Reversed Phase HPLC Solutions for Proteins and Peptides
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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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identify, Purify, and Analyze</td>
<td>1</td>
</tr>
<tr>
<td>Dependable Solutions</td>
<td>2</td>
</tr>
<tr>
<td>Guaranteed Performance, Lifetime, and Quality</td>
<td>3-4</td>
</tr>
<tr>
<td>Jupiter 300 – for Intact Protein Separation and Purification</td>
<td>5-10</td>
</tr>
<tr>
<td>Jupiter Proteo – for Peptide Mapping and Peptide Purification</td>
<td>11-13</td>
</tr>
<tr>
<td>Material Characteristics</td>
<td>14</td>
</tr>
<tr>
<td>Ordering Information</td>
<td>14</td>
</tr>
</tbody>
</table>
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 ≥ 10,000 MW
- Available with C18, C5, and C4 bonded phases
- Excellent peak shape and resolution of protein samples

### 90 Å column engineered for peptide mapping and peptide separations
- Separation of proteins and peptides ≤ 10,000 MW
- Identify post-translational modifications
- Increased peak capacity and resolution

### Purify Intact Proteins

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

- **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 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

### Peptide Maps of Cytochrome c Tryptic Digests

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 µm 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) for 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

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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

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

Analyze Reduced IgG
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

Purify Away Degradants
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

Proteins and Peptides

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

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 (ambient)
Sample: HSA Tryptic Digest

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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

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Column</th>
<th>Dimensions</th>
<th>Part No.</th>
<th>Mobile Phase</th>
<th>Gradient</th>
<th>Flow Rate</th>
<th>Temperature</th>
<th>Detection</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA</td>
<td>Jupiter 4 µm Proteo 90 Å</td>
<td>250 x 4.6 mm</td>
<td>00G-4396-E0</td>
<td>A) 5 mM modifier in Water, B) 5 mM modifier in Acetonitrile (see chromatograms for modifiers)</td>
<td>A/B (95:5, 5 µL/min) for 5 min, then to A/B (55:45, 5 µL/min) in 55 minutes</td>
<td>1 mL/min</td>
<td>22 °C</td>
<td>UV @ 210 nm</td>
<td>Myoglobin Tryptic Digest</td>
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</tbody>
</table>

Stability of Jupiter 300 C18 at pH 1.5 and 10

Test Conditions: Column flushed in 20 mM Na₂HPO₄ (pH 10.0) in Water/Acetonitrile (50:50) for 2500 hours at pH extremes.

Utilize pH for Method Development of Protein Separations

- pH 2.0
- pH 4.0
- pH 9.5

Column: Jupiter 300 5 µm C18 300 Å
Dimensions: 250 x 4.6 mm
Part No.: 00G-4053-E0
Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV @ 215 nm

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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

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

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.

High Mechanical Strength Silica Resists Sheering

Jupiter® 300
10 µm C18 300 Å

Virgin Bulk Media
After Packing in a DAC System

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

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

- 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 (ambient)
- Sample:
  1. Equine Cytochrome c
  2. Bovine Cytochrome c
  3. Canine Cytochrome c

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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

**Sharp, Symmetric Peaks 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

**Purify Key Proteins from One Another**

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

**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

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Jupiter 300 – for Intact Protein Separation and Purification

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
2. Ribonuclease
3. Acid Glycoprotein
4. Fibrinogen
5. Leptin

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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 µm 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
  2. Cyanocobalamin
  3. RNase
  4. Insulin
  5. Transferrin
  6. Trypsin Inhibitor

Wheat Protein Extract

(Data supplied by customer. Conditions proprietary.)
Column: 5 µm 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 µm 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.”

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Proven Performance (cont’d)

Compare PEGylated vs. Native Forms of Proteins

![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.](image)

Columns: Jupiter 300 5 μm C4 300 Å  
Dimensions: 150 x 4.6 mm  
Part No.: 00F-4167-EO  
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

![Protoporphyrins](image)

(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

![Protein Mix Purification](image)

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. Lysosome  
4. Lactalbumin  
5. Leptin

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**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 ≥ 10,000 MW
- For highly hydrophobic proteins

**Large Proteins on Jupiter 300 5 µm C4**

**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 ≤ 10,000 MW
- For hydrophilic proteins
- Most retentive phase

**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 ≥ 10,000 MW
- For highly hydrophobic proteins
- More retentive than C4, offering slightly different selectivity

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**Separation on Jupiter 300 5 µm C18**

**Column:** Jupiter 300 5 µm 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

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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**

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.

**Insulin Purification on 3 µm and 5 µm Materials**

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-ED
00F-4263-ED

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

**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

**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-ED
00F-4263-ED

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

**Jupiter 300 5 µm**
- Designed for optimized combination of efficiency and low backpressure
- High efficiency materials yields good resolution for most assays
- Recommended for majority of separations on analytical and capillary scale

**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

Scale up quickly between particle sizes is easier due to the fact that the larger particle sizes are identical to the 5 µm material and are not prep-variants.
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 a 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**

- Jupiter® Proteo 90 Å
- Vydac® MS C18 300 Å
- Zorbax® SB-C18 300 Å

**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

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<tr>
<th>Method</th>
<th>Threshold</th>
<th>Peak Width</th>
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<td>3.0</td>
<td>0.3</td>
<td>20.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

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.
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.

**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.
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 4 µm 90 Å
Zorbax® 5 µm SB-C18 300 Å
Vydac® 5 µm MS54 300 Å
Vydac® 5 µm TP54 300 Å

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

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
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
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:  

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 (ambient)

Sample:  Human Insulin
## Material Characteristics

<table>
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<tr>
<th>Packing Material</th>
<th>Particle Shape/Size (μm)</th>
<th>Pore Size (Å)</th>
<th>Pore Volume (mL/g)</th>
<th>Surface Area (m²/g)</th>
<th>Carbon Load (%)</th>
<th>Calculated Bonded Phase Coverage (μmol/m²)</th>
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<td>Spher. 5, 10, 15</td>
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<td>Spher. 5, 10, 15</td>
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<td>—</td>
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<td>C18</td>
<td>Spher. 3, 5, 10, 15</td>
<td>300</td>
<td>—</td>
<td>170</td>
<td>13.3</td>
<td>5.50</td>
<td>Yes</td>
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<tr>
<td>Protoo</td>
<td>Spher. 4, 10</td>
<td>90</td>
<td>—</td>
<td>475</td>
<td>15.0</td>
<td>—</td>
<td>Yes</td>
</tr>
</tbody>
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## ORDERING INFORMATION

### 4 μm & 5 μm Capillary Columns (mm)

<table>
<thead>
<tr>
<th>Phases</th>
<th>SecurityGuard™ Cartridges (mm)</th>
<th>/pk</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μm C4 300 Å</td>
<td>00B-4167-AC, 00G-4167-AC, 00B-4167-AF, 00F-4167-AF</td>
<td>/0 pk</td>
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<tr>
<td>5 μm C18 300 Å</td>
<td>00B-4053-AC, 00G-4053-AC, 00B-4053-AF, 00F-4053-AF</td>
<td>/0 pk</td>
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<tr>
<td>4 μm Protoo 90 Å</td>
<td>00B-4396-AC, 00G-4396-AC, 00B-4396-AF, 00F-4396-AF</td>
<td>/0 pk</td>
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### 3 μm, 4 μm & 5 μm Microbore and Minibore Columns (mm)

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<th>Phases</th>
<th>SecurityGuard™ Cartridges (mm)</th>
<th>/pk</th>
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<tbody>
<tr>
<td>5 μm C4 300 Å</td>
<td>00B-4167-A0, 00G-4167-A0, 00B-4167-B0, 00F-4167-B0</td>
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<tr>
<td>5 μm C5 300 Å</td>
<td>00B-4052-A0, 00G-4052-A0, 00B-4052-B0, 00F-4052-B0</td>
<td>/10 pk</td>
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<tr>
<td>5 μm C18 300 Å</td>
<td>00B-4053-A0, 00G-4053-A0, 00B-4053-B0, 00F-4053-B0</td>
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</tr>
<tr>
<td>4 μm Protoo 90 Å</td>
<td>00B-4396-A0, 00G-4396-A0, 00B-4396-B0, 00F-4396-B0</td>
<td>/10 pk</td>
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### 3 μm, 4 μm & 5 μm Analytical and Preparative Columns (mm)

<table>
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<tr>
<th>Phases</th>
<th>SecurityGuard™ Cartridges (mm)</th>
<th>/pk</th>
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</thead>
<tbody>
<tr>
<td>5 μm C4 300 Å</td>
<td>00B-4167-E0, 00G-4167-E0, 00B-4167-N0, 00F-4167-P0</td>
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<tr>
<td>5 μm C5 300 Å</td>
<td>00B-4052-E0, 00G-4052-E0, 00B-4052-N0, 00F-4052-P0</td>
<td>/10 pk</td>
</tr>
<tr>
<td>5 μm C18 300 Å</td>
<td>00B-4053-E0, 00G-4053-E0, 00B-4053-N0, 00F-4053-P0</td>
<td>/10 pk</td>
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<tr>
<td>4 μm Protoo 90 Å</td>
<td>00B-4396-E0, 00G-4396-E0, 00B-4396-N0, 00F-4396-P0</td>
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### 10 μm Analytical and Preparative Columns (mm)

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<tbody>
<tr>
<td>C4 300 Å</td>
<td>00G-4168-E0, 00G-4168-N0, 00G-4168-P0, 00G-4168-U0, 00G-4168-V0</td>
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<td>C5 300 Å</td>
<td>00G-4054-E0, 00G-4054-N0, 00G-4054-P0, 00G-4054-U0, 00G-4054-V0</td>
<td>/10 pk</td>
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<tr>
<td>C18 300 Å</td>
<td>00G-4055-E0, 00G-4055-N0, 00G-4055-P0, 00G-4055-U0, 00G-4055-V0</td>
<td>/10 pk</td>
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<tr>
<td>Protoo 90 Å</td>
<td>00G-4397-E0, 00G-4397-N0, 00G-4397-P0, 00G-4397-U0, 00G-4397-V0</td>
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### 15 μm Analytical and Preparative Columns (mm)

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<th>Phases</th>
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<tbody>
<tr>
<td>C4 300 Å</td>
<td>00G-4169-E0, 00G-4169-N0, 00G-4169-P0, 00G-4169-U0, 00G-4169-V0</td>
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<td>C5 300 Å</td>
<td>00G-4056-E0, 00G-4056-N0, 00G-4056-P0, 00G-4056-U0, 00G-4056-V0</td>
<td>/10 pk</td>
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<tr>
<td>C18 300 Å</td>
<td>00G-4057-E0, 00G-4057-N0, 00G-4057-P0, 00G-4057-U0, 00G-4057-V0</td>
<td>/10 pk</td>
</tr>
</tbody>
</table>

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*“SecurityGuard” Analytical Cartridges require holder, Part No.: AJ0-4281
**SemiPrep SecurityGuard” Cartridges require holder, Part No.: AJ0-7220
***PREP SecurityGuard” Cartridges require holder, Part No.: AJ0-8277

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- Consistent, reliable performance

Phenex Features

<table>
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<tr>
<th>Feature</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Low absorption</td>
<td>Low hold-up volume</td>
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<tr>
<td>Particulate-free</td>
<td>Certified quality</td>
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<tr>
<td>Maximum chemical compatibility</td>
<td>100 % integrity tested</td>
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<tr>
<td>Minimum extractables</td>
<td>Easy pore identification</td>
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<tr>
<td>Excellent flow rate</td>
<td>PVC-free</td>
</tr>
<tr>
<td>High total throughput</td>
<td>Bi-directional use</td>
</tr>
</tbody>
</table>

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3. Housing material is methacrylate butadiene styrene (MBS) polymerisate. Also known as Cryolite.
4. Cellulose acetate is surfactant-free.
5. 26 mm diameter.
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