Application note | 004331

Biopharma

Automated workflow for intact antibody drug conjugates (ADCs) and DAR analysis in Chromeleon CDS

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Keywords

Antibody-drug conjugate (ADC), drug-to-antibody ratio (DAR), native intact mass spectrometry, Orbitrap Exploris 240 mass spectrometer, Chromeleon CDS, intact deconvolution

Application benefits

- **Automated, high-throughput capable analysis** of intact antibody-drug conjugates (ADCs) and **drug-to-antibody ratio (DAR) determination** from sample to report.
- **Fully integrated solution** combining data acquisition with LC-MS instrument control, data processing, and a new, dedicated custom report in a single Chromatography Data System (CDS).
- **High confidence results** obtained from high resolution accurate mass data acquired under native conditions by determination of drug load distribution and DAR values from deconvoluted mass spectra.

Goal

Develop a report template for automated ADC analysis and DAR calculation to enable an automated and high-throughput capable workflow from sample to report in Thermo Scientific™ Chromeleon™ CDS.

Introduction

Antibody-drug conjugates have emerged as one of the most powerful modalities in targeted oncology therapeutics. By combining the selectivity of monoclonal antibodies (mAbs) with the potency of cytotoxic small molecules, ADCs enable the targeted delivery of chemotherapeutic agents to tumor cells while minimizing systemic toxicity.[1]

As of April 2025, 14 ADCs have received FDA approval, and more than 100 are currently in clinical development,[2] reflecting the rapid growth and scientific importance of this therapeutic class.

Comprehensive characterization of ADCs is essential throughout development and production to ensure product consistency, efficacy, and safety. Certain critical product attributes, such as the drug load distribution (DLD) and the average drug-to-antibody ratio (DAR) need to be monitored, since they can directly impact pharmacokinetics, pharmacodynamics, and stability. High-quality intact mass analysis under native conditions allows fast and direct determination of these attributes, providing insight into molecular heterogeneity and conjugation efficiency without requiring peptide-level analysis.

Traditional ADC characterization methods often involve multiple manual steps and time-consuming data processing, which can limit sample throughput and increase variability. As the number of ADC candidates and process development samples continues to rise, there is a growing need for high-throughput, automated analytical workflows that provide accurate and reproducible data with minimal analyst intervention. Such workflows reduce turnaround time, improve data integrity, and support rapid decision-making during biopharmaceutical development and quality control.

Here, we have developed an automated workflow in Chromeleon CDS for intact ADC analysis, including automated calculation and reporting of the DAR. Using short 3-minute LC-MS runs (4.5 minutes from injection to injection) under native conditions acquired on a Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer with the Thermo Scientific™ BioPharma Option, a total of 54 injections representing four different therapeutic ADCs and four blanks were analyzed, processed, and reported in under four hours. The workflow (Figure 1) represents an automated and reliable solution for high-throughput ADC analysis, delivering precise mass confirmation, glycoform profiling, and DAR determination within a single, integrated platform.

Experimental

Sample and consumables

Sample

Commercially available samples were used of Enhertu™ (Trastuzumab deruxtecan), Polivy™ (Polatuzumab vedotin), Adcetris™ (Brentuximab vedotin), and Aidixi™ (Disitamab vedotin), which include both lysine-linked and cysteine-linked ADCs.

Consumables

- Ultrapure 18.2 MΩ·cm water, generated using a Thermo Scientific™ Barnstead™ GenPure™ Pro UV TOC Water Purification System (Cat. No. 50131948)
- Invitrogen™ Ammonium acetate (5 M) (Cat. No. AM9071)
- Thermo Scientific™ NativePac™ OBE-1 Column (Cat. No. 43803-052130).

Sample preparation

Commercially available ADCs were dissolved using ultrapure water to a final concentration of 1.0 mg/mL.

Chromatography

The LC-MS system was controlled by Chromeleon CDS 7.3.2 MUe. (Note: The workflow described here is implementable in Chromeleon CDS 7.3.2 MUb and later versions.)

For chromatography, a Thermo Scientific™ Vanquish™ Flex Binary UHPLC System and NativePac OBE-1 SEC column were employed.[3]

- Column temperature was set to 25 °C and the autosampler temperature to 4 °C.
- Solvent A was 100 mM ammonium acetate. The flow rate was 100 µL/min with an isocratic elution of 100% A for 3 minutes (4.5 minutes injection to injection).
- Injection volume was 2.0–3.0 µL (2.0–3.0 µg).

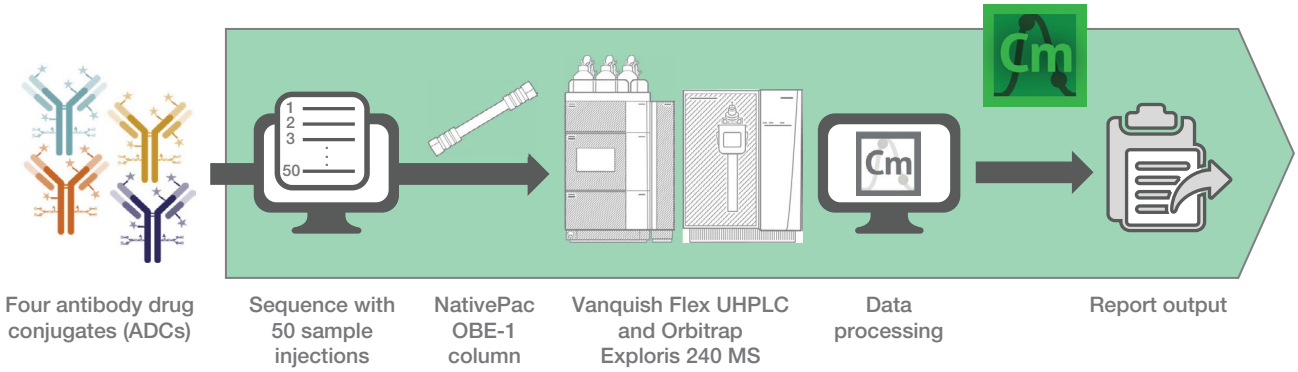


Figure 1. Automated workflow built on a tailored report template for ADC analysis and DAR calculation, fully supported by Chromeleon CDS.

The Vanquish Flex UHPLC system consisted of the following modules:

- Thermo Scientific™ System Base Vanquish™ Horizon/Flex (Cat. No. VF-S01-A-03)
- Thermo Scientific™ Vanquish™ Binary Pump F (Cat. No. VF-P10-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler FT (Cat. No. VF-A40-A-02)
- Thermo Scientific™ Vanquish™ Column Compartment C (Cat. No. VC-C10-A-03)
- Thermo Scientific™ Vanquish™ VWD-F (Cat. No. VF-D40-A)
- Thermo Scientific™ Viper™ MS Connection Kit for Vanquish™ LC Systems (Cat. No. 6720.0405A)

Mass spectrometry

An Orbitrap Exploris 240 mass spectrometer equipped with the BioPharma option was used for MS data acquisition. The MS parameter settings are provided in Table 1.

Table 1. MS settings for intact analysis under native conditions.

Parameter	Value
Ion source parameters	
Spray voltage positive ion (V)	3,600
Sheath gas (Arb)	35
Aux gas (Arb)	10
Sweep gas (Arb)	0
Ion transfer tube temp. (°C)	250
Vaporizer temp. (°C)	175
Application mode	Intact Protein
Pressure mode	High Pressure
Full Scan MS	
Orbitrap resolution	60,000
Scan range (*m/z*)	2,000–8,000
RF lens (%)	200
AGC target	Custom
Normalized AGC target (%)	300
Maximum injection time mode	Custom
Maximum injection time (ms)	200
Microscans	5
Data type	Profile
Polarity	Positive
Source fragmentation energy (eV)	120

Data processing

Intact protein deconvolution analysis was performed using Chromeleon CDS 7.3.2 MUe with the intact protein deconvolution plugin, using the Thermo Scientific™ ReSpect™ Deconvolution Algorithm. The *Default Native Sliding Windows ReSpect* intact deconvolution processing method template was used with minor modifications. See Table 2 for the exact deconvolution parameters used.

Table 2. Data processing parameters applied for intact mass deconvolution. Settings not mentioned are default settings in the processing method.

Component detection	
Chromatogram parameters	
m/z range	4,500 to 6,500
Source spectra parameters	
Source spectra method	Sliding Windows
RT range	1 min to 2 min
Target avg spectrum width	0.2 min
Target avg spectrum offset	% Offset = 25
Merge tolerance	30 ppm
Max RT gap	1 min
Min. number of detected intervals	3
Algorithm parameters	
Deconvolution algorithm	ReSpect (isotopically unresolved)
Output mass range	145,000 to 160,000
Deconvoluted spectra display mode	Isotopic profile (new)
Model mass range	145,000 to 160,000
Deconvolution mass tolerance	30 ppm
Choice of peak model	Intact protein
Resolution at 400 *m/z*	Raw file specific
Advanced settings (algorithm parameters)	
Charge (high and low)	50 and 5
Minimum adjacent charges (high and low)	4 and 4
Target peak mass	150,000

Results and discussion

To test the workflow for automated ADC analysis, 10 or 15 replicates of native LC-MS were performed for each of the four different ADCs, for a total of 50 injections of ADCs. LC-MS analyses were performed under native conditions to maintain the ADCs native structure and inter- and intramolecular interactions as much as possible, which is of particular relevance for the cysteine-linked ADCs studied here. In addition, compared to denatured intact mass analysis, native MS provides greater spectral spatial resolution of resulting features and less overlap of variants between adjacent charge states. Therefore, native conditions can improve the confidence in detection of the heterogeneous ADC components compared to a reversed-phase (RP) approach.

High-throughput processing of ADC intact mass data in Chromeleon CDS

In ADC analysis, assessing the variability of drug payload molecules conjugated to proteins is a critical attribute. This includes evaluating the drug load distribution (DLD) and the drug-to-antibody ratio (DAR) value. This new workflow in Chromeleon CDS automates and enables high-throughput identification of components and performs these calculations automatically. Users can assign the expected mass of the antibody and mass of the drug, and use a standard or custom list of *N*-glycoforms or other modifications. The workflow will automate the intact deconvolution processing, components identification, drug assignment, and calculations of the DAR for each injection. This is done by an automated list of calculated expected masses that are matched to the experiment results. The intensity-based average of the components with different numbers of drugs bound to them will be calculated as the DAR.

To enable automated and high-throughput intact analysis of ADCs, five custom columns are applied to the Chromeleon CDS sample sequence as detailed in Table 3. Expected glycan compositions are defined in the report for all ADCs. A standard list of expected glycans is prefilled in the report, but additional glycans can be added or removed from the list if required. The intact deconvolution processing and ADC report will automatically do the rest of the calculations.

Figure 2 illustrates a Chromeleon CDS example sequence created for the batch analysis of ADC analysis. The custom columns are all populated with sample specific information related to the four different ADCs (data only shown for one ADC). It is possible to paste directly from an Excel™ table to the Chromeleon CDS sequence or create a CSV-file for import into Chromeleon CDS to facilitate the sequence table creation.

Intact protein deconvolution was performed in Chromeleon CDS for all ADC sample injections of the four different ADCs. Chromeleon CDS was set to automatically perform intact protein deconvolution after finished acquisition of each injection to further improve the automation of the workflow.

Data from the analysis of the Aidixi sample are presented in Figure 3, demonstrating the high quality of the heterogeneous sample data. Part A shows the full scale mass spectrum with an insert of the UV trace, while Part B provides a zoomed-in view of the mass spectrum. Due to the analysis performed under native conditions, different drug amounts and glycan compositions could be separated by mass and identified, even in crowded areas of the MS spectra. This is evident in the range of *m/z* 5,520–5,560, where different charge states of the D2 and D6 forms overlap in mass but are still clearly resolvable even with only slightly different *m/z* values. Lastly, Part C shows the resulting mass spectrum obtained upon deconvolution from *m/z* to mass, allowing the calculation of the drug-to-antibody ratio distribution. For the most abundant glycan compositions, the mass accuracy was less than 10 ppm for the most abundant components, showing high mass accuracy of this system and workflow.

Table 3. Custom columns in Chromeleon CDS to enable automated and high-throughput intact analysis of ADCs.

AntibodyMass	Expected average mass of the antibody without glycosylation and conjugated drugs. As the mass of the antibody is used, knowing the amino acid sequence is therefore not required for this workflow.
DrugName	Name of the modification to be used in the report (e.g., name of linker and payload).
DrugMass	Expected mass increase for each drug conjugation, including the combined mass of both linker and payload.
MaxNumberDrugs	The maximum number of drugs/repeating masses the report can monitor is 23, which will be used if left empty. But a lower number can be selected to minimize false positives. This column is therefore not mandatory.
TargetNumberDrugs	The sequence overview of the report will ID the most intense component in the sample. Unlike the rest of the workflow, the ID here will only be based on the first three glycan combinations listed and ±2 drugs of the TargetNumberDrugs. If the sequence overview page of the report is not used, this column is not required.

#	UV_VIS_1	Name	Type	Position	Volume	*AntibodyMass	*DrugName	*DrugMass	*MaxNumber Drugs	*TargetNumber Drugs	Instrument Method	Processing Method
1		Enhertu_2ug_01	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
2		Enhertu_2ug_02	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
3		Enhertu_2ug_03	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
4		Enhertu_2ug_04	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
5		Enhertu_2ug_05	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
6		Enhertu_2ug_06	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
7		Enhertu_2ug_07	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
8		Enhertu_2ug_08	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
9		Enhertu_2ug_09	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact
10		Enhertu_2ug_10	Unknown	B:B1	2.00	145173.8900	DXd	1034.0500	10	8	OBE_60K_2ul_3min	ADC_Native_Intact

Figure 2. Chromeleon CDS sequence for ADC intact mass analysis consisting of 50 injections total, including the four ADC samples with blank injections between different samples. Only the first ten injections are shown here.

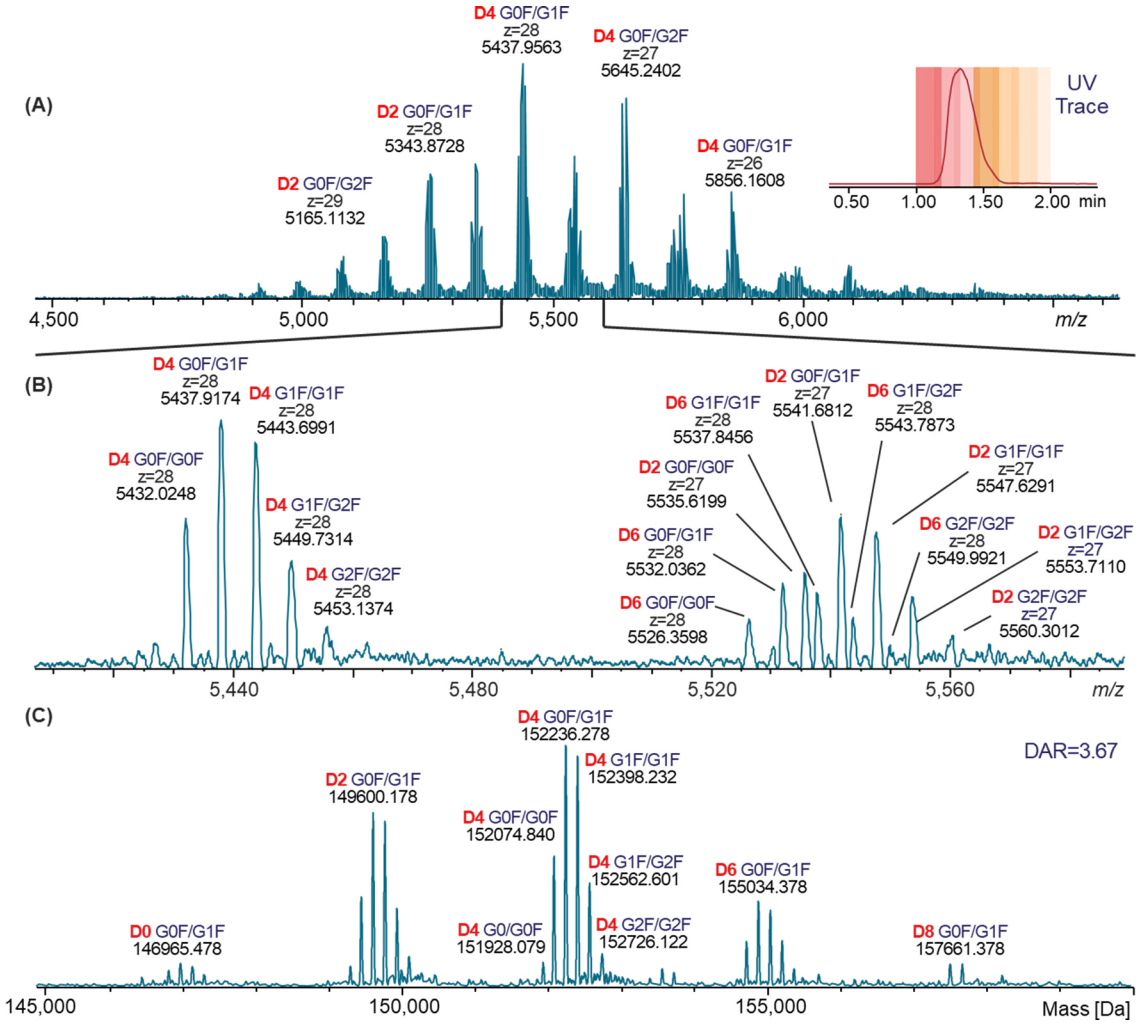


Figure 3. Native intact MS of Aidixi with peak assignments. (A) Full MS spectrum of native intact MS of Aidixi with the insert representing the UV trace. The shaded area of the UV chromatogram indicates the range used for averaging the mass spectrum. (B) Zoom-in of *m/z* range that illustrates sufficient resolution and spectral quality to assign drug load, glycan profile, and charge state, even for crowded areas as seen at *m/z* 5,520–5,560, with overlapping peaks for D2 and D6 at different charge states. (C) Deconvoluted mass spectrum, which shows D0 to D8 identified at different glycoforms as illustrated for D4 components, and the calculated DAR.

A specialized intact ADC report was developed to identify the intact deconvoluted components based on the AntibodyMass, DrugMass, and MaxNumberDrugs, as specified in Table 3, and the list of expected glycans or other modifications as specified in the report. A report page per injection is created, as shown in Figure 4. The report page presents the chromatogram, mass spectrum, deconvoluted spectrum, drug distribution, average DAR, and a component table with identifications. This developed report is a template that can be further customized to meet user specific requirements (e.g., by adding or removing data).

An INDEX MATCH spreadsheet-based formula is used to identify the deconvoluted components. The equation is considered a match when the identified component's mass falls within the Modifications tab's defined mass range for the specified modification combinations (antibody, glycans, drug) and meets the set mass error tolerance. If a component is identified, it will be named based on the assigned modifications, for instance, A2G0F/A2G1F 4xMMAE for the most abundant deconvoluted component for Aidixi as seen in the first line in the Deconvolution Results table in Figure 4. This name in the identification column reflects that the component was identified to carry an A2G0F and an A2G1F glycan in addition to four MMAE drugs (also referred to as D4 in Figure 3).

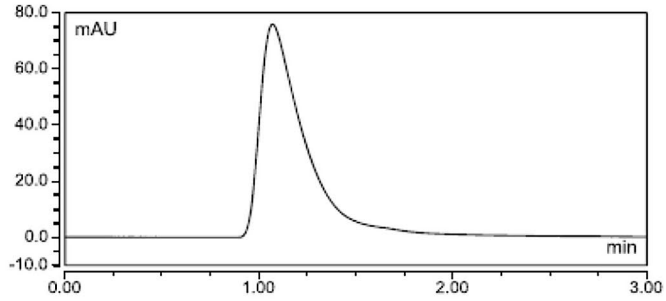
Mass accuracy was also determined and is presented in the report per component identified. It is possible to filter and only use results based on user defined criteria, for instance fractional abundance and intact deconvolution confidence score. Only components that are identified and not filtered out will be used for the intensity-based average DAR calculation. In this example, only components above 0.5% fractional abundance are shown in the table and used for calculations. The average DAR is calculated using intensity-weighted values: sum of (drugs × intensity) across all identified components, divided by the sum of their intensities.

ADC Deconvolution Summary

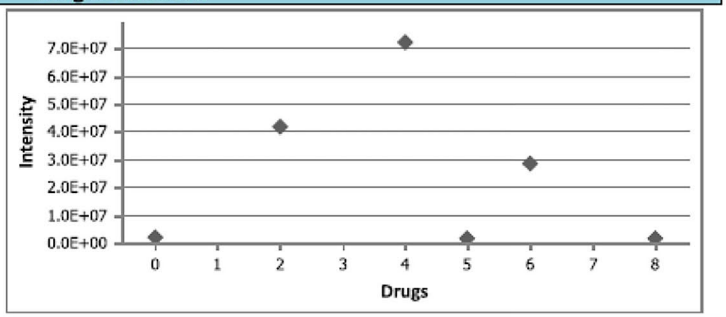
Injection Details

Injection Name:	Aidixi_3ug_07	Injection Date/Time:	2025-Jul-17 11:17
Vial Number:	B:C2	Processing Method:	ADC_Native_Intact
Injection Volume:	3.00	Report Template:	ADC_report_v1.0WIP
Instrument Method:	OBE_60K_2ul_3min		
Sequence Mass:	143912.58 Da	**ΔDAR:**	**MMAE 3.82**

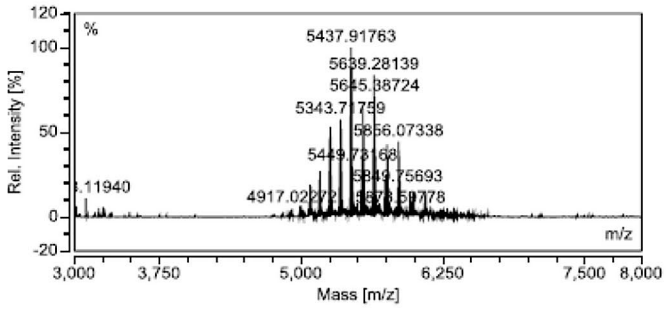
UV



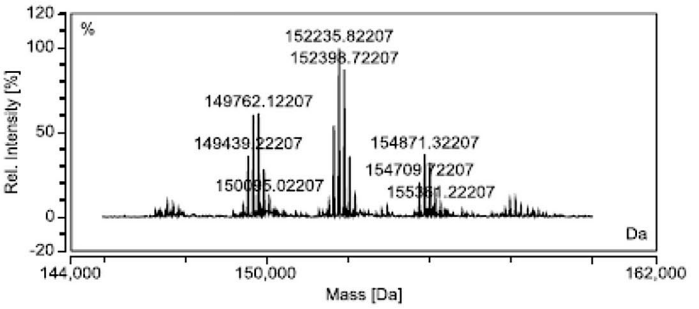
Drug Distribution



Mass Spectrum



Deconvoluted Spectrum



Deconvolution Results

Measured Mass Da	Delta Mass Da	Theoretical Mass Da	Mass Accuracy Da	Mass Accuracy ppm	Identification	RT min	Intensity counts	Fract. Abun. %	Drugs	Drugs* intensity	Score
152235.86	0.00	152235.93	0.07	0.5	A2G0F/A2G1F 4xMMAE	1.35	2.5E+07	8.74	4	1.0E+08	43.53
152398.72	162.86	152398.07	-0.64	-4.2	A2G1F/A2G1F 4xMMAE	1.37	2.2E+07	7.62	4	8.8E+07	46.67
149762.08	-2473.78	149762.81	0.72	4.8	A2G1F/A2G1F 2xMMAE	1.35	1.6E+07	5.35	2	3.1E+07	41.81
149600.67	-2635.19	149600.67	0.00	0.0	A2G0F/A2G1F 2xMMAE	1.35	1.5E+07	5.31	2	3.1E+07	40.19
152074.84	-161.01	152073.79	-1.05	-6.9	A2G0F/A2G0F 4xMMAE	1.37	1.4E+07	4.72	4	5.5E+07	41.84
154871.32	2635.46	154871.20	-0.12	-0.8	A2G0F/A2G1F 6xMMAE	1.37	9.4E+06	3.25	6	5.6E+07	42.09
149439.23	-2796.63	149438.53	-0.70	-4.7	A2G0F/A2G0F 2xMMAE	1.33	9.3E+06	3.20	2	1.9E+07	38.91
152560.82	324.96	152560.21	-0.61	-4.0	A2G1F/A2G2F 4xMMAE	1.35	8.8E+06	3.05	4	3.5E+07	41.15
155034.28	2798.42	155033.34	-0.94	-6.1	A2G1F/A2G1F 6xMMAE	1.37	8.3E+06	2.86	6	5.0E+07	40.66
154709.68	2473.82	154709.06	-0.62	-4.0	A2G0F/A2G0F 6xMMAE	1.35	5.3E+06	1.85	6	3.2E+07	43.22
155196.82	2960.96	155195.48	-1.34	-8.7	A2G1F/A2G2F 6xMMAE	1.37	3.7E+06	1.29	6	2.2E+07	37.31
151929.20	-306.66	151927.65	-1.55	-10.2	A2G0F/A2G0 4xMMAE	1.33	2.3E+06	0.79	4	9.2E+06	34.87
146963.05	-5272.80	146965.40	2.35	16.0	A2G0F/A2G1F 0xMMAE	1.40	2.1E+06	0.73	0	0.0E+00	26.87
149291.93	-2943.92	149292.39	0.45	3.0	A2G0F/A2G0 2xMMAE	1.37	1.9E+06	0.65	2	3.8E+06	32.13
155360.18	3124.32	155357.58	-2.60	-16.7	A2G2F/A2G2F 6xMMAE	1.37	1.9E+06	0.65	6	1.1E+07	32.72
157833.83	5597.97	157830.74	-3.09	-19.6	A2G1F/A2G2F 8xMMAE	1.37	1.7E+06	0.58	8	1.3E+07	27.40
153716.65	1480.80	153715.71	-0.95	-6.2	A2G1F/A2G1F 5xMMAE	1.35	1.6E+06	0.55	5	8.0E+06	31.86

Figure 4. Report example for a single injection of Aidixi ADC. The report includes a header with information about the injection, sequence, processing, report method, and calculated average DAR. The UV trace, mass spectrum, and deconvoluted spectrum are also shown in the report with a figure illustrating the drug load distribution. At the bottom of the report, a result table is shown that includes the measured mass for each component, identified components, mass accuracy, abundance, deconvolution score, etc. The Drugs*intensity row is the multiplication of the number of drugs and intensity for each component, which are used for calculating the intensity-based average DAR. A customizable filter has been applied to only show components above 0.50% fractional abundance.

Another part of the report is a summary of the sequence, as presented in Figure 5 for all 50 ADC injections in this study. This report page includes expected mass, most intense component, and the identity of the most intense component for each injection. This provides an overview of the full sequence and the confirmation of the ADC analyzed.

The Chromeleon CDS report template for version 7.3.2 MUb and later versions presented in this study as well as reference data can be accessed via the Thermo Scientific™ AppsLab™ Library: https://appslab.thermofisher.com/

Deconvoluted mass overview

No.	Injection Name	Antibody Mass	Drug Name	Drug Mass	Most Intense Component	Most Intense Component	ID Most Intense Component
		Da		Da	Mass (Da)	Intensity	
1	Enhertu_2ug_01	145173.890	DXd	1034.05	156339.581	9.8E+07	A2G0F/A2G0F 8 x DXd
2	Enhertu_2ug_02	145173.890	DXd	1034.05	156339.867	9.5E+07	A2G0F/A2G0F 8 x DXd
3	Enhertu_2ug_03	145173.890	DXd	1034.05	156338.999	9.1E+07	A2G0F/A2G0F 8 x DXd
4	Enhertu_2ug_04	145173.890	DXd	1034.05	156339.582	8.5E+07	A2G0F/A2G0F 8 x DXd
5	Enhertu_2ug_05	145173.890	DXd	1034.05	156339.519	8.1E+07	A2G0F/A2G0F 8 x DXd
6	Enhertu_2ug_06	145173.890	DXd	1034.05	156339.534	8.0E+07	A2G0F/A2G0F 8 x DXd
7	Enhertu_2ug_07	145173.890	DXd	1034.05	156338.997	8.3E+07	A2G0F/A2G0F 8 x DXd
8	Enhertu_2ug_08	145173.890	DXd	1034.05	156339.268	8.4E+07	A2G0F/A2G0F 8 x DXd
9	Enhertu_2ug_09	145173.890	DXd	1034.05	156339.290	8.8E+07	A2G0F/A2G0F 8 x DXd
10	Enhertu_2ug_10	145173.890	DXd	1034.05	156338.883	8.0E+07	A2G0F/A2G0F 8 x DXd
11	Blank				–	–	–
12	Polivy_2ug_01	145001.340	MMAE	1317.63	153161.936	1.1E+08	A2G0F/A2G0F 4 x MMAE
13	Polivy_2ug_02	145001.340	MMAE	1317.63	153161.957	1.0E+08	A2G0F/A2G0F 4 x MMAE
14	Polivy_2ug_03	145001.340	MMAE	1317.63	153161.930	1.0E+08	A2G0F/A2G0F 4 x MMAE
15	Polivy_2ug_04	145001.340	MMAE	1317.63	153162.179	9.8E+07	A2G0F/A2G0F 4 x MMAE
16	Polivy_2ug_05	145001.340	MMAE	1317.63	153162.200	1.0E+08	A2G0F/A2G0F 4 x MMAE
17	Polivy_2ug_06	145001.340	MMAE	1317.63	153162.224	1.0E+08	A2G0F/A2G0F 4 x MMAE
18	Polivy_2ug_07	145001.340	MMAE	1317.63	153161.870	9.8E+07	A2G0F/A2G0F 4 x MMAE
19	Polivy_2ug_08	145001.340	MMAE	1317.63	153161.637	1.0E+08	A2G0F/A2G0F 4 x MMAE
20	Polivy_2ug_09	145001.340	MMAE	1317.63	153162.464	9.0E+07	A2G0F/A2G0F 4 x MMAE
21	Polivy_2ug_10	145001.340	MMAE	1317.63	153161.735	9.1E+07	A2G0F/A2G0F 4 x MMAE
22	Blank				–	–	–
23	Adcetris_3ug_01	145191.520	MMAE	1317.63	153354.887	1.0E+07	A2G0F/A2G0F 4 x MMAE
24	Adcetris_3ug_02	145191.520	MMAE	1317.63	153354.755	9.5E+06	A2G0F/A2G0F 4 x MMAE
25	Adcetris_3ug_03	145191.520	MMAE	1317.63	153356.140	9.5E+06	A2G0F/A2G0F 4 x MMAE
26	Adcetris_3ug_04	145191.520	MMAE	1317.63	153515.978	9.0E+06	A2G0F/A2G1F 4 x MMAE
27	Adcetris_3ug_05	145191.520	MMAE	1317.63	153515.256	9.5E+06	A2G0F/A2G1F 4 x MMAE
28	Adcetris_3ug_06	145191.520	MMAE	1317.63	153354.194	8.7E+06	A2G0F/A2G0F 4 x MMAE
29	Adcetris_3ug_07	145191.520	MMAE	1317.63	153514.914	8.4E+06	A2G0F/A2G1F 4 x MMAE
30	Adcetris_3ug_08	145191.520	MMAE	1317.63	153356.190	9.3E+06	A2G0F/A2G0F 4 x MMAE
31	Adcetris_3ug_09	145191.520	MMAE	1317.63	150881.071	8.2E+06	A2G0F/A2G1F 2 x MMAE
32	Adcetris_3ug_10	145191.520	MMAE	1317.63	153516.036	9.6E+06	A2G0F/A2G1F 4 x MMAE
33	Adcetris_3ug_11	145191.520	MMAE	1317.63	153354.438	9.0E+06	A2G0F/A2G0F 4 x MMAE
34	Adcetris_3ug_12	145191.520	MMAE	1317.63	153354.971	8.1E+06	A2G0F/A2G0F 4 x MMAE
35	Adcetris_3ug_13	145191.520	MMAE	1317.63	153354.300	8.2E+06	A2G0F/A2G0F 4 x MMAE
36	Adcetris_3ug_14	145191.520	MMAE	1317.63	153515.728	8.1E+06	A2G0F/A2G1F 4 x MMAE
37	Adcetris_3ug_15	145191.520	MMAE	1317.63	153354.980	9.3E+06	A2G0F/A2G0F 4 x MMAE
38	Blank				–	–	–
39	Aidixi_3ug_01	143912.580	MMAE	1317.63	152236.230	3.1E+07	A2G0F/A2G1F 4 x MMAE
40	Aidixi_3ug_02	143912.580	MMAE	1317.63	152398.789	2.5E+07	A2G1F/A2G1F 4 x MMAE
41	Aidixi_3ug_03	143912.580	MMAE	1317.63	152235.400	2.6E+07	A2G0F/A2G1F 4 x MMAE
42	Aidixi_3ug_04	143912.580	MMAE	1317.63	152236.421	2.2E+07	A2G0F/A2G1F 4 x MMAE
43	Aidixi_3ug_05	143912.580	MMAE	1317.63	152235.518	2.4E+07	A2G0F/A2G1F 4 x MMAE
44	Aidixi_3ug_06	143912.580	MMAE	1317.63	152235.736	2.5E+07	A2G0F/A2G1F 4 x MMAE
45	Aidixi_3ug_07	143912.580	MMAE	1317.63	152235.858	2.5E+07	A2G0F/A2G1F 4 x MMAE
46	Aidixi_3ug_08	143912.580	MMAE	1317.63	152235.936	2.5E+07	A2G0F/A2G1F 4 x MMAE
47	Aidixi_3ug_09	143912.580	MMAE	1317.63	152236.226	2.3E+07	A2G0F/A2G1F 4 x MMAE
48	Aidixi_3ug_10	143912.580	MMAE	1317.63	152235.778	2.1E+07	A2G0F/A2G1F 4 x MMAE
49	Aidixi_3ug_11	143912.580	MMAE	1317.63	152235.791	2.5E+07	A2G0F/A2G1F 4 x MMAE
50	Aidixi_3ug_12	143912.580	MMAE	1317.63	152236.573	2.3E+07	A2G0F/A2G1F 4 x MMAE
51	Aidixi_3ug_13	143912.580	MMAE	1317.63	152235.829	2.1E+07	A2G0F/A2G1F 4 x MMAE
52	Aidixi_3ug_14	143912.580	MMAE	1317.63	152235.685	2.4E+07	A2G0F/A2G1F 4 x MMAE
53	Aidixi_3ug_15	143912.580	MMAE	1317.63	152236.000	2.5E+07	A2G0F/A2G1F 4 x MMAE
54	Blank				–	–	–

Figure 5. Sequence overview in the report that includes relevant metadata from the sequence as well as mass and ID of the most intense component identified for each injection.

Conclusions

Here, we showcase the implementation of a newly developed, customizable Chromeleon CDS report template that supports a fully automated workflow for intact ADC analysis and drug-to-antibody ratio calculation, delivering the following key benefits:

- **Fully integrated:** Chromeleon CDS workflow combines instrument control, data processing, and DAR reporting in one environment.
- **Automation:** End-to-end automation of acquisition, deconvolution, identification, and DAR calculation, eliminating the need for manual data handling.
- **High-throughput:** 50 injection samples in under four hours—including data acquisition, processing, and reporting—using 3-minute LC-MS runs.
- **Data quality:** Sensitive high-resolution native MS data enabling confident identification of ADC heterogeneity and drug load distribution.

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