Candidate reference measurement procedure for determination of urea in serum by liquid chromatography-tandem mass spectrometry

Liqiao Han, Xiaoting Huang, Lu Zhang, Qiaoxuan Zhang, Jianbing Wang, Haibiao Lin, Jun Yan, Junhua Zhuang, Xianzhang Huang*

Department of Laboratory Medicine, Guangdong provincial hospital of Chinese medicine, Guangzhou 510120, Guangdong, China

BACKGROUND

- Urea is a nitrogenous product of protein metabolism, produced mainly in the liver during the urea cycle from the conversion of ammonia, and excreted principally via the kidneys. serum urea measured as blood urea nitrogen (BUN) is an important indicator of liver and kidney function, and is one of the most frequently detected markers in the clinical laboratory.
- Reference measurement procedures (RMPs) provide accurate and traceable results against which routine methods may be calibrated and evaluated. This study describes a candidate RMP (cRMP) that utilizes LC-MS/MS accurately measuring serum urea. This method was well characterized, with good accuracy, precision, definitive uncertainty, and comparability with the recognized reference method of the JCTLM.

RESULTS AND CONCLUSIONS

1. Procedure of the LC-MS/MS method

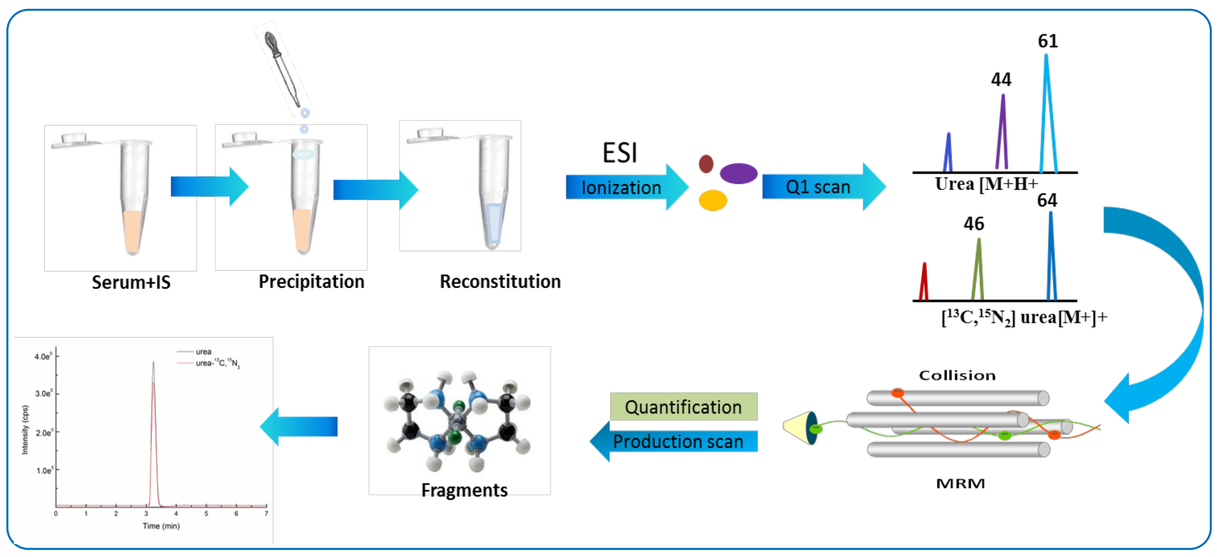

Fig 1. Schematic of the LC-MS/MS method of urea in serum

2. Results

2.1 Chemical structure, ion spectrum, and chromatogram for urea

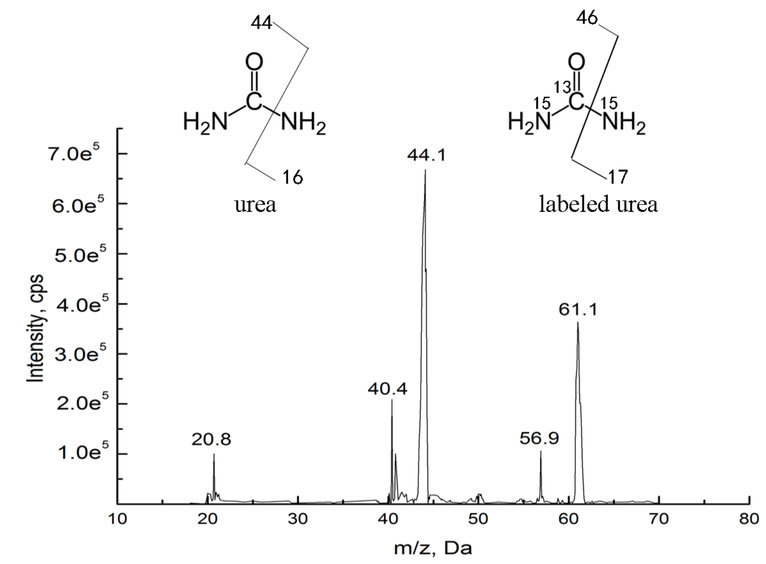

Fig 2. Product-ion spectra of urea by LC-MS/MS. The $[M+H]^+$ ion of *m/z* 61 was isolated, collision-activated, to produce the product-ion spectrum.

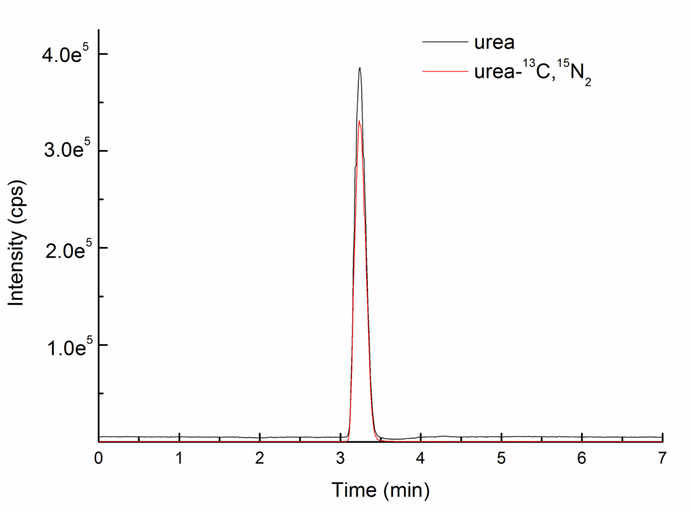

Fig 3. Selected ion chromatograms by LC-MS/MS for urea and urea isotope (urea-$^{13}C,^{15}N_2$) from a serum sample, at urea concentration of 10.4 mmol/L.

2.2 Intra-method and inter-laboratory comparisons

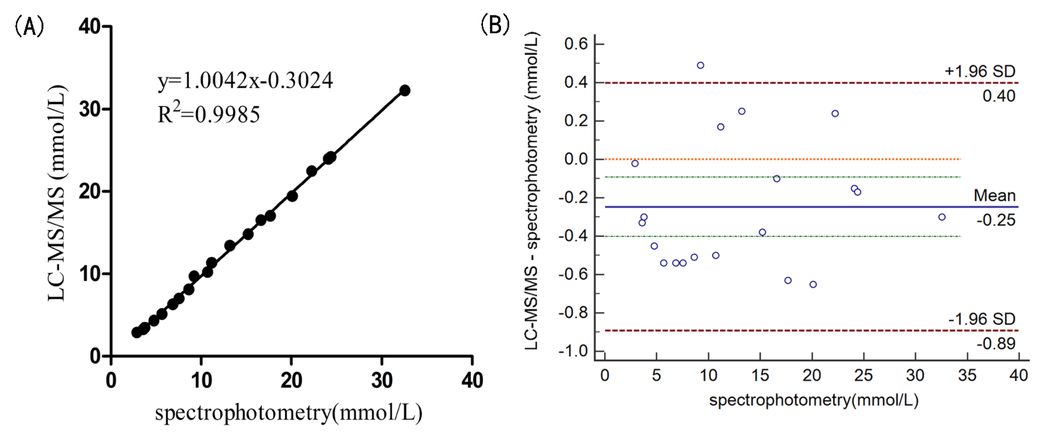

Fig 4. Comparisons of the LC-MS/MS method with the JCTLM reference method (spectrophotometry) for the measurement of serum urea, 2.87 mmol/L to 32.26 mmol/L; n = 20. (A) Correlation plot. (B) Bland-Altman plot.

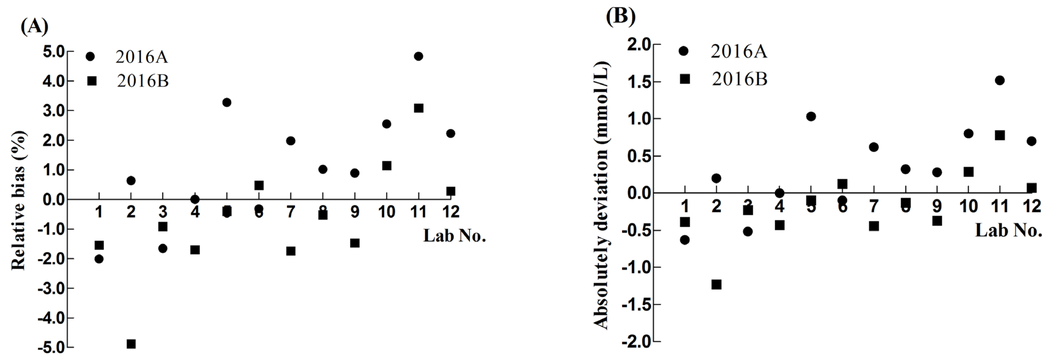

Fig 5. Inter-laboratory comparisons of the LC-MS/MS method with ID/GC/MS and enzyme-coupled spectrophotometry for detection of urea in RELA 2016A and 2016B. (A) Relative bias. (B) Absolute deviation.

2.3 Application in clinical laboratory by investigation of the accuracy of routine systems

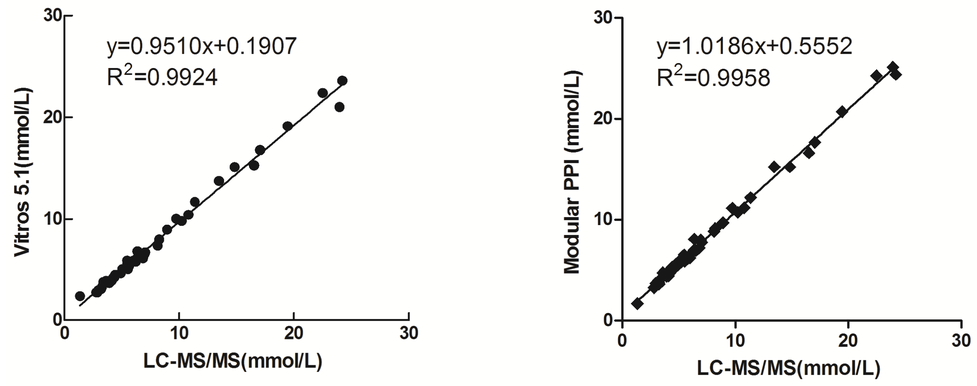

Fig 6. Linear regression of LC-MS/MS method with (A) Vitros 5.1 system and (B) Roche Modular PPI systems. Serum urea was between 1.3 and 24.2 mmol/L.

3. Conclusions

The LC-MS/MS method for the detection of urea in serum was well characterized and comparability with JCTLM recognized reference method. Protein precipitation was the only pretreatment required for the isolation of urea from serum, as derivatization was not necessary. This makes pre-processing samples very simple. In addition, this procedure can provide a base of accuracy for establishing the traceability of routine clinical systems.