Introducing 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

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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

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

Hydroxyzine

ProTain™ → Silica-C18
(1 cm) (15 cm)

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**
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
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.