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A sensitivity Colorimetric Method for Determination of Roxithromycin using Ferric ion

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Abstract

Roxithromycin is found to reacted with Iron(III)chloride in the presence of an acetic acid-sulfuric acid mixture to form a colored compounds having absorption band at 491 nm. Different variables affecting the reaction were studied. A good correlation coefficient 0.9950 were found between the absorbance's and the concentrations of the studied drug in the range of 6-60 µg ml^{-1} . The molar absopitivity is about 1.248×10^{4} L. mol^{-1} .cm^{-1}, a sandall sensitivity of ($6.7x10^{-5}$ µg cm^{-2}), a relative standard deviation of (0.147-4.20 %)depending on the concentration and the detection limit is 1.1 µg ml^{-1} . The method was applied successfully for the assay of the drug in pure and pharmaceutical dosage form as a tablets formulation with the rang of (95-99 %) recovery.

الخلاصة:

يتضمن البحث تطوير طريقة طيفية بسيطة ودقيقة لتقدير عقار روكسي ثرومايسين في المستحضرات الصيدلانية. اذ تعتمد الطريقة على تفاعل العقار مع ايون الحديد الثلاثي بوجود مزيج من حامض الخليك مع حامض الكبريتيك مكوننا معقد ملون والذي يمتلك اعلى امتصاص عند 491 نانوميتر. وقد تم دراسة العوامل التي تؤثر على الامتصاص . اذ تم الحصول على علاقة خطية بين 6-60 مايكروغرام/مل مع معامل خطية 0.9950 .اما معامل الامتصاص المولاري ودلالة ساندل فكانت 1.248×10^{4} لتر/مول$^{-1}$ سم$^{-1}$,6.7×10^{-5} مايكروغرام/سم$^{-2}$ على التوالي. اما الانحراف القياسي النسبي فكان (0.147-4.20)% اعتمادا على مستوى التركيز اما حد الكشف فكان 1.1 مايكروغرام /مل وقد طبقت الطريق بنجاح على المستحضر الصيدلاني بحالتة النقية و بهيئة اقراص فكان مقدار الاستيعادية (95-100)%.

Introduction

Roxithromycin Fig. 1 is a semisynthetic macrolide antibiotic derived from erythromycin . Roxithromycin was reported to be absorbed rapidly with the long elimination half time, giving higher plasma levels than erythromycin. Therefore, it can be effective at lower doses with less frequent administration, which is regarded as an advantage in clinical settings. Due to these advantage, it could be applied in human and veterinary[1,2].

Several methods have been reported for determination of Roxithromycin, were presented in the literature UV spectrophotometry[3-7],high performancy liquid chromatography[8-11], liquid chromatography –Mass[12,13]Electrochemical detection[14],chemluminsecs[15] and fluorescence[16].

The aim of the present work was to develop sensitive, selective and accurate colorimetric methods for the determination Roxithromycin in pharmaceutical preparations based on complex formation of the drug with Fe^{+3} . Analytical criteria including linearity, sensitivity, precision ,accuracy and recovery were discussed.

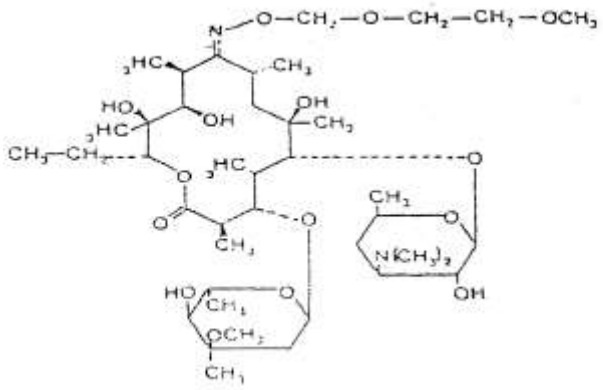


Fig. 1. Structure of roxithromycin.

Experimental:

Apparatus:

All spectral and absorbance measurement were carried out on a shimadzu UV-vis(200-900nm) 1800 digital double beam recording spectrophotometer using 1-cm quarts cells.

Reagents:

All chemicals used were of analytical reagent grade unless otherwise state , Roxithromycin Standard powder was provided from SDI-Iraq.

Roxithromycin tablets was provided from local market.

- Roxithromycin stock solution(1000 ppm):

A quantity of 0.1 gm Roxithromycin was dissolved in 100ml of glacial acetic acid.

- Ferric chloride (0.0031M):

Prepared by dissolving 0.08gm of $FeCl_3.5H_2O$ in 1ml of concentrated H_2SO_4 and made up to 100ml volumetric flask with glacial CH_3COOH.

Recommended procedure:

In to a series of 10ml volumetric flasks, Increasing volumes of (100ppm) Roxithromycin solution in the range of calibration curve(6-60ppm) were transfer , 5ml (0.0031M) of $FeCl_3$ was added and shake well., dilute the solution to the mark with glacial acetic acid , and allow the reaction to stand For 15min.Measure the absorbance at 491nm against a reagent blank prepared in the same way but containing no Roxithromycin. The color complex is stable for 30min after that a precipitate was observed.

Procedure for pharmaceutical preparations:

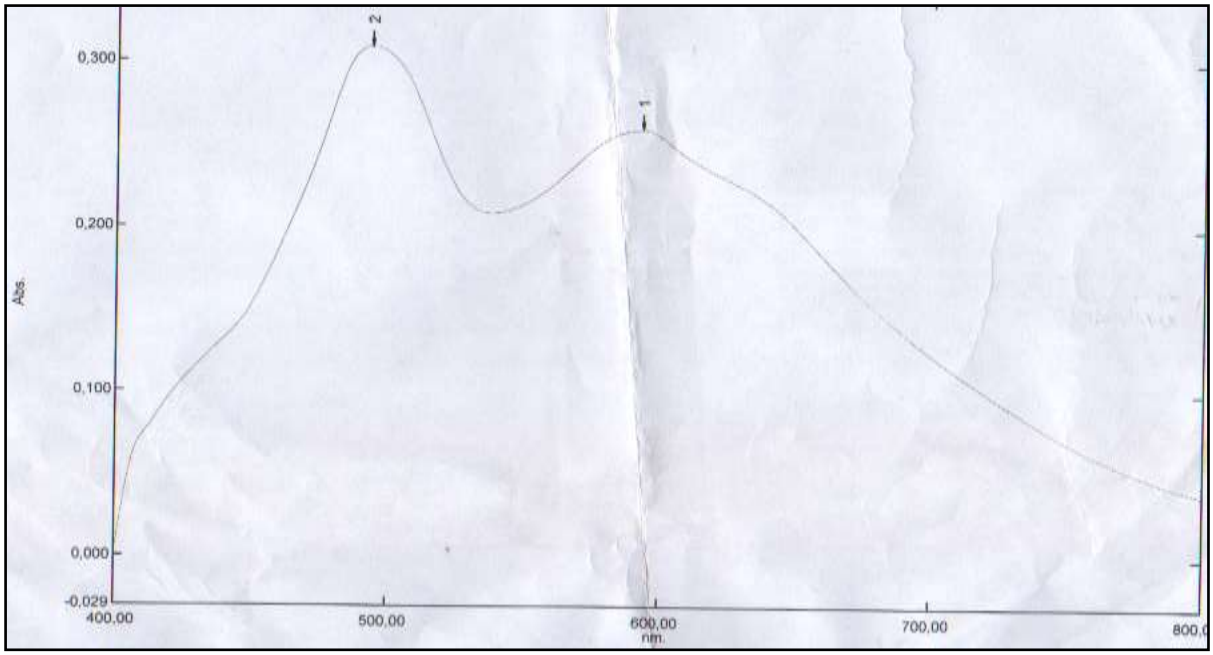
- Roxithromycin tablet **(150mg):**
- Twenty tablets were powdered and a quantity of the powdered(250mg) equivalent to (150mg Roxithromycin) was extracted by shaking with 60 ml acetone for 15 min with the aid of a magnetic stirrer. The mixture were filtered through

Whatman no.41 filter-paper and dry in water bath then dissolved in 100ml of glacial acetic acid in volumetric flask. Then 10ml from the above solution was diluted to 100ml in a volumetric flask with same solvent to obtain (1500ppm). This solution is used for the determination of the drug by recommended procedure.

Results and discussion:

Absorption spectra:

When a diluted solution of Roxithromycin is treated with Fe^{+3} ion in the presence of glacial acetic acid a color complex that have two a maximum absorption at λ_{max} = 491nm and 592nm and this agreement with some literature[17-19] compounds that contain micro ring in the presence of concentrated acetic and sulfuric acid under go dehydration and forming hemiketial and thus reacted with ferric chloride forming colored compound. The absorbance of color complex depends very much on the reaction conditions, therefore it is very important to optimize the reaction condition.



(**Fig.2:** Absorption spectrum of the complex.

Optimization of working conditions:

The optimum condition should be selected so that the maximum sensitivity, largest linear range and low blank readings are obtained together with the maximum correlation coefficient and precision. In order to find these optimum conditions, the influence of the variable affecting the reaction, such as temperature, reaction time ,pH,solvent,metal concentration and color stability were investigated. The effect of various parameters on the absorbance intensity of the complex formed were studied and the reaction conditions were optimized.

Effect of $FeCl_3$ concentration:

The effect of different concentration of Fe^{+3} ion was investigate in the range (0.1-2.5ml and made up to 10ml volumetric flask with glacial acetic acid) 0.0031M ,0.5ml(0.0015M) of $FeCl_3$ gave the highest absorbance as shown in Fig.3 , therefore it is chosen for further work.

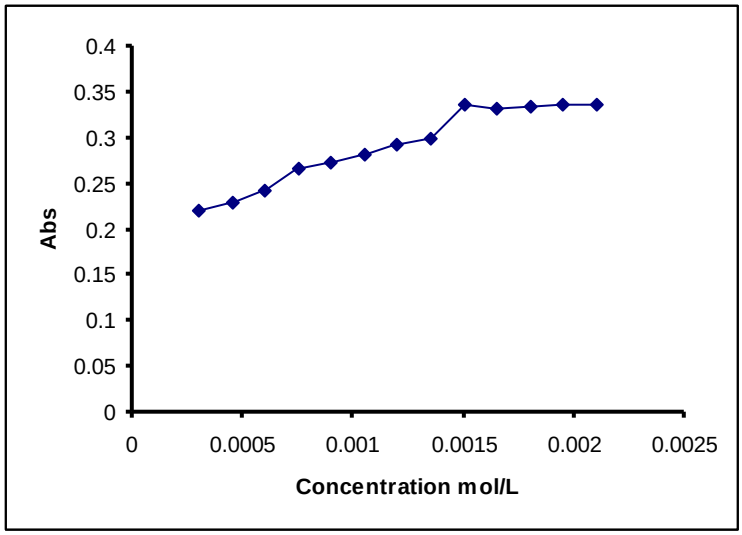


Fig.3: Effect of $FeCl_3$ concentration.

Effect of sulfuric acid concentration :

The effect of H_2SO_4 concentration in the preparation of $FeCl_3$ was also studied in the range (0.09-0.72M).The absorbance increased with increasing concentration up to 0.36M ,above which is stable as shown in Fig.4.

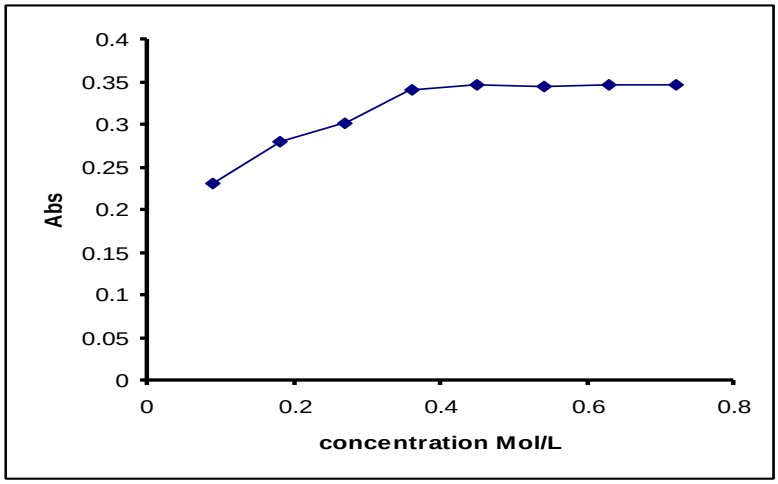


Fig.4: Effect of H_2SO_4 concentration.

Effect of water quantity :

The effect H_2O in the preparation of $FeCl_3$ was also studied in the range (0.5-5ml) and made up to 10ml volumetric flask with glacial acetic acid.The absorbance increased with increasing concentration up to 2ml ,above which is stable as shown in Fig.5.

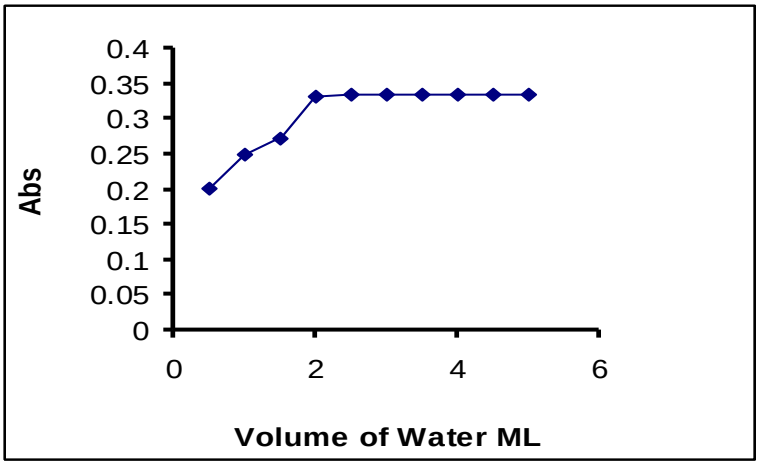


Fig .5 The effect of volume of H_2O

Effect of order of addition:

After fixing all other parameters, a few other experiment were performed to ascertain the influence of the order of addition of reactants. The order, drug : Fe^{+3} ion : CH_3COOH, after full color development gave a maximum absorbance and hence the same order was followed throughout the investigation as shown in table .1.

Table.1
Effect of order of addition for reactants

Arrangement	Absorbance
1- drug+Fe^{+3} +CH_3COOH	0.334
2- drug + CH_3COOH + Fe^{+3}	0.321
3- Fe^{+3}+ CH_3COOH + drug	0.327

Effect of temperature:

Table (2) shows, neither high temperature of 50^0C which caused precipitation, nor the ice bath of 5^0C which has reduced the absorbance. The optimum was found to be 25^0C .

Table.2
Effect of temperature

Temperature (^{0}C)	Absorbance
5	0.089
25	0.333
50	precipitate

Effect of reaction time and stability:

Fig .6 shows that the reaction between Roxithromycin with Fe^{+3} and CH_3COOH has reached the optimum after 10-15 min .Therefore measurements were taken after 15 min.

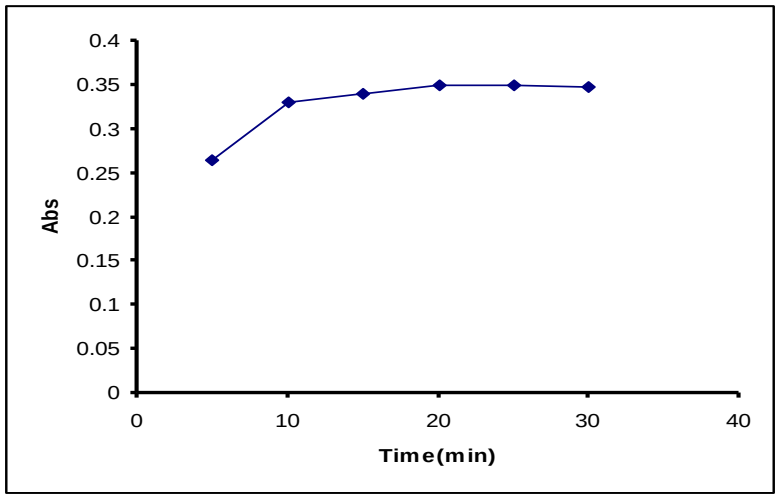


Fig.6: Effect of reaction time

The calibration curve:

The conditions described under the procedure, a liner calibration curve of Roxithromycin is obtained(Fig.7),which shows that Beer's law is obeyed over the concentration range 6-60ppm of Roxithromycin with a correlation coefficient of 0.9953.The molar absorptivity of the color complex was found to be 12485.25L mol^{-1} $cm^{-1}$with reference to Roxithromycin and sandell index ($6.7x10^{-5}$ µg ml^{-2}),with detection limit of 1.1 ppm.

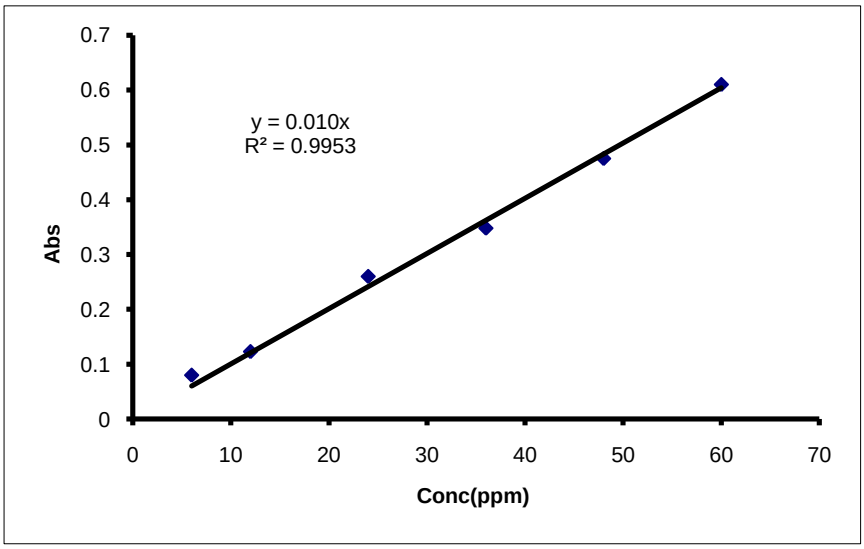


Fig. 7: Calibration curve of Roxithromycin.

Accuracy and precision:

In order to establish the validity and accuracy of the proposed method for the determination of pure drug solutions containing three different concentrations of Roxithromycin were prepared and analyzed in five replicates. The analytical results obtained from this study are summarized in table.3.

Table.3

Accuracy and precision of the proposed method

Concentration of Roxithromycin (ppm)		Recovery* %	R.S.D* %	Error* %
Taken	Found			
8.000	7.940	99.25	0.147	0.742
12.000	12.059	100.49	1.568	0.492
24.000	23.2871	97.024	4.20	-2.97
70.000	68.568	97.954	4.01	-2.03

*five replicated

Analytical application:

One tablet containing Roxithromycin (150mg) has been analyzed using the calibration curve in the range (6-60ppm) giving a very good accuracy and precision as shown in table.4.

Table.4
Application of the proposed method for the determination of Roxithromycin in pharmaceutical preparations

Drug sample	Amount of Roxithromycin (ppm)		Recovery*%	R.S.D* %	Error*%
Roxithromycin India	present	found			
	150	143	95.33	0.699	-4.66
Roxithromycin SDI	150	146	97.33	1.369	-2.66

*five replicated.

Conclusions:

On the basis of obtained data, we found a new analytical form and developed a new methodology for determining micro amounts of Roxithromycin using charge transfer complex. The technique has good metrological characteristics, high sensitivity, and is simple in use. No interference was observed from excipients and the validity of the procedure was tested by analyzing pharmaceutical formulation . Recovery were 95.0-100.0% .the concentration measurements are reproducible within a relative standard deviation of 0.147-4.20 % .

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