UV-Spectrophotometric Estimation of Olopatadine hydrochloride in Bulk and Pharmaceutical Dosage Form by Zero, First and Second Order Derivative Methods

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ABSTRACT

Simple and accurate UV spectrophotometric methods by Zero, First and Second order derivative method have been developed and validated for the estimation of Olopatadine hydrochloride in bulk and its pharmaceutical dosage form. The standard and sample solutions of Olopatadine hydrochloride were prepared in methanol and water. Olopatadine hydrochloride was estimated at 299, 289 and 267 nm for the derivative UV-spectrophotometric method. Beer’s law was obeyed in the concentration range of 20 to 120 μg / mL with coefficient of correlation value 0.9996, 0.999 and 0.999 for Zero, First and Second order derivative method. These methods were tested and validated for various parameters according to ICH and USP guidelines. The precision expressed as relative standard deviation were of less than 2 for the above three methods respectively. The proposed methods were successfully applied for the determination of Olopatadine hydrochloride in pharmaceutical dosage form. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Olopatadine HCl, UV-Visible spectrophotometry, Pharmaceutical Dosage forms, Derivative Spectroscopy, Method validation.

INTRODUCTION

The chemically Olopatadine hydrochloride is \((11Z)-11-[3-(\text{di-methyl-amin}) \text{propylidine}-6, 11-\text{dihydrodibenzo}[b,c] \text{oxepin-2-yl})\) acetic acid, hydrochloride. (Figure 1) corresponding to the molecular formula \((\text{C}_{22}\text{H}_{32}\text{NO}_{3}\text{HCl})\). Olopatadine HCl has a relative molecular mass of 373.873 g/mole. \([1, 2, 3, 4]\)

Olopatadine HCl is a selective histamine H1 receptor-antagonist activity and inhibits the release of histamine from mast cell. It is used to treat itching associated with allergic conjunctivitis. Its principal effects are inhibition of H1 receptors. They act on the bronchi, capillaries, and other smooth muscles.\([3, 1]\)

The literature survey reveals that numerous methods for determinations of Olopatadine HCl in single in pharmaceutical dosage forms, spectrophotometric methods in combination with other drugs \([7, 8]\), HPTLC \([9, 10]\), stability indicating spectrophotometric \([11, 12]\), HPLC methods in combination with other drug including HPLC \([13, 14]\) and UPLC method in bulk and pharmaceutical dosage form \([15]\). In this study we described very simple, sensitive, novel spectrophotometric methods. These methods show very simple, precise, cost effective and accurate approach for the analysis of Olopatadine HCl. For these methods there is no need of sophisticated instruments,
expensive solvents or a large number of samples.

**MATERIALS AND METHODS**

Pure sample of Olopatadine HCl was kindly supplied as a gift sample by Aurobindo Pharmaceuticals (Hyderabad, Maharashtra) India. All solvents and chemicals were of analytical grade. Marketed Tablet dosage form used in this research work was WINOLAP 5mg (SUN PHARMA) acquired from local market.

**Instruments**

Spectrophotometric measurements were carried out on Shimadzu UV 1800 double beam spectrophotometer. Infrared spectroscopic study was done on FTIR (Bruker, Japan).

**Preparation of standard stock solution**

Standard stock solution of Olopatadine Hydrochloride was prepared by accurately weighing 100 mg of Olopatadine Hydrochloride to 100 ml volumetric flask with 10 mL of methanol. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 μg/ml.

**Selection of analytical wavelength for zero order derivative method**

0.1 mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was then scanned in UV range between 200-400nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as water. The absorption maxima were found to be 299 nm and it is shown in Figure 2.

**Selection of analytical wavelength for First order derivative method**

The 0.1 mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was scanned in the wavelength range of 200 - 400 nm using UV spectrophotometer. The conversion of normal spectrum into first order derivative spectrum was done. The spectrum shows the sharp peak and maximum absorbance at 289 nm. The λmax 289 nm was selected for the first order derivative analysis and it is shown in Figure 3.

**Selection of analytical wavelength for Second order derivative method**

The 0.1 mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was scanned in the wavelength range of 200 - 400 nm using UV spectrophotometer. The conversion of normal spectrum into first order derivative spectrum was done. The spectrum shows the sharp peak and maximum absorbance at 267 nm. The λmax 267 nm was selected for the first order derivative analysis and it is shown in Figure 4.
Preparation of calibration curve for Zero, First and Second order derivative methods

Aliquots portion 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL was pipetted out from the standard stock solution and transferred to series of 10 mL volumetric flask and volume was made with water to get the concentration range from 20-120 μg/mL. The absorbance was measured three times for each concentration. Absorbance of each solution was measured against water as blank at 299 nm and 289 nm and 267 nm for zero order, first order and Second order methods respectively.

Table 1. Result for tablet analysis (Label Claim)

| Parameters | Tablet | Zero Order | First Order | Second Order |
|------------|--------|------------|-------------|--------------|
| Winolap (5 mg) | 10.135 | 100.28 | 98.92 |
| SD | 0.37 | 1.06 | 0.54 |
| % RSD | 0.36 | 0.06 | 0.55 |

Analysis of tablet formulation

Twenty tablets were weighed and finely powdered. Equivalent to 10 mg of Olopatadine HCl was weighed and transferred to a 100 mL volumetric flask containing with specific 10 mL of methanol and sonicated for 20 minutes. The solution was filtered through 0.45 μm Whatmann filter and volume was made up to mark with water to get concentration of 10 μg/mL of Olopatadine HCl. Recovery study of tablet formulation is shown in Table 1.

RESULTS AND DISCUSSION [16,17,18]

Method validation

The method of analysis was validated as per the recommendations of ICH and USP for the parameters like accuracy, linearity, precision, detection limit, quantitation limit, ruggedness and robustness.

\[
SD = \sqrt{\frac{\sum(X-X)^2}{N-1}}
\]

% RSD = \frac{SD}{\text{Mean}} \times 100

SD: Standard Deviation

% RSD: Relative standard deviation

N: Number of data values in dataset

X: Each of values of the data set

Linearity

The linearity of proposed derivative methods was evaluated by plotting the absorbance against concentrations of standard drug solutions. Stock solutions was consequently diluted with water to get 20, 40, 60, 80, 100, 120 μg/mL. The \( \lambda_{\text{max}} \) for first and second order derivative was obtained by converting the normal spectrum of zero order spectrum to first order spectrum and second order spectrum. The correlation coefficient was found to be 0.9996, 0.999 and 0.999 for zero order, first order and Second order derivative method. The result for calibration curve of zero, first and second order derivative spectrophotometric method shown in Figure 5, 6, 7.

Result for Linearity:

Preparation of calibration curve

![Figure 4: Second Order Derivative Spectra of Olopatadine HCl](image)

![Figure 5: Calibration curve for Olopatadine HCl by Zero Order spectrophotometric method](image)
**Figure 6**: Calibration curve for Olopatadine HCl by First order spectrophotometric method

**Figure 7**: Calibration curve for Olopatadine HCl by Second order derivative spectrophotometric method

**Precision**

The Precision study of analytical method validation express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of day precision of proposed methods was evaluated by analysing the three different independent concentrations i.e. 40, 60, 80 μg/ml in triplicate. These concentrations were evaluated for three consecutive days and the results are given in Table 2, 3 and 4 for zero order, first order and second order derivative spectrophotometric method.

**Table 2: Result for Precision Study for Intra-Day and Inter- Day (Zero Order).**

| Concentration (μg/ml) | Intra-Day Precision | Inter-Day Precision |
|-----------------------|---------------------|---------------------|
|                       | Amount Found (n=3)  | %RSD                |
|                       | 99.83±0.70          | 0.71                |
|                       | 99.02±0.93          | 0.94                |
| 40                    | 101.25±0.74         | 0.73                |
| 60                    | 101.34±0.24         | 0.23                |
| 80                    | 101.02±0.52         | 0.51                |

**Table 3 : Result for Precision Study for Intra-Day and Inter-Day (First Order)**

| Concentration (μg/ml) | Intra-Day Precision | Inter-Day Precision |
|-----------------------|---------------------|---------------------|
|                       | Amount Found (n=3)  | %RSD                |
|                       | 101.99±0.0006       | 0.23                |
|                       | 100.87±0.0020       | 0.81                |
| 40                    | 102.09±0.0034       | 0.95                |
| 60                    | 101.11±0.0037       | 0.81                |
| 80                    | 100.87±0.0015       | 0.33                |

**Table 4: Result for Precision Study for Intra-Day and Inter-Day (Second Order)**

| Concentration (μg/ml) | Intra-Day Precision | Inter-Day Precision |
|-----------------------|---------------------|---------------------|
|                       | Amount Found (n=3)  | %RSD                |
|                       | 101.64±0.0006       | 0.18                |
|                       | 100.95±0.0025       | 0.79                |
| 40                    | 99.53±0.0005        | 0.13                |
| 60                    | 99.04±0.0061        | 1.01                |
| 80                    | 99.72±0.0041        | 0.69                |

**Accuracy**

The accuracy of an analytical procedure expresses the results obtained by that method to the true value. The accuracy of the developed method was determined on the basis of recovery studies. The recovery tests were performed by adding known quantity of pure standard drug into the solution of tablet powder. The sample was then spiked with standard at levels 50%, 100% and 150% of tests concentrations. The resulting spiked sample solutions were analysed in triplicate. The result for accuracy shown in Table 5.

\[
y = 0.005213x + 0.038 \\
R^2 = 0.999
\]

\[
y = 0.00731x + 0.02213 \\
R^2 = 0.999
\]
Limit of Detection (LOD) Limit of Quantitation (LOQ)
The LOD and LOQ were evaluated from the data obtained from calibration curve. The LOD and LOQ for zero order derivative method was found to be 0.530μg/ml and 1.607μg/ml respectively, for first order derivative was found to be 0.863μg/ml and 2.877μg/ml and for second order derivative method was found to be 0.616μg/ml and 2.052μg/ml respectively.

Robustness
Robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness study of proposed method was evaluated by changing parameters like wavelength. In the proposed method the robustness study was studied by changing the wavelength (297 and 301) for zero order and (287 and 291) for first order derivative methods and (265 and 269) for Second Order derivative. The robustness testing for both the methods are given in Table 6, which indicates that, no significant difference was observed in results. Thus, it's demonstrated that the methods are robust.

Specificty
Specificity study is the ability to assess unequivocally the analyte in the presence of component which may be expected to be present. For the specificity study of proposed method the sample may be spiked with excipients or possible interfering components.

### Table 5: Result for accuracy (Recovery) Study

| Parameters | Zero Order | First Order | Second Order |
|------------|------------|-------------|--------------|
| Level of addition | % recovery (n=3) | Level of addition | % recovery (n=3) | Level of addition | % recovery (n=3) |
| 50% | 99.81±0.0026 | 50% | 100.180 | 50% | 100.82±0.0026 |
| 100% | 99.98±0.0011 | 100% | 99.993 | 100% | 99.33±0.0011 |
| 150% | 99.92±0.0012 | 150% | 99.049 | 150% | 101.17±0.0015 |

### Table 6: Result for Robustness Study

| Parameters | Zero Order | First Order | Second Order |
|------------|------------|-------------|--------------|
| Wavelength | Wavelength | Wavelength | Wavelength |
| 297 nm | 301 nm | 287 nm | 291 nm | 265 nm | 269 nm |
| Conc. (μg/ml) | 40 μg/ml | 40 μg/ml | 30 μg/ml | 30 μg/ml | 60 μg/ml | 60 μg/ml |
| Mean (n=5) | 0.280 | 0.277 | 0.158 | 0.186 | 0.532 | 0.614 |
| ±SD | 0.0016 | 0.0016 | 0.0016 | 0.0023 | 0.0049 | 0.0039 |
| % RSD | 0.5725 | 0.5787 | 1.012 | 1.2259 | 0.9265 | 0.6406 |

### Table 7: Result for Ruggedness Study

| Parameters | Zero Order | First Order | Second Order |
|------------|------------|-------------|--------------|
| Wavelength | Wavelength | Wavelength | Wavelength |
| 297 nm | 301 nm | 287 nm | 291 nm | 265 nm | 269 nm |
| Conc. (μg/ml) | Analyst I | Analyst II | Analyst I | Analyst II | Analyst I | Analyst II |
| 30 (μg/ml) | 30 (μg/ml) | 20 (μg/ml) | 20 (μg/ml) | 80 (μg/ml) | 80 (μg/ml) |
| Mean (n=5) | 0.213 | 0.275 | 0.121 | 0.119 | 0.531 | 0.576 |
| ±SD | 0.000752 | 0.00233 | 0.0008 | 0.0016 | 0.006 | 0.004 |
| % RSD | 0.3537 | 0.8491 | 0.6213 | 1.344 | 1.089 | 0.742 |

### Table 8: Result for Specificity Study

| Parameters | Std. drug conc. | Exipients Conc. | Total conc. | Zero Order | First Order | Second Order |
|------------|-----------------|-----------------|-------------|------------|-------------|--------------|
| Level of Addition | (μg/ml) | (μg/ml) | (μg/ml) | Abs. | % RSD | Abs. | % RSD | Abs. | % RSD |
| 50% | 20 | 40 | 60 | 0.150 | 0.38 | 0.145 | 0.39 | 0.177 | 0.33 |
| 100% | 40 | 40 | 80 | 0.279 | 0.35 | 0.247 | 0.40 | 0.312 | 0.67 |
| 150% | 60 | 40 | 100 | 0.429 | 0.23 | 0.351 | 0.59 | 0.461 | 0.13 |
In proposed method % RSD was found to be less than 2%. The method is highly sensitive and entirely suitable for routine analysis of Olopatadine HCl in bulk and solid dosage forms.

CONCLUSION

The developed analytical method for Olopatadine HCl by zero order derivative, first order derivative and second order derivative spectroscopic methods was found to be linear, specific, rapid and precise. The solvent used in proposed methods is methanolic water is an economical and cheap, which indicates that the proposed methods are economic and cost effective. The %RSD for all validation parameters studied.

Conflicts of Interest:

None.

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