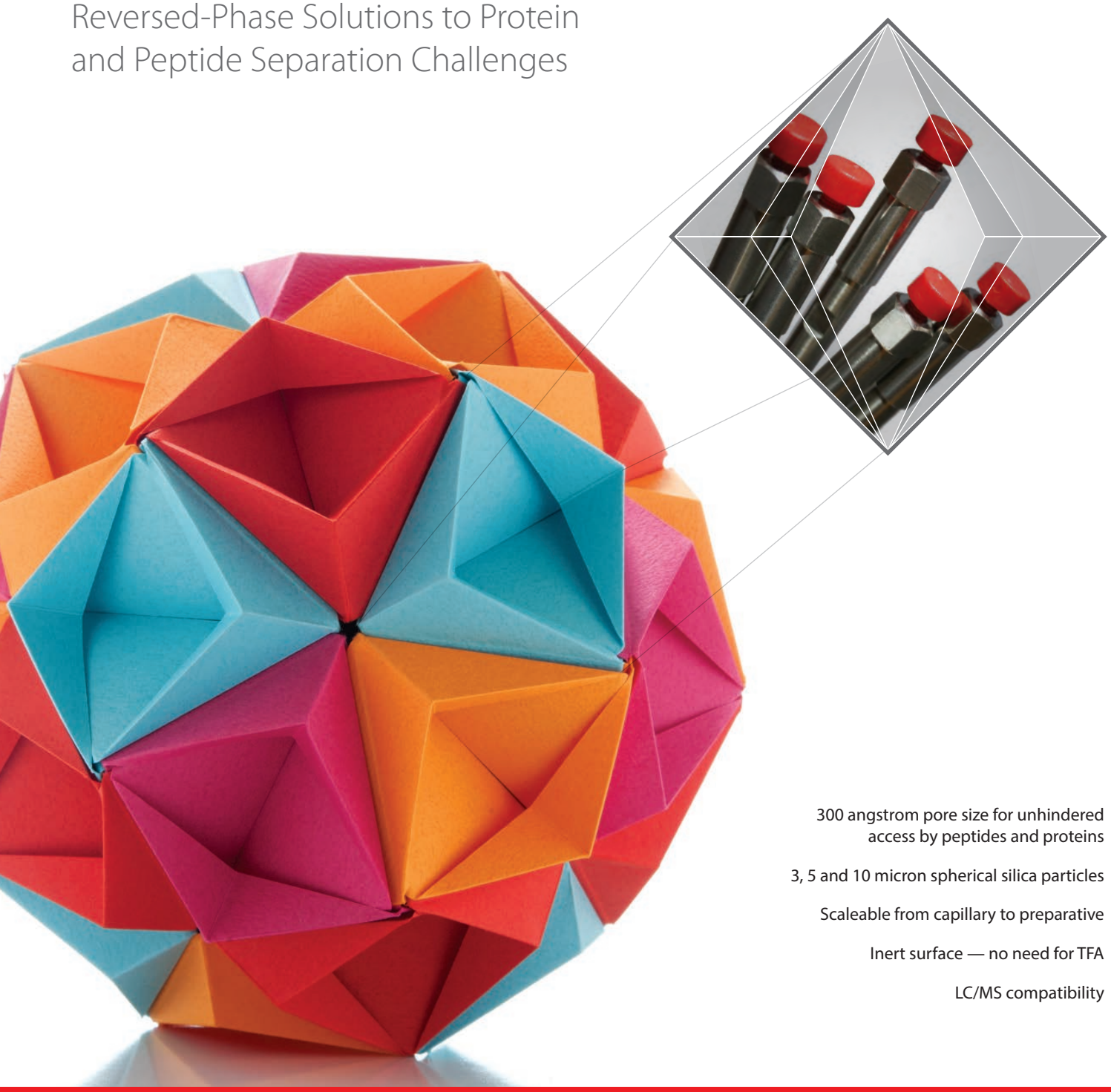
SUPELCO®
Solutions within.™

Discovery® BIO
Wide Pore HPLC Columns

Reversed-Phase Solutions to Protein and Peptide Separation Challenges



300 angstrom pore size for unhindered access by peptides and proteins

3, 5 and 10 micron spherical silica particles

Scaleable from capillary to preparative

Inert surface — no need for TFA

LC/MS compatibility

Discovery BIO Wide Pore Reversed Phase HPLC Columns

Meeting the challenges of protein and peptide separations

Many of the challenges facing researchers in the proteomics and biopharmaceutical fields are related to the need to obtain as much information as possible on very limited samples. Supelco designed Discovery BIO Wide Pore reversed-phase HPLC columns to address these challenges.

Discovery® BIO Wide Pore Reversed Phase HPLC columns and capillaries provide sensitive, stable, efficient, reproducible separations of proteins and peptides.

Octadecyl- (C18), octyl- (C8) and pentyl (C5) alkyl bonded phases provide unique selectivity to tailor to your separation needs.

Separations are directly scalable from analytical to preparative columns.

The low-bleed feature, and capillary column dimensions make them ideal for proteomics and other LC/MS applications.

Discovery BIO Wide Pore Reversed-Phase columns help you solve your protein and peptide separation challenges.

Separate Complex Protein or Peptide Mixtures

The selectivity and efficiency offered by Discovery BIO Wide Pore columns gives maximum power to resolve complex mixtures of proteins, natural or synthetic peptides, and peptide digests. Exceptional pH stability allows full use of mobile phase pH to adjust the separation.

Small Sample Volumes and Proteins at Low Concentrations or Low Copy Numbers

The efficiency of Discovery BIO Wide Pore reversed-phase columns maximizes the S/N ratio. Many Discovery BIO products are available in capillary and microbore dimensions.

The Need for Detailed Characterization

Because of sample complexity, biological samples often require multi-dimensional separations. Discovery BIO Wide Pore reversed phase columns are compatible with secondary separation or detection methods. If purified sample is required for further characterization, Discovery BIO phases are scalable from capillary to preparative column dimensions, each providing high sample recovery.

Large Number of Samples to Analyze

High sample throughput is achievable with the fast analysis times provided by short Discovery BIO Wide Pore columns packed with 3 micron particles.

Trouble-Free Operation

The stability and reproducibility of Discovery BIO Wide Pore reversed-phase columns permit reliable, trouble-free, and long term operation.

Reversed-Phase Solutions to Protein and Peptide Separation Challenges

High performance reversed-phase chromatography is the most widely used mode of chromatography for protein and peptide analysis and peptide purification. Discovery BIO Wide Pore reversed-phase columns provide sensitive, stable, efficient and reproducible separations of proteins and peptides.

The Discovery BIO Wide Pore family:

Highly-efficient, reversed-phase separations of proteins and peptides for proteomics, biotherapeutics, peptide mapping, and isolation and purification of natural or synthetic peptides.

Discovery BIO Wide Pore reversed-phase columns satisfy the need for efficiency, selectivity, LC/MS-sensitivity, stability, scalability, and reproducibility for reversed-phase HPLC analyses of proteins, peptides, and small biomolecules. Three phase chemistries, C18, C8, and C5, give unmatched selectivity and performance. Separations are completely scalable from analytical to preparative column dimensions. The low-bleed feature, inert surface chemistry, and microbore and capillary dimensions make them ideal for proteomics and LC/MS applications.

Significant benefits include:

- Better protein and peptide resolution compared to leading RP-HPLC columns
- High efficiency for peptide mapping
- Complementary selectivity choices with C5, C8, and C18 phase chemistries
- C5 has enhanced stability and lifetime compared to conventional C4 phases
- Excellent, no-bleed LC/MS properties
- Column dimensions from capillary to prep to cover all of your separation needs
- Guaranteed reproducibility run-to-run, column-to-column, batch-to-batch

In addition to Discovery BIO Wide Pore reversed-phase columns, the Discovery BIO product line also contains the Discovery BIO PolyMA line of ion exchange columns. Request publication T410079 or download at sigmaaldrich.com/bio-hplc.

Choosing a Discovery BIO Wide Pore Reversed Phase for Samples and Separation Modes

Sample or Usage	Separation Mode	Discovery BIO Product
Proteomics	Reversed-phase	Discovery BIO Wide Pore C18 in 0.18 to 0.5 mm I.D. capillaries
Peptide Mapping / Proteolytic Digests	Reversed-phase	Discovery BIO Wide Pore C18 Discovery BIO Wide Pore C8
Hydrophobic Peptides	Reversed-phase	Discovery BIO Wide Pore C5
Proteins	Reversed-phase	Discovery BIO Wide Pore C5

SUPELCO®
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Discovery BIO Wide Pore C18, C8, and C5

Highly-efficient, reversed-phase separations of proteins and peptides for proteomics, biotherapeutics, peptide mapping, and isolation and purification of natural or synthetic peptides.

Discovery BIO Wide Pore HPLC columns are packed with C5, C8, or C18 ligands bonded to 3, 5, or 10 µm, spherical, high purity silica particles containing 300 Å pores. All Discovery BIO Wide Pore products provide stable, efficient, and reproducible separations of proteins and peptides. The low-bleed character and excellent peak shape without TFA in the mobile phase makes Discovery BIO Wide Pore ideal for proteomics and other LC/MS applications and preparative purifications.

Discovery BIO Wide Pore Properties

	Discovery BIO Wide Pore C18	Discovery BIO Wide Pore C8	Discovery BIO Wide Pore C5
Bonded Phase	Octadecylsilane	Octylsilane	Pentylsilane
Endcap (yes / no)	Yes	Yes	Yes
Particle Platform	Silica	Silica	Silica
Particle Shape	Spherical	Spherical	Spherical
Particle Purity	<10 ppm metals	<10 ppm metals	<10 ppm metals
Particle Sizes (µm)	3, 5, 10	3, 5, 10	3, 5, 10
Pore Size (Å)	300	300	300
Surface Area (m^2/g)	100	100	100
% Carbon	~ 9	~ 5	~ 3.5
Coverage (µmoles/m^2)	~ 3.6	4.0	~ 4.5
pH range	2 to 8*	2 to 8*	2 to 8*
Temperature Range	up to 70 °C	up to 70 °C	up to 70 °C

* Recommended range is pH 2-8 but higher pH values are allowable using organic base buffer.

The Resolution Solution

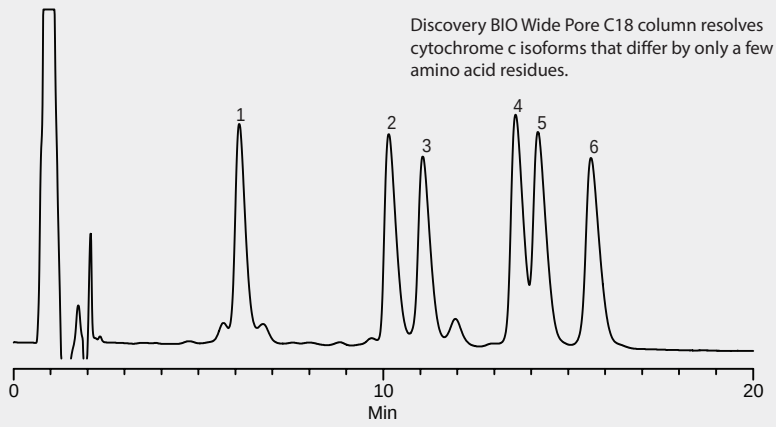
Reversed-phase HPLC (RP-HPLC) is the method of choice for many protein and peptide separations. New Discovery BIO Wide Pore columns provide state-of-the-art RP-HPLC technology.

Along with electrophoresis, ion exchange, and gel filtration, protein and peptide biochemists rely heavily on reversed-phase high performance liquid chromatography (RP-HPLC) based on wide pore silicas to perform many separations. RP-HPLC is a popular analytical tool for protein and peptide separations because it often provides better resolution over traditional ion-exchange and gel filtration methods. The power and utility of RP-HPLC is typified in the separation of cytochrome c isoforms shown in **Figure 1**.

Figure 1. Separation of Cytochrome C Isoforms on a Discovery BIO Wide Pore C18 Column

column: Discovery BIO Wide Pore C18, 15 cm x 4.6 mm, 5 µm (568222-U)
mobile phase: (A) 70:30, (0.1% TFA in water):(0.1% TFA in CH_3CN);
(B) 64:36, (0.1% TFA in water):(0.1% TFA in CH_3CN)
gradient: 0-100% B in 30 min
flow rate: 1.0 mL/min
column temp.: ambient
detector: UV, 220 nm
injection: 12 µL each at 0.8 mg/mL in 0.1%TFA

1. Horse cytochrome c
2. Rabbit cytochrome c
3. Cow cytochrome c
4. Pigeon cytochrome c
5. Chicken cytochrome c
6. Dog cytochrome c



Discovery BIO Wide Pore C18 column resolves cytochrome c isoforms that differ by only a few amino acid residues.

Meeting the Challenges of Protein and Peptide Separations

Efficiency and Inertness

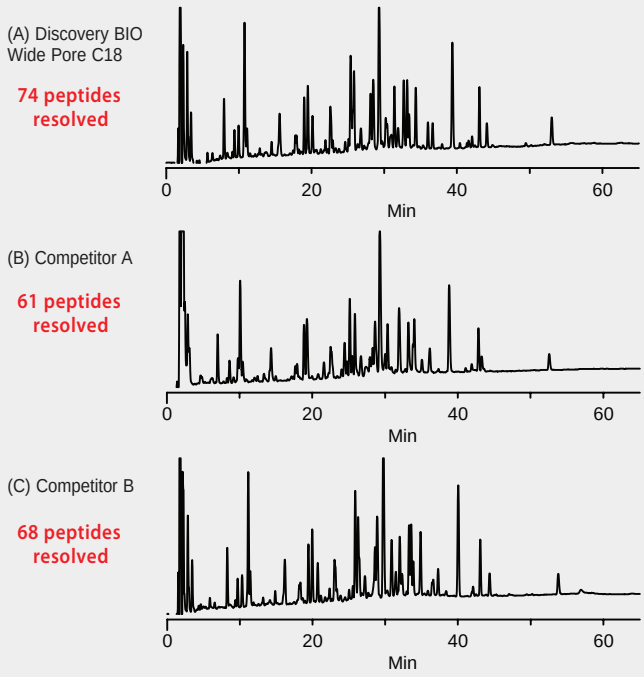
Discovery BIO Wide Pore columns provide the high efficiency required to obtain detailed fingerprints of peptide digests.

RP-HPLC is widely used for the separation of peptide fragments resulting from proteolytic digestion.

Peptide maps provide valuable information about protein structure, stability, and purity. The RP-HPLC column must be able to resolve a high percentage of peptides in the sample. The more peptide fragments, the better the information. As demonstrated in the tryptic digest of carboxymethylated apohemoglobin shown in **Figure 2,** the highly efficient Discovery BIO Wide Pore column provides impressive resolving power. The Discovery BIO Wide Pore C18 column resolved significantly more peptide fragments than the competitive columns under the same analysis conditions and detector settings. Resolution of the extra peptide fragments offers valuable protein structure sequencing information.

Figure 2. Tryptic Digest of Carboxymethylated Apohemoglobin on a Discovery Wide Pore C18 versus Competitive Columns

columns:	(A) Discovery BIO Wide Pore C18, 15 cm x 4.6 mm, 5 µm (568222-U),
	(B) and (C) Competitive protein and peptide C18, 15 cm x 4.6 mm, 300 Å, 5 µm;
mobile phase:	(A) 95:5, (0.1% TFA in water):(0.1% TFA in CH_3CN);
	(B) 50:50, (0.1% TFA in water):(0.1% TFA in CH_3CN)
gradient:	0-100% B in 65 min
flow rate:	1.0 mL/min
column temp.:	30 °C
detector:	UV, 215 nm
injection:	50 µL carboxymethylated apohemoglobin tryptic digest in 50 mM NH_4HCO_3



Note: The absolute number of peptides detected depends on the detector settings. In this comparison, the relative number of detected peptides is important, not the absolute number. The Discovery BIO Wide Pore C18 column detected more peptides relative to the competitive columns under the same conditions.

Challenge: Complex Protein or Peptide Mixtures

The efficiency, inertness, and selectivity offered by Discovery BIO Wide Pore gives maximum power to resolve complex mixtures of proteins, natural or synthetic peptides, and peptide digests. Because of the inert surface, **TFA is not required in the mobile phase to obtain good peak shape.**

Efficiency and Inertness

Discovery BIO Wide Pore C5 columns are more efficient than conventional C4 columns.

RP-HPLC provides structural information necessary to identify and assess purity and stability of biomolecules.

Proteins and polypeptides are subject to various types of molecular transformations that affect their biological activity and integrity. The RP-HPLC column must allow the scientist to see these degradation products and impurities.

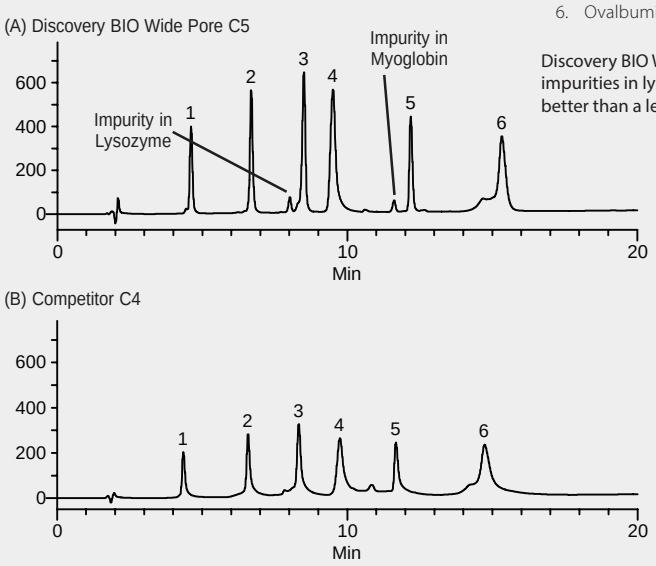
The Discovery BIO Wide Pore C5 column shown in **Figure 3** provides efficient, baseline resolution of six hydrophobic proteins and resolves impurities in lysozyme and myoglobin better than a leading competitive column.

Even without TFA in the mobile phase, Discovery BIO Wide Pore C5 gives acceptable peak shape and efficiency. (**Figure 4**).

Figure 3. Improved Separation of Proteins on Discovery BIO Wide Pore C5 versus a Competitive C4 Column

columns: (A) Discovery BIO Wide Pore C5, 15 cm x 4.6 mm, 5 µm (568422-U)
(B) Competitive protein and peptide C4, 15 cm x 4.6 mm, 300 Å, 5 µm
mobile phase: (A) 75:25, (0.1% TFA in water):(0.1% TFA in CH_3CN);
(B) 25:75, (0.1% TFA in water):(0.1% TFA in CH_3CN)
gradient: 0-100% B in 25 min
flow rate: 1.0 mL/min
column temp.: ambient
detector: UV, 220 nm
injection: 12 µL in 0.1 %TFA

1. RNase (13.7 kDa, 1 mg/mL)
2. Cytochrome c (12.4 kDa, 1 mg/mL)
3. Lysozyme (14.3 kDa, 1 mg/mL)
4. BSA (67.0 kDa, 2.5 mg/mL)
5. Myoglobin (18.8 kDa, 1 mg/mL)
6. Ovalbumin (45.3 kDa, 3.5 mg/mL)



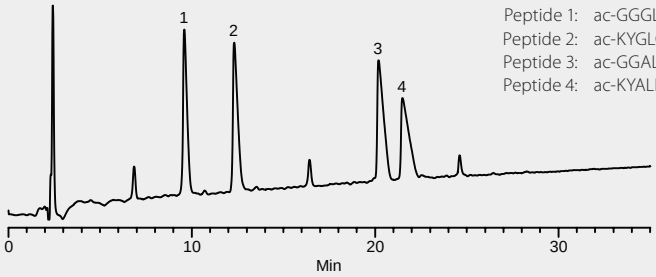
Discovery BIO Wide Pore C5 resolves impurities in lysozyme and myoglobin better than a leading competitive column.

Figure 4. Efficient Analysis of Basic Peptides on Discovery BIO Wide Pore C5 without TFA

column: Discovery BIO Wide Pore C5, 15 cm x 2.1 mm, 5 µm (568402-U)
mobile phase: (A) 25 mM HCOOH in water; (B) 50:50 (25 mM HCOOH in water) : (20 mM HCOOH in CH_3CN)
gradient:

Min	% B
0	15
35	50

flow rate: 0.208 mL/min
detector: UV, 215 nm
column temp.: 35 °C
inj.: 0.5 µL (~0.25 µg each peptide)
sample: RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)



Peptide 1: ac-GGGLGGAGGLK-amide
Peptide 2: ac-KYGLGGAGGLK-amide
Peptide 3: ac-GGALKALKGLK-amide
Peptide 4: ac-KYALKALKGLK-amide

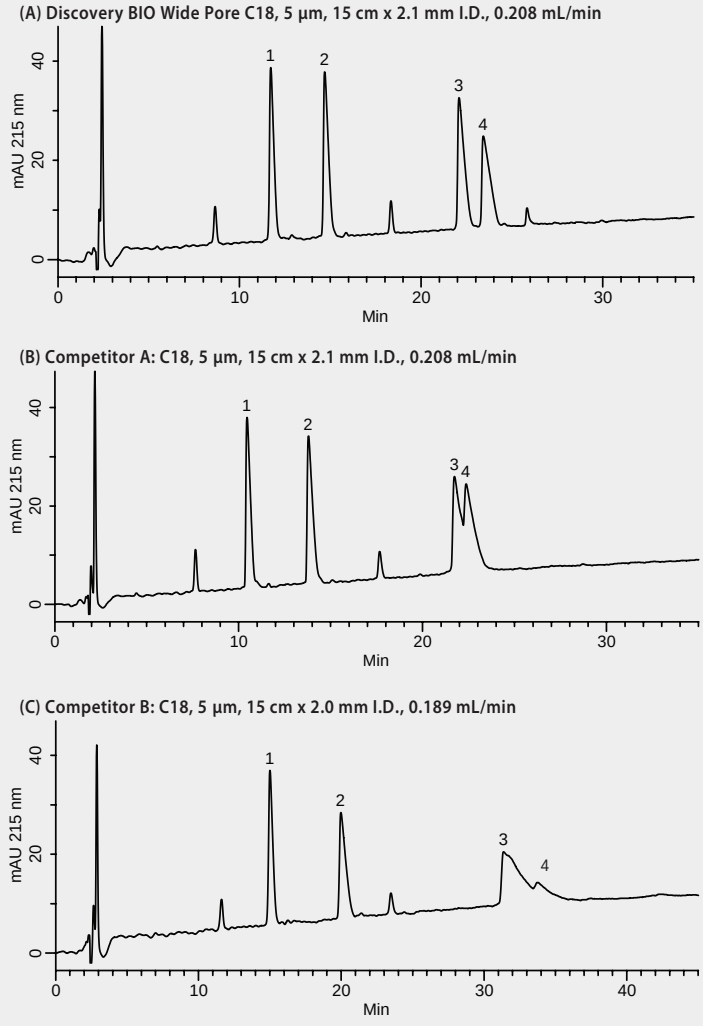
No TFA required — The inert surface of Discovery BIO Wide Pore phases gives excellent peak shape without suppressing the LC/MS signal.

Maximizing Peak Efficiency in LC/MS

When used at traditional concentrations, typically above 0.05% (v/v), TFA (trifluoroacetic acid) can effectively mask silanol interactions. Under those conditions, most modern RP-HPLC columns will give good peak shape. However, TFA also reduces the sensitivity of MS analyses. When TFA is absent or used at very low concentrations (for the purpose of maximizing MS sensitivity), the inertness, or lack of silanol activity, of the column becomes increasingly relevant to attaining the best chromatographic performance. Differences in column inertness are obvious under low- or no-TFA conditions. **Figure 5** shows the separation of four basic peptides on Discovery BIO Wide Pore C18 and two other modern wide pore C18 silica columns. Results show that under the same conditions, Discovery BIO Wide Pore C18 provides superior resolution of the most basic and hydrophobic peptides. This clearly illustrates that selecting the best column becomes a critical element for developing efficient and sensitive LC/MS methods.

Figure 5. Column Performance Differences toward Basic Peptides without TFA

columns:	(A) Discovery BIO Wide Pore C18, 15 cm x 2.1 mm I.D., 5 µm (568202-U) (B) and (C) Competitive protein and peptide C18, 300 Å, 15 cm x 2.0 or 2.1 mm I.D., 5 µm
mobile phase:	(A) 25 mM HCOOH in water; (B) 50:50 (25 mM HCOOH in water) : (20 mM HCOOH in CH_3CN)
gradient:	Min %B 0 15 45 60
flow rate:	0.208 (or 0.189) mL/min
detector:	UV, 215 nm
column temp.:	35 °C
injection:	0.5 µL (~ 0.25 µg each peptide)
sample:	RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)
Peptide 1:	ac-GGGLGGAGGLK-amide
Peptide 2:	ac-KYGLGGAGGLK-amide
Peptide 3:	ac-GGALKALKGLK-amide
Peptide 4:	ac-KYALKALKGLK-amide



Selectivity

The different selectivities of Discovery BIO Wide Pore columns over other reversed-phase columns benefits the resolution of natural and synthetic peptide mixtures.

Solid phase peptide synthesis relies on RP-HPLC

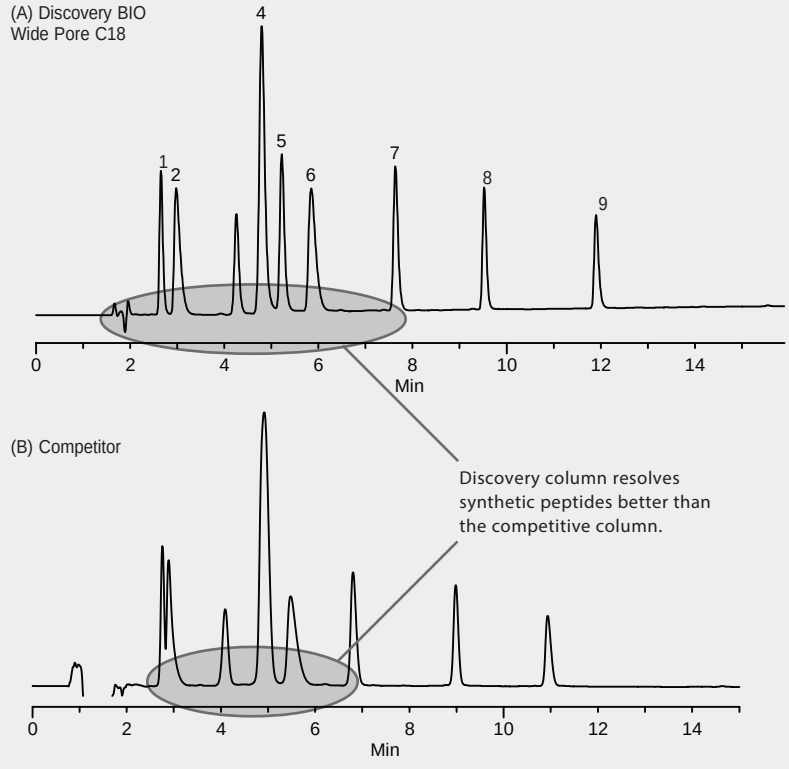
Solid phase synthesis is a common method to obtain novel peptides quickly and efficiently. Unintended side reactions are common and the RP-HPLC method must be capable of separating the peptides of interest from unwanted by-products. Discovery BIO Wide Pore columns are ideal for this application. **Figure 6** shows better resolution of a mixture of synthetic peptides on a Discovery BIO Wide Pore C18 column versus a leading, competitive C18 column.

Figure 6. Mixture of Synthetic Peptides on Discovery BIO Wide Pore C18 and a Leading Competitive Column

columns: (A) Discovery BIO Wide Pore C18, 15 cm x 4.6 mm, 5 µm (568222-U),
(B) Competitive protein and peptide C18, 15 cm x 4.6 mm, 300Å, 5 µm
mobile phase: (A) 80:20, (0.1% TFA in water): (0.1% TFA in CH_3CN);
(B) 66:34, (0.1% TFA in water): (0.1% TFA in CH_3CN)
gradient: 0-100% B in 14 min after 1 minute delay
flow rate: 1.0 mL/min
column temp.: 30 °C
detector: UV, 220 nm
injection: 10 µL, ~0.25 µg each peptide (Arg8- vasopressin, bradykinin (fragment 1-5), oxytocin, luteinizing hormone releasing hormone, Met-enkephalin, bradykinin, Leu-enkephalin, bombesin, Substance P) in 0.1% TFA. See sequence in **Figure 14**.

Peak	Peptide	Amino Acid Sequence
1	Arg8-vassopressin	CYFQNCPRG-amide; disulfide
2	Bradykinin, fragment 1-5	RPPGF
3	Oxytocin	CYIQNCPLG-amide; disulfide
4	LHRH*	pEHWSYGLRPG-amide **
5	Met-enkephalin	YGGFM
6	Bradykinin	RPPGFSPFR
7	Leu-enkephalin	YGGFL
8	Bombesin	pEQRLGNQWAVGHLM-amide **
9	Substance	P RPKPQQFFGLM-amide

* Luteinizing Hormone Releasing Hormone ** pE is pyroglutamate



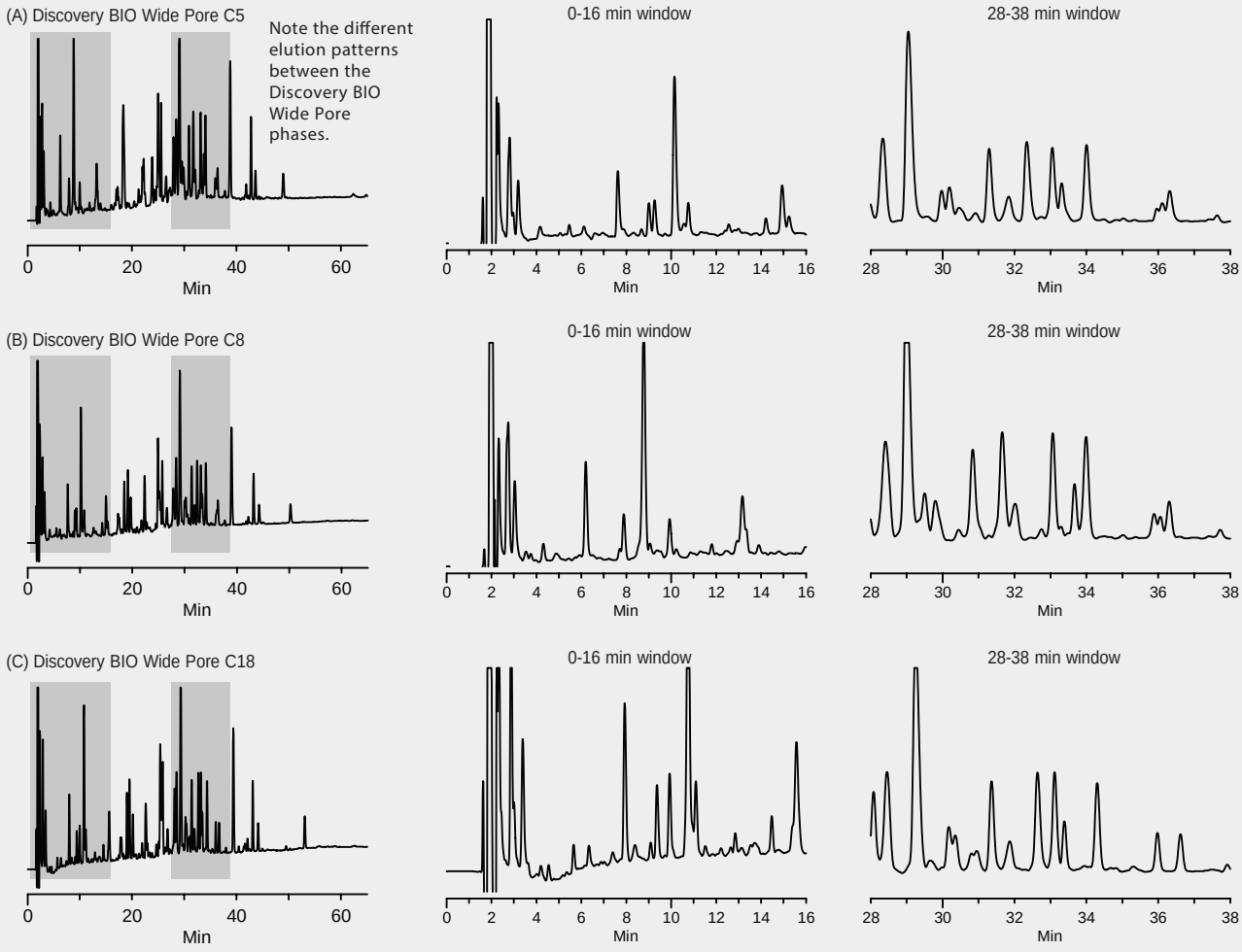
Each of the Discovery BIO Wide Pore phases offers a different profile of peptide digests allowing you to more accurately reconstruct the parent protein.

RP-HPLC stationary phase chemistry provides the selectivity and retention needed to resolve proteins and peptides. Discovery BIO Wide Pore columns maximize your choices in selectivity by offering C18, C8, and C5 chemistries.

The chain length (number of carbon atoms) of the bonded phase is important because it affects the type of proteins and peptides that can be analyzed, the mobile phase options, and the ultimate resolution of the separation. Having a choice is crucial. Supelco's Discovery BIO Wide Pore C5, C8, and C18 columns display different selectivity towards the peptide mixtures shown in **Figure 7**. Notable differences appear throughout the elution profiles in the expanded time ranges shown.

Figure 7. Each Discovery BIO Wide Pore Phase Gives Unique Elution Profiles of Carboxymethylated Apohemoglobin Peptide Fragments

columns: (A) Discovery BIO Wide Pore C5 (568422-U); (B) Discovery BIO Wide Pore C8 (568322-U); or (C) Discovery BIO Wide Pore C18 (568222-U), each 15 cm x 4.6 mm, 5 µm
mobile phase: (A) 95:5, (0.1% TFA in water):(0.1% TFA in CH_3CN); (B) 50:50, (0.1% TFA in water):(0.1% TFA in CH_3CN)
gradient: 0-100% B in 65 min
flow rate: 1.0 mL/min
column temp.: 30 °C
detector: UV, 215 nm
injection: 50 µL carboxymethylated apohemoglobin tryptic digest in 50 mM NH_4HCO_3



LC/MS Sensitivity: Capillary and Microbore Dimensions

Capillary and microbore dimensions of Discovery BIO Wide Pore provide enhanced sensitivity and lower sample consumption.

In the world of modern HPLC separations, smaller is often better. Columns with narrow I.D. can enhance sensitivity when dealing with a limited sample size. This makes them ideal for applications where the need is to detect compounds that exist at very low concentration in small sample volumes, in other words, when the sample components would be diluted beyond the point of detection on a standard bore column. The low flow rates and miniscule solvent consumption also makes narrow I.D. columns ideal for LC/MS applications because of the lower solvent desolvation volume.

The Trend Toward Smaller I.D. Columns

Proteomics and other areas of modern biological research often generate a large number of small volume samples that contain minute quantities of compounds which need to be analyzed in a minimal amount of time. When the concentration and the volume of the sample are sufficiently small, the employed means of detection may not be sensitive enough when using a conventional 4.6 mm I.D. or even a narrow bore 2.1 mm I.D. column. This may be the case whether detection is by UV absorption or mass spectrometry where inlet systems can be concentration dependent as well.

A direct approach to reducing the extent of dilution is to reduce the column volume. Compared to conventional ID columns, the flow rate and thus the linear velocity on narrower ID columns is lower by the square of the column diameter. The same is true for the peak volumes in which the sample components elute from the column. When only a small sample mass is available for analysis, the sensitivity on a smaller ID column will be higher than on a larger ID column as long as (1) the same sample mass is injected on both columns and (2) the injected sample mass does not cause overload on the smaller ID column. One can detect levels that are 100 or even 1,000 fold lower by decreasing the column I.D.. These principles are illustrated in **Tables 1** and **2** (next page), which all relate to each other by relative cross-sectional areas of the various column dimensions.

Figures 8 and **9** on this page show how column diameter, flow rate and mass injected relate to detection sensitivity. In **Figure 8**, sample mass was scaled down proportionally with the reduction in column I.D., while in **Figure 9** the same mass was injected on both columns. The graph in **Figure 9** represents the sample mass limited case.

Figure 8. Sensitivity as a Function of Column Diameter

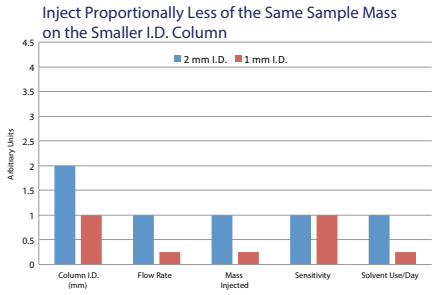
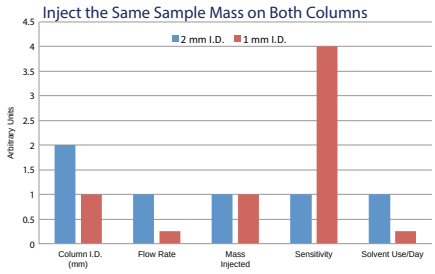


Figure 9. Sensitivity as a Function of Column Diameter



Challenge: Provide a Detailed Characterization of Proteins Present at Low Concentration in Small Sample Volumes

The efficiency of Discovery BIO Wide Pore provides sensitive analyses, especially when combined with capillary and microbore dimensions. TFA is a common mobile phase additive in protein and peptide separations. It improves peak shape on poor quality HPLC phases, but decreases the LC/MS signal. Because of the inert surface of Discovery BIO Wide Pore columns, TFA is not required in the mobile phase to obtain acceptable peak shape, thereby increasing LC/MS sensitivity. Discovery BIO Wide Pore phases will also provide bleed-free LC/MS analyses. Often purified sample is needed for further characterization. Discovery BIO Wide Pore phases are scalable from analytical to preparative column formats in a predictable fashion.

Increased Sensitivity Demonstrated

Figure 10 shows the observed behavior of increased sensitivity on columns of decreasing I.D. The same sample mass was injected onto Discovery BIO Wide Pore C18 columns of varying I.D. from 0.32 mm to 2.1 mm I.D., on the same chromatographic system. Linear velocity (L/t_0) was held constant, an important consideration when comparing columns of different diameters. The relative corresponding peak heights closely approximate what is mathematically predicted in **Tables 1** and **2**.

Figure 10. Comparison of Peak Height (Sensitivity) Between Columns of Different Internal Diameters

columns: Discovery BIO Wide Pore C18, 300 Å, 10 cm x 0.32 mm I.D., 0.50 mm I.D., 1.0 mm I.D. or 2.1 mm I.D., 3 µm
mobile phase: (65:35) H_2O:CH_3CN
flow rate: shown on figures
column temp.: ambient
detector: UV, 254 nm
injection: shown on figures
sample mass: acetophenone (0.1 µg), benzene (1 µg), toluene (1 µg)

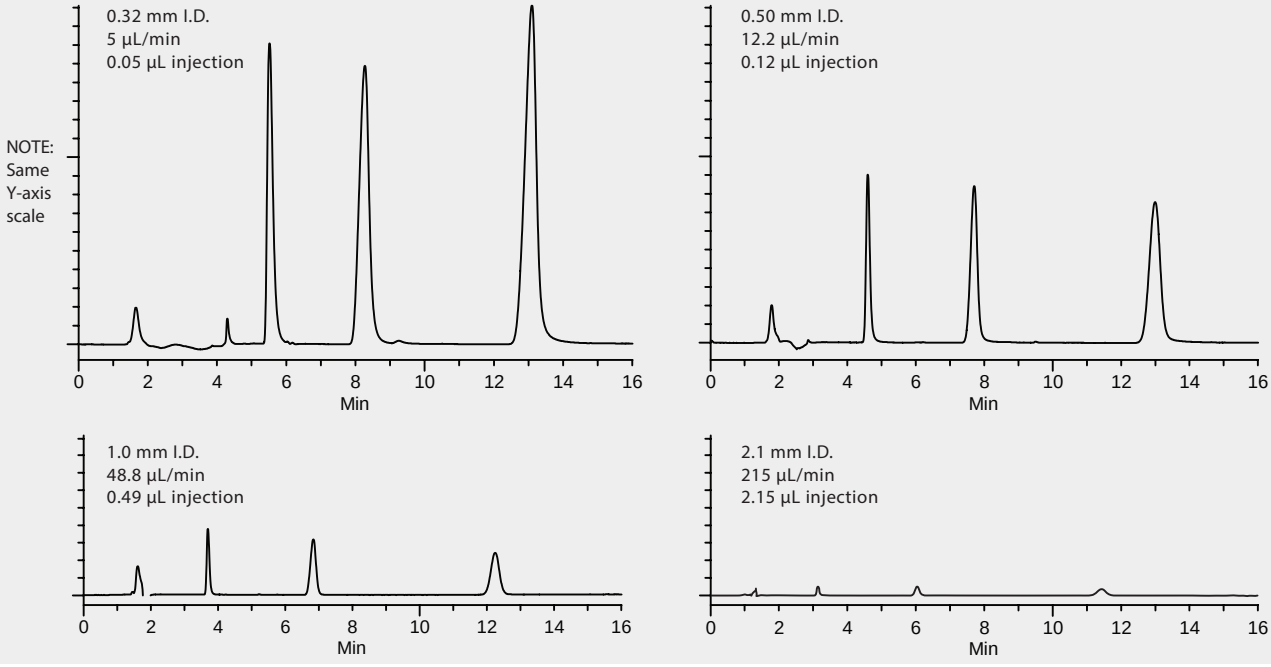


Table 1. Effect of Column Dimension on Sensitivity for a Limiting Sample Mass

Column I.D. (mm)	Relative Volumetric Flow*	Relative Sample Mass	Relative Sensitivity
4.6	1	1	1
3.0	0.42	1	2
2.1	0.21	1	5
1.0	0.047	1	21
0.50	0.012	1	85
0.32	0.0048	1	207
0.18	0.0015	1	653

Table 2. Effect of Column Dimension on Required Sample Mass for a Given Sensitivity

Column I.D. (mm)	Relative Volumetric Flow*	Relative Sample Mass	Relative Sensitivity
4.6	1	1	1
3.0	0.42	0.42	1
2.1	0.21	0.21	1
1.0	0.047	0.047	1
0.50	0.012	0.012	1
0.32	0.0048	0.0048	1
0.18	0.0015	0.0015	1

* Assumes constant linear velocity, equivalent column length and efficiency (plates/meter), and no significant extra-column volume nor mass overload on the column with the smallest internal diameter.

LC/MS Sensitivity: Capillary and Microbore Dimensions

Use Discovery BIO Wide Pore in capillary and microbore dimensions to obtain the maximum amount of information from a minimum amount of sample.

Conserve precious samples and detect very low levels of proteins and peptides on Discovery BIO Wide Pore capillary or microbore dimensions without sacrificing efficiency or resolution.

Proteomics researchers rely on peptide digests to help identify proteins and provide other critical information. Two challenges facing researchers in the proteomics field are the need to maximize the information from a very limited amount of sample and the need to detect proteins that exist at very low concentration in the sample. One solution to both of these needs is to use capillary or microbore HPLC column dimensions. Because samples are diluted over a smaller column volume, capillary and microbore columns give greater peak height (sensitivity) than columns with conventional internal diameters (*e.g.* 4.6 mm). Interfaced directly with a mass spectrometer, capillary columns help provide structural information on proteins or peptides at extremely low copy numbers in the cell. **Figures 11** and **12** show the utility of Discovery BIO Wide Pore C18 capillary columns for sensitive peptide analysis by LC/MS.

Figure 11. ß-Lactoglobulin Tryptic Digest on 0.5 mm I.D. Discovery BIO Wide Pore C18 Capillary

column: Discovery BIO Wide Pore C18, 15 cm x 0.5 mm I.D., 5 µm (65519-U)
mobile phase: (A) 0.1% TFA in water; (B) 0.1% TFA in CH_3CN
gradient: 5-40% B in 70 min
flow rate: 14 µL/min
column temp.: 30 °C
injection: 500 pmol (5 µL) ß-Lactoglobulin tryptic digest in 50 mM NH_4HCO_3
MS conditions: +ESI mode Capillary Temp 130 °C, Source Voltage 2.5 KV, Capillary Voltage 12 V

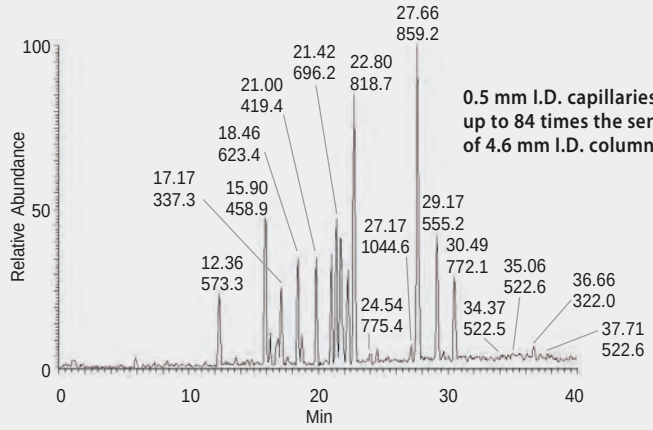
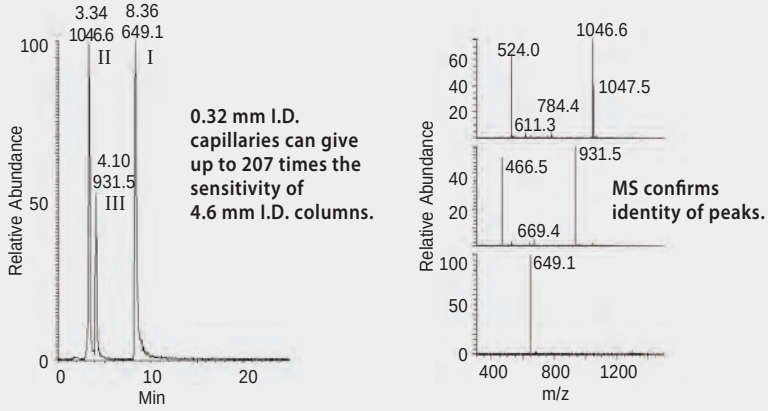


Figure 12. Angiotensins on 0.32 mm I.D. Discovery BIO Wide Pore C18 Capillary

column: Discovery BIO Wide Pore C18, 10 cm x 0.32 mm I.D., 3 µm (65527-U)
mobile phase: (A) 65:35, (10 mM NH_4OAc, pH 7):(50% CH_3CN in 20 mM NH_4OAc, pH 7)
(B) 25:75, (10 mM NH_4OAc, pH 7):(50% CH_3CN in 20 mM NH_4OAc, pH 7)
flow rate: 6 µL/min
column temp.: ambient
injection: 50 pmol in water
gradient: 0-100% B in 12.5 min
MS conditions: +ESI mode Capillary Temp 130 °C, Source Voltage 2.5 KV, Capillary Voltage 12 V



LC/MS Sensitivity: No TFA Needed

Discovery BIO Wide Pore phases improve sensitivity by giving symmetrical, efficient peaks without TFA in the mobile phase.

TFA (trifluoroacetic acid) is a commonly used mobile phase additive for reversed-phase HPLC (RP-HPLC) separations of proteins and peptides. The benefit of using TFA is improved peak shape for more basic peptides by forming an ion-pair and thus reducing the tendency of basic peptides to interact with residual silanol groups. However, TFA interferes with and significantly reduces the LC/MS signal, lowering sensitivity. The ideal column for modern RP-LC/MS analysis should provide symmetrical peak shape without TFA in the mobile phase. The highly inert surface of Discovery BIO Wide Pore silica results in columns that give symmetrical and efficient peaks for peptides without TFA for maximum LC/MS sensitivity.

While TFA has little effect on UV detection, it has serious disadvantages for LC/MS detection. First, typical concentrations of TFA (0.1% v/v) have high surface tension and prevent efficient spray formation (nebulization). Second, TFA ions in the gas phase ion-pair with the peptide's basic groups suppressing their ionization and reducing sensitivity. A demonstration of TFA's adverse effect on LC/MS sensitivity is shown in **Figure 13**. Without TFA, the MS is able to detect much lower concentrations of these peptides. An added benefit is that at low TFA concentrations, resolution is improved because small differences in peptide structure are no longer masked by the ion-pair formation. This is shown in the increased separation of peptides 1 and 2 as the TFA concentration is decreased. At 0.1% TFA, they coelute. Therefore, from the mobile phase standpoint, the best LC/MS method employs ionic additives other than TFA that are still volatile, can provide pH control, and do not strongly ionpair with the peptides.

Discovery BIO Wide Pore columns permit the use of mobile phases without TFA.

Figure 13. Effect of Chromatographic Conditions on MS Signals of Peptides

column: Discovery BIO Wide Pore C18, 15 cm x 2.1 mm, 3 µm (567202-U)
mobile phase: (A) A:0.1% TFA, B:0.1% TFA in 50:50 (CH_3CN:H_2O)
(B) A:0.01% TFA, B:0.01% TFA in 50:50 (CH_3CN:H_2O)
(C) A:25 mM formic acid in H_2O, B:50:50; (25 mM formic acid in H_2O):(20 mM formic acid in CH_3CN)[a]
flow rate: 0.208 mL/min[b]
detector: +ES
column temp.: ambient
injection: 1 µL or 3 µL
sample: RP Peptide Performance Standard, p/n RPS-P0010 (Alberta Peptide Institute)

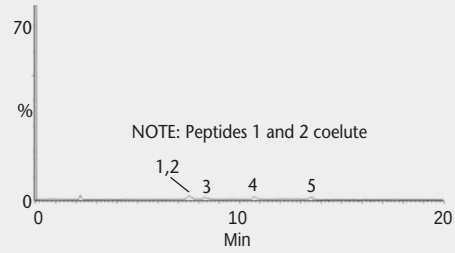
a) molarity of formic acid adjusted to provide minimum baseline drift
b) linear velocity equal to 1 mL/min on 4.6 mm I.D. columns

Peptide 1: ac-RGAGGLGLGK-amide
Peptide 2: ac-RGGGGLGLGK-amide
Peptide 3: ac-RGAGGLGLGK-amide
Peptide 4: ac-RGVGGLGLGK-amide
Peptide 5: ac-RGVVGLGLGK-amide

A: 0.1% TFA, 3 µL injection

Gradient:

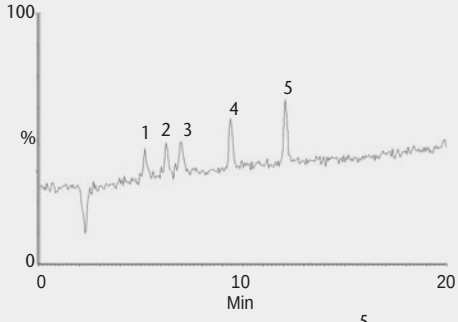
Min	%C	%D
0	80	20
20	40	60



B: 0.01% TFA, 3 µL injection

Gradient:

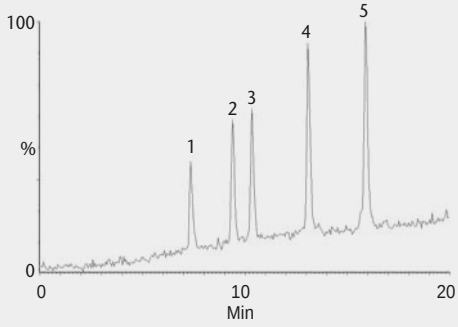
Min	%C	%D
0	80	20
20	40	60



C: 0% TFA (25 mM formic acid), 1 µL injection

Gradient:

Min	%A	%B
0	90	10
20	50	50



LC/MS Sensitivity: Bleed-free

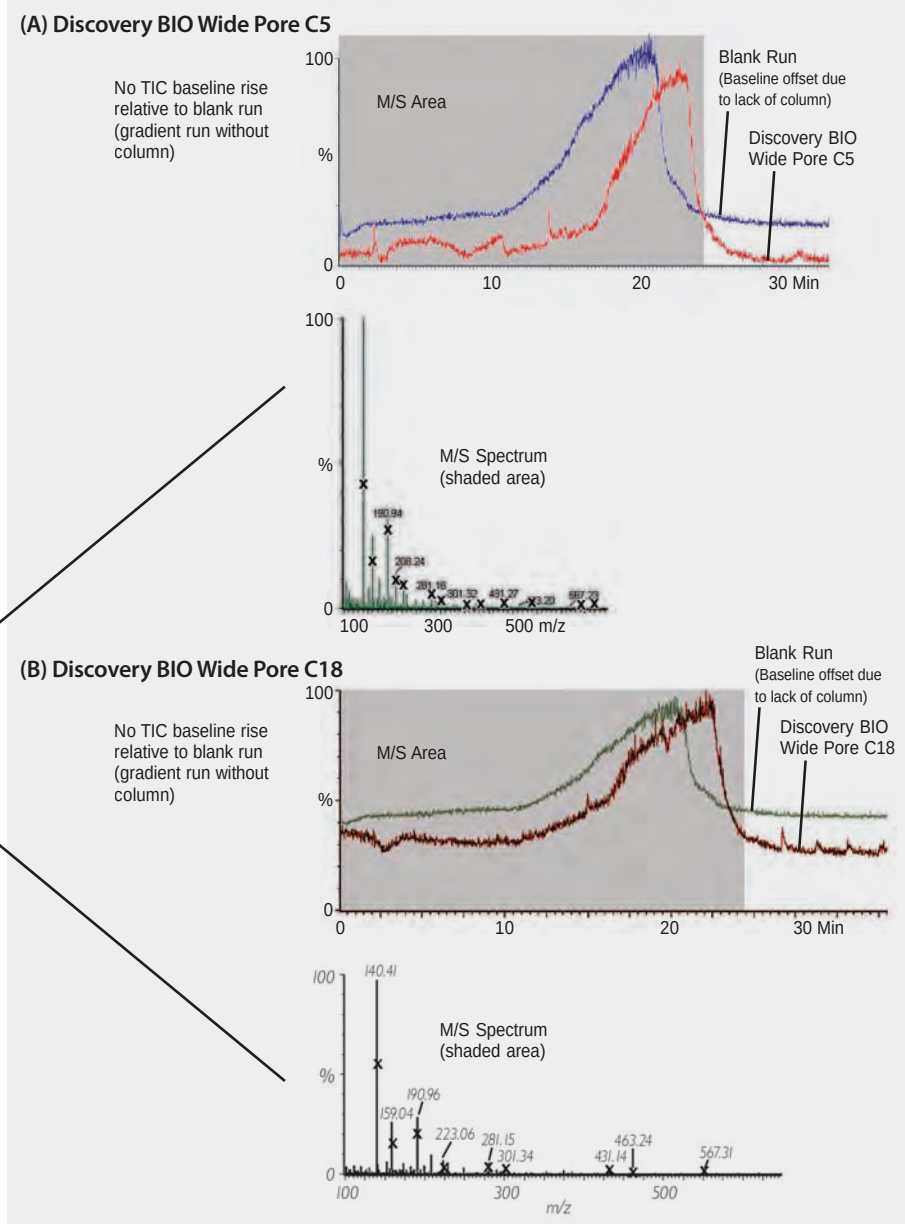
The chemical stability of Discovery BIO Wide Pore columns minimizes bleed in LC/MS analyses.

The quality and stability of all Discovery BIO Wide Pore phases make them ideal for proteomics and other LC/MS applications.

HPLC interfaced with mass spectrometry (LC/MS) provides valuable information about the sample, such as molecular mass, structural data, molecular conformation, and presence of impurities. LC/MS is an essential tool for proteomics researchers. In many cases, however, LC/MS information may be obscured or misleading if the column introduces residue (bleed) during the analysis. Discovery BIO Wide Pore columns were designed with LC/MS in mind. The LC/MS analyses in **Figure 14** shows minimal column bleed from Discovery BIO Wide Pore C5 or C18 columns.

Figure 14. Undetectable LC/MS Bleed on Discovery BIO Wide Pore C5 and C18 Columns

columns: Discovery BIO Wide Pore C5, 15 cm x 4.6 mm I.D., 5 µm (568422-U), or Discovery BIO Wide Pore C18, 15 cm x 4.6 mm I.D., 3 µm (567205-U)
mobile phase: (A) 0.1% TFA in water; (B) 0.1% TFA in CH_3OH
gradient: 0-100% B in 15 min, 100% B for 5 min, 0% B for 10 min
flow rate: 1.0 mL/min
column temp.: 30 °C



Essentially no m/z peaks generated from Discovery BIO Wide Pore C5 or Discovery BIO Wide Pore C18 compared to a blank run. "X" indicates peaks that were also seen in the blank run.

Scalability

Separations developed on Discovery BIO Wide Pore are completely scalable between 3, 5, and 10 µm particles, and from capillary to preparative column dimensions.

Bonded phase and silica chemistry are uniform across all Discovery BIO Wide Pore particle sizes.

Precious samples can be wasted during scale-up if the analytical and preparative columns do not give the same elution pattern.

Analytical separations that are developed on Discovery BIO Wide Pore 3 or 5 micron particles are completely scalable to preparative separations on Discovery BIO Wide Pore 10 micron particles and larger columns. Additionally, separations developed on 5 or 10 micron particles can be scaled down for fast analysis on 3 micron particles (**Figure 15**).

- Discovery BIO Wide Pore 10 micron particles in large column dimensions are ideal for isolating and purifying mg to gram amounts of proteins and peptides for further characterization.
- Discovery BIO Wide Pore 3 micron particles in short columns are ideal for rapid analysis and LC/MS applications.
- Discovery BIO Wide Pore 3 or 5 micron particles in long columns provide maximum resolution of complex mixtures of proteins and peptides.

The breadth of the offering of Discovery BIO Wide Pore column formats can be seen in the product listing at the end of this brochure.

Figure 15. Matched Selectivity from Analytical to Preparative on Discovery BIO Wide Pore C18

columns: (A) Discovery BIO Wide Pore C18, 15 cm x 4.6 mm I.D., 3 µm (567205-U)
(B) Discovery BIO Wide Pore C18, 15 cm x 4.6 mm I.D., 5 µm (568222-U
(C) Discovery BIO Wide Pore C18, 15 cm x 10 mm I.D., 10 µm (567208-U)
mobile phase: (A) 80:20, (0.1% TFA in Water):(0.1% TFA in CH_3CN),
(B) 66:34, (0.1% TFA in Water):(0.1% TFA in CH_3CN)
linear velocity: 6.02 cm/min
column temp.: 30 °C
detector: UV, 215 nm
sample: peptide mix (Arg8- vasopressin, bradykinin (fragment 1-5), oxytocin, luteinizing hormone releasing hormone, Met-enkephalin, bradykinin, Leu-enkephalin, bombesin, Substance P) in 0.1% TFA

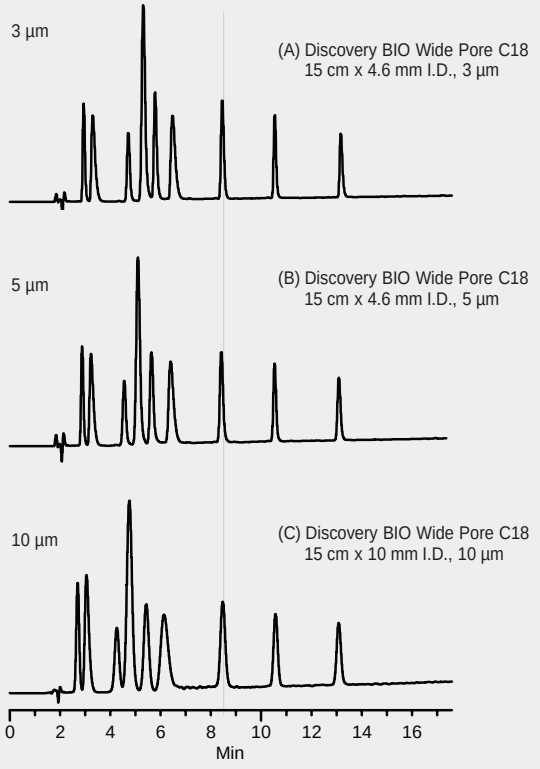
Column Parameters and Run Conditions:

Column	Column Volume (mL)	Injection (µL)	Flow (mL/min)
15 cm x 4.6 mm I.D., 3 µm	1.64	5.0	1.00
15 cm x 4.6 mm I.D., 5 µm	1.71	5.0	1.00
15 cm x 10 mm I.D., 10 µm	8.01	24.5	4.73

Gradient:

Column Volumes	%A	%B
0	100	0
2	100	0
9	0	100

Same selectivity on 3 µm, 5 µm, and 10 µm Discovery BIO Wide Pore particles.



Stability

To minimize downtime, the RP-HPLC method should be stable run-to-run over a wide range of mobile phase pH. Discovery BIO Wide Pore columns have exceptional pH-stability.

Consistent retention time and efficiency at acidic, neutral, and basic pH

The pH and ionic strength of the mobile phase are powerful tools to adjust the separation. However, they can affect the silica or bonded phase in the column resulting in retention time shifts or decreased column life. Discovery BIO Wide Pore was designed to allow the full use of pH and ionic strength.

Trifluoroacetic acid (TFA) at pH 2 is a commonly used mobile phase in RP-HPLC separation of proteins and peptides. A robust method dictates that the column is stable under these harsh conditions. Discovery BIO Wide Pore columns were developed to provide stable, reproducible separations at low pH. Selectivity and peak shape remain essentially unchanged on a Discovery BIO Wide Pore C18 after 40,000 column volumes of TFA mobile phase at 70 °C (See **Figure 16**).

Neutral or even alkaline pH mobile phases are occasionally used in protein and peptide separations. Although most silica-based RP-HPLC columns are destroyed at pH 8 and above, Discovery BIO Wide Pore's advanced bonded phase technology allows safe use under alkaline conditions using organic buffers, as demonstrated in **Figure 17**.

Figure 16. Stability of Discovery BIO Wide Pore C18 at pH 2 and 70 °C

column: Discovery BIO Wide Pore C18, 5 cm x 4.6 mm I.D., 5 µm (568220-U)
mobile phase: (A) 95:5, (0.5% TFA in water):(0.5% TFA in CH_3CN);
(B) 25:75, (0.5% TFA in water):(0.5% TFA in CH_3CN)
gradient: 2-24% B in 22 min, 8 min at 100% A
flow rate: 2.0 mL/min
column temp.: 70 °C
detector: UV, 220 nm
injection: 5 µL, 2.5 µg each peptide (Fluka Peptide Standard Mixture, H2016) in mobile phase A

1. Gly-Tyr
2. Val-Tyr-Val
3. Met-Enkephalin
4. Leu-Enkephalin
5. Angiotensin II

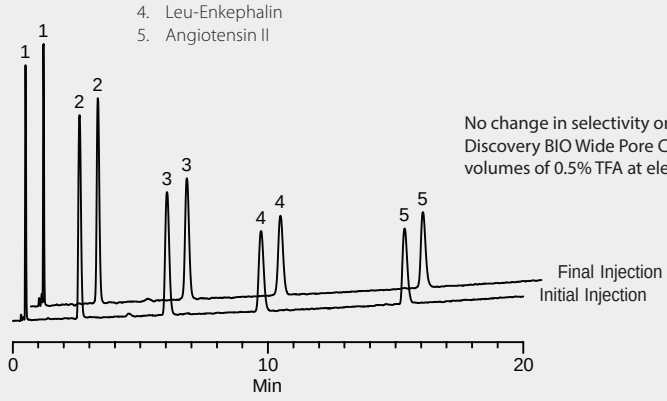
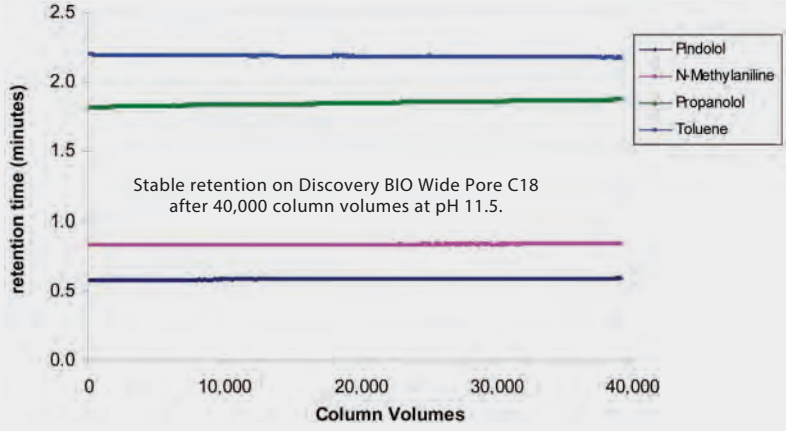


Figure 17. Discovery BIO Wide Pore C18 Stability at pH 11.5

column: Discovery BIO Wide Pore C18, 5 cm x 4.6 mm I.D., 5 µm (568220-U)
mobile phase: 65:35, 50 mM pyrrolidine HCl (pH 11.5):CH_3CN
flow rate: 2.0 mL/min
column temp.: 35 °C

1. Pindolol
2. N-Methylaniline
3. Propranolol
4. Toluene



Note: Stability was measured using small molecule probes because they are generally more sensitive to changes in the silica and bonded phase chemistry than peptides and proteins. If the retention and selectivity for the small molecule probes does not change, it is very likely that the retention and selectivity for proteins and peptides will be stable as well.

Challenge: Maintaining the Separation Over Time (Trouble-Free Operation)

The stability and reproducibility of Discovery BIO Wide Pore phases permit reliable, trouble-free operation from day-to-day.

Discovery BIO Wide Pore C5 is more stable than conventional C3 and C4 phases.

The majority of RP-HPLC protein separations are performed on C4 bonded phases. Discovery BIO Wide Pore C5 has similar selectivity to a C4, but greatly improved stability at high and low pH over C4 bonded phases.

Short chain alkyl bonded phases such as C3 and C4 are routinely used for RP-HPLC separation of proteins and hydrophobic peptides. However, both C3 and C4 phases hydrolyze at low and high pH resulting in short column life. Discovery BIO Wide Pore C5 gives similar selectivity to a C3 or C4, but greatly improved pH stability.

The data in **Figure 18** shows that subjecting a Discovery BIO Wide Pore C5 column to a pH 8 mobile phase for 14,000 column volumes resulted in minimal loss of retention for probes of low molecular mass.

The results of a separate stability experiment are shown in **Figure 19**, in which a Discovery BIO Wide Pore C5 and a Competitive C4 column were flushed with 25,000 column volumes of a mobile phase containing 5% acetonitrile 95% of a 0.1% TFA in water (pH < 1.0). Although both columns had lost efficiency at the end of the experiment, the C4 column (blue bars) lost 50%, while the C5 column (red bars) maintained 75% of its original efficiency.

The chemical stability of Discovery BIO Wide Pore columns allows you to employ acidic, neutral, or alkaline pH mobile phases to optimize your separation.

Figure 18. Stability of Discovery BIO Wide Pore C5 at pH 8

column: Discovery BIO Wide Pore C5, 5 cm x 4.6 mm I.D., 5 µm (568420-U)
mobile phase: 95:5, 25 mM potassium phosphate (pH 8):CH_3OH
flow rate: 2.0 mL/min
column temp.: 35 °C

1. Sorbic acid
2. Pyridine
3. Procainamide
4. Caffeine

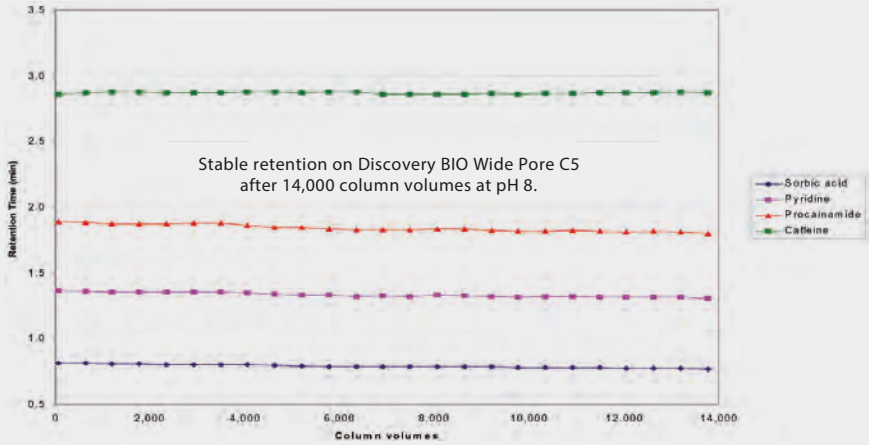
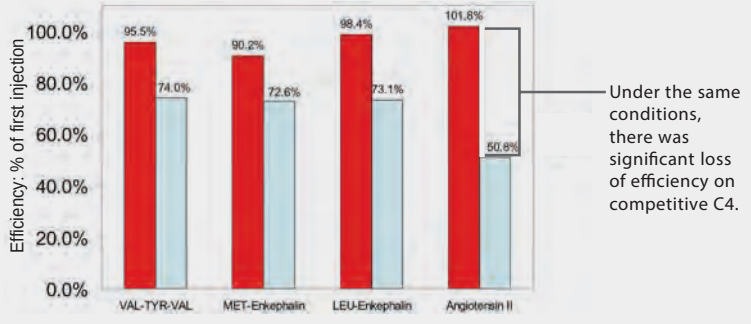


Figure 19. Comparison of Low pH Stability of Discovery BIO Wide Pore C5 versus a Competitive C4 Column

columns: Discovery BIO Wide Pore C5, 5 cm x 4.6 mm I.D., 5 µm (568420-U),
or (B) Competitive protein and peptide C4, 5 cm x 4.6 mm I.D., 300 Å, 5 µm
mobile phase: (A) 5:95, (0.5% TFA in water):(0.5% TFA in CH_3CN);
(B) 25:75, (0.5% TFA in water):(0.5% TFA in CH_3CN)
gradient: 2-24% B in 22 min, 8 min at 100% A
flow rate: 2.0 mL/min
column temp.: 30 °C
detector: UV, 220 nm
injection: 5 µL, 2.5 µg each peptide (Fluka Peptide Standard Mixture H2016) in mobile phase A



Reproducibility

Discovery BIO Wide Pore phases undergo rigorous testing to ensure their reproducibility.

Customers can depend on Sigma-Aldrich/Supelco to provide reproducible HPLC columns, from run-to-run, column-to column, and batch-to-batch. Discovery BIO Wide Pore columns are guaranteed to be reproducible.

An important factor for developing a robust analytical method is column reproducibility. Column selectivity should remain the same and the elution patterns of the proteins or peptides must be reproducible. Consistency in silica and bonded phase chemistry guarantees that Discovery BIO Wide Pore columns have exceptional reproducibility between injections, columns, and bonded phase lots (See **Figures 20** and **21**).

Figure 20. Lot-to-Lot Reproducibility of Discovery BIO Wide Pore C18

column: Discovery BIO Wide Pore C18, 15 cm x 4.6 mm I.D., 5 µm (568222-U)
mobile phase: 80:20, 10 mM NH_4OAc (pH 6.8):CH_3OH
flow rate: 1.0 mL/min
column temp.: 35 °C
detector: UV, 254 nm
injection: 5 µL

1. Uracil (8 µg/mL)
2. Procainamide (30 µg/mL)
3. Sorbic acid (4 µg/mL)
4. Pyridine (100 µg/mL)
5. Caffeine (40 µg/mL)
6. Phenol (240 µg/mL)

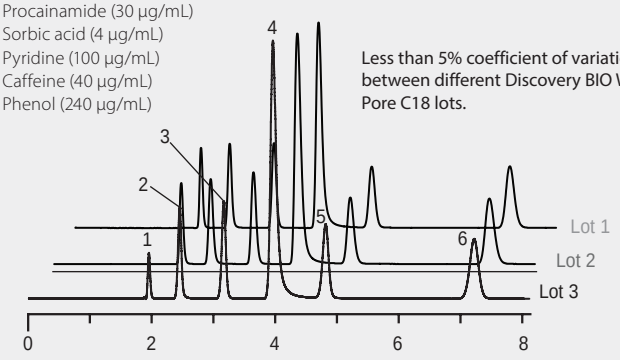


Figure 21. Lot-to-Lot Reproducibility of Discovery BIO Wide Pore C5

column: Discovery BIO Wide Pore C5, 15 cm x 4.6 mm I.D., 5 µm (568422-U)
mobile phase: (A) 0.1% PFPA (pentafluoropropionic acid) in water:CH_3CN (81:19);
(B) 0.1% PFPA in water:CH_3CN (62:38)
gradient: 0-100% B in 19 min
flow rate: 1.0 mL/min
column temp.: 30 °C
detector: UV, 215 nm
injection: 10 µL, ~0.25 µg each peptide in mobile phase A

	Peptide	Sequence
1.	Arg^8-vasopressin	CFQNCPRG-amide; disulfide
2.	Bradykinin, fragment 1-5	RPPGF
3.	Oxytocin	CYQNCPLG-amide; disulfide
4.	Met-enkephalin	YGGFM
5.	Luteinizing hormone releasing hormone	pEHWSYGLRPG-amide
6.	Leu-enkephalin	YGGFL
7.	Bradykinin	RPPGFSPFR
8.	Bombesin	pEQRLGNQWAVGHLM-amide
9.	Substance P	RPKPQQFFGLM-amide

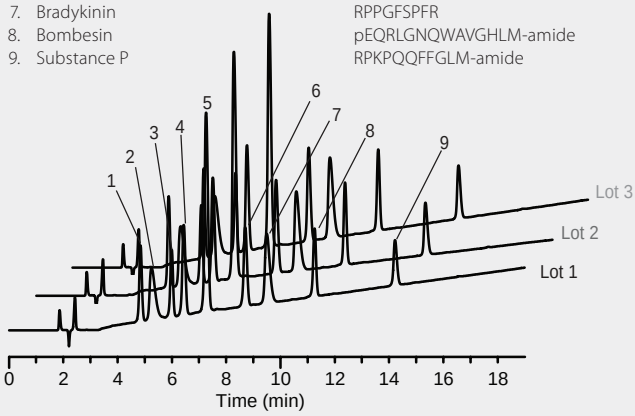


Table 3. Suggestions for Selecting the Appropriate Discovery BIO Wide Pore Column:

Application	Bonded Phases
Proteins	BIO Wide Pore C5
Hydrophobic peptides or proteins (e.g. membrane proteins)	BIO Wide Pore C5
Peptide mapping	BIO Wide Pore C18
Proteomics	BIO Wide Pore C18
Scouting	BIO Wide Pore C8 (because of its intermediate hydrophobicity between a C18 and C5)
Application	**Silica Particle Sizes**
LC/MS	3 micron or 5 micron
Fast analysis, or high-throughput applications	3 micron
Peptide mapping	3 micron or 5 micron
Analytical HPLC	3 micron or 5 micron
Preparative	10 micron
Application	**Column I.D.**
LC/MS	2.1 mm or smaller
Peptide mapping	4.6 mm, 4.0 mm, 2.1 mm
Analytical HPLC	4.0 mm, 4.6 mm
Preparative	10 mm, 21.2 mm
Low level detection or limited sample volume	0.18 mm, 0.32 mm, 0.5 mm, 1.0 mm

Discovery BIO Product Listing

Phase Type		Particle Size (micron)	Length (cm)	I.D. (mm)	Cat. No.
Discovery BIO Wide Pore C5					
Capillary		5	10	0.18	**65613-U**
		3	10	0.32	**65532-U**
		5	15	0.32	**65533-U**
Microbore		3	5	1	**65511-U**
		3	10	1	**65512-U**
		5	15	1	**65513-U**
Narrowbore		3	5	2.1	**567226-U**
		3	10	2.1	**567227-U**
		3	15	2.1	**567228-U**
		5	5	2.1	**568400-U**
		5	10	2.1	**568401-U**
		5	15	2.1	**568402-U**
		5	25	2.1	**568403-U**
Guards	Pk 2	3	2	2.1	**567278-U**
	Kit*	3	2	2.1	**567279-U**
	Pk 2	5	2	2.1	**568470-U**
	Kit	5	2	2.1	**568471-U**
Standard Analytical		5	10	4	**568411-U**
		5	15	4	**568412-U**
		5	25	4	**568413-U**
		3	5	4.6	**567229-U**
		3	10	4.6	**567230-U**
		3	15	4.6	**567231-U**
		5	5	4.6	**568420-U**
		5	10	4.6	**568421-U**
		5	15	4.6	**568422-U**
		5	25	4.6	**568423-U**
		10	25	4.6	**567232-U**
Guards	Pk 2	3	2	4	**567280-U**
	Kit	3	2	4	**567281-U**
	Pk 2	5	2	4	**568472-U**
	Kit	5	2	4	**568473-U**
Semi-preparative		5	25	10	**568430-U**
		10	5	10	**567233-U**
		10	15	10	**567234-U**
		10	25	10	**567235-U**
Preparative		10	5	21.2	**567236-U**
		10	15	21.2	**567237-U**
		10	25	21.2	**567238-U**
Guards		10	1	10	**567286-U**

Phase Type		Particle Size (micron)	Length (cm)	I.D. (mm)	Cat. No.
Discovery BIO Wide Pore C8					
Narrowbore		3	5	2.1	**567213-U**
		3	10	2.1	**567214-U**
		3	15	2.1	**567215-U**
		5	5	2.1	**568300-U**
		5	10	2.1	**568301-U**
		5	15	2.1	**568302-U**
Guards	Pk 2	3	2	2.1	**567274-U**
	Kit	3	2	2.1	**567275-U**
	Pk 2	5	2	2.1	**568370-U**
	Kit	5	2	2.1	**568371-U**
Standard Analytical		5	15	4	**568312-U**
		5	25	4	**568313-U**
		3	10	4.6	**567217-U**
		3	15	4.6	**567218-U**
		5	5	4.6	**568320-U**
		5	10	4.6	**568321-U**
		5	15	4.6	**568322-U**
		5	25	4.6	**568323-U**
		10	25	4.6	**567219-U**
Guards	Pk 2	3	2	4	**567276-U**
	Kit	3	2	4	**567277-U**
	Pk 2	5	2	4	**568372-U**
	Kit	5	2	4	**568373-U**
Semi-preparative		5	25	10	**568330-U**
		10	25	10	**567222-U**
Preparative		10	25	21.2	**567225-U**
Guards		10	1	10	**567284-U**
Discovery BIO Wide Pore C18					
Capillary		3	5	0.18	**65603-U**
		5	5	0.18	**65606-U**
		5	15	0.18	**65608-U**
		3	5	0.32	**65526-U**
		3	10	0.32	**65527-U**
		5	15	0.32	**65529-U**
		3	5	0.5	**65517-U**
		3	10	0.5	**65518-U**
		5	15	0.5	**65519-U**
Microbore		3	5	1	**65504-U**
		3	10	1	**65506-U**
		5	15	1	**65508-U**
		5	25	1	**65509-U**

Phase Type		Particle Size (micron)	Length (cm)	I.D. (mm)	Cat. No.
Discovery BIO Wide Pore C18					
Narrowbore		3	5	2.1	567200-U
		3	10	2.1	567201-U
		3	15	2.1	567202-U
		5	5	2.1	568200-U
		5	10	2.1	568201-U
		5	15	2.1	568202-U
		5	25	2.1	568203-U
Guards	Pk 2	3	2	2.1	567270-U
	Kit	3	2	2.1	567271-U
	Pk 2	5	2	2.1	568270-U
	Kit	5	2	2.1	568271-U
Standard Analytical		5	15	4	568212-U
		5	25	4	568213-U
		3	10	4.6	567204-U
		3	15	4.6	567205-U
		5	5	4.6	568220-U
		5	10	4.6	568221-U
		5	15	4.6	568222-U
		5	25	4.6	568223-U
		10	25	4.6	567206-U
Guards	Pk 2	3	2	4	567272-U
	Kit	3	2	4	567273-U
	Pk 2	5	2	4	568272-U
	Kit	5	2	4	568273-U
Semi-preparative		5	25	10	568230-U
		10	5	10	567207-U
		10	15	10	567208-U
		10	25	10	567209-U
Preparative		10	5	21.2	567210-U
		10	15	21.2	567211-U
		10	25	21.2	567212-U
Guards		10	1	10	567282-U

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BIOshell™
U/HPLC Columns

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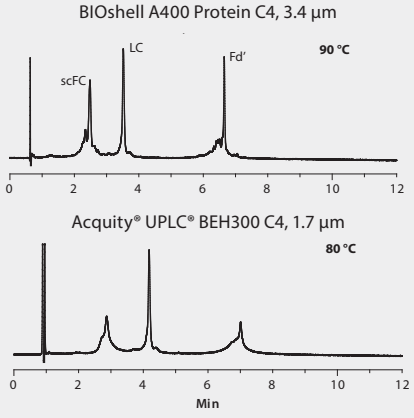
Figure 22. Analysis of Antibody Fragments on Wide Pore Reversed Phase Columns

column: as indicated, 10 cm x 2.1 mm I.D.
mobile phase A: 80:20, (water, 0.1% TFA): (acetonitrile, 0.1% TFA)
mobile phase B: 50:50, (water, 0.1% TFA): (acetonitrile, 0.1% TFA)
gradient: 30 to 70% B in 12 min
flow: 0.3 mL/min
column temp.: maximum temp., as indicated
detection: UV, 215 nm
injection: 1 µL, after sample diluted in mobile phase A



Features

- Peptides
  - 2.7 µm particles, 160Å pore size, C18 and CN functional groups
  - 5 µm particles, 160Å pore size, C18 and CN functional groups
- Proteins
  - 3.4 µm particles, 400Å pore size, C4 functional group
- High temperature stability (see Figure)

Particle Characteristics of BIOshell Fused-Core Columns

BIOshell Columns	Particle Size (µm)	Core Size (µm)	Shell Thickness (µm)	Pore Size (Å)	SBET (m^2/g)	Capacity vs. Porous*
2.7 µm A160 Peptide C18	2.7	1.7	0.5	160	80	75%
2.7 µm A160 Peptide CN	2.7	1.7	0.5	160	80	75%
5 µm A160 Peptide C18	4.7+	3.5	0.6	160	80	59%
5 µm A160 Peptide CN	4.7+	3.5	0.6	160	80	59%
3.4 µm A400 Protein C4	3.4	3.0	0.2	400	15	31%

+ Nominal particle size is 5 micron
* Calculated capacity based on value of core diameter and shell thickness

Bonded Phase and Operational Characteristics of BIOshell Fused-Core Columns

BIOshell Columns	Bonded Phase Ligand	End Cap	Max. Temp. (°C)#	pH Range	Pmax (bar)	Frit (µm)
2.7 µm A160 Peptide C18	di-isobutyl-octadecylsilane	No	100	1 - 8	600	2
2.7 µm A160 Peptide CN	di-isopropyl-cyanopropylsilane	Yes	80	1 - 8	600	2
5 µm A160 Peptide C18	di-isobutyl-octadecylsilane	No	100	1 - 8	600	2
5 µm A160 Peptide CN	di-isopropyl-cyanopropylsilane	Yes	90	1 - 8	600	2
3.4 µm A400 Protein C4	dimethylbutylsilane	Yes	90	2 - 9	600	2

Temperature at which each bonded phase type was tested for long term physical and chemical stability.

BIOshell Product Listing

Pore Size	Particle Size	I.D. (mm)	L (cm)	C4	C18	CN
BIOshell Fused-Core Peptide and Protein Columns						
400 Å	3.4 µm	2.1	5	66824-U	—	—
400 Å	3.4 µm	2.1	10	66825-U	—	—
400 Å	3.4 µm	2.1	15	66826-U	—	—
400 Å	3.4 µm	4.6	5	66827-U	—	—
400 Å	3.4 µm	4.6	10	66828-U	—	—
400 Å	3.4 µm	4.6	15	66829-U	—	—
160 Å	2.7 µm	2.1	3	—	66901-U	66965-U
160 Å	2.7 µm	2.1	5	—	66902-U	66966-U
160 Å	2.7 µm	2.1	7.5	—	66903-U	66967-U
160 Å	2.7 µm	2.1	10	—	66904-U	66968-U
160 Å	2.7 µm	2.1	15	—	66905-U	66969-U
160 Å	2.7 µm	3.0	3	—	66906-U	66970-U
160 Å	2.7 µm	3.0	5	—	66907-U	66971-U
160 Å	2.7 µm	3.0	10	—	66908-U	66972-U
160 Å	2.7 µm	3.0	15	—	66909-U	66973-U
160 Å	2.7 µm	4.6	5	—	66913-U	66974-U
160 Å	2.7 µm	4.6	10	—	66915-U	66975-U
160 Å	2.7 µm	4.6	15	—	66917-U	66976-U

Pore Size	Particle Size	I.D. (mm)	L (cm)	C4	C18	CN
BIOshell Fused-Core Peptide and Protein Columns						
160 Å	5 µm	2.1	3	—	67001-U	67061-U
160 Å	5 µm	2.1	5	—	67002-U	67062-U
160 Å	5 µm	2.1	7.5	—	67003-U	67063-U
160 Å	5 µm	2.1	10	—	67004-U	67064-U
160 Å	5 µm	2.1	15	—	67006-U	67065-U
160 Å	5 µm	3.0	3	—	67007-U	67066-U
160 Å	5 µm	3.0	5	—	67008-U	67067-U
160 Å	5 µm	3.0	10	—	67011-U	67068-U
160 Å	5 µm	3.0	15	—	67012-U	67069-U
160 Å	5 µm	4.6	5	—	67013-U	67071-U
160 Å	5 µm	4.6	10	—	67014-U	67080-U
160 Å	5 µm	4.6	15	—	67015-U	67081-U
BIOshell Fused-Core Peptide and Protein Guard Columns, pk. of 3						
400 Å	3.4 µm	2.1	0.5	66830-U	—	—
400 Å	3.4 µm	4.6	0.5	66831-U	—	—
160 Å	2.7 µm	2.1	0.5	—	66918-U	66977-U
160 Å	2.7 µm	3.0	0.5	—	66919-U	66978-U
160 Å	2.7 µm	4.6	0.5	—	66921-U	66979-U
160 Å	5 µm	2.1	0.5	—	67016-U	67082-U
160 Å	5 µm	3.0	0.5	—	67017-U	67083-U
160 Å	5 µm	4.6	0.5	—	67018-U	67084-U
BIOshell Fused-Core Peptide and Protein Capillary Columns						
400Å	3.4 µm	75 µm	5	67031-U	—	—
400Å	3.4 µm	75 µm	15	67032-U	—	—
400Å	3.4 µm	100 µm	5	67033-U	—	—
400Å	3.4 µm	100 µm	15	67034-U	—	—
400Å	3.4 µm	200 µm	5	67036-U	—	—
400Å	3.4 µm	200 µm	15	67037-U	—	—
400Å	3.4 µm	300 µm	5	67038-U	—	—
400Å	3.4 µm	300 µm	15	67039-U	—	—
400Å	3.4 µm	500 µm	5	67040-U	—	—
400Å	3.4 µm	500 µm	15	67041-U	—	—
400Å	3.4 µm	1.0 mm	5	67042-U	—	—
400Å	3.4 µm	1.0 mm	15	67045-U	—	—
160Å	2.7 µm	75 µm	5	—	67085-U	67150-U
160Å	2.7 µm	75 µm	15	—	67086-U	67152-U
160Å	2.7 µm	100 µm	5	—	67087-U	67153-U
160Å	2.7 µm	100 µm	15	—	67088-U	67155-U
160Å	2.7 µm	200 µm	5	—	67089-U	67157-U
160Å	2.7 µm	200 µm	15	—	67091-U	67158-U
160Å	2.7 µm	300 µm	5	—	67092-U	67159-U
160Å	2.7 µm	300 µm	15	—	67093-U	67160-U
160Å	2.7 µm	500 µm	5	—	67095-U	67161-U
160Å	2.7 µm	500 µm	10	—	67096-U	—
160Å	2.7 µm	500 µm	15	—	67097-U	67163-U
160Å	2.7 µm	1.0 mm	5	—	67098-U	67164-U
160Å	2.7 µm	1.0 mm	15	—	67099-U	67165-U
160Å	5 µm	75 µm	5	—	67201-U	67305-U
160Å	5 µm	75 µm	15	—	67202-U	67307-U
160Å	5 µm	100 µm	5	—	67203-U	67311-U
160Å	5 µm	100 µm	15	—	67204-U	67312-U
160Å	5 µm	200 µm	5	—	67205-U	67314-U
160Å	5 µm	200 µm	15	—	67206-U	67315-U
160Å	5 µm	300 µm	5	—	67207-U	67321-U
160Å	5 µm	300 µm	15	—	67208-U	67324-U
160Å	5 µm	500 µm	5	—	67209-U	67325-U
160Å	5 µm	500 µm	15	—	67212-U	67326-U
160Å	5 µm	1.0 mm	5	—	67215-U	67327-U
160Å	5 µm	1.0 mm	15	—	67219-U	67329-U

Related Product

Description	Cat. No.
BIOshell Guard Cartridge Holder	66841-U

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