Spectrophotometric Determination Of Tamoxifen Citrate Using Eosin G In Aqueous Media

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Abstract
The research included a description of a spectrophotometric method for the determination of tamoxifen citrate, which relied on a complex formation reaction in a reddish-pink color and in aqueous media, through the reaction of a microgram amount of the drug with the optimal amount of eosin G in the presence of hydrochloric acid in its optimal amount. The absorption was measured at 543 nm, and the molar absorption coefficient was 3.8. x 10^4 Liter. Mole.$^{-1}$ml.$^{-1}$ is an indication of the sensitivity of the method, and it was compliance with Beer's law for concentrations that ranged from (0.25-12.5) micrograms. ml^{-1}, the relative standard deviation was less than 1.8%, the method was distinguished as a result of not using the extraction and heating processes in simplicity and easy, and the method was applied to the pharmaceutical preparation of tamoxifen citrate`

Keywords: Tamoxifen citrate, Eosin G, Spectrophotometer.

Introduction

Tamoxifen Citrate

Tamoxifen citrate is a white powder that is used to treat breast cancer as it is an anti-estrogen, and it prevents the growth of cancer cells and has fat solubility(1), It has the following structural formula(2)

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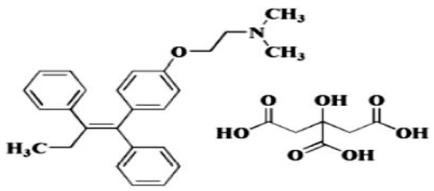

4-(1,2-diphenylbut-1-enyl) phenoxy]N,N-dimethylethanamine (3)[Z) 2- (M.Wt=563.6 g/mol Tamoxifen citrate was determined by spectrophotometric and chromatographic methods.(3-8)

Experimental Part:

Devices used:

To perform the spectral measurement of the solutions, a double –beam photometric device, "Shimadzu UV-1800 spectrophotometry was used , using cells made of "glass" with a width of 1 cm **.**The materials were weighed with a sensitive balance of the type "Ae ADAM", and the water bath of the type "Electro-mag" was used to conduct the process of heating the solutions. The pH was measured using the Lovibond Senso Direct 150 device.

Chemical solutions used:

All materials used were in high purity.

Tamoxifen citrate solution (50 µg.ml^{-1})

The solution was prepared by dissolving 0.005 grams of pure tamoxifen citrate in a small amount of distilled water, with shaking and heating to complete the dissolution then transferred to a 100 ml volumetric flask and completing the volume to the mark with distilled water.

Eosin G solution (100 µg.ml^{-1})

The solution was prepared by dissolving 0.01 g of the pure substance in a small amount of distilled water, then transferring it to 100 ml volumetric flask and filling the volume with distilled water to mark.

Approximate hydrochloric acid solution (0.1 M)
The acid solution was prepared at a concentration of 0.1 M by dissolving 0.85 ml of concentrated acid (concentration 11.7) with distilled water in a 100 ml volumetric flask.

Surface active material solutions
All solutions (Tween 20, SDS, CPC) were prepared by dissolving 0.1 g of each substance except (Triton X-114) which was prepared by diluting 0.1 ml of it with distilled water, then the solutions were transferred to volumetric flask and the volume was completed with distilled water to 100 ml.

Preliminary study:
It was found at a reactivity of 5 µg. ml^{-1} of the drug with 1.0 ml of eosin G (100 µg.ml^{-1}) and in the presence of 0.5 ml of 0.1 M hydrochloric acid in aqueous media formed a reddish-pink complex measured at 543 nm. Therefore, eosin G was considered a chromogenic reagent and was used in subsequent studies in the determination of tamoxifen citrate and its pharmaceutical formulation.

Set optimal conditions:
Effect of acid volume and pH:
The effect of the pH function on the intensity of absorption was studied, as increasing volumes (0.20-3.5) ml of 0.1 M hydrochloric acid were to 5 µg. ml^{-1} of tamoxifen citrate in the presence of 1.0 ml of eosin G at a concentration of (100 µg. ml^{-1}) and the absorption was measured at 543 nm. Table (1) shows that the highest absorption was with the addition of 0.5 ml of the acid.

Table (1) Effect of acid volume on absorption of complex formation

Volume of HCl (0.1) M	0.2	0.4	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Absorbance	0.134	0.173	0.335	0.313	0.292	0.277	0.270	0.265	0.261
pH	2.2	2.2	3.2	2.9	2.5	2.3	1.9	1.6	1.2

The effect of a number of buffer solutions pH = 3.2 on the intensity of absorption was studied, and the result was a decrease in the intensity of absorption, so it was excluded from the study. Table (2) shows the effect of buffer solutions.

Table (2) Effect of buffer solutions

Buffer solution	Without	Phthalate	Citrate	Acetate
Absorbance	0.173	0.129	0.084	0.062

Acid type effect:
To find out the best acid on the intensity of absorption, the effect of different acids was studied, where 0.5 ml were added at a concentration of 0.1 M of each acid to 5 µg.ml^{-1} of tamoxifen citrate in the presence of 1.0 ml of eosin G at a concentration of (100 µg.ml^{-1}) and the absorption was measured at 543 nm.

Table (3) Effect of acid type on complex absorption

Acid 0.1M	Without	HCl	H_2SO_4	CH_3COOH	HNO_3	H_3PO_4
Absorbance	0.173	0.334	0.246	0.290	0.286	0.301

Effect of Acid Concentration and Volume:
Different concentrations of hydrochloric acid were prepared, ranging from (0.05-5) M, and added to 5 µg.ml^{-1} of tamoxifen citrate in the presence of 1.0 ml of eosin G at a concentration of (100 µg. ml^{-1}) and the absorption was measured at 543 nm.
Figure (1) shows the effect of different concentrations of acid. Then, increasing volumes of the acid were taken, ranging from (0-1.5) milliliters, and following the method of work and the same additions in the previous step, it was found that 0.5 milliliters is the optimal volume. Figure (2) shows the optimal volume of acid .

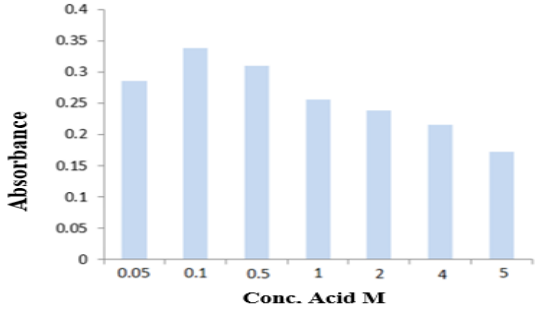

Figure (1) A study of the effect of different concentrations of hydrochloric acid

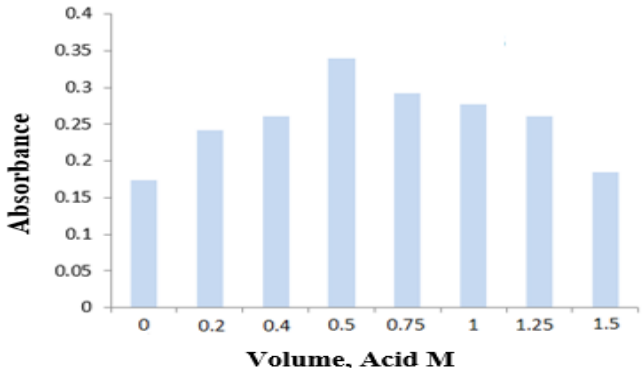

Figure (2) The effect of acid volume on the determination of tamoxifen citrate

The effect of G-eosin concentration and volume

Different concentrations of eosin G were prepared, ranging from (25-200) $\mu g.ml^{-1}$ and 1.0 ml of each concentration was added to 5 $\mu g\ ml^{-1}$ of the drug compound in the presence of the optimum amount of acid, and the absorption was measured at 543 nm. Table (4) shows that the concentration is 200 $\mu g\ ml^{-1}$ is optimal and was adopted in subsequent studies.

Table (4) The effect of the concentration of eosin G on the absorption of the formed complex

Conc.Eosin G (ppm)	25	50	100	150	200
Absorbance	0.171	0.247	0.334	0.357	0.391

To find out the best volume, increasing volumes (0-3.5) milliliters of G-eosin were studied at a concentration of 200 $\mu g.ml^{-1}$, Figure (3) shows that 1.5 ml is the optimal volume because it gave the highest absorption. Accordingly, it was adopted in subsequent studies.

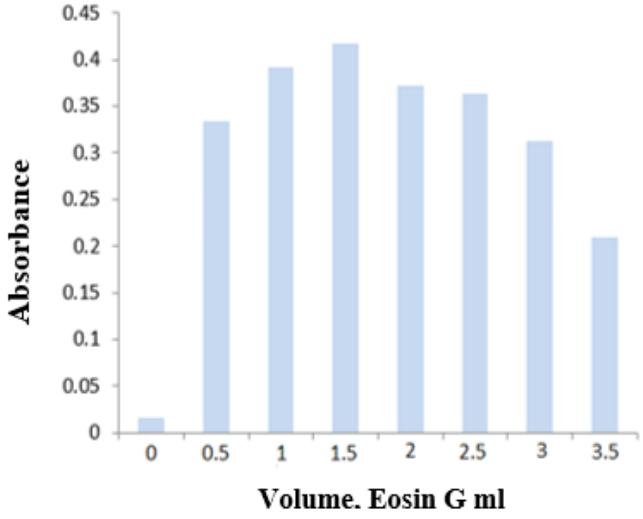

Figure (3) The effect of the size of eosin G on the absorption of the formed complex

The effect of surface active substances

The effect of different types (positive, neutral, and negative) was studied. Table (5) shows a decrease in the intensity of absorption, so it was excluded from the study.

Table (5) Effect of surface active substances on complex formation

Surfactant 0.1%(0.5ml)	Without	CPC	SDS	Tween-20	Triton x-114
Absorbance	0.417	-0.069	0.098	0.301	Turbid

Effect of temperature and time on the adsorption and stability of the complex

The effect of temperatures (25°C,40°C,50°C) on the absorption of the complex and its settling time was studied in the presence of a constant amount of tamoxifen citrate 5$\mu g\ ml^{-1}$,and the optimal amounts of acid and reagent, and measured absorbance at 543 nm, Figure (4) shows that the complex is formed directly and with a settling time of more than thirty-five minutes, and that the temperature of the laboratory (25°C) was the best as it gave the maximum absorption of the formed complex.

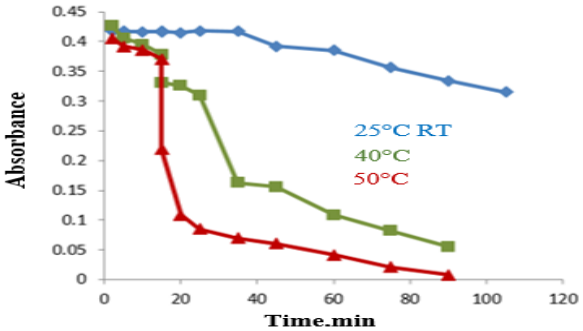

Figure (4) Effect of temperature and settling time of the formed complex

Addition sequence effect:

Three variable addition sequences were studied to obtain the best sequence that gives the highest absorption, and the results recorded in Table (6) indicate that the last sequence is the best.

Table (6) Effect of addition sequence

Order Number	Reaction of component	Absorbance
I	D + A +R	0.291
II	A + R + D	0.199
III	D + R + A	0.418

Tamoxifen (A), citrate (D), eosin G (R), hydrochloric acid

Final absorption spectrum:

After reaching the optimal conditions in the determination and stabilization of tamoxifen citrate, the final absorption spectrum was drawn, which gave the highest absorption at 543 nm, as shown in Figure (5).

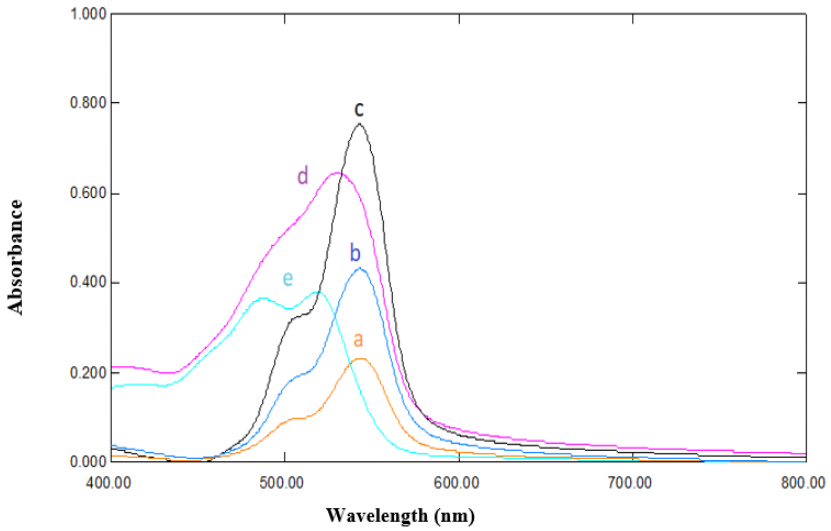

Figure (5) The final absorption spectrum of the tamoxifen citrate – eosin G complex

a: The reaction product of 2.5 µg.ml^{-1} of tamoxifen citrate with 1.5 ml of eosin G in the presence of hydrochloric acid against the blank solution.
b: The reaction product of 5 µg.ml^{-1} of tamoxifen citrate with 1.5 ml of eosin G in the presence of hydrochloric acid against the blank solution.
c: The reaction product of 10 µg.ml^{-1} of tamoxifen citrate with 1.5 ml of eosin G in the presence of hydrochloric acid against the blank solution.
d: The reaction product of 5 µg.ml^{-1} of tamoxifen citrate with 1.5 ml of eosin G in HCl against distilled water.
e: Blank solution against distilled water.

Modus operation of the Standard Curve of Tamoxifen Citrate:

Increasing amounts (0.05-2.5) ml of a 50 µg.ml^{-1} solution of the medicinal compound were added into a set of volumetric flasks with a capacity of 10 ml, followed by the addition of 1.5 ml of eosin G and 0.5 ml of hydrochloric acid, and the absorption was measured at 543 nm. Figure (6) shows the standard curve that complies with Beer's law with concentrations ranging from (0.25-12.5) µg. ml^{-1}.

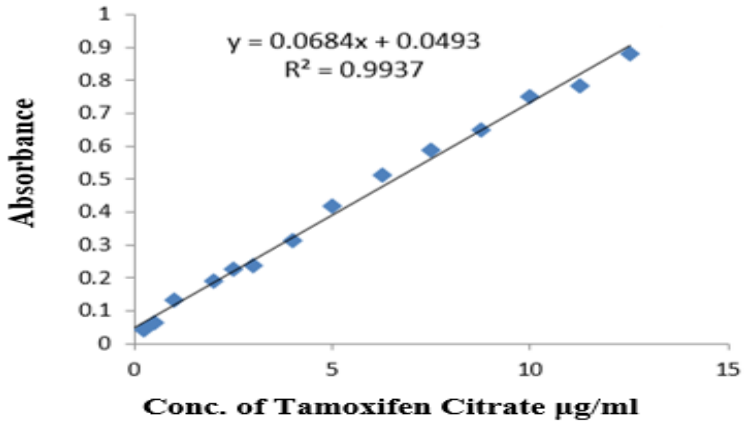

Figure (6) The standard curve of tamoxifen citrate

The molar absorptivity was 3.8×10^4 $l.m^{-1}.cm^{-1}$. This indicates that the method is sensitive, while the detection limit and the quantitative limit were 0.075 and 0.252 $\mu g.ml^{-1}$, respectively. Table (7) shows the results that have been reached.

Table (7) Linear specifications of tamoxifen citrate

Parameters	Ciprofloxacin Hydrochloride
Linearity range(µg/ml)	(0.25-12.5)
Intercept	0.0493
Slope	0.0684
Correlation coefficient(R^2)	0.9937
LOD ($\mu g.ml^{-1}$)*	0.075
LOQ ($\mu g.ml^{-1}$)*	0.252
Molar absorptivity ($L.mol^{-1}.ml^{-1}$)	3.8×10^4

*Average of ten determinations

Method accuracy and compatibility:

The accuracy and compatibility of the proposed method were studied by calculating the slop and relative standard deviation using three replicates for three different concentrations of tamoxifen citrate. The results noted in Table (8) show the accuracy and compatibility of the method.

Table (8) Accuracy and compatibility of the method

Compound	Amount added ($\mu g.ml^{-1}$)	Recovery* (%)	Average recovery (%)	RSD*
Tamoxifen Citrate	2	99.15	101.37	1.708
	5	102.82		0.251
	10	102.14		0.339

*Average of three determinations

Application of the method to pharmaceutical preparations:

Tamoxifen tablets (20 mg)

Ten tablets of tamoxifen were taken, weighed and grinded well, then the equivalent of the weight of one tablet was taken, dissolved in distilled water, filtered, and completed the volume to 100 ml using distilled water to obtain a concentration of 200 $\mu g.ml^{-1}$, and 25 ml were drawn from it to prepare a solution with a concentration of 50 $\mu g.ml^{-1}$ and different volumes were taken from it to obtain the concentrations of (2, 5, 10) $\mu g.ml^{-1}$, and the concentration of the drug compound in the tablets was found using the straight line equation of the standard curve of the pure substance, and the results were recorded in Table (9).

Table (9) Application of the method to pharmaceutical preparations

Pharmaceutical preparation	Certified Value (mg)	Amount present ($\mu g.ml^{-1}$)	Amount found ($\mu g.ml^{-1}$)	Recovery (%)	Average recovery (%)	Drug content (mg)
Tamoxifen	20	2	1.940	97.00	99.04	19.40
		5	5.024	100.48		20.09
		10	9.966	99.66		19.93

Average of three determinations . WOCKHARDT,UK (Tablet).*

Application of the standard addition method to pharmaceutical preparations:
To prove the efficiency of the proposed method and the extent of its success in estimation, and that it is free from the effect of interference with additives, the standard addition method was applied to the pharmaceutical preparation of tamoxifen citrate. The results are shown in Figure (7) and are listed in Table (11).

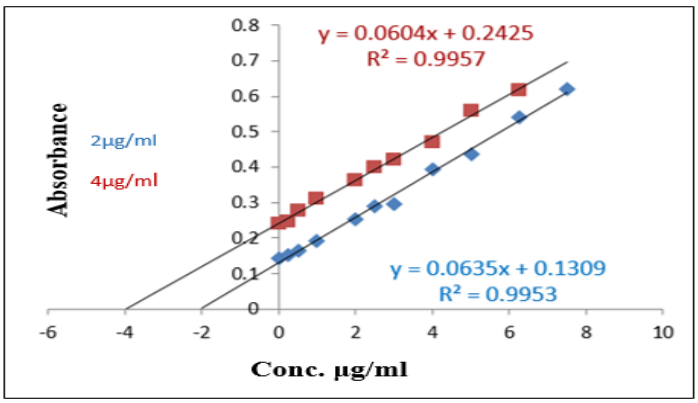

Figure (7) Standard addition curves for the estimation of tamoxifen citrate

Table (7) Linear specifications of tamoxifen citrate

Parameters	Ciprofloxacin Hydrochloride
Linearity range(µg.ml^{-1})	(0.25-12.5)
Intercept	0.0493
Slope	0.0684
Correlation coefficient(R^2)	0.9937
LOD (µg.ml^{-1})*	0.075
LOQ (µg.ml^{-1})*	0.252
Molar absorptivity (l.mol^{-1}.ml^{-1})	3.8×10^4

*Average of ten determinations

Study the nature of the colored product:
The study was carried out by following the two methods of continuous change (Joop's method) and the slope ratio (9-10) for the complex resulting from the reaction between tamoxifen citrate with eosin G in the presence of the optimal amount of hydrochloric acid. The results shown in Figure (8) and Figure (9) confirm that the output is formed in a ratio of 1:1.

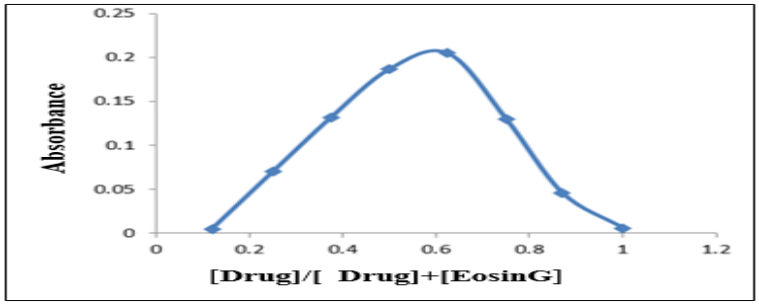

Figure (8) A diagram of the continuous changes of the complex consisting of tamoxifen citrate – eosin G

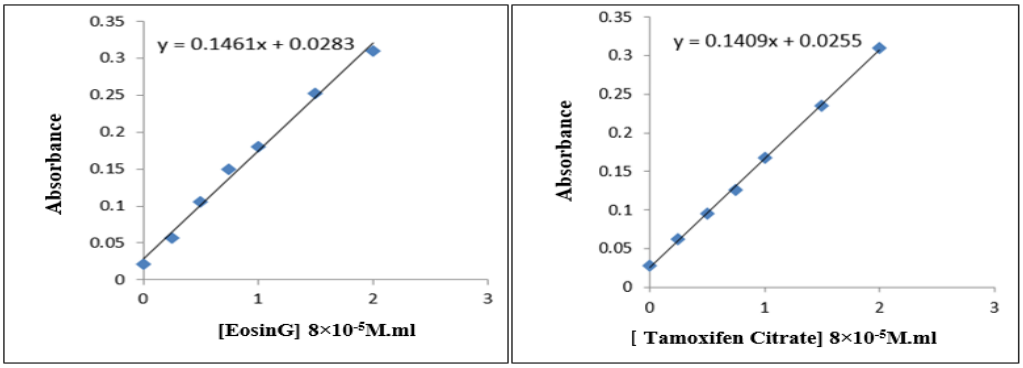

Figure (9) Graph of the slope ratio for the complex consisting of tamoxifen citrate-eosin G

suggested reaction:

By studying the nature of the formed complex, it was found that the ionic paired of the complex is formed in a ratio of 1:1 drug: Iosin G. The proposed method relied on the formation of the ionic paired complex, which is expected to be the result of ionic attraction between positive and negative ions. Figure (10) shows the proposed chemical reaction.

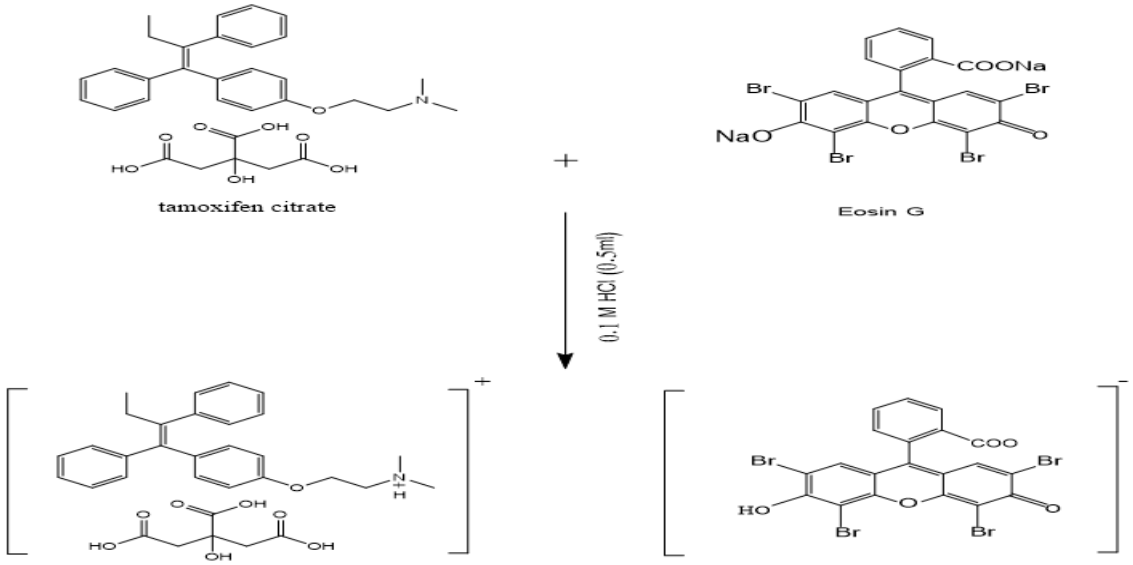

Figure (10) The suggested reaction of tamoxifen citrate‑eosin G

Conclusion:

A spectrophotometric method was described based on the reaaction of the formation of ionic complexes in aqueous media for the determination of tamoxifen citrate, It relied on the reaction of a microgram of the drug with the optimal amount of eosin G in the presence of hydrochloric acid in its optimal amount. The absorbance was measured at 543 nm, and the molar absorption coefficient was 3.8×10^4 $l.mole^{-1}ml^{-1}$, an indication of the sensitivity of the method and compliance with Beer's law for concentrations ranged from (0.25-12.5) $\mu g..ml^{-1}$, the relative standard deviation was less than 1.8% . This method was characterized by its simplicity and easy by not using the extraction and heating processes.

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