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Research Article

Simultaneous estimation of zinc carnosine and aceclofenac in bulk and tablet dosage form by HPLC method

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ABSTRACT

A simple, precise, rapid and accurate HPLC method developed for the simultaneous estimation of Zinc carnosine and Aceclofenac in tablet dosage form. An C-18 column with 250x4.6mm, 5µm particle size with mobile phase consisting of a mixture of potassium di-hydrogen phosphate buffer, Acetonitrile, methanol in the ratio of 50:30:20 v/v was used. The flow rate was 1.0 ml/min and detection carried out by using U.V visible detector at 215 and 275nm .The retention time were 2.258 for Zinc carnosine, and 6.690min for Aceclofenac. The detector response was linear for Zinc carnosine and Aceclofenac in the concentration range of 2 to 10µg/ml for both drugs. The limit of detection (LOD) for Zinc carnosine and Aceclofenac were found to be 33.4ng/ml and 101.3ng/ml respectively. The limit of quantification (LOQ) for Zinc carnosine and Aceclofenac were found to be 2.473ng/ml and 7.495ng/ml respectively. The percentage assay of Zinc carnosine and Aceclofenac was 99.82% and 99.92% respectively. The method was validated by determining its linearity, precision, accuracy, system suitability in accordance with ICH guidelines. The results of the study showed that the proposed HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Zinc carnosine and Aceclofenac in bulk drug and its pharmaceutical dosage forms.

Keywords: Zinc carnosine; Aceclofenac; HPLC; validation; Tablet

INTRODUCTION

Zinc carnosine is the combination of elemental Zinc and dipeptide L-carnosine. L- carnosine is the composition of β-alanyl-L-histidine (D.J.Miller et.al 1996). Zinc carnosine is chemically a zinc salt of (2*S*)-2-[(3-Amino-1-oxopropyl) amino]-3-(3*H*-imidazol-4-yl) propanoic acid (Masaru Odashima et.al 2006). It has anti-ulcer and anti-oxidant properties (Yoshikawa T et.al 1991). Aceclofenac is chemically 2-(2, 6-Dichlorophenyl) amino phenyl acetyl oxyacetic acid. It is anti-inflammatory and analgesic agent (Godse et.al 2009). Literature survey reveals that 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 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 precise RP-HPLC method has been developed for Simultaneous determination of Aceclofenac, Paracetamol and Chlorzoxazone from bulk drug and pharmaceutical Formulations (Uttam D.Pawar et.al2009). A sensitive and reproducible method for quantitative determination of Aceclofenac in pure form and in pharmaceutical formulation by U.V (Rohit shah et.al 2008& Singhvi et.al 2007). Development and validation of RP-HPLC method for simultaneous estimation of three tablet formulation containing Acetaminophen, Chlorzoxazone and Aceclofenac (Joshi R et.al 2008).

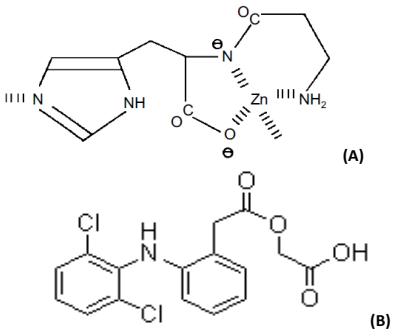

Figure 1: (A) Chemical structure of Zinc carnosine (B) Chemical structure of Aceclofenac

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of HPLC grade. Potassium di-hydrogen phosphates are obtained from S.D. fine chemicals, Mumbai, which is used for preparing the buffer. Acetonitrile, Methanol was obtained from E.Merck, Mumbai. Pure sample of Zinc carnosine

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Table 1: Assay for Zinc carnosine and Aceclofenac

Drugs	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

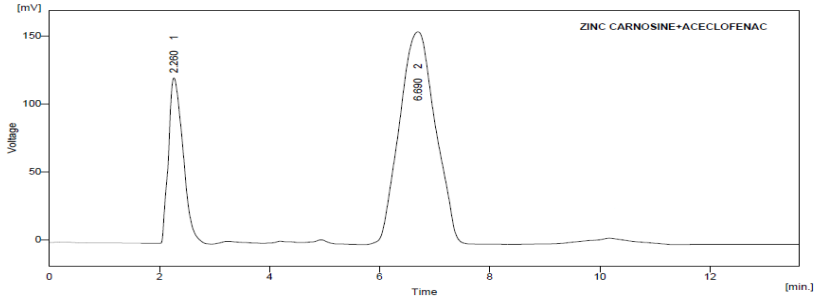

Figure 2: A typical chromatogram of Zinc carnosine and Aceclofenac standard

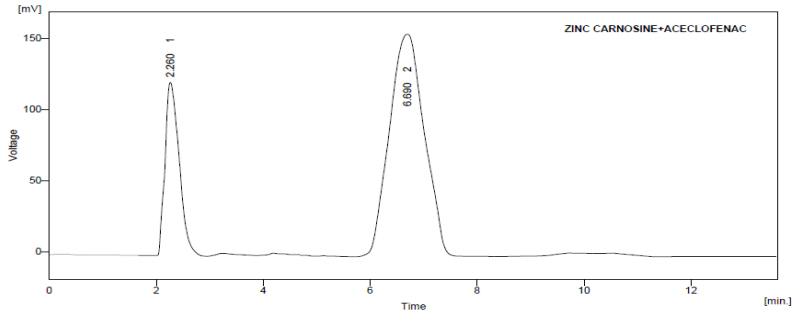

Figure 3: A typical Chromatogram of Zinc carnosine and Aceclofenac sample

Table 2: Zinc carnosine and Aceclofenac linearity results

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

and Aceclofenac was obtained as gift sample from Safe tab life sciences, Pondicherry. Commercial samples of tablets containing the drugs Zinc carnosine and Aceclofenac (Acenal safe) were purchased from the Regemix drugs Pvt Ltd, Chennai.

Instruments

The instrument used for the study was a High pressure liquid chromatography prominence model equipped with pump (isocratic LC-20 AT), inline degasser, U.V visible detector (SPD-20A) (Lloyd R et.al 2005).

Chromatographic Conditions

Column : Phenominex C-18(250x 4.6mm) x 5 µm particle size

Mobile phase : Potassium di-hydrogen phosphate buffer (0.02M, pH3.5): Acetonitrile: Methanol (50:30:20 v/v)

Flow rate : 1 ml/min

Injection volume : 20µl

Detection wave length : 215nm and 275nm

Pump model : Isocratic

Run time : 10minutes

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 (Acenol Safe) were weighed and finely powdered. Powder equivalent to 40.86 mg of zinc carnosine and 42.82 mg of Aceclofenac was transferred to

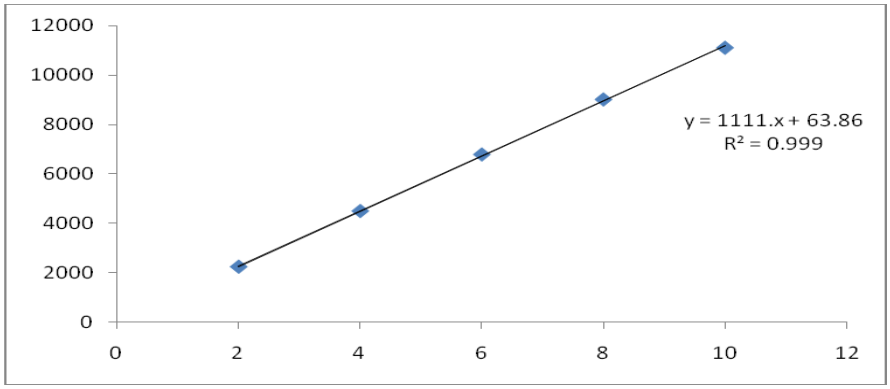

Figure 4: Linearity curve of Zinc carnosine

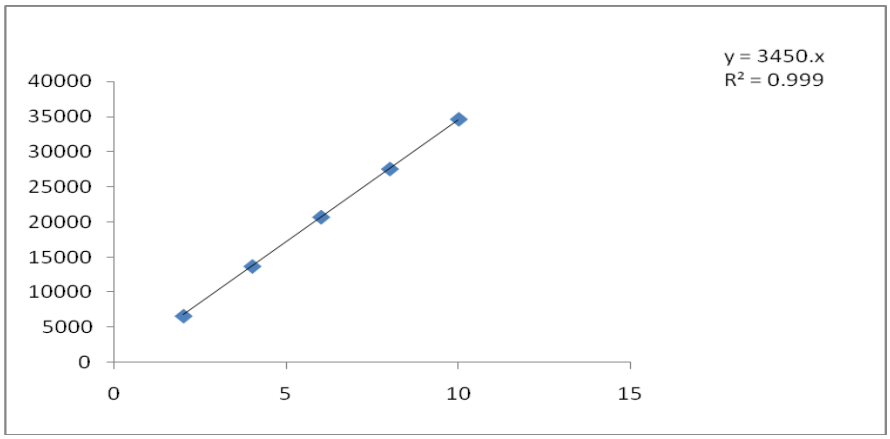

Figure 5: Linearity curve of Aceclofenac

Table 3: Acuracy of Zinc carnosine and Aceclofenac

Drug	Label claim in mg	Sample conc in mg/mL	Amount added in µg	Amount recovered 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	99.0 99.75 99.83	99.52

Table 4: System precision

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

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 (2µg/mL) and Aceclofenac (2µg/mL).

Procedure for Assay

Sample and standard solutions (2µg/mL) separately injected into the HPLC system. From the peak area the

Table 5: Method precision

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

amount of drugs in the sample and standard solution were computed and the Results shown in table no 1. The standard and sample chromatograms were shown in Figure no -2, 3

Method validation

The proposed method was validated for assay as per ICH guidelines by using following parameters. (Anonymous ICH guidelines, Q2 (B), 2003, 1996& Q2 (R1) 2005).

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 up to 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 no 2, Figure no.4,5.

Accuracy as recovery

It was done by recovery study (Raman M Singh et.al 2009& Zawilla .N et.al 2002). 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 no 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) was prepared as per test method and injected for 3 times. Results were shown in Table no 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 no 5.

Limit of detection and limit of quantification

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. Results were shown in Table no 6.

RESULTS AND DISCUSSION

HPLC has acquired most valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation. Literature survey revealed no HPLC was developed for the determination of Zinc carnosine and Aceclofenac. The composition of mobile phase for development of chromatographic method was optimized by using different solvent mixtures of varying polarity. The best result were obtained using Potassium di-hydrogen phosphate buffer (0.02M, pH3.5): Acetonitrile: Methanol (50:30:20 v/v). This mobile phase showed good resolution of Zinc carnosine and Aceclofenac peaks. Fig 2, 3 shows typical chromatograms of Zinc carnosine and Aceclofenac. By this proposed method the retention time of Zinc carnosine and Aceclofenac was 2.260, 6.690 minutes respectively and none of the impurities were interfering in its assay. 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 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

In the conclusion the proposed HPLC method was simple and precise because commonly used buffer, easier procedure. The proposed method is highly accurate which showed high percentage recovery and that the method was free from interference of excipients used in the formulations. It can be concluded that HPLC method was successfully developed for simultaneous estimation of combined dosage form containing Zinc carnosine and Aceclofenac.

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REFERENCES

Anonymous, ICH Guidelines: Validation of Analytical Procedures: Methodology Q2 (B), 2003.

D.J Miller and A. O' Dowd "Vascular smooth muscle actions of Carnosine as its Zinc complex Are mediated by Histamine H1 and H2 receptors" Journal of Physiology, 495(2), 1996, 535- 543.

Garg.G, Swarnlata Saraf, and S Saraf., "A Simultaneous estimation of Aceclofenac, Paracetamol and Chlorzoxazone in tablets by UV-Spectroscopy" Indian journal of pharmaceutical sciences, 69(5),2007, 692-694.

Godse VP.Deodhar M N, Bhosale A V, Sonawane R A, Sakpal P S, Borkar D D and Bafana Y S. "A simple, rapid and selective HPLC method has been developed for quantitation of Aceclofenac and Paracetamol from bulk drug and pharmaceutical formulations" Asian J. Research Chem. 2(1) 2009.

Joshi.R and Sharma.R "Development and validation of RP-HPLC Method for simultaneous Estimation of three tablet formulation containing Acetaminophen, Chlorzoxazone and Aceclofenac" Analytical letters.41 (18), 2008, 3297-3308.

Lloyd R. Snyder et al., practical HPLC method development. John Wiley & Son's publishers, 2nd Edition, 2005, Page no. 350-400.

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 , 79(24), 2006, 2245-2250.

Pawan K Saini, Raman M Singh, Chhoten L Jain, Satish C Mathur and Gyanendra N Singh "A Sensitive and selective RP-HPLC method for the determination of Lamivudine and stavudine in tablets" Journal of Pharmacy Research 2009, 2(10),1598-1600

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.

Rohit shah, Chandrakant magdum and Shital kumar patil "validated spectroscopic method for Estimation of Aceclofenac from tablet formulation" Research journal of pharm. And tech. 1(4) 2008, 430-432

Singhvi and Anju Goyal "Development of a Visible Spectrophotometric estimation of Aceclofenac and Indapamide from tablets using folin-ciocalteu reagent" Indian journal of pharmaceutical sciences, 69(1), 2007, 164-165.

Uttam D.Pawar, Abhijit V.Naik and Aruna V. Sulebhavikar "Simultaneous determination of Aceclofenac, Paracetamol and Chlorzoxazone by HPLC in tablet dosage form" E-journal of Chemistry, 6(1), 2009, 289-294.

Yoshikawa T., Naito Y., Tanigawa T., Yoneta T., Yasuda M., Ueda S., Oyamada H. and Kondo "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. 14(4), 1991, 289-296.

Zawilla .N. M. Abdul, Azim Muhammad. 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,27(1-2), 2002,243-251.