

Native State Charge Variant Analysis of Commercialized Monoclonal Antibodies in Minutes

モノクローナル抗体医薬品のネイティブ状態での電荷不均一性試験、数分間の迅速分離で。

Key Words : IgG 抗体、電荷不均一性、翻訳後修飾、迅速化、簡便化、チャージバリエント迅速分析用 CZE キット、PA800 Plus

抗体医薬品開発の迅速化と効率化（コスト軽減含む）が色々な側面から求められ、翻訳後修飾などに基づく電荷不均一性評価も、頑健性・安定性を維持したままにこれらの要求に応えなければなりません。

等電点電気泳動よりも迅速でよりネイティブ状態の評価法として、キャピラリーゾーン泳動法（CZE）が注目され、Sciex からチャージバリエント迅速分析用 CZE キット（製品番号 C44790）として導入されました。ほとんどの抗体について希釈のみのサンプル調製により、泳動条件検討無しで 10 分以内に分離結果が得られます。

PA800 Plus で UV 検出器 (214 nm) で、Herceptin® (trastuzumab, Fig.2)、Rituxan® (rituximab, Fig.3)、Remicade® (infliximab, Fig.4) を評価しました。その結果、Remicade® は 8 分、他は 5 分以内に良好に分離されました。3 種の抗体とも、各部分の補正面積値 % の再現性 (%RSD、10 回繰返し) はメイン部分で 0.5% 以内、酸性・塩基性部分では 2% 以内と良好でした (Table 1, 2, 3)。

以上の結果から、チャージバリエント迅速分析用 CZE キットは迅速かつ高再現性な電荷不均一性評価法として有用なことが認められました。現在の抗体医薬品開発／製造の状況に広く対応でき、条件検討がほとんど必要ありません。サンプル調製も大幅に簡便化できます。

このキットは、抗体医薬品純度試験のグローバルスタンダードとなっている SDS キャピラリーゲル泳動と同様に、PA800 Plus で運用可能です。キャピラリーやチップでの方法を含む等電点電気泳動法や、イオン交換 LC 法に比べても、より High-Throughput に対応できる技術です。

Technology

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CZE Rapid Charge Variant Analysis Kit

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Introduction

As biopharmaceutical companies face increasing pressure to decrease the overall drug development timeline, analytical researchers find themselves finalizing methods earlier in development with less material on hand. Of all the analytical methods to be developed, charge variant analysis methods used to monitor post-translational modifications (PTMs), require the longest time commitment. Quickly finding a robust, stability-indicating method that can monitor charge variants is an ongoing analytical challenge.

HPLC-based ion-exchange chromatography (IEX) methods commonly analyze biotherapeutics in their native state. These methods can be optimized with regard to column stationary phase, mobile phase pH, type of and concentration of buffer salts, gradient conditions, and a host of other parameters. Development of a suitable method for a single molecule can easily span weeks to months.

Capillary Isoelectric Focusing (cIEF) charge variant methods generally analyze biotherapeutics in a denatured state. Development time is often much shorter compared to IEX due to fewer parameters to optimize, including buffer additives, concentration and focusing time. However, when analyzed in a denatured state, generated stability data does not always trend as well as native state stability data.

Capillary Zone Electrophoresis (CZE) combines the benefits of a native state analysis with the speed and resolution expected of a capillary electrophoresis method. Using a widely universal buffer,¹ this technique requires little to no method development, has a simple sample preparation and a higher analytical throughput than IEX or cIEF.

The SCIEX CZE Rapid Charge Variant Analysis Kit (P/N C44790) provides high resolution, high sensitivity CZE separations.

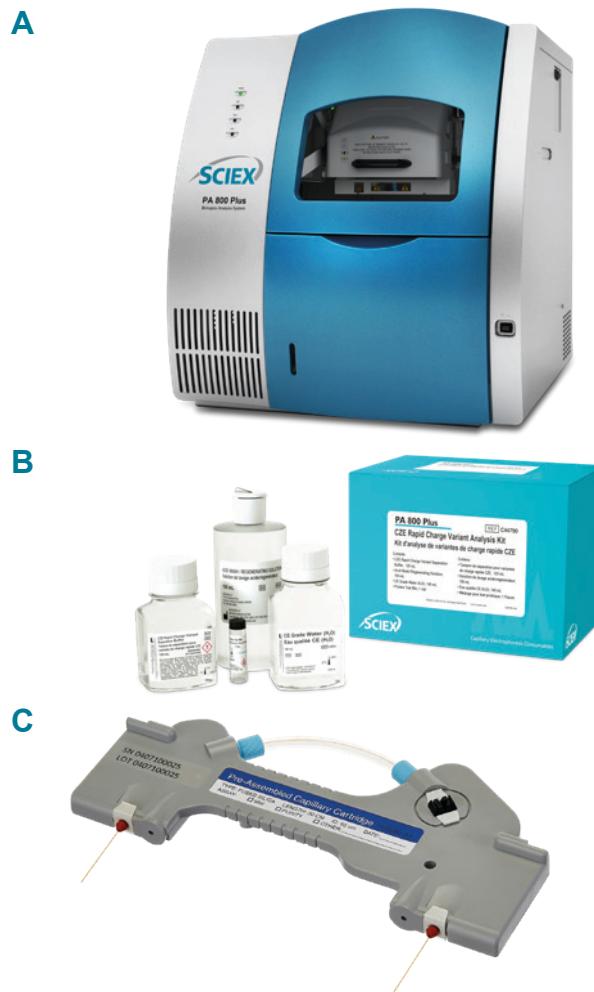


Figure 1. (A) The PA 800 Plus Pharmaceutical Analysis System, (B) the CZE Rapid Charge Variant Analysis Kit (P/N C44790) and (C) pre-assembled cartridge (P/N A55625).

Charge Heterogeneity in Minutes

- Quantify charge variants in their native state
- Little-to-no method development
- Dilute, shoot and prepare for your next CE application
- Platform capable methodology

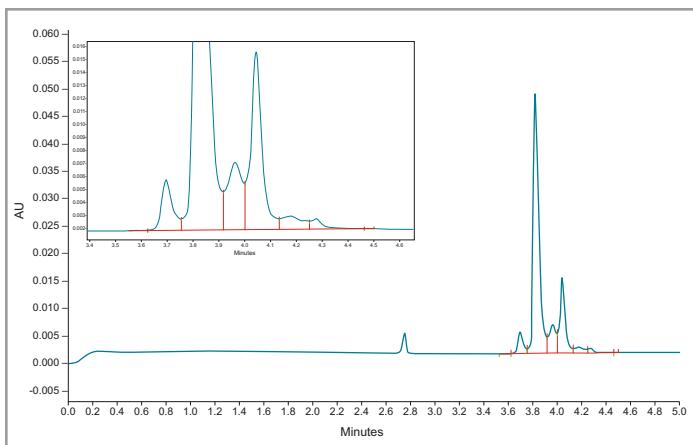


Figure 2. Fullview and zoomed electropherogram of Herceptin® (trastuzumab).

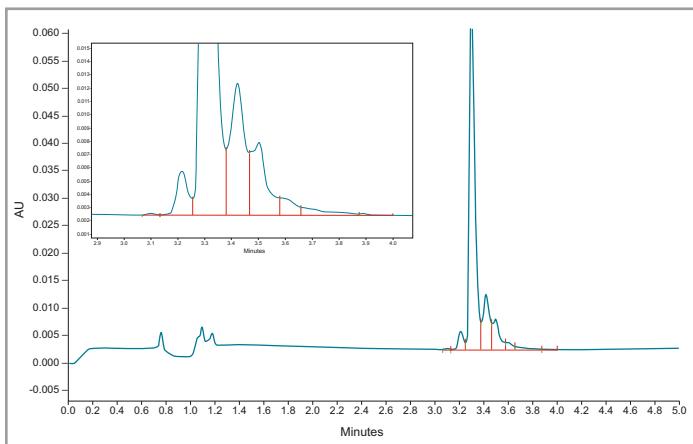


Figure 3. Fullview and zoomed electropherogram of Rituxan® (rituximab).

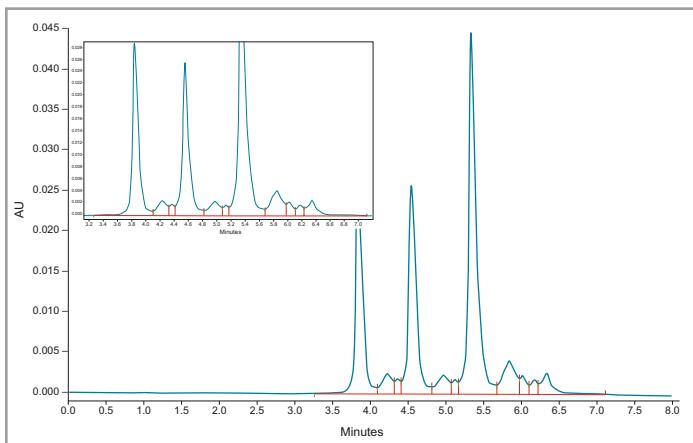


Figure 4. Fullview and zoomed electropherogram of Remicade® (infliximab).

Demonstrated here are the charge variant analyses of multiple commercially available monoclonal antibodies on the PA 800 Plus platform using this kit. This method can easily be applied to therapeutic proteins such as antibody-drug conjugates (ADCs), pre-clinical monoclonal antibodies (mAbs), and fusion proteins with little to no method development. Additionally, it can be easily modified to suit more specific user criteria.

Methods

Instrumentation

All experiments were performed on the PA 800 Plus Pharmaceutical Analysis System (SCIEX). A Pre-Assembled Cartridge (SCIEX P/N A55625) was used for the separation of all three commercially available monoclonal antibodies: Herceptin® (trastuzumab), Rituxan® (rituximab) and Remicade® (infliximab).

Reagents

Reagents used to prepare the samples and analysis buffers were provided in the CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C44790). The therapeutic proteins of Herceptin®, Remicade® and Rituxan® were purchased from Myoderm (Norristown, PA).

Sample Preparation

Herceptin®, Remicade® and Rituxan® were diluted to 1 mg/mL in CE Grade water (SCIEX P/N C48034).

Instrumentation

The capillary electrophoresis instrument used was a PA 800 Plus equipped with UV detection and a 214nm bandpass filter (SCIEX P/N 144437).

Separation and Analysis

Separations were performed at 1000 V/cm and injection was performed by pressure for 10 seconds at 0.5 PSI. The separation buffer used was from the CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C44790). Data acquisition and analysis was done using 32 Karat software V10.

Technology

Results:

To investigate the reproducibility of the assay, 10 injections of each molecule were made. The peak profile was stable and %RSDs of the total relative area percentages of acidic variants, basic variants and main peak were below 2% (tables 1-3).

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

Table 1. Statistics of Percent Total Acidic variants, Percent Total Main Peak, and Percent Total Basic Variants of Herceptin® (trastuzumab).

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

Table 2. Statistics of Percent Total Acidic variants, Percent Total Main Peak, and Percent Total Basic Variants of Remicade® (infliximab).

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

Table 3. Statistics of Percent Total Acidic variants, Percent Total Main Peak, and Percent Total Basic Variants of Rituxan® (rituximab).

Conclusions

- **Reproducible:** CZE separations are shown to be reproducible through both replicate injections of commercial mAbs and an inter-company collaboration.²
- **Platform capable:** The simplicity of separation and optimal pH of the buffer allows for application to molecules across a broad pI range above 7.0.²
- **Limited method development:** Additional resolution can easily be obtained through the use of a longer bare-fused silica capillary, lower capillary temperature or separation voltage. Further development has focused on buffer pH and levels of additives.³
- **High-Throughput:** High-resolution separation can be achieved in approximately 5 minutes or less. With only two minutes of buffer replenishment in between injections, total sample analysis time can be completed in under 10 minutes.

References

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