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Application Note

A Platform Method for the Molecular Mass Analysis of the Light Chains and Heavy Chains of Monoclonal Antibodies using the BioAccord System

Henry Shion, Ying Qing Yu, Weibin Chen

Waters Corporation

Abstract

The study demonstrates fast and routine mass analysis of reduced monoclonal antibodies based on a denaturing SEC-MS method using the Waters BioAccord System.

A denaturing SEC/UV/MS method has been developed that enables the inline combination of size exclusion chromatography with optical and MS detection, for quantitative and qualitative mAb analysis at the subunit level. This well established and robust separation technique could prove useful for scientists needing a higher throughput analytical solution to meet the demand to analyze a diverse array of mAbs without customized method development. UPLC-based sub-2-µm SEC separations enabled rapid antibody light and heavy chain separations under molecule and MS-friendly conditions. This separation based on size is complementary to RP chromatography, and when coupled with MS, offers a powerful, routine and platform solution for antibody analysis.

Benefits

A platform solution for rapid mass analysis of monoclonal antibodies light chain and heavy chain subunits using on-line denaturing SEC-MS.

Introduction

Therapeutic monoclonal antibodies (mAbs) still account for the largest percentage of biotherapeutics under development in the biopharmaceutical industry. Mass analysis of mAbs at the intact and subunit level is routinely conducted throughout the product development life cycle, serving to confirm sequence integrity, monitor product variations, and establish product identity. A simple and generic platform method for the mass measurement of mAbs with diverse physicochemical properties is highly desirable for improving the efficiency of analyses in the biopharmaceutical industry, particularly in the discovery and early development stages, where higher throughput screening of diverse mAb constructs is most common.

Size exclusion chromatography (SEC) is an isocratic protein separation mode, with selectivity predominantly based on the average stokes radius of the protein which correlates with the log molecular weight (MW) of the protein. Compared to reversed-phase (RP) based chromatography methods, SEC is inherently more generic as analyte recovery is not dependent on higher column temperature, and product peaks typically elute at predictable positions independent of the molecule's isoelectric point (pI) and hydrophobicity. Taking advantage of size-based separation capabilities, we developed a denaturing SEC method, employing mobile phases containing 30% acetonitrile, 0.1% trifluoroacetic acid (TFA), and 0.1% formic acid (FA) that achieved separation of reduced antibody light chain (LC) and heavy chain (HC) subunits. We transferred the SEC separation to the BioAccord System to obtain both UV and mass data for the reduced fragments. This method offers a robust workflow for mAb analysis, one highly tolerant of many buffers and formulants, and amenable to deployment as a platform analysis method.

Results and Discussion

The employment of an MS-friendly mobile phase (30% acetonitrile, 0.1% TFA, and 0.1% FA) facilitates the direct coupling of SEC to inline UV and ESI-MS detection. The SEC separation on an ACQUITY UPLC BEH SEC 200Å Column (1.7 µm, 4.6 mm x 150 mm, p/n 186005225) was achieved with an ACQUITY UPLC I-Class PLUS System at a flow rate of 0.40 mL/min. The robust BEH hybrid particle technology ensures chemical and physical stability of the column under these aggressive denaturing SEC conditions. The flow passed through an ACQUITY UPLC TUV Detector (280 nm) inline with the ACQUITY RDa MS Detector. The separation achieved using sub-2-µm UPLC BEH SEC particle in the low-dispersion ACQUITY UPLC I-Class PLUS System enabled 6-minute SEC runs that completely resolved glycosylated heavy chain (HC) from the later eluting light chain (LC).

Figure 1A shows the Total Ion Chromatograms (TIC) of 5 reduced mAbs subunits (heavy chains and light chains)

from denatured SEC-MS analysis. All the light chain and heavy chain peaks are baseline separated and eluted with the same retention times within the 6 minutes total runtime method. Figure 1B compares the TIC and TUV (280 nm) chromtagrams of the reduced NIST mAb subunits, which shows the late eluted salt species were switched to the waste to minimize potential contamination to the ACQUITY RDa MS Detector.

Figure 2 A and B shows the NIST mAb LC (A) and HC (B) summed raw spectra and deconvoluted spectra respectively. Good mass accuracies are observed from the automatically identified major LC and HC peaks as displayed in the components summary table in Figure 2C.

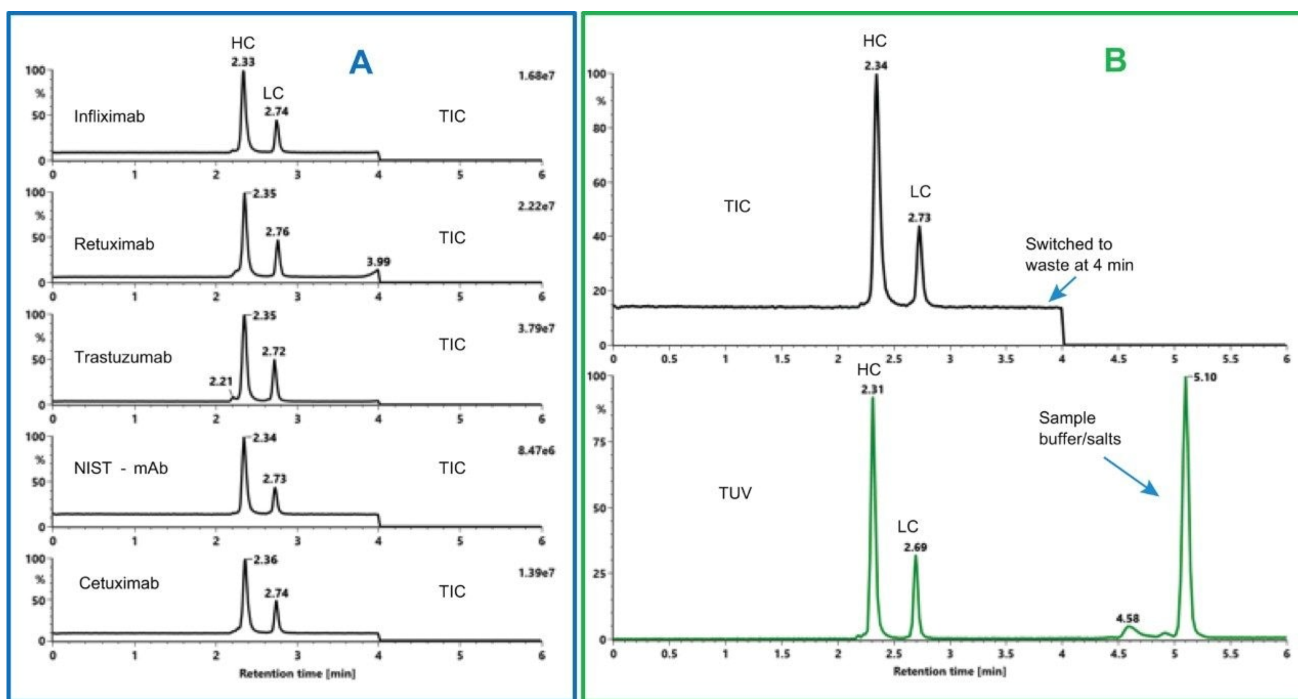
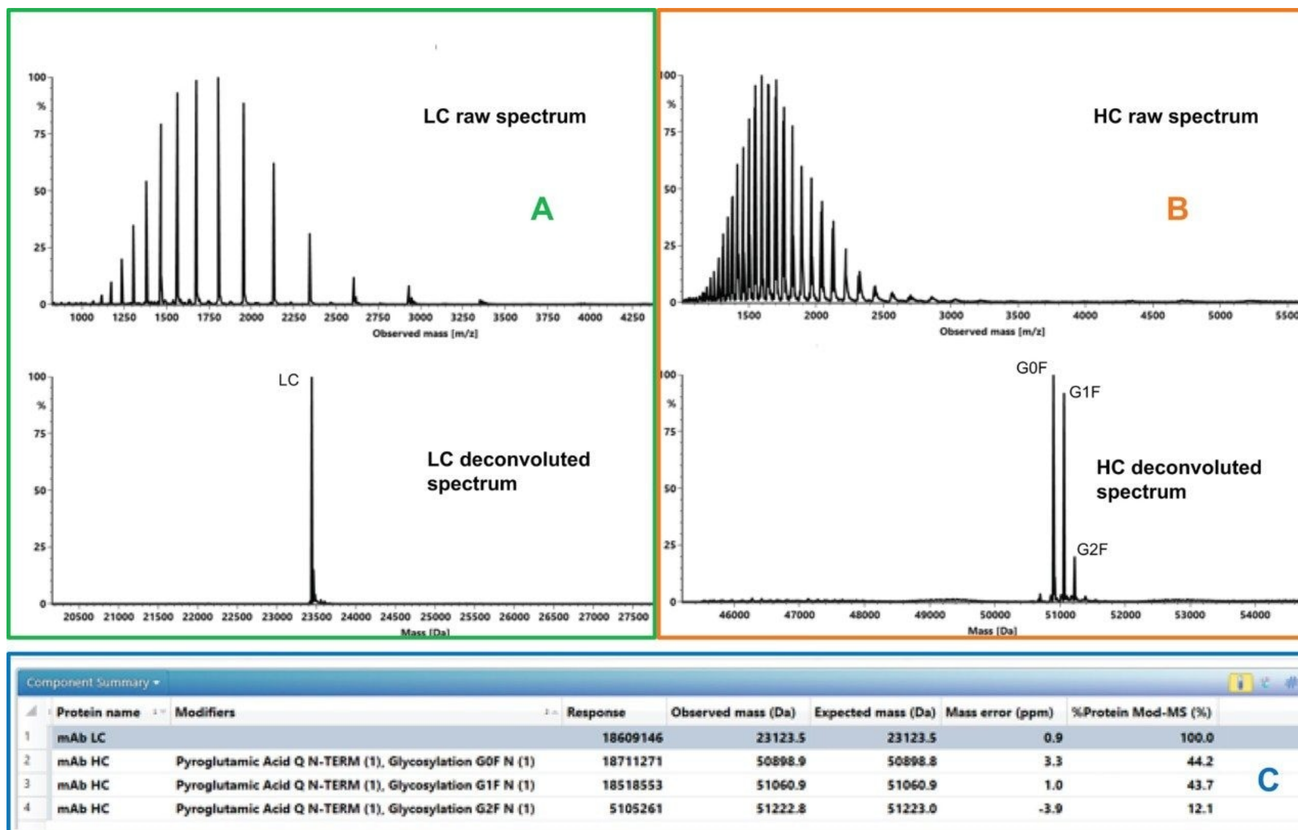


Figure 1. A. Total Ion Chromatograms (TIC) of multiple reduced mAbs subunits (heavy chains and light chains) from denatured SEC-MS analysis. B. TIC and TUV (280 nm) chromatograms of reduced NIST mAb subunits.



	Protein name	Modifiers	Response	Observed mass (Da)	Expected mass (Da)	Mass error (ppm)	%Protein Mod-MS (%)
1	mAb LC		18609146	23123.5	23123.5	0.9	100.0
2	mAb HC	Pyroglutamic Acid Q N-TERM (1), Glycosylation G0F N (1)	18711271	50898.9	50898.8	3.3	44.2
3	mAb HC	Pyroglutamic Acid Q N-TERM (1), Glycosylation G1F N (1)	18518553	51060.9	51060.9	1.0	43.7
4	mAb HC	Pyroglutamic Acid Q N-TERM (1), Glycosylation G2F N (1)	5105261	51222.8	51223.0	-3.9	12.1

Figure 2. NIST mAb LC (A) and HC (B) summed raw spectra and deconvoluted spectra are shown from the denatured SEC-MS analysis. Good mass accuracies are observed for the automatically identified major LC and HC peaks as displayed in the components summary table (C).

Conclusion

A denaturing SEC/UV/MS method has been developed that enables the inline combination of size exclusion chromatography with optical and MS detection, for quantitative and qualitative mAb analysis at the subunit level. This well-established and robust separation technique could prove useful for scientists needing a higher throughput analytical solution to meet the demand to analyze a diverse array of mAbs without customized method development. UPLC-based sub-2-µm SEC separations enabled rapid antibody light and heavy chain separations under molecule and MS-friendly conditions. This separation based on size is complementary to RP

chromatography, and when coupled with MS, offers a powerful, routine and platform solution for antibody analysis.

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BioAccord LC-MS System for Biopharmaceuticals <
https://www.waters.com/waters/nav.htm?cid=135005818>

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