Characterization of Intact Monoclonal Antibodies (mAb) and their fragments using Novel Reversed Phase Columns

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Introduction

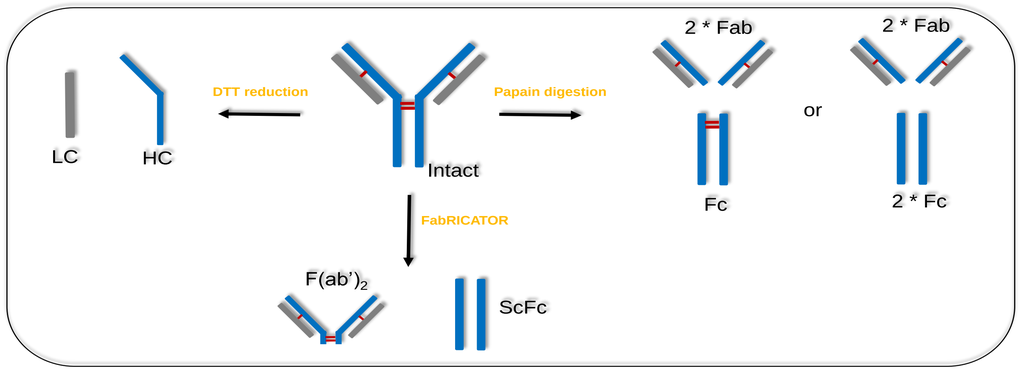
With the continued importance of monoclonal antibodies (mAbs) as biotherapeutics, comprehensive characterization is a prerequisite. It is critical at discovery, development and manufacturing stages to confirm that the antibody drug has the correct primary structure (number, location and order of amino acids) and to monitor variants and other potential post translational modifications that can impact safety and efficacy. Due to the complexity of the molecules, the characterization method with high resolution for mAb primary structure by reversed-phase liquid chromatography can be very lengthy and time consuming. In this presentation, the data analysis of intact mAb and fragments such as heavy/light chains and Fc/Fab regions with high speed and high resolution are presented. These data are performed by using the novel, unique bonding chemistries-C4, C8 and Diphenyl on a new particle design of 3.5 µm Poroshell technology. The data from the LC/UV and LC/MS methods will be included here in this presentation.

Experimental

Therapeutic monoclonal antibodies were purchased from a local pharmacy and stored according to the manufacturers' instructions.

LC/UV: For intact mAb analysis, mAb samples were diluted to 2 mg/mL using PBS. For the separation of the light and heavy chains, an aliquot of 0.5 M TCEP stock was added to the mAb samples to obtain a final concentration of 10 mM. The mixture was held at 60 °C for 30 minutes.

LC/MS: For intact mAb analysis, mAb samples were diluted to 1 µg/µL using 0.1% formic acid in 3% ACN, For the separation of the light and heavy chains, an aliquot of 1M DTT stock was added to the mAb samples and the mixture was held at 37 °C for 1 hr. Digestion with Papain and FabRICATOR was performed at 37 °C for 3 hr and 1 hr respectively.



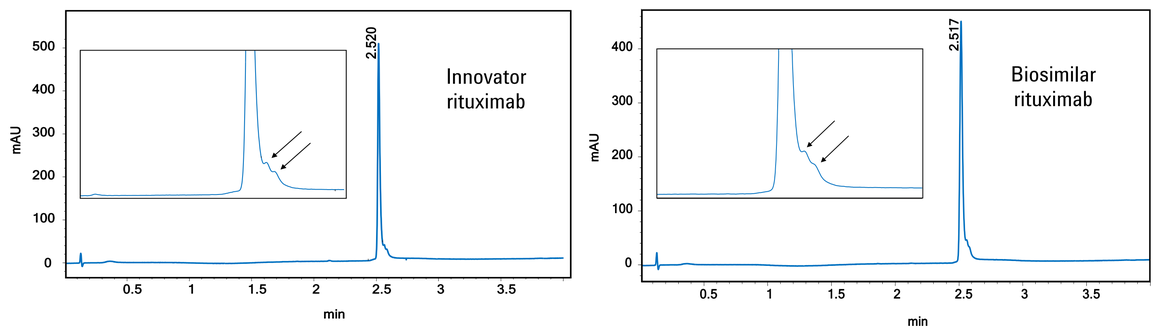
For LC/UV and LC/MS conditions, refer Agilent application notes:

LC/UV: 5991-6274EN

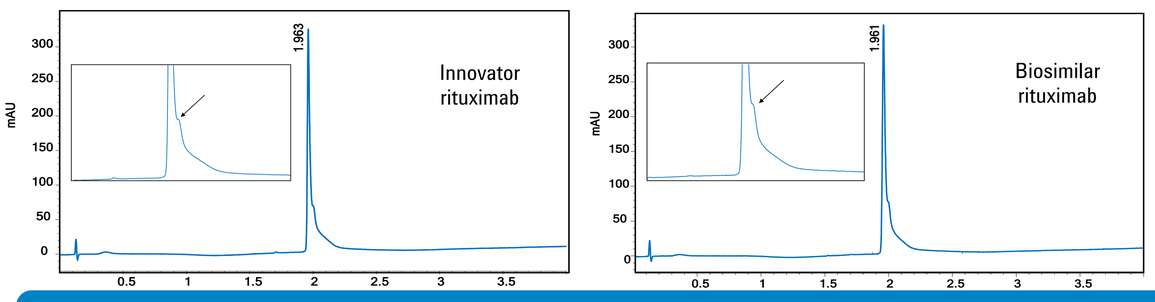
LC/MS : 5991-6296EN

LC-UV

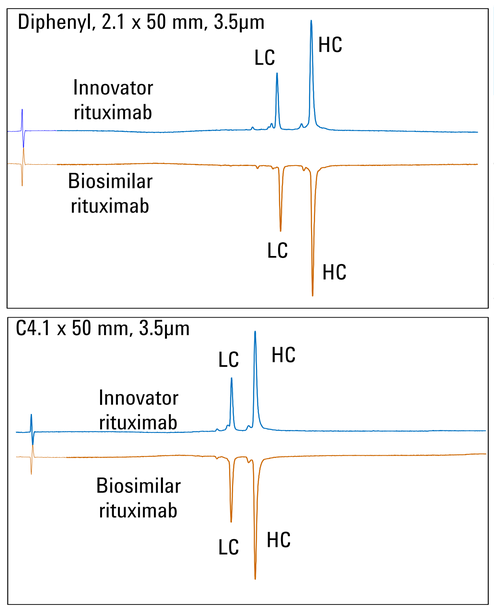
Intact mAb analysis on a AdvanceBio RP-mAb Diphenyl, 2.1 x 50 mm, 3.5µm column



Intact mAb analysis on a AdvanceBio RP-mAb C4, 2.1 x 50 mm, 3.5µm column



Reduced mAb analysis on a AdvanceBio RP-mAb column

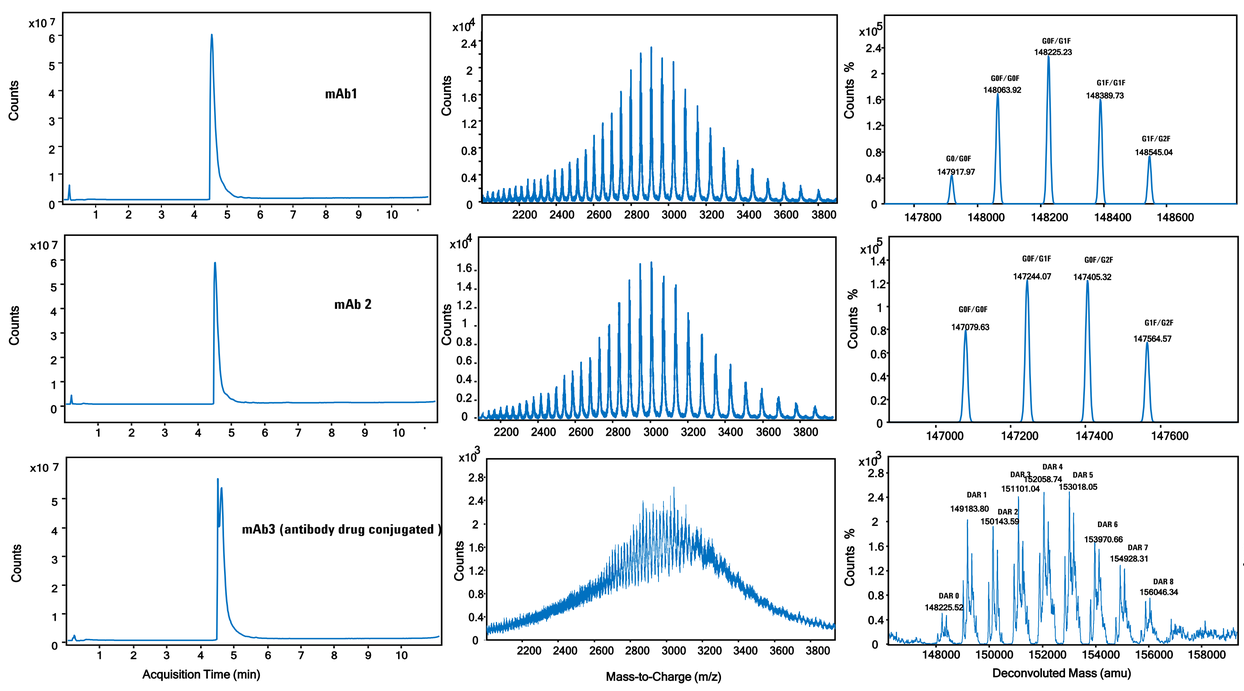


Samples	Retention time Mean (min)	RSD	Peak area Mean (mAU/min)	RSD
Agilent AdvanceBio RP-mAb, C4, 2.1 × 50 mm, 3.5 µm				
Innovator rituximab	1.96	0	71.61	1.98
Biosimilar rituximab	1.95	0.26	77.3	0.47
Agilent AdvanceBio RP-mAb, Diphenyl, 2.1 × 50 mm, 3.5 µm				
Innovator rituximab	2.51	0.20	66.7	0.458
Biosimilar rituximab	2.51	0	73.3	1.86

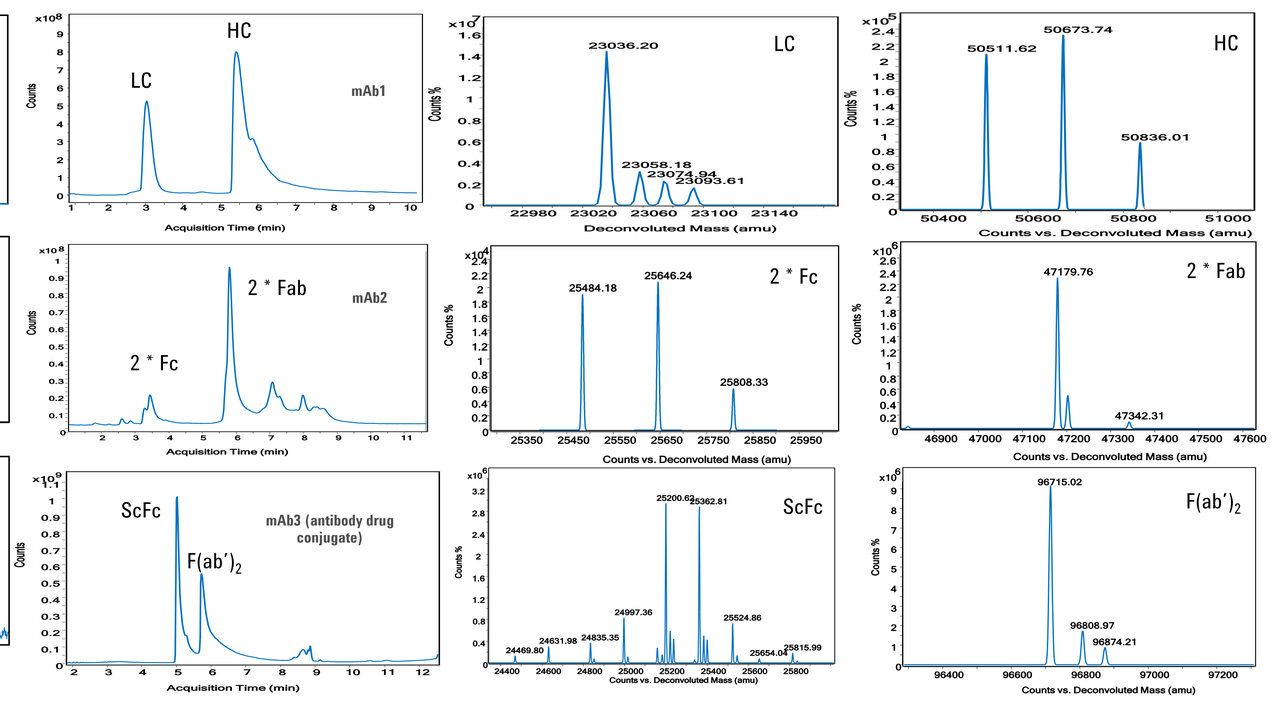
Samples	Retention time Mean (min)	RSD	Peak area Mean (mAU/min)	RSD
Agilent AdvanceBio RP-mAb, Diphenyl, 2.1 × 50 mm, 3.5 µm				
Innovator rituximab LC	2.32	0.60	19.71	4.24
Innovator rituximab HC	2.58	1.52	57.33	1.57
Biosimilar rituximab LC	2.32	0.07	23.56	3.25
Biosimilar rituximab HC	2.60	0.05	58.40	5.61
Agilent AdvanceBio RP-mAb, C4, 2.1 × 50 mm, 3.5 µm				
Innovator rituximab LC	1.83	0	21.5	1.4
Innovator rituximab HC	2.03	0.04	51.2	2.25
Biosimilar rituximab LC	1.83	0.03	24.47	3.84
Biosimilar rituximab HC	2.03	0.06	52.66	0.84

LC-MS

Intact mAb mass analysis on a AdvanceBio RP-mAb C4, 2.1 x 100 mm, 3.5µm column



mAb fragment analysis on AdvanceBio RP-mAb C4, 2.1 x 100 mm, 3.5µm column



Conclusions

- AdvanceBio RP-mAb columns: Fast and superior separation power for both intact and fragments of mAb
- Demonstration of LC-UV-based approach to define the molecular similarity between a biosimilar and its innovator reference
- Area and RT precision of intact and reduced analysis using AdvanceBio RP-mAb columns were excellent, and show the reliability of the method
- The C4 and Diphenyl AdvanceBio RP-mAb columns, with an MS-compatible method, delivered fast and high-resolution analysis for intact and ADC mAbs

Publication Number 5991-6626EN

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