Discovery® BIO Wide Pore

Solutions to Protein and Peptide Separation Challenges

HPLC Columns for Bio-Pharmaceutical Analysis and Purification

Leadership in Life Science and High Technology
Agenda:

• What is Discovery BIO Wide Pore
• Physical characteristics
• Why we developed it and for whom
• Performance demonstrations
• Choosing a column
What is Discovery BIO Wide Pore?

- Reversed-phase HPLC columns and capillaries
- 3, 5, and 10µm spherical silica particles
- 300Å pore diameter silica
- C5, C8, and C18 bonded phases
- Column IDs: 0.32mm to 21.2mm
- See handout for details on particle and bonded phase properties.
## Discovery BIO Wide Pore Silica

<table>
<thead>
<tr>
<th>Property</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape:</td>
<td>spherical</td>
</tr>
<tr>
<td>Type:</td>
<td>B (sil-gel process)</td>
</tr>
<tr>
<td>Size:</td>
<td>3µm (2.8-3.2µm)</td>
</tr>
<tr>
<td></td>
<td>5µm (4.5-5.1µm)</td>
</tr>
<tr>
<td></td>
<td>10µm (9.0-11.0µm)</td>
</tr>
<tr>
<td>Distribution profile:</td>
<td>Single mode (particle and pore size)</td>
</tr>
<tr>
<td>Pore size:</td>
<td>260-340Å</td>
</tr>
<tr>
<td>Pore volume:</td>
<td>1mL/g</td>
</tr>
<tr>
<td>Surface area:</td>
<td>80-120m²/g</td>
</tr>
<tr>
<td>Metals analyzed:</td>
<td>Al, Ti, Fe, Zr</td>
</tr>
<tr>
<td>Metal content:</td>
<td>&lt;10ppm, typically &lt;2ppm</td>
</tr>
</tbody>
</table>

Photomicrograph of Discovery BIO Wide Pore 5µm silica particles
<table>
<thead>
<tr>
<th></th>
<th>C5</th>
<th>C8</th>
<th>C18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silane:</td>
<td>pentyl</td>
<td>octyl</td>
<td>octadecyl</td>
</tr>
<tr>
<td>Endcap:</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
</tr>
<tr>
<td>%C:</td>
<td>3.2-3.8%</td>
<td>4.8-5.3%</td>
<td>9.0-9.5%</td>
</tr>
<tr>
<td>Coverage (µmole/m²):</td>
<td>4.1-5.0</td>
<td>3.8-4.3</td>
<td>3.3-4.0</td>
</tr>
<tr>
<td>Temp max:</td>
<td>70°C</td>
<td>70°C</td>
<td>70°C</td>
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<tr>
<td>Pressure max (bar):</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>pH range (phosphate):*</td>
<td>1 - 8</td>
<td>1 - 8</td>
<td>1 - 8</td>
</tr>
</tbody>
</table>

*using organic buffers, pH max is higher
Discovery BIO Wide Pore Dimensions

- Capillary (0.32, 0.5mm ID)*
- Microbore (1mm ID)
- Narrowbore (2.1mm ID)
- Standard Analytical (4.0, 4.6mm ID)
- Semi-Prep (10mm ID)
- Prep (21.2mm ID)*

*look for <0.32mm and >21.2mm ID
Why “Wide Pore?”

>80% of the surface area is inside the particle - where separation occurs.

Cross-section of Discovery BIO Wide Pore silica particle

Photomicrograph of Discovery BIO Wide Pore silica particles
What are its key features?

- Improves Resolution by providing:
  - Choices in selectivity
  - High efficiency
- Stable at high and low pH
- Reproducible
- Scalable from analytical to preparative
- LC-MS compatible (no-bleed, low TFA)
For whom was it developed?

Biochemists and researchers in proteomics or biopharmaceuticals who are:

- Separating native or recombinant proteins or peptides
- Working with synthetic peptides
- Using peptide maps to sequence proteins
- Employing LC-MS or conventional detectors
Why was it developed?

To meet the challenges of protein and peptide HPLC separations.

What are those challenges?

- Complex protein and/or peptide mixtures
- Small sample volumes and proteins at low concentrations or low copy numbers
- Need for detailed characterization
- Maintaining the separation (trouble-free operation)
#1 Complex Protein and/or Peptide Mixtures

“The selectivity and efficiency offered by Discovery BIO Wide Pore gives maximum power for resolving complex mixtures of proteins, natural or synthetic peptides, and peptide maps. Exceptional pH stability allows full use of mobile phase pH to adjust the separation.”

Demonstrations:

- BIO Wide Pore C5 has greater efficiency and resolution than competitive phases.
- Choices in selectivity of BIO Wide Pore C5, C8, C18 phases.
- Harness the power of mobile phase pH to alter selectivity.
Competitive RP-HPLC Columns

Waters Symmetry® 300 C18
Vydac 214TP, 218TP, 238TP
Zorbax® SB300-C18
Phenomenex Jupiter C18
Demonstrating Efficiency: Proteins on C5

Fig. 1 Conditions:

Conditions: C4 or C5 columns, 15cm x 4.6mm, 5µm,
Mobile Phase: (A) 75:25, H₂O:CH₃CN containing 0.1%TFA, (B) 66:34, H₂O:CH₃CN containing 0.1%TFA,
Flow Rate: 1mL/min,
Temp: ambient,
Detection: 220nm,
Sample: Protein mix
Gradient: 0-100%B in 25 mins

1. RNase (1mg/mL) (13.7kDa)
2. Cytochrome C (1mg/mL) (12.4 kDa)
3. Lysozyme (1mg/mL) (14.3 kDa)
4. Bovine Serum Albumin (2.5mg/mL) (67.0 kDa)
5. Myoglobin (1mg/mL) (18.8 kDa)
6. Ovalbumin (3.5mg/mL) (45.3 kDa)
Demonstrating Efficiency: Proteins on C5

BIO Wide Pore C5 has higher efficiency than popular competitive C4 phases

1. RNase (1mg/mL) (13.7kDa)
2. Cytochrome C (1mg/mL) (12.4 kDa)
3. Lysozyme (1mg/mL) (14.3 kDa)
4. Bovine Serum Albumin (2.5mg/mL) (67.0 kDa)
5. Myoglobin (1mg/mL) (18.8 kDa)
6. Ovalbumin (3.5mg/mL) (45.3 kDa)

Efficiency affects ability to see peaks
Demonstrating Efficiency: Peptide Maps

**Fig 2 Conditions BIO Wide Pore C18, peptide resolution**

**Conditions**: C18 columns, 15cm x 4.6mm, 5µm, 300Å,
**Mobile Phase**: (A) 95:5, H₂O:CH₃CN containing 0.1%TFA, (B) 50:50,
H₂O:CH₃CN containing 0.1%TFA,
**Flow Rate**: 1mL/min,
**Temp**: 30°C,
**Detection**: 215nm,
**Sample**: 50µL carboxymethylated apohemoglobin tryptic digest,
**Gradient**: 0-100%B in 65 mins
Demonstrating Efficiency: Peptide Maps

**BIO Wide Pore C18 resolves more peptides than competitive C18 phases**

- **Discovery BIO Wide Pore C18**
  - 74 peptides resolved

- **Competitor A C18**
  - 68 peptides resolved

- **Competitor B C18**
  - 61 peptides resolved

*Fig. 2*
Demonstrating Efficiency: Synthetic Peptides

Fig. 3 Conditions BIO Wide Pore C18 synthetic peptides resolution

Conditions: C18 columns, 15cm x 4.6mm, 5µm, 300Å,
Mobile Phase: (A) 80:20, H₂O:CH₃CN containing 0.1%TFA, (B) 66:34, H₂O:CH₃CN containing 0.1%TFA,
Flow Rate: 1mL/min,
Temp: 30°C,
Detection: 220nm,
Sample: 10µL Sigma peptide mix (P 2693),
Gradient: 0-100%B in 14 mins. after 1 min. delay
Demonstrating Efficiency: Synthetic Peptides

BIO Wide Pore C18 resolves these synthetic peptides better than competitive C18 phases

Discovery BIO Wide Pore C18

Competitor A C18
Demonstrating Selectivity

**Fig. 4 Conditions BIO Wide Pore C5, C8, C18 selectivity**

**Conditions:** Discovery BIO Wide Pore columns, 15cm x 4.6mm, 5µm, 300Å,

**Mobile Phase:** (A) 95:5, H₂O:CH₃CN containing 0.1%TFA, (B) 50:50, H₂O:CH₃CN containing 0.1%TFA,

**Flow Rate:** 1mL/min,

**Temp:** 30°C,

**Detection:** 215nm,

**Sample:** 50µL carboxylated apohemoglobin tryptic digest,

**Gradient:** 0-100%B in 65 mins
Demonstrating Selectivity

BIO Wide Pore C5, C8, C18 phases offer different selectivity
Demonstrating Power of pH on Selectivity

Fig. 5 Conditions Angiotensins at neutral pH

Conditions: Discovery BIO Wide Pore C18, 15cm x 4.6mm, 5µm, 300Å,
Mobile Phase: (A) 65:35, (10mM NH₄OAc, pH 7):(50% CH₃CN in 20mM NH₄OAc, pH 7), (B) 25:75, (10mM NH₄OAc, pH 7):(50% CH₃CN in 20mM NH₄OAc, pH 7),
Flow Rate: 1mL/min,
Temp: 30°C,
Detection: 215nm,
Sample: 6µL (10 µg) each peptide in H₂O,
Gradient: 0-100%B in 12.5 mins
Demonstrating Power of pH on Selectivity

Angiotensins resolved at neutral pH on Discovery BIO Wide Pore C18

1. Angiotensin II  DRVYIHPF
2. Angiotensin III  RVYIHPF
3. Angiotensin I   DRVYIHPFHL

at pH 2, II and III are not resolved
at pH 7 they are resolved
#2 Small Sample Volumes and Proteins at Low Concentrations or Low Copy Numbers

“The efficiency of Discovery BIO Wide Pore provides Sensitive analyses, especially when combined with capillary and microbore dimensions.”

Demonstrations:

- Higher efficiency than competitive phases.
- The microbore and capillary dimensions greatly enhance sensitivity, and conserve samples.
Demonstrating Efficiency: Proteins on C5

**Fig. 6 Conditions**

**Conditions:** C4 or C5 columns, 15cm x 4.6mm, 5µm,

**Mobile Phase:** (A) 75:25, H₂O:CH₃CN containing 0.1%TFA, (B) 66:34, H₂O:CH₃CN containing 0.1%TFA,

**Flow Rate:** 1mL/min,

**Temp:** ambient,

**Detection:** 220nm,

**Sample:** Peptide Mix (Sigma Cat. No. P2693),

**Gradient:** 0-100%B in 25 mins

1. RNase (1mg/mL) (13.7kDa)
2. Cytochrome C (1mg/mL) (12.4 kDa)
3. Lysozyme (1mg/mL) (14.3 kDa)
4. Bovine Serum Albumin (2.5mg/mL) (67.0 kDa)
5. Myoglobin (1mg/mL) (18.8 kDa)
6. Ovalbumin (3.5mg/mL) (45.3 kDa)
Demonstrating Efficiency: Proteins on C5

**BIO Wide Pore C5 has higher efficiency than popular competitive C4 phases**

Efficiency affects peak height, sensitivity, LOD

2X sensitivity

1. RNase (1mg/mL) (13.7kDa)
2. Cytochrome C (1mg/mL) (12.4 kDa)
3. Lysozyme (1mg/mL) (14.3 kDa)
4. Bovine Serum Albumin (2.5mg/mL) (67.0 kDa)
5. Myoglobin (1mg/mL) (18.8 kDa)
6. Ovalbumin (3.5mg/mL) (45.3 kDa)
Demonstrating Sensitivity: Cap/MB Dimensions

**These parameters vary with the square (or inverse square) of column radius.**

<table>
<thead>
<tr>
<th>Chromatographic parameters relative to 4.6mm ID columns</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow rates (volumetric)</strong></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>4.6 mm ID</td>
</tr>
<tr>
<td>3 mm ID</td>
</tr>
<tr>
<td>2.1 mm ID</td>
</tr>
<tr>
<td>1 mm ID</td>
</tr>
<tr>
<td>0.5 mm ID</td>
</tr>
<tr>
<td>0.32 mm ID</td>
</tr>
</tbody>
</table>

- uses less sample
- more sensitive
#3 Need for Detailed Characterization

“Discovery BIO Wide Pore phases are bleed-free and designed for LC-MS. Often purified sample is needed for further characterization. Discovery BIO Wide Pore phases are completely scalable from analytical to preparative for easy, reliable scale-up.”

Demonstrations:
- If you use LC-MS, there is no bleed, and you can use very low levels of TFA and have good peak shape.
- If you need to isolate and purify proteins or peptides, analytical separations are completely scalable on Discovery BIO Wide Pore preparative columns.
Demonstrating Sensitivity: LC-MS Compatible

**Fig. 7 Conditions Column bleed**

- **Conditions:** Columns, 15cm x 4.6mm,
- **Mobile Phase:** (A) 0.1% TFA in H₂O, (B) 0.1% TFA in CH₃OH,
- **Flow Rate:** 1mL/min,
- **Temp:** 30°C,
- **Gradient:** 0-100% B in 15 mins, 100% B 5 mins, 0% B 10 mins
Demonstrating Sensitivity: LC-MS Compatible

Discovery BIO Wide Pore phases are bleed-free

Fig. 7
Demonstrating Sensitivity: No TFA

Discovery BIO Wide Pore can be used without TFA, increasing LC-MS sensitivity.

Columns: 15cm x 2.1 (or 2.0) mm, 5µm, Mobile Phase A: water/25mM HCO2H B: 50:50, (water/25mM HCO2H) : (MeCN/20mM HCO2H), Flow: 0.208 (or 0.189) mL/min, Gradient: 15 to 60%B in 45 min

Basic peptides on Discovery BIO Wide Pore C18

Competitor C C18
Demonstrating Sensitivity: No TFA

The low surface activity of Discovery BIO Wide Pore is evident without TFA in the mobile phase, even compared to shielded phases.

Basic peptides on
Discovery BIO Wide Pore C18

Competitor D C18

Columns: 15cm x 2.1 (or 2.0) mm, 5µm, Mobile Phase A: water/25mM HCO$_2$H B: 50:50, (water/25mM HCO$_2$H) : (MeCN/20mM HCO$_2$H), Flow: 0.208 mL/min, Gradient: 15 to 60%B in 45 min
Demonstrating Scale-Up

Fig 10 Conditions Reproducibility of 3, 5 10um phases

**Mobile Phase:** (A) 80:20, H$_2$O:CH$_3$CN containing 0.1%TFA, (B) 66:34, H$_2$O:CH$_3$CN containing 0.1%TFA,

**Flow Rate:** 6.02cm/sec,

**Temp:** 30°C,

**Detection:** 215nm,

**Sample:** Peptide Mix (Sigma Cat. No. P 2693),

**Gradient:** 0-100%B in 9 column volumes
Demonstrating Scale-Up

Reproducible separations on 3, 5, and 10µm Discovery BIO Wide Pore

3 µm C18, 15cm x 4.6mm, 1mL/min

5 µm C18, 15cm x 4.6mm, 1mL/min

10 µm C18, 15cm x 10mm, 4.7mL/min

Leadership in Life Science and High Technology
#4 Maintaining the Separation (Trouble-Free Operation)

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

Proof:
• Run-to-run reproducibility and excellent column lifetime at low and high pH are characteristics of Discovery BIO Wide Pore phases.
• Batch-to-batch reproducibility is a very important concern. We have designed Discovery BIO Wide Pore phases to have guaranteed reproducibility.
Demonstrating Stability at pH 2

Enhanced stability at low pH of BIO C5 vs. popular C4

Change in efficiency after 25,000 column volumes (15L, 5 days) of 0.5% TFA in water:CH₃CN

- VAL-TYR-VAL: BIO C5 -26%, C4 -49%
- MET-Enkephalin: BIO C5 -27%, C4 -27%
- LEU-Enkephalin: BIO C5 -27%, C4 -27%
- Angiotensin II: BIO C5 101.8%, C4 50.8%
Demonstrating Column Lifetime at pH 2

**Fig. 12 Conditions: BIO Wide Pore C5 stability at pH 2**

**Discovery BIO Wide Pore C18 Stability**

**Conditions:** Discovery BIO Wide Pore C18, 5cm x 4.6mm, 5μm

**Mobile Phase:** (A) 5:95, H₂O:CH₃CN containing 0.5%TFA, (B) 25:75, H₂O:CH₃CN containing 0.5%TFA

**Flow Rate:** 2mL/min

**Temp:** 70°C

**Detection:** 220nm, **Sample:** Peptide Mix (Sigma Cat. No. H 2016)

**Gradient:** 2-24%B in 22 mins, 100%A for 8 mins

**Column volume (CV) calculation:**

CV = (0.7) πr²L = (0.7) π(0.23)²5 = 0.6mL

40,000 CV = 24,000mL

Time: (40,00mL)(1min/2mL)(1hr/60min)(1day/24hr) = 8 days
Demonstrating Column Lifetime at pH 2

*BIO Wide Pore C18 stability at pH 2, 70°C, 40,000 column volumes (24L, 8 days)*

No change in selectivity or peak shape on Discovery BIO Wide Pore C18 after 40,000 column volumes of 0.5% TFA at elevated temperature (70°C).

1. Gly-Tyr
2. Val-Tyr-Val
3. Met-Enkephalin
4. Lue-Enkephalin
5. Angiotensin II
Demonstrating Column Lifetime at pH 11.5

**Fig 13 Conditions: BIO Wide Pore C18 stability at pH 11.5**

**Discovery BIO Wide Pore C18 Stability**

Column #35136-03  
5μm, 50x4.6mm  
(65:35) 50mM **pH 11.5** Pyrrolidine-HCl : Acetonitrile  
2mL/min  
35°C  
UV254nm  
5 μL injection every 30 minutes

**Column volume (CV) calculation:**  
CV = (0.7) πr²L = (0.7) π(0.23²)5 = 0.6mL  
40,000 CV = 24,000mL  
Time: (24,000mL)(1min/2mL)(1hr/60min)(1day/24hr) = 8 days
Demonstrating Column Lifetime at pH 11.5

BIO Wide Pore C18 stability at pH 11.5, 40,000 column volumes (24L, 8 days)
Demonstrating Column Lifetime at pH 8

**Fig. 14 Conditions: BIO Wide Pore C5 stability at pH 8**

**Discovery BIO Wide Pore C5 Stability**
Column #11797
5µm, 50x4.6mm
(95:5) 25mM PO4 **pH 8.00**: MeOH
2mL/min
35°C
UV254nm
5 µL injection
950 psi

**Column volume (CV) calculation:**
CV = (0.7) πr²L = (0.7) π(0.23)²5 = 0.6mL
14,000 CV = 8,400mL
Time: (8,400mL)(1min/2mL)(1hr/60min)(1day/24hr) = 3 days
Demonstrating Column Lifetime at pH 8

BIO Wide Pore C5 stability at pH 8, 14,000 column volumes (8L, 3 days)

Retention Time (min) vs. Column Volumes graph showing the stability of Sorbic Acid, Pyridine, Procainamide, and Caffeine at pH 8.
Demonstrating Reproducibility

**Fig 15 Conditions Reproducibility of Discovery BIO Wide Pore phases**

<table>
<thead>
<tr>
<th>C18</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery BIO Wide Pore C18, 15cm x 4.6mm, 5µm,</td>
<td>Discovery BIO Wide Pore C5, 15cm x 4.6mm, 5µm,</td>
</tr>
<tr>
<td>Mobile Phase: (A) 80:20, 10mM NH₄OAc (pH 6.8):CH₃OH,</td>
<td>Mobile Phase: (A) 81:19, H₂O:CH₃CN containing 0.1% PFPA,</td>
</tr>
<tr>
<td>Flow Rate: 1mL/min,</td>
<td>(B) 62:38, H₂O:CH₃CN containing 0.1% PFPA,</td>
</tr>
<tr>
<td>Temp: 35°C,</td>
<td>Flow Rate: 1mL/min,</td>
</tr>
<tr>
<td>Detection: 254nm</td>
<td>Temp: 30°C,</td>
</tr>
<tr>
<td></td>
<td>Detection: 2154nm</td>
</tr>
</tbody>
</table>
Demonstrating Reproducibility

Reproducibility of Discovery BIO Wide Pore phases for both small and large molecules.

For small molecules:
1. Uracil
2. Procainamide
3. Sorbic acid
4. Pyridine
5. Caffeine
6. Phenol

For peptides:
1. Arg⁸-vasopressin
2. Bradykinin, frag 1-5
3. Oxytocin
4. Met-enkephalin
5. LHRH
6. Leu-Enkephalin
7. Bradykinin
8. Bombesin
9. Substance P

Fig. 15
Choosing a Discovery® BIO Phase

Discovery BIO Wide Pore C18
- Peptides
- Protein digests (mapping)
- Small proteins (<5kd)

Discovery BIO Wide Pore C8
- Intermediate hydrophobicity polypeptides
- Small proteins

Discovery BIO Wide Pore C5
- Large polypeptides (>20kd)
- Hydrophobic peptides
- Large proteins
## When to use a BIO column

<table>
<thead>
<tr>
<th>Application</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>C5</td>
</tr>
<tr>
<td>Hydrophobic peptides</td>
<td>C5 or C8</td>
</tr>
<tr>
<td>Peptide mapping</td>
<td>C18 or C8</td>
</tr>
<tr>
<td>Scouting (method development)</td>
<td>C8</td>
</tr>
<tr>
<td>LC-MS</td>
<td>3 micron or 5 micron</td>
</tr>
<tr>
<td>Fast analysis, or high-throughput applications</td>
<td>3 micron</td>
</tr>
<tr>
<td>Peptide mapping</td>
<td>3 micron or 5 micron</td>
</tr>
<tr>
<td>Analytical HPLC</td>
<td>3 micron or 5 micron</td>
</tr>
<tr>
<td>Preparative</td>
<td>10 micron</td>
</tr>
<tr>
<td>LC-MS</td>
<td>2.1mm or smaller</td>
</tr>
<tr>
<td>Peptide mapping</td>
<td>4.6mm, 4.0mm, 2.1mm</td>
</tr>
<tr>
<td>Analytical HPLC</td>
<td>4.0mm, 4.6mm</td>
</tr>
<tr>
<td>Preparative</td>
<td>10mm, 21.2mm</td>
</tr>
<tr>
<td>Low level detection or limited sample volume</td>
<td>0.32mm, 0.5mm, 1.0mm</td>
</tr>
</tbody>
</table>
Conclusion: The BIO Story

“Discovery BIO Wide Pore HPLC columns and capillaries provide sensitive, stable, efficient, reproducible separations of proteins and peptides. The different phase chemistries provide unique selectivity increasing your resolution options. Separations are completely scalable from analytical to prep. The low-bleed feature and microbore and capillary dimensions make them ideal for LC-MS applications.”

Discovery BIO Wide Pore meets the challenges of today’s protein and peptide separations.
“We are Committed to the Success of Our Customers through Science, Technology, and Service.”
Discovery® BIO Wide Pore Chemistry

- What: Alkylmethylsilyl on 300Å pore silica
- How: Hydrophobic (van der Waals, dispersive) interactions
- Why:
  - Specifically designed for protein/peptide analysis
  - Matched selectivity across particle sizes for ease of scalability
  - Exceptional resolution for peptide analysis and purification
  - Highly stable to ensure excellent run to run reproducibility and long column life
  - Ideally suited for LC-MS; no detectable bleed

\[
\begin{align*}
\text{Si} & \quad \text{O} & \quad \text{Si} & \quad -(\text{CH}_2)_n\text{CH}_3 \\
\text{CH}_3 & \quad & & \\
\end{align*}
\]

- C18: n=17
- C8: n=7
- C5: n=4
## Discovery BIO Wide Pore Column Testing

<table>
<thead>
<tr>
<th>ID (mm)</th>
<th>3µm</th>
<th>5µm</th>
<th>10µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.32*</td>
<td>&gt;95,000</td>
<td>&gt;70,000</td>
<td>n/a</td>
</tr>
<tr>
<td>0.50*</td>
<td>&gt;95,000</td>
<td>&gt;70,000</td>
<td>n/a</td>
</tr>
<tr>
<td>1.0</td>
<td>&gt;88,000</td>
<td>&gt;62,000</td>
<td>n/a</td>
</tr>
<tr>
<td>2.1</td>
<td>&gt;84,667</td>
<td>&gt;53,333</td>
<td>n/a</td>
</tr>
<tr>
<td>4.0</td>
<td>n/a</td>
<td>&gt;66,667</td>
<td>n/a</td>
</tr>
<tr>
<td>4.6</td>
<td>&gt;110,000</td>
<td>&gt;80,000</td>
<td>&gt;35,200</td>
</tr>
<tr>
<td>10</td>
<td>n/a</td>
<td>&gt;84,000</td>
<td>&gt;35,333</td>
</tr>
<tr>
<td>21.2</td>
<td>n/a</td>
<td>n/a</td>
<td>&gt;35,000</td>
</tr>
</tbody>
</table>

* the 5cm are >90,000pl/m
**Discovery® BIO Wide Pore C8**

Intermediate hydrophobicity between a C18 and C4/C5. Ideal for the analysis and purification of peptides, polypeptides, and small proteins.

**Resolution of Angiotensins at Neutral pH**

1. Angiotensin II (1.67g/L) (DRVYIHPF)
2. Angiotensin III (1.67g/L) (RVYIHPF)
3. Angiotensin I (1.67g/L) (DRVYIHPFHL)

**Resolution of Insulins from Various Species**

1. Bovine insulin (5µg)
2. Human insulin (5µg)
3. Porcine insulin (5µg)

**Conditions:** Discovery BIO Wide Pore C8, 15cm x 4.6mm, 5µm; Mobile Phase: (A) 10mM NH₄H₂PO₄/ NH₄OH, pH 7; (B) 50:50, (20mM NH₄H₂PO₄/ NH₄OH, pH 7):MeCN; Flow Rate: 1mL/min; Temp: 30°C; Detection: 215nm; Injection: 6µL; Gradient: 30-60% B in 15 min

**Conditions:** Discovery BIO Wide Pore C8, 15cm x 4.6mm, 5µm; Mobile Phase: (A) 71:29, (0.1% TFA in H₂O):(0.1% TFA in MeCN); (B) 68:32, (0.1% TFA in H₂O):(0.1% TFA in MeCN); Flow Rate: 1mL/min; Temp: 30°C; Detection: 215nm; Injection: 50µL; Gradient: 0-100% B in 30min
Resolution: The Separation Objective

\[ R_S = \left(\frac{1}{4}\right) \left\{ \frac{(\alpha - 1)}{\alpha} \right\} N^{1/2} \left\{ \frac{k}{1 + k} \right\} \]

To improve resolution between peaks we have three options:

>> Increase selectivity (peak spacing) -- by changing the chemistry of the phase and the types of interactions that can occur between it and the analytes

>> Increase efficiency (narrow the peaks) -- by reducing adsorption and dispersion that lead to band broadening

>> Increase retention -- by increasing time analyte spends on the bonded phase

*Discovery BIO Wide Pore leverages all three to maximize resolution.*