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

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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 are 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

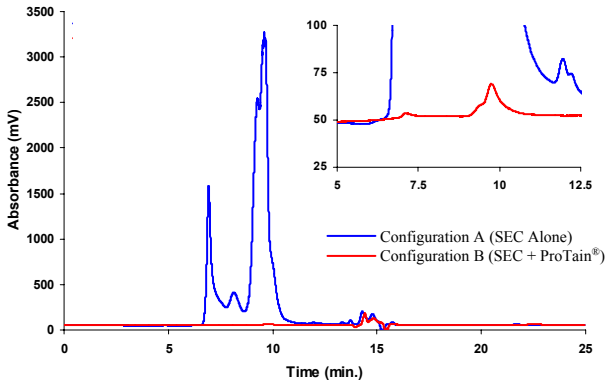

Figure 1. UV traces at 215 nm of the elution of BSA from a SEC column with and without ZirChrom's ProTain® system installed.

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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