INTRODUCTION

For several decades, amino acid analysis has been used in the study of a number of physiological processes. A correlation with the metabolic disturbances and secondary amines. Excess reagent reacts with the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. The 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reaction with water to form aminoisopropylamine (AMQ). Subsequently, AMQ can react with excess AQC reagent to form a bis derivative. Both of these side products do not interfere with the identification of any of the amino acids. The derivatives are stable for days, permitting batch-wise processing. The standard chromatogram shown in Fig. 2 is evaluated for its suitability based on peak identification and reliability of quantification. Identification is based on the retention time and the reproducibility of retention time is summarized in Table 1. Interrun variability of retention time is much less than the retention difference between adjacent peaks. Identification is therefore, not ambiguous. Reliable quantification is dependent on reproducibility, sensitivity and linearity. Inter-run quantitative variability averages less than 10% with internal standard as shown in Table 1. The limit of detection for the method has been found to be 0.5 µmol/L (Fig. 3). The limit of quantification is determined in the linearity experiments. Each individual amino acid exhibits a linear response from 1 µmol/L to 10 mM/L with a R² of >0.995.

RESULTS AND DISCUSSION

The MassTrak AAA Solution is utilized for the analysis of physiological amino acids in serum and plasma. Plasma is deproteinized with an equal volume of 10% sulfosalicylic acid prior to derivatization. Urine samples do not require deproteinization. For derivatization of plasma, 10 µL of supernatant, 20 µL of borate buffer, and 20 µL of reagent are mixed. For analysis of urine samples, 50 µL of urine is deproteinized with 50% sulfosalicylic acid (prior to derivatization. The MassTrak AAA Solution is compatible with several deproteinization procedures, including 100 µL of plasma or 50 nL of urine.

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As an example, the results are shown for Phe in Figure 6. For a complete mixture of the 42 amino acids linearity has been determined from 1 µmol/L to 500 µmol/L with a R² of greater than 0.995. These ranges exceed the commonly observed levels of the amino acids in physiological samples.

METHODS

DERIVATIZATION

The MassTrak AAA Solution is utilized for the analysis of physiological amino acids in serum and plasma. Plasma is deproteinized with an equal volume of 10% sulfosalicylic acid prior to derivatization. Urine samples do not require deproteinization. For derivatization of plasma, 10 µL of supernatant, 20 µL of borate buffer, and 20 µL of reagent are mixed. For analysis of urine samples, 50 µL of urine is deproteinized with 50% sulfosalicylic acid (prior to derivatization. The MassTrak AAA Solution is compatible with several deproteinization procedures, including 100 µL of plasma or 50 nL of urine.

SAMPLE PREPARATION

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CONCLUSION

The MassTrak AAA Solution provides a complete overview approach to the analysis of physiological amino acids for research use only.

- Stable derivatives are formed for both primary and secondary amino acids.
- The chromatographic separation provides unambiguous identification of the amino acids.
- Reliability of quantification is better than 2% CV.
- The demonstrated linearity exceeds the levels that are commonly observed in physiological samples.
- Analysis requires low microliter volumes of samples.
- Chromatographic method is compatible with electrospray MS detection.
- The MassTrak AAA Solution is a robust and reliable tool for the quantitative analysis of physiological amino acids for research use only.

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Figure 1. Reaction of AQC reagent with amino acids.

Figure 2. Chromatogram of physiological amino acids (250 µmol/L) with MassTrak AAA Solution. The amino acids include glutamine (Gln), asparagine (Asn), alanine (Ala), arginine (Arg), etc. (Asp, Glu), and taurine (Tau) as well as those found in the AminoAcids and Bases amino acids and nucleotides. Normal (Ref) is used as the control standard.

Figure 3. Limit of detection of the MassTrak AAA Solution. The limit of detection is 6.67 µmol/L. Region selected to show details.

Table 1. Interrun precision and accuracy. Residual Amino Acids Depicted, 100 µmol/L. Multiple derivatizations, duplicate injections, 5 runs for each concentration.

Figure 4. Calibration curve for Phe (5, 25, 100, 500, 1000 µmol/L).

Figure 5. Chromatogram of a pooled human plasma sample.

Table 2. Analysis of physiological samples

Figure 6. Overlay chromatogram of derivatized human urine sample and MassTrak AAA Standard (250 µmol/L). There is no difference in retention time between the sample and the standard.