DocID: https://connectjournals.com/03896.2020.20.4065

Biochem. Cell. Arch. Vol. 20, Supplement 2, pp. 4065-4070, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

SPECTROPHOTOMETRIC INVESTIGATION OF THYMOL BY UTILIZE OF OXIDATIVE COUPLING REACTION IN DIFFERENT SAMPLES OF MOUTH WASHES

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(Received 5 May 2020, Revised 7 July 2020, Accepted 12 July 2020)

ABSTRACT : A clear, sensitiveas well as quick spectrophotometric process is defined in various mouth washes samples to investigate the microgram amounts of thymol. In the existence of potassium ferricyanide ($K_3Fe(CN)_6$) and sodium hydroxide, the process is depended on the oxidative coupling reaction between orthotolidene (OTD) to for man extreme pink substance with 544nm strongly absorption. Beer's law is provided on the concentration scale between 0.5–16ppm with 1.3608×10^4 $l.mol^{-1}.cm^{-1}$ as molar absorptivity as well as 0.011µg.cm^{-2} as Sandal's sensitivity. The limit of detection was 0.198ppm. The optimal conditions for the color production are defined additionally utilizing the proposed method for determining thymolin bulk drug and Pharmaceutical preparations (Various Mouth Washes Samples) has been successfully applied. This approach was not interfered with by the usual excipients and additives.

Keys words : Thymol, oxidative coupling, spectrophotometric determination.

INTRODUCTION

Thymol(thy) is a dependably happening component identified in thyme. Plants as well as oil inclusiviy *Thymus vulgaris*. The studied compound is an antifungal additionally antiseptic agent which has been utilized for centuries in modern medicine, containing various regions. So, for example Iraq as well as China (Al-Bayati *et al*, 2009; Zekovic *et al*, 2000; British Pharmacopoeia, 2007). Thymol is a 2-isopropyl-5-methyl phenol, $C_{10}H_{14}O$, while its chemical structure is given in Fig. 1. (Liang *et al*, 2007).

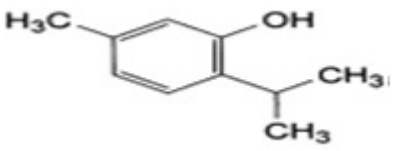

Fig. 1 : Thymol's chemical composition (thy).

Thymol has many applications as well, including perfumes, mouthwashing, food flavoring, cosmetics as well as also as a stabilizer for sundry medicinal agents containing halothane (Katzung, 1989; Neidle E A Yagida, 1989). A variety of analytical processes for determining thymol have been published, containing liquid chromatography with Electrochemical detection (Gao *et al*, 2010), the gas chromatography (Noall *et al*, 1975; Badertscher *et al*, 2010), high-performance liquid chromatography (Thompson *et al*, 1989; Ji *et al*, 2004; Kang *et al*, 2006) ultraviolet spectrometry (Korany *et al*, 1984), colorimetric analyses (Fibranz *et al*, 1985) additionally voltametry (differential pulse) (Lau *et al*, 1988). In the study process, in the existence of potassium ferricyanide ($K_3Fe(CN)_6$) in alkaline media, the steady ortho tolidene agent solution was suggested to evaluate thymolin bulk drugas well as the preparations of mouth wash through the reaction of the Oxidative coupling. The pink outcome was spectrophotometrically recording in 544 nm. The method of analyzing is accurate, fast as well as simple. It was extended to the investegation of thymol in bulk as well as satisfactorily in the preparations for mouth washing.

MATERIALS AND METHODS

Apparatus

- Both spectral and absorbance measurements were performed on 160 digital double - beam applied UV-Visible registration spectrometer.

- Water bath Warming as well as cooling (Haake, Fe3).
- pH meter, Jenway 3020.
- Susceptible balance (Sartorius BL 210S).

Material and agents

The chemical substances utilized were a considerable degree of purity prepared by the next :

1. Typical thymol powder has been supplied from the (SDI) (state company for drug industries and medical appliances Samara, Iraq).

The stock basic solution of (thy) with 250ppm concentration by going to dissolve 0.025 gm of absolute contentin 5 ml ethayl alcohol as well as weakening to the limit 100 ml Dionized water. After protecting well away from light, this solution is reliable for at minimum a month.

2. Potassium ferricyanide 0.01 M:- it was supplied from (BDH Chemicals Ltd, Laboratory Agent) to dissolve (0.328) gm of absolute content in 100 ml Dionized water.
3. Sodium hydroxide 1M:- it was supplied from merck company as well as the clarity was 98%. It was prepared by dissolution 4gm in Dionized water 100 ml.
4. ortho tolidene (OTD) 0.005 M:

It was dissolved 0.106 gm of absoluate material in 50 ml ethayl alchole from (percentage 99.9) by (BDH Chemicals Ltd, Laboratory Reagent) company.

Procedure suggested

In a sequence of 25 ml volumetric flasks, standard solutions aliquots of thymol with concentrations of 0.5 - 16 ppm were inserted separately In the final volume ccompanied by adding 1.8 ml) orthotolidene (0.005)M and (1.6 ml) potassium ferricyanide (0.01) M and adding (1.5 ml) sodium hydroxide (1 M), respectively. The components have been weakened to the sign with dionized water, the absorption was estimated at (544 nm) at room temperature and the calibration curve was built against the reagent blank.

Procedure for the pharmaceutical preparation of thymol assay

A test of mouth wash 25 ml is transported to a 100 volumetric flask as well as weakened with distilled water to the mark. An equal of this solution 1ml is inserted in avolumetric flask 25 ml, 1.8 ml ortho tolidene 0.005 M as well as 1.6 ml potassium ferricyanide 0.01 M and adding 1.5 ml sodium hydroxide (1 M), the absorption is then estimated at 544 nm. Athymol concentration is estimated utilizing the calibration curve that already developed additionally defined previously. This approach has been introduced to three commercial forms of mouth wash.

1. Listerine fresh burst-antiseptic mouth wash (USA) : The product label contains 0.064 percent thymol.

2. Listerine cool mint-antiseptic mouth wash (USA) : The product label contains 0.064 percent thymol.

3. Mestril–antiseptic mouth wash (JORDAN - MID PHARMA) : The product label contains 0.063 percent thymol According to the label and by the changed process, the percentage of thymol obtained is as exceeds.

RESULTS AND DISCUSSION

Examine the best requirements for the reaction : Various requirements have been tested that influence the strength of the dye being absorbed in order to develop.

1. Agent volume influence : The impact of reagent volume on strength absorption was tested. It was collected from 0.5 -4 ml of ortho tolidene reagent (OTD) at concentration 0.005 M with attendance 1.6 ml of oxidation agent additionally 1.5 ml of base solution. It has been noticed that 1.8 ml is the reagent's optimal volume, which gives optimum absorption, that was utilized in the tests below.

2. Oxidation agent volume influence : The impact of oxidation reagent volume in the absorption of strength was observed.

The concentration of potassium ferricyanide 0.5 -6ml 0.005 M was provided with the inclusion of the reagent 1.8ml as well as 1.5 ml of the basic solution. It has been noticed that 1.6 ml is the optimal the oxidizing agent volume, which provides the strongly absorption uitilzedin the corresponding tests.

3. Base influence : Base presence has been linked to increased the strength of the generated outcome, therefore some bases such as NH_4OH, KOH,NaOH, additionally several basic salt like Na_2CO_3 as well as CH_3COONa are being investigated. It was noticed that the color substance was absorbed by all of these bases; NaOH was chosen as well as discovered that 1.5 ml of this base gives a good sensitivity that was specified in corresponding tests.

4. Order of addition influence : The best order of addition was discovered to give the best absorption (D+R+O+B) whihe (D = drug ingredient, R = agent, O = oxidation agent, as well as B = basic solution) selected in consequent tests.

5. Temperature Influence : At various temperatures, the resulting component of the current

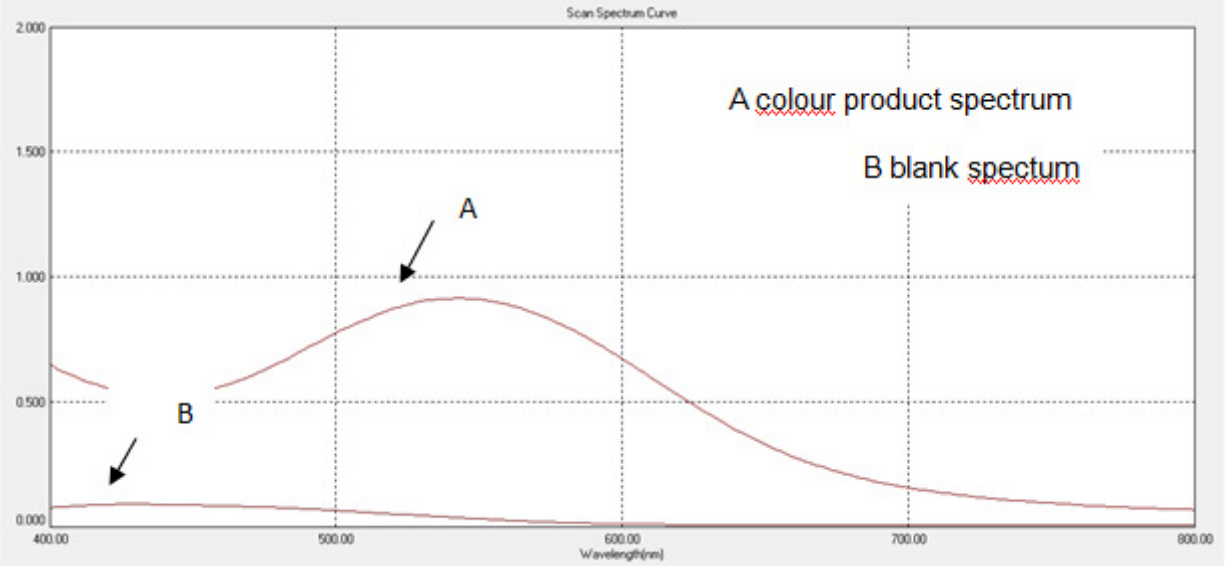

Fig. 2 : The spectrum of colored outcome created (A) as well as blank(B).

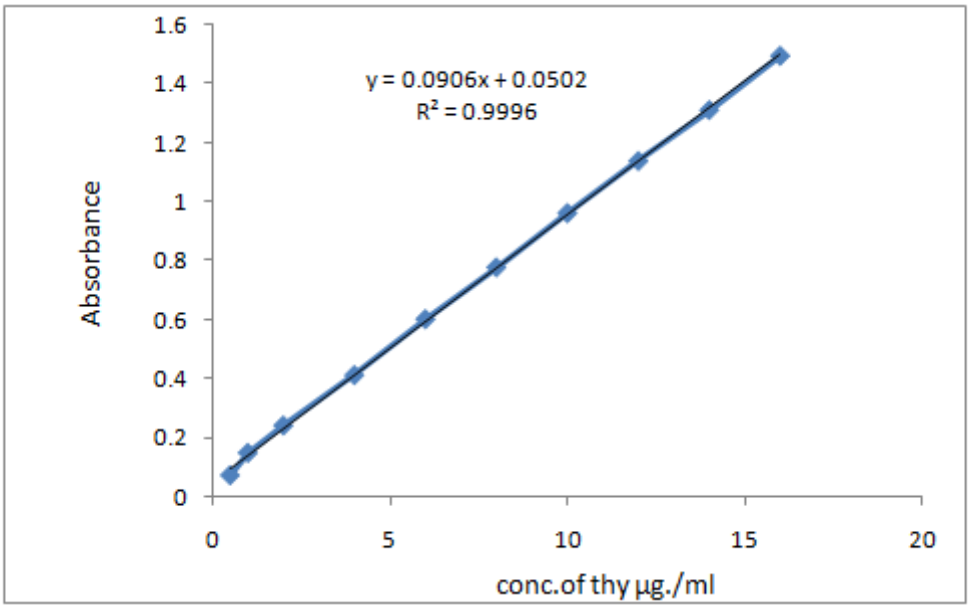

Fig. 3 : The (thy) Calibration curve.

method was examined. The results demonstrate an improvement in the absorbance levels in the temperature scale 0 - 80ºC. The colored product was temperature-stable 25ºC, which offered the strongest absorption. In this process, this temperature was chosen and used in corresponding tests.

5. Reaction time influence : The strength of the color achieved its limit after the drug (thy) was immediately reacted with the Reagent in the existence of potassium ferricyanide as well as after 15 minutes the specific solution was stabilized at 25°C. Thus, in the general process, 15 minutes implementation time was chosen as optimal. At 180 minutes, the color obtained was constant.

7. Absorption Spectra : The spectral analysis was undertaken for achieving stronger absorptionof the wavelength of the subsequent component after implementing the optimal requirements for reaction. A solution of the blank including the Oxidation reagent, the Reagent, as well as the uiltilizing NaOH (Fig. 2) displays maximal absorption at 544 nm, while (A) spectrum is the compound component of the reaction as well as (B) the blank spectrum the reaction.

8. The calibration curve : A linear calibration curve for thymol is obtained utilizing the conditionsdefined in the process (Fig. 3), which indicates that the law of Beer is observed over the concentration scale of (0.5-16) ppm with an intercept of 0.0502 as well as correlation coefficient of 0.9996. The substantial molar absorptivity

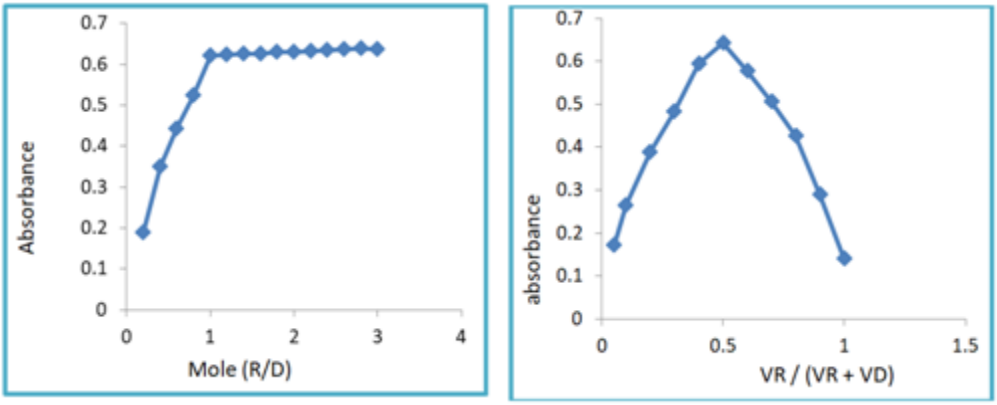

Fig. 4 : In the existence of ($K_3Fe(CN)_6$) and NaOH mole ratio and continuous variance plots for (thy) reaction.

Table 1 : The current approach is accurate and precise.

No	Error%	Recovery%	Conc. of thy (µg/ml)		R.S.D%
			Present	Found	
1	1	0.971	- 2.900	97.100	1.982
2	10	9.985	+ 0.150	100.150	1.235
3	14	14.201	+ 1.435	101.435	0.880

Table 2 : Color product spectrophotometric characteristics in differeent organic solvents.

Solvent	$\ddot{e}_{max}$,nm	å,L.mol^{-1}.cm^{-1}
Acetic acid	520	1.215×10^3
Dimethyl sulphoxide	420	0.918×10^3
Formic acid	430	2.457×10^3
2- propanol	470	1.985×10^3
chloroform		Two layers
Acetone	510	4.934×10^3
Dioxane	380	0.946×10^3
CCl_4		Two layers
Methanol	526	1.143×10^4
Ethanol	530	1.093×10^4
Dimethyl formamide	502	3.360×10^3
Teri butyl alcohol		Two layers
Benzene		Two layers
Di ethyl ether		Two layers
Pyridine		Turbid

of it was noticed that the color product shaped on 1.3608 x10^4 L.mol^{-1} cm^{-1}. Additionally, Sandell's sensitivity was 0.011 (ìg./cm^2). The slope was 0.0906.

9. Accuracy and precision : Three separate concentrations of thymol demonstrated the accuracy and precision of the process. The outcomes shown in the Table 1 demonstrate which the process is appropriate. Additionally have better precision as well as accuracy.

10. The reaction Stoichiometry : The thymol-reactive stoichiometry of the reaction was analyzed utilizing Job's method and molar ratio process; the findings were gathered (Fig. 4) indicate, which a color product of 1:1 was generated at 544 nm. The substance was soluble in water, the stabilization constant was determined with contrasting the solution absorption representing stoicheiometricthymol and the agent with the solution supplying the ideal quantity (1ml of 2.8×10^{-3} M) as well as another solution reagent the solution at the concentration with five times for the initial concentration. The median constant contenting stabilization of the color substance in water under the test conditions defined was 3.98×10^6l^2.mol^{-2}.

Therefore, the formulation of the product and the indicated reaction possibly takes part as describes in Fig. 5 (Morrison *et al*, 1973; Al-Abachi *et al*, 1988; Roberts and Caserio, 1964).

11. Solvents influence : Table (2) indicates the spectrophotometric features of the color component in different organic solvents. From either the viewpoint of economy as well as sensitivity, water is shown to be a better medium (solvent utilized to dilute in the flask to the mark.

12. Interference : The excipients were examined : starch, talc, lactose, Cross povidone, Sucrose, Glucose, Acacia, poly vinyl pirolidone (PVP) as well as magnesium stearate. For this analysis solution contained (thy) each one of the excipients was collected individually at concentrations tentimes greater than (thy) and tested in the Calibration Curve according to the same process. The extent of interference was deemed acceptable if the

Table 3 : In the presence of excipients, assurance of 10 ppm thymol.

Interference	% Error	% Recovery
Talc	-1.200	98.800
poly vinyl pirolidone (PVP)	1.160	101.160
Acacia	-0.670	99.230
lactose	-1.160	98.840
Sucrose	0.550	100.550
Cross povidone	-0.910	99.090
Glucose	-0.880	99.120
Starch	- 0.780	99.220

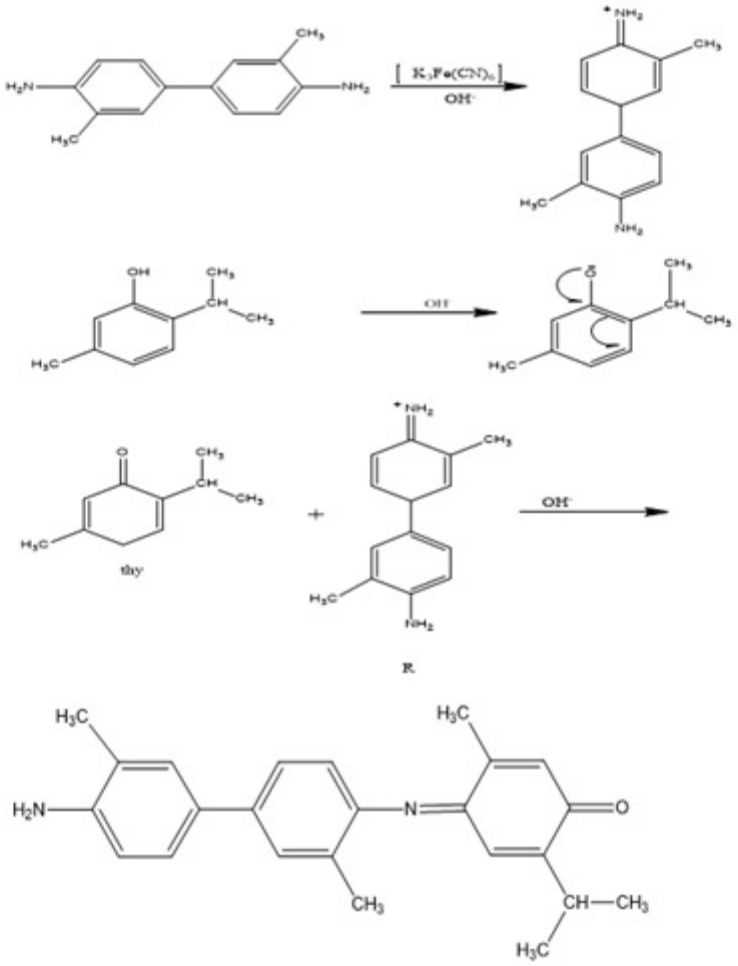

Fig. 5 : Plausible path of product creation.

Table 4 : Thymol assay in bulk and types of dose.

Pharmaceutical preparation	Average recovery %	
	Proposed method	Standard method (Ashutoshkar, 2005)
Thymol pure	100.5	100.2
Mestril	99.7	99.3
Listerine fresh burst	99.4	99.1
Listerine cool mint	100.4	99.7
Largactil tablets (25mg) Syria	98.9	98.7

error did not exceed ± 2% of the expected level No interference was found in the evaluation of thy in the existence for the examined excipients (median of three investigations) (Table 4).

13. Process application : The applicability of the pharmaceutical formulation check system has been studied. Table (5) summarizes the results of the test for appropriate formulations of thymol drugs.

Where the five determinations compare and British Pharmacopoeia (2009) took the standard method. The outcomes were replicable as well as the standard method verified the assay of formulations.

CONCLUSION

Aclear, fast, accurate and effective spectrophotometric process for determining trace levels of thymol in water solution was created established on its oxidative coupling reaction with para ortho tolidene as well as $K_3Fe(CN)_6$ In the presence of sodium hydroxide.

No temperature regulation or solvent extraction process is needed by the current process. The approach has been introduced.

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