Article

Determination of Amino Acids in Plasma Samples of Preeclampsia Patients by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry

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This work presents the application of a simple and sensitive method developed for the simultaneous analysis of the levels of twenty two underivatized free L-amino acids in plasma samples of preeclampsia patients by high performance liquid chromatography coupled to high-resolution mass spectrometer (LC-HRMS) using hydrophilic interaction liquid chromatography (HILIC) silica column. The linearity was evaluated in the range of 0.009 to 8.018 µmol L^{-1} showing coefficients of determination (R^2) in the range of 0.999 to 0.911 ($p < 0.05$). The intra- and inter-day precisions were above of 9.84 and 15.31, respectively. The limits of detection (LOD) and quantification (LOQ) were in the range of 0.003 to 0.683 µmol L^{-1} and 0.009 to 2.276 µmol L^{-1}, respectively. The results of the investigation of free L-amino acids showed that the levels of GABA, Arg, Ser, Leu, Ile, Met, Val, Ala, Gly, Bet, His and Lys in plasma of patients with preeclampsia were significantly different from healthy patients.

Keywords: L-amino acids, biomarkers, preeclampsia, LC-HRMS-ESI-IT-TOF

Introduction

The direct analyses of free amino acids (AA) is very important in metabolomics studies and have been an important implement of search for understanding, diagnostic and treatment of various diseases.[1-7] Amino acids are important metabolites for various metabolic pathways, contributing for several biochemical reactions that involves a wide metabolic network within living cell. Therefore, amino acids play an important role in connecting the diverse metabolic pathways since changing the level of one amino acid in response to environmental transformation and/or genetic variation originates disruptions in the levels of several other amino acids.[8] Various analytical methods were described in the literature for the determination of free amino acids in biological samples such as serum,[9] plasma,[1,10-14] sweat,[15] saliva,[5,16,17] tissue[18] or urine.[7] Chromatography methods as LC-HRMS (liquid chromatography-high resolution mass spectrometry) and GC-MS (gas chromatography mass spectrometry) are more frequently used due to their high sensitivity and selectivity.[16-20] GC-MS presents higher

sensitivity but free amino acids should be derivatized before analysis.[19,20] On the other hand, LC-MS (liquid chromatography mass spectrometry) is suitable for amino acids analysis due to better compatibility and application to bioassays involving high polar compounds, greater simplicity in sample preparation procedure without derivatization steps and shorter analysis time.[13] Besides this, LC-HRMS presents high selectivity and sensitivity, therefore high capacity to identify and detect coelutions not resolved by chromatography. Recently, Cocuron *et al.*[21] proposed a high sensitivity method to analyze amino acids by LC-MS method, nevertheless the proposed method can analyze only 19 AAs. The use of hydrophilic interaction liquid chromatography (HILIC) to amino acid analysis is growing up because it is efficient to separate very polar analytes quickly.[22-24] This work presents the development of a simple and sensitive method for the analysis of twenty two underivatized free L-amino acids using hydrophilic interaction through liquid chromatography coupled to a high-resolution ion trap and time-of-flight mass spectrometry (LC-HRMS-IT-TOF). This method was successfully applied to investigate the levels of free L-amino acids in plasma sample of humans' patients with preeclampsia.

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Experimental

Chemicals and reagents

Amino acids of high-purity (≥ 98%), L-tryptophan (Trp), L-phenylalanine (Phe), L-tyrosine (Tyr), L-leucine (Leu), L-isoleucine (Ile), L-methionine (Met), L-valine (Val), L-glutamic acid (Glu), γ-aminobutyric acid (GABA), L-aspartic acid (Asp), L-alanine (Ala), L-threonine (Thr), L-serine (Ser), L-glycine (Gly), L-proline (Pro), L-sarcosine (Sar), L-asparagine (Asn), L-glutamine (Gln), L-betaine (Bet), L-arginine (Arg), L-histidine (His), L-lysine (Lys) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) and formic acid 88% were purchased from J. T. Baker (Xalostoc, Edo do México, Mexico). Ultra-pure water was purified with a Purelab Classic from Elga (Woodridge, Illinois, USA). Other chemicals were of analytical grade.

Solutions preparation

Standard stock solutions of amino acids in water were prepared at 1161.4-13321.0 µmol L^{-1}. The mixed standard solutions and working solutions were obtained by sequential dilutions using the mixture acetonitrile (ACN):water (50:50, v/v) or ACN:water (90:10, v/v) with 0.1% of formic acid.

Sample preparation

Human plasma samples were provided by the Biomarker Laboratory of the Research Institute of the Santa Casa de Misericórdia of Belo Horizonte (Belo Horizonte-MG, Brazil). This study was approved by the Research Ethics Committees of the Santa Casa de Misericórdia of Belo Horizonte under No. 315034. Fourteen patients were recruited with preeclampsia and ten healthy pregnant women (control) who signed the informed consent form. The demographic data of the study group were: pregnant women with a mean age of 26.07 ± 5.23 years and 33.64 ± 4.48 weeks of gestation presenting systolic pressure of 143.40 ± 12.14 mmHg and diastolic pressure of 31.00 ± 4.64 mmHg. And healthy patients with a mean age of 27.50 ± 6.16 years and 34.80 ± 2.20 weeks of gestation presenting systolic and diastolic blood pressure of 109.80 ± 10.34 and 69.80 ± 6.70 mmHg, respectively. Venous blood samples from all patients were collected in tubes of the Vacuette system (Vacuette® do Brasil LTDA, São Paulo, SP, Brazil) containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The samples were centrifuged at 3000 rpm

for 15 min at room temperature. Blood plasma was collected and stored at –80 °C until use. In the chemistry laboratory of Universidade Federal de Minas Gerais, Brazil, all plasma samples were prepared using methanol iced to precipitate proteins. 100 µL of plasma sample was transferred to the vial and 900.0 µL of methanol at –10 °C were added. The mixture was centrifuged at 10,000 rpm for 10 min at room temperature. 100 µL of supernatant was transferred to Eppendorf tube for solvent evaporation at dryness under nitrogen. The solid was redissolved in 200.0 µL of ACN:water (90:10, v/v) with 0.1% formic acid and analyzed by LC-HRMS.

Instrumental analysis

All analysis were performed on a high performance liquid chromatography (HPLC) Promidence 20AC equipped with a system controller CBM-20A, an autosampler SIL-30AC, a binary solvent system LC-20AD, and a column heater CTO-20AC from Shimadzu Corp. (Tokyo, Japan). Chromatographic separation was performed with a HILIC silica column (2.1 × 150 mm, 3.0 µm particle size) and an Atlantis HILIC silica precolumn (2.1 × 10 mm × 3 µm), both from Waters Corp. (Milford, MA, USA). The conditions of chromatography were: sample injection volumes of 20.0 µL, column was maintained at 35.0 °C, flow rate of mobile phase at 0.2 mL min^{-1}. The mobile phase used was a binary system of 0.1% formic acid aqueous solution (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution started with 15% A, rinsing to 60% A in 13.0 min, which was then held for 2.0 min, then reduced to 15% in 15.5 min, and held for 4.5 min. The HRMS was an hybrid IT-TOF from Shimadzu Corp. (Tokyo, Japan) with mass accuracy (< 5 ppm) equipped with an electrospray ionization (ESI) source operating in positive mode under the conditions: N_2 nebulizing gas flow of 1.5 L min^{-1}; curved desorption line (CDL) interface temperature of 250 °C; detector voltage 2.00 kV; drying gas pressure of 114 kPa; octopole ion accumulation time of 100 ms; detection of the protonated ions $[M + H]^+$ was carried out in *m/z* range of 60-250 with an resolution power over 10,000.

Method performance

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to Eurachem Guide.[25] Ten replicates of solutions without amino acids in conditions identical of working standard solutions were analyzed. The intra-day precision was studied analyzing on the same day three concentration levels (low, medium, and high) in

replicates (n = 7) for each concentration level. The inter-day precision was performed analyzing on three different days three concentration levels in replicates (n = 7) for each concentration level.

Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) and Tukey test was achieved by Origin version 8.0.[26] Values of $p < 0.05$ were considered statistically significant in all statistical studies.

Results and Discussion

Optimization of chromatographic and mass spectrometric conditions

Figure 1 shows an overview of optimized method and Figure 2, the LC-HRMS chromatograms obtained for water solutions spiked at 5.0 µmol L^{-1}. Positive ionization mode increased the method sensitivity. The HILIC silica column increased separation efficiency of amino acids that are quite polar analytes.[23,24] Furthermore, the gradual increase of the mobile phase polarity provides analytes protonation. Table 1 shows mass spectrometer data for the amino acids obtained by the optimized method.

Figure of merits

Table 2 shows the figure of merits of optimized method. The proximity of the experimental measurement to the true value (exact mass) of protoned amino acid ions shows error lower than 5 ppm. The calibration curves were linear in the range of 0.009 to 8.018 µmol L^{-1}, presenting coefficients of determination (R^2) in the range of 0.999 to 0.985 for all amino acids except lysine (0.911), histidine (0.977) and arginine (0.978). The LODs and LOQs ranged from 0.003 to 0.683 µmol L^{-1} and 0.009 to 2.276 µmol L^{-1}, respectively. The relative standard deviation (% RSD) for

intra-day precision ranged from 4.2 to 16.2% and inter-day was from 7.0 to 19.1%.

Statistical analysis

The optimized method was applied to analyze plasma samples of preeclampsia (n = 14) patients and healthy controls (n = 10) groups, which results are shown in Table 3. All mean concentrations (Figure 3) were above LOQs. ANOVA followed by Tukey test were applied to the discrimination of the levels of amino acids of plasma from preeclampsia and healthy controls. The results from this test indicated that the difference between means is significant (p-value < 0.05) for GABA, Arg, Ser, Leu, Ile, Met, Val, Ala, Gly, Bet, His and Lys, suggesting these AAs as potential biomarkers. The differences in levels of twelve amino acids discriminated by Tukey test in two groups (preeclampsia and controls) can be visualized in the box plot (Figure 4).

Preeclampsia analyses

The method we developed for the simultaneous analysis of 22 underivatized amino acids in blood plasma using LC-HRMS was applied in analysis of amino acid levels in blood plasma samples from patients with preeclampsia. Preeclampsia, also known as hypertensive pregnancy disease, is a dangerous complication of pregnancy, characterized by high blood pressure and the presence of protein in the urine that appears after the 20th week of gestation. Although metabolic studies have already been conducted using advanced technologies,[27-34] the metabolic profile of patients with preeclampsia and biomarkers of the disease have not yet been elucidated for early identification of the disease and understanding of its pathogenesis. In this work a small contribution is made in metabolomics studies to identify possible biomarkers of preeclampsia. Through the statistical analysis of the results of the amino acid levels found in plasma samples from patients with preeclampsia, it was found that 12 of
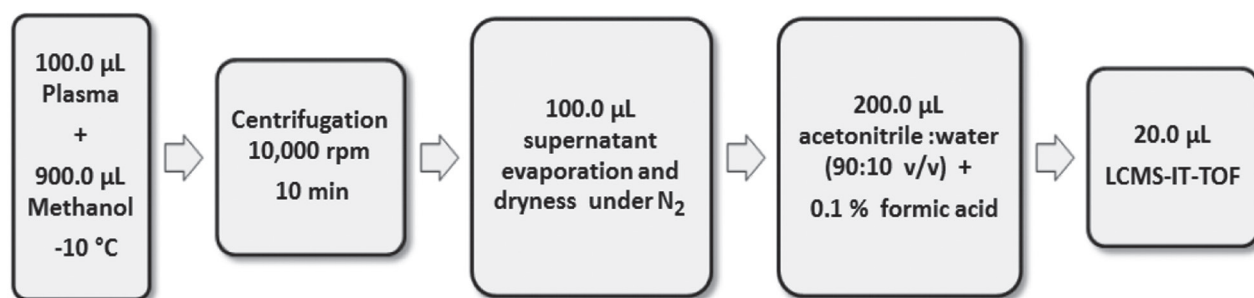
Figure 1. Overview of optimized method of LC-HRMS. LC conditions: mobile phase A, H$_2$O with 0.1% FA and B, ACN with 0.1% FA, flow rate was 0.2 mL min^{-1} in HILIC silica column. MS conditions: nebulizing gas flow, 1.5 L min^{-1}; CDL temperature, 250 ºC; heat block temperature, 250 ºC; detector voltage, 2.00 kV; drying gas pressure, 114.0 kPa. Positive-ion mode with detection of protonated ions in *m/z* range of 60-250.
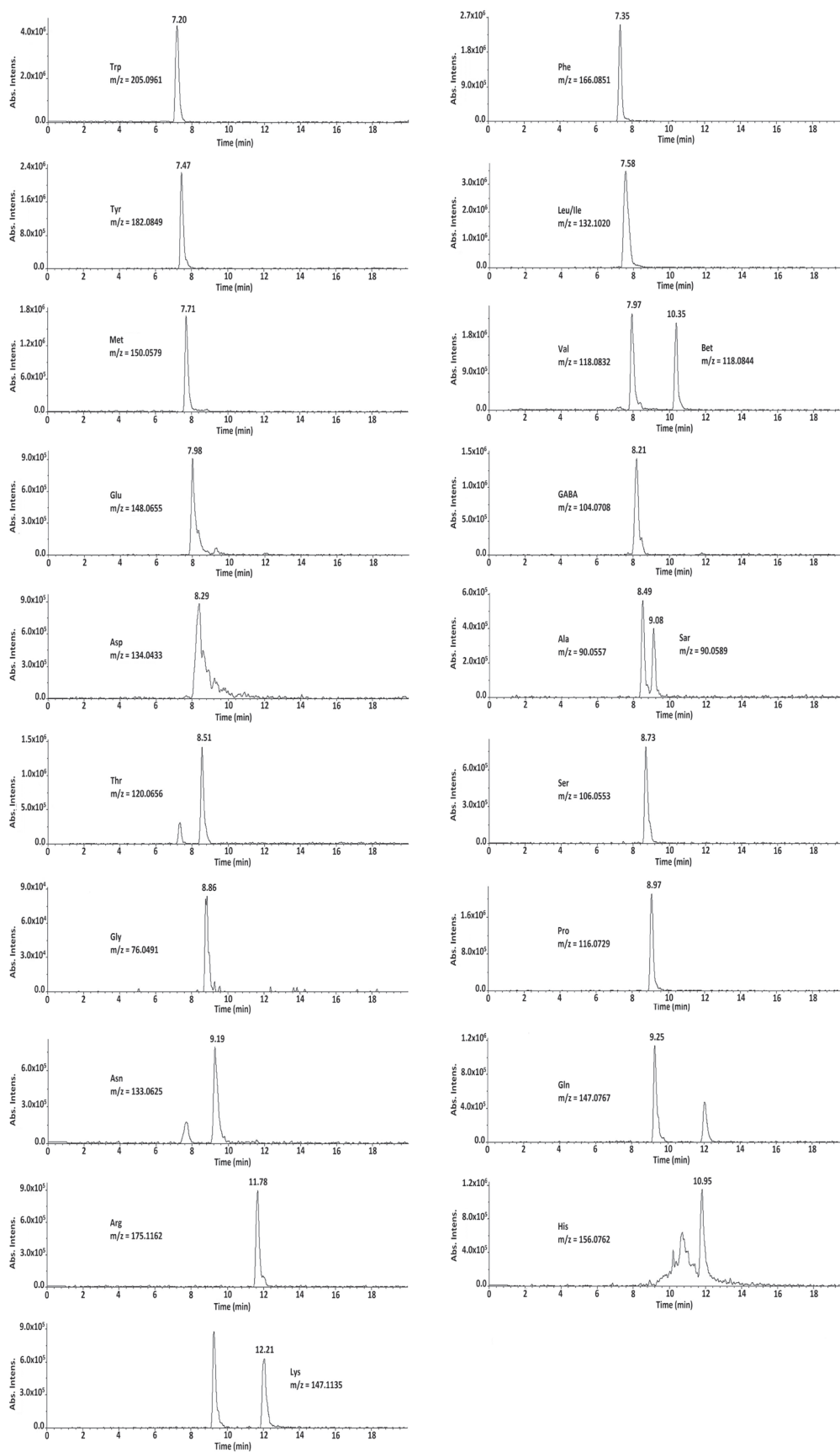
Figure 2. LC-HRMS chromatograms of underivatized L-amino acids aqueous solution spiked at 5.0 µmol L^{-1}.

Table 1. Mass spectrometer data for the amino acids

Amino acid	Molecular formula	Theoretical [M + H]⁺ *m/z*	Experimental [M + H]⁺ *m/z*	t_R / min
Trp	$C_{11}H_{12}N_2O_2$	205.0977	205.0961	7.20
Phe	$C_9H_{11}NO_2$	166.0868	166.0851	7.35
Tyr	$C_9H_{11}NO_3$	182.0817	182.0849	7.47
Leu	$C_6H_{13}NO_2$	132.1025	132.1020	7.58
Ile	$C_6H_{13}NO_2$	132.1025	132.1020	7.58
Met	$C_5H_{11}NO_2S$	150.0589	150.0579	7.71
Val	$C_5H_{11}NO_2$	118.0868	118.0832	7.97
Glu	$C_5H_9NO_4$	148.0610	148.0655	7.98
GABA	$C_4H_9NO_2$	104.0712	104.0708	8.21
Asp	$C_4H_7NO_4$	134.0453	134.0433	8.29
Ala	$C_3H_7NO_2$	90.0555	90.0557	8.49
Thr	$C_4H_9NO_3$	120.0661	120.0656	8.51
Ser	$C_3H_7NO_3$	106.0504	106.0553	8.73
Gly	$C_2H_5NO_2$	76.0399	76.0491	8.86
Pro	$C_5H_9NO_2$	116.0706	116.0729	8.97
Sar	$C_3H_7NO_2$	90.0555	90.0589	9.08
Asn	$C_4H_8N_2O_3$	133.0613	133.0625	9.19
Gln	$C_5H_{10}N_2O_3$	147.0764	147.0767	9.25
Bet	$C_5H_{10}NO_2$	118.0868	118.0844	10.35
Arg	$C_6H_{14}N_4O_2$	175.1190	175.1162	11.78
His	$C_6H_9N_3O_2$	156.0773	156.0762	10.95
Lys	$C_6H_{14}N_2O_2$	147.1128	147.1135	12.21

t_R: retention time; Trp: L-tryptophan; Phe: L-phenylalanine; Tyr: L-tyrosine; Leu: L-leucine; Ile: L-isoleucine; Met: L-methionine; Val: L-valine; Glu: L-glutamic acid; GABA: γ-aminobutyric acid; Asp: L-aspartic acid; Ala: L-alanine; Thr: L-threonine; Ser: L-serine; Gly: L-glycine; Pro: L-proline; Sar: L-sarcosine; Asn: L-asparagine; Gln: L-glutamine; Bet: L-betaine; Arg: L-arginine; His: L-histidine; Lys: L-lysine.

Table 2. Figure of merits for the analysis of amino acids by LC-HRMS method

Amino acid	Elemental composition	Retention time / min	Exact mass [M + H]⁺ *m/z*	Measured mass [M + H]⁺ *m/z*	Linear range / (μmol L⁻¹)	LOD / (μmol L⁻¹)	LOQ / (μmol L⁻¹)	R^2	Precision / (% RSD)	
									Intra-day (n = 21)	Inter-day (n = 21)
Trp	$C_{11}H_{12}N_2O_2$	7.20	205.0977	205.0961	0.059-1.210	0.018	0.059	0.9931	5.9	10.1
Phe	$C_9H_{11}NO_2$	7.35	166.0868	166.0851	0.009-1.500	0.003	0.009	0.9868	5.8	14.1
Tyr	$C_9H_{11}NO_3$	7.47	182.0817	182.0849	0.021-1.451	0.006	0.021	0.9904	6.1	9.5
Leu	$C_6H_{13}NO_2$	7.58	132.1025	132.1020	0.036-3.781	0.011	0.036	0.9697	4.2	13.8
Ile	$C_6H_{13}NO_2$	7.58	132.1025	132.1020	0.036-3.781	0.011	0.036	0.9697	4.2	13.8
Met	$C_5H_{11}NO_2S$	7.71	150.0589	150.0579	0.067-1.696	0.020	0.067	0.9897	8.1	14.3
Val	$C_5H_{11}NO_2$	7.97	118.0868	118.0832	0.040-2.120	0.012	0.040	0.9848	5.6	16.4
Glu	$C_5H_9NO_4$	7.98	148.0610	148.0655	0.047-2.469	0.014	0.047	0.9964	4.7	7.0
GABA	$C_4H_9NO_2$	8.21	104.0712	104.0708	0.053-3.440	0.016	0.053	0.9904	7.2	16.7
Asp	$C_4H_7NO_4$	8.29	134.0453	134.0433	0.106-2.743	0.032	0.106	0.9965	5.2	12.6
Ala	$C_3H_7NO_2$	8.49	90.0555	90.0557	0.153-2.787	0.046	0.153	0.9948	10.4	19.7
Thr	$C_4H_9NO_3$	8.51	120.0661	120.0656	0.323-5.837	0.097	0.323	0.9883	6.3	14.9
Ser	$C_3H_7NO_3$	8.73	106.0504	106.0553	0.344-6.789	0.103	0.344	0.9957	9.1	15.0
Gly	$C_2H_5NO_2$	8.86	76.0399	76.0491	0.587-6.661	0.176	0.587	0.9858	12.3	11.2
Pro	$C_5H_9NO_2$	8.97	116.0706	116.0729	0.079-6.377	0.024	0.079	0.9991	6.6	18.1
Sar	$C_3H_7NO_2$	9.08	90.0555	90.0589	0.366-8.018	0.110	0.366	0.9819	8.6	17.9
Asn	$C_4H_8N_2O_3$	9.19	133.0613	133.0625	0.261-1.903	0.078	0.261	0.9988	8.2	17.4
Gln	$C_5H_{10}N_2O_3$	9.25	147.0764	147.0767	0.143-4.858	0.043	0.143	0.9950	6.4	20.4
Bet	$C_5H_{10}NO_2$	10.35	118.0868	118.0844	0.115-2.149	0.035	0.115	0.9967	8.8	16.5
Arg	$C_6H_{14}N_4O_2$	11.78	175.1190	175.1162	0.059-2.000	0.018	0.059	0.9784	10.3	11.1
His	$C_6H_9N_3O_2$	10.95	156.0773	156.0762	2.276-6.487	0.683	2.276	0.9771	7.1	8.7
Lys	$C_6H_{14}N_2O_2$	12.21	147.1128	147.1135	0.473-2.371	0.142	0.473	0.9110	16.2	19.1

LOD: limits of detection; LOQ: limits of quantification; R^2: coefficients of determination; RSD: relative standard deviation; Trp: L-tryptophan; Phe: L-phenylalanine; Tyr: L-tyrosine; Leu: L-leucine; Ile: L-isoleucine; Met: L-methionine; Val: L-valine; Glu: L-glutamic acid; GABA: γ-aminobutyric acid; Asp: L-aspartic acid; Ala: L-alanine; Thr: L-threonine; Ser: L-serine; Gly: L-glycine; Pro: L-proline; Sar: L-sarcosine; Asn: L-asparagine; Gln: L-glutamine; Bet: L-betaine; Arg: L-arginine; His: L-histidine; Lys: L-lysine.

Table 3. Concentration of the amino acids in the human plasma samples of patients with preeclampsia and healthy controls

Amino acid	Preeclampsia — Concentration / (μmol L⁻¹)												Mean ± SD / (μmol L⁻¹)	Control — Concentration / (μmol L⁻¹)										Mean ± SD / (μmol L⁻¹)
Trp	0.99	0.16	0.48	3.60	0.46	0.20	0.47	0.79	2.31	3.09	0.80	1.04	1.14 ± 1.07	0.07	3.41	1.64	1.26	1.08	0.77	1.12	0.71	2.88	0.33	1.33 ± 1.06
Phe	3.54	2.76	3.47	3.87	4.46	3.66	4.14	2.54	4.36	5.55	5.14	3.62	3.81 ± 0.90	3.12	10.68	2.34	4.66	4.53	2.73	3.96	2.08	3.67	3.10	4.09 ± 2.47
Tyr	1.08	1.31	1.01	1.79	1.33	0.92	0.96	1.19	1.66	1.38	1.44	1.23	1.36 ± 0.43	2.47	2.16	0.91	1.26	1.29	1.36	1.23	1.18	1.96	0.79	1.46 ± 0.55
Leu	7.26	9.00	10.75	10.40	11.18	6.41	7.83	4.85	8.39	11.09	12.05	6.58	9.14 ± 2.97	11.72	33.66	13.72	14.16	17.60	8.71	13.39	9.91	10.76	7.00	14.06 ± 7.52
Ile	7.26	9.00	10.75	10.40	11.18	6.41	7.83	4.85	8.39	11.09	12.05	6.58	9.14 ± 2.97	11.72	33.66	13.72	14.16	17.60	8.71	13.39	9.91	10.76	7.00	14.06 ± 7.52
Met	0.87	0.28	0.02	0.48	0.94	0.45	2.09	0.23	0.55	0.50	2.40	0.55	0.79 ± 0.70	0.76	2.40	1.61	3.16	0.99	0.92	1.36	2.15	1.62	0.66	1.56 ± 0.81
Val	4.73	4.72	4.02	3.61	3.77	2.50	3.82	2.63	3.84	3.48	4.86	3.46	3.94 ± 1.03	5.32	10.31	6.83	8.69	5.85	4.47	6.32	5.10	3.75	3.76	6.04 ± 2.11
Glu	1.08	2.47	0.55	0.79	0.60	0.24	0.09	0.60	0.43	0.53	0.30	0.49	0.74 ± 0.60	0.71	0.35	0.37	0.94	1.11	0.51	0.37	0.20	0.37	0.48	0.54 ± 0.29
GABA	106.97	112.66	96.34	73.98	95.28	70.97	74.91	77.12	69.66	63.60	49.37	66.96	78.12 ± 18.47	131.45	161.47	191.12	146.02	173.89	117.45	122.82	102.37	83.59	107.09	133.73 ± 34.03
Asp	3.03	0.97	0.73	0.60	1.40	2.14	2.67	1.19	1.96	1.32	3.07	1.32	1.65 ± 0.82	3.01	3.62	1.57	3.54	1.71	1.45	2.45	1.51	1.75	1.75	2.24 ± 0.85
Ala	52.41	28.91	30.01	16.67	26.40	18.59	27.68	15.50	17.86	24.16	52.02	16.40	26.55 ± 11.95	25.99	78.82	45.92	57.30	36.10	33.20	35.45	28.56	20.30	32.88	39.45 ± 17.28
Thr	9.43	8.37	9.44	4.79	6.12	5.52	13.30	8.39	10.52	7.78	17.25	12.90	10.37 ± 4.00	9.70	22.86	11.26	17.37	19.62	7.88	13.12	7.59	8.07	4.29	12.18 ± 5.99
Ser	4.73	4.68	5.91	2.40	3.87	3.75	5.01	3.02	2.87	2.93	4.25	4.64	4.37 ± 1.34	7.92	8.23	6.93	7.61	11.98	6.37	8.15	4.63	4.05	5.70	7.16 ± 2.24
Gly	16.37	5.26	17.47	2.84	8.71	2.10	4.05	2.07	2.26	2.30	19.24	14.82	8.89 ± 6.64	13.91	29.48	13.68	30.84	28.56	14.00	15.63	10.99	6.29	8.42	17.18 ± 9.04
Pro	54.04	44.16	36.56	22.45	42.37	34.03	63.53	43.20	29.78	39.85	55.57	27.25	41.17 ± 11.22	21.30	116.26	53.59	71.24	70.53	47.77	51.57	49.11	29.63	37.41	54.84 ± 26.76
Sar	2.05	2.81	0.77	1.22	1.28	1.55	3.15	2.43	1.06	2.43	6.35	1.46	2.43 ± 1.49	5.54	2.45	2.49	0.24	1.37	1.93	2.37	6.42	3.45	2.60	2.89 ± 1.85
Asn	0.49	0.54	2.51	0.73	1.65	1.42	2.18	0.72	2.57	1.55	3.93	1.92	1.79 ± 0.98	1.91	3.91	2.93	3.57	2.52	2.03	2.02	1.41	1.32	0.96	2.26 ± 0.97
Gln	16.37	1.89	16.63	7.93	22.93	24.58	30.08	23.44	32.42	18.18	59.36	34.16	25.55 ± 14.54	33.12	48.91	40.31	28.78	20.86	20.28	21.87	21.04	18.03	17.74	27.09 ± 10.59
Bet	9.06	6.98	6.24	5.14	8.50	3.57	4.74	4.61	4.89	4.81	6.35	4.62	5.89 ± 1.63	5.39	21.68	18.15	9.68	10.28	9.83	16.58	10.12	5.37	9.38	11.65 ± 5.40
Arg	20.47	23.91	23.40	11.65	10.15	13.68	39.38	19.58	28.00	14.91	51.86	21.94	21.49 ± 11.96	11.44	12.20	2.10	1.90	8.78	8.12	6.48	7.30	18.98	8.40	8.57 ± 4.97
His	22.77	13.07	8.72	17.29	11.85	10.42	7.65	9.81	13.07	10.80	15.44	12.33	12.97 ± 4.10	84.11	29.54	12.36	25.72	17.77	21.35	29.80	14.29	22.30	10.05	26.73 ± 21.29
Lys	15.41	8.17	21.10	5.43	19.97	9.05	24.68	8.00	12.88	9.10	24.97	16.80	16.48 ± 9.00	26.00	51.43	20.19	30.61	19.80	16.02	28.98	26.17	19.28	11.48	25.00 ± 11.03

SD: standard deviation; Trp: L-tryptophan; Phe: L-phenylalanine; Tyr: L-tyrosine; Leu: L-leucine; Ile: L-isoleucine; Met: L-methionine; Val: L-valine; Glu: L-glutamic acid; GABA: γ-aminobutyric acid; Asp: L-aspartic acid; Ala: L-alanine; Thr: L-threonine; Ser: L-serine; Gly: L-glycine; Pro: L-proline; Sar: L-sarcosine; Asn: L-asparagine; Gln: L-glutamine; Bet: L-betaine; Arg: L-arginine; His: L-histidine; Lys: L-lysine.
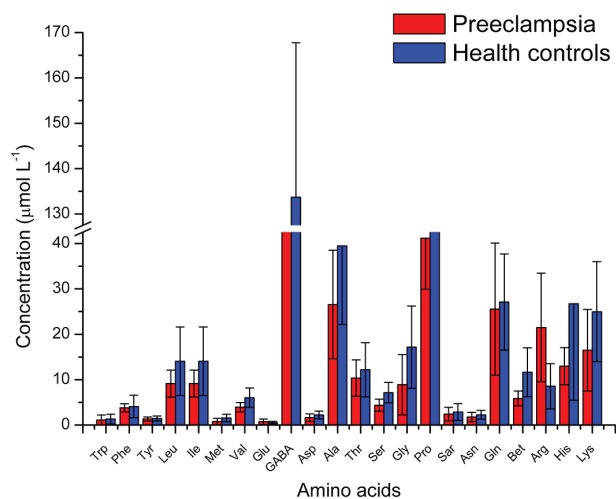
Figure 3. Mean concentrations of L-amino acids determinated in plasma samples of preeclampsia (n = 14) patients and healthy controls (n = 10).
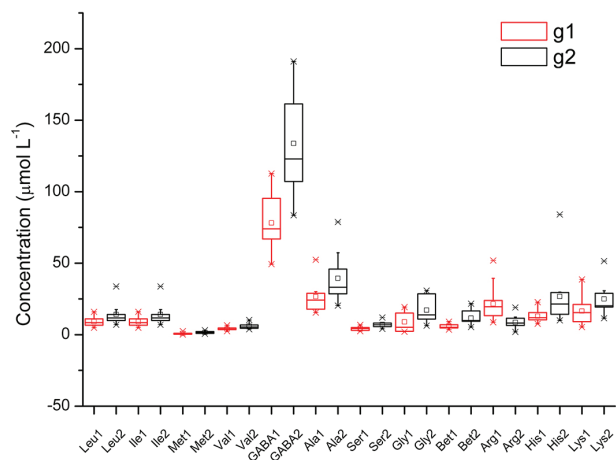
Figure 4. Box plot of the amino acids discriminated by Tukey test in plasma of preeclampsia patients and healthy controls.

the 22 amino acids studied showed significant changes when compared to the amino acids levels analyzed in the control samples of healthy patients. These results revealed that the levels of GABA, Arg, Ser, Leu, Ile, Met, Val, Ala, Gly, Bet, His and Lys were significantly lower in the group of preeclampsia compared to the control group of healthy patients.

Conclusions

An LC-HRMS optimized method to analyze twenty-two underivatized L-amino acids was developed. This method showed good linearity, repeatability, reproducibility, and low limits of detection and quantification. It was successfully applied to the determination of L-amino acids in plasma samples of patients with preeclampsia and healthy controls. The amino acids levels of GABA,

Arg, Ser, Leu, Ile, Met, Val, Ala, Gly, Bet, His and Lys presented significant differences between the samples of patients with preeclampsia and healthy controls. Therefore this study offers a tool to improve the understanding of preeclampsia.

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