



Spectrophotometric Determination of Catecholamine Containing Drugs Using Calcon Dye

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Abstract

Calcon dye has been used for spectrophotometric determination of catecholamine-containing drugs, namely, adrenaline, methyldopa and dopamine in their pure forms and pharmaceutical formulations. The method is based on the oxidation of the above drugs with an excess of N-bromosuccinimide (NBS) in an acidic medium. The residual oxidizing agent bleaches the blackish-brown color of calcon measured at 510 nm. The decolorization of the dye is proportional to the residual amount of NBS, which is proportional to the concentration of the drug. Linear calibration graphs were obtained in the concentration range 0.5-16.0, 2.0-40.0 and 1.036.0 $\mu\text{g mL}^{-1}$ with molar absorptivity values 1.10×10^4 , 3.2×10^3 and $4.3 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ for above drugs, respectively. The method is simple, sensitive, accurate, precise and free from excipients. The developed method was successfully applied to determine the drugs in their pharmaceutical formulations.

Keywords: Catecholamine drugs, Calcon, Oxidation, Spectrophotometry

Introduction

Catecholamines are a class of monoamines synthesized from tyrosine, which contain a catechol group and a side chain with an amino group in their structure [1]. Catecholamines-containing drugs are widely used to treat different disorders, such as playing a key role in the mechanisms of emotions, learning, memorization, and sleep, as well as in psychomotor activity and neural regulation [2].

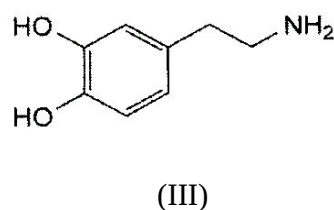
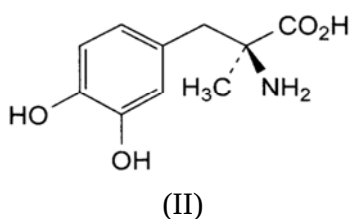
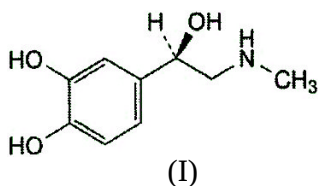
Adrenaline (Epinephrine) is a hormone that is involved in regulating visceral functions (such as respiration) [3,4]. It is chemically known as 4-[(1R)-1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol (I). It is a neurotransmitter for the mammalian central nervous system. Many diseases are associated with a change in catecholamine concentration.

It is an active principle of adrenal gland balm and is a drug used to treat nasal congestion, asthma, heart block, hypotension and cardiac arrest. A change in the concentration of adrenaline in biological fluids (blood, urine, cerebrospinal fluid) can serve as a reliable indicator of a violation of homeostasis [5,6].

Methyldopa, is in the family of alpha-2-adrenergic receptor agonists. Chemically it is named S-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid (II). It is an antihypertensive drug used to lower blood pressure and treat some hypertension and gestational hypertension cases in renal failure and complicated pregnancies [7,8].

Dopamine constitutes approximately 80% of the catecholamine content material

with inside the brain [9]. Chemically known as 2-(3, 4-dihydroxyphenyl) ethylamine (III), It is classified among the heart-stimulating drugs [10], which is a hormone secreted by the adrenal gland. It contributes to the fight or flight response processes [11] and the circulatory system (shock) due to myocardial infarction and trauma [12].



Different techniques have been described for determination of catecholamine drugs such as voltammetry [13-16], chromatography [17-21], amperometry [22], capillary electrophoresis [23,24] and fluorometry [25]. However, these techniques are expensive, need experience and are not available in all laboratories. The spectrophotometric technique is still the preferred method due to its simplicity. Various spectrophotometric methods using different reagents have been reported for the determination of catecholamine drugs. These methods include ion association using eosin Y and application of cloud point extraction technique [26], charge transfer complexes using bromanil [27], oxidation-reduction using

ferric ion [28-30], diazotisation using p-nitroaniline [31], 4-aminoantipyrine in the basic medium [32], complexation reaction using 4-aminoantipyrine and copper ion, ion-pair complex using triiodide ion [33] and using ferrous ion as a complexing agent in alkaline medium [34], oxidative coupling using p-toluidine in the presence of sodium periodide [35], 2, 6-diaminopyridine in the presence of potassium periodate [36] and 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate in the presence of potassium ferricyanide [37].

The aim of this study is to develop an accurate, simple, sensitive and cost-effective method for the spectrophotometric determination of catecholamine containing drugs. The method is based on the oxidation of drugs by N-Bromosuccinimide (NBS) in an acidic medium, and the residual oxidant, which is proportional to the drug concentration, bleach the color of calcon dye and reduction in absorbance is measured at 510 nm, which forms the basis of determination method.

Materials and Methods

Instruments: UV-Visible spectrophotometer type Shimadzu UV-1650 PC equipped with a 1.0-cm path length silica cell, pH-meter with a combined glass electrode type Philips PW (9421) was used for pH measurements. All calculations in the computing process were performed in Microsoft Excel format.

Reagents: Calcon reagent was prepared in a concentration of $500 \mu\text{g mL}^{-1}$ by dissolving 0.05 g in distilled water in a 100 mL volumetric flask. Oxidizing agent (NBS) was prepared in a concentration of 5×10^{-3} M by dissolving 0.0890 g in 100 mL distilled water. Hydrochloric acid was prepared in a concentration of 1 M by diluting an appropriate volume of conc. HCl.

Stock Solutions of Drugs: Stock solutions of adrenaline, methyl dopa and dopamine were prepared in a concentration of $100 \mu\text{g mL}^{-1}$ by dissolving 0.01 g of each drug in 100 mL distilled water in volumetric flasks. The solutions were kept in the refrigerator.

Sample Preparation

Aliquots containing 0.5-16.0, 2.0-40.0 and 1.0-36.0 $\mu\text{g mL}^{-1}$ of adrenaline, methyl dopa and dopamine standard solutions were added respectively, and separated into three series of 10 mL volumetric flasks, followed by adding 1 mL of 1 M HCl and 1.5 mL of 5×10^{-3} M NBS. The solutions were left for 10 min in a water bath adjusted at 30°C for completion of the oxidation process, then 2.6 mL of $130 \mu\text{g mL}^{-1}$ calcon was added. The solutions were diluted to the mark, and the absorbance was measured at 510 nm after 20 min for dopamine and 15 min for adrenaline and methyl dopa against respective reagent blank.

Procedure for Drug Formulations Injection

Adrenaline: The content of five ampoules of adrenaline (each ampoule contains 1.0 mg/1 mL adrenaline) or adrenaline darnitsa (each ampoule contains 1.8 mg/1 mL adrenaline) were mixed and then diluted to 50 mL with distilled water to obtain 100 or $180 \mu\text{g mL}^{-1}$ respectively. Different volumes of these solutions were taken to obtain concentrations 1.0, 4.0, 6.0 and $10.0 \mu\text{g mL}^{-1}$, and they were treated according to the general procedure. The concentration of adrenaline per ampoule was found using the standard calibration curve for the drug in its pure form.

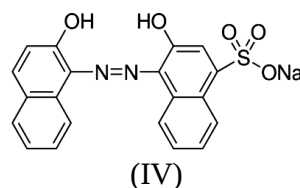
Dopamine HCl: The content of three dopadren ampoules (each ampoule contains 200 mg/5 mL of dopamine. HCl). 2 mL were mixed and diluted to 100 mL with distilled water to obtain a solution of $800 \mu\text{g mL}^{-1}$.

Then, a solution was prepared by dilution with a concentration of $100 \mu\text{g mL}^{-1}$. Different volumes of the solution were taken to obtain concentrations 4.0, 10.0, 16.0 and $24.0 \mu\text{g mL}^{-1}$. They were treated according to the general procedure. The dopamine concentration was found in the ampoule using the calibration curve of the drug in its pure form.

Tablet: Ten tablets of aldosa or aldoma (each tablet contains 250 mg methyl dopa) were carefully weighed, crushed and mixed well. The weight of the powder equivalent to one tablet was dissolved in 20 mL of distilled water and heated in a water bath for a few minutes to increase the solubility. The solution was allowed to cool and filtered in a 100 mL volumetric flask, then completed to the mark with distilled water to obtain $2500 \mu\text{g mL}^{-1}$. The solution was diluted to $100 \mu\text{g mL}^{-1}$, and 4.0, 10.0, 16.0 and $24.0 \mu\text{g mL}^{-1}$ treated according to the general procedure.

Results and Discussion

Calcon, called Eriochrome Blue Black R or Mordant Black 17. It is chemically known as 2-Hydroxy-1-(2-hydroxy-1-naphthylazo)naphthalene-4-sulfonic acid sodium salt (IV) that was proposed by Hildebrand and Reilly in 1958 to be used as an indicator for calcium ion [38, 39].



In the preliminary investigation, it was found that calcon dye has maximum absorption at 510 nm. Experimentally, a quantitative oxidation of calcon dye was noticed in the presence of the oxidizing agent NBS in the acidic medium. The absorbance of the dye decreased by increasing the

concentration of the oxidizing agent by bleaching its color. Based on this feature, the possibility of indirect spectrophotometric determination of the catechol amine drugs (adrenaline, methyl dopa and sopamine hydrochloride) has been studied. The increasing concentration of drugs leads to a decrease in the concentration of oxidizing agents for bleaching calcon color. It also leads to an increase of the absorbance at 510 nm, which is proportional to the drug concentration (Fig.1). However, the optimum conditions for the quantitative determination of the above drugs have been considered.

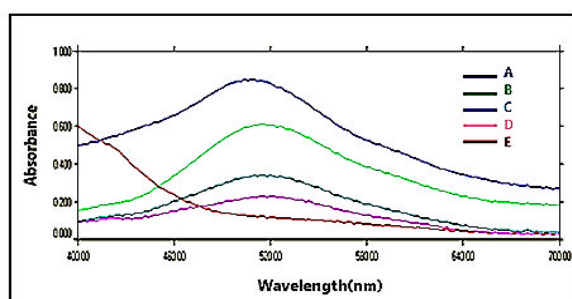


Figure 1. Absorption spectra of 130 $\mu\text{g mL}^{-1}$ calcon in acidic medium (A) in the presence of NBS and 10 $\mu\text{g mL}^{-1}$ adrenaline (B), 20 $\mu\text{g mL}^{-1}$ methyl dopa (C) and 15 $\mu\text{g mL}^{-1}$ dopamine (D) against reagent blank (E)

Optimization of Calcon Dye Concentration:

To select the optimum amount of calcon to be used for the determination of catecholamine drugs, increasing volumes (milliliters) of a dye solution at a concentration of 500 $\mu\text{g mL}^{-1}$ were added to a 10 mL volumetric flasks containing 1.0 mL of 1 M HCl. The volume was supplemented with distilled water to the mark, and the absorption was measured at 510 nm. It was found that the linear concentration range of calcon dye was 1-130 $\mu\text{g mL}^{-1}$. Therefore, the concentration was fixed at 130 $\mu\text{g mL}^{-1}$ for the estimation of the drug.

Optimization of Oxidizing Agent, Concentration and Volume: The effect of the oxidizing agents, including NBS, potassium dichromate, chloramine-T and cerium (IV) sulfate with a concentration of 5×10^{-3} M on

the bleaching of the dye in the medium of HCl was considered. The results, cited in Fig. 2, indicated that NBS was the most suitable in the oxidation of the dye, as it gave the lowest absorbance; therefore, it was adopted in the present method. The effect of NBS concentration and volume was studied. It was found that 1.5 mL of 5×10^{-3} M was the best for this method.

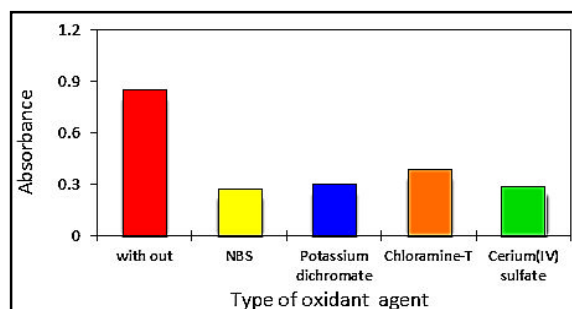


Figure 2. Effect of oxidizing agent on the bleaching of 130 $\mu\text{g mL}^{-1}$ calcon

Effect of Acid

Experimental results demonstrated that the oxidation of calcon dye and the studied drugs by NBS were present in an acidic medium and at room temperature (23°C) for adrenaline but at 30°C for methyl dopa and dopamine. Therefore, the effect of different acids of 1 M was examined for determination of the above drugs in the presence of 130 $\mu\text{g mL}^{-1}$ calcon dye separately. The results in Table 1 indicate that HCl was suitable acid. However, the concentration and volume of HCl were studied. It was found that 1 mL of 1 M gave maximum absorbance, Table 2 & Fig. 3. The conducted procedure was recommended in subsequent experiments.

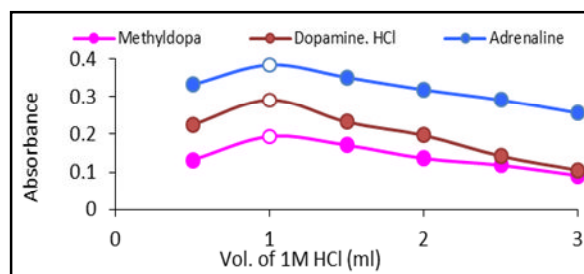


Figure 3. Effect of volume of 1 M HCl on the absorbance of drugs

Table 1. Effect of 1 mL of 1 M acid on the absorbance of drugs.

Drug	$\mu\text{g mL}^{-1}$	Absorbance				
		HCl	H ₂ SO ₄	H ₃ PO ₄	HNO ₃	CH ₃ COOH
Adrenaline	10	0.393	0.369	0.317	0.284	0.251
Methyldopa	20	0.192	0.177	0.136	0.117	0.106
Dopamine, HCl	15	0.285	0.226	0.185	0.147	0.131

Table 2. Effect of molar concentration of HCl on the absorbance of drugs.

Drug	$\mu\text{g mL}^{-1}$	Molarity of HCl /Absorbance					
		0.5	1.0	1.5	2.0	2.5	3.0
Adrenalin	10	0.331	0.385	0.349	0.296	0.252	0.221
Methyldopa	20	0.152	0.196	0.177	0.134	0.112	0.091
Dopamine, HCl	15	0.174	0.285	0.233	0.191	0.161	0.127

Effect of Time on the Oxidation and Bleaching:

This study was conducted to determine the time period required for the oxidation of the pharmaceutical compounds and the dye with NBS in hydrochloric acid at different times with shaking. The results in Fig. 4 showed that 10 min was sufficient time for the oxidation of

drugs before adding a calcon dye, 15 min for bleaching of a dye for adrenaline and methyldopa, and 20 min for dopamine hydrochloride. The reaction remained stable for at least 24 h for all drugs.

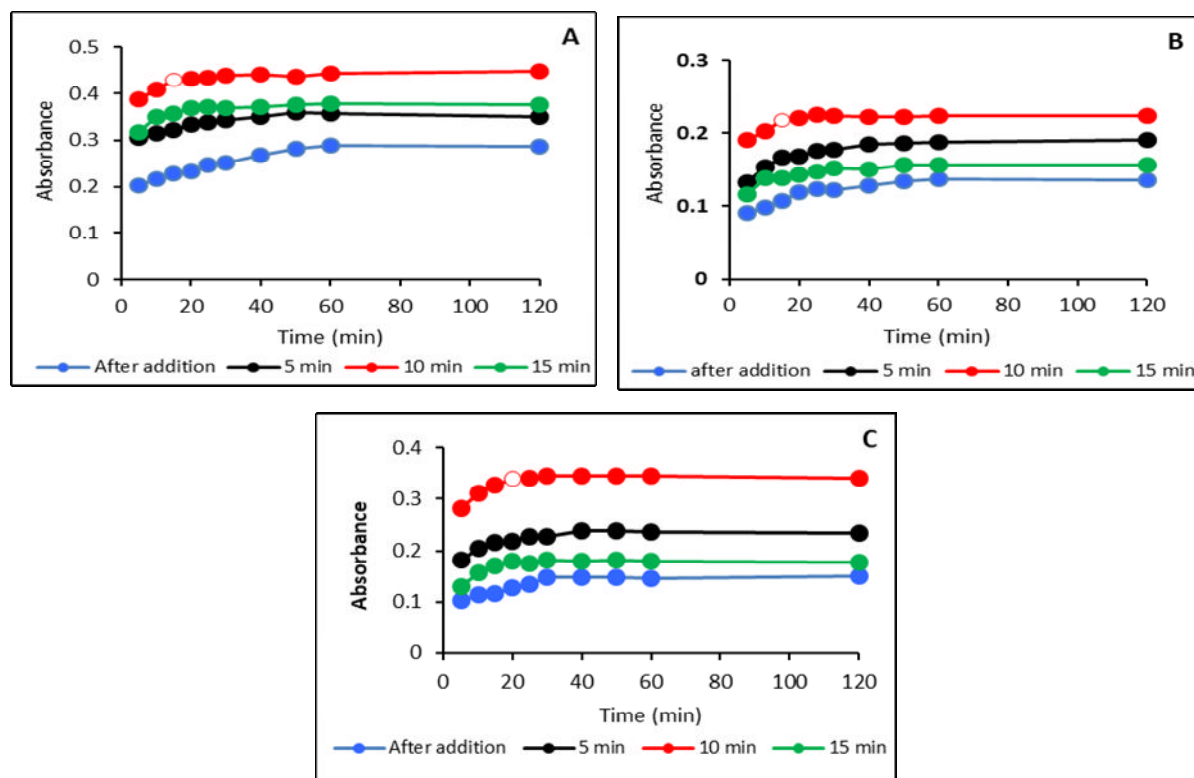


Figure 4. Effect of time on the oxidation of drugs and bleaching of calcon for (A) adrenaline, (B) methyldopa and (C) dopamine. HCl

Effect of Temperature on Oxidation Reaction and Stability:

The effect of the temperature and time on the oxidation of catecholamine drugs was studied in the range 23°C (Room temperature; RT) - 50°C using the optimum conditions and concentrations of the reagents as mentioned above. The absorbance of the dye was measured after 5 min intervals at 510 nm. The results showed in Fig. 5, A, B, and C indicated that high absorbance was reached after 30 min for dopamine and 20 min for adrenaline and methyl dopa at 30°C and remained constant for more than 3 h. Whereas higher temperature decreases the absorbance.

Effect of Addition Sequence: The addition sequence must follow the general procedure; otherwise, a decrease in absorbance was noticed.

Linearity and Calibration Graph: Under the optimum conditions mentioned above, standard calibration graphs for adrenaline, methyl dopa and dopamine. HCl with calcon dye was created by plotting the absorbance

versus concentration Fig. 6. The proposed methods obeyed Beer's law in the ranges 0.5-16.0, 2.0-40.0 and 1.0-36.0 $\mu\text{g mL}^{-1}$ with low intercept and good correlation coefficients for above drugs respectively. The molar absorptivity values were estimated, and they indicated that the method was sensitive (Table 3). Accuracy (average recovery %) and the relative standard deviation (RSD) for the analysis of three replicates of different concentrations for each of the above drugs explain that the method is accurate and precise. The detection limit (LOD) and quantitation limit (LOQ) [40] were calculated by the subsequent equations:

$$\frac{10\sigma}{h} \text{LOQ} =, \text{LOD} = \frac{3\sigma}{b}$$

Where: σ = standard deviation of the blank and b = slope of the calibration curve. The results, cited in Table 3, are below the lower limit of Beer's law range.

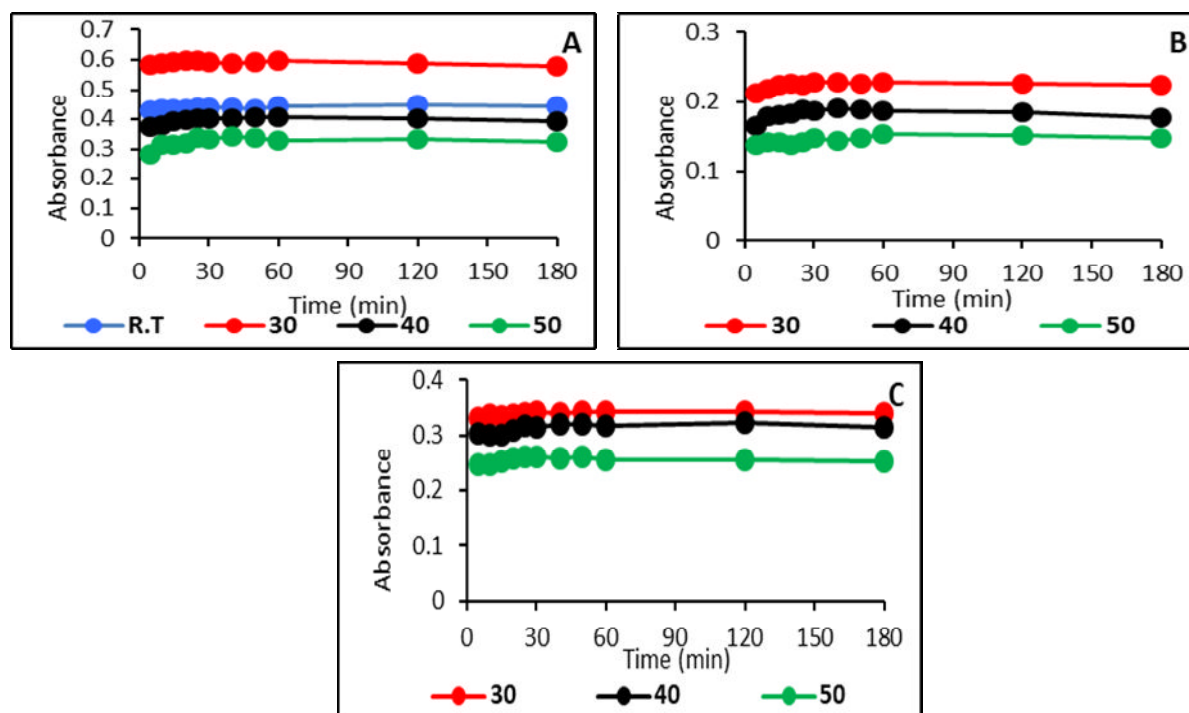


Figure 5. Effect of temperature on the absorbance and stability of the oxidation of adrenaline (A), methyl dopa (B) and dopamine (C)

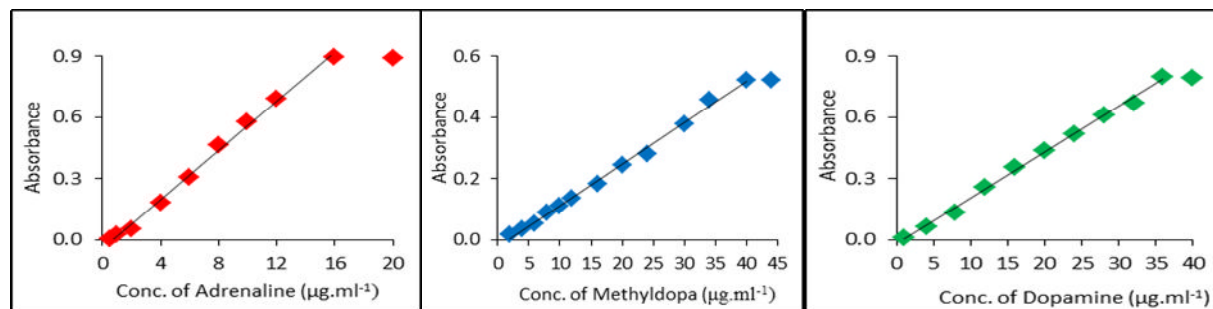


Figure 6. Calibration graphs of adrenaline, methyldopa and dopamine HCl

Table 3. Quantitative parameters and statistical data for assay of adrenaline, methyldopa and dopamine.

Parameters	Adrenaline	Methyldopa	Dopamine. HCl
Linearity range ($\mu\text{g mL}^{-1}$)	0.5-16.0	2.0-40.0	1.0-36.0
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.10×10^4	0.32×10^4	0.43×10^4
Sandell's Sensitivity ($\mu\text{g cm}^{-2}$)	16.67	74.07	44.44
Average recovery (%) [*]	101.42	99.83	100.06
LOD ($\mu\text{g mL}^{-1}$)	0.053	0.234	0.141
LOQ ($\mu\text{g mL}^{-1}$)	0.176	0.781	0.468
RSD [*]	0.38	0.85	0.35
Intercept	0.0423	0.0239	0.0216
Slope	0.0600	0.0135	0.0225
Correlation coefficient (R^2)	0.9968	0.9963	0.9970

^{*}Average for six determinations

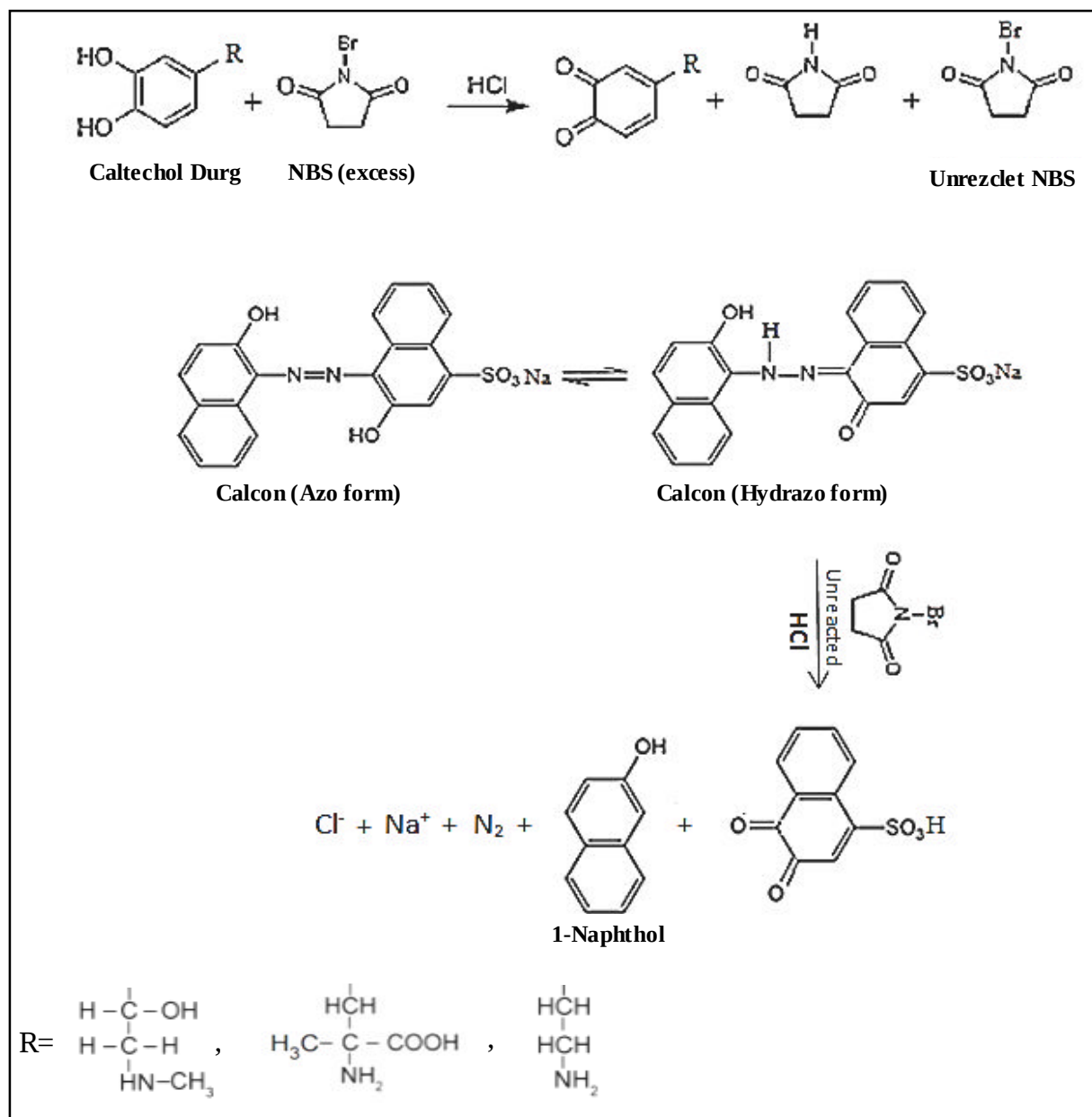
Suggested Mechanism: NBS is an oxidizing agent. It behaves as a bromination agent in the acidic medium for aliphatic and aromatic organic compounds [41-43]. This method assumed that NBS oxidizes the catecholamine drugs (adrenaline, methyldopa and dopamine hydrochloride) in an acidic medium. Then, the unreacted NBS oxidizes the known amount of calcon and bleach its color. The decrease of NBS concentration upon oxidation of known concentration of drugs leads to an increase in the absorbance of the dye at 510 nm, which is linearly proportional to the concentrations of the studied drug compounds. The suggested mechanism is explained in Scheme 1.

Analysis of Catecholamine Medicines in Commercial Formulations: The present method has been successfully applied for the determination of catecholamine drugs (Injection and tablet). The results mentioned in Table 4 indicate that the proposed method is accurate and reproducible.

Validation of the Method: For the purpose of demonstrating the efficiency of the developed method, its success in estimating catecholamine drugs, and its freedom from the effect of excipients in its pharmaceutical preparations, the standard addition technique has been applied to pharmaceutical

preparations for the above drugs. The method was summarized by adding increasing amounts of a standard solution of pure drug to a known quantity of the pharmaceutical preparation. By following the described

method, the absorption was measured at the wavelength of 510 nm, and the obtained results were included in Table 5, which indicates that the method has good selectivity.



Scheme 1. Suggested mechanism for oxidation of catecholamine drugs and calcon by NBS

Table 4. Analysis of the catecholamine medicines in commercial formulations.

Pharmaceutical preparation	Certified value	Amount present ($\mu\text{g mL}^{-1}$)	Drug content found* (mg)	Recovery ^a (%)	Average recovery (%)	Average recovery (mg)
Adrenaline ampoule ^b	1 mg	1	1.01	100.50	99.52	0.995
		4	0.98	98.04		
		6	1.00	99.81		
		10	1.00	99.72		
Adrenaline ampoule ^c	1 mg	1	0.99	98.83	99.20	0.992
		4	1.01	100.54		
		6	0.99	98.69		
		10	0.99	98.72		
Adrenaline ampoule ^d	1.8 mg	1	1.81	100.50	99.98	1.799
		4	1.79	99.29		
		6	1.80	100.08		
		10	1.80	100.05		
Aldosam tablet ^e	250 mg	4	244.90	97.96	99.34	243.350
		10	249.83	99.93		
		16	248.73	99.49		
		24	249.93	99.97		
Aldomac tablet ^f	250 mg	4	249.53	99.81	100.14	250.285
		10	251.67	100.67		
		16	251.04	100.42		
		24	249.15	99.66		
Dopadren ampoule ^g	200 mg	4	199.11	99.56	99.63	199.26
		10	200.52	100.26		
		16	199.78	99.89		
		24	197.62	98.81		

^aAverage of four determinationsManufactured by ^bLINCOLN Pharmaceuticals, India, ^cOSEL-Turkey, ^dDARNITSA Pharmaceuticals, Firm-Ukraine, ^eSDI-Iraq, ^fMACLEODS Pharmaceuticals Ltd-India and ^gVEM Pharmaceuticals-Turkey.

Table 5. Effect of excipient interferences by the standard addition procedure.

Pharmaceutical preparation	Certified value	Amount present ($\mu\text{g mL}^{-1}$)	Recovery (%)	Average recovery (%)	Drug content found (mg)
Adrenaline ampoule (India)	1 mg	4	97.49	98.78	0.987
		6	100.07		
Adrenaline ampoule (Turkey)	1 mg	4	97.05	98.36	0.983
		6	99.68		
Adrenaline ampoule (Ukraine)	1.8 mg	4	96.14	97.21	1.75
		6	98.28		
Aldosam tablet (Iraq)	250 mg	4	97.15	97.95	244.87
		10	98.75		
Aldomac tablet (India)	250 mg	4	99.00	99.73	249.32
		10	100.47		
Dopadren ampoule (Turkey)	200 mg	4	98.37	99.67	199.34

Table 6. Comparison between the proposed method and other methods.

Analytical parameter	Present method				Literature methods	
				[29] Dopamine	[44]	[36]
	Adrenaline	Methyldopa	Dopamine		Adrenaline	Methyldopa
Reagent		Calcon		Fe III-K ₃ Fe(CN) ₆	V ⁵⁺	Diaminopyridine- KIO ₄
Method		Oxidation		Prussian blue formation	Chelating complex	oxidative coupling
$\lambda_{\max}$ (nm)		510		735	488	478
Linearity ($\mu\text{g mL}^{-1}$)	1.0-36.0	2.0-40.0	0.5-16.0	0.05-6.00	0.5-140	2-24
Molar absorptivity $\text{L mol}^{-1}\text{cm}^{-1}$	1.10×10^4	0.32×10^4	0.43×10^4	3.2×10^4	2.015×10^3	1.0481×10^4
Temp. (C°)		30		R.T.	70	25
RSD	0.35	0.85	0.38	0.65	≤ 0.88	≤ 0.86
Applications	Injection	Tablet	Injection	Injection	Injection	Tablet

Comparison between the proposed method and other spectrophotometric methods: The suggested method for the determination of catecholamine drugs has been compared with other described spectrophotometric methods in the literature. The current method was distinguished by its sensitivity over some of the spectrophotometric methods listed in Table 6. This method is simple and could be applied to all catecholamine-containing drugs in their pure form and pharmaceutical preparations.

Conclusion

A new spectrophotometric method has been suggested for the determination of catecholamine-containing drugs. The method is dependent on the oxidation of drugs by NBS and subsequently reacted with calcon dye. The decolorization of the dye, measured at 510 nm, is proportional to the residual amount of NBS, which is proportional to the concentration of the drug. The method is simple, sensitive and accurate. The linearity range for calibration curves were 0.5-16.0, 2.0-40.0 and 1.0-36.0 $\mu\text{g mL}^{-1}$ with molar absorptivity values 1.10×10^4 , 3.2×10^3 and 4.3×10^3 $\text{L mol}^{-1}\text{cm}^{-1}$ for adrenaline, methyldopa and dopamine respectively. The method was applied successfully for the

determination of the intended drugs in their pharmaceutical formulations as tablets and injections with good recovery values.

Conflict of Interest

There is no conflict of interest in this research.

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