Fast and Easy Physiological Amino Acids Analysis in 15 Minutes

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Introduction
Due to the complex chemical composition of urine and serum samples, the analysis of physiological amino acids has often been difficult. Sample preparation remains a challenge and is perhaps the most time-consuming part of most published methodologies. In addition, traditional chromatographic separation of amino acids requires either a dedicated instrument or HPLC column, and no traditional assay provides both good selectivity and resolution of amino acids.

The patent-pending EZ:faast® kit from Phenomenex provides a low-cost, simple sample preparation procedure that results in fast analysis times and reliable, reproducible results. Interfering protein components in the urine or serum sample are easily separated. This simple sample clean-up and easy-to-follow derivatization procedure occurs in under 7 minutes. Since all necessary reagents for this rugged method are included in the kit, derivatized amino acids are quantitatively analysed via a gas chromatograph (GC) equipped with FID or NPD in less than 8 minutes. The total time for sample preparation and GC analysis is 15 minutes.

Instrumentation
An Agilent Technologies 6890 gas chromatograph equipped with a split/splitless injector and FID was used for the analysis of derivatized amino acids prepared by the EZ:faast® method.

Experimental Conditions
Sample preparation: Either a urine or a serum sample is first passed through a solid-phase extraction sorbent tip followed by a derivatization procedure and a liquid/liquid extraction step. Derivatized amino acids in the organic phase are then analysed by a GC equipped with FID or NPD.

Chromatographic conditions: EZ:faast® GC conditions: Initial oven temperature of 110 °C with 32 °C/min ramps to the final temperature of 320 °C. Inlet temperature of 250 °C under constant helium flow of 1.5 mL/min and the detector temperature of 320 °C is used for the analysis of derivatized amino acids.


Chromatogram for Hitachi amino acid analyser was reproduced from Hitachi literature.

Results
The advantages of the EZ:faast® technology lie in three main areas: easy sample clean-up, derivatization and fast analysis. Sample clean-up occurs with patent-pending SPE pipette tips that allow a user to rapidly extract amino acids from complex physiological fluids. Derivatization then occurs through the addition of two reagents at ambient temperature and the resulting organic phase is analysed via a GC equipped with FID or NPD. A comparative chromatographic analysis of amino acids is shown in Figure 1.

Firstly, the EZ:faast® technique requires no dedicated amino acid analyser or a dedicated HPLC column specific for amino acids. Secondly, EZ:faast® offers extremely fast analysis times and consequently high sample throughput as compared to other popular methods that require at least 50 to 75 minutes for completion. Thirdly, more than 33 different aliphatic and aromatic amino acids can be analysed in a single run. Finally, the resolution of the derivatized amino acids in the EZ:Faast® method is better than the other three popular methods shown.

In summary, the EZ:faast® kit provides a very simple sample preparation procedure and a fast assay method for amino acids with significant advantages over all published technologies. Over 50 aliphatic and aromatic amino acids as well as dipeptides may be identified without a dedicated HPLC column, instrument or an analyser. Furthermore, the EZ:faast® kit can also be used to analyse amino acid content in food and beverage, fermentation and nutritional products.
Sample preparation remains a challenge and is perhaps the most time-consuming part of most published methodologies. In addition, traditional chromatographic separation of amino acids requires either a dedicated instrument or HPLC column, and no traditional assay provides both good selectivity and resolution of amino acids.

Figure 1: Comparative chromatograms of EZ:faast® and other technologies for amino acid analysis.

(a) Ez:faast® – 8 min run time; (b) Dionex® BioLC – 52 min run time; (c) Hitachi® L-800 – 75 min run time; (d) Waters® Pico-Tag™ – 66 min run time.