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Anion exchange-based method for nucleotide sugar determination

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Keywords

Dionex CarboPac PA1 column, anion-exchange chromatography, protein glycosylation

Goal

To develop a method for nucleotide sugar determination using anion-exchange chromatography with a Thermo Scientific™ Dionex™ CarboPac™ PA1 column and manually prepared eluents for separation

Introduction

Nucleotide sugars are important biomolecules. Activated nucleotide sugars, such as UDP-N-acetylhexosamines [UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetygalactosamine (UDP-GalNAc)] and UDP-hexoses [UDP-glucose (UDP-Glc), UDP-galactose (UDP-Gal), and GDP-mannose (GDP-Man)], play a critical role in protein glycosylation as monosaccharide donor substrates of glycosyltransferases enzymes.[1] Nucleotide sugars must be available for the formation of glycosidic linkages. These are synthesized from monosaccharides and nucleoside triphosphates, followed by sugar interconversions.[2] Due to their role in protein glycosylation, nucleotide sugars influence important protein properties such as biological activity, *in vivo* half-life, and immunogenicity of recombinant protein therapeutics.[3,4]

The majority of the methods for nucleotide sugar determination reported in the literature have liabilities. HPLC is a widely used method for the quantification of nucleotide sugars.[5,6] However, studies using HPLC methods show that isobaric nucleotide sugars, such as UDP-GalNAc and UDP-GlcNAc, UDP-Gal and UDP-Glc, and GDP-Man, coelute and thus cannot be quantified individually.[5–9] Other methods

used include capillary electrophoresis,[10] but this method showed high variation in sample-to-sample measurements, with coefficient of variation values around 10%. Hydrophilic interaction chromatography has also been used for quantifying nucleotide sugars, but separation of isobaric nucleotide sugars is a challenge.[11] High-performance anion-exchange (HPAE) chromatography is a widely used technique for carbohydrate analysis.[12,13] However, reported methods used for nucleotide sugar determination suffer from poor resolution of analytes[14] or long run times[15].

Here, we have designed a method for nucleotide sugar determination using anion-exchange chromatography. This method uses a Dionex CarboPac PA1 column and manually prepared eluents for separation and UV absorbance at 262 nm for detection. The Dionex CarboPac PA1 column is an anion-exchange column that delivers high resolution separation. It is packed with a pellicular resin that provides excellent mass transfer resulting in fast gradient re-equilibration. This column is stable over a pH range of 0–14. Using a Dionex CarboPac PA1 column, a 34 min method that resolved seven analytes was designed. Results for method linearity, precision, and accuracy for sugar nucleotide quantification are discussed here.

Experimental

Equipment

- A Thermo Scientific™ Dionex™ ICS-6000 Reagent-Free Ion Chromatography (RFIC™) system, an integrated ion chromatograph that includes:
  - DP Dual Pump with degas option
  - DC detector compartment with single-temperature zone
  - DAD detector (P/N 5082.0010) with 13 µL volume analytical flow cell (P/N 6082.0400)
- 2.5 µL sample loop
- Thermo Scientific™ Dionex™ EGC 500 KOH eluent generator cartridge (P/N 075778)*
- Thermo Scientific™ Dionex™ CR-ATC 600 continuously regenerated trap column (P/N 088662)*
- Thermo Scientific™ Dionex™ AS-AP autosampler with tray cooling option (recommended)
- Sterile assembled microcentrifuge tubes with screw cap, 1.5 mL (Sarstedt P/N 72.692.005)
- 1.5 mL polypropylene autosampler vials, with caps and split septa (P/N 079812)
- 1.5 mL sample vials with septum, 0.3 mL capacity (P/N 055428)

[*Not required for the Dionex CarboPac PA1 column application]

Chromatography conditions: Dionex CarboPac PA1 column	
Columns	Dionex CarboPac PA1 2 × 250 mm analytical (P/N 057178) and 2 × 50 mm guard column (P/N 057179)
Eluents	A) 3 mM NaOH B) 1.5 M NaOAc in 3 mM NaOH
Elution gradient	Table 1
Flow rate	0.3 mL/min
Injection volume	2.5 µL full loop
Column temperature	30 °C
Detection	UV detection at 262 nm

Table 1. Elution program for the Dionex CarboPac PA1 column method

Time (min)	% Eluent A	% Eluent B	Comment
0	80	20	Start
2	80	20	Hold B at 20%
10	50	50	20 to 50% B
12	50	50	Hold B at 50%
13	40	60	50 to 60% B
22	40	60	Hold B at 60%
27	80	20	Requilibrium
34	80	20	Stop

Chromatography conditions: Dionex IonPac AS11-HC and AS20 columns	
Columns	1) Dionex IonPac AS11-HC 2 × 250 mm analytical (P/N 052961) and 2 × 50 mm guard column (P/N 052963) 2) Dionex IonPac AS20 2 × 250 mm analytical (P/N 063065) and 2 × 50 mm guard column (P/N 063066)
Eluents	KOH
Eluent source	Dionex EGC 500 KOH eluent generator cartridge
Flow rate	0.3 mL/min
Injection volume	2.5 µL full loop
Column temperature	30 °C
Detection	UV detection at 262 nm

Reagents and standards

- UDP-GlcNAc (Uridine 5'-diphospho-N-acetylglucosamine, Sigma P/N 670107)
- UDP-GalNAc (Uridine 5'-diphospho-N-acetylgalactosamine, Sigma P/N 670105)
- UDP-Glc (Uridine 5'-diphosphoglucose, Sigma P/N 670120)
- UDP-Gal (Uridine 5'-diphosphogalactose, Sigma P/N 670111-M)
- GDP-Man (Guanosine 5'-diphospho-D-mannose, Sigma P/N G5131)
- UDP-GlcA (Uridine 5'-diphosphoglucuronic acid, Sigma P/N U6751)
- GDP-Fuc (Guanosine 5'-diphospho-D-fucose, Sigma P/N G4401)
- BSA (Bovine serum albumin) for recovery experiment (Sigma P/N A7638)

Preparation of solutions and standards

30 mM sodium hydroxide and 3 mM sodium hydroxide

To 900 mL of degassed deionized (DI) water water, add 1.56 mL of 50% (w/w) sodium hydroxide solution. Transfer the solution to a 1 L volumetric flask and bring to volume with degassed DI water. Filter the solution through an alkaline-resistant nylon membrane with pore size of NMT 0.45 µm. Dilute 100 mL of this solution to 1 L to make 3 mM sodium hydroxide, immediately transfer it to the plastic eluent bottle on the HPAE-PAD system, and blanket it with nitrogen at 5 to 10 psi. See Technical Note 71 for more information on preparing eluents for HPAE of carbohydrates.[16]

1.5 M sodium acetate and 3 mM sodium hydroxide

Dissolve 123 g of anhydrous sodium acetate in 800 mL of degassed DI water. Vacuum filter this solution through a 0.2 µm Thermo Scientific™ Nalgene™ 1 L nylon filter to remove particles from the sodium acetate that can damage parts of the pump. Transfer the solution to a 1 L volumetric flask, add 100 mL of freshly prepared 30 mM NaOH, and bring to volume. Immediately transfer this solution to the plastic eluent bottle on the HPAE-PAD system and blanket it with nitrogen at 5 to 10 psi.

Nucleotide sugar stock solutions 10 mg/mL

Accurately weigh 10 mg of each sugar nucleotide and dissolve in 990 µL DI water in a 1.5 mL microcentrifuge tube. Prepare a 1,000 mg/L secondary stock solution by 10-fold dilution of the primary stock solution.

Calibration standards

On the day of the analysis, dilute the secondary stock solutions to prepare calibration standards with concentrations of 80, 50, 40, 20, 10, 5.0, 2.5, and 1.25 mg/L of each nucleotide. For example, for a 50 mg/L standard, perform a 1:20 dilution of each of the 1,000 mg/L standards by mixing 50 µL of 1,000 mg/L standard into a 1.5 mL microcentrifuge tube. Add 650 µL DI water to a final volume of 1,000 µL, cap, and store the tubes at 4 °C until needed.

Results and discussion

Separation

We surveyed the scientific literature to understand applications of nucleotide sugar analysis and which analytes are studied most. Table 2 shows a summary of our literature search. There are many nucleotide sugar structures, but when these are determined in target samples, such as in examples 1 to 3 in Table 2, only a few, such as UDP-Glc, UDP-Gal, GDP-Man, UDP-GalNAc, and UDP-GlcNAc are determined. When comprehensive analytical methods are being developed (Example 4 in Table 2), then as many nucleotide sugars as possible are included in the analysis. The following seven nucleotide sugars were identified as the most common across sample types: UDP-Glc, UDP-Gal, GDP-Man, UDP-GalNAc, UDP-GlcNAc, UDP-GluA, and UDP-Fuc (underlined in Table 2). Depending on sample type, there might be other nucleotide sugars in a sample. A suitable method should be able to resolve at least the seven common nucleotide sugars identified here.

Table 2. Nucleotide sugar applications and methods

No.	Analytes	Application	Matrix / technique	Reference
1	UDP-Glc and UDP-Gal	Galactosaemia detection – GALT activity	Blood / HPLC-UV	17
2	UDP-Glc, UDP-Gal, GDP-Man, UDP-GalNAc, UDP-GlcNAc	Protein glycosylation mechanism study	CHO cell culture / Ion pair HPLC	18
3	UDP-Glc, UDP-GlcNAc and UDP-N-acetyl-hexosamine	GPCR/signaling studies	Human epithelial cell lysate / LC-MS and other activity assays	19
4	CMP-NeuAc, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc, GDP-Man, GDP-Fuc and UDP-GlcUA	Method for simultaneous determination	CHO and MCF7 cell lysates / IP-HPLC	20
5	**Method capability:** CMP-Neu5Ac, CMP-Neu5Gc, CMP-KDN, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc, GDP-Fuc, GDP-Man **Found in samples:** UDP-GlcNAc, UDP-Gal, UDP-Glc, GDP-Fuc, and GDP-Man	Determination of nucleotides and sugar nucleotides involved in protein glycosylation	Insect and CHO cells / HPAE-PAD with a Dionex CarboPac PA1 column	14
6	UDP-GalNAc, UDP-Glu, UDP-GlcNAc, UDP-Gal, UDP-GluA	Determination of UDP-sugars in brain, heart, adipose, and liver tissues	Sugars in brain, heart, adipose and liver tissues / HPAE-PAD with a Dionex CarboPac PA1 column and borate acetate eluents	21
7	ADP-Glucose, UDP-GalNAc, UDP-Glu, UDP-GalNAc, UDP-Gal, UDP-GluA, UDP-Arabinose, UDP-Xylose, GDP-Fuc	Method development for determination of plant cell wall precursors	Plant cell extract / HPAE-MS/MS using a Dionex IonPac AS11 column and manually prepared NaOH eluent	22
8	UDP-GalNAc, UDP-Glc, UDP-GluA	Quantitative analysis of intracellular sugar phosphates and sugar nucleotides in encapsulated streptococci	Liquid culture of *Streptococcus zooepidemicus* / HPAE-PAD using a Dionex CarboPac PA10 column	15
9	UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc, GDP-Man, GDP-Fuc and UDP-GlcUA, GDP-Glc	Determination of nucleotides and nucleotide sugars in mammalian cells	CHO cell extract / HPAEC-PAD using a Dionex CarboPac PA1 column	23
10	UDP-Gal, UDP-Gal_f	Synthesis and assay for UDP-galactopyranose mutase	Bacterial cell lysate / HPLC using a Dionex CarboPac PA100 column, KH_2PO_4 eluent, and UV detection at 254 nm	24
11	UDP-GalNAc, UDP-Gal, UDP-GluA, UDP-Xylose, UDP-Galacturonic acid	Synthesis of UDP-galacturonic acid	Plant cell extract / HPAE with a Dionex CarboPac PA1 column and PAD and UV detection	25
12	UDP-GlcNAc, UDP-Gal	UDP-glucose pyrophosphorylase mechanistic study	Glycosomal cell lysate / IC-MS using a Dionex IonPac AS11 column	26
13	UDP-Glu, UDP-Gal	Determination of UDP-Glc and UDP-Gal	Red blood cells / HPAE-PAD using a Dionex CarboPac PA1 column, KH_2PO_4 eluent, and UV detection at 254 nm	27
14	UDP-GlcNAc, UDP-GalNAc	Glycosyl transferase mechanistic studies	HEK293 Cell extract / HPAE-PAD with a Dionex CarboPac PA1 column	28
15	UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc	Determination of UDP-sugars in bacterial and human red blood cell extracts	Bacterial and human red blood cell extracts / HPAE with a Dionex OmniPac PAX-100 column and conductivity detection	29

Good separation of the seven nucleotide sugars was achieved on a Dionex CarboPac PA1 column using a sodium acetate gradient of 200 to 900 mM in 34 min. Figure 1 shows this separation. As shown in Table 3, most of the resolutions are greater than 1.5. Only the resolution between UDP-GlcNAc and UDP-GalNAc is lower at 1.2. Though there are newer members of the Dionex CarboPac column family, some of which were used in the publications in Table 2, the publications in Table 2 convinced us to focus our efforts on the Dionex CarboPac PA1 column. It is possible that newer generations Dionex CarboPac columns, which have smaller particle sized packings, could offer better separations.

Two additional columns were tested to determine if improved resolution could be obtained for nucleotide sugars. These were the Dionex IonPac AS11-HC and IonPac AS20 columns. In both cases, electrolytically generated KOH was used for separation. The Dionex IonPac AS11-HC column-based method used isocratic elution with 45 mM KOH. The Dionex IonPac AS20 column-based method used a 40 to 55 mM KOH gradient over 22 min (0 to 22 min – 40 to 55 mM KOH, 22 to 30 min – 40 mM KOH). As shown in Figures 2 and 3, improved resolution could not be obtained on either of these columns. Peak resolution on the Dionex IonPac AS11-HC column could be improved slightly by lowering the KOH concentration (not shown). Though these two methods did not show improved resolution for all seven analytes, they can still be useful in some situations where fewer nucleotide sugars need to be resolved. These methods can be particularly beneficial for MS-based quantification. Due to the high acetate concentration used for separation, the Dionex CarboPac PA1 column-based method is not amenable to MS detection. The Dionex IonPac AS11-HC column-based method and the Dionex IonPac AS20 column-based method are suitable for MS detection as they use low KOH concentrations, which can be converted to water (suppressed). After the eluent passes through an electrolytic suppressor, the sugar nucleotide can be detected by suppressed conductivity detection prior to the MS detector.

This application note uses UV absorbance detection, as sugar nucleotides have large 260 nm extinction coefficients, which results in sensitive detection. This detection mode allows a greater variety of eluents to be used for separation than with either pulsed amperometric detection (PAD) or suppressed conductivity detection. Sugar nucleotides are also detected with good sensitivity by HPAE-PAD as shown by some of the publications in Table 2. PAD is especially useful if other carbohydrates need to be detected along with the sugar nucleotide. Stand-alone suppressed conductivity detection (i.e., without a MS detector) has also been used.[29]

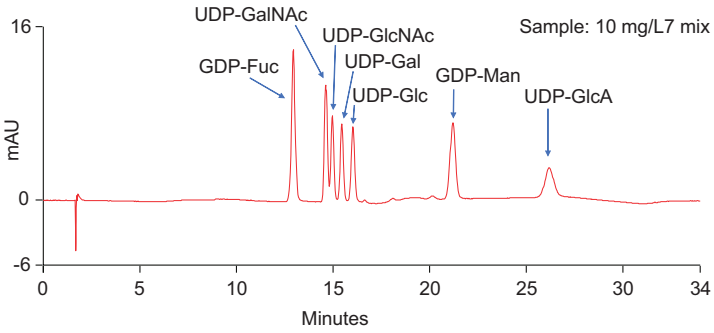

Figure 1. Nucleotide sugar separation on a Dionex CarboPac PA1 column

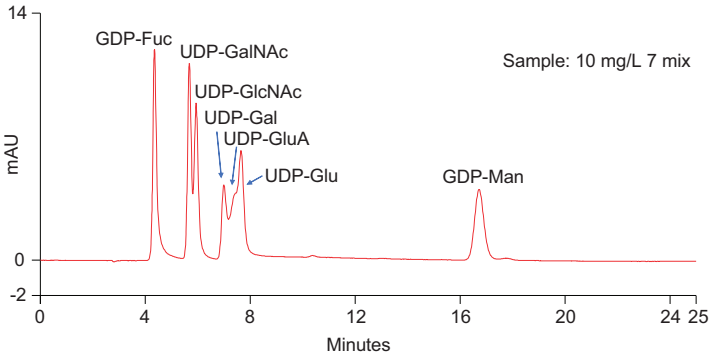

Figure 2. Nucleotide separation on an Dionex IonPac AS11-HC column using 45 mM KOH

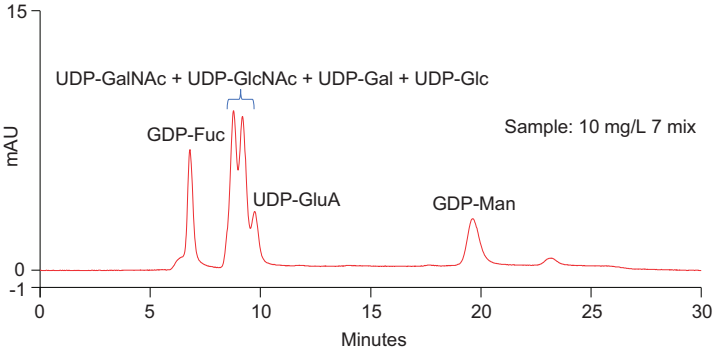

Figure 3. Nucleotide sugar separation on an Dionex IonPac AS20 column using a 40 to 50 mM KOH gradient

Table 3. Sugar nucleotide separation on a Dionex CarboPac PA1 column

Peak no.	Peak name	Resolution (at 20 mg/L)
1	GDP-Fuc	5.5
2	UDP-GalcN	1.2
3	UDP-GlcN	1.6
4	UDP-Glu	1.8
5	UDP-Gal	11.9
6	GDP-Man	7.2
7	UDP-GluA	—

Table 4. Retention time and peak area precision (n=3)

Conc. (mg/L)	Retention time RSD							Peak area RSD						
	GDP-Fucose	UDP-GalNAc	UDP-GlcNAc	UDP-Galactose	UDP-Glucose	GDP-Mannose	UDP-GluA	GDP-Fucose	UDP-GalNAc	UDP-GlcNAc	UDP-Galactose	UDP-Glucose	GDP-Mannose	UDP-GluA
3	0.06	0.07	0.06	0.06	0.06	0.06	0.13	1.41	1.15	1.45	0.60	1.85	0.69	1.14
15	0.07	0.07	0.06	0.06	0.07	0.07	0.11	0.58	1.09	2.32	1.88	0.56	1.28	1.21
75	0.09	0.09	0.1	0.09	0.13	0.08	0.14	0.84	0.36	0.92	1.35	1.43	0.36	0.73

Precision and linearity

Table 4 contains retention time as well as peak area RSDs for all seven nucleotide sugars. The highest RSD value observed was 2.32% for the UDP-GlcNAc peak, with all other RSDs below this value, indicating good method precision. Method linearity was determined using eight calibration standards ranging from 1.25 to 80 mg/L (50 mg/L for GDP-Fuc) for the seven nucleotide sugars. As shown in Table 5, coefficient of determination values ranged from 0.996 to 1, indicating good linearity over the nucleotide sugar concentration ranges tested.

Method sensitivity

Method sensitivity was determined by calculating signal-to-noise ratios. Figure 4 shows the chromatogram obtained using a sample containing 1.25 mg/L of each of the seven nucleotide sugars. Corresponding S/N values are shown in Table 6. Except for UDP-GluA, all other nucleotide sugars have LOQ (S/N=10) values well below 1.25 mg/L.

Method accuracy

Method accuracy was determined using a spike recovery experiment, performed as follows. To simulate the matrix effect, 300 µL of 10 mg/mL BSA (bovine serum albumin) was mixed with 300 µL acetonitrile and incubated over ice for 10 min. The samples were then dried in a speed vac overnight. The following day, the dried samples were dissolved in 300 µL DI water. A 100 µL aliquot of the matrix solution was mixed with 100 µL of nucleotide sugar standard solution. Three nucleotide sugar standard concentrations were used—150, 30, and 6 mg/L. These yielded final spike concentrations of 75, 15, and 3 mg/L for each nucleotide sugar in the three spiked solutions, respectively. The results of this study are shown in Table 7. All spike recoveries ranged from 98.7% to 117%, indicating good method accuracy.

Table 5. Method linearity

Peak no.	Peak name	Concentration levels	Coefficient of determination (n=3)
1	GDP-Fuc	1.25 to 50 mg/L (7 levels)	1.00
2	UDP-GalNAc	1.25 to 80 mg/L (8 levels)	0.996
3	UDP-GlcNAc		1.00
4	UDP-Glu		1.00
5	UDP-Gal		1.00
6	GDP-Man		1.00
7	UDP-GluA		1.00

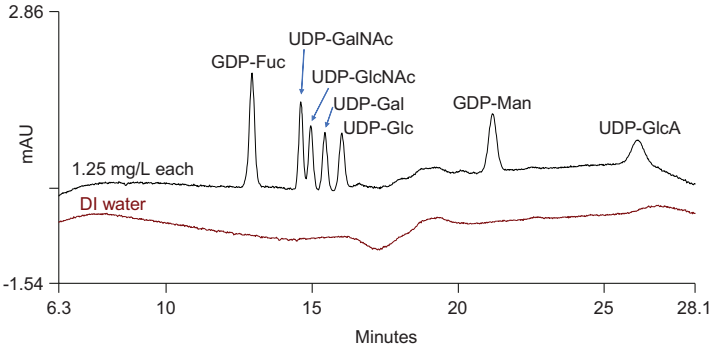

Figure 4. Method sensitivity

Table 6. Signal-to-noise ratios for sugar nucleotides at 1.25 mg/L concentration

GDP-Fuc	UDP-GalNAc	UDP-GlcNAc	UDP-Gal	UDP-Glu	GDP-Man	UDP-GluA
51.0	30.9	22.4	20.2	19.8	32.4	12.1

Table 7. Spike recovery (n=3)

Spike conc. (mg/L)	Spike recovery (%)						
	GDP-Fuc	UDP-GalNAc	UDP-GlcNAc	UDP-Gal	UDP-Glu	GDP-Man	UDP-GluA
3	115	107	107	106	112	104	114
15	107	103	102	102	103	101	102
75	105	101	100	100	101	100	98.7

Conclusion

This study describes a simple HPAE-based assay for accurate determination of nucleotide sugars. The nucleotide sugars were separated on an anion-exchange column and detected by UV absorbance detection in 34 min. Seven major nucleotide sugars were separated with good resolution. The assay was validated and shown to measure the nucleotide sugar content in a simulated sample accurately.

References

1. Grammatikos, S.I.; Valley, U.; Nimtz, M.; Conradt, H.S.; Wagner, R. Intracellular UDP-N-acetylhexosamine pool affects N-glycan complexity: a mechanism of ammonia action on protein glycosylation. *Biotechnol. Prog.* **1998**, *14*, 410–419.
2. Reiter, W. D. Biochemical genetics of nucleotide sugar interconversion reactions. *Curr. Opin. Plant Biol.* **2008**, *11*, 236–243.
3. Kochanowski, N.; Blanchard, F.; Cacan, R.; Chirat, F.; Guedon, E.; Marc, A.; Goergen, J.L. Intracellular nucleotide and nucleotide sugar contents of cultured CHO cells determined by a fast, sensitive, and high-resolution ion-pair RP-HPLC. *Anal. Biochem.* **2006**, *348*(2), 243–251.
4. Jefferis, R. Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action. *Trends Pharmacol. Sci.* **2009**, *30*(7), 356–362.
5. Räbinä, J.; Mäki, M.; Savilahti, E.M.; Järvinen, N.; Penttilä, L.; Renkonen, R. Analysis of nucleotide sugars from cell lysates by ion-pair solid-phase extraction and reverse-phase high-performance liquid chromatography. *Glycoconj. J.* **2001**, *18*, 799–805.
6. Ryll, T.; Wagner, R. Improved ion-pair high-performance liquid chromatographic method for the quantification of a wide variety of nucleotides and sugar—nucleotides in animal cells. *J. Chrom. B: Biomedical Sciences and Applications* **1991**, *570*(1), 77–88.
7. Nyberg, G.B.; Balcarcel, R.R.; Follstad, B.D.; Stephanopoulos, G; Wang, D.I.C. Metabolic effects on recombinant interferon-c glycosylation in continuous culture of Chinese hamster ovary cells. *Biotechnol. Bioeng.* **1999**, *62*, 336–347.
8. Gu, X.; Wang, D.I.C. Improvement of IFN-c sialylation in CHO cell culture by feeding of N-acetylmannosamine. *Biotechnol. Bioeng.* **1998**, *58*, 642–648.
9. Sweeney, C.; Mackintosh, D.; Mason, R.M. UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes. *Biochem. J.* **1993**, *290*, 563–570.
10. Lehmann, R.; Huber, M.; Beck, A.; Schindera, T.; Rinkler, T.; Houdali, B.; Weigert, C.; Haring, H. U.; Voelter, W.; Schleicher, E. D. *Electrophoresis* **2000**, *21*, 3010–3015.
11. Preinerstorfer, B.; Schiesel, S.; Lammerhofer, M.; Lindner, W. J. *Chromatogr. A.* **2010**, *1217*, 312–328.
12. Thermo Scientific Application Note 72580: An improved HPAE-PAD method for glycoprotein monosaccharide determination. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72580-hpae-pad-glycoprotein-monosaccharide-an72580-en.pdf (Accessed September 22. 2022).
13. Thermo Scientific Application Note 72210: Fast determination of biofuel sugars by HPAE-PAD. http://tools.thermofisher.com/content/sfs/brochures/AN-72210-IC-HPAE-PAD-Biofuel-Sugars-AN72210-EN.pdf (Accessed September 22. 2022).
14. Tomiya, N.; Ailor, E.; Lawrence, S.M.; Betenbaugh, M.J.; Lee, Y.C. Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. *Anal. Biochem.* **2001**, *293*(1), 129–137.
15. Marcellin, E.; Nielsen, L.K.; Abeydeera, P.; Krömer, J.O. Quantitative analysis of intracellular sugar phosphates and sugar nucleotides in encapsulated streptococci using HPAEC-PAD. *Biotechnol J.* **2009**, *4*(1), 58–63.
16. Thermo Scientific Technical Note 71: Eluent preparation for high-performance anion-exchange chromatography with pulsed amperometric detection. http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf (Accessed September 22. 2022).
17. Lindhout, M.; Rubio-Gozalbo, M.E.; Bakker, J.A.; Bierau, J. Direct non-radioactive assay of galactose-1-phosphate:uridyltransferase activity using high performance liquid chromatography. *Clin. Chim. Acta.* **2010**, *411*(13–14), 980–983.
18. Kochanowski, N.; Blanchard, F.; Cacan, R.; Chirat, F.; Guedon, E.; Marc, A. Goergen JL. Intracellular nucleotide and nucleotide sugar contents of cultured CHO cells determined by a fast, sensitive, and high-resolution ion-pair RP-HPLC. *Anal. Biochem. 2006*, *348*(2), 243–251.
19. Sesma, J.I.; Esther, C.R. Jr.; Kreda, S.M.; Jones, L.; O'Neal, W; Nishihara, S.; Nicholas, R.A.; Lazarowski, E.R. Endoplasmic reticulum/golgi nucleotide sugar transporters contribute to the cellular release of UDP-sugar signaling molecules. *J. Biol. Chem.* **2009**, *284*(18), 12572–12583.
20. Nakajima, K.; Kitazume, S.; Angata, T.; Fujinawa, R.; Ohtsubo, K.; Miyoshi, E.; Taniguchi, N. Simultaneous determination of nucleotide sugars with ion-pair reversed-phase HPLC. *Glycobiology* **2010**, *20*(7), 865–871.
21. Oikari, S.; Venäläinen, T.; Tammi, M. Borate-aided anion exchange high-performance liquid chromatography of uridine diphosphate-sugars in brain, heart, adipose and liver tissues. *J. Chromatogr. A* **2014**, *1323*, 82–86.
22. Alonso, A.P.; Piasecki, R.J.; Wang, Y.; LaClair, R.W.; Shachar-Hill, Y. Quantifying the labeling and the levels of plant cell wall precursors using ion chromatography tandem mass spectrometry. *Plant Physiol.* **2010**, *153*(3), 915–924.
23. del Val, I.J.; Kyriakopoulos, S.; Polizzi, K.M.; Kontoravdi, C. An optimized method for extraction and quantification of nucleotides and nucleotide sugars from mammalian cells. *Anal. Biochem.* **2013**, *443*(2), 172–180.
24. Lee, R.; Monsey, D.; Weston, A.; Duncan, K.; Rithner, C.; McNeil, M. Enzymatic synthesis of UDP-galactofuranose and an assay for UDP-galactopyranose mutase based on high-performance liquid chromatography. *Anal Biochem.* **1996**, *242*(1), 1–7.
25. Liljebjelke, K.; Adolphson, R.; Baker, K.; Doong, R.L.; Mohnen D. Enzymatic synthesis and purification of uridine diphosphate [14C] galacturonic acid: a substrate for pectin biosynthesis. *Anal. Biochem.* **1995**, *225*(2), 296–304.
26. Villafraz, O.; Baudouin, H.; Mazet, M.; Kulyk, H.; Dupuy, J.W; Pineda, E.; Botté, C.; Inaoka, D.K.; Portais, J.C; Bringaud, F. The trypanosome UDP-glucose pyrophosphorylase is imported by piggybacking into glycosomes, where unconventional sugar nucleotide synthesis takes place. *mBio.* **2021**, *12*(3), e0037521.
27. Palmieri, M.J.; Berry, G.T.; Player, D.A.; Rogers, S.; Segal, S.; The concentration of red blood cell UDP-Glucose and UDP-Galactose determined by high-performance liquid chromatography. *Anal. Biochem.* **1991**, *194*(2), 388–393.

28. Schumann, B.; Malaker, S.A.; Wisnovsky, S.P.; Debets, M.F.; Agbay, A.J.; Fernandez, D.; Wagner, L.J.S.; Lin, L.; Li, Z.; Choi, J.; Fox, D.M., Peh, J.; Gray, M.A.; Pedram, K.; Kohler, J.J.; Mrksich, M.; Bertozzi, C.R.; Bump-and-hole engineering identifies specific substrates of glycosyltransferases in living cells. *Mol. Cell.* **2020**, *78*(5), 824–834.

29. Hull, S.R.; Montgomery, R. Separation and analysis of 4'-epimeric UDP-sugars, nucleotides, and sugar phosphates by anion-exchange high-performance liquid chromatography with conductimetric detection. *Anal. Biochem.* **1994**, *222*(1), 49–54.

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