Spectrophotometric Determination of Some Phenolic Compounds by Formation of Copper(II) Complexes

Spectrophotometric Determination of Some Phenolic Compounds by Formation of Copper(II) Complexes

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Abstract

Simple and sensitive spectrophotometric method is described for the determination of phenolic compounds in both in pure form and in pharmaceutical preparations. The method is based on the formation of a new ligand from the reaction between 4-aminoantipyrine with phenolic compounds and then reacts with copper (II) to give a colored complex at room temperature. The maximum absorbance of the prepared complexes were measured at 450,500 and 480 nm for pyridoxine, resorcinol and phloroglucinol complexes respectively. Beer's law was obeyed in the concentration range of 1.5-20, 2.5-30 and 2.0-25 μg/ml^{-1}, the molar absorptivity values are 2.4778 x 10^4,1.6740x10^4 and 1.7001 x 10^4 L mol^{-1}cm^{-1} , the Sandal sensitivity values are 0.0501x10^{-3},0.1740x$10^{-3}$and 0.1228x10^{-3}μg cm^{-2} for Pyridoxine, resorcinol and phloroglucinol complexes respectively. The correlation coefficients were 0.9999, 0.9998 and 0.9999, the limits of detection(LOD) were 0.47793, 0.15125 and 0.01434μg ml^{-1} , the limits of quantification were (LOQ) 1.4482, 0.45833 and 0.04347 for pyridoxine, resorcinol and phloroglucinol complexes respectively. The stoichiometry of the complexes formed (1:2) was determined by Job's continuous variations method and molar ratio method. Furthermore the stability constant (K) and Gibbs free energy (ΔG) for the complexes were also calculated. The proposed methods were applied successfully for the determination of phenolic compounds in commercial tablets.

Keywords: 4-Aminoantipyrine; Copper(II)Complexes; Spectrophotometric determination, Phenolic compounds

1. Introduction:

Phenolic compound, are a class of chemical compounds consisting of a hydroxylgroup (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid COH. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule.[1] Some phenolic compounds are believed to be cancer chemopreventive, compounds that may decrease your risk of developing cancer. Epigallocatechin-3 gallate, for example, is a phenolic compound found in green tea and believed to be a cancer chemopreventive. [2,3]

A broad group of phenolic compounds called flavonoids are common in plants; according to a review in the "British Journal of Nutrition," there is evidence to suggest many flavonoids like anthocyanin may have anticancer effects. Phenolic compounds that act as antioxidants might also help promote healthy aging by minimizing DNA damage caused by free radicals[4].

As noted in a 2002 review in the journal "Free Radical Biology and Medicine," there is evidence to suggest some of the deterioration associated with aging is caused by oxidative damage to DNA; this hypothesis is called the oxidative stress or free-radical theory of aging.

According to these important of these phenolic compounds, we interested with three phenolic compounds, resorcinol, pyridoxine and phloroglucinol. Resorcinol is also used as a chemical intermediate for the synthesis of pharmaceuticals and other organic compounds it is also externalused, it is an antiseptic and disinfectant, and is used 5 to10% in ointments in the treatment of chronic skin diseases such as psoriasis,hid adenitissupportive, and eczema of a sub-acute character[5].Pyridoxine is used to treat or prevent vitamin B6 deficiency. It is also used to treat a certain type of anemia (lack of red blood cells) [6].

Pyridoxine injection is also used to treat some types of seizure in babies[7].Phloroglucinol is an organic compound that is used in the synthesis of pharmaceuticals and explosives. It is a phenol derivative with antispasmodic properties that is used primarily as a laboratoryreagent[8-9].

Different analytical methods have been used in the literature for the analysis of phenolic compound including, quantitative determinationsfluorometry[10], ion pair chromatography [11], High-performance liquid chromatography [12-13], flow Injection analysis with chemiluminescence detection [14,15].

The present research aims to developing a sensitive, simple and accurate spectrophotometric method for the determination of resorcinol, pyridoxine and phloroglucinol based on their coupling with 4-aminoantipyrine (4-AAP) to give a new ligand that reacts with copper (II) to give intense bright colored chelate. The method was applied for determining these compounds in pure and pharmaceutical formulations as tablet.

2. Experimental

2.1. Apparatus

Spectral and absorbance measurements were made with a Jena Model 1100, UV-Visible spectrophotometer(Germany) in Pharmaceutical Chemistry Department, College of Pharmacy, University of Basrah, Iraq .It was equipped with a quartz cell with a 10mm path length. The pH measurements are performed using HANNA PH 211 pH meter. E. Meter electrical balance is used for weighting the sample.

2.2. Reagents and solutions

All chemicals were of analytical reagent grade from Merck (Germany)and BDH. All standard and sample solutions were made up with double deionized water.

a. 4-Aminoantipyrine reagent solution (1.0%):

1.0 g of reagent was dissolved in 20 ml of ethanol then complete to 100 ml in a volumetric flask with distilled water.

b. Copper sulphate reagent solution(0.1%):

This solution was prepared by diluting 0.1g of copper sulphate (BDH) with distilled water in 100 ml volumetric flask.

c. Sodium nitrite solution (1% w/v):

1 g of sodium nitrite was dissolved in 20 ml of distilled water then complete to 100 ml in a volumetric flask.

d. Sodium hydroxide solution (0.05M):

0.2 g of sodium hydroxide was dissolved in 20 ml of distilled water then complete to 100 ml in a volumetric flask.

e. Phenolic compounds solutions:

A 1000 $\mu g.ml^{-1}$solution of Phenolic compounds Pyridoxine,resorcinol and Phloroglucinol(provided from Sammara drug industries;SDI, Iraq) is prepared by dissolving 0.01g of Phenolic compounds in small amount of ethanol (20 ml)and complete to 100 mla volumetric flask with distilledwater, the solution was stored in amber coloredbottle and kept in refrigerator.Working standard of Phenolic compounds solutions were prepared by simple dilution of the appropriate of the compound in distilled water completing the volume in a volumetric flask.

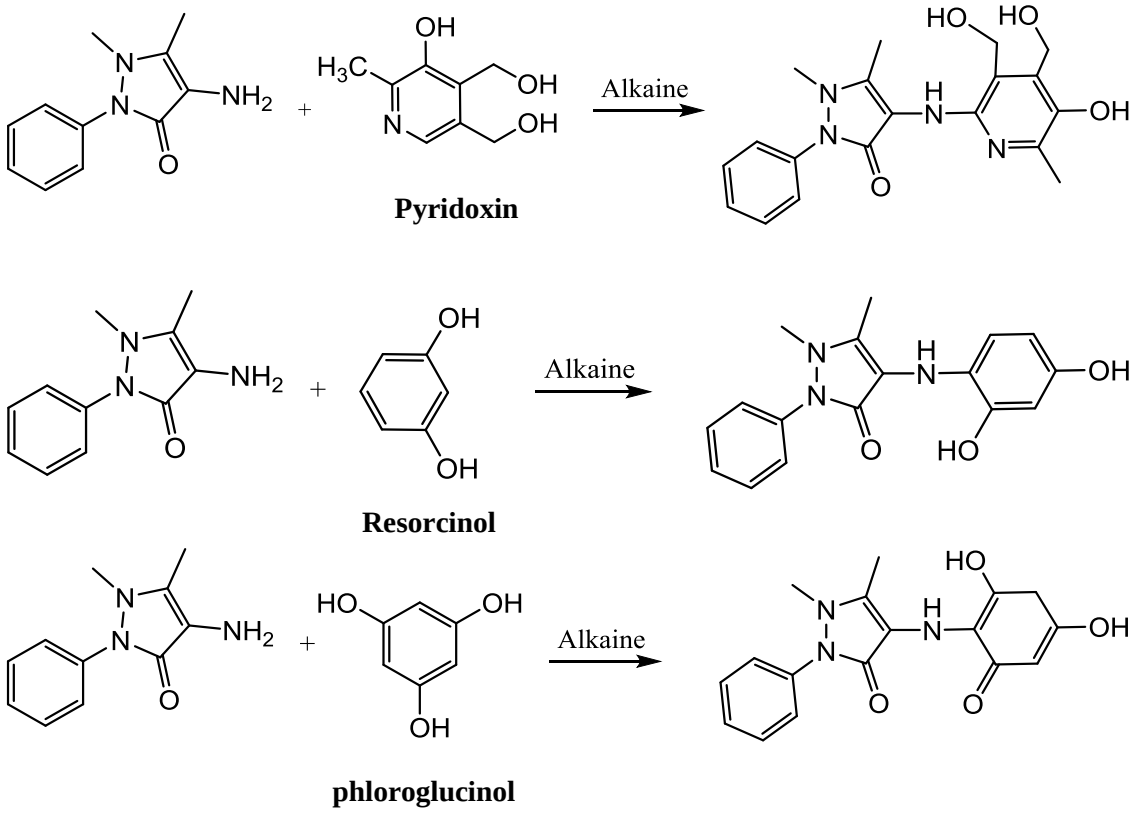
f. Preparation of tablet/capsule sample solution

Twenty tablets of Pyridoxine drug(samaitB6,Tablets SDI,Iraq,100mg)wereweighted, powdered and mixed thoroughly. Similarly, ten capsule of Pyridoxine,were carefully evacuated, and mixed. A quantity equivalent to 10 mg of each drug was transferred to 100 mL volumetric flask. The drugs were dissolved in water, shaken well, and made up to the volume with water. The resultant solutions were filtered and analyzed as described under general procedure.

2.3. General procedure[16]

Accurately measured suitable volume of Pyridoxine, Resorcinol and phloroglucinolwere transferred from working solution to 10 ml volumetric flasks, which could be diluted quantitatively to obtain 1.5-20, 2.5-30 and 2.0-25μg/mL for Pyridoxine, Resorcinol and phloroglucinol, respectively. To each flask containing drugs in the order mentionedabove, 1.0mL of 4-Aminoantipyrine reagent (1.0%) and 0.8 mL of copper sulphate (0.1%) wereadded in alkaline medium, as shown in Scheme (1).After10min with string,the red color hence developed was further make up the solution to 10 ml

with distilled water, the absorbance values were measured at 450,500 and 480nm against the reagent blank.



Scheme (1) Coupling reaction between phenolic compounds and 4-aminoantipurine

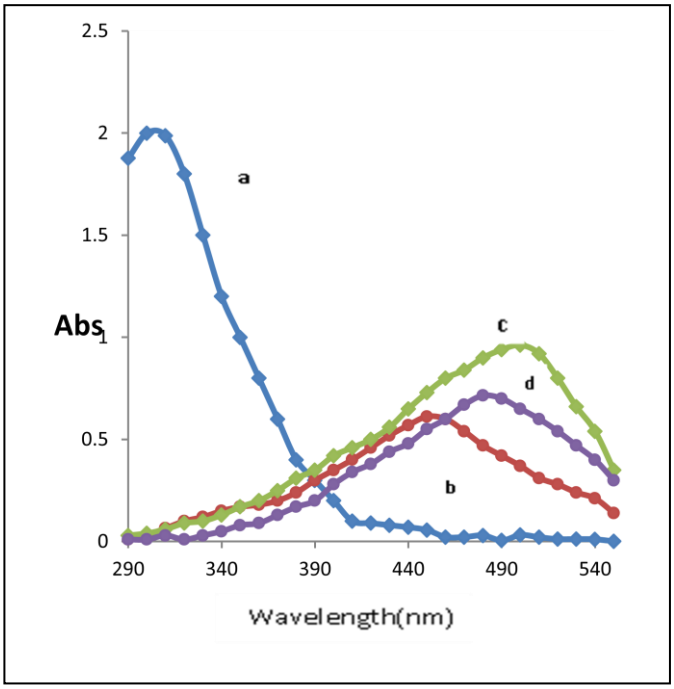
3. Results and Discussion

3.1. Optimization of Variables

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant.

3.2. Absorption spectra

The absorption spectrum of colored product show an absorption bands at 480, 500and 450 nm for Pyridoxine, Resorcinol and phloroglucinol, respectively. Whereas, the reagent blank give no absorption atthis wavelength (Fig .1).



Fig(1).Absorption spectra of (a) reagent blank against distilled water under optimum conditions,(b)B6 (20 µg/ml)complex,(c)Resorcinol(40µg/ml);(c) phloroglucinol (30 µg/ml)with 4-AAP and Cu(II) against reagent blank andEffect of 4-Aminoantipyrine reagent concentration

When various concentration of 4-Aminoantipyrine was added to afixed concentration of Pyridoxine, Resorcinol and phloroglucinol drugs, It was found that absorbance increases with increasing 4-AAP concentration and reached its maximum value on using 1.2 ml %1concentration of 4-AAP at maximum absorption 450,500 and 480nmof the Pyridoxine, Resorcinol and phloroglucinol, respectively (Fig.2).

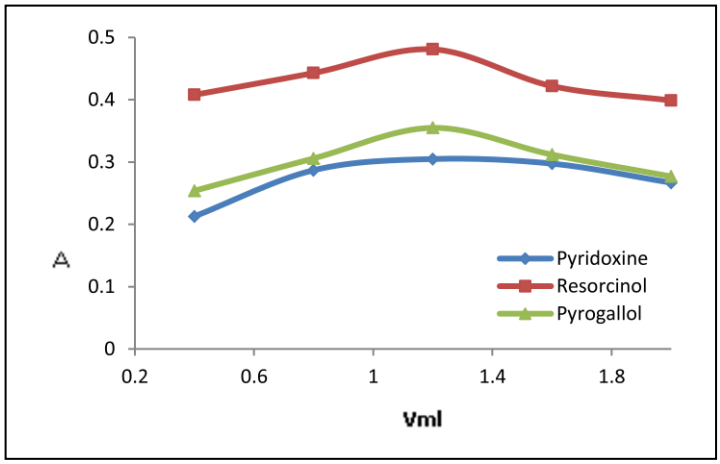


Fig.2:Effect of 4-Aminoantipyrine reagent volume on absorbance of complexes

3.3. Effect of Copper Sulphate Concentration

To obtain the optimum results, the amount of copper sulphate was studied. Various concentration of copper sulphate was added to fixed of 4-aminoantipyrine 1.0% and 0.05M Sodium hydroxide.0.8ml of 0.1% of $CuSO_4.5H_2O$ solution were added to Pyridoxine, and phloroglucinol mixture was sufficient to develop the color to its full intensity and gave minimum blank value and 1ml of 0.1% of $CuSO_4.5H_2O$ solution were added to Resorcinol solution sufficient to develop the color, above 1 ml, the absorbance of the blank value was increased causing a decrease in the absorbance of the sample was used in all subsequent experiments (Fig.3).

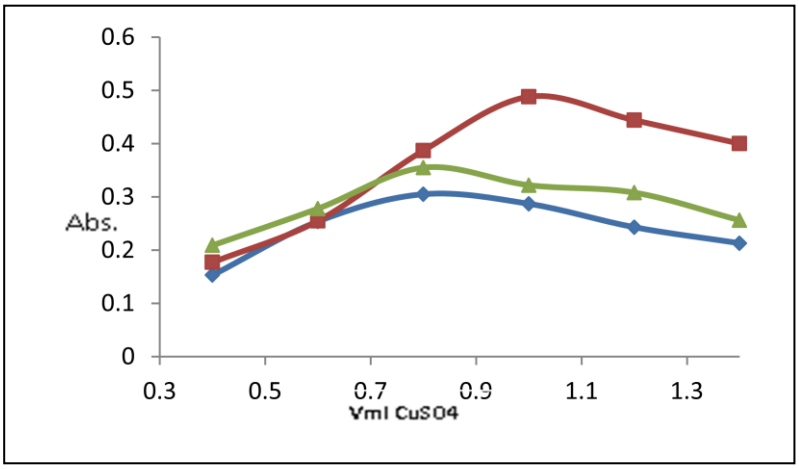


Fig.3.Effect of $CuSO_4$ $5H_2O$ concentration on the absorbanceof 10,20and 15 µg/mlfor Pyridoxine, Resorcinol and phloroglucinol, respectively in the presence of 4-AAP.

3.4. Effect of time on the complex formation

The results show that the complexes produced was stable between (5-45) minutes, absorbance value stable. The results were shown in fig.(4) . It is evident from (Fig4) that the formation of stable colored complexes for phenolic compounds was achieved after 15 min.

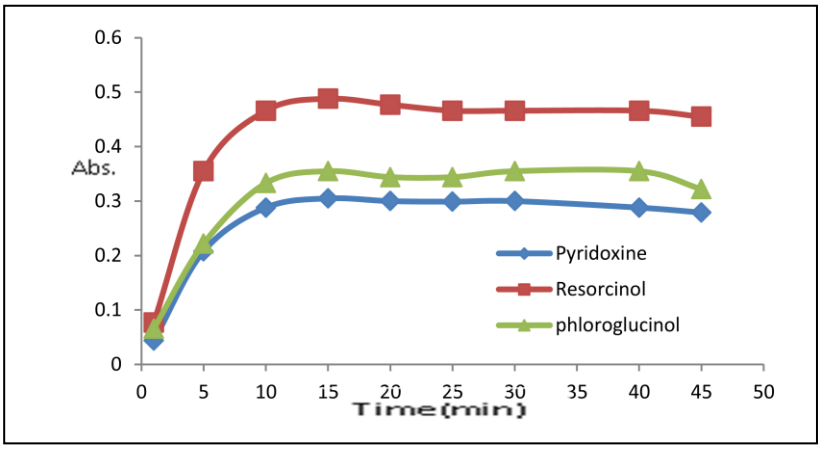


Fig.(4):Effect of time on the complex formation

3.5. Effect of temperature

The resulting complexes of the proposed method was studied at room temperature (25 ˚C), the absorbance values remain constant. Further heating of the solutions tends to decrease the absorbance. This is probably due to the dissociation of the complexes formed or the auto-degradation of the analysts, assisted by overheating.

3.6. Order of addition of Reagents

To obtain the optimum results, the order of addition of reagents should be followed as given by the procedure, otherwise, a loss in color intensity and stability are observed. The optimum concentration was chosen for complex solution gave rise to a constant (λ max).Table(1)shows the sequences of addition[17].

Table 1: Effect of order of addition on absorbance value of complex at temperature (25 ˚C)

Sequence of addition	Abs. of Pyridoxine phenolic drug	Abs. of Resorcinol phenolic drug	Abs. of phloroglucinolp henolic drug
phenolic drug - 4AAP - Cu(II)	0.309	0.588	0.355
4AAP - phenolic drug -Cu(II)	0.300	0.580	0.348
4AAP - Cu(II) - phenolic drug	0.298	0.567	0.342
Cu(II) - phenolic drug-4AAP	0.299	0.555	0.339

3.7. Effect of pH

The influence of pH on the intensity of the color reaction was also studied at pH range (5-10) and the absorbance- pH curves for complexes formation measured at certain (λ_{max}) were plotted. Figure(5) shows a selective pH- absorbance curves.It was found that the chelating complex was formed at pH7-8. The plateaus of the curves represent the completion of the reaction and consequently represent the optimum pH. Hence further analytical investigations were carried out in media of pH 7.However; the aqueous solution(pH7) has been used to studied and recommended in the subsequent experiments (Fig.5).

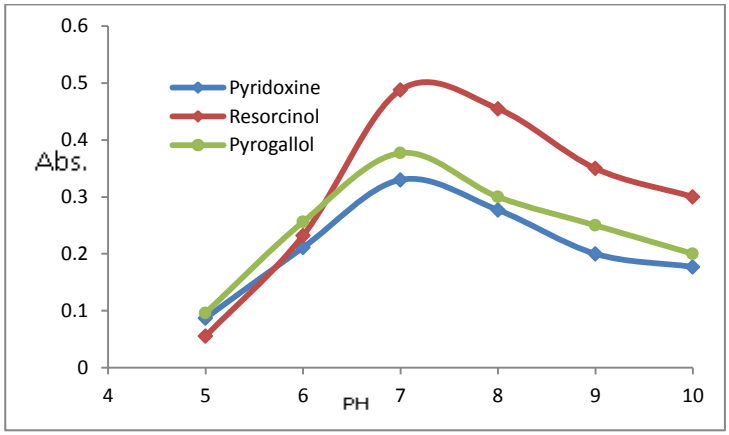


Fig.5:Effect of pH on the absorption intensity of PEH (25 µg/ ml)-4-AAP-Cu(II) complex.

3.8. Analytical characteristics of the method

Calibration graphs were constructed using standard solutions under optimum experimental condition. A linear relationship was observed between the absorbance and concentration of drugs from 1.5-20,2.5-30and2-25µg/ml for Pyridoxine, Resorcinol and phloroglucinol drugs respectively. The molar absorptivity and Sandal's sensitivity for each drug were calculated from beer's law. Figures(6-8) and Table(2)summarized the analytical parameters and the results of statistical analysis of the experimental data: regression equation computed from calibration graph, correlation coefficient (r), detection limit and quantitation limit. The high value of correlation coefficient for the proposed method indicated excellent linearity[18].

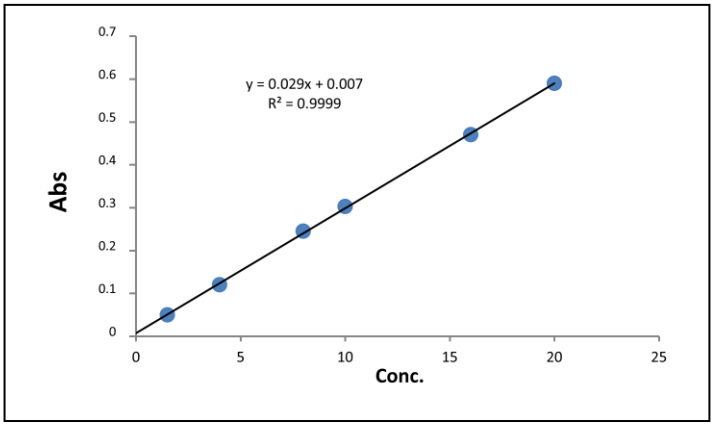


Fig. (6): Calibration curve for the determination of Pyridoxine

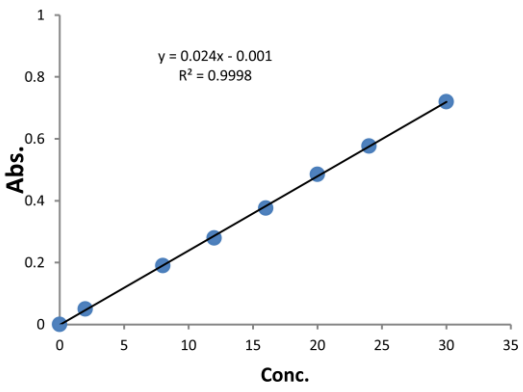


Fig. 7: Calibration curve for the determination of resorcinol

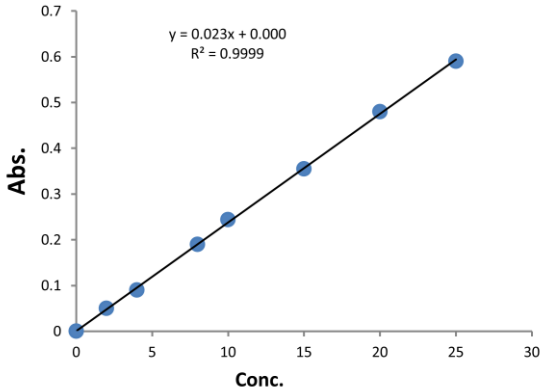


Fig. 8: Calibration curve for the determination of phloroglucinol

Table(2):Optical characteristics for the determination of Pyridoxine,Resorcinol and phloroglucinol drugs

Parameters	Value		
	Pyridoxine	Resorcinol	Phloroglucinol
Measurement wavelength (nm)	450	500	480
Linear range (µg ml^{-1})	1.5-20	2.5-30	2.0-25
Intercept	0.007	0.001	0.0001
Slope	0.029	0.024	0.023
Standard deviation	0.0042	0.0011	0.0001
Correlation coefficient (R^2)	0.9999	0.9998	0.9999
Limit of detection, LOD (µg ml^{-1})	0.47793	0.15125	0.01434
Limit of quantification, LOQ (µg ml^{-1})	1.4482	0.45833	0.04347
Molar absorptivity, e (1 mol^{-1} cm^{-1})	$2.4778x10^4$	$1.6740x10^4$	$1.70019x10^4$
Sandal sensitivity	$0.0501x10^{-3}$	$0.1740x10^{-3}$	$0.1228x10^{-3}$

3.9. Structure of the complex

The composition of the complexes formed in solution was determined by studying the complex formation in solution by mole ratio and job method sunder the optimum condition employed to obtain maximal and constant absorbance(λ_{max}). The results show that 1:2 Cu(II) to ligand complexes to all complexes were formed In the results reveals (1:2) metal to ligand ratio. Chosen plots were represented in Fig.(9,10). The solutions of the prepared complexes showed increase absorbance of the complexes solutions to get to the intersection point and the absorbance still constant at passing this point which indicate that the complex was formed[19].

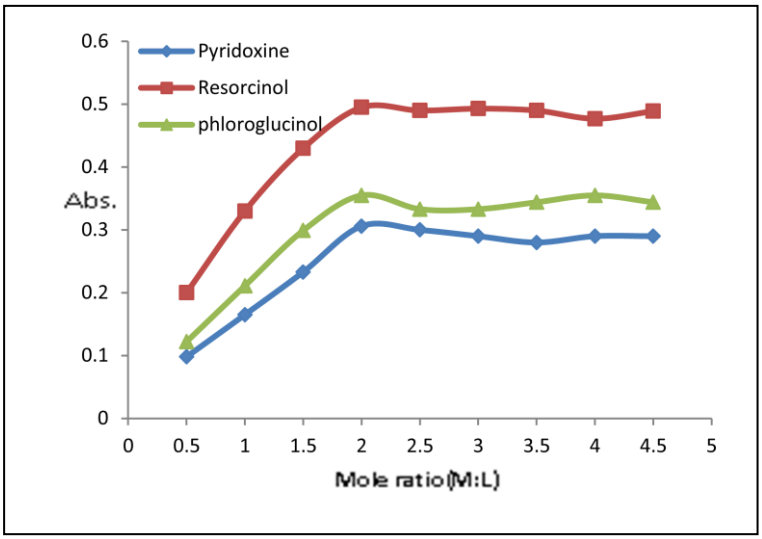


Fig.9: Mole ratio for complexes solutions at optimum conditions

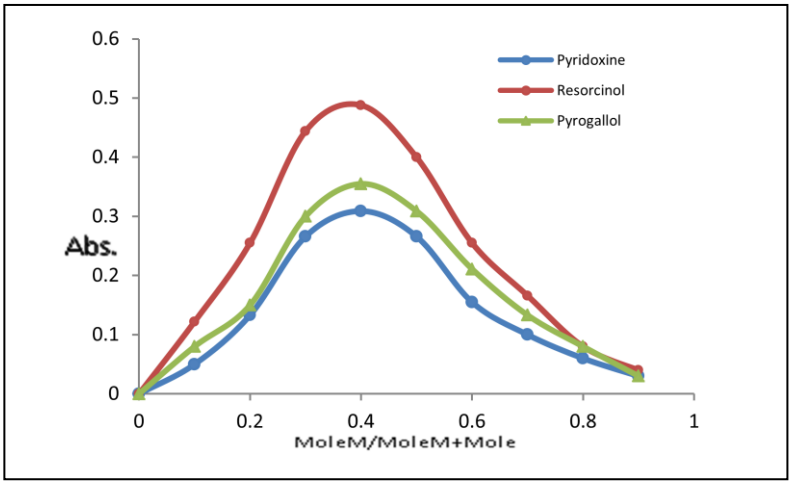


Fig.10: jobs methods for complexes solutions at optimum conditions

Therefore the formation of the product probably may be tetrahydral or octahydral shape as following figures 11-13

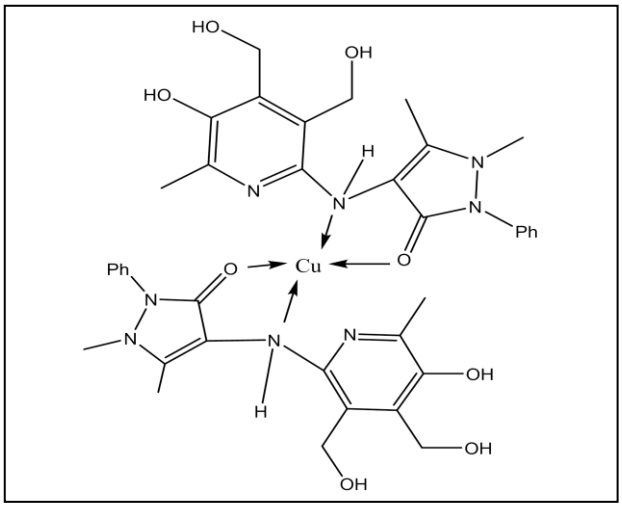


Fig.(11): Probably formation structure of 4-AAP-Pyridoxine-Cu complex

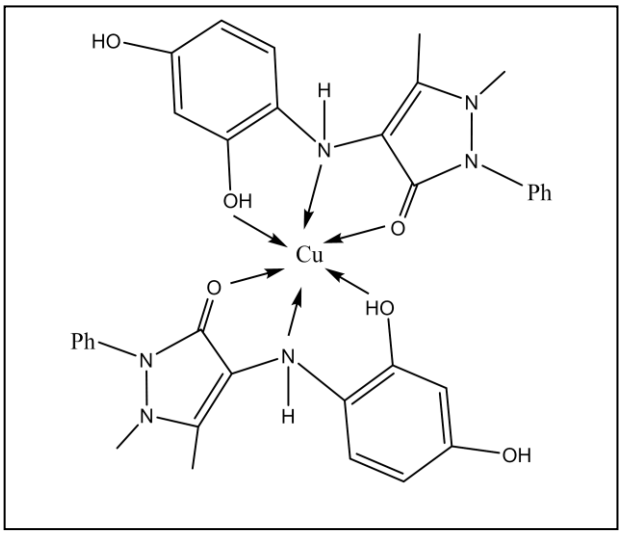


Fig.(12): Probably formation structureof 4-AAP-Resor.-Cu complex

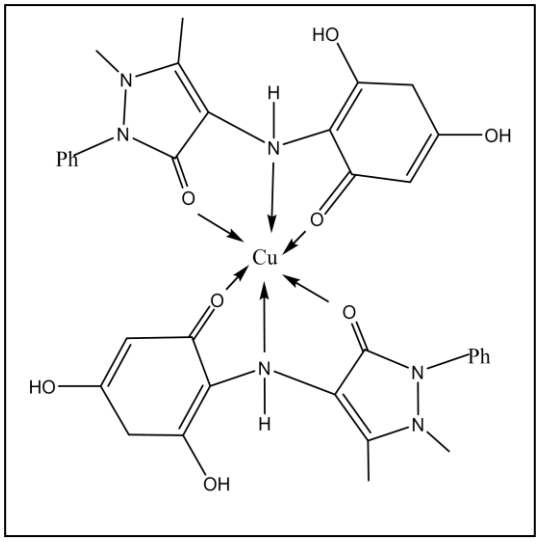


Fig.(13): Probably formation structure of 4-AAP-phloroglucinol.-Cu complex

3.10. Calculation of Complexes Stability Constant

In order to calculate the stability constant (K) for each (1:2) complexes, the spectroscopy method was used [20].All Data of (K) and log K are recorded in Table 3 by used the following equations:

$$K = \frac{1-\alpha}{4\alpha^3 C^2}$$

$$\alpha = \frac{A_m - A_s}{A_m}$$

Where, C is the molar concentration of the complex solution ; Am and As are the absorbance of the fully and partially formed chelating complex respectively at optimum condition and (max) of solution. The thermodynamic parameters of Gibbis free energy (ΔG) were also studied by the following equations.

$$\Delta G = - R\,T\,\ln K$$

Where, R =is gas constant =8.3 J.mol^{-1}.K : T is absolute temperature (Kelvin)

(Δ G) values were calculated and the negative value of (ΔG) (Table3) indicates that the interaction between ligand and cupper selected ion[21].

Table 3: The stability constant and Gibbs free energy for the ligand and itsselected ion Complexes

Compound	Log K	ΔG J.mol^{-1}
Pyridoxine-4AAP-Cu	7.602	-43294.393
Resorcinol-4AAP-Cu	8.598	-60355.906
pyrogallol-4AAP-Cu	5.115	-29131.705

3.11. Interference Studies

To assess the analytical potential of the proposed method, the effect of some common excipients; glucose, lactose, starch , Sodium chloride , Talc and acacia, were examined by carrying out the determination of 10 μg.ml^{-1}for pyridoxine, resorcinol and phloroglucinol phenolic compounds in the presence of above compounds. It was found that the studied excipients do not interfere in the determination of phenolic compounds in its dosage forms.

3.12. Precision and Accuracy

The precision, accuracy of the proposed methods were evaluated by measuring five samples of Pyridoxine, Resorcinol and phloroglucinol phenolic compounds in pharmaceutical preparations at 4, 8, and 12 μg/ml concentrations. Five replicate measurements were made for each concentration. The precision and accuracy were evaluated in terms of the standard deviation and percent recovery. The results are given in Table(4). The calculated relative standard deviations are around 2% for the concentrations studied, indicating excellent precision of the proposed methods. The percent recovery has been between 99.83%and 100.52%, indicating a close agreement between the measured and true values.The results in Table(4) shows acceptable values for accuracy and precision were obtained.

3.13. Analytical applications

The proposed methods will be effective for the determination of Pyridoxine,Resorcinol and phloroglucinol phenolic compounds in pharmaceutical formulations. The obtained results were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the standard method [22],at the95 % confidence level with five degrees of freedom, as cited in Table (5). The results showedthat the experimental t-test and F-test were less than the theoretical value (t=2.776, F=6.39),indicating that there was no significant difference between the proposed method and standardmethod. The results of the application of the proposed method that are given inTable 8 were satisfactory. The recovery was ranged from (100.42-98.89%) confirm that there is no interference of these excipients on the proposed methods.

Table (4): Evaluation of accuracy and precision for theproposed method.

Compounds name	Conc. Taken ($\mu g.ml^{-1}$)	Conc. Found ($\mu g.ml^{-1}$)	Recovery %	RSD %
Pyridoxine	4	4.005	100.13	2.022
	8	8.012	100.15	2.211
	12	11.98	99.83	2.098
Resorcinol	4	4.021	100.52	2.321
	8	8.003	100.04	2.221
	12	12.006	100.05	2.100
Phloroglucinol	4	3.997	99.93	2.335
	8	8.016	100.20	2.411
	12	12.031	100.26	2.055

Table (5): Application of the method to the determination of Pyridoxine,Resorcinol and phloroglucinol phenolic compounds in pharmaceutical formulations

Compound name	Conc. Taken ($\mu g.ml^{-1}$)	Conc. Found* ($\mu g.ml^{-1}$)	Recovery %	RSD %
Pyridoxine (B6)	5	5.005	100.10	2.140
	10	10.013	100.13	2.098
	15	15.053	100.35	2.113
Resorcinol (terbutaline)	5	5.021	100.42	2.209
	10	9.978	99.78	2.213
	15	15.006	100.04	2.289
Phloroglucinol(Spasfon 80mg)	5	4.997	99.95	2.096
	10	9.889	98.89	2.155
	15	15.035	100.23	2.182

*Average of five determinations.

4. Conclusion

The proposed methods are simple which preclude any use of harmful and costly solvents and reagents.It is not only cheap and safe but also is available in any analytical laboratory. The procedure of the proposed methods is simple and time saving. The proposed method offers good linearity and precision. The method is important for the assay of pharmaceutical preparations of Pyridoxine,Resorcinol and phloroglucinolphenolic compounds,and the results suggested that there is no interference with which are present in commercial dosage forms. The proposed method

is sensitive, accurate and reproducible, requires simple apparatus for its performance, and consequently is suitable for routine quality control of the drug.

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