Coupling Ion Exchange Chromatography with Native Mass Spectrometry for Charge Heterogeneity Characterization of Monoclonal Antibodies using new generation SCX column

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ABSTRACT
Purpose: To demonstrate online SCX-MS charge variant analysis of durvalumab using a MS compatible salt buffer system with a 2 × 50 mm, 2 μm monopropionate SCX column.

RESULTS
Durvalumab is a human immunoglobulin G1 lambda (IgG1L) monoclonal antibody that blocks the interaction of programmed cell death ligand 1 (PD-L1) with the PD-1. In this study nine peaks were successfully separated, including five acidic peaks, two basic peaks and main peak for subsequent online intact protein analysis by mass spectrometry. The UV profile is shown in Figure 1A and the acidic–basic chromatography (3–acidic peaks and 2 basic peaks) were separated from the main peak (Figure 1B).

Figure 1. SCX – UV and MS profile of durvalumab.

Thermal stressing of samples typically results in an increase in the number of acidic variants such as deamidation, the relative abundance of acidic peaks also increased. The peak area of basic peak1 increased because of lost GK, which was induced by thermal stress. It is not explained that new peaks appeared in thermal stressed samples, especially samples stressed for 6 days and 7 days. One new acidic peak was observed between acidic peak1 and main peak, results of deamination and oxidation. Three new basic peaks appeared because of oxidation. Main modifications in new peaks are displayed in Table5.

CONCLUSIONS
The ProPac® 3R SCX column provides excellent separation of these variant peaks and high resolution MS data gives more information in intact protein molecular weight and modifications. Figure 3 displays MS spectra and deconvolution result of non-stressed durvalumab. A, MS spectra and B, deconvolution result. Lysine on both heavy chain C-terminal are truncated.

System reproducibility evaluation
For consistent performance, two columns from different lots were used. Non-stressed durvalumab was loaded on 3 days. 3 injections for both columns, using same instrument method. Figure 4A displayed UV signal profile of 4 injections from two columns, four different days. Signal auto was offset for better display. Figure4B is comparison of peaks %area measured by two different columns, each bar was averaged from 3 injections across 3 days. Table 6 is average %area across 10 injections, from two columns. It proves that the ProPac® 3R SCX column can provide excellent column-to-column and lot-to-lot consistency using MS compatible salt solvent buffers.

Figure 4. System reproducibility test using durvalumab.

A. Overall UV profile of injections from two columns, four different days. B. Comparison of peak %area measured by two different columns, each bar was averaged from 3 injections across 3 days.

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MATERIALS AND METHODS
Sample preparation: mAb: Commercially available durvalumab is diluted to 1mg/mL using ddH2O. Thermal stressed samples were treated at 55°C for 1, 2, 3, 4, 5 and 7days, respectively.

HPHPLC Separation:
Two different lots ProPac® 3R SCX, 2 × 50 mm, 3 μm (HP4130-03205b) columns are used for separation. HPLC settings are listed in Table 1.

Mass Spectrometry:
A Thermo Scientific™ Q Exactive High-Resolution Orbitrap mass spectrometer was used for separation. UHPLC settings are listed in Table 1. Two different lots ProPac® 3R SCX, 3μm columns provide excellent separation of mAb samples.

Sample preparation:
Using a MS compatible salt buffer system with a 2 × 50 mm, 2 μm monopropionate SCX column.

Data Analysis
Thermo Fisher Scientific™ Xcalibur™ software.

Table 1. UPLC-MS parameters

Table 2. MS parameters

Table 3. Major components from each peaks in non-stressed sample.

Table 4. Mass accuracy of glycoforms 28:0 24:0, 28:0 23:0 peak1 is deamidation, the relative abundance of acidic peaks also increased. The peak area of basic peak 1 increased because of lost GK, which was induced by thermal stress. It is not explained that new peaks appeared in thermal stressed samples, especially samples stressed for 6 days and 7 days. One new acidic peak was observed between acidic peak 1 and main peak, results of deamination and oxidation. Three new basic peaks appeared because of oxidation. Main modifications in new peaks are displayed in Table 5.

Figure 2. SCX – MS profile of thermal stressed durvalumab.

A. SCX – MS profile of durvalumab on day 0. B. Zooming in areas. C, %area of peaks in all samples. New peaks were detected in stressed samples. D, %area measured by two different columns, each bar was averaged from 3 injections across 3 days.