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KINETIC METHOD FOR THE DETERMINATION OF SOME B-ADRENERGIC BLOCKING AGENTS IN BULK AND IN DRUG FORMULATION

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ABSTRACT

A simple, accurate and sensitive kinetic method for the determination of acebutolol HCl (I) and atenolol (II) in pure form and in pharmaceutical preparations was developed. The procedure is based upon a kinetic investigation of the reaction using alkaline potassium permanganate at room temperature for a fixed time of 20 min. The absorbance of the coloured manganate ion was measured at 609 and 608 nm respectively. The absorbance- concentration plots for both drugs were rectilinear over the range 8 - 24 and 12-40 μg ml^{-1} for (I) and (II), respectively.

The concentration of (I) and (II) were calculated using the corresponding calibration equation for fixed-time method. The determination of the studied drugs by fixed concentration and fixed absorbance methods were feasible with the calibration equation obtained to be more applicable. The procedure was applied successfully to pharmaceutical formulations and the results obtained were in good agreement with those obtained using official method.

INTRODUCTION

Acebutolol HCl (I) and atenolol (II) are two of a number of drugs collectively known as beta adrenergic antagonist used as antihypertensive, antianginal, anti-arrhythmic drug and myocardial infraction, where it acts preferentially upon the B-adrenergic receptors in the heart[1], the reported methods for the determination of this drugs including spectrophotometry[2-6], atomic absorption[7], polarographic[8], colorimetry[9], high performance liquid chromatography[10-14], first derivative spectrometric[15], capillary electrophoresis[16-19] and flourimetry[20].

In the present work, a kinetically based method is proposed for the determination of acebutolol HCl (I) and atenolol (II) by measuring the absorbance at 609 and 608 nm respectively after oxidation reaction with $KMnO_4$ in alkaline medium.

The aim of the present work was the development of a simple, accurate and sensitive analytical method for the assay of (I) and (II) in bulk and in pharmaceutical forms.

Our method was compared with the official method[21], showing a good agreement between the final results.

EXPERIMENTAL

Apparatus:

Shimadzu UV and visible recording spectrophotometer (UV. 260) with 1.0 cm quartz cells was used.

Materials and reagents:

All materials used were of analytical reagent grade.

Reference acebutolol HCl (Rhone, Poulence, Paris, France) was kindly supplied by Alexandria company for pharmaceuticals and chemical industries (Alexandria, Egypt). Reference atenolol (B. P.) obtained as gift sample from Eipico Company for pharmaceutical industries A. R. E.

- The commercial Sectral tablets used (batch no. 6512005), was manufactured by Alexandria company for pharmaceuticals and chemical industries, under license from Rhone, poulence, Paris, France, Containing acebutolol HCl equivalent to 200 mg acebutolol per tablet
- Ateno tablets (batch no 013796) was manufactured by Eipico Egyptian. International Pharmaceutical Industries company A.R.E., containing atenolol equivalent to 100 mg per tablet.
- Stock solutions of the reference compounds (1 mg ml^{-1}) were prepared by dissolving 100 mg in distilled water in 100 ml measuring flask.
- Potassium permanganate solution 5×10^{-3} M was prepared by dissolving an appropriate weight in distilled water in 500 ml measuring flask.
- Sodium hydroxide solution, 0.5 M aqueous solution was prepared.

Procedure:

A 1.0 ml of 0.5 M NaOH and 3ml of 5×10^{-3} M $KMnO_4$ were mixed and transferred in 25 ml calibrated flask.

The appropriate amount of drugs (8 - 40 μg) were added and the solution was diluted to the mark with distilled water, the flask with its contents was shaken gently at room temperature at fixed time of 20 min. The absorbance was measured directly at 609 and 608 min for (I) and (II) respectively against blank omitting drug. The concentration of drug was then calculated from the corresponding equation for the calibration graph for the fixed time method.

Procedure for tablets:

Twenty tablets were weighed and finally powdered. A portion of the powder equivalent to about 100 mg of (I) or (II) was weighed accurately, dissolved in methanol, the sample solution was filtered and then diluted in a 100 ml measuring flask with water. The above general procedure was then applied using the constructed calibration graph.

RESULTS AND DISCUSSION

Kinetics and optimization of the reaction condition:

The reaction between acebutolol HCl or atenolol with alkali potassium permanganate yields a green colour with maximum absorbance at 609 and 608 nm respectively (Fig. 1). As the intensity of colour increase with time, this behaviour was used as a useful method for the determination of above cited drugs in pure and in pharmaceutical preparations.

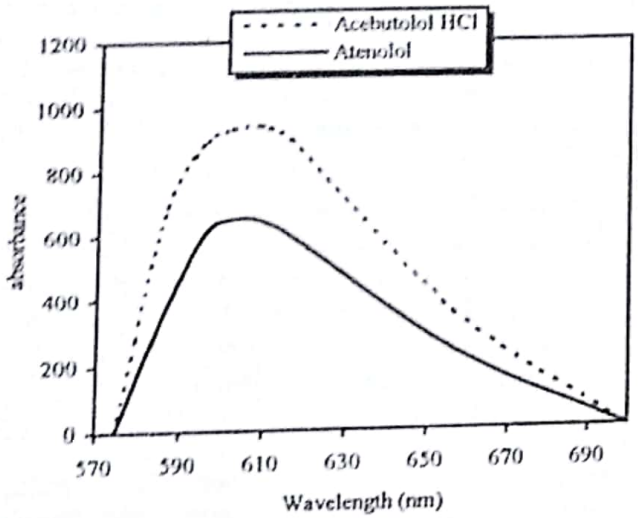

Fig. (1): Absorption spectrum of acebutolol HCl 16 µg m^{-1} (1) and atenolol 32µg m^{-1} (2) after reaction with 5×10^{-3} M $KMnO_4$ in presence of 0.5 M NaOH

The reaction rate increased substantially as the colour development increased. Therefore, room temperature was selected as the optimum temperature. Heating the solution was found to increase the rate of the reaction but Mno_2 was precipitated.

The reaction rate and maximum absorbance increased with time was investigated with increasing $KMnO_4$ concentration. It was found that 3.0ml of 5×10^{-3} M $KMnO_4$ was adequate for the maximum absorbance, using more than 3.0 ml no change in reaction rate and maximum colour intensity whereas less than the optimal (3.0 ml), the colour intensity not achieve its maximum value in addition to longer time to obtain.

The influence of NaoH concentration on the reaction rate was also studied using 0.5 - 3.0 ml of 0.5 M NaOH. It was found that increasing the volume of 0.5 M NaOH would increase the absorbance of the reaction product up to 1.0ml, after which further increase in the volume of 0.5 M NaOH resulting no change in the absorbance of the reaction product. Therefore, thus 1.0 ml of 0.5 M NaOH was found to be the most suitable concentration for maximum absorbance.

To summarize, the optimum working conditions for the Kinetic determination of (I) and (II), thus 1.0 ml of 0.5 M NaOH and 3.0 ml of 5×10^{-3} M $KMnO_4$ at room temperature were used.

The rate of the reaction was also found to be (I) and (II) concentration dependent. The rates were followed at room temperature with various concentrations range 8-24 and 12-40ug ml^{-1} for (I) and (II) respectively keeping $KMnO_4$ and NaOH constant at optimum concentration as described above.

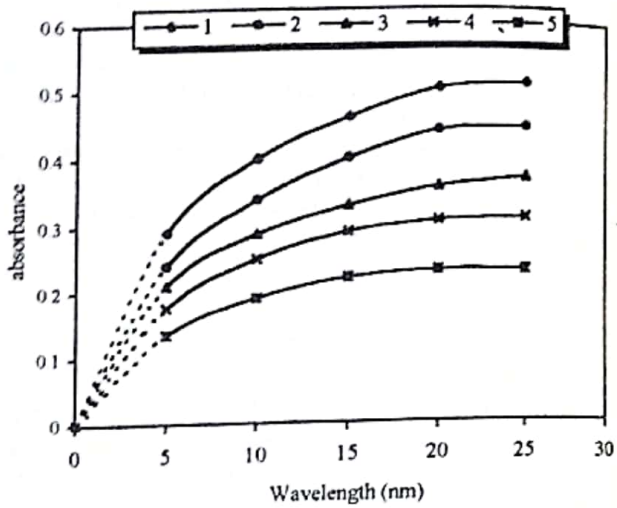

Fig. (2): Absorbance versus time graphs for the reaction between acebutolol HCl and potassium permanganate showing dependence of the reaction on acebutolol HCl concentration. Concentration of acebutolol HCl (1) 2.14×10^{-5}, (2) 3.21×10^{-5}, (3) 4.28×10^{-5}, (4) 5.36×10^{-5}, (5) 6.43×10^{-5} M. Sodium hydroxide 0.5 M and potassium permanganate 5×10^{-3} at room temperature.

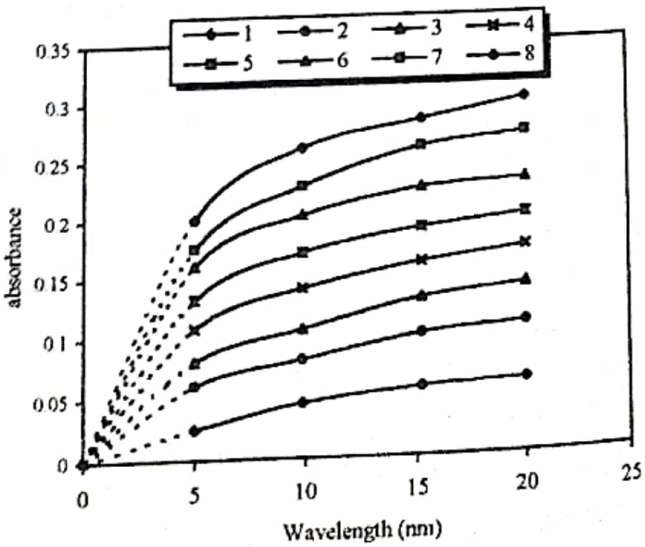

Fig. (3): Absorbance versus time graphs for the reaction between atenolol and potassium permanganate showing dependence of the reaction on atenolol concentration. Concentration of atenolol (1) 4.5×10^{-5}, (2) 6.01×10^{-5}, (3) 7.51×10^{-5}, (4) 9.02×10^{-5}, (5) 1.05×10^{-4} (6) 1.20×10^{-4} (7) 1.35×10^{-4} (8) 1.50×10^{-4} M. Sodium hydroxide 0.5 M and potassium permanganate 5×10^{-3} at room temperature.

The graph shown in (Figs. 2 and 3) were obtained from which it is clear that, the rate increase as the concentrations of drugs increases, indicating that the reaction rate obeys the following equation:

Rate = K' (drug)^n (1)

Where K' is the pseudo-order rate constant and n is the order of the reaction.

The rate of the reaction was estimated by the variable-time method measurement as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds.

Taking logarithms of rates and concentration (Table 1), equation (1) is transformed into.

$$\log \text{rate} = \log \frac{\Delta A}{\Delta t} = \log K' + n \log (\text{drug}) \quad (2)$$

Regression of log (rate) versus log (acebutolol HCl) gave the following regression equation.

log rate = - 0.517 + 1.04 log c (r = 0.9969)

Hence $K' = 0.304$ sec^{-1} and the reaction is first order (n = 1.04) with respect to acebutolol HCl concentration.

Regression of log (rate) versus log (atenolol) gave the following equation

log (rate) = 1.32 + 1.25 log c (r = 0.9842)

Hence $K' = 20.89$ sec^{-1} and the reaction is first order (n = 1.25), with respect to atenolol concentration.

Table (1): Logarithms of rates for different concentration of (I) and (II) at room temperature.

log $\Delta A/\Delta t$	log [acebutolol HCl]/M	log $\Delta A/\Delta t$	log [atenolol]/M
-3.996	-4.668	-4.488	-4.345
-3.802	-4.492	-4.825	-4.220
-3.679	-4.367	-4.371	-4.123
3.604	-4.270	-4.301	-4.044
-3.541	-4.191	-4.257	-3.977
--	--	-4.209	-3.919
--	--	-4.139	-3.868
--	--	-4.074	-3.822

Appraisal of kinetic method:

The determination of (I) and (II) under the optimum experimental conditions mentioned above, in which the potassium permanganate and sodium hydroxide concentrations were at least 40 times of the initial concentration of analyte, would result in a pseudo-zero order reaction with respect to $KMnO_4$, however, the rate will be directly proportional to drug concentration in a pseudo-first order rate equation as follows:

Rate = K' [drug] (3)

Where K' is the pseudo-first order rate constant.

Several experiments were run to obtain drug concentrations from rate data according to equation (3). Initial-rate, rate-constant, constant-absorbance and constant-time method[22,23] were used and the best method was chosen on basis of applicability, the slope of the calibration graph, intercept and correlation coefficient (r).

Initial-rate method (pseudo-zero-order-method): -

In this method, graphs of rate (at the beginning of the reaction) versus drug concentration were not easy to obtain because the first step of the reaction is not rate determining and is too fast to follow, so tangents to the curves at zero time are not easy to draw, this method was therefore abandoned.

Rate-constant method:

Graph of log (absorbance) versus time for (I) and (II) concentrations in range 2.3×10^{-5} - 7.1×10^{-5}M and 1.05×10^{-4} - 1.50×10^{-4} M respectively were studied and all were of straight lines. Pseudo-first order rate constant (K') corresponding to different concentrations of (I) and (II) were calculated from the slopes multiplied by - 2.203 and were presented in Table 2. Regression of C versus K' gave the equations.

$K' = -5.90 \times 10^{-5} + 0.133$ C (r = 0.8916) for (I)

$K' = -4.93 + 0.370$ C (r = 0.6012) for (II)

The poor linearity is probably due to changes in the rate constant (K') with the inevitable slight changes in the elevated temperature of the reaction.

Table (2): Value for K' calculated from slopes of log A versus t graphs multiplied by - 2.303 for different concentrations of drugs at constant concentration of 0.5 M NaOH and 5×10^{-3} M potassium permanganate.

K'/S^{-1}	[acebutolol HCl]/M	K/S^{-1}	[atenolol]/M
6.2×10^{-4}	2.14×10^{-5}	4.68×10^{-4}	1.05×10^{-4}
8.1×10^{-4}	3.21×10^{-5}	4.45×10^{-4}	1.20×10^{-4}
8.4×10^{-4}	4.28×10^{-5}	4.52×10^{-4}	1.35×10^{-4}
8.9×10^{-4}	5.36×10^{-5}	5.14×10^{-4}	1.50×10^{-4}
9.0×10^{-4}	6.43×10^{-5}	--	--

Fixed - absorbance method:

Reaction rates were recorded for different concentrations of (I) and (II) in range 2.14×10^{-5} - 5.36×10^{-5} M and 4.5×10^{-5} - 7.51×10^{-5} M , respectively. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time versus the initial concentration of drug (I) and (II) (Table 3) was plotted. And the following equations of the calibration graph was obtained

$1/t = 1.98\times10^{-3} + 9.7\times10^{-3}$ C (r = 0.9264) for I

$1/t = 3.720 + 0.023$ C (r = 0.9800) for II

Table (3): Value of reciprocal of time taken at fixed absorbance for different rates of variable concentration of drug at constant concentration of 0 5 M NaOH and 5×10^{-3} M potassium permanganate.

$1/ts^{-1}$	[acebutolol HCl]/M	$1/ts^{-1}$	[atenolol]/M
7.5×10^{-4}	2.14×10^{-5}	4.27×10^{-4}	4.5×10^{-5}
1.1×10^{-3}	3.21×10^{-5}	8.33×10^{-4}	6.01×10^{-5}
1.7×10^{-3}	4.28×10^{-5}	1.66×10^{-3}	7.51×10^{-5}
3.7×10^{-3}	5.36×10^{-5}	--	--

The range of drug (I) and (II) concentrations giving the most acceptable calibration graph with the above equation was limited (8 - 20 µg ml^{-1}), and (12 - 20 µg ml^{-1}), respectively which could be a disadvantages.

Fixed-time method:

Reaction rates were measured for different concentrations of (I) and (II). Calibration graphs of absorbance versus initial concentration of drugs were obtained at fixed time of 5, 10, 15, 20, and 25 min, with the calibration equations shown in (Table 4). Both the slops and intercepts increase with time and the best correlation coefficient was obtained for fixed time of 20 min, which was therefore chosen as the most suitable time for absorbance measurements. Acebutolol HCl and atenolol keeping sodium hydroxide and permanganate concentration constant at 0.5 M, 5×10^{-3} M at temperature.

Table (4): Calibration equations at different fixed times for acebutolol HCl and atenolol keeping sodium hydroxide and potassium permanganate concentration constant at 0.5 M, 5×10^{-3} M at room temperature.

drug	Time/ min	Calibration equation	Correlation coefficient
Acebutolol HCl	5	A = 0.071 + 0.0036C	0.9990
	10	A = 0.103 + 0.010C	0.9940
	15	A = 0.110 + 0.014C	0.9960
	20	A = 0.081 + 0.0175C	0.9999
	25	A = 0.099 + 0.0172C	0.9998
Atenolol	5	A = -0.04 + 6.20×10^{-3} C	0.9980
	10	A = -0.03 + 6.21×10^{-3} C	0.9911
	15	A = -0.03 + 6.86×10^{-3} C	0.9360
	20	A = -0.032 + 8.34×10^{-3} C	0.9999
	25	A = -0.033 + 8.04×10^{-3} C	0.9998

Mechanism:

From the kinetic data cited above and in the light of the mechanism of (I) and (II) oxidation through the common active site of the N-alkylethanolamine group. The hydroxy function is oxidised in two sequential steps, of which the second is rate determing via the radical to the ketone as shown in (Fig. 4)

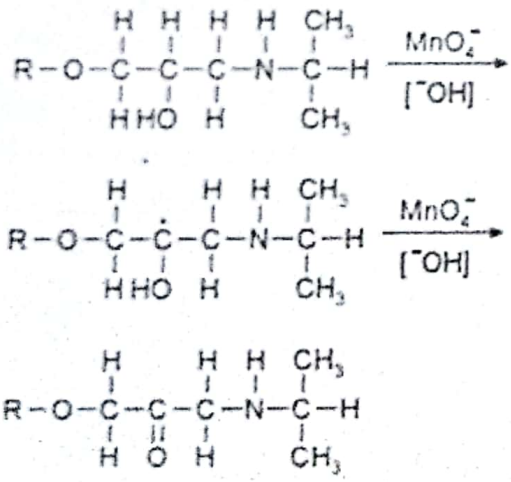

Fig. 4: Reaction mechanism

Applications:

The fixed-time method was applied to the determination of the studied drugs in commercially available drug formulations in tablet form. The concentration of (I) and (II), calculated using the corresponding calibration equation was shown in Table 5 at a fixed time of 20 min. The results obtained for the analysis of the drugs were compared statistically with those obtained using official B.P.[21]

Table (5): Determination of acebutolol HCl and atenolol in their tablets by suggested method and official methods[21]

Items	Sectral tablets	Ateno tablets
Kinetic method		
Mean	99.8	100.35
N	6	6
Variance	0.43	0.262
SD	0.66	0.51
SE	0.27	0.21
RSD	6.6×10^{-3}	5.1×10^{-3}
RSE	0.3	0.2
Official method		
Mean	99.9	100.3
N	5	5
Variance	0.38	0.29
SD	0.62	0.54
SE	0.28	0.25
RSD	6.2×10^{-3}	5.4×10^{-3}
RSE	0.31	0.27
F-value (5.19)*	1.13	1.10
t-value (2.26)*	0.26	0.16

*Theoretical value at p = 0.05

CONCLUSION

The results obtained for the analysis of cited drugs formulation employed were compared with those obtained with the official B.P. method (Table 5).

The student t-test and F-test values of the 95% confidence level did not exceed the theoretical values 2.26 and 5.19 respectively indicating no significant difference between the accuracy and the precision of the method. The method is also a direct method and sensitive.

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التقدير الكمى لبعض مركبات منبطات البيتا بالطرق الحركية فى المادة الفعالة والمستحضرات الطبية

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تـم اسـتحداث طـريقة حساسة ودقيقة لتقدير مـادتى الأسيبيوتولول هيدروكلوريد والأتينولول تعتمد على حركـية تفـاعل الأكسدة بينهما وبين برمنجنات البوتاسيوم فى الوسط القاعدى وذلك فى زمن ثابت قدره ٢٠ دقيقة حيـث يتم تقدير أيون المنجنات الناتج من التفاعل طيفياً عند طول موجه قدره ٦٠٩ و٦٠٨ نانوميتر على التوالى. ويـتم تقديـر تركيز كل منهم باستخدام معادلة التغيير فى طريقة الزمن الثابت وقد أمكن تطبيق هذه الطريقة بنجاح على الأقراص وقورنت النتائج إحصائيا مع نتائج دستور الأدوية البريطانى.

كذلـك بالدراسـة اتضـح أنه يمكن تقدير تركيز كل منهم بطريقة امتصاص الثابت وطريقة ثابت سرعة التفاعل باستخدام المعادلات الموضحة ولكن طريقة الزمن الثابت ثبت أنها أكثر الطرق الحركية إمكاني للتطبيق.