Charge Variant Analysis of Trastuzumab using a bioZen™ 6 µm WCX Column with a pH Gradient and Native MS Detection

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Overview

Charge variants of proteins commonly result from post translational modifications (PTMs) during recombinant production. These PTMs, including C-terminal lysine clipping, deamidation and glycosylation, give rise to changes in the acidic and basic charged residues relative to the native protein. The most common method to detect and assess these acidic and basic variants is cation-exchange chromatography (CEX), typically weak cation-exchange (WCX). Ion-exchange, unlike most other interactive chromatography mechanisms, is an on / off process and relies on the electrostatic interaction of analyte with stationary phase. The retention of an ionic compound depends on the number of charges as well as their location on the molecule. Elution is accomplished by increasing the concentration of salt when using a salt gradient or increasing the pH of the mobile phase when using a pH gradient. Optimization of the elution conditions is important to ensuring a good, reproducible method that gives consistent results. Ion exchange chromatography is a powerful technique for identifying charge heterogeneity of a monoclonal antibody. However, the presence of a previously undetected isoform typically leads to further analysis requirements which in themselves can be problematic. For example, the process used to identify unknown isoforms may create further post translational modifications. However, using native conditions for cation-exchange chromatography and high resolution mass spectrometry eliminates these issues. The utility of combining these techniques is reported in this application using Trastuzumab.

In this application note we show the separation of charge variants of Trastuzumab under native conditions using a pH gradient formed with volatile buffers. The novel, MS-friendly buffers used for the pH gradient are stable for at least one week at room temperature when an inlet air filter is used (ex. SecurityCAP™) on the reservoir.

The chromatogram generated with the pH gradient using volatile buffers is very similar to one acquired using a common, commercially available, non-volatile buffer, HEPES (4-(2 Hydroxyethyl)piperazine-1-ethanesulfonic acid), with a salt gradient.

A very detailed separation of isoforms was achieved using a longer column, bioZen 6 µm WCX 250 x 2.1 mm. Mass spectra from this detailed separation we were used to identify the main peak glycoforms as well as those of the lysine variant.

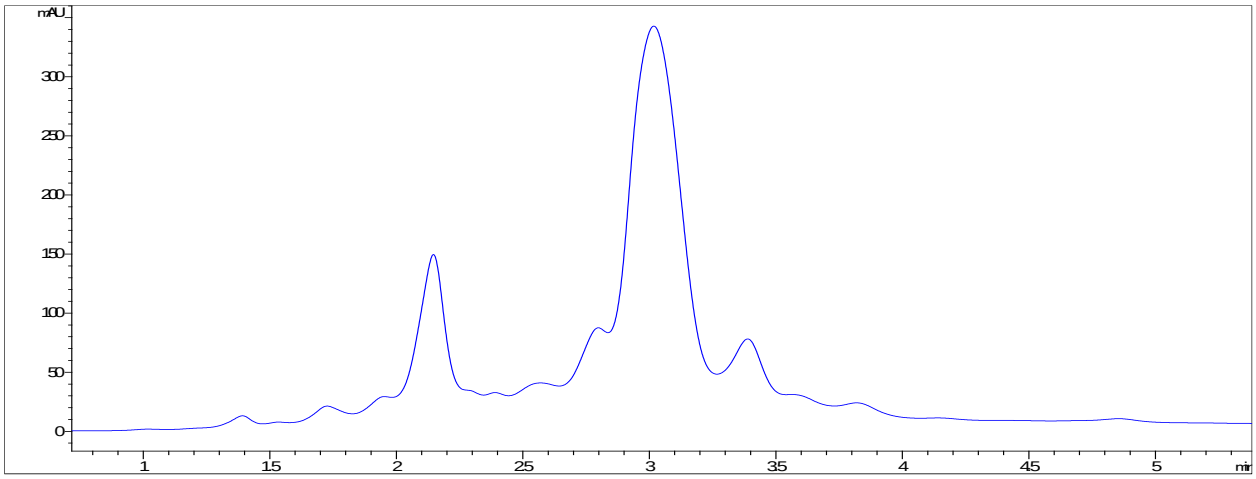
LC Conditions

Column: bioZen 6 µm WCX
Dimension: 50 x 2.1 mm
Part No.: 00B-4777-AN
Mobile Phase: **A:** 20 mM Ammonium Acetate, pH 5.2
B: 5 mM Ammonium Acetate, pH 10.2
Gradient: 20-50 % in 5 min
Flow Rate: 0.5 mL/min
Temperature: 30 °C
Detector: UV @ 280 nm
Sample: Trastuzumab, 50 µg

LC-MS/MS Conditions

Column: bioZen 6 µm WCX
Dimension: 250 x 2.1 mm
Part No.: 00G-4777-AN
Mobile Phase: **A:** 20 mM Ammonium Acetate, pH 5.2
B: 5 mM Ammonium Acetate, pH 10.2
Gradient: 20-50 % in 25 min
Flow Rate: 200 µL/min
Temperature: 30 °C
Detector: QTOF (SCIEX® X500B)
Sample: Trastuzumab, 50 µg

UV Chromatogram on 50 x 2.1mm bioZen™ 6 µm WCX using pH Gradient Buffer System



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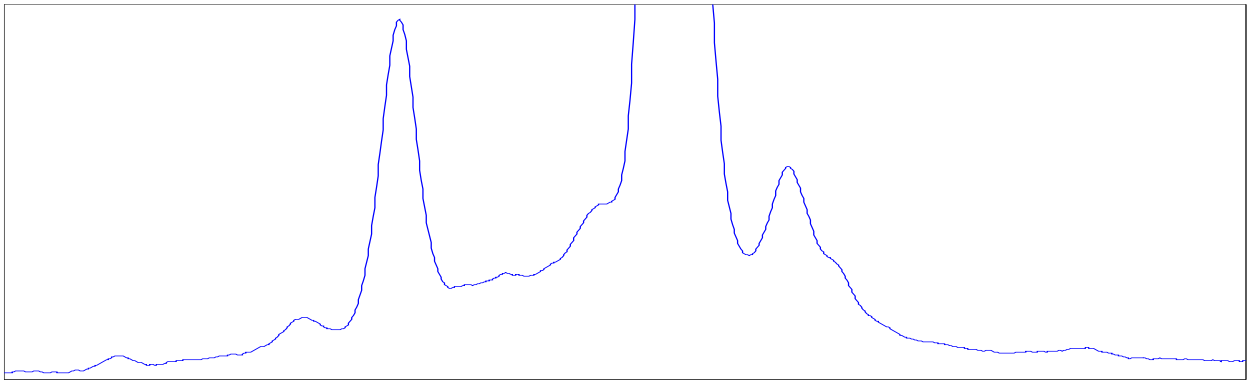
UV Chromatogram on 50 x 2.1mm bioZen 6 µm WCX using HEPES buffer with a salt gradient



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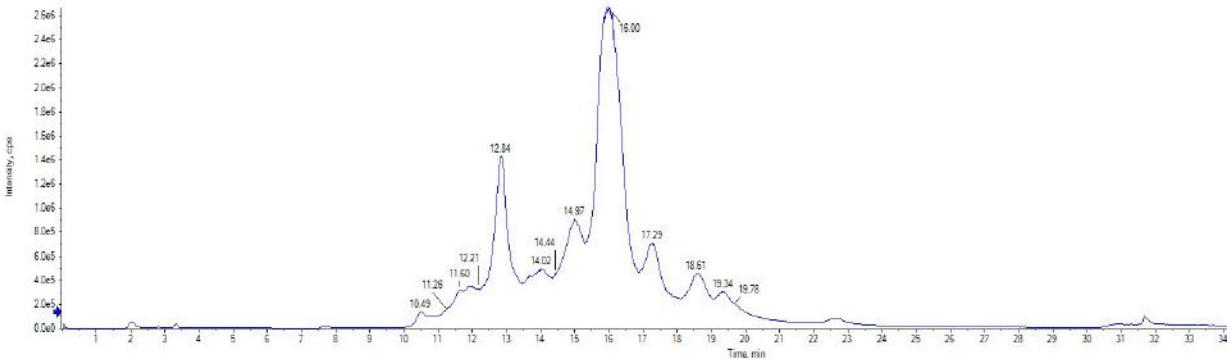
MS TIC Chromatogram on a 250 x 2.1mm bioZen 6 µm WCX using pH Gradient Buffer System



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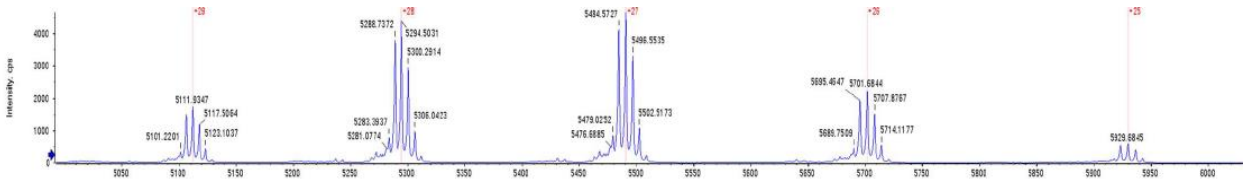
High Resolution Raw Mass Spectra for Main Peak



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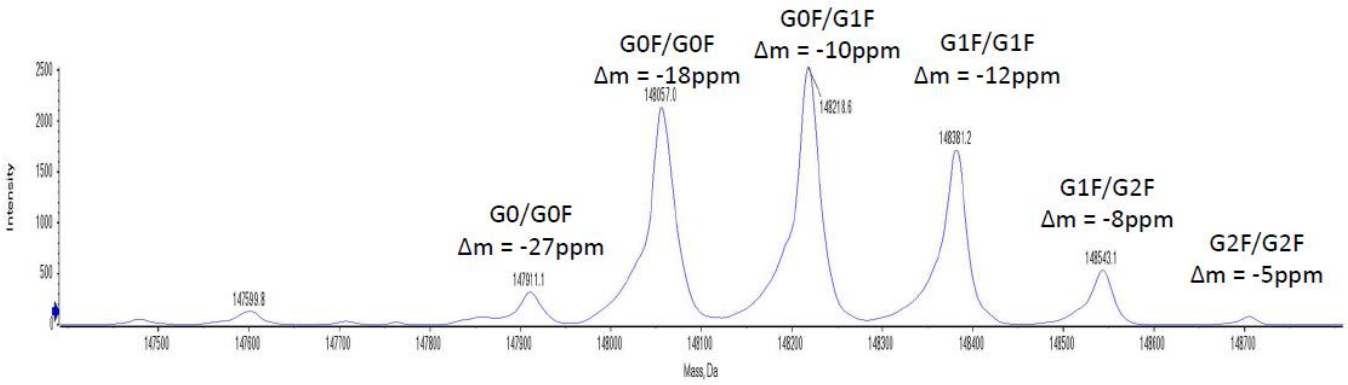
Main Peak Glycoforms



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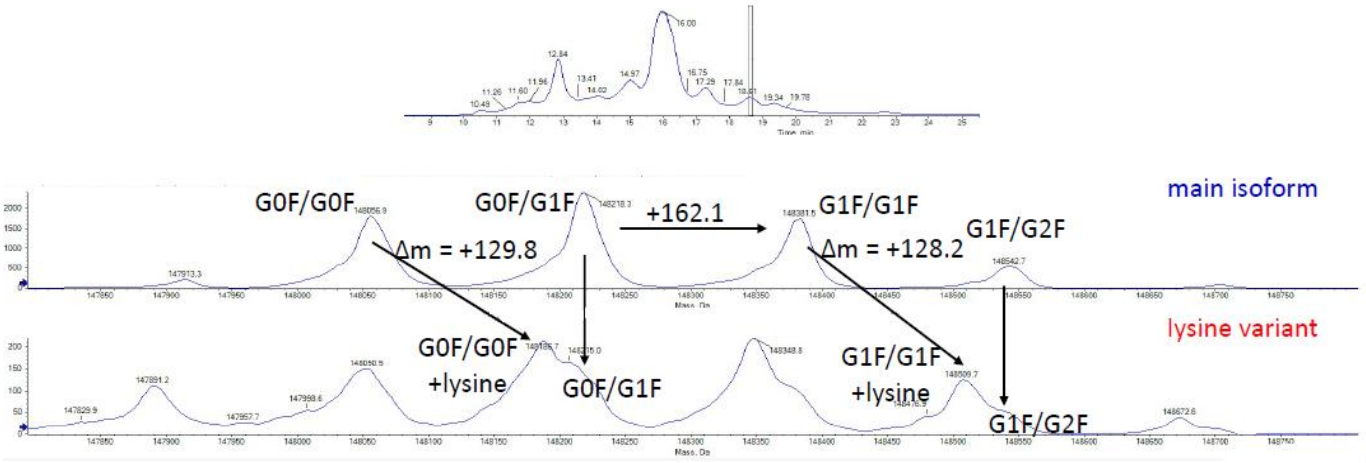
Lysine Variant



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