Intact Protein Analysis Using an Agilent 6550 Q-TOF Mass Spectrometer

Application Note

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Introduction

LC/MS has been widely used in the biopharmaceutical industry for therapeutic protein molecular weights confirmation. It is fast, accurate, and relatively quantitative. The accurate mass measurement helps to determine whether the correct protein sequence has been expressed with the expected post translational modifications (PTM). It also provides relative abundance of different proteins or PTMs present in the same sample. A high resolution and high analytical sensitivity mass spectrometer will facilitate this analysis. This application note presents an intact protein analysis using an Agilent 6550 Q-TOF mass spectrometer.

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Experimental

Sample

Monoclonal antibody (mAb) was diluted to 100 µg/mL using 0.1 % formic acid (FA) in 3 % acetonitrile (ACN) and 96.9 % water. A 1 µL (100 ng) amount was injected. Purified protein sample (P128) was obtained from GangaGen Biotechnologies Pvt. Ltd and was analyzed using 0.025 % trifluroacetic acid (TFA) in 3 % acetonitrile (ACN) and 96.9 % water.

Instrumentation

LC systems
Agilent 1290 Infinity LC System

MS systems
Agilent 6550 iFunnel Q-TOF with Agilent JetStream

Results and Discussion

Proteins form multiply charged ions during electrospray ionization. Large proteins such as monoclonal antibody have a charge distribution envelope as shown in Figure 1. The center of the envelope is charge 48. Multiple peaks can be observed, and these are the different glycoforms attached on the mAb. The spectrum is deconvoluted using a Peak Modeling deconvolution algorithm in Agilent MassHunter BioConfirm software. It converts the multiply charged spectrum to the zero charge mass spectrum. Figure 2 shows the result.

LC/MS Parameters

Parameter	Agilent 1290 LC System
Column	Agilent Poroshell 300SB C8 1.0 × 75 mm, 5 µm or Agilent ZORBAX RRHD 300 Diphenyl, 2.1 × 100 mm, 1.8 µm (p/n 858750-944)
Column temperature	80 °C or 60 °C
Sample thermostat	5 °C
Mobile phase A	0.1 % formic acid in water or 0.025 % TFA in water
Mobile phase B	90 % acetonitrile in water with 0.1 % formic acid
Gradient	0–1 minutes 3 %B 1–5 minutes 3–90 %B 5–6 minutes 90 %B 6–7 minutes 3 %B
Stop time	7 minutes
Flow rate	0.4 mL/min
Ion mode	Positive ion mode, ESI (Profile)
Drying gas temperature	290 °C
Drying gas flow	14 L/min
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Nebulizer	20 psi
Capillary voltage	5,000 V
Nozzle	2,000 V
Data analysis	The data obtained from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis software B.06 and Agilent MassHunter BioConfirm software B.06

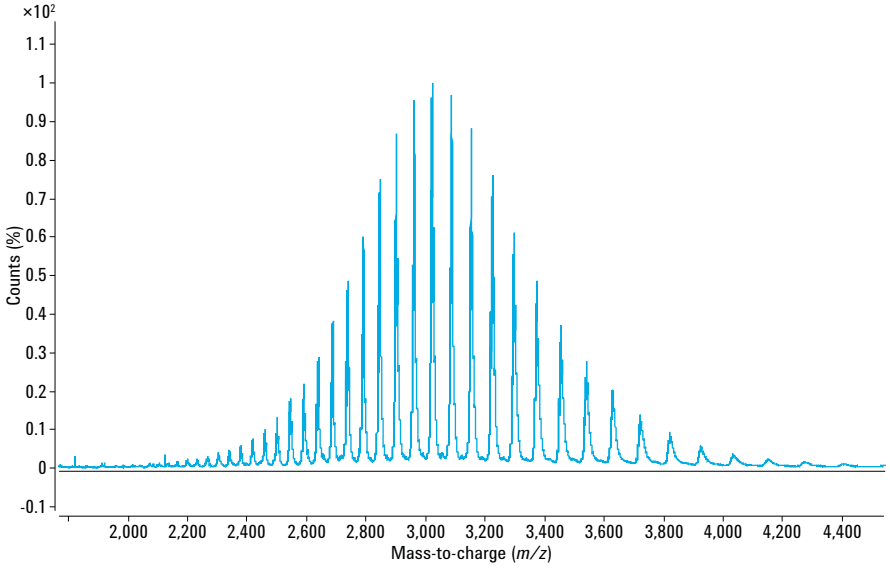

Figure 1. LC/MS raw data of charge envelope of intact mAb.

Five major glycoforms were observed on this mAb. Figures 3 and 4 show another example of a therapeutic protein P128 with a molecular weight of 26,490 Da.

Conclusions

- The analysis of therapeutic protein using an Agilent 1290 Infinity LC System coupled to an Agilent 6550 iFunnel Q-TOF has been demonstrated.
- The Agilent 1290 Infinity LC System provided fast and superior separation power, and the Agilent 6550 iFunnel Q-TOF delivered excellent resolution and sensitivity for intact protein analysis.
- The Agilent MassHunter BioConfirm software provided automated data extraction, deconvolution, and protein confirmation.

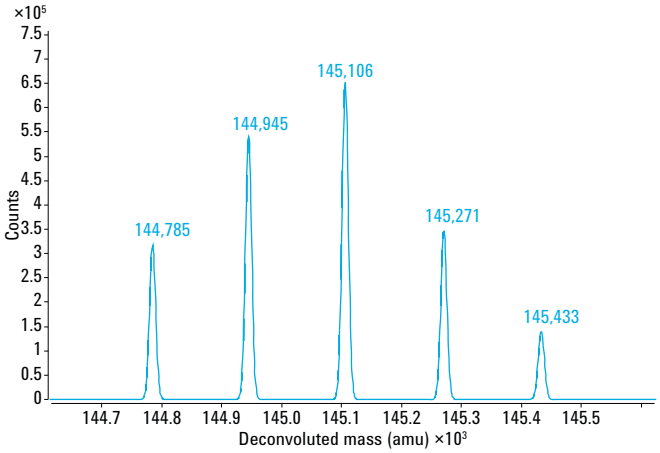

Figure 2. Deconvoluted spectrum of intact mAb. The major peaks are the major glycoforms on the mAbs.

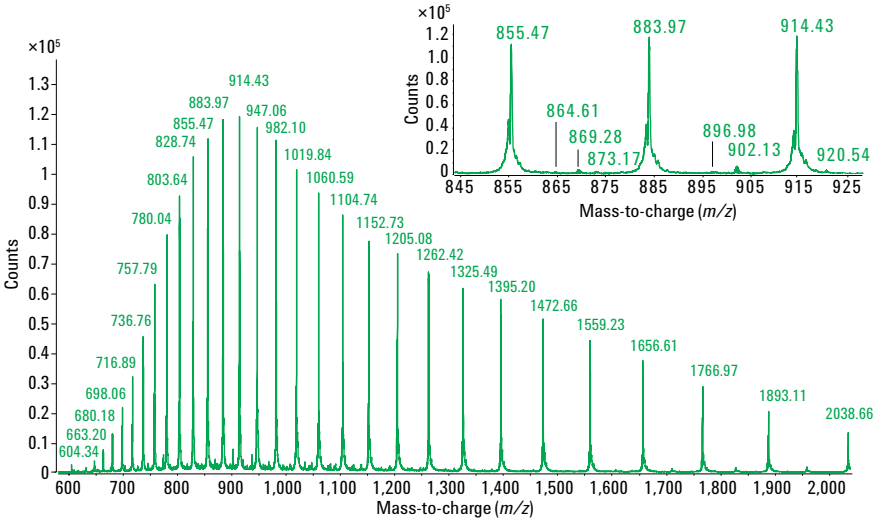

Figure 3. LC/MS raw data of charge envelope of P128 with inset showing zoom in of the charge states of P128.

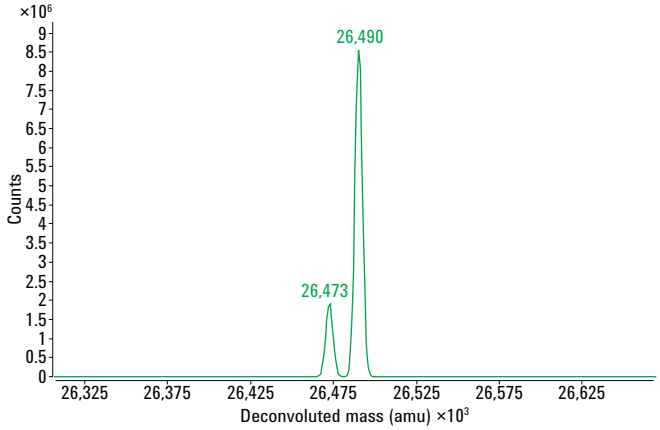

Figure 4. Deconvoluted spectrum of P128.

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