An integrated solution for N-glycan analysis

Introduction

Glycans are involved in a variety of biological and physiological processes, including cell recognition, regulatory functions, immunity, communication, and development [1,2]. They are often key biomarkers for human health and disease [1-3].

Complex, heterogeneous structures provide glycans with more structural diversity than any other class of biomolecules [4]. Variation in glycosylation patterns can result in dramatic differences in cell functions. It is these attributes that make glycosylation one of the critical quality attributes (CQAs) for biotherapeutics. Therefore, glycan characterization is essential during the development and manufacturing of biotherapeutics. Equally important is the development of an assay for biotherapeutic glycoproteins to help ensure reproducible production quality [5-7].

This application note demonstrates an integrated solution for N-glycan sample preparation and analysis. We analyzed National Institute of Standards and Technology monoclonal antibody (NISTmAb) glycans in under 1.5 hours using the Applied Biosystems™ GlycanAssure™ HyPerformance APTS Kit and the Thermo Scientific™ Vanquish™ Horizon UHPLC System.

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Materials and methods

The GlycanAssure HyPerformance APTS Kit was used to prepare 50 µg of NISTmAb glycans according to the product user guide. The kit workflow is summarized in Figure 1. Separation was carried out with a Thermo Scientific™ Accucore™ 150 Amide HILIC column. Data were analyzed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software. Separation conditions are described in Table 1.

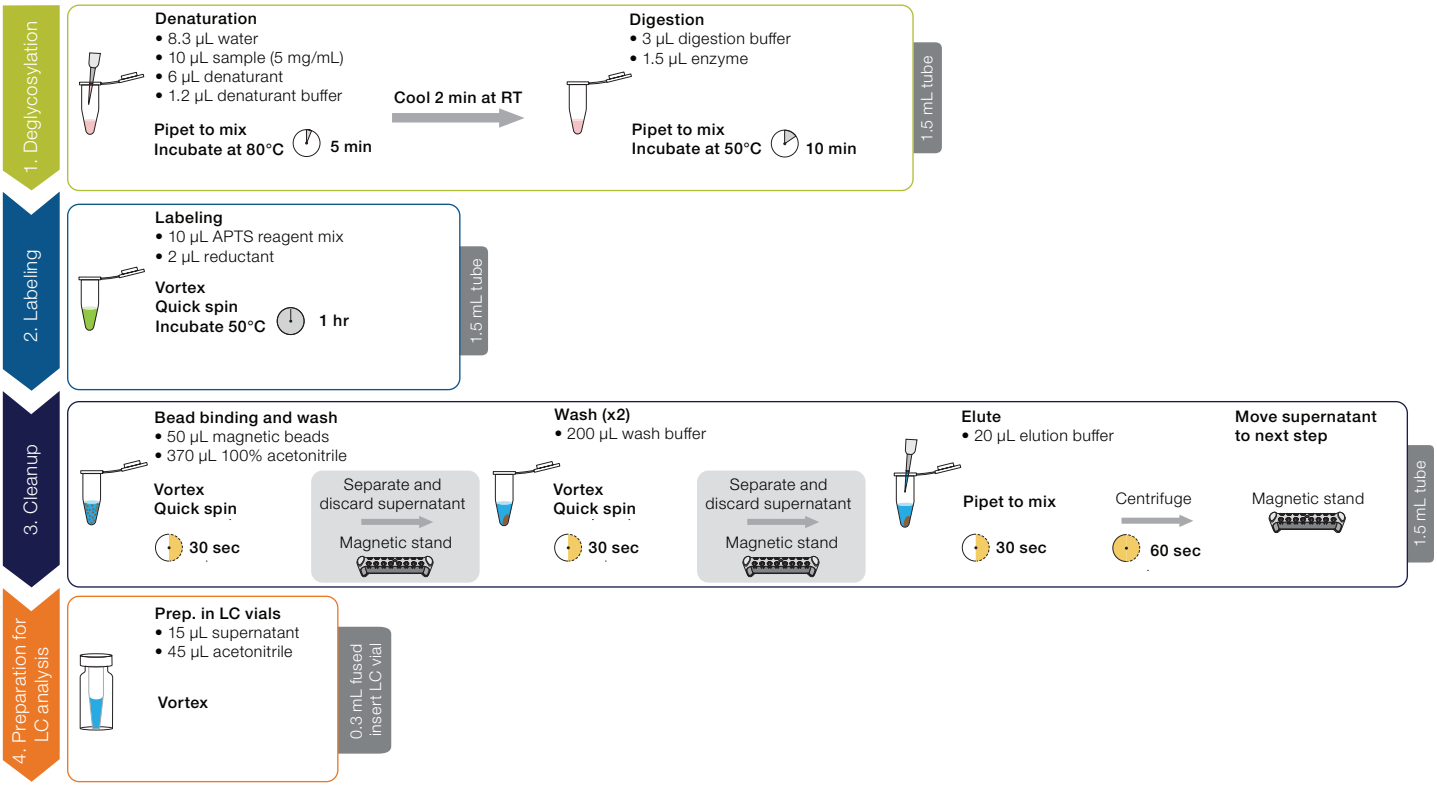

Figure 1. The workflow of the GlycanAssure HyPerformance APTS Kit.

Table 1. Separation conditions for NISTmAb glycan analysis.

Separation condition			
Instrumentation	Vanquish Horizon UHPLC System equipped with a Thermo Scientific™ Vanquish™ Fluorescence Detector (2 µL micro flow cell) Cat. No. IQLAAAGABHFAPUMZZZ, 6079.4330		
Column	Accucore 150 Amide HILIC, 2.6 µm, 2.1 x 150 mm, Cat. No. 16726-102130		
Mobile phase A	100 mM ammonium formate, pH 4.4		
Mobile phase B	100% acetonitrile		
Gradient	Time (min)	B (%)	A (%)
	0	68	32
	45	55	45
	45.5	40	60
	47	40	60
	47.5	68	32
	50	68	32
Flow rate	0.45 mL/min		
Column temperature	50°C		
Injection volume	15 µL		
Excitation wavelength	455 nm		
Emission wavelength	500 nm		
Sensitivity	7		
Lamp mode	High power		
Data collection rate	10 Hz		

Results

The chromatogram displayed 37 peaks of ≥0.03% relative area (RA) with high resolution (Figure 2). Excellent sample preparation repeatability of <5% was found for all peaks with RA ≥0.5% (Table 2). Increased signal heights were achieved without laborious vacuum drying, resulting in a lower limit of quantification (i.e., 0.1%).

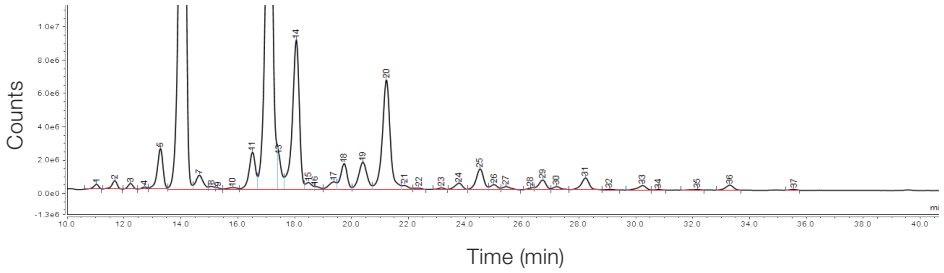

Figure 2. Chromatogram of labeled glycans from NISTmAb.

Table 2. Summary of relative area (RA) of 37 peaks.

Peak number	Average retention time (RT, min)	Relative standard deviation (RSD) of RT (%)	RA (%)			RSD of RA (%)
			Repeat 1	Repeat 2	Repeat 3	
1	11.03	0.09	0.25	0.21	0.23	8.70
2	11.68	0.06	0.39	0.40	0.42	3.79
3	12.23	0.17	0.25	0.25	0.25	0.00
4	12.72	0.07	0.10	0.10	0.11	5.59
5	13.28	0.11	2.07	2.10	2.15	1.92
6	14.07	0.07	37.45	37.74	38.12	0.89
7	14.65	0.07	0.92	0.92	0.94	1.25
8	15.07	0.13	0.15	0.14	0.15	3.94
9	15.29	0.05	0.05	0.05	0.05	0.00
10	15.84	0.29	0.10	0.11	0.12	9.09
11	16.52	0.07	2.13	2.12	2.10	0.72
12	17.11	0.08	27.82	27.66	27.40	0.77
13	17.46	0.04	1.26	1.25	1.24	0.80
14	18.07	0.09	9.37	9.26	9.16	1.13
15	18.50	0.12	0.37	0.39	0.37	3.07
16	18.74	0.28	0.14	0.13	0.13	4.33
17	19.39	0.18	0.51	0.49	0.50	2.00
18	19.75	0.10	1.53	1.54	1.53	0.38
19	20.40	0.08	2.04	2.01	1.98	1.49
20	21.23	0.08	7.74	7.66	7.50	1.60
21	21.87	0.16	0.24	0.24	0.23	2.44
22	22.37	0.03	0.08	0.09	0.09	6.66
23	23.17	0.03	0.07	0.07	0.08	7.87
24	23.79	0.09	0.42	0.41	0.41	1.40
25	24.54	0.11	1.39	1.38	1.34	1.93
26	25.02	0.06	0.33	0.32	0.31	3.13
27	25.45	0.12	0.22	0.23	0.21	4.55
28	26.38	0.28	0.09	0.10	0.09	6.19
29	26.73	0.09	0.64	0.65	0.61	3.29
30	27.22	0.09	0.19	0.22	0.19	8.66
31	28.24	0.06	0.83	0.87	0.81	3.65
32	29.09	0.05	0.04	0.04	0.04	0.00
33	30.26	0.10	0.32	0.34	0.34	3.46
34	30.81	0.04	0.03	0.03	0.03	0.00
35	32.17	0.04	0.05	0.05	0.05	0.00
36	33.31	0.05	0.35	0.36	0.35	1.63
37	35.57	0.09	0.05	0.05	0.05	0.00

applied
biosystems

Discussion and conclusions

Glycans from NISTmAb were released, labeled, and purified in under 1.5 hours. Separation was performed on the Vanquish Horizon UHPLC System equipped with a Vanquish Fluorescence Detector and an Accucore 150 Amide HILIC column. Data analysis was then performed using Chromeleon software. This process generated 37 peaks with ≥0.03% RA that were identified with high resolution and signal intensity. Excellent precision of ≤5% RSD for peaks of ≥0.5% RA was observed for three sample preparation replicates. These data demonstrate that the GlycanAssure HyPerformance kit offers a fully integrated workflow to provide rapid and high-quality analysis of N-glycans for biotherapeutics.

References

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