

Automated On-Line SDS Removal from Minute Proteinaceous Samples Prior to Capillary HPLC

INTRODUCTION

Reversed-phase high performance liquid chromatography is a widely applied method for the separation of complex protein and peptide mixtures. However, protein/peptide samples are often at low concentration and contain buffers and/or detergents, which hamper LC and LC/MS analysis. Commonly used purification and concentration techniques often fail in quantitatively recovering the compounds of interest and are therefore not accessible to minute sample volumes. Furthermore, these techniques are very time consuming and cannot be coupled easily with identification methods such as MS.

RESULTS AND DISCUSSION

Using a fully automated on-line microcolumn switching method (Figure 1), ionic detergents can be completely removed from proteinaceous samples. The system includes two micro precolumns: a specific ionic detergent trapping column and a preconcentration column. Loading and clean-up of the samples, as well as regeneration of the detergent trapping column, was performed at 50 $\mu\text{L}/\text{min}$, resulting in a sample preparation time of only 30 s.

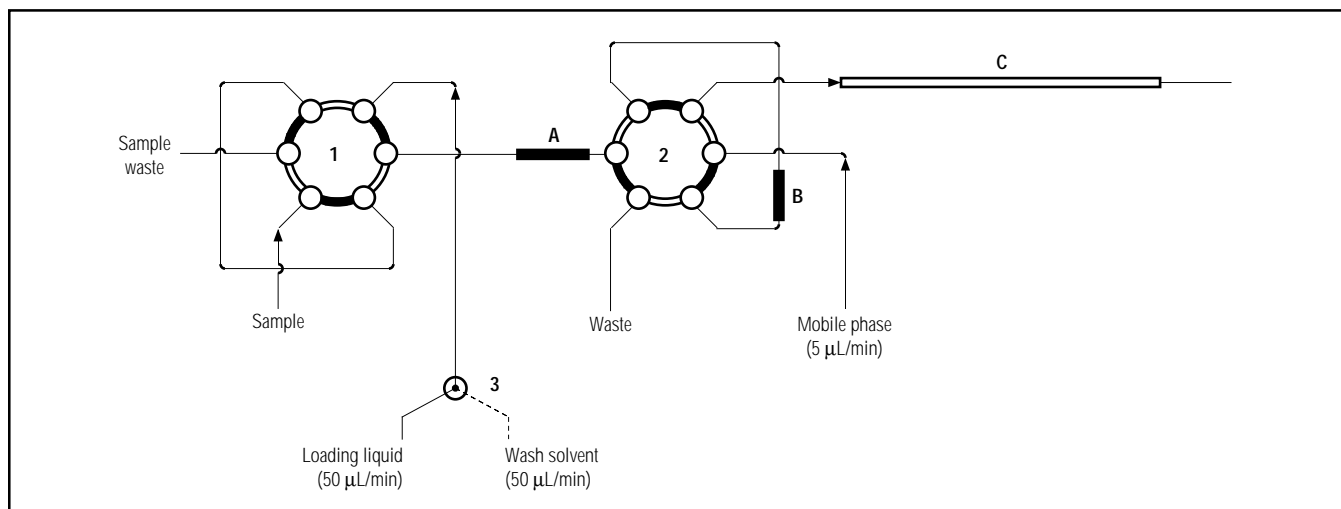


Figure 1. System set-up: 1) sampling valve, 2) pre-concentration valve, 3) solvent selection valve, A) SDS trapping column, B) preconcentration column, and C) micro analytical column.

Tryptic digested protein samples containing SDS were directly applied to the micro precolumns without any sample pretreatment. Characterization of the proteinaceous samples after removal of the ionic detergents was performed by Capillary liquid chromatography with UV absorption detection and electrospray mass spectrometry. The developed technique minimizes the risk of sample loss since no additional sample preparation steps are required.

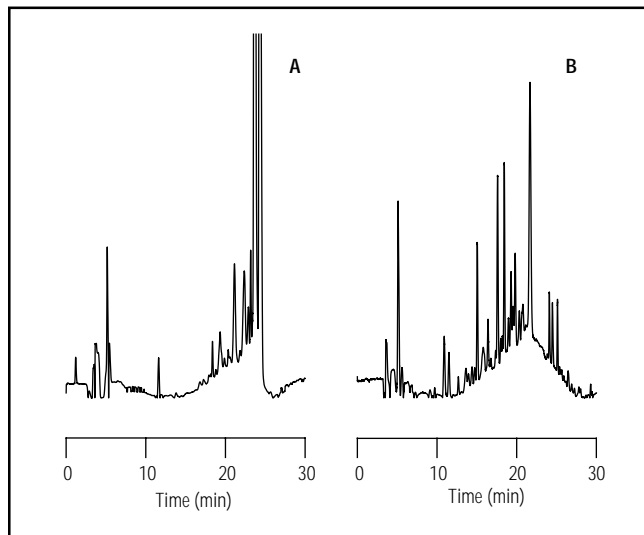


Figure 2. Tryptic digest of α -Lactalbumin B: A) in presence of SDS, and B) after removal of SDS

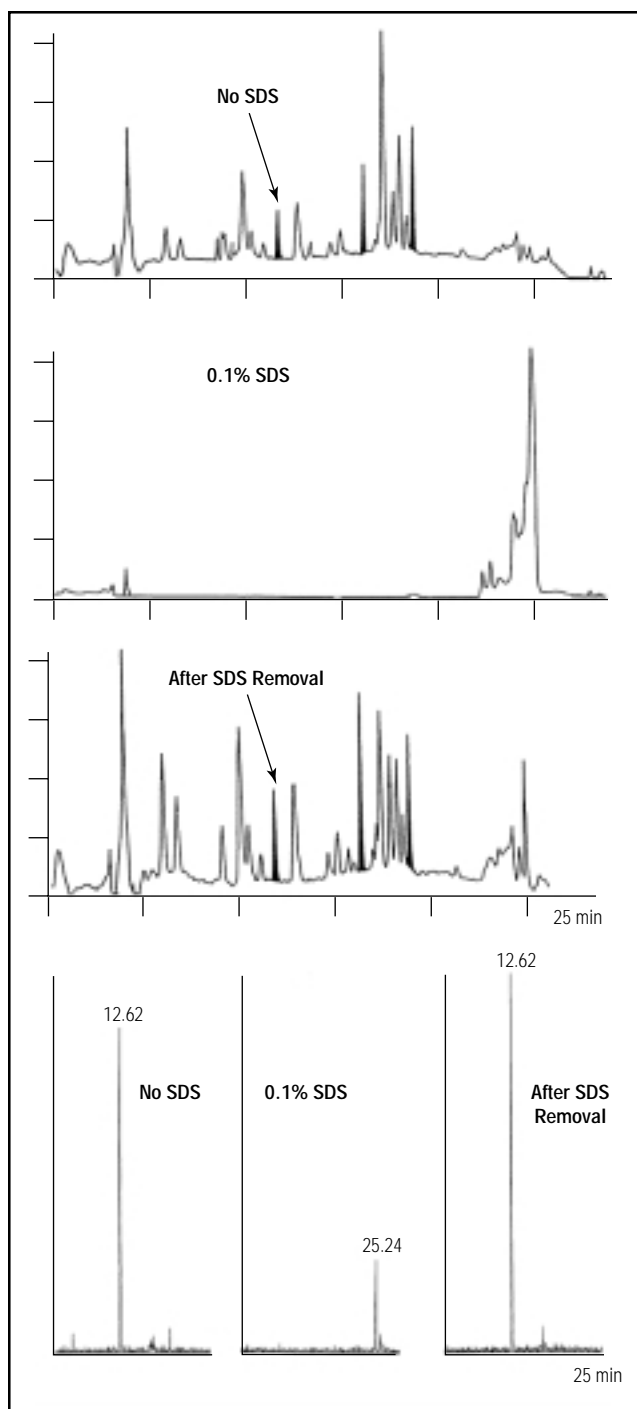


Figure 3. Sample clean-up prior to ESI/MS showing SDS removal of minute protein samples.

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