WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 8, Issue 4, 01-10 Research Article ISSN 2278 – 4357

DEVELOPMENT AND VALIDATION OF A SIMPLE RP-HPLC METHOD FOR THE ESTIMATION OF ATORVASTATIN IN SELF EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS)

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Article Received on
24 Jan. 2019,

Revised on 14 Feb. 2019,
Accepted on 06 March 2019

DOI: 10.20959/wjpps20194-13199

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ABSTRACT

A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of atorvastatin in self emulsifying drug delivery system (SEDDS) has been developed and validated. The chromatographic system consisted of a LC-20 AT pump, SPD-20 A UV/visible detector. The separation was achieved from C_{18} column at 30^0C with a mobile phase consisting of 0.025 M phosphoric acid solution: acetonitrile (60:40 v/v, pH 3.0 adjusted with 80% phosphoric acid) at a flow rate of 1.2 ml/min and the retention time was 2.737± 0.019 min. The method was found selective and able to resolve drug peaks from formulation excipients. The calibration curve was linear over the concentration range of 2-20 µg/ml (r^2 = 0.999). The proposed method was accurate with 100.14 ± 0.57% recovery and precise (%RSD of Intraday variation were 0.55% and 0.58% for Inter day variation). The method has been used to determine potency of prepared SEDDS. Potency in all the cases was found within 99.02%- 100.02%. Therefore, this method can be used as a more convenient and efficient option for the analysis of atorvastatin in self emulsifying drug delivery system (SEDDS).

KEYWORDS: Atorvastatin, Method Validation, HPLC Method, Quantitative Analysis.

INTRODUCTION

Atorvastatin calcium (ATV) is a synthetic lipid lowering agent which inhibits HMG–CoA reductase. Chemically ATV is [R-(R*, R*)]-2-(4-fluorophenyl)-ß, dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (Figure 1). Atorvastatin acts 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] The drug is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and apolipoprotein B in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease.

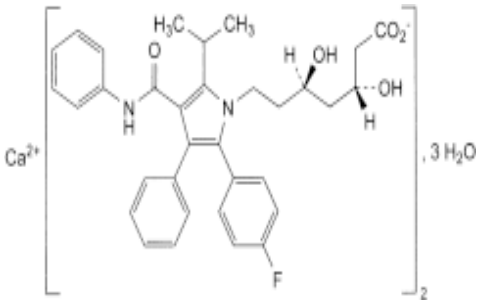

Figure. 1: Structure of atorvastatin calcium.

Atorvastatin is a BCS Class II,[2-3] less bioavailable[4-5] drug (only 12% after a 40 mg oral dose) due to low solubility[6-7], although its intestinal permeability is high at the physiologically relevant intestinal pH.[8-9] Different researchers tried to increase the bioavailability of ATV by self emulsifying drug delivery system (SEDDS) technology.[10-11] It is very difficult to determine amount of ATV in SEDDS formulation by previously developed simple and available UV or titration methods[12] as SEDDS contains different excipients like oils, surfactants etc. which hamper the analysis process. So it is essential to develop a precise, accurate and validated method suitable for analysis of ATV in SEDDS formulation.

Atorvastatin is official in EP and USP. Official methods utilizing HPLC Gradient methodology are reported in European Pharmacopoeia (EP) United State Pharmacopoeia (USP). Literature survey revealed that HPTLC and HPLC methods are available for atorvastatin analysis either in single formulation or in combination with another drugs.[13-15] HP-TLC is not available in all testing laboratory and the HPLC methods that are reported are

applicable for only bulk or tablet dosage form and they are not free from limitations. Moreover they are not tested for SEDDS formulation containing exceptional excipients like oils, surfactants etc. Based on the facts this study was aimed to develop and validate simple, economic and fast analytical method which can be easily applied in routine analysis for the determination of atorvastatin in SEDDS formulation.

So, the present work was undertaken with the aim to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method with high resolution according to ICH guideline. We used 0.025 M phosphoric acid solution: acetonitrile (60:40 v/v, pH 3.0 adjusted with 80% phosphoric acid). So far as we know this combination and ratio of mobile phase has not been reported in any literature for this drug and dosage form.

EXPERIMENTAL

Instrumentation: A Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A auto-sampler and CTO-10ASVP column oven was used. Ultraviolet detection was achieved with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analysis data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC.

System Suitability Study: In order to assess the system suitability of the method solution containing 100% target concentration of drug was injected in six replicates and various chromatographic parameters such as retention time, peak area, tailing factor and theoretical plates (Tangent) of the column were determined. The method was evaluated by analyzing the parameters.

Selectivity: Selectivity was determined by injecting the pressing drugs containing common excipients used in SEDDS formulation. Sample containing 100% nominal concentration was injected first. Then the samples of drug along with different oils and surfactants were injected to find out the selectivity of the method.

Linearity: Linearity of the method was determined by constructing calibration curve. Standard solutions of drug of different concentrations level (2-20 µg/ml) were used for this purpose. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were

plotted against the concentrations to obtain the calibration curve. The data were then subjected to regression analysis to calculate calibration equation and correlation coefficient.

Accuracy: Accuracy was determined by means of spike and recovery method. Drug at different level (50%, 100% and 150% of nominal concentration) were added to placebo formulations. The accuracy was calculated as the percentage of the drug recovered by the assay.

Precision: The precision of the method was determined by intra-day (repeatability) and inter-day (ruggedness) study. Intra-day precision (repeatability) was determined by performing four repeated analysis of the standard solutions (100% of nominal concentration) on the same day but at different times and inter-day precision (intermediate precision) of the method was assessed by carrying out the analysis of standard solutions on three different days in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

Robustness: Robustness of the method was determined by the analysis of the samples under a variety of conditions making small changes in the mobile phase component (± 0.5%), flow rate (± 0.05 ml/ min) and column temperature (±2 ^{0}C).

RESULTS AND DISCUSSION

Method Development and Optimization: RP-HPLC method with UV detection was developed for the determination of atorvastatin, the reversed-phase column, shim-pack CLC, ODS (C_{18}), 150 mm × 4.6 mm, 5µ was used for separation. The mobile phase was chosen after several trials with different buffers and organic solvents. 0.025 M phosphoric acid and acetonitrile in various proportions like 70:30, 60:40, 50:50, 40: 60, 30:70 and 35:65 at different pH values were tested for atorvastatin. Finally a mobile phase constituting 0.025 M phosphoric acid solution: acetonitrile (60:40 v/v, pH 3.0 adjusted with 80% phosphoric acid) was selected to achieve maximum separation and sensitivity. Different flow rates (0.50 to 2.0 ml /min) were studied. A flow rate of 1.2 ml/min flow rate was found suitable for atorvastatin for short retention time (2.737 min). Wave length for UV detection was determined by scanning individual standard of atorvastatin in the UV region. Then HPLC analysis of individual standard was measured at 245 nm. The developed method is summarized in the Table 1. For peak identification a blank sample was injected three times to observe the peak of the blank. No peak was observed. Then samples containing atorvastatin was then injected to identify peaks.

Table. 1: Summary of HPLC analytic method Validation.

Item	Value
Stationary Phase	Shim-pack C_{18}; 5µm, 4.6×150mm.
Mobile phase	0.025M OPA (pH 3.0) : acetonitrile 60:40
Temperature	30ºC
Flow rate	1.2 ml/minute.
Injection volume	10µl
Wave length	245 nm (λ_{max})
Run time	10 minutes.
R-Time (min)	2.737± 0.019

Peak area and retention time were found 325124 and 2.737 min for atorvastatin for 10µg/ml solution.

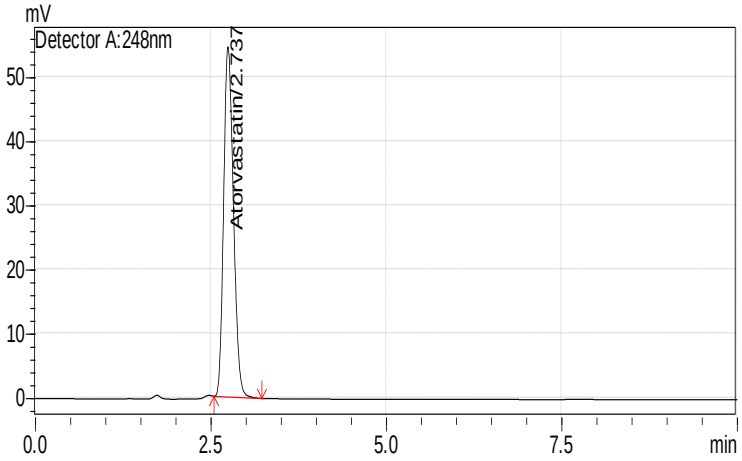

Figure. 2: Typical HPLC chromatograms of atorvastatin.

Validation of the Method

The methods were validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness.

System Suitability Test

System suitability tests were carried out on freshly prepared standard solution of drugs to evaluate the resolution and reproducibility of the system for the analysis and test result is summarized in Table 2. Average value, SD and % RSD of retention time, peak area, theoretical plates and tailing factor were calculated after six replicate injections. % RSD of retention time, peak area was within limit (< 2%) which indicates uniformity of test results. The tailing factor was found less than 1.5 which indicated symmetric nature of the column. High theoretical plate numbers suggested an efficient performance of the column.

Table. 2: System suitability test results of HPLC analytical method validation.

Parameters	Average ± SD, %RSD
Retention time	2.737 ± 0.003, 0.11
Area	325124 ± 500.18, 0.154
Theoretical plates	7255.5 ± 25.34, 0.349
Tailing factor	1.255 ± 0.0028, 0.223

Selectivity: The absence of additional peaks near the drug peak in the chromatogram indicated non-interference by the common excipients used in the SEDDS that indicates the selectivity and specificity of the method.

Linearity: The linearity of the method was determined at ten concentration levels .The plot of peak area of each sample against respective concentration of drug was found linear in the range of 2-20 µg/ml (Figure 3). Regression equation, correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) were also calculated and summarized in Table 3.

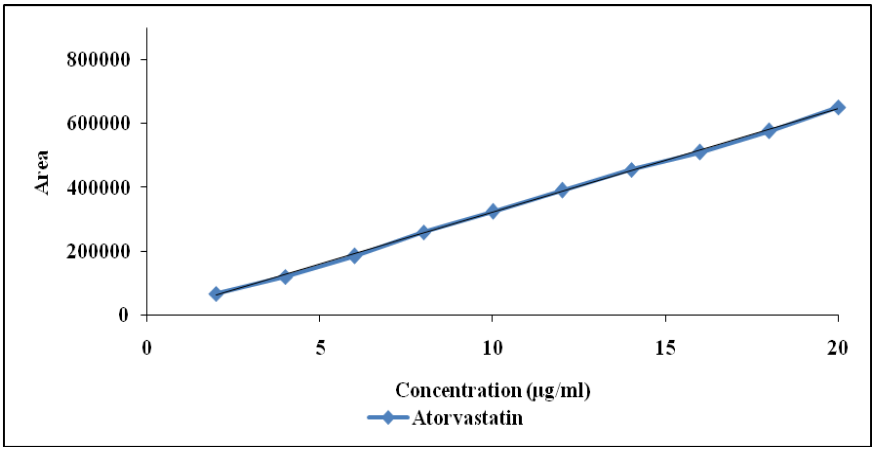

Figure. 3: Linearity curve of atorvastatin for HPLC analytical method validation.

Table. 3: Regression equation, correlation coefficients, LOD and LOQ of HPLC analysis method.

Parameters	Atorvastatin
Regression equation	y = 32480x - 2474.
Correlation coefficient	R^2 = 0.999
LOD (µg/ml)	0.184
LOQ (µg/ml)	0.614

Accuracy: Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to placebo formulations and the proposed method was followed to determine % recovery. 50%, 100% and 150% of

nominal concentration was included for this study. Six replicates of each concentration were measured. The percentage recovery value were 100.14 ± 0.57% which indicates the accuracy of the method and absence of interference from the excipients present in the samples.

Precision: The precision is a measure of ability of a method to generate reproducible results. The precision of the methods was determined by repeatability (intra-day) and Intermediate precision (inter-day) and reported as %RSD. For repeatability, four determinations of 100% test concentration were measured from 9.00 AM to 6.00 PM and %RSD was calculated. For intermediate precision the same work was done in three consecutive days and average %RSD value was calculated. The intra-day and inter-day precision measurements showed, good reproducibility with percent relative standard deviation (%RSD) values < 2%. This indicates that method was highly precise (Table 4).

Table. 4: Accuracy and precision results of HPLC method validation.

Validation parameters		**Result**
Accuracy	% Recovery	100.14 ± 0.57%
	%RSD	0.559
Precision (%RSD)	Repeatability (%RSD)	0.55
	Ruggedness (%RSD)	0.58

Robustness: Robustness was performed by small but deliberate variation in the chromatographic conditions. Robustness of the method was determined by the analysis of the samples under a variety of conditions making small changes in the mobile phase component (± 0.5%), flow rate (± 0.05 ml/ min) and column temperature (±2 ^{0}C). It was observed that there were no marked changes in the chromatograms, which demonstrates that the method was robust.

Analysis of Prepared Atorvastatin SEDDS

From pseudo-ternary phase diagrams optimal surfactant (**Tween 80)**, co-surfactant (**Transcutol)** and lipid (**Oleic Acid)** combinations were selected and SEDDS formulations **(Table 5)** were prepared by dissolving drug into mixture of surfactant and cosurfactant in a glass tube, heating at 40°C in a water-bath and using a vortex mixer to facilitate solubilization, then adding the required weight of lipid into the glass tube and mixing. The mixture was filled in capsules (Global Capsules Ltd., Bangladesh). The capsules were tightly sealed and stored at ambient temperature (25°C) until used.

Table. 5: Composition of SEDDS formulations.

Product	Drug	Oleic Acid	Peceol	Capryol 90	Tween 80	Labrasol	Transcutol
	(in gm)						
ATV SEDDS F-1	0.1	1			0.33		0.66
ATV SEDDS F-2	0.1	1			0.5		0.5
ATV SEDDS F-3	0.1	1			0.66		0.33
ATV SEDDS F-4	0.1	1			0.75		0.25

Drug from pre-weighed SEDDS was extracted by dissolving in 25 ml methanol. Drug content in the methanolic extract was analyzed by the developed validated HPLC method. Potency in all the cases was found within 99.02%- 100.02%.

CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of atorvastatin in SEDDS formulation. The measured signals were shown to be precise, accurate and linear over the concentration range tested with a acceptable correlation coefficient. Moreover, the lower solvent consumption along with the short retention time leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine sample analysis of atorvastatin in SEDDS. In this method, there was no interference from matrix sources. So it can be used for analysis of bulk drug and other dosage form.

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