------ Raf. J. Sci., Vol. 24, No.1 pp 84-96, 2013------

Indirect Spectrophotometric Determination of Oxymetazoline Hydrochloride

Nabeel S. Othman **Sahar A. Fathe**

Department of Chemistry
College of Science
University of Mosul

nsn20002004@yahoo.com sahar09a@yahoo.com

(Received 7/ 5 / 2012 ; Accepted 18 / 7 / 2012)

ABSTRACT

A simple and sensitive spectrophotometric method for the quantitative determination of trace amounts of oxymetazoline hydrochloride (OMZ) was developed .The method based on the reaction of (OMZ) with Fe(III) in acidic medium.The librated Fe(II) coupled with potassium ferricyanide, the reaction needs heating for 25 minutes at 50 ^{o} C, to form Prussian blue which is more stable by adding 1ml of 1% ethylendiaminetetraacetic acid. The maximum absorption of the product was measured at 766 nm .Beer's law is obeyed in the concentration range 5-125 µg/10ml with a molar absorptivity of $5.07x10^{4}$ l. $mol^{-1}.cm^{-1}$,with a relative error of +1.74 to +2.34 % and a relative standard deviation (RSD%) of the of ±1.272 to ±1.982 %.The method was applied successfully for the assay of OMZ in pharmaceutical preparation.

Keywords: Oxymetazoline hydrochloride; oxidation-reduction; spectrophotometry.

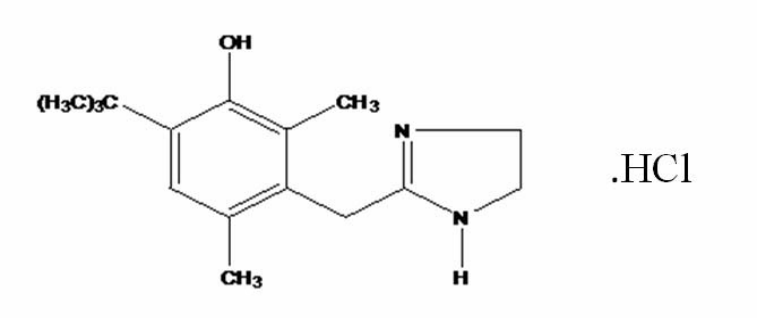
التقدير الطيفي غير المباشر لهيدروكلوريد الاوكسي ميتازولين

الملخص

تم تطوير طريقة طيفية بسيطة وحساسة للتقدير الكمي لكميات نزرة من هيدروكلوريد الاوكسي ميتازولين معتمدا على أكسدة هيدروكلوريد الاوكسي ميتازولين باستخدام الحديد الثلاثي في وسط حامضي. إذ يختزل الحديد الثلاثي بواسطة هيدروكلوريد الاوكسي ميتازولين إلى الحديد الثنائي والذي يقترن بدوره مع بوتاسيوم سيانيد الحديديك بالتسخين لفترة 25 دقيقة بدرجة 50 درجة مئوية لتكوين صبغة بروسيان. وتم الحصول على حساسية أعلى واستقرارية أفضل عند الطول ألموجي 766 نانوميتر بإضافة محلول اثيلين ثنائي الامين رباعي حامض الخليك (1%). كانت حدود تطبيق قانون بيير في مدى التركيز من 5 إلى 125 مايكرو غرام في حجم نهائي 10 مللتر وقيمة الامتصاصية المولارية 10^{4}x5.07 لتر. مول$^{-1}$.سم$^{-1}$ وتراوح الخطأ النسبي للمنحي القياسي بين 1.74+ و 2.34+ % وقيمة الانحراف القياسي النسبي بين ±1.272 و ±1.982 % اعتمادا على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير هيدروكلوريد الاوكسي ميتازولين في مستحضر دوائي.

INTRODUCTION

Oxymetazoline hydrochloride(OMZ) is 4-tert- butyl 2,6-dimethyl -3-hydroxy –benzyl-imidazoline hydrochloride (USP, 2009) (I).



(I) Oxymetazoline hydrochloride
M.wt.=296.8 g.mol^{-1}

OMZ is available as a topical decongestant in nasal spray, it is also used to treat epistaxis and eye redness (Wikipedia, 2008).

Different methods have been used for the spectrophotometric determination of oxymetazoline hydrochloride.

Different reagents have been used in the determination OMZ as a pure and in dosage forms such as: sodium cobalti nitrite in acetic acid medium (Shingbal *et al.*, 1983), 2,6-dichloroquinonchlorimide the method based on the formation of a coloured oxidative coupling product (Sankar *et al*., 1987), Fe (III) and 2,4,6-tris(2-pyridyl)-5-triazine (Sankar *et.al*,1988). The formation of ferrion complex which is measured at 510 nm. is produced by oxidation – reduction reaction between OMZ and ferric ion, (AL-Sabha and Rashed, 2011), 4-Aminoantipyrine in presence of potassium periodate in oxidative coupling reaction (Zakaria,2011), 3,5-dinitrosalicylicacid (AL-Neaimy, 2006).

The construction and analytical application of plastic membrane electrode (ion selective electrode) for the determination of OMZ was applied (Issa and Zayed, 2004), also a flow injection analysis of OMZ with inhibited chemiluminscent detection (Garcia *et al*., 2004; Wang *et al*., 2009)was also reported.The chromatographic method, high performance liquid chromatography technique has been used in the determination of OMZ (Sudsakorn *et al*., 2006; Hong, 2009; Hoffmaann,1989; Hayes,1995; Stanisz and Nowinki, 2000).

The British pharmacopoeia reported a potentiometric method using perchloric acid solution for the determination of OMZ (British Pharmacopeia, 2007)

The objective of the investigation reported in this paper is to evaluate a sensitive and accurate method for the assay of oxymetazoline in a pure form or in dosage forms. The method was based on the oxidation of OMZ using Fe (III) in acidic medium and the librated Fe (II) which subsequently complexes with potassium ferricyanide to form the Prussian blue complex.

EXPERIMENTAL

Instruments

Spectrophotometric measurements are performed using Shimadzu UV-160 UV-Visible recording spectrophotometer using 1.0cm quartz cells. The pH measurements are performed by using pH meter type HANNA 211 pH-ion meter. Waterbath (Memmert, Germany) has also been used.

Reagents

All chemicals used in this investigation are of analytical grade. Oxymetazoline hydrochloride standard material was provided from state company for Drug Industries and Medical Appliance (N.D.I), Ninavah-Iraq.

Working OMZ solution (50 µg.ml^{-1})

This solution is prepared by dissolving 0.005 g of OMZ in distilled water and the volume is completed to the mark with distilled water in 100ml volumetric flask.

Ferric chloride solution, 0.1%

This solution is prepared by dissolving 0.1 g of ferric chloride (Fluka) in distilled water and completed with distilled water to the mark in a 100ml volumetric flask.

Potassium ferricyanide ,0.1%

This solution is prepared by dissolving 0.1 g of potassium ferricyanide in distilled water and completed with distilled water to the mark in a 100ml volumetric flask .

Recommended procedure and calibration curve

To a series of 10ml calibrated flasks ,0.1 to 2.5 ml of 50 µg/ml OMZ solution were added followed by the addition of 3 ml of potassium ferricyaninde (0.1%), 3ml of Fe (III) (0.1%) and 0.5ml (1M) acetic acid were added. Then the solution stood for 25 minutes in water bath adjusted at 50^{o}C,then 1ml of EDTA(1%) was added and diluted to the mark with distilled water. The absorbance was measured at 766 nm against the reagent blank.

The calibration graph is linear over the concentration range of 5-125 µg /10 ml while higher concentrations show a negative deviation from Beer's law (Fig. 1), the apparent molar absorptivity referred to OMZ has been found to be 5.07x10^{4} l.mol^{-1}.cm^{-1}

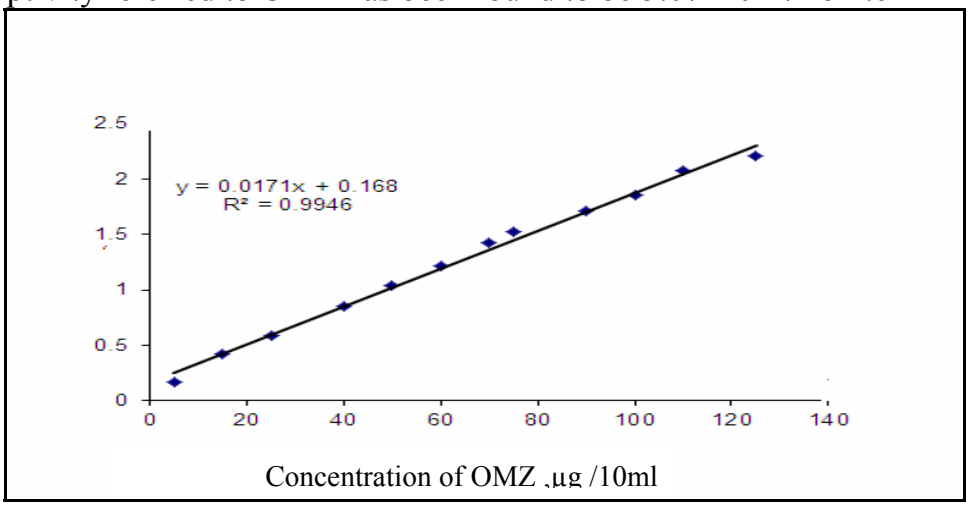


Fig.1: Calibration graph for OMZ determination using the proposed method.

Determination of OMZ in nasal drop

The contents of 3 containers of Oxymet drops were mixed well, a 5 ml which is equivalent to 0.0025 g OMZ was diluted to 50 ml with distilled water in a volumetric flask. A suitable aliquot of drug solution was analyzed as described in the recommended procedure.

RESULTS AND DISCUSSION

The effect of various variables on the color development of 12.5-100µg OMZ/10ml reacted with ferric chloride (0.1%) and potassium ferricyanide reagent (0.1%) in acidic medium was tested to establish the optimum conditions for the determination of OMZ by Prussian blue complex formation reaction.

Effect of the amount of ferric chloride

Increasing volumes of ferric chloride solution (0.1%) were added to the standard amounts of OMZ, the results indicated that 3 ml of ferric chloride solution gave the highest intensity of colored product at 720 nm, and this volume was considered as an optimum (Table 1).

Table 1: Effect of ferric chloride amount on absorbance

ml of Fe^{+3}(0.1%)	Absorbance/µg OMZ used					
	12.5	25	50	75	100	R^2
1	0.065	0.112	0.214	0.280	0.66	0.9951
2	0.073	0.139	0.260	0.377	0.473	0.9964
3	0.078	0.146	0.281	0.394	0.550	0.9974
4	0.057	0.125	0.242	0.377	0.506	0.9993

Effect of the amount of potassium ferricyanide

Various volumes of potassium ferricyanide solution (R) (0.1%) were added. The results indicated that using 3 ml of this solution gave maximum absorbance of colored product at 720 nm, and this volume was recommended in the subsequent experiments (Table 2).

Table 2: Effect of potassium ferricyanide amount on absorbance.

ml of R(0.1%)	Absorbance/µg OMZ used					
	12.5	25	50	75	100	R^2
1	0.057	0.119	0.258	0.328	0.497	0.9995
2	0.075	0.140	0.289	0.429	0.561	0.9995
3	0.071	0.144	0.290	0.442	0.576	0.9996
4	0.061	0.142	0.280	0.429	0.563	0.9995

Choice of acid and its amount

The effect of different acids(1M) on the absorbance of the colored product was studied. The results indicated that 0.5ml of 1M acetic acid solution (pH=2.70 in final dilution) was the optimum and recommended in the subsequent experiments according to the highest intensity of the colored product (Table 3).

Table 3: Selection of acid and its optimum volume

Type of acid, 1M solution	Absorbance /ml acid used					Final pH
	0.25	0.5	1	2	3	
CH_3COOH	0.304	0.320	0.280	0.246	0.259	2.88- 2.41
HCl	0.193	0.204	0.115	0.038	0.024	1.35- 0.65
H_2SO_4	0.029	0.034	0.014	-0.003	-0.22	1.28- 0.65
HNO_3	0.171	0.180	0.105	0.041	0.027	1.27- 0.57

Effect of buffer solution on absorbance and stability.

According to the optimum pH found in Table 3,different types of buffers of pH 2.70 have been tested. The results shown in Table 4 indicated that all these types of the buffer solutions used decrease the absorbance or forming a turbid solution, so that the use of a buffer solution is not recommended.

Table 4: Effect of buffers

Buffer (2 ml used)	Absorbance	pH
Glycine-HCl	0.335	2.71
KHphathalate	Turbid	2.70
Citric acid-NaOH	-0.027	2.75

Note: The absorbance using acetic acid =0.339

Effect of temperature

The rate of reaction is observed to be slow when carried out at room temperature. However, enhanced reaction rate is observed when the reaction mixture is heated. The effect of temperature on the absorbance of the final reaction mixture is shown in (Table 5).

Table 5: Effect of temperature on absorbance

Temp., ^oC	0	10	15	17	20	30	40	50	60
Absorbance	0.255	0.281	0.292	0.330	0.338	0.378	0.481	0.609	0.590

The results indicated that absorbance of the product increased with increasing temperature and heating to 50^oC was considered as an optimum temperature.

Effect of heating time

The time needed to complete the reaction had been studied at 50^oC. The results in Table 6 showed that a maximum intensity occurred at 25 minutes heating before the dilution of the solution with distilled water to the mark.

Table 6: Effect of heating time on absorbance

Time, min.	5	10	15	20	25	30
Absorbance	0.437	0.509	0.593	0.669	0.700	0.700

Effect of surfactant

The effect of different types of surfactants (positive, negative and neutral) on the color intensity was studied. The results showed that using Triton X-100 decreases the absorbance of the colored product whereas CPC gives a turbid solution and using SDS increases the absorbance but the solution becomes turbid after heating to 50^0C. Therefore, it has been recommended to eliminate the use of surfactants in the subsequent experiments.

The stability of product

The stability of the formed colored product was investigated under the optimum conditions for the determination of OMZ and compared with the stability of the formed colored complex after adding 1ml of NaF (1%) and EDTA(1%) (Table 7).

Table 7 : Effect of time on color stability.

Time/ min	Absorbance* of 50 µg	Absorbance** (EDTA,1%)	Absorbance*** (NaF, 1%)
0	0.686	1.143	0.872
5	0.687	1.140	0.867
10	0.683	1.140	0.869
15	0.678	1.140	0.867
20	0.678	1.140	0.867
25	0.673	1.141	0.870
30	0.672	1.141	0.876
35	0.667	1.142	0.883
40	0.671	1.142	0.890
45	0.665	1.138	0.891
50	0.658	1.138	0.894
60	0.656	1.138	0.899
$B_{\lambda max}$	417	401	420
$S_{\lambda max}$	794	762	722

*Absorbance without adding EDTA or NaF solutions
**Absorbance with 1ml EDTA solution (1%)
***Absorbance with 1ml NaF solution(1%)

The results indicated that adding EDTA or NaF solutions which act as masking agent for the unreacted amount of ferric ions causes an increase in the absorbance and also the resulting product becomes more stable, so that adding EDTA solution is recommend for the subsequent experiments with an optimum volume of 1ml (Table 8) and (Fig. 2)

Table 8: Effect of EDTA amount on stability.

Ml of EDTA(1%)	0.5	1	2
Absorbance	1.109	1.150	1.123
λ_{max}	762	761	765

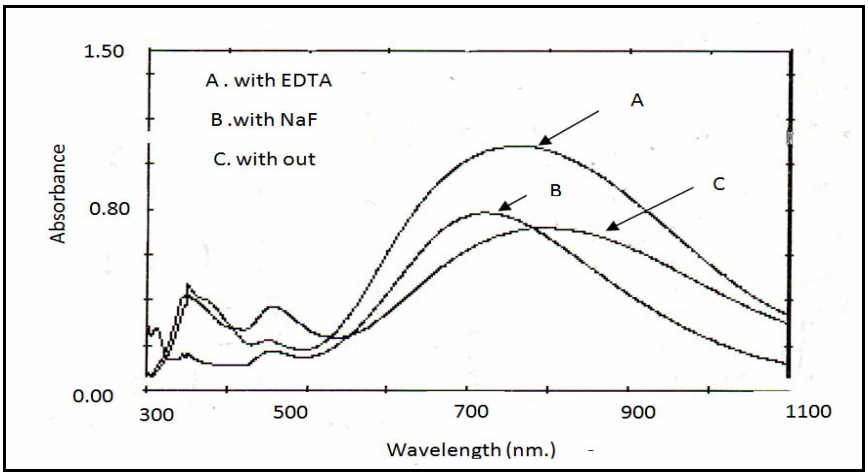


Fig. 2: The effect of EDTA and NaF on absorbance

Absorption spectra

The absorption spectra of the formed product showed a maximum absorption at 766 nm, against the corresponding reagent blank (Fig 3).

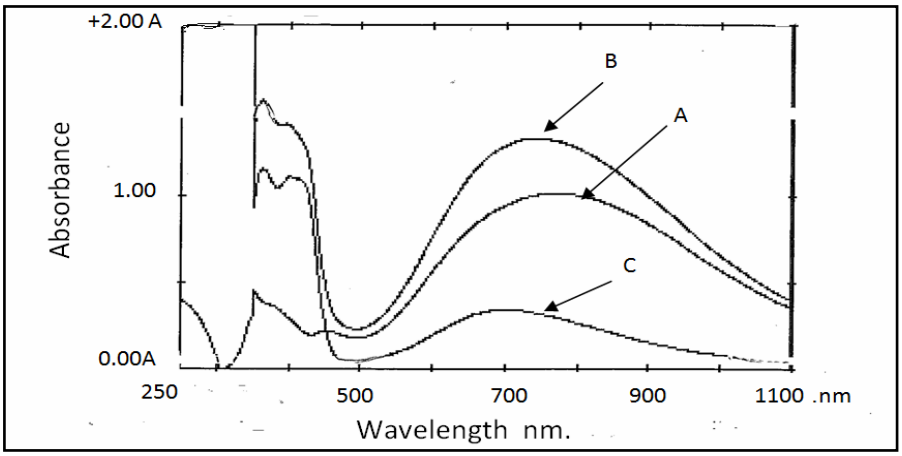


Fig. 3: Absorption spectra of 50µg OMZ treated according to the recommended procedure and measured against (A) blank,(B) distilled water and (C) blank measured against distilled water.

Accuracy and precision

To check the accuracy and precision of the calibration curve, OMZ was determined at two different concentrations. The results shown in Table 9 indicated that the results are satisfactory.

Table 9: Accuracy and precision of the method.

OMZ µg, taken	Relative error, %	Relative standard deviation,%	Recovery,%*
25	+ 1.74	±1.25%	98.25%
50	+ 2.34	±1.98%	97.65%

*Average of six determinations

The nature of the reaction product

Job's and mole-ratio methods (Delevie, 1997) have been used in determination of reaction ratio of OMZ with Fe(III). The obtained results (Fig. 4) and (Fig. 5) showed that a 1:3 OMZ to Fe (III) ratio is obtained.

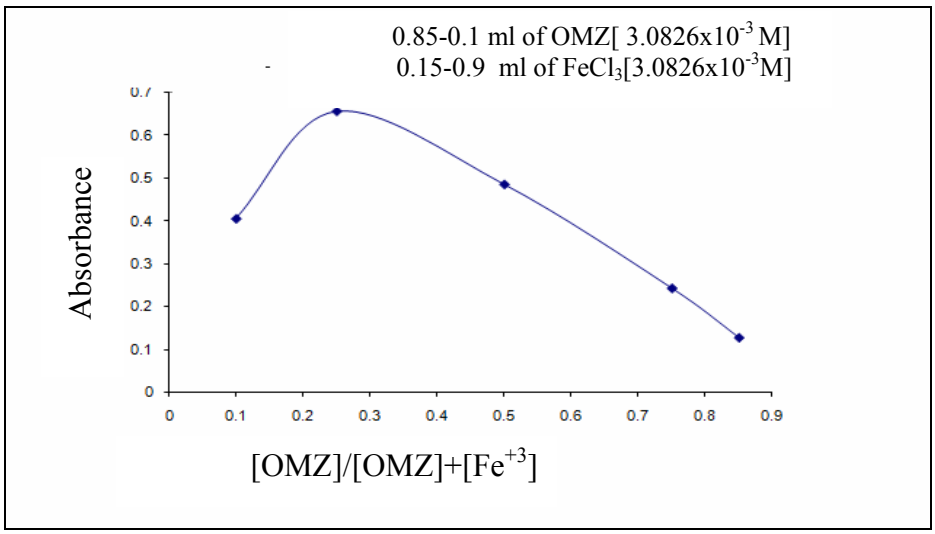


Fig. 4 : Job's plot for OMZ-Fe(III)

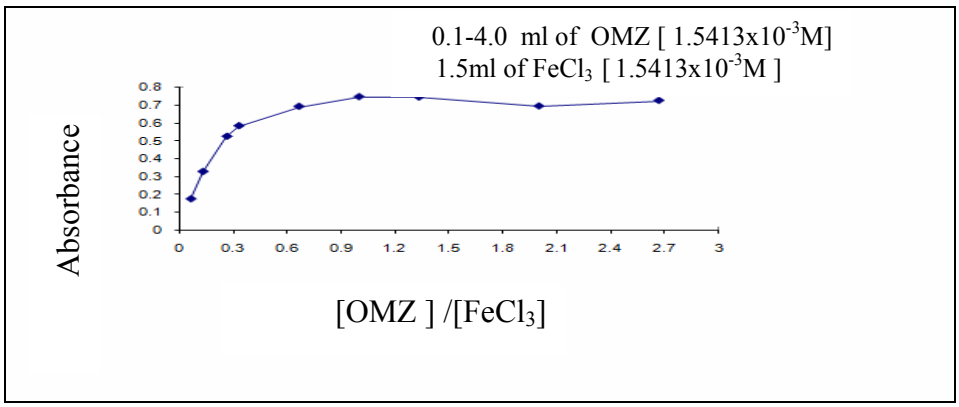
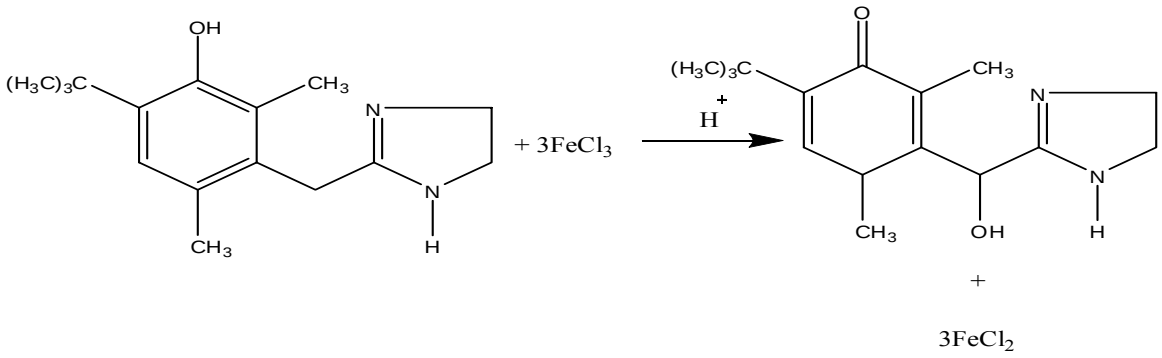


Fig. 5: Mole ratio plot for OMZ-Fe(III)

The probable mechanism of the reaction may be according to the following:



The ferrous ion produced from the above reaction was reacted with potassium ferricyanide to form the well-known product (KFe [$Fe(CN)_6$], Prussian blue) as in the following equation.

$$FeCl_2 + K_3Fe(CN)_6 \longrightarrow KFe[Fe(CN)_6] + 2KCl$$

Effect of dilution with organic solvents

Different organic solvents are examined in the dilution of the solution to evaluate their effects on the absorbance of the resulting colored complex and the data are shown in Table (10)

Table 10: Effect of dilution with organic solvents on the absorbance.

Solvent	λ_{max}	Absorbance
Distilled water	745	1.082
Methanol	Turbid	…….
Ethanol, absolute	Turbid	…….
N -propanol	745	1.082
Acetone	745	1.058
Acetic acid	745	1.053
Formic acid	755	1.024
DMF	762	1.095

Water is chosen in the subsequent experiments due to its availability, non-toxicity as well as from the sensitivity point of view.

Effect of interferences

In order to test the efficiency and selectivity of the proposed method, the effect of some foreign substances (e.g. gum acacia, glucose, lactose and starch) that usually present in dosage forms were studied by adding different amounts of foreign substances to 50 µg OMZ/10ml.

Table 11: Effect of additive and Excipients on determination of 50µgOMZ.

Excipient	Recovery (%) of 50µgOMZ in the presence of excipients added		
	100	500	1000
Gum acacia	127.13	151.39	149.7
Lactose monohydrate	98.25	94.15	89.52
Glucose	109.2	102.6	109.2
Starch	100.1	96.1	97.0

The results indicated that gum acacia and lactose monohydrate interference at high concentration in the determination of OMZ.

Application of the method

The proposed method was successfully applied in the determination of oxymetazoline hydrochloride in pharmaceutical preparation (nasal drops), the results which are shown in Table 12 indicate that good recoveries are obtained.

Table 12: Analytical application.

Drug	µg OMZ/10ml	Recovery*%	RSD%
Oxymet (0.05%) Pharonia-Egypt	25	98.25	±1.272
	50	97.65	±1.982

* Average of six determinations

Note : Added 0.004g of NaH_2PO_4 and 0.004g of Na_2HPO_4 to a standard solution.

Evaluation of the proposed method:

According to the difficulties of using the standard method for the determination of OMZ which includes potentiometric titration, so standard addition method was applied in order to prove that the proposed method is free from interference in the determination of OMZ in pharmaceutical preparations.(Table 13 and Fig. 6).

Table 13: The results of standard addition method

Drug	µg OMZ present/ml	µg OMZ measured/10ml	Recovery%
Oxymet 0.05% Pharaonia-Egypt	5	4.82	96.45%
	6.5	6.31	97.18%

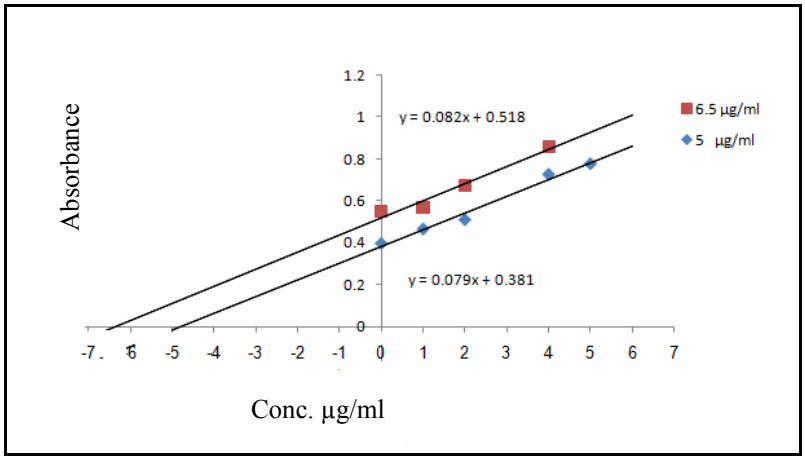


Fig. 6: Graph of standard addition plot for the determination of OMZ in oxymet.

The result in Table 13 and Fig. 6 indicated that the proposed method can be used to the determination OMZ in pharmaceutical preparation with a satisfactory result.

Comparison of the methods

Table (14) shows the comparison between some of analytical variables for the present method with that of other literature spectrophotometric methods.

Table 14: Comparison of the methods.

Variable	Present Method	Literature method (Al-Sabha and Rasheed, 2011)	Literature method (Zakaria, 2011)
Reagent	Potassium ferricyanide	1,10-Phenanthroline	4-Aminoantipyrine
Temperature ($^{\circ}$C)	50	70	70
pH	2.70	3.82	13.15
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	5.07×10^4	5.74×10^4	5.34×10^4
λ_{max} (nm)	766	510	480
Recovery (%)	98.25-97.65	100.53	98.33-100.33
RSD (%)	1.27-1.98	0.72-1.6	0.36-1.58
Beer's law range µg/ml	0.5-12.5	0.1-7	0.36-1.58

The results in Table 14 indicated that the suggested method was sensitive and accurate.

CONCLUSION

The proposed method for the determination of OMZ in pharmaceutical formulation is simple, sensitive, accurate and precise. The method is based on oxidation – reduction between OMZ and Fe (III), then the subsequent reaction of librated Fe (II) with potassium ferricyanide to form Prussian blue complex which is water soluble, stable and shows a maximum absorption at 766 nm. The proposed method has been applied successfully to the determination of the intended compound in its pharmaceutical formulation (nasal drop)

REFERENCES

AL-Sabha, T. N.; Rasheed, B. A. (2011). Spectrophotometric determination of oxymetazoline hydrochloride based on the oxidation reaction. *Jordan J. Chim*., **56**(4), 403-411.

AL-Neaimy, U. I. (2006). The use of 3,5-dinitrosalicylic acid in spectrophotometric determination of organic and drug compounds. Ph. D., Thesis, Mosul University., pp.82-104.

"British Pharmacopoeia on CD-ROM" (2007). 5th ed. System Simulation Ltd, The Stationary Office, London.

Delevie, R. (1997). "Principles of Quantitative Chemical Analysis". McGraw-Hill, Internation Ed., Singapore, 498 p.

Garcia-Campana, A. M.; Sendra, J. M. ; Vargas, M. P.; Baeyens, W.R. ; Zhang, X. (2004). Flow injection analysis of oxymetazoline hydrochloride with inhibited chemiluminesscent detection. *Anal. Chim. Acta*, **516**, 245-249.

Hayes, F.J.; Beaker, T. R.; Dobson, R. M.; Tueda, M. S. (1995). Rapid liquid chromatographic –mass spectrometric assay for oxymetazoline in whole rat blood. *J. Chromatogr. A*, **692**, 73-81.

Hoffmann, T.; Thompson R.; Seifert J. (1989). Determination of the nasal decongestant, oxymetazoline hydrochloride, in pharmaceutical formulations by HPLC. *Drug development and Industrial Pharmacy*, **15**(5), 743-757.

Hong, Y.Y. (2009). Content determination of oxymetazoline hydrochloride spray by HPLC. *J. Strait Pharm.*【DOI】: CNKI:SUN:HAIX.0.2009-09-017

http://www.wikikpedia.org./wiki/oxymetazoline, (2008).

Issa, Y. M.; Zayed, S. I. (2004). Construction and analytical application of plastic membrance electrode for oxymetazoline hydrochloride. *Anal. Sci.*, **20**, 297-300.

Sane, R.; Joshi, L. ; Latage, K.; Kothurkar, R. ; Bhate, V. (1990). High performance liquid chromatographic determination of oxymetazoline hydrochloride from nasal drop. *Ind. J .Pharm. Sci.*,**52**(1), 38-39.

Sankar, D. G.; Sastry, C. S. P. ; Reddy, M. N. ; Aruna, M. (1988). Spectrophotometric determination of adrenergic agents using iron (III) and 2,4,6-tris(2-pyridyl)-s-triazine. *Ind. J. Pharm. Sci,* **50**,178-180.

Sankar, D. G.; Sastry, C. S. P. ; Ready , M. N.; Akuna, M. (1987). Spectrophotometric determination of some a drenergic agents using 2,6-dichloroquinone –chlorimide. *Ind. J. Pharm. Sci*., **49**, 69-71.

Shingbal, D .M. ; Naik, S. D. (1983). Spectrophotometric determination of oxymetazoline hydrochloride. *East Pharm*., **26**, 201-202.

Stanisz, B.; Nowinski, W. (2000). Determination of oxymetazoline hydrochloride and decomposition products by high –performance liquid chromatography. *Acta Pol. Pharm. Drug Research*, **57**(6), 339-401.

Sudsakom, S.; Kaplan, L.; Williams, D. A. (2006). Simultaneous determination of triamcinolone acetonide and oxymetazoline hydrochloride in nasal spray formulations by HPLC. *J. Pharm. Biomed. Anal*. **40**(5), 1273-80.

"USP Pharmacopoeia on CD-ROM", (2009). The United States Pharmascopeial Convention.

Wang, N.N.; Shao Y.Q.; Tang, Y.H.; Yin, H.P.; Wu, X.Z. (2009). Flow injection chemiluminescen method. *Biological J. Chem. Iluminescence*, **24,** 178-182.

Zakaria, S. A. (2011). Spectrophotometric determination of oxymetazoline hydrochloride via oxidative coupling reaction with 4-aminoantipyrine in the presence of potassium periodate. *Raf. J. Sci.* , **22** (4), 97-108.