High-Throughput Determination of Urea and Glucose in Serum Samples by High Performance Liquid Chromatography Tandem Triple Quadrupole Mass Spectrometry

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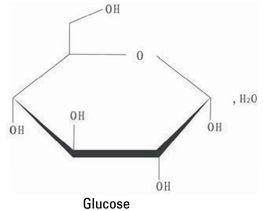
ASMS 2011
WP 685

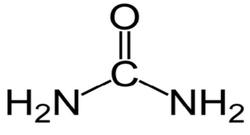
Agilent Technologies

Introduction

Traditional methods of quantifying urea and glucose in serum involve complicated sample preparation and result in low sensitivity. To date, simultaneous determination of these two bio-markers in serum samples at ng/ml (ppb) concentrations has not been reported. A high-throughput, ultra-sensitive method using normal-phase high performance liquid chromatography (HPLC) tandem triple quadrupole (QQQ) mass spectrometry was developed for the simultaneous determination of urea and glucose concentrations in serum. Quantification of the two bio-markers can be achieved within 3 minutes. This method demonstrates high sensitivity, excellent reproducibility and good linearity, providing a robust application for determination of urea and glucose suitable for clinical research use.



Glucose



Urea

Experimental

LC conditions

HPLC system: Agilent 1260 HPLC

Column: Zorbax RX-SIL (2.1 × 150 mm, 5 µm) / 30 °C

Injection volume: 10 µl; Flow rate: 0.4 mL/min

Mobile phase: A-H2O; B-ACN (A:B=1:9, *v/v*)

MS conditions

Mass system : Agilent 6460A

Ion source: ESI with Agilent Jet Stream; Nebulizer gas: N_2

Polarity: Negative/positive switching; Nebulizer pressure: 40.0 psi

Drying gas temperature: 350 °C; Drying gas flow rate: 5.0 L/min

Capillary HV : 3500 V; Sheath gas temperature: 400 °C

Sheath gas flow: 10.0 L/min; Nozzle voltage: 1500 V

MRM transition for urea is 61.1>44 (Quantifier) with 20 V collision energy (CE); and MRM transitions for glucose are 178.8>58.7 (Quantifier, CE = 10V) and 178.8>88.7 (Qualifier, CE = 2V)

Sample preparation

Human serum samples were used for this study, which were provided by Guangzhou Hospital (China). Samples were diluted 5000 times with water, filtered and then injected onto the LC-QQQ for analysis.

Results and Discussion

Chromatographic and MS optimization

Optimization was performed using normal-phase chromatographic separation due to the polar nature of urea and glucose. The Zorbax SIL-RAX column achieved rapid separation of urea and glucose within 3 min as shown in Figure 1. Mass parameters were optimized to ensure the best sensitivity. Only one product ion was produced for urea (MW = 61). For optimal results, Agilent's rapid positive/negative switching mode was used to allow for the simultaneous analysis of urea in positive mode and glucose in negative mode.

Due to excellent sensitivity, the serum sample treatment was very simple as compared to the literature. The 5000 times dilution greatly eliminated the interference of serum matrix, so that the external standard calibration method can ensure reliable quantification of urea and glucose based on the methodological validation above.

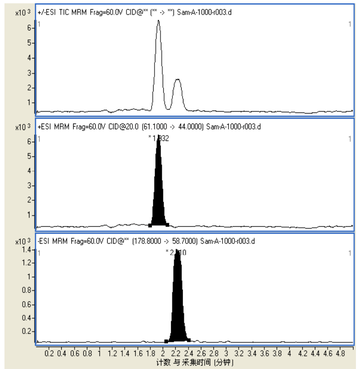


Figure 1. Simultaneous separation of urea and glucose by using Zorbax SIL-RAX column and with the positive/negative switching mode.

Repeatability validation

This established quantitative method demonstrates good inter-day reproducibility (RSD=5.1% and n=4 for urea; RSD=3.3% and n=4 for glucose) of serum samples. Figures 2 and 3 show good reproducibility of glucose at 100ng/ml and urea at 30 ng/ml.

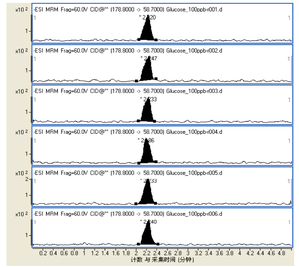


Figure 2. Consecutive six analysis of glucose at 100ng/ml

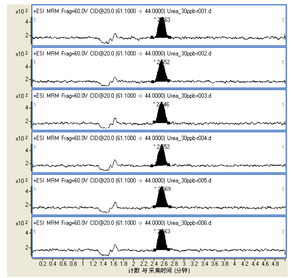


Figure 3. Consecutive six analysis of urea at 30ng/ml

Results and Discussion

Sensitivity of glucose and urea

The established method demonstrated excellent sensitivity (LOQ=40 ng/ml for urea; and LOQ=60 ng/ml for glucose) as shown in Figure 4-5.

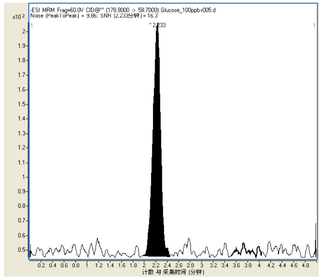


Figure 4. Detection of 100ng/ml glucose (S/N=16)

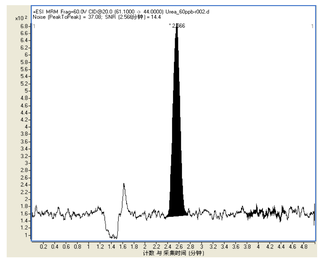


Figure 5. Detection of 60ng/ml urea (S/N=13)

Linearity test

This established quantitative method demonstrates good linearity (R^2>0.995 for two analytes) with satisfactory accuracy for as shown as below,

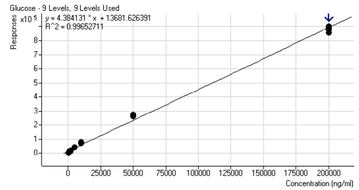


Figure 6. Glucose with the linear range from ng/ml to 216 µg/ml, (R^2 = 0.997)

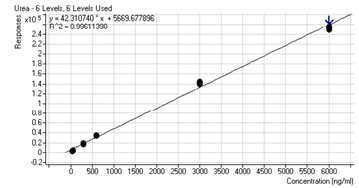


Figure 7. Urea with the linear range from 0.03 ng/ml to 6.0 µg/ml (R^2 = 0.996)

Conclusions

The established method provides a robust technique for simultaneously determining both urea and glucose with simple sample preparation, high-throughput and excellent sensitivity.