

APPLICATIONS

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

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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 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

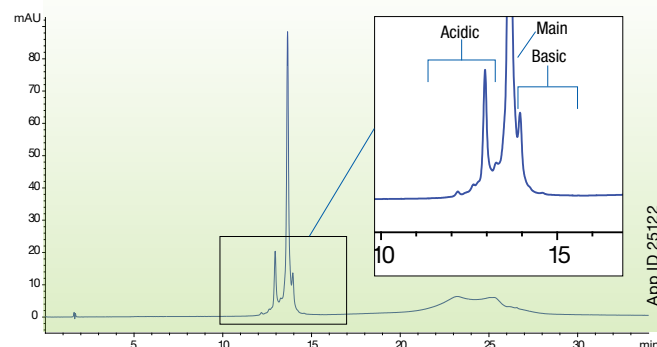
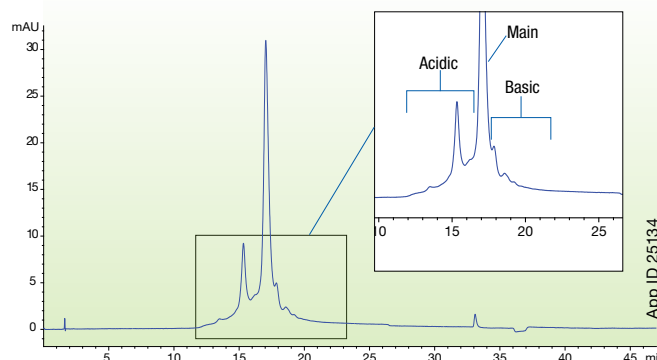


Figure 2.
Charge variant profile using salt gradient



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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

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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



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