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Oxidemetric Determination of Labetalol Hydrochloride with Potassium Permanganates in Acid Medium.

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ABSTRACT

A simple, convenient and sensitive validated visual (range 1.0mg – 3.0mg) end-point and Spectrophotometric titration (range 0.50 mg – 1.50 mg) methods have been developed for the determination of labetalol, a premier B.P drug [*Molecular Formula:* $C_{19}H_{24}N_2O_3$ HCl. *Molecular Weight:* 364.87] using potassium permanganate as an oxidizing agent for the first time with an accuracy of ± 0.7% and ± 0.8% respectively. The visual end-point method consists in heating the drug solution taken in 1-2M phosphoric acid medium to about 65-70^{0}C and then adding a known excess of a standard permanganate solution and titrating the excess permanganate at about 35-40^{0}C or at room temperature with a standard solution of Mohr's salt using diphenylamine as an indicator. In the spectrophotometric titration method, the drug solution taken into a medium of 0.5-1.0M sulphuric acid and 0.4% manganese(II) [which acts as catalyst], is titrated against a standard solution of potassium permanganate spectrophotometrically at 520nm at room temperature. The precision of both the methods has been determined by computing the pooled standard deviation and 95% confidence limits. In these methods the drug compound reacts with potassium permanganate in 1:1 mole ratio in a five electron change. The advantages of the present method over the earlier ones have been discussed.

Keywords: labetalol hydrochloride, potassium permanganate, manganese(II), phosphoric acid visual end-point method, spectrophotometric determination,.

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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, and is used in the treatment of hypertension. Now clinically, it is most commonly prescribed as a primary drug for the treatment of hypertensive disorders of pregnancy, including pre-eclampsia[1]. It is a moderately potent hypotensive drug and is especially useful in pheochromocytoma. Labetalol hydrochloride reduces blood pressure more rapidly than other receptor blockers and the drug is also used to lower blood pressure in myocardial infarction and in unstable angina. However, the over dosage of labetalol hydrochloride exhibits heptatoxicity and renal failure. Labetalol hydrochloride is also one of the well known doping agents in sports and hence it has been banned for Olympic players by International Olympic Committee (I.O.C) [2-4]. The drug is quite sensitive and even its small dosage gives sufficient blockage. Thus, the drug is found to be very much confined to the cardio protective effects. Hence, it is worthwhile to find the amount of labetalol hydrochloride in drug formulations. The drug is official in Martindale: The Extra Pharmacopoeia[5]. The assay procedure is cited in the monographs of the British Pharmacopoeia[6] and the United States Pharmacopoeia[7]. The former described a potentiometric titration method while the latter furnished a liquid chromatographic method. Therefore, it is most useful to develop simple, selective and accurate analytical method for the determination of labetalol hydrochloride in pharmaceutical formulations and biological fluids. However, a survey of literature revealed that only limited number of methods are available for the estimation of labetalol in bulk & tablet forms and in biological fluids. Most of the methods utilized the state of the art techniques like polarography[8], NMR spectroscopy[9], spectrofluorimetry[10-12], capillary iso-techophoresis[13], capillary electrophoresis[14-15], adsorptive voltametric method[16], ion-selective electrode[17], liquid-chromatography mass-spectrophotometry (LC-MS)[18], capillary liquid chromatography[19], micellar liquid chromatography[20], gas chromatography (GC)[21], thin layer chromatography (TLC)[22], and High performance liquid chromatography (HPLC)[23-25]. In addition, spectrophtometric determinations based on coupling reactions between the drug (labetalol) and the organic coupling reagents[26-28] [which forms a coloured complex] and measuring the absorbance at its λmax have been reported. Further, some of the spectrophotometric methods reported were based on the formation of coloured compounds between labetalol and some organic dyes/compounds[29-30] and measuring the absorbance at λ_{max}. Moreover, spectrophotometric methods using potassium permanganate[31] (kinetic determination), hexacyanoferrate(III)[32] (kinetic study) and ferric ammonium sulphate[33] as oxidants have been reported. Recently, we have reported an oxidemetric method for the determination of labetalol with hexacyanoferrate(III) using spectrophotmetric and visiual - end point techniques[34]. Evidently, these methods require the use of sophisticated and expensive instrumentation. Further, these methods are laborious and time consuming.

The present paper describes an indirect visual end- point method [by adding a known excess of potassium permanganate solution to the drug solution in 1–2M phosphoric acid medium and back titrating the excess with iron(II)]; and a spectrophotometric titration method[in 0.5-1.0M sulphuric acid medium and in presence of 0.4% manganese(II) as a catalyst]s for the determination of labetalol using potassium permanganate as an oxidizing agent. Further, the methods now developed have been extended for the estimation of labetalol hydrochloride in pure, commercial tablet forms and in spiked human urine. The methods now developed obviate most of the disadvantages of the earlier methods.

EXPERIMENTAL

Chemicals and Reagents

All chemicals used in this investigation were of analytical reagent grade. Double distilled water was used throughout the investigation.

Labetalol Drug solution: Labetalol hydrochloride as a reference standard was obtained from Sigma Aldrich Company. A standard solution of $5.0x10^{-3}$M labetalol solution was prepared in 1liter standard flask by dissolving required amount of the drug in double distilled water and diluted to the mark. From this solution a $2.5x10^{-4}$M solution and a $5.0x10^{-4}$M solution were also prepared by suitable dilution and utilized in spectrophotometric titration and visual methods respectively.

Iron(II) solution: An approximately 0.05M solution of iron(II) in 0.25M sulphuric acid medium was prepared by dissolving required amount of AR grade ferrous ammonium sulphate hexahydrate in double distilled water and standardized by titrating against a standard solution of potassium dichromate solution[35]. From this solution a 0.02N solution was prepared by suitable dilution and utilized in visual method.

Potassium Permanganate: A 0.02N of potassium permanganate solution was prepared by dissolving required amount of potassium permanganate crystals taken from an AR grade sample in doubled distilled water and standardized by titrating against a standard iron(II) solution(0.02N) using BDAS as an indicator.

Phosphoric acid: An approximately 10M phosphoric acid solution was prepared from an AR grade phosphoric acid using double distilled water.

Sulphuric acid: An approximately 5M sulphuric acid solution was prepared from an AR grade sample using double distilled water.

Carbonate Buffer Solution of P^H 9.4: Carbonate buffer solution of p^H 9.4 was prepared by dissolving required amounts of sodium carbonate and sodium bicarbonate in 500 ml distilled water[33].

Manganese(II) solution(catalyst): An approximately 10% manganese(II) solution has been prepared by dissolving 10g of an AR grade manganese(II) sulphate monohydrate sample in 100ml of distilled water.

BDAS Indicator: A 0.1% (w/v) solution of barium salt of diphenylamine sulfonate (BDAS) indicator was prepared by dissolving about 100mg of the salt in about 100 ml of double distilled water.

Apparatus: Shinadzu Double Beam Spectrophotometer (UV-800) has been utilized in the study.

Procedure for the estimation of labetalol by potassium permanganate (Visual - end point method):

An aliquot (5-15ml) of labetalol solution($5.0x10^{-4}$M) was taken in the titration cell, the acidification of the solution was followed by adding 5.0ml of 10M phosphoric acid, and the solution diluted to about 50ml (over all phosphoric acid concentration 1M). The titration cell with the content was heated to about 65-70^{0}C, then it was removed from heating and treated with 10 ml of standard potassium permanganate solution (0.02N) slowly while it is being stirred on a magnetic stirrer. The stirring was continued for 5 more minutes to ensure complete oxidation of labetalol by permanganate [during the time the temperature of the reaction mixture goes down to about 40^{0}C]. The excess permanganate in the reaction mixture is now titrated against a standard solution of iron(II) employing BDAS as an indicator. The colour change at the end-point is from colorless to bluish-violet.
Some of the typical results obtained have been shown in the Table 1

Table-1: Estimation of labetalol hydrochloride (LBT HCl) with potassium permanganate (Visual end-point method)

S.No	Amount of labetalol hydrochloride taken, mg	Amount of labetalol hydrochloride found*, mg $\bar{X}$	Sg	Pooled standard deviation $\frac{Sg \times 1.96}{\sqrt{n}}$	95% Confidence limits $\bar{X} \pm \frac{Sg \times 1.96}{\sqrt{n}}$
1	0.945	0.941			0.938 0.944
2	1.219	1.225			1.222 1.228
3	1.492	1.486			1.483 1.489
4	1.765	1.772	0.004	0.003	1.769 1.775
5	2.038	2.031			2.028 2.034
6	2.311	2.315			2.312 2.318
7	2.584	2.578			2.575 2.581

*Average of determinations.

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Procedure for the estimation of labetalol by potassium permanganate (spectrophotometric method):

A known aliquot (5-15ml) of labetalol solution ($2.5x10^{-4}$) is taken into a 150 ml beaker. To the solution about 5.0ml of 5M sulphuric acid 2.0ml of manganese(II) solution(10%) [overall concentrations 0.5M and 0.4% respectively in 50ml] are added and the solution diluted to about 50ml. To the solution, a small and equal installment of a standard solution of potassium permanganate (0.01N) is added and the solution stirred on a magnetic stirrer for about two minutes. After each addition the absorbance of the solution is recorded against its corresponding blank at 520nm. The titration is continued in this way till the absorbance values increase for the addition of each installment of permanganate and 4-5 such absorbances (after increase absorbance) have been recorded. The plot of absorbance verses the volume of permanganate added gives two straight lines; the point of interception gives the end-point.

Some of the typical results obtained by the method have been shown in Table 2

Table 2: **Procedure for the determination of labetalol hydrochloride using Potassium permanganate (spectrophotometric method)**

S.No	Amount of labetalol hydrochloride taken, mg	Amount of labetalol hydrochloride found*, mg $\bar{X}$	Sg	Pooled standard deviation $\frac{Sg \times 1.96}{\sqrt{n}}$	95% Confidence limits $\bar{X} \pm \frac{Sg \times 1.96}{\sqrt{n}}$
1	0.472	0.470			0.468 0.472
2	0.609	0.612			0.610 0.614
3	0.745	0.742			0.740 0.744
4	0.882	0.886	0.002	0.0016	0.884 0.888
5	1.019	1.021		(=0.002)	1.019 1.023
6	1.155	1.152			1.150 1.154
7	1.292	1.294			1.292 1.296

*Average of six determinations

Determination of labetalol hydrochloride in commercial tablets

Procedure for commercial tablets:

Table 3: Visual end-point method

Trade name	Amount of labetalol HCl found		RSD	% of labetalol present in tablet
	Reference method[29] mg	Proposed method* mg		
Labebet 100mg	1.015	1.020	0.004	91.92
	1.523	1.517	0.006	90.45
	1.994	1.988	0.008	91.52
	2.457	2.464	0.009	91.34
Lobet 100mg	1.086	1.091	0.003	91.93
	1.629	1.622	0.005	90.25
	2.137	2.130	0.007	91.52
	2.628	2.633	0.008	90.45

Several tablets (labeled 100mg per tablet) were weighed separately and mixed and then crushed into fine powder in a mortar. A suitable amount of this powder was dissolved in about 50ml of distilled water. After 15 minutes of continuous shaking the mixture was filtered through a Whatmann filter paper (No. 42), then the residue was washed with 10ml of distilled water twice. The filtrate and the washings were collected into a 100ml standard flask and diluted to the volume. This solution was preserved as a stock solution. The solution

was standardized (with respect to the drug content) according to the method described in official British pharmacopeia[1]. From this an approximately $2.5x10^{-4}$M and $5.0x10^{-4}$M solutions [with respect to labetalol content] were prepared and the drug content was determined by spectrophotometric and visual end–point methods respectively as per the recommended procedures. Some of the typical results obtained, as well as the percent of drug content present in the tablets have been shown in Tables 3&4

Table 4: Spectrophotometric titration method

Trade name	Amount of labetalol HCl found		RSD	% of labetalol present in tablet
	Reference method[29] mg	Proposed method* mg		
Labebet 100mg	0.677	0.674	0.003	91.81
	1.015	1.019	0.006	91.22
	1.332	1.336	0.007	90.94
	1.638	1.634	0.008	91.21
Lobet 100mg	0.684	0.681	0.004	91.89
	1.026	1.030	0.005	90.87
	1.347	1.344	0.007	91.32
	1.656	1.657	0.008	90.21

*Average of six determinations

Determination of labetalol hydrochloride in spiked human urine samples:

Aliquots of human urine samples were collected from healthy persons and needful precautions have been taken before using them for experiment. Known aliquot of urine sample was taken into a separating funnel and known amount of labetalol was spiked into the sample. To this about 5.0ml of carbonate buffer of p^H 9.4 was added and the sample was mixed well. The extraction of drug was carried out thrice by adding 5ml of diethyl ether each time and shaking the solution for about 20 minutes. The ether extract was collected into a beaker and evaporated on a water bath. The residue was dissolved in distilled water and diluted to volume in 100ml standard flask. The solution was then analyzed for labetalol content spectrophotometrically as per the recommended procedure.

Some of the typical results obtained have been shown in Table 5

Table 5: Determination of labetalol hydrochloride in spiked human urine samples

Amount of labetalol hydrochloride added, mg	Amount of labetalol hydrochloride found, mg		Recovery, %
0.473	0.475		100.38
0.645	0.642		99.38
0.837	0.834		99.64
1.010	1.013		100.29
1.201	1.196		99.58
1.365	1.369		100.29
		Mean	99.92

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RESULTS AND DISCUSSION

A survey of literature revealed that the redox analytical applications of potassium permanganate in alkaline medium are scarce while those in dilute sulphuric acid medium are in abundance; a few instances in phosphoric acid medium have also been reported. Therefore, the authors attempted to develop a convenient oxidemetric method for the determination of labetalol with permanganate in dilute sulphuric acid medium and found that the oxidation reaction is too slow at room temperature, but it is found to be catalyzed by manganese(II). However, because of the use of dilute solutions of permanganate in the determinations (as can be seen from the recommended procedure), the detection of the end point through the self colour of permanganate is found difficult. In fact it is absolutely imperative to make use of dilute solutions of oxidant in the determinations involving smaller quantities (1-2 mg/microgram quantities) of drugs or organic compounds. We tried the use of several redox indicators to detect the end point, but in vain, because, no indicator is found to give a sharp colour transition at the end point. However, we could successfully detect the end point through the spectrophotometric titration method as described in the recommended procedure in which manganese(II) functioned as a catalyst. The spectrophotometric titration is quite satisfactory in 0.5-1.0 M sulphuric acid medium in presence of about 0.4% manganese (II) which acts as a catalyst. The spectrophotometric titration can be carried out accurately even in sulphuric acid medium greater than 1M (up to 2.5M), however, we preferred to carry out the titration in low sulphuric acid medium of 0.5-1.0M.

The authors further investigated the possibility of developing a redox procedure involving both spectrophotometric and visual end-point methods for the determination of labetalol with permanganate in phosphoric acid medium and observed that the oxidation process is very slow at room temperature and thus it is found to have no analytical importance. We tried the use of manganese(II) as a catalyst in phosphoric acid medium similar to that in sulphuric acid; but it did not respond positively and found to form a brown turbid solution. Earlier authors[36] studied the effect of manganese(II) in phosphoric acid medium involving permanganate as an oxidizing agent and observed that manganese(II) decreases the redox potential of MnO_4^- /Mn(II) system so that it becomes a weaker oxidizing agent. It was also stated that in phosphoric acid medium manganese(II) ions are likely to react with permanganate ions forming manganese(III) which is generally reddish brown in colour. The authors contemplate that the brown coloured turbidity observed by them in the present redox process in phosphoric acid medium(1-2M) containing manganese(II) and permanganate is most likely a manganese(III) compound.

As the oxidation of labetalol by permanganate at room temperature (even in presence of catalyst) in phosphoric acid medium is found to be very slow, the authors studied the oxidation process at elevated temperatures. Our preliminary studies in phosphoric acid medium at higher temperatures indicated that the rate of oxidation of the drug with permanganate increases with increase in temperature up to 65-70^{0}C. At a temperature above 80^{0}C the oxidation reaction is found to have no analytical importance, as large volumes of permanganate solution are consumed (for a small quantity of drug solution taken) without any reproducibility in the titer values. Probably, at elevated temperatures, the drug is rapidly oxidized by permanganate yielding small fractions of drug compound of unknown composition. We further noticed that even at a temperature of about 65-70^{0}C, the oxidation of the drug by permanganate is not sufficiently fast to develop a visual end-point method employing a redox indicator. Therefore, the authors have added a known excess of a standard solution of permanganate(0.02N) to the drug solution taken in 1-2M phosphoric acid medium at 65-70^{0}C, the solution cooled to about 40^{0}C and then titrated the excess permanganate with a standard solution of iorn(II) using diphenylamine as a redox indicator. As stated earlier, one can not but utilize a redox indicator[37] to detect the end-point when dealing with dilute solutions of permanganate(0.02N) in analytical methods. Unlike in dilute sulphuric acid medium, the oxidation process in dilute phosphoric acid medium provided access to make use of barium salt of diphenylamine (BDAS) as a redox indicator. Several other redox indicators may be found suitable in the present method for titrating excess permanganate with iron(II). However, we did not pursue this aspect and confined ourselves to the use of BDAS as an indicator. Satisfactory results are obtained for the determination of labetalol with potassium permanganate in 1-2M phosphoric acid medium and following the recommended procedure. The indicator method is found feasible even in high phosphoric acid medium as high as 4M. But when the concentration of phosphoric acid is about 5M or above the indicator BDAS did not respond satisfactorily, so the titration is recommended to be carried out in a low phosphoric acid medium of 1-2M, which makes the medium less viscous and the method inexpensive.

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Stoichiometry of the reaction

In both the methods (spectrophotometric and indicator method) the drug labetalol is found to be oxidized by permanganate in a 5- electron oxidation step, thus one mole of labetalol reacts with one mole potassium permanganate [MnO_4^- ion is reduced to Mn^{+2} ion in a 5 electrons change]. However, we have not identified the oxidized products of the drug obtained after reacting with potassium permanganate. The identification of the oxidized products of the drug using the state of art technologies is under investigation.

The Absorption Spectra of the Reagents

In order to select an appropriate wave length for the spectrophotometric determination of labetalol with $KMnO_4$ in sulphuric acid medium the absorption spectra of the reactants and products involved in the reaction are needed. However, except $KMnO_4$ all the other reactants and products involved in the reaction have negligible absorbance in the visible region. Further, the author has measured the absorption spectra of $KMnO_4$ and labetalol and it has been shown presented in Fig 1. From the figure it may be seen that the most appropriate wave length for the spectrophotometric titration of labetalol with $KMnO_4$ is 520nm.

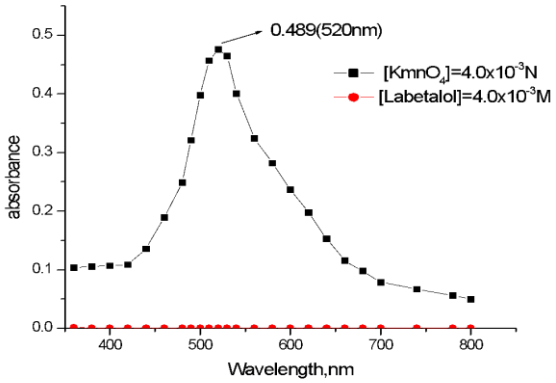

***Fig 1: Absorption Spectrum of* KMnO$_4$**

Beer's law

Labetalol is a colorless solution, hence it has negligible absorbance in the visible region. The concentration limit of labetalol in the spectrophotometric method is not possible to be found in the usual way. But, since the determination of the drug is carried out by titrating against a standard solution of $KMnO_4$ which is a colored solution, the adherence to Beer's law of the drug has been determined indirectly by measure the absorbance of the $KMnO_4$ in the following way: Varying volumes of $4.0x10^{-4}$N $KMnO_4$ solution are taken in different 50ml standard volumetric flasks and about 5ml of 5M H_2SO_4solutions are added to each one of them and diluted to the mark with distilled water. The absorbance of $KMnO_4$ was measured using the cell of 1cm path length at its λmax (520nm) against its corresponding blank. From such a study (the plot of concentration of $KMnO_4$ versus absorbance at 520nm), adherence to Beers law with respect to $KMnO_4$ has been found. Upon converting the concentration of $KMnO_4$ into the equivalent of labetalol, it has been found that labetalol obeyed the Beer's law up to 2.5mg/50ml of the solution.

CONCLUSIONS

The labetalol hydrochloride which is a major component in B.P drugs can be determined using potassium permanganate as oxidizing agent. The drug content can be found either spectrophotometrically (0.5 to 1.5 mg) (0.5-1.0M sulphuric acid medium and in presence of 0.4% manganese(II) as a catalyst) or using visual end-point method (1.0-3.0 mg) . In the latter procedure the drug content can be found by treating the solution with a known excess of potassium permanganate in 1-2M phosphoric acid medium at about 65 – 70^0C and the

excess permanganate back titrated against a standard iron(II) solution at room temperature using BDAS(Barium Salt of Diphenylamine Sulphonate) as redox indicator. The new method now developed can be extended for the determination of labetalol hydrochloride content in commercial tablets and in spiked human urine samples with good accuracy. It is found that in both the methods one mole of labetalol hydrochloride reacts with one mole of MnO_4^- ion in a five electron change process.

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