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

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ABSTRACT

Purpose: Develop a native intact mass analysis method for rapid therapeutic protein sample screening with high sensitivity by using the new developed online buffer exchange (OBE) column coupled to native mass spectrometry (nMS) to support the new protein drug development and process optimization.

Methods: A Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer coupled with a Thermo Scientific™ Vanquish™ Horizon UHPLC system was used for the method development. A NIST mAb sample was used as model therapeutic protein for the evaluation of the developed method. The Thermo Scientific™ NativePac OBE-1 Column was used for separating the NIST mAb and the salts. The intact mass of the on-line desalted native NIST mAb was directly detected by the Orbitrap Ascend Tribrid mass spectrometer. The MS data were analyzed using the Thermo Scientific™ BioPharma Finder™ 5.1 Software and beta version of OptiMSe software.

Results: The developed method allowed accurate intact mass measurement and relative quantification of glycoforms of the NIST mAb with high sensitivity and throughput.

INTRODUCTION

Native intact mass analysis has been routinely used to characterize the glycosylation and microheterogeneity of therapeutic proteins due to its ability to retain non-covalent interactions. A new developed online buffer exchange column allows rapid separation of proteins and non-volatile small molecules at a micro flow rate and can be used for direct screening of therapeutic proteins with increased throughput and sensitivity.[1] Here we used the online buffer exchange column for native intact NIST mAb analysis. The analytical time was less than 5 min. The sensitivity was improved significantly by using the micro flow rate (65µL/min) for separation and the Orbitrap Ascend Tribrid mass spectrometer for the high-resolution accurate intact mass detection. Down to 5 ng NIST mAb was measured with great mass accuracy.

MATERIALS AND METHODS

Sample Preparation

The NIST mAb was diluted with water at different concentration ranges (0.005µg/µl, 0.01µg/µl, 0.02µg/µl, 0.05µg/µl, 0.1µg/µl, 1 µg/µl).

HPLC conditions

A NativePac OBE-1 SEC column was used for the separation of the diluted NIST mAb samples with 50 mM ammonium acetate at a flow rate of 65 µL/min. The column temperature was 30 ºC (Table 1). Per each HPLC-MS run, 1 µl of mAb sample was injected.

Mass spectrometry

The Orbitrap Ascend Tribrid mass spectrometer equipped with HMRn+ option was used for native intact MS data collection. The MS parameters were settled based on the native intact mass method template with slight modifications (table 2).

Data Analysis

BioPharma Finder 5.1 software was used for the intact protein spectra deconvolution. OptiMSe software was developed for aotumated data analysis for high-throughput screening.

Table 1. HPLC settings

Mobile phase A	50 mM ammonium acetate	
Flow rate	65 µL/min	
Column temperature	30 ºC	
Gradient	Isocratic	
	Time (min)	%A
	0.0	100
	4.5	100

Table 2. ESI and MS settings

ESI Source Settings	
Sheath gas (a.u.)	25
Aux gas (a.u.)	7
Sweep gas (a.u.)	0
Spray voltage (+V)	3500
Capillary temp. (°C)	275
Vaporizer temp. (°C)	175
MS conditions	**Native Intact**
Method type	Full MS
Scan range (m/z)	4,000-12,000
Application mode	Intact
Pressure mode	High
Resolution	15000 at m/z 200
RF lens (%)	150
AGC target value	250
Max inject time (MS)	200
Microscans	10
Source fragmentation (V)	250
Sorce CID compensation scaling	0.02

Results

Online buffer exchange efficiency of the NativePac OBE-1 SEC column

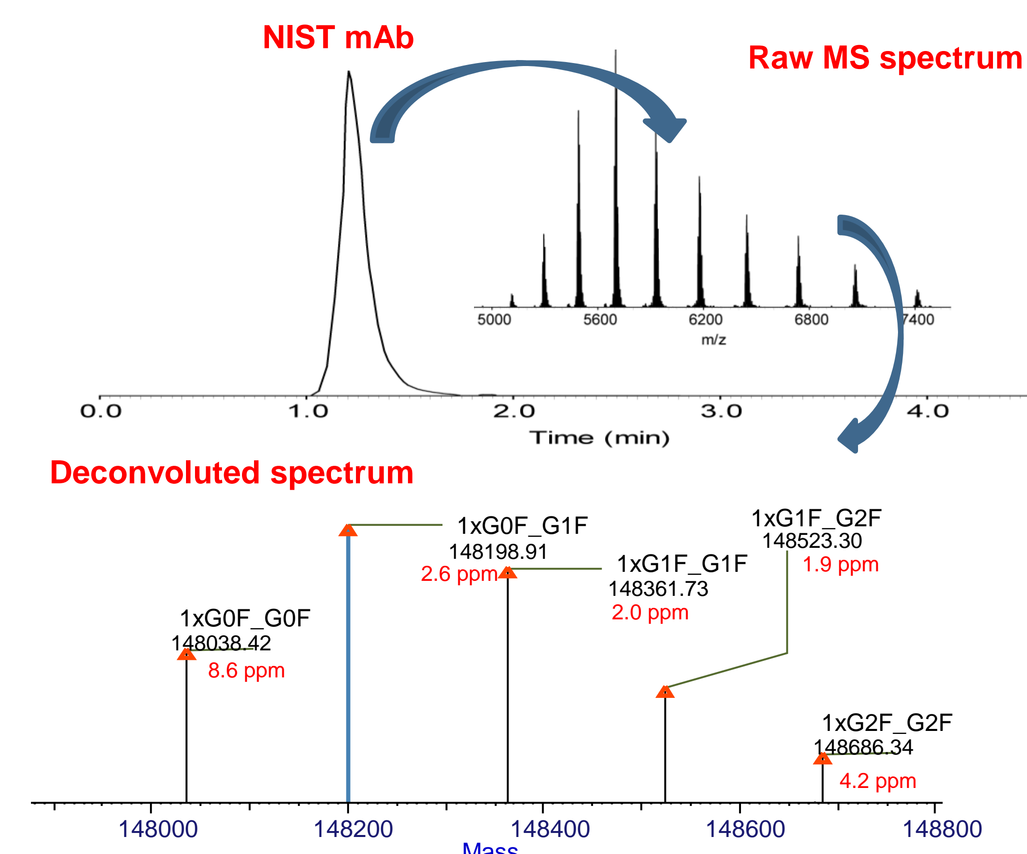
Native mass spectrometry (nMS) has emerged as a widely used technique for the characterization of intact proteins and noncovalent protein complexes. Online coupling of size exclusion chromatography (SEC) to nMS has been widely used for online desalting and additional dimension of separation for complex protein mixtures. Flow rate, sample volume, column length, and particle pore size are main factors in chromatographic resolution of SEC. Typically, SEC columns designed for protein separations have used large particle pore sizes and large column length to get better separation of protein mixture, yielding high flow rate and relatively long HPLC-MR running time for the SEC-native intact mass analysis. To increase the online buffer exchange throughput and sensitivity for Native-MS analysis of biomacromolecules, the new NativePac OBE-1 SEC column (2.1 mm x 50 mm, 80 Å, 3 µm) was developed. The small pores of the SEC stationary phase maximize separation of the non-volatile components from the sample of interest while the short column length minimizes analysis times.[1] Plus, the narrow column ID enables the use of low flow rates for high sensitivity detection and maximizes up time by minimizing buffer consumption. Figure 1 top shows a representative total ion chromatogram of the NIST mAb sample by using the OBE for separation. The buffer-exchanged NISTmAb was not retained on column and eluted quickly around 1 min, while the non-volatile components were eluted after the protein. Excellent quality of the intact mass data (**Figure 1** insert) was observed from the buffer exchanged NIST mAb, enabling great mass accuracy for the deconvoluted NIST mAb glycoforms (**Figure 1** bottom).

REFERENCES

1. Thermo Scientific Technical note #001259. Weijing Liu, et al., (2022) Solutions for high-throughput analysis of large biomolecules by native mass spectrometry.

Figure 1 top shows a total ion chromatogram of the NIST mAb by using OBE for separation. The buffer-exchanged NIST mAb was not retained on column and eluted quickly around 1 min, while the non-volatile components were eluted after the protein. Excellent quality of the intact mass data (**Figure 1** inset) was observed from the buffer exchanged NIST mAb, enabling great mass accuracy for the deconvoluted NIST mAb glycoforms (Figure 1 bottom).

Figure 1.Total ion chromatogram of NIST mAb using the OBE column at 65 µL/min. The right insert shows the raw intact mass spectrum of the NIST mAb, and the bottom shows the deconvoluted results.



Improved throughput and sensitivity for native NIST mAb analysis using NativePac OBE-1 SEC column

The OBE column separation enables higher throughput offered by the short analysis time and better sensitivity offered by the low flow rate for the native intact NIST mAb mass analysis. As proof of concept, we performed native intact mass analysis of a low concentration (50 ng on column) NIST mAb sample using the new on-line buffer exchange column with 50 mM ammonium acetate at the flow rate of 65 µL/min and a regular MabPac™ SEC-1 column with 50 mM ammonium acetate at the flow rate of 250 µL/min for sample separation, respectively. **Figure 2** shows the comparison of the elution profiles from two different SEC column separation runs. The 50 ng NIST mAb was eluted much quicker and detected with much higher signal to noise ratios with the on-line buffer exchange approach.

Figure 2. Comparison of elution profiles, 50 ng of NIST mAb on column

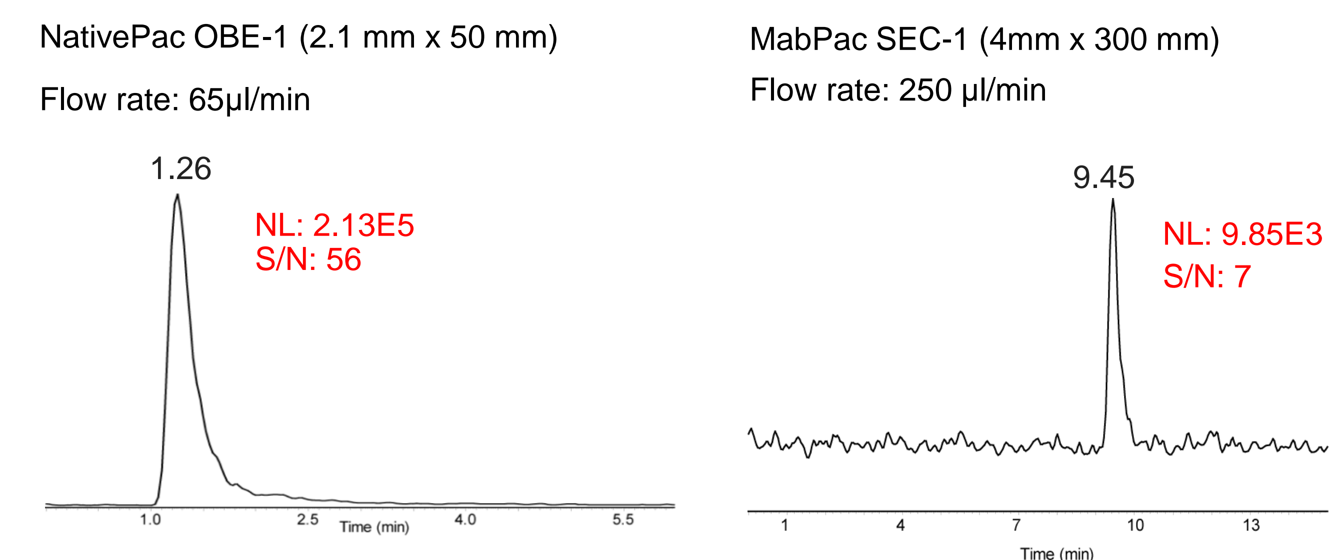
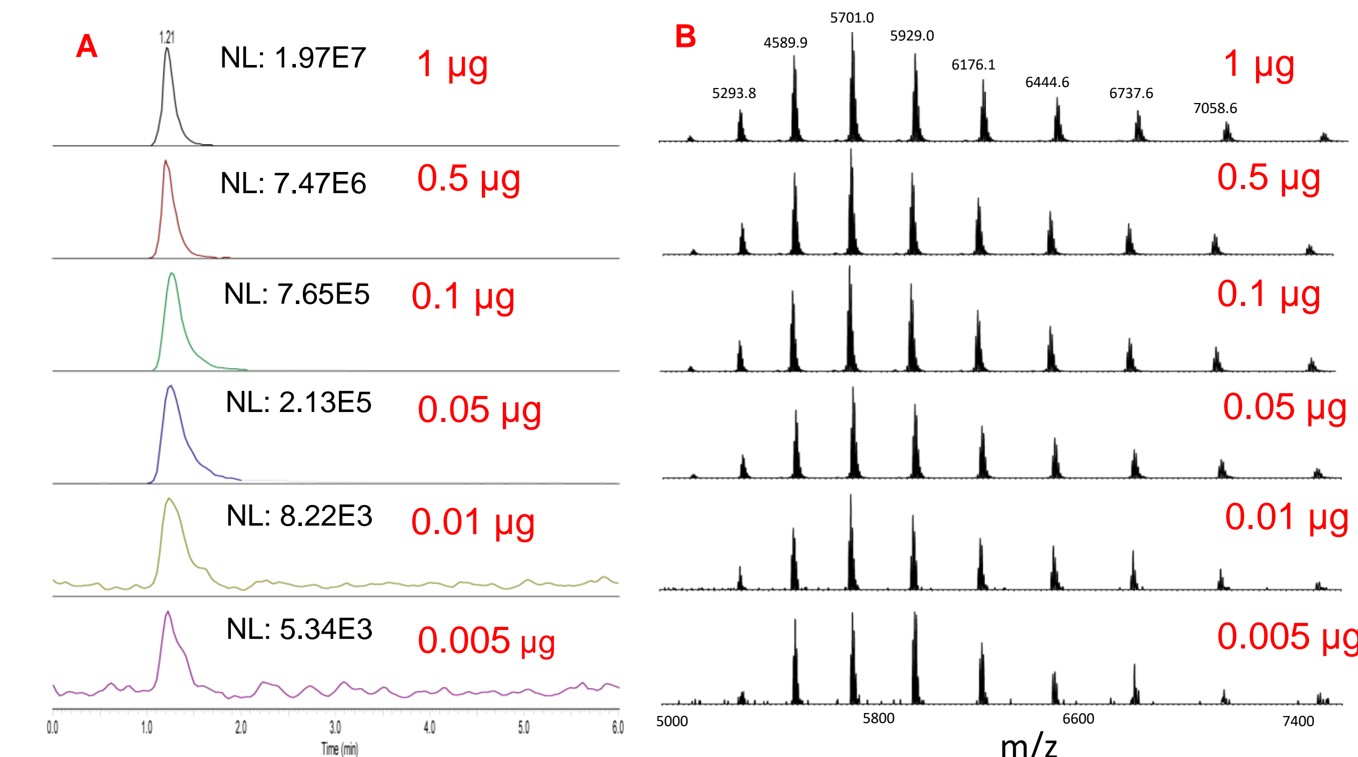


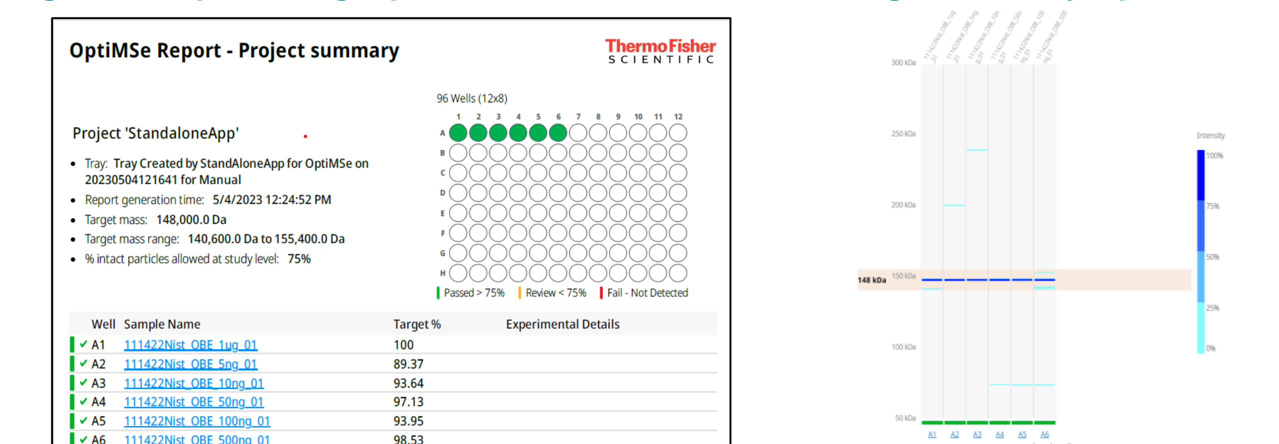
Figure 3 shows the selected ion chromatograms (m/z 5680-5740, Charge 26) (A) and the native intact mass spectrum (B) observed from the NIST mAB dilution series. Down to 5 ng NIST mAb on column was detected clearly with great quality of intact mass data.

Figure 3. TIC and RAW spectra of a dilution series of NIST mAb using OBE-nMS



The dilution series files (**Figure 3**) were processed using the OptiMSe software. The NIST mAb target was quickly identified from all the samples (**Figure 4**).

Figure 4. Report and gel-plot of NIST mAb dilution series generated by OptiMSe



CONCLUSIONS

- A high throughput, high sensitive native intact mass method was developed by coupling the on-line buffer exchange column to the Orbitrap Ascend Tribrid mass spectrometer.
- Each therapeutic protein sample could be analyzed in 4.5 min.
- Significantly sensitivity improvement for the native intact mass analysis (down to 5 ng on column for the NIST mAb) was observed with the developed method.
- High throughput data processing was enabled by using the OptiMSe software.

TRADEMARKS/LICENSING

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