ProTain™ – A New In-Line Protein Removal System for HPLC

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Outline

• 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

• Customer feedback

• Visit ZirChrom Separations at our booth - # 220
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
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 TFA in water, pH 1.6
B: 25mM TFA in ACN

Flow rate: 2.0 ml/min.

Temperature: 35 °C

Injection volume: 10 µl

Detection: UV at 254 nm
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 TFA in water, pH 1.6
  B: 25mM TFA in 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
Capacity Study

Purpose: To test the effect of pH and buffer type on the protein capacity of the Protain stationary phase.

Study Conditions:

• Cartridge/Holder: 20 mm x 4.6 mm i.d. (Part Number: PT01-0246/850-00-2)
• Mobile Phase: 50/50 ACN / 20 mM Indicated buffer and pH
• Temperature: 30 ºC
• Flow Rate: 1 ml/min.
• Injection Vol.: 5 µl
• Detection: UV at 280 nm
## Capacity Study Results

### Table: Loadability Matrix for the ProTain Media Inserts

<table>
<thead>
<tr>
<th>Mobile Phase pH</th>
<th>Buffer Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TFA</td>
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<tr>
<td>2</td>
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<tr>
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<td>7</td>
<td>+</td>
</tr>
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<td>9</td>
<td>+</td>
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</tbody>
</table>

**Figure 1:** Loadability Matrix for the ProTain Media Inserts

*Capacity:*  
- $+ = 0 - 0.2$ mg  
- $++ = 0.2 - 1.0$ mg  
- $+++ = 1.0 - 5.0$ mg  

*Black Areas:* Not tested due to lack of buffer capacity at pH
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.
- The type of buffer, specifically its strength as a Lewis base, and the pH of the mobile phase play a significant role in determining the actual protein binding capacity.