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Development and validation of a liquid chromatography–tandem mass spectrometric method for the quantification of 5-thio-D-glucose in rat and human plasma

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ABSTRACT

A highly selective, sensitive, and robust liquid chromatography–tandem mass spectrometric method for the determination of 5-thio-D-glucose concentrations in rat and human plasma was developed and validated. The sample preparation procedure involved protein precipitation and solid phase extraction, which efficiently removed sources of interference present in the plasma. Chromatographic separation was obtained using an NH_2-column with distilled water and acetonitrile as the mobile phase under gradient conditions. Detection was performed using tandem mass spectrometry equipped with an electrospray ionization interface in negative ion mode. The selected reaction monitoring (SRM) transitions for 5-thio-D-glucose and an internal standard (5-thio-D-glucose-$^{13}C_6$) were m/z 195 → m/z 105 and m/z 201 → m/z 108, respectively. The correlation coefficients of the calibration curves ranged from 0.9997 to 0.9999 over a concentration range from 10 to 3000 ng/mL plasma. The validated method was successfully applied to a pharmacokinetic study in rats.

1. Introduction

The potent antimetabolic glucose analogue 5-thio-D-glucose differs from D-glucose by the presence of a sulphur atom in place of an oxygen atom in its pyranose ring. 5-Thio-D-glucose reportedly has various effects including such as an increase of food intake [1,2], the enhancement of septopreoptic γ-aminobutyric acid (GABA) turnover [3,4], the regulation of male fertility [5,6], the inhibition of tumor cell growth in vitro [7], protection against substantial β-cell toxicity in streptozotocin-induced diabetes [8,9], and the inhibition of glucose transport [10]. However, a method for quantification of 5-thio-D-glucose in biological fluids has not been reported, with the exception of the use of radiolabeled (^{35}S or ^{3}H) 5-thio-D-glucose [11]. Therefore, a highly sensitive and selective method for quantification of 5-thio-D-glucose in biological samples is required.

The quantitative analysis of monomeric sugars in biological fluids is very difficult because of the high polarity, the poor UV absorption or ionization, and the numerous sources of interference within the matrix. In general, methods for detecting monomeric sugars include differential refractive index detection [12], fluorescence detection [13], and electrochemistry detection (pulsed amperometric detection [14,15]). However, the analytical performances of these methods often yield unsatisfactory results because of the lack of selectivity or sensitivity. Furthermore, laborious sample preparation procedures and time-consuming derivatization protocols are required for the analyses. Monomeric sugar levels in the serum or plasma samples are often measured using enzymatic methods with an assay kit [16] or assay devices [17]. However, these assays seem to be unreliable with regard to their selectivity or sensitivity, especially for concentrations measured on the order of ng/mL. Nowadays, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has proven to be an extremely sensitive and specific tool for the analysis of biological samples [18]. A few reports have described assays for iminosugars in biological samples using LC–MS/MS in the positive ion mode [19]. However, similar to D(+)-glucose and D(−)-fructose, which are richly present in plasma, 5-thio-D-glucose is ionized in the negative ion mode. Therefore, the chromatographic separation of 5-thio-D-glucose from other monomeric sugars and the efficient removal of sources of interference present in the plasma are important.

Abbreviations: SPE, solid phase extraction; CV, coefficient of variation; RE, relative error.

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The purpose of this study was to develop a selective, sensitive, and robust LC–MS/MS method for the determination of 5-thio-D-glucose concentrations in rat and human plasma.

2. Experimental procedure

2.1. Chemicals and materials

5-Thio-D-glucose was purchased from Fluka (a division of Sigma–Aldrich, St. Louis, MO, USA). The stable isotope labeled internal standard (IS) $^{13}C_6$-5-thio-D-glucose was synthesized at Taisho (Saitama, Japan). D(+)-Glucose and D(−)-fructose were purchased from Wako (Osaka, Japan). The chemical structures of 5-thio-D-glucose, $^{13}C_6$-5-thio-D-glucose (IS), D(+)-glucose and D(−)-fructose are shown in Fig. 1. Distilled water (HPLC-grade) was purchased from Kanto Chemical (Tokyo, Japan). Acetonitrile (HPLC-grade) and methanol (HPLC-grade) were purchased from Wako (Osaka, Japan). Blank rat and human plasma for the preparation of calibration standards and quality control (QC) samples were purchased from Kitayama Labes (Nagano, Japan) and COSMO BIO (Tokyo, Japan), respectively.

Ultrafree-MC Centrifugal Filter Units were purchased from Millipore Corporation (Bedford, MA, USA). Bond Elut DEA cartridges and SPE cartridges were purchased from Varian (Harbor City, CA, USA).

2.2. Instrument

The LC–MS/MS system consisted of a Waters 2795 Separations module (Milford, MA, USA) and an API4000 triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA, USA). The data were collected and processed using Analyst 1.3 software.

2.3. Chromatographic conditions

Chromatographic separation was achieved using an Asahipak NH2P-50 2D analytical column (150 mm × 2.0 mm I.D., 5 μm particle size; Showa Denko, Tokyo, Japan) with acetonitrile and distilled water as the mobile phase under a gradient condition at a flow rate of 0.2 mL/min. A linear gradient was applied as follows: decrease from 85% to 75% acetonitrile, 0–10 min; 75–25% acetonitrile, 10–11 min; hold for 1 min; increase from 25% to 85%, 12–13 min; hold for 5 min. The temperatures of the column and sample compartment were 40 °C and 5 °C, respectively. The LC eluate was directed, via a divert valve, into the mass spectrometer at between 5 and 10 min after injection.

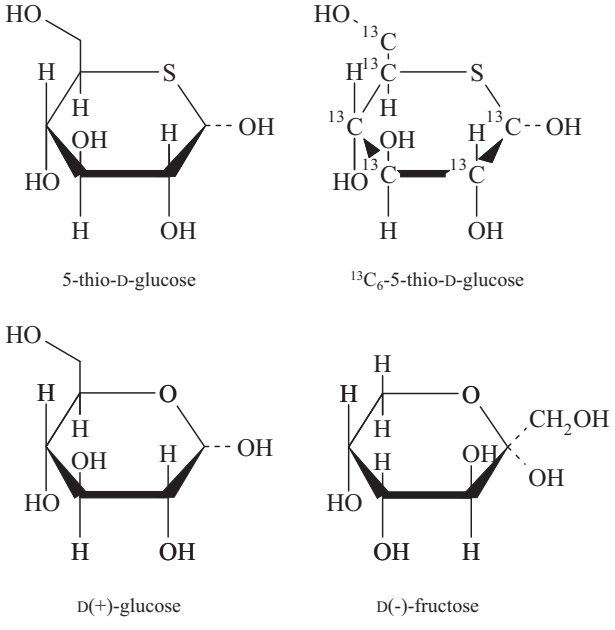

Fig. 1. Chemical structures of 5-thio-D-glucose, $^{13}C_6$-5-thio-D-glucose, D(+)-glucose and D(−)-fructose.

2.4. Mass spectrometric conditions

An API4000 triple quadrupole mass spectrometer with a TurboIonSpray® (TIS) interface was operated in the negative ionization mode for selected reaction monitoring (SRM) LC–MS/MS analyses. The mass spectrometric conditions were optimized for both the analyte and the IS as follows: TIS source temperature (TEM), 600 °C; TIS voltage (IS), −4500 V; curtain gas, 50 psi; nebulizing gas (GS1), 40 psi; TIS gas (GS2), 20 psi; collision gas, 2 units; declustering potential (DP), −10 V; entrance potential (EP), −60 V; collision energy (CE), −14 eV; collision cell exit potential (CXP), −5 V for 5-thio-D-glucose and −1 V for IS. Quantification was performed using the SRM mode with the following transitions: m/z 195 → m/z 105 for 5-thio-D-glucose and m/z 201 → m/z 108 for IS, with a dwell time of 500 ms.

2.5. Preparation of calibration standards and QC samples

Stock solutions of 5-thio-D-glucose (1000 μg/mL) and IS (100 μg/mL) were prepared by dissolving the compound in distilled water. Working solutions of 5-thio-D-glucose were prepared by serial dilution of the 5-thio-D-glucose stock solution in distilled water. A 1000-ng/mL IS working solution was prepared by diluting the IS stock solution in distilled water.

Calibration standards were prepared at 10, 30, 100, 300, 1000, and 3000 ng/mL as follows: 5 μL of the corresponding 5-thio-D-glucose working solution was added to 495 μL of blank rat plasma, or 10 μL of the corresponding working solution was added to 990 μL of blank human plasma. QC samples (10, 24, 240, and 2400 ng/mL) and dilution QC samples (2400 and 10,000 ng/mL) were similarly prepared using blank rat or human plasma.

2.6. Sample preparation

For the rat sample, 50 μL of plasma, 10 μL of IS working solution, and 300 μL of acetonitrile/methanol (90:10, v/v) were combined. For the human sample, 100 μL of plasma, 20 μL of IS working solution, and 600 μL of acetonitrile/methanol (90:10, v/v) were combined. The mixture was vortex-mixed for 30 s and then centrifuged at 10,000 × g for 5 min at 4 °C. The supernatant was transferred to a glass tube and evaporated to complete dryness under a stream of nitrogen in a waterbath at 60 °C. The residue was dissolved in 100 μL of distilled water. The reconstituted solution was applied to an SPE cartridge (Bond Elut DEA, 100 mg/1 mL) preconditioned with 1 mL of methanol and 1 mL of distilled water. 5-Thio-D-glucose and the IS were eluted with 800 μL of distilled water by centrifugation at approximately 200 × g for 5 min at 4 °C. The eluate was evaporated to complete dryness under a stream of nitrogen in a waterbath at 60 °C. The residue was dissolved in 100 μL of distilled water. The reconstituted solution was filtered through an Ultrafree-MC by centrifugation at 10,000 × g for 5 min at 4 °C. A 50-μL aliquot of the filtrate was combined with 50 μL of acetonitrile and vortex-mixed. An aliquot (rat: 5 μL, human: 3 μL) of the solution was then injected into the LC–MS/MS system.

2.7. Method validation

The method was validated for selectivity, sensitivity, linearity of the calibration curve, precision and accuracy, recovery, stability, and dilution integrity according to the FDA guidelines for the validation of bioanalytical methods [20].

2.8. Application of the method to a pharmacokinetic (PK) study

To investigate the pharmacokinetics of 5-thio-D-glucose in rats, the above-described quantification method was applied. In the studies, 5-thio-D-glucose was administered to male Sprague-Dawley rats (Charles River Laboratories, Japan). While administering multiple oral doses for 14 days (6.25, 12.5, 25 or 50 mg/kg/day), blood samples were collected at 1, 2, 4, 8, and 24 h after the first dosing, at 24 h after the seventh dosing, and before and 1, 2, 4, 8, and 24 h after the fourteenth dosing. The blood samples were collected into heparinized tubes and centrifuged. The resulting plasma samples were stored at −70 °C or below until analysis.

3. Results and discussion

3.1. Method development

To develop a method for the quantification of 5-thio-D-glucose in rat and human plasma, the following three points were considered: chromatographic separation, preparation of the biological samples, and robustness of the method for large number of samples.

The chromatographic challenge was to achieve adequate separation of 5-thio-D-glucose from other monomeric sugars found abundantly in plasma. The most abundant monomeric sugar is D(+)-glucose, the level of which often exceeds 1 mg/mL in plasma [21]; this level is 100,000 times higher than the LLOQ (10 ng/mL) of 5-thio-D-glucose. In the case of 5-thio-D-glucose (10 ng/mL) and D(+)-glucose (1 mg/mL) co-eluted, the peak area of 5-thio-D-glucose became about one-third of the value obtained when 5-thio-D-glocose was eluted alone. A Gelpack GL-C64Z column (sulphonated styrene-divinylbenzene copolymer column with zinc ions; Hitachikasei, Tokyo, Japan) or an Asahipak NH2P column (polymer-based amino column) with an optimized mobile phase provided good results. 5-Thio-D-glucose was clearly separated from D(+)-glucose and D(−)-fructose, with a good peak shape (Fig. 2). Furthermore, D(+)-galactose was confirmed not to interfere with the ionization of 5-thio-D-glucose (data not shown).

To achieve the efficient removal of interferences in plasma samples with a good recovery of 5-thio-D-gucose, several preparation methods were examined. To extract the analytes from the biological samples, preparation methods such as protein precipitation (PPT), liquid–liquid extraction, and SPE are often used. PPT is the simplest approach for removing plasma components [22] [23]; however, we could not obtain good results using PPT alone, since the peak response and retention time of 5-thio-D-glucose were unstable and the pressure of the analytical column increased rapidly with short-term use. These problems were thought to have been caused by the influence of interference sources remaining in the PPT extracts. The matrix effect in LC–MS/MS analysis is a well-known phenomenon that results in ion suppression [24] or the enhancement of target responses when interference sources are co-eluted with analytes [25]. To avoid this phenomenon further clean-up of the PPT extract was required; therefore, the use of SPE following PPT was examined. Various SPE cartridges were compared, and the Bond Elut DEA cartridge was selected because of its relatively high recovery and weaker matrix effect for 5-thio-D-glucose. By adding the Bond Elut DEA treatment, not only the peak response, but also the retention time of 5-thio-D-glucose stabilized because of the effective elimination of interference components, including anionic compounds.

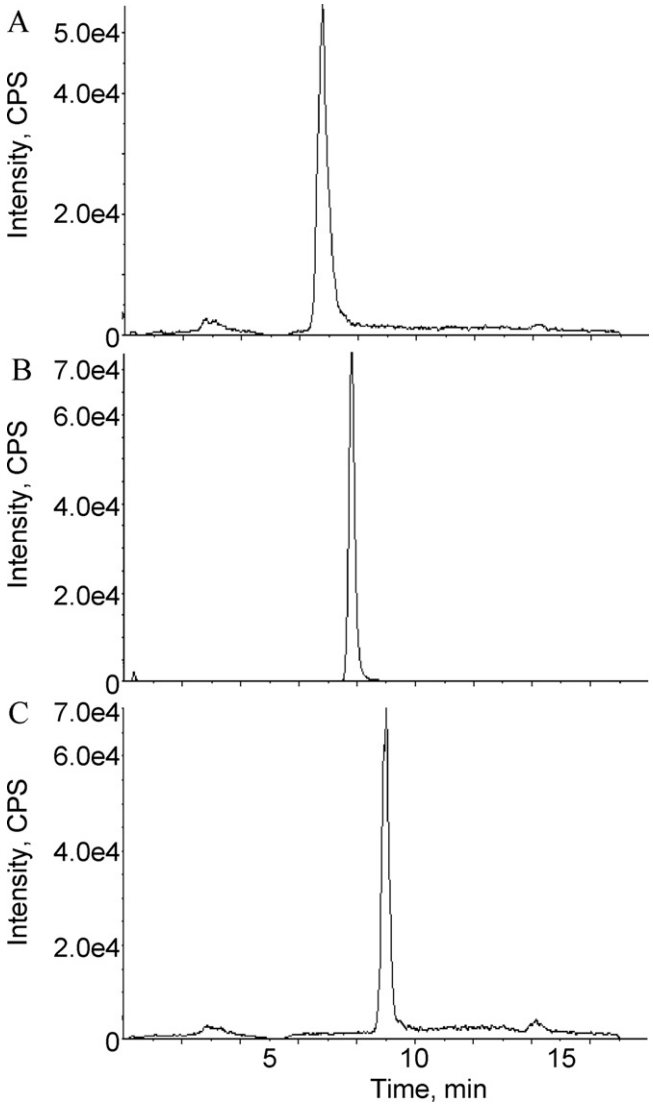

Fig. 2. Typical MRM chromatograms of synthetic compounds in aqueous solutions: (A) D(−)-fructose (m/z 179 → 89); (B) 5-thio-D-glucose (m/z 195 → 105) and (C) D(+)-glucose (m/z 179 → 89).

The sample preparation procedure was further optimized to achieve robustness. During the early stage of the method development, the eluate obtained by SPE following PPT was evaporated and dissolved in acetonitrile/distilled water (1:1, v/v). The solution was filtered and then injected into the LC–MS/MS system. This procedure provided acceptable results for the linearity of the calibration curve and the reproducibility of the QC samples, but the durability of the analytical column was unacceptable. Further development of the procedure was continued to resolve the brittleness issue. Since column durability usually depends on the cleanliness of the injection solution and 5-thio-D-glucose has a hydrophilic property, decreasing the organic solvent in a reconstitute solution that could remove lipophilic components, such as lipids, was investigated. Finally, the SPE eluate was completely evaporated to dryness, reconstituted with distilled water instead of acetonitrile/distilled water (1:1, v/v), and then filtered for LC–MS/MS analysis. This modification improved the durability of the column. To increase the

Table 1
Calibration curves for 5-thio-D-glucose in rat and human plasma.

	Nominal concentration (ng/mL)	Back-calculated concentration (ng/mL)			Relative error (%)		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day
Rat plasma	10	9.94	9.91	10.0	−0.6	−0.9	0.0
	30	30.6	30.6	29.8	+2.0	+2.0	−0.7
	100	100	102	100	0.0	+2.0	−0.2
	300	299	302	310	−0.3	+0.7	+3.3
	1000	993	972	982	−0.7	−2.8	−1.8
	3000	2980	2970	2980	−0.7	−1.0	−0.7
	Slope	0.00657	0.00634	0.00650			
	Y-intercept	−0.00545	0.00248	0.00317			
	r[a]	0.9999	0.9998	0.9998			
Human plasma	10	9.92	9.89	9.91	−0.8	−1.1	−0.9
	30	30.9	31.1	30.7	+3.0	+3.7	+2.3
	100	100	101	102	0.0	+1.0	+2.0
	300	292	297	299	−2.7	−1.0	−0.3
	1000	1010	977	999	+1.0	−2.3	−0.1
	3000	3000	3000	2920	0.0	0.0	−2.7
	Slope	0.00653	0.00673	0.00665			
	Y-intercept	−0.000342	−0.00288	−0.00273			
	r[a]	0.9998	0.9997	0.9998			

[a] Correlation coefficient.

robustness of the method, the Asahipak NH2P and Gelpack GL-C64Z columns were compared. As a result, the Asahipak NH2P column was adopted based on its durability.

3.2. Method validation

3.2.1. Selectivity

The presence of interfering peaks was investigated using SRM chromatograms of blank plasma samples from six different sources.

Typical SRM chromatograms for a single blank sample and calibration standards (10 and 3000 ng/mL) are shown in Fig. 3. 5-Thio-D-glucose and IS were eluted with good peak shapes. No interfering peaks were observed at each of the eluting position for 5-thio-D-glucose and IS in any of the blank rat and human plasma samples that were studied.

3.2.2. Linearity of calibration curve

Calibration standards including concentrations of 10, 30, 100, 300, 1000, and 3000 ng/mL of 5-thio-D-glucose in rat and human plasma ($n = 1$ for each level) were assayed on three different days. Calibration curves were then constructed using a $1/y^2$ weighted linear least-squares regression. The linearity (correlation coefficient: r) of the calibration curves and the accuracy (%RE) of the back-calculated values at each concentration were evaluated.

The parameters of the calibration curves are shown in Table 1. The calibration curves for 5-thio-D-glucose in rat and human plasma were linear over the concentration range from 10 to 3000 ng/mL with correlation coefficients of ≥0.9998 and ≥0.9997, respectively. The accuracy for each concentration ranged from −2.8% to +3.3% for rat plasma and from −2.7% to +3.7% for human plasma.

3.2.3. Precision and accuracy

The intra-assay precision and accuracy for this method were determined by analyzing the QC samples at four concentrations ($n = 5$ for each level). The inter-assay precision and accuracy were also determined by analyzing the QC samples in three batches on different days. The precision and accuracy were presented as %CV and %RE, respectively.

The intra-assay precision and accuracy ranged from 0.9% to 6.1% and from −2.8% to +5.4%, respectively, for rat plasma, and from 0.8% to 3.6% and from −3.3% to +1.7%, respectively, for human plasma. The inter-assay precision and accuracy ranged from 1.4% to 4.3% and from −4.0% to +0.8%, respectively, for rat plasma and from 0.8% to 3.0% and from −3.8% to +0.8%, respectively, for human plasma (Table 2).

3.2.4. Lower limit of quantification (LLOQ)

The LLOQ was defined as the lowest concentration on the calibration curve where the analyte can be measured with a precision of ≤20.0%, an accuracy of ≤±20.0%, and a signal-to-noise ratio of at least 5:1.

Based on the evaluation results for *Selectivity* (Fig. 3) and *Precision and accuracy* (Table 2), the LLOQ of 5-thio-D-glucose in rat and human plasma was determined to be 10 ng/mL.

3.2.5. Recovery

The extraction recovery of 5-thio-D-glucose from rat and human plasma was determined at three concentrations (24, 240, and 2400 ng/mL, $n = 5$ for each level) by comparing the peak area ratios (5-thio-D-glucose/IS) of the QC samples (spiked before extraction) with those of the corresponding samples spiked post-extraction. In a similar manner, the recovery of IS was also evaluated at 200 ng/mL, based on the peak area ratios (IS/5-thio-D-glucose).

The results for the recovery evaluation are shown in Table 3. The extraction recovery of 5-thio-D-glucose ranged from 61.4% to 64.7% with a CV ≤ 5.1% for rat plasma and from 61.0% to 67.4% with a CV ≤ 4.0% for human plasma. The IS recovery from rat and

Table 2
Precision and accuracy for the determination of 5-thio-D-glucose in rat and human plasma.

Nominal concentration (ng/mL)	Intra-assay ($n = 5$)		Inter-assay ($n = 15$)	
	%CV	%RE	%CV	%RE
Rat plasma				
10	6.1	−2.8	4.3	−4.0
24	0.9	+5.4	3.3	+0.8
240	1.2	0.0	1.4	+0.4
2400	1.0	+1.3	1.4	0.0
Human plasma				
10	3.6	−0.6	3.0	−0.6
24	1.5	+1.7	2.1	+0.8
240	0.8	−1.3	0.8	−1.3
2400	1.1	−3.3	1.4	−3.8

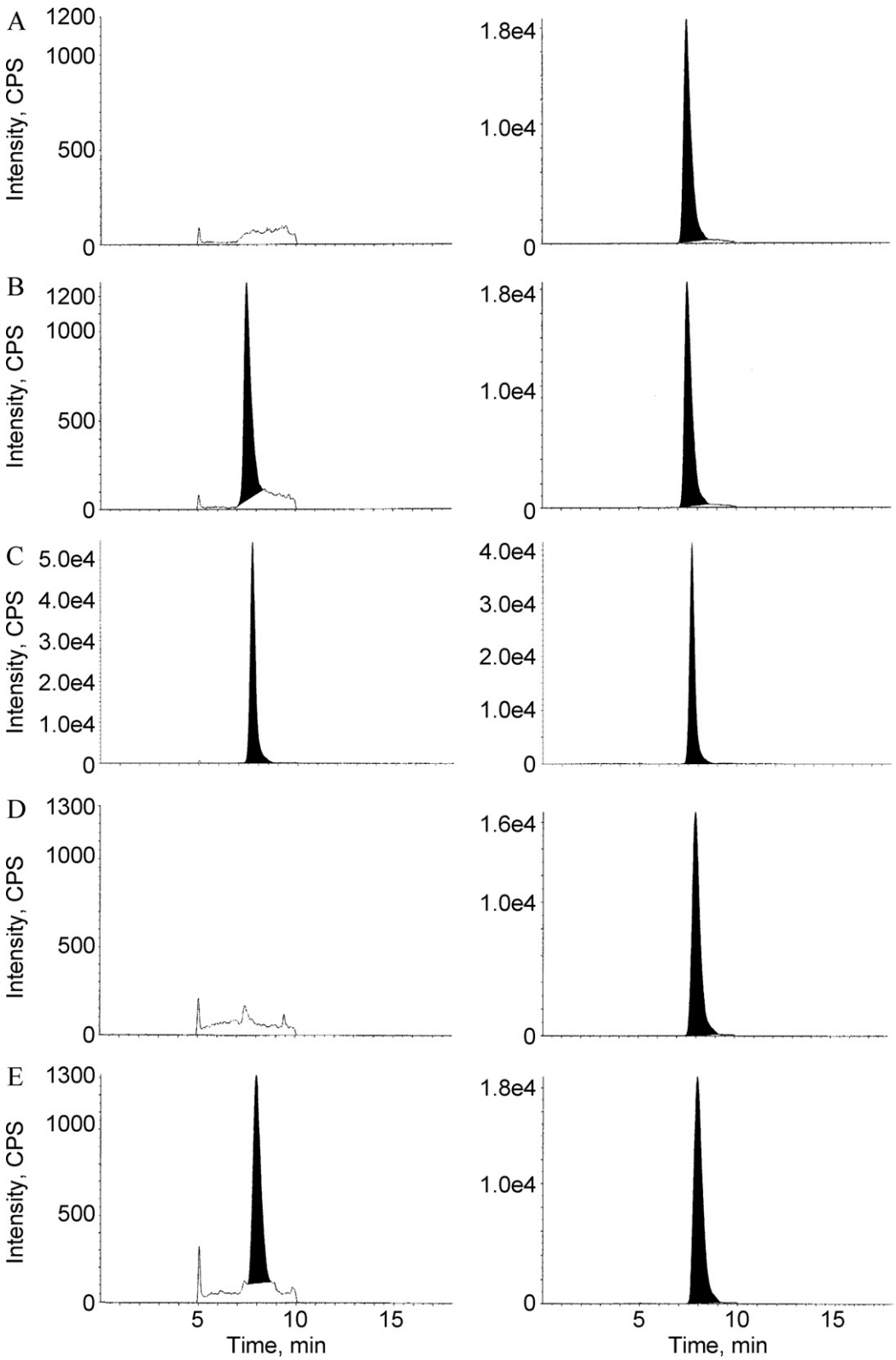

Fig. 3. Representative chromatograms of 5-thio-D-glucose and IS in rat plasma (A, single blank; B, 10 ng/mL LLOQ sample; C, 1-h post-dose sample following repeat oral doses of 5-thio-D-glucose) and human plasma (D, single blank; E, 10 ng/mL LLOQ sample). 5-Thio-D-glucose (left panel), IS (right panel).

human plasma was 67.2% and 67.7% with a CV of 3.6% and 3.4%, respectively.

3.2.6. Stability

The stability of 5-thio-D-glucose in rat and human plasma was assessed using QC samples at concentrations of 24 and 2400 ng/mL ($n = 3$ for each level) and was considered acceptable when the remaining percentage of the concentration was from 85% to 115% of the initial values.

The results for the stability of the plasma samples are shown in Table 4. In rat and human plasma, 5-thio-D-glucose was stable at room temperature for at least 4 h, at −20 °C and −70 °C for at

Table 3
Recoveries of 5-thio-D-glucose and IS from rat and human plasma.

	Nominal concentration (ng/mL)	Recovery (%)	SD	%CV
Rat plasma ($n=5$)				
5-Thio-D-glucose	24	62.6	2.5	4.0
	240	64.7	3.3	5.1
	2400	61.4	2.4	3.9
IS	200	67.2	2.4	3.6
Human plasma ($n=5$)				
5-Thio-D-glucose	24	61.0	1.3	2.1
	240	65.6	1.4	2.1
	2400	67.4	2.7	4.0
IS	200	67.7	2.3	3.4

Table 4
Stability of 5-thio-D-glucose in rat and human plasma.

	Storage conditions	Nominal concentration (ng/mL)	Remaining (%)
Rat plasma ($n=3$)	Freeze-thaw stability (6 cycles)	24	106.3
		2400	100.0
	Short-term stability (room temperature for 4 h)	24	98.6
		2400	110.9
	Long-term stability (−20 °C for 42 days)	24	105.9
		2400	102.7
	Long-term stability (−70 °C for 42 days)	24	107.7
		2400	102.3
	Post-preparative stability (5 °C for 72 h)	24	109.5
		2400	102.3
Human plasma ($n=3$)	Freeze-thaw stability (6 cycles)	24	99.6
		2400	97.8
	Short-term stability (room temperature for 4 h)	24	93.4
		2400	99.6
	Long-term stability (−20 °C for 43 days)	24	103.3
		2400	102.2
	Long-term stability (−70 °C for 43 days)	24	100.8
		2400	102.6
	Post-preparative stability (5 °C for 72 h)	24	102.1
		2400	99.6

least 42 days (for rat plasma) or 43 days (for human plasma), and throughout the course of six freeze/thaw cycles from −70 °C to room temperature. 5-Thio-D-glucose was also found to be stable in processed samples stored in the LC sample compartment at 5 °C for at least 72 h.

3.2.7. Dilution integrity

To investigate the ability to dilute plasma samples containing 5-thio-D-glucose, dilution QC samples at concentrations of 2400 and 10,000 ng/mL were diluted 10-fold and 100-fold with blank rat or human plasma and then analyzed in triplicate.

The precision and accuracy observed with the 10-fold and 100-fold dilutions were acceptable for both rat and human plasma, as shown in Table 5.

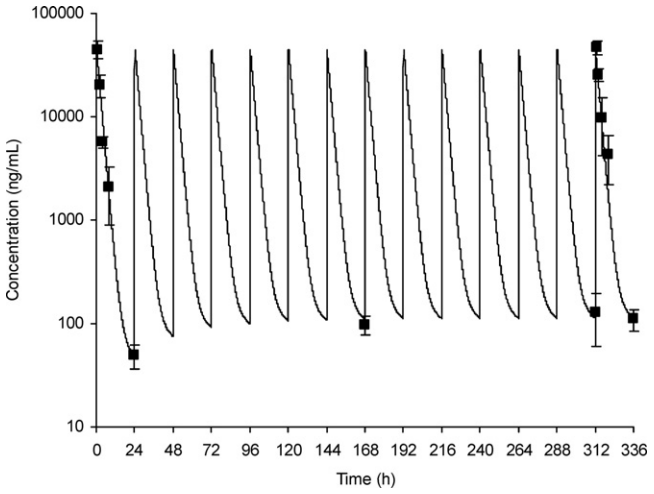

Fig. 4. Experimental values of plasma concentration (■) and WinNonlin modeling simulation (—) for 14 repeat oral doses of 5-thio-D-glucose (50 mg/kg/day).

3.3. Application of the method to a PK study

The LC–MS/MS method described above was applied successfully to PK studies of 5-thio-D-glucose in rats. All the QC samples were found to be within acceptable limits for precision and accuracy. The mean data for the 5-thio-D-glucose concentration after the first dose of 5-thio-D-glucose (6.25, 12.5, 25, or 50 mg/kg) are presented in Table 6. The plasma concentrations increased in an approximately dose-proportional manner.

The plasma concentration-time data for multiple oral of 5-thio-D-glucose doses over a 14-day treatment period were analyzed using WinNonlin software (Professional version 6.1; Pharsight Corporation, Mountain View, CA) using a two-compartment, first-order model. The model is shown in Eq. (1):

$$C_p = \frac{A \times Ka}{Ka - \alpha}\exp^{-\alpha \times t} + \frac{B \times Ka}{Ka - \beta}\exp^{-\beta \times t} - \left(\frac{A \times Ka}{Ka - \alpha} + \frac{B \times Ka}{Ka - \beta}\right)\exp^{-Ka \times t} \quad (1)$$

where C_p is the concentration of 5-thio-D-glucose in the plasma and α and β are rate constants for the distribution and elimination processes, respectively. The representative pharmacokinetic parameters (50 mg/kg) in Eq. (1) were estimated as follows: A, 48,600 (ng/mL); B, 80.1 (ng/mL); Ka, 18.0 (1/h); α, 0.42 (1/h); and β, 0.023 (1/h). T1/2α and T1/2β, the half-lives of the initial and terminal phases of disposition, were 1.6 h and 31 h, respectively. The plasma concentration curve simulated by equation 1 and the experimental plasma concentration values were well matched (Fig. 4).

The PK data for 5-thio-D-glucose in hamsters [11], mice [11], and rabbits [26] have already been reported, but these values were obtained using ^{35}S-labeled (hamster) and ^{3}H-labeled (mice and rabbits) 5-thio-D-glucose. The present PK data is the first to be reported for rats and to be obtained using non-radioisotope-labelled 5-thio-D-glucose. The data showed a long (31 h) terminal half-life and a good fit with the PK model equation. Since the rat is a useful experimental animal in pharmacological and toxicological investigations, this PK result is likely to be valuable for further 5-thio-D-glucose research.

Table 5
Influence of dilution with rat and human plasma.

	Nominal concentration (ng/mL)	Dilution factor	Mean of calculated value (ng/mL)	SD	%CV	%RE
Rat plasma ($n=3$)	2400	×10	2550	35.1	1.4	+6.3
	10,000	×100	10,500	416	4.0	+5.0
Human plasma ($n=3$)	2400	×10	2470	15.3	0.6	+2.9
	10,000	×100	10,100	100	1.0	+1.0

Table 6
Plasma concentrations of 5-thio-D-glucose in male rats given single oral administration of 5-thio-D-glucose.

Dose (mg/kg)		Plasma concentration (ng/mL) (n = 5)				
		1 h	2 h	4 h	8 h	24 h
6.25	Mean	5970	3000	980	210	6.74
	S.D.	773	520	119	65.6	6.16
12.5	Mean	11,200	4680	1540	566	16.4
	S.D.	907	879	651	328	5.90
25.0	Mean	22,600	9850	2730	1600	27.7
	S.D.	1540	1140	827	756	3.26
50.0	Mean	44,700	20,100	5670	2070	49.1
	S.D.	8750	5050	718	1170	12.4

4. Conclusion

An LC–MS/MS method for the quantification of 5-thio-D-glucose in rat and human plasma was developed and validated over a concentration range from 10 to 3000 ng/mL. This is the first quantitative method for 5-thio-D-glucose in biological matrices that does not require the use of radioisotope-labelled compounds. The method proved to be highly sensitive, selective, accurate, precise, rugged, and suitable for pharmacokinetic studies of 5-thio-D-glucose in rats and humans.

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