Original Research Article

DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHODS AND VALIDATION FOR ESTIMATION OF SIMVASTATIN IN BULK AND TABLET DOSAGE FORM BY ABSORBANCE MAXIMA AND AREA UNDER THE CURVE METHOD

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ABSTRACT

Introduction: To develop two simple UV spectrophotometric methods for simultaneous estimation of Simvastatin (SMV) in bulk and tablet dosage form and validate as per ICH guidelines.
Methods: Method A involved Absorbance maxima method which based on the measurement of absorbance of Simvastatin in methanol at λmax of Simvastatin 238 nm and Method B involved Area under the curve (AUC) method which based on the measurement of AUC in the range of 234-240 nm.
Results: The developed methods were validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. Both the methods were found to be linear within the conc. range of 4-32 µg/ml for Simvastatin.
Conclusion: The present methods were found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of Simvastatin in bulk and tablet dosage form.

***Keywords*:** Simvastatin (SMV), Absorbance ratio method, Area under curve method (AUC) and ICH guidelines.

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INTRODUCTION

Simvastatin belongs to a class of drugs called HMG-CoA reductase inhibitors commonly called statins that derived synthetically from fermentation products of Aspergillus terreus. [1] It is chemically known as (1S, 3R, 7S, 8S, 8aR)-8-{2-[(2r, 4r)-4-hydroxy-6-oxotetrahydro-2H-pyran-2yl]-ethyl}-3, 7-dimethyl-1, 2, 3, 7, 8.8a hexahydronapthalen-1-yl-2, 2-dimethylbutanoate (Fig. 1). All statins act by inhibiting 3-hydroxy-3-methylglutarylcoenzyme. A HMG-CoA reductase, the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolicpath way responsible for the endogenous production of cholesterol mainly used for the treatment of dyslipidaemia 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 glutarylCoenzyme A) enzyme, responsible for catalysing the conversion of HMG CoA to mevalonate a rate limiting step in the synthesis of cholesterol in liver. [2] The drug is officially listed in US pharmacopeia, British pharmacopeia and European pharmacopeia. Simvastatin can be estimated by UV

spectrophotometry [3-10, 18, 19], Derivative Ratio spectrophotometry [11-13], Stability Indicating RP-UPLC [15], Stability Indicating RP-HPLC [14-17] , RP-HPLC [19-30], Stability indicating HPTLC [31], HPTLC [32-34] and LC-MS/MS [35] alone or in combination with other drugs. Two official methods utilizing HPLC Gradient methodology are reported in European Pharmacopoeia (EP) [36] United State Pharmacopoeia (USP) [37].

Because of cost-effective and minimal maintenance, UV spectrophotometry is always preferred at small scale industries. Literature survey reveals that so far many UV spectrophotometric methods have been reported for the estimation of Simvastatin in alone or in combination with other drugs. But out of them only few methods included single estimation of Simvastatin. Therefore the main objective of the proposed methods were to develop simple, new and economic UV spectrophotometric methods for the estimation of Simvastatin in bulk and tablet dosage form and validate as per ICH guidelines.

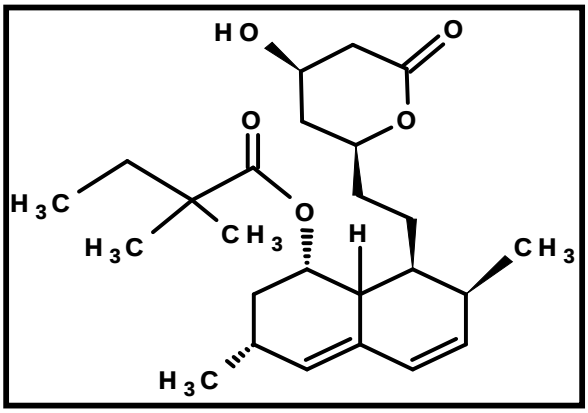

Figure 1. Chemical structure of Simvastatin

MATERIALS AND METHODS

Chemicals and Reagents

The pure API sample of Simvastatin was obtained as free gift samplefrom Gen Pharma Ltd; Pune respectively while solvent such as methanol used were of spectroscopy grade (E. Merck India) and double distilled water used for whole experiment. The marketed combined pharmaceutical dosage form ofSimvastatin (10 mg) i.e. Simvas (Micro Labs, India)was purchased from local market.

Instrumentation

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2nm) and 1-cm quartz cell was used for Spectral and absorbance measurements.

Preliminary solubility studies of drug

1 gm of Simvastatin was weighed and solubility was checked in 10 ml water, methanol, 0.1N NaOH and 0.1 N HCl. The drug was found to be freely soluble in methanol and practically poorly soluble in water, 0.1N NaOH and 0.1 HCl. Therefore methanol was selected as diluent and Simvastatin was also found to be stable in methanol for 48 hours in stability studies.

Preparation of standard stock solutions

Transfer 2.5 mg of pure Sitagliptin phosphate and Simvastatin in separate 25 ml of volumetric flask containing methanol as diluent and then sonicated for 15 minutes and final

volume made upto mark with same diluent to form 100µg/ml std. stock solution of Simvastatin.

Preparation of calibration curve

From above std. stock solution of Simvastatin (100 µg/ml), pippete out aliquots 0.4 to 3.2 ml of Simvastatin and transferred to series of 10 ml volumetric flasks and final volume made upto mark with methanol as diluent to form solutions of 4 to 32µg/ml of Simvastatin. These solutions were then scanned in the range of 200-400 nm against diluent as blank. The absorbance maxima (λmax) were found to be 238 nm for Simvastatin and then calibration curve was plotted as absorbance vs concentration.

Sample preparation for analysis of Tablet formulation

Twenty tablets (Simvas) containing 10 mg of Simvastatin weighed, average weight calculated and triturated to fine powder and then weight equivalent 10 mg of Simvastatin transferred to 100 ml of volumetric flask containing proposed diluent, then sonicated for 15 minutes and filtered through Whatman filter paper no. 42 to form 100µg/ml of Simvastatin stock solution of and final volume made upto mark with diluent. From this, 1 ml of aliquot transferred in 10 ml of volumetric flask containing diluent to form 10µg/ml of Simvastatin stock solution and scanned in the range of 200-400 nm against methanol as blank at 238 nm and then drug content of solution was calculated by using standard calibration curve.

Absorbance maxima method

For the selection of analytical wavelength, standard solution of Simvastatin was scanned in the spectrum mode from 200 nm to 400 nm separately. From the spectra of drug, λmax of SMV, 238 nm was selected for the analysis (Fig. 2). Aliquots of standard stock solution were made and calibration curve was plotted.

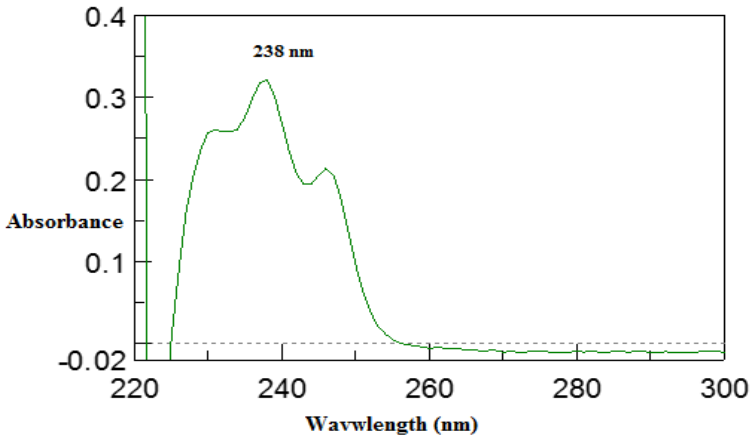

Figure 2. Absorption maxima of Simvastatin

Area under curve method

For the determination of Simvastatin using the area under curve (AUC) method, suitable dilutions of the std. stock solutions (100 µg/mL) of simvastatin were prepared in methanol and scanned in the range of 200 - 400 nm. For Area under curve method, the sampling wavelength ranges from 234-240 nm.(Fig. 3) selected for estimation of Simvastatin and area were integrated between these selected wavelength range, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations.

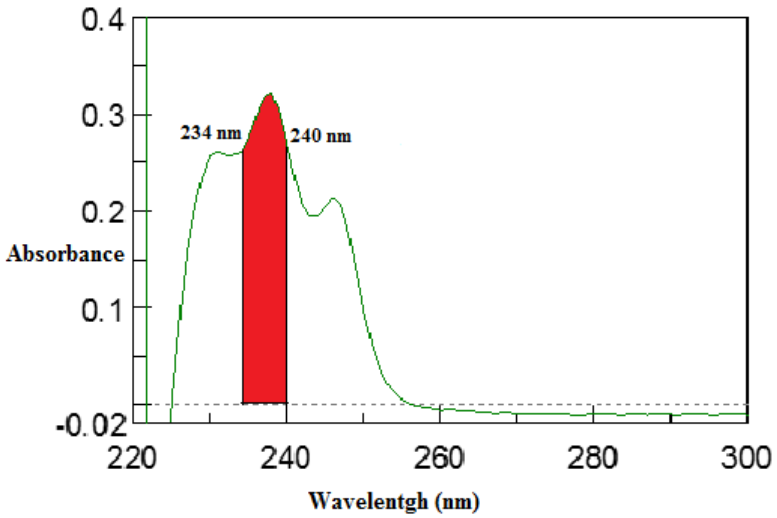

Figure 3. Area under the Curve Method

Validation

The present UV spectrophotometric methods were validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines [38] for estimation of Simvastatin in bulk and tablet dosage form.

Linearity

From std. stock solutions of Simvastatin (100 µg/ml), pippete out aliquots of 0.4 to 3.2 ml of Simvastatin transferred to series of 10 ml volumetric flasks and final volume made upto mark with methanol as diluent to form solutions of 4 to 32µg/ml of Simvastatin. These solutions were then scanned in the range of 200-400 nm against diluent as blank at λmax of Simvastatin and then calibration curve was plotted as absorbance vs concentration to check the linear relationship between absorbance and concentration of Simvastatin.

Precision

Precision study expressed by carrying out Repeatability (intraday precision) and interday precision. The intraday (Repeatability) and interday precision study were carried out by estimating corresponding responses three times on the same day and on the three different days for the three different concentration for (8, 12 and 16µg/ml) for Simvastatin. The results of precision study were reported in terms of % relative standard deviation

Accuracy

The accuracy of developed method was carried out by calculating the % recovery of Simvastatin by standard addition method at three different levels i.e. 80 %, 100 % and 120 %. Known amount of standard solutions of SMV (9.6, 12 and 14.4µg/ml) were added to prequantitated sample solutions of 12µg/ml of SMV).

LOD and LOQ

Limit of detection (LOD) is defined as lowest concentration of analyte that can be detected while limit of quantitation is defined as lowest concentration of analyte that can be quantitated. With suitable precision and linearity, LOD and LOQ can be calculated from the following formulas

$$LOD = 3.3^* r / S \quad \text{and} \quad LOQ = 10 * r / S$$

Where r is the Standard deviation of y-intercept of the regression line and S is slope of the calibration curve.

RESULTS AND DISCUSSION

Method development and optimization

The present study describes development and validation of two simple UV spectrophotometric methods for the estimation of Simvastatin in bulk and tablet dosage form using absorbance maxima method and area under the curve method. Solubility studies indicated that a Simvastatin shows better solubility in methanol solution as compared to solubility in distilled water and the λmax of Simvastatin was found to be 238 nm. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries as compared to other reported methods.

Validation

Linearity

Linearity was evaluated by analysis of Std. SMV at Six different concentrations. SMV found to be linear within conc. range of 4-32µg/ml with regression coefficient of 0.9995 by the method A and 0.9992 by method B. The results of regression analysis are summarized in (Table 1). A result shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration.

Table 1.Results of regression analysis of SMV

Simvastatin	Beer's Range (µg/ml)	Regression equation	Regression coefficient (r^2)
Method A	4-32	y = 0.0293x-0.0278	0.9995
Method B	4-32	y = 0.0146x+0.0078	0.9992

Table 2. Results of Intraday Precision Study

Simvastatin	Conc. taken (µg/ml)	Conc. found * (µg/ml)	% Amt. found	S.D.	% R.S.D.
Method A	8	7.85	98.88	0.218	0.220
	12	11.72	99.52	0.076	0.076
	16	15.49	99.36	0.081	0.081
Method B	8	7.82	97.44	1.071	1.099
	12	11.79	96.49	0.440	0.456
	16	15.42	98.07	0.722	0.737

*Average of three estimations, S.D. – Standard Deviation, R.S.D. - Relative Standard Deviation

Accuracy (Recovery Study)

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing9.6, 12 and 14.4µg/ml of SMV. The average % recoveries for three different concentrations was found to be 99.56for method A and 99.71 for method B. SMV using proposed UV spectrophotometric methods. The higher values

indicated that the proposed UV spectrophotometric method was accurate for the determination of SMV in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 4).

Table 3. Results of Interday Precision Study

Simvastatin	Conc. taken (µg/ml)	Conc. found * (µg/ml)	% Amt. found	S.D.	% R.S.D.
Method A	8	7.83	97.85	0.360	0.363
	12	11.63	96.91	0.417	0.419
	16	15.47	96.68	0.202	0.205
Method B	8	7.91	98.87	0.387	0.391
	12	11.78	96.91	0.404	0.408
	16	15.81	98.91	0.534	0.536

*Average of three estimations

Table 4. Results of Recovery Studies.

Drugs	Conc. of drug taken (µg/ml)		% Recovery *
	From Tablet	From API	
Method A	12	9.6	99.91
	12	12	99.80
	12	14.4	98.98
Method B	12	9.6	99.30
	12	12	99.83
	12	14.4	100.02

*Average of three estimations

LOD and LOQ

The limit of detection was found to be 2.09µg/ml and 2.82µg/ml for method A and for method B respectively. The limit of quantification was found to be 6.33µg/ml for method A and 8.56 µg/ml for method B respectively. Low values of LOD and LOQ indicates that the developed method was sensitive for the estimation SMV in bulk and tablet dosage form. Results of LOD and LOQ are summarized in (Table 5).

Table 5. Results of LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Method A	2.09	6.33
Method B	2.82	8.56

Assay

Analysis of sample of marketed tablet containing 10 mg Simvastatin was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery of Simvastatin were 99.48 for method A and 99.69for method B respectively and these values are complying with the assay specifications for active drug Simvastatin in the United States of Pharmacopoeia (90.0–110.0%) which are required to be met by most drug formulations. Results of tablet assay are summarized in (Table 6).

Table 6. Results of tablet Assay

Simvastatin	Label Claim (mg/tab)	Amount of Drug* Estimated (mg/tab)	% Assay
Method A	10 mg	99.66	99.48
Method B	10 mg	9.86	98.69

* Average of Six estimations

CONCLUSION

Simple UV spectrophotometric methods have been developed and validated for the determination of Simvastatin in bulk and tablet dosage form. The results of the validation parameters show that the UV spectrophotometric methods were found to be accurate, precise and sensitive. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of Simvastatin in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

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