Quantitative amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ reagents. Do we have a new gold standard?

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Results

Two built-in amino acids used as internal controls for the labeling reaction were detected with this assay (Fig. 1). Amino acid quantitation was obtained using 6-point external calibration with stable isotope dilution. There were 45 amino acid calibration curves generated with each batch of analysis. Four amino acids (sulfocysteine, argininosuccinic anhydrides, formiminoglutamic acid, and glycoproteins) were not detected, and estimated without an external calibration curve. For these amino acids accurate quantitation requires further analysis.

Discussion

Amino acid or a group of amino acids leads to disorders generally referred to as inherited disorders of amino acid metabolism. Accurately quantifying amino acids in biological fluids (plasma, urine, or cerebrospinal fluid) is essential for the diagnosis and follow up of aminoacidopathies, as well as useful for the nutritional assessment of patients with non-metabolic conditions. Amino acid analysis is conventionally performed on an ion-exchange chromatographic (IEX) based amino acid analyzer, which provides excellent separation and reproducibility with minimal sample preparation. The IEC method has several disadvantages: long run time, large sample volume, and lack of analyte specificity due to interfering substances. To address our large clinical load and improve specificity, we have optimized the aTRAQ™ method (AB SCIEX) with ion-pairing reverse-phase liquid chromatography and tandem mass spectrometry (IP-LC-MS/MS), and transferred the assay in our clinical lab.

Method

Samples were labeled with aTRAQ™ reagents prior to instrument analysis. Anserine and tandem mass spectrometry (IP-LC-MS/MS), and transferred the assay in our clinical lab.

Materials & Methods

Reagents

- Amino acid reagents were purchased from Sigma-Aldrich.
- Amino acid standard solutions were prepared in house by ARUP Reagent Lab.
- aTRAQ™ Internal standards were from AB SCIEX.
- mTRAQ® Reagent D2 was from AB SCIEX.

Sample preparation

- 25 uL of plasma or CSF and 0.1 mg creatinine equivalents were used for the analysis.
- SSA solution was used for protein precipitation.
- After mixing with a borate buffer, 10 uL sample was transferred to a second plate for labeling reaction with (TRAQ™) Reagent D2 at room temperature for 30 minutes.
- 15 uL of the labeled solution was added to stop the labeling reaction with incubation at room temperature for 15 minutes. An aliquot of unlabeled sample and aTRAQ™ Internal standards were added before the instrument analysis.

Table 1

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<th>Age-dependent reference intervals in plasma.</th>
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Figure 1. Extracted ion traces for the 49 amino acids monitored (x axis: minutes, y axis: intensity).

Figure 2. Deming regression model comparing results by IEC (x axis) to results by IP-LC-MS/MS (115 plasma samples).

Acknowledgement

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