THINK INDIA JOURNAL

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

Development and Validation of UV-Spectrophotometric Method For The Estimation Of Atorvastatin Calcium In Bulk and Marketed Dosage Form

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ABSTRACT

The current research procedure involved the evolvement of analytical method by UV spectrophotometry for precise, simple, accurate and rapid assessment of Atorvastatin calcium (solid dosage form) in marketed and bulk dosage form.This study involved checking the solubility of drug in methanol.The drug showedgood solubility in solvent, sothestock solution was prepared in methanol. This drug showed good absorbance at wavelength 250 nm. Five different concentrations were prepared from 2-10 µg/ml and Beer's law was obeyed by them. For the standard stock solution, correlation coefficient was 0.996. Level of percentage recovery was estimated at 80%, 100% and 120% and it was found to be 99.64%.No interference of any foreign particle or excipients was observed throughout the assessment of drug in the formulation. Moreover, this method of validation was found to be inexpensive and chemicals required during the entire procedurewere arranged easily.The validation of result of analysis was done as per ICH guidelines and it wasobserved that for the purpose of routine laboratory analysis and quality control check of pharmaceutical formulations, the proposed method can be utilised.

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KEYWORDS

UV-Spectrophotometry, Validation, Atorvastatin calcium, Beer's law, ICH guidelines.

INTRODUCTION

The most prominent precipitating factor for the initiation of atherosclerotic disease is hypercholesterolaemia. Atorvastatin is most commonly used drug in hypercholesterolaemia. It inhibits HMG-CoA reductase enzyme which is responsible for the synthesis of low-density lipoprotein (LDL) cholesterol and thereby decrease the plasma level of LDL. The clinical doseabout 10–80 mg/day can be given to the patient suffering from hypercholesterolaemia. It is significantly soluble, permeable and fully absorbed orally. The oral bioavailability, volume of distribution and protein binding are 14%, 381L and 98% respectively. It is metabolised in both the liver and gut throughglucuronidation, oxidation and lactonization. Its metabolites are eliminated from the body throughdirect secretion into the intestine from blood and biliary secretion. The half-life and total plasma clearance of atorvastatin acid is about 7 hoursand 625 mL/min respectively.The elimination through renal route is (<1%).When administered orally either in the morning or evening, itsrate of absorption is decreased by food intake[1].

Chemically Atorvastatin is (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid[2](Fig.1). Chemical formula for atorvastatin calcium is $C_{66}H_{68}CaF_2N_4O_{10}$;558.65 g/mol.

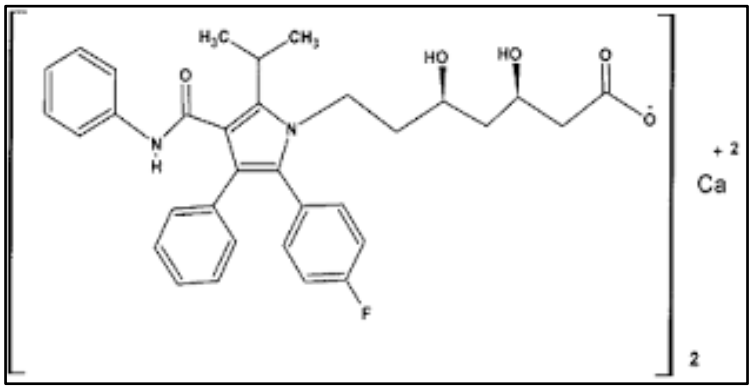

Figure 1: Atorvastatin calcium

For the purpose of analysis of Atorvastatin in bulk and formulation, a few UV-spectrophotometric methods were proclaimed, however, no method is available for quantitative determination using methanol. Literature review also revealed different bioanalytical methods like UPLC tandem mass spectrometry [3], LC-MS [4] and LC-

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MS/MS [5]to perform the analysis of Atorvastatin in biological samples like human and dog plasma.

So, themajor aim was to build up a simple, accurate, specific and rapid method through UV spectrophotometry to estimate theAtorvastatin in dosage forms (pharmaceutical and bulk) using methanol. The method was validated according to ICH Q2B recommendations for thevarious parameters such as sensitivity, accuracy, linearity and precision. The LOQ (limit of quantification) and LOD (limit of detection) values were also determined. The statistical and recovery studies were done to validate theresults of analysis[6,7].

MATERIAL AND METHODS

Atorvastatin was taken as a gift sample from Cadila Pharmaceuticals Limited. Atorvastatin tablets were purchased from local pharmacy. All the analytical grade reagents were used in study. A double beam Shimadzu UV-Visible Spectrophotometer having a spectral bandwidth of 1nm, a pair of 1 cm quartz cells and wavelength accuracy of ±0.5nm was used for the measurementof absorbance of the prepared solutions over a range of 200-400 nm.

Preparation of standard stock solution: In anErlenmeyer flask (25 ml), 25 mg of Atorvastatin calcium was taken and solubilizedin 25 ml of methanol to attain a concentration of 1000µg/ml. Different concentrations varyingfrom 2-10 µg/ml were prepared using methanol from this stock solution [8].

Determination of $\lambda_{max}$of Atorvastatin: Aworking solution of drug having 100µg/ml concentration was prepared and scanned on UV spectrophotometer using 200-400 nm range of wavelength. Atorvastatincalcium showed good absorbance at wavelength 250 nm. The absorbance of the prepared concentrations was taken at 250 nm andthe blank solution used was methanol. The calibration curve was drawn between concentration and observed absorbance.

Preparation of sample solution: In mortar and pestle, twenty tablets were weighed and crushed. The powdered content (equivalent to 10 mg of drug) was taken and dissolved in methanol (10 ml). The sonication was done on this solution for 5 minutes and then afterwards filtration was performedthrough Whatman filter paper. Different concentrations varying from 2-10 µg/ml were made using methanol. The amount of drug was determined by measuring absorbance of sample solution and plotting calibration curve.

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Validation of Analytical Methods: The term "validation" defines the action of providing accuracy. In pharmaceutical companies, validation of a particular analytical method is done to authenticatethe acceptability for its purpose, to acquire authentic or good and correct data.

Parameters of analytical method validation:

There are many parameters that are used for the validation of analytical methods. Some of these parameters are range, linearity,precision, accuracy, LOQ, LOD, ruggedness, robustness etc.

Linearity: In analytical method, linearity is explained as the capability to acquire test result and this result is related directly to the analyte concentration within given range(y=mx+c). The calibration graph made between the concentration and absorbance was found to be linear.

Range: Range is interim between upper concentration and lower concentration in the sample indicating that the method has acceptable precision, accuracy and linearity level.

Accuracy/Trueness*:* Accuracy can be defined as the agreement between the true values or reference value and determined the value. It is indicated as percentage recovery. To evaluate the correctness of the performance, recovery studies were carried out. Percentage recovery was determined for every concentration from the calibration curve. The formula for calculating percent recovery [9]is:

$$\text{Percentage recovery } = \frac{\text{Observed concentration}}{\text{total concentration}} \times 100 \ldots\ldots\ldots Eq.(1)$$

LOD and LOQ: Limit of detection can be explained as the minimum analyte concentration which can be sensed by instrument. Basically, it is the ratio of 3.3σ to the slope.

$$Limit\ of\ detection = \frac{3.3\,\sigma}{S} \ldots\ldots\ldots\ldots \text{Eq. (2)}$$

where, σ = The std. deviation (SD) of the response,

s = slope of line

There are 4 methods for the estimation of limit of detection as per ICHrecommendations namely signal-to-noise ratio, visual evaluation, SD of the response andSD of the slope of linearity plot.

LOQcan be defined as the minimum concentration of the analyte which can be quantitively estimated with suitable parameters such as accuracy and precision. It is the ratio of 10 σ to the slope.

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$$Limit\ of\ quantification = \frac{10\ \sigma}{S} \ldots\ldots\ldots\ldots \text{Eq. (3)}$$

Where, σ= SD of the response,

s= slope of calibration curve.

The specific calibration curve was studied to determine LOD and LOQ, using the analyte sample, in the range of detection and quantification limit [10,11].

Precision: By evaluating the drug at three different time points of the same day, intra-day precision was analysed and by analysing on different days (i.e. day 1, day 2 and day 3), inter-day precision was checked. %RSD was also determined [12-14].

Robustness: Robustness can be explained as if intentional change is done in the method parameter and that causes change in the assay capacity whichcould be measured in this parameter. During the development phase, the evaluation of robustness should be considered as it is dependent on the type of method adopted for study. It givesa hintfor the reliability of the procedure during normal usage.

Robustness was proved by analysing standard solutions 10 μg/ml of drugby two different wavelengths undersimilarenvironmental and experimental conditions. Under the optimised conditions from the standard detection wavelength, the parameter was checked by using changing detection wavelength (±2 nm).

Ruggedness: Ruggedness is conducted under variable conditions. This parameter is the level of reproducibility produced by the analysis of same sample under divergent conditions such asdifferent analyst, assay temperatures, days, instruments and laboratories [15].

Ruggedness of the developed method was evaluated by analysing the standard solution having 10 μg/ml concentration repeatedly, for six times by two different analysts under the same environmental and experimental conditions [16].

RESULTS AND DISCUSSION

The study was focussed to establishspecific, simple, accurate, and rapid UV spectrophotometric method to estimate Atorvastatin in pharmaceutical and bulk dosage forms using methanol. The pharmaceutical assay was carried out on atorvastatin calcium tablets by using UV-spectrophotometer [17].

The linearity studies of the drug were done by plotting a graph between different concentrations of standard solution against their respective absorbanceand linear regression

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analysis was then applied as shown in Table 1. The drug was observed to show linearity in 2-10µg/ ml range of concentration with correlation co-efficient0.996, and the calibration curve of the drugcomplied with Beer's law within the proposed concentration limits(Figure 2 and 3) [18].

Table2 shows the calibration curve of Atorvastatin calcium tablet in which correlation coefficient was 0.961.

LOQ and LOD of drug was observed to be 4.920 µg/ml and 1.623 µg/ml respectively which proved the sensitivity of method (Table 3).

To confirm the accuracy of this method, standard addition method was used to perform recovery studies.In this, standard addition was done at 80, 100, and 120% levels of drug concentration in the pre-analysed samples and percentage recoveries were also determined [19]. The mean % recovery for Atorvastatin calcium was found out to be 99.64% (Table 4).

The intraday precision was checked by analysing the drug at specific concentration 3 times a day at an interval of 3 hours at 10:00 am, 01:00 pm and 04:00 pm respectively. In the same way, the inter-day precision was determined by analysing the samples for 3succeeding days. The results attained from intraday and inter-day precision are shown in Table 5.

Robustness studies presumed that the minorchanges in any of the above variables did not majorly affect the observations [20]. The results obtained are shown in the Table 6 in which percentage relative standard deviation at two different wavelength 248 and 252 was found out to be 1.29 and 5.04%.

The results for ruggedness studies of Atorvastatin calcium (Table 7) did not show any major statistical difference between operators indicating ruggedness of this method. The Optimal characteristics of Atorvastatin have been shown in Table 8.

Table 1: Linearity curve of Atorvastatin

Sr.No.	Conc.(µg/mL)	Absorbance a	Absorbance b	Absorbance c	Mean of Absorbance
1	2	0.088	0.098	0.122	0.103
2	4	0.173	0.193	0.239	0.202
3	6	0.243	0.252	0.331	0.275
4	8	0.359	0.399	0.41	0.389

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5	10	0.427	0.501	0.513	0.48

Table 2: Calibration curve of Atorvastatin calcium tablet

Sr.No.	Concentration (ug/ml)	Absorbance
1	2	0.291
2	4	0.304
3	6	0.417
4	8	0.524
5	10	0.602

Table 3: LOD and LOQ of Atorvastatin calcium

Drug	LOD (µg/ml)	LOQ (µg/ml)
Atorvastatin calcium	1.623762	4.92049

Table 4: Accuracy studies of Atorvastatin calcium

Recovery Level	Initial conc.present (µg/ml)	Added drugconc. (µg/ml)	Total conc. present (µg/ml)	Total conc.recovered (µg/ml)	% Recovery
80	10	8	18	17.98	99.8
100	10	10	20	19.8	98.9
120	10	12	22	22.04	100.2
MEAN					99.64

Table 5: Precision studies of Atorvastatin Calcium

Intra Day Precision						
Concentration taken (µg/ml)	**Observed concentration (µg/ml) (*n=3)**			**Mean**	**SD**	**% RSD**
	10:00 am	**01:00 pm**	**04:00 pm**			
10	8.978	9.001	9.138	9.039	0.071	0.781
9	7.67	7.779	7.88	7.776	0.086	1.103
8	7.689	7.499	7.652	7.613	0.082	1.080

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Inter Day Precision						
Concentration taken (µg/ml)	Observed concentration (µg/ml) (µg/ml) (*n=3)			Mean	SD	%RSD
	Day 1	Day 2	Day 3			
10	9.309	9.352	9.465	9.375	0.066	0.702
9	8.291	8.349	8.35	8.330	0.028	0.331
8	6.481	6.563	6.609	6.551	0.053	0.808

Table 6: Robustness studies of Atorvastatin calcium

Conc. taken (µg/ml)	Absorbance at 248nm	Conc. observed(µg/ml) at 248nm	Absorbance at 252nm	Conc.observed(µg/ml) at 252 nm
10	0.487	10.2	0.419	8.8
10	0.479	10.1	0.417	8.7
10	0.484	10.1	0.425	8.9
10	0.491	10.3	0.468	9.8
10	0.495	10.4	0.422	8.8
Mean		10.28		9.01
Std. deviation		0.13		0.45
%RSD		1.29		5.04

Table 7: Ruggedness studies of Atorvastatin calcium

Conc. taken (µg/ml)	Conc. observed by analyst 1 (µg/ml)	Conc. observed by analyst 2 (µg/ml)
10	10.21	8.02
10	9.94	8.34
10	9.94	8.38
10	10.15	8.59
Mean	10.06	8.34
SD	0.144	0.24

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%RSD	1.43	2.85

Table 8: Optimal characteristics of Atorvastatin

Sr. No.	Parameters	Proposed Method
1	Absorbance maxima (λ_{max})	250 nm
2	Linearity Range	2-10 µg/ml
3	slope (m)	0.047
4	Correlation co-efficient (R^2)	0.996
5	Accuracy/trueness	99.63%
6	LOD (µg/ml)	1.62
7	LOQ (µg/ml)	4.92
8	Precision (%RSD) Intraday & Inter-day	 0.968 & 0.613
9	Robustness (%RSD) 249 nm 251 nm	 1.29 5.04
10	Ruggedness (%RSD) Analyst 1 Analyst 2	 1.43 2.85

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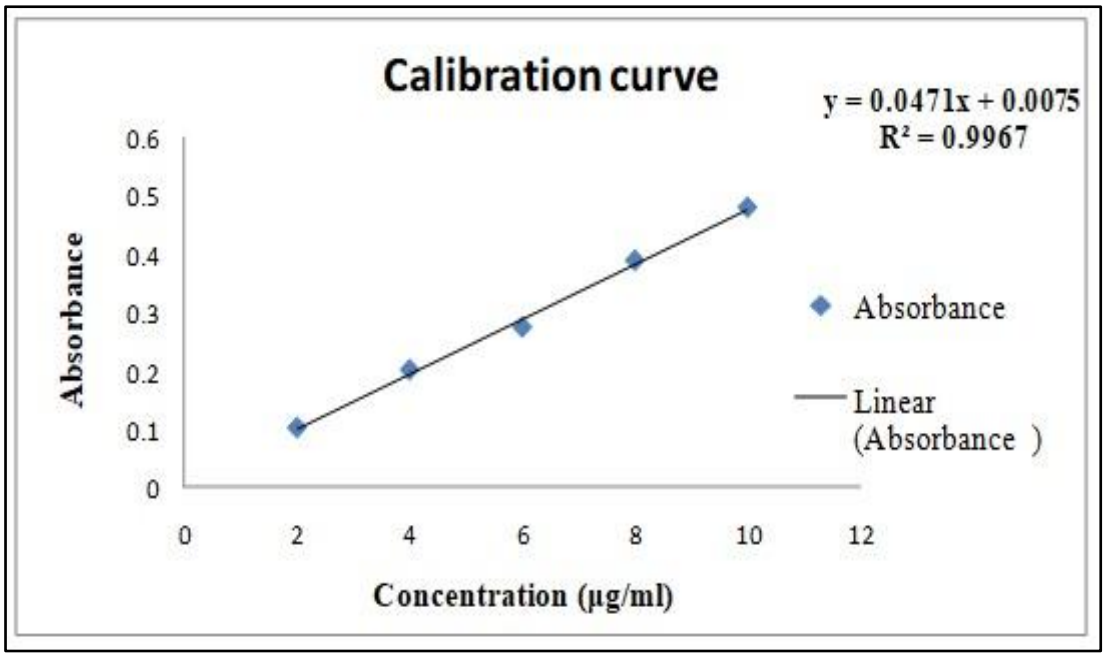

Figure 2: Calibration curve of Atorvastatin calcium at 250 nm

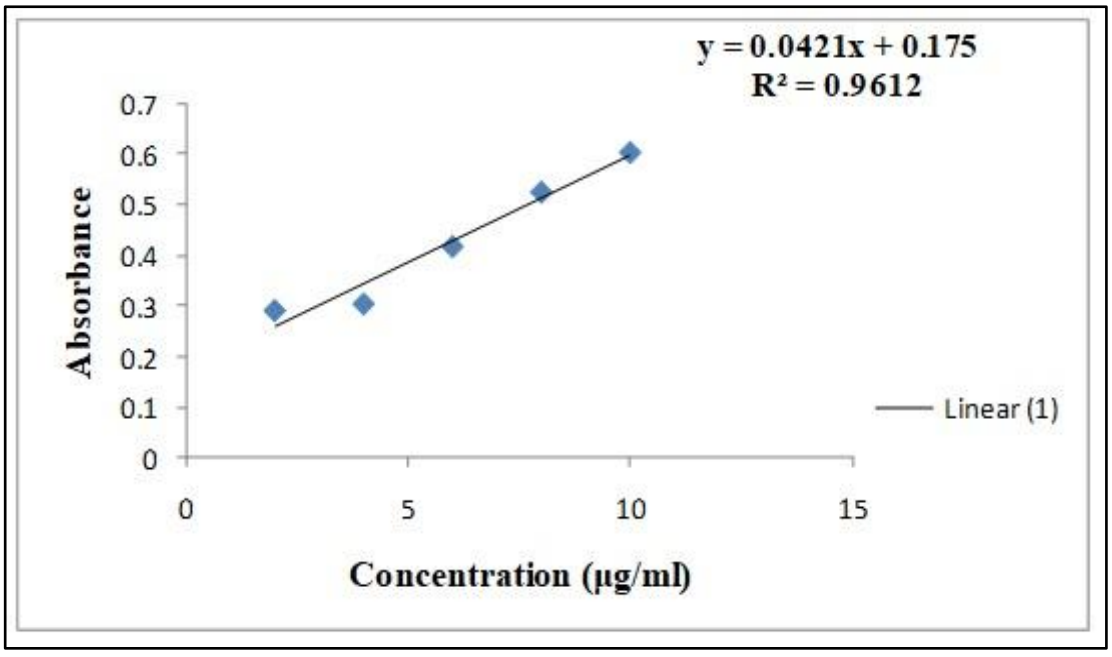

Figure 3: Calibration curve of Atorvastatin calcium tablet at 250 nm

CONCLUSION

For quantification of Atorvastatin calcium, an analytical method was developed and also validated.The analytical methodology was developed and validated following the guidelines as per ICH. The observations and results of the developed method were observed to be satisfactory and thus,this method of quantification of atorvastatin was validated in terms of linearity, accuracy, range, ruggedness, robustness andprecision. The validated method was

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observed to be economical and can be applied successfully for regular quality control procedures of Atorvastatin calcium incommercial pharmaceutical as well as bulk formulations.

CONFLICT OF INTEREST

Authors declare none.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Monica Gulati, Sr. Dean, School of Pharmaceutical Sciences, Lovely Professional University for providing the research facilities in the department.

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