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(RESEARCH ARTICLE)

Development and validation of HPLC-UV based bioanalytical method for the quantification of atorvastatin in rat plasma

Karan Wadhwa and A.C. Rana *

Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India.

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Abstract

A highly selective, sensitive, and fast HPLC based bioanalytical method was developed and validated for the estimation of atorvastatin in rat plasma which can be further employed in pharmacokinetic studies. The developed analytical procedure utilized protein precipitation approach to extract atorvastatin from rat plasma using ice-cold acetonitrile as an extraction solvent and diclofenac as IS. The chromatographic separation of atorvastatin was achieved on a LiChrospher RP C-18 column (250 × 4.6 mm, 5 µm) using isocratic elution of Methanol: water (containing 0.05 % glacial acetic acid) (70:30, v/v pH 3.0) as mobile phase with 1mL min^{-1} flow rate and further detected by UV absorbance at 248 nm. Atorvastatin eluted within 3.093 min, whereas IS eluted at 8.953 min. The developed method was validated as per USFDA guidelines. Linearity was observed in concentration between 15.62 ng mL^{-1} to 2000 ng mL^{-1} with r^2 equals to 0.9999 and LOQ 10.45 ng mL^{-1}. The developed method follows the all acceptance criteria concerning its linearity, accuracy, precision, selectivity, dilution integrity, and recovery. Also, atorvastatin was demonstrated to be stable in the battery of stability studies such as freeze-thaw, bench-top, autosampler, short-term, and long-term stability. Thus the developed bioanalytical assay can be used in pharmacokinetic studies.

Keywords: Atorvastatin; Bioanalytical Method; HPLC; Rat plasma; Validation

1. Introduction

Atorvastatin (Figure 1), is chemically (3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid [1] and clinically used as an antihyperlipidemic agent which belongs to the class of statins [2]. Physico-chemically, atorvastatin is a white crystalline powder, slightly soluble in methanol (MeOH), insoluble in water, and has a pKa value of 4.46 [1,3]. Atorvastatin has a molecular formula of $C_{33}H_{15}FN_2O_5$ with a molecular weight of 558.064 gm mol^{-1} [4] and exhibits two different structures, one is ring-structural lactone while another is open-structured hydroxyl acid. Atorvastatin induces its hypolipidemic potential by lowering the total cholesterol and LDL-cholesterol levels in the human body by competitively impeding the HMG-CoA reductase enzymes, which is accountable for the formation of mevalonate by HMG-CoA in the cholesterol synthesis pathway. Atorvastatin also decreases the count of LDL particles and the LDL production by increasing the catabolism and uptake of LDL and inhibiting the cholesterol synthesis in the liver. With a longer plasma half-life of 18-24 hrs, atorvastain has low oral bioavailability of 14% [5]. After the oral administration, 80% of total dose undergoes presystematic metabolism and converted into two main metabolite products (o-hydroxy atorvastatin and p-hydroxy atorvastatin) [6]. Atorvastatin is a substrate and inhibitor of P-glycoprotein and metabolized by CYP3A4 [7,8]. Clinically, it is primarily used for the prevention of arterial disease with elevated serum cholesterol concentration, especially if there are other risk factors for atherosclerosis such as diabetes or renal failure [9]. Atorvastatin also reduces the risk of angina [10] and revascularization procedures [11]. Along with this, atorvastatin is widely indicated to decrease cholesterol levels in patients having homozygous familial hypercholesterolemia and for the treatment of severe drug-resistant dyslipidemia

* Corresponding author: A.C. Rana
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India.

[9]. However, it is contraindicated during pregnancy and in nursing mothers and not administered with active liver disease or constantly increased serum-aminotransferase concentrations. Apart from runny nose, sneezing, and coughing as common side effects [12]; the overdose of atorvastatin also causes hepatotoxicity, myopathy, and skeletal muscle damage [9,13].

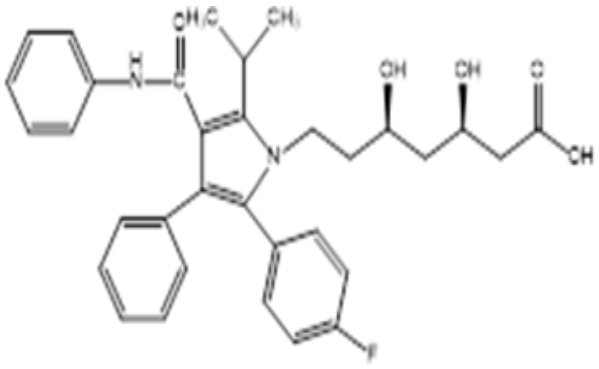

Figure 1 Chemical structure of atorvastatin

Numerous HPLC-UV based bioanalytical methods have been developed to analyze atorvastatin in various biological matrices [14–18]. The present study aims to design and validates a highly sensitive, sensitive, fast, and precise HPLC-UV based bioanalytical method for the determination of atorvastatin in rat plasma at a very low concentration of ng mL^{-1}. The developed method was validated in accordance with the standard protocols so as to apply this precise HPLC method for routine laboratory analysis of atorvastatin, alongside with determination of pharmacokinetic and drug-drug interaction studies of atorvastatin in human and animals.

2. Material and methods

2.1. Materials and reagents

Atorvastatin calcium (Yarrow chemicals, Mumbai), Diclofenac sodium (Sigma Aldrich, USA) Acetonitrile (ACN), Methanol (MeOH), Water (HPLC grade), Ethanol (EtOH), Glacial acetic acid (AcA), Formic acid (AR Grade) (Rankem, India), and Potassium dihydrogen phosphate (KH_2PO_4) Buffer (Himedia, India),

2.2. Instruments

HPLC system (Agilent 1200 Infinity, USA), UV Visible spectrophotometer (Shimadzu UV1800, Japan), Centrifuge, Ultrasonicator (INCO, India), pH meter, Weighing Balance (Mettler Toledo, Taiwan), and Vortex (Spinix).

2.3. Preparations of standard solutions

A 100 µg mL^{-1} stock solution of atorvastatin and 500 µg mL^{-1} stock solution of the internal standard (IS) were prepared in HPLC grade MeOH. The plasma calibration standards were prepared freshly by spiking the drug-free rat plasma matrix in atorvastatin containing solutions to obtain concentrations ranging from 15.62 to 2000 ng mL^{-1}, keeping IS concentration of 500 ng mL^{-1} in each sample.

2.4. Sample preparation or extraction of plasma samples

Direct injection of the plasma sample into the HPLC system blocks the column and affects the separation signals. Thus to prevent column blockage and to get good separation and signals in bioanalysis, sample preparation is a necessary step. Sample preparation commonly involves diluting or dissolving the spiked atorvastatin in a suitable solvent before the chromatographic separation to remove the interfering compounds which help to isolate atorvastatin and also to pre-concentrate it.

2.4.1. Procedure for sample preparation

200 µL of the plasma spiked samples were transferred to a 2 mL micro-centrifuge tube and 50 µL of IS (500 ng mL^{-1}) was added and vortexed for 2 min. Afterward, 750 µL of extraction solvent was mixed in solution to precipitate the plasma proteins and centrifuged at 12000 rpm for 12 min at 4°C. The supernatant obtained was filtered and relocated

into another labelled 2 mL micro-centrifuge tube and was further reconstituted with 500 µL of the mobile phase, vortexed thoroughly and 20 µL was injected into HPLC system for the final analysis.

2.4.2. Selection of extraction solvent

Different organic solvents like ACN, MeOH, and EtOH was tried as the extraction solvent and selected based on the higher level of recovery and reproducibility**.**

2.5. Development of HPLC method and optimization of different chromatographic conditions

Development of a fast, sensitive, and selective HPLC method to quantify atorvastatin in rat plasma involves the optimization of many parameters like mobile phase, flow rate, and sample preparation which were discussed below.

2.5.1. Determination of λmax

100 µg mL^{-1} stock solution of atorvastatin was diluted 10X using MeOH as solvent and aliquots were scanned for λmax between 200 to 400 nm using MeOH as a blank in a double beam UV Visible spectrophotometer, UV1800 Shimadzu.

2.5.2. Selection of mobile phase

The mobile phase for the chromatographic separation was selected on the basis of the trial and error method using different ratios of the HPLC grade organic solvents (MeOH and ACN), water, and buffers using isocratic elution system. After equilibrating the system for 30 min, atorvastatin 10 µg mL^{-1} was injected in replicates of three with a run time of 30 min and the best mobile phase was selected based on peak shape, retention time (R_t), resolution, asymmetry factor, and theoretical plate count.

2.5.3. Selection of stationary phase

After the mobile phase, the column used for quantification of atorvastatin in rat plasma was selected by hit and trial method using different stationary phases like C8 and C18 columns.

2.5.4. Selection of internal standard

Internal standard (IS) is an essential part of the bioanalytical method. Diclofenac and ibuprofen were tested as IS and selected based on chromatographic behaviour alike to the atorvastatin with good recovery from plasma.

2.5.5. Optimization of the final mobile phase

After selecting the IS, the composition of the mobile phase was optimized based on the R_t of the atorvastatin and IS to elude the IS and atorvastatin with a feasible peak separation.

2.5.6. Optimization of flow rate

After the final optimization of the mobile phase, the flow rate was finalized using different flow rates to ensure appropriate peak asymmetry, R_t, and resolution for both atorvastatin and IS in bioanalysis.

2.6. Method validation

The developed bioanalytical method was validated in compliance with USFDA guidelines [19].

2.6.1. Carryover effect

The carryover effect was determined by immediately injecting the blank plasma sample after the extracted highest quality control (HQC) sample and the % residual of IS and atorvastatin carried over by the neat (blank) plasma sample was calculated. The following procedure was followed in a set of six.

2.6.2. System suitability

System suitability was performed by calculating % RSD of peak area and R_t for IS (500 ng mL^{-1}) and atorvastatin (1800 ng mL^{-1}) in 6 consecutive injections and checked either they were below 5% and 2% respectively or not.

2.6.3. Selectivity and specificity

Selectivity and specificity of the developed method was analyzed by comparing the chromatographic intercession at the R_t of the IS and atorvastatin between the neat plasma samples and its corresponding plasma spiked samples obtained from six different sources.

2.6.4. Linearity

An 8-point calibration curve was prepared using concentrations of 15.62, 31.25, 62.5, 125, 250, 5000, 1000, and 2000 ng mL^{-1} for atorvastatin (n=3). The Y-intercept, slope, and coefficient of regression (r^2) were calculated using the linear regression analysis.

2.6.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOQ is the minimum amount of the analyte that can be calculated quantitatively with acceptable precision and accuracy. LOD was calculated as 3 times σ/s and LOQ was calculates as 10 times σ/s, where 'σ' is SD of intercepts attained from the standard linear curve and 's' is the slope obtained from the same linear curve.

2.6.6. Accuracy and precision

Inter and intra-day accuracy were obtained by analyzing 4 QC levels i.e. Lowest quality control (LQC), middle quality control (MQC), highest quality control (HQC), and LOQ (n=6) on three consecutive days and the similar day respectively and % accuracy was calculated as per Equation 1.

$$\text{Accuracy (\%)} = (\text{Measured concentration})/ (\text{Nominal concentration}) \times 100 \quad \text{(Equation 1)}$$

Whereas, intra-day precision was validated by assessing HQC, MQC, LQC, and LOQ samples in a set of six on the same day while on the three different days for inter-day precision. Inter and intra-day assay precision was calculated as % CV using Equation 2.

$$\text{Precision (\%CV)} = \text{SD/Mean} \times 100 \quad \text{(Equation 2)}$$

The acceptance basis includes accuracy within ±15% from the actual concentration, except for LOQ (not exceed ±20%) and precision less than 15% CV, but for LOQ it should not exceed 20%.

2.6.7. Ruggedness

The ruggedness of the developed method was assessed by examining the peak area of MQC (n=6) using two distinct C18 columns.

2.6.8. Recovery

Three QC concentrations (LQC, MQC, and HQC) were analyzed in a set of six to determine the total recovery of the atorvastatin from rat plasma. The % recovery was obtained by using Equation 3, whereas recovery for IS was done using a single concentration i.e. 500 ng mL^{-1}.

Recovery (%) = (Peak area ratio of extracted plasma sample)/ (Peak area ratio of unextracted samples) ×100 (Equation 3)

2.6.9. Stability

Freeze-thaw stability: Each QC sample (n=6) was undergo three consecutive freeze-thaw cycles at -20°C and room temperature, and analyzed for freeze-thaw stability by comparing with freshly processed samples.

Benchtop stability: Each QC samples of atorvastatin (n=6) were quantified after 8 hrs of exposure on the benchtop (25°C) to examine benchtop stability.

Autosampler stability: Processed QC samples were placed in autosampler at 4°C for 24 hrs and analyzed for stability after 24 hrs.

Long and short term stability: Short-term and long-term stability of atorvastatin in rat plasma was determined by assessing QC samples (n=6) when stored at -20°C for one day and 15 days respectively, and differentiated with freshly prepared samples.

Long and short term stability of stock solution: Short-term and long-term stability of atorvastatin stock solution in QC concentration was determined (n=6) when stored at for room temperature for 4 hrs and at -20°C for 30 days respectively.

Freshly prepared samples were used to analyze stability and acceptability criteria include limits of accuracy limits within ±15% SD and precision within ±15% RSD**.**

2.6.10. Dilution integrity

The dilution integrity test was investigated by preparing a 5X and 10X dilution of 2400 ng mL^{-1} by additional blank plasma and analyzed in a set of 6 to assess precision and accuracy.

2.7. Data analysis

All the data were analyzed using MS-Excel for the calculation of all means, SD, %CV, and %RSD.

3. Results and discussion

3.1. Sample preparation or extraction of plasma samples

Different organic solvents were investigated to extract atorvastatin from rat plasma. %recovery of atorvastatin and IS obtained from different precipitating agents was represented in Table 1**.**

Table 1 Extracting recovery of atorvastatin and IS from rat plasma using different extracting organic solvents.

Extracting organic solvent	% Recovery	
	Atorvastatin	IS
Methanol (MeOH)	70.65	80.22
Acetonitrile (ACN)	89.55	87.47
Ethanol (EtOH)	68.22	74.54

ACN extracted atorvastatin and IS effectively from the rat plasma with % recovery of 89.55% and 87.47% respectively. Furthermore, ice-cold ACN was explored to enhance the extraction efficacy and it was found that the use of ice-cold ACN improves the recovery of atorvastatin and IS by increasing it up to 96.87 % and 92.15% respectively. Thus, ice-cold ACN was used as a precipitating agent in the sample pre-treatment during bio-analysis.

3.2. Development and optimization of HPLC method for the quantification of atorvastatin in rat plasma

3.2.1. Determination of λmax

10 µg mL^{-1} solution of atorvastatin showed maximum absorbance (λmax) at 248 nm indicating the suitable detection wavelength for HPLC analysis of atorvastatin.

3.2.2. Selection of mobile phase

Numerous mobile phases in different concentrations of organic solvents with or without buffer solutions were examined to elute atorvastatin. The chromatogram obtained using the mobile phase composition of MeOH: water (containing 0.05 % glacial AcA) (70:30 v/v) had a well-shaped peak with R_t of about 3.007 min.

3.2.3. Selection of stationary phase

Both C8 and C18 columns were used with MeOH and water as the mobile phase in different ratios. It was observed that C18 column elute atorvastatin more quickly than the C8 column, so C18 was preferred for HPLC analysis of atorvastatin.

3.2.4. Selection of internal standard

After the selection of the mobile and stationary phases, different IS were investigated (Diclofenac and Ibuprofen) in the concentration of 500 ng mL^{-1}. Ibuprofen (R_t = 12.412 min) showed quite longer R_t than diclofenac (R_t = 8.933 min), thus diclofenac was selected as IS for analysis.

3.2.5. Optimization of the final mobile phase

After the selection of different chromatographic conditions, different pH of the mobile phase was evaluated to enhance resolution and R_t of atorvastatin and IS to get better chromatographic results. It was observed that the mobile phase at pH 3.0 enhanced the peak resolution of IS and atorvastatin.

3.2.6. Optimization of flow rate

After the hit and trial method, it is observed that the flow rate is inversely proportional to the asymmetry and the resolution of the peaks, thus the flow rate selected for the analysis was 1 mL min^{-1}.

3.2.7. Retention time of atorvastatin and IS

Rt for atorvastatin and IS in C18 column using MeOH: water (containing 0.05 % glacial acetic acid) (70:30, v/v pH 3.0) as mobile phase with a flow rate of 1 mL min^{-1} was found to be 3.093 min and 8.953 min respectively.

3.2.8. Final optimized chromatographic conditions

After the selection of all chromatographic parameters, the final optimized chromatographic conditions that were required for the analysis of atorvastatin were enlisted in Table 2.

Table 2 Final optimized chromatographic conditions

Column used	LiChrospher RP C-18 column (250 × 4.6 mm, 5µm)
Type of Method	Reverse phase
Mobile phase	MeOH: water (0.05 % glacial AcA) (70:30, v/v pH 3.0)
Type of elution	Isocratic elution
Sample injection	20µL
Column oven temperature	25°C
Autosampler temperature	4°C
Flow rate	1.0 mL min^{-1}
Detection wavelength	248 nm
Run time	10 min
Retention time	3.093 min

3.3. Method validation

The developed method was validated as per USFDA guidelines and discussed below.

3.3.1. Carryover effect

% carryover of the atorvastatin and IS was illustrated in Table 3 and no residual of IS and atorvastatin was carried over by the neat plasma sample.

3.3.2. System suitability

System suitability was checked by injected each sample at the start of the analysis and % RSD of peak area and R_t for IS (500 ng mL^{-1}) and atorvastatin (1800 ng mL^{-1}) was calculated. Both the parameters of the atorvastatin and IS had low % RSD (≥ 2%), thus ensuring the reproducibility of the present system towards the specific analytical run.

Table 3 % carryover of the atorvastatin and IS.

Sample	Peak area of atorvastatin	% carryover of atorvastatin	Peak area of IS	% carryover of IS
Mobile phase	0		0	
Aqueous HQC	620844±2497	0.00	167830±2056	0.00
Extracted blank plasma	0		0	
Extracted HQC	595395±3145	0.00	164241±2984	0.00

Data were expressed in Mean ± SEM.

3.3.3. Selectivity and specificity

The R_t of atorvastatin and IS was nearly 3.093 min and 8.953 min respectively. Chromatograms of blank rat plasma, plasma spiked with IS, and plasma spiked with IS and atorvastatin were represented in Figure 2, Figure 3, and Figure 4 respectively with no substantial interfering peaks.

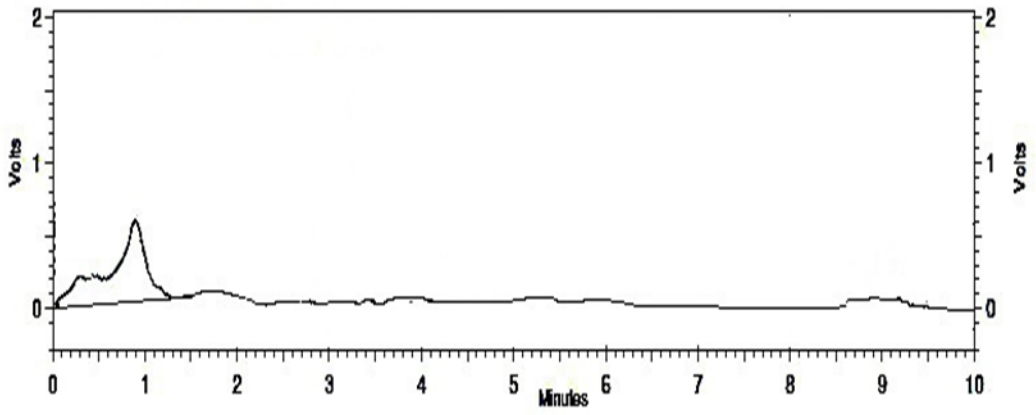

Figure 2 Chromatogram representing blank rat plasma (free of atorvastatin and IS)

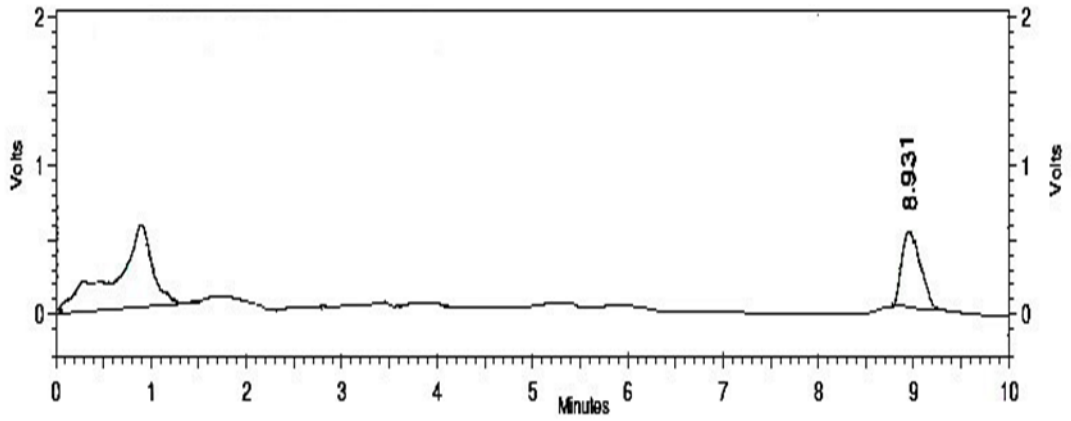

Figure 3 Chromatogram representing Diclofenac (IS) (500 ng mL^{-1}) spiked blank rat plasma

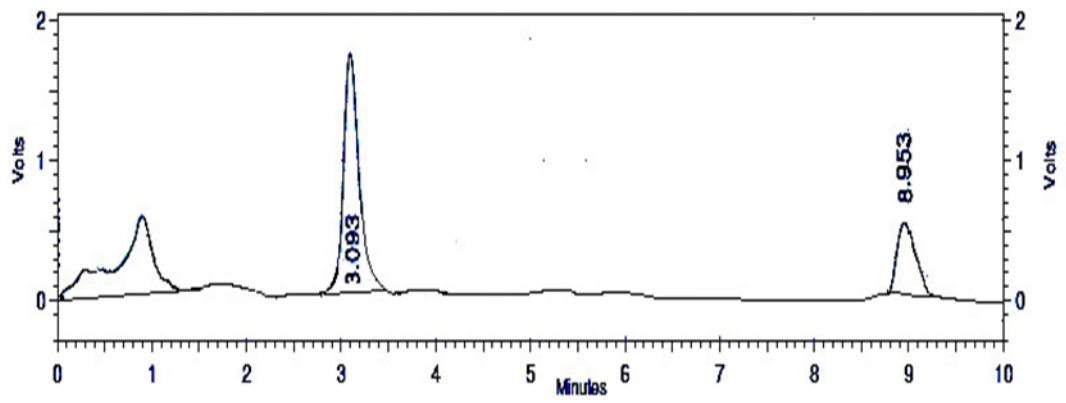

Figure 4 Chromatogram representing Atorvastatin (analyte) (2000 ng mL^{-1}) and Diclofenac (IS) (500 ng mL^{-1}) extracted from rat plasma

3.3.4. Linearity

The standard calibration curve was observed linear in the concentration range of 15.62 to 2000 ng mL^{-1} for the atorvastatin, as shown in Figure 5 and Y-intercept, slope, and coefficient of regression (r^2) (n=3) were found to be 0.0051, 0.002, and 0.9999 respectively using linear regression analysis.

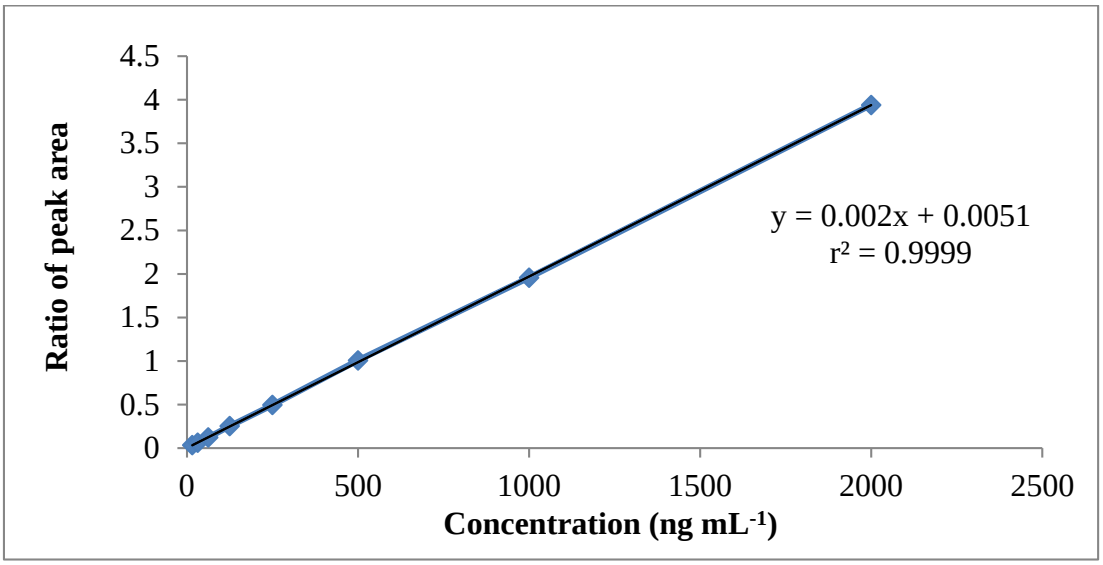

Figure 5 Calibration curve of plasma spiked concentrations of atorvastatin

3.3.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were found to be 3.45 ng mL^{-1} and 10.45 ng mL^{-1} respectively.

Table 4 Calibration curve parameters of the developed method

Calibration curve parameters	Batch ID			Mean	SD
	Batch 1	Batch 2	Batch 3		
Slope	0.002	0.0002	0.002	0.002	0
Y-intercept	0.0037	0.0075	0.0041	0.0051	0.00209
r^2	0.9999	0.9999	0.9999	0.9999	0

3.3.6. Accuracy and precision

Intra-day and inter-day % accuracy of all the 4 concentration levels was ranged between 94.17% to 100.20% and 99.27% to 100.12% respectively, whereas intra-day and inter-day precision were varied from 0.5381% to 5.6286 % and 1.0537 % to 3.5132% respectively (Table 5). The outcomes of accuracy and precision studies were under the acceptance criteria which suggest that the developed HPLC method was extensively accurate and precise to quantify atorvastatin from rat plasma.

Table 5 Intra-day and inter-day accuracy and precision of the developed method.

QC Samples	Observed Conc. (ng mL^{-1})	Precision (%CV)	% Accuracy
Intra-day (day 1)			
HQC	1796.358	0.5447	99.80
MQC	797.462	0.8487	99.68
LQC	49.892	3.7074	99.78
LLOQ	10.261	3.7033	98.20
Intra-day (day 2)			
HQC	1803.553	0.5381	100.20
MQC	787.973	1.7345	98.50
LQC	48.5233	3.5359	97.05
LLOQ	10.09	3.9049	96.55
Intra-day (day 3)			
HQC	1798.64	1.1405	99.92
MQC	769.453	2.6916	96.18
LQC	47.0833	5.6286	94.17
LLOQ	10.025	2.4586	95.93
Inter-day			
HQC	1793.263	1.0537	99.62
MQC	794.1583	1.5051	99.27
LQC	49.98	3.3812	99.96
LLOQ	10.463	3.5132	100.12

3.3.7. Ruggedness

The result of the ruggedness of the developed method demonstrated % RSD of 0 .4484% and 0.5991% for the column I and II respectively, indicating the no susceptible change in peak area ratios in both the columns.

3.3.8. Recovery

% Recovery of atorvastatin from rat plasma was 90.45% ± 0.78%, 97.67% ± 0.45%, and 96.17% ± 0.47% for LQC, MQC, and HQC samples respectively (Table 6), while % recovery of IS was analyzed at only one concentration (500 ng mL^{-1}) and found to be 105.68% ± 0.73%. Both % CV and % accuracy of all the 3 QC samples of atorvastatin and one sample of IS were under the acceptance criteria, thus the use of ice-cold ACN as an extraction solvent using PP technique was found to be decidedly efficient to extract atorvastatin from rat plasma.

3.3.9. Stability

The summary of different stability studies of atorvastatin in the biological matrix was presented in Table 7. The results demonstrated that atorvastatin was stable after three freeze-thaw cycles in rat plasma with 97.92% and 99.78%

stability in LQC and HQC samples. Similarly, analyte was stable at benchtop for 8 hrs at 25°C with %CV of 1.7452 and 0.4045 for LQC and HQC respectively and in autosampler for 24 hrs with %CV of 1.1671 and 0.6436 for LQC and HQC respectively. The experimental data from short term stability studies and long term stability studies also demonstrated the good stability of atorvastatin in rat plasma when stored under -20°C. Also, atorvastatin was found stable in stock solution for 4 hrs at room temperature and up to 30 days when stored at -20°C. Precision and accuracy of all the stability studies are under acceptable criteria as by USFDA.

Table 6 Extraction data of atorvastatin from rat plasma

Analyte	Conc.	% Recovery (Mean ±SEM)	Precision (%CV)	% Accuracy
Atorvastatin (HQC)	1800 ng mL^{-1}	96.17 ± 0.47	1.2118	99.78
Atorvastatin (MQC)	800 ng mL^{-1}	97.67 ± 0.45	1.122127	100.12
Atorvastatin (LQC)	50 ng mL^{-1}	90.45 ± 0.78	2.158909	88.07
IS	500 ng mL^{-1}	105.68 ± 0.73	4.09973	101.40

Table 7 Summary of stability data of atorvastatin in rat plasma

Stability Study	Observed conc. (ng mL^{-1}) (Mean ± SEM)	% Stability	Accuracy (%)	Precision (%CV)
LQC samples				
Freeze-thaw (3 cycles)	48.55 ± 0.356	97.92	97.10	1.7965
Bench-top (8hrs, 25°C)	48.252 ± 0.344	98.08	96.50	1.7452
Autosampler (24hrs, 4°C)	47.783 ± 2.23	97.46	95.56	1.1671
Short-term (1day, -20°C)	48.528 ± 0.30	97.96	97.05	1.5145
Long-term (15days, -20°C)	47.856 ± 0.323	97.34	96.40	1.6535
HQC samples				
Freeze-thaw (3 cycles)	1790.617 ± 6.296	99.78	99.47	0.8614
Bench-top (8hrs, 25°C)	1780.123 ± 2.940	99.39	98.89	0.4045
Autosampler (24hrs, 4°C)	1782.805 ± 4.684	99.44	99.04	0.6436
Short-term (1day, -20°C)	1787.827 ± 5.068	99.81	99.32	0.6943
Long-term (15days, -20°C)	1777.153 ± 4.913	99.33	98.73	0.6772

3.3.10. Dilution integrity

The results of the dilution integrity assay showed precision (2.5458 % and 2.9899 %) and % accuracy (98.03% and 98.38%) for both the dilution samples (5X and 10X respectively) under the acceptance limits, thus signifying no dilution effect on the quantification of atorvastatin from rat plasma up to 10 folds.

4. Conclusion

A highly selective, rapid, and simple HPLC-UV based bioanalytical method was developed for quantification of atorvastatin in rat plasma and validated as per USFDA protocols. The developed method can easily quantify the atorvastatin in ng mL^{-1} concentration with a short run time of 10 min. Thus the developed bioanalytical method can be widely used in routine laboratory analysis of atorvastatin in biological samples, alongside with determination of pharmacokinetics and drug-drug interaction studies of atorvastatin in humans and animals.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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