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
- Visit ZirChrom Separations at our booth - # 2203

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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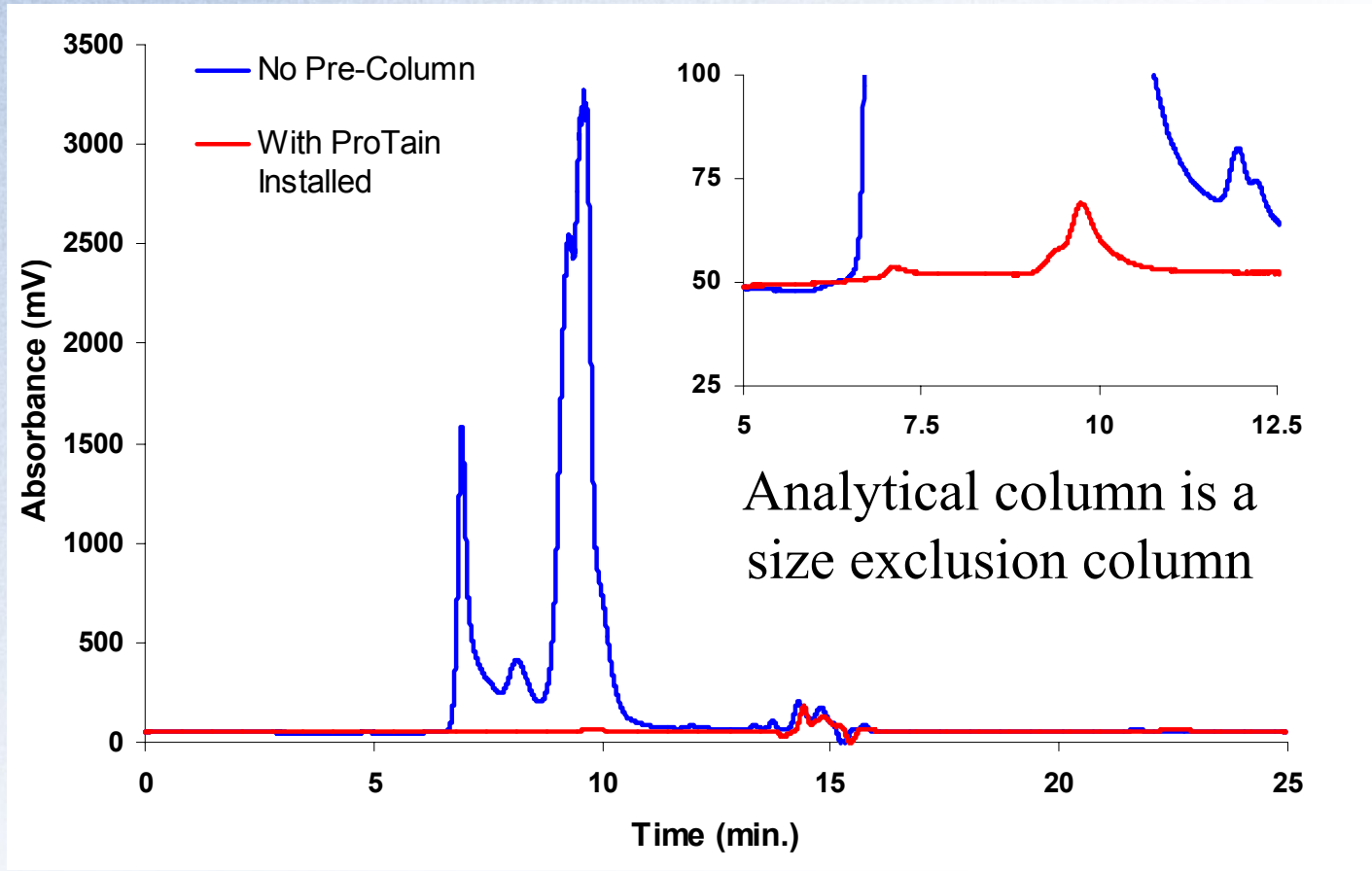
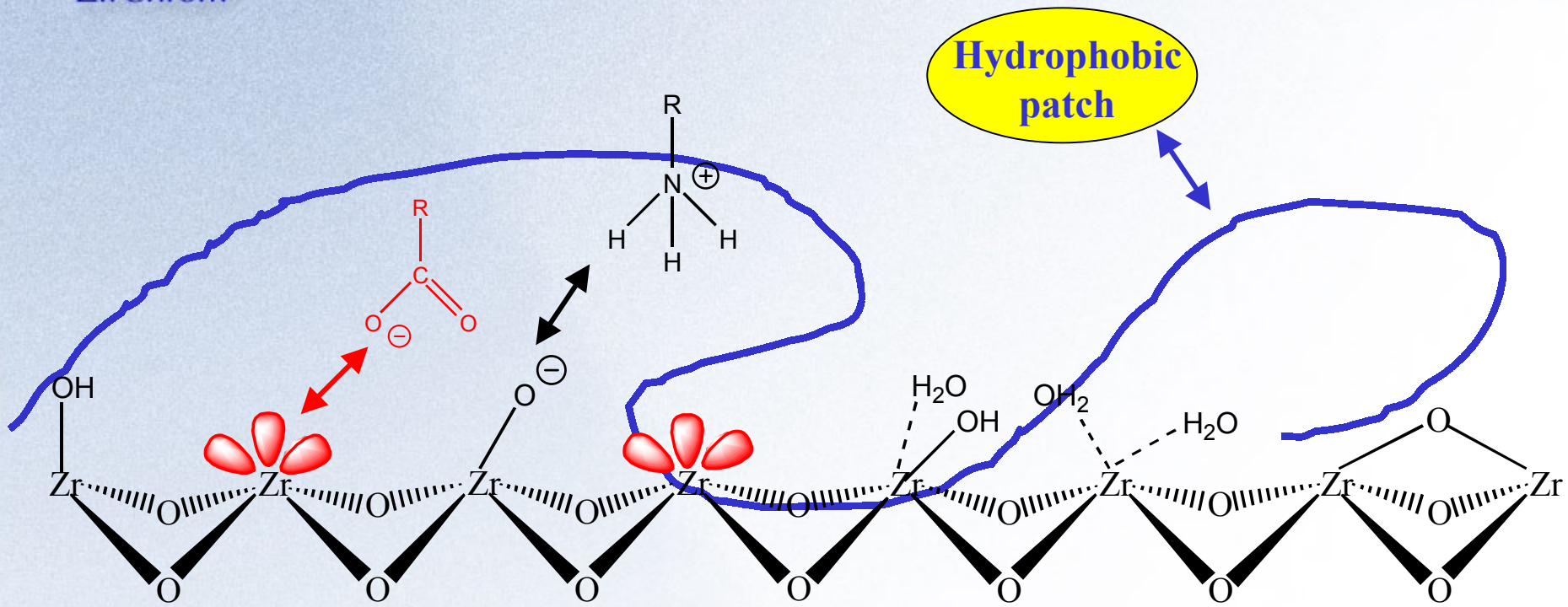
ProTain™ → SEC Column



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

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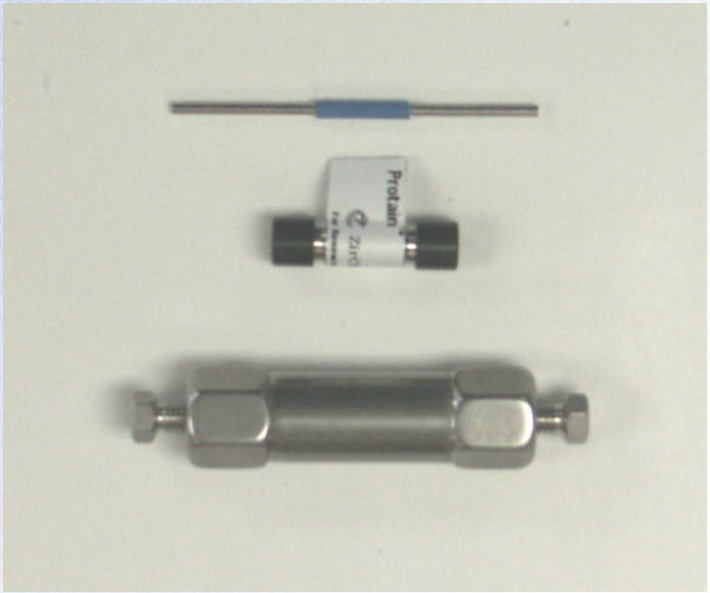
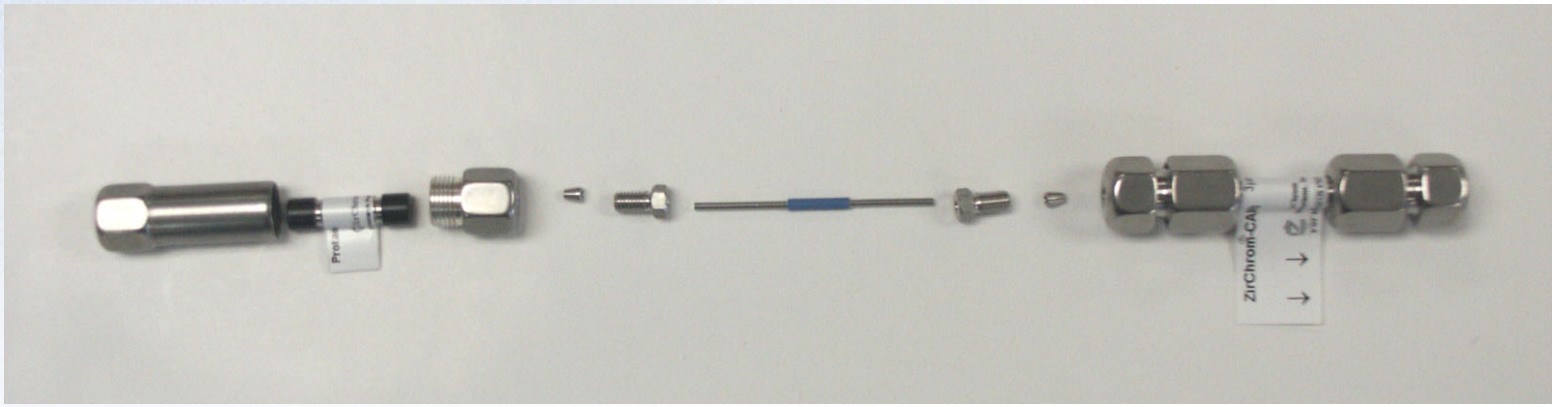
The ProTain™ System





Detection of Basic Pharmaceuticals in Serum by LC/UV

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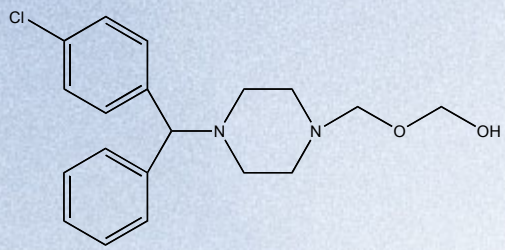
Hydroxyzine



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

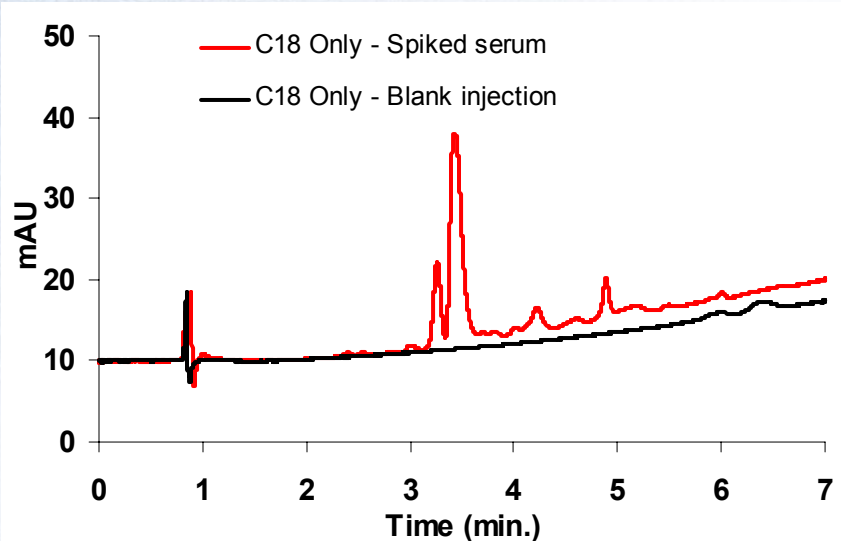
B: 25mM TFA in ACN

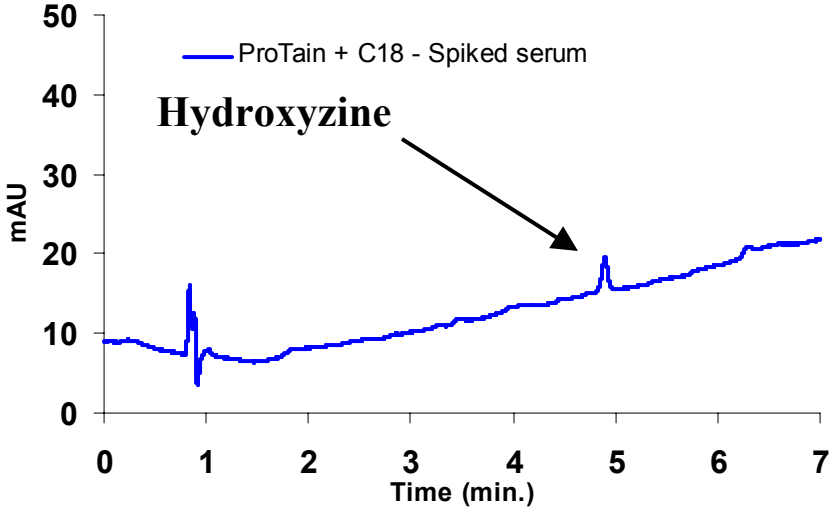
Flow rate: 2.0 ml/min.

Temperature: 35 °C

Injection volume: 10 µl

Detection: UV at 254 nm





Detection of Basic Pharmaceuticals in Serum by LC/UV

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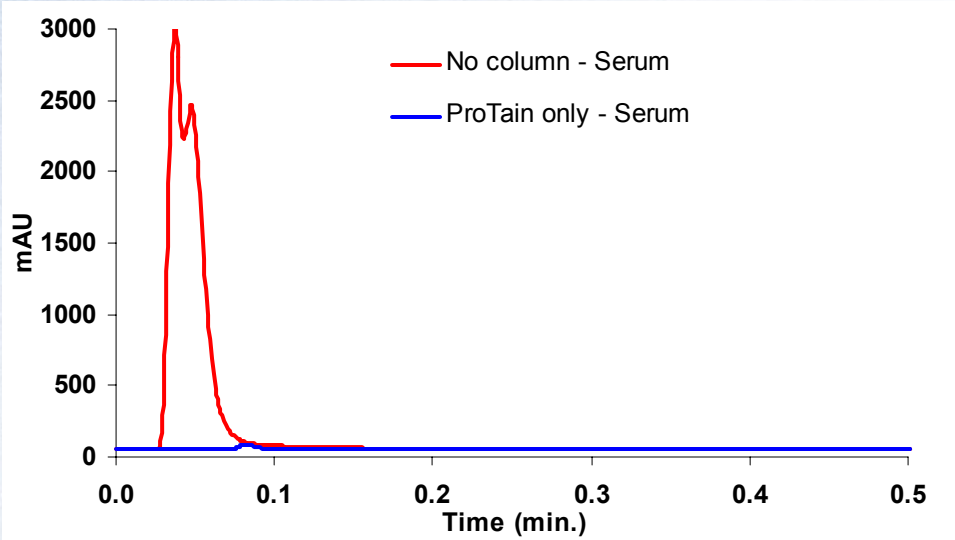
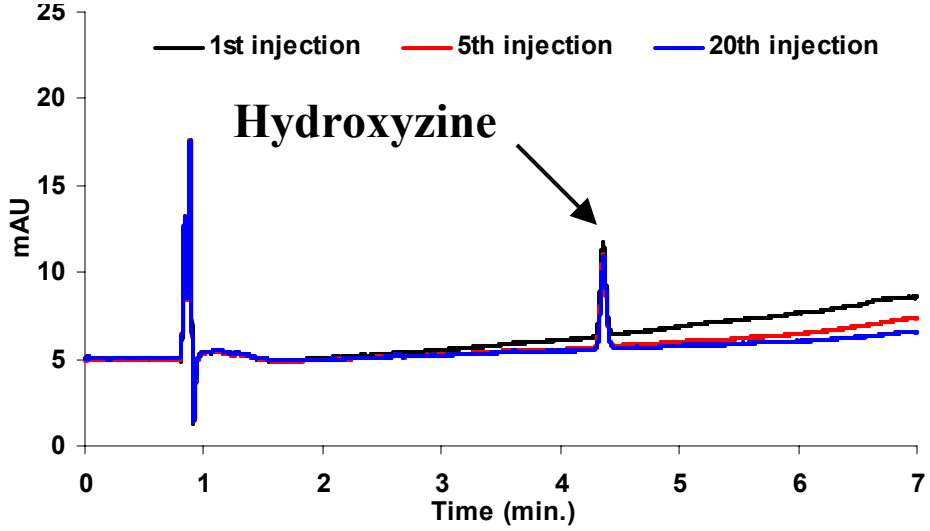
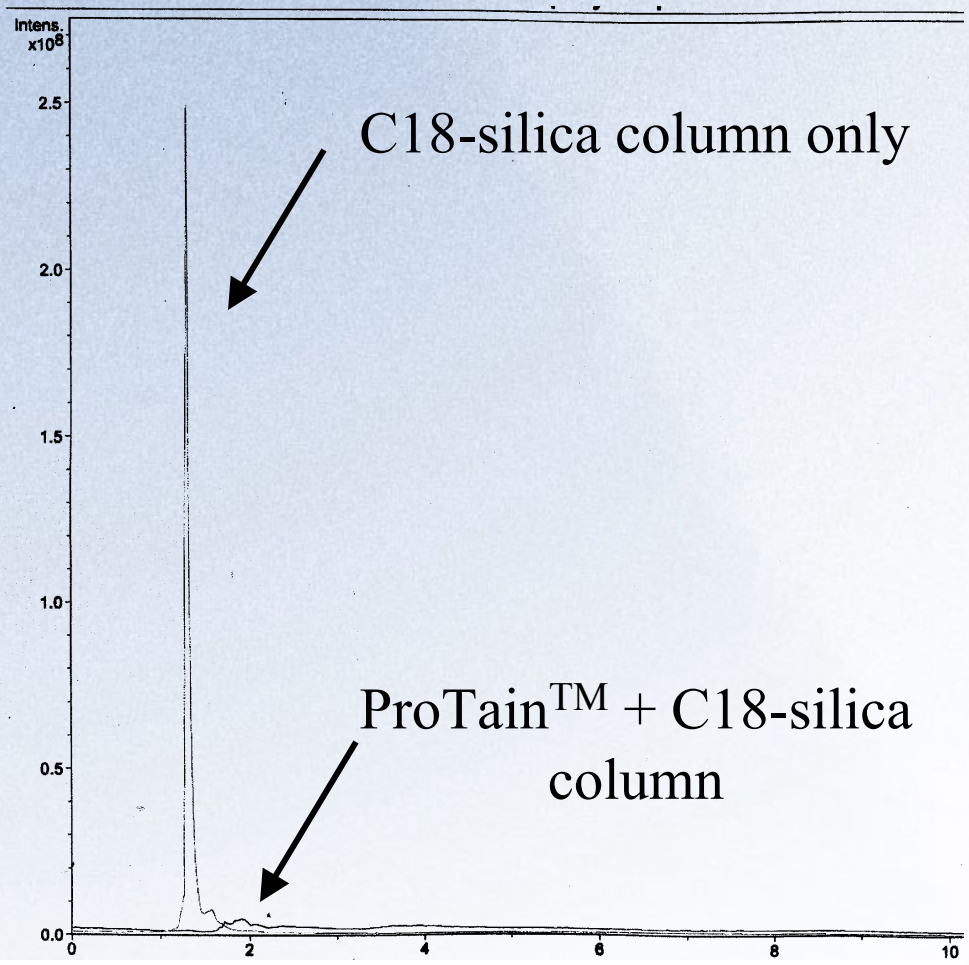




Reduction of Baseline Signal for LC/MS



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

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