Development And Validation of Uv Visible Spectrophotometric Method for Estimation of L -Arginine in Bulk and Pharmaceutical Dosage Form

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***Abstract*—The study focused on the development and validation of a simple, cost-effective, and reliable UV-visible spectrophotometric method for the quantitative analysis of L-arginine in bulk and tablet formulations. A solvent system of water and 0.1N HCl was employed owing to its solubility and economic feasibility. A stock solution of 1000 µg/mL was prepared, followed by suitable dilutions for analysis, and the absorption maxima (λ max) of L-arginine was found to be 207 nm. The method was validated according to ICH guidelines for parameters such as linearity, precision, accuracy, specificity, robustness, and sensitivity. Linearity was established in the range of 5–30 µg/mL with an excellent correlation coefficient (R^2 = 0.999). Precision studies demonstrated reproducibility with %RSD values below 2% for both intra-day and inter-day assays. Accuracy, evaluated using the standard addition method, showed recoveries within 100 ± 2%, confirming the method's reliability in the presence of excipients. Specificity was confirmed by the absence of spectral interference, and robustness testing through deliberate variations in wavelength and solvent volume indicated minimal influence on absorbance values. The method exhibited high sensitivity with an LOD of 0.058 µg/mL and an LOQ of 0.2 µg/ml. Assay of tablet formulations yielded a purity of 99.28%, well within acceptable limits. Overall, the validated method proves to be accurate, sensitive, specific, and robust, making it highly suitable for routine pharmaceutical quality control of L-arginine.**

***Index Terms*—Spectrophotometric, Validation, L-arginine, Linearity, Sensitivity, Specificity**

I. INTRODUCTION

L-arginine is chemically 2-Amino-5-guanidinopentanoic acid.

Molecular formula -$C_6H_{14}N_4O_2$

It is a White crystalline or powder form it is Synthesized from citrulline via the urea cycle

It acts as Precursor of nitric oxide (NO) via nitric oxide synthase; modulates vascular tone, immunity, and wound healing It participates in Absorption: Rapid; Metabolism: Liver; Excretion: Primarily renal Survey of the literature review revealed that few analytical methods are available for the estimation of water soluable L-arginine by using HPLC, HPTLC. Yet there is no method reported in the literature for the estimation of L-arginine by using UV Spectroscopy. This work aims to develop a method and validate for the estimation of L-arginine by using UV Spectrophotometer by using aqueous solvent system.

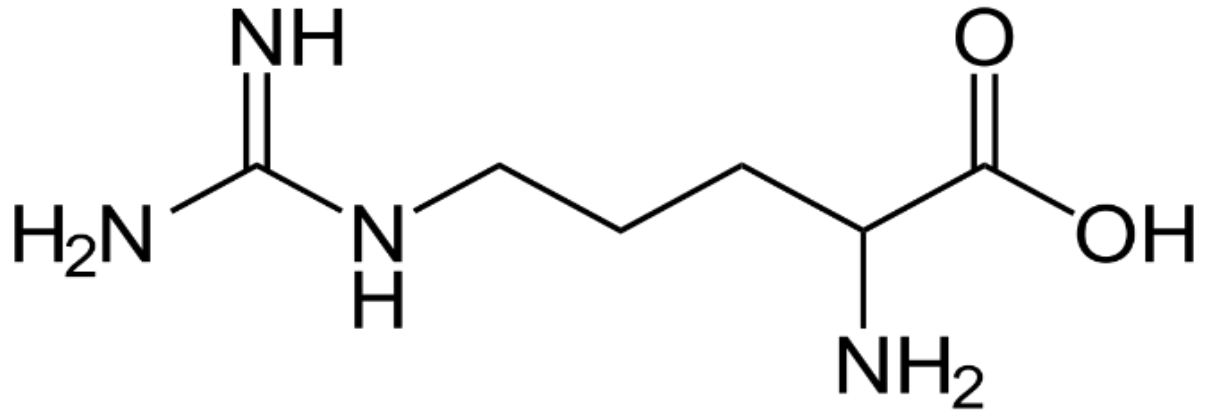

Figure-1: Chemical Structure of L-Arginine

II. MATERIAL AND METHOD

API of L-arginine by I Dreamz Health Care, No.274C, Road No.5A, Harohalli Industrial area, 2nd Phase, Harohalli, Karnataka 562112 as gift sample. L-arginine dosage form was purchased from Apollo pharmacy. Shimadzu (U-1900i) UV/Visible spectrophotometer coupled with UV- probe data acquisition software.

2.1 METHOD DEVELOPMENT

2.1.1 SELECTION OF SOLVENT

HCl, 0.1 NaOH, formic acid, methanol, and acetonitrile. The results showed that L-Arginine was highly soluble in water, 0.1N HCl, NaOH and 0.1% formic acid, while it exhibited less solubility in methanol and acetonitrile. Based on its cost-effectiveness and safety profile, Water and 0.1N HCl were chosen as the preferred solvents for dissolving the drug and drug product The study investigated the solubility of L-Arginine in different solvents, including water, 0.1N.

2.1.2 PREPARATION OF STOCK SOLUTION (1000µg/ml)

Accurately weighed quantity of pure L-Arginine (10mg) was transferred into 10 ml volumetric flasks, dissolved with the solvent system of water, and made up to 10 ml with the same solvent to give a solution containing 1000µg/ml. The solution was sonicated for 5 minutes.

2.1.3 PREPARATION OF STANDARD SOLUTION (20µg/ml)

1ml of each stock solution was taken and transferred to another separate 10ml volumetric flask and the same solvent system was added up to 10ml for additional dilution to give a solution containing 20µg/ml. From the above solution, 2ml was further diluted to 10ml to obtain 20 µg/ml of L-Arginine .

2.1.4 PREPARATION OF SAMPLE SOLUTION (20µg/ml)

The tablet powder equivalent to 10mg of L-Arginine was transferred into a 100 mL volumetric flask, diluted to 100ml with 0.1 N HCl. The resultant solution was further diluted with the water to obtain a solution containing 20µg/ml concentration of L-arginine. The sample solution was filtered through the 0.25µm Nylon filter.

2.1.5 DETERMINATION OF ABSORPTION MAXIMA (λ max)

30µg/ml of L-Arginine solution was scanned in the UV spectrophotometer from 200-400nm to determine the λ max of the given compounds. The λ max of L-Arginine was observed to be 207 nm

2.2 METHOD VALIDATION

Analytical method validation is the process of proving that an analytical method is suitable for its intended purpose and capable of producing reliable and consistent results. In pharmaceuticals, chemical manufacturing, and food safety industries, method validation is essential for ensuring product quality, safety, and efficacy. The main parameters evaluated during the validation process such as Accuracy, Precision, Specificity, Linearity, Sensitivity, Robustness

2.2.1 LINERITY

The method's linearity means concentration immediately affects test findings. The linearity of the current approach was tested by measuring absorbance 207nm for L-Arginine concentrations from 5 to 30 µg/ml. Finally, the concentration-absorbance linearity graph was plotted, and the regression coefficient (R^2) was calculated.

2.2.2 PRECISION

Precision studies were conducted to evaluate intraday and Interday variations of L-Arginine at 20µg/mL concentrations. The Stated concentration was subjected to analysis for 6 times. The % RSD can be evaluated for the obtained absorbance values.

2.2.3 ACCURACY

The recommended method's accuracy was verified through the standard addition method at 50%, 100%, and 150% concentrations. This involved adding stated concentrations of pure drug solutions to a predetermined amount of the L-Arginine tablet sample (20µg/mL) and measuring the absorbance at the respective wavelength. The analysis of the percentage recovery at each level was conducted.

2.2.4 SPECIFICITY

Specificity refers to the capability of the method to accurately detect the analyte in the presence of other potentially interfering components. The specificity of the developed method for determining L-arginine in tablet dosage form was assessed by comparing the spectral characteristics of the tablet solution to those of the standard solution. The sample spectrum was thoroughly examined to identify any potential interferences arising from the presence of excipients.

2.2.5 ROBUSTNESS

The maximum absorption wave length were purposely changed to test the method's resilience. After changing the wavelength maximum (± 2nm), % RSD can be evaluated for obtained absorbance values.

2.2.6 SENSITIVITY

Standard deviation equations were utilized to calculate LOD and LOQ.

LOD=3×σ/S

LOQ = 10×σ/S

Where,

σ is the standard (SD) of the intercept

S -slope of the linear plot

2.3 ASSAY OF DOSAGE FORM

The marketed 20 tablets were weighed, and the powder equivalent to 20mg of L-Arginine was transferred into a 100 mL volumetric flask, diluted to 100ml with 0.1N HCl. The resultant solution was further diluted with water to obtain a solution containing 20µg/ml L-Arginine. The sample solution was filtered through the 0.25µm Nylon filter. Both sample and standard solutions of L-Arginine were analyzed. Assay of the compound was analyzed by following formula.

$$\% \text{ Assay} = \frac{AT}{AS} x \frac{WS}{DS} x \frac{DT}{WT} x \frac{P}{100} x \frac{\text{AVG Wt}}{\text{LabelClaim}} x100$$

Where:

AT - Absorbance of sample (tablet) solution.

AS - Absorbance of standard solution.

WS - Weight of standard substance (mg)

DS -Dilution factor of standard solution

DT -Dilution factor of sample solution

WT - Weight of sample (tablet) powder (mg)

P - % purity of standard substance

AVG WT -Tablet weight in average (mg)

III. RESULTS AND DISCUSSION

Figure 2, shows that scanned spectra of L-arginine bulk and pharmaceutical dosage form show absorption between 200 to 250nm. The maximum absorption (λ max) scanned at 207nm.1 Determination of Absorption Maxima (λ max)

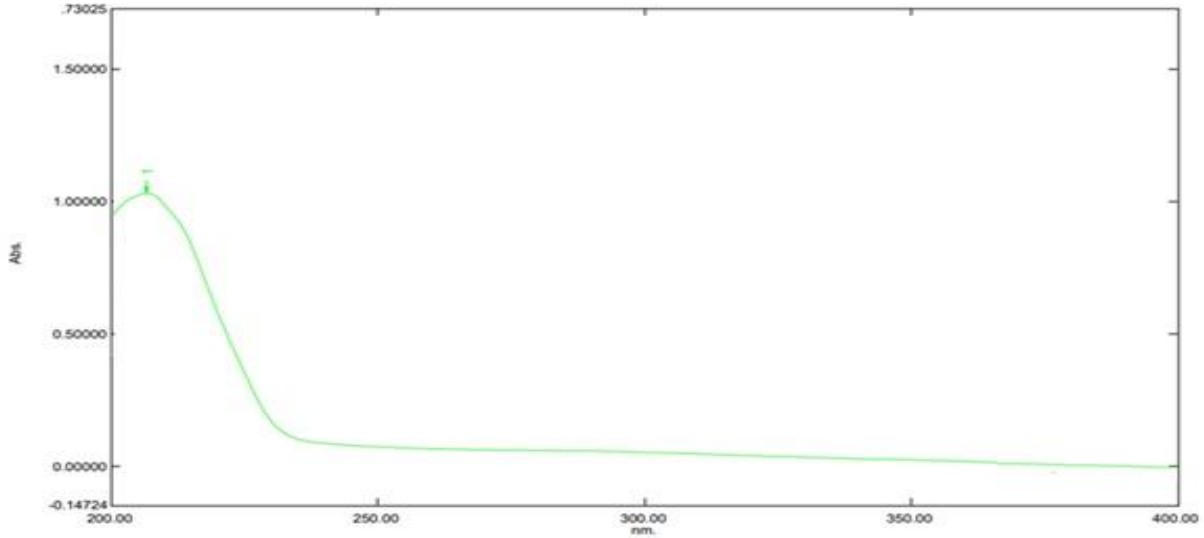

Figure-2: Absorption Maxima of L-Arginine

3.2 Method Validation

3.2.1 LINEARITY

The Calibration curve of L-arginine shows good Linearity at concentration ranging from 5-25µg/ml. The graph were plotted against Absorbance vs Concentration and fit a linear Regression line: y=mx+b The three trials was conducted as Set1, Set2, Set3 with same concentration. The Correlation co-efficient (R^2) is calculated. $r = n(\varepsilon xy) - (\varepsilon x)(\varepsilon y)$

√[nεx2 −(εx)2][nεy2 − (εy)2] The correlation coefficient (R^2) was was within the working concentration. The intercept and slope of L-arginine were respectively.

		Absorbance at 207 nm			
% Level	Concentration (µg/mL)	SET-1	SET-1	SET-1	AVG
50	5	0.17	0.19	0.18	0.180
100	10	0.35	0.4	0.32	0.350
125	15	0.51	0.49	0.56	0.520
150	20	0.71	0.63	0.69	0.670
175	25	0.83	0.81	0.87	0.830
150	30	0.96	0.97	0.99	0.977

Table 1: Linearity of L -Arginine

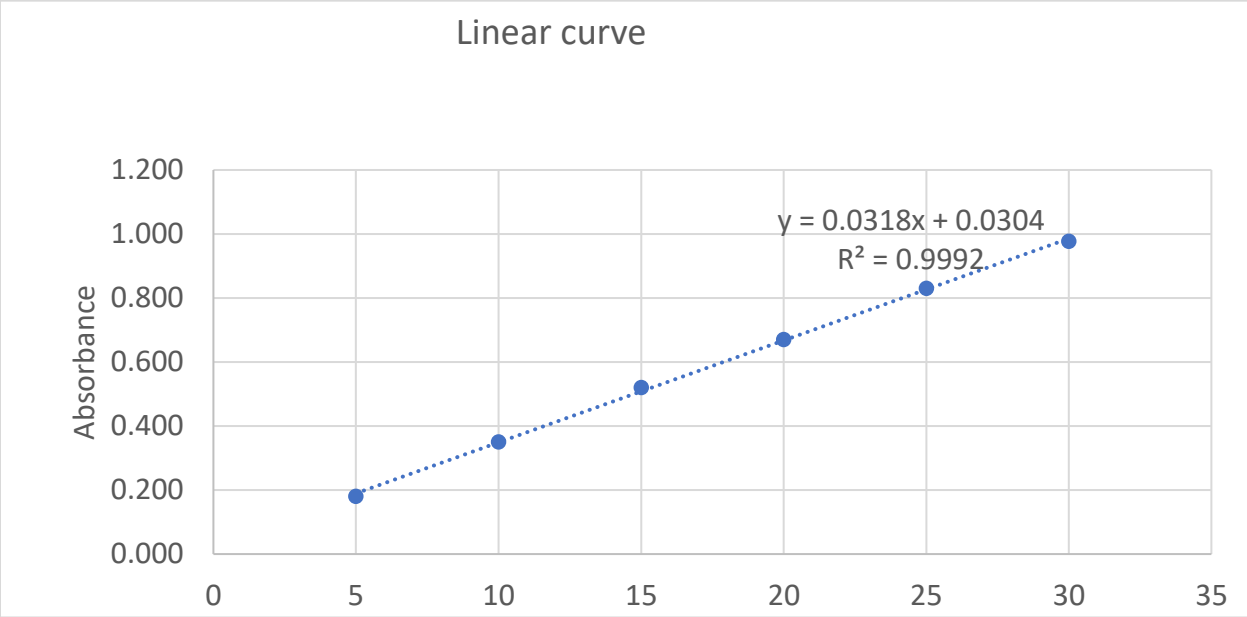

Figure-3: Linearity curve of L-Arginine at 207 nm

3.2.2 ACCURACY

Using known concentration, the measured absorbance and %recovery are presented in Tabel 2%Recovery=Observed value/True value × 100. This value portrays high reproducibility with accuracy was found to be satisfactory

Sample solution	% level	Amount added	Amount recovered	%Recovery	Acceptance limit
Set-1	50	10	9.96	99.6	98-102%
	100	20	19.98	99.49	
	150	30	28.63	95.43	
Set-2	50	10	10.04	100.4	
	100	20	19.89	99.45	
	150	30	30.05	100.16	
Set-3	50	10	9.89	98.9	
	100	20	19.97	99.85	
	150	30	29.95	99.83	

Table 2: Accuracy of L-Arginine

3.2.3 PRECESION

The intraday and interday precision for proposed method are presented in Table- 3 and table-4 . The Relative Standard Deviation (%RSD) for L-arginine Precision studies were conducted to evaluate intraday and interday variations of L-Arginine at 20µg/mL concentrations. The State concentration was subjected to analysis for 6 times. The % RSD can be evaluated for the obtained absorbance values. This value portrays high reproducibility with precision found satisfactory

	SI.NO	Absorbance at 207nm
10µg/ml	1	0.679
	2	0.667
	3	0.680
	4	0.69
	5	0.660
	6	0.689
	AVG	0.677
	SD	0.011
	%RSD	1.76

Table 3: Inter-day precision of L-Arginine

	SI.NO	Day-1 Absorbance	Day-2 Absorbance	Day-2 Absorbance
10µg/ml	1	0.679	0.671	0.682
	2	0.667	0.677	0.679
	3	0.68	0.69	0.683
	4	0.69	0.668	0.681
	5	0.66	0.697	0.689
	6	0.689	0.7	0.7
	AVG	0.677	0.683	0.685
	SD	0.011	0.013	0.007
	%RSD	1.763	1.99	1.135
	Average %RSD :1.63			

Table 4: Intra-day precision of L-Arginine

SI.NO	Absorbance at 205 nm	Absorbance at 207 nm	Absorbance at 209 nm
1	0.669	0.681	0.692
2	0.687	0.691	0.691
3	0.69	0.689	0.687
4	0.7	0.71	0.72
5	0.67	0.679	0.695
6	0.69	0.7	0.697
AVG	0.684	0.691	0.697
SD	0.012	0.011	0.011
%RSD	1.80	1.71	1.690

Tabel 5: Robustness results of L-Arginine at various wavelengths

3.2.4 SPECIFICITY

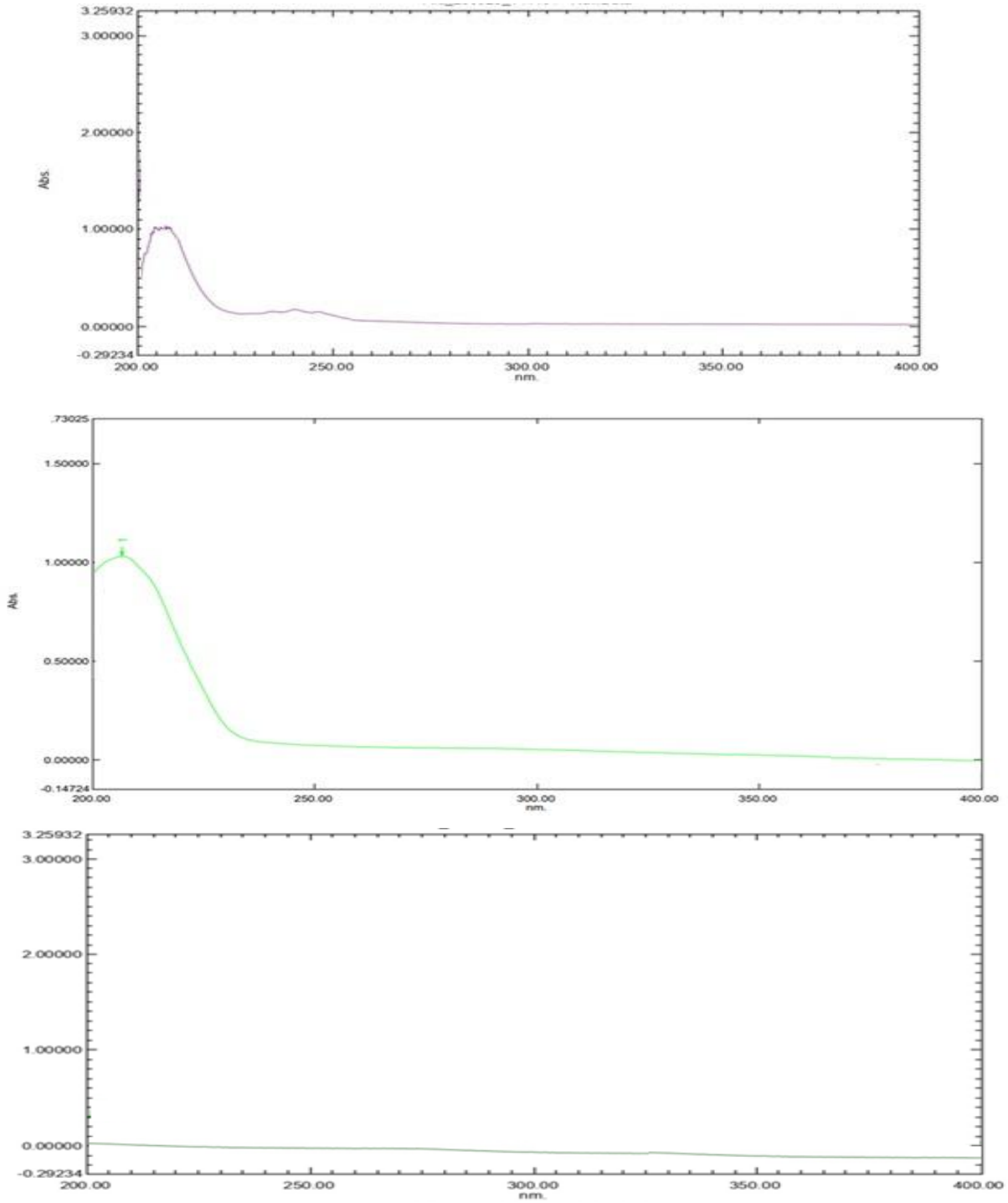

Figure-4: UV spectrum of the L-Arginine sample standard and blank

3.2.5 SENSITIVITY

Parameter	Drug name	Value
L-arginine	LOD (µg/ml)	0.058
	LOQ (µg/ml)	0.2

Tabel 6: Sensitivity of L-arginine

3.2.6 ASSAY

Label claim L-arginine: 1000mg

Tablet average weight-1.189mg=1189mg

$$\% \text{ Assay} = \frac{AT}{AS} x \frac{WS}{DS} x \frac{DT}{WT} x \frac{P}{100} x \frac{AVG\ Wt}{LableClam} x100$$

$$\% \text{ Assay} = \frac{0.692}{0.689} x \frac{10}{500} x \frac{500}{11.89} x \frac{99.28}{100} x \frac{1189}{1000} x100$$

$$= 99.281\%\ W/V$$

Acceptance limit: 100±2%

The optimized method for the estimation of L-arginine was carried out using a solvent system consisting of water and 0.1N HCl, with the absorption maximum (λ max) determined at 207 nm. These optimized conditions were employed to validate the proposed method in accordance with ICH Q2(R1) guidelines. The calibration curve constructed for L-arginine exhibited excellent linearity within the concentration range of 5–30 µg/mL, with a correlation coefficient (R^2) of 0.9992, confirming the reliability of the method for quantitative analysis. At the selected test concentration of 20 µg/mL, both intra-day and inter-day precision studies were performed, and the computed %RSD values were found to be well within the acceptable limits (<2%), thereby establishing the precision and reproducibility of the method (Tables 3 and 4). Accuracy of the method was further confirmed through recovery studies, wherein spiked solutions showed % recoveries within the acceptable range of 100 ± 2% (Table 2), indicating that the excipients present in the tablet formulation did not interfere with the estimation of L-arginine. The specificity of the method was demonstrated by the absence of significant spectral differences between the pure drug solution and the tablet sample solution, confirming that the method is specific for L-arginine. Robustness was verified by deliberately introducing small variations in the analytical parameters, such as slight changes in the wavelength, which did not significantly affect the %RSD values of absorbance (Table 5). Sensitivity of the method was assessed through the calculation of the limit of detection (LOD) and limit of quantification (LOQ), and the results (Table 6) demonstrated that the method is highly sensitive and capable of detecting and quantifying very low concentrations of L-arginine. The assay of marketed tablet formulations yielded a drug content of 99.28% w/v, which falls within the acceptable range for pharmaceutical formulations, further validating the method. Taken together, these results confirm that the developed UV spectrophotometric method is simple, accurate, precise, specific, sensitive, and robust, making it highly suitable for routine quality control and pharmaceutical analysis of L-arginine.

SUMMARY OF THE METHOD

Drug name	L-arginine
Solvent	Water (Pure drug) 0.1NHCl for tablet
Absorption Maximum (λmax)	207nm
Linearity range	5-30µg/ml
R^2	0.9992
Intraday precision (%RSD)	1.76
Inter day precision (%RSD)	1.63
Accuracy (% recovery)	94.4-100.46%
LOD	0.058
LOQ	0.2
%Assay	99.28%W/V

IV. CONCLUSION

A novel, simple, sensitive, cost-effective, and eco-friendly UV spectrophotometric method was developed for the estimation of L-arginine in bulk and tablet formulations, making it highly suitable for routine pharmaceutical analysis. The method was validated as per ICH guidelines and demonstrated robustness and reliability. Statistical analysis confirmed excellent linearity in the concentration range of 5–30 µg/mL with a correlation coefficient (R^2) of 0.999, ensuring consistent performance. Precision studies showed %RSD values below 2% for both intra-day and inter-day assays, highlighting good repeatability and reliability. Accuracy, confirmed by the standard addition method, yielded recoveries within 100 ± 2%, verifying the method's ability to estimate L-arginine accurately without interference from excipients. The method was highly sensitive with an LOD of 0.058 µg/mL and an LOQ of 0.2 µg/mL, while specificity was established by the absence of spectral interference from tablet excipients. Overall, this validated UV spectrophotometric method is reliable, accurate, and sensitive, making it an excellent choice for the routine quality control of L-arginine in pharmaceutical preparations.

BIBLIOGRAPHY

[1] Picollo M, Aceto M, Vitorino T. UV-Vis spectroscopy. Physical sciences reviews. 2019 Mar 26;4(4):20180008.

[2] Shinde G, Godage RK, Jadhav RS, Manoj B, Aniket B. A review on advances in UV spectroscopy. Research Journal of Science and Technology. 2020;12(1):47-51.

[3] Verma G, Mishra M. Development and optimization of UV-Vis spectroscopy review. World J. Pharm. Res. 2018 Apr 19;7(11):1170-80.

[4] Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Vol. I & II. New Delhi: CBS Publishers and Distributors; 2007.

[5] Atole DM, Rajput HH. Ultraviolet spectroscopy and its pharmaceutical applications: a brief review. Asian journal of pharmaceutical and clinical research. 2018;11(2):59-66.

[6] Br. Jay, J. Kelvin, and B. Pierre, Understanding and Implementing Efficient Analytical Methods Development and Validation, 2003.

[7] R.M. Christopher, and W.R. Thomas, Quality Systems approach to Pharmaceutical cGMP Development and validation of Analytical Methods, (1st Ed., 2005) 147-152.

[8] Zoughi S, Faridbod F, Alizadeh-Parchebaf K. Analysis of l-Arginine in sports supplements with a colorimetric apta-nanosensor. Optical Materials. 2024 Nov 1; 157:116144.

[9] Dr. Ameya Mulay, Mr. Mahendra Godase, Mr. Ashpak Inamdar, Mr. Sonal Gawali, Miss. Mukta Rankhamb Analytical Method Development and Validation of LArginine Effervescent Powder © March 2023| IJIRT | Volume 9 Issue 10 ISSN: 2349-6002

[10] Daniela Menichini, Lia Feliciello, Isabella Neri, Fabio Facchinetti , L-Arginine supplementation in pregnancy: a systematic review of maternal and fetal outcomes J Matern Fetal Neonatal Med, 2023 Dec;36(1):2217465 polyvinylpyrrolidone functionalized silver nanoparticles Journal of Molecular Liquids Volume 300, 15 February 2020, 112361

[11] Mariusz GFleszar , Jerzy Wisniewski, Malgorzata Krzystek-Korpacka , Blazej Misiak, Dorota Frydecka, Joanna Piechowicz, Katarzyna Lorenc-Kukula, Andrzej Gamian. Quantitative Analysis of l-Arginine, Dimethylated Arginine Derivatives, l-Citrulline, and Dimethylamine in Human Serum Using Liquid Chromatography–Mass Spectrometric Method 2018 Apr 21;81(6):911–921

[12] Rao PN, Bitla AR. Simultaneous determination of arginine, citrulline, and asymmetric dimethylarginine in plasma by reverse-phase high-performance liquid chromatography. Journal of Laboratory Physicians. 2017 Oct;9(04):243-8.

[13] Nataliya Stasyuk , Galina Gayda , Hasmik Yepremyan , Agnieszka Stepien , Mykhailo Gonchar. Fluorometric enzymatic assay of l-arginine 2017 Jan 5:170:184-90

[14] Kalaiyarasi Duraisamy , K S Jaganathan , Marothu Vamsi Krishna. Method development and validation of HPLC tandem/mass spectrometry for quantification of

perindopril arginine and amlodipine besylate combination in bulk and pharmaceutical formulations. Res Pharm Sci. 2017 Aug;12(4):307-314.

[15] Armaghan shafaei, Abdairahim. F.F.A, Mohammad jamshed Siddiqui, Zhari ismail. Analysis of L-citrulline and L-arginine in Ficus deltoidea leaf extracts by reverse phase high performance liquid chromatography January 2015Pharmacognosy Research 7(1):32-7 DOI:10.4103/0974-8490.147196

[16] Gayda G. Z., Stasyuk N. E., Gonchar M. V. THE METHODS OF L-ARGININE ANALYSIS ISSN 2410-7751

[17] Magdalena Paczkowska , Przemysław Zalewski , Piotr Garbacki , Alicja Talaczyńska , Anna Krause , Judyta Cielecka-Piontek The Development and Validation of a Stability-Indicating UHPLC-DAD Method for Determination of Perindopril l-Arginine in Bulk Substance and Pharmaceutical Dosage Form 2014;77(21-22):1497-1501

[18] Nalluri Naga Jyothi, Syed Imran pasha. Development and Validation of a New Rp-Hplc Method for Simultaneous Estimation of N-Acetylcysteine and L – Arginine in Combined Dosage form September 2014 Oriental Journal Of Chemistry 30(3):1371-1378

[19] Giradkar P A, Damdar P P, Virendra shende L-Arginine monohydrochloride analysis by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). International Journal of Life Sciences March 2021(Special Issue A15):15-22

[20] S. Balasurya, Asad Syed, Ajith Mesmin Thomas, Ali H. Bahkali, Abdallah M. Elgorban, Lija L. Raju, S. Sudheer Khan. Highly sensitive and selective colorimetric detection of arginine by

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

[22] Pravin Bhandare, P. Madhava, B.M. Rao, N. Someswar rao. Determination of arginine, lysine and histidine in drug substance and drug product without derivatization by using HILIC column LC technique J. Chem. Pharm. Res., 2010, 2(5):580-586

[23] Hassan Y. Aboul-Enein, Mohamed H, Hubert H.LC Determination of the Enantiomeric Purity of L-Arginine Using a Teicoplanin Chiral Stationary Phase June 2009 27(11):1681-1693

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