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Research Article

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR SIMVASTATIN IN BULK AND TABLET DOSAGE FORM BY USING AREA UNDER CURVE (AUC) METHOD.

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ABSTRACT

Simvastatin is used as hypolipidaemic agent for the treatment of congestive heart disease (CHD) and atherosclerosis. A simple, accurate, precise and economic spectrophotometric method was developed and validated for the estimation of simvastatin using area under curve method. Methanol was used as solvent and all parameters were validated as per ICH guideline Q2(R1). The wavelength of maximum absorption for Simvastatin was found to be at 238 nm. The method obeyed Beer-Lambert's law and indicated by the calibration curve in the range 2-12µg/ml. The regression equation was y= 0.0969x-0.0051. The Correlation coefficient was found to be 0.9995. LOD and LOQ were calculated as 0.0703µg/ml and 0.2130µg/ml respectively. The developed method can be used for quantitative estimation of simvastatin in bulk and pharmaceutical dosage forms.

Key-words: *Simvastatin, Area under curve, UV-spectrophotometry.*

INTRODUCTION

Statins are cholesterol-lowering agents that reversibly inhibit HMG-CoA reductase, which catalyzes a rate-limiting step in cholesterol biosynthesis [1].These agents are structural analogues of HMG-CoA(3-hydroxy-3-methylglutaryl-coenzyme A) [2]. As HMG-CoA reductase activity is maximum at midnight, all statins are administered at bed time to obtain maximum effectiveness [3].The liver is their target organ, and decreased hepatic cholesterol synthesis ultimately leads to increased removal of LDL particles from the circulation [4].

Hyperlipidaemia is one of the major clinical sequels for acute pancreatitis and atherosclerosis [2]. These agents decrease the risk of a first heart attack in subjects and In addition decrease clinical expression of heart disease. This category includes Lovastatin, Simvastatin, Pravastatin, Atorvastatin and Rosuvastatin [3].

Simvastatin is an inactive lactone prodrug (Fig. 1) that is hydrolysed in gastrointestinal tract to its active β-hydroxyl derivative. It is a butanoic acid derivative,chemically called as 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester. Simvastatin is a potent inhibitor of HMG-CoA .The inhibition of the HMG-CoA causes a decrease in LDL, triglycerides (10–20 %), while it increases HDL, high-density

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lipoprotein (5–15%) and LDL receptor expression. Oral absorption is better and first pass metabolism is extensive; $t_{1/2}$ is 2-3 hr.

Simvastatin is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopeia. The literature survey revealed that High performance liquid chromatography (HPLC) [5], High performance thin layer chromatography (HPTLC) [6] and UV-spectrophotometric method [7] has been developed to determine simvastatin in pharmaceutical dosage form.

But there is no method reported using Area under curve (AUC) for the UV-spectrophometric method for simvastatin. Hence, the aim of the present work is to develop a simple, rapid and precise Area under curve (AUC) method for the estimation of simvastatin in tablet dosage form.

MATERIAL AND METHODS

Apparatus and Instrumentation

A double beam UV visible spectrophotometer of Shimadzu Corporation, UV-1800 (Made in Japan) with two identical 1 cm quartz cells was used for all Spectrophotometric measurements. The absorption spectra of reference and test solution were taken over the range of 200-800 nm. Single pan electronic balance (Shimadzu, Type: AX 200) was used for weighing. Borosil® volumetric glass wares were used.

Chemicals and reagents

The pure drug (API), simvastatin was received as a gift sample from vesta pharmachem (P) Ltd., surat, (Gujrat) India. Methanol (AR-grade) was used as solvent and purchased from Merck India Ltd.

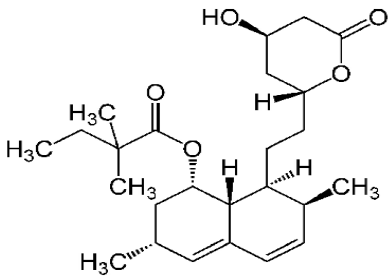


Fig. 1: Structure of Simvastatin

Preparation of standard stock solution

A stock solution of simvastatin was prepared by dissolving 10 mg of simvastatin in methanol and diluting it up to 10 ml in 10 ml volumetric flask. The final drug concentration was 1 mg/ ml or 1000 µg/ml.

Preparation of intermediate standard solution

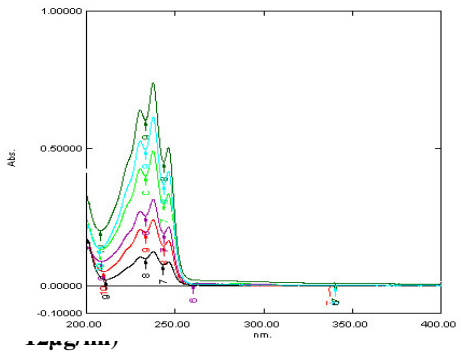
From the prepared stock solution (1000 µg/ml), 1 ml was pipette out in a 10 ml volumetric flask. It was diluted up to mark with methanol to give an intermediate solution having concentration of 100µg/ml.

Preparation of working solution

For the preparation of working solution 1.0 ml was taken from the intermediate standard solution and further diluted up to 10 ml with solvent methanol to obtain the concentration of 10µg/ml. This prepared solution was scanned between 400 nm to 200 nm in UV spectrophotometer against methanol as blank. For Area Under Curve method, the wavelength was selected at 238 nm (Fig. 2).

Preparation of calibration curve

For the preparation of working solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were pipette out from the intermediate standard solution and further diluted up to 10 ml with solvent methanol to obtain the concentration of 2, 4, 6, 8, 10 and 12µg/ml respectively. Above solutions were scanned from 400 nm to 200 nm to give absorbance in a linear range. The calibration curve was plotted between Absorbance against concentration (Fig.3).



12µg/ml)

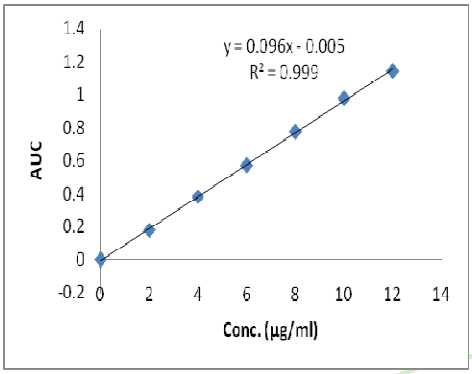


Fig. 3: Calibration Curve of Simvastatin (2-12 µg/ml)

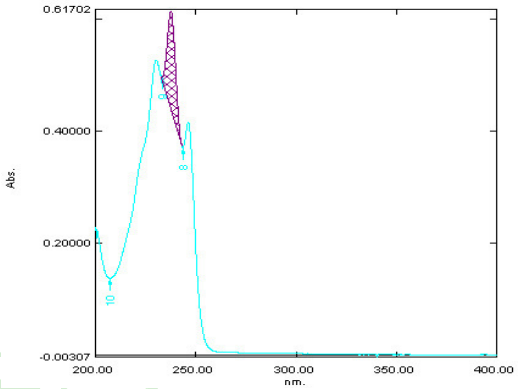


Fig. 4: AUC for Simvastatin (10µg/ml)

Assay of tablet formulation

Twenty tablets of simvastatin (SIMLO manufactured by Ipca Laboratories limited, Label Claim: 10 mg of simvastatin per tablet) were accurately weighed and powdered. Powder equivalent to 10 mg of simvastatin was accurately weighed and transferred into a 100 ml volumetric flask. About30 ml of methanol was added to dissolve the drug. The contents were sonicated and further diluted up to 100 ml with methanol. The solution was filtered with Whatmann filter paper No.41 and the first 5 ml of filtrate was discarded. The filtrate was further subjected for UV analysis. The procedure was repeated in triplicate. (Table No.1)

Table I: Assay of Tablet dosage form

Tablet solution containing Simvastatin(µg/ml)	*% Found*	*Mean % Found**	*% RSD**
10	99.80	99.86	0.21
10	99.69		
10	100.10		

*n=3

Method validation

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline [8].

Linearity and Range

The linearity was evaluated by linear regression analysis. The correlation coefficient was used to determine linearity of simvastatin (2-12 µg/ml). Calibration curve of Area under curve vs. Concentration was plotted and regression line equation and correlation coefficient were determined (Figure 3; Table 2).

Table II: AUC for Simvastatin (2-12µg/ml)

Conc.(µg/ml)	Wavelength(238±5nm)		Divisor	Area	Result
	Start	End			
2	233.00	243.00	1.000	0.180	0.180
4	233.00	243.00	1.000	0.383	0.383
6	233.00	243.00	1.000	0.570	0.570
8	233.00	243.00	1.000	0.776	0.776
10	233.00	243.00	1.000	0.978	0.978
12	233.00	243.00	1.000	1.145	1.145

Precision

Precision has been measured at three levels *viz.* repeatability, intraday precision and inter-day precision. The term standard deviation is used to express the precision of any analytical procedure. In repeatability, six different standard solutions were prepared each of having concentration of 10 µg /ml. The absorbance of each of these solutions was measured at 238 nm at three concentration levels and % R.S.D was calculated (Table 3).

For intraday precision nine different solutions were prepared of 8, 10 and 12 µg /ml with three replicates of each and their Area under curve was measured at 238 nm on the same day and % R.S.D was calculated. Three different solutions were prepared of 8, 10 and 12 µg/ml concentrations and their Area under curve was measured on three different days (For inter-day precision).

Table III: Precision for Simvastatin

Parameters	Intra-day precision	Inter-day precision
Sample solution concentration (µg/ml)	10	10
AUC (Mean ± S.D)*	0.09767±.0020	0.09752±0.0020
%RSD	0.2013	0.2093

Accuracy

The accuracy for the analytical procedure was determined at 80 %, 100 % and 120 % levels of standard solution. Area under curve was measured at 238 nm and results were expressed in terms of % recoveries (Table 4).

Table IV: Accuracy data of Simvastatin

Accuracy Level	Amount added (µg/ml)	% Recovery*	Mean % Recovery*	% RSD*
I (80%)	8	99.79±0.9672	99.83	0.035
II (100%)	10	99.86±0.2128		
III (120%)	12	99.83±0.3149		

*n=3

Limit of Detection (LOD)

Six sets of calibration curves were used to determine limit of detection and estimating the standard deviation (SD) of the response and slope of the calibration curve. The results were calculated as per equation 1.

*Equation 1: Limit of Detection (LOD) = 3.3 * σ/ S*

Where, σ is standard deviation of y-intercept of the calibration curves.

S is mean slope of six calibration curves.

Limit of Quantitation (LOQ)

The Quantitation limit was estimated using six sets of calibration curves and calculating the standard deviation of the response and slope of the calibration curve. The results were calculated as per equation 2.

*Equation 2: LOD = 10 *σ/ S*

Where, σ is standard deviation of y-intercept of the calibration curves

S is mean slope of six calibration curves.

Table V: Summary of Validation Parameters

Parameter	*Results*
λ max (nm)	238
Linearity Range (µg/ml)	2-12
Regression Equation (y=mx+c)	y= 0.0969x-0.0051
Slope (m) ± SD*	0.0969 ± 0.0002
Intercept (c) ± SD*	0.0051 ± 0.0021
Correlation Coefficient ($R^{2)}$	0.9995
Precision (% R.S.D*)	
Intraday	0.2013
Inter-day	0.2093
Accuracy (Mean % Recovery)	99.83 %
LOD	0.0703 µg/ml
LOQ	0.2130µg/ml

RESULTS AND DISCUSSION

The validation is carried out as per ICH guideline Q2(R1) for the proposed analytical spectrophotometric method. % R.S.D. values for intraday and inter-day precision were less than 2 %, which indicated that the method was precise. Good recoveries were obtained at each 80 %, 100 % and 120 % levels, which showed the accuracy of method. The LOD and LOQ were calculated as 0.0703µg/ml and 0.213µg/ml respectively. In case of Assay of simvastatin formulation, the amount of drug was found to be 99.86%, which was consistent with the labelled claim.(Table 5).

CONCLUSION

It can be concluded from the calculated results that the proposed area under curve method was accurate, precise and consistent for the measurement of simvastatin in dosage form. The developed method can be used for quantitative estimation of simvastatin in bulk and pharmaceutical dosage forms.

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