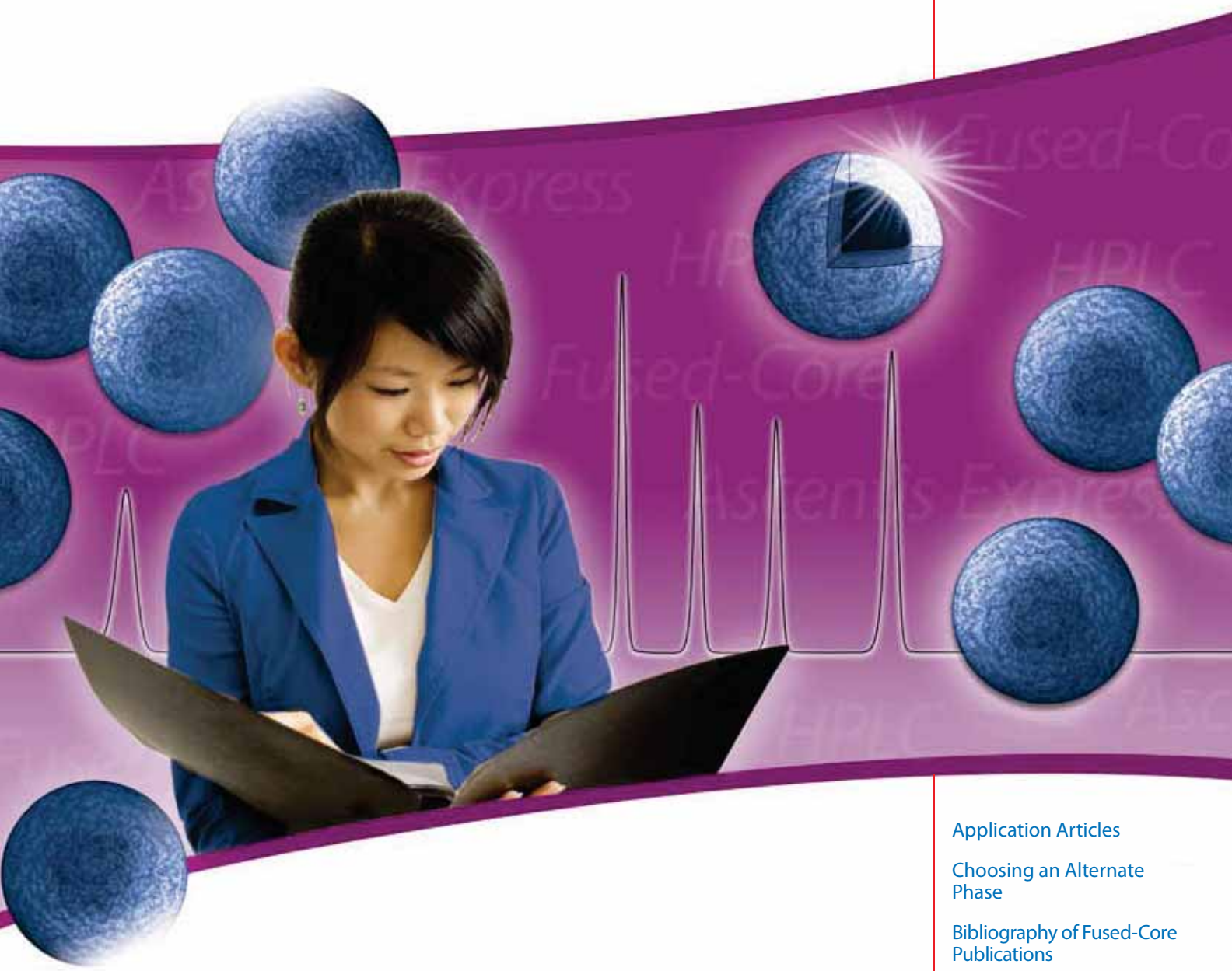
Ascentis® Express HPLC Resource Guide

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

phone: 800-359-3041 (US and Canada only)
814-359-3041

fax: 800-359-3044/814-359-5468

email: techservice@sial.com

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

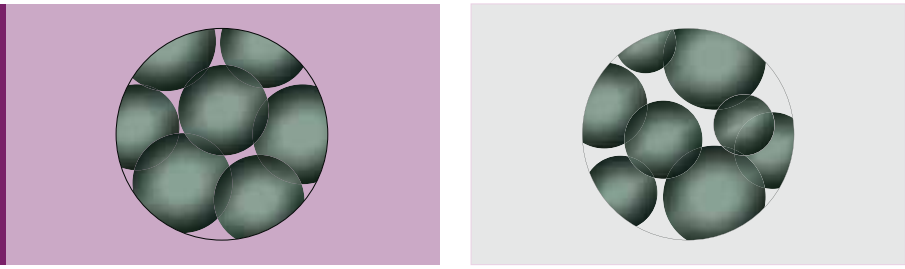
The Fused-Core™ Advantage

Extreme performance on any LC system

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

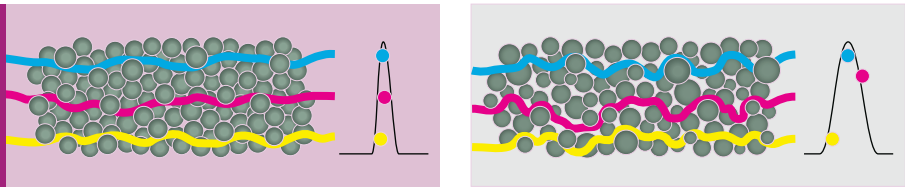
Narrow Particle Size Distribution

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



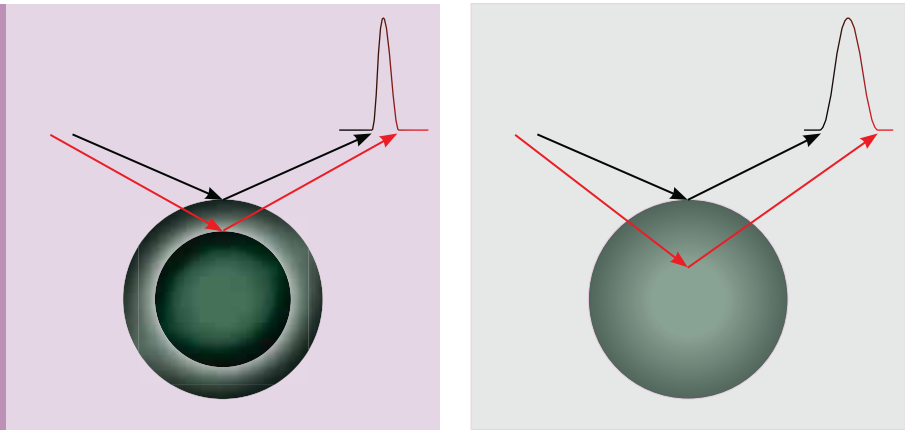
The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured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

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.*

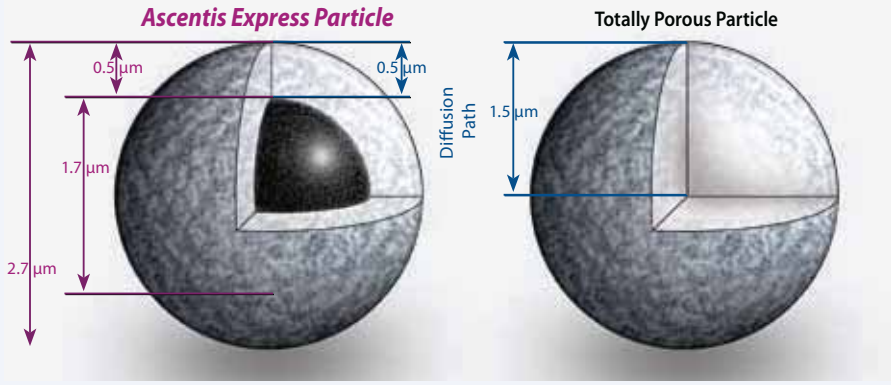
Compared to 3 µm Particles:

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.

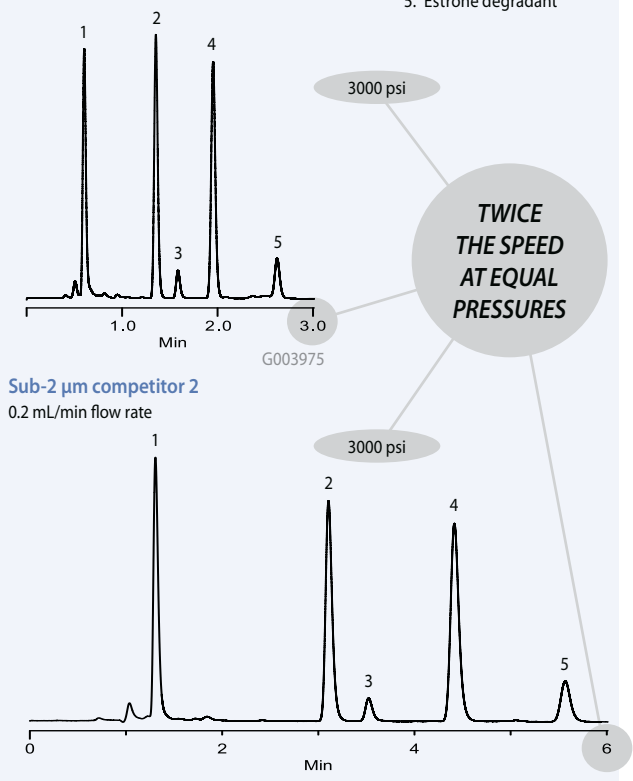
There are five distinct properties of Ascentis Express particles that account for their high performance and are worth emphasizing:

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

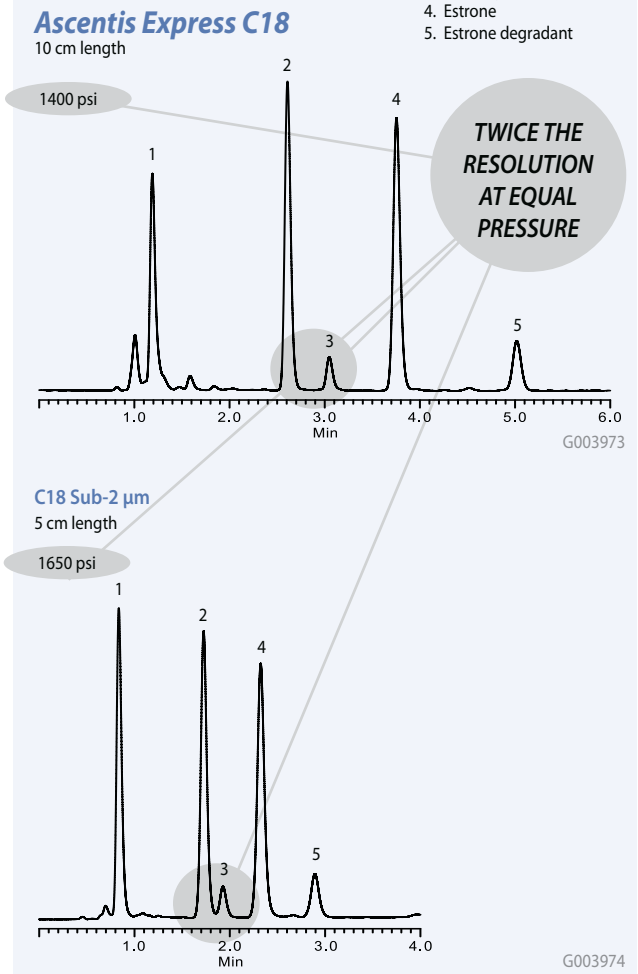
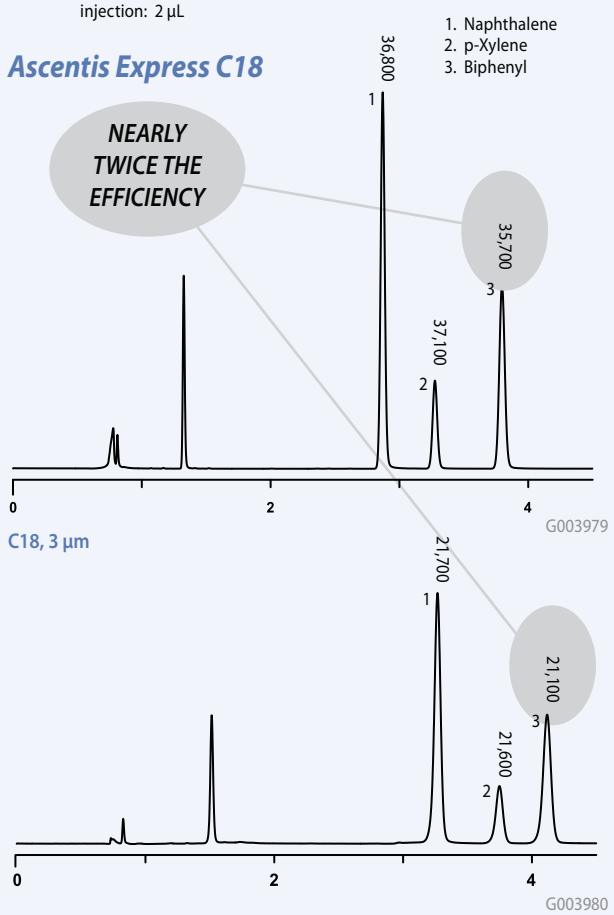


Figure 4. HD-Resolution on Ascentis Express Compared to 3 µm Particles

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
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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)

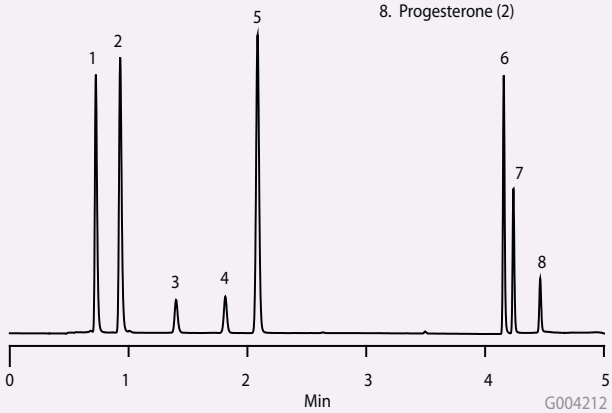
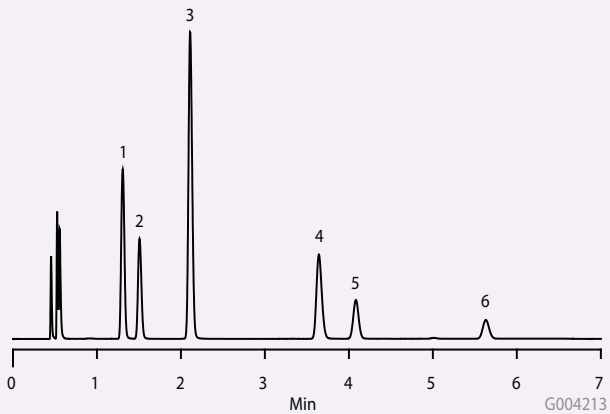


Figure 2. Basic Drug Panel

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).

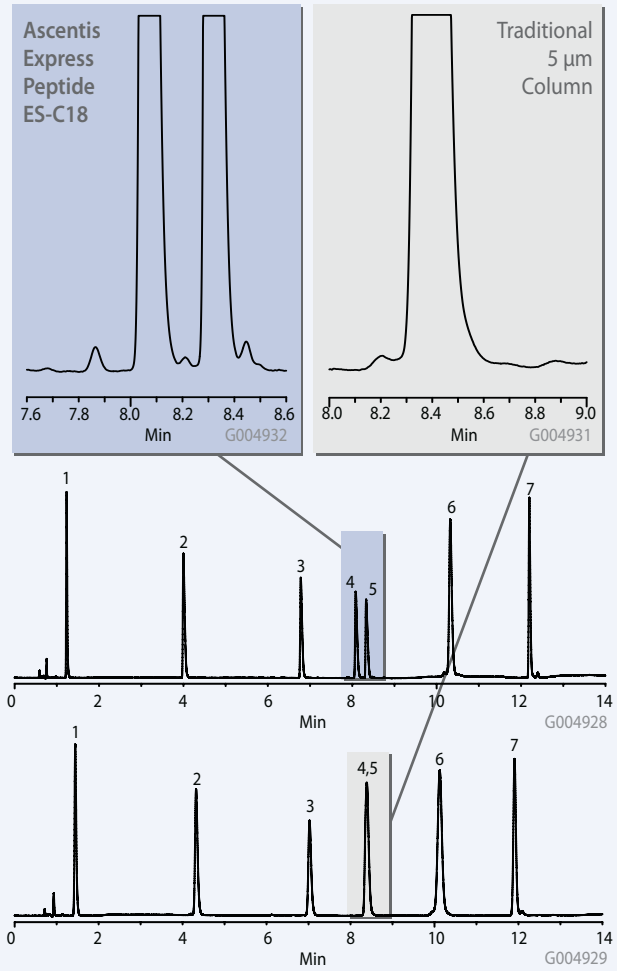
Shown in Figure 1 is the chromatographic separation of a peptide mix. The peptide mix contains a range of peptides in terms of molecular weight, basicity, and hydrophobicity. Excellent peak shape and peak width are achieved with a standard acetonitrile gradient and 0.1% TFA modifier. The resolution of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

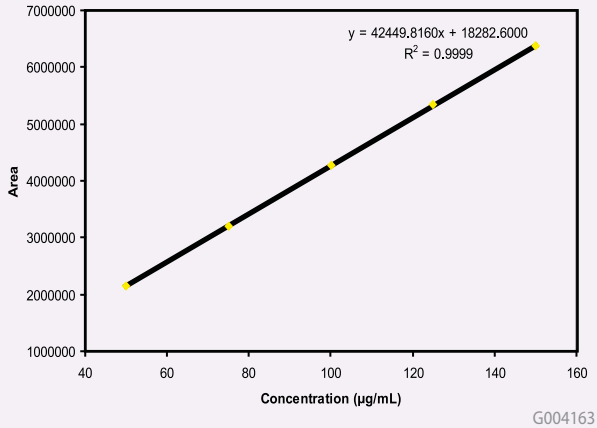

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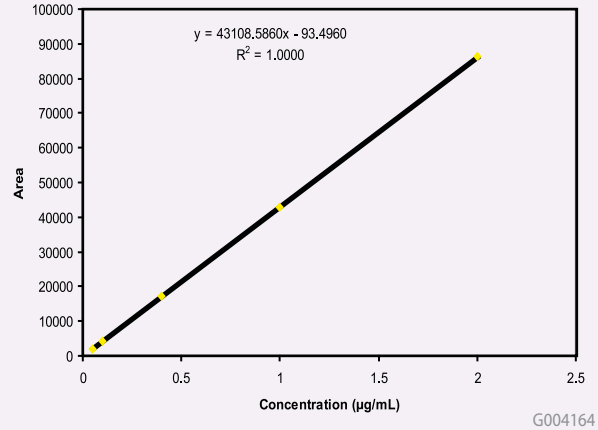
Figure 2. Linearity Curve from 0.05 to 2.0% Nominal Concentration



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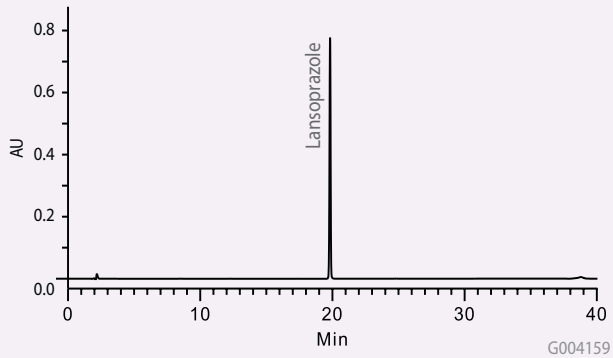
The new method was shown to be linear from 0.05% to 150% of nominal concentration of 100 mg/mL, with quantitation limit less than 0.05%. The broad range of linearity allows for simultaneous impurity and assay analysis. The linearity data are shown in Figures 1 and 2. The RSD of 5 replicate injections 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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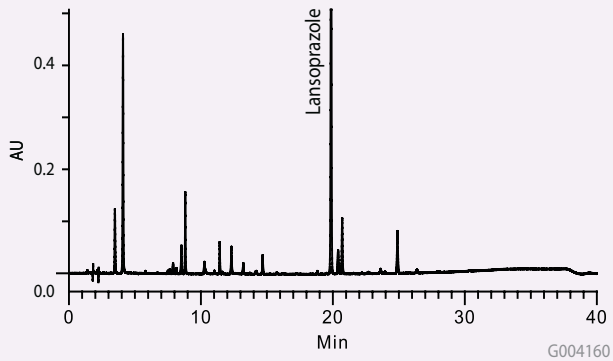
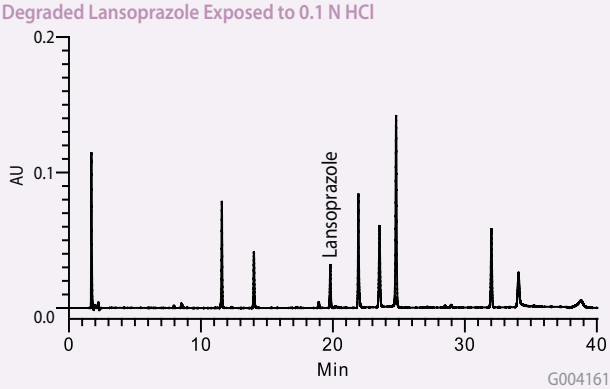
Aged Resolution Solution



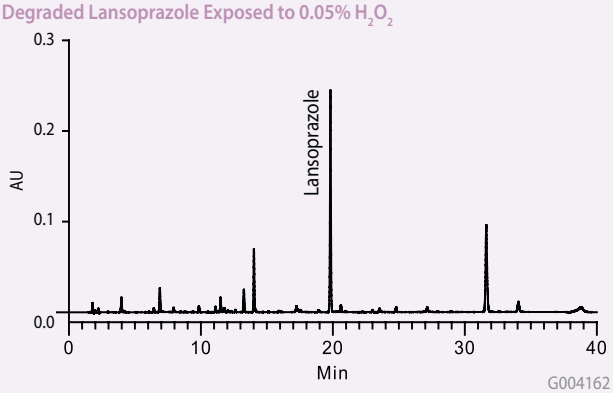
G004160

Degraded Lansoprazole Exposed to 0.1 N HCl



G004161

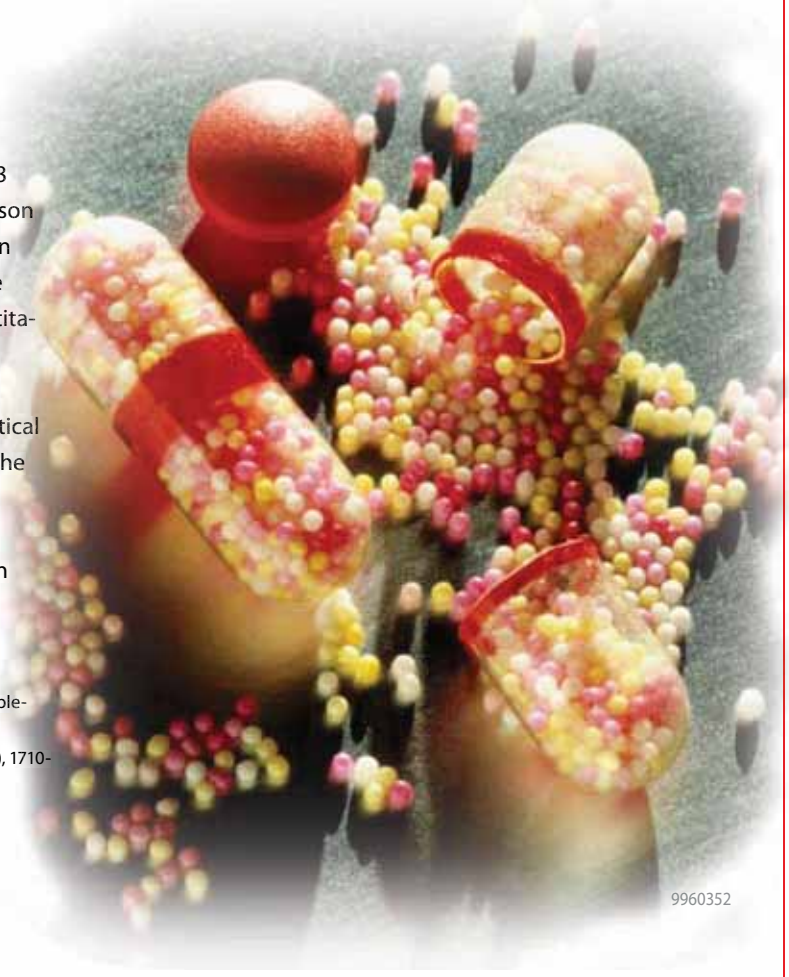
Degraded Lansoprazole Exposed to 0.05% H_2O_2



G004162

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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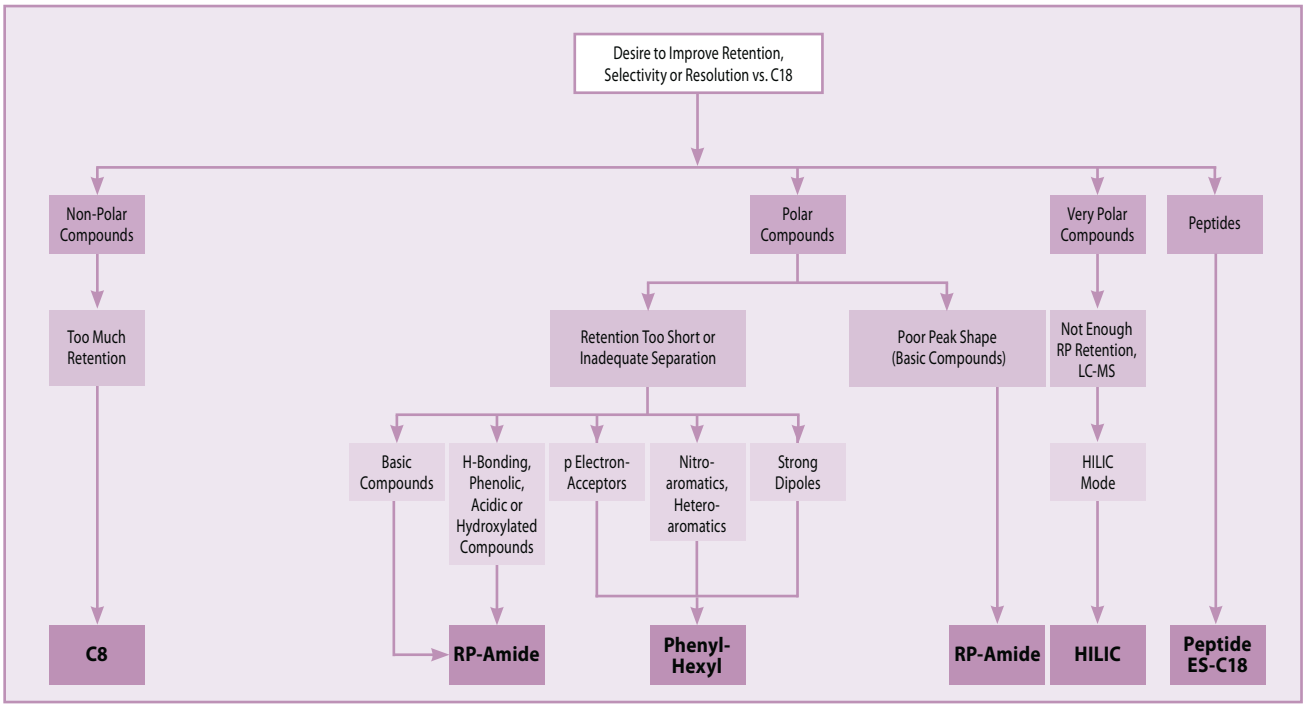
1. USP Monograph: Lansoprazole, USP 30-NF 25 through second supplement.
2. Pharmacopeial Forum, In-Process Revision, Lansoprazole, Vol. 32(6), 1710-1712 (2006).

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

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

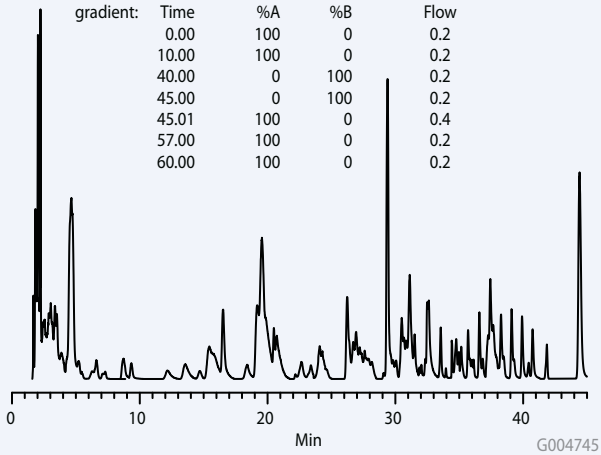
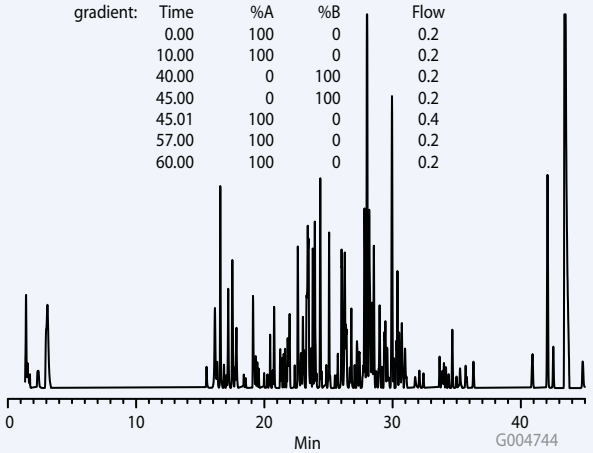


Figure 2. Component Chromatogram of Stevia Extract on Ascentis Express RP-Amide

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



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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
Affiliation: 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
Affiliation: 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
Affiliation: 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

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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
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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
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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
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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
Journal: Bioorganic & Medicinal Chemistry, 2008, 6116-6123

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Authors: Thomas L. Chester
Affiliation: Dept. of Chemistry, University of Cincinnati, Cincinnati, OH
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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

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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
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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

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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
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Affiliation: Analytical Applications Dept., Shimadzu Corp., Kyoto, Japan
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Authors: G. Ramis-Ramos, A. Micó-Tormos, E. F. Simó-Alfonso
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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

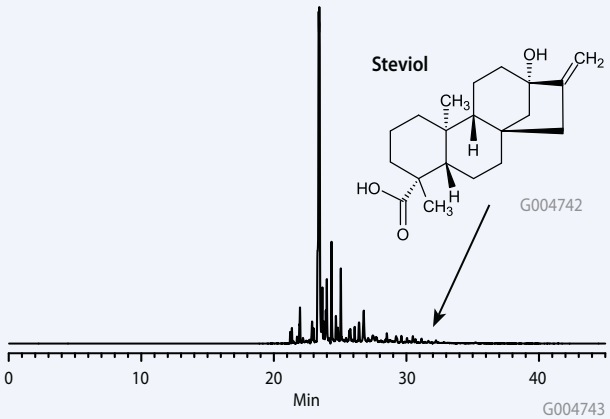
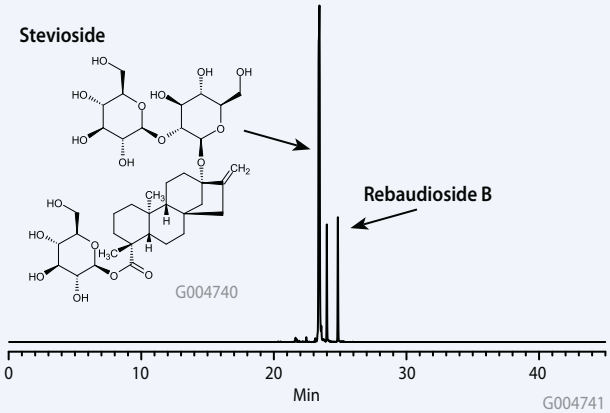


Figure 4. Stevia Extract on Ascentis Express RP-Amide, Extracted Ion Chromatogram for Stevioside



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

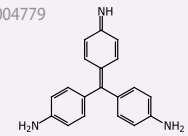
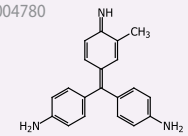
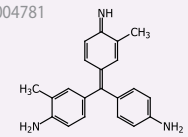
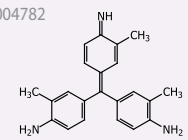
Table 1 contains a list of dyes added to one sample and dissolved in a mixture of methanol and acetonitrile. The sample was injected on an Ascentis Express C8 HPLC column under varying mobile phase conditions to determine the best separation parameters. Temperature, injection volume, detector settings,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

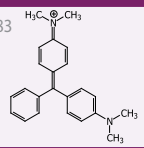
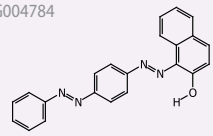
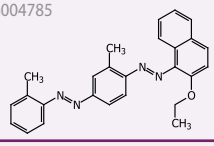
Peak No.	Structure	Name / Exact Mass
5	G004783 	Malachite Green $C_{23}H_{25}N_2$ 329.201773
6	G004784 	Sudan III $C_{22}H_{16}N_{4O}$ 352.132411
7	G004785 	Sudan 410 $C_{26}H_{24}N_{4O}$ 408.195011

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			Final Conditions		
solvents:	(A) water with 0.1% formic acid (B) acetonitrile			(A) water with 0.1% formic acid (B) acetonitrile:methanol (90:10)		
gradients:	Time	%A	%B	Time	%A	%B
	0.0	75	25%	0.0	75	25%
	1.5	75	25%	1.5	75	25%
	15.0	0	100%	15.0	2	98%
	22.0	0	100%	22.0	2	98%
	25.0	75	25%	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

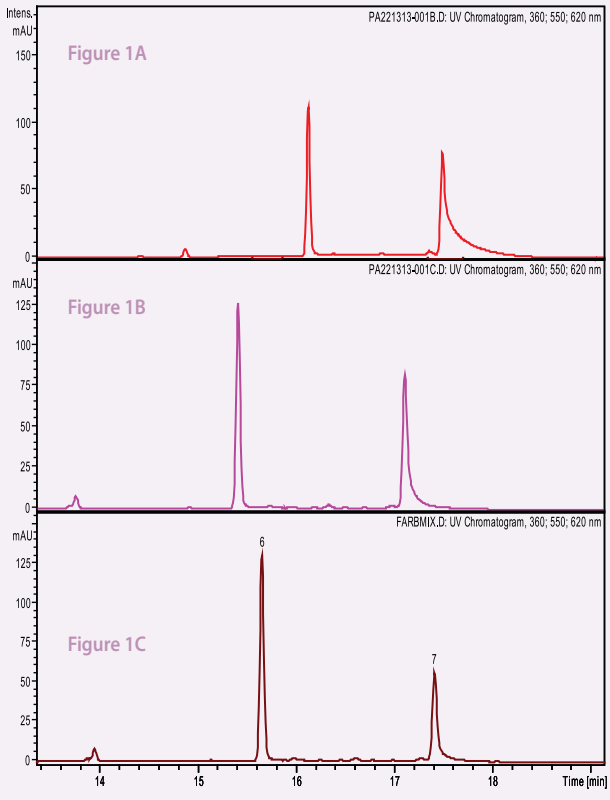
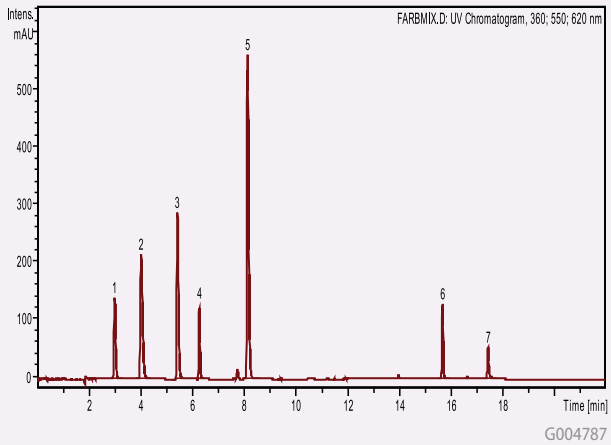


Figure 1 shows UV chromatograms of Sudan III (Peak 6) and Sudan 410 (Peak 7) after three optimization steps. An organic phase mixture of methanol:acetonitrile (10:90) and a final gradient composition of 98% organic mobile phase resulted in the best overall peak shapes with a minimum of tailing of compounds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

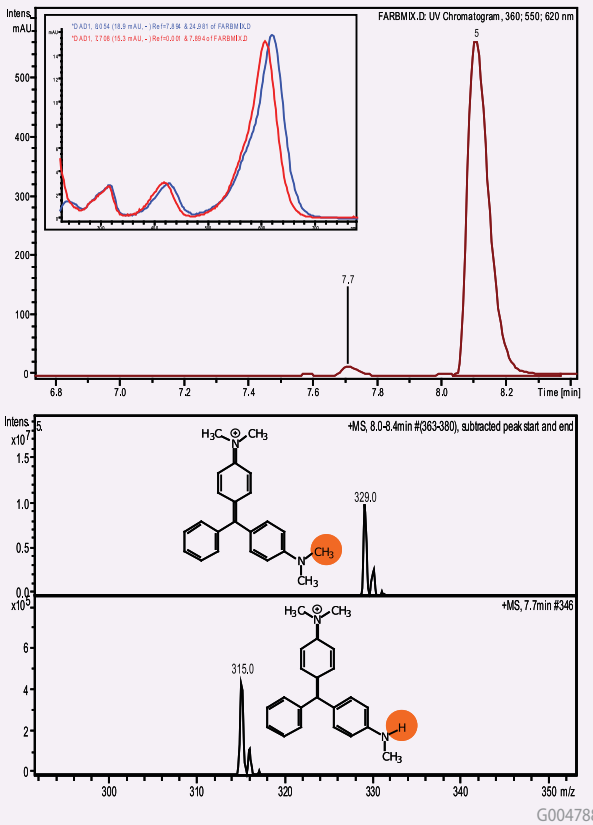


Ascentis Express HPLC Columns: High Efficiency and LC-MS Compatibility

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).



G004788

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

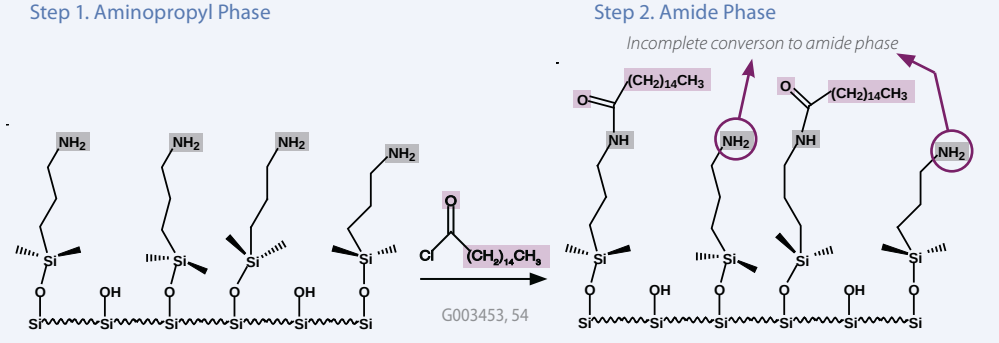
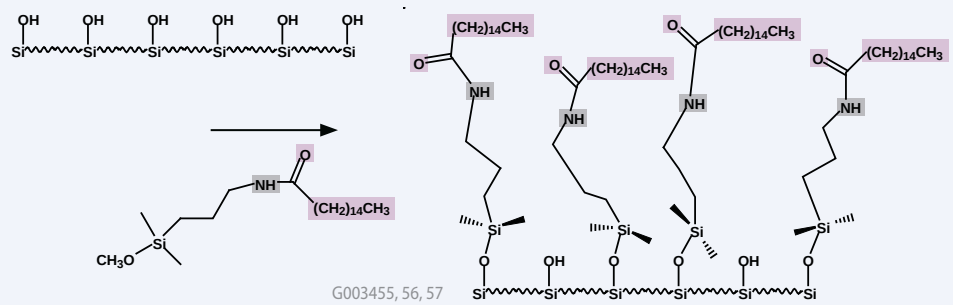


Figure 2. Next Generation One-step Bonding Process for EPG Amide Phases



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

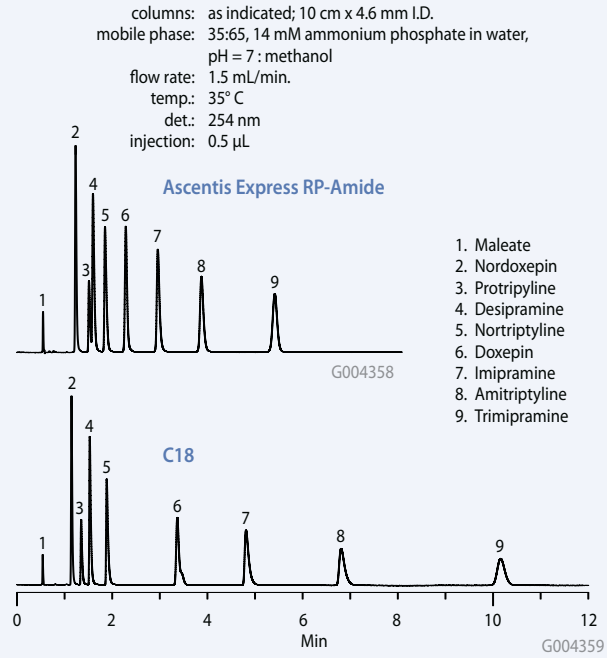


Figure 4. Asymmetry Factors for Difficult Bases on Ascentis Express RP-Amide and Conventional C18

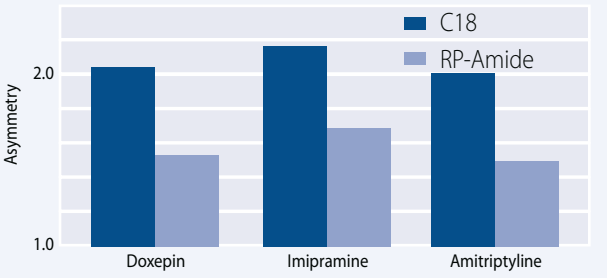
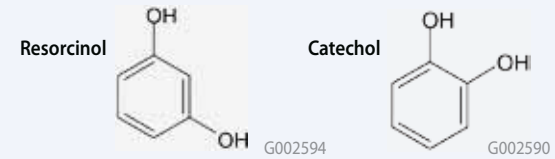


Figure 5. Separation of Phenolics on Ascentis Express RP-Amide & C18, & Waters BEH Shield RP18, 1.7 µm

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



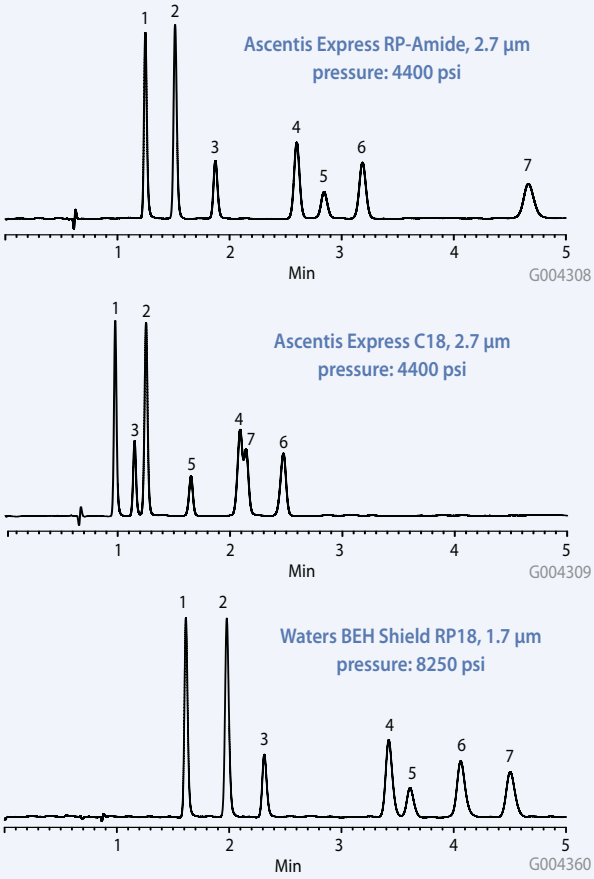
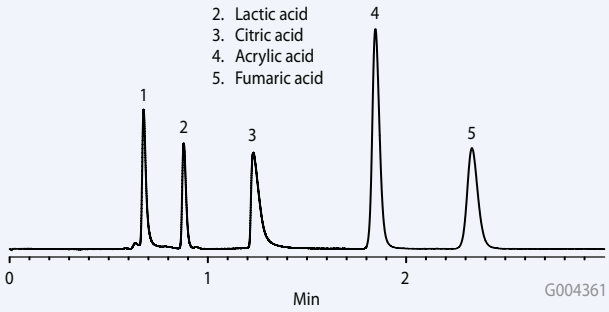


Figure 6. Separation of Small Organic Acids Under 100% Aqueous Conditions

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



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

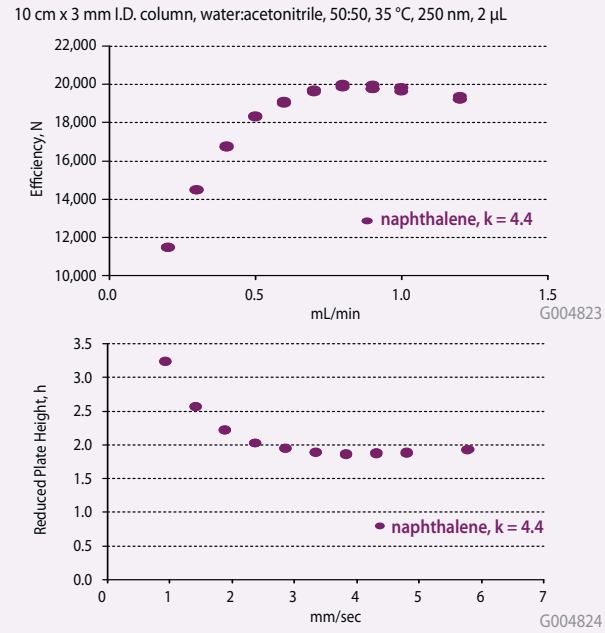


Figure 2. Benzodiazepine Structures

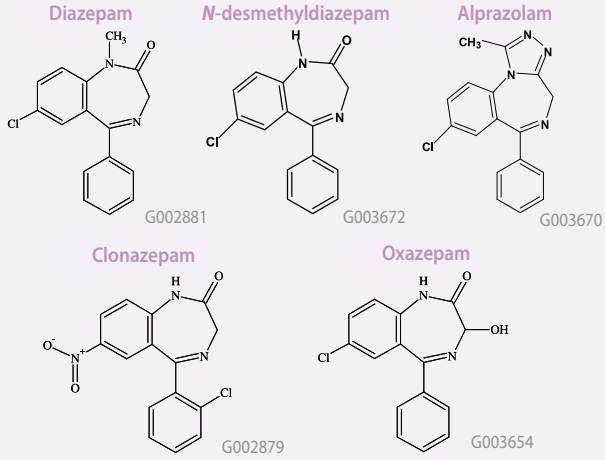
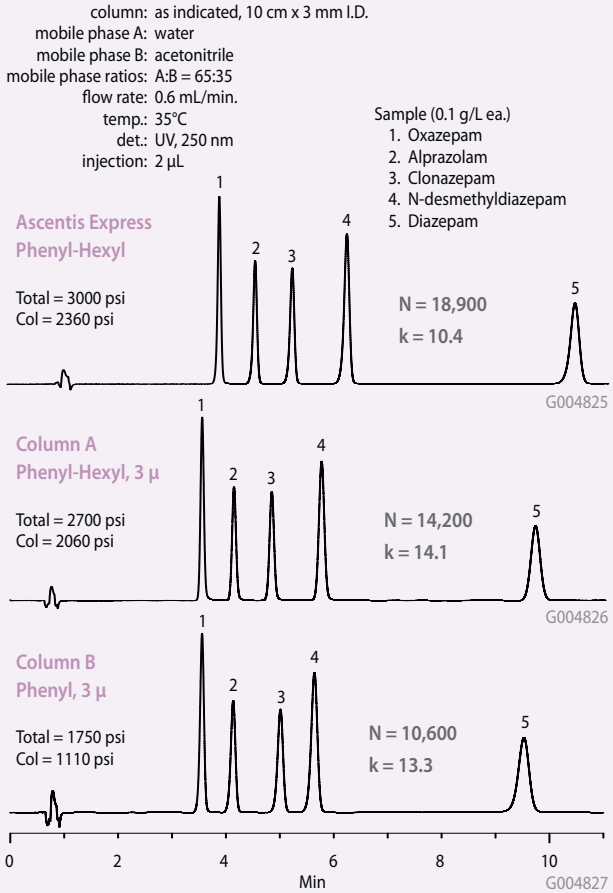


Figure 3. Comparison of Phenyl Column Selectivity for Benzodiazepines



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

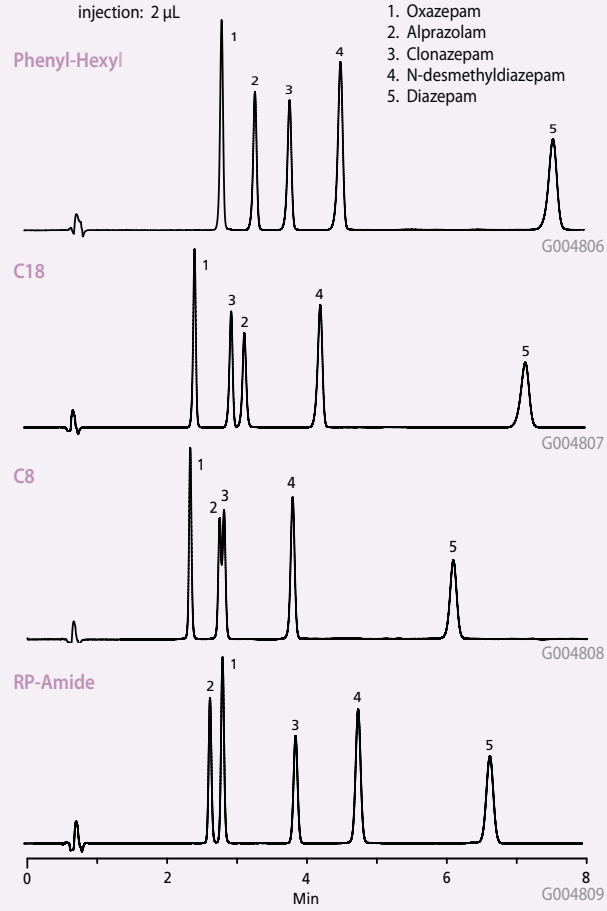
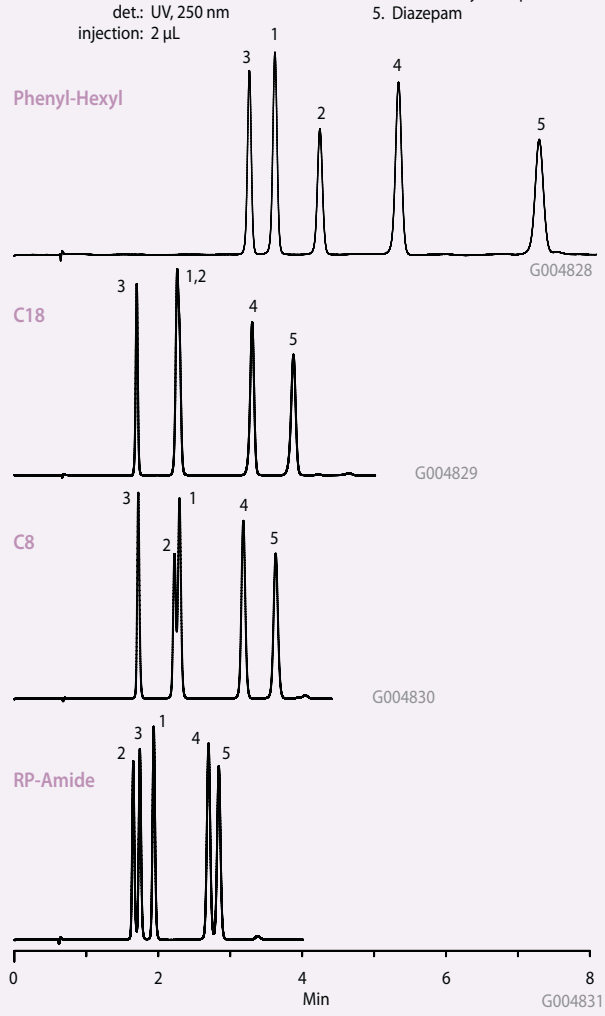


Figure 5. Benzodiazepines in 60% Methanol Mobile Phase with no Additive

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

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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