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

Stephane Houel¹, Aaron Bailey¹, Erin Redman², Scott Mellors², Eugen Damoc³, Kai Sheffler³, Jennifer Sutton¹, Chris Petty², Jonathan Josephs¹
¹Thermo Fisher Scientific, San Jose, CA, USA; ²908 Devices, Boston, MA, USA; ³Thermo Fisher Scientific, Bremen, Germany.

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 decomplexed. In this case, the work was done on a novel microfluidic separation system with a mass spectrometer equipped with High Mass Range (HMR) mode. Raw files were processed with Thermo Scientific™ BioPharma Finder™ 2.0 software. For data processing with BioPharma Finder, MS1 spectra were acquired with Thermo Scientific™ Q Exactive™ Plus or Q Exactive™ HF mass spectrometers. The mass spectrometer was set at 70,000. For data processing with BioPharma Finder, MS1 spectra were processed with Thermo Scientific™ BioPharma Finder™ 2.0 software.

RESULTS

For each acquisition, approximately 10 mg of sample was loaded and desalted online without pretreatment. The sample was analyzed without pretreatment therefore an average DAR value can be determined 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 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.

INTRODUCTION

Antibody-drug conjugates (ADCs) are an important class of therapeutics and, by their heterogeneous nature, 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 spectra. 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 acid in water

Column: Thermo Scientific™ Vanquish™ UPLC system

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

RESULTS

For each acquisition, approximately 10 mg of sample was loaded and desalted online without pretreatment. Data was acquired using 10 scans and the rejection power of the mass spectrometer was set at 70,000. For data processing with BioPharma Finder, MS1 spectra were processed with Thermo Scientific™ BioPharma Finder™ 2.0 software. The sample was analyzed without pretreatment therefore an average DAR value can be determined 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.

CONCLUSIONS

Lysine-linked ADCs are a heterogeneous mixture because a broad distribution of linker-drug addition is observed for each glycoform. Average DAR values for different glycoforms were calculated for different forms of trastuzumab emtansine are consistent with previous published data. 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).

REFERENCES - 18 PT, ARIAL BOLD


TRADEMARKS/LICENSING

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