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

Application Note

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.

Authors

Ravindra Gudihal and Suresh Babu CV
Agilent Technologies India pvt. Ltd.
Bangalore India

Ning Tang
Agilent Technologies, Inc.
Santa Clara, CA USA

Madhavi H.N and Uma Maheshwari
GangaGen Biotechnologies Pvt. Ltd.
Bangalore, India
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 % trifluoroacetic 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.

![Figure 1. LC/MS raw data of charge envelope of intact mAb.](image-url)
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.