Determination of ranitidine - HCl in pharmaceutical formulations by kinetic spectrophotometric and flow injection - activated chemiluminescence methods .

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Abstract

This study involves development of a simple kinetic spectrophotometric method and a new flow injection-activated chemiluminescence (FIA-CL) for the determination of ranitidine -HCl (R-HCl) in pharmaceutical preparations.

Spectrophotometric method was based on the oxidation of the R-HCl with alkaline potassium permanganate , the reaction is followed spectrometrically by measuring the rate of change of the absorbance at 600 nm. A fixed-time (at 30min) method is adopted for determining the drug concentration . The calibration graph was linear in the range of (1 - 7) $\mu g\text{-}ml^{-1}$ with a correlation coefficient of 0.9981,detection limit of 0.183 $\mu g\text{-}ml^{-1}$ and a relative standard deviation RSD% of 0.58-1.62%.

The method of FIA-CL was based on the activation of luminol-cobalt-H_2O_2 chemiluminescence by R-HCl. The linearity is (1-6) $\mu g\text{-}ml^{-1}$ with detection limit of (0.75) $\mu g\text{-}ml^{-1}$, and correlation coefficient was 0.9996 (n=6) and the relative standard deviation was (0.74-1.00)% .

The two methods were applied successfully to determine the content of R-HCl in pharmaceutical preparations with a recovery of 97-99 % .

الخلاصة

تضمنت هذه الدراسة على جزئين أساسيين ، ضم الجزء الأول تطوير طريقة طيفية لتقدير دواء رانتدين هيدروكلورايد تستند على أكسدة هذا الدواء مع برمنغات البوتاسيوم القاعدية ($KMnO_4$) و متابعة التفاعـل طيفياً من خلال قياس التغير بالامتصاص عند طول موجي 600 نانوميتر . وجد أن الزمن الـلازم لتماميـة التفاعل هو 30 دقيقة ومدى الخطية يتراوح بين (1 ـ 11) مايكرو غرام / مل ومعامل الارتباط 0.9981

، وحد الكشف مقداره 0.183 مايكرو غرام / مل وقيم الانحراف القياسي النسبي RSD% تتراوح بين (0.58 ـ 1.62)% .

أما الطريقة الثانية استندت على تحفيز البريق الكيميائي لنظام (لومينول ـ بيروكسيد الهيدروجين ـ كوبلت) بواسطة هذا الدواء وكانت الحدود الخطية للطريقة تتراوح بين (1.0 ـ 10.0) مايكرو غرام / مل وبمعامل أرتباط 0.9996 وانحراف قياسي نسبي مئوي يتراوح بين 0.74% ـ 1.00% وحد كشف (0.75) مايكرو غرام / مل .

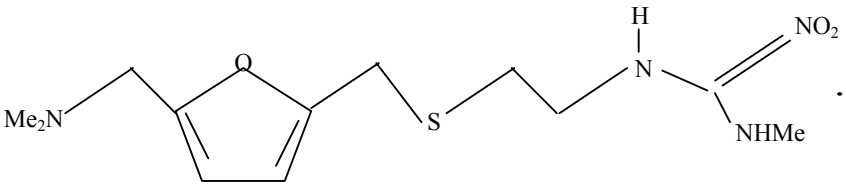
طبقت هاتين الطريقتين بنجاح على المستحضرات الصيدلانية وكان الاسترداد المئوي (97 ـ 99)% .

Introduction

Ranitidine[1]- HCl (R - HCl):

N,N - Dim ethyl – 5 – [2 (1- methyl amino - 2 - nitro vinyl amino) ethyl thio methyl] furfuryl amine is a white or pale yellow , crystalline powder , freely soluble in water and methanol .

The empirical formula for R-HCl is $C_{13}H_{22}N_4O_3S.HCl$ and its molecular weight is 350.9 , it is used as[2] inhibits gastric acid secretion and its structure is:



. HCl

This drug has been determined in tablets and ampoules by coluometric[3] and potentiometric[4] titrations . HPTLC[5,6] and HPLC have been used for the determination of R-HCl using C_{18} column at different mobile phases[7-9] .

De Almeida[10] and chattoraj[11] used UV/Visible spectrophotometry for the determination of (R-HCl)in tablets pharmaceutical preparation, the absorbance was measured at 314nm with linearity ranged from 2-30 g/ml.

The present paper describes a spectrophotometric method for the determination of (R-HCl)in pharmaceutical preparations based on the oxidation with alkaline potassium permanganate. This study also includes development of FIA-CL method

Experimental

A) spectrophotometer apparatus for determination the absorbance, the spectrophotometer used was Philips model pu 8720

uv/visible, with quartus cells of 1 cm width.

B) FIA Chemiluminescence apparatus

The FIA-CL configuration is outlined in figure 1 showing the system of chemical reaction used for the determination of (R-HCl).

Reagents

Reagents of analytical grade and distilled water were used through out the study Solutions were prepared by appropriate dissolution as shown in table 1.

Luminol was prepared by dissolving in a solution of (0.1 M Na_2CO_3) where as $KMnO_4$ was prepared and standardized with 0.1M $Na_2C_2O_4$.

Hydrogen peroxide solution (1M) was prepared by diluting 45.72ml of H_2O_2 (48%) in 1 L of distilled water and standardized against standard 0.1 M $KMnO_4$.

0.55 ml of sulfuric acid (96%) was diluted in 100 ml of distilled water and standardized against 0.1 M Na_2CO_3 . Cobalt ($100\mu g.ml^{-1}$) was prepared by dissolving 0.4039 gm of $CoCl_2.6H_2O$ with 20 ml of 5 $x10^{-3}$ M H_2SO_4 and diluted to 1L with distilled water. Finally , R-HCl stock solution ($100\mu g.ml^{-1}$) was prepared by dissolving 0.01 gm of R-HCl powder in 1L of ($0.7\mu g.ml^{-1}$) cobalt (II) solution . Solutions of lower concentrations were prepared by appropriate dilution .

Pharmaceutical preparation

Rantism tablets : provided from (SDI) Samara - Iraq .

Ten tablets were grinded well and a certain portion of the final fine powder was accurately weighed to give an equivalent to 150 mg of ranitidine – HCl and then dissolved with (25ml) of Methanol . The resulting solution was washing by shaking with methanol and filtered on Whatman filter paper No.4 to remove any suspended particles . The filterate and washing were evaporated to dryness at 60C and the residue was redissolved in distilled water forming a solution of 100 $\mu g.ml^{-1}$ concentration .

The same method was adopted for preparation of Zantac tablets, and the final dissolution was in ($0.7\ \mu g.\ ml^{-1}$) cobalt and ultrasonication was needed.

General procedure for the spectrophotometric method.

Initial rate method : Aliquots of 0.01M $KMnO_4$ solution (1.0ml) and 1.00M NaOH solution (2.0 ml) were transferred into a 10ml volumetric flask. An accurate volume of the

working solution of R- HCl (0.1 - 1.2) ml was added and diluted to volume with distilled water. The contents of the mixture were shaken well and immediately transferred to the spectrophotometric cell at room temperature. The absorbance was recorded at 600nm as a function of time against reagent blank.

In the second procedure, the absorbance was measured at a fixed time of (30min.) and was plotted against the final concentration of R-HCl and the content of the drug was calculated from either the calibration graph or regression equation .

Results and Discussion

Part(1) : Kinetic spectrophotometric determination of R- HCl in pharmaceutical preparation .

In an alkaline medium , potassium permanganate oxidizes R-HCl , resulting in the formation of manganate ion[13] , which showed an absorption peak at 600nm.

Because the intensity of the color increased with time , a kinetically based method was elaborated for the determination of R-HCl in dosage forms . Initial parameters for this study are given in table 2 .

The various experimental parameters affecting the formation of the reaction product were optimised as follows :

Effect of the $KMnO_4$ concentration

To study the effect of the $KMnO_4$ concentration , aliquots of R-HCl containing 100µg $.ml^{-1}$ were transferred into a series of 10ml volumetric flasks, followed by addition of varying volumes of 0.01M $KMnO_4$ (0.1-1.1) ml and 2.0 ml of 1.00M NaOH solution . The absorbance at 600nm was measured at a fixed time of 30 minutes . It is apparent from figure 2 that the absorbance increased with increasing volume of the $KMnO_4$ solution, and became constant at 0.9ml and so then 1.0ml of $KMnO_4$ was used as the optimal volume .

Effect of the NaOH concentration

The influence of the NaOH concentration on the formation of MnO_4^{2-} was examined critically. Figure3 shows that the maximum absorbance was obtained with 1.8ml of the 1.00M NaOH, so the optimum volume of 2.0ml was chosen.

Effect of oxidation time

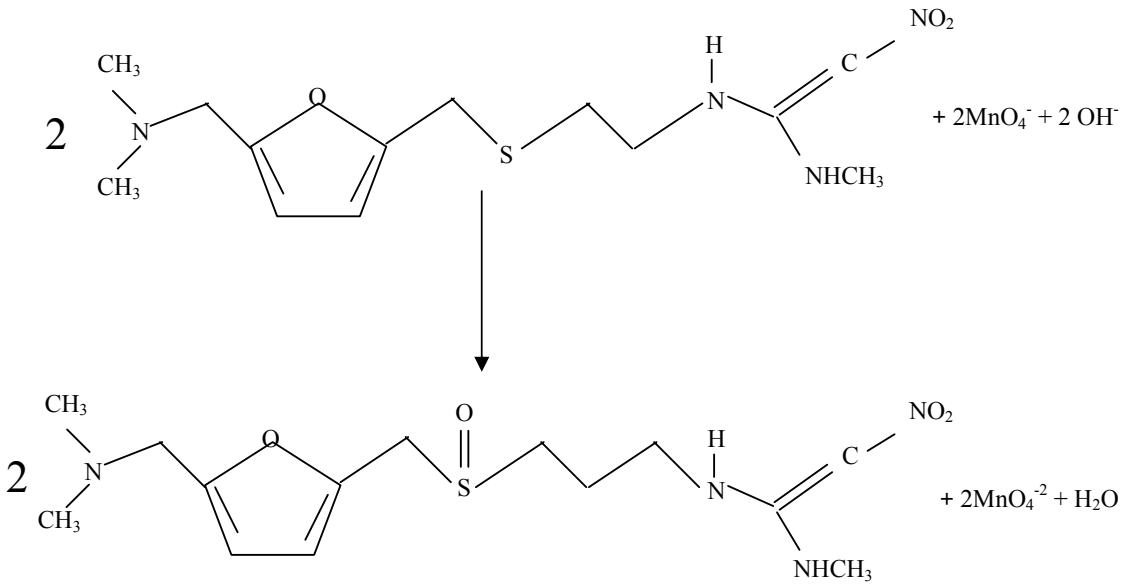
It was found that the most acceptable value was obtained at a fixed time of 30min., and therefore was considered to be the most

suitable time interval for measurements .

Final absorbance spectrum of Ranitidine – HCl

A clear peak of MnO_4^{2-} at 600nm (fig.5) was obtained after the oxidation of R-HCl by 0.01M $KMnO_4$ solution in an alkaline medium of 1M NaOH .

The Ranitidine-HCl contains thio ether linkage which has an ability to be oxidized to the corresponding sulfoxide[13] . The reaction mechanism is proposed and given in the following equations .



Recommended Analytical conditions

According to the results obtained , the optimum conditions for the determination of ranitidine-HCl using a kinetic spectrophotometric method are given in table 3 .

Calibration graph

A linear calibration graph for ranitidine -HCl (Fig.6) under the optimized conditions was obtained . Beers law is obeyed over the concentration range of 1.0- 7µg $.ml^{-1}$ with correlation coefficient of 0.9981 and molar absorbance $\in_{max}$ 2.3 x 10^4 $L.mol^{-1}.cm^{-1}$.

The relative standard deviation of the method was 1.62 based on 6 replicate determinations. Table 4 shows summary of analytical data for the determination of ranitidine -HCl using spectrophotometric method .

Pharmaceutical application

The proposed method was applied for the determination of ranitidine -HCl in tablets . Good precision and recovery were obtained . The method was successfully compared with the British pharmacopoeia standard method . The results obtained are summarized in table 5 .

Part (2) : Determination of ranitidine -HCl using a new FIA-CL method.

General procedure

The FIA-CL scheme is outlined in Figure1. Varying concentrations of R-HCl were prepared in Co^{2+} (0.7mg.ml^{-1}) solution . 150ml aliquot of each solution was injected through the sample loop into the stream of Co^{2+} solution , which then was combined with luminol and hydrogen peroxide streams in the flow cell which situated in front of the photomultiplier tube (PMT) .

Results and discussion

The chemiluminescence of luminol–hydrogen peroxide–Co (II) System is very intense[14]. However, in this work, trace amounts or R-HCl were found to be strongly activate chemiluminescence signal of this system .

Effect of reagents concentration

The effect of luminol concentration on the net chemiluminescence intensity was studied . Different concentrations of luminol (1×10^{-3} –1×10^{-5} M) are used to establish the best emission intensity – time profile that can be obtained. Figure 7 shows that (5×10^{-4} M) of luminol is the optimal concentration .

The effect of H_2O_2 concentration was investigated ; from the results of figure (8), the concentration of (1×10^{-2} M) H_2O_2 was selected to be the optimum concentration .

Effect of Sulphuric acid and Cobalt Concentrations

The effect of the acidity of cobalt Co^{2+} solution was also studied ; three concentration of cobalt Co^{2+} (0.2 , 0.5 , 0.7) µg . ml^{-1} with varing concentrations of (1×10^{-3} - 10×10^{-3}) M of H_2SO_4 were investigated . The best intensity was obtained at the concentration of ($5x10^{-3}$)M of H_2SO_4(fig.9).

The effect of cobalt concentration was also studied, figure (10) shows that the suitable concentration of Co^{+2} is 0.7 µg / ml^{-1}

It is worth noting here that as the concentration of H_2SO_4 increases the CL intensity increases , this is because of increasing the catalysed oxidation of luminol by H_2O_2 [16] . At $1x10^{-2}$ M H_2SO_4 the CL intensity decreases , this

can be attributed to the cleavage of the formed fluorescent compound .

Table 6 shows that as the concentration of H_2SO_4 increases the time of analysis decreases accompanied with decreasing band width .

Effect of flow rate

A flow rate ranged from (1-10) ml/ min was investigated . Table 7 shows that the CL intensity increased with the increase of flow rate . However , a flow rate of 3ml/min is recommended for all streams because of satisfactory CL intensity , less reagent consumption and short analysis time .

Recommended Analytical Conditions

A Summary of the optimum experimental conditions for the determination of R-HCl in pharmaceutical preparations is on table 8 .

Calibration graph

A calibration graph of relative chemiluminescence intensity against the ranitidine -HCl concentration was established by applying the optimal conditions . The regression equation is (Y = 7.7714 X + 0.1333) , and the linearity is in the range of (1.0 – 6) µg . ml^{-1} of R-HCl (fig. 11).The analytical data obtained from the calibration graph are summarized in table 9 .

Interferences

Ranitidine - HCl is usually formulated in tablets , and injections forms , therefore, the effect of some common excipient substances usually present in pharmaceutical preparation were investigated . The presence of 1µg.ml^{-1} of Aerosil , and 2 µg.ml^{-1} of Mg-stearale gave no significant interfering effect on the chemiluminescence intensity of 5.0 µg.ml^{-1} of ranitidine -HCl .

Application of developed new FIA-CL method for the determination of R-HCl in pharmaceutical preparations

Two types of tablets containing R-HCl were analysed using the developed method and the results were compared with the British pharmacopoeia standard method , Table 10 .

Comparison between the two methods

The two proposed methods were compared with other methods as shown in table (11). The value (0.55) of calculated F for FlA-CL / spectrophotometric methods is much

less than the value (4.95) for tabulated F which indicates good agreement .

Conclusion

In part (1) a simple kinetic spectrophotometric method was developed for the determination of ranitidine- HCl in pharmaceutical preparation by oxidation of the R-HCl with alkaline $KMnO_4$. The results show good precision and accuracy for the determination of R-HCl in the range of (1-7) $\mu g.ml^{-1}$ and correlation coefficient of 0.9981 with detection limit of 0.183 $\mu g.ml^{-1}$ and RSD% of 1.62% with 99.0 % recovery .

In part (2) the chemiluminescence was found to be activated by R-HCl and this is the base of the developed new method . The proposed method offers advantages of simplicity , rapidity , high sensitivity and low reagent consumption . The linearity of this method is (1-6) $\mu g .ml^{-1}$, correlation coefficient of 0.9996 (n=6) and RSD% was 1.00% with detection limit $0.75 \mu g.ml^{-1}$and recovery (98%) .

Table (1) preparation of some solutions

Substance	Molar Concentration (M)	Dissolved weight (gm)	Final Volume (ml)
NaOH	1.0	4.000	100
$NaCO_3$	0.1	10.599	1000
$Na_2C_2O_4$	0.1	13.390	1000
$KMnO_4$	0.1	15.800	1000
Luminol	1×10^{-3}	0.1772	1000

Table (2) : Initial Experimental parameters for the spectrophotometric method .

Item	Preliminary parameter	Value
1	Conc . of $KMnO_4$	0.01 M
2	Conc . of NaOH	1.00 M
3	Volume of $KMnO_4$	1.0 ml
4	Volume of NaOH	2.0 ml
5	Reaction Time	6 min
6	Wave Length λ_{max} (nm)	600 nm

Table (3): Optimum conditions for the detrmination of Ranitidine –HCl using the spectrophotometric method.

No.	Parameter	Value
1.	Conc. Of $KMnO_4$	0.01M
2.	Conc.of NaOH	1.00 M
3.	Fixed time	30min
4.	Vol . of $KMnO_4$	1.0ml
5.	Vol. of NaOH	2.0 ml
6.	Temperature	25C°

Table (4) : Analytical data for the determination of R-HCl by the spectrophotometric method.

Analytical data	Value
Linear range	1.0-7 μg. ml^{-1}
Correlation coefficient	0.9981
Regression equation	Y=0.0461x +0.7324
RSD %	1.62 %
Detection limit	0.183 μg. ml^{-1}
ε_{max}	2.3 $x10^4$ $L.mol^{-1}.Cm^{-1}$

Table (5) : Application of the proposed Spectrophotometric method for the determination of Ranitidine – HCl in Pharmaceutical Preparations .

Sample	Recovery %		RSD %
	Proposed method *	Standard method	
Pure R-HCl	99.6	99.5	1.63
Rantism Tablets	97.8	97.3	1.62
Zantac Tablets	98.4	98.6	1.60

- **Each result is the average of three determinations .**

Table (6) Effect of sulphuric acid concentration on band width and time of analysis .

H_2SO_4 Concentration (M)	Base band width (mm)	Time of analysis (sec)
$1x10^{-4}$	26.0	75.0
$5x10^{-4}$	22.0	67.0
$1x10^{-3}$	16.0	55.0
$5x10^{-3}$	10.0	45.0
$1x10^{-2}$	8.5	43.0

Table (7) Effect of flow rate on the emission intensity , analysis time and peak width using 0.7 µg. ml^{-1} of Co^{+2}.

Flow rate ml/min	Intensity (mv)	Analysis time (Sec)	Peak width mm
1.0	228	85	27
2.0	300	55	18
3.0	500	45	10
4.0	610	40	8
5.0	700	35	7
6.0	790	30	6
7.0	850	26	5
8.0	900	20	4
9.0	910	15	3.5
10.0	925	12	3.0

Table (8) The recommended analytical conditions for the determination of Ranitidine –HCl using FIA – Chemiluminescence system.

Parameter	Recommended value
Conc. Of luminal	$5x10^{-4}$ M
Conc. Of H_2O_2	$1x10^{-2}$ M
Conc. Of Co^{+2}	0.7 µg. ml^{-1}
Conc. Of H_2SO_4	$5x10^{-3}$ M
Flow rate	3 ml/ min
Volume of Co^{+2} injected	150 µl

Table (9) Analytical data for the determination of Ranitidine –HCl using FIA-CL method.

Analytical data	Value
Linear range	1.0-6 µg. ml^{-1}
Correlation coefficient	0.9996
Regression equation	Y = 7.7714 X + 0.1333
RSD %	1.0%
Average Recovery	99.0 %
Detection limit (D.L)	0.75 µg. ml^{-1}

Table (10) Application of the proposed method for the determination of Ranitidine –HCl in pharmaceutical preparations

Sample	Recovery %		RSD %
	Proposed method	Standard method	
Pure Ranitidine-HCl	99.0	99.3	1.76
Rantism Tablets	98.65	99.2	1.72
Zantac Tablets	98.30	99.12	1.69

Table (11) The statistical comparison of results for the spectrophotometric and FIA-CL method with other methods

The method	Regression equation	Linearity (µ g/ml)	Correlation Coefficient (r)	Recovery %	RSD %	t-table	Ref.
spectrophotometric	Y=0.0461 X+0.7321	1.0-7.0	0.9981	97-98	1.62	32.72	___
FIA-CL	Y= 7.7714 X+0.1333	1.0-6.0	0.9996	98-99	1.00	63.26	___
HPLC	_____	1.5-20	0.9978	93.00	4.50	_____	5
HPTLC	_____	5.0-10	0.9969	94.50	5.45	_____	8
Coulometric titration	_____	5.0-9.0	_____	92.70	1.40	_____	3
Polarography	_____	3.5-15	0.9970	98.00	2.30	_____	31

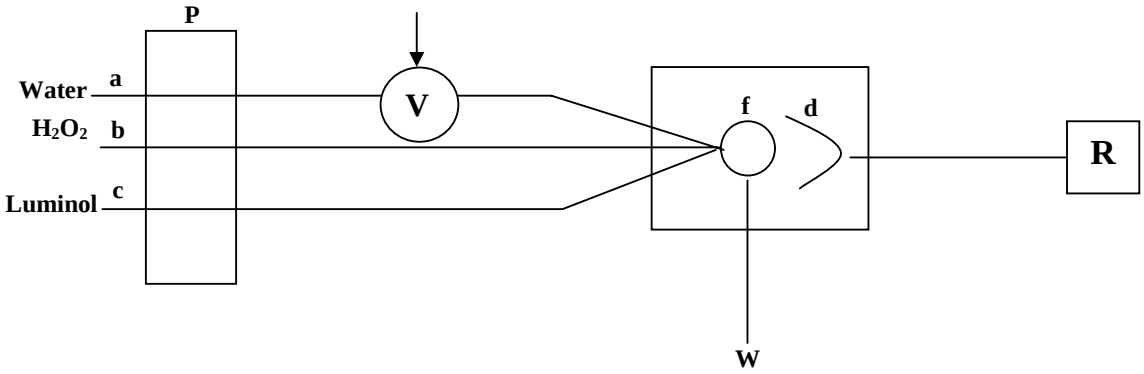


Fig (1): Chemical reaction manifold used for the Flow-Injection Chemiluminescence determination of Ranitidine-HCl.

P- Peristaltic pump V- Injection valve F- Flow cell d- Photomultiplier
R- Recorder W- Waste solution .

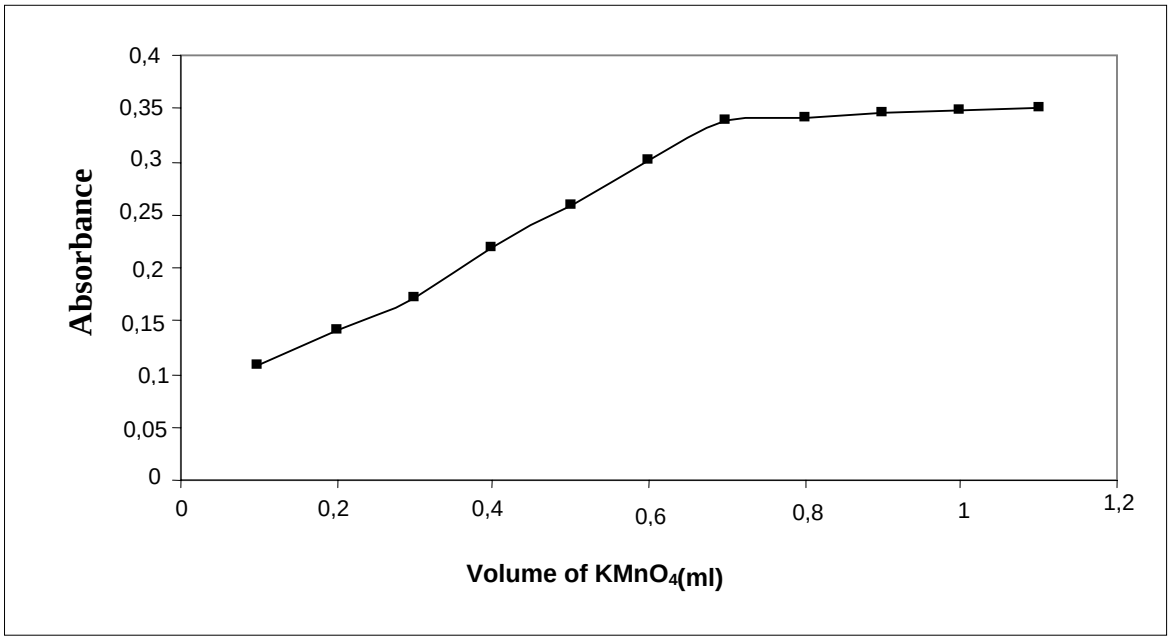


Fig (2) : Effect of the volume of 0.01M $KMnO_4$ on the intensity of color produced for the reaction (Ranitidine-HCl 10µg / ml , 2.0 ml of 1 M NaOH)

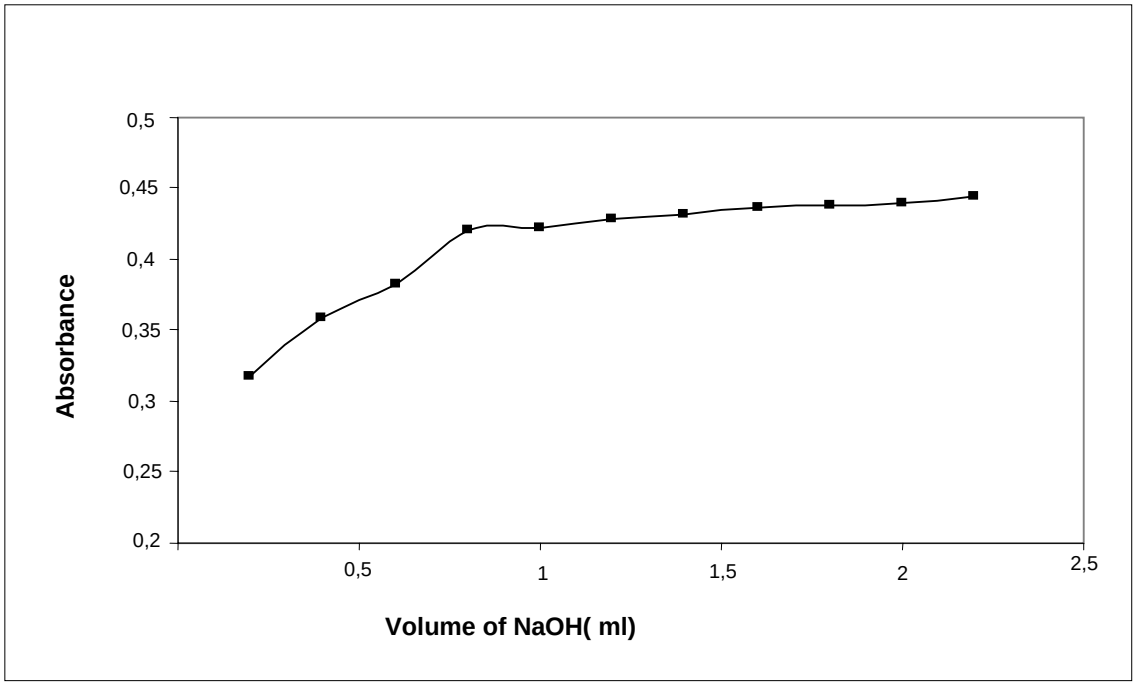


Fig (3) : Effect of the volume of 1M NaOH on the intensity of color produced for the reaction (Ranitidine-HCl 10 µg /ml , 1.0 ml of 0.01 M KMnO4)

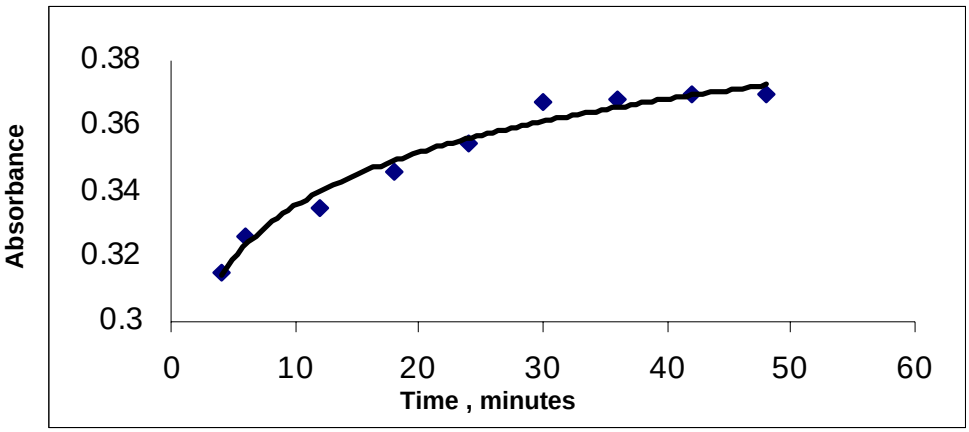


Fig (4) : Effect of time on the oxidation of R-HCl by 0.01M KMnO4

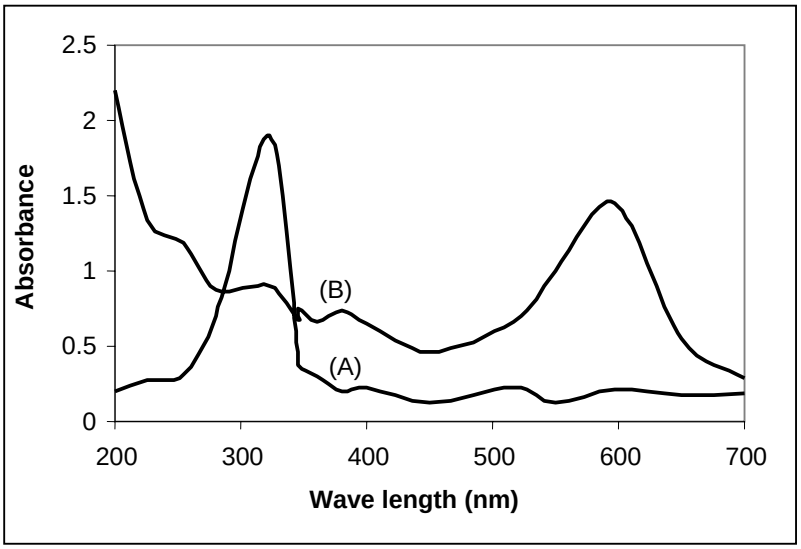


Fig (5) : A-absorption spectrum of Ranitidine-HCl before oxidation

B- absorption spectrum of Ranitidine-HCl after oxidation.

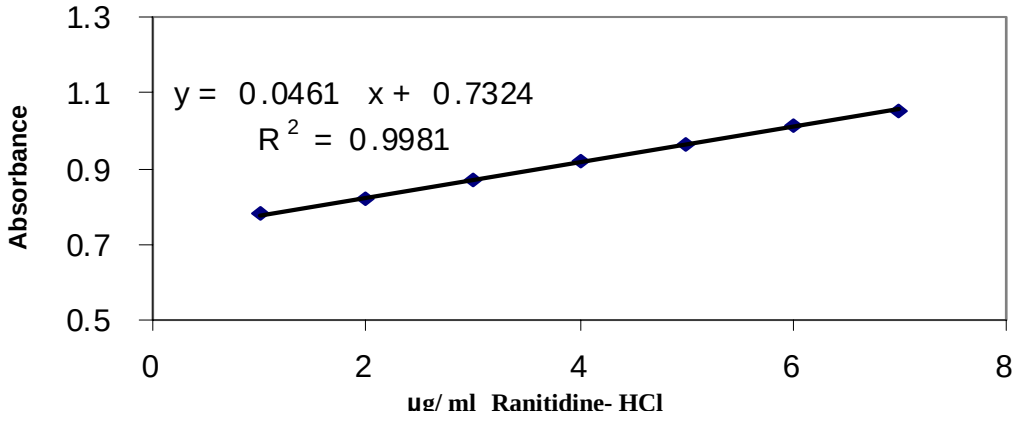


Fig (6) : Calibration graph for the determenation of Ranitidine- HCl by the spectrophotometric method

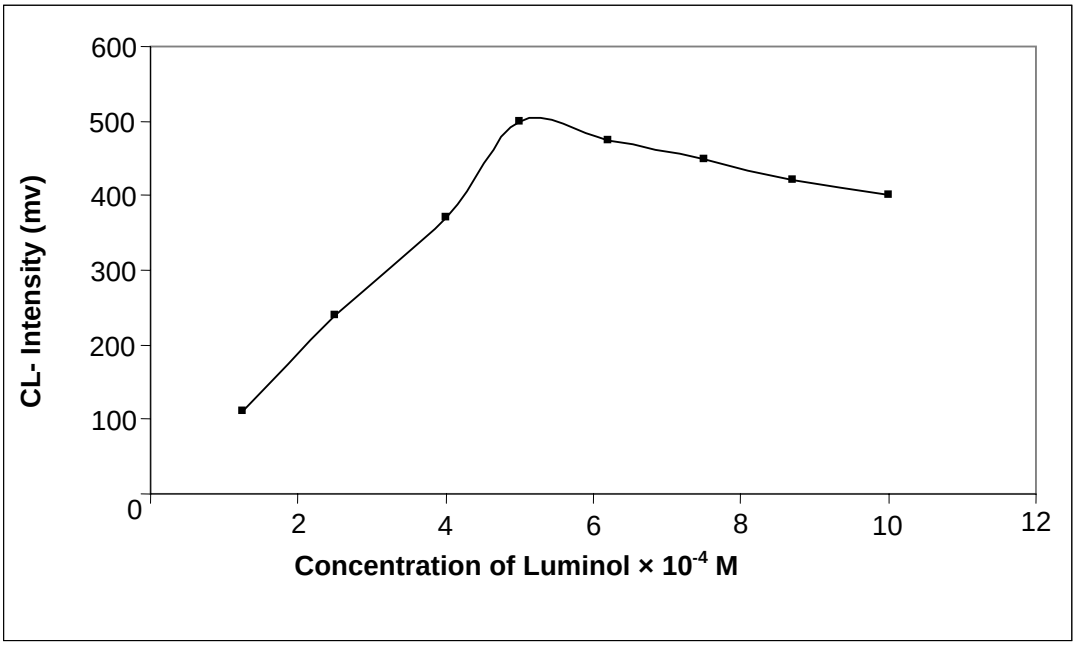


Fig (7)Effect of concentration of Luminol on the CL- intensity.

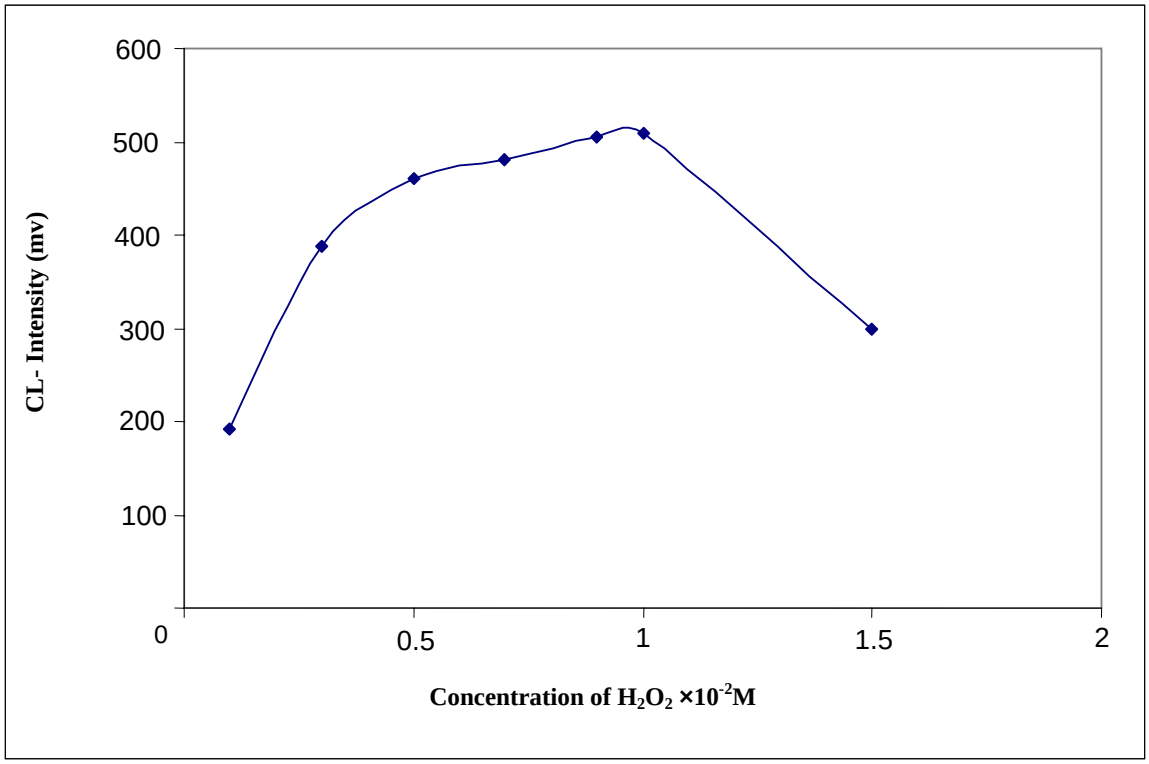


Fig (8) Effect of concentration of H2O2 on the CL- intens.

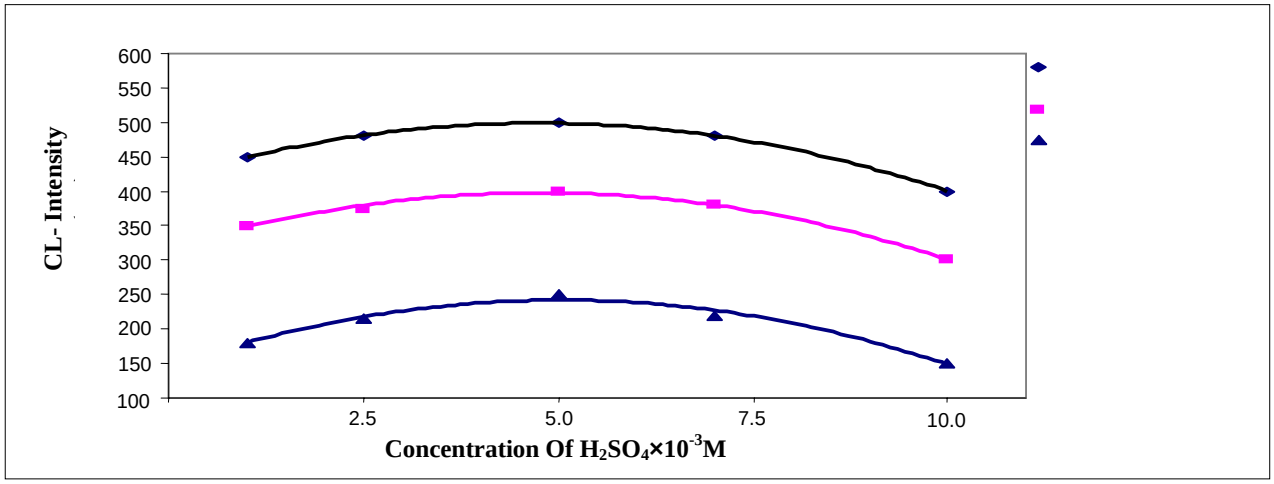


Fig (9) Effect of Concentration of H2SO4 on the CL-Intensity .

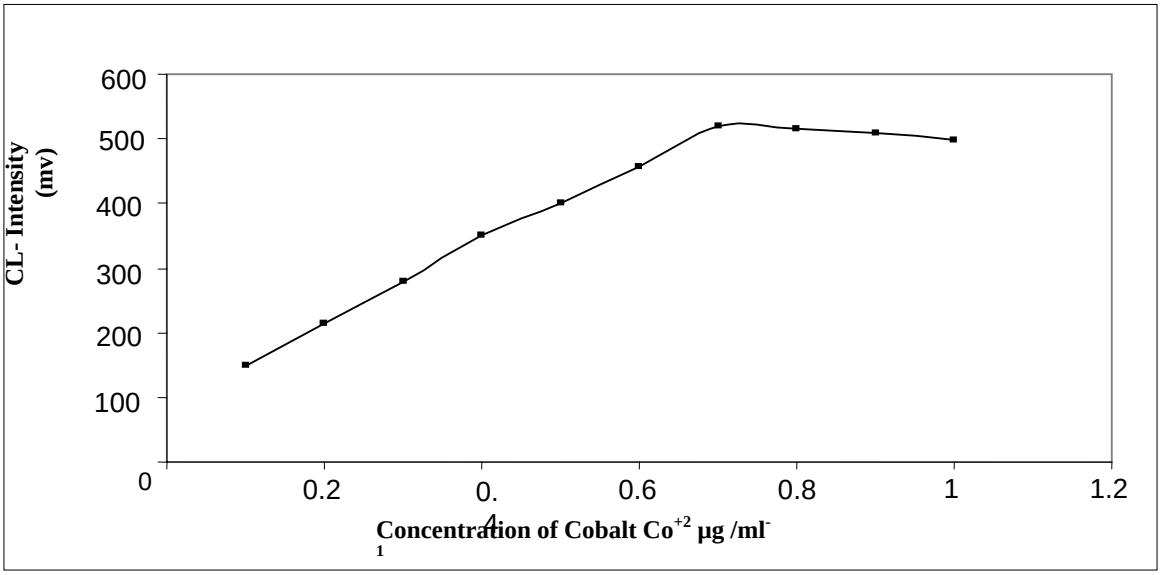
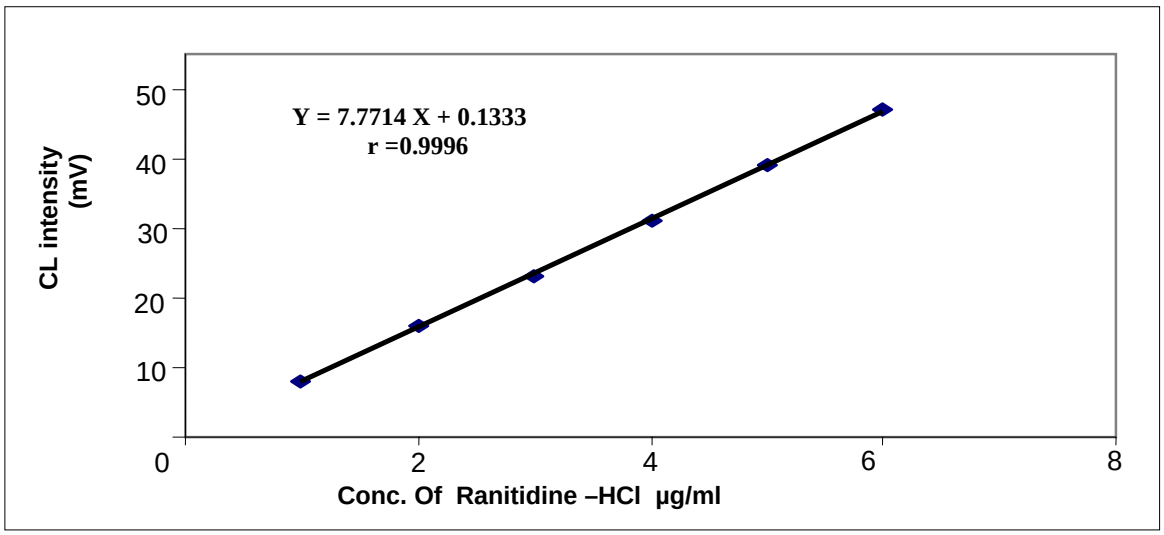


Figure (10) Effect of Cobalt concentration on the CL-intensity



Fig(11)calibration graph of Ranitidine –HCl by using FIA-CL method

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