INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.17.1.2023.13(25)

SPECTROPHOTOMETRIC DETERMINATION OF THYMOL IN MOUTHWASHES VIA SODIUM NITROPRUSSIDE REACTION WITH HYDROXIDE AMINE HYDROCHLORIDE

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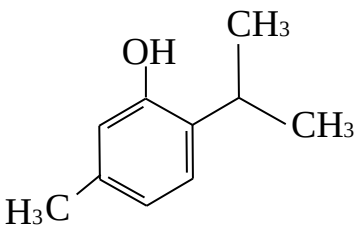
ABSTRACT

A reliable and efficient technique spectrophotometric method was developed for the determination of thymol. The method depends on its reaction with sodium nitroprusside and hydroxylamine hydrochloride in a regulated phosphate medium (pH12), to form a green product of $[Fe_2(CN)_{10}]^{-10}$ its maximum absorption was measured at a wavelength of 700 nm, estimated Microgram quantities ranged between 0.1 and 14 µg/mL with good accuracy and agreement, as the recovery rate was 100.94% and the relative standard deviation was less than 1%. The molar absorptivity was 27787 $L/mole^{-1}/cm^{-1}$ with alimit ofdetection of 6.0 ng/ml and a quantitative estimate of 20.0 ng/ml, which indicates that the method has high sensitivity. The developed method was applied successfully in the determination of thymol in pharmaceutical preparations (mouthwashes), and the results were in good agreement with the original content of the pharmaceutical preparation and the standard 4-amino antipyrine method. The validity of the analytical application of the method pharmaceutical preparations was also studied by calculating the t_{exp} and F values, which were less than the level values, which indicates that the developed method has good analytical application.

Keywords: Thymol, sodium nitroprusside and hydroxide amine hydrochloride.

INTRODUCTION

Thymol (thymol camphor) is a substituted crystalline phenol that has the chemical formula shown as follows[1].



2-Isopropyl-5-methylphenol
M.wt = 150.112 g/mol

It is obtained from the volatile oils of Thymus vulgris, Carum copticum, and other plants. Thymol has been widely used as an antiseptic in the medical fields, agriculture, cosmetics, and the food industry. To release inflammatory metabolites such as prostanoids, interleukins, and leukotrienes. Thymol was included in a number of ointments for the treatment of eczema and psoriasis, ulcers of the hands and feet, parasitic skin infections, and burns. Thymol was used in an oily solution (1.0% or 2.0%) in cases of catarrh of the respiratory tract in the form of a spray. Thymol is also added as a stabilizer to many therapeutic agents, which include halothane[2].

At the same time, it was found that thymol inhibits the exudation of calcium and potassium in cardiac cells of mammals and humans, and that little is known about its effects on mammalian skeletal muscles. toxicity with thymol may occur as a result

of its incorrect intake or use in anesthesia[3].

Besides, it was found that most of the studies discuss mainly to develop comprehensive methods for the estimation of phenolic compounds that enter the chemical and petrochemical industries and pesticides and apply them to industrial wastewater because they are a major source of pollution, as well as specialized methods for phenolic compounds that enter the pharmaceutical and food industries because thymol is effective and uncompensated in the Bara site can be estimated by a comprehensive methods, as well as the limited use of it previously in the medical fields, so it was found scarce in its specialized methods, and the following is a presentation of several methods:

The intermittent and continuous flow injection techniques were applied in the spectrophotometric determination of phenolic compounds (including thymol) by coupling it with the reagent 2,4,6-trimethylamine which is azoated in a medium with a pH of 9.5 and in the presence of sodium dodecyl sulphate to form an azo dye that has a maximum absorption at 380 nm, with a molar absorbance between 1000 and 7000 $Lmole^{-1}cm^{-1}$ for intermittent flow injection and for continuous flow injection 100-600 liters[3].Thymol and many phenolic compounds were determined by direct titration with iodine cyanide (ICN) and bromine cyanide (BrCN) in an anhydrous medium composed of acetic acid (10-100%) and a mixture of glacial acetic acid and acetic anhydride (1:1). It estimated 0.5 mg of phenol with a relative error of ± 0.25%[4].Thymol was also estimated by indirect titration by applying the amplification reaction. The method is based on the reaction of thymol with an excess of iodine in an alkaline medium to form a precipitate with a red color and to estimate the amount of unconsumed iodine with standard sodium thiosulfate in the presence of starch as a guide in an acidic medium. It was found that each molecule of thymol needs four atoms of iodine for precipitation[5]. Gas chromatography was used for the determination of thymol in biological fluids (after extraction of thymol from samples by diethyl ether), using a column filled with 5% of -25-OV, a flame ionization detector and eugenol as an internal reference. The limits of detection and quantification of thymol in plasma were 0.01 and 0.05 µg/mL, respectively[6].

Therefore, the aim of the workwas to develop a rapid, sensitive, and accurate spectrophotometric method for the determination of thymol in an aqueous solution and to apply it to mouthwashes by reacting with sodium nitroprusside and hydroxylamine hydrochloride in an alkaline medium.

MATERIAL AND METHODS

REAGENTS AND MATERIALS

Chemical name	Chemical structure	Company
Thymol	$C_{10}H_{14}O$	BDH
Sodium nitroprusside	$Na_2[Fe(CN)_5(NO)].2H_2O$	Riedel-dehaen AG
Hydroxylamine hydrochloride	$NH_2OH.HCl$	Fluka
Sodium dihydrogen phosphate	NaH_2PO_4	BDH
Sodium hydroxide	NaOH	BDH

Material solutions

Thymol solution

A thymol solution is prepared at a concentration of 100 micrograms /ml by dissolving 0.0100 g of thymol in 5 ml of ethanol, then adding volume to the mark with distilled water in 100 ml.

Hydroxylamine hydrochloride solution

The solution is prepared at a concentration of 4×2^{-10} molarity by dissolving 0.6949 g of the substance in 250 ml of distilled water.

Sodium nitroprusside solution

The solution is prepared at a concentration of 0.1 molar by dissolving 2.9705 g of the substance in 100 ml of distilled water.

Phosphate buffer solution (pH12)

It is prepared by mixing 100 milliliters of NaH_2PO_4 solution with a concentration of 0.1 M with 3.5 milliliters of sodium hydroxide with a concentration of 6.0 M.In a volumetric flask of 200 ml, then fill the volume to the mark with distilled water.

Solutions of surface-active substances

Prepared at concentrations of 0.1% and 1% in distilled water.

Interferential solutions

They are prepared at concentrations of 1.0 mg/ml in distilled water.

Apparatus

Spectral measurements were made by CE1021 CECIL single-beam spectrometer and Shimadzu UV-210 double-beam spectrophotometer, and 1 cm thick silica cells were used. Heating operations were carried out using a Grant water bath. The pH of the solutions was measured using a Philips PW 9420 pH meter connected to a pH 0-12 CEI electrode. Weighing operations were carried out using a sensitive balance of the Sartorius Management system.

RESULTS AND DISCUSSION

Spectrophotometric determination of thymol in aqueous solution: The absorption spectrum of the colored product formed from the reaction of thymol with sodium nitroprusside and hydroxylamine hydrochloride in phosphate medium (pH12) was studied by taking microgram amounts (100 micrograms /25 ml) of pure thymol solution and adding 1.0 ml of sodium nitroprusside solution at a concentration of 0.1M and 1. 0 ml of hydroxylamine hydrochloride solution at a concentration of 4 x 10^{-2} M, and 3.0 ml of phosphate buffer solution (pH12) and fill the volume with distilled water to the mark and leave the solution for 15 min at room temperature. The absorption of the green-colored produce was measured against the blank solution at wavelengths ranging from 410 to 850 nm, and it was found that the maximum absorption was given by the colored produce at the wavelength of 700 nm. **Fig.** (1).

Effect of the amount of sodium nitroprusside

It was found experimentally that the concentration of 0.1M of sodium nitroprusside solution is most appropriate in forming a colored and stable product with high sensitivity when thymol is estimated, so a study was conducted to choose the best volume of the solution (0.1- 4.0ml) and the results recorded in Table (1) showed that 0.5 milliliters It is the optimal size in estimation.

Effect of the amount of hydroxylamine hydrochloride

A study was conducted to stabilize the best amount of hydroxylamine hydrochloride solution, which gives maximum absorption of the colored product. Increasing volumes (0.1-3.0 ml) of hydroxylamine hydrochloride solution at a concentration of 4 x 10^{-2} M were added to equal concentrations of thymol, 0.5 ml of sodium nitroprusside solution at a concentration of 0.1 M, and 3.0 ml of phosphate buffer solution (pH12). The results listed in Table (2) indicate that 0.5 milliliters of the concentration used are the optimal volume of hydroxylamine hydrochloride.

Effect of the amount of buffer solution

The effect of different volumes of buffered phosphate solution with a pH of 12 has studied the absorption of the colored product. As it became clear from the results obtained in Table (3), the volume of 3.0 ml gave the best sensitivity.

Method and standard curve for the determination of thymol

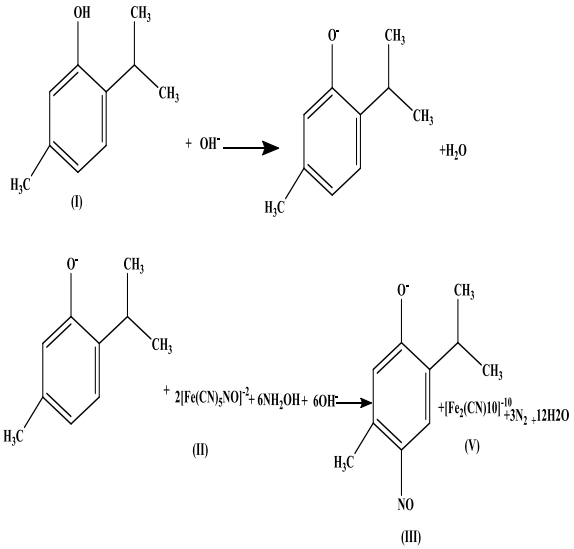
The standard curve was prepared for the determination of thymol in an aqueous solution, as follows:

Increasing volumes of pure thymol solution prepared at concentrations of 5, 50, and 100 µg/ml are added to a series of 25-ml volumetric vials to cover the range 2.5-350 µg/25ml, followed by the addition of 0.5 ml of sodium nitroprusside (0.1M) and 0.5 ml of sodium nitroprusside (0.1 M) of hydroxylamine hydrochloride solution (4×10^{-2} M) and 3.0 ml of phosphate buffer solution (pH12) and addition the volume to the mark with distilled water. The solutions were left for 15 minutes at room temperature and the absorbances were measured against the blank solution at a wavelength of 700 nm. Figure (2) shows the straight standard curve that follows Beer's law for the estimation of amounts ranging from 0.1 to 14 µg / ml, and there is a negative deviation from Beer's law after the upper estimated limits. The value of the correlation coefficient for the standard curve was more than 0.99, which indicates that it has excellent linear characteristics.

The molar absorptivity value was 27787 L. $mole^{-1}$ cm $^{-1}$and Sandell significance is 0.0054 µg/cm^2, while it was found that the values of LOD* and LOQ* were equal to 6.0 and 20.0 ng/ml, respectively, which indicates the high sensitivity of the method[7].

The accuracy and compatibility of the method were checked by calculating the recovery ratio and the relative standard deviation for three different concentrations of thymol. Table (4) includes the obtained results, which indicate that the method has good accuracy (recovery rate 100.94%) and good agreement (RSD less than 1.0%).

In addition, Thymol (I) was estimated based on the method used in the determination of phenol and its substitutes[8], in which phenol is reacted with sodium nitroprusside (II) and hydroxylamine hydrochloride in a regulated phosphate medium (pH12), to form a colored product that gives maximum absorption at 700 nm wavelength. The spectral studies showed by Kang[8], and his group have proven that the reaction depends on the presence of the phenolic hydroxyl group in the basic medium, which activates the aromatic ring towards the electrophilic compensation reactions, so the compensation occurs on the ring in the para site mainly, as the formed phenoxide reacts with The nitroso group (N = O) as the electrophile (provided by sodium nitroprusside) giving the ion 2-isopropyl-4-nitroso-5-methyl phenol (III), then the dimerization process takes place for the remaining part of the sodium nitroprusside to give a colored product green (V) and as shown in the proposed schematic below.



Application of the developed method to pharmaceutical preparations. The developed method was applied to the determination of thymol in its pharmaceutical preparations, which were in the form of mouthwashes from different origins, as shown in Table (5).

Mentoral mouthwash analysis

Prepare an initial solution with a concentration of 252 µg / ml by diluting 20 ml of the content of a mouthwash package (containing 0.063 g / 100 ml of thymol) to 50 ml with distilled water, then prepare a solution with a concentration of 100 µg /ml, as different volumes of the last solution were used to obtain concentrations of 4, 8, 10 and 12 micrograms/ml of thymol were treated according to the working method standard Thymol. The concentration of thymol from mouthwash was found using the standard curve of the compound in its pure form, and the results were included in Table (6).

Lastarime mouthwash analysis

Dilute 20 milliliters of the content of a mouthwash package (containing 0.06 g / 100 milliliters of thymol) to 50 milliliters with distilled water to obtain a solution with a concentration of 240 micrograms/milliliter, and a solution with a concentration of 100 micrograms/milliliter was prepared from it, and different volumes were taken from the solution to obtain concentrations 4 and 8, 10 and 12 micrograms/milliliter of thymol were treated in the same way as described for the standard solutions. The concentration of thymol in the mouthwash using the standard curve of the drug compound in pure form, and the obtained results were recorded in Table (6).

The results recorded in Table (6) indicate the accuracy of the method in estimating thymol in pharmaceutical preparations compared to the original content of pharmaceutical preparations, as the recovery rate for estimating thymol in Mentoral and Lastarime mouthwashes reached 98.86% and 101.83%, respectively[9].

Besides, A comparison was made between the proposed analytical method and the standard 4-amino antipyrine method[10], for the determination of thymol to find out the accuracy and validity of the analytical application of the proposed method using the F and t-tests for four estimates mentioned in paragraph above. The results of both methods were included in Table (7), including Variance values extracted for each of the proposed and standard methods.

It has been shown from the results of the above table that the experimental t value for Mentoral and Lastarime mouthwashes are equal to 0.34 and 2.10, respectively, which is less than the level t of 2.45 at the 95% confidence level and six degrees of freedom[11]. This indicates that the difference is not significant between the two proposed and standard methods, which indicates However, the proposed method has good applicability to

pharmaceutical preparations, and it was found that the experimental F value is equal to 2.26 and 2.13 for Mentoral and Lastarime mouthwashes, respectively, and it is less than its tabular value of 9.28 at the 95% confidence level and for three degrees of freedom, so we can judge that there is no difference, It is clear between the accuracy of the two methods and the standard deviation of both methods resulting from random errors[12].

Conclusion

In the present study, we demonstrate the development of a simple and sensitive spectrophotometric method for the determination of thymol. This method is based on the reaction between thymol, sodium nitroprusside, and hydroxylamine hydrochloride in a phosphate buffer medium (pH12) to form a green product that has an absorption maximum of 700 nm. Beer's Law is complied with over a concentration range of 0.1 - 14 µg/mL with good accuracy (mean recovery) of 100.94% and accuracy (RSD) of less than 1%. The molar absorbance is 27787 L. mol. cm^{-1} with a LOD of 6.0 ng/mL and a LOQ of 20.0 ng/mL. The proposed method has been successfully applied for the determination of thymol in pharmaceutical preparations. The analytical results are consistent with those obtained by the standard 4-AAP method.

ACKNOWLEDGMENT

We are thanking the University of Babylon, College of Science for Women-Iraq, for providing the necessary infrastructure.

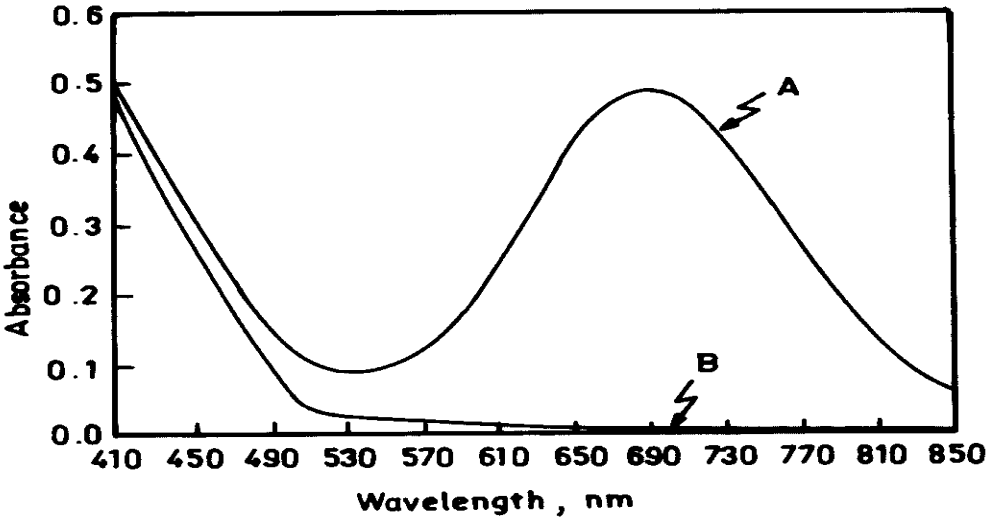


Fig. 1: Absorption spectra, A: stained product of thymol (4 µg/mL) vs. Blank reagent, B: Blank reagent versus distilled water

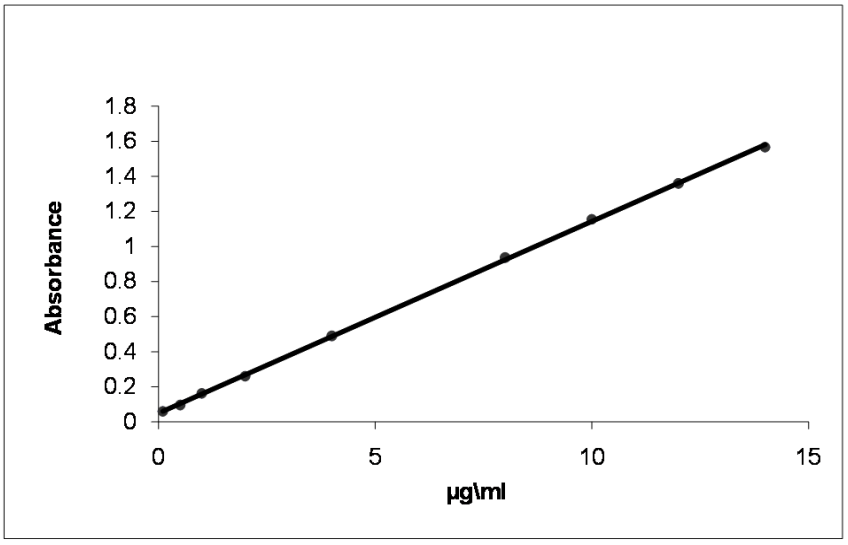


Fig. 2: Standard curve for the of thymol

Table 1: Effect of the amount of sodium nitroprusside

Volume of 0.1 M sodium nitroprusside (ml)	0.1	0.2	0.3	0.5	1.0	1.5	2.0	2.5	3.0	4.0
Absorbance	0.474	0.475	0.478	0.485	0.482	0.477	0.469	0.467	0.460	0.456

Table 2: The effect of the amount of hydroxylamine hydrochloride on the absorption intensity of the colored product

Volume of 4×10^{-2} M hydroxylamine hydrochloride(ml)	Absorbance
0.1	0.452
0.3	0.478
0.5	0.492
1.0	0.488
1.5	0.471
2.0	0.465
2.5	0.460
3.0	0.448

Table 3: Effect of the amount of buffered phosphate solution

Volume of phosphate buffer (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Absorbance	0.464	0.469	0.473	0.478	0.489	0.495	0.485	0.472

Table 4: Accuracy and compatibility of the method for the determination of thymol

Amount (µg/ml)		Recovery *(%)	Average recovery (%)	RSD*(%)
Added	Found			
1	1.03	103.00	100.94	0.78
4	4.03	100.75		0.89
14	13.87	99.07		0.25

***Average of six determinations**

Table 5: Pharmaceuticals used and their origins

Pharmaceutical preparation	Contains	Company
Mentoral antiseptic (Mouth wash)	Thymol 0.063%w/v	Amman pharmaceutical Industries Co.Ltd - Jordan
	Menthol 0.042% w/v	
	Ethanol (96%) 29% v/v	
	Benzoic acid 0.125 %w/v	
	Eucalyptol 0.091 %	
	Methyl salicylate 0.055	
Lastarime antiseptic	Benzoic acid 0.12%	Homs - Syria
	Eucalyptol 0.09 %	
	Menthol 0.04%	
	Methyl salicylate 0.05%	
	Thymol 0.06%	

Table 6: Determination of thymol in pharmaceutical preparations

Pharmaceutical preparation	Certified value (mg)	Amount present (µg / ml)	Recovery* (%)	Drug content found (mg)
Mentoral antiseptic	0.063	4	97.49	0.062
		8	98.84	0.062
		10	97.23	0.061
		12	101.42	0.064
Lastarime antiseptic	0.060	4	101.25	0.061
		8	102.25	0.061
		10	102.95	0.062
		12	100.88	0.061

***For three determinations**

Table 7: Comparison of the proposed method for the determination of thymol in pharmaceutical preparations with standard 4-amino antipyrine

Pharmaceutical preparation	Recovery (%) *		RE(%)*	$t_{exp.}$	F_{test}
	Present method	Standard method			
Mentoral antiseptic	98.86	98.48	0.39	0.34	2.26
Lastarime antiseptic	101.83	100.86	0.96	2.10	2.13

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