Monoclonal Antibody Charge Heterogeneity Analysis by CZE and CZE/MS

Comparison of charge variant profiles of Rituximab Innovator and Biosimilar mAbs

Application Note

Biopharmaceutical

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Introduction

During biosysnthesis, various post-translational modifications (PTMs) will lead to charge heterogeneity in monoclonal antibody (mAb) products. Due to these modifications, charge variants can affect the biological properties of mAbs as biotherapeutics. Hence it is very important to monitor this quality attribute during bioprocessing for lot-to-lot consistency and stability. Capillary electrophoresis (CE) with charge to mass based separation mechanism, is best situated for separation of charged molecules. Due to difference in net charge of the charge variants, their electrophoretic mobility differs, resulting in capillary zone electrophoresis (CZE) separation[1,2]. This Application Note demonstrates the feasibility of using CZE for monitoring the charge variants of innovator and biosimilar rituximab on Agilent 7100 CE system. Further, the basic charge variants of biosimilar rituximab were characterized by CZE/MS.

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Experimental

Instrumentation

- Agilent 7100 Capillary Electrophoresis System (G7100A)
- Capillary cassette (G7100-60002)
- Alignment interface for standard capillary ID 50 µm (G7100-60210)
- Fused silica capillaries (G1600-61232)
- PVA coated capillaries (G1600-61219)
- Agilent 6530 (option 200) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF)

Materials

Innovator and biosimilar rituximab were purchased from a local pharmacy and stored according to the manufacturer's instructions. A stock solution of 10 mg/mL rituximab was prepared by dissolving appropriate amount in water. ε-Amino-caproic acid (EACA), acetic acid, hydroxypropyl methyl cellulose (HPMC), and triethylenetetramine (TETA) were purchased from Sigma-Aldrich.

Software

Agilent MassHunter Workstation Software was used for data acquisition and data analysis. However, the standard software for Agilent 7100 CE is the Agilent OpenLAB (ChemStation C.01.07) that allows control of the standard Diode Array Detector as well as for Agilent Single Quadrupole (SQ) Mass Spectrometer or external detectors like laser-induced fluorescence (LIF)[3].

Capillary electrophoresis (CE)	
Capillary	FSC, 56 cm, 50 µm id (CE/UV); PVA, 65 cm, 50 µm id (CE/MS)
Sample	Rituximab, Innovator and Biosimilar mAbs
Injection	5 seconds at 50 mbar
Buffer	400 mM EACA–acetic acid pH 5.7 + 0.05 % HPMC + 2 mM TETA (CE-UV); 2 % acetic acid (CE/MS)
Voltage	30 kV
Temperature	20 °C
DAD	214 nm
Mass spectrometry (MS)	
MS	Agilent 6530 (option 200) Accurate-Mass Q-TOF LC/MS
Ionization mode	ESI
Acquisition mode	MS (mass range 1,000–6,000 *m/z*)
Sheath liquid	0.5 % acetic acid in 50 % methanol, 5 µL/min
Drying gas flow	5 L/min
Nebulizer	10 psi
Drying gas temperature	250 °C
Fragmentor	350 V
Vcap	3,500 V

Results and Discussion

Figure 1 demonstrates the excellent separation of charge variant profiles of rituximab innovator and biosimilar mAbs within 25 minutes. The peaks at 19.4 and 19.1 minutes in the electropherograms of innovator and biosimilar are designated as the main peaks. Early and late-migrating peaks were called basic and acidic variants, respectively.

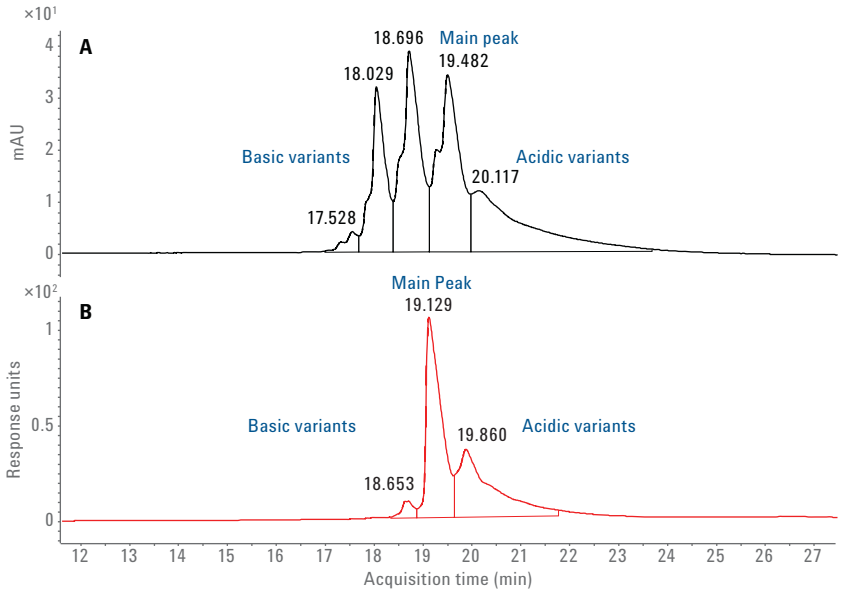

Figure 1. CZE separation of charge heterogeneity of (A) Biosimilar–Reditux and (B) Innovator–Ristova.

Precision of migration time and area are summarized in Table 1. The migration time and peak area RSDs for the main peaks are in the range of 0.12 %–0.58 % and 0.2 %–1.11 % respectively, which demonstrates excellent reproducibility of the method and thus the precision of the system.

The CZE electropherogram of biosimilar mAb showed different separation profiles indicating different degrees of basic and acidic variants compared to innovator mAb. This high-resolution separation enables calculation of percentage of basic and acidic variants using peak areas (Table 2). The major charge variant in biosimilar rituximab was found to be basic variants.

Further, biosimilar rituximab was characterized by CZE/MS[4]. Figure 2A and 2B shows the average mass spectrum and deconvoluted spectra of deglycosylated biosimilar rituximab respectively. The mass spectrum shows Gaussian distribution of the charge state envelope. Peak modeling (pMod) based deconvoluted spectra shows series of peaks with a mass difference of ~128 Da corresponds to c-terminal lysine truncation.

Table 1. Retention time and area RSD (%), n = 3.

	Retention time		Peak area	
	Mean (min)	RSD	Mean (mAU/min)	RSD
Biosimilar–Reditux	17.54	0.58	18.36	5.07
	18.03	0.57	468.94	0.52
	18.7	0.58	692.78	1.11
	19.5	0.58	549.02	0.2
	20.31	0.58		
Innovator–Ristova	18.62	0.13	148.34	6.22
	19.1	0.12	2216.61	1.11
	19.8	0.14	1757.58	3.63

Table 2. Charge variants quantification.

	Area %		
	Mean (%)	RSD	Variants
Biosimilar–Reditux	2.09	1.43	Basic
	18.14	0.58	Basic
	27.46	2.11	Basic
	28.91	2.12	Main
	23.82	3.86	Acidic
Innovator–Ristova	3.54	5.23	Basic
	54.36	2.18	Main
	42.08	2.46	Acidic

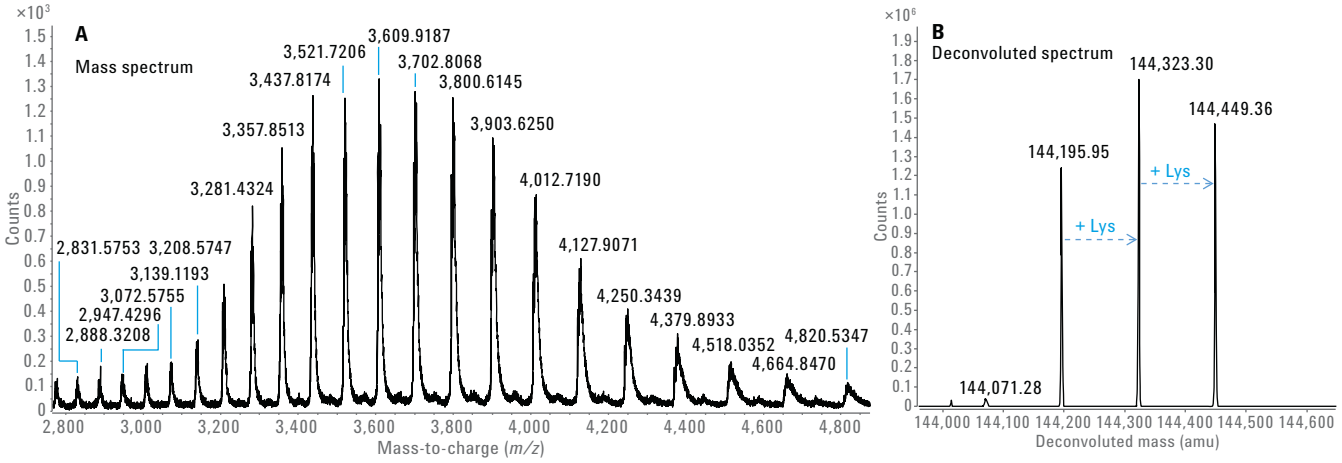

Figure 2. CZE/MS analysis of Biosimilar–Reditux.

Conclusion

This application note shows mAb charge variant analysis on Agilent 7100 CE system. The CZE analysis provided high resolution analysis of charge variants of innovator and biosimilar rituximab. CZE as method provides a fast and simple to use alternative to isoelectric focusing (IEF) methods that are often used for charge heterogeneity analysis. CZE/MS analysis provides detailed characterization of basic charge variants of biosimilar rituximab.

References

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