Validated Kinetic Spectrophotometric Method for the Determination of Metoprolol Tartrate in Pharmaceutical Formulations

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A kinetic spectrophotometric method has been described for the determination of metoprolol tartrate in pharmaceutical formulations. The method is based on reaction of the drug with alkaline potassium permanganate at 25±1 °C. The reaction is followed spectrophotometrically by measuring the change in absorbance at 610 nm as a function of time. The initial rate and fixed time (at 15.0 min) methods are utilized for constructing the calibration graphs to determine the concentration of the drug. Both the calibration graphs are linear in the concentration range of 1.46×10^{-6}—8.76×10^{-6} M (10.0—60.0 μg per 10 ml). The calibration data resulted in the linear regression equations of log (rate)=3.634+0.999 log C and $A=6.300\times10^{-4}+6.491\times10^{-2}$ C for initial-rate and fixed time methods, respectively. The limits of quantitation for initial rate and fixed time methods are 0.04 and 0.10 μg ml^{-1}, respectively. The activation parameters such as E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ are also evaluated for the reaction and found to be 90.73 kJ mol^{-1}, 88.20 kJ mol^{-1}, 84.54 J K^{-1} mol^{-1} and 63.01 kJ mol^{-1}, respectively. The results are validated statistically and through recovery studies. The method has been successfully applied to the determination of metoprolol tartrate in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Key words spectrophotometry; metoprolol tartrate; validation parameters; pharmaceutical formulation

Metoprolol tartrate is a selective β-adrenergic antagonist, which is used in the treatment of cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. The drug is quite sensitive, even a small dose of the drug gives sufficient blockade. Since the β-blockers are also misused as doping agents in sports and therefore these drugs have been added to the list of forbidden drugs by the International Olympic Committee (IOC).[1] Therefore the development of an analytical method for the determination of metoprolol tartrate is of great significance.

The assay of the drug is listed in the monograph of British Pharmacopoeia, which describes a potentiometric titration method.[2] Several analytical methods have been developed for the determination of metoprolol in biological fluids and pharmaceutical formulations based on high performance liquid chromatography,[3—11] gas chromatography,[12,13] capillary electrophoresis,[14] thin layer chromatography,[15,16] infrared spectroscopy,[17] and electrochemical methods.[18,19] The above mentioned techniques are sensitive but expensive and require laborious clean up procedure prior to analysis. Spectrophotometry is the technique of choice even today due to its inherent simplicity and therefore frequently used in the laboratories of the developing countries to overcome versatile analytical problem. The drug has been determined in the visible region based on ion-pair formation between drug and reagents like oxidized quercetin[20]; bromophenol blue, bromocresol purple, bromocresol green[21]; benzyl orange[22]; bromothymol blue[23]; and carbon disulfide-copper chloride.[24] The charge transfer complexation reactions of metoprolol tartrate with σ and π-acceptors[24,25] have also been utilized for its quantification in pharmaceutical formulations. Shingbal and Bhangle[26] have reported a spectrophotometric method based on the reaction of drug with 2,4-dinitrofluorobenzene in HCl/dioxan medium to form a colored chromophore, which absorbs maximally at 380 nm. The quantification of metoprolol tartrate was done on treatment of the drug with ammonium metavanadate,[27] $FeCl_3$,[28] *N*-bromosuccinimide,[29] and a mixture of KNO_3 and H_2SO_4 followed by addition of alkali to get colored chromophore.[30] The literature is still poor in analytical procedures based on kinetic spectrophotometry for the determination of drug in pharmaceutical preparations. There is, therefore, a need for a simple and sensitive kinetic spectrophotometric method for the determination of metoprolol tartrate in pharmaceutical formulations. It was found that potassium permanganate oxidizes the metoprolol in alkaline medium and this reaction has not been used before to quantify the drug spectrophotometrically.

This paper describes a simple and sensitive kinetic spectrophotometric method for the determination of metoprolol tartrate in drug formulations. The method involves the oxidation of drug with alkaline potassium permanganate at 25±1 °C and subsequent measurement of absorbance at 610 nm. The initial rate and fixed time methods are adopted for its determination in pharmaceutial formulations.

Experimental

Apparatus A Shimadzu UV–visible spectrophotometer (model-1601, Japan) with matched quartz cells was used to measure absorbance.

A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature for color development.

Reagents and Standards All reagents and chemicals used were of analytical or pharmaceutical grade. Aqueous solutions of 0.6 M sodium hydroxide and 0.015 M potassium permanganate (GR Grade, Merck Limited, Mumbai, India) were prepared in doubly distilled water. Potassium permanganate (GR Grade, Merck Limited, Mumbai, India) solution should be freshly prepared and its apparent purity was assayed by titrimetric method.[31]

The standard test solution of metoprolol tartrate (0.01%) was prepared in doubly distilled water. The formulated dosage forms of metoprolol tartrate such as betaloc (AstraZeneca Pharma India Ltd., Bangalore, India), metapro (Cardicare, Bangalore, India) and metolar (Cipla, Mumbai, India) were purchased from the local market.

Determination Procedures for Metoprolol Tartrate Initial-Rate Method: Aliquots of 0.1—0.6 ml of 0.01% metoprolol tartrate were pipetted into a series of 10 ml standard flasks. To each flask 2.0 ml of 0.60 M NaOH followed by 2.0 ml of 0.015 M potassium permanganate were added and then diluted with doubly distilled water at 25±1 °C. The contents of each flask

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were mixed well and the increase in absorbance was recorded as a function of time at 610 nm. The initial rate of the reaction (v) at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration graph was constructed by plotting the logarithm of the initial rate of reaction (log v) *versus* the logarithm of the molar concentration of the metoprolol tartrate (log C). The amount of the drug was computed either from the calibration graph or the regression equation.

Fixed-Time Method: The absorbance of each drug sample solution was measured at 610 nm against a reagent blank prepared similarly at a preselected fixed time of 15 min. The calibration curve was constructed by plotting the absorbance against the final concentration of the drug. The amount of the drug was computed either from calibration curve or regression equation.

Determination Procedure for Metoprolol Tartrate in Pharmaceutical Formulations Five tablets were weighed and powdered. The powder equivalent to 50 mg of active ingredient was weighed accurately, stirred well with doubly distilled water and filtered through Whatman No. 42 filter paper (Whatman International Limited, Kent, U.K.). The residue was washed well with doubly distilled water for complete recovery of the drug. The content of the drug was then diluted to 250.0 ml with doubly distilled water. It was further diluted according to the need and subjected to the determination procedures for metoprolol tartrate. The percent recovery of the metoprolol tartrate was calculated from the corresponding linear regression equations or calibration graphs.

Procedure for Reference Method Into a series of 10 ml standard volumetric flask, different volumes (0.25—2.5 ml) of 0.01% drug (0.1 mg ml^{-1}) solution were pipetted and diluted to volume with doubly distilled water. The absorbance was measured against the solvent blank at 224 nm. The amount of the drug in a given sample was computed from the calibration equation.

Results and Discussion

Spectral Studies The absorption spectrum of metoprolol tartrate solution in doubly distilled water shows two absorption bands peaking at 194 and 224 nm while that of potassium permanganate solution in the alkaline medium exhibits an absorption band peaking at 530 nm. The addition of potassium permanganate to the solution of pure drug produces a new characteristics band at 610 nm. This band is attributed to the formation of manganate ion, which resulted on reduction of potassium permanganate in alkaline medium. The intensity of the colored product increases with time and therefore, a kinetic method was developed for the determination of metoprolol tartrate in drug formulations. Moreover, potassium permanganate also oxidizes metoprolol in acid medium resulting in the formation of α-hydroxy metoprolol and Mn(II). In the presence of acid such as H_2SO_4, HCl and H_3PO_4, α-hydroxy metoprolol gives a violet color which has not been utilized for quantitative analysis.

Stoichiometry and Reaction Mechanism The stoichiometric ratio between metoprolol tartrate and potassium permanganate was established using limiting logarithmic method[32] by performing two sets of experiments. In the first set, the concentration of metoprolol tartrate was varied keeping a constant concentration of $KMnO_4$. In the second set of experiment, concentration of metoprolol tartrate 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 metoprolol tartrate or $KMnO_4$ (Figs. 1a, b). It is evident from the slopes of the two straight lines that the combining molar ratio between metoprolol tartrate and $KMnO_4$ is 1 : 1.

Horai *et al.* have suggested that the metoprolol tartrate undergoes oxidation[33] resulting in the formation of α-hydroxy metoprolol. In this study, the potassium permanganate oxidizes the metoprolol tartrate in alkaline medium producing

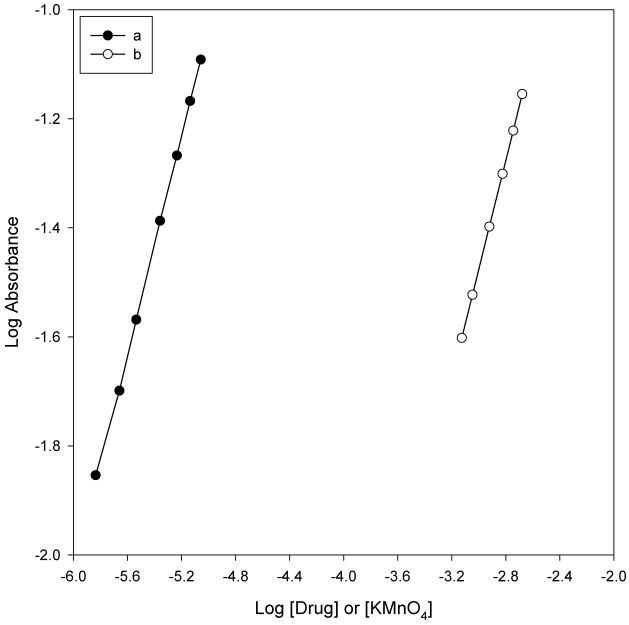

Fig. 1. Limiting Logarithmic Plot for Stoichiometric Ratio between Metoprolol Tartrate and $KMnO_4$ (a) log$A$ *vs.* log[drug] and (b) log$A$ *vs.* log[$KMnO_4$]

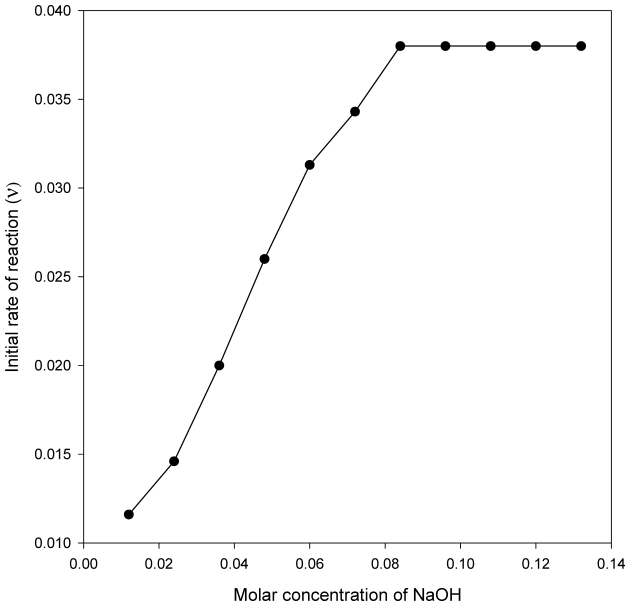

Fig. 2. Effect of the Molar Concentration of NaOH Solution on the Initial Rate of Reaction with 60.0 μg per 10 ml Metoprolol Tartrate in Doubly Distilled Water

α-hydroxy metoprolol and itself reduced to MnO_4^{2-}. The reaction product gives violet color on treating with formaldehyde-sulfuric acid reagent, which confirmed the formation of α-hydroxy metoprolol.[34]

Optimization of Variables The influence of the concentration of NaOH solution on the rate of reaction was studied by keeping the constant concentrations of metoprolol tartrate (8.76×10^{-6} M) and $KMnO_4$ (3.00×10^{-3} M) and varying the concentration of NaOH (1.20×10^{-2}—1.32×10^{-1} M) in a final volume of 10 ml solution. Figure 2 shows that the initial rate of reaction increased up to 8.4×10^{-2} M NaOH; beyond this concentration the initial rate of reaction remained con-

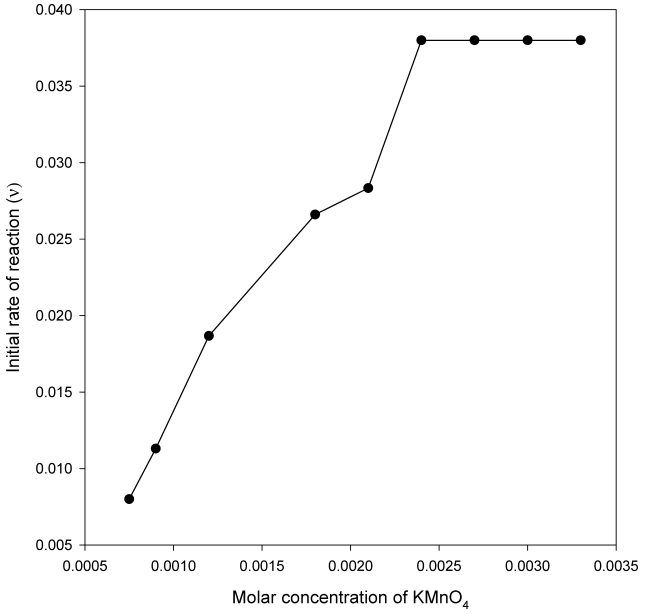

Fig. 3. Effect of the Molar Concentration of $KMnO_4$ Solution on the Initial Rate of Reaction with 60.0 μg per 10 ml Metoprolol Tartrate in Doubly Distilled Water

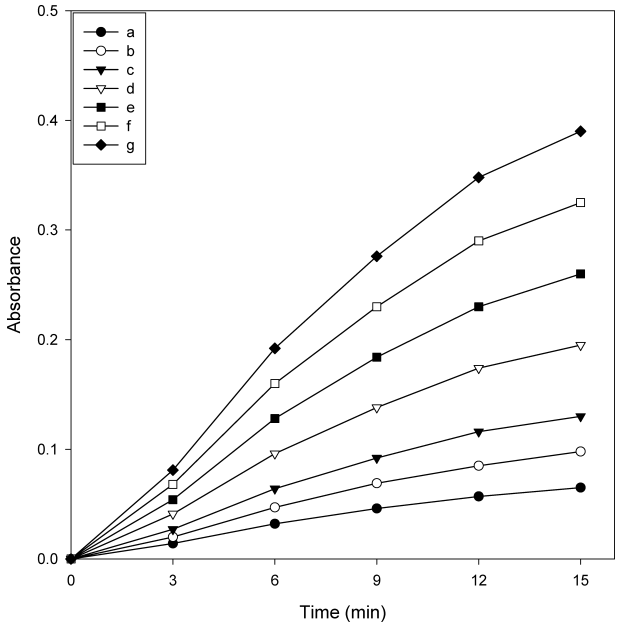

Fig. 4. Absorbance–Time Curves for the Reaction between Metoprolol Tartrate and $KMnO_4$ in Aqueous Medium: 2.0 ml of 0.015 M $KMnO_4$ and Metoprolol Tartrate: (a) 1.0, (b) 1.5, (c) 2.0, (d) 3.0, (e) 4.0, (f) 5.0 and (g) 6.0 μg ml^{-1}. Each Set is Diluted in 10 ml Standard Flask with Doubly Distilled Water

stant. Therefore, a concentration of 1.20×10^{-1} M NaOH was used throughout the experiment. The effect of the concentration of $KMnO_4$ solution on the initial rate of the reaction was studied in the range of 7.50×10^{-4}—3.30×10^{-3} M. The initial rate of reaction (Fig. 3) increased with increasing the concentration of $KMnO_4$ and became constant at 2.40×10^{-3} M. Thus, a concentration of 3.00×10^{-3} M $KMnO_4$ in the final solution proved to be sufficient for the maximum concentration of metoprolol tartrate used in the determination process. The effect of temperature on reaction rate was studied in the range of 298—308 K. The absorbance–time curves showed the temperature dependence of the reaction rate. It was observed that metoprolol tartrate reacts faster with potassium permanganate within the short period of 3—15 min, 3—10 min and 3—7 min. at 298, 303 and 308 K, respectively. At temperatures >308 K, the decomposition of the reaction product may take place. To avoid this and for the sake of good results, the optimum temperature of 298 K is selected for the determination process.

Analytical Data and Method Validation Under the optimized experimental conditions, a pseudo-order reaction condition was worked out by using a large excess of $KMnO_4$ and NaOH solution with respect to the initial concentration of metoprolol tartrate. As a result, a pseudo zero order condition was obtained with respect to the reagents, the overall concentration change of $KMnO_4$ and NaOH during the course of reaction would be negligible. The initial rates of the reaction were determined from the slopes of the initial tangent to the absorbance–time curves (Fig. 4) and are summarized in Table 1. The reaction would obey the following rate equation:

$$\text{rate}=k_\psi C^n$$

where k_ψ is the pseudo-order rate constant, C is the concentration of metoprolol tatrate, n is the order of the reaction. The logarithm form of the above equation is written as:

Table 1. Summary of Data of the Initial Rate of Reaction at Different Concentration of Metoprolol Tartrate and $KMnO_4$

[Drug] (mol l^{-1})	[$KMnO_4$] (mol l^{-1})	Initial rate of reaction, v (mol l^{-1} min^{-1})
1.460×10^{-6}	3.000×10^{-3}	6.250×10^{-3}
2.190×10^{-6}	3.000×10^{-3}	9.375×10^{-3}
2.920×10^{-6}	3.000×10^{-3}	1.270×10^{-2}
4.380×10^{-6}	3.000×10^{-3}	1.880×10^{-2}
5.840×10^{-6}	3.000×10^{-3}	2.500×10^{-2}
7.300×10^{-6}	3.000×10^{-3}	3.100×10^{-2}
8.760×10^{-6}	3.000×10^{-3}	3.800×10^{-2}
8.760×10^{-6}	7.500×10^{-4}	8.000×10^{-3}
8.760×10^{-6}	9.000×10^{-4}	1.130×10^{-2}
8.760×10^{-6}	1.200×10^{-3}	1.860×10^{-2}
8.760×10^{-6}	1.800×10^{-3}	2.660×10^{-2}
8.760×10^{-6}	2.100×10^{-3}	2.830×10^{-2}
8.760×10^{-6}	2.400×10^{-3}	3.800×10^{-2}

$$\log(\text{rate})=\log k_\psi+n\log C$$

The linear regression analysis using the method of least square treatment of calibration data was made to evaluate slope, intercept and correlation coefficient. Under the working experimental conditions, a calibration graph was constructed by plotting log of initial rate of reaction (log v) *versus* log of metoprolol tartrate concentration (log C), which showed a linear relationship over the concentration range of 10.0—60.0 μg per 10 ml. The regression of log rate *versus* log C gave the following linear regression equation:

$$\log(\text{rate})=3.634+0.999\log C$$

with a correlation coefficient (r) of 0.9999. The value of n in regression equation confirmed that the reaction is first order with respect to metoprolol tartrate. The confidence limits for

Table 2. Summary of Optical Characteristics and Statistical Data for the Fixed-Time Method

Parameters	Fixed-time method					Reference method
	3 min	6 min	9 min	12 min	15 min	
Beer's law limit (μg per 10 ml)	10.0—60.0	10.0—60.0	10.0—60.0	10.0—60.0	10.0—60.0	25.0—250.0
Molar absorptivity (l mol^{-1} cm^{-1})	9.133×10^3	2.192×10^4	3.151×10^4	3.973×10^4	4.453×10^4	2.028×10^4
Regression equation	$A=1.01\times10^{-3}+1.324\times10^{-2}C$	$A=6.9\times10^{-4}+3.178\times10^{-2}C$	$A=7.9\times10^{-4}+4.580\times10^{-2}C$	$A=-1.72\times10^{-3}+5.831\times10^{-2}C$	$A=6.3\times10^{-4}+6.491\times10^{-2}C$	$A=1.07\times10^{-3}+2.940\times10^{-2}C$
S_0 [a)]	5.80×10^{-4}	7.80×10^{-4}	6.30×10^{-4}	1.04×10^{-3}	6.60×10^{-4}	7.00×10^{-4}
Intercept	1.01×10^{-3}	6.9×10^{-4}	7.9×10^{-4}	-1.72×10^{-3}	6.3×10^{-4}	1.07×10^{-3}
S_a	4.70×10^{-4}	6.20×10^{-4}	5.10×10^{-4}	8.31×10^{-3}	5.30×10^{-4}	5.00×10^{-4}
$\pm tS_a$	1.208×10^{-3}	1.594×10^{-3}	1.311×10^{-3}	1.697×10^{-3}	1.363×10^{-3}	1.286×10^{-3}
Slope	1.324×10^{-2}	3.178×10^{-2}	4.580×10^{-2}	5.831×10^{-2}	6.491×10^{-2}	2.940×10^{-2}
S_b	1.30×10^{-4}	1.70×10^{-4}	1.40×10^{-4}	2.30×10^{-4}	1.50×10^{-4}	4.00×10^{-5}
$\pm tS_b$	3.342×10^{-4}	4.371×10^{-4}	3.599×10^{-4}	5.913×10^{-4}	3.857×10^{-4}	1.03×10^{-4}
Correlation coefficient (r)	0.9997	0.9999	0.9999	0.9999	0.9999	0.9999
Variance (S_0^2)	3.364×10^{-7}	6.084×10^{-7}	3.969×10^{-7}	1.082×10^{-6}	4.356×10^{-7}	4.900×10^{-7}
Detection limit (μg ml^{-1})	0.15	0.08	0.05	0.06	0.03	0.08
Quantitation limit (μg ml^{-1})	0.44	0.25	0.14	0.18	0.10	0.24

a) Calculated *t*-value, which is less than the theoretical value of *t* (2.776) for *n*-2 degrees of freedom.

the slope of the line of regression and intercept were computed using the relation $b\pm tS_b$ and $a\pm tS_a$[35] at 95% confidence level and found to be $0.999\pm1.45\times10^{-2}$ and $3.634\pm2.60\times10^{-2}$, respectively. This indicated the high reproducibility of the proposed method. The limits of detection (LOD) and quantitation (LOQ) were evaluated using the following equation:

$$\text{LOD}=3.3\times S_0/b \quad \text{and} \quad \text{LOQ}=10\times S_0/b$$

where S_0 is the standard deviation of the calibration line and b is the slope and found to be 1.3×10^{-2} and 4.0×10^{-2} μg ml^{-1}, respectively. The variance was calculated using the equation:

$$S_0^2=\frac{\sum(\log \nu_{\text{expt.}}-\log \nu_{\text{reg.}})^2}{n-2}$$

and found to be 1.576×10^{-5} μg ml^{-1}. The low value of variance indicated negligible scattering of the experimental data points around the line of regression.

In the fixed-time method, the absorbance of green colored solution obtained on interaction of different concentration of metoprolol tartrate with alkaline potassium permanganate was measured at a preselected fixed time. Calibration plots of absorbance versus initial concentrations of metoprolol tartrate were established at a fixed time of 3, 6, 9, 12 and 15 min. The molar absorptivity, regression equations, coefficient of correlation, limits of detection and quantitation and variance are given in Table 2. It is clear from Table 2 that the most acceptable values of molar absorptivity, limit of detection and quantitation were obtained at a fixed time of 15 min. Therefore, the fixed time of 15 min was adopted as the optimum time for the determination of metoprolol tartrate in pharmaceutical formulations. The important analytical parameters of conventional UV spectrophotometric method have been summarized in Table 2. It can be seen that the molar absorptivity of the fixed time method is higher than that of conventional UV spectrophotometric method whereas the limits of detection and quantitation of the proposed method are smaller. The performance of the initial rate method is better since the values of LOD and LOQ are smaller than that of the fixed time method and analysis can be completed in a shorter time.

Solution Stability and Selectivity The solution stability of metoprolol tartrate was checked by observing UV spectra of metoprolol tartrate for 5 d. The aqueous solution of the drug having two λ_{max}: 194 and 224 nm, showing no change in the absorption spectra of standard and sample solutions of drug for at least 5 d, when the solutions were stored at room temperature. To identify the metoprolol and α-hydroxy metoprolol, thin layer chromatography was performed. The standard solution, sample solution and reaction product were applied on TLC plates coated with silica gel and developed in ethyl acetate–methanol–ammonia (40 : 5 : 5 v/v/v) solvent system. The plates were air-dried and spots were detected in the iodine chamber. In the case of standard and sample solutions, a single spot was observed with $Rf=0.50$ corresponding to metoprolol, whereas reaction product also showed one spot with $Rf=0.65$. This corresponds to α-hydroxy metoprolol.[34] Thus, the proposed methods are selective as the major metabolite, α-hydroxy metoprolol does not interfere in the determination. However, other β-adrenergic antagonists such as propranolol, atenolol and labetalol react with potassium permanganate in alkaline medium resulting in the formation of green colored solution, which absorbs maximally at 610 nm.

Accuracy and Precision The accuracy and precision of the proposed methods was established by measuring the content of metoprolol tartrate in pure form at three different concentration levels (low, medium and high). The short-term (intra day assay) and the daily precisions (inter day assay) were performed by measuring five independent analyses at 1.5, 3.0 and 6.0 μg ml^{-1} concentration levels within 1 d and on 5 consecutive days, respectively (Table 3). The standard deviation, relative standard deviation and mean percent recoveries obtained by both the initial rate and fixed time methods can be considered to be very satisfactory.

The validity of the proposed methods was also checked by performing recovery experiments through standard addition method. For this, a known amount of the pure drug was added to preanalysed dosage forms and then the total amount

Table 3. Test of Precision of the Proposed Methods by Intra Day and Inter Day Assays

Proposed methods	Amount (μg ml^{-1})		Recovery (%)	SAE[b]	C.L.[c]
	Taken	Found±S.D.[a]			
Initial rate method					
Intra day assay	1.5	1.50±0.04	100.03	0.02	0.05
	3.0	3.00±0.06	100.06	0.03	0.07
	6.0	6.01±0.06	100.13	0.03	0.07
Inter day assay	1.5	1.50±0.04	100.14	0.02	0.05
	3.0	2.99±0.06	99.95	0.03	0.07
	6.0	6.00±0.06	100.07	0.03	0.07
Fixed time method					
Intra day assay	1.5	1.50±0.04	100.21	0.02	0.06
	3.0	2.99±0.05	99.93	0.02	0.06
	6.0	6.01±0.05	100.15	0.02	0.06
Inter day assay	1.5	1.50±0.06	100.01	0.03	0.07
	3.0	2.99±0.06	99.93	0.03	0.07
	6.0	6.00±0.07	100.04	0.03	0.08

a) Mean for five independent analyses. *b*) SAE, standard analytical error. *c*) C.L., confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

of metoprolol tartrate was determined following the recommended procedures and reference method. The results are summarized in Table 4, which showed recoveries in the range of 100.02—100.13%, 99.99—100.15% and 99.94—100.11% for initial rate, fixed time and reference methods, respectively. No interference from the common excipients was observed.

Robustness The conditions are very robust for the application of the proposed methods to determine the active drug in pharmaceutical formulations. Each operational parameter was checked and challenged for the robustness of the methods. The operational parameters investigated were:

- volume of 0.015 M $KMnO_4$ (±0.2 ml)
- volume of 0.60 M NaOH (±0.2 ml)

Under these conditions a sample solution containing 6.0 μg ml^{-1} (Metalor 25, Cipla) of active metoprolol tartrate was assayed five times by the initial rate and fixed time methods. The values of mean recovery, standard deviation and relative standard deviation represent good reliability of the proposed methods.

The effect of temperature on reaction rate is well known and important in understanding the various activation parameters of the reaction products. In order to evaluate the apparent activation parameters, the reaction rate was studied at 298, 303, and 308 K at [metoprolol]=1.46×10^{-6}—8.76×10^{-6} M, [$KMnO_4$]=3.00×10^{-3} M and [NaOH]=1.20×10^{-1} M.

Arrhenius curve (Fig. 5) was constructed by plotting $\log k$ *versus* $1/T$ and found to be linear with coefficient of correlation, $r=-0.9998$. Activation energy (E_a) can be calculated from the slope ($-E_a/2.303R$) and A from the intercept of the Arrhenius curve and found to be 90.73 kJ mol^{-1} and 4.75×10^{17}, respectively. The other activation parameters such as enthalpy, entropy and free energy of activation of the reaction product were calculated using Eyring equation:

$$\log\frac{k}{T}=\left[\log(k_b/h)+\frac{\Delta S^{\ddagger}}{2.303R}\right]-\frac{\Delta H^{\ddagger}}{2.303R}\frac{1}{T}$$

The plot of $\log k/T$ *versus* $1/T$ (Fig. 6) was linear with correlation coefficient of −0.9999. $\Delta H^{\ddagger}$ was evaluated from the slope ($-\Delta H^{\ddagger}/2.303R$) and $\Delta S^{\ddagger}$ from the intercept [$\log(k_b/h)+\Delta S^{\ddagger}/2.303R$] of the compiled Eyring plot. The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were found to be 88.20 kJ mol^{-1} and 84.54 J K^{-1} mol^{-1}, respectively. The Gibbs free energy of activation was determined by $\Delta G^{\ddagger}=\Delta H^{\ddagger}-T\Delta S^{\ddagger}$ at 298 K and found to be 63.01 kJ mol^{-1}.

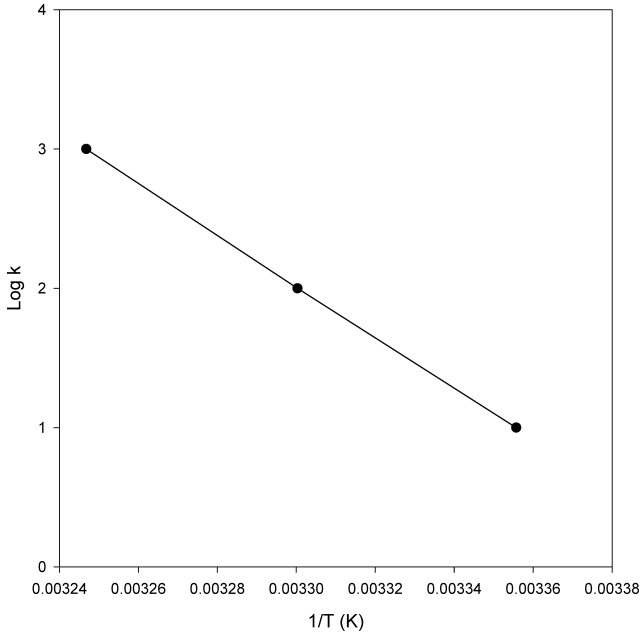

Fig. 5. Arrhenius Plot: $\log k$ *versus* $1/T$ for Activation Energy

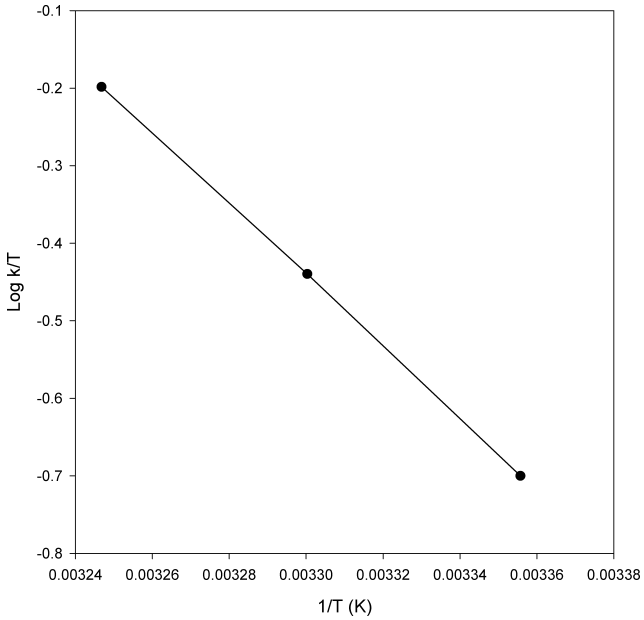

Fig. 6. Eyring Plot: $\log k/T$ *versus* $1/T$ for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$

Applicability of the Proposed Methods The proposed methods (initial rate and fixed time methods) were successfully applied to the determination of metoprolol tartarte in pharmaceutical formulations. The results of the proposed methods were statistically compared with those of the reference method using point hypothesis test. Table 5 shows that the calculated t- (paired) and F-values at 95% confidence

Table 4. Summary of Data for the Determination of Metoprolol Tartrate in Pharmaceutical Preparations by Standard Addition Method

Preparations[a]	Amount (μg ml^{-1})		Initial rate method				Fixed time method				Reference method			
	Taken	Added	Found±S.D.[a] (μg ml^{-1})	Recovery (%)	SAE	C.L.	Found±S.D.[a] (μg ml^{-1})	Recovery (%)	SAE	C.L.	Found±S.D.[a] (μg ml^{-1})	Recovery (%)	SAE	C.L.
Betaloc-25	3.0	1.0	4.00±0.06	100.05	0.03	0.07	3.99±0.06	99.99	0.03	0.07	4.00±0.05	100.11	0.02	0.06
(AstraZeneca)	3.0	3.0	6.00±0.08	100.02	0.04	0.10	6.01±0.05	100.09	0.02	0.06	5.99±0.05	99.94	0.02	0.06
Metapro-25	3.0	1.0	4.01±0.05	100.13	0.02	0.06	4.01±0.06	100.14	0.03	0.07	3.99±0.09	99.94	0.04	0.11
(Cardicare)	3.0	3.0	6.00±0.07	100.07	0.03	0.09	6.01±0.05	100.15	0.02	0.06	6.01±0.06	100.17	0.03	0.07
Metolar-25	3.0	1.0	4.00±0.05	100.06	0.02	0.06	3.99±0.05	99.99	0.02	0.06	4.00±0.05	100.11	0.02	0.06
(Cipla)	3.0	3.0	6.00±0.06	100.02	0.03	0.07	6.00±0.05	100.04	0.02	0.06	6.00±0.07	100.05	0.03	0.09

a) Mean for five independent analyses.

Table 5. Comparison of the Proposed Methods Using Point and Interval Hypothesis Tests with the Reference Method at 95% Confidence Level

Formulations[a]	Initial rate method						Fixed time method						Reference method	
	Recovery (%)	RSD[a] (%)	t-value[b]	F-value[b]	θ_L[c]	θ_U[c]	Recovery (%)	RSD[a] (%)	t-value[b]	F-value[b]	θ_L[c]	θ_U[c]	Recovery (%)	R.S.D.[a] (%)
Betaloc-25 (AstraZeneca)	100.02	0.99	0.23	1.55	0.994	1.008	100.15	1.14	0.55	2.06	0.992	1.012	99.94	0.80
Metapro (Cardicare)	100.07	1.16	0.23	1.53	0.988	1.010	100.04	0.82	0.37	1.32	0.990	1.008	100.17	0.94
Metolar (Cipla)	100.13	0.93	0.18	1.55	0.989	1.012	99.99	0.83	0.13	1.92	0.986	1.013	100.05	1.15

a) Mean for five independent analyses. *b*) Theoretical t-value (ν=8) and F-value (ν=4, 4) at 95% confidence level are 2.306 and 6.39, respectively. *c*) In pharmaceutical analysis, a bias, based on recovery experiments, of ±2% (θ_L=0.98 and θ_U=1.02) is acceptable.

level are less than the theoretical ones, confirming no significant differences between the performance of the proposed methods and the reference method. The previous investigations were also checked and confirmed by interval hypothesis tests.[36] The Canadian Health Protection Branch has recommended that a bias, based on recovery experiments, of ±2% is acceptable.[37] It is evident from Table 6 that the true bias of all samples of drug is smaller than ±2%.

Conclusion

Initial rate and fixed time methods are applied for the routine quality control analysis of metoprolol tartrate in pharmaceutical formulations. The proposed method does not require any laborious clean up procedure prior to analysis and therefore, can be frequently used in the laboratories of research, hospitals and pharmaceutical industries. It has extremely high sensitivity, selectivity and low limit of detection.

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