Healthcare & Life Sciences

SUPELCO®
Solutions within.™

Discovery BIO PolyMA

Ion exchange columns for protein and peptide separations

Hydrophilic surface eliminates protein adsorption for quantitative recovery

High capacity and excellent recovery

Efficient, reproducible and long lasting

Discovery® BIO PolyMA-SCX and PolyMA-WAX

Polymethacrylate polymer-based cation-exchange and anion-exchange columns provide efficient separations 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. 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 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

Table 1. Discovery BIO PolyMA Properties

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, monodisperse	Spherical, monodisperse
Particle Size (µm)	5	5
Pore Size (Å)	1000	1000
Coverage	0.3 meq/g	0.3 meq/g
pH range	1 to 13	2 to 10*
Temperature range	4 °C to 50 °C	4 °C to 50 °C
Pressure limit	5 MPa	5 MPa

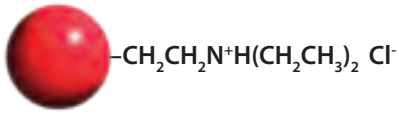
* Although a weak anion-exchange material, PolyMA-WAX can be used at high pH values but with reduced charge.

Table 2. Choosing Discovery BIO PolyMA-SCX or PolyMA-WAX

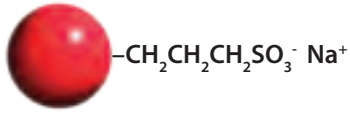
Particle	Separation Mode	When to Use
Discovery BIO PolyMA-SCX	Cation Exchange	Generally at a pH less than the protein pI; usually at pH less than 7
Discovery BIO PolyMA-WAX	Anion Exchange	Generally at a pH greater than the protein pI; usually at pH 7 or higher

Figure 1. 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 3**. 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 3. 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

High 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 better it resolves 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 2** 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.

Ion-exchange and reversed-phase often provide different, complementary elution patterns. This can be leveraged to extract more information from the sample. **Figure 3** shows the separation of three cytochrome c variants on Discovery® BIO PolyMA-SCX and Discovery BIO Wide Pore C18 columns. Note the difference in elution order.

Figure 2. Discovery BIO PolyMA-WAX columns have the efficiency to resolve hGH and its degradation products

column:	Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase A:	20 mM Tris-HCl, pH 8.0
mobile phase B:	20 mM Tris-HCl, 0.5 M NaCl, pH 8.0
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 254 nm
inj.:	10 µL
sample:	hGH (1 mg/mL in mobile phase A)
gradient:	5 to 70% B in 30 min. (linear)

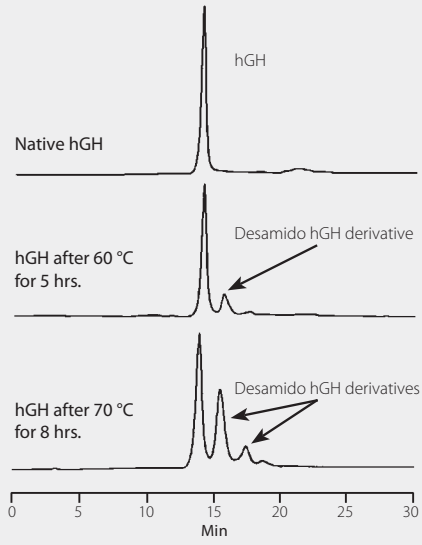
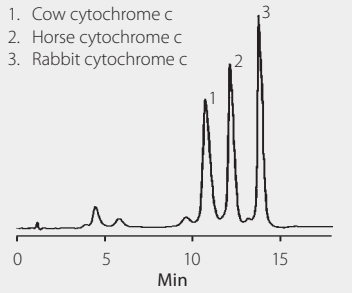


Figure 3. Discovery BIO PolyMA-SCX and wide pore C18 have the efficiency and selectivity to separate closely-related cytochrome c variants

Discovery BIO PolyMA-SCX

column:	Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
mobile phase A:	20 mM Bis-Tris HCl, pH 7.0
mobile phase B:	20 mM Bis-Tris HCl, 0.5 M NaCl, pH 7.0
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	10 µg each cytochrome c variant
gradient:	24 to 69% B in 20 min. (linear)

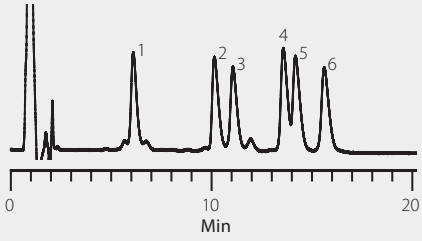
Note the different elution order compared to the Discovery BIO Wide Pore C18.



Discovery BIO Wide Pore C18

column:	Discovery BIO Wide Pore C18, 15 cm x 4.6 mm, 5 µm particles (568222-U)
mobile phase A:	70:30, 0.1% TFA in water:0.1% TFA in acetonitrile
mobile phase B:	64:36, 0.1% TFA in water:0.1% TFA in acetonitrile
flow rate:	0.5 mL/min.
temp.:	ambient
det.:	UV, 220 nm
inj.:	12 µL
sample:	each cytochrome c variant at 0.8 mg/mL in 0.1% TFA
gradient:	0 to 100% B in 30 min. (linear)

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



Meeting the Challenges of Today's Protein and Peptide Separations

Challenge: Complex Protein or Peptide Mixtures

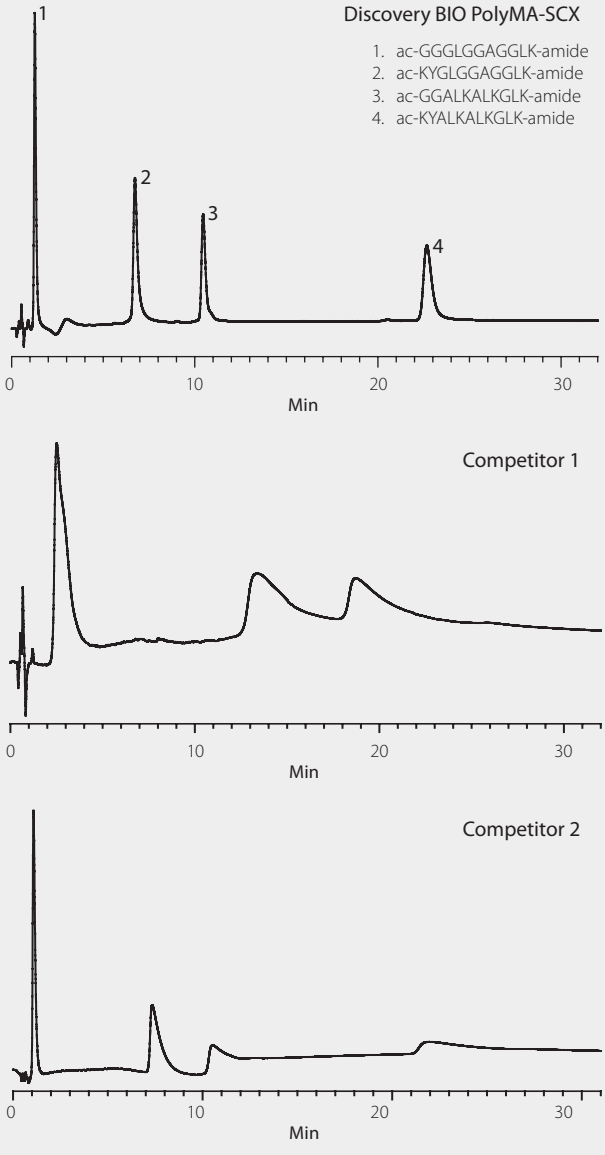
Efficiency and selectivity offered by Discovery BIO PolyMA-SCX and PolyMA-WAX give efficient ion-exchange separation of a wide variety of peptides and proteins.

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 4**. 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 4. Discovery BIO PolyMA-SCX columns have higher efficiency than competitive polymeric columns

column:	Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U) and competitive polymeric-SCX columns of comparable dimensions
mobile phase A:	5% acetonitrile in 20 mM ammonium carbonate, pH 3.5 with H_3PO_4
mobile phase B:	5% acetonitrile in 20 mM ammonium carbonate, 480 mM ammonium phosphate, pH 3.5 with H_3PO_4
flow rate:	0.2 mL/min.
temp.:	35 °C
det.:	UV, 215 nm
inj.:	10 µL
sample:	RP Peptide Ionic Interactions Standard, p/n RPS-10020 (Alberta Peptide Institute)
gradient:	0 to 100% B in 24 min. (linear)



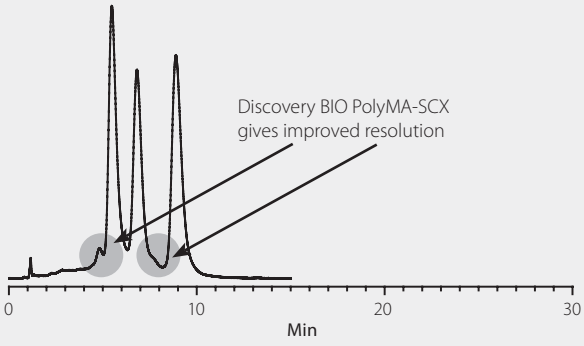
Higher column efficiency also translates to better sensitivity and the ability to detect lower levels of proteins or peptides. Further comparisons attesting to the efficiency of Discovery® BIO PolyMA-SCX and PolyMA-WAX are shown in **Figures 5 and 6**, respectively. The higher efficiency of the Discovery BIO PolyMA-SCX allowed it to resolve a small impurity of cytochrome c not resolved by the leading competitive columns. Note that flow rates were adjusted to achieve the same linear velocity and gradient slope for both columns.

Figure 5. Comparison of efficiency: cytochrome c variants

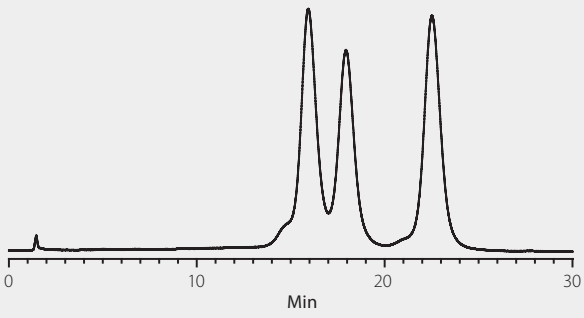
column: Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U) and competitive polymeric-SCX columns of comparable dimensions
mobile phase A: 50 mM MOPS/KOH, pH 7.0
mobile phase B: 50 mM MOPS/KOH, 0.5 M KCl, pH 7.0
flow rate: 3.01 cm/min. (flow rates appear in figure)
temp.: 35 °C
det.: UV, 280 nm
inj.: 10 µg each variant
gradient: 0.6% B per min. (see figure for details)

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

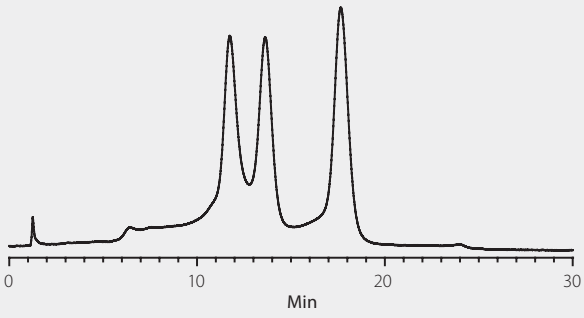
Discovery BIO PolyMA-SCX
5 cm x 4.6 mm, 0.5 mL/min., 28-37% B in 15 minutes



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



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



Meeting the Challenges of Today's Protein and Peptide Separations

Challenges: Small Sample Volumes, Low Concentrations, the Need for Detailed Protein 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.

Similarly, in **Figure 6** Discovery BIO PolyMA-WAX is shown to give better resolution and band spacing of hemoglobin variants than leading competitive columns. Note that flow rates were adjusted to achieve equal linear flow and gradient slope on the columns for proper comparison.

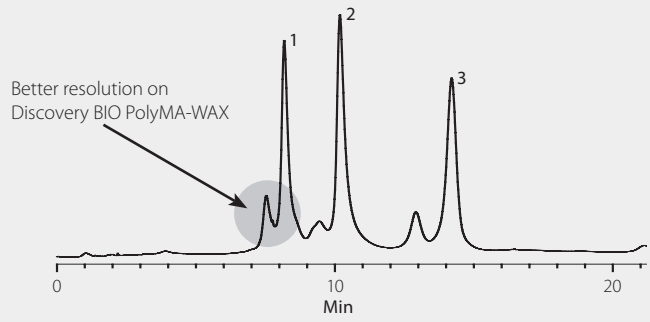
Figure 6. Comparison of Efficiency: Hemoglobin Variants

column: Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U) and competitive polymeric weak anion exchange columns
mobile phase A: 10 mM Tris, pH 8.0 with acetic acid
mobile phase B: 10 mM Tris, 0.25 M KCl, pH 8.0 with acetic acid
flow rate: 3.01 cm/min. (flow rates appear in figure)
temp.: 35 °C
det.: UV, 280 nm
inj.: 50 µg each variant
gradient: 1.6 % B per min. (see figure for details)

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

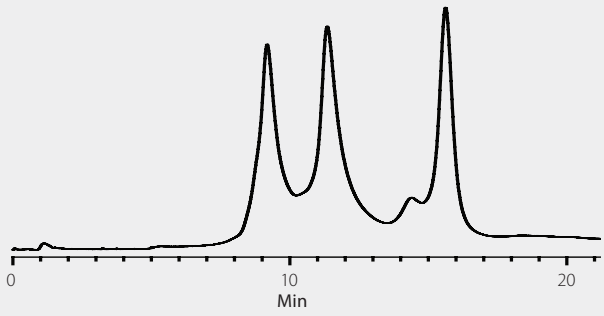
Discovery BIO PolyMA-WAX

5 cm x 4.6 mm, 0.5 mL/min., 0-32% B in 20 minutes



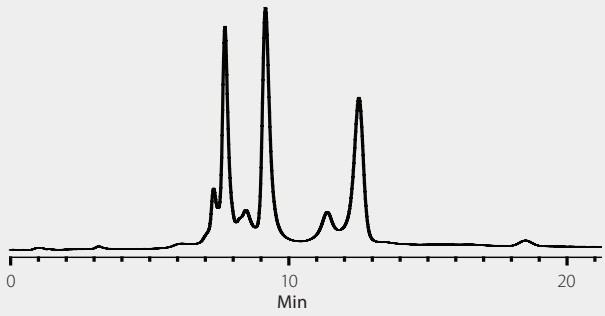
Competitor 1

5 cm x 5 mm, 0.59 mL/min., 0-32% B in 20 minutes



Competitor 2

5 cm x 5 mm, 0.59 mL/min., 0-32% B in 20 minutes



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 (1000 Å) of Discovery BIO PolyMA-SCX and PolyMA-WAX particles allows full access to globular proteins and aggregates up to a molecular mass of almost one million Da. **Figure 7** shows the separation of proteins that vary from 18 to 80 kDA. **Figure 8** shows the power of ion-exchange to resolve proteins with very little difference in molecular weight, an advantage over size-exclusion separations.

Figure 7. Discovery BIO PolyMA columns separate proteins of varying different molecular weight by ion-exchange

column:	Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase A:	10 mM Tris, pH 8.0 with HCl
mobile phase B:	10 mM Tris, 0.5 M NaCl, pH 8.0 with HCl
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	myoglobin (5 µg), conalbumin (5 µg), trypsin inhibitor (10 µg)
gradient:	5 to 100% B in 15 min. (linear)

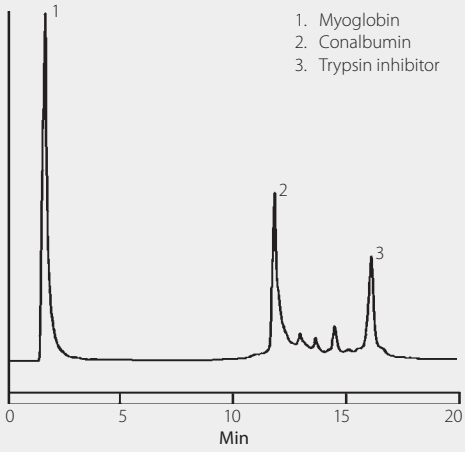
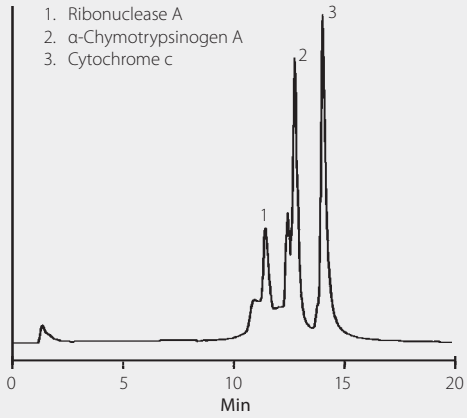


Figure 8. Discovery BIO PolyMA columns distinguish proteins of similar molecular weight

column:	Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
mobile phase A:	20 mM sodium phosphate, pH 7.0
mobile phase B:	20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	ribonuclease A (10 µg), α-chymotrypsinogen A (5 µg), cytochrome c (5 µg)
gradient:	5 to 100% B in 15 min. (linear)



The separations in **Figures 9 and 10** show Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

Figure 9. Discovery BIO PolyMA-SCX application: Elastase and human hemoglobin S

column:	Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
mobile phase A:	20 mM sodium phosphate, pH 7.0
mobile phase B:	20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	10 µL
sample:	elastase (6.5 mg/mL), human hemoglobin S (2 mg/mL)
gradient:	0 to 100% B in 30 min. (linear)

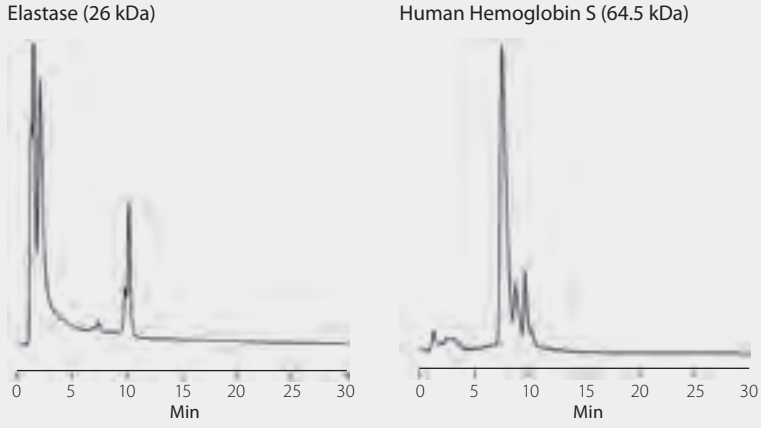
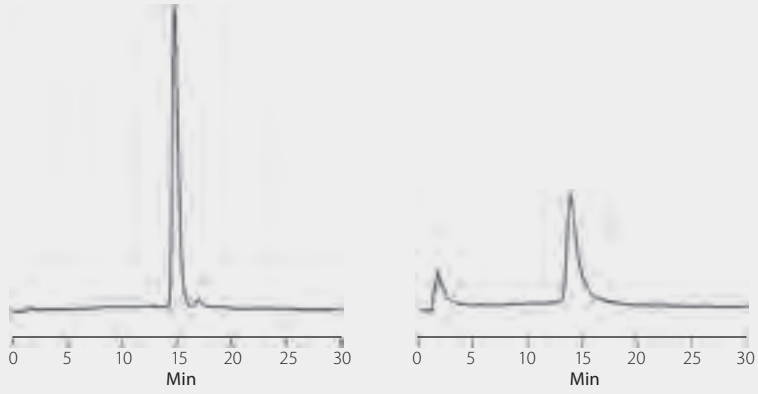


Figure 10. Discovery BIO PolyMA-WAX application: Superoxide dismutase and creatine kinase

column:	Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase A:	20 mM Tris, pH 8.0 with HCl
mobile phase B:	20 mM Tris, 0.5 M NaCl, pH 8.0 with HCl
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	superoxide dismutase (2 mg/mL), creatine kinase (2 mg/mL)
gradient:	0 to 10% B in 30 min. (linear)



Sample 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 4**. Although capacity is dependent on the protein under study and operating conditions, generally between 20 and 50 mg of total protein can be injected onto the Discovery BIO PolyMA-SCX and PolyMA-WAX columns.

Table 4. 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	40 mg	30 mg
Discovery BIO PolyMA-WAX	BSA	50 mg	40 mg

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

Protein 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 columns because they are completely covered with a covalently-bonded hydrophilic layer. This absence of non-specific interactions gives rise to the high recovery on Discovery BIO PolyMA-WAX and PolyMA-SCX columns as reported in **Tables 5 and 6** for fibrinogen and cytochrome c respectively.

Table 5. Recovery on Discovery BIO PolyMA-WAX vs. Competitor Column

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

Mobile Phase: Gradient of 0 – 0.5 M NaCl in 20 mM Tris-HCl (pH 8) over 30 minutes,
Flow: 1 mL/min., ambient temperature,
Sample: 40 µg fibrinogen in 20 µL starting mobile phase.

Table 6. Recovery on Discovery PolyMA-SCX vs. Competitor Column

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

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

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 11**, 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 12**.

Figure 11. Stability of Discovery BIO PolyMA-WAX Columns

column: Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase A: 20 mM Tris, pH 8.0 with HCl
mobile phase B: 20 mM Tris, 0.5 M NaCl, pH 8.0 with HCl
flow rate: 0.5 mL/min.
temp.: 25 °C
det.: UV, 280 nm
inj.: myoglobin (5 µg), conalbumin (5 µg), trypsin inhibitor (10 µg)
gradient: 5 to 100% B in 15 min. (linear)

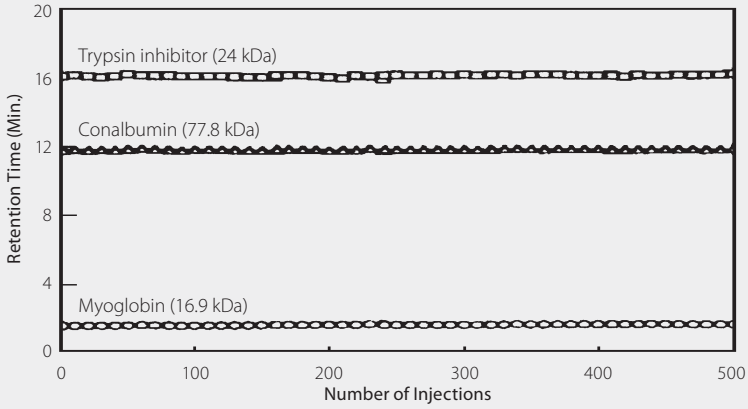
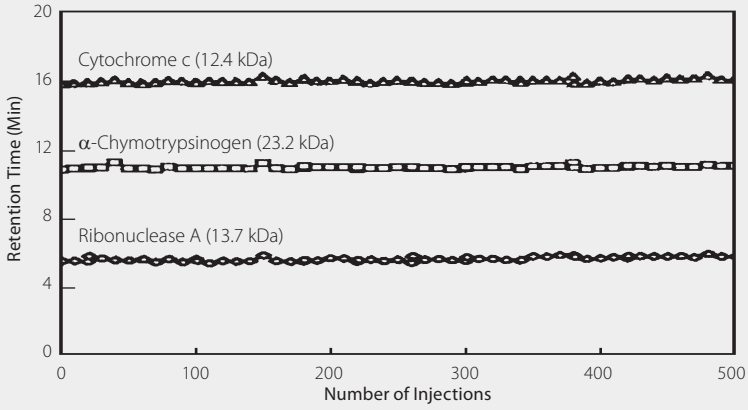


Figure 12. Stability of Discovery BIO PolyMA-SCX Columns

column: Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
mobile phase A: 20 mM sodium phosphate, pH 7.0
mobile phase B: 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
flow rate: 0.5 mL/min.
temp.: 25 °C
det.: UV, 280 nm
inj.: 10 µL
sample: ribonuclease A (10 µg), α-chymotrypsinogen A (5 µg), cytochrome c (5 µg)
gradient: 5 to 100% B in 15 min. (linear)



Meeting the Challenges of Today's Protein and Peptide Separations

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

BIO PolyMA-SCX and PolyMA-WAX permit reliable, trouble-free routine and long term operation.

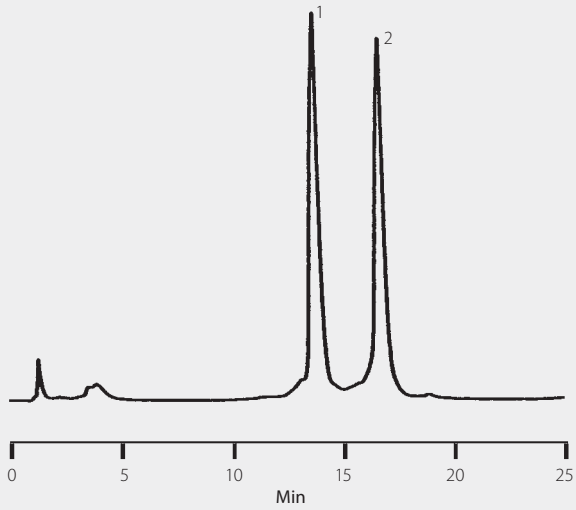
Discovery® BIO PolyMA-SCX and PolyMA-WAX columns are resistant to base hydrolysis and can be sanitized with caustic treatment

There are several reasons why it is advantageous to use mobile phases at basic pH values. The protein may not be stable at acidic pH, the separation may simply be better above pH 7, or strongly retained impurities may be removed at alkaline pH. 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 13** 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 0.1 N NaOH.

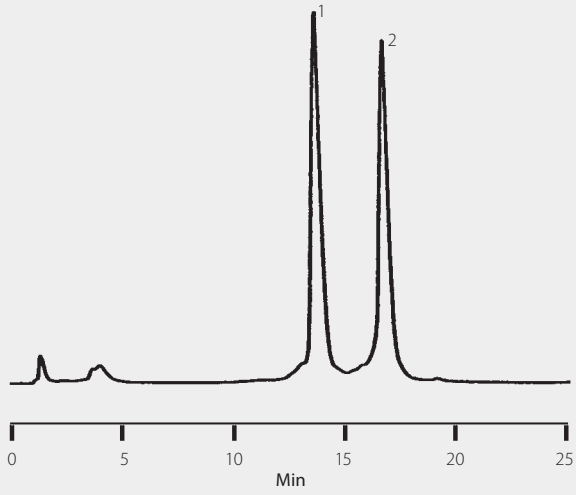
Figure 13. Stability of Discovery BIO PolyMA-SCX columns to caustic treatment

column:	Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
caustic treatment:	0.1 N NaOH at 0.5 mL/min., 100 column volumes
mobile phase A:	20 mM Bis-Tris, pH 8.0 with HCl
mobile phase B:	20 mM Bis-Tris, 0.5 M NaCl, pH 8.0 with HCl
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV, 280 nm
inj.:	10 µL
sample:	hemoglobin A and S (100 µg each)
gradient:	10 to 50% B in 20 min. (linear)

Before Caustic Treatment



After Caustic Treatment (0.1 N NaOH)



Organic Solvents and Mobile Phase Additives

Although polymer-based, the high degree of cross-linking allows Discovery BIO PolyMA-SCX and PolyMA-WAX columns 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 (5 MPa or 50 bar for each column type).

Reproducibility

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

As with any HPLC method, no separation is valuable if it is not reproducible. Discovery BIO PolyMA-SCX and PolyMA-WAX columns undergo rigorous testing to ensure reproducibility. **Tables 7 and 8** present the reproducibility data for several recent lots of Discovery BIO PolyMA-WAX and PolyMA-SCX columns.

Table 7. 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 %

Column: 5 cm x 4.6 mm, 5 µm, Mobile Phase: Gradient of 0–0.05 M NaCl in 20 mM Tris-HCl (pH 8.15) over 30 minutes, Flow: 0.5 mL/min., Temperature: 40 °C, Sample: hemoglobin S (100 µg), hemoglobin A0 (100 µg), Injection: 50 µL.

Table 8. 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 %

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

Ordering Information

Cat. No.	Phase	Separation Mode	Length (cm)	ID (mm)	Particle Size (µm)
59601-U	Discovery BIO PolyMA-SCX	Cation Exchange	5	4.6	5
59602-U	Discovery BIO PolyMA-WAX	Anion Exchange	5	4.6	5

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