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Introducing FDA Validation Guidelines for the Spectrophotometric Determination of Olopatadine Hydrochloride in Pure Form and Eye Drops

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ABSTRACT

A novel simple, accurate, sensitive and economical spectrophotometric method has been established and validated for the determination of the antihistaminic drug, olopatadine hydrochloride in both pure form and eye drops. The spectrophotometric method is divided into three procedures (A, B & C). The method is based on the oxidation of the studied drug by a known excess of potassium permanganate, followed by measuring the decrease in absorption (ΔA) of KMnO4 in acidic medium (A) or basic medium (B) or measuring the increase in absorption of added methyl orange in the same basic medium (C) at wavelengths of 526, 547 and 523 nm, respectively. The detection limit is reported to be 1.05, 0.62 and 0.40 µg/mL for procedures A, B and C respectively showing a high degree of sensitivity. The proposed method was successfully validated according to FDA guidelines for the determination of the drug in eye drops with a highly precise recovery and very low relative standard deviation. Finally, the method was compared statistically with a reference method showing equal accuracy, reproducibility and no significant difference with the reported one.

Keywords: Spectrophotometric, Olopatadine hydrochloride, $KMnO_4$, Methyl orange, FDA.

INTRODUCTION

Olopatadine hydrochloride (Figure 1), is a new antihistaminic drug and chemically, is 11-[3-(dimethylamino)propylidene]-6,11dihydrodibenzo [b,e]oxepin-2-yl acetic acid hydrochloride] [1]. It has a dual selective histamine H^1 receptor antagonist and mast cell stabilizer activity showing an excellent anti-allergic activity. This synergic block action of endogenous histamine release leads to a temporary relief of the negative symptoms brought on by histamine. As such, olopatadine is currently used to treat some allergic symptoms like allergic rhinitis, chronic urticaria, eczema, dermatitis and conjunctivitis (itching eyes) [2].

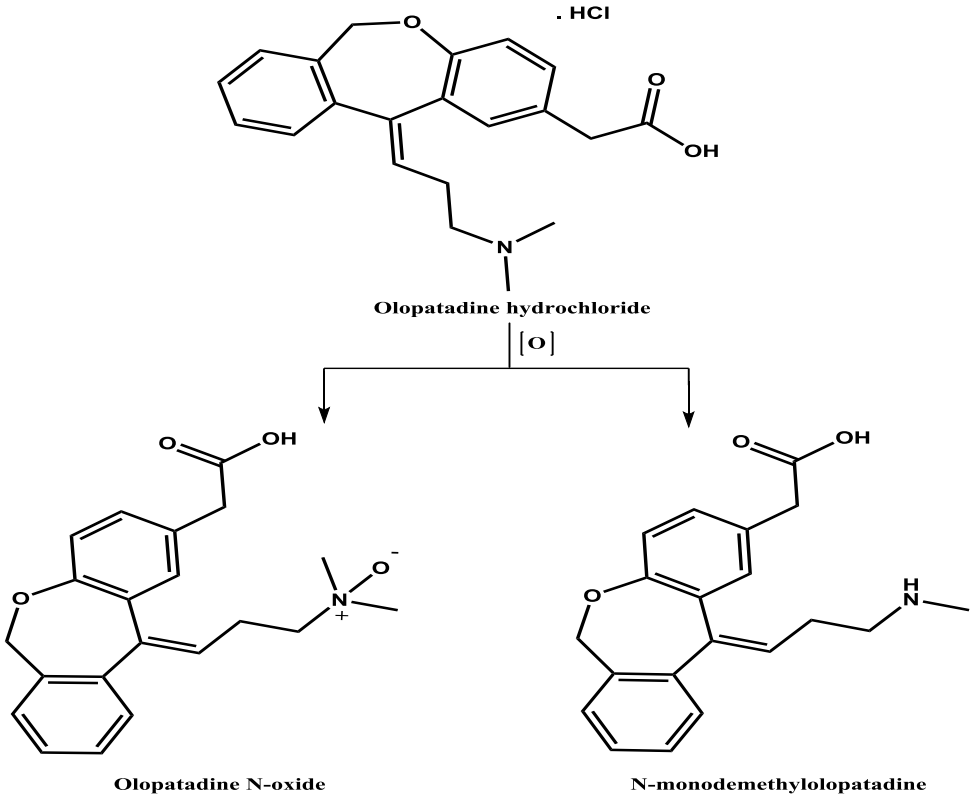

Figure 1: Proposed mechanism for chemical oxidation of olopatadine hydrochloride into olopatadine N-oxide and N-monodemethylolopatadine.

Literature survey demonstrated that few analytical techniques have been employed for the determination of olopatadine such as spectrophotometry [3-5] , derivative spectrophotometry [6,7], high performance liquid chromatography [8-11], high performance liquid chromatography with tandem mass spectrophotometry [12-14], high performance thin layer chromatography [15,16], capillary electrophoresis [17] and voltammetry [18].

In comparison to other instrumental analysis methods, spectrophotometric methods are better applied for routine analysis due to their economic, rapid, simple and maintenance free advantages without compromising on accuracy and precision [3].

To the best of our knowledge and comprehensive survey, olopatadine was not determined before spectrophotometrically based on any kind of oxidation-reduction reactions. As such, the present work introduces a simple, reproducible and sensitive spectrophotometric method for the determination of olopatadine relying on oxidation with $KMnO_4$ followed by measuring the decrease in absorption (ΔA) of $KMnO_4$ in acidic medium (A) or basic medium (B) or measuring the increase in absorption of added methyl orange in the same basic medium (C) at 526, 547 and 523 nm, respectively. This method was then validated to determine of olopatadine hydrochloride in pure form as well as in eye drops to ensure the quality and purity of the sample drug.

EXPERIMENTAL PROCEDURE

Apparatus

Labomed®

Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells and connected to windows compatible computer using UV Win 5 Software v5.0.5.

Materials and reagents

All solvents and reagents were of analytical grade and double distilled water was used throughout the work.

Olopatadine HCl

This was kindly provided by Egyptian Company for Pharmaceutical & Chemical Industries (EIPICO), 10th Of Ramadan City, Egypt. Standard solution of 200 µg.ml^{-1} was prepared by dissolving (0.02 g) of the pure drug in 100 ml double distilled water.

Methyl Orange

(Aldrich Chemical Co. Ltd., Dorset, England)

Standard solution of 3.0×10^{-3} M, It was prepared by dissolving (0.096 g) of the dye in 100 ml double distilled water. The solution was set at room temperature for about two weeks without any significant decay.

Potassium permanganate

(Aldrich Chemical Co. Ltd., Dorset, England) Standard solution of 5.0×10^{-3} M, was prepared by dissolving (0.079 g) of the $KMnO_4$ in 100 ml double distilled water and stored in dark bottle [19].

Sulfuric acid and sodium hydroxide

(El-Nasr chemical, Egypt) were prepared as 2 M and 0.1 M, respectively.

Olohistine® 0.1% eye drops

These were labeled to contain 1mg/5ml olopatadine HCl (EIPICO, Egypt).

Spectrophotometric procedures

Construction of the calibration curve using procedure A

Aliquot portions of 200 µg.ml^{-1} olopatadine HCl ranging from (0.2 - 1.4 ml) were transferred into a series of 10 ml measuring flasks. To these, 1.5 ml of 2 M sulfuric acid and 1 ml of 5 × 10^{-3} M potassium permanganate were added then the total volume was adjusted to 10 ml with double distilled water. The absorbance of a reagent blank (similarly prepared without the drug) was measured against each drug concentration at 526 nm.

Construction of the calibration curve using procedure B

Aliquot portions of 200 µg.ml^{-1} olopatadine HCl ranging from (0.2 - 1.2 ml) were transferred into a series of 10 ml measuring flasks. To these, 0.5 ml of 0.1 M sodium hydroxide and 1.5 ml of 5 × 10^{-3} M potassium permanganate were added then the total volume was adjusted to 10 ml with double distilled water. The absorbance of a reagent blank was measured against each drug concentration at 547 nm.

Construction of the calibration curve using procedure C

Aliquot portions of 200 µg.ml^{-1} olopatadine HCl ranging from (0.8 - 2.2 ml) were transferred into a series of 10 ml measuring flasks. To these, 0.5 ml of 0.1 M sodium hydroxide, 1.5 ml of 5 x 10^{-3} M potassium permanganate and 1 ml of 3 x 10^{-3} M methyl orange were added and the flasks were shaken and allowed to stand for 5 minutes at room temperature. The total volume was then adjusted to 10 ml with double distilled water and absorbance of methyl orange was measured at 523 nm, against a reagent blank.

Procedure for pharmaceutical preparation

For Olohistine® eye drops: An accurately volume of 1.8 ml of the eye drops (olopatadine 1mg/5ml) was transferred to a 10 ml measuring flask and completed to volume with double distilled water to give an equivalent final concentration of 200 µg.ml^{-1}. The procedures A, B & C were then conducted as mentioned above under the general procedures applying standard addition techniques.

RESULTS AND DISCUSSION

Chemistry of the oxidation product

The proposed method is based on the oxidation of olopatadine hydrochloride with potassium permenganate. Figure 1 is depicting the proposed scheme of olopatadine hydrochloride oxidation into olopatadine N-oxide and N-monodemethylolopatadine. Both compounds are expected to be the major oxidation products similar to the pathway of the cited drug metabolism in the body as reported by Jiro et al. [20].

Optimization of the reaction conditions

The optimum conditions for the method development were established by varying each specific parameter and keeping the others constant and observing the effect produced on the absorbance of the colored species. The optimum parameters are reported in Table 1.

Table 1: Analytical parameters and spectrophotometric characteristics of the proposed method for olopatadine hydrochloride determination.

Parameters	**Procedure A**	**Procedure B**	**Procedure C**
λ_{max}	526 nm	547 nm	523 nm
Volume of the media	1.5 ml 2 M H_2SO_4	0.5 ml 0.1 M NaOH	0.5 ml 0.1 M NaOH
Volume of the reagents	1 ml $KMnO_4$	1.5 ml $KMnO_4$	1.5 ml $KMnO_4$ + 1 ml methyl orange
Time of reaction between olopatadine and $KMnO_4$	Immediate		
Time after dye addition	------	------	5 minutes
Temperature	Ambient		

Absorption spectra

Absorption spectra of olopatadine HCl with the reagents were studied over a range of 400-800 nm. Potassium permanganate reacts with olopatadine HCl in acidic medium (A), or basic medium (B) and the decrease in absorption can be measured at 526 nm, 547 nm, respectively. Also, the increase in absorption of added methyl orange in the same basic medium (C) can be measured at 523 nm (Figure 2).

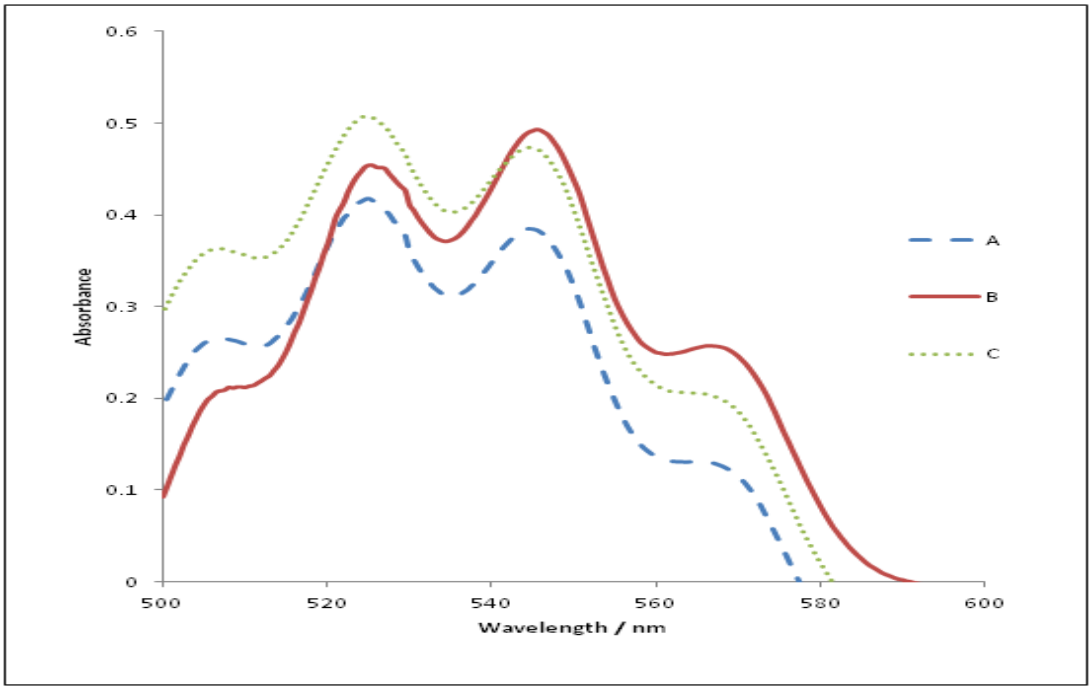

Figure 2: Absorption spectra of olopatadine HCl with potassium permenganate in acidic medium (A), in basic medium (B) and with methyl orange in the same basic medium (C) at 526 nm, 547 nm and 523 nm, respectively.

Effect of temperature

Effect of temperature was studied and results showed that there is no an evident effect of temperature on the reaction as increase in temperature is not accompanied with any increase in absorbance and so, optimum reaction was performed at room temperature.

Effect of addition sequence

Addition sequences were studied and results revealed that the most appropriate sequence was the drug then the added acid or base then the added potassium permenganate (procedures A&B) and finally the dye addition (procedure C).

Effect of time

The effect of time on the oxidation reaction was studied to obtain the highest and most stable absorbance. This absorbance can be achieved immediately after the reaction between the drug and potassium permenganate (procedures A&B) while the reaction between potassium permenganate and methyl orange was reported to be stable after 5 minutes (procedure C).

Effect of acidity and basicity

To study the effect of sulfuric acid and sodium hydroxide volumes, the reaction was performed in a series of 10 ml volumetric flasks containing different volumes (0.5 -3.5 ml) of both 2 M sulfuric acid and 0.1 M sodium hydroxide, separately. It was found that the maximum absorbance was obtained when using 1.5 ml of 2 M sulfuric acid (procedure A) or 0.5 ml of 0.1 M sodium hydroxide solutions (procedures B&C).

Effect of permenganate concentration

When studying the effect of potassium permanganate concentration referring to decrease of its color intensity (procedures A&B) or increase of methyl orange color intensity (procedure C) , it was observed that the absorbance reached its maximum when 1 ml of 5×10^{-3} M potassium permanganate was used in case of procedure A, while 1.5 ml of the same solution was sufficient for procedures B&C.

Effect of dye concentration

In order to ensure a linear relationship between the different concentrations of olopatadine hydrochloride and the increase in absorbance of methyl orange (procedure C), experiments were performed in 0.5 ml of 0.1 M sodium hydroxide, 1.5 ml of 5.0×10^{-3} M potassium permanganate and different volumes of methyl orange. It was found that 1.0 ml of 3.0×10^{-3} M methyl orange was enough to give a maximum absorbance at 523 nm.

Method Validation

The method validation was performed according to food and drug administration and international conference of harmonization guidelines (ICH) [21].

Linearity

Five different concentrations of olopatadine HCl for each procedure was prepared for linearity studies. The linearity ranges of absorbance as a function of drug concentration (Table 2) provided acceptable indication about sensitivity of reagents used. Linear regression equations of procedures A, B and C were found to be y = 0.013x + 0.044, y = 0.0204x + 0.0098, and y = 0.0115x - 0.0885, respectively and the regression coefficient values (R^2) were found to be 0.9995, 1 and 0.9992, respectively indicating a high degree of linearity for all procedures (Figure 3).

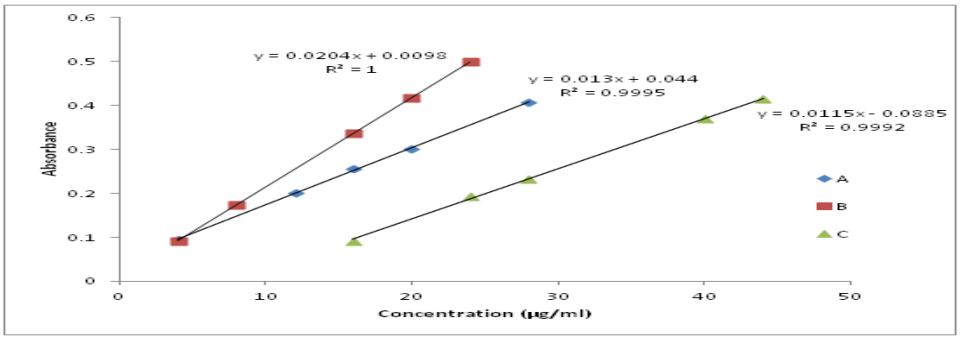

Figure 3: Calibration curves of olopatadine HCl with potassium permenganate in acidic medium (A), in basic medium (B) and with methyl orange in the same basic medium (C).

Table 2: Results of the analysis for determination of olopatadine hydrochloride sample using the proposed method.

Parameters	Procedure A			Procedure B			Procedure C		
	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	4	3.92	98.99	4	3.99	99.75	16	16.27	101.70
	12	12	100.0	8	8.02	100.36	24	24.43	101.81
	16	16.30	101.92	16	16.10	100.67	28	27.82	99.78
	20	19.76	98.91	20	20.04	100.24	40	39.82	99.56
	28	28	100.0	24	24.08	100.36	44	43.73	99.71
Mean			99.76			100.28			100.37
±SD			1.454			0.336			1.266
±SE			0.65			0.15			0.56
±RSD			1.457			0.335			1.261
Variance			2.11			0.11			1.60
Slope			0.013			0.020			0.011
LOD			1.05			0.62			0.40
LOQ			3.51			2.08			1.34

Accuracy

The accuracy of the method was determined by investigating the recovery of olopatadine HCl concentration levels covering the specified range using the standard addition technique. It was performed by adding a fixed standard drug concentration at different levels of the eye drops solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated and the results are shown in Table 3.

Table 3: Application of standard addition technique for the determination of Olohistine® eye drops using the proposed method.

parameters	Procedure A				Procedure B				Procedure C			
	Added pure Olo µg/ml	Taken µg/ml	Found µg/ml	Recovery %	Added pure Olo µg/ml	Taken µg/ml	Found µg/ml	Recovery %	Added pure Olo µg/ml	Taken µg/ml	Found µg/ml	Recovery %
	4	0	4.07	101.92	4	0	4.06	101.60	4	12	16.27	101.70
	4	8	12.00	100.00	4	4	8.07	100.98	4	20	24.26	101.08
	4	16	20.23	101.15	4	16	19.70	98.52	4	24	28.00	100.00
	4	20	24.46	101.92	4	20	24.13	100.57	4	36	39.82	99.56
	4	24	28.07	100.27	4	24	27.53	98.34	4	40	44.08	100.19
Mean				101.05				100.01				100.51
±SD				0.89				1.48				0.86
±SE				0.40				0.66				0.38
±RSD				0.89				1.48				0.86
Variance				0.80				2.19				0.75

Precision

Intraday precision and interday reproducibility were evaluated by calculating relative standard deviations and recoveries of three replicate determinations using three different concentrations of olopatadine HCl. It was found that the reproducibility of the method in the basic medium (%RSD ≈ 1.2) is much better than in the acidic medium (%RSD ≈ 2.3). However, the results obtained by the proposed method were found to be acceptable.

Specificity

The specificity studies revealed that the presence of the excipents in the eye drops formulation didn't show any kind of impurity interference, since the recoveries lied in the range of 98-102% as reported in Table 3.

Limits of detection and limits of quantification

The calculation of limits of detection and quantitation was based on the following equations: LOD = 3.3 S/K and LOQ = 10 S/K, respectively, where S is the standard deviation of the seven replicate values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. Limits of detection in case of procedures A, B and C were calculated to be 1.05, 0.62 and 0.40 $\mu g.ml^{-1}$ and limits of quantification were 3.51, 2.08 and 1.34 $\mu g.ml^{-1}$, respectively (Table 2).

Robustness

The robustness of the method was evaluated by making small changes (± 0.05 ml) in the volume of H_2SO_4, NaOH and methyl orange keeping the other conditions constant where the effect of the changes was studied on the percent recovery and standard deviation of 20 $\mu g.ml^{-1}$ olopatadine HCl. The changes had negligible influence on the results where SD values were in the acceptable range (≤ 1.93) as reported in (Table 4).

Table 4: Results of the robustness for 20 µg.ml-1 olopatadine hydrochloride sample using the proposed method.

Procedure / Parameter changes	**Mean recovery** ± SD	**CV (%)**	**% Accuracy**
A + 0.05 ml H_2SO_4	100.53 ± 1.83	1.80	2.69
A - 0.05 ml H_2SO_4	99.38 ± 1.93	1.95	- 3.07
B + 0.05 ml NaOH	99.44 ± 1.92	1.93	- 3.94
B - 0.05 ml NaOH	99.93 ± 0.85	0.86	- 1.47
B + 0.05 ml Methyl orange	99.64 ± 1.27	1.28	- 1.81
B - 0.05 ml Methyl orange	100.73 ± 1.88	1.87	3.62

Ruggedness

The ruggedness of the method was tested by measuring three concentrations of the standard working solution using a different double beam spectrophotometer (model Jenway 6500, UK). The absorbances in case of the three procedures for both instruments were exactly similar indicating that the method is fairly rugged. According to FDA-ICH guidelines, the obtained values indicated high sensitivity of the proposed method.

Statistical analysis of the pharmaceutical formulation

Olohistine® eye drops have been successfully analyzed by the proposed method. Results obtained were compared to those obtained by applying reference method [7] where Student's t-test and F-test were performed for comparison. Results are shown in Table 5 where the calculated t and F values were less than tabulated values at p= 0.05, which in turn indicate that there is no significant difference between proposed method and reference one relative to precision and accuracy.

Table 5: Statistical analysis of results obtained by the proposed method applied on Olohistine® eye drops compared with reference method.

Parameters	Procedure A	Procedure B	Procedure C	Reported method[7]
N	5	5	5	4
Mean Recovery	101.05	100.01	100.51	100.56
SE	0.36	0.33	0.28	0.09
Variance	1.01	0.69	0.58	4.43
Student-t**	0.69 (1.89)a	0.61 (1.89)a	0.09 (1.89)a	
F-test**	1.85 (6.59)b	1.46 (6.59)b	1.99 (6.59)b	

Note: a and b are the theoretical Student t-values and F-ratio at p= 0.05.

CONCLUSION

Unlike GC and HPLC techniques, spectrophotometry is simple and inexpensive. The proposed method requires reagents which are very cheap and readily available, no pH adjustment is required and the procedures do not involve any critical reaction conditions or tedious sample preparation. According to FDA guidlines, the method is simple, fast, accurate, sensitive, rugged and free from interference by common additives and excipients which makes it ideal for routine quality control analysis. The amounts obtained by the proposed method for the eye drops lied in the acceptable range of 98-102% and were statistically superior to the reference method with respect to both sensitivity and selectivity.

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REFERENCES

1. Ohshima, E., et al. Synthesis and anti-allergic activity of 11-(amino-alkylidene)-6,11-dihydrodibenz [b,e]oxepin derivatives. *J Med Chem.* **1992.** 35(11): 2074-2084.
2. James, M., A review of the use of olopatadine in allergic conjunctivitis. *Int Ophth.* **2004.** 25(3): 171-179.
3. Suddhasattya, D., et al. 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. *Int J Pharm Pharm Sci*. **2010.** 2(4): 212-218.
4. Annapurna, M., Bindu, G., and Divya, I., New analytical methods for the determination of olopatadine (an anti-allergic drug) in eye drops, *Drug Inv Tod.* **2012.** 4(8): 441-443.
5. Jain, D., and Basniwal, P., Spectrophotometric determination of olopatadine hydrochloride in eye drops and tablets**,** *J Pharm Res*, **2013.** 12(2): 48-52.
6. Annapurna, M., Bindu, G., and Divya, I., Derivative spectrophotometric methods for the determination of olopatadine in pharmaceutical dosage form, *Drug Inv Tod.* **2012.** 4(10): 540-542.
7. Rele, V., UV-Spectrophotometric estimation of olopatadine hydrochloride in bulk and pharmaceutical dosage form by area under curve and second order derivative methods. *Res J Pharm Tech.* **2015.** 8(3): 265-269.
8. Rele, R., and Warkar, B., Application of high performance liquid chromatographic technique for olopatadine hydrochloride and its impurity in ophthalmic solution. *Int J Chem Sci.* **2011.** 9(2): 601-614.
9. Parth, B., and Jawed, A., Development and validation of stability indicating RP-HPLC method for estimation of olopatadine hydrochloride in bulk drug and its formulations. *Int J Pharm Sci Rev Res.* **2011.** 9(2): 153-158.
10. Pawan, B., and Deepti, J., ICH guideline practice: Application of novel RP-HPLC-DAD method for determination of olopatadine hydrochloride in pharmaceutical products. *J Anal Sci Tech.* **2013.** 4 (12): 1-6.
11. Jovanović, M., et al. Chromatographic analysis of olopatadine in hydrophilic interaction liquid chromatography. *J Chromatogr Sci*. **2015.** 53(5): 680-686.
12. Magara, H., and Kobayashi, H., Determination of olopatadine, a new antiallergic agent, and its metabolites in human plasma by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl.* **1999.** 731(2): 345-352.
13. Koichiro, F., et al. Determination of some anti-allergic drugs in human plasma by direct-injection high-performance liquid chromatography-tandem mass spectrometry. *Forens Tox.* **2006.** 24(1): 8-16.
14. Zhu, P., et al. A rapid and sensitive liquid chromatography-tandem mass spectrometry method for determination of olopatadine concentration in human plasma. *J Anal Toxicol.* **2011.** 35(2): 113-118.
15. Mahajan, A., et al. A validated high performance thin layer chromatographic method for estimation of olopatadine hydrochloride as bulk drug and in ophthalmic solutions. *Int J Chem Tech Res.* **2010.** 2(3): 1372-1375.
16. Varghese, J., Kumar, M., and Ravi, K., Stability-indicating high-performance column liquid chromatography and high-performance thin-layer chromatography methods for the determination of olopatadine hydrochloride in tablet dosage form. *J AOAC Int.* **2011.** 94(6): 1815-1820.
17. Güray, T., et al. A validated capillary electrophoretic method for the determination of olopatadine and its application to a pharmaceutical preparation of eye drops. *J AOAC Int.* **2017.** 100(1): 206-211.

18. Sreedhar, Y., et al. Voltammetric determination of olopatadine hydrochloride in bulk drug form and pharmaceutical formulations. *Int J Pharm Sci Res*. **2012.** 3(7): 2320-2324.
19. Mendham, J., et al. Vogel qualitative analysis*" Pearson Education Limited.* (7th edn), **2000.**
20. Jiro, K., et al. Effects of olopatadine, a new antiallergic agent, on human liver microsomal cytochrome P450 activities. *Drug Metab Dispos.* **2002.** 30(12): 1504-1511.
21. https://www.fda.gov/downloads/drugs/guidances/ucm073384.pdf