Review Article Open Access

Edebi N. Vaikosen [1]*, Jeniffer Bioghele[1], Ruth C. Worlu[1], Benjamin U. Ebeshi[1]

Spectroscopic Determination of Two Beta-Blockers – Atenolol and Propanolol by Oxidative Derivatization Using Potassium Permanganate in Alkaline Medium

https://doi.org/10.1515/revac-2020-0103
Received April 03, 2020; accepted June 30, 2020

Abstract: A simple, rapid, sensitive, cheap and accurate oxidative method for two beta-blockers in pharmaceutical dosage forms was developed and evaluated. The method involved the oxidimetric treatment of atenolol and propanolol with 2 x 10-3 mol L-1 KMnO4 in alkaline medium (pH ≥ 9). Scanned spectra of oxidized complex showed distinctive absorptions at 460, 520, 540 and 570 nm. Arrays of colour changes were observed - from violet to blue; blue to bluish-green and yellow. Exhibited colours were due to ligand-metal charge transfer. An indirect spectrophotometric determination of atenolol and propranolol was done after 12-15 minutes at 520 nm. The optimum assay conditions showed linearity ranged from 0 – 15.0 µg mL-1 for both beta-blockers (R= 0.9997 – 0.9999). Molar absorptivity values were 4.79 x 103 and 4.88 x 103 L mol-1 cm-1 for atenolol and propanolol respectively, with corresponding Sandell's sensitivity values of 0.056 and 0.053 µg cm-2. Limits of detection and quantification were 0.50 and 1.65 µg mL-1 for atenolol respectively and 0.58 and 1.91 µg mL-1 for propanolol, while relative standard deviation for intra- and inter-day precision were < 2.0%. The applicability, accuracy and reliability of the method were demonstrated by the determination of atenolol and propanolol in tablet formulations. The recovery studies ranged from 93.33 - 103.00% for both beta-blockers and the amounts in brands were from 97.53 ± 2.68 to 100.84 ± 1.82%.

Key Words: Beta-blockers; Derivatization; Oxidimetric reaction; Pharmaceuticals; Visible spectrophotometry

***Corresponding author: Edebi N. Vaikosen**
email; vaikosen@yahoo.co.uk; edebi.vaikosen@ndu.edu.ng
Tel.: +2348034626596; ORCID: 0000-0002-2413-6127

[1] Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State. Nigeria

1 Introduction

Beta-blockers (also known as β-adrenergic receptor antagonists) embrace a group of synthetic drugs used for the management of cardiac arrhythmias, prevention of myocardial infarction or reduction of non-fatal re-infraction in survivors of acute myocardial infarction [1-3]. Amongst this group are atenolol and propanolol. Atenolol (ATL), 4-(2-hydroxy-3-[91-methylethyl) amino] propoxy benzeneacetamide (Figure 1a) is a cardio-selective β-blocker widely used in the treatment of hypertension and angina pectoris, while propanolol (PPN), 1-naphthalen-1-yloxy-3-(propan-2-ylamino) propan-2-ol (Figure 1b) has neither cardio-selective nor intrinsic sympathomimetic activity [3].

Both drugs are listed officially in the United States Pharmacopoeia [4] and the British Parmacopoeia [5], which describes potentiometric acid-base titration. A number of analytical techniques have been reported for ATL and PPN in pharmaceutical dosage forms – these includes, spectrophotometry [6-8], fluorimetry [9-11], voltammetry [12,13], high-performance thin layer chromatography [14], near-infrared spectroscopy [15], capillary electrophoresis [16], and high-performance liquid chromatography [17-19].

Absorption spectroscopy in the ultraviolet (UV) and visible (VIS) regions of the electromagnetic spectrum has been widely applied in pharmaceutical and biomedical analysis for qualitative and quantitative purposes [20]. It is versatile, robust, simple, quick and cheap for both research and routine analysis; and also found usefulness in the study of drug release from formulations and kinetics of drug degradation. The presences of chromophores in these two β-blockers make them easily assayed in the UV region. In addition, the presence of functional groups such as hydroxyl (-OH), amino (-NH-, -NH_2), carbonyl (-C = O), ether (- O -) and unsaturated aromatic ring make them highly susceptible to conjugation on reacting with appropriate chromogens to form derivatized coloured

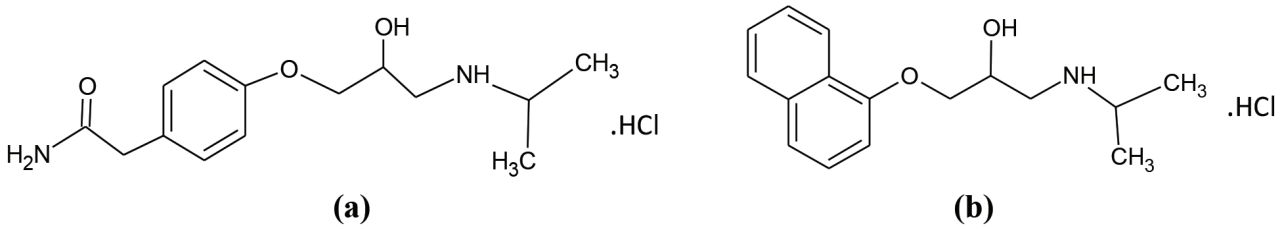


Figure 1 Structures of (**a**) Atenolol hydrochloride (**b**) Propanolol hydrochloride

complexes that absorb at higher wavelength in the visible region [20]. Spectrophotometric determinations of derivatized pharmaceuticals through oxidimetric reactions by metal ions precursors such as Fe^{3+}, Ce^{4+}, Mo^{5+}, Cu^{2+} and Mn^{7+} in acidic or alkaline medium have been reported [21-23], while, pharmaceutical organometallics containing Bi, Cu and Co have been reported quantified using synthesized chelating chromogenic compounds [24 - 26]. The use of potassium permanganate ($KMnO_4$) to oxidize aminoglycosides [27], 1,4-dihydropyridine drugs [28], metoclopramide [29], ampicillin [30] and in kinetic oxidative degradation and deamination reaction of ATL in alkaline medium [31], have been reported. However, the $KMnO_4$ oxidimetric quantification and evaluation of atenolol and other β-blockers in alkaline medium by spectrophotometry have not been reported. The proposed method is aimed at developing and evaluating a simple, quick, sensitive, accurate and cheap quality control assessment of atenolol and propranolol in tablet formulations, especially where modern and expensive equipment is not available.

2 EXPERIMENTAL

2.1 Apparatus

Absorption spectra were measured with JENWAY UV-Vis Spectrophotometer model 6305, while Electric Centrifuge Model 800, manufactured by B BRAN scientific & instrument company, England, was used to enhance the extraction of beta-blockers from solid dosage formulation. Analytical weighing balance (Sartorius) and Micro-pipettors (JENCONS scientific Ltd) were calibrated to be used.

2.2 Material and Reagents

The chemicals used were of analytical and spectroscopic grade. The methanol and ethanol were manufactured by Jiahua Chemiclas (JHC), China, while sodium hydroxide and potassium permanganate were from J.T Baker, USA and British Drug House (BDH) of England respectively. The pure atenolol hydrochloride and propanolol hydrochloride reference powder (99.5%) used as reference standards were obtained from Primal Nigeria Ltd, Lagos. Nigeria.

2.2.1 Preparation of reagents

All working reagents were prepared freshly in doubly distilled water.

Potassium permanganate ($KMnO_4$) solution, 2.0×10^{-3} mol L^{-1} was prepared by dissolving 0.079 g of $KMnO_4$ in 10 mL of warm double distilled water in a 250 mL volumetric flask, cooled and made to the mark, while 0.1 mol L^{-1} NaOH solution was prepared by dissolving 1.0 g of sodium hydroxide pellets in 50 mL distilled water in a 250 mL volumetric flask and then made up to volume.

Stock solutions of 100 μg mL^{-1} atenolol and propanolol standards, were prepared separately by dissolving 50 mg (equivalent of ATL and PPN) of each with 2 mL methanol in 50 mL calibrated flask and diluting to mark with distilled water (solution **A**). An aliquot of 1.0 mL solution **A** was further diluted to 10 mL in a calibrated flask with distilled water to obtain the stock concentration.

2.3 Samples

Ten (10) samples of beta-blockers comprising of 5 brands of atenolol; Betafil (ATL_1), Tenormin (ATL_2), Tenolol (ATL_3), Ratenol (ATL_4), Atenolol (ATL_5) and 5 brands of Varpranol (PPN_1), Junolol (PPN_2), Inderal (PPN_3), Alpranol (PPN_4), Propanolol (PPN_5) used for this study were purchased from reputable Pharmacies in Port Harcourt, Rivers State and Yenagoa, Bayelsa State, Nigeria.

2.4 General procedure and determination absorption spectra of beta-blockers and reference solution

Into one of two 10 mL volumetric flasks, 0.4 mL standard solution of drugs was transferred, 0.5 mL 0.1 mol L^{-1} NaOH was added and allowed to stand for 3 minutes, then 1

mL of 0.002 mol L^{-1} $KMnO_4$ was added, mixed gently and allowed to stand for 10 minutes screened from direct sunlight. The solutions were made to mark with distilled water and scanned in the visible region from 380 - 700 nm. Scanning was done against a reagent blank treated the same way without 0.002 mol L^{-1} $KMnO_4$ and drug. To the second volumetric flask, the procedure was repeated without the drugs (reference solution), diluted to mark with distilled water and also scanned.

2.5 Calibration and validation

2.5.1 Calibration graphs for ATL and PPN

A six-point calibration graph was prepared by transferring aliquots (0 – 0.80 mL) of stock solutions of reference standards into a series of 10 ml volumetric flasks using a micro-pipette to obtain a working concentration range of 0 – 8.0 μg mL^{-1}. To each flask, 0.5 mL mol L^{-1} NaOH was added, allowed to stand for 3 minutes, then 1 mL of 2 x 10^{-3} mol L^{-1} $KMnO_4$ was added and allowed to stand for 10 minutes, diluted to mark with distilled water. The absorbance of the resultant solution was measured at 520 nm against a blank.

2.5.2 Validation of proposed methods

In assessing and validating the method performance, criteria such as; precision, accuracy, specific interference, sensitivity, ruggedness and robustness were determined in addition to the calibration curve earlier mentioned. The recovery studies were evaluated for the proposed method using 5 μg mL^{-1} and 4 μg mL^{-1} of two brands each of atenolol tablets (ATL_1 and ATL_2) and propanalol (PPN_1 and PPN_2) respectively – these were spiked at three concentration levels with 2, 4 and 6 μg mL^{-1} of the reference standards. Percent recoveries of the spiked analytes were calculated, while statistical analyses with respect to data obtained in experimental results were also determined.

Replicate analysis of 5 concentrations - 5, 10, 15, 20 and 25 μg mL^{-1} for atenolol and 4, 8, 12, 16 and 20 μg mL^{-1} for propranolol were evaluated to determine the intra-day and inter-day precision. Both drugs under investigation were replicated thrice at each concentration on the same day, while using the same concentrations the inter-day assay was done on 3 days within a week.

The linearity of ATL and PPN under optimum experimental conditions and within working concentrations were ascertained from calibration curves while using the least-squares method the regression equations and other key parameters were derived from the data obtained.

The limit of detection (LOD) and the limit of quantification (LOQ) were used to measure the sensitivity of the method. The LOD and LOQ were determined by evaluating the lowest concentrations of each beta-blocker can be detected and measured, respectively. They were obtained using the expressions, LOD =3.3S_a/b; LOQ = 10 S_a/b (where S_a is the standard deviation of the intercept of regression line, and 'b' is the slope of the calibration curve) [32, 33].

2.6 Procedure for application of method to pharmaceutical preparations

An amount equivalent to 50 mg atenolol and propanolol were weighed from finely powdered tablets (20) of each and transferred into 25 mL of methanol in 50 mL volumetric flask. This was shaken gently and intermittently for 2 – 3 minutes and made to mark with the same solvent. Mixtures were filtered through a whatman no. 42 filter paper and the first 10ml portion of the filtrate was discarded. The filtrate, 1 mL was diluted to 10 mL with water (solution **B**). To 0.4 mL aliquot of solution **B**, 0.5 mL 0.1 mol L^{-1} NaOH was added, allowed to stand for 3 minutes, then 1 mL of 2 x10^{-3} mol L^{-1} $KMnO_4$ was added, allowed to stand for another 10 minutes, diluted to mark with distilled water. The absorbance of the resultant solution was measured at 520 nm against a reagent blank.

3 Results and discussion

3.1 Absorption spectra of beta-blockers

Figure 2, shows the spectra of oxidized drugs complex by $KMnO_4$ in alkaline medium (pH ≥ 9). The absorption spectra showed four distinctive peaks at 460, 520, 540 and 570 nm. The peaks at 520 and 540 nm were the most predominant, with the former being the maximum. The reference solution spectrum (i.e., 2 x10^{-3} mol L^{-1} $KMnO_4$, without drugs) showed the same spectra trend as the oxidized drugs, however, the absorbance of the predominant peaks were significantly higher when compared to those of the $KMnO_4$ – drug complex. The spectra were similar to those reported by Askal *et al.,* [28] for determination 1, 4-dihydropyridine drugs using $KMnO_4$ in an acidic medium. This implied that the spectra for both β-blockers were due to the unreacted (or excess) $KMnO_4$ solution after the formation of the complex [29, 30, 34]. Figure 3, confirms this trend as there were a significant

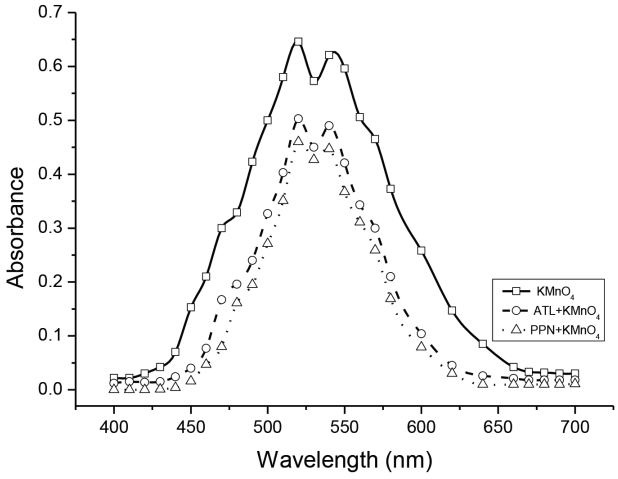


Figure 2 Absorption spectra of 2 x10^{-3} $KMnO_4$ (Reference chromogenic solution) against alkaline solution blank (Reagent blank); ATL+$KMnO_4$ and PPN+$KMnO_4$ complex against alkaline solution blank (Reagent blank)

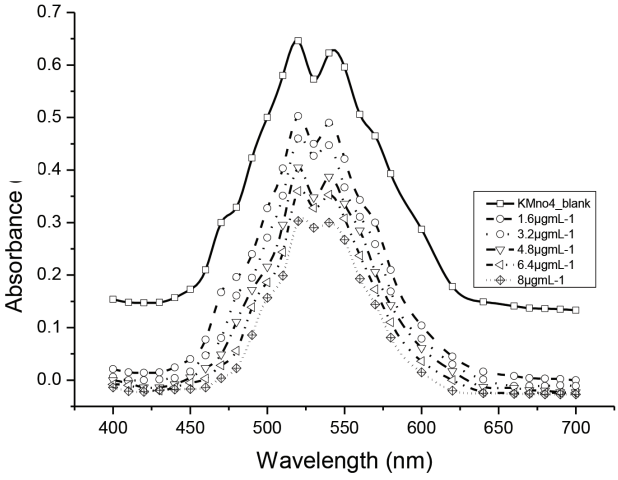


Figure 3 A typical spectra finger-printing of 2 x10^{-3} M $KMnO_4$ against alkaline solution blank (Reagent blank) and beta-blocker-$KMnO_4$ complex against alkaline solution blank (Reagent blank) (after reaction with, 1.6, 3.2, 4.8, 6.4 and 8.0 µg mL^{-1} of atenolol)

decline in the absorbance of $KMnO_4$-Drug complex as the concentration of beta-blocker increases from 0 – 8 µg mL^{-1}. This decrease was used as a measure for the quantification of beta-blocker drugs in their respective solutions.

3.2 Principle and Chromogenic property of potassium permanganate

3.2.1 Principle and chromogenic property

During the reaction three distinctive transitional colour changes were observed for drug-$KMnO_4$ complex; from violet to blue; blue to bluish-green, then to green; green to greenish-yellow and to yellow after 10 minutes – which was relatively stable. In the alkaline medium $KMnO_4$ was spontaneously reduced to form the blue/bluish-green coloured K_2MnO_4 with Mn (+6) oxidation state [30, 34], with a life span of 2 – 4 minutes, while the green/yellowish-green coloured complex emerges thereafter for a duration of 4 – 6 minutes. The reaction time for the formation of the green coloured complex agrees with previous spectrophotometric studies of β-blocker using $KMnO_4$ [35]. The exhibition of these colours may be due the presences of manganese (Mn) – a transitional metal that is capable of existing in different oxidation states [36] [Figure 4a]. In potassium permanganate, manganese exist in (+7) oxidation state, which is violet/purple in aqueous solution. The formation of Mn (+6) was evident by the decrease in the absorbance at 520 nm as the reaction progresses [30]. In addition, the deep purple/violet colour exhibited by $KMnO_4$ solution makes it absorbs the green or bluish-green and greenish-yellow colour between 500 -550 nm [36]. It is pertinent to mention that these colours exhibited by manganese are not due to d→d electronic transition but due to ligand-to-metal charge transfer (LMCT), since it electronic configuration has an empty d-orbital ($4d^o$).

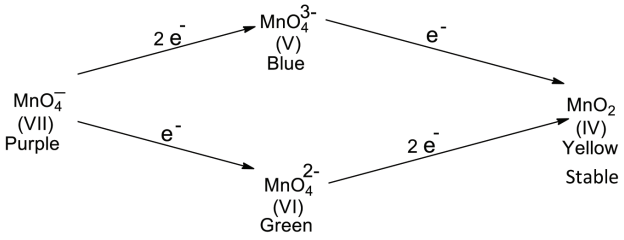


Figure 4a Manganese oxidation states and colour changes during reaction of $KMnO_4$ with β-blockers

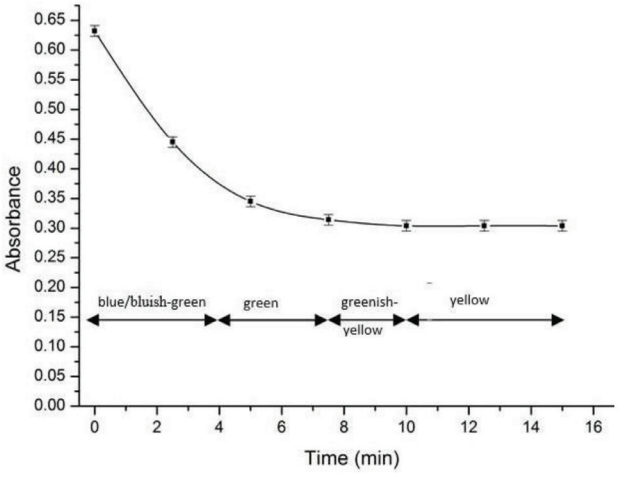


Figure 4b Reaction time and its effect on formed coloured-complex at 520 nm

3.2.2 Reaction time.

Figure 4b, shows the various colour changes and absorbance with respect to time. Prior to application of the proposed method for quantification of drugs, the time duration for the reaction to go to completion was determined, while absorbance of the formed complex were measured at 7 time amplitudes (0, 2.5, 5, 7.5, 10, 12.5 and 15 min) at 520 nm.

3.2.3 Reaction mechanism

The suggested equations for the oxidimetric reactions are presented in scheme 1, while the proposed reaction mechanism is shown in scheme 2. The stoichiometric equation for the reactions between each β-blocker and Mn (VII) was 1:2 molar ratio. This agrees with the previous report on the kinetic oxidative degradation and deamination of atenolol by aqueous alkaline permanganate [31].

3.3 Method validation

The proposed alkaline $KMnO_4$ oxidative spectrophotometric method was validated in accordance with ICH guidelines [37].

3.3.1 Linearity and sensitivity

Table 1, shows the experimental conditions and analytical characteristics associated with the proposed method. The calibration plots for ATL and PPN reference standards were found to be linear for concentrations ranging from 0 - 15.0 μg mL^{-1} (Figure 5). The regression equations were obtained with the aid of Microsoft Excel, using the least-squares method. The intercept and slope for both β-blockers were 0.571 and 0.082 respectively for ATL, with corresponding values for PPN being 0.556 and 0.084 for five concentrations of Beer's plot against absorbance (n = 5). The correlation coefficients (R) ranged were 0.9996 to 0.9999 for PPN and ATL respectively. These values implied high sensitivity of the proposed method. The degree of accuracy was further ascertained by obtaining the Sandell's sensitivity optimum concentrations for both β-blockers, with ATL and PPN being 0.056 and 0.053 μg cm^{-2} respectively. The LOD were 0.50 and 0.58 μg mL^{-1} for ATL and PPN respectively, with corresponding LOQ as 1.65 and 1.91 μg mL^{-1}. Both LOD and LOQ values simply confirmed the repeatability and reliability of the proposed methods.

Table 1 Analytical characteristics of the proposed method

PARAMETER	ATN	PPN
pH (0.1 M NaOH)	9.0 - 9.5	9.0 - 9.5
Maximum wavelength (λ_{max}) (nm)	520	520
Beer's linearity range (μg mL^{-1})	0 - 15.0	0 - 15.0
Sandell's sensitivity (μg cm^{-2})	0.056	0.053
Limit of detection (LOD)(μg mL^{-1})	0.50	0.58
Limit of quantification (LOQ)(μg mL^{-1})	1.65	1.91
Molar absorptivity ($Lmol^{-1}$ cm^{-1})	4.788 x 10^3	4.883 x 10^3
Regression equation		
Slope	0.030	- 0.031
Standard deviation of slope	± 0.082	± 0.084
CL (95%) for slope	± 0.261	±0.267
Intercept	0.571	0.556
Standard deviation of intercept	± 0.005	± 0.006
CL (95%) for intercept	± 0.016	± 0.019
Correlation coefficient (r)	0.9999	0.9997

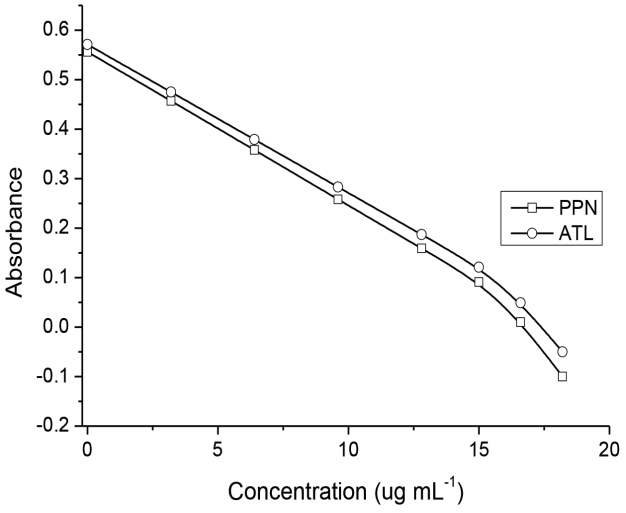


Figure 5 Beer's linearity range for ATL-$KMnO_4$ and PPN-$KMnO_4$ complexes against alkaline solution blank (Reagent blank)

3.3.2 Selectivity and interference

This study showed that the selectivity and interference in the proposed method were enhanced and negligible respectively. Firstly, drug samples are in tablet formulations, hence co-extractive chromogenic interferences from methanol soluble substances in tablets are expected to be absent. The presence of such co-extractives would contribute to the amount of the beta-blockers reacting with $KMnO_4$, thus decreasing further the residual $KMnO_4$ measured by spectrophotometry. Most solid dosage formulations are compressed with excipients that are mainly inorganics, which are also highly insoluble in organic solvents such as alcohols. In tablet formulations, the excipients and additives used are; sodium lauryl sulfate, magnesium

stearate, starch sodium glycolate, lactose spray dried, carboxymethylcellulose (CMC), talc, titanium dioxide, microcrystalline cellulose, hydroxypropylcellulose and pre-gelanitizated starch [38]. These substances are alcohol-insoluble and non-chromogenic. The absence of interference, therefore, implied high selectivity and applicability of the method for routine analysis in pure and solid dosage forms.

3.3.3 Reproducibility, precision and accuracy

The intra- and inter-day assay to ascertain the precision and accuracy of the $KMnO_4$ oxidative method at specified concentrations are shown in Table 2. The relative standard deviations (RSD) for both β-blockers ranged from 0.2 - 1.95 % for inter- and intra-day assays, with corresponding standard errors ≤ 0.12 for all runs. These values potrayed high reproducibility and satisfactory precision and accuracy of the method.

3.3.4 Recovery studies and ruggedness

Table 3, shows the assessment of the ruggedness of the proposed method under-recovery studies. This was done by studying the effect of minor changes on some experimental conditions using both β-blockers. The aforementioned study provided a measure of the reliability of the proposed method during routine work. Values obtained by varying some experimental parameters, such as different brands and spiked concentrations of beta-blockers showed any significant variations, therefore the proposed method could be adjudged reliable. Calculated percent recovery at different spiked concentrations ranged from 95.10 - 105.02% and 96.65 - 101.82% for ATL and PPN respectively, while variations were considered minor and insignificant, hence the reliability of the method.

Table 3 Recovery studies for atenolol and propanolol in tablet formulations

Beta-blocker	Amount of drug in tablets (μgmL^{-1})	Amount of pure drug spiked (μgmL^{-1})	Total quantity of drug found (μgmL^{-1})	Percent recovery of drug spiked (%)
Atenolol				
ATN_1	5	2	6.90 ± 0.10	96.83 ± 2.25
	5	4	9.00 ± 0.09	100.83 ± 1.39
	5	6	11.01 ± 0.13	103.00 ± 1.50
ATN_2	5	2	6.96 ± 0.10	99.50 ± 2.16
	5	4	8.95 ± 0.05	98.67 ± 1.23
	5	6	10.85 ± 0.09	97.44 ± 1.58
Propanolol				
PPN_1	4	2	5.92 ± 0.05	96.00 ± 2.48
	4	4	7.94 ± 0.13	98.42 ± 1.31
	4	6	10.03 ± 0.13	102.11 ± 1.73
PPN_2	4	2	5.87 ± 0.06	93.33 ± 2.78
	4	4	7.85 ± 0.14	96.33 ± 2.77
	4	6	9.88 ± 0.08	98.06 ± 1.31

Table 2 Precision and accuracy studies of proposed oxidative $KMnO_4$ spectrophotometric methods

		Intra-day determination (n = 3)			Inter-day determination (n = 3)		
Analyte Drug	**Theoretical concentration (μgmL^{-1})**	**Mean ±S d Content (μgmL^{-1})**	**RSD%**	**SEM**	**Mean ± Sd Content (μgmL^{-1})**	**RSD%**	**SEM**
Atenolol	5	4.89 ± 0.09	1.84	0.05	4.92 ± 0.06	1.30	0.04
	10	9.91 ± 0.16	1.63	0.09	9.89 ± 0.14	1.40	0.08
	15	15.05 ± 0.06	0.39	0.03	14.96 ± 0.09	0.59	0.05
	20	20.09 ± 0.10	0.49	0.06	20.08 ± 0.06	0.32	0.04
	25	25.04 ± 0.05	0.20	0.03	24.96 ± 0.18	0.70	0.10
	30	29.95 ± 0.07	0.24	0.04	30.00 ± 0.12	0.40	0.07
Propanolol							
	4	3.97 ± 0.08	1.94	0.04	3.96 ± 0.08	1.95	0.04
	8	7.94 ± 0.09	1.15	0.05	7.95 ± 0.07	0.88	0.04
	12	11.99 ± 0.06	0.50	0.03	11.84 ± 0.21	1.72	0.12
	16	16.19 ± 0.06	0.36	0.03	15.99 ± 0.17	1.05	0.10
	20	19.95 ± 0.15	0.73	0.08	19.94 ± 0.15	0.74	0.09
	24	23.96 ± 0.12	0.52	0.07	24.05 ± 0.19	0.80	0.11

RSD% - Relative standard deviation; SEM – Standard error of mean

3.4 Application to pharmaceutical formulations

The proposed oxidative procedure was applied to determine the drug contents in different formulated brands of two beta-blockers (ATL and PPN) of five brands each. The brands were ATL_1, ATL_2, ATL_3, ATL_4 and ATL_5 containing atenolol, while for propranolol the brands were – PPN_1, PPN_2, PPN_3, PPN_4, and PPN_5. The proposed method showed the inclination of being free from interferences likely to be caused by the aforementioned excipients and/or common degradation products. Table 4, shows that the amount of drugs found in tablet formulations were in good agreement with label claims for all brands of the β-blockers. The amount of ATL and PPN ranged from 98.13 ± 1.84% to 100.84 ± 1.82% and 97.53 ± 2.68 to 100.18 ± 1.61% respectively. The student-t tests for accuracy were all < 2.78 (tabulated) at 95% confidence level for 5 replicates - this suggested that no significant difference between label claim on brands of β-blockers and values obtained in applying the proposed method at 95% confidence level [38].

Table 4 Application of oxidative $KMnO_4$ spectrophotometric method to tablet formulation

Beta-blockers	Label claim (mg/tablet)	Amt. Found ± SD (mg/tablet)	SEM	Content (%)
Atenolol				
Betafil (ATL_1)	50	49.41 ± 0.70	0.31	98.82 ± 1.40 t = 1.88
Tenrmin (ATL_2)	50	49.31 ± 0.63	0.28	98.62 ± 1.26 t =2.44
Tenolol (ATL_3)	50	50.42 ± 0.91	0.41	100.84 ± 1.82 t = 1.03
Ratenol (ATL_4)	100	99.40 ± 0.78	0.35	99.40 ± 0.78 t = 1.72
Atenolol (ATL_5)	100	98.13 ± 1.84	0.82	98.13 ± 1.84 t = 2.27
Propanolol				
Varpranol (PPN_1)	40	39.50 ± 0.62	0.28	98.76 ± 1.54 t = 1.81
Junolol (PPN_2)	40	39.01 ± 1.07	0.48	97.53 ± 2.68 t = 2.06
Inderal (PPN_3)	40	40.07 ± 0.64	0.29	100.18 ± 1.61 t = 0.24
Alpranol (PPN_4)	40	39.77 ± 0.29	0.13	99.43 ± 0.74 t = 1.74
Propanolol (PPN_5)	40	39.93 ± 0.52	0.23	99.82 ± 1.31 t = 0.32

4 Conclusion

The present method is based on a redox reaction using $KMnO_4$ as an oxidizing agent and chromogen in inducing a simple ligand-to-metal charge transfer (LMCT) process in an aqueous alkaline medium (pH ≥ 9). The method has demonstrated that β-blockers can be assayed in bulk drugs and tablet formulations in the visible region of the spectrophotometer. The reagents used are cheap and readily available, while the instrument is affordable. In addition, the methodology is time-saving and not tedious. The method has also shown high precision, accuracy, selectivity - devoid of interferences and with high sensitivity in terms of molar absorptivities. These advantages enhance the application of this method in routine quality assessment of β-blockers in the pharmaceutical industry.

Author contribution

A – Designed the experiment; **B** – Carried them Out; **C –** Prepared the manuscript with contribution from all co-authors; **D -** Reading of manuscript

ENV – A, B, C & D
JB - B, C & D
RCW - C & D
BUE - C & D

References

[1] Patel DP, Sharma P, Sanyal M, Singhal P, Shrivastav PS. UPLC-MS/MS assay for the simultaneous quantification of CAR and its active metabolite 4′-hydroxyphenyl CAR inhuman plasma to support a bioequivalence study in healthy volunteers. Biomed Chromatogr. 2013;27(8):974–86.
[2] DiNicolantonio JJ, Fares H, Niazi AK, Chatterjee S, D'Ascenzo F, Cerrato E, et al. β-Blockers in hypertension, diabetes, heart failure and acute myocardial infarction: a review of the literature. Open Heart. 2015;2(1):1–12.
[3] Weiner P. C and Clifford Mason, C. Drugs for Pregnanat and Lactating Women. 3rd ed. Elsevier Publishers; 2018.
[4] The United States Pharmacopoeia XXVIII and NF XXV. Washington (DC): American Pharmaceutical Association; 2007.
[5] British Pharmacopoeia. The British Pharmacopoeia Commission, Monographs on Medicinal and Pharmaceutical Substances Market Towers 1 Nine Elms Lane London SW8 5NQ, 2009. I & II, 3407–13; 6433–37
[6] Vaikosen NE, Ebeshi BU, Joffa PP. Simple, Sensitive and Reproducible Acetous Perchlorate and Spectrophotometric Determination of Atenolol in Tablet Dosage Form. J. Pharm. Sci. & Res. 2012;4(10):1933–8.
[7] Lamie N. Simultaneous determination of binary mixture of amlodipine besylate and ATE based on dual wavelengths. Spectrochim Acta A Mol Biomol Spectrosc. 2015;149:201–7.

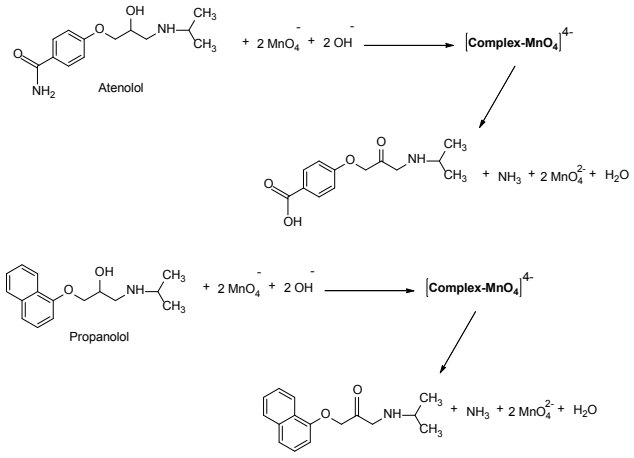
[8] Aboud MK, Mohammad A, Isbera M, Beesh M. Development and validation of UV spectrophotometric method for determination of atenolol in pure materials and pharmaceutical dosage. Indo American J Pharm Res. 2017;7(4):8179–84.
[9] Derayea MS, Omar AM, Abdel-Lateef AM, Hassan IA. Development and validation of a new spectrofluorimetric method for the determination of some beta-blockers through fluorescence quenching of eosin Y. Application to content uniformity test. Open Chem. 2016;14:258–66.
[10] Omar MA, Badr El-Din KM, Salem H, Abdelmageed OH. Spectrophotometric and spectrofluorimetric methods for determination of certain biologically active phenolic drugs in their bulk powders and different pharmaceutical formulations. Spectrochim Acta A Mol Biomol Spectrosc. 2017;5(192):108–16.
[11] Tabrizi BA, Yousefzadeh F. Spectrofluorimetric Determination of atenolol and carvedilol in pharmaceutical preparations after optimization of parameters using response surface methodology. Pharm Sci. 2019;25(3):262–7.
[12] Er E, Çelikkan H, Erk N. Highly sensitive and selective electrochemical sensor based on high-quality graphene/nafion nanocomposite for voltammetric determination of nebivolol, Sensor Actuat. Biol Chem. 2016;224:170–7.
[13] Khairy M, Khorshed AA, Rashwan FA, Salah GA, Abdel-Wadood HM, Banks CE. Simultaneous voltammetric determination of antihypertensive drugs nifedipine and ATE utilizing MgO nanoplatelet modified screen-printed electrodes in pharmaceuticals and human fluids. Sens Actuators B Chem. 2017;252:1045–54.
[14] Bhavar G, Chatpalliwar AV. Quantitative analysis of propranolol hydrochloride by high performance thin layer chromatography. Indian J Pharm Sci. 2008;70(3):395–8.
[15] Ayala C, Brunetto MR, Ovalles F, Gallignani M. Determination of atenolol in pharmaceutical dosages by fourier transform infrared spectrometry (FTIR). Rev. Téc. Ing. Univ. Zulia. 2009;32(3):238–48.
[16] Zhou S, Wang Y, De Beer T, Baeyens GR, Fei TG, Dilinuer M, et al. Simultaneous separation of eight β-adrenergic drugs using titanium dioxide nanoparticles as additive in capillary electrophoresis. Electrophoresis. 2008;29(11):2321–9.
[17] Yilmaz B, Arslan S. Determination of atenolol in human urine by using HPLC. J Sep Sci. plus. 2018;1:1–10.
[18] 18. Mahmoudi A, Rajabi M. Selective determination of some beta-blockers in urine and plasma samples using continuous flow membrane microextraction coupled with high performance liquid chromatography. J Chromatogra B. 2019;1128:1–8.
[19] El-Alfy W, Ismaiel AO, El-Mammli YM, Shalaby A. Determination of atenolol and trimetazidine in pharmaceutical tablets and human urine using a high performance liquid chromatography-photo diode array detection method. Inter J Anal Chem. 2019:1–8.
[20] Peraman R, Reddy PY. Application of Spectral Studies in Pharmaceutical Product Development. Basic Approach with illustrated Examples. Chapter 1, UV-Visible Spectroscopy. pp 1- 43. PharmaMed Press/BSP Books. 4-4-309/316, Giriraj Lane Sultan Bazar, Hyderabad - 500 095. Andhra Pradesh, INDIA. 2019.
[21] Shqair AI, Shakhshir MZ, Masharqah J, Odeh I, Jondi JW. Spectrophotometric determination of Cu^{2+} metal ions via complex formation with carboxylated tris(2-aminoethyl) amine. J. Advs Chem. 2106;12(3):4237–46.
[22] Prashanth NK, Kanakapura B. 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, 2014; 84(1):27–35.
[23] Vaikosen NE, Ebeshi UB, Worlu RC. Spectroscopic fingerprinting of aminoglycosides and determination of neomycin sulphate through oxidative ion-pair complex formation using ammonium molybdate. Curr Pharm Anal. 2019;15(5):487–96.
[24] Barache UB, Shaikh AB, Deodware SA, Dhale PC, Kamble GS, Lokhande TN, et al. Sensitive and selective liquid-liquid extractive spectrophotometric determination of Bismuth (III) from water, pharmaceuticals and synthetic mixtures. Groundw Sustain Dev. 2019;9:100221.
[25] Kamble GS, Kolekar SS, Anuse MA. Synergistic extraction and spectrophotometric determination of copper (II) using 1-(2′,4′-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol: analysis of alloys, pharmaceuticals and biological samples. Spectrochim Acta A Mol Biomol Spectrosc. 2011;78(5):1455–66.
[26] Barache UB, Shaikh AB, Lokhande TN, Kamble GS, Anuse MA, Gaikwad SH. An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper (II) incorporated with 4-(4′-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole. Analysis of food samples, leafy vegetables, fertilizers and environmental samples. Spectrochim Acta A Mol Biomol Spectrosc. 2018;189(15):443–53.
[27] El-Didamony A, Ghoneim KA, Amin SA, Telebany MA. Spectrophotometric determination of aminoglycoside antibiotics based on their oxidation by potassium permanganate. J. Korean Chem Soc. 2006;50(4):298–306.
[28] Askal HF, Abdelmeqeed HO, El-Hamd AM. Spectrophotometric and spectrofluorimetric determination of 1,4-dihydropyridine drugs using potassium permanganate and cerium (iv) ammonium sulphate. Bull Pharma Sci Assiut. 2010;4(33)2: 201–15.
[29] Devi ZO, Basavaiah K, Vinay BK. Application of potassium permanganate to spectrophotometric assay of metoclopramide hydrochloride in pharmaceuticals. J Appl Spectrosc. 2012;78:873–83.
[30] Khan PA, Mohd A, Bano S, Siddiqi SK, Asiri MA. Spectrophotometric methods for the determination of ampicillin by potassium permanganate and 1-chloro-2,4-dinitrobenzene in pharmaceutical preparations. Arab J Chem. 2015;8:255–63.
[31] Hiremath CG, Kulkarni MR, Nandibewoor TS. Kinetics of oxidative degradation and deamination of atenolol by aqueous alkaline permanganate. Indian J Chem. 2005;44A:245–50.
[32] Bohm DA, Stachel CS, Gowik P. Confirmatory method for the determination of streptomycin in apples by LC-MS/MS. Anal Chim Acta. 2010;672:103–6.

[33] Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci. 2011;2:21–5.
[34] Mendham J, Denney RC, Barnes JD, Thomas MJ. Vogel's Textbook of Quantitative Chemical Analysis. 6th ed. Pearson Education Ltd and Dorling Kindersley Publishing Inc.; 2006.
[35] Basavaiah K, Chandrashekar U, Prameela HC, et al., Titrimetric and spectrophotometric determination of propranolol hydrochloride using chloramine-T. J. Ind. drugs, 2004;41:303–5.
[36] Hamada ZY, Makoni N, Hamada H. Three very different UV-Vis absorption spectra of three different transition metals found in biological solutions. Electronic J Bio. 2016;S2:6–9.
[37] ICH. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, ICH Harmonized Tripartite Guideline, ICH Q2A. Text on validation of analytical procedures, November 2005. (accessed: 12 January, 2020).
[38] Karthik VV. Excipient used in the formulation of tablets. Research and Reviews: J. Chem. 2016;5(2):143–54.

Supporting Information

Table of Contents

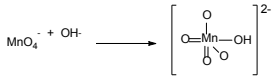
1 Equation of reaction



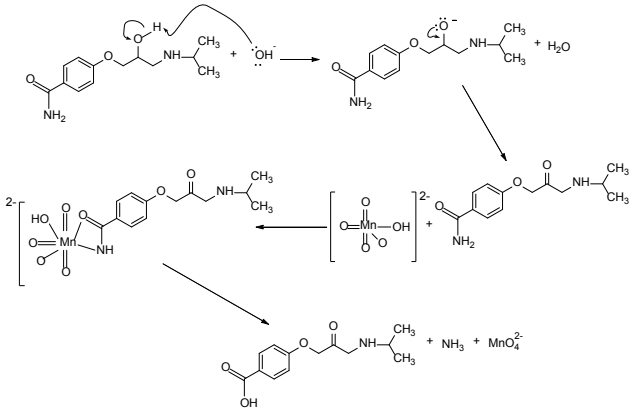
Scheme 1: Proposed equation of reaction for $KMnO_4$ oxidation of atenolol and propranolol in alkaline medium

2 Mechanism of reaction

Step 1: *Formation of manganate ion*



Step 2: *Abstraction of proton from OH functional group in ATL and complex formation Abstraction of proton from OH functional group in ATL and complex formation*



Scheme 2: Proposed mechanism for oxidation of β-blocker drugs (ATL and PPN) by $KMnO_4$ in alkaline medium