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Spectrophotometric determination of adrenaline in pharmaceutical preparations using Prussian blue reaction

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Abstract

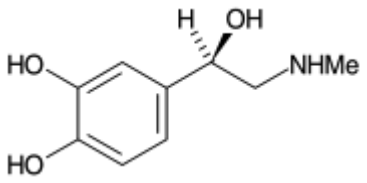
A simple ,sensitive and accurate spectrophotomeric method for the determination of adrenaline in pharmaceutical dosage forms has been developed. The method is based on the reaction of adrenaline with Fe^{+3} ion to produce Fe^{+2} which is upon further reaction with Potassium hexacynoferrate(III) to produce a Prussian blue color product that has a maximum absorption at λ_{max}=700nm.A graph of absorbance versus concentration shows that beer law is obeyed over the range 0.1-2.0 p.p.m with a molar absorpitivity of ($7.13x10^4$ $l.mole^{-1}.cm^{-1}$) ,a sandall sensitivity of($2.56x10^{-6}$ $\mu g.cm^{-2}$) ,correlation coefficient of 0.9990 , a relative standard deviation of (0.12-1.36%) and detection limit of 0.017$\mu g.ml^{-1}$ depending on the concentration. The optimum conditions for full color development are described and the proposed method was applied satisfactorily to pharmaceutical preparations containing adrenaline.

الخلاصة

يتضمن البحث الحالي تطويرطريقة طيفية بسيطه ذات دقة وحساسية عالية لتقدير دواء الادرينالين في المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الادرينالين مع ايون الحديد الثلاثي(III) الذي بدورة يتحول الى ايون الحديد الثنائي(II) . يتفاعل ايون الحديد الثنائي(II) مع المركب سداسي سيانيد الحديديك البوتاسوم (III) مكونا ناتج ذات لون ازرق(الصبغة البروسية) الذي يمتلك اعلى امتصاص عند طول موجي λmax = 700 نانوميتر. يشير الرسم البياني للامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التركيز (2.0-0.1) جزء لكل جزء بالمليون ,ومعامل الامتصاص المولاري مقدارة ($7.13x10^4$) لتر .مول$^{-1}$.سم$^{-1}$, ودالة ساندل مقدارها ($2.56x10^{-6}$) مايكروغرام.سم$^{-2}$, ومعامل الارتباط مقدارة 0.9990 والانحراف المعياري النسبي (0.12-1.36%) وحد الكشف مقدارة (0.017) مايكروغرام.مللتر$^{-1}$ اعتمادا على مستوى التركيز. تمت دراسة الظروف المثلى للطريقة المقترحة وتطبيقها على المستحضرات الصيدلانية الحاوية على الادرينالين .

Introduction

Adrenaline is 1-(3,4-dihydroxy phenyl)-2-methyl amino ethanol and it is used as beta-adrenoceptor agonist,used in treatment of glaucoma[1],has the following
Formula.



Various methods has been reported for the determination of adrenaline.These are include chromatographic[2,3], flow injection[4,5,6], spectrophotometric[7,8,9], and biosensors[10,11]. The formation of Prussian blue complex is a classical qualitative test used to detect $Fe^{(+2)}$ using hexacynoferrate(III)[12].
Many drugs has been determination using this test such as Amoxicilline[13], cephalosporine antibiotics[14],tinidazol[15],nifedipine[16] and folic acid[17].
The present study describes a new spectrophotometric method for the determination of Adrenaline in pharmaceutical preparations via formation a prussian blue color product that has a maximum absorption at λ_{max}= 700nm.

Experimental

Apparatus:

All spectral and absorbance measurement were carried out on a shimadzu UV-vis 1800 digital double beem recording spectrophotometer using 1-cm quarts Cells.

Reagents:

All chemicals used were of analytical reagent grade otherwisestate,adrenaline Standard powder was provided from cipla India,Mumbai,India.
Adrenaline injection ampule was provided from Aguettant.company – France.

- Adrenaline stock solution(1000 p.p.m):

0.1 gm of adrenaline was dissolve in 5ml(0.1M) HCl and made up to 100ml volumetric flask with distilled water.

- Ferric chloride (0.005M):

Prepared by dissolving 0.0811gm of $FeCl_3$ in 1ml of concentrated HCl and made up to 100ml volumetric flask with distilled water.

- Potissum hexacynoferrate(III) (0.001M):

Prepared by dissolving 0.0329gm of $K_3Fe(CN)_6$ in 100ml of distilled water.

Recommended procedure

In to a series of 25ml volumetric flask, transfer increasing volumes of adrenaline solution (10p.p.m) to cover the range of calibration curve(0.1-2.0p.p.m) ,added 1.6ml (0.005M) of $FeCl_3$ and shake well. Add 3ml(0.001M) of $K_3Fe(CN)_6$,dilute the solution to the mark with distilled water, and allow the reaction to stand for 15min in water bath at $50C^0$.Measure the absorbance at 700nm against a reagent blank prepared in the same way but containing no adrenaline. The color of the prussian Blue product is stable for 30min after that a blue precipitate was observed.

Procedure for pharmaceutical preparations

- Adrenaline ampoule($1mg.ml^{-1}$):

One ampoule content of adrenaline was transferred into a 100ml volumetric flask, add 5ml(0.1M) HCl and complete the volume to the mark with distilled water to obtained (10p.p.m) of adrenaline.The solution was suitable to analyzed by taking a convenient volumes in the range of calibration curve under general procedure.

Results and discussion

Absorption spectra:

When a diluted aqueous solution of adrenaline is treated with Fe^{+3} ion to produce Fe^{+2} which is in the presence of potissum hexacynoferrate(III) forms a prussian blue color product measurable at λ_{max} = 700nm(Fig.1). The absorbance of the Prussian blue color product is directly related to the concentration of adrenaline and can be used for it is spectrophotometric determination. The development of the color depends very much on the reaction Conditions, therefore it is very important to optimize the reaction conditions.

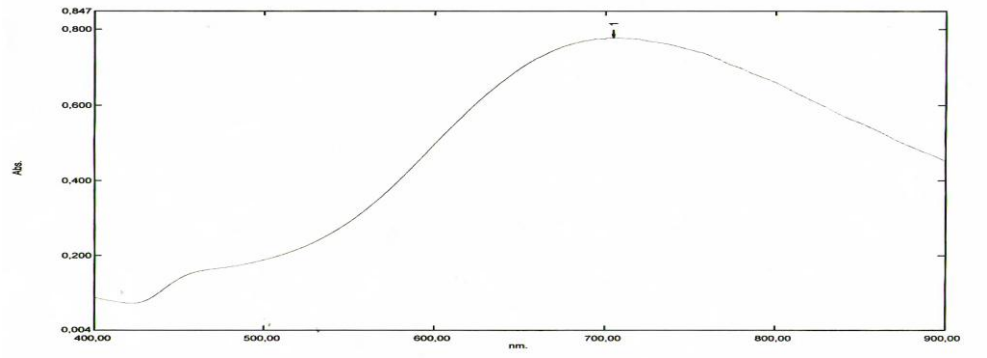


Fig.1: Absorption spectrum of the Prussian blue color product resulted from 2p.p.m of adrenaline with 1.6ml(0.005M) $FeCl_3$ and 3ml(0.001M) $K_3Fe(CN)_6$ after heating at $50C^0$ for 15min and measured against reagent blank.

Optimum reaction conditions

The effect of various parameters on the absorbance intensity of the product Formed were studied and the reaction conditions were optimized.

Effect of temperature:

Temperature greatly enhances the reaction, different temperature were tested from 5-$80C^0$,using both of ice bath and water bath.$50C^0$ gave the best result as shown in Fig.2.That is mean the reaction is endothermic.

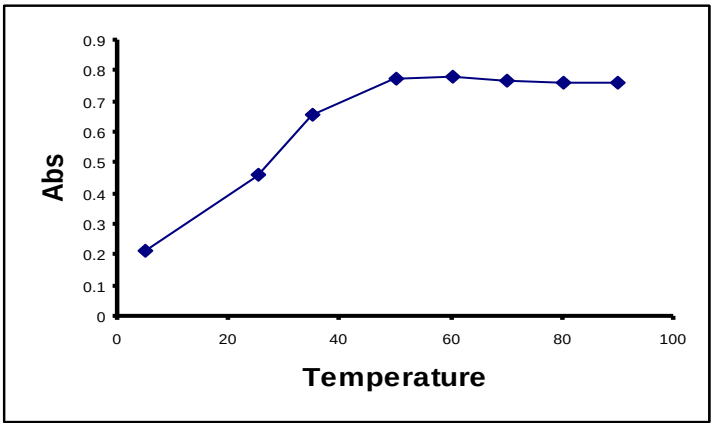


Fig.2: Effect of temperature on the absorbance of the Prussian blue color product.

Effect of Fe^{+3}ion concentration:

Fe^{+3} ion is reduced to Fe^{+2} ion by the adrenaline which is react with $K_3Fe(CN)_6$ to form a Prussian blue color product. The effect of different concentration was investigate in the range ($4x10^{-5}$ – $4x10^{-4}$M) in a final volume of 25ml , $3.2x10^{-4}$M of $FeCl_3$ gave the highest absorbance, therefore it is chosen for further use as shown in Fig.3

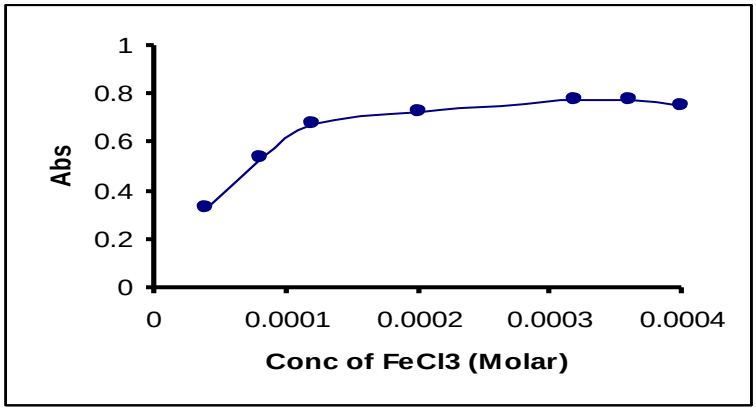


Fig.3: Effect of $FeCl_3$ concentration.

Effect of $K_3Fe(CN)_6$ concentration:

The effect of $K_3Fe(CN)_6$ concentration was similarly studied in the range ($2x10^{-5}$ – $1.8x10^{-4}$M) in a final volume of 25ml.The absorbance increased with increasing $K_3Fe(CN)_6$ concentration up to $1.2x10^{-4}$M ,above which is stable as shown in Fig.4.

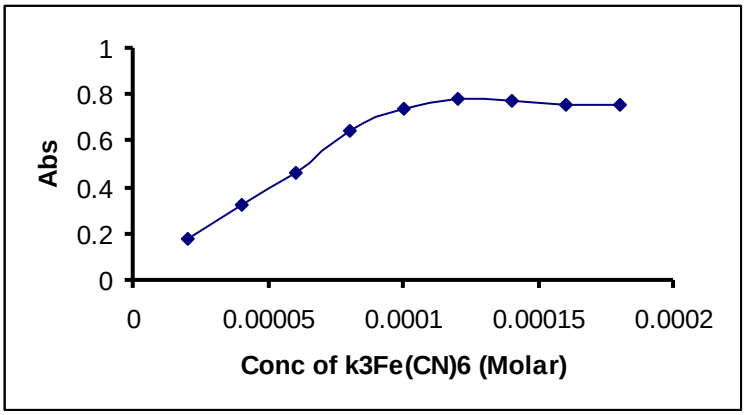


Fig.4: Effect of $K_3Fe(CN)_6$ concentration

Effect of order of addition:

A series of experiments were preformed to test the order of addition of reactants which gave the highest absorbance of the colored product. It was that the best order of addition is to add Fe^{+3} ion to adrenaline solution followed by the addition of $K_3Fe(CN)_6$. Because the adrenaline is reduced Fe^{+3} ion to Fe^{+2} ion which is upon further reaction with $K_3Fe(CN)_6$ to produce a Prussian blue color product as shown in table.1.

Table.1

Effect of order of addition for reactants

Arrangement	**Absorbance at λ_{max} = (700nm)**
1- adrenaline+Fe^{+3} +$K_3Fe(CN)_6$	0.778
2- adrenaline + $K_3Fe(CN)_6$ + Fe^{+3}	0.540
3- Fe^{+3}+ $K_3Fe(CN)_6$ + adrenaline	0.692

Effect of heating time:

The effect of heating time was studied in the range 5-60min as shown in Fig.5.At the beginning of the reaction, a green color is observed, the development of the Prussian blue color started after 10min and reached a maximum intensity in 15min in the water bath at $50C^0$ and is stable for 30min after that the absorbance started to decrease.

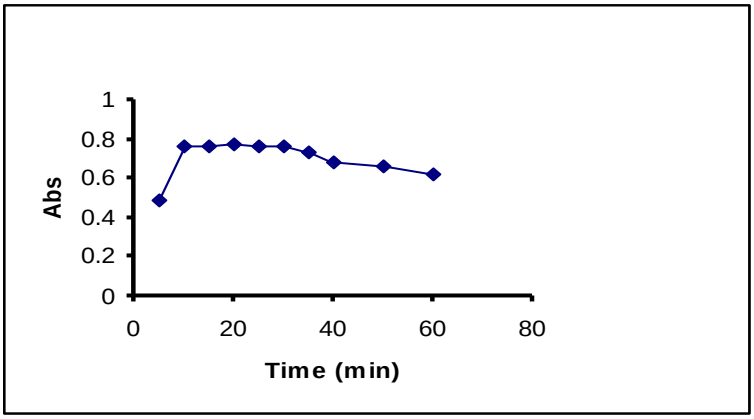


Fig.5: Effect of heating time

Calibration curve

Employing the conditions described under recommended procedure, a linear calibration curve for adrenaline is obtained(Fig.6),which shows that Beer law is obeyed over the concentration range(0.1-2.0p.p.m) of adrenaline with correlation coefficient of 0.9990 and an intercept 0.0184.
The conditional molar absorptivity of the Prussian blue color product was found to be (7.137×10^4 $l.mole^{-1}.cm^{-1}$) with reference to adrenaline and the sandell sensitivity was (2.566×10^{-6} $\mu g.cm^{-2}$).

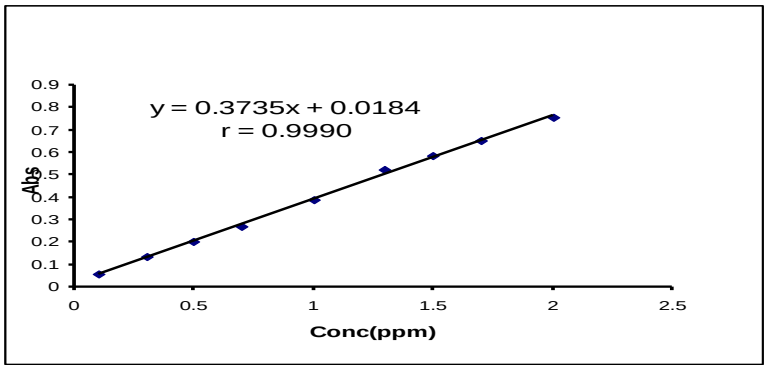


Fig.6: Calibration curve for adrenaline determination.

Accuracy and precision

To determine the accuracy and precision of the method,adrenaline was determined at three different concentration. The results obtained are shown in table.2.
A satisfactorily precision and accuracy could be obtained using the proposed method.

Table.2

Accuracy and precision of the proposed method for the determination of adrenaline

Amount of adrenaline p.p.m		Recovery* %	R.S.D* %	Error* %
Taken	Found			
0.5	0.504	100.8	1.36	0.8
1	0.981	98.1	0.36	-1.9
1.5	1.519	101.26	0.12	1.2

*Average of three determinations

Analytical application

One ampoule containing adrenaline($1mg.ml^{-1}$) has been analyzed using the calibration curve of adrenaline in the range (0.1-2.0p.p.m) giving a good accuracy and precision table.3.

Table.3

Application of the proposed method for the determination of adrenaline in pharmaceutical preparations.

Drug sample	Amount of adrenaline p.p.m		Recovery* %	R.S.D* %	Error* %
Adrenaline (Aguettant.co) France	Taken	found			
	0.5	0.48	97.6	0.70	-2.4
	1	0.99	99.4	0.36	-.50
	1.5	1.52	101.46	0.12	1.46

*Average of three determination.

Conclusions

The present method is proposed for the determination of adrenaline to be used in quantity analysis of the pharmaceutical preparations. The method is based on the oxidation of adrenaline with Fe^{+3}ion in the presence $K_3Fe(CN)_6$ to produced a Prussian blue color product. It has several advantages,

need neither long time or solvent extraction step. Therefore, the proposed method is considered to be suitable for the routine analysis.

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