Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[9] August 2020 : 17-21

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of New Simple, Sensitive and Validated UV Spectrophotometric Method For The Simultaneous Estimation of Simvastatin and Labetalol

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ABSTRACT
A simple, precise and highly selective analytical method was developed for simultaneous estimation of Simvastatin and Labetalol in tablet formulation. Estimation was carried out by multicomponent mode of analysis at selected wavelength of 239 and 222.4 nm for Simvastatin and Labetalol respectively in 0.25 N NaOH. The method was validated in terms of linearity, accuracy (% Recovery), Precision (Interday, intraday, and reproducibility)and robustness. Methods were linear (R2 = 0.991- 0.997 for UV method) and accurate (% recovery was 98.3%-98.2%). The method was also obtained precise (% RSD <2 %) . The linearity was obtained in the concentration ranges of 2-10 µg/ml for Simvastatin and 2-10 µg/ml for labetalol. The method was validated as per international conference of Harmonization (ICH) guidelines.
KEYWORDS: *Simvastatin, Labetalol, UV,ICH.*

Received 13.04.2020 Revised 28.05.2020 Accepted 26.07.2020

INTRODUCTION

Simvastatin is chemically is 2,2-Dimethyl butanoic acid(1S,3R,7S,8S,8aR)-1,2,3,7,8,8ahexahydro- 3,7-dimethyl-8-[2-[(2R,4R)- tetrahydro-4- hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester used as a HMGCoA reductase inhibitors. Simvastatin belongs to a class of drugs called HMG-CoA reductase inhibitors commonly called statins that derived synthetically from fermentation products of *Aspergillus terreus.* All statins act by inhibiting3-hydroxy-3-methylglutarylcoenzyme (HMG-CoA).A HMG-CoA reductase, the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolic pathway responsible for the endogenous production of cholesterol mainly used for the treatment of dyslipidemia and the prevention of cardiovascular diseases. Simvastatin is prodrug which is converted into its β- hydroxy which inhibits HMG CoA reductase(3-hydroxy-3-methyl glutaryl Coenzyme A) enzyme, responsible for catalysing the conversion of HMG CoA to mevalonate arate limiting step in the synthesis of cholesterol in liver.[2,3]

Labetalol HCl is a selective α1 and non-selective βadrenergic blocker used to treat high blood pressure.Chemically it is 2-hydroxy-5-{[1-hydroxy-2-(4 phenylbutane -2-lyl) amino]ethyl}benzamide. It has amolecular formula of C19H24N2O3.Hcl and a molecular weight of 328.40g/mol.[2,3]

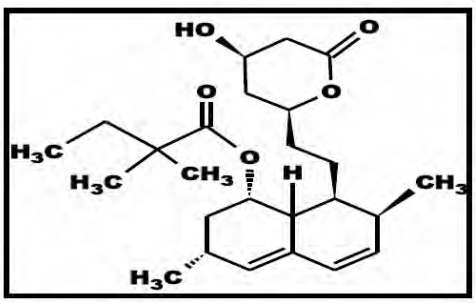

Fig.1 Chemical Structure of Simvastatin

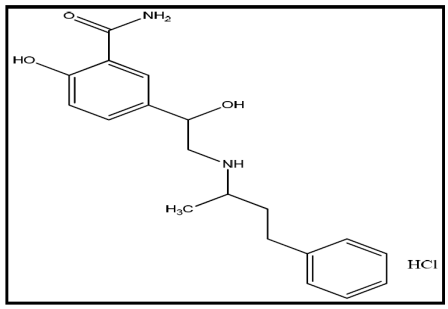

Fig.2 Chemical Structure of labetalol HCL

MATERIAL AND METHOD

A UV Visible double beam spectrophotometer (Shimadzu model UV 1800) attached to computer UV probe 2.33 with spectral width of 2 nm, wavelength accuracy 0.5 nm and pair of 1 cm matched quartz cell was employed. All weighing was done on analytical balance(Shimadzu AY220). A sonicator (Citizen, digital ultrasonic cleaner) was used in the study. Calibrated glass wares were used throughout the work.[2,3,4]

DETERMINATION OF λ_{MAX}

The standard solution of simvastatin and labetalol were separately scanned at different concentration in the range of 200-400 nm and the λ_{max} was determined. which showed maximum absorbance at 239.0 and 222.4 nm for Simvastatin and Labetalol respectively.[1,2,3,4]

4.PREPERATION OF STANDARD STOCK SOLUTION

An accurately weighed quantity of about 100 mg of simvastatin was taken in 100 ml volumetric flask dissolved in sufficient quantity of 0.25 N NaOH then sonicated for 15 min and diluted to 100 ml with the same solvent so as to get the concentration of 100µg/ml. An accurately weighed quantity of about 100 mg of labetalol was taken in 100 ml volumetric flask dissolved in sufficient quantity of 0.25N NaOH then sonicated for 15 min and diluted up to 100 ml with the same solvent so as to get the concentration of 100µg/ml. This stock solution is used for making dilutions for calibration curve.[2,3,4]

METHODOLOGY

The working standard solutions of Simvastatin and Labetalol were prepared separately in diluent 0.25 N NaOH having concentration of 10 µg/ml. They were scanned in the wavelength range of 200- 400 nm against diluent 0.25 N NaOH as blank. λmax of both the drugs were 239 nm and 222.4 nm for Paracetamol and Etodolac respectively.[2,3,4]

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[1]

Preperation of calibration curve

For each drug appropriate aliquots were pipette out from standard stock solution into the series of 10 mlvolumetric flask and the volume was made up to the mark with NaOH to get concentration of 2-10 µg/ml of simvastatin and 2-10 µg/ml of labetalol. Solutions of different concentrations for each drug were analysed at their respective wavelengths and absorbance's were recorded.[2,3,4]

Precision

The reproducibility of the proposed method was determined by performing the assay for the same day (intraday assay precision) and on three different days (inter day precision). Precision studies were performed by preparing nine determinations covering the specified range for the procedure (3 x 3 replicates for each concentration). Low % RSD shows that the method has good precision. The results of intraday and inter day precision were expressed in % RSD. [1,3,4,5]

Accuracy

The accuracy of the method was determined by calculating the recoveries of Simvastatin and Labetalol by the standard addition method. Known amounts of standard solutions of Simvastatin and Labetalol were added at 80 %, 100 % and 120 % level to prequantified sample solutions of Simvastatin and Labetalol. The amounts of Simvastatin and Labetalol were estimated by applying obtained observation values to the respective regression line. The results of accuracy were expressed in % Recovery.[1,2,5,6]

Limit of Detection and Limit of Quantification

The LOD and LOQ were separately determined based on the standard calibration curve. The residual standard deviation of y-intercept of regression lines may be used to calculate LOD and LOQ using following equations.

LOD = 3.3 * D/S

LOQ = 10 * D/S

Where, D = Standard deviation of the intercepts of regression line

S = Slope of the calibration curve.[1,2]

Robustness

Solutions of both the drugs in methanol were studied for their stability at ambient temperature for 24 h. Absorbance variation was found to be less than 1%.[5]

Table 1: Statistical parameter of the calibration curve Statistical Parameter

Statistical parameter	Simvastatin	Labetalol
λ_{max}	239 nm	222.4nm
Linearity range	2-10µg/ml	2-10µg/ml
Linearity equation	y = 0.0507x-0.0156	y = 0.0675x-0.0821
Slope	0.0507	0.0675
Intercept	0.0156	0.0821
Correlation co-efficient	0.9918	0.9977

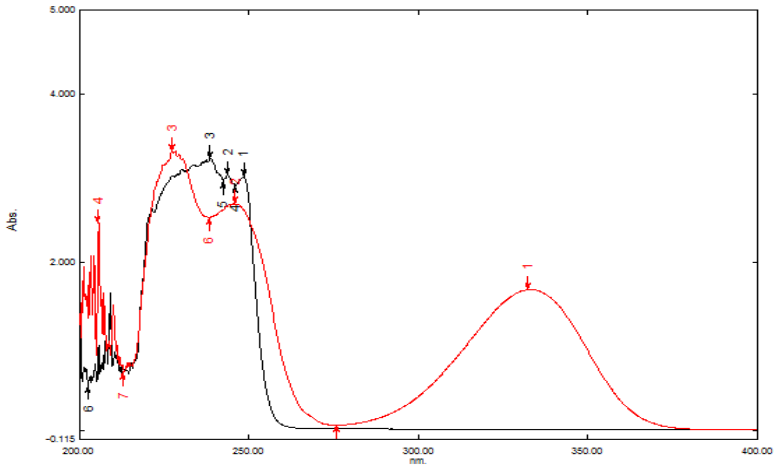

Fig.3 overlay spectra of simvastatin and labetalol

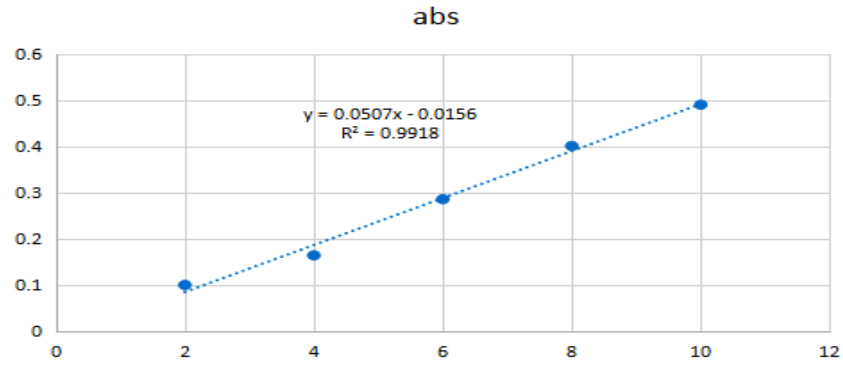

Fig. 4 calibration curve for simvastatin

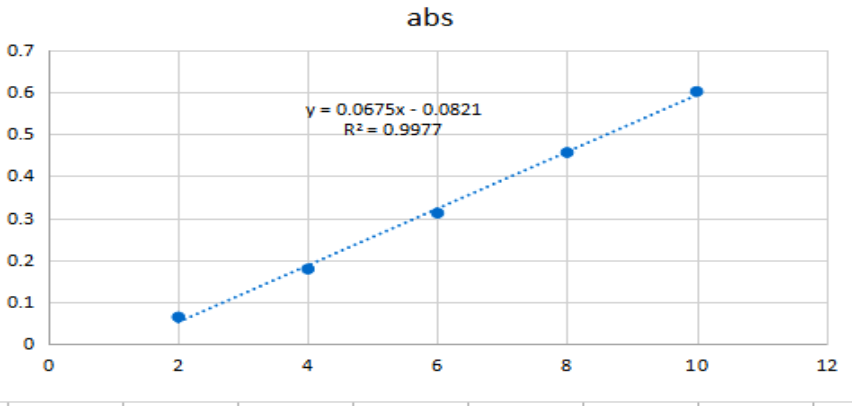

Fig.5 Calibration curve for labetalol

Table 2: Intraday precision data for simvastatin and labetalol

Simvastatin			**Labetalol**		
Conc. (µg/ml)	Mean absorbance at 239 nm (n=3)	% RSD	Conc. (µg/ml)	Mean absorbance at 222.4 nm (n=3)	% RSD
4	0.165	0.35	4	0.179	0.16
6	0.245	0.45	6	0.302	0.12
10	0.423	0.85	10	0.598	0.14

Table 3 : Inter day precision data for simvastatin and labetalol

Simvastatin			**Labetalol**		
Conc. (µg/ml)	Mean absorbance at 239 nm (n=3)	% RSD	Conc. (µg/ml)	Mean absorbance at 222.4 nm (n=3)	% RSD
4	0.145	0.92	4	0.135	0.60
6	0.215	0.87	6	0.285	0.52
10	0.411	0.75	10	0.498	0.89

Table 4 : Recovery study (% Accuracy)

Formulation	**Drug**	**Label claim (mg)**	**% Recovery estimated**
Tablet	Simvastatin	20 mg	98.3%
	Labetalol	50 mg	98.2%

Table 5 : Summery of validation parameter

Parameter	**Simvastatin**	**Labetalol**
Linearity range	2-10 µg/ml	2-10 µg/ml
Regression equation	y = 0.0507X-0.0156	y = 0.0675-0.0821
Correlation coefficient	0.9918	0.9977
Precision (%RSD) Intraday (n=3) Inter day (n=3)	 0.35-0.85 0.75-0.95	 0.12-0.16 0.52-0.89
Accuracy or recovery (%)	98.3%	98.2%
LOD	0.0070	0.0062
LOQ	0.0236	0.0356
Robustness	Robust	Robust

Table 6 : Assay result for tablet formulation.

Tablet	**Label claim (mg/tablet)**		**Assay**	
	Simvastatin	**Labetalol**	**Simvastatin**	**Labetalol**
	20 mg	50 mg	98.20%	99.1%

The calibration curves were constructed by plotting drug concentration versus the absorbance values. Standard calibration curves for Simvastatin and Labetalol were linear with Correlation coefficients (r2) values in the range of 0.9994 and 0.9983 respectively at the selected wavelengths and the values were average of five readings. The Statistical parameter of the calibration curve was shown in table 1.

Precision study showed co-efficient of variance (% CV) values less than 2 % for both Simvastatin and Labetalol respectively. Result for the intra-day and inter-day precision was shown in table 2 and 3.

RESULT AND DISCUSSION

The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 80 %, 100 %, 120 % recovery in the range of 98.3% – 98.2% justifies the accuracy of method. The overall summary of all validation parameter was shown in table 5 which was carried out as per ICH guidelines. The results of pharmaceutical dosage forms analysis of the combinations for simvastatin and labetalol 98.20% and 99.1% respectively. which showed good agreement with the labeled claim. There was no interference was observed from the presence of excipients in the amounts, which are commonly present in tablet dosage forms. From all the present work we can conclude that the proposed UV spectrometric method for quantitative determination of Simvastatin and Labetalol in combined dosage form was found

to be simple, rapid, precise, accurate and sensitive. The developed method was found to be more reproducible and sensitive.[1,3,4]

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CITATION OF THIS ARTICLE

S Kadam, A Shinde, S Jadhav, D Gaikwad, S Dhobale. Development and Validation of New Simple, Sensitive and Validated UV Spectrophotometric Method For The Simultaneous Estimation of Simvastatin and Labetalol. Bull. Env. Pharmacol. Life Sci., Vol 9[9] August 2020 : 17-21