Gas chromatography to determine amino acids in serum

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Amino acid analysis forms the basis for much of what is known about protein function and nutrition. The diverse chemistry of amino acids makes fast, accurate quantification of all amino acids challenging. Moreover, analyses of amino acids in physiological samples are further complicated by the increased number of compounds that must be separated. Gas chromatographic techniques have played an important role in amino acid analysis, offering advantages in resolution, sensitivity, speed, and cost. However, the development of satisfactory gas chromatographic procedures has been hindered as amino acids are not sufficiently volatile to permit direct analysis and must thus be converted into volatile derivatives prior to gas chromatography.

The EZ:Faast® gas chromatographic kit (Phenomenex) for amino acid analysis was promoted as a new method for the rapid clean–up, derivatisation and analysis of more than 33 amino acids and related compounds from complex mixtures of physiological fluids. The method takes 15 minutes, including the time for sample preparation. In our laboratory, amino acids have been analysed routinely using an ion exchange high–performance liquid chromatography system with *O*–phthaldialdehyde post–column derivatisation and fluorometric detection for various purposes. Excluding sample preparation, it takes 70 minutes to separate all amino acids using a dedicated HPLC column specific for amino acids. To explore the EZ:Faast® gas chromatographic kit, method validation was conducted in our laboratory.

A mixed standard solution in replicates of six and horse serum samples in re plicates of five were extracted and derivatised following the instructions and using the reagents supplied with the EZ:faast® Amino Acid Analysis Kit for calculation of accuracy and precision of the method, respectively. The common horse serum samples were repeated in 26 batches from which the inter–assay variation was derived. A tryptophan spike sample (tryptophan standard was added to the serum samples) in replicates of five was prepared as described above to calculate tryptophan recovery rate. Derivatised amino acids were separated on a Zebron–AA column, specifically developed for amino acid analysis (Phenomenex), which was connected to a gas chromatography analyser (GC–17A, Shimadzu Corporation, Japan) fitted with a flame ionization detector. High purity helium was used as a carrier gas at a flow rate of 1.5 ml/min. The initial column temperature of 110^{o}C, was increased at 32^{o}C/min to 320^{o}C and held for 1 minute. The injector and detector temperatures were 250^{o}C and 320^{o}C, respectively.

The accuracy and precision in our study were <9% for all amino acids measured (Table 1). The tryptophan recovery was 93%. The inter–assay coefficient of variation was less than 20% (Table 1), which is generally regarded as acceptable, but this will depend on analyte concentration. The advantages of the EZ:faast® technology are easy sample clean–up and derivatisation and fast, cost–effective analysis. The disadvantages of the system are that arginine, methyl histidine, citrulline and taurine can't be analysed.

Table 1 Accuracy, precision and inter–assay variation of amino acids measured using the EZ:faast® Amino Acid Analysis Kit.

Parameter	n	Leucine	Isoleucine	Phenylalanine	Tyrosine	Tryptophan
Accuracy (%)	6	–5.01	–1.04	0.79	–5.35	–8.50
Precision (% CV)	5	2.63	1.07	2.34	6.07	8.52
Inter–assay variation (% CV)	26	15.7	17.1	18.2	19.8	19.6