Original Research Article

STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF SIMVASTATIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Simvastatin (SMV) is antihyperlipidemic drug belonging to HMG-CoA reductase inhibitor and helps to reduce harmful cholesterol levels in blood. Simple and economic stability indicating UV spectrophotometric method of Simvastatin in bulk and tablet dosage form has been developed and validated as per ICH guidelines.The absorption maxima of Simvastatin shown at 237 nm and methanol was used as diluent. Stability studies of Simvastatin were carried out under acidic, basic, neutral, oxidative, thermal and photolytic conditions in developed method as per stability indicating assays and validated according to ICH guidelines for linearity, precision, accuracy, LOD and LOQ. Simvastatin found to be linearwithin the concentration range of 3-18 µg/ml with regression coefficient of 0.9998. The percentage RSD values of precision study were less than 2 percent while developed method capable of recovering good amount of drug (% Recovery) in presence of excipients and LOD and LOQ values of SMV were 0.73 µg/ml and 2.07 µg/ml respectively. The results of validation parameters indicates that the developed method was also found to be accurate, precise and sensitive and such simple & economic method can be used for the analysis of formulation of Simvastatin in quality control laboratories as stability indicating assay method.

Keywords: Simvastatin (SMV), Stability Indicating Method, UV Spectrophotometry and ICH Guidelines

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INTRODUCTION

Simvastatin belongs to a class of drugs called HMG-CoA reductase inhibitors commonly called statins. All statins act by inhibiting HMG-CoA reductase, a rate limiting enzyme in the synthesis of cholesterol in liver and used for the treatment of dyslipidemia and the prevention of cardiovascular diseases [1]. It is chemically known as (1, 3, 7, 8, 8a) -8-{2-[(2r, 4r)-4-hydroxy-6oxotetrahydro2H-pyran-2yl] ethyl}-3, 7-dimethyl-1, 2, 3, 7, 8.8a-hexahydronaphthalen-1-yl-2, 2-dimethyl butanoate (Fig.1). A HMG-CoA reductase (3-hydroxy-3-methylglutarylcoenzyme), the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolic path way responsible for the endogenous production of cholesterol. Simvastatin is prodrug which is converted into its β- hydroxy form which inhibits HMG CoA Reductase enzyme, a rate limiting enzyme in the synthesis of cholesterol in liver [2].

Simvastatin can be estimated by UV spectrophotometry [3-19], RP-HPLC [18-34] HPTLC [35-39] and LC-MS/MS [40-47] alone or in combination with other drugs. Two official methods utilizing HPLC Gradient methodology are reported in European Pharmacopoeia (EP) [48] United State Pharmacopoeia (USP) [49].

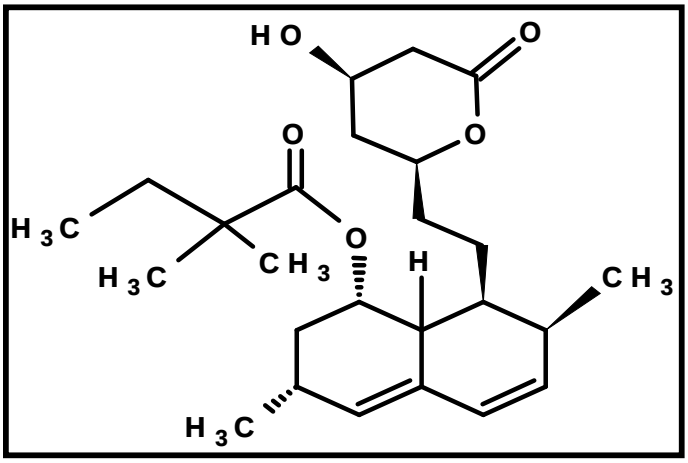

Figure 1: Chemical structure of Simvastatin

Because of cost-effective and minimal maintenance, UV spectrophotometry is always preferred at small scale industries. The literature survey reveals that till date there was no Stability indicating UV spectrophotometric method have been reportedand only Stability indicating RP-HPLC [18-34] and HPTLC [35-39] methods have been reported for Simvastatin in alone or in combined with other drugs but these methods requires more solvent preparation and long chromatographic runs. Therefore the main objective of this research work was to develop and validate simple, economic and rapid UV spectrophotometric method by absorption maxima method and its application to stability indicating assay method for Simvastatin in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

The pure API sample of Simvastatin was obtained as free gift sample from Gen Pharma Ltd; Pune while all spectroscopy grade solvent such as methanol and AR grade hydrochloric acid, sodium hydroxide, hydrogen peroxidewere purchased from E.MerckLtd; India and double distilled water was used for whole experiment. The marketed pharmaceutical tablet dosage form of Simvastatin i.e. **Simvas 10 (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 fond to be stable in methanol for 48 hours in stability studies.

Preparation of standard stock solution

Transfer 25 mg of pure simvastatin in 25 ml of volumetric flask containing methanol as diluent and then sonicated for 15 minutes and final volume made upto mark with methanol to form 1000 µg/ml std. stock solution. From this std. stock solution, aliquot of 10 ml transferred in 100 ml of volumetric flask and final volume made upto mark with methanol to form std. stock solution of 100 µg/ml.

Preparation of calibration curve

From above std. stock solution, pippete out aliquots of 0.5 to 3.0 ml and transferred to series of 10 ml volumetric flasks and final volume made upto mark with methanol to form solutions of 5 to 30 µg/ml. These solutions were then scanned in the range of 200-400 nm against

methanol as blank. The absorbance maxima was found to be 237 nm (Fig. 2) and then calibration curve was plotted as absorbance vs. concentration.

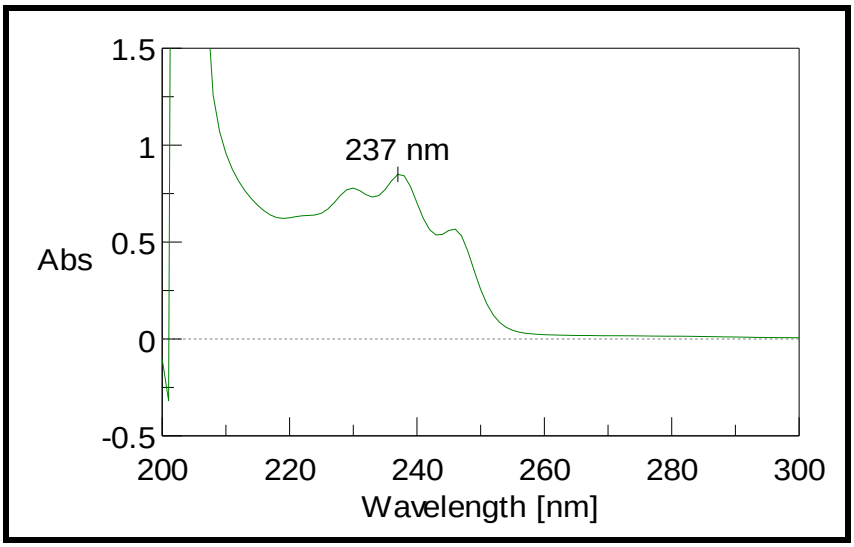

Figure 2: Absorption maxima of Simvastatin at 10 µg/ml

Sample preparation

Twenty tablets (Simvas 10) containing 10 mg of Simvastatin weighed, average weight calculated and triturated to fine powder and then weight equivalent to 10 mg of Simvastatin transferred to 100 ml of volumetric flask containing methanol, then sonicated for 15 minutes and filtered through Whatman filter paper no. 42 to form 100 µg/ml stock solution and final volume made upto mark with methanol. From this, 1 ml of aliquot transferred in 10 ml of volumetric flask containing methanol to form 10 µg/ml solution and scanned in the range of 200-400 nm against methanol as blank at 237 nm. The drug content of solution was calculated by using standard calibration curve.

VALIDATION

The present UV spectrophotometric method was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines[42]for estimation of Simvastatin in bulk and tablet dosage form.

Linearity

From std. 100 µg/ml stock solution, pippete out aliquots of 0.5 to 3.0 ml and transferred to series of 10 ml volumetric flasks and final volume made upto mark with methanol to form solutions of 5 to 30 µg/ml. These solutions were then scanned in the range of 200-400 nm against methanol as blank at 237 nm and then calibration curve was plotted as absorbance vs. concentration to check the linear relationship between absorbance and concentration of Simvastatin at 237 nm.

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 concentrations (5, 10 and 15 µg/ml) for Simvastatin. The results of precision study were reported in terms of % relative standard deviation.

Accuracy

To carry out accuracy study of proposed method, the recovery studies were carried out by standard addition method at three different levels (80, 100 and 120 %) of API sample of Simvastatin to the previously analysed solution of formulation containing 10 µg/ml of Simvastatin and the same procedure followed as described in assay. The results of precision study carried out in terms of % RSD.

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 and 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.

FORCED DEGRADATION STUDIES

ICH guidelines entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of active substance. Stability studies of Simvastatin was carried out under extreme conditions acidic, alkaline, hydrolytic, oxidative,thermolytic and photolytic as per stability indicating assays. In present study, Simvastatin was exposed to 0.1N acid, (0.1 to 5N) NaOH, 3 % H_2O_2, Distilled water, heat and sunlight at different time intervals till sufficient degradation of Simvastatin was not achieved.

Standard stock solution of Simvastatin

2.5 mg of pure Simvastatin was transferred in 25 ml of volumetric flask and dissolved in 15 ml of methanol, sonicated for 15 minutes and then final volume made upto mark with methanol to form std. stock solution of 100 µg/ml which was then filtered through 0.45 µ Whatman filter paper before used for forced degradation study.

Preparation of blank solution

In separate 10 ml volumetric flask,each containing 5 ml of solvents used for degradation such as 0.1 N HCl, 0.1 N NaOH, 3 % H_2O_2 and Distilled water, 5 ml of methanol was added and heated at 60°C. From this, 2 ml of aliquot taken in separate 10 ml of volumetric flask at different time intervals, which was then neutralized with proper solvent and final volume made upto mark with methanoland absorbance was recorded at 237 nm on UV spectrophotometer.

Acid induced degradation

In separate 10 ml volumetric flask containing 5 ml 0.1 N HCl, 5 ml Simvastatin std. stock solution was added and heated at 60°C.From this, 2 ml of aliquot taken at different time intervals in separate 10 ml of volumetric flasks till sufficient degradation of Simvastatin was not achieved, which was then neutralized with proper solventand final volume made upto mark with methanol to form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against blank.

Alkali induced degradation

In separate 10 ml volumetric flask containing 5 ml (0.1 to 5 N) of NaOH, 5 ml Simvastatin std. stock solution was added and heated at 60°C. From this, 2 ml of aliquot taken at different time intervals taken in separate 10 ml of volumetric flasks till sufficient degradation of Simvastatin was not achieved, which was then neutralized with proper solvent and final volume made upto mark with methanol to form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against blank.

Neutral hydrolysis

In separate 10 ml volumetric flask containing 5 ml Distilled water, 5 ml Simvastatin std. stock solution was added and heated at 60° C. From this, 2 ml of aliquot taken at different time intervals in separate 10 ml of volumetric flasks till sufficient degradation of Simvastatin was not achieved, which was then neutralized with proper solvent and final volume made upto mark with methanolto form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against blank.

Oxidation induced degradation

In separate 10 ml volumetric flask containing 5 ml 3% H_2O_2, 5 ml Simvastatin std. stock solution was added and heated at 60° C. From this, 2 ml of aliquot taken at different time intervals in separate 10 ml of volumetric flasks till sufficient degradation of Simvastatin was not achieved, which was then neutralized with proper solvent and final volume made upto mark with methanol to form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against blank.

Thermal degradation

0.25 mg of pure Simvastatin was exposed in an oven at 80 º C till sufficient degradation of Simvastatin was not achieved. From this, 0.1 mg of exposed Simvastatin was transferred in 10 ml of volumetric flask and final volume made with methanol to form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against methanol as blank.

Photo degradation

0.25 mg of pure Simvastatin was exposed in a sunlight till sufficient degradation of Simvastatin was not achieved. From this, 0.1 mg of exposed Simvastatin was transferred in 10 ml of volumetric flask and final volume made with methanol to form solution containing 10 µg/ml of Simvastatin and absorbance was recorded at 237 nm on UV spectrophotometer against methanol as blank.

RESULTS AND DISCUSSION

UV spectrophotometric method development

Generally for Stability Indicating method, HPTLC, HPLC with UV and MS can be used for stability indicating method which requires lot of sample preparation and solvent consumption and therefore time consuming. Since both techniques involves UV method for detection. Therefore directly going to HPLC or HPTLC methods, it is always better to go with simple, economic and time effective UV spectrophotometry method which at least gives idea about degradation and always helpful for planning to any stability indicating method by HPLC or HPTLC which reduces time.

The present study describes development and validation of UV spectrophotometric method for estimation of Simvastatin in bulk and tablet dosage form using absorption maxima method and its application to carry out stability indicating assay of Simvastatin by exposing it under different extreme conditions such as acidic, alkaline, oxidative, thermal and photolytic degradation. The Simvastatin given better solubility in methanol as compared to other solvents, therefore methanol was selected as diluent.

The present method was found to be simple and economic as compared to reported HPLC [15-30] and HPTLC [31-35] methods since it requires less sample preparation and solvent consumption. The literature survey also reveals that there was no any UV spectrophotometric method available.

Validation of UV spectrophotometric method

Results of validation parameters also proved that the present UV spectrophotometric method was found to be linear, accurate, precise and sensitive as follows.

Linearity

Linearity was evaluated by analysis of working standard solution of Simvastatin at six different concentrations. SMV found to be linear within conc. range of 5-30 µg/ml with regression coefficient of 0.9998. The results of regression analysis are summarized in (Table 1). Results shows that within the concentration range mentioned above, there was an excellent correlation between absorbance and concentration of Simvastatin (Fig. 3).

Table 1: Regression analysis of calibration graphs of Simvastatin for proposed UV Spectrophotometric method

Sr. No.	Parameters	Results
1	**Absorption maxima**	237 nm
2	**Beer's range**	3-18 µg/ml
3	**Regression equation**	Y= 0.0699X - 0.0636
4	**Correlation coefficient**	0.9998
5	**Slope**	0.0699
6	**Intercept**	-0.0636

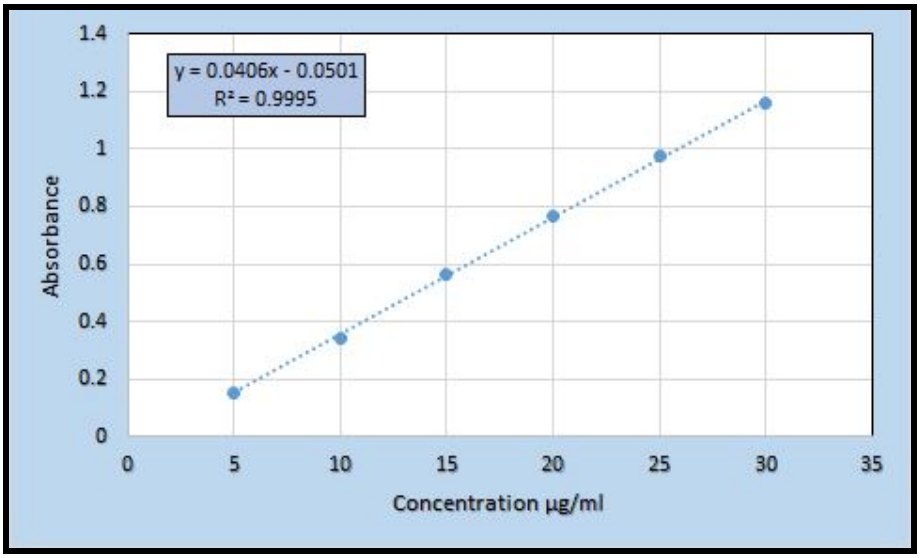

Figure 3: Calibration curve of Simvastatin

Precision

The repeatability (intra-days precision) is expressed as percentage relative standard deviations (% RSD) for the SMV at the concentration of 5, 10and 15µg/ml and their average % RSD value were0.350, 0.168 and 0.286 while for the time-different intermediate precision (inter-days precision) of the SMV at the concentration of 5,10 and 15 µg/ml, the % RSD values were 0.056, 0.216 and 0.450 respectively. The % RSD levels of intra-day and inter-day precision

were less than 2.0 in all cases, which indicated that there were no significantvariations in the analysis of SMV at the concentrations, which are shown in (Table 2 and 3).

Table 2. Result of Intraday (Repeatability) Precision studies

Parameters	% Amt. found		
	3 µg/ml	6 µg/ml	9 µg/ml
Morning	99.89	99.65	99.48
Afternoon	99.69	99.98	100.05
Evening	99.21	99.76	99.72
Mean	99.59	99.79	99.75
S.D.	0.349	0.168	0.286
% R.S.D.	0.350	0.168	0.286

S.D- Standard Deviation, % R.S.D. - % Relative Standard Deviation

Table 3. Result of Interday Precision studies

Parameters	% Amt. found		
	3 µg/ml	6 µg/ml	9 µg/ml
Day 1	99.46	99.25	99.28
Day 2	99.35	99.68	100.15
Day 3	99.42	99.46	99.52
Mean	99.41	99.46	99.65
S.D.	0.055	0.215	0.449
% R.S.D.	0.056	0.216	0.450

Accuracy

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 8,10 and 12 µg/ml of SMV. The average % recoveries for three different concentrations was found to be 99.88 %for SMV using proposed UV spectrophotometric method. The higher values indicate that the proposed method is accurate for the determination of SMV in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 4)

Table 4: Results of accuracy (Recovery) studies.

Level of % Recovery	Amount of drug taken from tablet (µg/ml)	Amount of standard drug Added (µg/ml)	% Recovery*	Standard Deviation	% R.S.D
80	6	4.8	101.48	0.528488	0.52
100	6	6	99.72	0.081445	0.08
120	6	7.2	98.44	0.060583	0.07

* Average of three estimations

LOD and LOQ

The limit of detection and limit of quantification were found to be 0.73 µg/ml and 2.07 µg/ml for Simvastatin respectively by proposed UV spectrophotometric method. Results of LOD and LOQ are summarized in (Table 5).

Table 5. Results of LOD and LOQ

Active Ingredient	LOD (µg/ml)	LOQ (µg/ml)
Simvastatin	0.73	2.07

Assay

Analysis of sample of marketed tablet containing 40 mg Simvastatin was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery of Simvastatin was 99.45 % and this value was 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

Active Ingredients	Label Claim (mg/tab)	Amt. of Drug Estimated (mg/tab)*	% Assay
Simvastatin	10	99.45	99.45

* Average of six estimations

Forced degradation study

The results of forced degradation study indicated that If considering the time span and stress condition, Simvastatin was found to be stable in alkaline (5 N NaOH upto 36 hours) and photolytic conditions (In sunlight upto 8 days) as compared to other conditions while drug was found to unstable in acidic, neutral, oxidative and thermal conditions which shown sufficient degradation of Simvastatin (upto 20 %)

and even thermal condition also shown change in λmax. Results of forced degradation study summarized in (Table 7).

Table 7: Results of Degradation study of Simvastatin at different stress conditions in developed UV Spectrophotometric method

Sr. No.	Stress Conditions	Conc. of Simvastatin solution (µg/ml)	% Degradation of Simvastatin
1	**Acidic (0.1 N HCl, 3 hrs)**	**10**	17.00
2	**Basic (5N NaOH, 36 hrs)**	**10**	13.50
3	**Oxidative (3% H_2O_2, 14 hrs)**	**10**	19.13
4	**Neutral (Dist. Water, 3 hrs)**	**10**	18.00
5	**Thermal (Oven, 4 hrs)**	**10**	17.78
6	**Photo (Sunlight, 4 hrs)**	**10**	01.50

Acid induced degradation

In stress degradation study under acidic conditions, Simvastatin exposed to 0.1N to 1N HCl at different time intervals till sufficient degradation was not achieved, but 0.1 N HCl upto 3 hours was found suitable for sufficient degradation of Simvastatin. The degradation was fond to be 17 % in this stress condition[Fig. 4].

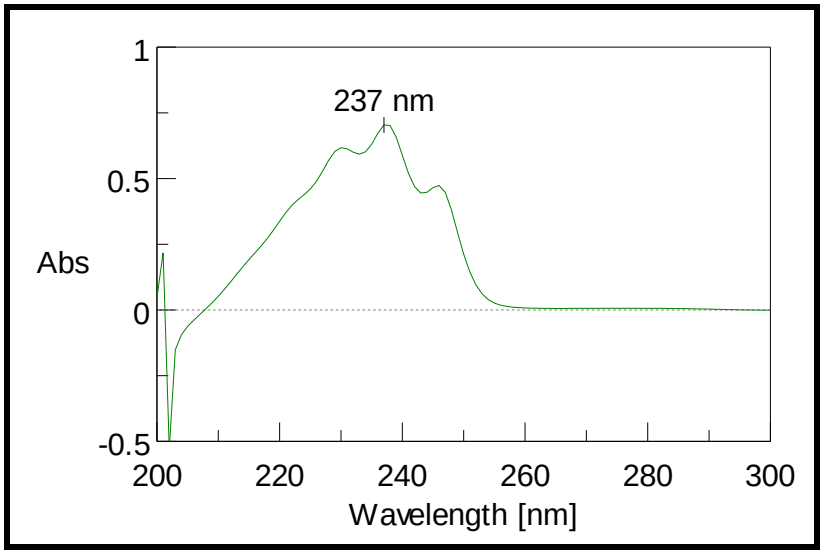

Figure 4: Acid induced degradation of Simvastatin at 10 µg/ml

Alkali induced degradation

In stress degradation study under alkaline conditions, Simvastatin exposed to 0.1N to 5N NaOH at different time intervals till sufficient degradation was not achieved, but 5 N NaOH upto 36 hours was

found suitable for sufficient degradation of Simvastatin. The degradation was fond to be 13.50 % in this stress condition [Fig. 5].

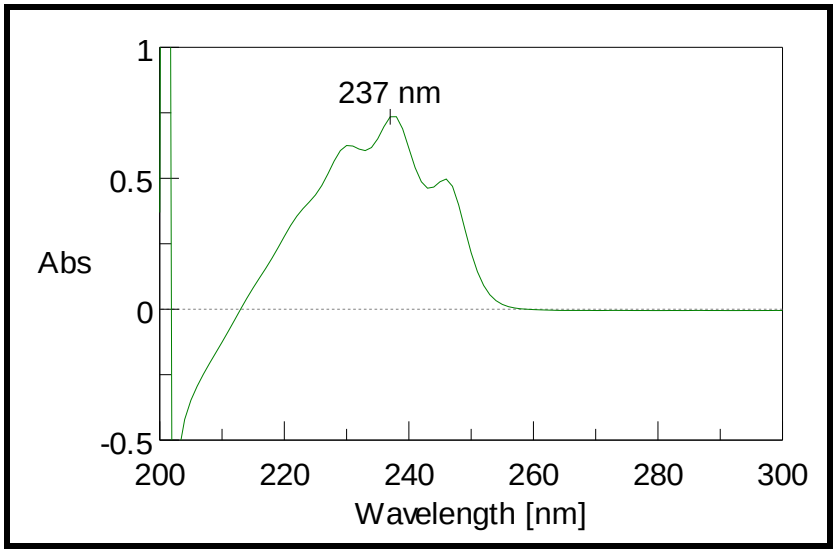

Figure 5: Alkali induced degradation of Simvastatin at 10 µg/ml

Neutral hydrolysis

In stress degradation study under neutral conditions, Simvastatin exposed to distilled water at different time intervals till sufficient degradation was not achieved, but 3 hours were foundsuitable for sufficient degradation of Simvastatin. The degradation was fond to be 18 % in this stress condition [Fig. 6].

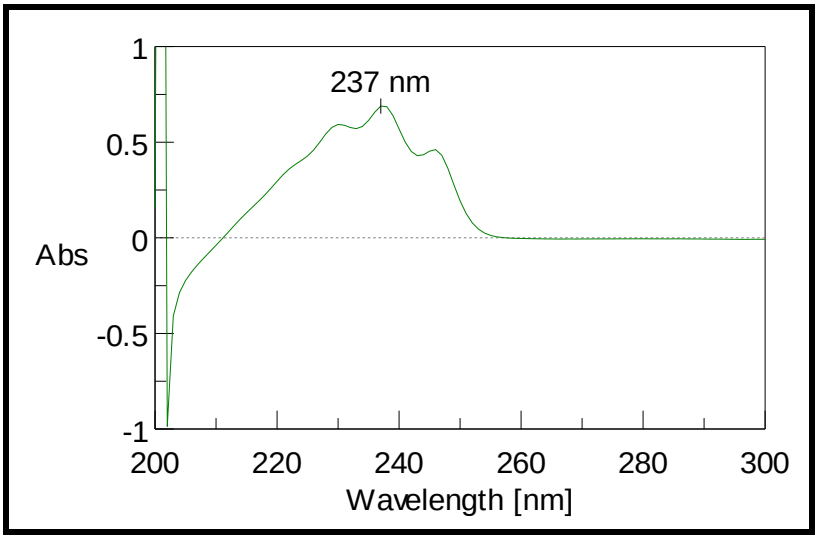

Figure 6: Neutraline induced degradation of Simvastatin at 10 µg/ml

Oxidation induced degradation

In stress degradation study under oxidative conditions, Simvastatin exposed to 3 % H_2O_2 at different time intervals till sufficient degradation was not achieved, but 14 hours were found suitable for sufficient degradation of Simvastatin. The degradation was fond to be 19.13 % in this stress condition [Fig. 7].

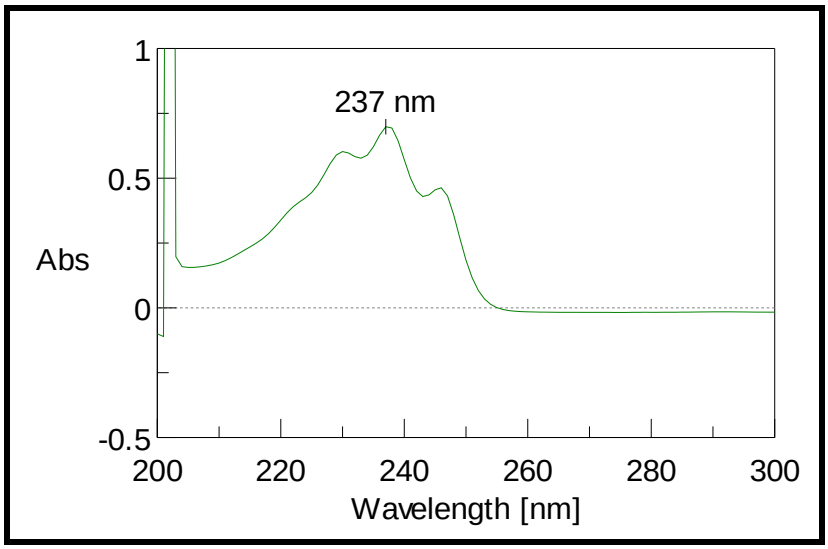

Figure 7: Oxidative induced degradation of Simvastatin at 10 µg/ml.

Thermal degradation

In stress degradation study under thermal conditions, Simvastatin exposed at 80º C at different time intervals till sufficient degradation was not achieved, but 4 hours were found suitable for sufficient degradation of Simvastatin. The degradation was fond to be 17.78 % in this stress condition with change in λmax (from 237 nm to 229 nm) [Figure 8].

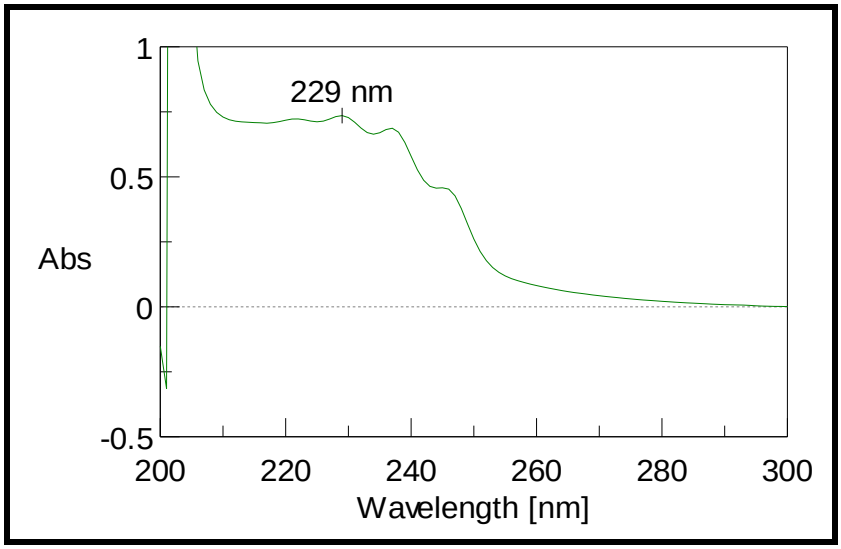

Figure 8: Thermal induced degradation of Simvastatin at 10 µg/ml with change in λmax

Photolytic degradation

In stress degradation study under photolytic conditions, Simvastatin exposed to sunlight till sufficient degradation was not achieved, but even 8 days were not found suitable for sufficient degradation of Simvastatin. The degradation was fond to be 01.50 % in this stress condition and therefore the Simvastatin was found to be stable in this stress condition [Fig. 9].

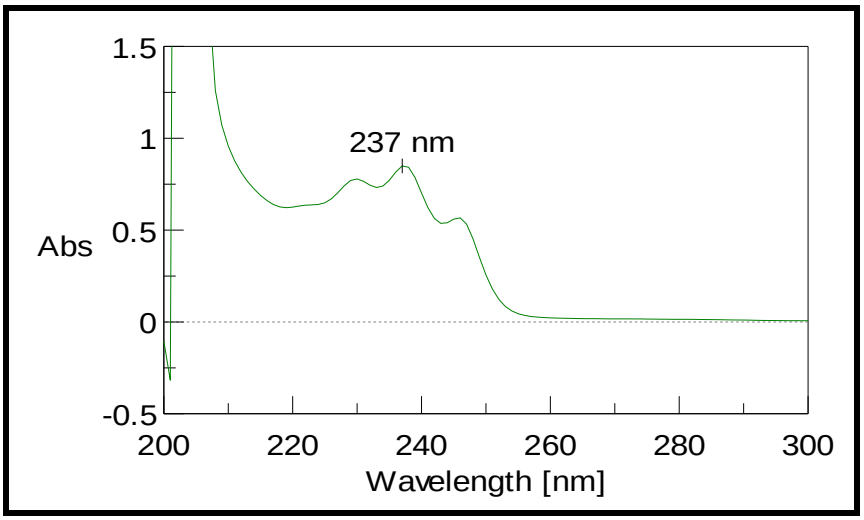

Figure 9: Photolytic induced degradation of Simvastatin at 10 µg/ml

Future Prospective

Since till date there was no any UV spectrophotometric method which has been used for stability indicating assay of Simvastatin, the present UV spectrophotometric method can be used in future before planning any HPTLC or HPLC method with UV or Mass detection for stability indicating assay which provides some idea about degradation and save the time

CONCLUSIONS

UV spectrophotometric method have been developed and validated using absorbance maxima method according to ICH guidelines.The present UV spectrophotometric method was found to be simple, rapid and economic as compared to reported RP-HPLC and HPTLC methodswhile results of validation parameters also showed that the present method was linear, accurate, precise and sensitive. The present method also used for stability indicating assay of Simvastatin by exposing it under extreme conditions such as acidic, alkaline, oxidative, thermal and photolytic degradation using forced degradation technique. The Simvastatin was found to be stable in presence of alkaline and photolytic stress conditions and found to be unstable in acidic, oxidative, neutral and photolytic stress conditions. The present method can be used for routine quality control study and for stability indicating assay of Simvastatin in fixed dose formulation before going to HPLC or HPTLC methods to save the time.

ACKNOWLEDGMENT

Authors are thankful to Gen Pharma; Pune (India) International Pvt. Ltd. for providing free gift sample of Simvastatin.

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