Highly sensitive method for quantification of iohexol

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BACKGROUND

Iohexol is used for accurate determination of the GFR in CKD patients. In order to minimize the iohexol dosage required for the GFR determination in humans, the development of a sensitive quantification method is essential. Therefore, the objective of our preclinical study was to establish and validate a simple and robust mass-spectrometry method using the multiple reaction monitoring mode for iohexol quantification.

METHODS

In order to test whether a significantly decreased amount of iohexol is sufficient for reliable quantification, a LC-ESI-MS approach was assessed. We analyzed the kinetic of iohexol in rats after application of different amounts of iohexol (15 mg-150 µg/rat). Blood sampling was conducted at four time points, at 15, 30, 60, and 90 min after iohexol injection. The analyte (iohexol) and the internal standard (iothalamic acid) were separated from serum proteins using centrifugal filtration device with a cut-off of 3 kDa. The chromatographic separation was achieved on an analytical Zorbax SB C18 column. The detection and quantification were performed on a high capacity trap mass spectrometer using positive ion ESI in the multiple reaction monitoring (MRM) mode. Furthermore, using real-time polymerase chain reaction (RTPCR) the effect of iohexol on early regulated gene expression in thyroid and renal cortex was tested to determine a threshold of physiological active iohexol concentrations.

RESULTS

The chemical structure of iohexol is given in Figure 1A and the internal standard iothalamic acid in Figure 2A. Since molecular structures of iothalamic acid and iohexol are highly comparable, iothalamic acid was used an internal standard in this study. The MRM transmission parameters were optimised for both iohexol and internal standard. The quantification was performed using MRM mode. The MRM transmissions for iohexol were m/z 821.8 -> 803.8 (Figure 1 B-D) and for internal standard m/z 614.7 -> 583.6 (Figure 2 B-D).

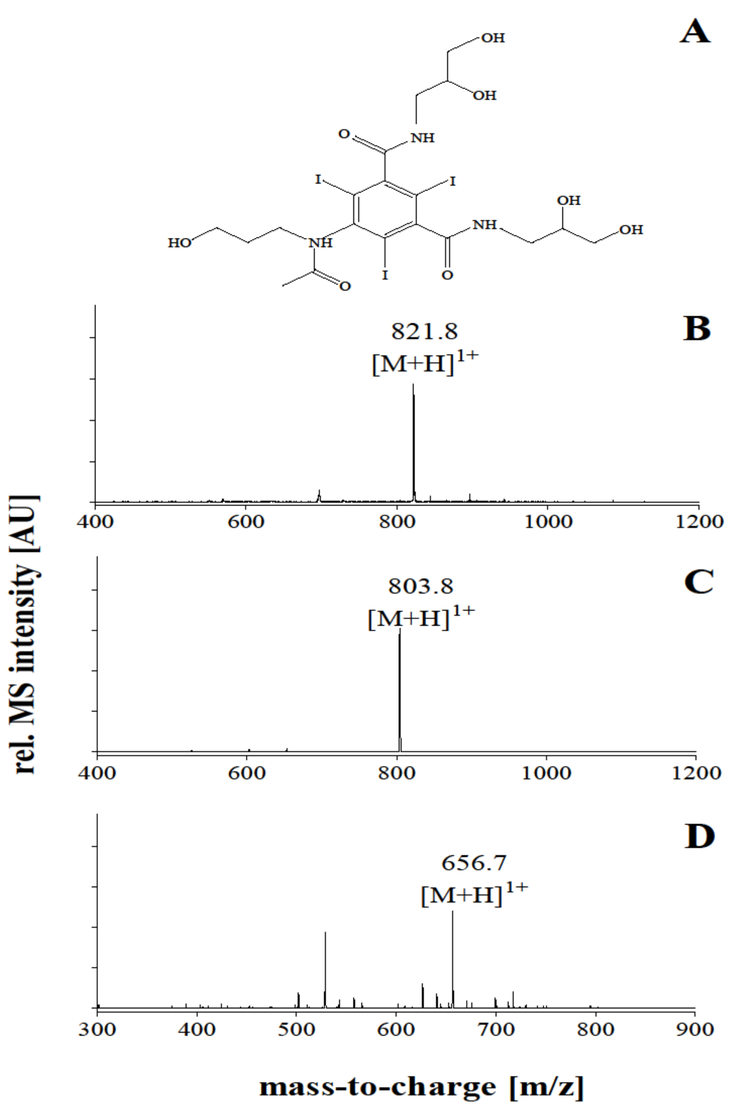

Figure 1: (**A**) Molecular structure of iohexol; (**B**) Representative positive ESI MS mass spectrum of iohexol; (**C**) Representative positive ESI MS2 mass spectrum of iohexol (parent ion: m/z 821.8); (**D**) Representative positive ESI MS3 mass spectrum of iohexol (transmission: m/z 821.8 -> 803.8)

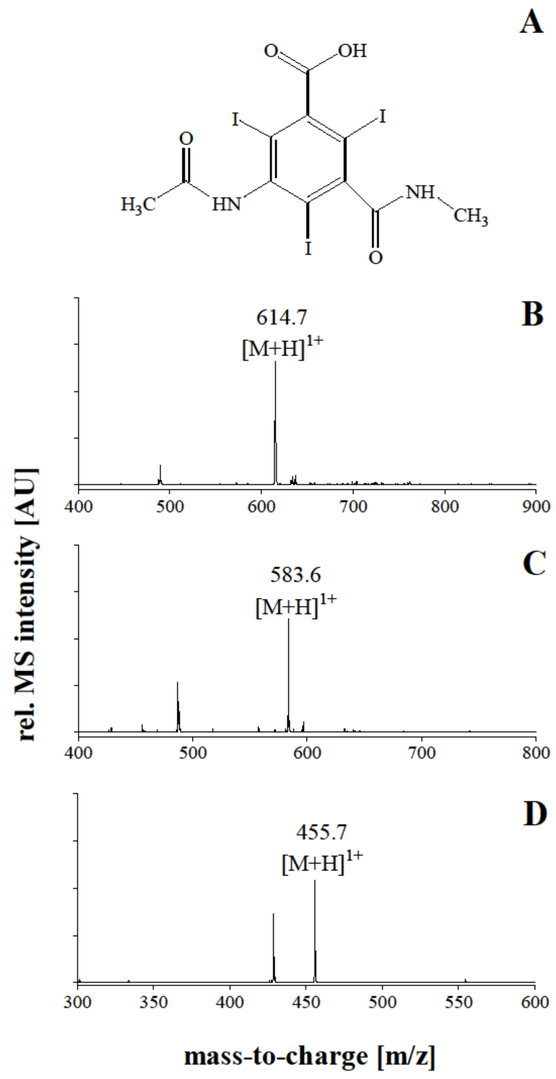

Figure 2: (**A**) The molecular structure of iothalamic acid, used as internal standard (**B**) Representative positive ESI MS mass spectrum of iothalamic acid (internal standard); (**C**) Representative positive ESI MS2 mass spectrum of iothalamic acid (internal standard) (parent ion: m/z 614.7); (**D**) Representative positive ESI MS3 mass spectrum of iothalamic acid (internal standard) (transmission: m/z 614.7 -> 583.6).

The schematic preparation protocol for all serum samples is given in Figure 3A. The method was validated by analysing an appropriately prepared calibration, and quality control standards in three consecutive batches to demonstrate acceptable intra- and inter-batch accuracy and precision over the desired range of concentration. Furthermore the method was validated in terms of selectivity and recovery. The calibration curve was determined in the range of 50 pg-40 ng for iohexol. The linear calibration curve of iohexol was calculated as y=0.004197x + 0.01197 (Figure 3B). The calibration curve showed a coefficient of determination (R2) of 0.998. The linear regression of the calibration curve was weighted by reciprocal y.

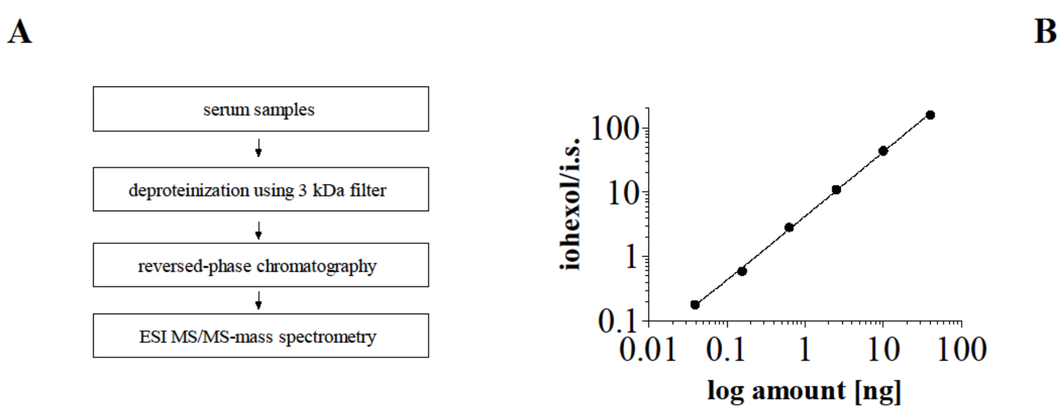

Figure 3: (**A**) Overview of the preparation steps for the iohexol quantification; (**B**) Representative extracted ion chromatograms of iohexol (m/z range: 656.7 ± 0.2 Da) and iothalamic acid (m/z range: 455.7 ± 0.2 Da); (**C**) Linear responses in iohexol/iothalamic acid area ratio versus iohexol amount in samples

CONCLUSIONS

This mass-spectrometric based method has been proved to be sensitive, selective and suitable for the quantification of iohexol in serum. Due to high sensitivity of this novel method the iohexol application dose as well as the sampling time in the clinical routine could be reduced in the future in order to further minimize side effects in humans.

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Disclosure Statement of Financial Interest: I, The authors have a financial interest or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this presentation

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