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Analytical Method Development and Method Validation of L- Carnosine in Tablet Dosage Form by UV Spectrophotometer

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Abstract: L-Carnosine is a naturally occurring dipeptide widely used in pharmaceutical and nutraceutical formulations due to its antioxidant and anti-aging properties. The present study focuses on the development and validation of a simple, rapid, and economical analytical method for the quantitative estimation of L-Carnosine in tablet dosage form using UV–Visible Spectrophotometry. The method was developed using a suitable solvent system and the absorbance of L-Carnosine was measured at its maximum wavelength. Then the developed method is applied on the quantification of marketed formulation. The developed method was validated according to the guidelines of ICH and the parameters such as linearity, solution stability, accuracy, precision, specificity, limit of detection, limit of quantification, and robustness. The results indicated that the method is reliable and reproducible for the routine analysis of L-Carnosine in tablet formulations by UV Spectrometry. This method is suitable for this drug in pharmaceutical industries.

Keywords: L-Carnosine; UV–Visible Spectrophotometry; Analytical Method Development; Method Validation

1. INTRODUCTION

Analytical chemistry is the branch of chemistry concerned with the development and application of methods to identify the chemical composition of materials and quantify the amounts of components in mixtures. Ultraviolet–visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. Being relatively inexpensive and easily implemented, this methodology is widely used in diverse applied and fundamental applications. The only requirement is that the sample absorb in the UV–Vis region, i.e. be a chromophore. Absorption spectroscopy is complementary to fluorescence spectroscopy. Parameters of interest, besides the wavelength of measurement, are absorbance (A) or transmittance (%T) or reflectance (%R), and its change with time. Method development starts with the documentation of the development studies. Standard analyte characterization, Method Requirements, Literature Search and prior Methodology, Choosing of Method, Instrumental Setup and Initial Studies, Optimization, Documentation of analytical figures, Evaluation of Method Development with actual Sample, Determination of Percent Recovery of Actual Sample and Demonstration of Quantitative Sample Analysis. From the literature survey conducted, it was found that there are some analytical methods reported for the L-Carnosine in RP-HPLC, LC-MS but not in UV spectrometry. But most of the works were conducted pharmacokinetic assessment and stability studies and no work was reported in UV spectrometry for quantitation. So the aim of present work is to develop simple UV spectrometry for the estimation of L-Carnosine in tablet dosage form. L-Carnosine is a dipeptide found in high concentrations in muscles and the brain, acting as a potent antioxidant, neuroprotective, and anti-aging compound. It is used to support cognitive function, improve behaviour in children with Autism, protect against diabetic complications, and aid in muscle performance by reducing lactic acid build-up. Validation is a process involving conformation or establishing by laboratory studies the method/ procedure/ system/ analayte give accurate and reproducible result for intended application in a proven and established range. That performance characteristics of (accuracy, precision, sensitivity, ruggedness etc) meets the requirement of intended analytical application.

2. AIM & PLAN OF WORK

[1] Method development by UV spectroscopy.
[2] Quantitative determination of the formulation.
[3] Validation of the developed method

2.1. MATERIALS AND METHODS

INSTRUMENTS

S.NO	NAME OF THE INSTRUMENT	MAKE	MODEL
1	UV-VIS Spectrophotometer	JASCO	V-730
2	BALANCE	SHIMADZU	AUW220D
3	SONICATOR	SOLTEC	A99-01141

Reagents And Chemicals - Sodium Hydroxide Distilled Water
Standard Drug- Gift Sample From Beref Pharma, Madurai
Marketed Formulation - Cognicare, Ignicar, Carniston

3. METHOD DEVELOPMENT AND OPTIMIZATION

Selection of wavelength and solvent: The L-carnosine of 10 mcg.100mcg and 1000 mcg were dissolved in distilled water, 0.1 M sodium hydroxide and 1m sodium hydroxide separately.The resulting solutions were then scanned between 200 to 400 nm and the peaks got for the drug in 0.1 M sodium hydroxide at 10 mcg shown good response and absorbance and it is shown in fig 1. The absorbances were noted down in the table .Maximum absorbance was found at 220nm and it was selected for the analysis of L-Carnosine.

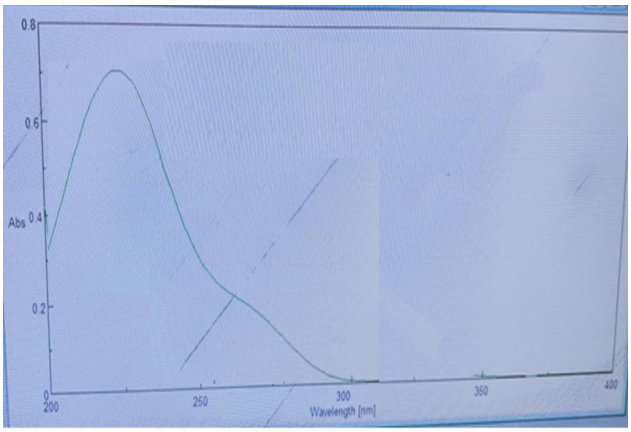


Fig 1: UV Graph of L-CARNOSINE

Wavelength in nm	Absorbance
220	0.6924
223	0.5168
225	0.4498
230	0.3011
243	0.2380
254	0.2596
273	0.2014
298	0.1011
308	0.0661
310	0.0212
315	0.0119

3.1. QUANTITATIVE DETERMINATION OF THE DRUG USING THE DEVELOPED METHOD

Sample : L- Carnosine
Carnosine Label claim : 200mg

Standard solution of L- Carnosine

100mg of L-Carnosine standard was accurately weighed and transferred into a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and the volume was made up to the mark. From the stock solution 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

3.2. Sample preparation

20 tablets were randomly selected and weighed. Then the average weight was calculated and the sample equivalent to 100mg was weighed and transferred into a 100ml

volumetric flask. 0.1M sodium hydroxide was added and the volume was made up to the mark. The solution was then shaken well to dissolve the contents. Then it was sonicated for 9 minutes to get dissolved. Then the resulting solution was filtered. 1ml of the filtrate was diluted to 100ml with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

The amount of L-Carnosine present in tablet formulations were calculated by comparing the absorbance of the standard and sample and is given in table 1

Table: 1 Quantitative Estimation

S.NO	Brand Name	Content	Absorbance of standard	Absorbance of sample	Amount present (mg)	Percentage content(%)
1	Cornicare	L-CARNOSINE	0.6894	0.6901	0.2001	100.05
2	Ignicar	L-CARNOSINE	0.6890	0.6884	0.1998	99.9
3	Carniston	L-CARNOSINE	0.6886	0.6881	0.1998	99.9

3.3. VALIDATION

LINEARITY AND RANGE

The linearity of the analytical method was determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range present in table no 3. Absorbance values were plotted graphically as a function of analyte concentration. Percentage curve fitting was also calculated.

3.4. Preparation of standard solution

100mg of L-Carnosine standard was accurately weighed and transferred into 100ml volumetric flask and 0.1M sodium hydroxide was added. The volume was then made up with 0.1M sodium hydroxide. From the above stock solution 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4. 4.5 and 5ml were transferred to 100ml volumetric flasks and the volumes were made up with 0.1M sodium hydroxide. The absorbance of the above solutions were measured and the calibration curve was plotted. From the calibration curve, the correlation coefficient and the percentage curve fitting equation were calculated using the formula.

The linearity data was shown in table 2 and the calibration curve was given in graph 2

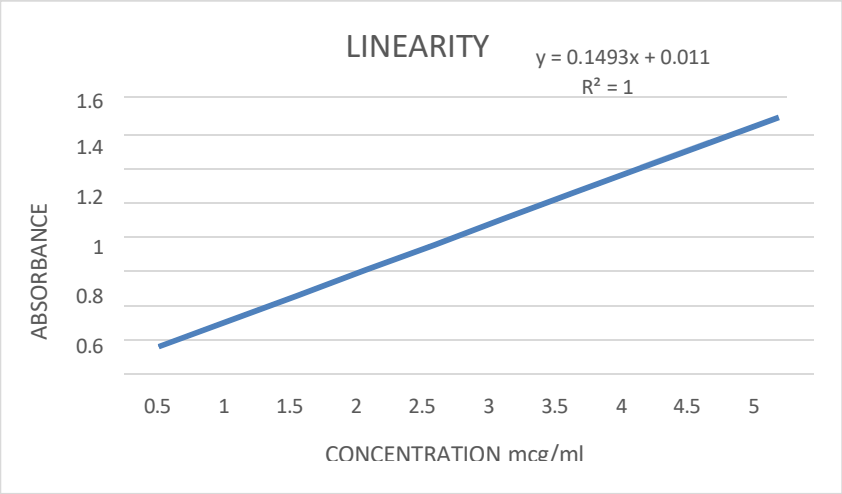
Table: 2 Linearity Data

S.NO	CONCENTRATION (mcg/ml)	ABSORBANCE
1	5	0.1599
2	10	0.3102
3	15	0.4579
4	20	0.6084
5	25	0.7559
6	30	0.9087
7	35	1.0598
8	40	1.2065
9	45	1.3541
10	50	1.5026

Table: 3 Analytical Performance Parameters

Sl.No	Drug	Linearity Range	Correlation Coefficient	Percentage curve fitting	Slope
1	L-Carnosine	5-50 mcg/ml	0.9999934	99,99%	0.1493

Graph: 2 LINEARITY GRAPH



3.5. SOLUTION STABILITY

To establish the stability of analytical solutions by measuring the absorbance of the standard and sample solutions at periodic intervals upto 24hrs.

Acceptance criteria: The %RSD of absorbance of both standard and sample solutions at periodic intervals should not be more than 2.0%

Procedure

The absorbance of the blank, standard and sample preparation were measured.

The %RSD for the absorbance were calculated and the values are given in Table 4

Table: 4 Stability Data

Sl. No	Time Intervals	Absorbance Of blank	Absorbance Of placebo	Absorbance Of Standard	Absorbance Of Sample
1	0	0	0	0.6876	0.6881
2	30min	0	0	0.6881	0.6869
3	1hr	0	0	0.6865	0.6875
4	1hr30min	0	0	0.6884	0.6885
5	2hr	0	0	0.6885	0.6886
6	2hr 30min	0	0	0.6892	0.6891
7	3hr	0	0	0.6872	0.6879
8	3hr 30min	0	0	0.6890	0.6875
9	4hr	0	0	0.6881	0.6885
10	4hr 30min	0	0	0.6895	0.6890
11	5hr	0	0	0.6872	0.6877
12	5hr 30min	0	0	0.6859	0.6874
13	6hr	0	0	0.6876	0.6880
14	6hr 30min	0	0	0.6891	0.6875
15	7hr	0	0	0.6878	0.6881

16	7hr 30min	0	0	0.6885	0.6878
17	24hr	0	0	0.6890	0.6892
MEAN				0.6881	0.6881
STANDARD DEVIATION				0.00096	0.00063
% RSD				0.1395	0.0915

4. ACCURACY

4.1. Determination

The accuracy of the analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

4.2. Procedure

Different concentrations of L-Carnosine were prepared by taking different weights and dissolved in 0.1M sodium hydroxide. The suitable dilutions were made to give concentrations of 80%, 100% and 120%. The absorbances of the solutions were then measured in triplicates and the values are given in table 5. Then the amount was calculated using the equation:

$$\text{Accuracy} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \text{Potency}$$

Table: 5 Accuracy study data of L-Carnosine

S.NO	SAMPLE - ID	Amount added (mg)	Absorbance observed	Amount found (mg)	Percentage Recovery(%)
1.	80%	80	0.5530	79.9	99.87
		80	0.5543	80.1	100.12
		80	0.5560	80.4	100.50
2.	100%	100	0.6876	99.4	99.4
		100	0.6892	99.6	99.6
		100	0.6898	99.7	99.7
	120%	120	0.8295	119.9	99.91
		120	0.8260	119.4	99.50
		120	0.8298	120	100

4.3. SPECIFICITY

The specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of compounds that may be expected to be present in the sample matrix.

4.4. Determination

The specificity of the analytical method was determined by analysing the placebo solution under the same experimental conditions as the assay.

4.5. Preparation of Placebo

Placebo was prepared by mixing all the excipients other than the active ingredients.

4.6. Procedure

About 100mg of placebo was accurately weighed and transferred to a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and the volume was made up to the mark. The solution was then filtered through Whatmann filter paper. 1ml of the above solution was pipetted into a 100ml volumetric flask and the

volume was made up with 0.1M sodium hydroxide. From this solution 1 ml was taken and it is made up to 10ml with 0.1M sodium hydroxide.

5. STANDARD SOLUTION OF L- CARNOSINE

100mg of L- Carnosine standard was accurately weighed and transferred into a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and the volume was made up to the mark. From the stock solution 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide. Preparation of standard + placebo 100mg of accurately weighed standard and 100mg of placebo was transferred to a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and made up to the mark. The solution was then filtered through Whatmann filter paper. 1ml of the above solution was pipetted into a 100ml volumetric flask and the volume was made up distilled water. From this solution 1 ml is taken and it is made up to 10ml with distilled water. The absorbance of the above solutions were measured and the values are given in table 6.

Table: 6 Specificity for L- Carnosine

S.NO	Sample	Absorbance obtained
1	Placebo	0.0020
2	Standard	0.6914
3	Standard + Placebo	0.6918

6. LIMIT OF DETECTION & QUANTITATION LIMIT OF DETECTION

Based on the standard deviation of the Response and slope:

A specific calibration curve was studied using samples containing an analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The detection limit may be expressed as: $LOD = \frac{3.3\,\sigma}{S}$

Where, σ = standard deviation of the response, S=slope of the calibration curve The slope S was estimated from the calibration curve of the analyte.

The data are given in table 7.

7. LIMIT OF QUANTITATION

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The quantitation limit may be expressed as: $LOQ = \frac{10\,\sigma}{S}$

Where, σ = standard deviation of the response, S=slope of the calibration curve The slope S may be estimated from the calibration curve of the analyte. The data is given in table no 8.

Table: 7 Limit Of Detection

S.NO	Absorbance
1	0.6961
2	0.6992
3	0.7041
4	0.6972
5	0.7012
6	0.6981
MEAN	0.6993
S.D	0.002923
% RSD	0.4179

LOD= 0.064 mcg

Table: 8 Limit Of Quantitation

S.NO	Absorbance
1	0.6961
2	0.6992
3	0.7041
4	0.6972
5	0.7012
6	0.6981
MEAN	0.6993
S.D	0.002923
% RSD	0.4179

LOQ= 0.19mcg

7.1. PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

7.2. Procedure

Standard solution of L-Carnosine

100mg of L-Carnosine standard was accurately weighed and transferred into a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and the volume was made up to the mark. From the stock solution 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

7.3. Sample preparation

20 tablets were randomly selected and weighed. Then the average weight was calculated and the sample equivalent to 100mg was weighed and transferred into a 100 ml volumetric flask. 0.1M sodium hydroxide was added and the volume was made up to the mark. The solution was then shaken well to dissolve the contents. Then it was sonicated for 9 minutes to get dissolved. Then the resulting solution was filtered. 1ml of the filtrate was diluted to 100ml with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

7.4. System precision

System precision was determined by preparing the standard solution for six times and measuring the absorbance of each of the prepared solutions. The absorbance values are given in table 9.

7.5. Method precision

The method precision was determined by preparing the sample of single batch of L-Carnosine tablets for six times and the absorbance of the six solutions were measured. The method precision data are given in table 10.

Table: 9 System Precision Data

S.NO	Absorbance
1	0.6961
2	0.6992
3	0.7041
4	0.6972
5	0.7012
6	0.6981
MEAN	0.6993
S.D	0.002923
% RSD	0.4179

Table: 10 Method Precision Data

S.NO	Standard Absorbance	Sample Absorbance	Amount Present in Tablet (mg)	Percentage Content(%)
1	0.6949	0.6916	0.1991	99.55
2	0.6896	0.6889	0.1998	99.9
3	0.6898	0.6892	0.1998	99.9
4	0.6916	0.6909	0.1998	99.9
5	0.6882	0.6883	0.2000	100
6	0.6921	0.6924	0.2001	100.05
MEAN			0.1997	99.88
STANDARD DEVIATION			0.00035	0.175119
%RSD			0.1752	0.1753

7.6. RUGGEDNESS

The ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness is normally expressed as a lack of influence on test results of operational and environmental variables of the analytical method.

7.7. Procedure

Standard solution of L- Carnosine

100mg of L-Carnosine standard was accurately weighed and transferred into a 100ml volumetric flask. Then 0.1M sodium hydroxide was added and the volume was made up to the mark. From the stock solution 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with 0.1M sodium hydroxide. From the resulting solution. 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

7.8. Sample preparation

20 tablets were randomly selected and weighed. Then the average weight was calculated and the sample

equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. 0.1M sodium hydroxide was added and the volume was made up to the mark. The solution was then shaken well to dissolve the contents. Then it was sonicated for 9 minutes to get dissolved. Then the resulting solution was filtered. 1ml of the filtrate was diluted to 100ml with 0.1M sodium hydroxide. From the resulting solution 1 ml was taken and diluted to 10ml with 0.1M sodium hydroxide.

8. METHOD

The standard stock solution and sample stock solution were prepared bydifferent analysts on different days and the absorbances of the resulting solution were measured. The ruggedness of the method and the reports are given in table 11-12.

Table: 11 Ruggedness of L-Carnosine Analyst 1 - SANGEETHA

S.No	Date of Analysis	Standard absorbance	Sample absorbance	Assay Value in (mg)	Percentage content(%)
1	05.01.2026	0.6894	0.6812	0.1976	98.8
2	06.01.2026	0.6918	0.6899	0.1996	99.8
MEAN STANDARD DEVIATION					99.3
% RELATIVE STANDARD DEVIATION					0.707106
					0.712

Table: 12 Ruggedness of L- Carnosine

Analyst 2- SANTHOSH

S.No	Date of Analysis	Standard absorbance	Sample absorbance	Assay Value in (mg)	Percentage content(%)
1	05.01.2026	0.6854	0.6848	0.1998	99.9
2	06.01.2026	0.6889	0.6826	0.1996	99.8
MEAN STANDARD DEVIATION					99.9
% RELATIVE STANDARD DEVIATION					0.070710
					0.070

9. RESULTS AND CONCLUSION

The developed method is a simple method which has the advantage of determination of L-Carnosine with simple solvent system. This provides shorter analysis time and conserves the solvent system. The method was validated based on USP and ICH parameters

Linearity of the drug was obtained in the range of 5-50µg/ml. The correlation coefficient is found to be 0.9999934 and slope is 0.1493. Stability of the method was determined by assays of the drug formulation up to 24hrs hours. There was almost no appreciable change in absorbance up to 24hrs. Percentage recovery of the assays was found to be within limits. Thus the developed method was found to provide high degree of stability Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug is well within acceptance limits (98 to 102%). Specificity of the method was found out through non-interference of the placebo in identical conditions of assay. This confirms the specificity of the developed method. Detection limit was done by calibration curve method. The limit of detection was found to be 0.064 mcg/ml. Quantitation limit was done by calibration curve method. The limit of quantification was found to be 0.19 mcg/ml. Precision of the method was determined by assays of drug formulations by replicate injection and precision of system was determined by using standard solution. %RSD of the assays is found to be within the limits of 2%. Thus the developed method is found to provide high degree of

precision and reproducibility. Ruggedness was determined by performing the same assay on different days, assay being carried out by different analysts. The test results were within the limits 98 to 102%. The result is found to be reproducible. Overall, the method was successfully validated according to USP and ICH guidelines and can be effectively used for the routine quantitative analysis of L-Carnosine in pharmaceutical formulations.

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