Intact Mass Analysis of Lysine-Linked Antibody-Drug Conjugates using Microfluidic Chip Electrophoresis and Size Exclusion Chromatography Coupled to an Orbitrap Mass Spectrometer

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ABSTRACT

Purpose: Intact mass analysis and DAR calculation of a lysine-linked antibody-drug conjugate.

Methods: The sample used was trastuzumab emtansine (trade name Kadcyla®) and it was not deglycosylated. In one case, the separation was done on a novel microfluidic electrophoresis device (ZipChip), and on the other case we used size exclusion chromatography. In both cases, the new Thermo ScientificTM Q ExactiveTM BioPharma mass spectrometer with the high mass range (HMR) mode was used. Raw files were processed with Thermo ScientificTM BioPharma Finder™ 2.0 software where the deconvolution, the assignment of the number of drug on the deconvoluted peaks, and the determination of the average DAR are done automatically.

Results: Using the ZipChip, for the glycoforms G0F/G1F, G1F/G1F and G1F/G2F of trastuzumab emtansine the average DAR values were respectively 3.46, 3.47 and 3.39, and the mass error of any deconvoluted peaks was less than 25 ppm. Similarly, for the size exclusion chromatography experiment, an average DAR value of 3.71, 3.64 and 3.65 was respectively calculated for the glycoforms G0F/G1F, G1F/G1F and G1F/G2F of trastuzumab emtansine. These average DAR values are in accordance with previously published data.

INTRODUCTION

Antibody-drug conjugates (ADCs) are an important class of therapeutics and, by their inherent heterogeneity, present analytical challenges. One of the critical attributes of ADCs is the determination of the average drug to antibody ratio (DAR) that can be calculated directly from the intact mass analysis. However, often the N-linked glycans of the monoclonal antibody are removed to reduce the sample complexity. This results in the loss of glycoform/DAR information. In this study no alteration was done to the sample and we were able to successfully determine the average DAR value for different glycoforms.

MATERIALS AND METHODS

Sample: Trastuzumab Emtansine

Electrophoresis: 908 Devices chip-based ZipChip

BGE: 10% isopropanol, 0.2% acetic acetic in water

LC: Thermo Scientific™ Vanquish™ UHPLC system

Column: Thermo Scientific™ MAbPac™ SEC-1, (150 mm * 4 mm; 5 mm)

Buffer: 50 mM ammonium acetate; flow rate: 300 mL/min

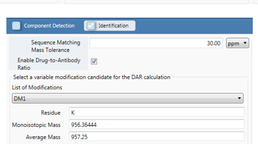
Mass Spectrometry: Thermo Scientific™ Q Exactive™ Plus or Q Exactive™ HF mass spectrometer with High Mass Range (HMR) mode

Data Analysis: Raw files were processed with BioPharma Finder 2.0 software.

FIGURE 1: Sliding windows (a) and drug to antibody (b) parameters used for the size exclusion chromatography experiment.

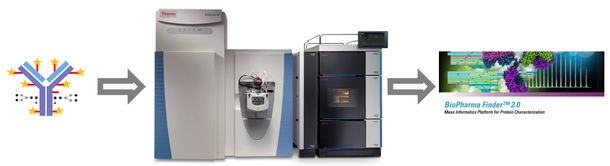
(a)

(b)



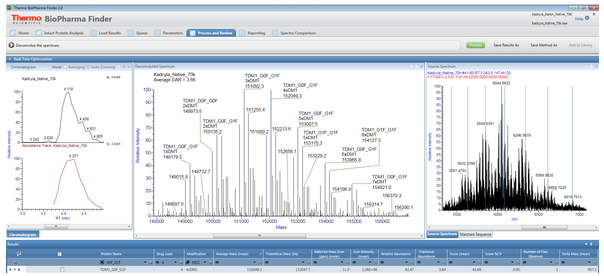
RESULTS

FIGURE 2: Workflow 1: Lysine-linked ADC analyzed by size exclusion chromatography coupled to a Q Exactive Plus and processed with Biopharma Finder 2.0



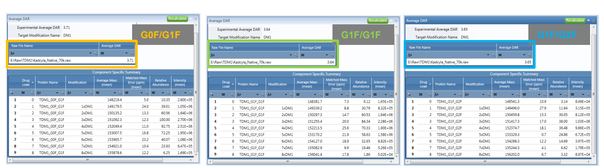
For each acquisition, approximately 10 mg of sample was loaded and desalted online without pretreatment. Data was acquired using 10 mscans and the resolving power of the mass spectrometer was set at 70,000. For data processing with BioPharma Finder, MS1 spectra were deconvoluted with the Respect algorithm in combination with the sliding window over a defined time range.

FIGURE 3: Data visualization in BioPharma Finder 2.0 of the lysine-linked ADC acquired by size exclusion chromatography



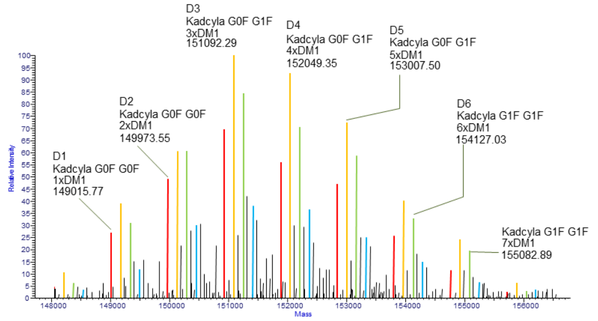
G0F/G1F glycoform of trastuzumab emtansine containing four conjugations was selected (bottom panel). On the left panel, an extract component chromatogram was automatically generated below the TIC and the selected component was highlighted in the annotated deconvoluted spectrum (middle panel) and in the raw data (right panel) .

FIGURE 4: Average DAR calculation for glycoforms G0F/G1F, G1F/G1F and G1F/G2F of trastuzumab emtansine



The sample was analyzed without pretreatment therefore an average DAR value can be calculated for different glycoforms.

FIGURE 5: Deconvoluted and annotated masses of trastuzumab emtansine

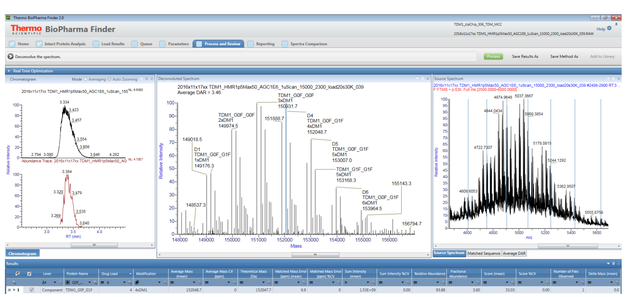


Lysine-linked ADCs are a heterogeneous mixture because a broad distribution of linker-drug additions is observed for each glycoform.

FIGURE 6: Workflow 2: Lysine-linked ADC analyzed by ZipChip based electrophoresis coupled to a Q Exactive HF and processed with Biopharma Finder 2.0

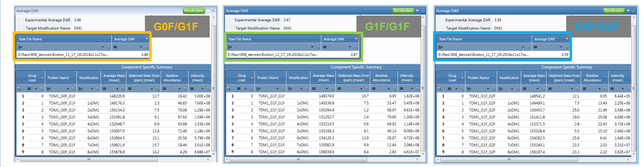
The ZipChip interface simply mounts onto your mass spectrometer (Fig.6) The ZipChip is a twenty two centimeter etched channel with a neutral hydrophilic coating with an integrated nanoelectrospray emitter. For each run, around 2.5 ng of sample ([sample] = 1mg/mL), was separated on the microfluidic channel, and data was acquired using 1 mscan at 35,000 resolving power.

FIGURE 7: Data visualization in BioPharma Finder 2.0 software of the Lysine-linked ADC acquired by ZipChip based electrophoresis



All of the different forms of trastuzumab emtansine were separated in less than 40 seconds, and the charge states distribution ranged from 27 to 33 (≈ 4600 *m/z* to 5600 *m/z*), which differs from the size exclusion workflow.

FIGURE 8: Average DAR calculation for glycoforms G0F/G1F, G1F/G1F and G1F/G2F of trastuzumab emtansine



For the glycoforms G0F/G1F, G1F/G1F and G1F/G2F, the average DAR values are respectively 3.45, 3.47 and 3.39, which is consistent with previous published data (Marcoux et al., Protein Sci. 2015 Aug;24(8):1210-23).

CONCLUSIONS

Lysine-linked ADCs are very heterogeneous samples and can be successfully analyzed without sample pre-treatment by size exclusion chromatography or ZipChip based electrophoresis.

Average drug to antibody ratio values for different glycoforms were automatically calculated with BioPharma Finder 2.0 software.

Average drug to antibody ratio values calculated for different glycoforms of trastuzumab emtansine are consistent with previous published data.

REFERENCES - 18 PT. ARIAL BOLD

1. Marcoux J, Champion T, Colas O, Wagner-Rousset E, Corvaïa N, Van Dorsselaer A, Beck A, Cianférani S. Native mass spectrometry and ion mobility characterization of trastuzumab emtansine, a lysine-linked antibody drug conjugate. Protein Sci. 2015 Aug;24(8):1210-23

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