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[Research article]

An Experimental Design Approach for Method Development and Impurity Profiling of Simvastatin by UV Spectrophotometric and RP–HPLC Methods

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ABSTRACT

Analytical method development is a vital part of pre-formulation and formulation development research. Development of validated, robust, cost-effective methodologies for routine drug estimations is the urgent need of the pharmaceutical R&Ds. Quality is an essential attribute in any pharmaceutical product and impurity profiling offers a broad scope with the changed perspectives of the research scenario. The present work aims to devise a validated UV-spectroscopic and RP–HPLC method for estimations of SVN in bulk and formulations and impurity profiling of SVN related impurities at the specification limit with the aid of response surface methodology. The percent assay for SVN determined by UV method was 100.4±0.15 and mean %recovery was achieved to be 99.87±0.19. The percent assay of SVN by RP-HPLC method was found to be 100.14±0.1. The mean percent recovery at different spike levels (50–150%) ranged from 97.3±0.01–100.5±0.02 and the %RSD of assays at lower and higher spike levels were 1.4 and 0.3 respectively. The linearity data of impurities (A–G) at different spike levels (25, 50, 100, 125 and 200 µg) showed a high correlation coefficient of 0.99 in all cases. Percent mean recovery of impurities (A–G) at different spike levels comply the acceptance criterion. All other validation parameters also comply within the range of acceptable limits. The impurities were well separated with good resolution and peak shape, good retention times. The robustness of the developed HPLC method and that of impurity profiling was optimized applying Box-Benkhen experimental design approach.

Keywords: Method development, Impurity profiling, Validation, Box-Benkhen, Experimental design.

INTRODUCTION

Analytical method development plays a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. It is an integral part of preformulation and formulation development research. Quality assurance and quality control departments of pharmaceutical industries are largely responsible in bringing out safe, effective dosage formulations. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of analytical methodologies which are simple, rapid, cost effective, and robust thus providing results with great accuracy and precision. Sophisticated hyphenated techniques are in vogue.

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But cost factor, analyte extraction from respective sample matrices and complicated sample preparation steps, time consumption, difficulty in operation and error in recovery limit their routine applications[1-3].Impurity profiling is a broad term which encompasses identification, quantitative determination and structural elucidation of impurities with the aid of spectroscopic or chromatographic techniques or the utilizations of latest developed hyphenated methods. ICH defines impurities as "any chemical compound of the medicinal product which is not the chemical entity defined as the active substance or as an excipient in the product." According to its guidelines, the threshold limit for any impurity is lower at 0.1% for drugs used in dosages more than 2 gm/day and those dosed at less than 2 gm/day, the limit is below 0.05%.

Quality is an essential attribute in any pharmaceutical product greatly determined by the content of active ingredient present in it. In the pharmaceutical world, impurities are considered as any material other than the active pharmaceutical ingredient (API) or excipients; may be of organic or inorganic origin, may arise from varied sources like process related drug substances (starting material, intermediate or drug product), impurity in starting materials, due to degradation of drug substances, or unwanted excipient-interactions, contaminations of some reagents and catalysts, presence of enantiomeric impurities and some impurities may be due to environmental factors. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products and can precipitate adverse and toxic drug reactions in patients after consumption.[1-5] With the tremendous advancements of analytical technologies and changed perspectives of the research scenario, not only detection of active constituents but a detailed profiling of impurities offers a broad scope of research in pharmaceutical and other bio-allied fields.

Simvastatin (SVN), chemically butanoic acid, 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-napthalenyl ester (**Fig.1**); a lipid lowering agent derived synthetically from fermentation products of *Aspergillusterreus*. Pharmacologically, SVN on oral ingestion is an inactive lactone, hydrolyzed to β-hydroxy acid leading to the inhibition of HMG-CoA reductase (3-hydroxy-3-methyl glutaryl coenzyme A) which catalyzes the conversion of HMG-CoA to mevalonate which is the early rate limiting step in cholesterol biosynthesis. SVN can be obtained in various synthesis pathways. During synthesis apart from the main reaction, unwanted side reactions are one of the major causes of impurities. In case of SVN, lovastatin and analogues are one of the major sources of impurities. The possible degradation pathways of SVN are provided in **Fig.2**. The major process related impurities of SVN which may be either due to synthetic or degradation pathways are presented in **Fig.3**. The impurities of simvastatin are: (**A**) (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxy heptanoic acid (hydroxyacid); (**B**) (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-(acetyloxy)-6-oxotetra hydro-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl2,2-dimethyl butanoate (acetate ester);(**C**) (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2H-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydro naphthalen-1-yl2,2-dimethylbutanoate (anhydro simvastatin); (**D**) (2*R*,4*R*)-2-[[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2H-pyran-4-yl(3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (dimer); (**E**) R1=CH3, R2=H: (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl(2*S*)-2-methylbutanoate (lovastatin);(**F**) R1=H, R2=CH_3:(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl(2*R*)-2-methylbutanoate (epilovastatin); and (**G**) (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxo tetrahydro-2H-pyran-2-yl]ethyl]-7-methyl-3-methylene-1,2,3,7,8,8a-hexahydronaphthalen-1-yl2,2-dimethylbutanoate.

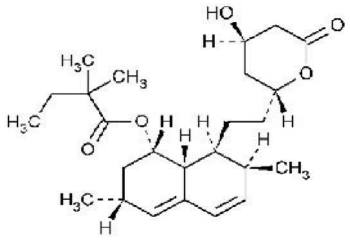

Fig.1: Structure of Simvastatin

Factorial experiments find extensive applications in all research fields which allows investigation of multiple factors simultaneously and also examination of one factor at different levels of the other factor or factors. The Box-Behnken experimental design (BBD), developed by Box and Behnken in 1980 is a useful response surface methodology, where the level of one of the factors is fixed at centre level while combinations of all levels of the other factors are applied.[6-8]

Extensive literature surveys have reported some of

the spectroscopic and chromatographic methods for estimations of SVN in bulk and formulations.[9-15] There are reporting's of impurity profiling of SVN in USP (United States Pharmacopeia) and EP (European Pharmacopeia). Some researchers reported about FTIR, UPLC and hyphenated techniques.[16,17] But current research aims to devise a validated UV-spectroscopic and RP–HPLC method for estimations of SVN in bulk and formulations and impurity profiling of SVN related impurities at the specification limit with the aid of response surface methodology.

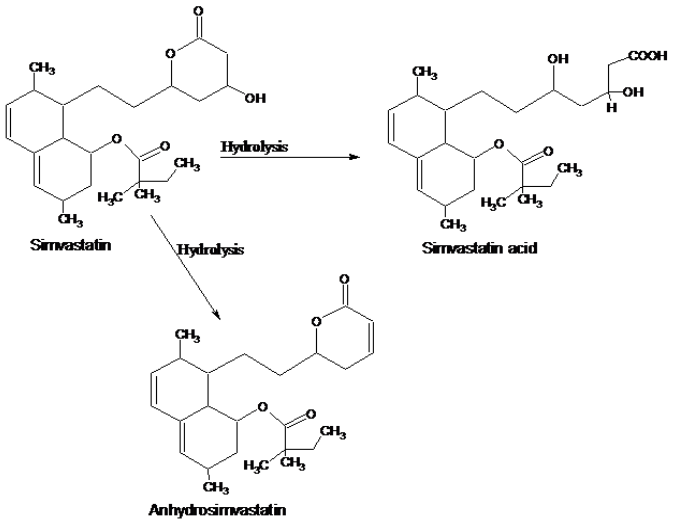

Fig.2: Possible degradation pathways of Simvastatin

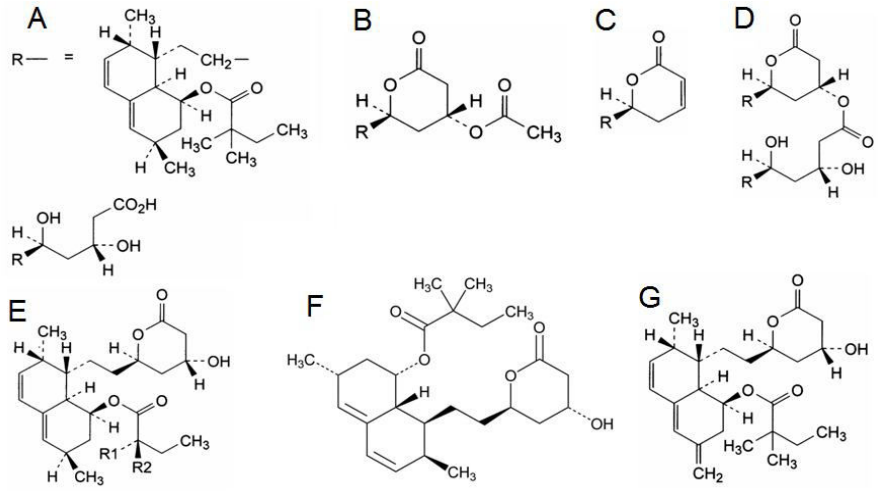

Fig.3: Impurities of simvastatin: (A) hydroxyacid (B) acetate ester; (C) anhydrosimvastatin (D) dimer; (E) lovastatin; (F) epilovastatin; and (G) dimethylbutanoate

MATERIALS AND METHODS

Reagents

Simvastatin (SVN) was a kind gratis of Salius Pharma Pvt. Ltd. Mumbai, India. Impurities (A–G) were obtained from Sigma Aldrich, Mumbai, India. Di Sodium hydrogen phosphate, potassium dihydrogen phosphate, acetonitrile, triple distilled water, methanol and other reagents used were either AR grade or HPLC grade, purchased from Merck (Mumbai) and Sigma (India).

Instruments

HPLC (Equipped with a Agilent technologies 1100 series VWD detector); UV-visible spectrophotometer (Thermo Scientific, Aquamate Plus, India), Electronic balance (Shimadzu, Japan), Sonicator (Cyber labs, India), pH meter (Datla instruments, DI-45, India), Syringe Filters (ZodiacLife Sciences, India), Nylon filters; water bath (Lab Companion, Mumbai, India).

Software

Experimental design, data analysis and surface plots were performed by using Design Expert Trial version 7.0 and Matlab version 12.0.

UV method development

For the UV estimation of SVN methanol was used as the main solvent for preparing standard and stock solutions. A buffer solution was used as the diluent. The buffer solution was prepared by mixing a solution of 13.6 gm of Potassium dihydrogen phosphate in 1000 mL of distilled water designated as solution A. Another solution-B was prepared by dissolving 35.8 gm of disodium hydrogen phosphate in 1000 mL distilled water. Next, 20 mL of solution-B and 1000 mL of solution-A was mixed which was used as diluent. The standard and stock solutions of SVN were prepared in methanol and further dilutions of working standards were achieved by dilutions with the above diluent. A solution of SVN of 10 µg/mL concentration was scanned in the wavelength range of 200–400 nm and the drug showed absorbance maxima at 238 nm. The standard calibration curve was prepared with aliquots in the concentration range of 2.5–15µg/mL using reagent as the blank.

Validation of the method

The above method was validated as per ICH guidelines in terms of linearity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ).

The linearity range for the estimation of SVN by UV was determined by preparing aliquots in the concentration range of 2.5–15 µg/mL and absorbances measured at 238 nm. Calibration curves (concentrations vs absorbance) were plotted and R^2 value not less than 0.99 was regarded as acceptance criterion.

Accuracy of the proposed method was ascertained by recovery studies using standard addition method where known quantity of standard drug was mixed with formulation sample at three different levels of 50, 100 and 150% and percent recovery for SVN in the range of 98–102% were regarded as acceptance criterion.The precision was studied by inter and intra-day variations in the test method of SVN and expressed as percent relative standard deviation (%RSD) where the values should not be greater than 2%.The LOD and LOQ parameters were determined from the calibration curves basing on the formulae:

LOD = 3.3 σ / S and LOQ = 10 σ / S

Assay of SVN

Ten tablets were weighed accurately and then triturated thoroughly. Accurately weighed portion of powder equivalent to 5 mg of SVM was transferred to 1000 mL volumetric flask; dissolved in 20 mL of methanol and diluted with diluent. It was centrifuged for 20 min. Absorbance was determined at 238 nm. Amount of SVN was determined using the formula:

$$\text{Amount present} = \frac{\text{Test O.D.} \times \text{Standard conc.} \times \text{Dissolution factor}}{\text{Standard O.D.}}$$

$$\% \text{ Purity} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

Estimation of SVN by RP–HPLC

The current research represents a new stability indicating, validated RP–HPLC method development. The buffer was prepared by dissolving 35.49 gm of disodium hydrogen phosphate in 1000 mL of distilled water, pH adjusted to 4.5 with dilute ortho phosphoric acid, filtered through 0.45µm nylon membrane filter and degassed. The diluents consisted of a mixture of methanol: water: buffer: BHA in the ratio of 63:35:1:1 well sonicated and degassed.

Optimization of chromatographic conditions

Before proceeding to the optimized RP–HPLC chromatographic conditions for SVN estimations, three trails were conducted with varying ratios of methanol: water: buffer mobile phase compositions. Amongst three trials injection volume (10 µL), flow rate (1 mL/min), column temperature (40°C), and detector wavelength (238 nm) were kept constant. Run time varied between 15–18 min. With mobile phase ratio of 40:40:20 (methanol: water: buffer), a comparatively long retention time was observed. On changing the ratio to 50:45:5, peak tailing was observed with delayed retention time. With mobile phase ratio of 55:40:5, peak tailing was observed. Satisfactory results were obtained with a mobile phase ratio of 60:36:4 with a run time of 15 min.

Thus the optimized chromatographic conditions for SVN estimations were obtained with isocratic separation mode in a C_{18} column (150 × 3.9 mm, 5µ) using a degassed mixture of methanol: water: buffer in the ratio of 60:36:4; injection volume (10 µL), flow rate (1 mL/min) and run time (15 min), at column temperature (40°C), and detector wavelength (238 nm).

The stock solution was prepared by dissolving 100 mg of SVN in 100 mL of methanol. From this solution, 10 mL was pipette into 100 mL volumetric flask, mixed well with 30 mL diluents and volume adjusted with the same. This served as the working standard solution.

Twenty tablets were accurately weighed and the average weight was calculated. The tablets were triturated well and power equivalent to 10 mg were transferred to 100 mL volumetric flask containing

30 mL of diluent. The solution was sonicated for about 20 min and volume adjusted with the diluent. Next the solution was centrifuged at 4000 rpm for 15 min. This centrifuged solution served as the test solution.

System suitability

The solution for testing system suitability was prepared by transferring 1 mg of Lovastatin (LVN) standard in 100 mL volumetric flask to which 10 mL of standard stock solution and 40 mL of diluents was added. The mixture was sonicated well and volume adjusted with diluents.

Acceptance criteria lies in the fact that the % RSD for the retention times of principal peak from 10 replicate injections of system suitability solution should be not more than 2.0 %; resolution between SVN and LVN should not be less than 3; theoretical plate number and tailing factor of SVN should not be more than 2000 and not less than 2 respectively.

Assay of SVN

The percent assay of SVN was determined using the formula:

$$\text{Assay } \% = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{Avg. Wt}}{\text{Label claim}} \times 100$$

where AT is area of the test substance, as is the area of standard substance, WS and WT are the weights of standard and test substance respectively and DS, DT are the dilutions of standard and test respectively; P is the percent purity. The assay of SVN should be not less than 97.0% and not more than 103.0%.

Method validation

The proposed RP–HPLC method was validated as per ICH guidelines. Linearity of the method was determined using solutions prepared in the concentration range of 50–150% from SVN working standard. Accuracy of the proposed method was ascertained by recovery studies using standard addition method, where the spike level of drug substance with placebo were in concentrations of 50, 75, 100, 125 and 150 µg and the acceptable criteria of percent recovery for SVN were set between 95–105%. Precision was studied in terms of repeatability (system precision), where six samples prepared on SVN tablets as per test procedure were assayed and %RSD of assay results were calculated which should not be more than 2.Intermediate precision study or ruggedness of experimentation was carried out by different analyst, on different instrument, using different columns and on different days. The %RSD of assay results should not be more than 2. Specificity of the method is tested by observing placebo interference at the retention time of SVN.

For measurement of robustness, BBD approach of RSM was employed to evaluate the effect of three independent factors, viz. flow rate (X_1), mobile phase composition (X_2) and pH of mobile phase (X_3) on responses like tailing factor of SVN peak (Y_1), resolution between LVN and SVN peak (Y_2), %RSD of peak areas of SVN after five replicate injections (Y_3) [8-10]. Basing on previous experimentations and knowledge, the ranges of values used in the design are as follows: flow rate (X_1): 0.8–1.2 mL/min; mobile phase composition (X_2): 90% and 110%; and buffer pH (X_3): 4.3–4.7. Compatibility with system suitability values under altered conditions was tested to check if they meet the acceptance criteria. The polynomial equation generated for the study was:

$$\begin{aligned} Y = {} & 0.12 - 0.025X_1 + 0.025X_2 + 0.025X_3 \\ & - 0.05X_1X_2 - 0.05X_1X_3 \\ & + 0.00X_2X_3 + 0.065X_1^2 \\ & + 0.065X_2^2 - 0.35X_3^2 \end{aligned}$$

whereX_1= mobile phase composition, X_2= flow rate, X_3= pH of mobile phase, X_4= variation in column oven temperature.

Bench top stability of SVN standard and test solutions were determined at initial, first, second and seventh day against freshly prepared standard each time. Difference in percent assay results not more than 3 from initial value are regarded as acceptable criteria. Similarity factors for standard preparations in range of 0.98–1.02 are the acceptance limit. Bench top stability of mobile phase tested initially, first and second day is considered stable if system suitability criteria are within acceptable limits.

Interference from the products of forced degradation studies conducted by acid hydrolysis, base hydrolysis, peroxide oxidation, degradation by sun light and UV radiations, heat degradation, humidity and water stress conditions were evaluated for peak purity. Acceptable criterion is satisfied if purity angle be less than purity threshold of SVN peak and should not have any flag in purity results.

Impurity profiling by RP–HPLC method

The optimized chromatographic conditions for impurity profiling include gradient elution through Lichrosphere RP18 column (250 × 4.6mm, 5µm) using o-phosphoric acid as mobile phase A and acetonitrile (ACN) as mobile phase B. A degassed mixture of buffer: ACN (20:80 v/v) was used as diluents. Injection volume was 20µL with a flow rate of 1.5 mL/min. Column temperature was maintained at 30°C, the detector wavelength set at 238 nm with a total run time of 50 min.

The buffer solution was prepared by dissolving 1.41 gm of potassium dihydrogen phosphate in 1000 mL water, well sonicated and the pH was

adjusted to 4 with dilute orthophosphoric acid. It was filtered through 0.45µ membrane filter. For preparation of sample solution, 20 tablets were weighed accurately and triturated well. A 50 mg of SVN was transferred to 50 mL of volumetric flask, 20 mL of diluent added, sonicated for 10 min and volume adjusted with diluent. The solution was centrifuged at 4000 rpm for 10 min and the supernatant was used for analysis.

The standard solution was prepared by dissolving 20 mg of SVN working standard in 15 mL of diluents taken in 100 mL of volumetric flask, sonicated for dissolution of SVN and volume adjusted with the diluent. The solution for placebo was prepared by dissolving placebo powder equivalent to 50 mg of SVN in 20 mL of diluent taken in 50 mL volumetric flask, sonicated for 10 min and volume adjusted with diluent. The solution was centrifuged at 4000rpm for 10min and the supernatant was used for further analysis.

The impurities stock solution were prepared by dissolving accurately weighed 20 mg of impurities in 20 mL of diluents taken in 50 mL of volumetric flask, sonicated well for 10 min and volume adjusted with the diluent. The spiked sample solution was prepared by transferring 20 mg of SVN working standard in 100 mL volumetric flask to which 3 mL of impurities stock solution was added, sonicated and volume adjusted with diluent. Next to inject separately 20µL of diluents (as blank) twice and standard, test and placebo preparations six times in to the HPLC system and the relative response factors (RRF) and relative retention time (RRT) of known impurities with respect to SVN were determined.

System suitability

The solution for system suitability was prepared by dissolving 20 mg of SVN working standard in 40 mL of diluent taken in 100 mL volumetric flask; sonicated for 10 min and volume adjusted with diluent. In another 25 mL volumetric flask, 12.5 mg of lovastatin working standard was dissolved in diluent, sonicated and volume adjusted with the same. A 1 mL of each of the two solutions were pipetted into another 25 mL volumetric flask, mixed well and volume adjusted with the diluent. The solution is injected into the HPLC system. The acceptance criteria complies when theoretical plate count of LVN and SVN peak is not less than 8000; resolution between SVN and LVN is not less than 6 and %RSD of LVN and SVN after six replicate injections should not be more than 2.

Method validation

The developed RP–HPLC method for detection and segregation of impurities A–G was validated as per ICH guidelines [4–7]. The linearity of the method for impurity profiling was studied by injecting impurities and SVN with concentration ranging from LOQ to 200% and R^2 value not less than 0.99 and %RSD of peak areas of the solution not more than 2% was regarded as the acceptance criterion. Accuracy of the proposed method ascertained by recovery studies was carried out in triplicate using standard addition method where the test preparation was spiked with impurities stock solutions in the concentrations of 50, 75, 100, 125 and 200 µg and the acceptable criteria for percent recovery of impurities were set at 85–115%.

System precision or repeatability of the method was evaluated by analyzing six samples prepared by spiking test preparations with impurity blend solutions to get each impurity target concentration and the %RSD of relative retention times(RRT) should not be more than 2%. Intermediate precision or ruggedness was carried out by different analyst, on different instrument and on different days.

The specificity of the method was studied to see if there is any interference of placebo or interferences from known impurities at the retention times of test impurities.Interference of degradation products due to acid hydrolysis, base Hydrolysis, peroxide oxidation, degradation by Sun light and UV radiations, thermal and humidity degradations were also conducted.

For measurement of robustness, BBD approach of RSM was employed to evaluate the effect of independent factors, viz.mobile phase composition (90% and 110%); flow rate (1.3 mL/min and 1.7 mL/min); and pH (3.8 and 4.2) on responses like resolution between LVN and SVN peak (Y_1); RSD of peak areas of LVN from six replicate injections (Y_2); RSD of peak areas of SVN from six replicate injections (Y_3). The polynomial equation generated for the study was:

$$Y = 0.22 - 0.0375X_1 + 0.125X_2 - 0.025X_3 - 0.075X_1X_2 + 0.00X_1X_3 - 0.05X_2X_3 - 0.0025X_1^2 - 0.0475X_2^2 - 0.0225X_3^2$$

whereX_1=flow rate, X_2=mobile phase composition, X_3=pH of mobile phase composition.

LOD and LOQ for SVN impurities and SVN were determined basing on signal to noise ratio and the S/N ratio near to 3.0 and 10.0 was considered for LOD and LOQ respectively.

RESULTS AND DISCUSSION

UV method development

SVN showed absorption maxima at 238 nm and linearity was achieved in the concentration range of 2.5–15 µg/mL with R^2 value of 0.99 (y=0.06x+0.000). The percent assay for SVN was determined to be 100.4±0.15. The mean %recovery was achieved to be 99.87±0.19. Precision studies of

intra and inter day assays expressed in terms of %RSD were found to be 0.2 and 0.3 respectively. The LOD and LOQ were found to be 0.165 µg/mL and 0.5 µg/mL respectively.

RP–HPLC method development

The linearity of the method was demonstrated in the concentration range of 18–225 µg/mL with R^2 value of 0.99 (**Fig.4**). The percent assay of SVN was found to be 100.14±0.1. Results of system suitability parameters have shown that tailing factor of SVN peak was 1.1; resolution between SVN and lovastatin (LVN) peak was 4.2 and %RSD of peak areas of SVN from five replicate injections were 0.1; values of all parameters falling within the acceptance criterion.

The mean percent recovery at different spike levels (50–150%) ranged from 97.3±0.01–100.5±0.02 and the %RSD of assays at lower and higher spike levels were 1.4 and 0.3 respectively. Regarding precision, the system precision or repeatability of the method showed %RSD of peak areas to be 0.2 and it is 0.1 in case of intermediate precision or ruggedness. In both cases the values are less than 2%. Results of method robustness and specificity due to physical and chemical degradation studies are presented in **Table 1 and 2**. The surface and contour plots of the method robustness is provided in **Fig.5-6**.

Bench top stability of SVN standard and test preparations have shown the initial and final assay results to be 102.3 and 103.0 respectively and the similarity factors for standard preparations is 0.99–1.00; all values coming within acceptance criterion. Considering the bench top stability of the mobile phase, initial and final values of %RSD of peak areas of SVN after five replicate injections were 0.1 and 0.2 respectively, the tailing factor value of 1.1 was same in both cases and the resolution of SVN and LVN peaks were 4.2 initially and 4.0 after 2 days. Thus all the system suitability parameters of bench top stability of mobile phase falls within the acceptance criterion.

Impurity profiling by RP–HPLC method

Results of system suitability parameters have shown that resolution between LVN and SVN peaks was 10, %RSD of peak areas of LVN after six replicate injections were 0.1 and the same following SVN injection was also 0.1; all values meeting the acceptance criterion. The linearity data of impurities (A–G) at different spike levels (25, 50, 100, 125 and 200 µg) showed a high correlation coefficient of 0.99 in all cases. Percent mean recovery of impurities (A–G) at different spike levels (50%, 75%, 100%, 150% and 200%) carried out in triplicate were found to be 106.25 ± 0.01, 108.19 ± 0.02, 107.31 ± 0.01, 107.81 ± 0.01, 105.05 ± 0.01 for impurity A at respective spike levels; for impurity B it was 100.65 ± 0.01, 100.66 ± 0.01, 98.18 ± 0.01, 98.29 ± 0.01, 97.96 ± 0.01; for impurity C values are 97.05 ± 0.01, 97.68 ± 0.01, 96.11 ± 0.02, 97.10 ± 0.02, 94.65 ± 0.02; for impurity D values are 91.44 ± 0.01, 92.88 ± 0.01, 90.85 ± 0.02, 94.32 ± 0.01, 91.26 ± 0.01; for impurity E it was 101.03 ± 0.01, 101.17 ± 0.01, 100.65 ± 0.01, 100.15 ± 0.01,100.68 ± 0.01; for impurity F it was 100.03 ± 0.01, 100.19 ± 0.01, 99.65 ± 0.01, 99.15 ± 0.01, 99.68 ± 0.01; for impurity G values are 92.65 ± 0.01, 91.15 ± 0.01, 91.74 ± 0.01, 92.78 ± 0.01, 93.15 ± 0.01. The values of the recovery studies in all cases comply within the range of acceptance criterion (85–115%).

The %RSD values of System precision or repeatability of the method for impurities (A–G) in terms of relative retention times (RRT) and %impurities were 0.02 and 0.98 respectively for impurity A; 0.116 and 0.47 respectively for impurity B; 0.119 and 0.32 respectively for impurity C; 0.153 and 0.43 respectively for impurity D; 0.033 and 0.4 respectively for impurity E; 0.114 and 0.41 respectively for impurity F; 0.017 and 0.5 respectively for impurity G.

Intermediate precision or ruggedness with two different analyst have shown that resolution between LVN and SVN peaks were 10 and 9 for analyst 1 and 2 respectively; %RSD of peak areas of LVN with two analysts were 0.1 in both cases; and %RSD of peak areas of SVN with two analysts were 0.1 in both cases. Thus in all cases the values comply with the standard acceptance criterion.

The LOD and LOQ values of impurities with corresponding signal to noise ratio is provided in **Table 3**. Specificity of the method in terms of interferences of the impurities and physical and chemical degradation studies are provided in **Tables 4 and 5**. Results of robustness of the impurity profiling method are presented in **Table 6** and the corresponding surface and contour plots in **Fig.7-8**.

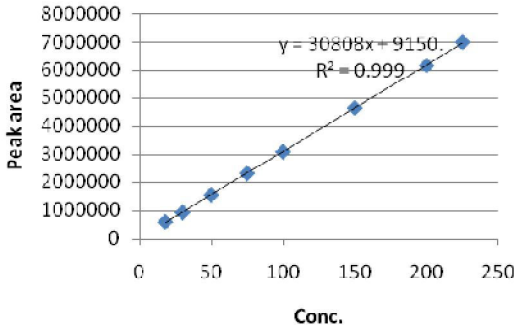

Fig.4: Calibration curve for HPLC method development

Table 1: Robustness of the RP–HPLC method development of simvastatin

Robustness parameters	System suitability parameters		
	TF of SVN peak	Resolution between SVN and LVN peak	%RSD of peak areas of SVN
Mobile phase composition			
90%	1.1	5.0	0.2
100%	1.1	4.2	1.0
110%	1.1	3.0	0.2
Flow rate (mL/min)			
0.8	1.1	4.8	0.1
1.0	1.1	4.1	0.1
1.2	1.1	4.5	0.1
pH of mobile phase			
4.3	1.1	3.0	0.2
4.5	1.1	4.1	0.1
4.7	1.1	3.0	0.2

Table 2: Specificity of the method by physical and chemical degradation studies

Stress condition	Purity angle	Purity Threshold	Purity Flag
UV light stress 7days	0.171	0.413	No
Acid Degradation (0.1N HCl at 60°C for 30 min)	0.132	0.331	No
Base degradation (0.1N NaOH at 60°C for 30 min)	0.126	0.325	No
Peroxide degradation (1% H_2O_2 at 60°C for 30 min)	0.107	0.309	No
Thermal degradation (at 105°C for 6 hr)	0.178	0.399	No
Humidity degradation (90% RH for 7 days)	0.191	0.419	No

Table 3: LOD and LOQ values of impurities

Name of the impurities	Parameters		Signal to Noise ratio	
	LOD % Impurity	LOQ % Impurity	LOD	LOQ
Impurity-A	1.1	1.2	4.097	11.28
Impurity-G	0.0028	0.0093	4.499	11.68
Impurity-E	0.0016	0.0053	4.413	12.09
Impurity-B	0.0013	0.0042	4.692	11.33
Impurity-C	0.0014	0.0048	4.169	10.54
Impurity-D	0.0026	0.0088	4.022	9.03
Simvastatin	0.0005	0.004	2.349	10.517

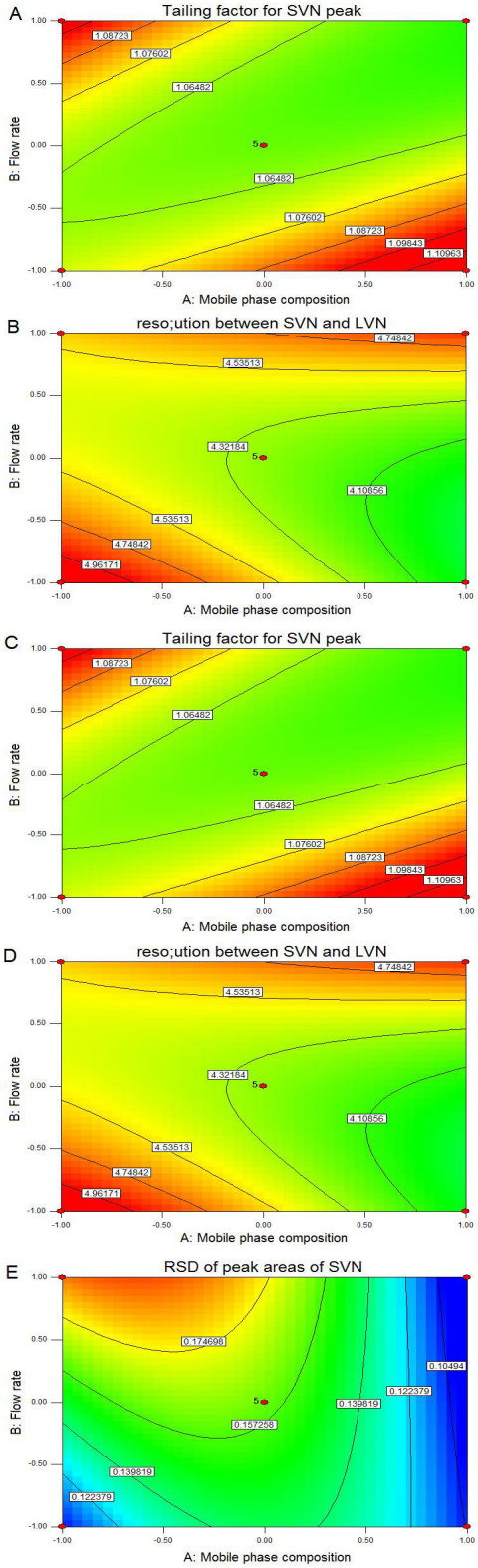

Fig.5: Contour plots of HPLC method robustness showing the responses of independent variables like mobile phase composition, flow rate and pH of mobile phase

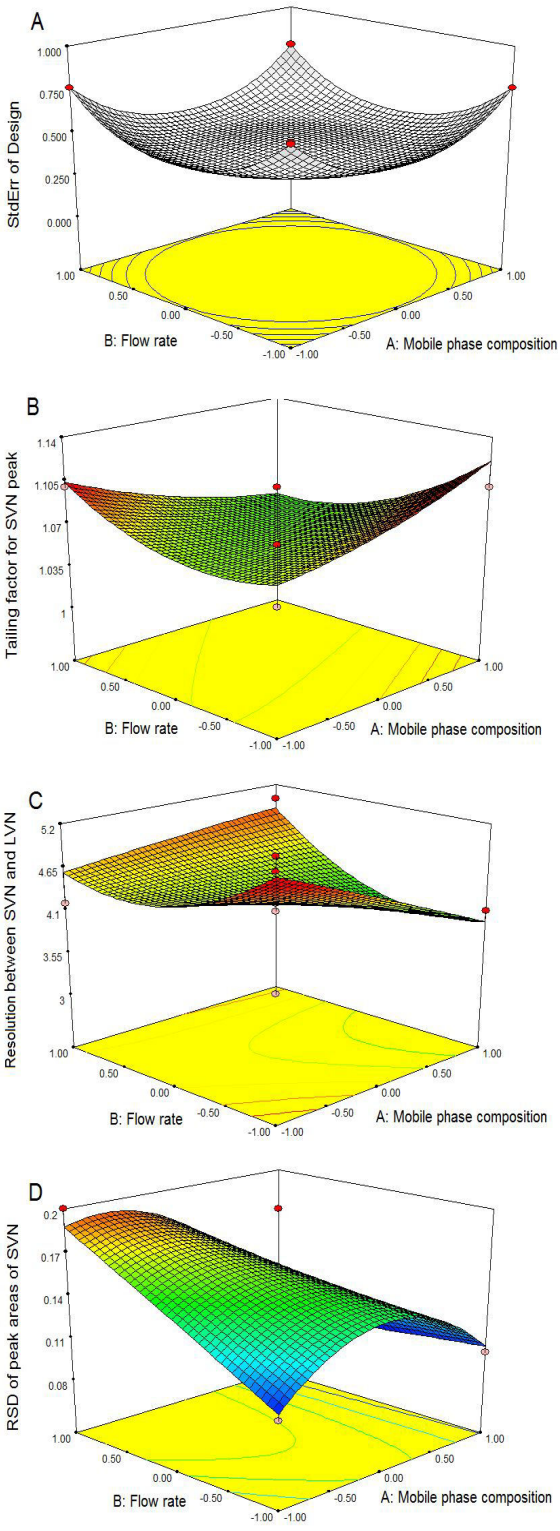

Fig.6: Surface plots of HPLC method robustness showing the responses of independent variables like mobile phase composition, flow rate and pH of mobile phase

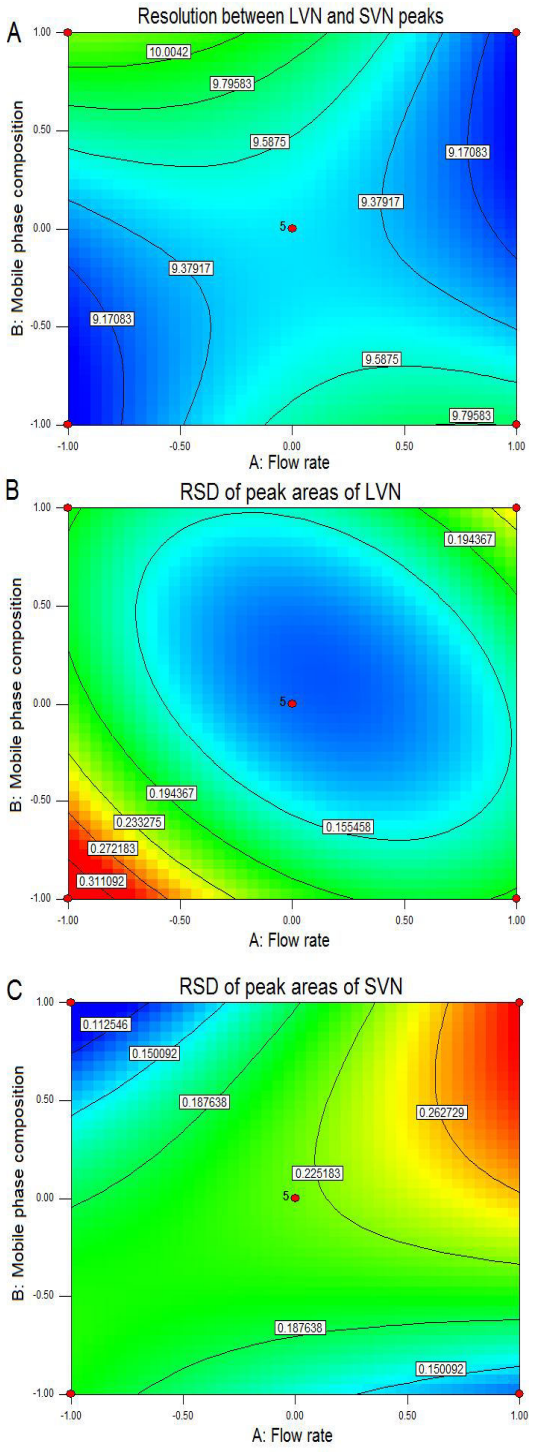

Fig.7: Contour plots of impurity profiling showing the responses (resolution between LVN and SVN peak; RSD of peak areas of LVN and RSD of peak areas of SVN) of independent variables (mobile phase composition, flow rate and pH of mobile phase).

Table 4: Specificity of the method due to impurity interference

Name of impurity	RT
Impurity A	8.207
Impurity-E	10.867
Impurity-F	11.178
Impurity-G	12.316
Impurity-B	23.520
Impurity-C	23.687
Impurity-D	37.123
Simvastatin	14.669

Table 5: Specificity of the impurity profiling method by physical and chemical degradation studies

Stress condition	Purity angle	Purity Thres-hold	Purity Flag
UV light stress (7 days)	0.318	0.761	No
Acid Degradation (0.1N HCl at 60°C for 30 min)	0.312	0.635	No
Base degradation (0.1N NaOH at 60°C for 30 min)	0.375	0.754	No
Peroxide degradation (1% H_2O_2 at 60°C for 30 min)	0.294	0.452	No
Thermal degradation (at 105°C for 6 h)	0.374	0.547	No
Humidity degradation (90% RH for 7 days)	0.302	0.712	No

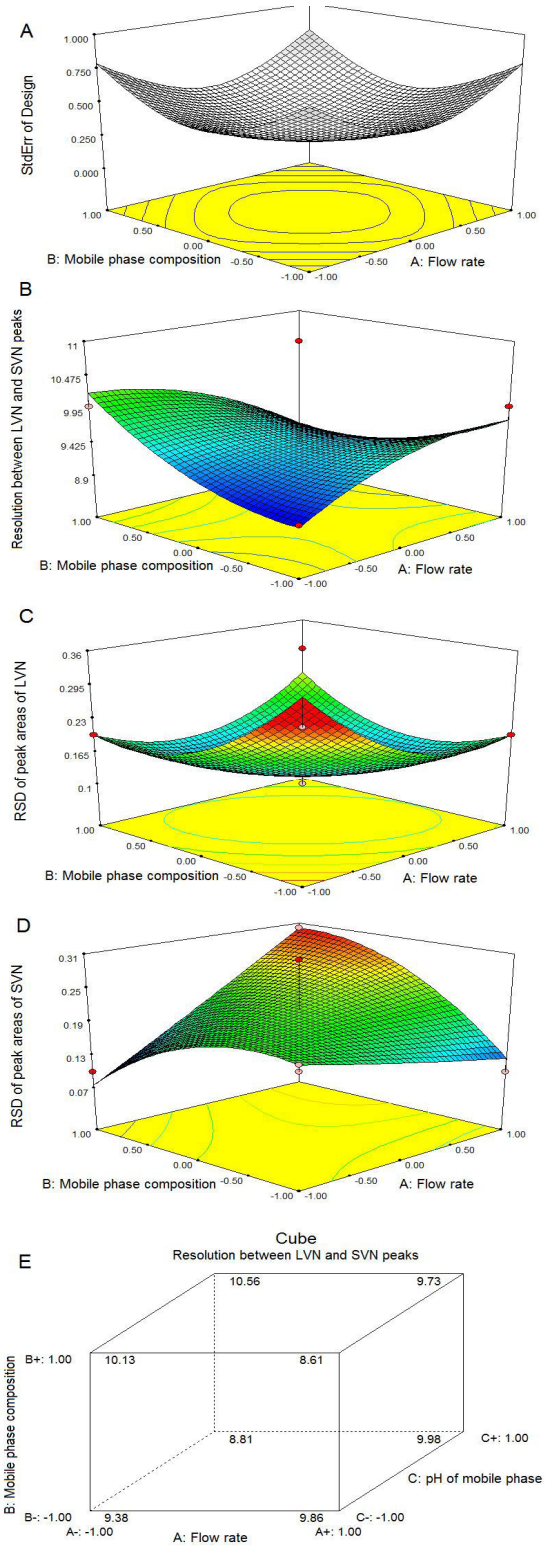

Fig.8: Surface plots of impurity profiling showing the responses (resolution between LVN and SVN peak; RSD of peak areas of LVN and RSD of peak areas of SVN) of independent variables (mobile phase composition, flow rate and pH of mobile phase

Table 6: Robustness of the RP–HPLC method for impurity profiling

Robustness parameters	System suitability parameters		
	%RSD of peak areas of SVN	Resolution between SVN and LVN peak	%RSD of peak areas of LVN
Mobile phase composition			
90%	0.2	9.0	0.12
100%	0.1	10	0.1
110%	0.1	9.3	0.15
Flow rate (mL/min)			
1.3	0.3	11	0.2
1.5	0.1	10	0.1
1.7	0.2	9	0.3
Mobile phase pH			
3.8	0.1	10	0.1
4.0	0.1	9	0.1
4.2	0.2	9	0.15

CONCLUSION

The UV and the RP-HPLC method developed were found to be simple, rapid and economical. The two validated methods were found suitable for the routine quality control of Simvastatin in bulk drugs and pharmaceutical dosage forms both by small and large scale pharmaceutical industries. Impurity profiling is of crucial importance in drug synthesis, quality control and storage, as depicted by ICH guidelines and stated in different Pharmacopeia specifications. The developed and optimized RP-HPLC methods by BBD approach was found to be simple, accurate, robust, precise, specific and suitable in terms of tailing factor, theoretical plate count. The impurities were well separated with good resolution and peak shape, good retention times finding applications in impurity profiling of simvastatin related substances.

CONFLICT OF INTEREST: Conflict of interest is nil.

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