thermoscientific

Characterization of a Cysteine-conjugated ADC on a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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ABSTRACT

Purpose: To completely characterized a special modified cysteine-linked ADC.

Methods: Molecular weight detection under native condition and peptide mapping of the ADC after digestion.

Results: This workflow provides a complete characterization method for a designed cysteine-linked ADC.

INTRODUCTION

The biopharmaceutical industry has continued its focus on the development of biotherapeutic monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs). In this study, we used a Thermo Scientific™ Q Exactive™ Plus MS with Biopharma option to measure the intact protein molecular weight of a cysteine-conjugated ADC under native condition. Online desalting size exclusion chromatography and High mass range (HMR) mode were employed, which are necessary for intact cysteine-conjugated ADC analysis, to preserve structurally-critical non-covalent binding between antibody chains and to observe the signals between ~5,000-7,000 m/z with high sensitivity.

Additionally, we have performed peptide mapping on the same instrument to generate complementary datasets for complete characterization.

MATERIALS AND METHODS

Sample Preparation

An ADC sample was prepared for peptide mapping (reduction, alkylation, and trypsin digestion) or intact analysis (no treatment).

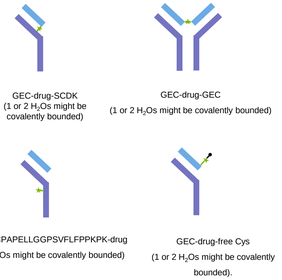
Test Method

For native LC-MS intact analysis, 15 µg of sample was desalted online using size exclusion chromatography (50 mM NH_4OAc isocratic elution) and directly introduced to the mass spectrometer. Peptide mapping was performed using 1 µg of sample separated with a 49 min gradient of 3-50% ACN in H_2O and 0.1% formic acid. A Q Exactive Plus instrument with the Biopharma option was used for both analyses.

Data Analysis

BioPharma Finder 2.0 was used for both intact and peptide mapping data analysis.

Figure 1. Four possible forms of conjugation. There might be different combinations in one ADC sample.

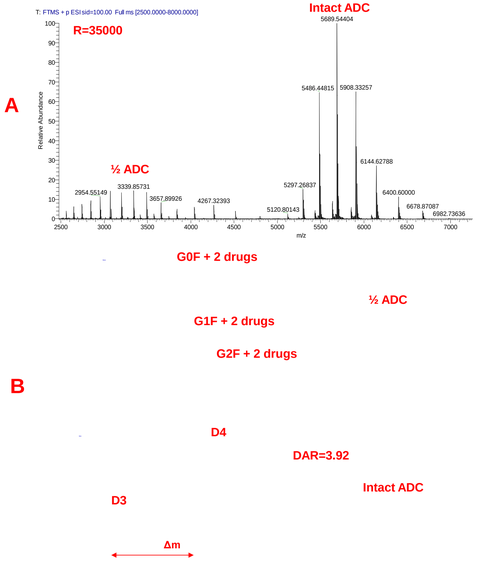


RESULTS

Molecular Weight Detection of ADC under Native Condition

In order to maintain non-covalent binding in the ADC molecule to achieve intact analysis. Native conditions were employed using high mass range (HMR) mode on a Q Exactive Plus with BioPharma Option (Fig. 2) .

Figure 2. ADC analyzed under native condition (A) and the deconvolution results (B).

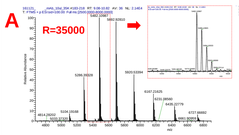


Half ADC appears in the native MS spectra due to the manufacturing procedure. It is clear that the main ingredient of this product is intact ADC and the non-covalent binding between chains remains due to the more gentle native condition. With a unique design of conjugation sites, populations with three and four drugs loaded onto the antibody were detected with a mass difference between peaks corresponding to the addition of one drug and linker (+1396.7642 Da). Higher mass accuracy is achieved due to the high spectral resolution of proteoforms and non-overlapping charge states. Therefore the drug-antibody ratio (DAR) can be determined accurately and an average DAR is 3.92 was determined.

Molecular Weight Detection of unmodified mAb under Native Condition

We also measured the molecular weight of the unmodified mAb under native condition. The instrument is Q Exactive Plus with BioPharma Option, using HMR mode. The results are shown in Fig.3.

Figure 3. mAb analyzed under native condition and the deconvolution results. A, MS spectrum; B, deconvoluted spectrum; C, summary table of deconvolution.



C

Modification	Average Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Relative Abundance
2xA2G0F,2xLys loss	147991.55	147992.45	6.1	100.00
1xA2G0F,1xA2G1F,2xLys loss	148153.23	148154.59	9.1	62.31
1xA2G0F,1xA2G2F,2xLys loss	148315.95	148316.73	5.2	44.06
1xA2G1F,1xA2G2F,2xLys loss	148477.91	148478.87	6.5	17.41
2xA2G2F,2xLys loss	148637.44	148641.01	24.0	4.36

As shown in Fig.3, the main glycoforms of the mAb could be identified. For the top 4 most abundant glycoforms, the mass difference between theoretical and measured mass are all less than 10ppm.

Peptide Mapping Results of the Products

Using standard mode of the Q Exactive Plus with BioPharma option, we finished peptide mapping analysis of digested ADC and mAb respectively. Fig.4 shows the peptide mapping results of the ADC 100% of sequence coverage could be easily achieved.

Figure 4. Peptide Mapping Results of the ADC. A, labeled chromatogram; B, sequence coverage map.

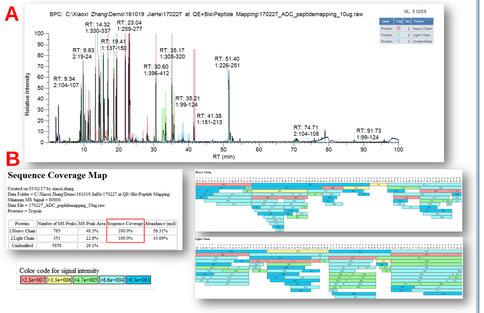
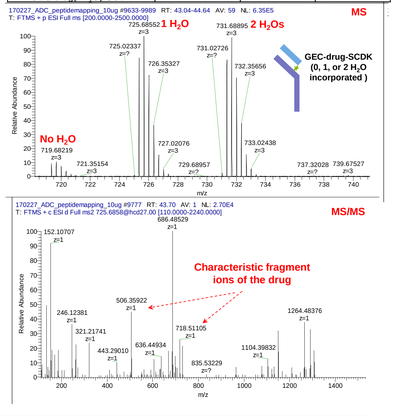


Fig.5 shows the identifications of the peptides conjugated with drugs. All possible modified sites are confirmed with high quality MS/MS spectra, even for short peptides and hinge region peptides. The proprietary conjugation chemistry (not shown) used here can result in the incorporation of one or two water molecules. This results in coleuting but distinctly modified peptides in the peptide map.

Figure 5. Drug modified sites identification and confirmation. A, modified peptide GEC-drug-SCDK; B, modified peptide GEC-drug-GEC; C, THTCPPCPAPELLGGPSVFLFPPKPK-drug. All conjugated peptides manifested as three different species with incorporation of 0, 1, or 2 H_2O

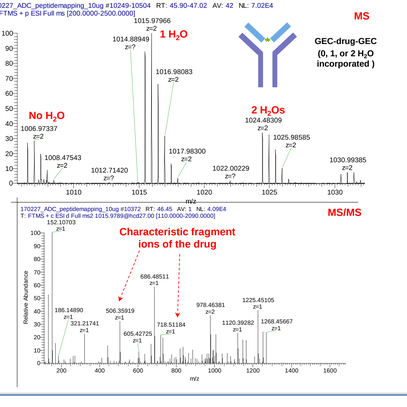
A

	MH+	MH2+	MH3+
GEC-drug-SCDK	2156.029	1078.5185	719.3483
GEC-drug(1H2O)-SCDK	2174.0396	1087.5238	725.3518



B

	MH+	MH2+	MH3+
GEC-drug-GEC	2011.9391	1006.4735	671.3183
GEC-drug(1H2O)-GEC	2029.9497	1015.4788	677.3218



C

	MH+	MH3+	MH4+	MH5+
THTCPPCPAPELLGGPSVFLFPPKPK-drug	4127.1788	1376.398	1032.550625	826.2421
THTCPPCPAPELLGGPSVFLFPPKPK-drug(1H2O)	4145.1894	1382.402	1037.053275	829.8442

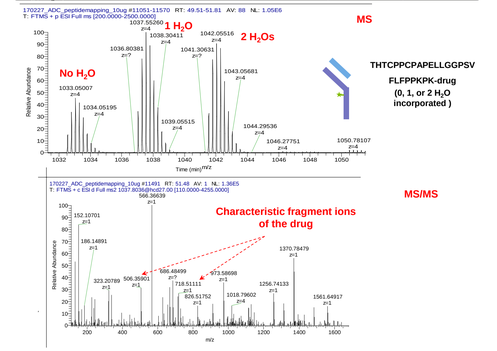


Figure 6. XIC, MS and MS/MS spectra of THTCPPCPAPELLGGPSVFLFPPKPK-drug with/without H_2Os. A~C, XIC of peptide-drug with no H_2O/1 H_2O/2 H_2Os; D~F, MS spectra of peptide-drug with no H_2O/1 H_2O/2 H_2Os; G~I, b4 ions of peptide-drug with no H_2O/1 H_2O/2 H_2Os. Each row represents a same peptide.

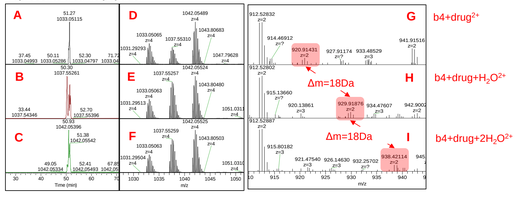


Fig. 6 shows that these peptide-drug conjugates with different incorporation of H_2O are very closely eluting but have distinct chromatographic profiles and product ion spectra consistent with covalent incorporation.

CONCLUSIONS

- This workflow provides a complete characterization method for a designed cysteine-linked ADC, including native MS, denatured MS and peptide mapping.
- We successfully measured the molecular weight and DAR of a designed Cys-conjugated ADC under native condition, and identified all possible modified sites in peptide mapping analysis.
- BioPharma option for Q Exactive Plus/HF mass spectrometers combined with BioPharma Finder software offers an integrated approach to biotherapeutic protein characterization.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING