Spectrophotometric Determination of Thymol in Pure and Lesterine Mouth washing

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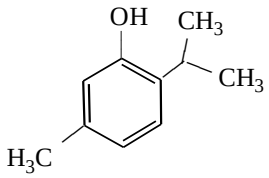
Abstract

A simple and sensitive spectrophotometric method for the determination of Thymol in pure and pharmacetical drugs form has been described**.** This Method was based on the diazotization of the drug by sodium nitrite in acidic medium at 5C° .This was followed by coupling with ortho-aminobenzoic acid to form yellow colored azo dye stabilized and measured at 390 nm. Beer's law was obey in the concentration range of 1-10µg ml^{-1}.The molar absorptivity and Sandell's sensitivity were $1.4x10^5$ L $mole^{-1}$ cm^{-1}, 0,009 µg cm^{-1} respectively. The detection limit and the limit of quantitation were 0,0082 µg ml^{-1}, 0,027 µg ml^{-1} respectively .All variables including the reagent concentration, reaction time, colour stability period, and mole ratio were studied in order to optimize the reaction conditions**.**No interferences were observed**.**Results of analysis were validated statistically and by recovery studies**.**These methods are successfully employed to determine thymol in some oral solution.The developed method is easy to use and accurate for routine studies relative to HPLC and other techniques.

Keywords: Determination Spectrophotometric;Thymol.

Introduction

Thymol is a phenolic compound (Camphor).It consists of white or colorless powder crystals , very slightly soluble in water, freely soluble in alcohol and in methanol[1] .The formula structure of the compound is :



Iso-propyl-m-cresol
(2-Iso propyl-5-methylphenol)
m.p : 50 – 51°C M.wt: 150.2

Thymol was initially registered as a pesticide in the United States in 1964 to be used as a repellent for domestic animals. Currently,thymol is listed by the Food and Drug Administration (FDA) as foods for human consumption, as well as food additives[2,3].

Thymol is a constituent of oil thyme, a naturally occurring mixture of compounds in the plant thymus vulgaris.L, or thyme[4]. Thymol is an active ingredient in pesticide products registered for use as animal repellents, fungicides/fungistats, medical disinfectants, tuberculocides, and virucides. These products are used on a variety of indoor and outdoor sites, to control target pests including animal pathogenic bacteria and fungi[5]. Products are liquids applied by spray, mop, brush-on,wipe-on dip, aerosol, immersion and spot treatment. Thymol also has many non-pesticidal uses, including perfumes, food flavorings, mouthwashes, pharmaceutical preparations, and cosmetics[6].

Most methods for the determination of thymol in cosmetics and pharmaceutical preparations are based on gas chromatography (GC)[7-9], high-pressure liquid chromatography (HPLC)[10-15] 'and colouremtric spectrometry[16].

In current study,thymol was determined spectrophotometry in pure and mouth washing drug, based on the diazotization of ortho-aminobenzoic acid by sodium nitrite in acidic medium at 5C° followed by coupling with thymol in alkali medium .

Experimental

Apparatus

All spectrophotometric measurements were carried out by using Computerized UV-Visible, Shimadzu.In addition silica glass cell was used throughout this study.

Materials : Thymol stock standard solution 1000 µg ml^{-1} was prepared by dissolving 0.1 g of pure thymol in distilled water and diluting them to the mark in 100 ml volumetric flask. Working standard solution 100 µg ml^{-1} was prepared by diluting 10 ml of this stock standard solution with distilled water in 100 ml volumetric flask.

- Sodium nitrite solution 1% w/v was prepared by dissolving 1 g of sodium nitrite in distilled water and diluting the mixture to the mark in 100 ml volumetric flask.
- Hydrochloric acid solution 1 M was prepared by diluting 43 ml of 11.64 M of concentrated hydrochloric acid (BDH) with distilled water in 500 ml volumetric flask.
- Ortho-aminobenzoic acid 100 µg ml^{-1} was prepared by dissolving 0.01 g of ortho-aminobenzoic acid in 5ml ethanol (BDH) and diluting them with distilled water to the mark in 100 ml volumetric flask
- Sodium hydroxide solution 1 M was prepared by dissolving 4 gm of sodium hydroxide in distilled water and diluting them to the mark in 100 ml volumetric flask.

Recommended Analytical 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 ortho-aminobenzoic acid and

0.5 ml of 1% sodium nitrite and 0.5 ml of 1M HCl.They were mixed in and completed with distilled water to the mark in10 ml volumetric flask shaked for 2 minutes, with shaking and cooling in ice bath at 5°C for 2 minutes. After 5 minutes the yellow color is completely developed and the absorbance measurement was carried out at a wavelength at 390nm against a blank solution prepared in the same method but without Thymol.

Analysis of Dosage Form

Oral Solution

Lesterine antiseptic**:.** 15.6 ml was taken from container containing 640mg of thymol in 100 ml,disolved with 5ml ethanol, transferred into 100 ml volumetric flasks, and diluted up to the mark with distilled water.

Results & Discussion

Absorption Spectra

A yellow colored oxidizing coupling product with absorption maximum at 390 nm. Figure 1 shows the spectra of orange product.

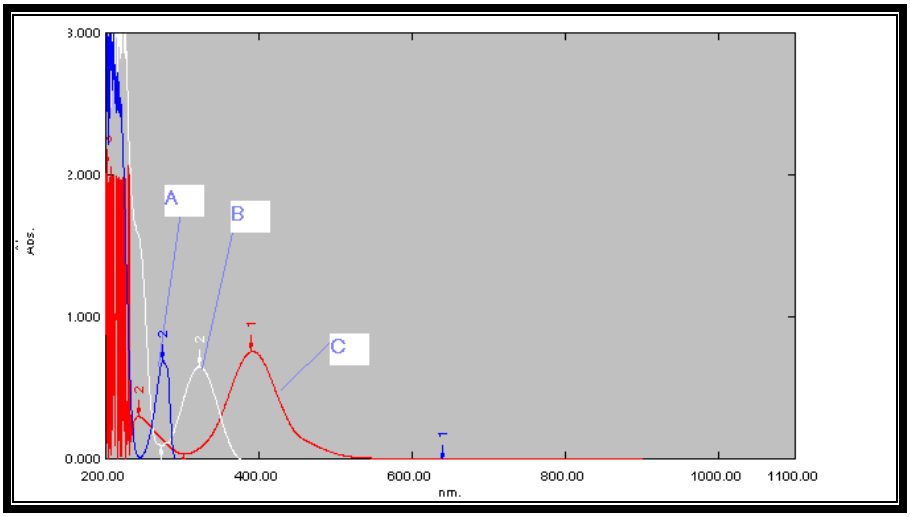


Figure 1. Absorption spectra of (a) Thymol versus distilled water, and (b) reagent versus distilled water (C) Azo Dye against reagent blank

Optimization of the Experimental Conditions

The effect of various variables on the color development was studied to get the optimum conditions to determined the thymol . In the subsequent experiments, 1ml of(100 µg ml^{-1}) thymol solution with varies volumes of 0.1% sodium nitrite solution.The optimum concentration of 0.1% sodium nitrite solution that gave maximum absorption at 390 nm.Whereas versus reagents blanks were found to be 0.5ml (Figure.2).

The effect of different volumes(0.1 – 1.0) ml of 1 M Hydrochloric acid solution , (0.1 – 1.0 ml) of 100 µg ml^{-1} ortho -aminobenzoic acid and(0.1 – 1 ml) of 1M sodium hydroxide solution were also examined.It was found that 0.5 ml of(1 M) hydrochloric acid solution, 0.6 ml of (100 µg ml^{-1}) ortho -aminobenzoic acid solution, and 0.6 ml of (1 M) sodium hydroxide solution were enough to obtain the maximum absorbance.

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

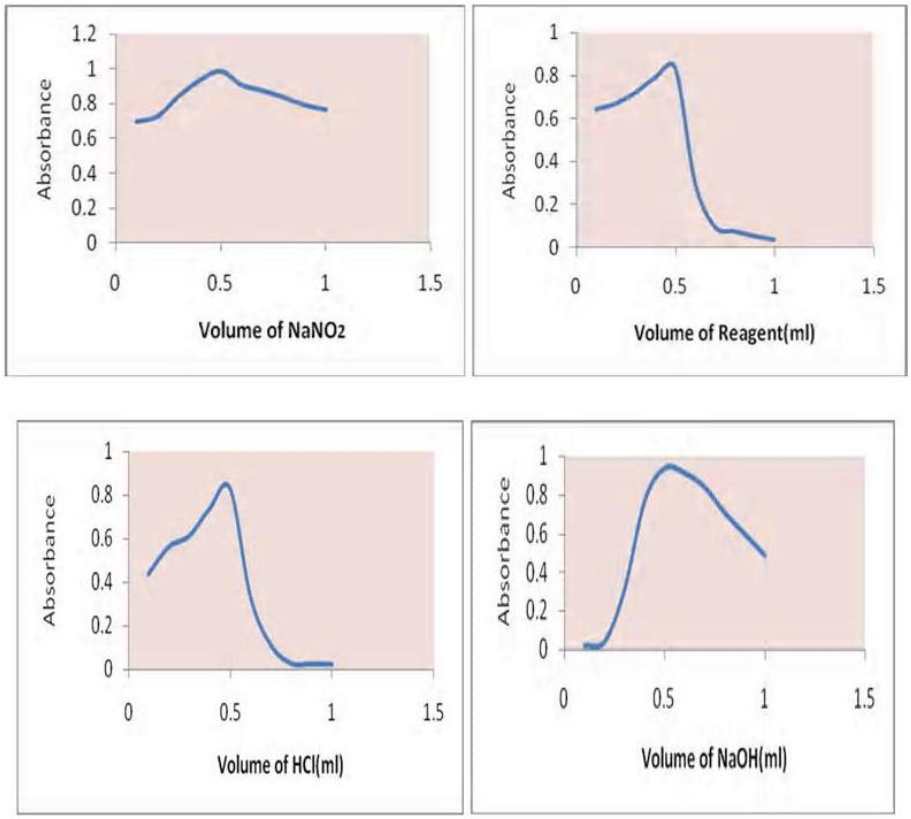


Figure. 2: Optimum conditions for determination of Thymol

The stability of the dye was studied for 1 hour following after mixing the reagents. The colored azo dye developed rapidly after mixing and attained maximum absorbance about 5 minutes at room temperature. The color was stable for 24 hours.

The effect of temperature on the diazotization and coupling reaction shows that the absorbance of the azo dye remains constant in the range 0 – 40ºC and decreases up to 40

ºC. Therefore, it has been recommended to carry out reaction at zero temperature.

Calibration Graph

Employing the conditions described in the procedure, a linear calibration graph of thymol is obtained.Figure 3 shows that Beer's law is obeyed over the concentration range of 1-10 μgml^{-1} with correlation coefficient of 0.9996 and an intercept of 0.0162. The conditional molar absorptivity of the yellow product formed was found to be $14x10^6$ $L.mol^{-1}.cm^{-1}$.

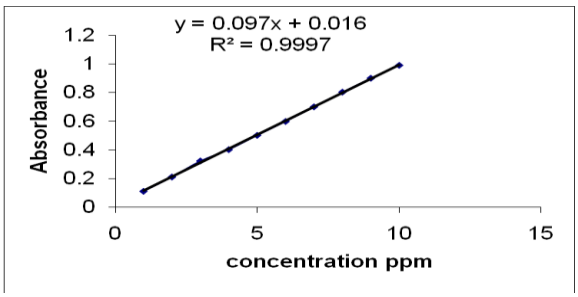


Figure 3. Calibration graph of Thymol

The Effect of Interference: The effects of some foreign ions which often accompany this drug in pharmaceutical products were studied by adding different amounts of foreign ions to 10µg/ml ofthymol. The color was developed following the recommended procedure described earlier. It was observed that the Arabic Gum, glucose, Fructose, sodium acetate, Urea, NaCl, and O- Cresol were not interfering with the determination at levels found in dosage form.

Structure of the Dye

The stoicheiometry of the reaction between Thymol and ortho -aminobenzoic acid was investigated using Job method[17] .The results obtained (figure 4) show that 1:1 drug to reagent was absorbed at 390nm.

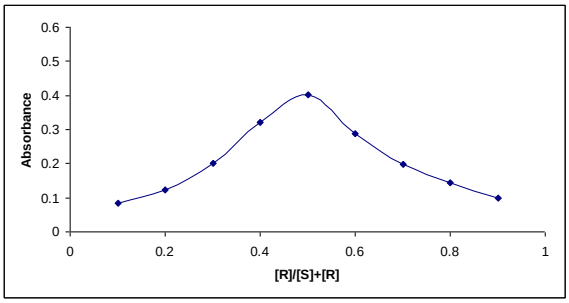


Figure 4. Jop method of Thymol (S) and ortho – aminobenzoic acid(R)

Therefore, the formation of the product probably occurs as follows (Figure5).

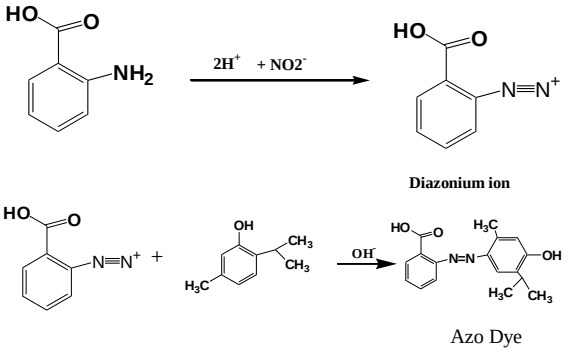


Figure 5. Probable product formation pathway.

The product formed was water soluble.The stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of Thymol with ortho –aminobenzoic acid.The average conditional stability constant of the dye in water under the described experimental conditions was 27 $x10^4$ $mole^{-2}.L^2$.

Precision and Accuracy

Thymol was determined at three different concentrations.(Table1).Precision and accuracy were obtained applying the proposed method.

Table 1. Accuracy and precision of the proposed method.

Thymol Taken	Thymol found	⋆ Recovery% Rec%	Average recovery% Rec%	Relative Standard Deviation⋆ RSD%
6	5.8	96.6	99	0.37
8	8.2	102.5		0.59
10	9.8	98		0.67

⋆ **Average of five determinations**

Analytical Application

Oral solution of drug containing thymol has been analyzed with good accuracy and precision. The results obtained were compared successfully with the official method (Table 2). F-test and t-test showed that there was no significant difference between the proposed method and the standard anti pyrien method[18].

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

Oral Solution	Thymol ppm		Recovery% Rec%	⋆ Average recovery % Rec%	Relative Standard Deviation⋆ % RSD%
[a] Lesterine antiseptic	Taken	Found			
	6	**5.8**	**96**	**98.3**	**0.58**
	8	**8.2**	**102**		**0.65**
	10	**9.7**	**97**		**0.75**

⋆ Mean of three determinations.

a Marketed by U.S.A

Evaluation of the Proposed Method:

For evaluating the results of the proposed method comparing with standard method to determine the efficiency and success in the estimate standard method in the British Pharmacopoeia. Standard method is unavailable in the British Pharmacopoeia, therefore, Standard addition method[19] was used to determine Thymol in Lesterine antiseptic preparation .The results shown in Figure (6) show that the results of standard addition method agree well with the proposed method, indicating that the method is selective and free from interference.

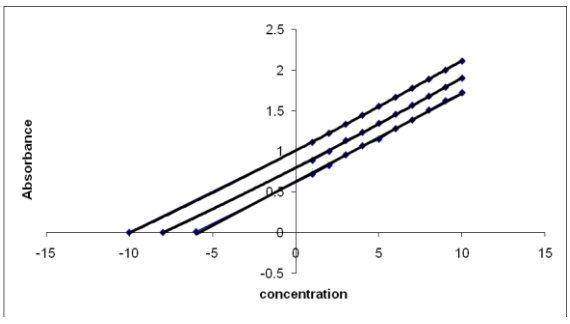


Figure 6. Standard addition method for determination of Thymol in Lesterine antiseptic.

The comparison sensitivity determination of Thymol applying the proposed method other than methods in literature.As showed in table (3)

Table 3: Comparison of Thymol determination the proposed method and other literature methods

Analytical parameters	Present method	Literature[16] method	Literature [13] method	Literature[9] method
Type of method	Azo coupling	Azo coupling	HPLC	GC-Mass
Reagent	Diazotized Anthralic acid	Diazotized p-phenyline ,di-amine Sodium per Iodate	___	___
λ_{maz}	390	550	274	___
Colour of the dye	yellow	Violate	___	___
Beer's law range (ppm)	0.1-10	0.4 – 24	0.04-1.23	0.098-0.196
Molar Absorptivity ($l.mol^{-1}.cm^{-1}$)	1.4×10^5	7.45×10^3	___	___
pH	10.76	11.3	___	___
Temperature(^oC)	R.T	___	___	300
Development time (min)	4	___	___	___
LOD (μg)	0.082	___	___	0.0015
Recovery(%)	99	101.5	99.02	98.1
RSD(%)	1>	2>	1.03	1.9
Analytical application	Mouth wash	Oral Solution	Inula nervosa wall herpes	Oral Solution

Conclusion

The proposed method was found to be simple, economical, selective, and sensitive.The statistical parameters and recovery study data clearly indicate no interference.Hence, this method could be considered the best method to determine of thymol in the quality control laboratories.

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التقدير الطيفي لدواء الثايمول في المادة النقية وفي دواء غسول الفم الليستيرين

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الملخص

أقترحت طريقة بسيطة و سريعة و حساسة لتحليل الثايمول النقي وفي المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الأزوتة لاورثو امينو حامض البنزويك مع نتريت الصوديوم في حامض الهيدروكلوريك لتكوين ملح الديازونيوم الذي يزدوج مع المحلول القاعدي للثايمول لتكوين صبغة آزو صفراء اللون عند 5°م، لتعطي أقصى امتصاص عند طول موجي 390نانومترً. والتي تطيع قانون بير عند مدى التركيز 1–10مايكروغرام مل$^{-1}$ وبحد كشف 0،082مايكروغرام مل$^{-1}$. وإن قيمة الامتصاصية المولارية و حساسية ساندل4،1×10^{5} لتر مول$^{-1}$ سم$^{-1}$ و 0،009 مايكروغرام سم$^{-2}$ على التوالي. وحد تقدير كمي 0.027 مايكروغرام.مللتر$^{-1}$.وقد طبقت الطريقة بنجاح وسهولة لتقدير الثايمول في المحاليل الصيدلانية وبدون تداخل من قبل المواد المعروفة التي تستعمل كمضافات للادوية وذلك بمقارنتها مع طرق التقدير الاخرى ككروماتوغرافيا السائل عالية الأداء وكروماتوغرافيا الغاز.