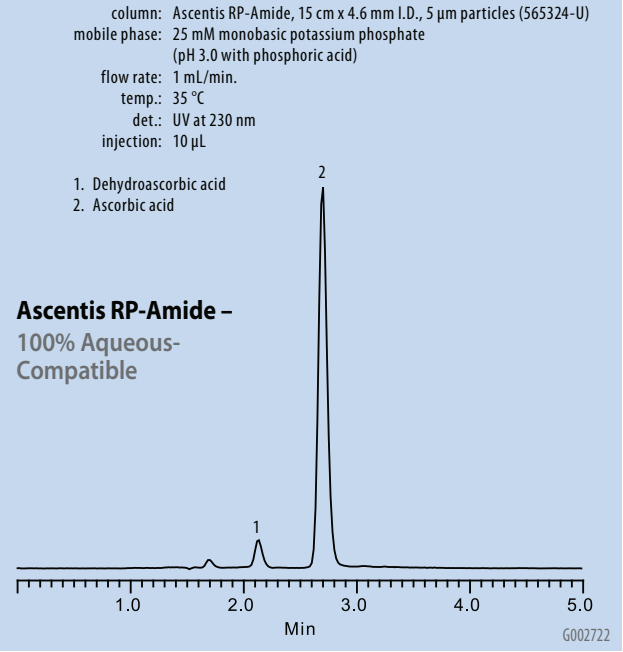


Figure 7. Separation of Organic Acids on Ascentis RP-Amide



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**

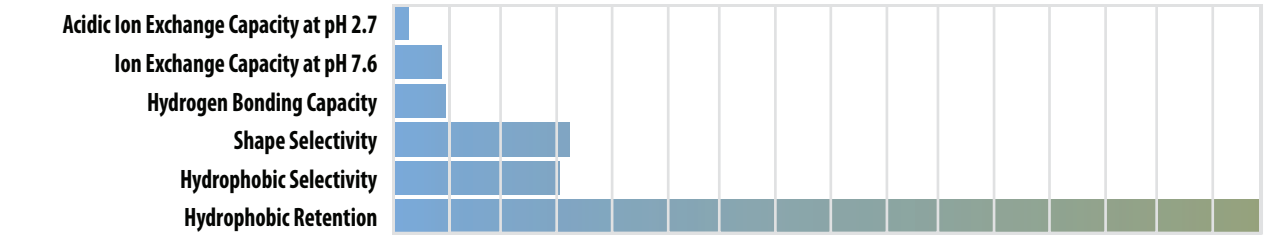
Key Applications:

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

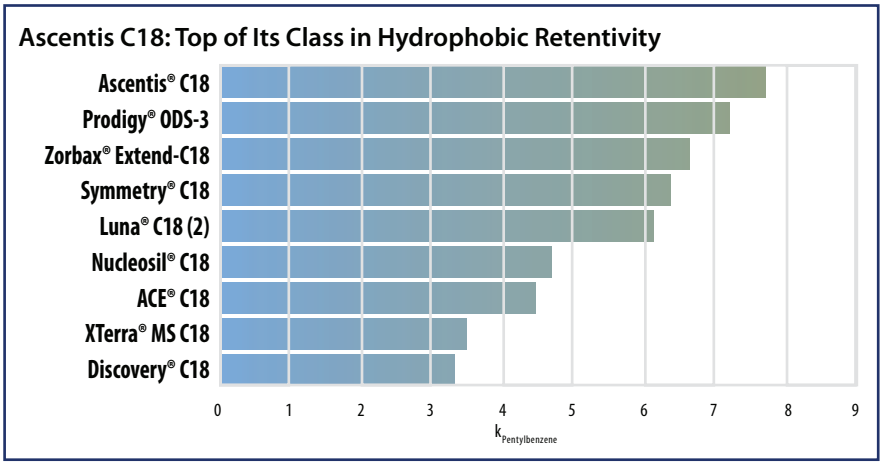
Ascentis C18 is low-bleed for clean ESI and APCI traces. The high retentivity means that the mobile phase can contain high levels of organic modifier that are more readily desolvated.

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.



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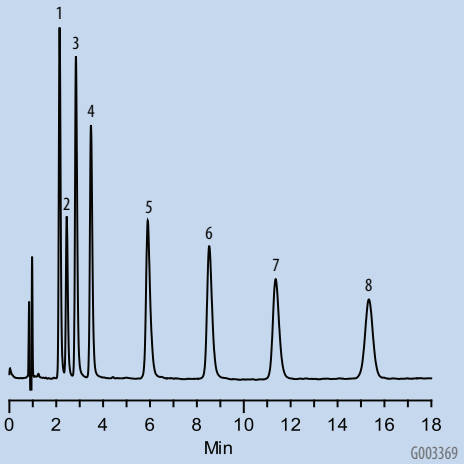
Analysis of basic compounds at neutral pH often gives longer retention compared to acidic mobile phases, but sometimes causes poor peak shape due to silanol interactions. The highly-inert surface of Ascentis C18, as with all Ascentis phases, permits analysis in neutral pH mobile phases. In this example,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

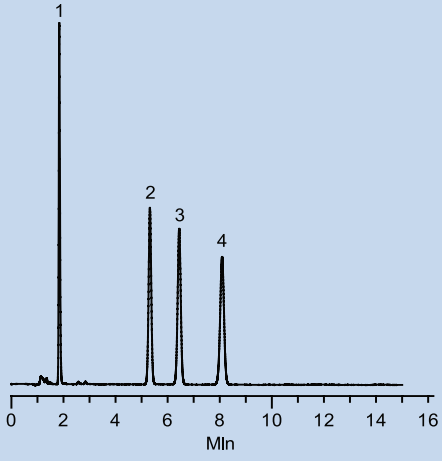


Ascentis C18 provides the selectivity and retention for a range of compounds including steroids. Ascentis C18 is a reliable first choice HPLC column that gives symmetric peak shape and excellent retention for even difficult compounds.

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**

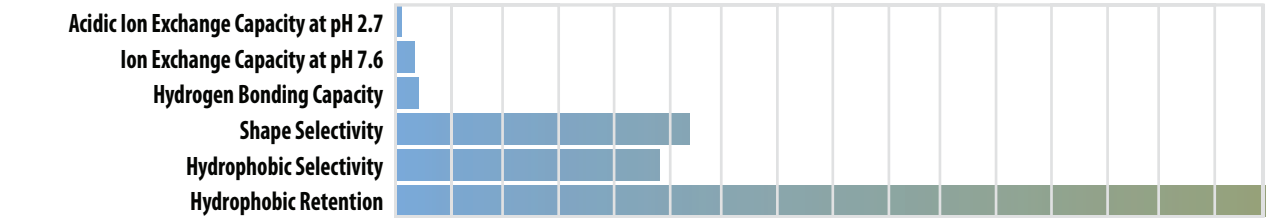
Key Applications

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



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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

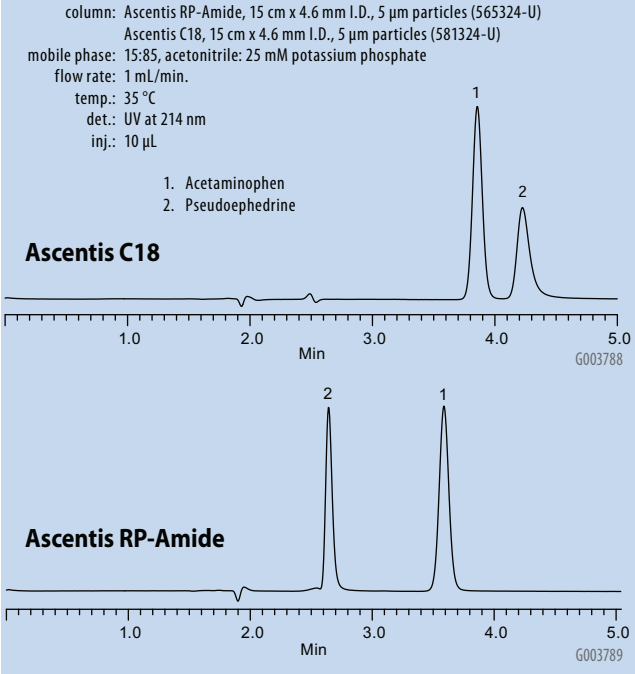
- Generally, acids are retained more and bases retained less on RP-Amide compared to C18 and C8 columns.
- Methanol can be comparable in elution strength to acetonitrile when compounds are retained by H-bonding mechanism on the RP-Amide phase.

* 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

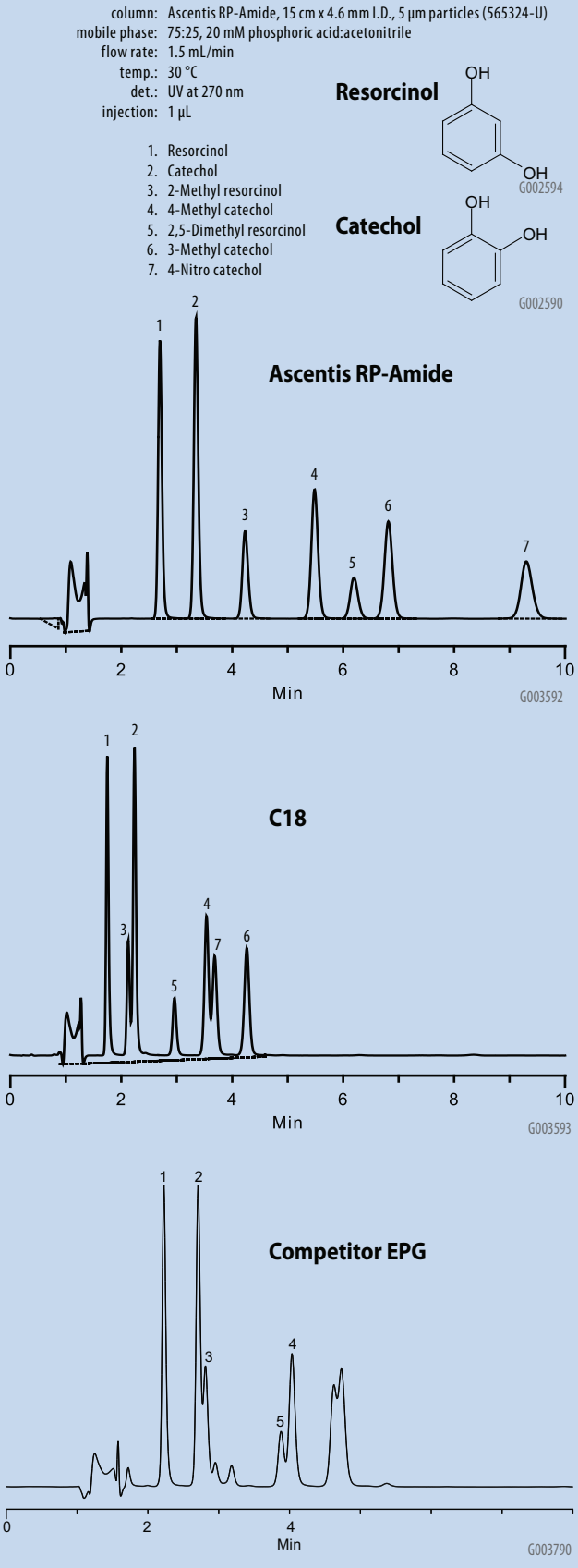
Because the amide group lies near the silica surface, it is believed to suppress tailing of basic solutes by electrostatic shielding (repulsion) or by interacting preferentially with the silanols (H-bonding between the phase and the substrate). The absence of unwanted silanol and other secondary interactions 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**

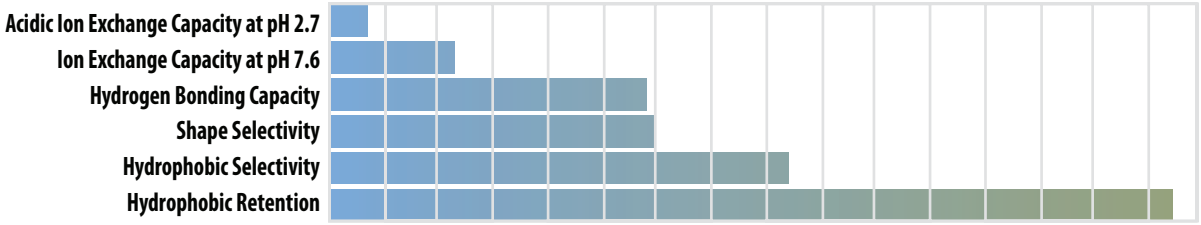
Key Applications

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:

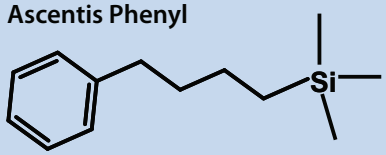
- hydrophobic bases (TCAs, tetracyclines)
- moderate bases (anesthetics and narcotic analgesics)
- benzodiazepines
- some acidic compounds such as ACE inhibitors and quinoline antibiotics
- nucleosides (e.g. cytidine)
- nitro, azide and sulfonyl compounds

Notes

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

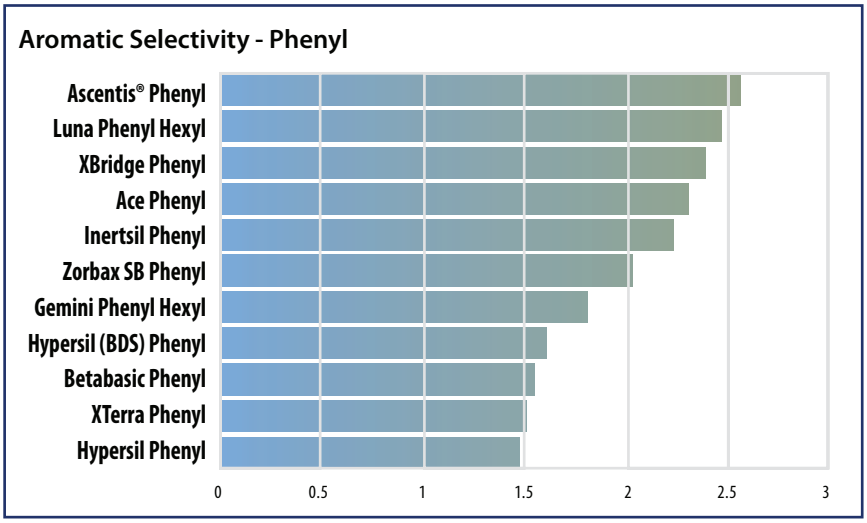
* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

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.

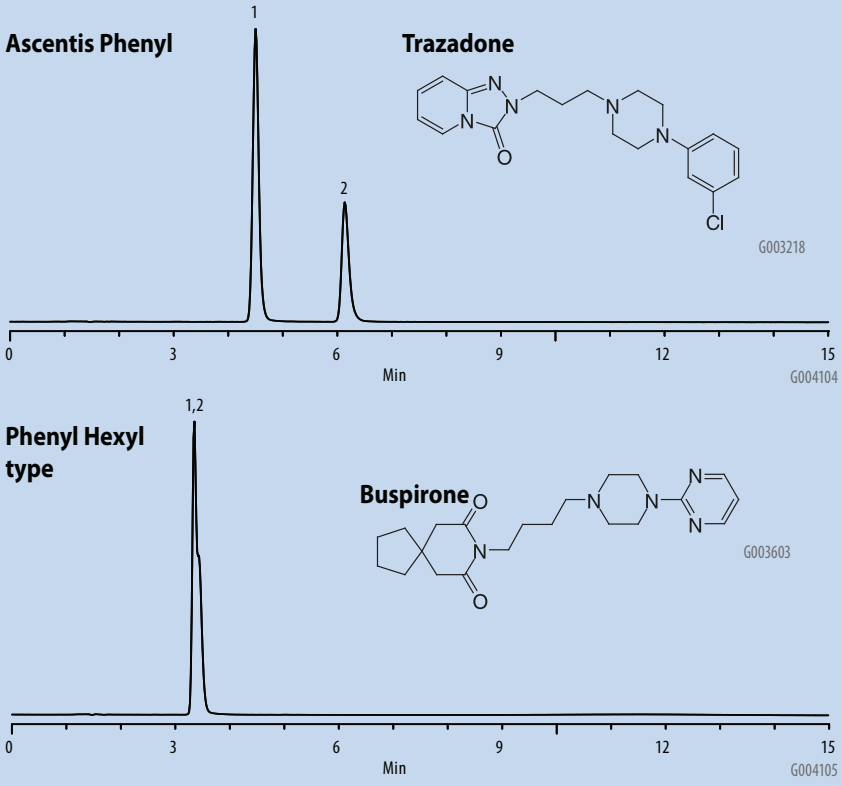


All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from 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

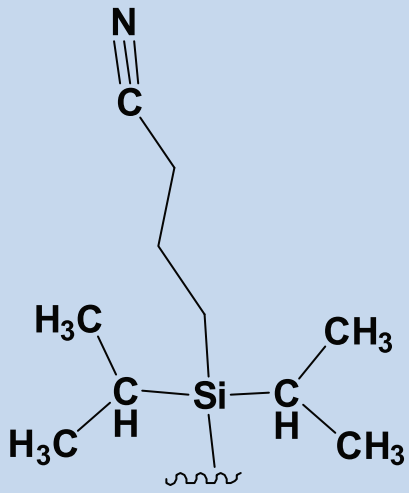
Use

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

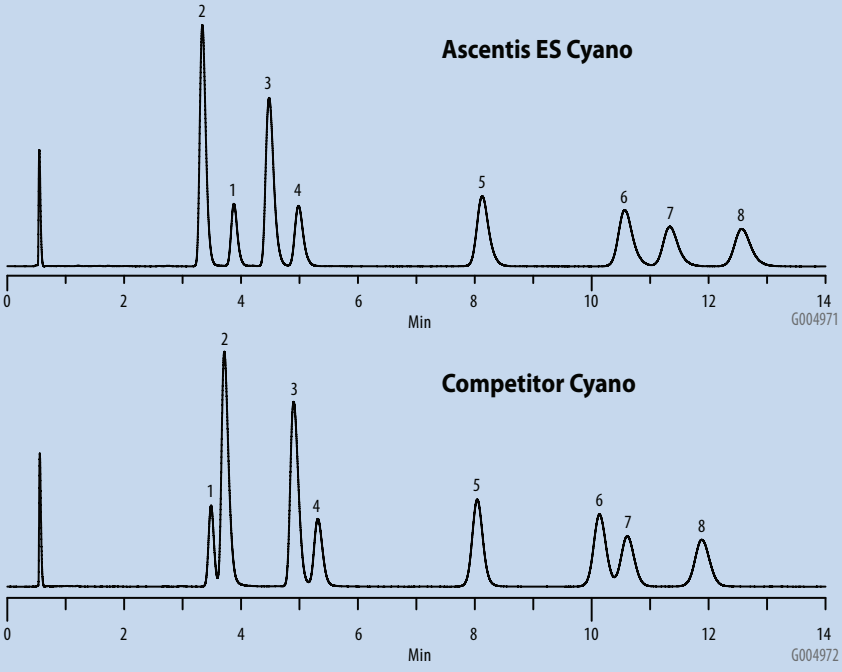
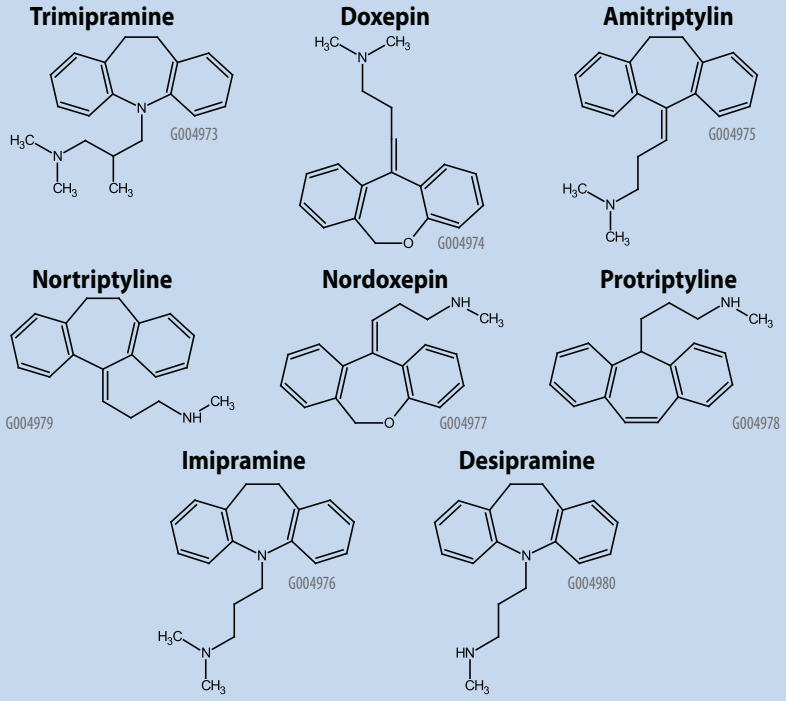


Figure 17. Structures of Tricyclic Antidepressants



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

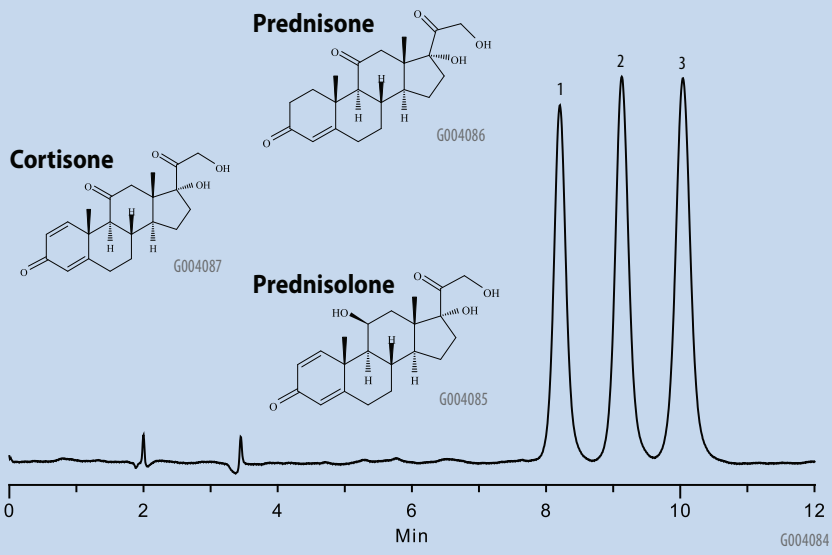
- Normal phase and HILIC HPLC modes
- Preparative chromatography
- Purification (organic synthesis)
- LC-MS

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

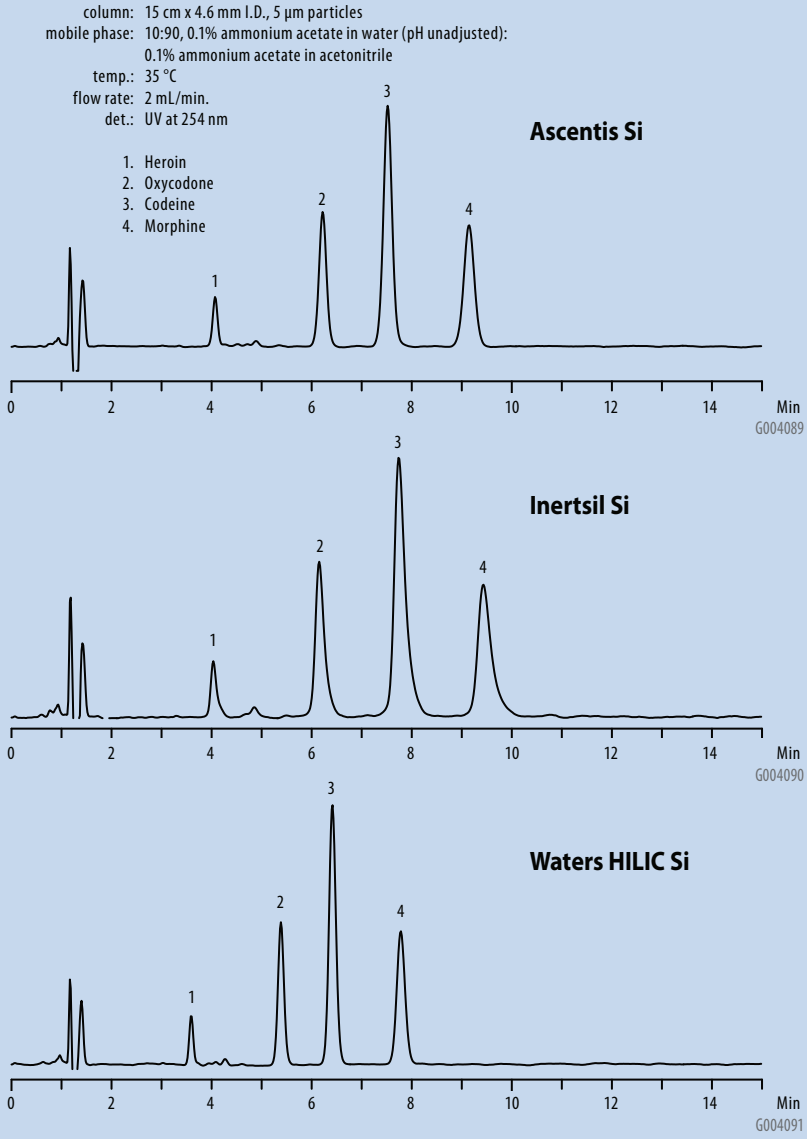


Ascentis Silica is used successfully in the aqueous normal-phase or HILIC mode. In this mode, water is the strong modifier and the organic is the weak modifier of the mobile phase. Like reversed-phase, HILIC offers the flexibility of using pH and ionic strength to control retention.

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

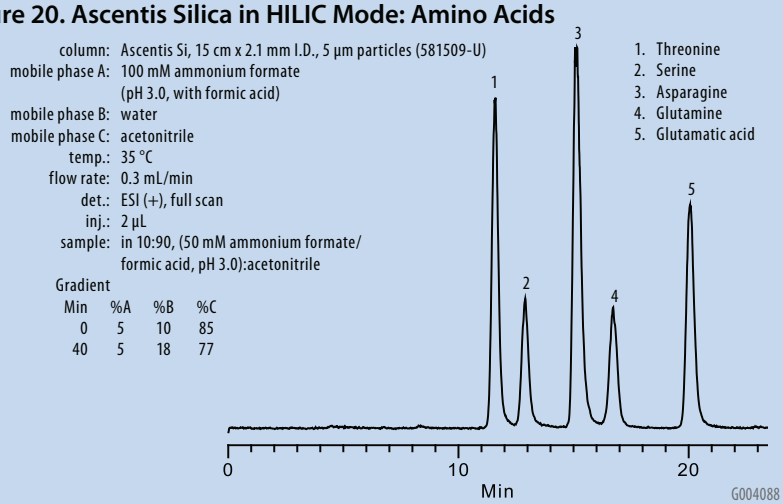
Elution order is generally opposite to that obtained under reversed-phase conditions. Figure 16 compares Ascentis Silica with two competing silicas, demonstrating better resolution by virtue of excellent peak shape and high retentivity.

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**

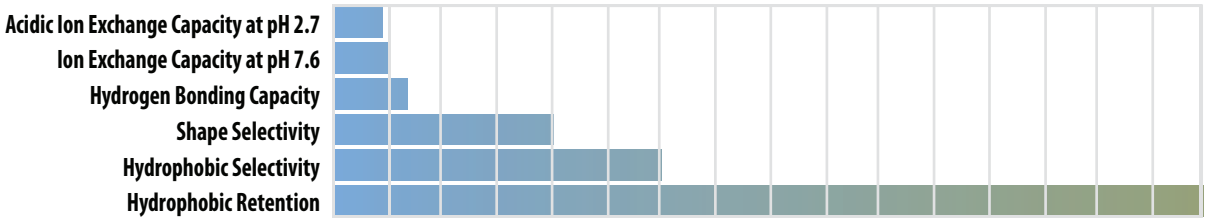
Key Applications:

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

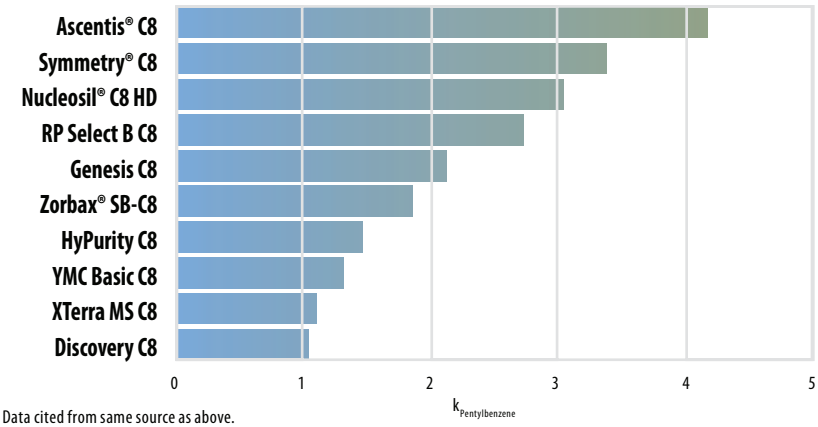


All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from 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.

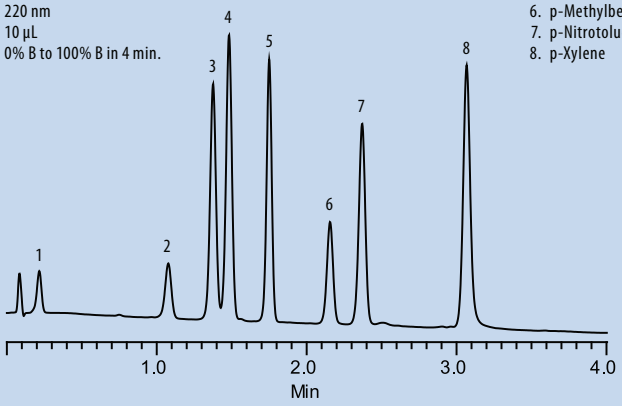
* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

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



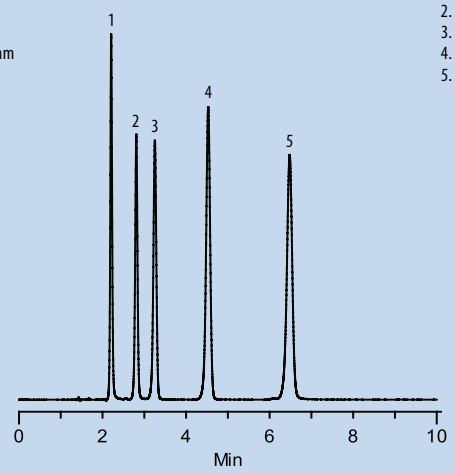
G003377

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



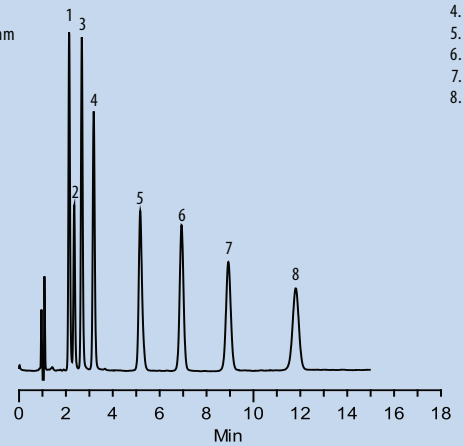
G003368

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**

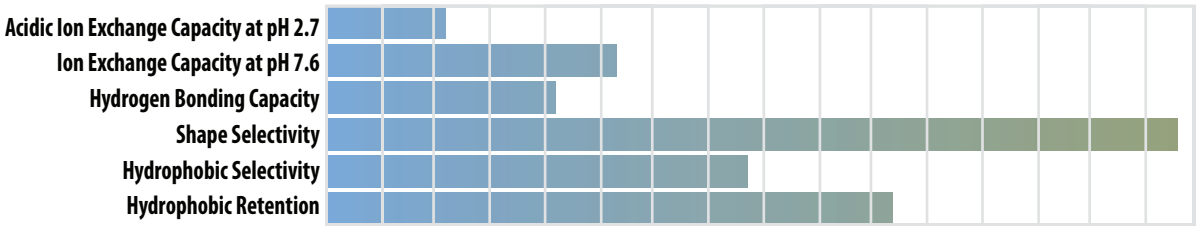
Key Applications

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



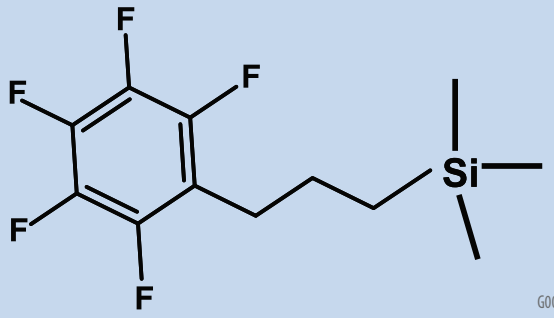
All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from 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



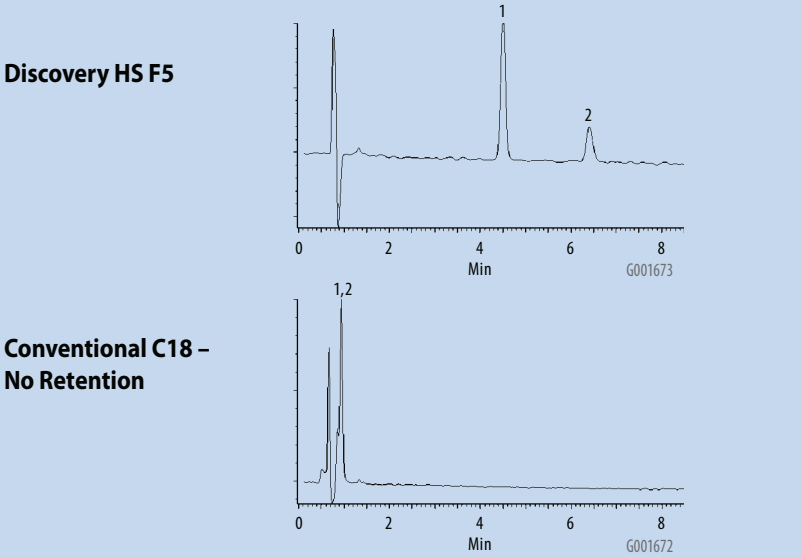
Guidelines for Transferring a C18 Method to Discovery HS F5

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)

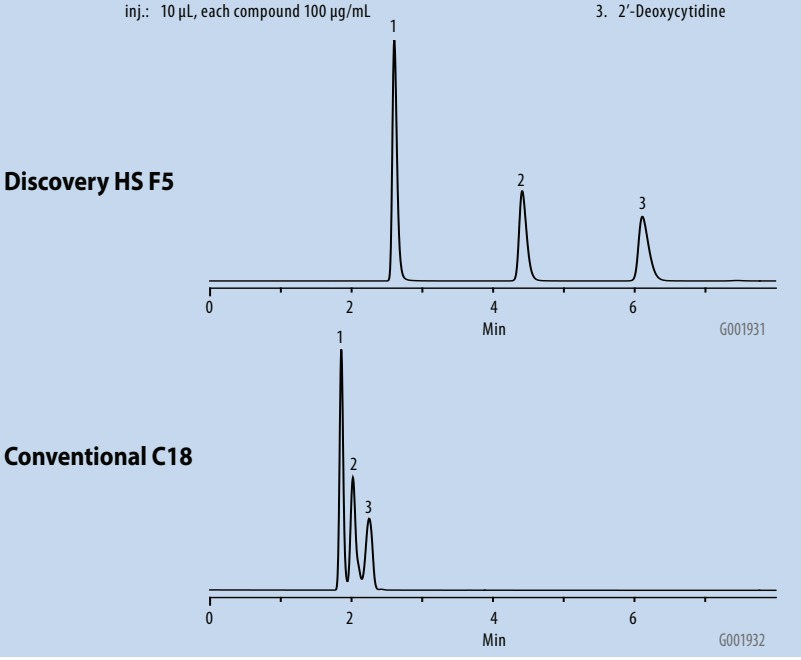


In Figure 25, cytidine and related compounds provide another example of the power of HS F5 to provide unique and valuable separations compared to a C18. An added benefit of the HS F5 is its resistance to phase collapse under 100% aqueous conditions.

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!

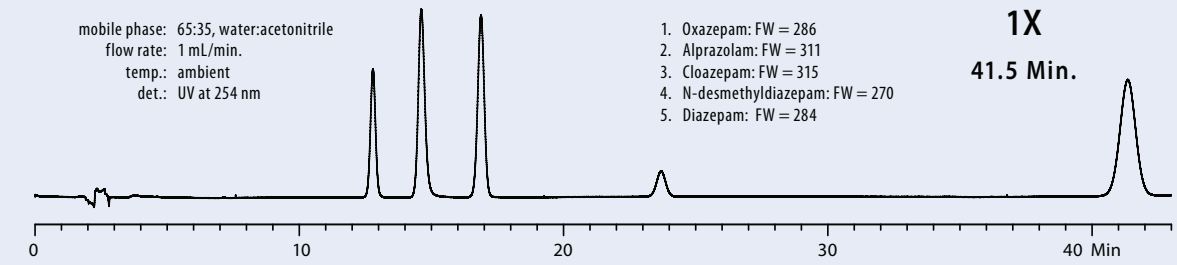
For more information on this exciting new technology, visit our website: ***sigma-aldrich.com/express***

Do More Work in Less Time without Changing your Method

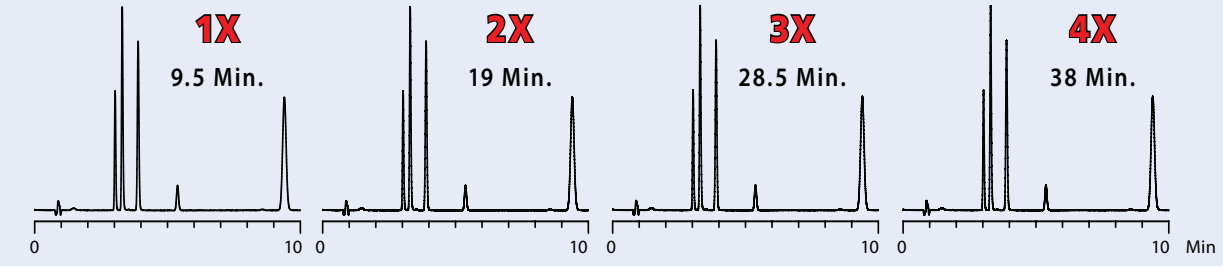
With identical conditions, the Ascentis Express C18 column performs **4X** as many separations as a standard C18 column in less time.

CONDITIONS						
Sample Prep	Flow Rate	System Pressure	HPLC System	Theoretical Plates	Standard C18 Throughput	**Ascentis C18 Throughput**
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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