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Research Article

VALIDATION OF HPLC METHOD FOR DETERMINATION OF L – ARGININE IN TONOTYL® SOLUTION

DANKA PETROVA OBRESHKOVA[1], DOBRINA DONCHEVA TSVETKOVA*[1], KALIN VALENTINOV IVANOV[2]

[1]Medical University – Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, [2]Medical University – Plovdiv, Faculty of Pharmacy. Email: dobrinkadoncheva30@yahoo.com

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ABSTRACT

An isocratic HPLC method with UV – detection for quality control of L – Arginine in Tonotyl® solution is validated in respect of analytical parameters selectivity, accuracy, precision and linearity. For model mixtures with added quantity of 80 mg, 100 mg and 120 mg L – Arginine, precision is estimated by standard deviation (SD) and relative standard deviation (RSD) (%): SD = 1.76, RSD = 2.20 % (80 mg); SD = 2.50, RSD = 2.50 % (100 mg); SD = 1.61, RSD = 1.32 % (120 mg). Accuracy is presented by the degree of recovery R (%) ± RSD (%): 99.66 % ± 0.71 (80 mg); 99.99 ± 2.20 (100 mg); 100.37 % ± 0.24 (120 mg). The obtained regression equation for L – Arginine is: $y = 2.10^8.x - 98532$, $R^2 = 0.996$. The applied HPLC analytical procedure can be used for quality control of food supplements, containing 100 mg L – Arginine, with great precision and accuracy in appointed linear interval.

Keywords: L – Arginine, HPLC, Accuracy, Precision, Linearity.

INTRODUCTION

Amino acids are of great importance for human health and espesially for football players [1] due to their different roles. L – Tyrosine is useful in following conditions: depression, Parkinson's disease, phenylketonuria and vitiligo 2. L – Lysine prevents of herpes and lowers the severity of osteoporosis by increasing the absorption and reducing the excretion of calcium 3. L – Leucine is involved in protein synthesis in the skeletal muscles 4.

L – Arginine is an amino acid, involved in numerous areas of human biochemistry, including: 1) changing into powerful neurotransmitter nitric oxide, which mediates it's biological effects by activating the soluble isoform of guanylyl cyclase and increasing the synthesis from GTP of secondary messenger cyclic GMP synthesis. Cyclic GMP can activate cyclic GMP – dependent protein kinase (PKG), causing by this way: a) smooth muscle relaxation and blood vessel dilatation; b) decreasing of blood pressure and platelet aggregation; c) improving of blood flow in the arteries of the heart [5], vascular function [6] and muscle metabolism; 2) reversing of endothelial dysfunction in hypertensive cardiac transplant recipients, hypercholesterolemic patients and in cigarette smokers; 3) ammonia detoxification; 4) regulation of growth hormone production; 5) enhancement of the spermatogenesis; 6) stimulation of: a) insuline secretion from pancreas, b) synthesis of the pituitary hormone vasopressin [7]; c) immune system, by increasing the output of T – lymphocytes from the thymus gland [8]; 7) prevention of wasting in people with critical illnesses [7]; 8) improvement of survival in gut – derived sepsis and peritonitis by modulating bacterial clearance 5, 9. Arginine vasopressin is nonapeptide, which regulates hypothalamus – pituitary adrenal system by enhancing the effects of corticotropine releasing factor on adrenocorticotropic hormone release 10. L-Arginine is used as a component of media for isolation of actinobacteria 11.

For the determination of L – Arginine are developed the following methods: 1) second derivative UV – spectrophotometry (in injections in combination with Cephradine) [12]; 2) VIS – spectrophotometry by measurement of the absorbance of derivative product of reaction between L – Arginine and thymol – sodium hypobromite reagent at λ_{max} = 440 nm) (in protein hydrolyzate) [13]; 3) indirect determination by graphite furnace atomic absorption spectrometry, after preconcentration on a nafion chemically modified tungsten coil [14]; 4) fluorimetry, after reaction with 2.3 – naphthalenedicarbaldehyde at $\lambda_{excitation}$ = 462 nm, $\lambda_{emission}$ = 520 nm (in serum) [15]; 5) isocratic RP HPLC with: a) UV – detection (with Ibuprofen) [16]; b) fluorescence detection by derivatization with naphthalene – 2.3 – dicarboxaldehyde (in human plasma) [17]; c) electrospray ionisation (ESI) in the positive mode (in human plasma) [18]; 6) gradient RP HPLC with: a) UV – detection at λ = 250 nm (in human plasma) [19]; b) chemiluminescence detection after the Sakaguchi reaction [20]; c) MS coupling with an atmospheric pressure chemical ionization (APCI) (in human urine) [21]; 7) Hydrophilic – Interaction Liquid Chromatography – MS (ESI) [22]; 8) High – Resolution Capillary Electrophoresis (combination with Ragaglitazar in tablets) 23.

The aim of current study to validate in respect of analytical parameters: precision, accuracy and linearity an isocratic HPLC method with UV – detection for quality control of L – Arginine in Tonotyl® solution and in food supplements, containing 100 mg L – Arginine.

MATERIALS

I) Reference standard (RS): L – Arginine. II) Reagents with analytical grade quality: 20 mM ammonia acetate, distilled water.

METHODS

HPLC

Chromatographic system

Liquid chromatograph Shimadzu (Japan) (LC – 10 Advp), equipped with: analytical column RP – 18 ODS (250 mm/4.6 mm i.d. /5 μm), column oven (CTO – 10 Asvp Shimadzu); isocratic pump (LC – 10 A); (UV – VIS – detector at fixed wavelength (SPD – 10 Avvp); 20 μl injector loop.

Chromatographic conditions

Mobile phase (MPh): 20 mM ammonia acetate; flow rate: 1.0 ml/min; column temperature: 40 ^{0}C; UV – detection at λ = 254 nm; volume for injection – 20 μl. Before using MPh was filtered throught membrane filter with pore size 0.45 μm.

Accuracy and precision (repeatability)

1) Preparation of model mixtures with L – Arginine

Three (3) equal homogenous model mixtures (MM) were prepared from all respective supplements (magnesium stearate, microcrystalline cellulose), by adding of RS L – Arginine, equivalent to 80 % (80 mg), 100 % (100 mg), 120 % (120 mg) of theoretical concentration. An average weight of MM were: 0.38 g (A_{80}), 0.4 g (A_{100}), 0.42 g (A_{120}). From each MM were prepared 3 samples, by dissolving in volumetric flasks an accurately weighed quantity, containing RS L – Arginine: 80 mg, 100 mg, 120 mg to 100.0 ml with MPh.

2) Preparation of standard solutions from RS L – Arginine

An accurately weighed quantity: 80 mg, 100 mg, 120 mg of RS L – Arginine was dissolved in MPh to 100.0 ml to obtain solutions with

concentration respectively: 8.10^{-4} g/ml; 1.10^{-3} g/ml; $1.2.10^{-3}$ g/ml L – Arginine.

Preparation of solutions for linearity

An accurately weighed quantity of RS L – Arginine: 80 mg, 100 mg, 120 mg was dissolved in MPh to 100.0 ml to obtain solutions with concentration correspondingly: 8.10^{-4} g/ml; 1.10^{-3} g/ml; $1.2.10^{-3}$ g/ml L – Arginine.

All solutions were filtered throught membrane filter with pore size 0.45 μm. The written HPLC method was applied and chromatograms were recorded.

RESULTS AND DISCUSSION

A) Validation of HPLC method for determination of L – Arginine for analytical parameters selectivity, accuracy, precision (repeatability) and linearity.

Selectivity

The "placebo" solution with all labeled in Tonotyl ® solution supplements (magnesium stearate, microcrystalline cellulose), without the active ingredients were prepared at the same manner, like reference standard of L – Arginine. The selectivity of the applied HPLC method was proved by the fact, that on chromatogram with "placebo" solution didn't exist peaks with t_R, corresponded to retention time: t_R = 2.9 min., obtained by reference standard L – Arginine and by L – Arginine in Tonotyl® solution.

Analytical parameters accuracy, precision and linearity for the validation procedure for HPLC method for determination of L – Glutamic acid are described in our previous work [24]. The current study is connected with the validation of the same parameters for the determination of L – Arginine in Tonotyl® solution.

Accuracy and precision (repeatability)

For model mixtures are summarized data for: 1) added RS L – Arginine: A_{80} (80 %), A_{100} (100 %), A_{120} (120 %) of labeled content; 2) weighed quantity of model mixtures: WA: WA_{80}, WA_{100}, WA_{120} (Table 1.); 3) high of peak (H): H A: H A_{80}, H A_{100}, H A_{120}; 4) Chauvenet's criterion for H (U H): U H A_{80}, U H A_{100}, U H A_{120} (Table 2.).

Table 1: Added content of RS L – Arginine in MM and weighed quantity of MM

N:	Added A_{80} [mg]	Weighed A_{80} [g]	Added A_{100} [mg]	Weighed A_{100} [g]	Added A_{120} [mg]	Weighed A_{120} [g]
1.	79.3	0.3875	99.8	0.4053	120.0	0.4200
2.	79.4	0.3886	100.3	0.4056	122.0	0.4270
3.	81.6	0.3890	100.5	0.4075	122.5	0.4288

Table 2: High of peak (H) for model mixtures of RS L – Arginine and Chauvenet's criterion for H

N:	H A_{80}	U H A_{80}	H A_{100}	U H A_{100}	H A_{120}	U H A_{120}
1.	55073	0.84	88887	0.75	131372	1.13
2.	55858	0.26	89814	0.38	136301	0.38
3.	57690	1.10	93670	1.14	137528	0.76
$\overline{X}$	56207		90790		135067	
SD	1343		2537		3258	
RSD [%]	2.39		2.79		2.41	

The content of L – Arginine is obtained by method of reference standard. On Table 3, are indicated: N – number of the individual measurements (1 ÷ 3); [A] – obtained quantity of L – Arginine in model mixtures: [A_{80}], [A_{100}], [A_{120}]; U [A] – Chauvenet's criterion for [A]: U[A_{80}], U[A_{100}], U[A_{120}]; R (%) – degree of recovery: R[A_{80}], R[A_{100}], R[A_{120}]; $\overline{X}$ – arithmetical mean; SD – standard deviation; RSD (%) – relative standard deviation; 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.

Table 3: Accuracy and precision for L – Arginine – estimation by method of calibration curve

N:	[A_{80}] [mg]	R[A_{80}] [%]	U[A_{80}]	[A_{100}] [mg]	R[A_{100}] [%]	U[A_{100}]	[A_{120}] [mg]	R[A_{120}] [%]	U[A_{120}]
1.	78.40	98.87	0.81	98.30	98.50	0.76	120.12	100.10	1.14
2.	79.29	99.86	0.31	99.25	98.95	0.38	122.58	100.48	0.39
3.	81.80	100.25	1.12	103.03	102.52	1.14	123.16	100.54	0.75
$\overline{X}$ ± SD	79.83 ± 1.76			100.19± 2.50			121.95 ± 1.61		
R̄ [%] ± RSD [%]		99.66 ± 0.71			99.99 ± 2.20			100.37 ± 0.24	
SD	1.76	0.71		2.50	2.20		1.61	0.24	
RSD [%]	2.20	0.71		2.50	2.20		1.32	0.24	
S $\overline{X}$	1.02	0.41		1.45	1.27		0.93	0.14	
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 $\overline{X}$	2.98	1.20		4.23	3.71		2.72	0.41	
$\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$	76.85 ÷ 82.81	98.46 ÷ 100.86		95.96 ÷ 104.42	96.28 ÷ 103.70		119.23 ÷ 124.67	99.96 ÷ 100.78	
E [%]	1.28	0.41		1.45	1.27		0.76	0.14	

For all of the obtained by the applied HPLC method data for high of peak and content of L – Arginine in every sample is necessary to estimate U, because when U for one value is higher than the relevant standard criterion (USt), the result must be removed as unexpected.

The relations: UH < 1.68 (Table 2.) and UC < 1.68 (Table 4.) show, that all experimental results for UH and UC are lower, than standard requirement: Umax = 1.68 (n = 3), and it isn't necessary to remove data for H and C.

For the assessment of 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.

Analytical parameter accuracy is presented by the degree of recovery R (%) and RSD (%) [25]. All results for R, suit relevant confidence inteval: [A_{80}]: 98.46 ÷ 100.86 (RSD = 0.71); [A_{100}]: 96.28 ÷ 103.70 (RSD = 2.20); [A_{120}]: 99.96 ÷ 100.78 (RSD = 0.24).

For the estimation of an analytical parameter precision (repeatability) is used the uncertainty of the result, which is determined by: SD, RSD and $\overline{X} \div t \,.\, S_{\overline{X}}$. From the assessment of precision [25], it is obvious, that the content of L – Arginine in model mixtures correspond to the relevant confidence inteval: [A_{80}]: 76.85 ÷ 82.81 (SD = 1.76, RSD = 2.20); [A_{100}]: 95.96 ÷ 104.42 (SD = 2.50, RSD = 2.50); [A_{120}]: 119.23 ÷ 124.67 (SD = 1.61, RSD = 1.32).

Linearity

For the investigation of analytical parameter linearity the prepared 3 solutions with decreasing concentration of reference standard L – Arginine are analyzed separately by the written HPLC method. The proportional accordance between the peak high (H) and concentration (C) in g/ml is found and the results are shown on Table 4.

Table 4: Results for analytical patameter linearity for L – Arginine

N:	Concentration [g / ml]	High of peak
1.	8.10^{-4}	55112
2.	1.10^{-3}	89246
3.	$1.2.10^{-3}$	131246

The results are putted into linearity regression analysis and the coefficient of regression (R) is calculated. The obtained regression equation, showing the proportional accordance H = f (C) is: y = 2.10^8.x – 98532, R^2 = 0.996. The calibration curve, presented the linearity of L – Arginine is illustrated on Fig. 1.

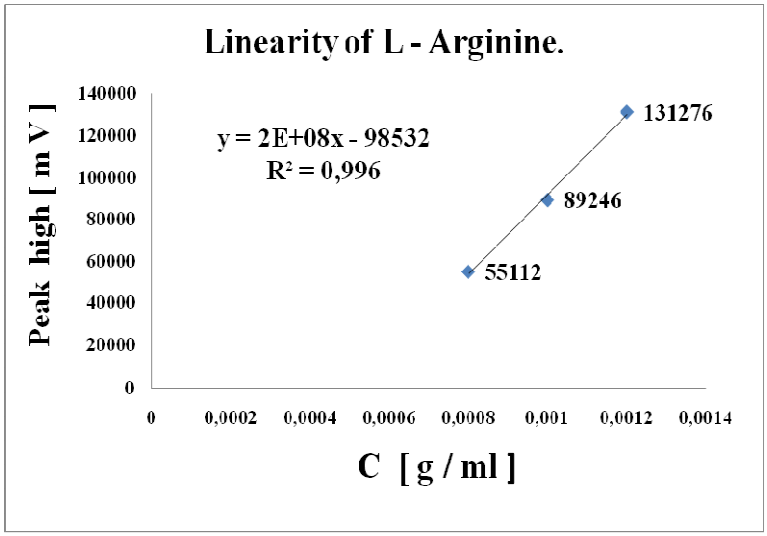

Fig. 1: Linearity for L – Arginine

CONCLUSION

All data for $\bar{R}$ [%] ± RSD (%) suit respective confidence intervals: A_{80}: 99.66 ± 0.71; A_{100}: 99.99 ± 2.20; A_{120}: 100.37 ± 0.24.

The obtained quantities of L – Arginine ($\overline{X}$ ± SD) in model mixtures are correspondingly: A_{80}: 79.83 ± 1.76; A_{100}: 100.19 ± 2.50; A_{120} : 121.95 ± 1.61.

The validated HPLC method is appropriate for determination of L – Arginine with great accuracy and precision in Tonotyl® solution and in other food supplements with the same content (100 mg) of L – Arginine.

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