Profiling and characterization of charge heterogeneity of biologics using capillary electrophoresis and mass spectrometry

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ABSTRACT

Biological therapeutics such as monoclonal antibodies (mAbs), engineered fusion bodies, and multi-specific antibodies have great potential in clinical applications. They are generally cell-originated and are subject to post-translational modifications (PTMs) that can introduce charge heterogeneity to the molecule. Monitoring of these variants is required throughout manufacturing to assess drug purity and heterogeneity to ensure the safety and efficacy of the drug.
Hence, robust analytical methods for reliable charge variant analysis are needed. In this study, we demonstrate a simple capillary zone electrophoresis (CZE) based methodology for charge variant profiling and monitoring on PA 800 Plus pharmaceutical analysis system. A seamless bridge for peak characterization via the ZipChip system coupled to the SCIEX TripleTOF 6600 system was achieved.

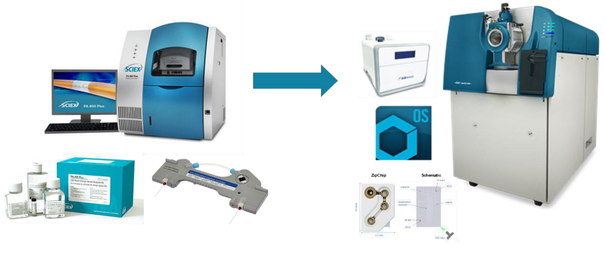
INTRODUCTION

Currently, multiple strategies are used for performing charge variant analysis for biological therapeutics, such as ion exchange chromatography (IEX), capillary isoelectrical focusing (cIEF) and capillary zone electrophoresis (CZE).[1,2] IEX and cIEF are common, but both methods are relatively slow, requires additional method development work for new molecules, and the peak identification often require development and validation of an orthogonal analytical technique. CZE can combine the benefits of native state analysis and platform capability. With the speed and high resolution, coupled with high-resolution mass spectrometry (MS), CZE can readily separate and identify different charge variants.

In this study, we demonstrate a simple methodology to quickly separate and quantify charge variants in various mAb samples, for monitoring and quality control purposes on the PA 800 Plus. Parallel charge variant characterization via the ZipChip system coupled to the SCIEX TripleTOF 6600 System demonstrated correlating peak profiles between CZE-UV and ZipChip-MS traces (Figure 1).

MATERIALS AND METHODS

All commercialized biologic therapeutics are purchased from Myonex. Proteins were diluted with water or buffer exchanged into water for a final concentration of 0.5- 1.0 mg/mL before analysis.
CZE-UV analysis of the proteins was carried out on a PA 800 Plus equipped with UV detector and a 214 nm bandpass filter. Separations were performed on a pre-assembled bare fused silica cartridge (SCIEX P/N A55625) at 1000 V/cm field strength unless noted otherwise. Sample was introduced into the capillary via pressure injection for 10 seconds at 0.5 psi. The separation buffer used was from the CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C44790). Instrument control and data acquisition were done using 32 Karat software V10.0.060



The CE-MS analysis was performed using the ZipChip system (908 Devices Inc.) with an autosampler coupled with SCIEX TripleTOF 6600 system. An HRN chip together with the Charge Variant TOF kit (includes protein dilute and separation BGE) were used here. For each analysis, 1 nL (1 ng) of the sample was injected onto the chip. The separation was performed at 500 V/cm with Pressure Assist turned on after 0.5 minutes. The total analysis time was set to 15 min. The ZipChip system is controlled with ZipChip software while the SCIEX TripleTOF 6600 system was controlled with Analyst TF. The detailed MS parameters can be found in Ref 3 and 5. BioPharmaView software (SCIEX) was used for MS data processing.

RESULTS

CZE and ZipChip-MS analysis were performed for traditional mAbs, such as trastuzumab (Figure 1) and infliximab (Figure 2), multispecific protein (Figure 3), and fusion proteins (Figure 4). In all instances, the CZE charge variant profiles aligned with the ZipChip-MS profiles.

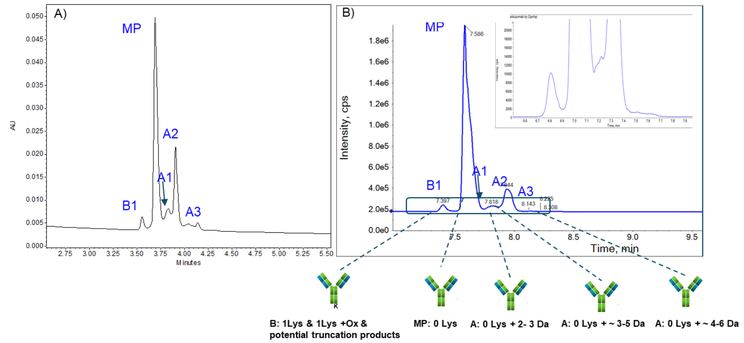


Figure 1: A side-by-side comparison between the charge variant separation with (A) CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus and (B) CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to a SCIEX TripleTOF 6600 system for trastuzumab , The highly sensitive 6600 TripleTOF system was able to differentiate and identify different species based on their masses for coeluting peaks.

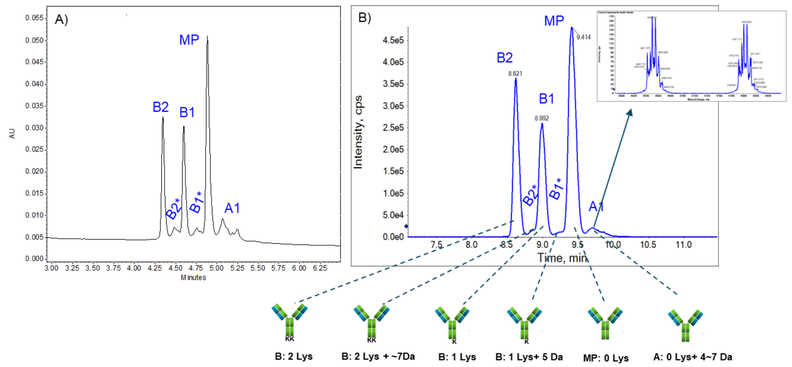


Figure 2: A side-by-side comparison between the charge variant separation with (A) CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus and (B) CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to a SCIEX TripleTOF 6600 system with the insert showing the raw spectrum of the acidic variant for Infliximab. The high resolution TripleTOF system was able to generate high quality spectra correlating with variant glycan modification.

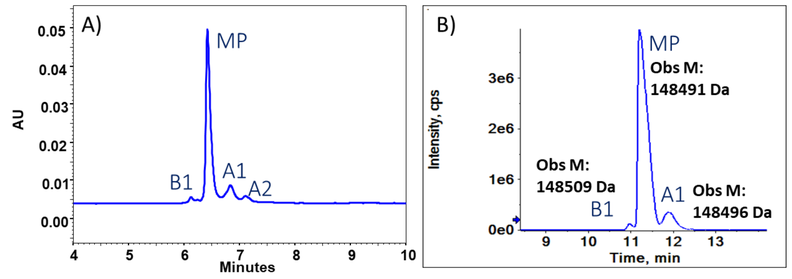


Figure 3: A side-by-side comparison between the charge variant separation with (A) CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus and (B) CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to a SCIEX TripleTOF 6600 system for emicizumab-kxwh.

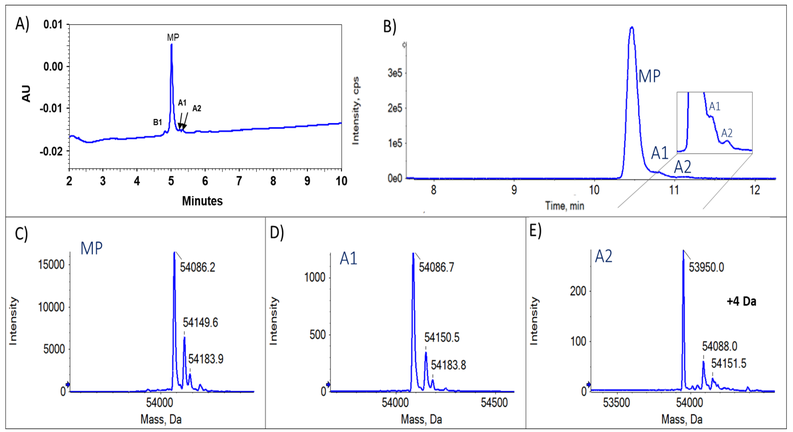


Figure 4. A side-by-side comparison between the charge variant separation with (A) CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus and (B) CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to SCIEX TripleTOF 6600 system (B) for blinatumomab.

CONCLUSIONS

- Rapid charge variant analysis times (<10 minutes) of the native forms of biologic therapeutic using CZE-UV
- Ready to use kits and pre-assembled cartridge enables streamlined analysis
- Seamless bridge from peak profiling with CZE-UV to peak characterization with ZipChip Charge Variant TOF kit with good data alignment
- Platform method for intact protein analysis with flexibility for method development and optimization
- Good profile correlation between CZE-UV and ZipChip-TOF
- Excellent MS sensitivity for identification of low abundant peaks

REFERENCES

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[5] SCIEX technical note, Charge heterogeneity characterization of next-generation antibodies using capillary electrophoresis and mass spectrometry. SCIEX technical note, RUO-MKT-02-13124-A.

TRADEMARKS/LICENSING