Spectrophotometric Determination of Promethazine Hydrochloride by the Oxidative Coupling Method Using Sulfamethaxazole as a Coupling Reagent

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Abstract:

This research involves the development of spectrophotometric method for determination of promethazine hydrochloride by oxidative coupling reaction with sulfamethaxazole in a neutral medium in the presence of potassium periodate to produce an intense green color, soluble in water, stable product and absorbs at 600.5 nm. Beer's law was in the linear range 3.125-43.75 µg/ml of promethazine hydrochloride, the molar extinction coefficint, Sandell's sensitivity index and detection limit were 7444.88 L. $mol^{-1}.cm^{-1}$, 0.0431 $\mu g.cm^{-2}$ and 0.0682 µg/ml respectively. The RSD value was (0.152-0.221)% depending on the concentration. This method was applied successfully to the determination of promethazine hydrochloride in pharmaceutical preparation(tablets) with recovery of not less than 99.93 % .

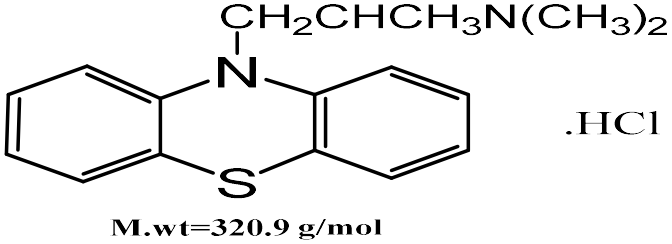
Key words: promethazine, sulfamethaxazole, oxidative coupling

The Introduction

Generic name for promethazine:

- (2-dimethylaminoproply)phenothiazine
- N,N&-trimethyl-10H-phenothiazine-10-ethanamine

The structural form



M.wt=320.9 g/mol

The molecular formula is: $C_{17}H_{20}N_2S_1.HCl$

It is a nerve depressant drug from the antihistamine class and belongs to the phenothiazine family .It is used to treat insomnia, help sleep, and treat symptoms of allergic rhinitis, runny nose, and sneezing [1].It is a white powder that dissolves quickly in water, alcohol and chloroform, but it is slightly soluble in acetone and ether
It turns from white to blue when exposed to moisture and air It shows the maximum absorption at the wavelength 249-297 nm [2], and it is odorless and its melting point is 222 ^oC [3].

Due to the medical importance of the compound under study, it was estimated by many different analytical methods Such as spectroscopic methods [4-14], flow injection methods [15-17], electrochemical methods [18-20], chemiluminescence methods [21], and chromatographic methods [22-25]. In this research, the drug was estimated in a simple and sensitive spectrophotometric manner by means of oxidative coupling reactions.

MATERIALS AND METHODS

Instruments

1-Shimadzu UV-Visible Spectrophotometer UV-160

2- Jenway pH/mv meter 3310

3-Ultrasonic with water bath, UNISONICS

4-Hot Plate with Magnetic Stirrer (BIOSAN MSH 300)

5-Sartorius BL210 S AG GOTTINGEN

chemicals

All the chemicals used were of high purity and supplied by Fluka and BDH.

material solutions used:
Promethazine standard solution 1000 µg (3.1162×10^{-3} M)1-

Prepared by dissolving 0.1000 grams of pure promethazine powder in a quantity of distilled water Then complete the volume to the mark in a 100 mL volumetric vial Solutions of less concentration were prepared by dilution.

2- Promethazine solution 250 µg.mL (77.90588×10^{-2}M).
This solution was prepared by drawing 25 ml of the solution prepared at a concentration of 1000 µg/ml and placing it in a 100 ml volumetric flask and filling the volume up to the mark with distilled water.

Sulfamethaxazole reagent solution 1 x 10^{-2} molar . 3-
The solution was prepared by dissolving 0.253 grams of the reagent in 2 milliliters of concentrated sulfuric acid Then complete the volume to the mark in a 100 ml volumetric vial with distilled water.

4- Potassium periodate solution 1 x 10 -2 M
This solution was prepared by dissolving 0.23 g of potassium periodate in distilled water using a water bathThen, after dissolving, add the volume up to the mark in a 100 ml volumetric vial with distilled water.

5- Sodium hydroxide solution on approximate day (1 M).
This solution was prepared by dissolving 4 gm of sodium hydroxide in a small amount of distilled water and then to the mark in a volumetric bottle of 100 ml with distilled water.

Interactants solutions 1000 µg/ml6-
It was prepared by dissolving 0.1000 g of each substance in distilled water and completing the volume to 100 ml in a volumetric vial of 100 ml with distilled water, from which dilute solutions were prepared.

7-Approximate hydrochloric acid solution (1M)
This solution was prepared by diluting 8.5 milliliters of concentrated HCl with a concentration of 11.8 M with distilled water .the volume was brought up to 100 ml, using a 100 ml volumetric vial from which the diluted solutions were prepared.

8-Pharmaceutical solutions 250 micrograms / ml

This solution was prepared from the preparation prepared by (Brussels Laboratories Pvt. Ltd) in India, where each tablet contains 5 mg, equivalent to 0.05 grams.It is prepared from grinding 20 grains well, the average weight of one grain is (0.6939), and the weight of (0.025) gm is taken from it and dissolved in the same way as the standard solution.then

the solution was filtered and the precipitate was washed several times, after that the filtrate was transferred to a volumetric vial of 100 ml and the volume was filled with distilled water to obtain a solution with a concentration of 250 µg/ml.

Preliminary Investigations

It was observed that when the promethazine solution was mixed with the sulfamethaxazole reagent solution in the presence of potassium periodate with slight shaking, a dark green compound was formed.It showed maximum absorption at a wavelength of 600.5 nm, while the photo solution showed little absorption at the above wavelength.Therefore, the optimum conditions for the coupling reaction were studied to obtain the best possible results in order to develop a simple and sensitive spectrophotometric method for the determination of promethazine.

Optimization of the Experimental Conditions

Subsequent experiments were carried out using 1 ml of the oxidizing agent solution, 1 ml of the used reagent solution, and 1 ml of promethazine solution with a concentration of 250 µg/ml in a final volume of 20 ml. The absorbance of the solutions was measured at 600.5 nm against the mock solution.

Choosing the best coupling reagent

A number of chemical compounds that can be used as coupling reagents have been used.Such as (2,4-dinitrophenylhydrazine, pyrocatechol, para-phenylenediamine, para-aminophenol, orthotolydine, 1,4-diaminobenzene, sulfamethaxazole) at a concentration of 1 x 10^{-2} molar in determination of promethazine In the presence of the oxidizing agent potassium periodate at a concentration of 1 × 10^{-2} M, the absorbance of each sample against its mock solution was measured in the wavelength range between 200-800 nm.And sulfamethaxazole was chosen as a coupling agent because it gave the highest absorption of the colored product at the wavelength of 600.5 nm.

Choosing the best oxidizing agent

Several oxidizing agents were used such as (potassium periodate, N-bromo succinamide, ammonium sulfate, potassium ferric cyanide, cerium ammoniac sulfate, ferric chloride) At a concentration of 1 x 10^{-2} M each was added to 1 ml of promethazine at a concentration of 250 µg/ml, then 1 ml of sulfamethaxazole reagent was added at a concentration of 1 × 10^{-2} M in a volumetric vial of 20 ml.The volume was supplemented with

distilled water to the mark. Then, the absorbance of each sample was measured against its mock solution in a wavelength range between 200-800 nm, and it was found that the best oxidizing agent is potassium periodate.Because it gave the highest absorption at wavelength 600.5 and was used in subsequent experiments.

The effect of the size of the oxidizing agent

The volume effect of the oxidizing agent potassium periodate (KIO4) was studied at a concentration 1×10^{-2} molar by adding volumes of (0.2-1.8) ml of it to volumetric vials of 20 ml capacity containing 2 ml of promethazine solution at a concentration of 250 µg / ml And then add 1 ml of the sulfamethaxazole reagent solution at a concentration of 1×210 M and complete the volume to the mark with distilled water and the results are as shown in Figure (1)

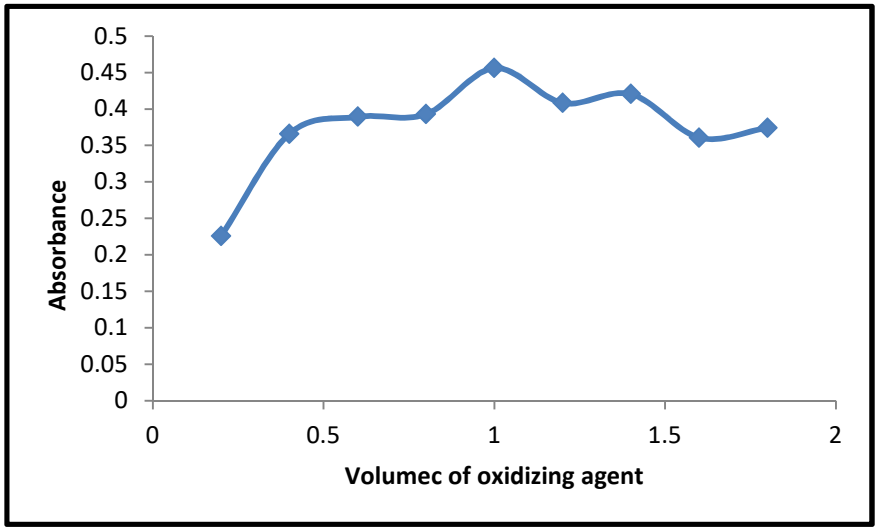


Figure (1) The effect of the size of the oxidizing agent

It was found from the results shown in Figure (1) that the best volume of the oxidizing agent solution that gives the best absorption is 1.0 ml, which was used in the subsequent experiments.

Detector size effect

The effect of the volume of the conjugation reagent was studied by taking a volume of 2 ml of promethazine at a concentration of 250 µg/ml in volumetric flasks (20 ml) and adding a volume of 1 ml of the oxidizing agent potassium pyridate KIO4 at a concentration of 1 × 10-2 mol and adding different volumes of sulfamethaxazole reagent starting from (2 - 0.2) milliliters volume is completed to the mark with distilled water,And the absorption was measured against the dummy solution for each of them, and it was found that the volume of 1.8 gave the best absorption,

and it was used in subsequent experiments, and the results are shown in Figure (2)

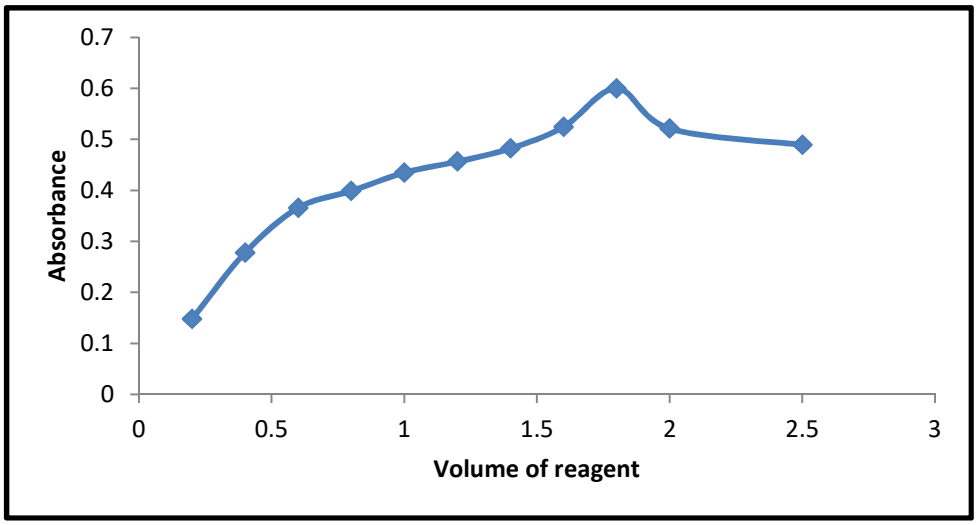


Figure 2: Effect of detector size

The effect of acid volume

The effect of acid volume on absorption was studied by adding different volumes (0.2-1.8) milliliters of hydrochloric acid solution with a concentration of 1 M to a series of volumetric bottles of 20 milliliters.Each contains 2 ml of promethazine at a concentration of 250 µg/ml and 1 ml of the oxidizing agent potassium periodate at a concentration of 1×10-2 M.The bottles were left for 5 minutes to complete the oxidation process, then 1.8 ml of sulfamethaxazole reagent was added at a concentration of 1×10-2M, after that the solutions were diluted with distilled water.Then the absorbance was measured at a wavelength of 600.5 nm against its mock solutions The acidity function was measured and it was found through the absorptions that there was no effect of adding the acid on the absorption, so the addition of the acid was excluded in the subsequent experiments. The results are shown in Table (1).

Table (1) the effect of acid volume on absorption

Volume of acid, ml	Absorbance
0.0	0.5997
0.2	0.5783
0.4	0.5364
0.6	0.5253
0.8	0.5098
1	0.4866
1.2	0.4498

1.4	0.4067
1.6	0.3856
1.8	0.3512

The effect of oxidation time

The time required for the oxidation of promethazine by potassium periodate was studied by taking a series of volumetric vials of 20 ml . It contains 2 milliliters of promethazine solution at a concentration of 250 µg/ml, to which 1 milliliter of potassium periodate solution at a concentration of 1×10-2 M was added.The solutions were left for different periods of time, then .81 ml of the sulfamethaxazole reagent solution was added at a concentration of 1×10-2M,Then it was diluted with distilled water to 20 milliliters.The absorbance of the solutions was measured at a wavelength of 600.5 nm against their mock solutions, and the results are shown in Table (2).

Table (2) Effect of oxidation time on absorption

Time minutes	Direct	5	10	15	20	25	30	35
Absorbance	0.3631	0.5527	0.5079	0.4998	0.4454	0.4099	0.4038	0.4026

It is noted from the above table that 5 minutes is sufficient to complete the oxidation process and was adopted in subsequent experiments.

Sequence effect additions

The effect of changing the sequence of additives to the solutions used in the reaction was studied Since the sequence of addition has an effect on the intensity of the color of the resulting compound, so a number of experiments were conducted with different sequences of additions.Note that all the volumes and concentrations of the materials used were the same in all cases. It was noted from the results obtained in Table (3) The

first order gives the highest absorption, so it was used in subsequent experiments.

- Promethazine (C)
- KIO_4(O)
- Sulfamethoxazol (R)

Table (3) Effect of sequence of additions on absorption

No	Order of additions	Absorbance
1	C + O + R	0.5676
2	O + R + C	0.2257
3	C + R + O	0.4193
4	R + O + C	0.3152

stability of the resulting product

The stability of the resulting product was tracked using the optimal conditions obtained in previous experimentsThis is done by taking a volume of 2 ml of promethazine solution at a concentration of 250 µg/ml, and adding 1 ml of the oxidizing agent potassium periodate at a concentration of 1×10-2 M.The bottles were left for 5 minutes to complete the oxidation process, then 1.8 ml of the sulfamethaxazole reagent solution was added at a concentration of 1×10-2 M Then fill the volume up to the mark with distilled water in volumetric vials of 20 milliliters ,After that, the absorbance of the colored solutions was measured after leaving them for certain periods of time in minutes at a wavelength of 600.5 nm against their mock solutions, and the results are shown in Table (4).

Table (4) stability of the product formed

Time (min)	Absorbance
5	0.5111
10	0.5654
15	0.5469
20	0.5623
25	0.5698
30	0.5738
35	0.5768
40	0.5791
45	0.5584
50	0.5582
55	0.5873
60	0.5743

It is clear from the results in Table (4) that the stability of the colored product has been proven after 5 minutes and for at least 60 minutes, which is a sufficient period for conducting measurements .

temperature effect

The effect of temperature on the absorption and stability of the formed colored product was studied using temperatures of 5-50°C. The results are shown in Table (5).

Table (5) Effect of temperature on absorption

Temperature ^{o}C	Absorbance
5	0.4956
10	0.4704
15	0.5044
20	0.5405

25	0.5637
30	0.5109
35	0.4725
40	0.4515
45	0.4436
50	0.4329

Table (5)shows that the optimum temperature is 20-30 °C The absorption decreases as the temperature increases, so 25°C was used in subsequent experiments.

influence of solvent type

After adding all the reaction components according to the optimal values in the previous experiments,Various solvents were used to complete the volumes to the mark in 20 ml volumetric vials.The results are as shown in Table (6).

Table (6) Effect of solvent type

Solvent	λ_{max}(nm)	Absorbance
Water	600.5	0.5930
Ethanol	604	0.4603
Acetone	604.5	0.3819
Diethyel ether	Turbid	Turbid
Methanol	600	0.4222

The results are shown in Table (6) that the use of distilled water gives the highest absorption of the resulting solution compared to the solvents used, and therefore it was used as a solvent in all subsequent experiments .

final absorption spectrum

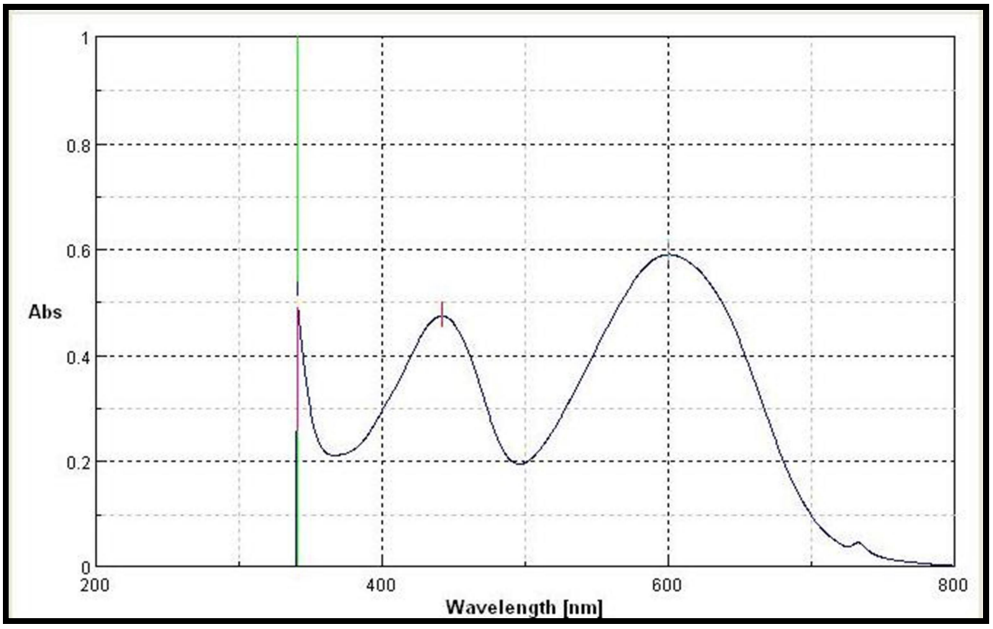
Through the results of previous experiments, the optimal conditions for the determination of promethazine were summarized as shown in Table (7).

Table (7) is a summary of the optimal conditions for the determination of promethazine

Experimental Conditions	The value
λ_{max} (nm)	600.5
Amount (ml) of 1 $\times 10^{-2}$ M potassium periodate	1.0
Amount (ml) of 1 $\times 10^{-2}$ M sulfamethoxazol	1.8
Oxidation time	min5
Temperature (°C)	25
Solvent	Water

The wavelength of the highest absorption was confirmed under optimal working conditions for the determination of promethazine by measuring the absorption spectrum of the resulting solution, and Figure (3) was obtained.It turns out that the wavelength of the highest absorption is 5.600 nm,And according to what was found in the preliminary tests. While the photo solution did not show any absorption at the aforementioned wavelength, Figure (4).

Figure (3): Absorption spectrum of the product formed against the plank



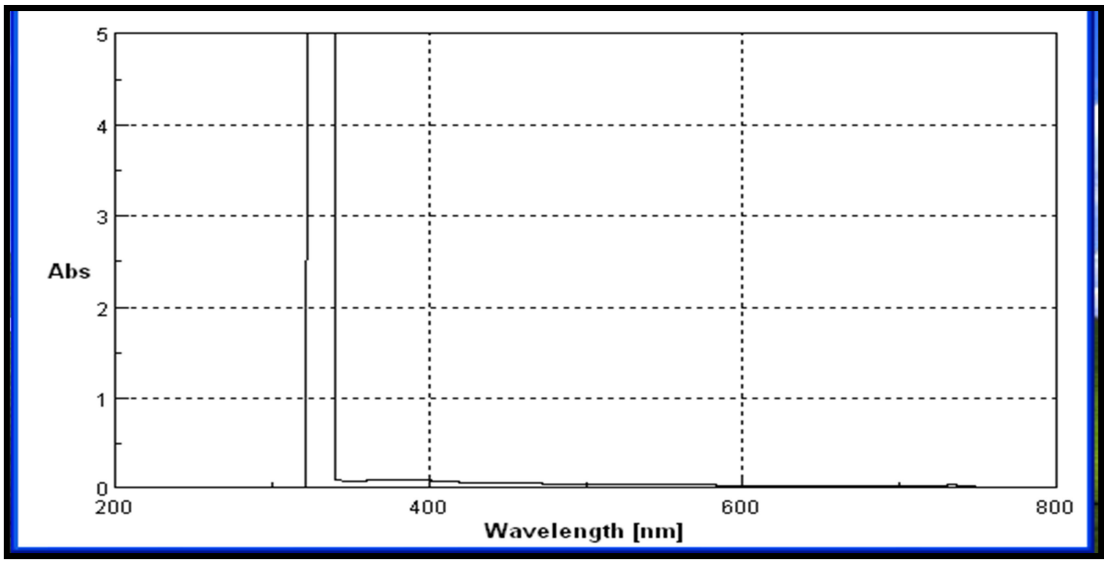


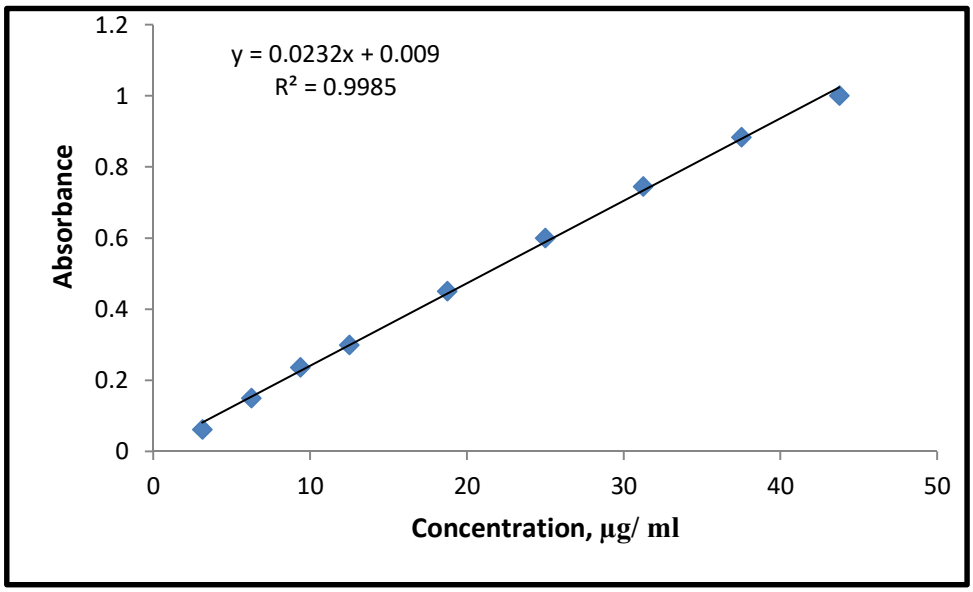
Figure 4: Absorption spectrum of planck solution versus distilled water

Approved working method and calibration curve preparation

After fixing the optimal conditions for the determination of promethazine, the standard curve was prepared according to the following:Incrementing volumes (0.25-5) mL of a 250 µg/mL promethazine solution were added to a series of 20 mL volumetric vials,1 ml of the oxidizing agent potassium periodate solution was added to it at a concentration of $^{2-}10$ x 1 M, and the solutions were left for 5 minutes to complete the oxidation reaction.Then 1.8 ml of the sulfamethaxazole reagent solution was added at a concentration of 1 x 10^{-2} M, then the volume was added to the mark with distilled water.The absorbance of all solutions was measured at .5600 nm against the mock solution for each.

Figure (5) represents the standard curve that follows Beer's law for a range of concentrations between (3.125 - 43.75) µg/ml of promethazine.The molar absorbance of the method was 7444.88 L.mol^{-1}.cm^{-1}, and Sandel's significance was 0.0431 µg.cm^{-2}.

Figure 5: The standard curve for the determination of promethazine



Method accuracy and compatibility[26]

The accuracy and compatibility of the proposed method for the determination of promethazine were calculated under the optimal conditions indicated in the working method.This was done by calculating the recall, and the relative standard deviation for two different concentrations of promethazine (6.25 and 9.375) µg/ml by taking an average of six readings for each.The recovery rate was 99.935%, and the relative standard deviation did not exceed 0.1868%.That is, the method has high accuracy and satisfactory agreement, and the return, its mean, and the relative standard deviation were calculated mathematically as follows :

RE % =O-T/T×100

RE = relative error

O = practical value

T = true value

: The(recovery) value is calculated from the following law

Recovery % =RE % + 100

As for calculating the percentage value of the relative standard deviation, the following law is applied:

$$RSD = \frac{S}{\overline{X}} \times 100$$

S = standard deviation

rate of reads= $\overline{X}$

.)The results are shown in Table (8

Table (8) method accuracy and compatibility

Amount of Promethazine taken µg /ml	RE, %	Recovery, %	Average recovery,%	RSD, %
6.25	+0.1336	99.86	99.935	0.221
9.375	+0.0141	100.01		0.1526

detection limit and quantity limit

The quantitative and qualitative limits of detection were calculated by measuring the absorbance of 10 solutions of the lowest concentration (3.125 µg / ml) in the calibration curve within the limits of Beer's law and at optimal conditions.The qualitative and quantitative detection limits were respectively 0.0682 µg/ml and 0.2066 µg/ml, as shown in Table (9), and it was calculated from the following mathematical relationship [27].

LOD=3.3S/B

LOQ=10S/B

Since::

DOD = Qualitative Detection Limit

LOQ = Limit of Quantitative Detection

S = standard deviation of the lowest concentration

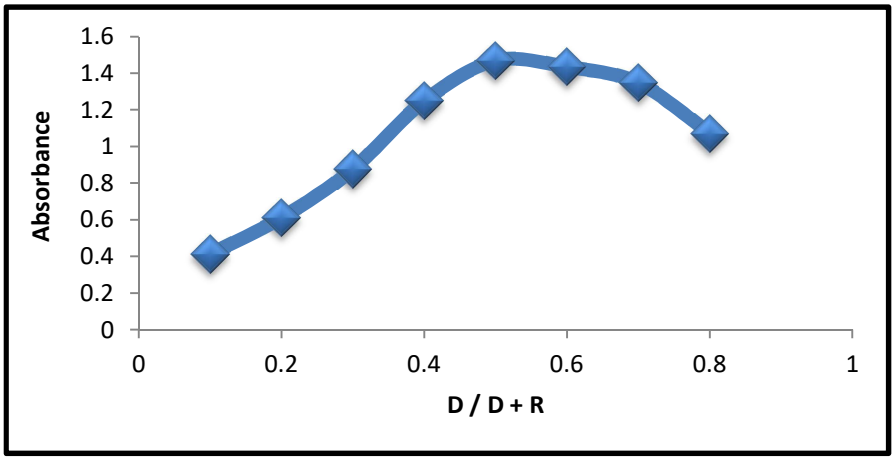
B = slope

Conncentration µg /ml	*B*	*S*	*LOD µg /ml*	*LOQ µg /ml*
3.125	0.0232	0.0004795	0.0682	0.2066

The nature of the resulting product

To find out the nature of the product formed and the ratio of the drug's bond with the reagent, the methods of continuous changes (Jopp's method) and the molar ratio method were applied.In both methods, the concentration of both the promethazine solution and the sulfamethaxazole reagent solution is in the same concentration, which is 2-10×1 molar.In (Job's method) (28) different volumes of the drug solution ranging from 0.9-0.1 milliliters were placed in volumetric vials with a capacity of 20 milliliters, and the supplements of these sizes were added to the volume of 1 milliliter of the reagent solution, then 1 milliliter of potassium periodate solution with a concentration of 10-2 was added. × 1 molar and diluted with distilled water to the mark After that, the absorbance of these solutions was measured at a wavelength of 5.600 nm against their mock solutions. Figure (6) shows that the ratio is 1:1 between promethazine and the reagent.

Figure(6) Curve of the method of continuous changes (Jopp's method)


Absorbance
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
D / D + R

To ensure that the reaction ratio between promethazine and the reagent is 1:1, the molar ratio method (28) was used.1 ml of promethazine drug solution was placed in a series of 20 ml volumetric bottles, and the reagent solution was added to it in different volumes (3.0-3.5) ml, then the rest of the additions were completed with the optimal sizes. It was diluted with distilled water to the mark, and the absorbance of these solutions was measured at a wavelength of 5.600 nm against the mock solution for each of them.It was found that the molar ratio agrees with the method of changes. Figure (7) confirms that the ratio is 1:1 between promethazine: sulfamethaxazole.

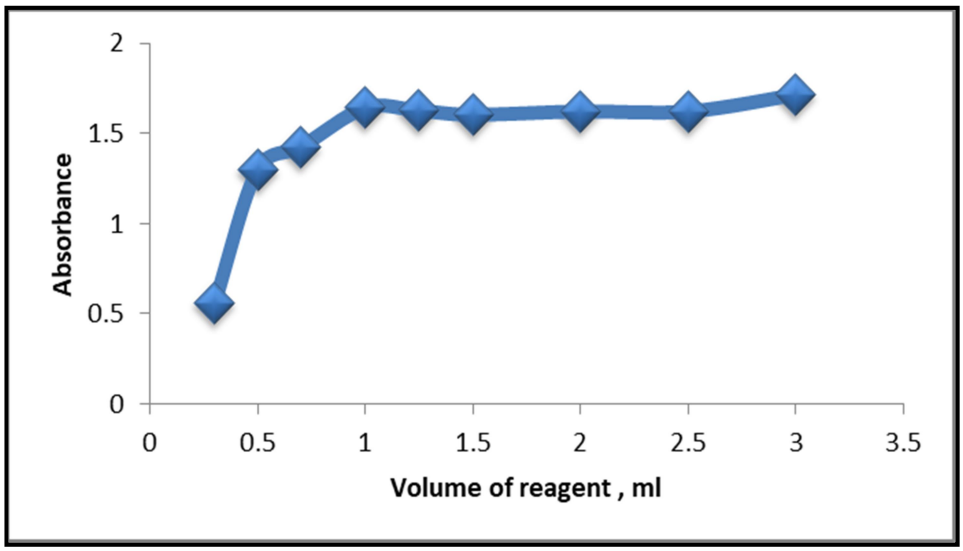
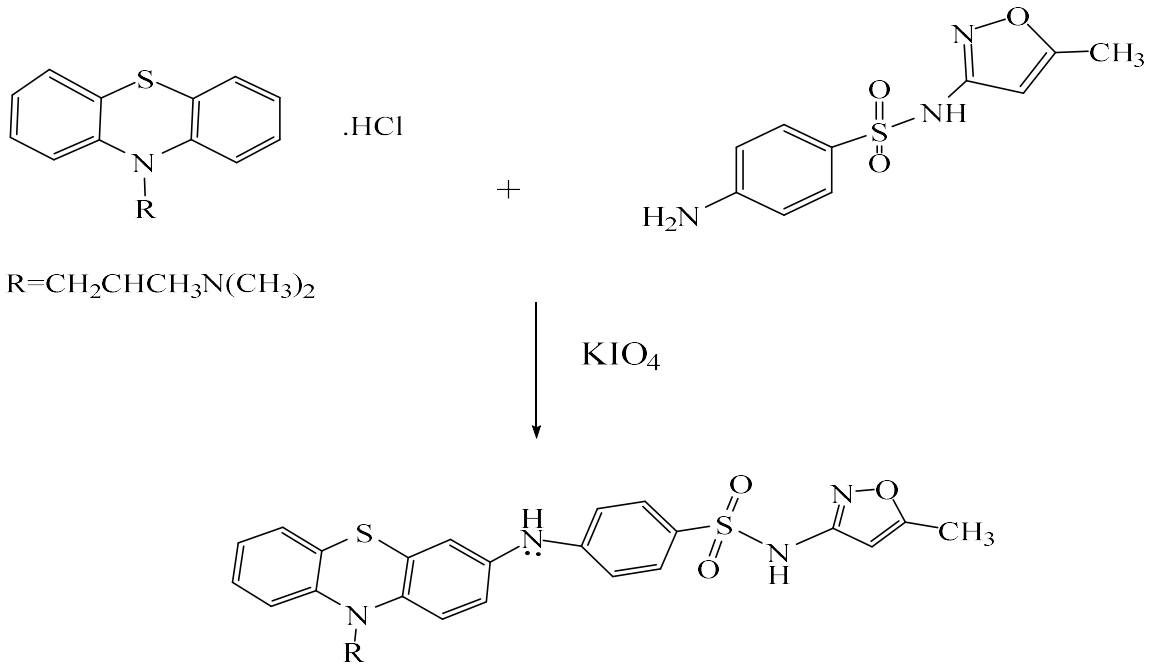


Figure (7) The curve of the molar ratio method

Therefore, the proposed reaction equation is as follows:

Foreign Compound	Recovery (%) of 500 µg Promethazine / µg and Foreign Compound Added		
	100	200	300



Green Color Complex

interference effect

For the purpose of examining the selectivity of the method and its applicability to pharmaceutical preparations,The effect of the interactions was studied by adding different volumes of these interactions (2,4,6) ml at a concentration of 1000 µg/ml to a series of 20 ml volumetric vials containing 2 ml of promethazine solution and 1 ml of oxidizing agent at a concentration of

1×10^{-2} molar

The solutions were left for 5 minutes to complete the reaction, then 1.8 ml of the sulfamethaxazole reagent solution was added at a concentration of

1×10^{-2}.After that, it was diluted with distilled water to the mark, then the absorbance was measured for all solutions at a wavelength of 600.5 nm against their mock solutions, and by calculating the recovery for each addition, it was found that there was no effect of the interventions used on the absorption, which makes the possibility of applying the method to pharmaceutical preparations, and the results are

Foreign Compound	Recovery (%) of 500 µg Promethazine / µg and Foreign Compound Added		
	100	200	300
Maltose	100.01	99.93	100.03
Glucose	99.74	99.69	99.79
Lactose	99.89	99.84	99.78
Sucrose	96.68	99.74	99.78
Mannose	99.87	99.83	100.07

Table (10) Effect of Interferometers on Absorption

The applied part

Determination of promethiazine in a 5 mg tablet by direct method

Different volumes (1, 2, 3) milliliters of the prepared 5 mg tablet solution were taken to obtain concentrations of 12.5, 25, 0.37.5 µg/ml. These volumes were treated according to the optimal working method, where these volumes were placed in three volumetric vials with a capacity of 20 ml. To each vial, a volume of 1 ml of the oxidizing agent potassium periodate at a concentration of 0.01 M was added, and 1.8 ml of the reagent sulfamethaxazole at a concentration of 0.01 M was added.The absorbance (an average of six readings) was measured for each solution against its mock solution at a wavelength of 600.5 nm, and the retrospective and RSD were calculated, and the results are shown in Table (11)

Table (11) Determination of Promethazine in Pharmaceutical Tablets (5mg) by the direct method

Amount of promethazine taken µg /ml	RE, %	Recovery*, %	Average recovery,%	RSD, %
12.5	-1.373	98.59	99.50	0.7138
25	-0.10	99.89		0.4670
37.5	0.0339	100.03		0.2883

* Average of five determinations

The results shown in Table (11), confirmed the success of the proposed method in the determination of promethazine in the studied drug tablets.

Conclusions:

A new spectrophotometric method for the determination of promethazine, simple and with high sensitivity, was developed It is based on the direct determination of promethazine by the method of oxidative coupling reactions with the reagent sulfamethaxazole acid 1×10^{-2} M in the presence of the oxidizing agent potassium periodate at a concentration of 1×10^{-2} M,

The reaction was carried out in a neutral medium and a water-soluble and stable product appeared that was green in color and gave the highest absorption at the wavelength of 600.5 nm. The method follows Beer's law in the range of 3.123-43.75 µg/ml .A deviation occurred at higher concentrations, and the molar absorptivity was 7444.88 liters. $Mall^{-1}$. CM^{-1} and the Sandal significance is 0.0431 µg. -cm2, relative standard deviation (0.152-0.221), with a detection limit of 0.0682 µg/mL,The method was successfully applied in the determination of promethiazine in pharmaceutical preparations (tablets), with a recovery of 99.93%.

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