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Rapid and Simple Spectrophotometric Analysis of Labetalol Hydrochloride in Pharmaceutical, Urine and Blood Samples

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Abstract: There is an increasing interest in new strategies to determine as well as assessment of drugs in medical and pharmaceutical sciences. Herein we describe a simple, rapid, sensitive, and selective spectrophotometric method for determination of labetalol (LBT) hydrochloride based on the oxidation of the drug with ferric ammonium sulphate that yields a green colored product. The increase in absorbance of colored product is measured at 535 nm. All experimental parameters affecting the development of color are investigated and optimized. Under the optimum conditions, Beer's law is obeyed over the concentration range 10 - 200 µg/mL with molar absorptivity of 2.13 $\times 10^3$ L mol^{-1} cm^{-1}. The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed method are 2.71 and 8.22 µg/mL, respectively. The intra–day and inter–day precisions and accuracy of the proposed method are acceptable with low values of standard analytical error. The data are in agreement with values obtained by standard British Pharmacopeia (BP) method. The method is successfully applied to the determination of LBT in both pharmaceutical formulations and biological samples (e.g., human urine and plasma) *in vitro.*

Keywords: Labetalol; Spectrophotometry; Ferric ammonium sulphate; Biological samples

1. INTRODUCTION

Labetalol (LBT) hydrochloride (mixed α- and β- adrenoreceptor blocking agent) is considered as one of the major therapeutic drugs for the treatment of hypertension. LBT hydrochloride is also used to induce hypotension during surgery as it reduces blood pressure more rapidly than other receptor blockers.

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Besides these important pharmacological activities, LBT hydrochloride therapy exhibits hepatotoxicity and renal failure due to overdose. 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 [1-3]. Therefore, it is required to develop sensitive, selective, rapid and simple analytical method for determination of LBT hydrochloride in both pharmaceutical and biological samples.

Various analytical techniques including spectrofluorimetry [4-6], thin layer chromatography (TLC) [7] high performance liquid chromatography (HPLC) [8-10], liquid- chromatography mass-spectrophotometry (LC-MS) [10] , gas chromatography (GC) [11], micellar liquid chromatography [12], capillary liquid chromatography [13], capillary electrophoresis [14,15] capillary isotachophoresis [16], NMR spectroscopy [17], ion-selective electrode [18], and adsorptive voltammetric method [19] have been employed to estimate the concentration of LBT hydrochloride in pharmaceutical preparations and/ or biological fluids. Though these systems have high selectivity and enough sensitivity for LBT hydrochloride, almost all of them require extended analysis times, tedious pretreatment of the samples, high cost, and laborious clean up procedures prior to analysis. It is therefore difficult as well as not feasible to determine LBT with these systems in the third world/non-developed countries where operating cost and simple procedure are considered the main factors prior to analysis.

UV-Visible spectrophotometry is the technique of choice even today in the third world countries due to its low cost, ease of operation, high sensitivity, and simplicity of the instrumentation used for the technique. Several spectrophotometric methods based on the reaction of LBT with sodium nitroprusside and hydroxylamine hydrochloride [20], p-N,N-dimethylphenylenediamine hydrochloride [21], suprachen violet 3 B [21], diazotized 4-aminobenzene sulfonic acid [22], potassium permanganate [23], diazotized benzocaine in presence of trimethylamine or diazotized p-nitroaniline in the presence of sodium carbonate [24], and ferric 1, 10 – phenanthroline [25] have been developed to determine the concentration of LBT in bulk drug and pharmaceutical formulations effectively. Among these, only diazotized based method has been used to analyze LBT in biological samples [24]. However, a harmful solvent, trimethylamine as well as a number of solvents are required for this assay, which are costly and need extra safety caution to handle. Thus, at present, a considerable attention has been focused on the development of the low cost, solvent free and safe spectrophotometric method which is amenable to estimate LBT hydrochloride in both pharmaceutical and biological samples.

This paper reports a simple and accurate spectrophotometric method for the determination of LBT hydrochloride. The principle of this method is that the ferric ammonium sulphate oxidizes LBT hydrochloride at pH 3.49 resulting in the formation of phenoxyl radical which on coupling yields green colored dimerization product at 535 nm. The reaction sequence is shown in Scheme 1. All experimental parameters affecting the development of color are optimized. The developed method is then applied in the analysis of the LBT hydrochloride in pharmaceutical formulations. In addition, the method is applied to estimate LBT hydrochloride in biological samples (e.g., human plasma and urine) *in vitro* (in which LBT hydrochloride was spiked prior to analysis) and provided quantitative data that was in agreement with values obtained by official British Pharmacopeia (BP) method [26].

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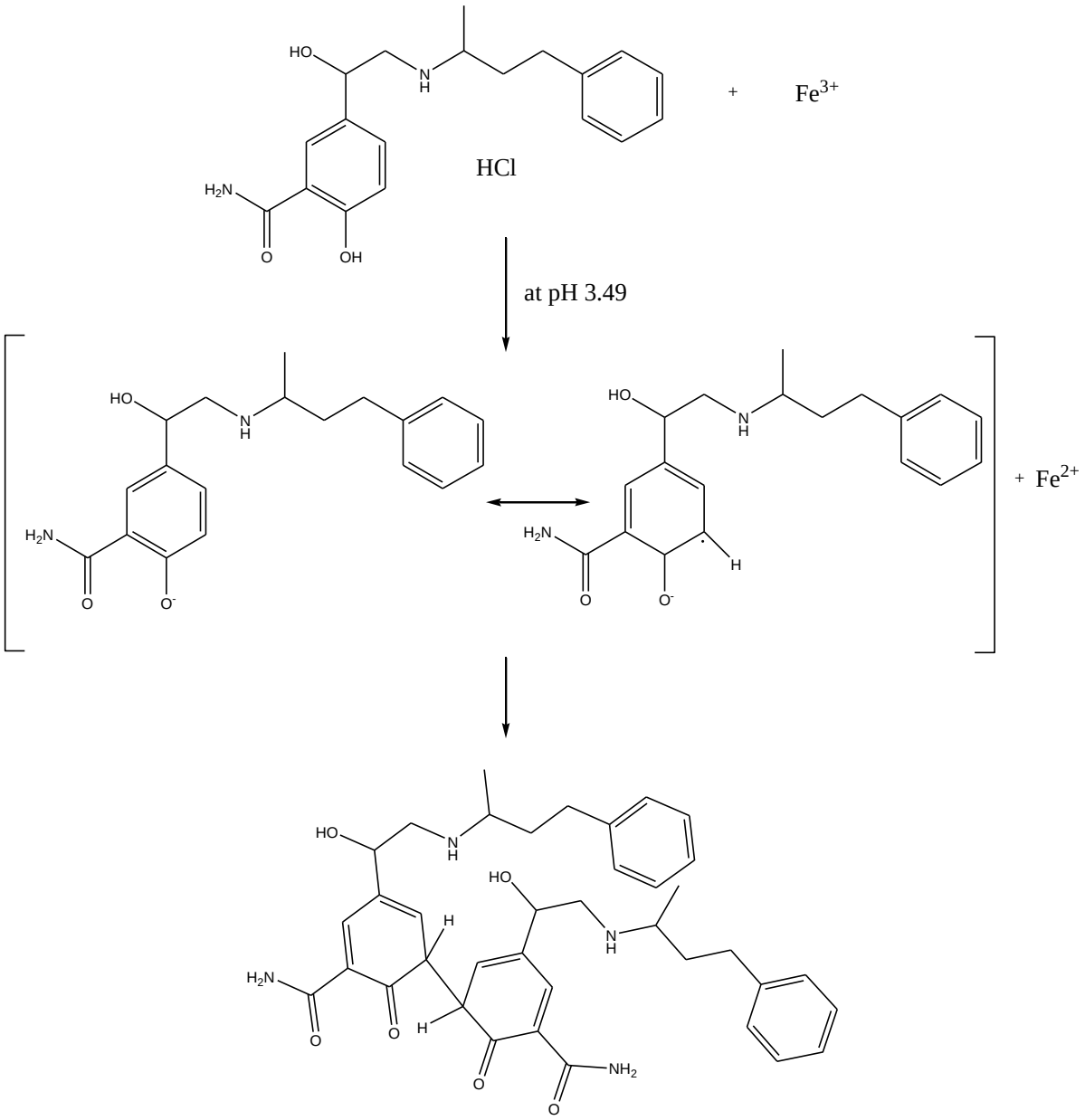

Dimerization Product

Scheme 1. The reaction sequence of labetalol (LBT) hydrochloride with a mild oxidizing agent, ferric ammonium sulphate. Ferric ammonium sulphate oxidizes LBT hydrochloride resulting in the formation of phenoxyl radical which on coupling yields green coloured dimerization product.

2. EEPERIMENTAL

2.1. Apparatus

A Spectronic 20 D^+ spectrophotometer (Milton Roy, USA) with 1 cm glass cell was used for recording spectra and absorbance measurements. The pH values of the solutions were measured with an Elico LI-120 pH meter (Hyderabad, India).

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2.2. Chemicals and Solutions

All the reagents were of analytical grade. Reference standard sample of labetalol (LBT) hydrochloride was obtained from Sigma (USA). The commercial pharmaceutical preparations of LBT hydrochloride such as lobet 100 (Samarth Pharma., India) and gravidol 100 (Mercury Lab., India) were purchased from local market. Ferric ammonium sulphate was obtained from Loba Chemie Pvt. Ltd, India.

Sodium acetate-hydrochloric acid buffer (pH 3.49) was prepared by mixing 50 mL of 1.0 M sodium acetate solution with 46.25 mL of 1.0 M HCl and diluted to 250 mL with distilled deionized water. Carbonate buffer (pH 9.4) was prepared by dissolving 26.5 gm sodium carbonate and 21.0 gm sodium bicarbonate in 500 ml distilled deionized water. Distilled deionized water was obtained from a Milli-Q Synthesis A10 water purification system. A standard solution of LBT hydrochloride (1 mg/mL) was prepared in distilled deionized water. The solution was used to prepare calibration curves and quality control samples. Quality control samples prepared at three concentration levels: 40.0, 110.0 and 180.0 µg/mL. Ferric ammonium sulphate of 4.15×10^{-3} M was prepared in distilled deionized water.

2.3. Construction of LBT Hydrochloride Standard Curve and Determination of LBT Hydrochloride

Appropriate volumes of standard solution (1.0 mg/mL) of LBT hydrochloride in the concentration range of 10-200 µg/mL were pipetted into a series of 10 mL volumetric flasks. To each flask, 1.8 mL acetate – hydrochloric acid buffer of pH 3.49 was added followed by 2.8 mL of 4.15×10^{-3} M ferric ammonium sulphate solution and completed to volume with distilled deionized water (total reaction mixture is 10 mL). The mixture was shaken well and allowed for 2 min at room temperature to complete the reaction. Absorbance was measured at 535 nm as a function of time against the reagent blank. Prior to this, wavelength scanning of labetalol (LBT) hydrochloride was conducted using a spectrophotometer (Milton Roy, USA) in the range of 300 to 620 nm with a reaction mixture composed of 1 ml of 100 µg/mL lebatalol hydrochloride, 1.8 mL acetate – hydrochloric acid buffer (pH 3.49), 2.8 mL of 4.15×10^{-3} M ferric ammonium sulphate solution and 4.4 mL distilled deionized water. The calibration curve was then constructed by plotting the absorbance values vs. the initial concentration of LBT hydrochloride. The amount of drug, LBT hydrochloride was calculated either by using the calibration curve or the corresponding regression equation.

2.4. Optimization of Variables

In order to develop an efficient analysis technique, all the necessary parameters including pH of reaction mixture, volume of acetate – hydrochloric acid buffer, and amount of ferric ammonium sulphate were optimized based on single factor experiments (vary one parameter and fix others). The effect of pH on reaction was performed over the pH range 3.09 – 5.20 using the acetate – hydrochloric acid buffer with different volume. The effect of volume of 4.15×10^{-3} M ferric ammonium sulphate solution was examined in the range 0.1 – 3.1 mL.

2.5. Determination of LBT Hydrochloride in Pharmaceutical Formulations

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Ten tablets (100 mg of LBT hydrochloride per tablet) were finely grounded using a homogenizer and then added into 100 mL distilled deionized water. After 10 min of shaking, the solution was filtered through Whatmann 42 filter paper (Whatmann International Limited, Kent, UK) in a 500 mL volumetric flask. The residue was washed three times with 10 mL of distilled deionized water and then diluted with 100 mL distilled deionized water. It was further diluted according to the need and then analyzed following the proposed procedure.

2.6. Determination of LBT Hydrochloride in Biological Fluids

Aliquot volumes of human serum and urine samples were collected from local hospital and stored in a refrigerator at 0-5^{o}C before using them for experiment. Both the samples were transferred into a small separating funnel, separately. Prior to analysis, a known amount of LBT hydrochloride was spiked into the samples. Carbonate buffer (5 mL, pH 9.4) was added and mixed well with each sample. The LBT hydrochloride extraction from both biological fluids was done three times using 5 ml of diethyl ether with hand shaking the funnel for 20 min (liquid-liquid extraction). The ether extract (supernatant) was collected and then evaporated the ether using a Fume hood at room temperature. Since boiling point of ether is 34.6^{o}C, it evaporates quickly at room temperature. The residue was then dissolved in 5 ml of distilled deionized water and the procedure mentioned above was then followed to analyze LBT hydrochloride. The nominal content of LBT hydrochloride was estimated from the corresponding regression equation.

2.7. Statistics

Mean (X) was measured from five independent determinations for all data points. Standard deviation (SD), relative standard deviation (RSD), standard analytical error (SAE), confidence limit (C.L.) at 95%, and interval hypothesis test were calculated in order to validify the experimental data. Microsoft Excel was used for all statistical data analysis.

3. RESULTS AND DISCUSSION

It is well known that labetalol (LBT) hydrochloride possesses phenolic group (-OH) in its moiety and hence oxidized by Fe^{3+} resulting in the formation of phenoxyl radical which on dimerization yields highly colored product. The colored product absorbs maximally at 535 nm (supplementary Fig. 1). It is important to note that phenols are susceptible to oxidation as they readily donate electron (s) to the oxidizing agents. Strong oxidizing agents such as potassium permanganate causes the rupture of the benzene ring. The interaction of milder oxidizing agents such as Fe^{3+} with the phenols leads to the formation of resonance stabilized phenoxyl radical. This radical soon undergoes dimerization resulting in the formation of highly colored product. The formation of colored product has been utilized to develop a spectrophotometric method for the determination of LBT hydrochloride in both commercial dosage forms and biological fluids by measuring the absorbance at 535 nm. The reaction stoichiometry between Fe^{3+} and LBT hydrochloride has been evaluated by Job's method of continuous variations. Figure 1 shows that the absorbance is reached to a maximum value at a mole fraction of 0.5 indicating that the stoichiometric ratio of the maximum formation of complex between Fe^{3+} and LBT hydrochloride is 1:1. The results also indicated that the absorbance decreases after the mole fraction of 0.5. This is due the lesser formation of complex, which is in good agreement with previous literature report [27].

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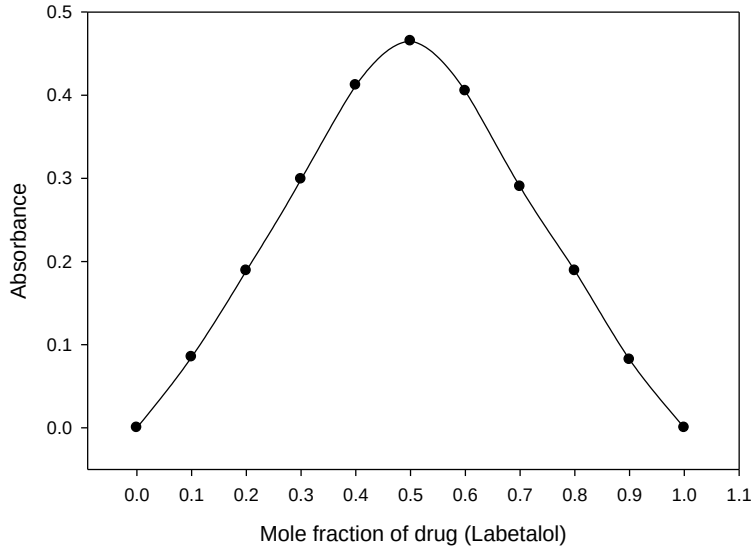

Figure 1. Job's plot for stoichiometric ratio between labetalol (LBT) hydrochloride and ferric ammonium sulphate (4.11×10^{-3} M each). Absorbance was measured at 535 nm.

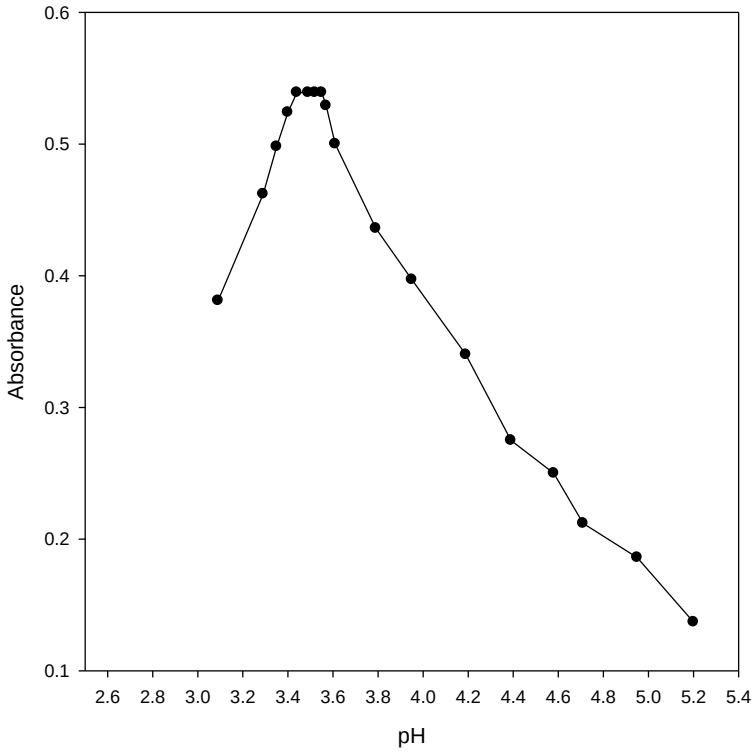

Figure 2. Effect of pH on the absorbance (at 535 nm) of the green colored dimerization product. The product is formed due to the coupling reaction between labetalol (LBT) hydrochloride and ferric

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ammonium sulphate.

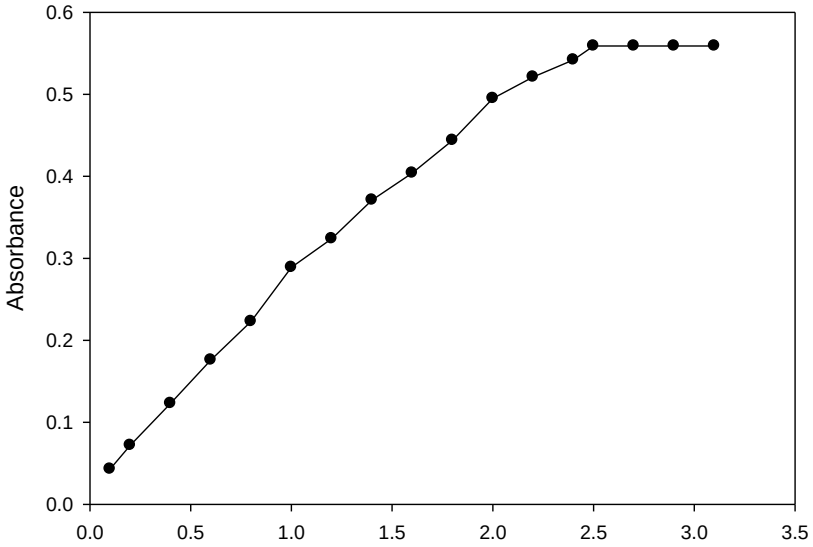

Figure 3. Effect of volume of 4.15 × 10^{-3} M ferric ammonium sulphate on the absorbance (at 535 nm) of the green colored dimerization product. The product is formed due to the coupling reaction between labetalol (LBT) hydrochloride and ferric ammonium sulphate.

3.1. Optimizations of Reaction Variables

For optimization of experimental conditions affecting the development of color such as pH, volume of sodium acetate-hydrochloric acid buffer, and volume of 4.15 ×10^{-3} M ferric ammonium sulphate solutions have been studied. The reaction was performed over the pH range 3.09 – 5.20 using the sodium acetate – hydrochloric acid buffer system. The maximum and constant absorbance was found in the pH range 3.44 – 3.55, which is shown in Figure 2. Thus, a buffer solution of pH 3.49 was used in all subsequent studies. Concerning the effect of volume of sodium acetate – hydrochloric acid buffer solution of pH 3.49, it was found that 1.5 mL gave the maximum absorbance; higher volumes did not affect the color intensity (data not shown). Therefore, 1.8 mL of the pH 3.49 buffer solution was taken as optimum volume. The effect of volume of 4.15 × 10^{-3} M ferric ammonium sulphate solution was examined in the range 0.1 – 3.1 mL. The maximum absorbance was obtained with 2.5 mL; higher volumes caused no change in the absorbance value. This is because the total volume of reaction mixture was fixed and the stoichiometric ratio for maximum formation of complex between Fe^{3+} and LBT was 1:1, which is mentioned in the previous section. Our observations are in agreement with the results of previous studies [27,28]. Thus, 2.8 mL of 4.15 × 10^{-3} M ferric ammonium sulphate was taken as a optimum value throughout the experiment (Fig. 3).

3.2. Validation of Assay Performance in Pharmaceutical Formulations

Under the described experimental conditions, a calibration curve was constructed by plotting absorbance against the initial concentration of LBT hydrochloride, which is shown in supplementary Figure 2. The

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absorbance was linearly related to the drug concentration over the range 10 – 200 µg/mL with molar

Table 1: Test of precision of the proposed method for determination of labetalol (LBT) hydrochloride.

	Concentration (µg/ml)		RSD[a] (%)	SAE[b]	C.L.[c]
	Theoretical	Found ± SD[a]			
Intra day assay					
	40.0	40.019 ± 0.171	0.43	0.077	0.213
	110.0	110.019 ± 0.143	0.13	0.064	0.178
	180.0	179.985 ± 0.195	0.11	0.087	0.242
Inter day assay					
	40.0	40.053 ± 0.223	0.56	0.100	0.277
	110.0	109.985 ± 0.195	0.18	0.087	0.242
	180.0	179.985 ± 0.094	0.05	0.041	0.116

[a] Mean for five independent determinations.

[b] SAE, standard analytical error.

[c] C.L., confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

Table 2. Determination of labetalol (LBT) hydrochloride in commercial tablets by proposed method and official BP method.

Proposed method	Recovery (%)	RSD (%)	Reference method	
			Recovery (%)	RSD (%)
Formulation				
Lobet 100	99.85	1.96	100.19	1.77
	θ_L = 0.993	θ_U=1.004		
	t = 0.246	F= 1.200		
Gravidol 100	100.53	1.70	99.89	1.48
	θ_L = 0.989	θ_U=1.003		
	t = 0.400	F= 1.335		

Theoretical values: t = 2.447 (degree of freedom = 6) and F = 9.28 at 95 % confidence level

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Table 3. Application of the proposed spectrophotometric method for the determination of labetalol hydrochloride in urine samples.

Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)
10.0	10.02	100.20
20.0	20.03	100.15
30.0	29.12	97.08
40.0	40.05	100.03
50.0	49.88	99.76
100.0	99.20	99.20
Mean (X)		99.40
RSD		1.34

Table 4. Application of the proposed spectrophotometric method for the determination of labetalol hydrochloride in low concentrated plasma samples.

Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)
10.0	9.82	98.20
20.0	19.62	98.10
30.0	29.50	98.33
40.0	39.21	98.03
50.0	50.10	100.20
60.0	58.90	98.16
Mean (X)		98.50
RSD		0.93

absorptivity of 2.07 $\times 10^3$ L moL^{-1} cm^{-1}. The detection and quantitation limits were found to be 1.14 and 3.45 µg/mL, respectively. The linearity of the calibration curve is validated by the high value of correlation coefficient ($r^2 = 0.9998$) of the regression equation.

The precision of the proposed method was established by determining the content of LBT hydrochloride in quality control samples at three different concentration levels: 40, 110 and 180 µg/mL. The intra–day

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precision was evaluated by carrying out five independent analyses at each concentration level within 1 day. Similarly, the inter–day precision was also performed through replicate analysis at each concentration level for five consecutive days, which is shown in Table 1. The data clearly showed the high precision of the proposed method.

The developed method was applied to the determination of LBT hydrochloride in commercial tablets, namely Lobet 100 and Gravidol 100. The commercial tablets also contain the ingredients such as corn starch, hydroxypropyl methylcellulose, lactose, magnesium stearate, methyl paraben, sodium benzoate, talc and titanium dioxide. The results of the proposed method were compared with those obtained by the reference, British Pharmacopeia (BP) method [26], which is shown in Table 2. British Pharmacopeia (BP) method was just selected as a reference method because it is more popular and widely used in Asia. The calculated t- and F- values did not exceed the theoretical values at 95 % confidence level and therefore, we can conclude that the proposed method does not differ significantly from the reference method. The interval hypothesis [29] test has also been performed to compare the results of the proposed method with those of reference method at 95 % confidence level. In pharmaceutical analysis, a bias based on recovery experiments of ± 2 % is acceptable. Thus the limit of acceptance interval is within θ_L= 0.98 and θ_U = 1.02. The results presented in Table 2 shows that the true bias of both the samples is less than ± 2 %, indicating the compliance of regulatory guidelines [30]. This study also suggested that the excipients present in tablets did not interfere with the assay performance.

3.3. Validation of Assay Performance in Biological Samples

The proposed method was further extended to the *in vitro* determination of labetalol (LBT) hydrochloride in human plasma and urine samples in the proposed linearity range. For this, a known amount of LBT hydrochloride was spiked into the sample prior to analysis. The results of analysis of urine and plasma samples are summarized in Table 3 and Table 4, respectively. The data obtained by the method for both cases indicated that the mean recoveries and RSD values were in the range of 98.5 – 99.4% and 0.93 - 1.34 %, respectively. In addition, the data clearly showed that the complicated biological matrix did not interfere the signal. Overall, the proposed method was satisfactorily accurate and precise.

4. CONCLUSION

In this study, a simple and rapid spectrophotometric method for LBT hydrochloride estimation has been proposed since it does not require any pretreatment of the drug and tedious extraction procedure. The method obeys Beer's law in the concentration range 10 – 200 µg/mL with good accuracy and precision. The interval hypothesis test has revealed that the bias based on recovery experiments is lower than ± 2 %. The statistical data for the determination of LBT hydrochloride in both drug formulations and biological samples demonstrated that the proposed method is accurate, precise and linear. We therefore conclude that the present technique could be a cheap, simple, rapid, and sensitive pivotal platform, especially for the third world counties in order to make routine analysis of LBT hydrochloride in pharmaceutical industries and hospitals.

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