Rapid, On-Line Desalting of Intact Proteins prior to Characterization by Mass Spectrometry

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Abstract

Therapeutic proteins can undergo structural modification during production, storage, and stability testing. Alterations can affect safety and efficacy, therefore reliable determination and continuous monitoring of the protein properties is essential. Since structural changes are reflected in molecular weight, measuring this property is one convenient way to monitor modifications. Methods including electrophoresis, chromatography, and ultracentrifugation have been used; however, these methods measure average relative errors from 10-100%. This large error range is due to these methods being affected by properties other than molecular weight such as conformation, Stokes' radius, and hydrophobicity. Mass spectrometry is a technique whereby more exact molecular weight information can be obtained. However, the proteins are often dissolved in buffers containing non-volatile salts. These salts cause ion suppression and adduct formation, which complicates molecular weight determination. Many methods have been developed for the purpose removing salts from protein. These methods include SEC, centrifugal filtration and buffer exchange, yet these techniques are limited to off-line use only. An alternative technique has been developed that can be used either off-line or in an on-line approach coupled directly to a mass spectrometer. This 2.1 x 10 mm device, packed with polymer sorbent, was evaluated using acidic, (bovine serum albumin), basic, (cytochrome c), and large globular (monoclonal IgG1) protein samples.
Parameters such as column carryover, loading, and lifetime were investigated. The results of this study show no sample carryover and excellent lifetime for repeated sample analysis under the conditions tested. This technique yields accurate direct measurement of molecular weight of biopharmaceutical proteins.

Sample preparation:

Bovine serum albumin (BSA), cytochrome c and monoclonal IgG1, were obtained from Sigma Chemical Co. St. Louis, Mo. All proteins were dissolved in phosphate buffered saline (PBS).

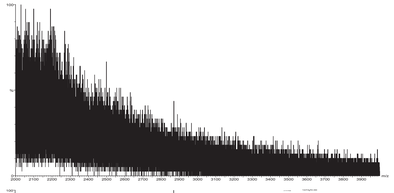
Instrumentation:

HPLC System:	Waters Alliance® Bioseparations Module
Needle Wash Solution:	35:5:60 H_2O:IPA:ACN
Number of Needle Washes:	Single
Needle Wash Time:	15 sec needle exterior / 3 sec inject port
Mass Spectrometer:	Waters Micromass® Q-Tof micro
Ionization mode:	ES +
Capillary voltage:	3300 V
Cone voltages:	30 V for BSA and 35 v for MAb
Source temp:	150 °C
Desolvation temp:	350 °C
Gas flow:	500 L/Hr
Cone gas:	50 L/Hr

On-Line Desalting Method:

Eluent A:	H_20 with 0.1% Formic Acid
Eluent B:	Acetonitrile with 0.1% Formic Acid
Flow:	0.4 mL/minute
Gradient elution:	5-80 %B in1.5 minutes
Column:	MassPREP™ On-Line Desalting Cartridge 2.1 x 20 mm

A



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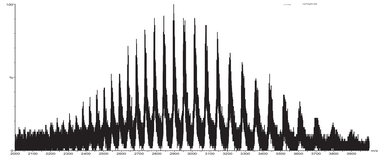
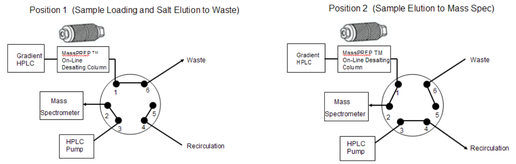


Figure 1A: Protein spectrum of infused monoclonal IgG in PBS.

Figure 1B: Protein spectrum of on-line desalted monoclonal IgG in PBS.



Time	Eluent A	Eluent B	Valve position
0	95	5	1
0.5	95	5	1
2.0	20	80	2
3.0	95	5	1

Figure 2: Diversion valve position timing and plumbing for the on-line protein desalting method.

On-Line Desalting of Proteins

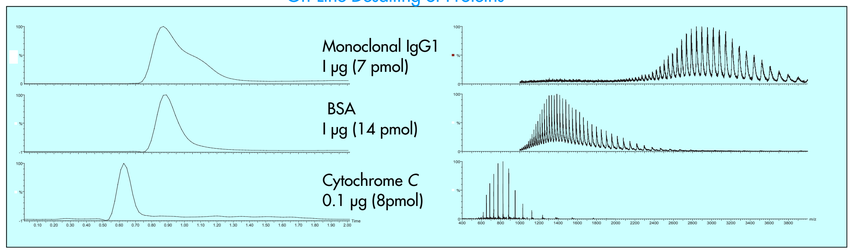


Figure 3: Total ion chromatograms and summed spectra of desalted proteins. Acidic (BSA), basic (cytochrome c), and large globular (monoclonal IgG1) proteins were all successfully desalted.

Carryover and Lifetime

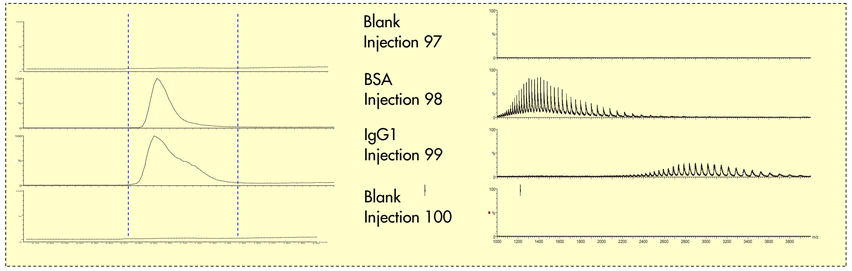


Figure 4: Total ion chromatograms and summed spectra of indicated region, showing no detectable carryover and lifetime after 100 injections.

Monoclonal IgG1Loading Capacity and Carryover

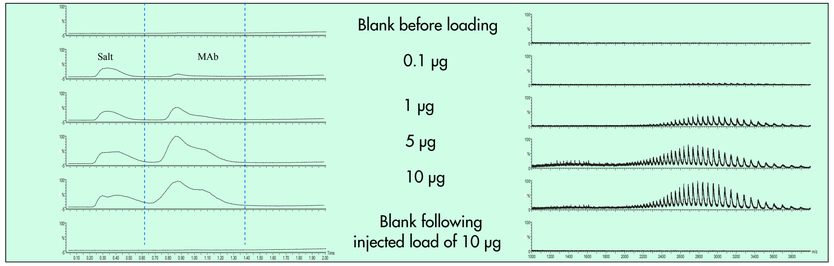


Figure 5: Total ion chromatograms and summed spectra for indicated region demonstrating loading capacity and carryover of monoclonal IgG1. Blanks were injected between increasing loads of IgG1 to measure carryover. Data not shown.

Sorbent Background and Bleed

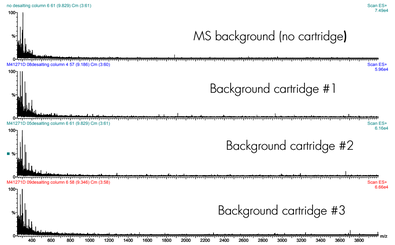


Figure 6: Background spectra (m/z 250-4000) of three desalting cartridges versus MS background without cartridge.

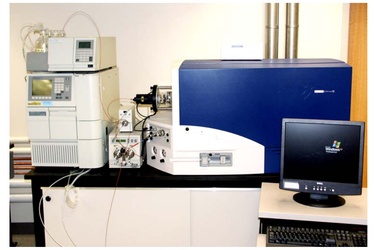


Figure 7: On-line desalting system configuration.

CONCLUSIONS

The data presented shows that MassPREP™ On-Line Desalting Cartridges are fully capable of desalting a wide variety of proteins including acidic, basic, and large globular species.

Excellent lifetime and the absence of protein carryover after 100 consecutive injections were demonstated.

Loading of up to 10 µg of monoclonal IgG1(over-loading conditions) was accomplished without the detection of protein carryover in a following blank injection.

No sorbent bleed was observed.