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Original Research Article

Quantitative Determination of L-Carnitine Tablet Formulation by A Validated Stability-Indicating Reversed-Phase HPLC Method

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Abstract: A rapid and stability-indicating RP-HPLC method was developed for determination of L-Carnitine in tablets dosage form. The method was developed on C18 column (250 x 4.6 mm ID) using the mobile phase composition as 0.05M phosphate buffer (pH = 3.2) Methanol: buffer (5:95 V/V). The flow rate was set as 0.9ml/minute and the maximum absorption was observed at 225 nm. The method was validated for specificity, selectivity, linearity, precision, accuracy, and robustness. The L-Carnitine drug showed a precise and good linearity at the concentration ranges of 70-1120 µg/ml. The RP-HPLC, assay showed the highest purity ranging 99.74% to 99.84 % for L-Carnitine tablet formulation and 99.80 % was the mean percentage purity. The L-Carnitine retention time was found to be 7.7 minutes. The method accuracy was showed by statistical analysis. The developed RP-HPLC method can be adopted for the routine analysis of L-Carnitine pharmaceutical dosage forms in quality control laboratories. The developed method was validated according to the ICH guidelines. Accordingly, the proposed validated and rapid procedure was proved to be suitable for routine analyzing and stability studies of L-Carnitine in tablets.

Keywords: L-Carnitine, Methanol, Water, HPLC and UV.

INTRODUCTION

L-Carnitine((R)-3-carboxy-2-hydroxy-N,N,N-trimethyl-1- propaminium hydroxide inner salt, is a vitamin like amino acid derivative, which is an essential factor in fatty acid metabolism as acyltransferase cofactor and in energy production processes, such as interconversion in the mechanisms of regulation of ketogenesis and thermogenesis [1, 2]. Lack of L-Carnitine leads to lipid accumulation in the cytosol and impaired energy production from long-chain fatty acids, especially during periods of fasting or stress. L-Carnitine pharmaceutical preparations, including injections, syrups, tablets, and capsules, are used in the therapy of primary and secondary carnitine deficiency, and in other diseases such as dis-lipo proteinemia and Alzheimer's [1-3]. A detailed literature survey revealed that there are few analytical methods reported for the estimation of L-Carnitine in pharmaceutical formulations. The US Pharmacopeia (USP) provides two HPLC methods for quantitation of L-Carnitine in oral solution and tablet formulations. The method for tablets involves an amino propylsilane-bonded [4-6].

A stability study ensures the maintenance of product quality, safety, and efficacy throughout its shelf life. Stress testing can help identify degradation products and provide important information about the intrinsic stability of drug substances [7]. Regulatory agencies recommend the use of stability-indicating assay methods for the analysis of stability samples [8]. With the advent of the International Conference on Harmonization (ICH) guidelines [8-10], requirements for the establishment of stability-indicating assay methods have become more clearly mandated [11]. Taking ICH guidelines into consideration, the present study describes a simple, validated, and stability-indicating analytical method for determination of L-Carnitine in tablets. Also, the calculation of the measurement uncertainty which is based on the validation of the analytical procedures in a laboratory is presented. Moreover, the performances of the method were evaluated and its potential for the determination of L-Carnitine in tablets was investigated.

The proposed aim of this study was to develop simple, accurate, specific and precise RP-HPLC method for the L-Carnitine estimation in the pharmaceutical tablet formulation.

MATERIALS AND METHODS

Chemicals

The L-Carnitine reference standard (RS) was purchased from Sigma-Aldrich, USA. The marketed L-Carnitine 500mg tablet marketed by Optimum Nutrition, purchased from, local Pharmacy from USA. The HPLC grade a methanol was purchased from Fisher, USA. HPLC-grade water was obtained through a Milli-Q system (Millipore, Milford, MA, USA) and was used to prepare all solutions

RP-HPLC instrumentation

Shimadzu LC-20 AT HPLC system, SPD- M20A, Japan). The Chromatographic separation was carried out on a C18 column [Agilent ODS UG 5 column, 250 mm x 4.5 mm]. The column temperature was maintained at a 45ºC and the flow rate was 0.9ml/min. The sample injection volume is 20µl and the wavelength was set as 225nm, the HPLC run time was set for 15 minutes.

Preparation of Mobile phase

0.05M phosphate buffer (pH = 3.2). Equal volumes of HPLC grade methanol and buffer were mixed in the ratio of (5:95 V/V), filtered through a 0.45µm membrane filter and sonicated for 15 minutes.

Preparation of L-Carnitine stock solution

Standard L-Carnitine solution

L-Carnitine (70 mg) weighed accurately and transferred to 100 ml volumetric flask and mixed with 100 ml of water, the resulting solution were kept in the sonicator for 5 minutes. The concentration of 70-1120 µg/ml was achieved by diluting the standard stock solution with mobile phase. L-Carnitine powder was freely soluble in water.

Preparation of L-Carnitine tablet solution

500 mg of marketed sample of L-Carnitine tablet was analyzed by this method. Ten tablets were accurately weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 10 mg was transferred into 25ml volumetric flask and dissolved with 25 ml of water and filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

Solution stability

The prepared drug solution stability was analysed during the time of analysis and also repeated the same analysis method on same day with different time intervels. The same analysis was repeated after 24 hrs by keeping the drug solution under laboratory temperature (37 ± 1°C) and in refrigeration (5 ± 1°C).

Method validation

The proposed method was preceded to achieve a new, sensitive and easy method for estimation of L-Carnitine by RP-HPLC. The experimental analysis was validated according to the ICH (Q2 B) guidelines, recommendations and USP-30.

System suitability

The resolution, retention time, tailing factor and column theoretical plates parameters were performed by six replicates of standards and three replicates of sample preparation.

RESULTS AND DISCUSSION

Method optimization

Chromatogram with good shape peaks and good retention time shows good resolution for L-Carnitine. The typical RP-HPLC conditions are presented in Table 1. The good separation of L-Carnitine shows the success of the method. The HPLC chromatogram of L-Carnitine standard and L-Carnitine tablet is presented in figure 1 and 2.

Table-1: RP-HPLC conditions for estimation of L-Carnitine.

Parameters	Description
Column	Agilent ODS UG 5 column, (250 mm x 4.5 mm)
Mode of operation	Isocratic
Column temperature	45 ± 1°C
Mobile phase	Methanol: Buffer (5:95 V/V).
Detection	UV 225 nm
Injection volume	20 µl
Flow rate	0.9 ml min^{-1}

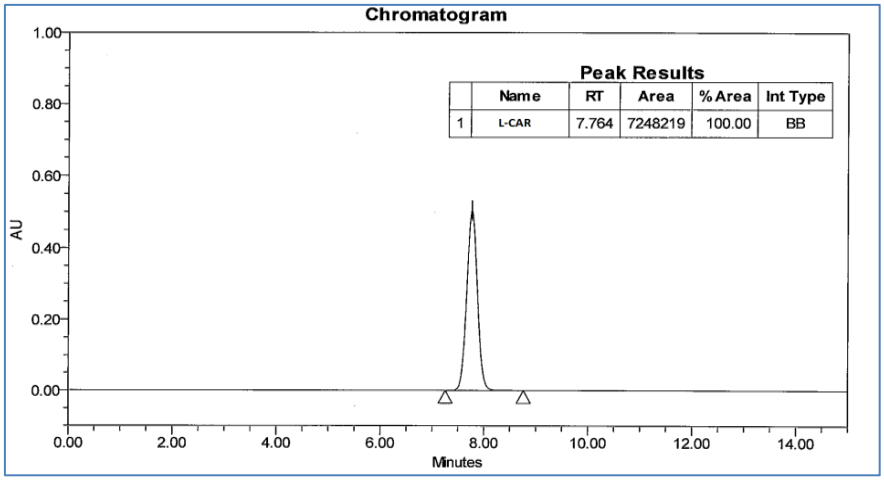

Fig-1: A Chromatogram of L-Carnitine Standard

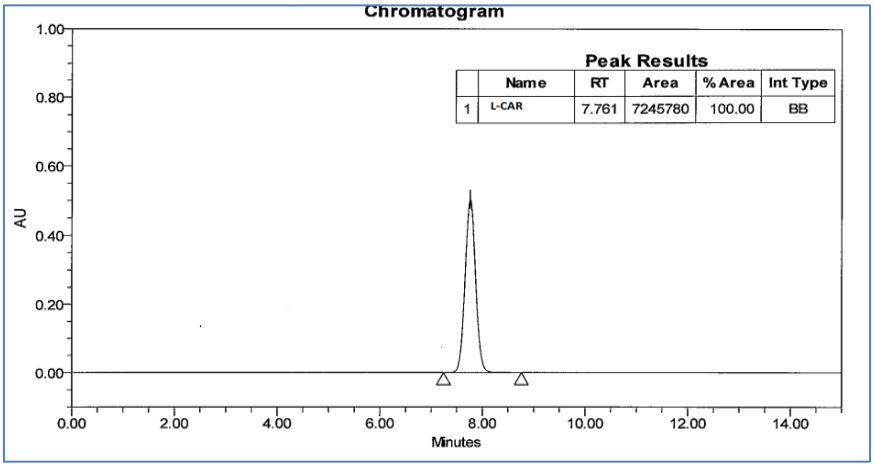

Fig-2: A Chromatogram of L-Carnitine tablet formulation

Linearity

The proposed method linearity was examined for five concentrations. The concentration ranges from 70-1120 µg/ml. The L-Carnitine standard linearity was determined by the plotting graph concentration vs absorbance. By absorbance as a functional of analyte concentration linearity was evaluated for L-Carnitine. The linearity graph presented in figure 3, and data presented in Table 2. The system suitability is demonstrated by the linearity analysis.

Table-2: RP- HPLC linearity for L-Carnitine

Concentration (µg/ml)	Peak area
70	7245780
140	14491912
280	28987120
560	57967457
1120	115765432

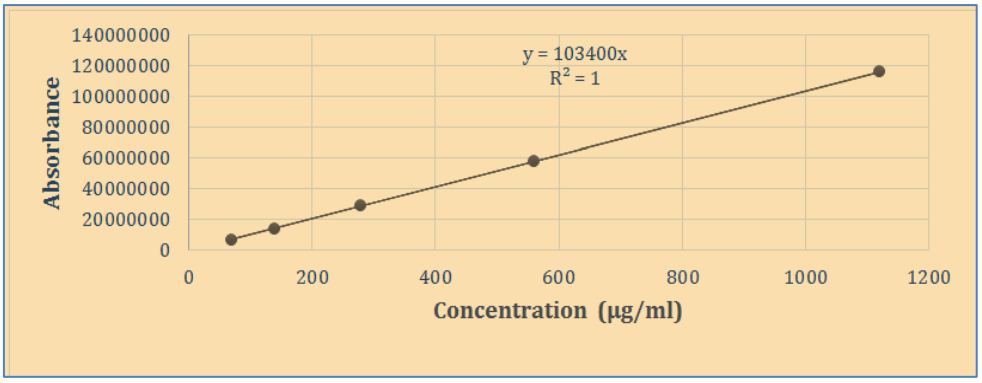

Fig-3: Calibration graph of L-Carnitine 70-1120 µg/ml precision

Accuracy

The recovery experiment shows the accuracy of the method. The good recovery shows the method was accurate. The analysis for recovery was performed by known amount of L-Carnitine working standard added to pre-analyzed solution of formulation in the test concentration range of (80%, 100% and 120 %). For each recovery level three samples were prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The L-Carnitine tablet formulation recovery results are presented in Table 3.

Table-3: Recovery studies of L-Carnitine tablet formulation

Recovery Level (%)	Amount added (µg/ml)		Amount Found (µg/ml)	% Recovery	Mean recovery
	Standard	Test			
80	56	5	60.98	99.96	99.78
100	70	5	74.96	99.41	
120	84	5	88.98	99.97	

Precision

The proposed method precision (repeatability) experiment results of are shown in Table 4. In the proposed method intraday and intraday precision was examined by analysing the responses of the sample on the same day for 4 repetitions and 3 alternate days for 70µg/ml concentration range of L-Carnitine. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in Table 5.

Table-4: Method precision data of L-Carnitine by RP-HPLC method

L-Carnitine 20µg/ml (n=4)	Retention time	Area
1	7.76	7245780
2	7.76	7245128
3	7.74	7243211
4	7.79	7247658
Mean	7.76	7245444
S.D [a]	0.0123	0.1132
% CV[b]	0.67	1.43

n=4 observations

Table-5: Intermediate precision data of L-Carnitine by RP-HPLC method

L-Carnitine µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)
70	7242281± 1.09	0.0562	7247286± 1.02	0.0565
140	14471932±1.42	0.0517	14462221±1.62	0.0617
280	28707140±1.11	0.0712	28766770±1.01	0.0706

n^c = 4 observations

Specificity

The standard reference and the drug formulation show specificity of the method. The RP-HPLC chromatogram of L-Carnitine tablet formulation are presented in figure 1, 2. The tablet formulation retention time were found to be 7.7

minutes. For the tablet formulation there was no excipient interference was detected, which shows the specificity of the method. The proposed method shows the ability to determine the analyte in presence of excipients.

Limit of detection and quantitation

The limit of detection and quantification for L-Carnitine is presented in table 6. Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were examined by minimum detectable peak area by injecting known concentration of drug solution. As per the International Conference on Harmonization guidelines the results are multiplied thrice to get LOD and 10 times to get LOQ. LOD and LOQ were found at concentrations of 0.217µg/mL and 1.103µg/mL respectively. The limit of detection and quantification for L-Carnitine is presented in table 6.

Table-6: Limit of detection and quantification

Parameters	**Results** (µg/ml)
Limit of detection (LOD)	0.217
Limit of quantification (LOQ)	1.103

System suitability

For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in table 7. The Assay data of L-Carnitine is presented in table 8.

Table-7: Results of system suitability parameters

SNo	**Parameters**	**L-Carnitine**
1.	Theoretical plates	10107
2.	Tailing factor	0.776
3.	Resolution factor	1.01
4.	Retention time	7.7± 0.1
5.	Calibration range or Linear dynamic range	70-1120 µg/ml

Table-8: Quantitative estimation (Assay) data of L-Carnitine

Drug	**Label claim (mg)**	**Amount found (mg)**	**Mean amount found (mg /ml)**	**Percentage purity (% w/w)**	**Mean purity (%w/w)**	**% Deviation**
L-Carnitine	500.0	498.7 499.2 498.9 499.1 499.2	499.02	99.74 99.84 99.78 99.82 99.84	99.80	+0.2 +0.1 +0.1 +0.1 +0.2

n= 4 observations

Statistical parameters

The obtained assay results are subjected to the coefficient of variation; statistical analysis, regression equation and standard deviation are presented in table 9.

Table-9: Results of statistical parameters

SNo	**Parameters**	**L-Carnitine**
1.	Standard deviation (SD)	1.07
2.	Relative standard deviation (RSD)	0.0476
3.	% RSD	0.216
4.	Standard error (SE)	0.02721
5.	Correlation Coefficient (r)	0.9988
6.	Slope (a)	3318.8
7.	Intercept (b)	1767.2

CONCLUSION

The proposed and validated RP-HPLC method was performed according to the guidelines of International Conference on Harmonization (ICH), the developed RP-HPLC method shows the accuracy, sensitive and stability indicating. The developed method is rapid, reproducible. The developed method can be used for the routine analysis for L-Carnitine tablet formulations.

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REFERENCE

1. Dąbrowska, M., & Starek, M. (2014). Analytical approaches to determination of carnitine in biological materials, foods and dietary supplements. Food chemistry, 142, 220-232.
2. Fu, L., Huang, M., & Chen, S. (2013). Primary carnitine deficiency and cardiomyopathy. Korean circulation journal, 43(12), 785.
3. de Andrés, F., Castañeda, G., & Ríos, Á. (2010). Achiral liquid chromatography with circular dichroism detection for the determination of carnitine enantiomers in dietary supplements and pharmaceutical formulations. Journal of pharmaceutical and biomedical analysis, 51(2), 478-483.
4. Mancuso, C., Siciliano, R., Barone, E., & Preziosi, P. (2012). Natural substances and Alzheimer's disease: from preclinical studies to evidence based medicine. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1822(5), 616-624.
5. Kakou, A., Megoulas, N. C., & Koupparis, M. A. (2005). Determination of L-carnitine in food supplement formulations using ion-pair chromatography with indirect conductimetric detection. Journal of Chromatography A, 1069(2), 209-215.
6. He, G. X., & Dahl, T. (2000). Improved high-performance liquid chromatographic method for analysis of L-carnitine in pharmaceutical formulations. Journal of pharmaceutical and biomedical analysis, 23(2-3), 315-321.
7. De Witt, P., Deias, R., Muck, S., Galletti, B., Meloni, D., Celletti, P., & Marzo, A. (1994). High-performance liquid chromatography and capillary electrophoresis of L-and D-carnitine by precolumn diastereomeric derivatization. Journal of Chromatography B: Biomedical Sciences and Applications, 657(1), 67-73.
8. Kamata, K., Takahashi, M., Terasima, K., & Nishijima, M. (1994). Liquid chromatographic determination of carnitine by precolumn derivatization with pyrene-1-carbonyl cyanide. Journal of Chromatography A, 667(1-2), 113-118.
9. El-Ragehy, N. A., Hassan, N. Y., Abdelkawy, M., & Tantawy, M. A. (2014). Stability-indicating chromatographic methods for the determination of sertindole. Journal of chromatographic science, 52(6), 559-565.
10. Bakshi, M., & Singh, S. (2002). Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis, 28(6), 1011-1040.
11. Ermer, J., & Ploss, H. J. (2005). Validation in pharmaceutical analysis: Part II: central importance of precision to establish acceptance criteria and for verifying and improving the quality of analytical data. Journal of Pharmaceutical and Biomedical Analysis, 37(5), 859-870.

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