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Evaluation of chromatographic phases for separation of differentially labeled glycans from erythropoietin and trastuzumab

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Keywords

NIBRT, Biopharmaceutical, Bioproduction, QA/QC, Biotherapeutic, IgG, Monoclonal antibody (mAb), Glycans, Glycosylation, Erythropoietin, Trastuzumab, Vanquish Flex UHPLC, Accucore 150 Amide HILIC column, GlycanPac AXH-1 column, GlycanPac AXR-1 column, Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer with Biopharma Option

Application benefits

- Demonstrate the benefit of using alternative chromatographic methodologies to HILIC phase columns for glycan analysis of different therapeutic proteins
- Demonstrate applicability of Thermo Scientific™ GlycanPac™ mixed mode chromatographic columns for analysis of mixtures of highly sialylated glycan species
- Method outlined for glycan identification by LC-MS

Goal

To evaluate the effect of various fluorescent labels and column chemistries for glycan analysis of different biopharmaceutical substances, i.e. erythropoietin and the monoclonal antibody trastuzumab. To demonstrate the applicability of mixed mode analytical columns for *N*-glycan analysis of therapeutic proteins, in particular for those that are known to contain highly branched, sialylated glycan structures.

Introduction

Glycosylation is a critical quality attribute that may affect the pharmacodynamic and pharmacokinetic behavior of therapeutic proteins. Hence, detailed characterization of glycans attached to therapeutic drug substances is a regulatory requirement. Furthermore, characterization of

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biosimilar products is required to demonstrate the comparability of an innovator therapeutic protein and related biosimilar candidate. Combined, these requirements have resulted in an increased demand for reliable and robust analytical technologies for the comprehensive characterization of protein glycosylation.

Analysis of glycan moieties is commonly performed subsequent to their release from a therapeutic protein and derivatization with an appropriate fluorophore, followed by liquid chromatography (LC) or capillary electrophoresis separation and fluorescence (FLD) or mass spectrometry (MS) detection. Hydrophilic interaction liquid chromatography (HILIC) is the most frequently applied separation strategy for glycan analysis during which glycan retention increases with increasing glycan size and associated reduction in hydrophobicity. While HILIC columns are an excellent choice for separation of glycan pools, especially if the majority of glycans present are neutral, they often result in inadequate separation for glycans harboring two or more charged moieties are present (e.g. sialylated glycans). Furthermore, the structural complexity of glycan mixtures including closely related isomeric species represents a major analytical challenge with commonly occurring partial or incomplete liquid phase separation.

Introduction of an additional and complementary offline separation dimension has been employed to increase the separation of oligosaccharides; however, the inclusion of extended sample handling steps may introduce selective sample alterations or loss of glycoforms. Hybrid phases, such as anion exchange-HILIC or anion exchange-reversed phase chromatography, have been developed that combine multiple separation strategies on a single column. The Thermo Scientific™ GlycanPac™ AXH 1 column has both weak anion exchange and HILIC properties, which enables the separation of molecules based on charge, polarity, and size, creating an added dimension for separation of highly charged glycoforms. The Thermo Scientific™ GlycanPac™ AXR-1 mixed mode column also offers an alternative separation strategy to traditional HILIC columns. Separation is achieved by first resolving glycans into different charge groups and then subsequently separating glycans in each group based on size and isomerization. Both alternative strategies may increase resolution of complex glycan mixtures enabling separation and identification of glycan structures that are not observed by more traditional methodologies.

In addition to consideration of the chromatographic separation phases, multiple fluorophores are now available for labeling of released *N*-glycans species by reductive amination prior to UHPLC analysis with FLD. Different fluorophores impart different properties on labeled glycan samples: chemical derivatization with 2-aminobenzoic acid (2-AA) imparts one negative charge to glycan moieties; labeling with 9-aminopyrene-1,4,6-trisulfonic acid (APTS) imparts three negative charges onto glycan species; derivatization with 2-aminobenzamide (2-AB) has no impact on the charge of glycan species; while derivatization with 2-aminoacridone (2-AMAC) changes the hydrophobicity of oligosaccharides. Hence, choice of fluorescent label may have a large impact on selectivity depending on the mode of chromatographic separation employed. Therefore, in addition to evaluation of the column for *N*-glycan analysis, it is also imperative to consider the choice of label used in combination with the chromatographic stationary phase as both the properties imparted on glycan samples from derivatization with different fluorophores and separation capabilities of different analytical columns may be manipulated to enable better separation of oligosaccharides.

In this application, *N*-glycans released from erythropoietin (EPO) were used to evaluate the effect of labeling and chromatographic chemistry on the separation of glycan species. EPO was chosen as a model protein as it is highly glycosylated and exhibits high heterogeneity due to the inherent inclusion of various highly branched sialylated *N*-glycan structures.[1] *O*-glycosylation was not examined herein due to the reported minor biological significance of *O*-glycans on EPO. EPO was derivatized using three different labels, namely 2-AA, 2-AB, and 2-AMAC. Subsequently, labeled glycans were analyzed using a Thermo Scientific™ Accucore™ Amide-150 HILIC, 2.1 × 150 mm (HILIC), a GlycanPac AXH-1 column (AXH), and a GlycanPac AXR-1 column (AXR). In addition to sample analysis using a Thermo Scientific™ Vanquish™ Flex UHPLC system with fluorescence detection, identification of individual glycan species was performed using a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer with the BioPharma option. Due to the prevalence of monoclonal antibody (mAb) drug substances in the biopharmaceutical industry, released glycans from the therapeutic mAb trastuzumab were also analyzed using the three chromatographic columns. Due

to the neutral nature of the glycans present on this mAb, they were only derivatized with the commonly used 2-AA label.

Experimental

Recommended consumables

- Deionized water, 18.2 MΩ·cm resistivity
- Acetonitrile, Optima™ LC-MS grade (Fisher Chemical) (P/N 10001334)
- Water, Optima™ LC-MS grade (Fisher Chemical) (P/N 10505904)
- Formic acid, Optima™ LC-MS grade (Fisher Chemical) (P/N 10596814)
- Ammonium hydroxide (Honeywell Fluka) (P/N 44273)
- PNGase F (New England BioLab) (P/N P0705L)
- Amicon® Ultra 0.5 mL centrifugal filters MWCO 10 +6kDa (Fisher Scientific) (P/N 10088753)
- Sodium cyanoborohydride (Acros Organics) (P/N 168550100)
- Glacial acetic acid (Fisher Chemical) (P/N 10394970)
- Dimethylsulfoxide (DMSO) (Thermo Scientific, Pierce) (P/N 13494279)
- 2-Aminobenzamide (2-AB) (Acros Organics) (P/N 10569610)
- 2-Aminobenzoic acid (2-AA) (Honeywell Fluka) (P/N 10678)
- 2-Aminoacridone (2-AMAC) (Sigma-Aldrich) (P/N 06627)
- Urea (Fisher Chemical) (P/N 10687422)
- Tris-HCl (Fisher Chemical) (P/N 10060390)
- Dithiothreitol (DTT) (Fisher Bioreagents) (P/N 10386833)
- Iodoacetamide (IAA) (Acros Organics) (P/N 10408660)
- Ammonium bicarbonate (Acros Organics) (P/N 10207183)
- Sodium Hydroxide (NaOH) (Fisher Chemical) (P/N 10488790)
- Thermo Scientific GlycanPac AXH-1, 1.9 µm, 2.1 × 150 mm (P/N 082472)
- Thermo Scientific GlycanPac AXR-1, 1.9 µm, 2.1 × 150 mm (P/N 088136)
- Thermo Scientific Accucore 150 Amide HILIC column, 2.1 × 150 mm (P/N 16726-152130)
- Thermo Scientific™ Virtuoso™ vial, clear 2 mL kit with septa and cap (P/N 60180-VT405)
- Thermo Scientific™ Virtuoso™ vial identification system (P/N 60180-VT100)

Instrumentation

- Thermo Scientific Vanquish Flex UHPLC system, including:
  - Quaternary Pump F (P/N VF-P20-A)
  - Column Compartment H (P/N VH-C10-A)
  - Split Sampler FT (P/N VF-A10-A) with 25 µL (V = 50 µL) sample loop
  - Fluorescence Detector F (P/N VF-D50-A)
  - System Base Vanquish Flex (P/N VF-S01-A)
- Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with the BioPharma option
- Thermo Scientific™ SpeedVac™ Concentrator (P/N SPD121p)

Sample preparation

Buffer preparation

- Ammonium formate (50 mM, pH 4.4): 1.8 g of formic acid was dissolved in 1 L of DI water. The pH was adjusted to 4.4 with ammonium hydroxide solution.
- 8 M urea in 0.1 M Tris-HCl, pH 8.5 (UA buffer): 1.57 g of Tris-HCl was dissolved in 100 mL DI water and the pH was adjusted to 8.5 with 1 M NaOH. 24 g of urea was dissolved in 50 mL 0.1 M Tris-HCl, pH 8.5.
- Ammonium bicarbonate (50 mM, pH 7.8): 0.39 g of ammonium bicarbonate was dissolved in 100 mL of DI water.

Release of N-glycans from proteins

For each label utilized in this study, 500 µg of protein was denatured using 8 M urea in 0.1 M Tris buffer pH 8.0 (UA solution) and subsequently reduced and alkylated using 10 mM DTT and 50 mM IAA prepared in UA solution. Following buffer exchange into 50 mM ammonium bicarbonate using 10 kDa MWCO filters, *N*-glycan release was performed by incubation of the reduced and

alkylated sample with 2500 units of PNGase F overnight at 37 °C. The released glycans were collected from the deglycosylated proteins by centrifugation through 10 kDa MWCO filters and subsequently reduced to dryness via vacuum centrifugation. The dried glycans were reconstituted in 50 µL of 1% (v/v) aqueous formic acid to ensure complete conversion to the reducing sugar form prior to derivatization and subsequently reduced to dryness.

2-AA labeling of released N-glycans

1. 2-AA labeling reagent (100 µL) was prepared by dissolving 2-aminobenzoic acid (5 mg) and sodium cyanoborohydride (6 mg) in 70/30 DMSO/glacial acetic acid.
2. 10 µL of 2-AA labeling reagent solution was added to the dried *N*-glycans.
3. The solution was incubated at 65 ºC for 5 hours.

2-AB labeling of released N-glycans

1. 2-AB labeling reagent (100 µL) was prepared by dissolving 2-aminobenzamide (5 mg) and sodium cyanoborohydride (6 mg) in 70/30 DMSO/glacial acetic acid.
2. 10 µL of 2-AB labeling reagent solution was added to the dried *N*-glycans.
3. The solution was incubated at 65 ºC for 2 hours.

2-AMAC labeling of released N-glycans

1. 2-AMAC labeling reagent (100 µL) was prepared by dissolving 2-aminoacridone (2 mg) and sodium cyanoborohydride (6 mg) in 85/15 DMSO/glacial acetic acid.
2. 12 µL of 2-AMABC labeling reagent solution was added to the dried *N*-glycans.
3. The solution was incubated at 37 ºC for 16 hours.

Cleanup of fluorescently labeled N-glycans

Excess labeling dye removal was carried out by HILIC purification. Samples were loaded in 80% acetonitrile, 20% 50 mM ammonium formate pH 4.4 (v/v) (for 2-AA and 2-AB labeled *N*-glycans) or in 82.5% acetonitrile, 17.5% 50 mM ammonium formate pH 4.4 (v/v) (for 2-AMAC labeled *N*-glycans) onto an Accucore 150-Amide-HILIC 2.1 × 50 mm column at 0.5 mL/min for 2.5 minutes. Labeled glycans were eluted in 20% aqueous acetonitrile for 2.5 minutes, monitored by fluorescence detection, $\lambda_{ex/em}$ = 330/420 (for 2-AB labeled *N*-glycans), $\lambda_{ex/em}$ = 350/425 (for 2-AA labeled *N*-glycans), or $\lambda_{ex/em}$ = 429/525 (for 2-AMAC labeled *N*-glycans) and evaporated to dryness.

Sample preparation for analysis using UHPLC-FLD and UHPLC-FLD-MS

1. 25 µL of purified labeled *N*-glycans resuspended in DI water (at 2.5 µg/µL) was diluted with 75 µL of acetonitrile (for analysis using a HILIC or AXH mixed mode column) or 75 µL of water (AXR mixed-mode column).
2. The total solution was transferred to the autosampler vial for analysis.

Analytical conditions for UHPLC analysis

Analytical conditions for UHPLC analysis are listed in Tables 1-4.

Table 1. UHPLC parameters used for all analyses.

Parameter	Condition
Mobile phase	A: Ammonium formate 50 mM, pH 4.4 B: Acetonitrile D: Water
Flow rate	0.4 mL/min
Column temperature	30 ºC
Autosampler temperature	10 ºC
Sample volume	25 µL*
FLD excitation/emission wavelength	342 / 423 nm (2-AA-labeled glycan samples) 348 / 432 nm (2-AB-labeled glycan samples) 429 / 525 nm (2-AMAC-labeled glycan samples)
Mobile phase gradient	Refer to Tables 2-4

*Note: Due to sample limitation, 7 µL of AMAC-labeled EPO *N*-glycans were injected on both the AXH and AXR columns.

Table 2. Mobile phase gradient for analysis performed using an Accucore Amide-150 HILIC column.

Time (min)	% A	% B	% D	Curve
0	25	75	0	5
30	50	50	0	5
30.5	60	40	0	5
32	60	40	0	5
32.5	25	75	0	5
40	25	75	0	5

Table 3. Mobile phase gradient for analysis performed using a GlycanPac AXR-1 column.

Time (min)	% A	% B	% D	Curve
0	15	0	85	5
1	15	0	85	5
25	30	0	70	5
70	60	10	40	5
70.5	15	0	85	5
80	15	0	85	5

Table 4. Mobile phase gradient for analysis performed using a GlycanPac AXH-1 column.

Time (min)	% A	% B	% D	Curve
0	22	78	0	5
30	30	70	0	5
35	40	60	0	5
40	50	50	0	5
40.5	22	78	0	5
50	22	78	0	5

Analytical conditions for MS analysis

Glycan samples were injected into a Q Exactive Plus MS with the BioPharma option equipped with a HESI ion source (Table 5) by using a Vanquish Flex UHPLC system to enable identification of glycan species.

Table 5. MS parameters utilized for all LC-MS analyses.

Parameter	Setting
Method duration	40 min (HILIC); 80 min (AXR); 50 min (AXH)
Polarity	Negative
In-source CID	20 eV
Default charge state	2
Full MS	
Number of microscans	1
Resolution	70,000 Da
AGC target	3.00E+06
Maximum IT	50 ms
Scan range	380–2000 *m/z*
dd-MS2 / dd-SIM	
Number of microscans	3
Resolution	17,500
AGC target	1.00E+05
Maximum IT	120 ms
TopN	3
Isolation window	2.0 *m/z*
Scan range	200–2000 *m/z*
(N)CE/stepped (N)CE	NCE: 30, 42, 72
dd settings	
Minimum AGC target	1.00E+03
Intensity threshold	8.30E+03
Charge exclusion	Unassigned, 1, 5-8, >8
Peptide match	Preferred
Exclude isotopes	On
Dynamic exclusion	10.0 s
Tune file settings	
Spray voltage	3.5 kV
Capillary temperature	320 °C
Sheath gas pressure	40
Aux gas pressure	10
Probe heater temp.	400 °C
S-lens RF value	50

Data processing and software

Thermo Scientific™ Xcalibur™ software 2.2 SP1.48 was used for UHPLC-FLD-MS data acquisition and analysis.

Results and discussion

N-glycosylation of EPO is known to have a dramatic impact on the therapeutic efficiency of the drug substance due to its effect on serum half-life. The *N*-glycan profile of EPO is structurally complex, incorporating closely related isomeric species, and thereby representing a major analytical challenge with partial or incomplete liquid separation commonly occurring with a singular chromatographic method. Introduction of an additional and complementary offline separation dimension is a common approach to ensuring sufficient peak capacity to enable resolution of glycan species. In this application note, hybrid chromatographic phases (AXR, AXH) were compared to the standard HILIC phase for separation of EPO *N*-glycans derivatized with different fluorescent labels. Alternative labels impart different physicochemical characteristics to glycan species and hence, may affect the selectivity of the analytical columns for separation of the samples.

Figures 1, 2, and 3 depict the chromatographic separation of EPO *N*-glycans labeled with 2-AA, 2-AB, and 2-AMAC, on a HILIC, AXR, and AXH column, respectively. Chromatographic separation of glycan species was complemented by MS detection for identification of glycan species based on mass. A corresponding list of identified *N*-glycans is shown in Table 6.

HILIC separates molecules based on increasing polarity. Similar glycan profiles were observed for EPO *N*-glycans derivatized with each fluorophore following analysis using a HILIC column. However, the increased hydrophobic properties of *N*-glycans resulting from derivatization with 2-AMAC give rise to a slightly early elution time for 2-AMAC labeled *N*-glycans. *N*-glycan species identified were in agreement with previously published data, with high levels of highly sialylated species observed.[1]

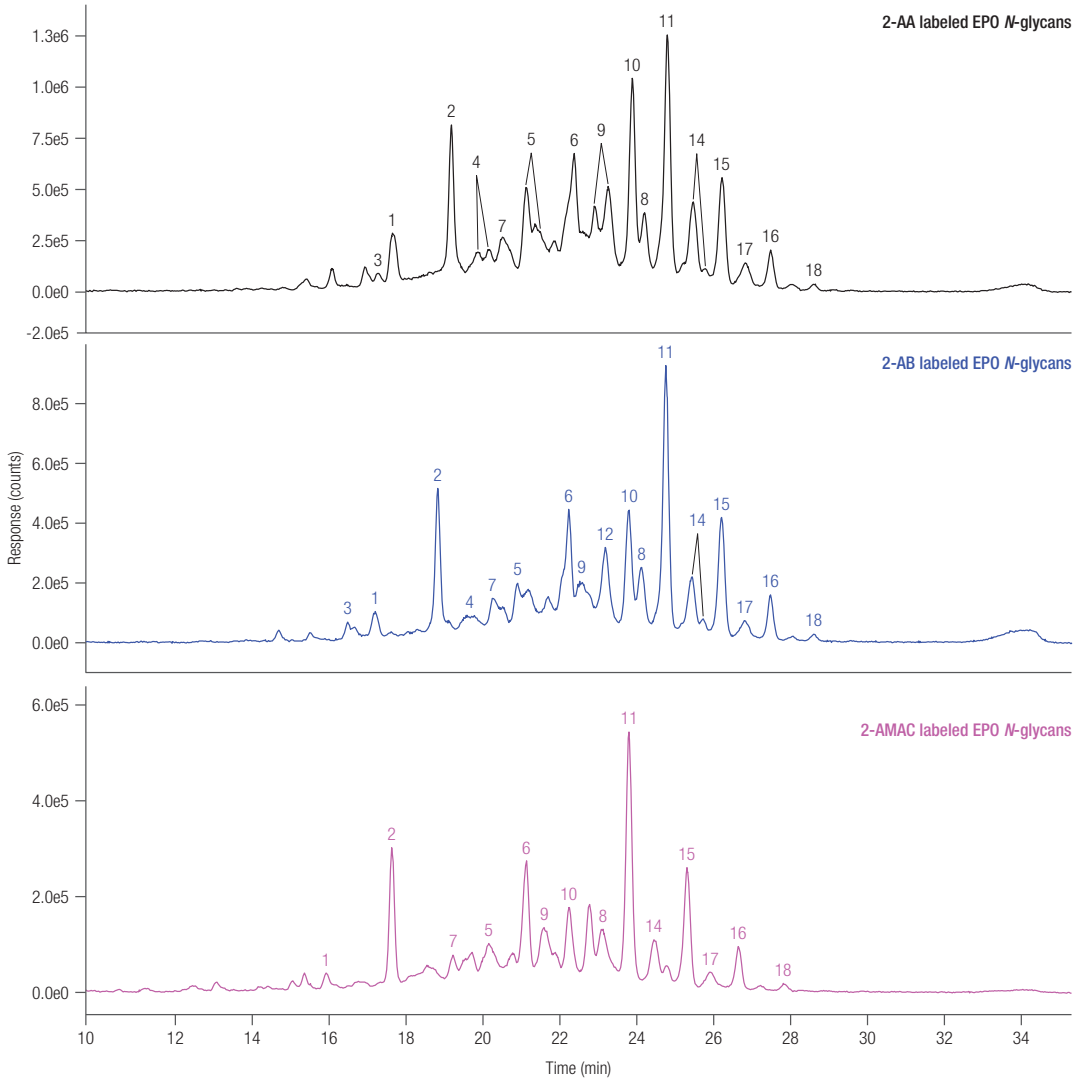


Figure 1. Chromatographic separation of *N*-glycans released from EPO labeled with 2-AA, 2-AB, and 2-AMAC on an Accucore Amide-150 HILIC column with UHPLC-FLD detection.

The GlycanPac AXR-1 column separates glycan species based on their polarity and charge and is a powerful tool for separation of sialylated or other negatively charged glycans. A glycan elution profile from an AXR column consists of early elution of neutral glycans, followed by monosialylated, disialylated, trisialylated, and tetrasialylated species. Glycans within each of these groups are then further separated according to their isomeric structure, polarity, and size by reversed-phase mechanisms.

Of the three columns evaluated, the AXR column has the most dramatic impact on the separation profile of differentially labeled EPO *N*-glycans (Figure 2). A greater number of isomeric species could be resolved using the AXR column when compared to the other column chemistries. The highest number of individual species were detected for 2-AB labeled EPO *N*-glycans (26 structures) and 2-AA labeled EPO *N*-glycans (23 structures). As expected, the *N*-glycans were found to elute in order of increasing sialic acid content, while *N*-glycans containing the same number of sialic acid molecules were also sufficiently separated to enable detection and identification. 2-AA imparts a single negative charge to glycans upon labeling and resulted in later elution times for all glycans analyzed when compared to 2-AB labeled glycans, which does not change the charge of the glycan moieties.

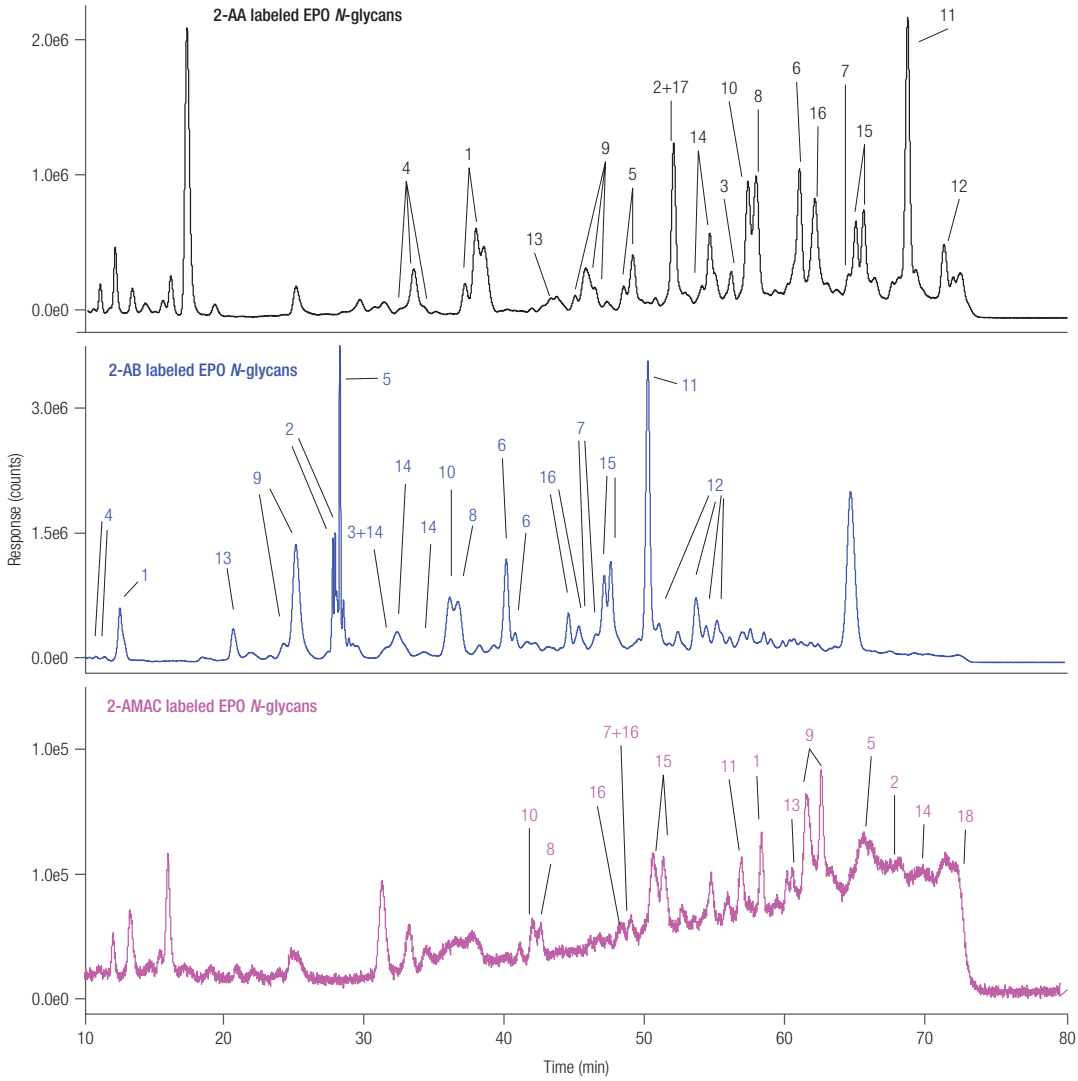


Figure 2. Chromatographic separation of *N*-glycans released from EPO labeled with 2-AA, 2-AB (25 µL), and 2-AMAC (7 µL) on a GlycanPac AXR-1 column with UHPLC-FLD detection.

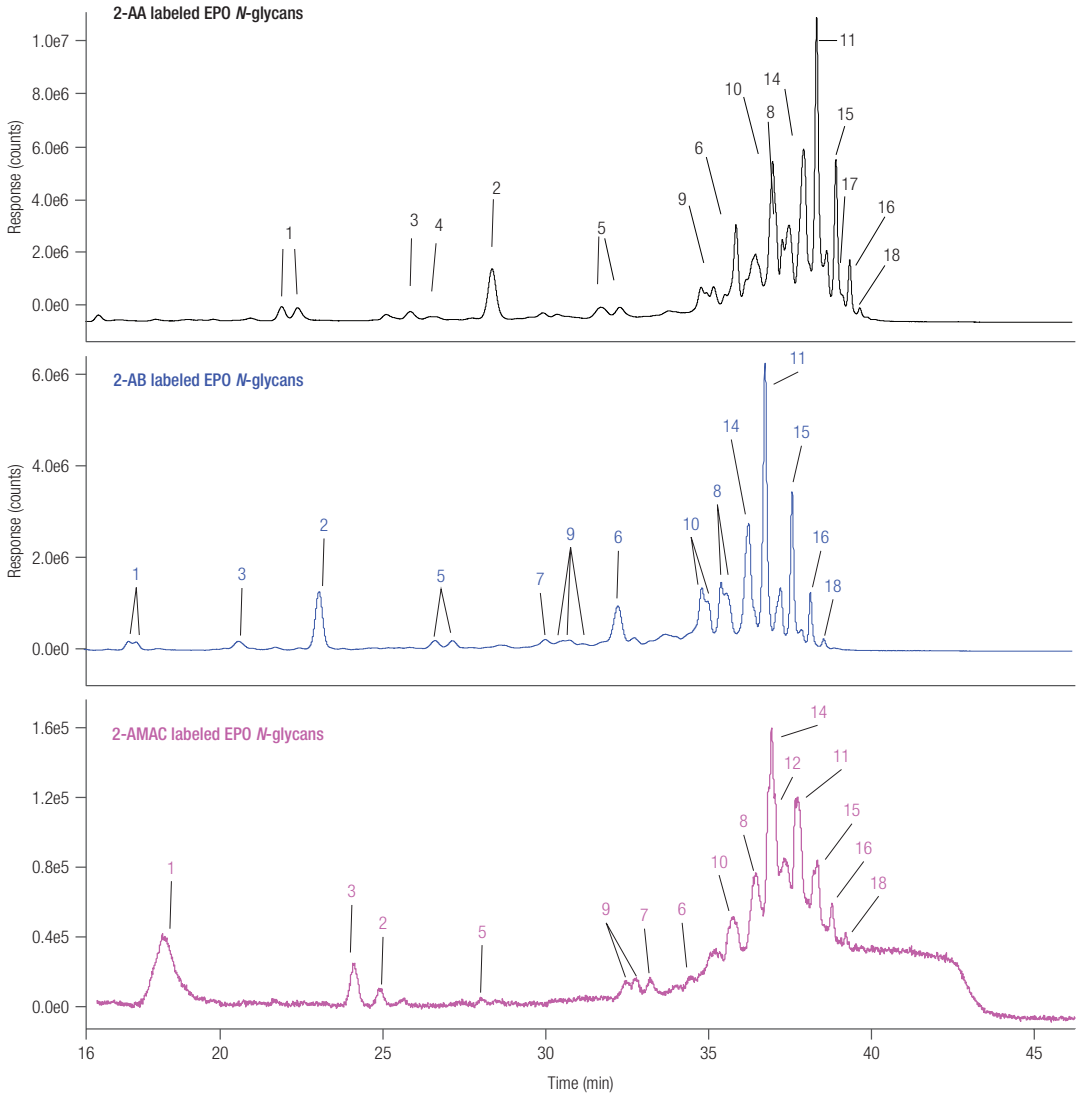


Figure 3. Chromatographic separation of *N*-glycans released from EPO labeled with 2-AA, 2-AB (25 µL), and 2-AMAC (7 µL) on a GlycanPac AXH-1 column with UHPLC-FLD detection.

The GlycanPac AXH-1 column separates glycans based on charge, size, and polarity, enabling an additional separation dimension over HILIC phases, which are unable to resolve glycan structures based on their different charge states. The AXH column resulted in excellent separation of smaller glycan moieties. Larger, more polar structures could also be clearly detected.

The ability of the AXH stationary phase to resolve smaller *N*-glycans, harboring fewer charges, suggests the potential of this chromatographic stationary phase for separation of *N*-glycan pools containing a majority of smaller, less sialylated glycan species.

Table 6. Structural identification of differently labeled *N*-glycans from EPO.

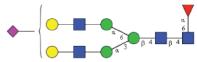
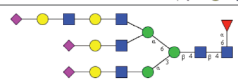
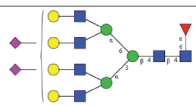
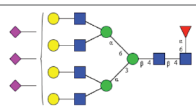
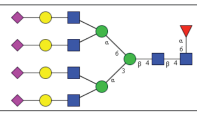
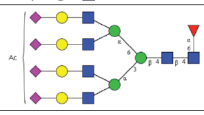
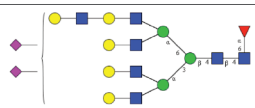
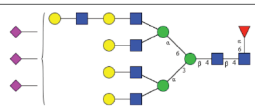
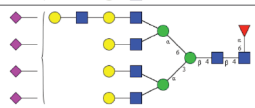
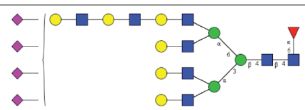
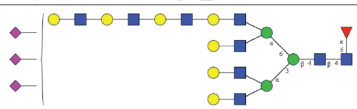
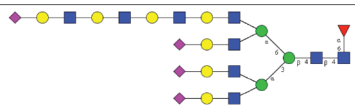
Peak number	Glycan Annotation	Glycan structure
1	FA2G2S1	
2	FA2G2S2	
3	FA2G2S2 (acetylated)	
4	FA3G3S1	
5	FA3G3S2	
6	FA3G3S3	
7	FA3G3S3 (acetylated)	
8	FA3G3Lac1S3	
9	FA4G4S2	
10	FA4G4S3	
11	FA4G4S4	
12	FA4G4S4 (acetylated)	
13	FA4G4Lac1S2	
14	FA4G4Lac1S3	
15	FA4G4Lac1S4	
16	FA4G4Lac2S4	
17	FA4G4Lac3S3	
18	FA4G4Lac3S4	

Table 7. *N*-glycans from EPO identified following derivatization with 2-AA, 2-AB, or 2-AMAC fluorescent labels and analyzed using different column chemistries. Green annotation represents glycans identified with < 3 ppm mass error, while red areas denote the *N*-glycans that were below the limit of detection.

Glycan Annotation	Peak Number	HILIC			AXR			AXH		
		2-AA	2-AB	2-AMAC	2-AA	2-AB	2-AMAC	2-AA	2-AB	2-AMAC
FA2G2S1	1									
FA2G2S2	2									
FA2G2S2 (acetylated)	3									
FA3G3S1	4									
FA3G3S2	5									
FA3G3S3	6									
FA3G3S3 (acetylated)	7									
FA3G3Lac1S3	8									
FA4G4S2	9									
FA4G4S3	10									
FA4G4S4	11									
FA4G4S4 (acetylated)	12									
FA4G4Lac1S2	13									
FA4G4Lac1S3	14									
FA4G4Lac1S4	15									
FA4G4Lac2S4	16									
FA4G4Lac3S3	17									
FA4G4Lac3S4	18									
Total number of *N*-glycans identified (including isomers)		**20**	**18**	**14**	**23**	**26**	**14**	**17**	**19**	**16**

Due to the importance of mAb therapeutics in the biopharmaceutical industry, the ability of the different chromatographic phases investigated to separate mAb *N*-glycans was also evaluated. In general, *N*-linked glycans released from mAbs are predominantly neutral species and are thus often derivatized with 2-AA and analyzed using a HILIC column. Figure 4 shows the separation profiles of *N*-glycans released from trastuzumab, derivatized with 2-AA, and analyzed using a HILIC, AXR, and AXH column before UHPLC-FLD-MS detection. Detected and identified *N*-glycan structures are displayed in Table 8. Excellent separation of the predominantly singly negatively charged species (due to 2-AA derivatization of neutral species) was observed following analysis using a HILIC column.

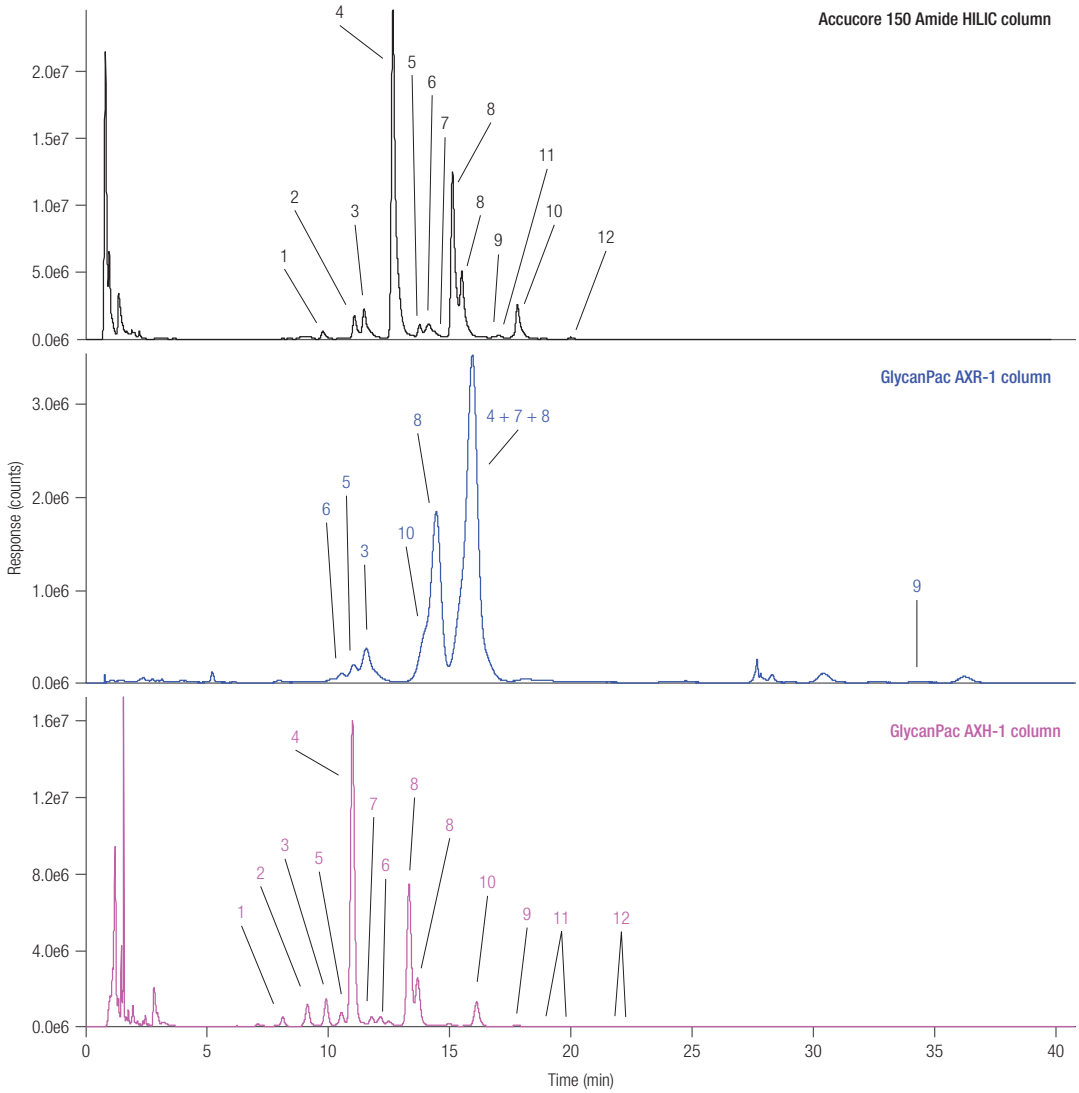
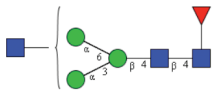
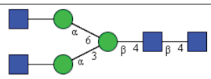
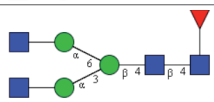
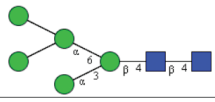
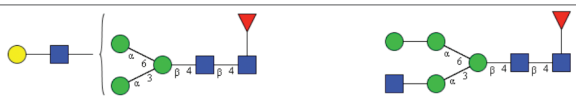
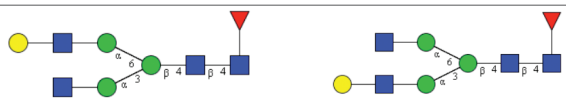
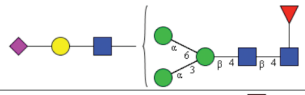
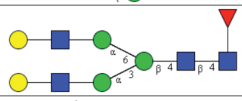
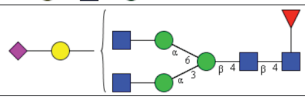
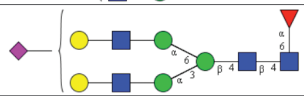


Figure 4. Chromatographic separation of *N*-glycans released from trastuzumab labeled with 2-AA an Accucore Amide-150 HILIC, 2.1 x 150 mm column, a GlycanPac AXH-1 column, and a GlycanPac AXR-1 column with UHPLC-FLD detection.

The AXR column displayed poor resolution of non-sialylated glycan structures. However, the sample highlights how AXR is most suitable for resolution of sialylated species, as the sialic acid containing structure is well separated from the non-sialylated species (peak 9). Of the three column chemistries evaluated, the AXH column enabled superior separation of mAb *N*-glycans. The elution profile for trastuzumab *N*-glycans on the AXH column was similar to the widely applied HILIC column; however, the AXH column also enabled the resolution of the closely related isomeric forms of FA2G1S1 and FA2G2S1. This data highlights the potential for AXH to compliment traditional amide-based HILIC chromatography as the chromatographic method of choice for glycosylation analysis of mAbs.

Table 8. Structural identification of differently labeled *N*-glycans from trastuzumab.

Peak number	Glycan Annotation	Glycan structure
1	A1	
2	FA1	
3	A2	
4	FA2	
5	MAN5	
6	A2G1	
7	FA1G1, FMAN4A1	
8	FA2G1	
9	FA1G1S1	
10	FA2G2	
11	FA2G1S1	
12	FA2G2S1	

Conclusions

- An LC-MS method has been outlined that may be used for identification of *N*-glycans from therapeutic proteins using a Thermo Scientific Vanquish Flex UHPLC system coupled to a Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with the BioPharma option.

- Mixed-mode analytical columns, such as the Thermo Scientific GlycanPac AXH-1 and the Thermo Scientific GlycanPac AXR-1 columns, offer an additional separation dimension that may be utilized for resolution and detection of *N*-glycans in a mixture.

- The Thermo Scientific AXH-1 column may be a powerful alternative to HILIC chromatographic phases for glycosylation analysis of therapeutic mAbs.

References

1. Bones, J., et al. 2D-LC analysis of BRP 3 erythropoietin N-glycosylation using anion exchange fractionation and hydrophilic interaction UPLC reveals long poly-N-acetyl lactosamine extensions. *Anal. Chem.*, **2011**, *83* (11), 4154-62.

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