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Research Article

Colurimetric Determination of Olopatadine Hydrochloride Oxidation-Reduction Products in Pure form and Eye Drops

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Abstract

A novel simple, accurate, sensitive and economical colurimetric 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 four procedures (A, B, C & D). The method is based on the oxidation of the studied drug by a known excess of $KMnO_4$, followed by measuring the decrease in absorption (ΔA) of $KMnO_4$ in 2M H_2SO_4 (A), in 0.1M NaOH (B) both at 504nm or measuring the increase in absorption of added methyl orange in 0.1M NaOH (C) at 443 nm or measuring the change of $KMnO_4$ into green colour in 1M NaOH (D) at 605nm. The detection limit is reported to be 1.05, 0.62, 0.40 and 0.60µg/ml for procedures A, B, C and D, 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: Colurimetric; 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 H1 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].

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 eectrophoresis [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) at 504nm or measuring the increase in absorption of added methyl orange in the same basic medium (C) at 443nm or measuring the change of $KMnO_4$ color in higher basic medium concentration at 605nm. 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

Apparatus

- Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1cm 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** was kindly provided by Egyptian Company for Pharmaceutical & Chemical Industries (EIPICO), 10th Of Ramadan City, Egypt. Standard solution of 200µg/ml was prepared by dissolving (0.02g) of the pure drug in 100ml double distilled water.

- **Methyl Orange** (Aldrich Chemical Co. Ltd., Dorset, England). Standard solution of $3.0x10^{-3}$M, was prepared by dissolving (0.096g) of the dye in 100ml double distilled water. The solution was set at room temperature for about two weeks without any significant

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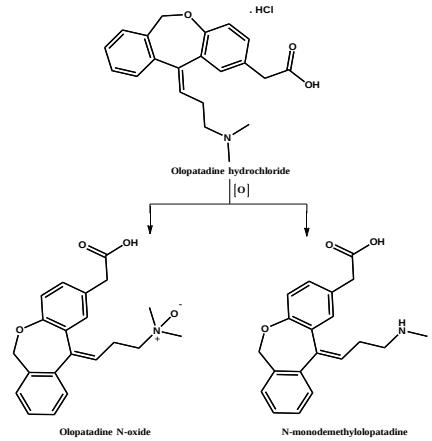

Figure 1: Proposed mechanism for chemical oxidation of olopatadine hydrochloride into olopatadine N-oxide and N-monodemethylolopatadine.

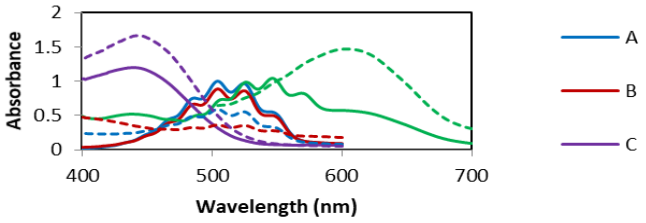

Figure 2: Absorption spectra of potassium permenganate in 2M H_2SO_4 (A), in 0.1M NaOH (B), with methyl orange in 0.1M NaOH (C) & in 1M NaOH (D) ____ and the effect of olopatadine HCl (20µg/ml) addition on each spectrum ------- at 504nm (A & B), 443nm (C) & 605nm (D), respectively.

decay.

- **Potassium Permanganate** (Aldrich Chemical Co. Ltd., Dorset, England). Standard solution of $5.0x10^{-3}$M, was prepared by dissolving (0.079g) of the $KMnO_4$ in 100ml double distilled water and stored in dark bottle [19].

- **Sulfuric Acid** (El-nasr chemical, Egypt) was prepared as 2M concentration for procedure A.

- Sodium Hydroxide (El-nasr chemical, Egypt) was prepared as two different concentrations of 0.1M for procedures B & C and 1M for procedure D.

- **Olohistine**® 0.1% eye drops was labeled to contain 1mg/5ml olopatadine HCl (EIPICO, Egypt).

Spectrophotometric procedures

Construction of the calibration curve using procedure A: Aliquot portions of 200µg/ml olopatadine HCl ranging from (0.2-1.4ml) were transferred into a series of 10ml measuring flasks. To these, 1.5ml of 2M sulfuric acid and 1 ml of 5×10^{-3}M potassium permanganate were added then the total volume was adjusted to 10ml with double distilled water. The absorbance of a reagent blank (similarly prepared without the drug) was measured against each drug concentration at 504nm.

Construction of the calibration curve using procedure B: Aliquot portions of 200µg/ml olopatadine HCl ranging from (0.2-1.2ml) were transferred into a series of 10ml measuring flasks. To these, 0.5ml of 0.1M sodium hydroxide and 1.5ml of $5x10^{-3}$M potassium permanganate were added then the total volume was adjusted to 10ml with double distilled water. The absorbance of a reagent blank was measured against each drug concentration at 504nm.

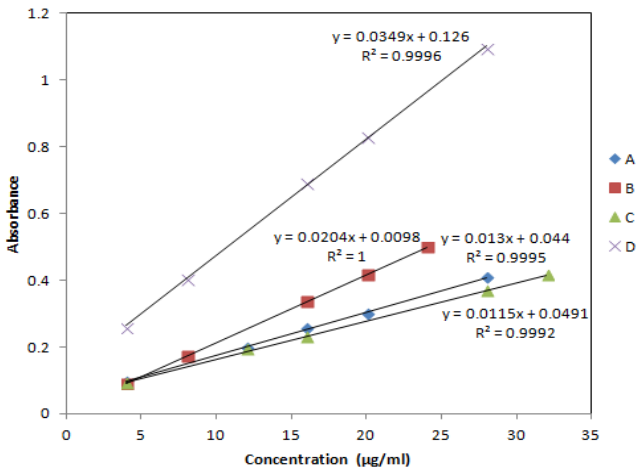

Figure 3: Calibration curves of olopatadine HCl with potassium permenganate in 2M H_2SO_4 (A), in 0.1M NaOH (B), with methyl orange in 0.1M NaOH (C) & in 1M NaOH (D).

Construction of the calibration curve using procedure C: Aliquot portions of 200µg/ml olopatadine HCl ranging from (0.8-2.2ml) were transferred into a series of 10ml measuring flasks. To these, 0.5ml of 0.1M sodium hydroxide, 1.5ml of 5×10^{-3}M potassium permanganate and 1ml of $3x10^{-3}$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 10ml with double distilled water and absorbance of methyl orange was measured at 443nm, against a reagent blank.

Construction of the calibration curve using procedure D: Aliquot portions of 200µg/ml olopatadine HCl ranging from (0.2-1.4ml) were transferred into a series of 10ml measuring flasks. To these, 2ml of 1M sodium hydroxide and 3ml of $5x10^{-3}$M potassium permanganate were added then the total volume was adjusted to 10ml with double distilled water. After 25 minutes, the absorbance of each drug concentration was measured at 605nm against reagent blank.

Procedure for pharmaceutical preparation

For Olohistine® eye drops: An accurately volume of 1.8ml of the eye drops (olopatadine 1mg/5ml) was transferred to a 10ml measuring flask and completed to volume with double distilled water to give an equivalent final concentration of 200µg/ml. The procedures A, B, C & D 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

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

Parameters	Procedure A	Procedure B	Procedure C	Procedure D
λ_{max}	504nm	504nm	443nm	605nm
Volume of the media	1.5ml 2M H_2SO_4	0.5ml 0.1M NaOH	0.5ml 0.1M NaOH	2ml 1M NaOH
Volume of the reagents	1ml $KMnO_4$	1.5ml $KMnO_4$	1.5ml $KMnO_4$ + 1ml methyl orange	3ml $KMnO_4$
Time of reaction between olopatadine and $KMnO_4$	immediate			25 minutes
Time after dye addition	------	------	5 minutes	------
Temperature	Ambient			

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

para-meters	Procedure A			Procedure B			Procedure C			Procedure D		
	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Taken µg/ml	Taken µg/ml	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	101.7	4	3.94	98.5
	12	12	100	8	8.02	100.36	24	24.43	101.81	8	8.14	101.78
	16	16.3	101.92	16	16.1	100.67	28	27.82	99.78	16	16.31	101.96
	20	19.76	98.91	20	20.04	100.24	40	39.82	99.56	20	20.28	101.42
	28	28	100	24	24.08	100.36	44	43.73	99.71	28	27.85	99.48
Mean			99.76			100.28			100.37			100.63
±SD			1.454			0.336			1.266			1.54
±SE			0.65			0.15			0.56			0.69
±RSD			1.457			0.335			1.261			1.53
Variance			2.11			0.11			1.6			2.38
Slope			0.013			0.02			0.011			0.034
LOD			1.05			0.62			0.4			0.6
LOQ			3.51			2.08			1.34			2.01

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 K. 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.

Absorption spectra: Absorption spectra of olopatadine HCl with the reagents were studied over a range of 400-800nm. Potassium permanganate reacts with olopatadine HCl in 2M acidic medium (A), or in 0.1M basic medium (B) and the decrease in absorption can be measured at 504nm. Also, the increase in absorption of added methyl orange in the same basic medium (C) can be measured at 443nm or the change of $KMnO_4$ color into green in 1M basic medium (D) can be measured at 605nm as shown in Figure 2.

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 & D) 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) or after 25 minutes (procedure D) in higher basic concentration 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 10ml volumetric flasks containing different volumes (0.5-3.5ml) of 2M H_2SO_4, 0.1M NaOH, or 1M NaOH, separately. It was found that the maximum absorbance was obtained when using 1.5ml of 2M H_2SO_4 (procedure A), 0.5ml of 0.1M NaOH (procedures B&C) or 2ml 1M NaOH (procedure D) .

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) or change in $KMnO_4$ colour (procedure D), it was observed that the absorbance reached its maximum when 1ml of $5x10^{-3}$M potassium permanganate was used in case of procedure A, 1.5ml was sufficient for procedures B&C, while 3ml was optimum for procedure D.

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

para-meters	Procedure A				Procedure B				Procedure C				Procedure D			
	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 %	Added pure Olo µg/ml	Taken µg/ml	Found µg/ml	Recovery %
	4	0	4.07	101.92	4	0	4.06	101.6	4	12	16.27	101.7	4	0	4.05	101.49
	4	8	12	100	4	4	8.07	100.98	4	20	24.26	101.08	4	4	7.97	99.62
	4	16	20.23	101.15	4	16	19.7	98.52	4	24	28	100	4	12	15.88	99.25
	4	20	24.46	101.92	4	20	24.13	100.57	4	36	39.82	99.56	4	16	19.61	98.05
	4	24	28.07	100.27	4	24	27.53	98.34	4	40	44.08	100.19	4	24	28.41	101.49
Mean				101.05				100.01				100.51				99.98
±SD				0.9				1.48				0.87				1.49
±SE				0.4				0.66				0.38				0.66
±RSD				0.89				1.48				0.86				1.49
Variance				0.8				2.19				0.75				2.22

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

Procedure / Parameter changes	Mean recovery ± SD	CV (%)	% Accuracy
A + 0.05ml H_2SO_4	100.53 ± 1.83	1.8	0.53
A - 0.05ml H_2SO_4	99.38 ± 1.93	1.95	-0.62
B + 0.05ml NaOH	99.44 ± 1.92	1.93	-0.56
B - 0.05ml NaOH	99.93 ± 0.85	0.86	-0.07
C + 0.05ml Methyl orange	99.64 ± 1.27	1.28	-0.36
C - 0.05ml Methyl orange	100.73 ± 1.88	1.87	0.73
D + 0.05ml $KMnO_4$	101.32 ± 1.68	1.65	1.32
D - 0.05ml $KMnO_4$	100.33 ± 1.93	1.92	0.33

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	Procedure D	Reported method [7]
N	5	5	5	5	4
Mean Recovery	101.05	100.01	100.51	99.98	100.56
SE	0.36	0.33	0.28	0.66	0.09
Variance	1.01	0.69	0.58	2.22	4.43
Student-t**	0.69 (1.89)a	0.61 (1.89)a	0.09 (1.89)a	0.63 (1.89)a	
F-test**	1.85 (6.59)b	1.46 (6.59)b	1.99 (6.59)b	1.49 (6.59)b	

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

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 0.5ml of 0.1M sodium hydroxide, 1.5ml of 5.0 $x10^{-3}$M potassium permanganate and different volumes of methyl orange. It was found that 1.0ml of 3.0 $x10^{-3}$M methyl orange was enough to give a maximum absorbance at 443nm.

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, C and D were found to be y = 0.013x + 0.044, y = 0.0204x + 0.0098, y = 0.0115x - 0.0885, and y = 0.0349x + 0.126, respectively and the regression coefficient values (R^2) were found to be 0.9995, 1, 0.9992 and 0.9996, respectively indicating a high degree of linearity for all procedures (Figure 3).

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.

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 ≈ 1.9) 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, C and D were calculated to be 1.05, 0.62, 0.40 and 0.60µg/ml and limits of quantification were 3.51, 2.08, 1.34 and 2.01µg/ml, respectively (Table 2).

Robustness: The robustness of the method was evaluated by making small changes (± 0.05ml) in the volume of H_2SO_4, NaOH, methyl orange and $KMnO_4$ keeping the other conditions constant where the effect of the changes was studied on the percent recovery and standard deviation of 20µg/ml 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.

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 four 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 has 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.

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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