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Original Research Article

Analysis of Simvastatin using a Simple and Fast High Performance Liquid Chromatography-Ultra Violet Method: Development, Validation and Application in Solubility Studies

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Abstract

Purpose: To develop and validate an accurate, rapid and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) analytical method for the lipid lowering drug, simvastatin, and to apply the developed method to study the solubility of the drug in various oils and surfactants.

Methods: Isocratic RP-HPLC system with a UV-vis detector, and a column with dimensions 4.6 mm x 150 mm and 5 μ particle size, was employed. The mobile phase consisted of methanol and 0.01M KH₂PO₄ phosphate buffer (80:20) at pH 5.5 adjusted with phosphoric acid (2M) and pumped at a flow rate of 1 ml/min. Validation parameters, viz, limit of detection (LOD), limit of quantification (LOQ) linearity, accuracy, precision, and sensitivity, were established. Solubility study was performed in various oils and surfactants at 25 °C and the developed HPLC method was applied to analyze all samples.

Results: The developed HPLC method showed good linearity ($R^2 = 0.9958 \pm 0.0040$). The intra- and inter-day % accuracy was more than 98 %. LOQ and LOQ were 0.160 and 0.484 μ g/ml respectively. Simvastatin showed the highest solubility in sesame oil (15 mg/ml) and in Tween 80 (11 mg/ml) at 25 °C.

Conclusion: An accurate, rapid and robust HPLC-UV method has been developed, validated and applied successfully to determine the solubility of simvastatin in oils.

Keywords: Simvastatin, Validation, Solubility, Sesame oil, Tween 80.

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INTRODUCTION

Simvastatin is a (1S,3R,7S,8S,8aR)-8-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl)-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate that belongs to the statin drug family; the members of which are used as cholesterol-lowering agents for patients with

hypercholesterolemia. This semi synthetic drug exhibits a very important hepatic first-pass metabolism by inhibiting 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA) and reduces low-density lipoproteins [1,2]. Structure of simvastatin has been shown in Figure 1.

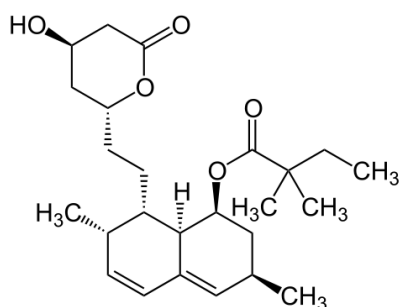


Figure 1: Structure of simvastatin

Various methods such as HPTLC [3], electrokinetic chromatography and voltametry [4] have been reported. Based on different techniques several methods like high performance liquid chromatography/mass spectrometry (LC/MS) [5-9], Gas chromatography/mass spectrometry (GC/MS) [10], high performance liquid chromatography (HPLC) [11-14] and high performance thin layer chromatography (HPTLC) [15] have been developed but these methods utilize expensive reagents or buffers as the mobile phase or complex procedures adopted in sample preparation.

Therefore, the aim of this study was to develop a simple and rapid HPLC-UV method to investigate the solubility of simvastatin in various oils and surfactants. The study focus was to explore the solubility profile of simvastatin in various oils and surfactants.

EXPERIMENTAL

Chemicals and reagents

Simvastatin was received as a gift from Sami Pharmaceutical (Pvt) Ltd Karachi, Pakistan. All other chemicals used were purchased from Merck, Germany.

Instrumentation

HPLC system of Sykam S 3210 consisted of isocratic pump (Sykam GmbH HPLC system, Germany) with UV-Detector (Sykam UV/VIS Germany), column (with dimensions of 4.6 mm x 150 mm, 5 μ particle size) and computer software (Clarity operating software) with Microsoft Windows XP Professional, ultrasonic bath (Fisher Scientific FS 28 H (Germany), centrifuge machine (model 4000 (China), column thermostat (Sykam 4011, Thermo controller, Germany), injection syringe (20 μ l, Rheodyne Injector, Germany), magnetic stirrer (Gallenkamp, England), membrane filter (Sartorius, 0.45 μ m pore size, Germany).

Preparation of standard solutions

The stock standard solution of simvastatin was prepared by dissolving the accurately weighed simvastatin in Methanol. The stock standard solutions were then diluted with methanol to achieve standard working solutions at concentrations of 0.612, 1.25, 2.5, 5, 10, 20, 40, 80, 160, 320 and 640 μ g/ml of simvastatin.

Calibration curve

To construct a calibration curve, standard stock solution of simvastatin was diluted to give concentrations of 2.5, 5, 10, 20, 40, 80, 160, 320 and 640 μ g/ml. Separate labeled glass centrifuge tubes were used for all the dilutions. Chromatograms for these concentrations were obtained by injecting 20 μ l of standard solutions. Regression analysis was carried out. A typical chromatogram of simvastatin standard is given in Figure 2.

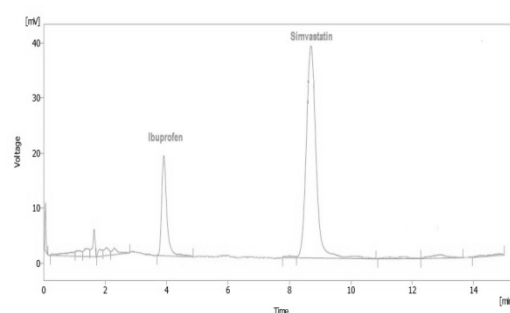


Figure 2: Representative chromatogram of ibuprofen (internal standard) and simvastatin

High performance liquid chromatography

An isocratic pump with flow rate of 1ml/min and UV-detector set at a wavelength of 238nm, column (with dimensions of 4.6 mm x 250 mm 5 μ particle size) and Clarity software for data analysis were employed. The mobile phase consisted of methanol and 0.01M KH₂PO₄ phosphate buffer (80:20) and pH was maintained at 5.5 with 2M phosphoric acid.

Linearity determination

Linearity of the assay method was determined by constructing calibration curve to find out the relationship between instrument response and known concentrations of the analytes. The number of standards used in constructing a calibration curve was selected to the anticipated range of analytical values of simvastatin in the samples of interest. Each drug concentration in three replicates was run in the HPLC system (Sykam S 3210 series) and the data plotted to determine the parameters of standard curve.

Determination of precision and accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by true value (concentration) of the analyte. Accuracy was determined by triplicate based on analysis of low, medium and high concentrations of samples containing known amounts of the analyte. Accuracy in present method was measured by using a minimum of three concentrations in the range of expected concentrations (intra-day). The deviation of mean from the true value served as the measure of accuracy. The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogenous volume of biological fluid (inter-day). Precision was measured in triplicate at three concentrations in the range of expected concentrations.

Assessment of quantification limits

LOD and LOQ values were calculated by injecting the solutions with known decreased concentrations of simvastatin into the HPLC system. The limit of detection (LOD) and quantification (LOQ) were then measured by calculating the minimum level at which the simvastatin can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1).

Application of method

The developed HPLC-UV method was applied to determine the solubility of simvastatin in various oils (canola oil, sesame oil, sunflower oil, oleic acid, olive oil, arachis oil and soybean oil), surfactants (Tweens 20 and 80, and Span 85), and co-surfactants (propylene glycol and polyethylene glycol 400). An excess amount of simvastatin was added to separate capped glass vials containing 2 ml of each of the vehicles. After sealing, the mixture was heated at 40 °C in a water-bath to facilitate solubilization using a vortex mixer. The mixtures were shaken in a shaker apparatus at 25 °C for 48 h. After reaching equilibrium, each vial was centrifuged at 4000 rpm for 10 min, filtered using a membrane filter (0.45 µ, 13 mm, Whatman, USA) and the filtrate suitably diluted prior to injecting into the chromatograph. The concentration of simvastatin was quantified by HPLC as described above.

RESULTS

Calibration curve parameters of simvastatin have been shown in Figure 2. Mean correlation

coefficient (R^2) slope and intercept values were obtained as 0.9958, 228.5 and 45.145 respectively. The developed method showed good linearity with mean correlation coefficient of 0.9958 ± 0.004 . Limits of quantitation (LOQ) and of detection (LOD) of simvastatin were 0.160 and 0.484 µg/ml, respectively. Percent coefficient of variation (CV%) for both intra- and inter-day was < 2 % which is well within the range of 15 % for lowest and 20 % for highest concentration based on FDA criteria for biological fluids [16]. Intra-day and Inter-day accuracy and precision are shown in Table 1.

Table 1: Intra-day and Inter-day accuracy and precision

Curve code	LQC	MQC	HQC
	5 µg/mL	25 µg/mL	50 µg/mL
Intra-day			
Batch-01	4.97	24.8	49.88
	4.98	24.75	49.52
	4.89	24.62	49.48
Batch-02	4.87	24.72	49.67
	4.93	24.82	49.76
	4.87	24.76	49.69
Batch-03	4.98	24.98	49.98
	5.00	24.75	49.96
	4.97	24.80	49.97
Mean	4.94	24.78	49.77
S.D.	0.05	0.10	0.19
% CV	1.04	0.39	0.39
% accuracy	98.80	99.11	99.54
Inter-day			
Batch 01	4.97	24.97	49.96
Batch 02	4.98	24.88	49.78
Batch 03	4.96	24.78	49.98
Batch 04	5.01	24.75	49.95
Batch 05	4.98	24.98	49.84
Batch 06	4.88	24.77	49.97
Mean	4.96	24.86	49.91
S.D.	0.04	0.10	0.08
% CV	0.89	0.42	0.17
% accuracy	99.27	99.42	99.83

The proposed method was applied to assess the solubility profile of simvastatin in various oils and surfactants. Simvastatin showed the highest solubility in sesame oil and (15 mg/ml) and was more soluble in this oil than in Tween 80 (11 mg/ml). The solubility profile of simvastatin in various media, mainly oils and surfactants, is shown in Table 2.

In the development process, different compositions of mobile phase with several combinations of buffer and organic phases, including acetonitrile, methanol and buffer in

varying ratios, were tested. The mobile phase, consisting of KH_2PO_4 and methanol in the ratio of 20:80 (%v/v) and with a pH of 5.5 was chosen. This mobile phase was found to be suitable to achieve the desired objectives of not absorbing at low wavelength and sufficient in concentration to avoid peak tailing since silica-based particles are unstable at low pH (< 2).

Table 2: Solubility of simvastatin in various media

Medium	Solubility (mg/mL)
Propylene glycol	4.930
Canola oil	2.257
Sesame oil	14.986
Sunflower oil Oleic acid	1.228 1.033
Tween 20	9.172
Olive oil	4.556
Span 85	6.486
Arachis oil	2.035
Soya been oil	1.745
Tween 80	11.139
Polyethylene glycol 400	7.361

DISCUSSION

Flow rate of 1.0ml/min. was found appropriate for peak resolution with a short retention time. Similarly, different stationary phases of suitable dimensions were also tried to achieve retention and separation. The run time was short requiring only 15 min While the retention time for simvastatin was 8.5 min.

The higher solubility of simvastatin in sesame oil and than in Tween 80 is noteworthy. The results suggest that both of these media can be utilized to prepare lipid-based delivery systems to enhance the solubility of simvastatin and thus ultimately increase the bioavailability of the drug,

CONCLUSION

The findings of this study are significant two major ways, First, an accurate, reproducible and rapid analytical method utilizing commonly available chemicals and technique was successfully developed and validated. Second, the solubility data generated for simvastatin in various various media could be helpful in formulating lipid-based formulation of the drug to enhance its solubility of this poorly soluble agent. It is seems feasible therefore that micro-emulsions of simvastatin can be developed to improve bioavailability based on the solubility data generated in this study.

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