A NEW SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ADRENALINE IN PHARMACEUTICAL PREPARATIONS

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Abstract

A sensitive and simple spectrophotometric method for the 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 from the colored complex which absorb at λ_{max} = 488 nm. A graph of absorbance versus concentration shows that Beer 's low is obeyed over concentration range of (0.5–140) μg adrenaline ml^{-1} with a molar absorpitivity of (2.015×10^3 Lit. $mol^{-1}.cm^{-1}$), a sandell sensitivity of (0.09 $\mu g.cm^{-2}$) ,LOD (0.46$\mu g.ml^{-1}$) , Recovery % (101.16± 0.97) , E_{rel} % (1.17± 0.97) .

The mole–ratio method (1:2) approved that ADH- V (V) as a structure of the complex. The optimum conditions for full colour development are described and the proposed method was satisfactorily applied for the determination of ADH in pharmaceutical preparations. The response surface method (RSM) was applied for optimization of experimental condition and data obtained were found similar.

Key words: Adrenaline determination; Spectrophotometry; Vanadium; chelating complex; Basic media.

Introduction

Adrenaline, also called epinephrine (EP), is a component of neural transmission media that has an effect on the transmission of nerve impulse, and is an important hormone secreted by the medulla of the adrenal glands. It is also served as a chemical mediator for conveying the nerve pulse to efferent organs [1,2].

As a medicine, EP is used to stimulate heartbeat and to treat emphysema, bronchitis, bronchial asthma and other allergic conditions, as well as in the treatment of the eye disease, glaucoma [3,4].

Therefore, performing the research of EP has an important significance to medicine and life science. Adrenaline 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 [5,6]. Hormones acted as chemical messengers controlling the activity of living things. In humans, they were released in small amounts in the blood stream from a number of vital sites especially some brain regions. Dopamine was recognized as neurotransmitter in its own right but the demonstration of its non-uniform distribution in the brain suggested that it might had a specific functional role of dopamine [7]. It had an important role in the pathogenesis of drug treatment of certain brain diseases, e.g. Parkinson's disease and schizophrenia [8].

A variety of methods have been used for the determination of adrenaline in pharmaceutical products, including specrophtometric[9-11],flow injection[12-15], Thermogravimetric analysis coupled to FTIR[16], HPLC[17,18]capillary electrophoresis[19,20], fluorometry [21,22], chemiluminescence [23-26].

A few authors have tried to quantify the adrenaline spectrophotometry: Al-Abachi et al[27] determined the adrenaline using 1mM from the chloranil in basic medium (pH= 9) and after heating to 60^oc , which absorb at 350 nm, LDR (0.4–28 μg. ml^{-1}), sandell sensitivity of (0.02589 $\mu g.cm^{-2}$) and RSD% (2.3).

Kothari and srinvasulu [28] 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 475 nm with a molar absorpitivity of (3500 Lit.mol^{-1}.cm^{-1}), liner dynamic range(1.5–22 μg ml^{-1}) and sandell sensitivity of (0.052 μg.cm^{-2}).

Rodriguez-Dopazo et al[10] reported the determination adrenaline in acidic medium by mixing the adrenaline with iodine in chloroform and after extraction , the complex was measured at 375 nm, yielding LOD of 1.5 μg. 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 coloured product formed, etc.

The idea of the present method is to provide simple, sensitive and rapid spectrophotometer determination of ADH in pharmaceutical products and the method is environmentally- Friendly, as no organic solvent is needed.

Experimental

Instruments

The absorption spectra were obtained with a cintra 5 spectrophotometer. The pH readings were obtained the experimentation using pH 211 HANNA instruments.

Reagents

Analytical–grade reagents and deionized water were used in the preparation and dilution of solutions; adrenaline standard material was provided from the BDH, $NaVO_3.H_2O$ from BDH.

Procedure

Solutions

Stock solution of adrenaline was prepared by dissolving 0.1 g of ADH in deionized water and diluted to 100 ml and vanadium stock solution was dissolved 0.2747 g of $NaVO_3.H_2O$ in 5 ml of hydrochloride acid (3N) and diluted to 100 ml with deionized water.

Absorption spectra

I- Adrenaline stock solution

0.1 ml of (1000 μg.ml^{-1}) adrenaline 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 200 to 500 nm using water as the reference. Fig. (1) shows the three absorption maxima for the adrenaline was 203, 220, and 278 nm.

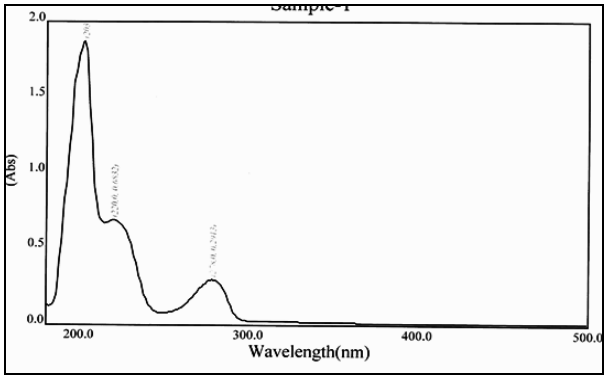

Fig. (1) : Absorption spectrum of Adrenaline.

II- Vanadium (V) stock solution

0.1 ml of (1000 μg.ml^{-1}) vanadium (V) 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 400 nm using water as the reference. Fig. (2) shows the two absorption maxima for the vanadium (V) were at 196 and 207 nm.

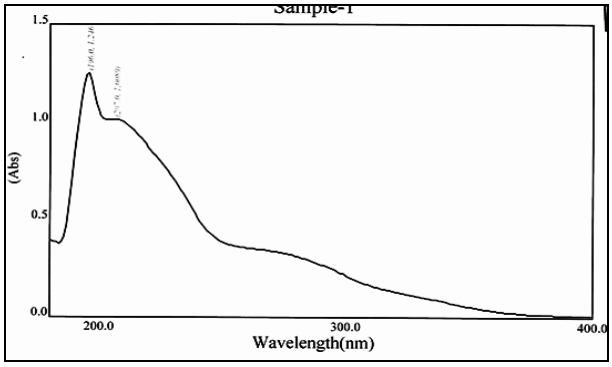

Fig. (2) : Absorption spectrum of vanadium.

III- Brown-Radish complex of ADH with vanadium (V).

The absorption spectrum of complex was measured in the region (300-1100 nm) using water as the reference. Fig. (3) shows that a wavelength maximum was 488 nm.

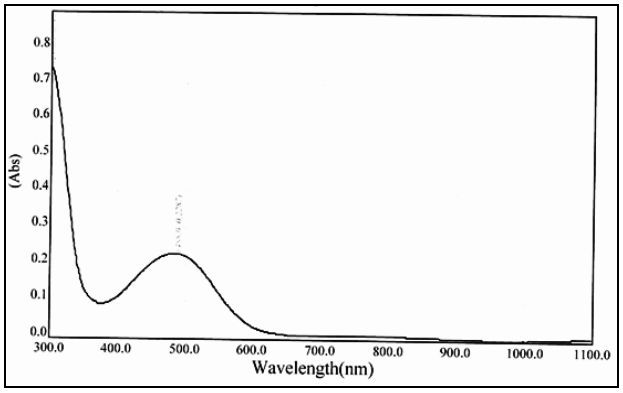

Fig. (3): Absorption spectrum of ADH-V (V).

Preparation of Epinephrine drug

Epinephrine injection containing 1 mg adrenaline per 1 ml. This solution was then diluted to 25 ml with water.

Direct Calibration

Preparation of working calibration solutions in (0.5-140 μg adrenaline ml^{-1}) : A volume in range of 0.025-7 ml of 500 $\mu g.ml^{-1}$ standard adrenaline solution into 25 ml volumetric flasks , then 2.5 ml of 500 $\mu g.ml^{-1}$ of vanadium standard solution was add to each flask and after adjusting the pH (2), each flask was diluted to mark with water. Solutions were immersed in water bath at temperature of 70 ^{o}C for 4 min. These solutions were set aside for 25 min, then the absorbance of solutions was measured at (λ_{max}=488 nm) against blank.

The calibration graph was constructed by regression Fig.(4) from which the concentration of adrenaline in epinephrine drug sample was determination by regression.

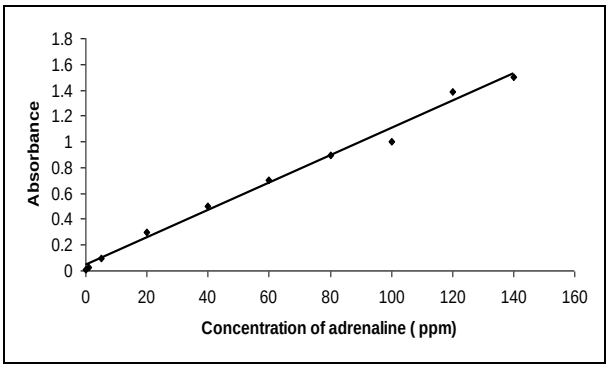

Fig. (4) : Calibration graph for ADH-V (V).

Results and discussion

Optimization of experimental conditions

1- Effect of concentration of vanadium (V)

It was found that the absorbance of ADH-V (V) complex increases linearly as the concentration of vanadium (V) ion increases and the deviation from this linearity was appared by curve bending towards the vanadium concentration axis Fig.(5).

Consequently, the optimum concentration of V (V) of 50 $\mu g.ml^{-1}$ was selected for complete formation of chelating complex.

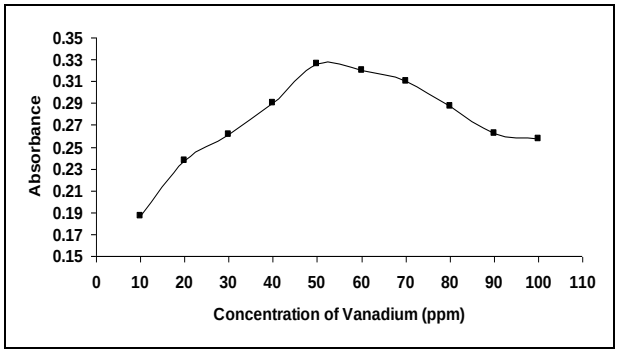

Fig (5) : Effect of Con. of Vanadium on the determination of ADH.

2- Effect of temperature

The reaction of V (V) with ADH was very slow and took about one hour, consequently, the effect of temperature was studied and found that the best temperature was 70 ^{o}C Fig.(6) to obtain maximum absorbance and decreased thereafter due to the decomposition of the complex.

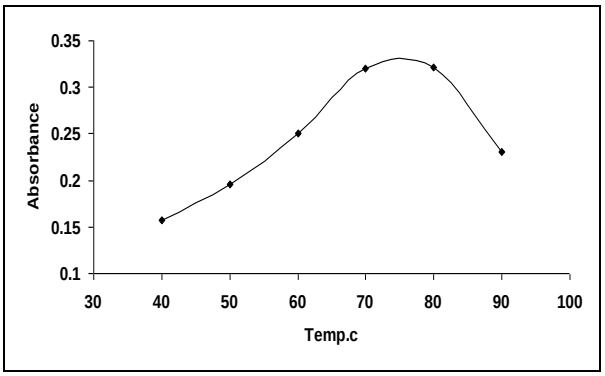

Fig. (6) : Effect of Temperature.

3- Effect of pH values

The effect of pH on the formation of ADH-V (V) complex is shown in Fig. (7); from which it appears that the best pH occur (2) for the formation of chelate complex.

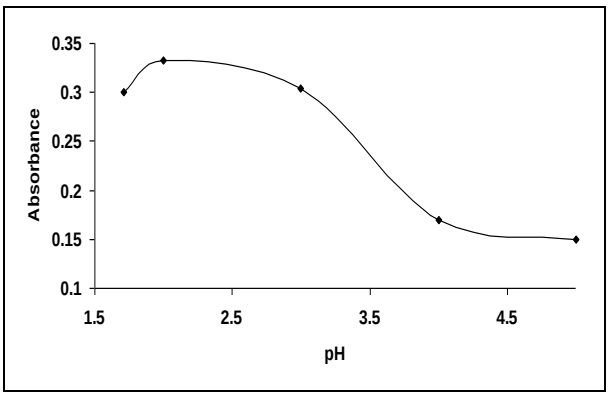

Fig. (7) : Effect of pH.

4- Effect of reaction time

Fig. (8) refers that a reaction time of (25 min.) is enough for complete complex formation.

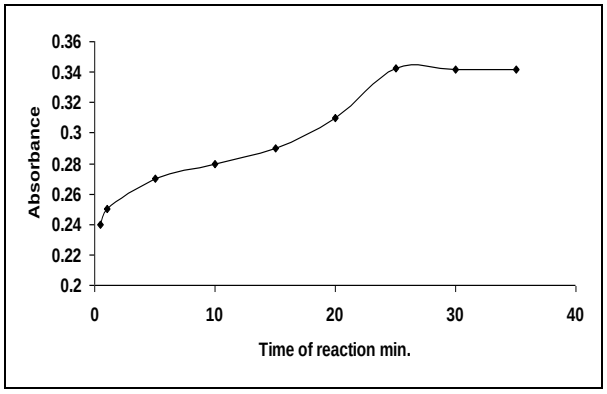

Fig. (8) : Effect of reaction time.

Structure of the complex

Molar- ratio methods have been used to elucidate the structure of ADH-V (V) complex formed at optimal conditions and show Fig. (9). The data revealed that a 1:2 complex.

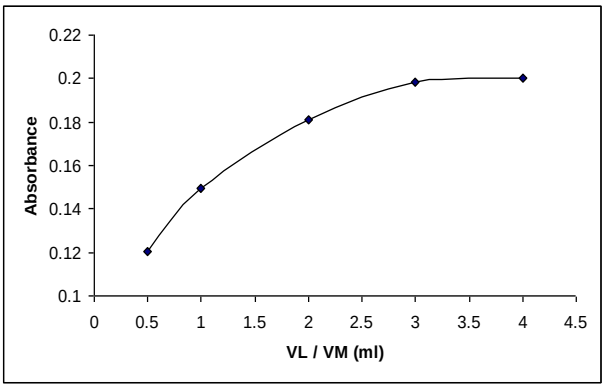

Fig. (9) : Molar ratio for ADH-V (V).

Optimization and Experimental Design

Response Surface Methods (RSM)

Response surface methods are very useful in order to quantify and interpret the relationships between responses and factor effects. The Response Surface Method (RSM) using Screening Design (SD) was also applied to estimate the effects of factors for the extraction of chelating complex on statistical basis.

Three main factors were selected such as the concentration of vanadium (V) ions (Cppm), the pH and temperatures. Table (1) shows the coding of these factors at two levels and Table (2) represents the 2^3-screeing design and factor levels for the estimation of the above mentioned factors.

The factor effects were calculated as the difference between the responses of a factor at high and low level. These differences were then tested against the experimental error expressed by the standard deviation multiplied by the student's t-value.

The factor effects were evaluated according to the relationships described elsewhere [29], and the results were shown in Table (3).

Data have shown that the comparison of the experimental error with absoult differences reveal that the main factors pH and the concentration of vanadium (V) ions (Cppm) show a significant effect (DpH and Dcppm are higher than 0.0188), while the effect of temperature can be neglected in the studied ranged between 80 and 90 ^{o}c (i.e there is a minimal influence by the temperature.

From the above study, the factors pH and Cppm were found to be significantly influenced on the extraction of the chelating complex ADH-V (V).A design at three levels, a Box- Behnken design was run at optimal temperature in order to study the relationship between the response and the significant two factors.

Table (4) shows the coding of the two factors at three levels, and Table (5) describes the factors at three levels according to Box-Behnken. The response surfaces were drawn graphically Fig.(10 and 11).

It can be concluded that the curved dependences in the direction of both factors lead to a maximum absorbance at coded level

of pH and Cppm to the range close to the optimal values.

Then, the surface startes to fall-off slightly in the case of increasing factor value from the optimal limit.

However, the response surface was observed to be depressed extremely toward the least factor value , hence , inferring that it is necessary to maintain the pH at level higher than 2 and lower than 5, and the same situation for concentration of vanadium (V).

Table (1)
Coding factors at two levels..

Factor	+1	–1
pH	5	2
Temp. ^{o}C	90	80
C_{ppm}	100	50

Table (2)
2^3-Screeing design and factor levels for estimation of the factors pH-values, the temperature and the concentration of vanadium(V).

Run	pH	$C_{ppm.}$	Temp.	pH.$C_{ppm.}$	pH.Temp.	$C_{ppm.}$Temp.	Response (Absorbance)
1	–1	+1	+1	–1	–1	+1	0.2577
2	–1	–1	+1	–1	+1	–1	0.3200
3	-1	–1	1	+1	+1	+1	0.3400
4	–1	+1	1	+1	–1	–1	0.2500
5	+1	+1	+1	+1	+1	+1	0.1300
6	+1	1	+1	+1	–1	–1	0.1700
7	+1	1	–1	–1	–1	+1	0.1360
8	+1	+1	–1	–1	+1	–1	0.1400

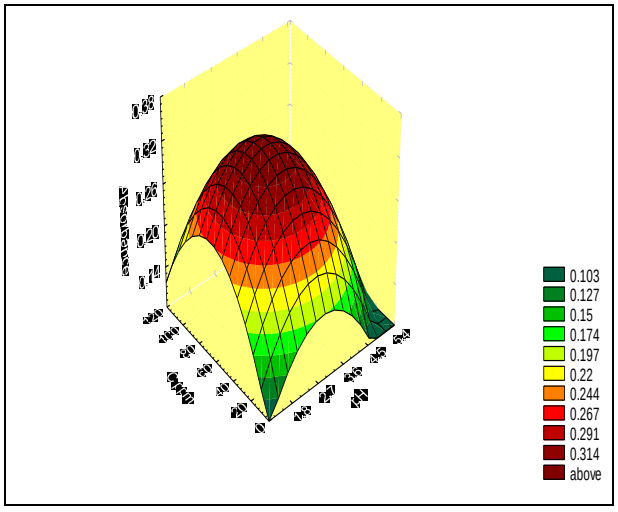

Fig. (10) : Screening surface plot of absorbance versus the factors pH and Cppm.

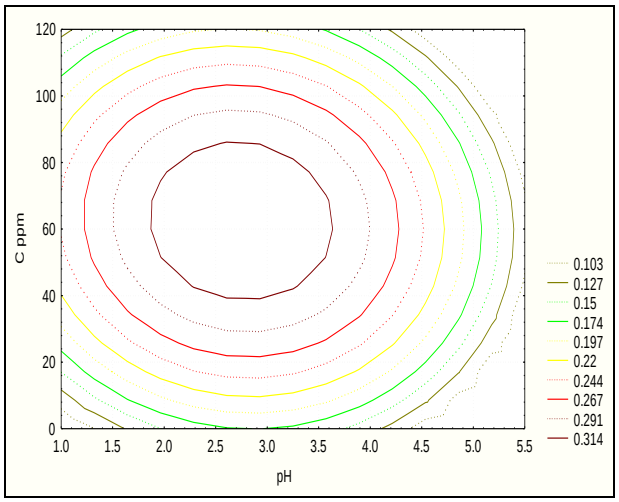

Fig. (11) : Contour plot of absorbance versus the factors pH and Cppm.

Table (3)
The comparison of the experimental error with the absolute Differences.

Factor	value	T(95% C.I,n=4)S.D
D_{pH}	0.2958	0.0188
D_{Cppm}	0.0941	0.0188
$D_{Temp.}$	0.0058	0.0188
$D_{pH.Cppm.}$	0.0581	0.0188
$D_{pH.Temp.}$	0.0150	0.0188
$D_{Cppm.Temp.}$	0.0081	0.0188

Table (4)
Coding the two factors at three levels.

Factor	Level		
	+1	0	–1
pH	5	2	1.7
$C_{ppm.}$	100	50	20

Table (5)
Factor levels and Box-Behnken design for studing the ADH determination by spectrometric.

Box-Bbehnken level			
Run	pH	C_{ppm}	Response (Absorbance)
1	+1	+1	0.1300
2	+1	–1	0.1000
3	–1	+1	0.2500
4	+1	0	0.1500
5	–1	0	0.3000
6	0	+1	0.2500
7	0	–1	0.1866
8	–1	–1	0.1800
9	0	0	0.3430

Calibration graph

Fig. (1) shows a calibration graph of ADH established by plotting the absorbance of complex vs. concentration and shows that beer's law is obeyed over the range concentration of (0.5-140 μg adrenaline ml^{-1}) at wavelength 488nm.

Statistical calculations

All measurement can be characterized statistically Table (6) shows the liner range of ADH-V(V) and detection limit, molar absorptivity (ε), sandell sensitivity (s) and confidence limits for the concentration and the absorbance .Table (7) reveals that the test statistic t =72.97 is higher than critical value (2.31) in regression analysis (r = 0.9992).

This means that the predications based on the estimated regression line Y=0.0412+0.011X should be acceptable therefore, all concentration ADH in the analyzed sample was determined from this relationship.

Table (8) shows the accuracy test in term of recovery.Recovery% was shown to be acceptable and found to be 101.16±0.97 Good precision as E_{rel} % of the method was achieved and found to be 1.17±0.97.

Table (6)
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 ($\mu g.ml^{-2}$)	Conf. Limit. Conc. ($\mu g.ml^{-1}$) 95%C.I	Conf. Limit. Abs. 95%C.I	ε ($L.mol^{-1}.cm^{-1}$)
488	0.5-140	0.46	6.36	0.09	60.33±1.86	0.705±0.02	2.015×10^3

*** *Experimental,*
** *Theoretical*

Table (7)
Regression equation , correlation coefficient (r) two tailed t-test and confidence limit for the slope for 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=0.011X+0.0412	0.9992	72.97	2.31	0.011±0.0022	0.0412±0.0082

Table (8)
Shows the relative standard deviation RSD%, $Erel_{\%}$, recovery Rec% of the proposed method.

Amount of ADH Taken ($\mu g.ml^{-1}$)[1]	Amount of ADH found ($\mu g.ml^{-1}$)	% Rec.	% Erel.	%RSD n=5	Mean %Rec.+S.D	Mean %Erel.
40	40.89	102.22	2.22	0.86	101.16±0.97	1.17±0.97
80	80.77	100.96	0.96	0.78		
120	120.4	100.3	0.33	0.88		

Table (9)
Application of proposed method for determination of adrenaline in pharmaceutical preparation.

Name of pharmaceutical	Manufacturer	Stated conc. ($\mu g.ml^{-1}$)	Found direct calb ($\mu g.ml^{-1}$)	Rec. %	RSD % n=5	E_{rel} %
Epinephrine (INJ.)	Life phama italy	1000	990.8	99.8	0.98	0.92
Epinephrine (INJ.)	Renaudin france.	1000	1000.9	100.9	0.45	0.09
Adrenaline (INJ.)	Rotex medica Tittau.Germany	1000	1010.32	101.32	0.88	1.032
Adrenaline (INJ.)	Germany medicince	1000	940.67	94.67	1.1	5.93
Epinephrine (INJ.)	Ciplea-india	1000	980.8	98.2	0.76	1.92

Analytical applications

Five types of pharmaceutical preparations containing adrenaline (injection) have been analyzed and they gave a good accuracy and precision.

The proposed method was also applied successfully on five types of injection and the results obtained are given in Table (9).

Conclusion

A simple accurate and sensitive spectrophotometric method has been developed for the determination of trace amounts of adrenaline in aqueous solutions based on its reaction between adrenaline with vanadium. The proposed method does not require the solvent extraction step. The method was applied successfully on different pharmaceutical samples.

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الخلاصة

يتضمن البحث استحداث طريقة طيفية لتقدير الكميات النزرة من دواء الأدرينالين في المحاليل المائية باستخدام المطياف الفوتوميتري . اعتمدت الطريقة على تفاعل الأدرينالين مع الفناديوم في وسط حامضي حيث يتكون معقد ذو لون جوزي محمر وأعطت أعلى قمة امتصاص عند طول موجي(٤٨٨ نانوميتر) وأشار الرسم البياني الخطي للامتصاص مقابل التركيز بأن قانون بير ينطبق ضمن مدى التركيز(٠.٥ – ١٤٠ مايكروغرام من الأدرينالين لكل مل) . أما قيمة الامتصاصية المولارية كانت مساوية إلى (2.015×10^3 لتر.مول$^{-1}$.سم$^{-1}$) وقيمة حساسية ساندل (٠.٠٩ مايكروغرام.سم$^{-2}$) مع خطأ نسبي مقدار (١.١٧ ± ٠.٩٧%) واستردادية (0.97 ±101.16%) اعتمادا" على مستوى التركيز المراد تحديده ، تمت دراسة الظروف المثلى للتفاعل وجرى تطبيق الطريقة في تعين الأدرينالين في المستحضرات الصيدلانية. كذلك تمت دراسة طريقة سطح الاستجابة (RSM) في هذه الطريقة لتقويم العوامل وإيجاد الظروف المثلى للعمل إحصائيا" وكانت النتائج مقاربة للنتائج التجريبية.