Automated Sample Concentration and Buffer Exchange Platform enables High-Throughput Biotherapeutics Analysis with Mass Spectrometry

SampleStream platform coupling with Bruker timsTOF Pro 2 mass spectrometer delivers automated and rapid buffer exchange of monoclonal antibodies

Abstract

Mass spectrometry has been widely used to evaluate the heterogeneity profile of therapeutic proteins. However, the throughput of intact mass analysis can be limited by the incompatibility of formulation buffers with LC-MS or chromatographic issues such as recovery or stability in usual reverse phase eluents. Here we describe a new analytical platform that enables automated high-throughput sample concentration and buffer exchange to a wide range of eluents. The SampleStream platform coupled with the Bruker timsTOF Pro 2 mass spectrometer has demonstrated a linear response across a large concentration range, high reproducibility and low carryover. In this case study, NISTmAb is analyzed with a throughput of 2 min/sample to evaluate the platform method performance.

Keywords: timsTOF Pro 2, monoclonal antibody, intact mass analysis, subunit mass analysis, biopharma characterization

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Introduction

Mass analysis of intact biotherapeutic antibodies and their reduced subunits has become a standard characterization method to gain insights into a wide range of quality attributes; including primary sequence, disulfide bonding, glycosylation, biotransformation, and more. However, reducing agents, stabilizing agents, and ligands which are frequently added to minimize protein oxidation and aggregation during the production and purification process could form adduct complexes with proteins in the gas phase and reduce the quality of the mass spectra [1]. To ensure an efficient electrospray ionization process, desalting and exchanging the biomolecule solutions into a MS-compatible buffer is imperative [2]. Sample preparation and desalting methods vary widely based on applications. Offline buffer exchange, including dialysis and centrifugation, has been traditionally used for sample preparation that is labor-intensive and time-consuming. Online buffer exchange involving various liquid chromatography techniques reduces throughput and typically limits the desalting buffer to the starting eluent of the HPLC separation.

Here we take advantage of a microfluidic channel-based platform, SampleStream (Integrated Protein Technologies, Figure 1), that uses a molecular weight cutoff (MWCO) membrane for automated buffer exchange and sample enrichment to address these limitations. The platform is compatible with a wide range of matrices and solvents and can process samples up to a rate of 15 s/sample [3]. In addition, it can be easily coupled with an ultra-high resolution Bruker timsTOF Pro 2 instrument for the online MS analysis and quantification of antibody and its subunits.

Experimental

Materials and Instrumentation

The NISTmAb RM 8761, humanized IgG1κ, was purchased from NIST (Gaithersburg, MD), consisting of 1 unit containing 800 µL of 67 µM (10 mg/mL) IgG1κ in 12.5 mmol/L histidine, 12.5 mmol/L histidine HCl (pH 6.0). Aliquoted antibody stored at -80°C was thawed at room temperature and diluted in 30% acetonitrile with 0.1% formic acid before intact analysis. The IdeS protease was purchased from Genovis in the format of FabRICATOR 8x100 units. LC-MS grade water, acetonitrile, guanidine hydrochloride solution and formic acid were purchased from Sigma-Aldrich (St. Louis, MO).

For subunit analysis, FabRICATOR enzyme was reconstituted in phosphate buffered saline (PBS buffer, pH = 7.5) and then mixed with NISTmAb for incubation at 37°C. The mixture was then reduced with 4 M guanidine and 100 mM DTT at 50°C for 45 min. The reaction was quenched with 1% TFA solution and further diluted in water to 0.05 mg/mL for analysis.

The buffer exchange method was developed on an Elute HT UHPLC (Bruker GmbH & Co. KG) augmented with a prototype SampleStream module (IPT) with a 3 kDa MWCO membrane for subunit and a 10 kDA MWCO membrane for intact NISTmAb. Elution buffer was 30% acetonitrile with 0.1% formic acid.

Figure 1: SampleStream Platform

The SampleStream platform was coupled with a timsTOF Pro 2 trapped ion mobility QTOF instrument (Bruker) for mass analysis. Method and sequence creation as well data acquisition with this setup was performed under full Hystar control.

Data Processing

The data was processed with DataAnalysis and BioPharma Compass® 2021b for data visualization and automated deconvolution. Maximum entropy deconvolution for subunit NISTmAb was performed with 800-1500 *m/z* input range and 20000 to 30000 Da deconvoluted mass range. For intact NISTmAb, it was performed with 5300-6300 *m/z* input range and 140,000 to 160,000 Da mass range.

Result and discussion

NISTmAb and its reduced subunits were used as a model protein for the study. During the focusing mode of SampleStream operation, the protein was retained by the MWCO membrane and concentrated in the flow cell while the buffer and small molecules passed through to waste. With an elution flow rate of 50 µL/min, rapid buffer exchange and elution to the MS was achieved in less than 2 min.

Here the deconvoluted spectrum (Figure 2) reveals the three expected species of the NISTmAb subunits, Fc/2, LC and Fd'. For all three species, the level of sodium and potassium adduct formation was low and indicated that the SampleStream platform is effective for buffer exchange and desalting of proteins even for a sample in a high ionic strength denaturing buffer. The non-volatile salts in the reduced sample were comprehensively removed during the focusing mode of the SampleStream run as evidenced by the absence of guanidine adducts (+59 Da). Compared with traditional reverse phase chromatography, only the cleaned sample was introduced to the mass spectrometer during the elution mode negating the need for a divert valve setup. Sub-ppm mass accuracy was observed for all major peaks in the spectrum, which supports that the SampleStream platform is suitable for protein identity verification with higher throughput than liquid chromatography.

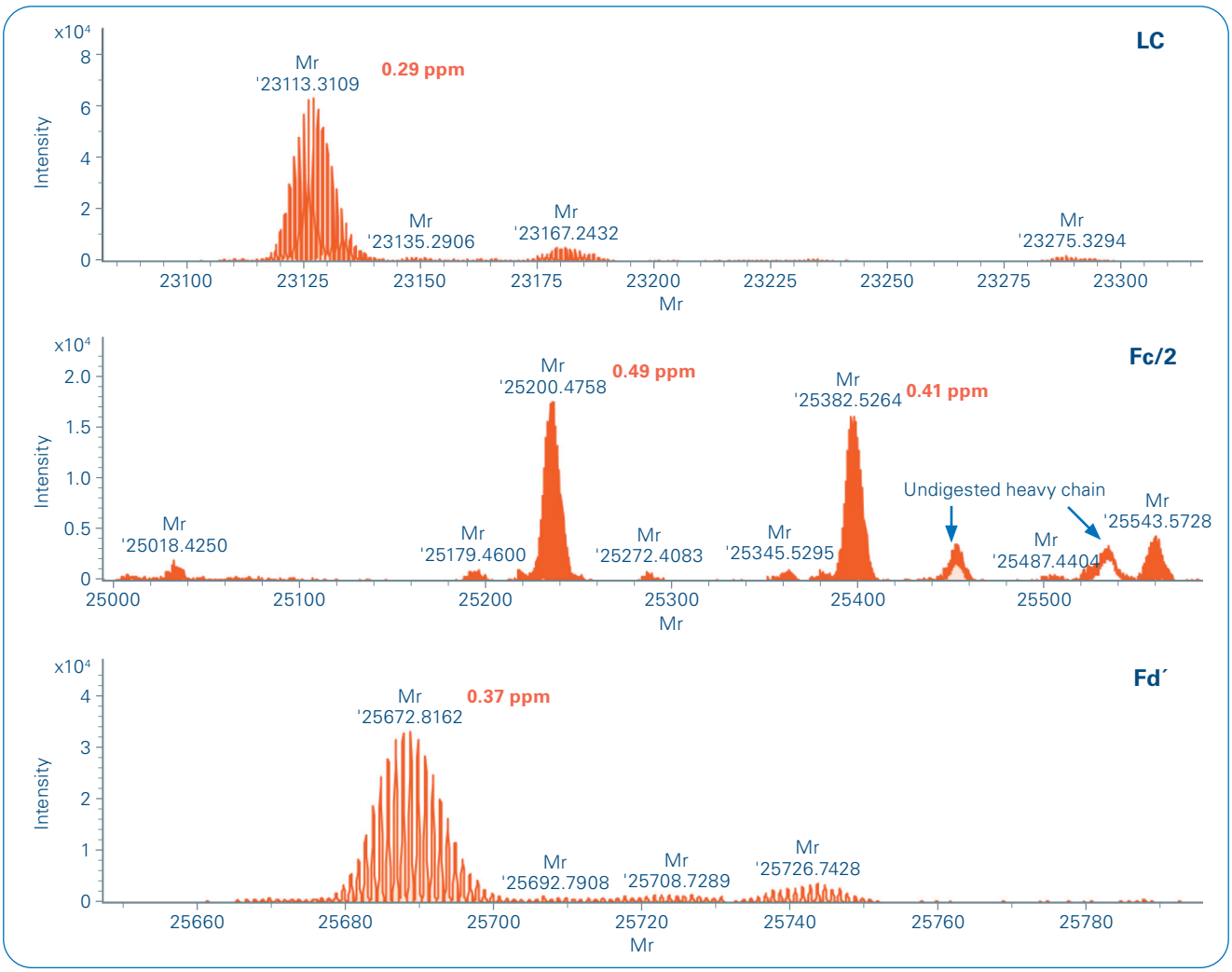

Figure 2: Deconvoluted spectrum of subunit of NISTmAb (top: Light chain; middle: Fc/2; bottom: Fd')

Intact NISTmAb was then used to systematically characterize the linearity, limit of quantification, repeatability, and recovery performance of the SampleStream platform. Peak symmetry in the deconvoluted spectrum suggested a low level of adduct formation and effective buffer exchange. As shown in Figure 3, all major glycoforms of NISTmAb were detected at a concentration of 0.5 µg/mL with high mass accuracy. A linear response was observed over the range of 0.5 µg/mL to 10 µg/mL and the LOQ was determined to be 5.6 ng for NISTmAb (Figure 4). The low LOQ would allow the potential for utilizing the SampleStream system coupled with timsTOF for biotransformation studies where the sample concentration is usually low. The repeatability and robustness of the SampleStream platform were assessed by evaluating the consistency of the peak intensity of the most abundant glycoform over 6 injections of the NISTmAb with

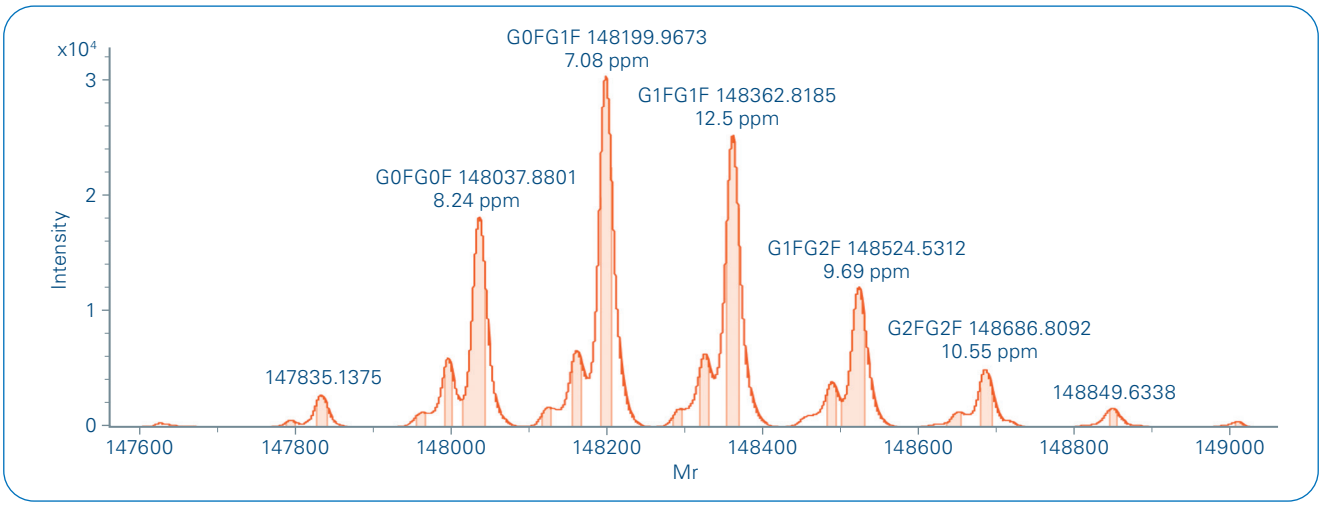

Figure 3: Deconvoluted spectrum of intact NISTmAb

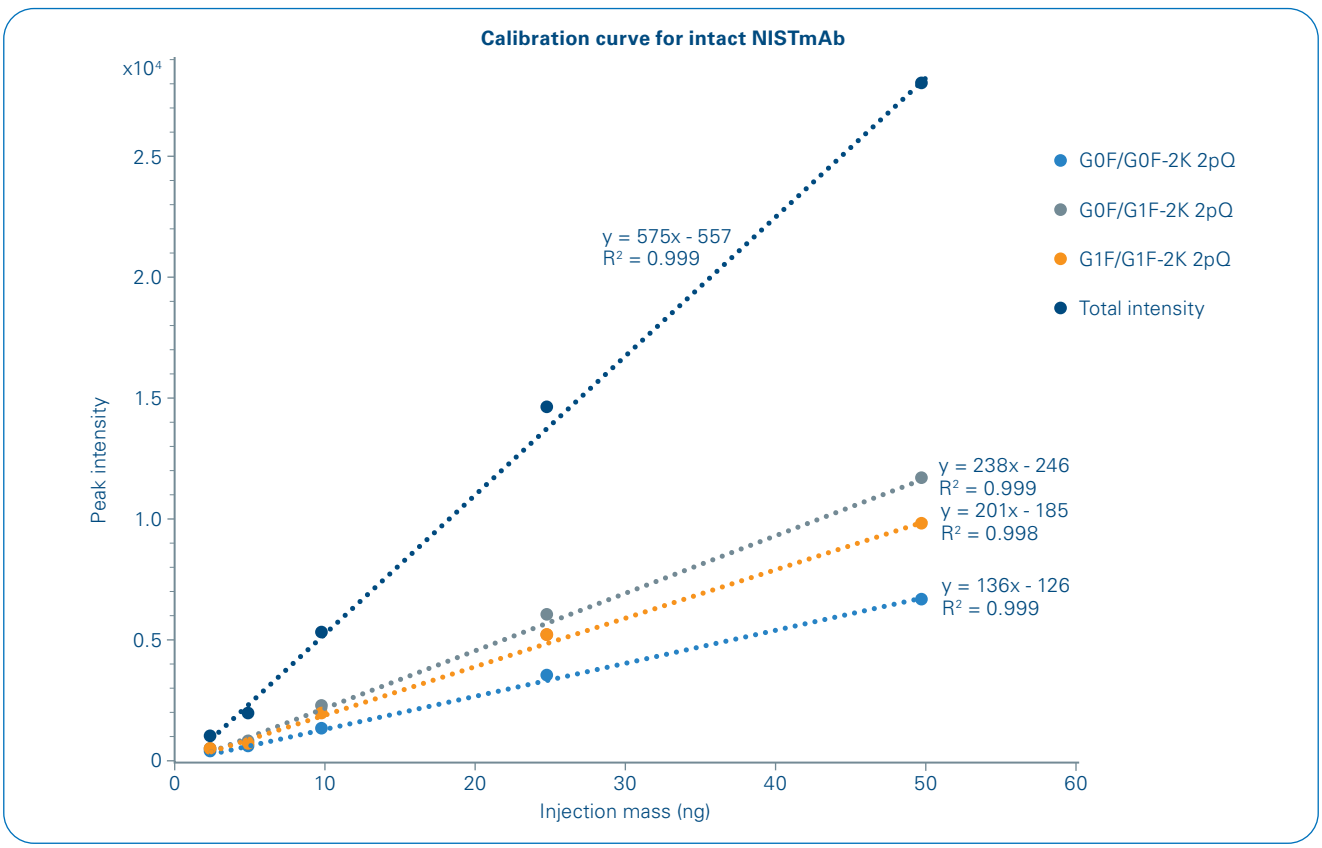

Figure 4: Calibration curve for intact NISTmAb

varying the focusing volume and elution flow rate. The average peak intensity, standard deviation and %RSD are summarized in Table 1. Small standard deviation demonstrates the high reproducibility of the SampleStream platform. Varying the focusing volume from 250 µL to 500 µL and the elution flow rate from 50 µL to 100 µL has no significant impact for NISTmAb quantification. The carryover effect was studied by injecting two blanks following the standard injection of 250 µg of NISTmAb. No carryover from the previous run was observed which demonstrated the unique advantage of SampleStream, which has no stationary phase. Carryover from HPLC columns can require extensive washing which hinders the throughput. The SampleStream platform method performed well across all characteristics evaluated here, which indicated that this approach could be applied for routine, high-throughput analysis of biotherapeutic antibodies.

Table 1: Summary for condition of repeatability and robustness test for SampleStream

	Focusing volume	Elution flow rate	Peak intensity
1	250	50	1.172E4
2	250	50	1.141E4
3	250	50	1.156E4
4	250	75	1.241E4
5	250	100	1.141E4
6	500	50	1.218E4
Avg. intensity	1.178E4		
Std. Dev.	0.042E4		
%RSD	3.57%		

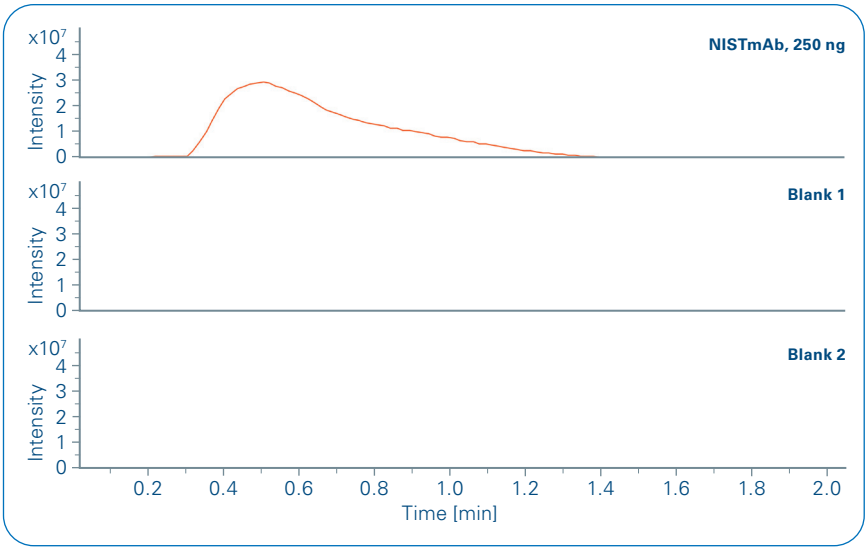

Figure 5: EIC for 250 ng NISTmAb injection and two following blank injections

Conclusion

- Automated high-throughput buffer exchange and MS analysis of the intact and subunit NISTmAb samples was achieved in less than 2 min on the SampleStream platform. Coupling with Bruker timsTOF Pro 2, a low level of adducts was observed for both intact and subunit NISTmAb demonstrating the potential of the method for identity testing.
- A linear quantitative performance with LOQ of 5.6 ng was observed for intact NISTmAb analysis using SampleStream. High reproducibility and robustness and low carryover was demonstrated for the system.

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