



Reversed Phase HPLC Solutions
for Proteins and Peptides

IDENTIFY
PURIFY
ANALYZE





ABOUT PHENOMENEX... Founded in 1982, Phenomenex is extremely dedicated to the development, manufacturing, and supply of innovative separation and purification products for the life science/pharmaceutical industries. However, we are even more dedicated to providing the highest level of customer support and satisfaction in the separation science industry. By personalizing science, we are able to ensure that every customer has the opportunity to work with energetic and extremely knowledgeable Phenomenex employees and distributors to address day-to-day responsibilities such as method development, product evaluations, and troubleshooting. Our “customer first” policy means that we deliver the same degree of support and service to the small, independent lab or university as well as major corporations. An extensive global presence through offices located in 12 countries worldwide and a vast network of distributors in over 60 countries enables Phenomenex to successfully supply and support the work of scientists worldwide. No matter where you are located in the world, you can always count on Phenomenex.

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Identify, Purify, and Analyze

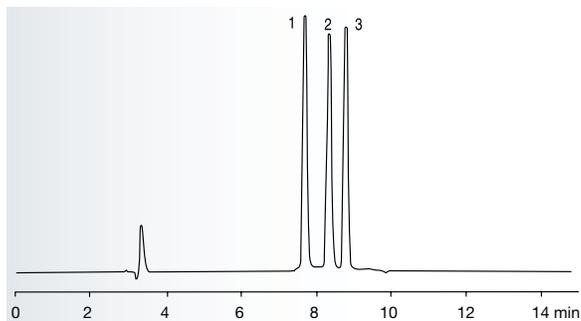
The Jupiter HPLC column portfolio, including Jupiter 300 and Jupiter Proteo, offers optimized reversed phase solutions for protein characterization and purification. With these columns, one can identify, purify, and analyze almost any protein.



300 Å column designed for intact protein purification and analysis

- Separation of proteins $\geq 10,000$ MW
- Available with C18, C5, and C4 bonded phases
- Excellent peak shape and resolution of protein samples

Purify Intact Proteins



App ID 8715

The excellent resolving power of Jupiter 5 μ m 300 C18 has the ability to separate proteins with very similar compositions and chemical properties.

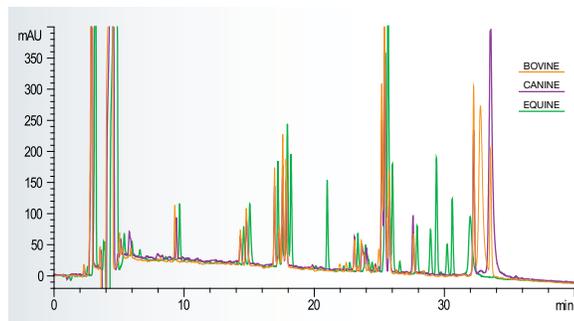
- Column:** Jupiter 300 5 μ m C18 300 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4053-E0
- Mobile Phase:** A) 0.1 % TFA in Water
B) 0.1 % TFA in Acetonitrile
- Gradient:** A/B (75:25) to A/B (45:55) in 15 min (2 % B/min)
- Flow Rate:** 1 mL/min
- Temperature:** Ambient
- Detection:** UV @ 220 nm
- Sample:** 1. Equine Cytochrome *c*
2. Bovine Cytochrome *c*
3. Canine Cytochrome *c*



90 Å column engineered for peptide mapping and peptide separations

- Separation of proteins and peptides $\leq 10,000$ MW
- Identify post-translational modifications
- Increased peak capacity and resolution

Peptide Maps of Cytochrome *c* Tryptic Digests



App ID 14682

Identify differences between peptide maps of similar proteins, which are typically difficult to resolve, due to the high peak capacity feature of Jupiter Proteo.

- Column:** Jupiter 4 μ m Proteo 90 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4396-E0
- Mobile Phase:** A) 0.12 % TFA in Water
B) 0.1 % TFA in Acetonitrile
- Gradient:** A/B (95:5) to A/B (45:55) in 50 min, then to A/B (5:95) in 5 min, then hold at A/B (5:95) for 5 min, then hold at A/B (95:5) for 5 minutes
- Flow Rate:** 1 mL/min
- Temperature:** Ambient
- Detection:** UV @ 210 nm
- Sample:** Tryptic digest of Cytochrome *c* genetic variants – see chromatogram for species

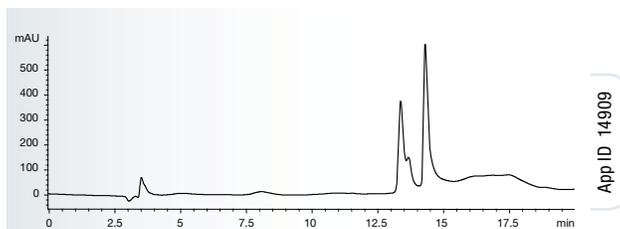
Dependable Solutions

Jupiter HPLC columns and bulk material are used throughout the life science industry in a variety of departments and applications. Phenomenex offers support and solutions in all areas of protein research and manufacturing, especially in characterization, purification, and proteomics/biomarker discovery.

Protein Characterization

- Identify post-translational modifications
- Analyze intact antibodies and fragments
- Study PEGylated proteins

Analyze Reduced IgG



App ID 14909

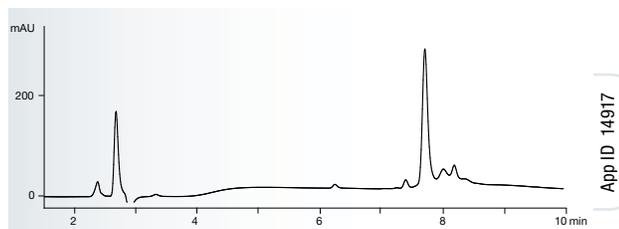
Baseline separation between heavy and light chains of IgG is achieved on Jupiter 5 µm 300 C4.

- Column:** Jupiter 300 5 µm C4 300 Å
- Dimensions:** 150 x 2.0 mm
- Part No.:** 00F-4167-B0
- Mobile Phase:** A) 0.1 % TFA in Water/Acetonitrile (95:5)
B) 0.085 % TFA in Acetonitrile/IPA/Water (75:20:5)
- Gradient:** A/B (80:20) to (5:95) in 20 minutes
- Flow Rate:** 0.25 mL/minute
- Detection:** UV @ 220 nm
- Sample:** IgG Dog Reduced

Protein/Peptide Purification

- Separate target compound from impurities
- Purify antibodies
- Separate protein components from one another
- Easy, direct scale-up to preparative and process scales

Purify Away Degradants



App ID 14917

Due to its unique C12, 90 Å chemistry, Jupiter Proteo is able to purify insulin from its degradants ensuring high sample purity.

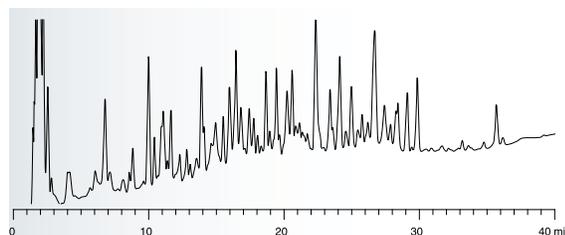
- Column:** Jupiter 4 µm Proteo 90 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4396-E0
- Mobile Phase:** A) 0.1 % TFA in Water
B) 0.085 % TFA in 95:5 Acetonitrile/Water
- Gradient:** A/B (80:20) to A/B (20:80) in 15 minutes
- Flow Rate:** 1 mL/min
- Detection:** UV @ 220 nm (ambient)
- Sample:** Human Insulin

Proteomics/ Biomarker Discovery

- Perform peptide mapping for differential proteomics
- Identify low level proteins using capillary columns with increased sensitivity
- Excellent for 2nd dimension of 2D-HPLC

- Column:** Jupiter 4 µm Proteo 90 Å
- Dimensions:** 150 x 0.5 mm
- Part No.:** 00F-4346-AF
- Mobile Phase:** A) 0.01 % TFA in Water
B) 0.008 % TFA in Acetonitrile
- Gradient:** A/B (95:5) for 5 min, then to A/B (55:45) in 55 minutes
- Temperature:** 40 °C
- Flow Rate:** 15 µL/min
- Detection:** UV @ 210 nm
- Sample:** HSA Tryptic Digest

Human Serum Albumin



App ID 14335

Jupiter Proteo resolves many peaks at higher efficiencies, which is critical for peptide maps with a large number of generated peptides.

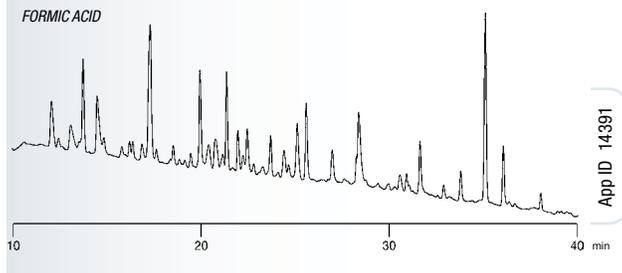
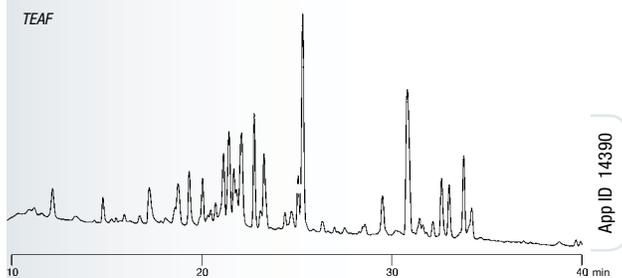
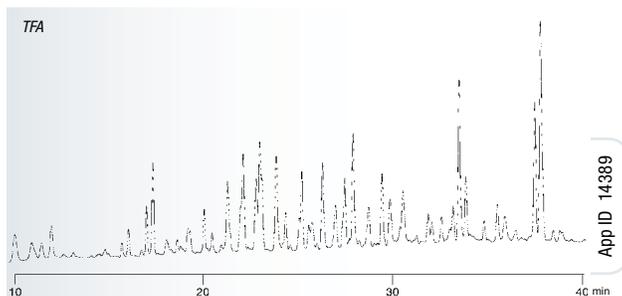
Guaranteed Performance, Lifetime, and Quality

It is difficult to compete with Jupiter standards. Jupiter is an extremely robust column with extended pH stability that undergoes rigorous quality testing and has extensive QC documentation. Each column has consistent specifications and thus consistent performance.

Long Lifetime and Method Development Opportunities

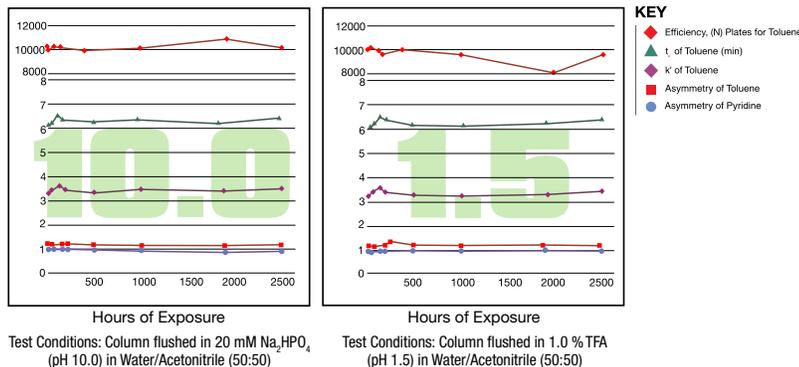
- pH 1.5 – 10 stability
- Stable for over 2500 hours at pH extremes
- Compatible with various LC/MS buffers
- Excellent resolutions down to 0.01 % TFA

Effect of LC/MS Modifiers on Selectivity

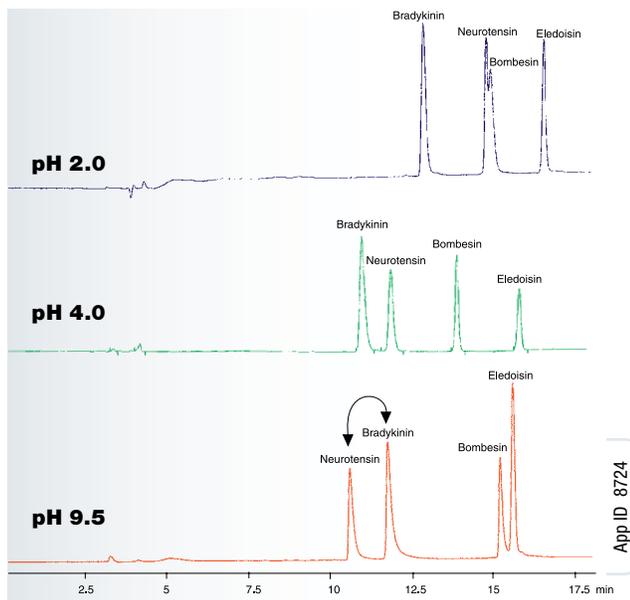


Columns: Jupiter 4 μ m Proteo 90 \AA
Dimensions: 250 x 4.6 mm
Part No.: 00G-4396-E0
Mobile Phase: A) 5 mM modifier in Water
 B) 5 mM modifier in Acetonitrile
 (see chromatograms for modifiers)
Gradient: A/B (95:5) for 5 min, then
 to A/B (55:45) in 55 minutes
Flow Rate: 1 mL/min
Temperature: 22 $^{\circ}$ C
Detection: UV @ 210 nm
Sample: Myoglobin Tryptic Digest

Stability of Jupiter 300 C18 at pH 1.5 and 10



Utilize pH for Method Development of Protein Separations



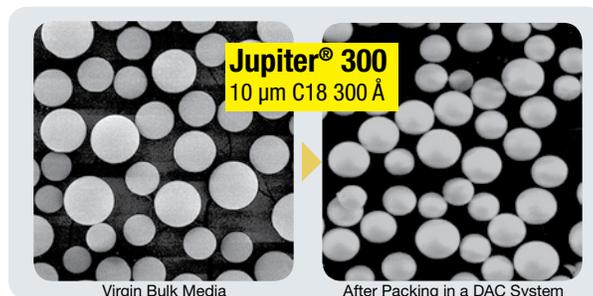
Column: Jupiter 300 5 μ m C18 300 \AA
Dimensions: 250 x 4.6 mm
Part No.: 00G-4053-E0
Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV @ 215 nm
Sample: 1. Bradykinin 3. Neurotensin
 2. Bombesin 4. Eleudoisin

Guaranteed Performance, Lifetime, and Quality (cont'd)

Easy Scale-up to Preparative Columns and Bulk Material

- Identical bonding and base silica technology used in both analytical and preparative materials
- Large loading capacity
- Resistance to silica sheering and fine formation at high packing pressures

High Mechanical Strength Silica Resists Sheering



Quality Proven

- Traceability assured throughout the manufacturing process
- Over 25 individual quality control tests performed on every batch of Jupiter material
- Materials Validation Document (MVD) accompanies every Jupiter column

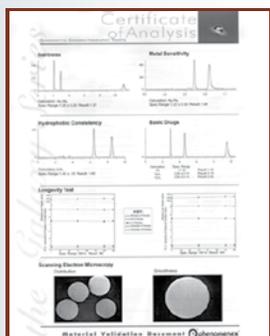
Reproducibility Assured

- Guaranteed batch-to-batch and column-to-column reproducibility
- Tight control maintained over silica particle consistency, size, and smoothness

Material Characterization and Control

Silica Physical Tests and Specifications

Pore size, particle size and distribution, metal content, surface area, carbon load and surface coverage specifications and results are all reported.

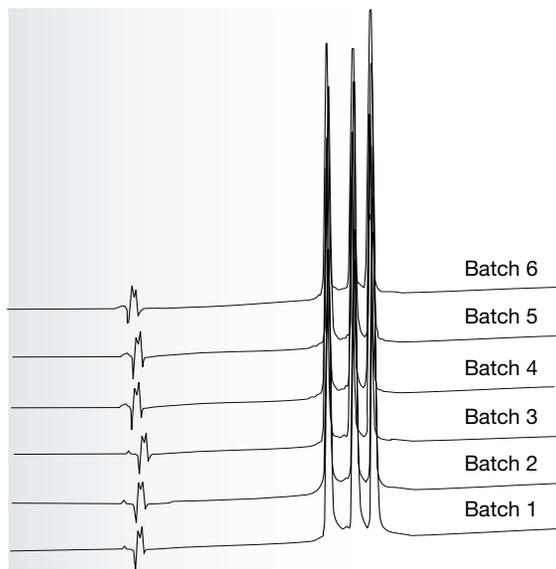


Diagnostic Chromatography Tests
Monitoring chromatographic specifications for silanol activity, hydrogen bonding capacity, hydrophobicity and peptide standards.

pH Stability
Every batch goes through pH 1.5 and 10.0 testing before release, the results of which are reported on each MVD.

SEM Analysis
Scanning Electron Microscopy (SEM) photos show surface smoothness and particle consistency as well as a visual representation of particle size distribution.

Batch-to-Batch Reproducibility of Jupiter 300 5 µm C18



App ID 16658

- Column:** Jupiter 300 5 µm C18 300 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4053-E0
- Mobile Phase:** A) 0.1 % TFA in Water
B) 0.1 % TFA in Acetonitrile
- Gradient:** A/B (75:25) to A/B (45:55) in 15 minutes
- Flow Rate:** 1 mL/min
- Temperature:** Ambient
- Detection:** UV @ 220 nm
- Sample:** 1. Equine Cytochrome c
2. Bovine Cytochrome c
3. Canine Cytochrome c

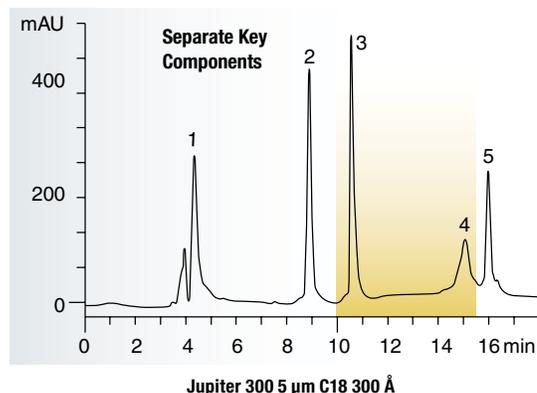
Jupiter 300 – for Intact Protein Separation and Purification

Reversed phase chromatography is a widely used HPLC technique for the separation, purification, and study of proteins as well as the discovery and development of biopharmaceuticals. The popularity of the method can be attributed to the speed and efficiencies typically achieved in its use. Jupiter 300 has proven its performance to chromatographers worldwide as a leading 300 Å solution.

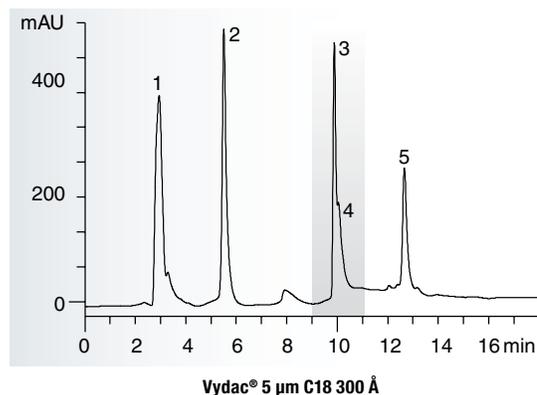
Achieve Baseline Resolution Between Proteins of Interest

- Super-smooth, high-mechanical strength silica reduces silica fine formation during the packing process ensuring highly efficient columns, thus improving resolution
- Ability to eliminate subsequent purification steps
- Easier method validation

Purify Key Proteins from One Another



App ID 14924



App ID 14925

Jupiter 300 5 µm C18 has excellent resolving power as seen above. Resolution is extremely important, especially when impurities can differ by only a few amino acids.

Columns: Jupiter 300 5 µm C18 300 Å
Vydac 5 µm C18 300 Å (238EV52-Everest)

Dimensions: 250 x 2.0 mm

Mobile Phase: A) 0.1 %TFA/ 95 % Water/ 5 % Acetonitrile
B) 0.085 % TFA/ 95 % Acetonitrile/ 5 % Water

Gradient: A/B (80:20) to A/B (15:85) in 15 minutes

Flow Rate: 0.2 mL/min

Temperature: Ambient

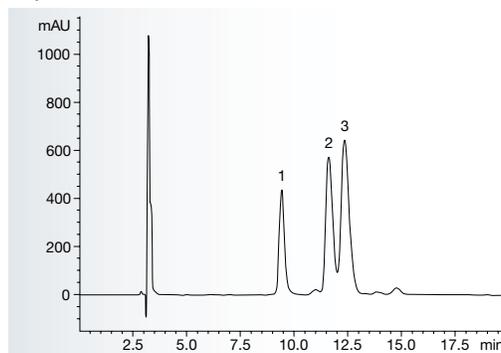
Detection: UV @ 220 nm

Sample: 1. Aprotinin 4. Fibrinogen
2. Ribonuclease 5. Leptin
3. Acid Glycoprotein

Sharp, Symmetric Peak Shape for Easier Quantitation

- Ultra-pure (99.99 %, metal-free) silica and dense bonded phase coverage provides sharp peaks for your sample by decreasing the number of non-specific interactions
- High peak efficiency, which enables separation of more components

Separation of Insulin Genetic Variants



Bovine, human, and porcine insulin, proteins with very similar structures, are purified away from each other due to the strong resolving power of Jupiter 300.

Column: Jupiter 300 5 µm C18 300 Å

Dimensions: 250 x 4.6 mm

Part No.: 00G-4053-E0

Mobile Phase: A) 0.1 % TFA in Water
B) 0.1 % TFA in Acetonitrile

Gradient: A/B (70:30) to A/B (68:32) in 20 minutes

Flow Rate: 1 mL/min

Temperature: Ambient

Detection: UV @ 210 nm

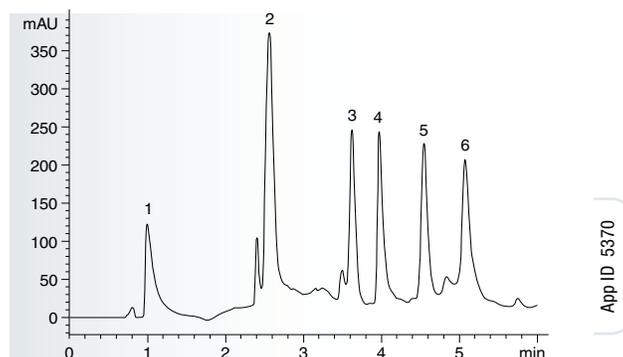
Sample: 1. Bovine Insulin
2. Human Insulin
3. Porcine Insulin

Jupiter 300 – for Intact Protein Separation and Purification (cont'd)

Proven Performance

- Sharp peaks enable improved resolution for easier quantitation
- Rugged material ensures long column lifetime and excellent reproducibility
- Easily separate complex protein mixtures

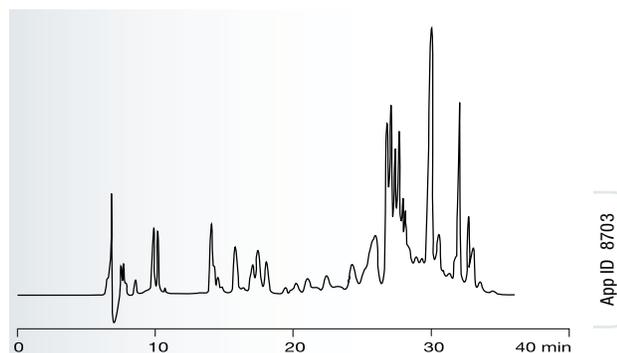
Separation of Protein Mix



Jupiter 300 is capable of separating samples with a wide mixture of proteins that vary in size, chemistry, and concentration.

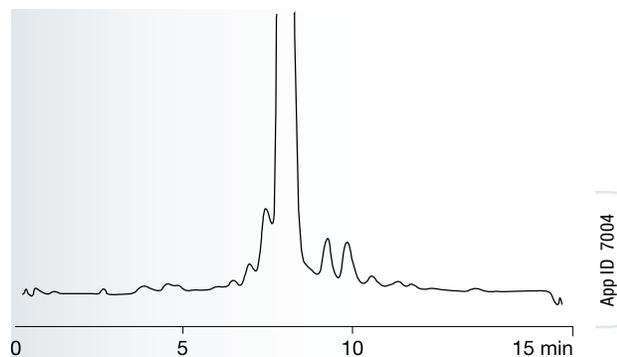
- Column:** Jupiter 300 5 μ m C4 300 Å
- Dimensions:** 50 x 4.6 mm
- Part No.:** 00B-4167-E0
- Mobile Phase:** A) 0.1 % TFA in Water
B) 0.1 % TFA in Acetonitrile
- Gradient:** a) A/B (100:0) to A/B (80:20) in 1 min (20 % B/min)
b) A/B (80:20) to A/B (65:35) in 1.5 min (10 % B/min)
c) A/B (65:35) to A/B (53.5:46.5) in 1.5 min (7.67 % B/min)
d) A/B (53.5:46.5) to A/B (53.5:46.5) for 2 min (constant B)
- Flow Rate:** 1 mL/min
- Temperature:** Ambient
- Detection:** UV @ 220 nm
- Sample:** 1. Alkaline Phosphatase 4. Insulin
2. Cyanocobalamin 5. Transferrin
3. RNase 6. Trypsin Inhibitor

Wheat Protein Extract



(Data supplied by customer. Conditions proprietary.)
Column: 5 μ m C18, 300 Å, 250 x 4.6 mm. "We purchased the Jupiter 300 C18 300 Å column a few months ago and have been quite impressed with its performance. The Jupiter 300 column provides better separation of the proteins. As for reproducibility, the control profiles have not changed since day one of its use."

Recombinant Proteins

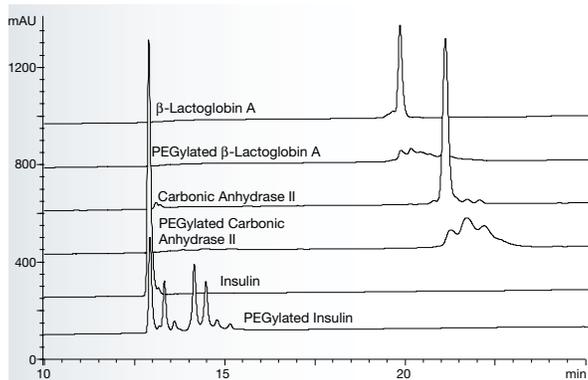


(Data supplied by customer. Conditions proprietary.)
Column: 5 μ m C4 300 Å, 250 x 4.6 mm "In comparison to another C4 column for the analysis of a recombinant protein, the Jupiter was much more rugged: typically hundreds of injections."

Jupiter 300 – for Intact Protein Separation and Purification (cont'd)

Proven Performance (cont'd)

Compare PEGylated vs. Native Forms of Proteins



App ID 16191

Reversed phase separation of PEGylated and native proteins on a Jupiter 300 C4 column. Note the good resolution of multiple PEGylated forms for all proteins tested.

Columns: Jupiter 300 5 µm C4 300 Å

Dimensions: 150 x 4.6 mm

Part No.: 00F-4167-E0

Mobile Phase: A) 2 % Acetonitrile / 0.1 % TFA in Water
B) 70 % Acetonitrile / 20 % IPA / 0.08 % TFA in Water

Gradient: A/B (85:15) to A/B (30:70) in 25 min

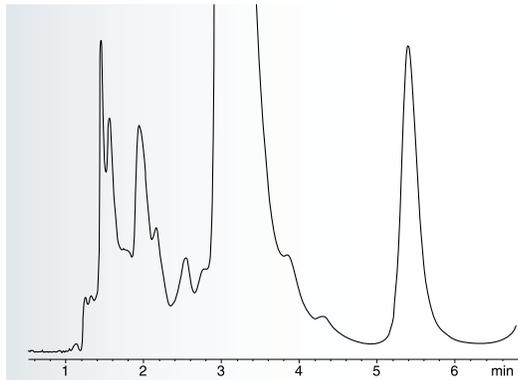
Flow Rate: 1 mL/min

Temperature: 45 °C

Detection: UV @ 214 nm

Sample: PEGylated and Native Proteins

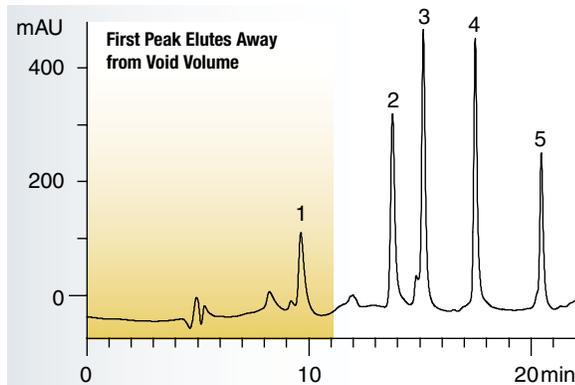
Protoporphyrins



App ID 9034

(Data supplied by customer. Conditions proprietary.) 10 µm C4, 300 Å, 150 x 4.6 mm "I found significant improvement in peak shape and symmetry. This was true not only for small peaks, but also for peaks 30 times larger as well."

Protein Mix Purification



App ID 14934

Aprotinin is typically very difficult to retain. Due to the fact that Jupiter 300 is a high surface material, it has the hydrophobic ability to pull proteins away from the void volume.

Columns: Jupiter 300 5 µm C4 300 Å

Dimensions: 250 x 2.0 mm

Part No.: 00G-4167-B0

Mobile Phase: A) 0.1 % TFA/ 95 % Water/ 5 % Acetonitrile
B) 0.085 % TFA/ 95 % Acetonitrile/ 5 % Water

Gradient: A/B (85:15) to A/B (15:85) in 21 minutes

Flow Rate: 0.2 mL/min

Temperature: Ambient

Detection: UV @ 220 nm

Sample: 1. Aprotinin
2. Ribonuclease
3. Lysozyme
4. Lactalbumin
5. Leptin

Jupiter 300 – for Intact Protein Separation and Purification (cont'd)

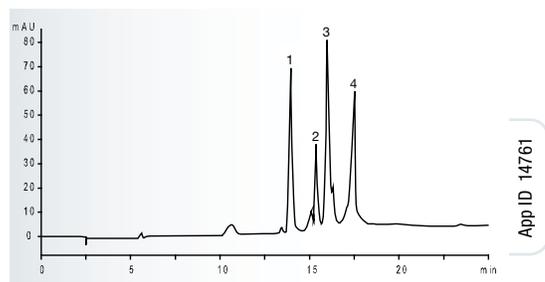
Selecting the Appropriate 300 Å Phase

Jupiter 300 C4

This low hydrophobicity phase is less likely to cause irreversible adsorption of “sticky” proteins and allows the use of shallow gradients along with lower concentrations of organic solvent.

- For proteins $\geq 10,000$ MW
- For highly hydrophobic proteins

Large Proteins on Jupiter 300 5 μ m C4



Column: Jupiter 300 5 μ m C4 300 Å

Dimensions: 150 x 4.6 mm

Part No.: 00F-4167-E0

Mobile Phase: A) 0.1 % TFA in Water
B) 0.08 % TFA in Acetonitrile

Gradient: A/B: (95:5) to A/B: (20:80) in 20 minutes

Flow Rate: 1 mL/min

Temperature: 22 °C

Detection: UV @ 280 nm

Injection Volume: 25 μ L

Sample: 1. Bovine Serum Albumin
2. Glutamic Dehydrogenase
3. β -Galactosidase
4. Ovalbumin

Jupiter 300 C5

This bonded phase imparts greater pH stability compared to traditional C4 phases. One can expect longer column lifetimes and more stable, reproducible retention times because of the bonded phase's increased stability to hydrolysis.

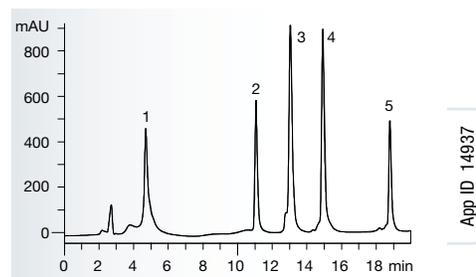
- For proteins $\geq 10,000$ MW
- For highly hydrophobic proteins
- More retentive than C4, offering slightly different selectivity

Jupiter 300 C18

Excellent for polar as well as non-polar proteins; most retentive of Jupiter 300 phases, allowing one to separate proteins with slight differences in hydrophobicity.

- For proteins $\leq 10,000$ MW
- For hydrophilic proteins
- Most retentive phase

Separation on Jupiter 300 5 μ m C18



Column: Jupiter 300 5 μ m C18 300 Å

Dimensions: 150 x 2.0 mm

Part No.: 00F-4053-B0

Mobile Phase: A) 0.1 %TFA/ 95 % Water / 5 % Acetonitrile
B) 0.085 % TFA/ 95 % Acetonitrile/ 5 % Water

Gradient: A/B (88:12) to A/B (15:85) in 21 minutes

Flow Rate: 0.2 mL/min

Temperature: Ambient

Detection: UV @ 220 nm

Sample: 1. Aprotinin
2. Ribonuclease
3. Lysozyme
4. Lactalbumin
5. Leptin

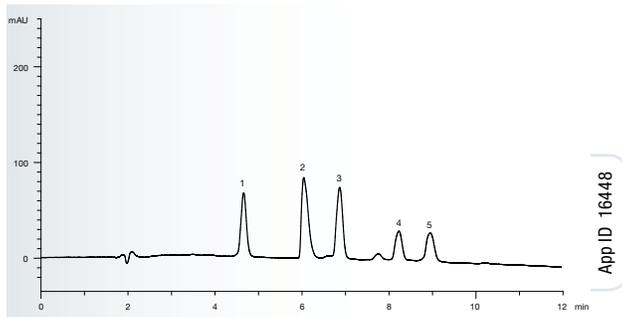
Jupiter 300 – for Intact Protein Separation and Purification (cont'd)

Selecting a Suitable Particle Size

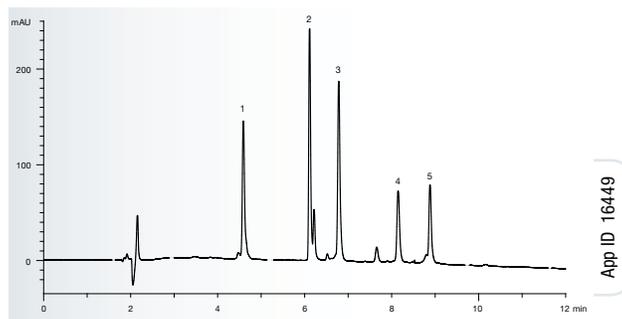
Jupiter 300 3 μm

- Most efficient material currently available in C18 phase
- Ensures sharp, symmetric peaks resulting in excellent resolution
- Recommended when 5 μm materials don't give adequate baseline separation or peak shape

Separation of Protein Mixture on 3 μm and 5 μm Materials



App ID 16448



App ID 16449

Though the Jupiter 5 μm material provides good separation and peak shape, the 3 μm material dramatically improves resolution, sensitivity, and peak shape. This is critical when proteins closely elute.

Columns: Jupiter 300 5 μm C18 300 Å
Jupiter 300 3 μm C18 300 Å

Dimensions: 150 x 4.6 mm

Part No.: 00F-4053-E0
00F-4263-E0

Mobile Phase: A) 0.1 % TFA and 5 % Acetonitrile in Water
B) 0.08 % TFA and 90 % Acetonitrile in Water

Gradient: A/B (80:20) to A/B (15:85) in 15 min (2 % B/min)

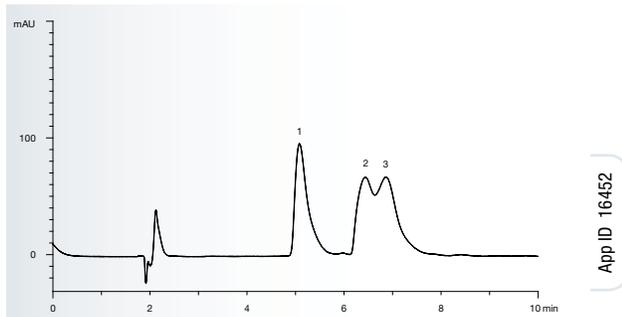
Flow Rate: 1 mL/min

Temperature: 40 °C

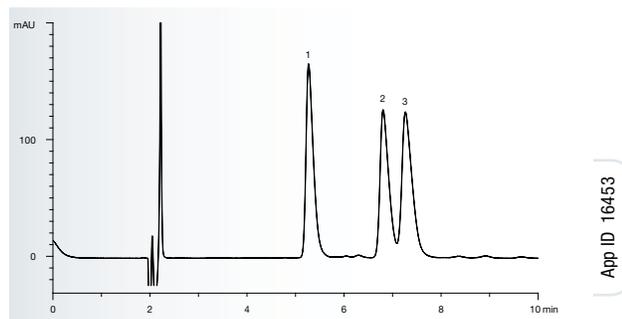
Detection: UV @ 214 nm

Sample: 1. Ribonuclease A
2. Bovine Insulin
3. Lysozyme
4. Trypsin Inhibitor
5. β -Lactoglobulin A

Insulin Purification on 3 μm and 5 μm Materials



App ID 16452



App ID 16453

The highly efficient 3 μm material is able to fully resolve genetic variants of insulin, which is sometimes difficult to resolve on 5 μm materials.

Columns: Jupiter 300 5 μm C18 300 Å
Jupiter 300 3 μm C18 300 Å

Dimensions: 150 x 4.6 mm

Part No.: 00F-4053-E0
00F-4263-E0

Mobile Phase: A) 0.1 % TFA and 5 % Acetonitrile in Water
B) 0.08 % TFA and 90 % Acetonitrile in Water

Gradient: A/B (70:30) to A/B (68:32) in 15 min

Flow Rate: 1 mL/min

Detection: UV @ 214 nm

Sample: 1. Bovine Insulin
2. Human Insulin
3. Porcine Insulin

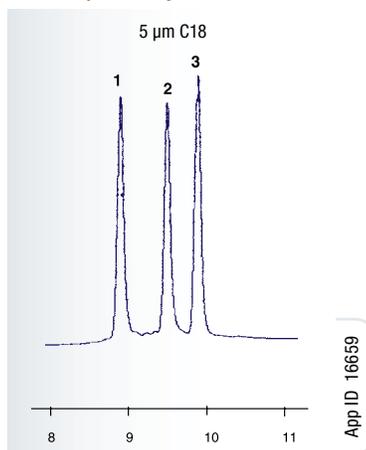
Jupiter 300 – for Intact Protein Separation and Purification *(cont'd)*

Selecting a Suitable Particle Size *(cont'd)*

Jupiter 300 5 µm

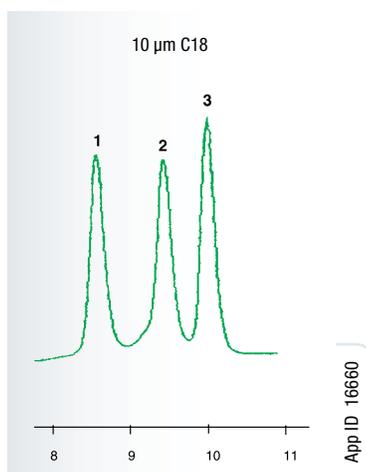
- Designed for optimized combination of efficiency and low backpressure
- High efficiency material yields good resolution for most assays
- Recommended for majority of separations on analytical and capillary scale

Scale-Up Quickly Between Particle Sizes



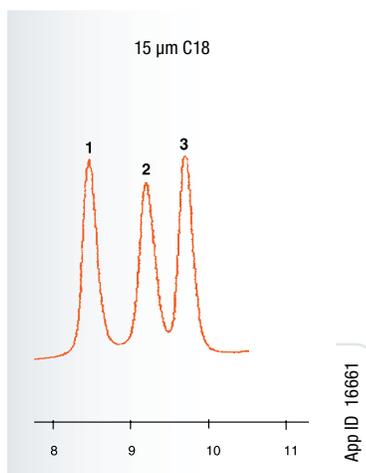
Jupiter 300 10 µm

- Designed for preparative applications
- Highest efficiency for preparative applications
- Recommended when maximum resolution is required on the prep scale



Jupiter 300 15 µm

- Designed for preparative applications
- Best combination of performance and economy
- Recommended for most preparative/process applications



Conditions same for all columns:

- Dimensions:** 250 x 4.6 mm
- Mobile Phase:** A) 0.1 % TFA in Water
B) 0.1 % TFA in Acetonitrile
- Gradient:** A/B (75:25) to A/B (45:55) in 15 min
- Flow Rate:** 1 mL/min
- Detection:** UV @ 220 nm
- Sample:** 1. Equine Cytochrome *c*
2. Bovine Cytochrome *c*
3. Canine Cytochrome *c*

Scaling up from 5 µm to preparative grades is easier due to the fact that the larger particle sizes are identical to the 5 µm material and are not prep-variants.

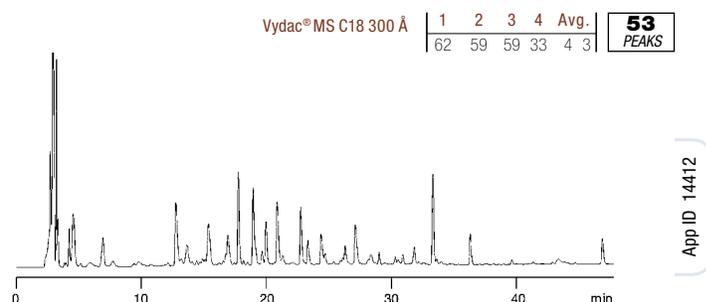
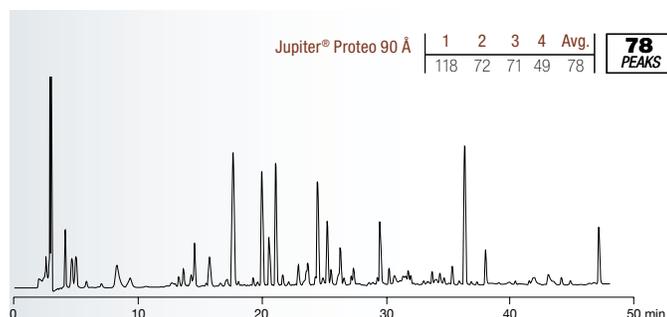
Jupiter Proteo – for Peptide Mapping and Peptide Purification

Traditionally, 300 Å columns were used for peptide purification and mapping. 300 Å silica materials are excellent tools for intact proteins, but do not provide the column resolution and peak capacity required for peptide mapping. Jupiter Proteo breaks all traditions and optimizes column parameters to provide optimal performance for peptides. A 90 Å, high surface area silica maximizes stationary phase interaction, a 4 µm particle provides high column efficiencies, and a unique C12 bonded phase with proprietary endcapping sharpens peak symmetry and increases peak capacity.

Identify More Peaks with Increased Peak Capacity

- 4 µm particles produce column efficiencies similar to 3 µm materials, but backpressure of 5 µm particles
- C12 phase bonded onto an ultra-high surface area (475 m²/g) silica increases bonded phase/sample interaction and column capacity
- Less peak tailing, due to endcapping, means sharper peaks and better separation between closely eluting peptides

Myoglobin Tryptic Digest



Due to the unique material characteristics of Jupiter Proteo, peak capacity increases by 40-60 %. An increase in capacity is essential to identifying more peptides in enzymatic digest samples.

Columns: Jupiter 4 µm Proteo 90 Å
Vydac 5 µm MS C18 300 Å
Zorbax 5 µm SB-C18 300 Å

Dimensions: 250 x 4.6 mm

Part No.: 00G-4167-E0

Mobile Phase: A) 0.012 % TFA in Water
B) 0.01 % TFA in Acetonitrile

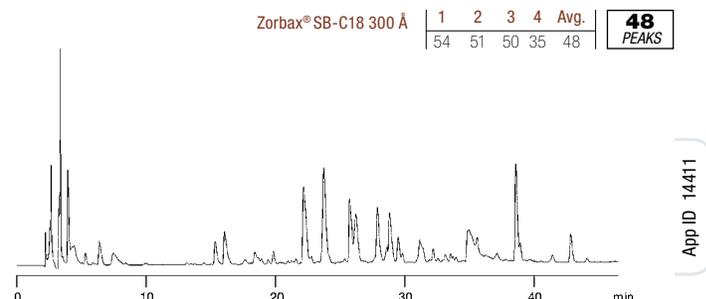
Gradient: A/B (95:5) for 5 min, then to A/B (60:40) in 55 minutes

Flow Rate: 1 mL/min

Temperature: 22 °C

Detection: UV @ 210 nm

Sample: Myoglobin Tryptic Digest



Method	Threshold	Peak Width	Min Area	Min Height
1	1.0	0.1	10.0	20.0
2	2.0	0.2	10.0	20.0
3	3.0	0.3	20.0	10.0
4	3.0	0.3	20.0	50.0

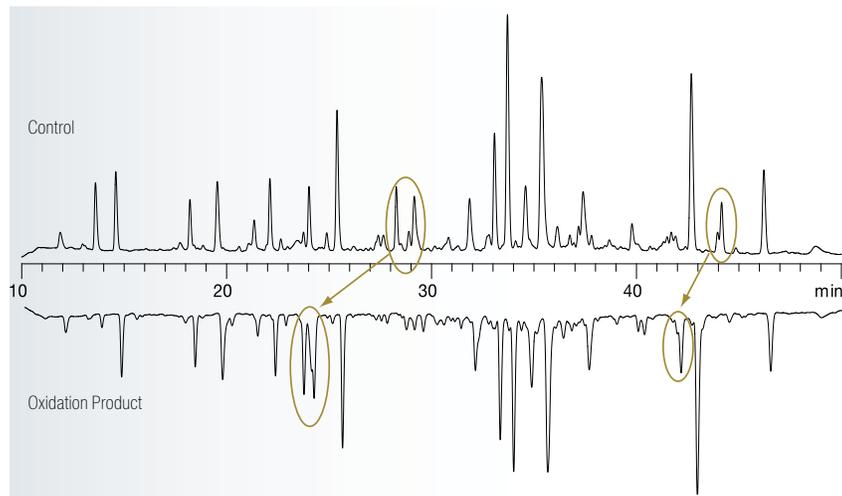
Determining peak counts - The large number of peaks in a given tryptic digest makes counting peaks visually both inaccurate and subjective. For a more accurate approach, peak counting was performed using Agilent Technologies (HP) ChemStation™ software. Four different integration parameters at different sensitivity settings were used in calculating the number of peaks and an average. The parameters changed within each method were: minimum peak area, minimum peak height, peak width, and threshold. The table describes the parameters used for each calculation.

Jupiter Proteo – for Peptide Mapping and Peptide Purification (cont'd)

Better Identification of Post-translational Modifications (PTMs)

- Better resolution enables easier identification of shifts in peak location to determine if a PTM has occurred
- 90 Å, 4 µm, C12 Jupiter Proteo materials are engineered for peptide mapping and peak identification

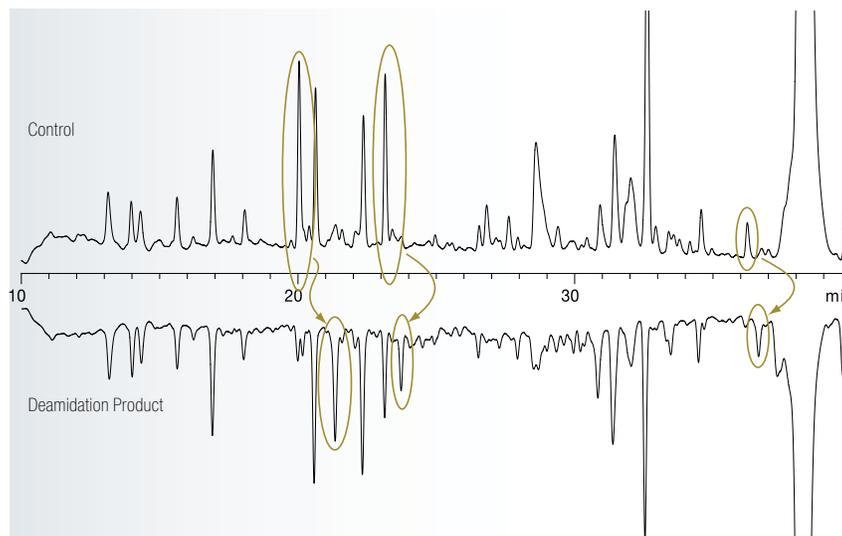
Oxidation of β-Lactoglobulin



Oxidation is commonly seen with methionine due to its readily oxidized sulfur group. A tryptic digest of β-Lactoglobulin on Jupiter Proteo easily reveals retention time changes due to the oxidation of methionine containing peptides.

- Columns:** Jupiter 4 µm Proteo 90 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4396-E0
- Mobile Phase:** A) 0.012 % TFA in Water
B) 0.01 % TFA in Acetonitrile
- Gradient:** A/B (95:5) for 5 min, then to A/B (60:40) in 55 minutes
- Flow Rate:** 1 mL/min
- Temperature:** 22 °C
- Detection:** UV @ 210 nm
- Sample:** Top Chromatogram – β-Lactoglobulin tryptic digest
Bottom Chromatogram – Oxidized β-Lactoglobulin tryptic digest

Deamidation of RNase



Deamidation of asparagine to aspartic acid and glutamine to glutamic acid can occur in protein products. A tryptic digest of RNase shows several examples of deamidation.

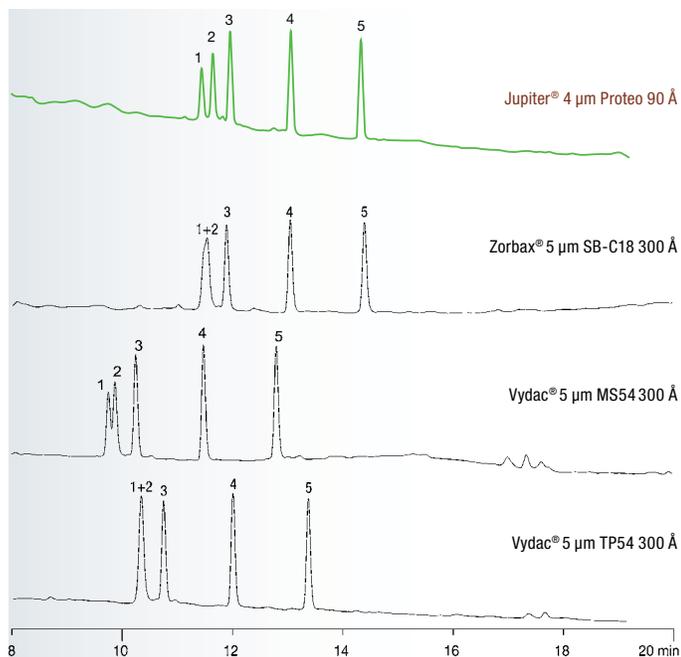
- Columns:** Jupiter 4 µm Proteo 90 Å
- Dimensions:** 250 x 4.6 mm
- Part No.:** 00G-4396-E0
- Mobile Phase:** A) 0.012 % TFA in Water
B) 0.01 % TFA in Acetonitrile
- Gradient:** A/B (95:5) for 5 min, then to A/B (60:40) in 55 min
- Flow Rate:** 1 mL/min
- Temperature:** 22 °C
- Detection:** UV @ 210 nm
- Sample:** Top Chromatogram – RNase tryptic digest
Bottom Chromatogram – Deamidated RNase tryptic digest

Jupiter Proteo – for Peptide Mapping and Peptide Purification (cont'd)

Selectivity to Improve Resolution

- Jupiter Proteo is engineered with respect to efficiency, selectivity, and resolution to effectively purify peptides
- Resolve peptides of only 1-2 amino acid differences
- Purify contaminants from target peak

Resolve Peptides with Similar Hydrophobicity



Jupiter Proteo is able to fully resolve peptides that differ in hydrophobicity by one methyl group.

Columns: Jupiter 4 µm Proteo 90 Å
 Zorbax 5 µm SB-C18 300 Å
 Vydac 5 µm MS54 300 Å
 Vydac 5 µm TP54 300 Å

Dimensions: 250 x 4.6 mm

Part No.: 00G-4167-E0

Mobile Phase: A) 0.1 % TFA in Water
 B) 0.085 % TFA in Acetonitrile

Gradient: A/B (95:5) to A/B (55:45) in 20 minutes

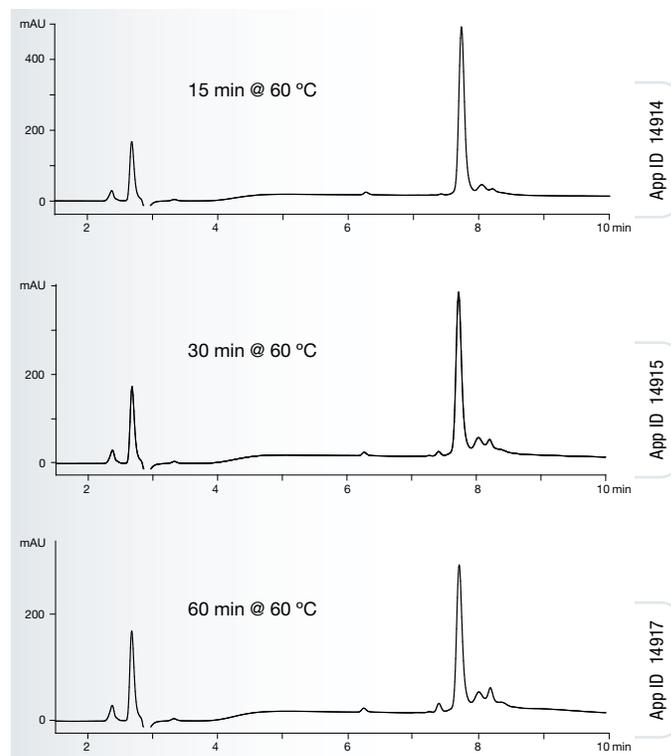
Flow Rate: 1 mL/min

Temperature: 22 °C

Detection: UV @ 214 nm

Sample: 1. NH₂-Arg-Gly-Gly-Ala-Gly-Gly-Leu-Gly-Leu-Gly-Lys-Amide
 2. Ac-Arg-Gly-Gly-Gly-Gly-Gly-Leu-Gly-Leu-Gly-Lys-Amide
 3. Ac-Arg-Gly-Ala-Gly-Gly-Gly-Leu-Gly-Leu-Gly-Lys-Amide
 4. Ac-Arg-Gly-Val-Gly-Gly-Gly-Leu-Gly-Leu-Gly-Lys-Amide
 5. Ac-Arg-Gly-Val-Val-Gly-Gly-Leu-Gly-Leu-Gly-Lys-Amide

Insulin Purified from its Degradants



HPLC separations on Jupiter Proteo at different time points of insulin incubated under basic conditions at 60 °C. Time points were taken at 15, 30, and 60 minutes. As seen above, there was an increase of numerous degradation products with extended exposure at elevated temperature that Jupiter Proteo was able to separate.

Columns: Jupiter 4 µm Proteo 90 Å

Dimensions: 250 x 4.6 mm

Part No.: 00G-4396-E0

Mobile Phase: A) 0.1 % TFA in Water
 B) 0.085 % TFA in 95:5 Acetonitrile/Water

Gradient: A/B (80:20) to A/B (20:80) in 15 minutes

Flow Rate: 1 mL/min

Detection: UV @ 220 nm

Sample: Human Insulin

Material Characteristics

Packing Material	Particle Shape/Size (µm)	Pore Size (Å)	Pore Volume (mL/g)	Surface Area (m ² /g)	Carbon Load %	Calculated Bonded Phase Coverage (µmole/m ²)	End Capping
C4	Spher. 5, 10, 15	300	—	170	5.0	6.30	Yes
C5	Spher. 5, 10, 15	300	—	170	5.5	5.30	Yes
C18	Spher. 3, 5, 10, 15	300	—	170	13.3	5.50	Yes
Proteo	Spher. 4, 10	90	—	475	15.0	—	Yes



If Jupiter does not provide you with at least an equivalent separation as compared to a column of similar phase, particle size and dimension, send in your comparative data within 45 days and keep the Jupiter column for FREE.

ORDERING INFORMATION

4 µm & 5 µm Capillary Columns (mm)

	50 x 0.30	150 x 0.30	250 x 0.30	50 x 0.50	150 x 0.50	250 x 0.50
Phases						
5 µm C4 300 Å	00B-4167-AC	00F-4167-AC	00G-4167-AC	00B-4167-AF	00F-4167-AF	00G-4167-AF
5 µm C18 300 Å	00B-4053-AC	00F-4053-AC	00G-4053-AC	00B-4053-AF	00F-4053-AF	00G-4053-AF
4 µm Proteo 90 Å	00B-4396-AC	00F-4396-AC	00G-4396-AC	00B-4396-AF	00F-4396-AF	00G-4396-AF

**SecurityGuard™ Analytical Cartridges require holder, Part No.: KJO-4282*
†SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-7220
***PREP SecurityGuard™ Cartridges require holder, Part No.: AJ0-8223*
**PREP SecurityGuard™ Cartridges require holder, Part No.: AJ0-8277*

3 µm, 4 µm & 5 µm Microbore and Minibore Columns (mm)

	50 x 1.0	150 x 1.0	250 x 1.0	50 x 2.0	150 x 2.0	250 x 2.0	SecurityGuard™ Cartridges (mm)
Phases							4 x 2.0* /10 pk
5 µm C4 300 Å	00B-4167-A0	00F-4167-A0	00G-4167-A0	00B-4167-B0	00F-4167-B0	00G-4167-B0	AJO-4329
5 µm C5 300 Å	00B-4052-A0	00F-4052-A0	00G-4052-A0	00B-4052-B0	00F-4052-B0	00G-4052-B0	AJO-4326
5 µm C18 300 Å	00B-4053-A0	00F-4053-A0	00G-4053-A0	00B-4053-B0	00F-4053-B0	00G-4053-B0	AJO-4320
4 µm Proteo 90 Å	00B-4396-A0	00F-4396-A0	00G-4396-A0	00B-4396-B0	00F-4396-B0	00G-4396-B0	AJO-6073
							/10 pk
3 µm C18 300 Å	—	—	—	00B-4263-B0	00F-4263-B0	—	AJO-4320

for ID: 2.0-3.0 mm

3 µm, 4 µm & 5 µm Analytical and Preparative Columns (mm)

	50 x 4.6	150 x 4.6	250 x 4.6	250 x 10	250 x 21.2	SecurityGuard™ Cartridges (mm)
Phases						4 x 3.0* /10 pk
						10 x 10† /3 pk
						15 x 21.2** /ea
5 µm C4 300 Å	00B-4167-E0	00F-4167-E0	00G-4167-E0	00G-4167-N0	00G-4167-P0	AJO-4330
5 µm C5 300 Å	00B-4052-E0	00F-4052-E0	00G-4052-E0	00G-4052-N0	00G-4052-P0	AJO-4327
5 µm C18 300 Å	00B-4053-E0	00F-4053-E0	00G-4053-E0	00G-4053-N0	00G-4053-P0	AJO-4321
4 µm Proteo 90 Å	00B-4396-E0	00F-4396-E0	00G-4396-E0	00G-4396-N0	00G-4396-P0	AJO-6074
						/10 pk
3 µm C18 300 Å	—	00F-4263-E0	00G-4263-E0	—	—	AJO-4321

for ID: 3.2-8.0 mm

9-16 mm

18-29 mm

10 µm Analytical and Preparative Columns (mm)

	250 x 4.6	250 x 10	250 x 21.2	250 x 30	250 x 50	SecurityGuard™ Cartridges (mm)
Phases						4 x 3.0* /10 pk
						10 x 10† /3 pk
						15 x 21.2** /ea
						15 x 30.0‡ /ea
C4 300 Å	00G-4168-E0	00G-4168-N0	00G-4168-P0	00G-4168-U0	00G-4168-V0	AJO-4330
C5 300 Å	00G-4054-E0	00G-4054-N0	00G-4054-P0	—	00G-4054-V0	AJO-4327
C18 300 Å	00G-4055-E0	00G-4055-N0	00G-4055-P0	00G-4055-U0	00G-4055-V0	AJO-4321
Proteo 90 Å	00G-4397-E0	00G-4397-N0	00G-4397-P0	00G-4397-U0	00G-4397-V0	AJO-6074

for ID: 3.2-8.0 mm

9-16 mm

18-29 mm

30-49 mm

15 µm Analytical and Preparative Columns (mm)

	250 x 4.6	250 x 10	250 x 21.2	250 x 30	250 x 50	SecurityGuard™ Cartridges (mm)
Phases						4 x 3.0* /10 pk
						10 x 10† /3 pk
						15 x 21.2** /ea
						15 x 30.0‡ /ea
C4 300 Å	00G-4169-E0	00G-4169-N0	00G-4169-P0	00G-4169-U0	00G-4169-V0	AJO-4330
C5 300 Å	00G-4056-E0	00G-4056-N0	00G-4056-P0	—	00G-4056-V0	AJO-4327
C18 300 Å	00G-4057-E0	00G-4057-N0	00G-4057-P0	00G-4057-U0	00G-4057-V0	AJO-4321

for ID: 3.2-8.0 mm

9-16 mm

18-29 mm

30-49 mm

Other Dimensions available upon request.
 Bulk Material available.

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Ordering Information

Membrane Type/Size	4 mm Diameter for < 2 mL sample volumes			15 mm Diameter for 2 - 10 mL sample volumes			25 mm Diameter for 10 - 100 mL sample volumes		
	Part No.	Unit	Price	Part No.	Unit	Price	Part No.	Unit	Price
0.45 µm									
Phenex-RC (Regenerated Cellulose)	AF0-3103-12	100/Pk		AF0-2103-12	100/Pk		AF0-8103-12 ⁵	100/Pk	
	AF0-3103-52	500/Pk		AF0-2103-52	500/Pk		AF0-8103-52 ⁵	500/Pk	
Phenex-PES ³ (Polyethersulfone)	—	—		AF2-5108-12 ¹	100/Pk		AF0-8108-12 ⁵	100/Pk	
	—	—		—	—		AF0-8108-52 ⁵	500/Pk	
Phenex-PTFE ⁶ (Polytetrafluoroethylene)	AF0-3102-12	100/Pk		AF0-2102-12	100/Pk		AF0-1102-12	100/Pk	
	AF0-3102-52	500/Pk		AF0-2102-52	500/Pk		AF0-1102-52	500/Pk	
Phenex-NY (Nylon)	AF3-3107-12	100/Pk		AF2-5107-12 ¹	100/Pk		AF0-1107-12	100/Pk	
	AF3-3107-52	500/Pk		AF2-5107-52 ¹	500/Pk		AF0-1107-52	500/Pk	
Phenex-GF/CA ^{2,3,4,9} (Glass Fiber/Cellulose Acetate)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a CA membrane. Excellent for filtration of tissue culture media, general biological sample filtration and clarification.						AF0-8B09-12	100/Pk	
							AF0-8B09-52	500/Pk	
0.20 µm									
Phenex-RC (Regenerated Cellulose)	AF0-3203-12	100/Pk		AF0-2203-12	100/Pk		AF0-8203-12 ⁵	100/Pk	
	AF0-3203-52	500/Pk		AF0-2203-52	500/Pk		AF0-8203-52 ⁵	500/Pk	
Phenex-PES ³ (Polyethersulfone)	—	—		—	—		AF0-8208-12 ⁵	100/Pk	
	—	—		—	—		AF0-8208-52 ⁵	500/Pk	
Phenex-PTFE ⁶ (Polytetrafluoroethylene)	AF0-3202-12	100/Pk		AF0-2202-12	100/Pk		AF0-1202-12	100/Pk	
	AF0-3202-52	500/Pk		AF0-2202-52	500/Pk		AF0-1202-52	500/Pk	
Phenex-NY (Nylon)	AF3-3207-12	100/Pk		AF2-5207-12 ¹	100/Pk		AF0-1207-12	100/Pk	
	AF3-3207-52	500/Pk		AF2-5207-52 ¹	500/Pk		AF0-1207-52	500/Pk	
Phenex-GF/CA ^{2,3,4,9} (Glass Fiber/Cellulose Acetate)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a CA membrane. Excellent for filtration of tissue culture media, general biological sample filtration and clarification.						AF0-8A09-12	100/Pk	
							AF0-8A09-52	500/Pk	
1.20 µm									
Phenex-GF ² (Glass Fiber)	Prefiltration of heavily contaminated or highly viscous samples. When used in-line preceding a membrane filter, clogging of the membrane filter is prevented and sample clean up is optimized.						AF0-8505-12	100/Pk	
							AF0-8505-52	500/Pk	



1. 17 mm diameter.
2. Glass fiber filters are 26 mm diameter and made of borosilicate. They will remove 90 % of all particles >1.2 µm.
3. Housing material is methacrylate butadiene styrene (MBS) polymerisate. Also known as Cryolite.
4. Cellulose acetate is surfactant-free.
5. 26 mm diameter.
6. Hydrophobic membrane. Can be made hydrophilic by pre-wetting with IPA.
7. Additional dimensions and membrane types are available. Please contact your local Phenomenex technical consultant or distributor for availability or assistance.
8. Larger quantity purchases at significant savings are available.
9. Surfactant-free



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