Detergent removal using Agilent Bond Elut OMIX SCX pipette tips

Application Note

Authors

William C Hudson and
Jennifer M Massi

Agilent Technologies, Inc.

Introduction

Ion suppressing salts and detergents can severely compromise MS analyses. Pipette tip clean-up is a simple and effective method for removing these interferences from samples prior to mass spectrometry. Reversed-phase resins like C18 are very effective for salt removal, but can be ineffective in removing detergents. Strong cation exchange (SCX) materials are much more proficient in removing detergents, in addition to providing an effective alternative for salt removal and sample concentration.

Effectiveness of detergent removal was investigated using various methanol washes on Bond Elut OMIX SCX 10 µL pipette tips. The methods described offer a simple and efficient solution for removing detergents from protein digests. Digests or other samples containing detergents, salts, or other ion-suppressing interferences can be prepared in less than five minutes. The monolithic nature of Bond Elut OMIX provides good capacity of the peptides of interest while maintaining superb flow characteristics.

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Method

For each extraction, 200 fmols of bovine serum albumin tryptic digest (Michrom Bioresources, Inc.) were re-suspended to a final volume of 10 µL in 0.1% TFA and 0.5% of either Tween20, CHAPS, or TritonX-100 (Sigma Aldrich). For each sample, a Bond Elut OMIX pipette tip was conditioned and equilibrated by setting the pipettor at 10 µL, securely attaching a Bond Elut OMIX SCX 10 µL tip, and aspirating and expelling 10 µL of 0.1% TFA washing solution. This step was performed a total of three times with fresh 0.1% TFA washing solution each time. With the pipette plunger still depressed, samples were loaded by aspirating and dispensing four times. The tips were then washed four times with washing solution containing either 100%, 50%, 30% or no methanol (see materials section), using fresh solution for each wash cycle. Samples were eluted with 2 µL of 5% ammonium hydroxide/30% methanol elution solution and analyzed by MALDI-TOF MS.

Table 1. *Bond Elut OMIX SCX 10 µL tip method*

Pretreat sample	Adjust sample to ~ 0.1% trifluoroacetic acid (TFA) concentration.
Condition and equilibrate tip	Aspirate 10 µL of 0.1% TFA and discard solvent. Repeat 4 times.
Bind sample	Aspirate up to 10 µL of pre-treated sample into tip. Dispense and aspirate sample 3 to 5 cycles for maximum efficiency. Up to 10 cycles may be used for improved binding.
Purify	Aspirate 10 µL of 0.1% TFA in 50 to 100% methanol and discard solvent. Repeat 3 to 4 times.
Elute	For MALDI-TOF analysis: Aspirate 2 to 10 µL of 5% ammonium hydroxide in 30% methanol and dispense directly onto a MALDI plate. Add matrix. For LC/MS/MS analysis: Aspirate 2 to 10 µL of 5% ammonium hydroxide in 30% methanol and dispense. Dilute with acid or dry down and reconstitute in mobile phase before analysis.

Materials

Bond Elut OMIX SCX pipette tips (part number A5700410 or A5700410K)
10 µL pipettor

Washing solutions:	0.1% trifluoroacetic acid (TFA) 30% methanol, 0.1% TFA in Milli-Q grade water 50% methanol, 0.1%TFA in Milli-Q grade water 0.1% TFA in 100% methanol
Elution soution:	25% ammonium hydroxide/30% methanol in Milli-Q grade water

Results

BSA tryptic digest samples containing 0.5% detergent were directly spotted with no clean-up and analyzed by MALDI-TOF mass spectrometry followed by a SwisProt protein database search using Mascot. As expected, interference from the detergents made protein identification impossible (Figure 1).

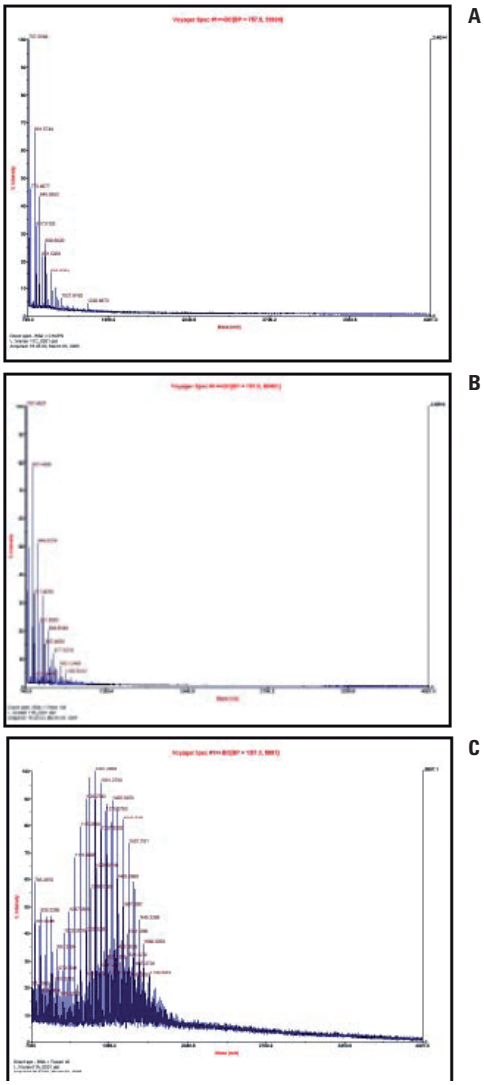

Figure 1. *BSA digest analysis by MALDI MS with no clean-up. 9 µL of a 200 fmol/µL BSA tryptic digest was mixed with 1 µL of 5% detergent and spotted onto a MALDI plate. (A) 0.5% CHAPS, (B) 0.5% TritonX-100, (C) 0.5% Tween20. Results were searched against the SwisProt database using Mascot. As expected, interference from the detergents made protein identification impossible.*

The same samples were extracted using 10 µL Bond Elut OMIX SCX pipette tips and various methanol washes. While using a 30% methanol washing solution led to correct protein identification from the CHAPS and TritonX-100 samples, a 50% methanol washing solution more effectively removed the detergents, resulting in better Mascot scores and sequence coverage (Figure 2).

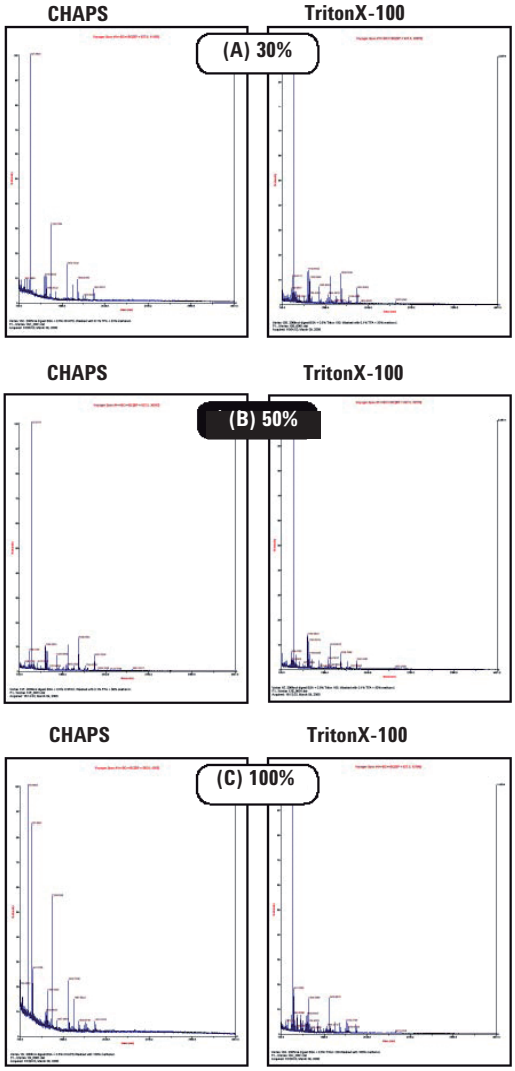

Figure 2. *Effect of methanol concentration in washing solution on detergent removal and protein identification. 200 fmols of BSA tryptic digest were re-suspended to a final volume of 10 µL in 0.1% TFA and 0.5% of either CHAPS or TritonX-100. The samples were purified on Bond Elut OMIX SCX tips using various washing solutions. (A) 30% methanol washing solution, (B) 50% methanol washing solution, (C) 100% methanol washing solution. The protein was correctly identified in all cases. Sequence coverages for the CHAPS samples washed with 30%, 50%, and 100% methanol washing solution, respectively, were 26%, 42%, and 20%. For the TritonX-100 samples, coverages were 44%, 53% and 38%, respectively. Mascot scores for the CHAPS samples washed with 30%, 50% and 100% methanol washing solution, respectively, were 73, 63, and 39. For TritonX-100, Mascot scores were 78, 90, and 47.*

The protein was also successfully identified using a 50% methanol-containing washing solution on the Tween20 samples. However, best removal of Tween20 was achieved with a 100% methanol washing solution (Figure 3).

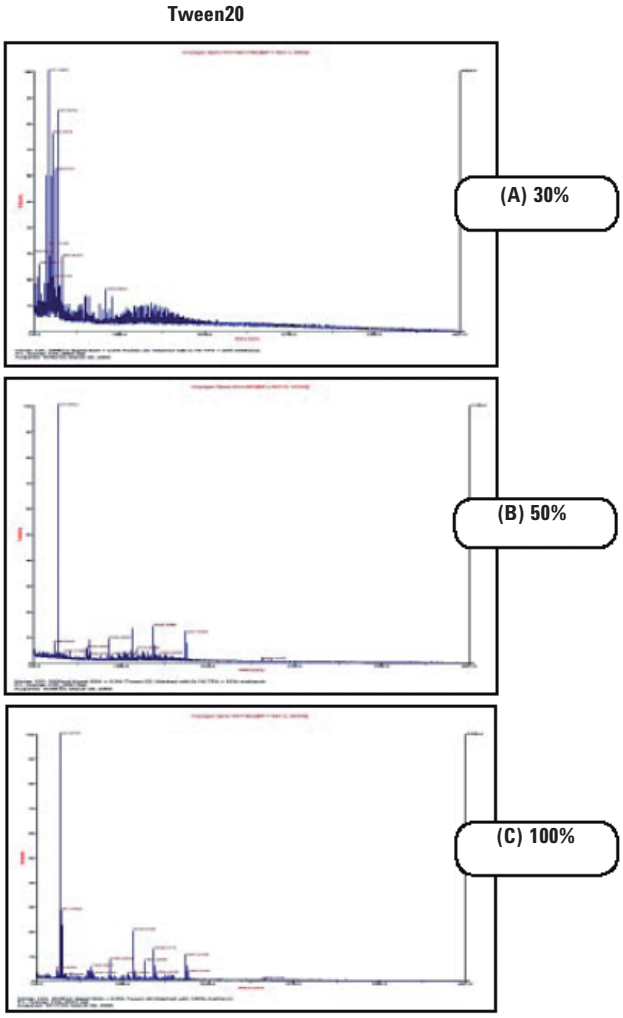

Figure 3. *Effect of methanol concentration in washing solution on Tween20 removal and protein identification. 200 fmols of BSA tryptic digest were re-suspended to a final volume of 10 µL in 0.1% TFA and 0.5% of Tween20. The samples were purified on Bond Elut OMIX SCX tips using various washing solutions. (A) 30% methanol washing solution, (B) 50% methanol washing solution, (C) 100% methanol washing solution. The protein was correctly identified after 50% and 100% methanol washes, but not after a 30% methanol wash. Sequence coverages for the samples washed with 50% and 100% washing solution were 28% and 57% respectively. Mascot scores for the 50% methanol and 100% methanol samples were 35 and 50, respectively.*

Conclusion

Bond Elut OMIX SCX 10 µL pipette tips successfully remove interfering detergents from protein digests. By utilizing the ionic nature of the peptides in the BSA digest, the peptides could be properly removed from the interfering non-ionic and zwitterionic detergents with a simple methanol wash. Sequence coverages of 42% to 57% were achieved from samples, which are otherwise impossible to analyze, by MALDI MS. The proper washing solution can vary depending on the protein or the detergent, but washes containing 50% to 100% methanol gave excellent results. Larger sample volumes can be purified using Bond Elut OMIX SCX 100 µL tips.

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This information is subject to change without notice.

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