Spectrophotometric methods for estimation of simvastatin in bulk drug and its dosage form

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ABSTRACT

Simple, accurate, precise, sensitive and selective spectrophotometric methods were developed for the estimation of simvastatin. The estimation of simvastatin was carried out by various solvents like ethanol (method I) at 238 nm, methanol (method II) at 235.8 nm. And these methods were found to be linear in the range of 5-30μg/ml and 2-10 μg/ml of simvastatin for method I and II, respectively. The percent amounts of simvastatin estimated by method I and method II were found to be 99.12% and 98.94%, respectively. The developed method was validated according to ICH guidelines and it found to be accurate and precise. Thus the proposed method can be successfully applied for simultaneous determination of simvastatin and in routine analysis work. © 2016 Trade Science Inc. - INDIA

INTRODUCTION

Simvastatin (SST) is butanoic acid, (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (Figure 1). The empirical formula of SST is $C_{25}H_{38}O_5$ corresponding to molecular weight of 418.57[1].

SST is a prodrug, used for hyperlipidemia. It belongs to 'statin' class of drugs. Statin drugs are more effective to reduce blood levels of low-density lipoprotein (LDL) cholesterol ("bad cholesterol"). So it is called 'a lipid-lowering drug'[2, 3].

SST is a synthetic derivative of a fermentation product of *Aspergillus terreus*. The drug is in the form of an inactive lactone that is hydrolyzed after ingestion to produce the active agent. So after oral administration, SST is hydrolyzed to its active β-hydroxyacid form, simvastatin acid (as it is a prodrug). SST is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the biosynthetic pathway for cholesterol. In addition, SST reduces VLDL (very-low-density lipoprotein) and TG (Triglycerides) and increases HDL-C (high-density lipoprotein cholesterol) [4, 5].

The primary uses of SST are for the treatment of dyslipidemia and the prevention of cardiovascular disease[6].
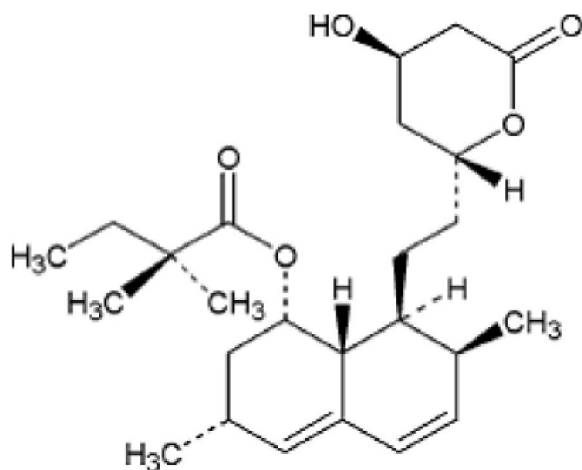
It is recommended that, SST should be used with

Figure 1 : Chemical structure of simvastatin

exercise, diet, and weight-loss to control elevated cholesterol, or hypercholesterolemia[7].

The statins contain chromophoric groups in their chemical structure, which are responsible for giving sharp spectra in UV-visible region. So the drug can be directly assessed by dissolving in suitable solvents using UV spectrophotometry. Therefore there is no need to derivatise the drug.

SST is practically insoluble in water, and freely soluble in chloroform, methanol and ethanol. So here, ethanol and methanol are selected to dissolve the drug for further analysis.

The drug is officially listed in 2004 United States Pharmacopocia and the official method of its determination is high-performance liquid chromatography[8].

Several methods have been reported so far for analysis of SST by various analytical techniques. Some of them include liquid chromatography with UV detection (LC–UV)[9-11], liquid chromatography with fluorescence detection[12], gas chromatography–

mass spectrometry (GC–MS)[13], HPLC[14-19], HPTLC[20]. And there are very few UV-visible spectrophotometric methods reported as per extensive literature survey.

So a successful attempt was made to develop, validate and compare simple, precise and accurate spectrophotometric methods for the estimation of SST in bulk and its pharmaceutical formulation using ethanol and methanol as solvents. The methods were validated according to ICH guidelines[21].

EXPERIMENTAL

Instrumentation

A double beam UV visible spectrophotometer (UV-2450, 'Shimadzu', Japan) connected to computer loaded with spectra manager software 'UV probe' with 10 mm quartz cells were used. The spectra were obtained with the instrumental parameters as: wavelength range (200-400 nm), scan speed (medium), sampling interval (1 nm), spectral slit width (1 nm).

A high precision analytical balance, Model-GR-202 (AND Instrument India Pvt. Ltd., Gurgaon, India) of sensitivity 0.1 mg was used to weigh samples.

Materials/ Chemicals

SST was obtained as a gift sample from Cipla Ltd. It was used without further purification. A tablet formulation 'Simcard' of Cipla Ltd. containing 10 mg of SST was purchased from local market.

All chemicals used were of analytical grade (AR grade).

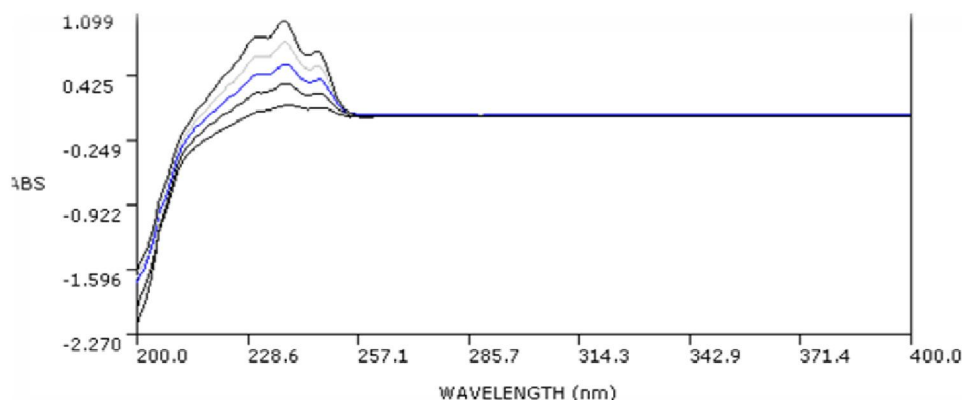### Preparation of standard stock solution

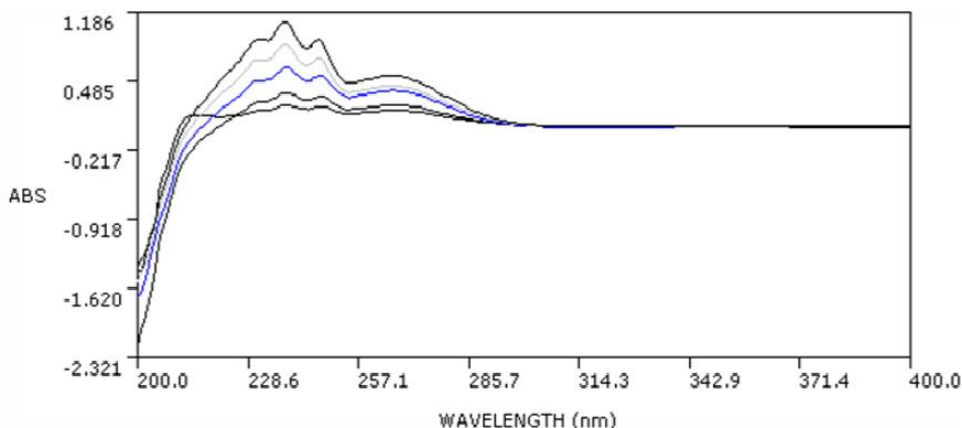**Figure 2 : UV overlain spectrum of SST in ethanol**

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Figure 3 : UV overlain spectrum of SST in methanol

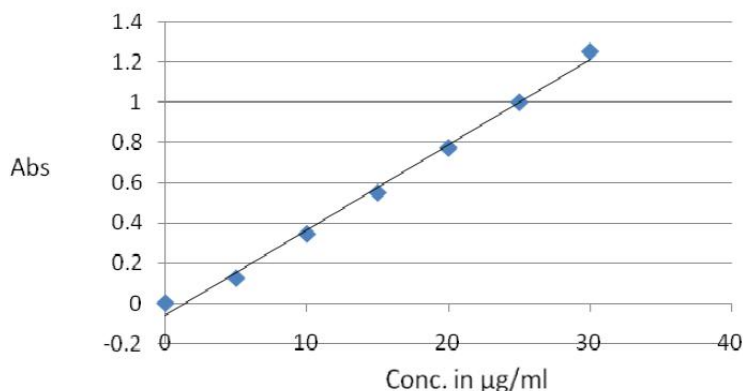$y = 0.042x - 0.058$
$R^2 = 0.994$

Figure 4 : Calibration curve for method I

For method I, an accurately weighed quantity 5 mg of SST was taken in a 50 ml volumetric flask and was dissolved in around 20 ml of ethanol, finally made up the volume up to the mark with ethanol to get the stock solution of 100 µg/ml. Suitable aliquots from this solution were taken and further diluted to obtain the working solutions using water.

Same procedure had been carried out for method II using methanol as a solvent, in place of ethanol for the preparation of standard stock solution. In this case also, further dilutions were made using water.

Procedure

Construction of Calibration Graphs

Method I:

Aliquots of working standard solution of SST (prepared for Method I) were further diluted to obtain the concentrations in the range of 5-30 µg/mL using water.

Then the absorbance of the samples were measured spectrophotometrically at 238 nm against respective reagent blank i.e. ethanol. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equation was derived (Figure 2 and 4).

Method II:

In the same way, aliquots of working standard solution of SST (prepared for Method II) were further diluted to obtain the concentrations in the range of 2-10 µg/mL using water.

Then the absorbance of the samples were measured spectrophotometrically at 235.8 nm against respective reagent blank i.e. methanol. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equation was derived (Figure 3 and 5).
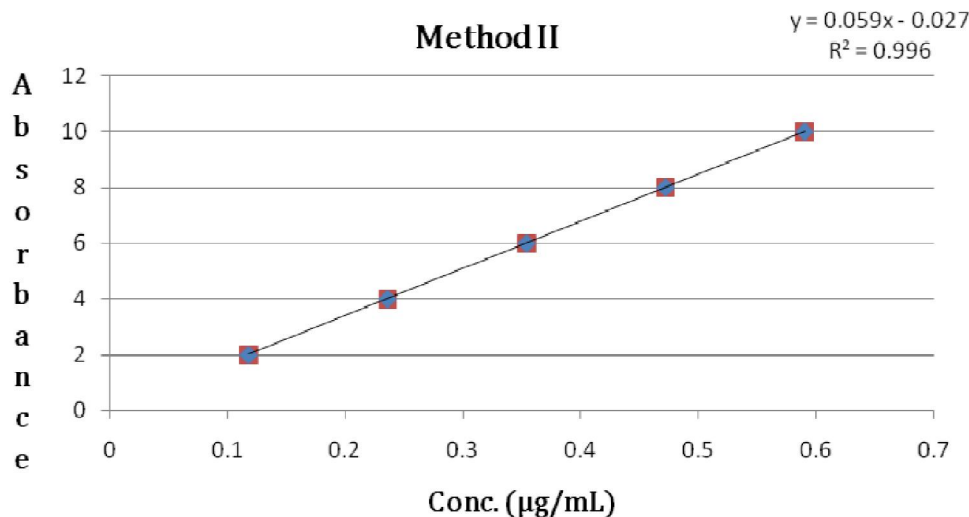
Figure 5: Calibration curve for method II

TABLE 1 : Optical characteristics of SST

Sr. No.	Parameters	Method I (using ethanol)	Method II (using methanol)
1.	λmax (nm)	238 nm	235.8 nm
2.	Beer-Lambert's range(μg/mL)	5 – 30 μg/mL	2 – 10 μg/mL
3.	Regression Equation, y=mx+c	y = 0.042x - 0.058	y = 0.059x - 0.00027
4.	Slope (m)	0.042	0.059
5.	Intercept (c)	0.058	0.027
6.	Correlation coefficient (r^2)	0.994	0.996
7.	Limit of detection (μg/mL)	0.84	0.48
8.	Limit of quantitation (μg/mL)	2.42	1.66

Assay procedures for tablets

Twenty tablets were weighed and powdered. Average weight was determined. Powder equivalent to 5 mg of SST was weighed accurately and transferred to the 50 ml volumetric flask. To it, 20 ml of ethanol was added and ultrasonicated until the drug is dissolved. The solution was filtered and made up the volume up to the mark with ethanol. This solution was suitably diluted to obtain the required concentration.

Same procedure had been followed for method II using methanol as a solvent, in place of ethanol.

Validation

Validation of the developed methods were done according to ICH guidelines[21].

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (μg/mL). A linear correlation was found between the absorbance and the concentration of SST. Beer's law was obeyed. The regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (m), the intercept (c) and the correlation coefficient (r) and the calculated values are given in TABLE 1. The graphs showed maximum intercept as described by the regression equation, y = mx + c, where y is the absorbance and x is the concentration in μg/ml.

Limits of detection and limits of quantitation

The limit of detection (LOD) is a lowest concentration of analyte which can be detected and limit of quantitation (LOQ) is a lowest concentration of analyte which can be quantitated. The results are summarized in TABLE 1. LOD and LOQ can be calculated according to the following equations

LOQ = 10 Sc/m

LOD = 3.3 Sc/m

Where Sc is the standard deviation of the intercept of regression line, and m is the slope of the regres-

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TABLE 2: Accuracy

Sr. No.	Initial amount (µg/mL)		% amount of drug added	Method I		Method II	
	Method I	Method II		% Recovery	% RSD	% Recovery	% RSD
1.	5	20	80	99.77	0.38	100.56	0.32
2.	5	20	100	98.90	0.48	99.43	0.41
3.	5	20	120	101.02	0.62	100.32	0.24

TABLE 3: Intraday and interday precision data for method I and II

Methods		Intraday Precision		Interday Precision	
	Concentrations (µg/mL)	Intraday ±S. D.	C. V.	Interday ± S. D.	C. V.
Method I	10	9.98 ± 0.09	0.60	9.95 ± 0.11	0.73
	15	15.01 ± 0.06	0.29	14.99 ± 0.02	0.10
	20	20.07 ± 0.07	0.24	20.02 ± 0.14	0.35
Method II	3	2.98 ± 0.05	0.33	3..23 ± 0.14	0.91
	6	6.09 ± 0.14	0.65	5.86 ± 0.12	0.60
	9	9.11 ± 0.08	0.32	8.78 ± 0.23	0.92

sion line.

Accuracy

The accuracy of the proposed methods was studied by calculating mean % recovery performed at three different levels i.e. 80 %, 100% and 120 %. To the pre-analyzed sample solution a known amount of SST bulk drug was added at 80 % to 120 % and the re-analyzed the SST by proposed methods.

Precision

Intraday and Interday precision were expressed as the S. D. and C. V. of a series of measurements by analyzing the drug concentrations of 10 µg/mL, 15 µg/mL and 20 µg/mL for method I and 3 µg/mL, 6 µg/mL and 9 µg/mL for method II.

Specificity

The specificity of the methods was investigated by observing any interference encountered from the common tablet additives. These additives did not interfere with the proposed methods.

Ruggedness

Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot 20 µg/mL (method I) and 6 µg/mL (method II) by different analysts under similar operational and environmental conditions.

Comparison of the two developed methods

The comparison of the two developed spectro-

photometric methods for the determination of SST leads to the following advantages/disadvantages:

Method II is more sensitive than Method I.

The linearity for method I lies in 5 – 30 µg/mL whereas that for method II in 2 – 10µg/mL.

Both methods are selective for the determination of the analyte in its pharmaceutical formulations.

HPLC technique also gives good results but, because of the low cost and ease of carrying out the spectrophotometric methods, the proposed procedures are likely to be very suitable for the quality control of SST in tablet dosage form.

RESULTS AND DISCUSSION

In method I and II, SST followed linearity in the concentration range of 5 – 30 µg/ mL and 2 -10 µg/ mL, respectively. The details of optical characteristics are summarized in TABLE 1. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r)obtained from different concentrations are given in TABLE 1.

LOD and LOQ were found to be 0.84 µg/mL and 2.42 µg/mL respectively for method I and 0.48 µg/ mL and 1.66 µg/mL, respectively for method II (TABLE 1).

The % amount of SST estimated by method I and II was found to be 99.17 % and 98.26%, respectively. Results signified that there was no in-

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	% label claim		Mean	± S. D.	% RSD
	Method I	Method II			
1st analyst	99.86	98.89	99.38	0.68	0.69
2nd analyst	99.69	100.81	100.25	0.79	0.79
3rd analyst	100.21	98.98	99.59	0.87	0.87

terference from the excipients generally occurs in tablet formulation.

The developed methods were validated for accuracy, precision and ruggedness as per ICH guidelines.

The accuracy methods were studied as mean % recovery and found to be in the range of 98.90-101.02 (method I) and 99.43 -100.56 (method II), respectively (TABLE 2).

The precision of the methods was determined as intra-day and inter-day study. An appropriate concentration 10 µg/mL, 15 µg/mL and 20 µg/mL in method I and 3 µg/mL, 6 µg/mL and 9 µg/mL (method II) were selected and instrumental responses were determined (TABLE 3).

Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot 20 µg/mL (method I) and 6 µg/mL (method II) by different analysts under similar operational and environmental conditions. The results are reported in terms of % RSD, shown in TABLE 4.

CONCLUSION

Two simple, accurate and precise and reasonably sensitive spectrophotometric methods were developed for the determination of SST in bulk drug and in tablets. The developed spectrophotometric methods were validated for estimation of SST using linearity, range, accuracy, precision and ruggedness.

Moreover, the developed methods are less time-consuming as the methods require simple dilutions. The technique employed is inexpensive but was demonstrated to provide the sensitivity comparable to the expensive technique like HPLC. Thus, the methods are suitable for routine analysis in quality control laboratories.

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