Spectrophotomeric Estimation of Thymol in Pure and Pharmaceutical Formulation with Diazotized 4-aminoacetophenone

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Abstract:

A spectrophotometric method for the determination of trace amount of thymol in pure form and in mouth wash preparation has been proposed in this study. The method is based on the coupling reaction of thymol with diazotized 4-aminoacetophenone in alkaline medium to form an intense red water-soluble dye that is stable and has a maximum absorption at 487 nm. A graph of absorbance versus concentration show that Beer s law is obeyed over the concentration range of 10-400 µg /25mL (0.4- 16 ppm) with a molar absorptivity of 2.77×10^4 $L.mol^{-1}$ cm^{-1} and Sandell s sensitivity index of $0.005\mu g/cm^2$. The method does not require neither temperature control nor solvent extraction step. The proposed method is successfully applied to the determination of thymol in mouth wash preparation.

Keywords:Spectrophotometric determination; Diazotization; Thymol; 4-aminoacetophenone.

التقدير الطيفي للثايمول بشكله الحر وفي مستحضره الصيدلاني مع الكاشف المؤزوت 4ـ امينو اسيتوفينون

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ملخص البحث:

يتضمن البحث تطوير طريقة طيفية لتقدير كميات متناهية في الصغر من الثايمول بشكله الحر وفي غسول الفم، تعتمد الطريقة على اقتران الثايمول مع الكاشف المؤزوت 4- امينواسيتوفينون بوجود هيدروكسيد الصوديوم، لتكوين صبغة آزوية ذات لون احمر التي تكون ذائبة في الماء ومستقرة وتعطي أعلى امتصاص عند الطول الموجي 487 نانوميتر.

كانت حدود قانون بير في مدى 10–400 مايكروغرام ثايمول في حجم التفاعل النهائي 25 مللتر(0.4– 16 جزء/مليون) ، وكانت الامتصاصية المولارية 2.77×10^4 لتر. مول$^{-1}$. سم$^{-1}$ ودلالة ساندل 0.005 مايكرو غرام/سم^2 . بالإضافة الى ان الطريقة لاتحتاج الى السيطرة على درجات الحرارة أو الاستخلاص بالمذيب. تم تطبيق الطريقة بنجاح في تقدير الثايمول في غسول الفم.

Introduction:

Thymol (2-isopropyl-5-methylphenol) is widely used as a general antiseptic in the medical practice, agriculture, cosmetics and food indusrty[1-3]. Due to its potent fungicide, bactericide and antioxidant properties, it is applied primarily in dentistry for the treatment of oral infections[4-6]. It is added as a stabilizer to several therapeutic agents, including halothane[7]. Thymol is a white crystalline substance of pleasant aromatic odor and strong antiseptic properties[8-10].

A number of analytical methods have been reported for the determination of thymol, these included: High performance liquid chromatography[11-14], liquid chromatography[15],gas chromategraphy[16,17] and flow injection analysis[18]. Thymol has been determined spectrophoto metrically via oxidative coupling reaction[19-21] , azo-dye formation reaction [22-24] and charge transfer complex formation reaction [25] .

The present study describes a simple spectrophotometric method for determination of thymol using diazotized 4- aminoacetophenone.

The Experiment

Apparatus

The spectrophotometric measurements are carried out on ShimadzuUV-Visible Recording Spectrophotometrer UV-210, using 1-cm silica cells.

Reagents and materials

All chemicals used are of analytical grade reagents.

Working thymol solution, 100 μg / mL:

A 0.0100g amount of thymol (BDH) is dissolved in 5mL of absolute ethanol and then volume is completed to 100 mL in a volumetric flask with distilled water. The solution is stable for about two weeks.

Diazotized 4-aminoacetophenone (0.003M) reagent solution:

This reagent solution is prepared by dissolving 0.0405g of 4-aminoacetophenone (Fluka) in 5 mL of absolute ethanol and 20 mL distilled water (heating is required to hasten dissolution) then 2mL of 0.8 M HCl is

added, the mixture is then cooled to 0 - 5°C in an ice– bath, and a 0.0207g sodium nitrite is added and stirred vigorously. After 5 minutes the solution is made up to volume in 100 mL volumetric flask with cooled distilled water, and is kept in a brown bottle in a refrigerator. This solution is prepared freshly each day.

Sodium hydroxide solution, 1N: This solution is prepared by appropriate dilution of the concentrated solution (Fluka) with distilled water and then transferred to a plastic bottle.

Thymol solution:

A stock solution of thymol is prepared in the concentration of 256µg /mL by diluting 20 mL of Listerine (antiseptic original mouth wash from Warner - Lambert Pharmaceutical Co.) which is certified to contain 64 mg thymol/ 100 mL to 50 mL in a volumetric flask, then 5 mL of absolute ethanol is added followed by dilution to the mark with distilled water . A 100 µg/mL solution of thymol is prepared by simple dilution of the stock solution.

Procedure and calibration graph

To a series of 25 mL volumetric flasks 10 – 500 µg (0.4 – 20 ppm) of thymol is added, followed by 1.5mL of diazotized 4-aminoacetophenone (0.003M) and 1mL of 1N NaOH and the volumes are completed to the mark with distilled water. After 10 minutes the absorbances are read against a reagent blank (prepared in the same manner but without thymol) at 487 nm using 1-cm cell. The calibration graph is linear over the range 10 – 400 µg/25mL (0.4–16ppm) (Fig.1). The molar absorptivity is found to be 2.77×10^4 $L.mol^{-1}.cm^{-1}$

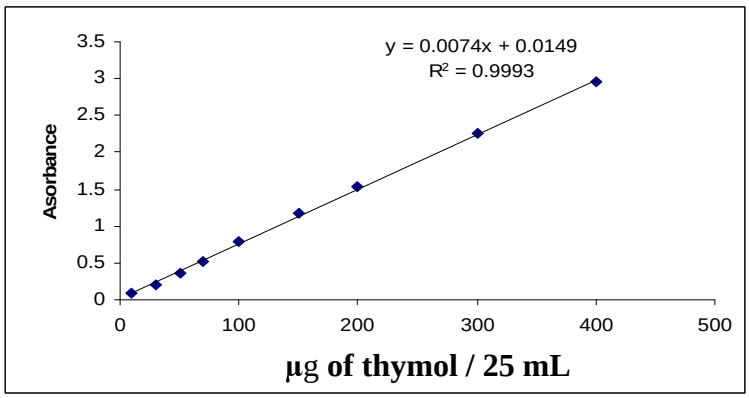

Fig.1. Calibration graph of thymol determination

Results and Discussion

For the subsequent experiments 100 μg of thymol are taken and final volumes are brought to 25 mL with distilled water.

Final absorption spectrum

When thymol in aqueous solution is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 487 nm. The reagent blank shows no absorption at this wavelength (Fig.2).

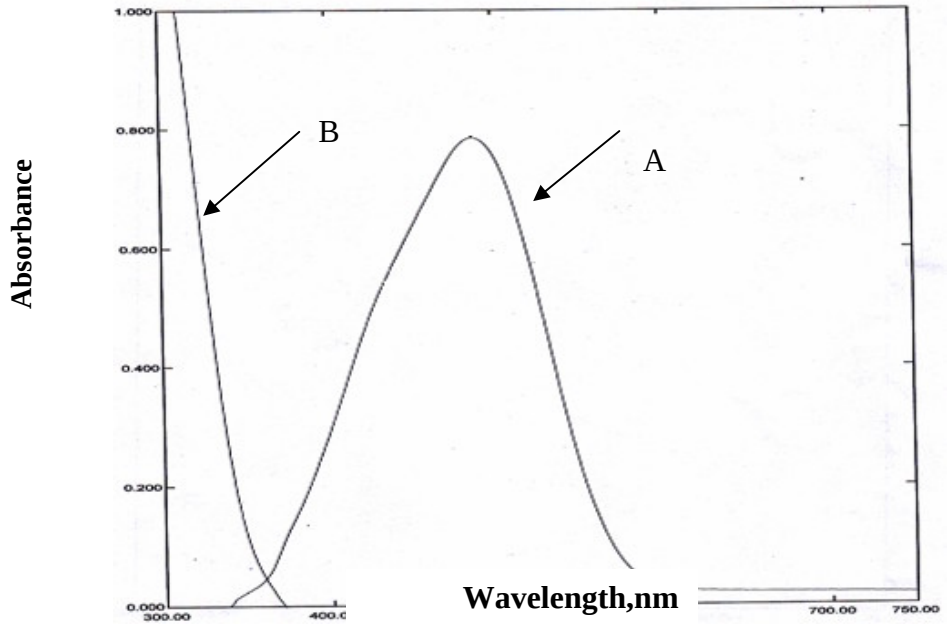

Fig.2:Absorption spectrum of 100μg thymol/25mL treated according to the recommended procedure.(A) the azo dye against blank, (B) blank against distilled water

Study of the optimum reaction conditions

The effects of various parameters on the optical properties of the azo dye have been studied and the optimum conditions are taken.

Choice of the base and its amount

The preliminary experiments showed that the azo dye develops only completely in alkaline medium. Different bases (strong and weak) have been used (Table1).

Table 1: Selection of the base

1 mL of 1N Base	Absorbance	$\Delta\lambda^*$
NaOH	0.770	211.5
KOH	0.760	211.0
Na_2CO_3	0.240	208.5
$NaHCO_3$	0.219	208.5

$\Delta\lambda^* = \lambda^S{}_{max} - \lambda^B{}_{max}$; where S = the dye, B = blank

The results in Table 1 indicate that NaOH gives the highest color intensity of the product and the best color contrast. The effect of different volumes (0.3-2.0 mL) of 1N NaOH solution on the color intensity is studied then, a 1 mL of 1N NaOH with a final solution pH of 11.65 gives the best intensity of the product formed therefore it is used in the subsequent experiments (Table 2).

Table 2: Effect of base amount on absorbance.

mL of 1N NaOH	0.3	0.5	0.7	1.0	1.5	2.0
Absorbance	0.745	0.757	0.767	0.774	0.775	0.773
pH	11.41	11.56	11.61	11.65	11.70	11.74

Effect of diazotized 4-amino-acetophenone reagent amount

0.3-3.0 mL volumes of 0.003M the diazotized 4-aminoaceto-phenone are tested, the results indicate that using 1.5 mL of diazotized 4-aminoacetophenone reagent solution gives maximum absorbance of the complex at 487 nm this volume is considered as an optimum value (Table 3).

Table 3: Effect of diazotized4-aminoacetophenone reagent amount on the absorbance

ML of diazotized4-aminoacetophenone reagent solution (0.003M)	Absorbance
0.3	0.735
0.5	0.752
1.0	0.772
1.5	0.779
2.0	0.770
3.0	0.746

Effect of surfactant

The effect of different surfactants on the color intensity are studied by using 1 mL of various types of surfactants. The results reveal that none of the surfactants gives useful results from the analytical point of view. Therefore, it has been recommended to eliminate their use in the subsequent experiments (Table 4).

Table 4: Effect of surfactant

1mL Surfactant solution	Absorbance	Δλ *
****CTAB, 1×10^{-3} M**	0.647	178.5
*****SDS, 1×10^{-3} M**	0.781	187.5
******Triton x-100, 1%**	0.723	195
With out	0.779	

*$\Delta\lambda=\lambda^{S}_{max}\lambda^{B}_{max}$

**Cetyltrimethylammonium bromide

***Sodium dodecyl sulphate

**** iso-Octylphenoxypolyethoxyethanol

Effect of the order of addition

The effect of different orders of reagent addition on the absorbance of the dye are studied under the optimum experimental conditions (Table 5).

Table 5: Effect of the order of addition

Order number	Order of addition	Absorbance
I	S+R+B	0.781
II	S+B +R	0.752
III	B+ R+S	0.734

Assuming that: S = sample, R = reagent, B = base

From the results above, it is found that the order of reagents addition which is followed as given under the general procedure gives highest color intensity, otherwise a loss in color intensity takes place.

Effect of time on the color development

The effect of time on the development and stability period of the formed dye is investigated under the optimum conditions of the reaction.

The maximum color intensity is reached immediately after mixing the components of the reaction, and the absorbance of the formed dye remained constant for at least 2 hours, this stability period is sufficient for many measurements (Table 6).

Table 6: Effect of time on the absorbance

µg of thymol present	Absorbance / minute standing time									
	0	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**120**
50	0.344	0.344	0.342	0.342	0.341	0.341	0.341	0.342	0.344	0.344
100	0.777	0.777	0.777	0.776	0.775	0.776	0.775	0.774	0.776	0.776
200	1.491	1.502	1.511	1.510	1.510	1.510	1.510	1.509	1.509	1.509

Accuracy and precision

To determine the accuracy and precision of the method, thymol is determined at three different concentrations. The results shown in Table 7 indicate that the method is satisfactory.

Table 7: Accuracy and precision

Thymol(µg/ 25mL)	Relative error, %*	Relative standard deviation, %*
50	-0.16	± 0.52
100	-0.07	± 0.25
200	-0.63	± 0.98

*Average of five determinations

The nature of the dye

Job's (Fig.3) and mole-ratio (Fig.4) methods indicate that the dye has a composition of 1:1 thymol to diazotized 4-aminoacetophenone reagent at 487 nm.

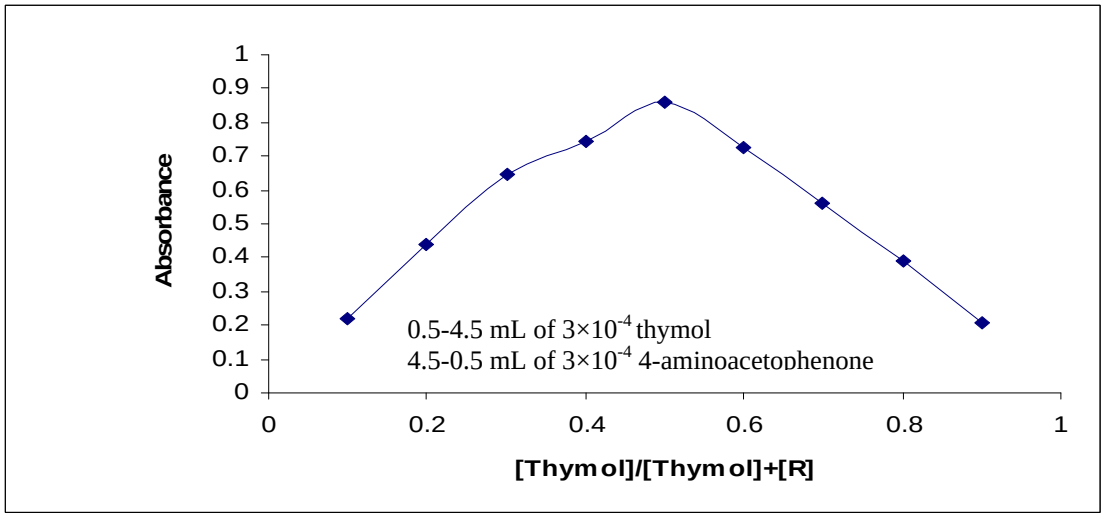

Fig.3: Job's plot for thymol – diazotized 4-aminoacetophenone

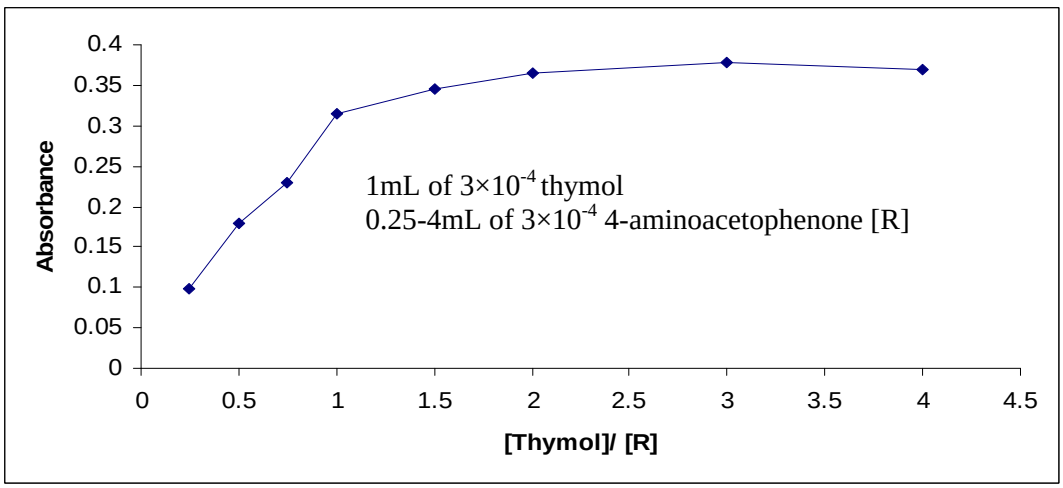

Fig.4: Mole ratio's plot for thymol – diazotized 4-aminoacetophenone

Therefore, the structure of the formed dye may be written as follows:

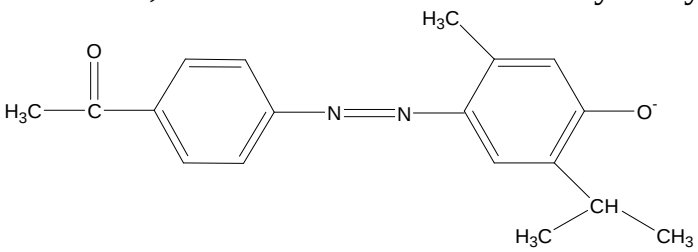

Fig. 5: The possible structure of the red azo dye

Interferences

In order to assess the possible analytical applications of the present proposed method, the interfering of excipients at various levels on the

determination of 100 µg of thymol by the proposed method have been examined and the results are given in (Table 8).

Table 8. Effect of foreign compounds on the assay of thymol

Foreign compound	Recovery (%) of 100µg Thymol per µg foreign compound added		
	100	500	1000
Glucose	100.0	103.3	100.7
Furctose	100.1	100.2	100.0
Starch	98.0	98.2	97.3
Gum Arabic (Acacia)	101.9	99.0	97.3

The results in Table 8 indicate that the excipients do not interfere in the determination of thymol using the proposed method.

Analytical applications

The proposed method is successfully applied for the determination of thymol in its pharmaceutical preparation as a mouth wash. The performance of the proposed method is assessed by the application of t-test in comparison with the standard method[26] for 95% confidence level for six degrees of freedom. The results in Table 9 have been shown that the t-values is less than the critical value of 2.447 indicating that no significant difference between the proposed and standard method for the determination of thymol .

Table 9:Application of methods

Drug	µg thymol present/25 mL	Presence method Recovery ,%	Standard method Recovery, %	t-value
Listerine	100	99.4	100.9	± 0.95

*Average for four determinations

Comparison of methods

Table 10 gives the comparison between the present method and other spectrophotometic methods.

Table 10: Comparison of the methods

Analytical parameters	Present method	Literature method[21]	Literature method[22]
pH	11.65	Basic medium	Basic medium
Temperature (C°)	Room temperature	Room temperature	Room temperature
$\lambda_{max\ (nm)}$	487	550	513
Reagent	Diazotized 4-aminoacetophenone	p-phenylenediamine	Diazotized p-nitroaniline
Beer's law range (ppm)	0.4-16	0. 4-24	0.04-12
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	2.77×10^4	7.45×10^3	2.48×10^4
Type of reaction	Diazo- coupling	Oxidative coupling	Diazo- coupling
Nature of the dye	1:1	1:1	1:1
Application of the method	Pharmaceutical preparation	Pharmaceutical preparations	Pharmaceutical, oil and waters

Conclusion

4-aminoacetophenone is a sutiable chromogenic reagent for the determination of thymol in pure form or in its pharmaceutical preparations.The present method is more sensitive than the literature methods and it has an application part.

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