Article

J. Mex. Chem. Soc. **2011**, *55(2),* 105-112

ISSN 1870-249X

Determination of Labetalol Hydrochloride by Kinetic Spectrophotometry Using Potassium Permanganate as Oxidant

Nafisur Rahman,[1]* Nishat Anwar[2] Mohammad Kashif[3] Md. Nasrul Hoda[4] and Habibur Rahman[1]

[1] Analytical Research Division, Department of Chemistry, Aligarh Muslim University, Aligarh (U.P.) 202 002 India. cht17nr_amu@yahoo.com.
[2] Anand Engineering College, Keetham Agra U.P. India.
[3] National Dope Testing Laboratory, Ministry of Youth Affairs and Sports, Government of India, CGO Complex, New Delhi-110 003, India.
[4] Department of Neurology, Medical College of Georgia, 1120 15th Street, Augusta GA 30912 USA.

Received November 13, 2010; Accepted March 10, 2011

Abstract. A simple and sensitive kinetic spectrophotometric method was developed for the determination of labetalol (LBT) hydrochloride. The method was based on the kinetic investigation of the oxidation of the drug with alkaline potassium permanganate at room temperature (25 ± 1°C). The increase in absorbance of coloured manganate ions was measured at 605 nm. All experimental variables affecting the development of the colour were investigated and optimized. The initial rate and fixed time (at 6 minute) methods were adopted for determining the drug concentration. The calibration graphs were linear in the concentration ranges of 2-14 μg mL^{-1} and 1-10 μg mL^{-1}, using the initial rate and fixed time methods, respectively. The method was successfully applied to the determination of labetalol in laboratory made tablets and commercial tablets. The results were validated statistically and through recovery studies.
Keywords: Labetalol hydrochloride, kinetic spectrophotometry, initial rate and fixed time method.

Resumen. Un método simple y sensible de espectrofotometría cinética se desarrolló para la determinación del clorhidrato de labetalol (LBT). El método se basó en la investigación de la cinética de oxidación de la droga con permanganato de potasio alcalino a temperatura ambiente (25 ± 1°C). El aumento de la absorción de iones de manganato coloridos se midió a 605 nm. Se investigaron y optimizaron todas las variables experimentales que afectan al desarrollo del color. Asimismo, se adoptaron la velocidad inicial y el tiempo fijado (a los 6 minutos) de los métodos para la determinación de la concentración del fármaco. Las curvas de calibración fueron lineales en los intervalos de concentración de 2-14 μg mL^{-1} y 1-10 μg mL^{-1}, haciendo uso de la velocidad inicial y de los tiempos fijados de los métodos, respectivamente. El método se aplicó con éxito a la determinación en el laboratorio de labetalol realizados en tabletas y tabletas comerciales. Los resultados se validaron estadísticamente y mediante estudios de recuperación.
Palabras clave: Clorhidrato de Labetalol, espectrofotometría cinética, velocidad inicial y tiempo fijado del método.

Introduction

Labetalol (LBT) hydrochloride: 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino) ethyl] salicylamide hydrochloride, is the first adrenergic antagonist capable of blocking both α and β receptors. It is a moderately potent hypotensive and is especially useful in pheochromocytoma. The drug is used to lower blood pressure in myocardial infarction and unstable angina. Beside these important pharmacological activities, labetalol therapy exhibits hepatotoxicity and renal failure due to over dosage.

Labetalol is listed in British Pharmacopoeia [1] and United States Pharmacopoeia [2], which recommend non-aqueous titration and HPLC methods for the assay of its content in pharmaceutical formulations. Several analytical methods such as TLC [3, 4], HPLC [5-8], LC-MS [9-13], capillary electrophoresis [14-16], polarography [17], Voltammetry [18], NMR spectroscopy [19] and spectrofluorimetry [20-23] have been reported for the determination of the drug in its pure and commercial dosage forms. In addition, some spectrophotometric methods have also been developed for the quantitation of the labetalol. Labetalol has been determined in bulk and tablets spectrophotometrically [24] based on the reaction of the drug with folin-ciocalteau's reagent, 4-aminophenazone, 2,6-dichloroquinone chlorimide and ferric ammonium sulphate with Beer's law limit of 8-40, 2.5-15, 5-25, and 50-250 μg mL^{-1} respectively. Spectrophotometric methods [25] for the assay of labetalol have been described; two methods are based on the coupling reaction of labetalol with p-N, N-dimethylphenylenediamine and 3-methyl-2-benzothiazoline hydrazone hydrochloride in the presence of sodium hypochloride and ceric ammonium sulphate as oxidant, respectively and the third method depends on the formation of ion- association complex of labetalol hydrochloride with Suprachen Violet 3B at pH 1.3. Another extractive spectrophotometric method [26] for assay of labetalol for pharmaceutical formulation has been described based on chloroform extractable ion-association complex of drug with phenazine dye and wool fast blue BL. Belal and coworkers [27] have developed two spectrophotometric methods based on the phenolic group of the drug. In the first method, labetalol was coupled with diazotized benzocaine in the presence of trimethylamine whereas second method depends on the coupling of the drug with diazotized *p*-nitroaniline in presence of sodium carbonate. Another method [28] based on coupling reaction of positive diazonium ion of 4-aminobenzenesulphonic acid with phenolate ion of labetalol form coloured azo compound. However, some of these methods are tedious and/or time consuming. Therefore, a kinetically based spectrophotometric method is

presented with the advantage of simplicity, reliability and less time of analysis.

Results and Discussion

It has been reported [29] that labetalol undergoes oxidation with periodate in alkaline medium resulting in the formation of 2-hydroxy-5-formylbenzamide, 1-methyl-3-phenylpropylamine and formaldehyde. In the present study, labetalol is oxidized by potassium permanganate in alkaline medium producing the same products: 2-hydroxy-5-formylbenzamide, 1-methyl-3-phenylpropylamine and formaldehyde. The potassium permanganate itself reduced to MnO_4^{2-}. The permanganate ion is monitored spectrophotometrically at 605 nm. On the basis of our experimental findings and literature background a tentative reaction sequence is shown in scheme 1.

Optimization of Variables

The various experimental variables affecting the development of the reaction products were optimized by changing each variable in turn, while keeping all others constant. In the study of optimization of reaction variables, the absorbance (λ_{max} 605 nm) was measured at a fixed time of 30 min. At room temperature (25 ± 1°C), the rate of the reaction increased substantially and to avoid decomposition and for the sake of good results, room temperature (25 ± 1°C) is selected for the determination process.

Effect of $KMnO_4$ Concentration

The effect of the concentration of potassium permanganate on the intensity of the colour developed was studied in the range 3.16×10^{-4} M-1.90×10^{-3} M. As shown in figure 1, the absorbance value increased with the increase in the concentration of $KMnO_4$ due to formation of MnO_4^{2-} and became constant at 1.42×10^{-3} M. Therefore, a concentration of 1.58×10^{-3} M $KMnO_4$ in the final solution proved to be optimum for the maximum concentration of labetalol hydrochloride in the determination process.

Effect of NaOH Concentration

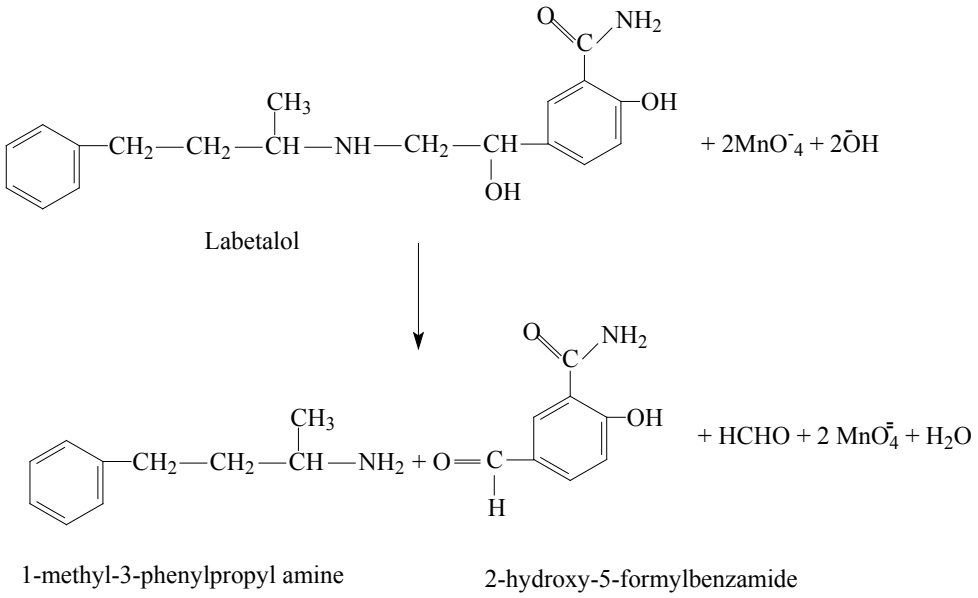
The influence of concentration of NaOH was examined in the range 0.025 M-0.2 M. It is apparent from figure 2 that increasing the concentration of NaOH would increase the absorbance of the reaction product up to 0.1 M; after which further increase in concentration resulted in no change in the absorbance. Thus, 0.1 M NaOH was chosen as an optimum value for maximum absorbance.

Analytical Data

The Initial Rate Method

The initial rates of the reaction were determined by measuring the slopes of the initial tangents to the absorbance-time curves (Figure 3). Under the optimized experimental conditions, the concentrations of $KMnO_4$ and NaOH were at least 37 times and 2900 times higher than the initial concentration of labetalol. Under this condition, the pseudo-zero order reaction conditions with respect to the reagents concentration were obtained and the rate of the reaction increases with increase in labetalol concentration. Under pseudo order reaction, the rate equation can be written as

$$\text{Rate} = K_{\psi}\,[\text{labetalol}]^n$$



Scheme 1.

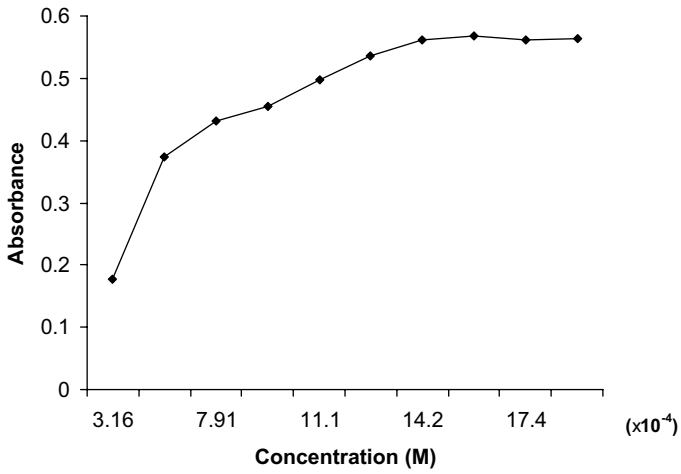


Fig. 1. Effect of Concentration of $KMnO_4$ on the Absorbance at 605 nm (LBT: 6.09×10^{-4}M).

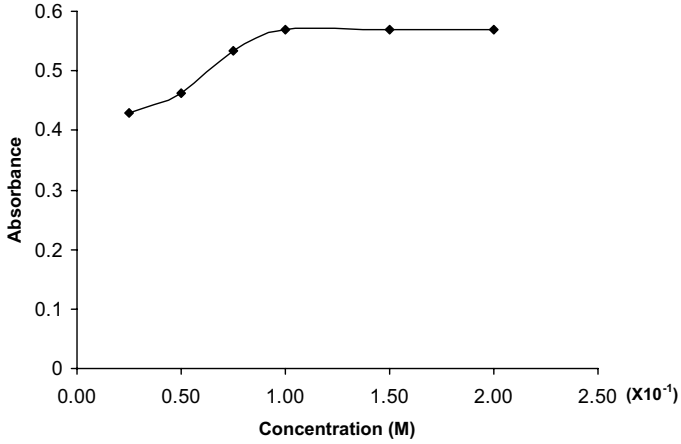


Fig. 2. Effect of Concentration of NaOH on the Absorbance at 605 nm (LBT: 6.09×10^{-4}M).

where K_ψ is the pseudo order rate constant and 'n' is the order of the reaction. The reaction can be written in logarithmic form as

$$\log (\text{rate}) = \log K_\psi + n \log [\text{labetalol}]$$

The regression analysis yielded the following regression equation

$$\log (\text{rate}) = 2.147 + 0.902 \log [\text{labetalol}]$$

with a correlation coefficient of 0.9969. The value of 'n' suggested that the rate of reaction with respect to the labetalol concentration is one. Under the pseudo-first order reaction condition, the plot of log rate vs log [labetalol] was linear over the concentration range of 2-14 μg mL^{-1}. The confidence limits for slope and intercept were calculated at 95% confidence level and found to be 4.388×10^{-2} and 4.168×10^{-2}, respectively. The

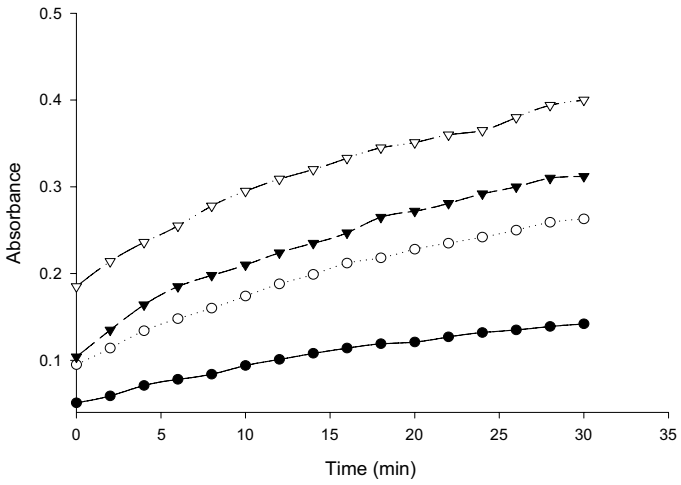


Fig. 3. Absorbance *vs*. Time Graphs for the Reaction of Labetalol and $KMnO_4$ Showing the Dependence of the Reaction on Labetalol Concentration. Labetalol; (•) 1.218×10^{-5}M, (o) 2.436×10^{-5}M, (▼) 3.045×10^{-5}M, (▽) 3.654×10^{-5}M.

limit of detection, limit of quatitation [30] and variance were evaluated and found to be 4.27×10^{-2} μg mL^{-1}, 1.41×10^{-1} μg mL^{-1} and 5.293×10^{-4}, respectively. The good linearity of the calibration graph and negligible scatter of experimental data points around the line of the regression are clearly evident by the value of correlation coefficient and variance about the regression.

Fixed Time Method

In this method, the absorbance of a green coloured solution containing varying amount of labetalol was measured at a pre-selected fixed time. Calibration plots of absorbance *vs.* initial concentration of the labetalol were established at a fixed time of 2, 4, 6, 8 and 10 min. The regression equations, coefficients of correlation, limits of detection and quantitation and variances were evaluated and are given in Table 1. It was observed that the most acceptable values of correlation coefficient, intercept, detection limit, quantitation limit and variance were obtained at a fixed time of 6 minute and therefore a fixed-time of 6 minute was adopted for measurement. The calibration graph was linear over the concentration range of 1-10 μg mL^{-1}.

The instrumental signal derived from any test material is subject to random errors. Therefore, the following formula [31] can be used to determine the error in the corresponding concentration:

$$S_c = \frac{S_0}{b}\left[1 + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \Sigma (C_0 - \bar{C})^2}\right]^{1/2}$$

where y_0 is the experimental value of y from which the concentration value C_0 is to be determined, S_c is the estimated standard deviation of C_0, S_0 is the standard deviation about regression; b, slope of the line of regression and n, number of

Table 1. Optical and regression characteristics of fixed time method.

Parameters	2 minutes	4 minutes	6 minutes	8 minutes	10 minutes
Linear dynamic range (μg mL^{-1})	1-10	1-10	1-10	1-10	1-10
Slope (b)	1.439×10^{-2}	1.619×10^{-2}	1.979×10^{-2}	1.931×10^{-2}	2.069×10^{-2}
Intercept (a)	-1.677×10^{-3}	5.342×10^{-3}	1.742×10^{-3}	7.205×10^{-3}	9.890×10^{-3}
$\pm tS_a$[a]	1.05×10^{-2}	1.12×10^{-2}	0.61×10^{-2}	1.01×10^{-2}	0.83×10^{-2}
$\pm tS_b$[b]	1.34×10^{-3}	1.43×10^{-3}	0.78×10^{-3}	1.30×10^{-3}	1.06×10^{-3}
Correlation Coefficient (r)	0.9957	0.9961	0.9992	0.9977	0.9987
Detection limit (μg mL^{-1})	0.70	0.65	0.29	0.50	0.38
Quantitation limit (μg mL^{-1})	—	—	0.96	—	—
Variance (S_o^2) about regression	2.70×10^{-5}	3.08×10^{-5}	0.92×10^{-5}	2.52×10^{-5}	1.70×10^{-5}

[a] Confidence interval of the intercept at 95% confidence level.
[b] Confidence interval of the slope at 95% confidence level.

samples. The confidence limits can be calculated as $C_0 \pm t_p S_c$ with (n-2) degrees of freedom. Figures 4 and 5 showed graphs of S_c *vs.* concentration of labetalol for both the initial rate and fixed-time methods. It is apparent from the figures that the error is minimum at 6.8 μg mL^{-1} & 5 μg mL^{-1} of labetalol for initial rate and fixed time methods, respectively.

The uncertainty (%) on the concentration is taken as $t_p S_c/C_0$ (%) [32], which is plotted against the concentration, C_0 at 95% and 99% confidence levels (Figures 6 and 7). Figures 6 and 7 gave useful information about the level of precision for determination of labetalol over the concentrations examined.

The accuracy and precision was determined by measuring the labetalol at three different concentration levels, each six times, within one day by both initial rate and fixed time methods. Intraday data for the precision and accuracy are given in (Table 2) which indicate RSD = 0.68-1.52% and SAE = 0.02-0.04 for initial rate method whereas RSD = 0.68-0.82% and SAE = 0.01-0.20 for fixed time method. The interday accuracy and precision was evaluated at three different concentration levels for six consecutive days. The interday RSD and SAE values for initial rate and fixed time methods were ranged from 0.58-1.11%; 0.68-0.82%; and 0.02-0.03; 0.02-0.05, respectively. The intraday and interday data indicated the high accuracy and precision of the proposed methods.

In order to test the reliability of the present method, recovery studies were performed. For this, a known amount of pure drug was added to the laboratory made tablets at three different concentration levels and total amount was determined by the proposed method. Each level was repeated six times. The results (Table 3) obtained for both the methods showed that the mean recoveries and relative standard deviations were in the range of 99.49-100.87% and 0.52-1.35%, respectively.

The initial rate and fixed time methods have been applied to determine the labetalol in laboratory made tablets and commercial tablets. The concentration of the drug was computed

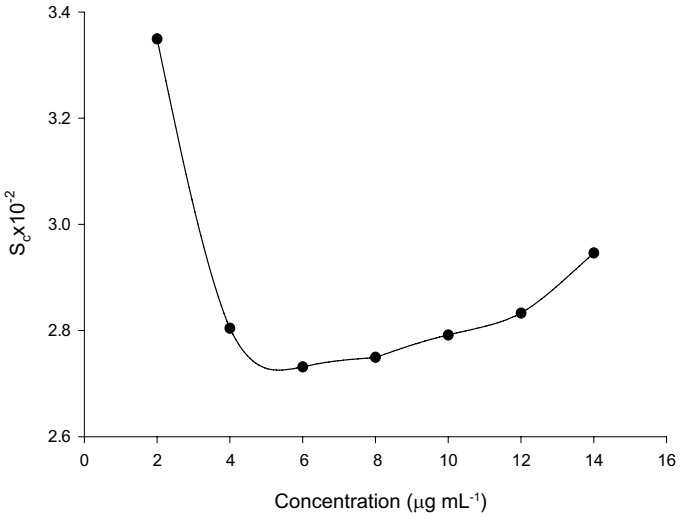


Fig. 4. Error in the Determination of the Concentration of Labetalol Using Initial Rate Method.

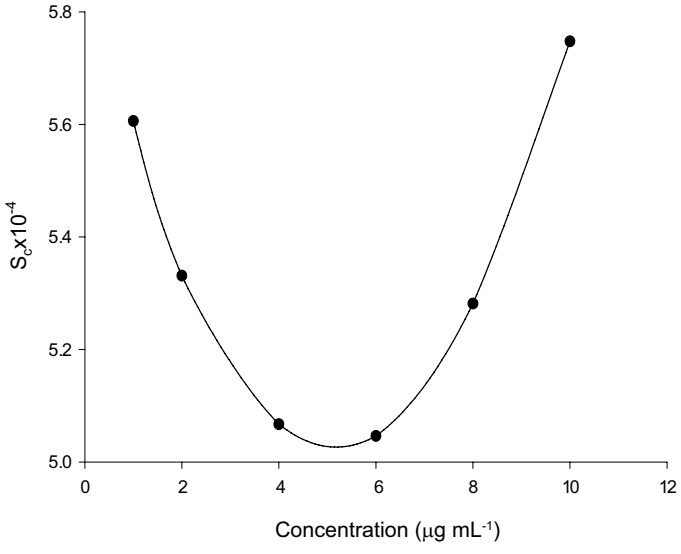


Fig. 5. Error in the Determination of the Concentration of Labetalol Using Fixed-Time Method.

Table 2. Accuracy and precision of within and between run analyses for the determination of labetalol hydrochloride by initial rate and fixed-time method.

Nominal Concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})							
	Initial rate method				Fixed-time method			
	Found ± SD	RSD	SAE[a]	CL[b]	Found ± SD	RSD	SAE[a]	CL[b]
Intraday (n=6)								
6.0	5.96 ± 0.09	1.52	0.04	0.08	6.02 ± 0.03	0.82	0.20	0.01
8.0	8.00 ± 0.05	0.68	0.02	0.11	7.99 ± 0.05	0.71	0.02	0.04
10.0	9.98 ± 0.07	0.76	0.03	0.06	9.91 ± 0.07	0.68	0.03	0.14
Interday (n=6)								
6.0	5.96 ± 0.06	1.11	0.03	0,06	6.01 ± 0.05	0.82	0.05	0.010
8.0	8.00 ± 0.05	0.58	0.02	0.09	7.99 ± 0.06	0.79	0.02	0.04
10.0	9.98 ± 0.07	0.76	0.03	0.06	9.95 ± 0.07	0.68	0.03	0.14

[a]Standard analytical error
[b]Confidence limit at 95% confidence level

from corresponding regression equations. The results of the proposed method (initial rate or fixed-time) were compared with those of the reference method [27] using point and interval hypotheses [33]. In interval hypothesis, the lower and upper acceptance limits can be calculated using the following equation:

$$\theta^2\left(\overline{X}_1^2 - S_P^2\, t_{tab}^2 / n_1\right) - 2\theta\,\overline{X}_1\,\overline{X}_2 + \theta^2\left(\overline{X}_2^2 - S_P^2\, t_{tab}^2 / n_2\right) = 0$$

The values of θ_L and θ_U of the confidence interval were obtained as:

$$\theta_L = -b - (b^2 - 4ac)^{1/2} / 2a$$

$$\theta_u = -b + (b^2 - 4ac)^{1/2} / 2a$$

Where

$$a = \overline{X}_1^2 - S_P^2 t_{tab}^2 / n_1$$

$$b = -2\,\overline{X}_1\,\overline{X}_2$$

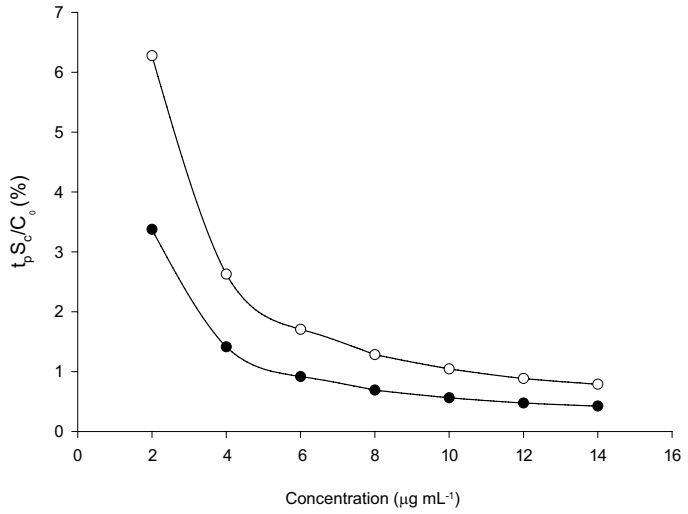


Fig. 6. Variation of the Confidence Limit at 95% (•) and 99% (o) Confidence Levels in the form of Uncertainty (%) in Concentration of Labetalol Using Initial Rate Method.

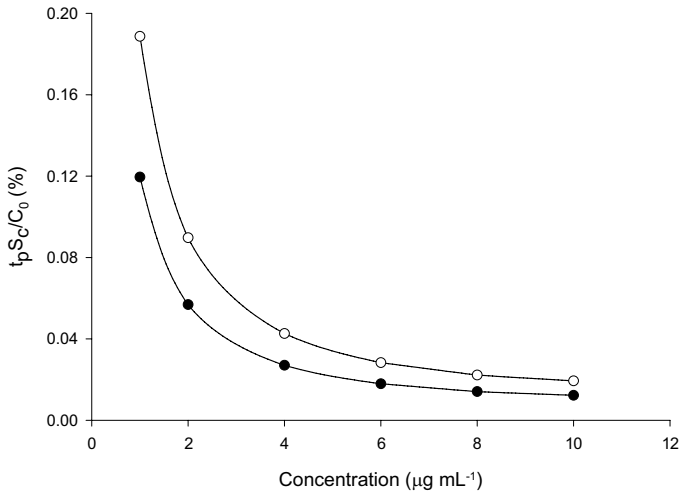


Fig. 7. Variation of the Confidence Limit at 95% (•) and 99% (o) Confidence Levels in the Form of Uncertainty (%) in Concentration of Labetalol Using Fixed-Time Method.

Table 3. Standard addition method for the determination of labetalol hydrochloride in dosage forms by initial rate and fixed-time method.

Formulation	Initial rate method						Fixed-time method					
	Amount (μg mL^{-1})			Recovery ± RSD[a] (%)	SAE[b]	CL[c]	Amount (μg mL^{-1})			Recovery ± RSD[a] (%)	SAE[b]	CL[c]
	Taken	Added	Found ± SD				Taken	Added	Found ± SD			
Tablet A	2.0	2.0	3.98 ± 0.05	99.53 ± 1.20	0.20	0.10	1.0	1.0	1.99 ± 0.03	99.87 ± 1.35	0.01	0.05
	3.0	5.0	8.01 ± 0.04	100.05 ± 0.56	0.02	0.09	1.0	3.0	4.01 ± 0.04	100.36 ± 0.99	0.02	0.08
	10.0	4.0	13.99 ± 0.08	99.95 ± 0.55	0.03	0.16	5.0	3.0	8.06 ± 0.05	100.87 ± 0.61	0.02	0.10
Tablet B	2.0	2.0	4.02 ± 0.04	100.42 ± 1.11	0.02	0.09	1.0	1.0	2.01 ± 0.03	100.01 ± 1.30	0.01	0.53
	3.0	5.0	7.98 ± 0.06	99.71 ± 0.65	0.02	0.10	1.0	3.0	4.01 ± 0.03	100.11± 0.80	0.01	0.06
	10.0	4.0	13.98 ± 0.09	99.89 ± 0.66	0.04	0.19	5.0	3.0	7.99 ± 0.05	99.95 ± 0.57	0.02	0.09
Tablet C	2.0	2.0	4.02 ± 0.03	100.46 ± 0.86	0.01	0.07	1.0	1.0	1.99 ± 0.02	99.59 ± 1.04	0.01	0.04
	3.0	5.0	7.98 ± 0.04	99.78 ± 0.52	0.02	0.08	1.0	3.0	3.99 ± 0.04	99.90 ± 0.95	0.04	0.08
	10.0	4.0	13.93 ± 0.09	99.49 ± 0.64	0.04	0.18	5.0	3.0	7.99 ± 0.06	99.95 ± 0.69	0.02	0.11

[a]Mean ± SD for six independent determinations
[b]Standard analytical error
[c]Confidence limit at 95% confidence level and 10 degrees of freedom (t =1.812)

$$c = \overline{X}_2^2 - S_P^2 t_{tab}^2 / n_2$$

The results are summarized in Table 4, which revealed that the calculated t and F values are less than the theoretical ones at 95% confidence level confirming no significant difference in the performance of the two methods with regard to accuracy and precision. θ_L and θ_u values are less than ± 2.0% (Table 4). It is concluded based on θ_L and θ_u values that the proposed method is not biased because the true bias is smaller than ±2.0%; indicating the compliance of regulatory guidelines [34].

Experimental

Apparatus

The absorbance measurement was made on a Spectronic 20 D^+ spectrophotometer (Milton Roy, USA) with 1 cm glass cells.

Material and Reagents

All the chemicals used were of analytical reagent grade. Doubly distilled water was used throughout the experimental work.

Labetalol hydrochloride (Sigma, USA, 5.48×10^{-4}M) was prepared by dissolving 20 mg in 100 mL doubly distilled water. Potassium permanganate solution (GR Grade, Merck Limited Mumbai, India, 6.32×10^{-3}M) was prepared freshly in doubly distilled water and its purity was checked titrimetrically. Sodium hydroxide solution (GR Grade, Merck Limited Mumbai, India, 1M) was prepared in doubly distilled water and kept as stock solution.

Procedure for Determination

Aliquots of 2.5 mL of 6.32×10^{-3}M $KMnO_4$ solution and 1.25 mL of 1 M NaOH solution (were transferred into a 10 mL volumetric flask. An accurate volume of the working solution of labetalol (0.10-0.7 mL) was added and diluted to volume with doubly distilled water. The mixture was shaken well and immediately transferred in spectrophotometric cells. The absorbance was measured at 605 nm as a function of time against the reagent blank. The initial rates of the reaction at different concentrations were obtained from the slopes of the initial tangents to the absorbance time curves. The calibration curve was constructed by

II. plotting the logarithm of initial rate of reaction *vs.* the logarithm of molar concentration of labetalol and
II. the absorbance at a fixed time of 6 minutes *vs.* the initial concentration of labetalol. The amount of the drug was computed either from the calibration curves or the corresponding regression equations.

Procedure for Pharmaceutical Formulations

Laboratory Made Tablets

Tablets A, B and C have been prepared in the laboratory. The tablets of labetalol hydrochloride were prepared by dry granulation technique. Accurately weighed quantities of presieved drug and intragranular materials (colloidal silicon dioxide, lactose anhydrous, microcrystalline cellulose, sodium starch glycolate, corn starch, stearic acid) were mixed and slugs were prepared, and then passed with multi mill screen (1.5”). The granules were sieved through #20 sieves. The final granules

Table 4. Application of the proposed method to the determination of labetalol hydrochloride in laboratory made tablets and commercial tablets.

Formulations		Proposed method		Reference method [27]
		Initial rate	Fixed-time	
Laboratory made tablets				
Tablet A	Recovery (%)	99.91	100.72	100.20
	RSD (%)	0.84	0.94	0.62
	t-value	0.69	1.12	
	F-value	1.81	2.29	
	θ_L	0.989	0.996	
	θ_U	1.004	1.013	
Tablet B	Recovery (%)	100.23	100.49	100.17
	RSD (%)	0.70	0.97	0.90
	t-value	0.14	0.42	
	F-value	1.67	3.66	
	θ_L	0.992	0.993	
	θ_U	1.009	1.011	
Tablet C	Recovery (%)	100.13	100.12	100.13
	RSD (%)	0.61	0.93	0.61
	t-value	0.83	0.21	
	F-value	2.04	1.07	
	θ_L	0.988	0.989	
	θ_U	1.004	1.008	
Commercial tablets				
Lobet	Recovery (%)	100.05	100.12	100.17
	RSD (%)	0.58	0.93	0.90
	t-value	0.30	0.10	
	F-value	2.47	1.03	
	θ_L	0.997	0.989	
	θ_U	1.005	1.009	
Normadate	Recovery (%)	100.23	100.12	100.20
	RSD (%)	0.69	0.93	0.62
	t-value	0.10	0.18	
	F-value	1.20	1.49	
	θ_L	0.992	0.990	
	θ_U	1.006	1.010	

t-value ($\nu = 10$) and *F*-value ($\nu = 5, 5$) at 95% confidence level are 1.812 and 5.05, respectively.

were blended with extragranular materials (lactose anhydrous, sodium starch glycolate and magnesium stearate) and compressed using 12.7 mm round flat standard concave punches on 12-station rotatory tablet press (Lab press, CIP Machineries Pvt. Ltd. Ahmadabad, India). Composition of the prepared tablet is shown in Table 5.

The laboratory prepared dosage forms were further finely grounded and dissolved in distilled water. It was shaken well and filtered through tightly packed glass wool bed into 100 mL volumetric flask. The whole content was made up to mark with washing and analyzed following the recommended procedures.

Commercial Tablets

The finely grounded contents of five tablets of 100 mg strength (Lobet, Samarth Pharma Pvt. Ltd. Mumbai, India and Normadate, Glaxo Smithkline Pharmaceutical Ltd., Mumbai, India) were taken in 100 mL distilled water and left for 10 min for complete dispersion and then filtered through Whatman No.

Table 5. Composition of laboratory made tablets.

Ingredients	Tablets		
	A	B	C
Labetalol hydrochloride (mg)	100	100	100
Colloidal silicon dioxide (mg)	10	15	20
Corn starch (mg)	15	15	15
Lactose anhydrous (mg)	180	170	160
Microcrystalline cellulose (mg)	60	60	60
Sodium starch glycolate (mg)	06	12	16
Stearic acid (mg)	03	03	03
Magnesium stearate (mg)	06	05	06
Tablet weight (mg)	380	380	380

42 filter paper (Whatman International Limited, Kent, UK) in a 500 mL standard volumetric flask. The residue was washed well with distilled water for complete recovery of the drug and then diluted up to the mark with distilled water. It was further diluted according to the need and subjected to determination procedure for labetalol hydrochloride.

Acknowledgements

The authors are grateful to Department of Chemistry, Aligarh Muslim University, Aligarh, for providing necessary research facilities.

References

1. The British Pharmacopoeia, HMSO, London, **2004,** Vol. I.
2. The United Sates Pharmacopoeia XXVII. The USP convection Rockville,MD, USA, **2004,** 1060-1062
3. Witek, A.; Hopkala, H.; Matysik, G. *Chromatographia* **1999,** *50*, 41-44
4. Witek, A.; Hopkala, H.; Prazyborowski, L. *Chem. Anal.* **1998,** *43*, 817-822
5. Dakers, J. M.; Boulton, D. W.; Fawcett, J. P. *J. Chromatogr. Sect. B.* **1997,** *704*, 215-220
6. Cenicerors C., Maguregui M. I., Jimenez R. M., Alonso R. M. *J. Chromatogr. Sec. B* **1998,** *705*, 97-103
7. Zhao, H.; Li, H.; Qui, Z. *Sepu* **1999,** *17*, 369-371
8. Magali, D.; Charlotte, D.; Francois, P.; Philippe, D.; Jean-Claude, A. *Forensic Sci. Int.* **2004,** *141*, 23-31
9. Umezawa, H.; Lee, X. P.; Arima, Y.; Hasegawa, C.; Izawa, H.; Kumazawa, T.; Sato, K. *Biomed. Chromatogr.* **2008,** *22*, 702-711
10. Wu, J.; Lord, H. L.; Pawliszyn, J.; Kataoka, H. *J. Microcolumn Sep.* **2000,** *12*, 255-266
11. Gergov, M.; Robson, J. N.; Duchoslov, E.; Ojanpera, I. *J. Mass Spectrum.* **2000,** *35*, 912-918
12. Mario, T.; Georg, O.; Wilhelm, S. *Biomed. Chromatogr.* **2001,** *15,* 393-402
13. Maurer, H. H.; Tenberken, O.; Kratzsch, C. *J. Chromatogr. A,* **2004,** *1058*, 169-181
14. Le Potiera, I.; Tamisier- Karolak, S. L.; Morin, Ph.; Megel, F.; Taverna, M. *J. Chromatogr. A* **1998,** *829*, 341-349.
15. Tamisier- Khalendi, S. L.; Stanger, M. A.; Bommart, A. *Electrophoresis* **1999,** *20*, 2656-2663.
16. Wang, F.; Khalendi, M. G. *Eletrophoresis* **1998,** *19*, 2095-2100.
17. Salonies, H.; Luukkanen, L.; Knutila, R.; *J. Pharm. Biomed. Anal.* **1989,** *7*, 1447-1451
18. Radi, A.; El-Sherif, Z.; Wassel, A. *Chem. Papers*, **2004,** *58*, 242-246.
19. Iorio, M. A.; Farina, A. M.; Doldo, A. *J. Pharm. Biomed. Anal.* **1987,** *5*, 1-10.
20. Mohamed, M. E. *Pharmazie* **1983,** *38*, 784-785.
21. Belal F., Al-Shaboury A., Al-Tamrah A. S. *J. Pharm. Biomed. Anal.* **2002,** *30*, 1191-1196.
22. El-Wasseef, D. R.; El-Ashry, S. M.; Abu-El-Enien, M. A.; Moustafa, M. A. A. *J. Food Drug Anal.* **2006,** *14*, 133-140.
23. Rahman, N.; Manirul, H. Sk. *Int. J. Biomed. Sci.* **2008,** *4*, 140-146.
24. Sane, R. T.; Chandrashekhar, T. G.; Nayak, V. G. *Indian Drugs* **1986,** *23*, 565-568.
25. Sastry, C. S. P.; Krishna, D. M. *Microchim. Acta.* **1996,** *122*, 87-93.
26. Sastry, C. S. P.; Rao, S. G.; Shrinivas, K. R. *Indian Drugs* **1998,** *35*, 594-596.
27. Belal, F.; Al-Shaboury, S.; Al-Tamra, A. S. *IL Farmaco* **2003,** *58*, 293-299.
28. Rahman, N.; Rahman, H.; Azmi, S. N. H. *J. Chin. Chem. Soc.* **2007,** *54*, 185-196.
29. Salomies, H.; Luukkanen, L.; Knuutila, R. *J. Pharm. Biomed. Anal.* **1989,** *7*, 1447-1451.
30. International Conference on Harmonisation, ICH Harmonised Tripartite Guideline-Text on Validation of Analytical Procedures, Fed Regist. **1995**; 60:11260.
31. Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry;* 4th ed., Pearson Education: England, **2000**; p 118.
32. Morelli, B. *J. Pharm. Biomed. Anal.* **2003,** *32*, 257-267.
33. Hartmann, C.; Verbeke, J. S.; Penninckx, W.; Heyden, Y. V.; Vankeerberghen, P.; Massart, D. L. *Anal. Chem.* **1995,** *67*, 4491-4499.
34. *Acceptable methods in Drugs Directorate Guidelines*, Canada Health Protection Branch, Ministry of National Health and Welfare, Draft, Ottawa, Canada, **1992**.