Micro Determination Study and Organo physical properties Of Resorcinol with Thiamine in the Presence of Potassium Dichromate.

دراسة تقديرية دقيقة للخواص (الفيزيائية والعضوية) لـ الريزورسينول مع الثيامين في وجود ثنائي كرومات البوتاسيوم .

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الخلاصة :
الهدف : تهدف الدراسة إلى تقدير الريزورسينول (٣- هيدروكسي فينول).
المنهجية : دراسة تم من خلالها تطوير طريقة بسيطة وذات حساسية و انتقائية لتقدير الريزورسينول (٣- هيدروكسي فينول) وتعتمد هذه الطريقة على تفاعل الريزورسينول (٣- هيدروكسي فينول) مع الثيامين (فيتامين ب١) وثنائي كرومات البوتاسيوم عند الدالة الهيدروجينية ٣.٥. بحيث أعطت التفاعلات نواتج ملونة ذائبة في الماء ولها أقصى امتصاص عند الطول الموجي ٥١٠ نانومتر .
النتائج : أظهرت هذه الدراسة أن معامل الامتصاص المولاري لـ ٣- هيدروكسي فينول هو (٠.١٤٥ X ١٠ ^٤ لتر.مول^-١.سم^-١) وكذلك كانت العلاقة بين الامتصاص عند الطول الموجي الأقصى والتركيز خطية في مدى التركيزات بين (١- ٩مايكروغرام\مل) لـ ٣- هيدروكسي فينول والدقة (نسبة الخطأ أفضل من ٠.٢-%) أما نتائج التكرارية (الانحراف القياسي النسبي أفضل من ١.٢%). وكانت النسبة المولية للناتج الملون هي ١:١ـ (٣- هيدروكسي فينول : ثيامين) وبلغ ثابت الاستقرارية للمعقد المتكون تحت الظروف المثلى عند درجة حرارة الغرفة (٣.٢٥ X ١٠^٥ لتر.مول^-١).
التوصيات : توصي الدراسة بتعيين المركبات الفينولية أو المشتقات الفينولية الأخرى بالاعتماد على هذه الطريقة أي استخدام الثيامين والعامل المؤكسد نفسه (ثنائي كرومات البوتاسيوم).
مفتاح الكلمات : الثيامين، الريزورسينول، ثنائي كرومات البوتاسيوم، القياس الطيفي.

Abstract:
Objective: This study aims to estimate Resorcinol (3- hydroxy phenol).
Methodology : The study was through the development of a simple and high sensitivity and selectivity to estimate Resorcinol (3- hydroxy phenol) and The method is based on the reaction of Resorcinol (3-hydroxyphenol) with Thiamine (Vitamin B_1) and potassium Dichromate at pH 3.5. So The reactions gave an intense water soluble color products that have maximum absorption at 510 nm.
Results: This study showed that the ε max for 3- hydroxy phenol is (0.145 $X10^4$ liter. $Mol^{-1}.cm^{-1}$), A linear correlations (1-9 µg ml^{-1}) for 3-hydroxyphenol compound was found between absorbance at λmax and concentration. The accurate (relative error was better than -0.2 %) and precise (RSD was better than 1.2%). The colored product was found to be 1:1 (3-hydroxyphenol : Thiamine) . The stability constant of the reaction under optimized conditions at room temperature was 3.25 $X10^5$ $L.mole^{-1}$.
Recommendations: The study recommends the appointment of phenolic compounds or other phenolic derivatives are relying on this method any use of thiamine and oxidized worker himself (potassium dichromate).
Key words: Thiamine, Resorcinol, potassium Dichromate, Spectrophotometry

INTRODUCTION:

Resorcinol is the 1,3-isomer of benzenediol with the formula C6H4(OH)2. Benzene-1,3-diol is the name recommended by the International Union of Pure and Applied Chemistry (IUPAC) in its 1993 Recommendations for the Nomenclature of Organic Chemistry.[1] It is produced when any of a large number of resins (e.g., galbanum, asafoetida, etc.) are melted with potassium hydroxide, or by the distillation of Brazilwood extract. It may be prepared synthetically by melting 3-iodophenol, phenol-3-sulfonic acid, or benzene-1,3-disulfonic acid with potassium carbonate; by the action of nitrous acid on 3-aminophenol or on 1,3-diaminobenzene.[2] Many ortho- and para-compounds of the aromatic series (for example, the bromophenols, benzene-para-disulfonic acid) also yield resorcinol on fusion with potassium hydroxide. Resorcinol is one of the main natural phenols in argan oil.[3] Its Used externally, it is an antiseptic and disinfectant, and is used 5 to 10% in ointments in the treatment of chronic skin diseases such as psoriasis, hidradenitis suppurativa, and eczema of a sub-acute character. It is present in over-the-counter topical acne treatments at 2% or less concentration, and in prescription treatments at higher concentrations.[4] In large doses, it is a poison, causing giddiness, deafness, salivation, sweating, and convulsions. It is also worked up in certain medicated soaps. Monoacetylresorcinol, C6H4(OH)(O-

COCH3), is used under the name of euresol.[5] Resorcinol is also used as a chemical intermediate for the synthesis of pharmaceuticals and other organic compounds. It is used in the production of diazo dyes and plasticizers and as a UV absorber in resins. An emerging use of resorcinol is as a template molecule in supramolecular chemistry. The -OH groups on resorcinol form hydrogen bonds to target molecules, holding them in the proper orientation for a reaction. Many such reactions are able to be carried out in the solid state, thereby reducing or eliminating the use of solvents that may be harmful to the environment. (see Green chemistry).

Thiamine is a colorless compound with a chemical formula C12H17N4OS. Its structure contains a aminopyrimidine ring and a thiazole ring with methyl and hydroxyethyl side chains linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether, chloroform, and benzene. It is stable at acidic pH, but is unstable in alkaline solutions.[6][7] Thiamine in foods can be degraded in a variety of ways. Sulfites, which are added to foods usually as a preservative,[8] will attack thiamine at the methylene bridge in the structure, cleaving the pyrimidine ring from the thiazole ring.[9] The rate of this reaction is increased under acidic conditions. Thiamine is degraded by thermolabile thiaminases (present in raw fish and shellfish[6]). Some thiaminases are produced by bacteria. Bacterial thiaminases are cell surface enzymes that must dissociate from the membrane before being activated; the dissociation can occur in ruminants under acidotic conditions. Rumen bacteria also reduce sulfate to sulfite, therefore high dietary intakes of sulfate can have thiamine-antagonistic activities. Plant thiamine antagonists are heat-stable and occur as both the ortho- and para-hydroxyphenols. Some examples of these antagonists are caffeic acid, chlorogenic acid, and tannic acid. These compounds interact with the thiamine to oxidize the thiazole ring, thus rendering it unable to be absorbed. Two flavonoids, quercetin and rutin, have also been implicated as thiamine antagonists.[9] Thiamine derivatives and thiamine-dependent enzymes are present in all cells of the body, thus a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism.[10] Thiamine deficiency commonly presents subacutely and can lead to metabolic coma and death. A lack of thiamine can be caused by malnutrition, a diet high in thiaminase-rich foods (raw freshwater fish, raw shellfish, ferns) and/or foods high in anti-thiamine factors (tea, coffee, betel nuts)[11] and by grossly impaired nutritional status associated with chronic diseases, such as alcoholism, gastrointestinal diseases, HIV-AIDS, and persistent vomiting.[12] It is thought that many people with diabetes have a deficiency of thiamine and that this may be linked to some of the complications that can occur.[13],[14]

Oxidative coupling reactions have been long used for the determination of many drugs such as amoxicillin[15], folic acid[16] , sulphonamide[17] and phenols[18],[19]. Spectrophotometric methods often suffer form limitations in sensitivity and selectivity but are widely used due to both the resulting experimental rapidity and simplicity. Therefore the objective of the investigation reported in this paper was to evaluate a spectrophotometic determination of 3-hydroxyphenol with vitamin B_1 in the presence of potassium dichromate **.**

EXPERIMENTAL:

Apparatus:

All spectral and absorbance measurements were carried out on a shimadzu UV-visible 1700 double beam spectrophotometer using 1cm glass cells. A digital pH meter was used for pH measurements. All Kinetic measurements were made on TRUV 754 UV-visible spectrophotometer.

REAGENTS:

All chemicals used were of analytical grade supplied from B.D.H and Fluka companies. Standard 3-Hydroxyphenol solution ($4.54X10^{-4}$) was prepared by dissolving 0.0025g of 3-Hydroxyphenol in 50 ml of distilled water. Standard solution of 3-Hydroxyphenol were prepared by simple dilution of the appropriate volume of the standard 3-Hydroxyphenol ($4.54X10^{-4}$) with distilled water.

VITAMIN B1 (1X10-3M):

0.084g of Vitamin B_1 was dissolved in 250ml of distilled water.

POTASSIUM DICHROMATE SOLUTION (0. 1M):

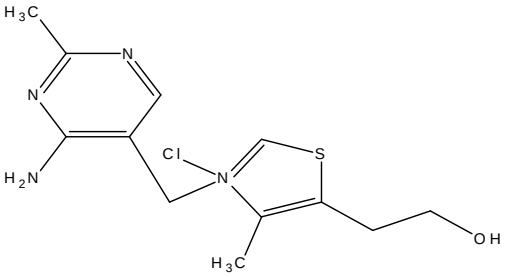
1.4710g of potassium Dichromate was dissolved in 50 ml of distilled water .

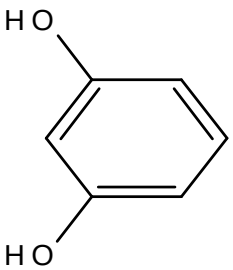
GENERAL PROCEDURE:

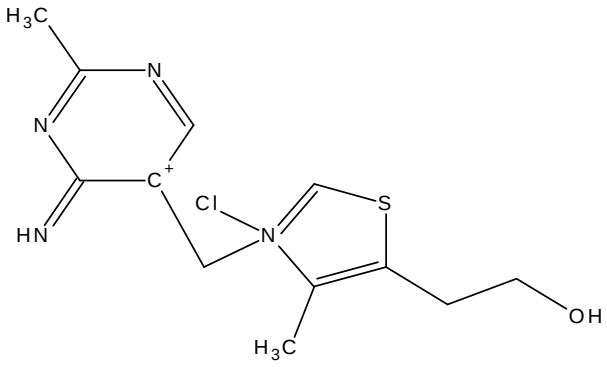
An aliquot of samples containing 10-100 µg of 3-Hydoxyphenol was transferred into a series of 10 ml standard flasks. A volume of 3 ml (1×10^{-3}M) Vitamin B_1 solution, 1 ml of 0.1M of potassium dichromate and 1 ml of H_2SO_4 were added. The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 7min. The absorbance was measured at 510 nm for 3-Hydoxyphenol against reagent blanks containing all materials except 3-Hydoxyphenol for determination of 3-Hydoxyphenol.

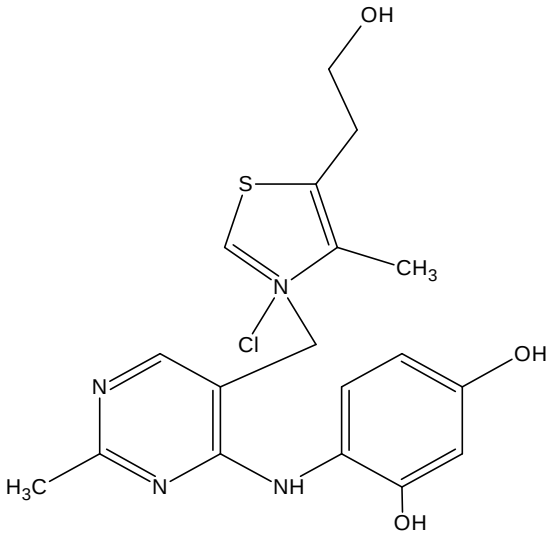
REACTION MECHANISM OF THE METHOD:

3-Hydroxyphenol form colored products with Vitamin B_1 in the presence of potassium dichromate in acidic medium. Under the reaction conditions, Vitamin B_1 upon oxidation with potassium dichromate loses two electrons and one proton, forming an electrophilic intermediate which is an active coupling species. The intermediate undergoes electrophilic substitution with the phenolic moieties of 3-Hydroxyphenol to from a colored product [17] according to scheme 1.









Scheme 1 : proposed mechanism of the reaction 3-Hydroxyphenol with Vitamin B_1 .

RESULTS AND DISCUSSION:

The result of this investigation indicated that the reaction between 3-Hydroxyphenol with Vitamin B_1 in the presence of potassium Dichromate and sulfuric acid in the pH 3.5 yield highly soluble colored condensation products which can be utilized as a suitable assay procedure for 3-Hydroxyphenol. This colored product have maximum absorption at 510 nm The blank at this wave length shows zero absorbance Fig (1) .

The influence of various reaction variables on the color development was tested to establish the most favorable conditions and these are :

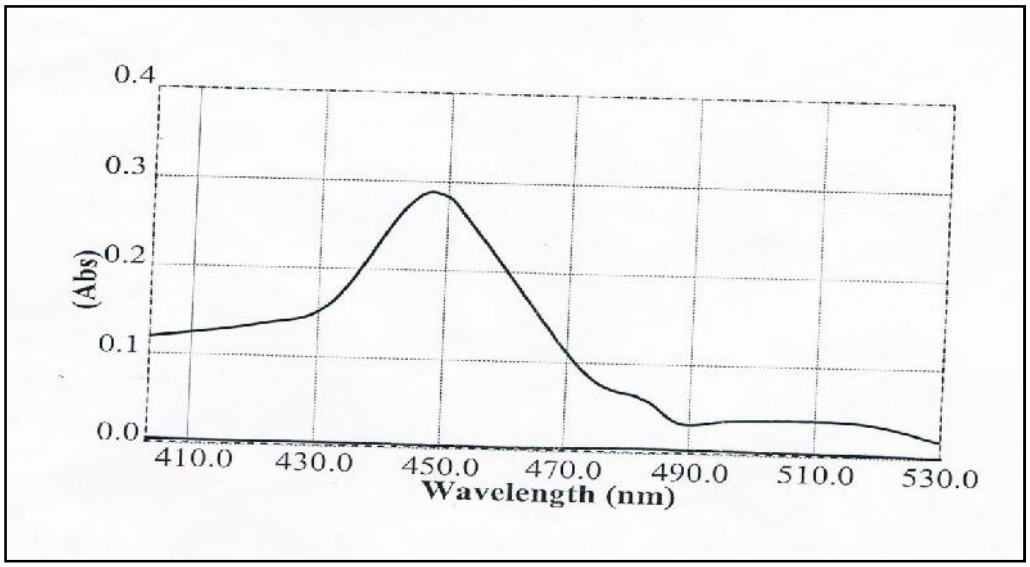


Fig.1 : (A) Absorption spectra of reagent blank with potassium dichromate.

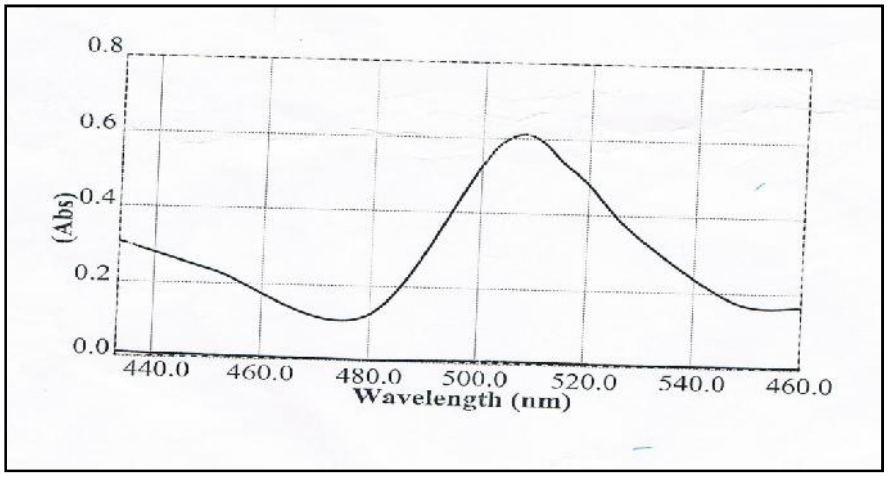


Fig.1 : (B) Absorption spectra of 3-hydroxyphenol complex in the presence of potassium dichromate.

OPTIMIZATION OF REAGENT CONCENTRATION:

The effect of various concentrations of Vitamin B_1 were investigated. 3 ml of (1×10^{-3}M) for 3-Hydroxyphenol was found necessary for developing the colored products and increase their stability Fig 2 .

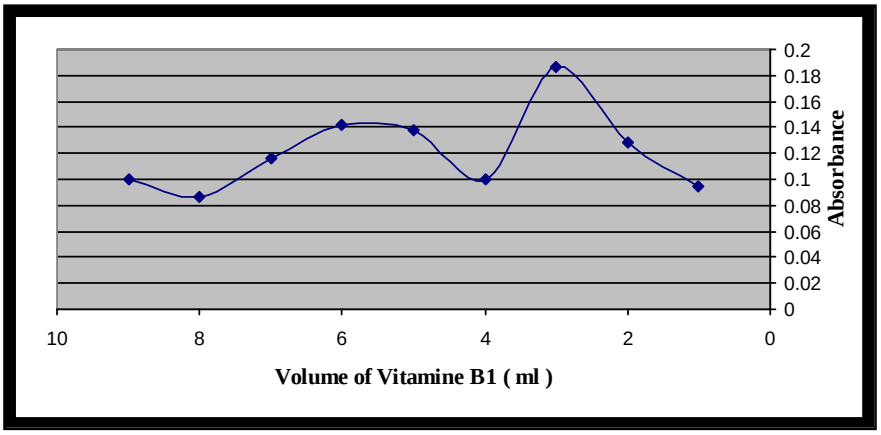


Fig 2: Effect of the reagent volume

EFFECT OF OXIDANT CONCENTRATION :

Various concentrations of potassium dichromate solutions were added to a fixed amount of 3-Hydroxyphenol, 1 ml of (0. 1M) potassium dichromate was used in the procedure since it gives high sensitivity Fig 3.

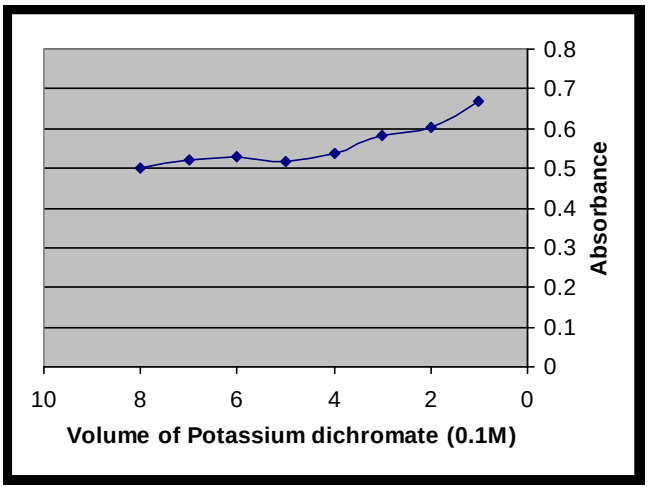
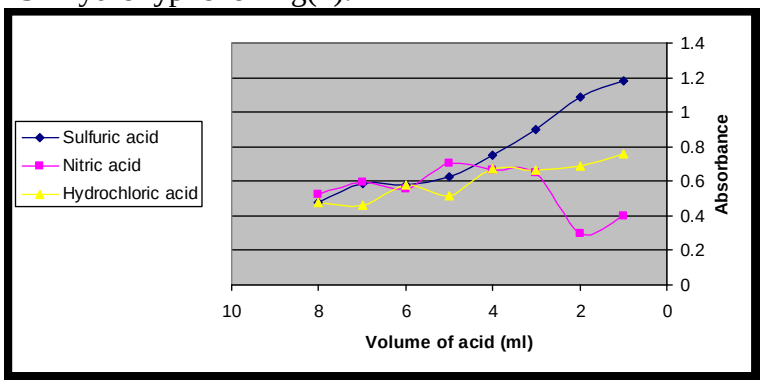


Fig 3: Effect of the oxidizing agent volume.

EFFECT OF ACIDS :

It was found experimentally that the colored product was formed only in acidic medium. Different acids were examined these include Hydrochloric, Sulfuric and Nitric acid only Sulfuric acid was found optimum since it gives a high sensitivity, minimum blank value and high stability of the colored product . The effect of the

Amount of Sulfuric acid was also tested and 1 ml of 0.1M was selected used indetermination of 3- Hydroxyphenol Fig(4).



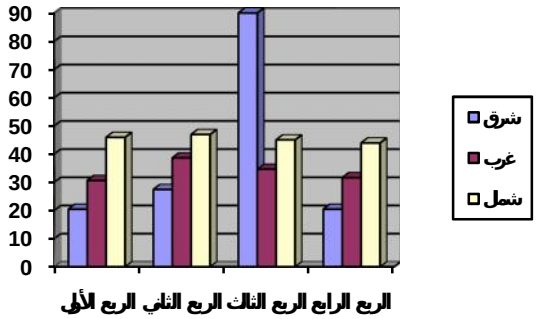


Fig 4: Effect of acids volume in the 3-Hydroxyphenol.

CALIBRATION CARVES :

The calibration curves were constructed at their respective absorption maxima and these were linear over concentration range at optimum conditions as listed in Table.1 for

phenolic compound. The molar absorptivity are given in table 1. In this method , Beer's law is obeyed in the inverse manner . The calibration graphs are described by the equation : Y = a + bX (where Y = absorbance , a = intercept , b = slope and X = concentration in µg / ml) obtained by the method of least squares with correlation coefficient , (r) 0.9960 and standard deviation of intercept 0.018.

Table 1 : Analytical data of determination of 3-Hydroxyphenol.

Characteristic	Resorcinol
Absorption maxima (nm)	510
pH	3.5
Beer's 1aw range (µg/ml)	(1-9)
Molar absorptivity ($L.mol^{-1}cm^{-1}$)	0.145×10^{4}

ORDER OF ADDITION OF REAGENTS:

To obtain the optimum results, the order of addition of reagents should be followed as given by the procedures, otherwise, a loss in color intensity and stability are observed.

DEVELOPMENT TIME AND STABILITY PERIOD:

The color intensity reached maximum after Resorcinol had been reacted with Thiamine in the presence of potassium dichromate solutions for 7 min. The color obtained was stable for at least 2hr and this stability, period was sufficient to allow several measurements to be performed sequentially.

COMPOSITION OF THE COMPLEX:

The composition of the complex was studied by the mole ratio method. A break of 1:1 suggested the formation of 3-Hydroxyphenol with Thiamine Fig(5).The apparent stability constant was calculated by comparing the absorbance of solution containing stoichiometric amounts of 3- Hydroxyphenol with that of a solution containing a five-fold excess of Thiamine reagent. The average conditional stability constants of the dyes in water, under the described experimental conditions is 3.25×10^{5} L. $mole^{-1}$.

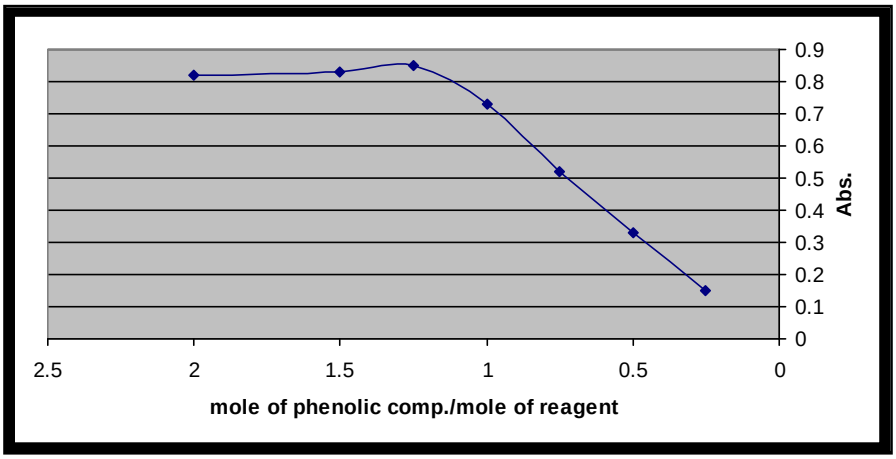


Fig 5: Mole ratio of the 3-Hydroxyphenol complex.

INTERFERENCES :

The effect of diverse ions on the determination of 3- Hydroxyphenol were studied in detail. To test of diverse ions were determined by the general procedure, in the presence of their

respective ions . 3- Hydroxyphenol can be determined with out serious interferences in the presence of a 10 fold excess of cations Tables 2.

Table (2) : Effect of foreign ions.

Foreign ions	Amount added p.p.m	3- Hydroxyphenol E%
Na^{+}	10	+0.03
K^{+}	10	-0.02
Ca^{++}	10	-0.13
Mg^{++}	10	+0.11
Mn^{++}	10	0.00
Cr^{++}	10	+0.07
Ni^{++}	10	-0.05
Cu^{++}	10	+0.10
Zn^{++}	10	+0.08

ACCURACY AND PRECISION :

To determine the accuracy and precision of the method, 3- hydroxyphenol was determined at four different concentration. The results are shown in Table 3 indicate that satisfactory precision and accuracy could be attained with the proposed method .

Table(3) : Accuracy and precision of the method .

Amount of 3-Hydroxyphenol taken ppm	%E of 3-hydroxyphenol	* %RSD of 3-Hydroxyphenol
``	+0.18	1.20
4	-0.09	0.65
6	-0.12	0.82
8	-0.20	1.63

* for five determinations.

CONCLUSIONS:

The present study demonstrates an excellent approach for the development of spectrophotometric method for determination of 3-hydroxyphenol high selectivity and excellent sensitivity for the oxidative coupling reaction of 3-hydroxyphenol is achieved with thiamine.

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