Spectrophotometry-Based Oxidative Coupling Method for Determining Thymol Utilizing a Coupling Agent

Batool Mansour Zayan[1*], Israa Talib Humeidy[2], and Mohamed Salem Abdel Aziz[1]**

[1]*Department of Chemistry, College of Education for Girls, University of Tikrit, Tikrit 34001, Iraq*

[2]*Department of Chemical Engineering, College of Engineering, University of Tikrit, Tikrit 34001, Iraq*

* ***Corresponding author:***

email: batolaldoury@gmail.com; israatalib@tu.edu.iq***

Received: June 19, 2023
Accepted: March 20, 2024

DOI: 10.22146/ijc.85933

Abstract: *Developing a spectroscopic approach to assess the medicinal substance thymol was one of the research's objectives. Using an oxidative coupling reaction between thymol solution and* N,N-*dimethyl*-p-*phenylenediamine dihydrochloride solution (*N,N-*DMPPDADH) in alkaline media with potassium periodate as an oxidizing agent, the current technology forms a blue-colored soluble product. The wavelength at which a colored product exhibits maximum absorption is 600 nm. According to Beer's law, the concentration range covered by the approach under investigation is 1.25–20.00 µg/mL of thymol. The specific molar absorbance value of 10725.71 L/mol cm indicates the method's sensitivity. The Sandell significance value was 0.014 µg/cm², which represents sensitivity per unit length. The precision of the method is demonstrated to be commendable, and the low relative standard deviation value of 0.16% supports this. This method's accuracy in identifying thymol at such low quantities is demonstrated by its confirmed detection limit of 0.0124 µg/mL. The developed technique has been useful in screening thymol in pharmaceutical products, with mouthwash being the focus of particular attention. Thymol content in real samples was accurately determined using the approach, as evidenced by the reported 101.13% recovery rate of thymol.*

Keywords: *thymol;* N,N-*dimethyl-p-phenylenediamine dihydrochloride; oxidative coupling*

■ INTRODUCTION

Thymol (2-isopropyl-5-methylphenol) is a derivative of monoterpenoid phenol and is commonly present in natural sources and botanicals such as oregano and thyme (Fig. 1). Its molecular weight is 150.2 g/mol. The stabilization of solutions and serum samples is expected to have a broad application in the chemical industry [1]. The substance in question is a crystal lacking in color, exhibiting solubility in ethanol and essential oils, and possessing a limited degree of solubility in glycerin [2]. The utilization of this substance as a disinfectant in the medical domain has been extensively documented.

Dentistry employs various techniques and materials for the maintenance and treatment of oral health, while the agricultural, cosmetic, and food industries utilize these materials for their respective purposes [3]. Thymol has been added to a number of ointments for the treatment

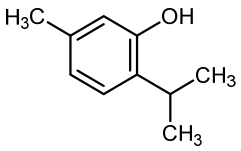

Fig 1. Thymol structure

of hand ulcers, psoriasis, and eczema. It has been used to treat respiratory system catarrh at concentrations of 1.0% or 2.0% in an oily formulation. Thymol has also been identified as a stabilizing agent for a variety of chemicals, such as halothane [4]. The results of the investigation showed that thymol can stabilize the exudation of potassium and calcium in heart cells. Inadequate intake or anesthetic administration can result in thymol toxicity [5]. Because of its potential medical use, the molecule under inquiry was examined using a range of analytical techniques, such as spectrum methods [6-11], flow injection methods [12-13], high-

performance liquid chromatography methods [14-19], and gas chromatography [20-24]. Oxidative coupling processes were used in this work to measure the drug in a simple and accurate way through oxidative coupling with *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride in the presence of potassium periodate as an oxidative agent, the drug was estimated in both pure forms and liquid pharmaceutical formulations.

■ EXPERIMENTAL SECTION

Materials

High-purity analytical chemicals and reagents were utilized in this work, including thymol (99.9%, Samarra Pharmaceutical), potassium periodate (99.0%, BDH), *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (99%, BDH), sodium hydroxide (98%, Fluka), hydrochloric acid (37%, Fluka), ethanol (99%), methanol (99%), diethyl ether (98%), and acetone (99.0%).

Instrumentation

For all spectrum measurements, the following equipment was used: a Shimadzu UV-vis 160 spectrophotometer (Japan), sensitive balance precision (XR-205gm, Sweden), pH meter (WTW 720, Germany), and water bath (Memmert, Germany).

Procedure

Chemical materials

The present methodology involved the dissolution of 0.1 g of high-purity thymol in 3 mL of ethanol. The solution was then made up to a total volume of 100 mL with distilled water in a volumetric flask. Subsequently, the diluted solutions were prepared from this stock solution 1000 µg/mL (6.657789×10^{-3} M). *N-N*-dimethyl-*p*-phenylene diamine dihydrochloride at a concentration of 0.01 M was utilized. The reagent was dissolved in distilled water (0.209 g) and subsequently diluted to the 100.00 mL mark in a volumetric flask using distilled water. The present methodology involved the dissolution of KIO_4 (0.23 g) in distilled water under controlled conditions using a water bath. Upon complete dissolution, the solution was made up to the mark in a 100 mL volumetric flask with distilled water to reach a concentration of 10^{-2} M.

The present solution was formulated by dissolving a precise quantity of 4 g of NaOH in a limited volume of distilled water, followed by filling up to the calibrated mark on a 100 mL volumetric flask with distilled water to reach a concentration of 1 M. The experimental procedure involved the dissolution of 0.1 g of each substance in distilled water, followed by the addition of distilled water to a volumetric flask to achieve a final volume of 100 mL. Dilute solutions were subsequently prepared from the resulting solution to reach a 1000 µg/mL concentration.

The present study involved the preparation of the HCl solution (1 M) through the dilution of 8.5 mL of concentrated HCl with a concentration of 11.8 M, using distilled water in a volumetric flask (100 mL). To get a solution with a concentration of 240 µg/mL of Listerine mouthwash, dilute 20 mL of the mouthwash bottle's contents (which comprise 0.6 g/L of thymol) with 50 mL of distilled water. After that, make a solution that has 100 µg/mL concentration of the prepared solution.

Preliminary investigations

The method's basic idea is to oxidize thymol in a basic medium using potassium periodate, an oxidizing agent. The resulting product is then mixed with the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent, producing a greenish-blue solution that exhibits the highest absorption at 600 nm in comparison to the mock solution, which exhibits no absorption at this wavelength.

Optimization of the experimental conditions

Adding 1 mL of KIO_4 10^{-2} M solution to 1 mL of thymol 100 µg/mL solution was the initial step in the experiment. The observation was made thereafter. A volume of 1 mL was subjected to oxidation for a duration of 15 min, following which 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent was introduced. The solution was subjected to the addition of 1 mL of NaOH 10^{-2} M. The resulting solution exhibited a blue-green coloration. Subsequently, the solution was diluted with distilled water to reach the 20 mL mark on a volumetric flask. The colored product was then subjected to spectral analysis, which revealed the highest absorption at a specific wavelength. At a wavelength of 600 nm, the mock solution exhibits no

absorption, whereas the observed sample displays absorption.

Choosing the best coupling reagent

Several chemical compounds (2,4-dinitrophenylhydrazine, 2,4-dichloroaniline, 4-bromoaniline, and *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride) have been utilized as coupling reagents at 10^{-2} M concentration and 1 mL of volume to determine the presence of thymol during the addition of KIO_4 10^{-2} M and the amount of 1 mL of NaOH. The selection of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride as the optimal coupling agent was based on its ability to yield the greatest absorption of the colored product at a wavelength of 600 nm.

Choosing the best oxidizing agent

Various oxidizing agents, including potassium periodate, *N*-bromo succinamide, ammonium sulfate, potassium iodate, ferric chloride, and potassium dichromate, were utilized at 10^{-2} M each. These agents were added to 1 mL of thymol solution, which had a 100 µg/mL concentration. The solutions were then allowed to sit for 15 min. Following this, 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent 10^{-2} M was added, along with 1 mL of NaOH 10^{-2} M in a 20 mL volumetric vial. The volume was augmented by the addition of distilled water up to the designated mark. Subsequently, the absorbance of each sample was assessed against its corresponding mock solution within the wavelength range of 200–800 nm. The results indicated that KIO_4 exhibited the most effective oxidizing properties, as evidenced by its highest absorption rate at the 600 nm wavelength. Consequently, this oxidizing agent was utilized in all subsequent experiments.

■ RESULTS AND DISCUSSION

The Phenomenon of Base Volume Effect

To investigate the effect of base volume on absorption, a series of 20 mL volumetric flasks were filled with different amounts (0.05–2.00 mL) of a 1 M NaOH solution, along with 1.6 mL of an oxidizing agent (10^{-2} M) and 1 mL of thymol at a concentration of 100 µg/mL. After 10 min of oxidation, 1.2 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride 10^{-2} M reagent was added to the flasks. The solutions were then diluted with purified water. At 600 nm, their absorbance was measured in comparison to their mock solutions, as can be seen in Fig. 2.

The Oxidizing Agent Volume's Impact

By introducing 0.4–2.2 mL of oxidizing agent solution (KIO_4) at a concentration of 10^{-2} M to a series of volumetric flasks holding 1.0 mL of thymol solution at a concentration of 100 µg/mL, the effect of oxidizing agent quantity on absorption was investigated. To complete the reaction, the solutions were given for 15 min. After adding 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, which had a concentration of 10^{-2} M, the containers were filled to 20 mL with distilled water. In comparison to its mock solutions, each solution's absorbance was lastly measured at a wavelength of 600 nm. Fig. 3 displays the results. Based on the findings depicted in Fig. 3, it was

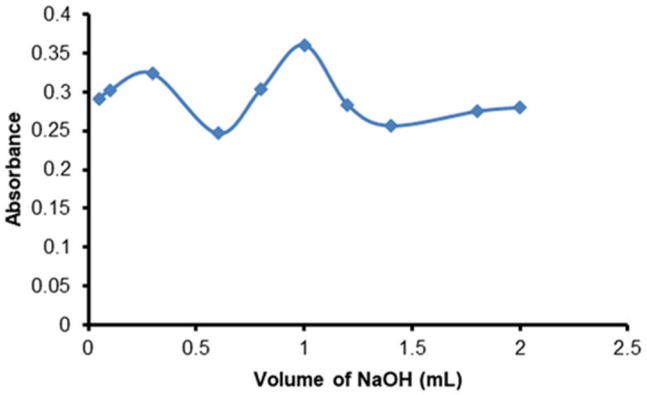

Fig 2. The effect of the volume of the base

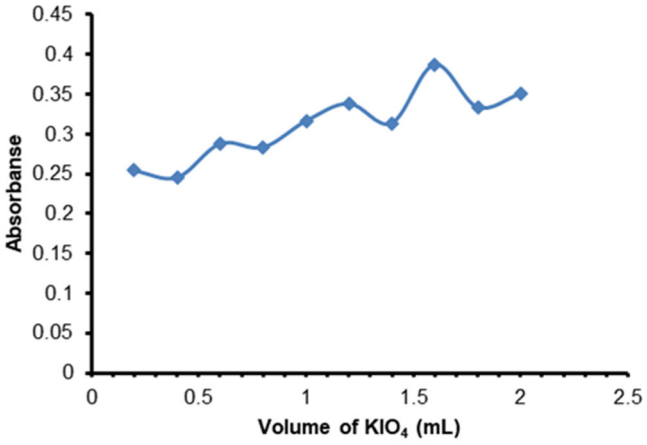

Fig 3. The effect of the volume of the oxidizing agent

determined that the optimal quantity of the oxidizing agent solution resulting in the highest absorption is 1.6 mL. This value was subsequently employed in subsequent experimental procedures.

Impact of Volume of the Coupling Reagent

The volume of the coupling reagent and its effect on the reaction was examined by adding escalating quantities (0.4–2.2 mL) of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride 10^{-2} M to a volumetric flask (20 mL) holding 1 mL of thymol 100 µg/mL and 1.6 mL of KIO_4 10^{-2} M. After giving the solutions 25 min to finish the reaction, the volume was increased to include the marker and the absorbance of each solution was measured at 600 nm in comparison to its mock solution. The outcomes are displayed in Fig. 4.

The Impact of Oxidation Duration

The duration required for KIO_4 to oxidize thymol was examined. This was achieved by using a set of volumetric vials with a capacity of 20.0 mL, each holding 1 mL of thymol solution at a 100 µg/mL concentration. After adding 1.6 mL of KIO_4 10^{-2} M solution to this solution, the mixture was left to stand for different amounts of time. Next, 1.2 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride 10^{-2} M solution and 1.0 mL of NaOH 1 M solution were added to the mixture. Then, a final volume of 20.0 mL was achieved by diluting the resultant combination with distilled water. At 600 nm, the absorbance of the final solutions was measured and contrasted with the corresponding mock solutions. The obtained results are presented in Table 1. Table 1 indicates that 20–30 min duration is adequate for accomplishing the oxidation process. In the subsequent experiments, a time frame of 25 min was utilized.

Additions Sequence Effect

The present study investigated the impact of altering the order of additives in the solutions employed during the reaction process. It was observed that the sequence of addition significantly influenced the chromatic intensity of the resultant compound. Consequently, a series of experiments were performed utilizing various additive sequences while ensuring uniformity in the volumes and concentrations of all materials employed across all trials. The findings presented in Table 2 indicate that the first order exhibits

Table 2. Effect of sequence of additions on absorption

No	Order of additions	Absorbance
1	D + O + R+B	0.4193
2	O + D+R+B	0.3603
3	D + R + O+B	0.3831
4	R + D+O+B	0.3299

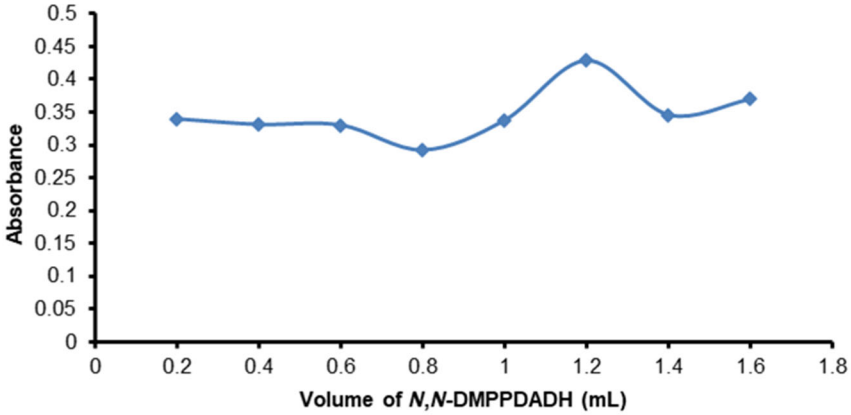

Fig 4. Effect the volume of coupling reagent

Table 1. Effect of oxidation time

Time (min)	Direct	5	10	15	20	25	30	35
Absorbance	0.1950	0.2655	0.2679	0.3651	0.4092	0.4192	0.4141	0.4782

the most significant absorption. Therefore, it was employed in subsequent experiments: thymol (D), KIO_4 (O), *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (R), and NaOH (B).

Stability of the Resulting Product

The stability of the product was monitored under optimal conditions, as determined in prior experiments. Specifically, a 1.0 mL of thymol solution with a concentration of 100 µg/mL was combined with 1.6 mL of KIO_4 10^{-2} M. The mixture was allowed to react for 20 min before analysis. The experiment involved an oxidation process followed by the addition of 1.2 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent solution 10^{-2} M and 1 mL of NaOH 1 M solution. The solution was then diluted with distilled water in 20 mL volumetric vials. The absorbance of the resulting-colored solutions was measured at a wavelength of 600 nm against their respective mock solutions after a specific time in minutes. The obtained results are presented in Table 3. The results presented in Table 3 show that the stability of the colored product was confirmed for a minimum of 60 min, which is considered sufficient for measurements.

Temperature Effect

The impact of temperature on the absorption and stability of the resultant chromatic compound was investigated by subjecting it to a range of temperatures spanning from 5 to 50 °C. The outcomes of this study are presented in Table 4. The data presented in Table 4 indicates that the temperature range of 20–30 °C is optimal, with a decrease in absorption observed as temperature increases. Therefore, subsequent experiments were conducted at a temperature of 25 °C.

Influence of Solvent Type

Different solvents were employed to bring the quantities to the target in volumetric vials with a 20 mL capacity after all the reaction ingredients had been added in accordance with the ideal values from the previous tests. The findings are reported in Table 5. The results are shown in Table 5, and they indicate that ethanol has the highest absorption of the solution when compared to the other solvents. However, water was used in subsequent experiments because it also had a good absorption rate, was inexpensive, readily available, and had a minimal negative environmental impact.

Final Absorption Spectrum

The ideal circumstances for determining thymol were compiled using the findings of earlier research, as indicated in Table 6. Analyzing the absorption spectra of the resultant solution confirms the wavelength of the maximum absorption under ideal working circumstances for the estimate of thymol. According to the results of the first tests, Fig. S1 was produced, and it was discovered that the wavelength for the maximum

Table 3. Stability of the formed product

Time (min)	Absorbance
5	0.4190
10	0.4193
15	0.4195
20	0.4191
25	0.4196
30	0.4189
35	0.4185
40	0.4190
45	0.4193
50	0.4187
55	0.4191
60	0.4197

Table 4. Effect of temperature on absorption

Temperature (°C)	Absorbance
5	0.3005
10	0.3126
15	0.3813
20	0.4190
25	0.4191
30	0.4189
35	0.3965

Table 5. Effect of solvent type

Absorbance	λ_{max} (nm)	Solvent
0.4196	600	Water
0.6394	617	Ethanol
0.2360	552	Acetone
Turbid	Turbid	Diethyl ether
0.3088	343	Methanol

Table 6. Summary of optimal conditions for the determination of thymol

Experimental Conditions	Value
λ_{max} (nm)	600.0
Volume of KIO_4 10^{-2} M (mL)	1.6
Volume of *N,N*-DMPHDAH 10^{-2} M (mL)	1.2
Oxidation time (min)	25.0
Temperature (°C)	25.0
Solvent	Water
NaOH (mL)	1.0

absorption is 600 nm and the absorbance is 0.4196. As for the blank, it does not give any absorption at the same wavelength as in Fig. S2.

Approved Working Method and Calibration Curve Preparation

The ideal conditions for thymol determination were established, and the standard curve was created using the following guidelines: Following the addition of 1.6 mL of the oxidizing substance KIO_4 solution 10^{-2} M, increasing volumes (0.25–4.00 mL) of thymol solution with a concentration of 100 g/mL were added to a series of volumetric vials with a capacity of 20 mL. The solutions were then left for 20 min. The volume was then filled to the identity with distilled water, and the absorbance of all of the solutions was measured at 600 nm versus a blank solution. To complete the reaction, 1.2 mL of the substance solution, *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, and 1.0 mL of the base solution NaOH were added. The standard curve for a range of thymol concentrations between 1.25 and 20 g/mL is shown in Fig. 5 and follows Beer's law. The technique has a 10725.708 L/mol cm molar absorbance, and Sandel's importance is 0.014 g/cm^2.

Accuracy and Compatibility of the Method

By calculating the recall and the corresponding standard deviation of two distinct concentrations of thymol from 2.50–3.75 g/mL by taking an average of six readings for each, the precision and compatibility of the suggested approach for the determination of thymol were calculated under the ideal conditions shown in the work method. The retrospective rate was 99.93%, relative standard deviation was less than 0.16%, and agreement was good, indicating great accuracy and reliability of the approach. The following are the mathematical calculations made to determine the retrospectiveness, its mean, and its relative standard deviation (Eq. (1)).

$$RE\% = O - \frac{T}{T} \times 100 \quad (1)$$

where RE is a relative error, O is the practical value, and T is the true value [25].

The recovery value is calculated from the following law (Eq. (2)).

$$Recovery\% - RE\% + 100 \quad (2)$$

As for calculating the percentage value of the relative standard deviation, the following law is applied in Eq. (3):

$$RSD = \frac{S}{\bar{X}} \times 100 \quad (3)$$

where S is the standard deviation and $\bar{X}$ is the rate of reads. The results are shown in Table 7.

Detection Limit and Quantity Limit

Concentration (1.25 µg/mL) in the calibration curve within the limits of Beer's law and under optimal conditions, the quantitative and qualitative detection limits were calculated by measuring the absorbance of 10 minimum solutions. From the following mathematical relationship (Eq. (4) and (5)) [26];

$$LOD = \frac{3.3S}{b} \quad (4)$$

$$LOQ = \frac{10S}{b} \quad (5)$$

where LOD is the qualitative detection limit, LOQ is the quantitative detection limit, S is the standard deviation of

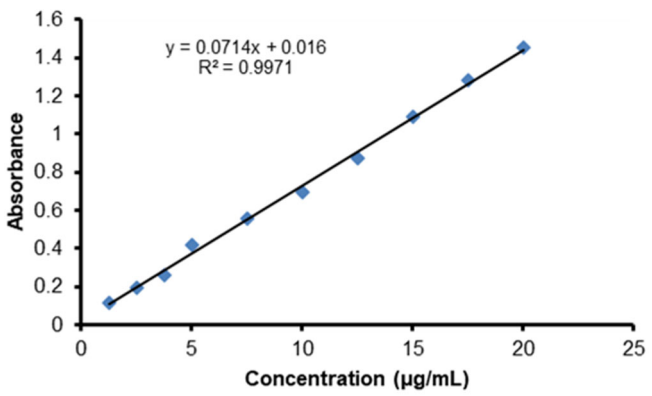

Fig 5. Standard curve for the determination of thymol

Table 7. Accuracy and compatibility of the method

Amount of promethazine taken (µg/mL)	RE (%)	Recovery (%)	Average recovery (%)	RSD (%)
2.50	−0.05	99.94	99.93	0.14
3.75	−0.06	99.93		0.18

Table 8. Limit of detection

Concentration (µg/mL)	B	S	LOD (µg/mL)	LOQ (µg/mL)
0.25000	0.07140	0.00027018	0.01248	0.03784

the lowest concentration, and b is the slope. The results are shown in Table 8.

The Nature of the Resulting Product

The two techniques of continuous changes (Job's approach) and the molar ratio method were used to determine the kind of product created and the proportion of the drug's bond with the reagent. The thymol solution and the reagent solution in both procedures contain the same amount of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride 10^{-2} M. According to Job's approach, various amounts of the drug solution, ranging from 0.1–0.9 mL, were put into volumetric vials with 20 mL capacity. Supplements were added to 1.0 mL of the reagent solution, and the remaining additions were finished using the recommended volumes in accordance with the work technique. The absorbance of these solutions was then measured at a wavelength of 600 nm in comparison to their blank solutions after diluting with distilled water to the proper concentration. Fig. 6 demonstrates that the thymol to reagent [27] ratio is 1:1.

The molar proportion method, in which 1.0 mL of the thymol drug solution was set in a series of 20 mL volumetric flasks and the solution of the reagent had been added to it at various amounts (0.3–3.5 mL), was used to ensure that the reaction ratio that occurred between thymol and the reagent was 1:1. The remaining additions were then completed with the optimal volumes, was used to ensure this. It was diluted to the appropriate strength with distilled water, and the absorbance of each of the resulting solutions was determined at a wavelength of 600 nm in comparison to the blank solution (Fig. 7) [27]. Therefore, the proposed equation is shown in Fig. 8.

Interference Effect

The impact of interactions was investigated to evaluate the selectivity of the method and its suitability for pharmaceutical preparations. This was achieved by introducing varying volumes of each interaction, ranging from 2.4 to 6.0 mL, with a concentration of 1000 µg/mL, into a set of 20.0 mL volumetric vials. These vials contained 1.0 mL of thymol solution and 1.6 mL of thymol. The experiment involved the addition of an

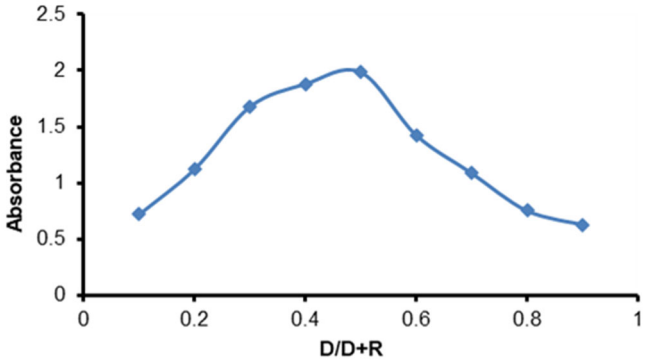

Fig 6. The curve of the method of continuous changes (Job's method)

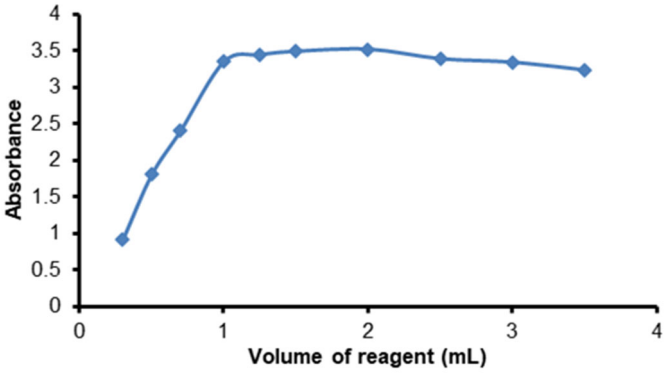

Fig 7. Molar ratio curve

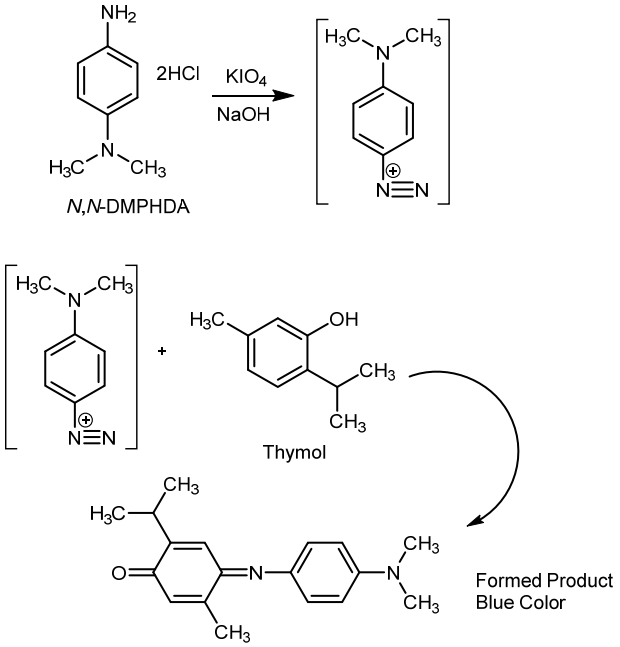

Fig 8. The blue product's suggested equation

oxidizing agent at a concentration of 10^{-2} M, followed by the addition of 1.2 mL of a reagent solution at the same concentration and 1.0 mL of a NaOH 1 M solution. The resulting solution was then diluted with distilled water to the mark. The absorbance of all solutions was measured at a wavelength of 600 nm and compared to mock solutions. Recovery was calculated for each addition, and it was determined that the interferences used did not have an effect on absorption. This suggests that the method can be applied to pharmaceutical preparations. The results are presented in Table 9.

Determination of Thymol in Mouthwash by the Direct Method

A solution with a concentration of 240 µg/mL was obtained by diluting 20 mL of the mouthwash package content, which contains 0.6 g/L of thymol, with 50 mL of distilled water. From this solution, a solution with a concentration of 100 µg/mL was prepared. Various volumes were extracted from this solution to achieve concentrations of 2.5, 5.0, and 7.5 µg/mL of thymol. The solutions were subjected to an optimal working method involving their placement in three volumetric vials with a capacity of 20 mL each. An oxidizing agent, specifically KIO_4, was added to the volumes at a concentration of 10^{-2} M, in a quantity of 1.6 times the volume of the original solution. The mixture was allowed to react for a period of 20 min until completion. Subsequently, the oxidation process was supplemented with 1.2 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent, which was present at a concentration of 10^{-2} M. Following this, 1.0 mL of NaOH was introduced, and the absorbance was gauged (averaging six readings) for each solution against its corresponding blank solution at a wavelength of 600 nm. The retrograde and RSD were then computed, and the outcomes are presented in Table 10. The findings presented in Table 10 validate the efficacy of the proposed technique for quantifying thymol in the analyzed pharmaceutical tablets.

Table 9. Effect of interferences on absorption

Foreign compound	Recovery (%) of 50 µg/mL of sulfacetamide sodium per µg/mL of another compound added		
	100	200	300
Maltose	97.52	99.76	99.88
Glucose	102.35	100.02	99.53
Lactose	101.47	100.22	100.07
Sucrose	98.61	97.98	97.54
Mannose	97.66	102.79	100.43

Table 10. Determination of thymol in mouthwash by direct method

Amount of thymol taken (µg /mL)	RE (%)	Recovery* (%)	Average recovery (%)	RSD (%)
2.5	−0.1536	99.84		0.21723
5	−0.0476	99.95	99.91	0.12152
7.5	−0.0536	99.94		0.30951

*Average of five determinations

■ CONCLUSION

A spectrophotometric method for determining thymol has been established, which exhibits high sensitivity. This method involves the oxidative coupling reaction between thymol and *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride reagent, with a concentration of 10^{-2} M, in the presence of KIO_4, an oxidizing agent, at a concentration of 10^{-2} M. A solution containing NaOH 10^{-2} M was introduced, resulting in the formation of a stable, water-soluble product exhibiting a blue hue. This product displayed maximum absorption at a wavelength of 600 nm. The present study employed Beer's law within the concentration range of 1.25–20.00 µg/mL. However, a deviation was observed at higher concentrations. The molar absorbance was determined to be 10725.708 L/mol cm, while the sandal index was found to be 0.014 µg/cm^2. The relative standard deviation did not exceed 0.16%, and the detection limit was calculated to be 0.0124 µg/mL. The proposed method was successfully implemented for the measurement of thymol in pharmaceutical products, specifically mouthwash, with a recovery rate of not less than 99.91%.

■ ACKNOWLEDGMENTS

We appreciate the Department of Chemical Engineering, College of Engineering, and University of Tikrit for enabling us to finish the research in the instrumental analysis laboratory.

■ CONFLICT OF INTEREST

The authors have no conflict of interest with any organization with a direct or indirect financial interest.

■ AUTHOR CONTRIBUTIONS

Israa Talib Humeidy and Mohamed Salem Abdel Aziz made contributions to the study's conceptualization and design. Batool Mansour Zayan handled the data collecting, analysis, and material preparation. Israa Talib Humeidy wrote the initial draft of the manuscript. All writers read and approved the final manuscript.

■ REFERENCES

[1] Nagoor Meeran, M.F., Javed, H., Al-Taee, H., Azimullah, S., and Ojha, S.K., 2017, Pharmacological properties and molecular mechanisms of thymol: Prospects for its therapeutic potential and pharmaceutical development, *Front Pharmacol.*, 8, 380.

[2] Barrak, M.H., Al-Rufaie, M.M, and Motaweq, Z.Y., 2021, Thymol quantitative analysis in medicinal formulation types through employing of nano-technology and antimicrobial activity in some pathogenic bacterial isolates, *Nusantara Biosci.*, 13 (1), 129–137

[3] Gao, S.S., Yon, M.J.Y., Chen, K.J., Duangthip, D., Lo, E.C.M., and Chu, C.H., 2019, Utilization of a mobile dental vehicle for oral healthcare in rural areas, *Int. J. Environ. Res. Public Health*, 16 (7), 1234.

[4] Sancheti, J., Shaikh, M.F., Chaudhari, R., Somani, G., Patil, S., Jain, P., and Sathaye, S., 2014, Characterization of anticonvulsant and antiepileptogenic potential of thymol in various experimental models, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 387 (1), 59–66.

[5] Pérez-Sánchez, A., 2023, Relaxant effect of thymol on smooth muscle, *Mex. J. Med. Res.*, 11 (22), 23–29.

[6] Fadhil, G., 2014, Spectrophotometric determination of thymol in pharmaceutical preparation via oxidative coupling reaction with 2,4-dinitrophenylhydrazine in the presence of potassium periodate, *Iraqi J. Sci.*, 55 (1), 27–34.

[7] Al-Ramadhan, S.T., 2014, Spectrophotometric determination of thymol in pharmaceutical preparation via diazotization reaction with 4-aminoacetophenone, *Rafidain J. Sci.*, 25 (3), 44–56.

[8] Waheeb, A.S., Abd Al-Kadhum, Z.T., Salman, N.S., Award, M.A., and Abd Al-Kadhum, D.T., 2020, Spectrophotometric investigation of thymol by utilize of oxidative coupling reaction in different samples of mouth washes, *Biochem. Cell. Arch.*, 20 (Suppl. 2), 4065–4070.

[9] Al-Majidi, M., 2023, Spectrophotometric determination of thymol in mouthwashes via sodium nitroprusside reaction with hydroxide amine hydrochloride, *Int. J. Res. Pharm. Chem.*, 13 (1), 101–107.

[10] Salian, M., and Agnihotri, J., 2023, Development and validation by UV spectrophotometric method for simultaneous estimation of fluconazole and thymol in formulation, *Int. J. Res. Appl. Sci. Eng. Technol.*, 11 (VI), 2175–2183.

[11] Zakaria, S.A., Zakaria, R.A., and Othman, N.S., 2018, Spectrophotometric determination of thymol in pure and pharmaceutical formulation via diazo–coupling with diazotised 4-aminoantipyrine, *Int. J. Enhanced Res. Sci. Technol. Eng.*, 7 (1), 38–43.

[12] Al-Abachi, M.Q., Hadi, H., and Fadhil, G., 2015, Flow injection determination of thymol in pharmaceutical samples via oxidative coupling reaction with 2,4-dinitrophenylhydrazine, *J. Al-Nahrain Univ.*, 18 (2), 18–24.

[13] Al-Abachi, M.Q., and Al-Najjar, N.A., 2015, Flow Injection spectrophotometric determination of thymol using 4-aminoantipyrine and copper(II) nitrate, *Baghdad Sci. J.*, 12 (2), 332–339.

[14] Dedić, M., Bečić, E., Imamović, B., Žiga, N., Medanhodžić-Vuk, S., and Šober, M., 2018, HPLC method for determination the content of thymol and carvacrol in Thyme tincture, *Glas. Hem. Tehnol. Bosne Hercegovine*, 50, 1–6.

[15] Angelo, T., Pires, F.Q., Gelfuso, G.M., da Silva, J.K.R., Gratieri, T., Cunha-Filho, M.S.S., 2016, Development and validation of a selective HPLC-UV method for thymol determination in skin permeation experiments, *J. Chromatogr. B*, 1022, 81–86.

[16] Louchard, B.O., Costa, L.C., e Silva, A.R.A., and Leal, K.A.M., 2017, Validation of a high performance liquid chromatography method to quantify thymol in nanocapsules of bioactive essential oil from *Lippia sidoides*, *Int. J. Complementary Altern. Med.*, 10 (2), 902–907.

[17] Ahmed, I., Foudah, A.I., Shakeel, F., Alqarni, M.H., Ali, A., Alshehri, S., Ghoneim, M.M., and Alam, P., 2022, Determination of thymol in commercial formulation, essential oils, traditional, and ultrasound-based extracts of *Thymus vulgaris* and *Origanum vulgare* using a greener HPTLC approach, *Molecules*, 27 (4), 1164.

[18] Chang, X., Sun, P., Ma, Y., Han, D., Zhao, Y., Bai, Y., Zhang, D., and Yang, L., 2020, A new method for determination of thymol and carvacrol in *Thymi herba* by ultra-performance convergence chromatography (UPC^2), *Molecules*, 25 (3), 502.

[19] Muniz, V.M., Chaves Júnior, J.V., Aragão, C.F.S., de Souza, F.S., and Sampaio, F.C., 2023, A HPLC method for simultaneous quantification of chlorhexidine and thymol using Box-Behnken design for robustness of the method assessment, *J. Liq. Chromatogr. Relat. Technol.*, 46 (6-10), 168–179.

[20] Bernal, J., Nozal, M.J., Bernal, J.L., and Ares, A.M., 2020, Determination of carvacrol and thymol in honey by using a simple and efficient headspace-gas chromatography-mass spectrometry method, *Food Anal. Methods*, 13 (11), 2138–2146.

[21] Ares, A.M., Nozal, M.J., Bernal, J.L., and Bernal, J., 2020, Simultaneous determination of carvacrol and thymol in bee pollen by using a simple and efficient solvent extraction method and gas chromatography-mass spectrometry, *J. Pharm. Biomed. Anal.*, 181, 113124.

[22] Lona-Ramírez, F.J., Hernández-López, N.L., González-Alatorre, G., Flores-Flores, T.C., Patiño-Herrera, R., and Louvier-Hernández, J.F., 2023, Thymol and carvacrol determination in a swine feed organic matrix using headspace SPME-GC-MS, *Rev. Mex. Cienc. Pecu.*, 14 (1), 78–93.

[23] Kast, C., Fracheboud, M., and Fuchsmann, P., 2022, Quantitation of 1,4-dichlorobenzene and thymol in beeswax using dynamic headspace vacuum transfer in trap extraction prior to gas chromatography-mass spectrometry, *Molecules*, 27 (17), 5367.

[24] Helaliy, R., Alrouh, F., Trefi, S., and Bitar, Y., 2020, Determination thymol in thyme extract and its pharmaceutical forms by using gas chromatography method, *Res. J. Pharm. Technol.*, 13 (9), 4055–4060.

[25] Humeidy, I.T., 2019, Spectrophotometric method for determination of sulfamerazine using 2,4-dinitrophenylhydrazine reagent, *J. Phys.: Conf. Ser.*, 1294 (5), 052022.

[26] Humeidy, I.T., 2021, Spectrophotometric determination of cefotaxime sodium in pharmaceutical formulations, *Mater. Today: Proc.*, 47, 6043–6049.

[27] Humeidy, I.T., Salman, S.A., and Hashim, K.K., 2020, Spectrophotometric determination of methyldopa with 2,6-diaminopyridine reagent using oxidative coupling reaction, *J. Eng. Sci. Technol.*, 15 (3), 1824–1839.