

APPLICATIONS

Released N-Glycan Analysis of Infliximab-dyyb including Sialylated Glycans using a bioZen™ 2.6 μm Glycan Column

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Overview

Glycosylation of biologics during recombinant synthesis must be fully characterized for therapeutic development. A common procedure to determine glycosylation identity is through chromatography in HILIC mode after an enzymatic cleavage and labeling sequence. One challenge with this method is the separation of sialylated glycans. Employing a bioZen Glycan LC column for a HILIC-mode separation of released N-glycans is an effective protocol. Sample preparation involves the enzymatic cleavage of N-glycans with PNGase F. Subsequent labeling with Instant-PC™ dye followed by filtration to remove excess dye prepares the sample for HPLC analysis. For the chromatographic analysis, the unique amide polyol stationary phase combined with a superficially porous core-shell particle provides excellent separation of the released N-glycans (**Figure 1**). Moreover, the separation of the sialylated glycans is outstanding.

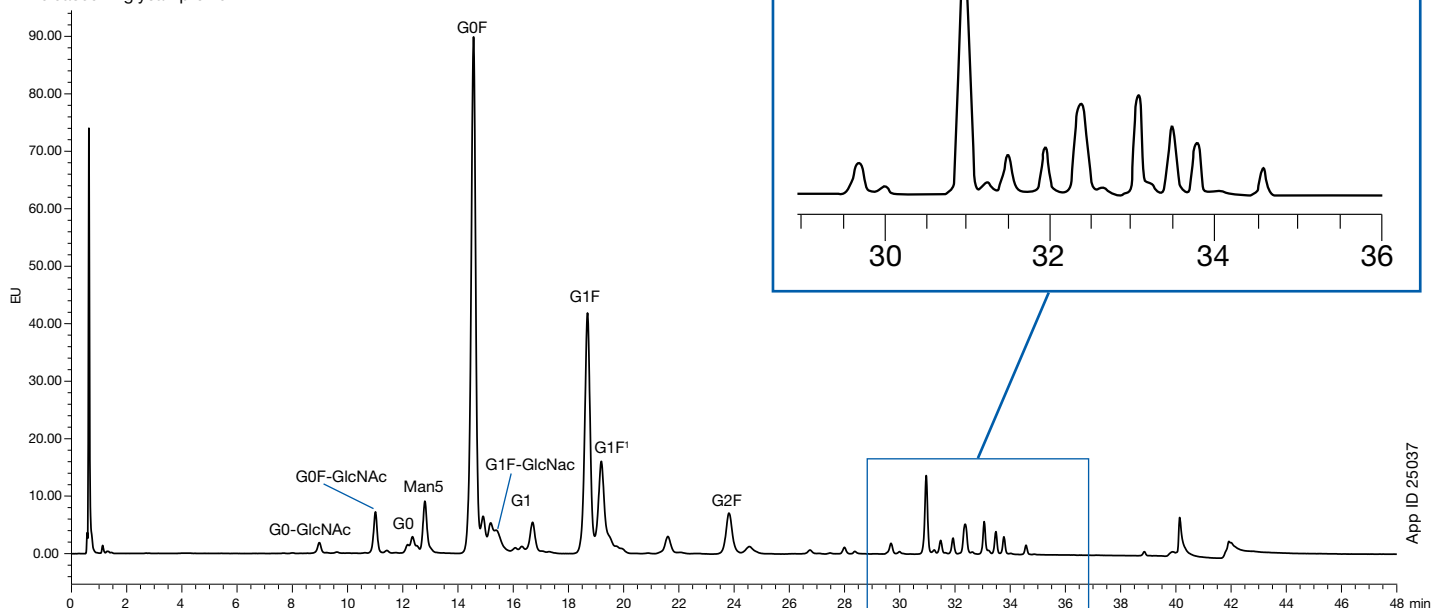
LC-FLD Conditions

Column:	bioZen 2.6 μm Glycan																		
Dimensions:	150 x 2.1 mm																		
Part No.:	00F-4773-AN																		
Mobile Phase:	A: 100 mM Ammonium Formate, pH 4.5 B: Acetonitrile																		
Gradient:	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr><td>0</td><td>78</td></tr> <tr><td>10</td><td>74.5</td></tr> <tr><td>24</td><td>72</td></tr> <tr><td>38.5</td><td>55.9</td></tr> <tr><td>38.6</td><td>40</td></tr> <tr><td>40.6</td><td>40</td></tr> <tr><td>40.7</td><td>78</td></tr> <tr><td>48</td><td>78</td></tr> </tbody> </table>	Time (min)	% B	0	78	10	74.5	24	72	38.5	55.9	38.6	40	40.6	40	40.7	78	48	78
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24	72																		
38.5	55.9																		
38.6	40																		
40.6	40																		
40.7	78																		
48	78																		
Flow Rate:	0.5 mL/min																		
Detection:	FLD ex/em 285/345 nm																		
LC:	Waters® ACQUITY® UPLC® I-Class																		
Temperature:	50 °C																		
Injection Volume:	1 μL																		
Sample:	Infliximab biosimilar																		

Sample Preparation

To prepare infliximab-dyyb for chromatographic analysis, the Gly-X™ N-Glycan Rapid Release and Labeling with InstantPC kit from ProZyme® was utilized. The protein was first denatured with the denaturation reagent provided and incubated at 90 °C for 3 min. Subsequent enzymatic digestion with N-Glycanase® (PNGase F) at 50 °C for 5 min provided the released glycans. Immediate labeling with InstantPC dye for 1 min at 50 °C followed by cleanup through a Gly-X clean up plate provided the labeled N-glycans. The bioZen N-Glycan Clean-Up plate (Part No.: 8M-S009-NGA) can be used as an alternative 96-well clean up plate within this method.

Figure 1.
Released N-glycan profile



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	00B-4773-AN	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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