DEVELOPMENT OF HPLC METHOD FOR THE DETERMINATION OF ZINC CARNOSINE

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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 potassium di-hydrogen ortho phosphate buffer and Acetonitrile (76:24 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 5.700 min. The method produced linear responses in the concentration range 0.5 to 60 μg/mL of zinc carnosine. The LOD and LOQ values for HPLC method were found to be 1.29 and 3.90 μg/mL respectively. The method was found to be applicable for determination of the zinc carnosine.

KEYWORDS: HPLC, Zinc carnosine, Estimation, validation.

INTRODUCTION
Zinc carnosine is a zinc salt of (2S)-2-[(3-Amino-1-oxopropyl)amino]-3-(3H-imidazol-4-yl)propanoic acid. It is the prescribed drug for the treatment of ulcer. As few 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.[1-4]

MATERIALS AND METHODS
Apparatus
High performance liquid chromatography including a Jasco instrument equipped with rhynodyne manual sampler, UV detector, Borwin software and HiQ-sil C18HS, 5 μm column having dimensions 4.6mm x 250mm was used.
MATERIALS AND REAGENTS
Zinc carnosine was obtained as a gift sample from Puneet Laboratory Pvt. Ltd, Mumbai. HPLC grade methanol, acetonitrile and water used was purchased from S.D. Fine Chemicals (Mumbai, India).

Chromatographic condition
A mobile phase consisted of acetonitrile: 0.2M phosphate buffer pH-3 (76:24, v/v) was pumped at a flow rate of 1 mL/min. The elution was monitored at 215 nm and the injection volume was 20 μL. The validation of the method was done following the ICH guidelines.[23]

Preparation of mobile phase
A freshly prepared 76:24 v/v mixture of 0.2M Potassium di-hydrogen ortho phosphate (3.0 pH) and Acetonitrile was used as the mobile phase. Buffer solution was prepared by dissolving 2.7 g of potassium dihydrogen orthophosphate in 1000 mL of water. To this add 0.48 g of 1-decane sulphonic salt and adjust the pH to 3.0 with orthophosphoric acid. Both Potassium 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 wavelength was chosen 215 nm for detection.

Preparation of the standard solution
Accurately weighed 10 mg of zinc carnosone working standard was transferred to 100 mL volumetric flask to this 20 ml of 0.1N HCL and then made upto mark with water and then sonicate for 5 minute. From this solution, concentrations of 0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0 mL were taken in 10 ml volumetric flasks and diluted upto the mark with water : 0.1N HCl (80:20) such that the final concentration of Zinc carnosine in the range of 0.5 to 60 μg/mL.

Analysis of marketed formulation
Assay of marketed capsules formulation containing zinc carnosine 75 mg was performed by preparing the sample solutions as described earlier in the preparation of the sample. Six injections of above prepared sample and standard solutions were injected. The assay of the commercial sample was calculated by comparing the areas of standard and sample peaks.
Validation of the method

Specificity.- Specificity of the method was studied by injecting blank, standard and sample solutions.

Calibration curve (Linearity of HPLC method)

Calibration curve was constructed by plotting concentrations of zinc carnosine vs. peak areas, and the regression equations were calculated. The linearity of this method was investigated by using the concentrations 5, 10, 15, 20, 25, 30, 40, 50 and 60 μg/mL for zinc carnosine. These concentrations were prepared by diluting appropriate volume of working standard with water: 0.1N HCl (80:20). The retention times of zinc carnosine was 5.69 (± 0.56) min respectively.

System suitability study

For this study first upon a 20 μL of blank solution (water : 0.1N HCl (80:20)) was injected and run for 10 minutes. After this 20 μL of standard solutions in 6 replicates were injected and the % relative standard deviation (% RSD) of the response peak areas was calculated.

Accuracy (% recovery).- Accuracy of the method was studied by recovery experiments using standard addition method at three different levels (80%, 100% and 120%). The known amounts of standard solutions containing zinc carnosine (16, 20 and 24 μg) were added to prequantified sample solutions to reach the 80, 100 and 120 % levels. These samples were analyzed by injecting the sample solution and recovery was calculated.

Precision (Repeatability)

Precision of the assay method was demonstrated by injecting six different sample solutions containing zinc carnosine equivalent to 20 μg/mL and RSD of mean assay value was calculated.

Intermediate Precision (Ruggedness)

Intermediate Precision of the method was demonstrated by carrying out the experiment on different day, by different analyst and on different instrument using different C-18 column.

Robustness

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.9 mL/min and also from 1.0 mL/min to 1.1 mL/min. The composition of mobile phase was changed.
from acetonitrile: 0.2M phosphate buffer pH-3 (24:76, v/v) to acetonitrile: phosphate buffer pH-3 (25.2:74.8, v/v) and also from acetonitrile:phosphate buffer pH-3 (24:76, v/v) to acetonitrile: phosphate buffer pH-3 (22.8:77.2, v/v). The solutions for robustness study were applied on the column in triplicate and the responses were determined.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

LOD and LOQ of escitalopram oxalate and etizolam were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \quad \text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma = \) Standard deviation of response, \( S = \) Slope of regression equation.

**RESULT AND DISCUSSIONS**

Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained by using the mobile phase containing acetonitrile: 0.2M phosphate buffer pH-3 (24:76, v/v). Quantification was achieved with UV detection at 215 nm based on peak area.

A representative chromatogram is shown in Figure 1.

![Figure 1: Chromatogram of zinc carnosine (R.T 5.92± 0.56) at 215 nm.](image)

System suitability tests were carried out on freshly prepared standard solutions (n = 6) containing zinc carnosine. System suitability parameters obtained with 20μL injection volume are summarized in Table 1.
Table 1: System suitability test parameters for zinc carnosine.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time of zinc carnosine</td>
<td>5.69 ± 0.538</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>2793.06±1.32</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.25±0.09</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
</tr>
</tbody>
</table>

Specificity studies indicated that there is no interference from excipients, impurities and degradation products and assured that the peak response was due to zinc carnosine only.

Linearity regression data is summarized in Table 2 which shows a good linear relationship between concentration and peak areas over a concentration range of 5-60 μg/mL (Figure 2). The correlation coefficient (R^2) was found to be 0.9996 for zinc carnosine. The limit of detection was found to be 1.29 μg/mL and the limit of quantification was found to be 3.90 μg/mL for zinc carnosine. These values indicate that the method is sensitive.

![Figure 2: Calibration curve diagram for zinc carnosine.](image)

Table 2: Regression analysis of the calibration curves for zinc carnosine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zinc carnosine (± %RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range(μg/mL)</td>
<td>5-60</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=26984x+12306</td>
</tr>
<tr>
<td>Correlation coefficient(R2)</td>
<td>0.9996</td>
</tr>
<tr>
<td>Slope</td>
<td>27298</td>
</tr>
</tbody>
</table>

In the precision studies, RSD of mean assay values was found to be 1.09 for zinc carnosine. These values indicate that the repeatability of this method is satisfactory.
Intermediate precision (Ruggedness) study reveals that the method is rugged with RSD values 0.94 and Accuracy studies indicate that the mean percent recovery of the added standard drug to found be 100.50% for zinc carnosine.

Robustness study signified that the results of the method remained unaffected by small, deliberate changes in the flow rate and mobile phase composition. The RSD of mean assay values was found to be 0.08 with a flow rate of 0.9 mL/min. The RSD of mean assay values was found to be 0.63 for escitalopram oxalate and 0.37 with a flow rate of 1.1 mL/min. Also RSD of mean assay values was found to be 0.10 with mobile phase composition of acetonitrile: 0.2M phosphate buffer pH-3 (22.8:77.2,v/v) and 0.07 respectively with mobile phase composition acetonitrile: 0.2M phosphate buffer pH-3 (25.2:74.8, v/v). Results obtained for various validation parameters are summarized in Table 3. The assay results obtained by using the proposed method for the analysis of marketed capsule formulation containing zinc carnosine 75 mg per capsule was in good agreement with the labeled amounts of zinc carnosine. The average contents of zinc carnosine was 76.22 mg per capsule (101.63%) respectively.

Table 3: Summary of validation parameters for the proposed HPLC method for zinc carnosine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>zinc carnosine (± % RSD )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (μg/ml)</td>
<td>1.29</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>3.90</td>
</tr>
<tr>
<td>Accuracy( % recovery)</td>
<td>100.50</td>
</tr>
<tr>
<td>Precision</td>
<td>101.63±1.09*</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>99.73±1.14*</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile: 0.2M phosphate buffer pH-3 (22.8:77.2,v/v)</td>
<td>101.60±0.91†</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile: 0.2M phosphate buffer pH-3 (25.2:74.8, v/v)</td>
<td>100.65±0.49†</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
<tr>
<td>(0.9 ml/min Flow rate )</td>
<td>100.65±0.04†</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
<tr>
<td>(1.1 ml/min Flow rate )</td>
<td>101.19±0.75†</td>
</tr>
</tbody>
</table>

* mean assay values of 6 replicates
† mean assay values of 3 replicates
CONCLUSION
The proposed RP-HPLC method is accurate, precise, sensitive, selective and rapid for the determination of zinc carnosine in a formulation.

ACKNOWLEDGEMENT
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