Increased sensitivity and throughput for native intact mass analysis of mAb and ADCs using an online buffer exchange column

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Abstract

Purpose: To develop a native intact mass analysis method for rapid ADC sample screening with high sensitivity by using the online buffer exchange NativePac OBE-1 SEC column coupled to native mass spectrometry.

Methods: A Vanquish Flex UHPLC coupled to a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometry equipped with BioPharma option were employed.

Results: Demonstrated the capability of this rapid method in accurate intact mass and DAR measurement of polatuzumab vedotin and trastuzumab deruxtecan with high sensitivity and throughput.

Introduction

Antibody-drug conjugates (ADCs) are heterogeneous mixtures of chemically distinct molecules that vary in both drugs/antibodies and conjugation sites. Most of ADCs on market or in clinical trials are lysine-linked, cysteine-linked or site-specific ADCs. For cysteine-linked ADC, the interchain disulfide bonds are reduced followed by linker-payload conjugation. Therefore, native intact mass analysis has been routinely used in cysteine-linked ADC characterization due to its ability to retain non-covalent interactions. Here we used the NativePac OBE-1 SEC column and the Orbitrap Exploris 240 mass spectrometry for native intact analysis of two cysteine-linked ADCs, polatuzumab vedotin and trastuzumab deruxtecan and tried to determine the limit of detection (LOD) and linear dynamic range(LDR) of each sample. Trastuzumab was also analyzed as reference.

Materials and methods

Sample Preparation

Commercially available polatuzumab vedotin, trastuzumab deruxtecan (schematic shown in Figure 1) and trastuzumab were diluted at different concentration ranges (0.01,0.1 and 1mg/mL) using ddH_2O.

UHPLC Separation

Thermo Scientific™ NativePac OBE-1 SEC (P/N 43803-052130) column was used, with 100 mM ammonium acetate at a flow rate of 100μL/min. The column temperature was set at 25℃. Total LCMS run time per injection was 3 minutes.

Mass Spectrometry

A Thermo Scientific™ Vanquish ™ Flex UHPLC system coupled to a Orbitrap Exploris™ 240 mass spectrometry with Biopharma option were used for data acquisition. The MS method parameters are detailed in Figure 2.

Data Analysis

Data analysis was performed using Thermo Scientific™ BioPharma Finder™ 5.2 software.

Figure 1. The Schematic of polatuzumab vedotin and trastuzumab deruxtecan [1]

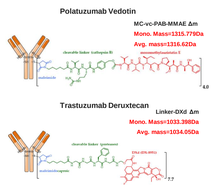
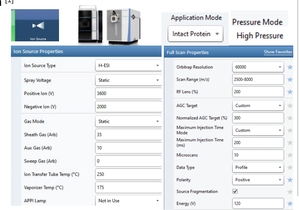


Figure 2. MS method parameters

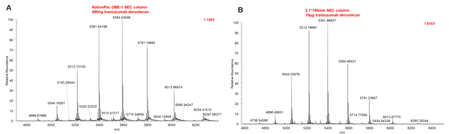


Results

Improved sensitivity for ADC analysis using NativePac OBE-1 SEC column

In this study we evaluated the sensitivity of OBE-1 column for the native intact mass analysis of ADCs and mAb. The linker-payload conjugation induces not only structure heterogeneity, but also lower MS signal level compares to naked mAb. Therefore, sensitivity plays even more important roles in native intact mass analysis of ADCs than mAbs. The low flow rate (50-100μL/min) for OBE column separation provides better sensitivity compares to regular SEC columns. In this study we observed 500ng trastuzumab deruxtecan on OBE-1 column gives same level MS intensity as 10μg on a 2.1*150mm SEC column (Figure3).

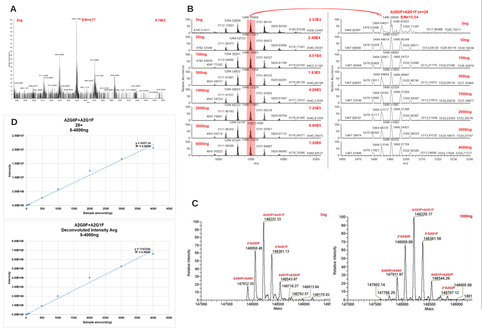
Figure 3. Native intact MS spectra of tratuzumab deruxtecan using different columns with different sample loading amount. A, 500ng trastuzumab deruxtecan on OBE-1 column. B, 10μg trastuzumab deruxtecan on a 2.1*150mm SEC column.



LOD and LDR of native mAb and ADCs using NativePac OBE-1 SEC column

Figure 4A shows the native intact mass spectra of 2ng trastuzumab on column. Native intact mass spectra of trastuzumab dilution series can be observed from Figure4B. Down to 2ng trastuzumab was detected with MS S/N>3 and 5ng trastuzumab on column was measured with MS S/N>10.

Figure 4. LOD and LDR of native trastuzumab using NativePac OBE-1 SEC column. A, 2ng trastuzumab on OBE-1 column. B, Native intact mass spectra of trastuzumab dilution series (5ng-4000ng). C, deconvoluted mass spectra of 5ng trastuzumab and 1000ng trastuzumab on column. D, LDR of trastuzumab, calculated based on MS signal intensity of A2G0F+A2G1F(z=+28) and deconvoluted A2G0F+A2G1F intensity, respectively.



The LOD of polatuzumab vedotin is 5ng and trastuzumab deruxtecan is 10ng (Figure 5A and 6A). Both payload and N-glycosylation distributions can be detected even with 5~10ng ADC sample loading. Figure 5B and C show native intact mass spectra of polatuzumab vedotin dilution series and deconvolution results(10ng and 2000ng). The linear dynamic range calculated on MS signal intensity or deconvoluted intensity are displayed in Figure 5D. Native intact mass spectra of trastuzumab deruxtecan dilution series, deconvolution results and linear dynamic range are shown in Figure 6B-D.

Figure 5. LOD and LDR of native polatuzumab vedotin using NativePac OBE-1 SEC column. A, 5ng polatuzumab vedotin on OBE-1 column, three replicate injections. B, Native intact mass spectra of polatuzumab vedotin dilution series(10ng-2000ng). C, deconvolution results of 10ng polatuzumab vedotin and 2000ng polatuzumab vedotin on column. D, LDR of polatuzumab vedotin, calculated based on MS signal intensity of 2*A2G0F (D2 and D4, z=+26) and deconvoluted intensity of 2*A2G0F (D2 and D4), respectively.

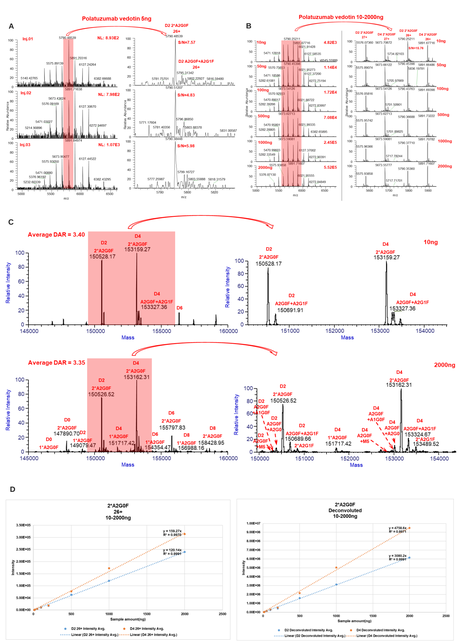
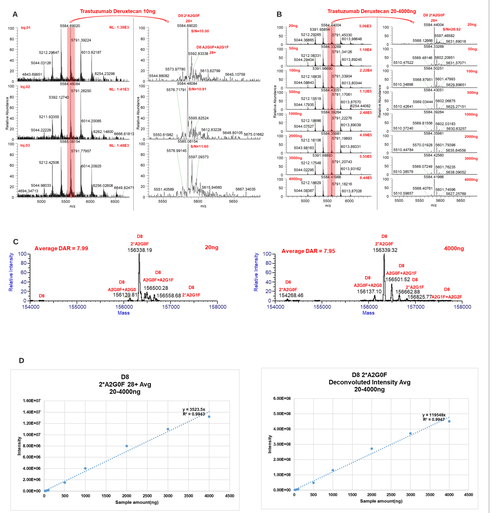


Figure 6. LOD and LDR of native trastuzumab deruxtecan using NativePac OBE-1 SEC column. A, 10ng trastuzumab deruxtecan on OBE-1 column, three parallel injections. B, Native intact mass spectra of trastuzumab deruxtecan dilution series(20ng-4000ng). C, deconvolution results of 20ng trastuzumab deruxtecan and 4000ng trastuzumab deruxtecan on column. D, LDR of trastuzumab deruxtecan, calculated based on MS signal intensity of 2*A2G0F (D8, z=+28) and deconvoluted intensity of 2*A2G0F (D8), respectively.



Nowadays there are rising requirements of LOD, LOQ and LDR of mAb and ADCs at native intact level, since immuno-enrichment step may introduce sample denaturing and can't preserve the cysteine ADC structure. Also, sensitivity can be critical for native intact ADC detection and quantification. In this study, LODs for both mAb and ADCs are less or equal than 10ng, benefitted from the OBE-1 column and Orbitrap platform. Also, LDRs for-both mAb and ADCs are across three orders with R^2>0.99.

The OBE column can also improve throughout by decreasing analytical time. In this study, the analytical time was 3 minutes for each run, much shorter than regular SEC columns, which usually takes 15~20 minutes per run. The sensitivity and throughout were improved significantly compares to previous SEC-native mass analysis workflow, providing benefits in fast sample screening during drug development and process optimization.

Conclusions

- In this study we demonstrated the utility of NativePac OBE-1 SEC on-line buffer exchange column on Vanquish Flex UHPLC couple to Orbitrap Exploris 240 mass spectrometer with Biopharma Option enabled for high throughput and ultra high sensitive native intact mass analysis of mAb and ADCs.
- Each sample was analyzed in 3 minutes.
- Significantly sensitivity improvement for the native intact mass analysis of mAb and ADCs (LOD down to 2ng on column for trastuzumab, 5ng for polatuzumab vedotin and 10ng for trastuzumab deruxtecan) was achieved.
- Excellent linear dynamic range with R^2>0.99 of each sample was determined using MS signal intensity and deconvoluted mass intensity, respectively (5-4000ng for trastuzumab, 10-2000ng for polatuzumab vedotin and 20-4000ng for trastuzumab deruxtecan).

References

1. Pharmaceuticals 2020, 13, 245; doi:10.3390/ph13090245.

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