SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF ADRENALINE IN PHARMACEUTICAL DOSAGE FORM USING METHYLTHIAZOLYLDIPHENYL-TETRAZOLIUM-BROMIDE (MTT) [+]

Saladdin M.AL-Talib [*] Thabit S.Al-Ghabsha [**] Theia'a N. Al-Sabha [***]

Abstract:

A sensitive and simple spectrophotometric method is developed for the determination of adrenaline. The method is based on the reaction of adrenaline with methylthiazolyldiphenyl- tetrazolium- bromide (MTT) in basic medium and the increase of reagent's color intensity is measured at 536 nm against reagent blank. Beers law was valid over the concentration range of 0.1 – 3.0µg.ml^{-1} with molar absorptivity of 1.179 × 10^{5}l.mol^{-1}.cm^{-1}, and Sandell sensitivity of 0.00156 µg. cm^{-2}. The relative error is less than ± 3%, and the relative standard deviation of better than ± 1.8% depending on the concentration. The proposed method was successfully applied to the assay of the adrenaline in dosage form.

Keyword: Spectrophotometry, Adrenaline, Methylthiazolyldiphenyl- tetrazolium-bromide (MTT).

التقدير الطيفي للأدرينالين باستخدام الكاشف مثيل ثيازولايل ثنائي فنيل تترازوليوم بروميد

صلاح الدين محمد الطالب ثابت سعيد الغبشة ضياء نجم الصبحة

المستخلص:

تم تطوير طريقة طيفية سهلة وحساسة لتقدير كميات مايكروغرامية من الادرينالين .تعتمد الطريقة على تفاعل الادرينالين مع مثيل ثيازولايل ثنائي فنيل تترازوليوم بروميد (MTT) بوسط قاعدي وقياس الزيادة في شدة لون الكاشف عند طول موجي 536 نانوميتر مقابل المحلول الصوري . لقد اتبعت الطريقة قانون بير عند مدى تركيز 0.1–3.0 مايكروغرام . مللتر$^{-1}$وبامتصاص مولاري 1.17× 10^{5} لتر.مول$^{-1}$. سم$^{-1}$ وحساسية ساندل 0.00156 مايكرو غرام. سم$^{-2}$ وخطا نسبي اقل من ± 3% وانحراف قياسي نسبي أفضل من ±1.8% اعتمادا على التركيز. امكن تطبيق الطريقة بنجاح في تقدير كمية الأدرينالين بمستحضره الصيدلاني

Introduction:

Adrenaline [(R)-1-(3.4-dihydroxyphenyl)-2-methylaminoethanol] (I) belongs to a group of catechol amines, which affected the constriction of blood vessels and control tissue metabolism by increasing the glucose and lactic levels [1]

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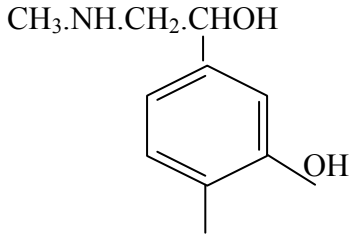
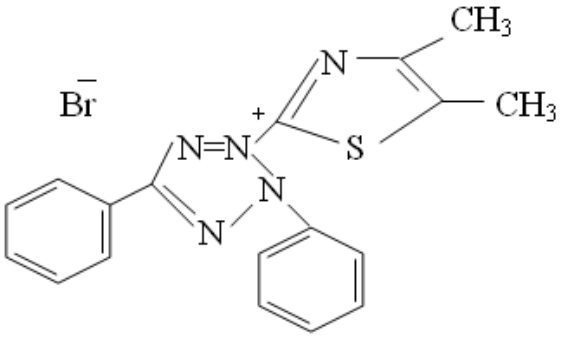
[*] Professor / Technical Institute / Mosul
[**] Professor / College of Education / Mosul University
[***] Assistant Prof. / College of Education / Mosul University



(I)

Many spectrophotometric methods have been proposed for the determination of adrenaline using several reagents such as chloranil [2], iron (III) in the presence of potassium hexacynoferrate(III) [3] and in the presence 1,10-phenanthroline [4], Tyramine in the presence of potassium metaperiodate [5], semicarbazide hydrochloride in the presence of potassium persulfate [6], 2,6-dichloroquinone-chlorimide [7], 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [8]. Other techniques have also been reported for the determination of adrenaline such as fluorimetry [9], elctrochemical [10] and high performance liquid chromatography [11]. Methylthiazolyldiphenyl–tetrazolium-bromide (MTT)(II) have been extensively evaluated as indicators of cell viability in this high-flux in vitro antitumor drug screening effort. The validity of MTT assay depends, in part, on the assumption that only viable cells reduce tetrazolium salts to colored formazan derivatives which can be quantitated spectrophotometrically[12].



(II)

The present work describes a simple, sensitive and accurate spectrophotometric method for the determination of adrenaline using Methylthiazolyldiphenyl- tetrazolium- bromide (MTT) in alkaline medium.

Experimental:

Apparatus:

All spectral absorbance measurements were carried out on single beam spectrophotometer Cecil (UV-VIS) using 1-cm
silica cell.

Reagents:

All chemicals used were of analytical reagent grade.

Stock solution of adrenaline (200 µgml^{-1}): Prepared by dissolving 0.02 g of pure adrenaline, provided from Sammara drug industries; SDI, Iraq, in distilled water in the presence of 0.5 ml of 0.1 M NaOH and the solution was made up to 100 ml in volumetric flask with distilled water and stored in amber colored bottle in a refrigerator . Working standard solutions were prepared by further dilution of stock solution.

Thiazolyl blue tetrazolium bromide (MTT) solution (2x10^{-4} M): Prepared by dissolving 0.0166 g of MTT in 10 ml of acetone and the solution was made up to 200 ml in a volumetric flask with distilled water.

Sodium hydroxide solution 0.1 M.

Recommended procedure for calibration:

Into a series of 25-ml calibrated flasks 5 ml of 2x10^{-4} M (MTT) solution were transferred, then an accurately measured volume containing 0.1 – 3 µg / ml of adrenaline were added and followed by the addition of 3 ml of 0.1 M sodium hydroxide, then the volume was completed to the mark with acetone and allowed to stand for 30 minutes at room temperature. The intensity of the of bluish- violet color was measured at 536 nm against reagent blank solution.

Procedure for the assay of adrenaline hydrochloride injection:

An accurately measured volume of the mixed contents of 3 ampoules (each contains 1 mg/ml of pure adrenaline), and a volume equivalent to one injection content was diluted with water and a suitable aliquot was treated as described under the recommended procedure.

Results and Discussion:

A bluish-violet colored product with an absorption maximum at 536 nm was formed when adrenaline was allowed to react with MTT in the presence of sodium hydroxide. The absorption spectrum of the resulting product is shown in Fig. 1.

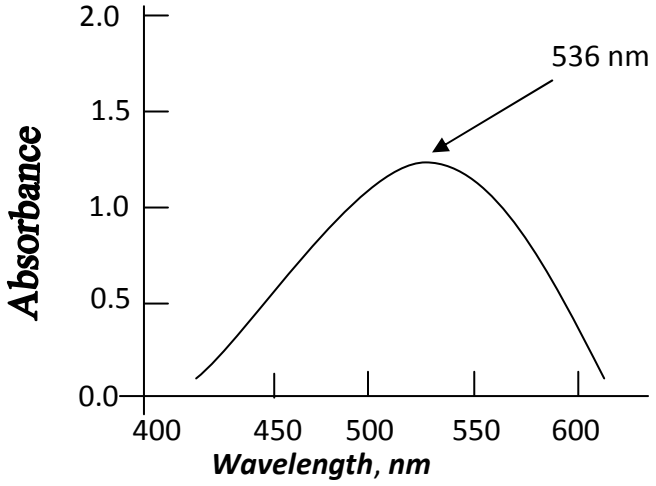


Figure 1: Absorption spectra of adrenaline (3 µg ml^{-1}) MTT reagent (2x10^{-4} M) against reagent blank

Optimum reaction condition:

The optimum reaction conditions for quantitative determination of adrenaline were established doing a number of preliminary experiments. The absorbance of a series of solutions were measured by varying one parameter and fixing the other parameters at 536 nm.

Effect of solvent on the absorbance and stability of the product:

The effects of different solvents on the color intensity of the reaction product were studied. The results obtained (Table 1) indicated that acetone is the suitable solvent and showed that the product needed 30 min to attain maximum absorbance and it remained stable for 45 min time, after that a slight decrease in absorbance was observed .

Table 1: Effect of solvent on the absorbance and stability of the product.

solvent	Measurment	Absorbance/ min standing time						
		5	15	30	45	60	75	90
Methanol	S vs. W	1.131	1.146	1.162	1.255	1.250	1.239	1.236
	B vs. W	0.251	0.355	0.467	0.664	0.708	0.811	0.894
	S vs. B	0.880	0.791	0.695	0.591	0.542	0.428	0.342
Ethanol	S vs. W	1.556	1.586	1.582	1.582	1.579	1.577	1.546
	B vs. W	1.248	1.320	1.420	1.438	1.440	1.453	1.466
	S vs. B	0.308	0.266	0.162	0.144	0.139	0.124	0.080
Acetone	S vs. W	1.320	1.358	1.336	1.338	1.334	1.328	1.271
	B vs. W	0.686	0.640	0.606	0.604	0.603	0.601	0.558
	S vs. B	0.634	0.718	0.730	0.734	0.731	0.727	0.713
Water	S vs. W	1.088	1.053	0.958	0.903	0.847	0.801	0.764
	B vs. W	0.270	0.282	0.297	0.314	0.337	0.362	0.402
	S vs. B	0.818	0.771	0.661	0.589	0.510	0.439	0.362

5 ml $2x10^{-4}$ M MTT , 1 ml $2x10^{-4}$ M Adrenaline ,
3 ml 0.1 M sodium hydroxide, R.T, λ max 536
S= sample, B=blank, W= water

Effect of MTT concentration:

The effect of changing the MTT concentration on the absorbance was studied. From figure 2 it is evident that the absorbance is increased with increasing MTT concentration and reached maximum absorption when using 5 ml of 2×10^{-4}M MTT. Therefore , this volume has been used in all subsequent experiments.

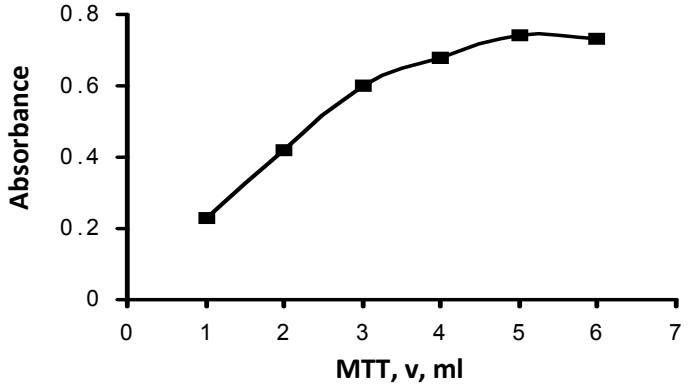


Figure 2. Effect of MTT volume ($2x10^{-4}$ M) on the absorption of 1 ml adrenaline ($2x10^{-4}$M) in the presence of NaOH (0.1M) at room temperature

Effect of bases:

The colored product was formed in alkaline medium, so; the effect of different bases with increasing volumes of 0.1 M were examined. As shown in table 2, the product was formed in strong basic medium of NaOH and found that 3.0 ml of 0.1 M solution gave maximum color intensity.

Table 2: Effect of bases on the absorption of 1ml MTT of $2x10^{-4}$M

0.1 M of base	ג max	Absorbance / ml base				
		1	2	3	4	5
Sodium hydroxide	536	0.261	0.680	0.730	0.722	0.301
Ammonium hydroxide	500	0.013	0.025	0.056	0.061	0.069
Sodium carbonate	529	0.144	0.173	0.248	0.259	0.261
Disodium tetra borate	500	0.012	0.019	0.064	0.068	0.074

Effect of temperature and reaction time:

The reaction time was determined by following the color development at room temperature and at different temperatures in thermostatically controlled water-bath. The absorbance was measured against reagent blank treated similarly. It was observed that formation of colored complex was achieved maximum absorbance after 30 min. at room temperature (Table 4)

Table 3 : Effect of temperature on the absorbance and stability of the product

Time (min.)	Absorbance		
	0ºC	R.T	60ºC
5	0.741	0.638	0.546
15	0.735	0.719	0.633
30	0.730	0.732	0.645
45	0.728	0.733	0.652
60	0.724	0.730	0.659
75	0.720	0.731	0.664
90	0.726	0.725	0.661

Effect of order of addition:

To obtain optimum results the order of addition of reagents should be followed as given under the recommended procedure. Otherwise a loss in color intensity was observed (Table 5).

Table 4 : Order of addition

Reaction compound	λ_{max}	Absorbance
MTT+ Sodium hydroxide +Adrenaline	535	0.620
Adrenaline+ sodium hydroxide+MTT	532	0.357
MTT+adrenaline+ sodium hydroxide	536	0.731

Quantification.

Under the experimental conditions described in recommended procedure standard calibration graph was constructed by plotting absorbance versus concentration of adrenaline-MTT product (Fig 3). The correlation coefficient , Beer's law limits , molar absorptivity and Sandell sensitivity values were evaluated and are given in Table 6 which are indicating the good linearity and high sensitivity of the method .

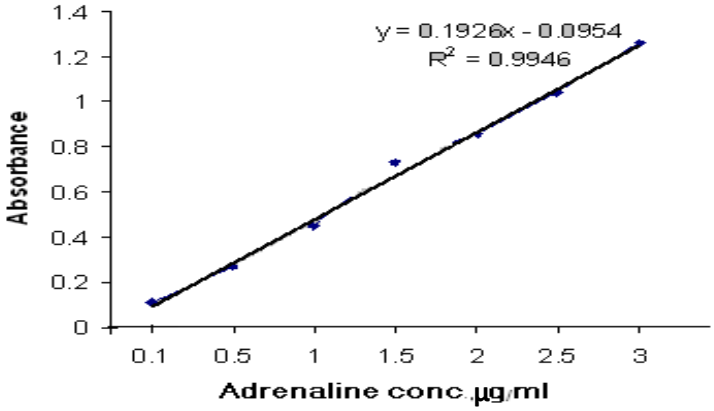


Figure 3: Calibration graph for determination of adrenaline

Table 5 : Optical characteristics and statistics for the proposed method .

Parameters	Values of the method
ג max (nm)	536
Linearity range (µ g.ml^{-1})	0.1-3.0
Molar absorptivity (l.mol^{-1}.cm^{-1})	1.179x10^{5}
Sandell sensitivity (µ g.ml^{-1})	0.00156
Slope	0.1926
Intercept	-0.0954
Correlation coefficient	0.9946

Accuracy and precision:

To estimate the accuracy and precision of the method, six replicate measurements were performed at three different concentrations of adrenaline. The results indicated that the proposed method is accurate and precise (Table 7).

Table 6: Accuracy and precision data of the proposed method

Amount of adrenaline taken (µ g.ml^{-1})	Relative error (%)*	RSD (%)*
0.5	+1.3	±1.7
1.5	+2.8	±0.9
2.5	-0.5	±0.6

*** Mean of six determinations.**

Application:

The proposed method was applied for analyzing a commercial formulation of adrenaline (injection) and comparing the results obtained with British pharmacopeia method [9] . Satisfactory agreement between the results was obtained with an acceptable range of error (Table 8)

Table 7 : Assay of adrenaline in pharmaceutical preparation

Pharmaceutical formulation	Certified value (mg.ml^{-1})	Present method ** (mg.ml^{-1})	British pharmacopoeia method ** (mg.ml^{-1})
Injection *	1.0	1.03	1.01

*** Miser -Co- Egypt.**

**** Mean of three determinations.**

Conclusion:

The proposed method for the determination of adrenaline un pharmaceutical formulation reported in this paper is rapid (30 min) , precise (better than ± 1.8%) , accurate (better than±3.0 %) and it could be used for routine analysis .

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