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

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ZirChrom Separations, Inc.

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 intereferences 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

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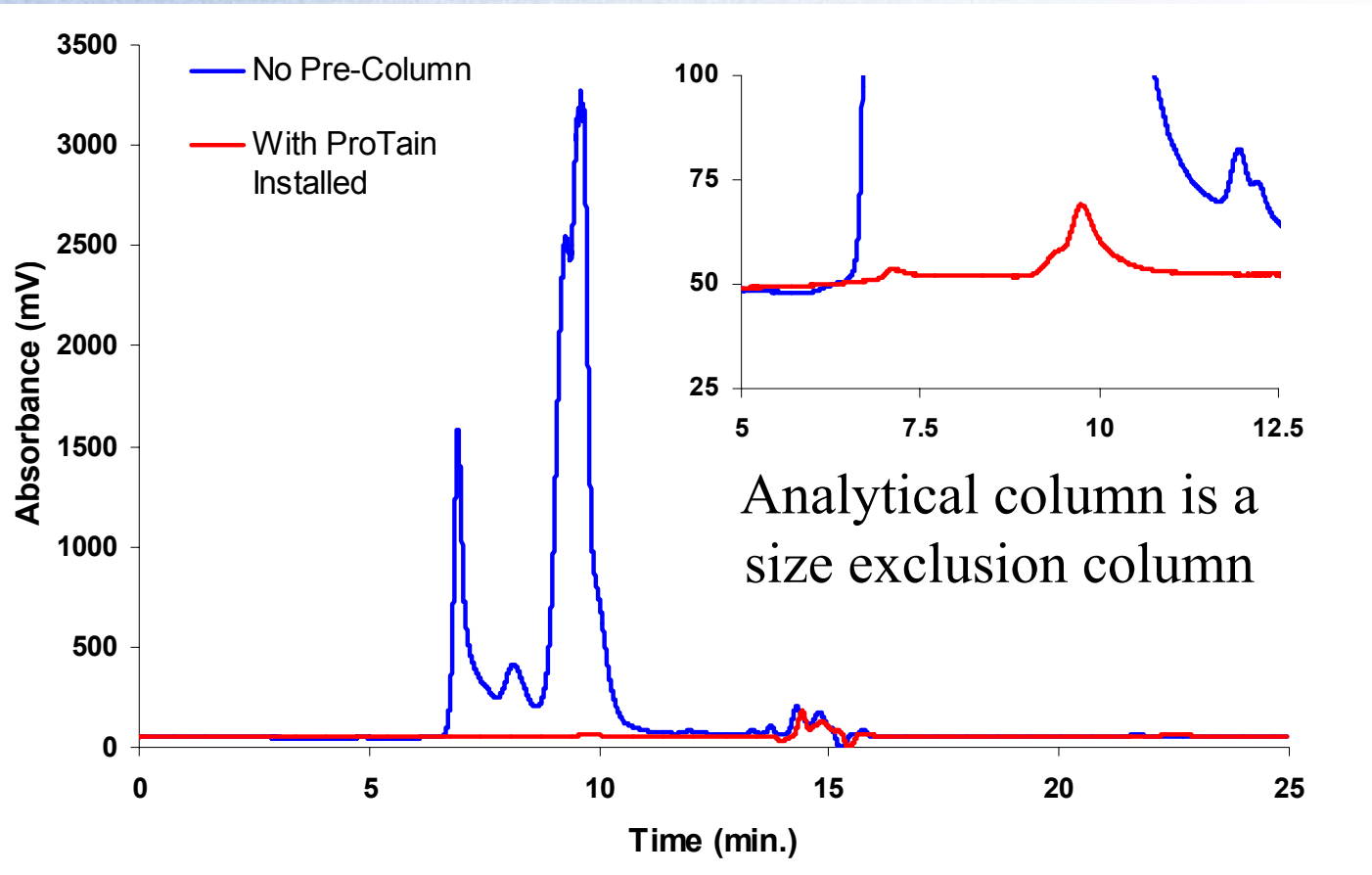
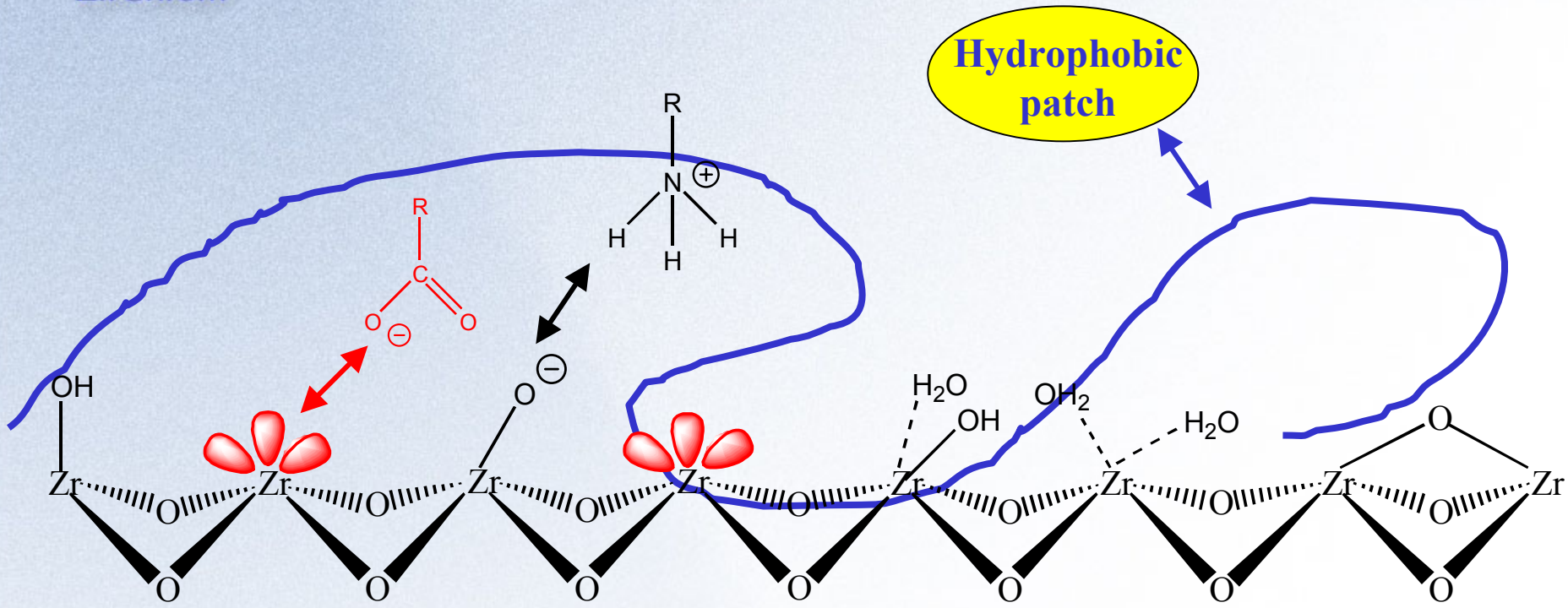
ProTain™ → SEC Column



LC Conditions: Mobile phase, 20mM phosphate buffer, pH 6.8; Flow rate, 1.0 ml/min.; Temperature, ambient; Injection volume, 10 µl.

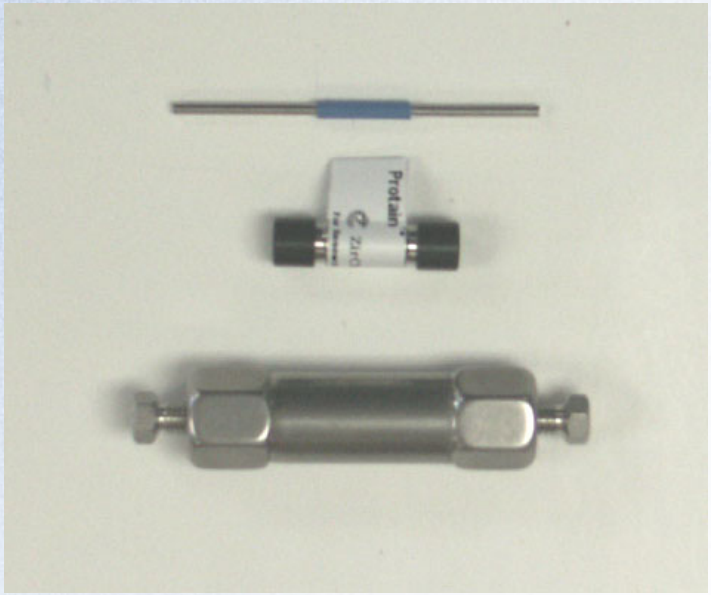
Interactions of Proteins with Polymer Coated Zirconias

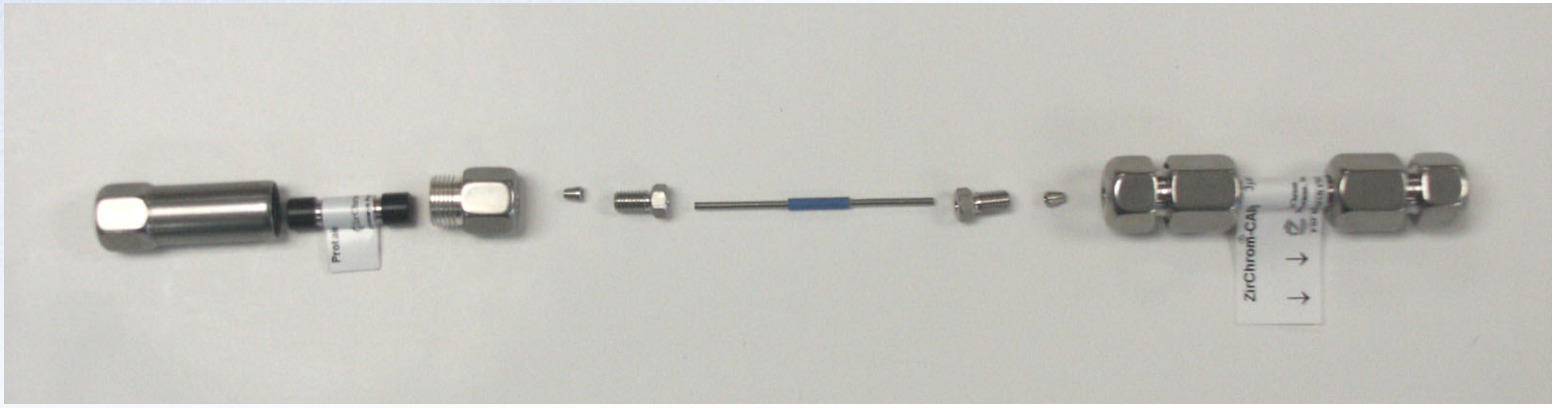


Three interactions acting simultaneously lead to irreversible adsorption of proteins on polymer coated zirconia materials

- Hydrophobic, electrostatic, and ligand exchange interactions

The ProTain™ System

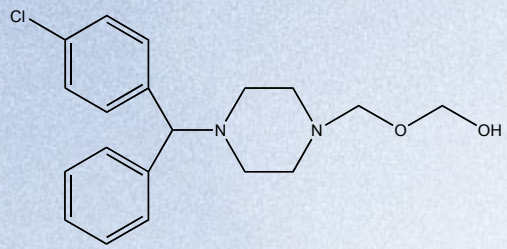




Detection of Basic Pharmaceuticals in Serum by LC/UV

ZirChrom®

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

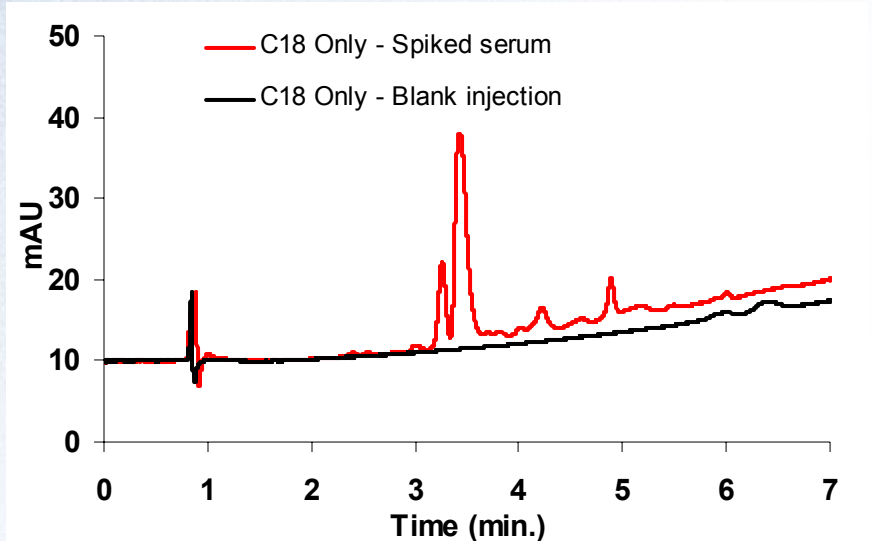
B: 25mM TFA in ACN

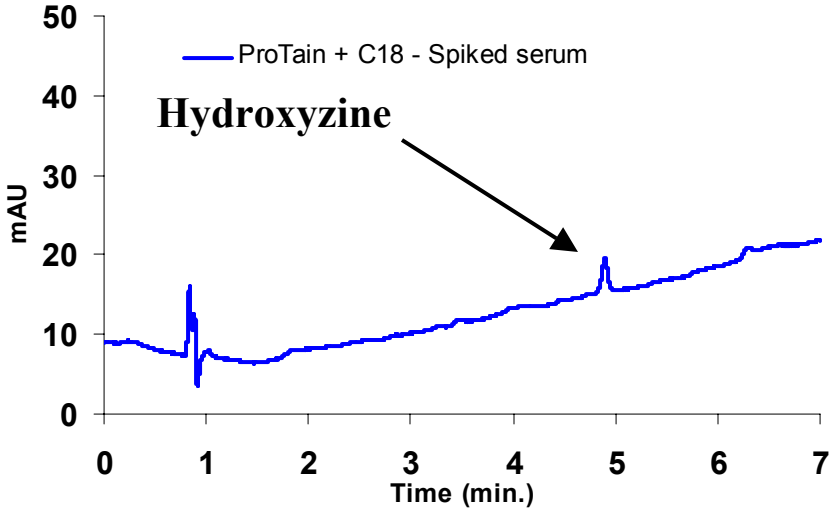
Flow rate: 2.0 ml/min.

Temperature: 35 °C

Injection volume: 10 µl

Detection: UV at 254 nm





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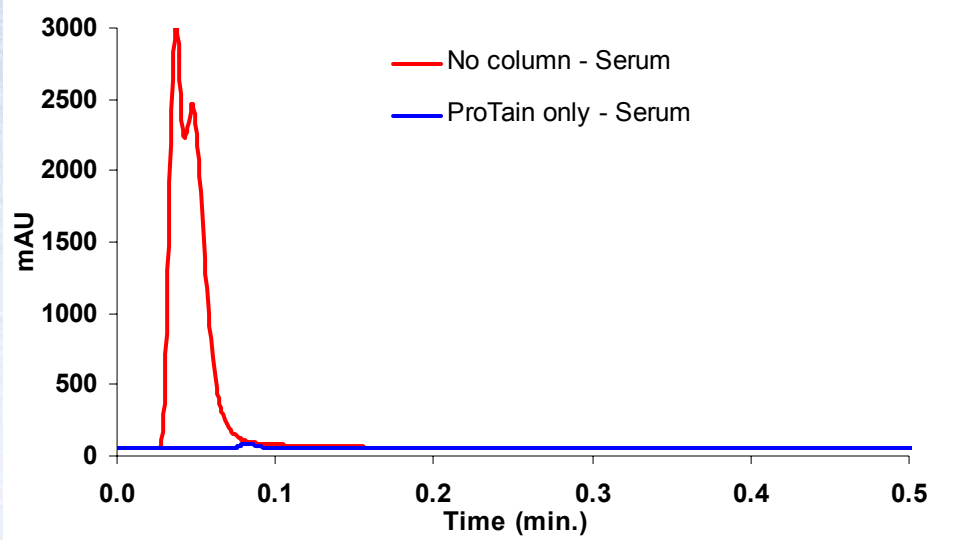

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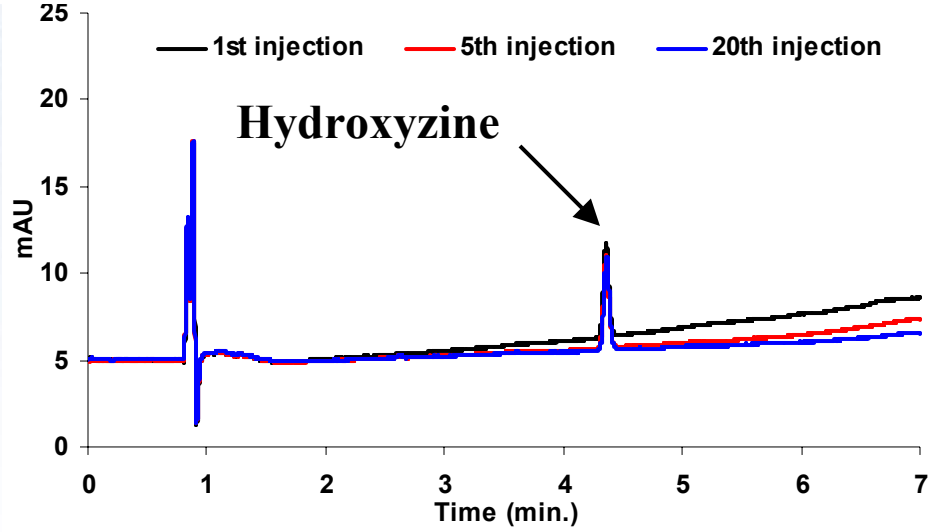
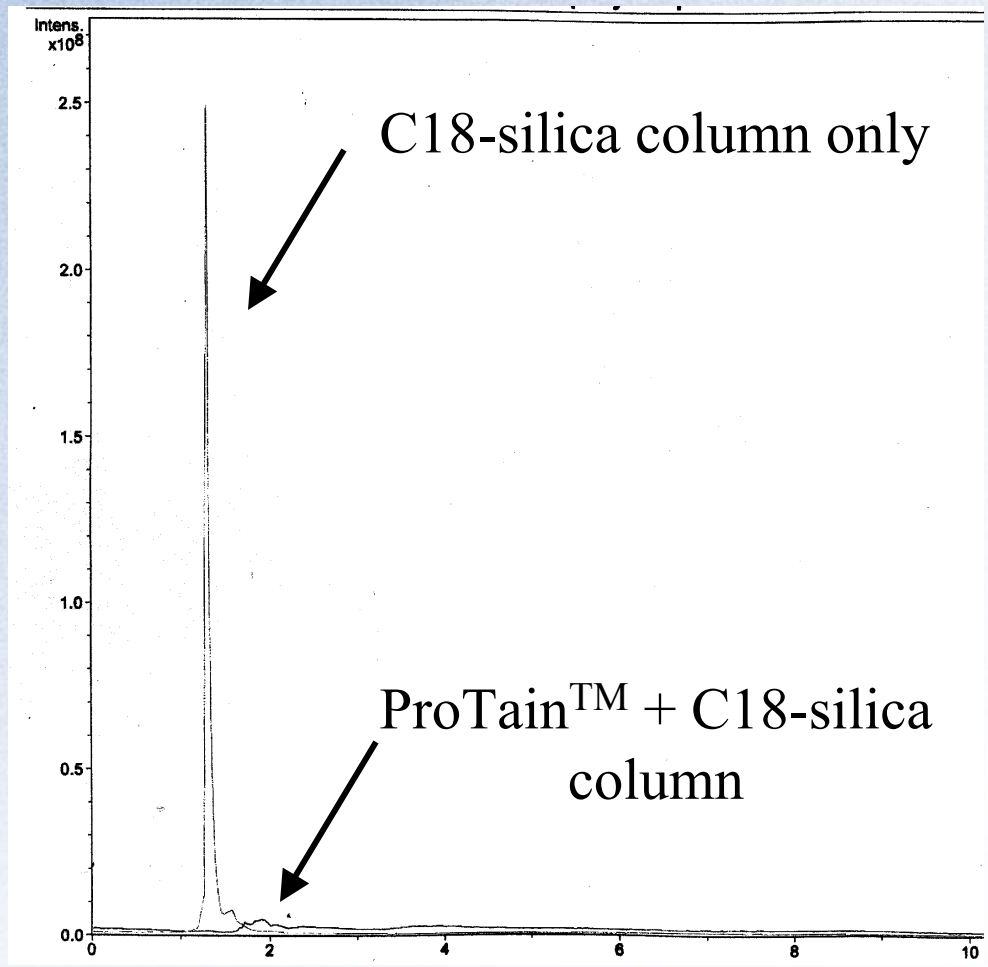


Reduction of Baseline Signal for LC/MS



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

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

		Buffer Type			
		TFA	*Acetate*	*Phosphate*	*Carbonate*
Mobile Phase pH	*2*	+		+ +	
	3		+	+ +	
	5		+ + +	+ + +	
	7		+ + +	+ + +	+
	9		+	+	+

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

User 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.**
- **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.**