307312: Rapid Desalting of Proteins for Direct Infusion Intact Mass Analysis using an Optimized SEC Tip

Donna Osterman, **Jennifer M. Nguyen,** Wenjing Li, Anna Boardman, Mingcheng Xu, Matthew A. Lauber
Waters Corporation, Milford, MA, USA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Introduction

Intact mass analysis of proteins is commonly performed using direct infusion or liquid chromatography coupled to electrospray ionization mass spectrometry (ESI-MS). While direct infusion grants higher throughput, extremely pure samples are required. Size exclusion chromatography (SEC) can be employed to remove MS-unfriendly substances (salts). However, conventional spin columns or plates require extra instrumentation, tedious equilibration, and cannot be easily automated to improve sample preparation throughput.

To combat this, an optimally designed desalting device based on a pressure-driven tip configuration can grant high protein recovery and near-complete removal of salts for both manual use and automation. This tip, packed with a modified dextran-based particle, can desalt intact mAbs with efficient sample clean up prior to both optical and MS detection.

Experimental

Figure 1. Desalting tips.

A 1 mg/mL concentration of NIST Reference Material 8671 in 6M guanidine hydrochloride was prepared. Desalting was performed using 100 µL sample loads with 1.2 mL tips packed with a superfine SEC resin or a commercially available spin column that is suitable for 30-130 µL sample load. Analysis was performed with a quadrupole time of flight spectrometer for direct infusion ESI-MS after diluting the eluant in Milli-Q™water. MS detection settings were adjusted to a capillary voltage of 2.25 kV, sampling cone and source offset at 140, a source temperature of 150 ◦C, a desolvation temperature of 600 ◦C, a cone gas flow at 0 L/h, desolvation gas flow set at 600 L/h, and collision energy set at 6 eV with data collected over 1 minute. Mass spectra were manually processed using MaxEnt1 deconvolution with a resolution of 20,000 over a range of 2250–4500 or 3200-6000 m/z at a rate of 10 Hz. Duplicate sets of columns and injections were analyzed. To quantify the salt concentration, a conductivity assay was performed using a chromatographic assay coupled to a GE Healthcare Monitor and a 4.6 x 150 mm column packed with a 200 Å, diol-bonded stationary phase. Fractions were collected from the chromatographic separation for quantitation.

Results

Device	Area	Guanidine Conc. (mM)
Tip # 1 (n=2 injections)	undetectable	N/A
Tip #2 (n=2)	undetectable	N/A
Spin column #1 (n=2)	110745	35.01
Spin column #2 (n=2)	57035	18.90

Table 1. Salt concentrations from elution volumes of 100 µL after desalting with tips or spin columns. No salt detected when desalting tips are used.

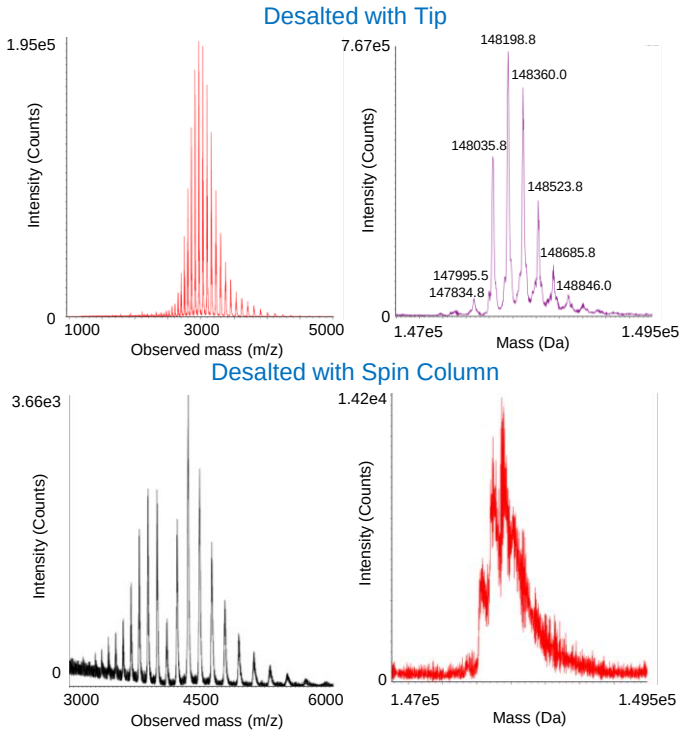

Figure 2. Mass spectra and deconvoluted MS of NIST mAb, as obtained from tip-based or spin column desalting and direct infusion. Higher intensities suggest greater protein recovery may be achieved using desalting tips.

Proteoform	Theoretical Mass (Da)	Observed Mass (Da)	Mass Accuracy (ppm)
G0F/G0F-GlcNAc	147834.0	147834.8	5.41
G0F/G1F-GlcNAc	147996.1	147995.5	-4.05
G0F/G0F	148037.2	148035.8	-9.46
G0F/G1F	148199.3	148198.8	-3.37
G1F/G1F	148361.4	148360.0	-9.44
G1F/G2F	148523.6	148523.8	1.35
G2F/G2F	148685.7	148685.8	0.67
G2F/G2F + Hex	148847.9	148846.0	-12.76

Table 2. Summary of deconvoluted MS data of NIST mAb, as obtained from tip-based desalting and direct infusion.

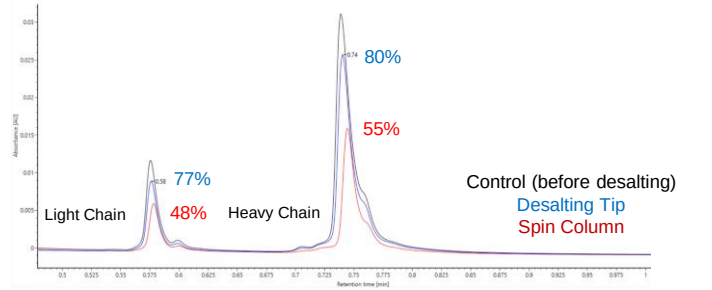

Figure 3. Overlaid UV chromatograms of the light and heavy chains of reduced NIST mAb as obtained before desalting (control, full recovery assumed) and from samples desalted using tips or spin columns.

Conclusions

The results presented herein show that high throughput intact mass analysis through direct infusion for proteins can be achieved without the use of column injections by utilizing a specially designed tip configuration for desalting. The pressure-driven tip proves to be highly advantageous in both manual and automation-based workflows, with the optimized SEC resin offering removal of species up to 5000 Da. Versus typical sample preparation using centrifugal filters, this device offers more efficient removal of salts, higher protein recoveries, and may contribute to more optimal unfolding of proteins.

Milli-Q is a trademark of Merck KGAA