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Spectrophotometric Determination of Oxymetazoline Hydrochloride Via Oxidative Coupling Reaction with 4-Aminoantipyrine in the Presence of Potassium Periodate

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ABSTRACT

A simple spectrophotometric method is developed for the determination of oxymetazoline hydrochloride (OMCl) as pure and in its pharmaceutical preparations. The method is based on the oxidative coupling reaction of OMCl with 4-aminoantipyrine (4-A.A.P) in the presence of potassium periodate as oxidizing agent in an alkaline medium to produce a coloured water soluble product that is stable and has a maximum absorption at 480 nm. Beer's law is obeyed in a concentration range 20 -400µg OMCl/20 ml with a molar absorptivity of 5.34 x 10^3 $1.mol^{-1}.cm^{-1}$, a relative error of -0.50 to -1.47% and a relative standard deviation of ± 0.36 to ± 1.58%, depending on the concentration level. The optimum conditions for full colour development are described and the proposed method was applied successfully to the assay of OMCl in two pharmaceutical preparations.

Keywords : spectrophotometry ; oxidative coupling ; oxymetazoline ; 4-aminoantipyrine.

التقدير الطيفي للاوكسيمتازولين هيدروكلوريد باستخدام تفاعل الاقتران التأكسدي مع 4-امينوانتي بايرين بوجود بيريودات البوتاسيوم

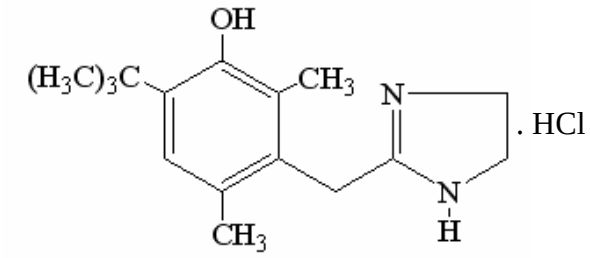
المخلص

تم تطوير طريقة طيفية لتقدير الاوكسيمتازولين هيدروكلوريـد بـشكله الحـر وفـي مستحـضراته الصيدلانية. تعتمد الطريقة على تفاعل الاقتران التأكـسدي بـين الاوكـسيمتازولين هيدروكلوريـد و 4-امينوانتي بايرين بوجود العامل المؤكسد بيريودات البوتاسيوم في وسط قاعدي لإعطاء ناتج ملون يكون ذائباً في الماء ومستقراً ويعطي أعلى شدة امتصاص عند الطول الموجي الأعظم 480 نـانوميتر. كانـت حـدود تطبيق قانون بير في مدى التركيز 20-400 مايكروغرام في حجم نهائي 20 مـل، وقيمـة الامتـصاصية المولارية 5.34 ×10^3 لتر . مول$^{-1}$. سم$^{-1}$ وتراوح الخطأ النـسبي بـين 0.50- و 1.47- % وقيمـة الانحراف القياسي النسبي بين 0.36 ± و 1.58 ± اعتمادا على مستوى التركيز. تمت دراسـة الظـروف

المثلى للتفاعل وطبقت الطريقة بنجاح في تقدير الاوكسيمتازولين هيدروكلوريد في مستحضرين دوائيين محضرين.

INTRODUCTION

Oxymetazoline hydrochloride is 3- [(4,5 – dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimethyl ethyl)-2,4-dimethyl-phenol hydrochloride(OMCl) ,it is a white, crystalline powder, freely soluble in water and in alcohol, practically insoluble in ether (British Pharmacopia, 2000) ,its chemical formula and structure are as follows :



$C_{16}H_{24}N_2O.HCl$
M.wt =296.8g/mol

OMCl is available as a topical decongestant in nasal sprays, it is also used to treat epistaxis and eye redness due to minor irritation, OMCl was developed by German patent Fruhstofer in 1961 (Wikipedia, 2008).

The large doses of OMCl may cause hypotension, presumably because of a central clonidine-like effect (Katzung, 2004).

Different methods have been used for the determination of OMCl such as a titrimetric method using ammonium metavandate as a reagent in acidic medium (Dwivedi *et al*., 2006), ion selective membrane electrode (Issa and Zayed, 2004), also, a flow injection method employing chemiluminescence detection (Garcia-Campana *et al*., 2004),the chromatographic methods are often the most analytical methods used such as high performance liquid chromatography (HPLC) (Stanisz and Nowinski, 2000 and Sundsakom *et al*., 2006), also, reversed-phase-HPLC (RP-HPLC) (Sane *et al*., 1990) and RP-HPLC used ion pair (Hoffmann *et al*., 1989) have been used in the determination of OMCl in pharmaceutical preparations.

Finally, a few spectrophotometric methods have been described for the determination of OMCl, these methods included oxidation of OMCl with iron(III) and the librated iron(II) reacts with either (2,4,6-tris(2-pyridyl)-5-triazine to produce a maximum absorption at 595 nm (Snakar *et al*., 1988) or the ferrion complex which is measured at 510 nm is produced by oxidation-reduction between OMCl and ferric ion, (Al-Abd Alazeez, 2009),also, OMCl has been determined in pharmaceuticals by using 3,5-dinitrosalicylic acid ,(Al-Neaimy, 2006) and 2,6-dichloroquinone-chlorimide as a reagent (Snaker *et al.*, 1987).

The literature review revealed that up today, nothing has been published concerning the use of 4-A.A.P as coupling reagent in the presence of potassium periodate in oxidative coupling reaction for determination of OMCl so that it is used in the present work to satisfactorily be applied for the determination of OMCl in dosage forms.

EXPERIMENTAL

Instruments :

Spectrophotometric measurements are performed using Shimadzu UV-160 UV-Visible Recording Spectrophotometer and CEClL-CE 7200 UV-visible spectrophotometer using 1-cm silica cells. The pH measurements are performed on pH meter type HANNA 211 pH-Ion meter.

Reagents :

All chemicals used in this investigation are analytical grade reagent.

Working OMCl solution, (200 μg/ml). A 0.02 g of OMCl is dissolved in distilled water and the volume is completed to the mark with distilled water in a 100-ml volumetric flask, the solution is kept in a brown bottle, where it is stable for at least one week.

4-Aminoantipyrine reagent, ($1.25x10^{-3}$M). This solution is prepared by dissolving 0.025 g of pure 4-A.A.P reagent in distilled water and the volume made up to the mark in a 100-ml volumetric flask with the same solvent.

Sodium periodate solution, (0.015M). This solution is prepared by dissolving 0.3448 g of sodium periodate in distilled water and made up to 100 ml in a volumetric flask with the same solvent.

Sodium hydroxide solution, (4N). This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water to 250 ml in a volumetric flask and then transferred to a plastic bottle.

Nazordin drops, (200 μg/ml). Provided from the State company for drug industries and medical appliances (SDI), Sammara-Iraq.

A three containers of drug (each contains 10 ml of 0.05% OMCl) are mixed, then 20 ml of the above solution was diluted with distilled water to 50 ml in a volumetric flask to prepare a solution of 200 μg/ml OMCl.

Oxymet drops, **(200 μg/ml).** Provided from pharaonia pharmaceuticals **.**

A three containers of drug (each contains 15 ml of 0.025% OMCl) are mixed, then 40 ml of the above solution was diluted with distilled water to 50 ml in a volumetric flask to prepare a solution of 200 μg/ml OMCl.

RESULTS AND DISCUSSION

The effect of various variables on the colour development of 200μg of OMCl ,1ml of 4-A.A.P and 2ml of KIO_4 in alkaline medium(1ml 4N NaOH)was tested to establish the optimum conditions.

Choice of oxidizing agent with its concentration

Different types of oxidizing agents were used to select the best one which will give the highest colour intensity (Table 1).

Table 1 : Selection of oxidizing agent.

2ml Oxidizing agent 0.015M	Absorbance*	$\Delta\lambda^{**}$
KIO_4	0.129	186.5
KIO_3	0.122	13
K_2CrO_4	No colour contrast	
$K_2Cr_2O_7$	No colour contrast	
N-chlorosuccinimide	0.116	13

*****The flasks left on water bath for 30 minutes at 60°C.

** $\Delta\lambda = \lambda^{S}_{max} - \lambda^{B}_{max}$; where S = the coloured product, B = blank.

The results illustrated in Table 1 indicated that KIO_4 give the highest intensity of coloured product and a good colour contrast.

The effect of different volumes (0.5-6 ml) of KIO_4 solution (0.015M) on the colour intensity has been studied, it was observed that 4 ml of KIO_4 is the most suitable amount, since it gives the highest intensity of the formed product therefore it is chosen for further studies (Table 2).

Table 2 : The effect of KIO_4 amount on absorbance.

Ml of KIO_4 solution (0.015M)	0.5	1	2	3	4	5	6
Absorbance	0.049	0.103	0.137	0.160	0.203	0.184	0.151

Effect of 4-A.A.P concentration

Various volumes of 4-A.A.P ($1.25x10^{-3}$M) were tested, the results indicated that using 2 ml of 4-A.A.P solution gives maximum absorbance of the coloured product at 480 nm and the volume was considered as an optimum value(Table3).

Table 3 : Effect of reagent amount.

Ml of 4-A.A.P ($1.25x10^{-3}$M)	0.5	1	2	3	4	5
Absorbance	0.109	0.126	0.135	0.127	0.116	0.102

Choice of base and its amount

The preliminary experiments have shown that OMCl can give high intensity of coloured dye with (4-A.A.P) in the presence of potassium periodate in alkaline medium,so that different types of bases are examined (Table 4).

Table 4 : Selection of base.

1ml of 4N Base	Absorbance	$\Delta\lambda^{*}$
NaOH	0.202	202.5
KOH	0.189	191
Na_2CO_3	0.117	42.5
$NaHCO_3$	0.201	7.5

*$\Delta\lambda = \lambda^{S}_{max} - \lambda^{B}_{max}$; where S = the coloured product, B = blank.

The results shown in Table 4 indicated that NaOH gives the highest colour intensity of product and a good colour contrast. Also, the effect of different volumes (0.5-4 ml) of 4N NaOH solution on the colour intensity has been studied,a 1 ml of 4N NaOH with a final solution pH (13.15) gives the highest intensity of the formed product therefore it is used in subsequent experiments (Table 5).

Table 5 : Effect of base amount on absorbance.

ml of 4N NaOH	0.5	1	2	3	4
Absorbance	0.183	0.200	0.173	0.161	0.152
pH	12.92	13.15	13.37	13.50	13.67

Effect of surfactant

The effects of different surfactants on the colour intensity were studied in four orders by using 3 ml of various types of surfactant. The results showed that no effect of the surfactant had on the intensity (Table 6).

Table 6 : Effect of surfactant.

3 ml Surfactant Solution	**Absorbance*/order** of addition**							
	I		**II**		**III**		**IV**	
	A	**Δλ*** **	**A**	**Δλ**	**A**	**Δλ**	**A**	**Δλ**
CPC $1x10^{-3}$M	Turbid	----	Turbid	---	Turbid	---	Turbid	---
SDS $1x10^{-3}$M	0.199	186.9	0.190	188.7	0.193	187	0.191	189
Triton X-100 1%	0.086	189	0.089	187	0.081	190.2	0.075	191.7

* Absorbance without surfactant (s) = 0.205

** I. OMCl + S + 4-A.A.P + KIO_4 + NaOH
II. OMCl + 4-A.A.P + S + KIO_4 + NaOH
III. OMCl + 4-A.A.P + KIO_4 + S + NaOH
IV. OMCl + 4-A.A.P + KIO_4 + NaOH + S

*** $\Delta\lambda = \lambda_{max}$ S – λ_{max} B.

Order of addition

The effect of different orders of reagents addition were studied(Table 7).It was found that the order of reagents addition be followed as given under the general procedure give highest colour intensity, otherwise a loss in colour intensity takes place.

Table 7 : Order of addition.

Order of addition	Order number	Absorbance
OMCl + 4-A.A.P + KIO_4+OH	I	0.205
OMCl + KIO_4 + 4-A.A.P + OH	II	0.198
KIO_4 + 4-A.A.P + OMCl + OH	III	0.194
OH + 4-A.A.P + OMCl + KIO_4	IV	0.203
OH + OMCl + 4-A.A.P + KIO_4	V	0.205
OH + OMCl + KIO_4 +4-A.A.P	VI	0.162
OH + KIO_4 + 4-A.A.P + OMCl	VII	0.201

Effect of temperature

The effect different of temperatures on the colour intensity of resulting complex were investigated. The results indicated that the absorbance of complex increased with increasing temperature, the high value of absorbance was obtained at 70^{o}C then a decrease in colour intensity was observed as the temperature increase above70^{o}C (Table 8).

Table 8 : Effect of temperature.

Temperature (^{o}C)	40	50	60	70	80	90
Absorbance	0.098	0.171	0.205	0.238	0.229	0.219

The effect of the time needed to complete the oxidative coupling reaction had been studied at 70^{o}C and the results showed that a maximum intensity occurred at 25 minutes before dilution of the flask with distilled water (Table 9).

Table 9 : The effect of time on absorbance at 70C^{0}.

Time (min.)	0	5	10	15	20	25	30	35	40
Absorbance	0.042	0.098	0.120	0.211	0.240	0.242	0.232	0.226	0.223

Development time and stability period

The stability time of the formed coloured complex is investigated under the optimum conditions for the determination of OMCl, the experimental results (Table 10) showed that the coloured complex formed is complete after 15 minutes from removing of the flasks from water bath and the absorbance remained constant at least for one hour.

Table 10 : Effect of colour stability time.

µg of OMCl present	**Absorbance/min. standing time**							
	5	**10**	**15**	**20**	**30**	**40**	**50**	**60**
100	0.145	0.145	0.145	0.145	0.145	0.145	0.145	0.145
200	0.241	0.241	0.241	0.241	0.241	0.240	0.239	0.239
300	0.323	0.323	0.323	0.323	0.323	0.323	0.322	0.320

Final absorption spectrum

When OMCl was treated according to recommended procedure, the absorption spectrum showed a maximum absorption at 480 nm versus the reagent blank (Fig. 1).

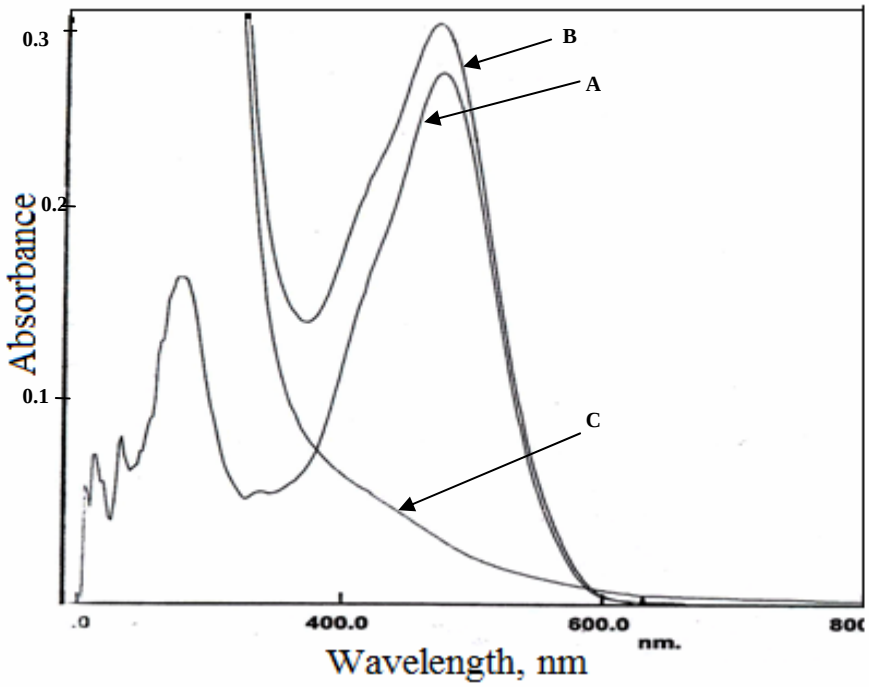


Fig1 : Absorption spectrum of (A) the coloured product (from 200µg OMCl) against blank, (B) complex against distilled water and (C) blank against distilled water.

Recommended procedure and calibration curve

To a series of 20-ml volumetric flasks, 0.1-3 ml of 200 µg.ml^{-1} OMCl solution are transferred then 2 ml of 4-A.A.P reagent (1.25x10^{-3}M) and 4 ml of potassium periodate (0.015 M) are added. After that a 1 ml of 4N sodium hydroxide solution was added. The solutions were left for 25 minutes in water bath adjusted at 70^{o}C, then the volumes were completed to the mark with distilled water and left to stand for 15 minutes at room temperature, after that the absorbances are measured at 480 nm against the reagent blank.

The calibration graph is linear over the concentration range of 20-400 µg /20 ml (Fig.2). The apparent molar absorptivity referred to OMCl, has been found to be 5.34x10^{3} 1.mol^{-1}.cm^{-1}.

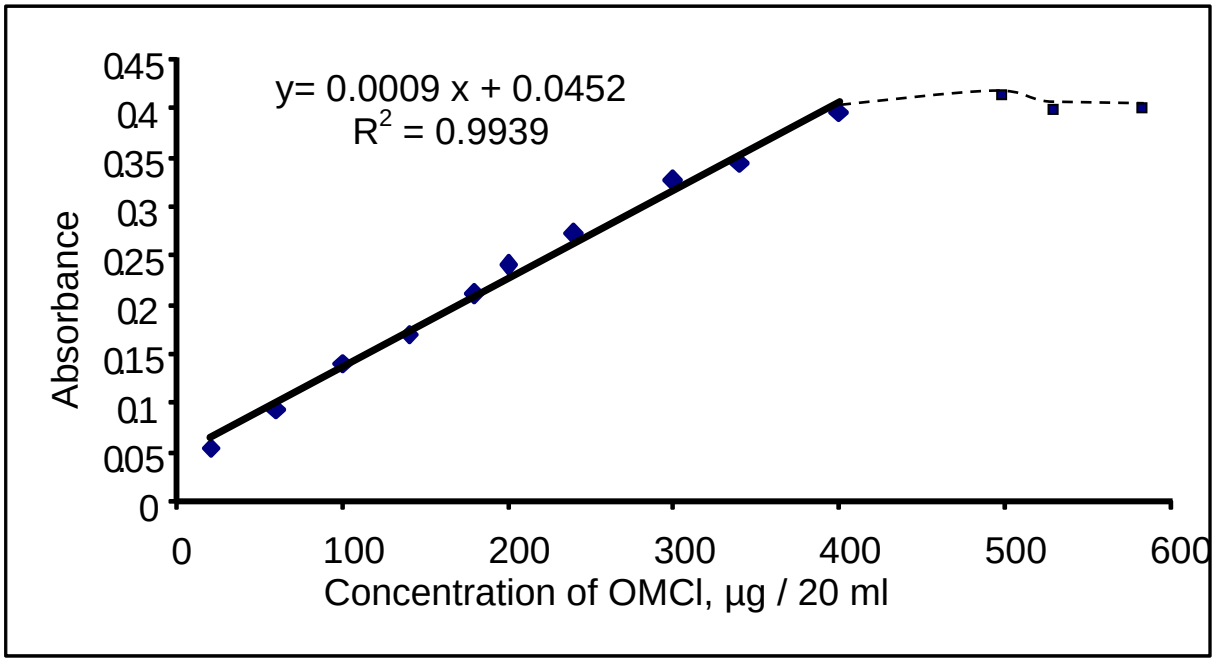


Fig. 2**:** The calibration curve of OMCl determination.

Accuracy and precision

To determine the accuracy and precision of the method, OMCl was determined at three different concentrations. The results shown in table (11), indicate that a satisfactory accuracy and precision could be obtained with the proposed method.

Table 11 : Accuracy and precision of the proposed method.

OMCl (µg/20ml)	Relative error, %	Relative standard deviation %*
100	-1.476	± 1.580
200	-0.826	± 0.869
300	-0.507	± 0.365

* Average of five determinations.

The nature of the reaction product

Job's method of the continuous variations (Fig.3) indicates that the coloured product has a compostion of 1:2 OMCl to 4-A.A.P reagent at 480 nm.

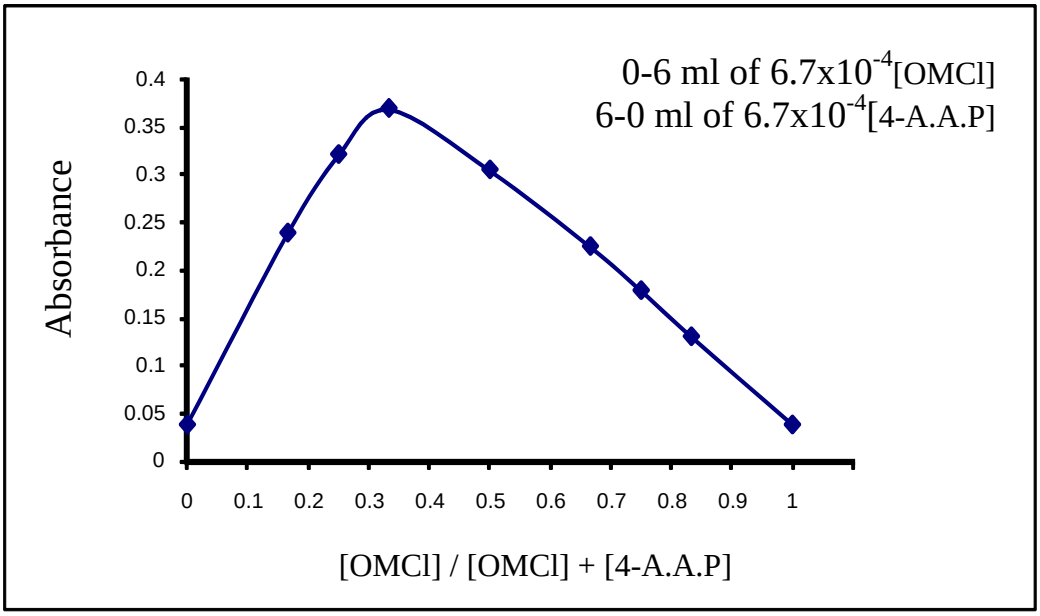
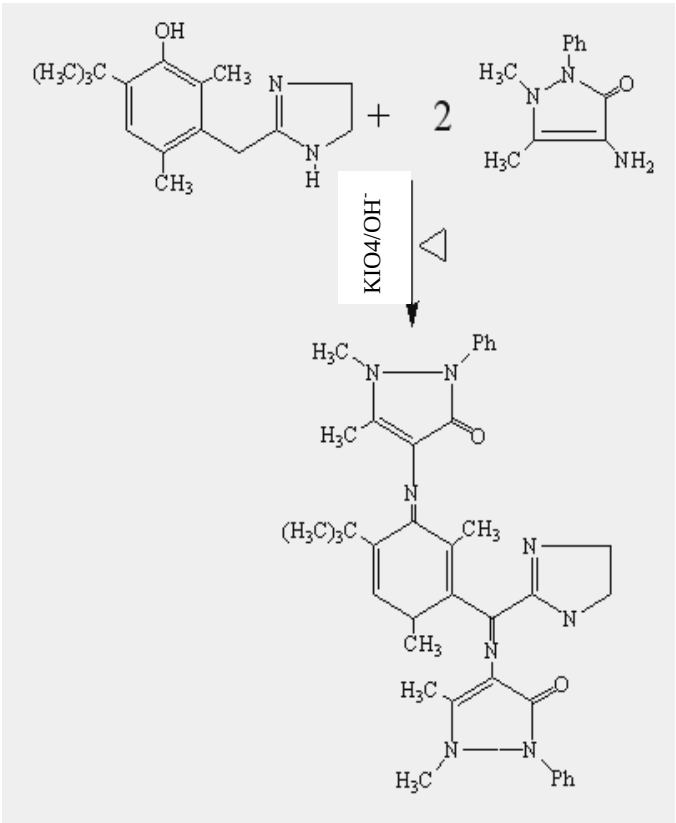


Fig. 3 : Job's plot for OMCl -4-A.A.P coloured product.

Therefore, the probable reaction path might be written as follows:



Evaluation of the proposed method :

Because the standard method for the determination of OMCl included potentiometric titration, according to difficulties of availability of using it, so that standard addition method was used in order to prove that the proposed method can be applied to determination of OMCl in pharmaceutical preparations. (Table 12 and Fig. 4).

Table 12 : The results of standard addition method.

Drug	µg OMCl present/20 ml	µg OMCl measured/20 ml	Recovery*, %
Nazordin 0.05% S.D.I-Iraq	80	79	98.75
	120	118	98.33
Oxymet 0.025% Pharaonia (Egypt)	80	82	102.50
	120	124	103.33

* Average of three determinations.

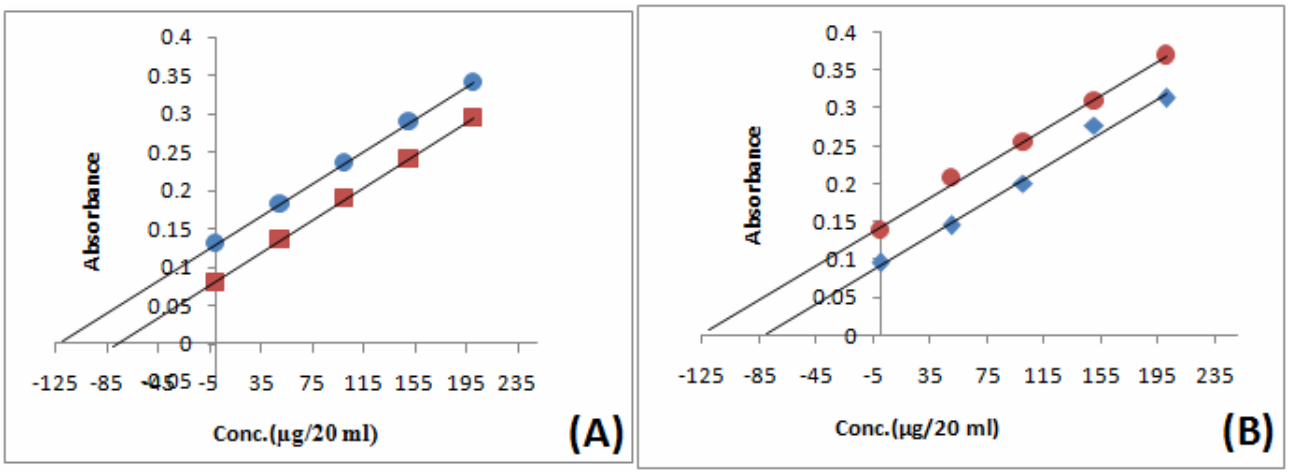


Fig 4: Calibration standard addition graph for the determination of OMCl in Nazordin (A) and Oxymet (B)

The results in Table 12 and Fig.4 indicated that the proposed method can be used to determine OMCl in pharmaceutical preparation with satisfactory results.

Table 13 shows the comparison between some of analytical variables obtained from the present method with that of a recent spectrophotometric method.

Table13: Comparision of the method.

Conditions	Present Method	Literature Method*
λ_{max}(nm)	480	510
Temperature (^{o}C)	70	70
Beer's law	1-20	0.1-7
Molar absorptivity $1.mol^{-1}.cm^{-1}$	$5.34x10^{3}$	$5.74\ x10^{4}$
Stability of the colour	At least one hour	……
RSD(%)	0.36-1.58	0.72-1.6
Type of reaction	Oxidative coupling	Oxidation-reduction
Applications	Nazordin 0.05% Oxymet 0.025%	Nazoden 0.025% Oxymet 0.05%

*Al-Abd Alazeez, B.A.R. (2009), M.Sc. Thesis, Mosul University, pp. 93-108.

The results indicate that the proposed method is sensitive and stable compared with the literature method .

CONCLUSION

A simple and sensitive spectrophotometric method for the determination of OMCl in aqueous solution based on the reaction of OMCl with 4-A.A.P in the presence of KIO_4 is developed. The proposed method has been successfully applied to assay OMCl in pharmaceutical preparations.

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