Ampule-sealed Acidolysis for Monosaccharide Composition Analysis of Serum or Plasma Samples

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Method Article

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Abstract

This protocol describes the procedures where an ampule-sealed acidolysis is used for releasing glycan monosaccharides from serum or plasma samples. The monosaccharide composition analysis is subsequently obtained by a traditional HPLC method that separates and quantifies all 1-phenyl-3-methyl-5-pyrazolone (PMP)-labeled monosaccharides in 10 μl serum in 55 minutes. The described workflow takes approximately 2 days, up to 26 serum samples can be analyzed with one HPLC instrument per day. Each step of the experimental procedures has been optimized with repeated tests of monosaccharide standards and serum samples.

Introduction

Glycans are abundantly present in tissues and blood circulation in the form of glycoproteins or glycolipids. Glycome is estimated to be 10^4 times larger than the proteome[1-4]. Glycan biosynthesis has no templates and its structures are influenced by genes, nutrition, and other environmental factors in time and space[5]. Over 125 congenital disorders of glycosylation (CDG) discovered in humans are associated with multi-systems disfunctions [6]. It has been well documented that total serum O- or N-glycans are different among healthy individuals and patients suffering different type of cancers [7-10]. Thus, abnormal glycan structures are common features for both inherited and environment-related diseases.

Indeed, most clinically used cancer biomarkers established during the past 40 years are either specific glycan structures or glycoproteins [11, 12]. However, these clinically used cancer biomarkers have low sensitivity and specificity as biomarkers [13] due to limited glycan information content [11]. In contrast, current glycomics approaches depend on profiling complicated glycan structures, which are technically difficult for clinical applications [14, 15].

Despite there are many different types of glycans [11], all human glycans consist of up to 10 monosaccharides, i.e. sialic acid, N-acetyl galactosamine, N-acetyl glucosamine, galactose, mannose, fucose, glucose, xylose, glucuronic acid, and iduronic acid. However, few methods have been developed to quantify glycan contents or monosaccharide compositions in human sera or plasmas for biomarker development.

This protocol was originally developed from the corresponding author's laboratory at Washington University in St. Louis for glucosamine- and galactosamine-based, serum- or animal tissue-derived glycosaminoglycan (GAG) quantification purposes [16-20]. We then discovered that significantly different quantity and compositions of glucosamine and galactosamine are present in the plasmas of human patients suffering lung, breast, and pancreatic cancers, respectively, [17, 21]. Since the major glycans in human sera/plasmas are N-linked and O-linked glycans instead of GAGs, we subsequently developed a HPLC method that can quantify all other monosaccharides in addition to glucosamine and galactosamine released from serum/plasma glycans simultaneously [22-25] for biomarker development.

Releasing monosaccharides from glycans is the bottleneck of monosaccharide composition analysis [26, 27]. The conventional hydrolysis assay for releasing monosaccharides from glycans of either plant or animal origin is conducted in a sealed glass ampoule at 105–120 °C for 1–6 h [27, 28] or in a PicoTag station[17]. Thus, we have optimized the ampule-sealed acidolysis assay for human serum or plasma analysis. The monosaccharide compositions are then obtained by PMP-labeling and HPLC analysis.

Reagents

Ultrapure water (Simplicity UV Ultrapure water preparation system, MILLIPORE, Germany)

High-purity water (AQUELIX high-purity water preparation system, MILLIPORE, Germany)

PMP (1-phenyl-3-methyl-5-pyrazolone, Analytical pure, Sigma Aldrich, USA)

Methanol (CH_3OH, Chromatographic pure, Merck)

Acetonitrile (HCN, Chromatographically pure, Merck)

Glycerol (Tianjin Komeo Chemical Reagent)

Trifluoroacetic acid (TFA, Analytical pure, Sinopharm Chemical Reagent)

Hydrochloric acid (HCl, Analytical pure, Sinopharm Chemical Reagent)

Sodium hydroxide (NaOH, Analytical pure, Sinopharm Chemical reagent)

Chloroform (CHCl_3, Tianjin Kemeou Chemical Reagent)

Acetic acid (HAc, Sinopharm Chemical Reagent)

Ammonium acetate (NH_4Ac, Sinopharm Chemical Reagent)

Anhydrous ethanol (CH_3CH_2OH, Tianjin Kemeou Chemical Reagent)

Glucose (Glc, 99% purity, Sigma Aldrich, USA)

Mannose (Man; 99% purity, Sigma Aldrich, USA)
Fucose (Fuc; 99% purity, Sigma Aldrich, USA)
Galactose (Gal; 99% purity, Sigma Aldrich, USA)
Glucosamine (GIN; 99% purity, Sigma Aldrich, USA)
Xylose (Xyl; 99% purity, Sigma Aldrich, USA)
Galactosamine (GalN; 99% purity, Sigma Aldrich, USA)
Glucuronic acid (GlcA; 99% purity, Sigma Aldrich, USA)
Rhamnose (Rha; 99% purity, Sigma Aldrich, USA)

REAGENT SETUP

**Sera** Each serum sample is stored at -80°C before use.

**PMP derivative reagent** Weigh the correct amount of PMP powder and dissolve in methanol to form 0.5 M PMP derivative reagent. CRITICAL Prepare the PMP derivative reagent before use.

**TFA (2M)** Take 15mL of 6mol/L TFA and add double distilled water to 45mL to dilute to 2mol/L, mix well, and prepare for immediate use. CRITICAL when working with concentrated TFA, fume hoods must be used and proper protective measures need to be taken according to all relevant workplace regulations.

**HCl (0.3M)** Prepare by careful dilution from concentrated HCl. It can be stored at 20-25 °C. CRITICAL When working with concentrated HCl, fume hoods must be used and proper protective measures need to be taken according to all relevant workplace regulations.

**NaOH (0.3M)** Prepare sodium hydroxide particles with an analytical balance according to the standard of 0.3 mol/L NaOH, dissolve them in double-distilled water, seal, and store at room temperature.

**Monosaccharides standard stock solution (10 mg/mL)** Weigh the correct amount of monosaccharides standard, dissolve in double-distilled water, aliquot into tubes and store at -20 °C.

**Mobile phase A (100 mM NH₄Ac-HAc)** Add the correct amount of NH₄Ac into 800 mL double-distilled water and adjust the pH to 5.5 with HAc or NH·HO.

**Mobile phase B** HPLC-grade acetonitrile.

**Equipment**

- Column, Infinity Lab ZORBAX XDB-C18 (150 mm length × 4.6 mm inner diameter, 5um particle size, Agilent)
- 2 mL ampoule (Anyang Nipro Chanda Pharmaceutical)
- 1.5 mL centrifuge tube (CRNING, NEST; USA)
- Ultrapure Water Preparation System (Simplicity UV Ultrapure Water Preparation System, MILLIPORE, Germany)
- High-purity water preparation system (AQUELIX high-purity water preparation system, MILLIPORE, Germany)
- Oil bath (DF-101Z, Zhengzhou Great Wall Technology Industry)
- Alcohol blowtorch (Shanghai Longtuo Equipment)
- Centrifugal concentrator (RVC2-18HCL, Germany CHRIST)
- Digital display constant bath water temperature pot (HH-2, Guohua Electric)
- Vortex oscillator (VG 3 S25, VORTEX GENIUS3, IKA equipment, Germany)
- High-speed centrifuge (HERAEUS LABOFOUGE 400R Centrifuge, Thermo Scientific, USA)
- The Agilent 1260 infinity HPLC system (quaternary pump G1311C, high-performance auto-sampler G1367E, thermostatic column compartment G1316A, UV detector DAD, G1315D)
Acidolysis of serum samples

Take 10 μL serum sample into a 2 mL ampoule, add 1 mL of 2M trifluoroacetic acid, place it in oil bath at 105°C, react for 6 h, transfer the sample to 1.5 mL centrifuge tube, and dry it in centrifugal concentrator.

PMP derivatization

Add 0.3 M sodium hydroxide solution, 0.5 M PMP methanol solution in turn, react for 90 minutes at 70°C in a water bath, then neutralize with hydrochloric acid.

HPLC system setup

The LC system should be optimized in order to minimize dead volumes. Gradients should be optimized for the samples at hand, and the post sample column washing and re-equilibration should be adjusted to the system in use. As an example, below is a table of the specific setup used in our laboratory on an Agilent 1260 Series HPLC.

- **Reversed Phase Octadecylsilyl (C18) column**: 150 mm length × 4.6 mm inner diameter, 5.0 μm particle size; Agilent
- **Column temperature**: 37 °C
- **Mobile phases**: A: 100 mM NH₄Ac-HAc; B: Acetonitrile
- **Flow rate**: 1 mL/min
- **DAD setup**: 254nm
- **Separation program**: Time gradient, 0→40→40.1→55min; corresponding to the concentration gradient of B, 85%→78%→85%→85%.
- **Sample injection volume**: 20 μL

Procedure

Serum sample collection. TIMING ~ 50 min for 60 samples

1. Collecting leftover serum samples after their clinical tests at clinical lab of the hospital.
2. Storage: each serum sample was divided into 3 aliquots in 1.5 mL EP tubes and store at -80°C. Serum can be stored at -80°C for more than one year without affecting monosaccharide compositions.

Acidolysis. TIMING 24 h for up to 72 samples

4. Serum samples are thawed on ice and 10 μL serum sample is transferred into 2 mL ampoule.
5. Add 10 μL Rha (1 mg/mL) and 1 mL TFA (2M) into each ampoule.
6. Use Oil bath to conduct acidolysis, which takes 6 h. Transfer the sample to 1.5 mL centrifuge tube, and dry it in centrifugal concentrator

   - **Problem**: Extra residues in hydrolyzed serum samples.
   - **Solution**: Add 200 μL of chromatographic methanol and then vortex. After fully mixed, concentrate and dry, repeat three times to obtain the cleaner hydrolyzed samples.

Sample derivatization. TIMING 90 min for up to 72 samples

7. Add 40 μL NaOH (0.3M) and 60 μL PMP (0.5M) into each sample. Vortex for 5 s and centrifuge for another 10 s.

   **CRITICAL STEP** At this point, the 0.3M NaOH offer the basic environment required for PMP derivation. The proper derivative pH is between 11-13.

   - **Problem**: PMP-derivatives show brown color.
   - **Solution**: The preparation of 0.3 M NaOH should be careful. Excessive alkali produces the colored byproducts.

8. PMP derivatization reaction is conducted at 70°C in a water bath for 90 minutes, then neutralize with hydrochloric acid.

   **CRITICAL STEP** In alkaline conditions, non-reactive PMP will exist in the form of salt in the system, which will reduce the efficiency of subsequent extraction, and lead to an increasing background for HPLC analysis.

9. Add 500 μL CHCl₃ into each sample tube, and vortex for 5 s.
10. Centrifuge for 10 min at 13,000 g/min and transfer the supernatant to HPLC vials.

**PAUSE POINT** The PMP-labeled monosaccharides can be stored at 4 °C and analyzed within 3 days.
Monosaccharide standard derivatization.

11. Preparation of monosaccharide standards: Firstly, dilute the stock solution of Man\(\text{Glc\text{N}}\)\(\text{Gal\text{N}}\)\(\text{Glc\text{U}}\)\(\text{Glc\text{Gal}}\)\(\text{Xyl\text{Fuc}}\), Rha to 1.0 mg/mL. Then mix the 9 monosaccharide standards together and dilute 2 times. The final range of working standard solution is 0.5 mg/mL to 0.0005 mg/mL.

12. Add 40 \(\mu\)L 0.3 M NaOH into each standard mixture to adjust pH to 12-13, and add 60 \(\mu\)L 0.5 M PMP for monosaccharide labeling as described in Steps 7 to 10.

HPLC analysis. TIMING~20 min per sample

13. Tuning of the LC system. Assure that the LC is working appropriately according to the manufacturers’ recommendations or the standard operating procedure (SOP) of the respective laboratory.

14. Set up the HPLC system to separate PMP-labeled monosaccharides as described under EQUIPMENT SETUP.

· Problem: High background; No signal; Weak signal or impurity peaks.

· Solution: The extraction conditions described in Steps 9-10 need to be followed, a high background is usually caused by leftover PMP in samples; ensure that the LC-MS system is working properly and the wavelength of DAD is set at 254 nm; Check pH value of Step 7. The efficiency of the PMP-derivatization will be decreased if the pH is lower than 11 or higher than 13.

Characterization of glycan monosaccharide compositions in serum samples. TIMING It takes 55 min per sample. Up to 26 samples can be analyzed by one HPLC instrument in 24 h.

15. Calculating monosaccharide contents is based on the regression equation of the monosaccharide standards.

16. Statistical analysis of serum glycan monosaccharide compositions in specific disease.

Troubleshooting

Time Taken

Steps 1-3, serum collection and storage: ~50 min for 96 samples

Steps 4-6, serum glycan acidolysis: at least overnight to removing 1mL TFA in each of 72 samples

Steps 7-10, PMP derivatization: ~ 90 min for 72 samples

Steps 11-12, monosaccharide standard derivatization along with the samples: ~120 min

Steps 13-15, HPLC analysis: ~55 min per sample

Steps 16, a single HPLC instrument can analyze up to 26 samples per day. Our lab is equipped with 4 Agilent 1260 series HPLC systems and has the capacity of analyzing 104 samples per day.

Anticipated Results

Methodological investigation

Using rhamnose as an internal quality control monosaccharide. The glycans assembled in human cells consist of 10 monosaccharides, i.e. N-acetyl galactosamine, galactose, N-acetyl glucosamine, glucose, mannose, fucose, sialic acid, xylose, glucuronic acid, and iduronic acid. After hydrolysis, 8 out of 10 monosaccharides, including galactosamine (GalN), galactose (Gal), glucosamine (GlcN), glucose (Glc), mannose (Man), fucose (Fuc), xylose (Xyl), glucuronic acid (GlcA) could be detected by the PMP-derivatization and HPLC separation and detection method. Human fasting blood has no detectable rhamnose (Rha). Thus, we have added the Rha to each serum sample and during the assay optimizing process. Figure 1 showed the baseline separation of the 9 monosaccharide standards using the optimized HPLC method.

Linear relationship investigation

Each monosaccharide standard solution of 1mol/L was derivatized, diluted 2, 4, 6, 8 and 10 times respectively, and then analyzed by HPLC. The concentration of monosaccharide (mol/L) was taken as the abscissa and the corresponding peak area was taken as the ordinate. Coordinates, calculate the linear regression equation, the square of the linear correlation coefficient \(R^2\) is greater than 0.990 as a good linear relationship. Mannose, glucosamine, galactosamine, glucuronic acid, glucose, galactose, xylose and fucose have a good linear relationship.

Standard concentration curve of the eight monosaccharides

Regression equation and Correlation coefficient \(R^2\)
Man: \( y = 84680x + 448.84, R^2 = 0.9994 \); GlcN: \( y = 72933x + 348.8, R^2 = 0.9991 \); GalN: \( y = 67796x - 75.11, R^2 = 0.9993 \); Glc: \( y = 90330x + 525.0, R^2 = 0.9990 \); GlcA: \( y = 52893x + 6.7293, R^2 = 0.9995 \); Gal: \( y = 82851x + 528.91, R^2 = 0.9992 \); Fuc: \( y = 98532x - 125.05, R^2 = 0.9996 \); Xyl: \( y = 12054x - 0.0683, R^2 = 0.9993 \).

Precision experiment

**Precision of the method for monosaccharide standards.** After the 9 monosaccharides standard mixture have gone through the optimized hydrolysis and derivatization procedure, the analysis was repeated 5 times with expected procession of a typical HPLC method. The relative standard deviation (RSD %) of both elution times and peak area were summarized in Figure 2.

**The RSD for each of the monosaccharide standard analyzed 5 times by HPLC**

**Retention time/min and RSD%**
- Man: 15.37±0.055, 0.36; GlcN: 17.15±0.048, 0.28; GalN: 22.08±0.060, 0.27; Glc: 29.53±0.067, 0.23; GlcA: 24.27±0.059, 0.24; Gal: 31.25±0.072, 0.27; Fuc: 36.83±0.079, 0.22; Xyl: 33.33±0.074, 0.22.

**Peak area and RSD%**
- Man: 12026.16±155.40, 1.29; GlcN: 10230.9±89.90, 0.88; GalN: 8157.62±18.91, 0.23; Glc: 9709.5±52.74, 0.54; GlcA: 5813.18±62.27, 1.07; Gal: 12099.24±138.69, 1.14; Fuc: 13672.16±52.42, 0.92.

Repetitiveness experiment

**Repeatability of the method for a serum sample.** After the serum sample has gone through the optimized hydrolysis and derivatization procedure, the analysis was repeated 6 times with expected procession of a typical HPLC method. The relative standard deviation (RSD %) of both elution times and peak area were summarized in Figure 3.

**The RSD for a serum sample analyzed 5 times by HPLC**

**Retention time/min and RSD%**
- Man: 15.38±0.013, 0.09; GlcN: 17.25±0.006, 0.04; GalN: 22.22±0.012, 0.05; Glc: 29.49±0.022, 0.07; GlcA: 24.32±0.017, 0.07; Gal: 31.18±0.023, 0.07; Fuc: 36.84±0.021, 0.05.

**Peak area and RSD%**
- Man: 7458.3±132.23, 1.77; GlcN: 7409.06±66.13, 0.89; GalN: 4972.23±12.27, 0.25; Glc: 6639.26±163.54, 2.46; GlcA: 1703.36±49.13, 2.88; Gal: 7245.1±112.71, 1.56; Fuc: 5672.16±52.42, 0.92.

Stability experiment

**Stability of a hydrolyzed and derivatized serum sample before HPLC analysis.** After the serum sample has gone through the optimized hydrolysis and derivatization procedure, the HPLC analysis was conducted immediately (0 h), 2 h, 4 h, 8 h, 16 h, 24 h, respectively. The relative standard deviation (RSD %) of both elution times and peak area were summarized in Figure 4. The hydrolyzed and derivatized serum sample showed excellent stability. Based on above experiment, all the serum samples after derivatization were analyzed in the same day.

**The RSD for a serum sample analyzed at 0, 2, 4, 6, 8, 16, and 24 hours after derivatization by HPLC**

**Retention time/min and RSD%**
- Man: 15.39±0.02, 0.15; GlcN: 17.23±0.03, 0.21; GalN: 22.18±0.05, 0.25; Glc: 29.49±0.03, 0.10; GlcA: 24.37±0.06, 0.25; Gal: 31.18±0.0, 0.19; Fuc: 36.75±0.06, 0.17; Xyl: 33.22±0.06, 0.18.

**Peak area and RSD%**
- Man: 2128.55±2.77, 0.13; GlcN: 2733.2±5.42, 0.2; GalN: 603.3±1.16, 0.19; Glc: 2214.65±4.28, 0.19; GlcA: 151.91±0.93, 0.62; Gal: 1923.61±5.40, 0.28; Fuc: 808.35±1.25, 0.1; Xyl: 530.86±0.56, 0.11.

Loss rate experiment

Since the properties of the eight monosaccharides in human serum are different, the effects of hydrolysis of trifluoroacetic acid will also be different. In order to explore the degree of influence of the hydrolyzed serum with trifluoroacetic acid on the eight monosaccharides, we conducted a hydrolysis method (Table 5). Exploratory test of the loss rate caused. We took 100 ml of serum from each of 10 healthy controls and 10 endometrial cancer patients and made a serum mixture. We then added monosaccharide standard into the sera to test the loss rate of monosaccharide standard using the optimized experimental procedure by HPLC analysis.

**The loss rate of monosaccharide standards when added to the mixture of serum samples**

**Peak areas of Serum plus Monosaccharide standards / Serum alone / Monosaccharide standards alone**
- Man: 14123.4/9794.7/4599.3; GlcN: 18419.6/14381.2/4079.0; GalN: 4117.75/780.3/3343.2; Glc: 15247.1/11561.5/4830.7; GlcA: 1039.55/71.85/2049.3; Gal: 12745 Xyl: 3627.4/80.85/5135.1.
The loss rate of monosaccharide standards/%

\[ \text{Man: 5.882; GlcN: 0.995; GalN: 0.1708; Glc: 23.70; GlcA: 52.78; Gal: 6.028; Fuc: 13.16; Xyl: 30.94.} \]

Typical monosaccharide composition analysis of a serum sample

Using the optimized method, Figure 5 showed that baseline separation of 8 monosaccharides were achieved from a serum sample of a cancer patient in that 3 x 10^5 μL sample were independently hydrolyzed, PMP-labeled, and analyzed (Figure 5A-C) in comparison to a mixture of 8 monosaccharide standards (Figure 5D). Almost identical retention time and peak area for each monosaccharide were observed for three independent analysis of the same serum patient (Figure 5A-C), which showed that this method was dependable in performing monosaccharide quantification and monosaccharide compositional analyses of serum samples.

References

1. Cummings, R.D. and J.M. Pierce, *The challenge and promise of glycomics*. Chem Biol, 2014. 21(1): p. 1-15.
2. Zhang, L., *Glycosaminoglycans in development, health and disease*. Preface. Prog Mol Biol Transl Sci, 2010. 93: p. xvii-xviii.
3. Schachter, H. and H.H. Freeze, *Glycosylation diseases: quo vadis?* Biochim Biophys Acta, 2009. 1792(9): p. 925-30.
4. Hart, G.W. and R.J. Copeland, *Glycomics hits the big time*. Cell, 2010. 143(5): p. 672-6.
5. Varki, A., *Biological roles of glycans*. Glycobiology, 2017. 27(1): p. 3-49.
6. Ng, B.G. and H.H. Freeze, *Perspectives on Glycosylation and Its Congenital Disorders*. Trends Genet, 2018. 34(6): p. 466-476.
7. He, Y., et al., *Liquid chromatography mass spectrometry-based O-glycomics to evaluate glycosylation alterations in gastric cancer*. Proteomics Clin Appl, 2016. 10(2): p. 206-15.
8. Hatakeyama, S., et al., *Serum N-glycan alteration associated with renal cell carcinoma detected by high throughput glycan analysis*. J Urol, 2014. 191(3): p. 805-13.
9. Kim, J.H., et al., *Mass spectrometric screening of ovarian cancer with serum glycans*. Dis Markers, 2014. 2014: p. 634289.
10. Nouso, K., et al., *Clinical utility of high-throughput glycome analysis in patients with pancreatic cancer*. J Gastroenterol, 2013. 48(10): p. 1171-9.
11. Hu, M., et al., *Glycan-based biomarkers for diagnosis of cancers and other diseases: Past, present, and future*. Prog Mol Biol Transl Sci, 2019. 162: p. 1-24.
12. Zhang, L., *Glycans and Glycosaminoglycans as Clinical Biomarkers*. Preface. Prog Mol Biol Transl Sci, 2019. 163: p. xvii-xviii.
13. Tang, Y., et al., *The sensitivity and specificity of serum glycan-based biomarkers for cancer detection*. Prog Mol Biol Transl Sci, 2019. 162: p. 121-140.
14. Kunej, T., *Rise of Systems Glycobiology and Personalized Glycomedicine: Why and How to Integrate Glycomics with Multiomics Science?* OMICS, 2019. 23(12): p. 615-622.
15. Rojas-Macias, M.A., et al., *Towards a standardized bioinformatics infrastructure for N- and O-glycomics*. Nat Commun, 2019. 10(1): p. 3275.
16. Frazier, S.B., et al., *The Quantification of Glycosaminoglycans: A Comparison of HPLC, Carbazole, and Alcian Blue Methods*. Open Glycosci, 2008. 1: p. 31-39.
17. Studelska, D.R., et al., *Quantification of glycosaminoglycans by reversed-phase HPLC separation of fluorescent isoindole derivatives*. Glycobiology, 2006. 16(1): p. 65-72.
18. McDowell, L.M., et al., *Inhibition or activation of Apert syndrome FGFR2 (S252W) signaling by specific glycosaminoglycans*. J Biol Chem, 2006. 281(11): p. 6924-30.
19. Studelska, D.R., et al., *High affinity glycosaminoglycan and autoantigen interaction explains joint specificity in a mouse model of rheumatoid arthritis*. J Biol Chem, 2009. 284(4): p. 2354-62.
20. Lu, H., et al., *Glycosaminoglycans in Human and Bovine Serum: Detection of Twenty-Four Heparan Sulfate and Chondroitin Sulfate Motifs Including a Novel Sialic Acid-modified Chondroitin Sulfate Linkage Hexasaccharide*. Glycobiol Insights, 2010. 2010(2): p. 13-28.
21. Pan, J., et al., *Glycosaminoglycans and activated contact system in cancer patient plasmas*. Prog Mol Biol Transl Sci, 2010. 93: p. 473-95.
22. He, Y.L., et al., *Optimizing microwave-assisted hydrolysis conditions for monosaccharide composition analyses of different polysaccharides*. International Journal of Biological Macromolecules, 2018. 118: p. 327-332.
23. Zhang, L. and Y. Liu, Detection of free mannose and glucose in serum using high performance liquid chromatography US16/618,033, U.P. office, Editor. 2020.

24. Zhang, L., et al., Application of a method for detecting monosaccharides hydrolyzed from blood samples for cancer detection, S.I.P.O.o.t. P.R.C, Editor. 2015: China. p. 11.

25. Zhang, M., et al., Using a PCR instrument to hydrolyze polysaccharides for monosaccharide composition analyses. Carbohydrate Polymers, 2020. 240.

26. Gasilova, E.R., et al., Association of kappa-carrageenan subjected to deep alkaline hydrolysis. Biopolymers, 2018. 109(9): p. e23236.

27. Wang, Q.C., et al., Influences of acidic reaction and hydrolytic conditions on monosaccharide composition analysis of acidic, neutral and basic polysaccharides. Carbohydr Polym, 2016. 143: p. 296-300.

28. Zhu, H., et al., Acidolysis-based component mapping of glycosaminoglycans by reversed-phase high-performance liquid chromatography with off-line electrospray ionization-tandem mass spectrometry: evidence and tags to distinguish different glycosaminoglycans. Anal Biochem, 2014. 465: p. 63-9.

Figures

Figure 1

Using rhamnose as an internal quality control monosaccharide.
Figure 2

Precision of the method for monosaccharide standards.
Figure 3

Repeatability of the method for a serum sample.

Figure 3
Figure 4

Stability of a hydrolyzed and derivatized serum sample before HPLC analysis.

Figure 5

HPLC separation of PMP-labeled monosaccharides from a cancer patient serum (A-C) and eight monosaccharide standards (D). Ten μL of serum sample from endometrial cancer was acid-hydrolyzed, PMP-labeled, and the final product was dissolved in 70 μL of ddH2O. The HPLC analysis was done by injecting 20 μL of the final products three times and UV absorbance of PMP-labeled products was monitored at 245 nm (See details in Materials and Methods) (A-C: 3 parallels) or 20 μL of a mixture of 8 monosaccharide standards containing 3 nmol of each monosaccharide. Abbreviation: fucose (Fuc), galactose (Gal), galactosamine (GalN), glucose (Glc), glucuronic acid (GlcA), glucosamine (GlcN), mannose (Man), xylose (Xyl).