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Spectrophotometric Assay of Phenylephrine Hydrochloride Using 4-Aminoantipyrine and Copper (II)

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Abstract

A new spectrophotometric method is proposed for determination of phenylephrine hydrochloride. The method is based on the coupling of 4-aminoantipyrine (4-AAP) with phenylephrine hydrochloride (PEH) to give a new ligand that reacts with copper (II) in the presence of sodium tetraborate buffer solution of pH 9.00 at 50 °C to give an intense red colored chelate having maximum absorption at 480 nm. The optimization of the experimental conditions is described. The method has been used for the determination of 2.0–50.0 μg/ml of PEH. The molar absorptivity is 5.34×10^3 $L.mol.^{-1}cm.^{-1}$ and the accuracy of the method is achieved by the value of average recovery (101.28 %) and the precision is supported by relative standard deviation (RSD=1.25 %) values. The results of the method was compared with those of the standard method. The interference of excipients was studied. The mechanism of the chemical reaction has been proposed. The proposed method was successfully applied for the determination of the PEH in pharmaceutical syrup formulations.

Keywords: Phenylephrine; 4-Aminoantipyrine; Copper(II); Spectrophotometry.

Introduction

Phenylephrine hydrochloride [PEH, (R) -1-(3-hydroxyphenyl) – 2 - (methylamino) ethanol hydrochloride, $C_9H_{13}O_2N$. HCl], is a white crystalline powder, and belongs to the group of medicines called sympathomimetics. It acts stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for non-specific and allergic conjunctivitis, sinusitis and nasopharyngitis [1]. PEH syrup is used for relieving congestion, cough and preventing or treating symptoms such as runny nose, sneezing, itching of the nose and throat, watery eyes due to colds, flu, or hay fever [2].

Various analytical techniques have been reported in the literature for the analysis of PEH including, titrimetry [3] fluorometry [4], ion pair chromatography [5], High-performance liquid chromatography [6-8], micellar liquid chromatography [9], micellar electrokinetic chromatography [10], capillary zone electrophoresis [11,12] and flow Injection analysis with chemiluminescence detection [13].

Different spectrophotometric procedures have been reported for determination of PEH including the formation of ion-pair complexes between the drug and alizarine, alizarine red S, alizarine yellow G or quinalizarine [14], ninhydrin in sulfuric acid [15], nitrobenzene derivates in acetonitrile medium [16], oxidative coupling with 4-aminoantipyrine in the presence of potassium ferricyanide or sodium periodate [17,18], diazotized p-nitroaniline or 2-aminobenzothiazol in alkaline medium [19,20], forming a charge transfer complex with chloranil or haematoxylin in alkaline medium [21,22] and uranyl (II) ion forming a complex at pH ≤ 7 [23].

The present research aims to developing a

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sensitive, simple and accurate spectrophotometric method for the determination of PEH based on its coupling with 4-aminoantipyrine (4-AAP) to give a new ligand that reacts with copper (II) to give intense red colored chelate. The method was applied for determining PEH in pure and pharmaceutical formulations as syrup.

Experimental

Apparatus

All absorption measurements were made on double-beam spectrophotometer Shimadzu (UV-160A) and 1-cm optical silica cells. The pH of the solutions were measured by HANNA (pH_{211}) Microprocessor pH meter. Heating of solutions is carried out on a water bath.

Reagents

All reagents were of analytical grade and obtained from Fluka and BDH companies. A 1000 µg/ml solution of phenylephrine hydrochloride (PEH) (provided from Sammara drug industries; SDI, Iraq)) is prepared by dissolving appropriate amount of PEH in small amount of ethanol and diluted to the appropriate volume with distilled water, the solution was stored in amber colored bottle and kept in refrigerator. The solution was diluted as needed. Solutions of copper sulphate of 0.1 %, 4-aminoantipyrine (4-AAP) of 1 % and sodium hydroxide of 0.05 M concentrations were prepared by dissolving accurate weight in distilled water in addition to the borate buffer solution of pH 9.00.

Procedure

Aliquots containing 2–50 µg/ml of PEH, in final dilution, were transferred into a series of 10-ml volumetric flasks, followed by addition of 3 ml of 1 % 4-AAP, 1 ml of 0.1 % copper sulphate and 0.75 ml buffer solution of pH 9.00. The red colored mixture was placed in water bath adjusted at 50 °C for 25 min, cooled and make up the solution to 10 ml with distilled water, the absorbance values were measured at 480 nm against the reagent blank solution.

Analysis of syrup

The appropriate volume of the syrup containing PEH equivalent to 5 mg was transferred into a 100 ml measuring flask, diluted, filtered and made up to the mark with distilled water. An aliquot of the solution was analyzed, as described earlier.

Results and Discussion

Optimum reaction conditions affecting the reaction of phenylephrine with 4-AAP and copper sulphate were studied.

Absorption spectrum

PEH reacts with 4-AAP and copper sulphate in the presence of sodium hydroxide when heated for 25 min at 50 °C to give a red colored complex. The absorption spectrum under optimum conditions shows a maximum at 480 nm, whereas the reagent blank give no absorption at this wavelength (Fig. 1).

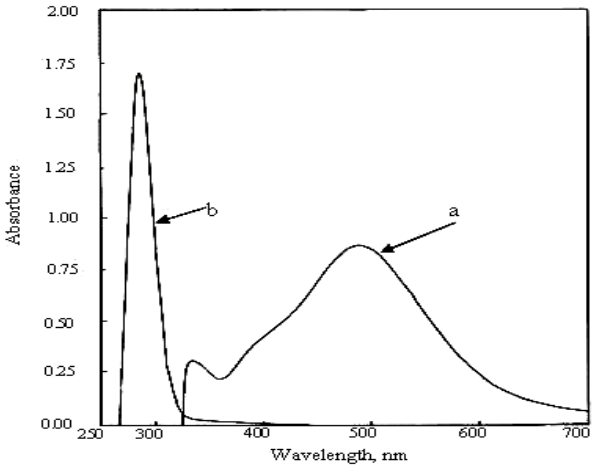


Figure 1. **Absorption spectra of (a) PEH (35 µg/ml) complex with 4-AAP and Cu(II) against reagent blank and (b) reagent blank against distilled water under optimum conditions.**

Effect of pH

The effect of pH on the absorption of the complex formed by the reaction of PEH with 4-AAP and Cu(II) was studied at different pH of HCl or NaOH in the range 2.70-11.45. It was found that the chelating complex was formed at

pH 9.00 (Fig. 2). Therefore different buffers of pH 9.00 were prepared using carbonate, bicarbonate and borate buffers to investigate the sensitivity of the 4-AAP-PEH–Cu(II) complex. It was found that borate buffer solution increased the sensitivity of the complex. However; the optimum amount of borate buffer solution of pH 9.00 has been studied and (Fig. 3) shows that 0.75 ml is the optimum amount which is recommended in the subsequent experiments.

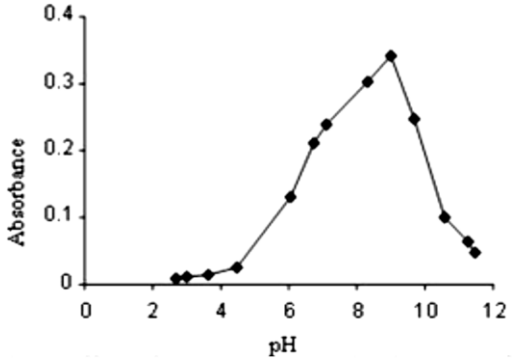


Figure 2. **Effect of pH on the absorption intensity of PEH (25 µg/ ml)-4-AAP-Cu(II) complex.**

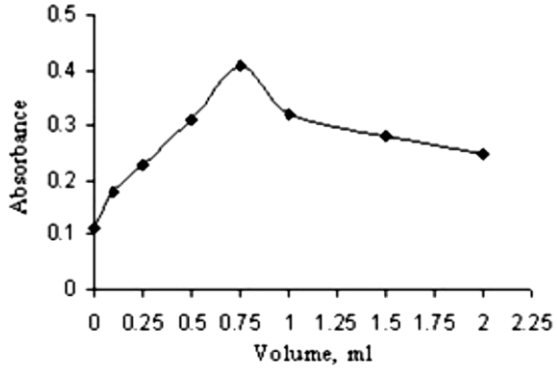


Figure 3. **Effect of borate buffer solution (pH9) amount on the intensity of PEH (25 µg/ml)-4-AAP-Cu(II) complex.**

Effect of 4-AAP reagent concentration

The effect of changing the 4-AAP reagent concentration on the absorbance of solution keeping a fixed amount of the drug, Cu(II) and pH 9.00 was studied. It was found that absorbance increases with increasing 4-AAP concentration and reached its maximum value on using 3 ml of 1 % 4-AAP (Fig. 4). This condition is used in the subsequent experiment.

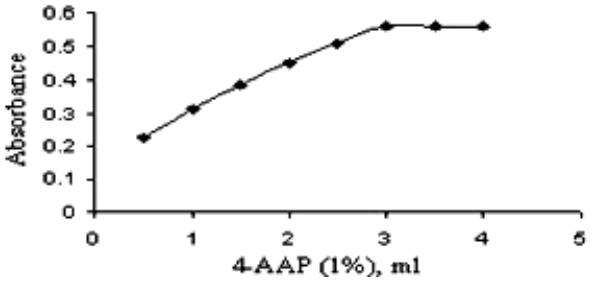


Figure 4. **Effect of 1% 4-AAp reagent amount on the absorption intensity of 25 µg/ml PEH in the presence of Cu(II).**

Effect of $CuSO_4.5H_2O$ concentration

The chelating complex formation reached its maximum when 1ml of 0.1% of $CuSO_4.5H_2O$ solution were added to a mixture containing a fixed amount of PEH, 4-AAP and pH 9.00, (Fig. 5), therefore, this amount was used in the procedure since it gives high sensitivity and minimum blank value.

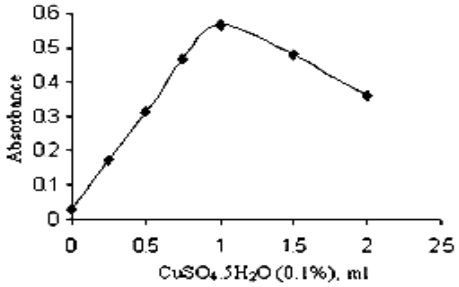


Figure 5. **Effect of $CuSO_4$ $5H_2O$ concentration on the absorbance of 25 µg/ml PEH. In the presence of 4-AAP.**

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and at different temperatures ranged between 22–60°C in thermostatically controlled water-bath. The absorbance was measured at 5 and 10 min intervals against reagent blank treated similarly. It is evident from (Fig. 6) that the formation of stable colored complex for PEH was achieved after 50 min. at 40 °C. Hence, this temperature and time was selected for further investigations.

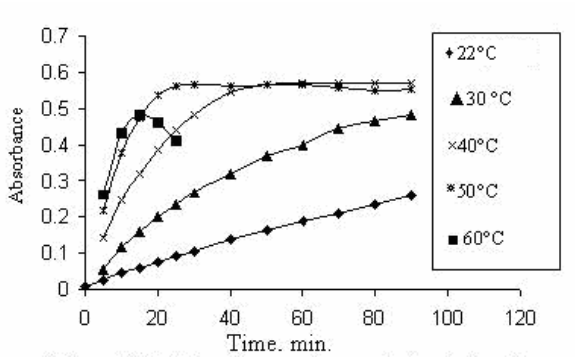


Figure 6. **Effect of temperature and developing time on the absorbance of 20 µg/ml PEH.**

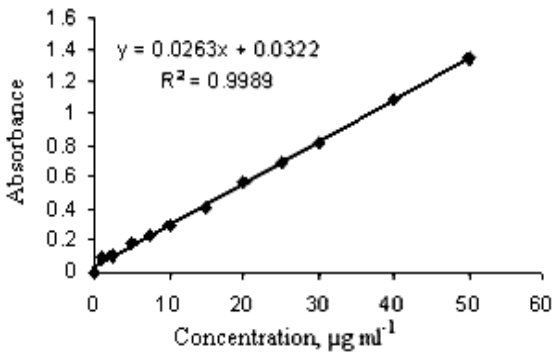


Figure 7. **Calibration graph of PEH drug.**

Effect of order of addition

From the experiments in which the reagent was added in all possible sequences, it was concluded that the maximum absorbance is attained only with the following order: PEH - 4AAP - Cu(II) – pH 9.00.

Quantification

In order to investigate the range in which the colored complex adhere to Beer's law, the absorbance of the complex was measured at λ_{max} for a series of solutions containing increasing amounts of PEH drug (Fig. 7) at optimum conditions. The validity of Beer's law, molar absorptivity and Sandell's sensitivity values were evaluated and are given in (Table1), indicated that the method is sensitive. The proposed method showed excellent linearity for the determination of PEH drugs with a good correlation coefficient (0.9989). The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of six replicates of each three different concentrations of PEH (7.5, 25 and 40 µg/ml) indicated that the method is precise and accurate. Limit of quantitation (LOQ) is determined by taking into account the ratio of standard deviation of the blank with respect to water and the slope of calibration curve multiplied by a factor of 10. However, LOD is well below the lower limit of Beer's law range.

Table 1. **Summary of optical characteristics and statistical data for the proposed method.**

Parameter	Values of method
Beer's law limits (µg.ml^{-1})	2-50
Molar absorptivity (l.mol^{-1}. cm^{-1})	5.357×10^3
LOD (µg/ml)	0.1251
LOQ (µg/ml)	0.3812
Average recovery (%)**	101.28
Correlation coefficient	0.9989
Regression equation (*Y*)*	
Slope, *a*	0.0322
Intercept, *b*	0.0263
RSD**	1.25
Average recovery %	99.78

* $Y = ax + b$, where x is the concentration of PEH in µg/ml.
** Average of six determinations.

Interferences

The extent of interfering by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 20 µg/ml of PEH and various amounts of diverse species in a final volume of 10 ml. It was found that the studied excipients do not interfere in the determination of PEH in its dosage forms. Vitamin C showed an interference effect when present in a large excess, this may be attributed to the reduction of Cu (II) to Cu (I) by vitamin C [24]. An error of 5.0 % in the absorbance readings was considered tolerable. Typical results are given in Table 2.

Table 2. **Effect of excipients for assay of PEH.**

Excipients	Recovery %* of 20 μg/ml of PEH per μg/ml excipients added in			
	25	*50*	*100*	*250*
Glucose	99.25	103.75	101.25	101.00
Lactose	98.75	100.50	100.62	100.75
Starch maize	98.25	99.00	99.30	102.00
Acacia	102.00	100.87	103.75	105.50
Talc	99.00	100.62	103.50	103.00
Sodium chloride	102.50	100.62	100.75	100.57
Glycerin	103.25	103.07	103.37	105.75
Vitamin C	100.75	81.25	65.00	53.75

* Average for three determinations

Analytical applications

The proposed method was successfully applied to determine PEH in pharmaceutical syrup preparations. 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 [25] (depending on potentiometric titration of pure drug dissolved in anhydrous acetic acid with perchloric acid) at the 95 % confidence level with five degrees of freedom, as cited in (Table 3). The results showed that 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 standard method. However; there is no method described in British Pharmacopoeia for the assay of PEH in syrup preparations. The proposed method is comparable with other reported methods as shown in (Table 4).

Table 3. **Assay of PEH in pharmaceutical preparations using the proposed method and comparison with the standard method.**

Preparation[b]	Nominal Value	Recovery % ± RSD[a]	
		Present method	Standard method[c]
Tussiram (syrup)	5.0mg/10ml	105.00 ± 1.82 t =1.28 F =3.91	
Tussilet (syrup)	2.5mg/5ml	101.12 ±1.47 t =1.86 F=2.28	102.15±1.85
Pulmocodin (syrup)	5.0mg/5ml	98.86±2.12 t =2.16 F=3.20	

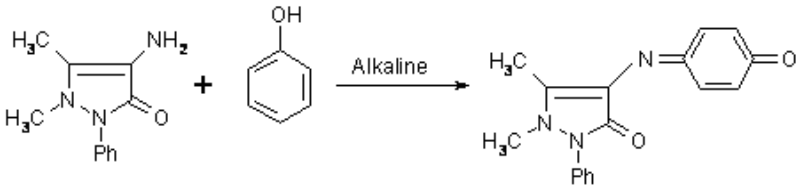
[a] Average of six determinations.
[b] Marketed by S.D.I Iraq
[c] Standard method was applied for determination of pure drug.

Table 4. **Comparison of results for the determination of PEH by the proposed method and the reported methods.**

Reagent used	λ_{max} (nm)	Beer's Law (μg/ml)	Molar absorptivity (L mol^{-1} cm^{-1})	Application	Remarks
m-Dinitrobenzene	560	12-175	1.59 ×10^3	Capsule, ampoule	Involves organic medium [16]
4-AAP–$K_3[Fe(CN)_6]$	503	0.5-17.5		eye and nasal drops	Involves automated sequential injection and condensation reaction [17]
Haematoxylin	620, 640	0.5-5	2.38 ×10^4	Eye drops	Involves heating at 65°C and using of organic solvent [21]
4-AAP-Cu(II)	480	2-50	5.357 ×10^3	syrup	Proposed method

Composition of complex

Different colorimetric methods described for phenol determination [26,27] are based on the reaction between phenols and 4-AAP to form antipyrine dyes where 4-AAP is found to be the most sensitive, fast, and precise colorimetric reagent. 4-AAP reacts with phenolic-type compounds according to the reaction shown in Scheme 1. The reaction product may be any color from red to purple depending on the type of phenolic compounds. In the present work, it was found that PEH reacted with 4-AAP in 1:1 ratio forming a new ligand having low sensitivity at 480 nm. This sensitivity has been increased in its complexation with Cu(II) to give intense red colored chelate as shown in Figure 5. The stoichiometric ratio of the 4-AAP–PEH ligand and Cu(II) was investigated applying the continuous variation (Job's) and mole ratio methods [28] using equimolar solutions of the new ligand and 1×10^{-3} M Cu(II) (Fig. 8). It was found that phenylephrine forms a dye-coupled product with 4-AAP in the 2:1 ratio. The reaction may proceed as given in Scheme 2.



Scheme 1. Coupling reaction between phenol and 4-AAP.

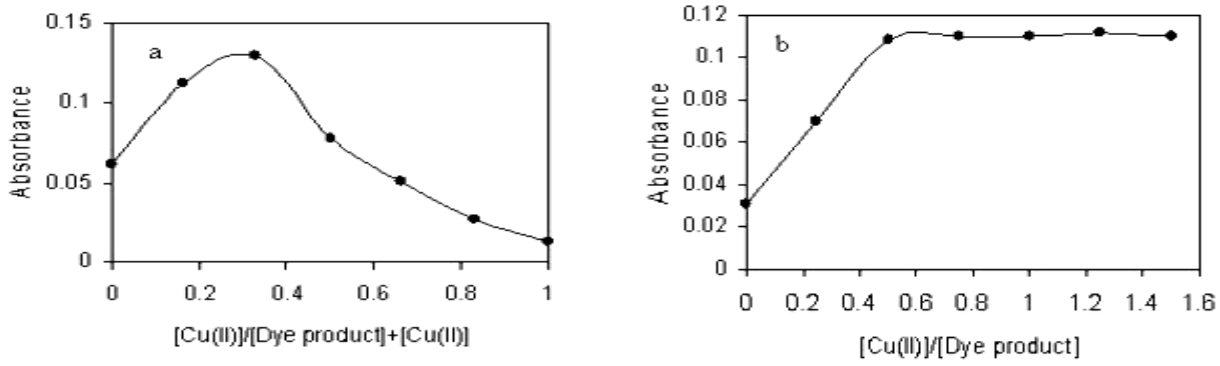
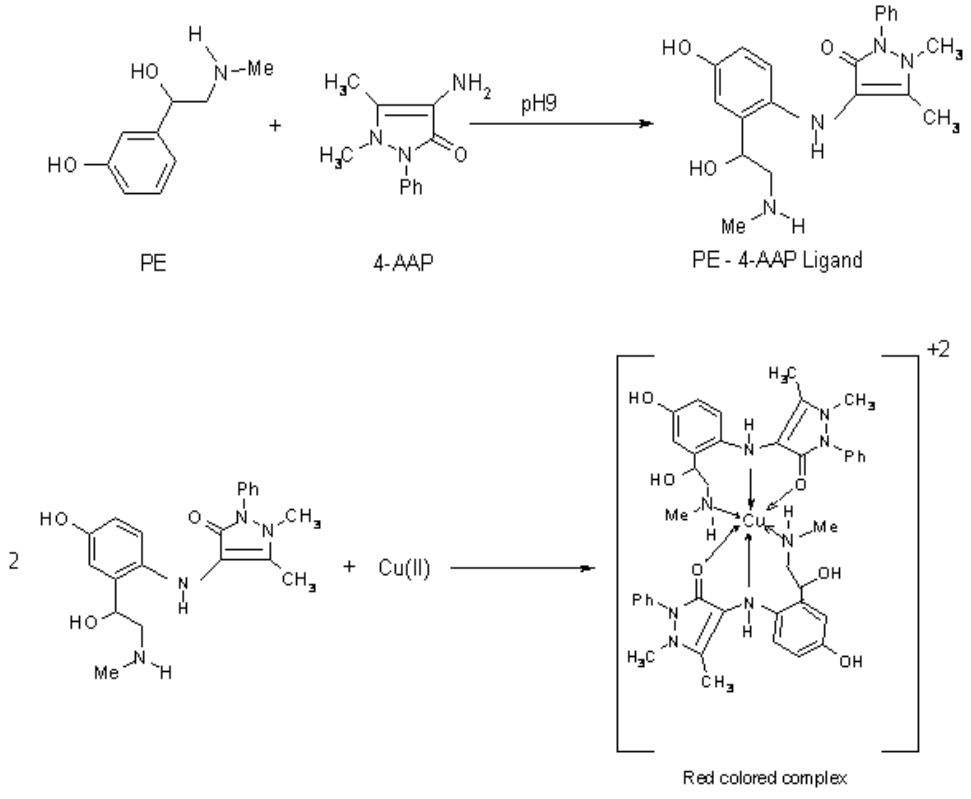


Figure 8. **Continuous variation (a) and mole ratio (b) plots for the dye product of 4-AAP-phenylephrine (1×10^{-3}M) with Cu(II) (1×10^{-3}M) under the optimum conditions.**



Scheme 2. Probable mechanism reaction for complex of PE with 4AAP and Cu (II)

Conclusion

The proposed method is simple, fairly sensitive and economical when compared with already reported methods especially those which are based on non-aqueous medium and expensive technique such as chromatographic instruments and do not require any pretreatment of the drugs. It has a good accuracy and precision. The method is important for the assay of pharmaceutical preparations of PEH as syrup, and the results suggested that there is no interference with which are present in commercial dosage forms except of vitamin C present in excessive amount.

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