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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, 3 µm monodisperse SCX column.

Methods: A Vanquish Flex UHPLC coupled with HRAM Orbitrap mass spectrometer was used for analyzing protein charge variants at intact level.

Results: Successfully separated and measured the molecular weight and relative abundance of durvalumab charge variants.

INTRODUCTION

Charge heterogeneity is considered a critical quality attribute in therapeutic monoclonal antibodies (mAbs) and needs to be thoroughly characterized and monitored throughout the drug development stages. Ion exchange chromatography (IEX) is one of the main techniques used to assess the overall charge heterogeneity of mAbs. Mass spectrometry (MS) based tools have played a critical role in charge variants characterization. Recent advances in both instrumentation and methodology have enabled the online coupling of multiple charge-based separation techniques with direct MS detection.

In this study, we developed and optimized pH gradient based cation exchange chromatography coupled with native MS (CEX-MS) methods and explored its utility in charge heterogeneity characterization of non-stress/thermal stressed durvalumab, using the new Thermo Scientific™ ProPac™ 3R SCX column.

MATERIALS AND METHODS

Sample preparation:
mAb: Commercially available durvalumab is diluted to 1mg/mL using ddH_2O. Thermal stressed samples were treated at 50℃ for 1, 2, 3, 6, and 7days, respectively.

UHPLC Separation:
Two different lots ProPac™ 3R SCX, 2×50 mm, 3 µm (PN43103-052068) columns are used for separation. UHPLC settings are listed in Table 1.

Mass Spectrometry:
A Thermo Scientific™ Q Exactive™ Plus Biopharma mass spectrometer was used for data acquisition. All settings are listed in Table 2.

Data Analysis
Data analysis was performed using Thermo Scientific™ Biopharma Finder™ software.

Table 1. UHPLC parameters

Time(min)	Flow (ml/min)	%B	Time(min)	Flow (ml/min)	%B
0.0	0.3	0.0	30.0	0.3	75.0
1.0	0.3	0.0	33.0	0.3	0.0
3.0	0.3	15.0	40.0	0.3	0.0
28.0	0.3	75.0			
Buffer A - 25mM ammonium bicarbonate and 30 mM acetic acid (pH 5.3) Buffer B - 10 mM ammonium hydroxide (pH 10.18) Column temperature: 30℃ sample load=20µg					

Table 2. MS parameters

Full MS – HMR mode, trapping gas pressure setting=1.0	
Sheath gas: 30Arb	In-source CID: 100eV
Aux gas: 10 Arb	S-lens: 200
Spray voltage: 3.6kV	Microscans: 10
Capillary temp.: 275℃	Resolution: 35,000
Aux gas temp.: 200 ℃	Scan range: 2500-8000

RESULTS

ProPac™ 3R SCX column separation and real-time intact protein analysis of durvalumab charge variants

Durvalumab is a human immunoglobulin G1 kappa (IgG1κ) 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, three basic peaks and main peak for subsequent online intact protein analysis by mass spectrometry. The UV profile is shown in Figure 1A and B. Multiple distinct chromatographic peaks (5 acidic peaks and 3 basic peaks) were separated from the main peak (Figure 1B).

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

A, SCX – UV profile of durvalumab. B, Zooming-in SCX-UV profile to show details of charge variant peaks.

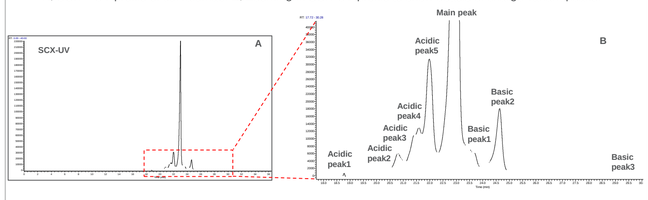


Table 3 shows the major components from each chromatographic peaks in non-stress sample, based on the deconvolution results of the high resolution native intact mass data. Acidic peak1 is partial mAb, mass range between 100~110kDa. Dominant modification from acidic peak2 to acidic peak5 is deamidation, and A2S1G0F/A2S1G1F start to be eluted in acidic peak5. In basic peak1, K truncation at one heavy chain C-terminal and GK truncation at the other heavy chain C-terminal can be observed, with three oxidation. In basic peak2, K truncation and GK truncation at heavy chain C-terminal, and K truncation just at one heavy chain C-terminal can be detected. In basic peak3, two types of variants appears, both heavy chain keep lysine at C-terminal, or both chain lost GK.

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

A1-A5, acidic peak1-5. B1-B3, basic peak1-3. K loss, heavy chain C-terminal lysine truncated. GK loss, heavy chain C-terminal glycine and lysine truncated.

Peak	A1	A2	A3	A4	A5
Modifications	Fragments, 100~110kDa	2*deamidation 2*K loss	2*deamidation 2*K loss	3*deamidation 2*K loss	1*deamidation A2S1G0F/A2S1G1F 2*K loss
Peak	**Main peak**	**B1**	**B2**	**B3**	
Modifications	2*K loss	1*K loss, 1*GK loss, 3*oxidation	1*K loss, 1*GK loss 1*K loss	2*GK loss 0*K loss	

Thermal stress test of durvalumab

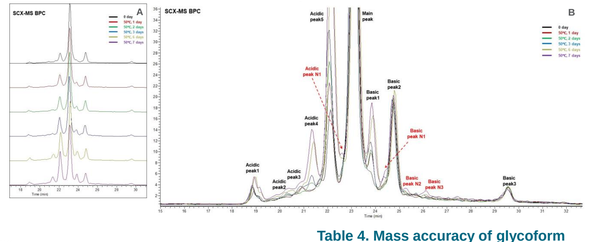
To illustrate the ability of the ProPac 3R SCX column to differentiate samples with irregular variant profiles, we compared the separation of native forms of durvalumab against respective samples that have been stressed at 50℃ for 1/2/3/6/7days. The chromatograms in Figure 2 demonstrate clear differences in the peak profile due to an overall increase in the peak intensity and relative area of the variant peaks for stressed samples (colored traces).

The ProPac 3R SCX column provides excellent separation of these variant peaks and high resolution MS data gives more information on intact protein molecular weight and modifications. Figure 3 displays MS spectra and deconvolution result of main peak in non-stress sample. Table 4 shows the mass accuracy of glycoform 2*A2G0F, 2* K loss detected in all samples are less than 6ppm compared to their theoretical mass.

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 oxidation% grows, which was induced by thermal stress. It is not surprised that new peaks appeared in thermal stressed samples, especially samples stressed for 6 and 7 days. One new acidic peak was observed between acidic peak5 and main peak, results from deamidation and oxidation. Three new basic peaks appeared because of oxidation. Main modifications in new peaks are displayed in table5.

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

A, SCX – MS profile of durvalumab at 50℃ for 0/1/2/3/6/7days. B, Zooming-in spectra, overlaid. Increasing trend of acidic peaks and basic peak1 can be observed. One new acidic peak and three new basic peaks appeared in stressed samples, which are marked as red. C, %area of peaks in all samples. New peaks were not included in because they were not detected in every condition.



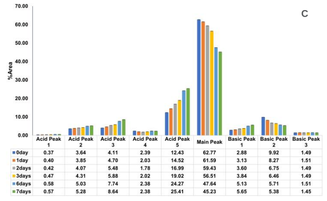


Table 4. Mass accuracy of glycoform 2*A2G0F, 2* K loss from main peak in all samples.

Condition	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)
0day	148954.97	148955.38	-2.75
50℃, 1day	148954.56	148955.38	-5.49
50℃,2days	148954.70	148955.38	-4.54
50℃,3days	148954.78	148955.38	-4.04
50℃,6days	148955.30	148955.38	-0.56
50℃,7days	148955.23	148955.38	-0.98

Figure 3. MS spectra and deconvolution result of non-stressed durvalumab.

A, MS spectra. B, deconvolution result. Lysine on both heavy chain C-terminals are truncated.

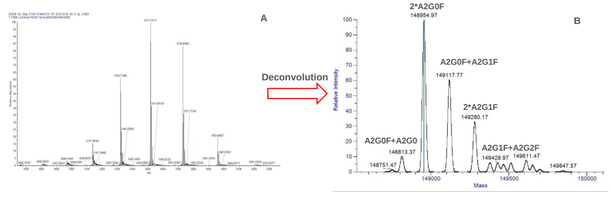


Table 5. Major components from new peaks in stressed samples.

K loss, heavy chain C-terminal lysine truncated. GK loss, heavy chain C-terminal glycine and lysine truncated.

Peak	Acidic peak N1	Basic peak N1	Basic peak N2	Basic peak N3
Modifications	1*deamidation, 2*K loss; A2G0F/A2G1F+A1G1F, 2*K loss, 2*oxidation	1*K loss, 1*GK loss, 3*oxidation	1*K loss, 1*GK loss, 4*oxidation	2*GK loss, 8*oxidation

System reproducibility evaluation

For consistent performance, two columns from different lots were used. Non-stress durvalumab was loaded on 3 days, 3 injection/day for both columns, using same instrument method. Figure 4A shows overlaid UV profile of 4 injections from two columns, four different days. Signal axis was offset for better display. Figure4B is comparison of peaks %area measured by two different columns, each bar was averaged from 9 injections across 3 days. Table 6 is average %area across 18 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, Overlaid UV profile of 4 injections from two columns, four different days. B, comparison of variant peaks %area measured by two different columns, each bar was averaged from 9 injections across 3 days.

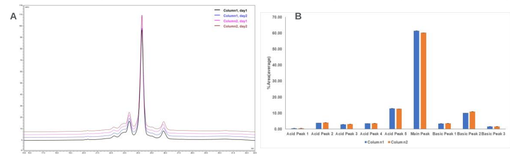


Table 6. Average %area across 18 injections, from two columns.

Peak	%Area Avg.	CV%
Acid Peak 1	0.45	4.62
Acid Peak 2	3.90	3.28
Acid Peak 3	2.99	4.96
Acid Peak 4	3.50	3.59
Acid Peak 5	12.80	1.41
Main Peak	60.87	1.09
Basic Peak 1	3.45	3.03
Basic Peak 2	10.45	4.89
Basic Peak 3	1.59	2.46

CONCLUSIONS

ProPac 3R SCX 3µm columns provide excellent separation of durvalumab and associated charge variants using a MS compatible pH gradient to give high resolution, robust performance and excellent reproducibility.

Thermal stress induced modification% changes can be observed. High resolution MS data provides modification details.

This online SCX-MS workflow can be used for charge variant analysis in biopharma industry.

TRADEMARKS/LICENSING

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