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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF OLOPATADINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS AND ITS STRESS DEGRADATION STUDIES USING UV-VIS SPECTROPHOTOMETRIC METHOD

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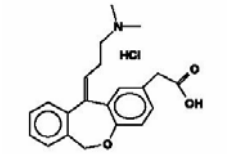
ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Olopatadine, an antihistaminic, with selective H1-receptor antagonist activity, in bulk and pharmaceutical dosage form. The solvent used was methanol and 0.1N HCl (50:50) and the λmax or the absorption maxima of the drug was found to be 206nm. A linear response was observed in the range of 2-10µg/ml with a regression coefficient of 0.9999. The method was then validated for different parameters as per the ICH (International Conference on Harmonization) guidelines. This method can be used for the determination of Olopatadine in quality control of formulation without interference of the excipients. Olopatadine Hydrocloride was subjected to stress degradation under different conditions recommended by ICH. The samples so generated were used for degradation studies using the developed method.

Keywords: Olopatadine, H1-receptor antagonist, λmax, ICH, UV-VIS spectroscopy.

INTRODUCTION

Olopatadine Hydrochloride, chemically[1] is {(11*Z*)-11-[3-(dimethylamino) propylidene]-6, 11-dihydrodibenzo [*b,e*] oxepin-2-yl}acetic acid.



Olopatadine is an anti-histaminic, with selective H1 -receptor antagonist activity. Its principal effects are mediated via inhibition of H1 receptors. Olopatadine hydrochloride was patented on 1st December 2007 by Kyowa Hakko Kogyo Company Ltd, Japan. These drugs selectively bind to H1 receptors there by blocking the actions of endogenous Histamine. They act on the bronchi, capillaries, and other smooth muscles. Olopatadine is an inhibitor of the release of Histamine from the mast cell and a relatively selective H1 receptor antagonist that inhibits the *in vivo* and *in vitro* type 1 immediate hypersensitivity reaction including inhibition of histamine induced effects on human conjunctival epithelial cells.

Literature survey indicated that estimation of Olopatadine was done by using HPTLC & HPLC[2]. It includes quantitative determination of Olopatadine in Human Plasma by HPLC-MS[3]. No UV-VIS Spectrophotometric method was proposed for the estimation of Olopatadine in bulk and Pharmaceutical dosage form. The literature survey indicated that no stability indicating Spectrophotometric method was proposed for Olopatadine. Only estimation of Olopatadine was done by using HPTLC & HPLC[5]. Literature survey also says that quantitative determination of Olopatadine in Human Plasma by HPLC-MS[4]. The aim of this work is to develop and validate an analytical method by using UV-VIS spectrophotometry for the estimation of olopatadine in bulk and pharmaceutical dosage forms and also perform stress degradation studies on the drug as per ICH Guidelines using the developed method.

MATERIALS AND METHODS

The instrument used for the study was an UV-VIS double beam spectrophotometer (Model T60, Analytical Technologies Limited) with 1cm matched pair quartz cells. The solvent used was methanol, double distilled water and HCl which was of AR grade, purchased from SD Fine Chemicals Limited, India.

METHOD DEVELOPMENT

Solubility Test: Solubility test for the drug olopatadine was performed by using various solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, 0.1 N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH) and Chloroform. However, Methanol and 0.1N HCl in a ratio 50:50 was chosen as a solvent for developing the method.

Determination of λ_{max}:

Preparation of Stock Solution: Standard stock solution of olopatadine hydrochloride was prepared by dissolving 10mg of olopatadine hydrochloride in 10ml of methanol and 0.1N HCl (50:50) to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken and then diluted up to 10ml by using methanol and 0.1N HCl (50:50) to produce a concentration of 100µg/ml which is the standard stock solution.

Preparation of Working Standard Solution: From the above stock solution, 0.4ml was pipetted into a 10ml volumetric flask and the volume was made up to the mark with methanol and0.1N HCl (50:50) to produce a concentration of 4µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using methanol and 0.1N HCl (solvent system) as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 206nm(fig.1).

Preparation of Calibration Curve: 1ml of the 100µg/ml solution was diluted to 10ml by using solvent system to produce 10µg/ml solution. 0.2ml, 0.4ml and 0.6ml and 0.8ml of 100µg/ml solution were diluted to 10ml using methanol and 0.1N HCl (solvent system) to produce 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 2-10µg/ml. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in fig.2). The curve showed linearity in the concentration range of 10-50µg/ml. The correlation coefficient (r^2) was found to be 0.9999.

Assay of Olopatadine Eye drops (wino lamp, PATANOL Eye Drops -5mg/ml):

A quantity of powder equivalent to 50mg of Olopatadine eye drop was taken in a 100ml volumetric flask and it was dissolved and

diluted up to the mark with solvent system. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in solvent system to obtain the desired concentration (10µg/ml). This solution was then analyzed in UV and the result was indicated by % recovery given in table 1.

METHOD VALIDATION[4]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity: Various aliquots were prepared form the stock solution (100µg/ml) ranging from 2-10µg/ml. The samples were scanned in UV-VIS Spectrophotometer using methanol and 0.1N HCl as blank. It was found that the selected drug shows linearity between the 2-10µg/ml (Table 3&1).

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation(wino lamp, PATANOL Eye Drops -5mg/ml) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (Table 1 & 5).

Precision: Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 4µg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (Table no.6 & 7).

In the interday variation study, solutions of same concentration 4µg/ml were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table no.1 & 8).

Specificity: 10mg of Olopatadine was spiked with 50%(5mg), 100%(10mg), and 150% (15mg) of excipient mix (Magnesium Stearate) and the sample was analysed for % recovery of Olopatadine (Table no.1 & 9).

Robustness: Robustness of the method was determined by carrying out the analysis of 4µg/ml at two different temperatures i.e. at room temperature and at 18^0c. The respective absorbances were noted and the result was indicated by % RSD (Table no.1 & 10).

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts at 4µg/ml and the respective absorbances were noted. The result was indicated by % RSD (Table no.1 & 10).

Limit of Detection (LOD): The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value (Table no.1).

Limit of Quantification (LOQ): The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (Table no.1).

Degradation Studies[5]

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. The aim of this work was to perform the stress degradation studies on the olopatadine using the method developed.

Stress degradation by hydrolysis under acidic condition: To1ml of stock solution(1000µg/ml) of olopatadine, 1 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with solvent system{methanol and 0.1N HCl (1:1)}. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, 1 ml of solution was pipetted out from this flask, neutralised and diluted with solvent system in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (4µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with solvent system in 10 ml of volumetric flask (Table no.2 & fig. no.3)

Stress degradation by hydrolysis under alkaline condition: To 1 ml of stock solution of at Olapatadine,1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with solvent system{methanol and 0.1N HCl (1:1)}. Volumetric flask was kept at normal condition for 90 min. After 60 min time interval, 1 ml of solution was pipetted out from this flask, neutralized and diluted with solvent system in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (4µg/ml). The solution was then taken in cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH diluted with solvent system in 10 ml of volumetric flask. After, 90 minutes 1ml of solution was again pipetted out from the flask and the above procedure was repeated (Table no.2 & fig. no.4 & 5).

Dry heat induced degradation: Olopatadine sample was taken in a petriplate and exposed to a temperature of 70°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with solvent system {methanol and 0.1N HCl (1:1)} in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (4µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis (Table no.2 & fig. no.6).

Oxidative degradation: To 1 ml of the stock solution of Olopatadine (1000µg/ml), 1 ml of 30 % w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with solvent system {methanol and 0.1N HCl (1:1)}. The volumetric flask was then kept at room temperature for 15 min. For the blank, 1 ml of the 30 % w/v of hydrogen peroxide was kept at normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on boiling water bath to remove the excess of hydrogen peroxide. Finally, after 15 minutes dilutions were made from the stock solution to achieve the required concentration (4µg/ml). The solution was then taken in a cuvette and analyzed (Table no.2 & fig. no.7).

Photolytic degradation: Sample of Olopatadine was exposed to near ultraviolet lamp in photo-stability chamber providing illumination of not less than 1.2 million lux hours. 10mg of sample was dissolved in solvent system {methanol and 0.1N HCl (1:1)} made up to 10 ml volume. From this solution appropriate dilution (4µg/ml) was made using solvent system and taken in cuvette for the U.V. analysis (Table no.2 & fig. no.8).

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (98.9% to 101.9%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.98% to 101.9%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of Assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.8%). Summary of validation parameters of proposed Spectrophotometric method is shown in table 1. The stress degradation studies showed that olopatadine undergoes degradation in acidic and alkaline conditions whereas it is relatively stable when exposed to dry heat, alkaline and photolytic conditions. Summary of the results of stress degradation studies of olopatadine are shown in the table.

Table 1: summary of validation

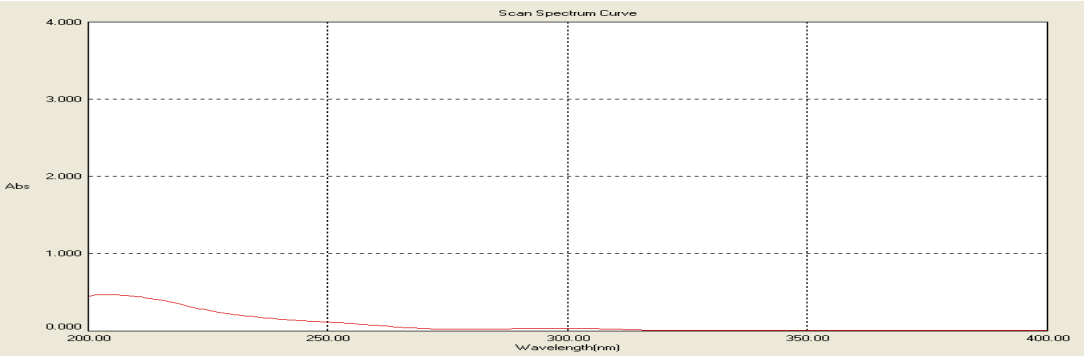
Parameter	Result
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	0.313
Accuracy indicated by % recovery	98.9-101.9%
Specificity indicated by % recovery	98.98-101.9%
Limit of Detection	0.5µg/ml
Limit of Quantification	1.65µg/ml
Range	2-10µg/ml
Linear regression equation	y = 0.099x + 0.009
Robustness indicated by %RSD	0.355
Assay indicated by % recovery	101.8%

VALIDATION: Table for linearity:

Table 2: Linearity of Olopatadine hydrochloride in working standard

Concentration (µg/ml)	Absorbance
2	0.207
4	0.422
6	0.601
8	0.808
10	0.989

Determination of λ_{max}:



λmax of Olopatadine hydrochloride showing at 206nm (fig. no. 1)

Peak 2: 206.00- 0.422

Preparation of calibration curve

conc.	Abs
0	0
2	0.207
4	0.422
6	0.601
8	0.808
10	0.989

Calibration Curve of Olopatadine hydrochloride

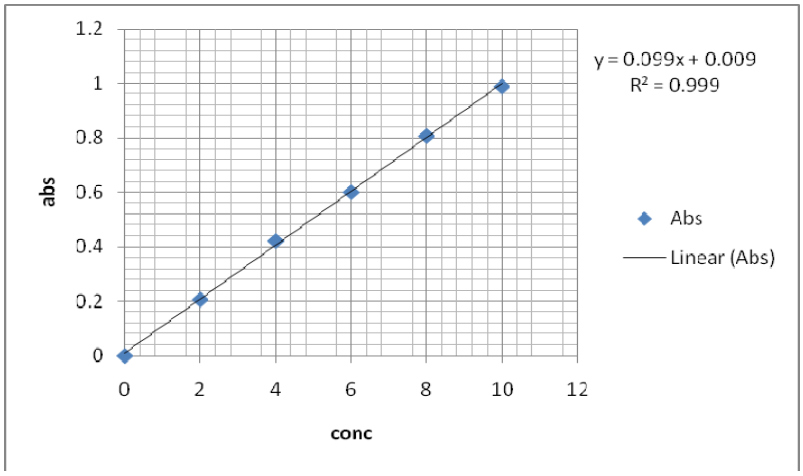


Fig. 2: Calibration curve of Olopatadine hydrochloride

Table 3: Optical characteristics

Beer's Law limit (µg/mL)	2-10µg/ml
Molar extinction coefficient (1 mole-1 c.m-1)	9.86×10^2
Correlation coefficient	0.999
Regression equation (Y*)	y = 0.099x + 0.009
Slope (a)	0.099x
Intercept (b)	0.009

Accuracy:

Table 4: Accuracy Readings of Olopatadine hydrochloride

Observation / Results						
No. of preparations	**Concentration (µg/ml)**		**% Recovery**	**Statistical Results**		
	Formulation	**Pure Drug**		**Mean**	**SD**	**%RSD**
S_1 : 80 %	10	8	100.1	100.4	0.7	0.69
S_2 : 80 %	10	8	99.9			
S_3 : 80 %	10	8	101.2			
S_4 : 100 %	10	10	101.2	100	1.153256	1.15
S_5 : 100 %	10	10	99.9			
S_6 : 100 %	10	10	98.9			
S_7 : 120 %	10	12	100.9	100.9	1	0.99
S_8 : 120 %	10	12	99.9			
S_9 : 120 %	10	12	101.9			

Precision:

Table 5: Precision results showing repeatability of Olopatadine hydrochloride

Concentrations (µg/ml)	**Absorbance**	**Statistical Analysis**
4	0.423	
4	0.422	
4	0.423	
4	0.422	Mean =0.4218
4	0.421	SD = 0.001135
4	0.420	%RSD =0.269
4	0.422	
4	0.423	
4	0.422	
4	0.420	

Table 6: Intra-assay precision:

Concentrations (µg/ml)	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Average %RSD**
4	0.420	0.422	0.419	
4	0.420	0.423	0.420	
4	0.422	0.420	0.420	
4	0.422	0.419	0.420	
4	0.423	0.424	0.422	
4	0.422	0.420	0.422	
4	0.422	0.423	0.423	
4	0.423	0.422	0.423	
4	0.420	0.420	0.422	
%RSD	**0.29%**	**0.41%**	**0.35%**	**0.35%**

Table 7: Inter-assay precision

Concentrations (µg/ml)	**%RSD**			**Average %RSD**
	Day 1	**Day2**	**Day3**	
4	0.39	0.34	0.24	0.32%

Test for specificity

Table 8: Test for Specificity showing no effect of excipient

Sample No.	**Excipient Conc.(%)**	**Olopatadine Input (mg)**	**Olopatadine Recovered (mg)**	**Olopatadine Recovered (%)**	**Mean Recovered (%)**	**S.D.**	**%R.S.D.**
1	100%	10	9.82	98.2%			
2	50%	10	10.05	100.5%	100.2%	1.868154	1.86%
3	150%	10	10.19	101.9%			

Ruggedness & Robustness

Table 9: Results showing ruggedness of method for Olopatadine hydrochloride

Analyst-1				**Analyst-2**		
Conc. (µg/ml)	Abs.	Statistical Analysis		Conc. (µg/ml)	Abs.	Statistical Analysis
4	0.422	Mean = 0.421667		4	0.420	Mean = 0.422
4	0.423			4	0.422	
4	0.420	SD = 0.001633		4	0.422	SD = 0.001414
4	0.424			4	0.424	
4	0.421			4	0.421	
4	0.420	%RSD = 0.38		4	0.423	%RSD = 0.33
Room Temperature				**Temp. 18°C**		
Conc. (µg/ml)	Abs.	Statistical Analysis		Conc. (µg/ml)	Abs.	Statistical Analysis
4	0.420	Mean = 0.422		4	0.422	Mean= 0.4216
4	0.423			4	0.420	
4	0.422	SD =0.00141		4	0.423	SD = 0.001633
4	0.421			4	0.421	
4	0.424			4	0.420	
4	0.422	%RSD = 0.33		4	0.424	%RSD = 0.38

Limit of Detection (LOD)

The LOD for Olopatadine hydrochloride was found to be 0.5µg/ml.

Limit of Quantification (LOQ)

The LOQ for Olopatadine hydrochloride was found to be 1.65µg/ml.

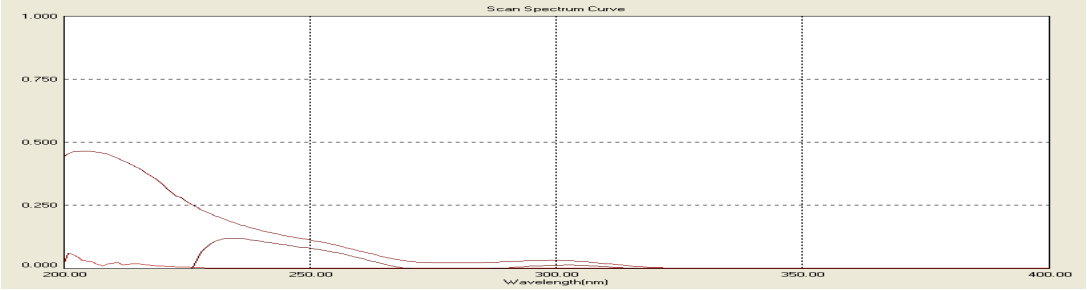
Table 11: summary of result of stress degradation studies:

Condition	**Time**	**%Degradation**
0.1N NaOH(1ml)	60min	4.26%
	90min	6.39%
3N HCl(1ml)	60min	λmax shifted
30% Hydrogen Peroxide(1ml)	15min	λmax shifted
Dry Heat 70°	48hr	35.7%
Photolytic	3hr	22.9%

Degradation studies

Stress degradation by hydrolysis under acidic condition:

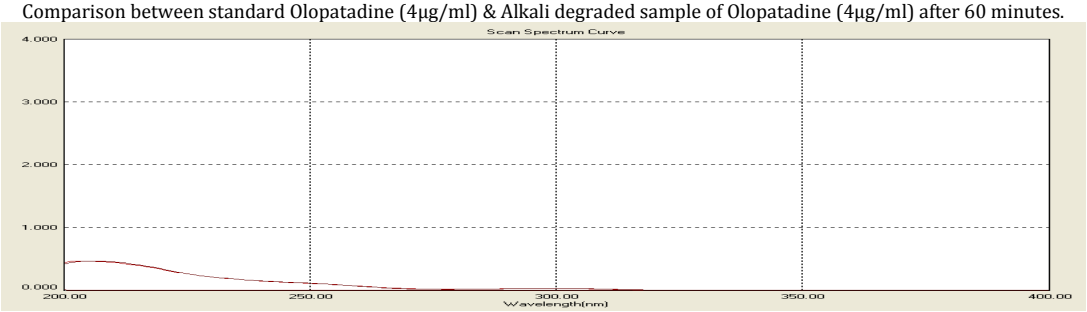
Comparison between standard Olopatadine (4µg/ml) & Acid Degraded sample of Olopatadine (4µg/ml) after 60 minutes.



Drug got degraded completely and λmax Shifted (fig. no.3)

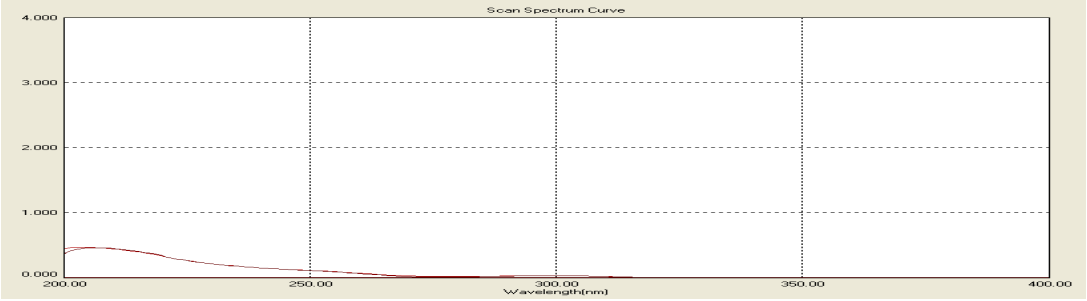
Stress degradation by hydrolysis under alkaline condition:

Comparison between standard Olopatadine (4µg/ml) & Alkali degraded sample of Olopatadine (4µg/ml) after 60 minutes.



Drug got degraded 4.26% after exposing for 60min. to the alkaline condition (fig. no.4)

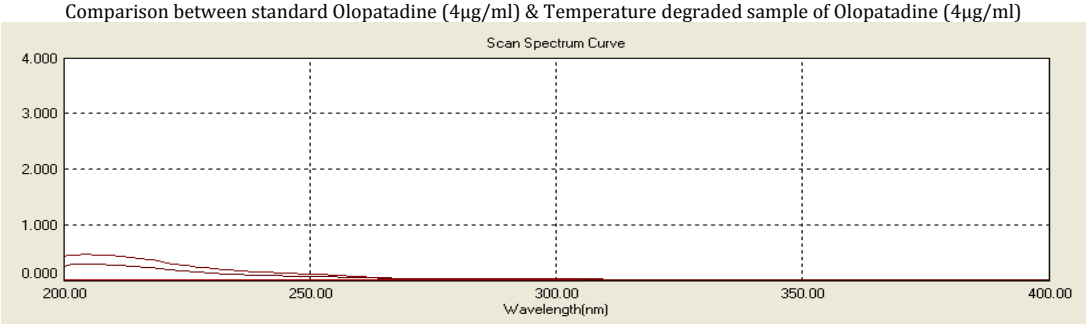
Comparison between standard Olopatadine (4µg/ml) & Alkali degraded sample of Olopatadine (4µg/ml) after 90 minutes.



Drug got degraded by 6.39% after exposing for 90min. to the alkaline condition (fig.no.5)

Dry heat induced degradation:

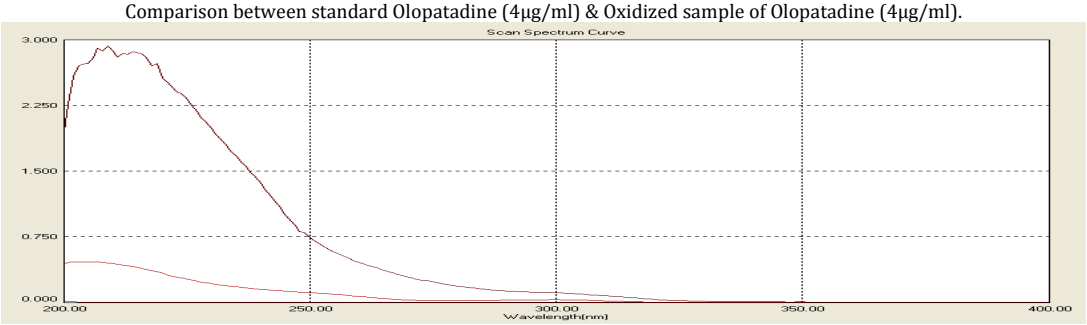
Comparison between standard Olopatadine (4µg/ml) & Temperature degraded sample of Olopatadine (4µg/ml)



Drug got degraded by 35.7% when exposed to a temp of 70°c for 48 hours (fig. no.6)

Oxidative degradation:

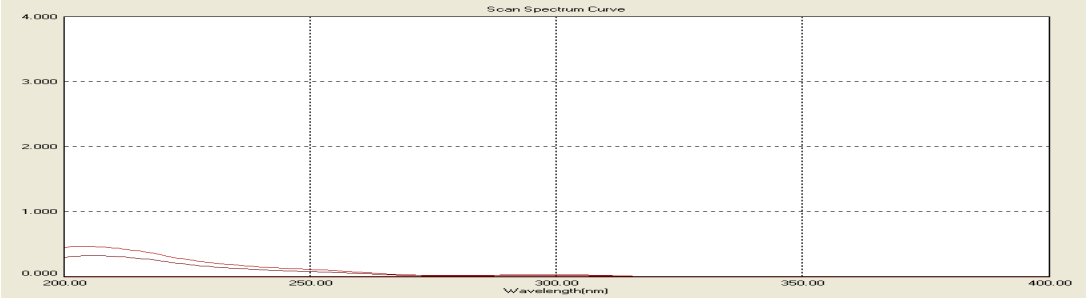
Comparison between standard Olopatadine (4µg/ml) & Oxidized sample of Olopatadine (4µg/ml).



Drug got degraded completely and λmax Shifted (fig. no.7)

Photolytic degradation:

Comparison between standard Olopatadine (4µg/ml) & UV degraded sample of Olopatadine (4µg/ml) after 3 hours



Drug when exposed to UV light for 3hrs, got degraded by 22.9% (fig. no.8)

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