DIONEX

Application Update 180

Direct Determination of Sialic Acids in Glycoprotein Hydrolyzates by HPAE-PAD

INTRODUCTION

Sialic acids are critical in determining glycoprotein bioavailability, function, stability, and metabolism.[1] Although over 50 natural sialic acids have been identified,[2] two forms are commonly determined in glycoprotein products: *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). Because humans do not generally produce Neu5Gc and have been shown to possess antibodies against Neu5Gc, the presence of this sialic acid in a therapeutic agent can potentially lead to an immune response.[3] Consequently, glycoprotein sialylation, and the identity of the sialic acids, play important roles in therapeutic protein efficacy, pharmacokinetics, and potential immunogenicity.

Sialic acid determination can be performed by many methods. Typically, sialic acids are released from glycoproteins by acid hydrolysis or by enzymatic digestion before analysis. Once the sialic acids are liberated, there are many options for quantification. Numerous spectroscopic methods exist, although interferences in these methods can cause overestimation of the concentration of sialic acids in many samples. Therefore, chromatographic methods that separate the sialic acids from potentially interfering compounds are preferred.[4] Among the chromatographic methods, there are thosethat require further sample derivatization for analyte detection, such as fluorescent labeling followed by high-performance liquid chromatography (HPLC), and direct detection methods such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD).[5] Of these methods, HPAE-PAD offers the advantage of direct analysis without sample derivatization.

In this work, sialic acids are determined in five representative glycoproteins by acid hydrolysis followed by HPAE-PAD. Sialic acid determination by HPAE-PAD on a CarboPac® PA20 column is specific and direct, eliminating the need for sample derivatization after sample preparation. The use of a disposable gold on polytetrafluoroethylene (Au on PTFE) working electrode simplifies system maintenance compared to conventional gold electrodes while providing consistent response with a four-week lifetime. The rapid gradient method discussed separates Neu5Ac and Neu5Gc in under 10 min with a total analysis time of 16.5 min, compared to 27 min using the CarboPac PA10 column by a previously published method.[6,7] By using the CarboPac PA20 column, the total analysis time is reduced, eluent consumption and waste generation are reduced, and sample throughput is improved.

EQUIPMENT

Dionex ICS-3000 or ICS-5000 Ion Chromatography system including:

SP Single Pump or DP Dual Pump module

DC Detector/Chromatography module

AS Autosampler

ICS-3000/5000 ED Electrochemical Detector (Dionex P/N 061719)

Electrochemical Cell (Dionex P/N 061757)

Disposable Gold Working Electrode, Au on PTFE (Dionex P/N 066480)

Reference Electrode (Ag/AgCl) (Dionex P/N 061879)

Chromeleon® 7 Chromatography Workstation

Polypropylene injection vials, 0.3 mL, with caps (Dionex P/N 055428)

Polypropylene injection vials, 1.5 mL, with caps (Dionex P/N 061696)

Nalgene® 1000 mL 0.2 µm nylon filter units (VWR P/N 28198-514)

Polypropylene microcentrifuge screw-cap tubes, 1.5 mL (Sarstedt P/N 72.692.005)

Dry block heater (VWR P/N 13259-005)

REAGENTS AND STANDARDS

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better

Sodium hydroxide, 50% (w/w) (Thermo Fisher P/N SS254-500)

Sodium acetate, anhydrous (Dionex P/N 059326)

Acetic acid (JT Baker P/N 9515-03)

N-Acetylneuraminic acid (Neu5Ac, NANA), Ferro Pfanstiehl

N-Glycolylneuraminic acid (Neu5Gc, NGNA), Ferro Pfanstiehl

Micro BCA™ Protein Assay Kit (Thermo Scientific P/N 23235)

α (2→3,6,8,9) Neuraminidase, proteomics grade from *Arthrobacter ureafaciens* (Sigma P/N N3786)

SAMPLES

Five glycoproteins were selected for analysis:

Calf fetuin (Sigma P/N F2379)

Bovine apo-transferrin (b. apo-transferrin) (Sigma P/N T1428)

Human transferrin (h. transferrin) (Sigma P/N T8158)

Sheep α_1-acid glycoprotein (s. AGP) (Sigma P/N G6401)

Human α_1-acid glycoprotein (h. AGP) (Sigma P/N G9885)

CONDITIONS

Columns:	CarboPac PA20, 3 × 150 mm (P/N 060142) CarboPac PA20 Guard, 3 × 30 mm (P/N 060144)
Eluent Gradient:	70–300 mM acetate in 100 mM NaOH from 0–7.5 min, 300 mM acetate in 100 mM NaOH from 7.5–9.0 min, 70 mM acetate in 100 mM NaOH from 9.0–9.5 min, 7 min of equilibration at 70 mM acetate in 100 mM NaOH.
Eluents:	A: NaOH, 100 mM B: Sodium acetate, 1.0 M, in 100 mM NaOH
Flow Rate:	0.5 mL/min
Temperature:	30 °C (column and detector compartments)
Inj. Volume:	10 µL
Detection:	Pulsed amperometric, disposable Au on PTFE electrode
Background:	18–25 nC (using the carbohydrate waveform)
Noise:	~15–30 pC
System Backpressure:	~3000 psi

Carbohydrate 4-Potential Waveform for the ED

Time(s)	*Potential (V)*	*Gain Region**	*Ramp**	*Integration*
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.40	+0.1	Off	On	Off
0.41	-2.0	Off	On	Off
0.42	-2.0	Off	On	Off
0.43	+0.6	Off	On	Off
0.44	-0.1	Off	On	Off
0.50	-0.1	Off	On	Off

*Settings required in the ICS-3000/5000, but not used in older Dionex systems.

Reference electrode in Ag mode (Ag/AgCl reference). See Application Update 141 for more information.[6]

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solutions

Prepare 1 L of 100 mM sodium hydroxide by adding 5.2 mL of 50% (w/w) NaOH to 994.8 mL of degassed DI water.

Prepare 1 L of 1 M sodium acetate in 100 mM sodium hydroxide by dissolving 82.0 g of anhydrous sodium acetate in ~800 mL of DI water. Filter and degas the acetate solution through a 0.2 µm nylon filter unit. Transfer the solution to a 1 L volumetric flask, add 5.2 mL of 50% (w/w) NaOH, and fill the flask with degassed DI water. See Dionex Technical Note 71 for detailed information on eluent preparation for HPAE-PAD applications.[8]

Acetic Acid, 4 M

Transfer 22.5 mL of glacial acetic acid to a polyethylene bottle containing 77.5 mL of DI water.

Sodium Acetate Buffer, 0.1 M, pH 5 for Neuraminidase Digestions

Prepare a 0.3 M sodium acetate stock solution by dissolving 12.31 g of sodium acetate in 500 mL of DI water. Transfer 68.21 g (68.3 mL) of 0.3 M sodium acetate to a 250 mL polypropylene bottle. Add 1.8 mL of 4 M actetic acid to the solution. Dilute to a total of 249.62 g (250 mL).

Stock Standard Solutions

Dissolve 149.8 mg of Neu5Ac in 50 mL DI water and 41.0 mg Neu5Gc in 50 mL of DI water. This results in 9.68 mM and 2.52 mM stock solutions, respectively. Dilute 500 µL of 9.68 mM Neu5Ac and 130 µL of 2.52 mM Neu5Gc to 48.4 mL total with DI water. Aliquot this mixed stock of 0.10 mM Neu5Ac and 6.8 µM Neu5Gc into 1.5 mL cryogenic storage vials and store at -40 °C.

Working Standard Solutions

Prepare calibration standards by diluting the standard stock solution as detailed in Table 1. For example, 10 µL of the stock solution were added to 990 µL of DI water to prepare a calibration standard of 1.0 µM Neu5Ac, or 10 pmol/10 µL injection. Prepare standards daily from the stocks stored at -40 °C.

Protein Stock Solutions, 4.0 mg/mL Nominal

Dissolve 3.44 mg of sheep α_1-acid glycoprotein in 860 µL of DI water. Gently swirl to thoroughly mix the solution. Prepare 200 µL aliquots of the solution in microcentrifuge vials to minimize freeze/thaw cycles when the stock is needed. Store all protein solutions at -40 °C. Repeat this process as follows. Dissolve 8.8 mg (b. apo-transferrin), 8.6 mg (h. transferrin), and 8.1 mg (fetuin) of the glycoprotein in individual aliquots of 2.0 mL DI water. Dissolve 2.2 mg of h. AGP in 0.56 mL of DI water. Each glycoprotein will be at a nominal concentration of 4 mg/mL.

Working Stock Protein Solutions

Pipet 250 µL of a protein stock solution into 1750 µL DI water to prepare a working stock solution. Aliquot 400 µL of the working stock into individual microcentrifuge tubes and store the working stock solutions at -40 °C. Protein may be lost both during freeze/thaw cycles and by adsorption to surfaces. Therefore, it is important to measure the working stock

Table 1. Sialic Acid Standards Used for Sample Analysis

Volume of Combined Stock Standard (µL) Diluted to 1000 µL	Neu5Ac Concentration (nM)	Neu5Gc Concentration (nM)	Neu5Ac Amount (pmol/10µL)	Neu5Gc Amount (pmol/10 µL)
1.0	100	7.8	1.0	<LOQ*
2.5	250	20	2.5	<LOQ*
5.0	500	39	5.0	0.39
10	1000	78	10	0.78
25	2500	200	25	2.0
50	5000	390	50	3.9
75	7500	580	75	5.8
100	10000	780	100	7.8

*Not used for Neu5Gc calibration

Table 2. Protein Hydrolyzate Concentrations						
Protein	**BCA Measured Working Soln. Conc. (µg/mL)**	**Volume Protein (µL)**	**Amount of Protein (µg)**	**Volume DI Water (µL)**	**Volume 4M Acetic Acid (µL)**	**Protein Conc. (µg/µL)**
Fetuin	280	50	14	50	100	0.07
h. Transferrin	400	50	20	50	100	0.10
b. apo-Transferrin	500	50	25	50	100	0.12
h. AGP	260	50	13	50	100	0.06
s. AGP	140	50	7.0	50	100	0.04

protein concentrations before hydrolysis by using a colorimetric BCA protein assay kit. Values listed in Table 2 are results from BCA assay of the working stock solutions.

Acetic Acid Hydrolysis of Proteins

Add 14 µg (fetuin), 20 µg (h. transferrin), 25 µg (b. apo-transferrin), 13 µg (h. AGP), and 7 µg (s. AGP) of the glycoprotein to individual 1.5 mL microcentrifuge vials with a total of 200 µL of 2 M acetic acid as detailed in Table 2. For example, pipet 50 µL of the working fetuin stock, 50 µL of DI water, and 100 µL of 4 M acetic acid to prepare the solution for hydrolysis. Hydrolyze the protein solutions for 2 h by the method of Varki et al.[9] to preserve *O*-acetylated sialic acids for comparison to UHPLC-FLD method using DMB derivatization.[10] After hydrolysis, lyophilize and resuspend 50 µL of sample hydrolyzates in 500 µL of DI water; prepare more concentrated hydrolyzates (0.70 µg/µL of protein) by diluting the hydrolyzate 1:80 with DI water. Note that this acid hydrolysis method may not be optimized for complete release of all sialic acids without degradation of the free sialic acids. Optimization of the hydrolysis conditions for a given sample and analysis method is highly recommended. Additional hydrolysis conditions may be found in Technical Note 41.[7]

Neuraminidase Digestion of Proteins

Add 50 µL of DI water to a 25 mU vial of neuraminidase. Add 2 µL of this stock to 148 µL of 0.1 M sodium acetate buffer to prepare a 1 mU/mL neuraminidase solution. Add 14 µg, 20 µg, 25 µg, 13 µg, and 7 µg of fetuin, h. transferrin, b. transferrin, h. AGP, and s. APG, respectively, to individual 1.5 mL microcentrifuge vials with this solution and incubate at 37 °C for 18 h. After incubation, centrifuge the samples and dilute them with an additional 300 µL of DI water prior to analysis.

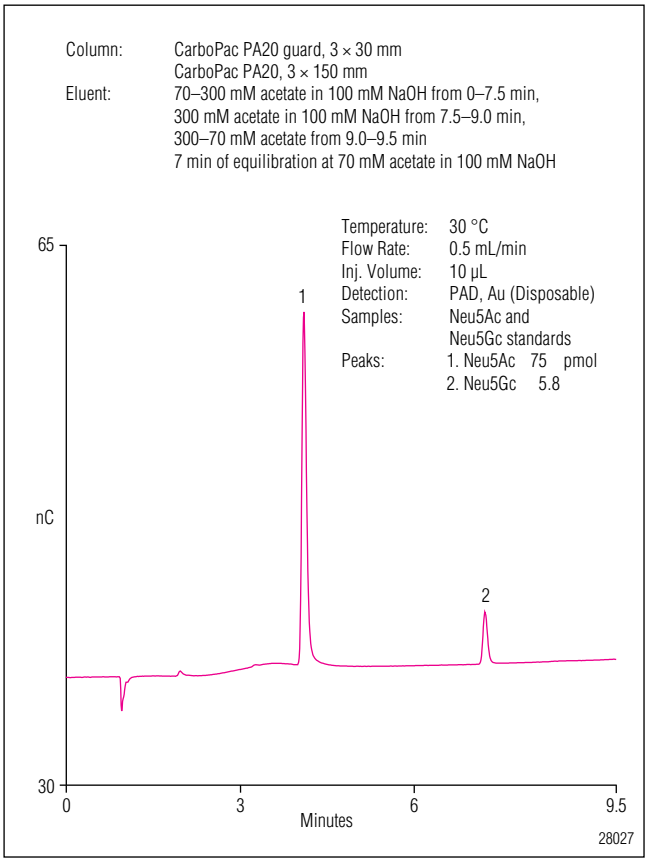

Figure 1. Separation of sialic acid standards on the CarboPac PA20 column.

RESULTS AND DISCUSSION

Figure 1 shows the separation of Neu5Ac and Neu5Gc on the CarboPac PA20 column with a 70–300 mM acetate gradient in 100 mM NaOH. The peaks are well separated and easily quantified. Additionally, the Neu5Ac peak is well separated from the void, which is an important consideration when analyzing acid hydrolyzed samples that may contain additional poorly retained compounds.

Table 3. Linearity, LOD, LOQ, and Precision of Sialic Acid Determination							
Analyte	**Range (pmol)**	**Coeff. of Determination (r^2)**	**RT (min)**	**RT Precision (RSD)**	**Peak Area Precision[a] (RSD)**	**LOQ[b] (pmol)**	**LOD (pmol)**
Neu5Ac	1.0–100	0.9997	4.08	0.18	1.08	0.5	0.17
Neu5Gc	0.39–7.8	0.9995	7.18	0.09	1.01	0.3	0.08

[a]Precision was measured by 7 injections of 25 pmol Neu5Ac, 2.0 pmol Neu5Gc.

[b]LOD and LOQ are confirmed by injections at the concentrations listed and measuring response at 3× and 10× the noise, respectively.

Linear Range, Limit of Quantification, Limit of Detection, and Precision

Table 3 shows the calibration results for Neu5Ac and Neu5Gc. In both cases, response is linear for the range studied. The limit of detection (LOD) and limit of quantification (LOQ) were confirmed by standard injections that resulted in a response of 3× and 10× the noise, respectively. Neu5Ac was determined to have an LOD of 0.17 pmol on column and an LOQ of 0.50 pmol. Similarly, Neu5Gc limits were found to be 0.08 pmol and 0.30 pmol. During this work, 2 and 3 mil gaskets were installed with the disposable electrodes and evaluated in terms of analyte linearity and LOQ. The results listed were determined with a 3 mil gasket. Injections of LOD and LOQ standards with a 2 mil gasket installed yielded equivalent results. When establishing an assay, standardize on one gasket size and specify it in the standard operating procedure.

Retention time and peak area precisions of standards were determined by seven injections of a mid-range standard. In both cases, precision was excellent, with an RSD of <0.2 for retention time for both sialic acids and peak area RSDs of 1.08 and 1.01 for Neu5Ac and Neu5Gc, respectively.

Sample Analysis, Precision, and Accuracy

Figure 2 shows the separation of hydrolyzed and lyophilized protein samples. In each case, Neu5Ac is well separated from early eluting components of the hydrolyzed sample. In each case, Neu5Ac is present, and as expected, Neu5Gc is not detected in human forms of the glycoproteins. Using fetuin as an example, 0.07 µg/µL of protein were hydrolyzed. After lyophilizing 50 µL of hydrolyzate and dissolving it in 500 µL of DI water (a 10-fold dilution), a 10 µL full-loop injection loads the equivalent of 0.07 µg of protein on the column. After preparation by dilution, 10 µL of 0.75µg/µL hydrolyzate are diluted to a total of 800 µL of sample with the

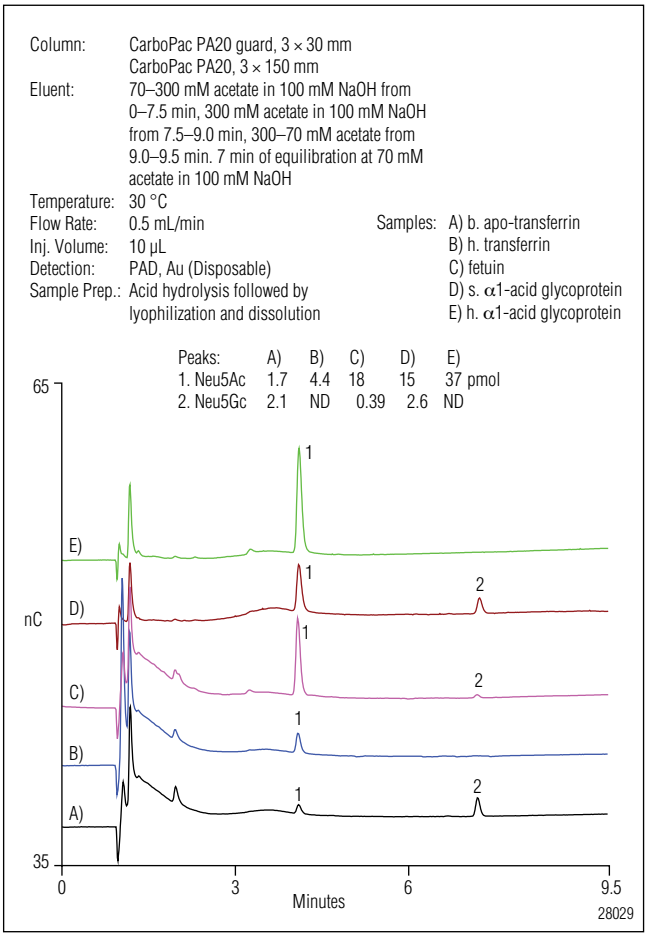

Figure 2. Sialic acid determination of five glycoprotein acid hydrolyzates. A 10% signal offset has been applied.

equivalent of 0.09 µg of protein injected with 10 µL. In both cases, there is enough sample for multiple sets of triplicate full-loop injections.

For glycoproteins that are highly glycosylated, such as α_1-acid glycoproteins, the amount of protein that is hydrolyzed can easily be reduced. In the example of s. AGP, 0.04 µg/µL of protein were hydrolyzed. Because of the high degree of glycosylation, the average determined amount for a single day of sample analysis

was 20 pmol of Neu5Ac (Table 4). In comparison, an average of 19 pmol of Neu5Ac was determined in fetuin hydrolyzates, even though nearly twice as much protein was hydrolyzed. Based on the amount of Neu5Gc in the fetuin hydrolyzate and the determined LOQs for Neu5Ac and Neu5Gc, a lower concentration of fetuin is not recommended. However, the concentration of s. AGP can be reduced by a factor of 10 before reaching the LOQ of Neu5Gc and by a factor of 40 before reaching the LOQ of Neu5Ac. Depending on the degree of sialylation, the amount of protein hydrolyzed can be reduced to low ng/µL concentrations and still allow efficient sialic acid determination. Designing experiments that routinely release amounts of analyte near the LOQ is not recommended. However, this evaluation of potential conditions highlights both the sensitivity of the method and the importance of considering the approximate protein sialylation amount when designing acid hydrolysis experiments.

Table 4 presents the results from one day of triplicate analysis. Retention time precision was similar to that determined by injecting standards, with retention time RSDs

Table 4. Results from Analysis of Triplicate Protein Acid Hydrolysis, n=3 per Hydrolyzed Sample

Sample (replicate #)	Analyte	Amount (pmol)	Peak Area (nC*min)	Peak Area Precision (RSD)	RT (min)	RT Precision (RSD)
Fetuin (1)	Neu5Gc	0.45	0.017	1.76	7.18	0.13
	Neu5Ac	20	0.404	2.76	4.04	0.24
Fetuin (2)	Neu5Gc	0.54	0.020	2.20	7.18	<0.01
	Neu5Ac	23	0.479	0.54	4.04	<0.01
Fetuin(3)	Neu5Gc	0.35	0.014	1.44	7.19	0.12
	Neu5Ac	15	0.313	2.23	4.06	0.21
h. Transferrin (1)	Neu5Gc	ND				
	Neu5Ac	4.4	0.095	2.39	4.08	0.20
h. Transferrin (2)	Neu5Gc	ND				
	Neu5Ac	4.5	0.096	2.66	4.08	<0.01
h. Transferrin (3)	Neu5Gc	ND				
	Neu5Ac	3.9	0.084	2.25	4.07	0.12
b. Transferrin (1)	Neu5Gc	2.6	0.099	0.59	7.20	0.08
	Neu5Ac	2.0	0.044	2.66	4.09	0.14
b. Transferrin (2)	Neu5Gc	2.2	0.083	2.34	7.21	0.07
	Neu5Ac	1.6	0.036	2.22	4.09	0.12
b. Transferrin (3)	Neu5Gc	2.4	0.090	1.01	7.21	0.07
	Neu5Ac	1.8	0.039	2.19	4.10	0.12
h. AGP (1)	Neu5Gc	ND				
	Neu5Ac	42	0.876	1.55	4.10	<0.01
h. AGP (2)	Neu5Gc	ND				
	Neu5Ac	41	0.820	3.52	4.10	<0.01
h. AGP (3)	Neu5Gc	ND				
	Neu5Ac	42	0.865	1.60	4.10	<0.01
s. AGP (1)	Neu5Gc	3.7	0.139	1.31	7.21	0.07
	Neu5Ac	21	0.431	1.39	4.10	<0.01
s. AGP (2)	Neu5Gc	3.4	0.128	0.86	7.21	0.07
	Neu5Ac	19	0.396	0.73	4.10	<0.01
s. AGP (3)	Neu5Gc	3.4	0.131	0.52	7.21	0.07
	Neu5Ac	19	0.403	0.38	4.10	0.12

Table 5. Triplicate Sample Analysis Results Between-Day Precision Over 3 Days, n=3 per Hydrolyzed Sample					
Sample	**Analyte**	**Acid Hydrolysis Average (mol analyte/ mol protein)**	**Intraday Precision Between Replicates (RSD)**	**Between-Day Precision (RSD)**	**Neuraminidase Digestion Average (mol analyte/ mol protein)**
Fetuin	Neu5Gc	0.32	22	14	0.30
	Neu5Ac	14	21	13	19
h. Transferrin	Neu5Gc	ND	ND	ND	ND
	Neu5Ac	3.4	7.8	8.6	4.8
b. apo-Transferrin	Neu5Gc	1.6	8.6	7.9	1.4
	Neu5Ac	1.2	9.5	9.4	1.9
h. AGP	Neu5Gc	ND	ND	ND	ND
	Neu5Ac	25	1.7	8.9	30
s. AGP	Neu5Gc	4.5	4.6	12	3.1
	Neu5Ac	26	4.6	13	25

ranging from <0.01–0.24. Variability in the absolute retention time may be expected based on the batch of manual eluent prepared. Peak area precision for triplicate injections, as measured by RSD, is generally good, ranging from 0.38–3.52.

Table 5 shows the calculated results of sialic acid analysis for the proteins studied, as well as intraday precision for one day of analysis, as in Table 4, and between-day precision for three days of triplicate analysis. Variability between sample replicates can be large, with RSDs ranging from 1.7 to 22; therefore, optimization of the digestion for individual glycoproteins is highly recommended. Between-day precision, as RSD, ranges from 7.9 to 14, with an average of 11.

Comparison of the determined amounts between acid hydrolysis and neuraminidase digestion suggest that either the hydrolysis is not complete using the mild hydrolysis or that acid degradation of the free sialic acids has occurred. Acid hydrolysis is a complex balance between release of the sialic acids from the glycoprotein and degradation of the released analytes. The efficiency of the hydrolysis will depend on the hydrolysis temperature, acid concentration, type of sample being hydrolyzed, and the relative concentrations of acid and the sample. Because of these interdependent factors—which can impact the hydrolysis—variability between sample preparations can be expected. For the best accuracy, either optimized acid hydrolysis or neuraminidase digestion is recommended. For methodology to optimize acid hydrolysis, see Fan et al.[11] However, the amounts of sialic acids determined in the protein samples are consistent with literature results for the glycoproteins.[12-16]

Table 6. Recoveries, Triplicate Hydrolyzes				
Sample	**Analyte**	**Amount Added (pmol)**	**Average Recovery (Dilution) (%)**	**Average Recovery (Lyophilization) (%)**
Reagent blank	Neu5Gc	0.50	92.7	75.9
	Neu5Ac	5.0	91.3	78.7
Fetuin	Neu5Gc	0.60	99.0	86.4
	Neu5Ac	25	94.7	81.6
h. Transferrin	Neu5Gc	0.50	99.8	74.9
	Neu5Ac	5.0	77.4	74.6
b. apo-Transferrin	Neu5Gc	2.5	76.3	84.9
	Neu5Ac	2.5	83.2	78.9
h. AGP	Neu5Gc	0.50	98.8	74.9
	Neu5Ac	50	102	74.6
s. AGP	Neu5Gc	5.0	88.9	84.9
	Neu5Ac	30	87.2	78.9

Method accuracy was investigated by spiking protein acid hydroylzates with known amounts of Neu5Ac and Neu5Gc equal to the determined amounts. For human glycoproteins, which did not contain Neu5Gc, 0.38 pmol of Neu5Gc was added. Recoveries were evaluated for both sample preparation by lyophilization and by dilution. Recoveries for Neu5Ac ranged from 83–103% by dilution and 75–82% by lyophilization (Table 6). Recoveries for Neu5Gc were similar, ranging from 76–100% by dilution and 75–86% by lyophilization. Where protein amounts are not limited, dilution is recommended for both ease of sample preparation and improved recoveries.

Glycoprotein Hydrolyzate Stability

A set of glycoprotein hydrolyzates was re-analyzed after 14 days of storage at -40 °C. These samples were stored in solution after lyophilization. The comparative results of these stored samples quantified with freshly prepared working standards are shown in Table 7. Values across replicates can be more variable after storage; however, overall, the determined amounts are generally within 10% of the original values.

Table 7. Stability of Samples Stored at -40 °C				
Sample (Replicate)	**Analyte**	**Initial Determined Amount (pmol)**	**Amount After 14 Days of Storage at -40 °C (pmol)**	**Difference (%)**
h. AGP (1)	Neu5Gc	ND	ND	
	Neu5Ac	42±0.6	45±0.8	7.4
h. AGP (2)	Neu5Gc	ND	ND	
	Neu5Ac	41±1.7	38±0.8	-6.9
h. AGP (3)	Neu5Gc	ND	ND	
	Neu5Ac	42±0.7	35±1.4	-15
s. AGP (1)	Neu5Gc	3.7±0.5	3.6±0.04	-2.3
	Neu5Ac	21±0.3	20±0.2	-4.4
s. AGP (2)	Neu5Gc	3.3±0.03	3.2±0.03	-3.8
	Neu5Ac	19±0.1	18±0.07	-4.4
s. AGP (3)	Neu5Gc	3.4±0.02	3.4±0.03	<0.1
	Neu5Ac	19±0.07	19±0.3	<0.1

CONCLUSION

In this work, sialic acids are determined in five representative glycoproteins by acid hydrolysis release and HPAE-PAD. Determination of the sialic acids Neu5Ac and Neu5Gc by HPAE-PAD on a CarboPac PA20 column is specific and direct. After sample hydrolysis or enzymatic treatment, there is no need for further sample derivatization. Disposable Au on PTFE working electrodes with a four-week lifetime simplify system maintenance compared to conventional gold electrodes. The gradient method discussed separates Neu5Ac and Neu5Gc with a total analysis time of 16.5 min, which is faster than previous methods, allowing greater sample throughput.

SUPPLIERS

VWR, 1310 Goshen Parkway, West Chester, PA 19380 U.S.A. Tel: 800-932-5000. www.vwr.com

Fisher Scientific, One Liberty Lane, Hampton, NH 03842 U.S.A. Tel: 800-766-7000. www.fishersci.com

Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178 U.S.A. Tel: 800-325-3010. www.sigma-aldrich.com

Ferro Pfanstiehl, 1219 Glen Rock Avenue, Waukegan, IL 60085 U.S.A. Tel: 800-383-0126. www.ferro.com

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