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Sensitive Analytical Method Development and Validation of Simvastatin Bulk Drug by RP-HPLC

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ABSTRACT

This paper describes the analytical method suitable for validation of Simvastatin by reversed Phase High Performance liquid chromatography (RP-HPLC) method. The method utilized RP-HPLC (Younglin HPLC with UV-detector) model and a column, 150mm 4.6 mm, 5µ (Symmetry, ODS- 3V, 150mm × 4.6mm, 5µ). The mobile phases were comprised of Acetonitrile and (0.02M) Buffer pH 3.5 (60:40 v/v). Validation experiments were performed to demonstrate System suitability, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-150 µg/ML^{-1}. The method showed good recoveries (98.2 – 104.3%).

Key words: RP-HPLC, Simvastatin, Analytical method, Quality control, validation.

INTRODUCTION

Simvastatin (SMV) is -(+)-{1*S*,3*R*,7*S*,8*S*,8*aR*)-1, 2, 3, 7, 8, 8*a*-hexahydro-3,7-dimethyl-8-[2-(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-I-naphthyl-2,2-dimethyl butanoate [Fig. 1]. It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into â-hydroxy acid of Simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver [1].

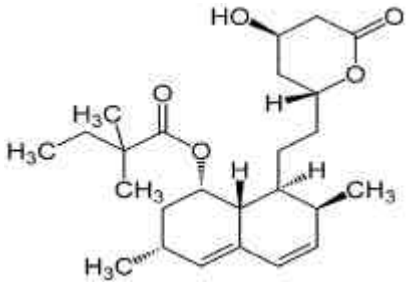

Fig. 1: Structure of Simvastatin

SMV alone can be estimated by various methods reported in the literature such as high performance liquid chromatography (HPLC) with UV detection [2], liquid chromatography coupled with tandem mass spectroscopy [3], UV spectrophotometry [4]. Reports are available which describe a stability indicating method for determination of SMV as well as for EZE with its degradation products and impurities [5, 6]. Simultaneous determination of SMV and EZE from pharmaceutical dosage form by dual mode gradient liquid chromatography was also reported [7]. The method involved use of C8 column and elution was accomplished by the application of a dual-mode solvent and flow-rate gradient system. Detection was carried out using a diode-array detector set at 240 nm. A Stability indicating HPTLC method for simultaneous estimation of SMV and EZE was also reported [8].

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Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness [9, 10], and validation as per ICH guidelines [11].

MATERIALS AND METHOD

Apparatus:
The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 744 (metrohm), the HPLC used is of Younglin with UV-detector. Column used in HPLC is of 150mm × 4.6mm 5(Symmetry, ODS-3V, 150mm ×4.6mm, 5µ is suitable) with a flow rate of 1.2ml/min (Isocratic). The mobile phase consists of the mixture of Acetonitrile and the Buffer pH (3.5) at different proportions A & B which are degassed in a sonicator for about 10 minutes the injection volume is 20µL and the ultra violet detection was at 240 nm.

Reagents and solutions:
Pure sample of Simvastatin (USP) and other ingredients such as Acetonitrile and water used were of HPLC grade. All other chemicals like Glacial acetic acid, Potassium Di-hydrogen phosphate used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

Standard preparation:
Accurately weigh and transfer 10mg of Simvastatin into a 10 mL of volumetric flask, add about 10 mL of diluent, sonicate to dissolve, make up to volume with diluent. Transfer 1.0 mL of the above solution into 10 mL volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45 µm filter.

Table - 1 Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Younglin with UV-detector)
Column	Symmetry ODS-3V, 150mm×4.6mm, 5µ is suitable
Mobile Phase*	Acetonitrile and Buffer (60 : 40 v/v)
Flow rate	1.2ml/min
Detection	UV at 240 nm
Injection volume	20µl
Temperature column	Ambient

Accuracy:
Accuracy for the assay of Simvastatin tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Simvastatin standard is added at different levels (80%, 100% and

120%). The sample were filtered through 0.45µm membrane filter and injected into the chromatographic system.

Precision:
The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as %RSD. The %RSD was found to be 0.06% in the results of precision.

Linearity & Range:
The Linearity of detector response is established by plotting a graph to concentration versus area of Simvastatin standard and determining the correlation coefficient. A series of solution of Simvastatin standard solution in the concentration ranging from about 1to 150 µg/ml levels of the target concentration (100µg/ml of Simvastatin) were prepared and injected into the HPLC system.

RESULTS AND DISCUSSION

Simvastatin standard having concentration 100µg/ml was scanned in UV-region between 200-400 nm. λ_{max} of Simvastatin was found to be at 240 nm. The Simvastatin peak in the sample was identified by comparing with the Simvastatin standard and the Retention time was found to be around 12.033 minutes.

The estimation Simvastatin was carried out by RP-HPLC using Mobile phase having a composition volumes of phosphate buffer, 60 volumes of acetonitrile and 40 volumes of buffer (60: 40 v/v). The ratio pH was found to be 3.5. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was C18 Symmetry ODS 3V (150 mm x 4.6 mm x 5 µ particle size). Flow rate of Mobile phase was 1.2ml/min, System suitability parameters such as RSD for six replicate injections were found to be 0.06%, theoretical plates – 7313.72, and tailing factor – 1.12.

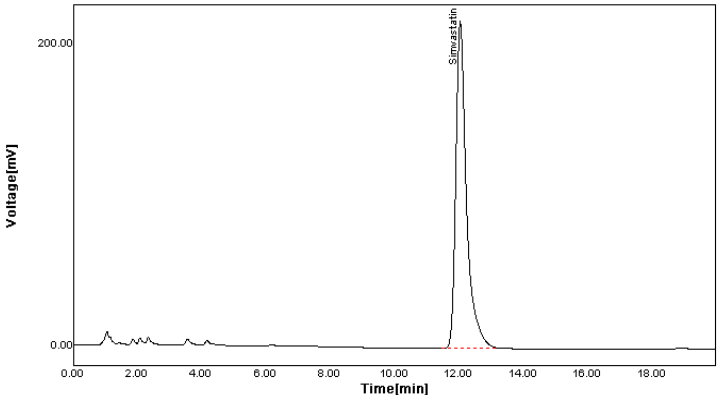

Fig. 2: Standard Chromatogram of Simvastatin

The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.06% which shows that the method is repeatable.

The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.09% which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 1 to 150 µg/ml of target concentration for Simvastatin standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.999, which shows that the method is capable of producing good response in UV-detector.

The Accuracy limit is the % recovery should be in the range of 98.2% to 104.3%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

Table - 2 . System Suitability Parameters

Parameter	Simvastatin
Calibration range (µg/ml)	1-150
Theoretical plates	7313.72
Tailing factor	1.12
Correlation Coefficient(r^2)	0.999
% Recovery	98.2% - 104.3%
System Suitability %RSD	0.06%
Method Repeatability %RSD	0.09%

Fig. 3: Linearity of Simvastatin

CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Simvastatin is done by RP-HPLC. The mobile phase consists of buffer (volumes of phosphate buffer, 60 volumes of Acetonitrile and 40 volumes of buffer. The ratio pH was found to be 3.5. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using UV-detector set at 240nm. The solutions are chromatographer at the constant flow rate of 1.2ml/min. The Retention time for Simvastatin was around 12.033 minutes. Linearity range for Simvastatin is 1 to 150µg/ml.

The quantitative estimation was carried out on the API by RP-HPLC taking a concentration of 100µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 98.2% to 104.3% for Simvastatin.

Regression analysis of the calibration curve for Simvastatin showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.999 in all the curves assayed.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer's law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

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