International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 7 Issue 1, January-2018, Impact Factor: 4.059

Spectrophotometric Determination of Thymol in Pure and Pharmaceutical Formulation Via Diazo-coupling with Diazotised 4-aminoantipyrine

Safaa A. Zakaria[1], Rawaa A. Zakaria[2], Nabeel S. Othman[3]*

[1]Chemistry Dept. College of Science, Mosul University
[2]Chemistry Dept. College of Education, Mosul University
*Chemistry Dept. College of Science, Mosul University

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 was based on the coupling reaction of thymol with diazotized 4-aminoantipyrine in alkaline medium to form an intense yellow water-soluble dye, that was stable and has a maximum absorption at 450 nm. A graph of absorbance versus concentration showed that Beer's law was obeyed over the concentration range of 10-250 µg /25ml (0.4-10ppm) with a molar absorptivity of 1.276×10^4 $L.mol^{-1}$ cm^{-1} and Sandell's sensitivity index of $0.011\mu g/cm^2$. 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; Diazo- coupling; Thymol; diazotized 4-aminoantipyrine.

HOW TO CITE THIS ARTICLE

Safaa A. Zakaria, Rawaa A. Zakaria, Nabeel S. Othman, "Spectrophotometric Determination of Thymol in Pure and Pharmaceutical Formulation Via Diazo-coupling with Diazotised 4-aminoantipyrine", International Journal of Enhanced Research in Science, Technology & Engineering, ISSN: 2319-7463, Vol. 7 Issue 1, January-2018.

INTRODUCTION

Thymol is a component of thyme essential oil, which has been reported to possess interesting antimicrobial effects on various micro-organisms because of its phenolic structure. It is a natural monoterpene derivative of cymene, isomeric with cavacrol[1,2]. Thymol is also shown to have strong anti-inflammatory action by decreasing the release of inflammatory metabolites like prostanoids, interleukins and leukotrienes. Thymol is only slightly soluble in water at neutral pH, but it is extremely soluble in alcohols and other organic solvents. It is also soluble in strongly alkaline aqueous solutions due to deprotonation of the hydroxyl group of phenol [3,4].

Thymol and some other phenolic compounds in volatile oils and plants have been determined spectrophotometrically via oxidative coupling reaction[5-7],diazo-coupling reaction[8-10]and charge transfer complex formation reaction[11] . Also, several analytical methods have been reported for the estimation of thymol including high performance liquid chromatography[12-14], liquid chromatography with electro-chemical detection[15,16],gas chromatography[17],GS-MS[18],cyclic voltammetry and DPPH [19],voltammetry using various electrodes [20-22],electro kinetic capillary chromatography method[23], ,and liquid-liquid micro-extraction-GC[24].

Experimental
Apparatus

The spectrophotometric measurements are carried out on Shimadzu UV-Visible Recording SpectrophotometrerUV-210, using 1-cm quartz 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) was dissolved in 5 ml of absolute ethanol and then volume is completed to 100 ml in a volumetric flask with distilled water.

Diazotized 4-aminoantipyrine (0.0025M) reagent solution: This solution was prepared by dissolving 0.0500 g of 4-aminoantipyrine (Fluka) in 60 ml of distilled water (heating was required to hasten dissolution), then 3 ml of concentrated hydrochloric acid was added, the mixture was then cooled to 0 - 5°C in an ice– bath, then a 0.0170 g sodium nitrite was added. After 5 minutes the solution was made up to volume in 100 ml volumetric flask with cooled distilled water, the solution was kept in a brown bottle, and the prepared diazotized solution was stable for at least five days.

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

Listerine-antiseptic original mouth wash: A stock solution of thymol was prepared at the concentration of 256 μg /ml by diluting 20 ml of Listerine (antiseptic original mouth wash from Warner - Lambert Pharmaceutical Co.) which was certified to contain 64 mg thymol/ 100 ml to 50 ml in a volumetric flask, then 5 ml of absolute ethanol was added followed by dilution to the mark of the flask with distilled water. A 100 μg/ml solution of thymol was prepared by simple dilution of 3.9 ml of the stock solution to 100 ml in a volumetric flask.

Procedure and calibration graph

To a series of 25 ml volumetric flasks 10– 400 μg (0.4 – 16 ppm)of thymol was added, followed by1.0 ml of diazotized 4-aminoantipyrine (0.0025M) and 0.5 ml of 1MNaOH and then the volumes were completed to the mark with distilled water. Then the absorbance was read against the blank (prepared in the same manner but without thymol) at 450 nm using 1-cm quartz cells. The calibration graph was linear over the range10 – 250 μg/25ml (0.4–10ppm).

RESULTS AND DISCUSSION

For the subsequent experiments 100 μg of thymol was taken and final volumes were brought to 25 ml with 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.

Effect of diazotized 4-aminoantipyrine reagent amount

Various volumes from 0.5 to 2.0 ml of 0.0025M of diazotized 4-aminoantipyrine were tested; the results indicate that using 1.0 ml of diazotized 4-aminoantipyrine (D 4-AAP) reagent solution showed maximum absorbance of the formed azo dye at 450 nm, this volume was considered as an optimum value (Table1).

Table 1: Effect of D 4-AAP reagent amount on the absorbance.

Amount of 0.0025M D 4-AAP solution (ml)	Absorbance
0.5	0.209
1.0	0.281
1.5	0.120
2.0	0.098

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 (Table2).

Table 2: Selection of the base.

1 ml of 1N Base	Absorbance	Δλ*
NaOH	0.285	164.5
KOH	0.279	164.0
Na2CO3	0.179	69.0
NaHCO3	0.135	54.0

Δλ*= Color contrast = λS max_ λBmax; where S = the dye, B = blank

The results in (Table 2) indicated that NaOH gives the highest color intensity of the product and the best color contrast. The effect of different volumes (0.3-1.5 ml) of 1MNaOH solution on the color intensity was studied, a 0.5ml of 1MNaOH with pH of a final solution equal to 12.03 gives the best intensity, therefore it was used in the subsequent experiments (Table 3).

Table 3: Effect of base amount on absorbance.

Amount of 1M NaOH (ml)	0.3	0.5	0.7	1.0	1.5
Absorbance	0.233	0.435	0.359	0.287	0.256
pH	11.87	12.03	12.27	12.48	12.63

Effect of the order of addition

The effect of different orders of reagents addition on the absorbance of the dye was studied under the optimum experimental conditions. The results indicated that the order of reagents addition which was followed as given under the general procedure (Thymol+D4AAP+NaOH)gives highest color intensity, otherwise (D4AAP+NaOH+Thymol or Thymol+NaOH+D4AAP)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 was investigated under the optimum conditions of the reaction. The maximum color intensity is reached immediately after mixed the components of the reaction, and the absorbance of the formed dye remained constant for at least 2 hours, this stability period was sufficient for many measurements.

Final absorption spectrum

When thymol in an aqueous solution was treated according to the recommended procedure, the absorption spectrum showed a maximum absorption at 450 nm. The reagent blank shows very low absorption at above wavelength (Fig.1).

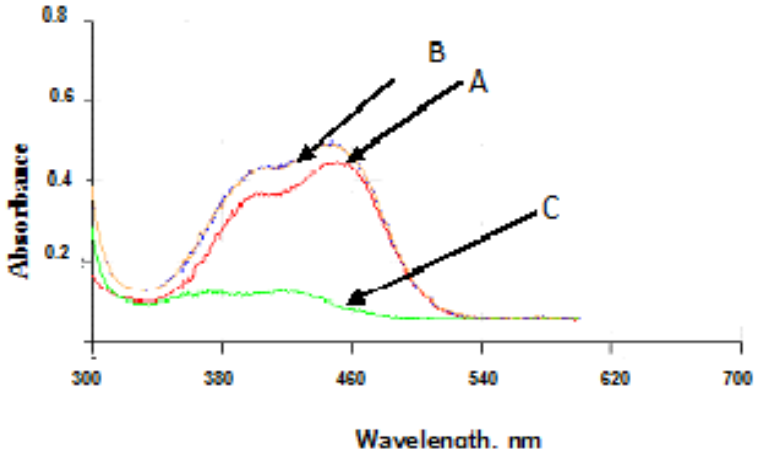
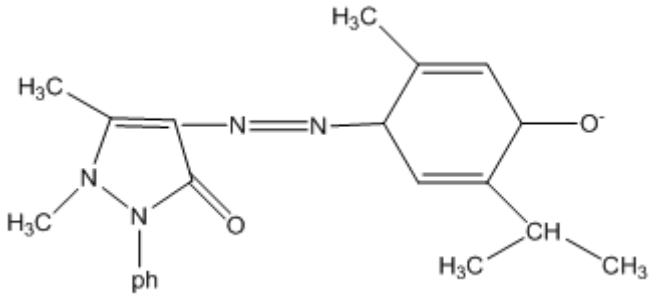


Fig.1:Absorption spectrum of 100µg thymol/25ml treated according to the recommended procedure.(A) the dye against blank, (B) the azo dye against distilled water (C) blank against distilled water

The nature of the dye

Job's method[25] indicated that the dye has a composition of 1:1 thymol to diazotized 4-aminoantipyrine reagent. Therefore, the structure of the formed dye may be written as follows:



The possible structure of the yellow Azo-dye:

Analytical data

Optical characteristics such as Beer's law range, molar absorptivity, Sandell's sensitivity, slope, intercept, of the suggested method are illustrated in (Table 4).

Table 4: Optical characteristics and precision data

Parameter	Proposed method
Beer's law limits(µg.25 ml-1)	10 -250.
Molar Absorptivity(l. mol.-1cm-1)	1.27 x 104
Sandell's Sensitivity(µg.cm-2)	0.011
λmax (nm)	450
Regression Equation (Y= sx* +c) Slope= s Intercept = c	 0.0034 0.0853
Determination Coefficient (R2)	0.9989
Precision(RSD%)	Not more than 0.25%

* x= Concentration of thymol in µg.25 ml-1

Pharmaceutical application

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 was 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 5 have been shown that the t-values are 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 5: Application of the proposed and standard methods for the determination of thymol in its pharmaceutical formulation

Drug	µg thymol present/25 ml	Presence method			Standard method Recovery, %	t-value
		Recovery* ,%	RE, %	RSD, %		
Listerine	100	101.27	+0.13	± 0.25	101.52	± 0.21

*Average for four determinations

Comparison of the methods

(Table 6), showed a comparison between the proposed method and that of other literature spectrophotometric methods throughout some measured analytical parameters.

Table 6: Comparison of the some analytical parameters of the proposed method with the same parameter for other spectrophotometric methods

Analytical parameters	Present method	Literature method[6]	Literature method[10]
pH	12.03	Alkaline	Alkaline
Temperature (C°)	Room temperature	Room temperature	Room temperature
λmax (nm)	450	550	467
Reagent	Diazotized 4-aminoantipyrine	p-Phenylenediamine	Diazotized p-phenylenediamine
Beer's law range (ppm)	0.4-10	0. 4-24	0.5-7
Molar absorptivity (l.mol-1.cm-1)	1.27×104	7.45×103	1.438×104
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 preparation	Pharmaceutical formulation

The results in (Table 6) are indicated that the method is sensitive and has an application part.

CONCLUSION

The proposed method is simple, sensitive and does not require temperature control or solvent extraction step. The method has been successfully applied to the determination of thymol in its pharmaceutical preparation as a mouth wash.

REFERENCES

[1]. M.D. Sasso; M. Culici, P.C. Braga, E.E. Guffanti and M., Mucci. "Thymol inhibitory activity on ESCHENICHIAeschenichia coli and staphylococcus aureus adhesion to human vaginal cells", J. of Essential Oil Research, (2006), 18.
[2]. B. G., Katzung "Basis and clinical pharmacology". 4th Edn., Prentice – Hall International Inc., London, pp.614-615, (1989).
[3]. K. Skold, S. Twetman, A. Hallgren, T. Lindberg and T. Modeer. "Effect of a chlorhexidine/ thymol containing varnish on prostaglandin E2 levels in gingival crevicular fluid". Eur. J. Oral. Sci., (1998), 106.
[4]. T. Lindberg, S. Twetman, K. Larsson and T. Modeerb. " Effect of an antibacterial dental vanish on the levels of prostanoids, leukotriene B4 andinterieukin-1 beta in gingival crevicular fluid". Acta Odontologica Scandinavica, (1999), 57, 23.
[5]. N.R. Ahmed and W. Aesa. "Spectrophotometric determination of thymol in smicks ointment." J.Edu. Sci., (2004),16(1), 52.
[6]. I. Mohammad."Spectrophotometric determination of thymol in pharmaceutical formations via oxidative coupling reaction". J.Edu.Sci., (2005), 17(2), 41.
[7]. M. Q. Al-Abachi, H. Hadi and G. Fadhil."Flow Injection determination of thymol in pharmaceutical samples via oxidative coupling reaction with 2,4- dinitrophenylhydrazine ."Journal of Al-Nahrain University (2015), 18 (2),18 .
[8]. J.S. Romero., L.A. Rodriguez, M.C. Coque and G.R. Ramos. "Spectrophotometric determination of phenols by coupling with diazotized 2,4,6-trimethylaninline in a micellar medium". Analyst, (1994),119, 1381.
[9]. W., Pucher and L.A. Burd."A new method for the colorimetric determination of small concentrations of thymol". Exp. Biol. Med., (1924), 21, 565.
[10]. A. Dhahir, S. Muhammad, M. Naz and T. Adday. "New diazo-coupling reactions for visible spectrophotometric determination of thymol in pharmaceutical preparations with phenylenediamine as the coupling reagent". Middle East J. of Int. Med., (2012), 5, 36.
[11]. M.S. Al-Enizzi, T.N. Al-Sabha and T.S. AL- Ghabsha". Use of charge transfer complex formation reaction in spectrophotometric micro determination of some drugs" JJC., (2012), 7(1),87.
[12]. H. Hajimehdipoor, M. Shekarchi, M. Khanavi, N. Adib and M.Amri." A validated high performance liquid chromatography method for the analysis of thymol and carvacrol in thymus vulgaris L. volatile oil". Pharmacogn. Mag., (2010), 6(23), 154.
[13]. LI. Kang, JA. Yuan and SU. Weiwei."Determination of liquidritin, naringin, hesperidin, thymol,imperatorin, honokiolisoimperatorin and magnolol in the traditional chines medicinal preparation huoxiang-zhengqi liquid using high performance liquid chromatography".Yakugaku Zasshi, (2006),126(11), 1185.

[14]. L. Ji, Y. Wang, Y. Tong, X. Li, X. Feng, L. Hang and G. Zhou." Determination of carvacrol and thymol in moslachinensis by HPLC". Zhongguo Zhong Yao Za Zhi, (2004), 29, 1030.
[15]. A. Cantalapiedra, M. J. Gismera, M. T. Sevilla and J. R. Procopio , Phytochemical. "Sensitive and selective determination of phenolic compounds from aromatic plants using an electrochemical detection coupled with HPLC method". Phytochemical Analysis,(2014), 25(3), 247. .
[16]. H. Gao, W. Cao, Y. Liang, N. Cheng, B. Wang, and J. Zheng. "Determination of thymol and phenol in honey by LC with electrochemical detection". Chromatographia, (2010), 72, 316.
[17]. C. Kohlert, G. Abel, E. Schmid and M. Veit. "Determination of thymol in human plasma by automated headspace solid-phase micro extraction gas chromatographic analysis".J. Chromatogr. B Anal. Technol. Biomed. Life Sci., (2002),767(1), 11.
[18]. R. Badertscher, V. Kilchenmann, A. Liniger and P. Gallmann."Determination of 1, 4- dichlorobenzene,naphthalene and thymol residues in honey using static headspace coupled with GC-MS". J.Apiproduct & ApiMedical Sci., (2010), 2(3), 78.
[19]. F. A Jesús., R. M. Mercedes, P. Alberto and A. Gema, .,"Comparison of the "Simple cyclic voltammetry (CV) and DPPH assays for the determination of antioxidant capacity of active principles". Molecules (2012), 17(5), 5126.
[20]. OW. Lau, SF. Luk, and WC. Wong. "Simultaneous determination of methyl salicylate and thymol in various pharmaceutical formulations by differential-pulse voltammetry using a glassy carbon electrode". Analyst, (1988), 113, 865.
[21]. D. Stanković ." Sensitive voltammetric determination of thymol in essential oil of Carum copticum seeds using boron-doped diamond electrode". Anal Biochem. (2015),486,1.
[22]. G. Ziyatdinova, E. Ziganshina, P. Cong, H. Budnikov".Voltammetric determination of thymol in oregano using CeO2-modified electrode in brij® 35 micellar medium".Food Analytical Methods, (2017),10 (1), 129-136
[23]. M. Lopez, , J. Vilarino, M.V. Rodriguez, , L.F. Losada. ,"Development, validation and application of micellar electrokinetic capillary chromatography method for routine analysis of catechins, quercetin and thymol in natural samples"., Microchemical J. (2011), 99(2), 461.
[24]. H. Sereshti, Y. Izadmanesh, S. Samadi. " Optimized ultrasonic assisted extraction-dispersive liquid-liquid micro extraction coupled with gas chromatography for determination of essential oil of Oliveria decumbens Vent". J. of Chromatography A ,(2011),1218(29), 4593.
[25]. R. Delevie, "Principles of Quantitative Chemical Analysis", McGraw-Hill, International Edn., Singapore, (1997), p. 498
[26]. "Standard methods for the examination of water and wastewater",(1975), 14th Edn., American Public Health Association Washington, 582.