RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF Q- ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND LABETALOL HCL IN COMBINED DOSAGE FROM

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ABSTRACT : A simple, precise, accurate and economical methods without any extraction step' The solvent used was 0.25N sodium hydroxide. Two wavelengths 246.00nm (λ max of Labetalol HCl) and 243.48nm (Isoabsorptive point) were selected for estimation of Labetalol HCl and Simvastatin for Q-absorbance ratio method. The concentrations of a drugs were determined by a using ratio of Q absorbances at isoabsorptive points and at the λ-max of Labetalol HCl methods was a successfully applied to pharmaceuticals dosage forms.

Keywords : *Labetalol HCl, Simvastatin, 0.25N sodium hydroxide.*

INTRODUCTION : [5,6]

Simavastatin is 2,2-Dimethyl butanoicacid(1S,3R,7S, 8S,8aR)-1,2,3,7,8,8ahexahydro- 3,7- dimethyl-8-[2-[(2 R,4R)- tetrahydro-4-hydroxy-6 oxo2H pyran-2yl]eth yl]1-napthalenyl ester 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 inhibiting HMG-CoA enzymes. A 3-hydroxy-3- methyl glutaryl reductase coenzyme, that decides the rate 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 Coen zyme A) enzyme, responsible for catalysing the conversion of HMG CoA to mevalonate a rate limiting step in the synthesis of cholesterol in liver. Labetalol HCl is a selective α1 and non-selective beta blocker used to treat a hypertension (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 C19H24N2O3.Hcl and a molecular weight of 328.40g/mol.[5,6]

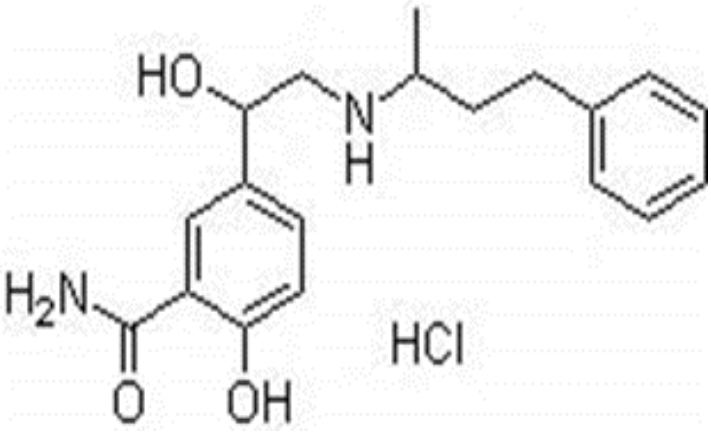

Fig 1: Chemical Structure of labetalol HCL

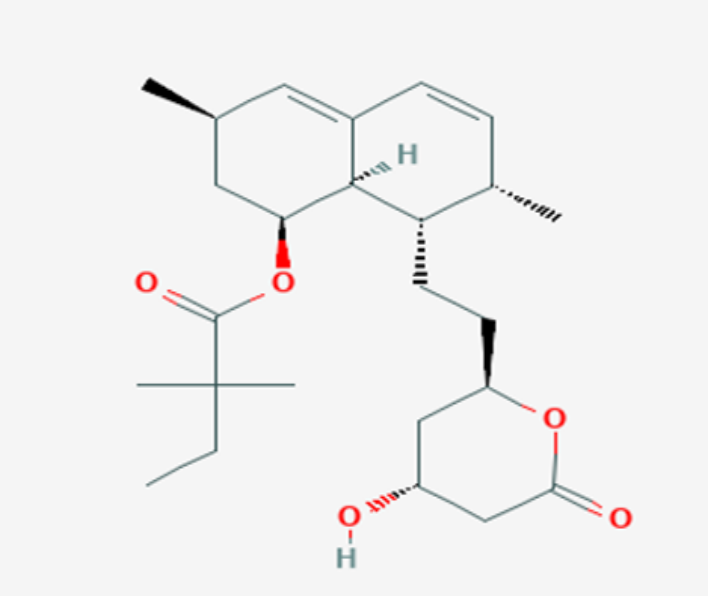

Fig 2: Chemical Structure of Simvastatin

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Q-ABSORBANCE RATIO METHOD

Let it be one drug X and Y

According to Q-Absorbance ratio methods use the ratio of absorbance at two a selected wavelengths. One is aiso-absorptive points and other being the λ-max of one and two components.

Two equations are formed, using the relationship ax1=ay1 at λ1 and L=1. Equations are ;

at λ1 A1 = ax1Cx + ax1Cy (ax1=ay1) …. (1)

at λ2 A2 = ax1Cx + ay2Cy ..... (2)

Dividing equation (2) by (1), we get A2/A1 =

(ax2Cx + ay2Cy)/(ax1Cx + ax1Cy) …… (3)

Let Cx/(Cx+Cy)=Fx& Cy/(Cx+Cy)=Fy

Dividing Equation (3) by Cx+Cy, we get

A2/A1 = (ax2Fx+ay2Fy)/(ax1Fx+ax1Fy)

But Fy =1 - Fx

A2/A1 = (ax2Fx+ay2-ay2Fx)/ax1 …… (4)

A2/A1 = (ax2Fx/ax1) – (ay2Fx/ ay1) + (ay2/ay1) (because ax1=ay1)

Let ax1 / ax2 = Qx, ay2/ay1 = Qy& A2 / A1 = QM

So, QM = FxQx – FyQy + Qy

Fx = (QM-Qy)/(Qx-Qy) …… (5)

This equation it gives a fractions of mixture that a determine the absolute the concentrations of X and Y.

Cx/(Cx=Cy) = (A2/A1)-(ay2/ay1)/(ax2/ax1)-(ay2/ ay1) …… (6)

Both equations (5) & (6) gives a fraction, rather than the concentrations of a X and consequently of a Y in the mixture the term of absolutely ratio. As, these are independent of the concentrations only approximate rather than accurate.

If a absolute concentrations of X & Y than rearrange equations (1), we gets.

Cx+Cy = A1/ax1 .. …… (7)

From equation (6) & (7), we get

Cx/(A1/ax1) = (QM-Qy)/(Qx-Qy)

Cx= {(QM-Qy)/(Qx-Qy)}* (A1/ax1) …… (8)

& Cy= {(QM-Qx)/(Qy-Qx)}* (A1/ay1) ….....(9)

Finally equation [8 & 9] gives the absolute concentration value of drug X & Y.[7,8,9]

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.Kindly gifted reference standard of simvastatin and labetalol HCL (Glen mark pharmaceutical) were used for study.[5,9]

PREPARATION OF STANDARD STOCK SOLUTION

A 100 mg weighed quantity of simvastatin was taken in a 100 ml volumetric flask and take sufficient amount of 0.25N NaOH and dissolve on it after this sonicated this mixture for 15 min. After sonication dilute this mixture with 100 ml of same solvent so as to get the concentration of a 100100µg/ml. Accurately weighed 100mg of labetalol was a taken in 100ml 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.[5]

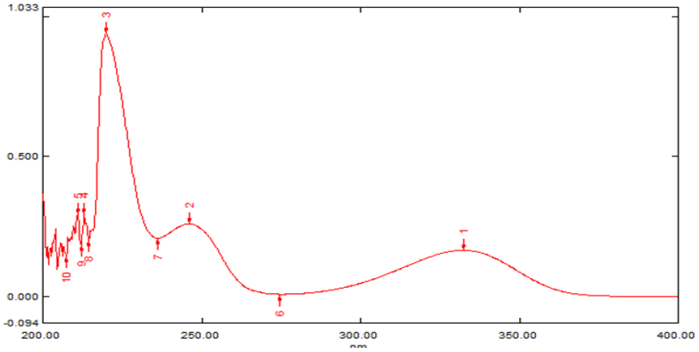

Fig 3: Absorption spectra of labetalol HCL

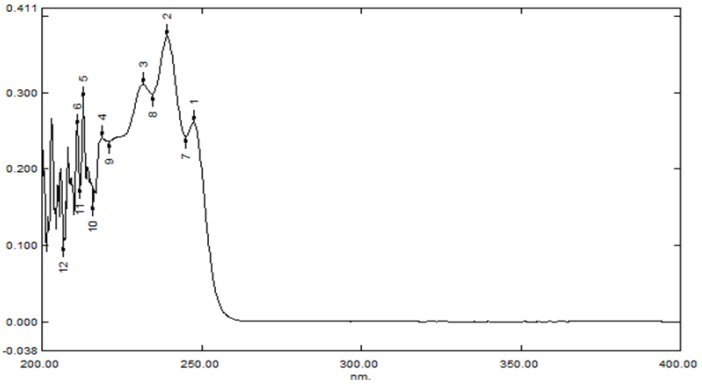

Fig 4: Absorption spectra of simvastatin

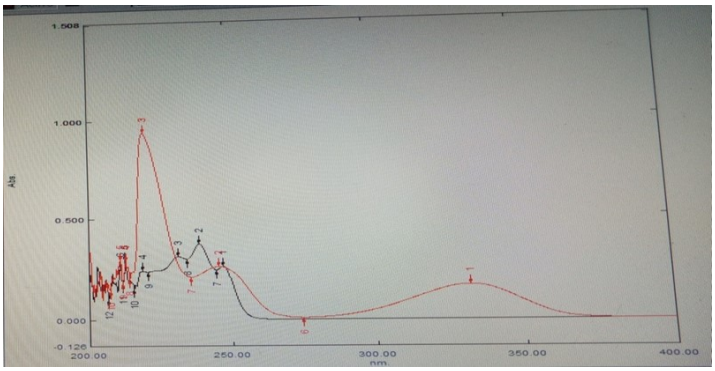

Fig 5: Overlain spectra of Simvastatin and Labetalol HCl

The maximum absorption (λmax) of Labetalol HCl was found at 246.00 nm and iso-absorptive point at 243.48 nm. Absorption for a series of standard solutions were recorded at selected wavelength.[7,10]

METHODOLOGY:

Q Absorbance ratio method was used ratio of absorbance of two a selected waveleng λ-max. From the overlain spectra of two drugs (as shown in figure 5), it shows that Simvastatin and Labetalol HCl having iso-absorptive point at 243.48 nm. The second wavelength used is 246.00 nm, which is the λmax of Labetalol HCl.(9) Working standard solutions having concentration 2, 4, 6, 8, 10 ppm and 10 µg/ml for Simvastatin and Labetalol HCl were prepared in 0.25N NaOH and the absorbance at 243.48 nm (iso-absorptive point) and 246.00 nm (λmax of Labetalol HCl) were measured.[5]

The concentrations of two drug in the mixture can be calculating by using the equations (8 & 9), we gets,

$$Cx = \{(QM-Qy)/(Qx-Qy)\} * (A1/ax1)$$

$$Cy = \{(QM-Qx)/(Qy-Qx)\} * (A1/ay1)$$

where, A1 and A2 are the absorbance of mixture at 243.48 nm and 246.00 nm; ax1 and ay1 are absorptivities of Simvastatin and Labetalol HCl at 243.48 nm; ax2 and ay2 are absorptivities of Simvastatin and Labetalol HCl at 246.00 nm; QM = A2/A1, Qx= ax2/ ax1, Qy = ay2/ay1.[8,9]

VALIDATION OF PROPOSED METHOD:

Linearity:

Linearity was evaluated by preparing different concentration in the range of 2-10 µg/ml for both the drugs and absorption was measured. Each measurement was carried out in triplicate.

Accuracy (Recovery studies):

Accuracy of an analysis was determined methods, recovery study was a carried out by taking the standard mixtures solutions of both Simvastatin and Labetalol HCl (as shown in Table 1).[5,7,8,9,10]

Method Precision (Repeatability):

The precision of the instrument was checked by epeated scanning and measurement of absorbance of solutions (n = 3) for Simvastatin and Labetalol HCl (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method (as shown in Table 2).[8,9]

RESULTS AND DISCUSSION

Q-Absorbance ratio method the primary for developing a method for analysis is that the wavelengths, was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 243.48 nm (iso-absorptive point) and 246

Table 1: Calibration Curve of Simvastatin

Conc. µg/ml	Absorbance
2	0.049
4	0.065
6	0.084
8	0.111
10	0.133

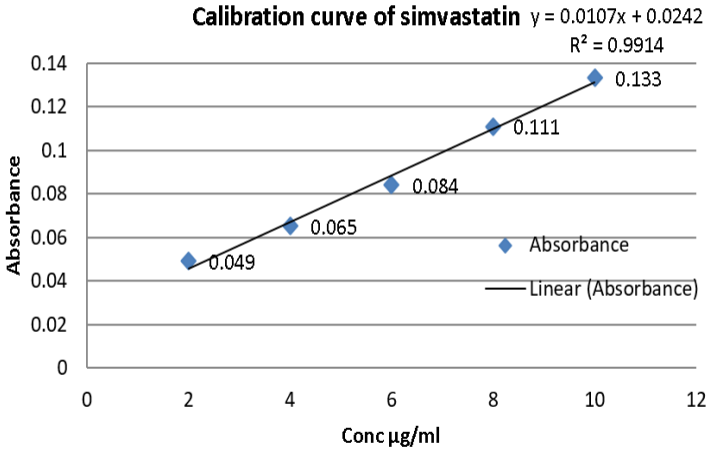

Fig. 6: Calibration Curve for Simvastatin HCL at 246.00nm.

Table 2: Calibration Curve of Labetalol

Conc. µg/ml	Absorbance
2	0.122
4	0.132
6	0.139
8	0.149
10	0.161

Fig. 7: Calibration Curve for Labetalol HCL at 243.48nm

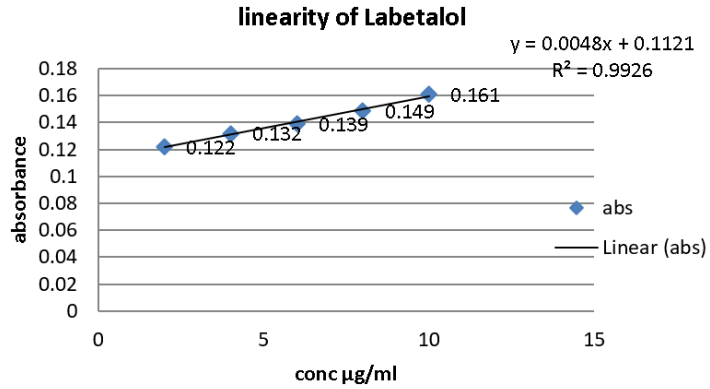

Table 1: Recovery study data of simvastatin and labetalol HCl [5,9]

Simvastatin(µg/ml)	Labetalol HCl(µg/ml)	Simvastatin(% Recovery)	Labetalol HCl(% Recovery)
2	2	99.83%	99.52%
4	4	99.32%	99.28%
6	6	99.65%	98.65%
8	8	98.59%	96.45%
10	10	98.35%	95.07%

Table 2: Regression analysis data

Parameter	Simvastatin	Labetalol HCl
Wavelength(nm)	239.20	246.00
Beer's law limit(µg/ml)	2-10	2-10
Regression Equation (Y= MX + C)	y = 0.010x + 0.024	y = 0.004x+ 0.112
Slope(m)	0.010	0.004
Intercept(c)	0.024	0.112
Correlation coefficient(R^2)	0.999	0.992
Precision(n=3)	9.5	8.0

nm (λ-max of Labetalol HCl) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of Simvastatin (239.20 nm) and Labetalol HCl (246.00 nm) showing iso-absorptive point (243.48 nm) in 0.25N NaOH is shown in Figure 5. The validation parameters was study at all the wavelengths for the proposed method.

Accuracy was determined by calculating the recovery and the mean was determined (as shown in Table 1). Precision was calculated as repeatability for both the drugs (as shown in Table 2). Hence, the method can be employed for the routine analysis of these two drugs in combined dosage form.[5,6,9]

CONCLUSION

The Spectrophotometer provides versatile techniques for analyse drug in multicomponent pharmaceutical formulation in presence of various interferences. The present work describes simple, economical and non-interfering spectrophotometric method for the estimation of simvastatin and labetalol using Absorbance ratio method. The method was found to be economic, simple, precise, accurate and reproducible during analysis of drug formulations containing the two drugs.[1,2,3]

ACKNOWLEDGEMENTS

Authors are grateful to Glenmark Pharmaceutical, for providing the gift samples of simvastatin and labetalol.

[2,3,4]

CONFLICT OF INTREST:

Authors do not have any conflict of interest.

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