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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF OLOPATADINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate, economical and reliable UV spectrophotometric method has been developed for the estimation of Olopatadine hydrochloride in bulk and its tablet dosage form. The drug shows maximum absorption (λmax) at 231 nm in methanol and obeys Beer's law in the concentration range of 3-15 µg/ml with correlation coefficient (R^2=0.999). The accuracy was found to be 97.2-99.6%. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.047µg/ml and 0.144µg/ml respectively. The relative standard deviation was found to be <2.0% in all cases. The proposed spectrophotometric method was validated as per ICH Q2 (R1) guidelines. Statistical analysis proved that the method is repeatable and specific for the determination of the said drug. The proposed method can be used for the reliable quantification of Olopatadine hydrochloride in bulk form and routine analysis of pharmaceutical formulations.

KEYWORDS

Olopatadine hydrochloride, UV Spectrophotometer and Method Validation.

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INTRODUCTION

Olopatadine Hydrochloride is a selective histamine H1 receptor antagonist and mast cell stabilizer with anti-allergic activity. This drug also prevents histamine-induced pain and itching of mucous membranes. It is usually used in the treatment of allergic rhinitis, hives, and itch associated with conjunctivitis[1]. Chemically it is 2-[(11Z)-11-[3-(dimethylamino) propylidene]-6H-benzo[c][1] benzoxepin-2-yl] acetic acid; hydrochloride.

Literature survey revealed that OLP has been estimated by spectrophotometric technique[2-4]. Several HPLC[4,5], HPTLC[6] and derivative spectroscopy[7] methods have been reported for the determination of Olopatadine. All of these methods are very expensive because these methods require long and tedious pretreatment of the samples, laborious clean up procedures (including extraction with solvent for the analysis of OLP. So, the aim of present study is to develop and validate[8] a simple, precise, efficient and selective UV Spectrophotometric method for the analysis of Olopatadine hydrochloride in bulk and tablet dosage forms.

MATERIAL AND METHODS

Instruments

Shimadzu UV-visible double beam spectrophotometer- 1800 with 1 cm matched quartz cells was used for measuring absorbances. For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used.

Material

Pure standard Olopatadine hydrochloride was obtained as a gift sample from Ajanta pharma limited. Olopatadine tablet was procured from the local market. Tablet containing Olopatadine hydrochloride equivalent to Olopatadine 5 mg (Patadin-5) was purchased from the market. Methanol of analytical grade was used as the solvent.

UV Spectroscopic Method

Solvent Selection

Solubility test of Olopatadine hydrochloride was performed by using various solvents like water, methanol, and acetonitrile. However, the drug is freely soluble in methanol. So, for a good result methanol was used as the solvent.

Preparation of Standard Stock Solution

The standard stock solution of Olopatadine hydrochloride (OLP) was prepared by transferring accurately weighed 10mg of Olopatadine hydrochloride separately into 10ml volumetric flask and dissolved in methanol. Then volume was made up to the mark by using methanol to give a concentration of 1000µg/ml. From this, 1ml of the solution was transferred to a 10 ml volumetric flask and make up the volume with methanol to give a concentration of 100µg/ml, which was a standard stock solution and it was further diluted with methanol to get concentration of 10 µg/ml of Olopatadine hydrochloride (OLP).

Determination of absorption maxima

The prepared standard solution (10µg/ml) of OLP was scanned over a wavelength range of 200-400 nm in the UV-VIS spectrophotometer. It was observed that the drug showed maximum absorbance (λmax) at 231 nm which was selected as the wavelength for detection.

Procedure for assay of Tablet Dosage Form

Twenty tablets (Patadine-5) were weighed accurately and powdered. Powder equivalent to 5mg Olopatadine hydrochloride was weighed and transferred to a 10 ml volumetric flask, dissolved in methanol and sonicated for 15min. The volume was then made up to the mark using same solvent. Then it was filtered through 0.45µ Whatman filter paper to remove some un-dissolved excipients. After filtration, from this filtrate 1ml was taken and diluted to 10 ml with methanol which gives 50µg/ml of OLP and further 1ml was diluted to 10 ml with methanol to get a final concentration of 5µg/ml. Absorbance of this sample solution was recorded at 231nm.

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

Linearity

From the 'Std stock OLP' solution (100µg/ml), 0.3, 0.6, 0.9, 1.2, 1.5 ml were transferred in a series of 10 ml volumetric flasks. The volume was made up to the mark with methanol to obtain conc. of 3, 6, 9, 12, 15µg/ml of OLP.

The absorbances of the spectra were measured at 231nm. The calibration curve was constructed by plotting the Absorbance of OLP v/s Conc. Of OLP and the correlation coefficient (r^2) of least square linear regression for OLP was calculated.

Range

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

Accuracy

Recovery study was carried out by the standard addition method by adding a known amount of OLP to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1ml from 50µg/ml of tablet solution was transferred to 3 different 10ml volumetric flasks (labelled 80%, 100%, 120%) separately and 8, 10, 12µg/ml standard solution was added respectively and the volume was made up to the mark with methanol. Absorbances were noted for these samples. Accuracy is reported as % recovery, which was calculated from the expression as equation given below:

% Recovery = Observed value / True value ×100

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions.

The Intraday precision of the developed UV method was determined by analyzing the six samples of same concentration (12µg/ml) for 3 times in a day and the absorbance was noted. From the absorbance result mean, standard deviation and % RSD was calculated.

Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (12µg/ml). Absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method, the wavelength of analysis was changed and the absorbance was measured. The effect of detection wavelength was studied at ±5 nm.

Ruggedness

Ruggedness was determined by carrying out analysis by three different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection (LOD)

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of OLP (12µg/ml) and LOD calculated by equation below:

$LOD = 3.3 \times (SD/S)$

Where, SD- standard deviation; S= slope of the curve

Limit of Quantification

Quantification limit was determined based on the standard deviation of absorbance of same concentration that is standard solution OLP (12µg/ml) and LOQ calculated by following equation:

$LOD = 10\times (SD/S)$

Where, SD= standard deviation; S= slope of Curve.

RESULTS AND DISCUSSION

Linearity

The linearity of this method was determined at ranges from 3-15µg/ml. The regression equation was found to be.

Accuracy

The accuracy of the analytical method for OLP was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 231nm. Results were expressed in terms of % recoveries.

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

Preliminary Analysis of Olopatadine hydrochloride

Preliminary analysis of Olopatadine hydrochloride such as description, solubility was performed.

UV-Spectrophotometry for Olopatadine hydrochloride

OLP being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in methanol, stock solutions and working standards were prepared in methanol. The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was found to be 97.2-99.6%. The proposed method showed absorption maxima at 231 nm and obeyed Beer's law in the concentration range of 3-15µg/ml. The limit of detection (LOD) was found to be 0.047µg/ml and limit of quantification (LOQ) to be 0.144µg/ml respectively. All statistical data prove validity of the proposed method, which can be applied for routine analysis of Olopatadine hydrochloride.

Assay of Tablet formulation

Amount of drug present in tablet formulation was calculated using equation *Y=0.052x-0.126* at 231 nm. Amount of Olopatadine hydrochloride was found to be 103% of label claim. This method can be employed for routine analysis of Olopatadine hydrochloride.

Summary and conclusion

Summary of UV Spectrophotometric Method for Olopatadine hydrochloride.

Table No.1: Result of analysis of tablet dosage form

S.No	Drug	Label Claim (mg / tab.)	Amount found (mg)	% Drug found
1	OLP	5	5.15	103%

Table No.2: Linearity of Olopatadine hydrochloride

S.No	Conc.(µg/ml)	Absorbance
1	3	0.030
2	6	0.186
3	9	0.348
4	12	0.506
5	15	0.655
Regression equation: *Y=0.052x-0.126*		
R^2= 0.999		

Table No.3: Result for Accuracy

S.No	Drug	Amount present(µg/ml)	Level of addition (%)	Amount of standard drug added (µg/ml)	Amount found (µg/ml	%Recovery
1	OLP	5	80	8	12.9	98
		5	100	10	14.86	97.2
		5	120	12	16.98	99.6

Intra-day Precision

Table No.4: Intra-day Precision

S.No	OLP	
	Concentration (µg/ml)	Absorbance
1	12	0.507
2	12	0.507
3	12	0.506
4	12	0.507
5	12	0.506
6	12	0.508
	% RSD	0.148

Inter-day Precision

Table No.5: Inter-day Precision

S.No	OLP	
	Concentration (µg/ml)	Absorbance
1	12	0.506
2	12	0.507
3	12	0.507
4	12	0.508
5	12	0.506
6	12	0.507
	% RSD	0.149

Repeatability

Table No.6: Repeatability Study

S.No	OLP	
	Concentration (µg/ml)	Absorbance
1	12	0.507
2	12	0.506
3	12	0.506
4	12	0.508
5	12	0.507
6	12	0.507
	% RSD	0.148

Limit of Detection

Table No.7: For Limit of Detection

LOD (µg/ml)	Conc.
OLP	0.047µg/ml

Limit of Quantification

Table No.8: For Limit of Quantification

LOQ (µg/ml)	Conc.
OLP	0.144µg/ml

Robustness and Ruggedness

Table No.9: Result for Robustness and Ruggedness

S.No	Method	Condition	Conc.(µg/ml)	Abs.	%RSD
1	Robustness	Wavelength=229nm	10	0.524	1.83
		Wavelength= 230nm	10	0.525	
		Wavelength=232nm	10	0.508	
2	Ruggedness	Analyst 1	10	0.531	0.108
		Analyst 2	10	0.530	
		Analyst 3	10	0.531	

Table No.10: For Summary

S.No	Parameters	OLP
1	Beer's Law limit (µg/ml)	3-15
2	Absorption maxima (nm)	231
3	Standard regression equation	*Y=0.052x-0.126*
4	Correlation coefficient (R^2)	0.999
5	Accuracy	97.2-99.6%
6	Precision (% RSD) Repeatability	0.148
7	LOD (µg/ml)	0.047
8	LOQ (µg/ml)	0.144
9	Robustness (%RSD)	1.83
10	Ruggedness(%RSD)	0.108
11	Assay (%)	103

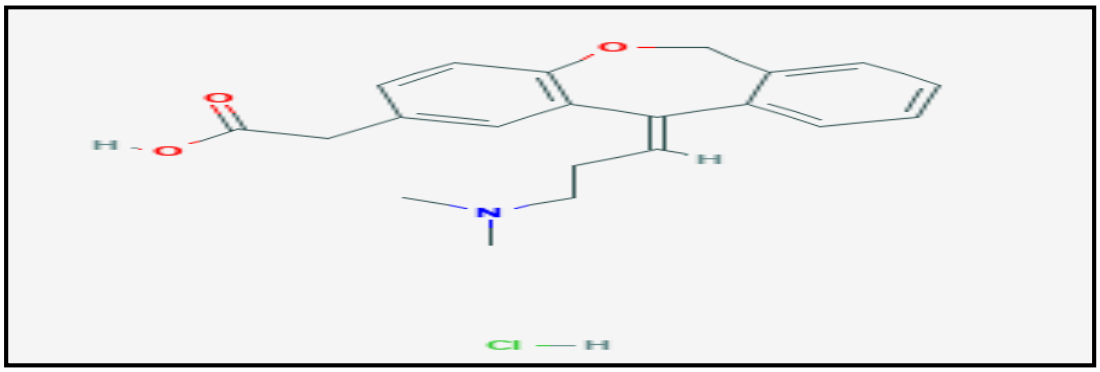

Figure No.1: Structure of Olopatadine hydrochloride

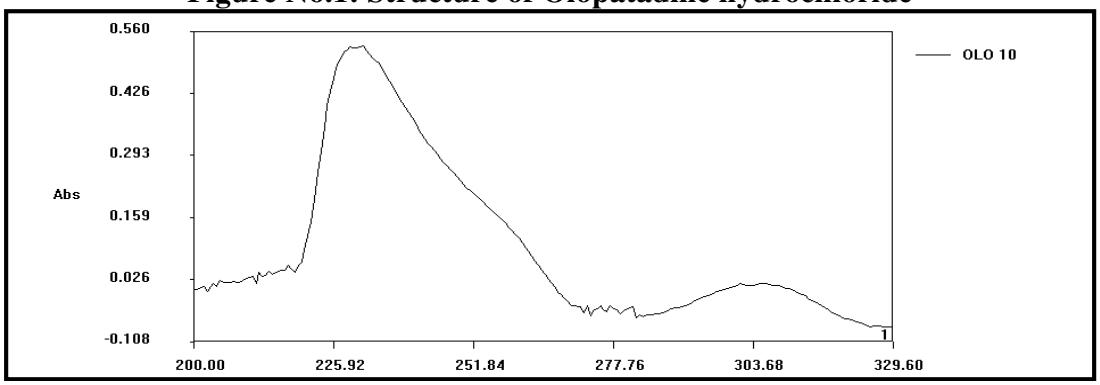

Figure No.2: Absorption maxima of OLP at 231nm

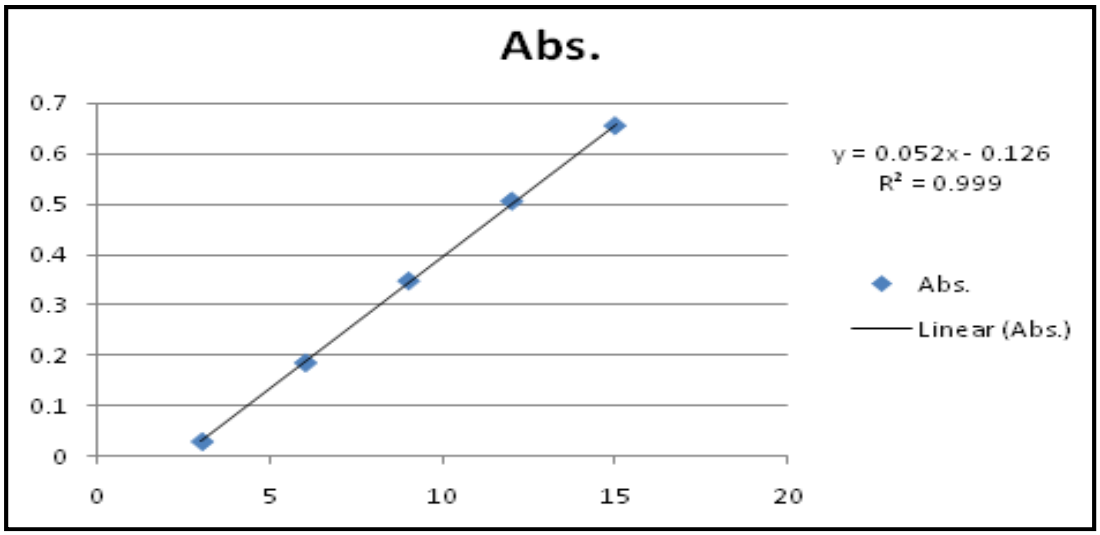

Figure No.3: Linearity of OLP

CONCLUSION

The UV-Spectrophotometric method was developed and it was found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Olopatadine hydrochloride in API and its dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Olopatadine hydrochloride in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS

UV-Ultra Violet
API- Active Pharmaceutical Ingredient
OLP- Olopatadine hydrochloride
LOD- Limit of Detection
LOQ- Limit of Quantification

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. National Centre for biotechnology information. Pub Chem Compound Database; CID 5282402. [Cited 2019 April2]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/olopatadine_hydrochloride.
2. Jain D, Basniwal K. Spectrophotometric determination of olopatadine hydrochloride in eye drops and tablets, *J Pharma Res,* 12(2), 2013, 48-52.
3. Sucheta R, Vijay S. Development and Validation of UV Spectrophotometric method for simultaneous estimation of montelukast sodium and olopatadine hydrochloride in bulk and formulated dosage form, *Int J Pharma Res Dev,* 5(3), 2013, 83-87.
4. Bhanu M, Thangabalan B. Estimation of olopatadine hydrochloride by RP-HPLC and UV spectrophotometry method in pure and pharmaceutical formulation, *Int J Pharma Anal Res,* 3(4), 2014, 434-444.
5. Rajan R. Reversed phase high performance liquid chromatography method for determination of olopatadine hydrochloride from active pharmaceutical dosage form, *Der Pharmacia Lettre,* 7(1), 2015, 258-262.

6. Anand M *et al*. Validated high performance thin layer chromatographic method for estimation of olopatadine hydrochloride as bulk drug and in ophthalmic solutions, *Int J chem. Tech Res,* 2(3), 2010, 1372-1375.
7. Furquan K *et al*. Zero order derivative spectroscopic estimation of olopatadine hydrochloride from eye drops, *J Innov Pharma Bio Sci,* 3(2), 2016, 10-107.
8. Validation of Analytical Procedure: Text and Methodology, *Q2(R1), ICH, Quality Guidelines/ICH Guidelines,* 2005.

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