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Oxidemetric determination of Labetalol Hydrochloride with Hexacyanoferrate(III) in alkaline medium

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ABSTRACT

*A Simple, convenient and accurate spectrophotometric titration and a visual end - point method [employing a redox indicator] have been developed for the determination of Labetalol[LBT], a premier B.P drug (*Molecular Formula: $C_{19}H_{24}N_2O_3$ *HCl.* Molecular Weight *364.87), using hexacyanoferrate(III) [HCF(III)] as an oxidizing agent for the first time. The spectrophotometric method consists of titrating the drug solution in about 4M alkaline medium (sodium hydroxide) using a standard solution of hexacyanoferrate(III) at 420nm. In the case of visual end - point method, however, the drug solution in about 4M sodium hydroxide medium, is treated with a known excess of HCF(III) solution and the excess HCF(III) is titrated against a standard solution of iron(II) solution in phosphoric acid medium [about 9M] and in presence of zinc sulphate using diphenylamine as a redox indicator. The drug labetalol in the range 0.32mg – 0.97mg (by spectrophotometric method) and in the range of 0.63mg – 1.89mg (by visual end - point method) have been determined with an accuracy of ± 0.7% and ± 0.8% respectively. The precision of both the methods have been assessed by computing pooled standard deviation and 95% confidence limits. In these methods the drug is oxidized by HCF(III) in a two electron oxidation step. The oxidation products of the drug have been identified and confirmed by IR-spectrum. The advantages of the present methods over the earlier ones have been discussed.*

Keywords: Labetalol, Hexacyanoferrate(III), Spectrophotometric, Diphenylamine, Iron(II), Phosphoric acid

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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. It is listed in British Pharmacopoeia BP[1], United States Pharmacopoeia[2] and the European Pharmacopoeia[3]. 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. LBT hydrochloride is also used to induce hypotension during surgery as it reduces blood pressure more rapidly than other receptor blockers. Besides these important pharmacological activities, labetalol therapy exhibits hepatotoxicity and renal failure due to over dosage. LBT 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[4-6]. Therefore, it is worthwhile 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 there are only a handful of methods available for the estimation of labetalol in bulk & tablet forms and in biological fluids. These methods are based on techniques like : High performance liquid

chromatography (HPLC)[7-9], thin layer chromatography (TLC)[10], liquid-chromatography mass-spectrophotometry (LC-MS)[11], gas chromatography (GC)[12], capillary liquid chromatography[13], micellar liquid chromatography[14], spectrofluorimetry[15-17], adsorptive voltametric method[18], ion-selective electrode[19], NMR spectroscopy[20], capillary electrophoresis[21,22], capillary iso-techophoresis[23], and polarography[24]. Evidently, these methods require the use of sophisticated and expensive instrumentation. In addition, some spectrophotometric determination methods are available. These methods are based on forming a colored complex of labetalol using a coupling reaction between the drug (labetalol) and some organic reagents[25-27] and measuring the absorbance at its λmax. Spectrophotometric methods using potassium permanganate[28] (kinetic spectrophotometric method), ferric ammonium sulphate[29] etc., as oxidants have been reported. In addition, spectrophotometric methods based on the formation of a colored compounds between labetalol and some organic compounds/dyes[30,31] and measuring the absorbance at their λmax were available.

All these methods are laborious and time consuming. Though hexacyanoferrate(III) is well known as a good oxidizing agent in alkaline medium and utilized for the determination of several organic compounds, and in the kinetic studies of labetalol[32], it [HCF(III)] has not so far been utilized for the determination of the drug. Further, so far, no satisfactory visual end – point or spectrophotometric method has been reported for the determination of the drug.

Therefore, the authors of the present paper made an attempt in these lines and developed a visual end- point method and a spectrophotometric titration method for the determination of labetalol utilizing HCF(III) in alkaline medium and presented the results of these investigations in this paper. Further, the methods now developed have been extended for the estimation of labetalol hydrochloride in pure, tablet forms and in spiked human urine.

EXPERIMENTAL SECTION

All chemicals used in this investigation were of analytical reagent grade. All the solutions were prepared in double distilled water.

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 $2.0x10^{-4}$M solution and a $3.0x10^{-4}$M solution were also prepared by suitable dilution and utilized in spectrophotometric titration and visual methods respectively.

Hexacyanoferrate(III) solution: An approximately 0.01M solution of HCF(III) was prepared by dissolving the required amount of AR grade potassium hexacyanoferrate(III) salt in double distilled water. The strength of the solution is checked iodometrically[33]**.** The solution was transferred into an amber colored bottle and stored in dark place. From this solution a $1.0x10^{-3}$M and $1.5x10^{-3}$M solutions were prepared by suitable dilution and utilized in both spectrophotometric and visual end – point methods respectively.

Iron(II) solution: An approximately 0.05M solution of iron(II) in 0.5N sulphuric acid medium was prepared by dissolving required amount of AR grade ferrous ammonium sulphate hexahydrate in double distilled water and standardized[33**]** by titrating against a standard solution of potassium dichromate solution. From this solution a $1.0x10^{-3}$M solution was prepared by suitable dilution and utilized in visual method.

Hexacyanoferrate(II) solution: An approximately 0.05M solution of HCF(II) was prepared by dissolving the required amount of AR grade potassium hexacyanoferrate(II) salt in double distilled water and it is standardized[33]. From this solution a $1.0x10^{-3}$M solution was prepared and utilized in recording its absorbance spectra.

Sodium hydroxide solution: An approximately 10M solution of sodium hydroxide solution was prepared by dissolving required amount of sodium hydroxide pallets in double distilled water.

Phosphoric acid: syrupy phosphoric acid of AR grade has been utilized in this investigation.

Zinc sulphate solution: A 3.0 M solution of zinc sulphate was prepared by dissolving required amount of AR grade $ZnSO_4$. $7H_2O$.in distilled water.

Carbonate Buffer Solution of P^H 9.4: Carbonate buffer solution of p^H 9.4 was prepared by dissolving required amount of sodium carbonate and sodium bicarbonate in 500 ml distilled water[29].

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 has been utilized in the study.

Procedure for the determination of labetalol hydrochloride by HCF(III) (visual-end point method):
To an aliquot (5-15ml) of labetalol solution ($3.0x10^{-4}$M) taken in a titration cell, about 12ml of sodium hydroxide (10M) solution was added and the solution diluted to about 30ml so that the overall concentration of the sodium hydroxide is about 4M. The solution is now treated with a known volume of $1.5x10^{-3}$M HCF(III) solution such that its concentration is about twice that required for the complete oxidation of the drug content present in the solution. The solution is then stirred for about 5 minutes, 40 ml of syrupy phosphoric acid and about 2ml of 2% (w/v) zinc sulphate solution were added. The excess HCF(III) is then back titrated against a standard solution of iron(II) ($1.0x10^{-3}$M) as described by Raju and Co-workers[34] using BDAS as a redox indicator.

Some of the typical results obtained have been shown in the Table1

Table-1: Determination of labetalol hydrochloride[LBT HCl] with HCF(III) (Visual end - point method)

S.No	Amount of LBT HCl taken, mg	Amount of LBT HCl 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	0.6006	0.6042			0.6032 0.6052
2	0.7644	0.7605			0.7595 0.7615
3	0.9282	0.9309			0.9299 0.9319
4	1.092	1.088	0.0013	0.0010	1.087 1.089
5	1.255	1.257			1.256 1.258
6	1.419	1.416			1.415 1.417
7	1.638	1.641			1.631 1.751

*****Average of six determinations*

Procedure for the determination of labetalol hydrochloride using HCF(III) by Spectrophotometric titration method:
To an aliquot of labetalol (5-15ml) solution ($2x10^{-4}$M) taken in a reaction vessel, about 12ml of 10N solution of sodium hydroxide was added and the solution diluted to about 30ml (over all concentration of sodium hydroxide in the reaction medium is about 4M). The solution is now titrated against a standard solution of [$1.0x10^{-3}$M] HCF(III) solution spectrophotometrically by adding a small and equal installments of [HCF(III)] solution, while the solution is being stirred on a magnetic stirrer. After each addition, the absorbance of the solution is measured against its corresponding blank using 1cm cell at 420nm. The titration is continued in this way until the absorbance of the solution increases linearly. The plot of absorbance versus volume of HCF(III) solution added gives two straight lines, the point of interception of which corresponds to the end point.

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

Table 2: **Procedure for the determination of labetalol hydrochloride [LBT HCl] using HCF(III) (spectrophotometric method)**

S.No	Amount of LBT HCl taken, mg	Amount of LBT HCl found*, mg $\bar{X}$	S_g	Pooled standard deviation $\frac{Sg \times 1.96}{\sqrt{n}}$	95% Confidence limits $\bar{X} \pm \frac{Sg \times 1.96}{\sqrt{n}}$
1	0.4004	0.4012			0.4020 0.4044
2	0.5096	0.5060			0.5048 0.5072
3	0.6188	0.6225			0.6213 0.6239
4	0.6552	0.6512	0.0015	0.0012	0.6500 0.6524
5	0.7644	0.7682			0.7670 0.7694
6	0.8736	0.8612			0.8680 0.8704
7	0.9828	0.9867			0.9855 0.9879

*****Average of six determinations*

Determination of labetalol hydrochloride in commercial tablets

Procedure for commercial tablets:

Several tablets (labeled 100mg per tablet) were weighed separately and mixed and crushed into fine powder in a morter. 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 $2.0x10^{-4}$M and $3.0x10^{-4}$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 3: Determination of labetalol hydrochloride [LBT HCl] in commercial tablets Visual end-point method

Trade name	Amount of LBT HCl found		RSD	% of labetalol present in tablet
	Reference method[29] mg	Proposed method* mg		
Labebet 100mg	0.5896	0.5854	0.0006	89.81
	0.8845	0.8889	0.0008	90.14
	1.1790	1.1820	0.0010	90.09
Lobet 100mg	1.4740	1.4710	0.0015	89.59
	0.5896	0.5929	0.0006	89.94
	0.8947	0.8991	0.0009	91.17
	1.1930	1.1870	0.0012	90.47
	1.4910	1.4960	0.0016	90.60

Table 4: Spectrophotometric titration method

Labebet 100mg	0.3911	0.3931	0.0003	89.11
	0.5896	0.5919	0.0005	90.72
	0.7838	0.7862	0.0008	90.32
Lobet 100mg	0.9808	0.9828	0.0010	89.33
	0.3959	0.3976	0.0004	90.40
	0.5935	0.5965	0.0006	91.60
	0.7953	0.7992	0.0007	91.94
	0.9923	0.9942	0.0009	89.94

Average of six determinations

Determination of labetalol hydrochloride in spiked human urine samples:

Aliquot 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 then 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 [LBT HCl] in spiked human urine

Amount of LBT HCl added, mg	Amount of LBT HCl found, mg	Recovery of %
0.5462	0.5487	100.49
0.7644	0.7605	99.50
0.9828	0.9867	100.39
1.201	1.196	99.58
1.419	1.423	100.28
1.638	1.641	100.18
	Mean 100.07	

RESULTS AND DISCUSSION

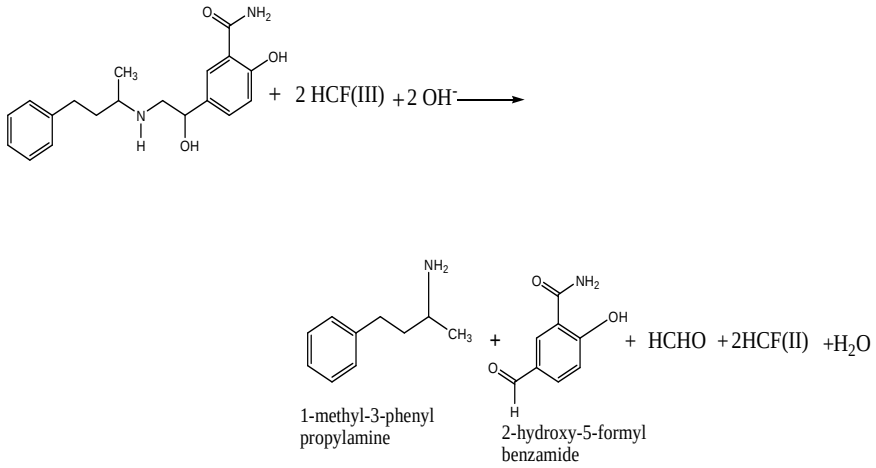
From the survey of literature as well as the procedures described above, it is evident that for the first time, hexacyanoferrate(III) has been utilized as a satisfactory oxidimetric titrant for the determination of labetalol. The advantages associated with the oxidant are that its aqueous solutions are quite stable for a long time, it acts as a good oxidizing agent especially in alkaline medium and it has high molecular weight.

Through some preliminary investigations the authors observed that the rate of oxidation of labetalol by HCF(III) is faster in alkaline medium than in acid/neutral conditions. In alkaline medium, the rate of oxidation is found to increase with increase in alkaline concentration. Even in the alkali medium, the rate of reaction is too slow to develop a visual end – point method whatever high may be the alkali concentration. However, the authors could develop an indirect visual end – point method in which a known excess of a standard HCF(III) solution is added to labetalol solution in alkaline medium(4M) and back titrating the excess HCF(III) by iron(II) in phosphoric acid medium(9M) and in presence of a small amount of zinc sulphate using BDAS as a redox indicator[34].

The determination of HCF(III) by iron(II) in phosphoric acid medium (containing small amount of zinc sulphate) was thoroughly studied and the reaction conditions have been well explained by Raju et.al[34].

It has been observed that the oxidation products of the drug obtained in alkaline medium did not undergo any change when they were brought into phosphoric acid medium (9M). Further, it has been found that for a satisfaction titration and completion of oxidation of the drug by HCF(III), one has to wait at least for 3-4 minutes even after the addition of an excess of HCF(III) in alkaline medium.

Stoichiometry of the reaction: Labetalol gets oxidized by HCF(III) in two electron process, while HCF(III) is reduced to HCF(II) in a one electron reduction step. The expected oxidation products of labetalol have been shown in Eq.1. All these products have already been confirmed by us through IR spectrum. However, formaldehyde was confirmed by chromotropic acid test while exploring the kinetic studies of labetalol by HCF(III) in alkaline medium[32].



Equation 1: Stoichiometry of the reaction

The Absorption Spectra of the Reagents

In order to select an appropriate wave length for the spectrophotometric determination of labetalol with HCF(III) in alkaline medium the absorption spectra of the reactants and products involved in the reaction are needed. However, except HCF(III) and HCF(II), all the other reactants and products involved in the reaction have negligible absorbance in the visible region. However, the author has measured the absorption spectra of HCF(III), HCF(II) and labetalol and presented in Fig:1. From the figure it may be seen that the most appropriate wave length for the spectrophotometric titration of labetalol with HCF(III) is 420nm.

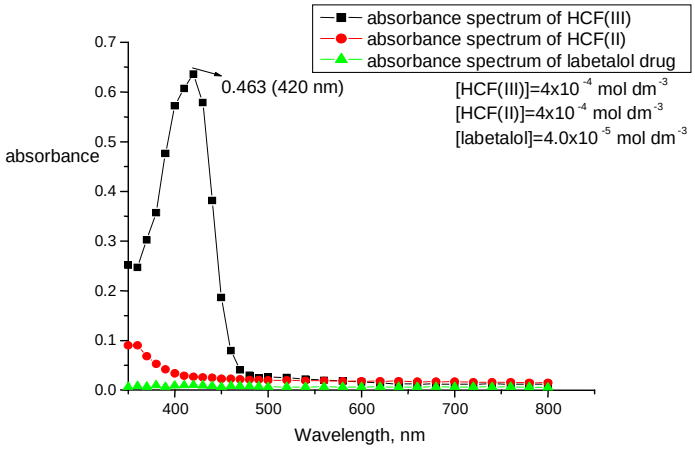


Fig 1: Absorption spectrum of HCF(III)

Beer's law

Since labetalol is a colorless solution and has negligible absorbance in the visible region, the concentration limit of labetalol in the spectrophotometric method is not possible to be determined in the usual way. But, since the determination of the drug is carried out by titrating against a standard solution of HCF(III) which is a colored solution, the adherence to Beer's law of the drug has been determined indirectly by measure the absorbance of the HCF(III) in the following way: Varying volumes of $1.0x10^{-3}$M HCF(III) solution are taken in different 50ml standard volumetric flasks and about 20ml of NaOH(10M) solutions are added to each one of them and diluted to the mark with distilled water. The absorbance of HCF(III) was measured using the cell of 1cm path length at its λmax (420nm) against its corresponding blank. From such a study (the plot of concentration of HCF(III) versus absorbance at 420nm), adherence to Beers law with respect to HCF(III) has been found. Upon converting the concentration of HCF(III) into the equivalent of labetalol, it has been found that labetalol obeyed the Beer's law up to 2.0mg/50ml of the solution.

CONCLUSION

The premier B.P drug labetalol hydrochloride (content) can be estimated using hexacyanoferrate(III) as an oxidemetric titrant in alkaline medium(4M) employing visual end – point and spectrophotometric titration techniques. The drug is oxidized by HCF(III) in a two electron change. These methods can be satisfactorily applied for the determination of drug content in commercial tablets and in spiked human urine. The methods are simple, accurate, convenient, rapid and inexpensive.

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