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Original Article

DIRECT SPECTROPHOTOMETRIC DETERMINATION OF ATENOLOL AND TIMOLOL ANTI-HYPERTENSIVE DRUGS

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ABSTRACT

Objective: Direct and sensitive spectrophotometric method is described for the quantitative determination of some anti-hypertensive drugs such as atenolol (ATN) and timolol (TIM) in pure forms as well as in their dosage forms.

Methods: The proposed method is based on the redox reaction between the selected drugs and $KMnO_4$ in alkaline medium. The method involves treating the aqueous solution of the selected drugs with $KMnO_4$ in alkaline medium and measuring the bluish-green product at 610 nm. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized.

Results: The fixed-time method is adopted for constructing the calibration curves, which were found to be linear over the concentration ranges of 2.0–14 µg/ml and 2.0–28 µg/ml for ATN and TIM, respectively. The determination of the studied drugs by initial rate, variable time and rate constant method was workable with the calibration equations obtained but the fixed time method has been found to be more applicable.

Conclusion: The applicability of the proposed method was demonstrated by the determination of the selected drugs in both pure and in commercial dosage forms and has met the validation requirements.

Keywords: Atenolol, Timolol, Dosage forms, Spectrophotometry, Potassium permanganate

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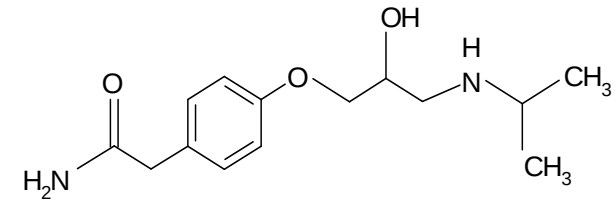
INTRODUCTION

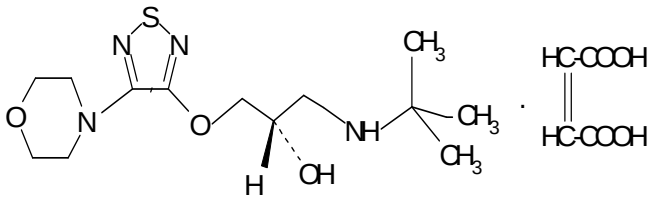
β-Blockers (or β-adrenergic antagonists) are a group of drugs widely used in the treatment of cardiovascular diseases (CVD), namely, arterial hypertension, cardiac arrhythmias, and angina pectoris as well as other types of pathologies such as anxiety or glaucoma [1]. The therapeutic effects of beta blockers are normally explained by their capacity to block the beta-adrenoceptors, hindering access of the endogenous agonist's noradrenaline and adrenaline. Atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide (fig. 1a), which in therapeutics is known as a β-blocker and is widely used in the management of hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction [2].

The drug is official in the Indian pharmacopoeia [3] and in the British pharmacopoeia [4]. Several analytical methods have been reported for the determination of atenolol in human plasma, urine, or pharmaceutical preparations, such as high-performance liquid chromatography [5–9], gas chromatography [10], liquid chromatography [11, 12]. Other techniques include voltammetric [13–15], electrophoresis [16, 17], chemiluminescence [18-20], spectrofluorimetric [21], ultraviolet and visible spectrophotometry [22–27].

Timolol maleate, (–)-(S)-1-*tert*-butylamino-3-(4-morpholino-1, 2, 5-thiadiazol-3-yloxy)-2-propanol (fig. 1b), is used as an antihypertensive and an antiglaucoma agent. Literature survey revealed that few methods have been reported for the determination of TIM in pharmaceutical preparations such as spectrophotometric methods [28–30]. Here, also, derivative ultraviolet [31, 32], high-performance liquid chromatography [33, 34], liquid chromatography [35], chemiluminescence [36] and electrophoresis [37]. Timolol is officially recognised in the USP [38] and BP [39]. There are few spectrophotometric methods for the determination of atenolol and timolol. Some reported methods suffer from one or more disadvantages such as critical dependence on acid/pH condition, heating and/or extraction step, use of use of organic solvents, longer contact time, less stable coloured species and expensive chemicals as indicated in table 1. For these reasons, develop a new simple, spectrophotometric method for the determination of ATN and TIM in their pharmaceutical dosage forms using eco-friendly chemicals and free from the use of organic solvents.



a- Atenolol



b- Timolol maleate

Fig. 1: Chemical structure of a-atenolol and b-timolol maleate

Table 1: Comparison of Beer's law limits of reported methods with the proposed method

Method	λ_{max}, nm	Linear range, µg/ml		Ref
		ATN	TIM	
Derivative spectroscopy	225.5	1–15		22
Correction methods	250	5–70		
Bromate–bromide mixture	520	0.5–4.0		23
bromate–bromide mixture				
A	540	1–20		24
B	445	2–40		
C	630	1–8.0		
Ion-pair				25
BPB	415	1–8	1–10	
BTB		1–9	1–10	
bromate–bromide mixture				
I_2	360	0.5–9		26
Starch–I_2	570	0.3–6		
DDQ	590	3–48		
2,4-Dinitrphenol	420	2–24		27
Picric acid	420	1.5–18		
Vanadometric spectrophotometric	504		2–20	28
Drug-metal	369.4		20–200	29
Ion-pair	552.2		1.6–16	
Absorbance subtraction	272.8		5–60	30
Amplitude modulation			5–60	
First derivative UV	313		5–85	31
Second derivative UV			2–35	
Kinetic spectrophotometric	610	2–14	2–28	Proposed method

MATERIALS AND METHODS

Chemicals and instruments

All chemicals and reagents used were of analytical grade. High purity double distilled water was used throughout. Pharmaceutical grade atenolol and timolol maleate were received from Egyptian Pharmaceutical Industries (EIPICO), 10th of Ramadan City, Egypt; which were reported to be 99.8% purity, as a gift and were used as received. A stock standard solutions containing 10 mg of ATN and TIM were prepared by dissolving appropriate weight of pure drugs in distilled water and made up to the mark in a 100 ml calibrated flask for obtaining working concentration (100 µg/ml) for ATN and TIM. NaOH (BDH, UK), 1.0M aqueous solution was prepared by dissolving 4.0 g of the chemical in 100 ml of water. A stock solution of 5.0×10^{-3}M $KMnO_4$ (Aldrich) was prepared by dissolving an accurate weight in 10 ml of warm distilled water, then completed to the mark in a 100 ml calibrated flask and standardized using sodium oxalate and kept in the dark bottle.

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190–1100 nm, spectral bandwidth 2 nm, with 10 mm matched quartz cells. A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature for color development.

Analytical procedure

Appropriate volumes of ATN or TIM stock solution (100 µg/ml) were transferred into a series of 10 ml standard flasks. To each flask, 2.0 ml of 1.0M NaOH followed by 2.0 ml of 5×10^{-3}M $KMnO_4$ were added. The volume was made up to the mark using distilled water, mixed well for 25 min at room temperature. Afterwards, the absorbance of the solutions was measured at 610 nm against a reagent blank which was treated similarly. The calibration graph was then constructed by plotting the final concentration of each drug against the absorbance values which were measured at a fixed time. Alternatively, the corresponding regression equation was derived.

Procedure for the tablets

At least ten tablets of blokium 100 mg/tablet (Pharco, Egypt) were powdered and a quantity of the powder equivalent to 10 mg ATN was extracted by shaking with 10 ml distilled water. The extracts were filtered into a 100 ml calibrated flask and then diluted to the mark. The assay for ATN content was completed as described under procedures for calibration curves.

Procedure for eye drops

The TIM pharmaceutical preparations were cusimolol eye drops, Sterile Ophthalmic solution, 5 mg/ml (Rameda, 10th of Ramadan City, Egypt). An accurate measured volume equivalent to 10 mg of drug was transferred into a 100 ml calibrated flask; diluted to the mark with distilled water. The assay for TIM content was completed as described under procedures for calibration curves.

Validation methods

Validation parameters of atenolol (ATN) and timolol (TIM) in pure and pharmaceutical formulations assay which were tested were included linearity, Sandell's sensitivity, accuracy, precision, range, limit of detection (LOD) and the limit of quantitation (LOQ) [40].

RESULTS AND DISCUSSION

Optimization of the reaction conditions

Potassium permanganate as a strong oxidizing agent was utilised extensively for the determination of several drugs. The proposed method is concerned with the treatment of the investigated drugs with a known excess amount of $KMnO_4$ in NaOH medium for certain time at room temperature. The reaction between studied drugs with $KMnO_4$ in alkaline solution yields a bluish-green color as a result of the manganate (VI) species, peaking at 610 nm (fig. 2). The absorbance of the colored solution increase with time and hence, a kinetically-based spectrophotometric method was elaborated for their assay in pharmaceutical formulations. The extent of formation of the manganate (VI) species depends on the reactants, alkalinity of the medium, order of addition of reactants, diluting solvent and temperature. Therefore, various experimental parameters affecting the development and stability of the reaction product were optimized by changing each variable in turn while keeping all others constant.

Effect of $KMnO_4$ concentration

To study the effect of $KMnO_4$ concentration, aliquots of the studied drugs were transferred into a series of 10 ml volumetric flasks as cited in (table 2), followed by varying volumes of 5×10^{-3}M of $KMnO_4$

(0.2–2.2.5 ml) and 2.0 ml of 1.0M NaOH solutions. The absorbance at 610 nm was measured at a fixed time of 25 min. The reaction increased substantially with increasing the concentration of $KMnO_4$. Maximum absorbance was obtained when 2.0 ml of 5×10^{-3}MKMnO$_4$ solution was used. Further increase in the concentration had no effect of the reaction.

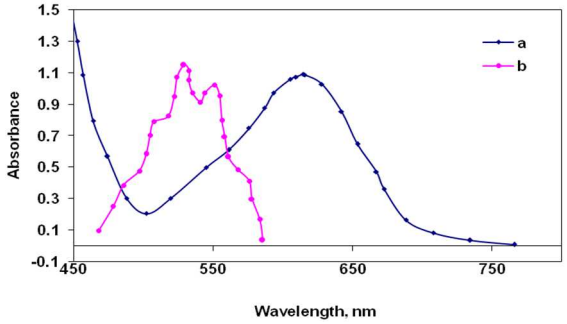


Fig. 2: Absorption spectra of the reaction product of (a) 14 µg/ml atenolol after reaction with alkaline $KMnO_4$ system against (b) reagent blank of 5×10^{-3}M $KMnO_4$

Effect of NaOH concentration

Effect of NaOH concentration on the reaction rate was studied using 0.2–3.0 ml of 1.0M NaOH. It was found that increasing the volume of 1.0M NaOH, would increase the absorbance of the reaction product up to 2.0 ml. It was also observed that there was no significant difference in the absorbance of reactant solutions at NaOH concentrations above 1.5 ml, while decreasing NaOH concentration resulted in lower absorbance values. Therefore, 2.0 ml of 1.0M NaOH was found to be the most suitable concentration for maximum absorbance.

Effect of temperature and time

At room temperature the reaction rate increases substantially with time, although heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated. However, 25±2 °C was selected as the optimum temperature due to the low reproducibility of absorbance values obtained at higher temperatures. At room temperature, the reaction increased substantially with time, as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graph indicating high analytical sensitivity. The intensity of the color produced increased gradually and reached its maximum after 25 min, where it remained stable for at least 1.0 h.

Order of addition

The experimental parameters were fixed and further experiments were performed to test the influence of the order of the addition of reactants. It was found that the order drug, $KMnO_4$ and NaOH, results in maximum absorbance. Addition orders, other than that described in the procedure, gave lower results.

Stoichiometric ratio

The stoichiometric ratio between the studied drugs and potassium permanganate was determined by the limiting logarithmic method [41] by performing two sets of experiments. In the first set, the concentration of drugs was varied keeping a constant concentration of $KMnO_4$. In the second set of experiment, the concentration of drugs was kept constant while varying the concentration of $KMnO_4$. The logarithm of the absorbance was plotted against the logarithm of the respective varied concentration of drug or $KMnO_4$. The slopes of the two straight lines were calculated and found to be unity in each case. Thus the stoichiometric ratio between each drug and potassium permanganate was found to be 1: 1.

Analytical parameters

Under the experimental conditions described, standard calibration curves for ATN and TIM were constructed by plotting absorbance versus concentration (fig. 3). Conformity with Beer's law was evident in the concentration range of the final dilution cited in table 2. The calibration graphs are described by the equation:

$$A = a + bX$$

(Where A= absorbance, a = intercept, b = slope and X = concentration in µg/ml) obtained by the method of least squares. The correlation coefficient, intercept and slope for the calibration data are summarised in table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification are calculated and compiled in table 2 and are indicative of the excellent sensitivity of both methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formula:

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

Where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

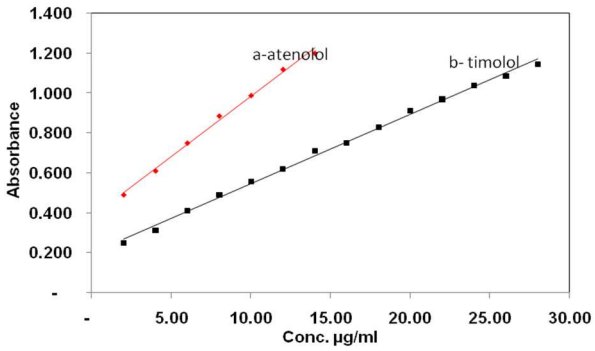


Fig. 3: Calibration curve of (a) atenolol (2-14 µg/ml) and (b) timolol (2-28 µg/ml), 2.0 ml of 1.0 M NaOH, 2.0 ml of 5×10^{-3}M $KMnO_4$ and mix for 25 min

Table 2: Analytical parameters for the determination of atenolol and timolol in pure form

Parameters	ATN	TIM
λ_{max} (nm)	610	610
Temp. °C	25±2	25±2
Beer's law limit, µg/ml	2-14	2-28
Molar absorptivity, l mol^{-1} cm^{-1}	2.03×10^4	1.74×10^4
Sandell's sensitivity, ng/cm^2	13.1	24.8
Correlation coefficient (r)	0.9999	0.9998
Linear regression equation*		
Intercept (a)	0.340	0.201
Slope (b)	0.057	0.038
$S_{y/x}$	0.046	0.026
SD of slope (S_b)	0.002	0.003
SD of intercept (S_a)	0.015	0.019
LOD, µg/ml	0.081	0.181
LOQ, µg/ml	0.351	0.789

*A= a+bC, where A is the absorbance and C is the concentration of drug in µg/ml, ATN: atenolol and TIM: timolol

Accuracy and precision

The accuracy and precision of the proposed method were evaluated by performing five replicate analyses on pure drug solution at three different concentration levels (within the working ranges). The relative error (%), an indicator of accuracy was within 0.3 and within day precision, also called the repeatability, expressed as relative standard deviation (RSD %) was less than 2.7 indicating high accuracy and repeatability of the method. The results of the study are given in table 3.

Table 3: Evaluation of Intra-and inter-day precision and accuracy of the proposed method using alkaline $KMnO_4$

Frequency of analysis	Drugs	Drug taken µg/ml	Recovery[a], %	RSD, %	RE [b], %	SE [c]
Intra	ATN	4.0	99.99	1.584	-0.025	0.004
		8.0	99.96	1.349	-0.088	0.017
		12	99.98	0.595	-0.015	0.009
Inter		4.0	99.97	1.428	-0.095	0.008
		8.0	99.96	2.424	-0.178	0.005
		12	99.99	0.377	-0.025	0.007
Intra	TIM	4.0	99.95	2.418	-0.201	0.038
		12	99.98	1.048	-0.075	0.007
		20	99.99	0.125	-0.007	0.005
Inter		4.0	99.96	2.661	-0.265	0.039
		12	99.97	1.053	-0.108	0.013
		20	99.99	0.173	-0.015	0.007

[a]Mean value of five determinations, [b]RE: Relative error, [c]SE: standard error, ATN: atenolol and TIM: timolol

Analysis of pharmaceutical formulations

The fixed-time method has been successfully applied to determine ATN in tablets and TIM in eye drops.

The concentrations of the drugs were calculated using the corresponding regression equations at a fixed time of 25 min for both ATN and TIM. The results obtained are presented in table 4.

Table 4: Recovery of the studied drugs in formulations using the standard addition method using alkaline $KMnO_4$

Drugs	Drug taken µg/ml	Drug found, µg/ml	Recovery[a], %	RSD, %	RE[b], %
Blokium[c], 100 mg/tablet	4.0	4.02	100.01	0.493	0.025
	12	11.99	99.999	0.574	-0.008
	14	13.99	99.999	0.869	-0.005
Cusimolol eye drops[d], 0.5 %	4.0	3.99	99.999	0.526	-0.025
	12	11.99	99.999	1.348	-0.008
	20	20.02	100.04	1.191	-0.031

[a]Mean value of five determinations, [b]RE: Relative error, [c]Medical Union Pharmaceuticals, Ismailia, Egypt, [d]Egyptian Int Pharmaceutical Industries Co. (EIPICo) 10th of Ramadan, Egypt

Evaluation of the kinetic method

The quantitative determination of ATN and TIM under the optimized experimental conditions outlined above would result in a pseudo-first order reaction with respect to their concentration where, $KMnO_4$ concentration was at least 25 times of the concentration of each drug, and NaOH concentration was at least 300 times the initial concentration of each drug. However, the rates will be directly proportional to drugs concentration in a pseudo-first order rate equation as follows:

$$\text{Rate} = K + [C]^n \quad (1)$$

Equation (1) was the basis for several experiments, which were carried out to obtain drug concentration. The rate constant, fixed-concentration, and fixed time methods [42] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (*r*), and the intercept. Taking logarithms of rates and concentrations (table 5), the above equation becomes:

$$\log K = \log \Delta A/\Delta t = \log k' + n \log C$$

Where A is the absorbance, t is the time in seconds and K is the pseudo-first order rate constant. Regression of log (K) versus log [C] gave the regression equations:

Log K = log ΔA/Δt=-0.0077+0.6847 log C, r =0.9964 for ATN

Log K = log ΔA/Δt= 1.2287+0.9502 log C, r =0.9968 for TIM

A straight line with slope values of ($n \approx 1$) was obtained confirming that the reaction was first order.

Table 5: Values of logarithms rates and concentrations of the studied drugs with alkaline $KMnO_4$

Drugs	log ΔA/Δt	Log [Drug]	Regression equation log ΔA/Δt=log k'+n log C	Correlation coefficient (r)
ATN	-3.316	-4.823	log ΔA/Δt=-0.008+0.684 log C	0.9964
	-3.087	-4.523		
	-2.987	-4.346		
	-2.909	-4.221		
	-2.825	-4.124		
TIM	-3.565	-5.034	log ΔA/Δt= 1.228+0.950 log C	0.9968
	-3.258	-4.733		
	-3.080	-4.557		
	-2.989	-4.432		
	-2.902	-4.334		

ATN: atenolol and TIM: timolol

Fixed-time method

Reaction rates were determined for different concentrations of the investigated drugs. At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graph of absorbance versus initial concentration of drugs was established at fixed time of 2, 5, 7, 11, 14, 17, 20, 25 and 30 min (fig. 4, 5) with the regression equation assembled in table 6. It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 25 min, which was therefore chosen as the most suitable time interval for measurement.

The analytical parameters for the determination of drugs in pure form by fixed time method are shown in table 6. After optimising the reaction conditions, the fixed time method was applied to the determination of the studied drugs in pure form over the concentration range 2–14 and 2–28 µg/ml for ATN and TIM, respectively.

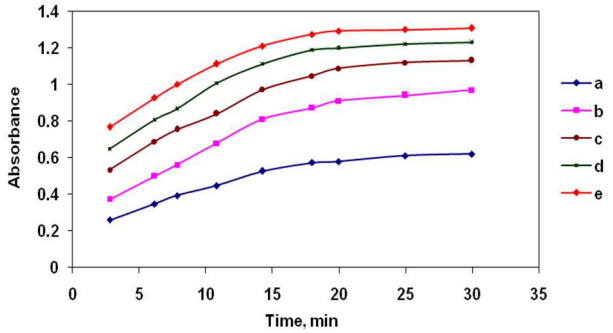


Fig. 4: Absorbance versus time graphs for the reaction of atenolol and alkaline potassium permanganate. Concentration of atenolol: (a) 1.50×10^{-5}, (b) 3.00×10^{-5}, (c) 4.51×10^{-5}, (d) 6.01×10^{-5} and (e) 7.50×10^{-5}M

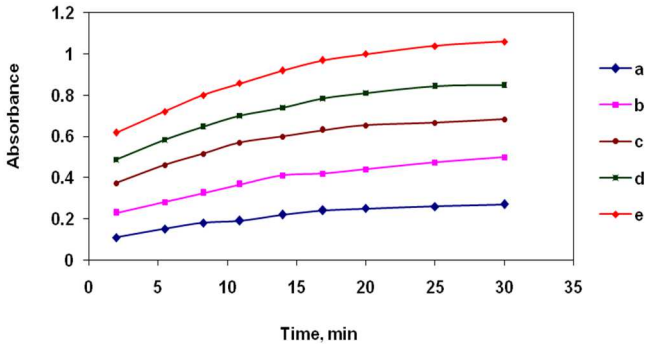


Fig. 5: Absorbance versus time graphs for the reaction of timolol and alkaline potassium permanganate. Concentration of timolol: (a) 9.25×10^{-6}, (b) 1.84×10^{-5}, (c) 2.77×10^{-5}, (d) 3.69×10^{-5} and (e) 4.62×10^{-5}M

Table 6: Regression equations for the studied drugs of different concentrations at different time intervals using fixed time method

Drugs	Time, min	Regression equation*, A = a+bC	Correlation coefficient (r)
ATN	2	A= 0.074+0.011 C	0.9912
	5	A= 0.136+0.020 C	0.9867
	7	A= 0.155+0.027 C	0.9868
	11	A= 0.178+0.036 C	0.9776
	14	A= 0.110+0.048 C	0.9966
	17	A= 0.195+0.046 C	0.9888
	20	A= 0.223+0.051 C	0.9892
	25	A= 0.415+0.043 C	0.9999
TIM	2	A=-0.028+0.021 C	0.9986
	5	A= 0.001+0.027 C	0.9879
	7	A= 0.012+0.030 C	0.9991
	11	A= 0.045+0.033 C	0.9894
	14	A= 0.090+0.031 C	0.9859
	17	A= 0.095+0.033 C	0.9889
	20	A= 0.100+0.033 C	0.9853
	25	A= 0.083+0.038 C	0.9998

*A is the absorbance at 610 nm and C is the concentration in µg/ml, ATN: atenolol and TIM: timolol

Rate constant method

Graphs of log (absorbance) versus time for ATN concentrations in the range of 1.5×10^{-5} to 7.51×10^{-5}M and TIM concentrations in the range of 9.23×10^{-6} to 4.62×10^{-5}M were plotted and all appeared to be rectilinear. Pseudo-first-order rate constants (K) corresponding to different concentrations of the investigated drugs [C] were calculated from the slopes multiplied by-2.303 (table 7). Regression of K values versus [C] gave the equations:

$K=-2.03\times10^{-4}+24.47$ log C, r = 0.9999 for ATN

$K=-1.61\times10^{-4}+24.78$ C, r = 0.9998 for TIM

Where A is the absorbance at 610 nm and C is the molar concentration. The method suffered from poor linearity as indicated from rvalue, therefore this method was excluded.

Fixed absorbance method

Reaction rates were determined for different concentrations of the investigated drugs. A pre-selected absorbance value was fixed at 0.5 for both ATN and TIM, for different concentrations of the studied drugs, in the range of 1.50×10^{-5} to 7.51×10^{-5}M for ATE and 9.23×10^{-6} to 4.62×10^{-5}M for TIM and the time required for each concentration to reach the preselected absorbance value was measured in seconds. The reciprocal of time (1/t) versus drug concentrations was plotted and the following equations were obtained by linear regression:

1/t =-0.00031+59.52C, r = 0.9988 for ATN

1/t =-0.00075+83.76C, r = 0.9707 for TIM

The concentration ranges giving the most satisfactory calibration graphs were limited, therefore this method was abandoned.

Table 7: Values of K calculated from slopes of log A versus t graphs multiplied by-2.303 for different concentration of the studied drugs

Drugs	[Drug]	K	Regression equation	Correlation coefficient (r)
ATN	1.50×10^{-5}	-1.61×10^{-3}	$K=-2.037\times10^{-4}+24.47 \log C$	0.9999
	3.00×10^{-5}	-1.38×10^{-3}		
	4.51×10^{-5}	-1.15×10^{-3}		
	6.01×10^{-5}	-9.21×10^{-4}		
	7.51×10^{-5}	-6.90×10^{-4}		
TIM	9.23×10^{-6}	-1.38×10^{-3}	$K=-1.608\times10^{-4}+24.78 \log C$	0.9998
	1.84×10^{-5}	-1.15×10^{-3}		
	2.77×10^{-5}	-9.21×10^{-4}		
	3.69×10^{-5}	-6.90×10^{-4}		
	4.62×10^{-5}	-4.61×10^{-4}		

ATN: atenolol and TIM: timolol

CONCLUSION

The proposed spectrophotometric method is appreciable with a view that the oxidation of drugs can be exploited for the routine quality control analysis of ATN and TIM in pharmaceutical formulations. The method is sensitive with a simple calibration system that does not require any laborious cleanup procedure prior to analysis. Moreover, the present technique has the advantage of using inexpensive and easily available reagents and therefore can frequently be used in the laboratories of research, hospitals and pharmaceutical industries.

CONFLICT OF INTERESTS

Declared none

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