Ion-Exchange Chromatography for Charge Variant Analysis of Cetuximab 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). Cetuximab, marketed under the trademark Erbitux®, is a common monoclonal antibody in the biopharmaceutical industry. The determination of charge variants in cetuximab 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 51 % acidic variants and 22 % basic variants. For the salt gradient, morpholino ethane sulfonic acid (MES) in combination with increasing NaCl (300 mM) was utilized (**Figure 2**). This approach provided 48 % acidic variants and 29 % basic variants. Both gradients sufficiently separate the acidic and basic variants from the neutral cetuximab with the use of the bioZen WCX ion-exchange column.

Materials and Methods

Cetuximab (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:	Cetuximab

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:	15-45 % B in 30 min
Flow Rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Injection Volume:	15 µL
Samples:	Cetuximab

Results

Figure 1.
Charge variant profile using pH gradient

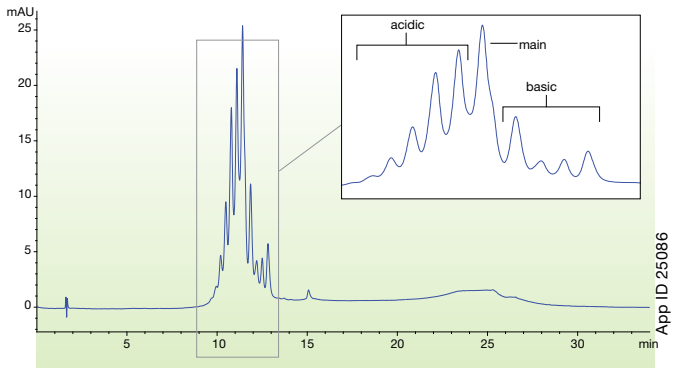
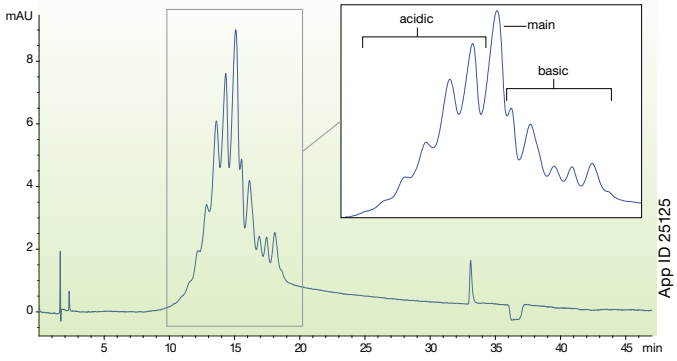


Figure 2.
Charge variant profile using salt gradient



APPLICATIONS

bioZen™ Ordering Information

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	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	AJ0-9800	—	AJ0-9000
						/3pk		ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJ0-9803	—	AJ0-9000
						/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJ0-7605	AJ0-7606	KJ0-4282
						/3pk		ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJ0-9806	—	AJ0-9000
						/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	AJ0-9806	AJ0-9808	AJ0-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJ0-9812	AJ0-9814	AJ0-9000

	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	—	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
							/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJ0-9400	KJ0-4282

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