

A Novel LC-MS/MS Quantification Method for Amino Acids in Human Plasma, Including Alloisoleucine, without Ion Pairing or Derivatization.

Aurora JAFFUEL¹, Alban HUTEAU¹, 1. SHIMADZU France, Noisiel, FRANCE.

Overview

We here present a new method for the quantification of 47 amino acids, including the separation of leucine, isoleucine and alloisoleucine (specific marker for MSUD). Additional amino acids may be added upon request. Total cost per sample is reduced from 13€ to 2€ compare to current methods. Certified plasma controls showed good accuracies and repeatability.

1. Introduction

Amino acids are routinely assayed to diagnose inherited metabolism disorders. As they are highly polar compounds, they are hardly retained onto reverse phase columns. Derivatization or addition of ion pairing reagent in the mobile phase is required. For easier and rugged analysis of amino acids, there was a need for a new solution not using pre-mentioned reagents. Recently, a new LC-MS/MS method was developed for the simultaneous high sensitive quantification of amino acids, using a mixed-mode column and typical volatile mobile phase suitable for LC-MS/MS (1). However a separate injection was still needed for the quantification of alloisoleucine. Alloisoleucine is of much interest for newborn screening as it is currently the most specific and most sensitive diagnostic marker for all forms of maple syrup urine disease (MSUD). We here present a new analytical method enabling the quantification 47 amino acids, in a unique analytical run, including the separation and specific quantification of leucine, isoleucine and alloisoleucine. Sample preparation is very simple. Plasma is precipitated and supernatant is directly injected. All amino acids were separated in 25 minutes. Particular attention was paid to separate isobaric amino acids chromatographically or by using specific MRM transitions. Plasma calibrators as well certified plasma controls showed good repeatability and excellent accuracy values.

2. Materials and Methods

The new method was developed using LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation) coupled to the Nexera X2 high performance liquid chromatography (Shimadzu Corporation). The separation was performed using Intrada Amino Acid 150mm column (Imtakt Company). An acetonitrile, tetrahydrofuran and ammonium formate buffer with 0.3% of formic acid was used as mobile phase A, and an acetonitrile and ammonium formate buffer mix was used as mobile phase B, in gradient mode. Injection volume was 1µL, reducing the matrix effect. Certified plasma samples from Recipe were used as calibrators and certified plasma samples from MCA Laboratory were used as quality control samples (QC). The sample preparation consisted of a protein precipitation followed by a centrifugation.



Figure 1. Sample workflow overview for the quantification of amino acids in human plasma.

3. Results

3-1. Typical chromatogram

A total of 47 amino acids, including alloisoleucine, were simultaneously quantified in an analysis time of 25 min. MRM transitions and source parameters were optimized using LabSolutions Connect software (Shimadzu Corporation) and specific MRM were chosen for isobars when hardly resolved by chromatography. Typical chromatogram in human plasma is showed below.

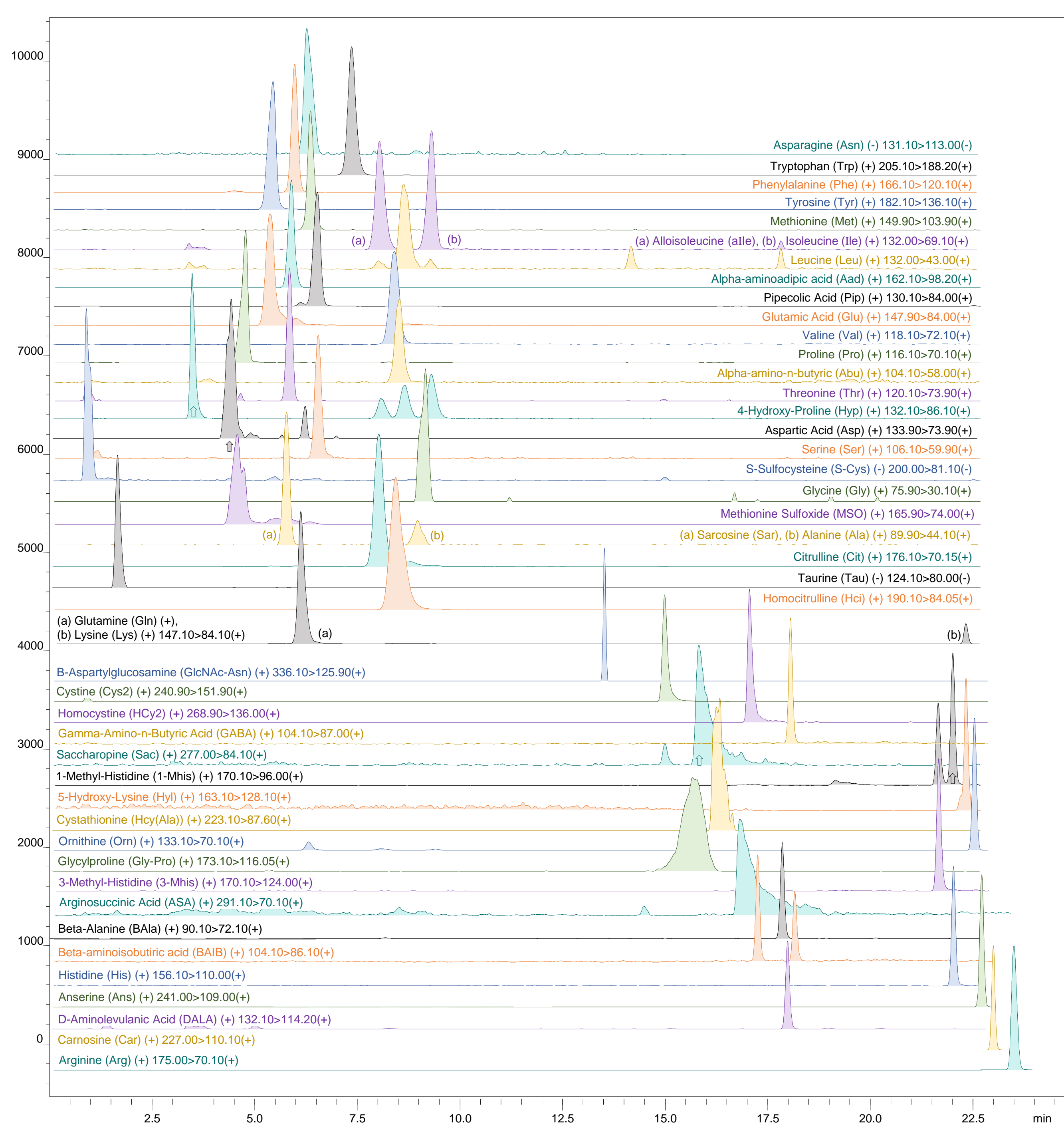


Figure 2. Typical Chromatogram for the quantification of amino acids in human plasma.

3-2. Alloisoleucine specific quantification

The chromatographic separation of leucine, isoleucine and alloisoleucine enables their specific quantifications in a single run, already including a wide number of amino acids. This specific quantification is reinforced by the use of specific transitions. A typical chromatogram is presented below.

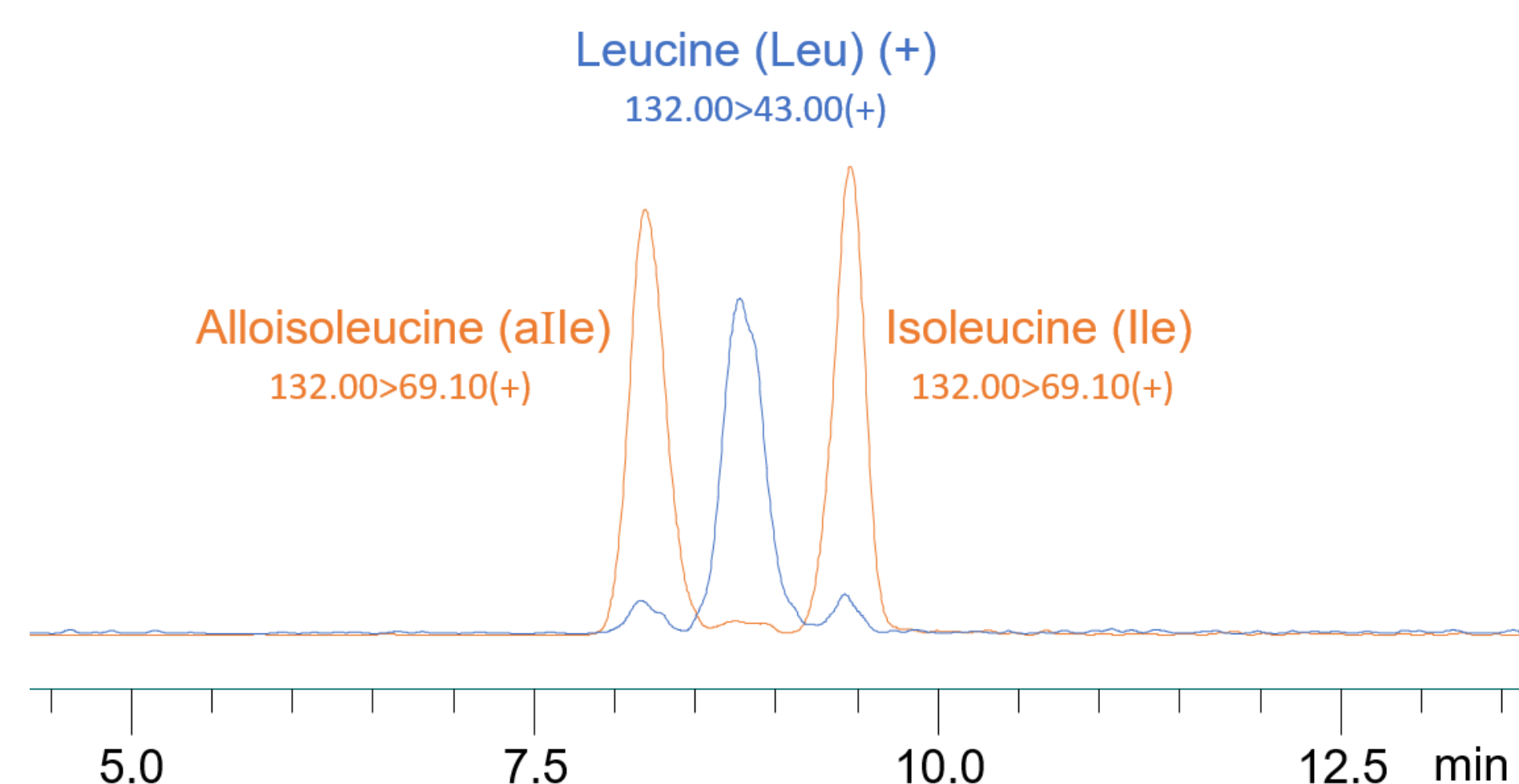


Figure 3. Typical chromatogram for the separation of leucine, isoleucine and alloisoleucine.

3-3. Method Performances

Analytical performances of the method were monitored using certified plasma samples from Recipe as calibrators and certified plasma controls from MCA Laboratory as QC samples. Accuracies of calibrators and QC samples were comprised between 80 and 120% for all analytes, and RSD values were all below 15% (n=3 intra-day, over 3 days for inter-day, n=9).

Amino Acid	Calibrator level 1				Calibrator level 2			
	Conc. (µM)	Acc. (%) (n=9) min-max	RSD intra day (% n=3)	RSD inter day (% n=9)	Conc. (µM)	Acc. (%) (n=9) min-max	RSD intra day (% n=3)	RSD inter day (% n=9)
Alanine (Ala) (+)	413	84 - 115	5.0	12	692	82 - 120	2.4	15
Alpha-amino-n-butyric (Abu) (+)	15	81 - 115	8.2	13	62	83 - 119	1.0	11
Arginine (Arg) (+)	29	94 - 106	1.9	4.2	256	95 - 107	0.5	4.5
Asparagine (Asn) (-)	40	81 - 119	13	14	185	83 - 117	12	14
Aspartic Acid (Asp) (+)	10	83 - 120	9.3	8.8	105	80 - 119	15	13
Citrulline (Cit) (+)	24	83 - 118	2.3	13	120	94 - 107	0.6	5.3
Glutamic Acid (Glu) (+)	104	91 - 115	8.8	8.1	207	89 - 115	3.5	8.0
Glutamine (Gln) (+)	449	87 - 112	3.4	9.4	945	96 - 106	1.7	4.0
Glycine (Gly) (+)	192	81 - 120	7.4	15	667	87 - 115	3.8	11
Histidine (His) (+)	77	93 - 112	4.9	6.1	170	97 - 108	1.8	3.5
Isoleucine (Ile) (+)	83	95 - 108	3.8	3.8	131	90 - 108	4.8	6.1
Leucine (Leu) (+)	148	94 - 114	2.4	6.0	291	89 - 110	4.9	6.8
Lysine (Lys) (+)	170	96 - 104	2.5	3.1	270	96 - 102	1.4	2.6
Methionine (Met) (+)	29	82 - 110	2.4	11	67	86 - 115	8.4	12
Ornithine (Orn) (+)	139	85 - 116	4.3	10	334	86 - 120	2.5	13
Phenylalanine (Phe) (+)	79	95 - 105	2.1	3.4	421	97 - 102	0.6	1.7
Proline (Pro) (+)	231	99 - 101	0.3	0.7	461	99 - 102	0.9	0.8
Serine (Ser) (+)	100	89 - 113	3.7	7.5	291	96 - 104	2.6	3.0
Taurine (Tau) (-)	45	83 - 118	3.9	12	190	96 - 105	1.6	2.8
Threonine (Thr) (+)	113	84 - 119	11	13	210	80 - 119	5.3	13
Tryptophan (Trp) (+)	48	96 - 104	1.9	2.6	215	98 - 104	0.2	2.5
Tyrosine (Tyr) (+)	65	98 - 102	1.2	1.4	200	97 - 102	0.9	1.9
Valine (Val) (+)	239	96 - 105	2.3	2.6	433	97 - 102	0.4	1.5

Table 1. Human plasma calibrators : accuracies and RSD values.

Amino Acid	Quality control level 1				Quality control level 2			
	Conc. (µM)	Acc. (%) (n=9) min-max	RSD intra day (% n=3)	RSD inter day (% n=9)	Conc. (µM)	Acc. (%) (n=9) min-max	RSD intra day (% n=3)	RSD inter day (% n=9)
Alanine (Ala) (+)	313	90 - 119	4.3	9.4	880	91 - 120	1.6	10
Alpha-amino-n-butyric (Abu) (+)	29	80 - 106	1.1	9.1	92	84 - 110	4.9	8.5
Arginine (Arg) (+)	27	81 - 94	1.3	5.1	507	97 - 110	0.5	4.3
Asparagine (Asn) (-)	37	84 - 114	8.2	11	205	80 - 119	3.0	8.9
Aspartic Acid (Asp) (+)	18	82 - 118	14	13	98	84 - 120	13	14
Citrulline (Cit) (+)	20	87 - 107	8.8	6.8	406	68 - 72	1.1	2.2
Glutamic Acid (Glu) (+)	74	82 - 113	1.2	13	240	85 - 106	0.8	8.5
Glutamine (Gln) (+)	531	90 - 99	4.1	3.7	1073	102 - 110	0.3	3.0
Glycine (Gly) (+)	291	81 - 117	8.8	13	948	92 - 117	7.8	7.0
Histidine (His) (+)	97	95 - 119	5.6	8.5	380	92 - 118	0.9	8.6
Isoleucine (Ile) (+)	31	96 - 120	2.7	7.0	377	99 - 105	1.3	1.9
Leucine (Leu) (+)	76	84 - 111	7.5	10	839	99 - 112	5.4	3.9
Lysine (Lys) (+)	76	87 - 116	0.3	2.3	467	87 - 117	0.7	1.8
Methionine (Met) (+)	9	83 - 113	10	13	226	82 - 96	3.3	5.1
Ornithine (Orn) (+)	62	80 - 103	6.4	10	614	84 - 112	2.4	9.7
Phenylalanine (Phe) (+)	74	97 - 100	1.7	1.4	922	101 - 104	0.6	1.2
Proline (Pro) (+)	191	99 - 102	0.9	1.0	565	104 - 108	0.8	1.0
Serine (Ser) (+)	51	80 - 94	3.8	4.6	448	89 - 104	2.7	4.8
Taurine (Tau) (-)	48	82 - 119	7.8	7.8	398	81 - 111	3.9	3.5
Threonine (Thr) (+)	100	81 - 99	7.5	7.7	402	92 - 116	8.7	7.8
Tryptophan (Trp) (+)	146	81 - 90	1.6	4.1	320	86 - 94	0.7	2.6
Tyrosine (Tyr) (+)	51	96 - 116	4.6	6.2	838	106 - 112	0.4	1.6
Valine (Val) (+)	151	90 - 94	1.0	1.8	804	101 - 105	0.8	1.0

Table 2. Human plasma quality controls : accuracies and RSD values.

Conclusions & Discussion

A novel LC-MS/MS method was developed for the simultaneous and high sensitive quantification of 47 amino acids in human plasma, with no need of derivatization or ion pairing agent, and a very simple sample preparation. The method directly includes the quantification of alloisoleucine, which is essential for the newborn diagnostic of maple syrup urine disease (MSUD). Additional amino acids may be added upon request. The total average cost per sample is reduced from 13€ to less than 2€ per sample compare to current methods with derivatization. Amino acids were quantified in a total analysis time of 25 min with a good distinction between isobars. Plasma control sample injections showed good repeatability and accuracy. The method proved its fits for purpose to support diagnosis and may be used also in clinical studies to monitor drug effects on metabolism.

(1) A. Jaffuel and M. Levi, "A novel fast quantification method for amino acids in human plasma by LC-MS/MS, without ion pairing or derivatization" poster presented at the 3rd MSACL EU Conference on Mass Spectrometry, Salzburg, Austria, 2015