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CONFERENCE

American Society for Mass Spectrometry

TITLE (QPS 2010-002)

LC-MS/MS Method for Simultaneous Determination of L-Arginine (ARG), L-Citrulline (CIT), and Asymmetric Dimethylarginine (ADMA) in Human Plasma

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ABSTRACT

Introduction

The important regulator in cardiovascular system, nitric oxide (NO), is synthesized under physiological conditions through the oxidation of ARG by the nitric oxide synthase (NOS) with CIT as by-product. One of the methylated arginines, ADMA, is the known potent inhibitor of NOS, while symmetric dimethylargine (SDMA) is inactive. Although quantification of ARG/CIT/ADMA in human plasma is clinically important, it has been challenging (endogenous, difficult to retain under reversed phase LC conditions) to simultaneously analyze them. Most of the reported LC-MS/MS methods involve pre-column derivatization using o-phthalaldehyde (OPA) to increase sensitivity and retention on reversed-phase columns. Here we present a reliable LC-MS/MS method for the simultaneous quantitation of ARG, CIT and ADME in human plasma without need of derivatization.

Methods

50µL of samples were mixed with 50µL of stable isotope labeled internal standard (IS) working solution first, then protein-crashed with 900µL of pre-chilled MeOH:FA/100:0.1(v:v). 8µL of supernatant was injected onto LC-MS/MS. An Atlantis HILIC Silica (3µm, 2.1mmx50mm, Waters) column was used to chromatographically separate ARG, CIT and ADMA as well as other endogenous components with gradient mobile phases of Water:FA/100:0.5(v:v) and ACN:FA/100:0.5 (v:v). The MS/MS detection of the analytes and internal standards was performed on a Sciex API-4000 tandem mass spectrometer under positive Turbo Ion Spray ionization mode. An ADMA specific fragment was chosen to differentiate it from SDMA. The analytical run time was ~7 min.

Preliminary Data

Phosphate buffered saline (pH7.4) with bovine serum albumin (1mg albumin/mL buffer) was used as surrogate matrix to prepare calibration standards and quality control (QC) samples at 4 concentration levels. One pooled human plasma with measured endogenous ARG/CIT/ADMA level also served as QC. We demonstrated the surrogate matrix is comparable to the human plasma for ARG/CIT/ADMA. Each background-corrected analyte/IS peak area ratio in plasma samples was within 7% different from the peak area ratio in surrogate matrix samples when 25000/12500/500ng/mL of ARG/CIT/ADMA and certain amount of IS were spiked into plasma and urrogate matrix, respectively. The 3-in-1 assay was validated over linear calibration ranges of 1-50µg/mL for ARG, 0.5-20µg/mL for CIT and 20-1000 ng/mL for ADMA. Protein precipitation was used to prepare samples with extraction efficiencies of 98.0%, 97.4% and 98.1% for ARG/CIT/ADMA, evaluated using their stable isotope labeled IS. The impact of matrix effect on the ionization was minimized. Assay precision and accuracy was evaluated through 3 consecutive validation runs on five QC samples (n=6) spanning the assay range for each analyte. The intra-day precision was ≤5.5%/4.0%/5.6%CV for ARG/CIT/ADMA, respectively. Inter-day precision (per daily mean values) was measured as ≤5.5%CV for all 3 analytes. The intra-day accuracy was -7.9 to 8.4% RE and inter-day accuracy of -5.7 to 4.3%RE for all 3 analytes. The method was sensitive, selective and simple. Stability of the analytes was evaluated in both the surrogate matrix and in human

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plasma (endogenous level) under conditions of 6 hours bench-top exposure at room temperature and 5 freeze/thaw cycles etc. All 3 analytes were found stable. 10-fold dilution integrity was demonstrated by diluting plasma samples with surrogate matrix. The reinjection reproducibility was good for the processed samples after storage at 4^{o}C for 100 hours.

Novel Aspect
A simple and reliable LC-MS/MS method for ARG/CIT/ADMA in human plasma without need of derivatization.