Sample Purification for Static Nanospray MS Using Wall-Coated Pipette Trap'nTips™

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Introduction

While highly suitable for MALDI-MS, in-pipette sample purification is difficult to implement with static nanospray methodology. Geometric constraints of pipette tips limit sample transfer efficiency into glass nanospray emitters, and conventional loading/extraction requires either pressurized back-loading into a pipette or coupling one pipette tip to a longer pipette tip.

The Trap'nTip™ (New Objective), a novel gel-loader pipette tip containing reverse-phase sorbent-coated walls, eliminates high pressure/vacuum requirements for loading a packed bed. Trap'nTip geometry (Figure 1) permits sample aspiration from the pipette tip and direct loading of static nanospray emitters. Trap'nTips demonstrate concentration, desalting, and excellent MS signal for 0.05 uM bovine serum albumin (BSA) tryptic digest solutions containing ammonium bicarbonate (NH_4HCO_3). Manual gradients using eluents of successively increasing organic concentrations display excellent peptide-separation ability.

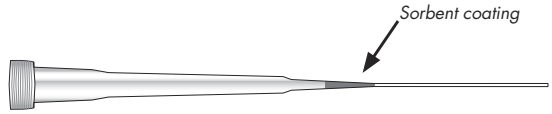

Figure 1 Anatomy of a Trap'nTip™

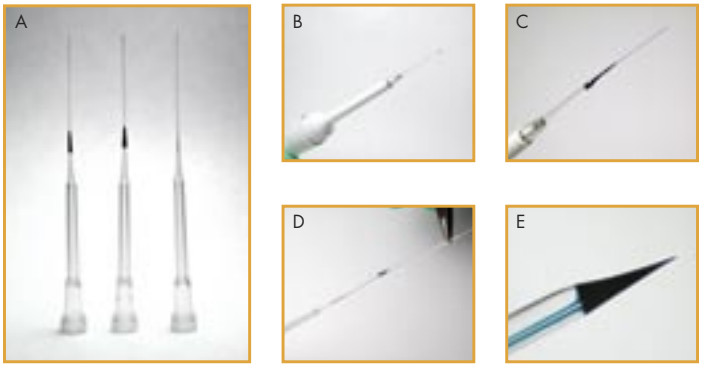

Figure 2 *Trap'nTips™: (A) Trap'nTips with carbon, carbon/C18 mixture, and C18 coated walls, (B) Trap'nTip on pipette, (C) Trap'nTip filled with sample, (D) Sample loading from Trap'nTip into conductively coated glass emitter, (E) Emitter tip filled with sample (shown in uncoated tip for clarity)*

Methods & Materials

Instrumentation and Components

- Ion trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Nanospray source (PicoView® 150, New Objective, Inc., modified for offline analysis)
- A 0.5 - 10 uL Eppendorf® Single-Channel Research Pipette
- Reverse-phase sorbent-coated pipette tips containing C18, carbon, and mixed C18/carbon sorbents (Glygen, Inc.)

BSA Sample Preparation

- 50 µL commercially available 500 pmol BSA standard were diluted in 950 µL 2% ACN to assess Trap'nTips™ concentration and elution ability
- BSA solutions of 0.05, 0.1, and 0.25 µM concentrations were prepared in 50 mM NH_4HCO_3 /2% acetonitrile (ACN) to test the desalting capacity of Trap'nTips

Trap'nTip™ Conditioning and Sample Loading

- Trap'nTips™ were first flushed with 5 aspiration/expulsion cycles of 10 µL 60% ACN
- Trap'nTips were then flushed with 5 aspiration/expulsion cycles of 10 µL 2% ACN
- The BSA analyte was loaded onto the Trap'nTip coating through 10 aspiration/expulsion cycles of 10 µL volumes each
- Loaded samples were desalted by 10 aspiration/expulsion cycles using 10 µL of 2% ACN

Offline Emitter Preparation

- BSA sample was loaded into the distal end of an Econo12 glass nanospray emitter (New Objective) via a single aspiration/expulsion of 2 µL 60% ACN eluent from the Trap'nTip™ (Figure 2)
- Carryover was assessed for samples not containing NH_4HCO_3 via 2 µL secondary eluent aspiration/expulsions into nanospray emitters

Trap'nTip™ Chromatographic Performance

- A 0.5 µM BSA tryptic digest sample was loaded onto the Trap'nTip using the above methodology
- A 2 µL aliquot of 10% ACN eluent was aspirated and expelled through the Trap'nTip and into an offline emitter
- For the same Trap'nTip, 2 µL aspiration/expulsion cycles were repeated for 20, 40, and 60% ACN eluents into separate offline nanospray emitters

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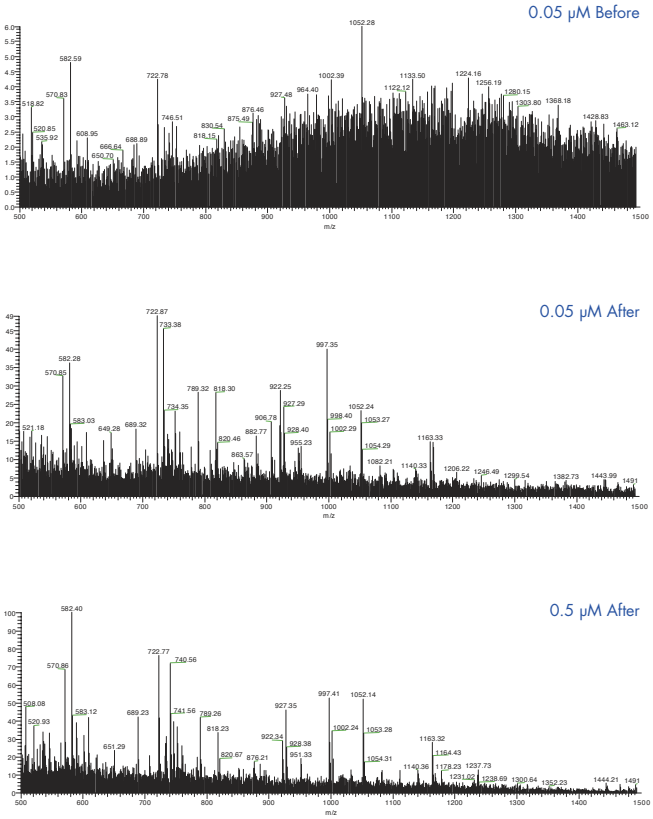

Figure 3 *BSA before and after using a carbon/C18 Trap'nTip™*

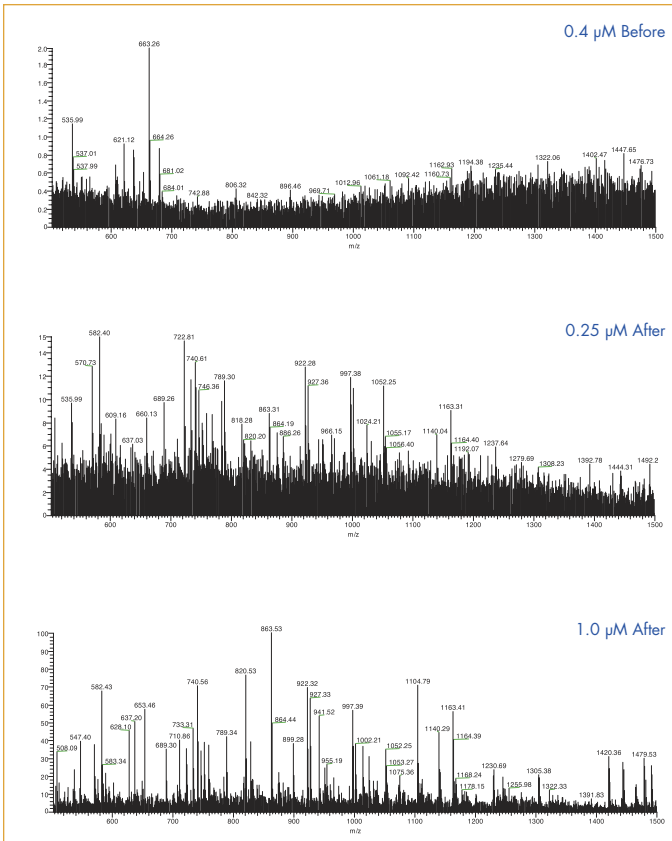

Figure 4 *BSA with NH_4HCO_3 before and after using a carbon/C18 Trap'nTip™*

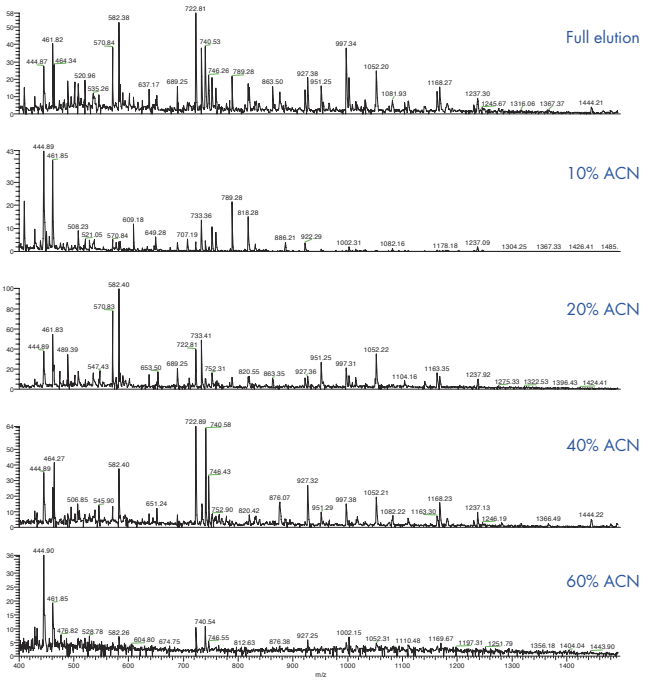

Figure 5 *Chromatography in a tip using a carbon/C18 Trap'nTip™ with BSA*

Results

Loading, washing, and elution onto each coating material produced significant signal enhancement for concentrations as low as 0.05 µM without NH_4HCO_3 and 0.1 µM with NH_4HCO_3. Representative spectra for mixed-sorbent Trap'nTips™ are displayed in Figures 3 and 4. Two 0.40 µM BSA digests, one containing 50 µM NH_4HCO_3, served as concentrated reference samples; for these references, no MS signal was observed in the presence of NH_4HCO_3, and decreased S/N ratio was observed for the BSA solution containing 2% ACN without NH_4HCO_3. In addition, the Trap'nTips provided excellent chromatographic separation from consecutive aspiration/expulsion cycles with eluent compositions ranging from 10-60% (Figure 5).

Conclusions

- BSA samples eluted from Trap'nTips™ containing three different sorbents revealed detectable MS signal in the presence of NH_4HCO_3
- Improved MS signal was observed for same without added salt
- The Trap'nTips offer the requisite purification, concentration, and separating facility for identifying complex peptide mixtures via offline analysis

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