Application of Agilent AdvanceBio Desalting-RP Cartridges for LC/MS Analysis of mAbs

A One- and Two-dimensional LC/MS Study

Application note

Biotherapeutics and Biologics

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Abstract

This application note describes how Agilent AdvanceBio Desalting-RP cartridges were used for effective desalting of LC-separated monoclonal antibodies (mAbs) before characterization by mass spectrometry. Reproducible mass spectra and total ion chromatograms (TIC) demonstrate a consistent performance of the AdvanceBio Desalting-RP cartridges. The Agilent 1290 Infinity 2D-LC/MS solution enabled online desalting and characterization of mAbs using heart-cutting techniques.

Introduction

Monoclonal antibodies (mAbs) represent a major category of therapeutic proteins. Various analytical tools are required to monitor mAb heterogeneity. Liquid chromatography/mass spectrometry (LC/MS) is a routine technology used for characterization of these biomolecules. For preparation and storage, mAbs often contain nonvolatile salts and solubilizing agents. The presence of these reagents causes adverse effects in mass spectrometry, suppressing ionization, which limits LC/MS application. Hence, it is important to remove these salts and detergents before MS analysis. In this study, a new desalting cartridge was evaluated for one- and two-dimensional LC/MS-based protein applications. The reversed-phase LC method for desalting was developed using polymeric-based desalting material. For effective desalting performance, salt-containing samples (1D), and two multidimensional separation experiments (ion exchange chromatography with reversed-phase chromatography) were tested. The desalting approach described in this application note demonstrates how coupling these desalting cartridges to mass spectrometry improves the protein mass spectra from samples containing salt and detergent in solution.

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Experimental

Instrumentation

Figures 1A and 1B show instrument and column configurations. Online desalting was performed in 1D and 2D modes. Figure 1A shows the MS valve switching for effective sample loading onto the column, and elution of the salt to waste before MS analysis. Figure 1B illustrates the 2D-LC/MS setup, where the second-dimension column is the desalting column coupled to the MS valve switching mode.

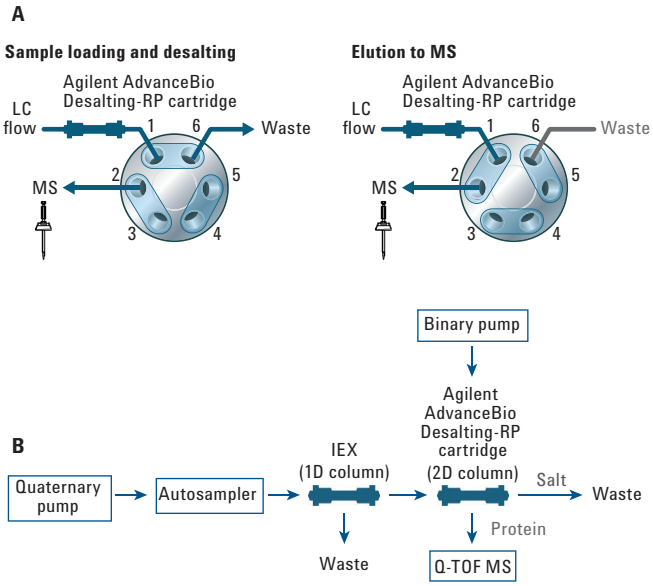


Figure 1. A) MS valve switching. B) Configuration for 2D-LC/MS.

Samples and solvents

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

Methods

1D LC/MS

Column:	Agilent AdvanceBio Desalting-RP cartridge, 2.1 × 12.5 mm, 10 µm, 1,000 Å (p/n PL1612-1102)
Flow rate:	0.75 mL/min
Solvent A:	0.1% FA
Solvent B:	0.1% FA in ACN
Flow rate:	0.4 mL/min

Gradient:

Time (min)	%B
0	5
0.5	5
3.0	80
4.0	80
4.1	5
6.0	5

Column temperature:	Room temperature
Injection volume:	5 µL
Sample temperature:	5 °C

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

2D LC/MS (IEX → Desalting RP)

First-dimension pump (IEX)

Solvent A:	Water
Solvent B:	NaCl (850.0 mM)
Solvent C:	NaH_2PO_4 (41.0 mM)
Solvent D:	NaH_2PO_4 (55.0 mM)
Flow rate:	0.75 mL/min

Gradient:

Time (min)	%A	%B	%C	%D
0	30.3	0.0	59.6	10.1
2	26.0	5.0	56.9	12.1
8	21.5	10.0	54.9	13.6
20	13.3	19.0	51.9	15.8
35	30.3	0.0	59.6	10.1

Post time:	10 min

Second-dimension pump (RP)

Solvent A:	0.1% FA
Solvent B:	0.1% FA in ACN
Flow rate:	0.4 mL/min

Gradient:

Time (min)	%B
0	5
0.5	5
3.0	80
4.0	80
4.1	5
6.0	5

^{2}D gradient stop time:	6.0 min
2D cycle time:	6.1 min

Autosampler	
Injection volume:	5 µL
Sample temperature:	5 °C
Columns (IEX → Desalting RP)	
First-dimension column:	Agilent Bio MAb NP5, 4.6 × 250 mm, PEEK (p/n 5190-2407)
Second-dimension column:	Agilent AdvanceBio Desalting-RP cartridge, 2.1 × 12.5 mm, 10 µm, 1,000 Å (p/n PL1612-1102)
Thermostatted column compartment	
First-dimension column:	Room temperature
Second-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	
First dimension DAD parameters:	Wavelength 280 nm/4 nm, Ref.: 360 nm/100 nm
Second 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
Timing of LC stream:	0 min to waste 10.8 min to MS 14.6 min to waste

Results and Discussion

In the first experiment, the mAb sample was analyzed using 2D-LC/MS with and without the AdvanceBio Desalting-RP cartridge in the second dimension, to determine whether desalting works effectively. Figure 2 shows the results of the experiment. The red profile shows significant suppression of the MS signal caused by salts entering the MS instrument. In contrast, the blue profile shows a high-quality MS signal that was achieved by removing salts with the AdvanceBio Desalting-RP cartridge in the second dimension. The experiment proved that desalting reduces suppression of the MS signal effectively.

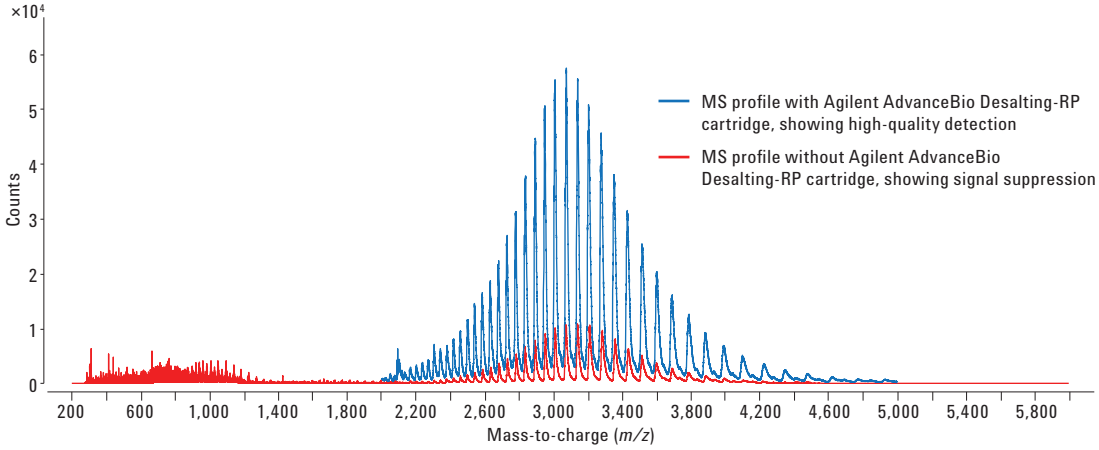
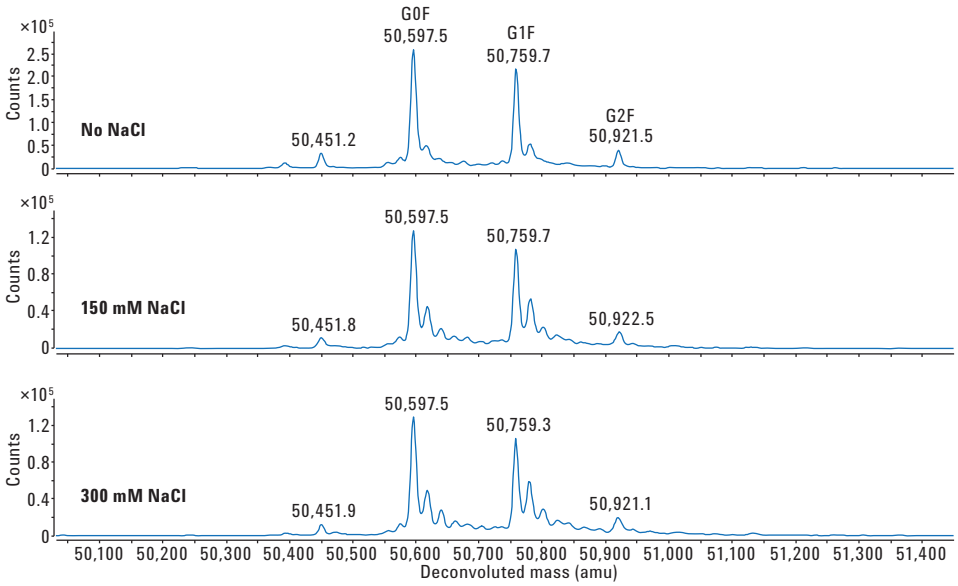


Figure 2. Analysis of the mAb sample using 2D-LC/MS (IEX → Desalting RP) showing the obtained mass spectrum profiles with (blue) and without (red) desalting, with an Agilent AdvanceBio Desalting-RP cartridge in the second dimension.

To determine the effect of salt concentration on the desalting process, mAb samples were mixed with different concentrations of NaCl, reduced with dithiothreitol (DTT), then analyzed with the desalting column in one-dimensional mode. Figure 3 shows the deconvoluted spectra of the heavy chain, where different glycoforms (G0F, G1F, and G2F) can be seen. Despite the signal decreasing, and the appearance of Na-adducts in the samples that contain higher salt concentration, the relative quantification between the samples is similar, regardless of the presence or absence of NaCl. This finding demonstrates the effective desalting achieved using an AdvanceBio Desalting-RP cartridge.

To evaluate the desalting procedure, 2D-LC/MS experiments were performed using the formulated mAb samples. An ion exchange (IEX) column was used in the first dimension, and an AdvanceBio Desalting-RP cartridge was used in the second dimension. The first-dimension chromatographic peak region was heart-cut (highlighted with the red box in Figure 4), transferred to the second-dimension column, then directed to the MS. Figure 4 shows the total ion chromatogram (TIC) peak, *m/z* spectrum, and deconvoluted spectra. This data show that the AdvanceBio Desalting-RP cartridge effectively removed the salts and provided high-quality mass spectra in a 2D setup.



Relative quantification

Glycoform	No 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 3. Deconvoluted spectra of the heavy chain. NaCl-containing mAb samples were analyzed on an Agilent AdvanceBio Desalting-RP cartridge following reduction using DTT. Relative quantification of glycoforms is similar between samples in the presence or absence of NaCl.

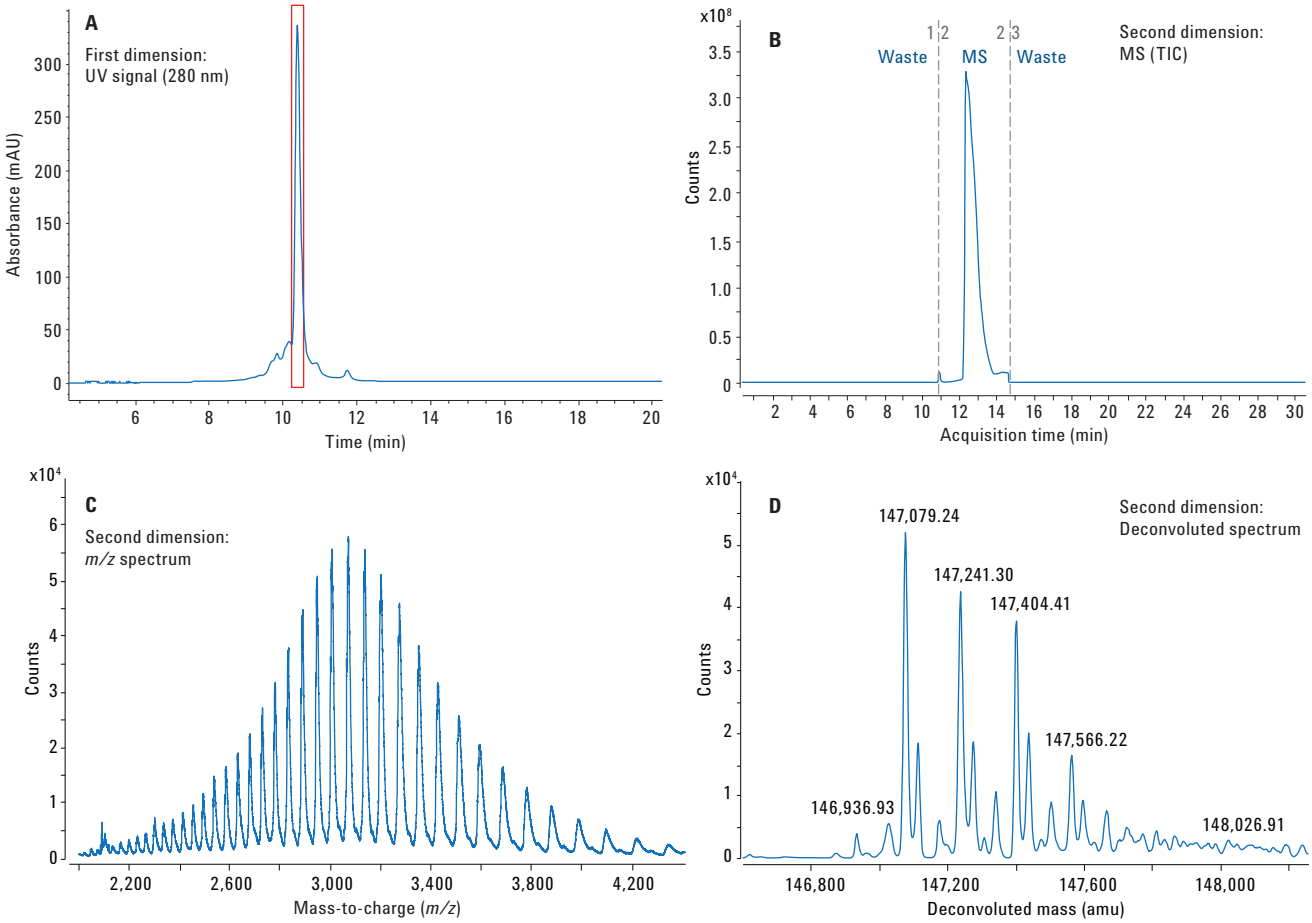


Figure 4. 2D-LC/MS of mAb profiles using an Agilent Bio MAb NP5 (IEX) as the first-dimension column and an Agilent AdvancedBio Desalting-RP cartridge for the second-dimension column. The selected main peak in the first-dimension chromatogram was captured and transferred to the AdvanceBio Desalting-RP cartridge that effectively removed salts and provided high-quality MS results.

To test the repeatability of the desalting procedure, three repetitive injections of mAbs were analyzed in a 2D setup. Ion exchange chromatography was used in the first dimension, and the desalting cartridge in the second dimension. Figure 5 shows that the extracted ion chromatogram (EIC) peaks and mass spectra were repeatable regarding height, shape, and *m/z* signal intensities. This demonstrates that the injection-to-injection repeatability underpins the consistent performance of the AdvanceBio Desalting-RP cartridge.

Conclusions

The Agilent AdvanceBio Desalting-RP cartridge can be used for effective desalting of LC-separated monoclonal antibodies before characterization by mass spectrometry. Injection-to-injection reproducibility supports consistent performance of the AdvanceBio Desalting-RP cartridge. The Agilent 1290 Infinity 2D-LC/MS solution enabled online desalting and characterization of mAbs using heart-cutting techniques.

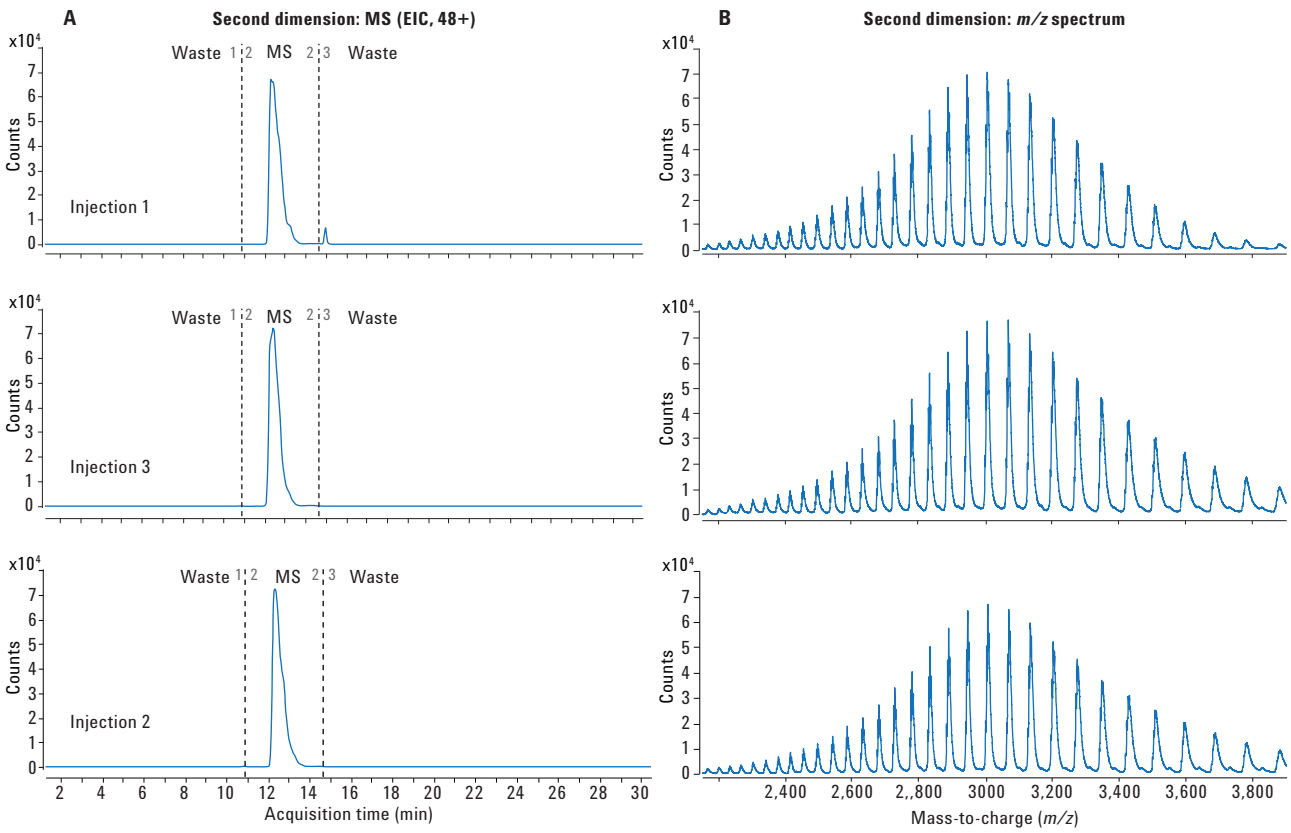


Figure 5. 2D-LC/MS of mAb profiles using an Agilent Bio MAb NP5 (IEX) as the first-dimension column and an Agilent AdvancedBio Desalting-RP cartridge for the second-dimension column. The second-dimension EIC (A) and *m/z* spectrum (B) profiles are shown.

For More Information

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