Waters™

Application Note

The INTACT Mass Application in waters_connect™ Platform Streamlines ADC DAR and Drug Distribution Analysis

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Application Brief

This is an application brief and does not contain a detailed Experimental section.

Abstract

The INTACT Mass App within the waters_connect Platform integrates data acquisition, processing, review, and reporting for automated mass deconvolution of biotherapeutic LC-MS data. This application brief demonstrates the INTACT Mass App 1.9 implementation of drug-to-antibody ratio (DAR) analysis for antibody-drug conjugates (ADCs). This application brief utilizes FDA-approved lysine-conjugated and cysteine-conjugated ADC samples to demonstrate these DAR analysis tools.

Benefits

- INTACT Mass App Ver 1.9 enhancements include automated DAR and drug distribution calculations for streamlined monoclonal antibody (mAb) conjugate analysis
- The compliance-ready waters_connect Informatics Platform enables this ADC analysis workflow to be utilized from product characterization to product release testing

Introduction

The waters_connect Platform is an app-based compliance-ready software containing a variety of application-based workflows for biopharmaceutical analysis. The INTACT Mass App represents a tool for rapid automated mass deconvolution, mass confirmation, and related purity assessments. It has been showcased for a variety of applications including, but not limited to, intact proteins,[1] oligonucleotides,[2–3] and synthetic peptides.[4] Over the years since its debut, new functionality has been incorporated into the App, including customizing deconvolution settings and peak integration events. The newest addition to INTACT Mass App functionality, ADC analysis, has been increasingly requested, as this class of drug has found renewed interest as a treatment for cancer and blood disorders. Calculation of DAR and drug distribution was previously only possible via the UNIFI™ App workflows and utilized a set of custom calculations to arrive at the DAR value. These processing enhancements to the INTACT Mass App significantly simplify these calculations, reducing the chance for calculation errors, and maintaining the ability to execute these analyses on a compliance-ready platform.

Results and Discussion

A critical quality attribute of ADCs and other protein conjugates is the assessment of the variability of drug payload molecules that have been successfully conjugated to the protein. This is reflected in both drug distribution (the relative percentage of each conjugate species) and DAR value (a single value derived from the weighted average of each species). With the release of the INTACT Mass App version 1.9, these calculations have been automated for the user. When creating the processing method, the drug/payload modification is indicated with a special tag, called “Drug”. During mass spectral data processing, the software automatically matches observed masses to theoretical masses of conjugated species, based on the indicated expected masses for the

unconjugated protein and drug modifier. The relative MS responses for matched components with a "Drug" tag attached (in conjunction with any unconjugated protein) are then used for the DAR and drug distribution calculations.

Analysis of a Cystine Conjugated ADC, ENHERTU™ (fam-trastuzumab-deruxtecan-nxki)

ENHERTU (fam-trastuzumab-deruxtecan-nxki) is a cysteine-conjugated ADC with DAR of 7–8, which is used to treat various breast, gastric, and gastroesophageal cancers.[5] In order to achieve fully intact ADC analysis via LC-MS, cysteine-conjugated ADCs that may lack interchain disulfide bonds connecting heavy and light chains must be analyzed using non-denaturing separation conditions and gentler source conditions. Therefore, a native size exclusion chromatography-mass spectrometry (SEC-MS) approach on the BioAccord™ System, as described previously,[6] was used for the analysis of ENHERTU (fam-trastuzumab-deruxtecan-nxki).

The deconvoluted mass spectrum from the native SEC-MS analysis (Figure 1A) indicates that the majority of species present are matched to N-glycoforms of trastuzumab with eight deruxtecan drug payloads conjugated (labeled as "D8"). Only a small percentage of the total species attributed to trastuzumab with six drug payloads ("D6"), as shown in the Results Tab of INTACT Mass App (Figure 1B). This Results Table lists all species detected, with their respective expected and observed masses, mass error (ppm), and relative percentage calculated from MS response. The new INTACT Mass App Version 1.9 Calculation Results Tab (Figure 1C) reveals the calculated DAR value and overall drug distribution. As expected, the DAR value for ENHERTU (fam-trastuzumab-deruxtecan-nxki) is 7.97, comparable to the analysis performed using the UNIFI App and from previous reports.[5,7] Based on relative MS response, about 98.7% of the sample contains eight drug payload molecules conjugated to the antibody.

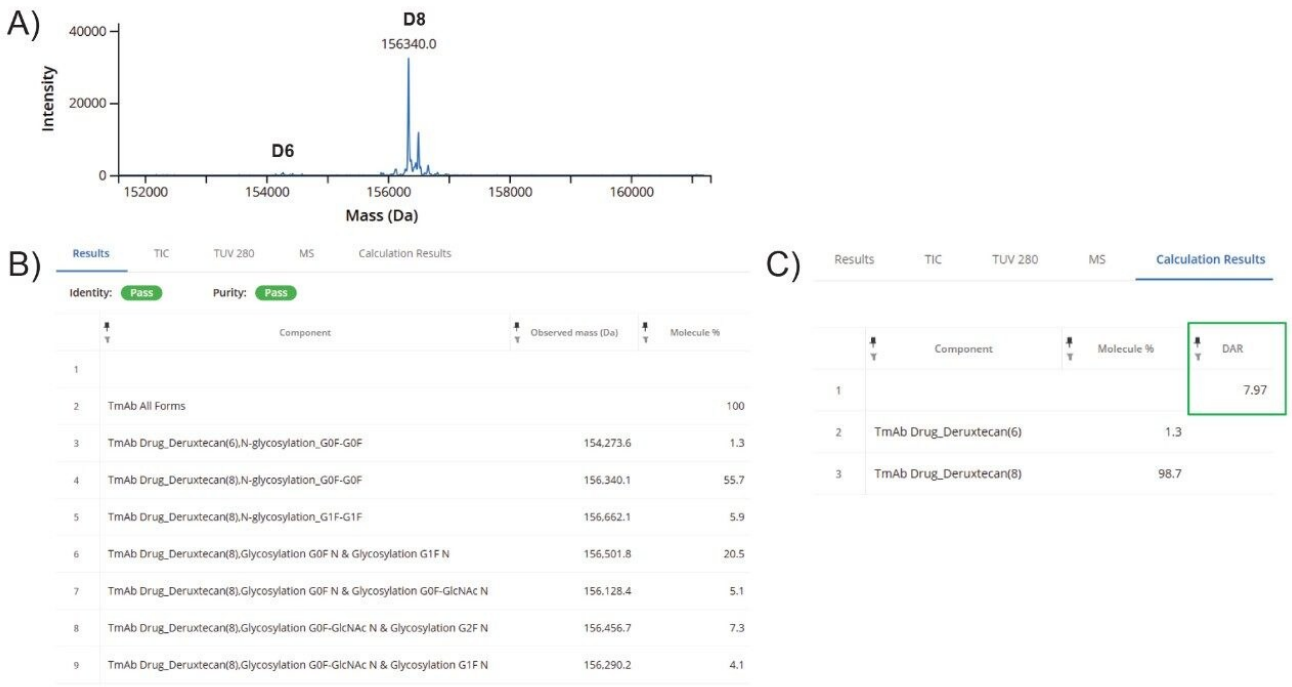


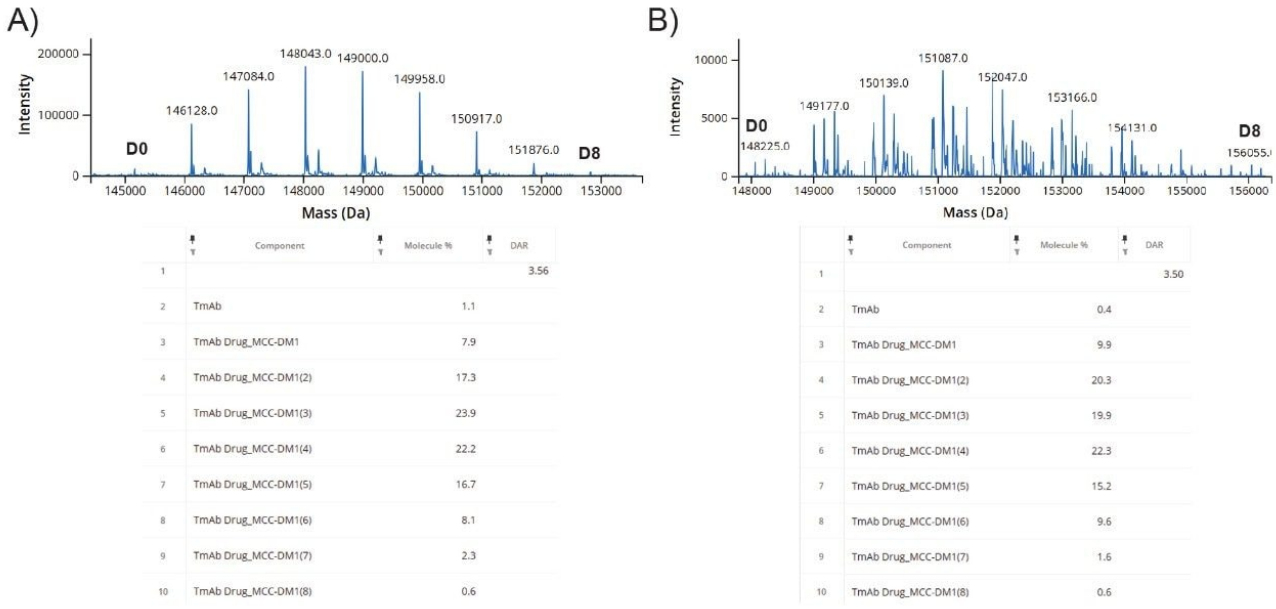
Figure 1. LC-MS Analysis of ENHERTU (fam-trastuzumab-deruxtecan-nxki) ADC in INTACT Mass App, showing A) the deconvoluted mass spectrum, B) Results Tab with all detected species, and C) Calculation Results Tab showing drug distribution and calculated DAR value.

Analysis of a Lysine Conjugated ADC, KADCYLA™ (ado-trastuzumab-emtansine)

KADCYLA (ado-trastuzumab-emtansine), a well-studied lysine-conjugated ADC with expected DAR of ~3.5 is primarily used for treatment of breast cancer.[5–8] Lysine-conjugated ADCs utilize solvent-exposed lysine residues for conjugation, which produces a very heterogeneous mixture of conjugated species. In the case of KADCYLA (ado-trastuzumab-emtansine), a range of zero to eight payload molecules has been detected in previous studies, with the majority possessing three or four payload molecules. As the lysine conjugation process does not disrupt the interchain disulfide bonds of the mAb, we can analyze KADCYLA (ado-trastuzumab-emtansine) using both denaturing or non-denaturing LC-MS conditions. Analysis with denaturing reversed-phase liquid chromatography-mass spectrometry (RPLC-MS) conditions, where the sample will have much higher, and more closely spaced, charge states, it is prudent to deglycosylate KADCYLA (ado-trastuzumab-emtansine) with PNGase F enzyme to reduce the complexity of the overall MS pattern.[8]

The resulting deconvoluted mass spectrum from the deglycosylated ADC sample analyzed with RPLC-MS on the BioAccord System and processed in INTACT Mass App (Figure 2A) is shown with an accompanying table showing the relative percentages of each species, from zero to eight drug molecules conjugated (D0-D8). The overall calculated DAR value from INTACT Mass App was 3.56, which is consistent with previous analyses conducted using the UNIFI App with a reported DAR value of 3.5.[5–8]

KADCYLA (ado-trastuzumab-emtansine) has also been successfully analyzed with native SEC-MS without the need for deglycosylation. For comparison, this sample was also analyzed with native SEC-MS on the BioAccord System, as described previously,[6] and processed with the INTACT Mass App. The resulting deconvoluted mass spectrum (Figure 2B), is more complex, with all of the N-glycoforms present. The INTACT Mass App was able to deconvolute the raw spectral data, match the glycosylated and conjugated species, and collate to the multiple forms of each DAR species to calculate the average DAR value and drug distribution. The table (Figure 2B) shows the calculated relative percentage abundance of each species from D0 to D8, as well as the calculated average DAR value of 3.50, as expected from published sources[5–8] and previous analysis in the UNIFI Software. Thus, users have flexibility in selecting separation methods that best fit their experimental goals while expecting comparable DAR and drug distribution results for their ADC analyses.



	Component	Molecule %	DAR
1			3.56
2	TmAb	1.1	
3	TmAb Drug_MCC-DM1	7.9	
4	TmAb Drug_MCC-DM1(2)	17.3	
5	TmAb Drug_MCC-DM1(3)	23.9	
6	TmAb Drug_MCC-DM1(4)	22.2	
7	TmAb Drug_MCC-DM1(5)	16.7	
8	TmAb Drug_MCC-DM1(6)	8.1	
9	TmAb Drug_MCC-DM1(7)	2.3	
10	TmAb Drug_MCC-DM1(8)	0.6	

	Component	Molecule %	DAR
1			3.50
2	TmAb	0.4	
3	TmAb Drug_MCC-DM1	9.9	
4	TmAb Drug_MCC-DM1(2)	20.3	
5	TmAb Drug_MCC-DM1(3)	19.9	
6	TmAb Drug_MCC-DM1(4)	22.3	
7	TmAb Drug_MCC-DM1(5)	15.2	
8	TmAb Drug_MCC-DM1(6)	9.6	
9	TmAb Drug_MCC-DM1(7)	1.6	
10	TmAb Drug_MCC-DM1(8)	0.6	

Figure 2. Comparison of KADCYLA (ado-trastuzumab-emtansine) DAR analysis from A) A deglycosylated denaturing RPLC-MS ADC analysis and B) An untreated native SEC-MS ADC analysis. Note that the drug distribution and calculated DAR values are comparable in both analyses.

Conclusion

The enhancements introduced in the waters_connect Platform INTACT Mass App version 1.9 improve the capabilities of this tool for automated analysis of conjugated molecule LC-MS data, from early characterization studies to the validated assays used for product release. The integration of automated DAR and drug distribution calculations streamlines ADC analysis, providing an efficient, automated workflow that can be utilized by analysts with a wide range of LC-MS experience.

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