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Multi-attribute monitoring of aggregates and charge variants of monoclonal antibody through native 2D-SEC-MS-WCX-MS

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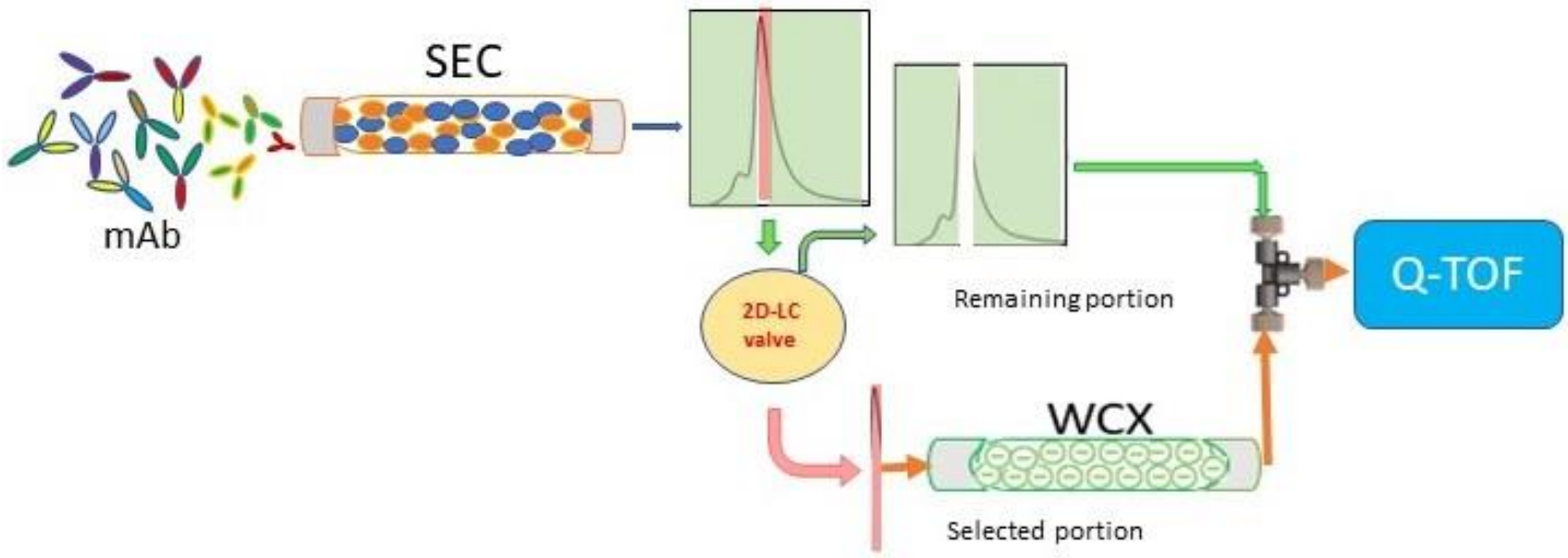
Introduction

Monitoring of critical quality attributes such as size and charge-related heterogeneities is essential for biopharmaceutical manufacturers. Size-exclusion chromatography (SEC) is the preferred analytical technique for quantification of aggregates and fragments in the product, whereas weak-cation exchange chromatography (WCX) is widely used for characterization of charge variants of biotherapeutic products, in particular monoclonal antibodies (mAbs). (Goyon et al., 2017; Trappe et al., 2018). Over the years, several studies have shown how to employ 2D-LC to monitor critical quality attributes (CQAs) using a combination of ProA, IEX, hydrophobic interaction chromatography (HIC), and SEC in the first dimension and RP, SEC, and IEX in the second dimension (Verscheure et al., 2022; Sarin et al., 2022). In the present study, a novel 2D-SEC-MS/WCX-MS workflow has been proposed, in which chromatography of both dimensions (D1 and D2) were directly coupled with mass spectrometry, through which size-related and charge-related variants of monoclonal antibody mAb A were analyzed simultaneously in their native form.



Experimental

Size variant analysis of mAb A was carried out using an AdvanceBio SEC column (300Å, 4.6 x 150 mm, 2.7 µm, Agilent Technologies) in the first dimension (D1) the fraction of the main peak in the SEC was transferred to the second dimension (D2) WCX column by a heart-cut method using 2D-LC Active Solvent Modulation (ASM) valve (G1170A) with ASM factor 5 to reduce salt concentration of peak by five folds.

Charge variant analysis of mAb A was performed using an Agilent Bio MAb column (NP5, 2.1 x 250 mm, 5 µm, Agilent Technologies) on a 1290 Infinity II UHPLC system (Agilent Technologies, Waldbronn, Germany). For native MS analysis, the 2D-LC system was coupled to 6545XT AdvanceBio LC/Q-TOF system (Agilent Technologies, USA) equipped with Dual Agilent JetStream electrospray ionization (AJS-ESI) source.

Figure 1: Agilent 2D-LC 1290 Infinity II UHPLC system

Figure 2: Agilent 6545XT AdvanceBio LC/Q-TOF system

2D-SEC-MS-WCX-MS method

In the present 2D-LC workflow, SEC-UV chromatography was used in the first dimension D1 and WCX chromatography was used in the second dimension D2. Additional to the typical 2D-LC method, the outlets (waste) of the D1 and D2 were directly coupled with mass spectrometry through a 'T' shape union. In analysis of mAb A using this 2D-LC method, a fraction of main peak (monomer) of SEC profile was transferred to the second dimension WCX column through the heart-cut method and the rest of the main peak was analyzed by mass spectrometry.

Run time	MS acquisition	D^1 flowrate (ml/min)	D^2 flowrate (ml/min)
0- 2	Waste	0.3	0.0
2-6	MS	0.3	0.0
6-10	waste	0.3	0.15
10-15	waste	0.0	0.15
15-25	MS	0.0	0.15

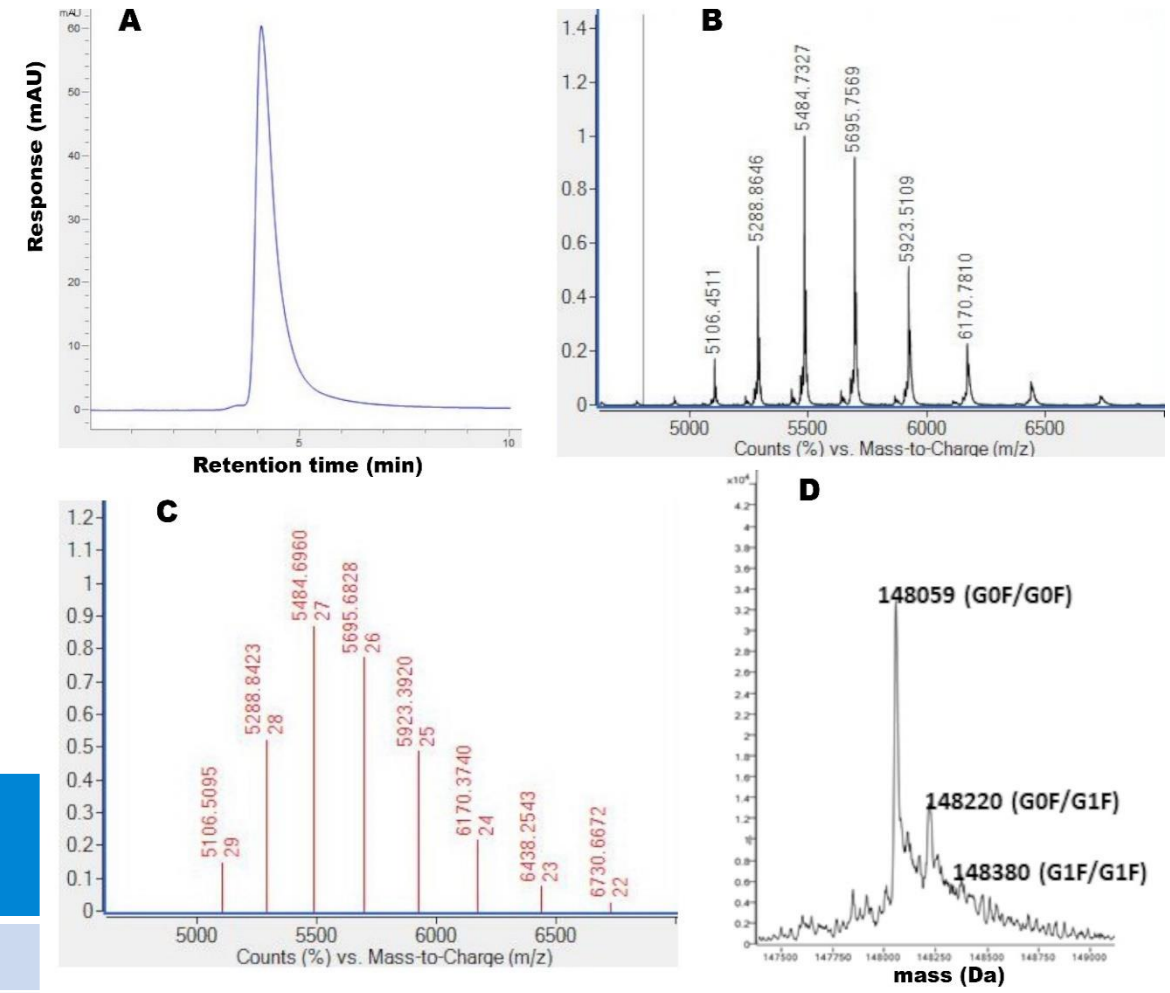


Figure 3: (A) SEC chromatogram of mAb A with UV detection; (B) Ionization mass spectra of mAb A to confirm ionization under native state; (C) Charge state pattern of native mAb A; (D) Deconvolution result of SEC main peak

Table 2: Summary of deconvoluted masses for mAb A from WCX-MS analysis

mAb A	Mass (Da)	PTM
Acidic A1	148061	3X deamidation
Acidic A2	148060	2X deamidation
Acidic A3	148059	1X deamidation
Acidic A4	148220	Glycation
Main (M)	148058	Unmodified
Basic B1	148074	1X oxidation
Basic B2	148094	2X oxidation

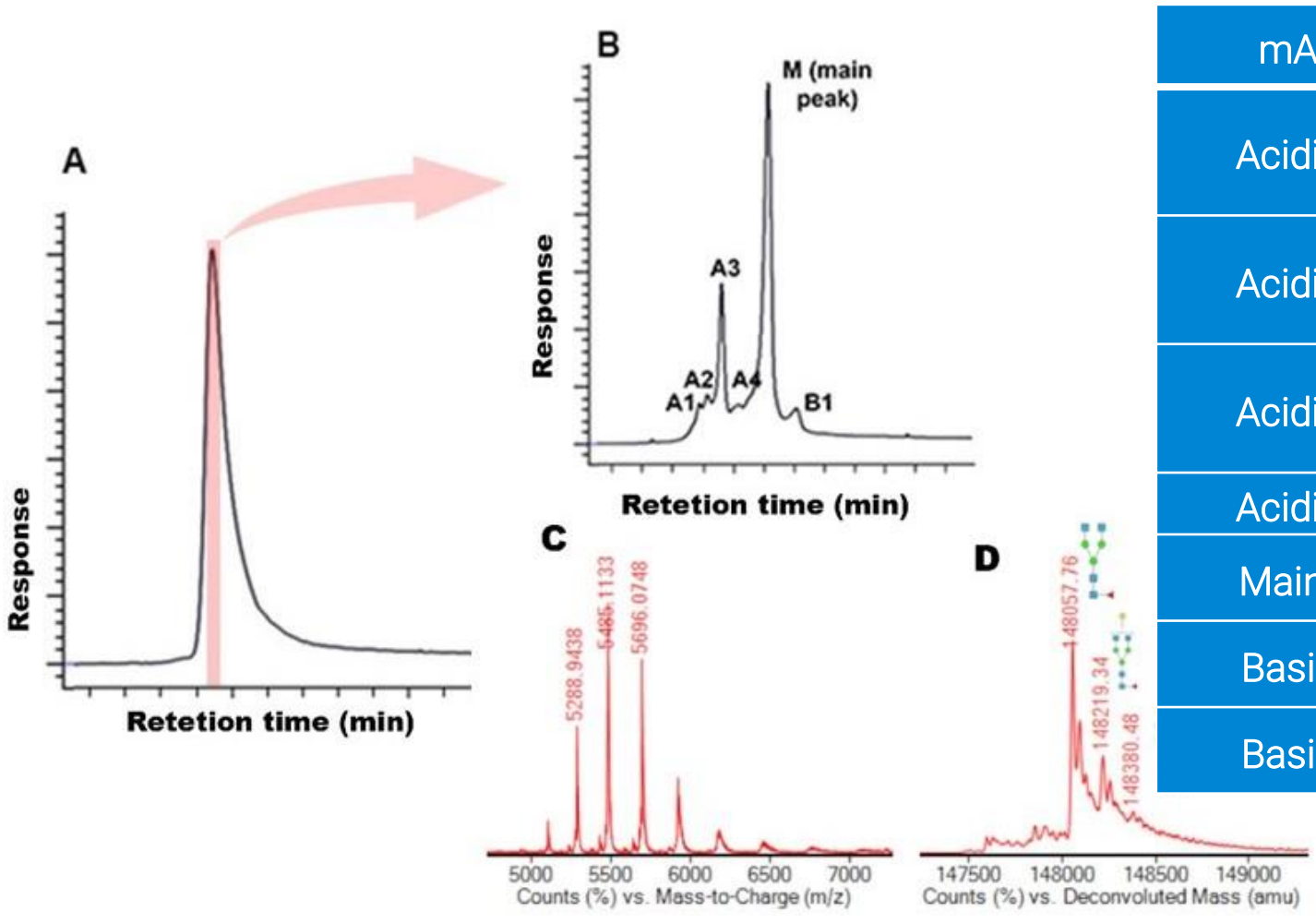


Figure 4: (A) SEC profile of mAb A in the D1 analysis; (B) WCX chromatogram of selected peak fraction of D1 transferred to D2 ; (C) MS spectra of main peak of mAb A; (D) Deconvolution result of main peak of mAb A.

Results and Discussion

Case Study I : The present native 2D-SEC-MS-WCX-MS method was applied for the quantification and intact mass analysis of size and charge variant species generated during the elevated temperature/photo-stress condition in mAb A.

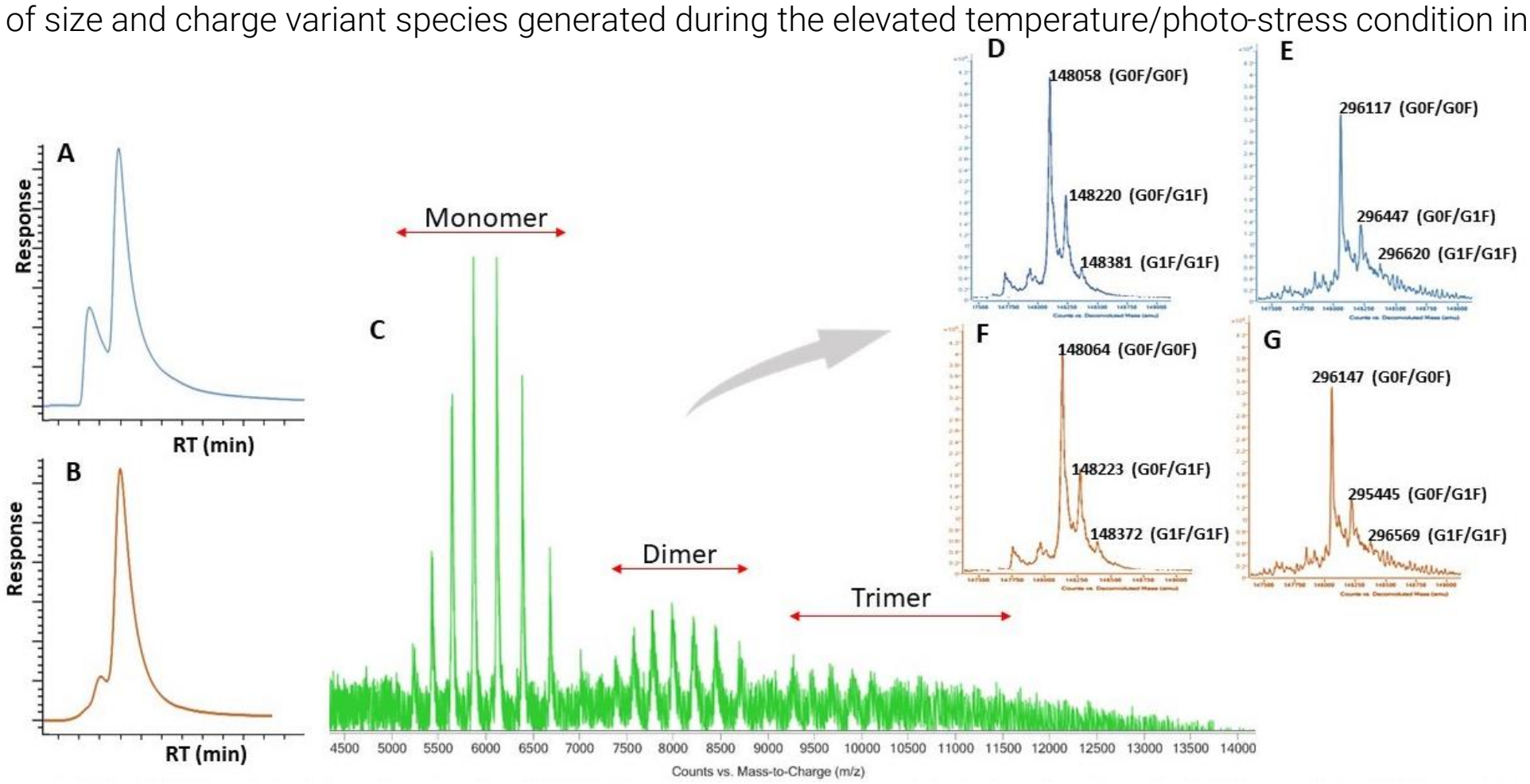


Figure 5: Native SEC-MS analysis of stressed samples: (A and B) SEC chromatograms of temperature and photo stressed samples under the UV, respectively; (C) Charge-state pattern of stressed sample to confirm ionization under native form; (D & E) Deconvolution of monomer and dimer peaks of thermally stressed sample; (F&G) Deconvolution of monomer and dimer peaks of photo stressed sample

Conclusions

The present study describes a novel native 2D-SEC-MS-WCX-MS workflow that enables us to characterize and quantify aggregates and charge variants of mAb A through native mass spectrometry. The present method required a shorter analysis time of 25 minutes as compared to stand-alone methods which required 90 minutes to analyze size and charge variants individually. In addition, when compared to traditional 2D-LC method, this method has the leverage for intact mass analysis in first dimension by mass spectrometry without the need of manual intervention and peak collection for sample processing which required 60 minutes additional time. The proposed MAM method overcomes the limitation of intact mass analysis of first dimension chromatography and offers the possibility of simultaneous analysis in both dimensions using mass spectrometry in a single workflow.

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