Determination of Thymol in Pure and Pharmaceutical Preparation by Diazotization – Coupling Method with 2,4- Dichloroaniline as the Coupling Agent

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الخلاصة

أقترحت طريقة بسيطة و سريعة و حساسة لتحليل الثايمول النقي و في المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الأزوتة ل٢و٤- ثنائي كلورو أنيلين مع نتريت الصوديوم في حامض الهيدروكلوريك لتكوين ملح الديازونيوم الذي يزدوج مع الثايمول بوجود هيدروكسيد الصوديوم لتكوين صبغة آزو،حيث يحصل أقصى امتصاص عند طول موجي 419 نانومتراً. و التي تطيع قانون بير عند مدى التركيز (1-10) مايكروغرام مل$^{-1}$ للثايمول. و إن قيمة الامتصاصية المولارية $2.3X\ 10^{-4}$ لتر مول$^{-1}$ سم$^{-1}$. طبقت الطريقة بنجاح في تقدير الثايمول في المستحضرات الصيدلانية بدون تداخل من قبل المواد المعروفة التي تستعمل كمضافات للدواء. كانت نتائج الطريقة متوافقة معنوياً مع طريقة القياسية المعتمدة.

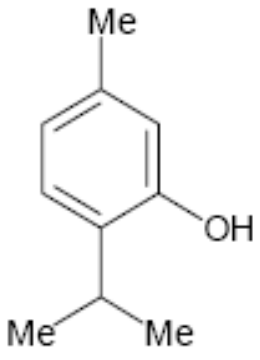
Abstract

Simple, rapid and sensitive spectrophotometric method was proposed for the analysis of thymol in pure form as well as in pharmaceutical tablets. The method is based on the diazotization reaction of 2,4-dichloroaniline with sodium nitrite in hydrochloric acid medium to form diazonium salt, which is coupled with thymol in sodium hydroxide medium to form azo dye, showing absorption maxima at 419 nm. Beer's law is obeyed in the concentration range of ١٠-١ μg mL^{-1} of thymol. The molar absorptivity is 2.3×10^4 L mol^{-1} cm^{-1} The method was successfully applied to the determination of thymol in pharmaceutical without any interference from common excipients used as additives in tablets. The results agree favorably with standard method .

Keywords: *Spectrophotometric;; thymol; Diazotization, 2,4-dichloroaniline*

Introduction

Thymol use Antimicrobial preservative; antiseptic. Thymol is 5-methyl-2-(methylethyl)phenol. it is colorless crystals with an aromatic odour; melting point, about 50° with formula explain below [1,2].



Mouthwashes containing four phenol-related essential oils (thymol, eucalyptol, menthol and methyl salicylate in up to 26% alcohol) claim to penetrate the plaque biofilm and thus kill micro-organisms that cause gingivitis. These mouthwashes display broad spectrum antimicrobial activity, prevent bacterial aggregation, slow bacterial multiplication, retard plaque maturation and decrease plaque mass and pathogenicity[3,4].

Thymol is found in the naturally occurring herb Thyme *(Thymus vulgaris)*. Products containing thymol were originally registered in 1964 for use as repellents for domestic animals. Subsequently, thymol-containing pesticides were approved for use as aciricides, fungicides and anti-microbial [5,6].

In 1997, the Biochemical Classification Committee determined that thymol is a biochemical pesticide because it is naturally occurring and it is used in foods and pharmaceuticals[7].Herba Thymi contains not less than 1.0% volatile oil [8,9], and not less than 0.5% phenols. Volatile oil is quantitatively determined by water/steam distillation[10], and the percentage content of phenols expressed as thymol is determined by spectrophotometric analysis [10]. Thin-layer chromatographic analysis [10, 11]. In literature there are many worker detection and determine thymol by different analytical techniques, liquid chromatography[12],Spectrophotometric methods[13-15]and titrimetric method[16]. The purpose of current study is to describe the simple Spectrophotometric analytical methods for determining , thymol.

Experimental

Apparatus

All spectral and absorbance measurements were by using a Computerize UV-Visible, ,shimadzu T60U Spectrophotometer, with 1cm matched quartz cells.

Reagents

Working thymol standard material was provided from state company for Drug Industries and Medical appliance (SDI) Sammara-Iraq.of (99% purity) and stander solution of 100 µg ml^{-1} was freshly prepared by dissolving 0.01gm of thymol in 20ml absolute ethanol and then diluted with distilled water to the mark with 100 ml volumetric flack. 2,4-dichloro aniline of (98.0% purity) was obtained from (Merck) a stander solution of 100 µg ml^{-1} was freshly prepared by dissolving 0.01gm of 2,4-dichloro aniline in 10ml absolute ethanol and then diluted with distilled water to 100 ml.

Sodium nitrite (99.8 purity) from (BDH) and stander solution of 1% was prepared. Sodium Hydroxide of (98% purity) from (RDL), solution of 1M was prepared by dissolving 4 gm in 100 ml distilled water, 100 ppm of varies interferences and 1M both of HCl, sulfuric acid and phosphoric acid were used These solutions are stable for a period of 7 days when refrigerated (4 °C).

General procedure

The 0.5 ml of thymol standard solution 100µgml $^{-1}$ and 0.5 ml of 1M sodium hydroxide solutions were added to 0.5 ml of of 2,4-dichloroaniline and 0.5 ml of

1% sodium nitrite and 0.5 ml of 1M HCl were mixed in and completed with distilled water to the mark in10 ml volumetric flask and shaked for 2 minutes, with shaking and cooling ice bath for 2 minute, after 5 minutes the orange color is completely developed and the absorbance measurement was carried out at a wavelength at 419 nm, against a blank solution prepared in the same method but without thymol

Procedure for the assay of mouth washing solution Lastarim antiseptic (100 µg ml^{-1})

The average concentration of thymol in Lastarim antiseptic drug container was (0.06gm in 100 ml) so, 40 ml from container was dissolved in distilled water into a 100 ml volumetric flask, and finally diluted to the marked with distilled water to obtain 240. µg ml^{-1}

Further appropriate solution (100 µg ml^{-1}) was made by using distilled water. Two different concentrations of this mouth washing solution were analyzed in five replicate by analytical spectrophotometric procedure.

Results and Discussion

The result of this investigation indicated that the reactions between thymol with 2,4-dichloroaniline in the presence of sodium nitrite and hydrochloric acid yield highly soluble colored condensation products which can be utilized as a suitable assay procedures for thymol The orange colored product have maximum absorption at 419 nm and. The blank at these wave lengths shows zero absorbance. (Figures 1,C). The influence of various reaction variables on the color development was tested to establish the most favorable conditions

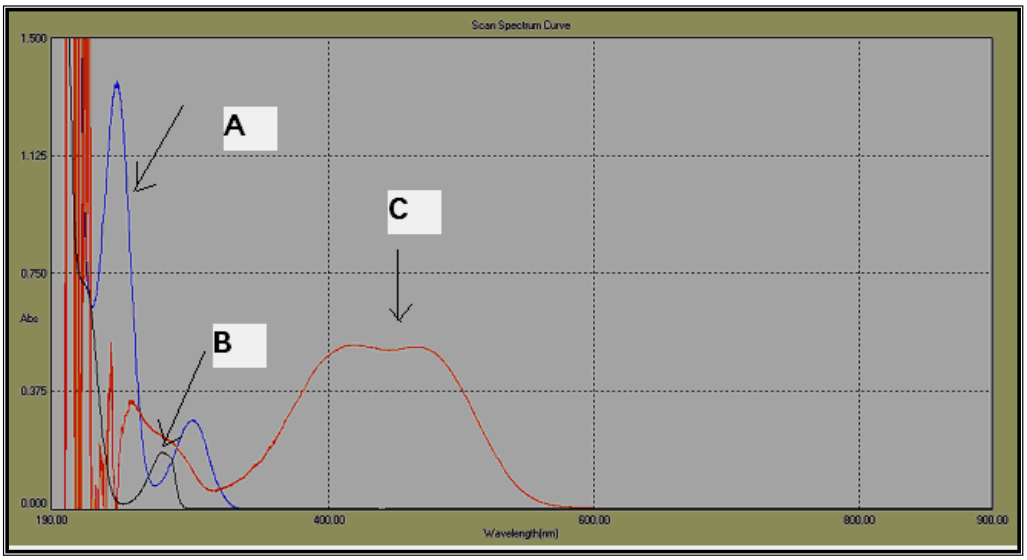


Fig. 1: (A) Absorption spectrum of thymol against distilled water , (B)Absorption spectrum of: 2,4-dichloroaniline against distilled water (C)Absorption spectrum of the azo dye formed against reagent blank .

Optimization of the experimental conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of thymol.

Effect of Base

It was found that the presence of a base led to increase the intensity of the produced product so;1M of NaOH was selected which was found that the best volume equal to 0.25 ml of this base give high sensitivity which selected in subsequent experiments .Fig. 2

Effect of acid

It was found experimentally that the colored products were formed stable by. using of the amount of 0.25 ml of (1M)hydrochloric acid and then used in determination of thymol Fig 2.

Effect of sodium nitrite

The optimum concentration of sodium nitrite solution that gave maximum absorption was found to be 1.75ml of 1% of sodium nitrite solution. Fig.2 explained these results.

Effect of reagent concentration:

The effect of various concentrations of 2,4-dichloroaniline were investigated using the proposed procedure and adding 0.25-3 ml of 100µg ml^{-1} 2,4-dichloroaniline. It was found necessary for developing the colored products 2 ml of 100µg ml^{-1} 2,4-dichloroaniline solution in final volume of 10 ml. Fig. 2 explained these results.

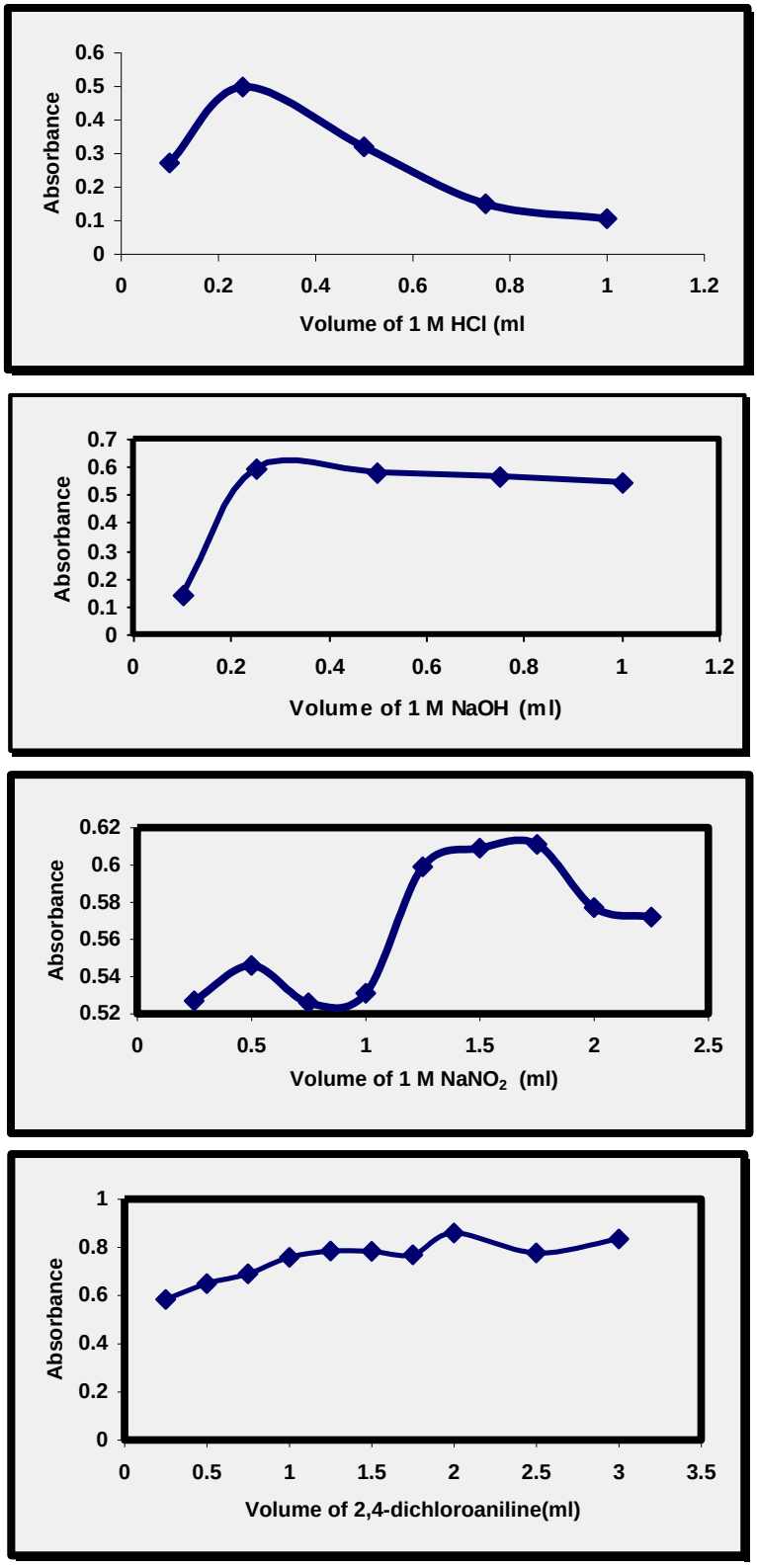


Fig. 2: Optimum conditions for determination of Thymol

The orange color azo dye was only formed in alkaline medium. Therefore, the effects of different alkaline solutions were studied such as sodium carbonate, potassium hydroxide, sodium hydroxide and ammonium hydroxide. It was found that sodium hydroxide is the most suitable alkaline medium to produce a maximum absorbance and was used in all subsequent experiments.

The stability of the dye was studied for 24 h following the mixing of the reagents. The colored azo dye developed rapidly after mixing and attained maximum absorbance about 2 min at room temperature. The color was stable for a period of 24 h.

The effect of temperature on the diazotization and coupling reaction show that the absorbance of the azo dye remains constant in the range 0 – 30ºC and decreases up to 30 ºC. Therefore, it has been recommended to carry out reaction at room temperature

Calibration carves

Employing the conditions described in the procedure, a linear calibration graph for thymol is obtained Fig. 3, which shows that Beer's law is obeyed over the concentration range of **1-10-** μg ml^{-1} with correlation coefficient of 0.9994 and The conditional molar absorptivity of the orange product formed was found to be $2.3x10^4$ $L.mol^{-1}.cm^{-1}$.

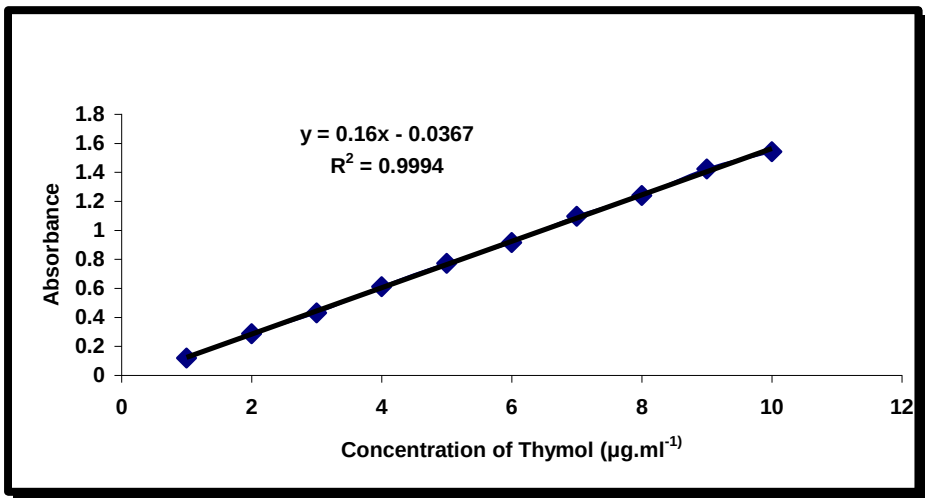


Fig. 3: The calibration curve of Thymol.

Effect of Organic solvents

The effete of organic solvents such us methanol, ethanol, propanol, acetone, and distill water were studied by using in the dilution and measuring the absorbance the absorbance were found 1.95,1.63,1.7, 1.65 and 1.437 respectively Distill water found to be the best. , Cheap, and available solvent.

Interference

The effect of some foreign compounds, which often found in pharmaceutical products, were studied by adding different amounts organic compounds to 1ml of 100μg/ml of thymol. The color was developed following the recommended procedure described earlier.. It was observed that the Glucose, Benzoic acid,Starch,$NaHCO_3$were not interfering with the determination at levels found in dosage form.

Effect of Order of Addition

To obtain optimum results the order of addition of reagents should be followed as given under the procedure, otherwise a loss in colour intensity was observed.

Structure of the Dye

The stoicheiometry of the reaction between thymol and 2- chloro-4-nitroaniline was investigated using mole ratio method[17]; the results obtained figure 4 show that 1:1 drug to reagent was formed at 419 nm.

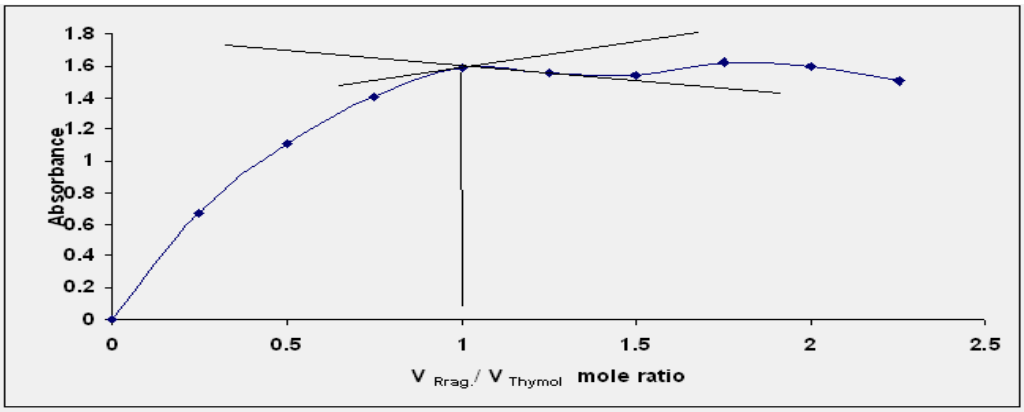


Fig. 4. Molar ratio method of thymol and 2- chloro-4-nitroaniline

Therefore the formation of the product probably occurs as follows Figure5.

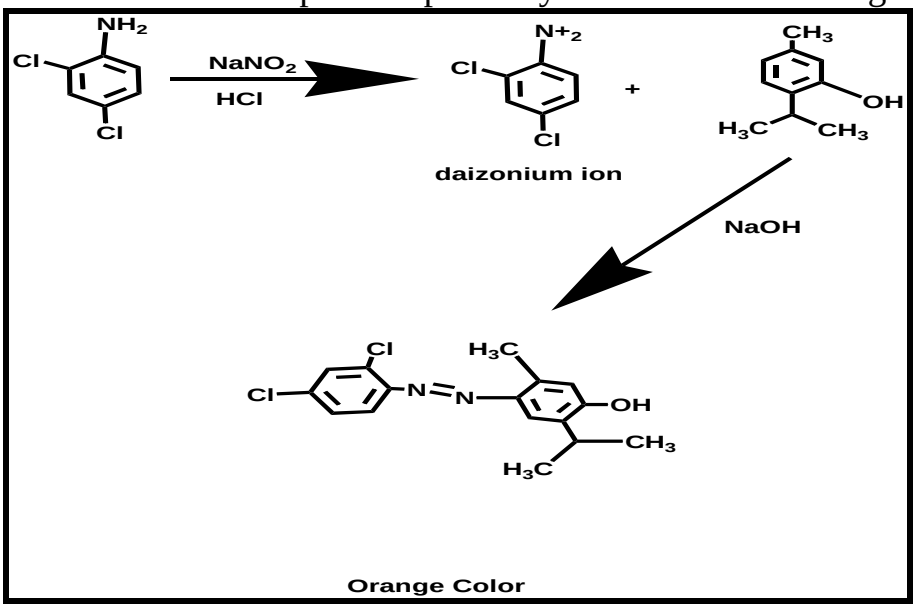


Fig. 5: Probable product formation pathway.

The product formed was water soluble, the stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of thymol and 2- chloro-4-nitroaniline The average conditional stability constant of the dye in water under the described experimental conditions was $1.5x10^4$.

Precision and Accuracy

To evaluate the accuracy and precision of the methods, pure drug analyzed, each determination being repeated five times. at three different concentrations.

Table1:Accuracy and precision of proposed methods

Thymol Taken	Thymol found	*Recovery% Rec%	%E Relative Standard error	Relative Standard Deviation*RSD%
2.5	2.48	99,2	2	1.12
5	5.01	100.2	1	1.18
10	9.98	99.8	2	1.002

* Average of five determinations

The results shown in Table 1 indicate that satisfactory precision and accuracy could be attained with the proposed method .The RE (%) and RSD (%) values were less than1.2%which indicate the high accuracy.

Analytical application

Proposed method have been used Lastarim antiseptic drug containing thymol (mouth washing) and it gave good accuracy and precision as showen in table 2, the proposed method was compared with standard method[18] ,since F-test,T-test showed that there was no significant differences between the proposed methd and standard method,the results obtained were tabulated in Table 3.

Table 2: Application of the proposed method and pharmaceutical preparations for determination of thymol drug.

Thymol Sample	Thymol ppm		Recovery% Rec%	* Average recovery% Rec%	Relative Standard Deviation* % RSD%
	Taken	Found			
Lastarim[a] antiseptic	5	5.11	102.2	100.95	1.6
	10	9.97	99.7		1.7

a Marketed by India..

Table 3: Comparison of thymol determination in pharmaceutical preparations by the proposed method and comparison with standard method

SBS Sample	Recovery Rec%	
	Standard method	Proposed method
Lastarim[a] antiseptic	99.7	100.95

Table 4: Comparison of thymol determination in the proposed method and other literature methods.

Reagent	λ_{max} nm	ε ,L mole^{-1} cm^{-1}	Linear range µg.ml^{-1}	Ref.
N,N –diethyl-p-phenylen diamine hydrochloride	605	1.9×10^3	0.4-11	13
Sodium nitroprusside	700	2.77×10^4	0.1-14	14
Para phenylen diamine	550	7.45×10^3	0-4-24	15
2,4-dichloroaniline	419	2.3×10^4	1-10	Proposed methods

Conclusions

The proposed method was found to be simple, rapid, economical, selective, sensitive and good color stability for determination of thymol and high selectivity and excellent sensitivity than other spectroscopic methods in literature for the oxidative coupling reaction of thymol .as table (4) The proposed method does not require temperature control or the solvent extraction step; the method was applied successfully on pharmaceutical samples .

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