Original Article

VALIDATED HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ZINC CARNOSINE AND ACECLOFENAC IN BULK AND TABLET DOSAGE FORM

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Abstract

A validated HPLC method for simultaneous estimation of Zinc carnosine and Aceclofenac in pharmaceutical dosage forms. Chromatography was carried out on a C18 column [250mm, 4.6m, 5µm] using a mixture of potassium di-hydrogen phosphate buffer: Acetonitrile: methanol (50:30:20 v/v) as the mobile phase at a flow rate of 1 ml/min. Detection was carried out by using PDA detector. The retention time of the drug was 2.258 and 6.690 min. The method produced linear responses in the concentration range of 2 to 10µg/ml for both drugs. The LOD and LOQ values were found to be 33.4, 101.3 and 2.473, 7.495 ng/ml respectively. The method was validated for linearity, precision, accuracy, LOD &LOQ in accordance with ICH guidelines. The proposed method was found to be applicable for determination of the drug in tablet dosage forms.

Keywords: HPLC, Zinc carnosine & Aceclofenac method development, validation, Tablet.

Introduction

Zinc carnosine is a zinc salt of (2S)-2-[(3-Amino-1-oxopropyl)amino]- 3 -(3H-imidazol-4-yl) propanoic acid. Aceclofenac is 2-[2-[2-(2,6-Dichlorophenyl) aminophenyl] acetyl] oxyacetic acid. These are the prescribed drugs for Antiulcer, NSAID. There is no HPLC method have been reported for the determination of zinc carnosine and Aceclofenac. 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. A simple, rapid and selective HPLC method has been developed for quantitation of aceclofenac and paracetamol from bulk drug and pharmaceutical formulations. Sensitive and reproducible methods for quantitative determination of aceclofenac in pure form and in pharmaceutical formulation by UV. Development and validation of RP-HPLP Method for simultaneous estimation of three tablet formulation containing Acetaminophen, Chlorzoxazone and Aceclofenac.

Materials and Methods

Chemicals and solvents

Potassium di hydrogen phosphate (AR grade, Qualigens) was used for preparing the buffer. HPLC grade acetonitrile, methanol was used.

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Pure sample of Zinc carnosine and Aceclofenac was a gift sample from a local pharmaceutical industry. Commercial samples of tablets containing the drug Zinc carnosine and Aceclofenac (Acenal safe) was 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 mixture of potassium di-hydrogen phosphate buffer (0.02M,pH-3.5): Acetonitrile: methanol (50:30:20 v/v) used as the mobile phase. Buffer solution was prepared by dissolving 6.8gms of potassium dihydrogen phosphate in 1000ml of water. Adjust the pH to 3.5 with phosphoric acid. Mobile phase was 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 PDA detector.

Preparation of the mixed standard solution

Accurately weighed 10 mg of both drugs (zinc carnisone and Aceclofenac).Weighed powder of both drugs were accurately transferred to a same volumetric flask of 10mL and made volume up to the mark with diluent (Acetonitrile:Methanol) to obtain a mixed standard stock solution (solution A) of Zinc carnosine (10mg/mL) and Aceclofenac (10mg/mL). Acurately measured solution A of 0.02 ml was transferred to volumetric flask of 10mL and made volume up to the mark with diluent to obtain a mixed standard stock solution (solution B) of Zinc carnosine (2µg/mL) and Aceclofenac (2µg/mL).

Preparation of Sample solution
Twenty tablets were weighed and finely powdered. Powder equivalent to 40.86 mg of zinc carnosine and 42.82 mg of Aceclofenac was transferred to 10ml volumetric flask and made volume up to the mark with diluent to obtain solution of Zinc carnosine (10mg/mL) and Aceclofenac (10mg/mL). From this solution 0.2mL was transferred to 100mL volumetric flask and made volume up to the mark with diluent to obtain solution of Zinc carnosine (2mg/mL) and Aceclofenac (2mg/mL).

Method validation
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 0.02, 0.04, 0.06, 0.08 and 1.0 mL were taken in 10ml volumetric flasks and diluted upto the mark with diluent (Acetonitrile : Methanol). 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 five 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, Fig.1,2.

Assay
Acurately weighed the powder equivalent to 40.86 mg of zinc carnosine and 42.82 mg of Aceclofenac was transfered to 10ml volumetric flask and made volume up to the mark with diluent to obtain solution of Zinc carnosine (10mg/mL) and Aceclofenac (10mg/mL). From this solution 0.2mL was transferred to 100mL volumetric flask and made volume up to the mark with diluent to obtain solution of Zinc carnosine (2mg/mL) and Aceclofenac (2mg/mL). The chromatogram was shown in Figure-3.

Accuracy as recovery
It 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 3 times. Results are shown in Table 4.

Method precision
Three samples were Prepared and analyzed as per the test method on same day and three different days and calculated the % RSD for Assay of five preparations. Results are shown in Table 5.

Limit of detection and limit of quantization
The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. Results are shown in Table 6.

Results and discussion

Zinc carnosine and Aceclofenac, indicated for the treatment of ulcer and relief of pain. Literature scan revealed no HPLC was developed for the determination of Zinc carnosine and Aceclofenac. Fig 2 shows typical chromatograms of Zinc carnosine and Aceclofenac. The retention time of Zinc carnosine and Aceclofenac was 2.260 , 6.690 min. The calibration curve was linear over the range 2 - 10 µg/ml for the determination of Zinc carnosine and Aceclofenac. 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 and Aceclofenac were found to be 33.4, 101.3 and 2.473, 7.495 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-4,5), indicates good repeatability and low inter day variability. The analytical recovery at five different concentrations of Zinc carnosine and Aceclofenac was determined and the recovery results were in the range for Zinc carnosine of 99.5-100.36% , for Aceclofenac 99-99.83%.Therefore proposed validated method was successfully applied to determine Zinc carnosine and Aceclofenac in tablet dosage forms.

Conclusion

For the determination of Zinc carnosine and Aceclofenac, 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 and Aceclofenac.

Acknowledgement

The authors are grateful to the Management of school of pharmaceutical sciences, VELS University, Chennai, for their continuous support and encouragement and for providing the necessary facilities.

Linearity

Table-1
Zinc carnosine and Aceclofenac

S.No	Concentration (µg/ml)	Peak Area	
		Zinc Carnosine	Aceclofenac
1.	2	2250.42	6564.134
2.	4	4501.42	13656.37
3.	6	6791.41	20684.72
4.	8	9016.72	27567.86
5.	10	11109.42	34668.14

Assay

Table-2

Formulation	Labeled amount (mg)	Observed amount*(mg)	%Amount found	%RSD
Zinc carnosine	37.5	74.87	99.82	0.013
Aceclofenac	100	99.92	99.92	0.015

Accuracy

Table-3
Accuracy of Zinc carnosine and Aceclofenac

Drug	Label claim in mg	Sample conc in mg/mL	Amount added in µg	Amount added in µg	% Recovery	Average recovery (%)
Zinc carnosine	37.5	10	2 4 6	1.99±1.32 4.03±1.7 6.02±1.1	99.5 100.83 100.36	100.23
Aceclofenac	100	10	2 4 6	1.98±0.01 3.99±0.152 5.99±0.025	99 99.75 99.83	99.52

System precision

Table -4

Drug	Injections	Peak Area	Mean	S.D	%R.S.D
Zinc carnosine	1 2 3 4 5	2255.366 2252.729 2251.518 2253.327 2254.303	2253.448	1.471	0.065
Aceclofenac	1 2 3 4 5	6564.134 6565.421 6564.243 6564.234 6563.432	6564.307	0.824	0.012

Method Precision

Table-5

Concentrations	Inter-day precision		Intra-day precision	
	Mean ± S.D	%R.S.D	Mean ± S.D	%R.S.D
2	99.81±0.616	0.617	99.73±0.123	0.123
4	99.53±0.225	0.226	99.44±0.572	0.575
6	99.31±0.415	0.417	99.72±0.323	0.323

Table-6
Characteristics of HPLC method

Drug	Parameters Determined	Obtained Value
Zinc carnosine	Linearity range (µg/ml)	2-10
	Slope	0.1120
	Intercept	63.86
	Regression Coefficient(r^2)	0.999
	LOD(ng/ml)	33.4
	LOQ(ng/ml)	101.3
Aceclofenac	Linearity range(µg/ml)	2-10
	Slope	3505.52
	Intercept	-406.105
	Regression Coefficient(r^2)	0.999
	LOD(ng/ml)	2.473
	LOQ(ng/ml)	7.495

Figure-1
Zinc carnosine

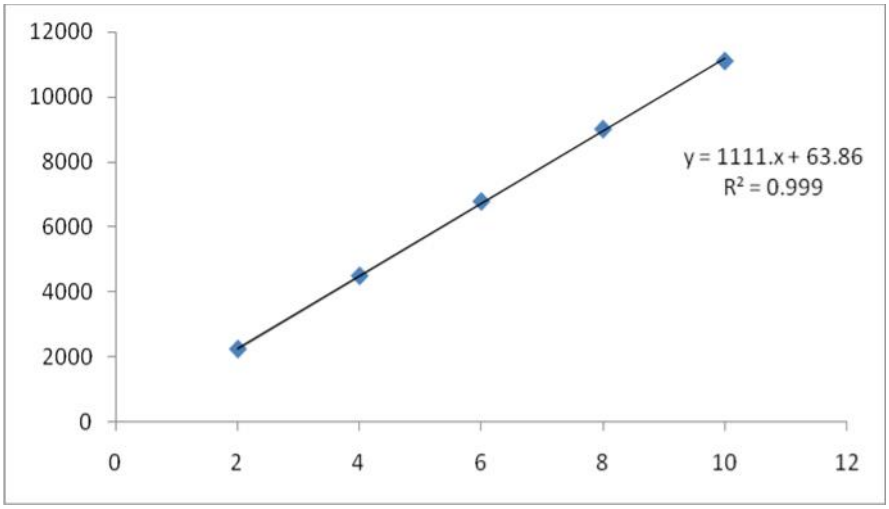


Figure-2
Aceclofenac

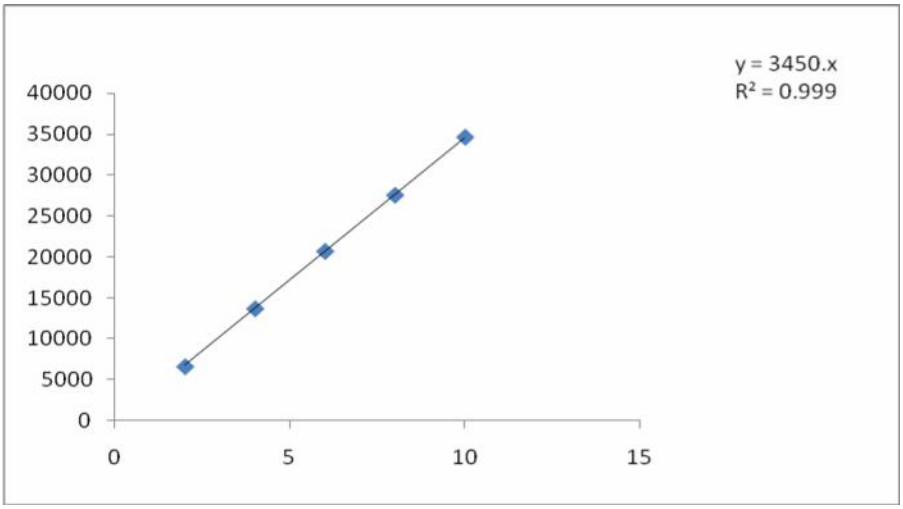


Figure-3
Standard chromatogram of Zinc carnosine and Aceclofenac

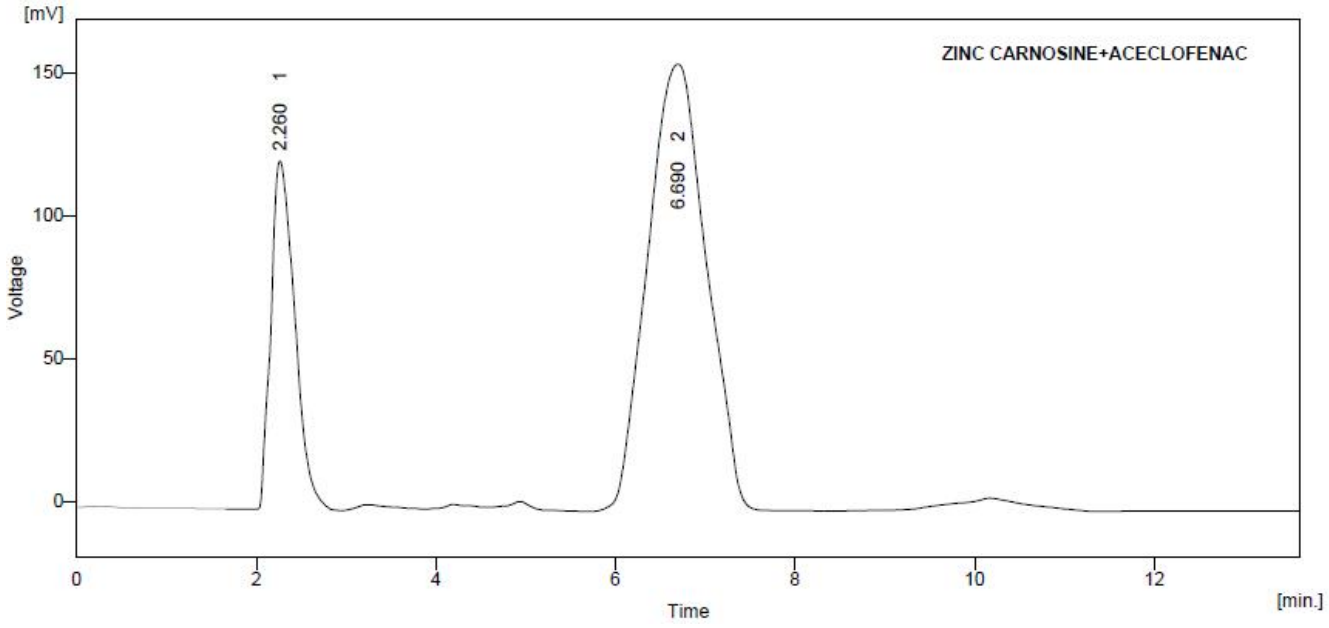
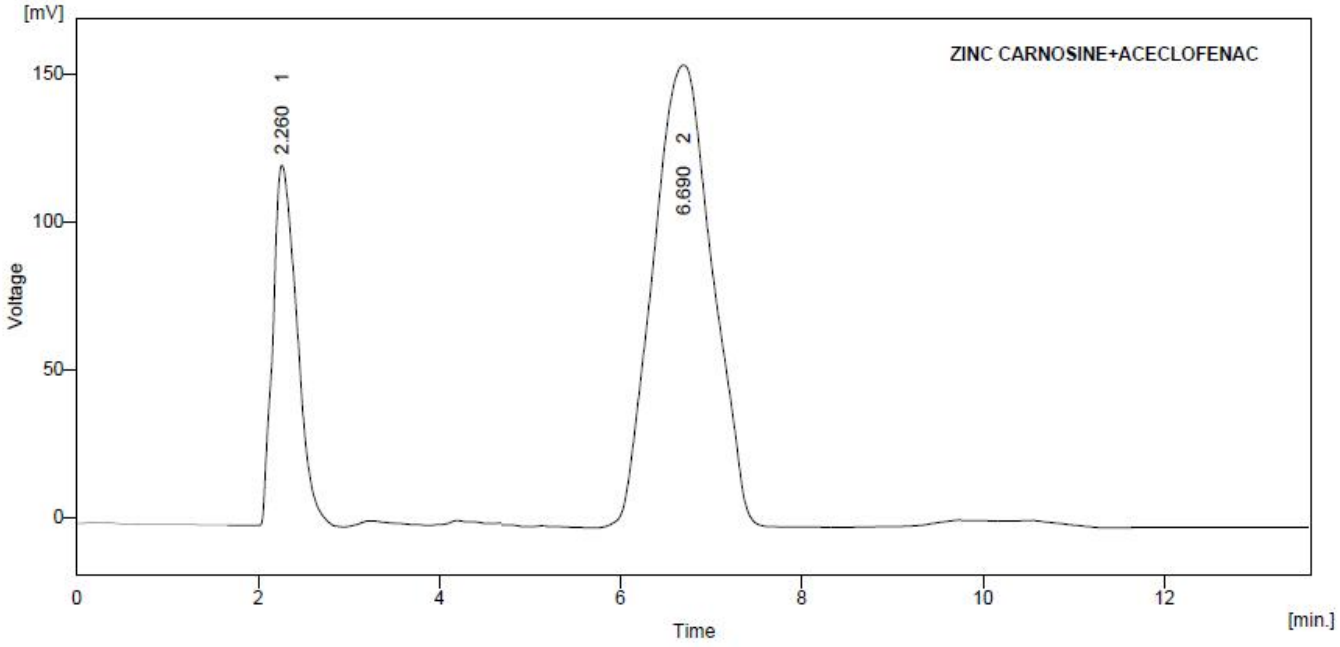


Figure-4
Sample chromatogram of Zinc carnosine and Aceclofenac



References

1. Yoshikawa T., Naito Y., Tanigawa T., Yoneta T., Yasuda M., Ueda S., Oyamada H. and Kondo M., Effect of Zinc-Carnosine Chelate Compound (Z-103), A Novel Antioxidant, on Acute Gastric Mucosal Injury Induced by Ischemia - Reperfusion in Rats, Free Radical Research, 1991, 14, 4,289-296.
2. Matsukura T. and Tanaka H., Applicability of Zinc Complex of L-Carnosine for Medical Use Biochemistry (Moscow), 2000, 65, 7, 817-823.
3. Masaru Odashima, Michiro Otaka, Mario Jin, Isao Wada, Youhei Horikawa, Tamotsu Matsuhashi, Reina Ohba, Natsumi Hatakeyama, Jinko Oyake and Sumio Watanabe Zinc L-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-κB activation Life Sciences , 2006,79, 24,2245-2250.
4. Razinah sharif, Philip Thomas, Peter Zalewski, Robin D. Graham and Micheal Fenech The effect of zinc sulphate and zinc carnosine on genome stability and cytotoxicity in the WIL2-NS human lymphoblastoid cell line Mutation Research/Genetic Toxicology and Environmental Mutagenesis 2011, 720, 1-2, 22-33.
5. Godse VP. Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD and Bafana YS. A simple, rapid and selective HPLC method has been developed for quantitation of aceclofenac and paracetamol frombulk drug and pharmaceutical formulations. Asian J. Research Chem. 2(1) 2009.
6. Zawilla .N. M. Abdul ,Azim MohammadN. M. El Kousy and S. M. El-Moghazy Aly. A sensitive and reproducible methods for quantitative determination of aceclofenac in pure form and in pharmaceutical formulation by UV. Journal of Pharmaceutical and Biomedical Analysis. Volume 27, Issues 1-2, 1 January 2002, Pages 243-251.
7. Joshi.R,Sharma.R., Development and validation of RP-HPLP Method for simultaneous estimation of three tablet formulation containing Acetaminophen ,Chlorzoxazone and Aceclofenac. Analytical letters.volume-41,issue-18,2008,3297-3308.
8. Singhvi, Anju Goyal .Developed aVisible spectrophotometric estimation of aceclofenac and indapamide from tablets using folin-ciocalteu reagent.Indian journal of pharmaceutical sciences, 2007, Volume -69, Issue-1, 164-165.
9. Garg.G,Swarnlata Saraf, S Saraf., A Simultaneous estimation of aceclofenac, paracetamol and chlorzoxazone in tablets by UV-Spectroscopy. Indian journal of pharmaceutical sciences, 2007, Volume -69, Issue -5, 692-694.
10. Q2 (R1) 2005 – Text on Validation of analytical procedures, step-4, Consensus guideline, ICH Harmonized Tripartite Guidelines. ICH Guidelines Q2B, Validation of Analytical Procedure: Definitions, published in March 1996, Geneva, Switzerland.
11. Lloyd R. Snyder et al., practical HPLC method development. John wiley & son's publishers, 2nd edition, Pg. no 350-400.
12. http://www.wikipedia.com. & www.Rxlist.com. April9