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Development of Indirect Spectrophotometric Method for the Estimation of Oxymetazoline Hydrochloride as Pure Form and in its Pharmaceutical Preparations

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Abstract

This method involves the development of a highly sensitive spectrophotometric procedure for estimating oxymetazoline hydrochloride in pure form and pharmaceutical formulations. The method involved the oxidation of oxymetazoline hydrochloride with an excess amount of cerium sulfate ($CeSO_4$) in an acidic medium. The liberated cerium ion Ce(III) was then treated with arsenazo III reagent to form a colored complex with maximum absorption at 652 nm. The linearity of the standard calibration curve follows Beer's law in the concentration range of 1.25 to 12.5 µg/mL of oxymetazoline hydrochloride with a correlation coefficient of 0.9993 and molar absorptivity of 2.25×10^{4} $L.mol^{-1}.cm^{-1}$. The Sandell's sensitivity index was estimated and found to be 0.0147 $\mu g/cm^{2}$. The limit of detection (LOD) and limit of quantitation (LOQ) were also calculated and found to be 0.3036 and 0.9824 µg/mL, respectively. The precision (RSD%) and the error percentage were also estimated and found to be in the ranges of ±0.446 to ±0.659 and -1.03% to 0.58%, respectively. The recommended procedure has been applied to estimate oxymetazoline hydrochloride in obtainable dosage forms (drops and sprays).

Keywords: Oxymetazoline hydrochloride, Cerium ion (IV), Arseneazo III, Spectrophotometry

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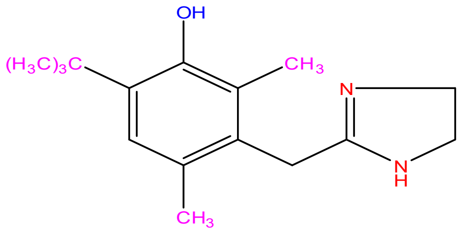
Introduction

Oxymetazoline hydrochloride (OXMH) Scheme 1. is a white or yellowish-white powder readily soluble in water and alcohol. It is chemically named [6-tert-butyl-3(2-imidazolin-2-ylmethyl)-2-4-dime-th-ylphenol monohydrochloride] [1].



Scheme 1. Structure of OXMH

OXMH is found in several nasal spray decongestants and ophthalmic drops. It is a topical decongestant that directly affects alpha-adrenergic receptors by constricting blood vessels supplying the nasal mucosa and conjunctiva, thereby producing vasoconstriction and decreasing congestion [2]. OXMH is used to treat eye redness and epistaxis because of minor irritation. It is preferable not to use oxymetazoline hydrochloride for more than three days, as it causes a return of congestion and swelling of the nose [3].

A literature survey revealed that various analytical techniques were reported for the quantitative estimation of OXMH in pharmaceutical forms such as, HPLC [4], potentiometric method using CeO_2 nanoparticles [5], flow injection analysis using a modified sensor unit via a green method of FI/MZ system [6], chemiluminescence [7], core-shell reversed-phase liquid chromatography [8], first derivative synchronous fluorescence spectroscopic (FDSFS) [9], ion-selective electrode [10], spectrofluorimetry using colloidal silver nanoparticles (AgNPs) [11] and electrochemical method using Titania (TiO_2) nanoparticles and carboxyl group functionalized multi-walled carbon nanotubes [12]. These methods require an experienced technician, expensive reagents, cultured instruments, apply derivatization reactions and several manipulation steps.

Several direct and indirect spectrophotometric methods have also been employed for assaying OXMH in bulk and in its pharmaceutical forms using various reagents such as potassium ferricyanide [13], metoclopramide [14] 1,10-phenanthroline-Fe(III) [15], oxidation reaction with potassium permanganate [16], 2,2-bipyridyl and ferric ion (III) [17], 2,4-dinitrophenylhydrazine [18], cerium sulfate (IV) [19], 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole [20], 4-aminoaceto-phenone [21], diazotized sulphanilic acid [22]. However, some of these methods suffer from some limitations, for instance, low sensitivity, low product stability, high temperatures, expensive materials, and the long time needed to complete the reaction. Others required heating, applicable to higher concentrations of OXMH.

In the present investigation, cerium sulphate was used to develop a simple, swift, and cheap visible spectrophotometric method for the assay of OXMH in pure form and in some pharmaceutical formulations.

Material and Methods

Chemical and reagents

High purity chemical materials and reagents were used in research paper experiments. The standard material of OXMH was provided by the drug industries , Samarra-Iraq.

Instruments

A professional Benchtop pH meter (BP3001) made in China was used to determine the acidity function (pH) and a double beam UV-visible spectrophotometer (JASCOV-630) with 1.0 cm quartz cells , made in Japan was utilized for absorption measurements.

Working standard solution of OXMH (100 µg/mL= 3.84×10^{-4} M): A 0.01 g of pure OXMH was dissolved in 5 mL of ethanol. The solution was then transferred to a volumetric flask of 100 mL and made to the mark with distilled water (DW).

Cerium ion [Ce(IV)] solution (5×10^{-4} M): In a small amount of 0.05 M sulphuric acid solution, 0.0166 g of cerium sulphate was dissolved, and the volume was then completed to 100 mL with DW.

ArsenazoIII (AZIII) solution (2×10^{-4} M): In about 10 mL of DW, 0.0155 g of AZ(III) reagent was dissolved, with the same solvent diluted to 100mL 25.5 mL of acetic acid (0.2M) and 24.5 mL of sodium acetate (0.2M) are combined to create an acetate buffer solution (pH 3.4). After being moved to a 100 mL volumetric flask, the solution was diluted by DW to the appropriate level [23].

Surfactant solutions (1% m/v): A 1.0 g of surfactant substance was prepared by dissolved in 100 mL of DW. Sulphuric acid (H_2SO_4) 0.05 M was also prepared by dissolving 2.4525 g in 500 mL of DW.

General Procedure

An aliquot of OXMH working standard solution (100 g/mL) containing 25-250 µg of OXMH was pipetted into a series of 20 mL volumetric flasks. To each flask, 0.6 mL of 0.05 M sulphuric acid and 5.0 mL of 5×10^{-4} M Ce^{+4} ion solution were added and mixed well. Solution was kept for 10 minutes at ambient temperature (25 ^{o}C)and 0.3 mL of acetate buffer solution (pH=3.4) was added, the solutions were mixed well, then, 2.5 mL of 2×10^{-4} M AZ(III) reagent was added and shaken thoroughly. The volumes of all solutions in volumetric flask were made upto the mark with DW and kept for for 10 minutes before using, and the absorbance was measured against the blank reagent solution at wavelength 652 nm.

Analysis of OXMH in its drugs

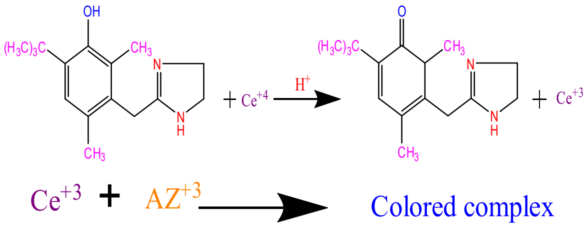
Oxymet drops solution (100 µg/mL). The containers of three bottles of oxymet drops 0.05% were mixed and shaken well. A 20 mL of the drug OXMH solution was diluted to 100 mL with DW using a volumetric flask.

Nasaclearsprayer 0.05% solution (100 µg/mL). This solution was also prepared in the same way as oxymet drops.

Results and Discussion

The Principle of the Proposed Reaction

The proposed reaction includes two steps. The first step is reducing Ce(IV) ions with OXMH in an acidic medium to liberate Ce(III) ions, as shown in the equation shown in Scheme 2. In the second step, the resulting Ce(III) reacts with an excess of AZ(III) reagent to produce a colored complex that is soluble in water and exhibits maximum absorption at wavelength 652 nm.



Scheme 2. Proposed reaction equations

Optimum Reaction Conditions

Effect of pH

The effect of pH on the color intensity of [Ce(III)-AZ(III)] is investigated. in Fig.1, The results reveal that the optimum pH range for the complex formation was 3.4 at wavelength 652 nm.

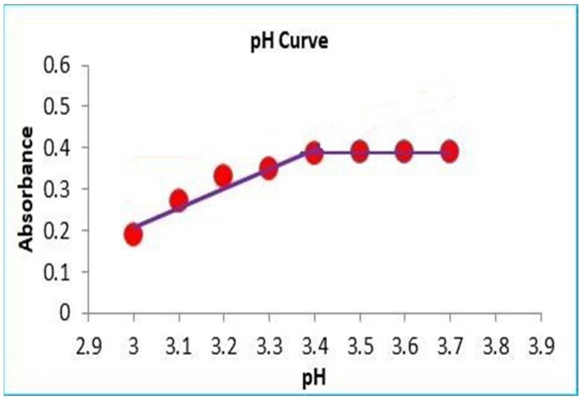


Figure 1. **Effect of pH on the absorbance of the complex**

Therefore, various buffer solutions of pH 3.4 were prepared [23], and their efficiencies and effects on the absorbance of the complex formed [Ce(III)-AZ(III)] were examined. The experimental results indicated that the acetate buffer solution at pH 3.4 was the best due to its maximum absorbance and clear color contrast.

The influence of different amounts of the acetate buffer solution (pH 3.4) was also investigated. The results in Fig. 2 show that 0.3 mL of the acetate buffer solution was the optimum. Therefore, it has been selected for the next experiments.

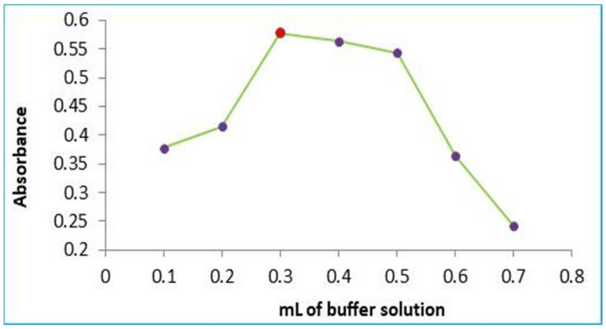


Figure 2. **Effect of the amount of acetate buffer solution pH 3.4 on absorbance**

Effect of Oxidizing Agent Ce(IV) Amount

The influence of different amounts of 1.0 - 5.0 mL of the oxidizing agent Ce(IV) with a concentration of 5×10^{-4} M on the formation of the Ce(III)-AZ(III) complex was investigated (Table 1). The results listed indicated that the volume of 5 mL of 5×10^{-4} M Ce(IV) reagent solution was optimal because it has high sensitivity and a good determination coefficient (R^2 = 0.9992). Therefore, 5 mL of Ce(IV) solution was chosen for the subsequent experiments.

Table 1. **Effect of the amount of the oxidizing agent Ce(IV) on absorbance.**

mL of Ce(IV) solution (5×10^{-4} M)	Absorbance/μg of OXMH added					R^2
	30	50	100	150	200	
1.0	0.0532	0.0783	0.1832	0.2481	0.3132	0.9897
2.0	0.0583	0.0933	0.2260	0.3343	0.4883	0.9957
3.0	0.0661	0.1191	0.2861	0.3952	0.5271	0.9918
4.0	0.0882	0.1622	0.3342	0.4533	0.5961	0.9921
5.0	0.1122	0.2010	0.4091	0.5921	0.7872	**0.9992**
6.0	0.1014	0.1941	0.4031	0.5780	0.7832	0.9987

Effect of the Oxidation Time and Temperature

The influence of time and temperature (5 - 70 ºC) on the oxidation OXMH with oxidizing agent Ce^{+4} was studied and the results are shown in Fig. 3.

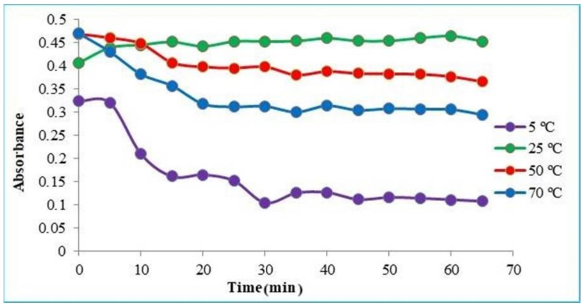


Figure 3. **Effect of the oxidation time and temperature on absorbance**

In the above figure, 10 minutes at room temperature (25±2 °C) were chosen to complete the oxidation process before adding the AZ(III) reagent. The resulting complex was highly sensitive at this time of oxidation.

Effect of Amount of AZ(III) Reagent

The absorbance of the Ce(III)-AZ(III) complex was investigated using different amounts of 0.5-5.0 mL of $2\text{x}10^{-4}$ M AZ(III) reagent. The experimental results in Fig. 4 indicate that 2.5 mL of $2\text{x}10^{-4}$ M AZ(III) reagent solution can be considered the optimum because it gives a good color contrast. Hence, they adopted it within the working procedure.

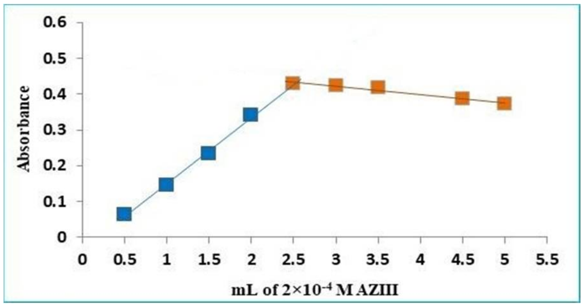


Figure 4. **Effect of AZ(III) reagent amount on absorbance**

Effect of Surfactants

To increase the sensitivity of the method, the effect of different amounts (0.5–2.0 mL) of various surfactant solutions, including cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), sodium dodecyl sulphate (SDS), and triton X-100 [24], on the absorbance of the colored complex, has been studied. The results in Table 2 show that the addition of surfactant solutions causes no effect on the absorbance values of the colored product, so the substances were excluded.

Table 2. **Effect of surfactants on absorbance.**

Surfactant solution*	**Absorbance / mL of surfactant added**				
(1%)	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**
Triton X-100	0.4282	0.4081	0.4054	0.4072	0.4004
SDS		0.3983	0.3991	0.3922	0.3841
CTAB		0.3911	0.3901	0.3893	0.3864
CPC		0.4014	0.3912	0.3944	0.3802

* Triton $_X$-100:Isoocytylphenoxypolyethoxyethanol

Effect of Temperature and Time on the Formation of Complex

The influence on the formation [Ce(III)-AZ(III)] and its stability was investigated. The reaction was carried out at different temperatures 5, 25, 50, and 70 °C using a water bath with other periods. Table 2 shows the results achieved. The results in Fig. 5 illustrate that when the reaction was conducted at temperatures 5, 50, and 70 C°, as time increased, observable unstable results in the complex's absorbance values were seen. While at 25 °C (room temperature), the absorbance values showed good stability, therefore, the room temperature was adopted in subsequent experiments.

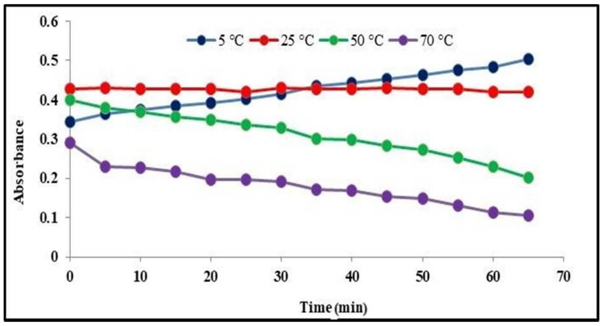


Figure 5. **Effect of time and temperature on the absorption of the complex formed**

Sequence of Additions

The order of additions in the reaction was investigated, and the experimental results in Table 3 show that the (order No. I) was the best and thus chosen for the following experiments at wavelength 651 nm.

Table 3. **Effect of Sequence of additions on absorbance.**

Order No.	**Sequence of additions***	**Absorbance**
I	OXMH + H^+ + Ce(IV) + B + AZ(III)	0.4253
II	OXMH + Ce(IV) + H^+ + B + AZ(III)	0.4061
III	Ce(IV) + OXMH + H^+ + B + AZ(III)	0.384
IV	H^++ Ce(IV)+ OXMH + B + AZ(III)	0.3216

*Hydrochloric acid (H^+), Buffer solution pH 3.4 (B)

Effect of excipients (Interferences)

The effect of excipients (interfering) was studied and it was found that they had no significant effect on the proposed method, as shown in Table 4, by calculating the relative error.

Table 4. Effect of foreign compounds for assay of OXMH.

Interference	**Error (%) of 10 µg /mLOXMH / µg of Interference added**		
	50	**100**	**200**
Starch	-0.64	-0.98	-1.56
Glucose	-0.39	-2.73	-2.56
Arabic gum	-0.59	0.03	0.01
Sucrose	-0.38	0.78	-0.69
Lactose	-0.63	0.11	-0.38

Absorption Spectrum

On using an acetate buffer solution of pH 3.4, the liberated Ce(III) reaction with the AZ(III) reagent produces a colored complex under ideal conditions. The producing complex shows maximum absorption at 652 nm versus the blank solution (Fig. 6).

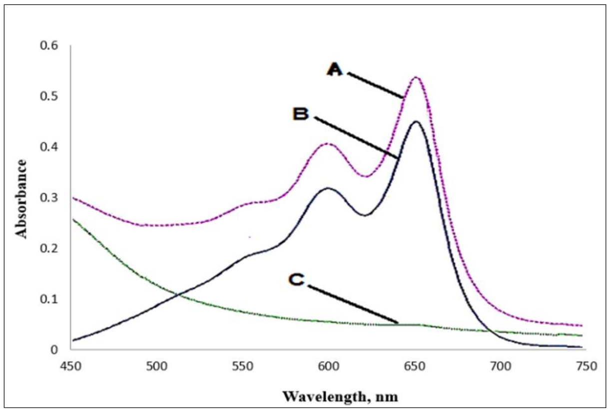


Figure 6. **Absorption spectrum of 5 µg/mLOXMH against DW (A), blank (B), and blank Vs. DW (C).**

Reproducibility and Validity of Beer's Law

A linear calibration graph had been established that obeyed Beer's law throughout the concentration range between 1.25-12.5 µg/mL of OXMH with determination coefficient (R^2=0.9993) (Fig. 7). The molar absorptivity value and Sandell's sensitivity index were 2.25×10^4 L/mol/cm and 0.0147 µg/cm^2, respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated and found to be 0.3036 and 0.9824 µg/mL, respectively.

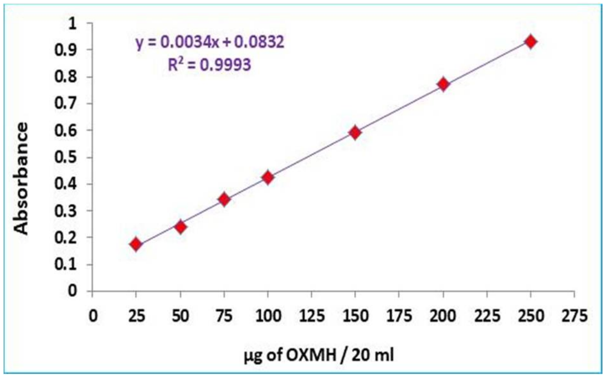


Figure 7. **Calibration curve of OXMH estimation**

Accuracy and Precision

The accuracy and precision of the proposed method were studied using two different amounts (100 and 200 µg) of OXMH and the results are illustrated in Table 5.

Table 5.**Accuracy and precision of the method.**

Amount of OXMH µg/20 mL present	Amount of OXMH µg/20 mL found	Recov-ery (%)*	relative standard deviation (RSD%)*
100	98.97	98.97	±0.659
200	201.16	100.58	±0.446

***Average of five estimations**

The results in Table 4 indicate that the proposed method has good accuracy and precision.

Application

The recommended procedure has been successfully applied to determine the drug in the available pharmaceutical preparations (drops and sprays). Calculating a high recall rate, it is clear from Table 6 that the method gives good accuracy and acceptable repeatability.

Table 6. Determination of OXMH in drops and sprays.

Drug	OXMH Present (µg)	OXMH Found* (µg)	Recovery (%)*	RSD* %	t-exp.*
Oxymet drops 0.05% (Egypt)	50	50.40	100.80	0.87	-
	70	70.66	100.94	1.21	-
	100	101.26	101.26	1.05	1.609
Nasaclear sprayer 0.05% (Syria)	50	49.34	98.80	1.03	-
	70	69.86	99.66	0.62	-
	100	97.26	97.26	0.75	1.927

***Average of four estimations,** $\#t\pm=(\bar{x}-\mu)\frac{\sqrt{N}}{s}$

According to Table 5's findings, all samples' experimental "t-test" values were lower than the calculated "t-test" value for three degrees of freedom and at the 95% confidence level, which was 3.182. [25-26]. The results of the t-test proved that the developed method is suitable for assaying OXMH in pharmaceutical forms (drop and spray) with acceptable results.

Evaluation of the Proposed Procedure

The standard additions method was used to test the selectivity of the proposed method and its success in estimating OXMH in its pharmaceutical preparations. The method was based on the addition of a fixed amount of Oxymet drops and Nasaclear sprayer 0.05% solutions (100 µg/mL) separately to a series of 20 mL volumetric flasks containing an increasing amount of 0–150 µg of OXMH working standard solution. The results are illustrated in Fig. 8 and Table 7 shows that the traditional adds approach for determining OXMH correlates well with the results of the suggested method within an acceptable margin of error.

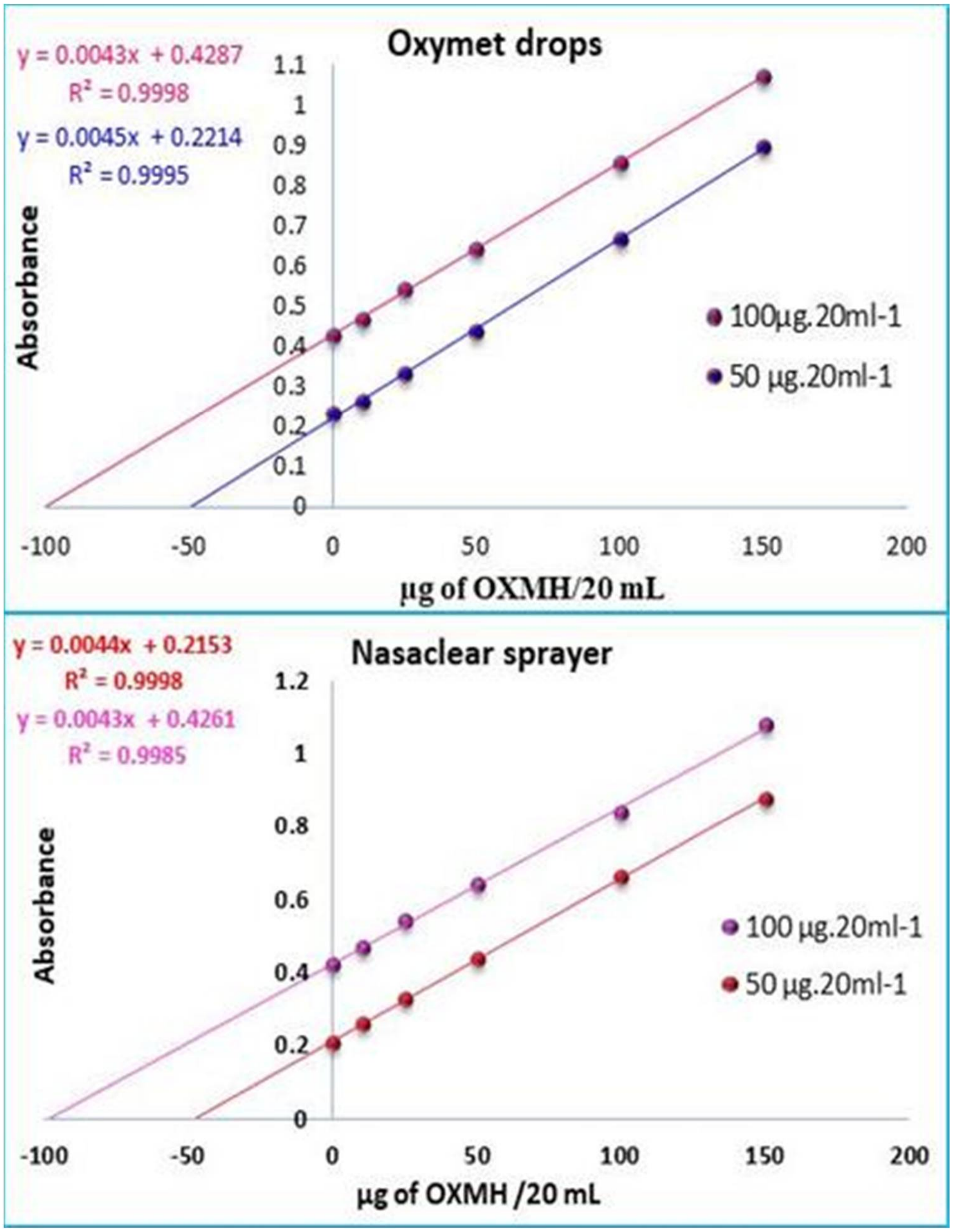


Figure 8. **Calibration graphs of the standard addition method for assaying OXMH in Oximet drops and Nasaclear sprays**

***Table 7.* The results of standard additions method for assay of OXMH.**

Drug	OXMH Present (µg)	OXMH Measured (µg)	Recovery (%)
Oxymet drops 0.05% (Egypt)	50	49.2	98.4
	100	99.69	99.69
Nasaclearsprayer 0.05% (Syria)	50	48.93	97.86
	100	99.09	99.09

Comparison of the Proposed Method

For the purpose of ensuring the accuracy and sensitivity of the method, a comparison was made with previous methods as shown in Table 8. The results showed that the current method was good.

***Table 8.* Comparison of some analytical spectroscopic variables of the proposed method with other spectrophotometric methods.**

Parameters	Present method	Literature method [14]	Literature method [15]	Literature method [22]
Type of reaction	Oxidation and Reduction	Diazo coupling	Oxidation and Reduction	Diazo coupling
Reagent	Arsenazo III	Metoclopramide	1,10-Phenanthroline	Diazotisedsulphanilic acid
λ_{max} (nm)	652	510	510	496
Medium of reaction	Acidic	Basic	Acidic	Basic
Temperature (°C)	At room temperature	97 °C	70 °C	At room temperature
Beer's law range (µg.mL^{-1})	1.25-12.5	0.05-17	0.1-7	0.8-16
Molar absorptivity (l.mol^{-1}.cm^{-1})	2.25×10^{4}	2.7×10^{4}	5.74×10^{4}	2.30×10^{4}
RSD (%) range	0.62 – 1.21	1.78 – 5.34	0.72-1.6	-
Recovery (%) range	97.26 – 101.26	98.95 – 105.75	97.43 – 103.08	97.6 – 105.2
Relative error (%) range	- 2.74 to 1.26	-0.91 to 0.73	-	- 2.4 to 5.2
Sandell's sensitivity (µg.cm^{-2})	0.0147	9.6×10^{-3}	0.005	-
LOD (µg.mL^{-1})	0.3036	0.047	-	-
LOQ (µg.mL^{-1})	0.9824	0.158	-	-
Application of the method	Nasal drops and sprays	Nasal drops	Nasal drops	Nasal drops

Conclusion

A sensitive and selective indirect spectrophotometric approach has been developed for the analysis of OXMH in bulk and its pharmaceutical forms. The proposed approach is based on the reduction of Ce(IV) ion with OXMH drug to produce Ce(III) ion in an acid medium. The producing Ce(III) ion is then combined with AZ(III) reagent at pH 3.4 to form a colored complex that exhibited maximum absorption at a wavelength of 652 nm and obeyed Beer's law within the concentration range 1.25-12.5 µg/mL. The molar absorptivity, recovery percentage range, and RSD% range are calculated and found to be 2.25×10^4 $\mathrm{l.mol^{-1}.cm^{-1}}$, 97.26% − 101.26 % and 0.62% – 1.21%, respectively. The proposed procedure is simple, fast, and can be mainly used for drug quality control.

Conflict of Interest

The authors declare that they have no conflict of interest

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