Spectrophotometric determination of thymol in pharmaceuticals with Gibb's reagent

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الخلاصة

تم وصف طريقة طيفية بسيطة وسريعة لتقدير الثايمول بهيئته النقية وفي المستحضرات الصيدلانية . تعتم د الطريقة على التفاع ل اللوني بين الثايمول و 2،٦- ثنائي كلوروكوينون -٤- كلورايميد (كاشف جبس) في وسط البورات المنظم (pH 10) لتكوين صبغة الاندوفينول الزرقاء و يقاس اقصى شدة امتصاص لها عند طول موجي ٦٠٥ نانوميتر . ينطبق قانون بير ضمن مدى التراكيز ٠.٠٤-١٦ مايكروغرام . مللتر $^{-١}$ وبامتصاصية مولارية 18430 لتر .مول $^{-١}$.سم $^{-١}$ ودلالة ساندل للحساسية ٠.٠٠٨ مايكروغرام .سم $^{-٢}$.اظهرت النتائج ان الطريقة ذات توافق (الانحراف القياسي النسبي) أفضل من ١ % ودقة (معدل نسبة الاسترجاعية) ١٠٠.٥٠% . إن الطريقة لا تحتاج الى السيطرة على درجة الحرارة او الاستخلاص بالمذيب. تم تطبيق الطريقة بنجاح في تقدير الثايمول في غسولات الفم وقورنت النتائج مع طريقة ٤-امينو انتي بليرين القياسية.

Abstract

A simple and rapid spectrophotometric method for the determination of thymol in pure form and in pharmaceutical preparations is described .The method is based on the color reaction between thymol and 2,6-dichloroquinone-4- chloroimide (Gibb's reagent) in borate buffer medium (pH 10) to form a blue indophenol dye with maximum absorption 605 nm. Beer's law is obeyed over the concentration range of 0.04–16 μg. $ml^{-1}$with a molar absorptivity of 18430 l. $mol.cm^{-1}$and sandell sensitivity index of 0.008

μg.cm. The results obtained were both precise (RSD) better than 1% and accurate (average recovery) 100.50%. The method does not resort to temperature control or to solvent extraction .The method has been applied further, successfully to the determination of thymol in mouthwashes and the results obtained are comparable with those given by the standard 4-aminoantipyrine method.

Introduction

Thymol is a component of thyme essential oil, which has been reported to possess interesting antimicrobial effects on various microorganisms, hence thymol is used in the case of female urogenital infections, bacterial vaginosis, urinary tract infections and vaginitis (1). Thymol has also many uses, including perfumes, food flavoring, mouthwashes, cosmetics and also as stabilizer to several therapeutic agents including halothane (2,3).

Thymol has been determined spectrophotometrically via oxidative coupling with N,N-diethylphenylenediamine in the presence of N-bromosuccinamide in alkaline medium (4) and p-phenylenediamine in the presence of sodium metaperiodate in alkaline medium(5) or by coupling with diazotized 2,4,6-trimethylaniline reagent in borate buffer solution (6) and diazotized p-nitroaniline in basic medium (7), in addition of its spectrophotometric determination by reacting with sodium nitroprusside and hydroxylamine hydrochloride in phosphate buffer solution (8). Also, several analytical methods have been reported for the estimation of thymol including high–performance liquid chromatography (9) and gas chromatography (10, 11).

Gibb's reagent (2,6-dichloroquinone-4- chloroimide) has numerous applications as analytical reagent .It has been used for the determination of hallogenated derivatives of 8-hydroxyquinoline (12), some adrenergic drugs (13), aminosalicylate sodium (14), salbutamol (15).

The aim of this work is to develop a new simple spectrophotometric procedure for accurate and rapid analysis of thymol using 2,6-dichloroquinone-4- chloroimide as a coloring reagent.

Apparatus

Absorption spectra and absorbances were measured in a Shimadzu UV 150-02 double beam spectrophotometer using 1-cm glass cells.

Reagents

All chemicals used were of analytical reagent grade.

Thymol solution, 100 µg.ml^{-1}

This solution was prepared by dissolving 0.01 g of thymol (BDH) in 5ml of absolute ethanol and then the volume was completed to 100ml with distilled water. The solution was stable at least for two weeks.

Gibb's reagent, 5 x 10^{-3} M

The solution was prepared freshly by dissolving 0.1052 g (Fluka) of 2,6-dichloroquinone -4- chloroimide (DCQ) in ethanol and diluted to 100ml in volumetric flask with the same solvent and kept in a dark bottle.

Borate buffer solution (pH 10)

This solution was prepared by mixing 50ml of 0.025M $Na_2B_4O_7$ .10 H_2O with 18.3ml of 0.1M sodium hydroxide then the volume was completed to 100ml with distilled water.

Procedure for calibration

To a series of 25ml calibration flasks, increasing volumes of thymol working standard solution were transferred to cover the range (0.04–16)µg.ml^{-1} in final dilution. 3ml of borate buffer solution (pH 10) followed by 3ml of DCQ reagent solution were added. The mixture was well mixed, allowed to stand for 5 min at room temperature and the absorbance was measured at 605 nm. A reagent blank was run simultaneously.

Assay procedure for a drug

Determination of thymol in listerine antiseptic original mouthwash :

Thymol was prepared in the concentration 256µg.ml^{-1} by diluting 20ml of Listerine (from Warner – Lambert Pharmaceutical Co. which was certified to contain 64mg thymol/100ml) to 50ml with distilled water and 100 µg.ml^{-1} of thymol solution was prepared from this above solution, then different volumes used from this solution containing the concentration range 0.04 – 16µg.ml^{-1}. After that the calibration procedure described above was carried out.

Determination of thymol in septica effervescent tablets :

Septica is effervescent tablets from avicenna labs. Damascus. Each tablet contains 4 mg thymol. Twenty tablets were weighed, powdered, mixed and an amount of the powder equivalent to five tablets was transferred into 100ml calibrated flask, dissolved in distilled water and the volume was completed with the same solvent, then 100µg.ml^{-1} was prepared from this above solution and the calibration procedure was carried out.

Results and discussion

Absorption spectra

Thymol was reacted with DCQ in basic medium producing a blue colored product with maximum absorption at 605 nm, while the reagent blank shows no absorption at this wavelength (Fig 1).

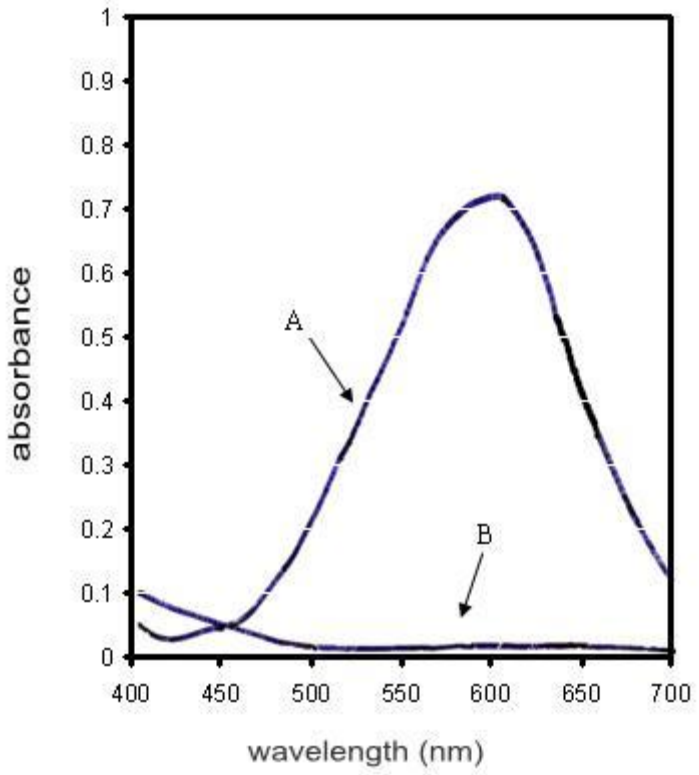


Fig (1) : Absorption spectra :

A : Thymol (6 µg . ml^{-1}) – DCQ (5x10^{-3}) product versus reagent blank.
B : Reagent blank versus D.W.

Effect of pH and buffers

The effect of pH on the sensitivity of the colored reaction product was investigated in the range of 4-13. The results obtained showed that the optimum pH value was 10. Therefore different buffer solutions of pH 10 were prepared. The results shown in Table 1 and 2 indicate that borate buffer solution gives clear blue color with maximum intensity and it was found that the optimum amount of borate buffer was 3ml.

Table (1): Effect of buffer solution on the absorbance

Buffer solution pH 10	Phosphate	Borate	Carbonate
Absorbance	0.485	0.516	0.443

Table (2): Effect of borate buffer amount on the absorbance

pH	1	2	3	4	5
Absorbance	0.513	0.540	0.601	0.552	0.536

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in a thermostatically controlled water bath adjusted at 0,40 and 50C°. The absorbance was measured at 5min intervals against reagent blank treated similarly. It was observed that the absorbance reached maximum after 5 min at room temperature and remained constant for at least 3 hours and the absorbance decreased slowly thereafter. Hence, room temperature and reaction time (5 min) were chosen for color development (Table 3) .

Table (3): Effect of temperature and reaction time

Temp (C°)	Absorbance / min standing time										
	0	5	10	20	30	40	50	60	90	180	240
0	-`	0.581	0.579	0.575	0.578	0.574	0.568	0.568	0.563	0.543	0.540
Room temp	0.600	0.603	0.603	0.603	0.603	0.603	0.603	0.603	0.603	0.603	0.580
40	-	0.587	0.579	0.575	0.570	0.569	0.568	0.560	0.554	0.532	0.501
50	-	0.586	0.570	0.571	0.570	0.569	0.560	0.549	0.540	0.530	0.513

Effect of 2,6-dichloroquinone-4-chlorimide (DCQ) concentration

The effect of various DCQ concentrations on the absorbance of solution containing 6 $\mu g.ml^{-1}$ thymol was studied, it is evident that the absorbance increases with increasing DCQ concentration and reached maximum on using 3ml of $5x10^{-3}$ M DCQ .Therefore, this volume was used in all subsequent work (Table 4).

Table (4): Effect of DCQ concentration

ml of DCQ solution 5 x 10^{-3}M	1	2	3	4	5
Absorbance	0.539	0.602	0.729	0.573	0.551

Effect of surfactant

The effect of different types of surfactants were used for the improvement of the absorption but the results shown in Table 5 confirm that there is no improvement in the absorption, therefore they were excluded.

Table (5): Effect of surfactant

Surfactant*	Absorbance
Cetyltrimethylammonium bromide (0.1 %)	0.692
Sodium dodecyl sulphate (0.1%)	0.686
Triton X-100 (1.0 %)	0.699
Without surfactant	0.731

* 1 ml of surfactant

Analytical data

Under the proposed experimental conditions linear relation between the absorbance and the concentration of thymol was observed over the concentration range 0.04-16µg.ml^{-1} (Fig 2) with a correlation coefficient of 0.9996 and intercept of 0.015. A negative deviation from Beer's law was observed above 16 µg.ml^{-1} concentration of thymol .The molar absorptivity was 18430 l.mol^{-1}.cm^{-1}.

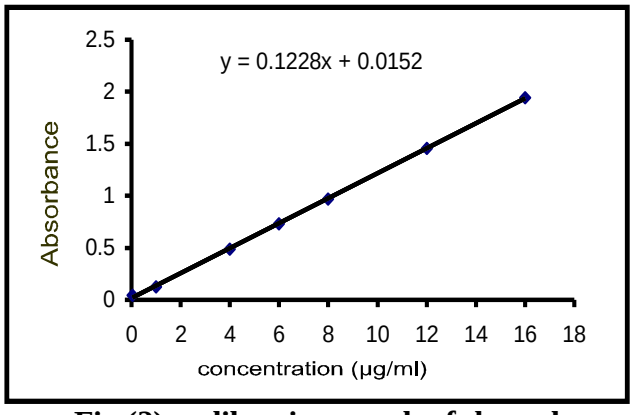


Fig (2): calibration graph of thymol

Accuracy and precision

To determine the accuracy and precision of the calibration graph, thymol was determined at three different concentrations. The results shown in Table 6 indicate a satisfactory precision and accuracy.

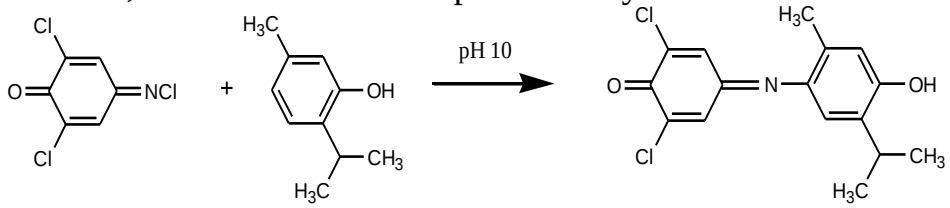
Table (6): Accuracy and precision of proposed method

Amount of thymol taken (µg.ml^{-1})	Recovery(%)*	Relative standard deviation (%)*
2	101.23	0.88
6	100.19	0.21
12	100.08	0.12

* Average for six determinations

Nature of product and reaction mechanism

To establish the composition (ratio of thymol to DCQ reagent) of the blue indophenol dye formed, Job's method of continuous variations and mole-ratio method have been used. The resulting data show that 1:1 thymol to reagent are reacted and the color reaction does not require any oxidant (16). Therefore, the formation of the product may be illustrated as follows:



The stability constant of the product was estimated and found to be $5x10^6$ l.mol^{-1}.

Effect of interferences

In order to assess the possible analytical application of the proposed method, the effect of some common excipients usually present with drug in pharmaceutical formulation was studied by analyzing synthetic sample solution containing 100µg of thymol and (10–fold excess) of each excipient, none of these substances interfered seriously (Table 7).

Table (7) :Interference effect of excipients (1000 µg) on the recovery of 100 µg/25 ml of thymol

Excipients	Recovery %
Starch	102.56
Glucose	101.31
Acacia	104.11
Glycerol	102.55
Arabic gun	103.13

Application of the method

The proposed method was applied to the determination of thymol in pharmaceutical formulations. Good recovery was obtained and the results compared with the standard 4- aminoantipyrine (17) (Table 8 and Table 9). The experimental t-test found to be 1.55 and 0.24 for listerine and septica, respectively. While the tabulated value is 2.776 at the 95% confidence limit for four degrees of freedom, which indicate that this method is reliable for application.

Table (8): Assay of thymol in mouthwash Listerine

Present method		Standard method	
Drug content found* (mg/100ml)	**Recovery* (%)**	**Drug content found* (mg/100ml)**	**Recovery* (%)**
63.60	**99.37**	**64.06**	**100.10**
65.50	**102.34**	**65.41**	**102.20**
65.20	**101.87**	**64.43**	**100.67**

Certified value 64 mg / 100 ml

* Average for three determinations

Table (9): Assay of thymol in septica tablet

Present method		Standard method	
Drug content found* (mg)	**Recovery* (%)**	**Drug content found* (mg)**	**Recovery* (%)**
4.07	**101.75**	**4.03**	**100.75**
3.97	**99.25**	**3.92**	**98.00**
4.02	**100.50**	**4.08**	**102.00**

Certified value 4 mg

*Average for three determinations

Comparison of methods

Table 10 gives the comparison between the present method and another spectrophotometric method.

Table (10): comparison of the present method with other method

Analytical parameter	Present method	literature method (7)
λ max (nm)	605	513
Temp C ° .	Room temperature	Room temperature
pH	Basic medium	Basic medium
Type of reaction	coupling	Diazo-coupling
Reagent	2,6-dichloroquinone-4-chloroimide	Diazotized p-nitroaniline
Beer's law range (μg.ml^{-1})	0.04-16	0.04-12
Molar absorptivity (l.mol^{-1}.cm^{-1})	18430	24800
Color of the dye	Blue	Pinkish – red
Composition of the dye	1:1	1:1
Analytical application	Pharmaceutical preparations	Pharmaceutical, oil and waters

CONCLUTION

DCQ is a sutiable chromogenic reagent for the determination of thymol in pure form or in its pharmaceutical preparations. The suggested method is simple, time saving, sensitive and reproducible.

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