LC-ESI-MS/MS methodology for determination of amino acids

The aTRAQ Kit for Amino Acid Analysis of Physiological Fluids was purchased from Sciex (Framingham, MA, USA). It consisted of amine-modifying labeling aTRAQ reagent Δ8, aTRAQ internal standard set of amino acids labeled with the aTRAQ reagent Δ0, 10 % sulfosalicylic acid, borate buffer of pH 8.5, 1.2 % hydroxylamine and mobile phase modifiers – formic acid and heptafluorobutyric acid. HPLC gradient grade methanol was purchased from J.T. Baker (Center Valley, PA, USA). Deionized water obtained from Millipore Simplicity UV water purification system (Waters Corporation, Milford, MA, USA) was used.

The following protocol was used for preparation of both serum and urine samples. An aliquot of 40 µl of the sample was added to 10 µl of 10% sulfosalicylic acid in order to precipitate proteins. After mixing and centrifugation (10 000 x g for 2 min) the supernatant was mixed with 40 µl of borate buffer. Next an aliquot of 10 µl the obtained solution was labeled with aTRAQ reagent solution (aTRAQ reagent Δ8), mixed and centrifuged. After 30 min of incubation at room temperature the labeling reaction was stopped by addition of 5 µl 1.2% hydroxylamine solution and the sample was incubated at room temperature for 15 min. In the next step 32 µl of the internal standard solution was added to the sample. After mixing and centrifugation the sample was evaporated in a vacuum concentrator for 15 min in order to reduce volume to about 20 µl. Then the residue was diluted with 20 µl of water. Each determined amino acid had its corresponding internal standard (the same amino acid labeled with the aTRAQ reagent Δ0). Two non-proteinogenic amino acids (norleucine and norvaline) were used to evaluate the labeling efficiency and recovery.

The determination of free amino acid levels was conducted using an HPLC instrument 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) combined with a 4000 QTRAP mass spectrometer (Sciex, Framingham, MA, USA) with an electrospray ionization source. The chromatographic separation was achieved on Sciex C18 column (5 µm, 4.6 mm x 150

mm) maintained at 50 °C with a flow rate of 800 µL/min. A mobile phase gradient of eluent A (0.1% formic acid and 0.01% heptafluorobutyric acid in water) and eluent B (0.1% formic acid and 0.01% heptafluorobutyric acid in methanol) was applied. A gradient profile was the following: from 2% to 40% of B from 0 till 6 min, maintained at 40% of B for 4 min, then increased to 90% of B till 11 min and held at 90% of B for 1 min. After 12 min the gradient decreased to 2% of B. From 13 to 18 min the mobile phase composition was unaltered. The injection volume was set at 2 µl. The ion source settings were: curtain gas, 20 psig; ion spray voltage, 4500 V; source temperature, 600 °C; ion source gas 1, 60 psig and ion source gas 2, 50 psig. The mass spectrometer operated in positive ionization mode with the following parameters: entrance potential, 10 V; declustering potential, 30 V and collision cell exit potential, 5 V. Collision energy of 30 eV was applied with the exception of cystathionine, cysteine, homocysteine, argininosuccinic acid, hydroxylysine, lysine and ornithine (50 V). The list of measured MRM transitions was contained in Table S1. Scheduled multiple reaction monitoring mode was used with nitrogen as a collision gas. A system suitability test was conducted before each batch of the samples (analysis of a standard mixture) to warm up the LC-MS/MS system and check the inter-day performance of the system. Data acquisition and processing were performed using the Analyst 1.5 software (Sciex, Framingham, MA, USA).

Table S1. MRM transitions for each amino acid and its corresponding internal standard.

Amino acid	MRM transition (Q1 > Q3)			
	Analyte		Internal standard	
	Q1	Q3	Q1	Q3
1-methylhistidine	318.2	121.1	310.2	113.1
3-methylhistidine	318.2	121.1	310.2	113.1
alanine	238.2	121.1	230.2	113.1
anserine	389.2	121.1	381.2	113.1

arginine	323.2	121.1	315.2	113.1
argininosuccinic acid	439.2	121.1	431.2	113.1
asparagine	281.2	121.1	273.2	113.1
aspartic acid	282.1	121.1	274.1	113.1
carnosine	375.2	121.1	367.2	113.1
citrulline	324.2	121.1	316.2	113.1
cystathionine	519.3	121.1	503.3	113.1
cystine	537.2	121.1	521.2	113.1
ethanolamine	210.2	121.1	202.2	113.1
glutamic acid	296.2	121.1	288.2	113.1
glutamine	295.2	121.1	287.2	113.1
glycine	224.1	121.1	216.1	113.1
histidine	304.2	121.1	296.2	113.1
homocitrulline	338.2	121.1	330.2	113.1
homocystine	565.3	121.1	549.3	113.1
hydroxyproline	280.1	121.1	272.1	113.1
isoleucine	280.2	121.1	272.2	113.1
leucine	280.2	121.1	272.2	113.1
lysine	443.3	121.1	427.3	113.1
methionine	298.2	121.1	290.2	113.1
ornithine	429.3	121.1	413.3	113.1
phenylalanine	314.2	121.1	306.2	113.1
phosphoethanolamine	290.1	121.1	282.1	113.1
phosphoserine	334.1	121.1	326.1	113.1
proline	264.2	121.1	256.2	113.1
sarcosine	238.2	121.1	230.2	113.1
serine	254.2	121.1	246.2	113.1
taurine	274.1	121.1	266.1	113.1
threonine	268.2	121.1	260.2	113.1
tryptophan	353.2	121.1	345.2	113.1
tyrosine	330.2	121.1	322.2	113.1
valine	266.2	121.1	258.2	113.1
α-aminoadipic acid	310.2	121.1	302.2	113.1
α-amino-n-butyric	252.2	121.1	244.2	113.1
β-alanine	238.2	121.1	230.2	113.1

β-aminoisobutyric	252.2	121.1	244.2	113.1
γ-amino-n-butyric	252.2	121.1	244.2	113.1
δ-hydroxylysine	459.3	121.1	443.3	113.1