Evaluation of polymeric based desalting cartridge for LC/MS based biopharmaceutical applications

Anne Blackwell[1], Timothy Rice[1], Suresh Babu C.V.[2], and Koen Sandra[3]
[1]Agilent Technologies, Inc. Wilmington, DE 19808; [2]Agilent Technologies, Inc. Bangalore, India; [3]
Research Institute for Chromatography (RIC), President Kennedypark 26, 8500 Kortrijk, Belgium

Agilent Technologies

Introduction

Monoclonal antibodies (mAbs) represent a major category of therapeutic proteins. A number of bioanalytical tools are required to monitor the antibody heterogeneity. Liquid chromatography/mass spectrometry (LC/MS) is routine technology nowadays applied for characterization of these biomolecules. Often the preparation and storage reagents of mAbs contains nonvolatile salts, detergents, and solubilizing agents. The presence of these reagents causes adverse effects in mass spectrometry, suppressing ionization which in turn limits LC/MS application. Hence it is very important to remove these salts and detergents prior to MS analysis. In the present study, a new desalting cartridge is evaluated for 1D and 2D-LC/MS based protein applications. The reverse phase LC method for desalting was developed using a polymeric based desalting material. For effective desalting performance, (a) salt containing samples (1D), and (b) two multidimensional separation experiments (IEX → RP and affinity chromatography → RP) were tested. The desalting approach described in this work demonstrates that coupling of these desalting columns to mass spectrometry improves the protein spectra from salt and detergent contaminated solutions.

Experimental

Monoclonal antibodies mAb1, and mAb2 were purchased from a local pharmacy and stored according to the manufacturers' instructions. All solvents used were LC grade.

First-dimensional pump (IEX)	
Solvent A	Water
Solvent B	NaCl (850.0 mM)
Solvent C	NaH_2PO_4 (41.0 mM)
Solvent D	Na_2HPO_4 (55.0 mM)
Flow rate	0.75 mL/min
Gradient	0 minutes: 30.3 %A, 0.0 %B, 59.6 %C, 10.1 %D 2 minutes: 26.0 %A, 5.0 %B, 56.9 %C, 12.1 %D 8 minutes: 21.5 %A, 10.0 %B, 54.9 %C, 13.6 %D 20 minutes: 13.3 %A, 19.0 %B, 51.9 %C, 15.8 %D 35 minutes: 30.3 %A, 0.0 %B, 59.6 %C, 10.1 %D
Post time	10 minutes
First-dimensional pump (Protein A)	
Solvent A	20 mM sodium phosphate buffer, pH 7.4
Solvent B	Acetic acid (500.0 mM)
Flow rate	1 mL/min
Gradient	0 to 0.5 minutes: 0%B (binding) 0.6 to 1.7 minutes: 100%B (elution) 1.8 to 3.5 minutes: 0%B (regenration)
Second-dimensional pump	
Solvent A	0.1% FA
Solvent B	0.1% FA in ACN
Flow rate	0.4 mL/min
Gradient	0 minutes - 5 %B, 0.5minutes – 5 %B, 3minutes – 80 %B, 4 minutes – 80 %B, 4.1 minutes – 5 %B, 6 minutes - 5%B
2D gradient stop time	
2D cycle time	
Autosampler	
Injection volume	5ul
Sample temperature	5 °C
Columns (IEX→RP) (Protein A → RP)	
1st -dimension column	Agilent Bio MAb NP5, 4.6 × 250 mm, PEEK
1st -dimension column	Agilent Bio-Monolith Column Protein A
2nd-dimension column	Agilent AdvanceBio RP desalting cartridges , 2.1 x 12.5 mm;
Thermostatted column compartment	
1st -dimension column	Room temperature
2nd-dimension column	Room temperature
Multiple heart-cutting	
Operation mode	• Time-based multiple heart-cutting was performed with the heart-cuts (2D time segments) set according to the first-dimension retention times. • Heart-cutting of impurities was conducted with a sampling time of 0.04 minutes (loop filling of > 200 %).
Detection	
1st - dimension	UV Wavelength 280 nm/4 nm, Ref.: 360 nm/100 nm
2nd - dimension	MS parameters
	Gas temperature: 350 °C
	Sheath gas temperature: 400 °C
	Gas flow: 8 L/min
	Sheath gas flow: 11 L/min
	Nebulizer: 35 psi
	Vcap: 5,000 V
	Nozzle: 1,000 V
	Fragmentor: 200

Time (min)	LC stream to
0	Waste
10.8	MS
14.6	Waste

Results and Discussion

Instrument and column configuration

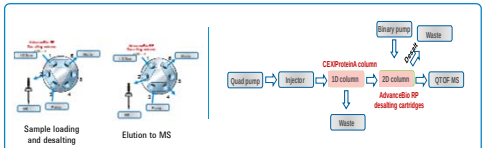


Figure 1: MS valve switching and 2D LC/MS configuration

High quality MS with AdvanceBio RP desalting cartridges

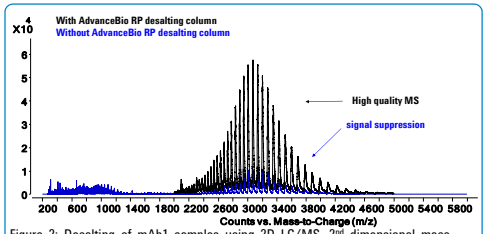


Figure 2: Desalting of mAb1 samples using 2D LC/MS. 2nd dimensional mass spectrum profiles with and without AdvanceBio RP desalting cartridges. Use of desalt column provides improvement in signal intensity (6X greater MS signal).

On-line desalting 2D LC/MS (IEX → RP): mAb1

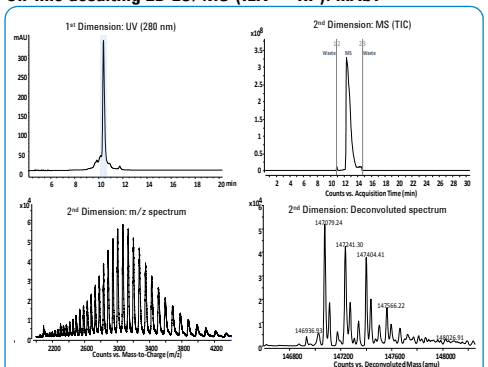


Figure 3: 2D LC/MS of mAb1 profiles. 1D column: IEX; 2D column: Desalt cartridges. The selected main peak in the 1D chromatogram is captured and transferred to the AdvanceBio desalting cartridges that effectively remove salts and provide high quality MS results.

AdvanceBio RP desalting cartridges: reproducibility

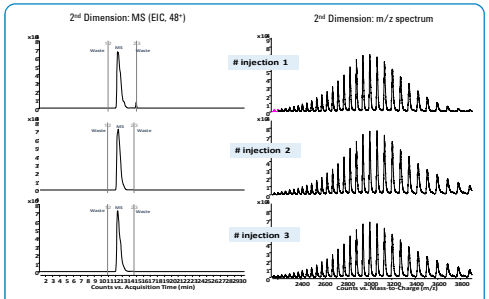
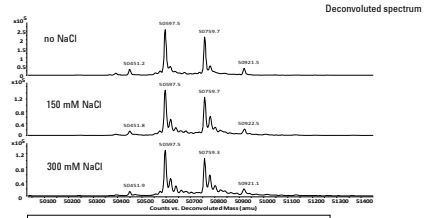


Figure 4: 2D LC/MS of mAb1 profiles. 1D column: IEX; 2D column: Desalting cartridges. 2nd dimension EIC (left) and m/z (right) profiles. Injection to injection repeatability shows consistent performance of AdvanceBio RP desalting cartridges.

Results and Discussion

Desalting of reduced mAb with different concentrations of NaCl (1D experiments)



Reduced Herceptin in the presence of			
Glycoform	0 mM NaCl	150 mM NaCl	300 mM NaCl
% G0F	49.6	50.9	50.0
% G1F	41.5	41.2	41.5
% G2F	8.9	7.9	8.5

Figure 5: Deconvoluted spectrum of Herceptin heavy chain. NaCl containing mAb samples were analyzed on the AdvanceBio RP desalting cartridges following reduction using DTT. The signal decreases slightly when samples contain more salt, but relative quantification is similar between samples in the presence or absence of NaCl.

On-line desalting 2D LC/MS (IEX → RP): mAb2

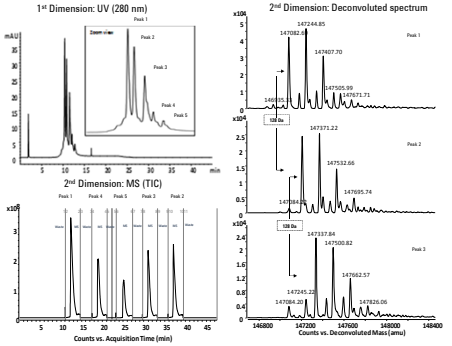


Figure 6: 2D LC/MS of mAb 2 profiles. 1D column: IEX; 2D column: Desalt cartridges. Selected peaks (multiple heart-cutting) in the 1D chromatogram was captured and transferred to the AdvanceBio desalting cartridges that effectively removed salts and provided high quality MS results. The 3 main peaks (peaks 1,2,3) corresponds to C-terminal lysine variants.

On-line desalting 2D LC/MS (Protein A → RP): mAb1

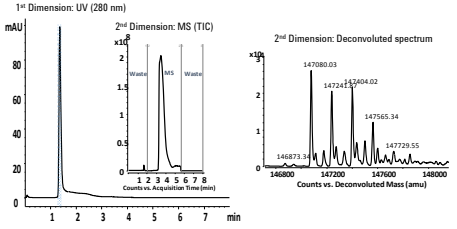


Figure 7: 2D LC/MS of mAb1 profiles. 1D column: Protein A; 2D column: Desalt cartridges. Selected peaks in the 1D chromatogram was captured and transferred to the AdvanceBio desalt cartridges that effectively removed salts and provided high quality MS results.

Conclusions

- AdvanceBio desalting cartridges effectively remove salts for better MS sensitivity.
- AdvanceBio desalting cartridges provide reproducible MS results.
- AdvanceBio desalting cartridges allow automated 2D LC in Multiple Heart-Cutting 2D-LC/MS methods for characterization of mAbs.