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Capillary Isoelectric Focusing (cIEF)

As a Platform Method for the Evaluation of Monoclonal Antibody Charge Variants

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Abstract

The determination of charge variants of therapeutic proteins is a regulatory requirement for pharmaceutical companies. This application note demonstrates the suitability of capillary isoelectric focusing (cIEF) to assess the charge heterogeneity of different types of monoclonal antibodies. Here, we analyzed the charge variants of 3 different IgGs, including an innovator molecule and its candidate biosimilar, using the PA800 Plus Pharmaceutical Analysis System from Sciex. Our results prove that the developed cIEF method can be used as a platform to obtain unique profiles for different IgGs and effectively resolve their charge variants, with enough sensitivity to identify differences between the innovator and its biosimilar.

Introduction

Monoclonal antibodies (mAbs) are large, complex molecules which contain a certain degree of natural heterogeneity. Slight differences in a mAbs structure can cause variations in the overall charge of the molecule to emerge. Charge variants can be caused by modifications, such as C-terminal lysine truncation, deamidation and other post-translational modifications. These components are normally referred to as acidic or basic depending on how their isoelectric point (pI) compares to the main species. Modification of the charge variant ratios induced during manufacture, storage and transport, can potentially shift the pI of the protein, leading to detrimental effects on drug activity[1]. Analytical methods that can monitor charge variant ratios during manufacture and lot release are therefore essential to ensure drug safety and efficacy.

Capillary Isoelectric Focusing (cIEF) is a technique with high peak capacity and high-resolution power based on the separation of amphoteric molecules according to their pI, which can effectively distinguish between molecules that have minor differences in the net charge. This application note describes a versatile, broad pH cIEF method for the assessment of charge variant profiles of five different marketed mAbs, including an innovator molecule and one of its approved biosimilars.

The assay utilizes a Sciex PA 800 Plus instrument, which is the industry standard for GMP-compliant testing of protein biotherapeutics.

Assay Principle

The cIEF method used in this application is a two-step technique. During the first step or focusing, a mix of ampholytes, catholyte, anolyte and the sample are introduced into a fused-silica capillary. On applying a high voltage, a pH gradient is established across the capillary, and the different components of the sample are distributed according to their pI. In the second stage, the catholyte solution is swept by a chemical mobilizer, which disrupts the pH gradient and provides the analytes with charge. This forces the analytes to move through the capillary until reaching the UV detector

Experimental

Samples

The samples were added to a mastermix made of Urea-cIEF gel solution and containing cathodic and anodic stabilizers, a broad range carrier ampholytes and three synthetic peptides as pI markers.

Instrument

The instrument used for this analysis was a Sciex PA 800 Plus Pharmaceutical Analytical System, set-up with a UV detector and a 50 µm ID Neutral Coated Capillary with a total length of 30.2 cm.

Data Analysis

Data was collected and processed using 32 Karat 10.3 software.

Results and Discussion

The electropherograms obtained for three of the mAb samples can be seen in Figure 1. A distinctive peak profile can be seen for the different mAb samples, each having peaks in different migration time and pI ranges. Three areas can be distinguished for all samples: the main peak, the basic variant area, with the peaks eluting before the main peak, and the acidic area, with the peaks eluting after the main peak. The relative distribution of acidic, main and basic variants was established from the corrected area of the peaks for each analyzed mAb sample and listed in Table 1.

Sample	mAb subclass	% Basic	% Main Peak	% Acidic
Infliximab (innovator)	IgG1	50.3	35.8	13.9
Infliximab (biosimilar)	IgG1	41.6	39.3	19.1
Trastuzumab	IgG1	2.7	32.2	65.1
Denosumab	IgG2	5.3	56.5	38.2
Nivolumab	IgG4	23.1	46.5	30.4

Table 1: Calculated percentage of charge variant ratio for each mAb sample

The electropherograms in Figure 1 also show the three pI markers included in the mastermix. The comparison of the migration time of the pI markers in all samples and replicates was highly reproducible, with RSDs lower than 1% for all pI markers 10, 9.5 and 4.1.

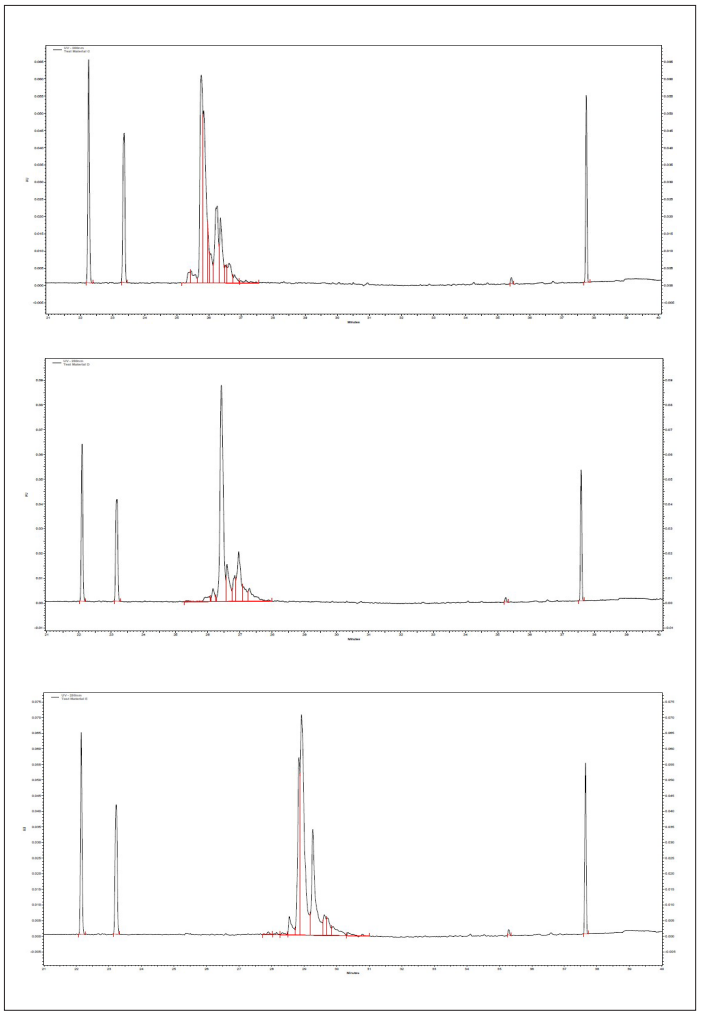

Fig. 1: Electropherograms for Trastuzumab (top), Denosumab (middle) and Nivolumab (bottom). Three pI markers with pI 10. 9.5 and 4.1 can be seen at both sides of the electropherograms. A peak profile with different migration time and pI ranges was obtained for each sample.

Figure 2 shows the overlaid electropherograms for Infliximab and its biosimilar. The figure shows the differences in peak profile, consisting of a variation between the first basic species, as well as the acidic species. As shown in Table 1, this represents an almost 10% difference between the basic and acidic relative content between the samples. These differences are consistent with those previously reported in the literature using an orthogonal technique, however they have been found to have no effect on the clinical efficacy for these two drugs[2-3].

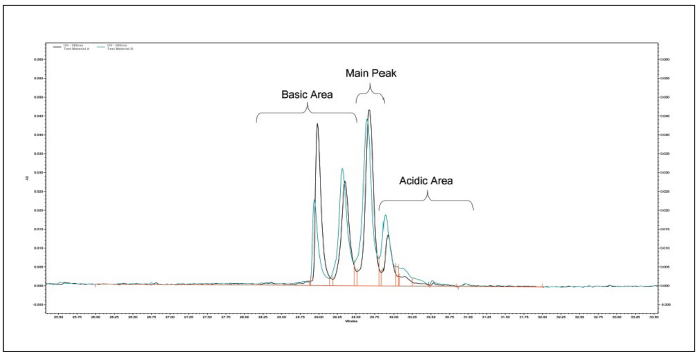

Fig. 2: Overlay of the electropherograms for Infliximab innovator (black) and its approved biosimilar (green), showing the differences between the acidic and basic regions between the molecules.

Conclusion

The results presented here demonstrate the versatility of cIEF as a platform method for the analysis of monoclonal antibodies. From the five mAb samples analyzed, three were IgG1, one was an IgG2 and one was an IgG4. Each sample presented a unique peak profile with a different pI range, it also showed the pI markers at highly reproducible migration times. The high resolution obtained for each mAb allowed the distribution of charge variants to be established and analyzed.

In addition to resolution, the method demonstrated to be sensitive enough to identify relatively small differences in charge profiles, which provides biosimilar developers with a strong analytical comparability tool to assess charge variant differences and to potentially reduce this variance through process development optimization, where required.

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