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Consumables Application Note 2109 Protein desalting and concentration for MS analysis using OMIX® C4 Pipette Tips

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Introduction

It is important to remove salts and other ion-suppressing interferences from samples before MS analyses or the results can be significantly compromised. Pipette tip clean-up is a popular and effective method for removing these interferences from samples before MALDI and LC MS. C18 is typically used for protein digests, but is not suitable for larger intact proteins. Intact proteins are more hydrophobic than the peptides in protein digests. They therefore bind too tightly to C18 and are difficult or impossible to elute, leading to little or no recovery. Less hydrophobic C4 membranes, however, should allow for successful purification and concentration of the proteins.

The method described is a simple and efficient solution to purifying and concentrating higher molecular weight proteins (20,000 – 70,000 Da). In less than 5 minutes, a protein sample containing salts and other ion suppressing interferences can be prepared.

Materials and Reagents

OMIX C4 pipette tips – 10 µL, 100 µL or 10 µL MB (minibed)

Varian Part Numbers:

10 µL tips	A5700910 or A5700910K
100 µL tips	A57009100 or A57009100K
10 µL MB tips	A57009MB or A57009MBK

10 µL or 100 µL pipettor

Washing Solution: 0.1% Trifluoroacetic acid (TFA)

Conditioning Solution: 50:50 Acetonitrile:water

Elution Solution: 20 mg/ml Sinnapinic Acid in 75% acetonitrile, 0.1%TFA, 24.9% Milli-Q grade water

Method

1 pmol Bovine Serum Albumin (66kDa) in 0.1% trifluoroacetic acid and 2 M guanidine HCL was desalted using a 10 µL OMIX C4 pipette tip and the method described in Table 1. For elution, Elution Solution was aspirated to completely immerse the tip bed in solution (1 – 2 µl), the sample was eluted directly onto the MALDI-TOF target, and analyzed by MALDI-TOF MS.

1 pmol BSA was also extracted on OMIX C4 100 µL and 10 µL minibed tips using the method described in Table 1.

Results and Conclusions

Bovine Serum Albumin containing 2M guanidine HCL was directly spotted with no clean-up and analyzed by MALDI-TOF MS. As expected, the signal to noise ratio was poor. The signal improved significantly after desalting with an OMIX C4 10 µL tip (Figure 1). BSA was also extracted successfully on OMIX 10 µL minibed tips with an elution volume of 0.5 µL and on OMIX 100 µL tips with an elution volume of 5 µL (Figure 2).

While C18 tips are best suited for peptides, OMIX C4 tips can efficiently desalt, purify, or concentrate protein samples from 20 to 70kDa, allowing for significantly improved MS results. Three tip sizes allow for sample and elution volumes as little as 0.5 µL or as much as 100 µL.

Thanks to Dario Miranda, Trisdan Williams and the Burnham Institute for the use of their MALDI-TOF mass spectrometer

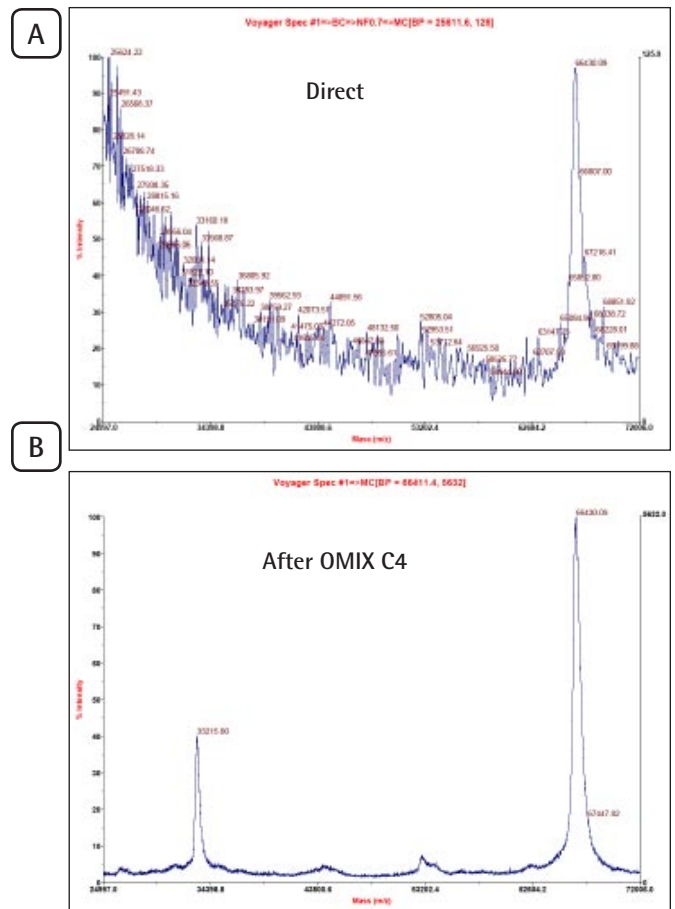


Figure 1. 1 pmol Bovine Serum Albumin in 2M guanidine analyzed by MALDI TOF both before and after clean-up with OMIX C4 10 µL tips.

Table 1 – OMIX C4 Tip Method	10 μ L tip	10 μ L MB tip	100 μ L tip
Pretreat sample	Adjust sample to ~ 0.1% trifluoroacetic acid (TFA) concentration		
Condition tip	Set pipettor at 10 μ L and securely attach an OMIX® C4 tip. Aspirate 10 μ L of conditioning solution and discard solvent. Repeat. Keep pipette plunger depressed and move to Equilibration step.		Set pipettor at 100 μ L and securely attach an OMIX® C4 tip. Aspirate 100 μ L of conditioning solution and discard solvent. Repeat. Keep pipette plunger depressed and move to Equilibration step.
Equilibrate tip	Aspirate 10 μ L Washing Solution and discard. Repeat. Keep pipette plunger depressed and move to Sample Binding step.		Aspirate 100 μ L Washing Solution and discard. Repeat. Keep pipette plunger depressed and move to Sample Binding step.
Bind Sample	Aspirate up to 10 μ L of pre-treated sample into tip. Dispense and aspirate sample 3-5 cycles for maximum efficiency. Up to 10 cycles may be used for improved binding. Dispense sample into a waste vial. Keep pipette plunger depressed and move to Purification step.		Aspirate between 10 and 100 μ L of pre-treated sample into tip. Dispense and aspirate sample 3-5 cycles for maximum efficiency. Up to 10 cycles may be used for improved binding. Dispense sample into a waste vial. Keep pipette plunger depressed and move to Purification step.
Purify / Desalt	Aspirate 10 μ L of Washing Solution and discard solvent. Repeat two to four times. Keep pipette plunger depressed and move to Elution step.		Aspirate 100 μ L of Washing Solution discard solvent. Repeat two to four times. Keep pipette plunger depressed and move to Elution step.
Elute	<u>For MALDI analysis:</u> Aspirate 2 - 10 μ L Elution solution, with or without matrix. Elute directly onto the MALDI-TOF target if desired. <u>For LC/MS analysis:</u> Elute with 2 - 10 μ L 0.1% formic acid or 0.1% acetic acid in 75-95% acetonitrile or methanol.	<u>For MALDI analysis:</u> Aspirate 0.5 - 2 μ L Elution solution, with or without matrix. Elute directly onto the MALDI-TOF target if desired. <u>For LC/MS analysis:</u> Elute with 0.5 - 2 μ L 0.1% formic acid or 0.1% acetic acid in 75-95% acetonitrile or methanol.	<u>For MALDI analysis:</u> Aspirate at least enough Elution solution, with or without matrix, to completely immerse tip bed (~5 μ L) or up to 100 μ L. Elute directly onto the MALDI-TOF target if desired. <u>For LC/MS analysis:</u> Elute with 5 - 100 μ L 0.1% formic acid or 0.1% acetic acid in 75-95% acetonitrile or methanol.

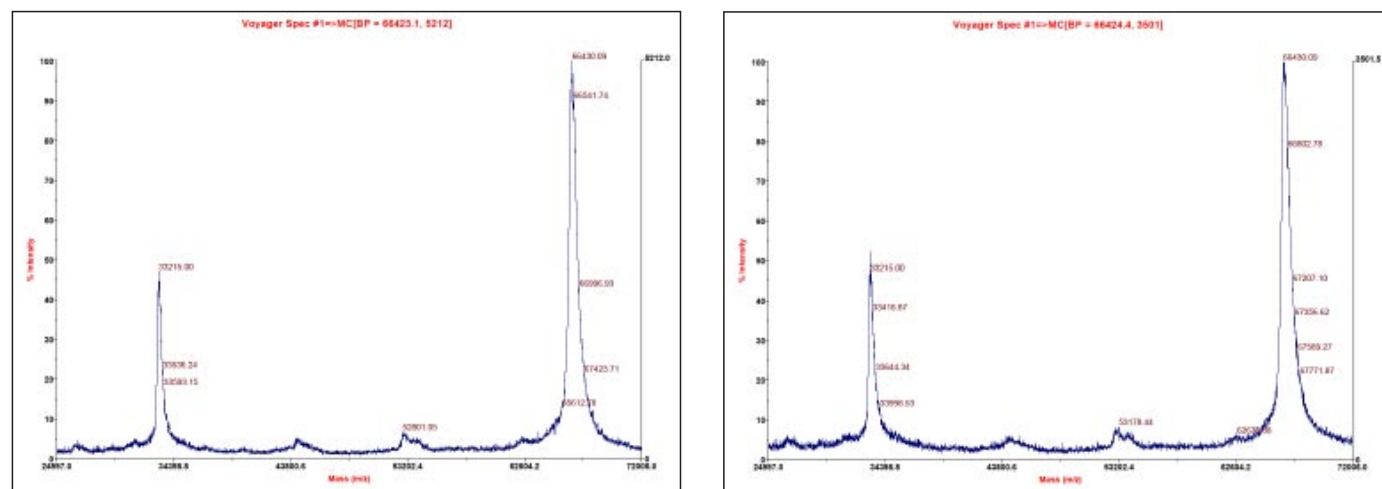


Figure 2. 1 pmol Bovine Serum Albumin after clean-up with OMIX C4 10 μ L minibed and 100 μ L tips. A) OMIX C4 10 μ L minibed. B) OMIX C4 100 μ L

These data represent typical results.

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