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

Carl Sanchez[1] and Helen Whitby Ph.D.[2]
[1] Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2] Phenomenex, Ltd., Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN UK

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.

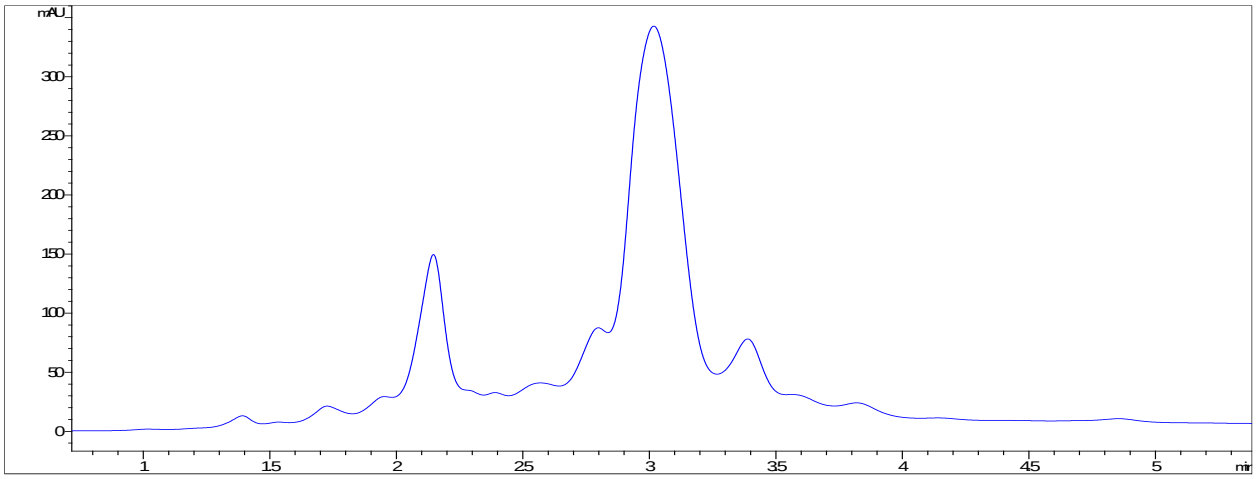
LC Conditions

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

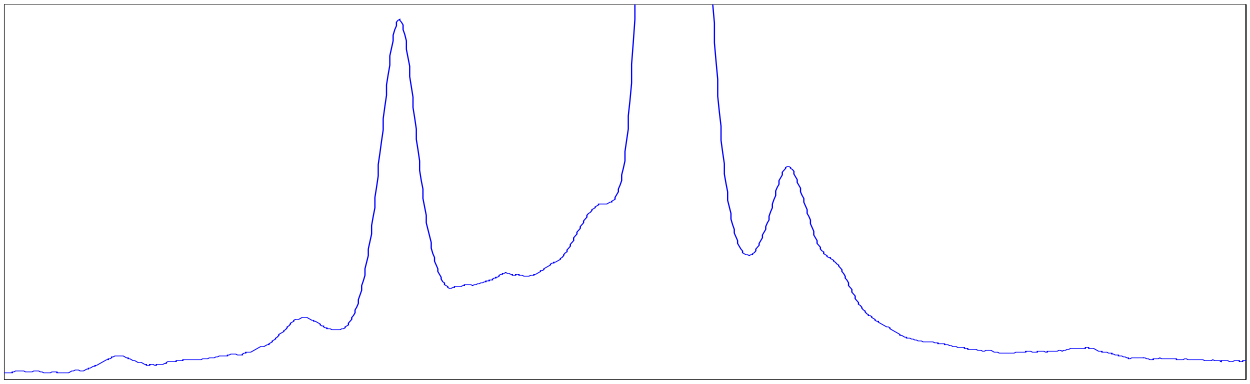
Column: bioZen 6 µm WCX
Dimension: 250 x 2.1 mm
Part No.: 00G-4777-AN
Mobile Phase: **A:** 20 mM Ammonium Acetate, pH 5.2
B: 5 mM Ammonium Acetate, pH 10.2
Gradient: 20-50 % in 25 min
Flow Rate: 200 µL/min
Temperature: 30 °C
Detector: QTOF (SCIEX® X500B)
Sample: Trastuzumab, 50 µg

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



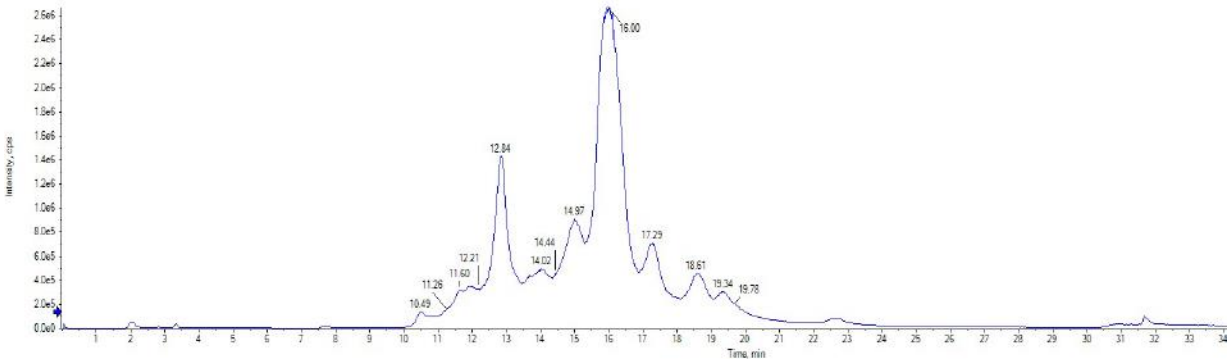
App ID 25587

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



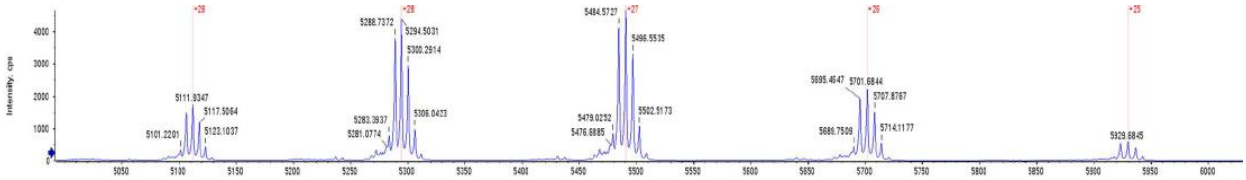
App ID 25587

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



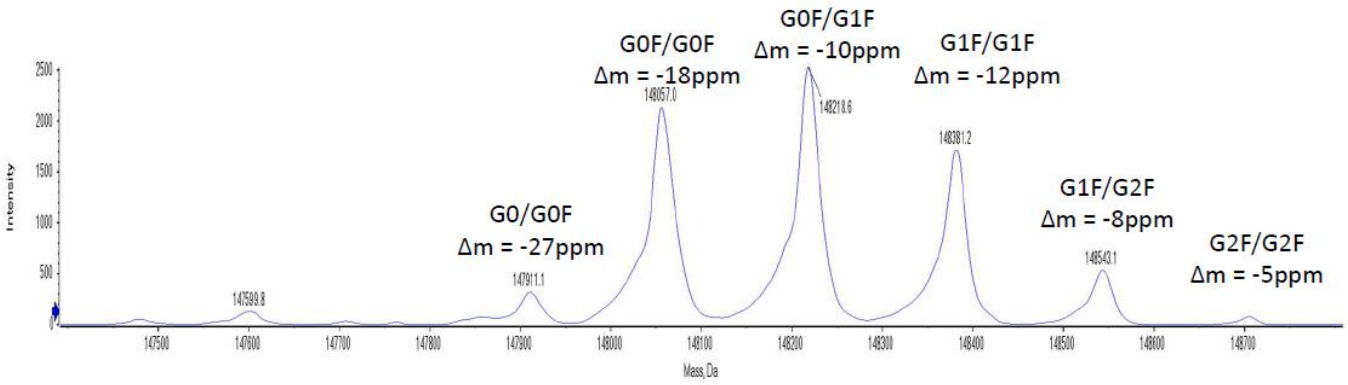
App ID 25587

High Resolution Raw Mass Spectra for Main Peak



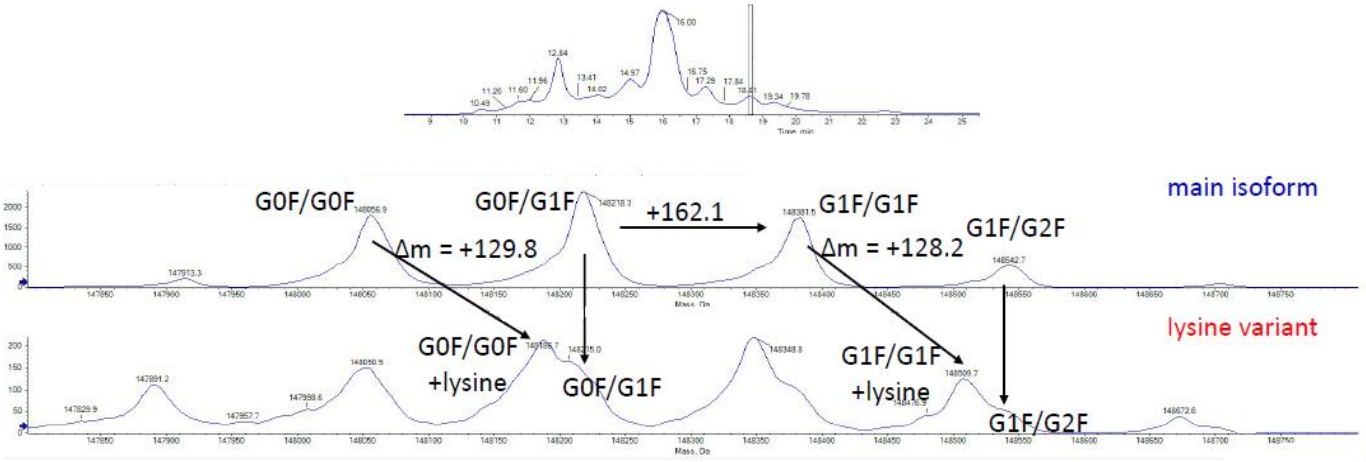
App ID 25587

Main Peak Glycoforms



App ID 25587

Lysine Variant



App ID 25587

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN69260719_W