ZirChrom®

... For Peak Performance

ZirChrom's ProTain® in-line protein removal system is made up of a holder and a disposable ProTain media insert. The ProTain in-line protein removal system should be installed up-stream from your analytical column.

Parts Needed for Zirchrom's ProTain In-Line Protein Removal System :

ZirChrom part #PT01-0246 or -0221

1 - ProTain® Media Insert

Holder and Assembly Items
ZirChrom part #852-00-2 or 850-00-2

1 - Two Piece Holder
2 - Ferrules
2 - 1/16" Nuts
1 - 2" length of 1/16" OD x 0.01" ID Stainless Steel Capillary Tubing

To order additional inserts or column holders, see reverse.

Assembly Instructions:

1. Remove nuts and ferrules from the ends of the two piece holder (see insert in figure 1). Position items as shown in figure 1.

2. Place ProTain® media insert into the threaded cap (B) of the holder. Insert is bi-directional. Screw threaded cap (B) of holder into the body portion of the holder (A) until finger-tight (see figure 2). Using two 1/2" wrenches tighten holder 30 degrees. ***Note: Overtightening may damage holder threads and will void the system warranty.***

3. Place one 1/16" nut and one ferrule (wide end towards nut) on each end of the capillary tubing (see figure 2).

4. Place one end of the assembled capillary tubing into the threaded cap of the holder. Holding tubing firmly seated into threaded cap tighten nut until finger-tight (See figure 3). Using a 1/2" wrench and a 1/4" wrench tighten connection to leak-tight. Loosen connection and remove to ensure that the ferrule has been seated correctly. Return connection to holder and re-tighten. Repeat procedure with opposite end of the capillary tubing and the inlet of analytical column.

5. As shown in figure 4, connect and appropriately tighten tubing from the injector into the inlet of the Protain® In-Line Protein Removal system (tubing should be 1/16" OD and compatible with analyte and mobile phase). Connect and appropriately tighten tubing from the detector to the outlet of the analytical column.

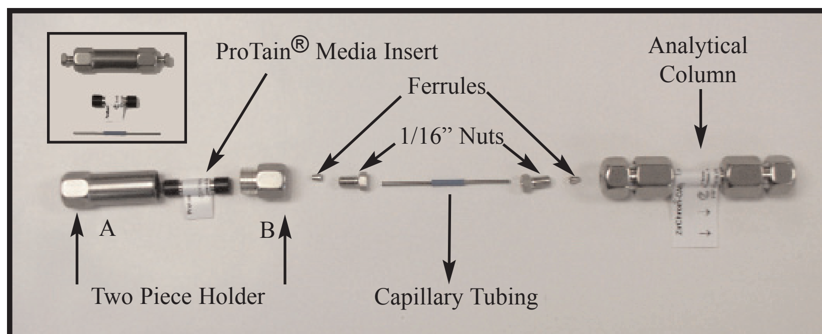


Figure 1: Description of Parts

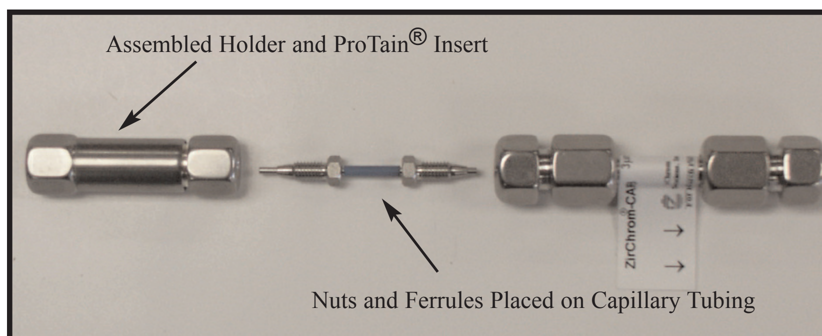


Figure 2: ProTain System Assembly

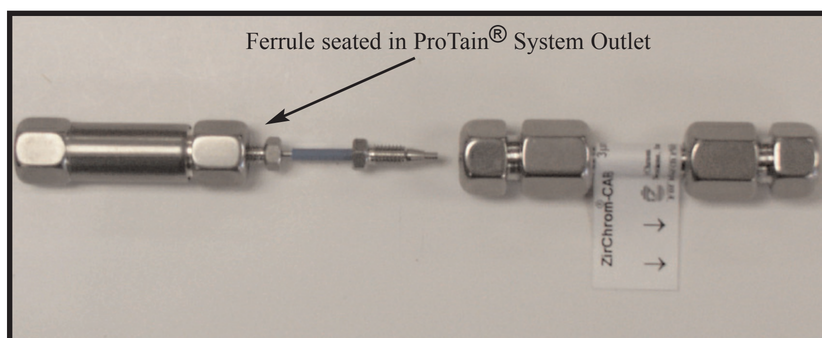


Figure 3: Setting Ferrule into Holder

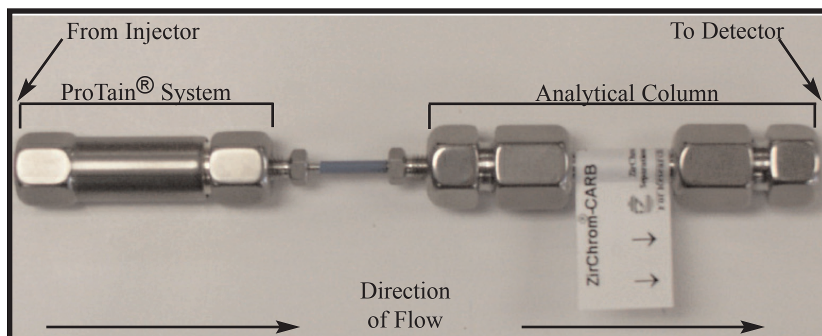
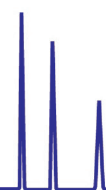


Figure 4: Final Installation



The Effect of Buffer Type and pH on the Capacity of ProTain® as an In-Line Protein Removal System

Clayton McNeff, Ph.D. and Dwight Stoll
ZirChrom Separations, Inc.

Technical Bulletin # 291

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 of the ProTain® system inserts. In this work, a range of pH of 2 - 9, was examined with the following buffers: trifluroracetate, acetate, phosphate, and carbonate.

Introduction

The unique surface chemistry of porous zirconia allows for a strong, three faceted interaction with serum proteins[1]. Electrostatic, ligand exchange, and hydrophilic/hydrophobic interactions all play a role in the material's capacity to absorb proteins. These mole cular interactions are governed both by buffer type and the pH of the mobile phase. Control of both the mobile phase type and pH is imperative in the process of maximizing the capacity of the material. To further investigate these effects, a range of pH, from 2 to 9, was examined with the following buffers: trifluroracetate, acetate, phosphate, and carbonate. Please note that buffers were tested only at pH conditions where there was sufficient buffer capacity. Want more details about buffering capacity? Visit www.zirchrom.com to use our free buffer wizard.

Experimental

A ProTain® 20 mm x 4.6 mm i.d. cartridge and holder was tested without an analytical column on an Agilent 1100 HPLC. After conditioning the cartridge with the desired mobile phase for 15 minutes, repeated 5 µl injections of bovine serum (protein load = 3.5 mg/ml) were made until a protein peak was observed eluting from the cartridge using UV detection. The injections were continued until the protein peak area became constant. The peak area of all of the injections was then used to back calculate how much protein had become adsorbed onto the ProTain® Cartridge. The experimental conditions were as follows:

Column:	ProTain® Cartridge/Holder, 20 mm x 4.6 mm (Part Number: PT01-0246/850-00-2)
Mobile Phase:	50/50 ACN / 20 mM Indicated buffer and pH
Temperature:	30 ºC with Metalox® 200-C column heater
Flow Rate:	1 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 280 nm

Figure 1 shows that the combination of acetate or phosphate and pH conditions between 5 and 7 result in the highest protein capacity. The maximum protein capacity for a 20 mm x 4.6 mm i.d. ProTain® cartridge, under the conditions tested, was 5 mg.

		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 condition.

ZirChrom's ProTain® system can be incorporated in front of any type of analytical column to offer a selective, cost effective, and simple method of reducing matrix interferences for the HPLC analysis of small molecules in bio-samples. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for further details.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

References

1) Sun, L.; Carr, P. W. *Analytical Chemistry* **1995**, *67*, 2517-2523

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Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.

In-Line Removal of Matrix Proteins in HPLC Analysis of Small Molecules

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Technical Bulletin #275

The HPLC analysis of small molecules in matrices containing proteins of variable origin is often problematic because of poor resolution between the analyte of interest and the matrix constituents and potential fouling of the analytical column by matrix proteins and debris. This application note demonstrates the simple but highly effective removal of matrix proteins from samples using ZirChrom's ProTain® in-line protein removal system installed in front of an analytical HPLC column.

Introduction

It is known that zirconia-based reversed-phase materials adsorb polypeptides and proteins in an irreversible manner through multimodal interactions including hydrophobic, electrostatic and Lewis acid-base interactions (1). These effects make it difficult and often impractical to perform reversed-phase analyses of proteins and large peptides using zirconia-based reversed-phase supports. This application note demonstrates the ability to capitalize on what was once considered a drawback of zirconia-based material to remove matrix proteins from samples containing small molecules of interest to simplify their analysis by HPLC.

Matrix interferences due to proteins in the analysis of small molecules is a problem in reversed-phase HPLC both with UV/Vis detection, and with detection by mass spectrometry. In the case of UV/Vis detection, matrix proteins often elute early in the chromatogram and obscure the rest of the chromatogram, either hiding small peaks for analytes of interest, or interfering with quantitation in general. When using mass spectrometry detection, the elution of matrix proteins during the elution of analytes of interest can potentially cause ion-suppression effects, ultimately leading to serious problems with quatitation. The elimination of these effects often requires intensive off-line sample preparation to try to remove as many undesirable matrix constituents as possible. The method described here allows for the nearly quantitative removal of bovine serum albumins in an in-line process that does not require any off-line sample preparation.

Experimental

Samples of Bovine Serum Albumin (BSA) were prepared in a phosphate buffer at pH 6.8 and injected onto two column configurations:

A: TSK G3000 Size Exclusion Column

B: ZirChrom's ProTain® in-line protein removal system (Holder - part # 850-00-2; Set of three inserts- part # PT01-0246) installed in front of the TSK G3000 SEC column

A mobile phase consisting of 10mM Phosphate buffer at pH 6.8 was pumped through the columns at 1.0 ml/min. while monitoring the effluent with a UV detector at 215 nm. The chromatograms in Figure 1 show that the injection of BSA onto

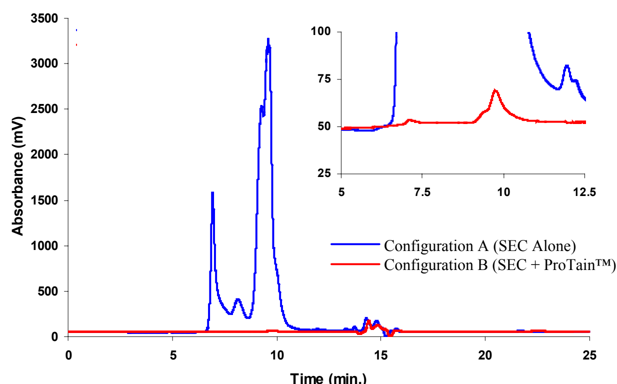

Figure 1. UV traces at 215 nm of the elution of BSA from a SEC column with and without ZirChrom's ProTain® system installed.

the SEC column yields large protein peaks in the 6-11 minute range, with smaller solvent disturbances observed at approximately 14-15 minutes. It is clear from these traces that the majority of the BSA is removed with ZirChrom's ProTain® system installed as indicated by the flat region of the red trace from 6-11 minutes. The same figure is shown in the inset plot where the absorbance scale has been expanded to allow for a closer view of the baseline. As shown in the expanded view it is apparent that the BSA has been almost completely removed as only one very small peak is observed at 10 minutes.

In summary, the incorporation of ZirChrom's ProTain® system in front of any type of analytical column offers a selective, cost effective, and simple method of reducing matrix interferences for the HPLC analysis of small molecules in bio-samples. Be sure to contact ZirChrom's technical support specialists for more information on the use of this new approach to in-line sample preparation.

References

(1) Sun, L.; McCormick, A. V.; Carr, P. W. *J. Chrom. A* **1994**, *658*, 465-473

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