ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 23, No. 2 (2022) 247 – 258

http://doi.org/10.21743/pjaec/2022.12.07

Spectrophotometric Determination of Catecholamine Containing Drugs Using Calcon Dye

Ikram K. Othman and Theia'a N. Al-Sabha*

Chemistry Department, College of Education for Pure Science, Mosul University, Mosul, Iraq.

**Corresponding Author Email: dr_theiaa@yahoo.co.uk*

Received 28 January 2022, Revised 03 July 2022, Accepted 27 September 2022

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 μgmL^{-1} with molar absorptivity values 1.10×10^4, 3.2×10^3 and 4.3×10^3 $Lmol^{-1}cm^{-1}$ 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

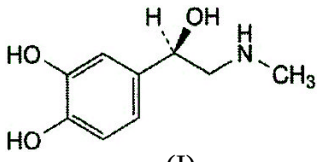
Catecholamines are a class of monoamines synthesized from tyrosine, which contain a catechol group and a side chain with an amino group in their structure [1]. Catecholamines-containing drugs are widely used to treat different disorders, such as playing a key role in the mechanisms of emotions, learning,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

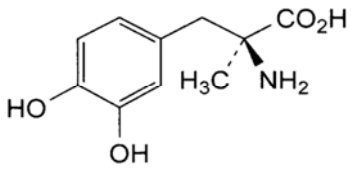
Methyldopa, is in the family of alpha-2-adrenergic receptor agonists. Chemically it is named S-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid (II). It is an antihypertensive drug used to lower blood pressure and treat some hypertension and gestational hypertension cases in renal failure and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

Dopamine constitutes approximately 80% of the catecholamine content material

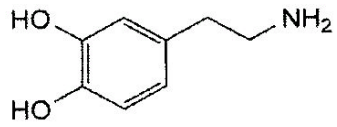
with inside the brain [9]. Chemically known as 2-(3, 4-dihydroxyphenyl) ethylamine (III), It is classified among the heart-stimulating drugs [10], which is a hormone secreted by the adrenal gland. It contributes to the fight or flight response processes [11] and the circulatory system (shock) due to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



(I)



(II)



(III)

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 μgmL^{-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, methyldopa and dopamine were prepared in a concentration of 100 μgmL^{-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 μgmL^{-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 μgmL^{-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 methyldopa against respective reagent blank.

Procedure for Drug Formulations Injection

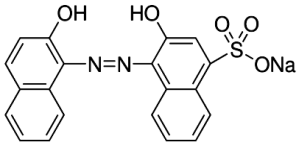
Adrenaline: The content of five ampoules of adrenaline (each ampoule contains 1.0 mg/1 mL adrenaline) or adrenaline darnitsa (each ampoule contains 1.8 mg/1 mL adrenaline) were mixed and then diluted to 50 mL with distilled water to obtain 100 or 180 μgmL^{-1} respectively. Different volumes of these solutions were taken to obtain concentrations 1.0, 4.0, 6.0 and 10.0 $\mu gmL^{-1,}$ 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 μgmL^{-1}. Then, a solution was prepared by dilution with a concentration of 100 μgmL^{-1}. Different volumes of the solution were taken to obtain concentrations 4.0, 10.0, 16.0 and 24.0 μgmL^{-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 aldosam or aldomac (each tablet contains 250 mg methyldopa) 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 μgmL^{-1}. The solution was diluted to 100 $\mu gmL^{-1,}$ and 4.0, 10.0, 16.0 and 24.0 μgmL^{-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 Reilley in 1958 to be used as an indicator for calcium ion [38, 39].



(IV)

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, methyldopa 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.

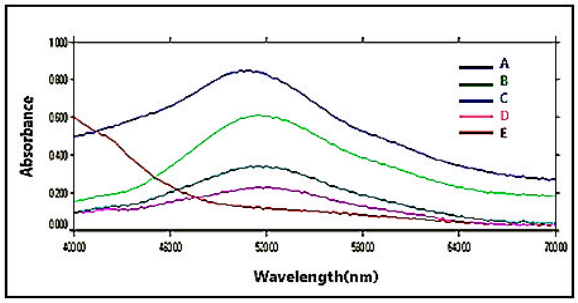


Figure 1. **Absorption spectra of 130 µgmL^{-1} calcon in acidic medium (A) in the presence of NBS and 10 µgmL^{-1} adrenaline (B), 20 µgmL^{-1} methyldopa (C) and 15 µgmL^{-1} dopamine (D) against reagent blank (E)**

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 µgmL^{-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 µgmL^{-1}. Therefore, the concentration was fixed at 130 µgmL^{-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.

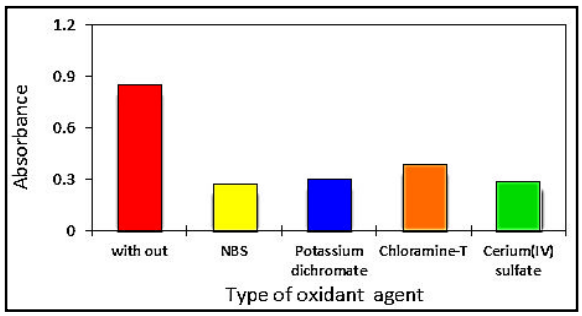


Figure 2. **Effect of oxidizing agent on the bleaching of 130 µgmL^{-1} calcon**

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 methyldopa and dopamine. Therefore, the effect of different acids of 1 M was examined for determination of the above drugs in the presence of 130 µgmL^{-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.

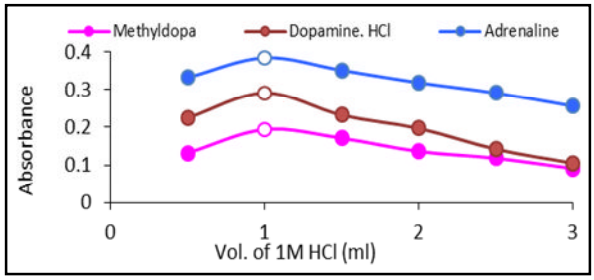


Figure 3. **Effect of volume of 1 M HCl on the absorbance of drugs**

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

Drug	µgmL^{-1}	Absorbance				
		HCl	*H_2SO_4*	*H_3PO_4*	*HNO_3*	*CH_3COOH*
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	µgmL^{-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.

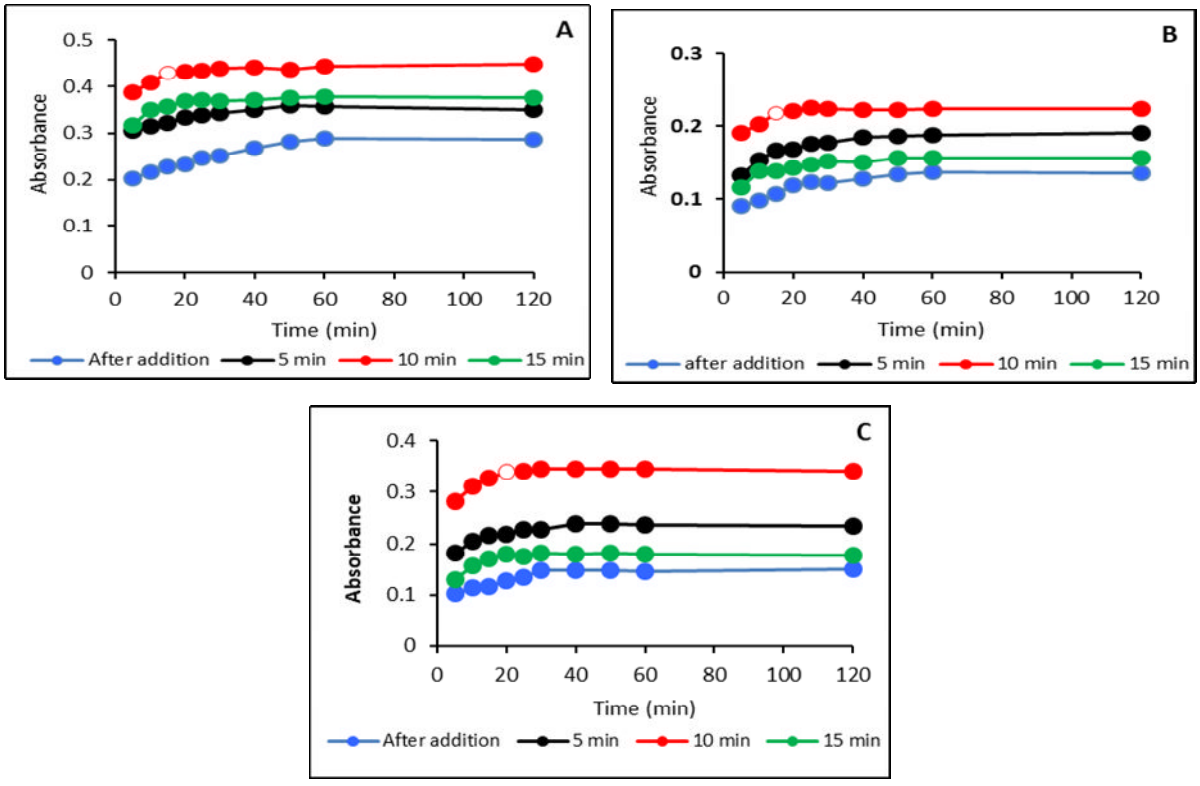


Figure 4. **Effect of time on the oxidation of drugs and bleaching of calcon for (A) adrenaline, (B) methyldopa and (C) dopamine. HCl**

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 methyldopa 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, methyldopa 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 μgmL^{-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:

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

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.

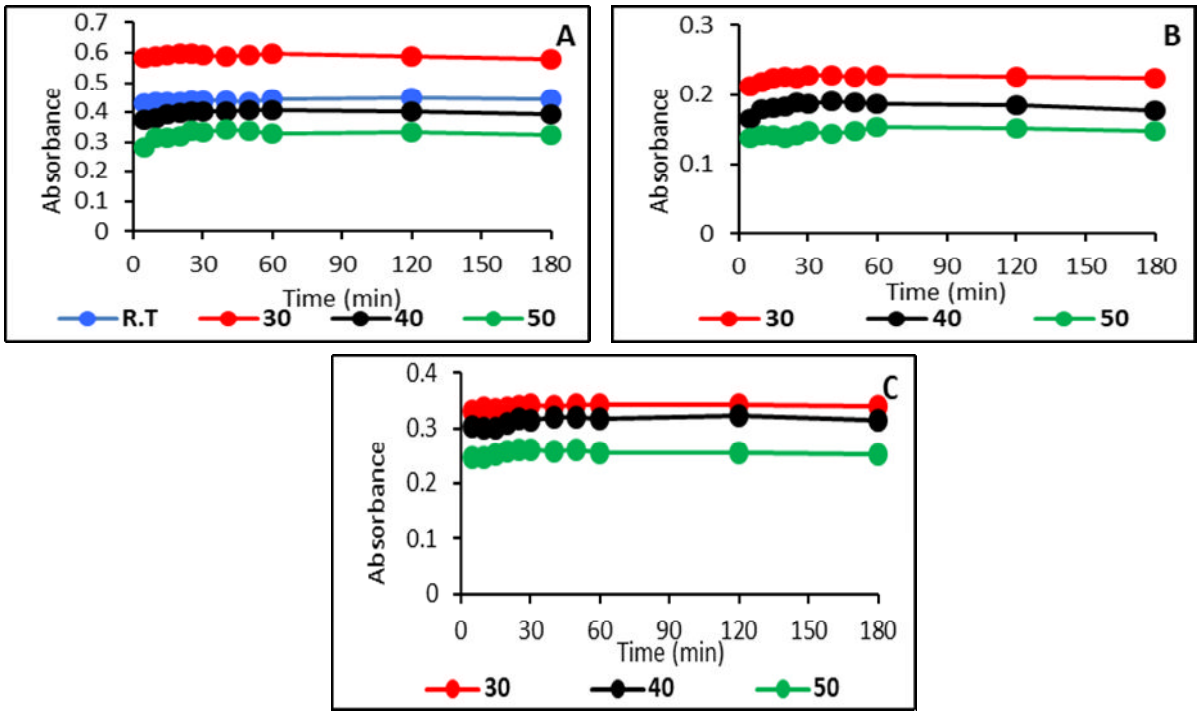


Figure 5. **Effect of temperature on the absorbance and stability of the oxidization of adrenaline (A), methyldopa (B) and dopamine (C)**

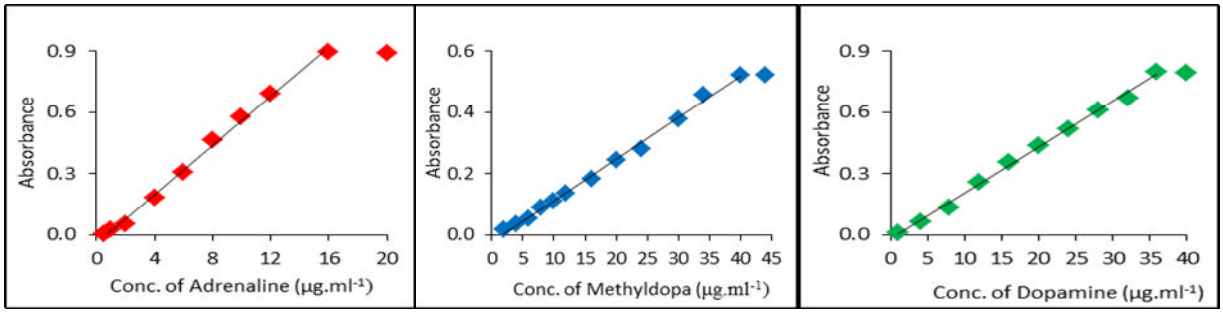


Figure 6. **Calibration graphs of adrenaline, methyldopa and dopamine. HCl**

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

Parameters	Adrenaline	Methyldopa	Dopamine. HCl
Linearity range (μgmL^{-1})	0.5-16.0	2.0-40.0	1.0-36.0
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	1.10×10^4	0.32×10^4	0.43×10^4
Sandell's Sensitivity (μgcm^{-2})	16.67	74.07	44.44
Average recovery (%)*	101.42	99.83	100.06
LOD (μgmL^{-1})	0.053	0.234	0.141
LOQ (μgmL^{-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

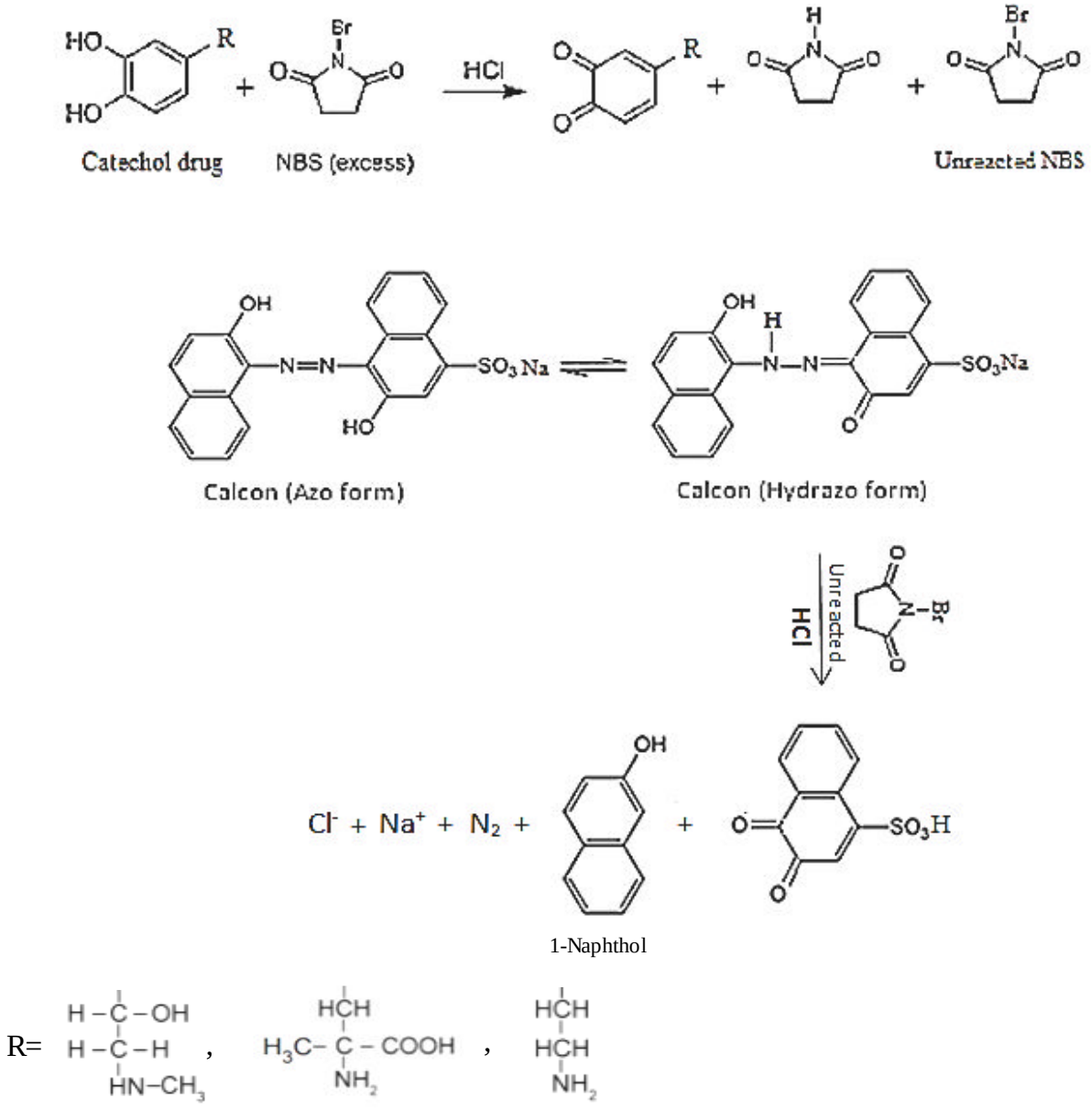
*Average for six determinations

Suggested Mechanism: NBS is an oxidizing agent. It behaves as a bromonation 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 (μgmL^{-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		

[a]Average of four determinations
Manufactured by [b]LINCOLN Pharmaceuticals, India, [c]OSEL-Turkey, [d]DARNITSA Pharmaceuticals, Firm-Ukraine,[e]SDI-Iraq,[f]MACLEODS Pharmaceuticals Ltd-India and [g]VEM Pharmaceuticals-Turkey.

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

Pharmaceutical preparation	Certified value	Amount present (μgmL^{-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		
				[44]	[36]	[29]
	Adrenaline	*Methyldopa*	*Dopamine*	*Adrenaline*	*Methyldopa*	*Dopamine*
Reagent		Calcon		V^{+5}	Diaminopyridine-KIO_4	Fe III-$K_3Fe(CN)_6$
Method		Oxidation		Chelating complex	oxidative coupling	Prussian blue formation
$\lambda_{max\,(nm)}$		510		488	478	735
Linearity (μgmL^{-1})	1.0-36.0	2.0-40.0	0.5-16.0	0.5–140	2-24	0.05–6.00
Molar absorptivity $Lmol^{-1}cm^{-1}$	1.10×10^4	0.32×10^4	0.43×10^4	2.015×10^3	1.0481×10^4	3.2×10^4
Temp. (C°)		30		70	25	R.T.
RSD	0.35	0.85	0.38	≤0.88	≤0.86	0.65
Applications	Injection	Tablet	Injection	Injection	Tablet	Injection

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 μgmL^{-1} with molar absorptivity values 1.10×10^4, 3.2×10^3 and 4.3×10^3 $Lmol^{-1}cm^{-1}$ 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.

References

1. P. Nagaraja, A. K. Shrestha, A. Shivakumar, N. G. S. Al-Tayar and A. K. Gowda, *Quim. Nova*, 34 (2011) 373. http://dx.doi.org/10.1590/S0100-40422011000300002
2. M. Lieberman, A. Marks and A. Peet. Marks' Basic Medical Biochemistry: A Clinical Approach (4th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, (2013) 175. ISBN 9781608315727.
3. R. C. Malenka, E. J. Nestler and S.E. Hyman, "Chapter 6: Widely Projecting Systems: Monoamines, Acetylcholine, and Orexin". In A. Sydor, R.Y. Brown (eds.). Molecular Neuropharmacology: A Foundation for clinical neuroscience (2nd ed.). New York, USA: McGraw-Hill Medical, (2009) 157. ISBN 9780071481274

5. N. Djelić, M. Radaković, S. Borozan, V. Dimirijević-Srećković, N. Pajović, B. Vejnović, N. Borozan, E. Bankoglu, H. Stopper and Z. Stanimirović, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 843 (2019) 8. doi: 10.1016/j.mrgentox.2019.01.013
6. S. Y. Hassan, *Cardiovasc. Revasc. Med.*, 20 (2019) 907. doi:10.1016/j.carrev.2018.10.026
7. T. Easterling, S. Mundle, H.Bracken, S. Parvekar, S. Mool, L. A. Magee, P. Dadelszen, T. Shochet and B. Winikoff, *Lancet*, 394 (2019) 1011. doi: 10.1016/S0140-6736(19)31282-6.
8. S. Braunthal and A. Brateanu, *SAGE Open Medicine*, 7(2019)1. doi: 10.1177/2050312119843700
9. K. C. Berridge, *Psychopharmacology*, 191 (2007) 391. doi:10.1007/s00213-006-0578-x
10. P. Sokoloff1 and B. L. Fol, *Eur. J. Neurosci.*, 45 (2017) 2. doi.org/10.1111/ejn.13390
11. A. Bjorklunda and O. Lindvallb, *J. Parkinson's Dis.*, 7 (2017) S21. doi.org/10.3233/jpd-179002
12. S. A. A. Touroutogloua, T. B. Rudya, S. Salcedoa, R. Feldmanc, D. J. M. Hookera, B. C. Dickersona, C. Catanaa and L. F. Barrett, *Proc. Natl. Acad. Sci.*, 114 (2017) 2361. doi.org/10.1073/pnas.1612233114
13. A. Kutluay, S. Karabulut and S. Abbasoglu, *J. Chin. Chem. Soc.*, 55 (2008)794. doi: 10.1002/jccs.200800119
14. X. Zhu, X. Ge and C. Jiang, *J. Fluorescence*, 17 (2007) 655. http://dx.doi.org/10.1007/s10895-007-0206-0
15. B. Perez-Mella, A. Alvarez-Lueje, *Electroanalysis*, 25 (2013) 2193. doi.org/10.1002/elan.201300241
16. N. F. Atta, A. Galal and R. A. Ahmed, *J. Electrochem. Soc.*, 158 **(**2011) F52. http://dx.doi.org/10.1149/1.3551579
17. M. Dolezalova and M. Tkaczykova, *J. Pharm. Biomed. Anal.*, 19 (1999) 555. doi.org/10.1016/s0731-7085(98)00257-x
18. J. Le, T. Sun, R. Peng, . Yuan, Y. Feng, S. Wang and Y. Li, *Mikrochim. Acta*, 187 (2020) 165. doi: 10.1007/s00604-020-4145-7
19. K. Murtada, F. Andrés, I. Galván, Á. Ríos and M. Zougagh, *J. Chromatogr. B*, 1136, (2020) 121878. https://doi.org/10.1016/j.jchromb.2019.121878
20. J. Britto-Júnior, N. J. Antunes, R. Campos, M. Sucupira, G. D. Mendes, F. Fernandes, M. O. Moraes, M. E. A Moraes and G. D. Nucci, *Biomed. Chromatigr.*, 35 (2021) e4978. doi: 10.1002/bmc.4978.
21. F. Chen, B. Fang and S. Wang, *J. Anal. Meth. Chem.*, ID 8821126 (2021)1. doi.org/10.1155/2021/8821126
22. M. P. dossantos, A. Rahim, N. Fattori, L. T. Kubota and Y. Gushikem, *Sensors Actuators B*, 171-172 (2012) 712. doi.org/10.1016/j.snb.2012.05.061
23. S. Xiong, H. Han, R. Zhao, Y. Chen and G. Liu, *Biomed. Chromatogr.*, 15 (2001) 83. https://doi.org/10.1002/bmc.37
24. L. A. Kartsova, E. A. Bessonova, A. A. Sidorova, V. A. Kazakov, I. A. Tver'yanovich and L. I. Velikanova, *Russ. J. Appl. Chem.*, 77 (2004) 1150. http://dx.doi.org/10.1023/B:RJAC.0000044165.62665.85
25. A. Moslehipour, *J. Chem. Rev.*, 2 (2020) 130. doi: 10.33945/SAMI/JCR.2020.3.1
26 I. A. Shihab and T. N. Al-Sabha, *Pak. J. Anal. Environ. Chem.*, 21 (2020) 10. http://doi.org/10.21743/pjaec/2020.06.02
27. M. M. Al-Sharook, *J. Edu. Sci.*, 19(2), (2007) 1. doi.org/10.33899/edusj.2007.5781
28. T. S. Al-Ghabsha, M. S. Al-Enizzi and Z. Z. Al-Abdaly, *J. Edu. Sci.*,19(3), (2007) 1. doi.org/10.33899/edusj.2007.51320

29. L. G., Y. Zhang, Q. Li., *Anal. Sci*., 25 (2009) 145. doi.org/10.2116/analsci.25.1451
30. B. S. Nagaralli, J. Seetharamappa and M. B. Melwanki, *J. AOAC Int.*, 85 (2002) 1288. doi.org/10.1093/jaoac/85.6.1288
31. P. Nagaraja, R. A. Vasantha and K. R. Sunitha, *J. Pharm. Biomed. Anal*., 25 (2001) 417. doi.org/10.1016/s0731-7085(00)00504-5
32. A. J. M. Al-Ogaidi, *J. Eng. Stud. Res.*, 22 (2016) 7. http://dx.doi.org/10.29081/jesr.v22i4.250
33. F. A. Nour El-Dien, M. A. Zayed, G. G. Mohamed and R. G. El-Nahas, *J. Biomed. Biotechn.*, (2005) 1. doi.org/10.1155/jbb.2005.1
34. N. j. Alaallah, S. A. Dhahir and H. H. Ali, *IOP Conf. Series: Mater. Sci. Eng.*, 871 (2020) 012033. doi:10.1088/1757-899X/871/1/012033
35. L. Rahman, A. M. Al-Abachi and M. H. Al-qaissy, *Anal. Chim. Acta*, 538 (2005) 331. doi:10.1016/j.aca.2005.02.045
36. I. T. Humeidy, *J. Eng. Sci. Techn*., 15 (2020) 1824.
37. S. Ashour, *Drug Anal. Res.*, 5 (2021) 51. doi.org/10.22456/2527-2616.113058
38. G. P. Hildebrand and C. N. *Reilley, Anal. Chem.*, 29 (1957) 258. doi.org/10.1021/ac60122a025
39. R. Belcher, R. A. Close and T. S. West, *Talanta*, 1 (1958) 238. doi.org/10.1016/0039-9140(58)80039-9
40. M. Valcarcel, "Principles of Analytical Chemistry: A textbook", *Springer* (2000) 67. Berlin, Germany. doi: 10.1007/978-3-642-57157-2
41. K. Basavaiah, B. C. Somashekar and V. Ramakrishna, *Acta Pharm*., *57* (2007) 87. doi:10.2478/v10007-007-0007-7
42. Zahraa J. Al-Gubouri and Theia'a N. Al-Sabha, *J. Edu. Sci*., 29 (2020) 246. doi: 10.33899/edusj.2020.126846.1060
43. K. B. Vinay, H. D. Revanasiddappa, O. Z. Devi, P. J. Ramesh and K. Basavaiah, *Braz. J. Pharm. Sci*., 47 (2011) 251. doi.org/10.1590/s1984-82502011000200006
44. A. S. Al-Ayash, *J. Al-Nahr. Univ*., 11 (2008) 54. doi.org/10.22401/jnus.11.3.05