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응용 자료

Routine LC-MS Analysis of Intact Antibodies

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Abstract

This application note describes in detail the routine LC-MS analysis of intact antibodies using the Waters mAb Mass Check Standard.

LC-MS intact mass analysis of antibodies becomes a routine and high throughput analysis in light of the advancements made in LC-MS technologies and informatics. Using the provided LC-MS experimental conditions as well as data processing settings, high quality intact mAb data can be obtained routinely for mAb identification and relative glycoform quantitation.

Benefits

A reference for system qualification or troubleshooting guide for routine LC-MS intact mass analysis when using the mAb Mass Check Standard on the Xevo G2-XS QTof Mass Spectrometer.

Introduction

Intact mass analysis of monoclonal antibodies (mAbs) via LC-MS methods can be accomplished in minutes. It is

typically used to confirm the mass of the target mAb, and can also be used to profile product-related variants such as glycosylations.

This application note offers experimental details for routine LC-MS analysis of mAbs. The Xevo G2-XS QTof System was used for data collection. An antibody reference compound, the Intact mAb Mass Check Standard was used as a test sample to illustrate the process.

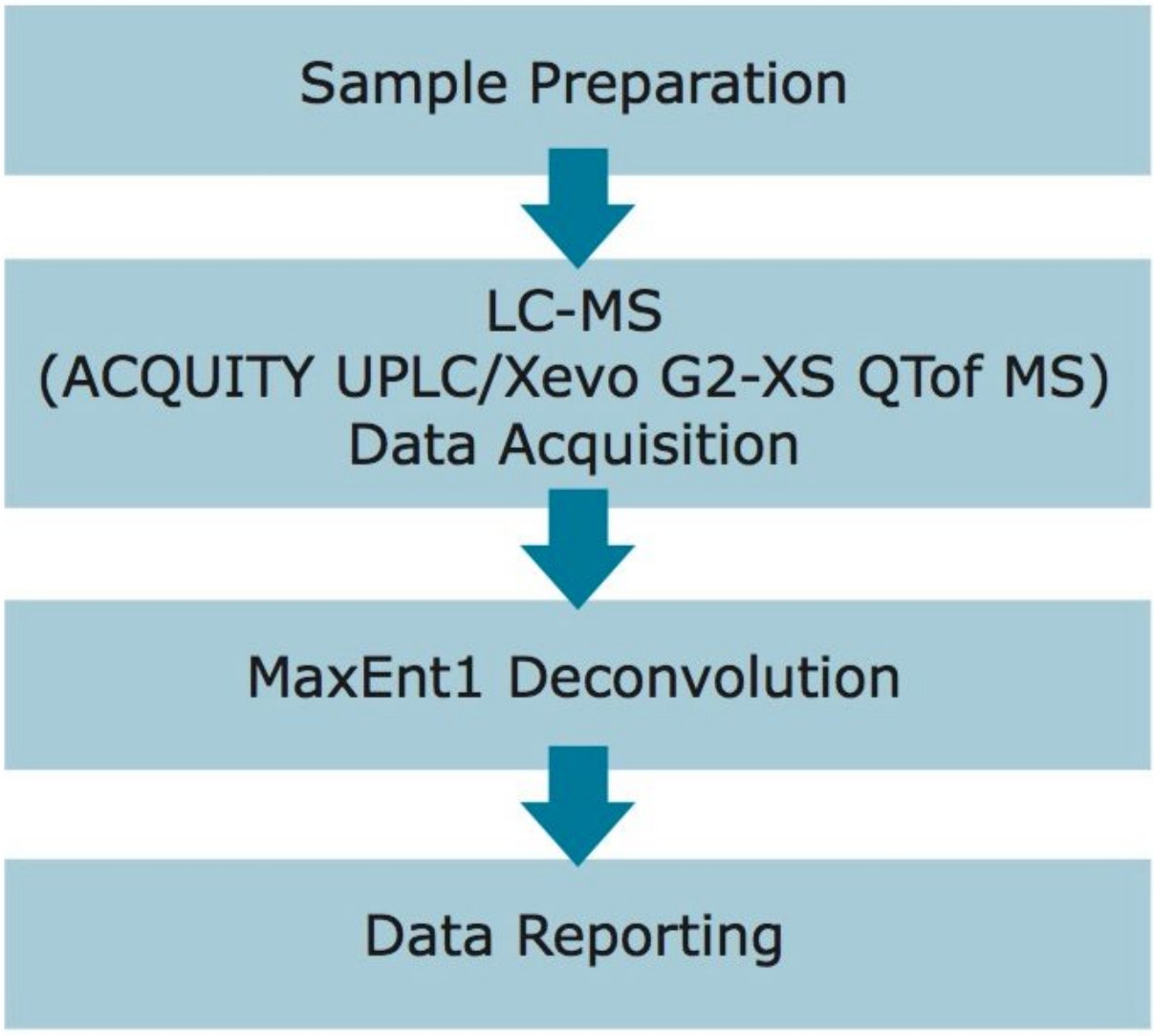

Figure 1. Workflow of Routine LC-MS Intact Mass Analysis.

Experimental

Sample preparation

Reference Standard: The Intact mAb Mass Check Standard was used for this application.

Xevo G2 QTof MS

Add 100 µL of DI water to the Max Recovery Vial that contains 1 mg of the mAb standard. Sonicate for 5 minutes. The mAb concentration of this solution is 10 mg/mL or 67 µM. Dilute the solution 20 fold with Eluent A* of the LC mobile phase to prepare a solution containing 3.3 µM (0.5 µg/µL) mAb.

Xevo G2-S QTof MS and Xevo G2-XS QTof MS

Add 100 µL of DI water to the Max Recovery Vial that contains 1 mg of the mAb standard. Sonicate for 5 minutes. The mAb concentration of this solution will be 10 mg/mL or 67 µM. Dilute this solution 100 fold with Eluent A* of the LC mobile phase to prepare a solution containing 0.67 µM mAb (0.1 µg/µL) mAb.

50 mM ammonium bicarbonate or ammonium acetate can be used as an alternative diluent instead of DI water.

LC-MS system

ACQUITY UPLC H-Class Variants

TUV optical detector*

Xevo G2 QTof, Xevo G2-S QTof, or Xevo G2-XS QTof, MassLynx. MaxEnt1, and or UNIFI Scientific Information System

**It is not recommended to have a PDA optical detector inline for intact mass analysis. For optical detection needs, a TUV detector is recommended.*

UPLC conditions

Column:	ACQUITY UPLC BEH C_4, 300Å, 2.1 mm x 50 mm Column

Column temp.:	80 °C
Mobile phase A:	0.1% formic acid in water*
Mobile phase B	0.1% formic acid in acetonitrile*
Detection:	UV 280 nm
Total run time:	6.5 min, injection volume 5 µL*

** LC-MS grade water, acetonitrile and formic acid are highly recommended for*

mobile phase preparations. Injection volume could be 10 µL as well.

** Recommended run time is 6.5 min.*

Gradient

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.4	95	5	6
1.00	0.4	95	5	6
1.01	0.2	95	5	6
3.50	0.2	5	95	6
3.70	0.4	5	95	6
4.00	0.4	95	5	6

Time (min)	Flow rate (mL/min)	%A	%B	Curve
4.50	0.4	5	95	6
5.00	0.4	95	5	6
5.50	0.4	95	5	6

MS conditions

Capillary:	3 kV
Sampling cone:	150V
Source offset:	80 V
Source temp.:	150 °C
Desolvation temp.:	500 °C
Cone gas flow:	0 L/Hr
Desolvation gas flow:	800 L/Hr
Data acquisition mass range:	m/z = 500 to 4000 amu
Suitable lockmass profile:	Glu Fibrinopeptide B (or other, if preferred)
Vacuum read back:	ToF vacuum is very important to intact mass analysis. A better vacuum will help to generate higher quality data.

** Please note that for different proteins (either varied by structure, or molecular weight), MS parameters may be changed to get better MS spectra quality. Here are the MS settings that can be adjusted within:*

Capillary: 1.5 to 3.5 KV, Sampling cone: 120 to 195V, source offset: 80 to 120 V, source temperature: 135 to 150 °C, desolvation temperature: 350 to 550 °C, cone gas flow: 0 to 100 L/Hr, and desolvation gas flow: 600 to 1000 L/Hr.

Results and Discussion

Routine LC-MS analysis for intact antibodies was conducted using a fast and simple desalting method described above. This section shows typical experiment results and discusses practices for achieving optimal results.

TIC and TUV Chromatograms

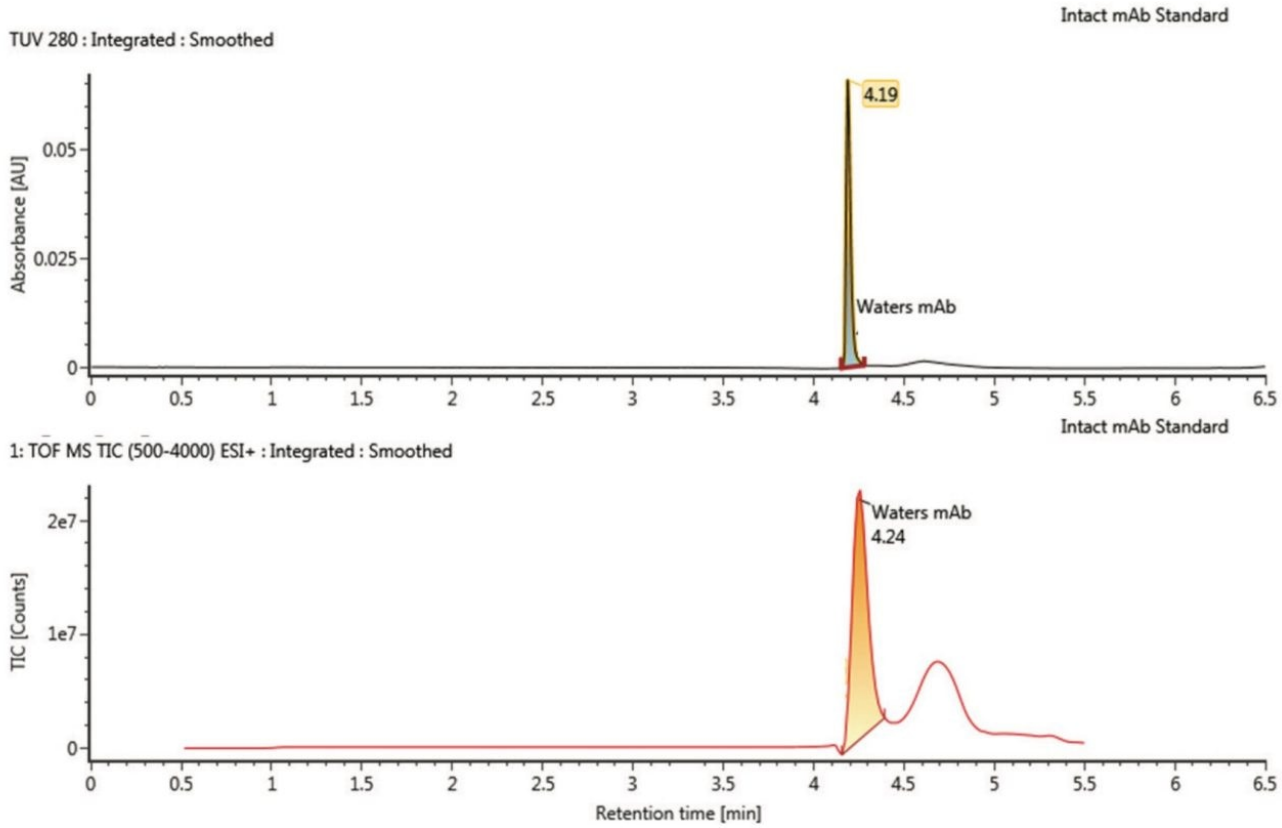

Figure 2. Typical TIC and TUV chromatograms (screen capture from UNIFI) from a routine LC-MS intact mass analysis.

Chemical background signals

Less than 1,000 ion counts (1 second scan with the mass range of m/z =500 to 4000) should be expected for background ion signal intensity, as shown in Figure 3.

A high chemical background signal will affect the raw spectrum quality (spectrum resolution and mass accuracy) in intact mass analysis. A high chemical background signal is very often attributed to contaminations from the LC system. The most commonly observed contaminants are polymers (e.g. polyethylene glycol (PEG) or PEG related materials). Residual TFA used as mobile phase additive from previous analysis could also interfere with new analysis that uses different mobile phase additive (such as formic acid).

Figure 4 shows what PEG polymer peaks look like in the mass spectrum. Figure 5 shows an example of the TFA adduct ion series (TFA+Na with 136 Da mass differences among the peaks) as the background contamination peaks.

If the LC system is deemed contaminated, the LC system must be cleaned. A cleanup procedure is included in Appendix A.

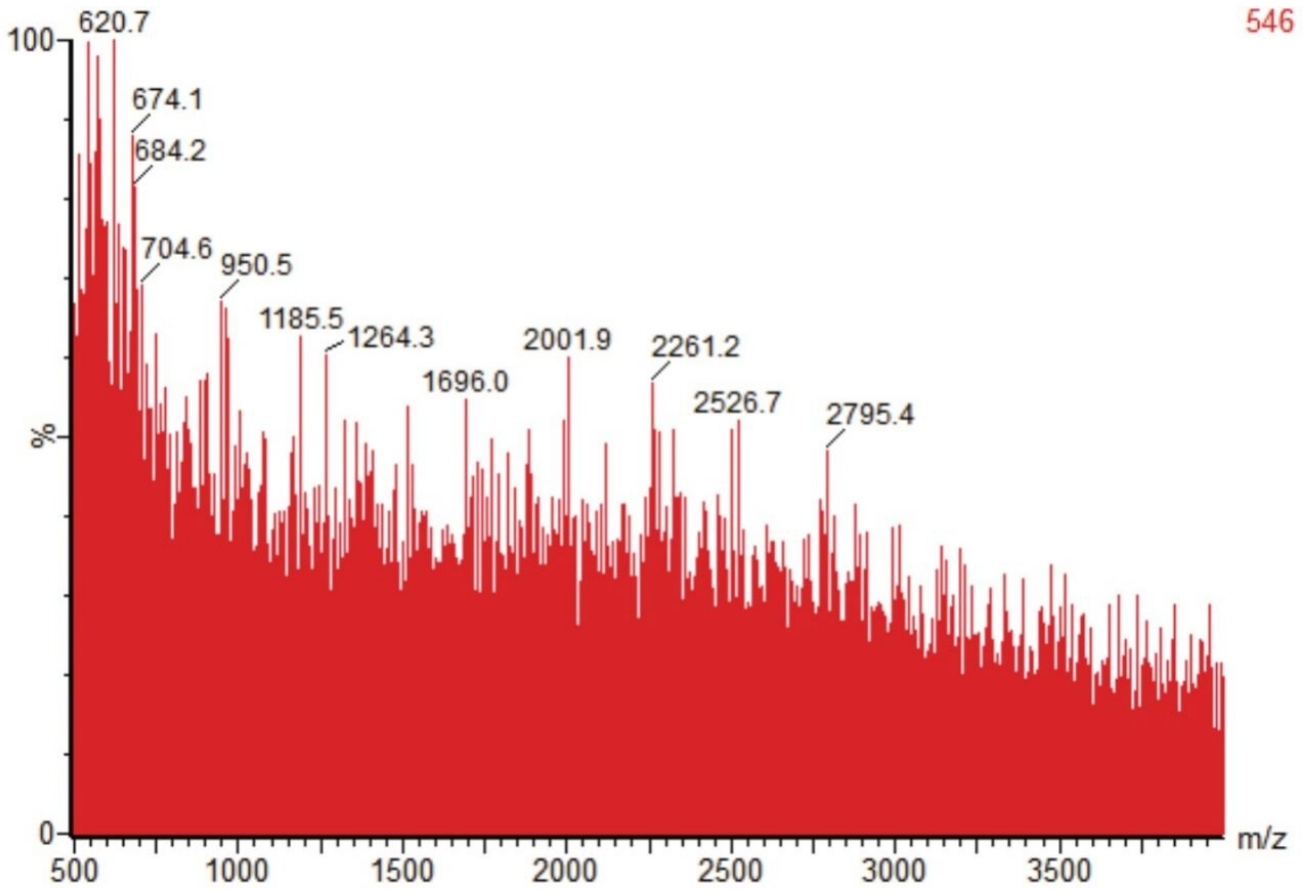

Figure 3. Normal single scan MS spectrum chemical background signal level.

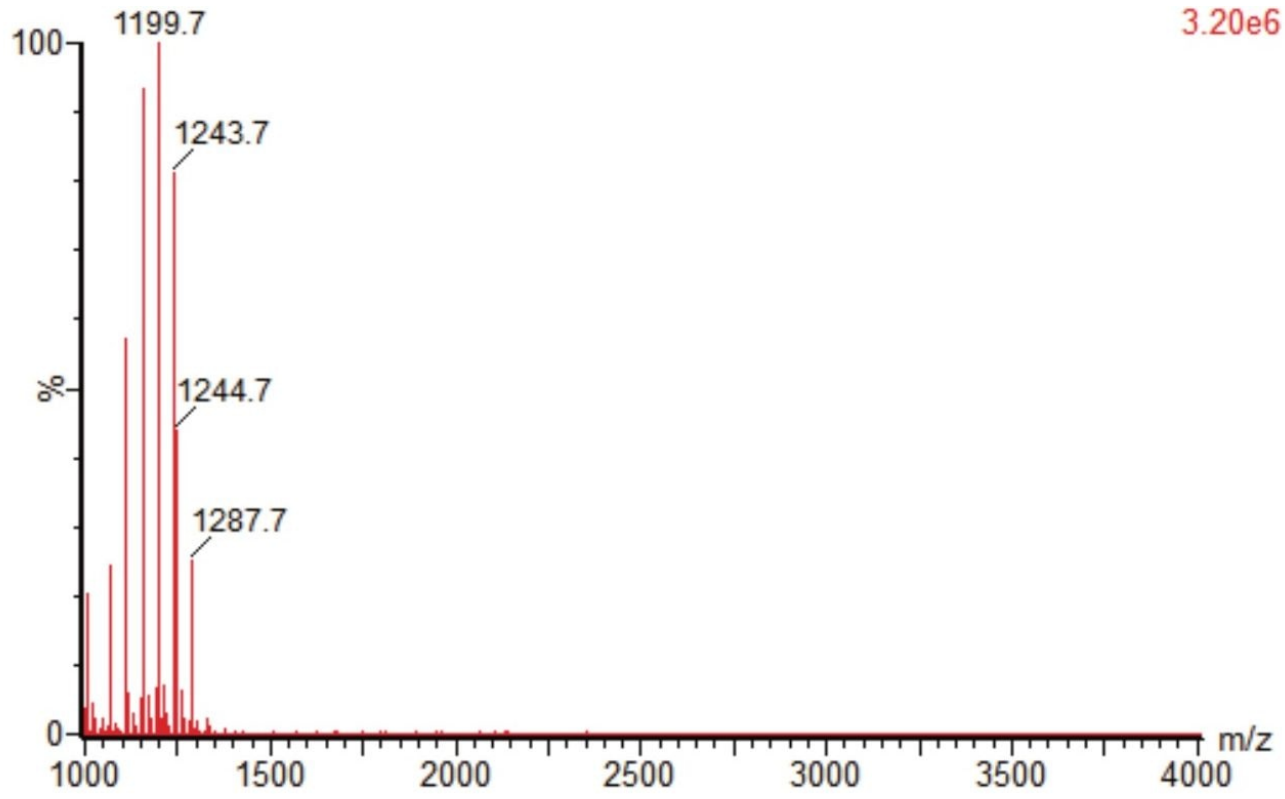

Figure 4. Example of PEG (+44 Da mass differences between the peaks) contaminations.

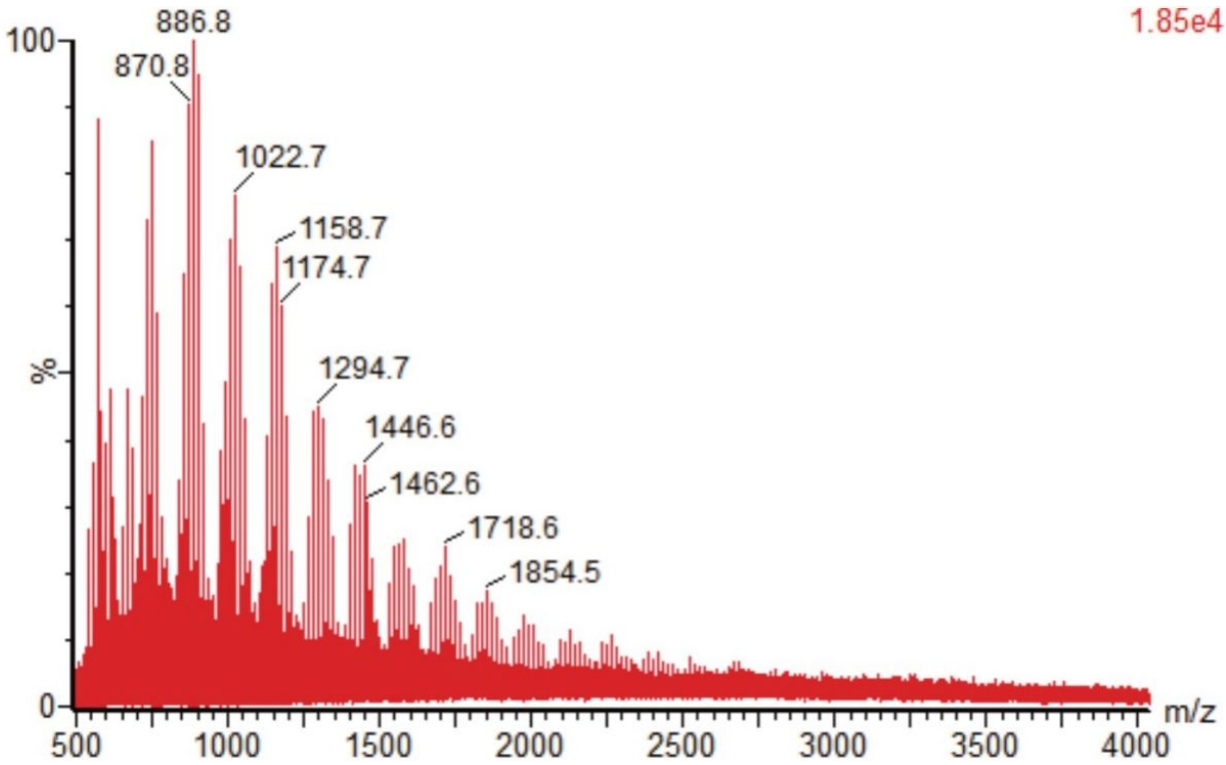

Figure 5. Example of TFA (+136 Da mass differences between the peaks) contaminations.

Raw spectrum

Figure 6 shows a typical example of a high quality raw spectrum (combined data, showing m/z range from 1900 to 4000 amu). The peak valley (as shown in Figure 7) between the first and the second glycoforms should be roughly 30% of the base peak height. Figure 7 shows a spectrum with the zoomed mass region, which shows the valley between each glycoform is approximately 27% of the base peak. This meets the criteria of the quality standard. The quality of the deconvoluted spectra, the measured mass accuracy of the glycoforms as well as their peak intensity is greatly improved by having a low valley between glycoforms in the raw spectrum.

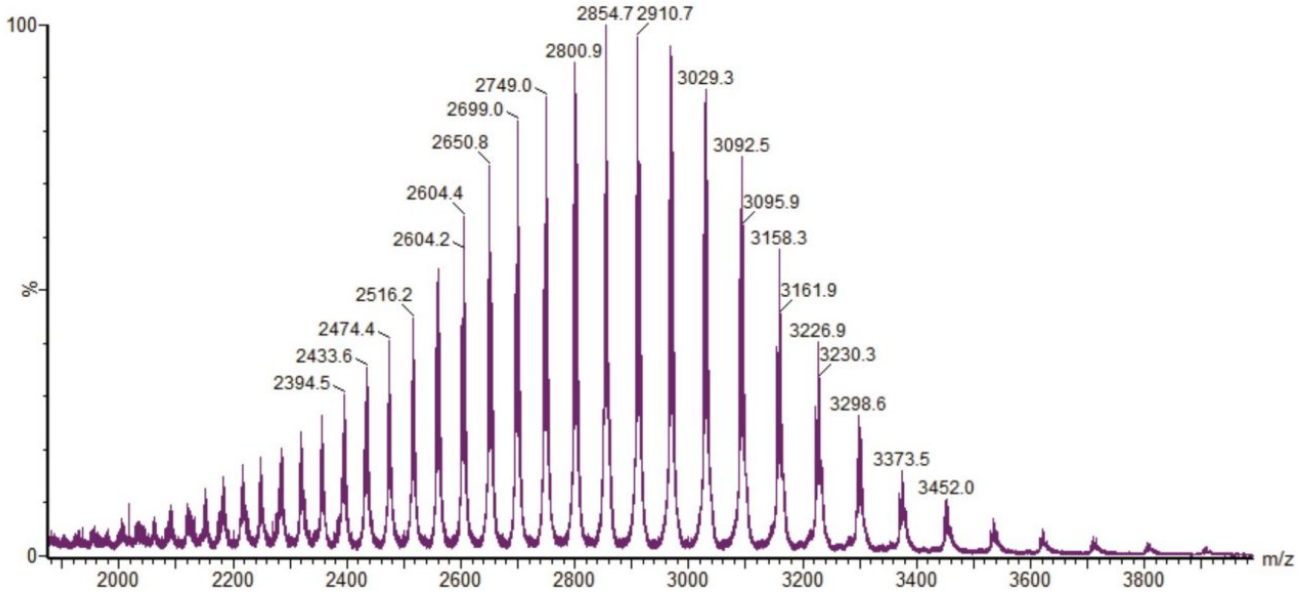

Figure 6. Waters mAb standard combined raw spectrum.

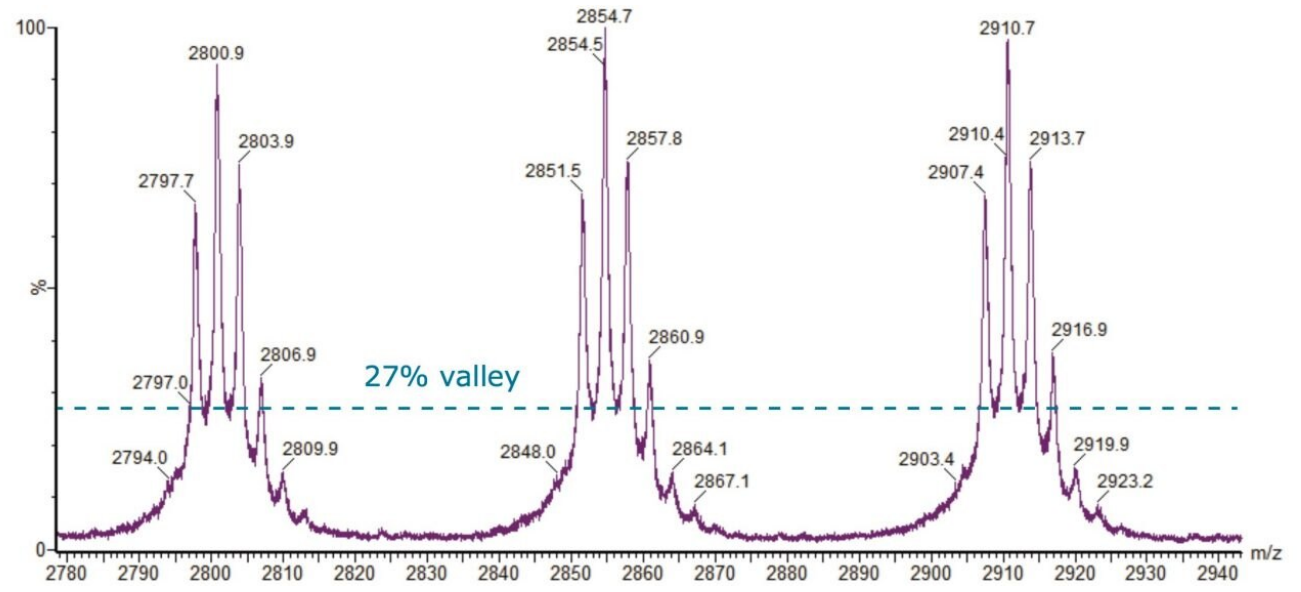

Figure 7. Waters mAb standard combined raw spectrum, zoomed in region.

Deconvolute combined raw spectrum using MaxEnt1

Figure 8 shows the MaxEnt1 settings in MassLynx to deconvolute combined raw spectrum (similar settings can

be used in UNIFI software for deconvolution).

When deconvoluting raw intact mass data by MaxEnt1, the peak width setting at half height and the number of iterations have the greatest impact on the final quality of the deconvoluted spectrum. Figure 9 shows the deconvolution spectrum from the raw spectrum in Figure 6.

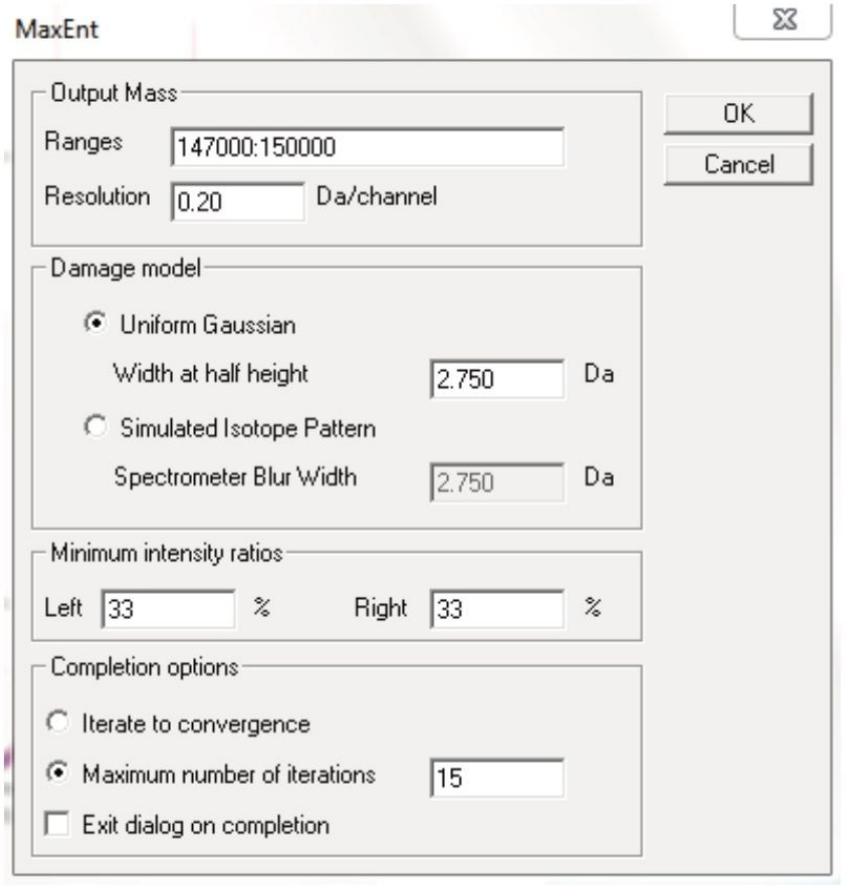

Figure 8. Recommended MaxEnt1 parameter settings were used to deconvolute combined raw spectrum in MassLynx (similar settings can be used in UNIFI Software).

The expected masses for the five major glycoforms are listed in Table 1.

The mass accuracy for the 5 major glycoforms is typically less than 50 ppm. Better mass accuracy (5 to 20 ppm) can be achieved with high quality mass spectrum, which is achievable following the recommendations given in

this application note.

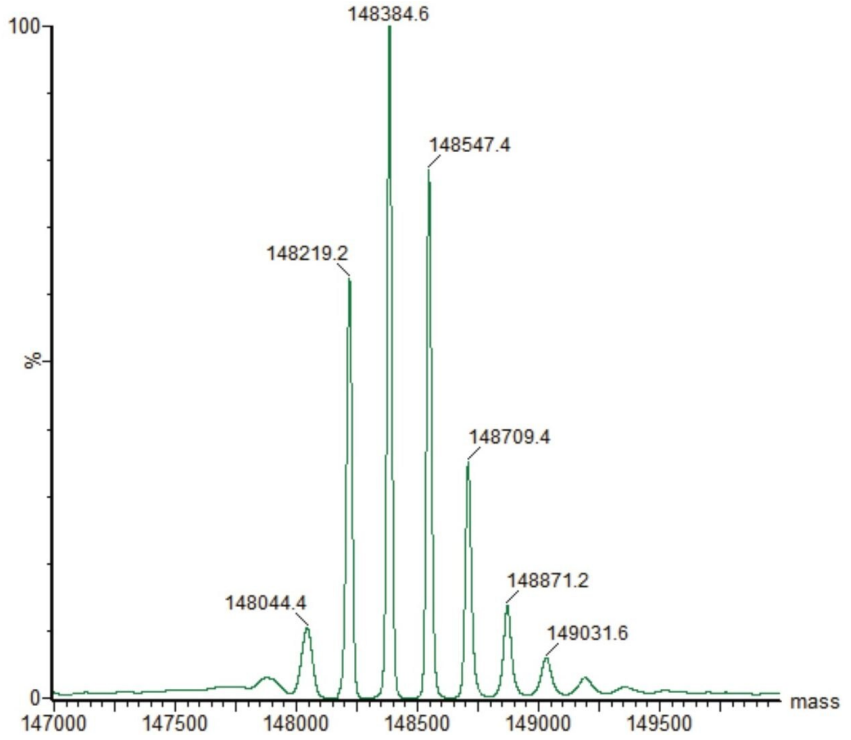

Figure 9. Deconvoluted spectrum, resulted from MaxEnt1 parameter settings as displayed in Figure 8.

Calculated MW of the Major Glycoforms of the Waters mAb		
Peak number	**mAb Glycoform**	**Expected mass (Da)***
1	M+G0F+G0F	148220.4
2	M+G0F+G1F	148382.5
3	M+G1F+G1F	148544.7
4	M+G1F+G2F	148706.8
5	M+G2F+G2F	148869.0

**expected mass (Da) was calculated in UNIFI.*

Table 1. Expected average masses of the five major glycoforms (from the Mass Calculator tool in UNIFI).

Relative abundance of the major glycoforms

Deconvoluted MS ion counts are widely used for glycoprofiling for intact mAbs. The relative abundance for this particular sample batch is shown in Table 2 (calculated automatically in UNIFI):

Relative Abundance of the Major Glycoforms of the Waters mAb		
Peak Number	**mAb Glycoform**	**Relative Abundance %**
1	M+G0F+G0F	25.65
2	M+G0F+G1F	30.99
3	M+G1F+G1F	23.92
4	M+G1F+G2F	12.98
5	M+G2F+G2F	6.45

Table 2. Calculated relative abundance of the five major glycoforms (processed and calculated in UNIFI).

Beyond the information that is provided in this application note, additional information on LC-MS analysis for intact proteins using the Biopharmaceutical Platform Solution with UNIFI can be found in the Waters application note listed under References.

Conclusion

LC-MS intact mass analysis of antibodies becomes a routine and high throughput analysis in light of the advancements made in LC-MS technologies and informatics. Using the provided LC-MS experimental conditions as well as data processing settings, high quality intact mAb data can be obtained routinely for mAb identification and relative glycoform quantitation.

References

1. Shion, H. and Chen, W. Streamlining Compliant and Non-Compliant Intact Mass Analysis of Biotherapeutic mAbs with the Biopharmaceutical Platform Solution with UNIFI. 2013 Waters Application Note. 2013 (P/N 720004617EN).
2. Intact mAb Mass Check Standard Product Solution Brochure. Waters Brochure. 2013 (P/N 720004610EN).
3. Intact mAb Mass Check Standard Care and Use Manual. Waters Brochure. 2013 (P/N 720004420EN).

Featured Products

ACQUITY UPLC H-Class Bio <https://www.waters.com/10166246>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometer <https://www.waters.com/134798222>

Biopharmaceutical Platform Solution with UNIFI <https://www.waters.com/10195515>

Available for purchase online:

Intact mAb Mass Check Standard <
https://www.waters.com/waters/partDetail.htm?partNumber=186006552>

ACQUITY UPLC Protein BEH C4, 300Å, 1.7 µm, 2.1 mm X 50 mm Column <
http://www.waters.com/waters/partDetail.htm?partNumber=186004495>

Appendix A

The ACQUITY UPLC System wash protocol*

1. Replace column (if there was one) with a clean union.
2. Place all of the mobile phase channel tubing into wash solvent** bottle number one (Bottle 1), which should contain 50/50, H2O/Methanol (wash solution number one). Run blank (H2O) injection every 3 min, with LC flow change (step or linear flow gradient) from 0.1 mL/min to 0.5 mL/min, for 4 hours.
3. Move all of the mobile phase channel tubing into wash solvent bottle number two (Bottle 2), which should contain 100% IPA (wash solution number two). Run blank (H2O) injection every 20 min, with LC flow change

(step or linear flow gradient) from 0.1 mL/min to 0.5 mL/min, for 4 hours.

4. Move all of the mobile phase channel tubing into wash solvent bottle number one (Bottle 1), which should contain 50/50 H2O/Methanol (wash solution number one). Run blank (H2O) injection every 20 min., with LC flow change (step or linear flow gradient) from 0.1 mL/min to 0.5 mL/min, for 2 hours.
5. Move all of the mobile phase channel tubing into wash solvent bottle number three (Bottle 3), which should contain 100% H2O (wash solution number three). Run blank (H2O) injection every 10 min, with LC flow change (step or linear flow gradient) from 0.1 mL/min to 0.5 mL/min, for 4 hours.

Solvent Bottle Table		
Bottle 1	Bottle 2	Bottle 3
50/50=H_2O/MeoH	100% IPA	100% H_2O

Appendix Table 1. Solvent bottle contents.

**This wash protocol can be used to clean up contaminates, such as TFA and PEGs, in Waters ACQUITY UPLC systems.*

***Please notice all the solvents used in the protocol should be LC-MS compatible.*

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