Spectrophotometric Determination of Thymol in Pharmaceutical Preparations Via Oxidative Coupling Reaction with 2,4-dinitrophenylhydrazine in the Presence of Potassium Periodate

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Abstract

A new, simple and sensitive spectrophotometric method for the determination of Thymol in pure and mouth wash preparations has been proposed in this study. The method was based on oxidation of 2,4-dinitrophenylhydrazine with potassium periodate and coupling with Thymol in alkaline medium to form an intense violet water-soluble dye that is stable and has a maximum absorption at 570 nm. A graph of absorbance versus concentration shows that Beer's law was obeyed over the concentration range of 0.25-10 $\mu g.mL^{-1}$ of Thymol, with detection limits of 0.063 $\mu g.mL^{-1}$. All experimental parameters that affect the development and stability of the colored product were carefully studied and the proposed method was successfully applied to the determination of Thymol in mouth wash preparations.

Key words: Thymol, 2, 4-dinitrophenylhydrazine, potassium periodate, spectrophotometry.

التقدير الطيفي للثايمول في المستحضرات الصيدلانية بوساطة تفاعل الازدواج التاكسدي مع 4,2- ثنائي نيتروفنيل هيدرازين بوجود بيرايودات البوتاسيوم

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الخلاصة

تم تطوير طريقة تحليلية جديدة للتقدير البسيط و الحساس للثايمول بصورته النقية وفي غسولات الفم. اعتمدت الطريقة على اكسدة كاشف 4,2- ثنائي نيترو فنيل هيدرازين بوساطة بيرايودات البوتاسيوم ثم مفاعلته مع الثايمول في الوسط القاعدي لتكوين ناتج مستقر بنفسجي اللون يعطي أقصى امتصاص عند طول موجي 570 نانومتر. يشير الرسم البياني للامتصاص مقابل التركيز بان قانون بير ينطبق ضمن المدى 0,25- 10 مكغم مل$^{-1}$ من الثايمول وبحد كشف 0,063 مكغم مل$^{-1}$.تم دراسة الظروف المثلى للتفاعل بدقة و تم تطبيق الطريقة المقترحة بنجاح في تقدير االثايمول في غسولات الفم.

Introduction:

Thymol is a 5-methyl-2-(methyl ethyl) phenol,$C_{10}H_{14}O$, whereas its chemical structure is shown in figure 1-[1]:

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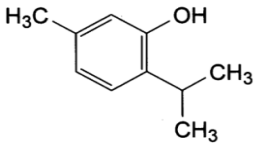


Figure 1- Structure of Thymol

Thymol is a monoterpenoid phenol derivatives. It is found in natural substances, spice plants. It is found in thyme, oregano and wolf's bane. Thymol is widely used in the chemical industry to stabilize and to store solutions and serum samples [2].

Thymol resembles phenol in its action, but owing to its insolubility in the fluids of the body, it is absorbed much more slowly; it is also less irritant to wounds, while its germicidal action is greater than that of phenol, though less than that of naphthol. In alcoholic solution, it penetrates the skin and produces local anaesthesia. It is used as an antiseptic lotion and mouth wash (1 in 1000), or as Liquor Thymolis Composites; as a paint in ringworm (1 in 10 of alcohol, or alcohol and ether); and as an ointment (1 in 24 of soft paraffin, the Thymol being dissolved with the aid of heat) in eczema, psoriasis, broken chilblains, parasitic skin affections, and burns.

An ointment perfumed with oil of lavender, is used to keep off mosquitoes. Thymol in oily solution (1 or 2 per cent.) is applied to the respiratory passages by means of a spray in nasal catarrh and it's also used to medicate absorbent gauze and wool for use as surgical dressings.

A number of analytical methods have been reported for the determination of Thymol, these included high pressure liquid chromatography [3-8], liquid chromatography with electrochemical detection [9], gas chromatography [10-15], differential-pulse voltammetry [16], spectrometry [17-19], colorimetric analysis [20],TLC[21,22] and Flow injection spectrophotometry[23]. However, some of these methods are time consuming and/or require expensive equipment and conditions.In this work, rapid and sensitive method using spectrophotometric detection at 570nm was proposed for the determination of Thymol in pharmaceutical preparations. The method is based on an oxidation of 2, 4-dintrophenyhydrazin with potassium periodate and reaction with Thymol in alkaline medium. The analytical procedure is safe, simple, fast and accurate. It has been satisfactorily applied to the determination of Thymol in pure and mouth wash preparations.

Experimental Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan) and using 1 cm quartz cells.

Preparation of solutions

Thymol stock solution(1000 µg.mL^{-1}): prepared by dissolving 0.100 gm amount of pure Thymol (BDH) in 5 mL of ethanol then complete to 100 mL in a volumetric flask with distilled water. Thymol working solution (100 µg.mL^{-1}), was prepared by dilution of 10 mL of the stock solution to 100 mL volumetric flask with distilled water.

2,4-dinitrophenylhydrazine (2,4-NPH) (1 × 10^{-3} M): was prepared by dissolving 0.019814 g of 2,4,DNPH (BDH) in 2mL of concentrated sulfuric acid then complete to 100mL in a volumetric flask with distilled water.

Potassium periodate (5×10^{-3}M) : A 0.09544g of potassium periodate (BDH) was dissolved in distilled water and diluting to mark in 100 mL volumetric flask.

Sodium hydroxide (0.5M) : was prepared by dissolving 5 g of sodium hydroxide (BDH) in distilled water and diluting to mark in 250 mL volumetric flask.

Pharmaceutical preparations of Thymol

Pharmaceutical preparations were obtained from commercial sources. 1-Listerine antiseptic (USA): containing 0.063% Thymol. 2-Breath Rx (mouth rinse-anti bacterial-USA):cntaining 0.060% Thymol**.**

General procedure for calibration

An aliquot of sample containing 0.025-2.5 mL of pure Thymol(100µg.mL^{-1}) was transferd into a series of 25 mL standard flask. Add 1 mL of potassium periodate (5×10^{-3} M), and 1 mL of 2,4-DNPH(1×10^{-3} M),then 5mL of sodium hydroxide(0.5M), the contents of the flasks were diluted to the mark with distilled water, mixed well and left for 15 min at room temperature, the absorbance of the violet compound formed was measured at 570 nm against a reagent blank containing all materials

except Thymol. A calibration graph was drawn and the regression equation was calculated. For the optimization of conditions a solution of 2mL of pure Thymol(100µg.ml^{-1}) was used in a final volume of 25mL.

Procedure for Mouth wash:

Two types of mouth wash were analyzed by the developed methods, these include:-

1-Breath Rx (mouth rinse-anti bacterial-USA),this type of mouth wash containing 0.060% Thymol. Transfer 20 mL of the mouth wash preparation to a 50 mL volumetric flask, add 5 mL of ethanol and dilute to the mark with distilled water. The concentration of this solution was (240 µg.ml^{-1}) stock solution. Working solution of 100 µg.mL^{1} was prepared by simple dilution of the stock solution with distilled water.

2-Listerine-antiseptic(USA): containing 0.063% Thymol. Transfer 20 mL of the mouth wash preparation to a 50 mL volumetric flask then add 5 mL of ethanol and dilute to the mark with distilled water. The concentration of this prepared solution was (252µg.mL^{-1}) stock solution, (100 µg.mL^{-1}) solution was prepared by simple dilution of the stock solution with distilled water.

Results and discussion Absorption spectra

When a diluted aqueous solution of Thymol was added to the oxidized 2,4-DNPH in alkaline medium, an intense violet product formed immediately, which became stable after 15 min. The violet product has a maximum absorption at 570nm. Figure 2- shows the spectra of the product formed and of the reagent blank, the maximum absorption at 570 nm produce from mixing [1mL of Thymol (100µg.mL^{-1}), 1mL of potassium periodate (5 × 10^{-3} M) ,0.5 mL of 2,4-DNPH (1 × 10^{-3} M) and 5mL of sodium hydroxide(0.5M) and diluted to 25mL with distilled water] measured versus reagent blank [it contains all components above except of Thymol] which has negligible absorbance at this wavelength.

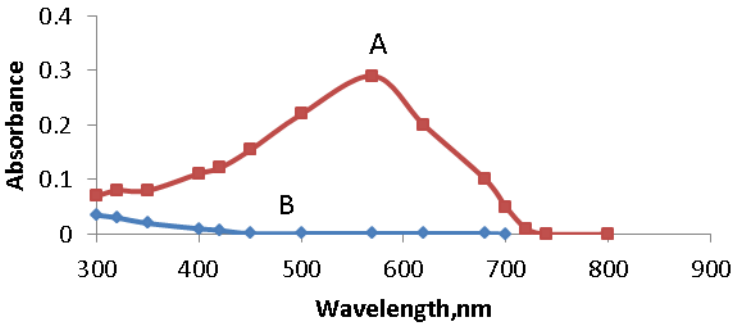


Figure 2- Absorbance spectra of thymol (4µg mL^{-1}) treated as described under procedure above and measured against blank(A), The reagent blank contains all components except Thymol measured against distilled water(B).

Effect of order of addition

The order of addition of the reagents is an essential part of the experiment, it was found that the order of addition of the reagent cited under general procedure gave a maximum color intensity and a minimum absorbance of the blank and was used in all subsequent experiments.

Optimization of the experimental condition

The effects of various parameters on the absorption intensity of the formed products were optimized. The effects of different alkaline solutions (0.5 M) were studied such as sodium hydroxide, sodium carbonate, potassium hydroxide and ammonium hydroxide. It was found that sodium hydroxide was the most suitable alkaline medium for a maximum absorbance and was used in all subsequent experiments. The effect of different volumes of sodium hydroxide (0.5M) were studied in the range of (2-8mL) and found that the greatest absorbance intensity was obtained with 5mL figure 3. Similarly, the effect of different volumes of the reagent 2,4-DNPH (1×10^{-3} M) were studied on the maximum absorbance by varying the volume of 2,4-DNPH between (0.1-1.5mL) .It was found that 1mL of 2,4-DNPH (1 × 10^{-3} M) gave the highest absorbance figure 4. Effect of different volumes (0.1–2 mL) of potassium periodate (5 × 10^{-3} M) was examined on the maximum absorbance of the

formed product. figure 5- Shows that 1 mL of potassium periodate solution was enough to obtain a maximum absorbance.

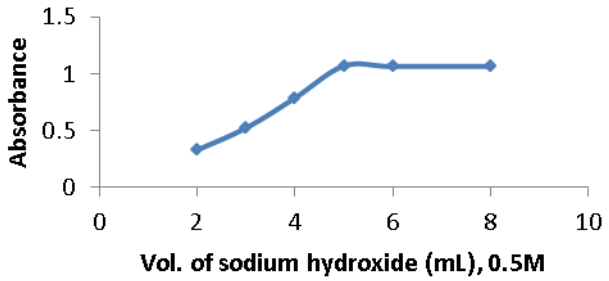


Figure 3- Effect of the volume of NaOH(0.5M) for determination of Thymol (8µg.mL^{-1}).

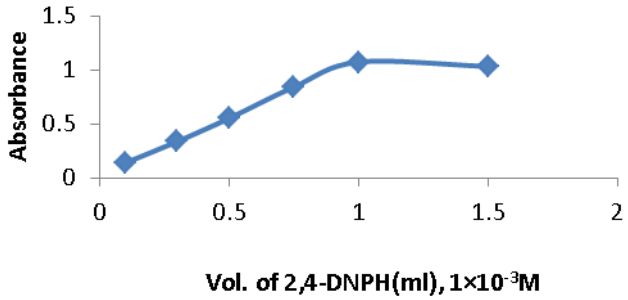


Figure 4- Effect of the volume of 2,4-DNPH(1×10^{-3}M) for determination of Thymol (8µg.mL^{-1}).

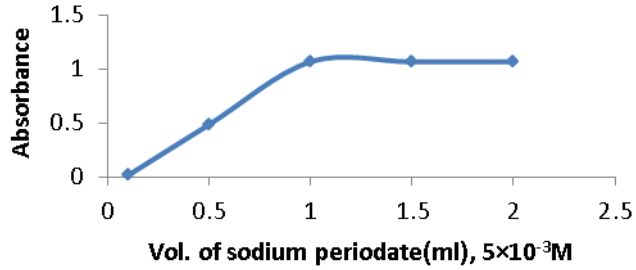


Figure 5- Effect of the volume of sodium periodate (5×10^{-3}M) for determination of Thymol (8µg.mL^{-1}).

Effect of reaction time

Experimental results revealed that the colour intensity reaches a maximum after 2,4-DNPH solution had been oxidized with potassium periodate and reacted with Thymol in alkaline medium for 15min, therefore, a 15 min development time was suggested as the optimum reaction time and remain stable for 120 min.

Structures of the products

The stoichiometry of the reaction between Thymol and 2,4-DNPH was investigated using both continuous variation and molar ratio methods respectively, The results obtained figure (6 and 7) show that a (1:1) was formed between Thymol and 2,4-DNPH .

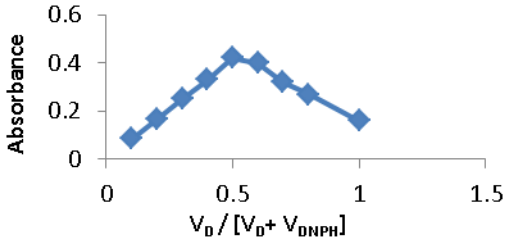


Figure 6- Continuous variation plot

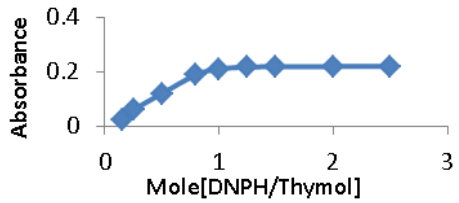
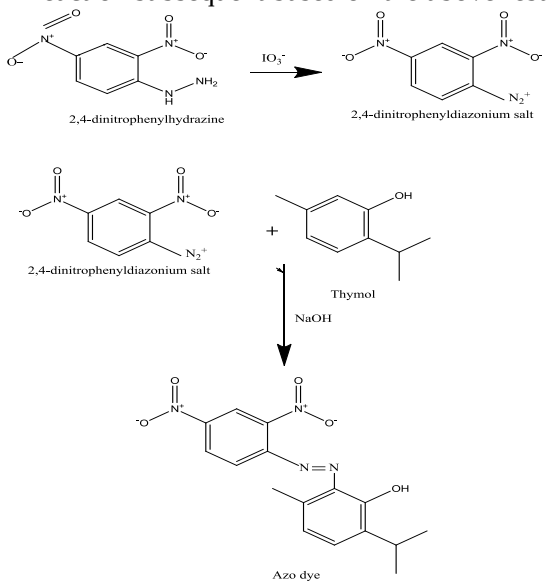


Figure 7- Mole ratio plot

A reaction subsequent based on the above results is shown in Scheme1.[24]



Scheme 1- Proposed mechanism of the reaction between Thymol and 2,4DNPH.

The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stiochiometric amount of Thymol (6.65×10^{-4} M) (A_S) with that of a solution containing a five – fold excess of 2,4-DNPH reagent (A_m) and according to analytical procedure. The average stability constant (K)= 5.4×10^{5} $L.mol^{-1}$, where is [$K=(1-\alpha)/\alpha^2C$] and $\alpha=Am-As/Am$ [25].

Analytical characteristics of spectrophotometric method

For the proposed method, a calibration graph, were obtained by the procedure described previously and a series of standard solutions was analyzed in triplicate to test the linearity figure 8. The molar absorptivity (ε), the Sandell's sensitivity (S), the slope (a) and the intercept (b) were determined and are included in Table 1. The accuracy and precision of the proposed methods were tested by analyzing five replicate of Thymol using the proposed spectrophotometric method for three different concentrations of thymol. The values of relative standard deviation RSD% and relative error E_{rel}% are summarized in the same table. These values indicated a high accuracy and precision of the proposed method. The limit of detection (LOD) was determined by taking the ratio of the standard deviation (SD) of the blank with respect to water and the slope of the calibration curve multiplied by a factor of three[26].

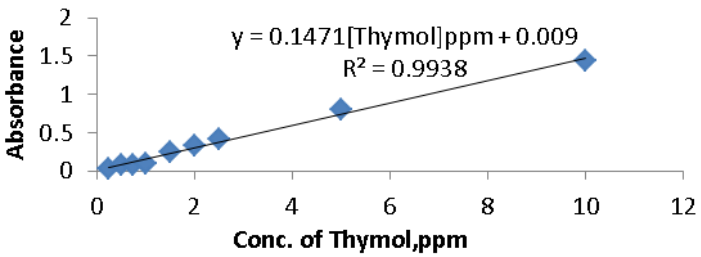


Figure 8- Calibration graph of Thymol

Table 1- Analytical parameters of spectrophotometric method

Parameters	**Value**
$\lambda_{max\ (nm)}$	570
Linearity range, µg ml^{-1}	0.25-10
Molar absorbtivity (L mol^{-1} cm^{-1})	2.2×10^{4}
Sandell's sensitivity(µg ml^{-1})	0.67×10^{-2}
Regression equation	Y=0.1471X + 0.009
Linearity (r)	0.9969
Limit of detection(L.O.D*) (µg ml^{-1})	0.063
Relative standard deviation (RSD%)*	1.55
Average of recovery%	101.12
E_{rel} %	1.12
Molar ratio (D:R)	1:1

***L.O.D= ($3\sigma_B$ /S), σ_B =standard deviation of blank S=slope**

Pharmaceutical application

The suggested methods were applied to the quantitative determination of Thymol in mouth wash formulation. Two types of mouth wash preparations containing Thymol were analyzed and they gave a good accuracy and precision as shown in Table 2. The proposed method was compared successfully with the official method [27].

Table 2- Application of the proposed and official methods for the determination of mouth wash containing Thymol

Mouth wash samples	Proposed method		Official method
	Recovery, %	RSD%	Recovery, %
Breath Rx	99.6	1.569	99.48
Listerine	99.2	4.181	100.86

F-test and T-test showed that there was no significant difference between the proposed method and the official method using 4-aminoantipyrine (4-AAP) and potassium ferricyanide Table 3.

Table 3- The comparison of the proposed method with standard method using t- and F-statistical tests

The pharmaceutical preparations for 2.0 µg.ml^{-1}	The proposed method		The official method	
	Rec.%	$(Xi-\bar{Xi})_1^2$	Rec.%	$(Xi-\bar{Xi})_2^2$
Pure Thymol	101.12	1.322	98.00	2.073
Breath Rx	99.6	0.136	99.48	0.001
Listarine	99.2	0.592	100.86	2.016
at 95% confidence level	$(\bar{Xi})_1$=99.97	$\Sigma(Xi-\bar{Xi})_1^2$= 2.050 S_1= (1.01) F calculated=S_2^2/S_1^2 = 2.004 F theoretical =19.0 F theoretical > F calculated	$(\bar{Xi})_2$= 99.44 S_2 = (1.43) t calculated = 1.239, t theoretical = 2.776 t theoretical > t calculated	$\Sigma(Xi-\bar{Xi})_2^2$= 4.090

A standard additions method was used to avoid and correct the chemical interferences that present in mouth wash preparations. It involves adding increment volumes (0-1.5mL) of standard solution of

100µg.mL^{-1} to a fixed volume sample(0.2 mL of 100µg.mL^{-1}) and employing the conditions described under procedure. It gave a good accuracy and precision Table 4.

Table 4- Application of the standard additions method and official methods for the determination of mouth wash containing Thymol

Mouth wash samples	[Thymol] depend on st. addition*	Standard additions method		Official method
		Recovery, %	RSD%	Recovery, %
Breath Rx	0.80	100.0	1.47	99.48
Listerine	0.81	101.25	1.93	100.86

***Standard additions**

Conclusions

The proposed method was found to be very simple, rapid, low cost, and fairly selective than some of the reported methods. They had an advantage of being accurate, did not require the removal of excipients, any chemical sample pretreatment, temperature control, pH control, solvent extraction step, and expensive reagents and solvents. The proposed method was applied to the analysis of Thymol in mouth wash and can be used for the routine analysis .

References:

1. British Pharmacopoeia on CD-ROM. **1998**. Version 5, 3rd Ed., Vol.1, Copyright by System Simulation Ltd., *The Stationery Office Ltd*., London,1288.
2. Szentandrassy, N. **2003**. Effects of thymol on cardiac and skeletal muscle. Ph.D.Thesis. Department of Physiology, Medical School, Medical and Health Science Center, University of Debrecen.
3. Solinas, V. and Gessa, C. **1981**. High-performance liquid chromatographic analysis of carvacrol and thymol in the essential oil of Thymus capitatus, *J. Chromatogr*, 219,pp: 332-339.
4. Rafiul, M.D., Ansari, S.H., Abdulkalam, N. and Kamran, J.N.**2012**. Validated HPLTC analysis method for quantification of thymol content in trachyspermum ammiand polyherbal unani formulation arq zeera, *Int J Pharm.Sci*, 4,pp: 478-484.
5. Poor, H.H., Shekarchi, M., Khanavi, M., Adib, N. and Amir, M. **2010** . A validated HPLC method for the analysis of thymol and carvacrol in thymus vulgarisL. volatile oil , *Pharmacognosy Magazine*, 6, pp:154-158.
6. Thompson, R.D. and Carlson, M**. 1989**. Determination of Thymol in halothane anaesthetic preparations by high-performance liquid chromatography, *J. Pharm. Biomed. Anal*., 7 pp:1199-1206.
7. Ji, L., Wang, Y.Y., Tong, Y, Li, X.D., Feng, X.F., Hang, L.Q. and Zhou, G.P. **2004**. Determination of carvacrol and Thymol in Mosla chinensis by HPLC, *Zhongguo Zhong Yao Za Zhi*, 29 pp: 1030-1032.
8. Kang, L.I., Jinsong, Y. and Weiwei, S.U. **2006**. Determination of Liquiritin, Naringin, Hesperidin, Thymol, Imperatorin, Honokiol, Isoimperatorin, and Magnolol in the Traditional Chinese Medicinal Preparation Huoxiang-zhengqi Liquid Using High-performance Liquid Chromatography, *Yakugaku Zasshi*, 126 pp:1185-1190.
9. Gao, H., Cao, W., Liang, Y., Cheng, N., Wang, B. and Zheng, J. **2010**. Determination of Thymol and Phenol in Honey by LC with Electrochemical Detection, *Chromatographia*, 72 pp: 316-363.
10. Glaudia, K., Gudrun, A., Eleonora ,S. and Markus, V.**2002**.Determination of thymol in human plasma by automated headspace solid-phase micro extraction-gas chromatographic analysis, *J. of Chromatogr.B,*767 pp:11-18.
11. Tran-Thi, N.T.,Herve, C. and Marie, F.G.**2006**. Deuterium/hydrogen ratio analysis of thymol,carvacrol, γ-terpinene and p-cymene in thyme savory and oregano essential oils by gas chromatography-pyrolysis-isotope ratio mass spectrometry, *J. of Chromatogr.A,*1142 pp:219-227.
12. Kohlert, C., Abel ,G., Schmid, E. and Veit ,M. **2002**. Determination of Thymol in human plasma by automated headspace solid-phase microextraction-gas chromatographic analysis, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 767 pp:11-8.

13. Tsigouri, A., Passaloglou-Katrali, M. and Sabatakou, O. **2008**. Determination of eucalyptol camphor menthol and Thymol in Greek thyme honey by GC-FID", Greek thyme honey by GC-FID, *Acta Alimentaria*, 37 pp: 181-189.

14. Badertscher, R., Kilchenmann, V., Liniger ,A. and Gallmann, P. **2010**. Determination of 1,4-dichlorobenzene, naphthalene and Thymol residues in honey using static headspace coupled with GC-MS, *J. ApiProduct & ApiMedical Sci*., 2 pp: 78-92.

15. Giovana, M.F., Pierina, S.B., Maria, P., Silvia, H.. and Pereira, A. **2013**. Determination of Thymol and Carvacrol in Plasma and Milk of Dairy Cows using Solid-Phase Microextraction, *J. Braz. Chem. Soc*., 24 pp: 837-846.

16. Lau, O.W., Luk, S.F. and Wong, W.C. **1988**. Simultaneous determination of methyl salicylate and Thymol in various pharmaceutical formulations by differential-pulse voltammetry using a glassy carbon electrode, *Analyst*,113 pp: 865-868.

17. Al-Neaimy, U.I.S. **2009**. Spectrophotometric determination of thymol in pharmaceuticals with Gibb's reagent, *J. Edu. and Sci*., 22 pp:125-136.

18. Al-Enizzi, M.S., Al-Sabha ,T.N. and A l-Ghabsha, Th. S. **2012**. Use of charge transe for complex formation reaction in spectrophotometric microdetermination of some drugs,*Jordan J. of Chem.*,7 pp:87-102.

19. Korany, M.A., Seif El-Din, A.A. and Abdel-Salam, N.N. **1984**. Application of second derivative ultraviolet spectrometry Part III : Determination of eugenol, Thymol and anethole in volatile oils, *Anal. Lett.,* 17 pp: 483.

20. Fibranz, L., Blake, M.I. and Miller, C.E. **1985**. Colorimetric determination of Thymol in thyme oil, *J. Am. Pharm. Asso*.,47 **pp:** 133–135.

21. Abou Basha, L. I., Rashed ,M. S. and Aboul-Enein ' H. Y. **1995**. TLC assay of thymoquinone in black seed oil (nigella sativa linn) and Identification of dithymoquinone and thymol, *J. of Liquid Chromatogr*.,18 pp:105-115.

22. Bazilko, A. and Strzelecka, H. **2000**.Quantitative determination of phenol derivatives from oleum thyme,*Chromatographia*,52 pp:112-114.

23. Al-Abachi, M.Q. and Al-Ward, H.S. **2012**. Batch and flow-injection spectrophotometric determination of thymol using procaine hydrochloride as a new chromogenic reagent, *J. Baghdad for Sci.*, 9 pp: 302-310.

24. Al-Abachi, M.Q. and Subhi, S. **2013**. Flow injection spectrophotometric determination of salbutamol sulphate and pyridoxine hydrochloride using 2,4-dinitrophenylhydrazine, *Iraqi J. of Sci.*,54 pp:6-16.

25. Al-Abachi, M.Q. and Al-Gabsha, T.S. **1983**. *Fundamentals of analytical chemistry*, Press of Mousl University, pp: 343 – 346.

26. Sanders, D.H. and Murph, A..F. **1976**. *Statistics*, Mc.Graw-Hill, New York.

27. Bigley, F.P. and Grob, R.L. **1985**. Determination of phenols in water and wastewater by post-column reaction detection high-performance liquid chromatography, *J. Chromatogr*, 350 pp: 407-416.