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METHOD DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF LABETALOL HYDROCHLORIDE IN PURE AND TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise and sensitive spectroscopic method was developed for the estimation of Labetalol hydrochloride in the pure and tablet dosage forms. The estimation of Labetalol hydrochloride was carried out at the maximum absorbance at 246 nm. The method was found to be linear and obeys beers law in the concentration range 1-10µg/ml with a correlation coefficient 0.999. The developed method was validated as per ICH guidelines and was found to be accurate and precise. Thus the proposed method can be successfully applied for the estimation of labetalol hydrochloride in pure and tablet dosage form.

KEYWORDS

Spectrophotometric, Labetalol Hydrochloride, Validation and ICH guidelines.

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INTRODUCTION

Labetalol HCl is a selective α_1 and non-selective β adrenergic blocker used to treat high blood pressure. Chemically it is 2-hydroxy-5-{[1-hydroxy-2-(4 phenyl butane -2-lyl) amino]ethyl}benzamide. It has a molecular formula of $C_{19}H_{24}N_2O_3$.Hcl and a molecular weight of 328.40 g/mol and its structure was given in Figure No. 1.

Literature survey revealed, few analytical methods which include A Validated stability indicating HPTLC method for determination of labetalol HCl in combined dosage forms, [1]Determination of Labetalol HCl by chromatography[2], Stability indicating HPTLC

Determination of Labetalol HCl and its impurity by TLC Method, Validation HPLC method for the Estimation of Labetalol HCl and Aspirin in combined tablet Formulation, Simultaneous Determination on of Labetalol HCl and Aspirin in tablet formulation by HPLC and HPTLC. The present work deals with estimation of Labetalol HCl in pure API and tablets by UV- Spectrophotometry.

MATERIALS AND METHOD

Authentic drug sample of Labetalol HCL was given as a gift sample by MERCURY LABORATORIES Ltd, Hyderabad. Tablets of Labetalol HCL were procured from local Pharmacy, Distilled water was used for the preparation of solutions.

Instrument

Lab India -3000+ UV / Vis double beam spectrophotometer with a fixed slit width (2 nm) and 10 mm quartz cell was used to obtain spectrum and absorbance measurement.

Preparation of stock solution

100 mg of standard labetalol HCl drug was weighed, transferred to a 100 ml volumetric flask and dissolved in distilled water. The flask was shaken and volume was made up to the mark with distilled water to give a solution containing 1000mcg/ml.

Determination of absorbance maxima

From the above stock solution 10ml is taken in 100ml volumetric flask and made up to 100ml volumetric flask and made upto 100ml to give 100mcg/ml concentration and further dilution done and subjected for scanning between 200-400nm. Effect of dilution on the absorbance maxima was studied by diluting above Solution to 1-14mcg/ml and scanned from 200-400 nm, shown in Figure No.2.

For the standard solution analytical concentration range was found to be 1-14mcg/ml and those values were reported in Table No.1.

Appropriate volumes of aliquots from standard Labetalol HCl stock solution were transferred to different volumetric flacks of 10ml capacity. The volume was adjusted to the mark with distilled water to obtain concentration of 1, 2, 3, 4, 5, 6, 8, 10, 12, 14mcg/ml. Absorbance spectra of each solution against distilled water as blank were measured at 246 nm and the graphs of absorbance against concentration was plotted and are shown in Figure No.2. The regression equation and correlation coefficient was determined.

ANALYSIS OF MARKETED FORMULATION

20 tablets of labetalol hydrochloride were weighed, pulverized and the powder equivalent to 0.5 gm of labetalol was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in water. This solution was filtered through Whatmann filter paper number 40. 1 ml of above was diluted to 10 ml with water to obtain a solution of 100 µg / ml. Again 1 ml of above test solution was diluted to 10 ml with water in 10 ml standard volumetric flask to produce the concentration 10 µg/ ml. The concentration of labetalol hydrochloride in marketed formulation was determined in Table No.5.

Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of standard Labetalol HCl drug to the sample solution of the tablets. Precision for assay were determined by repeatability, interday, intraday precision for drug (each in three replicate).

Limit of Detection (LOD) and limit of Quantification (LOQ)

The LOD and LOQ of Labetalol HCl are determined by using calibration standards. Value of LOD is determined by using the formula: 3.3 σ / S and the Value of LOQ is determined by: 10σ/s where 'σ' is the standard deviation of the y intercept of the regression equation and 'S' is the slope of calibration curve.

RESULTS AND DISCUSSION

The absorbance maximum was recorded at wavelength of 246 nm which is shown in Figure No.3. Beers law range was confirmed by linear curve of labetalol hydrochloride, shown in Figure No.3. Linearity for labetalol hydrochloride is shown at concentration range of 1-10 µg/ml.

From the above studies the optical characteristics such as linearity range (1-12µg/ml), correlation coefficient (0.999), slope (0.018) and intercept (+0.008) were calculated and results were found to be satisfactory. Quantitative data subjected to statistical analysis. The % RSD values < 2 indicate the precision of

methodology. The accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 99.72 % - 100.81%. The values are given in recovery was confirmed and shown in the Table No.4.

Table No.1: Results of Labetalol Hydrochloride Calibration Curve at 246nm for by UV Spectroscopy

S.No	Conc. (µg / ml)	Absorbance at 246nm
1	1	0.026
2	2	0.047
3	3	0.064
4	4	0.085
5	5	0.104
6	6	0.121
7	7	0.142
8	8	0.159
9	9	0.179
10	10	0.196

Table No.2: Calibration curve points of the proposed method for the estimation of labetalol hydrochloride

S.No	Parameters	Values
1	λ_{max} (nm)	246
2	Beer's law limits (µg/ml)	1-10
3	Regression equation (Y*)	Y = 0.018X+0.008
4	Slope (b)	0.018
5	Intercept (a)	+0.008
6	Correlation coefficient(r^2)	0.999
7	% RSD**	< 2%
8	Limit of Detection (µg/ml)	0.10584
9	Limit of Quantitation (µg/ml)	0.32075

*Y= mX + c,

Where X is the concentration of labetalol hydrochloride in mcg/ml, Y is the absorbance at the respective λ_{max}
c = Intercept, **Average of six determinations.

Table No.3: Determination of Precision Results for labetalol hydrochloride at 246 nm

S.No	Concentration µg/ml	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
1	LQC (2µg/ml)	0.0463 ± 0.00057735	1.246	0.0546 ± 0.00057735	1.057
2	MQC (5µg/ml)	0.1043 ± 0.00057735	0.553	0.1083± 0.00057735	0.533
3	HQC (8µg/ml)	0.1586± 0.00057735	0.363	0.1666± 0.00057735	0.346

**Average of six determinations.

Table No.4: Assay of Labetalol Hydrochloride Formulation

S.No	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
1	Gravidalol	100mg	40.003±0.43	96.00%	1.246

Table No.5: Determination of accuracy results for labetalol hydrochloride at 246 nm

S.No	Brand name	Spiked level	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD**
1	Gravidol	80%	1	0.8	1.79	99.44 ±0.026
2	Gravidol	100%	1	1	1.99	99.50 ± 0.061
3	Gravidol	120%	1	1.2	2.18	99.09 ±0.080

**Average of six determinations

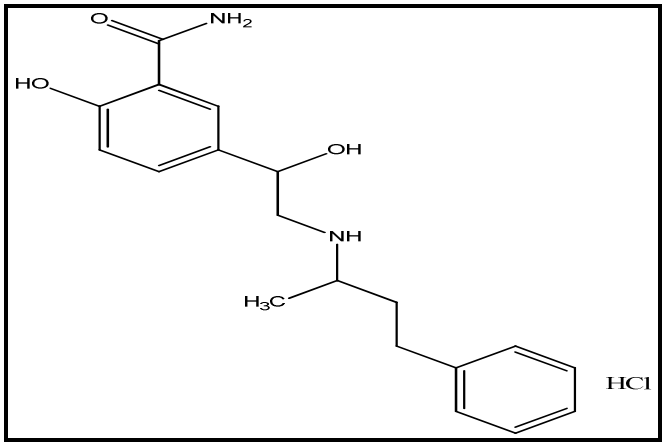

Figure No.1: Structure of Labetalol HCl

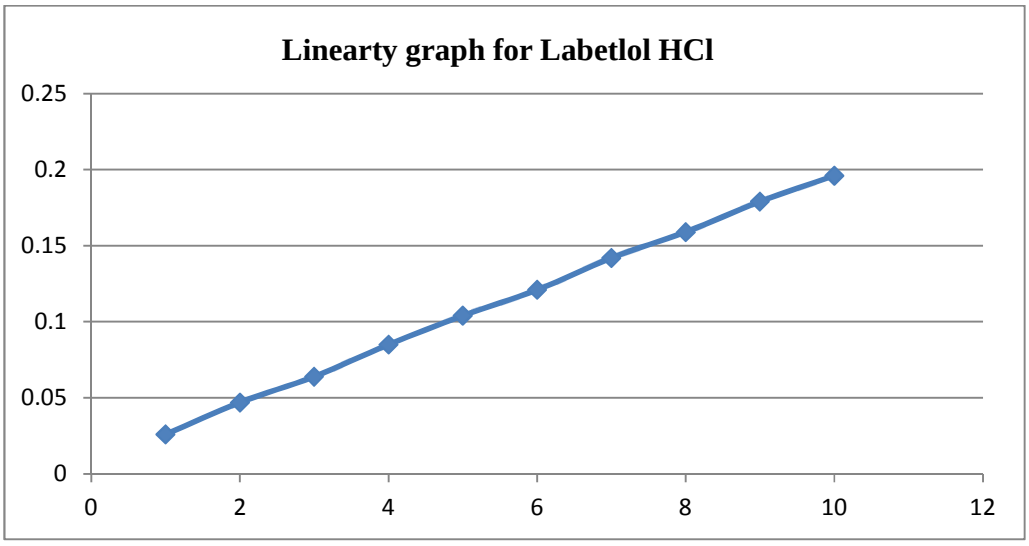

Figure No.2: Linearity Curve for Labetalol Hydrochloride At 246 nm

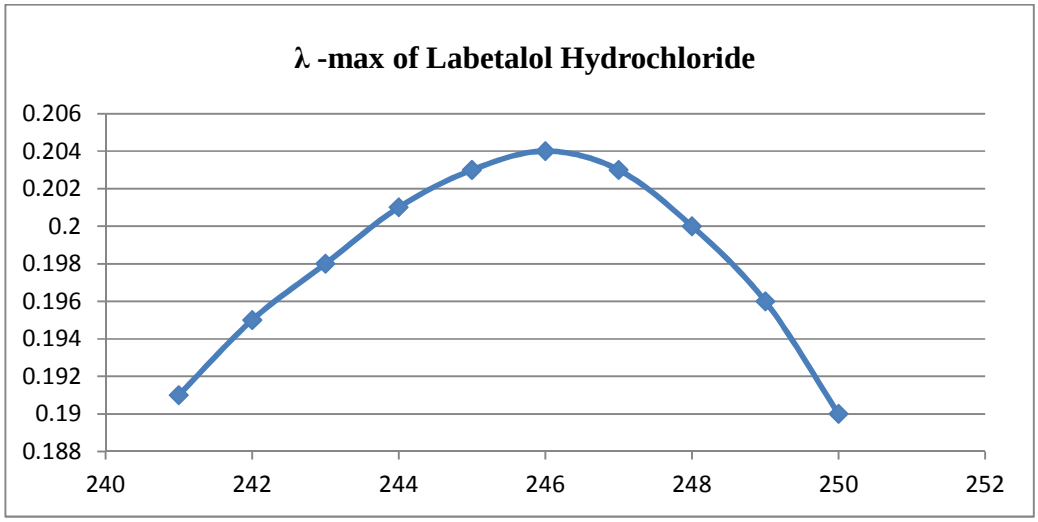

Figure No.3: Spectra of Labetalol HCl

CONCLUSION

All the above parameters combined with simplicity and ease of operation ensures that the application of proposed method form UV spectrometric method for estimation of labetalol hydrochloride was found to be useful with high accuracy, precision. It can be used for routine analysis of labetalol hydrochloride in bulk and pharmaceutical dosage form.

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CONFLICT OF INTEREST

None declared.

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