

APPLICATIONS

Ion-Exchange Chromatography for Charge Variant Analysis of Trastuzumab under pH and Salt Gradients using a bioZen™ 6 µm WCX Column

M. Christina Malinao and Chad Eichman, Ph.D.
Phenomenex, 411 Madrid Avenue, Torrance, CA 90501 USA

Overview

Charge variants of proteins commonly result from post translational modifications (PTMs) during recombinant production. These PTMs, including C-terminal lysine clipping and glycosylation, result in acidic and basic charged residues relative to the native protein. The most common method to detect and assess acidic and basic variants is through ion-exchange chromatography (IEX), specifically weak cation-exchange (WCX). Trastuzumab, marketed under the trademark Herceptin®, is a common monoclonal antibody in the biopharmaceutical industry. The determination of charge variants in trastuzumab is assessed on the bioZen 6 µm WCX column using both a pH and salt gradient. For the pH gradient, CX-1 gradient buffers from Thermo Fisher Scientific® were employed on a 0-100 % linear pH gradient over 20 min (**Figure 1**). This approach provided 23 % acidic variants and 13 % basic variants. For the salt gradient, N-morpholino ethane sulfonic acid (MES) in combination with increasing NaCl (300 mM) was utilized (**Figure 2**). This approach provided 27 % acidic variants and 12 % basic variants. Both gradients sufficiently separate the acidic and basic variants from the neutral trastuzumab with the use of the bioZen WCX ion-exchange column.

Sample Preparation

Trastuzumab (2 mg/mL) was injected directly onto the column.

pH Gradient Conditions (Figure 1)

Column: bioZen 6 µm WCX
Dimensions: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: CX-1 pH Gradient Buffer A (pH 5.6)
 B: CX-1 pH Gradient Buffer B (pH 10.2)
Gradient: 0-100 % B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm
Temperature: 30 °C
Injection Volume: 15 µL
Samples: Trastuzumab

Salt Gradient Conditions (Figure 2)

Column: bioZen 6 µm WCX
Dimensions: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: 20 mM MES (pH 5.6)
 B: 20 mM MES + 300 mM NaCl (pH 5.6)
Gradient: 20-50 % B in 30 min
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm
Temperature: 30 °C
Injection Volume: 15 µL
Samples: Trastuzumab

Figure 1.
Charge variant profile using pH gradient

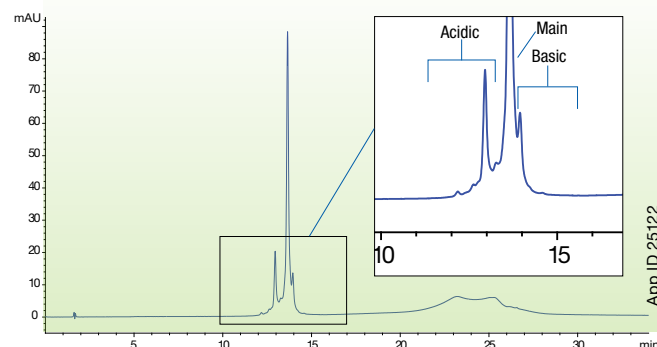
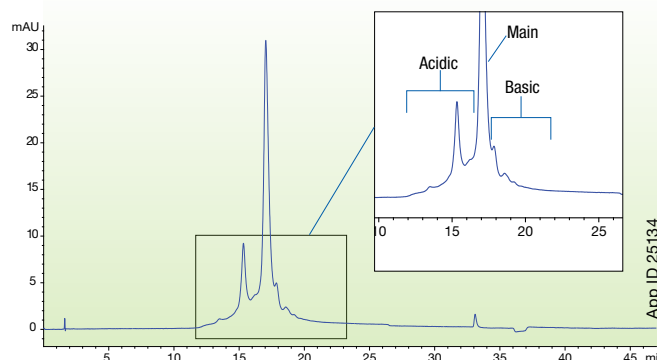


Figure 2.
Charge variant profile using salt gradient



APPLICATIONS

Ordering Information bioZen™

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
						/3pk		ea
bioZen 2.6 µm Glycan	50 x 2.1	00D-4773-AN	00F-4773-AN	—	—	AJO-9800	—	AJO-9000
						/3pk		ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJO-9803	—	AJO-9000
						/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJO-7605	AJO-7606	KJO-4282
						/3pk		ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJO-9806	—	AJO-9000
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJO-9806	AJO-9808	AJO-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJO-9809	AJO-9811	AJO-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJO-9812	AJO-9814	AJO-9000
	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	300 x 4.6	for 4.6 mm		Holder
							/3pk	ea
bioZen 1.8 µm SEC-2	—	—	00F-4769-E0	—	00H-4769-E0	—	AJO-9850	AJO-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJO-9851	AJO-9000
							/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJO-9400	KJO-4282

Australia
t: +61 (0)2-9428-6444
auiinfo@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 61 692 20 20
swissinfo@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

Sample Preparation

bioZen Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit
bioZen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box



BE-HAPPY™
guarantee

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
bioZen is a trademarks of Phenomenex. Herceptin is a registered trademark of Genentech, Inc. Thermo Fisher Scientific is a registered trademark of Thermo Fisher Scientific, Inc.
FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2018 Phenomenex, Inc. All rights reserved.