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Absolute quantification of amino acids in plasma using stable isotope dilution LC-MS/MS – application to a reference material for metabolomics

Mark S. Lowenthal; Gauthier Eppe; Elizabeth A. McGaw; Nathan G. Dodder; Karen W. Phinney

National Institute of Standards and Technology, Gaithersburg , MD

Novel Aspect: This is the first metabolomics SRM produced and first serum or plasma-based amino acid SRM produced from NIST.

Introduction

The human blood plasma metabolome consists of a complex, changing matrix of small molecules, currently poorly characterized and unquantified. Included in this classification are free amino acids which represent a significant part of the metabolome and could provide useful diagnostics to physiological health. A major focus at NIST is developing reference methods and Standard Reference Materials (SRMs) for characterizing and quantifying analytes of clinical interest, thereby providing the scientific community with the means to demonstrate traceability to a higher-order reference standard. We have quantified concentrations of 29 amino acids and amino acid derivatives from human blood plasma using selective ion monitoring and stable isotope dilution mass spectrometry in an ongoing effort to characterize NIST SRM 1950 (Metabolites in Human Plasma).

Methods

A human plasma pool (NIST SRM 1950) was obtained from a representative mix of healthy male and female donors. Samples were depleted of high molecular weight analytes using a standard technique of methanol precipitation. The remaining fraction, consisting of enriched metabolites, was quantified using a definitive method of isotope dilution involving isotopically-labeled standard peptides spiked into both the sample and calibrants. Unique internal standards were used for quantification of every measurand of interest. Multiple-reaction monitoring of 29 amino acids (and derivatives) was performed using a mixed-mode analytical column (ion-exclusion and reversed-phase) with liquid chromatographic separation on an Agilent 1200 LC system coupled in-line with ABI 5000 triple quadrupole mass spectrometer equipped with a standard microflow source.

Preliminary results

A definitive method is being developed to accurately quantify amino acid concentrations using stable isotope dilution and multiple-reaction monitoring of liquid chromatographically-separated analytes. The plasma samples have been prepared from a donor pool with no associated gravimetry measurement, thus an orthogonal measurement procedure (GCxGC-MS) will be used to validate the results of LC-MS/MS analyses prior to certification of the SRM. These results will be combined with statistical validation and later defined as certified values for NIST SRM 1950. Sampling includes aliquots in triplicate from four unique ampoules over the SRM lot analyzed on consecutive days for every measurand. Estimated native levels of amino acids in human blood plasma range from less than 1 µg/g for aspartic acid to greater than 30 µg/g for glutamine. Preliminary experiments estimate the precision as the coefficients of variation of the measurands ranging from 0.4% to 2.3%. Expanded uncertainties (Type A and B uncertainties) of the measurement precision range from 2.9% to 5.5% among amino acids. Due to the complexity of the sample matrix, we require quantitative agreement between two discrete fragmentation transitions for each measured ion. This well-characterized ID LC-MS/MS method for quantifying amino acids in a complex matrix will serve as a qualified reference measurement procedure to be used for characterization of analytical methods and to serve as a standard for higher order measurement traceability. Development of a certified reference material for amino acid analysis provides the scientific community essential metrological traceability to routine analyses, establishing a common, stable and accurate baseline for instrument calibration, and thus linking exploratory and clinical analyses to an established higher-order standard.

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Submitting Author:

MARK LOWENTHAL
National Institute of Standards and Technology
100 Bureau Drive
Stop 8392
Gaithersburg , MD 20899
UNITED STATES
301.768.6005
301.977.0685
mark.lowenthal@nist.gov

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