Native State Charge Variant Analysis of Commercialized Monoclonal Antibodies

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This poster described how a High resolution charge variant analysis can be done in minutes using commercial kits and is :-

High resolution charge variant analysis done in minutes using commercial kits

Reproducible: Separations are shown to be reproducible through both replicate injections of commercial mAbs and an inter-company collaboration[1]

Platform capable: The simplicity of separation allows for application to molecules across a broad pI range between 7.0 and 9.5[1]

High-throughput: High resolution separation can be achieved in less than 5 minutes with only two minutes of buffer replenishment in between injections, making the total sample analysis time under 10 minutes

Versatile: Additional resolution can easily be obtained through use of a longer bare-fused silica capillary, lower capillary temperature or separation voltage. Further development is possible by modifying buffer pH and levels of additives[2]

Results of Application Tests

To test the application and the new CZE chemical kit, 4 different commercial mAbs were obtained. Figures 1 through 4 show the charge variant profile of Remicade, Herceptin, Rituxan and Drozitumab biosimilar obtained with the SCIEX CZE Rapid Charge Variant Analysis Kit. The insert in each figure represent the zoomed-in view of the baseline. Ten injections of each molecule were also made to show the reproducibility and robustness of the assay. The peak profiles were stable and the %RSDs of relative area percentages of most acidic variants, basic variants and main peaks were below 2% (shown in Tables 1-4).

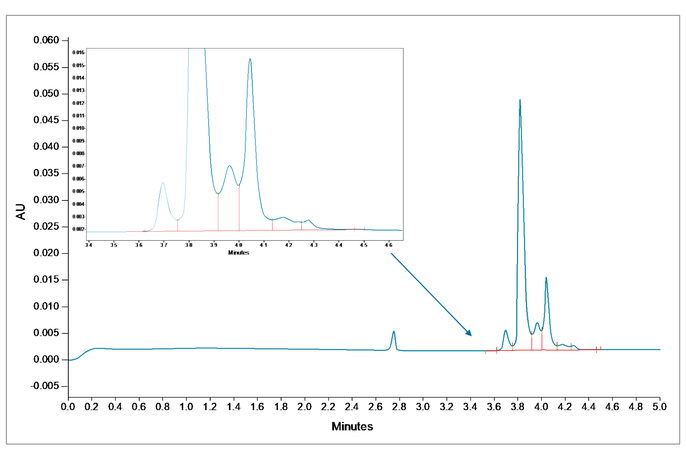

Figure 1. Charge variant analysis of Herceptin (trastuzumab).

Table 1. Statistics for variant analysis of Herceptin (trastuzumab).

	Total Acidic Variants (%)	Total Main Peak (%)	Total Basic Variants (%)
Avg	28.7	66.1	5.2
STDEV	0.2	0.2	0.1
%RSD	0.8	0.4	1.4

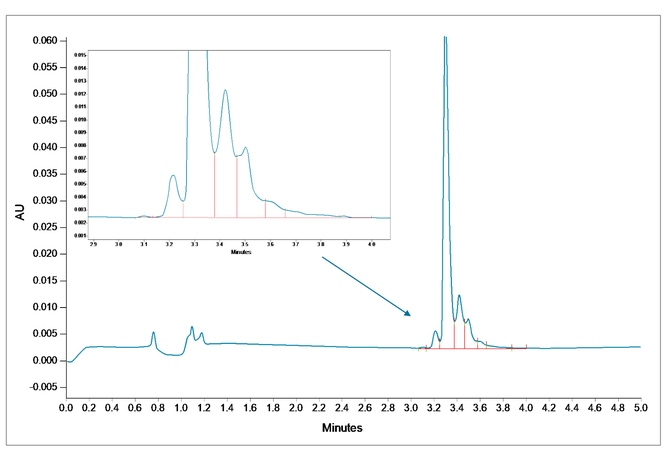

Figure 2. Charge variant analysis of Rituxan (rituximab).

Table 2. Statistics for variant analysis of Rituxan (rituximab).

	Total Acidic Variants (%)	Total Main Peak (%)	Total Basic Variants (%)
Avg	12.0	78.7	9.4
STDEV	0.2	0.5	0.5
%RSD	1.7	0.6	5.5

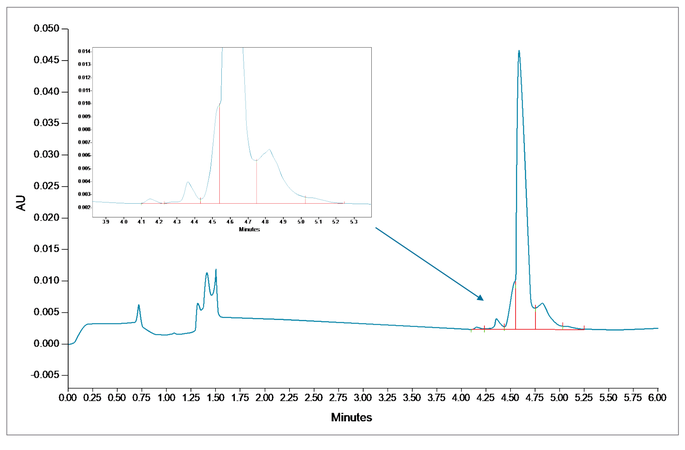

Figure 3. Charge variant analysis of Drozitumab biosimilar.

Table 3. Statistics for variant analysis of Drozitumab biosimilar.

	Total Acidic Variants (%)	Total Main Peak (%)	Total Basic Variants (%)
Avg	24.2	72.1	3.7
STDEV	0.4	0.4	0.1
%RSD	1.8	0.5	1.5

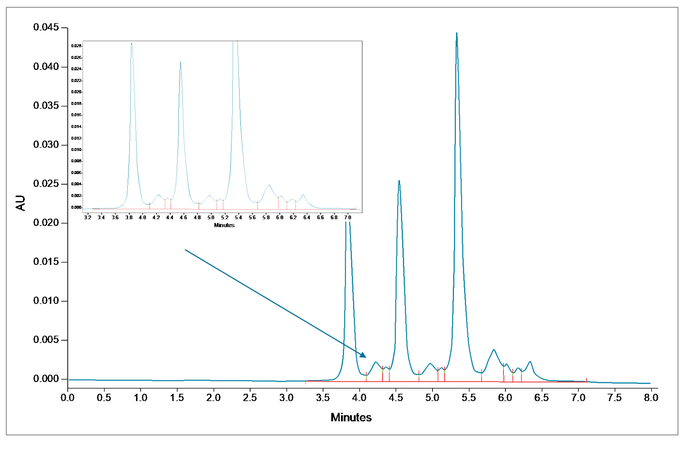

Figure 4. Charge variant analysis of Remicade (infliximab).

Table 4. Statistics for variant analysis of Remicade (infliximab).

	Total Acidic Variants (%)	Total Main Peak (%)	Total Basic Variants (%)
Avg	11.1	38.4	50.5
STDEV	0.2	0.2	0.1
%RSD	1.5	0.4	1.3

Project Background

Biopharmaceutical companies are under increasing pressure to finalize analytical methods earlier in drug development using less material. Finding a robust, stability-indicating method that can monitor charge variants is an ongoing analytical challenge. Traditional method development approaches for native proteins involving ion-exchange chromatography (IEX) can span weeks or months. Capillary isoelectric focusing (cIEF), although quicker than IEX, analyzes proteins in denatured forms that might not perform the same way as native proteins. Capillary zone electrophoresis (CZE) combines the benefits of a native state analysis with the speed and resolution expected of a capillary electrophoresis method. CZE is the simplest form of CE and separates based on the differences in a molecule's electrophoretic mobility, which is directly related to the charge on the protein. Using a widely used buffer, this technique requires little to no method development, has a simple sample preparation and a higher analytical throughput than IEX or cIEF. In this project, a commercial available CE system is used, fitted with a UV detector, a prebuilt cartridge and a new **CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C4479)** designed to be used for charge variant analysis of proteins, to analyze 4 commercial monoclonal antibodies which have been prepared in **CE Grade water (SCIEX P/N C48034**).

REFERENCES

1. Moritz, B. et.al. *J. Chromatogr.* B 2015, 983–984, 101–110.
2. Moritz, B. et. Al. J., *Electrophoresis* 2017, 38, 3136-3146.

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