Waters™

Applikationsbericht

Analysis of Antibody Drug Conjugates (ADCs) by Native Mass Spectrometry on the BioAccord System

Henry Shion, Ying Qing Yu, Weibin Chen

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the performance of the BioAccord System for the analysis of antibody drug conjugates (ADCs) under native conditions.

Benefits

Drug-to-Antibody Ratio (DAR) determination of Lys and Cys conjugated ADCs was accomplished using analytical scale size exclusion chromatography (SEC) and the BioAccord System for native LC-MS analysis.

Introduction

Drug-to-Antibody Ratio (DAR) is a critical quality attribute (CQA) for ADCs because it directly affects their therapeutic efficacy and pharmacokinetics. Determination (and monitoring) of DAR is essential across the ADC development process and within commercial manufacturing operations. Native electrospray mass spectrometry (native MS) has emerged as a powerful tool in the analysis of covalent complex therapeutic proteins and non-covalent protein complexes. Under native MS conditions, proteins are subject to electrospray ionization using a nondenaturing MS-friendly buffer system.

These conditions for LC-MS analysis enable many proteins to remain in their folded states that demonstrate characteristically low charge states, requiring sensitivity over a broader and higher mass to charge (*m/z*) range than that for the analysis of the denatured proteins. Native MS faces several unique challenges including the need for extensive sample clean-up before analysis if infusion MS is attempted, and greater operator skill to produce and interpret experimental results. Previously, we have made efforts to simplify the acquisition of native MS data by coupling inline SEC with existing MS technologies[1,2] to facilitate the sample desalting and buffer exchange for the study of the population of cysteine-conjugated ADCs. In this study, we employ the BioAccord System to improve accessibility to an analytical solution for native MS analysis of both cysteineconjugated and lysine-conjugated ADCs.

The BioAccord System is a small footprint, high performance bench top system that was designed and developed with simplified user interface as well as automatic system setup and self-diagnostic capabilities.

Results and Discussion

The BioAccord System is physically comprised of an ACQUITY UPLC I-Class PLUS System configured with an optical detector (TUV or FLR) coupled in-line to an ACQUITY RDa Detector (compact oa-TOF MS). The system is operated under a UNIFI Scientific Information System that enables streamlined workflow solutions for regulated and non-regulated laboratories with the combination of automated data acquisition, processing, and reporting, including automating the DAR calculation for ADC characterization.

Cysteine conjugated ADC analysis

Native mass spectrometry of cysteineconjugated ADCs analysis requires non-denaturing conditions to maintain the non-covalently linked ADC molecules intact to determine DAR values and the drug loading distribution for

the ADC samples. In this study, the BioAccord System was directly coupled with an analytical scale SEC column (ACQUITY UPLC Protein BEH SEC Column, 200Å, 1.7 µm, 2.1 mm x 150 mm, p/n=186008471) with isocratic elution (50 mM ammonium acetate (NH_4OAc) over a 10-minute run).

Under these SEC conditions the ADC subunits maintain a quazi-native state, maintaining quaternary structural interaction, and producing a surface area much smaller than that of its denatured, unfolded forms found with the acidic-organic mobile phases typical of reversed-phase separations. Proteins under native electrospray mass spectrometry conditions will carry fewer charges than in reversed-phase conditions, and the smaller charge envelope appears at a higher *m/z* range in the resulting mass spectra. As shown in Figure 1, the extended mass range (up to *m/z* 7,000) of the BioAccord System meets the need of high *m/z* measurement and demonstrates the detection of complex non-deglycosylated cysteineconjugated ADCs. These results are consistent with spectra from the ADC sample previously analyzed on other high-resolution MS systems,[1,2] with comparable DAR values obtained between the BioAccord and the previous QTof MS systems (Figure 1 and Table 1).

The combined raw spectral charge state envelope (Figure 1, left), a zoomed-in single charge state (center), and the deconvoluted spectra (right) are shown for the naked antibody mAb, and three levels (low, moderate, and high) of conjugated ADCs on the BioAccord System. The glycosylation pattern displayed in the naked mAb spectrum is repeated for each conjugation form (0, 2, 4, 6, and 8) across all three levels of conjugated samples. The combined integrated peak areas of each of the glycoforms from the deconvolued spectra were used for automated calculation of the total average DAR, and the drug loading distribution, within the UNIFI data processing workflows, as previously described in detail.[1]

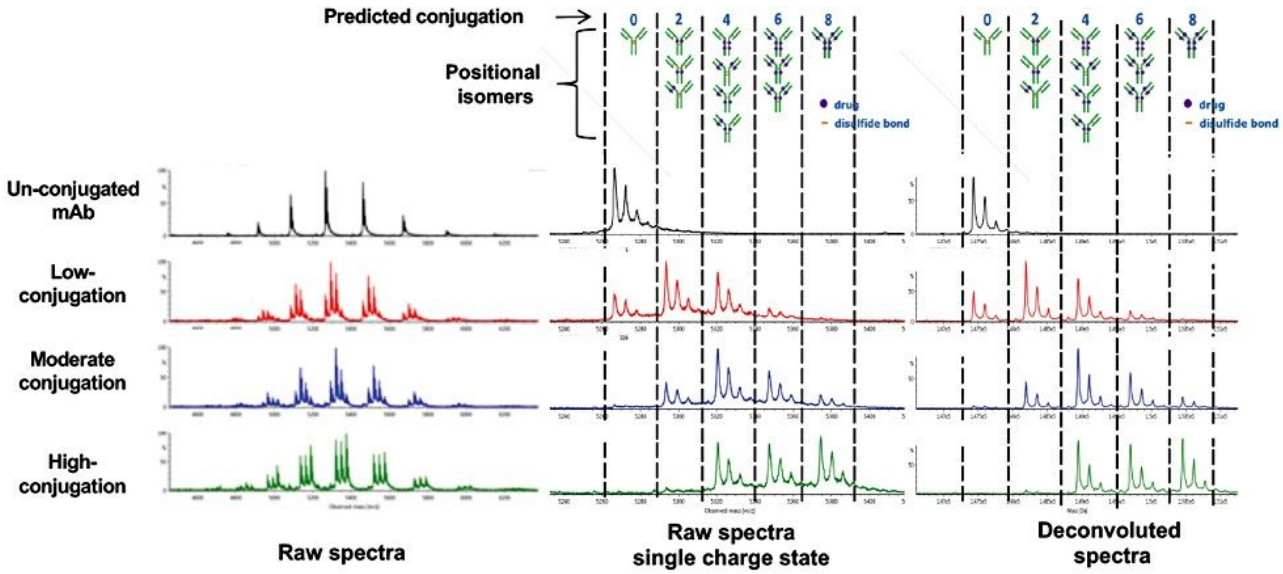

Figure 1. The combined raw spectra from multiple charge state envelope (left), the zoomed-in region (single charge state) of the combined raw spectra (center), and the deconvoluted spectra (right) of the reference materials (mAb), the low, moderate, and high conjugation level cysteine-conjugated ADC samples without deglycosylation treatment from the BioAccord System native LC(SEC)-MS analysis. Drug distribution was compared for three different cysteine-conjugated ADC samples with increasing drug load.

Cysteine-conjugated ADCs drug loading distribution and DAR													
		Low				Moderate				High			
		HIC	QTof1	QTof2	Tof	HIC	QTof1	QTof2	Tof	HIC	QTof1	QTof2	Tof
ADC 2		0.81	0.74	0.64	0.68	0.38	0.41	0.35	0.36	0.07	0.09	0.05	0.05
ADC 4		1.14	1.17	1.37	1.36	1.67	1.57	1.81	1.82	1.23	1.11	1.19	1.15
ADC 6		0.75	0.60	0.64	0.65	1.61	1.45	1.51	1.47	1.72	1.72	1.86	1.85
ADC 8		0.12	0.21	0.05	0.10	0.78	0.97	0.70	0.75	2.95	3.05	2.98	2.96
DAR		2.83	2.72	2.70	2.79	4.44	4.40	4.37	4.40	5.97	5.97	6.07	6.01

QTof 1	deglycosylated samples, run on Xevo G2-S in 2014
QTof 2	non-deglycosylated samples, run on Vion in 2017
Tof	non-deglycosylated samples, run on BioAccord in 2018

Table 1. Total average DARs and drug distribution comparison amongst the HIC (UV) and the three native SEC-MS experiments exhibit agreement across all three drug loading levels. The results indicated that DAR measurements can be measured consistently using orthogonal approaches (HIC vs MS), or across different QTof or Tof MS systems (Xevo G2-S, Vion IMS QTof MS, and the BioAccord System). With its streamlined workflow for automated data acquisition, processing, and reporting of DAR calculated results, the BioAccord System proved effective for native LC(SEC)-MS analysis of ADCs to determine lot to lot, batch-to-batch comparability.

Lysine conjugated ADC data

The raw and deconvoluted spectra of LC(SEC)- MS analysis of lysine-conjugated ADC Kadcyla (Trastuzamab Entansine (T-DM1)) were obtained without the need of deglycosylation of the sample (Figure 2). The detailed benefits of using native MS approach for covalent lysine-conjugated ADC analysis were described previously.[2] The number of conjugated drugs detected is labeled on the deconvoluted spectrum peaks. The DAR value calculated by UNIFI was 3.46, which is in good agreement with DAR of 3.50 reported by the drug manufacturer.[3] Again, results were comparable to a previous study using a Vion IMS QTof system.[2]

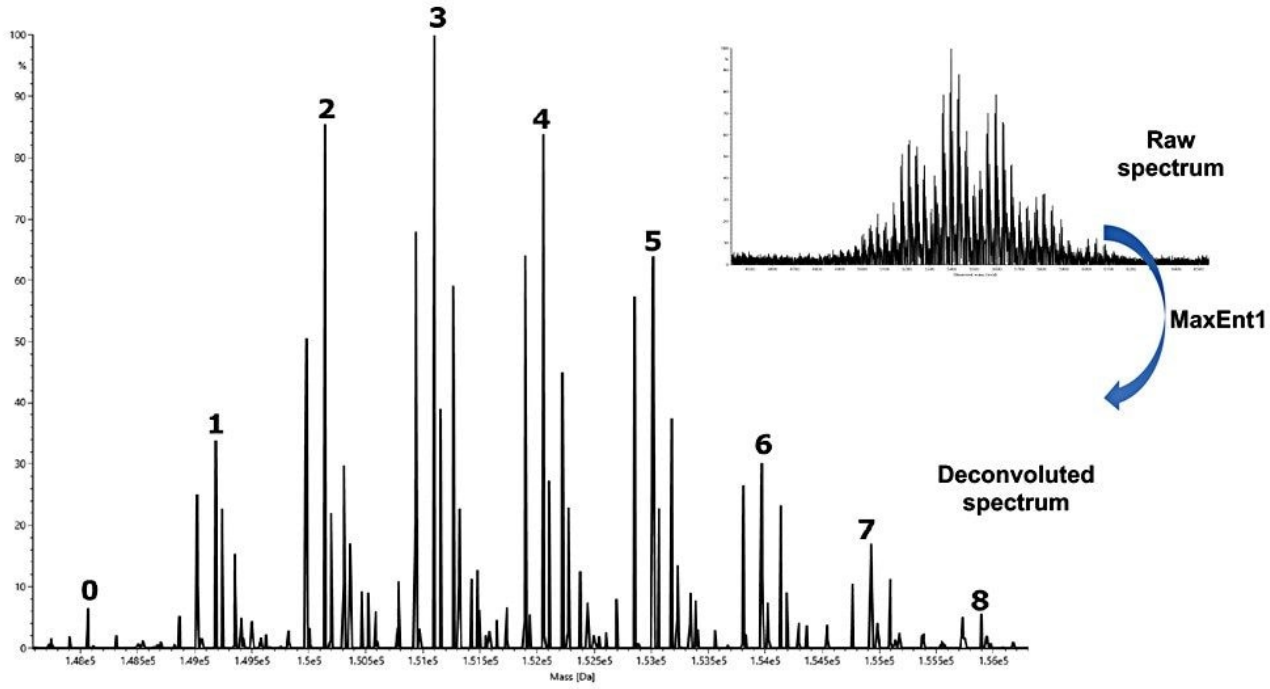

Figure 2. Raw and deconvoluted spectrum of LC(SEC)-MS analysis of Lysine conjugated ADC, Kadcyla (Trastuzumab Entansine (T-DM1)), by BioAccord System. The number labeled on the deconvoluted spectrum represents the detected number of drugs that are conjugated to the protein Trastuzumab in the intact level. The calculated average DAR is 3.46, vs. the published DAR of 3.50.[3] The experiment was conducted without deglycosylation of the ADC drug.

Conclusion

In this application brief, we have demonstrated that the BioAccord System is well suited for routine LC(SEC)-native MS analysis of both cysteine and lysine conjugated ADCs. The average DARs and drug loading distribution results were comparable to that generated from Hydrophobic Interaction Chromatography (HIC) separation (with TUV detection), and results from previous generations of QTof MS systems. Simplified system operation and configurable compliance features will enable the BioAccord System to be readily adopted by scientists with less MS experience, which will allow organizations to more readily deploy the mass spectrometry

in supporting ADC across development and manufacturing processes. The capabilities demonstrated in this tech brief and other published applications using the BioAccord System4-8 show the breadth of the BioAccord System for supporting routine analysis of biotherapeutic product quality attributes.

References

1. H. Shion *et al*, Development of Integrated Informatics Workflows for the Automated Assessment of Comparability for Antibody Drug Conjugates (ADCs) Using LC-UV and LC-UV/MS. Waters Application Note. 720005366EN. 2015.
2. H. Shion *et al*, Analytical Scale Native SEC-MS for Antibody-Drug Conjugates (ADCs) Characterization. Waters Application Note. 720006368EN. 2018.
3. 3. L. Chen *et al*, In-depth structural characterization of Kadcyla (ado-trastuzumab emtansine) and its biosimilar candidate. *MAbs*. 2016 Oct; 8(7): 1210–1223.
4. H. Shion *et al,* Enabling Routine and Reproducible Intact Mass Analysis When Data Integrity Matters. Waters Application Note. 720006472EN. 2019.
5. N. Ranbaduge et al, Routine Peptide Mapping Analysis using the BioAccord System. Waters Tech Brief. 720006466EN. 2019.
6. X. Zhang *et al*, Released N-linked Glycan Analysis Using the BioAccord System. Waters Tech Brief. 7200006474EN. 2019.
7. H. Shion *et al*, A Platform Method for the Molecular Mass Analysis of the Light Chains and Heavy Chains of Monoclonal Antibodies using the BioAccord System. Waters Tech Brief. 7200006529EN. 2019.
8. X. Zhang. *et al*, Increasing Productivity and Confidence for N-linked Glycan Analysis of Biosimilars Using the BioAccord System. Waters Tech Brief. 7200006545EN. 2019.

Featured Products

BioAccord LC-MS System for Biopharmaceuticals <
https://www.waters.com/waters/nav.htm?cid=135005818>

UNIFI Scientific Information System <https://www.waters.com/134801648>

720006570, May 2019