Enhanced QbD approaches in unravelling mysteries of charge heterogeneity of biotherapeutics

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1. Introduction

The charge variant profile of biotherapeutic products plays a crucial role in its efficacy, safety and quality. Qualitative or quantitative differences of product charge variants may affect its biological functions, pharmacokinetics and pharmacodynamics.

2. cIEF

cIEF uses a pH gradient generated in a capillary to separate proteins based on their isoelectric point(pI), where a protein has no net charge [1].

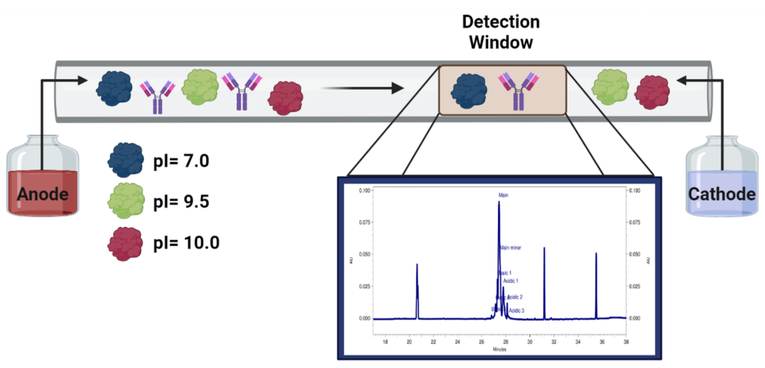

Figure 1: cIEF: Working Principles of Charge Variant Analysis

3. IEX-HPLC

Ion Exchange - High Pressure Liquid Chromatography (IEX-HPLC) uses a column packed with an ion exchange resin to separate proteins according to their charge. It separates proteins according to their charge by gradually changing pH or salt concentration of the eluent [1, 2, 3].

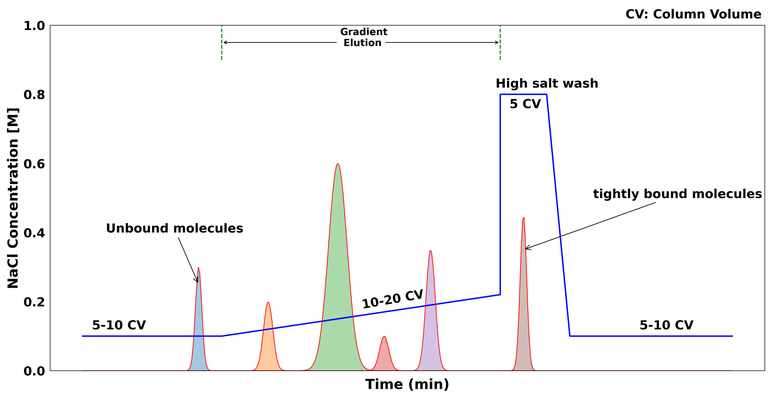

Figure 2: The ideal IEX separation: target proteins well resolved by gradient elution

4. Analytical Workflow

The monoclonal antibody product was subjected to charge variant analysis using both IEX-HPLC and cIEF techniques. QbD principles were applied to evaluate the suitability of these techniques. The design of experiments (DOE) approach was used to establish the relationship between critical process parameters (CPPs) and CQAs. The CPPs evaluated for IEX-HPLC were column temperature, pH, and salt concentration, while for cIEF, they were sample preparation, pH, and voltage.

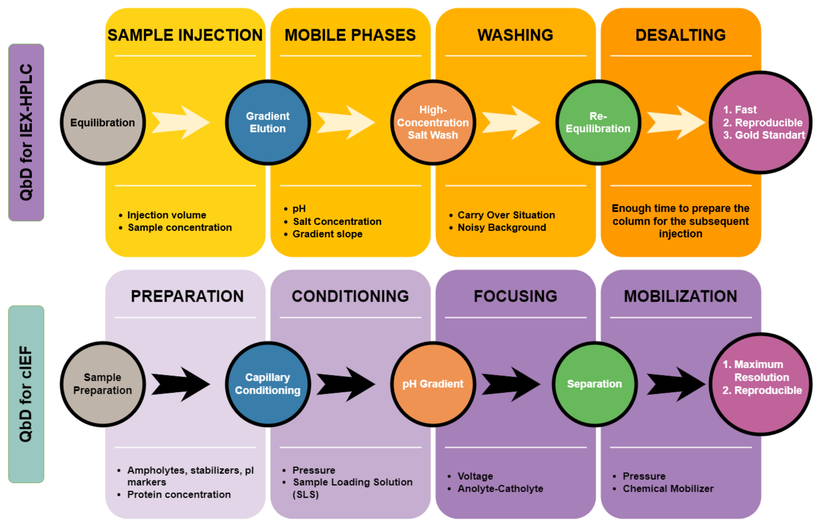

Figure 3: QbD Workflows

5. Statistical Comparison

In this study, the consistency between IEX-HPLC and cIEF methods was evaluated using Bland-Altman statistics. The analysis results showed a coherence between IEX-HPLC and cIEF methods for acidic, basic, and main variants. Overall results revealed that both methods provided high accuracy and repeatability.

$$mean\ diff = \frac{1}{n}\sum_{i=1}^{n}(x_i - y_i) \quad and \quad SD\ diff = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i - mean\ diff)^2}{n-1}}$$

Here, x_i and y_i represent the results measured with two different methods. "mean diff" represents the mean difference, and "SD diff" represents the standard difference.
Statistical comparison plots are shown below.

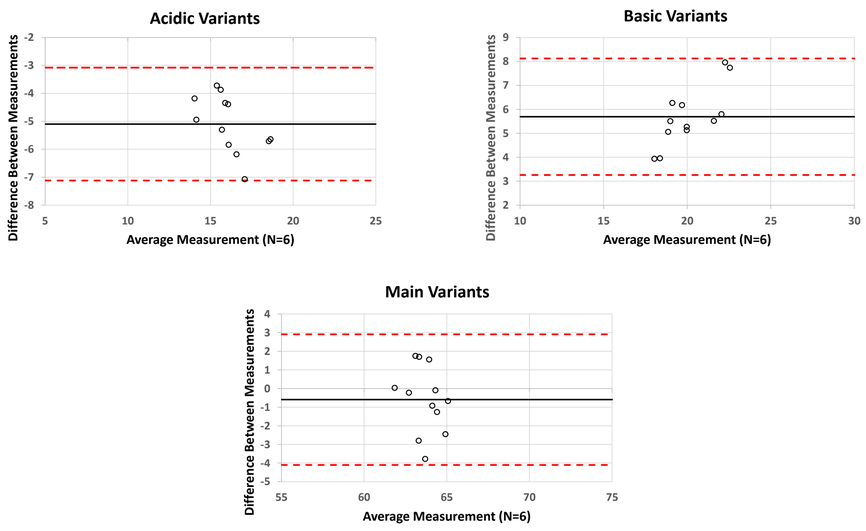

Figure 3: Bland-Altman Statistics Result (It includes double injection data for each sample.)

6. Conclusions

This study demonstrated that different orthogonal methods can be used interchangeably, as shown by Blant-Altman statistics. Different methods with similar performance characteristics can increase flexibility in method selection, and potentially reduce the time and consumables required for development and validation. Therefore, the demonstration of interchangibility between IEX-HPLC and cIEF have significant implications on creating analytical target product profile and the life cycle management of the method. Furthermore it increases the confidence in the results obtained from testing during process development. In conclusion, this study is novel in that it showed the comparison of different methods as emphasized for Analytical Procedure Lifecycle (ICH Q14)[4], in the Turkish biotechnological companies with advanced statistics.

7. References

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