Analytical Method Development and Validation of L-Arginine Effervescent Powder

Dr. Ameya Mulay, Mr. Mahendra Godase, Mr. Ashpak Inamdar, Mr. Sonal Gawali, Miss. Mukta Rankhamb

SciTech Specialities Pvt. Ltd., A-3, STICE, Musalgaon, Tal. Sinnar, Nashik-422 112

Abstract-A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the L-Arginine effervescent powder. The method was carried out on a Inertsil C8 (250 x 4.6 mm x 5 µm)) column with a mobile phase consisting of Solution A: Acetonitrile (920:80) where solution A contain 500 mg of octane sulphonic acid in 1000 ml of phosphate buffer (pH 3.5) at a flow rate of 1.0 ml/min. Detection was carried out at 215 nm. The retention time of L-Arginine was found to be 7.5 minutes. The developed method was validated in terms of system suitability, linearity, accuracy& %recovery, precision, robustness, specificity, intermediate precision.

The proposed method can be used for the estimation of L-Arginine.

The proposed method is optimized and validated as per ICH guidelines.

Index Terms-Analytical method development, RP-HPLC, L-Arginine etc.

INTRODUCTION

The L-arginine is classified as semi essential or conditionally essential amino acid, that plays an important function in the metabolism of an organism. Discovery says that nitric oxide (NO) is synthesized from L-arginine (ARG) by nitric oxide synthase (NOS) and works diastolic for vessels, has changed the perception of ARG role in many physiological and pathological processes and it had an influence on increased interest in this amino acid role in biochemistry, physiology and nutrition of human and animals. NO is an important signal-transduction molecule that plays a significant role in the regulation of cardiovascular functions.

ARG is the precursor for the synthesis of proteins and other molecules of great biological importance. Amino acid analysis is an important technique for detection of different disorders in organism. Amino acid plays an important role in physiology including cellular proliferation, vasodilation, neurotransmission, calcium and immunity.

Literature survey reveals that analytical HPLC method for L-arginine have been reported in combination with other drugs in dosage forms. Assay of L-arginine was reported but from biological sample and from urine sample. The method for L-arginine was not developed from pharmaceutical dosage forms. Method development and validation of L-arginine effervescent powder not reported yet. Therefore, it was thought to develop a novel, specific, accurate and robust method.

Working standard

Name of material	code	Assay / % purity
L-Arginine	WS/L-ARGININE/22-23	99.62%

List of instruments required

Sr. No.	Name of instrument	Make
1.	Analytical Balance	Aczet private limited
2.	HPLC	Shimazdu
3.	Sonicator	C-Abhaykumar
4.	UV Spectrophotometer	Shimazdu
5.	pH meter	Lab India

List of glassware:

1. Beaker (1000 ml, 2000 ml, 120 ml,)
2. Glass Rod
3. Bulb Pipette (5 ml, 10 ml, 15 ml, 20 ml, 25 ml)
4. Measuring Cylinder (1000 ml)
5. Mobile Phase Reservoir
6. Volumetric Flask (100 ml, 50 ml)

List of Chemicals

Sr.No.	Name of chemical	Grade
1.	Sodium phosphate monobasic dihydrate	HPLC

2.	Phosphoric acid	AR
3.	Water	HPLC
4.	Acetonitrile	HPLC
5.	Octane sulphonic acid sodium salt monohydrate	AR

Chromatographic conditions
Column – Inertsil C8 (250mm x 4.6mm x 5µm) packing L7
Flow Rate – 1 ml per minute
Detector – UV detector
Wavelength – 215
Injection volume - 10µl
Column oven temperature – 30
Run time – 15 minutes
Diluent – phosphate buffer pH 3.5
Mobile phase – solution A: Acetonitrile (920:80)

Preparation of mobile phase
Buffer solution Weigh accurately 13.8 gm monobasic sodium dihydrate, transfer into beaker. Add 2000 ml of water sonicate for 3 minutes. Adjust the pH of solution 3.5 with phosphoric acid.

Solution A weigh accurately 500 mg of octane sulphonic acid, transfer into beaker. Add 1000 ml of above prepared buffer solution and sonicate foe 5 minutes.

Mobile Phase
Solution A: Acetonitrile (920:80)

Standard preparation
Weigh accurately about 150 mg of L-Arginine working standard in a 100 ml volumetric flask add 70 ml of buffer solution and sonicate for 10 minutes further make up the volume with buffer solution.

Sample preparation
Weigh accurately about a sample equivalent to 1500 mg of L-Arginine i.e., 2625 mg L-Arginine in a 100 ml volumetric flask, add 70 ml of buffer solution and sonicate for 10 minutes further make the volume with buffer solution. Further dilute 10 ml of this solution to 100 ml of buffer solution.

Validation Parameters
1.System suitability

System suitability test is used to verify that the chromatographic system is suitable for the intended analysis

Procedure Prepare the standard solution as per given method. Inject 5 replicate injection of standard solution.

Observation Table I

Sr.No.	Standard area of L-Arginine	Tailing factor	Retention time	Theoretical plates
1.	457711	1.20	7.575	6117
2.	458957	1.23	7.567	6619
3.	459527	1.22	7.575	6151
4.	458354	1.23	7.575	6196
5.	457945	1.18	7.583	6264
AVG	458499	1.212	7.575	6269
STDEV	744.22	0.02167	0.01	NA
%RSD	0.1613	0.0003	0.0747	NA

Acceptance criteria
Relative standard deviation for all the 5 injections should be NMT 2.0 %
Relative standard deviation for retention time of all the 5 injections should be NMT 2.0 %
Theoretical plates should be not less than 2000
Tailing factor should be not more than 2.0

Conclusion
As per above observation table, asymmetry factor, and number of theoretical plates and %RSD of system suitability Solution is found within the limit, so that the given Procedure of analysis is suitable on above mentioned Chromatographic conditions.

2.Linearity
The linearity of analytical procedures is its ability to obtain test results which are directly proportional to the concentration of analyte in sample.

Procedure Prepare five different concentration solutions of L-Arginine working standard having final concentration of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm to that of the 1500 ppm decide optimum concentration of the standard solution.
Plot the graph of concentration Vs peak area and calculate Correlation coefficient (r^2).
Linearity curve

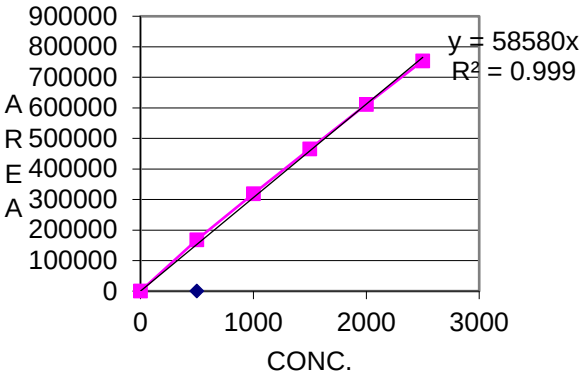

Fig. I Linearity Curve

Observation Table II

Conc. Level (ppm)	Area of L-Arginine
0	0
500	167671
1000	319192
1500	465406
2000	611460
2500	754150
Slope	300
Intercept	11432
Co – relation coefficient	0.996

Acceptance criteria
Correlation coefficient should be NLT 0.99
Conclusion over a selected concentration range.

Conclusion
From above observation table, the procedure of analysis is linear over a selected range of concentration. The test results are directly proportional to the concentration of the analyte.

3.Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be expressed on samples spiked with known amounts of impurities.

Procedure
Prepare three samples spike L-Arginine with different concentrations like 80%, 100% and 120%, to that of specification limit and analyze each concentration in duplicate as per suggested analytical procedure.

Observation Table III

Sample / %Recovery	Sample With 80% Conc.	Sample With 100% Conc.	Sample with 120% conc.
Assay of sample-1	80.41	99.70	120.72
Assay of sample-2	80.51	98.93	120.18
Assay of sample-3	80.80	99.53	120.29
Mean Assay	80.57	99.39	120.29
STDEV	0.2026	0.4045	0.2854
%RSD	0.25	0.41	0.24
% Recovery of sample-1	100.51	99.70	10.6
% Recovery of sample-2	100.64	98.93	100.15
% Recovery of sample-3	101.0	99.53	100.24
Mean % recovery	100.71	99.53	100.33
STDEV	0.2538	0.4045	0.2381
% RSD	0.25	0.41	0.23

Acceptance Criteria
% Recovery for each concentration stage should be between 98-102 %
%RSD for each concentration stage should be NMT 2.0%.

Conclusion
From the above observation table, recovery assays are within specified limits, which indicates that the procedure for analysis is accurate for the given set of chromatographic conditions.

4.Specificity
Specificity is the ability to asses unequivocally the analyte in the presence of components which may be expected to be present

Procedure
Prepare set of 6 samples of same selected batch spiking concentration of placebo. Samples should be spike with 10% placebo, spike with 20% placebo, spike with 30 % placebo, spike with 50 % placebo and spike with 100% placebo. This ensures the identity of analyte present in the designed formulation.

Observation Table IV

Sample	% Assay	Mg/sachet
Sample with 10% placebo spike	99.27	148.90

Sample with 20% placebo spike	98.63	147.94
Sample with 30% placebo spike	98.08	147.12
Sample with 40% placebo spike	98.42	147.63
Sample with 50% placebo spike	98.93	148.40
Sample with 100% placebo spike	98.73	148.09
Sample without API spike	0	0
Average	98.68	148.01
STDEV	0.4106	0.6151
% RSD	0.42	0.42

Acceptance criteria
The %RSD of 6 samples should not be more than 2.0%.

Conclusion:
As per above observation table, %RSD of standard and %assay of L-Arginine Content is NMT 2.0%.
There is no interference of placebo hence it is concluded that the proposed method is specific.

5.Precision (Repeatability)
Precision of the analytical method is the closeness of agreement between the series of measurements from multiple sampling of the same homogeneous sample under prescribed condition.

Procedure
Prepare and analyze the 6 different samples from uniform mixed blend. Calculate the % RSD of all sample.

Observation Table V

Sample	% Assay	Mg/sachet
Sample – 1	98.44	3937.63
Sample – 2	99.07	3962.88
Sample – 3	99.38	3975.24
Sample – 4	99.67	3986.94
Sample – 5	99.72	3988.62
Sample --6	99.10	3963.97
Average	99.14	3958.58
STDEV	0.5271	19.1696
%RSD	0.53	0.48

Acceptance criteria
% RSD for all samples should not be more than 2.0%

Conclusion
From the above observation table, %RSD of all samples is not more than 2.0% hence it is concluded the proposed method is precise

6.Intermediate precision
Intermediate precision expresses within laboratories variations: different days different analysts, different equipment, etc.

Procedure
Prepare 3 different samples as per designed MOA and calculate the content on above mentioned chromatographic conditions on different days.
Different days on same instruments

Observation Table VI

Test	Sample	% Assay	Mg/sachet
Day-I	Sample-1	100.78	4031.18
	Sample -2	101.12	4044.89
	Sample-3	100.33	4.13.08
	Average	100.74	4.29.72
	STDEV	0.3963	15.9554
	%RSD	0.39	0.40
Day-II	Sample-1	99.74	3989.62
	Sample-2	99.52	3980.72
	SAMPLE-3	99.92	3996.61
	AVERAGE	99.73	3988.98
	STDEV	0.2003	7.9641
	%RSD	0.20	0.20
%RSD of all assay values of day-I and day-II		0.29	0.3

Acceptance criteria
%RSD of individual day should not more than 2.0%
%RSD of both days should not more than 2.0%.

Conclusion
From above observation table it was concluded that %RSD of individual day should not more than 2.0% and %RSD of both days should not more than 2.0%.

7.Robustness
Robustness determines the reliability of an analytical method with deliberate variations in the method like change in Ph of the mobile phase, change in flow rate and change in temperature of column oven etc.

Variation in flow rate
Prepare 3 samples from homogenous blend of same samples and analyze on different flow rate of the mobile phase i.e. 0.8ml/min, 1.0ml/min and 1.2ml/min.

proposed flow rate of the methos is 1.0 ml/min according to USP variation is +- 50 %.

Observation Table VII

	% Assay
0.8ml/min	100.89
1.0ml/min	100.78
1.2ml/min	100.77
Average	100.81
STDEV	0.0666
%RSD	0.07

	% Assay
0.8ml/min	101.17
1.0ml/min	101.12
1.2ml/min	101.11
Average	101.11
STDEV	0.0603
%RSD	0.06

	% Assay
0.8ml/min	101.12
1.0ml/min	100.33
1.2ml/min	100.83
Average	100.76
STDEV	0.3996
%RSD	0.4

Acceptance criteria

%RSD of %Assay of each sample on different flow rate should not be more than 2.0%.

Conclusion

From the above observation table, % RSD of %Assay of each sample on different flow rate should not be more than 2.0%.

Result and discussion (Table VIII)

Sr no.	Parameter	Results
1	System suitability	Complies
	%RSD of area	0.1613
	%RSD of retention time	0.0747
	Theoretical plate	6269
	Asymmetry factor	0.0003
2	Linearity correlation coefficient	0.996
3	Accuracy	complies
	%RSD For 80% concentration	0.28
	%RSD For 100% concentration	0.41
	%RSD For 120% concentration	0.23
4	Specificity	0.42
5	Precision	0.53
6	Intermediate precision (%RSD of both days)	0.29
7	Robustness	complies
	For sample one on different flow rate	0.07
	For sample two on different flow rate	0.06
	For sample three on different flow rate	0.4

Conclusion

On the basis of analytical results obtained during analytical method validation of given analytical method of assay of L-Arginine effervescent powder. It is observed that all the results of different validation parameters like system suitability, linearity, accuracy, specificity, precision, intermediate precision and robustness are within the specified limits. Hence it is concluded that, the given analytical method of assay of L-Arginine effervescent powder is validated and can be used for routine laboratory analysis. Given method for L-Arginine effervescent powder is linear, precise, suitable, specific and robust for its intended purpose

Chromatograms

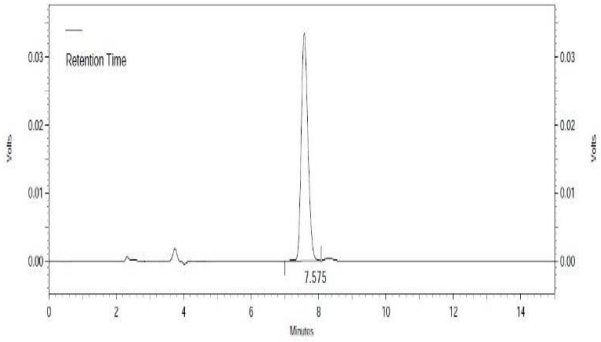

Fig. II chromatogram of standard

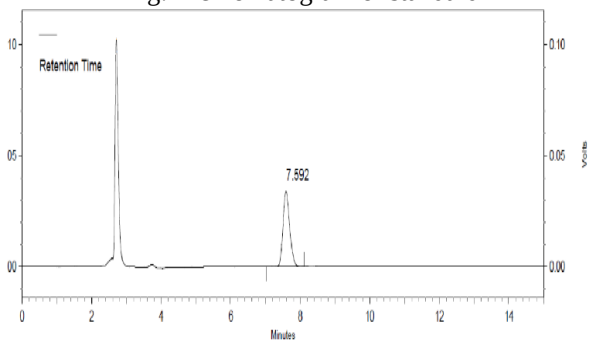

Fig. III Chromatogram of sample

REFERENCE

[1] Indian Pharmacopoeia (2018). Government of India, Ministry of Health and Family Welfare, Indian Pharmacopoeia Commission, Ghaziabad, India. 2, 324-324.

[2] Sharma S., Goyal S., Chauhan k., (2018). A review on analytical method development and validation review article 10(2), 120-125

[3] Ahuja, S., and amp; Scypinski, S. (2001). Handbook of Pharmaceutical Analysis Academic Press. 6, 48-147.

[4] Teerlink, T. (2007). HPLC analysis of ADMA and other methylated L-arginine analogs in biological fluids, Journal of Chromatography B, 851(1-2), 21-29.

[5] Jyothi, N., and amp; Pasha, S. (2014). Development and Validation of a new RP-HPLC method for simultaneous estimation of N-acetylcysteine and L-arginine in combined dosage form. Oriental Journal of Chemistry, 30(3), 1731-1778.

[6] E. Rech, A., P. Farina, C., C. Lukasewicz Ferreira, P., & Miron, D. (2017). Determination of arginine and ascorbic acid in effervescent tablets by multidimensional chromatography. Drug Analytical Research, 1(2), 44–52.

[7] ICH guideline Q2A (R1). Validation of analytical procedures: text and methodology. In International Conference on Harmonization, Geneva, Switzerland,2005.

[8] Sabir, A., Moloy, M. (2013); HPLC Method Development and Validation; International Research Journal of Pharmacy, 39-46.

[9] USP-NF (1225) validation of Compendial Procedures 2022.

[10] USP-NF (621) Chromatography 2022.

[11] Alkaitis, M. S., Nardone, G., Chertow, J. H., & Ackerman, H. C. (2015). Resolution and quantification of arginine, monomethylarginine, asymmetric dimethylarginine, and symmetric dimethylarginine in plasma using HPLC with internal calibration. Biomedical Chromatography, 30(3), 294–300.

[12] Kalamb, V. S. (n.d.). (2022). Review of HPLC Method Development and Validation: A Review, 2(2), 752-760.

[13] Margaret E. LA Course, William R. LA Course, General instrumentation in HPLC**,** University of Maryland, Baltimore County, MD, United States.

[14] Ridwan, R., Abdul Razak, H. R., Adenan, M. I., & Md Saad, W. M. (2018). Development of isocratic RP-HPLC method for separation and quantification of L-citrulline and L-arginine in watermelons. International Journal of Analytical Chemistry, 2018, 1–9.

[15] British Pharmacopoeia (2022). In British Pharmacopoeia Commission (Ed.), Stationary office.