DISCOVERY BIO

Discovery BIO HPLC Columns and Capillaries:

Solutions to Protein and Peptide Separation Challenges

Features:

- PolyMA-SCX and WAX ion-exchange columns
- LC/MS compatibility
- Scaleable from capillary to preparative
- Inert surface - no need for TFA

sigma-aldrich.com/supelco

SUPELCO

Discovery BIO

Meeting the challenges of today's protein and peptide separations

Meeting the challenges of today's

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

The different phase chemistries and separation modes provide unique selectivity increasing your resolution options.

Separations are completely scalable from analytical to preparative.

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

Discovery BIO PolyMA-SCX and Discovery BIO PolyMA-WAX columns provide efficient, high-recovery, non-denaturing ion-exchange separations of proteins and peptides.

Discovery BIO is your solution to protein and peptide separation challenges.

HPLC Columns

protein and peptide separations

The Challenges of Today's 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 HPLC columns to address these challenges.

Separate Complex Protein or Peptide Mixtures

The selectivity and efficiency offered by Discovery BIO gives maximum power for resolving complex mixtures of proteins, natural or synthetic peptides, and peptide maps. 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 provides sensitive analyses. Many Discovery BIO products are available in capillary and microbore dimensions.

The Need for Detailed Characterization

Because of the sample complexity, many biomolecule separations are multi-dimensional. Discovery BIO columns are designed to be compatible with secondary separation or detection methods. If purified sample is required for further characterization, many Discovery BIO phases are scalable from capillary to preparative, and have high sample recovery.

Large Number of Samples to Analyze

High sample throughput is achievable with the short analysis times provided by Discovery BIO in small particles and short columns.

Trouble-Free Operation

The stability and reproducibility of Discovery BIO phases permit reliable, trouble-free routine and long term operation.

Welcome to Discovery BIO

Solutions to Protein and Peptide Separation Challenges

Discovery BIO phases provide sensitive, stable, efficient, reproducible separations of proteins and peptides. The different separation modes and bonded phase chemistries provide unique selectivity increasing your resolution options.

The continually expanding Discovery BIO family currently comprises:

Discovery BIO Wide Pore C18, C8, and C5 (pgs 6-21)

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 satisfy the needs of 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. 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 phases
- Great 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
- Scalable from capillary to preparative dimensions

Discovery BIO PolyMA-SCX and PolyMA-WAX (pgs 22-33)

Polymer-based, ion-exchange columns for non-denaturing separations of proteins, polypeptides, and other biotechnology-derived products.

Discovery BIO PolyMA ion-exchange columns are specifically designed for the separation of proteins, peptides, and other biotechnology-derived products such as fragments of DNA, RNA, oligonucleotides, and antibodies. Proteins and polypeptides that exhibit similar hydrophobic characteristics but have different ionic charges can be separated on the Discovery PolyMA ion-exchange resins. The proprietary hydrophilic surface chemistry of Discovery PolyMA ion-exchange resins offers subtle ionic selectivity characteristics that are not available from the typical polystyrene-divinylbenzene (PS-DVB) or standard polymethacrylate based ion-exchange resins currently available in the market. Discovery PolyMA can be run from pH 1 to pH 14 without loss of retention or performance.

Significant benefits include:

- Excellent separations of protein isoforms
- High resolution at low sample load
- Quantitative recovery – a hydrophilic surface eliminates protein adsorption
- High efficiency

Choosing a Discovery BIO 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 0.5mm ID 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
Proteins / Peptides	Cation-Exchange	Discovery BIO PolyMA-SCX
Proteins / Peptides	Anion-Exchange	Discovery BIO PolyMA-WAX

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 and capillaries are packed with C5, C8, or C18 bonded 3, 5, or 10µm, spherical, 300Å pore diameter, high purity silica. All Discovery BIO Wide Pore products provide stable, efficient, reproducible, and scalable analytical to preparative 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	<10ppm metals	<10ppm metals	<10ppm 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
%C	~ 9	~ 5	~ 3.5
Coverage (µmoles/m^2)	~ 3.6	~ 4	~ 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.

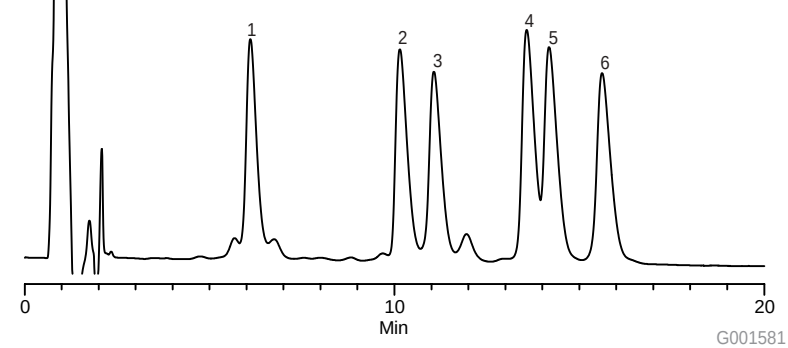
Along with electrophoresis, ion exchange, and gel filtration, protein and peptide biochemists rely heavily on reversed-phase high performance liquid chromatography (RP-HPLC) based on wide pore silicas to perform many separations. RP-HPLC is a popular analytical tool for protein and peptide separations 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, 15cm x 4.6mm, 5µm (Cat. No. 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)
Flow Rate: 1.0mL/min
Temp: ambient
Detection: 220nm
Injection: 12µL each at 0.8mg/mL in 0.1%TFA
Gradient: 0-100%B in 30 min

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.



G001581

Meeting the Challenges of Today's Protein and Peptide Separations

Challenge: Complex Protein or Peptide Mixtures

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

Efficiency and Inertness

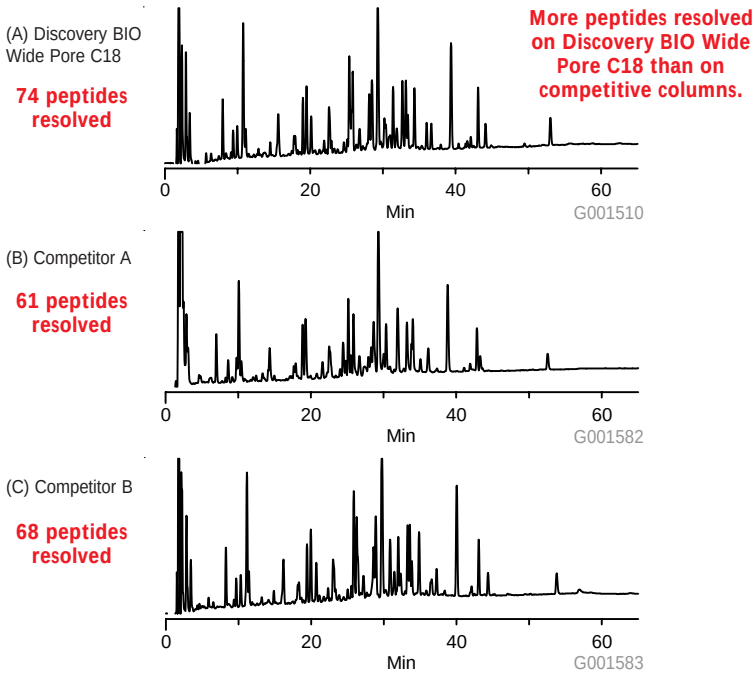
Discovery BIO Wide Pore phases provide the high efficiency required to capture the detail of peptide maps

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 the necessary resolving power. The Discovery BIO Wide Pore C18 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, 15cm x 4.6mm, 5µm (Cat. No. 568222-U), (B) and (C) Competitive protein and peptide C18, 15cm x 4.6mm, 300Å, 5µm;**
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)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 215nm
Injection: 50µL carboxymethylated apohemoglobin tryptic digest in 50mM NH_4HCO_3
Gradient: 0-100%B in 65 min



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.

Efficiency and Inertness

Discovery BIO Wide Pore C5 has higher efficiency compared to conventional C4 phases.

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.

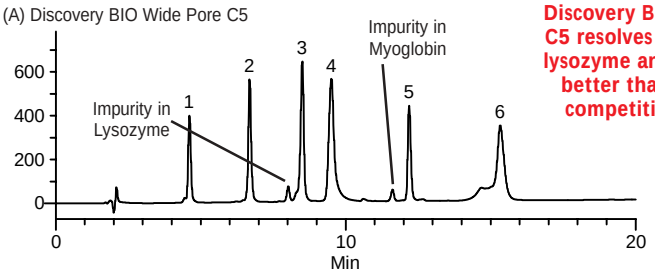
Even without TFA in the mobile phase, Discovery BIO Wide Pore C5 gives efficient, symmetrical peaks (Figure 4).

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

Columns: **(A) Discovery BIO Wide Pore C5, 15cm x 4.6mm, 5µm (Cat. No. 568422-U)**
(B) Competitive protein and peptide C4, 15cm x 4.6mm, 300Å, 5µm
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)
Flow Rate: 1.0mL/min
Temp: ambient
Detection: 220nm
Injection: 12µL in 0.1%TFA
Gradient: 0-100%B in 25 min

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

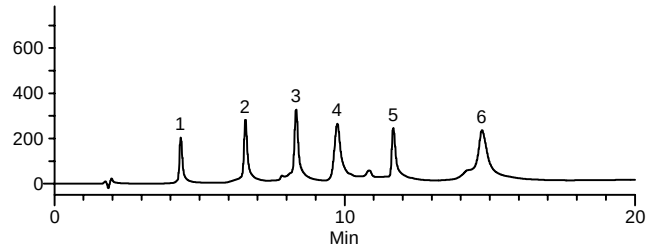
(A) Discovery BIO Wide Pore C5



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

G001488

(B) Competitor C4



G001553

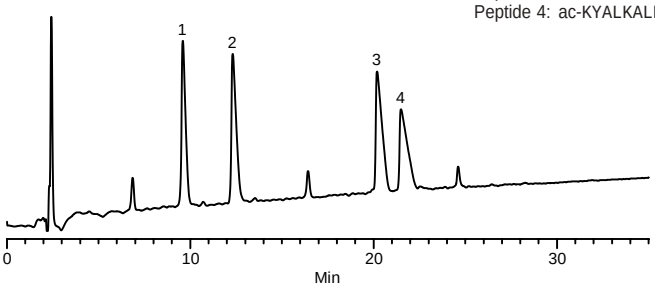
Figure 4: Efficient Analysis of Basic Peptides on Discovery BIO Wide Pore C5 without TFA

Column: **Discovery BIO Wide Pore C5, 15cm x 2.1mm, 5µm (Cat. No. 568402-U)**
Mobile Phase: (A) 25mM HCOOH in water
(B) 50:50 (25mM HCOOH in water) : (20mM HCOOH in CH_3CN)
Flow Rate: 0.208mL/min
Det.: 215nm
Temp.: 35°C
Inj.: 0.5µL (~0.25µg each peptide)
Sample: RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

Gradient:

Min	%B
0	15
35	50

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



G001882

Efficiency and Inertness

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

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 choosing the best column becomes a critical element for developing efficient and sensitive LC/MS methods.

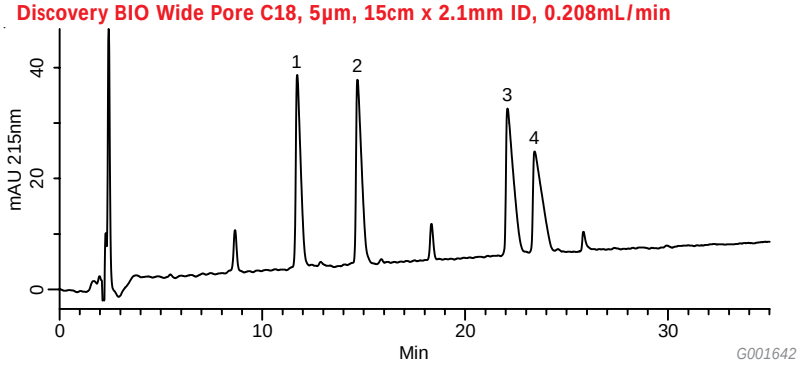
Figure 5: Column Performance Differences toward Basic Peptides without TFA

Columns: **C18, 300Å, 15cm x 2.1 or 2.0mm ID, 5µm**
Mobile Phase: (A) 25mM HCOOH in water;
(B) 50:50 (25mM HCOOH in water) : (20mM HCOOH in CH_3CN)
Flow Rate: 0.208 (or 0.189) mL/min
Det.: 215nm
Temp.: 35°C
Inj.: 0.5µL (~0.25µg each peptide)
Sample: RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

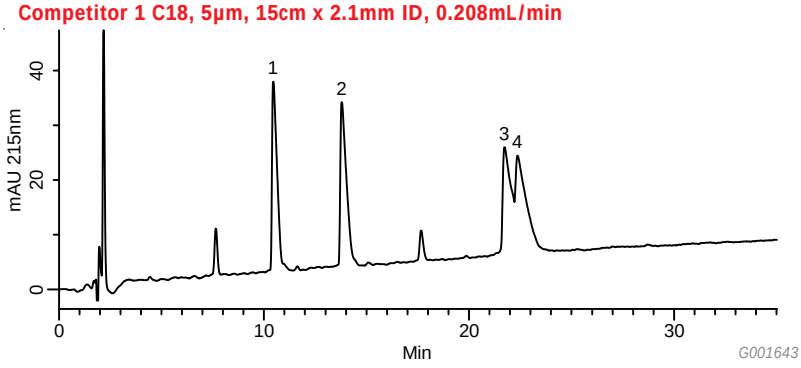
Gradient:	Min	%B
	0	15
	45	60

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

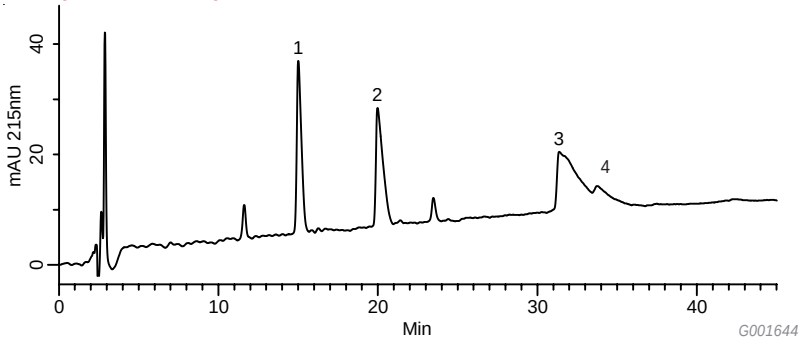
Discovery BIO Wide Pore C18, 5µm, 15cm x 2.1mm ID, 0.208mL/min



Competitor 1 C18, 5µm, 15cm x 2.1mm ID, 0.208mL/min



Competitor 2 C18, 5µm, 15cm x 2.0mm ID, 0.189mL/min



Selectivity

Discovery BIO Wide Pore phases have different selectivity than other reversed-phase columns increasing the resolution of natural and synthetic peptide mixtures.

Solid phase peptide synthesis relies on RP-HPLC

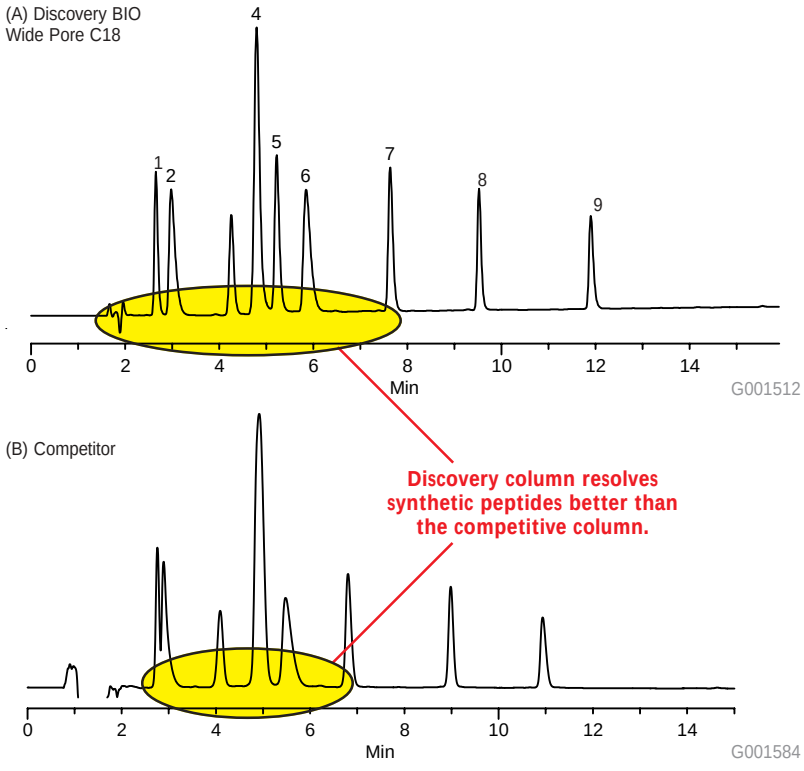
Solid phase synthesis is a common method to obtain novel peptides quickly and efficiently. Unintended side reactions are common and the RP-HPLC method must be capable of separating the peptides from unwanted by-products. Discovery BIO Wide Pore columns are ideal for this application. Figure 6 shows better 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, 15cm x 4.6mm, 5µm (Cat. No. 568222-U), (B) Competitive protein and peptide C18, 15cm x 4.6mm, 300Å, 5µm**
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)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 220nm
Injection: 10µL, ~0.25µg each peptide (Sigma Peptide Mix, Cat. No. P 2693 containing Arg^8-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.
Gradient: 0-100%B in 14 min after 1 minute delay

Peak	Peptide	Amino Acid Sequence
1	Arg^8-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



Selectivity

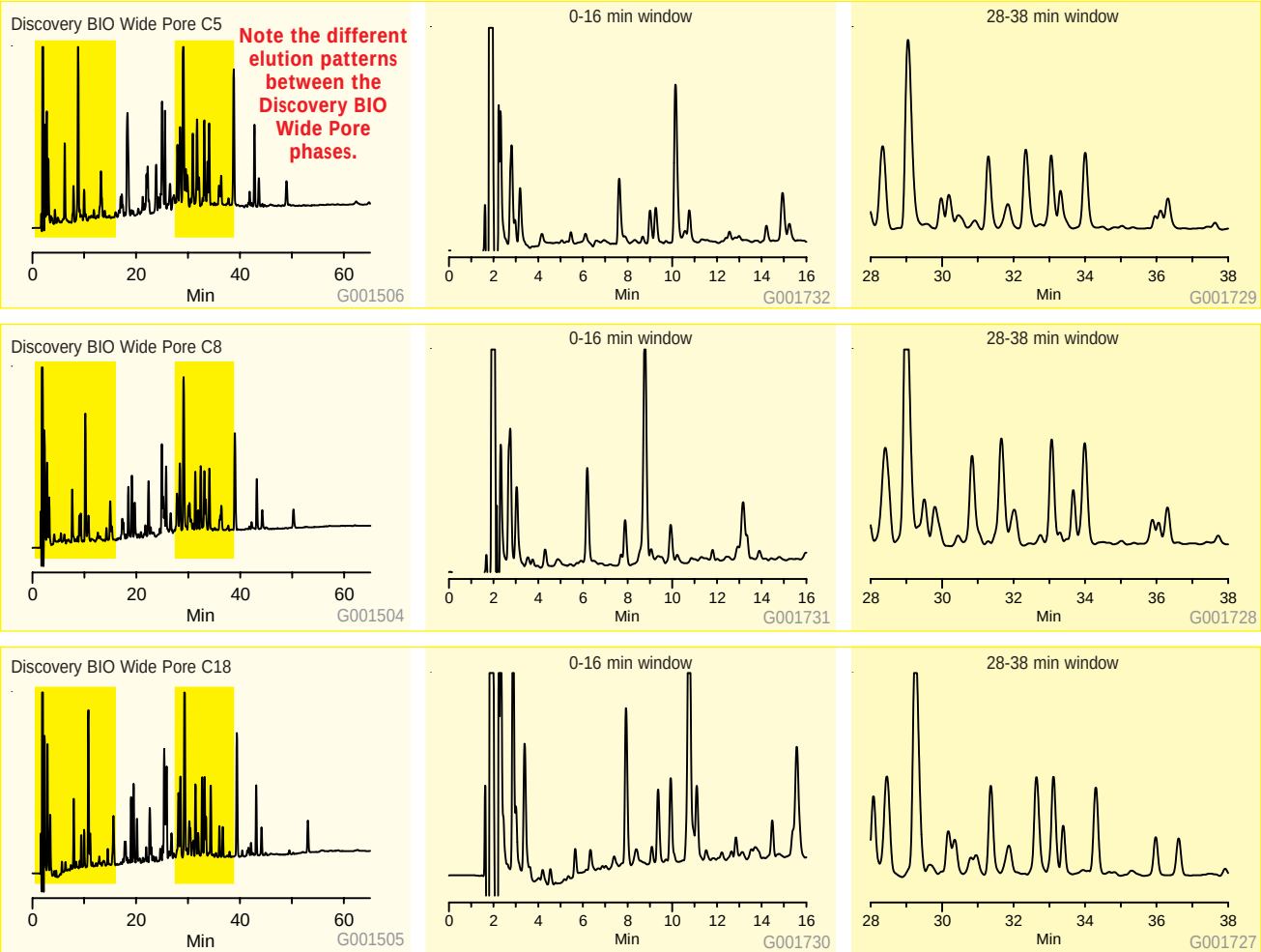
Each of the Discovery BIO Wide Pore phases offers a different profile of peptide maps allowing you to more accurately fingerprint and identify the parent protein.

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 peptides 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 (Cat. No. 568422-U); (B) Discovery BIO Wide Pore C8 (Cat. No. 568322-U); or (C) Discovery BIO Wide Pore C18 (568222-U), each 15cm x 4.6mm, 5µm**
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)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 215nm
Injection: 50µL carboxymethylated apohemoglobin tryptic digest in 50mM NH_4HCO_3
Gradient: 0-100%B in 65 min



Challenge: Small Sample Volumes and Proteins at Low Concentrations or Low Copy Numbers and the Need for Detailed Characterization

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, TFA is not required in the mobile phase to obtain good 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 completely scalable from analytical to preparative for easy, reliable scale-up.

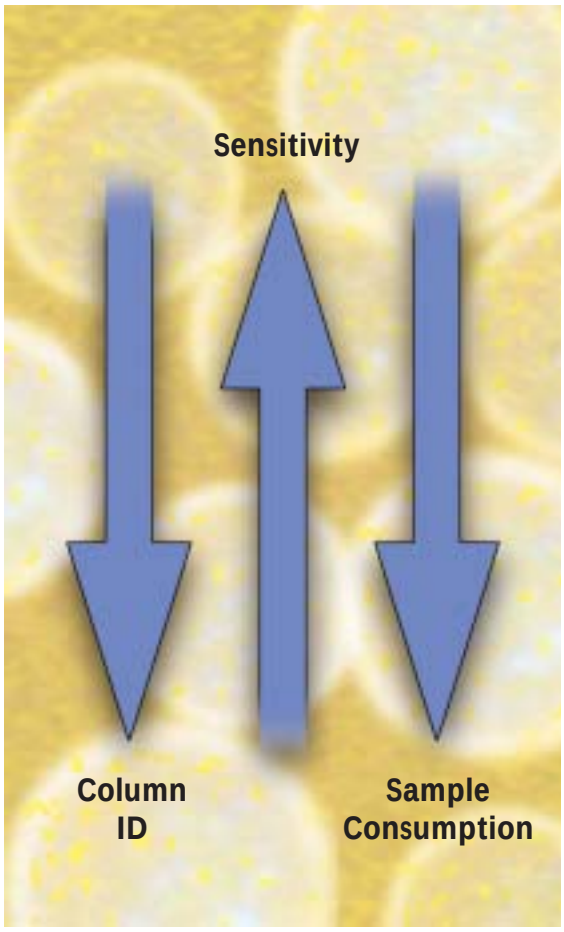
LC/MS Sensitivity: Capillary and Microbore Dimensions

Capillary and Microbore dimensions of Discovery BIO Wide Pore give greatly enhanced sensitivity and lower sample consumption.

In the world of modern HPLC separations, smaller is often better. Columns with narrow ID can enhance sensitivity when dealing with a limited sample size. This makes them ideal for applications where the need is to see compounds that exist at very low concentration in small sample volumes. The low flow rates and miniscule solvent consumption also makes narrow ID columns ideal for LC/MS applications because of the lower desolvation volume.

The Trend Toward Smaller ID Columns

Proteomics and other areas of modern biological research often generate large numbers of samples containing very small volumes that need to be analyzed in a minimal amount of time. Additionally, compounds of interest in these samples may exist at very low concentrations. When sample concentrations and volumes are sufficiently small, injection onto conventional internal diameter (ID) columns (4.6mm), and even narrowbore (2.1mm), immediately reveals that current means of detection lack adequate sensitivity for satisfactory analysis. This may be the case whether detection is by UV light absorption or mass spectrometry where inlet systems can be concentration dependent as well. This problem of detection sensitivity with conventional ID columns is a simple result of sample dilution within the relatively large volume comprised by the column and tubing. A direct approach to reducing the extent of dilution and to increase sensitivity is to reduce the column volume. As long as the linear velocity is constant, and for a given limiting sample mass, peak volumes are correspondingly reduced for narrower ID columns. One can detect levels thousands of times lower by decreasing the column ID. These principles are illustrated in Tables A and B, which all relate to each other by relative cross-sectional areas of the various column dimensions.



Increased Sensitivity Demonstrated

Figure 8 shows the observed behavior of increased sensitivity on columns of decreasing ID. The same sample was injected onto Discovery BIO Wide Pore C18 columns of equal length (10cm) but varying ID from 0.32mm to 2.1mm, 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 A and B.

Figure 8: Comparison of Peak Height (Sensitivity) Between Columns of Different Internal Diameters

Columns: **Discovery BIO Wide Pore C18, 300Å, 10cm x 0.32mm ID, 0.50mm ID, 1.0mm ID or 2.1mm ID, 3µm**
Mobile Phase: (65:35) H_2O:CH_3CN
Flow Rate: shown on figures
Temp.: ambient
Det.: UV, 254nm
Inj.: shown on figures
Sample Mass: acetophenone (0.1µg), benzene (1µg), toluene (1µg)

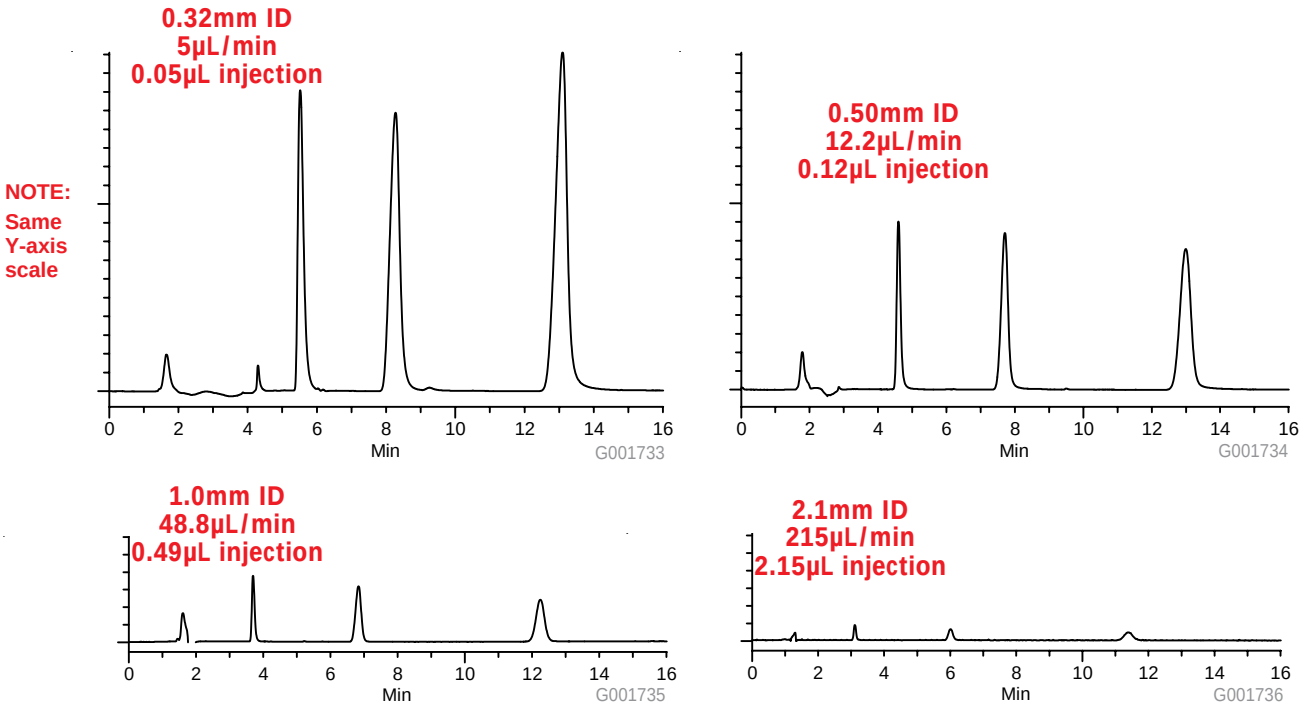


Table A: Effect of Column Dimension on Sensitivity for a Limiting Sample Mass

Column ID (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 B: Effect of Column Dimension on Required Sample Mass for a Given Sensitivity

Column ID (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.

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 the 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 maps to help identify proteins and provide other critical information. Two challenges facing researchers in the proteomics field are the need to obtain a lot of information from a very limited amount of sample and the need to detect proteins that exist at very low concentrations 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.* 4.6mm). 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 9 and 10 show the utility of Discovery BIO Wide Pore C18 capillary columns for sensitive peptide analysis by LC/MS.

Figure 9: ß-Lactoglobulin Tryptic Digest on 0.5mm ID Discovery BIO Wide Pore C18 Capillary

Column: **Discovery BIO Wide Pore C18, 15cm x 0.5mm, 5µm (Cat. No. 65519-U)**
Mobile Phase: (A) 0.1% TFA in water; (B) 0.1% TFA in CH_3CN
Flow Rate: 14µL/min
Temp: 30°C
Injection: 500pmol (5µL) ß-Lactoglobulin tryptic digest in 50mM NH_4HCO_3
Gradient: 5-40%B in 70 min
MS conditions: +ESI mode Capillary Temp 130°C, Source Voltage 2.5KV, Capillary Voltage 12V

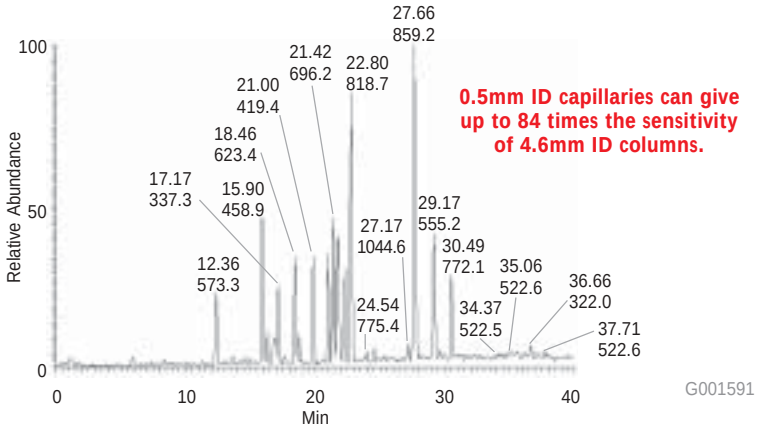
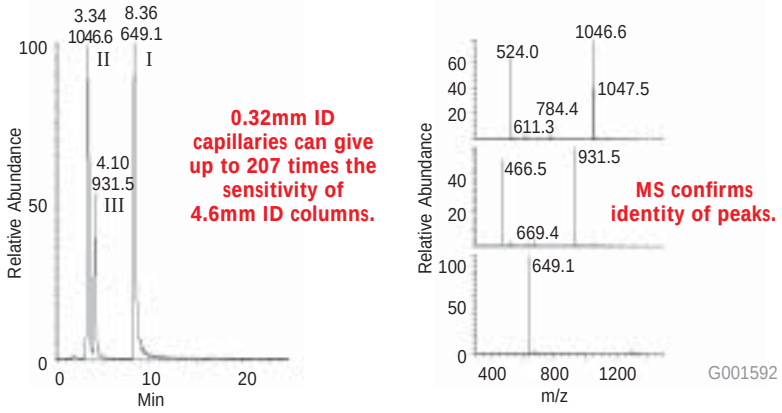


Figure 10: Angiotensins on 0.32mm ID Discovery BIO Wide Pore C18 Capillary

Column: **Discovery BIO Wide Pore C18, 10cm x 0.32mm, 3µm (Cat. No. 65527-U)**
Mobile Phase: (A) 65:35, (10mM NH_4OAc, pH 7):(50% CH_3CN in 20mM NH_4OAc, pH 7)
(B) 25:75, (10mM NH_4OAc, pH 7):(50% CH_3CN in 20mM NH_4OAc, pH 7)
Flow Rate: 6µL/min
Temp: ambient
Injection: 50pmol in water
Gradient: 0-100%B in 12.5 min
MS conditions: +ESI mode Capillary Temp 130°C, Source Voltage 2.5KV, Capillary Voltage 12V



LC/MS Sensitivity: No TFA Needed

Discovery BIO Wide Pore phases improve sensitivity by giving symmetrical, efficient peaks without TFA-containing mobile phases.

TFA (trifluoroacetic acid) is a commonly used mobile phase additive for reversed-phase HPLC (RP-HPLC) separations of proteins and peptides. 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 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 11. 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 retention are not masked. 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 ion-pair with the peptides.

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

Figure 11: Effect of Chromatographic Conditions on MS Signals of Peptides

Column: Discovery BIO Wide Pore C18, 15cm x 2.1mm, 3µm (Cat. No.: 567202-U)
Mobile Phase: (A1) A:25mM formic acid in H_2O,
B:50:50; (25mM formic acid in H_2O):(20mM formic acid in CH_3CN)[a]
(A2) A:0.01% TFA, B:0.01% TFA in 50:50 (CH_3CN:H_2O)
(A3) A:0.1% TFA, B:0.1% TFA in 50:50 (CH_3CN:H_2O)
Flow Rate: 0.208mL/min[b]
Det.: +ES
Temp.: ambient
Inj.: 1µL or 3µL
Sample: RP Peptide Performance Standard, p/n RPS-P0010 (Alberta Peptide Institute)

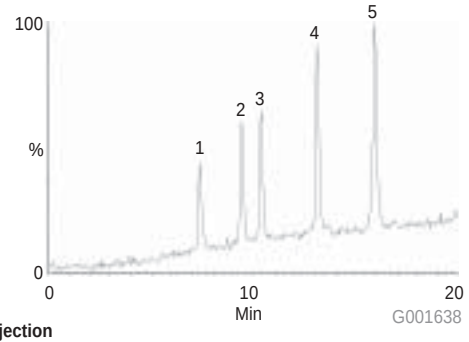
a) molarity of formic acid adjusted to provide minimum baseline drift
b) linear velocity equal to 1mL/min on 4.6mm ID columns

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

A1: 0% TFA (25mM formic acid), 1µL injection

Gradient:

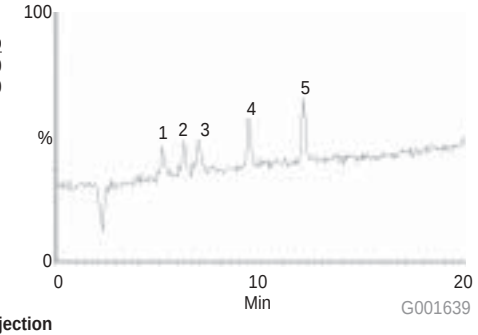
Min	%A	%B
0	90	10
20	50	50



A2: 0.01% TFA, 3µL injection

Gradient:

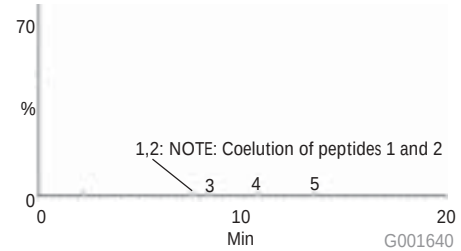
Min	%C	%D
0	80	20
20	40	60



A3: 0.1% TFA, 3µL injection

Gradient:

Min	%C	%D
0	80	20
20	40	60



LC/MS Sensitivity: Bleed-free

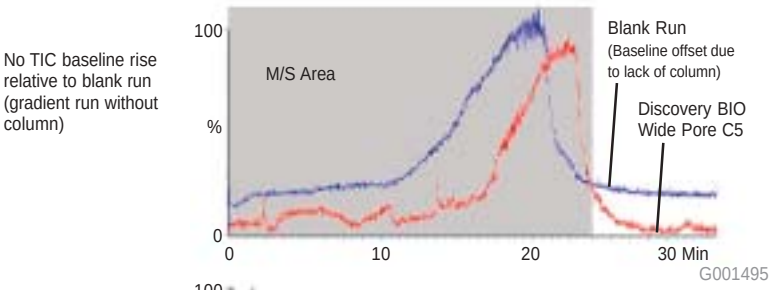
The bonded-phase stability of Discovery BIO Wide Pore provides no-bleed LC/MS analyses.

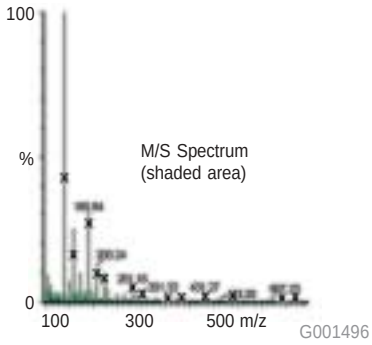
The quality and stability of all our Discovery BIO Wide Pore phases make them ideal for proteomics and other LC/MS applications.

HPLC interfaced with mass spectrometry (LC/MS) provides valuable information about the sample, such as molecular weight, 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 12 shows no evidence of column bleed from Discovery BIO Wide Pore C5 or C18.

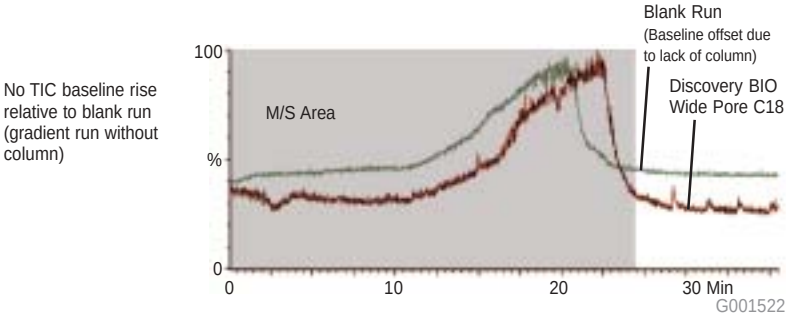
Figure 12: Undetectable LC/MS Bleed on Discovery BIO Wide Pore C5 and C18 Columns

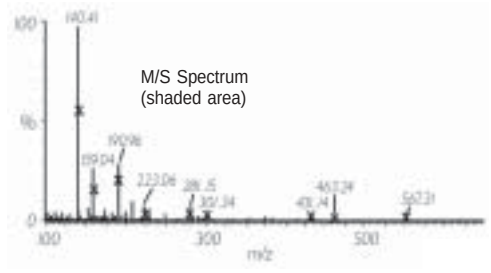
Columns: **Discovery BIO Wide Pore C18, 15cm x 4.6mm, 3µm (Cat. No. 567205-U), or Discovery BIO Wide Pore C5, 15cm x 4.6mm, 5µm (Cat. No. 568422-U)**
Mobile Phase: (A) 0.1% TFA in water;
(B) 0.1% TFA in CH_3OH
Flow Rate: 1.0mL/min
Temp: 30°C
Gradient: 0-100%B in 15 min, 100%B for 5 min, 0%B for 10 min





Essentially no m/z peaks generated from Discovery BIO Wide Pore C5 (above) or Discovery BIO Wide Pore C18 (below) 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 10µm particles, and 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 13).

- 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 Discovery BIO Wide Pore column dimension offering can be seen in the product listing at the end of this brochure.

Figure 13: Matched Selectivity from Analytical to Preparative on Discovery BIO Wide Pore C18

Columns: **Discovery BIO Wide Pore C18, 15cm x 4.6mm, 3µm (Cat. No. 567205-U)**
Discovery BIO Wide Pore C18, 15cm x 4.6mm, 5µm (Cat. No. 568222-U
Discovery BIO Wide Pore C18, 15cm x 10mm, 10µm (Cat. No. 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: 6.02cm/min
Temp: 30°C
Detection: 215nm
Sample: Sigma Peptide Mix (Sigma Cat. No. P 2693) in 0.1% TFA

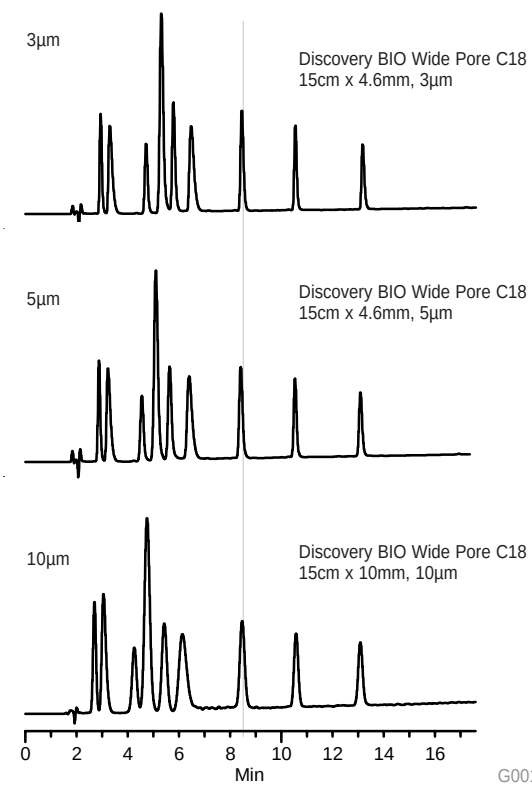
Column Parameters & Run Conditions:

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

Gradient:

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

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



G001512, 13, 11

Challenge: **Maintaining the Separation (Trouble-Free Operation)**

The stability and reproducibility of Discovery BIO Wide Pore phases permit reliable, trouble-free routine and long term operation.

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 14).

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 seen in Figure 15.

Figure 14: Stability of Discovery BIO Wide Pore C18 at pH 2 and 70°C

Column: Discovery BIO Wide Pore C18, 5cm x 4.6mm, 5µm (Cat. No. 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)
Flow Rate: 2.0mL/min
Temp: 70°C
Detection: 220nm
Injection: 5µL, 2.5µg each peptide (Sigma Peptide Mix, Cat. No. H 2016) in mobile phase A
Gradient: 2-24%B in 22 min, 8 min at 100%A

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

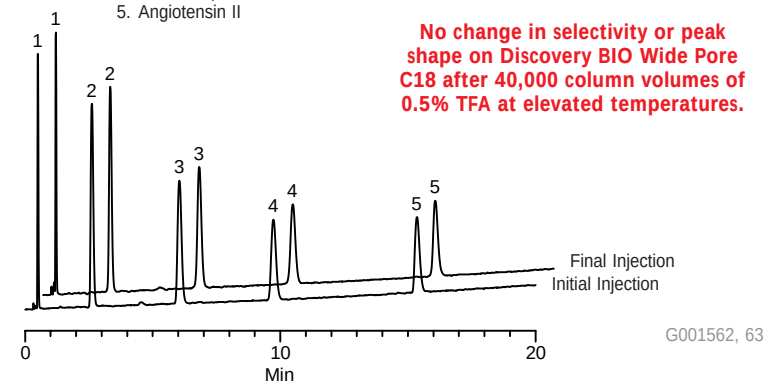
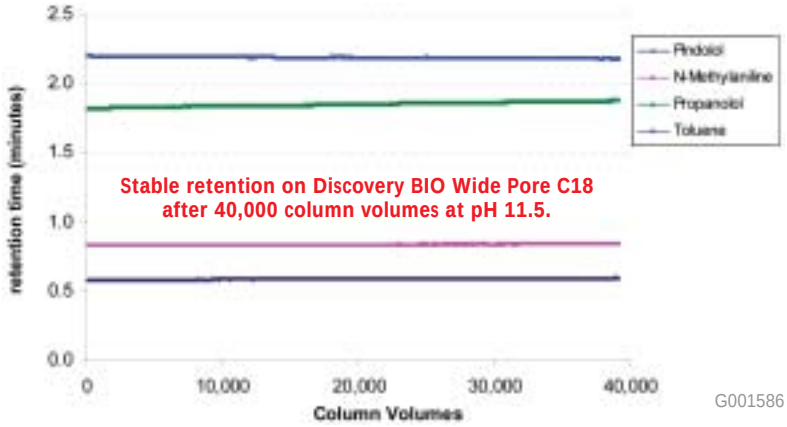


Figure 15: Discovery BIO Wide Pore C18 Stability at pH 11.5

Column: Discovery BIO Wide Pore C18, 5cm x 4.6mm, 5µm (Cat. No. 568220-U)
Mobile Phase: 65:35, 50mM pyrrolidine HCl (pH 11.5):CH_3CN
Flow Rate: 2.0mL/min
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 protein or peptide separations will be stable as well.

Stability

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.

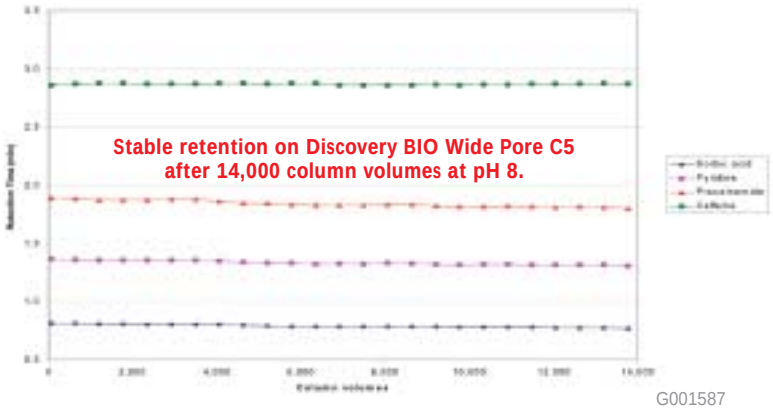
Short chain alkyl bonded phases such as C3 and C4 are routinely used for RP-HPLC separation of proteins and hydrophobic peptides. However, both C3 and C4 phases hydrolyze at low and high pH resulting in short column life. Discovery BIO Wide Pore C5 gives similar selectivity to a C3 or C4, but greatly improved pH stability (See Figures 16 and 17).

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 16: Stability of Discovery BIO Wide Pore C5 at pH 8

Column: **Discovery BIO Wide Pore C5, 5cm x 4.6mm, 5µm (Cat. No. 568420-U)**
Mobile Phase: 95:5, 25mM potassium phosphate (pH 8):CH_3OH
Flow Rate: 2.0mL/min
Temp: 35°C

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

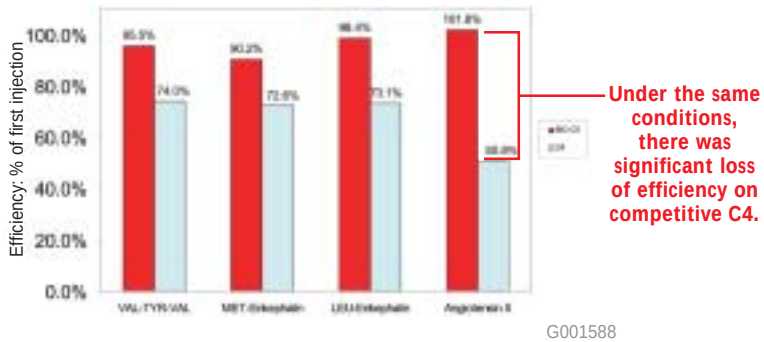


G001587

Figure 17: Comparison of Low pH Stability of Discovery BIO Wide Pore C5 versus a Competitive C4 Column

Columns: **Discovery BIO Wide Pore C5, 5cm x 4.6mm, 5µm (Cat. No. 568420-U), or (B) Competitive protein and peptide C4, 5cm x 4.6mm, 300Å, 5µm**
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)
Flow Rate: 2.0mL/min
Temp: 30°C
Detection: 220nm
Injection: 5µL, 2.5µg each peptide (Sigma Peptide Mix, Cat. No. H 2016) in mobile phase A
Gradient: 2-24%B in 22 min, 8 min at 100%A

Efficiency on Discovery BIO Wide Pore C5 is stable even after 25,000 column volumes (222 gradient cycles).



G001588

Reproducibility

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

No HPLC method is valuable if it is not reproducible, run-to-run, column-to-column, batch-to-batch. Discovery BIO Wide Pore columns are guaranteed to be reproducible.

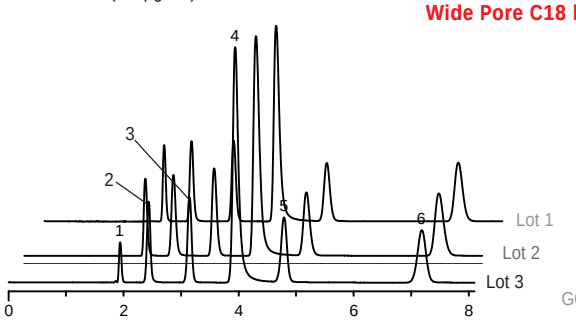
An important factor for developing a robust analytical method is column reproducibility. Column selectivity should remain the same and the elution patterns of the proteins or peptides must be reproducible. Consistency in silica and bonded phase chemistry guarantees that Discovery BIO Wide Pore columns have exceptional reproducibility between injections, columns, and bonded phase lots (See Figures 18 and 19).

Figure 18: Lot-to-Lot Reproducibility of Discovery BIO Wide Pore C18

Column: **Discovery BIO Wide Pore C18, 15cm x 4.6mm, 5µm (Cat. No. 568222-U)**
Mobile Phase: 80:20, 10mM NH_4OAc (pH 6.8):CH_3OH
Flow Rate: 1.0mL/min
Temp: 35°C
Detection: 254nm
Injection: 5µL

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

Less than 5% coefficient of variation between different Discovery BIO Wide Pore C18 lots.

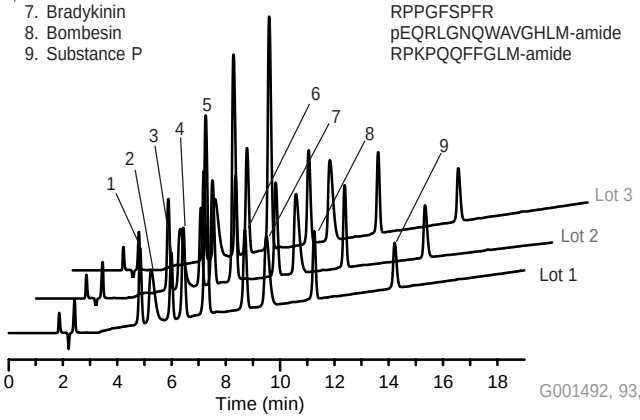


G001514, 15, 16

Figure 19: Lot-to-Lot Reproducibility of Discovery BIO Wide Pore C5

Column: **Discovery BIO Wide Pore C5, 15cm x 4.6mm, 5µm (Cat. No. 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)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 215nm
Injection: 10µL, ~0.25µg each peptide (Sigma Peptide Mix, Cat. No. P 2693) in mobile phase A
Gradient: 0-100%B in 19 min

	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



G001492, 93, 94

Suggestions for Choosing a 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 ID
LC/MS	2.1mm or smaller
Peptide mapping	4.6mm, 4.0mm, 2.1mm
Analytical HPLC	4.0mm, 4.6mm
Preparative	10mm, 21.2mm
Low level detection or limited sample volume	0.18mm, 0.32mm, 0.5mm, 1.0mm

The complete list of Discovery BIO Wide Pore phases appears at the back of this brochure.

Discovery BIO PolyMA-SCX and Discovery BIO PolyMA-WAX

Polymethacrylate polymer-based cation-exchange and anion-exchange columns provide efficient separation of proteins, peptides, and other biomolecules.

Discovery BIO PolyMA polymer-based ion-exchange particles have discriminating hydrophilic surface chemistry making them ideally suited for separating proteins, peptides, and other biotechnology-derived products. Differing from reversed-phase separations, ion-exchange separates proteins and peptides that may have similar hydrophobic characteristics but have different degrees of ionization (charge). Two ion-exchangers, Discovery BIO PolyMA-SCX for cation-exchange, and Discovery BIO PolyMA-WAX for anion-exchange, complement the Discovery BIO silica-based materials. The proprietary hydrophilic surface chemistry of Discovery PolyMA ion-exchange particles offers subtle ionic selectivity characteristics that are not available from the typical polystyrene-divinylbenzene (PS-DVB) and standard polymethacrylate based ion-exchange resins currently on the market. In contrast to silica-based packings, Discovery BIO PolyMA is resistant to chemical degradation at acidic and basic pH extremes. Significant benefits include:

- Excellent separations of protein isoforms
- High resolution at low sample load
- Quantitative recovery – a hydrophilic surface eliminates protein adsorption
- High efficiency
- Wide pH range

Type	PolyMA-SCX Strong cation exchange	PolyMA-WAX Weak anion exchange
Bonded Phase	SP (sulfopropyl)	DEAE (diethylaminoethyl)
Counter ion (as supplied)	Na^+	Cl^-
Particle Platform	Polymethacrylate	Polymethacrylate
Particle Shape	Spherical, monodispersed	Spherical, monodispersed
Particle Sizes (µm)	5	5
Pore Size (Å)	1000	1000
Coverage	0.3meq/g	0.3meq/g
pH range	1 to 13	2 to 10*
Temperature range	4°C to 50°C	4°C to 50°C

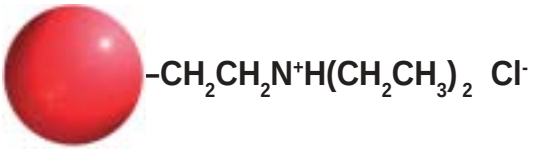
* Although a weak anion-exchange material, PolyMA-WAX can be used at high pH values but with reduced charge.

Choosing Discovery BIO PolyMA-SCX or PolyMA-WAX

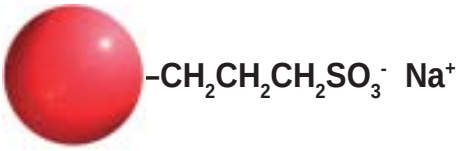
Particle	Separation Mode	When to Use
Discovery BIO PolyMA-SCX	Cation Exchange	Generally at a pH less than the protein pI; usually at pH less than 7
Discovery BIO PolyMA-WAX	Anion Exchange	Generally at a pH greater than the protein pI; usually at pH 7 or higher.

Figure 20: Structures of Discovery BIO PolyMA-SCX and PolyMA-WAX

Discovery BIO PolyMA-WAX:



Discovery BIO PolyMA-SCX:

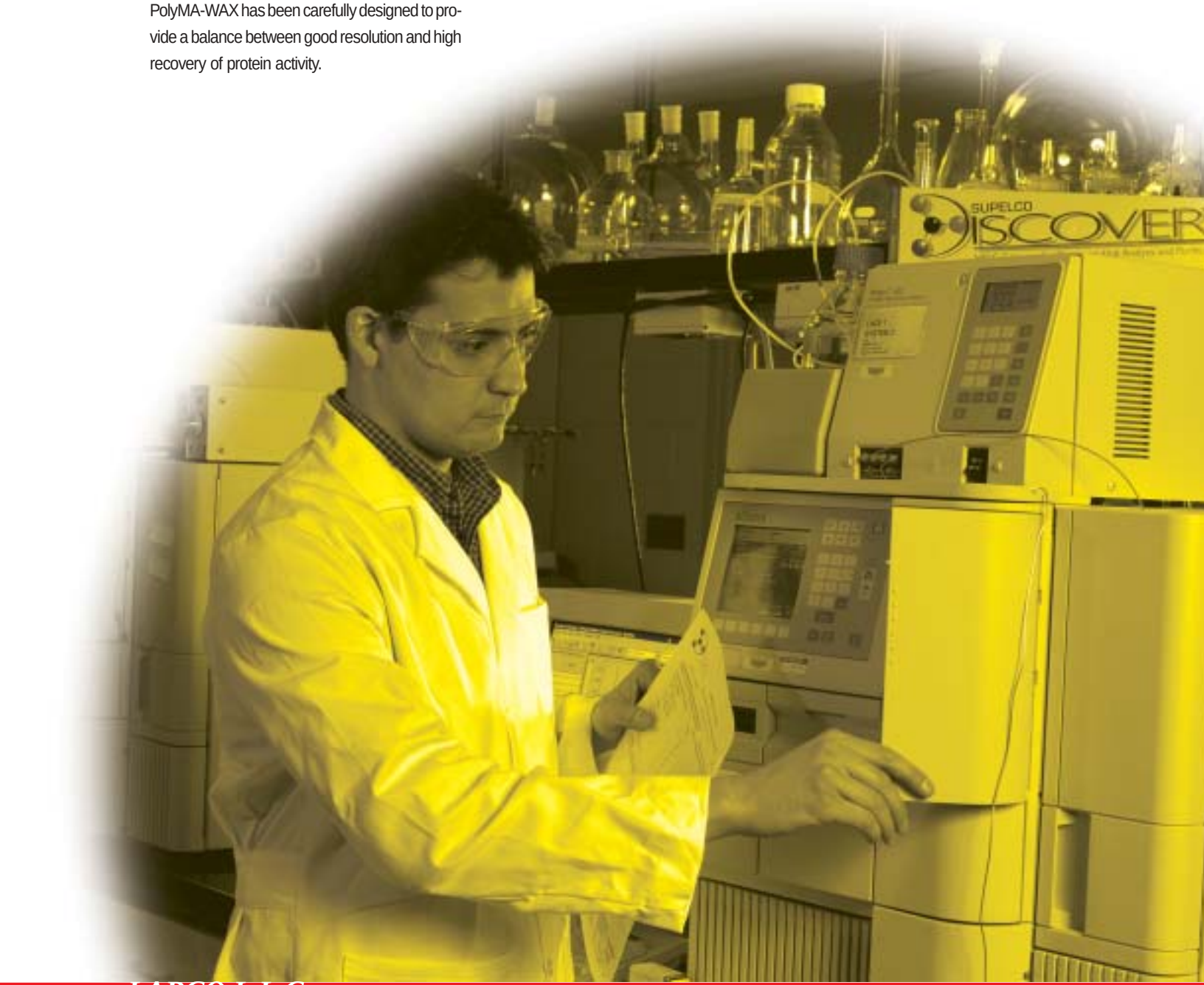


Ion-Exchange Analysis of Biomolecules on Polymeric Supports

Complementary to the silica-based reversed-phase materials, ion-exchange on non-silica supports is often used in the analysis and upstream processing of proteins, peptides, and other biotechnology-derived products. Discovery BIO PolyMA-SCX and PolyMA-WAX particles are based on polymethacrylate (PolyMA) particles that have distinct advantages over other particle types for HPLC of proteins and peptides, as listed in Table C. The degree of ion-exchange functionalization on Discovery BIO PolyMA-SCX and PolyMA-WAX has been carefully designed to provide a balance between good resolution and high recovery of protein activity.

Table C: Benefits of Polymethacrylic Polymers over other HPLC Particles

Competitive Particle	Benefits of Hydrophilic-coated Polymethacrylate (BIO PolyMA)
Polystyrene	BIO PolyMA is less hydrophobic, reducing the amount of secondary, non-specific interactions that can cause low protein recovery
Cross-linked polysaccharides	BIO PolyMA is more mechanically stable, increasing column lifetime and operating flow rates
Silica	BIO PolyMA is more chemically stable, increasing the range of pH available to alter selectivity, or regenerate with base
Standard Polymethacrylate	BIO PolyMA hydrophilic coating gives better protein recovery



Meeting the Challenges of Today's Protein and Peptide Separations

Challenge: Complex Protein or Peptide Mixtures

Efficiency and selectivity offered by Discovery BIO PolyMA-SCX and PolyMA-WAX give efficient ion-exchange separation of a wide variety of peptides and proteins.

Efficiency

Discovery BIO PolyMA-SCX and PolyMA-WAX provide the efficiency needed to resolve closely-related proteins and peptides.

Ion-exchange separations are sensitive to slight differences in protein structure if those changes affect the net charge or distribution of charges on the protein. These differences can occur as a result of chemical or enzymatic degradation, or they may naturally exist in the protein or peptide population under study. The higher the efficiency of the ion-exchange column, the more power it has to resolve proteins and peptides with small charge-related structural differences. Discovery BIO PolyMA-SCX and PolyMA-WAX columns have the high efficiency necessary to resolve complex protein and peptide mixtures. Figure 21 shows the degradation products of human growth hormone (hGH) well-separated on a Discovery BIO PolyMA-WAX anion-exchange column. The difference between the resolved compounds is the conversion of protein amide(s) to carboxylate(s), demonstrating both the power of the ion-exchange technique, and the efficiency of the Discovery BIO PolyMA-WAX column.

Figure 22 shows the separation of three cytochrome c variants on Discovery BIO PolyMA-SCX. Note the difference in elution order compared to the reversed-phase separation in Figure 1, page 6 on Discovery BIO Wide Pore C18.

Figure 21: Discovery BIO PolyMA-WAX Columns Have the Efficiency to Resolve hGH and its Degradation Products

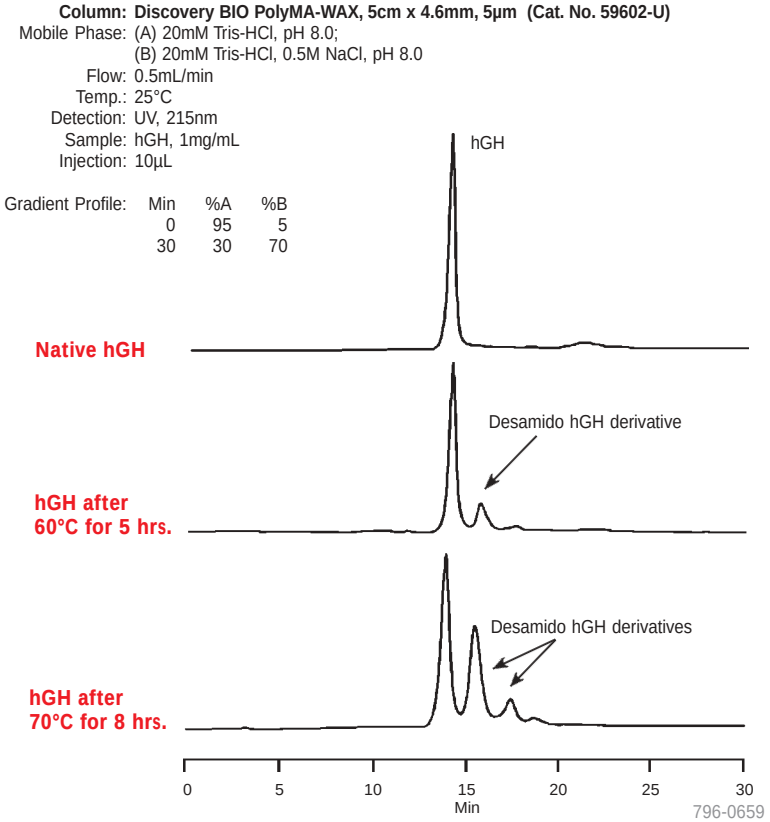
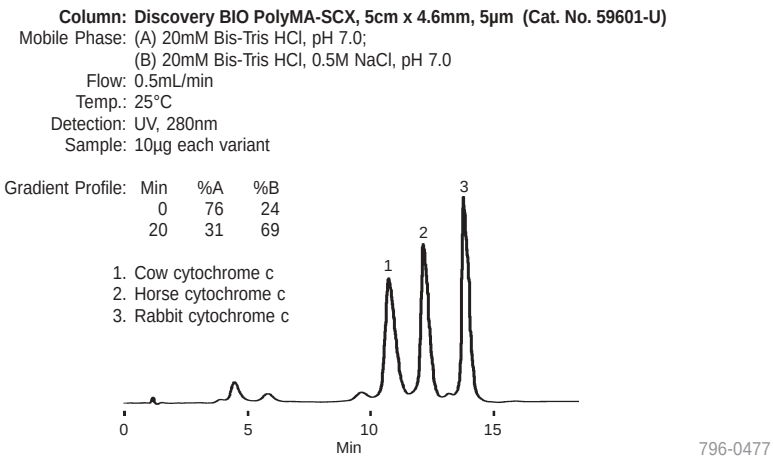


Figure 22: Discovery BIO PolyMA-SCX has the Efficiency to Separate Closely-Related Cytochrome c Variants



Efficiency

Discovery BIO PolyMA-SCX and PolyMA-WAX have higher efficiency than competitive polymeric ion-exchange materials.

Polymer particles are frequently used in bioseparations. Their advantages over inorganic particles, however, are often offset by generally lower efficiency. Discovery BIO PolyMA-SCX and PolyMA-WAX particles offer the benefits of polymeric particles, but have higher efficiency and better resolution than competitive particles. An example of the high efficiency of Discovery BIO PolyMA-SCX and PolyMA-WAX is shown in Figure 23. The Discovery BIO PolyMA-SCX column gives efficient, well-resolved separation of a four-component peptide mixture compared to two leading polymeric SCX columns.

Figure 23: Discovery BIO PolyMA-SCX Columns Have Higher Efficiency than Competitive Polymeric Columns

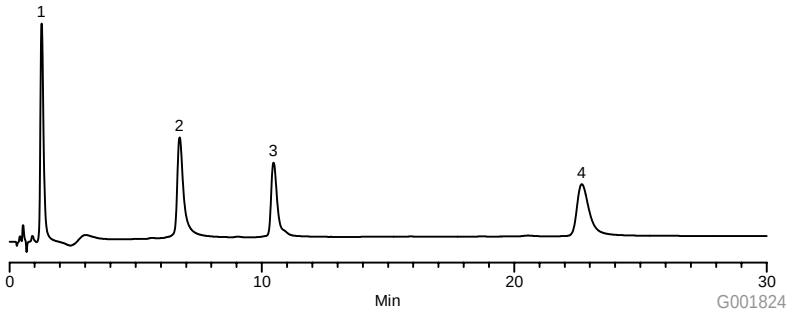
Columns: **Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U)**
Competitive polymeric-SCX columns of comparable dimensions
Mobile Phase: (A) 5% CH_3CN in 20mM NH_4HCO_2/H_3PO_4, pH 3.5;
(B) 5% CH_3CN in 20mM NH_4HCO_2, 480mM NH_4HPO_3/H_3PO_4, pH 3.5
Flow: 0.208mL/min
Temp.: 35°C
Detection: UV, 215nm
Sample: RP Peptide Ionic Interactions Standard, p/n RPS-10020 (Alberta Peptide Institute)
Injection: 10µL

Gradient Profile:

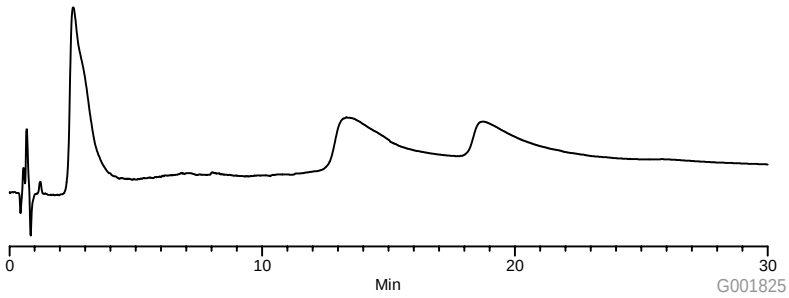
Min	%A	%B
0	100	0
24	0	100

1. ac-GGGLGGAGGLK-amide
2. ac-KYGLGGAGGLK-amide
3. ac-GGALKALKGLK-amide
4. ac-KYALKALKGLK-amide

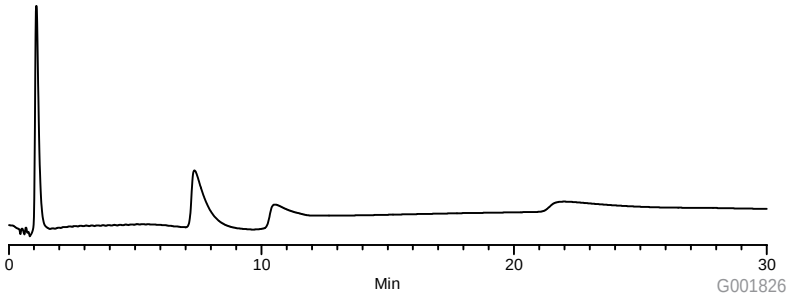
Discovery BIO PolyMA-SCX



Competitor 1



Competitor 2



Efficiency

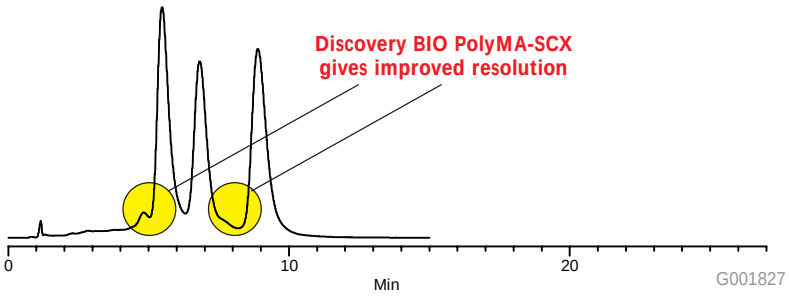
Higher column efficiency also translates to better sensitivity and the ability to detect lower levels of proteins or peptides. Further comparisons attesting to the efficiency Discovery BIO PolyMA-SCX and PolyMA-WAX are shown in Figures 24 and 25, respectively. The higher efficiency of the Discovery BIO PolyMA-SCX allowed it to resolve a small impurity of cytochrome c not resolved by the leading competitive columns.

Figure 24: Comparison of Efficiency: Cytochrome c Variants

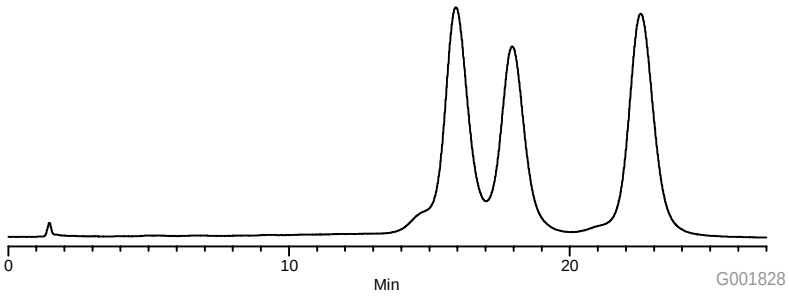
Columns: **Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U) and Competitive polymeric-SCX columns of similar dimensions**
Mobile Phase: (A) 50mM MOPS/KOH, pH 7.0;
(B) 50mM MOPS/KOH, 0.5M KCl, pH 7.0
Flow: 3.01cm/min (flow rates appear in Figure)
Temp.: 35°C
Detection: UV, 280nm
Sample: 10µg each variant
Gradient profile: 0.6% B per minute. See Figure for details.

1. Cow cytochrome c
2. Horse cytochrome c
3. Rabbit cytochrome c

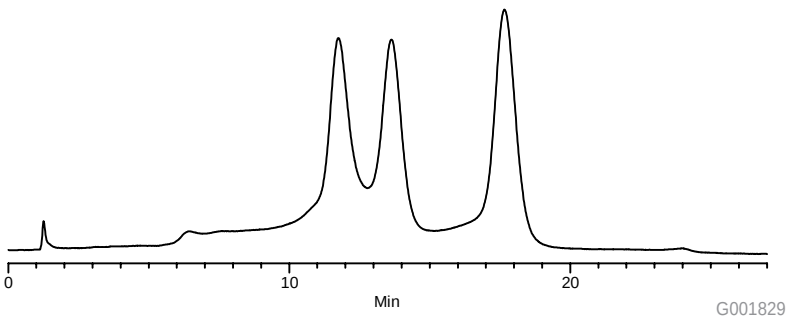
Discovery BIO PolyMA-SCX (5cm x 4.6mm, 0.5mL/min, 28-37%B in 15 minutes)



Competitor 1 (5cm x 5mm, 0.59mL/min, 28-46%B in 30 minutes)



Competitor 2 (5cm x 5mm, 0.59mL/min, 28-43%B in 25 minutes)



Efficiency

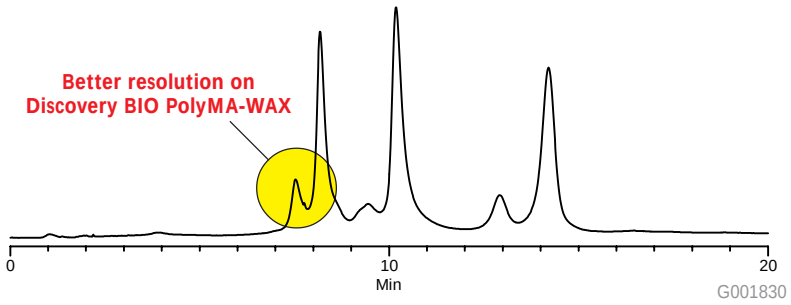
Similarly, in Figure 25 Discovery BIO PolyMA-WAX is shown to give better resolution and band spacing of hemoglobin variants. Note that flow rates were adjusted to achieve equal linear flow and gradient slope on the columns for proper comparison.

Figure 25: Comparison of Efficiency: Hemoglobin Variants

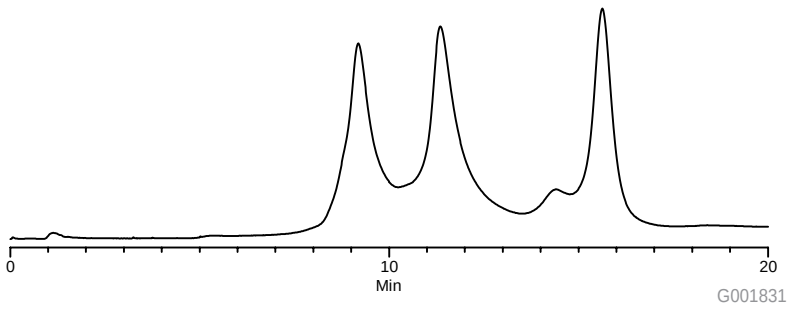
Columns: **Discovery BIO PolyMA-WAX, 5cm x 4.6mm, 5µm (Cat. No. 59602-U) and Competitive polymeric weak anion exchange columns**
Mobile Phase: (A) 10mM Tris/HOAc, pH 8.0;
(B) 10mM Tris/HOAc, 0.25M KCl, pH 8.0
Flow: 3.01cm/min (flow rates appear in Figure)
Temp.: 35°C
Detection: UV, 280nm
Sample: 50µg each variant
Gradient profile: 1.6% B per minute. See Figure for details.

1. Hemoglobin A_2
2. Hemoglobin S
3. Hemoglobin A_0

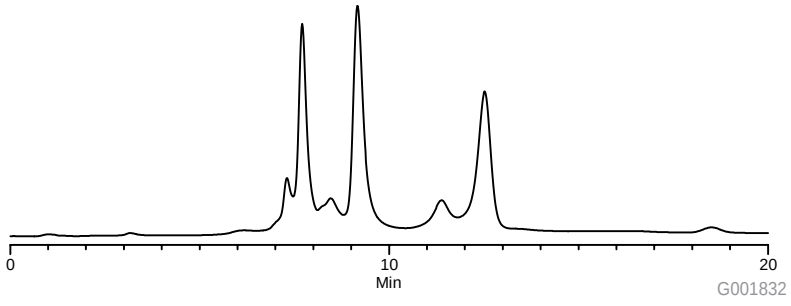
Discovery BIO PolyMA-WAX (5cm x 4.6mm, 0.5mL/min, 0-32%B in 20 min.)



Competitor 1 (5cm x 5mm, 0.59mL/min, 0-32%B in 20 min.)



Competitor 2 (5cm x 5mm, 0.59mL/min, 0-32%B in 20 min.)



Wide Applicability

Discovery BIO PolyMA-SCX and PolyMA-WAX have porosity needed to separate proteins over a wide molecular weight range.

The large pore diameter of Discovery BIO PolyMA-SCX and PolyMA-WAX particles allows full access to small peptides and very large proteins and protein aggregates. Figure 26 shows the separation of proteins that vary from 18 to 80kDA. Figure 27 shows the power of ion-exchange to resolve proteins with very little difference in molecular weight, an advantage over size-exclusion separations.

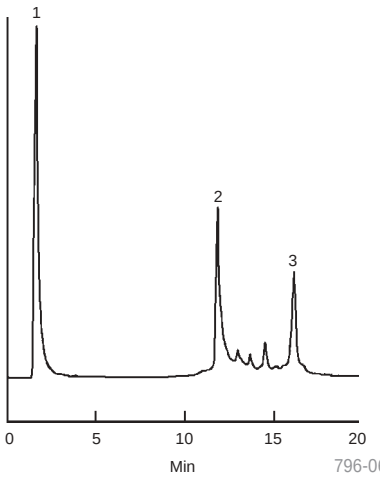
Figure 26: Discovery BIO PolyMA Columns Separate Proteins of Varying Different Molecular Weight by Ion-Exchange

Column: Discovery BIO PolyMA-WAX, 5cm x 4.6mm, 5µm (Cat. No.59602-U)
Mobile Phase: (A) 20mM Tris-HCl, pH 8.0;
(B) 20mM Tris-HCl, 0.5M NaCl, pH 8.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Myoglobin (5µg), conalbumin (5µg), trypsin inhibitor (10µg)

Gradient Profile:

Min	%A	%B
0	95	5
15	0	100

1. Myoglobin
2. Conalbumin
3. Trypsin inhibitor



796-0657

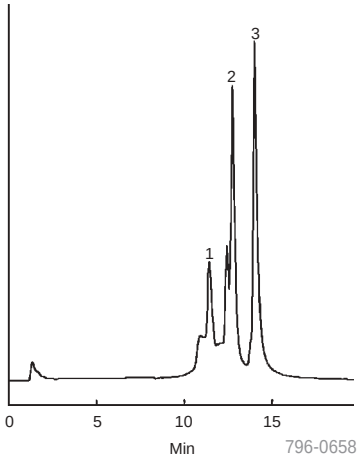
Figure 27: Discovery BIO PolyMA Columns Distinguish Proteins of Similar Molecular Weight

Column: Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U)
Mobile Phase: (A) 20mM sodium phosphate, pH 7.0;
(B) 20mM sodium phosphate, 0.5M NaCl, pH 7.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Ribonuclease A (10µg), α-chymotrypsinogen A (5µg), cytochrome c (5µg)

Gradient Profile:

Min	%A	%B
0	95	5
15	0	100

1. Ribonuclease A
2. α-Chymotrypsinogen A
3. Cytochrome c



796-0658

Wide Applicability

The selectivity and efficiency of Discovery BIO PolyMA-SCX and PolyMA-WAX is demonstrated in these protein separations.

The separations in Figures 28 and 29 show Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

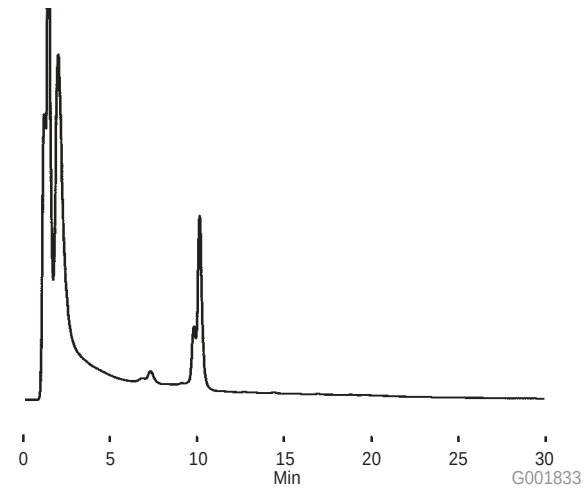
Figure 28: Discovery BIO PolyMA-SCX Application: Elastase and Human hemoglobin S

Columns: **Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U)**
Mobile Phase: (A) 20mM sodium phosphate, pH 7.0;
(B) 20mM sodium phosphate, 0.5M NaCl, pH 7.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Elastase (6.5mg/mL), human hemoglobin S (2mg/mL)
Injection: 10µL

Gradient Profile:

Min	%A	%B
0	100	0
30	0	100

Elastase (26 kDa)



Human hemoglobin S (64.5 kDa)

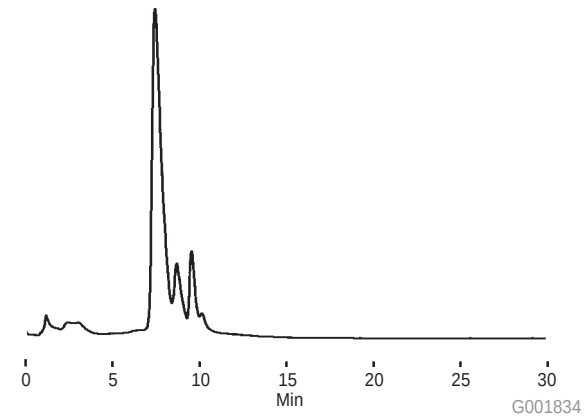


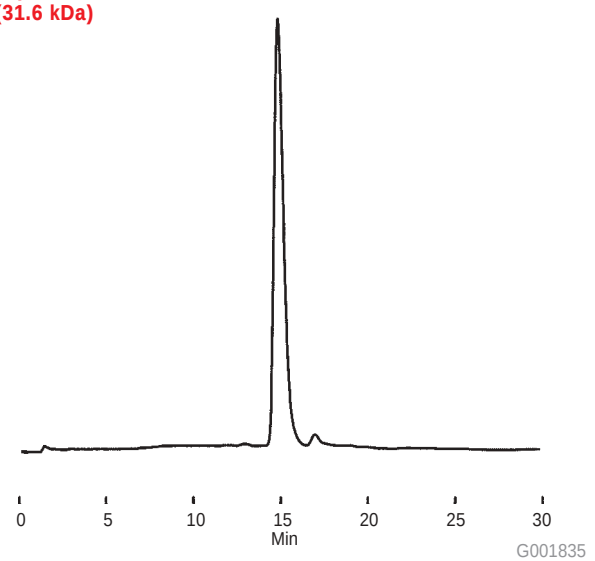
Figure 29: Discovery BIO PolyMA-WAX Application: Superoxide dismutase and Creatine kinase

Columns: **Discovery BIO PolyMA-WAX, 5cm x 4.6mm, 5µm (Cat. No. 59602-U)**
Mobile Phase: (A) 20mM Tris-HCl, pH 8.0;
(B) 20mM Tris-HCl, 0.5M NaCl, pH 8.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Superoxide dismutase (2mg/mL), creatine kinase (2mg/mL)
Injection: 10µL

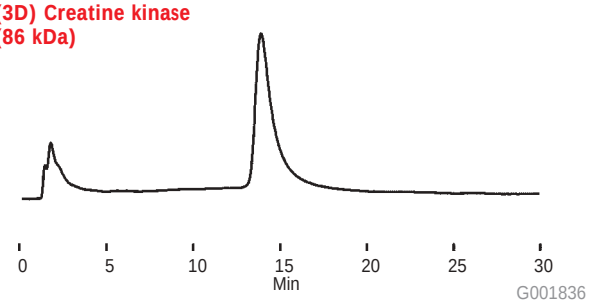
Gradient Profile:

Min	%A	%B
0	100	0
30	90	10

Superoxide dismutase (31.6 kDa)



(3D) Creatine kinase (86 kDa)



Challenge: Small Sample Volumes and Proteins at Low Concentrations or Low Copy Numbers, Need for Detailed Characterization

The features that are *status quo* for a protein separation media are resolution, recovery, capacity, mechanical strength, and chemical stability. Discovery BIO PolyMA-SCX and PolyMA-WAX meet these requirements.

Capacity

Discovery BIO PolyMA-SCX and PolyMA-WAX are designed to balance capacity and recovery.

The capacity of Discovery BIO PolyMA-SCX and PolyMA-WAX columns for two model proteins is shown in Table D. Although capacity is dependent on the protein under study and operating conditions, generally between 20 and 50mg of total protein can be injected onto the Discovery BIO PolyMA-SCX and PolyMA-WAX columns.

Table D: Capacity of Discovery BIO PolyMA-SCX and PolyMA-WAX

Column	Protein	mg per mL of column volume	mg per column
Discovery BIO PolyMA-SCX	Lysozyme	40mg	30mg
Discovery BIO PolyMA-WAX	BSA	50mg	40mg

Loading studies were run at 240cm/hr (0.67mL/min) using 0-1M NaCl salt gradients in either 20mM Tris-HCl, pH 8 (for BSA), or 20mM sodium phosphate, pH 7 (for lysozyme). Protein concentration was 2mg/mL in starting mobile phase.

Recovery

Full recovery of injected sample mass and activity is assured on Discovery BIO PolyMA-SCX and PolyMA-WAX.

Loss of protein can occur when it strongly binds to the particle surface by non-specific, hydrophobic interactions. Many polymer-based HPLC packings have this serious disadvantage. High protein recovery is guaranteed on Discovery BIO PolyMA-SCX and PolyMA-WAX because it is completely covered with a covalently-bonded hydrophilic layer. This absence of non-specific interactions gives the high recovery on Discovery BIO PolyMA-WAX, and PolyMA-SCX as reported in Tables E and F.

Table E: Recovery on Discovery BIO PolyMA-WAX vs. Competitive Column

Column	Fibrinogen Recovery		
	1st Injection	2nd Injection	5th Injection
Discovery BIO PolyMA-WAX (5cm x 4.6mm, 5µm)	88.9%	89.9%	92.9%
Competitive polymethacrylic DEAE column (7.5cm x 7.5mm, 10µm)	59.1%	75.7%	82.1%

Mobile Phase: Gradient of 0 – 0.5M NaCl in 20mM Tris-HCl (pH 8) over 30 minutes, Flow: 1mL/min, Ambient Temperature, Sample: 40µg fibrinogen (Sigma part no. F-4753) in 20µL starting mobile phase.

Table F: Protein Recovery on Discovery PolyMA-SCX vs. Competitive Column

Column	Cytochrome c Recovery		
	1st Injection	2nd Injection	5th Injection
Discovery BIO PolyMA-SCX (5cm x 4.6mm, 5µm)	99.5%	98.6%	100.2%
Competitive poymethacrylic SP column (7.5cm x 7.5mm, 10µm)	59.0%	76.1%	90.6%

Mobile Phase: Gradient of 0 – 1M NaCl in 20mM citrate (pH 4) over 30 minutes, Flow: 0.5 mL/min, Ambient Temperature, Sample: 40µg cytochrome c in 20µL starting mobile phase.

Challenge: Maintaining the Separation (Trouble-Free Operation)

The stability and reproducibility of Discovery BIO PolyMA-SCX and PolyMA-WAX permit reliable, trouble-free routine and long term operation.

Chemical Stability and Column Life

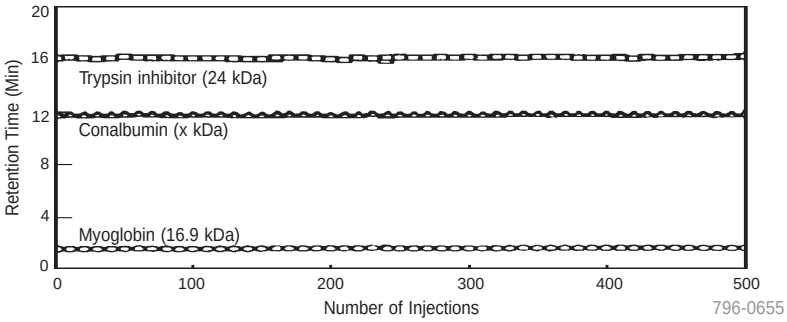
Chemical stability gives reliable separations and long column life on Discovery BIO PolyMA-SCX and PolyMA-WAX.

Column stability is important from quality and economy standpoints. Stable columns give reliable results. Stable columns cost less per injection and cause less system down-time. Results obtained on Discovery BIO PolyMA-SCX and PolyMA-WAX will be reproducible injection after injection because of exceptional column stability. In Figure 30, the Discovery BIO PolyMA-WAX columns are shown to give stable retention of three proteins after 500 injections, with no sign of deterioration. The same high degree of stability is shown for Discovery BIO PolyMA-SCX columns using three different proteins in Figure 31.

Figure 30: Stability of Discovery BIO PolyMA-WAX Columns

Column: Discovery BIO PolyMA-WAX, 5cm x 4.6mm, 5µm (Cat. No. 59602-U)
Mobile Phase: (A) 20mM Tris-HCl, pH 8.0;
(B) 20mM Tris-HCl, 0.5M NaCl, pH 8.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Myoglobin (5µg), conalbumin (5µg), trypsin inhibitor (10µg)

Gradient Profile:	Min	%A	%B
	0	95	5
	15	0	100

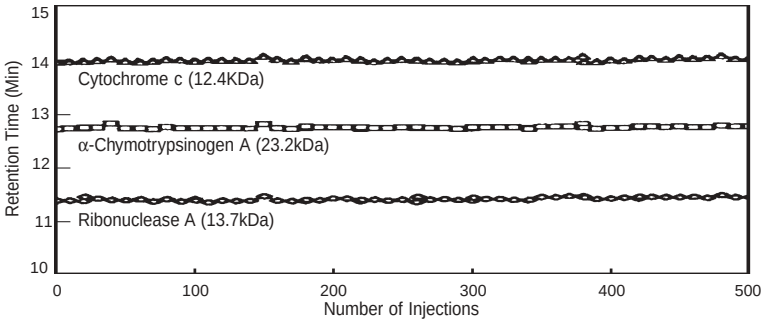


796-0655

Figure 31: Stability of Discovery BIO PolyMA-SCX Columns

Column: Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U)
Mobile Phase: (A) 20mM sodium phosphate, pH 7.0
(B) 20mM sodium phosphate, 0.5M NaCl, pH 7.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Ribonuclease A (10µg), α-chymotrypsinogen (5µg), cytochrome c (5µg)

Gradient Profile:	Min	%A	%B
	0	95	5
	15	0	100



796-0656

Chemical Stability and Column Life

Discovery BIO PolyMA-SCX and PolyMA-WAX are resistant to base hydrolysis and can be sanitized with caustic treatment.

There are several reasons why it is advantageous to use mobile phases with basic pH values (>pH 7). Among them may be altered ionic character of the protein, different selectivity of the separation, stability of the protein, and the need to clean the column between injections. To be certain that residual protein and other contaminants are removed from the column, a caustic wash of 0.1N NaOH is commonly employed. Silica-based materials cannot withstand this treatment. However, the polymeric backbone of Discovery BIO PolyMA-SCX and PolyMA-WAX allows them to be treated with caustic agents or run in high pH mobile phases without damage. This feature is demonstrated in Figure 32 which shows the separation of hemoglobin A and S on a Discovery BIO PolyMA-SCX column before and after treatment with 100 column volumes of 0.1N NaOH.

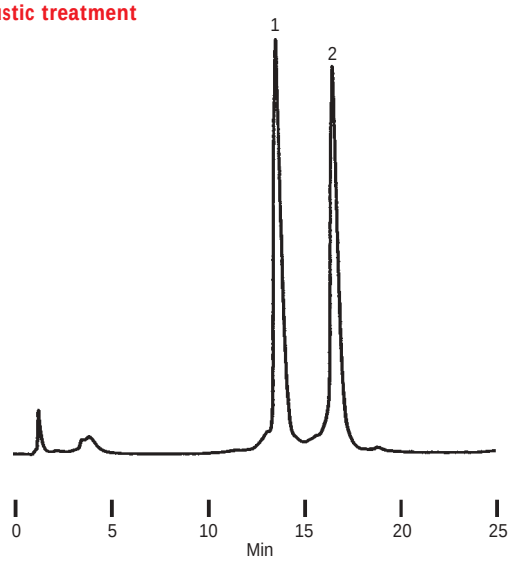
Figure 32: Stability of Discovery BIO PolyMA-SCX Columns to Caustic Treatment

Column: Discovery BIO PolyMA-SCX, 5cm x 4.6mm, 5µm (Cat. No. 59601-U)
Caustic Treatment: 0.1N NaOH at 0.5mL/min, 100 column volumes
Mobile Phase: (A) 20mM Bis-Tris HCl, pH 6.0;
(B) 20mM Bis-Tris HCl, 0.5M NaCl, pH 8.0
Flow: 0.5mL/min
Temp.: 25°C
Detection: UV, 280nm
Sample: Hemoglobin A and S (100µg each)
Injection: 10µL

Gradient Profile:	Min	%A	%B
	0	90	10
	20	50	50

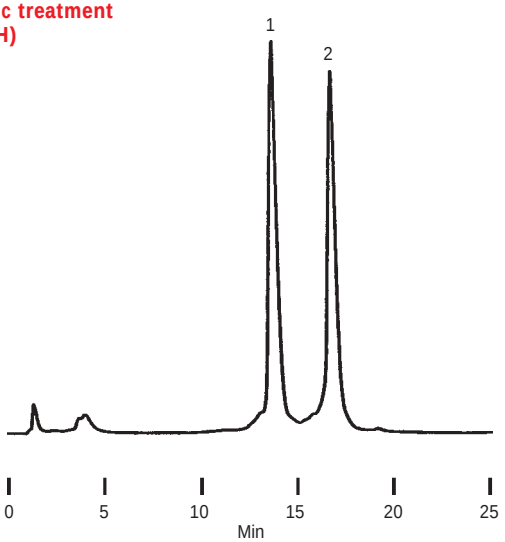
1. Hemoglobin A
2. Hemoglobin S

Before caustic treatment



G001837

After caustic treatment (0.1N NaOH)



G001838

The use of organic solvents and other mobile phase additives with Discovery BIO PolyMA-SCX and PolyMA-WAX columns

Although polymer-based, the high degree of cross-linking allows Discovery BIO PolyMA-SCX and PolyMA-WAX to be used with 100% organic solvent. Other polymeric particles shrink or swell in organic solvents. However, since mobile phases used in protein and peptide separations always contain a salt or buffer, the concentration of organic modifier must be kept low to prevent salt precipitation.

Other mobile phase additives for protein separations fall into two categories: those that maintain or enhance the solubility of the protein, and those that improve the separation by reducing hydrophobic interactions. Compounds such as ethylene glycol, glycerol, CHAPS, CHAPSO, and 6M urea belong to the former category. These are often added to the mobile phase, especially with membrane or other hydrophobic proteins. Methanol and acetonitrile are most common additives in the latter category. None of these additives at levels typically found in separations of biomolecules affects the stability or durability of Discovery BIO PolyMA-SCX and PolyMA-WAX columns. One caveat is that the additive must not cause the pressure to exceed the column limits.

Reproducibility

Low lot-to-lot variation guarantees reproducible separations now and into the future

As with any HPLC method, no separation is valuable if it is not reproducible. Discovery BIO PolyMA-SCX and PolyMA-WAX undergo rigorous testing to ensure reproducibility. Tables G and H present the reproducibility data for several recent lots of Discovery BIO PolyMA-WAX and PolyMA-SCX.

Table G. Reproducibility of Discovery BIO PolyMA-WAX Lots (n=7 lots)

t_R (min.)	Hemoglobin S	Hemoglobin A_0
Ave	12.97	20.56
Max	13.69	21.27
Min	12.19	19.72
%c.v.	3.78%	2.72%

Columns: 5cm x 4.6mm, 5µm, Mobile Phase: Gradient of 0 – 0.05M NaCl in 20mM Tris-HCl (pH 8.15) over 30 minutes, Flow: 0.5mL/min, Temperature: 40°C, Sample: Hemoglobin S (100µg), Hemoglobin A_0 (100µg), Injection: 50µL.

Table H. Reproducibility of Discovery BIO PolyMA-SCX Lots (n=5 lots)

t_R (min.)	Ribonuclease A	Cytochrome C
Ave	10.05	12.99
Max	10.49	13.59
Min	9.86	12.63
%c.v.	2.29%	2.62%

Columns: 5cm x 4.6mm, 5µm, Mobile Phase: Gradient of 0 – 0.5M NaCl in 20mM phosphate buffer, pH 6.0 over 20 minutes, Flow: 0.5mL/min, Temperature: 40°C, Sample: Bovine ribonuclease A (10µg), Bovine cytochrome c (5µg), Injection: 10µL.

The list of Discovery BIO PolyMA-SCX and PolyMA-WAX columns appears at the back of this brochure.

Product Listing

Phase Type		Particle Size (micron)	Length (cm)	ID (mm)	Cat. No.
Discovery BIO Wide Pore C5					
Capillary		3	5	0.18	65609-U
		3	10	0.18	65611-U
		5	5	0.18	65612-U
		5	10	0.18	65613-U
		5	15	0.18	65614-U
		3	5	0.32	65531-U
		3	10	0.32	65532-U
		5	15	0.32	65533-U
		3	5	0.5	65520-U
		3	10	0.5	65521-U
		5	15	0.5	65522-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	5	4	568410-U
		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)	ID (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
		5	25	2.1	568303-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	5	4	568310-U
		5	10	4	568311-U
		5	15	4	568312-U
		5	25	4	568313-U
		3	5	4.6	567216-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	5	10	567220-U
		10	15	10	567221-U
		10	25	10	567222-U
Preparative		10	5	21.2	567223-U
		10	15	21.2	567224-U
		10	25	21.2	567225-U
Guards		10	1	10	567284-U

* All guard column kits contain a holder and one replaceable cartridge.

Phase Type		Particle Size (micron)	Length (cm)	ID (mm)	Cat. No.
Discovery BIO Wide Pore C18					
Capillary		3	5	0.18	65603-U
		3	10	0.18	65604-U
		5	5	0.18	65606-U
		5	10	0.18	65607-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
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	5	4	568210-U
		5	10	4	568211-U
		5	15	4	568212-U
		5	25	4	568213-U
		3	5	4.6	567203-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

Phase Type	Particle Size (micron)	Length (cm)	ID (mm)	Cat. No.
Discovery BIO PolyMA-SCX				
	5	5	4.6	59601-U
Discovery BIO PolyMA-WAX				
	5	5	4.6	59602-U

Trademark

Discovery — Sigma-Aldrich Co.

ARGENTINA
Sigma-Aldrich de Argentina SA
Av. Pueyrredon 2446
5 "B"
C1119ACU Buenos Aires
Tel.: 54-11-4807 0321
0810-888-7446
Fax: 54-11-4807 0346
Email:cservice@sigma-aldrich.com.ar

AUSTRALIA
Sigma-Aldrich Pty. Ltd.
PO Box 970
Castle Hill, NSW 1765
Tel.: (612) 8853 5555
Fax: (612) 8853 5500
Free Tel.:1 800 800 097
Free Fax:1 800 800 096
Email:ausmail@sial.com

AUSTRIA
Sigma-Aldrich Handels GmbH
Favoritner Gewerbering 10
A-1100 Wien
Tel.: 01 605 81 10
Fax: 01 605 81 20
Email:sigma@sigma.co.at

BELGIUM
Sigma-Aldrich N.V./S.A.
K. Cardijnplein 8
B-2880 Bornem
Tel.: 03 899 1301
Fax: 03 899 1311
Free Tel.: 0800 14747
Free Fax: 0800 14745
Email:becustsv@eurnotes.sial.com

BRAZIL
Sigma-Aldrich Química Brasil Ltda.
Rua Ari Aps 83 Jd. Pinheiros
05594-010
São Paulo, SP Brasil
Phone:55 11 3733 2900
Fax: 55 11 3733 5151
Email:sigmabr@sigma-aldrich.com.br

CANADA
Sigma-Aldrich Canada Ltd.
2149 Winston Park Drive
Oakville, Ontario L6H 6J8
Tel.: 905 829 9500
Fax: 905 829 9292
Free Tel.:800 565 1400
Free Fax:800 265 3858
Email:canada@sial.com

CHINA
Sigma-Aldrich China Inc., Shanghai Rep. Office
Unit B, 22nd Floor, China Overseas Building
No. 398 Huai Hai Zhong Road
Shanghai 200020
P.R.China
Tel.: (86-21) 6386-2766
Fax: (86-21) 6386-3966
Email: china@sial.com

CZECH REPUBLIC
Sigma-Aldrich s.r.o.
Pobrezni 46
186 00 Praha 8
Tel.:00 420 2 2176 1310
Fax:00 420 2 2176 3300
Email:CZECustSV@eurnotes.sial.com

DENMARK
Sigma-Aldrich Denmark A/S
Vejlegaardsvej 65B
DK-2665 Vallensbaek Strand
Tel.: +45 43565900
Fax: +45 43565905
Email:DenOrder@eurnotes.sial.com

FINLAND
Sigma-Aldrich Finland
Y-A Kemia Oy
Teerisuonkuja 4
00700 Helsinki
Tel.: (09) 350 9250
Fax: (09) 350 92555
Email:finorder@eurnotes.sial.com

FRANCE
Sigma-Aldrich Chimie S.a.r.l.
L'Isle d'Abeau Chesnes - B.P. 701
38297 St. Quentin Fallavier Cedex
Tel.: 04 74822920
Fax: 04 74956808
Free Tel.: 0800 211408
Free Fax: 0800 031052
Email:fradvsv@eurnotes.sial.com

GERMANY
Sigma-Aldrich Chemie GmbH
Eschenstr. 5
82024 Taufkirchen
Tel.: 089 / 6513-1130
Fax: 089 / 6513-1161
Free Tel.:0800 / 5155 000
Free Fax:0800 / 6490 000
Email: DeOrders@eurnotes.sial.com

GREECE
Sigma-Aldrich (o.m.) Ltd.
72 Argonafton Str.
16346 Ilioupoli, Athens
Tel.: +30 210 9948010
Fax: +30 210 9943831
Email:GRCustSV@SIALEUROPE

HUNGARY
Sigma-Aldrich Kft.
1399 Budapest
Pf. 701/400
Magyarorszag
Tel.: 06-1-235-9055
Fax: 06-1-235-9050
Free Tel.:06-80 355355
Free Fax:06-80 344344
Email:info@sigma.sial.hu

INDIA
Sigma-Aldrich Chemicals Private Limited
Survey No. 31/1, Sitharamapalaya
Mahadevapura P.O.
Bangalore 560 048
Tel.: 91-80-5112-7272
Fax: 91-80-5112-7473
Email: india@sial.com
sigmaindia@vsnl.com

IRELAND
Sigma-Aldrich Ireland Ltd.
Airton Road
Tallaght
Dublin 24
Tel.: (01) 4041900
Fax: (01) 4041910
Free Tel.:1 800 200 888
Free Fax:1 800 600 222
Email:ElCustsv@eurnotes.sial.com

ISRAEL
Sigma-Aldrich Israel Ltd.
Park Rabin
Rehovot 76100
Tel.: 08 9484 222
Fax: 08 9484 200
Free Tel.:1 800 70 2222
Email:sigisr@sigma.co.il

ITALY
Sigma-Aldrich S.r.l.
Via Gallarate, 154
20151 Milano
Tel.: 02 33417310
Fax: 02 38010737
Free Tel.: 800 827018
Email:itorder@eurnotes.sial.com

JAPAN
Sigma-Aldrich Japan K.K.
Supelco Division
Tennouzu Central Tower 4F
2-2-24 Higashi Shinagawa Shinagawa-ku
Tokyo 140-0002
Tel.: 81-3-5796-7350
Fax: 81-3-5796-7355

KOREA
Sigma-Aldrich Korea Ltd.
PO Box 36, Yongin, 449-600
Tel.: 031 329 9000
Fax: 031 329 9090
Tel.: 080 023 7111
Fax: 080 023 8111
Email:supelco@sial.co.kr

MALAYSIA
Sigma-Aldrich (M) Sdn. Bhd.
No: 7 Jalan PJS 7/21, Bandar Sunway
46150 Petaling Jaya
Selangor Darul Ehsan
Tel.: 603-56353321
Fax: 603-56354116
Email:sigalm@po.jaring.my

MEXICO
Sigma-Aldrich Química, S.A. de C.V.
Calle 6 Norte No. 107
Parque Industrial Toluca 2000
50200 Toluca, Méx.
Tel.: (7) 276 1600
Fax: (7) 276 1601
Free Tel.:01 800 007 5300
Free Fax:01 800 712 9920
Email:mexico@sial.com

THE NETHERLANDS
Sigma-Aldrich Chemie B.V.
Stationsplein 4 E
Postbus 27
NL-3330 AA Zwijndrecht
Tel.: 078 6205411
Fax: 078 6205421
Free Tel.:0800 0229088
Free Fax:0800 0229089
Email:nlcustsv@eurnotes.sial.com

NEW ZEALAND
Sigma-Aldrich Pty. Ltd.
PO Box 12423, Penrose
Auckland
Tel.: (612) 8853 5555
Fax: (612) 8853 5500
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Email:ausmail@sial.com

NORWAY
Sigma-Aldrich Norway AS
Postboks 188 Leirdal
1011 Oslo
Tel.: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10
Email:norsigma@sial.com

POLAND
Sigma-Aldrich Sp. z o.o.
Szelagowska 30
61-626 Poznan
Tel.: (061) 8232481
Fax: (061) 8232781
Email:plcustsv@eurnotes.sial.com

PORTUGAL
Sigma-Aldrich Química, S.A.
Sucursal em Portugal
Apartado 131
2711-901 Sintra
Tel.: 21 9242555
Fax: 21 9242610
Free Tel.: 800 20 21 80
Free Fax: 800 20 21 78
Email encomendas: poorders@eurnotes.sial.com

RUSSIA
Sigma-Aldrich Russia
TechCare Systems, Inc.
Makarenko Str. 2/21
Building 1, Flat 22
Moscow 103062
Tel.: 7-095 9753321
Fax: 7-095 9754792
Email:techcare@online.ru

SINGAPORE
Sigma-Aldrich Pte., Ltd.
102E Pasir Panjang Road
#08-01 Citilink Warehouse
Singapore 118529
Tel.: 65-271 1089
Fax: 65-271 1571
Email: sapl@sial.com

SOUTH AFRICA
CNR Kelly & Ackerman Streets
Southern Life Industrial Park Unit
Unit 16/17
Jet Park 1459
Tel.: 27 11 397 8886
Fax: 27 11 397 8859
Free Tel.: 0800 110075
Free Fax: 0800 110079
Email: rsa@sial.com

SPAIN
Sigma-Aldrich Química, S.A.
Ronda de Poniente 3, 2 ª Planta
PO Box Correos 278
28760 Tres Cantos
Madrid Spain
Tel.: 91 6619977
Fax: 91 6619642
Free Tel.: 900 101376
Free Fax: 900 102028
Email: pedidos:esorders@eurnotes.sial.com

SWEDEN
Sigma-Aldrich Sweden AB
Solkraftsvägen 14C
135 70 Stockholm
Tel.: 08-742 42 00
Fax: 08-742 42 43
Free Tel.: 020-350510
Free Fax: 020-352522
Email:sweorder@eurnotes.sial.com

SUPELCO SWITZERLAND
c/o Fluka Chemie GmbH
Industriestrasse 25
P.O. Box 260
9471 Buchs
Tel.: 081 755 25 11
Fax: 081 755 28 15
Free Tel.:0800 80 00 80
Email: Fluka@sial.com

UNITED KINGDOM
Sigma-Aldrich Company Ltd.
Supelco UK
Fancy Road, Poole
Dorset BH12 4QH
Tel.: 01747 833000
Fax: 01747 833313
Free Tel.:0800 717181
Free Fax: 0800 378785
Email:ukorders@eurnotes.sial.com

UNITED STATES
Supelco
595 North Harrison Road
Bellefonte, PA 16823-0048
Tel.: 814 359 3441
Fax: 814 359 5459
Free Tel.:800 247 6628
Free Fax:800 447 3044
Email:supelco@sial.com

sigma-aldrich.com/supelco

Order/Customer Service 800-247-6628, 800-325-3010 ● Fax 800-325-5052 ● E-mail supelco@sial.com
Technical Service 800-359-3041, 814-359-3041 ● Fax 800-359-3044, 814-359-5468 ● E-mail techservice@sial.com

SUPELCO ● 595 North Harrison Road, Bellefonte, PA 16823-0048 ● 814-359-3441

ISO 9001 REGISTERED

We are committed to the success of our Customers, Employees and Shareholders through leadership in Life Science, High Technology and Service.

The SIGMA-ALDRICH Family SIGMA ALDRICH Fluka SUPELCO

T402038B-00
GAU