USING P-ANISIDINE FOR SPECTROPHOTOMETRIC DETERMINATION OF THYMOL BY OXIDATION COUPLING REACTION

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Abstract: *A rapid and sensitive spectroscopic method was developed for the determination of thymol in its pure form and in its pharmaceutical preparations. The method relied on oxidative coupling reactions. With the oxidizing agent N-chlorosuccinimide and the p-anisidine reagent in a basic sodium hydroxide medium to form a stable, blue-colored product that gives maximum absorption at a wavelength of 603 nm. The molar absorptivity was 1.35 x 10^4 l/mol.cm, and the concentrations followed Beer's law within the linear range 0.5-20 µg/ml. The accuracy of the method was 103.76%, while the compatibility was less than 0.28%. The LOD and LOQ values were 0.0300 and 0.100, respectively. The rate of the stability constant for the formed complex was 7.18 × 10^6 L/mol, which indicates the good stability of the compound. The method was successfully applied to pharmaceutical preparations in the form of mouthwash and ointment.*
Keywords: *Spectrophotometric determination, Thymol, NCS, p-Anisidine*
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Introduction

Thymol (thyme camphor) is a crystalline substituted phenol extracted from the volatile oils of thyme and other plants. Thymol has multiple uses, including in the medical field, as it is added to mouthwash due to its effectiveness against fungi and germs, and it is prescribed as an antiseptic to sterilize wounds [1-3]. Thymol is also used as a stabilizer in many pharmaceutical formulations, including halothane [4]. It has been reported to inhibit the maturation of calcium and potassium channels in cardiac cells in humans and other mammals [5]. In addition to these applications, thymol is employed in chemistry—particularly in analytical chemistry—either as an analyte to be determined [6] or as a reagent for the determination of pharmaceutical compounds [7]. Thymol has the chemical formula $C_{10}H_{14}O$, and its chemical structure is shown in Fig. 1.

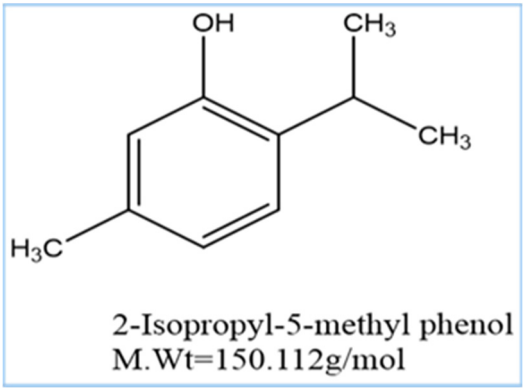

Fig. 1. Chemical structure of thymol

Thymol, both in its pure form and within pharmaceutical preparations, has been determined spectrophotometrically using various reagents [8–16], as well as by chromatographic methods [17–22].

Experimental part

Apparatus Used. A Shimadzu UV-1800 double-beam spectrophotometer was used for absorbance measurements with matched 1 cm glass cells. A sensitive balance (type AE ADAM) was employed for weighing. An electro-magnetic water bath was used to perform the heating process.

Solutions of Chemicals Used. All chemicals used were of high purity grade. The preparation procedures for the chemical compounds and the thymol drug solution used in the experiments are presented in Tables 1 and 2.

Table 1. Preparing the chemical compounds involved in the experiments

Chemicals	Conc.	Preparation
Thymol	100 µg/ml	The solution was prepared with 0.0100 grams of weight, dissolved in 2 ml of ethanol, and was transferred to a 100 ml volumetric flask, and the volume was completed to limit the mark with distilled water.
P-Anisidine	250 µg/ml	The solution was prepared by weighing 0.025 grams of compound, dissolving it with distilled water, and transferring it to a 100 ml volumetric flask.
N-chlorosuccinimide	1×10^{-2}M	The oxidizing agent, N-chloro-succinimide, was prepared by weighing 0.1335 grams, dissolving it with distilled water, and transferring it to a 100 ml volumetric flask.
Sodium hydroxide	1M	4 grams of sodium hydroxide were dissolved with distilled water and transferred to a 100 ml volumetric flask.

Table 2. Preparation of drug solution (Thymol) from the pharmaceutical preparation

Listerine mouthwash	A 20 ml aliquot of mouthwash was transferred into a 50 ml volumetric flask, followed by the addition of 5 ml of ethanol. This yielded a solution with a concentration of 240 µg/ml, from which a 100 µg/ml solution was subsequently prepared. The concentration of the drug compound in the mouthwash was determined using the straight-line equation obtained from the standard calibration curve of the pure substance
Smacks ointment	One gram of ointment containing 0.113% thymol was dissolved in 80 ml of hot triethanol in a beaker with continuous stirring and heating to ensure complete dissolution. The resulting solution was filtered and transferred into a 100 ml volumetric flask. From this stock, a solution with a concentration of 100 µg/ml was prepared. The concentration of thymol in the ointment was determined using the straight-line equation obtained from the standard calibration curve of the pure substance.

Result and discussion

The final absorption spectrum of thymol with p-anisidine, in the presence of the oxidizing agent NCS and sodium hydroxide, is presented in Fig. 2.

Preparation of the Standard Calibration Curve. Following the optimal conditions described in Table 6, the standard curve was prepared by transferring increasing volumes (0.05–2.0 ml) of pure thymol solution (100 µg/ml) into 10 ml volumetric flasks and diluting to the mark. The absorbance of each solution was measured at 603 nm. As shown in Fig. 3, the method obeys Beer's law in the concentration range of 0.5–20 µg/ml for thymol, with a molar absorptivity of 1.35×10^4 $L\cdot mol^{-1}\cdot cm^{-1}$.

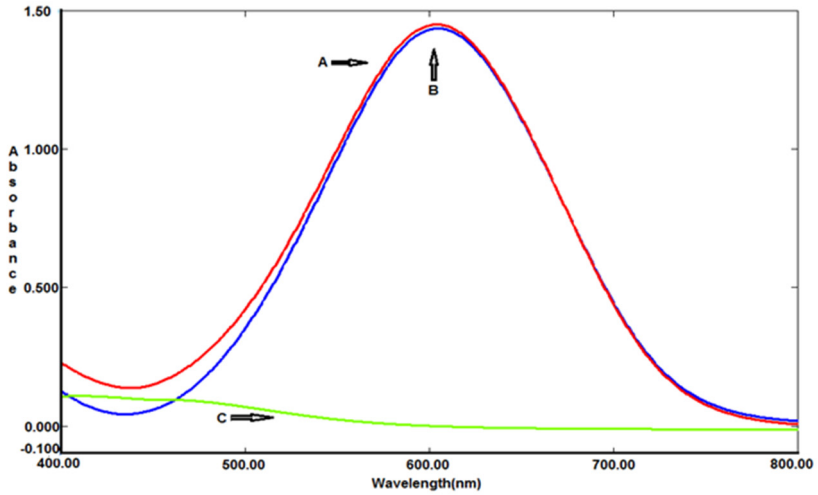

Fig. 2. Final absorption spectrum of thymol (17.5 µg/ml). Absorption spectrum of thymol complex vs. distilled water (A); absorption spectrum of thymol complex vs. the blank solution (B); absorption spectrum of blank solution vs. distilled water (C)

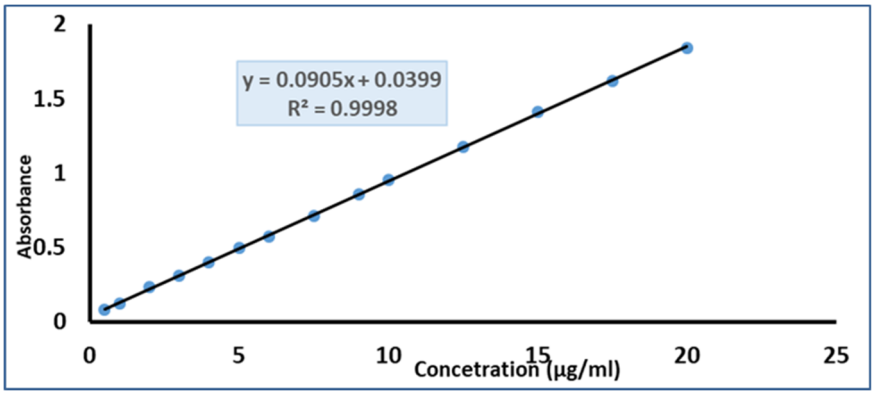

Fig. 3. Standard curve for the thymol (0.5-20) µg/ml

Study of Optimal Conditions. The optimal conditions were determined by adding the tested components to thymol at a concentration of 1×10^{-2} M in order to enhance absorption sensitivity. The experiments were conducted in the presence of fixed amounts of *p*-anisidine reagent and sodium hydroxide. As shown in Table 3, the results indicate that the oxidizing agent N-chlorosuccinimide provides the highest absorption and was therefore selected for subsequent experiments.

Table 3. Effect Different types of oxidizing agents

Type Of Oxidant(1×10^{-2} M) (1ml)	$NaIO_4$	KIO_3	$FeCl_3$	**NCS**	$CuSO_4.5H_2O$	$KMnO_4$	NBS
Absorbance	0.255	0.140	0.085	**0.899**	0.125	0.050	0.422
ʎmax(nm)	433	269	277	**603**	320	239	560

Effect of Different Amounts of the Oxidizing Agent. The optimal volume of the oxidizing agent was determined by adding increasing amounts (0.25–2.0 ml) of an N-chlorosuccinimide solution at a concentration of 1×10^{-2} M. As shown in Fig. 4, the maximum absorbance was obtained when 1.0 ml of the oxidizing agent was used. This volume was therefore selected for subsequent experiments.

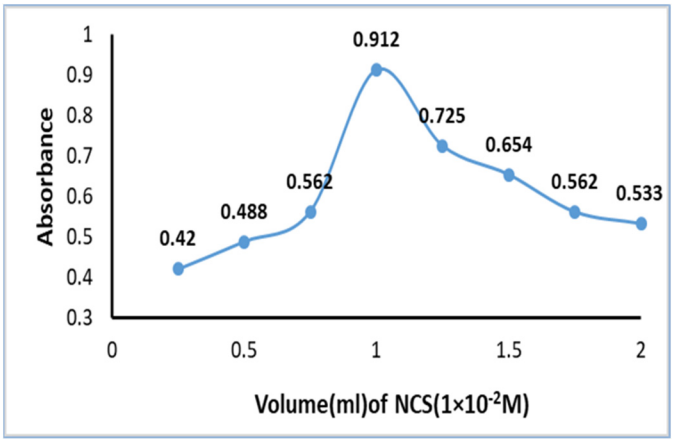

Fig. 4. Effect of different amounts of oxidizing agent

Effect of Different Amounts of Reagent. The effect of varying the volume of P-anisidine reagent was investigated by adding different amounts (0.25–1.5 ml) of a 250 μg/ml solution. As presented in Table 4, the maximum absorbance was obtained when 1.0 ml of P-anisidine was used. This volume was selected as the optimal value for subsequent experiments.

Table 4. Effect of different quantities of reagent.

Volume(ml)of p-ansidin (250μg/ml)	0.25	0.5	0.75	**1.0**	1.25	1.5
Absorbance	0.255	0.352	0.488	**0.915**	0.820	0.785

Effect of Different Types of Bases. The influence of various bases on the reaction was studied at a concentration of 1 M to determine the one providing the highest absorbance. Measurements were carried out at a wavelength of 603 nm. As shown in Fig. 5, sodium hydroxide produced the maximum absorbance and was therefore selected as the optimal base for subsequent experiments.

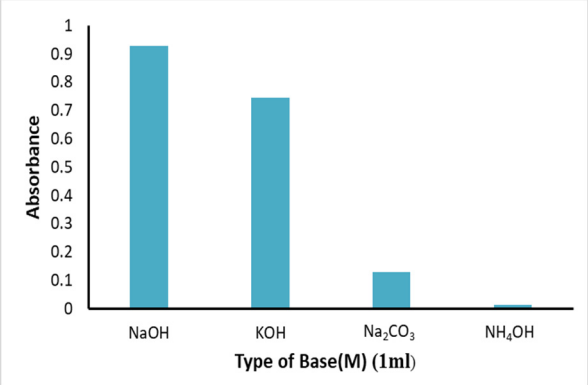

Fig.5. Effect of different kinds of bases

Effect of Different Amounts of Base (NaOH). The effect of increasing volumes of sodium hydroxide (0.25–1.5 ml, 1 M) on the absorbance of the conjugation product of thymol with *p*-anisidine was investigated (Table 5). The results showed that 1.0 ml of sodium hydroxide provided the highest absorbance and was therefore chosen as the optimal volume for further experiments.

Table 5. Effect of different amounts of sodium hydroxide.

NaOH (1M) Volume in ml	Absorbance
0.25	0.412
0.5	0.488

0.75	0.538
1	**0.920**
1.25	0.810
1.5	0.765

Effect of Oxidation Time. The effect of oxidation time was examined by adding the oxidizing agent to a series of 10 ml volumetric flasks containing 10 µg/ml of thymol solution and allowing the reaction to proceed for different time intervals (1–10 minutes). As shown in Fig. 6, an oxidation time of 5 minutes provided the highest and most stable absorbance, and was therefore selected as the optimal condition.

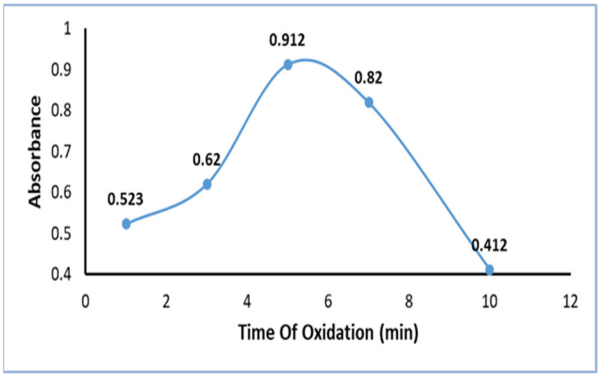

Fig. 6. Effect of oxidation time

Effect of Temperature. The effect of different temperatures (20–50 °C) on the oxidation of thymol was investigated. As shown in Fig. 7, at the laboratory temperature of 20 °C, the reaction required 15 minutes for complete formation and remained stable for more than 60 minutes.

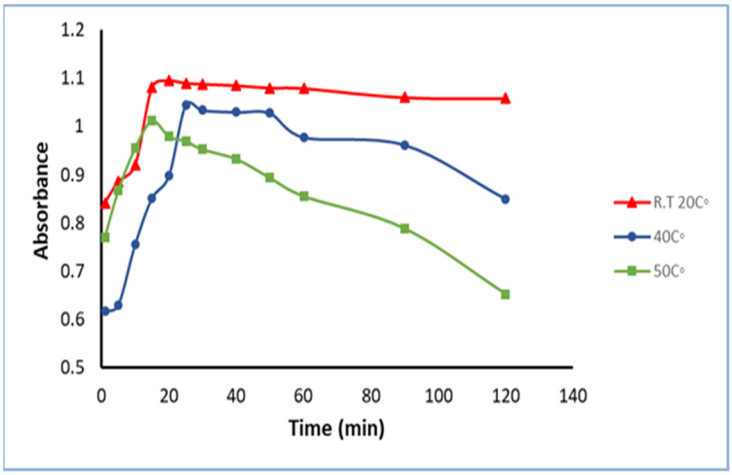

Fig.7. Effect of temperature

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

Experimental condition	**Concentration**	**Optimum amount (ml)**
Thymol	100 ppm	1
p-ansidin	250 µg/ml	1
NCS	1×10^{-2} M	1
Sodium hydroxide	1M	1
ʎ max(nm)	603	

Colour	Blue
Oxidation time(min)	5
Temperature(C◦)	20
Development Time(min)	15
Stability period(min)	>60

Accuracy and Precision of the Method. The accuracy and precision of the proposed method were evaluated by determining the recovery rate and relative standard deviation (RSD) from five replicate measurements at three different thymol concentrations. The results, summarized in Table 7, demonstrate that the method provides good accuracy and precision.

Table 7. Accuracy and precision of the method

Compound	Ammount added(µg/ml)		Recovery %	Average recovery %	RSD %
	Taken	found			
Thymol	2	2.098	104.9	103.76	0.36
	6	6.208	103.4		0.34
	15	15.45	103		0.14

Study of the Nature of the Resulting Product. The nature of the product formed between thymol and *p*-anisidine was investigated in the presence of N-chlorosuccinimide (NCS) as an oxidizing agent under basic conditions, using both Job's method and the molar ratio method [22].

Continuous Variation Method. The continuous variation method was applied to determine the molar ratio between the drug and the reagent. Equal molar concentrations of thymol and *p*-anisidine (6.65×10^{-4} M) were used, and the absorbance of the reaction mixture was measured at 603 nm. As shown in Fig. 8, the obtained results indicate a 1:1 molar ratio between thymol and *p*-anisidine.

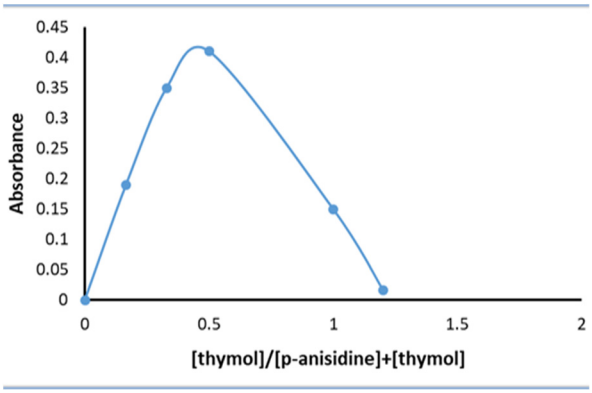

Fig. 8. Continuous Variation Method of thymol/p-anisidine

Molar Ratio Method. To confirm the validity of the results obtained by the continuous variation method, the molar ratio method was also employed. As shown in Fig. 9, the molar ratio between thymol and p-anisidine is 1:1, which corroborates the findings from the continuous variation method.

Proposed Chemical Reaction. Thymol reacts with p-anisidine in the presence of the oxidizing agent N-chlorosuccinimide (NCS) under basic conditions at a 1:1 molar ratio, resulting in the formation of a blue-colored product. Fig. 10 illustrates the reaction mechanism between thymol and p-anisidine.

The complex stability constant. The stability constant of the product formed in a 1:1 ratio between the drug compound and the reagent was calculated using the following law:

$$K_{st} = 1\text{-}\alpha / \alpha^2 c$$

The results in Table 8 show the high stability of the product formed between the drug compound and the reagent.

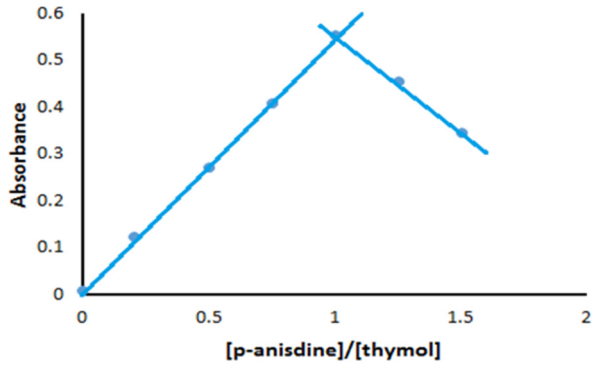

Fig. 9. Molar ratio method for thymol / p-anisidine

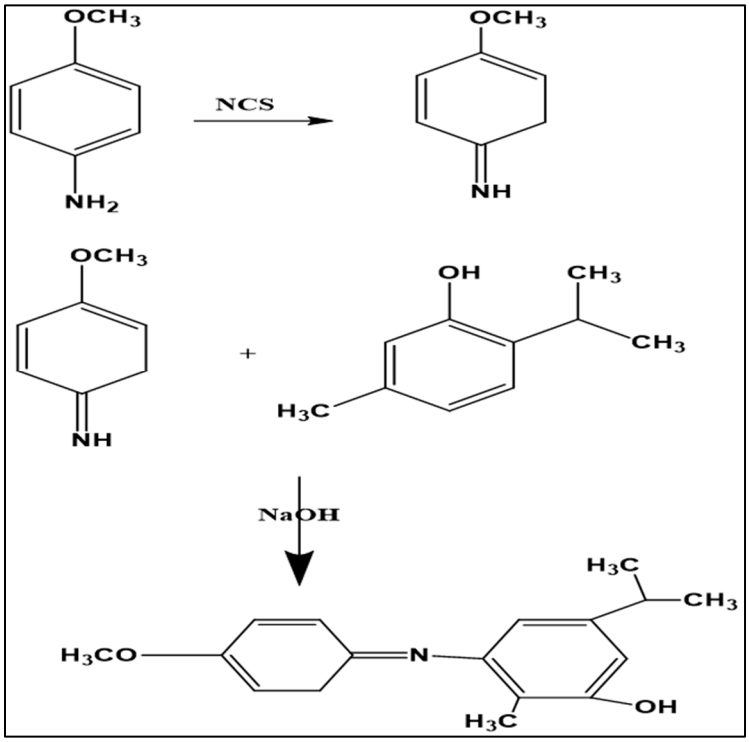

Fig. 10. Proposed chemical reaction

Table 8. Stability constant of the resulting thymol and p-anisidine products

Compound	Conc. mol/l	Absorbance		α	Kst	Average K_{st} l/mol
		As	Am			
Thymol	7.555×10^{-5}	0.2054	0.3096	0.19121	5.86×10^{4}	7.18×10^{6}
	1.511×10^{-4}	0.4166	0.474	0.12109	3.96×10^{5}	
	2.2665×10^{-4}	0.6082	0.6922	0.12135	2.63×10^{5}	

Application of the suggested method to pharmaceutical preparations. The suggested method for determining thymol was applied to pharmaceutical preparations that were in mouthwash and ointment form, as shown in Table 9.

Table 9. Pharmaceutical preparations used and their origin

Drug	Pharmaceutical preparation	Content %	Company

Thymol	Listerine Mouthwash	0.06	Johnsonc&Johnson-Spain
	Smicks Ointment	0.113	SDI,Samarra-Iraq

Table 10. Application of the developed method to pharmaceutical preparations

Pharmaceutical Preparation	Certified value %	Amount present µg/ml		Drug Content Found%	Recovery %	Average Recovery %
		Taken	Found			
Listerine Mouthwash	0.06	2	1.939	0.05814	96.9	97.7
		6	5.773	0.05772	96.2	
		15	15.01	0.06	100	
Smicks Ointment	0.113	2	1.974	0.111531	98.7	99.3
		6	5.985	0.112661	99.7	
		15	14.494	0.112548	99.6	

The outcomes evaluation of the developed method with the standard addition method. To prove the efficiency of the developed technique and its success in estimation, the standard addition method was applied to estimate thymol. It can be concluded from Fig. 11 that the results obtained are in good agreement with the developed technique, and this indicates that the technique has good selectivity.

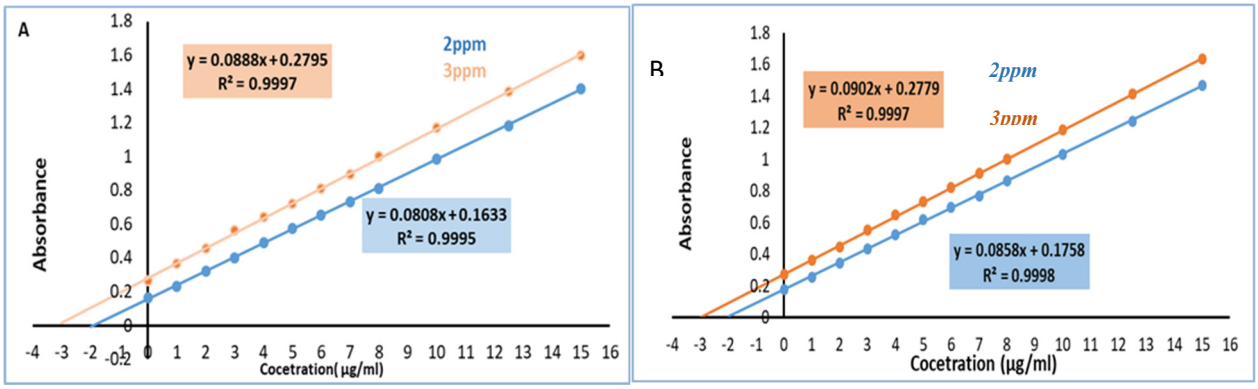

Fig. 11. Applcation of the standard addition method to the pharmaceutical preparation (A) Listerine (mouthwash); (B) Simicks(ointment)

Table 11. Standard addition method to estimate the thymol compound

Pharmaceutical preparation	Certified Value mg	Amount present µg/ml	Recovery %	Drug content found (mg)
Listerine Mouthwash	0.06	2	102.9	0.06174
		3	101	0.0606
Smicks Ointment	0.113	2	102.6	0.115938
		3	102.4	0.115712

Compare the developed method with another technique. The technique was compared with another spectroscopic method (Table 12), demonstrating higher sensitivity and a more favorable wavelength.

Table 12. Comparison of the suggested method with another spectroscopic methods

Analytical Parameters	Present Method	Literature Method [6]	Literature Method [11]	Literature Method [23]

λ_{max} (nm)	603	410	450	550
Reaction	Oxidative coupling	Charge Transfer Complex	Diazotization Coupling	Oxidative coupling
Reagent	p-anisidin	O-chloranil	4-Aminoantipyrine	p-phenylenediamine
Oxidizing agent	NCS	……..	…….	$NaIO_4$
Beer's law (µg/ml)	(0.5-20)	2.5-80	0.4-10	(0.4-24)
Molar absorptivity (l/mol.cm)	1.35×10^4	1140	1.27×10^4	7.45×10^3
Recovery %	103.76	…..	……	101.5
RSD %	< 0.28	< 4.39	……	< 2

Conclusion

A sensitive, rapid, and simple spectroscopic method was developed for the determination of thymol in its pure form and in pharmaceutical preparations. The method is based on an oxidative coupling reaction, where thymol is oxidized by N-chlorosuccinimide (NCS) in the presence of p-anisidine under basic conditions using sodium hydroxide. This reaction produces a blue-colored product with maximum absorbance at 603 nm. A linear relationship between concentration and absorbance was observed in the range of 0.5 to 20 µg/mL, with a molar absorptivity of 1.35×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$. The technique was successfully applied to pharmaceutical formulations, demonstrating its accuracy and suitability for routine analysis.

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