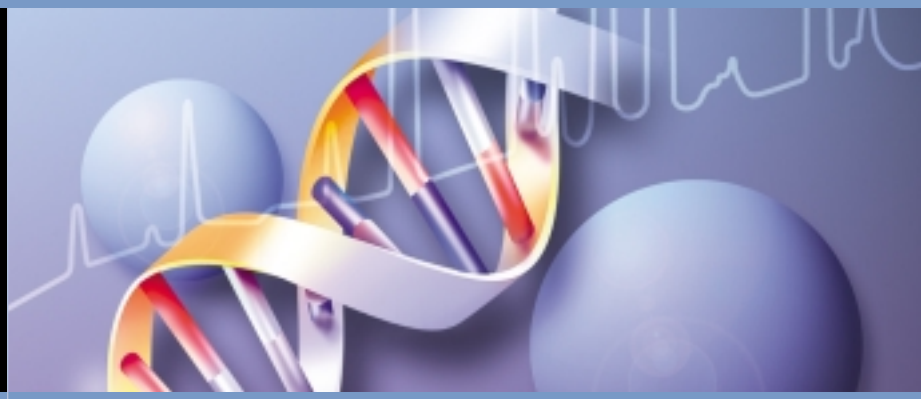
BioBasic™ HPLC Columns Technical Guide



The Complete Answer for Separating Biomolecules

Analyze • Detect • Measure • Control™

Thermo ELECTRON CORPORATION

The BioBasic Family of Columns

Improved Performance for Biomolecules

Introduction

BioBasic columns are designed specifically for chromatography of proteins, peptides and nucleic acids. The 300Å pore size, high purity silica and stable bonding chemistry of BioBasic packings makes them ideal for life science applications. Available in a range of reversed phase, anion exchange, cation exchange and size exclusion columns, BioBasic columns meet all of your protein and peptide separation needs from sample clean-up to analysis, including proteomics and LC/MS applications.

- **A range of phases for reversed phase, ion exchange and size exclusion chromatography**
- **Highly pure silica in tailored pore sizes for superior performance**
- **Improved resolution, efficiency, reproducibility and column lifetimes**
- **Hardware options include biocompatible PEEK™ columns, KAPPA™ capillary columns, and other designs for LC/MS**

BioBasic SEC Columns: Improved Performance

BioBasic SEC columns are available in four pore sizes and are ideal for molecular weight determination of peptides, proteins and water soluble polymers (Figure 1). They can also be used for sample clean-up prior to analysis. BioBasic SEC columns, designed to elute proteins with high recoveries, can be used for direct automated analysis of serum or urine samples. BioBasic SEC columns, based on silica with a proprietary polymeric coating, offer the mechanical stability of silica-based size exclusion columns with higher efficiencies than that of polymer-based columns. See page 4 for more information.

BioBasic Reversed Phase Columns: Analyze and Detect

BioBasic columns provide superior chromatography, run after run, column after column. The extra dense bonding chemistry used for BioBasic reversed phase packings gives a highly stable, reproducible surface for reliable results. Figure 2 demonstrates the reproducible performance for 19 batches of BioBasic 18 packing materials. BioBasic reversed phase packings are available in several chemistries, including C18, C8, C4, phenyl and cyano. See page 7 for more information.

Phase	Particle Size	Pore volume	Pore size	V_i/V_o	Silica Type
BioBasic SEC 60	5µm	0.7 cc/g	60Å	1.40	high purity hydro-link coated
BioBasic SEC 120	5µm	1.0 cc/g	120Å	1.77	high purity hydro-link coated
BioBasic SEC 300	5µm	0.9 cc/g	300Å	1.16	high purity hydro-link coated
BioBasic SEC 1000	5µm	0.9 cc/g	1000Å	0.96	high purity hydro-link coated

Table 1: BioBasic SEC Phase Specifications

Phase	Particle size	Carbon Load	Pore Size	End-capping	Silica type
BioBasic 18	5µm	9%	300Å	Yes	high purity base deactivated
BioBasic 8	5µm	5%	300Å	Yes	high purity base deactivated
BioBasic 4	5µm	4%	300Å	Yes	high purity base deactivated
BioBasic CN	5µm	N/A	300Å	Yes	high purity base deactivated
BioBasic Phenyl	5µm	N/A	300Å	Yes	high purity base deactivated
BioBasic AX	5µm	N/A	300Å	N/A	high purity base deactivated
BioBasic SCX	5µm	N/A	300Å	N/A	high purity base deactivated

Table 2: BioBasic Reversed Phase and Ion Exchange Specifications

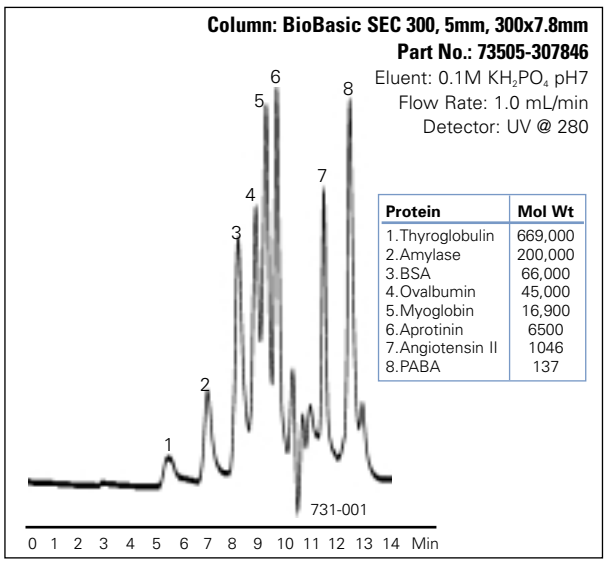


Figure 1: Protein Separation on a BioBasic SEC 300 Column

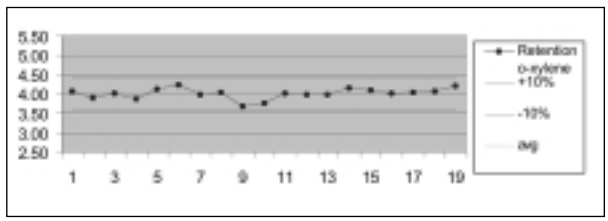


Figure 2: Reproducibility of BioBasic 18 Media

BioBasic Ion Exchange Columns: Versatile Protein and Peptide Analysis

The BioBasic family includes BioBasic AX and BioBasic SCX columns for ion exchange. BioBasic AX columns can be used for multiple modes of chromatography including HILIC and normal phase. Both phases provide superior performance for proteins, peptides and nucleic acids (Figure 3) using protein-friendly ion exchange conditions. BioBasic ion exchange columns are designed to give superior reproducibility, both column-to-column and batch-to-batch. The 5µm, 300Å silica provides significantly higher efficiency than typical polymer-based ion exchangers. All BioBasic SCX and AX columns are carefully prepared to provide superb efficiency and ensure robust and reproducible performance as shown in Figure 4.
See page 10 for more information.

BioBasic Columns for LC/MS: KAPPA™ Capillary Columns

The BioBasic KAPPA line has been designed to meet all the sensitivity needs of demanding LC/MS separations. High efficiency capillaries are available in internal diameters ranging from 500µm all the way down to 75µm ID, and lengths of 50mm to 250mm.

The BioBasic KAPPA line is ideal for all LC/MS analyses, especially proteomics separations with small sample concentrations.
See page 13 for more information.

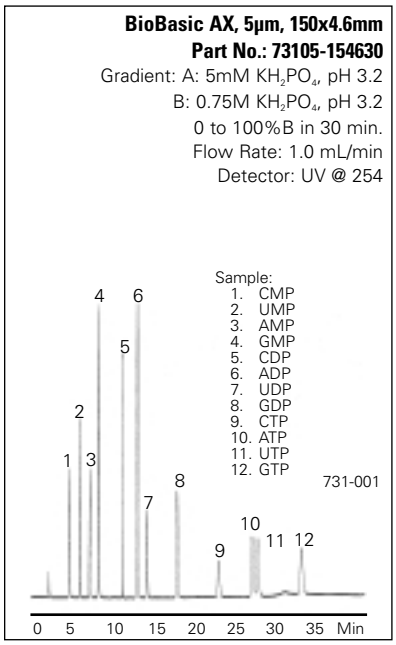


Figure 3: Nucleotides

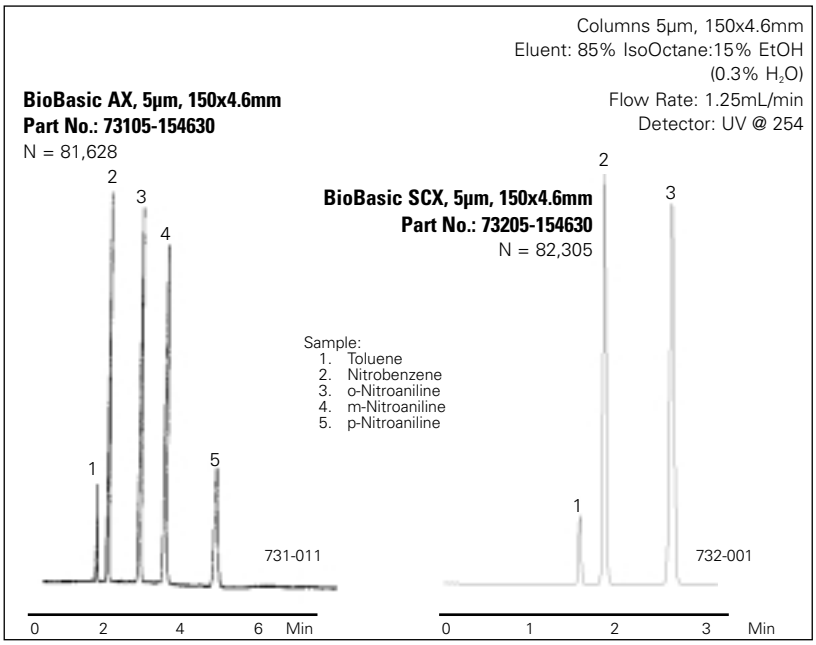


Figure 4: Efficiency of BioBasic Ion Exchange Columns

BioBasic SEC Columns

Introduction

Size exclusion chromatography (SEC) is a noninteractive technique which separates solutes according to their molecular size in solution. It is often used as the first step in isolation of a protein from a crude sample. When used with standards for calibration it is possible to determine the molecular mass of proteins and the molecular weight distribution of water soluble polymers. Other applications include polyethylene glycols/oxides, polysaccharides, and pullulans.

BioBasic SEC columns provide high efficiency separations for a wide range of samples, from 100 to 10,000,000 molecular weight. The columns are offered in a range of pore sizes (60, 120, 300 and 1000Å) and employ proprietary coated silica to ensure highest efficiency, good recoveries and accurate molecular weight data.

- **A silica-based media for superior resolution of water soluble compounds**
- **Pore sizes from 60 to 1000Å for a wide range of sample molecular weights**
- **Fast, easy, straightforward method development due to minimal secondary interactions**
- **Superior column lifetimes and efficiencies, particularly when protected by a guard column**
- **Ideal for sample clean up**

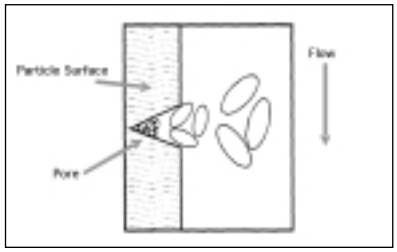
SEC Analysis of Whole Proteins and Water Soluble Polymers

Advantages of Silica SEC Columns

Polymer-based columns are popular for size exclusion separations. However, polymeric media is susceptible to compression, shrinking or swelling with changes in mobile phase composition. Because of this, polymer based columns are limited in their operating pressures and often separation efficiency is affected. In contrast, BioBasic SEC columns are based on chromatographic silica, which is mechanically rigid, does not swell or shrink with changes in solvent and shows higher efficiencies than polymer-based columns (typically greater than 70,000 plates/meter).

How Separation is Achieved

To provide accurate data, size exclusion columns must separate sample molecules strictly on the basis of their size in solution. Secondary ionic or hydrophobic interactions must be minimized, as they will degrade the size-based separation. BioBasic SEC columns employ highly base deactivated 5µm silica, which is coated with a "hydro-link" polymer to ensure separation occurs only on the basis of sample size. BioBasic SEC columns are ideal for high efficiency gel filtration separation of proteins and other biological molecules where the absence of secondary interactions, such as adsorption, is essential for accurate analysis.



In size exclusion, the elution volume is determined by the accessibility of the sample molecule to the pores. Maximum elution volume occurs if the sample can fully access the pores. Minimal elution volume occurs if the sample is larger than the pores. Hence, samples elute in order of size, with the highest molecular weight samples eluting first. If the analyte cannot enter the pores it passes through the column in the channels between the particles. Analytes that can enter the pores, either partially or completely, elute later. Since molecules are eluted based on their size in solution, linear or rod-like molecules will elute before globular molecules of the same molecular weight.

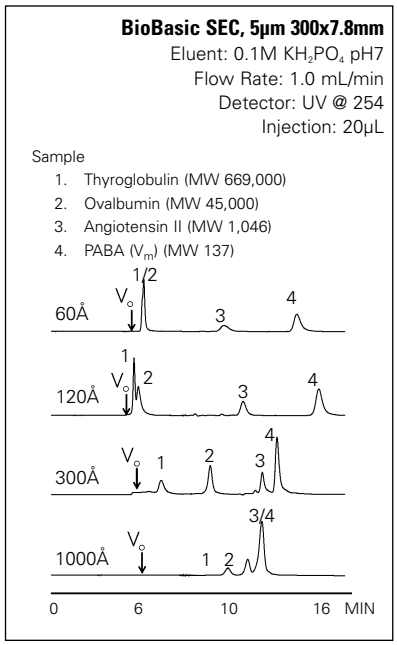


Figure 5: Effect of Pore Size on Resolution

Column Selection

Method development consists of selecting a mobile phase compatible with the sample type, and a column or columns with pore sizes that provide resolution for the molecular weight range of the sample.

Column selection should be lead by sample molecular weight, as the elution volume has a linear relationship to the log of molecular weight for a series of molecules of similar shape. Figure 5 shows the effect of various pore sizes in separating a set of proteins with a wide range of molecular weights.

The smallest pore size (60Å) gives the highest resolution between the smallest pair of peptides, but fails to resolve the largest two proteins. The larger pore size (1000Å) resolves the large proteins, while giving less resolution for the smaller molecules. Table 3 shows the recommend molecular weight range for each BioBasic SEC pore size.

Molecular Weight Calibration

To determine molecular weights of unknown samples, the log of the molecular weight is plotted against elution volume to create a distribution coefficient (calibration curve). The curve is usually linear for distribution coefficients between 0.2 and 0.8. The slope of the curve is sharp near the exclusion limit of large molecules and near total permeation of small molecules. To determine the molecular weights of unknown samples, the elution volume of the sample can be compared directly with that of standards of known molecular weight.

A smooth calibration curve is achieved for a homologous series when the separation is based only on sample size. Secondary interactions of the sample with the silica surface will increase sample retention (higher elution volume) more than otherwise expected from molecular weight calculations, causing a deviation from linearity. Figure 6 illustrates typical calibration curves for BioBasic SEC 300 for proteins, pullulans and polyethylene glycols/oxides over a broad range of molecular weights.

Column Reproducibility

Column-to-column reproducibility is of key importance to guarantee robust method development. Resolution in SEC is determined by the volume of pores with diameters between the inclusion and exclusion limits of the analytes. In order to manufacture a reproducible product, it is extremely important to maintain tight quality control on the physical properties of the silica. Every BioBasic SEC silica lot is tested chromatographically with a range of proteins to confirm accuracy of retention volumes. Every column also receives an efficiency test to confirm compliance with efficiency specifications. Each column is shipped with a test certificate containing both the silica lot test and the column efficiency test data.

Column Lifetime and Performance Stability

BioBasic SEC columns have been shown to be stable over 6000 to 7000 column volumes before a loss in efficiency is observed using a 0.1M KH2PO4 mobile phase at pH 7. The number of injections performed before column performance deteriorates can be significantly increased by using a guard column to protect the analytical column from mobile phase contaminants, as well as sample impurities and particulates (Figure 7).

Sample Clean up From Biological Matrices

BioBasic Size Exclusion Chromatography (SEC) columns in 60 or 120Å pore sizes can be used to exclude proteins from biological fluids in an aqueous environment. The proteins can then be diverted to waste allowing a protein free sample to be further separated prior to analysis.

Direct Serum Injection

Small pore size columns can be used for drug analysis with direct serum injection. Figures 8 through 11 demonstrate the use of a single SEC column and column switching techniques to separate protein serum matrices from smaller drug compounds.

Figure 8 shows a single column application, where the slight hydrophobic nature of the polymer coating and the large surface area of the small pore size are used to retain small, moderately polar drugs.

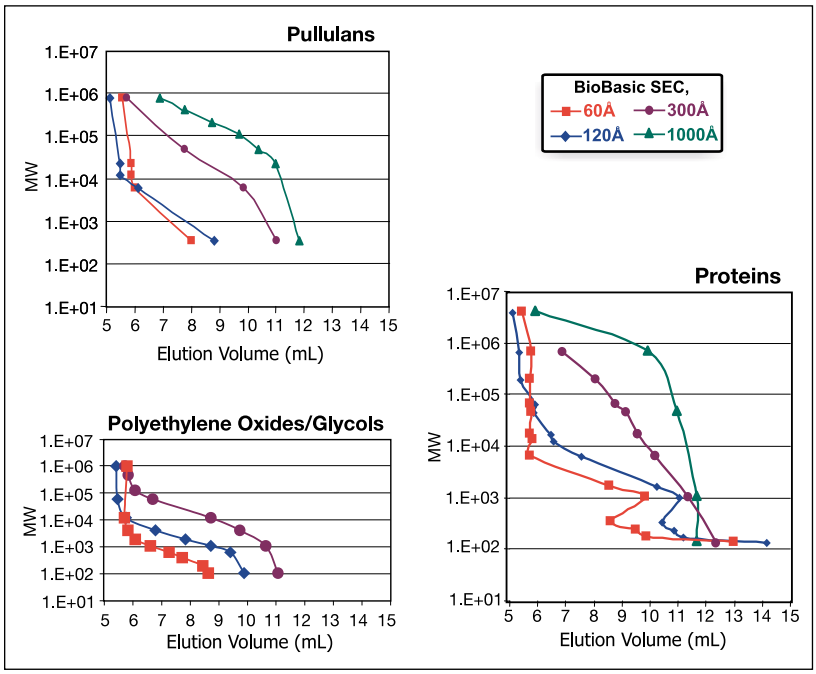


Figure 6: Molecular Weight Calibration Curves

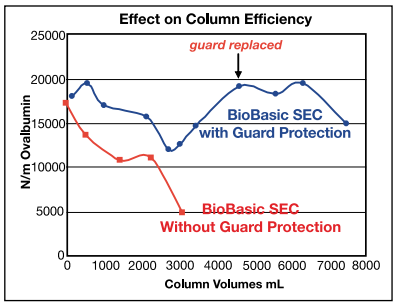


Figure 7: Effect of Guard Column on Lifetime

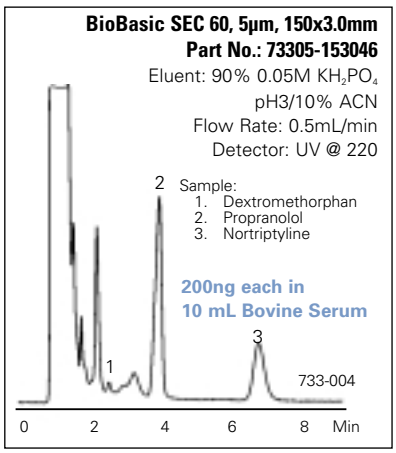


Figure 8: Direct Serum Injection Drug Analysis

Phase	Pore Size	Proteins	Pullulans	Polyethylene Oxides/Glycols
BioBasic SEC 60	60Å	0.1-6	0.3-6	0.1-4
BioBasic SEC 120	120Å	0.3-12	0.3-50	0.4-10
BioBasic SEC 300	300Å	1-500	1-100	2-100
BioBasic SEC 1000	1000Å	20-4000	20->1000	Not Recommended

Table 3: Molecular Weight Ranges for BioBasic SEC Columns (KDaltons)

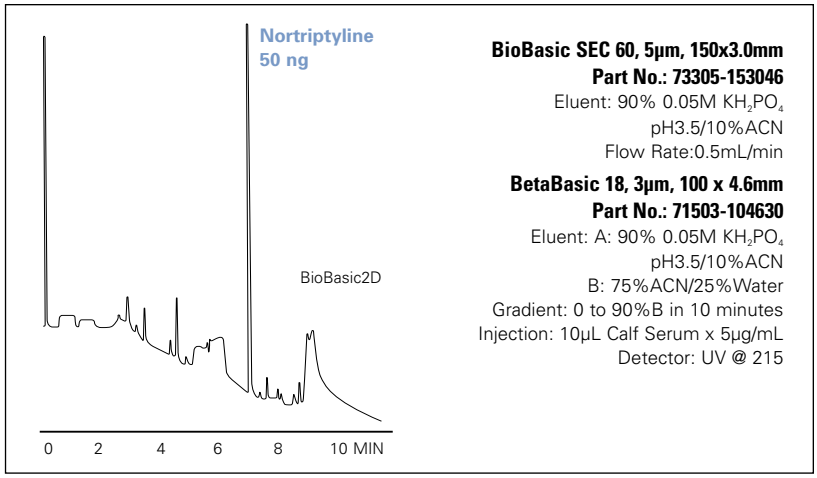
Two-Dimensional (2-D) Sample Clean-Up

Alternatively, as in Figure 9, column switching can be used to transfer the desired drug fraction eluted from the BioBasic SEC column onto a reversed phase column, where gradient elution is used to analyze the sample. For the more hydrophobic drug (nortriptyline), the fraction eluted from the BioBasic SEC 60 column with a high aqueous mobile phase is refocused onto a BetaBasic 18 (C18) column prior to gradient elution. This combination of size exclusion fractionation and reversed phase chromatography provides a more sensitive analysis of the nortriptyline as compared to a direct serum injection onto the reversed phase column.

This 2-D technique can also be used online for mass spectrometer screening of drug candidates and their metabolites. Figure 10 is a graph of peak area for three tricyclic antidepressants in whole serum. Over the course of 40 injections there is no significant degradation of signal intensity. Without the SEC clean up, signal intensities started to decrease after less than 10 injections.

Stability is further exhibited in Figure 11, which shows chromatograms and mass spectra for the first and last injections of the antidepressants. Chromatographic resolution and signal intensity are both maintained.

Please note that while serum proteins are eluted to waste, both columns should be protected by the corresponding guard column to prevent contamination and maximize analytical column lifetimes.



BioBasic SEC 60, 5µm, 150x3.0mm
Part No.: 73305-153046
Eluent: 90% 0.05M KH_2PO_4
pH3.5/10%ACN
Flow Rate:0.5mL/min

BetaBasic 18, 3µm, 100 x 4.6mm
Part No.: 71503-104630
Eluent: A: 90% 0.05M KH_2PO_4
pH3.5/10%ACN
B: 75%ACN/25%Water
Gradient: 0 to 90%B in 10 minutes
Injection: 10µL Calf Serum x 5µg/mL
Detector: UV @ 215

Figure 9: 2D SEC Sample Clean-Up

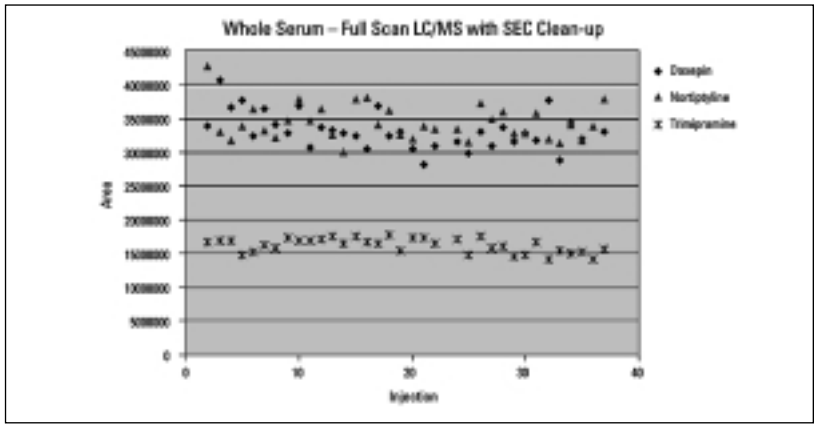


Figure 10: Whole Serum-Full Scan LC/MS with SEC Clean-Up

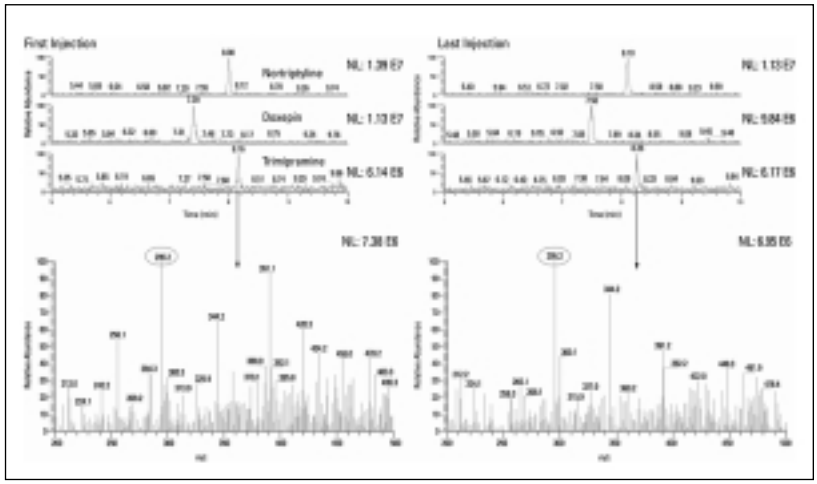


Figure 11: Extracted Ion Chromatograms and Full Scan MS Spectra

5µm BioBasic SEC Columns

Description	Dimensions (mm)	Part Number
BioBasic SEC 60	300x7.8 150x7.8 30x7.8 (guard)	73305-307846 73305-157846 73305-037821
BioBasic SEC 120	300x7.8 150x7.8 30x7.8 (guard)	73405-307846 73405-157846 73405-037821
BioBasic SEC 300	300x7.8 150x7.8 30x7.8 (guard)	73505-307846 73505-157846 73505-037821
BioBasic SEC 1000	300x7.8 150x7.8 30x7.8 (guard)	73605-307846 73605-157846 73605-037821

BioBasic Reversed Phase Columns

BioBasic Reversed Phase for Proteins and Peptides

Introduction

BioBasic reversed phase columns provide exceptional reliability for separations of proteins, peptides, and other biomolecules through a combination of high purity silica and reproducible, stable bonding. The proprietary bonding procedure ensures high phase coverage that gives improved resolution with shorter column lengths when compared to other 300Å columns. BioBasic packings are also designed to give longer lifetimes and withstand harsh mobile phase and clean-up conditions.

- **300Å pore size for improved performance for biomolecules**
- **High performance stationary phases offering different selectivity**
- **Outstanding reproducibility, efficiency and column lifetimes**
- **Ideal for LC/MS applications**

Pore Size and Chemistry

In the reversed phase mode, retention of protein analytes usually occurs through an adsorption/desorption type mechanism (small molecule retention is usually a partitioning type mechanism). This mode of retention means that the hydrophobic "foot" of the protein reversibly adsorbs to the bonded phase at the head of the column. As mobile phase conditions are changed, usually with an increase in organic composition, the protein is desorbed and eluted. Pore size plays an integral role because the majority of the bonded phase ligands (i.e. C4, C8, C18) are located inside the pore. Due to the larger size of proteins, it is important that the pore size be large enough to accommodate the protein.

Figure 12 shows the improved resolution with the 300Å pore size BioBasic 18 column for a tryptic digest separation compared to a 150Å pore size C18 column. In particular, the peptide fragments eluting near 8 and 13 minutes show much higher resolution on the BioBasic 18 column. This suggests that the larger pore size allows greater access to the stationary phase. A similar observation is made in Figure 13, where a comparison of a 300Å BioBasic 4 column and a 150Å C4 column is carried out for the separation of 3 proteins. The BioBasic 18 column provides taller, sharper peaks with less tailing.

Figure 14 demonstrates the changes in selectivity that occur due to differences in bonded phase chain length. In this example, BioBasic 18 and BioBasic 4 columns show a reversal of elution order for the two myoglobin peaks, which are not separated on the BioBasic 8 column.

General Recommendations for Column Selection

Start with a 5µm particle size, highly deactivated alkyl chain bonded phase. A short chain, wide pore column like BioBasic 4 is a good first choice for proteins. A longer chain, intermediate pore size column like BetaBasic™ 18 or HyPURITY™ C18 is a good initial selection for most peptides.

Start with short columns (50mm) since most protein and peptide separations occur by an adsorption/desorption mechanism in which retention is largely independent of column length.

When alternate selectivity is desired, consider changing pH, mobile phase modifiers or the separation temperature. Changing the bonded phase will also lead to changes in selectivity. Consider trying a BioBasic CN or BioBasic Phenyl column.

Stainless steel column hardware is suitable for most protein and peptide applications. Consider PEEK™ column hardware as an alternative when sample recovery or trace metal concentrations make stainless steel unsuitable. Remember that most of the surface area of the column hardware is in the frits. BioBasic media is available in PEEK columns and guard systems with a completely inert flow path.

Other Applications

The 300Å pore size of the BioBasic family is not just for large molecules. BioBasic columns also give outstanding results for small molecule separations such as food additives, aromatic acids and small organic acids (Figure 15).

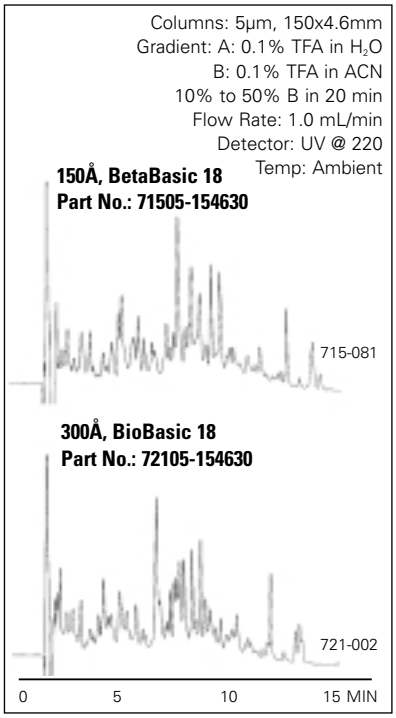


Figure 12: Effect of Pore Size on Tryptic Digest Separation

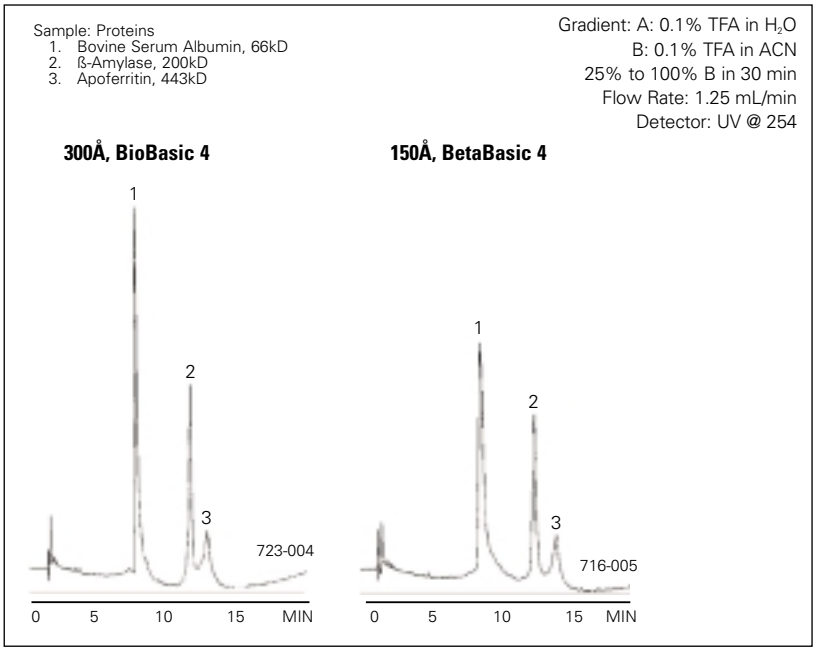


Figure 13: Effect of Pore Size on Protein Separation

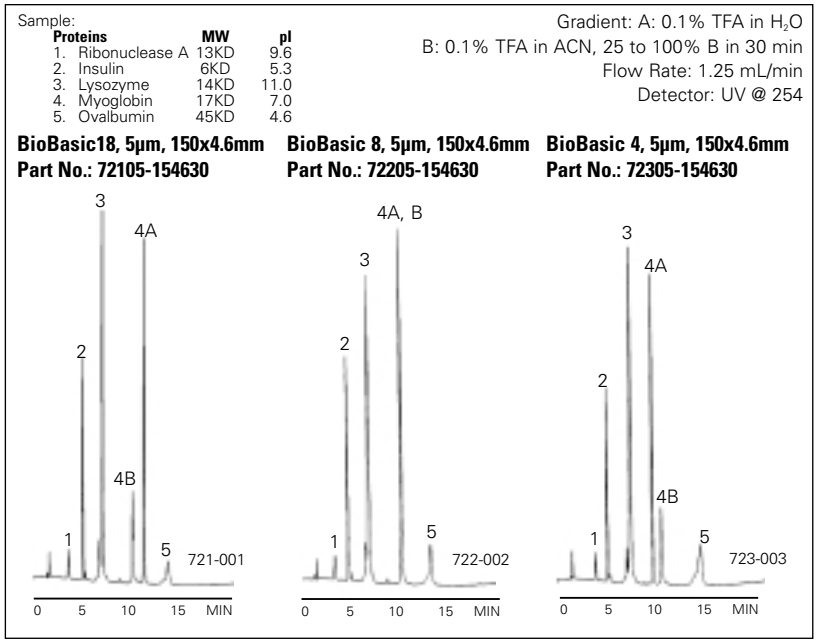


Figure 14: Effect of Bonded Phase Chain Length on Protein Separation

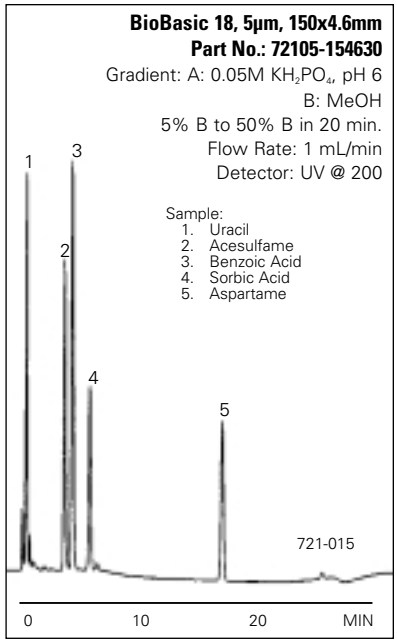


Figure 15: Food Additives

5µm BioBasic Reversed Phase Columns

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
BioBasic 18	30	72105-034630	72105-034030	72105-033030	72105-032130	72105-031030
	50	72105-054630	72105-054030	72105-053030	72105-052130	72105-051030
	100	72105-104630	72105-104030	72105-103030	72105-102130	72105-101030
	150	72105-154630	72105-154030	72105-153030	72105-152130	72105-151030
	250	72105-254630	72105-254030	72105-253030	72105-252130	72105-251030
BioBasic 8	50	72205-054630	72205-054030	72205-053030	72205-052130	72205-051030
	100	72205-104630	72205-104030	72205-103030	72205-102130	72205-101030
	150	72205-154630	72205-154030	72205-153030	72205-152130	72205-151030
	250	72205-254630	72205-254030	72205-253030	72205-252130	72205-251030
BioBasic 4	50	72305-054630	72305-054030	72305-053030	72305-052130	72305-051030
	100	72305-104630	72305-104030	72305-103030	72305-102130	72305-101030
	150	72305-154630	72305-154030	72305-153030	72305-152130	72305-151030
	250	72305-254630	72305-254030	72305-253030	72305-252130	72305-251030
BioBasic CN	50	72905-054630	72905-054030	72905-053030	72905-052130	72905-051030
	100	72905-104630	72905-104030	72905-103030	72905-102130	72905-101030
	150	72905-154630	72905-154030	72905-153030	72905-152130	72905-151030
	250	72905-254630	72905-254030	72905-253030	72905-252130	72905-251030
BioBasic Phenyl	50	72405-054630	72405-054030	72405-053030	72405-052130	72405-051030
	100	72405-104630	72405-104030	72405-103030	72405-102130	72405-101030
	150	72405-154630	72405-154030	72405-153030	72405-152130	72405-151030
	250	72405-254630	72405-254030	72405-253030	72405-252130	72405-251030

Other column dimensions are available, including preparative columns. BioBasic 18 is available in a 10µm particle size. Please call Customer Service for more information.
To order standard columns with integral guard (COLUMNPLUS Guard or CPG), please change the last 2 digits of the part number above to 31.

5µm BioBasic Reversed Phase Drop-In Guard Cartridges

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
BioBasic 18	10	72105-014001	72105-014001	72105-013001	72105-012101	72105-011001
BioBasic 8	10	72205-014001	72205-014001	72205-013001	72205-012101	72205-011001
BioBasic 4	10	72305-014001	72305-014001	72305-013001	72305-012101	72305-011001
BioBasic CN	10	72905-014001	72905-014001	72905-013001	72905-012101	72905-011001
BioBasic Phenyl	10	72405-014001	72405-014001	72405-013001	72405-012101	72405-011001
UNIGUARD Direct-Connect Drop-in Guard Cartridge Holder		850-00	850-00	852-00	852-00	851-00

BioBasic PEEK™ Bio-Inert Columns

Description	Length (mm)	4.6 mm ID	2.1 mm ID
BioBasic 18	100	72105-104668	72105-102168
	150	72105-154668	72105-152168
	250	72105-254668	72105-252168
BioBasic 8	100	72205-104668	72205-102168
	150	72205-154668	72205-152168
	250	72205-254668	72205-252168
BioBasic 4	100	72305-104668	72305-102168
	150	72305-154668	72305-152168
	250	72305-254668	72305-252168

Other phases and column dimensions are available in bio-inert column hardware.
Please call Customer Service for more information.

5µm BioBasic PEEK Guard Cartridges

Description	Length (mm)	4.6 mm ID	2.1 mm ID
BioBasic 18	10	72105-014003	72105-012103
BioBasic 8	10	72205-014003	72205-012103
BioBasic 4	10	72305-014003	72305-012103
Bio-inert Guard Holder	10	C270-01	inquire

Other Hardware Designs for BioBasic Reversed Phase Columns

PIONEER™ Columns		Direct connection columns
KAPPA™ Columns		Highly efficient capillary columns
Javelin Express™ Columns		Economical, not individually tested columns
PicoFrit™ Columns		Nanobore fused silica columns for LC/MS
Javelin™ Guard Columns		Direct connection column protection
SLIPFREE™ Column Connectors		Easy to use, void and leak free connectors
Preparative Columns		Available in 10, 21.2, 30, 40, 50 and 100 mm ID

BioBasic Ion Exchange Columns

Introduction

BioBasic ion exchange columns are a new generation column that makes use of polymeric ion exchange ligand technology bound to a high-quality base deactivated silica. This unique combination gives rise to a highly efficient and stable polymeric coating that is very robust and reproducible. The columns can be used in any one of several modes of operation: ion exchange, normal phase or BioBasic reversed phase columns provide exceptional reliability for separations of proteins, peptides, and other biomolecules, through a combination of high purity silica and reproducible, stable bonding. The proprietary bonding procedure ensures high phase coverage that gives improved resolution with shorter column lengths when compared to other 300Å columns. BioBasic packings are also designed to give longer lifetimes and withstand harsh hydrophilic interaction chromatography.

- **BioBasic AX for anion exchange and HILIC chromatography**
- **BioBasic SCX for cation exchange chromatography**
- **300Å pore size for enhanced protein and peptide separations**
- **Superb stability under demanding pH conditions**
- **Exceptional efficiency from 5µm silica particles**

Ion Exchange Mechanism

In ion exchange chromatography, molecules bind by the reversible interaction of electrostatic charges located on the outer surface of the solute molecule with clusters of groups with an opposite charge on the ion exchanger. To maintain neutrality, the charges on both the molecules of interest and the ion exchanger are associated with ions of opposite charge, termed counterions. Because a solute must displace the counterions on the stationary phase to attach to it, the technique is termed "ion exchange". Because all proteins and peptides have the ability to exist as a charged species, ion exchange is an excellent choice for protein and peptide characterization (Figure 16).

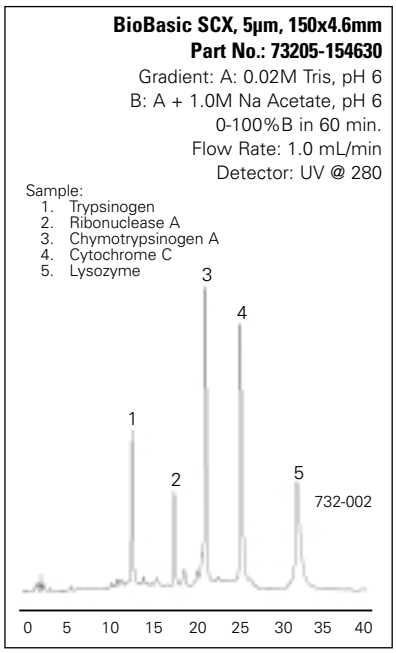


Figure 16: Proteins

Phase	Particle Size	Pore Size	Ion Exchange Ligand	Ion Exchange Capacity	Structure
BioBasic SCX	5µm	300Å	Sulfonic Acid	0.07mEq/gram	
BioBasic AX	5µm	300Å	PEI – Polyethyleneimine	0.22mEq/gram	

Table 4: BioBasic Ion Exchange Particle Properties

The BioBasic family of ion exchange columns make use of a proprietary polymeric ion exchange ligand technology bound to highly pure base deactivated silica. This unique combination gives rise to a highly efficient and stable polymeric coating that is very robust and reproducible. Figures 17a and 17b demonstrate the stability of both the BioBasic AX and SCX packings. In addition to providing superb stability, the columns can be used in any one of several modes of operation: ion exchange, normal phase, and hydrophilic interaction chromatography. Table 4 provides the ion exchange particle properties for the BioBasic AX and SCX phases.

Ion Exchange Chromatographic Characteristics

Most ion exchange packings fall into two groups: those which contain acid groups, such as sulfonic acid or carboxylic acid, for the separation of cationic compounds, and those which contain a basic group, such as an amine or quaternary amine, for the separation of anionic compounds. It is important to buffer the mobile phase in this mode of chromatography to control the ionization of both the analyte and the stationary phase, since the ionic state of both affects the acid-base equilibrium between analyte and ion exchange packing.

In ion exchange chromatography conditions are employed that permit the sample components to move through the column at different speeds. The higher the net charge of the analyte, the higher the ionic strength needed to bring about desorption. At a certain high level of ionic strength, all the sample components are fully desorbed and move down the column with the same speed as the mobile phase (Figure 18). Conditions that lie somewhere in between total adsorption and total desorption will provide the optimal selectivity for a given pH value of the mobile phase, as shown in Figure 19.

BioBasic AX for Normal Phase and Hydrophilic Interaction Chromatography

BioBasic AX media contains an amine on the bonded phase, which makes it susceptible to hydrogen bonding interactions. These interactions can be taken advantage of to give the BioBasic AX more chromatographic options, such as retention through either normal phase chromatography, or hydrophilic interaction chromatography.

Normal phase chromatography is governed by the ability of the analyte to hydrogen bond with the stationary phase. Retention is controlled by adding small quantities of a competing solvent with hydrogen bonding properties to the mobile phase (e.g. ethyl acetate).

Hydrophilic Interaction Chromatography (HILIC) is similar to normal phase chromatography. In this mode however, portions of the mobile phase are aqueous. Retention increases with the polarity of the analyte and retention decreases with the polarity of the eluent. The stationary phase easily adsorbs water from the mobile phase making it very hydrophilic. A separation using the BioBasic AX column in HILIC mode is shown in Figure 20.

Other Applications

BioBasic ion exchange columns can also be used for the analysis of nucleotides, aromatic acids, sugars, phospholipids, antibiotics, and vitamins.

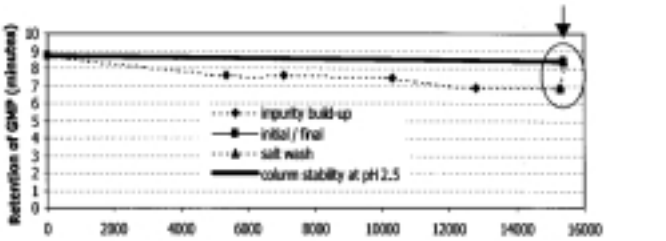
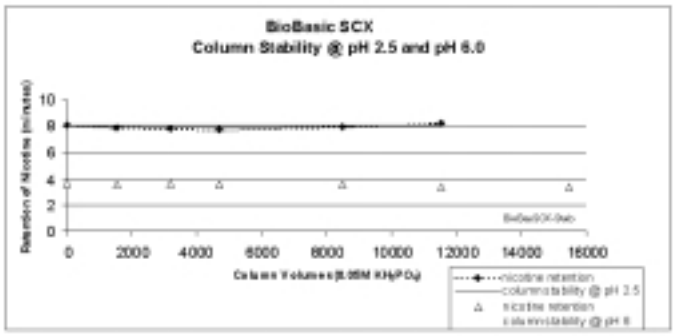
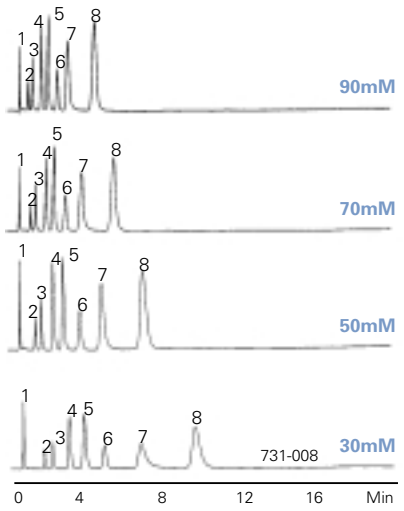


Figure 17a: pH Stability of BioBasic AX Media



Nicotine retention for stability study at pH 6 is significantly less due to use of NH_4 formate buffer (pH 3.1) as opposed to NH_4 acetate buffer (pH 4.5) in the mobile phase.

Figure 17b: pH Stability of BioBasic SCX Media



BioBasicAX, 5µm, 50x4.6mm
Part No.: 73105-054630
Eluent: A: 95% NH_4 Acetate, pH 5.7 / 5% ACN
B: ACN 95%A / 5%B
Flow Rate: 1.0 mL/min
Detector: UV @ 225

Sample:
1. Uracil
2. Shikimic Acid
3. Ascorbic Acid
4. Phenylacetic Acid
5. Sorbic Acid
6. Benzoic Acid
7. Vanillic Acid
8. p-Coumeric Acid

Figure 18: Effect of Buffer Concentration on Retention

BioBasic SCX, 5µm, 150x4.6mm
Part No.: 73205-154630
Eluent: A: 5mM NH_4 Formate
B: A + 1M NaCl 95% A / 5% B
Flow Rate: 1.0 mL/min
Detector: UV @ 210

Sample:
1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Leucine Enkephalin

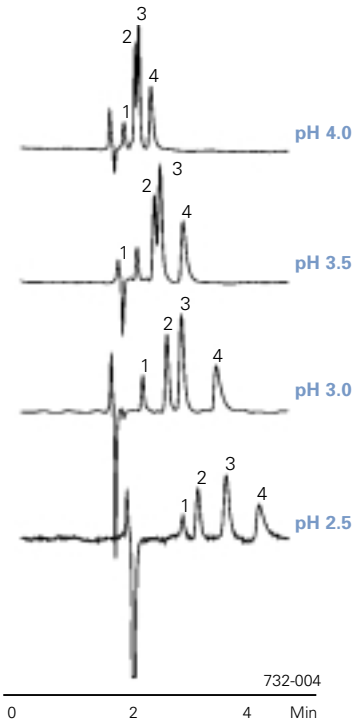


Figure 19: Effect of pH on Retention

BioBasic AX, 5µm, 150x4.6mm
Part No.: 73105-154630
Gradient: A:95% ACN / 5% 5mM NH_4 Formate, pH 6.13
B:80% ACN / 20% 5mM NH_4 Formate, pH 6.13
0% B 0-5 min, 0 to 100%B 5-15 min
Flow: 1.0 mL/min
Detector: ELSD

Sample:
1. L-erythro-Sphingosine
2. L-α-Phosphatidyl choline, egg
3. Phosphatidyl ethanolamine, bovine
4. lyso-Lecithin, egg

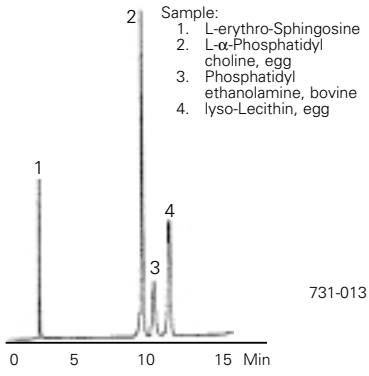


Figure 20: BioBasic AX Column used in HILIC Mode

5µm BioBasic Ion Exchange Columns

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
BioBasic AX	50	73105-054630	73105-054030	73105-053030	73105-052130	73105-051030
	100	73105-104630	73105-104030	73105-103030	73105-102130	73105-101030
	150	73105-154630	73105-154030	73105-153030	73105-152130	73105-151030
	250	73105-254630	73105-254030	73105-253030	73105-252130	73105-251030
BioBasic SCX	50	73205-054630	73205-054030	73205-053030	73205-052130	73205-051030
	100	73205-104630	73205-104030	73205-103030	73205-102130	73205-101030
	150	73205-154630	73205-154030	73205-153030	73205-152130	73205-151030
	250	73205-254630	73205-254030	73205-253030	73205-252130	73205-251030

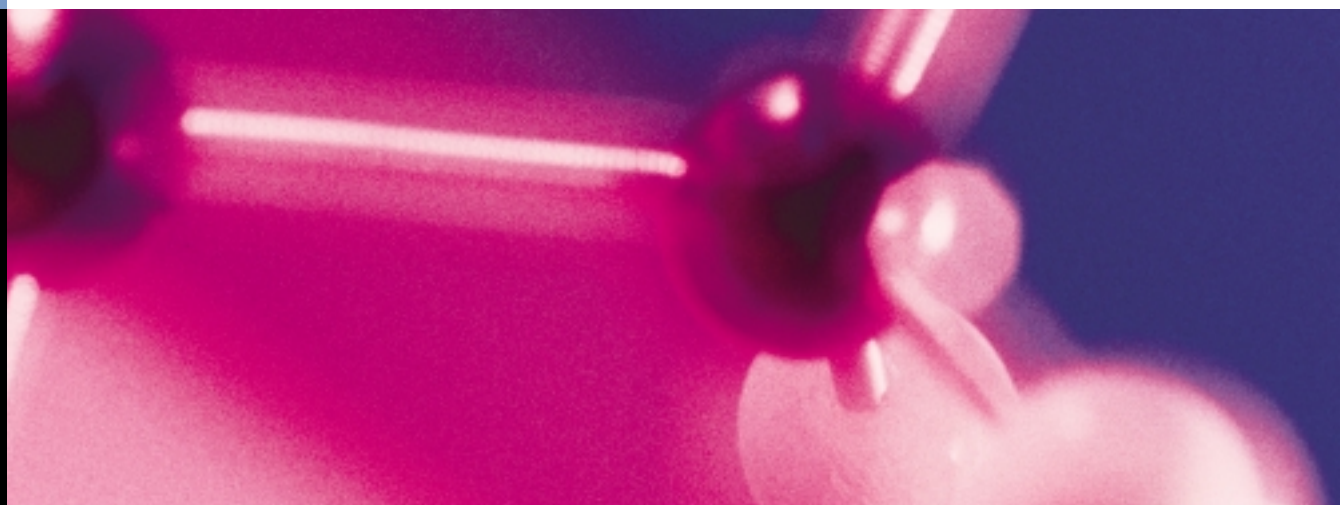
Other column dimensions are available. Please call Customer Service for more information. To order standard columns with integral guard (COLUMNPLUS Guard or CPG), please change the last 2 digits of the part number above to 31.

5µm BioBasic Ion Exchange Drop-In Guard Cartridges

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
BioBasic AX	10	73105-014001	73105-014001	73105-013001	73105-012101	73105-011001
BioBasic SCX	10	73205-014001	73205-014001	73205-013001	73205-012101	73205-011001
UNIGUARD Direct-Connect Drop-in Guard Cartridge Holder		850-00	850-00	852-00	852-00	851-00

Other Hardware Designs for BioBasic Ion Exchange Columns

PIONEER™ Columns		Direct connection columns
KAPPA™ Columns		Highly efficient capillary columns
SLIPFREE™ Column Connectors		Easy to use, void and leak free connectors



BioBasic Columns for LC/MS

Introduction

The BioBasic family offers a range of phases, as well as column dimensions, for all of your LC/MS needs. From small peptides to large biopolymers, the BioBasic family provides optimal analysis capabilities for the most sensitive protein and peptide separations.

- **BioBasic phases offer maximum sensitivity for biomolecules**
- **KAPPA capillary columns ideal for LC/MS analysis**
- **500 to 75µm ID columns for reduced sample concentration**
- **Multi-dimensional LC/MS analyses for very complex samples**

Proteomics

Proteomics is the study of the structure and function of proteins and their interactions. The causes of many diseases can be traced back to disruptions of normal biophysical regulatory processes, which may involve proteins and their interactions. Proteomics is an important step in the drug discovery process since over 95 percent of all pharmaceuticals target protein intervention points. Since the human genome was fully sequenced, accelerated protein research becomes the next logical step, assisted by rapid improvements in the analytical technologies. Much of the work involves analyzing very small quantities of sample. Quite often this will require the use of columns with diameters much smaller than those typically used in analytical liquid chromatography.

BioBasic KAPPA capillary columns have been designed to meet the demanding sensitivity requirements of high performance proteomics.

Single and multidimensional LC/MS methods play an important role in the field of proteomics. Atmospheric pressure ionization (API) is by far the easiest interface between the LC column and the mass spectrometer.

Atmospheric Pressure LC/MS

There are two popular types of atmospheric ionization modes:

- Atmospheric pressure chemical ionization (APCI)
- Electrospray ionization (ESI)
  - *Microelectrospray*
  - *Nanoelectrospray*

Typically, more polar compounds such as amines, peptides and proteins are best analyzed by ESI, and apolar compounds, such as steroids, are best analyzed by APCI.

Electrospray Ionization

The ESI mode typically produces mass spectra consisting of multiply charged ions (for proteins and peptides) depending on the structure of the analyte and the mobile phase.For example, the resulting mass spectrum of a higher molecular weight protein or peptide typically consists of a distribution of multiply charged analyte ions. The resulting mass spectrum can be mathematically manipulated to determine the molecular weight of the sample.

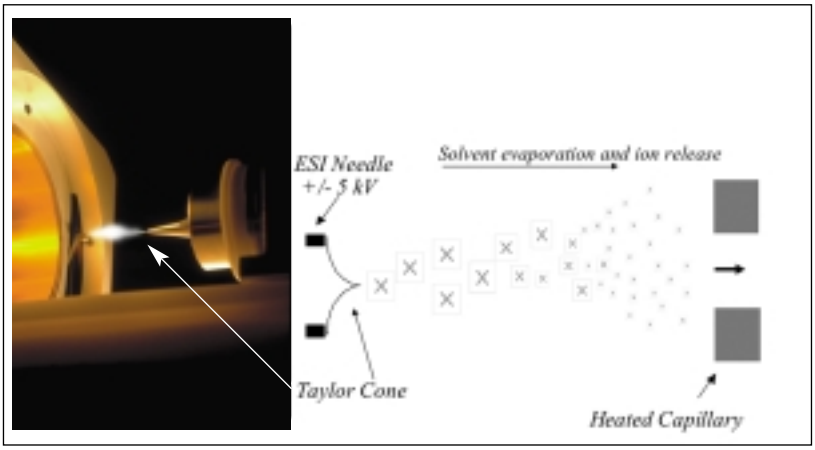


Figure 21: Electrospray Process

The ESI mode transfers ions in solution into the gas phase (Figure 21). Many samples that previously were not suitable for mass analyses (heat-labile or high molecular weight compounds) can be analyzed by ESI. ESI can be used in positive or negative ion polarity mode to analyze any polar compound that makes a preformed ion in solution. Molecular weights greater than 100,000 can be analyzed with ESI due to multiple charging. ESI is especially useful for the mass analyses of polar compounds, which include proteins, peptides, glycoproteins, nucleotides, pharmaceuticals, metabolites and industrial polymers.

The ESI process is affected by droplet size, surface charge and tension, solvent volatility and ion solvation strength. Large droplets with high surface tension, low volatility, strong ion solvation, low surface charge and high conductivity prevent good electrospray. Higher ionization efficiencies are achieved at lower flow rates. HPLC columns with 2.1mm and smaller diameters are recommended.

Mixed organic/aqueous solvent systems that include solvents such as methanol, acetonitrile and isopropyl alcohol are superior to water alone. Volatile acids and bases are good, but salts above 20mM are not recommended.

Figure 22 shows the total ion chromatogram (TIC) and the individual spectra of five whole proteins on a Finnigan™ LCQ™ Duo ion trap. The 5µm BioBasic 18 100x0.180mm column provides excellent peak shape and separation of all five proteins. Using the biomass deconvolution module it is then possible to identify these proteins by their distribution of multiply charged ions (Figure 23).

Micro- and Nanoelectrospray

Microelectrospray is an ionization method that uses a sheath gas and heat to assist in aerosol formation. Typically this method is used with capillaries ranging from 320 to 180µm ID with flow rates from 1 to 10µL/min. An example of a microelectrospray application, an angiotensin separation, is illustrated in Figure 24. Nanoelectrospray is an ionization method that utilizes low flows in the nL/minute range emitted via a spray tip.

A major benefit of this technique over microspray is increased sensitivity. This increase in sensitivity is often needed when analyzing low femtomole level peptides. There is an increase in signal because it is easier to produce a stable spray at these low flow rates. No sheath gas or heat is required because of the small amount of solvent used. Only a voltage is required to produce a stable spray. Nanospray also allows for longer analysis time for MS/MS.

Table 5 lists some recommendations for low flow LC/MS. The BioBasic KAPPA line is an excellent choice for any type of electrospray work. Figure 25 shows the sensitivity that can be achieved with small internal diameter columns, a 30 femtomole phosphorylase B digest on a 100x0.075mm BioBasic 18 column. This method identified nearly half of all the peptides contained in the digest.

Column ID (mm)	Flow Rate (µL/min)	Sample Amount	Ionization Technique
2.1	200	>25pmole	electrospray
1.0	50	>25pmole	electrospray
0.50	12	>25pmole	electrospray
0.32	6	2-50pmole	microelectrospray
0.18	2	0.1-5pmole	microelectrospray
0.10	0.50	0.05-1pmole	nanoelectrospray
0.075	0.35	<200fmole	nanoelectrospray

Table 5: LC/MS considerations

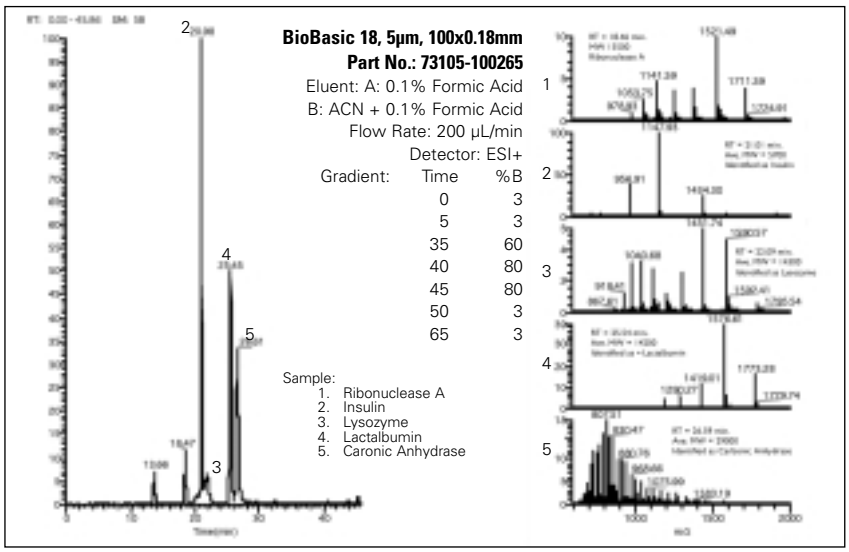


Figure 22: Whole Proteins Analysis using an Ion Trap

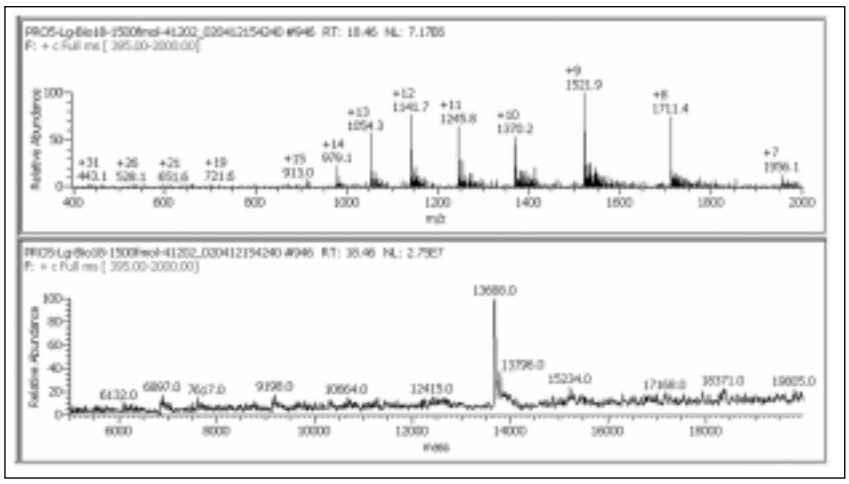
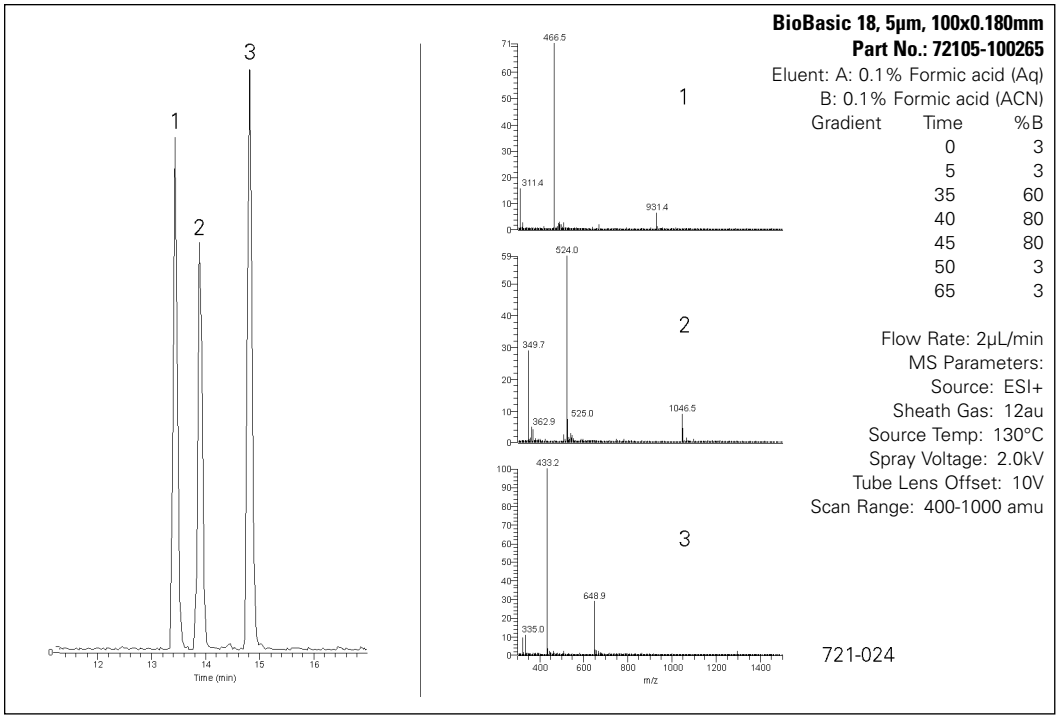


Figure 23: Component 1 - Ribonuclease A Biomass Deconvolution



BioBasic 18, 5µm, 100x0.180mm
Part No.: 72105-100265

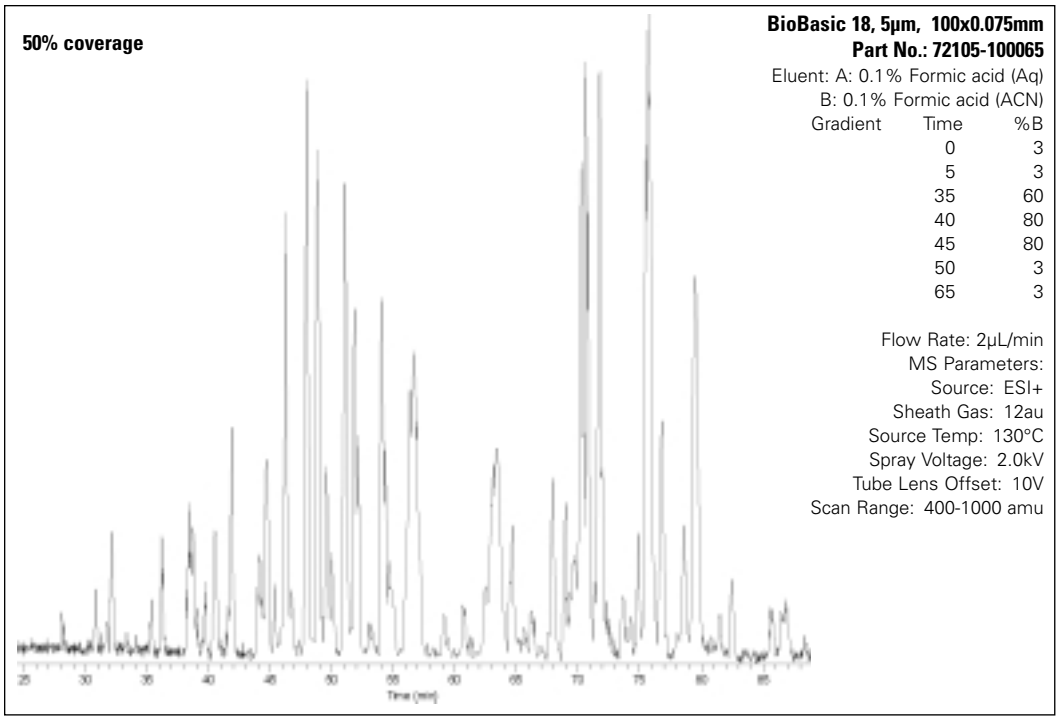
Eluent: A: 0.1% Formic acid (Aq)
B: 0.1% Formic acid (ACN)

Gradient Time	%B
0	3
5	3
35	60
40	80
45	80
50	3
65	3

Flow Rate: 2µL/min
MS Parameters:
Source: ESI+
Sheath Gas: 12au
Source Temp: 130°C
Spray Voltage: 2.0kV
Tube Lens Offset: 10V
Scan Range: 400-1000 amu

721-024

Figure 24: Angiotensin Separation on a 100x0.18mm BioBasic 18 KAPPA Capillary Column



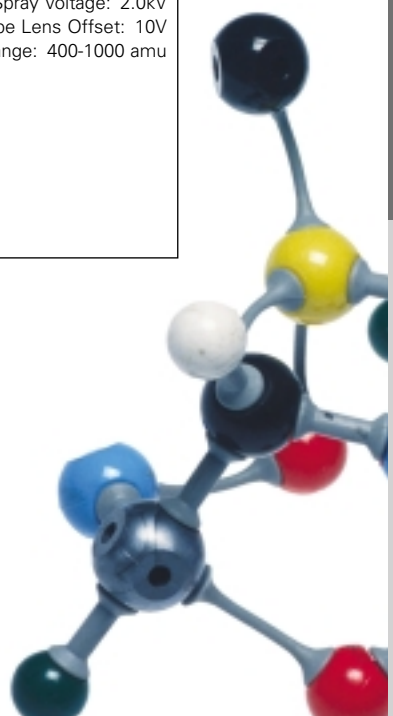
BioBasic 18, 5µm, 100x0.075mm
Part No.: 72105-100065

Eluent: A: 0.1% Formic acid (Aq)
B: 0.1% Formic acid (ACN)

Gradient Time	%B
0	3
5	3
35	60
40	80
45	80
50	3
65	3

Flow Rate: 2µL/min
MS Parameters:
Source: ESI+
Sheath Gas: 12au
Source Temp: 130°C
Spray Voltage: 2.0kV
Tube Lens Offset: 10V
Scan Range: 400-1000 amu

Figure 25: 30 femtomole Phosphorylase B digest



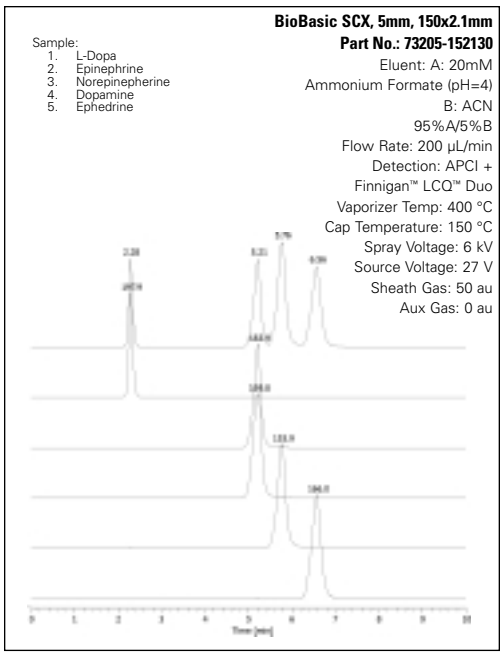


Figure 26: Catecholamines by Cation Exchange LC/MS

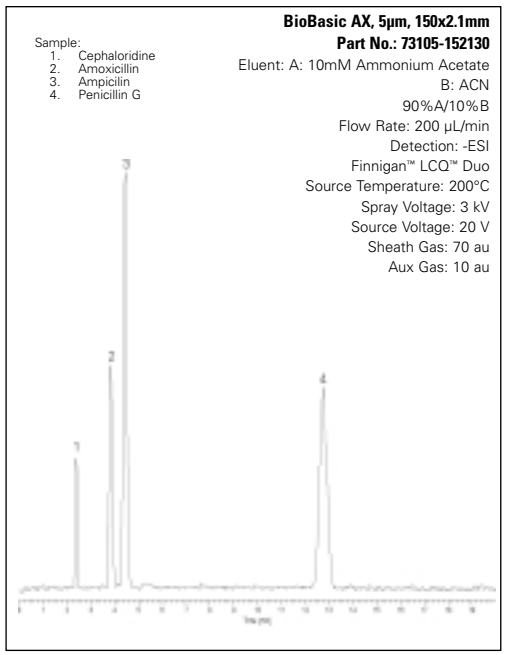


Figure 27: Antibiotics by Anion Exchange LC/MS

5µm BioBasic KAPPA Capillary Columns

Description	Length (mm)	500µm ID	320µm ID	180µm ID	100µm ID	75µm ID
BioBasic 18	50	72105-050565	72105-050365	72105-050265	72105-050165	72105-050065
	100	72105-100565	72105-100365	72105-100265	72105-100165	72105-100065
	150	72105-150565	72105-150365	72105-150265	72105-150165	72105-150065
	250	72105-250565	72105-250365	72105-250265	inquire	inquire
BioBasic 8	50	72205-050565	72205-050365	72205-050265	72205-050165	72205-050065
	100	72205-100565	72205-100365	72205-100265	72205-100165	72205-100065
	150	72205-150565	72205-150365	72205-150265	72205-150165	72205-150065
	250	72205-250565	72205-250365	72205-250265	inquire	inquire
BioBasic 4	50	72305-050565	72305-050365	72305-050265	72305-050165	72305-050065
	100	72305-100565	72305-100365	72305-100265	72305-100165	72305-100065
	150	72305-150565	72305-150365	72305-150265	72305-150165	72305-150065
	250	72305-250565	72305-250365	72305-250265	inquire	inquire
BioBasic CN	50	72905-050565	72905-050365	72905-050265	72905-050165	72905-050065
	100	72905-100565	72905-100365	72905-100265	72905-100165	72905-100065
	150	72905-150565	72905-150365	72905-150265	72905-150165	72905-150065
	250	72905-250565	72905-250365	72905-250265	inquire	inquire
BioBasic Phenyl	50	72405-050565	72405-050365	72405-050265	72405-050165	72405-050065
	100	72405-100565	72405-100365	72405-100265	72405-100165	72405-100065
	150	72405-150565	72405-150365	72405-150265	72405-150165	72405-150065
	250	72405-250565	72405-250365	72405-250265	inquire	inquire
BioBasic AX	50	73105-050565	73105-050365	73105-050265	73105-050165	73105-050065
	100	73105-100565	73105-100365	73105-100265	73105-100165	73105-100065
	150	73105-150565	73105-150365	73105-150265	73105-150165	73105-150065
	250	73105-250565	73105-250365	73105-250265	inquire	inquire
BioBasic SCX	50	73205-050565	73205-050365	73205-050265	73205-050165	73205-050065
	100	73205-100565	73205-100365	73205-100265	73205-100165	73205-100065
	150	73205-150565	73205-150365	73205-150265	73205-150165	73205-150065
	250	73205-250565	73205-250365	73205-250265	inquire	inquire

Replacement Columns for Finnigan™ ProteomeX™ System

Description	Dimensions (mm x µm)	Finnigan ProteomeX Part Number	Replacement Part Number
5µm BioBasic 18 Flexible KAPPA	100 x 180	00109-00508	72105-100266
5µm BioBasic SCX KAPPA	100 x 320	00109-00510	73205-100365

In addition to the Thermo Electron offices listed below, we have an extensive network of authorized global distributors for Thermo Electron HPLC columns. For the list of international distributors, please contact columninfo.uk@thermo.com.

Australia
+61 2 9898 1244 • analyze.au@thermo.com
Austria
+43 1 333 50340 • analyze.at@thermo.com
Belgium
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Canada
+1 800 532 4752 • analyze.ca@thermo.com
China
+86 10 5850 3588 • analyze.cn@thermo.com
France
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Germany
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South Africa
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Spain
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UK
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USA
+1 800 532 4752 • analyze.us@thermo.com

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