Investigating a new isotope dilution liquid chromatography tandem mass spectrometry method for quantifying glucose in frozen human serum SRM 965c

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Background

- Blood sugar levels are impacted by diabetes, which puts individuals at risk for heart disease, kidney disease, nerve damage and other complications
- Accurately measuring glucose promotes proper treatment of hypoglycemia and hyperglycemia to prevent these complications
- To support measurement accuracy the community relies on higher order reference measurement procedures (RMP) approved by the Joint Committee for Traceability in Laboratory Medicine (JCTLM)
- Currently, NIST uses a JCTLM approved isotope dilution (ID) gas chromatography mass spectrometry (GC-MS) method for measuring glucose in serum, which provides SI traceability using SRM 917c D-Glucose as a calibrator
- GC-MS RMP has complicated, lengthy two-step derivatization process after an overnight equilibration, as well as an extensive use of harsh chemicals

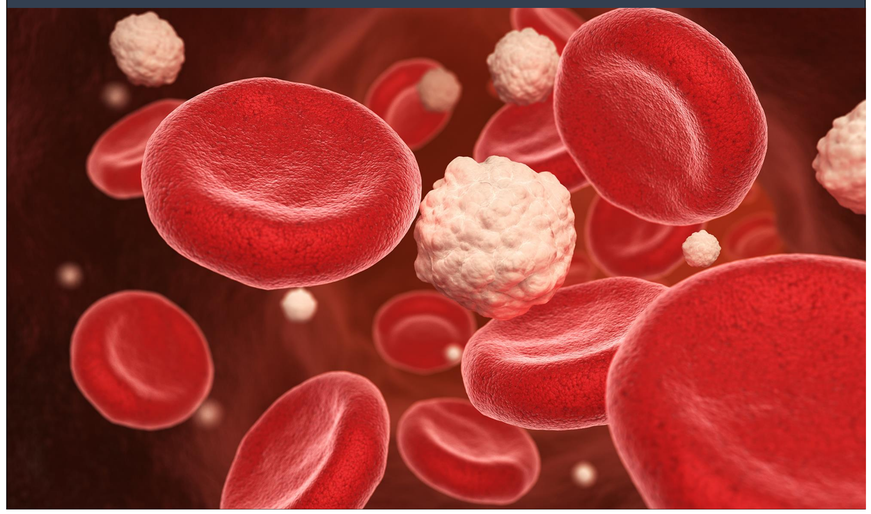
Methods

Two JCTLM approved ID-LC-MS/MS methods were assessed for their accuracy by comparing the results to the GC-MS derived certified values in SRM 965b Glucose in Frozen Human Serum

Methodology	GC-MS	LC-MS/MS Method 1	LC-MS/MS Method 2
Reference	Prendergast *et al.*, 2010	Chen *et al.*, 2012	Zhang *et al.*, 2016
Isotope Equilibration	Overnight	2 hours	1 hour
Derivatization	Multi-step	None	One-step
Deproteinization	Ethanol	Acetonitrile	Ethanol
Drying Step	Nitrogen (> 4 hours)	Nitrogen (> 4 hours)	None
Chromatography	Gradient	Isocratic	Gradient
Column	DB5-MS capillary (30 m)	Amino (2 mm x 250 mm)	C-18 (2 mm × 150 mm)
Precursor Ions	*m/z* 297, 303	*m/z* 225, 231 $[M+HCOO]^-$	m/z 511, 517 $[M+H]^+$
Method & Mode	SIM	MRM negative	MRM positive

UHPLC-HRMS/MS Method 3:
Sample processing was the same as Method 2, but instrumentation was a Vanquish UHPLC coupled to a Q Exactive orbitrap mass spectrometer and the gradient was modified

LC-MS/MS can be used for a faster accurate serum glucose analysis



Goal

Investigate alternative reference methods that utilize the strengths of liquid chromatography for measuring glucose in human serum

Results

LC-MS/MS Method 1
- The chromatography did not provide reliable retention times
- Changed method from isocratic to gradient to obtain consistent results
- Column length caused longer run times

LC-MS/MS Method 2
- One-step derivatization of glucose allows for shorter chromatography and use of C-18 column which typically produces sharper peaks
- Reproducible and accurate method using SRM 965b (**Figure 1**)

UHPLC-HRMS/MS Method 3:
- Reproducible and accurate using SRM 965b (**Figure 2**)
- Skyline was used to integrate selected peaks and to obtain a summed peak area for the following transitions (**Figure 3**):
  - **Glucose** m/z 511.2 → 373.16, 241.1, 217.09, 187.09, 175.09
  - **Labeled Glucose** m/z 517.2 → 375.17, 245.11, 219.1, 188.09, 175.09
- Using Method 2 and 3, structural analogues fructose, mannose, and glucose were very well chromatographically resolved

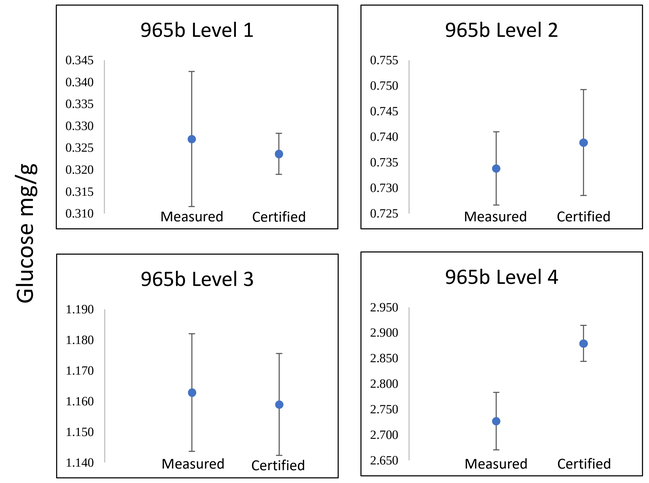


Figure 1. Comparison of SRM 965b certified values to measured values using Method 2

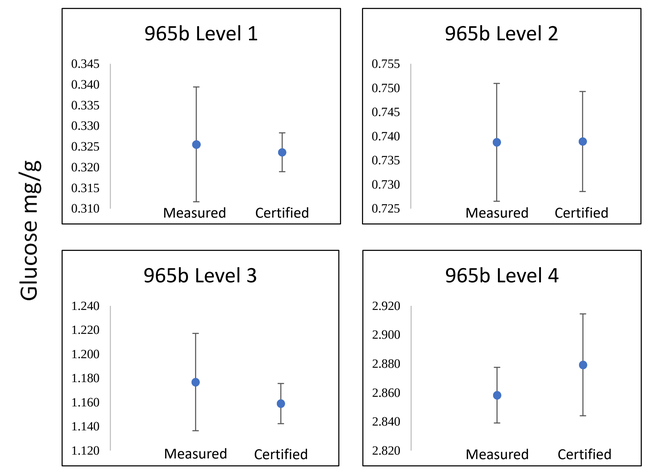


Figure 2. Comparison of SRM 965b certified values to measured values using Method 3

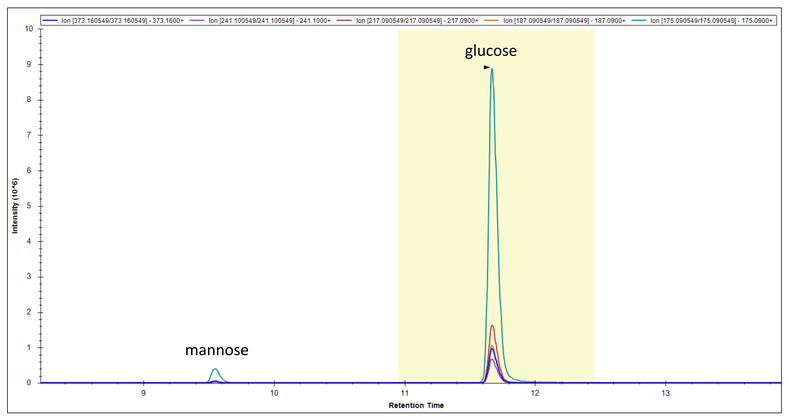


Figure 3. Representative chromatogram of unlabeled glucose transitions in SRM 965c using Method 3

Conclusions

- SRM 965c is currently being certified using method 2, which will save time and resources while providing a high accuracy targeted method for a critical measurement in the clinical community
- The high-resolution method was also successful and contains potential for additional separation of glucose from structural analogue, galactose

References:
1. Prendergast J.L., Sniegoski L.T. Welch M.J., Phinney K.W. (2010) Modifications to the NIST reference measurement procedure (RMP) for the determination of serum glucose by isotope dilution gas chromatography/mass spectrometry. Anal and Bioanal Chem, 397, 1179-1785.
2. Chen Y., Liu Q., Yong S., Lee T.K. (2012) High accuracy analysis of glucose in human serum by isotope dilution liquid chromatography-tandem mass spectrometry. Clincal Chimica Acta, 413, 808-813.
3. Zhang T., Zhang C., Zhao H., *et al*. (2016) Determination of serum glucose by isotope dilution liquid chromatography-tandem mass spectrometry: a candidate reference measurement procedure. Anal and Bioanal Chem, 407, 7403-7411.