Egypt. J. Chem. **Vol 63**, No. 10 pp. 3767 - 3777 (2020)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Spectrophotometric Method For Indirect Determination Of Antihypertensive Drugs In Pharmaceuticals

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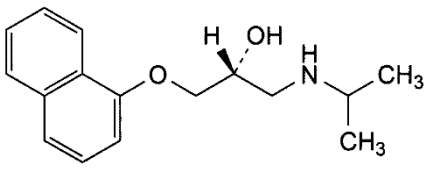
Abstract

A spectrophotometric method is proposed for the determination of antihypertensive drugs namely Propranolol and Methyldopa in their pure forms and in pharmaceutical formulations, based on the oxidation of the drugs with an excess of N-Bromo succinimide and the residual oxidizing agent bleaches the red colored eriochrom black-T (EBT) which is measured at 530 nm at room temperature. Linear calibration graphs were obtained in the concentration range 0.1-10 and 0.1-9 µg ml^{-1} with molar absorptivity 6.06×10^4 and 4.60×10^4 $L.mol^{-1}.cm^{-1}$ for above drugs respectively. No interference was observed coexistent substance. The proposed method was applied successfully for the determination of the drugs in their pharmaceutical formulations.

Keywords: Spectrophotometry, EBT, Antihypertensive drugs

1. introduction

Hypertension is commonly known as a silent killer disease which increases the risk of heart disease and stroke, and considered as one of factors for death and inability over the world [1]. Propranolol hydrochloride, 1-[isopropylamino-3-[1-naphthyloxy]-2-propanol hydrochloride (I), is β-blocker drug and used to control hypertension, cardiac arrhythmia, myocardial and hyperthyroidism diseases [2-5]. Methyldopa, α-methyl-3,4-dihydroxyphenylalanine (II) is used as an antihypertensive agent and effective inhibitor for the dopa-decarboxylase enzyme to lower blood pressure [6, 7].



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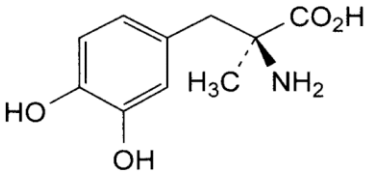

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Different analytical methods and techniques have been described for determination of the above drugs in pure form and pharmaceutical formulations, including spectrophotometric[8-14] , near infra-red and chemometric [14], HPLC [4, 15-17], titrimetric [14, 18], voltametric [4, 19-22] and electrochemical [4, 22] techniques. However; most of these techniques are expensive instruments and need experience. Some of the other methods have low sensitivity and tedious or uneconomical. EBT is anionic azo dye compound and known as a complexometric indicator which is used in complexometric titrations. According to literature information, it's known that EBT interacts with drugs, resulting in the formation of ion-pair complexes, which are extracted by organic solvent [23-25]. The color of this indicator can be bleached by the strong oxidizing agent [26]. The aim of the present work is to develop a simple and sensitive method for determination of propranolol and methyldopa drugs depending upon oxidation of these drugs and decolorization of EBT colored reagent.

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EJCHEM use only: Received date: 12 October 2019; revised date: 24 February 2020; accepted date: 3 March 2020
DOI: 10.21608/EJCHEM.2020.18096.2102

2. EXPERIMENTAL

2.1 Apparatus

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

2.2 Reagents

All reagents were of analytical-reagent grade which were provided by BDH and Fluka companies. Stock solutions of Propranolol and Methyldopa were prepared in a concentration of 100 μg 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. EBT was prepared in concentration of 500 μg ml^{-1} by dissolving 0.05 g in methanol in 100 ml volumetric flask. N-Bromo succinimide (NBS) was prepared in a concentration 2×10^{-3}M by dissolving 0.0356 g in 100 ml distilled water. Hydrochloric acid was prepared in a concentration of 5 M by diluting of an appropriate volume of conc. HCl.

3. General procedure

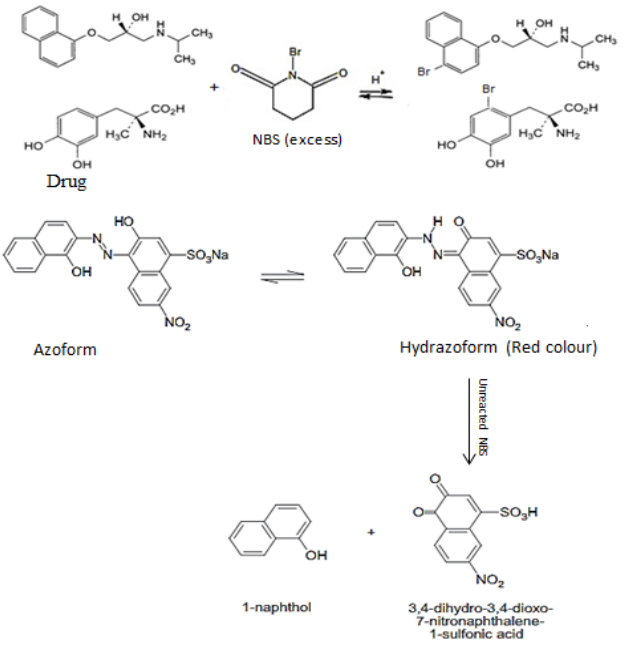
Into two series of 10-ml volumetric flasks aliquots of solutions containing 0.1-10 and 0.1-9 μg ml^{-1} of Propranolol and Methyldopa respectively, were added separately, followed by addition of 1.5 ml of 5M HCl and1.5 ml of 5×10^{-3}M NBS to each flask. The solutions were gently shaken and left for 10 min at room temperature for oxidation. A 2 ml of 500 μg ml^{-1} EBT were added to the solutions. The contents were diluted to the mark with distilled water and mixed well. The absorbance was measured at 530 nm after 5 min at room temperature against reagent blank.

4. Analysis of tablet

Weighed and finely powdered 7 tablets Propranolol (each tablet containing 40 mg Propranolol) and aldosam (each tablet containing 250 mg Methyldopa). an accurately weighed amount of powder, equivalent to one tablet, was dissolved in 20 ml distilled water and filtered into 100 ml calibrated flask, then the solution was made to the volume with the distilled water. A suitable volume was diluted with distilled water and followed the recommended procedure.

5. Results and Discussion

EBT is a dark blue dye as powder and its solution at pH< 6.3 become red color. It was found that the red color of EBT can be bleached by strong oxidizing agents [24]. According to this observation, a simple method has been developed for the determination of some antihypertensive drugs involves the oxidation of these drugs by NBS in acidic medium and the residual oxidant bleaches the red color of EBT. When the known volume of NBS is added to an increasing amount of drug, there was a decrease in the concentration of oxidant and increasing the absorbance of EBT. The proposed reaction mechanism is presented in Scheme 1.



Scheme 1: Proposed mechanism for determination of drugs

EBT showed an absorption band with λ_{max} at 530 nm in an aqueous medium (Fig. 1). The upper limit concentration of EBT was selected by plotting the absorbance of increasing amounts of dye against distilled water at λ_{max} and was found 100 µg ml^{-1} (Fig. 2) which was selected in subsequent experiments.

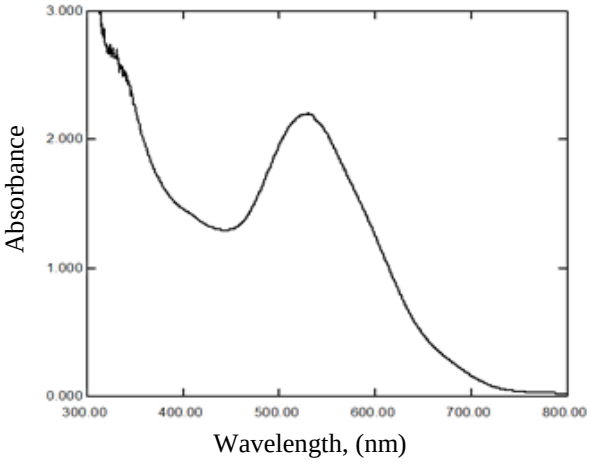


Figure 1: Absorption spectrum of 100 µg ml^{-1} EBT against distilled water

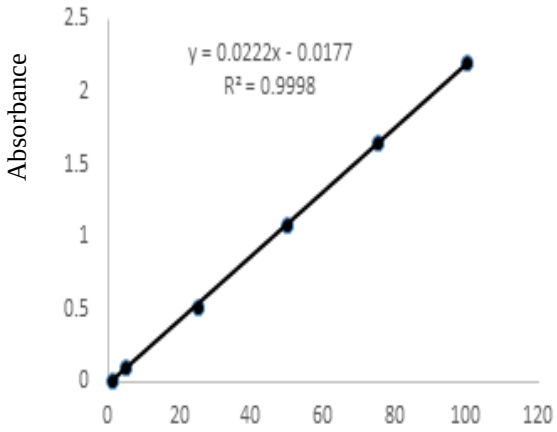


Figure 2: Calibration graph of EBT. EBT conc., µg ml^{-1}

ion of conditions

In order to establish the experimental conditions for high sensitivity of the method, effect of various parameters such as solvent, oxidizing agent, acid, temperature and time were studied and optimized by setting all parameters constant and optimizing one at a time.

5.1.1 Effect of solvent

To select the solvent which gives the highest absorbance for EBT, different solvents were

tested such as acetone, methanol, ethanol, propanol, dimethylformamide (DMF) in addition to water. It was found, as shown in Figure 3, that water and methanol were optimum solvents and gave high stability and absorbance at 530 nm. However; water was selected in subsequent experiments.

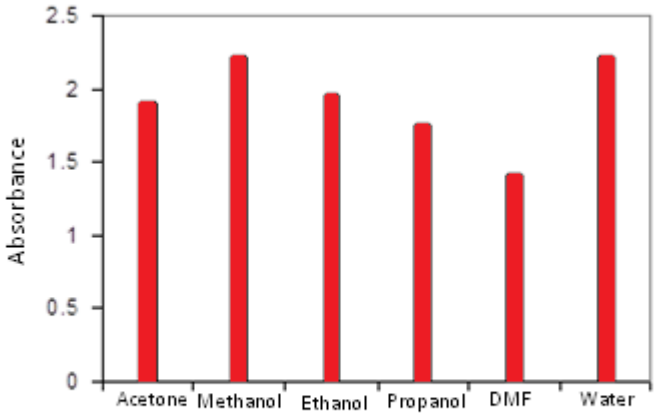


Figure 3: Effect of solvent on the absorbance of 100 µg ml^{-1} EBT against solvent

5.1.2 Effect of oxidant

Various oxidizing agents such as potassium chromate, potassium permanganate, potassium iodate, N-chlorosuccinimide and NBS with a concentration of 2×10^{-3}M have been tested for bleaching of EBT in the presence of 1 ml of 1M HCl. It was found that NBS is the best oxidant and 1.5 ml is sufficient to bleach the dye to colorless (Fig. 4) which is recommended in subsequent experiments.

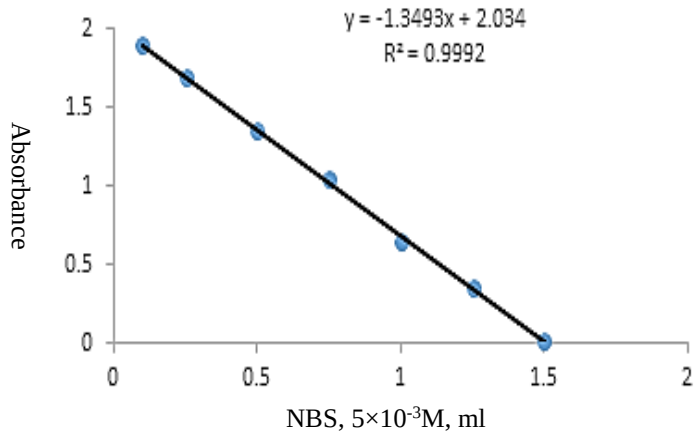


Figure 4: effect of NBS on the bleaching of 100 µg ml^{-1} EBT

5.1.3 Effect of acid

The oxidation of drugs and dye take place in acidic medium. Various acids such as HCl, H_2SO_4, HNO_3 and CH_3COOH of 1 M have been tested, using 2 µg ml^{-1} of each drug, to obtain high sensitivity. It was found that HCl is the best acid for the system (Fig. 5). and was found 1.5 ml of 5M HCl gave high sensitivity for each drug which is recommended in this method.

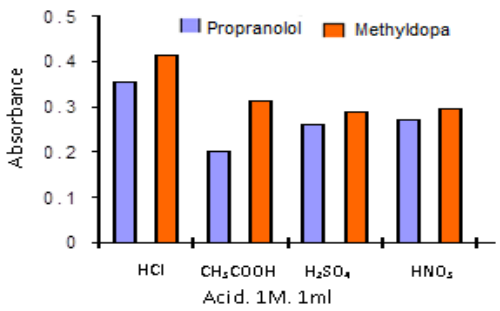


Figure 5: Effect of acid on the sensitivity of the method using 2 µg ml^{-1} of each drug

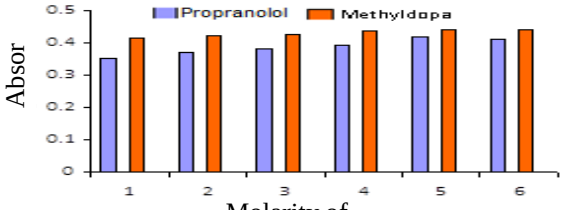


Figure 6: Effect of concentration of HCl on the sensitivity of the method using 2 µg ml^{-1} of each

5.1.4 Effect of oxidation period

The effect of oxidation period of Propranolol and Methyldopa drugs was studied by addition of 1.5 ml of 2×10^{-3}M NBS to 2 µg ml^{-1} for each drug in the presence of 1.5 ml of 5M HCl. The solutions were shacked and left at room temperature for different periods of time. Then 100 µg ml^{-1} EBT were added to each drug and the solutions were shacked and diluted to 10 ml in calibrated flasks. The absorbance of the residual EBT was measured after 5 min standing time at 535 nm against distilled water. The results obtained in Table 1 indicated that 10 min is sufficient for the oxidation of drugs and the absorbance remain constant for more than 2 hours.

Table 1: Effect of time on the oxidation of drugs and EBT

Standing time before dilution (min.)	Absorbance / standing time (min)								
	5	10	20	30	40	50	60	120	Over night
Propranolol									
Immediately	0.242	0.249	0.249	0.250	0.248	0.247	0.250	0.248	
5	0.412	0.421	0.421	0.420	0.420	0.413	0.409	0.409	0.407
10	0.410	0.410	0.408	0.408	0.408	0.406	0.406	0.406	
15	0.401	0.400	0.400	0.400	0.400	0.398	0.398	0.398	
25	0.410	0.402	0.400	0.400	0.400	0.397	0.397	0.390	
Methyldopa									
Immediately	0.431	0.431	0.431	0.431	0.43	0.43	0.43	0.0.429	
5	0.440	0.441	0.44	0.44	0.44	0.44	0.44	0.438	0.437
10	0.439	0.439	0.438	0.439	0.439	0.437	0.437	0.437	
15	0.432	0.433	0.432	0.431	0.43	0.43	0.43	0.43	
25	0.43	0.43	0.429	0.429	0.429	0.429	0.429	0.429	

5.1.5 Effect of temperature for oxidation and stability

The color produced must be stable so as to allow accurate readings to be taken. The period over which maximum absorbance remains constant must be long enough for precise measurement to be made. Stability of the color is affected by experimental conditions like temperature and time. However; the effect of the temperature and time on the oxidation of Propranolol as a model drug was studied in the range 28°(RT)- 45°C with the optimum of the concentrations of the reagents. Then EBT was added and diluted to 10 ml with distilled water in the calibrated flask. The absorbance of the dye (after addition) was measured after 5 min. The results showed that high absorbance was reached after 10 min oxidation of drugs at room temperature and remain constant for more than 6 hours. Whereas higher temperature values decrease the absorbance, (Fig. 7). The same results were obtained for Methyldopa. However; 10 min at room temperature was selected for further study.

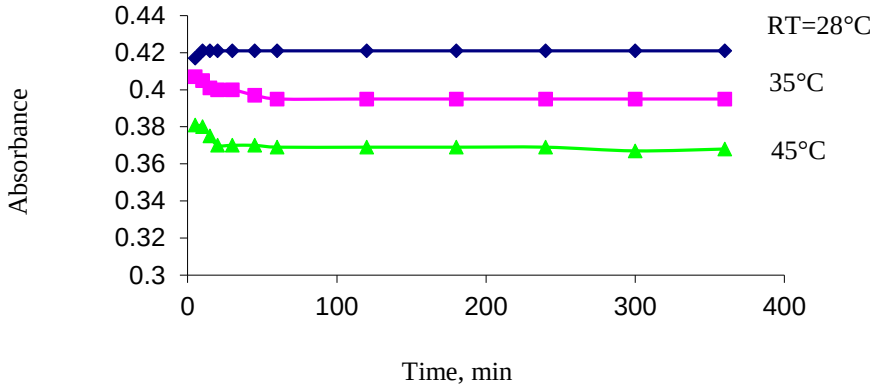


Figure 7: Effect of temperature and time for oxidation of 2 µg ml^{-1} Propranolol in the presence of 190 µg ml^{-1} EBT

5.1.6 Effect of sequence addition: As shown in table 2, there is no significant difference in absorbance when changing the sequence addition of reagents

Table 2: Effect of sequence addition for reagent and drug

Sequence addition	Propranolol	Methyldopa
D + H + NBS + EBT	0.422	0.442
H + NBS + D + EBT	0.420	0.439
NBS + EBT + D + H	0.421	0.440

D = drug, H = HCl.

6. Quantitation

Under the described experimental conditions, standard calibration curves for, Propranolol and Methyldopa with EBT were constructed by plotting absorbance against concentration (Fig.8). Beer's law limits and molar absorptivity values were evaluated and given in Table 3, which indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of three replicates of each three different concentrations for each drug indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

$$LOD = 3.3\sigma/b \text{ and } LOQ = 10\sigma/b$$

where σ is the standard deviation of five reagent blank determinations and b is the slope of the

calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range, (Table3).

Table3: Summary of optical characteristics and statistical data for the proposed method

Parameter	Propranolol	Methyldopa
Linearity range (µg/ml)	0.1-10	0.1-9
Molar absorptivity ($l.mol^{-1}. cm^{-1}$)	6.06×10^4	4.60×10^4
LOD ($\mu g.ml^{-1}$)	0.042	0.066
LOQ ($\mu g.ml^{-1}$)	0.120	0.192
Average recovery [a] (%)	100.83	100.05
Correlation coefficient (R)	0.9991	0.9990
Regression equation (Y)[b] Slope, a Intercept, b	 0.2429 -0.0031	 0.1932 0.0451
RSD [b]	≤ 1.0	≤ 1.0

[a]Average of three determinations
[b]Y = a X + b, where X is the concentration of each compound in µg ml^{-1}.

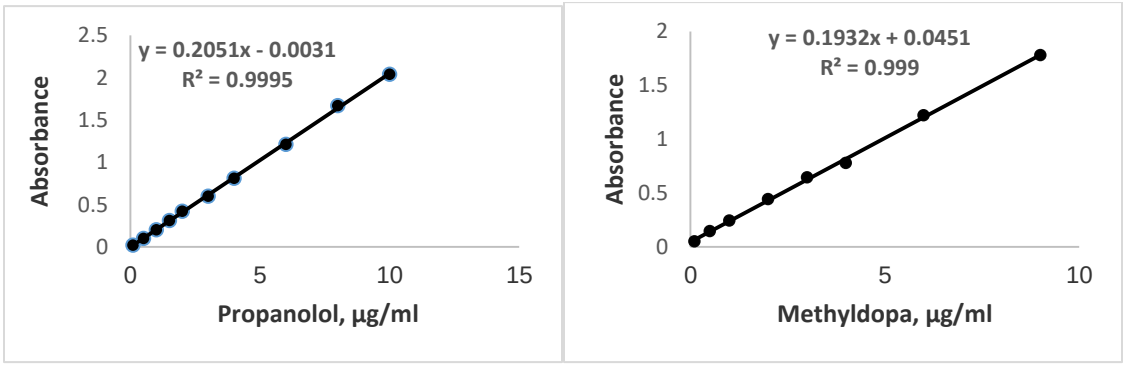


Figure 8: Calibration graphs of Propranolol and Methyldopa

7. Selectivity

The selectivity of the method was investigated by observing any interference encountered from the common excipients of the pharmaceutical formulations by measuring the absorbance of solutions containing a fixed amount of drug separately, and various amounts of diverse species, up to 500 µg/ml, in a final volume of 10 ml. It was found that the studied excipients did not interfere seriously, (Table 4).

Table 4: Effect of excipients on the determination of drugs

Excipient	Recovery (%) of 3 µg/ml of drug per µg/ml of excipient added			
	Propranolol		Methyldopa	
	250	500	250	500
Sucrose	98.87	96.23	97.23	98.65
Fructose	98.21	97.23	98.57	98.23
Lactose	98.85	96.36	96.25	97.98
Sodium chloride	97.68	98.89	98.58	97.87
Starch	95.53	98.00	95.23	98.80
Potassium chloride	98.50	96.23	96.85	98.21
Glucose	97.25	97.12	98.89	97.01

8. Analysis of pharmaceutical formulations

The proposed method was successfully applied to determine the intended drugs in their commercial tablets of Propranolol and Methyldopa, (Table 5). The results given in Table 6 indicated that the method is a reproducible and accurate.

Table 5: Industrial companies of drug formulations

Pharmaceutical preparation	Content	Company
Inderal tablets	40 mg Propranolol	Abbott - USA
Aldosam tablets	250 mg methyldopa	Samra-Iraq

Table 6: Assay of the drugs in some pharmaceutical formulations by the proposed method and standard addition procedure.

Pharmaceutical preparation	Certified Value	Amount present ($\mu g.ml^{-1}$)	Recovery* (%)	Average recovery (%)	Drug content found (mg)
Propranolol tablet	40 mg	2	98.57	99.04	39.428
		4	101.12		40.448
		6	97.45		38.98
Methyldopa Tablet	250 mg	2	101.22	100.48	253.05
		4	101.54		253.62
		6	98.77		246.92

* Average for four determinations

9. Validity of the method

The proposed method was compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the official method [24] for intended drugs formulations at the 95% confidence level with six degrees of freedom. The results showed in Table 7 that the experimental t-test and F-test were less than the theoretical value (t=2.45, F=6.39), indicating that there was no significant difference between the proposed method and official method.

Table 7: Comparison of the proposed method with official method for determination of antihypertensive drugs in pharmaceutical preparation

Pharmaceutical preparation	Recovery (%)		t_{exp}	F_{test}
	Present method	Official method		
Propranolol tablets	101.12	100.75	1.25	1.05
Methyldopa tablets	101.54	100.18	0.98	1.012

10. **Comparison of the proposed method**

Many spectrophotometric methods have been described for the determination of propranolol and methyldopa, but all of these methods suffer from limitations (table 8) for instance, these involve extraction, heating or have low sensitivity.

Table 8: Comparison of the proposed method with official method for determination of antihypertensive drugs with the previous works

Reagent used	**λ_{max} (nm)**	**Beer's law (µg.ml^{-1})**	**Molar absorptivity/10^4 (l.mol^{-1}.cm^{-1})**	**Remark**	**Ref. no.[a]**
Alizarine red-s	515	200-25	0.0961	Involved extraction	[26]
Erythrosine-b	525	10-80	0.163	Involved extraction	[26]
Chloramine-T in phosphate buffer of 7	555	40-5	0.486	Involved heating to 70 ˚c for 5 min	[27]
NBS+ promethazine	513	0.2-16	2.05	Has low sensitivity	[28]
Molybdate	410	50-200	1.13×10^3	Has low sensitivity	[29]
4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole	470	1.6-17.6	1.9337	Has low sensitivity	[28]
Proposed regent	530	0.1-10,0.1-9	6.06, 4.60	Sensitive and does not involve heating or extraction	[31 ,30]

a 26-28 refer to propranolol and 29-31 refer to methyldopa

11. Conclusion

The proposed method represents simple, rapid, precise, accurate and highly sensitive. The validity of the proposed method is well demonstrated by analyzing the dosage form of Propranolol and Methyldopa tablets. Moreover, the method is free from interference by common additives and excipients. The method depends on the use of simple and cost-effective chemicals and techniques but provide sensitivity in comparison to that achieved by sophisticated and expensive techniques like HPLC and voltammetry. Thus, the method can be used as alternatives for routine determination of bulk sample and pharmaceutical formulations

12. References

1. Khorshed, A.A., M. Khairy, and C.E. Banks, *Electrochemical determination of antihypertensive drugs by employing costless and portable unmodified screen-printed electrodes.* Talanta, 2019. **198**: p. 447-456.
2. Banerjee, S., et al., *Pharmacological Property of Pentacyclic Triterpenoids.* Egyptian Journal of Chemistry, 2019. **62**(Special Issue (Part 1) Innovation in Chemistry): p. 13-35.
3. Sartori, E.R., et al., *Square-wave voltammetric determination of propranolol and atenolol in pharmaceuticals using a boron-doped diamond electrode.* Talanta, 2010. **81**(4-5): p. 1418-1424.

4. Al-Majed, A.A., et al., *Propranolol,* in *Profiles of drug substances, excipients and related methodology*. 2017, Elsevier. p. 287-338.
5. Garfein, O.B., *Pharmacology of commonly used antiarrhythmic drugs and comments on the use of therapeutic drug monitoring.* Therapeutic drug monitoring, 1982. **4**(1): p. 1-14.
6. Molla Ali Akbari, S., et al., *Effects of Maternal Alpha Methyldopa Administration on Memory of Rat Offspring during Growing Age.* Iranian Journal of Toxicology, 2017. **11**(1): p. 43-47.
7. Adrover, R., et al., *Hepatotoxicity from Alpha-Methyldopa During Pregnancy: Two Case Reports.* Journal of Clinical Gastroenterology and Treatment, 2016. **2**(3): p. 1-3.
8. Zayed, M., et al., *Spectrophotometric assay of levodopa and alpha-methyldopa in pharmaceutical products and in biological samples of schizophrenic patients using copper complex and tri-iodide reagent.* Egyptian Journal of Chemistry, 2005. **48**(4): p. 437.
9. Madrakian, T., et al., *Spectrophotometric determination of catecholamines based on their oxidation reaction followed by coupling with 4-aminobenzoic acid.* Journal of the Brazilian Chemical Society, 2006. **17**(7): p. 1259-1265.
10. Hussein, A.F., M.A. AL-Da'amy, and M.H. AL-Fatlawy, *Spectrophotometric determination of Levo-dopa in pharmaceutical preparation via oxidative coupling organic reaction.* Karbala journal of pharmaceutical sciences, 2013(4): p. 145-154.
11. Sharma, D., J. Singh, and P. Raj, *Spectrophotometric determination of propranolol hydrochloride and metoprolol tartrate in pharmaceutical dosage forms, spiked water and biological fluids.* Int J Pharm Pharm Sci, 2018. **10**: p. 107-15.
12. Prashanth, K.N. and K. Basavaiah, *Quantitative spectrophotometric determination of propranolol hydrochloride in pharmaceuticals using cerium (IV) sulphate as oxidimetric reagent.* Proceedings of the National Academy of Sciences, India Section A: Physical Sciences, 2014. **84**(1): p. 27-35.
13. Haddi, H., R. Sinan, and M.Q. Al-Abachi, *Spectrophotometric determination of methyldopa and dopamine hydrochloride in pharmaceutical preparations using flow injection analysis.* Iraqi National Journal of Chemistry, 2009(36): p. 597-604.
14. Bagheri, A. and I. Mesgarzadeh, *Titrimetric and Spectrophotometric Methods for the Determination of β-blockers in Pharmaceutical Dosage Form.* Journal of Applied Chemical Research, 2012. **6**(2): p. 53-64.
15. Marques Junior, J.M., et al., *Determination of propranolol hydrochloride in pharmaceutical preparations using near infrared spectrometry with fiber optic probe and multivariate calibration methods.* Journal of analytical methods in chemistry, 2015. **2015**.
16. Emara, S., et al., *An eco-friendly direct injection HPLC method for methyldopa determination in serum by mixed-mode chromatography using a single protein-coated column.* Journal of chromatographic science, 2015. **53**(8): p. 1353-1360.
17. Abdel-Hamid, M.E., *Comparative LC–MS and HPLC analyses of selected antiepileptics and beta-blocking drugs.* Il Farmaco, 2000. **55**(2): p. 136-145.
18. Kumar, V. and I. Shukla, *New oxidative determination of some new Antihypertensive drugs in pure form and in their pharmaceutical preparations with Cu (III) reagent.*
19. Zilberg, R.A., et al., *A voltammetric sensory system for recognition of propranolol enantiomers based on glassy carbon electrodes modified by polyarylenephthalide composites of melamine and cyanuric acid.* Electroanalysis, 2018. **30**(4): p. 619-625.
20. Rezaei, B., N. Askarpour, and A.A. Ensafi, *Adsorptive stripping voltammetry determination of methyldopa on the surface of a carboxylated multiwall carbon nanotubes modified glassy carbon electrode in biological and pharmaceutical samples.* Colloids and Surfaces B: Biointerfaces, 2013. **109**: p. 253-258.
21. Ensafi, A.A., et al., *Differential pulse voltammetric determination of methyldopa using MWCNTs modified glassy carbon decorated with NiFe 2 O 4 nanoparticles.* Ionics, 2015. **21**(5): p. 1435-1444.
22. Ensafi, A.A., N. Kazemifard, and B. Rezaei, *Development of a nano plastic antibody for determination of propranolol using CdTe quantum dots.* Sensors and Actuators B: Chemical, 2017. **252**: p. 846-853.
23. El-Didamony, A.M., S.M. Hafeez, and I. Ali, *Extractive spectrophotometric method for the determination of some antipsychotic drugs using eriochrome black T.* Appl. Pharm. Sci, 2015. **5**: p. 26-33.
24. El-Didamony, A.M. and M.A. Moustafa, *Spectrophotometric determination of diphenhydramine hydrochloride in pharmaceutical preparations and biological*

fluids via ion-pair formation. Arabian Journal of Chemistry, 2010. **3**(4): p. 265-270.
25. Sivasubramanian, L., et al., *Visible spectrophotometric determination of levofloxacin in tablet dosaae forms.* Indian journal of pharmaceutical sciences, 2004. **66**(6): p. 799-802.
26. Manjunatha, A.S. and A. Sukhdev, *Oxidative decolorisation of E riochrome B lack T with C hloramine-T: kinetic, mechanistic, and spectrophotometric approaches.* Coloration Technology, 2014. **130**(5): p. 340-348.
27. Commission, B., *British pharmacopoeia 2015.* Stationery Office, London, 2014.
28. Gowda, B.G., J. Seetharamappa, and M.B. Melwanki, *Indirect spectrophotometric determination of propranolol hydrochloride and piroxicam in pure and pharmaceutical formulations.* Analytical sciences, 2002. **18**(6): p. 671-674.
29. Sajjan, A.G., J. Seetharamappa, and S.P. Masti, *Spectrophotometric Determination Of Propranolol Hydrochloride In Phramaceutical Preparations.* Indian journal of pharmaceutical sciences, 2002. **64**(1): p. 68.
30. Ribeiro, P., L. Pezza, and H.R. Pezza, *Spectrophotometric determination of methyldopa in pharmaceutical formulations.* Ecletica Quimica, 2005. **30**(3): p. 23-28.
31. Rashid, Q.N., M.H. Bakir, and S.O. Baban, *Spectrophotometric determination of Methyldopa in pure form and in the pharmaceutical preparations.* Tikret Journal of Pharmaceutical Sciences, 2016. **11**(1): p. 67-77.