Part II – ProTain™ – A New Approach for the In-line Removal of Matrix Proteins

- The general problem – Matrix interferences in biological samples lead to quantitation problems in HPLC

- A new solution – ProTain™ – A new in-line protein removal system
  - The chemistry of polymer coated zirconia makes it an ideal protein adsorbent
  - ProTain™ hardware
  - Demonstration of the removal of BSA
  - Detection of basic pharmaceuticals in serum by LC/UV
  - Reduction of baseline signal for LC/MS

- ProTain capacity and applicability matrix

- Customer feedback
The General Problem

- Matrix proteins can cause fouling of the analytical column
- Matrix proteins can interfere with detection of small organic molecules by either UV/Vis or mass spectrometry
- Matrix interferences can lead to inaccurate and irreproducible quantitation
- In the worst case, interferences can completely mask the elution of analytes of interest, and/or ruin the analytical column
A New Solution - ProTain™

- ProTain™ is an in-line protein removal system
- ProTain™ uses a polymer coated zirconia material to effectively serve as a protein sponge, while allowing small molecules of interest to pass through to the analytical column
- ProTain™ can be used in-line with any type of silica, polymer, or zirconia-based analytical column
Demonstration of the Removal of BSA

ProTain™ → SEC Column

Analytical column is a size exclusion column

LC Conditions: Mobile phase, 20mM phosphate buffer, pH 6.8; Flow rate, 1.0 ml/min.; Temperature, ambient; Injection volume, 10 µl.
Interactions of Proteins with Polymer Coated Zirconias

Three interactions acting simultaneously lead to irreversible adsorption of proteins on polymer coated zirconia materials

- Hydrophobic, electrostatic, and ligand exchange interactions
The ProTain™ System
Detection of Basic Pharmaceuticals in Serum by LC/UV

Sample: 1 µg/ml hydroxyzine in serum diluted 1:10 with acetate buffer

Mobile phase: 30-70% B in 6 minutes

A: 25mM ammonium acetate, pH 5.0
B: ACN

Flow rate: 2.0 ml/min.
Temperature: 35 °C

Injection volume: 10 µl

Detection: UV at 254 nm
Reduction of Baseline Signal for LC/MS

Sample: Serum diluted 1:1 with water
Mobile phase: 20-95% B in 10 minutes
  A: water
  B: ACN
Flow rate: 0.5 ml/min.
Temperature: 35 °C
Injection volume: 10 µl
Detection: MS TIC

C18-silica column only

ProTain™ + C18-silica column
Up to 5 mg of bovine serum proteins can be loaded onto a 20 mm x 4.0 mm i.d. cartridge depending on the mobile phase conditions.

<table>
<thead>
<tr>
<th>Mobile Phase pH</th>
<th>Buffer Type</th>
<th>TFA</th>
<th>Acetate</th>
<th>Phosphate</th>
<th>Carbonate</th>
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<tbody>
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</table>

+ = 0 – 0.2 mg  ++ = 0.2 – 1.0 mg  +++ = 1.0 – 5.0 mg
### ProTain™ Applicability Matrix

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Buffer Type</th>
<th>TFA</th>
<th>Acetate</th>
<th>Phosphate</th>
<th>Carbonate</th>
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</thead>
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<tr>
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<td>Acetate</td>
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<tr>
<td>Basic</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Acidic analytes currently require specific (hard Lewis base) buffers.
Customer Feedback

- ProTain™ is currently being used in validated methods for the determination of small pharmaceuticals molecules in protein-containing samples
- ProTain™ has provided sufficient versatility in method development for different sample types
- ProTain™ is a cost-effective alternative to other clean-up procedures such as liquid-liquid extraction and solid-phase extraction
Summary – Benefits of ProTain™

- ProTain™ is an in-line protein removal system that does not require extra handling of samples prior to analysis.
- ProTain™ uses a polymer coated zirconia material to effectively serve as a protein sponge, while allowing small molecules of interest to pass through to the analytical column.
- ProTain™ can be used in-line with any type of silica, polymer, or zirconia-based analytical column.