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Rapid analysis of titer, aggregate and intact mass of antibody therapeutics using multi-dimensional liquid chromatography coupled with native mass spectroscopy

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Introduction

In recent years, multiattribute monitoring (MAM) is becoming increasingly popular, as it involves combining two or more analytical approaches into a single workflow to monitor multiple attributes in a time efficient manner. Two-dimensional liquid chromatography (2D-LC) has been a key component of MAM applications and it involves transfer of separated fractions from first-dimension chromatography (D1) for further separation by orthogonal second dimension chromatography (D2) column without manual intervention (Stoll et al., 2017). In the last 5 years, multiple researchers have demonstrated use of 2D-LC to monitor critical quality attributes (CQAs) via a combination of ProA, IEX, hydrophobic interaction chromatography (HIC), and SEC in the first dimension and RP, SEC and IEX in the second dimension (Ehkirch et al., 2018; Verscheure et al., 2022; Sarin et al., 2022). This study proposes a novel, fast online 2D-LC workflow (8 minutes analysis time) that can analyze for titer using ProA column in the first dimension and aggregates and intact mass analysis of size variants through SEC-MS in the second dimension.

Experimental



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

Experimental

The monoclonal antibodies mAb A and mAb B were purified by Bio-Monolith recombinant Protein A (rProtein A) column (4.95 × 5.2 mm, Agilent Technologies) in the first dimension and size variants analysis was performed using an AdvanceBio SEC column (300Å, 4.6 × 150 mm, 2.7 μm, Agilent Technologies) in the second dimension 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. The MS parameters were drying gas at 350 °C, drying gas flow 13 L/min, nebulizer pressure 35 psi, sheath gas temperature at 300 °C, sheath gas flow at 12 L/min, nozzle voltage 5500 V, and Fragmentor voltage 150 V. MS data was collected in profile mode at a rate of 1 spectrum per sec with a 2500-20000 *m/z* range. Data was acquired using MassHunter Workstation LC/MS Data Acquisition 10.0 software (Agilent Technologies).



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

2D-ProA-SEC-MS method

In the present study, a MS-compatible 2D-LC/MS method was employed, in which protein-A affinity chromatography was used in the first dimension for the purification and estimation of titer, whereas characterization of size variants of mAb A were performed in the second dimension using SEC-MS method. The elution peak of mAb A in protein-A (D1) analysis was transferred to the second dimension (D2) through the heart-cut 2D-LC method (Figure 3 A&B). mAb A showed a mass spectra of m/z range from 5000 to 7000 with charge state range of +22 to +28, indicating a non-denature form of mAb A, and intact mass analysis of main peak showed a deconvoluted mass of ~148059 Da which was equal to a mass of monomer (Figures 3 C&D). The deconvoluted result of main peak showed 3 major glycovariants G0F/G0F (~148058 Da), G0F/G1F (~148219 Da) and G1F/G1F (~148380 Da) in mAb A.

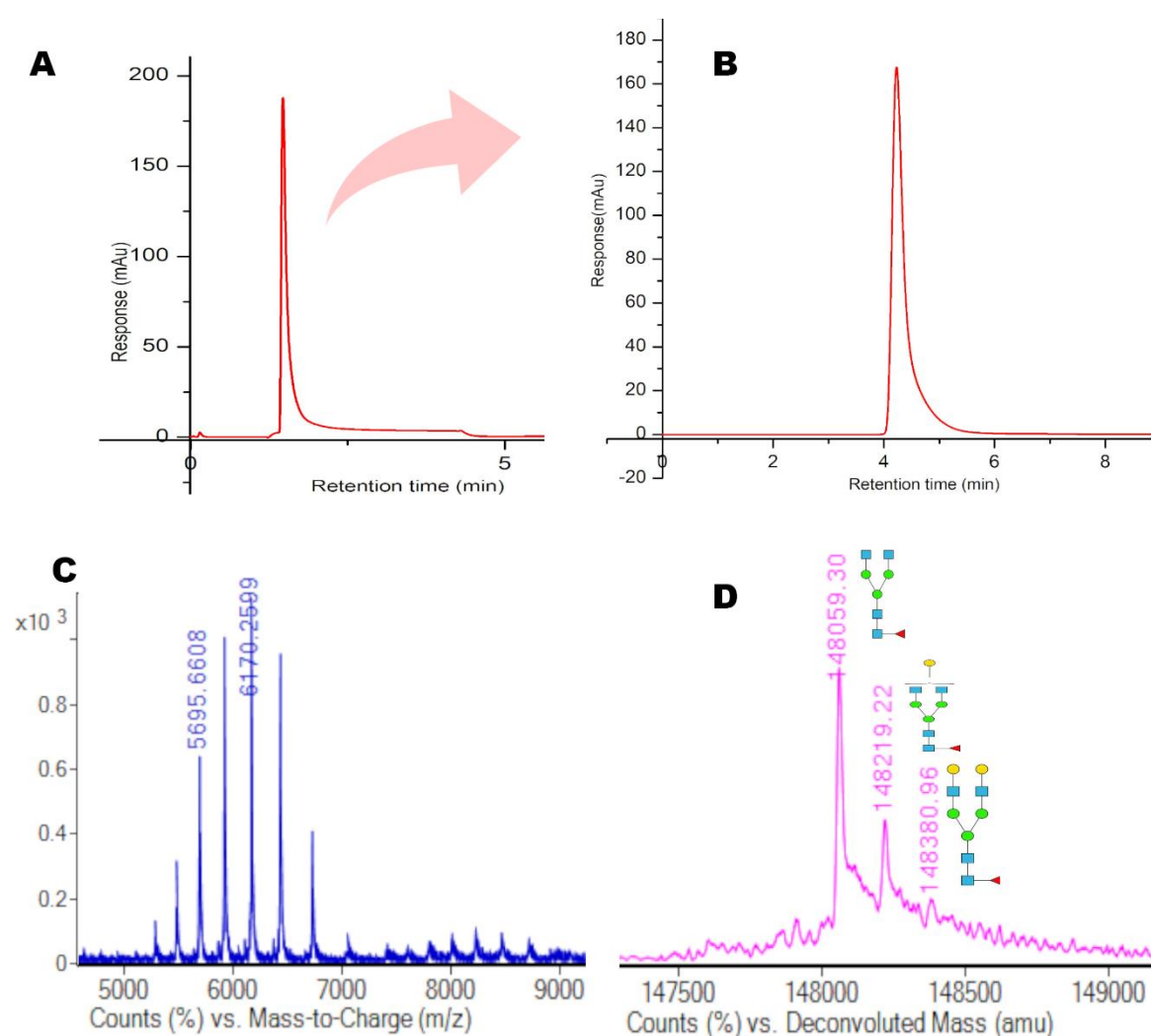


Figure 3: (A) Protein A profile of mAb A in the D1 analysis; (B) SEC chromatogram of selected peak fraction of D1 transferred to D2; (C) MS spectrum of main peak of SEC; (D) Deconvolution result of main peak of mAb A.

Case Study I

This 2D method was further employed to another mAb (mAb B). The mAb B showed a single elution peak in the D1 analysis (Figure 4A), but this peak separated into HMWs and monomer peaks in the D2 analysis (Figure 2B). mAb B showed ~22% HMWs and ~78% monomer product in the SEC profile (Figure 4 B). Moreover, during SEC-MS analysis of mAb B, monomer peak revealed a mass spectra range of 5000–7000 m/z with a charge state range of +21 to +29 which has shown a deconvoluted mass of ~149201 Da, equal to the mass of mAb B monomer. The deconvoluted result of the HMWs peak contained deconvoluted masses of ~298402 Da, ~298723 Da, ~299048 Da, and ~299372 Da which correspond to the masses of G0F/G0F, G0F/G1F, G1F/G1F, and G1F/G2F glycovariants, respectively (Figure 4C). Likewise, the deconvoluted mass of the monomer peak showed glycovariant masses of ~149201 Da, ~149363 Da, ~149524 Da, and ~149685 Da (Figures 4D).

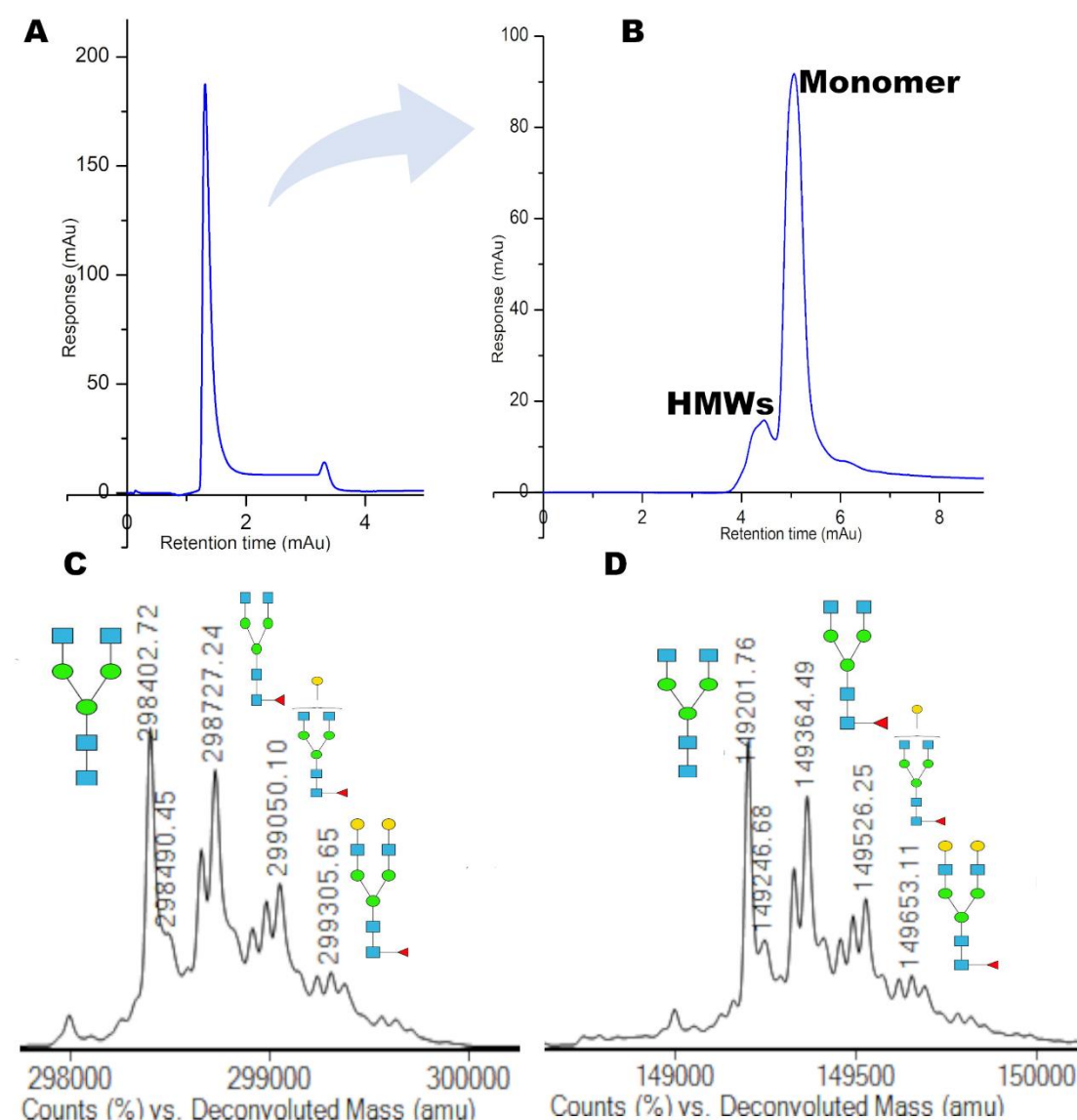
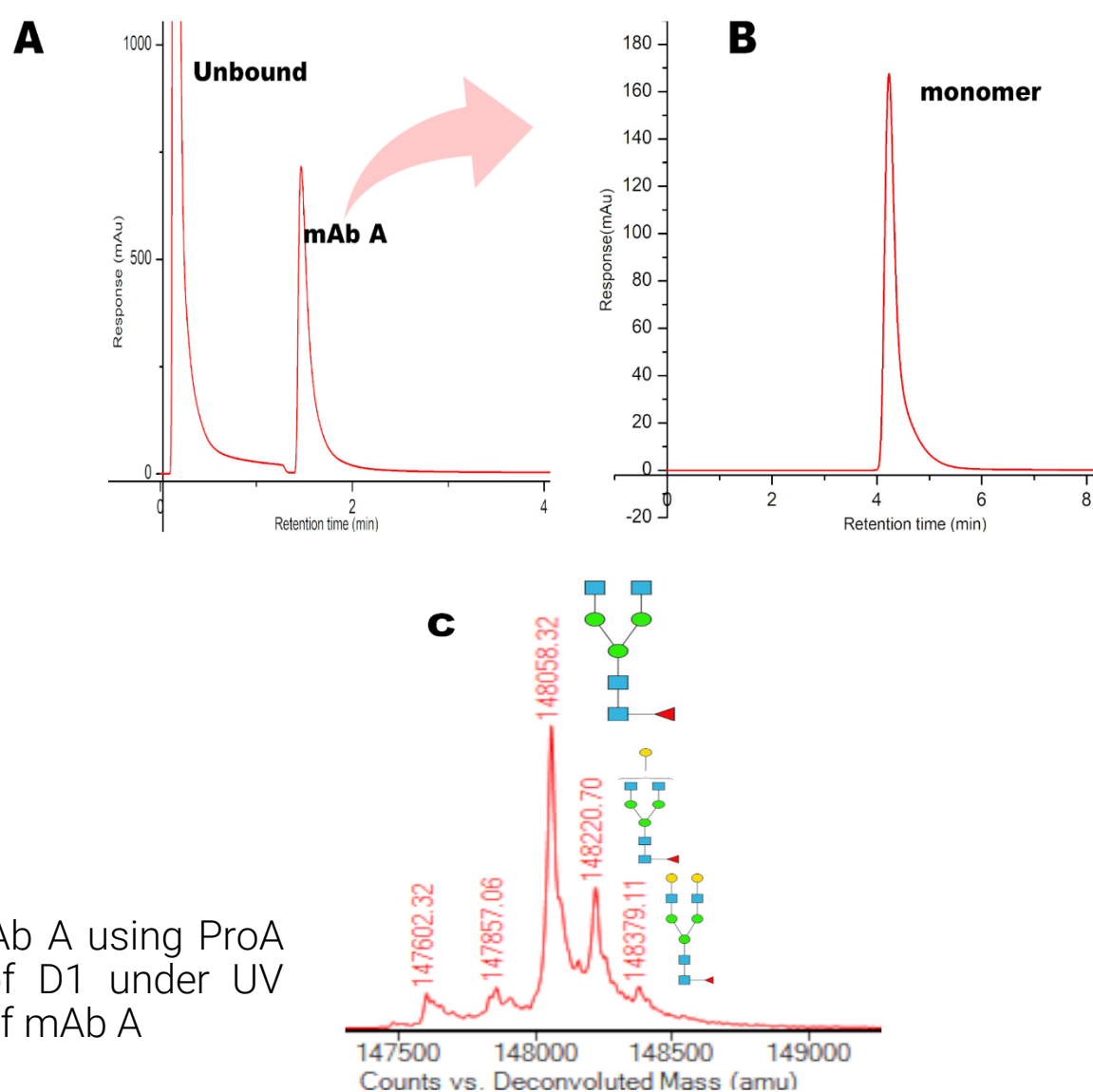


Figure 4: (A) Protein A profile of mAb B in the D1 analysis; (B) SEC chromatogram of selected peak fraction of D1 transferred to D2; (C) Deconvolution result of HMWs peak of mAb B; (D) Deconvolution result of main peak of mAb B.

Case Study II

The method with same 2D-set up was employed for analysis of harvested cell culture sample of mAb A to monitor such attributes. During purification of mAb A in the first dimension, a distinct elution peak of mAb A was observed after the unbound cell harvest peak (Figure 5A). The elution peak of mAb A was transferred to the second dimension using heart cut method which was further resolved into HMWs and monomer peaks in the SEC method (Figure 5B). In SEC analysis, mAb A showed ~ 0.9% HMWs and ~ 99% monomer species. The native mass analysis of the mAb A monomer peak showed glycovariants G0F/G0F (~148058 Da), G0F/G1F (~148220 Da) and G1F/G1F (~148378 Da) (Figure 5C).

Figure 5: (A) D1 analysis of cell culture harvest of mAb A using ProA column; (B) SEC chromatogram of peak fraction of D1 under UV detection; (C) Deconvolution result of monomer peak of mAb A



Conclusions

The coupling the ProA-SEC method with MS offers advantages to provides insight into multiple, additional CQAs such as intact mass and major glycoforms. As shown in this study, the present method revealed 3 glycovariants in the mAb A and 4 glycovariants in mAb B, with the analysis completed in less than 8 minutes with requirement of only a small sample amount (10-15 μg) without the need of any manual manipulations. A standalone SEC analysis typically takes up to 2-3 hours for peak collection of ProA followed by buffer exchange to a mass compatible buffer with the risk of sample loss, degradation, induced modifications, and manual handling which is likely to negatively impact reproducibility. The proposed method offers applications for quality control (QC) testing as well as for process analytical technology (PAT) applications in upstream and midstream processing.

References

1. Stoll, D. R., Carr, P. W., Two-Dimensional Liquid Chromatography: A State of the Art Tutorial. *Anal. Chem.* 2017, 89, 519–531.
2. Ehkirch, A., Goyon, A., Hernandez-Alba, O., Rouviere, F., D'Atri, V., Dreyfus, C., Haeuw, J. F., Diemer, H., Beck, A., Heinisch, S., Guillarme, D., Cianferani, S., A Novel Online Four-Dimensional SEC \times SEC-IM \times MS Methodology for Characterization of Monoclonal Antibody Size Variants. *Anal. Chem.* 2018, 90, 13929–13937.
3. Verscheure, L., Vanhoenacker, G., Schneider, S., Merchiers, T., Storms, J., Sandra, P., Lynen, F., Sandra, K., 3D-LC-MS with 2D Multimethod Option for Fully Automated Assessment of Multiple Attributes of Monoclonal Antibodies Directly from Cell Culture Supernatants. *Anal. Chem.* 2022, 94, 6502–6511.
4. Sarin, D., Kumar, S., Rathore, A. S., Multiattribute Monitoring of Charge-Based Heterogeneity of Recombinant Monoclonal Antibodies Using 2D HIC-WCX-MS. *Anal. Chem.* 2022, 94(43):15018-15026.

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