LC/MS of Intact Therapeutic Monoclonal Antibodies Using Agilent AdvanceBio RP-mAb

Application Note

Biologics and Biosimilars

Author

Suresh Babu C.V.

Agilent Technologies, Inc.

Introduction

Therapeutic proteins such as monoclonal antibodies (mAbs) are gaining much attention in the biopharmaceutical industry. Post translational modifications during the mAb manufacturing process commonly result in microheterogeneity. Hence, it is important to monitor and analyze these changes to ensure drug efficacy. Reversed-phase LC/MS is the routine method for the analysis of mAbs at intact or fragment level, which provides accurate molecular mass. The correct choice of LC column and method is critical to achieve fast analysis times and reproducible high-resolution separations.

We used Agilent AdvanceBio RP-mAb columns for intact mAb analysis to demonstrate the fast analysis time and highly reproducible chromatographic separation provided by these columns. AdvanceBio RP-mAb columns with C4, SB-C8, and Diphenyl chemistries are based on Agilent Poroshell technology with superficially porous particles (3.5 µm) with wide pores (450Å) [1]. These columns deliver higher resolution and faster run times compared to columns packed with fully porous particles of the same size. In this work, multiple therapeutic mAbs, including an antibody drug conjugate (ADC), were analyzed using AdvanceBio RP-mAb columns in an LC/MS method. This approach delivered fast analysis times and accurate mass determination of intact mAbs.

Agilent Technologies

Experimental

Samples

Therapeutic monoclonal antibodies mAb1, mAb2, and antibody drug conjugate mAb3 were purchased from a local pharmacy and stored according to the manufacturers' instructions. mAb1, mAb2, and mAb3 (lys-conjugated ADC) were diluted to 1 µg/µL using 0.1% formic acid in 3% ACN, and 1 µL was injected.

Instrumentation

LC: Agilent 1290 Infinity LC

MS: Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) with Agilent Jet Stream ion source

Conditions

Parameter	Agilent 1290 Infinity LC
Column	AdvanceBio RP-mAb C4, 2.1 × 50 mm, 3.5 µm (p/n 799775-904) AdvanceBio RP-mAb Diphenyl, 2.1 × 75 mm, 3.5 µm (p/n 799775-944)
Inj vol	1 µL
Sample thermostat	5 °C
Mobile phase A	0.1% Formic acid in water
Mobile phase B	80% IPA:10% ACN: 9.9% water with 0.1% formic acid
Gradient	At 0 min → 20% B At 4 min → 20% B At 5 min → 40% B At 10 min → 70% B At 11 min → 90% B At 11.1 min → 20% B
Stop time	11.1 min
Post time	4 min
Column temp	80 °C
Flow rate	0.6 mL/min
Parameter	**Agilent 6530 Accurate-Mass Q-TOF LC/MS**
Ion mode	Positive ion mode, dual AJS ESI (profile)
Drying gas temp	350 °C
Drying gas flow	8 L/min
Sheath gas temp	400 °C
Sheath gas flow	11 L/min
Nebulizer	35 psi
Capillary voltage	5,500 V
Fragmentor voltage	380 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Acquisition parameters	
MS mode	Data acquired at 1 GHz, MS only mode, mass range 2,000 to 6,000 *m/z*
Data analysis	Data from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis software and Agilent MassHunter BioConfirm software. Max entropy and pMod deconvolution algorithms were used to obtain zero-charge spectra of the drug conjugated antibody (mAb3) and mAb1/mAb2, respectively.

Results and Discussion

Determination of therapeutic protein molecular weights and glycosylation profiles is best established by LC/MS. Achieving good chromatographic peak and high signal-to-noise ratio *m/z* mass spectra is a tradeoff when working with high molecular weight proteins such as mAbs [2]. In addition, for high-throughput mAb screening in a batch-to-batch variation study, fast and reproducible LC/MS methods are required. AdvanceBio RP-mAb columns are packed with superficially porous particles. This particle design reduces diffusion distances, which allows high linear velocities to be used while still maintaining high chromatographic efficiency. This approach delivers high-speed and high-resolution separations. To improve resolution, different C4, SB-C8, and Diphenyl bonded phases are available for flexible method development.

Figures 1 and 2 show the LC/MS data for the three intact mAbs with AdvanceBio RP-mAb C4 and Diphenyl columns, respectively. Both columns provided excellent total ion chromatogram peak shapes with narrow peak width (0.5 min at base) using an isopropanol:acetonitrile:water mobile phase (Figures 1A and 2A). The charge-state envelope ranged from 2,000 to 4,000 *m/z* with a Gaussian distribution (Figure 1B and 2B). The raw mass spectra were converted to zero-charge mass spectra using the maximum entropy and peak modeling (pMod) deconvolution algorithms in Agilent MassHunter BioConfirm software. The deconvoluted spectra are shown in Figures 1C and 2C. Five major glycoforms are seen in the mAb1 deconvoluted spectrum, while four major glycoforms are evident in the mAb2 spectrum. Due to the strong heterogeneity of mAb3, split peaks were observed with both C4 and Diphenyl columns (Figure 2A). These split peaks corresponded to the mAb with different payloads. Deconvoluted mass spectra for mAb3 (Figure 2C) showed increasing payload trend in steps of one drug load with eight major drug conjugations (D0, D1, D2...D8).

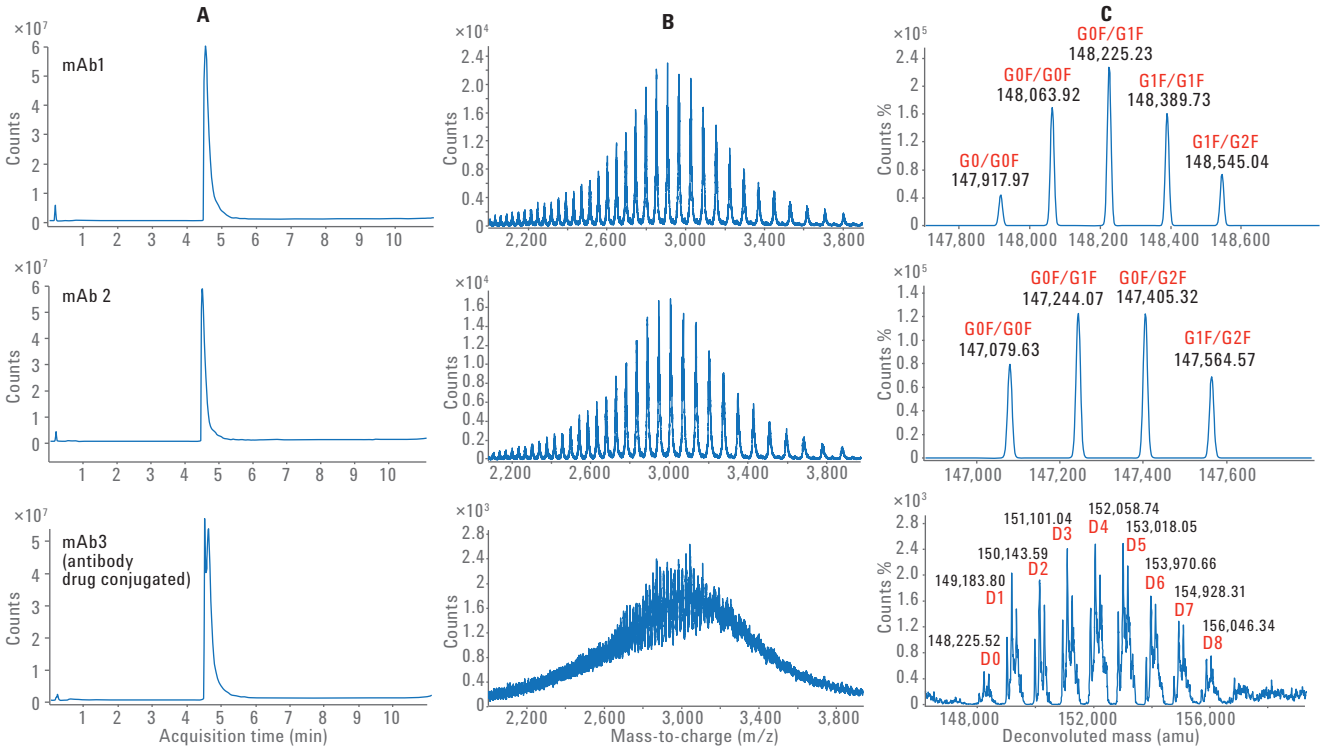

Figure 1. Intact mAb mass analysis on an Agilent AdvanceBio RP-mAb C4, 2.1 × 50 mm, 3.5 µm column. A) Total ion chromatogram, (B) mass spectrum, (C) deconvoluted spectrum.

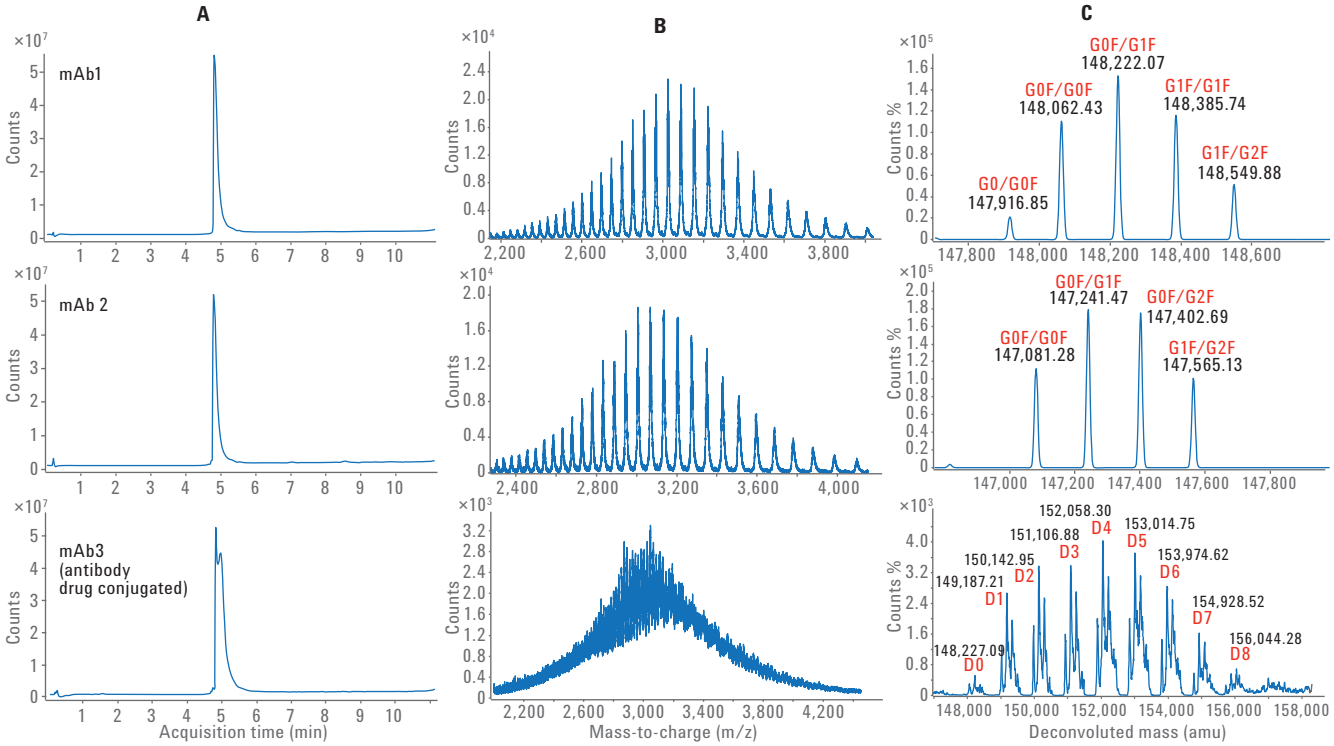

Figure 2. Intact mAb mass analysis on an Agilent AdvanceBio RP-mAb Diphenyl, 2.1 × 50 mm, 3.5 µm column. A) Total ion chromatogram, (B) mass spectrum, (C) deconvoluted spectrum.

Conclusions

We successfully analyzed therapeutic mAbs using Agilent AdvanceBio RP-mAb columns coupled to an Agilent 6530 Accurate-Mass Q-TOF LC/MS. The C4 and Diphenyl AdvanceBio RP-mAb columns, with an MS-compatible method, delivered fast and high-resolution analysis for intact and ADC mAbs.

References

1. Gritti, F. *Chromatog. Today* **June 2012**, 4-11.
2. Gudihal, R.; Suresh Babu C. V.; Tang, N. *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, Agilent Technologies, Inc. Publication number 5991-4266EN, **2014**.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
October 5, 2015
5991-6296EN

Agilent Technologies