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

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ABSTRACT

A new spectrophotometric method is proposed for the 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 pH9 at 50°C with an intensely red colored chelate having maximum absorption at 480 nm. The method has been used for the determination of 2.0–50.0 µg ml-1 of PEH with molar absorptivity of 5.357×103 L.mol-1cm-1, average recovery of 101.28 % and Relative Standard Deviation (RSD) of 1.25%. The results of the method were compared with those of the official method. 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], (Scheme1), is a white crystalline powder, and belongs to the group of medicines called sympathomimetics. It acts on 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 and 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], flow Injection analysis with chemiluminescence detection[13].

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Different spectrophotometric procedures have been reported for the 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 derivatives 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 mainly aims at developing a 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 intensely 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-

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beam spectrophotometer Shimadzu (UV-160A) and matched 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 of frost instruments, LTD.

Reagents

All reagents used were of analytical grade and were obtained from Fluka and BDH companies.

Copper sulphate ($CuSO_4.5H_2O$) solution (0.1%) was prepared by dissolving 0.1 g of $CuSO_4.5H_2O$ with distilled water and diluted to 100 ml in calibrated flask.

4-Aminoantipyrine (4-AAP) *solution (1%)* was prepared by dissolving 1.0 g of 4-AAP in a small amount of ethanol and then diluted to the mark in a 100 ml-volumetric flask with distilled water.

Sodium hydroxide (0.05M) *solution* was prepared by dissolving 0.2 g of sodium hydroxide in distilled water and diluted to 100 ml in a calibrated flask.

Borate buffer solution (pH9) was prepared by dissolving 9.5 g di-sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) in 1L distilled water and the pH adjusted to 9 by the addition of mls 0.1M boric acid.

Standard solution of Phenylephrine Hydrochloride (PEH) (1000ppm) was prepared by dissolving 0.1 g of pure PEH hydrochloride, provided from Sammara Drug Industries (SDI), in 5 ml absolute ethanol and diluted to the mark in 100 ml-volumetric flask with distilled water, the solution was stored in amber colored bottle and kept in the refrigerator. The solution was diluted as needed.

Recommended Procedure

Aliquots containing 2–50 μg ml^{-1} of PEH, in final dilution, were transferred into a series of 10-ml volumetric flasks, followed by the addition of 3 ml of 1% 4-AAP, 1 ml of 0.1% copper sulphate and 0.75 ml pH9. The red colored mixture was placed in a water bath adjusted at 50°C for 25 min, cooled and completed to 10 ml with distilled water, the absorbance values were measured at 480 nm against the reagent blank solution.

Analysis of Syrup

A requisite 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.

Result and Discussion

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

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, as shown in Figure 1, the absorption spectrum of which under optimum conditions shows a maximum at 480 nm, whereas the reagent blank gave no absorption at this wavelength.

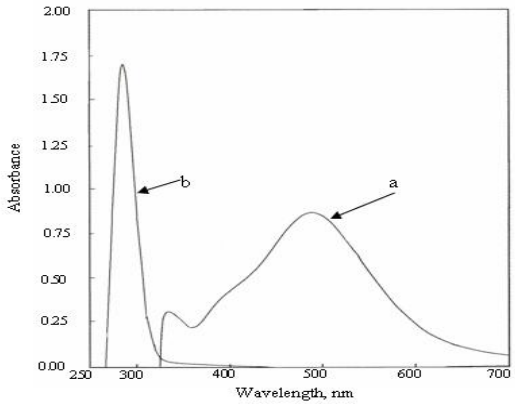


Figure 1. Absorption spectra of (a) PEH (35µg/ml) complex with 4-AAP and Cu(II) against reagent blank and (b) reagent blank against distilled water under optimum conditions.

Effect of pH and Buffer Solutions

The effect of pH on the absorption of the complex

formed by the reaction of PEH with 4-AAP and Cu(II) were studied using different pHs of HCl or NaOH in the range 2.70-11.45. It was found that the chelating complex was formed in the final pH of 9 by addition of NaOH solution (Figure 2). Therefore, different buffers of pH9 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 for this complex. However, the optimum amount of borate buffer solution of pH9 has been studied and Figure 3 shows that 0.75 ml is the optimum amount for which is recommended in the subsequent experiments.

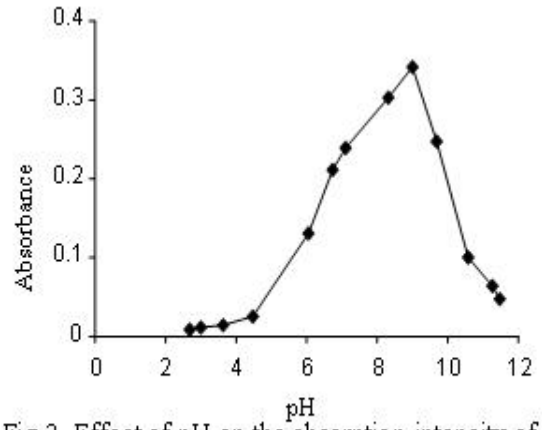


Fig.2. Effect of pH on the absorption intensity of PEH (25µg ml^{-1})-4-AAP-Cu(II) complex

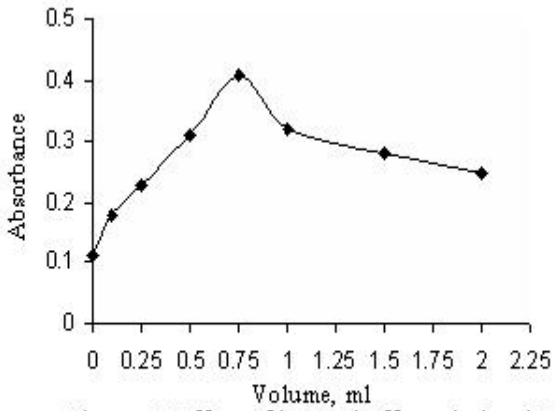


Figure 3. Effect of borate buffer solution (pH9) amount on the intensity of PEH (25µg ml^{-1})-4-AAP-Cu(II) complex

Effect of 4-AAP Reagent Concentration

The effect of changing the 4-AAP reagent concentration on the absorbance of solution containing a fixed amount of the drug, Cu(II) and pH9 was studied. It was found, as shown in Figure 4, that absorbance increases with increasing 4-AAP concentration and reached its maximum value on using 3 ml of 1% 4-AAP which is used in subsequent experiments.

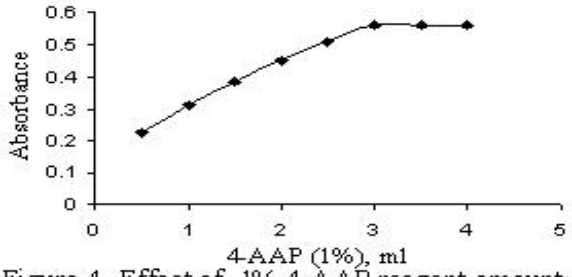


Figure 4. Effect of 1% 4-AAP reagent amount on the absorption intensity of 25 µg ml^{-1} PEH in the presence of Cu(II).

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 pH9, (Figure 5), therefore, this amount was used in the procedure since it gives high sensitivity and minimum blank value.

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 30±1° C and 60±1° C in thermostatically controlled water-bath. The absorbance was measured at 5 and 10 r blank treated similarly. As shown in Figure 6, it was observed that the formation of colored complex for PEH was achieved maximum after 25 min at 50° C and stable for 35 min after which it began fading slowly.

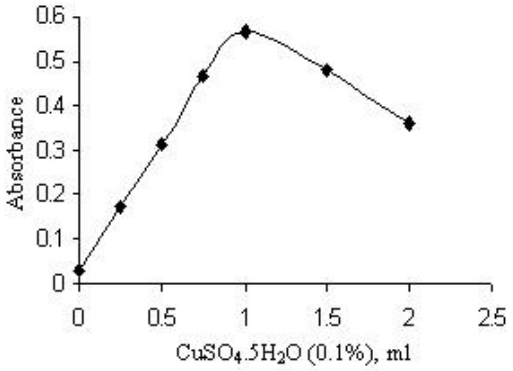


Figure 5. Effect of $CuSO_4.5H_2O$ concentration on the absorbance of 25 µg ml^{-1} PEH In the presence of 4-AAP.

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) - pH9.

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} value after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of PEH drug (Figure 7). The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in Table(1), which indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for the PEH determined by the proposed method represents excellent linearity. 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^{-1}) 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. This means that LoQ is approximately 3.3 times Limit of Detection (LoD). Naturally, the LoQ slightly crosses the lower limit of Beer's law range. However, LoD is well below the lower limit of Beer's law range.

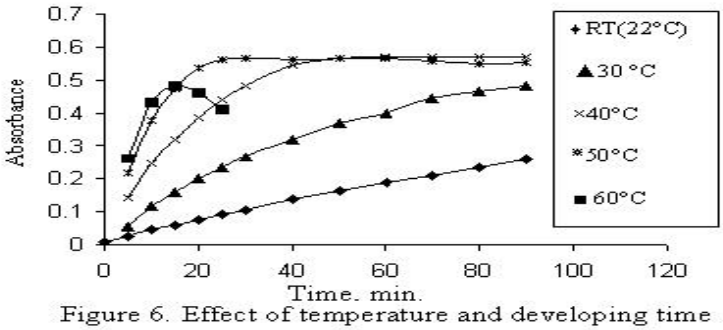


Figure 6. Effect of temperature and developing time on the absorbance of 20 µg/ml PEH

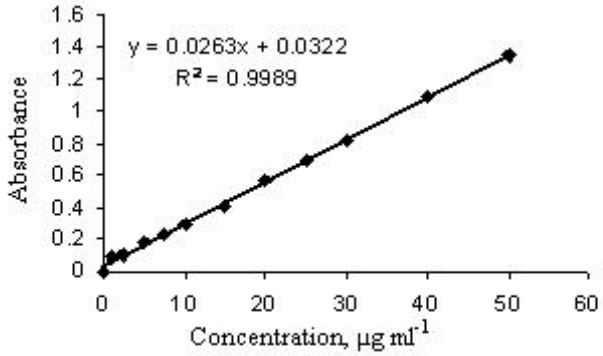


Figure 7. Calibration graph of PEH drug

Table 1. Summary of optical characteristics and statistical data for 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^{-1})	0.1251
LOQ (µg.ml^{-1})	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.

Interference

The extent of interferences by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 20 µg ml^{-1} 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 20µg/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

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

Preparation[b]	Nominal Value	Recovery % ± RSD[a]	
		Present method	Official 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.

Analytical Applications

The proposed method was successfully applied to determine PEH in syrup pharmaceutical 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 official 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 official method. However, there is no method described in the British Pharmacopoeia for the assay of PEH in syrup preparations. The proposed methods are compared favorably with other reported methods as shown in (Table 4).

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 ($\mu g ml^{-1}$)	Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	Application	Remarks
m-Dinitrobenzene (16)	560	12–175	1.59×10^3	Capsule, ampoule	Involves organic medium
4-AAP–$K_3[Fe(CN)_6]$ (17)	503	0.5-17.5	…….	eye and nasal drops	Involves automated sequential injection and condensation reaction
Haematoxylin (21)	620, 640	0.5-5	2.38×10^4	Eye drops	Involves heating at 65°C and using of organic solvent

Reagent used	λ_{max} (nm)	Beer's law ($\mu g ml^{-1}$)	Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	Application	Remarks
4-AAP-Cu (II)	480	2-50	5.357×10^{3}	syrup	Proposed method

[b] Marketed by S.D.I Iraq
[c] Official method was applied for determination of pure drug.

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 2. The reaction product may be any color from red to purple depending on the phenolic-type compounds involved. In the present work, it was found that PEH reacted with 4-AAP at ratio 1:1 forming a new ligand having low sensitivity at 480 nm. This sensitivity has been increased in its complexation with Cu (II) to give intensely red colored chelate as shown in Figure 5. However, 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 Cu (II) ($1x10^{-3}$M). As seen in Figure 8, it was found that phenylephrine forms a dye-coupled product with 4-AAP in the ratio 2 : 1 dye product : Cu (II) and the reaction may proceed as given in Scheme 2.

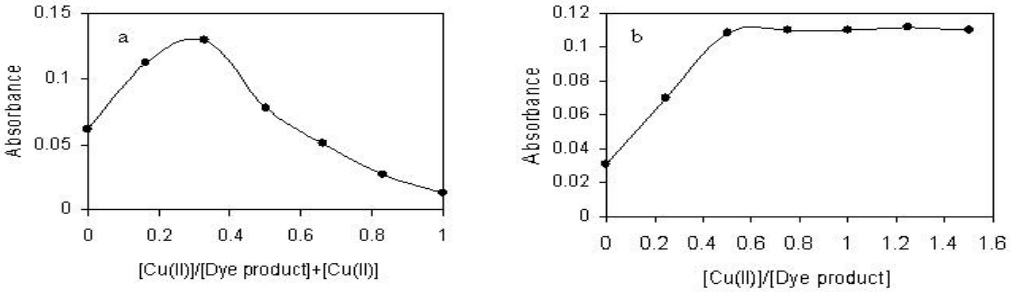
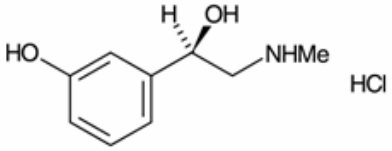
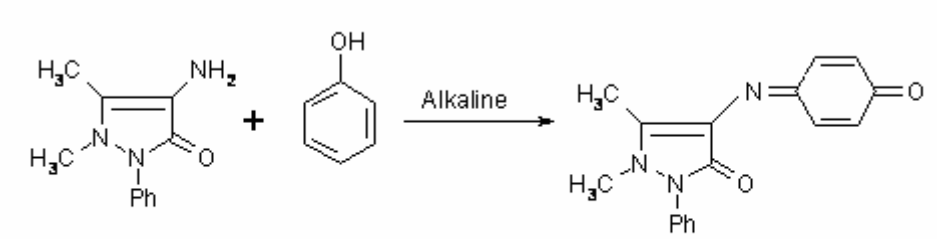


Figure 8: Continuous variation (a) and mole ratio (b) plots for the dye product of 4-AAP—phenylephrine (1×10^{-3}M) with Cu(II) (1×10^{-3}M) under the optimum conditions.

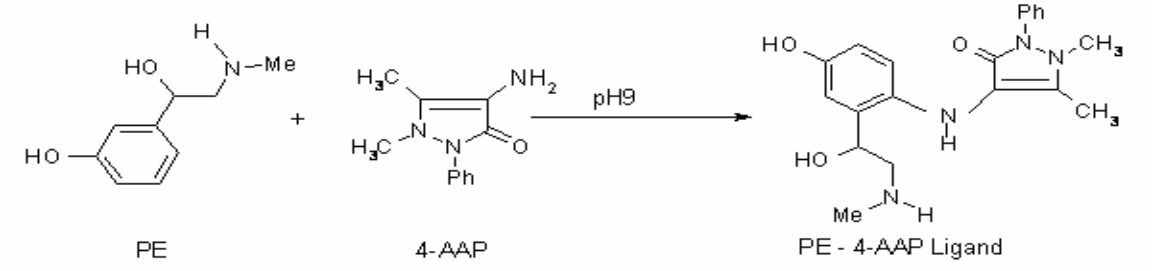


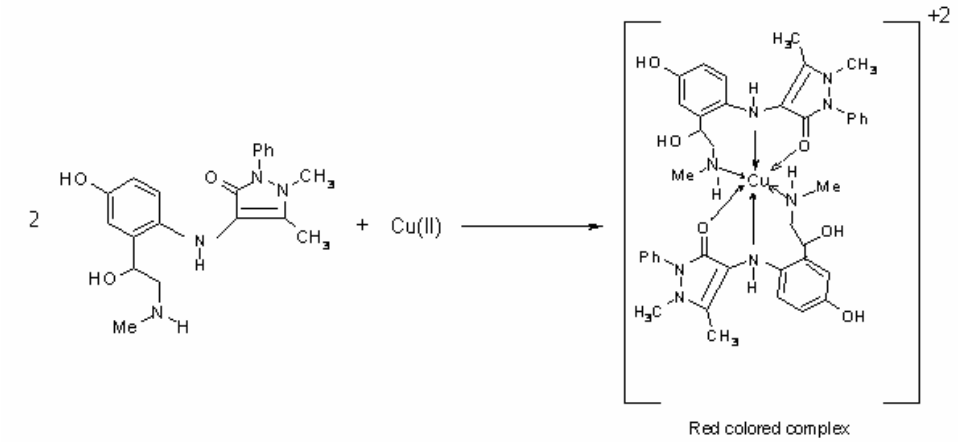
M.Wt.= 203.7 g Mol^{-1}

Scheme 1. Chemical structure of phenylephrine hydrochloride



Scheme 2. Coupling reaction between phenol and 4-AAP





Scheme3. Probable mechanism reaction for complex of PE with 4-AAP and Cu(II)

Conclusion

The proposed method is simple, fairly sensitive and economic when compared with already reported methods especially those based on non-aqueous medium and expensive technique such as chromatographic instruments and do not require any pretreatment of the drugs or extraction procedure and 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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تحليل الفينيل افرين هايدروكلورايد باستخدام طريقة تحليل الطيف الضوئي وباستعمال مادة 4-الامينوانتبارين والنحاس

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ملخـص

تم استخدام طريقة تحليل الطيف الضوئي لتحديد كمية الفنيل افرين هايدروكلورايد . تعتمد الطريقة على التفاعل ما بين 4-الامينوانتبايرين مع الفينيل افرين حيث تتكون مادة جديدة يتم تفاعلها مع النحاس بوجود الصوديوم تارتريت مع درجة حموضة "9 "ودرجة حرارة 50 هم– حيث يتكون لون احمر غامق يمتص الضوء على 480 نانوميتر . استعملت هذه الطريقة – لتحليل 2 – 50 مايكروغرام ومادة الفينيل افرين حيث ان المولار ابسوربتيفيتي كانت (5.357 x 103) وكانت قيمة ما تم استخلاصه حوالي (101.28 %) مع انحراف معياري قيمته 1.25 % . تم مقارنة النتائج التي تم التوصل اليها مع ما هو منشور رسمياً .
هذا ولقد تم توضيح وشرح ميكانيكية التفاعل وتم تطبيق عملي لهذه الطريقة لتحديد كمية الفينيل افرين في احدى الوصفات الصيدلانية والتي هي على شكل شراب .

الكلمات الدالة: فينيل افرين، 4-امينوانتبابرين، نحاس، الطيف الضوئي.

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