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STABILITY INDICATING RP – HPLC METHOD FOR DETERMINATION OF LABETALOL HCL IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of Labetalol HCL in pure and tablet form.. The method showed a linear response for concentrations in the range of 10-50µg/ml using Methanol: Water solution in the ratio (70:30) as the mobile phase with detection at 246 nm and a flow rate of 1 ml/min and retention time 6.3min. The value of correlation coefficient, slope and intercept were, 0.999, 15.30and7.019, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability indicating one.

KEYWORDS: labetalol HCL, RP-HPLC, Degradation studies.

INTRODUCTION

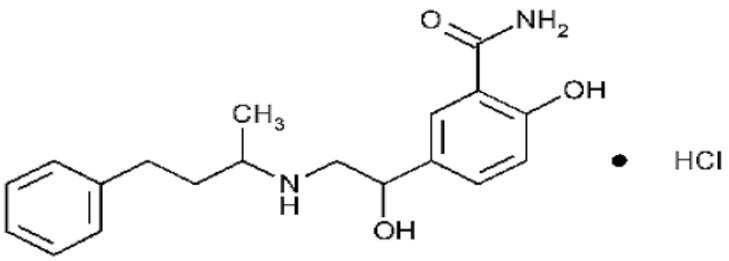

Fig.1 Chemical structure of labetalol HCL

Labetalol hydrochloride, 2-hydroxy- 5- [1- hydroxy- 2- (1- methyl - 3 phenyl propyl amino) ethyl] benzamide hydrochloride, is an adrenergic β-receptor blocking agent used in the treatment of hypertension, which exhibits both α- and β-adrenoceptor blocking activity[1]. 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, 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. Therefore, it is required to develop sensitive, selective, rapid and simple analytical method for determination of LBT hydrochloride in pharmaceutical samples Various analytical techniques including spectrofluorimetry , thin layer chromatography (TLC) high performance liquid chromatography (HPLC) , liquid-chromatography masss pectrophotometry (LC-MS) , gas chromatography (GC), micellar liquid chromatography, capillary liquid chromatography, capillary electrophoresis capillary isotachophoresis, NMR spectroscopy, ion-selective electrode, and adsorptive voltammetric method 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.[2]

Labetalol is extensively prescribed in the market and so there is a great need to develop a rapid simple analytical method for the determination of Labetalol in pharmaceutical preparation to be used in routine quality control laboratories. In the present work an accurate, sensitive and selective reversed-phase liquid chromatographic method for the determination of Labetalol HCL was developed. Different chromatographic conditions were carefully studied in an attempt to optimize the parameters for the evaluation of the studied compound in pure form and in tablets. The stability-indicating capability of the method was also investigated.

EXPERIMENTAL

MATERIALS AND METHODS

Gift sample of Labetalol (LBT) was received from flemingo. Commercial formulations LOPIH^{TM} containing 100mg of Labetalol were purchased from the local market.

INSTRUMENTATION

The analysis of the drug was carried out on a Younglin (S.K) Gradient System UV Detector. Equipped with a Reverse phase (Thermo) C18 Column (4.6mm x 250 mm; 5µm), a SP930D pump, a 20µl injection loop and a UV730D Absorbance detector and running on Autochro-3000 software.

Chromatographic conditions

Mobile phase consist mixture of methanol-water in the ratio of 70:30%v/v (PH was adjusted with 0.05% OPA). The mobile phase was pumped from the solvent reservoir to the column at flow rate 1ml/min. column temperature was maintained at Ambient. UV Detection performed at 246nm.the mobile phase was degassed by an ultrasonic water bath for 5min.filter through 0.45µ filter under vacuum filtration. The column was equilibrated for at least 30min with mobile phase flowing through the system. Mobile phase used as diluents during the standard and test sample preparation.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 246 nm Wavelength was selected.

Preparation of Standard Solutions

Accurately weigh and transfer 10mg of LABETALOL HCL working standard into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent To get 1000µg/ml standerd (Stock solution) Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents to get 20µgml solution. Mix well and filter through 0.45µm filter**.** The simple chromatogram are shown in fig no. 2

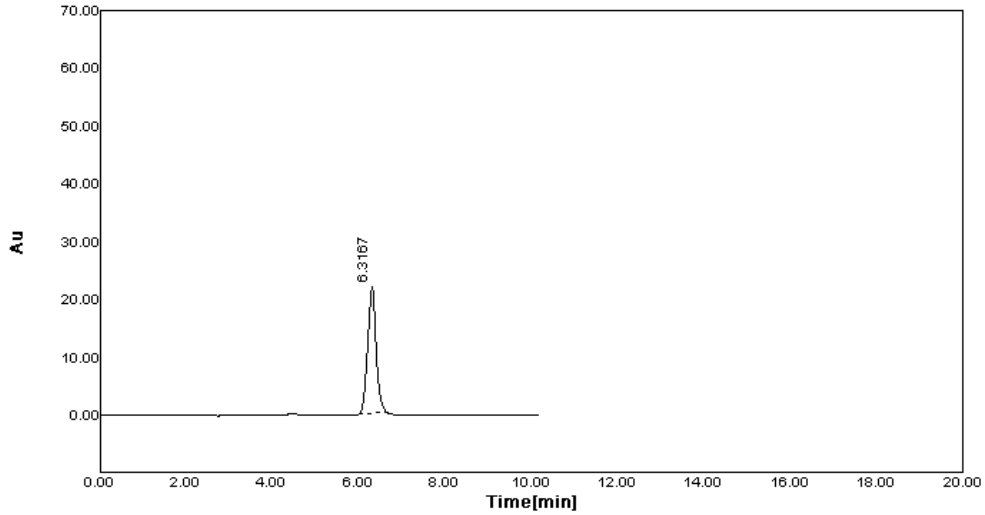

Fig No.2 The Simple Chromatogram of Standard Labetalol HCL.

Preparation of calibration graph

The linearity of response for labetalol HCL assay method was determined by preparing and injecting solutions with concentrations of about 10,20, 30,40,50µg/ml of labetalol HCL. Linearity curve of labetalol HCL shown if fig. no.3.

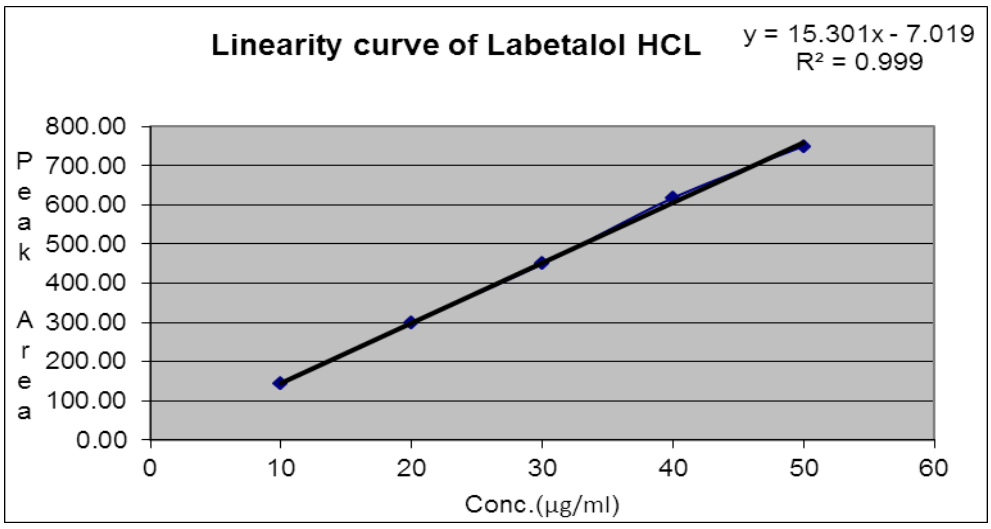

Fig.No.3 Linearity curve of labetalol HCL

Sample Solution Preparation

Weigh 20 labetalol Tablets and calculate the average weight. accurately weigh and transfer the sample equivalent to 10 mg of labetalol into a 10 ml volumetric flask. Add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4ml of the

above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.(40µg/ml).The simple chromatogram of test labetalol HCL shown in fig. no.4.

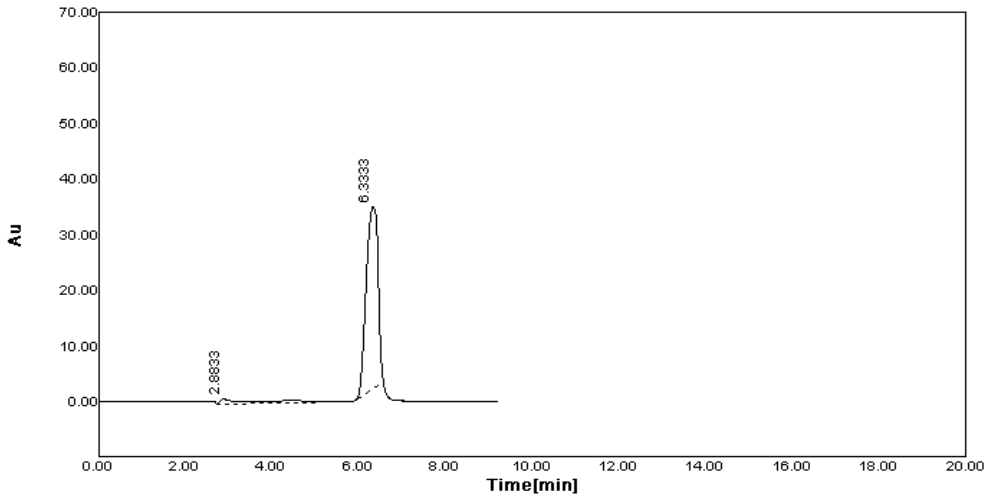

Fig. no.4 Simple Chromatogram of test Labetalol HCL

Forced degradation studies

In order to determine whether the analytical method and assay were stability-indicating, LBT tablets and LBT active pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 1N HCl), base hydrolysis (using 0.1N NAOH), oxidative degradation (using 3.0% H2O2) and thermal treatment (heated at 80°C for two days) to evaluate the ability of the proposed method to separate LBT from its degradation products. LBT at a concentration of 20µg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

Acid Degradation

Forced degradation in acidic media was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 1N HCl to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 1 hr and neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 20µg/ml. chromatograms for acid degradation studies were shown in fig 5.

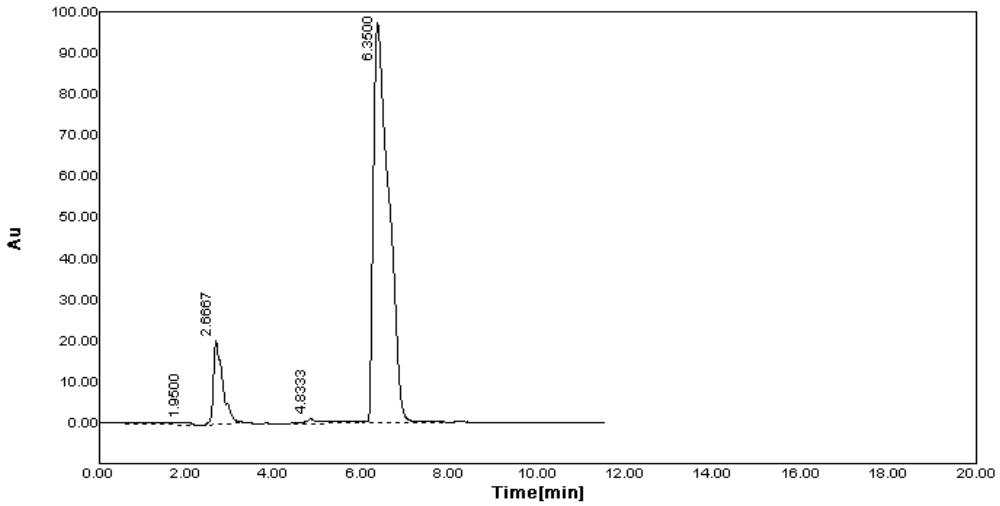

Fig no.5 Chromatogram of Acid Degraded Sample

Base Degradation

Forced degradation in alkaline media was performed using 0.1N NaOH. The representative Chromatograms for alkaline degradation studies were shown in fig-6 respectively.

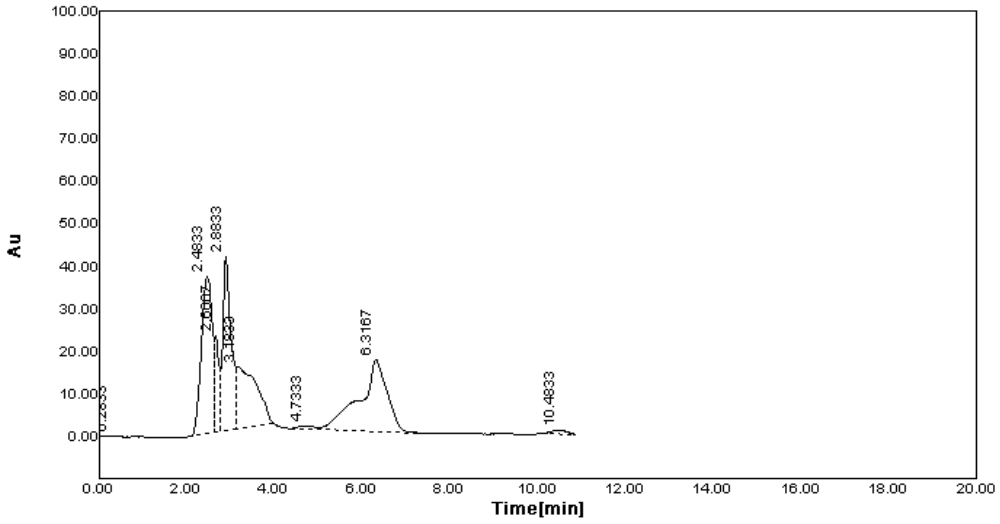

Fig no.6 Chromatogram of Base Degraded sample

Neutral Degradation

Neutral degradation in alkaline media was performed using water. The representative chromatograms for Neutral degradation studies were shown in fig-7 respectively.

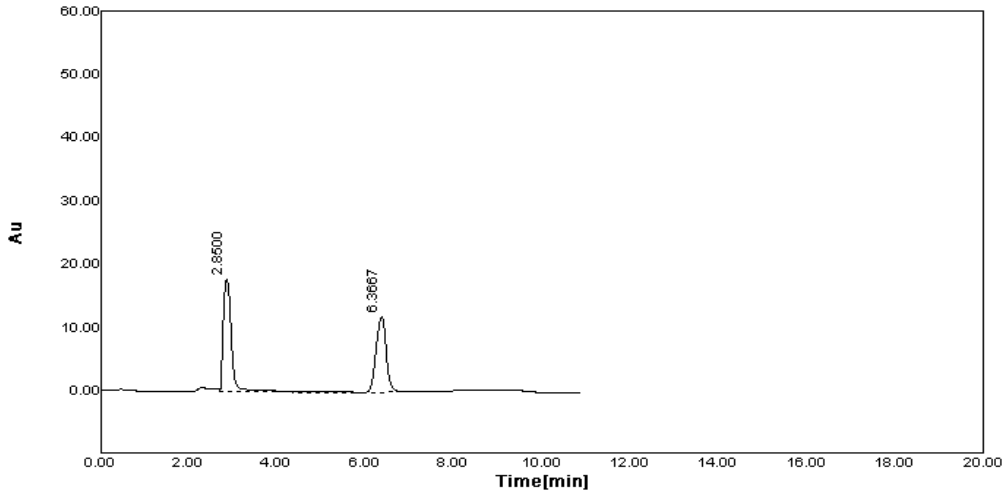

Fig no. 7 Chromatogram of Neutral Degraded sample

Oxidative degradation

Oxidative degradation was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 3%w/v of hydrogen peroxide to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 1hr. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 20µg/ml. The representative Chromatogram was shown in fig-8.

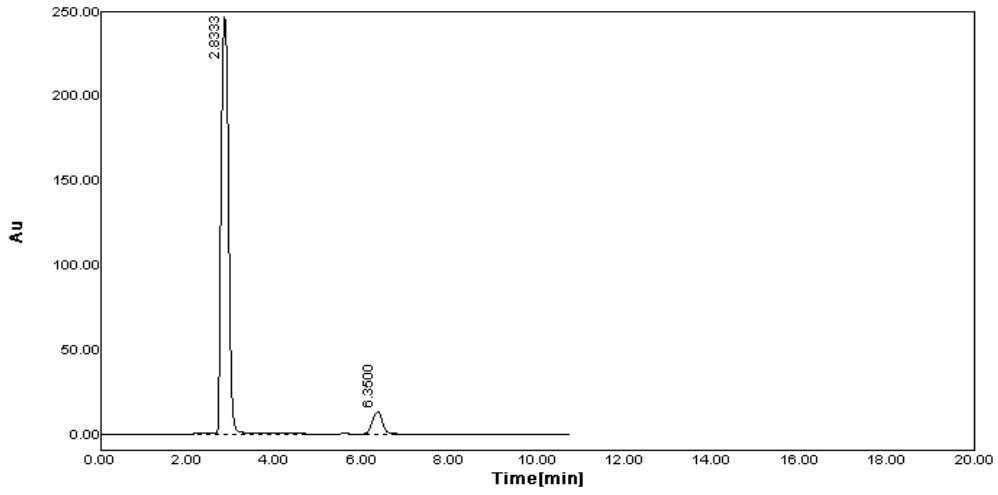

Fig. no. 8 Chromatogram of Oxidative degraded sample

Thermal stress studies

For thermal stress, LBT - API, tablet powder and solutions of both were prepared and exposed to a controlled-temperature oven at 80^{o}C for 3 hours. The representative chromatogram was shown in fig-9.

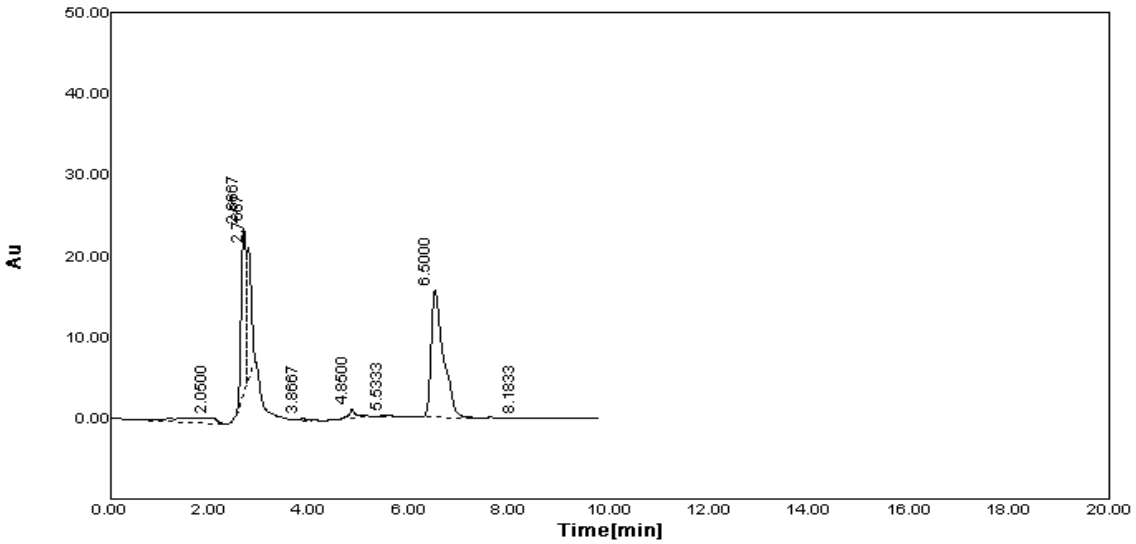

Fig no. 9 Chromatogram of thermal stress

Table No. 1 : Stress Study of Labetalol HCL.

Stress Condition	Time in hr	Mean peak area	% recovery of analyte	Retention time of analyte
Acid hydrolysis (0.1N HCL)	1hr	263.014	79.42	6.3500
Base hydrolysis (0.5N NAOH)	1hr	738.82	243.74	6.3167
Oxidation (3%H2o2)	1hr	211.2263	14.26	6.3500
Neutral	1hr	191.2933	12.96	6.3667
Thermal (80^0c)	3hr	267.5669	89.73	6.500

Validation of the Method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery and robustness/ruggedness.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve Shown in fig no.2.The constructed calibration curves were linear over the concentration range of 10– 50µg/ml. Peak areas of LBT HCL was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve Correlation coefficient (n=3) was found to be more than 0.999 with %RSD values were less than 2% across the concentration ranges studied. Typically, the regression equation was: $y = 15.30x - 7.019$ ($R^2 = 0.999$).

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no.2.

Table No.2 Repeatability

Sr. no.	Conc. (µg/ml)	Area	Amt found	% Label Claim
1	20	299.5751	20.04	100.19
2	20	294.6315	19.72	98.58
3	20	298.5757	19.97	99.87
4	20	295.6323	19.78	98.91
5	20	299.7575	20.05	100.25
6	20	298.6464	19.98	99.89
7	20	299.5751	20.04	100.19
8	20	294.6315	19.72	98.58
9	20	298.5757	19.97	99.87
10	20	295.6545	19.78	98.91
	Mean	297.52553	19.90	99.52
	S.D.	1.99	0.13	0.66
	%RSD	0.7145	0.6622	0.6622

Limit of Detection and Limit of Quantitation

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (S) of the calibration plot using the formula LOD = 3.3 σ/S and LOQ =10 σ/S. The limit of quantitation (LOQ) of the present method was found to be 0.72 µg/ml with a resultant %RSD of 0.29% (n = 5). The limit of detection (LOD) was found to be 0.24µg/ml.

Precision

Precision was evaluated in terms of Intraday and Interday precisions. Intraday precision was determined by analyzing sample solutions of LBT from formulations at three levels covering low, medium, and higher concentrations of calibration curve for five times on the same day. Inter day precision was determined by analyzing sample solutions of LBT at three levels covering low, medium, and higher concentrations over a period of seven days (n=5). The peak areas obtained were used to calculate mean and %RSD (relative SD) values.

Table No.3 Precision

Conc.(µg/ml)	Inter day			Intra Day		
	Mean± S.D.	Amt. Found	% Amt. Found	Mean± S.D.	Amt. Found	%Amt. Found
20	299.57±0.00	20.04	100.19	299.65±0.00	20.04	100.22
30	447.84±0.55	29.73	99.10	448.72±0.57	29.79	99.29
40	618.91±0.08	40.91	102.28	616.27±0.41	40.74	101.85

*mean of each 3 reading

Accuracy

Accuracy data for the assay following the determination of the compound of interest is summarized in Table-4.Accuracy was determined by interpolation of replicate (n=5) peak areas of three accuracy standards of different concentration, from a calibration curve that had been prepared as previously described. In each case, the percent relevant error and accuracy was calculated in Table no.4. Accuracy data (n=5)

Table No.4 Accuracy

Sr. no.	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
1	80	10	8	239.617 ± 0.698	8.12 ± 0.05	101.50± 0.57
2	100	10	10	299.4775± 0.21	10.03± 0.01	100.32 ± 0.14
3	120	10	12	359.576± 0.06	11.96± 0.00	99.67 ± 0.03

Specificity

The results of stress testing studies in addition to that of monitoring standard solutions of the drug in the presence of their impurities indicated a high degree of specificity of this method. The degradation product(s) of the parent compound was found to be similar for both the tablets and API powder. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating.

Ruggedness/Robustness

As recommended in the ICH Guidelines, a robustness assessment was performed during the development of the analytical procedure. The ruggedness of the method is assessed by comparison of the intra- and inter-day assay results that has been performed by two analysts. The %RSD values for intra- and inter-day assays of LBT tablets performed in the same laboratory by two analysts did not exceed 1.8%, indicating the ruggedness of the method. In addition, the robustness of the method was investigated under a variety of conditions including changes of flow rate, wavelength, mobile phase composition and column temperature,. The degree of reproducibility of the results obtained as a result of small deliberate a variations in the method parameters has proven that the method is robust and the data was summarized in Table no. 5.

Table No. 5 Ruggedness/Robustness

Parameters	Conc.	Amount of labetalol detected(mean ±SD)	%RSD
Mobile phase composition-(69:31)	30	370.1235 ± 0.80	0.21
Mobile phase composition-(71:29)	30	447.37 ± 1.01	0.22
Wavelength change247nm	30	419.6 ± 0.39	0.09
Wavelength Change 245nm	30	419.73 ± 0.34	0.08
Flow rate change(1.1ml)	30	466.00 ± 1.31	0.28
Flow rate change(0.9ml)	30	538.99 ± 0.84	0.16

Table no.6 System suitability parameter

SR.NO.	Parameters	Result
1	Range(µg/ml)	10-50µg/ml
2	Detection wavelength	246nm
3	Mean R^2 value	0.999
4	Slop(m)	15.30
5	Intercepts(c)	7.019
6	Run Time (min.)	15
7	Retention Time (min.)	6.33
8	Theoretical plate (N)	4096.6
9	Tailing Factor	0.9567
10	LOD	0.24
11	LOQ	0.72

Assay

The validated method was applied to the determination of Labetalol HCL in commercially available LOPIH™ tablets. Figure 2 and Figure 4 illustrates two typical HPLC chromatograms obtained from Labetalol HCL standard solution and from the assay of LOPIH™ tablets respectively. The results of the assay (n = 9) undertaken yielded 100.98% (%RSD = 0.13%) of label claim for LBT HCL. The observed concentration of LBT HCL was found to be 40.38±0.52µg/ml (mean±SD). The mean retention time of Labetalol was 6.33 min. The results of the assay indicate that the method is selective for the analysis of Labetalol HCL without interference from the excipients used to formulate and produce these tablets.

Table no.7 Label Claim

Sample	Label Claimed	% Label Claim* ± SD	% RSD
LOPIH™	Labetalol HCL 100mg	100.98 ± 0.13	0.13

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Labetalol HCL preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Methanol and water were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol : Water 70:30(v/v). The retention time of Labetalol HCL was found to be 6.333 min, respectively. The RSD values for accuracy and precision studies obtained were less than 2%which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 6. The average recovery was found to be 100.49% for Labetalol HCL indicating that the proposed method is highly accurate. The LOD and LOQ were found to be 0.72µg/ml and 0.24µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. LBT HCL were found to be relatively stable following photolysis and Thermal degradation. Considerable degradation was observed for both in oxidation, acid and base hydrolysis. The validated method was applied to the determination of LBT HCL in commercially available LOPIH^{TM} tablets. The results of the assay indicate that the method is selective for the analysis of Labetalol HCL without interference from the excipients used to formulate and produce these tablets.

CONCLUSIONS

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of Labetalol HCL in API and tablet dosage forms. The results of stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate the analyte from their degradation products, related substances; excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

ACKNOWLEDGEMENTS

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