Analytical and Bioanalytical Chemistry (2023) 415:6863–6871
https://doi.org/10.1007/s00216-023-04964-5

RESEARCH PAPER

Development of a rapid simultaneous assay of two urinary tetrasaccharide metabolites using differential ion mobility and tandem mass spectrometry and its application to patients with glycogen storage disease (type Ib and II)

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Received: 30 July 2023 / Revised: 27 August 2023 / Accepted: 18 September 2023 / Published online: 29 September 2023

Abstract

Glucose tetrasaccharide (Glc_4) and maltotetraose (M_4) are important biomarkers for Pompe disease and other glycogen storage diseases (GSDs). With the development of new treatments for GSDs, more specific and sensitive bioanalytical methods are needed to determine biomarkers. In recent years, differential mobility spectrometry (DMS) has become an effective analytical technique with high selectivity and specificity. This study aimed to develop an efficient analytical method for the two urinary tetrasaccharide metabolites using DMS and apply it to patients with GSDs (type Ib and II). Urine samples were directly diluted and injected into liquid chromatography-differential mobility spectrometry tandem mass spectrometry (LC-DMS-MS/MS). Chromatographic separation was performed on an Acquity™ UPLC BEH Amide column (2.1 × 50 mm, 1.7 μm) with a short gradient elution of 2.6 min. DMS-MS/MS was used to detect two urinary tetrasaccharide metabolites in a negative multiple reaction monitoring mode with isopropanol as a modifier. A total of 20 urine samples from 6 healthy volunteers and 10 patients with GSDs (type Ib and II) were collected for analysis. The method was linear over a concentration range of 0.5~100.0 μg/mL for each urinary tetrasaccharide ($r \geq 0.99$). The intra- and inter-day precision RSD% were less than 14.3%, and the accuracy RE% were in the range of −14.3~13.4%. The relative matrix effect was between 86.6 and 114.3%. No carryover or interference was observed. Patients with GSDs (type Ib and II) had significantly higher median urinary Glc_4 (P=0.001) and M_4 (P=0.012) excretion than healthy subjects. The developed method was simple, rapid, sensitive, and specific. It was successfully applied to healthy volunteers and patients with GSDs (type Ib and II). DMS technology greatly improved analysis efficiency and provided high sensitivity and specificity.

Keywords Biomarkers · Differential mobility spectrometry · Glucose tetrasaccharide · Maltotetraose · Glycogen storage diseases

Introduction

Glycogen storage disease (GSD) is a group of rare inherited metabolic disorders caused by dysfunction of an enzyme or transport protein that play important roles in the synthesis and/or degradation of glycogen. GSD includes 16 types based on the defective enzyme or transport protein, the GSD I and GSD II are the most prevalent [1]. GSD I, also known as Von Gierke's disease and glucose-6-phosphatase deficiency, includes two main subtypes: Ia and Ib. GSD I patients generally have poor fasting tolerance, growth retardation, and hepatomegaly [2]. In GSD Ib, neutrophil dysfunction occurs frequently and further leads to infections, recurrent aphtous gingivostomatitis, and inflammatory

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bowel disease [2]. GSD II, also known as Pompe disease, is a glycogen metabolism disorder caused by a deficiency of acid alpha-glucosidase (GAA) enzyme [3]. GSD II is characterized by cardiopulmonary dysfounction and skeletal muscle weakness. Pompe disease includes rapidly progressive infantile-onset Pompe disease (IOPD) and highly variable later-onset Pompe disease (LOPD) [4, 5]. IOPD occurs shortly after birth, and infants often die of respiratory failure within 1 year without treatment. LOPD progresses slowly and develops symptoms at any time from infancy to late adulthood [6–8].

Treatment for Pompe disease and other GSDs was palliative until the Food and Drug Administration (FDA) approved the enzyme replacement therapy (ERT) [9]. However, there remains a substantial unmet medical need, mainly due to the inability of ERT to penetrate blood-brain barrier and its immunogenicity. In recent years, gene therapy products based on adeno-associated virus (AAV) have developed rapidly with low immunogenicity [10]. In order to comprehensively monitor the treatment response and disease progression in Pompe disease and other GSDs, efficient bioanalytical methods with high specificity and sensitivity for the analysis of biomarkers are currently in great demand.

Glucose tetrasaccharide (Glc_4, Fig. 1) is derived from the degradation of glycogen by α-amylase and GAA, and secreted in plasma and urine. It is reported that urinary excretion of Glc_4 is an important biomarker for Pompe disease and other GSDs [11–14]. Elevated urinary Glc_4 excretions were observed in GSD I, GSD II, GSD III, and GSD IV patients, suggesting that urinary Glc_4 may be important to determine treatment response or monitor disease progression for these diseases [14]. Maltotetraose (M_4), an isomer of Glc_4 (shown in Fig. 1), is also released during the degradation of glycogen by GAA [15]. In previous studies, M_4 was rarely detected in urine samples of Pompe disease patients. Therefore, it did not receive enough attention and was generally considered an interference of Glc_4 [13, 16]. In our study, we found that M_4 was rapidly degraded in urine, but it could be avoided by adjusting the pH of urines to around 9.50. Therefore, urinary M_4 excretion may be a potential biomarker for Pompe disease and other GSDs that has not been widely investigated due to its instability.

In the past few decades, several methods have been developed to quantify Glc_4 and M_4 in urine samples, including thin-layer chromatography [17], high-performance liquid chromatography (HPLC) [16, 18], and liquid chromatography-mass spectrometry (LC-MS) [13, 19, 20]. However, it was the total amount of urinary Glc_4 and M_4 that most methods detected due to lack of specificity, and only the HPLC assay developed by An Y et al. [16] and the LC-MS method established by Sluiter et al. [13] could distinguish Glc_4 and M_4. The HPLC assay required a time-consuming derivatization step with 1-phenyl-3-methyl-5-pyrazolone and long chromatographic runtime (35 min), which were unacceptable in clinical laboratories [16]. The LC-MS did not require complicated sample preparations and the runtime was reduced to 10 min, which was still long for an efficient analytical method [13]. Meanwhile, the LC-MS method could not quantify M_4, which may cause M_4 to lose its potential as a biomarker [13].

In recent years, modifier-assisted differential mobility spectrometry (DMS) has become an effective analytical technique for isobaric ion separation [21]. In DMS, organic modifiers such as methanol, acetonitrile, and isopropanol are introduced into the DMS cell to amplify the separation. A radio frequency asymmetric voltage, also known as separation voltage (SV), is applied to two planar-parallel electrodes and provides a dynamic high and low electric field condition [22]. Ions move between the two electrodes, and as the SV increases, ions take a larger "Z" orbit repeatedly until they fly off or hit the electrodes. In addition, the off-axis offset of the trajectory increases non-linearly with increasing SV. To be successfully captured by mass spectrometers, a compensation voltage (CV) is needed to correct their off-axis offset [21]. In summary, DMS enables accurate quantification of isomers without chromatography separation, which may require a long runtime. In addition, it also reduces noise and interferences in biological matrices, making sample preparation simple [23, 24]. However, to our knowledge, there are

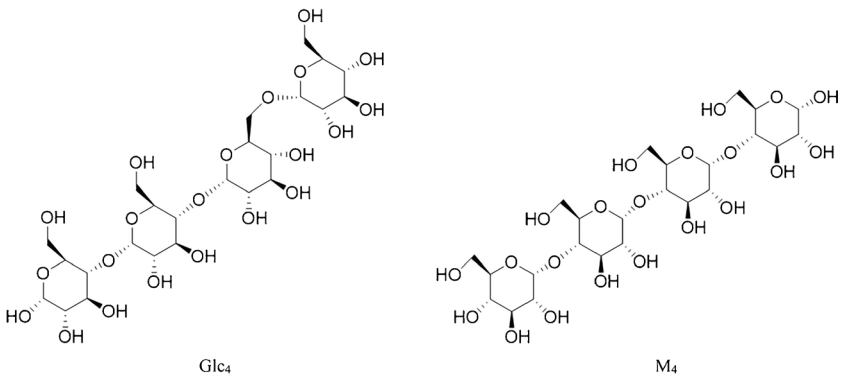

Fig. 1 The chemical structure of Glc_4 and M_4

hardly bioanalytical methods developed for Glc_4 and M_4 in human urine based on DMS technology.

Therefore, this study aimed to develop an efficient analytical method for the two urinary tetrasaccharide metabolites using liquid chromatography-differential mobility spectrometry tandem mass spectrometry (LC-DMS-MS/MS). The method did not require complicated derivatization and could quantify Glc_4 and M_4 simultaneously in 2.6 min without mutual interference. Ultimately, it was successfully applied to the clinical urine samples obtained from healthy volunteers and patients with GSDs.

Materials and methods

Chemicals and reagents

Glc_4 (6-α-D-glucopyranosyl-maltotriose, 97.0%) and its internal standard (IS) $^{13}C_6$-Glc_4 ($^{13}C_6$-6-α-D-glucopyranosyl-maltotriose, 96.0%) were obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). M_4 (maltotetraose, 99.2%) was purchased from ANPEL-TRACE Standard Technical Services Co., Ltd (Shanghai, China). HPLC-grade acetonitrile and isopropanol were purchased from Honeywell (Morris Plains, NJ, USA). Ammonia solution (A.R. grade) was bought from Xilong Scientific Co., Ltd (Shantou, Guangdong, China). Deionized water was purified with a Milli-Q® Ultrapure water system (Millipore Corporation, Bedford, MA, USA). Artificial urine was obtained from Dongguan Chuangfeng Automation Technology Co., Ltd. (DongGuan, Guangdong, China).

LC-DMS-MS/MS conditions

The liquid chromatographic analysis was performed using a LC-30A UPLC instrument (Shimadzu, Japan) with two solvent delivery units (LC-30AD XR), degasser (DGU-30A3R), communication bus module (CBM-30A), autosampler (SIL-30AC XR), and column oven (CTO-30AC). Chromatographic separation was achieved on an ACQUITY™ UPLC BEH amide column (2.1 × 50 mm, 1.7 µm, Waters Corp., Milford, MA, USA) with 0.1% ammonia solution as mobile phase A and ACN as mobile phase B. The gradient elution program was optimized as follows: 0.0–0.2 min, 70% (B); 0.2–1.0 min, from 70% (B) to 50% (B); 1.0–1.8 min, 50% (B); 1.8–1.9 min, from 50% (B) to 70% (B); 1.9–2.6 min, from 70% (B). The flow rate was 0.4 mL/min. The autosampler temperature was set to 10 °C and the column temperature was set to 40 °C with an injection volume of 3.0 µL.

DMS-MS/MS analysis was performed using a SelexION® DMS system mounted in the atmospheric-pressure region between an electrospray ionization source and the sampling orifice of a QTRAP® 5500 system (SCIEX, Concord, ON, Canada). The quantification of urinary tetrasaccharide isomers was conducted in negative multiple reaction monitoring (MRM) mode with isopropanol as a modifier. The optimized instrument parameters are listed in Table 1. Data acquisition and processing were performed on Analyst software (version 1.7.1, Applied Biosystems, USA).

Stock solutions, calibration standards, and quality controls

Accurately weighed Glc_4, M_4, and IS were separately dissolved in methanol-water (v/v, 1:1) to prepare the 1.0 mg/mL stock solution. The calibration standards were prepared by serially diluting stock solutions with artificial urine at the concentrations of 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/mL. The lower limit of quantitation (LLOQ) sample was prepared at the concentration of 0.5 µg/mL in artificial urine. The spiked LLOQ, low quality control (LQC), medium quality control (MQC), and high quality control (HQC) samples were spiked at the concentrations of 0.5, 1.5, 7.5, and 75 µg/mL in pooled real urine, respectively. The IS was diluted to 25.0 µg/mL in acetonitrile. All stock solutions, calibration standards, QC samples, and IS solutions were stored at − 80 °C until analysis.

Sample preparation

Urine samples were thawed at room temperature. A total of 20-µL aliquot was transferred to a 1.5-mL Eppendorf tube. Then, 20 µL IS and 40 µL acetonitrile were added. After vortex mixing and centrifuging at 17,000g for 10min, 3.0

Table 1 Optimized parameters of DMS-MS/MS

Parameters	Glc_4	M_4	$^{13}C_6$-Glc_4
Curtain gas (N_2, psi)	35	35	35
Collision gas (CAD)	Medium	Medium	Medium
Ionspray voltage (V)	− 4500	− 4500	− 4500
Ionization temperature (°C)	550	550	550
Gas1 (N_2, psi)	55	55	55
Gas2 (N_2, psi)	55	55	55
m/z transition	665.2–178.9	665.2–161.0	671.2–185.0
Declustering potential (V)	− 115	− 180	− 200
Collision energy (eV)	− 38	− 30.5	− 35
DMS temperature (°C)	150	150	150
DMS resolution enhancement	Low	Low	Low
DMS separation voltage (V)	3700	3700	3700
DMS compensation voltage (V)	− 3.0	− 0.5	− 3.0

μL of the supernatant was injected into LC-DMS-MS/MS system for analysis.

Method validation

The method was validated in accordance with Clinical and Laboratory Standards Institute guideline (CLSI 62-A) [25] and Food and Drug Administration guideline [26], including linearity, sensitivity, precision, accuracy, selectivity, relative matrix effect, carryover, and stability. The linearity of calibration curve was assessed by plotting the peak area ratios of the analytes to IS at eight levels against the nominal concentration with weighted ($1/X^2$) least squares linear regression. The precision and accuracy were assessed by analyzing six replicates of spiked LLOQ, LQC, MQC, and HQC samples in three different days. The precision was calculated as the percent relative standard deviation (RSD%), while the accuracy was expressed as recovery (calculated as the percent relative error, RE%). The RE% should be within ± 15% (± 20% for LLOQ), and the RSD% should be less than 15% RSD (20% for LLOQ). Sensitivity was evaluated by analyzing the accuracy and precision of six replicates of LLOQ. Selectivity was investigated by comparing six different double blank artificial urine samples with their corresponding spiked LLOQ samples. Glc_4 and M_4 were endogenous substance, and it is difficult to find a blank urine matrix without them. Therefore, we evaluated the relative matrix effect through a mixing experiment. The solution matrices were prepared at the concentrations of 1.5 and 75.0 μg/mL in artificial urine. Six independent urine matrices were separately mixed with two concentrations of solution matrix in a 1:1 ratio to prepare the corresponding mixed matrix, and each in triplicate. The relative matrix effect was the percentage ratio of the concentration of mixed matrices to the mean concentration of the urine matrices and the solution matrices. The relative matrix effect should be less than ± 15% and the RSD% of the relative matrix effect for the three concentrations should be less than 15%. In each batch, a double blank sample after the highest calibration standard sample was analyzed to assess the carryover, and it should be less than 20% of the response of the analytes in LLOQ and 5% for IS. The stability was assessed by analyzing six replicates of spiked low and high QC samples. The short-term stability was assessed after the QC samples were kept at room temperature for 24 h. The autosampler stability was tested after the processed QC samples were placed in an autosampler (10 °C) for 24 h.

Method application

The validated LC-DMS-MS/MS method was applied to healthy volunteers and patients with GSD Ib and Pompe disease from Peking Union Medical College Hospital for the assessment of treatment response. A total of 20 random urine samples were collected from anonymous healthy volunteers ($n = 6$) and GSD Ib ($n = 12$) and Pompe disease ($n = 2$) patients before or during treatment. After sample collection, the pH of urine was adjusted to around 9.50, and the obtained samples were store at − 80 °C until analysis. The study complied with the Declaration of Helsinki and was approved by the Peking Union Medical College Hospital Ethics Committee (I-22PJ394). Due to high intra- and inter-individual variation in random urine collected, the tetrasaccharide metabolite concentration was corrected by urinary creatinine, which was detected based on the LC-MS/MS method reported by Dziadosz et al. [27].

Results and discussion

DMS-MS/MS conditions

The stock solutions (1.0 mg/mL) of the analytes and IS were diluted separately to 100.0 ng/mL with acetonitrile–water (v/v, 1:1). The obtained solutions were then injected into the mass spectrometer to obtain ion transitions and optimize ionization parameters (Table 1). Despite sharing the same fragment ions, the fragment ion at *m/z* 178.9 showed the highest signal intensity for Glc_4, while that for M_4 was the fragment ion at *m/z* 161.0 (see Electronic Supplementary Material Fig. S1). Therefore, the fragment ions at *m/z* 178.9 and 161.0 were selected for the quantification of Glc_4 and M_4, respectively. Then, the ionization conditions were optimized for the maximum ion intensity (Table 1).

The single standard solutions (100 ng/mL) of Glc_4, M_4, and IS were separately injected into the DMS-MS/MS system using a syringe pump at the flow rate of 10 μL/min to obtain optimal DMS-MS/MS conditions (Table 1). Due to the high similarity in chemical structure of Glc_4 and M_4, high SV of 3200, 3500, and 3700 V were tested for the resolution of isomers and sensitivity. As the SV increased, the CoVs of Glc_4 and M_4 shifted positively, but none of the SV provided sufficient separation of the two tetrasaccharide isomers in the absence of modifiers (data not shown). Therefore, 2-propanol was introduced to the DMS cell as a chemical modifier to enhance the separation with a high modifier composition (3.0%). In addition, the DMS temperature seemed to have a limited effect on the separation of the two tetrasaccharide isomers, and low temperature (150 °C) was enough. The DMS enhanced resolution was a compromise between separation and sensitivity, high resolution meant improved separation and poor response. Balancing the separation and sensitivity, the DMS enhanced resolution was set to low mode with the optimized SV of 3700 V. Under these conditions, the compensation voltages were optimized to ensure that the separated ions fly off the electrodes and

are successfully captured by the mass spectrometer. Without the use of a modifier, there was no appropriate compensation voltage for Glc_4 and M_4 to avoid mutual interference between the two (see Electronic Supplementary Material Fig. S2). However, as shown in Fig. 2, with 2-propanol as a modifier, the maximum response was observed at a compensation voltage of − 3.0 V for Glc_4, followed by 1.0 V. In addition, the maximum response was observed at a compensation voltage of − 0.5 V for M_4, at which the response of Glc_4 was negligible. Therefore, the compensation voltage was set − 3.0 V for Glc_4, and − 0.5 V for M_4 to ensure sufficient sensitivity and specificity.

LC conditions

Glc_4 and M_4 are target analytes with the same fragment ions, and the MRM mode cannot distinguish them in the absence of chromatography separation. Due to the highly similar chemical structure (Fig. 1), chromatographic separation of Glc_4 and M_4 often requires complicated derivatization steps and/or long runtime, which are undesirable in clinical laboratories [13, 16, 18–20]. Three different chromatographic columns (Acquity™ UPLC BEH C_{18}, Acquity™ UPLC HSS T3, Acquity™ UPLC BEH Amide, Eschborn, Germany) were tested to acquire optimal separation. Glc_4 and M_4 were barely retented on C_{18} and T3 columns. Amide columns showed great retention for Glc_4 and M_4 with baseline separation achieved on the 2.1 × 100 mm 1.7 µm amide column, but the runtime required 10 min and more to equilibrate the column adequately (see Electronic Supplementary Material Fig. S3), which was consistent with Sluiter et al. [13].

In order to further reduce the runtime, DMS technology was introduced, which eliminated the need to separate Glc_4 and M_4 in the LC section. And the role of the LC section was to retent Glc_4 and M_4 to avoid their co-elution with interfering substances in the urine matrix, which was achieved on a BEH amide column (2.1 × 50 mm, 1.7 µm) in 2.6 min. Methanol and acetonitrile were evaluated, and acetonitrile provided better performance. Different mobile phases additives (ammonium formate, formic acid, ammonia solution) were investigated, and good peak shape and retention were observed under a gradient elution of 2.6 min with 0.1% ammonia solution as mobile phase A and acetonitrile as mobile phase B (Fig. 3).

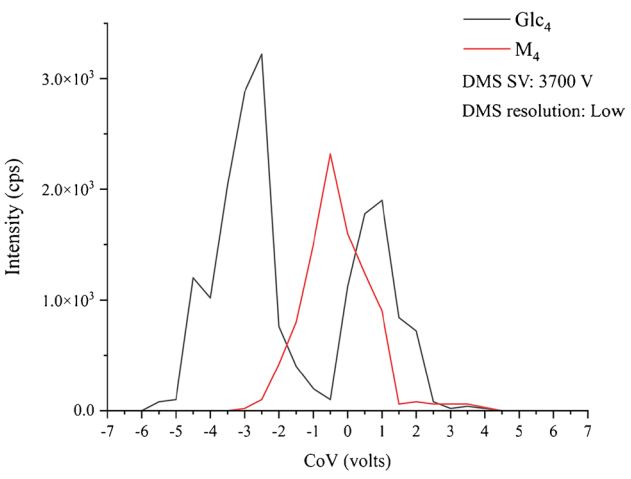

Fig. 2 Overlaid CoV optimization figure of two separated tetrasaccharide isomers

Verification of the LC-DMS-MS/MS method

The single standard solutions of Glc_4, M_4, and IS were separately detected by the optimized LC-DMS-MS/MS method to verify the separation efficiency of DMS. Glc_4 and M_4 were co-eluted in the LC system with a BEH amide column (2.1 × 50 mm, 1.7 µm), but were completely separated in DMS cell. The optimized LC-DMS-MS/MS method could accurately quantify Glc_4 and M_4 simultaneously without mutual interference (Fig. 3).

Sample preparation

Urine contains a large number of electrolytes and metabolites with great variability, and proper preparation is necessary to remove matrix interference. With great sample purification ability, DMS technology allows simple and rapid preparation. Different preparation methods (direct dilution, liquid-liquid extraction, and solid-phase extraction) were investigated, and the direct dilution was adopted due to its combination of simplicity, speed, and sensitivity. In order to get good peak shape, acetonitrile was selected as the diluent and was added to urine samples at a ratio of 3:1. Then, the urine-acetonitrile mixture was centrifuged at 17,000g for 10 min, and the supernatant was injected into the instrument for analysis. It was tested that more than 30 samples could be prepared in 30 min, which further proved the high efficiency of the preparation method.

Method validation

Linearity

The calibration curves showed good linearity between 0.5 ~ 100.0 µg/mL, and the regression coefficient (r) was no less than 0.99 (see Electronic Supplementary Material Table S1). In each batch, at least 75% of the calibration standards had a RE% within ± 15% (LLOQ of ± 20%).

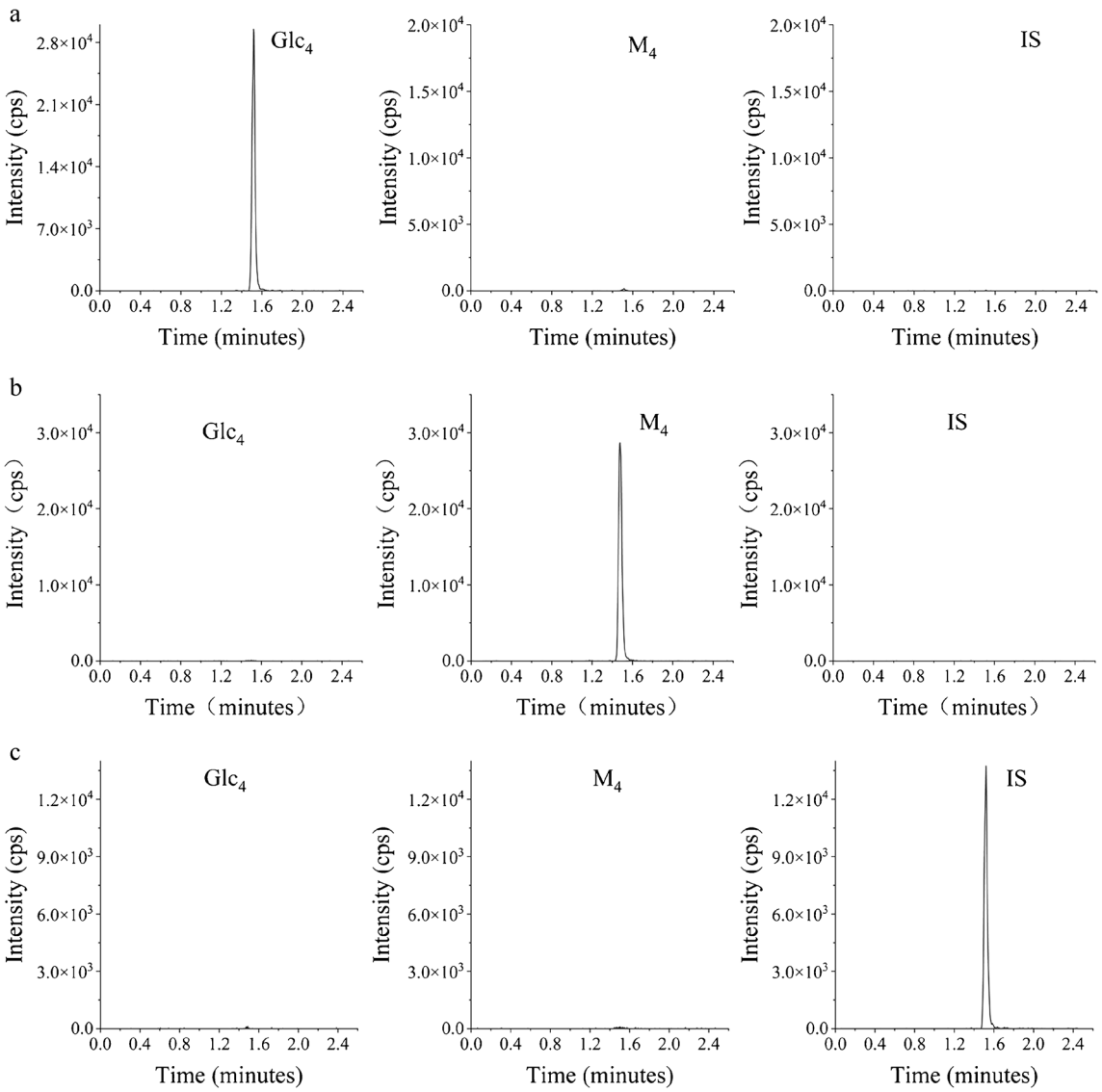

Fig. 3 Representative chromatograms of single standard solutions of Glc_4 (**a**), M_4 (**b**), and IS (**c**) by the developed LC-DMS-MS/MS

Selectivity

No significant interfering peak at retention times of the analytes or IS was found in double blank samples (see Electronic Supplementary Material Fig. S4).

LLOQ, precision, and accuracy

The LLOQ and intra-day and inter-day precision and accuracy results of the two tetrasaccharide isomers are summarized in Electronic Supplementary Material Table S2. The RE% was between − 14.3 ~ 13.4% (− 12.6 ~ 12.0% for LLOQ), and the RSD% was less than 14.3% (17.3% for LLOQ). These results demonstrated that the method was reliable and reproducible for the simultaneous assay of urinary Glc_4 and M_4.

Matrix effects

The results of relative matrix effect are summarized in Electronic Supplementary Material Table S3. The relative matrix effects of Glc_4 and M_4 at low and high concentrations were in the range of 86.6 ~ 114.3%, and the RSD% of relative matrix effect between high and low concentrations was less than 4.5%, revealing that the matrix interference from human urine was negligible.

Table 2 Summary of urinary Glc_4 and M_4 levels in subjects

Subject	Conditions	Sample collection	Glc_4 (μg/mg creatinine)	M_4 (μg/mg creatinine)
1	Health	None	4.8	0.0
2	Health	None	13.3	0.0
3	Health	None	9.5	0.0
4	Health	None	4.2	0.0
5	Health	None	7.6	0.0
6	Health	None	4.0	0.0
7	GSD Ib	After treatment	12.9	0.0
8	GSD Ib	Before treatment	13.7	0.0
8	GSD Ib	After treatment	17.3	0.5
9	GSD Ib	After treatment	11.7	0.0
10	GSD Ib	After treatment	8.4	0.7
11	GSD Ib	After treatment	14.6	0.0
12	GSD Ib	Before treatment	92.4	5.5
12	GSD Ib	After treatment	154.4	7.5
12	GSD Ib	After treatment	58.2	1.8
13	GSD Ib	After treatment	70.3	2.9
13	GSD Ib	After treatment	75.0	4.5
14	GSD Ib	After treatment	189.1	9.7
15	GSD II	Before treatment	101.3	5.4
16	GSD II	Before treatment	321.7	14.9

Carryover

No obvious peak was observed at the retention time of the two urinary tetrasaccharide isomers in double blank samples following the highest calibration standard samples (see Electronic Supplementary Material Fig. S5).

Stability

The short-term stability and autosampler stability results are shown in Electronic Supplementary Material Table S4. The RSD% was less than 14.3%, and the RE% ranged from − 14.3 to 11.3%, indicating that the two urinary tetrasaccharide isomers were stable at room temperature for 24 h before preparation and in the autosampler for 24 h after preparation.

Method application

The developed LC-DMS-MS/MS method was successfully applied to detect urinary excretion of Glc_4 and M_4 in healthy individuals and patients with GSDs. The results were corrected with urine creatinine and presented in Table 2. Patients with GSDs (Ib and II) had significantly higher median urinary Glc_4 (Mann-Whitney $U = 4.0$, $P = 0.001$) and M_4 (Mann-Whitney $U = 4.0$, $P = 0.012$) excretion than healthy subjects, which initially confirmed the potential of Glc_4 and M_4 as biomarkers. In addition, urinary M_4 in patients with GSDs accounted for less than 8.0% of urinary tetrasaccharides, which was consistent with the study reported previously [28].

In the past few decades, several assays have been developed to detect Glc_4 and M_4 in urine samples, including TLC [17], HPLC [16, 18], and LC-MS [13, 19, 20]. Compared with these methods, the developed method had several unique advantages (see Electronic Supplementary Material Table S5). Firstly, the runtime of this method was reduced to 2.6 min, which greatly improved the analysis speed. Secondly, with great sample purification ability, DMS technology allows simple and rapid preparation, which further improved the analysis efficiency. Thirdly, DMS technology provided higher sensitivity and specificity for the two tetrasaccharide isomers. Finally, this method could accurately quantify M_4 in urine, which has not been reported in previous methods, and can provide support for exploring the potential of M_4 as a biomarker for Pompe disease and other GSDs.

The collected clinical samples were also analyzed using the optimized LC-MS/MS method based on the assay reported by Sluiter et al. [13], which allowed simultaneous quantification of Glc_4 and M_4. And the results were compared with those detected using the LC-DMS-MS/MS method developed in this study to assess the consistency of

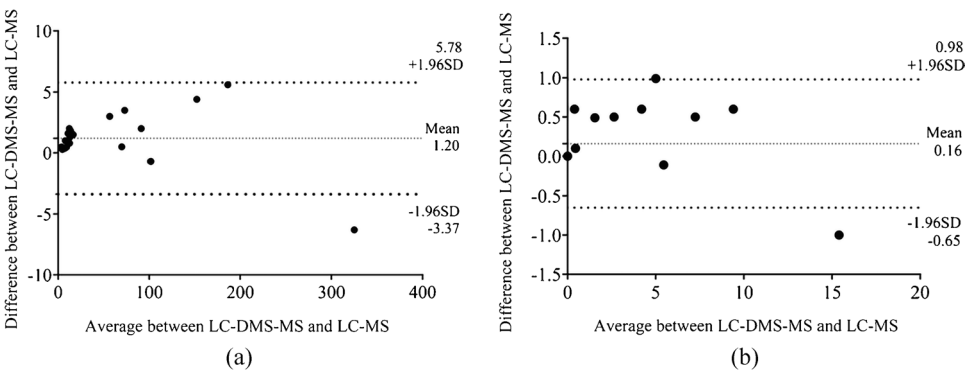

Fig. 4 The Bland-Altman diagram of Glc_4 (**a**) and M_4 (**b**) quantified by the developed LC-DMS-MS and the LC-MS

the two analytical methods. As shown in Fig. 4, the mean difference of Glc_4 and M_4 was 1.20 and 0.16, respectively. At least 95% of points fall within the clinically acceptable limit of agreement, suggesting good consistency.

Conclusion

A LC-DMS-MS/MS method was developed and validated for the simultaneous determination of two urinary tetrasaccharide metabolites in human urine. And the method was simple, rapid, efficient, and robust. Ultimately, the method was successfully applied to clinical samples from healthy volunteers and patients with GSD Ib and Pompe disease. To our knowledge, this is the first validated method for the detection of urinary tetrasaccharide metabolites using DMS technology. Compared with traditional LC separation, DMS technology reduces the runtime by 74%, greatly improves analysis efficiency with higher sensitivity and specificity.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00216-023-04964-5.

Author contribution Conceptualization: Xiaohong Han and Xin Zheng. Methodology: Jianwei Ren. Validation: Jianwei Ren. Writing—original draft preparation: Jianwei Ren. Writing—review and editing: Xin Zheng and Yufang Ma. Sample collection: Mingsheng Ma, Juan Ding, Jingjing Jiang. Funding acquisition: Xiaohong Han and Mingsheng Ma. The submitted manuscript has been approved by all authors, who accept responsibility for its entire content.

Funding The research was funded by National High Level Hospital Clinical Research Funding (grant numbers, 2022-PUMCH-B-033 and 2022-PUMCH-A-093) and The National Key Research and Development Program of China (grant number, 2022YFC2703100).

Data availability Not applicable.

Declarations

Ethical approval The study was approved by the Peking Union Medical College Hospital Ethics Committee (I-22PJ394).

Competing interests The authors declare no competing interests.

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