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A simple oxidative coupling spectrophotometric method for the determination of promethazine hydrochloride in pharmaceutical tablets with preliminary biological activity evaluation

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Abstract

A simple, sensitive, and cost-effective spectrophotometric method was developed for the determination of promethazine hydrochloride in pharmaceutical tablet formulations. The proposed method is based on an oxidative coupling reaction in which promethazine hydrochloride is oxidized by ferric chloride and subsequently coupled with potassium ferricyanide to form a stable blue-violet colored complex. The absorbance of the formed complex was measured at 682 nm using distilled water as the solvent under mild experimental conditions. The analytical procedure was optimized with respect to reagent concentration, reaction time, temperature, and solvent to achieve maximum color intensity and stability. Under the optimized conditions, Beer's law was obeyed over a concentration range of 1-40 µg/mL, with a high correlation coefficient, indicating good linearity. The method exhibited satisfactory sensitivity, precision, and accuracy, with low limits of detection and quantification. The proposed method was successfully applied to the determination of promethazine hydrochloride in commercial tablet formulations, and the results were in good agreement with the labeled amounts. In addition, a preliminary biological activity study was conducted to investigate the antimicrobial behavior of promethazine hydrochloride and its oxidative coupling complex. The results revealed noticeable antifungal activity, particularly against *Candida albicans*, while moderate antibacterial activity was observed against selected bacterial strains. The observed differences in biological activity between the free drug and the formed complex suggest that chemical complexation may influence biological behavior. The proposed method offers a practical analytical alternative for routine quality control analysis of promethazine hydrochloride in pharmaceutical preparations and provides additional insight into the effect of oxidative coupling on biological activity.

Keywords: Promethazine hydrochloride, oxidative coupling, spectrophotometric analysis, pharmaceutical tablets, antimicrobial activity

1. Introduction

Promethazine hydrochloride is a first-generation antihistamine with strong H₁-receptor antagonistic and antiemetic activities, and it is well known for its sedative properties. It is widely used for the prevention of motion sickness and for the management of nausea and vomiting, including postoperative conditions. Promethazine is also prescribed as a sedative or sleep aid, particularly in patients who require symptomatic relief, and it is used to alleviate allergic manifestations such as pruritus, rhinorrhea, sneezing, lacrimation, urticaria, and allergic skin eruptions. After intramuscular administration (25 mg) or oral dosing (25-50 mg), peak plasma concentrations are typically reached within 2-3 h; therefore, due to its sedative effect, nighttime administration is often preferred for allergic symptom control ^[1]. From a chemical perspective, promethazine hydrochloride (Promethazine HCl) is 10-[2-(dimethylamino) propyl] phenothiazine monohydrochloride (Figure 1) ^[2]. Its molecular formula is C₁₇H₂₀N₂S·HCl with a molecular weight of 320.88 g/mol, and it melts at approximately 230-232 °C.

Promethazine exhibits absorption maxima in the ultraviolet region, typically within 249-297 nm^[3]. The drug appears as a white to slightly yellowish crystalline powder, is highly soluble in water and readily soluble in alcohol, and is practically insoluble in methylene chloride; it should be stored in a tightly closed container protected from light^[4]. In pharmaceutical analysis, reaction-based spectrophotometric methods remain attractive because they are simple, inexpensive, and can provide high sensitivity. Among these approaches, oxidative coupling reactions are

particularly useful due to their operational simplicity and the formation of intensely colored products suitable for visible or near-visible detection^[5, 6]. Several methods have been reported for promethazine hydrochloride determination, including spectrophotometric procedures^[7, 8], chromatographic techniques^[9, 10], and electrochemical sensors^[11]. However, many of these methods may require sophisticated instrumentation, extended sample preparation, or expensive materials, which can limit their routine application in resource-limited laboratories.

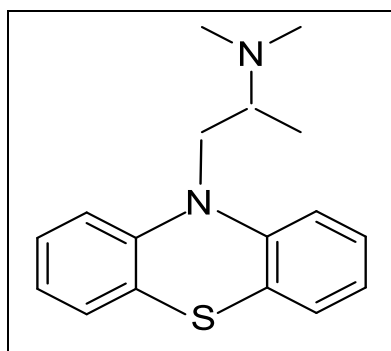
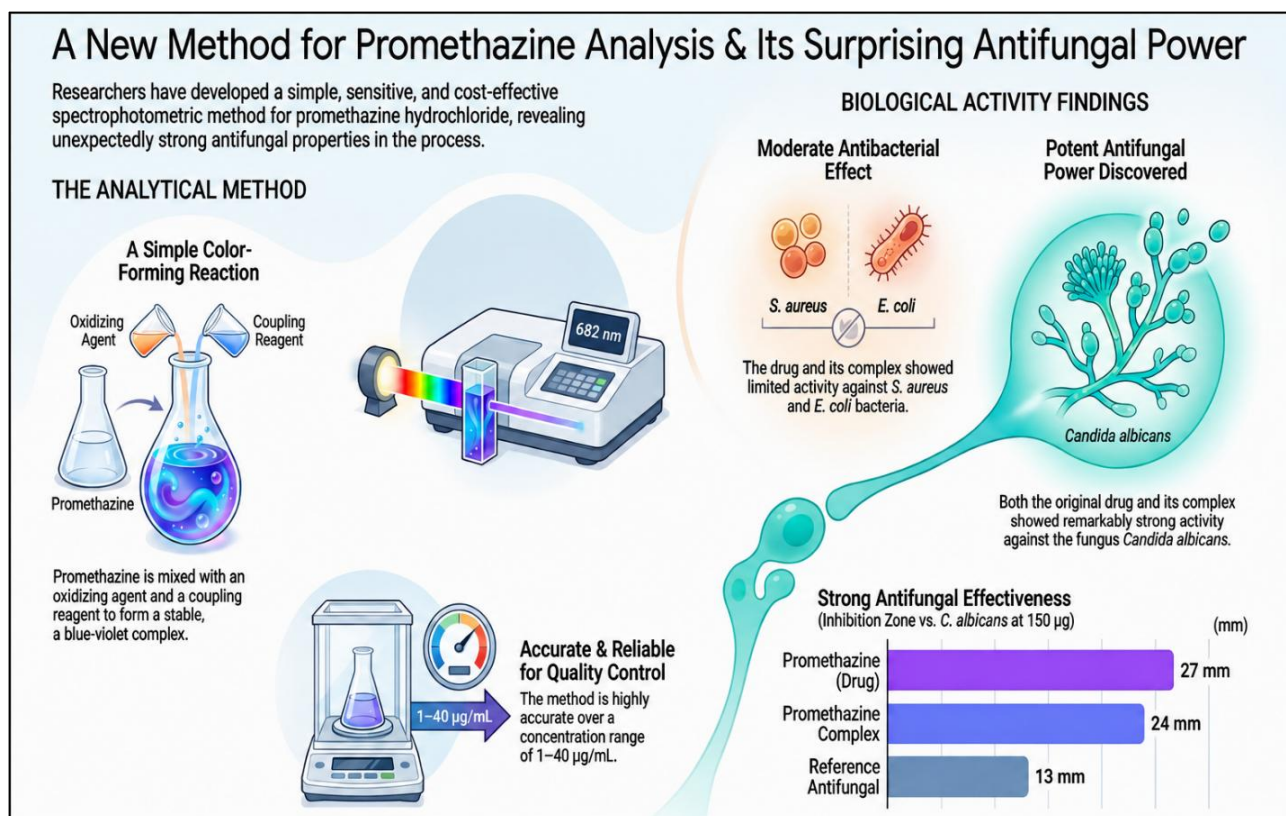


Fig 1: Chemical structure of promethazine^[2]

The present study aims to develop a simple spectrophotometric method for the determination of promethazine hydrochloride in pharmaceutical tablets based on an oxidative coupling reaction using ferric chloride (FeCl_3) in the presence of potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$). In addition, a preliminary biological activity evaluation is performed for both the free drug and the formed complex to explore whether complexation influences antimicrobial behavior. In recent years, increasing attention has been directed toward the development of simple and reliable analytical methods for routine quality control of pharmaceutical compounds.

Spectrophotometric techniques remain among the most widely used methods in pharmaceutical analysis due to their accessibility, rapid execution, and minimal instrumental requirements. These methods are particularly valuable in laboratories where advanced chromatographic or mass spectrometric systems are not readily available. Reaction-based spectrophotometric assays, which rely on chemical derivatization to produce colored species, offer additional advantages by improving selectivity and sensitivity through controlled chemical interactions.

1.1 Reaction mechanism of the oxidative coupling process

The oxidative coupling reaction proposed for the spectrophotometric determination of promethazine hydrochloride is illustrated in Figure 2. As shown in the scheme, the reaction proceeds through an initial oxidation step in which promethazine hydrochloride is oxidized by ferric chloride (FeCl_3). This step generates a reactive intermediate species within the phenothiazine ring system, which is known to be susceptible to oxidation due to its electron-rich sulfur and tertiary amine functionalities. The formation of this intermediate facilitates subsequent chemical interaction with the coupling reagent. In the second step, the oxidized promethazine intermediate reacts with potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), leading to

the formation of a stable blue-violet colored charge-transfer complex, as depicted in Figure X. The interaction between the oxidized drug species and ferricyanide ions results in an extended conjugated system, which is responsible for the appearance of a strong absorption band in the visible region. The formed complex exhibits a characteristic absorption maximum at 682 nm, enabling sensitive spectrophotometric measurement. The intensity of the developed color is directly proportional to the concentration of promethazine

hydrochloride under the optimized experimental conditions. The reaction proceeds efficiently under mild conditions without the need for heating or harsh reagents, and the resulting complex remains stable for a sufficient period to allow accurate absorbance measurements. The proposed mechanism explains the selectivity and sensitivity of the method and supports its suitability for routine analytical determination of promethazine hydrochloride in pharmaceutical formulations.

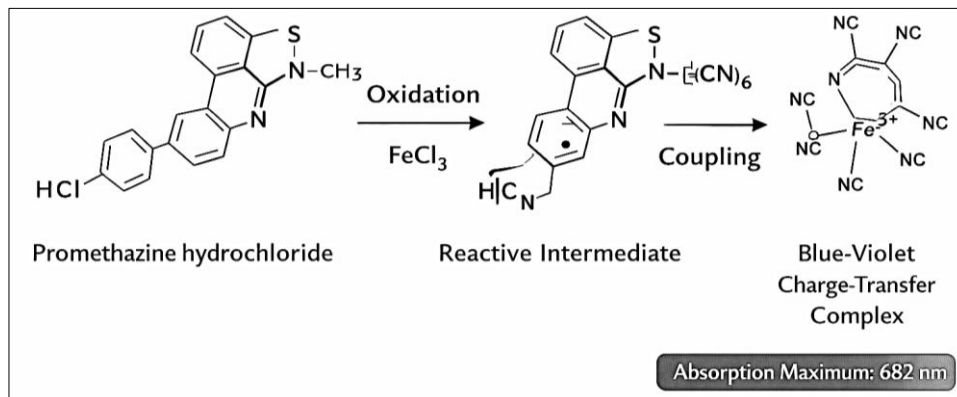


Fig 2: Proposed reaction mechanism for the oxidative coupling of promethazine hydrochloride with ferric chloride and potassium ferricyanide, leading to the formation of a blue-violet charge-transfer complex exhibiting maximum absorbance at 682 nm.

2. Materials and Methods

2.1 Chemicals and reagents

Promethazine hydrochloride reference standard was obtained from a certified pharmaceutical source and used as received without further purification. Ferric chloride (FeCl_3) and potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), both of analytical reagent grade, were purchased from reliable commercial suppliers and used without additional treatment. Hydrochloric acid and sodium hydroxide solutions were prepared at appropriate concentrations using distilled water for pH adjustment where necessary. All reagents employed in this study were of analytical grade, and distilled water was used throughout for the preparation of solutions and dilutions.

2.2 Instrumentation

Spectrophotometric measurements were carried out using a UV-Visible spectrophotometer equipped with matched 1 cm quartz cells. Absorbance readings were recorded against a reagent blank prepared under identical conditions. pH measurements were performed using a calibrated digital pH meter. Reaction temperature was controlled using an ice bath to maintain the solution temperature at approximately $10 \pm 1^\circ\text{C}$ during the oxidative coupling process. An analytical balance with a sensitivity of ± 0.1 mg was used for accurate weighing of reagents and samples. An ultrasonic bath was employed during tablet sample preparation to ensure complete dissolution and extraction of the active pharmaceutical ingredient.

2.3 Preparation of standard solutions

2.3.1 Solution of Standard Promethazine (1000 $\mu\text{g/mL}$)

The solution was prepared by dissolving 1 g of promethazine in a given volume of distilled water in a 1000 ml volumetric flask and then completing the volume to the mark with the same solvent so that the concentration of the solution became 1000 $\mu\text{g/ml}$ as working solution.

2.3.2 Potassium ferricyanide reagent solution (1000 $\mu\text{g/mL}$)

The solution was prepared by dissolving 1 g of dye in a given volume of water in a 1000 ml volumetric flask and then completing the volume to the mark with the same solvent so that the concentration of the solution became 1000 $\mu\text{g/ml}$ as working solution.

2.3.3 Ferric Chloride Standard Oxide Solution 1000 ($\mu\text{g/mL}$)

The solution was prepared by dissolving 1 g of iron (III) chloride in a specific volume of water in a 1000 ml volumetric flask and then completing the volume to the mark with the same solvent so that the concentration of the solution became 1000 $\mu\text{g/ml}$ as a working solution.

2.3.4 Hydrochloric acid solution with concentration (0.01 M)

The solution was prepared by diluting 0.08 mL of 11.86 M concentrated acid in a 100 mL volumetric flask, then filled to the mark with distilled water.

2.3.5 Sodium hydroxide solution with approximate concentration (0.01 M)

The solution was prepared by dissolving 0.04 g of solid NaOH in a given volume of distilled water in a 100 mL volumetric flask, then filling the volume up to the mark with the same solvent.

2.3.6 Coldin pharmaceutical solution (50 $\mu\text{g/ml}$)

10 pills of the pharmaceutical product Coldin produced by the State Company for Pharmaceutical Industry and Medical Appliances / Samarra were ground, which gave a weight of 6.1281 gm. The average weight of one tablet of 0.6128 g was taken, which contained 5 mg of promethazine. The powder was placed in a volumetric flask of 100 ml and dissolved in a certain volume of distilled water. The bottle was shaken and placed in an ultrasound water bath for 5

minutes, the volume was completed to the mark with the same solvent, and the solution was filtered with Whatman No.42 filter paper. The filtrate containing 50 µg/ml of promethazine was used in the next experiments.

2.4 Initial Investigations

It was observed that when 1 ml of PMTZ solution of 100 µg/ml was mixed with 2.5 ml of PFE reagent solution of concentration of 1000 µg/ml with 3 ml of FeCl₃ solution of concentration of 1000 µg/ml as an oxidant agent, a blue violet-coloured compound was formed. The spectrum showed the maximum absorption at a wavelength of 682 nm.

2.5 Optimization of the experimental condition

2.5.1 Select Best coupling reagent

Several experiments were conducted to choose the most appropriate reagent at which the complex formed gives the highest absorption. All reagents were used with the same concentration of 1000 µg/ml and a volume of 1 ml, as follows: Bromothymol blue, potassium ferricyanide, 2-aminopyridine, 4-Aminoantipyrine. It was found that the potassium ferricyanide reagent is the most suitable as it gave a colorful complex, the highest wavelength and the greatest absorption, while the other reagents had lower absorption values than the chosen reagent as shown in Table ^[1].

Table 1: Choosing the optimal reagent for PMTZ

Reagent	λ_{max}	Absorbance
Bromothymol blue	3.4	0.2352
Potassium ferricyanide	682	0.5358
2-Aminopyridine	502	0.3403
4-Aminoantipyrine	506	0.3581

2.5.1 Effect of Reagent Concentration

A study was conducted to select the best volume of potassium ferricyanide reagent solution with a concentration of 1000 µg/ml, where increasing volumes ranged between (0.5-4) ml were used and transferred to a series of volumetric flasks (eight flasks) with a capacity of 10 ml, which contain 1 ml of PMTZ solution. With a concentration of 100 µg/ml and 1.5 ml of the oxidizing agent solution FeCl₃ with a concentration of 1000 µg/ml, then the volume was completed to the mark with distilled water, and the absorption was measured at the wavelength 682 nm in comparison with the table as in the blank solution as shown in Table 2.

Table 2: Effect of reagent volume on the absorption of the complex formed by PMTZ

Volume (ml) of 100 (µg/ml)	Absorbance
	PFR
0.5	0.1257
1	0.3144
1.5	0.3590
2	0.4076
2.5	0.5358
3	0.3653
3.5	0.3567

It was found that the volume of 2.5 ml of (1000 µg/ml) of PFE was the best as it gave the highest absorption, which was adopted in the next experiments.

2.5.3 Selecting the best oxidant reagent

Several oxidizing agents were used to choose the best one, which gives the best peak and the highest absorption, as the results showed that the oxidizing agent that gives the best results is iron(III) chloride, while the rest of the oxidizing agents gave low absorption values as shown in Table 3.

Table 3: Choosing the best oxidizing agent

Oxidizing Agent	λ_{max}	Absorbance
Ce (SO ₄) ₂	513	0.3343
FeCl ₃	538	0.5657
K ₂ Cr ₂ O ₇	352	0.3691
KHSO ₅	530	0.0947

2.5.4 Concentration of oxidant reagent

The best volume of iron trichloride solution with a concentration of 1000 µg/mL was studied, where increasing volumes ranged between (0.5-4.5) ml and transferred to a series of volumetric flasks (seven flasks of capacity 10 mL). On 1 ml of PMTZ solution of 100 µg/ml and 2.5 ml of PFE reagent solution of 1000 µg/ml, then the volume was completed to the mark with distilled water, and the absorption was measured at the wavelength of the 682 nm against blank solution as per Table 4.

Table 4: The effect of the size of the oxidizing agent on the coloured absorption product of PMTZ

Volume (ml) of 1000 (µg/ml) FeCl ₃	Absorbance
0.5	0.2033
1	0.4002
1.5	0.5745
2	0.6523
2.5	0.7457
3	0.7879
3.5	0.7667
4	0.6425
4.5	0.5623

From Table 4 it is clear that the volume of 3 ml of the oxidizing agent is the best, as it gave the highest absorption, which was selected in the subsequent experiments.

2.5.5 Effect of Time Oxidative

The time required for oxidation of PMTZ drug was studied from the moment of reaction to 30 minutes, where a fixed volume of 1 ml of PMTZ solution with a concentration of 100 µg/ml was added to a series of 10 ml volumetric flasks, and then 3 ml of oxidant agent which is 1000 µg/ml was added at different times, before adding the PFE reagent, where it was noted that 5 minutes is a sufficient time to complete the drug oxidation process, which gives the best absorption of the formed product, as shown in Table 5.

Table 5: Effect of oxidation time on the absorption of the complex formed for PMTZ

Time (min)	Absorbance
0	0.8223
5	0.8732
10	0.8541
15	0.8213
20	0.7956
25	0.7634
30	0.7241

2.5.6 Effect of pH

The effect of the PH on the complex was studied by taking different volumes of acid and base, and this study was conducted at different PH values, which ranged between, (2.63-3.87), and the absorption values of the complex formed at each of these values were recorded, as shown in Table 6.

2.5.7 Effect of Addition Acid

HCl Incremental volumes were added by different series from a solution of hydrochloric acid of concentration 0.01 M to a series of a 10 ml volumetric flasks contains 1 ml from BRO drug. The concentration was 100 µg/ml, 3 ml of the oxidizing agent was 1000 µg/ml and 2.5 ml of the PFE reagent solution was 1000 µg/ml, and the absorption was measured at the wavelength of 682 nm.

2.5.8 Effect of Addition Base

Incremental volumes were added with different sequences from the 0.01 M NaOH base solution to a series of volumetric flasks of 10 ml that contain 1 ml of PMTZ with a concentration of 100 µg/ml, 3 ml of oxidizing agent with a concentration of 1000 µg/ml and 2.5 ml of PFE reagent solution with a concentration of 1000 µg/ml. The absorption was measured at the wavelength of 682 µm.

Table 6: The effect of the pH on the absorption of the complex formed for PMTZ

Addition	Volume (ml)	Absorbance	pH
HCl (0.01)M	0.1	0.6023	2.67
	0.2	0.6216	2.44
	0.3	0.7840	2.34
	0.4	0.7939	2.25
Without addition	-----	0.8841	2.99
NaOH (0.01)M	0.1	0.68921	3.62
	0.2	0.6462	3.66
	0.3	0.6174	3.69

Through Table 6 it was shown that the addition of the base and the acid led to a gradual decrease in absorption, so it was not adopted in the experiment, and the normal pH was adopted without any addition in the subsequent experiments.

2.6 Order of Additions

A number of laboratory experiments were carried out with different sequences of additions to obtain the order that gives the best absorption values for the complex formed, as 1 ml of PMTZ solution with a concentration of 100 µg/ml and 3 ml of the oxidizing agent FeCl₃ at a concentration of 1000 µg/ml with 2.5 ml of PFE reagent at a concentration of 1000 µg/ml in different sequences and depending on the optimum reaction conditions. The absorption values were recorded for it at the wavelength of 682 nm in each case and Table 7 illustrates this.

Table 7: The effect of the addition sequence on the absorption of the complex formed for BRO

No	Order of addition	Absorbance
1	D+O+R	0.8932
2	D+R+O	0.8325
3	O+D+R	0.9235
4	R+D+O	0.9575
5	R+O+D	1.2436
6	O+R+D	1.3774

Through Table 7, it was found that the best absorption of the formed product is the order: O + R + D, as (D) represents the drug, (O) the oxidizing agent and (R) the reagent, so it was adopted in next experiments.

2.6.1 Select the solvent

After fixing the appropriate conditions, all materials were added from PMTZ solution with a concentration of 100 µg/ml 3 ml of FeCl₃ oxidizing agent at a concentration of 1000 µg/ml with PFE reagent at a concentration of 1000 µg/ml, several solvents were used to complete the volume to the mark in volumetric flasks. The volumetric capacity of 10 ml and its absorption was measured to select the best solvent for the complex formed, and the results are shown in Table 8.

Table 8: Effect of the type of solvent used on the complex formed for PMTZ

Solvent	λ _{max}	Absorbance
Acetone	643	0.1853
Ethanol	675	0.9675
Ethanol	675	0.9675
Methanol	654	0.5915
Water	682	1.3774

2.6.2 Effect of temperature

The effect of temperature on the adsorption of the formed complex was studied. The degrees ranged between (5-50) °C. 1 ml of PMTZ solution with concentration of 100 µg/ml, 3 ml of FeCl₃ oxidant solution of 1000 µg/ml and 2.5 ml of PFE reagent solution of 1000 µg/ml were added, then completed the volume to the mark, with distilled water. The absorption was measured at a wavelength of 682 nm against the blank from the moment of interaction up to 50 °C, as shown in Table 9.

Table 9: The effect of temperature on the complex formed

Temperature °C	Bsorbance A
5	1.3635
10	1.3774
15	1.3484
20	1.3289
25	1.3101
30	1.2900
35	1.2784
40	1.2732
45	1.2447
50	1.2394

The complex was cooled down to 5 °C. It was observed that the decrease in temperature led to an increase in the absorption of the complex, especially at 10 °C. After that, the complex absorption decreased with increasing temperature. The best absorption was adopted at 10 °C in next experiments.

2.6.3 Stability of the Reaction Output

The stability time of the formed complex product was studied where 1 ml of PMTZ solution with a concentration of 100 µg/ml and 3 ml of the oxidizing agent FeCl₃ at a concentration of 1000 µg/ml, after 5 minutes of oxidation, 2.5 ml of PFE reagent at a concentration of 1000 µg/ml was added, and Complete the volume to the mark with distilled water, then the absorption of the complex formed at the wavelength 682 nm was measured, after different time periods, and the results are shown in Table 10.

Table 10: Effect of time on the stability of the complex formed for PMTZ

Time (min)	Bsorbance A
0	0.5987
5	1.250
10	1.315
15	1.311
20	1.312
25	1.178
30	1.071

Through Table 10, it was found that the complex formed is stable from 10 to 20 minutes, after which the absorbance gradually decreases, so this time was adopted in subsequent experiments.

2.6.4 Stoichiometric Ratio of Complex

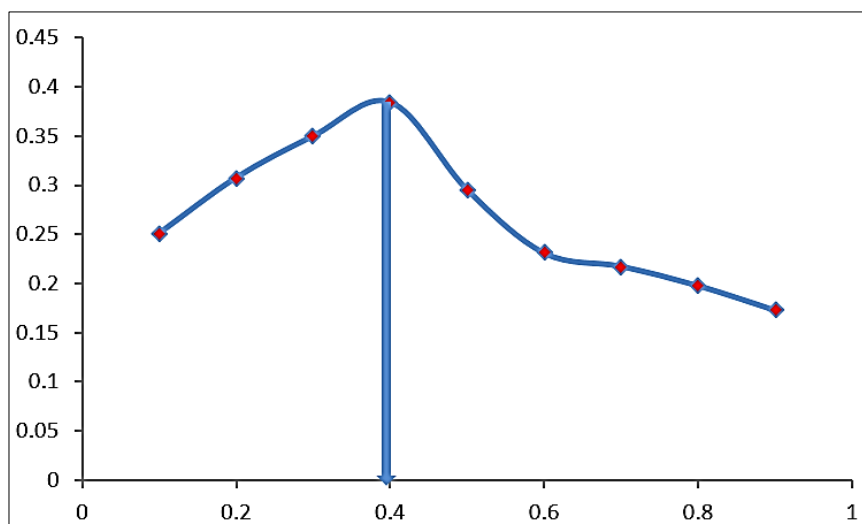
To determine the correlation ratio of PMTZ drug with the PEF reagent in the proposed method, the continuous changes method (Job's method) was adopted and using equal concentrations of the drug and the reagent, where a concentration of (0.1-0.9) ml of Bro solution of 100 µg/ml was transferred to 9 volumetric flasks of 10 ml contains 2.5

ml of a solution of 1000 µg/ml Ferric Trichloride, 3 ml of 100 µg/ml PEF reagent solution was added, then complete the volume to the mark with distilled water, and the absorbance was measured for each solution at the wavelength of 682 nm against the blank solution, as shown in Figure 2 and Table 11.

Table 11: Percentage of the complex binding ratio of PMTZ

V _D	V _R	Absorbance	V _D /(V _D +V _R)
0.1	0.9	0.2511	0.1
0.2	0.8	0.3076	0.2
0.3	0.7	0.3498	0.3
0.4	0.6	0.3842	0.4
0.5	0.5	0.2950	0.5
0.6	0.4	0.2308	0.6
0.7	0.3	0.2173	0.7
0.8	0.2	0.1976	0.8
0.9	0.1	0.1726	0.9

Through Table 11 and Figure 2, it is clear from the Job method that the complex formed under optimal conditions is composed of a molar ratio of 2:1 for each of the drug and reagent, consequently.

**Fig 2:** Correlation between recruitment process effectiveness and workforce productivity

3. Results and Discussion

3.1 Calibration curves of complexes

After fixing the optimal conditions for the complex, a standard Calibration curve solution was prepared and as follows.

A series of 10 ml volumetric flasks were prepared and increasing volumes (0.1-4) ml of PMTZ solution of 100

µg/ml were added to a series of volumetric flasks of 10 ml, then 3 ml of FeCl₃ solution was added to it with a concentration of 1000 µg/ml and 2.5 ml of PEF reagent solution with a concentration of 1000 µg/ml and then complete the volume to the mark limit with distilled water, and the absorption of each solution was measured against the solution and the blank linear range was 682 nm 1-40 µg/ml, as shown in Figure 3.

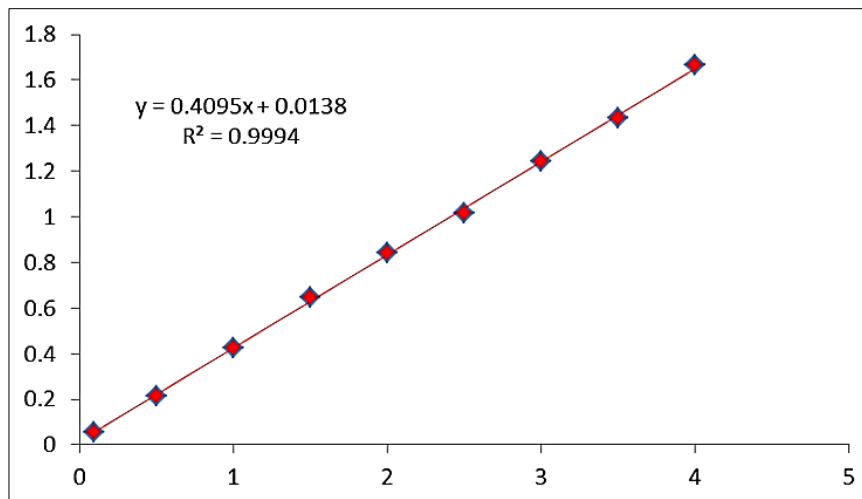


Fig 3: Calibration curve of the oxidation and conjugation complex of PMTZ

To find out the accuracy of the method's compatibility, a study was conducted by calculating the return value, the percentage for expressing the results, the relative deviation coefficient RSD% to express the compatibility of the results, and the different concentrations of the calibration curve (10,20,30), and six readings were made for each concentration, and the Rec values ranged % ranged between (100.5833-101.8730) %, while the RSD % values were between (0.0639-0.0923) %, and Table 12 explains this.

Table 12: Accuracy and compatibility of the oxidation complex and conjugation of PMTZ

Conc of PMTZ Taken µg/ml	Absorbance	Conc of PMTZ Found µg/ml	Rec%	RSD%
10.0000	0.4232	10.092	100.92	0.0923
20.0000	0.8438	20.097	100.485	0.0841
30.0000	1.2825	30.532	101.773	0.0639

Limit of detection and limit of quantification

The detection limit (L.O.D) and the quantitative limit (L.O.Q) were calculated for the determination of PMTZ by the oxidation and conjugation method and at a wavelength of 682 nm, by measuring the absorbance of the lowest concentration taken from the calibration curve, which is 1 µg / ml, with an average of six readings. The L.O.D value was 0.0304 µg/mL and the L.O.Q value was 0.0921 µg/mL, as shown in Table 13.

Table 13: The detection limit and the quantitative limit for the oxidation and conjugation complex of PMTZ

Concentration µg/ml	$\bar{X}$	S	L.O.D, µg/ml	L.O.Q, µg/ml
1	0.4232	0.00039	0.0304	0.0921

3.2 Applications direct method

Method The proposed method was applied to the Coldin product. Different volumes were drawn, which are (1,2,3) ml of the product with a concentration of 100 micrograms / milliliter, then 3 ml of ferric chloride solution of 1000 micrograms / milliliter were added to each vial and 2.5 ml of the reagent solution, potassium phthalate, with a concentration of 1000 micrograms / milliliter, then complete the volume up to the mark with distilled water to obtain concentrations of (10,20,30) micrograms / milliliter of the medicinal product, and this was done by conducting six readings for each measurement. The absorbance of each solution was measured against that of the blank solution at the wavelength of 682 nm. The straight line equation of the calibration curve was applied to know the concentration of the active substance in one pill, and the accuracy of the results was expressed using Rec% and it was between (96.9383-103.5017) %, and to express the agreement of the results, the RSD% was used and it was between (0.0743-0.0990)% and From which it was found that the method has good accuracy and control, as shown in Table 14.

Table 14: Application of the direct method of the oxidation complex and conjugation of the drug PMTZ

Conc. of PMTZ Taken µg/ml	Absorbance	Conc. of PMTZ Found µg/ml	Rec%	RSD%
15.0000	0.6374	10.3501	103.5017	0.0990
25.0000	1.1169	14.5407	96.9383	0.0862
35.0000	1.4325	19.7072	98.5361	0.0743

* Average of six readings

Multi Standard Additions Method

To estimate the PMTZ drug in its Coldin pharmaceutical preparation using the method of multiple standard additions, 0.5 ml of the pharmaceutical preparation with a concentration of 100 µg / ml was added to each vial, and increasing volumes of the standard solution PMTZ with a concentration of 100 µg / ml were added, ranging from 0.2-1.2 ml, and left. The seventh bottle without addition. Then,

2.5 ml of ferric chloride solution of 1000 µg/ml and 3 ml of potassium phthalate solution of 1000 µg/ml (for 6 vials), then fill the volume up to the mark with distilled water, where concentrations of 2-12 µg were obtained. /mL, then the concentration of the complex formed was plotted against the blank solution at the wavelength of 682 nm, which is shown in Figure 4.

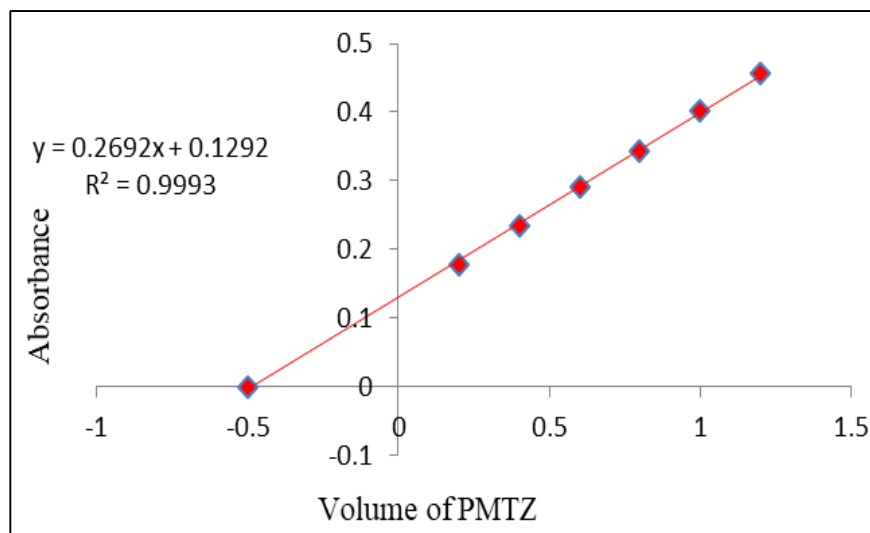


Fig 3: Standard addition curve of the oxidation and conjugation complex of PMTZ

3.3 Final Absorption Spectrum

After optimizing the experimental conditions, 1 mL of PMTZ solution at a concentration of 100 µg/mL was mixed with 3 mL of the oxidizing agent FeCl_3 at a concentration of 1000 µg/mL. After allowing 5 minutes for the oxidation process, 2.5 mL of the PFE reagent at a concentration of

1000 µg/mL was added, and the final volume was completed to the mark with distilled water. The resulting complex was then cooled to 10 °C. The final absorption spectrum of the complex was recorded against the blank at a wavelength of 682 nm, as illustrated in Figure 4.

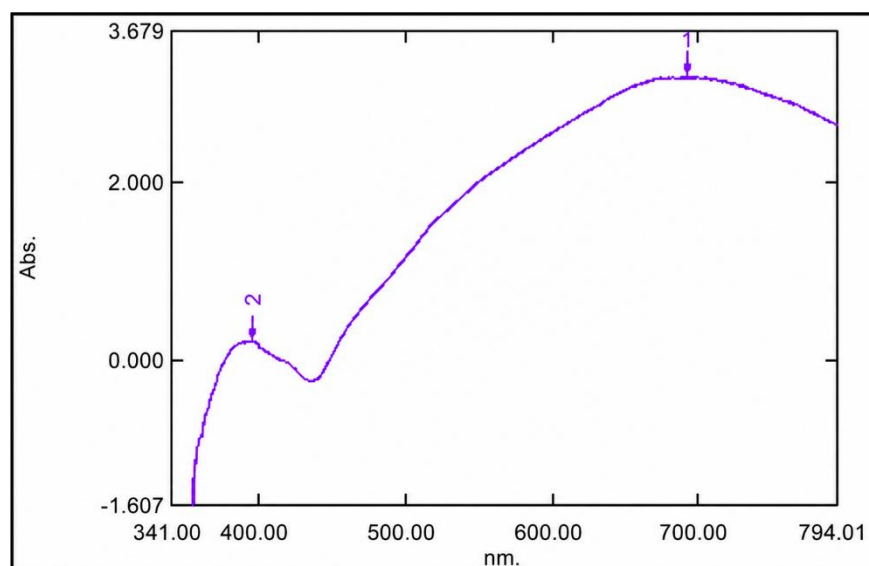


Fig 4: Absorption spectrum of the oxidation-coupling complex of the PMTZ drug measured against the blank solution

Comparison of Analytical characteristics of the proposed method: The proposed method has been compared with another spectroscopic method, as shown in Table 15.

Table 4-18: Comparison of the proposed method with another spectroscopic method

Parameters	Present Method	Other Method ^[26]
max (nm)λ	682	360
Beer's law range (µg/ml)	40 –1	11.17 –0.55
T (°C)	25 –5	35 –5
L.O.D (µg/ml)	0.0304	0.063
L.O.Q (µg/ml)	0.0921	0.190
Correlation coefficient (R2)	0.9994	0.9995
Sandell's index (µg/cm2)	0.0574	0.0413
(L/mol.cm)ε	2.104×103	2.23×103
Average Rec%	100.5833	-----
RSD%	0.0923	3.4

Table 15 shows that the proposed method has a higher molar absorption coefficient than the compared method, which indicates the sensitivity of the proposed method, and it is better in terms of the linear range of concentrations, in addition to the low values of the detection limit and the quantitative limit, and it is characterized by a good correlation coefficient.

3.4 Biological Activity

Petri dishes of 90 mm diameter were prepared with Tryptone Soya Agar for bacterial cultures and Sabouraud Dextrose Agar for fungal cultures. A total of 100 µL of bacterial and fungal suspensions, adjusted to a half-McFarland turbidity equivalent in culture medium, were inoculated. The samples were dissolved in D. water at specific concentrations, and 70 µL of each sample was injected into the wells. The plates were incubated at 37 °C

for 24 hours for bacterial strains and at 31-32 °C for 48 hours for fungal strains. Antimicrobial activity was evaluated for each microorganism by measuring the diameter of the inhibition zones (in millimeters) ^[12].

3.4.1 Activity against *Staphylococcus aureus*

The parent compound promethazine ("St") showed no inhibition zones at 50 µg and 100 µg concentrations, while at 150 µg it produced a modest inhibition zone of 14 mm. This indicates that *S. aureus* is relatively resistant to promethazine at lower concentrations, and noticeable activity appears only at higher doses. The complex exhibited improved antibacterial activity compared with the free drug, with inhibition zones of 10, 13, and 17 mm at 50, 100, and 150 µg, respectively. Nevertheless, its effect remained slightly lower than that of the reference antibiotic (20 mm). These findings suggest that both the free and complex forms of promethazine possess limited activity against *S. aureus*, and higher concentrations are required to achieve appreciable inhibition.

3.4.2 Activity against *Escherichia coli*

Promethazine ("St") exhibited no inhibitory effect at 50 µg and 100 µg, while a measurable zone of 15 mm was obtained at 150 µg. Similarly, the complex displayed no inhibition at lower concentrations but showed an inhibition

zone of 17 mm at 150 µg. The reference antibiotic produced a significantly larger inhibition zone (24 mm), indicating superior efficacy. The relatively lower activity of both compounds against *E. coli* could be attributed to the presence of an outer lipopolysaccharide membrane typical of Gram-negative bacteria, which limits the penetration of many antimicrobial agents. These results confirm that higher concentrations are necessary for both compounds to exert antibacterial effects against *E. coli*.

3.4.3 Activity against *Candida albicans*

In contrast, both compounds exhibited remarkable antifungal activity against *C. albicans*. Promethazine ("St") produced inhibition zones of 19, 21, and 27 mm at 50, 100, and 150 µg, respectively, indicating strong and concentration-dependent antifungal activity. The complex also displayed substantial activity with inhibition zones of 16, 19, and 24 mm at the same concentrations. Interestingly, both the free drug and its complex showed superior antifungal activity compared to the reference antibiotic (13 mm). This suggests that the presence of nitrogen and sulfur atoms in promethazine, along with possible chelation in the complex, may enhance its ability to interact with fungal cell wall components or membrane-associated enzymes, leading to effective growth inhibition as shown in Table 16 and Figure 5 ^[13, 14].

Table 16: Antimicrobial activity of some chemical compounds (All Numbers in MM)

	St			Ref. Antibiotic	Complex			Ref. Antibiotic
	1, 50 µg	2, 100 µg	3, 150 µg		1, 50 µg	2, 100 µg	3, 150 µg	
<i>Staphylococcus aureus</i> (Gram-positive bacteria)	---	---	14	20	10	13	17	20
<i>Escherichia coli</i> (Gram-negative bacteria)	---	---	15	24	---	---	17	24
<i>Candida albicans</i> (fungi)	19	21	27	13	16	19	24	13

*(---) = No inhibition detected

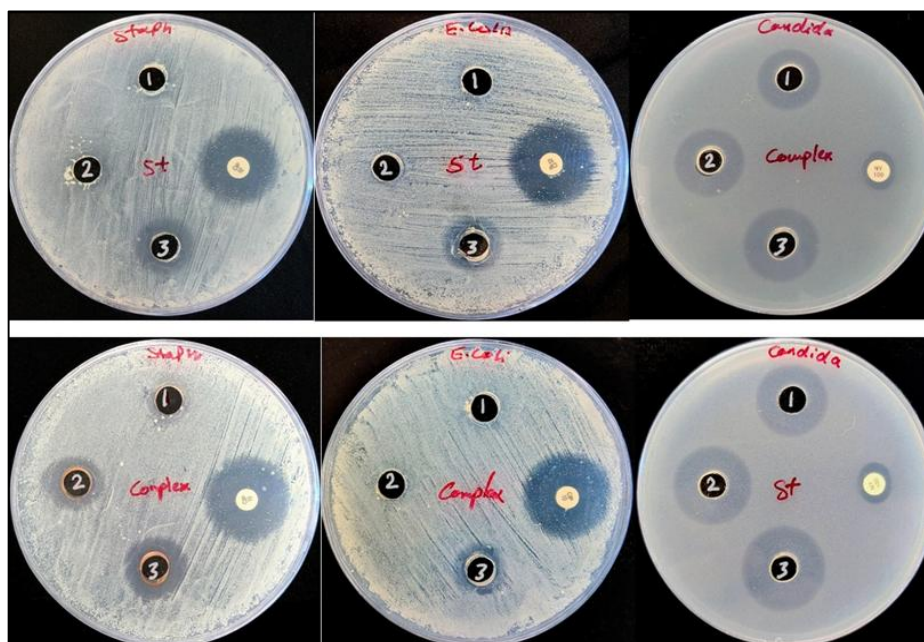


Fig 5: Petri dishes showing the results of the biological activity assay

4. Conclusions

The method of oxidation and conjugation was applied to estimate the drug PMTZ in its pure form and its pharmaceutical preparation Coldin, as the method included the interaction of PMTZ with the oxidizing agent FeCl₃ and then its coupling with the detector potassium phthalate to

give a complex with a purple color and gave its highest absorption at a wavelength of 682 nm, and through the results that It was obtained for the values of the percentage recall, the relative standard deviation, the detection limit and the quantitative limit that the method has good accuracy and

agreement, which indicates the success of the proposed method. 3-6-9 Future Works

- The use of colorimetric methods for the determination of promethazine, such as the ion aggregation method and the dye restriction method, and applying them to pharmaceutical preparations.
- The use of other oxidizing reagents and agents in the formation of oxidation and coupling complexes for the determination of promethazine in its pure form and in its pharmaceutical preparations.

4.1 Overall Interpretation

The obtained results clearly indicate that *C. albicans* is the most sensitive microorganism to both compounds, followed by *S. aureus*, while *E. coli* was the least sensitive. The enhanced activity of the complex, particularly at higher concentrations, may be explained by increased lipophilicity upon complexation, which facilitates diffusion through microbial cell membranes. The data thus confirm that both the free promethazine and its complex possess notable antifungal properties, while their antibacterial activities are moderate and concentration-dependent.

4.2 Future Works

- Using colorimetric methods to estimate the promethazine drug such as the ionic aggregation method and dye restriction spectroscopy, and applying it on its pharmaceutical preparation.
- Use of reagents and other oxidizing agents in the formation of oxidation and coupling complexes to estimate the promethazine in its pure form and pharmaceutical preparation.

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