

Determination of Promethazine Hydrochloride in pharmaceutical forms by Spectrophotometric Method

تقدير البروميثازين هيدروكلوريد في المستحضرات الصيدلانية بطريقة طيفية

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ABSTRACT

This research contains the development of a sensitive spectrophotometric method for determination Promethazine Hydrochloride in aqueous solution based on reduction of Fe^{3+} to Promethazine Hydrochloride . The Fe^{2+} formed is complexed with 1,10-Phenanthroline at pH 3.10 to produce a red-colour , water soluble and stable complex, which exhibits maximum absorption at 514 nm. Beer's law obeyed in the concentration range from 2-16 $\mu\text{g/ml}$ of Promethazine Hydrochloride . The molar absorptivity is $34015.4\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and Sandell's sensitivity index of $0.0094\ \mu\text{g}\cdot\text{cm}^{-2}$, a relative standard deviation is no more than 1.34%, and D.L 0.138 $\mu\text{g/ml}$. The method has been successfully applied to the determination of Promethazine Hydrochloride in tablets.

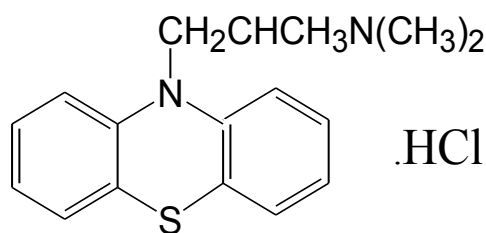
Key words: Promethazine hydrochloride determination, Promethazine hydrochloride absorption.

المخلص:

تصمّم تطوير طريقة طيفية جديدة وحساسة لتقدير البروميثازين هيدروكلوريد في الوسط المائي، تعتمد الطريقة على اختزال ايون الحديد الثلاثي بواسطة البروميثازين هيدروكلوريد إلى ايونات الحديد الثنائي ، في وسط دالته الحامضية 3.10 ، ثم تفاعل ايون الحديد الثنائي مع الكاشف 1,10-فينانثرولين يعطي معقداً احمر اللون مستقراً وذائباً في الماء تعتمد شدة امتصاصه على تركيز البروميثازين في المحلول ويعطي أعلى امتصاص عند الطول الموجي 514 نانوميتر ، كانت حدود قانون بير في مدى التراكيز 2-16 مايكروغرام/ مل البروميثازين ، وكانت الامتصاصية المولارية 34015.4 لتر.مول⁻¹.سم⁻¹ ، معدل الاسترجاعية 100.264 والانحراف القياسي النسبي لا يتجاوز 1.34%، وبحد كشف 0.138 مايكروغرام/مل، وتم تطبيق الطريقة بنجاح في تقدير البروميثازين هيدروكلوريد في مستحضرات صيدلانية على شكل اقراص.

Introduction

Its composition formula



Molecular formula: $C_{17}H_{20}N_2S \cdot HCl$, M.Wt 320.9 g/mol, UV max: 294 - 297nm

A white or faintly yellowish, crystalline powder, is very soluble in water, freely soluble in alcohol and in methylene chloride. It melts at about 222°C with decomposition, store in a well-closed container, protected from light^[1,2]. It is used as anti-histamine, antagonist, anti-emetic^[3]. Promethazine hydrochloride is determined by various analytical methods including the following [4,5], spectral methods^[6,7], flow injection methods [8, 9], other methods[10, 11].

The Aim of the Research

A sensitive and rapid spectral method was developed to estimate promethazine hydrochloride based on the method of reduction of Fe^{3+} tri-iron ion by promethazine and then complex formation of Fe^{2+} -Phen. It is soluble in water and stable for a period of time of at least 60 minutes and has maximum absorption at a wavelength of 514 nm. The standard calibration curve shows that the linear range 2-16 $\mu g / ml$, the value of the standard deviation was less than the mean (0.761), the recovery is 100.264%, molar absorptivity $34237.9 (L \cdot mol^{-1} \cdot cm^{-1})$, and Sandell Index $0.0094 \mu g \cdot cm^{-2}$. The analytical parameter indicates that the method accurate, sensitive, so reliable and could be applied to pharmaceutical analysis successfully.

Experimental Works

Preliminary investigation

Accurately measured portion (1-4)ml of (100) $\mu g / ml$ of promethazine hydrochloride standard solutions were transferred into series of 25ml volumetric flasks. 0.5 ml of (1622 $\mu g/ml$) of ferric chloride standard solution was added to each of the above flasks and left the mixture for 20 minutes. After the oxidation process was completed, 2ml of (1802 $\mu g / ml$) of detector solution was added to all flasks. The absorbance of the colored solution was measured at 514nm versus blank solution. Figures

Apparatus

1-Spectral and absorbance measurements are carried out using Shimadzu UV-160, UV-Visible 2-computerized double-beam spectrophotometer, Japan. In all measurements, 1cm matched silica cells are used.

3-The pH measurements are carried out using Philips PW 9421 pH meter.

Sensitive balance model Sartorius.

Hot plate with magnetic stirrer model Jenway, Wite Germany.

Reagents and chemicals used

The chemicals and analytical reagents used are all on a high degree of purity.

Preparation of the Standard Solutions

promethazine hydrochloride solution (1000 $\mu g/ml$).

This solution was prepared by dissolving 0.1000 g of promethazine hydrochloride (SDI, Iraq) powder in distilled water and the volume was completed to 100 ml with distilled water in a volumetric flask. This solution was kept away from light in dark volumetric flask.

Working promethazine hydrochloride solution (100 µg/ml).

This solution was prepared by diluting 10 ml of the above stock promethazine hydrochloride solution to 100 ml in a volumetric flask with distilled water.

Ferric(III) solution (1×10^{-2} M)(2703 µg/ml)

This solution is prepared by dissolving 0.1622g of ferric chloride (*Fluka, Italy*) in which volume distilled water and then 1 ml of it was diluted in 100ml of distilled water to get 0.01M of an acid solution.

1,10-Phenanthroline solution (1×10^{-2} M)(1802.1 µg/ml)

This solution was attended by dissolving 0.0400g of pure substance(*Fluka, Italy*) in 100ml of distilled water.

Hydrochloric acid solution (0.01M) (365 µg/ml)

Hydrochloric acid solution (1M) is prepared by diluting 8.4 ml of hydrochloric acid solution (1M)(*Fluka, Italy*)with purity (96%) from concentrated acid (11.8M) in 100 ml of distilled water then diluting 1 ml from it in 100ml distilled water to get 0.01M acid solution in a volumetric flask 100 ml.

Sodium hydroxide solution approximate (0.01M)(400 µg/ml)

Attended by dissolving 0.0400 grams of pure substance(*BDH, UK*) in 100 ml of distilled water in a volumetric flask 100 ml .

Pharmaceutical solution of Tablet 100 µg/ml

The proposed method has been applied on the preparation promethazine hydrochloride(SDI, Iraq) in tablets . Each tablet contains 100 mg promethazine hydrochloride , has been weight of 10 tablets each tablet alone and crushed well and then calculated the required weight to prepare a solution with concentration of 1000µg/ml , dissolved in amount of distilled water directly with shaking. The volume is completed up to 100 ml in a volumetric flask with distilled water ,then the solution was filtered and the solution of 100µg/ml was prepared.

Results and discussion

Study of optimum reaction conditions

The conducted subsequent experiments using 2 ml of 100 µg / ml of promethazine hydrochloride solution to a final volume 25 ml and measuring the absorption of solutions at a wavelength of 514 nm versus blank solution for each.

effect of Acid

To see the effect of the acid, different volumes ranging from 0 - 3 ml of hydrochloric acid with a concentration of 0.01 M were added to the complex and the results are recorded in Table(1)

Table (1) effect of Acid

ml of 0.01M HCl	Absorbance	pH
0.0	0.456	3.10
0.2	0.440	3.03
0.5	0.399	2.40
1.0	0.292	2.38
1.5	0.201	2.30
2	0.191	2.20
2.5	0.157	2.07
3	0.132	1.98

From the above Table shows that adding acid leads to a decrease in the absorption of the colored product so we should avoid its use in the subsequent experiments.

Effect of Base

To see the effect of the base, different volumes ranging from 0.1 – 1.5 ml of sodium hydroxide with a concentration of 0.01 M were added to the complex and the results are recorded in Table(2)

Table (2) effect of Base

ml of 0.01M	Absorbance	pH
0.0	0.451	3.10
0.1	0.444	3.12
0.3	0.368	3.13
0.5	0.330	3.14
0.7	0.318	3.21
1.0	0.292	3.25
1.5	0.268	3.29

The above previous two experiments (using effect the acid solution at concentration of 0.01 M hydrochloric acid and the effect of the base using a 0.01M solution of sodium hydroxide) we find that the pH 3.10 gives the highest absorbance of colored product at wavelength 514 nm and so should be adopted in the subsequent experiments.

effect of Buffer solution at pH 3.10

To adjust and maintain acid functionality, a buffer solution (Potassium Phthalate – Hydrochloric acid) with pH=3.10 was prepared [12] and its effect on absorption was studied and the results were shown in Table (3).

Table (3) effect of Buffer solution

ml of Buffer solution	Absorbance	pH
0.0	0.456	3.10
0.5	0.172	3.31
1	0.165	3.55
1.5	0.150	3.60
2	0.131	3.73

The results of Table (3) showed that the addition of a buffer solution resulted in a decrease in absorption with an increase in the value of the acidic function, so it should be adopted in subsequent experiments.

Effect of the amount of oxidizing agent(1×10^{-2} M) (1622 $\mu\text{g/ml}$)

The effect of ferric chloride volume has been studied by using different volume ranging from 0.3 - 3 ml of (1×10^{-2} M) (1622 $\mu\text{g/ml}$)of ferric chloride solution and leave the solution for 20 minutes to complete the oxidation. Then 1 ml of (1×10^{-2} M) (1802 $\mu\text{g/ml}$) of 1,10-Phenanthroline solution was added. The absorption of the solutions was measured at 514 nm wavelength versus blank solution for each of them and the results are recorded in Table (4).

Table (4) Effect of the amount of oxidizing agent

ml of 1×10^{-2} M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Absorbance
0.3	0.621
0.5	0.728
1	0.457
1.5	0.223
2	0.192
2.5	0.183
3	0.134

It was noted from the results shown in the Table above that the best volume of ferric chloride solution is in 0.5 ml, therefore it will be used in the subsequent experiments.

Effect of 1,10-Phenanthroline solution volume (Effect of Detector Volume(1×10^{-2} M)(1802 $\mu\text{g/ml}$))

This study has been used different volumes ranging 0.5 - 4 ml of (1×10^{-2} M)(1802 $\mu\text{g/ml}$) of a 1,10-Phenanthroline solution to different volumes from the drug, then the absorption of the solutions was measured at 514 nm wavelength versus blank solution for each of them and the results are recorded in Table (5).

Table (5)Effect of Detector Volume

ml 1×10^{-2} M of 1,10-phenanthroline	Absorbance, $\mu\text{g/ml}$ promethazine. HCl					R^2	Slope
	2	4	6	8	10		
0.5	0.152	0.263	0.287	0.377	0.458	0.986	0.036
1	0.250	0.293	0.448	0.688	0.703	0.957	0.063
1.5	0.276	0.329	0.580	0.719	0.901	0.993	0.087
2	0.301	0.384	0.643	0.725	1.133	0.960	0.106
2.5	0.216	0.367	0.522	0.683	0.972	0.991	0.085
3	0.201	0.228	0.480	0.636	0.661	0.959	0.069
3.5	0.168	0.183	0.384	0.517	0.573	0.958	0.053
4	0.130	0.164	0.323	0.388	0.490	0.973	0.043

It was noted from the results shown in the Table above that the best volume of for the solution of 1-10 phenanthroline is in 2 ml , therefore it will be used in the subsequent experiments.

effect of temperature

The effect of temperature on the absorption of the colored product formed and stability using temperatures ranging from 15 - 60 ° C and the results are recorded in Table (6) .

Table (6) effect of temperature

Temperature $^{\circ}\text{C}$	15	20	25	30	35	40	50	60
Absorbance	0.725	0.730	0.735	0.717	0.703	0.697	0.668	0.631

The results have shown that the temperature of 25⁰C gives the highest absorption of the product and room temperature is adopted in subsequent experiments.

Effect of time on stability of the colored product

The stability of the colored product has been studied by using different volumes (0.5, 1.0, 1.5 ml) of (3.1×10⁻⁴) (100 µg / ml) of the drug. And then the optimal values of the solutions were added to a volumetric flask of 25 ml and diluted to the mark with distilled water. The absorption of the solutions was measured at the highest wavelength and the results are shown in Table (7).

Table (7) Effect of time on stability of the colored product

ml of PRZH 100µg/ml	Absorbance standing time/ min.								
	5	10	15	20	25	30	40	50	60
1	0.370	0.373	0.375	0.375	0.377	0.379	0.378	0.376	0.377
2	0.728	0.733	0.750	0.753	0.755	0.755	0.756	0.756	0.758
3	1.287	1.295	1.317	1.321	1.322	1.324	1.325	1.324	1.326

It was observed from the results shown in the above table that absorption of the product increases after 15 minutes of dilution and then stabilizes for at least 50 minutes so it is a sufficient time for many experiments.

Effect of solvent

The effect of solvents on the resulting complex spectra was studied by diluting solutions with these solvents rather than distilled water. The absorbance spectra of these solutions were measured versus blank solutions and the results are shown in Table 8 and Figure 4.

Table (8) Effect of solvent

Solvent	λ _{max} (nm)	Absorbance	
Water	514	0.747	A
Acetone	510	0.589	B
2-Propanol	514	0.809	C
Ethanol	510	0.345	D
Methanol	510	0.322	E
DMSO	512	0.579	F

The results shown in Figure 8 and Table indicate that 2-propanol and water is a good medium of reaction and give high absorption value at 514 nm wavelength, As well as present study recommend to use distilled water because its availability and cheap price and therefore it was used as the best solvent.

The final absorption spectrum

After the optimal conditions of the reaction were established, the absorption spectra of the red-colour solution versus the photoluminescence were measured at a range of wavelengths of 200-800 nm. The colour output showed the highest absorption value at 514 nm while the photoreceptor gave weak absorption at the same wavelength.

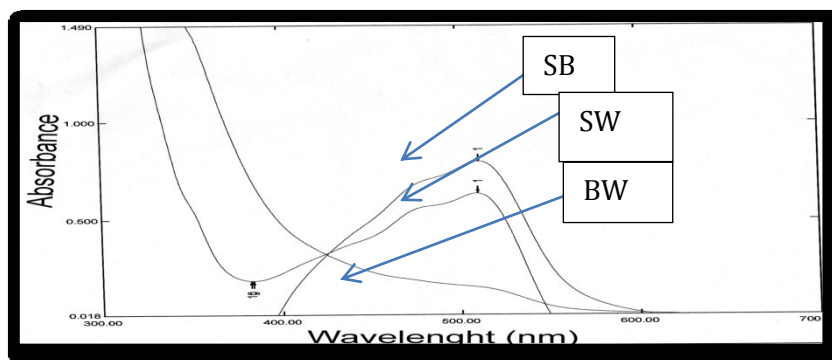


Figure (1) Final absorption spectrum

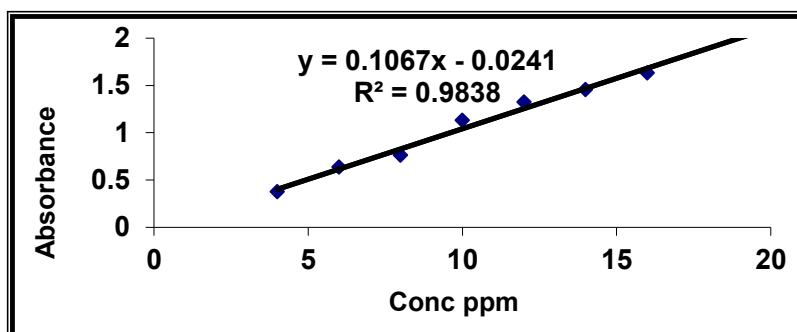
SW : the absorption spectrum promethazine hydrochloride & distilled water

SB : the absorption spectrum promethazine hydrochloride & Blank.

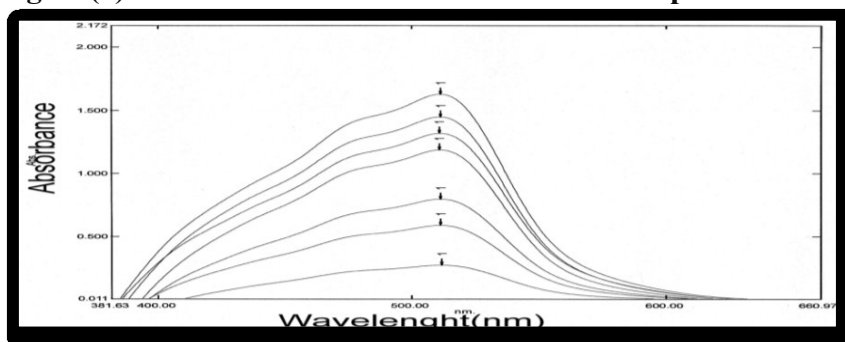
BW: the absorption spectrum Blank & distilled water

Preparation of Calibration curve

In series volumetric flask of 25 ml has been taking a different volume (1,1.5,2,2.5,3,3.5 and 4 ml) of 100 µg / ml promethazine hydrochloride solution with a different concentration of (10,15,20,25,30,35 and 40 µg / ml, respectively) and then add 0.5 ml of ferric chloride solution with concentration in (1×10^{-2} µg / ml)(2703 µg/ml) and leave the solution for 20 minutes to complete the oxidation then add 2 ml of detector solution with concentration in(1×10^{-2} µg / ml)(1802.1 µg/ml) to all flasks , measuring the absorption of solutions at a wavelength of 514 nm versus blank solution for each of them, Figures(2,3) shows the calibration curve



Figure(2) Calibration curve for determination of promethazine



Figure(3)Absorption spectrum for determination of promethazine

Precision and accuracy

The optimum conditions were used to test the accuracy of the calibration curve and its compatibility. Six readings of three different amounts of promethazine hydrochloride solution were taken within the limits of the Beer law in the calibration curve, From recovery and relative error was found that high-accuracy method[13] (recovery 100.2647%) and high precision (relative standard deviation[14] less than the mean 0.761%). Results are reported in Table (9).

Table (9) Precision and accuracy

Conc. of PRZH µg/ml	RE* %	Recovery* %	Average recovery%	RSD* %
4	-0.3999	99.6001	100.2647	1.340
10	+ 1.1943	101.1943		0.462
16	-0.0003	99.9997		0.481

*Average of six determinations

Detection limit

The detection limit was calculated by measuring the absorption of eleven solutions for the lowest concentration (4 µg / ml) in the calibration curve within the limits of the law and at the optimum condition , was the detection limit 0.138µg/ml.

The nature of product formed

In order to determine the nature of the red product and the molar ratio of the correlation with the detector, the method of Job and the percentage method were applied. In both methods, the concentration of promethazin hydrochloride solution and reagent solution is equal to (2.4×10^{-5} molar), in the job’s method[15]in a 25 ml volumetric flask. Different volumes of the solution were placed between 1 - 9 ml and 0.5 ml of oxidized agent solution at (1×10^{-2} molar)(2703 µg/ml) molar concentration. The solutions were left for 20 minutes to complete the oxidation. The supplementation was added to 10 ml of the reagent solution and was dilution to the mark with distilled water. The absorption of these solutions was measured at 514 nm wavelength and Figure (4) shows that the ratio is 1:1.

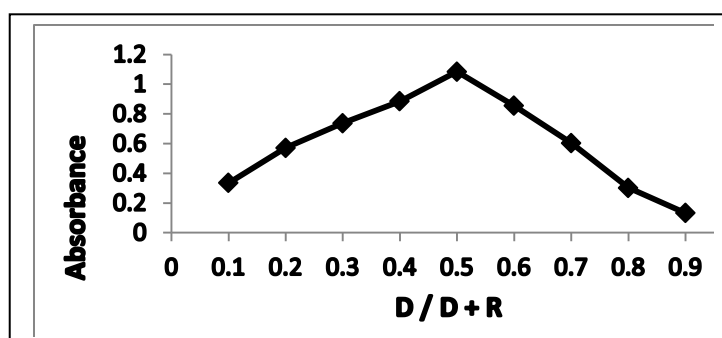


Figure (4) Job methods

In molar ratio method , 2 ml of the drug solution in series volumetric flask of 25 ml,. The reagent solution was added in volumes ranging from 0.5 - 4.5 ml and 0.5 ml of oxidized agent solution at 1×10^{-2} molar was added. The solutions were left for 20 minutes for oxidation completion then add 2 ml of 1×10^{-5} molar of the reagent solution. and completed the volume to mark with distilled water. The absorption of these solutions were measured at the wavelength of 514 nm versus blank solution. We found that the molar ratio should be consistent with the Job method and check the ratio 1:1

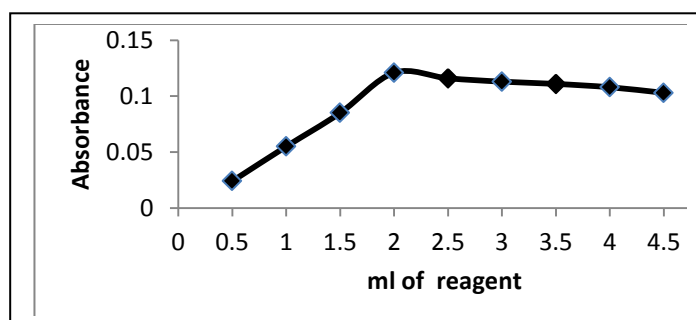


Figure (5) molar ratio method

Applications

The method was applied to pharmaceutical preparations containing promethazine hydrochloride, a promethazine drug, in 100 µg / ml tablets

Direct method

Three different concentrations of the solution were taken 4, 10 and 16 µg / ml. The solutions were treated with the same steps as the calibration curve. The absorption was measured at the 514 nm wavelength compared to the solution. The ratio of five measurements per concentration was calculated. In Table 10.

Table (10) Direct method

Drug	Type of pharmaceutical	Conc., of PRZH µg/ml	RE* %	Recovery* %	Average recovery* %
PRZH	Tablet	4.0	+1.7703	101.7703	101.1774
		10	+0.7575	100.7575	
		16	+1.0050	101.0050	

Average of five determinations

The results of the above Table show the success of the proposed method in the determination of promethazine hydrochloride in pharmaceutical preparation with high recovery 101.1774% for the preparation of promethazine Tablet.

Standard addition method

Apply the standard addition method. It was based on concentrations of the drug by taking 1 ml of 100 µg / ml solution of promethazine solution into six 25 ml volume bottles, then adding increasing volumes of standard solution promethazine (1 - 5 ml) with the remaining 6 bottles remaining without addition. The solutions were then treated in the same way as the calibration curve and then measured at 514 nm. Figure 8 and Table 11 show the results obtained when applying the standard addition method.

Table (11) Accuracy of standard addition method

Drug	Pharmaceutical preparation	µg/ml Promethazine HCl present	µg/ml Promethazine HCl measured	Recovery* %
Promethazine HCl	Tablet	10	10.4	100.4

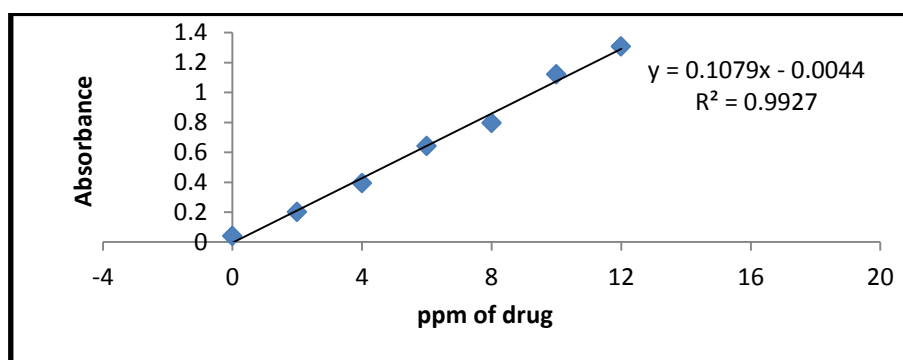
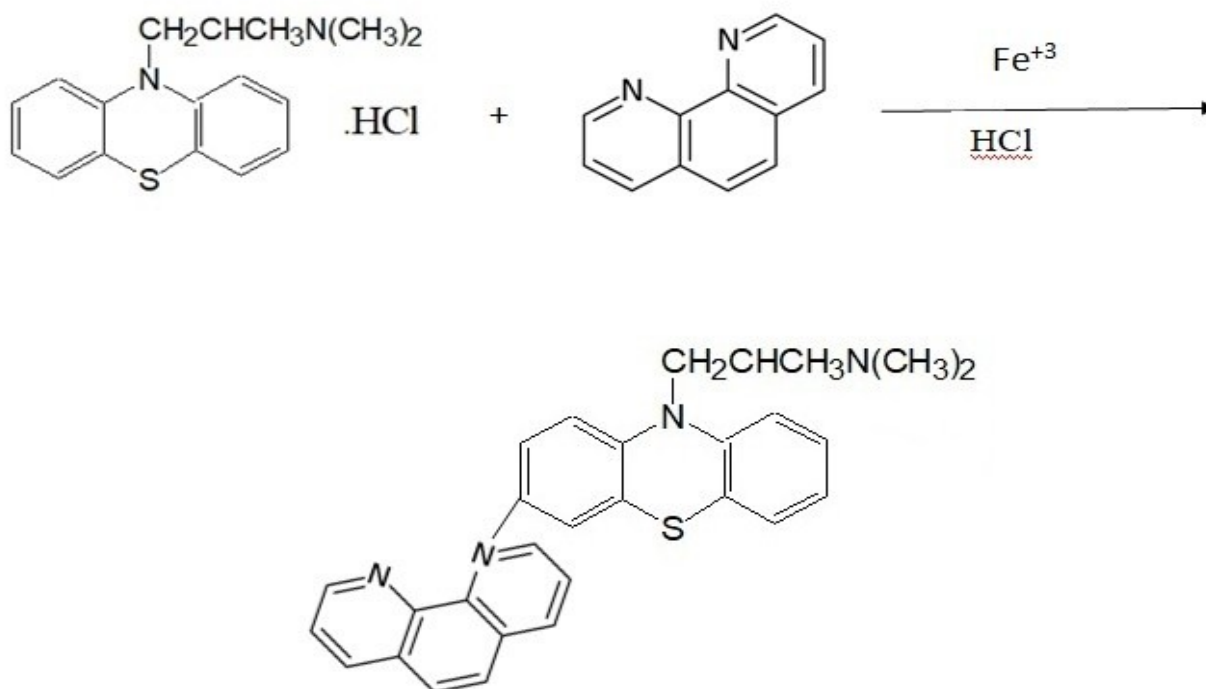


Figure (6) Regression line for standard addition method

The results shown in Table (11) is accurate and Figure (6) shows that the standard additions method is well in agreement with the proposed method for determination promethazine hydrochloride in pharmaceutical its preparation. Therefore the proposed reaction equation is as follows :



Conclusion

A sensitive and rapid spectral method was developed to estimate promethazine hydrochloride based on the method of reduction of Fe^{3+} tri-iron ion by promethazine and then complex formation of Fe^{2+} -Phen. It is soluble in water and stable for a period of time of at least 60 minutes and has maximum absorption at a wavelength of 514 nm. The standard calibration curve shows that the linear rang 2-16 $\mu\text{g} / \text{ml}$, the value of the standard deviation was less than the mean(0.761), the recovery is 100.264%, molar absorptivity 34237.9(L.mol⁻¹.cm⁻¹), and Sandell Index 0.0094 $\mu\text{g}.\text{cm}^{-2}$. The analytical parameter indicates that the method accurate , sensitive , so reliable and could be applied to pharmaceutical analysis successfully.

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