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Novel Approach of Sakaguchi's Reaction for Quantitative Determination of L-Arginine *via* Flow Injection Analysis-Merging Zones with Spectrophotometric Detection

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A new batch and flow injection analysis-merging zones method characterized by simplicity, accuracy and speed for determination of L-arginine in pure material and in pharmaceutical formulations. The methods was based on oxidation-condensation for L-arginine with α-naphthol was added in urea, in presence of sodium hydroxide as a medium. For amino acid reaction, using sodium hypobromite as oxidizing agent to form an intense red water-soluble dye that is stable and has a maximum absorption at 501 nm. The optimum parameters were 5.5 mL min^{-1} flow rate using distilled water as a carrier, 42.19 µL sample volume (35 µg mL^{-1}), 43.175 µL [α-naphthol (1.4×10^{-3} M) in 10 % urea], 54.95 µL NaOBr (0.25 M) for L_1, L_2 and L_3 respectively and open value for the purge of sample segment and other chemicals. A graphs of absorbance *versus* concentration show that Beer's law is obeyed over the concentration range of 1-45 and 3-1400 µg mL^{-1} of L-arginine with detection limits of 0.5 and 0.1 µg mL^{-1} of L-arginine for batch and flow injection analysis-merging zones methods respectively. The optimized flow injection analysis system is able to determine L-arginine through put 45 h^{-1}. The correlation coefficient (r) was 0.992 and percentage linearity (% r^2) was 98.4 %, RSD % for the repeatability (n = 8) was 0.5-1.5 % for determination of arginine with concentration 15 and 30 µg mL^{-1}. The proposed method was applied successfully for determination of arginine in pharmaceutical formulations. The newly developed method has been statistically evaluated with official method and there were no significant differences between either methods. The proposed method can be accepted as an alternative analytical method for determination of L-arginine in pharmaceutical and biological samples.

Keywords: L-Arginine, α-Naphthol, Oxidation-condensation, Flow injection analysis-merging zones, Sakaguchi reaction.

INTRODUCTION

Amino acids are of great importance for human health and especially for football players due to different roles [1]. Tyrosine is useful in depression, Parkinson's disease, phenylketonurea and vitiligo [2]. Lysine prevents of herpes and lowers the severity of osteoporosis by increasing the absorption and reducing the excretion of calcium [3]. Leucine is involved in protein synthesis in the skeletal muscles [4]. L-Arginine (Arg.), Fig. 1, chemically known as 2-amino-5-guanidino pentanoic acid [5], involved in numerous area of human biochemistry, including:

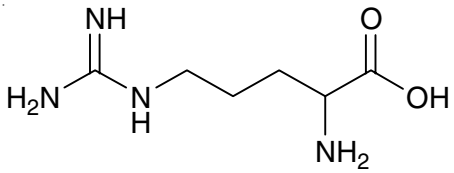


Fig. 1. Structure of L-arginine

(A) Changing into powerful neurotransmitter nitric oxide, which mediates its 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. Causing by this way [5] (1) smooth muscle relaxation and blood vessel dilatation. (2) Decreasing of blood pressure and platelet aggregation. (3) Improving of blood flow in the arteries of the heart, vascular function [6] and muscle metabolism. (B) Ammonia detoxification. (C) Enhancement of the spermatogenesis. (D) Reversing of endothelial dysfunction in hypertensive cardiac transplant recipients, hypercholesterolemic patients and in cigarette smokers. (E) Regulation of growth hormone production. (F) Stimulation of a) Insuline secretion from pancreas. b) Synthesis of the pituitary hormone vasopression [7]. c) Immune system by increasing the output of T-lymphocytes from the thymus gland [8]. (G) Prevention of wasting in people with critical illnesses [7], (H) Improvement of survival in gut-derived sepsis and peritonitis by modulating bacterial clearance [5,9].

L-Arginine is used as a component of media for isolation of actinobacteria [10]. L-Arginine vasopression is nonapeptide

which regulates hypothalamus-pituitary a drenal system by enhancing the effects of corticotropine releasing factor on adrenocorticotropic hormone release [11].

Several analytical methods had been developed for the determination of L-arginine and the majorities of them were applicable to microgram amounts. The reported methods are include indirect determination by graphite furnace atomic absorption spectrometry [12], fluorimetry [13], isocratic RP-HPLIC [14-16] and gradient RP-HPLC [17-19], capillary electrophoresis [20], flow injection analysis/chemiluminescence [21,22], ion-exchange [23] and UV-visible spectrophotometry [24-29]. Most of the present methods are expensive or required ion pair reagent, derivatization or laborious sample preparation procedures. The novelty of present method is analysis of L-arginine based on Sakaguchi reaction using FIA/mergining zones techniques without derivatization and without ion pair reagent. Through the reaction between L-arginine in basic solution with α-naphthol using sodium hypobromite as oxidizing agent in cooling place at 0 °C, gave a high intensity red product under effecting of urea were spectrophotometrically measured at 501 nm. The advantages of this method as compared to present methods is simple, fast and accurate. This method has been satisfactorily applied for the determination of L-arginine in pure material and dosage forms, the reaction can be carried out in batch and FIA and the two approaches were compared with official method.

EXPERIMENTAL

All spectral and absorbance measurements were performed on a Optima visible SP-300 digital single beam recording spectrophotometer (Japan), for the absorbance measurements as peak height through Kompensograph C1032, Siemens or absorbance with digital multimeter (DT9205A, China). Inside the detection unit, there is a flow cell (quartz, 1 cm) with 80 µL internal volume. For batch procedure, was carried out on Shimadzu UV-1800 (Japan) double-beam spectrophotometer and quartz cuvette with an optical path length of 1 cm. A one channel manifold was employed for the FIA/merging zones-spectrophotometer determination of L-arginine. A peristaltic pump (Master flex C/L, USA) with power supply (Yaxun, 1501 AD, china) used for pumping the carrier stream of distilled water through the valve (homemade, six-three injection valve (merging zone version), which moves at 90° and three loops (Teflon) were loaded with chemicals and the reagents solutions. This injection valve was employed to provide appropriate injection volumes of standard solutions and samples.

Flexible vinyl tubing of 0.3 mm internal diameter was used for the peristaltic pump. Reaction coil was made of glass with internal diameter of 2 mm. The whole components of the Merging Zone-FIA unit was shown in the Fig. 3. Distilled water as carrier was combined with injected sample [L-arginine in basic solution (NaOH), L_1] and they merged with the reagent (α-naphthol with urea, L_2) and (sodium hypobromite, L_3) as oxidizing agent, then mixed in reaction coil (RC) with length of 50 cm placed in cooling media using ice, injection sample 42.19 µL, flow rate of carrier of 5.5 mL min^{-1}, the absorbance as peak height (mV) was measured at 501 nm.

Analytical reagents grade chemicals and distilled water were used throughout. A standard solution of L-arginine ($C_6H_{14}N_4O_2$, 174.2 g mol^{-1}, BDH) (2000 µg mL^{-1} = 1.15 × 10^{-2} M) was transferred to a 200 mL volumetric flask and was dissolved and completed to the mark with distilled water, more dilution were made when it were necessary.

A stock solution of α-naphthol (m.w. = 144.17 g mol^{-1}, BDH) (1.4 × 10^{-3} M) was prepared by dissolving 0.02 g of α-naphthol in 95 mL of ethanol, shacked well and completed to 100 mL in volumetric flask with distilled water. Taking 20 mL of this standard solution and diluted with distilled water in 100 mL volumetric flask.

A stock solution of sodium hydroxide (40 g mol^{-1}, BDH) (2.5 M) was prepared by dissolving 10 g of NaOH in 100 mL volumetric flask in distilled water.

A 10 % urea was prepared by dissolving 10 g of urea (BDH) in 100 mL of distilled water in calibrated flask to prepare 1.67 M urea.

Sodium hypobromite (0.25 M NaOBr): was prepared by dissolving 5 g of NaOH (1.25 M) in 100 mL distilled water, then added 0.64 mL Br_2 (wt = 2 g, density = 3.103 g cm^{-3}).

Pharmaceutical preparations of L-arginine: Dosage forms were obtained from commercial sources available tablet by selecting 13 tablets from three types companies were analyzed by the developed methods. The names of the different suppliers, these included: (1) Argi powder (1500 mg of L-arginine HCl), OLIMP sport nutrition, 120 caps., EU. (2) Arginine powder (800 mg of USP L-arginine supplement facts), ULTIMATE NUTRITION, 100 caps., USA. (3) Ezerex, (2500 mg of L-arginine HCl), 30X6.4 g sachets, Italy.

The tablets were weighted an accurately, crushed and grinded using motor until fine powder, a 0.05 g of the each sample was weighing, equivalent to 500 µg mL^{-1} solution of active ingredient for each pharmaceutical formulation. The amount of L-arginine was dissolved in distilled water followed by filtration to remove any insoluble residue affecting on the response. The filtrate was transferred to a 100 mL volumetric flask and completed to the mark with distilled water to obtained 500 µg mL^{-1} (2.87 × 10^{-3} M) further dilute solutions were prepared to allocate the concentration within the linearity of the calibration curve.

General procedures for calibration

Batch method: An increasing concentration (1-45) µg mL^{-1} of 20 µg mL^{-1} L-arginine was prepared into a series of 25 mL standard flask, kept in ice, then put 1 mL of sodium hydroxide (2.5 M) and 1 mL of α-naphthol (1.4 × 10^{-3} M), mix well for (2 min), the added 0.1 mL of sodium hypobromite (0.25 M). The standard solutions were shaken for 4 s, 1 mL of urea (1.67 M) was added and thoroughly mixed. After 10 min, the absorbance of coloured product was measured at λ_{max} = 501 nm against the corresponding reagent blank.

Flow injection analysis method: A L-arginine solution in the range (3-1400) µg mL^1 was prepared from the standard working solution of 2000 µg mL^{-1}. The injection volumes of (42.19 µL (L_1) 43.175 µL (L_2) and 54.95 µL (L_3) are consist of 5 mL of Arg. and 1 mL of sodium hydroxide (2.5 M) was injected in loop 1, while of 1 mL of urea (1.67 M) and 1 mL of α-naphthol (1.4 × 10^{-3} M) was injected in loop 2. Sodium

hypobromite (0.25 M) was injected in Loop 3. Distilled water as carrier the sample and other chemicals of each loop were carried out with flow rate was 5.5 mL min^{-1}.

The resulting absorbance of the red product was measured at 501 nm and a calibration curve were constructed. Optimization of conditions were performed on 35 μg mL^{-1} of L-arginine.

RESULTS AND DISCUSSION

Preliminary studies (Sakaguchi reaction): L-arginine treated with sodium hypobromite and α-naphthol were placed in an ice bath (0 °C), developed an intensive red colour in an alkaline medium, were shaken a few second, then urea were added, an intense red product formed. This product has a maximum spectrum at 501 nm *versus* reagent blank which has negligible absorbance at the same wavelength (Fig. 2).

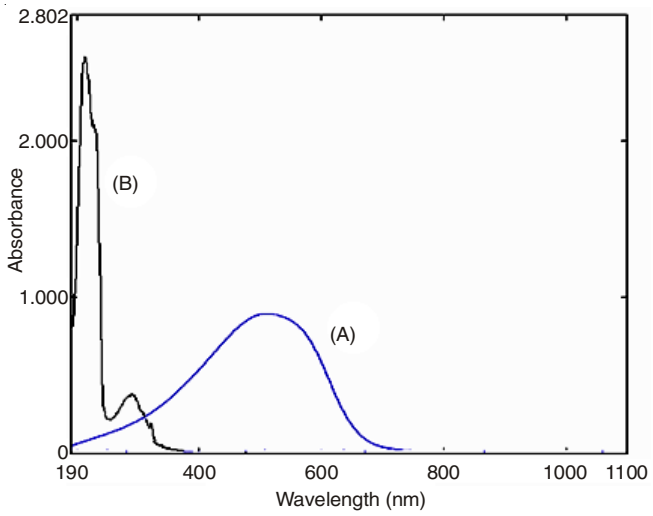


Fig. 2. Absorption spectra of the coloured product, 35 μg mL^{-1} of L-arginine against reagent blank (A) and blank against distilled water (B)

The factors affecting on the sensitivity and stability of the coloured product resulting from the oxidation of the amino acid (Arg.) with α-naphthol reagent in basic medium using sodium hypobromite as oxidizing agent, then add urea to the solution to remove the excess of sodium hypobromite and to stabilize the coloured product [30].

Optimization of the experimental conditions: The effects of various parameters on the absorbance intensity of the formed product were optimized.

Batch spectrophotometric determination: In the subsequent experiments, 20 μg mL^{-1} of arginine was taken in 25 mL final volume and performed by varing one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

Arginine concentration: Various concentrations of arginine (5, 10, 15, 20, 25, 30 μg mL^{-1}) were used with stabilized the other parameters, 20 μg mL^{-1} arginine seems to be optimum (Fig. 3).

Concentration of sodium hypobromite: The effect of various concentration of sodium hypobromite was investigated. A concentration of 0.1 M sodium hypobromite gave the highest absorbance and was chosen for further experiments (Fig. 4).

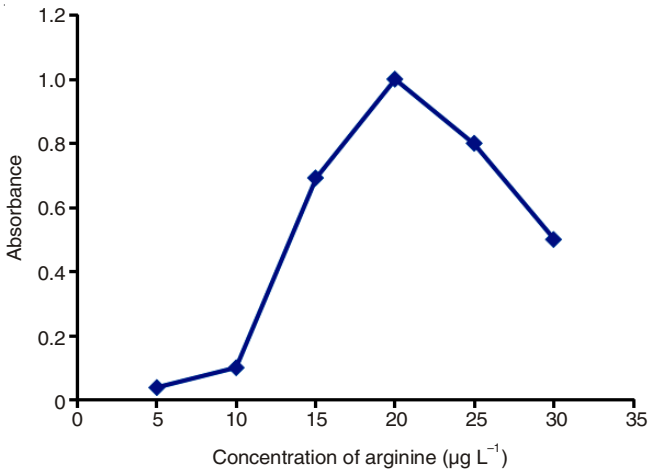


Fig. 3. Effect of the concentration of arginine

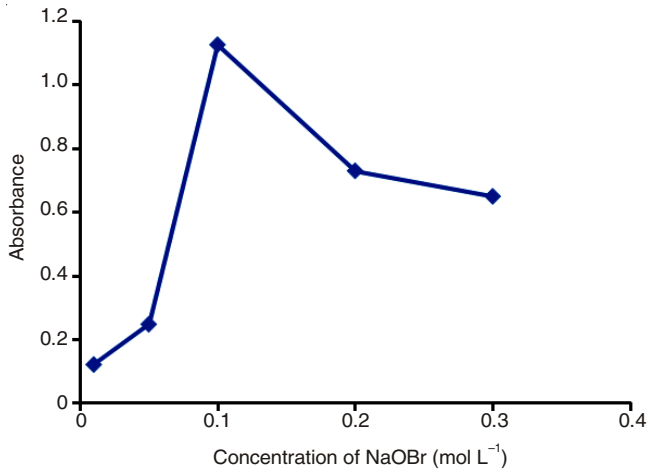


Fig. 4. Effect of concentration of oxidizing agent

Concentration of sodium hydroxide: The effect of concentration of sodium hydroxide was investigated by carring out the reaction using different volumes of NaOH ranging from (0.1-1.2 M). The maximum absorbance was obtained upon 1 mol L^{-1} was preferred (Fig. 5).

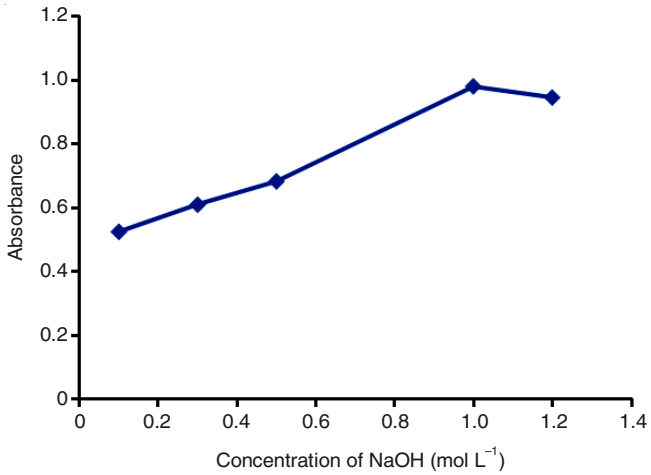


Fig. 5. Effect of concentration of NaOH

α-Naphthol concentration: The effect of using different concentration of α-naphthol was examined on the maximum

formation of the coloured product. Fig. 6 shows that 8×10^{-4} M was enough to obtain the maximum absorbance.

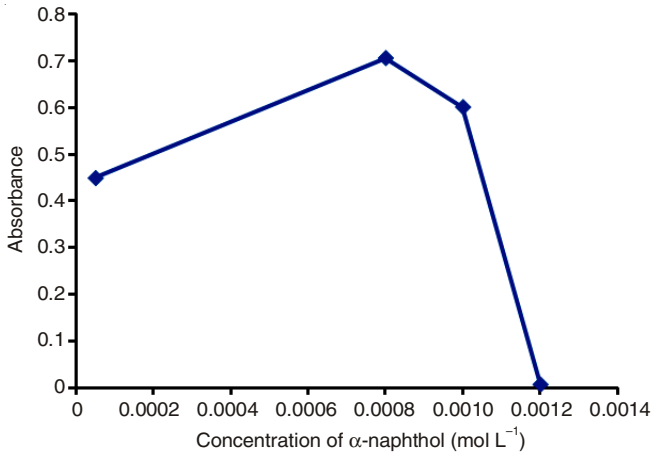


Fig. 6. Effect of α-naphthol concentration

Urea concentration: The effect of different concentrations of urea on the absorbance was studied, the highest absorbance at 0.3 M was found necessary for complete the reaction and it was adequate for the optimum (Fig. 7).

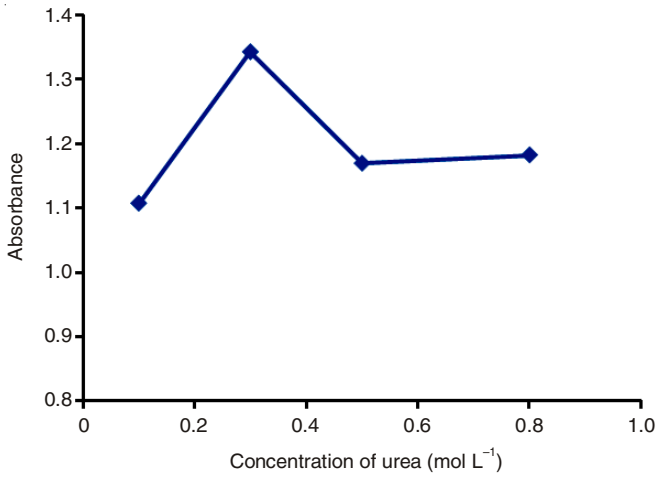


Fig. 7. Effect of urea concentration

Structure of product formed: The stoichiometry of the reaction between L-arginine and α-naphthol was investigated using continuous variation method [31]. In this method, Job's method was applied by putting 1 to 9 mL of 20 μg mL^{-1} of arginine in a series of 25 mL volumetric flask and put in ice then adding 1 mL of 2.5 M sodium hydroxide and 9 to 1 mL of 1.4×10^{-3} M α-naphthol, mix well for 2 min, then added 0.1 mL of sodium hypobromite (0.25 M), the solution was shaken for 4 s, then putting 1 mL of urea (1.67 M) and thoroughly mixed, after 10 min the absorbance was measured *versus* reagent blank at λ_{max} 501 nm (Fig. 8).

Using a mole ratio method, an increased volumes of (0.1-2) mL of (8×10^{-4} M), α-naphthol were added to a 1 mL of (20 μg mL^{-1}) L-arginine which was oxidation by 0.1 mL sodium hypobromite (0.1 M) and reacted with α-naphthol (8×10^{-4} M) and the coloured produced was stabilized by adding 1 mL of urea of 0.3 M (Fig. 9).

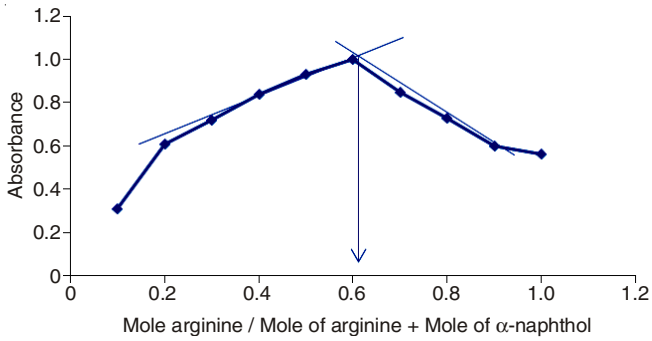


Fig. 8. Continuous variation plot of reaction between L-arginine and α-naphthol using batch procedure

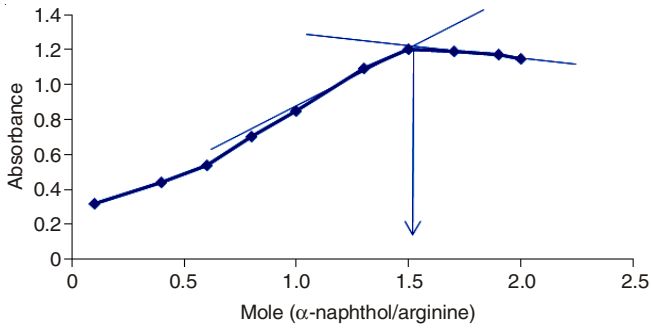


Fig. 9. Molar ratio for the reaction of arginine with α-naphthol at λ_{max} 501 nm using batch procedure

The results obtained from Figs. 8 and 9 show that 1:2 coloured product was formed and measured at 501 nm. The proposed mechanism that sodium hypobromite oxidizes the guanidine group of arginine and the resulting compound condenses with α-naphthol- a coloured solution, shown in **Scheme-I**.

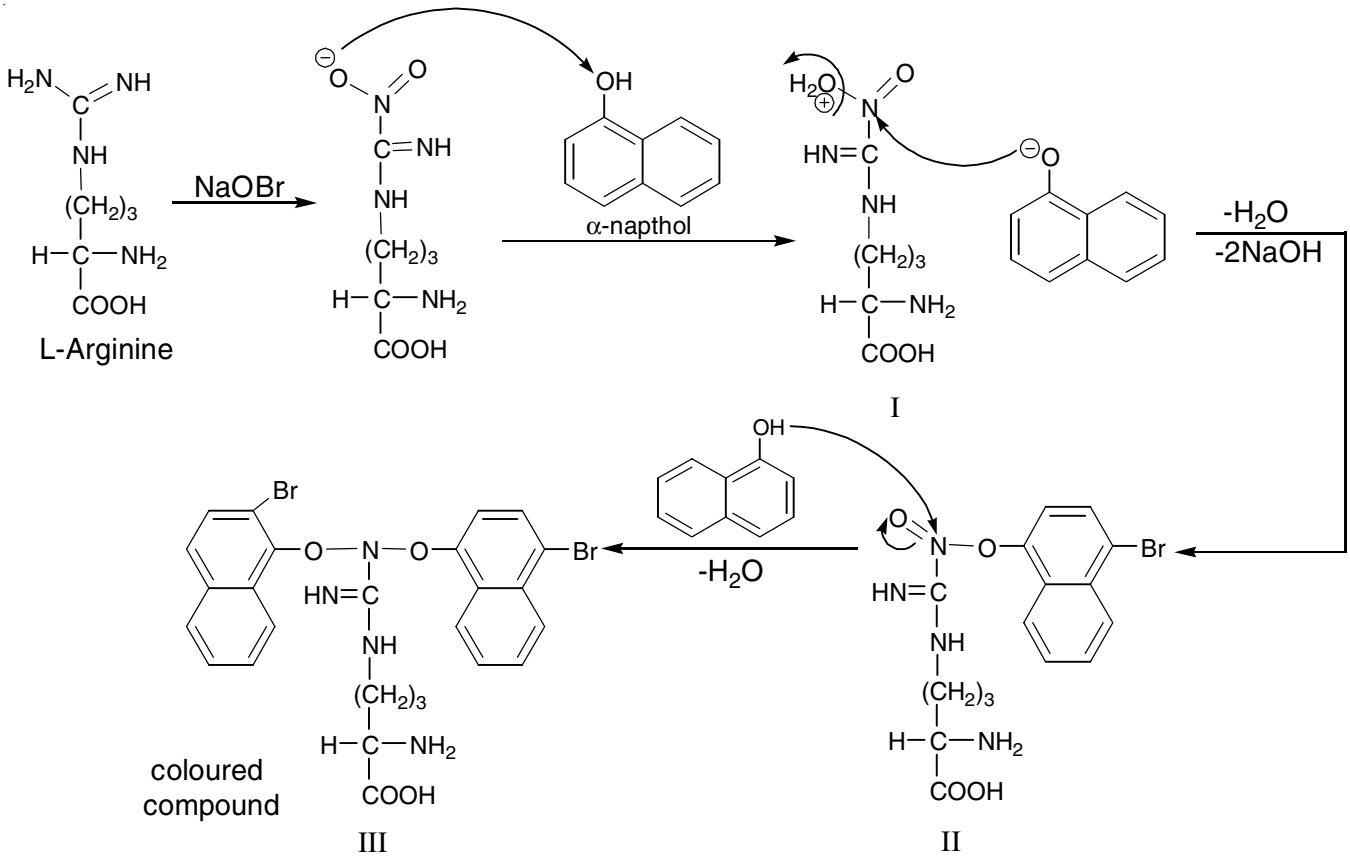
The product formed was soluble in water, the apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of L-arginine (1.148×10^{-4} M) and α-naphthol (A_s) using sodium hypobromite as oxidizing agent with that of a solution containing a five-fold excess of -naphthol reagent (A M) and according to analytical procedure.

The average of stability constant, $K = (1-\alpha)^2/4\alpha^3C^2$; α = Am-As/Am] [31] C=M(Arg.)] of the product in water under the described experimental of condition was 7.403×10^3 L mol^{-1}.

The regression equation obtained and the analytical features of the procedure are summarized in Table-1. It summarized the main performance of the flow/merging zones procedure developed for L-arginine determination in order to make an effective comparison between the two approaches.

FIA-merging zones spectrophotometric determination: The batch method for the determination of L-arginine was adopted as a basis to develop a FIA procedure.

Optimization of experimental conditions: A series of experiments were conducted to establish the conditions for the production of maximum repeatable response with good sensitivity for the reaction product. The manifold used for the determination of L-arginine was designed to provide different reaction conditions for magnifying the absorbance signal generated by reaction of L-arginine with (α-naphthol and urea)



Scheme-I: Proposed mechanism of the reaction between L-arginine with α-naphthol in alkaline medium

TABLE-1
EFFECT OF α-NAPHTHOL CONCENTRATION ON THE MEASUREMENT OF PEAK HEIGHT FOR [ARGININE-α-NAPHTHOL-OBr^-] SYSTEM

Concentration of [α-naphthol] M	Absorbance as peak height ($\bar{x}$) (n = 3) mV	Standard deviation (σ_{n-1})	Repeatability % RSD	Confidence interval of the mean $\bar{x} \pm t_{0.05}\frac{\sigma_{n-1}}{\sqrt{n}}$
1.0×10^{-4}	49	1.00	2.04	49 ± 5.16
5.0×10^{-4}	102	1.80	1.76	102 ± 4.45
8.0×10^{-4}	120	0.00	0.00	120 ± 0.00
1.0×10^{-3}	168	1.28	0.76	168 ± 5.60
1.2×10^{-3}	171	1.16	0.68	171 ± 2.90
1.3×10^{-3}	127	2.31	1.88	127 ± 4.76
1.4×10^{-3}	130	0.00	0.00	130 ± 0.00
1.5×10^{-3}	121	0.00	0.00	121 ± 0.00

in an ice bath for 10 min, then added hypobromite as oxidizing agent. Maximum absorbance intensity expressed as peak height in mV (n = 3) was obtained when the sample (35 μg mL^{-1} arginine with 2.5 M of sodium hydroxide), the reagent (1.4×10^{-3} M of α-naphthol in 1.67 M of urea) and 0.25 M of hypobromite were injected into L_1, L_2 and L_3, respectively into a carrier of distilled water with flow rate 5.5 mL min^{-1} (Fig. 10).

The chemical variables such as concentration of reagents used for oxidation and condensation reaction, pH of the reaction medium and physical variables including (injection time, flow rate, sample and reagents volumes, reaction coil length and temperature) that affecting on the absorbance of the coloured product were investigated.

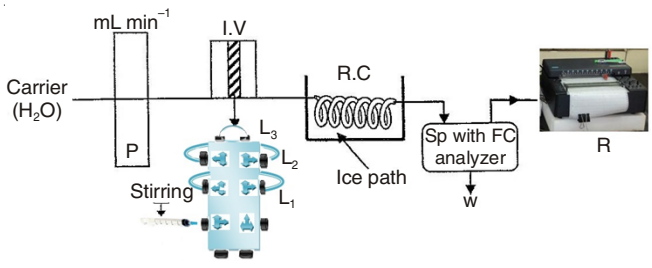


Fig. 10. Schematic diagram of FIA-merging zones system where, Sp with FC; spectrophotometry with flow cell, R.C.; reaction coil, I.V.; injection valve, P.; Peristaltic pump; R.; recorder and w.; waste (L_1, Arg. in NaOH) (L_2, α-naphthol with urea) (L_3, NaOBr)

Chemical parameters: The effect of various concentrations of sodium hydroxide (0.1-3 M) was studied for the

reaction between L-arginine and α-naphthol with urea in an ice bath (5 °C) using sodium hypobromite as oxidizing the guanidine group [32] of L-arginine and the resulting compound coudenses with α-naphthol which described above procedure, 2.5 M NaOH seems to be optimum (Fig. 11).

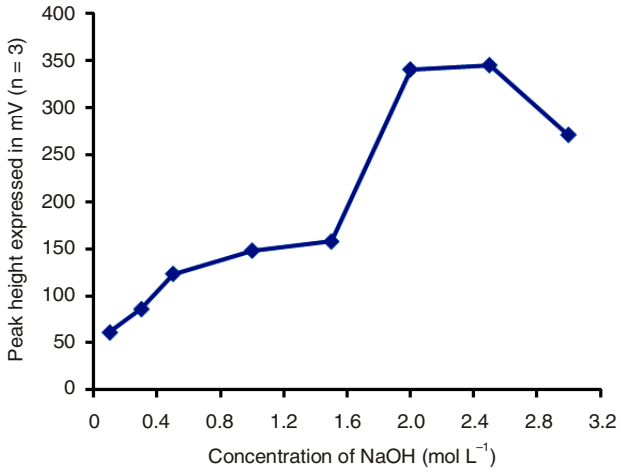


Fig. 11. Effect of the concentration of sodium hydroxide

The effect of various concentrations of α-naphthol which is soluble in ethanol (99 %) (1×10^{-4} to 1.5×10^{-3} M) was investigated. A concentration (1.4×10^{-3} M) of α-naphthol gave the highest response and was chosen for further experiments as shown in Table-2 and Fig. 12. One of the most important advantages of the FIA proposed system that reduce the number of channels of the manifold to get simple design (one line).

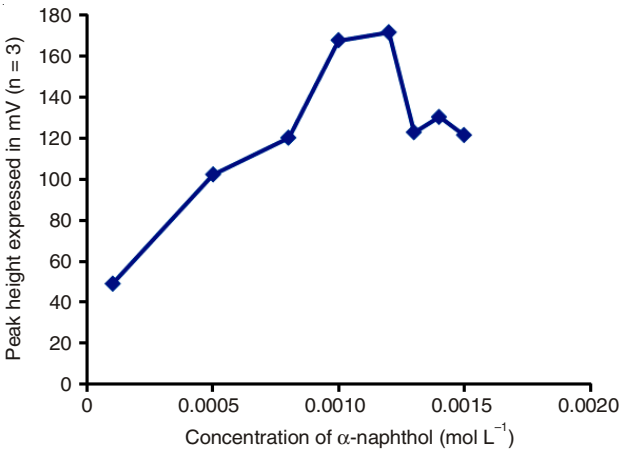


Fig. 12. Effect of concentration of α-naphthol reagent in (95 mL) ethanol (99 %)

The increase in the concentration of α-naphthol might be cause decrease in oxidization reaction of L-arginine. A concentration of 1.4×10^{-3} M α-naphthol gave the highest absorbance and was chosen for further experiments. Urea was added to the reaction, it mixed with α-naphthol was loaded in L_2 to increase the time of stability of the complex formed, therefore the effect of different concentrations of urea was studied and 1.67 M seems to be optimum (Fig. 13).

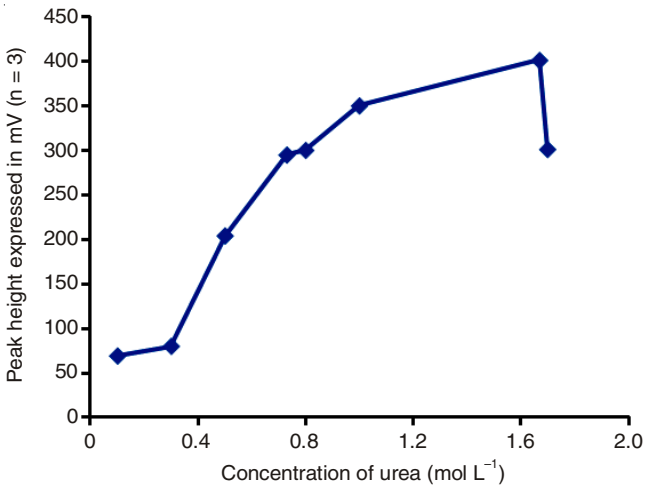


Fig. 13. Effect of concentration of urea

Effect of various concentration of sodium hypobromite was investigated. The concentration of 0.25 M was found to be the optimum value as oxidizing agent of L-arginine with α-naphthol in the presence of urea at ice path (5 °C) (Fig. 14).

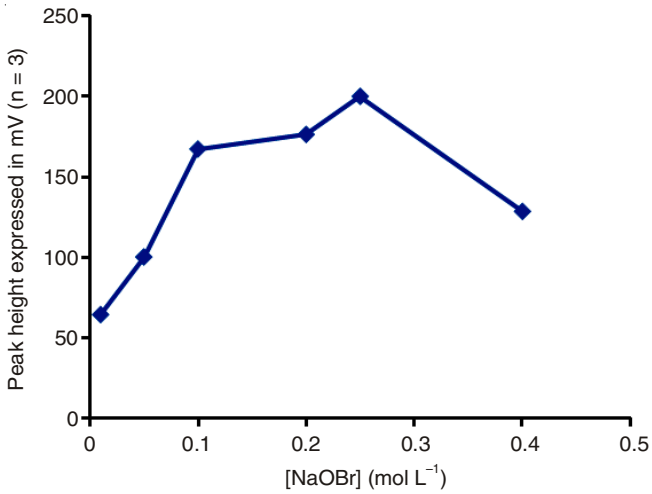


Fig. 14. Effect of variation of NaOBr concentration on peak height intensity

TABLE-2
EFFECT OF THE VARIATION OF FLOW RATE ON THE MEASURING OF PEAK HEIGHT

Pump speed indication approximate	Flow rate (mL/min) (carrier)	Average peak height (n = 3) mV	σ_{n-1}	% RSD	Confidence interval of the mean $\bar{x} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
5.5	1.1	163	2.30	1.40	163 ± 3.540
6.0	2.0	364	0.00	0.00	364 ± 0.000
6.5	2.9	323	1.06	0.32	333 ± 0.810
7.0	4.5	328	0.23	0.07	328 ± 0.178
7.5	5.5	291	1.10	0.38	291 ± 0.960
8.0	6.3	269	0.61	0.23	269 ± 0.580

The effect of using sodium hydroxide as carrier of the reaction was studied, Fig. 11 shows the optimum concentrations of α-naphthol (1.4×10^{-3}) in (10 % urea) with 35 μg mL^{-1} arginine and 0.25 M NaOBr. A preliminary physical condition was used 42.19 μL as sample volume (L_1), 43.175 μL α-naphthol in urea (L_2) and 54.95 μL of NaOBr (L_3). Fig. 11 explained a stability of the coloured product with decrease in response height when increase NaOH concentration. Therefore, it was concluded that distilled water can be used as carrier compared with the base was used. Distilled water was preferred as a carrier stream with high sensitivity.

Optimization of physical parameters: The influence of different physical parameters on the response profile of the coloured product was optimized through reducing the number of channels of the manifold (Fig. 10) to get a simple design with high sensitivity in determination. The variables studied under the optimum concentration of the reactant; α-naphthol (1.4×10^{-3} M), (10 % urea), (2.5 M NaOH), (0.25 M NaOBr) and preliminary concentration of L-arginine (35 μg mL^{-1}) in an ice bath through addition of oxidizing and condensation reaction.

The effect of total flow rate on the sensitivity of the coloured reaction product was investigated in the range of 1.1-6.3 mL min^{-1}. The result obtained showed that a total flow rate of 5.5 mL min^{-1} distilled water as carrier gave the highest response as shown in Fig. 15. The results are tabulated in Table-2. It was noticed that at low flow rates, there were an increase in peak base width, this mostly attributed to the dispersion and dilution which causes to an extended length of sample segment through crossing in FIA system.

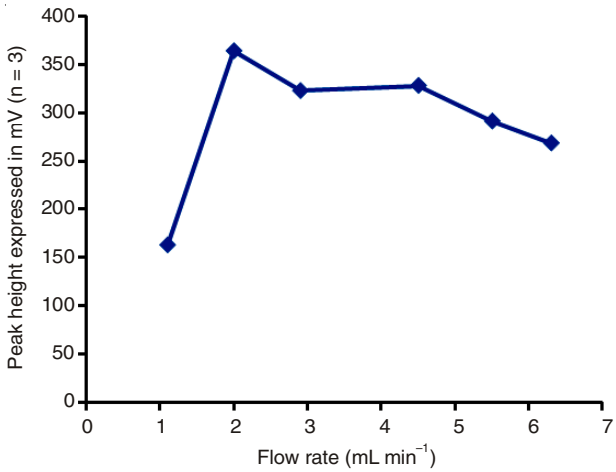


Fig. 15. Effect of total flow rate (mL min^{-1})

The effect of reaction coil length was evaluated through the use of a coil length 50, 100, 150 and 200 cm with i.d. 2 mm that was connected after injection valve directly in flow system (Fig. 10). The optimum concentration was used for oxidization reaction of L-arginine (35 μg mL^{-1}) with α-naphthol (1.4×10^{-3} M) and urea (10 %) as the injected sample in sodium hydroxide, with optimum volume of sodium hypobromite. The results obtained showed that a 50 cm coil gave the highest response (Fig. 16) and was used in all subsequent experiments.

The volumes of the injection sample and injection reagents varied (42.19, 43.175, 54.95 and 70.25) μL and (42.19, 43.175, 54.95 and 70.25) μL for sample and reagent volume,

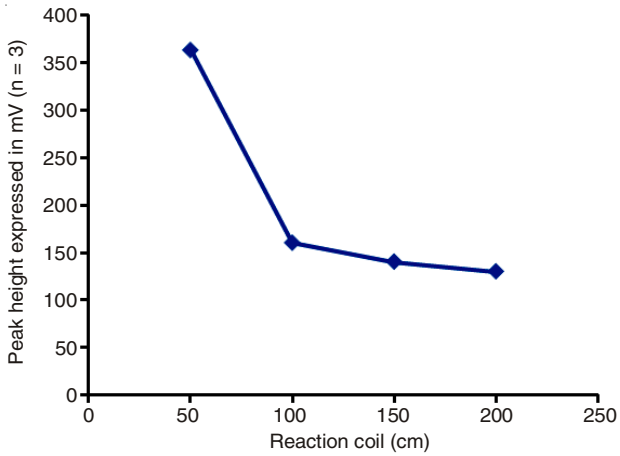


Fig. 16. Effect of length of the reaction coil (cm)

respectively using different lengths of loops and using open valve mode. The results showed that injected volumes of 42.19, 43.175 and 54.95 μL, respectively gave the best response (Fig. 17).

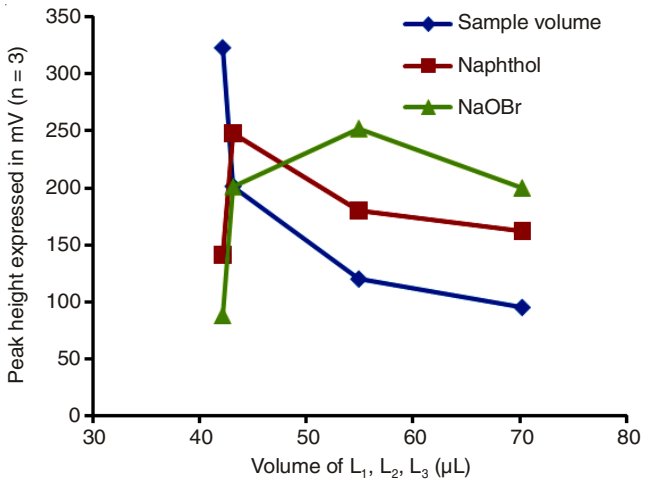


Fig. 17. Effect of variation of sample and reagent volumes of (a) L-arginine in NaOH; (b) α-naphthol with urea; (c) sodium hypobromite

Fig. 17 showed that an increasing of sample volume up to 42.19 μL lead to a significant decrease in response height, the reaction between L-arginine and α-naphthol with urea using sodium hypobromite as oxidizing agent in base medium was found to be instantaneous. However, the reaction is completed within 65 s in an ice bath (5 °C), but 80 s was sufficient to get maximum intensity and stability colour, after the addition of urea.

The effect of temperature on the coloured product was studied at 5, 25 and 45 °C. The results showed that the oxidation of L-arginine was occurred at 5 °C in an ice bath with high stability of the coloured product at least 30 min, as shown in Fig. 18.

The injection time is also an important parameters that affected on the sample throughput and was investigated by calculating the interval time between the sample injection and the appearance of the end of the signal. The reaction time of each sample was 80 s, therefore the sample through put were 45 samples per hour.

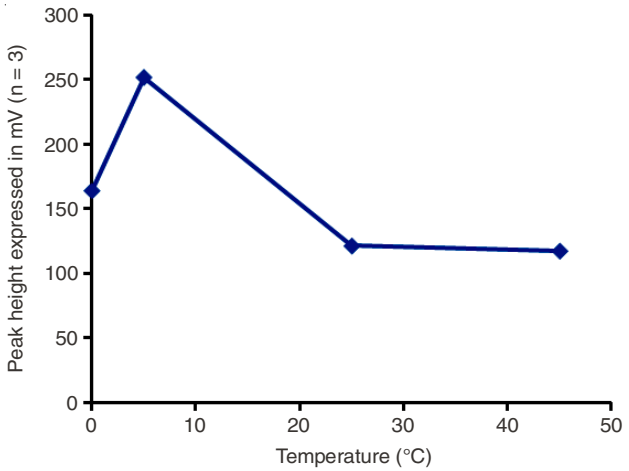


Fig. 18. Effect of temperature (°C) on peak height of coloured product

Analytical parameters: Under the optimum conditions described above, calibration graph for L-arginine (Fig. 19) was constructed by plotting average peak height (n = 3) in mV as a function of the analyte concentration. Linear calibration graph for the individual determination in the range of 3-1400 μg mL^{-1} for L-arginine. Analytical characteristics such as detection limit, linear range, relative standard deviation and correlation coefficient of each method were determined [30] (Table-3). Table-3 demonstrates the high sensitivity of the developed procedure.

Statistical evaluation of regression line gave the value of standard deviation for residuals ($S_{y/x}$) slope (S_b) and intercept (S_a) at 95 % confidence limits for (n-2) are demonstrated in Table-3.

These small points were referred to high repeatability and reproducibility of the developed FIA compared with the batch procedure. The FIA-merging zone is more convenient than the former method because of its speed (sample through put of 45 injection h^{-1}), wider linear range of calibration graph and good recovery were obtained.

Analysis of pharmaceutical samples: The suggested methods were applied for the quantitative determination of L-arginine in pharmaceutical formulations. Three types (different origins of these preparations) containing L-arginine were analyzed and Table-4 summarizes the results obtained for these

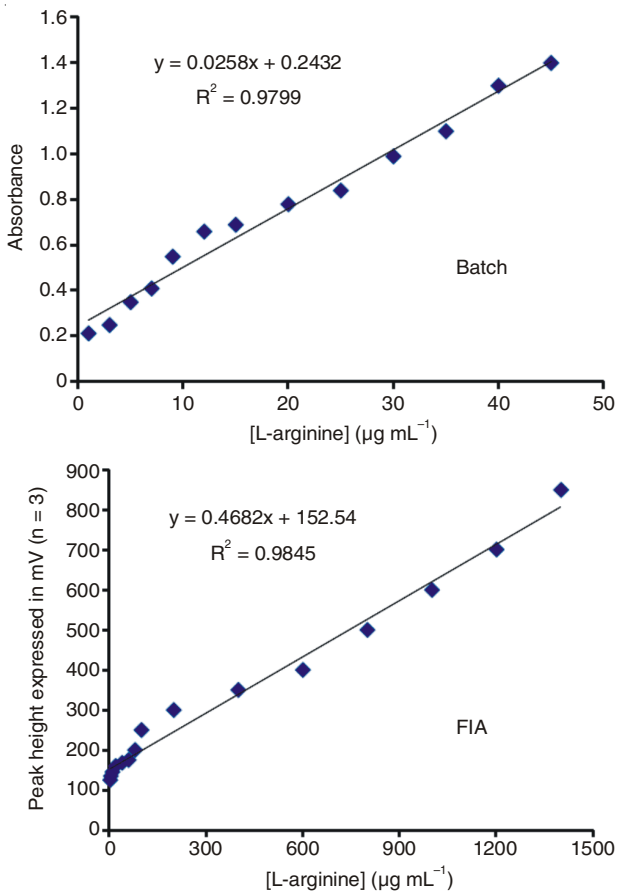


Fig. 19. Calibration graph for L-arginine

preparations, they gave a good accuracy and precision. There is no interference from the excipients in accordance with those obtained by official method [33]. The result obtained by the proposed methods by applying the F-test and t-test at 95 % confidence limits. The calculated valued for F-test were (5.459) and (3.45), t-test values were (0.553) and (0.613) for the batch and FIA methods, respectively, did not exceed the critical values of F-test = 19.01 and t-test = 2.770 ($n_1 + n_2 - 2 = 4$). These confirming that there are no significant differences between the proposed method and the official method with respect to precision and accuracy in determination of L-arginine in pharmaceutical formulations (Table-4).

TABLE-3
ANALYTICAL AND REGRESSION PARAMETERS OF THE PROPOSED METHOD FOR THE DETERMINATION OF L-ARGININE

Parameters	Batch method	FIA method
Linear range (μg mL^{-1})	1-45	3-1400
Regression equation	y = 0.025x + 0.243	y = 0.468x + 152.5
Correlation coefficient (r)	0.978	0.984
Linearity (r^2 %)	97.9	98.4
Relative standard deviation (RSD %)	0.0145 (at 15 ppm)	0.057 (at 60 ppm)
Slope (b), (μg mL^{-1})	0.025	0.468
Intercept (a)	0.243	152.5
Confidence limit of intercept (a) = a ± tS_a	0.2432 ± 0.0342	152.5 ± 5.8423
Confidence limit of slope (b) = b ± tS_b	0.0258 ± 0.00136	0.468 ± 0.0433
Standard deviation of slope (S_b)	3.9×10^{-3}	0.0185
Standard deviation of intercept (S_a)	0.19	25.39
Average of recovery (%)	99.5	100.025
Limit of detection (LOD)	0.468	0.12
Limit of quantification (LOQ)	4.68	1.186
Sample through put (h^{-1})	4	45

TABLE-4
APPLICATION OF THE PROPOSED METHODS AND OFFICIAL METHOD FOR THE DETERMINATION OF L-ARGININE IN PHARMACEUTICAL PREPARATIONS

Pharmaceutical preparations	Proposed methods						Official method recovery (%)
	Batch			FIA-merging zones			
	Present conc. (μg mL^{-1})	Rec.* (%)	RSD* (%)	Present conc. (μg mL^{-1})	Rec.* (%)	RSD* (%)	
Argi Power (2.5g L-arginine HCl) MEGA CAPS, EU	12	99.99	0.06	40	99.99	1.71	100.2
	30	100.01	0.023	100	99.54	0.249	
Arginine Power (0.8g L-arginine supplemenmts facts), USA	12	99.58	0.00	40	100.00	2.5	100.5
	30	101.7	0.023	100	98.34	0.587	
Ezerex (2.5g L-arginine, sachets) (Sigma-Tau), Italy	12	99.00	0.06	40	99.15	1.45	100
	30	99.93	0.033	100	100.99	0.99	

*Average of five determinations.

Conclusion

A batch and FIA-merging zones methods were described for the determination of L-arginine, based on oxidation-condensation reaction of arginine in basic medium with α-naphthol in urea using sodium hypobromite as oxidizing agent in an ice bath (5 °C). Although very few methods are available for the determination of arginine by spectrophotmetric analysis [23,34]. The suggested method is simple, rapid and offers the advantages of sensitivity more than all reported spectrophotometric methods, which needed a high temperature, high cost materials and reagents and have low linear range compared with the proposed methods that obeyed Beer's law and gave a good application for the pharmaceutical preparation. The wide applicability of the new method for routine quality control is well established by analyzing the assay of L-arginine at trace concentration level in dosage forms.

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