Indirect Spectrophotometric Determination of Phenylephrine Hydrochloride in Pharmaceutical Preparations

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Abstract
A simple and sensitive spectrophotometric method was developed for determination of phenylephrine-HCl in pharmaceutical preparations . The procedure is based on the oxidation of phenylephrine-HCl with Fe (III) in acidic medium to produce Fe (II), then reaction of Fe(II) with 2,2'-bipyridyl to produce a red complex which is water-soluble, stable, and has a maximum absorption at 523 nm against the reagent blank with a molar absorptivity of 7.1295×10^4 $l.mol^{-1}.cm^{-1}$.The variables affecting the intensity of complex were studied and optimized. Under the optimum conditions, the calibration graph was linear over the range 2.5-80 µg phenylephrine-HCl/25 ml (0.1-3.2ppm), with a relative error of +0.79 to +0.82% and a relative standard deviation of ±3.44 to ±3.57% depending on concentration level. The proposed method was applied to the determination of phenylephrine-HCl in two pharmaceutical preparations (nose drop and syrup) . The amount of phenylephrine-HCl found is very similar to that obtained by a standard method.

Introduction
Phenylephrine hydrochloride [3-(hydroxy phenyl)-2-(methylamino) ethanol] hydrochloride was widely used as a decongestant drugs(1) and available as an oral medicine or as a nasal spray. Phenylephrine is rarely used as a vasopressor to increase the blood pressure in unstable patients with hypotension.(2)
Different spectrophotometric methods have been applied for determination of phenylephrine using different reagents such as 4-aminoantipyrine in presence of periodate (1) or alkaline ferricyanide (3), nitroso acid in presence of copper ion (4), chloranil (5), ninhy-drin in sulphuric acid (6), periodate(7), methylbenzothiazoline-2-one hydrazine in presence of iron (Fe^{+3}) (8), haematoxline (9) 4-aminophenol (10), chlorimine (11), N, N - dimethylaniline hydrochloride with potassium ferricyanate (12), cupric ion with hydroxyl ammonium chloride (13), sodium borate (14), fluorodinitrobenzene (15), bromoth-ymol blue (16) and diazotized p-nitroaniline (17).
The flow injection methods have been also used in determination of phenylephrine with spectrophotometric detection (18-20).
Also the high performance liquid chromatography technique has been applied in determination of trace amount of phenylephrine (21-23).
The objective of investigation reported in this paper is to evaluate a sensitive and accurate method for the assay of phenylephrine in an aqueous medium , either in pure form or in pharmaceutical preparations. The method based on the oxidation of phenylephrine with ferric ion in acidic medium and subsequent complication of ferrous ion with 2,2'-bipyridyl reagent to produce red complex of ferrous ion-2,2'-bipyridyl which its intensity proportional to amount of phenylephrine.

Experimental
Apparatus
Shimadzu UV-160 Spectrophotometer was used with 1.0 cm quartz matched cells.
Reagent
All Chemicals used were of analytical-reagent grade .
A 50 $\mu g.ml^{-1}$ solution of phenylephrine-HCl (obtained from SDI, Samarra, Iraq) was prepared by dissolving 0.005g of phenylephrine-HCl in 100 ml distilled water.The Fe(III) solution at concentration 1.5×10^{-2} $mol.l^{-1}$ was prepared by dissolving 0.3028 g of ferric nitrate nanohydrate (Fluka).
A 4.99×10^{-2} $mol.l^{-1}$ of 2,2'-bipyridyl was prepared by dissolving 0.3904 g of 2,2'-bipyridyl in 5 ml ethanol and completed into 50 ml distilled water. All other analytical –reagent grade (Fluka) and solutions were prepared in distilled water.
Recommended procedure for calibration
To a set of 25 ml volumetric flasks, increasing volumes (0.05-2.4 ml) of standard solution of the phenylephrine- HCl (50ppm) were quantitatively transferred, so as to contain the drug within the concentration range 2.5 -120 µg /25 ml.To each flask 0.5 ml of Fe(III) and 1.0 ml of 2,2'-bipyridyl were added, the solutions were left for 40 minutes in water bath, adjusted at 80 C^o after that the solutions left to stand for 10 minutes at room temperature before completed to the mark with distilled water then the absorbance of the red coloured product are measured at 523 nm against the reagent blank. A linear calibration graph is obtained over the concentration range of 2.5-80 µg phenylephrine-HCl/25ml.
Above 80 µg/25ml gives a negative deviation (Fig.1).
The molar absorptivity has been found to be 7.1295×10^4 $l.mol^{-1}.cm^{-1}$

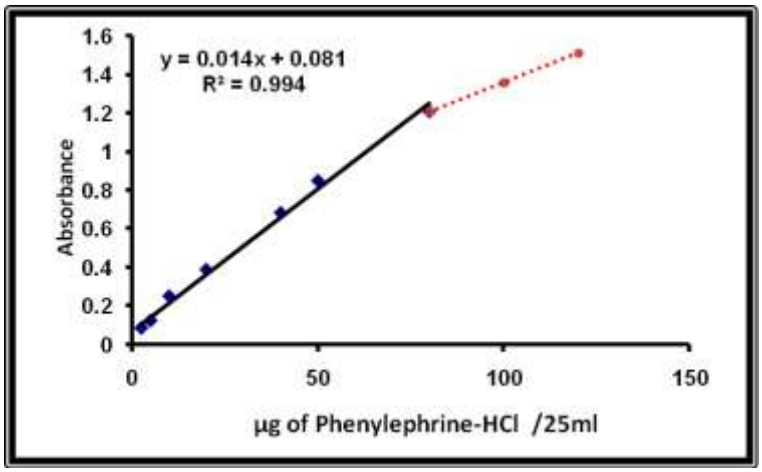


Fig. 1: Calibration graph of phenylephrine-HCl determination

Procedure for the phenylephrine - HCl nose drop

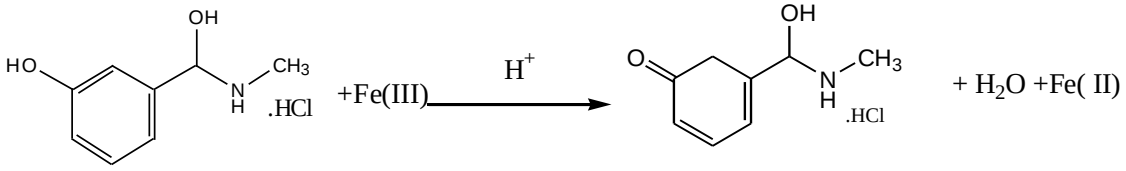
The contents of three containers phenylephrine -HCl nose drop (nasal drop, SDI, Samaraa, Iraq) were mixed.An accurate volume containing 0.005mg phenylephrine-HCl was transferred to a 100 ml volumetric flask, and the volume adjusted to the mark with distilled water. After necessary dilution, the solution
was assayed as stated before under procedure for calibration graph.

Procedure for phenylephrine-HCl syrup

A 10 ml of phenylephrine-HCl syrup (SDI. Iraq) (each 5ml contain 2.5 mg of phenylephrine-HCl) is diluted to 100 ml with distilled water in a volumetric flask. After necessary dilution, the solution was assayed as stated before under procedure for calibration graph. **Results And Discusion**

Principle of the method

Iron (III) ion can act as an oxidant and reacts with phenylephrine-HCl to produce quantitatively iron(II) ion . The amount of iron (II) ion can be determined by using 2,2'-bipyridyl reagent and the intensity of the red complex (as is shown in the following equations) can be used to develop a spectrophotometric method for the determination of phenylephrine - HCl in different pharmaceutical preparations.



$$Fe(II) + 3(2,2'\text{-bipyridyl}) \longrightarrow [Fe(II)\text{-}(2,2'\text{-bipyridyl})_3]^{2+}$$

Red complex

Effect of pH

The effect of pH on colour intensity is examined firstly. The extent of complex formation, and hence the absorbance of the final solution, is often a function of hydrogen ion concentration. Different amount of hydrochloric acid has been used and the results indicate that the pH =3.47 was the most suitable pH to produce the highest colour intensity (Table 1).

Table 1: Effect of pH on absorbance

ml of(0.05M) HCl	Absorbance*	pH
0	0.731	3.47
0.5	0.479	3.21
1.0	0.360	2.89
1.5	0.231	2.77
2.0	0.155	2.66

* Absorbance =0.621 with 0.5 ml NaOH (0.05M)

Effect of temperature

The effect of temperature on the colour intensity of the resulting complex is investigated, by adding 1 ml of Fe(III)($1.5x10^{-2}$M) and 1 ml of 2,2'-bipyridyl reagent solution ($4.99x10^{-2}$M) to 50 μg phenylephrine- HCl in each one of the flask (25ml) and left for 30 minutes in water bath adjusted at 50,60,70,80 and 90 ^{o}C, then each solution was left to stand for 10 minutes at room temperature before the volume completed to the mark with distilled water. The results indicated that absorbance of the complex increased with increasing temperature (Table 2).

Table 2: Effect of temperature

Temperature (C°)	50	60	70	80	90
Absorbance	0.258	0.485	0.724	0.766	0.772

From Table 2,whether 80 ^{o}C or 90 ^{o}C is used the absorbance almost the same, and 80 ^{o}C is therefore recommended for the subsequent experiments.

Effect of ferric nitrate nanohydrate concentration

In order to select the best volume of Fe(II) for a good sensitivity, the effect of different volumes of Fe(III) (1.5×10^{-2}M) was studied. Table 3 shows that 0.5 ml of Fe(III) gives the highest absorbance with a coefficient of determination (r^2 =0.968208) over a range of phenylephrine-HCl concentration of 10-80 μg /25ml .

Table 3. The effect of ferric nitrate nanohydrate amount on absorbance

ml of Ferric nitrate nanohydrate solution (0.015M)	**Absorbance / μg Phenylephrine-HCl in 25 ml**					**r^2**
	10	**20**	**40**	**50**	**80**	
0.25	0.192	0.404	0.501	0.537	0.588	0.772488
0.5	0.214	0.347	0.684	0.775	1.023	0.968208
1.0	0.170	0.340	0.605	0.716	0.977	0.980888

Effect of time on oxidation-reduction reaction

The effect of time needed to complete the oxidation-reduction reaction is studied by allowing the solutions to stand in water bath for different times, after adding the component of reaction (phenylephrine-HCl, ferric nitrate nanohydrate and 2,2'-bipyridyl), then the absorbances measured against the reagent blank at 523 nm(Table4).

Table 4. Effect of time on oxidation- reduction process

Time, minutes	5	10	15	20	25	30	35	40	50	60
Absorbance	0.345	0.536	0.599	0.652	0.681	0.693	0.749	0.776	0.783	0.773

Table 4 shows that highest absorbance of complex occurred after 40 minutes. Therefore, the standing time 40 minutes is recommended for the subsequent experiments.

Effect of 2,2'-bipyridyl reagent amount

The effect of different amounts of 2,2'-bipyridyl reagent on the absorbance of solution containing different amounts of phenylephrine-HCl (10-80 μg/25ml) is studied. The results indicated that the absorbance increases with increasing reagent concentration and reached maximum on using a volume of 1 ml of 4.99×10^{-2}M 2,2'-bipyridyl which also gives the highest value of coefficient of determination (Table 5).

Table 5. The effect of reagent amount on absorbance

ml of 2,2'-bipyridyl solution (4.99×10^{-2}) M	**Absorbance / μg of phenylephrine-HCl in 25 ml**					**r^2**
	10	**20**	**40**	**50**	**80**	
0.5	0.15	0.304	0.503	0.608	0.811	**0.977387**
1	0.209	0.379	0.615	0.751	1.034	**0.989650**
2	0.189	0.358	0.634	0.712	0.98	**0.977770**

Effect of surfactant

The effect of several types of surfactants on colour intensity of the complex has been investigated. The results indicate that addition of surfactants give no useful effect [increasing the intensity or improving the colour contrast (Δλ)],therefore it has not been used in the subsequent experiments (Table6).

Table(6) Effect of surfactant

Surfactant solution	Absorbance / order* of addition							
	I		II		III		IV	
	A	Δλ	A	Δλ	A	Δλ	A	Δλ
CPC** (1×10^{-3}M)	0.318	218.5	0.348	218	0.310	215	0.284	217
SDS*** (1×10^{-3}M)	Turbid	…	Turbid	…	Turbid	…	Turbid	…
Triton X-100 (1%)	0.209	219	0.209	218.5	0.525	218	0.213	219

Not. Absorbance without surfactant=0.785 and Δλ = 219
Colour contrast(Δλ) =λmax S - λ max B Where S=Sample and B=Blank

* I.Phenylephrine-HCl (PE)+Surfactant (S) +2,2'-bipyridyl (R) + $Fe(NO_3)_3.9H_2O$ (O)
II. PE +R+ S + O
III. PE + R+ O + S
IV. PE + O + R + S

**Cetylpyridinium chloride.
***Sodium dodecyl sulphate

Order of addition

The effect of the order of addition was studied by preparing six solutions with different addition orders (Table 7). The order No. I was selected because it gave the highest sensitivity.

Table 7.The order of addition

Reaction component*	Order number	Absorbance
PE + O + R	I	0.883
O + PE + R	II	0.868
R + PE + O	III	0.874
O + R + PE	IV	0.859
PE + R + O	V	0.809
R + O + PE	VI	0.833

*Phenylephrine-HCl (PE) , $Fe(NO_3)_3.9H_2O$ (O), 2,2'-bipyridyl (R)

Effect of time

The effect of time on the stability of the complex were also studied. Table 8 shows that the complex colour was stable up to 48 hours, after emerging the flasks from water bath (Table 8)

Table 8. Effect of time on absorbance

Time/min	Absorbance/µg of Phenylephrine-HCl	
	50	80
0	**0.850**	**1.113**
5	**0.850**	**1.114**
10	**0.851**	**1.113**
15	**0.852**	**1.113**
20	**0.852**	**1.114**
25	**0.852**	**1.114**
30	**0.852**	**1.115**
35	**0.853**	**1.115**
40	**0.853**	**1.115**
45	**0.853**	**1.117**
50	**0.853**	**1.118**
55	**0.853**	**1.118**
60	**0.853**	**1.118**
120	**0.858**	**1.120**
24h	**0.870**	**1.140**
48h	**0.890**	**1.159**

Final absorption spectrum

When phenylephrine-HCl is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 523 nm, characteristics of the Iron(II)-bipyridyl complex in contrast to the reagent blank (Fig. 2).

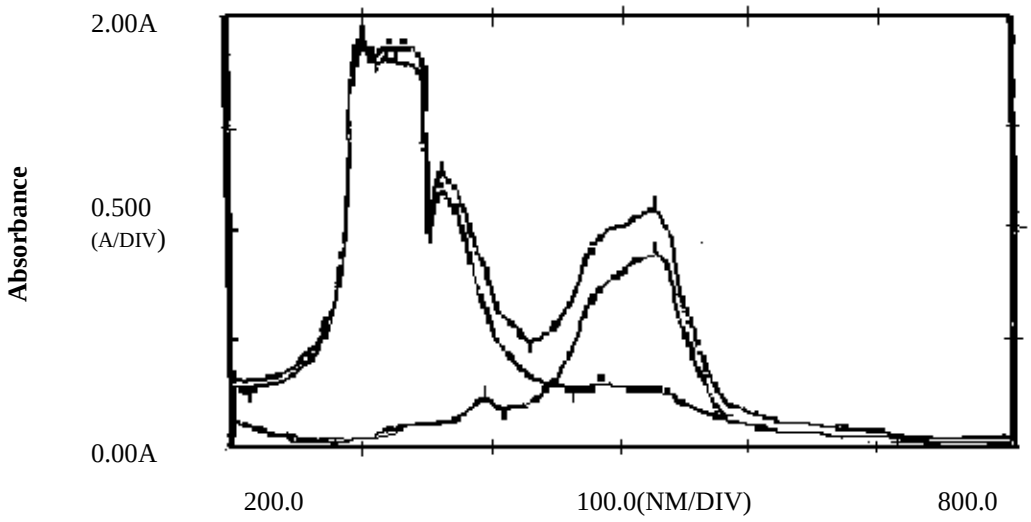


Fig. 2: Absorption spectra of 50μg phenylephrine-HCl/25ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Interference

The criterion of interference was an error of not more than ± 5.0% in the absorbance. To test the efficiency and selectivity of the proposed analytical method, a systematic study of additives and excipients (e.g., glucose, lactose, gum Arabic and starch) that usually present in dosage forms. Results showed that there was no interference from additives or excipients up to 250μg/25ml for the examined method as shown in Table 9.

Table 9.Effect of foreign compounds for assay of phenylephrine-HCl

Foreign compound	Recovery (%) of 50μg phenylephrine-HCl per μg foreign compound added			
	100	**250**	**500**	**1000**
Glucose	99.29	104.12	91.31	81.57
Lactose	97.76	100.00	93.54	91.31
Gum Arabic	98.70	100.12	100.00	94.47
Starch	99.17	102.96	92.94	91.29

Accuracy and precision

To check the accuracy and precision of the method, phenylephrine-HCl was determined at two different concentrations. The results illustrated in Table 10 indicated that the method was satisfactory.

Table 10. The accuracy and precision

μg Phenylephrine -HCl/25ml	Relative error, %*	Relative standard deviation, %*
40	+0.79	±3.57
80	+0.82	±3.44

*Average of four determinations

Stoichiometry of the reaction

The stoichiometry of the product formed from the reaction of phenylephrine [PE] with ferric ion [Fe^{+3}] was investigated by applying the continuous variations method (Job's method). The results indicate that the product was formed in the ratio of 1phenylephrine: 1 Fe^{+3} (Fig.3).

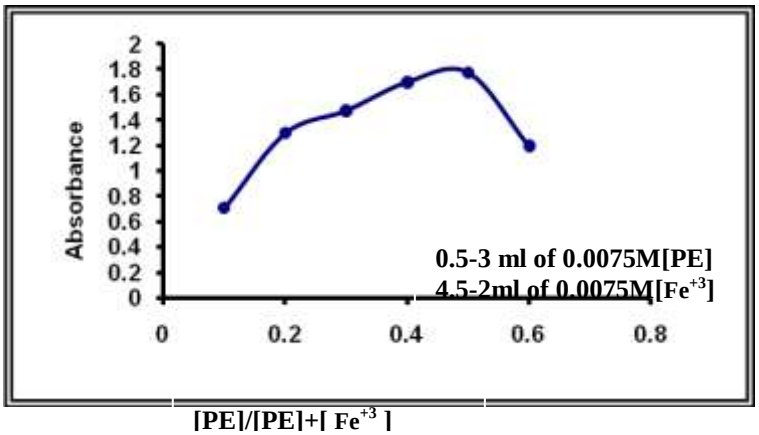
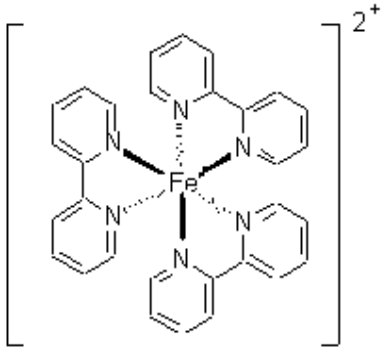


Fig.3: Job's plot for phenylephrine-Fe^{+3}

The ferrous ion produced is chelated by 2,2'-bipyridyl to form the well-known red chelate [1 Fe^{+2} : 3(2,2'-bipyridyl)] (24).



Iron(II) trisbipyridyl complex

Analytical Applications

The proposed method was successfully applied to determine phenylephrine in its pharmaceutical preprations. The performance of the proposed methods was assessed by calculation of the t-test compared with the standard method (25) (potentiometric titration with sodium hydroxide) for 95% confidence level with four degrees of freedom. The results (Table 11) showed that the t-values (±0.194 and ±0.180 for nose drop and syrup respectively) was less than the critical value (2.776), indicating no significant difference between the proposed and standard method for the determination of phenylephrine.

Table 11. Analytical applications of the proposed method and t-values expremental

Pharmaceutical preparation	µg phenylephrine-HCl present/25ml	Recovery% (presence method)	Recovery % (standard method)	t-value
Nasophrine Nasal Drop(0.25%) SDI - Iraq	80	100.08	99.60	±0.194
Tussilet syrup, 2.5mg phenylephrine-HCl/ 5ml)***S.D.I. Iraq***(	80	101.16	102.40	±0.180

Comparison of the methods: Table 12 shows the comparison between some of analytical variables for the present method with that of another literature spectrophotometric method (1).

Table 12. Comparison of the method with the literature method

Analytical parameters	Present method	Literature method(1)
pH	3.47	……..
Temperature (°C)	80	Room temperature
λ_{max} (nm)	523	500
Reagent	2,2'-bipyridyl	4-aminoantipyrine
Beer's law range (ppm)	0.1-3.2	1-36
(ε, l.mol^{-1}.cm^{-1})	7.1295×10^4	1.26×10^4
Stability(minutes)	2880	90
Application of the method	Nose drop and syrup	Nose drop

The results indicate that the proposed method is more sensitive than the literature method and has a wide application part in determination of drug under investigation in its pharmaceutical preparations, but the present method needs heating and longer time of analysis.

Conclusion

The proposed method is more sensitive than the published methods. The proposed method was advantageous over some of reported visible spectrophotometric methods with respect to their higher sensitivity, reproducibility, precision, accuracy and stability of the coloured species for 48 h. The proposed method is suitable for the determination of phenylephrine in pure form and in nose drop and syrup formulations without interference from excipients .

References

1. Al-Abachi, M.Q. and Al-Ward,H.S. ,National J. Chem., 2002, 6 , 221**.**

2. **http://www.medic8.com/medicines/phenylephrine.htm**

3. Hiskey, C.F. and Levin,N., J. Pharm. Sci, 1960, 50, 393.

4. Yahia, M.D. and Laila, N. G., Analyst,1976, 101, 717.

5. Amer , M.M.; Taha, A.M.; El-Shabouri, S.R. and Khashaba, P.Y. ,J. Assoc. of Anal. Chem., 1982,65, 894 .

6. Muszalska, I.; Zajac, M.; Wrobel, G. and Nogowska, M. Acta-Pol-Pharm.,2000 ,57 ,247.

7. Neil ,H.B. and Gelenn ,A.P., J. Pharm.Sci.,1971, 60(8) ,1229.

8. Gala ,B.; Gomez-Hens, A. and Perez-Bendito, D.,J. Anal. Chem. ,1994 ,349(12),824.

9. Ibrahim, S. A. and Alaa, S. A., J. Molecular Liquids, 2007 , 130
(1-3) ,84 .

10.Sane, R.T. and Narkar, V.S., Indian Drugs, 1980, 18(1), 23 .

11.Szekeres , L. ; Harmon ,E. and Gupta , S. K. , Micro Chem. J. ,1973 , 18 ,583 .

12. Tatsuzawa ,M. and Shimoda ,M., Bunseki Kagaku, 1968, 17(5) ,
551.;Chem.Abstr.,69,46091h (1969).

13.Deodhar, R.D. and Mehta, R. C., ndian J. Pharm. Sci., 1978, 40(5), 167; Anal. Abstr., 37 ,1E56 (1979).

14. Doulakas, J., Pharm. Acta Helv., 1975, 50(3) ,66; Anal. Abstr.,
29,4E44,(1975).

15. Tammilehto, S., Farmaseuttinen Aikak, 1975, 84(2), 53 ; Anal.Abstr.31,3E33(1976).

16.Matthew,W. R.; Browne, H.C. nd Weber, J. B., J.Ass. off. Anal. Chem., 1972, 55(4), 789. Anal.Abstr., 24, 1088, (1973).

17.Auerbach,M. E., J. Am. Pharm Assoc., 1950, 50(2), 39; Anal. Abstr. 3210d (1950).

18.Yolanda , F.M. ; Luis, L.Z. and Jose, M.C., J.AOAC Int. ,2001, ___ 84(1), 13 .

19.Knochen , M. and Giglio, J., Talanta, 2004, 64(5), 1226 .

20.Beyene, N. W. and Vanstaden, J.F., Talanta, 2004 , 63(3), 599.

21.Amer, S.M.; Abbas, S.S.; Shehata, M.A. and Ali, N.M., J. AOAC Int. , 2008 ,91(2) ,276 .

22.Marin, A. and Barbas , C.J. Pham. Biomed . Anal. , 2004, 35(4), 769.

23.Senyuva , H. and Ozden, T., J.Chromatogr.Sci., 2002 , 40(2) , 97.

24. Foster, D.S. "photometric and fluorometric methods of analysis metals", 1978 , part 1, John Wiley & Sons, Inc. , New York ,750.

25. " ***British Pharmacopeia on CD-ROM***", 3rd Edn., System simulation Ltd, the stationary office, London, (2000).

التقدير الطيفي غير المباشر للفنيل افرين في مستحضراته الصيدلانيه

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الملخص

يتضمن البحث تطوير طريقة طيفية بسيطة وحساسة لتقدير كميات متناهية في الصغر من الفينل افرين. تعتمد الطريقة على أكسدة الفينل افرين هيدروكلوريد بوساطة الحديد الثلاثي في الوسط ألحامضي ومفاعلة الحديد الثنائي الناتج مع الكاشف٢،٢'- ثنائي البريدين لينتج معقد احمر ذائب في الماء ومستقر ويعطي أعلى شدة امتصاص عند الطول ألموجي 523 نانومتر مقابل المحلول الصوري، وكانت قيمة الامتصاصية المولارية 7.1295×10^4 لتر. مول$^{-}$.سم$^{-}$ ^{١}. تم دراسة جميع العوامل المؤثرة على شدة امتصاص المعقد المتكون. كانت العلاقة الخطية في مدى التركيز من 2.5 إلى 80 مايكروغرام فنيل افرين في حجم نهائي 25 مل، والخطأ النسبي تراوح بين 0.79+ و0.82+ % والانحراف القياسي النسبي بين3.44± و 3.57± % اعتمادا على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير الفنيل افرين هيدروكلوريد في مستحضراته الصيدلانية (قطرة للانف ، شراب التوسيليت) وكانت نسبة الاستعادة متوافقة مع نتائج ألطريقه القياسية.