Spectrophotometric Determination of Epinephrine in Pharmaceutical Preparations Using Praseodymium as Mediating Metals

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Abstract:

A simple, accurate and sensitive spectrophotometric method for the determinaion of epinephrine is described . The method is based on the coordination of Pr (III) with epinephrine at pH 6. Absorbance of the resulting orange yellow complex is measured at 482 nm .

A graph of absorbance versus concentrations shows that beer 's low is obeyed over the concentration range (1-50)μg.ml^{-1} of epinephrine with molar absorpitivity of (2.180x10^{3} L.mol^{-1}.cm^{-1}), a sandell sensitivity of (0.084 μg.cm^{-2}), a relative error of (-2.83%) , a corrolation coffecient (r= 0.9989) and recovery % (97.03 ± 0.75) depending on the concentration.This method is applied to analyse EP in several commercially available pharmaceutical preparations using direct methods .All statistical calculations are implemented via a Minitab software version 11.

Key words: Epinephrine, Spectrophotometric, Praseodymium.

Introduction:

Epinephrine[1-(3,4-dihydroxyphenyl)-2-methyloaminoethanol] is an active principle of the medulla of the suprarenal gland and is a drug used in treatment of cardiac arrest,heart block, asthma, nasal congestion, hypotension etc[1-3].

Medically, EP has been used as a common emergency healthcare medicine [4]. Also, low levels of EP have been found in patients with Parkinson's disease[5] .Epinephrine and dopamine are very important catecholamine neurotransmitters in the mammalian central nervous system. Catecholamine drugs are also used to treat hypertension, bronchial asthma and organic heart disease, and are used in cardiac surgery and myocardial infraction[6-9] .

Accordingly, the determination of the parent Epinephrine and their metal complexes necessitates the establishment of an accurate, rapid and reliable method.Various procedures have been described for estimation of epinephrine,. These include specrophptometry [10-12], flow injection[13-16], Thermogravimetric analysis coupled to FTIR[17], HPLC [18,19] capillary electrophoresis [20,21], fluorometry [22,23], chemiluminescence [24-27]. Authors have tried to quantify the Epinephrine spectrophotometry: Al-Ayash [28] estimation of adrenaline (ADH) in either pure form or in pharmaceutical preparations. The method is based on the reaction of adrenaline with vanadium (V) in acidic solution to

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from the colored complex which absorb at λ_{max} = 488 nm. A graph of absorbance versus concentration shows that Beer's low was obeyed over concentration range of (0.5–140)μg adrenaline mL^{-1} with a molar absorpitivity of ($2.015\times10^3 L.mol^{-1}.cm^{-1}$) , a sandell sensitivity of(0.09 μg. cm^{-2}) ,LOD (0.46μg.mL^{-1}),Recovery % (101.16± 0.97) , E_{rel} % (1.17± 0.97). Al-Abachi et al[29] determined the adrenaline using 1mM from the chloranil in basic medium (pH=9) and after heating to 60°c,which absorb at 350 nm, LDR (0.4–28μg.mL^{-1}), sandell sensitivity of (0.02589μg.cm^{-2}) and RSD%(2.3). Kothari and srinvasulu [30] used mixture of $NaNO_2$ and ammounium molybadate to assay the adrenaline in acidic medium (pH=3.7) and after one hour, which absorb at 475nm with a molar absorpitivity of (3500 $L.mol^{-1}.cm^{-1}$), liner dynamic range (1.5–22μg.mL^{-}) and sandell sensitivity of (0.052μg.cm^{-2}). Rodriguez - Dopazo et al[11] reported the determination adrenaline in acidic medium by mixing the adrenaline with iodine in chloroform and after extraction , the complex was measured at 375nm, yielding LOD of 1.5μg.mL^{-1} and RSD% of 2.1%.Most of spectrophotometric methods reported suffer from the disadvantages like the use of non-aqueous solvent, long time for reaction to complete and stability of the coloure product formed, etc,show table(5).

The proposed method does not require solvent extraction step and can be applied successfully to pharmaceutical preparations containing epinephrine

Material and Methods:

Apparatus

The absorption spectra were obtained with a cintra 5 spectrophotometer(180-1100) nm. The pH readings were obtained pH 211 HANNA instruments.

Reagents

Analytical – grade reagents and deionized water were used in the preparation and dilution of solutions; Epinephrine pure material and Pr_6O_{11} were provided from the BDH.

Procedure

Solutions

Stock solution of Epinephrine (1000 μg.mL^{-1}) was prepared by dissolving 0.1 g of EP in water and diluted to 100 mL and Praseodymium stock solution was dissolved 0.1214 g of Pr_6O_{11} in 5 mL of hydrochloride acid (5N) and diluted to 100 mL with water.

Absorption spectra

I- Epinephrine stock solution

2 mL of (100 μg.mL^{-1}) Epinephrine standard solution, was transferred to 5 mL volumetric flask, and diluted to the mark with water, 4 mL of this solution was transferred to absorbance cell, and then the absorption spectrum of this solution was measured in the region between 190 to 1100 nm using water as the reference. Fig (1,a) shows the three absorption maxima for the Epinephrine at 203, 220, and 278 nm.

II- Praseodymium(III) stock solution

2.5 mL of (100 μg.mL^{-1}) Praseodymium(III) stock solution, was transferred to 5 mL
volumetric flask, and diluted to the mark with water, 4 mL of this solution was transferred to absorbance cell, and then the absorption spectrum of this solution was measured in the region between 200 to 1100 nm using water as the reference. Fig (1,b) shows the absorption maxima for the Praseodymium(III) was at 198nm.

III- The complex of EP with Praseodymium(III)

The absorption spectrum of complex was measured in the region (200-1100nm)

using water as the reference. Fig (1,c) shows that the wavelength maximum was at

482 nm.

Preparation of Epinephrine drug

Epinephrine injection containing 1 mg Epinephrine per 1 mL was diluted to 25 mL with water.

Direct Calibration

Preparation of working standard solutions in (1-50 μg Epinephrine mL^{-1}) : A volume in range of 0.1-5 mL of 250 $\mu g.mL^{-1}$ standard Epinephrine solution into 25 mL volumetric flasks , then 4 mL of 250 $\mu g.mL^{-1}$ of Praseodymium standard solution was add to each flask and after adjusting the pH(6), each flask was diluted to mark with water. Solutions were immersed in water bath at temperature of $80^{o}c$ for 10 min.These solutions were set aside for 3 min, then the absorbance of solutions was measured at (λ_{max}=482 nm) against blank.

The calibration graph was constructed by regression (Fig.2) from which the concentration of epinephrine in drug samples were determination by regression.

Results and Discussion:

Optimization of experimental conditions

1- Effect of concentration of Praseodymium

It was found that the absorbance of Pr(III)-EP complex increases linearly as the concentration of praseodymium ion increases, the optimum concentration of Pr(III) of 40 $\mu g.mL^{-1}$ was selected for complete formation of complex (Fig.3).

2- Effect of temperature

The reaction of Pr(III) with EP was very slow , consequently, the effect of temperature was studied and it was found that the best temperature was $80^{o}c$ (Fig.4).

3- Effect of pH values

The effect of pH on the formation of Pr(III)-EP complex is shown in Fig (5); from which it appears that the best pH occur (6) for the formation of complex.

4- Effect of reaction time

Fig (6) refers that a reaction time of (3min.) is enough for complete complex formation.

Structure of the complex

Molar- ratio method have been used to elucidate the structure of Pr(III)-EP complex formed at optimal conditions and show in Fig (7). The data revealed that a 1:2 complex formed , the following equation of the complex was suggested:

$$\text{Adrenaline}(H_2LH^+)+\text{Pr(III)} \longrightarrow \text{Pr}(H_2LH^+)_2$$

Calibration Graph

Fig (2) shows a calibration graph of EP established by plotting the absorbance of complex vs. concentration and shows that beer's law is obeyed over the EP concentration of (1-50 μgmL^{-1}) at wavelength (482 nm).

Statistical Treatments

All measurment can be characterized statistically. Table (1) shows the linear range of Pr(III)-EP,detection limit, molar absorptivity(ε), sandell sensitivity(s) and confidence limits for the concentration and the absorbance .

Table (2) reveals that the test statistic t = 44.67 is higher than critical value (2.74) in regression analysis (r=0.9989). This means that the

predications based on the estimated regression line Y = 0.0119X + 0.0197 shoud be acceptable.Therefore , all concentration of EP in the analyzed sample was determind from this relationship. Table (3) shows the accuracy test in term of recovery. Recovery % was shown to be acceptable and found to be 97.03 ± 0.75. Good precision as E_{rel} of the method was achieved and found to be -2.83%.

Analytical applications

eigth types of pharmaceutical preparations containing adrenaline (injection) have been analyzed and they gave a good accuracy and precision as in(Table.4).The proposed method was also applied successfully on eigth types of injection.

Conclusions:

This study has shown that the method described allows a rapid determination of Epinephrine. The analytical scheme of the proposed system is simpler than that of other conventional procedures.

The analytical results obtained for the determination of EP in pharmaceuticals have shown a good agreement with the given labeled quantity. The complex formed have a stoichiometric ratio of 1: 2.

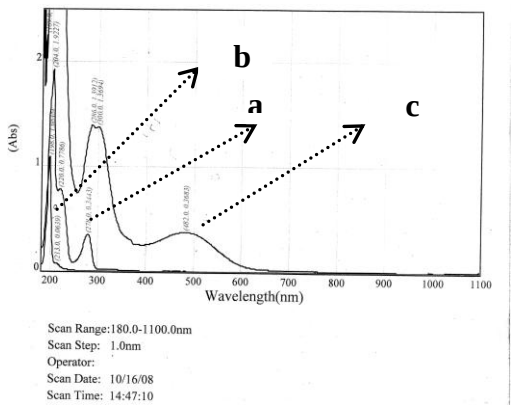

Fig. (1): Absorption spectrum (a) Drug (b) Ion (c) complex

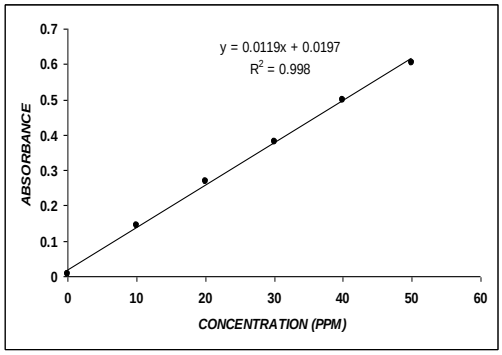

Fig. (2) Calibration graph for Pr(III)- Epinephrine

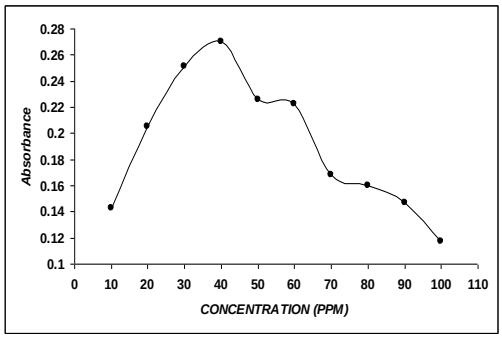

Fig. (3) Effect of Con. of Praseodymium on the determination of EP

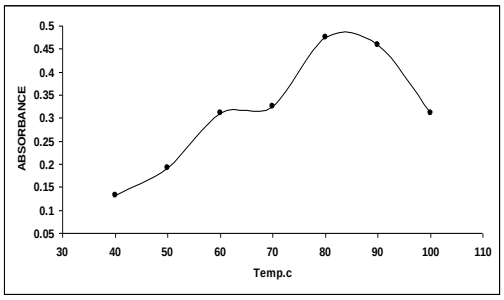

Fig. (4): Effect of Temperature

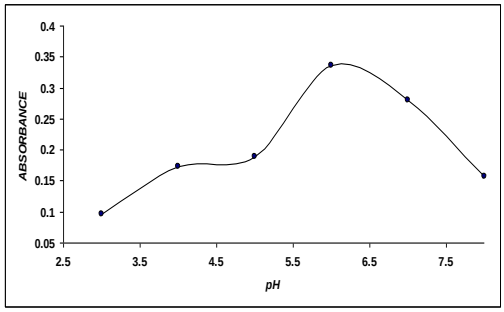

Fig. (5): Effect of pH

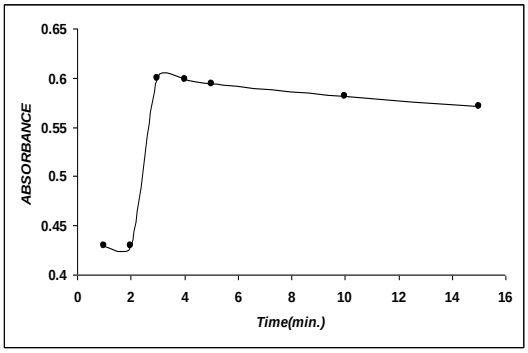

Fig. (6): Effect of reaction time

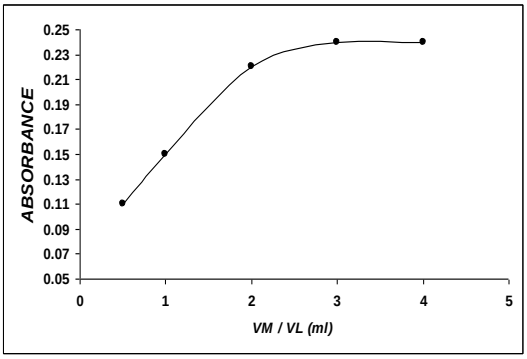

Fig. (7) Molar ratio for Pr(III)-EP

Table (1) : analytical characteristics of result

λ_{max} (nm)	Linearity ($\mu g.mL^{-1}$)	D.L.*** ($\mu g.mL^{-1}$) (n=13)	D.L.T** ($\mu g.mL^{-1}$)	S ($\mu g.cm^{-2}$)	Conf. Limit. Conc.($\mu g.mL^{-1}$) 95% C.I	Conf. Limit. Abs. 95% C.I	ε ($L.mol^{-1}.cm^{-1}$)
482	1-50	1.216	2.87	0.084	29.22 ± 3.182	0.3675 ± 0.023	$2.180x10^3$

*** **Experimental**

** **Theoretical**

Table (2) : Regression equation , correlation coefficient (r) two tailed t-test and confidence limit for the slope and the intercept at 95% confidence level and (n – 2) degree of freedom for the calibration graph .

Regre. Eq. Y=BX+A	Corr. Coef. (r)	t- test statistic	Tabulated t- test two tailed (n-2) 95% C.I	Conf. Limit. for the slope $b \pm t_{sb}$	Conf. Limit for the intercept $a \pm t_{sa}$
Y=0.0119X+0.0197	0.9989	44.67	2.78	0.0119 ± 0.007	0.0197 ± 0.021

Table (3) : shows the accuracy and precision of the proposed method

Amount of EP taken ($\mu g.mL^{-1}$)	Amount of EP found ($\mu g.mL^{-1}$)	%Rec.	%Erel.	%RSD n = 5	Mean %Rec. ±S.D	Mean %Erel.
20	19.3	96.5	-3.23	3.84	97.03 ± 0.75	-2.83
30	29	97.56	-2.43	3.54		

Table (4): Application of proposed method for the determination of Epinephrine in the pharmaceutical preparations

Name of pharmaceutical	Manufacturer	Stated conc. ($\mu g.mL^{-1}$)	Found direct calb. ($\mu g.mL^{-1}$)	Rec. %	RSD % n = 5	E_{rel} % n = 5
Adrenaline (INJ.)	Germany medicince	1000	955.53	95.53	2.70	-4.460
Adrenaline (INJ.)	Rotex medica Tittau.Germany	1000	1017.65	101.76	2.60	1.760
Epinephrine (INJ.)	Renaudin france	1000	1001.86	100.18	3.15	0.186
Epinephrine (INJ.)	Life phama italy	1000	987.88	98.75	2.80	-1.212
Epinephrine (INJ.)	Ciplea-india	1000	984.55	98.45	3.05	-1.545
Epinephrine (INJ.)	Global Parma UAE	1000	990.88	99	2.78	-1
Epinephrine (INJ.)	Holland medicines company	1000	996.76	99.67	3.44	-0.324
Epinephrine (INJ.)	Rowa tinex Germany	1000	960.88	96.08	3.08	-3.912

Table (5): Comparison between Proposed method with other spectrophotometer methods for determination epinephrine

Parameter	Spector.[W1] (µg ml-1)	Spector. [31] (µg ml-1)	Spector. [30] (µg ml-1)	Spector. [28] (µg ml-1)
Linear range	1-50	0.25-7	1.5-22	0.5-140
Corr. Coef.(r)	0.9989	0.9984	-	0.9992
pH	6	Acidic	3.7	2
Rec.%	97.03 ± 0.75	98.85± 0.65	-	101.16 ± 0.97
D.L.	1.21	0.15	-	0.46
λmax (nm)	482 nm	510	475	488 nm
Heating	80 °C	-		70 °C
Molar absorbtivity ($L.mol^{-1}.cm^{-1}$)	$2.180x10^3$	$2.13 x10^3$	3500	2.015×10^3

W1 : Proposed method

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تقدير دواء ايبفرين في بعض المستحضرات الصيدلانية بطريقة الاطياف المرئية وباستخدام عنصر البراسوديميوم كوسيط

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الخلاصة:

نظرا" لأهمية دواء ايبفرين وتأثيراته في الفعاليات البايولوجية حتى في التراكيز الاثرية فقد تضمن البحث استحداث طريقة تحليلية جديدة في تقدير المركب الدوائي ايبفرين Ep بطريقة الامتصاص الطيفي الجزيئي.

تم تقدير الدواء بتكوين المعقد Pr(III)-EP بعد تحديد الظروف العملية المثلى وهي الرقم الهيدروجيني (pH=6) وتركيز الايون Pr(III) (40 ميكروغرام.مل$^{-1}$) وافضل درجة حرارة لاكمال التفاعل 80c^{o} وزمن التفاعل 3 دقائق وتم التقدير عند الطول الموجي (482 نم) وتمت معرفة نسبة الاتحاد المولية بين الدواء والبراسوديميوم وهي (2:1).

أما مديات التركيز في تعيين الدواء فكانت (1-50 ميكروغرام.مل$^{-1}$) ومعامل الارتباط (0.9989 = r) وحساسية ساندل (0.084 ميكروغرام.سم$^{-2}$) والممتصية المولارية (2.180 x 10^{3} لتر.مول$^{-1}$.سم$^{-1}$) وحد الكشف (1.21 ميكروغرام.مل$^{-1}$) والخطأ النسبي المئوي (2.83-) والاسترددادية (97.03 ± 0.75) .كما تم تعيين الدواء في بعض المستحضرات الصيدلانية الموجودة في الاسواق المحلية .