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Development of HPLC Method for the Determination of Zinc Carnosine in Bulk and Dosage Forms

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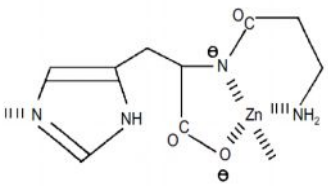
Abstract: A reverse phase HPLC method is developed for the determination of zinc carnosine in pharmaceutical dosage forms. Chromatography was carried out on a C18 column [250mm, 4.6m, 5µm] using a mixture of dipotassium di hydrogen ortho phosphate buffer and Acetonitrile (80:20 v/v) as the mobile phase at a flow rate of 1 ml/min. Detection was carried out at 215 nm .The retention time of the drug was 2.260 min. The method produced linear responses in the concentration range of 2 to 10µg/ml of zinc carnosine. The LOD and LOQ values for HPLC method were found to be 33.4 and 101.3 ng/ml respectively. The method was found to be applicable for determination of the drug in tablets.
Key words: HPLC, Zinc carnosine, Estimation, tablets.

INTRODUCTION:

Zinc carnosine is a zinc salt of (2*S*)-2-[(3-Amino-1-oxopropyl)amino]-3-(3*H*-imidazol-4-yl)propanoic acid. It is the prescribed drug for the treatment of ulcer. As no HPLC method have been reported for the determination of zinc carnosine an attempt was made to report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy, linearity, LOD & LOQ. The method has been satisfactorily applied to the determination of zinc carnosine in pharmaceutical preparations. Yoshikawa, has done the pharmacological work on Zinc carnosine ,A Novel Antioxidant, on Acute Gastric Mucosal Injury Induced by Ischemia-Reperfusion in Rats[1]. Matsukura was developed Applicability of Zinc Complex of L-Carnosine for Medical Use Biochemistry[2]. Masaru Odashima has developed the Pharmacological work, Zinc L-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-κB activation[3]. Razinah sharif was developed the effect of zinc sulphate and zinc carnosine on genome stability and cytotoxicity in the WIL2-NS human lymphoblastoid cell line[4].



Zinc carnosine

EXPERIMENTAL:

Chemicals and solvents:
Dipotassium di hydrogen ortho phosphate and orthophosphoric acid (AR grade, Qualigens) were used for preparing the buffer. HPLC grade acetonitrile (Qualigens) was used for diluent preparation. Pure sample of Zinc carnosine was a gift sample from a local pharmaceutical industry. Commercial samples of tablets containing the drug zinc carnosine were purchased from the local pharmacy.

Chromatographic Conditions
A High pressure liquid chromatography (Shimadzu LC-2010HT) with variable wavelength programmable UV-Visible detector and phenomenex C-18 column [250mm, 4.6m, 5µm] was used. The HPLC system was equipped with the soft ware Class VP series version 5.03 (Shimadzu). A freshly prepared 80:20 v/v mixture of Dipotassium di hydrogen ortho phosphate (3.0 pH) and acetonitrile was used as the mobile phase. Buffer solution was prepared by dissolving 6.8 gms of dipotassium dihydrogen orthophosphate in 1000ml of water. To this add 1.2gms of 1-decane sulphonic salt and adjust the pH to 3.0 with orthophosphoric acid. Both Dipotassium di hydrogen ortho phosphate and acetonitrile were filtered through a 0.45 µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1ml/min. The detection was carried out by UV detector at 215 nm.

Preparation of the standard solution:
Accurately weighed 10 mg of zinc carnisone working standard was transferred to 100 ml volumetric flask to this 5 ml of 10%v/v HCL and then made upto mark with water : acetonitrile (70:30) and then sonicate for 5 mins. From this solution, concentrations of 2, 4, 6, 8 and 10 µg/ml were made in 100 ml volumetric flasks.

Application of proposed method to tablets
Accurately powdered and weighed amount of tablet equivalent to 40.86 mg of zinc carnisone was transferred to 100 ml volumetric flask to this add 5 ml of 10%v/v HCL and then made upto mark with water : acetonitrile (70:30) and then sonicate for 5 mins. Aliquot of this solution were diluted with water: acetonitrile (70:30) at concentration of 2µg/ml. Results are shown in Table 4.

Method validation[5,6]
T he proposed method was validated as per ICH guidelines. The drug solutions were prepared as Per the earlier adopted procedure given in the experiment.

Linearity study
Linearity was performed by taking from stock solution aliquots of 2, 4, 6, 8 and 10 ml were taken in 100ml volumetric flasks and diluted upto the mark with mobile phase such that the final concentration of Zinc carnosine in the range of 2 to 10 µg/ml. Volume of 20 µl of each sample was injected in six times for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown in Table 1, 2, Fig.1.

Accuracy as recovery
I t was done by recovery study. Sample solutions were prepared by spiking at about 50 %, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in Table 3.

System precision
Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Standard solution of (2µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table 5.

Method precision
Six samples were Prepared and analyzed as per the test method on 3 different days and calculated the % RSD for Assay of six preparations. Results are shown in Table 6.

Limit of detection and limit of quantitation: The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

Table1: Linearity

Concentration (µg/ml)	Peak Area
2	2250.42
4	4501.42
6	6791.41
8	9016.72
10	11109.42

Fig 1: linearity of zinc carnosine

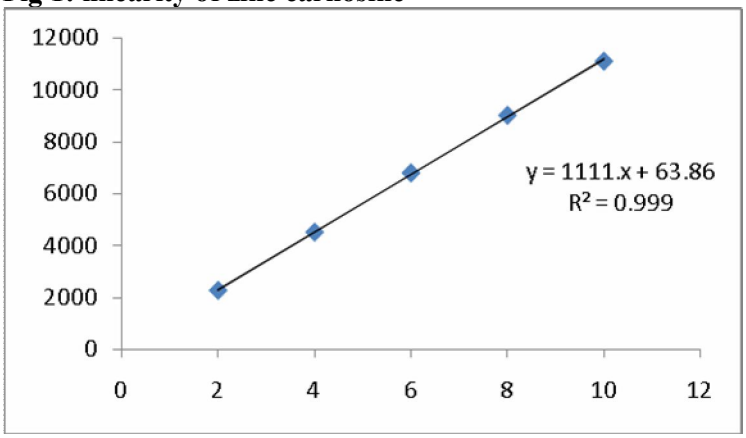


Table 2: Characteristics of HPLC method

Parameters Determined	Obtained Value
Linearity range(µg/ml)	2-10
Slope	0.1120
Intercept	63.86
Regression Coefficient(r2)	0.999
LOD(ng/ml)	33.4
LOQ(ng/ml)	101.3

Figure 2: Chromatogram of standard

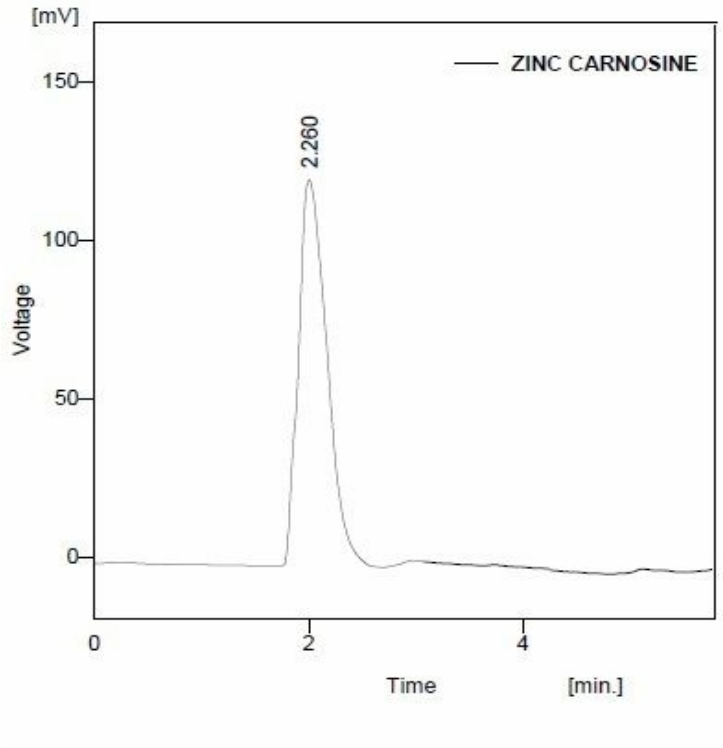


Figure 3: Chromatogram of sample

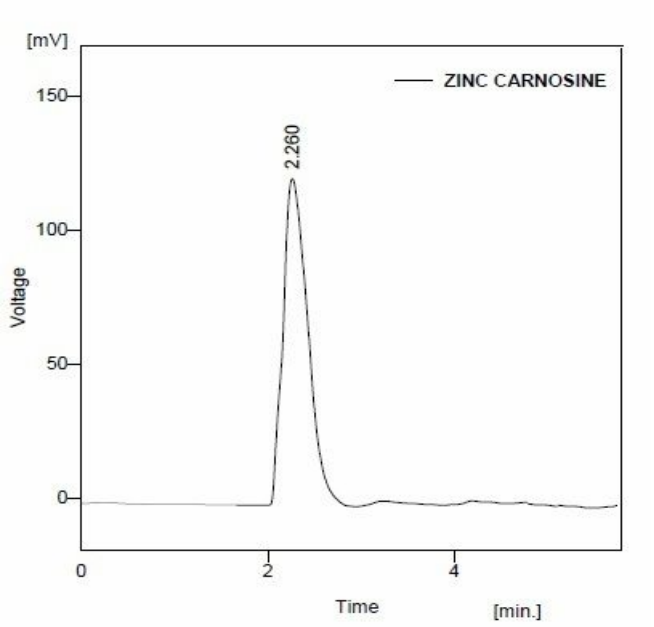


Table 3: Accuracy

Label claim	sample conc (µg)	Amount added in µg	Amount Recovered* in µg	% Recovery*	Average recovery (%)	%RSD
75 mg	10	2 4 6	1.99 ± 1.32 4.03 ± 1.7 6.02 ± 1.1	99.5 100.83 100.36	100.23	0.67

Table 4: Assay

Formulation	Labeled amount (mg)	Observed amount*(mg)	%Amount found	%RSD
Carno zin	75	74.87	99.83	0.015

Table 5: System precision

inject ions	Peak Area
1	2255.366
2	2252.729
3	2251.518
4	2253.327
5	2254.303
6	2252.199
Mean	2253.24
S.D	1.411
%R.S.D	0.063

Table6: Method precision

Concentrations	Inter-day precision		Intra-day precision	
	Mean ± S.D	%R.S.D	Mean ± S.D	%R.S.D
2	99.83±0.015	0.015	99.87±0.046	0.046
4	99.62±0.29	0.291	99.727±0.093	0.093
6	99.44±0.34	0.342	99.6±0.171	0.172

RESULTS AND DISCUSSION

Zinc carnosine, indicated for the treatment of ulcer. Literature scan revealed no HPLC was developed for the determination of Zinc carnosine. Fig 2 shows typical chromatograms of Zinc carnosine. The retention time of Zinc carnosine was 2.260 min. The calibration curve was linear over the range 2 - 10 µg/ml for the determination of Zinc carnosine. The linearity of method was statistically confirmed. The correlation coefficients (r^2) for calibration curves were not less than 0.999. The LOD and LOQ values of zinc carnosine were found to be 33.4 ng ml^{-1} and 101.3 ng ml^{-1} respectively. The Precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Precision was expressed as the RSD of the results. The values obtained for the precision studies presented (Table 5, 6), indicates good repeatability and low inter day variability. The analytical recovery at six different concentrations of Zinc carnosine was determined and the recovery results are in the range of 98-102% .Therefore proposed validated method was successfully applied to determine Zinc carnosine in tablet dosage form.

CONCLUSION

For the determination of Zinc carnosine, the proposed HPLC method was found to be superior due to high percentage recovery which shows that the method was free from interference of excipients used in the formulations. The results of the study indicate that the proposed HPLC method of analysis can be used in quality control department with respect to routine analysis for the assay of the tablets containing Zinc carnosine.

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