Spectrophotometric Determination of Thymol in Pharmaceutical Formulations Via Oxidative Coupling Reaction

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الخلاصة

يشمل البحث وصف طريقة طيفية ، بسيطة وسريعة وحساسة لتقدير الثايمول. تعتمد على تفاعل الاقتران التأكسدي للثايمول مع كاشف بارا-فينيلين ثنائي امين بوساطة بيريودات الصوديوم في وسط قاعدي لتكوين صبغة الاندوانيلين ذات اللون البنفسجي والتي تعطي اقصى امتصاص عند الطول الموجي 550 نانوميتر. أمكن تقدير الثايمول بوساطة الطريقة المقترحة ضمن مدى التراكيز 0.4-24 مايكروغرام/مللتر. وأظهرت النتائج ان الطريقة ذات توافق (الانحراف القياسي النسبي) أفضل من 2% ودقة (معدل نسبة الاسترجاعية) 101.5% وامتصاصية مولارية 7.45×10^3 ($L.mol^{-1}.cm^{-1}$) بالاضافة الى قيمة دلالة ساندل 0.02 ($\mu g\ cm^{-2}$).

تم تطبيق الطريقة بنجاح في تقدير الثايمول في المستحضرات الصيدلانية (غسولات الفم والكريم) حيث وجد ان نتائج الطريقة تتفق مع المحتوى الاصيل للمستحضرات الصيدلانية والطريقة القياسية المعتمدة في الدستور البريطاني لتقدير الادوية. كما أظهرت النتائج عدم حدوث تداخل في الطريقة المطورة من قبل مواد السواغ بوصفها مضافات في المستحضرات الصيدلانية.

ABSTRACT

A simple, rapid and sensitive spectrophotometric method was described for the determination of thymol. The method involves the oxidative coupling reaction of thymol with p-phenylenediamine reagent by sodium metaperiodate in alkaline medium leading to the formation of violet colored product (indoaniline) of maximum absorbance (λmax = 550nm). The proposed method allows the measurement of 0.4-24 $\mu g\ ml^{-1}$. The results obtained were both precise (RSD.) better than 2% and accurate (average recovery)101.5% and Molar absorptivity 7.45×10^3($L.mol^{-1}.cm^{-1}$) as well as Sandell's sensitivity 0.02 ($\mu g\ cm^{-2}$).

The method was successfully applied for the determination of thymol in mouth-washes and creams of pharmaceutical preparation and the results were in good agreement with the certified values and standard method. The common excipients used as additives in pharmaceutical do not interfere in the proposed method.

INTRODUCTION

Thymol is a more powerful disinfectant than phenol, but its use is limited by its low solubility in water. Thymol used chiefly as a deodorant in antiseptic mouth washes and gargles mixed with camphor. It is used in dentistry to prepare cavities before filling. Externally, thymol has fungicidal properties and has been used in dusting-powders for the treatment of fungous skin infections (1).

Major components of thymus vulgaris and thymus capitatus oil were thymol (2).

Several methods have been proposed for the determination of thymol in biological speciments or pharmaceutical formulation. Gas liquid chromatographic analysis with flame ionization detection of creams containing thymol were investigated (3). A gas chromatographic method to determine thymol, eucalyptol, menthol and camphor residues in honey and beeswax is proposed(4). HPLC procedure has been developed for the isolation and quantification of thymol astabilizing agent present in halothane a naesthetic preparations(5). A reliable and sensitive method was developed for determination of thymol in human plasma by automated headspace solid-phase micro extraction (SPME)(6). Thymol in biological samples is analyzed by gas chromatography (7). Another determination of phenols and thymol in nonaqueos media was studied (8). Spectrophotometric method of phenolic compound (includes thymol) were determined via oxidative coupling by using N-N-diethylphenyl enediamine in the presence of N-bromosuccinamide in alkaline medium(9).

In the present investigation we suggested a simple, sensitive, accurate and low cost spectrophotometric method for the determination of thymol in drugs using PPD. as coupling agent and sodium metaperiodate as oxidizing agent in alkaline medium to give an indoaniline dye which can be determined spectrophotometrically at λmax 550nm.

EXPERIMENTAL

Apparatus:

A Shimadzu UV-210 A digital double beam spectrophotometer with 1-cm matched quartz cells were used for all spectral and absorbance measurements.

Reagents and Solutions:

All solutions were prepared from analytical-reagent grade materials. Freshly prepared solution of thymol was used as the standard solution for analytical purpose. Stock solution (100μg ml^{-1}) of thymol (BDH) was prepared by dissolving 0.01 gm of thymol in 5ml ethanol and diluted to 100 ml with water. Solutin of 0.01M ethenolic PPD. (Fluka), 0.015M sodium metaperiodate (Fluka) and 0.1M sodium carbonate were used.

Recommended procedure for calibration:

To a series of 25ml calibrated flasks, transfered increasing volumes of thymol working solution to cover the range of calibration graph added 0.5ml of PPD. (0.01M) and 0.5ml of sodium carbonate (0.1M) followed by 4ml of sodium metaperiodate (0.015M). Diluted the solutions to the mark with distilled water and allowed the reaction mixture to stand for 15 minutes. Measured the absorbance at λmax 550nm against reagent blank, Fig (1).

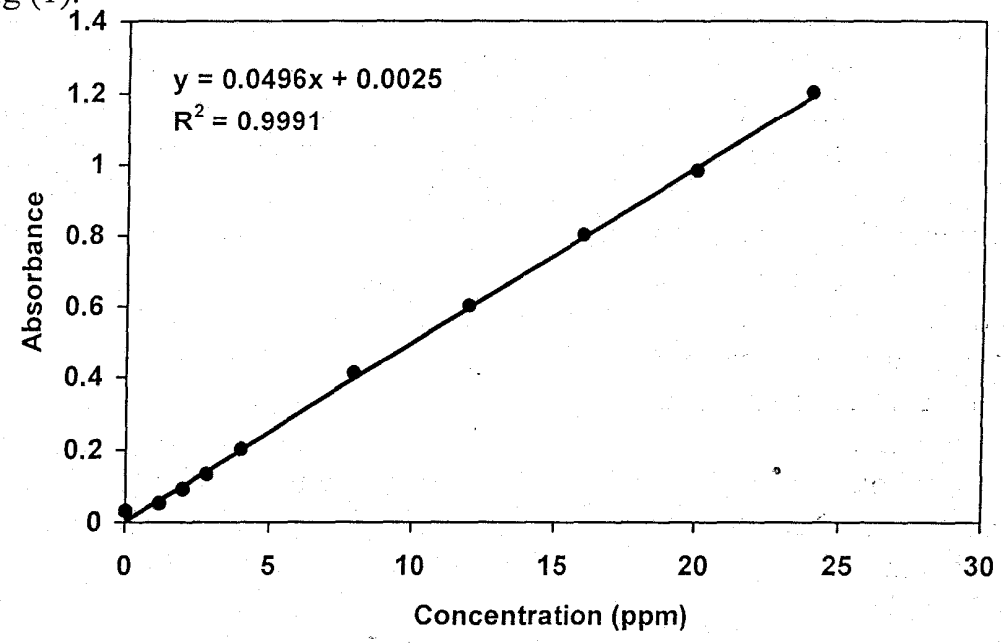


Fig (1): Calibration graph of thymol

Procudure for the assay of thymol in pharmaceutical formations:
Mouth-wash:

Mentrol mouth wash 20ml (0.063% thymol) and Lastarime antiseptic 20ml (0.06% thymol) was diluted with water to get the required concentration of the drug, and then the recommended procedure was followed.

Smicks ointement:

A weighted amount (10gm) of the cream was dissolved in ethanol and diluted with the same solvent up to 100 ml. Then the solution was treated as described a bove under recommended procedure.

RESULTS AND DISCUSSION

The results of this investigation indicate that the reaction between thymol and PPD in presence of suitable oxidant $NaIO_4$ and sodium carbonate to yield highly colored products can be utilized as a suitable assay procedure for thymol. The absorption spectra of the resulting colored product are shown in Fig (2).

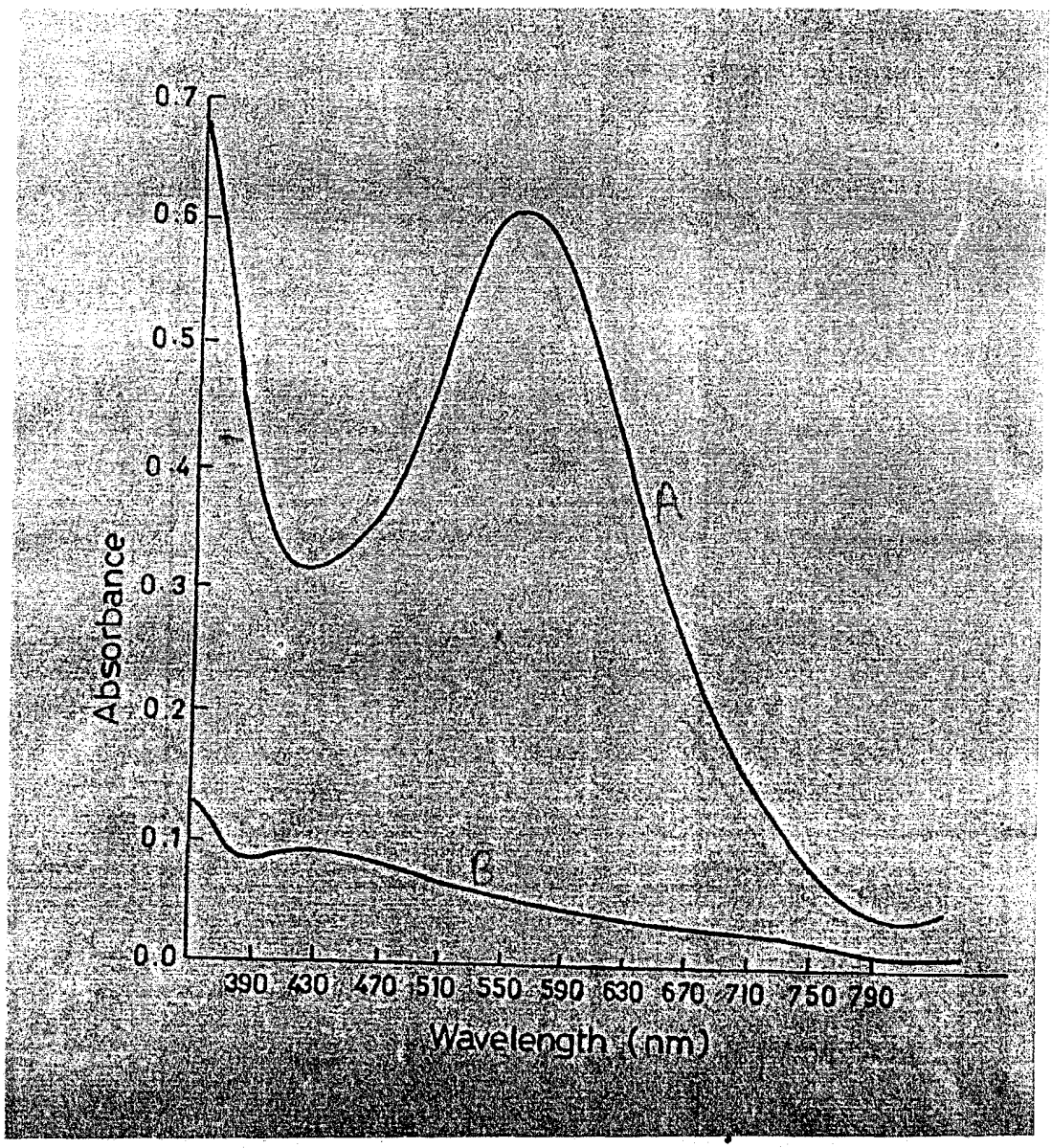


Fig (2): Absorption spectra of the colored product against blank (A) and blank against D.W. (B).

Effect of reagent concentration:

When various concentrations of PPD solutions were added to a fixed concentration of thymol, 0.5 ml of 0.01M PPD solution was sufficient to develop the color to its full intensity and give minimum blank value, therefore 0.5 ml of PPD was used for all experiments within the concentrations range.

Effect of oxidant concentration:

The dye formation reached a maximum absorbance with about 4 ml of 0.015M of sodium metaperiodate in presence of 0.5 ml of 0.1M sodium carbonate, therefore we used this solution to give high sensitivity and quantitative determination at the upper limit of the calibration graph.

Effect of pH:

To establish the optimum conditions the effect of pH in the range from 2-13 was studied. As a result 0.1M sodium carbonate (pH 10) was found to be selected as a suitable alkaline solution and optimum. The amount of base, 0.5 ml was the best recommended value of base for thymol.

Effect of temperature:

The effect of temperature on the color intensity of the dye was studied in the range (0-60 °C). The result indicated that the colored product was formed at room temperature as a suitable temperature.

Order of addition of reagents:

The reactant involved in the reactions were mixed in various sequences the best results were obtained when they were mixed as follows: Thymol, PPD, sodium carbonate and sodium metaperiodate.

Development time and stability period:

The color intensity of the reaction product reached a maximum for 5-15 min. the color obtained was stable for an additional 90 min with stability constant 4.4×10^{6} ($L.mol^{-1}$).

Evaluation of the method:

The linearity of the calibration graph was excellent (correlation coefficient > 0.99). The relative standard deviation (RSD%) was better than 2% (n = 6). The recovery experiments on solutions from pure samples of thymol were performed. A mean recovery of 101.5% was found. The molar absorptivity was 7.45×10^{3} ($l.mol^{-1}.cm^{-1}$) sandell index 0.02 ($Mgcm^{-2}$)

Interferences:

To show the selectivity of the method, the interfering effects of various compounds were examined by determining 20 μg/ml of thymol in the presence of 5,10-fold excess of each of the interfering compounds which found with thymol in pharmaceutical formulations such as glucose, sucrose, lactose, EDTA, sodium bicarbonate, sodium chloride, magnesium stearate, benzoic acid, methyl salicylate, glutamic acid, potassium chloride, sodium acetate. None of these substances interfered seriously. Results are shown in Table 1:

Table 1: Recoveries of 20μgml^{-1} of thymol with various additives used as excipients.

Additive	Concentration ratio (additive:thymol)	Recovery (%)
Glucose	5	100.10
	10	100.50
Sucrose	5	100.23
	10	100.10
Lactose	5	102.90
	10	103.50
EDTA	5	103.25
	10	104.50
Sodium bicarbonate	5	101.50
	10	103.00
Sodium chloride	5	101.20
	10	100.00
Magnesium stearate	5	100.74
	10	98.25
Benzoic acid	5	101.50
	10	103.50
Methyl salicylate	5	98.90
	10	96.20
Glutamic acid	5	103.00
	10	101.20
Potassium acid	5	102.10
	10	101.50
Sodium acetate	5	102.50
	10	103.75

Applications:

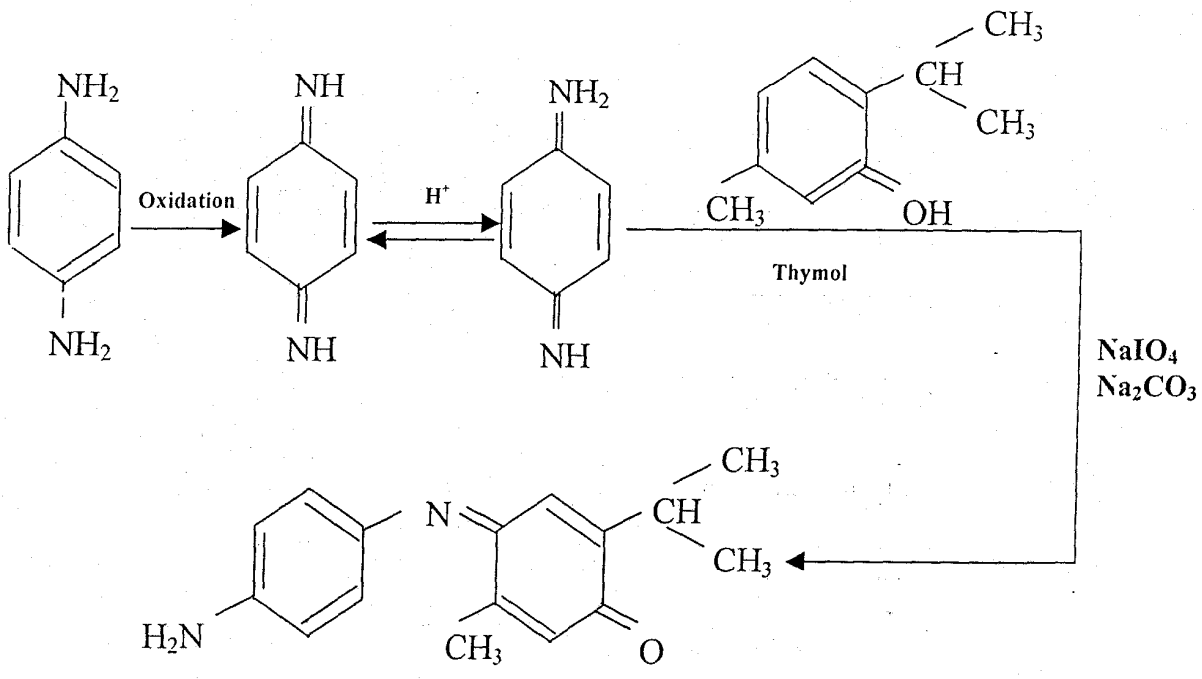
The proposed method was satisfactority applied to the determination of thymol in pharmaceutical formulations. The

concentrations of the thymol were calculated by direct measurement on the appropriate calibration graph. Similar results were obtained when the standard methods (11,12) was used indicating that the method is free from interferences. The proposed method was compared successfully with the British pharmacopoeia and standard method, since F-test and T-test showed that there was no significant differences between the proposed method and the two standard methods.

Commercially available formulations were analyzed and the results obtained are summerized in table 2 and 3. As can be seen, the assay results were in good agreement with certified values for all the formulations.

Proposed reaction sequence:

The stoichiometry of the reaction between thymol and PPD. was investigated by Job's method(13). The results obtained showed the existence of a 1:1 thymol:PPD reagent at 550 nm, therefore the reaction sequence may be postulated as in followed scheme (14):



Indo aniline dye

CONCLUSION

A spectrophotometric method is proposed for the determination of thymol to be used in the control analysis of the pharmaceutical preparations.

The procedure is simple, fast and sensitive. The method may be suitable for routing analysis. Common excipients present in the pharmaceutical preparations do not interfere.

Table 2: Assay of thymol in pharmaceutical formulation.

Pharmaceutical formulation	Certified value (mg)	Present method		British pharmacopoeia[11] or standard method [12]	
		Amount present (µg/ml)	Recovery* %	Amount present (µg/ml)	Recovery* (%)
Mentoral mouth wash [a]	0.063	4.0	100.3	4.0	100
		8.0	102.7	8.0	99.9
		20.0	101.5	20.0	101.3
Lastarime antiseptic [b]	0.06	8.0	103.9	8.0	100.0
		12.0	104.0	12.0	102.0
		20.0	104.1	20.0	102.5
Smicks ointement [c]	0.10	4.0	101.0	4.0	101.7
		8.0	102.1	8.0	101.9
		20.0	101.7	20.0	102.1

a-Marked by Amman Pharmaceutical industries.
b-Marked by Mediotic LABS. Homs Syria.
c-Marked by S.D.I. Iraq.
*Average of three determinations.

Table 3: Assay of thymol in pharmaceutical formulation using British pharmacopoeial [11], standard method , and present method.

Pharmaceutical formulation	Certified value (mg)	Present method* µg/100ml	British pharmacopoeia or standard method (mg)*	RE_1 ** %	RE_2 *** %
Mentoral mouth wash	0.063	0.0639	0.0632	+1.43	+1.11
Lastarime antiseptic	0.06	0.0624	0.0609	+4.00	+2.46
Smicks ointement	0.10	0.1016	0.1019	+1.60	-0.29

*Average of three determinations.
**Oxidative coupling versus certified value.
***Oxidative coupling versus pharmacopoial or standard method value.

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