Quantification of 22 plasma amino acids combining derivatization and ion-pair LC-MS/MS

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Abstract
Time efficient and comprehensive quantification of amino acids continues to be a challenge. We developed a sensitive and precise method for quantitative analysis of amino acids from very small plasma and serum volumes. Ion-pair chromatography of amino acid butyl esters proved to provide an optimal combination of selectivity, sensitivity and robustness. 10_L of plasma or serum are added to precipitation reagent containing stable isotope standards. After protein precipitation, the supernatants is dried and incubated with 3N butanolic HCl for improving chromatographic separation and ionization efficiency. Amino acid butyl esters are separated using ion-pair (heptafluorobutyric acid) reversed-phase chromatography coupled to triple quadrupole mass spectrometry. The established method enables quantitative analysis of 22 amino acids, all 20 proteinogenic amino acids, ornithine and citrulline. Cysteine is measured as cystine. The combination of precipitation, derivatization and chromatographic separation effectively avoids ion suppression and coelution. Simultaneous with quantification, analyte identity is verified in each sample using qualifier ions. The micro-method is very sensitive and accurate. The intraassay precision for the analysis of plasma was 2.6-10.1%. Absolute accuracy as determined by comparison of external reference samples was 82-117.7%. Excellent linearity of detection response was demonstrated for all compounds in the range representative for clinical samples from infants and adults. Lower limits of quantification were in the range of 1_mol/L for all analytes. In conclusion, the method is ideally suited for cost-effective high-throughput analysis of large numbers of samples in clinical studies and metabolomics research.

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