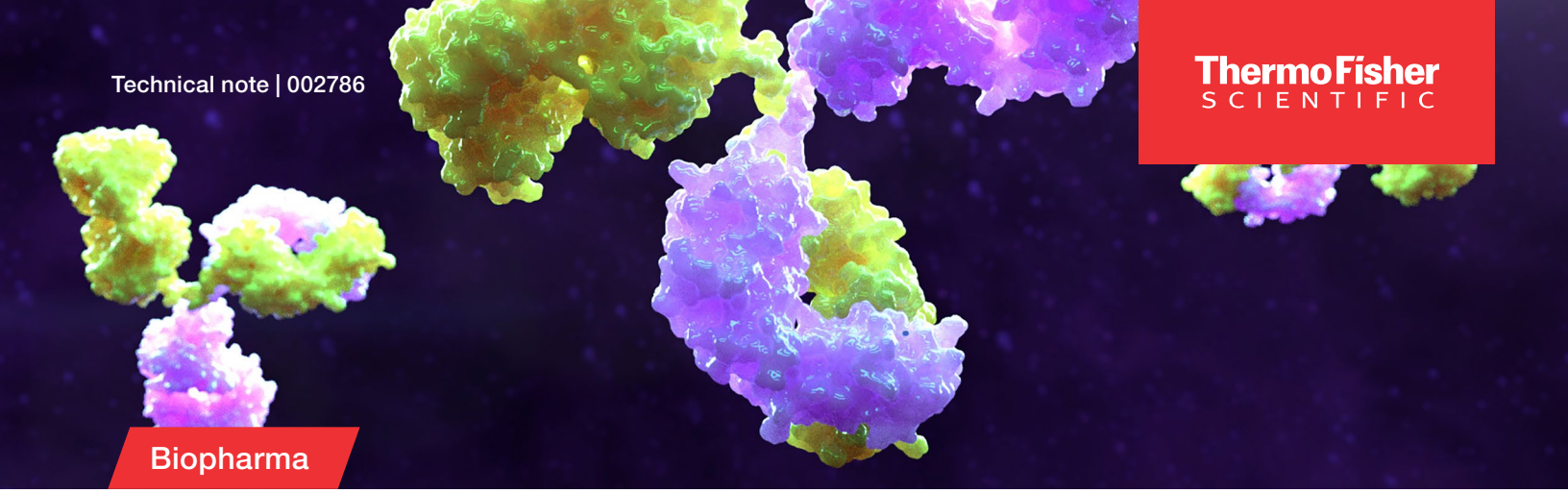

Technical note | 002786

Biopharma

Increased sensitivity and throughput for native intact mass analysis using a NativePac OBE-1 SEC column and an Orbitrap Ascend Tribrid mass spectrometer

Authors

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Keywords

Monoclonal antibodies (mAb), native mass spectrometry, Orbitrap Ascend Tribrid mass spectrometer, online buffer exchange column (OBE column), BioPharma Finder 5.2 software, OptiMSe 1.0 software

Application benefits

- Rapid native intact mass analysis of monoclonal antibodies at a rate of 4.5 min per sample.
- Minimized sample preparation using an online buffer exchange (OBE) column to desalt and buffer exchange the sample.
- Predefined system templates in the method editor on the Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer ensure operational simplicity with method setup.
- High sensitivity and spectral quality are achieved for confident native intact mass analysis with low sample loads (down to 5 ng mAb on column).
- Automated data acquisition, analysis, and result reporting are accomplished in real time with the newly developed Thermo Scientific™ OptiMSe™ software.

Goal

Implementation of a newly commercialized online buffer exchange (OBE) column with a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to an Orbitrap Ascend Tribrid mass spectrometer for fast, high-throughput native mass analysis of protein therapeutics including mAbs.

Introduction

Native intact mass analysis has been used routinely to characterize the glycosylation and microheterogeneity of therapeutic proteins due to its ability to retain non-covalent interactions. Online coupling of size exclusion chromatography (SEC) to native MS is widely used for online desalting and as an additional dimension of separation for complex protein mixtures. Typically, SEC columns designed for protein separations use large particle pore sizes and long column lengths to get better separation of the

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protein mixture, yielding high flow rates and relatively long LC-MS run times. However, shorter LC-MS run times, more sensitive MS detection, and automated data processing are desired for high-throughput native intact mass analysis to better support bioprocess development and optimization, such as cell line and culture media development, with less sample consumption and quicker turnaround time. OBE allows rapid separation of proteins and non-volatile small molecules at microflow rates and can be used for direct screening of therapeutic proteins with increased throughput and sensitivity.[1] The newly developed OptiMSe software can be further used for automated data acquisition and data processing. As a proof of concept, we used the Thermo Scientific™ NativePac OBE column and the OptiMSe software for native intact mass analysis of NISTmAb. Compared to using conventional flow, such as 250 µL/min, the sensitivity was improved significantly by using microflow (65 µL/min) for the separation and the Orbitrap Ascend Tribrid mass spectrometer for high-resolution accurate intact mass detection. Down to 5 ng NISTmAb was detected with good spectral quality in less than 5 minutes of run time. Automated deconvolution and reporting for the NIST mAb native intact mass analysis experiments were achieved using the OptiMSe 1.0 software.

Experimental

Sample preparation

The commercially available NISTmAb (RM 8671) was diluted in water serially to achieve the following concentrations: 5 ng/µL, 10 ng/µL, 50 ng/µL, 100 ng/µL, 1,000 ng/µL.

Chromatography

The Thermo Scientific™ Vanquish™ Horizon UHPLC system consisting of the following modules was used for the protein separation.

- System Base Vanquish Horizon/Flex (P/N VF-S01-A-02)
- Vanquish Binary Pump H (P/N VH-P10-A-02)
- Vanquish Split Sampler HT (P/N VH-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-03)

A Thermo Scientific™ NativePac OBE-1 SEC column (P/N 43803-052130, 2.1 x 50 mm, 80 Å, 3 µm) was used for the separation of the diluted NISTmAb samples with 50 mM ammonium acetate (99.999% trace metals basis) in water at a flow rate of 65 µL/min. The column temperature was 30 °C (Table 1). A regular Thermo Scientific™ MAbPac™ SEC-1 column (P/N 074696, 4 × 300 mm, 300 Å, 5 µm) was also used for the separation of a diluted NISTmAb sample (50 ng/µL) with 50 mM ammonium acetate (99.999% trace metals basis) in water at a flow rate of 250 µL/min (Table 1). Per each LC-MS run, 1 µL of mAb sample was injected.

Table 1. HPLC condition settings

Parameter	Value	
Mobile phase A	50 mM ammonium acetate	
Flow rate	NativePac OBE-1 column (2.1 × 50 mm, 80 Å, 3 µm) 65 µL/min	
Flow rate	MAbPac SEC-1 column (4 × 300 mm, 300 Å, 5 µm) 250 µL/min	
Column temperature	30 °C	
Gradient	Isocratic Time (min) 0.0 4.5	 %A 100 100

Mass spectrometry

The Orbitrap Ascend Tribrid mass spectrometer equipped with the HMR^n+ option was used for the native intact mass data collection. The settings of the ESI and MS parameters are shown in Table 2.

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)	3,500
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	15,000 at *m/z* 200
RF lens (%)	150
AGC target value	250
Max inject time (MS)	200
Microscans	10
Source fragmentation (V)	250
Source CID compensation scaling	0.02

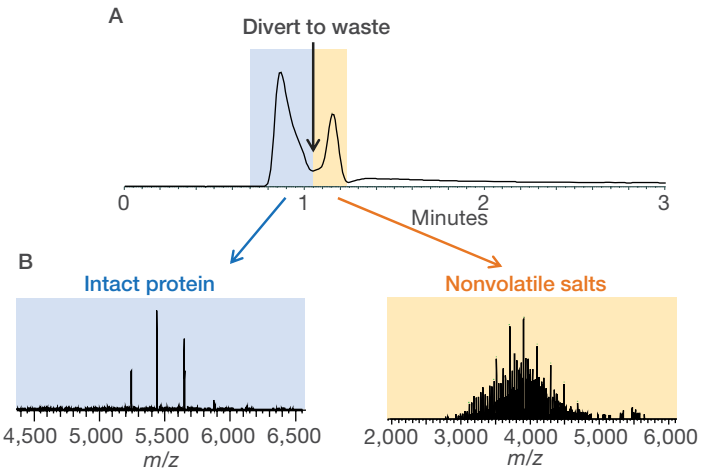
Data processing

Off-line data analysis was performed using the ReSpect™ algorithm in Thermo Scientific™ BioPharma Finder™ 5.2 software. OptiMSe 1.0 software was used for the automated data analysis workflow, which applies novel in-house developed algorithms for larger isotopically unresolved and smaller isotopically resolved protein species. The algorithm and parameters are selected automatically based on user-defined protein target mass, and run data is processed concurrently on existing instrument hardware so that results are ready shortly after the last run is completed.[1]

Results and discussion

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 is widely used for online desalting and as an additional dimension of separation for complex protein mixtures. Flow rate, sample volume, column length, and particle pore size are the main factors in chromatographic resolution of SEC. A newly designed SEC column (NativePac OBE-1, 2.1 × 50 mm, 80 Å, 3 µm) utilizes smaller pores in the SEC stationary phase to maximize separation of the non-volatile components from the sample of interest, short column length to minimize analysis time, and narrow column ID to enable the use of low flow rates for high sensitivity detection. During analysis on a NativePac OBE-1 column, proteins undergo buffer-exchange and will be eluted prior to the total column void volume where small-sized contaminants from the sample solvent will be eluted and can be diverted to waste, in order to reduce MS instrument contamination. A typical LC chromatogram (A) and raw spectra (B) from the OBE method is shown in Figure 1.



*Adapted from Technical Note 001259, Liu et. al.[1]

Figure 1. A typical chromatogram and raw spectra from the OBE method*. (A) Total ion chromatogram; (B) averaged spectra across the RT range highlighted in panel A.

The representative OBE-native NISTmAb (1 µg/µL) intact mass analysis result is shown in Figure 2. The NISTmAb sample was loaded directly onto the column without using the divert valve as it does not contain high concentrations of salt or other nonvolatile additives. The top section (A) shows the representative total ion chromatogram, and the bottom section (B) shows the corresponding deconvoluted result of the NISTmAb sample: (1 µg on column) using the ReSpect algorithm in BioPharma Finder 5.2 software. The buffer-exchanged NISTmAb was eluted quickly around 1 min, while the non-volatile components were eluted after the protein. Excellent quality of the intact mass data (Figure 2A inset) was observed from the online buffer exchanged NISTmAb, enabling great mass accuracy for the deconvoluted NISTmAb glycoforms (Figure 2B).

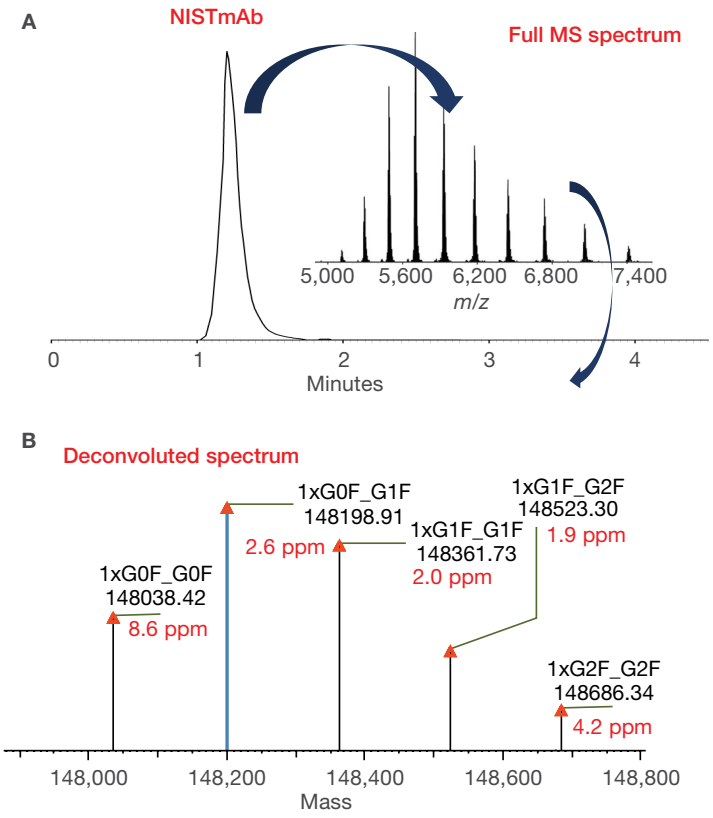


Figure 2. (A) The total ion chromatogram of NISTmAb nMS analysis using the OBE column at the flow rate of 65 µL/min with the averaged Full MS spectrum of the intact NISTmAb shown in the inset; (B) the deconvoluted spectrum shows mass accuracies below 10 ppm for all major glycoforms.

Improved throughput and sensitivity for native NISTmAb analysis using a NativePac OBE-1 SEC column

The OBE column separation allows higher throughput enabled by the short analysis time and better sensitivity permitted by the low flow rate for the native intact NISTmAb mass analysis. As a proof of concept, we performed native intact mass analysis of a low amount (50 ng on column) NISTmAb sample using the OBE column with 50 mM ammonium acetate at the flow rate of 65 µL/min and a regular MAbPac SEC-1 column (4 × 300 mm, 300 Å, 5 µm) with 50 mM ammonium acetate at the flow rate of 250 µL/min for sample separation. Figure 3 shows the comparison of the elution profiles using two different SEC columns for the NISTmAb sample separation. The 50 ng NISTmAb were eluted in less time and detected with a much higher signal-to-noise ratio using the OBE column.

The improved sensitivity allows high quality MS analysis of mAbs using lower sample amounts. Figure 4 shows the representative analysis results for the NISTmAb dilution series. High spectral quality of native MS data was observed across the injection range, even at the lowest 5 ng load.

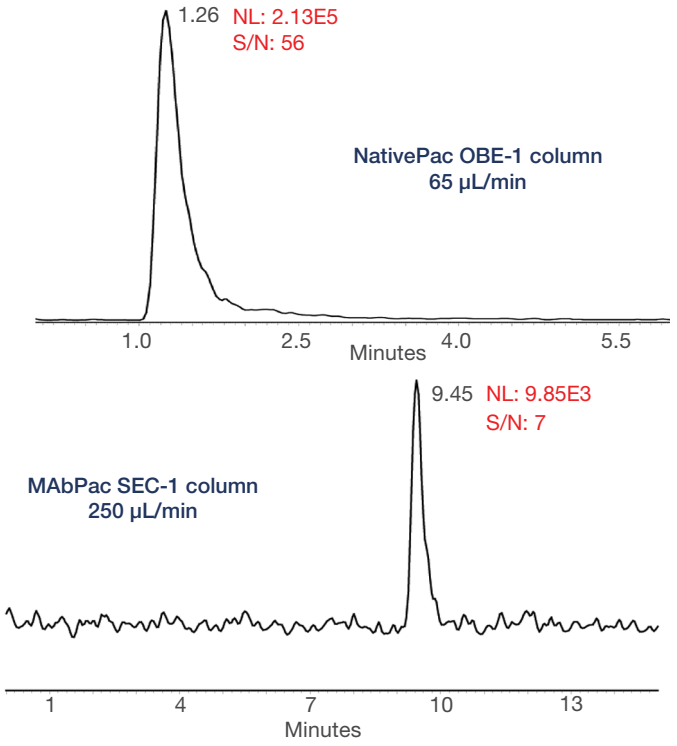


Figure 3. Comparison of elution profiles, 50 ng of NISTmAb on column

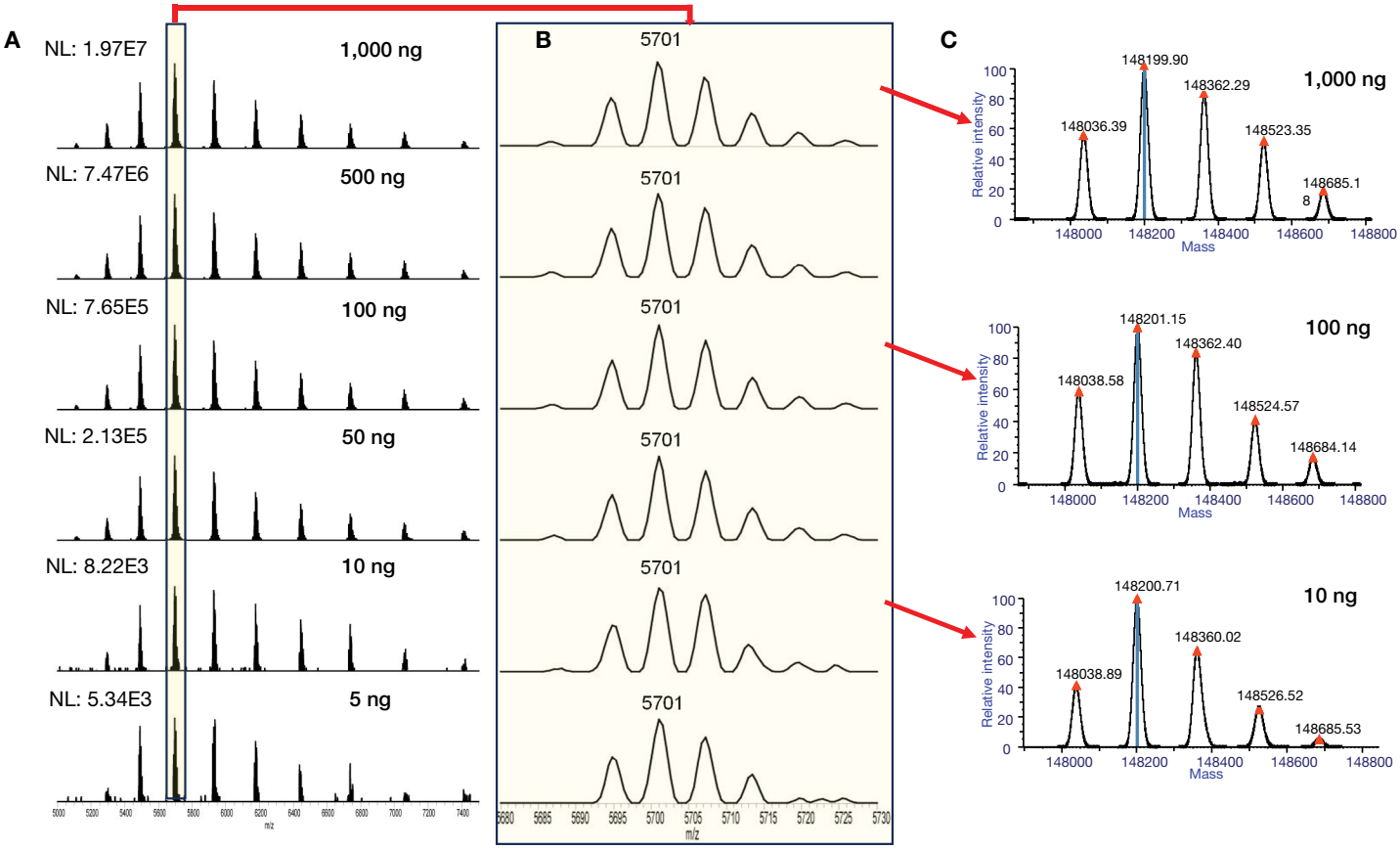


Figure 4. (A) Raw MS spectra and representative deconvolution results of the NISTmAb sample dilution series (5 ng – 1,000 ng on column); (B) zoom into the 26+ charge state detected between *m/z* 5680 and *m/z* 5740; (C) representative results obtained upon deconvolution of the mass spectra from 10, 100, and 1,000 ng NISTmAb injected on column

Automated data analysis using OptiMSe 1.0 software

In the previous section, we demonstrated that therapeutic proteins such as NISTmAb can be characterized with high throughput and high sensitivity by using the OBE column and the Orbitrap Ascend Tribrid mass spectrometer. To fully take advantage of the less than 5 min LC-MS run time for screening large batches of therapeutic protein samples, it is desirable to process each raw file and generate the report on the fly. The OptiMSe 1.0 software [1,3] is an optional software that can be controlled by Thermo Scientific™ Xcalibur™ software and can be used to address this data processing throughput challenge.[1]

OptiMSe 1.0 software includes automated data analysis and simplified reporting. It uses the post-acquisition program feature in Xcalibur software to trigger data analysis as data are acquired and applies novel in-house developed deconvolution algorithms for both larger isotopically unresolved and smaller isotopically resolved protein species. The algorithm and parameters are automatically selected based on the user-defined protein target mass. Data analysis is performed in real time upon completion of the data acquisition for a sample in parallel to the data acquisition for the next sample in the sequence. After data acquisition and data processing for the last sample in the injection sequence has completed, the report generation is triggered and an easy-to-interpret result is generated and stored on the local PC in PDF format.

The automated workflow including data acquisition, data processing, and reporting is shown in Figure 5. To showcase OptiMSe 1.0 software, the NISTmAb dilution series consisting of six samples was applied to the workflow, resulting in the project summary presented in Figure 6. The NISTmAb target was identified from all the samples, and no aggregation/dissociation was detected.

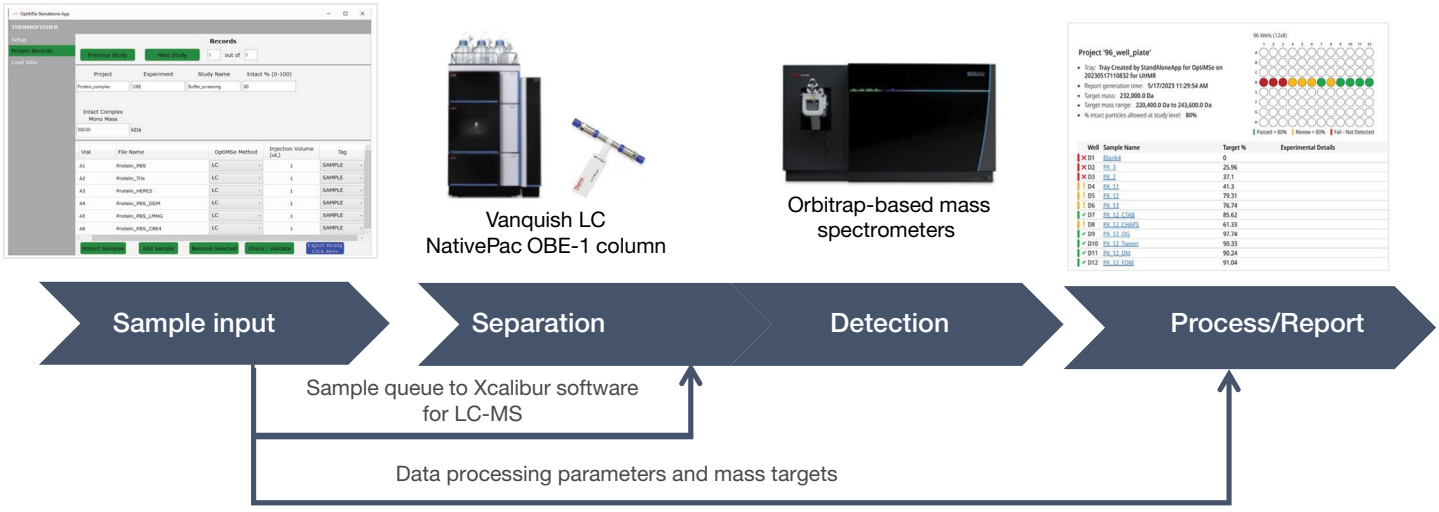


Figure 5. Automated protein screening workflow using OptiMSe software[1]

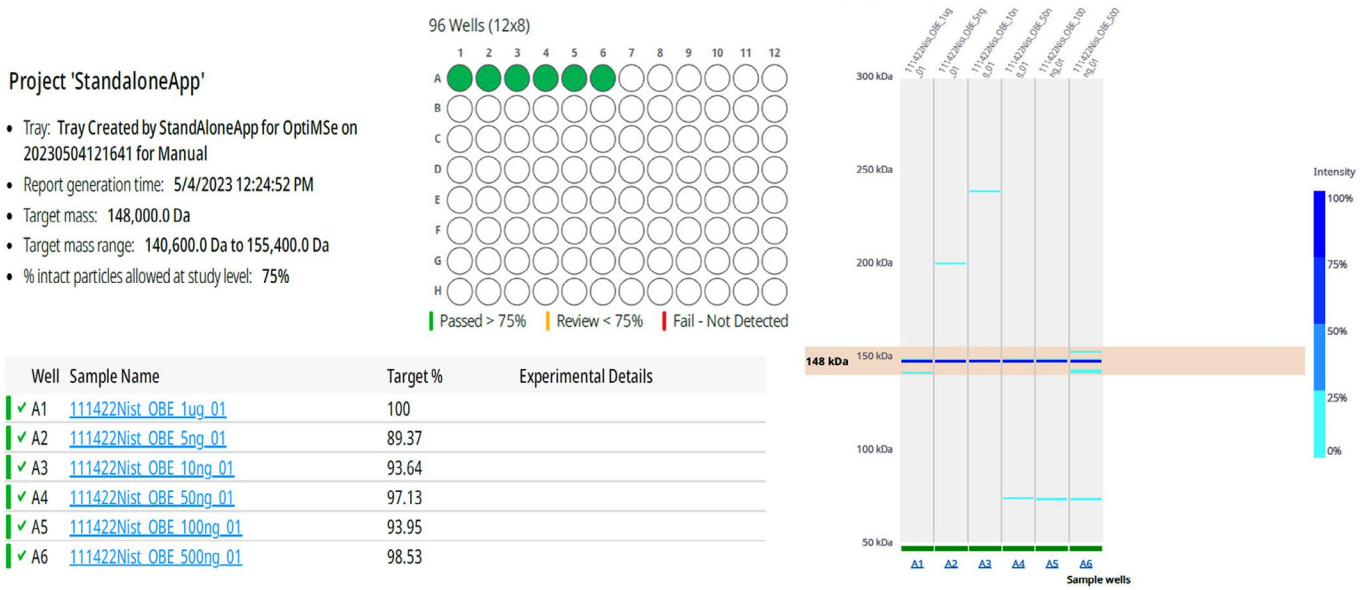


Figure 6. Project summary of the NISTmAb dilution series samples by the OptiMSe software

Conclusions

In summary, increased throughput and sensitivity for the native intact mAb mass analysis were achieved using the OBE-native LC/MS analysis on the Orbitrap Tribrid Ascend MS instrument.

- Each NISTmAb sample could be analyzed in less than 5 min.
- Significant sensitivity improvement for the native intact mass analysis data was achieved by using a short length SEC column with smaller pores and a highly sensitive Orbitrap detector.
- Automated data processing and reporting was enabled by using the OptiMSe 1.0 software.

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