

Establishment and Validation of A Surrogate Derivative LC/MS/MS Method for the Determination of Glucose in Human Plasma with [¹³C₆]D-Glucose as a Tracer

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Overview

A Surrogate Derivative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method capable of quantifying D-Glucose by using [¹³C₆]D-glucose as a tracer in human plasma has been successfully validated.

In this method, 0.1 mL of plasma was used. The analytes were extracted from plasma using a protein precipitation method. The dried extracts were reconstituted with 50 μL of water, followed by the addition of 150 μL of derivatization reagent. The separation was performed on a reverse phase C18 column. Detection was achieved using an AB SCIEX API-3000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation of [¹³C₆]D-glucose was 0.1 mg/mL.

Introduction

For development of drugs to treat diseases like diabetes, it is important to monitor the levels of glucose or other monosaccharides in human plasma. In recent years, many analytical methods for quantitation of glucose have been developed for pharmacokinetic studies. Besides general analysis with UV, evaporative light-scattering detector, refractive-index, fluorometric detectors, and GC-MS, analysis with LC-MS/MS has become more popular. In order to improve the MS sensitivity, a negative APCI ion source with post column addition of special reagent has been applied. Here we report a sensitive and convenient method capable of quantifying D-glucose from 100 μL of human plasma at a lowest level of 0.1 mg/mL. In this method, a simple high yield derivatization is performed, resulting in a good chromatography and reliable ionization in the positive ion mode.

Experimental

An aliquot (100-μL) of plasma containing [¹³C₆]D-glucose, and the internal standard ([¹³C₆,D₅]D-glucose) was deproteinized by adding 400 μL of methanol, vortex-mixing for 3 min, and centrifugation at 4,000 rpm for 5 min. The supernatant (50 μL) was evaporated to dryness under nitrogen at 35 °C. The dried extracts were reconstituted with 50 μL nanopure water, and 150 μL of the derivatization reagent (a mixture of 0.385 g butyl-PABA, 0.625 g sodium cyanoborohydride, 0.375 mL acetic acid, and 4.63 mL methanol). The samples were then incubated at 37 °C for 2 hours, and diluted for about 80 fold with acetonitrile:water (15:85).

Structures

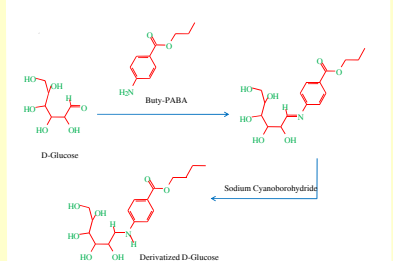


Figure 1. The D-Glucose derivatization flow-chart

LC-MS/MS Conditions

Liquid Chromatography:
 HPLC System Shimadzu LC-10AD (or LC-20AD)
 Analytical Column: Luna C18 column, 2.0 x 50 mm, 5 μm
 Mobile Phase A) 10 mM ammonium formate in water pH 3.2
 Mobile Phase B) Acetonitrile
 Gradient
 Flow rate: 0.6 mL/min
 Column Temperature: Ambient
 Injection Volume: 5 μL

Mass Spectrometry
 MS System: AB Sciex API-3000
 Condition: LC(+ESI-MS/MS (MRM)
 MRM Transition:
 Derivatized D-Glucose: 358 → 284
 Derivatized [¹³C₆]D-glucose: 364 → 290
 Derivatized [¹³C₆,D₅]D-glucose: 371 → 297

Results and Discussion

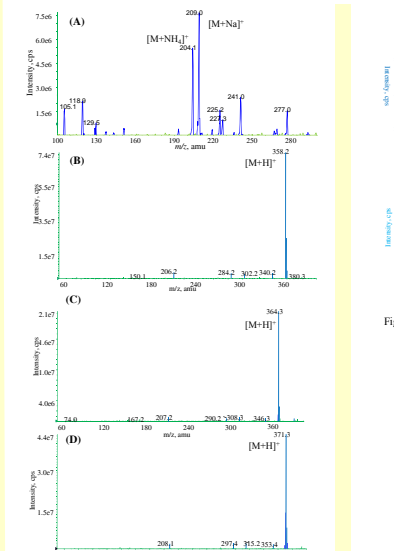


Figure 2. Full scan mass spectra of D-Glucose (A), derivatized D-Glucose (B), derivatized [¹³C₆]D-glucose (C), and derivatized [¹³C₆,D₅]D-glucose (D)

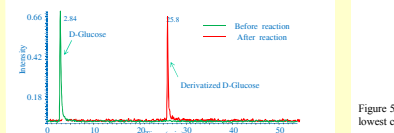


Figure 3. LC-RAM chromatograms of ¹⁴C D-Glucose before and after the derivative reaction.

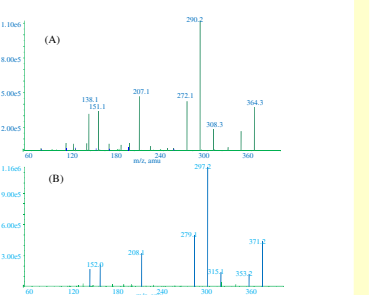


Figure 4. Product ion spectra of derivatized [¹³C₆]D-glucose (A), and derivatized [¹³C₆,D₅]D-glucose (B)

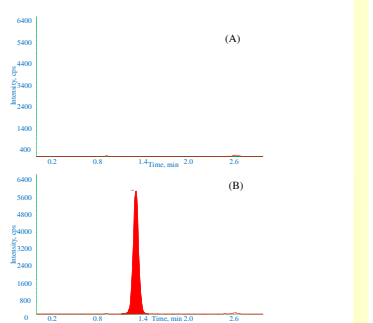


Figure 5. LC-MS/MS (MRM) ion chromatograms of blank plasma (A), and lowest calibration standard with 0.1 mg/mL (B)

Table I. Validation data summary of [¹³C₆]D-glucose in human plasma

Calibration Range				
0.1000 – 100.0000				
Correlation coefficient (R ²)				
0.9992 (n=10)				
Accuracy & Precision				
QC	Conc. (mg/mL)	Accuracy	Precision	
Inter-Run (n=3)	LLOQ	0.100	1.00	1.52
	Low	0.300	10.00	2.87
	Medium	2.00	2.00	2.42
Intra-Run (n=3)	Low	0.300	10.00	2.87
	High	4.00	2.50	3.00
Linearity				
R ² = 0.9992				
Accuracy				
83%				
Freeze-Thaw				
3 Cycles, -20°C				
-2.61 – 2.61				
Stability				
24 hrs, Room Temperature				
-2.61 – 2.61				
7 Days, Room Temperature				
-1.50 – 0.500				
Long Term Storage Stability				
-0.00 – 0.00				

In a couple of published methods, LC(-)APCI-MS/MS with post column addition of methanol containing CH₂Cl₂ was applied for measuring chloride adduct [M – H + Cl]⁻. In these methods, a long run time (~10 min) is applied to separate the analyte from the isomers such as fructose, sorbose, galactose, mannose, and mannitol. Matrix suppression is also very high which affects the method sensitivity and chloride-adduct stability.

The unique derivatization procedure employed in our method only quantitatively converts glucose to the derivative product, while the recovery of other monosaccharides is very low. In order to check the derivatization recovery, [¹⁴C]-Glucose with and without derivatization reaction was monitored by LC-RAM as shown in Figure 4. The derivatization yield was >95%.

The mass sensitivity of the derivative was also dramatically increased (Figure 5), so that the sample could be diluted almost 100-fold, significantly minimizing matrix effects. Excellent linearity was obtained with correlation coefficient (R²) ≥ 0.999 (Table I). The inter-day precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ were ≤3.08% and ≤2.50%, respectively. Three freeze/thaw cycles and ambient temperature storage QC samples for up to 24 hours prior to analysis, appeared to have little effect on the quantitation.

Conclusion

A simple surrogate derivative LC-MS/MS method was developed and validated for quantifying D-Glucose in human plasma. The lower limit of quantitation was 0.100 mg/mL using a plasma sample volume of 0.1 mL.