Automated High-throughput Sample Concentration and Buffer Exchange Platform Enhances Rapid Flow Analysis of Antibody Drug Conjugates by UHR-QTOF

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Introduction

Antibody drug conjugates (ADCs) as novel protein drug modalities have made considerable progress through the last decades with ten ADCs approved by FDA to date. The structural complexity of ADCs, as well as formulation in MS incompatible buffers, imposes challenges on analytical method development, especially for in-vivo biotransformation samples, which typically require extensive sample preparation and high sensitivity detection. Here we take advantage of a microfluidic channel-based platform, SampleStream (Integrated Protein Technologies, Fig 1), that uses a molecular weight cutoff (MWCO) membrane for automated buffer exchange and sample enrichment. The platform could be easily coupled with an ultra-high resolution QTOF instrument for the MS analysis and DAR measurement of intact ADCs under native and denaturing conditions.

Methods

For this study, intact NISTmAb and trastuzumab emtansine (T-DM1, Kadcyla) were measured on Bruker timsTOF Pro 2 mass spectrometer. The buffer exchange method was developed on a prototype stage SampleStream system with a 10 kDa MWCO membrane. Elution buffer for denaturing and native mode was 30% acetonitrile with 0.1% FA and 20 mM NH_4OAC with 10% IPA, respectively.

Data was processed in DataAnalysis and BioPharma Compass 2021 (Bruker) for mass deconvolution and DAR calculation.

Fig. 1 SampleStream Platform

Results

NISTmAb was used as a model protein for the initial study. During the focusing mode of SampleStream operation, the protein was retained by the MWCO membrane and accumulated at the center while the buffer and small molecules passed through. With an elution flow rate of 50 µL/min, rapid buffer exchange was achieved in less than 2 min. Deconvoluted spectrum (Fig 2) of NISTmAb in both denaturing and native mode reveals the expected heterogeneities.

Peak symmetry suggested a low level of adduct formation and indicates that the SampleStream platform is effective for buffer exchange and desalting of protein. Due to the removal of stationary phase interaction, a low concentration of salt is required in native conditions compared with traditional methods which is beneficial for system maintenance. This represents a clear benefit for the characterization of noncovalently linked species.

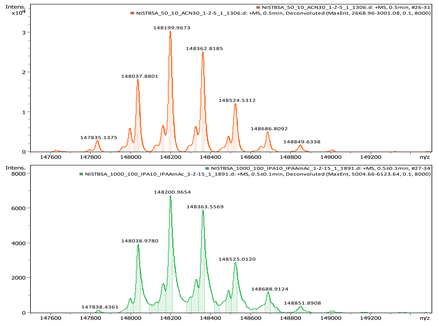

Fig. 2 Deconvoluted spectrum of NISTmAb in both denaturing (top) and native (bottom) mode

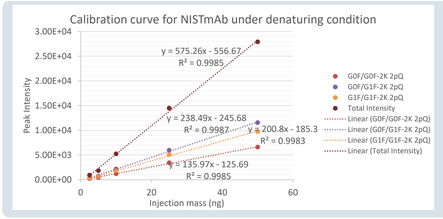

Fig. 3 Calibration curve for NISTmAb under denaturing condition

All major glycoforms of NISTmAb were detected at a concentration of 0.5 µg/mL. A linear response was observed and the LOQ was determined to be 5.6 ng for NISTmAb (Fig 3). The low LOQ would allow the potential for utilizing the SampleStream system coupled with UHR-QTOF for biotransformation study where the sample concentration is usually low.

The optimized method was applied for DAR characterization of T-DM1. Deconvoluted spectrum of T-DM1 (Fig 4) shows all major DAR species ranging from 0 to 8 were identified in both denaturing and native mode. The average DAR calculated from different sample concentrations is consistent and agrees with the published theoretical value (Fig 5). Furthermore, an additional peak with an MW difference of 221 Da suggests the presence of the free MCC linker in T-DM1. Further investigation could be conducted to study the cause of the DM1 drug loss.

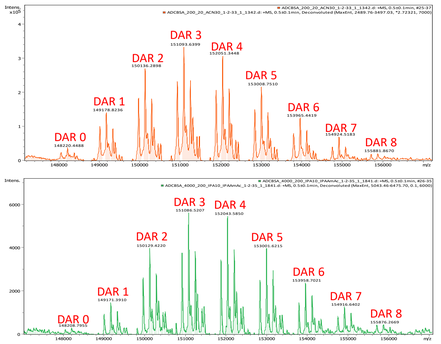

Fig. 4 Deconvoluted spectrum of T-DM1 in both denaturing (top) and native (bottom) mode

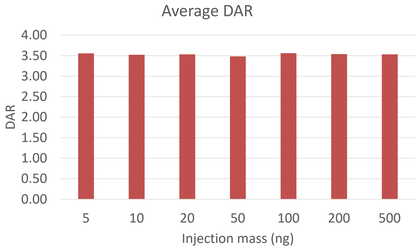

Fig. 5 Average DAR calculated for different sample concentrations

Conclusions

- Automated high-throughput buffer exchange of the intact protein sample was achieved in less than 2 min on the SampleStream platform.
- Coupling with Bruker timsTOF Pro 2, a low level of adduct and linear quantitative performance was observed for mAb and ADC in both denaturing and native mode.
- The average DAR measured from different sample concentrations is consistent and agrees with the theoretical value. The SampleStream workflow allows rapid flow analysis of the drug loading profile of antibody drug conjugates by UHR-QTOF.

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