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Research Article

DEVELOPMENT AND VALIDATION OF SIMVASTATIN IN MICROEMULSION FORMULATION USING RP-HPLC

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ABSTRACT

Aim: The present study is aimed to develop and validate stability indicating RP-HPLC method for Simvastatin in micro emulsion formulation for the treatment of hypercholesteremia.

Materials and Method: A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for estimation of Simvastatin in bulk and microemulsion formulation. The method was carried out using (Phenomenex Luna C18 5µm 4.6×250mm (i.d) column) with mobile phase comprised of 0.1% Triethylamine buffer (pH 7.5): Acetonitrile (20:80v/v). The flow rate was set at 1.0 ml/min and effluent was detected at 238nm.

Results: The retention time of Simvastatin was found to be 8.6 minute. The method developed was validated for specificity, accuracy, precision, linearity and limit of detection, limit of quantification, robustness and stability. The calibration curve was linear in the concentration range of 200-600 ng/ml with correlation coefficient of 0.999. LOD and LOQ were found to be 5ng/ml and 40ng/ml respectively. The percentage recovery for the Simvastatin was found to be 99.15% to 100.53% and the % RSD was found to be 0.980% (i.e <1%). The method developed is simple, fast, accurate and precise and can be applied for routine quality control analysis of Simvastatin in bulk and its microemulsion dosage form.

Keywords: Simvastatin, Acetonitrile, Triethylamine, Microemulsion, RP-HPLC, Validation.

INTRODUCTION

Simvastatin is chemically (1S,3R,7S,8S,8aR) - 1, 2, 3, 7, 8, 8a-Hexahydro-3, 7- dimethyl-8-{2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl] ethyl}-1-naphthyl-2,2-dimethyl butyrate[1] is obtained from the fermentation of Aspergillus terreus. This compound, acts as a highly potent and effective cholesterol-lowering agent, is being used in the control of hypercholesterolemia. It exhibits a very important hepatic first-pass metabolism, acting by blocking the 3-hydroxy-3- methylglutaryl coenzyme A reductase (HMG-CoA), and thereby reducing the low-density lipoproteins. Simvastatin is a potent inhibitor of HMG-CoA reductase, which is a rate limiting enzyme in cholesterol bio-synthesis[2].

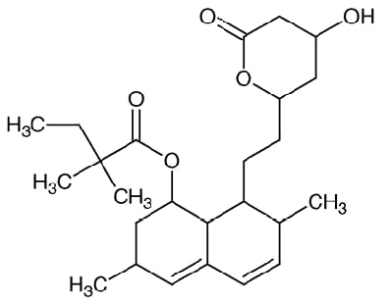

Fig. 1: Chemical Structure of Simvastatin

Several methods based on different techniques have been reported for the determination of Simvastatin in biological fluids. These methods include high-performance liquid chromatography/mass spectrometry (LC/MS) [4-8] and gas chromatography/mass spectrometry (GC/MS) (9) and high performance liquid chromatography (HPLC) [10-16] and high performance thin layer chromatography (HPTLC) [17]. Although these methods are sensitive to permit their use in determination of Simvastatin in urine, plasma or serum, but only few methods are reported for assay of Simvastatin in pharmaceutical formulations. Among them, HPLC methods have been described using expensive reagents or buffers in the mobile phase [18-22]. Based on the facts the study was aimed to develop, validate and compare simple, economic and fast analytical methods which can be easily applied in routine analysis for the determination of Simvastatin in lipid based systems.

MATERIALS AND METHODS

Chemicals

Simvastatin working standard (API) was a gift sample from Bright labs, Hyderabad, India. And prepared Simvastatin microemulsion, claimed to contain 10 mg of Simvastatin in 1ml of micro-emulsion composed of 19% Oleic acid, 33% Water, and 48% Cremophore RH40: Transcutol P as surfactant/co-surfactant mixture (S/Cos) in ratio of 1:1. HPLC grade Acetonitrile and Triethylamine was purchased from sdfine chemicals, Mumbai, India. HPLC grade water prepared by using SG-LABOSTAR™ 3 TWF-UV ultra pure water system, all chemicals was of analytical grade.

Instrumentation

Chromatographic separation was performed on a chromatographic system equipped with a LC-20AD pump; variable wavelength programmable UV/VIS detector, SPD-20A and rheodyne injector with 20µl fixed loop (LC-20AD Shimadzu).

Chromatographic conditions

Columns	**:** Phenomenex Luna C18 5µm 4.6×250mm (i.d) column
Mobile phase: A	**:** 0.1% Triethylamine buffer (PH 7.5)
B	**:** Acetonitrile
Isocratic	**:** 20:80 v/v
Flow rate	**:** 1000 µL/min
Detector	**:** UV, D2 lamp, 238 nm
Column Temperature	**:** Controlled room temperature (25°C)
Injection	**:** 20 µL sample loop.

Mobile phase

Selection of mobile phase

Mobile phase was selected from different mobile phase systems with different ratios were tried, among which 0.1% triethylamine buffer and acetonitrile (pH adjusted to 7.5 using glacial acetic acid) gave symmetrical peaks with good resolution, and hence fixed for further studies. And parameters were depicted in table1.

Table 1: Selection of mobile phase system

S. No	Mobile phase conditions	Observations
1	Water : Acetonitrile	Tailing, broad peak
2	Potassium dihydrogen phosphate buffer : Acetonitrile	Tailing, broad peak
3	Ammonium acetate buffer : Acetonitrile	Tailing, split peak
4	0.1% Triethylamine buffer : Acetonitrile	Good symmetric peak

Preparation of pre-mixed mobile phase

The mobile phase was prepared by using 0.1% triethylamine buffer pH 7.5 combined with HPLC grade acetonitrile in the ratio of 20:80 v/v. Further the solution was filtered through a membrane filter (0.45µm X 13 mm, Whattman, USA) unit and degassed by using sonicator under vacuum for 5min. Finally the solution was transferred to solvent reservoir bottle of the LC-20AD pump and purged the solvent line with 30ml of fresh mobile phase. The permutation and combinations of mobile phase were used and finally 0.1% triethylamine buffer pH 7.5: acetonitrile 20:80 v/v was selected as an appropriate mobile phase for method development, which gave good resolution and acceptable system suitability parameters (Table 2).

Table 2: Method development conditions for Simvastatin formulation

Condition	Mobile phase- A	Mobile phase- B	pH ofMobile phase	Ratio ofA/B	Simvastatin*Rt	Tailingfactor
1	0.1% TEA	Acetonitrile	7.5	35/65	20.6 min	1.101
2	0.1% TEA	Acetonitrile	7.5	30/70	13.8 min	1.198
3	0.1% TEA	Acetonitrile	7.5	25/75	10.6 min	1.080
4	0.1% TEA	Acetonitrile	7.5	20/80	8.6 min	1.056

* Rt- Retention time, TEA-triethylamine

Procedure

Preparation of standard solution

A standard stock solution was prepared by weighing about 100 mg of simvastatin standard and transferred into 100 ml volumetric flask; to the contents 25 ml of mobile phase was added and sonicated for about 5 minutes for complete solubility and was made up to volume with mobile phase to obtain a final concentration of 1mg/ml of Simvastatin.

Calibration curve

From the stock solution, measured volumes of working standards were prepared in the concentration range of 200 – 600 ng/ml. 20µL injections were made for each concentration in triplicate and were analyzed under optimized chromatographic conditions. A calibration curve was plotted by using the concentration versus peak area of drug (figure 2). Regression analysis was calculated. A typical chromatogram of simvastatin standard was represented in figure 3.

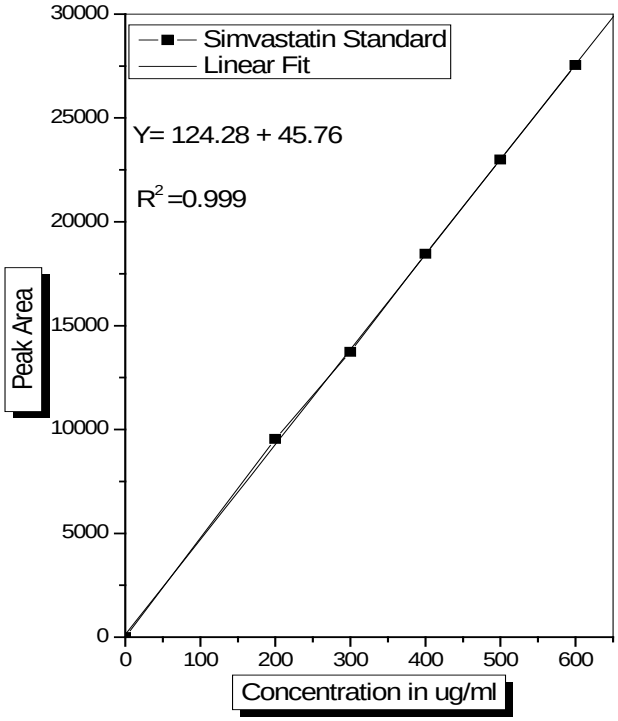

Fig. 2: Standard graph of simvastatin showing concentration vs. peak area

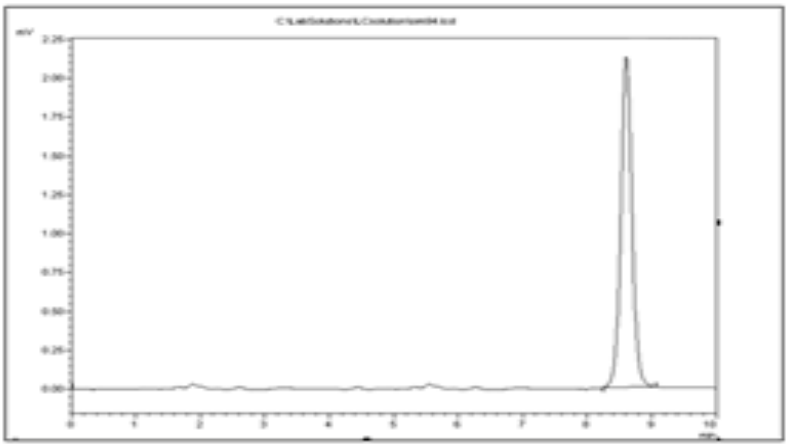

Fig. 3: The typical chromatogram representing the standard simvastatin drug

Preparation of simvastatin microemulsion

A microemulsion composed of 19% Oleic acid, 33% Water, 48% cremophoreRH40: transcutol P as surfactant/co surfactant mixture(S/Cos) in ratio of 1:1 was prepared. The composition of the microemulsion was chosen according to the ternary phase studies (Figure 4). Oleic acid and S/Cos mixture were mixed in the chosen concentrations, and then water was added portion wise with continuous stirring. The system was stored in a tightly closed glass container and left for 3 days to attain equilibrium before analysis.

50 mg of simvastatin was incorporated in the 5ml of prepared microemulsion. Simvastatin was mixed first with Oleic acid by using magnetic stirrer, then the S/Cos mixture was added, and the mixture was stirred till the drug was completely dissolved, then water was added and stirring was continued for about further 10 min. The composition of microemulsion was selected based on the solubility of Simvastatin. The solubility data were tabulated in table 3.

Table 3: Solubility of simvastatin in various oils and surfactants

Vehicle	Solubility mg/ml
Oleic acid	22.5
Isopropyl myristate	10.4
Meglyoil	13.2
Soya oil	7.6
Olive oil	5.5
Cremophore RH40	82.7
Transcutol P	75.5
Tween 80	71.3
PEG 400	55.0
Propylene glycol	46.1

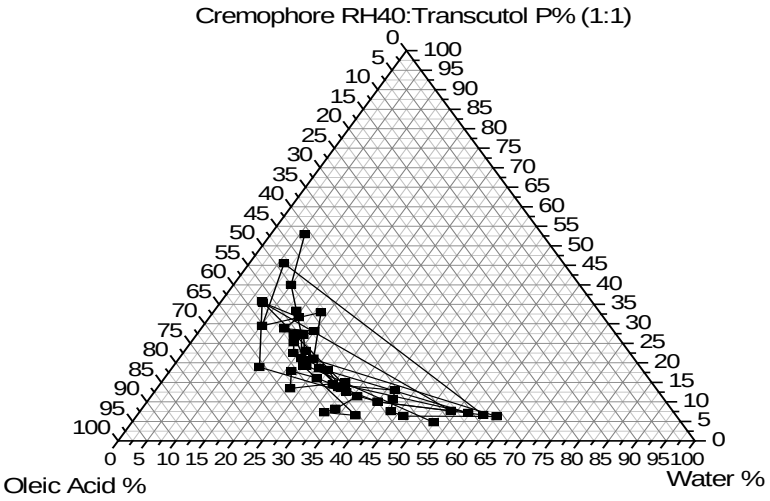

Fig. 4: Ternary phase diagram from which the composition of micro- emulsion was selected

Solubility studies

The solubility study was used to identify the suitable oil and surfactant that possess good solubilizing capacity for Simvastatin. One ml of each of the selected vehicle was added to each vial containing an excess of Simvastatin. After sealing the mixture was heated at 40^{0}c in a water bath to facilitate the solubilization. Mixtures were then shaken by using orbital shaking incubator (VIGNAN-0SR30) at 25°c for 48hrs, after reaching equilibrium the solution were centrifuged at 4000rpm for 15 min(REMI RM 12c micro centrifuge) and were filtered using a membrane filter (0.45µm x 13mm, Whatman, USA). The concentration of simvastatin in each vehicle was quantified by RP-HPLC and was graphically represented in Figure-5.

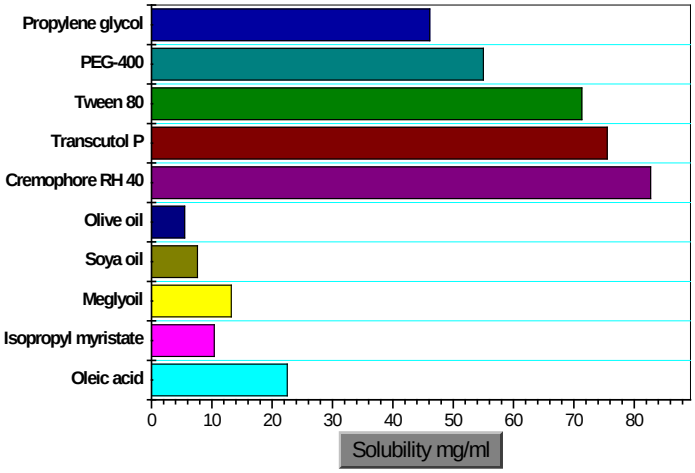

Fig. 5: Graph representing solubility of simvastatin in various oils and surfactants

Procedure for analysis of microemulsion

1 ml of micro emulsion was measured and an accurately measured portion of this microemulsion equivalent to 10mg of simvastatin was transferred to a 100 ml volumetric flask containing 25 ml of mobile phase. The content of the flask was allowed to stand for 15 minutes with intermittent sonication to ensure complete solubility of the drug and made up to volume with mobile phase. Then above solution was filtered through membrane filter. 1.0 ml of filtrate was diluted to 10 ml with mobile phase. From this solution appropriate dilutions were made with mobile phase to obtain concentration in calibration range and this solution was used for estimation. With the optimized chromatographic conditions, a steady baseline was recorded, the mixed working standard solution was injected and the chromatogram was recorded. The retention time of Simvastatin was found to be 8.6 min. The proposed method was found to be specific and no interference from common micro emulsion vehicles such as S/Cos mixture, Oleic acid etc. was observed. The response factors of the standard solutions and sample solutions were calculated. The

assay was calculated from the equation of regression line. The assay procedure was repeated for 6 times and the percentage of drug in the formulation was calculated. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation (Table 4). A typical chromatogram of Simvastatin microemulsion formulation was represented in figure 6.

Table 4: Label Claim of micro emulsion formulation

Formulation	Analyte	Label claim(mg)	% Label claimestimated
Microemulsion	Simvastatin	10	98.54

(*mean of six samples)

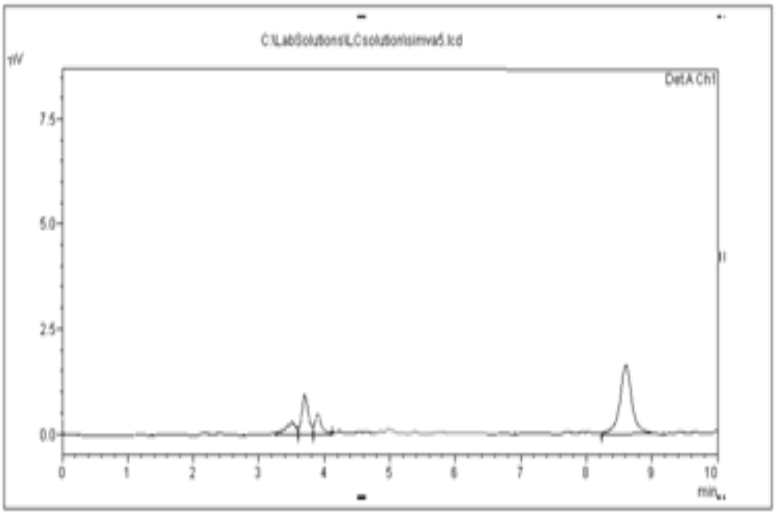

Fig. 6: The typical chromatogram of simvastatin microemulsion formulation

Method validation

Linearity

The method was linear in the concentration range of 200 to 600 ng/ml for simvastatin standard and results of linear regression data were depicted in table 5. The method validation parameters were summarized in table 6.

Precision

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies the solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. Where as in the inter day variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated.

Accuracy

The accuracy of the proposed method was determined by using recovery studies. It was determined by adding the known amounts of the Simvastatin reference standard to the test sample at the beginning of the process and all solutions were prepared in triplicate. For recovery studies, proportions of Simvastatin in microemulsion formulation were made 1:1 by adding reference standard Simvastatin into microemulsion formulation.

Table 5: Linear regression data for calibration curves

Parameters	Simvastatin
Linearity range (ng/ml)	200-600ng/ml
Correlation coefficient	0.999
Regression equation	Y=45.75x+124.2
Slope	45.75
Intercept	124.2

Table 6: Summary of validation parameters

Parameters	Simvastatin
LOD (ng/ml)	5ng/ml
LOQ (ng/ml)	40ng/ml
Mean % recovery	99.84
Precision (% RSD)	
Interday (n=3)	0.980
Intraday (n=3)	0.602
Robustness	Robust
Retention time	8.6 min
Theoretical plates	11805.854
Tailing factor	1.056

Limit of Detection and Limit of Quantification

The Limit of detection and quantification were calculated using standard deviation of the response and slope of calibration curve. The LOD and LOQ for Simvastatin were observed as 5 and 40ng/ml.

Robustness

Robustness of the method was checked by making slight changes in chromatographic conditions such as mobile phase ratio and pH of buffer.

Stability of solutions

In order to obtain reliable experimental results, it is essential to evaluate the stability of standard solution. The stability of the solution was validated as per ICH guidelines [3]. Standard solution was stored at room and refrigerated temperatures during three consecutive days (intermediate precision) and injections were made at every 3 hrs interval.

RESULTS AND DISCUSSIONS

The present developed method is novel for the determination of Simvastatin in microemulsion formulation. The retention time of standard Simvastatin drug was 8.6 minutes under optimized chromatographic conditions. In our study the retention time of Simvastatin in microemulsion formulation was 8.6 minutes. The method was found to be specific as excipients in the formulation did not interfere in the estimation of Simvastatin in microemulsion formulation (fig-6). The developed method was linear in the concentration range of 200 -600 ng/ml (fig-2). Accuracy of the method was indicated by recovery studies and it was in agreement with the

standard Simvastatin (100.53%). The LOD and LOQ for Simvastatin were found to be 5 and 40ng/ml respectively. The results showed that the amount of drug was in good agreement with label claim of developed microemulsion formulation and tabulated in Table 4.

It was observed that there were no marked changes in chromatogram, which demonstrated that the method developed was robust. The stability study was performed under stress degradation conditions as per ICH guidelines. The results indicated that the developed solutions were stable up to 12 hours which was sufficient for completing the analytical procedures. The developed method was specific and reproducible for the quantitative determination of Simvastatin in microemulsion formulation with a good resolution and high sensitivity.

CONCLUSION

There are no methods developed for quantitative determination of Simvastatin in microemulsion formulation. Precision and accuracy for Simvastatin were comparable with other HPLC methods previously described in the literature. The standard deviation and %RSD calculated for the proposed method are low, indicating high degree of precision. Hence, it can be concluded that the proposed chromatographic method was accurate, precise, and selective can be employed successfully for the determination of Simvastatin in bulk and microemulsion formulation.

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