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

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Introduction

This technical note illustrates a rapid profiling method for antibody charge variants during product development, production, stability and release testing using capillary zone electrophoresis (CZE). Subsequent analysis of the variants using ZipChip® system coupled to the SCIEX TripleTOF® 6600 MS System provides a deeper characterization of the molecule.

Biologics such as monoclonal antibodies (mAb) are cell-originated and are subject to post-translational modifications (PTMs) including sialylation, deamidation, oxidation, glycation, and clipping of C-terminal lysine. Monitoring of these variants is required throughout manufacturing to assess drug purity and heterogeneity to ensure the safety and efficacy of the drug.

Hence, there is a need for enhanced analytical methods for reliable profiling and characterization of charge variants and its degradation products. Currently multiple strategies are used for performing charge variant analysis for mAbs, 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, require additional method development work for new molecules, and the peak identification often require development and validation of an orthogonal analytical techniques. CZE can combine the benefits of native state analysis, platform capability. With the speed and high resolution and when coupled with high-resolution mass spectrometry (MS), CZE can readily separate and identify different charge variants.

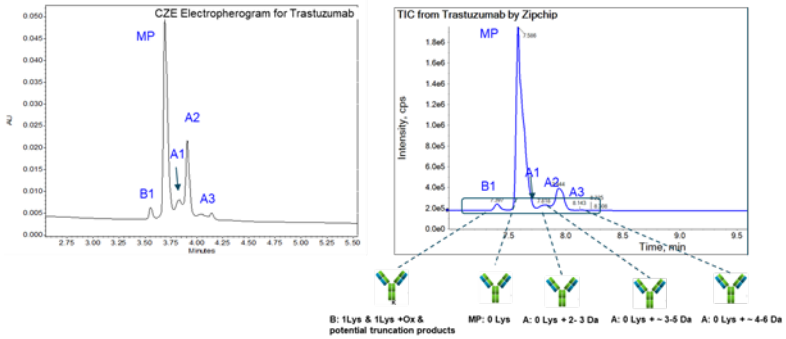

Figure 1. Comparison between the charge variants separation with CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System (left) and CZE-MS assay using Charge Variant TOF kit on a ZipChip coupled to SCIEX TRIPLETOF 6600 LC MS/MS system (right) for Trastuzumab.

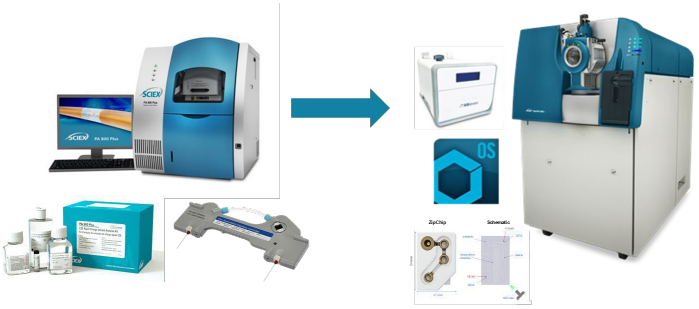

Figure 2. Quick charge heterogeneity profiling of mAb therapeutics followed by identification of peaks using ZipChip system couple to TripleTOF 6600.

In this study, we demonstrate a simple to quickly separate and quantify charge variants in various mAb samples for monitoring and quality control purpose on PA800 Plus Pharmaceutical Analysis system. Parallel charge variant characterization via the ZipChip® system coupled to the SCIEX TripleTOF® 6600 MS System [3] demonstrated correlating peak profiles between CZE-UV and ZipChip-MS traces (Figure 1).

Key features

- Reliable, high resolution, rapid charge variant profiling (<10 minutes) of the of mAb using CZE under native conditions
- Seamless bridge from peak profiling using CZE-UV for routine product monitoring followed by deep peak characterization with ZipChip TOF MS providing good profile alignment.
- Easy and ready to use kits for both platforms without complex sample preparation.

Methods

Sample preparation:

In this study, 5 monoclonal antibodies: NIST mAb reference material 8671 (10 µg/µL), trastuzumab (20 µg/µL), adalimumab (50 µg/µL), infliximab (10 µg/µL), and rituximab (10 µg/µL) were used. All mAb samples were diluted to a concentration of 1 µg/µL with CE grade water before loading onto instruments for either CZE_MS analysis using ZipChip with the TripleTOF 6600 system or CZE-UV Rapid Charge Variant Analysis using PA 800 Plus Pharmaceutical Analysis System.

Capillary electrophoresis-UV:

The capillary electrophoresis instrument used was a PA 800 Plus equipped with UV detector and a 214 nm bandpass filter (SCIEX P/N 144437). 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 was done using 32 Karat softwareV10.0.060

Capillary electrophoresis-mass spectrometry:

The CE-MS analysis was performed using the ZipChip system (908 Devices Inc.) consisting of an optional autosampler, coupled with SCIEX TripleTOF 6600 system. A “high resolution native” (HRN) chip together with the Charge Variant TOF kit, containing premixed buffers were used here. For each analysis, 1 nL (1 ng) of the mAb sample was injected onto the chip. The separation was performed at 500 V/cm with pressure assistance turned on after 0.5 minutes. If autosampler is used, minimum 20 µL of the diluted sample is loaded into sample vial for injection. 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. A detailed MS parameters can be found in Table 1.

Table 1. MS Parameters.

Parameter	Setting
Scan Mode	*TOF MS positive*
Gas 1	*4 psi*
Gas 2	*0 psi*
Curtain Gas	*10 psi*
Time Bins to Sum	*100*
Interface Heater Temperature	*50 °C*
Accumulation Time	*0.5 sec*
Mass Range	*1,000 - 6,000 m/z*
Declustering Potential	*160 V*
Collision Energy	*80 eV*

Data processing:

All CZE_UV data was processed using 32 Karat software V10.0.060 and the MS data was processed using SCIEX OS 2.0 with BioTool kit add on.

Results and discussion

CZE_UV rapid charge variant analysis repeatability

High-resolution separation of intact mAbs can be achieved by CZE in less than 10 minutes using the Rapid Charge Variant Analysis kit. Analysis of trastuzumab shows ~65% of the sample is the main product (at 3.8 min), ~5% (left of main peak) is coming from the basic variants and ~30% from acidic variants (right of main peak). Triplicate analysis of the sample was performed with good repeatability as shown in the overlays of the electropherograms in Figure 3. As shown in table 2, the standard deviation of the migration time of the MP from the triplicate injections was only 0.01 mins.

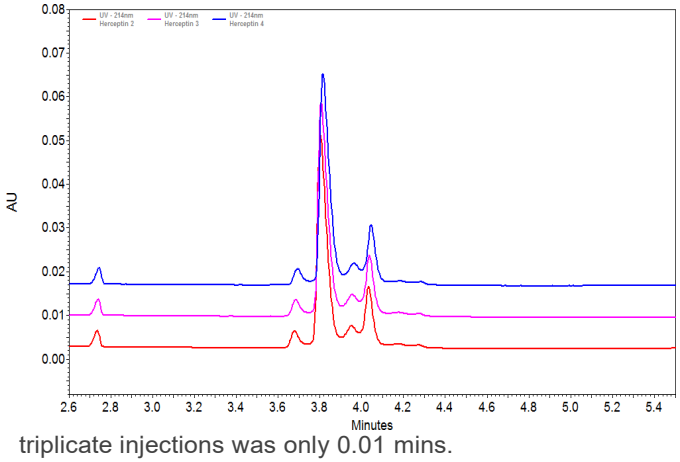

Figure 3. Overlay electropherograms of triplicate injections of Trastuzumab analyzed using CZE Rapid Charge Variant Analysis Kit wth UV detection at 214 nm.

Table 2. Migration time of the main peak (MP) of trastuzumab and the %distribution of the main peak (MP), total acidic variants and total basic variants.

	MP MT (min)	%Basic	%MP	%Acidic
Inj #1	3.80	4.97	65.11	29.91
Inj #2	3.81	5.06	64.94	29.99
Inj #3	3.82	4.86	65.33	29.80
Average	**3.81**	**4.96**	**65.13**	**29.90**
STDEV	**0.01**	**0.10**	**0.20**	**0.10**

CZE-MS charge variant analysis/identification repeatability

For further characterization of the peaks observed by CZE-UV, the trastuzumab was then analyzed using the ZipChip HRN chip coupled with SCIEX Triple TOF 6600 MS system using the Charge Variant TOF kit. The peak profile observed aligns well with the results from the CZE-UV analysis (figure 3). In both cases, there was a basic variant that migrated early followed by the main product and trailed by 3 acidic variants. Replicate analysis of the sample shows good repeatability of this technique (Figure 4), despite slightly bigger shift in migration time. Statistics are provided in table 3.

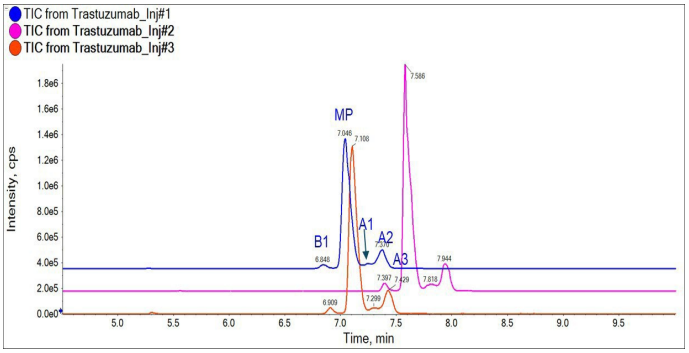

Figure 4. Overlay total ion electropherogram (TIC) of the separation of different trastuzumab charge variants using ZipChip HRN chip coupled with SCIEX TripleTOF 6600 LC MS/MS system.

	MP MT (min)	%Basic	%MP	%Acidic
Inj #1	7.11	2.44	81.78	15.79
Inj #2	7.59	2.59	81.03	16.37
Inj #3	7.05	2.14	82.15	15.71
Average	**7.25**	**2.39**	**81.66**	**15.96**
STDEV	**0.24**	**0.19**	**0.46**	**0.30**

Table 3. Migration time of the main peak (MP) of trastuzumab and the %distribution of the main peak (MP), total acidic variants and total basic variants.

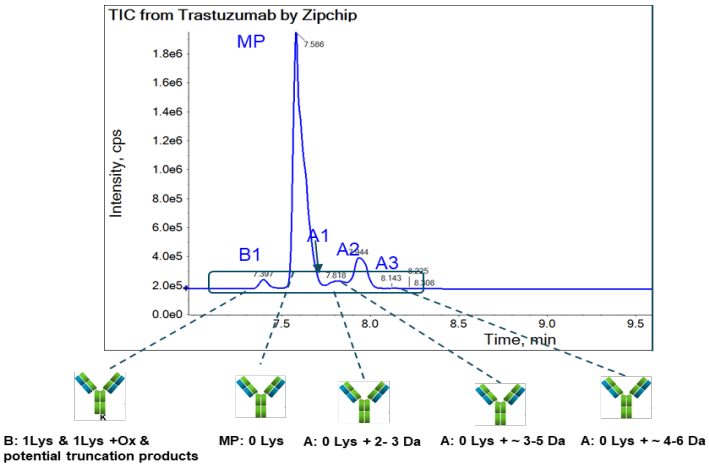

Figure 5: CZE-MS assay using Charge Variant TOF kit on a ZipChip coupled to SCIEX TripleTOF 6600 system (right) for trastuzumab.

Mass spectral interpretation of the peak profile shows that the basic peak (B1) consists of multiple truncated variants including a variant with loss of one lysine as well as its oxidized form. The acidic peaks (A1-3) ranges from 2- 6 Da addition to the molecular weight of the main product (MP). This may correspond to various deamidated forms of the product (Figure 5). Further site specific analysis such as peptide mapping of the mAb can be performed to identify the localization of these modification sites.

Similarly, CZE and ZipChip-MS analysis were performed for NIST, adalimumab, rituximab and infliximab with the results shown in figure 6-9. In all instances, the CZE charge variant profiles aligned with the ZipChip-MS profiles. In some cases, the lower abundant peaks were not well separated with the ZipChip-MS compared to the CZE. However, the highly sensitive 6600 TTOF was able to differentiate the variants based on differences in masses. One such example are the 2 basic variants, B1 and B2 shown in figure 8 for infliximab. Using CZE, the less abundant B2* is well resolved from the more abundant peak B1, however they are less resolved with the ZipChip-MS. The mass spectra of the B1 peak shows the presence of a different variant in addition to the more abundant variant with the loss of one lysine.

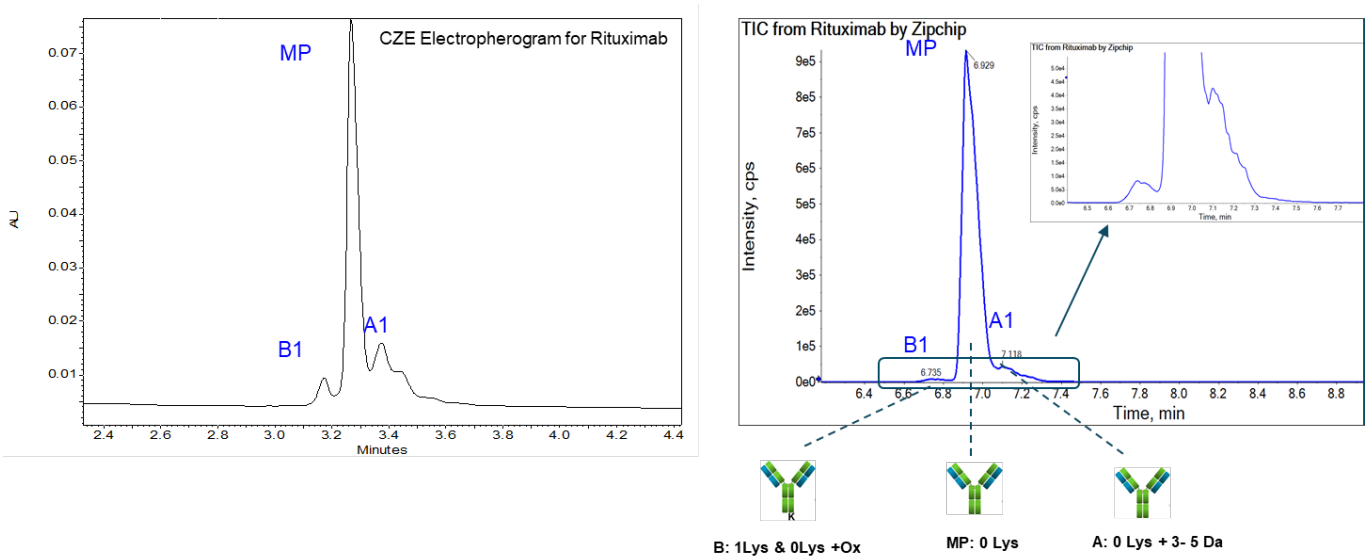

Figure 6. A side by side comparison between the charge variants separation with CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System (left side) and CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to SCIEX TripleTOF 6600 MS system (right side) for rituximab.

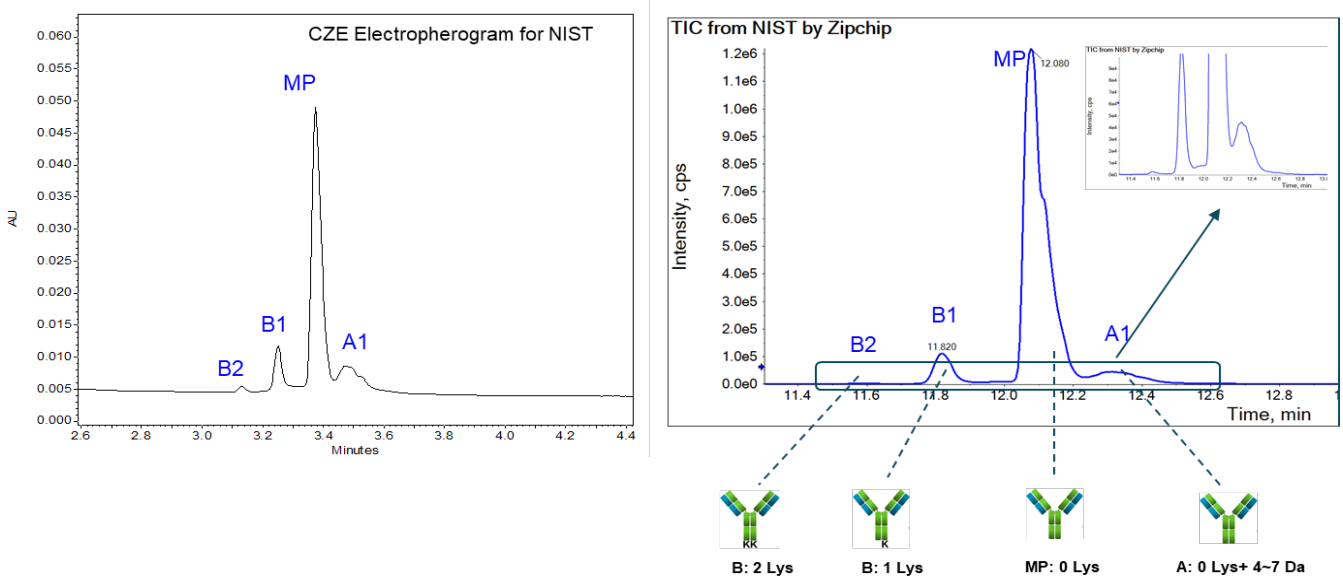

Figure 7. A side by side comparison between the charge variants separation with CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System (left side) and CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to SCIEX TripleTOF 6600 MS system (right side) for NIST mAb.

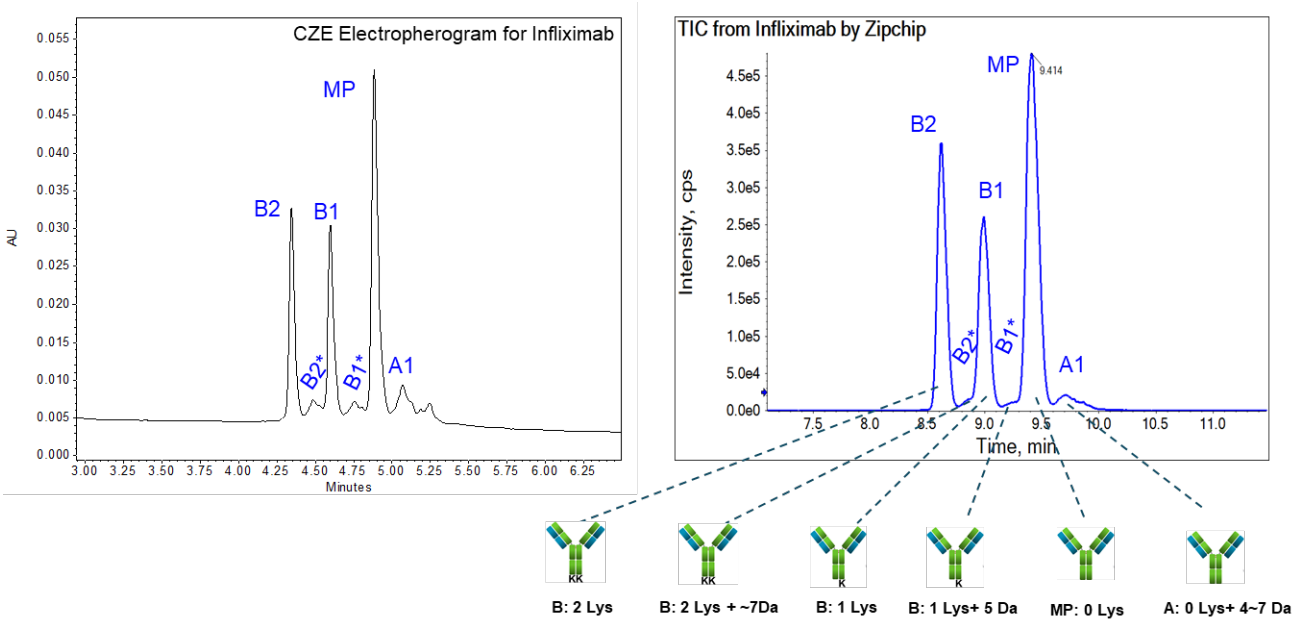

Figure 8. A side by side comparison between the charge variants separation with CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System (left side) and CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to SCIEX TripleTOF 6600 MS system (right side) for infliximab

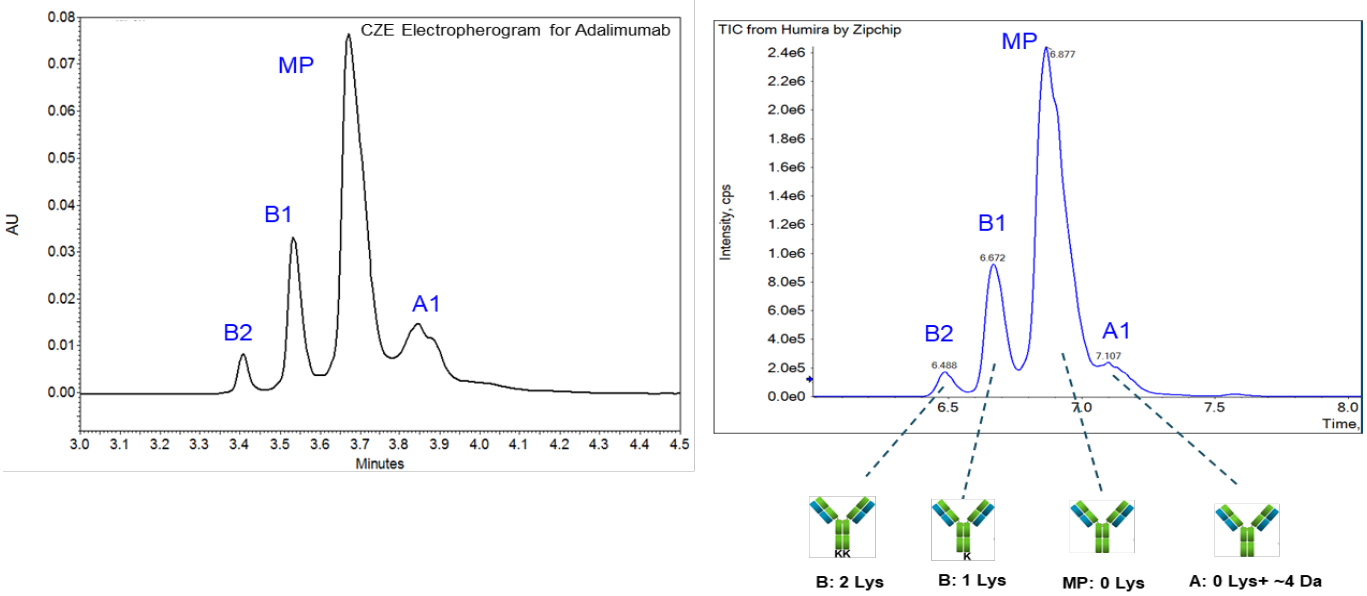

Figure 9. A side by side comparison between the charge variants separation with CZE-UV assay using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System (left side) and CZE-MS assay using Charge Variant TOF Kit on a ZipChip coupled to SCIEX TripleTOF 6600 MS system (right side) for adalimumab

Conclusions

- Rapid charge variant analysis times (<10 minutes) of the native forms of mAb 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 Native 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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2. Sosic, Z., et al., Application of imaging capillary IEF for characterization and quantitative analysis of recombinant protein charge heterogeneity. Electrophoresis, 2008. 29(21): p. 4368-76.
3. Characterization of charge heterogeneity of monoclonal antibodies using capillary electrophoresis (CE) coupled to mass spectrometry (MS), SCIEX, RUO-MKT-02-10949-A

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