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 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

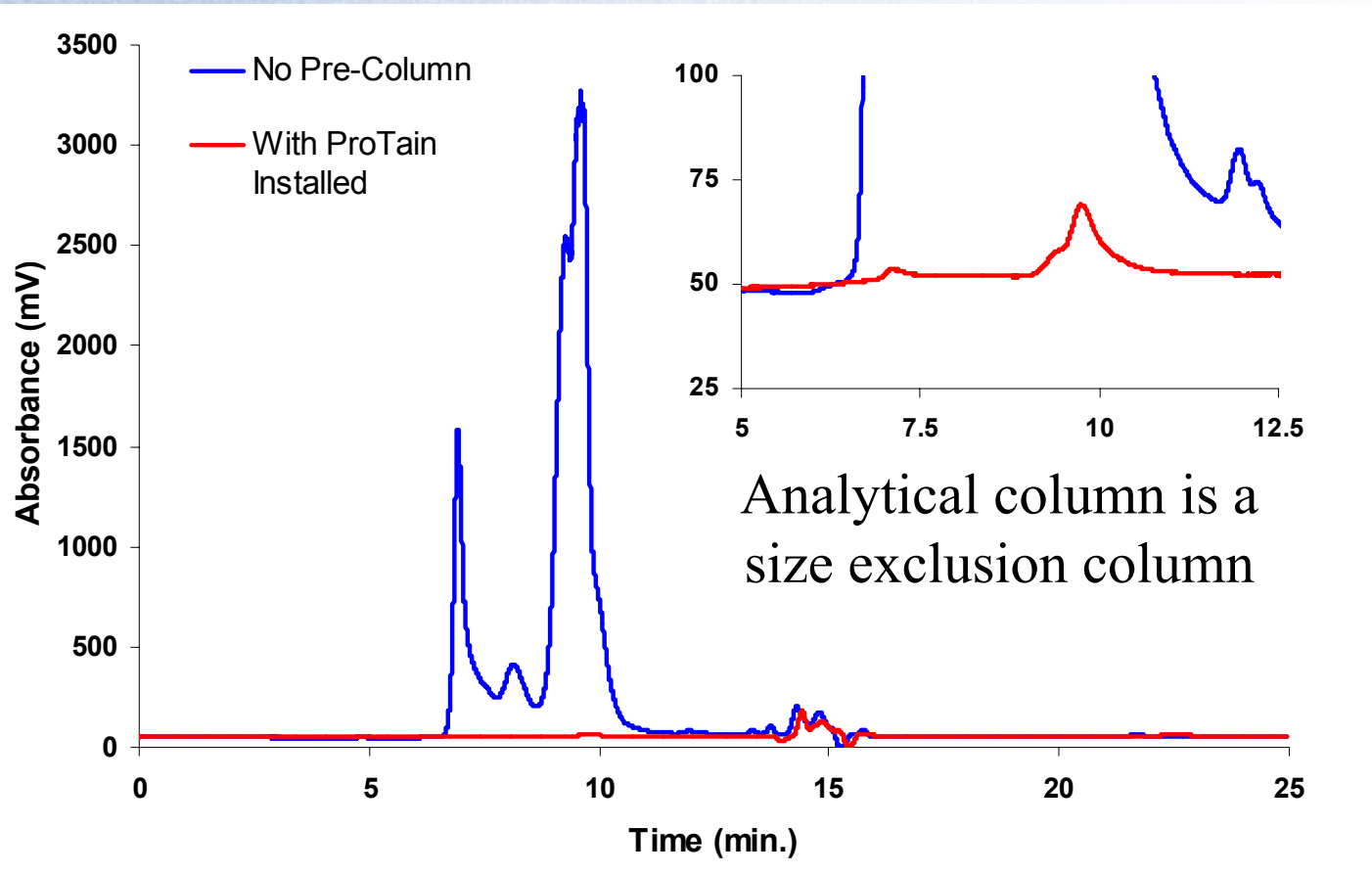
A New Solution - ProTain™

- 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

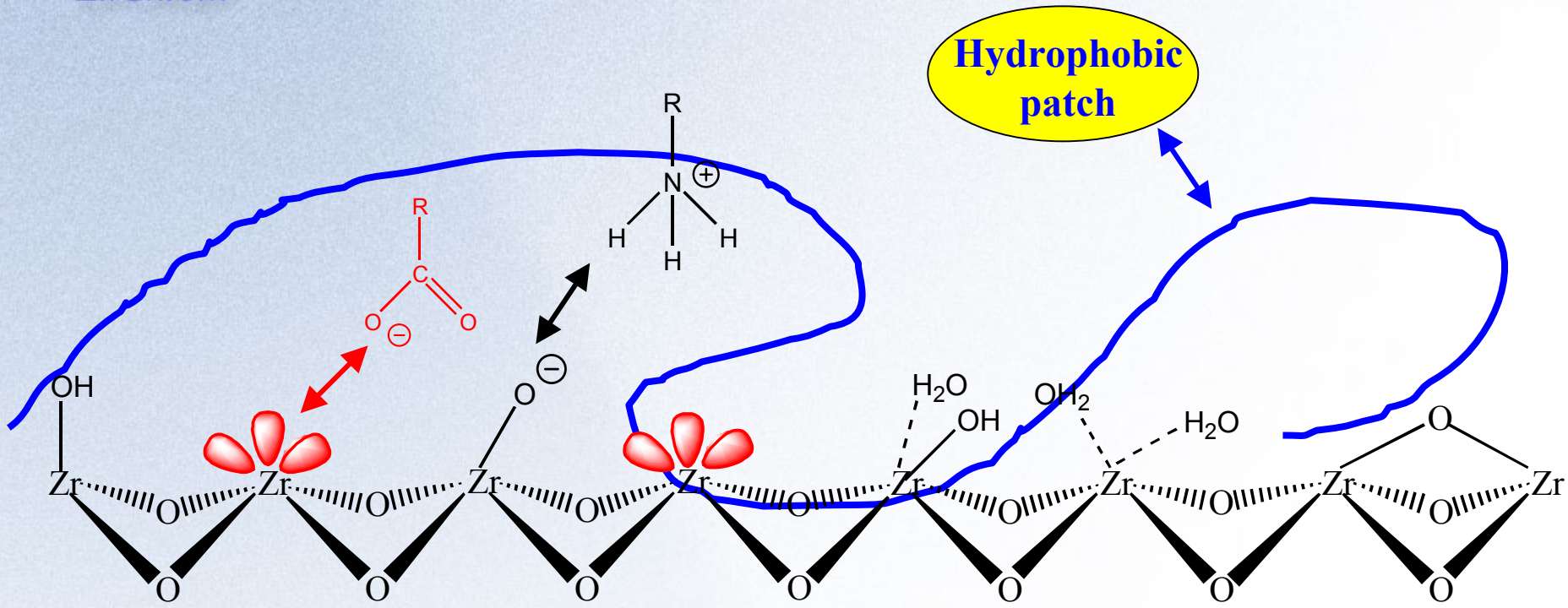
ZirChrom®

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.

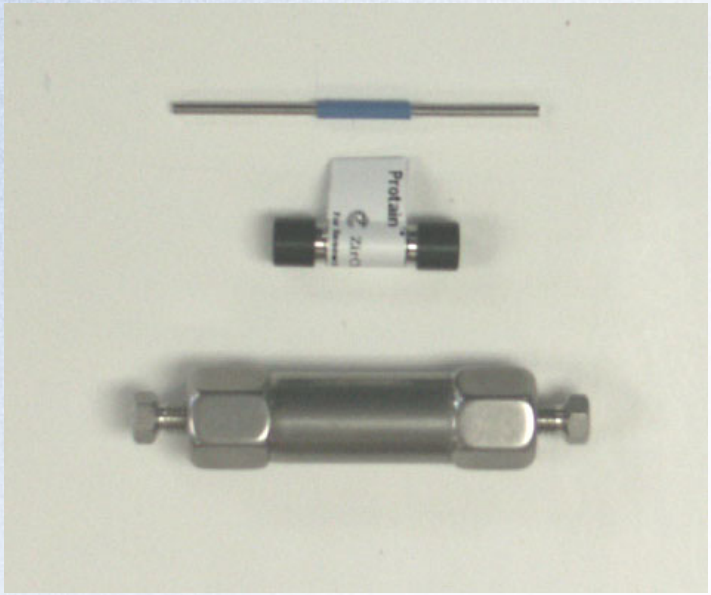
Interactions of Proteins with Polymer Coated Zirconias

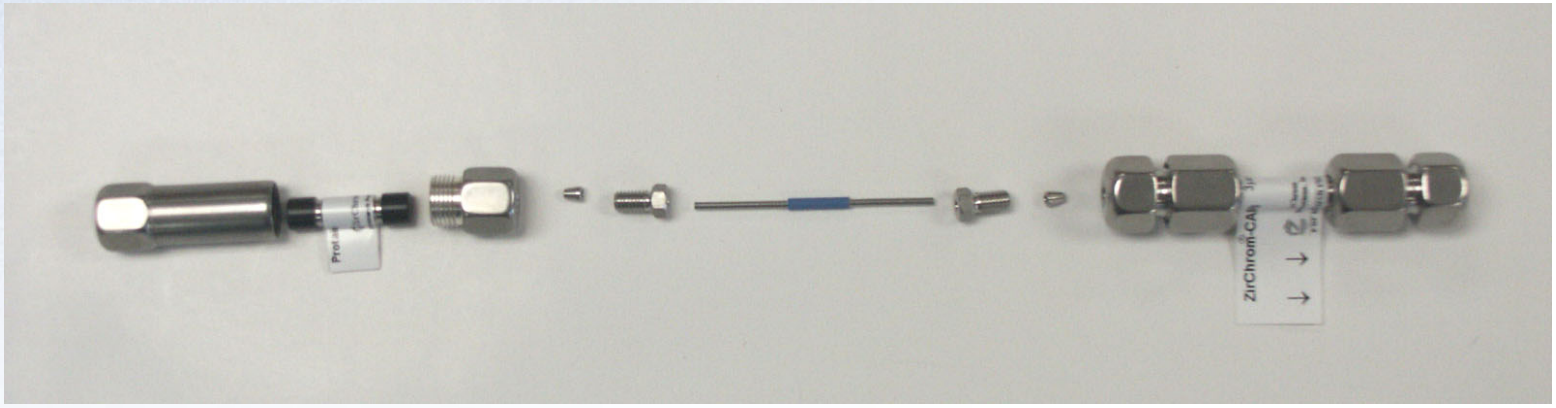


Three interactions acting simultaneously lead to irreversible adsorption of proteins on polymer coated zirconia materials

- Hydrophobic, electrostatic, and ligand exchange interactions

The ProTain™ System



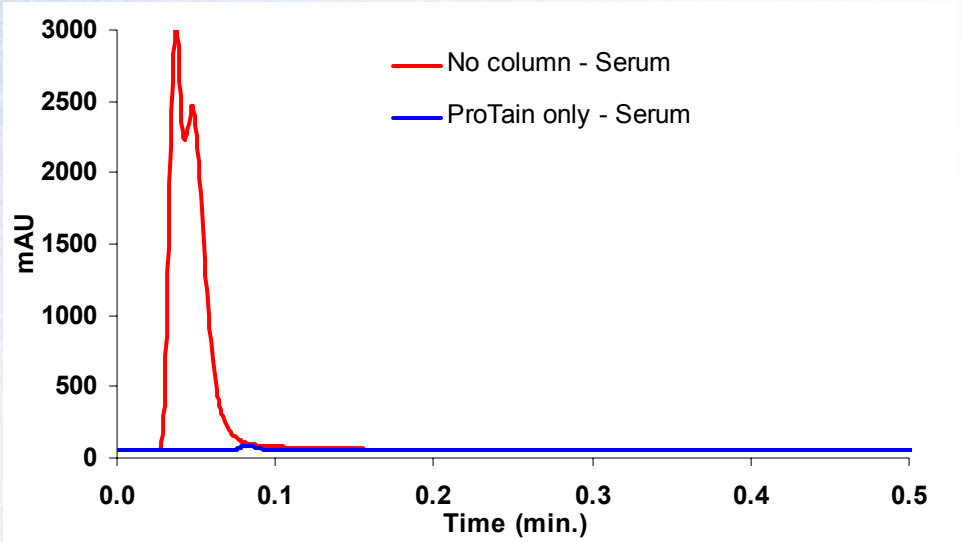


Detection of Basic Pharmaceuticals in Serum by LC/UV

ZirChrom®

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 ammonium acetate, pH 5.0

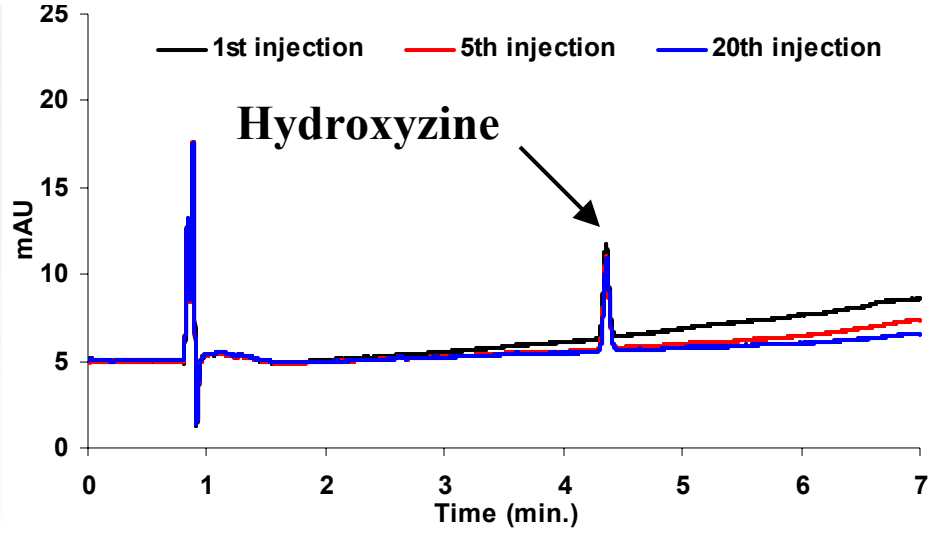
B: ACN

Flow rate: 2.0 ml/min.

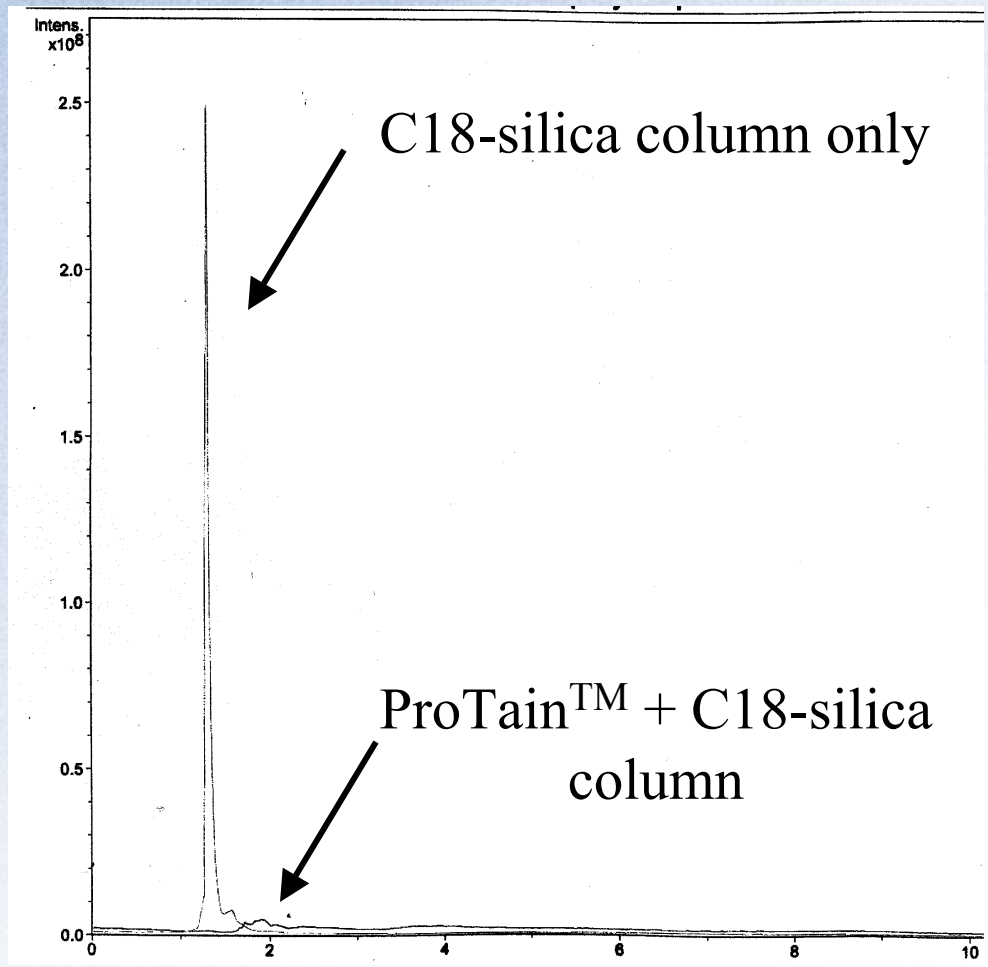
Temperature: 35 °C

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

ProTain™ Loadability Matrix

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.

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

+ = 0 – 0.2 mg ++ = 0.2 – 1.0 mg +++ = 1.0 – 5.0 mg

ProTain™ Applicability Matrix

		Buffer Type			
		TFA	Acetate	Phosphate	Carbonate
Analyte Type	Acidic			✓	✓
	Basic	✓	✓	✓	✓

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