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SPECTROPHOTOMETRIC DETERMINATION OF OLOPATADINE HYDROCHLORIDE IN EYE DROPS AND TABLETS

Jain D [1] and Basniwal P K * [1,2]

[1]School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal – 462033, Madhya Pradesh, India

[2]LBS College of Pharmacy, Udai Marg, Tilak Nagar, Jaipur – 302004, Rajasthan, India.

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ABSTRACT

Optimized and validated three UV spectrophotometric methods have been proposed for the determination of olopatadine hydrochloride in different dosage forms (eye drop and tablets) and bulk drug. The developed methods viz. linear regression equation (LRE), standard absorptivity (SA) and first order derivative (FOD) method were validated for linearity, accuracy, precision and robustness according to ICH guideline to assure reliability of the developed methods. The methods follow Beer-Lambert's law for the concentration of 10-50 ìg mL^{-1}. Validated methods were applied for determination of olopatadine hydrochloride content in tablet dosage forms as well as in eye drops with non-significant difference ($P > 0.05$) and can be successfully applied for routine analysis like assay, dissolution studies, bio-equivalence studies etc. in pharmaceutical industries.

Keywords: *Olopatadine HCl; Eye drop; Tablets; UV spectrophotometry.*

INTRODUCTION

Quality control and quality assurance department develop and follow standard internal operating procedures directed toward assuring the quality, safety, purity and effectiveness of the drug supply[1]. In comparison to chromatographic methods, spectrophotometric methods are better applied for routine analysis as these are: economic (solvents and instruments costs are key factor), rapid (UV-analysis time is less), simple (not required much training to operate), maintenance free (no washing and special precaution), without compromising on accuracy and precision. Keeping the view of quality assurance, the present work was designed to develop and validate spectrophotometric methods for determination of olopatadine hydrochloride in bulk drug as well as in dosage form to ensure the quality and purity of the drug.

Olopatadine (OLO) is a new selective histamine H_1 receptor antagonist. It is a tricyclic drug containing an alkylamino moiety; chemically, it is 11-((*Z*)-3-(dimethylamino)propylidene)-6,11-dihydro-dibenz[*b,e*]oxepin-2-acetic acid[2] (Figure 1), used to treat allergic conjunctivitis (itching eyes)[3–7]. A radioimmunoassay (RIA) method for the determination of olopatadine in plasma has been developed previously to support the pharmacokinetic studies in humans[8] and some animals [9]. This RIA method was very sensitive with a lower limit of quantitation (LLOQ) of 0.1 ng mL^{-1} in plasma. However, it had several problems, such as cross-reactivity of metabolites and low precision, and so it was considered that plasma concentrations of olopatadine measured by RIA needed to be conûrmed by an alternative method. Recently, high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS–MS) has been widely applied to the determination of a number of compounds in biological ûuids[10-12]. Assay methods for olopatadine and its metabolites in human plasma by HPLC with electrospray ionization tandem mass spectrometry (LC–ESI-MS–MS) were also reported[13-14].

But hitherto, there is no UV spectrophotometric method reported in literature for the determination of olopatadine hydrochloride in commercial formulations. Thus, the objective of present work was to develop spectrophotometric assay methods for analysis of olopatadine hydrochloride in the bulk drug and in marketed formulations, and to compare the developed assay methods by statistical analysis.

EXPERIMENTAL

Instrument s, reagent s and chemicals

Ultraviolet spectrophotometer (1700 series and 1800 series Shimadzu) with 1 cm matched quartz cells were used for the measurement of absorbance. Shimadzu-Ax-200 electronic balance was used for weighing and class volumetric glassware were used. Olopatadine hydrochloride WS (working standard) was procured from Ranbaxy Laboratories Ltd. Gurgaon, New Delhi, as a gift sample. Analytical grade methanol and sodium hydroxide were procured from CDH, Mumbai, India. Olodin eye drop (FDC Limited, Aurangabad, India) and tablets (Olopat, Ajanta Pharma, Mumbai, India) were purchased from local market. Distilled water was obtained from a Milli-Q water puriûcation system (Millipore, Bedford, MA, USA).

*Correspondence : pawanbasniwal@gmail.com; deepti2515@yahoo.com

Linear regression equation (LRE) method

Stock A of 500 g/mL was prepared from accurately weighed 50 mg olopatadine hydrochloride WS in 50% aqueous methanol. It was diluted with the diluents (50% aqueous methanol) to get stock B of 50 µg mL^{-1} and aliquots of stock B were further diluted to get concentration of 10, 20, 30, 40, and 50 µg mL^{-1} of drug. Dilutions were scanned from 400 to 200 nm against diluent as blank, and the absorbance maxima ($ë_{max}$) was observed at 301 nm. The linear regression equation was calculated.

Standard absorptivity (SA) method

Five dilutions (10, 20, 30, 40, and 50 µg mL^{-1}) of OLO WS were prepared in triplicate and the absorbance were measured at 301 nm against 50% aqueous methanol as blank. From above observations the standard absorptivity A (1%, 1cm) and molar extinction coefficient å were calculated, which would be used to determine the drug content of dosage forms.

First order derivative (FOD) method

To avoid interference of excipients of dosage form in determination of drug content, the first order derivative spectrophotometric method was developed, as interference of one analyte in absorbance of another analyte may be nullified in the derivative mode. Standard dilutions of concentrations 10, 20, 30, 40, and 50 µg mL^{-1} of drug were prepared, derivatized to first order. The absorbances were measured at 262 nm and linear regression equation was calculated for the FOD method.

Validation of methods

As per ICH guidelines[15-16], five dilutions in triplicate were used to validate all three methods for linearity, accuracy (by recovery studies, standard addition to pre-analysed samples), repeatability (within day), intermediate precision (days, analyst and instrument variation) and robustness (methanol variation: 48, 50 and 52 %), and statistical parameters were calculated for them. The LOD (limit of detection) and LOQ (limit of quantitation) for all three methods were calculated as 3.3 ó/S and 10 ó/S, respectively (ó = standard deviation of standard concentration and S = slope of standard curve).

Dosage form analysis

Eye drop

An accurately measured volume of ophthalmic solution (Olodin; FDC Limited, Aurangabad, India), equivalent to about 10 mg of olopatadine hydrochloride was transferred to 100 mL volumetric flask, diluted with diluent up to 100 mL. The solution was mixed and sonicated for 10 min. An appropriate dilution was prepared from stock solution and filtered (stock A, 100 µg mL^{-1}). Aliquots of stock A were diluted to get sample concentrations (20, 30 and 40 µg mL^{-1}) in the range of linearity for spectrophotometric methods (LRE, SA and FOD). The absorbance of sample solutions was observed in multi-point calibration curve of quantitative mode at selected wavelength to get concentration by all the three methods.

Tablets

Twenty olopatadine hydrochloride tablets (Ajanta Pharma, Mumbai, India) were weighed and finely powdered; a quantity equivalent to 50 mg of olopatadine hydrochloride was dissolved in 100 mL of 50 % aqueous methanol and filtered through Whatman filter paper No. 41 to give Stock P. Aliquots of stock P were diluted to obtain sample concentrations (20, 30 and 40 µg mL^{-1}) in the range of linearity. The absorbance values of these sample solutions were observed in a multipoint calibration curve of quantitative mode at the selected wavelength to obtain test sample concentration.

RESULTS AND DISCUSSION

Optimization of solvent selection

OLO was soluble in organic solvents such as methanol, acetonitrile and dimethylformamide (DMF) etc., and very slightly soluble in water. Various percentages of methanol was tried to make the method more eco friendly and economic. Mixture of methanol and 0.1 N HCl (50:50) did not show ëmax at 206 nm and it is not preferred wavelength for UV spectrophotometric methods. Aqueous methanol (50%) was found optimum for the above spectrophotometric method. Solubility problem of drug has been encountered when it was reduced to lower percentage of methanol (45% and 40%). Methanol was preferred over acetonitrile due to its cost effectiveness. The shape of Gaussian spectra of OLO in mixture of methanol and water (50:50) was acceptable (Figure 2).

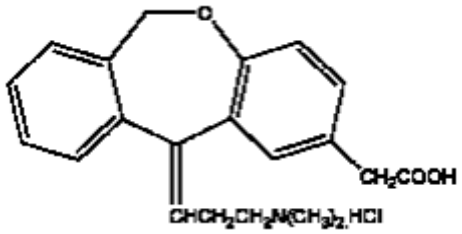

Fig. 1: *Structure of olopatadine hydrochloride.*

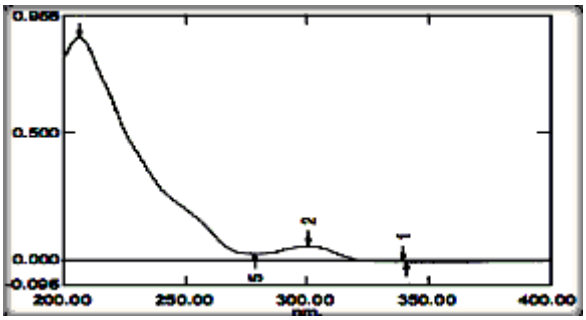

Fig. 2: *Gaussian spectrum of olopatadine HCl in 50% methanol.*

LRE Method

The two $\ddot{e}_{max}$ of the olopatadine hydrochloride was found but the first was near 200 nm which is near to cut off wavelength of methanol; so the second $\ddot{e}_{max}$ (301 nm) was selected for the LRE method (Figure 2). The six replicates were processed for the linear regression method and the linear regression equation was found to be y = 0.0306 x – 0.0047 with correlation coefficient R^2 = 0.9999. The Beer-Lambert's law limit was found to be 10-50 µg mL^{-1}.

SA Method

Three replicates of five serial dilutions were used to determine standard absorptivity [A (1%, 1cm)] and molar extinction coefficient ( ), which were found to be 304.54 $dlg^{-1}cm^{-1}$ and 10275.41 $Mol^{-1}cm^{-1}$, respectively (Table 1). Now, the concentration of any sample can be easily determined by using formula of the standard absorptivity specified in Table 1, without preparing calibration curve. Thus, it provides single step determination of the olopatadine hydrochloride; by measuring the absorbance and determining the concentration of the sample.

Table 1: *Standard absorptivity A (1%,1cm) and molar extinction coefficient ( )*

Conc. (µg mL^{-1})	Absorbance at 301 nm			Standard Absorptivity [A (1%, 1cm) = A/bc]		
	I	II	III	I	II	III
10	0.303	0.308	0.309	303.0	308.0	309.0
20	0.603	0.611	0.609	301.5	305.5	304.5
30	0.908	0.901	0.905	302.7	300.3	301.7
40	1.214	1.216	1.212	303.5	304.0	303.0
50	1.535	1.541	1.531	307.0	308.2	306.2
A (1%, 1 cm)*				304.54 $dlg^{-1}cm^{-1}$		
**				10275.41 $Mol^{-1}cm^{-1}$		

** Mean of 15 above standard absorbtivities determination.*
***Molar extinction coefficient = A (1%, 1cm) x Molecular weight/10.*

FOD Method

First order derivative of Gaussian spectrum of olopatadine hydrochloride was successfully applied to determine the drug content (Figure 3). Zero crossing was found at 280 nm and 301 nm in this mode. When the different solutions viz. degraded samples, diluted eye drop solution, filtered tablet solution were scanned in derivative mode, the zero crossing did not deviate from the 280 nm and 301 nm. No deviation from these values indicates, there was no interference of any excipient in absorbance of Gaussian spectrum of the olopatadine hydrochloride. The linear regression equation was found to be y =0.0228x–0.1301 with correlation coefficient R^2=0.9998. The Beer-Lambert's law limit was found to be 10-50 µg mL^{-1}.

Validation of methods

The linearity for all the methods (LRE, SA and FOD method) determined was having standard deviation 0.038, 0.062 and 0.072 respectively, which are comparatively acceptable. Accuracy was determined

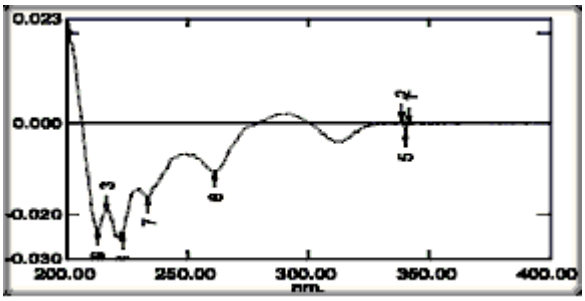

Fig. 3: *First order derivative of Gaussian spectrum of olopatadine HCl in 50% methanol.*

by recovery method and found to be 101.03%, 100.63% and 101.46% respectively, with less than one unit of standard deviation. The precision was studied under the head of repeatability and intermediate precision. The repeatability of the methods was around 100% with less than one unit of standard deviation for all the methods. The intermediate precision was studied for variation in day of analysis, analyst-to-analyst and instrument-to-instrument. The results for all variations were within 99.74-101.27% limit for all methods. Methanol variation in solvent system was used to study the robustness of the methods. The olopatadine hydrochloride was determined in different composition (48, 50 and 52% methanol) as 99.79%, 100.98% and 100.43% respectively (Table 2). LOD were found as 1 µg mL^{-1}, 1 µg mL^{-1} and 1.5 µg mL^{-1}, respectively, which is lowest level of analyte that can be detected, but not necessarily quantitated as an exact value; while LOQ were as 3 µg mL^{-1}, 3 µg mL^{-1} and 4 µg mL^{-1}, respectively. Both LOD and LOQ had higher values for FOD method, which may be due to the conversion of Gaussian spectra into derivative mode and absorbance in derivative mode is lower than Gaussian spectra. Thus, these parameters were validated and all validation parameters were found within limits of standard deviation which assure the reliability of the developed methods.

Table 2: *Results of validation parameters for all three methods*

Validation parameter	Value* ± SD		
	LRE method	SA method	FOD method
Linearity	100.49 ± 0.038	99.62 ± 0.062	99.85 ± 0.072
Accuracy	101.03 ± 0.061	100.63 ± 0.068	101.46 ± 0.027
Precision			
I. Repeatability	101.59 ± 0.052	100.34 ± 0.076	100.35 ± 0.039
II. Intermediate Precision			
a. Days	101.04 ± 0.029	100.28 ± 0.047	99.74 ± 0.086
b. Analysts	100.58 ± 0.093	101.27 ± 0.092	100.93 ± 0.095
c. Instruments	101.05 ± 0.039	100.73 ± 0.057	100.36 ± 0.047
Robustness	99.79 ± 0.097	100.98 ± 0.064	100.43 ± 0.034
LOD	1 µg mL^{-1} ± 0.264	1 µg mL^{-1} ± 0.305	1.5 µg mL^{-1} ± 0.475
LOQ	3 µg mL^{-1} ± 0.193	3 µg mL^{-1} ± 0.261	4 µg mL^{-1} ± 0.413

** mean of six dilutions in three replicates. SD = standard deviation.*

Dosage form analysis

Olopatadine hydrochloride was determined by all validated methods at three different levels (20, 30 and 40 µg mL^{-1}) in eye drop and tablets. Eye drop solution

was used as such to determine the drug content i.e. the solution was diluted and absorbance was measured to get concentration and powdered tablet was dissolved in 50% methanol and filtered to get stock solution. The dilutions of dosage forms show identical $\ddot{e}_{max}$ in Gaussian spectrum (301 nm) and first order derivative mode of Gaussian spectrum (262 nm) were found; confirming that there is no interference of any excipient.

In eye drops the drug content was found in between 100.1-100.8% (Table 3) with standard deviation of less than 0.77 by all three methods at all concentration levels. The P value (P trend) for all three methods was found to be 0.585, 0.719 and 0.528 respectively, which were within acceptable limits. The P values (Pearson Chi Square) for three concentration levels (20, 30 and 40 µg mL^{-1}) were found to be 0.847, 0.259 and 0.228 respectively. (Table 3).

Table 3: *Analysis of olopatadine hydrochloride eye drop*

Batch ↓	Determined % of olopatadine hydrochloride in eye drop								
	LRE method			SA method			FOD method		
Conc. (µg mL⁻¹) →	20	30	40	20	30	40	20	30	40
I	100.14	100.29	100.02	100.03	100.42	101.36	100.26	100.45	99.93
II	99.89	100.48	99.98	100.45	100.29	100.39	100.25	99.87	100.42
III	100.01	100.29	101.05	100.02	101.03	101.01	100.33	101.02	101.15
IV	100.21	101.04	101.10	101.36	100.04	100.87	100.24	99.87	100.21
V	100.48	100.42	101.04	100.42	101.11	101.14	100.42	100.54	100.42
VI	99.38	99.38	100.52	100.23	100.27	100.01	100.42	100.27	99.67
Mean	100.50	100.49	100.68	100.36	100.36	100.80	100.50	100.39	100.36
SD	0.372	0.461	0.483	0.351	0.358	0.461	0.297	0.592	0.572
P value*		0.585			0.719			0.528	
Conc. (µg/ml) →		20			30			40	
P value**		0.843			0.259			0.228	

** P value (P trend) of all three concentration levels for the method.*
*** P value (Pearson Chi Square) of all three methods for the concentration.*

Olopatadine hydrochloride content in tablets by all methods ranged between 99.36-101.28% with acceptable standard deviation (0.41 – 0.69). The P value (P trend) at all concentration levels was found to be 0.375, 0.304 and 0.895 respectively; which were non-significant. The P values (Pearson Chi Square) for three concentration levels (20, 30 and 40 µg mL^{-1}) were found to be 0.773, 0.518 and 0.418 (Table 4). These P values are non-significant; showing suitability of all method for determination of olopatadine hydrochloride at all these concentration levels without any significant difference.

CONCLUSION

Hence, three spectrophotometric methods were developed and validated viz. linear regression equation (LRE), standard absorptivity (SA) and first order derivative (FOD) and successfully applied to determine olopatadine hydrochloride in dosage forms. All methods have proved equally applicable by P values. Thus, all methods may be applied to determine olopatadine hydrochloride in bulk drugs, different dosage forms, dissolution studies, bioequivalence studies, degradation studies and in routine pharmaceutical industries.

Table 4: *Analysis of olopatadine hydrochloride tablets*

Batch ↓	Determined % of olopatadine hydrochloride in tablets								
	LRE method			SA method			FOD method		
Conc. (µg/ml) →	20	30	40	20	30	40	20	30	40
I	100.76	99.85	100.34	100.63	101.09	100.84	99.96	100.93	100.01
II	99.92	100.67	101.12	100.92	100.02	100.06	101.05	99.87	99.93
III	101.28	99.79	99.75	99.92	100.13	99.79	99.78	100.16	101.02
IV	100.39	100.73	99.68	100.06	99.48	101.07	100.76	100.73	99.97
V	100.82	100.75	100.29	100.26	100.16	101.01	100.47	99.85	100.06
VI	99.36	100.47	100.02	99.89	99.78	100.73	99.99	99.93	100.81
Mean	100.42	100.37	100.20	100.28	101.11	100.58	100.30	100.24	100.30
SD	0.690	0.441	0.525	0.411	0.543	0.537	0.501	0.478	0.485
P value*		0.375			0.304			0.895	
Conc. (µg/ml) →		20			30			40	
P value**		0.773			0.518			0.418	

** P value (P trend) of all three concentration levels for the method.*
*** P value (Pearson Chi Square) of all three methods for the concentration.*

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CONFLICT OF INTEREST

Authors have no financial gain from any related pharmaceutical company or trademark. Authors have no conflict of interest with anyone.

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