

## Determination The Drug Adrenaline in Pharmaceutical Forms By Spectrophotometric Method Using N-Bromo succinimide and p-Anisidine Reagents

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### Abstract

In order to determine the amount of adrenaline [ADR] in pure and pharmaceutical formulations, this research attempts to create an indirect, easy to understand sensitive, and cost-effective spectrophotometric approach. The suggested technique is predicated on the oxidation of ADR in an acidic medium with a certain amount of N-bromosuccinimide [NBS]. After oxidation is complete, the remaining NBS reacts with the reagent p-Anisidine, resulting in a colored brominated product with the maximum absorbance at 516 nm, which is inversely proportional to the amount of ADR in the sample. Plotting the method's standard curve revealed a linear relationship with a determination coefficient of 0.9995 for the concentration range of 1.0–12.0 µg/mL. The Sandell sensitivity index was 0.0203 µg/cm<sup>2</sup>, and the molar absorption coefficient was 9.01 × 10<sup>3</sup> L/mol.cm. The quantification [LOQ] and detection [LOD] limits were 0.493 and 0.178 µg/mL, respectively. With respectable accuracy and sensitivity, this approach has been effectively used to estimate adverse drug effects in pharmaceutical preparations and human blood serum.

### Introduction

Pure Adrenaline [ADR] is a white to gray odorless crystalline substance that is soluble in water, and it is insoluble in methanol, ethanol, and diethyl ether, but it dissolves well in dilute solutions of hydrochloric acid or acetic acid. Its molecular and structural formula is [C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub>] with molecular weight equal to 183.204 g/mol: [1].

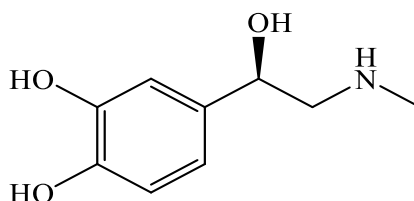


Fig. 1. Chemical Structure of Adrenaline

ADR, also known as epinephrine, is a hormone and neurotransmitter secreted by the adrenal medulla and a small number of neurons in the medulla oblongata. Its secretion is part of the body's response to emergency situations such as fear or stress, as ADR plays a direct role in activating the nervous system [2].

The release of ADR increases the heart rate and dilates the bronchi, which increases blood flow to the muscles, all of which helps the body quickly respond to sudden situations[3].

In addition to its physiological function in emergency situations, adrenaline is used medically in the treatment of anaphylaxis and cardiac arrest [4], and is also prescribed to treat acute asthma attacks [in emergency rooms]. It is recently used in ophthalmology [eye surgeries], while research is currently underway on its use in neurology [5].

Because of the importance of adrenaline, a large number of analytical methods have been developed to estimate adrenaline in therapeutic doses or biological fluids, such as spectrophotometric methods [6-8], UHPLC/MS-MS [9], HPLC [10-12] also by electrochemical methods [13-17].

The majority of laboratories in the world have an accessible instrument called a spectrophotometer. This apparatus has been applied in numerous investigations to estimate medicinal substances, such as metronidazole [18], and paracetamol, ibuprofen, and caffeine [19].

This study proposed a rapid and simple spectrophotometric method to measure the amount of pure adrenaline in human serum, and in pharmaceutical dosage substances.

## Excremental Part Chemical Reagents

All reagents and chemicals used in this research are of high purity and supplied by reputable companies (Table 1).

**Table 1:** The main chemicals used in present study

Name of substance	Chemical formula	Molecular weight g/mol	Company
Adrenaline	C <sub>9</sub> H <sub>13</sub> NO <sub>3</sub>	183.204	S.D.I*
N-Bromosuccinimide	C <sub>4</sub> H <sub>4</sub> BrNO <sub>2</sub>	177.98	Fluka
p-Anisidine	C <sub>7</sub> H <sub>9</sub> NO	123.15	Aldrich
[Acetic acid [99.5%	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	60.052	BDH

\* General Company for Medicines and Medical Appliances, Samarra, Iraq

**Working solution of ADR at 50 µg/mL [2.73×10<sup>-4</sup> M]:** In a volumetric flask, 0.005 g of pure ADR was dissolved in 10 mL of distilled water, and the volume was then increased to 100 mL using distilled water.

**N-Bromosuccinimide solution 600 µg/mL:** Using a volumetric flask, 0.06 g of NBS was dissolved in a small amount of distilled water while being shaken, and the volume was then increased to 100 mL.

**Solution of p-Anisidine [p-AN] 1000 µg/mL:** In a volumetric flask, 0.10 g of pure p-AN was dissolved in 10 mL of ethanol, shaken, and heated briefly before the volume was increased to 100 mL with distilled water.

**Stock solution of acetic acid 0.1 M:** In a 100 mL volumetric flask, 0.57 mL of concentrated hydrochloric acid [17.4 M] was diluted with distilled water.

### Pharmaceutical solutions

**Adrenaline injection solution [50 µg/mL]:** 1 mL of injection [provided by Vitalone Pharma – India, each mL contain 1mg ADR] were transferred to 20 mL volumetric flask, and the volume was completed with distilled water to the mark.

**Solution of sandralin injection [50µg/ mL]:** 1 mL of injection [Contains 1mg/ml from ADR, provided by SANJIVANI - India] were transferred to 20 mL volumetric flask, and the volume was completed with distilled water to the mark.

**Human blood serum sample:** Blood samples were obtained from healthy people who were not undergoing treatment, especially those taking drugs that contain adrenaline. The blood samples were centrifuged at 4,000 rpm for 15 minutes, resulting in serum separation [20]. Samples were used without pretreatment.

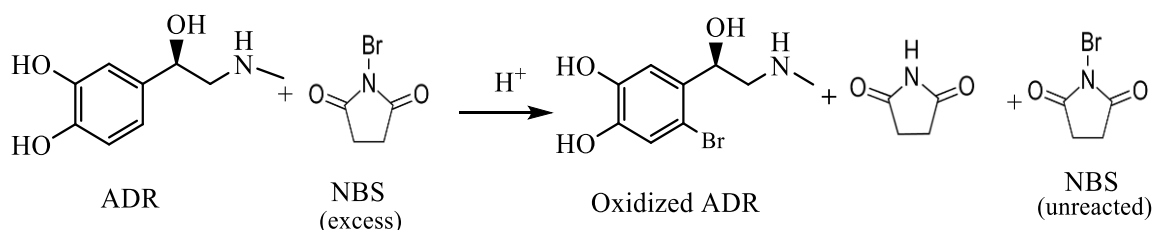
### Apparatus

All the measurements and absorption spectra were recorded using a Shimadzu UV-Vis. 1900I double-beam [Japan] spectrophotometer equipped with glass cells measuring 1.0 cm. pH measurements were performed using BP3001[Boeco, Germany] professional bench top pH-meter.

## Results and Discussion

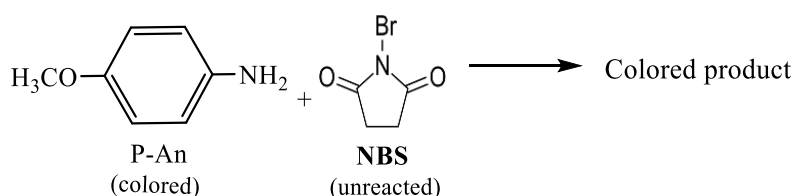
### The principle of the methods and suggested chemical reactions

The method's and the suggested chemical reaction's principle: As shown in Scheme 1, the working technique consists of two steps. In the first step, an excess of NBS in an acidic media causes the oxidation reaction of adrenaline.



**Scheme 1:** The reaction of NBS with ADR

In the second step, the remaining NBS reacts with p-AN to form a colored product with maximum absorption at 516 nm, inversely proportional to the amount of ADR present in the solution, as shown in Scheme 2.



**Scheme 2:** The reaction of NBS with P-AN

### The Optimum Reaction Conditions

All experiments were performed using 1 mL of ADR working solution [50 µg/mL] in a final volume of 10 mL. The resulting-colored spectrophotometers were performed at 516 nm

and the  $\Delta A$  value was found by measuring the absorbance of the sample and blank against distilled water and then subtracting the sample absorbance value from the blank absorbance value.

### Preliminary study

A preliminary method was tested by adding 1 mL of 600  $\mu\text{g/mL}$  of NBS oxidizing agent to a 10 ml volumetric flask containing 1 mL working solution of ADR and 1 mL of 0.1 M acetic acid with shaking and waiting for 5 minutes. Then, 1 mL of 1000  $\mu\text{g/mL}$  p-AN solution was added and standing for 5 minutes, then the volume was completed with distilled water to the mark [ Solution A], another solution was prepared containing all components except ADR [solution B], inhibition in the absorbance [at wavelength=516 nm] of Solution B according to adding of ADR. Decrease in absorbance of the complex depends on the amount of ADR.

### The effect of an acidic medium

Each of the available acids was added in 1 mL at a concentration of 0.1 M for this experiment. Acetic acid it produced the best results, as indicated by the experimental data in Table 2.

**Table 2:** Selection of acidic medium

Acid* [0.1 M]	$\Delta A^{**}$	Final pH
Acetic acid	0.161	4.12
H <sub>3</sub> PO <sub>4</sub>	0.108	3.16
H <sub>2</sub> SO <sub>4</sub>	0.031	2.33
HNO <sub>3</sub>	0.013	2.47
HCl	0.016	2.56

\*1 mL used

\*\* $\Delta A$  =Absorbance of solution B vs distilled water - Absorbance of solution A vs distilled water

### Effect of volume of the acetic acid

Various volumes of acetic acid were added at a concentration of 0.1 M in order to determine the ideal volume. The ideal volume of 0.5 mL of acetic acid was determined by the experimental results in Table 3, and this value was maintained in the tests that followed.

**Table 3:** Choosing the ideal acetic acid volume

mL of acetic acid [0.1M]	$\lambda$	$\Delta A$
0.25	498	0.118
0.5	516	0.184
0.7	523	0.163
1.0	531	0.160
1.5	532	0.159

### Selection of the Oxidizing Agent

The effect of several available oxidizing agents was studied: NBS [N-bromosuccinimide], potassium hypochlorite [KOCl], potassium iodate [KIO<sub>3</sub>], and potassium dichromate [K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>]. To obtain the best measurement value, 1 mL of each oxidizing agent at a concentration of 600 µg/mL [after adding acid] was added to 1mL of ADR [50 µg/ML], followed by 1 mL of 1000 µg/mL p-AN solution. The reaction was then waited for a short period of time [minute], and the volume was completed with distilled water up to the mark. Spectrophotometric scans were then performed. The results shown in Table 4 clearly show that NBS was the best reagent, giving the highest absorbance value at a wavelength of 516 nm, and was therefore chosen for subsequent experiments [A solution contain ADR and solution B without ADR in addition to other reaction components].

**Table 4:** Selection of the oxidizing agent

Type of Oxidative agent	Absorbance/Solution vs DW.		ΔA
	Solution A	Solution B	
NBS	0.596	0.805	0.209
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	0.438	0.463	0.025
NaOCl	0.176	0.320	0.144
KIO <sub>3</sub>	0.020	0.057	0.037

### Amount of NBS

Using various volumes of its solution at a concentration of 600 µg/mL, the ideal amount of NBS was investigated. The volume of 1.5 mL provided the best ΔA, as demonstrated by the data clearly displayed in Table 5, and as a result, it was incorporated into the working procedure.

**Table 5:** Selection the best volume of NBS

mL of 600 µg/mL NBS	ΔA
0.5	0.143
1	0.232
1.5	0.244
2	0.231

### Amount of p-AN reagent

In this experiment, different volumes of 1000 µg/mL p-AN solution was used versus 1.5 mL of 600 µg/mL NBS, and Table 6's findings indicate that the ideal volume of p-AN was 2 mL, that give better absorption of the reaction result.

**Table 6:** Effect of volume of p-AN

mL of 1000 µg/mL p-AN	ΔA
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<b>0.5</b>	0.207
<b>1</b>	0.241
<b>1.5</b>	0.247
<b>2</b>	<b>0.252</b>
<b>2.5</b>	0.249

### The oxidation time's effect

In order to get the optimum results, several amounts of time were waited before adding p-AN and after adding the oxidizing agent [NBS] to the ADR solution [50 µg/mL] in the presence of acid. By waiting varying amounts of time after adding p-AN, the proper amount of time was also established to finish the reaction of the remaining NBS with p-AN. According to Table 7, 10 minutes was the ideal amount of time to fully oxidize ADR and the right amount of time to finish the reaction between NBS and p-AN.

**Table 7:** The impact of oxidation time

Minimum standing time prior to P-AN addition.	$\Delta A$ / Minimum standing time prior to dilution				
	Immediately	5	10	15	20
<b>Immediately</b>	0.251	0.254	0.253	0.252	0.253
<b>5</b>	0.254	0.255	0.253	0.250	0.254
<b>10</b>	0.262	0.268	<b>0.274</b>	0.273	0.270
<b>15</b>	0.260	0.264	0.262	0.261	0.266
<b>20</b>	0.268	0.265	0.264	0.257	0.250

### Effect of temperature

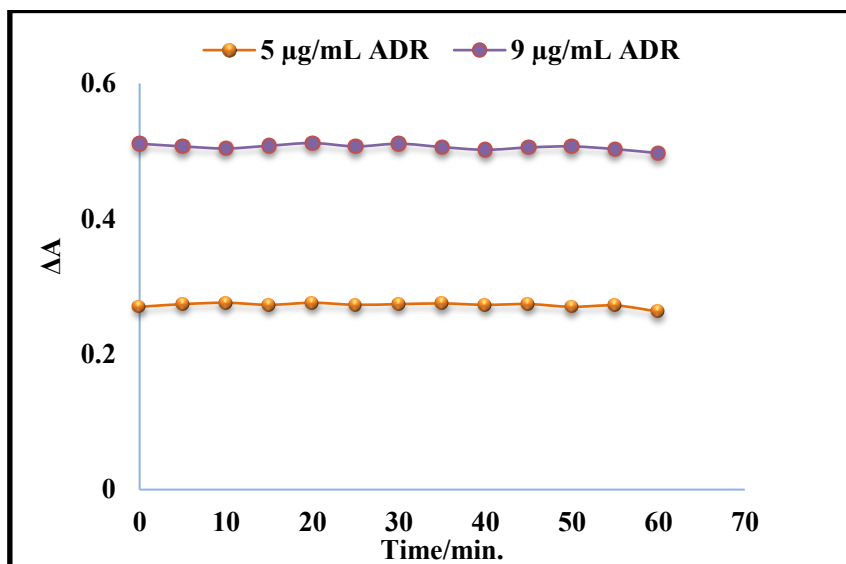
At various temperatures [10–40°C], the impact of temperature on the colored product's absorbance value[ $\Delta A$ ] was investigated. Table 8's findings indicate that high  $\Delta A$  values at 40, but this temperature caused distortion and blue shift as well as instability of the wavelength at which the colored product is absorbed. Therefore, room temperature [RT = 23±2°C] was chosen because the measurements were stable for a longer period, and thus the room temperature was fixed within the optimum conditions for the method.

**Table 8:** Effect of temp.

Temp, C°	$\Delta A$
10	0.241
RT	0.273
40	0.282

### Stability of colored product

$\Delta A$  values were monitored over time using two concentrations of ADR, and the results in Figure 2 show that  $\Delta A$  values remained constant for 60 minutes, which is a significant factor in the time required to perform the measurements.



**Fig 2:** Stability of colored product

### Effect of surfactants

The effect of adding 1 mL for each of four types of different surfactants [neutral, negative, and positive], namely SDS\*, CPC\*, CTAB\*, and Triton X-100\*, at  $1 \times 10^{-3}$  M on the  $\Delta A$  values for the colored product, was studied. The results listed in Table 9, Since they had no beneficial impact on the colored product's absorbance values, they were left out.

\*SDS: Sodium dodecyl sulphate.

\*CPC: Cetylpyridinium chloride.

\*CTAB: Cetyltrimethylammonium bromide.

\*Triton X-100: Iso-octyl phenoxy poly ethoxy ethanol.

**Table 9:** Effect of surfactants

Surfactant $1 \times 10^{-3}$ M	$\Delta A$ /mL of surfactants	
	0.0	1
Triton X-100		0.251
SDS	0.275	0.257
CPC		0.232
CTAB		0.233

### Effect of addition sequence

The proposed method was applied using a number of different addition sequences with the aim of obtaining the best addition sequence. As seen in Table 10, the initial addition sequence [I] was selected for further testing because it was the best.

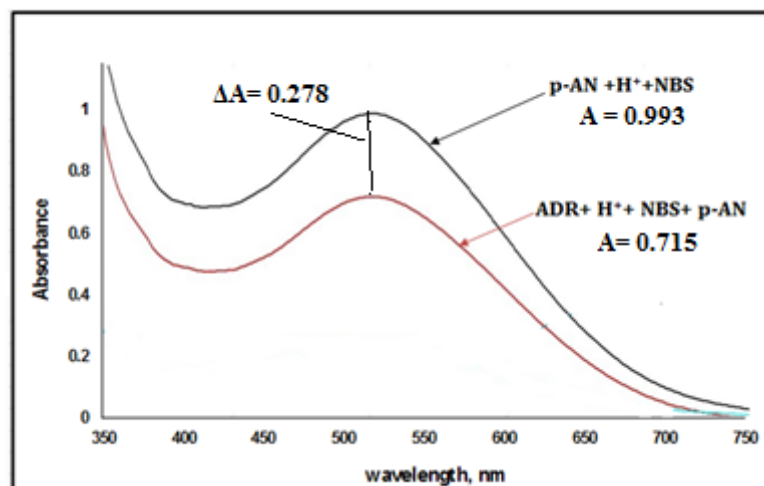
**Table 10:** The impact of the addition sequence

Order	Components *	$\Delta A$
I	ADR + H <sup>+</sup> + NBS + p-AN	0.275
II	H <sup>+</sup> + NBS + ADR + p-AN	0.266
III	ADR + NBS + H <sup>+</sup> + p-AN	0.262

\*Adrenaline [ADR], Acetic acid [ $\text{H}^+$ ], N-bromosuccinimide [NBS], p-ANisidine [p-AN]

### Final Absorption Spectrum

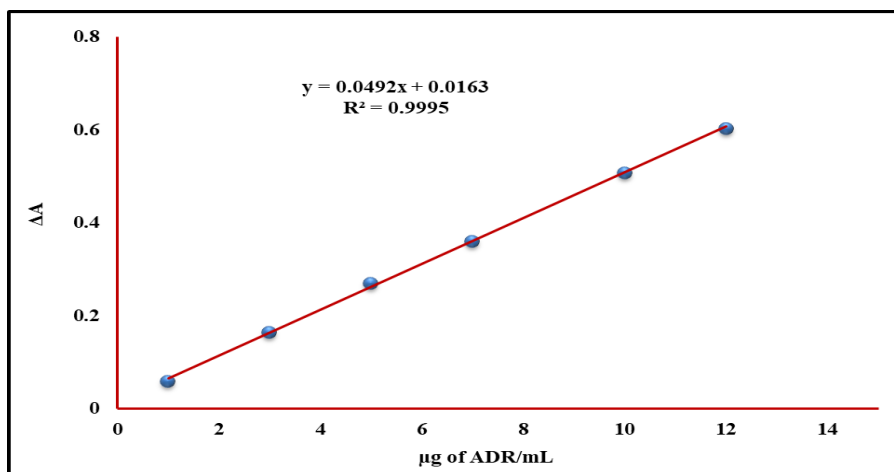
Figure 3 displays the final absorption spectra of the suggested approach, which was used with 1 mL of ADR working solution [ $50 \mu\text{g/mL}$ ] in a 10 mL volumetric flask at ideal circumstances demonstrated in earlier tests.



**Fig 3:** Final Absorption Spectrum for  $50 \mu\text{g}$  of ADR according proposed method

### Adopted procedure and calibration curve

To cover the concentration range of  $0.5\text{--}15 \mu\text{g/mL}$ , pour varying quantities [ $0.1\text{--}2.4 \text{ mL}$ ] of ADR standard solution [ $50 \mu\text{g/mL}$ ] into multiple 10 mL volumetric flaskls containing 0.5 mL of 0.1 M acetic acid. Next, add 1 mL of  $600 \mu\text{g/mL}$  NBS. Add 2 mL of  $1000 \mu\text{g/mL}$  p-AN solution and wait with shaking for 10 minutes after the ADR has oxidized for 10 minutes. Then, make up the volume with distilled water to the mark, and measure the  $\Delta A$  at 516 nm. The standard curve of the method was plotted using the difference in absorbance between the blank and sample absorbance values [ $\Delta A$ ], giving a linear relationship over a range of concentrations from 1.0 to  $12 \mu\text{g/mL}$ . At doses greater than  $12 \mu\text{g/mL}$ , a negative departure from Beer's law was noted. The method's standard curve is displayed in Figure 4. According to calculations, the method's molar absorbance was  $9.01 \times 10^3 \text{ L/mol.cm}$ , and its Sandell index was  $0.0203 \mu\text{g/cm}^2$ . It was discovered that the limits of quantification [LOQ] and detection [LOD] were 0.493 and  $0.178 \mu\text{g/mL}$ , respectively.



**Fig. 4:** Calibration curve of ADR according proposed method

### Accuracy and Precision

The accuracy and precision of the proposed method were tested using two different concentrations [5 and 9 µg/mL] and by preparing five replicates for each concentration, then figuring out the standard deviation, relative error, and recovery percentage. The study's findings, which are displayed in Table 11, reveal that the approach has good precision and accuracy.

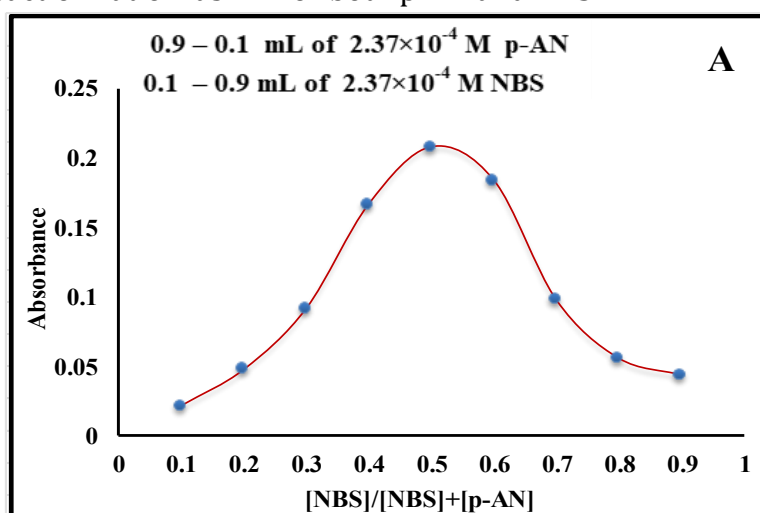
**Table 11:** The method's accuracy and precision

ADR µg/mL taken	ADR µg/mL found	Recovery*, %	Re*%	RSD*%
5	5.08	101.6	1.6	1.88
9	9.19	102.1	2.1	2.24

\*Average of five determinations

### The nature of the interaction

Under ideal conditions, the molar ratio method [21] and the continuous variation method [Job's method] were used to determine the reaction rate between the oxidizing agent N-bromosuccinimide [NBS] and adrenaline [ADR] using equal concentrations of both substances at a concentration of  $2.37 \times 10^{-4}$  M. However, the method failed because a turbid solution formed, so the reaction ratio of p-AN and NBS was examined, and the results [Figure 5] show that the reaction ratio was 1:1 for both p-AN and NBS.



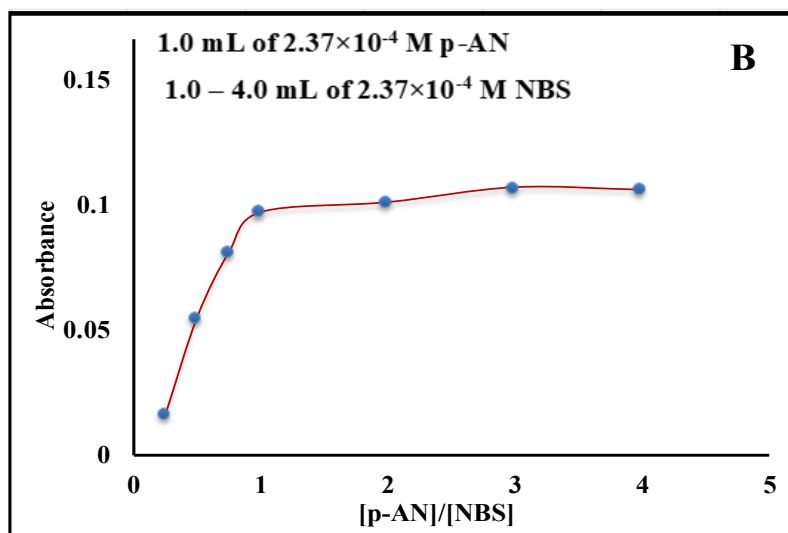


Fig. 5: [A] Continuous variation and [B] Mole ratio plots for ADR

### Application of the procedure

The proposed method was applied to the estimation of adrenaline in its available pharmaceutical formulations [injections] using two different concentrations [5 and 9  $\mu\text{g}/\text{mL}$ ] of ADR and preparing five replicates for each concentration. The results shown in Table 12.

Table 12: Application of the method

Pharmaceutical preparations of ADR	Present amount of ADR $\mu\text{g}/\text{mL}$	Found amount of ADR $\mu\text{g}/\text{mL}$	Recovery %	RE%*	RSD%*
Adrenalone injection 1 mg/mL	5	4.91	98.2	-1.8	1.78
Vitalone Pharma - India	9	8.87	98.6	-1.4	2.43
Sandralin injection 1 mg/mL	5	5.04	100.8	0.8	1.58
SANJIVANI - India	9	9.15	101.6	1.6	2.36

\*Average of five determinations

Referring to the results in Table 12, we find that the proposed method is applicable to pharmaceutical preparations with acceptable accuracy and precision.

### Application in the human blood serum

The proposed method was applied to the estimation of two different concentrations of ADR in 1.0 mL of human serum, and Table 13 presents the findings.

Table 13: Results of application in human blood serum

$\mu\text{g}/\text{mL}$ ADR taken	$\mu\text{g}/\text{mL}$ ADR found	Rec.* %	Re* %	RSD* %
5	4.93	98.6	-1.4	2.19
9	8.77	97.4	-2.6	1.82

\*Average of five determinations

After reviewing the results in Table 13, we find that the proposed method is applicable and has good accuracy and precision.

### Assessment of the suggested approach using standard addition

Using readily accessible medicinal solutions and the conventional addition approach [22], the suggested method's selectivity was investigated. The findings in Figure 6 and Table 14 show that there is no additive interference and that the standard addition method and the suggested method accord satisfactorily.

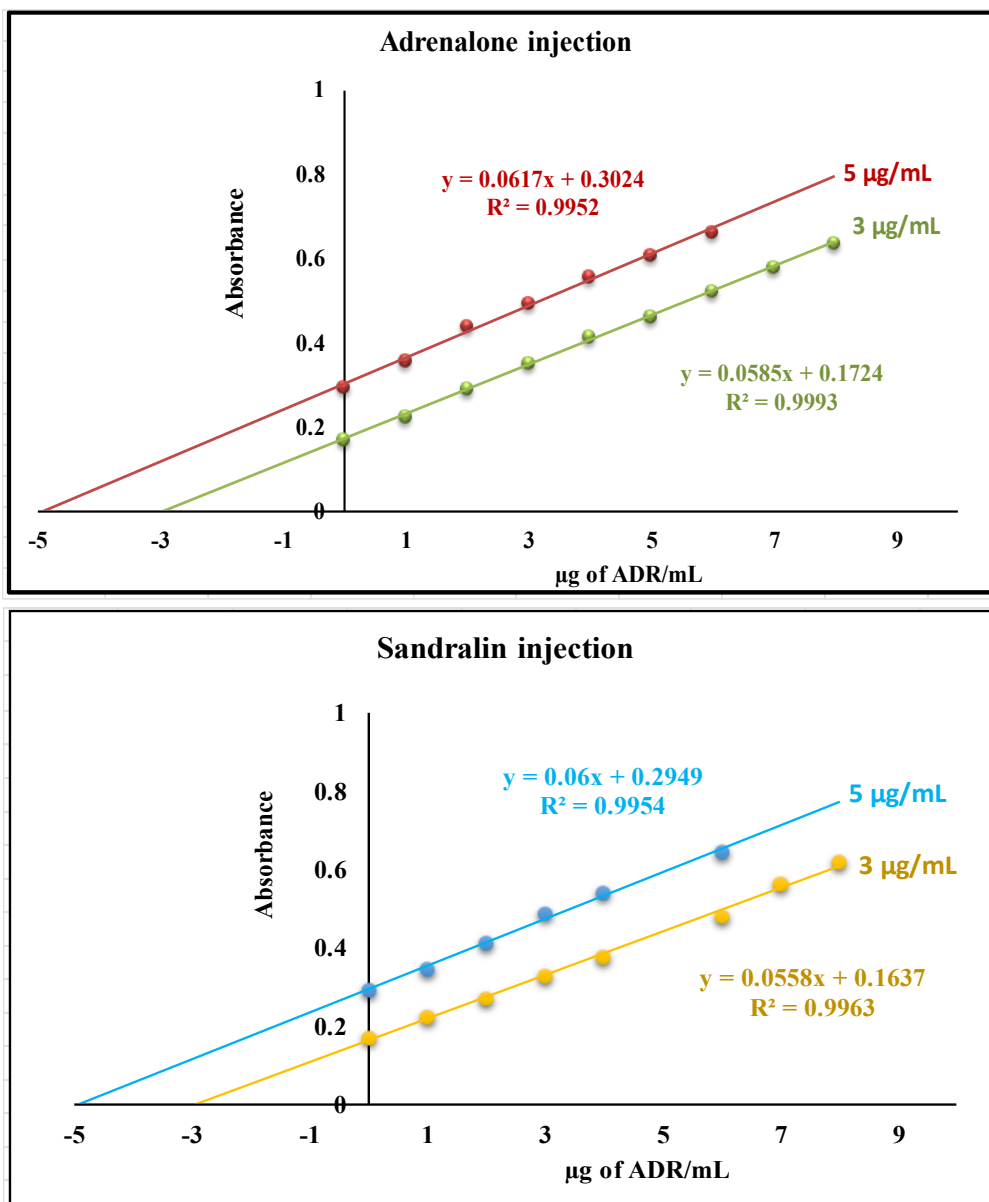


Fig. 6: Curves for the standard addition method

**Table 14:** Outcomes of the conventional additive technique

Pharmaceutical preparation	Taken amount of ADR $\mu\text{g/mL}$	Measured amount of ADR $\mu\text{g/mL}$	Recovery %
Adrenalone injection 1 mg/mL	3	2.95	98.33
Vitalone Pharma - India Sandralin injection 1 mg/mL	5	4.90	98.00
SANJIVANI - India	3	2.93	97.66
	5	4.91	98.20

### Comparison of the method

Table 15 contains some of analytical variable of present method compared with the same from literature methods.

**Table 15.** Comparisons of proposed method with other analytical methods

Type of reaction	$\lambda$ max, $[\text{nm}]$	Linearit y $\mu\text{g/mL}$	$\epsilon$ L/ mol.cm	Reference
Oxidation and reduction, Safranin O dye	535	0.1–16	$2.84 \times 10^4$	[6]
Complexation, 2,6-dichloroquinone-4-chloroamide	475	0.3–9.5	$1.46 \times 10^4$	[23]
Complexation, Iron [III]	648	20 – 214	$1.59 \times 10^4$	[24]
Complexation, Iodic acid	520	1.143 – 142.9	$3.69 \times 10^3$	[25]
Proposed method	516	1-12	$9.01 \times 10^3$	.....

The outcomes in Table above show that the proposed method is effective and has accepted sensitivity for determination of adrenaline in pharmaceutical preparation [injection].

### Conclusion

In this research paper, a new spectrophotometric method was developed for the determination of ADR in therapeutic doses as well as in blood serum. It is easy, rapid, and inexpensive, can be applied at room temperature, and requires no prior separation steps. The suggested technique is predicated on oxidizing ADR with a particular excess of NBS. After the oxidation process is complete, the remaining oxidizing agent [NBS] reacts with a specific amount of p-AN reagent to form a colored product with the highest absorbance value at 516 nm, inversely proportional to the amount of ADR present in the sample. The method has been successfully applied to the estimation of ADR in therapeutic preparations and human blood serum, with acceptable accuracy and sensitivity.

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## تقدير الأدرينالين في المستحضرات الصيدلانية بطريقة طيفية وباستخدام الكاشف N - بروموسكسينيميد و بارا-أنيسيدين

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### الخلاصة:

يهدف هذا البحث إلى تطوير طريقة طيفية غير مباشرة، بسيطة، حساسة، واقتصادية لتحديد الأدرينالين [ADR] في المستحضرات النقية والصيدلانية. تعتمد الطريقة المقترحة على أكسدة الأدرينالين باستخدام كمية ثابتة من NBS في وسط حامضي. بعد اكتمال الأكسدة، يتفاعل NBS المتبقي مع الكاشف بارا-أنيسيدين، مكوناً ناتج مبروماً ملوناً تتناسب كميته عكسياً مع كمية الأدرينالين الموجودة في العينة، ويعطي أعلى قيمة للامتصاص عند طول موجي 516 نانومتر، وقد رُسم المنحنى القياسي للطريقة، موضعاً علاقة خطية على مدى تراكيز يتراوح بين 1.0 و 12.0 مايكروغرام/ملتر، وبمعامل ارتباط قدره 0.9995، وبلغ معامل الامتصاص المولاري  $10 \times 9.01$  لتر/مول.سم، وكانت دلالة حساسية ساندل تساوي 0.0203 مايكروغرام/سم. بلغت حدود الكشف [LOD] والقياس الكمي [LOQ] 0.178 و 0.593 مايكروغرام/ملتر. وقد طبقت هذه الطريقة بنجاح في تقدير الأدرينالين في المستحضرات العلاجية ومصل الدم البشري، بدقة وحساسية مقبولتين.

### معلومات البحث:

تأريخ الاستلام:

تاريخ التعديل:

تأريخ القبول:

تاريخ النشر:

### الكلمات المفتاحية:

تقدير طيفي، الأدرينالين، التحديد، ن- بروموسكسينيميد، بارا-أنيسيدين

### معلومات المؤلف

الايمل:

الموبايل: