Analysis of Monoclonal Antibody (mAb) Using Agilent 1290 Infinity LC System Coupled to Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF)

Application Note

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Abstract

This Application Note describes the use of an Agilent 1290 Infinity LC System coupled to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) for intact and reduced monoclonal antibodies (mAb) analysis. It presents a method that gives high resolution total ion chromatogram and the mass spectrum. The software features of Agilent MassHunter Bioconfirm are highlighted to show the utility of comparing two mAbs in terms of intact and fragment mass using the mirror plot feature of the software. This feature is useful during comparability studies or biosimilar comparisons.

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Introduction

The mAb market is growing at a rapid pace, and biopharma companies have invested heavily in these biomolecules[1]. There is a need to achieve good chromatographic separation and mass spectra with powerful software features to analyze these biomolecules in detail, especially in the areas of biosimilars and comparability studies. High resolution chromatography and mass spectrometry help in quick quality check (QC) and impurity analysis for mAbs. This Application Note describes an LC/MS method that gives high resolution chromatography as well as mass spectra for two mAbs. The method was established using an Agilent 1290 Infinity LC System coupled to an Agilent 6530 Accurate-Mass Q-TOF. The LC/MS method was developed using isopropanol/water/acetonitrile with formic acid as the solvent system. Among various mobile phases tested, this solvent system gave good chromatography as well as good mass spectra. Narrow peak width in total ion chromatogram (TIC) for the intact mAbs was observed and well-separated peaks for light and heavy chain were also achieved for the reduced mAb samples. The data were analyzed using features of Agilent MassHunter Bioconfirm Software to compare differences between these two mAbs. Mirror plot of the two mAbs provides fast and visual comparison of the samples. This method is useful for the analysis of intact and reduced heavy and light chains of mAbs.

Experimental

Sample

mAbs—IgG1 and IgG2 were diluted to 100 µg/mL using 0.1 % formic acid in water; 1 µL (100 ng) was injected. For reduction of mAb, the antibodies were reduced using dithiothreitol (DTT) at 60 °C for 1 hour.

Instrumentation

LC system

Agilent 1290 Infinity LC System

MS systems

Agilent 6530 Accurate-Mass Q-TOF with Agilent JetStream ion source.

LC and MS parameters for intact mAb analysis

Parameter	Value
Agilent 1200 Infinity LC System	
Column	Agilent ZORBAX Poroshell 300SB-C8, 2.1 × 75 mm, 5 µm (p/n 660750-906)
Injection volume	1 µL (Needle with wash, flush port active for 5 seconds)
Sample thermostat	5 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	70 % IPA/20 % ACN/10 % water with 0.1 % formic acid
Gradient (segmented)	At 0 minutes → 15 % B At 4 minutes → 20 % B At 5 minutes → 75 % B At 10 minutes → 100 % B At 10.1 minutes → 15 % B
Stop time	10.1 minutes
Post time	4 minutes
Column temperature	60 °C
Flow rate	1.2 mL/min from an Agilent 1290 Infinity Series Binary Pump (p/n G4220A)
Agilent 6530 Accurate-Mass Q-TOF LC/MS	
Ion mode	Positive ion mode, Dual AJS ESI (Profile)
Drying gas temperature	350 °C
Drying gas flow	8 L/min (nitrogen)
Nebulizer	45 psi
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	5,500 V
Fragmentor voltage	380 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Acquisition parameters MS mode	Data were acquired at 1 GHz, MS only mode, mass range 2,000–5,000 *m/z*.
Data analysis	The data obtained from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis Software and Agilent MassHunter BioConfirm Software. Peak Modeling (pMod) deconvolution algorithm was used for obtaining zero-charge spectrum of mAb.

LC and MS parameters for reduced mAb analysis

Parameter	Value
Agilent 1200 Infinity LC System	
Column	Agilent ZORBAX Poroshell 300SB-C8, 2.1 × 75 mm, 5 µm (p/n 660750-906)
Injection volume	1 µL (Needle with wash, flush port active for 5 seconds)
Sample thermostat	5 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	70 % IPA/20 % ACN/10 % water with 0.1 % formic acid
Gradient (segmented)	At 0 minutes → 20 % B At 1 minutes → 25 % B At 5 minutes → 28 % B At 8 minutes → 40 % B At 9 minutes → 40 % B At 10 minutes → 80 % B At 10.1 minutes → 20 % B
Stop time	10 minutes
Post time	1 minute
Column temperature	60 °C
Flow rate	1.2 mL/min from Agilent 1290 Infinity Series Binary Pump (p/n G4220A)
Parameter	Value
Agilent 6530 Accurate-Mass. Q-TOF LC/MS	
Ion mode	Positive ion mode, Dual AJS ESI (Profile)
Drying gas temperature	350 °C
Drying gas flow	8 L/min (nitrogen)
Nebulizer	40 psi
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	4,200 V
Fragmentor voltage	300 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Acquisition parameters MS mode	Data were acquired at 2 GHz, MS only mode, mass range 300–3,200 *m/z*.
Data analysis	The data obtained from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis Software and Agilent MassHunter BioConfirm Software. Peak Modeling (pMod) deconvolution algorithm was used for obtaining zero-charge spectrum of mAb.

Results and Discussion

LC/MS analysis of intact mAbs IgG1 and IgG2 is shown in Figure 1. Excellent peak shapes with narrow peak width (0.5 minutes at base) is achieved using IPA/ACN/H_2O mobile phase with a ZORBAX Poroshell 300SB-C8 column (Figures 1A and 1D). Figures 1B and 1E show the Gaussian distribution of the charge state envelope for the mAbs. The deconvoluted spectrum are shown in Figures 1C and 1F. The spectrum is deconvoluted using the peak modeling (pMod) deconvolution algorithm in Agilent MassHunter BioConfirm Software. The pMod deconvolution algorithm first deconvolutes the raw spectrum using maximum entropy deconvolution. In addition, it automatically allows different peak models to fit and validate the maximum entropy deconvolution results[2].

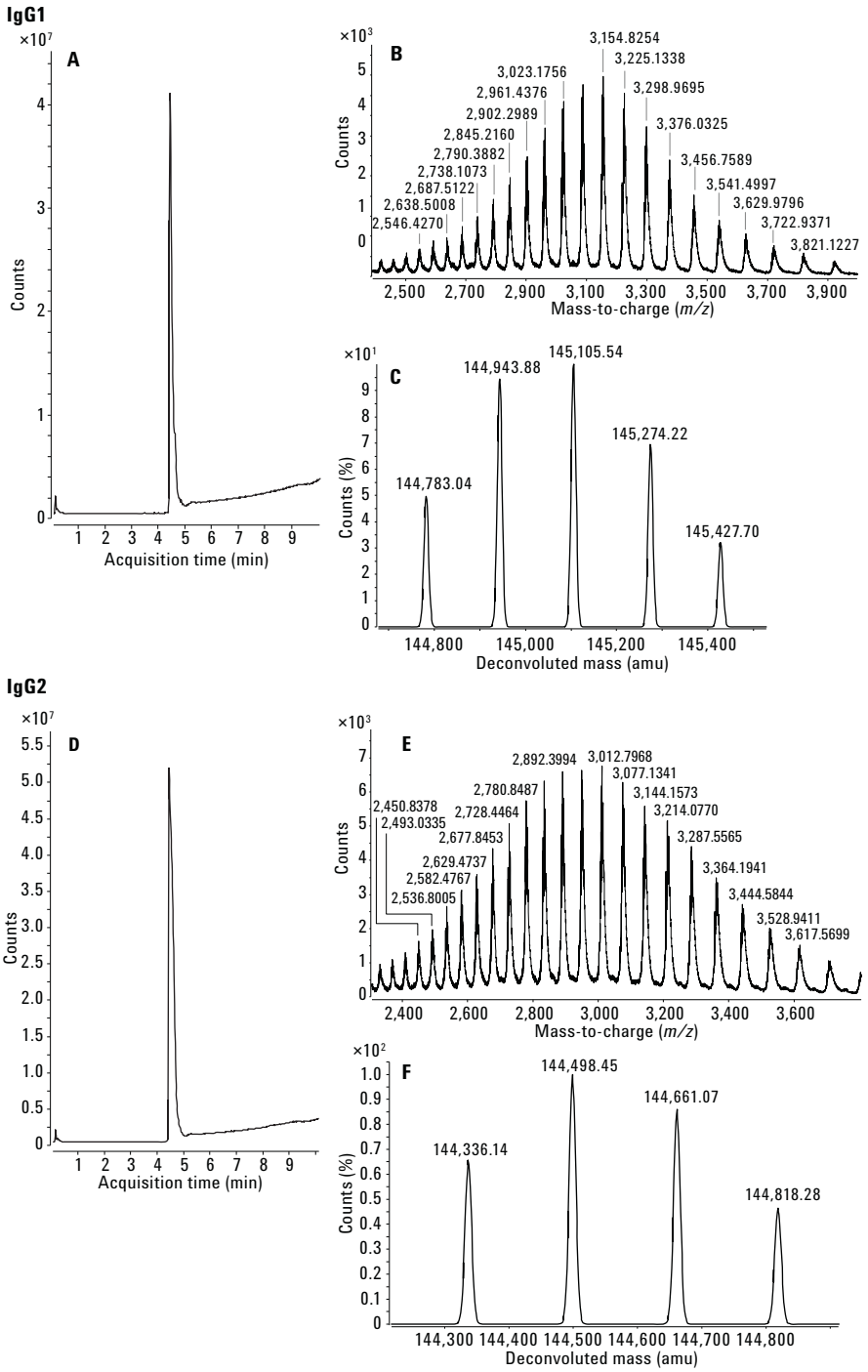

Figure 1. TIC of intact mAbs (A,D), charge envelope (B,E), and their respective deconvoluted masses (C,F) (A,B,C are IgG1 and D,E,F are IgG2).

Multiple mass peaks are observed corresponding to different glycoforms attached to the mAbs. The intact mAb analysis reveals that these two antibodies have different masses. IgG1 has a major mass peak at 144,943 Da, and IgG2 has one at 144,498 Da. Five major glycoforms are observed in the IgG1 deconvoluted spectrum, while four major glycoforms are observed in the IgG2 spectrum. To further understand the difference in details between these two mAbs, reduction of disulfides was performed to produce light and heavy chains of mAbs. Figure 2 shows the result of reduced mAb analysis using LC/MS. Light chains (LC) and heavy chains (HC) are separated very well with good peak shapes using the 10 minute gradient. Figure 2 also shows the deconvoluted spectrum for LC and HC for both mAbs. Inspection of the deconvoluted spectrum clearly shows the difference between the HCs of the two mAbs. IgG1has mass peaks ranging from 49,475 Da to 49,799 Da, whereas IgG2 ranges from 49,255 Da to 49,581 Da. The LC of both mAbs have identical masses.

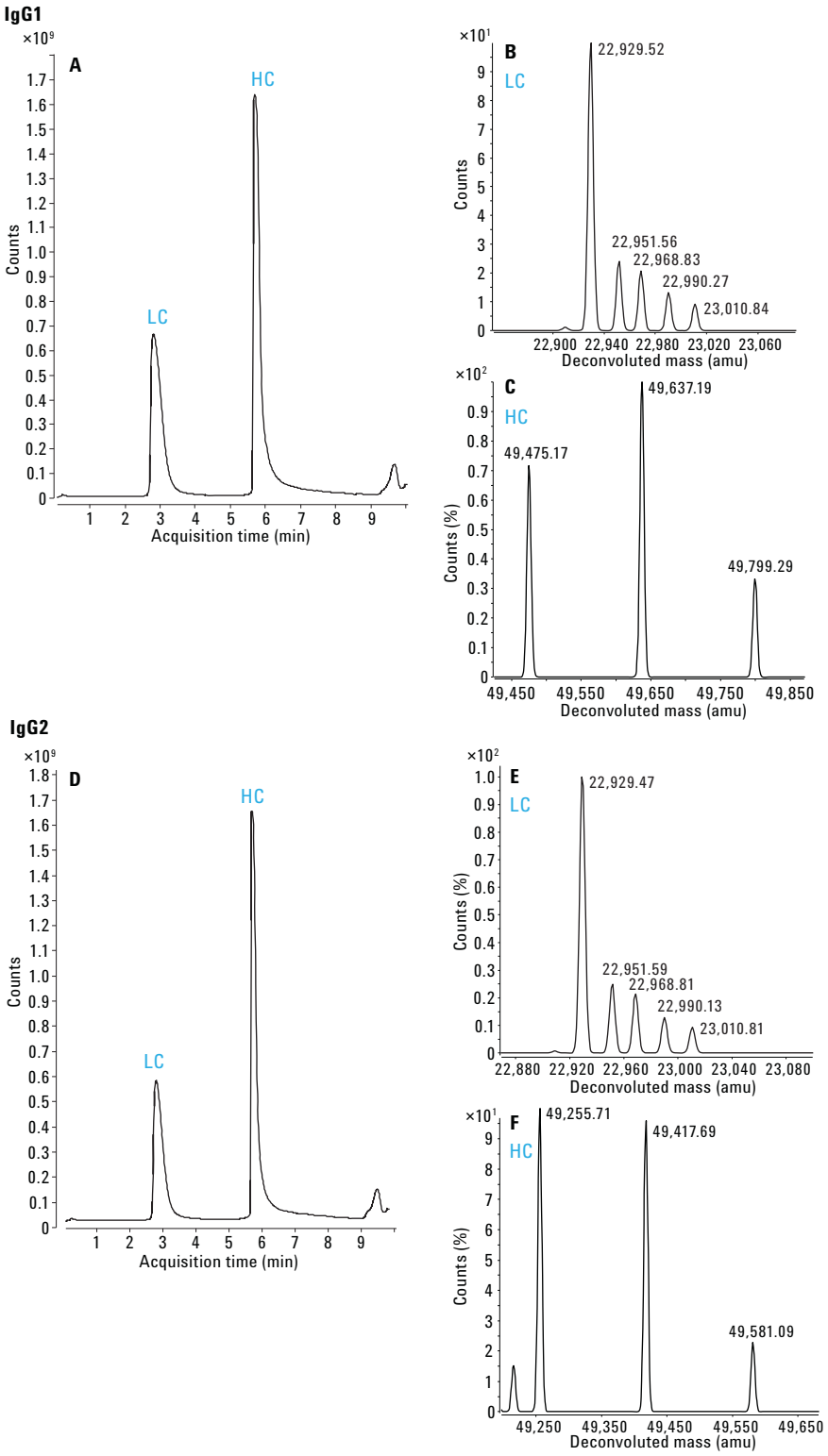

Figure 2. Total ion chromatogram (TIC) of reduced mAbs (A,D) into light chain (LC) and heavy chains (HC) and their respective deconvoluted masses (B,C,E,F) (A,B,C are IgG1 and D,E,F are IgG2).

Mirror plots of IgG1 and IgG2 are shown in Figure 3. It shows that the difference in the intact mAb comes from the difference in the HC chain, while the LC in both antibodies is identical in mass (~22,929 Da). The satellite peaks found near the main LC peak correspond to salt adducts of LC in both antibodies.

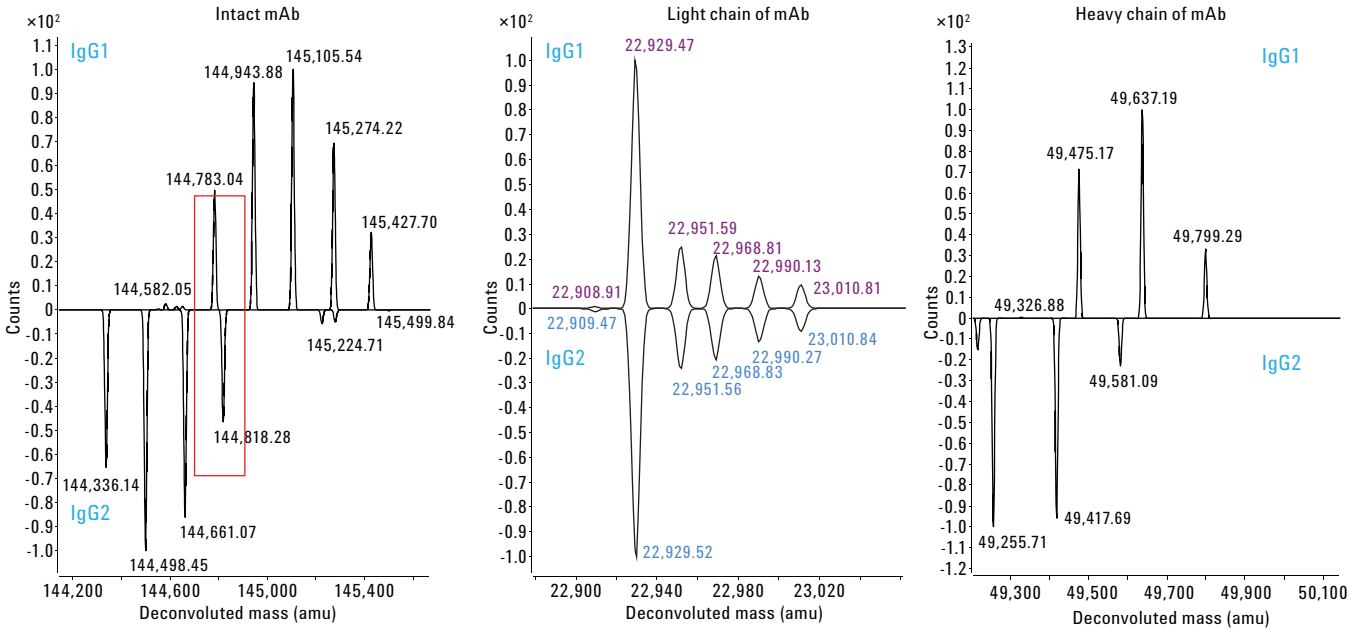

Figure 3. Mirror plots for IgG1 and IgG2 comparing intact mass, light chain, and heavy chain masses. The red box shown on intact mass mirror plot shows the power of the pMod algorithm to separate very close deconvoluted masses[2].

Conclusions

- The analysis of mAbs using an Agilent 1290 Infinity LC System coupled to an Agilent 6530 Accurate-Mass Q-TOF has been demonstrated with excellent performance.

- An Agilent 1290 Infinity LC System with an Agilent ZORBAX Poroshell 300SB-C8 column provided fast and superior separation power for both intact and reduced fragments.

- Agilent MassHunter BioConfirm software provided data extraction, deconvolution and mirror plots for finding the differences between the mAbs.

- This method is useful for comparability studies and biosimilar comparisons.

References

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   Accessed March, 2014

2. pMod – An Advanced Protein Deconvolution Algorithm with Automated Peak Modeling for Charge Deconvolution of Mass Spectrometry Data, Agilent publication 5991-2225EN.

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