

A Rapid Method for Removal of Detergents from Proteins and Protein Digests in a Spin Column Format

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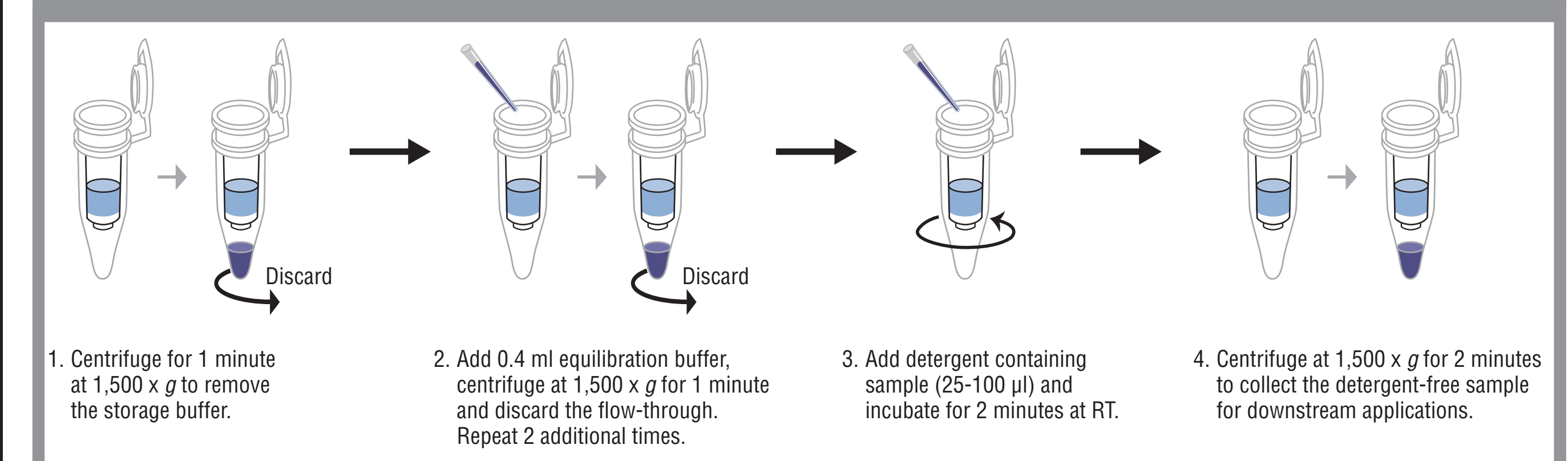
Abstract

We have developed a high-performance resin that offers exceptional detergent removal for proteins and peptides. The easy-to-use spin format dramatically improves results over standard drip column or batch methodologies with >95% removal from 1-5% solutions of detergents such as SDS, sodium deoxycholate, CHAPS, Triton X-100, Triton X-114, NP-40, Brij-35, octyl glucoside, octyl thioglucoside and lauryl maltoside with high recovery of proteins and peptides. Post-column LC-MS/MS analysis of BSA and Hela cell lysate trypsin digests followed by analysis of the data using MASCOT revealed excellent sequence coverage, and the high MASCOT scores obtained indicate successful removal of detergent from the digested peptides. MALDI-MS analysis of unprocessed and processed samples further confirmed efficient removal of detergents. The advantages of this method include speed (less than 15 minutes), efficient detergent removal, and high recovery of proteins/peptides.

Introduction

Detergents or surfactants play a significant role in protein chemistry, principally to solubilize and stabilize proteins and to disaggregate protein complexes. Membrane-bound proteins, in particular, frequently require detergent treatment for solubilization. However, the presence of excess unbound detergent interferes with many downstream applications like ELISA, isoelectric focusing and mass spectrometry. Protein chemists are often faced with the challenge of removing detergents from protein/protein digest samples prior to quantitative analysis via ESI or MALDI. The presence of detergents usually causes a deleterious effect by forming adducts with peptides and proteins, producing a shift in m/z values or noisy baselines. Sample clean-up before mass spectrometry is essential to avoid the expense of cleaning the ion source and substantial instrument down time. We describe a simple, efficient and rapid method for the removal of detergents from protein/protein digest solutions. The method involves a 2-minute centrifugation on Thermo Scientific Pierce Detergent Removal Resin in a microcentrifuge tube.

Protocol Summary for Thermo Scientific Pierce Detergent Removal Resin



PART I : Removal of Detergents from Proteins

Detergent Removal Efficiency and Protein Recovery

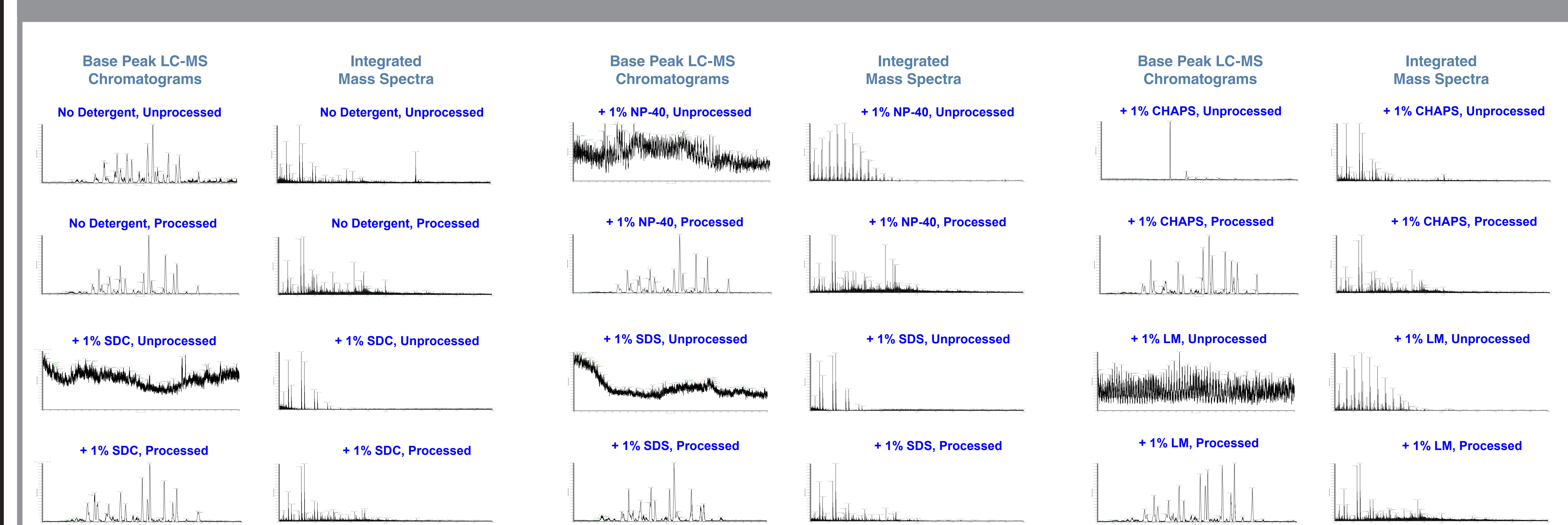
| Detergent and Removable Concentration (%) | Detergent Removal (%) | BSA Recovery (%) |
|---|-----------------------|------------------|
| SDS (2.5) | 99 | 95 |
| Sodium Deoxycholate (5) | 99 | 100 |
| CHAPS (3) | 99 | 90 |
| Octyl Glucoside (5) | 99 | 90 |
| Octyl Thioglucoside (5) | 99 | 95 |
| Lauryl Maltoside (1) | 98 | 99 |
| Triton X-100 (2) | 99 | 87 |
| Triton X-114 (2) | 95 | 100 |
| NP-40 (1) | 95 | 91 |
| Brij-35 (1) | 99 | 97 |

| Protein | SDS | | Triton X-100 | | CHAPS | |
|---------------------------------|-------------|----------------------|--------------|----------------------|-------------|----------------------|
| | Removal (%) | Protein Recovery (%) | Removal (%) | Protein Recovery (%) | Removal (%) | Protein Recovery (%) |
| Insulin (5.7 kD) | 96 | 84 | 98 | 89 | 99 | 84 |
| α -Lactalbumin (14.2 kD) | 96 | 92 | 97 | 86 | 99 | 84 |
| Carbonic Anhydrase (29 kD) | 96 | 92 | 100 | 89 | 99 | 88 |
| Hela Cell Lysate | 98 | 85 | ND | ND | ND | ND |

Each sample (0.1 ml) containing protein (1mg/ml) + detergent in 0.15 M NaCl, 0.05% sodium azide solution was processed through 0.5 ml of Pierce Detergent Removal Resin as described in the protocol. Residual SDS was measured by the colorimetric method using Stains-All dye [1]; Triton X-100, Triton X-114 and NP-40 were measured by absorbance at 275 nm (protein absorbance was subtracted); Sodium Deoxycholate, CHAPS, Octyl Glucoside, Octyl Thioglucoside and Lauryl Maltoside were measured by colorimetric method using concentrated sulfuric acid and phenol [2]. Removal of Brij-35 was monitored by LC-MS/MS and MALDI-MS analysis. The Thermo Scientific Pierce BCA Protein Assay was used for protein determination. Values in parenthesis represent maximum removable detergent concentration.

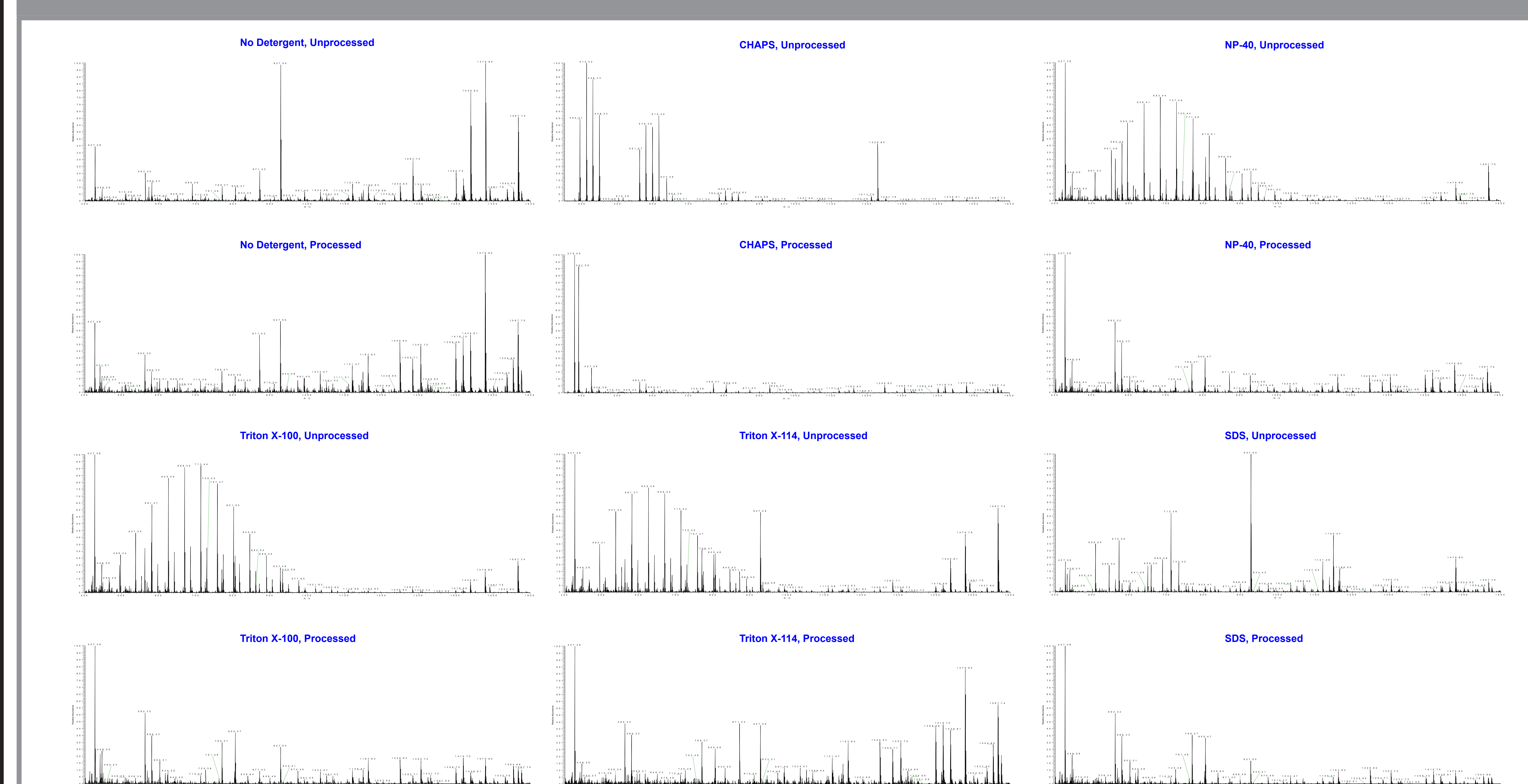
PART II: Removal of Detergents from Protein Digests for Mass Spectrometry Analysis

LC-MS/MS Analysis of BSA Tryptic Peptides



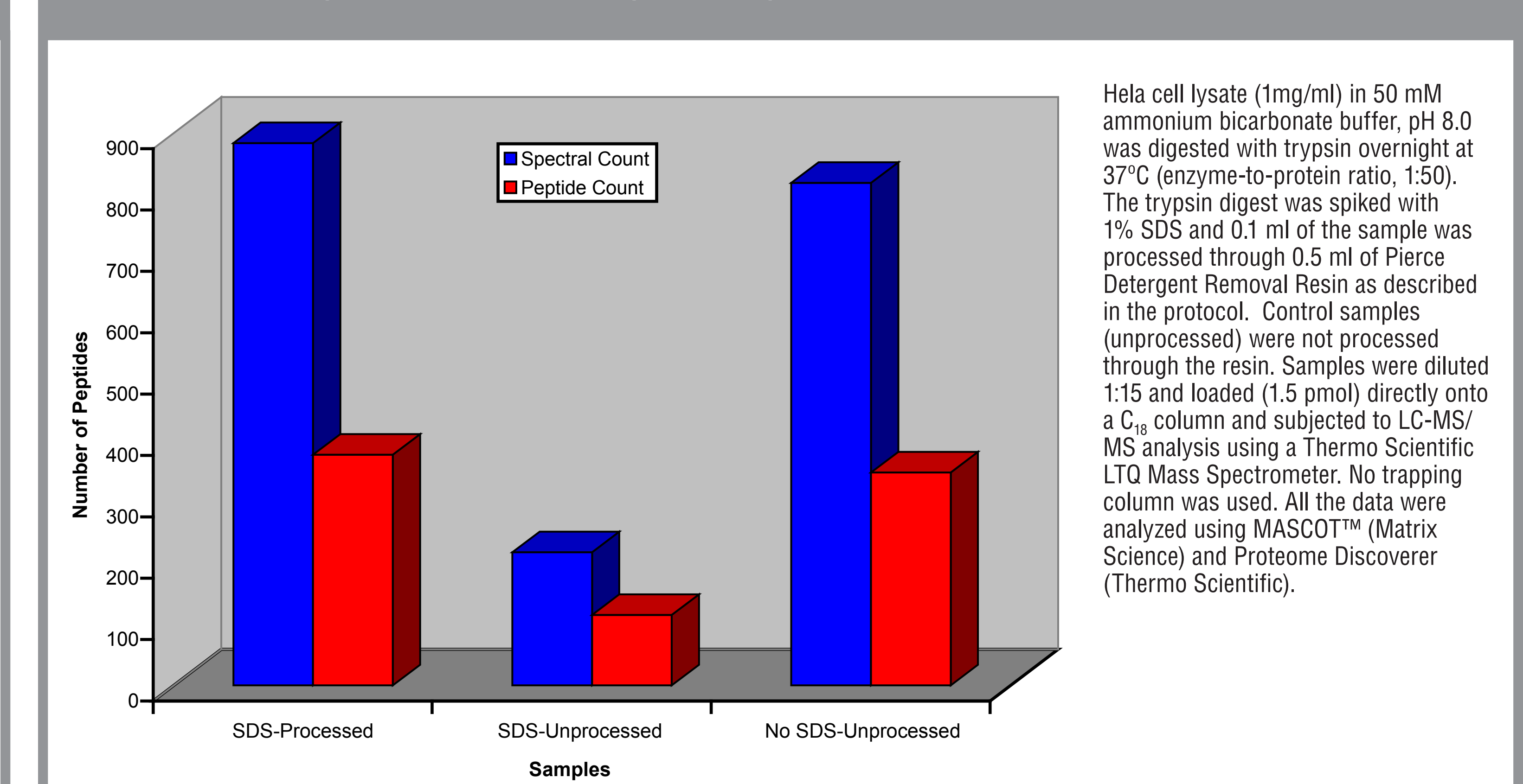
BSA (1mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin overnight at 37°C (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. Each digested sample (0.1 ml) containing the detergent was processed through 0.5 ml of Pierce Detergent Removal Resin as described in the protocol. Control samples (unprocessed) were not processed through the resin. Samples were diluted 1:15 and loaded (1.5 pmol) directly onto a C_{18} column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. No trapping column was used. All the data were analyzed using MASCOT™ (Matrix Science). The base peak LC-MS chromatograms are shown on the left panel and the integrated mass spectra are shown on the right panel.

MALDI-MS Spectra of BSA Tryptic Peptides



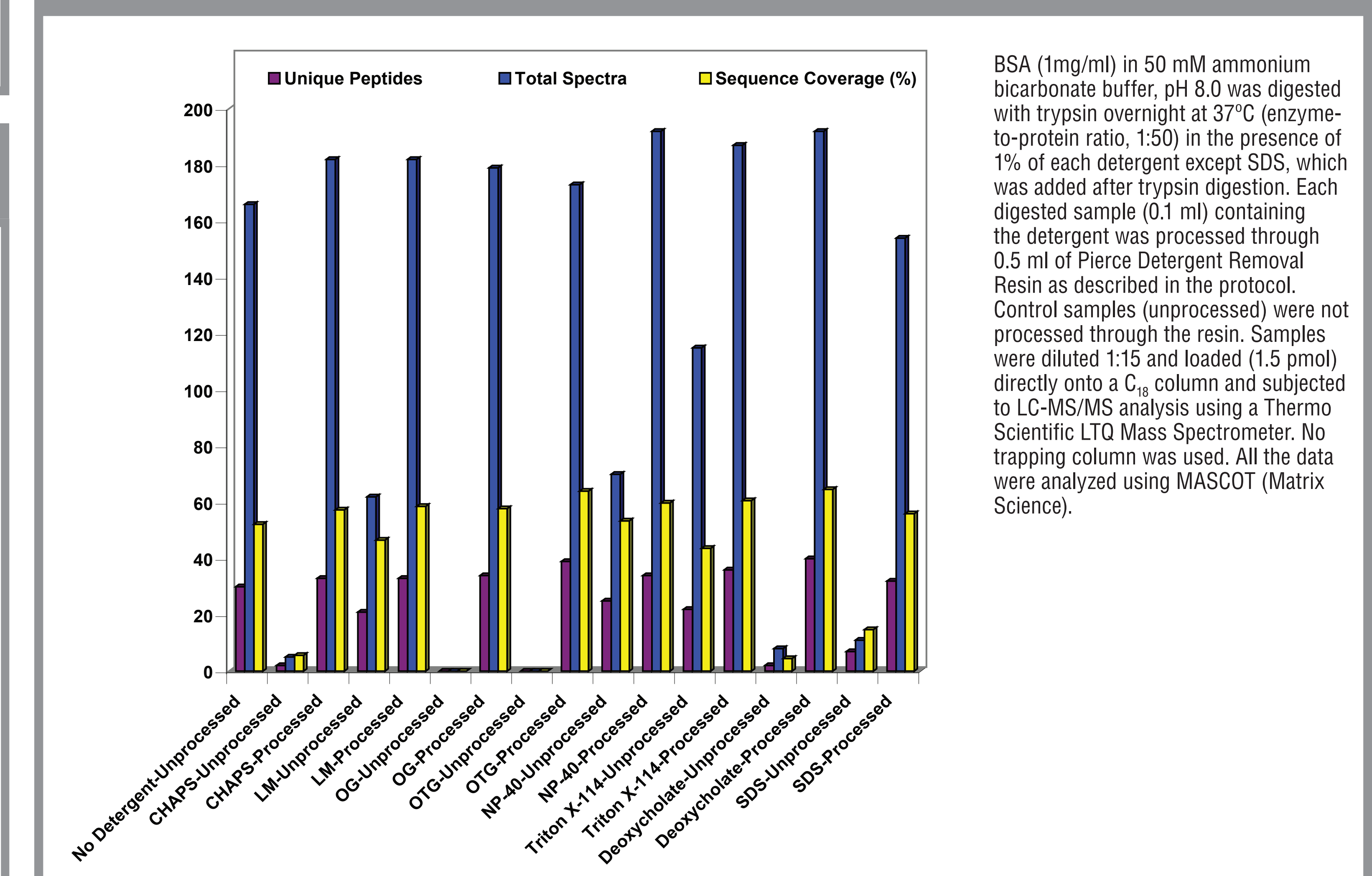
BSA (1mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin overnight at 37°C (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. Each digested sample (0.1 ml) containing the detergent was processed through 0.5 ml of Pierce Detergent Removal Resin as described in the protocol. Control samples (unprocessed) were not processed through the resin. Samples were diluted 1:15 and loaded (1 pmol) on to a Thermo MALDI-Orbitrap Mass Spectrometer. Apha-cyano 4-hydroxy cinnamic acid (5 mg/mL) was used a matrix with acetonitrile/water/ 0.1% TFA as a co-solvent. Data were processed with Xcalibur Qual Browser 2.0.

LC-MS/MS Analysis of Hela Cell Lysate Tryptic Peptides



Hela cell lysate (1mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin overnight at 37°C (enzyme-to-protein ratio, 1:50). The trypsin digest was spiked with 1% SDS and 0.1 ml of the sample was processed through 0.5 ml of Pierce Detergent Removal Resin as described in the protocol. Control samples (unprocessed) were not processed through the resin. Samples were diluted 1:15 and loaded (1.5 pmol) directly onto a C_{18} column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. No trapping column was used. All the data were analyzed using MASCOT™ (Matrix Science) and Proteome Discoverer (Thermo Scientific).

LC-MS/MS Analysis of BSA Tryptic Peptides – Comparison Between Processed and Unprocessed Samples



BSA (1mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin overnight at 37°C (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. Each digested sample (0.1 ml) containing the detergent was processed through 0.5 ml of Pierce Detergent Removal Resin as described in the protocol. Control samples (unprocessed) were not processed through the resin. Samples were diluted 1:15 and loaded (1.5 pmol) directly onto a C_{18} column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. No trapping column was used. All the data were analyzed using MASCOT (Matrix Science).

Conclusions

The Thermo Scientific Pierce Detergent Removal Resin:

- Efficiently removes detergents (>95%) from proteins and peptides samples
- Provides high recovery of proteins and peptides
- Eliminates detergent interference in down stream applications like ELISA, isoelectric focusing and mass spectrometry
- Removes detergents in sample volumes ranging from 10 μ l to 1 ml
- Spin-column format is simple, efficient and fast, taking less than 15 minutes for the entire process

Acknowledgements

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References

1. F. Rusconi, E. Valton, R. Nguyen, E. Dufoure, Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal Biochem.* 295 (2001) 31-37.
2. A. Urbani and T. Warne, A colorimetric determination for glycosidic and bile salt-based detergents: applications in membrane protein research. *Anal Biochem.* 336 (2005) 117-124.

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