



SPECTROPHOTOMETRIC DETERMINATION OF OXYMETAZOLINE HYDROCHLORIDE VIA CHARGE TRANSFER REACTION USING 2,3-DICHLORO-5,6-DICYANO- 1,4-BENZOQUINONE (DDQ) REAGENT

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Simple, sensitive and reproducible spectrophotometric method was developed for estimation of oxymetazoline hydrochloride via charge transfer complex formation reaction. The method is based on the reaction of oxymetazoline as n-donor with DDQ reagent as π -acceptor in basic aqueous medium of pH 9.79. The maximum absorption is 421 nm. Beer's law was obeyed in the range 0.4-4.0 $\mu\text{g mL}^{-1}$ with molar absorptivity $2.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0093 and 0.028 $\mu\text{g mL}^{-1}$ respectively. The stability constant has been determined and the mechanism of the reactions was proposed. The proposed method was applied to determine the oxymetazoline hydrochloride in dosage form as drop, and the results were statistically compared with official BP method.

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Introduction

Oxymetazoline hydrochloride¹ (OMZH), 3-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride (Figure 1), belongs to non-selective adrenergic drugs and has been used as eye and nose drops and acting on adrenergic receptors causing a strong vasospasm leading to an increase of a blood pressure.² Oxymetazoline is used to treat epistaxis and eye redness due to minor irritation.^{3,4}

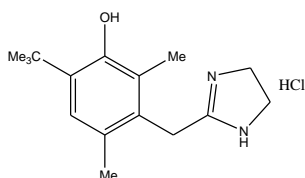


Figure 1. Oxymetazoline hydrochloride.

Various analytical techniques, including high performance liquid chromatography,^{2,5-8} liquid chromatographic-mass spectrometry,⁹ chemiluminescence¹⁰ and potentiometry^{1,11} have been used for the determination of OMZH. These techniques require sophisticated instruments and expensive reagents, and involve several manipulation steps and derivatization reactions. However, spectrophotometric techniques continue to be the most preferred method for routine analytical work due to their simplicity and reasonable sensitivity, along with significant economical advantages. Literature survey revealed that three spectrophotometric methods have been used for determination of OMZH. These methods are based on the reduction of ferric to ferrous ions by OMZH in acidic

medium and followed by complexation with 1,10-phenanthroline,¹² potassium ferricyanide¹³ and 2,4,6-tris(2-pyridyl)-5-triazine reagent.¹⁴ Other methods used 2,6-dichloroquinone-chlorimide in the presence of an oxidant,¹⁵ 4-aminoantipyrine reagent in the presence of potassium periodate as oxidizing agent¹⁶ and sodium cobaltinitrite in acetic acid medium.¹⁷ Some of these methods are time-consuming, involve extraction procedures or heating and require strictly controlled reaction conditions. Others are less sensitive. The aim of the present work was to provide a simple, sensitive, and rapid spectrophotometric method for determining of OMZH in pure form as well as in pharmaceutical drop formulations via charge transfer reaction using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) reagent.

Experimental

Apparatus

All spectrophotometric measurements are made on a Perkin-Elmer, Lambda 25 U.V- visible double beam spectrophotometer with 1 cm matched silica cells.

Reagents

All chemicals used are of the highest purity available. The following solutions were prepared.

DDQ solution ($1 \times 10^{-3} \text{ M}$) was prepared freshly by dissolving 0.0227g of DDQ in absolute methanol and diluted to the mark in 50 mL volumetric flask with the same solvent. This solution was prepared daily and used immediately. Standard solution of OMZH ($100 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.01 g of oxymetazoline.HCl in 5 mL methanol and diluted to the mark with distilled water in 100 mL volumetric flask. This solution was further diluted with water as needed.

Sodium hydroxide solution (1×10^{-2} M) was prepared by dissolving 0.04 g of NaOH in 100 mL of distilled water. Surfactant solutions (0.1 %) were prepared by dissolving of 0.1g of different surfactants (positive, neutral and negative) in 100 mL absolute ethanol.

Recommended procedure

Aliquots of the working solution of OMZH ($100 \mu\text{g mL}^{-1}$) were transferred into a series of 25 mL calibrated flasks. Then, 1.4 mL of 1×10^{-3} DDQ and 0.5 mL of 0.01 M NaOH were added and the solutions were diluted to the mark with distilled water. The absorbance was measured at 421.79 nm at room temperature against reagent blank.

Procedure for OMZH assay in Iliadin drops

Five Iliadin drop containers were mixed well and an accurately measured volume solution, equivalent to 5 mg of OMZH was transferred into a 100 mL calibrated flask and diluted to the mark with distilled water. The drug solution was analysed as described in the recommended procedure.

Results and discussion

Spectral characteristics

The proposed method involves the reaction of OXZH as n-donor with DDQ reagent as π -acceptor in the presence of NaOH to form a red colored charge transfer complex having maximum absorption at 421.79 nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 2. The corresponding reagent blank has low absorbance at this wavelength.

Optimum reaction conditions

In order to optimize the proposed spectrophotometric method, the effect of some experimental variables was studied. The effects of reagents were studied by measuring the absorbance of solutions containing $3 \mu\text{g mL}^{-1}$ of OMZH and varied amounts of the reagent separately.

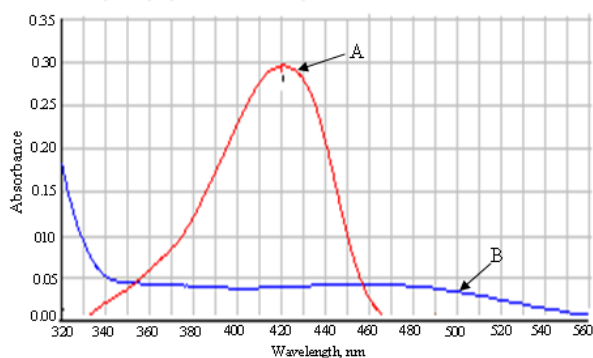


Figure 2. Absorption spectra of (A) OXZH ($3 \mu\text{g mL}^{-1}$) complex with DDQ reagent (1×10^{-3} M) in the presence of 0.01 M NaOH against reagent blank and (B) reagent blank against distilled water, under optimum conditions.

Effect of solvent

Different solvents such as methanol, ethanol, acetonitrile, acetone and water as medium for the reaction, between OMZH and DDQ, in the presence of NaOH, have been tried in order to achieve maximum sensitivity and complex stability. The absorbance of solutions was measured against corresponding blank after 5 min at room temperature. As shown in Table 1, it was found that on using water as solvent for OMZH and methanol as solvent for DDQ and dilution with water were gave maximum color intensity at 421.79 nm.

Table 1. Effect of solvent on colour intensity of OMZH – DDQ complex.

OMZH solvent	DDQ solvent	Diluent	λ_{max} (nm)	Absorbance
Water	Methanol	Water	421.79	0.216
Water	Methanol	Methanol	408.99	0.114
Methanol	Methanol	Water	421.83	0.174
Methanol	Methanol	Methanol	413.08	0.071
Water	Ethanol	Water	418.19	0.216
Water	Ethanol	Ethanol	421.95	0.103
Ethanol	Ethanol	Water	422.06	0.132
Ethanol	Ethanol	Ethanol	432.24	0.168

Effect of pH and buffer solution

The effect of pH on the absorption of the complex was studied in basic medium in the range of 8.64-11.31, by using different volumes of 1×10^{-2} M NaOH. It was observed that the complex was formed with low sensitivity at pH 8.64, in the absence of NaOH, but this sensitivity was increased by addition of NaOH and reached its maximum absorption at pH range 9.79-10.86 in the presence of 0.5-1.5 mL NaOH, (Figure 3). Different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and ammonium hydroxide with fixed volume (0.5 mL) and a concentration of 0.01M were examined. It was found that sodium hydroxide gave maximum color intensity (Figure 4). However; different buffers of pH 9.79, such as borate ($\text{Na}_2\text{B}_4\text{O}_7 + \text{HCl}$), carbonate ($\text{Na}_2\text{CO}_3 + \text{NaOH}$), bicarbonate ($\text{NaHCO}_3 + \text{NaOH}$) and phosphate ($\text{Na}_2\text{HPO}_4 + \text{NaOH}$) were prepared to examine the sensitivity. A negative effect was observed on the color intensity in comparison with NaOH, (Figure 5).

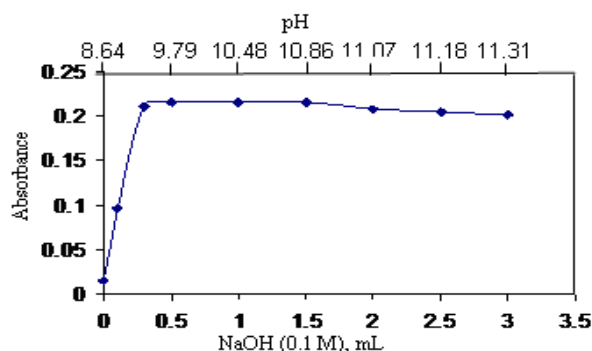


Figure 3. Effect of pH and NaOH on the absorption of $3 \mu\text{g mL}^{-1}$ OMZH complex with DDQ

Effect of reagent concentration

The effect of changing the DDQ concentration (0-3 mL of $1 \times 10^{-3} \text{ M}$) on the absorbance of a solution containing a fixed amount of OMZH ($3 \mu\text{g mL}^{-1}$) in the presence of NaOH was studied. It was observed that the absorbance increases with increasing DDQ concentration and reached maximum on using 1.4 mL of $1 \times 10^{-3} \text{ M}$ solution (Figure 6). Therefore, this much volume of the solution was used in the subsequent work.

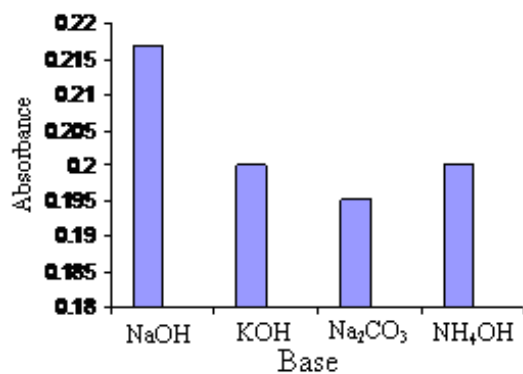


Figure 4. Effect of bases on the absorption of $3 \mu\text{g mL}^{-1}$ OMZH complex with DDQ.

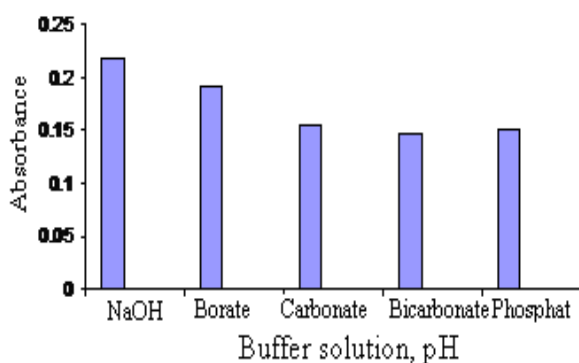


Figure 5. Effect of different buffer solutions on the absorption of $3 \mu\text{g mL}^{-1}$ OMZH complex with DDQ.

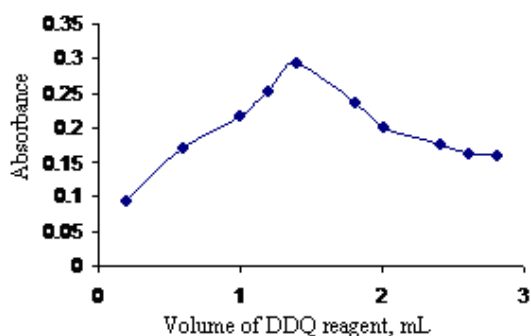


Figure 6. Effect of amount of DDQ reagent on the absorption of $3 \mu\text{g mL}^{-1}$ OMZH

Effect of the order of addition

In order to obtain the high color intensity, the order of the addition of reagents should be followed as given in the recommended procedure, otherwise a loss in color intensity was observed.

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in a thermostatically controlled water-bath at different temperatures up to 50°C . The absorbance was measured at 5 min intervals against reagent blank treated similarly. It was observed that the complex was formed after addition of DDQ reagent immediately at room temperature and remained constant for about 15 min after which the absorbance faded (Figure 7).

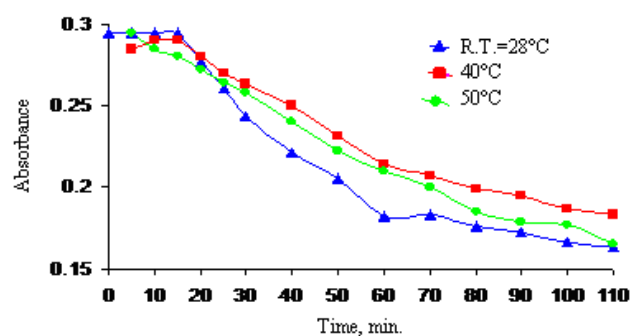


Figure 7. Effect of the time and temperature on the absorbance of $3 \mu\text{g mL}^{-1}$ OMZH.

Quantification

In order to investigate the range in which the colored complex adhere to Beer's law, the absorbance of the complex was measured at 421.79 nm after developing the colour by following the suggested procedure for a series of solutions containing increasing amounts of OMZH drug. The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in Table 2, which indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for the OMZH determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of five replicates of each three different concentrations of OMZH indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following eqns. (1) and (2).

$$\text{LOD} = 3.3\sigma/b \quad (1)$$

$$\text{LOQ} = 10\sigma/b \quad (2)$$

where σ is the standard deviation of five reagent blank determinations and b is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range, (Table 2).

Table 2. Summary of optical characteristics and statistical data for the recommended method.

Parameter	Value
Beer's law limits	0.5-3.2 $\mu\text{g mL}^{-1}$
Molar absorptivity	$2.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
LOD	0.0093 $\mu\text{g mL}^{-1}$
LOQ	0.028 $\mu\text{g mL}^{-1}$
Average recovery (%)**	99.488
Correlation coefficient	0.9825
Slope, <i>a</i>	0.0935
Intercept, <i>b</i>	0.0406
RSD**	≤ 1.47

* $Y = aX + b$, where *X* is the concentration of OMZH in $\mu\text{g mL}^{-1}$.

** Average of five determinations

Interference

The extent of interferences by some excipients which are often present in pharmaceutical preparations were studied by measuring the absorbance of solutions containing $3 \mu\text{g mL}^{-1}$ of OMZH and various amounts of diverse species in a final volume of 25 ml. It was found that the studied excipients do not interfere in the determination of OMZH in its dosage forms. An error of 5.0 % in the absorbance readings was considered tolerable. Typical results are given in Table 3.

Table 3. Effect of excipients on the assay of OMZH.

Excipient	Recovery (%) of OMZH ($3 \mu\text{g mL}^{-1}$) in the presence of excipients ($\mu\text{g mL}^{-1}$)			
	50	100	150	200
NaCl	95	96	98	105
Lactose	95	94	104	98
Glucose	105	108	103	99
Starch	103	95	96	95
Acacia	95	104	103	101

Analytical application

The proposed method was successfully applied to determine OMZH in its pharmaceutical preparation as nasal drops. The obtained results were compared statistically by a Student's *t*-test for accuracy with the official method¹ (depending on potentiometric titration for pure drug using 0.1 M perchloric acid) at the 95 % confidence level with three degrees of freedom. The results showed that the experimental *t*-test and *F*-test with three degrees of freedom are 1.37 and 4.31 respectively, which are less than the theoretical value ($t = 4.303$, $F = 9.28$), indicating that there was no significant difference between the proposed method and official method, (Table 4).

Stoichiometry, stability constant and reaction mechanism

The molar ratio of the complex formed between OMZH and DDQ reagent was investigated by applying the mole ratio and continuous variation (Job's) methods.¹⁸ The results indicated that complex was formed in the ratio of 1:2 OMZH : DDQ (Figure 8). This finding supports that the $n-\pi^*$ CT complex is formed through an interaction of hydroxyl and amino groups.^{19,20}

According to the results described above, the apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of each OMZH and DDQ (A_s) to one containing an excess of DDQ reagent (A_m). The average conditional stability constant of the complex was calculated by applying the following equations.

$$K_{st} = 1 - \alpha / 4\alpha^3 C^2 \quad (3)$$

$$\alpha = A_m - A_s / A_m \quad (4)$$

where K_{st} is the stability constant, α the dissociation degree and *C* is the concentration of the complex which is equal to the concentration of OMZH.

Table 4. Assay of OMZH in pharmaceutical nasal drops using the proposed method, standard addition procedure

Procedure applied	Name	Amount present ($\mu\text{g mL}^{-1}$)	Recovery (%)	Average recovery (%)*	Drug found (mg)	Stated value (mg)
Proposed method	Iliadin drops	1	101.6	99.48	2.32	2.5
		2	97.7			
		3	99.1			
Standard Addition method	Iliadin drop	1	102.0	100.5	2.51	2.5
		2	99.0			
British Pharmacopiea method			100.9	102.0	204	200
		200 mg	103.1			
			102.0			

The results shown in Table 5 indicate that the complex is stable.

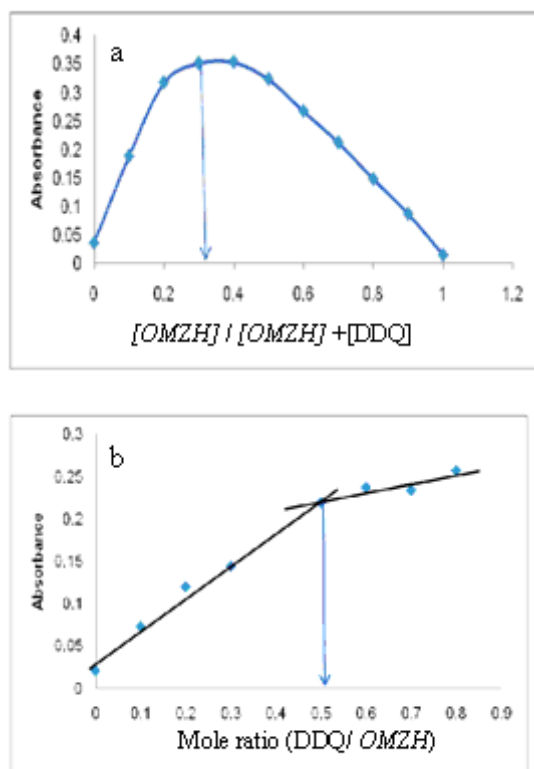


Figure 8. Continuous variation (a) and mole ratio (b) plots for OMZH-DDQ complex under the optimum conditions.

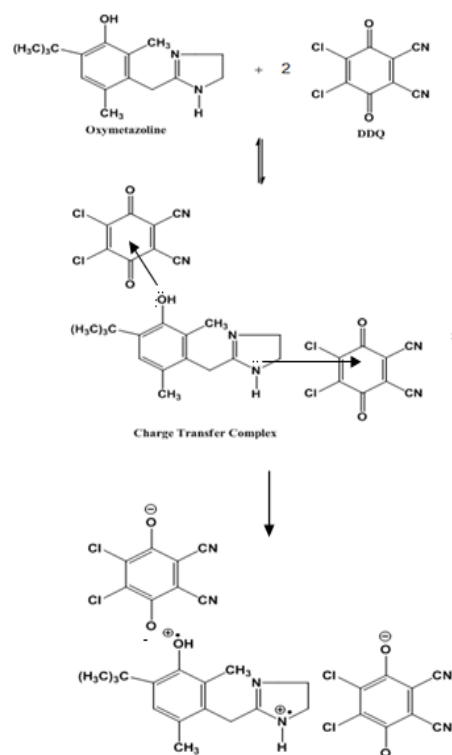
Table 5. Stability constant of OMZH-DDQ complex.

Volume, mL	Conc. mM	Absorbance		α	Average K_{st} , $L \text{ mol}^{-1}$
		A_s	A_m		
0.4	0.032	0.175	0.280	0.375	9.01×10^9
0.8	0.064	0.310	0.388	0.201	
1.2	0.096	0.355	0.399	0.110	

The interaction of OMZH, as n-donor with DDQ, as π -acceptor is a charge-transfer complexation, followed by the formation of a radical ions. Complete electron transfer from the donor to the acceptor moiety took place with the formation of intensely colored radical ions with high molar absorptivity values. However, the probable reaction mechanism is given in Scheme 1.

Comparison with the reported methods

Table 6 shows the comparison between some of analytical variables obtained from present method with that of the recent spectrophotometric methods. From the table, the proposed method is more sensitive than some other methods, simple, carry on at room temperature and take short time for color development.



Scheme 1. Proposed reaction mechanism for assay of OMZH

Table 6. Comparison of the present method with reported spectrophotometric methods.

Parameters	Methods		
	DDQ	Ferroin ¹²	4-Amino-antipyrine ¹⁶
λ_{max} , nm	420	510	480
pH	9.79	---	13.15
Solvent	water	water	water
Temp. °C	R.T.	70	70
Development time, min	5	40	25
Stability period, min	15	60	60
Beer's law range, $\mu\text{g}\cdot\text{ml}^{-1}$	0.5-3.0	0.1-7.0	1.0-20.0
Molar absorptivity, $L \text{ mol}^{-1} \text{ cm}^{-1}$	2.5×10^4	5.74×10^4	5.34×10^3
Recovery (%)	99.48	100.53	~ 99
RSD (%)	≤ 1.47	≤ 1.6	0.36-1.58
Type of reaction	Charge transfer	Oxidation-reduction	Oxidative coupling
Applications	Iliadin Drop 0.025%	Nazordin 0.05% Oxymet 0.025%	Nazordin 0.05% Oxymet 0.025%

Conclusion

The proposed method is simple and more sensitive than most of the previously reported spectrophotometric methods. The statistical parameters and the recovery test data indicate the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing *OMZH* showed no interference from common additives and auxiliary substances. Hence, this method can be considered for the determination of *OMZH* in both pure form and in pharmaceutical preparations.

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