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At-line N-linked glycan profiling for monoclonal antibodies with advanced sample preparation and high-performance liquid chromatography

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N-linked glycosylation is a post-translational modification that occurs on many proteins during biosynthesis. The profile of different glycans on the protein is a critical quality attribute of some recombinant biopharmaceutical proteins including monoclonal antibodies (mAbs). Methods for profiling glycan should be robust, fast, and sensitive. Isolating glycans from proteins and tagging a label on glycans is the most commonly used technique for glycan profiling. Currently, existing protocols for sample preparation can be complicated, time-consuming, and expensive, which can limit the wide adaptation of glycan profiling methods. As a further barrier to use, an expensive ultra-high-pressure liquid chromatography (UHPLC) system is frequently required for the profile. In this article, a low cost and easily-used workflow of sample preparation is coupled with a standard high-performance liquid chromatography (HPLC) system to achieve comparable results to UHPLC. The number of steps required in the protocol and the time, as well as the cost associated with the sample preparation, is significantly reduced, while maintaining robust analytical performance. We describe the creation and validation of a human serum IgG glycan library to be used as the calibration standard, and successful profiling of glycoforms from a variety of mAbs.

[**Key words:** Monoclonal antibody; Critical quality attribute; N-linked glycosylation; Sample preparation; High-performance liquid chromatography; High resolution profile]

N-linked glycosylation is a typical post-translational process that occurs with many proteins during biosynthesis, including monoclonal antibodies (mAbs), enzymes and peptide hormones (1–6). The heterogeneity of glycoforms for protein biologics is typically observed from biopharmaceutical processes, and is a critical quality attribute for efficacy and safety (7–9). Therefore, achievement of a consistent N-linked glycoform profile for biologic products is needed, and studies to understand cell culture impact on N-linked glycans are in high demand (1,10–15).

Different techniques are currently available for glycan profiling. These techniques include analyses at a variety of levels, including intact proteins, glycopeptides, and isolated glycans (16–18). To obtain a quantitative profile of a glycan, the most common method is releasing the glycan from proteins, followed by attachment of a fluorescence tag on the isolated glycan, and a chromatographic procedure to separate and measure the various glycoform structures (19). Several derivatization methods are available for fluorescence labeling of isolated glycans, extensively reviewed in several articles (20,21). 2-Aminobenzamide (2-AB) is one of the most common labeling reagents, providing satisfactory sensitivity, stability and enables good glycan separation by high-performance liquid chromatography (HPLC). The elution profile of 2-AB labeled glycans, using a hydrophilic interaction chromatography (HILIC)-based chromatographic method, has been thoroughly documented and thus can be used as a reference profile (22–24).

The process for preparing fluorescence-labeled glycans from a cell culture supernatant requires a number of intermediate reactions and purification steps. Currently, commercial kits that provide the reagents and materials associated with these steps are quite expensive. The procedures can demand a large investment of time and effort. In addition, ultra-high-performance liquid chromatography (UHPLC) is often specified to achieve high-resolution separations. These prerequisites pose limitations for researchers who operate with limited budgets, and lack access to UHPLC.

To address these limitations, this study was designed to produce a fast, simple, and more affordable operation of glycan preparation, as well as a method that employs standard HPLC systems to achieve glycan separation comparable to UHPLC. In the sample preparation workflow, the intermediate steps of drying and glycan purification after enzymatic reaction are removed; incubation time is reduced, and the expense associated with process material is minimized.

To understand the importance of changes in the analysis conditions, the impacts of process parameters including reaction time, reagent amount, and material selection on the analysis outcome were studied. The reliability of the method was ultimately verified. Even though protocol development for glycan profiling is not uncommon in research literature, consideration of methods' robustness has not been consistently evaluated. Since glycan preparation goes through several steps, a biased result can be possibly caused at the endpoint if intermediate procedures do not generate consistent

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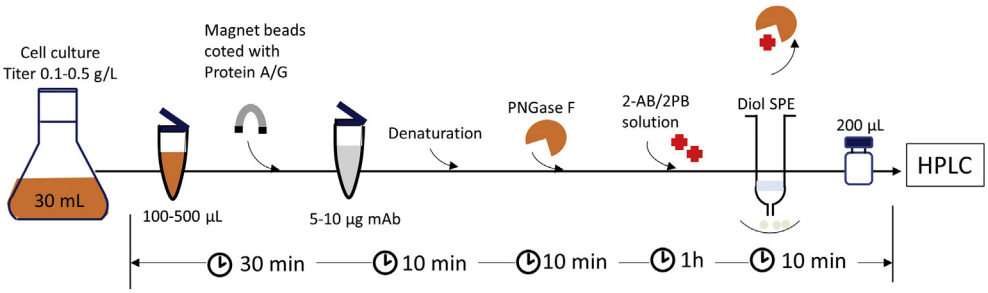

FIG. 1. Procedure for preparation of labeled glycan.

results. Therefore, each parameter was examined in detail at all steps in the procedure.

The protocol in this study demonstrates the satisfactory separation of a complex glycan mixture using a conventional HPLC system (maximum pressure, 400 bar), with the results comparable to published studies employing UHPLC. This information will especially benefit laboratories without access to UHPLC (24–26). The sensitivity and reproducibility of the methods allows accurate profiling with IgG samples at the 5-µg scale. The assay therefore, can be adapted to projects over a broad range of magnitude, from micro-culture to large batch culture. This is especially of value for studies with limited sample size, including low titer producing cell cultures, and scaled-down culture systems such as Ambr 250 multi-parallel bioreactors (27).

MATERIALS AND METHODS

Materials Human serum IgG, 2-AB, 2-picoline borane complex (2PB), ammonium formate and HPLC-grade acetonitrile (ACN), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Magnetic beads coated with protein A and protein G were from Thermo Fisher Scientific (Waltham, MA, USA). The PNGase F enzyme kit (containing 10× denaturing buffer, NP-40 and 10× reaction buffer) was from New England Biolabs (Ipswich, MA, USA) and stored as instructed by the manufacturer. Diol SPE cartridges were from either HyperSep Diol SPE (Thermo Fisher Scientific) or Bond Elute Diol (Agilent Technology, Santa Clara, CA, USA). Glycoclean S cartridges were from Prozyme (Hayward, CA, USA). The 0.2 µm inline filter was from Restek Corporation (Bellefonte, PA, USA) and the BEH Amide column (2.1 × 50 mm, 1.7 µm) was from Waters (Waters Corporation, Milford, MA, USA). Cell lines producing different mAbs were donated by collaborators.

Protein purification from the culture supernatant From a shake culture with titer of 0.1–0.5 g/L, 100–500 µL cell culture samples are collected, transferred to 1.5 mL Eppendorf tubes and centrifuged at 1000 rpm for 5 min to pellet the cells. The supernatants containing the IgG are transferred to clean 1.5 mL Eppendorf tubes. The procedure of using magnetic beads to isolate IgG is modified from the manufacturer's instructions. Specifically, an aliquot of 10 µL magnetic bead slurry is added to one microtube. The beads are washed 2 times with 250 µL binding buffer, using the magnetic stand at each wash to separate the beads from the buffer, which is discarded after each washing step. A volume of culture supernatant containing about 20–50 µg mAb is then added to the Eppendorf tube, vortexed gently and incubated on a shaker for 15 min. The beads, with the IgG attached to the surface, are subsequently collected by placing the tube on the magnetic stand and removing the supernatant. The beads are washed by two consecutive wash using 250 µL binding buffer and one wash using 250 µL water. After the wash step, beads are suspended into 25 µL elution buffer and incubated for 10 min. The tube is then placed on the magnetic stand and the supernatant (free of magnetic beads) is transferred to a new Eppendorf tube that is pre-loaded with 8 µL neutralization buffer. The reagents used in the above procedures are: (i) binding buffer: 50 mM sodium phosphate, pH 7; (ii) wash buffer: PBS; (iii) elute buffer: 100 mM sodium phosphate, pH 2.5; (iv) neutralizing buffer: 0.5 M sodium phosphate dibasic pH 8.

Enzymatic digestion of N-linked glycans from glycoproteins The concentration of human serum IgG solution or purified mAbs is measured using a Nanodrop One spectrophotometer (Thermo Fisher Scientific). An aliquot of purified IgG/mAbs, typically containing 5–20 µg of protein in 30 µL volume, is denatured by addition of 4 µL of 10× denaturing buffer (New England Biolabs) and 6 µL H_2O, and heating at 100 °C for 10 min. After cooling, 5 µL of 10 × PNGase F reaction buffer, and 5 µL of NP-40 in H_2O are added to the sample to reach a final 50 µL reaction volume. The PNGase F enzyme is added at 1 µL (500,000 units/mL) or other volumes as specified in the Results. The digestion is carried out at 37 °C, for intervals that range from 10 min to 24 h.

2AB labeling with 2PB reduction The 2AB labeling solution is prepared fresh for each reaction and used within 1 h. The labeling solution contains 0.35 M of 2AB and 1 M of 2PB in 70% DMSO + 30% acetic acid. The enzymatically-digested protein solution from the previous step (50 µL) is mixed with 50 µL labeling solution, and incubated at 65 °C for 1 h, or other intervals as described in Results. The reaction is protected from light by aluminum foil.

Removal of excess 2AB To use Diol SPE for 2AB removal, Diol SPE cartridges are attached to a vacuum manifold equipped with a vacuum pump. The cartridges are first washed with 1 mL of H_2O, and then conditioned with 4 washes with 1 mL 100% ACN. The samples of labeled glycan from the previous step are diluted in ACN at a ratio of 1:9 (e.g., 100 µL sample +900 µL ACN), and then loaded and pumped through the cartridge. Two washes with 500 µL ACN are applied to remove unbound compounds including excess 2-AB. At the elution step, clean microtubes are placed under the cartridges, and two consecutive aliquots of 100 µL water are applied to elute the labeled glycans. The total volume of the eluent is 200 µL. If diol cartridges are to be reused, 3 mL of water is used to pre-wash the cartridge.

During the development of the method, we also evaluated the use of HILIC material for 2AB cleanup. For this evaluation, the GlykoCleanS cartridge (HILIC-based) was used. The cartridge is primed with 1 mL water, 5 mL 30% acetic acid and 3 mL ACN. An aliquot of 10 µL of crude reaction mixture after equilibration at room temperature is loaded onto the cartridge membrane, followed by a 15 min incubation. The cartridge is washed with 1 mL 100% ACN, followed by 5 washes with 1 mL of 96% ACN/4% H_2O. Labeled glycans are finally eluted with 3 consecutive volumes of 0.5 mL water (total 1.5 mL). To concentrate the samples, the volume of samples is reduced to 250 µL using a Speed Vac operating at ambient temperature (Thermo Fisher Scientific).

HPLC method The HPLC runs are carried out on an Agilent 1100 gradient system (Agilent Technologies) with a Model 1329 A fluorescence detector. A calibration standard of labeled glycans from human serum IgG should be prepared and used to verify stable HPLC performance. The separation is accomplished with a Waters BEH amide column (2.1 × 50 mm, 1.7 µm). A 0.2 µm inline filter (Restek no. 25809, Restek, Bellefonte, PA, USA) is installed at the front of the column to prevent clogs caused by sample impurities. Mobile phase A is 100 mM ammonium formate, pH 4.5 and mobile phase B is 100% ACN. Analytical conditions are as follows: 25%–35% A (0–20 min); 35%–40% A (20–25 min); 40%–25% A (25–26 min), 25% A (26–32 min). The last 6 min are required to re-equilbrate the column to the starting conditions of 25% A (the column should be carefully conditioned with 75% ACN before use for glycan profiling, to make sure the retention profile is the same in consecutive runs). It should be noted that the HPLC system used in this work has a 1 mL pulse dampener and 2-min autosampler lag; with HPLC systems with different settings, the gradient parameters used in the method may need to be slightly adjusted. The time of each run is 32 min. The flow rate is 0.4 mL/min and the column temperature is 50 °C. Fluorescence detector settings used are excitation 350 nm, emission 420 nm, and PMT gain = 12. An injection volume of 1 or 2 µL is used. Due to the

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TABLE 1. Time and material cost associated with the protocol.

Step	Operation time (min)	Material	Dose/sample	Cost/sample (US dollar)	Reusability
IgG extraction	30	A/G coated magnetic beads (ThermoFisher no. 88802)	10 μL	1	Yes
Denaturation	10	PNGaseF kit (NEB no. P0704S)	5 μL	Negligible	NA
De-glycosylation	10	PNGaseF (NEB no. P0704S)	1 μL (10 time diluted from original stock)	0.60	NA
Derivatization	60	2AB and 2PB (Sigma–Aldrich nos. A89804 and 3999-38-0)	50 μL	Negligible	NA
Cleanup	10	Diol-SPE (Agilent no. 12102009)	1 piece	2	Yes
HPLC	30[a]	Human serum IgG used as test standard (Sigma–Aldrich I2511-10 mg)	10 μg	Negligible	NA

[a] Operation time for HPLC is min/run.

low internal volume of the HPLC column (100 μL), injection volumes should be kept small. Large injection volumes (>20 μL) were found to cause peak broadening. If necessary, samples can be concentrated before injection. Because the gradient is very sensitive to H_2O-content of the mobile phase, the reservoir containing ACN should be kept well-sealed, to avoid the absorption of water vapor by the ACN.

RESULTS

Protocol overview A brief flow of the protocol developed in this work is shown in Fig. 1 and the details are described in the Method section. The sample preparation begins with IgG extraction from culture supernatant using magnetic beads coated with protein A/G, followed by rapid digestion by PNGase F for as little as 10 min, and another incubation with 2AB for 1 h at 65 °C, to form the fluorescent derivatives. The resulting sample is purified with the Diol SPE unit and ready for HPLC analysis. The complete protocol, starting from culture supernatant, can be completed within 2 h, and could be adapted to a high-throughput platform. The time and material costs associated with the protocol are dramatically decreased from commercial kits (as shown in Table 1).

The separation of glycoforms is based on an HPLC system and a 50 mm HILIC column, as described in the Method. The optimal flow rate was found to be 0.4 mL/min, after the evaluation of flow rates between 0.2 and 0.8 mL/min (Fig. S1). The entire HPLC analysis is complete within 26 min, with another 6 min for the column re-conditioning before the next injection for a total run time of 32 min per sample.

Protocol robustness during process change In this section, the effects of parameters used in sample preparation on analysis accuracy were assessed. This effort examines the consistency of assay results during the optimization of the parameters at different intermediate steps of sample preparation and ensures that variable intermediates are not formed to cause non-uniform outcomes of glycoform profiles.

First, the effect of the amount of enzymes used in the de-glycosylation step was evaluated. An aliquot of PNGase F enzyme stock solution (initial concentration, 500,000 units/mL) was diluted 10 times in water (final concentration 50,000 units/mL). A volume of 10 μL, 5 μL, 2 μL and 1 μL of the diluted PNGase F, corresponding to 500, 250, 100, 50 units of enzyme, respectively, was used to digest 16 μg human serum IgG. Each incubation was conducted for 10 min. After completing the remaining steps in the protocol, the results of the distribution of four major glycans (G0F, G1F, G2F, and G2FS1) in the IgG are shown in Fig. 2A, and complete chromatograms in Fig. S2. The results demonstrate that the smallest amount of 1 μL PNGase F tested (containing 50 units of enzyme) obtains the same result for the glycan profile as the result using 500 units of the enzyme.

Next, the effect of different incubation periods with the enzyme was evaluated. It is commonly recommended to conduct an overnight or 24 h incubation with PNGase F (28). However, with IgG this reaction time might be longer than required. To examine this question, 100 μg of denatured human IgG was digested by 1 μL of PNGase F (500,000 units/mL). Intermediate samples during the course of a 24 h incubation were collected at 10 min, 20 min, 30 min, 40 min, and 24 h, and analyzed following completion of the reaction protocol. The results of four major glycans are shown in Fig. 2B and complete chromatograms in Fig. S3. The results suggest that a 10 min incubation achieves the same glycoform profiles as a 24 h incubation, therefore, the digestion of glycans from a denatured human serum IgG can be accomplished very rapidly.

Third, to determine the time required for the 2AB labeling reaction, glycans from 100 μg of IgG were incubated with 2-AB and reducing agents for 1, 2, and 3 h. As shown in Fig. 2C (chromatograms in Fig. S4), consistent glycoform profiles are obtained for all these incubation times. Therefore, incubation times as short as 1 h for 2AB labeling can be employed.

Finally, Diol SPE cartridges were investigated in this protocol as an economical replacement for the HILIC cartridges that have been commonly used in many established protocols. After the 2AB labeling reaction, a step to remove 2AB is needed since an excess of this reagent injected on the HPLC creates a large early-eluting peak that interferes with measurement of glycans at low molecular weight. To compare the performance of Diol- and HILIC- based cartridges, two aliquots of 2AB labeled human serum IgG glycan were taken from the labeling reaction mixture and purified in parallel using either Diol SPE or GlykoCleanS cartridge (HILIC material). The patterns of glycoform profiles obtained from the Diol and HILIC treatment were found comparable (Fig. 2D). In fact, the Diol SPE achieved a better resolution by clearly showing minor glycan peaks such as G2FS2 (Fig. S5). It was also confirmed that Diol SPE cartridges can be regenerated by washing the resin with water and used for purifying additional samples, with minimal carryover in the eluent between uses.

Application in human serum IgG and different mAb biosimilars The developed protocol was first applied to a sample of human serum IgG, for which the pattern of its 20–30 major glycoforms has been documented in the literature (22–24). Following the sample preparation, a good separation of the glycoforms from human serum IgG was generated on HPLC and shown in Fig. 3A. The peak IDs are assigned according to the reference pattern of published human IgG glycan libraries. A total of 26 glycoforms are annotated, including high mannose, hybrid, and sialylated glycoforms. Most glycoforms are eluted within a shallow gradient over 75%–65% ACN (mobile phase B). The more complex glycan structures are eluted during the gradient from 65% to 60% ACN. The profile of this material was also used later as a calibration standard to annotate glycans from unknown samples.

We then compared the quantitative results of glycan distribution obtained for human serum IgG glycoform profiles to published studies. Two reference glycoform distribution data of human serum IgG glycan library were obtained from the literature (Table S1) (22,23), and it was noted that variation occurs with

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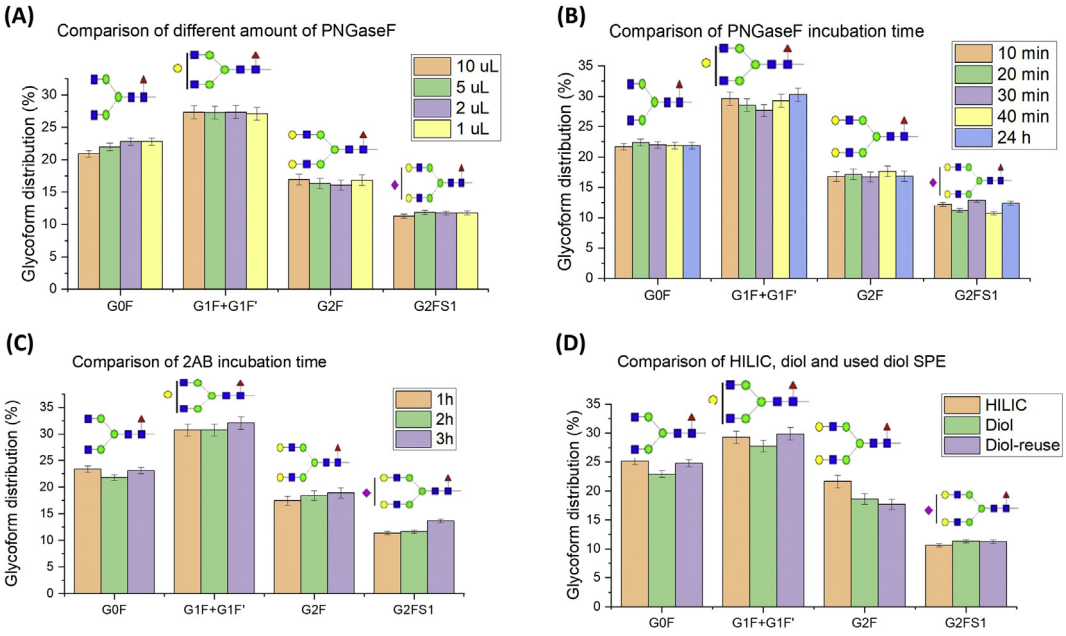

FIG. 2. Evaluation of sample preparation steps. (A) Comparison of glycoform profiles using different amount of PNGaseF at an incubation time of 10 min; (B) comparison of glycoform digested by PNGaseF given different amount of incubation time; (C) comparison of the glycoform profiles given different amount of incubation time at 2AB labeling; (D) comparison between using HILIC and Diol SPE in the process for removing excess 2AB.

published glycoform distributions. This could be due to the variations in the starting material used across different laboratories, or methodological differences. Our data fall within the range of the published data.

To further test the application of our protocol, results were obtained for five different mAb biosimilars produced from CHO cell culture. Using human serum IgG as the calibration standard, peaks of glycoforms in the mAbs were aligned and annotated (Fig. 3B). The results show that the glycan profiles of human serum IgG can be used as a reference to obtain glycoform IDs for unknown samples. Most of the glycoforms in the mAbs could be identified, except a few glycoforms that were not observed in the standard, and those would need further characterization using techniques such as mass spectrometry.

Application in cell culture study with varying galactose concentrations Finally, we applied the method to examine the impact of galactose levels in culture media on mAb glycan changes. We used four small volume (30 mL) cultures of a CHO cell line producing Adalimumab biosimilars with a titer <0.5 g/L. Three cultures were respectively supplemented at 72 h with 2 mM, 50 mM, and 100 mM galactose, while one culture remained non-supplemented as control. One hundred microliter samples were taken at 144 h from each culture and analyzed. As shown in Fig. 4, the titer was around 0.35 g/L. The G0F is the most abundant glycan in this mAb, and G1F is the second most abundant. The results show that at higher levels of galactose in the feed medium (up to 100 mM), there is a progressive increase in the G1F component on mAbs. The corresponding changes with the UDP-galactose, which is the substrate of galactosylation, are shown in our other published study (15). Thus far, the analytical method developed in this work has successfully enabled the study with small cost of material and sufficient resolution.

Method sensitivity and reproducibility To demonstrate the sensitivity of the protocol, a glycan sample prepared from 30 μg purified IgG in 200 μL H_2O was injected on the HPLC with a series of volumes: 2 μL, 0.5, 0.25, 0.125, 0.063 and 0.032 μL (Fig. S6). An injection of 0.25 μL, which corresponds to 1 pmol of glycan from 38 ng of IgG, provided a good signal/noise ratio. This result shows that the protocol can be used to process culture supernatant that contains a very small amount of IgGs.

In order to further test if starting with different amounts of IgGs for the protocol will lead to biased results, complete sample preparation was carried out with IgG samples ranging from 5 μg to 50 μg. Consistent results are obtained (Table 2). The consistency demonstrates that the sample preparation protocol is robust to changes in the starting amount of IgG or mAbs. Further investigation was made for the reproducibility across HPLC analyses. Five replicate runs of human serum IgG were tested. The peak areas are calculated for the four most abundant glycoforms (G0F, G1F, G2F, and G2FS1). As shown in Table 3, the results show stable retention times and peak areas over multiple injections. Column performance was shown to be consistent throughout the study, with stable backpressure (a range of ± 2 bars) and consistent results over 200 injections. When testing the method with a second column from the same supplier, the same result was obtained, as shown in Fig. S7. To this point, both HPLC and sample preparation have demonstrated satisfactory consistency and reliability.

DISCUSSION

Our simplified protocol allows rapid preparation of glycans from mAbs for HPLC analysis, and purification is only required after glycans' labeling with a fluorescent tag. Most established protocols have recommended purifying the isolated glycans following enzymatic removal from the proteins, whereas, our method shows that the compounds such as SDS (an ingredient of the denaturation buffer), NP-40, and the de-glycosylated protein accumulated from the de-glycosylation step cause little interference to 2AB derivatization. As a result, we have greatly streamlined the workflow for preparation of labeled glycans for HPLC analysis.

The use of 2PB as a reducing agent confers several advantages. This reagent, which converts the initial Schiff base between 2-AB and the glycan to a stable amide linkage, is active under aqueous conditions, and can be mixed directly with the 2-AB in the DMSO/acetic acid solvent mixture used for labeling reaction, to form a

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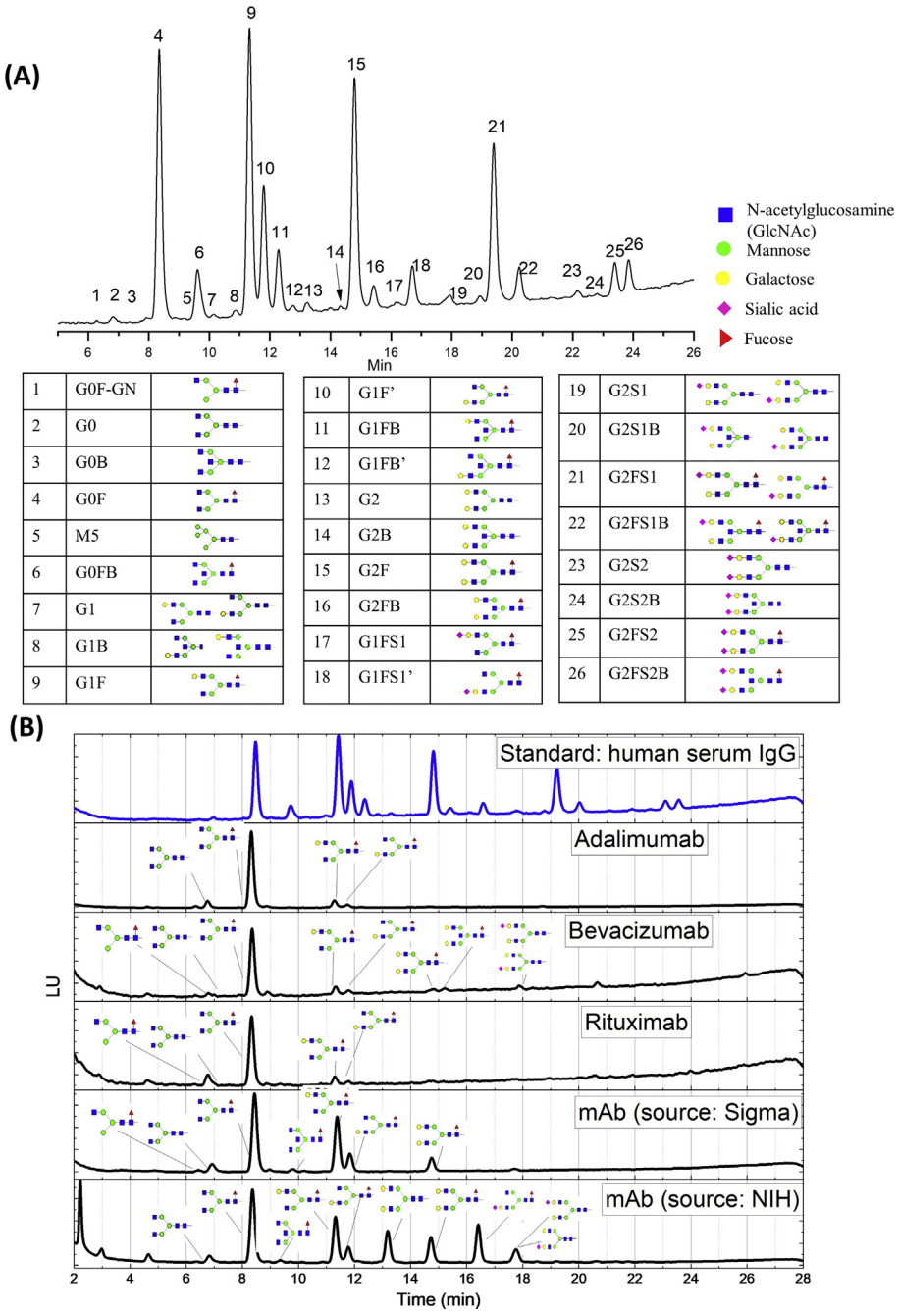

FIG. 3. Application of the method to profiling glycans of human serum IgG and different mAb biosimilars. (A) Chromatogram of 2AB-labeled human serum IgG glycans. The sample was derived from 20 μg of human serum IgG. At the final step, the glycans were eluted from the Diol SPE cartridge in 200 μL water, and 1 μL was injected on the HPLC. (B) Profiling different mAb biosimilars. The human serum IgG glycan (first plot) was used as the calibration standard and the unknown peaks in the subsequent mAbs were aligned and identified. The IDs of human serum IgG glycan are shown in Fig. 3A.

single reagent mixture. This 2-PB is used to replace potentially hazardous sodium cyanoborohydride, which has the further disadvantage of requiring anhydrous reaction conditions. Adaptation of 2PB both enhances the safety of the protocol and removes a laborious drying step from the workflow. Only two previous publications have reported the use of 2PB (23,28).

We were able to get stable, consistent glycan profiles with shorter enzymatic de-glycosylation intervals and use of smaller amounts of PNGase F enzyme reagent. These modifications of commonly-used protocols did not result in any changes in glycan profiles, including the sialylated glycoforms which are known to be highly sensitive to analytical conditions. These findings indicate that our protocol is appropriate for analysis of glycans on IgG and mAb; however, for glycoproteins that are more complex than mAbs, the enzymatic digestion period and quantity of PNGase F enzyme need to be carefully evaluated. The use of Diol-SPE cartridge post 2AB derivatization can decrease analysis costs. Diol-SPE ($1–$2 each) is an appropriate substitute for the widely used HILIC SPE cartridges and demonstrates comparable performance (23,29). The option of re-using Diol SPE cartridges is also feasible.

For the first time, we demonstrated high resolution results for glycoforms using only a standard pressure (400 bar) HPLC system and a 50 mm long HILIC column. Due to the short column length, the method can be operated at the flow rate of 0.4 mL/min, while

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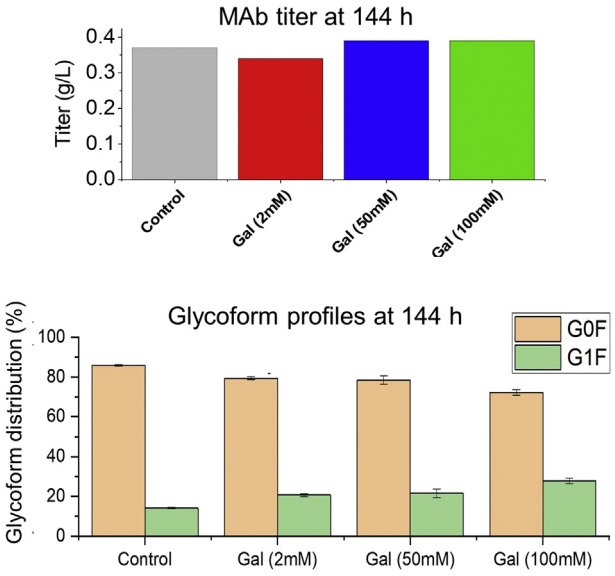

FIG. 4. Application in glycoform profiles of mAbs from an experiment showing the impact of galactose feeds on glycoform changes. The culture conditions include one control and three levels of galactose feed (2 mM, 50 mM, and 100 mM). The culture working volume is 30 mL and the samples are from 144 h. The glycoforms of each sample are profiled with triplicate measurements. The titer data from each culture at 144 h is shown at the top.

TABLE 2. Reproducibility of results from multiple processes (n = 3).

Glycoform	Mean (%)[a]	SD	CV%
G0F	20.88	0.79	3.77
G1F + G1F′	28.32	1.11	3.90
G2F	16.85	0.08	0.46
G2FS1	12.04	0.70	5.82

[a] Data represent the glycoform distribution (%).

TABLE 3. Reproducibility of retention time and peak area from multiple injections (n = 5).

Glycoform	Retention time			Peak area		
	Mean	SD	CV%	Mean	SD	CV%
G0F	5.17	0.15	2.99	11.38	0.29	2.53
G1F + G1F′	7.00	0.15	2.12	17.08	0.62	3.65
G2F	9.24	0.15	1.60	9.98	0.49	4.94
G2FS1	12.19	0.15	1.24	6.93	0.18	2.62

generating low backpressure (< 200 bar), and thus completes an HPLC run within about 30 min, including equilibration. These conditions make the method suitable for many laboratories. Our results with IgG prepared from human serum are comparable to UHPLC results (22,24) and an improvement over several published HPLC results (28,30,31).

The method shows very good sensitivity. One-pmol glycan (obtained from 38 ng IgG) injected on the HPLC can generate satisfactory values of S/N. Many methods specify the use of 200 μg or more of IgG or mAb (17,28). In this protocol, a quantity of 5 μg IgG at the start of the protocol provide excellent analytical sensitivity, when final dilution volume is 200 μL and injection volume is 1 μL. The sensitivity may vary between different fluorescence detectors, but the sensitivity generally enables a small amount of material (5 μg or less purified protein) to achieve a reliable glycan profile. This sensitivity enables routine glycan profiling from small culture volumes, and low-yield cell lines (e.g., as small as 100 μL per sample, for a culture with titer as low as 0.1 g/L).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2020.04.009.

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