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Research Article

RP-HPLC Method Development and Validation for Determination of Labetalol in Bulk and Marketed Formulation.

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ABSTRACT
The objective of present work is to develop, simple efficient and reproducible method by RP-HPLC for labetalol and marketed formulation. The RP-HPLC method development and validation for determination of labetalol are carried by intersil ODS C 18 (250mm X 4.6 mm) column using methanol phosphate 70:30 PH 6.5 as a mobile phase, flow rate 1 ml /min ,column temperature is ambient detection of wavelength was carried by UV visible spectrometer (Schimadzu) 302 nm. The method was validated in terms of specificity, linearity, Limit of detection, Limit of quantification values of known impurities were found between 0.260 µg /ml and 0.788 µg/ml and percentage recovery value 101% at different concentration level. The correlation coefficient was found <0.999.

KEYWORDS
Labetalol, method, development, RP-HPLC.

1. INTRODUCTION

Labetalol is mixed α/β-adrenergic antagonist, which is used to treat high blood pressure. The minimum requirement for adrenergic agents is a primary or secondary amine separated from a substituted benzene ring by one or two carbons. This configuration results in strong agonist activity. As the size of the substituent attached to the amine becomes greater, particularly with respect to a t-butyl group, then the molecule typically is found to have receptor affinity without intrinsic activity and is, therefore, an antagonist. Labetalol, with its 1-methyl-3-phenylpropyl substituted amine, is greater in size relative to a t-butyl group, and therefore, acts predominantly as an antagonist. The overall structure of labetalol is very polar. This was created by substituting the isopropyl group in the standard β-blocker structure with an aralkyl group, including a carboxamide group on the meta position, and by adding a hydroxyl group on the para position structure of labetalol is shown below.

Fig 1.**Structure of labetalol**

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 mass spectrophotometry (LCMS) , 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 we develop a method validation for labetalol and its marketed formulation.

The importance of anti-hypertensive drugs is increasing day by day for management & control of hypertension. α1, β1, & β1 blocker are generally used for prevalent disease hypertension. Labetalol is α1, β1, & β1 blocker and used as a life saving molecules for the treatment of hypertension. Quantification of drug molecule is important task for routine analysis of API in its

finished product analysis. Literature survey revealed very few analytical procedures for routine analysis of Labetalol. In addition the reported methods are complex, time consuming and costly. Therefore, the present work is proposed to develop and validate simple, precise and economic method for routine analysis of Labetalol in bulk and tablet dosage form.

1.1. Objectives of the study

1. To develop RP-HPLC method for Labetalol
2. To validate the developed method as per ICH guidelines (Q_2R_1)
3. To explore the applicability of the method for quantitative estimation of Labetalol in dosage form during routine analysis

2. MATERIALS AND METHODS

Labetalol of pharmaceutical grade were used without further purification (95-110%w/w) methanol ,water ,potassium phosphate having HPLC grade are purchased from purchased from Merck (Worli, Mumbai). Labetalol 100 gram (Labamate, sunpharma) the chromatographic analysis was performed using LC2010CHT, company shimadzu on packed intersil ODS (250mmX4.6 mm C18 column in addition, wenser high precision balance (Model PGB-100, PH meter (Elico 127 Hyderabad India) and a sonicator Wenser ultra sonicator (WUC-4L)

2.1. Preparation of phosphate buffer

Accurately weighed 0.850 gm of potassium di-hydrogen phosphate (HPLC grade) and transferred to 500ml volumetric flask containing some amount of water and the volume was made up to the mark after the complete dissolution of potassium di-hydrogen phosphate. The pH of solution was adjusted to 6.5 using OPA (HPLC grade). The resulting mobile phase was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.

2.2. Preparation of Stock solution of Labetalol

Accurately weighed 10mg of Labetalol and transferred to 100ml volumetric flask containing a mixture of methanol and phosphate buffer in the ratio of 70:30. The volume was made up to the mark using same combination of mobile phase to obtain the final concentration of100 µg/ml. The resulting stock solution was filtered through 0.45µ membrane filter and degassed by ultra-sonication for three cycles each of 10 min.

2.3. Preparation of Working Solution

0.5ml stock solution was pipette out and diluted up to 10ml to obtain resultant solution of 5µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.

2.4. Method Optimization

Six replicates of this solution were injected and results were recorded for retention time (RT), area, tailing factor (symmetry factor) and theoretical plates. Mean, SD and % RSD were calculated for the results obtained as well as other parameters were also verified for acceptability level.

The column efficiency for Labetalol peak should not less than 2000 theoretical plates.

a. The tailing factor for peak, should not more than 2.0
b. % RSD for area shall not be more than (NMT) 2 and for RT NMT 0.5%

2.5. HPLC Method Validation

Determination of wavelength maxima (λ_{max})

Initially, 10µg/ml solution of Labetalol was prepared in the mixture of methanol: buffer of pH 6.5 (70: 30). This solution was subjected to UV analysis in qualitative mode to determine the absorption maxima (λmax). The UV spectrum obtained was as given in Figure 1 below and showed the absorption at different wavelengths as given in Table 3 below. The wavelength of 302 nm was selected for quantitative determination of Labetalol as given in further sections.

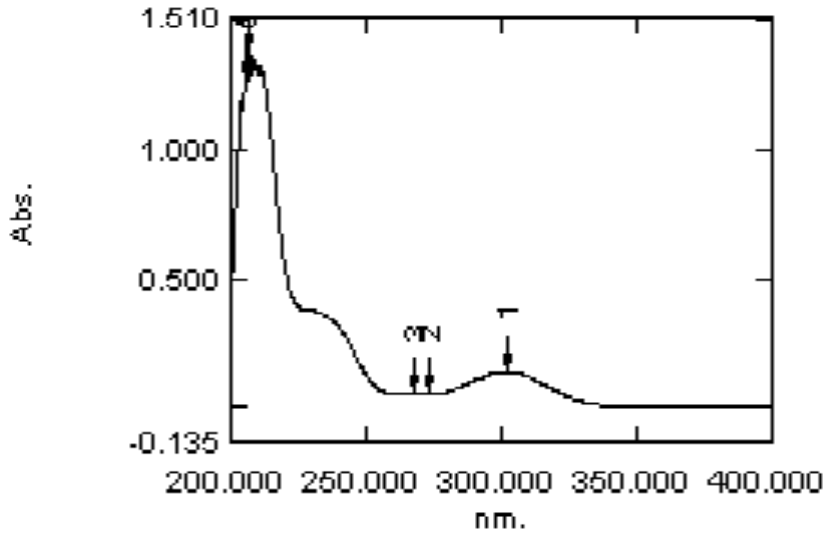

Fig 2. U.V. Spectrum of labetalol

Table1: Absorbance values obtain from UV spectrum of Labetalol

Sr. no	Wavelength (nm)	Absorbance
1.	302.10	0.440
2.	212.54	0.080

2.6. System suitability testing

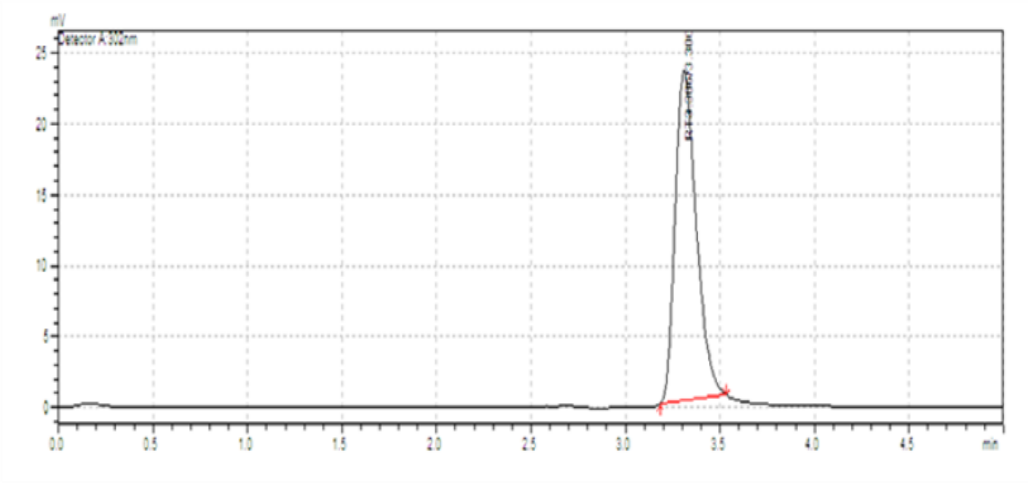

Fig 3. Result of system suitability testing

Table 2. Results of system suitability testing.

Sr. no	System suitability parameter	Mean*Observation	Limits	Inference
1.	Retention time (RT)in min	3.33	NLT 2.0	Passed
2.	Peak area in mV	25481.50	NLT 2000	Passed
3.	Theoretical plate	3162	NLT 2000	Passed
4.	Tailing factor	1.28	NMT 2.0%	Passed
5.	% RSD of RT	0.33	NMT 0.5%	Passed
6.	% RSD Of mean area	0.80	NMT 2.0%	Passed

2.7. Linearity

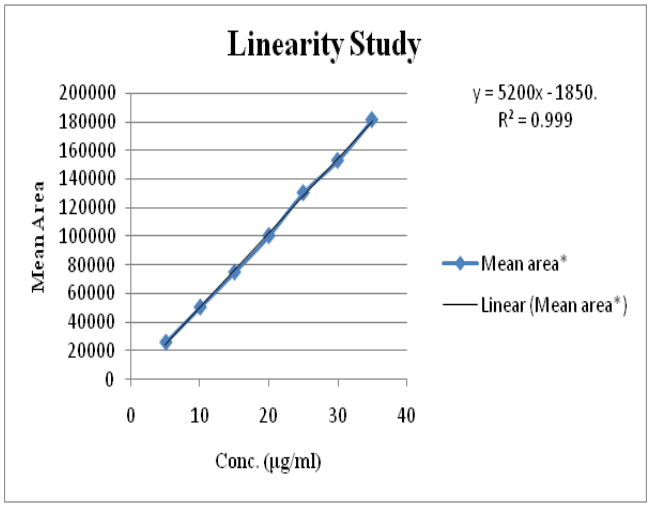

Fig 4. Calibration curve for labetalol

From stock solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5ml were pipetted out and diluted up to 10 ml to obtain 5, 10, 15, 20, 25, 30 and 35 µg/ml resultant solutions respectively. Each of these solutions was injected under optimized chromatographic conditions and area corresponding to the each concentration was determined. Calibration curve was constructed between concentrations versus peak area. From the calibration curve, the equation of line, slope, correlation coefficient and intercept were determined.

Y=mX+c ---- 1

Table 3. Observations obtained in determination of Linearity

Concentration (µg/ml)	**Mean area* (mV)**
5	25592
10	50211
15	74651
20	100206
25	130257
30	152784
35	181339

2.8. Precision

From the calibration range three quality control (QC) standards were decided viz. 7, 22 and 33 µg/ml as LQC, MQC and NQC respectively. The solutions for QC standards were prepared by diluting stock solution (100 µg/ml) of 0.7, 2.2, and 3.3 ml solutions up to 10ml. Area of each QC standard was recorded for intra-day and inter-day precision in six replicates as per ICH guidelines Q2R1. Results were recorded to calculate mean, standard deviation (SD) and percent relative standard deviation (%RSD).

Table 4. Results obtained for Precision study of the method

Sr. no	**Conc. (ug/ml)**	**Intra day**		**Inter day**	
		Mean Area* ± %	**RSD**	**Mean Area *± %**	**%RSD**
1.	7	38829.67± 199.70	0.51	3812O.78+ 409.89	1.08
2.	22	116489.67±732.76	0.37	116569.33+506.92	0.43
3.	33	171864.33±637.17	0.37	173401.00+340.27	0.20

*Mean of six replicate injections

2.9. Accuracy

Percent accuracy was determined using observations of precision study using following formula. Limit for % accuracy is NMT 5% RSD.

$$\textbf{\% Accuracy} = \frac{\text{Mean conc.} - \text{Nominal conc.}}{\text{Nominal conc.}}$$

Table 5. Results% accuracy determination from the precision data

Sr. no	Nominal conc.(ug/ml)	*Mean area	Mean measured conc.(ug/ml)	% Accuracy(w/w)	Inference
1.	7	38120.78	6.98	99.64	Passed
2.	22	116569.33	22.06	100.28	Passed
3.	33	173401.00	32.99	99.97	Passed

***** Mean of six replicate injections

2.10. Robustness

5µg/ml solution was selected for robustness study for the parameters like mobile phase proportion, and flow rate. Six replicates for parameters given in Table were injected and area for each of the parameter was recorded. The variation should not be more than 5% RSD. One factor was changed at time to estimate

Table 6. Results obtained robustness testing of the method

Sr. no	Conc. (ug/ml)	Robustness parameter	Variation	Mean RT	Mean area*(Mv)	% Assay	Limit (98-102%)
1.	5	Mobile phase composition	69.31	3.36	27678	99.33	Passed
2.	5	_	71.29	3.31	27403	98.28	Passed
3.	5	Flow rate (ml/min)	1.05	3.33	27387	98.21	Passed
4.	5	_	0.95	3.37	28145	101.13	Passed

***** Mean of three replicate injections the effect

2.11. Recovery

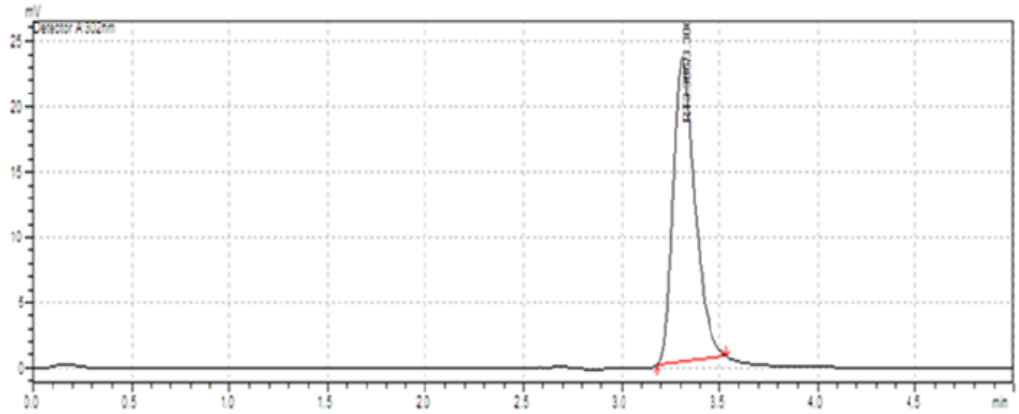

Fig. 5. HPLC Chromatogram for percentage recovery

Table 7. Results obtained in percent recovery study

Recovery level	Conc. Taken (ug/ml)	Amount added	Mean area*	Amount recovered	%Recovery.
80%	10	8	92396	18.12	100.69.
100%	10	10	101863	19.94	99.72
120%	10	12	115938	22.66	102.96

2.12. Preparation of stock solution of Labetalol

Accurately weighed 10mg of Labetalol (API) was added in volumetric flask containing some amount of mobile phase and volume was made up to the mark using mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. From the stock solution 1.0ml of stock was pipette out in triplicate and kept in three different volumetric flasks, cleaned previously and diluted up to 10ml by using mobile phase to obtain resultant solution of 10µg/ml. This solution was injected for given chromatographic system in triplicate and mean area was determined.

2.13. Preparation of stock solution of Labetalol Tablet

Twenty Tablets (Label claim 10 mg of Labetalol, Labamate, Sunpharma) were weighed, average weight was determined and powdered. Powder equivalent to 10mg (0.184gm) was transferred to 100 ml of Mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. From the stock 0.8, 1.0, 1.2 ml solutions were pipette out and diluted up to 10ml using mobile phase to obtain resultant solution of 8, 10 and 12µg/ml respectively.

2.14. Preparation of test solution for % recovery by spike method

10µg/ml solution of Labetalol (API) was spiked into each of above dilutions of 8, 10 and 12µg/ml to obtain solutions at 80%, 100% and 120% respectively. Each of these three levels was injected in triplicate and mean area for each level was determined. The mean area obtained on

API injection was subtracted from the mean area of each of these three levels to obtain area corresponding to test solutions. % recovery was determined from the calibration curve

2.15. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Table 8. Results for LOD and LOQ determination

Drug	LOD(ug/ml)	LOQ (ug/ml)
Labetalol	0.260	0.788

3. RESULTS AND DISCUSSION

Accurate, precise RP HPLC method is developed and validated as per ICH guidelines, for labetalol in bulk and its dosage form in this project

Table 9. Optical characteristics, precision and accuracy of the proposed method

Parameter	Values	ICH Guidelines
λ_{max}	302	--
% RSD	0.56	NMT 2.0%
LOD (ug/ml)	0.260	S/N>2 or 3
LOQ (ug/ml)	0.788	S/N > 10
Y = mx + c	5200x-1850 Slope: 5200	It should be linear, the %Y intercept as obtained from the linearity data should be within the $\pm$2
Correlation coefficient (R^2)	0.999	NMT 0.999
% Recovery	101.1	95-105%
Standard deviation	419.0	NMT 2%
% Accuracy	99.54	98-102%

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