QUALITY CONTROL OF AMINOACIDS IN ORGANIC FOODS AND FOOD SUPPLEMENTS

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ABSTRACT

Background: The variety of manufactured and proposed on the market food supplements, containing L – Glutamic acid and L – Arginine, set the pattern for the necessity of the developement of method, which can provide the possibility for their simultaneous determination with great selectivity, accuracy and precision. In accordance with this requirements, the aim of current study is to validate an isocratic HPLC method with UV – detection, which can be appropriate for simultaneous quality control of this aminoacids in different dosage food supplements.

Results. The data for the degree of recovery (R) are: L – Glutamic acid: 98.15 % (G_{80}), 97.60 % (G_{100}), 104.7 % (G_{120}); L – Arginine: 100.15 % (A_{750}), 100.13 % (A_{1000}), 100.28 % (A_{1250}). For the obtained quantity of aminoacids, SD is lower than 0.01 (L – Glutamic acid) and 0.08 (L – Arginine). Linearity is presented by the regression equations, which show proportional accordance AUC = f (C) in respective concentration ranges: $y = 2.10^9.x - 458796$ ($R^2 = 0.9956$) for L – Glutamic acid in $8.10^{-4} \div 1.2.10^{-3}$ g/ml and $y = 9.10^8.x + 699739$ ($R^2 = 0.9977$) for L – Arginine in $2.25.10^{-3} \div 3.75.10^{-3}$ g/ml.

Conclusion. The applied HPLC method with UV – detection is appropriate for quality control of L – Glutamic acid and L – Arginine with great accuracy and precision in food additives – tablets and capsules.

Keywords: L – Glutamic acid, L – Arginine, HPLC, Accuracy, Precision, Food supplements.

INTRODUCTION

Food supplements, containing aminoacids are very important for human health: L – Tyrosine, as a precursor of catecholamines [1]; L – Tryptophan, as a precursor of serotonin, melatonin and niacin [2]; L – Glutamic acid, as a fuel for the brain 3. L – Glutamic acid improves mental capacities and cognitive functions like memory and learning, shows promise in the treatment of neurological diseases, epilepsy, Parkinson, mental retardation, personality disorders, muscular dystrophy, ulcers, hypoglycemic come, benign prostatic hyperplasia 3, 4. L – Glutamic acid is important for the detoxification of body and brain of ammonia [3] and by high affinity system [5] helps in the transportation of potassium across the blood – brain barrier 6.

For the determination of L – Glutamic acid are applied the following methods: 1) HPLC [7]; 2) pre – or post – column derivatization with o – phthalaldehyde [8], followed by HPLC; 3) HPLC with fluorescence detection [9]; 4) piezoelectric quartz crystal sensor detection 10.

L – Arginine is a mediator in nonadrenergic and noncholinergic neurotransmission and is important for neuroprotection, learning and memory, synaptic plasticity and dilatation of small blood vessels in brain. The supplements with L – Arginine offers benefits in: 1) nitrogen storage [11]; 2) improving of: a) endothelial [12], cardiovascular [13] and renal function in patients with chronic heart failure; b) athletic performance; 3) increasing of: a) exercise tolerance in stable coronary artery disease patients; b) human growth and hormone levels; 4) boosting the immune system; 5) reducing the pulmonary resistance and healing time of injuries; 6) decreasing: a) mental capacity in the elderly with senile dementia [14]; b) high blood pressure in patients with systemic hypertension by stimulation of synthesis of nitric oxide [12] and during pregnancy (pre – eclampsia) [15]; 7) normalizing the platelet aggregation in hypercholesterolemic humans [16]; 8) preventing the inflammation of the digestive tract in premature infants; 9) regulating of hormones and blood sugar; 10) promoting lymphocyte production; 11) helping for liver detoxification by neutralizing ammonia 14.

L – Arginine is used in combination with: 1) conventional chemotherapy drugs for therapy of breast cancer; 2) aminoacids for treating weight loss in people with AIDS; 3) fish oil for reducing infections, improving wound healing and shortening recovery time after surgery 14. Arginine vasopressin regulates hypothalamus – pituitary adrenal system 17. Arginine is applied as a component of media for actinobacteria isolation 18.

The most widely used methods for the determination of L – Arginine are: 1) gradient RP HPLC with: a) UV – detection at λ = 254 nm in serum and tissue (with L – Citrulline) [19]; b) fluorescence detection ($\lambda_{excitation}$ = 340 nm, $\lambda_{emission}$ = 455 nm), after derivatization with o – phthalaldehyde in plasma, serum, urine, cell culture medium and tissues [20]; c) MS detection, coupling with an atmospheric pressure chemical ionization (APCI) in human urine [21] and human erythrocytes [22]; 2) second derivative UV – spectrophotometry in injections (with Cefepime) [23]; 3) capillary electrophoresis 24.

In our previous works was validated HPLC UV method for analysis of L – Glutamic acid and L – Arginine in Tonotyl solution®. The aim of current study is to validate HPLC method with UV – detection for simultaneous determination of L – Glutamic acid and L – Arginine in different trade dosage products, by using the method of calibration curve. 25

MATERIALS

I) Reference standards (RS): L – Glutamic acid, L – Arginine. II) Reagents: methanol, distilled water.

METHODS

HPLC with UV – detection

Chromatographic system: Liquid chromatograph Shimadzu (Japan) (LC – 10 Advp), equipped with: column Spherisorb ODS RP – C18 (250 mm/4.6 mm i.d./5 μm), column oven (CTO – 10 Asvp); isocratic pump (LC – 10 A); 20 μl injector loop; UV – VIS – detector at fixed wavelengths (SPD – 10 Avvp).

Chromatographic conditions: stationaty phase: Spherisorb ODS RP – C_{18}; mobile phase : methanol : distilled water = 1 : 1 v/v (before using is filtered throught membrane filter with pore size 0.45 μm); flow rate – 1.0 ml/min; column temperature – 25 ºC; analytical wavelength λ = 210 nm.

Preparation of solutions of RS L – Glutamic and RS L – Arginine for linearity.

An accurately weighed quantity: 0.08 g, 0.1 g, 0.12 g of RS L – Glutamic acid and 0.75 g, 1.0 g, 1.25 g of RS L – Arginine, was dissolved with mobile phase: methanol : distilled water = 1 : 1 v/v and was diluted with the same solvent in volumetric flask of 100.0 ml, From all solutions with L – Arginine an aliquot sample of 3.0 ml was diluted in volumetric flask to 10.0 ml with the mobile phase. Solutions with the following concentrations were obtained: 8.10^{-4}

g/ml; 1.10^{-3} g/ml; $1.2.10^{-3}$ g/ml (L – Glutamic acid) and $2.25.10^{-3}$ g/ml; 3.10^{-3} g/ml; $3.75.10^{-3}$ g/ml (L – Arginine).

Preparation of solutions of RS L – Glutamic acid and RS L – Arginine for estimation of accuracy and precision.

For each of the examined aminoacids, separately were prepared three (3) different homogenous model mixtures (MM), containing respectively: 1) for L – Glutamic acid: RS L – Glutamic acid, equivalent to 80 %, 100 % and 120 % of theoretical concentration (0.1 g) and all supplements in food additives: calcium hydrogen phosphate, crospovidone, glucose, hypromellose, microcrystalline cellulose, macrogol 400, magnesium stearate, silicon dioxide, stearic acid, titanium dioxide; 2) for L – Arginine: RS L – Arginine, equivalent to 75 %; 100 % and 125 % of theoretical concentration (1.0 g) and all supplements in food additives: dicalcium phosphate, magnesium stearate, microcrystalline cellulose, silicon dioxide, stearic acid. From each model mixture were prepared 3 samples as follows: an accurately weighed quantity equivalent to 80 %, 100 %, 120 % of RS L – Glutamic acid and 75 %, 100 %, 125 % of RS L – Arginine, was dissolved in mobile phase: methanol : distilled water = 1 : 1 v/v and was diluted with the same solvent in volumetric flask of 100.0 ml. From all solutions with L – Arginine an aliquot sample of 3.0 ml was diluted in volumetric flask to 10.0 ml with the mobile phase.

From each solution 20 μl were injected in chromatograph, after filtration through membrane filter with pores 0.45 μm. All solutions were analyzed by the written HPLC method and chromatograms were recorded.

RESULTS AND DISSCUSSION

Validation of HPLC method with UV – detection for determination of L – Glutamic acid and L – Arginine in food additives

For the development of the validation procedure for HPLC method are investigated the analytical parameters: selectivity, linearity, accuracy, precision.

Selectivity

For the assessment of analytical parameter selectivity, at the same manner, like RS, the ”placebo” solutions were prepared with all labeled in tablets supplements, without the active ingredients. The selectivity of the applied HPLC method is proved by the fact, that on chromatograms with ”placebo”solutions aren't exist peaks with t_R, corresponded to t_R of the respective RS: t_R L – Glutamic acid = 5.333 min., t_R L – Arginine = 2.4 min.

Linearity

The data for the concentrations of L – Glutamic acid and L – Arginine and area under the curve AUC are shown on Table. 1.

Table 1: Concentrations [g/ml] and AUC for L – Glutamic acid and L – Arginine.

N :	L – Glutamic acid		L – Arginine	
	C [g/ml]	AUC [mV]	C [g/ml]	AUC [mV]
1.	8.10^{-4}	1075846	$2.25.10^{-3}$	2767317
2.	1.10^{-3}	1507625	3.10^{-3}	3520934
3.	$1.2.10^{-3}$	1850570	$3.75.10^{-3}$	4158587

The dependences: concentration (C) (g/ml)/AUC (mV) in respective concentra – tion range are putted into linearity regression analysis. On Table 2. are presented the obtained regression equations and the calculated coefficients of regression (R^2), which are higher than 0.995.

Table 2: Regression equations and coefficients of regression (R^2) for L – Glutamic acid and L – Arginine.

N :	Aminoacid	Concentration range [g/ml]	Regression equation	R^2
1.	L – Glutamic acid	$8.10^{-4} \div 1.2.10^{-3}$	$y = 2.10^9.x - 458796$	0.9956
2.	L – Arginine	$2.25.10^{-3} \div 3.75.10^{-3}$	$y = 9.10^8.x + 699739$	0.9977

On Fig. 1. (L – Glutamic acid) and Fig. 2. (L – Arginine) are illustrated the calibration curves for analyzed aminoacids.
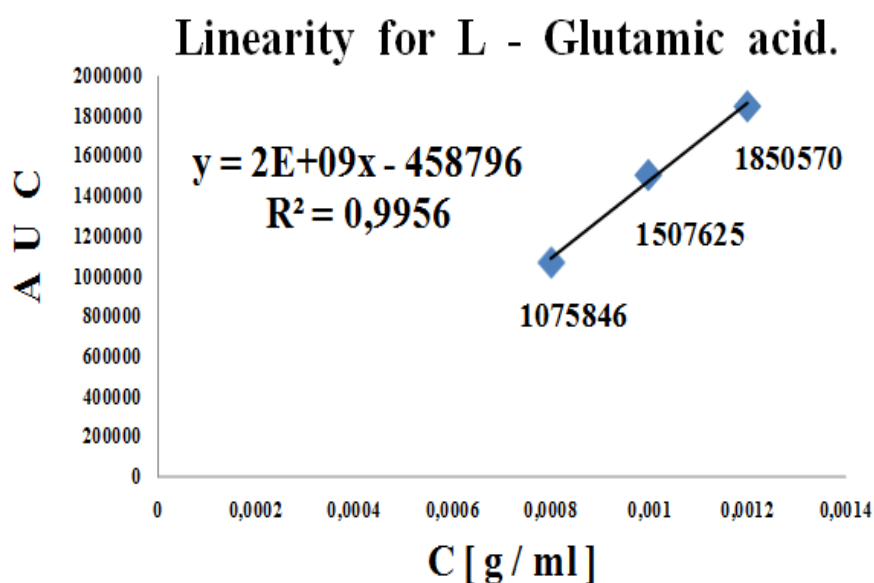

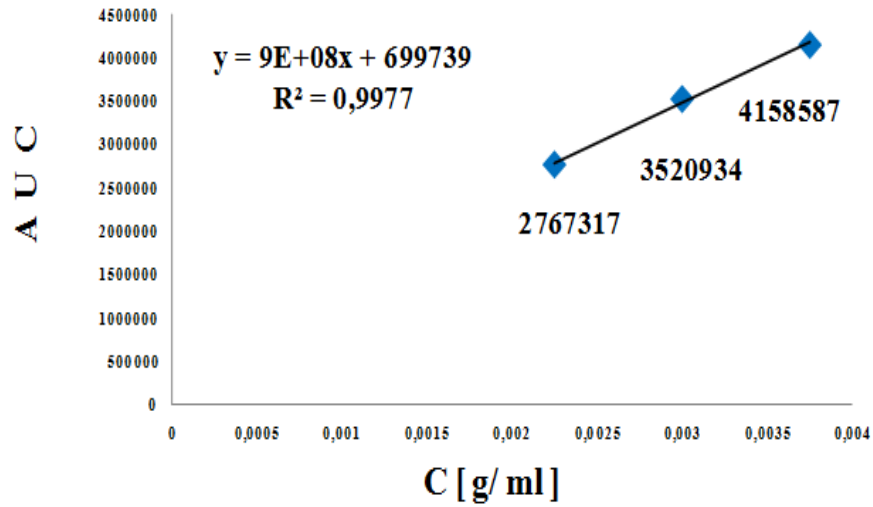**Fig. 1: Calibration curve for linearity of L – Glutamic acid**

Fig. 2: Calibration curve for linearity of L – Arginine

Accuracy and precision (repeatability).

The data for added content of L – Glutamic acid (G) and L – Arginine (A) and for weighed quantity (WG, WA) of the respective model mixtures (MM) are pointed on Table 3. for L – Glutamic acid: (G$_{80}$, WG$_{80}$), (G$_{100}$, WG$_{100}$), (G$_{120}$, WG$_{120}$) and on Table 4. for L – Arginine: (A$_{750}$, WA$_{750}$), (A$_{1000}$, WA$_{1000}$), (A$_{1250}$, WA$_{1250}$). The average weights of model mixtures are: for L – Glutamic acid: 0.12 g (G$_{80}$), 0.15 g (G$_{100}$), 0.18 g (G$_{120}$) and for L – Arginine: 0.9 g (A$_{750}$), 1.25 g (A$_{1000}$), 1.5 g (A$_{1250}$).

Table 3: Added content of RS L – Glutamic acid and weighed quantity of model mixtures.

N:	G$_{80}$ [g]	W G$_{80}$ [g]	G$_{100}$ [g]	W G$_{100}$ [g]	G$_{120}$ [g]	W G$_{120}$ [g]
1.	0.0784	0.1176	0.0974	0.1461	0.1182	0.1773
2.	0.0798	0.1197	0.0986	0.1479	0.1220	0.1830
3.	0.0802	0.1203	0.1008	0.1512	0.1234	0.1851

Table 4: Added content of RS L – Arginine and weighed quantity of model mixtures.

N:	A$_{750}$ [g]	W A$_{750}$ [g]	A$_{1000}$ [g]	W A$_{1000}$ [g]	A$_{1250}$ [g]	W A$_{1250}$ [g]
1.	0.745	0.8940	1.060	1.2720	1.260	1.5120
2.	0.760	0.9120	1.085	1.3020	1.300	1.5600
3.	0.785	0.9420	1.130	1.3560	1.340	1.6080

On Table 5. for L – Glutamic acid and on Table 6. for L – Arginine are summarized the results for: 1) area under the curve (AUC): L – Glutamic acid: AUC G$_{80}$, AUC G$_{100}$, AUC G$_{120}$; L – Arginine: AUC A$_{750}$, AUC A$_{1000}$, AUC A$_{1250}$; 2) Chauvenet's criterion for AUC (U AUC): L – Glutamic acid: U AUC G$_{80}$, U AUC G$_{100}$, U AUC G$_{120}$; L – Arginine: U AUC A$_{750}$, U AUC A$_{1000}$, U AUC A$_{1250}$.

Table 5: AUC and Chauvenet's criterion for AUC (U AUC) for model mixtures with L – Glutamic acid.

N:	AUC G$_{80}$	U AUC G$_{80}$	AUC G$_{100}$	U AUC G$_{100}$	AUC G$_{120}$	U AUC G$_{120}$
1.	1075846	1.12	1340470	1.11	1850570	1.14
2.	1108672	0.33	1507625	0.29	2162585	0.41
3.	1119315	0.80	1571602	0.82	2228860	0.73
$\overline{X}$	1101278		1473232		2080672	
SD	22658		119343		202010	
RSD [%]	2.06		8.1		9.71	

Table 6: AUC and Chauvenet's criterion for AUC (U AUC) for model mixtures with L – Arginine.

N:	AUC A$_{750}$	U AUC A$_{750}$	AUC A$_{1000}$	U AUC A$_{1000}$	AUC A$_{1250}$	U AUC A$_{1250}$
1.	2628734	1.01	3520934	0.77	4047534	0.82
2.	2767317	0.02	3591783	0.36	4158587	0.3
3.	2899640	0.99	3845930	1.13	4458158	1.11
$\overline{X}$	2765230		3652882		4221426	
SD	135465		170896		212402	
RSD [%]	4.9		4.68		5.03	

For all of the obtained by the applied HPLC method data for AUC of aminoacids L – Glutamic acid and L – Arginine in every sample of model mixtures is necessary to estimate the Chauvenet's criterion (U), because when U for one value is higher than the relevant standard criterion (USt), the result must be removed as unexpected. The relations: 1) L – Glutamic acid: U AUC G_{80} < 1.68; U AUC G_{100} < 1.68; U AUC G_{120} < 1.68; 2) L – Arginine: U AUC A_{750} <

1.68; U AUC A_{1000} < 1.68; U AUC A_{1250} < 1.68, show that all values for Chauvenet's criterion for AUC for L – Glutamic acid and L – Arginine are lower, than the standard Chauvenet's criterion for 3 samples: USt = 1.68 (N = 3), which proves, that there aren't unexpected values. The content of L – Glutamic acid (Table 7.) and L – Arginine (Table 8.) in model mixtures is obtained by method of calibration curve.

Table 7: Obtained quantity of L – Glutamic acid in model mixtures – estimation by method of calibration curve.

N:	$[G_{80}]$ [g]	R $[G_{80}]$ [%]	U $[G_{80}]$	$[G_{100}]$ [g]	R $[G_{100}]$ [%]	U $[G_{100}]$	$[G_{120}]$ [g]	R $[G_{120}]$ [%]	U $[G_{120}]$
1.	0.0767	97.83	1.3	0.09	92.40	1.10	0.1155	97.72	1.15
2.	0.0784	98.25	0.4	0.0983	99.70	0.28	0.1311	107.46	0.41
3.	0.0789	98.38	0.9	0.1015	100.69	0.82	0.1344	108.91	0.74
$\overline{X}$ ± SD	0.078 ± 0.001			0.0966 ± 0.006			0.127 ± 0.01		
R̄ [%] ± RSD [%]		98.15 ± 0.3			97.60 ± 4.64			104.7 ± 5.82	
SD	0.001	0.29		0.006	4.53		0.01	6.09	
RSD [%]	1.28	0.3		6.21	4.64		7.87	5.82	
$_S\overline{X}$	0.001	0.17		0.003	2.62		0.006	3.52	
P [%]	90.0	90.0		90.0	90.0		90.0	90.0	
t	2.92	2.92		2.92	2.92		2.92	2.92	
$_{t.S}\overline{X}$	0.003	0.5		0.009	7.65		0.018	10.28	
$\overline{X}_{+t.S}\overline{X}$ ÷ $\overline{X}_{-t.S}\overline{X}$	0.075 ÷ 0.081	97.65 ÷ 98.65		0.0876 ÷ 0.1056	89.95 ÷ 105.25		0.109 ÷ 0.145	94.42 ÷ 114.98	
E [%]	1.28	0.17		3.11	2.68		4.72	3.36	

Table 8: Obtained quantity of L – Arginine in model mixtures by method of calibration curve.

N:	$[A_{750}]$ [g]	R $[A_{750}]$ [%]	U $[A_{750}]$	$[A_{1000}]$ [g]	R $[A_{1000}]$ [%]	U $[A_{1000}]$	$[A_{1250}]$ [g]	R $[A_{1250}]$ [%]	U $[A_{1250}]$
1.	0.714	95.84	1.02	1.045	98.58	0.82	1.240	98.41	0.80
2.	0.766	100.79	0.02	1.071	98.71	0.38	1.281	98.54	0.29
3.	0.815	103.82	1.0	1.165	103.10	1.18	1.392	103.88	1.10
$\overline{X}$ ± SD	0.765 ± 0.05			1.094 ± 0.06			1.304 ± 0.08		
R̄ [%] ± RSD [%]		100.15 ± 4.02			100.13 ± 2.57			100.28 ± 3.11	
SD	0.05	4.03		0.06	2.57		0.08	3.12	
RSD [%]	6.54	4.02		5.48	2.57		6.13	3.11	
$_S\overline{X}$	0.03	2.33		0.03	1.49		0.05	1.80	
P [%]	90.0	90.0		90.0	90.0		90.0	90.0	
t	2.92	2.92		2.92	2.92		2.92	2.92	
$_{t.S}\overline{X}$	0.09	6.8		0.09	4.35		0.15	5.26	
$\overline{X}_{+t.S}\overline{X}$ ÷ $\overline{X}_{-t.S}\overline{X}$	0.675 ÷ 0.855	93.35 ÷ 106.95		1.004 ÷ 1.184	95.78 ÷ 104.48		1.154 ÷ 1.454	95.02 ÷ 105.54	
E [%]	3.92	2.33		2.74	1.49		3.83	1.79	

On Table 7. and Table 8. are indicated: N – number of the individual measurements (1 ÷ 3); obtained quantity of the examined aminoacids in model mixtures: L – Glutamic acid: $[G_{80}]$, $[G_{100}]$, $[G_{120}]$; L – Arginine: $[A_{750}]$, $[A_{1000}]$, $[A_{1250}]$; R (%) – recovery for the obtained content of aminoacids in model mixtures: L – Glutamic acid: R$[G_{80}]$, R$[G_{100}]$, R$[G_{120}]$; L – Arginine: R$[A_{750}]$, R$[A_{1000}]$, R$[A_{1250}]$; U – Chauvenet's criterion for the obtained quantity of aminoacids in model mixtures: L – Glutamic acid: U$[G_{80}]$, U$[G_{100}]$, U$[G_{120}]$; L – Arginine: U$[A_{750}]$, U$[A_{1000}]$, U$[A_{1250}]$; $\overline{X}$ – arithmetical mean; SD –

standard deviation; RSD (%) – relative standard deviation; $\overline{S\overline{X}}$ mean quadratic error; P – confidence possibility (%); t – coefficient of Student; $\overline{X}$ ± t.S $\overline{X}$ = $\overline{X}$ + t.S $\overline{X}$ ÷ $\overline{X}$ – t.S $\overline{X}$ – confidence interval (CI); E (%) – relative error.

For all of the obtained by the applied HPLC method results for the content of aminoacids in every sample in model mixtures is necessary to compare the respective value of Chauvenet's criterion with the relevant standard criterion for 3 samples: USt = 1.68 (N = 3).

From the obtained relations: 1) L – Glutamic acid (Table 7.): U$[G_{80}]$ < 1.68; U$[G_{100}]$ < 1.68; U$[G_{120}]$ < 1.68; L – Arginine (Table 8.): U$[A_{750}]$ <

1.68; U[A_{1000}] < 1.68; U[A_{1250}] < 1.68, it is obvious, that all values for Chauvenet's criterion for the quantity of the examined aminoacids are lower, than standard Chauvenet's criterion: USt = 1.68 (N = 3), which proves, that there aren't unexpected values.

For the assessment of analytical parameters accuracy and precision is calculated sample standard deviation (SD), by the applying of the Bessel's correction, in which the denominator N – 1 (degrees of freedom) is used instead of N and in this case (S $\overline{X}$)^2 is an unbiased estimator for (SD)^2.

Accuracy, presented by the recovery R (%) ± RSD (%) [26] at P = 90.0 % (t = 2.92, N = 3) is in the following ranges: for L – Glutamic acid: 97.60 ÷ 104.7 (G_{80} ÷ G_{120}); L – Arginine: 100.13 ÷ 100.28 (A_{750} ÷ A_{1250}). Precision is estimated by the uncertainty of the result,

determined by SD, RSD and $\overline{X}$ ± t.S $\overline{X}$ = $\overline{X}$ + t.S $\overline{X}$ ÷ $\overline{X}$ – t.S $\overline{X}$ [26]. All data for the obtained quantity of aminoacids at P = 90.0 % (t = 2.92, N = 3) correspond to the respective confidence interval and the values for t.S $\overline{X}$ for L – Glutamic acid: 0.001 ÷ 0.006 (G_{80} ÷ G_{120}) are lower than the respective data for L – Arginine 0.09 ÷ 0.15 (A_{750} ÷ A_{1250}).

For the obtained quantity of aminoacids, all data for SD for L – Glutamic acid: 0.001 ÷ 0.01 are lower than values for SD for L – Arginine: 0.05 ÷ 0.08. The results are clustered closely around the average value and the respective narrow confidence intervals implies high precision.

Table 9: Food additives, containing 0.1 g L – Glutamic acid and 1.0 g L – Arginine.

N:	L – Glutamic acid 0.1 g	L – Arginine 1.0 g
Tablets		
1.	Bromalain Papain, 60 tabl.	Good N Natural 1000, 100 tabl.
2.	Cobal – M, 10 tabl.	GNC 1000, 180 tabl.
3.	Country Life – Calcium Magnesium Zinc with L – Glutamic acid, 250 tabl.	Jarrow Formula 1000, 100 tabl.
4.	Gastro Digest 11 Ver Vita, 90 tabl.	Kal 1000, 60 tabl.
5.	Nutifacts Complete, 10 tabl.	Natrol 1000, 50 tabl.
6.	Orthoplex L. M. 1, 60 tabl.	Nature's Bounty 1000, 50 tabl.
7.	Orthoplex hydrozyme, 120 tabl.	Now Foods 1000, 120 tabl.
8.	Swiss Herbal Glutamic acid, 90 tabl.	NSI L – Arginine HCl 1000, 300 tabl.
9.	Trophic Glutamic acid, 180 tabl.	Total nutrition, 90 tabl.
10.	Ultimate Nutrition Super Complete Multi Vitamin Formula, 135 tabl.	Thompson Nutritional 1000, 30 tabl.
11.	Ultimate Nutrition Super Complete Formula, 270 tabl.	21 Sentury INC 1000, 100 tabl.
12.	Ultimate Nutrition Daily Complete Formula, 180 tabl.	Source naturals 1000, 100 tabl.
Capsules		
1.	Acida – Plex Acida – Zyme, 90 caps.	Fitness Pro L – Arginine 1000, 100 caps.
2.	Basic Pygeum Herbal, 60 caps.	GNC 1000, 90 caps.
3.	Recall Biocare, 30 caps.	Max Strength AKG Swanson Ultra, 90 caps.
Solutions with 1.0 g L – Arginine		
1.	Amino Fuel Liquid Orange	
2.	Liquid L – Carnitine, Co Enzyme Q –10 and L – Arginine	

CONCLUSION

All data for the degree of recovery and for the obtained quantity of aminoacids suit respective confidence interval. The applied HPLC method with UV – detection is appropriate for analysis of L – Glutamic acid and L – Arginine in food additives – tablets and capsules (Table 9.) with great accuracy and precision: SD < 0.01 (L – Glutamic acid), SD < 0.05 (L – Arginine).

REFERENCES

1. Draganov GB, Peikov PT, Obreshkova DP. Food supplements containing L – tyrosine as a precursor of catecholamines in human health. Eur J Drug Metab Pharmacokinet 2009;34:33.
2. Peikov PT, Obreshkova DP, Draganov GB. Food supplements containing L – tryptophan and 5 – hydroxytryptophan as a precursor of serotonin, melatonin and niacin in human health. Acta Medica Bulgarica 2009;36(2):61-4.
3. Balch PA. Prescription for nutritional healing: a practiacal A to Z reference to drug – free remedies using vitamins, minerals, herbs and food supplements. 4th ed. New York: Avery Publishing Groop; 2006,35-6.
4. Braverman ER. The healing nutrients within. New Canaan: Keats Publishing Inc; 1997,212-29.
5. Shigeri Y, Seal RP, Shimamoto K. Molekular pharmacology of glutamate transpoters, EAATs and VGLUTs. Brain Res Rev 2004;45:250-65.
6. Smith QR. Transport of glutamate and other amino acids at the blood – brain barrier. J Nutr 2000;130:1016-22.
7. Shih FF. Analysis of glutamine, glutamic acid and pyroglutamic acid in protein hydrolysates by high – performance liquid chromatography. J Chromatography A 1985;322:248-56.
8. Gardner WS, Miller WH. Reverse – phase liquid chromatographic analysis of amino acids after reaction with o – phthalaldehyde. Anal Biochem 1980;101:60-5.
9. Swanepoel E, de Villiers MM, du Preez JL. Fluorimetric method of analysis for D – norpseudoephedrine hydrochloride, glycine and L – glutamic acid by reversed – phase high – performance liquid chromatography. J Chromatography A 1996;729(1-2):287-91.
10. Liu D, Lai Y, Nie L, Yao Sh. Rapid detection of L – Glutamic acid using a series – electrode piezoelectric quartz crystal sensor. Anal Chimica Acta 1995;313(3):229-36.
11. Llácer JL, Fita I, Rubio V. Arginine and nitrogen storage. Curr Opin Struct Biol 2008;18(6):673-81.
12. Palloshi A, Fragasso G, Piatti P, Monti LD, Setola E, Valsecchi G et al. Effect of oral L – arginine on blood pressure and symptoms and endothelial function in patients with systemic hypertension, positive exercise tests, and normal coronary arteries. Am J Cardiol 2004;93(7):933-35.
13. Wu G, Meininger CJ. Arginine nutrition and cardiovascular function. J Nutr 2000;130:2626-29.
14. Grillo, MA, Colombatto S. Arginine revisited: Minireview article. Amino Acids 2004;26:345-51.
15. Staff AC, Berge L, Haugen G, Lorentzen B, Mikkelsen B, Henriksen T. Dietary supplementation with L – DK – arginine or placebo in women with pre – eclampsia. Acta Obstet Gynecol Scand 2004;83(1):103-07.
16. Wolf A, Zalpour C, Theilmeier G, Wang BY, Ma A, Anderson B et al. Dietary L – Arginine Supplementation normalizes platelet

aggregation in Hypercholesterolemic Humans. J Am Coll Cardiol 1997;29:479-85.

17. Gilhotra N, Dhingra D. Neurochemical modulation of anxiety disorders. International Journal of Pharmacy and Pharmaceutical Sciences 2010;2(1):1-6.

18. Karthik L, Kumar G, Rao KVB. Diversity of marine actinomycetes from nicobar marine sediments and it's antifungal activity. International Journal of Pharmacy and Pharmaceutical Sciences 2010;2(1):199-203.

19. Mao H-M, Chen B-G, Wang W-M, Zhuang P, Zong M, Xu Z-G. Simultaneous analysis of citrulline and arginine in serum and tissue. Microchem J 2011;97:291-95.

20. Wu G, Meininger CJ. Analysis of citrulline, arginine, and methylarginines using high – performance liquid chromatography. Methods Enzymol 2008;440:177-89.

21. Vishwanathan K, Tackett RL, Stewart JT, Bartlett MG. Determination of arginine and methylated arginines in human plasma by liquid chromatography – tandem mass spectrometry. J Chromatography B 2000;748:157-66.

22. Squellerio I, Tremoli E, Cavalc V. Quantification of arginine and it's metabolites in human erythrocytes using liquid chromatography – tandem mass spectrometry. Anal Biochem 2011;412:108-10.

23. Rodenas V, Parra A, Garcia – Villanova J, Gomez MD. Simultaneous determination of cefepime and L – arginine in injections by second – derivative spectrophotometry. J Pharm Biomed Anal 1995;13:1095-99.

24. Cobb KA, Novotny MV. Selective determination of Arginine and Tyrosine – containing peptides using capillary electrophoresis and laser – induced Fluorescence detection. Anal Biochem 1992;200:149-55.

25. Obreshkova DP, Tsvetkova DD, Iivanov KV. Validation of HPLC method for determination of L – Arginine in Tonotyl ® solution. International Journal of Pharmacy and Pharmaceutical Sciences 2012;4,Suppl 1:429-32.

26. Dimov NP. Method validation of a chromatographic method. Industrial Pharmacy – science and technology series. Volume 1: Chromatography in Pharmaceutical Analysis. 1st ed. Bulgaria, Sofia: Chemical Pharmaceutical Research Institute (NIHFI);1999;205-29.