ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the estimation of Olopatadine hydrochloride, using a Inertsil-ODS 3V column and a mobile phase composed of Buffer: Methanol: Triethylamine (55:45:0.1, %v/v), pH 3.0 adjusted with o-phosphoric acid. The retention time of Olopatadine hydrochloride was found to be 6.3 min. Linearity was established for Olopatadine hydrochloride in the range of 35-65 µg/ml. The percentage recovery of Olopatadine hydrochloride was found to be in the range of 98.70-100.40%. The drug was subjected to acid and alkaline hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated Olopatadine hydrochloride showed degradation in acid, alkaline and H₂O₂ while it was found stable in photolytic and in presence of dry heat. The degradation products of Olopatadine hydrochloride in acidic, alkaline and oxidative conditions were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for the quantitative analysis of Olopatadine hydrochloride in bulk drugs and formulations.

Keywords: Olopatadine hydrochloride, stress testing, degradation products, stability indicating method.

INTRODUCTION

Olopatadine hydrochloride 11-((Z)-3-(Dimethyl-amino) propylidene)-6, 11- dihydrodibenz [b, e] oxepin-2-acetic acid, is a white crystalline powder, freely soluble in water and alcohol. It is a histamine H1 receptor-antagonist and is used as an antiallergic and anti-inflammatory agent.

Literature survey indicated that estimation of Olopatadine was done by using HPTLC. It includes quantitative determination of Olopatadine in Human Plasma by HPLC-MS. The literature survey does not reveal any stability indicating assay method for the estimation of Olopatadine in bulk and Pharmaceutical dosage form which gives information about the degradation products as well as separation of degradation products. The literature survey indicated that no stability indicating RP-HPLC method was proposed for Olopatadine Hydrochloride.

The International Conference on Harmonization (ICH) guideline entitled “Stability testing of new drug substances and products” requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine Olopatadine Hydrochloride in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought necessary to study the stability of Olopatadine Hydrochloride under acidic, alkaline, oxidative, photolytic and dry heat conditions. This paper reports validated stability-indicating HPLC method for simultaneous determination of Olopatadine Hydrochloride in presence of their degradation products. The proposed method is simple, accurate, reproducible, stability-indicating and suitable for routine determination of Olopatadine Hydrochloride in its dosage form. The method was validated in compliance with ICH guidelines.

MATERIALS AND METHODS

Olopatadine Hydrochloride of working standard grade was kindly supplied as gift sample by MSN Laboratories, Hyderabad, India and was certified to contain 99.6% (w/w), on as is basis. Olopatadine Hydrochloride Active Pharmaceutical Ingredient (API) was kindly supplied as gift sample by Zyodus Cadila Healthcare Ltd., Ahmedabad, India and was certified to contain 99.6% (w/w), on as is basis. Methanol and water used were of HPLC grade and were purchased from Spectrochem Pvt. Ltd. Mumbai, India. The tablet formulation (Allelock Tablet) containing 5 mg of Olopatadine Hydrochloride was procured from local market and used for analysis of marketed formulation. Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC (Double pump) with Rheodyne 7725i type injector with 100µl loop capacity and SPD M20A, Prominence Diode Array Detector. The wavelength of detection chosen was 299 nm. A reverse phase Inertsil ODS-3V column (150 mm x 4.6 mm, 5 µm) was used for the analysis. The mobile phase comprising of a mixture of phosphate buffer, methanol and triethylamine with pH 3.0 adjusted with o-phosphoric acid in the ratio of 55: 45: 0.1 % v / v with a flow rate of 2 ml/min. The injection volume was 20 µl. In addition, an electronic balance (Shimadzu AX200), a pH meter (Systronics model E3MK VI), a sonicator (Spectra Lab, model UCB 40), a hot air oven (Labhosp) were used in this study.
Preparation of Mobile Phase

13.6 gm of potassium dihydrogen phosphate dissolved in 1000 ml of water. 1ml of triethylamine was added. The pH of mixture was adjusted to 3.0 with o-phosphoric acid. This phosphate buffer was mixed with methanol in the ratio of 55:45%v/v. This mixture was sonicated for 10 min and filtered through 0.22 µm membrane filter and used as mobile phase.

Preparation of Stock Solution

Accurately 50 mg of Olopatadine HCl was weighed into 100 ml of volumetric flask. The compound was first dissolved in few ml of methanol. The volume was then made up to 100 ml with methanol to get a concentration of 500 µg/ml.

The solution was further diluted with the same solvent to obtain final concentrations of 50µg/ml. The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of Buffer: Methanol: Triethylamine (55:45:0.1, %v/v), pH 3.0 adjusted with o-phosphoric acid on Inertsil ODS-3V column (150×4.6 mm, 5 µm particle size) with 2 ml/min flow rate at 299 nm using UV detector.

Analysis of Marketed Formulations

10 tablets were accurately weighed and transferred the intact tablets to a 100 ml volumetric flask. Around 50 ml of methanol was added and the mixture was sonicated for 45 min in an ultrasonic bath. The volume was then made up to mark with methanol to obtain a concentration of 500 µg/ml. Above solution was filtered using What man filter paper No 1. Appropriate volume of the aliquot was transferred to a 50 ml volumetric flask and the volume was made up to the mark with methanol to obtain a solution containing 50 µg/ml of Olopatadine HCl. A 20 µl volume of above sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions.

Method Validation

The method of analysis was validated as per the recommendations of ICH6 and USP7 for the parameters like accuracy, linearity, precision, specificity, detection limit, quantitation limit, ruggedness, robustness and solution stability.

Linearity

Every 20 µl of the working standard solution of Olopatadine HCl in the mass concentration range of 35 to 65 µg/ml, were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Olopatadine HCl was obtained by plotting the peak area ratio versus the applied concentrations of Olopatadine HCl (fig. 1).

![Calibration curve of Olopatadine HCl showing linearity](image)

**Figure 1**: Calibration curve of Olopatadine HCl showing linearity

Calibration curve with Concentration on X-axis and Area on Y-axis.

Accuracy

The accuracy of the method was determined by calculating percentage recovery of Olopatadine HCl. Recovery studies were carried out by applying the spiking method in which known amount of Olopatadine HCl corresponding to 70, 100 and 130% of label claim was added (standard addition method) to the placebo. At each level of the amount three determinations were performed and the results obtained were compared.

Precision

The method precision was done by preparing six different sample preparations by one analyst under the same conditions and analyzing them.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve.

System Suitability

System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 50 µg /ml Olopatadine HCl to check the reproducibility of the system.

Specificity

To carryout specificity test injection of placebo solution was injected to check whether there is no carry over in blank solution as well as the placebo does not interferes with the drug peak. The drug peak was also checked for its peak purity in presence of excipients.

Robustness

For robustness evaluation of HPLC method a few parameters like flow rate, column oven temperature, percentage of methanol in the mobile phase and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor
selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 50 µg/ml for Olopatadine HCl.

Ruggedness

Ruggedness was determined between two different analysts, instruments and columns to see whether the method provides reproducible results with the changes.

Solution Stability

The standard and sample solutions were injected at different time intervals for 36 hrs to observe % difference in chromatographic responses relative to freshly prepared solutions.

Forced degradation studies

Forced degradation studies of both the drugs were carried out under conditions of acid-alkali hydrolysis, dry heat, oxidation and photolysis. Olopatadine HCl was weighed (50 mg) and transferred to 100 ml volumetric flasks and added 10 ml of methanol. For sample tablets powdered tablets weighed equivalent to 50 mg of Olopatadine HCl and transferred to 100 ml volumetric flask and added 10ml methanol. Three such sets were prepared for acid, alkali and peroxide degradation.

- Forced degradation in basic media was performed by adding 5ml of 5N NaOH to above prepared mixtures in one set of flasks and these mixtures were heated for up to 2h at 80°C. Then the solution was cooled and neutralized with 5ml 5N HCl.
- Forced degradation in acidic media was performed by adding 5ml of 5N HCl to above prepared mixtures in one set of flasks and these mixtures were heated for up to 2h at 80°C. Then the solution was cooled and neutralized with 5ml 5N NaOH.
- Forced degradation in peroxide was performed by adding 5 ml of 10% H₂O₂ to above prepared mixtures in one set of flasks and these mixtures were heated for up to 2 h at 80°C.
- For dry heat degradation, solid drugs (50 mg Std. and Tablet powder equivalent to 50 mg) were kept in Petri dish in oven at 100°C for 24 h. Thereafter, they were transferred to 100 ml volumetric flasks and diluted up to the mark with methanol.
- For photostability, solid drugs (50 mg Std. and Tablet powder equivalent to 50 mg) were kept in Petri dish in direct sunlight in summer days for 5 h on a wooden plank. Thereafter, they were transferred to 100 ml volumetric flasks and diluted up to the mark with methanol.

For HPLC analysis, all the degraded sample solutions were diluted with methanol to obtain concentration of 500µg/ml of Olopatadine HCl and further diluted to obtain final concentration of 50µg/ml of Olopatadine HCl.

Besides, solutions containing 50 µg/ml of drug separately were also prepared without being performing the degradation of both the drugs. Then 20 µl solution of above solutions were injected into HPLC system and analyzed under the chromatographic condition described earlier.

RESULTS AND DISCUSSION

The mobile phase consisting of Buffer: Methanol: Triethylamine (55:45:0.1, % v/v), pH 3.0 adjusted with orthophosphoric acid, at 2ml/min flow rate was optimised which gave sharp peak with minimum tailing factor for Olopatadine HCl (fig. 2). The retention time for Olopatadine HCl was 6.3 min. UV spectra of Olopatadine HCl showed the λ_{max} at 299 nm, so this wavelength was selected as the detection wavelength.

![Chromatogram of Olopatadine HCl standard](image)

The calibration curve for Olopatadine HCl was found to be linear over the range of 35-65 µg/ml. The graph gave a regression coefficient of 0.9998. The data of regression analysis of the calibration curves is shown in Table 1 & 2. The calibration curve was plotted as Area vs. Concentration.

![Chromatogram of Olopatadine HCl standard](image)

The accuracy results show that the method is accurate to practically achieve added amount at three different levels within 98%-102% with RSD less than 2%. The average %recovery was found to be 99.3%. The results are shown in Table 3.
Table 3: Results of Accuracy Data of Olopatadine HCl

<table>
<thead>
<tr>
<th>Level of Recovery</th>
<th>Area</th>
<th>Added Amount (mg)</th>
<th>Recovered Amount (mg)</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
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<td>70%</td>
<td>146339</td>
<td>34.90</td>
<td>35.09</td>
<td>100.5</td>
<td>100.4</td>
<td>0.3</td>
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<tr>
<td></td>
<td>145541</td>
<td>34.87</td>
<td>34.90</td>
<td>100.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>146661</td>
<td>34.96</td>
<td>35.17</td>
<td>100.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>203638</td>
<td>49.60</td>
<td>48.83</td>
<td>98.4</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>202023</td>
<td>49.40</td>
<td>48.44</td>
<td>98.1</td>
<td></td>
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<td>130%</td>
<td>271647</td>
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<td>100.0</td>
<td>99.0</td>
<td>0.9</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>266260</td>
<td>64.74</td>
<td>63.84</td>
<td>98.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: Accuracy was checked at three levels viz. 70%, 100% and 130%

The method precision gave results obtained within 2% RSD suggesting the method is precise. The results are shown in Table 4.

Table 4: Results for Method Precision and Ruggedness

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Olopatadine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Assay</td>
</tr>
<tr>
<td>Method Precision</td>
<td>98.3</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>98.5</td>
</tr>
</tbody>
</table>

a: Mean of six determinations; b: Ruggedness studies were carried out using different analysts, instruments and columns.

The LOD for Olopatadine HCl was found to be 0.6 µg/ml while LOQ was 1.83 µg/ml.

The developed method was also found to be specific, since there was no interference of excipients and the purity of the peak was >0.999 (fig. 3).

Figure 3: Chromatogram of market formulation of Olopatadine HCl

Olopatadine hydrochloride with t_R of 6.3 min.

The results for ruggedness evaluation give RSD below 2% suggest the method is rugged to changes. The results are shown along with method precision in Table 4.

The results for system suitability parameters are shown in Table 5.

Table 5: System Suitability Data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Olopatadine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td>209450.167</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>2110.52</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>6.23</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.00</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.4</td>
</tr>
</tbody>
</table>

a: Mean of six determinations

Results for robustness evaluation for drug are presented in Table 6. Insignificant differences in peak areas and less variability in retention times were observed. The results mentioned are of area obtained.

The results for solution stability show that the standard and sample solutions were found to be stable up to 36hrs.

The results for validation and system suitability test parameters are summarized in Table 7.

The proposed method was successfully applied to the determination of Olopatadine HCl in its dosage form. The results for the combination were comparable with the corresponding labelled amounts.

The degradation study indicated that Olopatadine HCl was susceptible to acid, base and H_2O_2 while it was stable in direct sunlight and dry heat under experimental conditions (fig. 7, 8). Olopatadine HCl gets degraded into one to three degradation products in the stress conditions of acid-alkali hydrolysis as well as peroxide oxidation (fig. 4, 5, 6), while the drug showed no degradation at 0 h in all the degradation conditions.

Figure 4: Chromatogram of Olopatadine HCl degraded under acidic conditions

Olopatadine hydrochloride with t_R of 6.3 min and shows degradation product at t_R of 3.9 min.
Figure 5: Chromatogram of Olopatadine HCl degraded under basic conditions

Olopatadine hydrochloride with t<sub>R</sub> of 6.3 min shows decrease in peak area, but no additional degradation product is observed.

Figure 6: Chromatogram of Olopatadine HCl degraded under peroxide conditions

Olopatadine hydrochloride with t<sub>R</sub> of 6.3 min and degradation products with t<sub>R</sub> 5.25 min, 7.51 min and 14.58 min, respectively.

Figure 7: Chromatogram of Olopatadine HCl degraded under thermal degradation conditions

Olopatadine hydrochloride with t<sub>R</sub> of 6.3 min and no degradation found.

Figure 8: Chromatogram of Olopatadine HCl exposed to direct sunlight

Olopatadine hydrochloride with t<sub>R</sub> of 6.3 min and no degradation found.

Table 6: Results of Robustness Study

<table>
<thead>
<tr>
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<td>205882</td>
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<td>212568</td>
<td>210463</td>
<td>215645</td>
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<tr>
<td>6</td>
<td>209623</td>
<td>204587</td>
<td>202458</td>
<td>220996</td>
<td>196558</td>
<td>213058</td>
<td>210430</td>
<td>216584</td>
<td>211582</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Concentrations level used for robustness evaluation was 50 µg/ml. Three factors were slightly changed at three levels (-1, 0, 1). The results show area obtained.

Table 8: Summary of Degradation Studies for Olopatadine HCl

<table>
<thead>
<tr>
<th>Degradation Condition</th>
<th>Time(h/day)</th>
<th>% Degradation</th>
<th>t&lt;sub&gt;R&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (min) of degradation products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid, 5N HCl</td>
<td>2 hr</td>
<td>25.08%</td>
<td>3.904 min</td>
</tr>
<tr>
<td>Base, 5N NaOH</td>
<td>2 hr</td>
<td>33.42%</td>
<td>------ **</td>
</tr>
<tr>
<td>Oxidative, 10% H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2 hr</td>
<td>18.75%</td>
<td>5.25, 7.51 &amp; 14.58 min</td>
</tr>
<tr>
<td>Dry Heat (100°C)</td>
<td>24 hr</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>------</td>
</tr>
<tr>
<td>Direct sunlight(photolysis)</td>
<td>6 hr</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>------</td>
</tr>
</tbody>
</table>

<sup>a</sup>: t<sub>R</sub> stands for retention time; b: ND represents no degradation observed; ** Represents that no rise of additional degradation peak was observed.
The chromatogram of the acid degraded sample of Olopatadine HCl showed one additional peak at $t_r$ 3.904 min (fig. 4) and chromatogram of peroxide degraded sample showed three additional peaks at $t_r$ 5.25, $t_r$ 7.51 and $t_r$ 14.58 min, respectively (fig. 5). In alkali degradation, the drug degrades as shown by the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug, without giving any additional degradation peaks (fig. 6). Per cent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drug under non degradation condition. Summary of degradation studies of the drug is given in Table 8.

**CONCLUSION**

In the proposed study, stability-indicating HPLC method was developed for the estimation of Olopatadine HCl and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise, and repeatable. The developed method was found to be simple, sensitive and selective for analysis of Olopatadine HCl without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results for dosage form using proposed method showed 98.7±0.40 % of Olopatadine HCl. The results indicated the suitability of the method to study stability of Olopatadine HCl under various forced degradation conditions viz. acid, base, peroxide, dry heat and photolytic degradation. It can be concluded that the method separates the drugs from their degradation products; it may be employed for analysis of stability samples of Olopatadine HCl.

**Acknowledgements:** The authors thank Zydus Cadila Healthcare Ltd., Ahmedabad and MSN Laboratories, Hyderabad, India for providing Olopatadine hydrochloride as gift sample for this work. They also thank the management of Jaipur National University for providing required facilities to carry out this research work.

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