Analyzing the Efficacy of Different Gas Chromatography-Mass Spectrometry Methods in Determining Glucose Concentrations

Aaron J. Oates, Mohsin Rahim, Jamey Young
Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN

KEYWORDS. Glucose Cycling, Gas-Chromatography-Mass Spectrometry (GC-MS), Diabetes, total ion abundance screening

BRIEF. Measuring multiple glucose concentrations using a Gas Chromatography-Mass Spectrometry based technique.

ABSTRACT. High blood glucose levels have been linked to several chronic health issues, including Type II Diabetes. Recently, a stable isotope method was developed that determined the enzyme Glucose-6-phosphatase 2 (G6PC2) has a significant impact on the rate of glucose cycled in pancreatic mouse islets. Although G6PC2's function in humans is not yet clear, the previously described method must first be optimized before use in future human studies. Particularly, it is currently unclear whether the use of scan mode in Gas Chromatography-Mass Spectrometry (GC-MS) provided more accurate concentration results than Selective Ion Monitoring (SIM) mode. To determine this, we evaluated the relative effectiveness of a GC-MS based method in both modes when determining glucose concentrations from unlabeled glucose samples. Samples were derivatized into di-O-isopropylidene propionate and analyzed using similar specifications as previously described. We found scan mode exhibited a higher total ion abundance data than SIM. However, determined glucose concentrations from both modes remained within range of actual concentrations, indicating both modes' potential use.

INTRODUCTION.

Diabetes is the seventh leading cause of death in the United States, with approximately 1.7 million Americans diagnosed each year [1]. Type II Diabetes has been consistently linked to individuals with high fasting blood glucose (FBG) [2]. Independently, high blood glucose levels can lead to other chronic health issues, including heart and kidney disease [2]. Glucose levels are heavily dependent on the process of glucose cycling in cells. Glucose cycling is defined as the percentage glucose that is converted back from glucose-6-phosphate by pancreatic β-cells under hyperglycemic and euglycemic conditions [1,3]. Previously, a study by Wall *et al*. utilized a novel stable isotope technique to identify Glucose-6-phosphatase 2 (G6PC2) to have a significant role in the rate of glucose cycling in pancreatic mouse islets [3]. G6PC2 is currently understood to hydrolyze glucose-6-phosphate into glucose, which is then exported back into the bloodstream (Figure 1) [3,4].

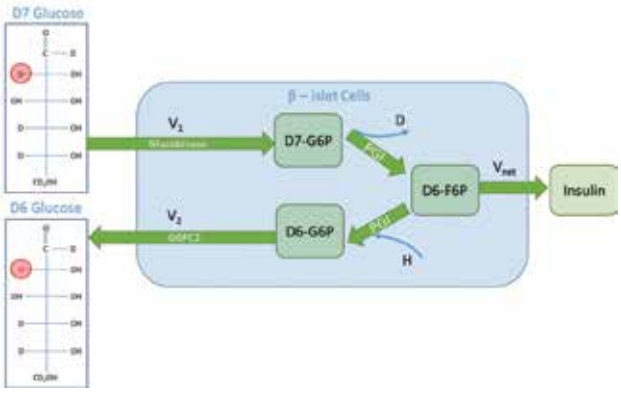


Figure 1. Diagram of isotopic glucose cycling in pancreatic β islets. Glucose is hydrolyzed into glucose-6-phosphate and fructose-6-phosphate before alternatively being converted back into glucose-6-phoshate and glucose by phosphoglucose isomerase (PGI) and glucose-6-phosphatase 2 (G6PC2) respectively [2]. During this process, the original deuterium atom in the second position is exchanged with a hydrogen atom gained from water [2].

However, the full enzymatic functions of G6PC2 in humans are not yet clear, and could have a large impact on glucose cycling rates in humans and on glucose stimulated insulin secretion (GSIS) [4]. Interestingly, genome wide association studies (GWAS) have previously indicated G6PC2 polymorphisms of the gene are associated with higher FBG levels and an increased risk of diabetes [5,6].

Preceding studies regarding glucose cycling have used radio-isotopic methodologies to measure the impact of G6PC2 in pancreatic islets. While most had suggested glucose cycling was elevated when G6PC2 was expressed, the full extent was unclear [7,8]. Unlike previous studies, the method reported by Wall *et al.* employed a stable isotope method to determine the metabolic rate of glucose cycling in mouse islets. As described, samples were measured using Gas Chromatography-Mass Spectrometry (GC-MS) in full scan mode with splitless parameters [3]. However, it is not yet clear whether full scan mode produces as accurate a total ion count when compared to the alternatively used Selective Ion Monitoring (SIM) mode. GC-MS analysis using SIM mode could be significantly more useful in determining glucose concentrations due to its ability to specifically scan for ions of interest, even in extremely low concentrations. This mode differs significantly from scan, which alternatively scans for all ions within an identified mass/charge (m/Z) value range. To contrast either method's use, we compared glucose concentrations and determined total ion abundance from both full scan and SIM mode, which allowed us to objectively investigate the previously described stable isotope technique.

MATERIALS AND METHODS.

Di-O-isopropylidene Propionate Derivatization.

Three sample pairs of both high glucose concentration (11 mmol/L) and low concentration (5.5 mmol/L) were created to use as comparison references. To serve as the internal standard, 20 μL of 5mmol/L [U-$^{13}C_6$; 1,2,3,4,5,6,6-$^2H_7$] glucose (from Cambridge Laboratory) was injected into 20 μL of each sample. A calibration curve was also established with four samples of 1.5, 3, 6 and 12 mmol/L. Samples were then derivatized to increase volatility using the protocol described in [9] to form the di-O-isopropylidene propionate derivative [3,9]. Derivatized glucose was then dissolved in 100 μL ethyl acetate for GC-MS analysis.

Gas Chromatography-Mass Spectrometry.

After derivatization, one microliter of each sample was injected into an Agilent Gas Chromatography System through a HP-5ms capillary column at 270°C. The vaporized sample was pushed through the column at a constant helium flow of 0.88 mL/min. Following a 5 min solvent delay, mass charge (m/Z) data for samples were analyzed in both full scan and SIM mode. In scan mode, all m/Z data was collected between a range of 300-320 m/Z values. Lower m/Z charges between 301-310 were quantified as the di-O-isopropylidene propionate derivative, while m/Z charges ranging from 311-317 were quantified as the [U-$^{13}C_6$; 1,2,3,4,5,6,6-$^2H_7$] internal standard. In SIM mode, each ion specified was measured 15 different times with an average dwell time of approximately 26 ms per ion, for a total retention time of 6600 ms. The same samples were run through the GC-MS in scan and SIM mode using a splitless liner, so that in both modes none of the sample was purged for dilution. Data was collected as a network Common Data Form (netCDF) file format and analyzed using a custom graphic user interface in Matlab (9). Using Eq.1 for the selected calibration samples, a linear relationship was established between sample ion abundance and sample glucose concentrations. We then used this linearity to compute the concentrations of the 5.5 mmol/L and 11 mmol/L samples.

$$\frac{[Sample\ concentration]}{[Internal\ Standard\ Concentration]} = m * \frac{[Sample\ Abundance]}{[Internal\ Standard\ Abundance]} + b \quad (1)$$

RESULTS.

Total Ion Abundance Screening.

In both full scan and SIM mode, the presence of oversaturation was measured visually at each sample point on custom graphic user interface on Matlab, as would be indicated by the lack of a Gaussian peak. Total ion abundance was available at each sample point, allowing us to confirm the ion count collected with each GC-MS mode.

Scan with Splitless.

Significant total ion abundance of di-O-isopropylidene propionate was reported in all one microliter samples measured using scan, with no evidence of oversaturation present. As mentioned, we used four 1.5, 3, 6, and 12 mmol/L samples to create the linear calibration curve ($y=1.11x+0.01$, $R^2=0.99865$).

SIM with Splitless.

Significant total ion abundance of di-O-isopropylidene propionate was reported in all one microliter samples measured in SIM, with no oversaturation evident. However, total ion abundance for each sample was reported to have significantly less total ion abundance than in scan. Calibration samples were plotted to illustrate the linear correlation used to determine sample concentrations ($y=1.2532x-0.1004$, $R^2=0.99367$).

Total Ion Abundance in Scan and SIM Mode.

After establishing calibration curves, we compared the total ion abundance reported between scan and SIM mode. Using our four calibration samples, we performed a chi-square analysis to determine whether the total ion abundances in SIM were significantly different from those reported in full scan mode. As noted earlier, total ion abundance in SIM was found to be approximately a magnitude of ten greater than scan in each reported sample, and chi-square analysis indicated total ion abundance in scan mode was significantly higher than in SIM mode ($\chi= 3.68*10_6$, $p<0.0001$) (Figure 2).

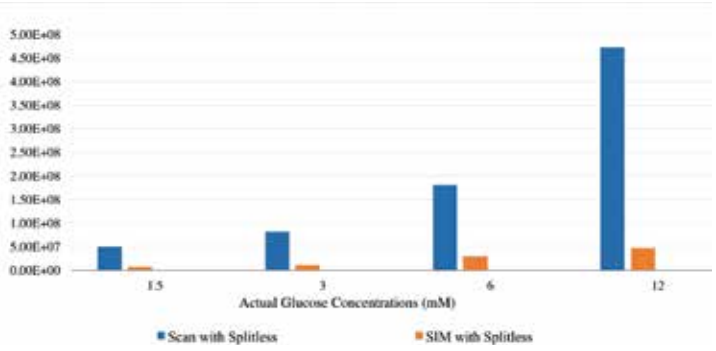


Figure 2. Chi-squared analysis performed to determine similarity between total ion abundances of full scan and SIM mode. Total ion abundance at each sample was found to be significantly different between full scan and SIM mode ($\chi=3.68*10^6$, $p<0.0001$).

Determined Glucose Concentrations between Scan and SIM.

Determined glucose concentrations from 5.5 and 11 mmol/L samples in scan and SIM mode were then compared. As described, glucose concentrations at each sample were determined based on our established concentration curve. While total ion abundances of scan and SIM mode were significantly different, determined glucose concentrations in both modes remained within comparable range, or within a 1.5 mM difference. (Figure 3).

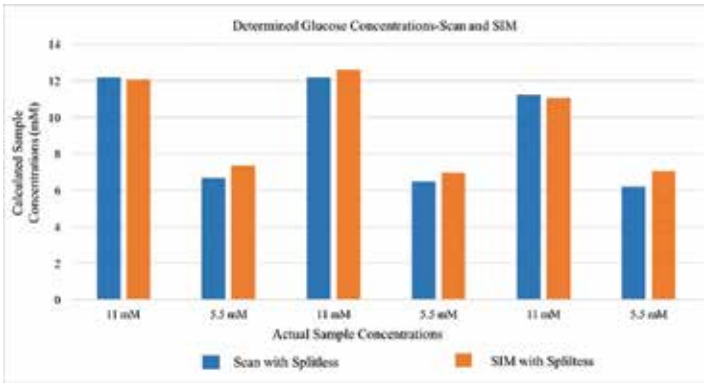


Figure 3. Determined glucose concentrations of 5.5 and 11 mmol/L samples based on total ion abundances collected by GC-MS in scan and SIM mode. In most samples, concentrations reported were within 1.5 mmol/L of their actual concentrations, indicating high accuracy of determined sample concentrations.

DISCUSSION.

We analyzed the accuracy of a stable isotope method using two different GC-MS modes. Our results indicate that scan mode with splitless liner produced a higher overall total ion abundance in all samples than SIM with splitless and determined glucose concentrations slightly more accurately than SIM. However, both GC-MS modes produced total ion abundances within the range of detection and oversaturation was not present in any samples. Importantly, in SIM mode the average dwell time for each ion was determined to be approximately 26 ms. This allowed for each ion within the peak to be counted at least 15 separate times, increasing overall ion count precision. Significantly lower ion abundances in SIM indicates established dwell time was not long enough to fully count all ions present. While dwell time could be increased, the total times cycled across the ion peak would inversely fall. Also, two other glucose derivatives (aldonitrile and methyloxime pentapropioate) not measured here have previously been used in the stable isotope method in conjunction with di-O-isopropylidene propionate, but only to specifically determine hydrogen placement [3]. We chose to focus solely on the di-O-isopropylidene propionate derivative as it is the only one critically needed to determine glucose cycling in pancreatic islets and preserves the entire glucose molecule. While only one derivative is truly necessary to determine rates of glucose cycling, future studies should focus on other derivatives to determine the efficacy of SIM mode. Sample glucose concentrations of 5.5 mmol/L and 11 mmol/L were chosen due to the normal range of FBG levels used to diagnose diabetes, with 5.5 mmol/L representing euglycemia and 11 mmol/L signifying hyperglycemia [2].

CONCLUSION.

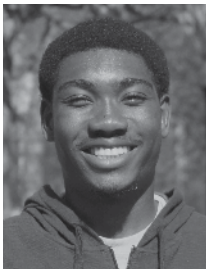
We demonstrated the importance of the selective ion monitoring mode of GC-MS to accurately measure glucose concentrations when using a stable isotope technique. Future human islet studies on glucose cycling will rely on a reproducible method of analyzing glucose concentrations, making it critical to understand and evaluate the previously used techniques. While our study found lower overall total ion abundance in SIM with splitless liner than in scan, it is important to note both modes eluted similar concentration amounts not significantly different from the actual value. Furthermore, we were able to examine the stable isotopic method overall using fixed glucose concentrations similar to levels typically found in human blood. Overall, our study was designed to help bridge the gap between former glucose cycling studies performed in mouse models to future research involving measuring the glucose cycling rates in human islets. Human islets express significant variability and recently, five unique GSIS pancreatic islet profiles have been reported [10]. Our work in this paper will help minimize any experimental variation that may cause erroneous results in already varying human islet samples. This work will help identify the underly-ing factors contributing to differences in pancreatic profiles and allow a correlation between individual's islet profiles and their rate of glucose cycling to be determined. Future investigation into the causes of high glucose concentrations in individuals is critical to our understanding of the underlying factors that contribute to several metabolic diseases.

ACKNOWLEDGEMENTS.

We would like to thank the Young Lab for providing access to materials and the Gas Chromatography-Mass Spectrometry machine. We would also like to thank research assistant Irina Trenary for her help throughout the research. We would also like to acknowledge the School for Science and Math at Vanderbilt for their support, and in particular Dr. Christopher Muller for providing guidance and a unique insight.

REFERENCES.

1. Centers for Disease Control and Prevention. *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States*, 2014. Atlanta, GA: U.S. Department of Health and Human Services, 2014, pp. 1-4.
2. National Institute of Diabetes and Digestive and Kidney Diseases. *Diagnoses of Diabetes and Prediabetes*, Bethesda, MD: National Diabetes Information Clearinghouse (NDIC), 2014, pp. 1-3.
3. M.L. Wall, *et al*, Novel Stable Isotope Analyses Demonstrate Significant Rates of Glucose Cycling in Mouse Pancreatic Islets. *Diabetes* 64, 2129-2137 (2015).
4. J.C. Hutton and R.M. O'Brien, Glucose-6-Phosphatase Catalytic Subunit Gene Family. *The Journal of Biological Chemistry* 284, 29241-29245 (2009).
5. N. Bouatia-Naji, *et al*, A Polymorphism Within the G6PC2 Gene is Associated with Fasting Plasma Glucose Levels. *Science* 320, 1085–1088 (2008).
6. W.M. Chen, *et al*, Variations in the G6PC2/ABCB11 Genomic Region are Associated with Fasting Glucose Levels. *Journal of Clinical Investigation* 118, 2620–2628 (2008).
7. A. Khan, *et al*, Evidence for the Presence of Glucose Cycling in Pancreatic Islets of ob/ob Mouse. *The Journal of Biological Chemistry* 264, 9732-9733 (1989).
8. J. Katz, P.A. Wells, R. Rognstad, Glucose Phosphorylation, Glucose-6-Phosphatase, and Recycling in Rat Hepatocytes. *The Journal of Biological Chemistry* 10, 253 (1978).
9. M.R. Antoniewicz, J.K. Kelleher, and G. Stephanopolous, Measuring Deuterium Enrichment of Glucose Hydrogen Atoms by Gas Chromatography/Mass Spectrometry. *Analytical Chemistry* 83, 3211-3216 (2011).
10. N.S. Kayton, *et al*, Human Islet Preparations Distributed for Research Exhibit a Variety of Insulin-Secretory Profiles. *American Journal of Physiology, Endocronilogy and Metabolism* 308, E592-E602 (2015).



Aaron J. Oates is a student at Martin Luther King Jr. Academic Magnet High School in Nashville, TN; he participated in the School for Science and Math at Vanderbilt.