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Research Article

Development and Validation of a UV-Spectrophotometric Method for Quantification of Atorvastatin in Tablets

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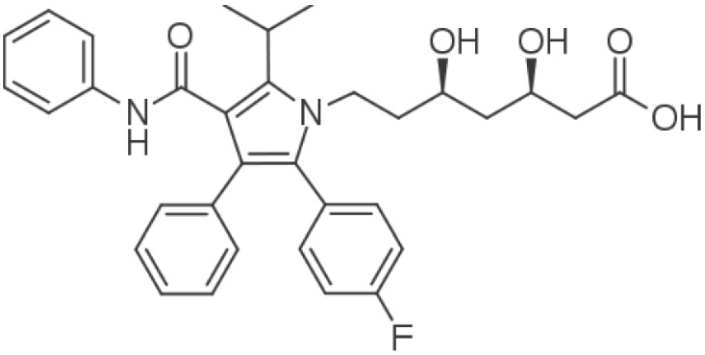
Abstract

A UV-spectrophotometric method has been developed for the quantitative estimation of atorvastatin in tablet. The present study describes development and validation of UV-spectroscopic method for estimation of atorvastatin in tablet dosage form and validation of newly developed method. During development of analytical method methanol: phosphate buffer (20:80), methanol: water (20:80), methanol: water (50:50), acetonitrile: water (10:90) were tried but drug was found to be soluble in methanol: water (50:50). Standard stock solution was prepared in methanol: water (50:50). The detection eave length (λmax) was found to be 248nm. Calibration curves were prepared. The proposed method obeys Beer's law in the range of 5-15µg/ml. Absorption maxima was determined with 10 µg/ml by scanning in the range of 200-400nm. Percent recovery studies are in the range of 98.78%-100.36%. The method was validated in terms of specificity, linearity, accuracy, range, precision, repeatability, robustness, system suitability, stability of analytical solution. Results of analysis were validated statistically and by specificity studies. From that it was observed that there is no interference of blank, excipients during the estimation of drug in formulation. This shows the adaptability of the method for routine estimation of atorvastatin in tablet dosage form.

***Keywords*:** Atorvastatin, method development, validation, spectrophotometry

Introduction

Atorvastatin is a member of the drug class known as statins, used for lowering blood cholesterol. Chemically, it is (3R, 5R)-7-(2-(4-flurophenyl)-3-phenyl-4-(phenylcarbamoy l)-5-propan-2-ylpyrrol-1-yl)-3, 5-dihydroxyheptanoicacid. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. The primary uses of atorvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease.[1] It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels.[1] It is used in hypercholesterolemia[2] (heterozygousfamilial and nonfamilial) and mixed dyslipidemia (Fredrickson types IIa and IIb) to reduce total cholesterol, low density lipoprotein-C[3] Apolipoprotein-B,[4] triglycerides[5] levels, and C-reactive protein[6] as well as increase lipoprotein levels. The drug has bioavailability of 12%, half-life of 14h and undergo hepatic metabolism.



Materials and methods[7-11]

Materials

Atorvastatin is obtained from Dr. Reddys Laboratory Limited., Hyderabad. HPLC grade Methanol (Merck), AR grade sodium hydroxide (Rankem), Potassium dihydrogen phosphate (Rankem) and Bransted HPLC water was sued in this study.

Interference due to blank, placebo with analyte (specificity)

The diluent was prepared by mixing methanol and water (50:50) and was this was used as a blank. To prepare standard solution (10ppm), 10 mg of Atorvastatin was weighed and transferred into a 100ml of volumetric flask. The drug was dissolved in 50 ml of methanol and volume was made up to100ml with water. The concentration of drug was 100ppm. 5ml of this solution was taken

in a 50 ml volumetric flask and volume was made up to the mark with diluents. Thus atorvastatin of strength 10ppm was obtained.

For the placebo solution, 10mg equivalent of atorvastatin placebo powder was weighed and transferred to a 100ml volumetric flask and dissolved in 50 ml of methanol and sonicated for 10 mins. Then it was shaken and the volume was adjusted up to 100 ml with water. Then, the solution was filtered and from the filtrate 10 ml filtrate was withdrawn and diluted up to 20 ml with the diluents. From this, additional 10 ml solution was withdrawn and diluted up to 50 ml with diluents.

10mg atorvastatin standard was taken and 10mg equivalent of atorvastatin placebo powder was weighed and transferred to a 100ml volumetric flask. Then 50ml methanol was added to it and sonicated for 15min, and then 40ml water was added to it and sonicated for the second time for another 5min. Then, the volume was made up to 100ml by water. Then, the solution was filtered. After filtration, withdraw 10ml sample and transferred in a 20ml volumetric flask and the volume was made up to 20ml with the diluents. From this solution, 10ml sample was withdrawn and diluted to 50ml with the diluent.

Accurately, 10mg equivalent atorvastatin powder was taken and dissolved in 50ml of methanol and was sonicated for 15min. Then, it was shaken and volume was adjusted up to 100 ml with water. Then, the solution was filtered and from the filtrate, 10 ml sample was withdrawn and diluted to 20 ml with diluents. Further, 10 ml diluted sample was withdrawn and the volume was adjusted to 50 ml with the diluents.

System precision and system suitability

Weight accurately 10 mg of atorvastatin was transferred into a 100ml of volumetric flask. The drug was dissolved in 50 ml of methanol and volume was made up to 100ml with water. The concentration of drug was 100ppm. 5ml of this solution was taken in a 50 ml volumetric flask and volume was made up to the mark with diluents. Thus, Atorvastatin of strength 10ppm was obtained. From the standard, the readings were taken 6 times for system precision and 5 times for system suitability and % RSD was determined.

Method precision

The absorbance of the standard solution (10 ppm) was measured five times and averaged. Six test samples (10 ppm) were prepared and absorbance was taken. The assay value was determined with respect to standard for the individual six samples and % RSD was calculated for the six samples.

Intermediate precision (analyst and day variation)

Accurately, 10mg equivalent atorvastatin powder was taken and dissolved in 50ml of methanol and was sonicated for 15min. Then, it was shaken and volume was adjusted up to 100 ml with water. Then, the solution was filtered and from the filtrate, 10 ml sample was withdrawn and diluted to 20 ml with diluents. Further, 10 ml diluted sample was withdrawn and the volume was adjusted to 50 ml with the diluents (10 ppm). Simultaneously, six samples were prepared from the same batch. Six sample preparations of Atorvastatin (10mg) were analyzed as per the method. By variability of analyst and day, the percentage assay of label claim was calculated.

Precision at different levels, linearity and range

Weight accurately 25 mg of atorvastatin was transferred into a 50 ml of volumetric flask. The drug was dissolved in 25 ml of methanol and volume was made up to 50 ml with water. The concentration of drug was 500 ppm. 10ml of this solution was taken in a 50 ml volumetric flask and volume was made up to the mark with diluents. Thus atorvastatin of strength 100 ppm was obtained.

Preparation of Level (50%): 2.5 ml of this 100 ppm stock solution diluted up to 50ml with diluents.

Preparation of Level (75%): 3.75 ml of this 100 ppm stock solution diluted up to 50ml with diluents.

Preparation of Level (100%): 5 ml of this 100 ppm stock solution diluted up to 50ml with diluents.

Preparation of Level (125%): 7.5 ml of this 100 ppm stock solution diluted up to 100 ml with diluents.

Preparation of Level (150%): 7.5 ml of this 100 ppm stock solution diluted up to 50ml with diluents.

For precision at different level, (50, 100 and 150%) six absorbances were taken and % RSD was calculated. For linearity concentration vs. absorbance for the five levels was plotted and correlation coefficients were calculated. In order to comply with the Lambert-Beers law, the concentration range was obtained from linearity plot (5-15 ppm). Recovery at Level (50%, 100% and 150%)

Accuracy

Accuracy at level 50%

5 mg atorvastatin standard was taken and 10mg equivalent of atorvastatin placebo powder was weighed and transferred to a 100ml volumetric flask. Then 50ml methanol was added to it and sonicated for 15min, and then 40ml water was added to it and sonicated for the second time for another 5min. Then, the volume was made up to 100ml by water. Then, the solution was filtered. After filtration, withdraw 10ml sample and transferred in a 20ml

volumetric flask and the volume was made up to 20ml with the diluents. From this solution, 10ml sample was withdrawn and diluted to 50ml with the diluents. Three samples were prepared.

Accuracy at level 100%

10 mg atorvastatin standard was taken and 10mg equivalent of atorvastatin placebo powder was weighed and transferred to a 100ml volumetric flask. Then 50ml methanol was added to it and sonicated for 15min, and then 40ml water was added to it and sonicated for the second time for another 5min. Then, the volume was made up to 100ml by water. Then, the solution was filtered. After filtration, withdraw 10ml sample and transferred in a 20ml volumetric flask and the volume was made up to 20ml with the diluents. From this solution, 10ml sample was withdrawn and diluted to 50ml with the diluents. Three samples were prepared.

Accuracy at level 150%

15 mg atorvastatin standard was taken and 10mg equivalent of atorvastatin placebo powder was weighed and transferred to a 100ml volumetric flask. Then 50ml methanol was added to it and sonicated for 15min, and then 40ml water was added to it and sonicated for the second time for another 5min. Then, the volume was made up to 100ml by water. Then, the solution was filtered. After filtration, withdraw 10ml sample and transferred in a 20ml volumetric flask and the volume was made up to 20ml with the diluents. From this solution, 10ml sample was withdrawn and diluted to 50ml with the diluents. Three samples were prepared.

The recovery at different levels was determined with respect to standard solution (10 ppm) and averaged at individual level.

Stability of analytical solution at room temperature

The standard and sample solution (10 ppm) was analyzed initially and the absorbance of the same sample was taken at 2 h interval up to 12h. Percentage differences were calculated with respect to the initial reading.

Robustness

The absorbance of standard and sample solution (10ppm) was taken at 247nm and 249nm. The assay value was determined. The assay value at 248nm was also determined. The deviation at 247 and 249nm from the assay value at 248 nm was calculated.

Results and Discussion

In order to detect the specificity of the spectrophotometric analysis, the blanks, standard, placebo, placebo in combination with the analyte and test solution of atorvastatin was prepared. The samples were scanned in UV-Vis spectrophotometer (Jasco V-530, USA) from 200nm to 400nm.

The blank sample showed an absorbance value of 0.001 at wavelength of 287.042nm. However, the placebo rendered an absorbance value of 0.006 at 238.259nm wavelength. The analysis of standard, test and placebo with analyte revealed that there was no significant difference in the absorbance values at λ_{max} close to 248nm (Table 1). Therefore, it was concluded that there was no interference in the analysis of atorvastatin due to placebo and blank.

Table 1. Scanning of 10ppm atorvastatin solution in methanol: water (50:50)

Sample	λ	Absorbance
Blank	287.042	0.001
Placebo	238.259	0.006
Standard	248.004	0.523
Sample	248.016	0.525
Placebo and analyte	248.006	0.523

In order to detect the system suitability of the spectrophotometric analysis, the standard of atorvastatin was prepared and simultaneously, five absorbances were taken for same standard at 248 nm in UV-Vis spectrophotometer. Percentage RSD for five of standard preparation is 0.28. It is not more than 2.0. The result is well within the acceptance criteria and the study concludes the suitability of analytical system for the analysis.

In order to detect the system suitability of the spectrophotometric analysis, the standard of atorvastatin was prepared and simultaneously, six absorbances were taken for same standard at 248 nm in UV-Vis spectrophotometer. Percentage RSD for six of standard preparation is 0.19. It is not more than 2.0. The result is well within the acceptance criteria and the study concludes the suitability of analytical system for the analysis.

Six sample preparations were assayed at 248 nm in UV-spectrophtometer. The method precision was evaluated by computing the percentage and relative standard deviation of the assay results. Percentage RSD for assay of six sample preparations was 0.36 (Table 2). It is not more than 2.0. The results are well within acceptance criteria and the % RSD observed for assay values indicates the precision of the method.

Table 2. Percentage of assay six atorvastatin sample

Sample	%Assay
Sample-1	100.57
Sample-2	100.19
Sample-3	99.80
Sample-4	100.00
Sample-5	100.19
Sample-6	100.78
Mean	100.26
Standard Deviation	0.362
%RSD	0.36

The six sample preparations were assayed by different analyst and in different day at 248 nm in UV-spectrophtometer. The method precision was evaluated by computing the percentage and relative standard deviation of the assay results. Percentage RSD for assay of six test preparations was 0.17 and is shown in Table 3. It was not more than 2.0 and cumulative percentage RSD of twelve determinations (method and intermediate precision) was 0.29 and is shown in Table 4. It is not more than 2.0. The results are well within acceptance criteria and the % RSD observed for % assay indicates the precision of the method.

Table 3. Percentage of assay six atorvastatin sample by different analyst in different day

Sample	%Assay
Sample-1	100.15
Sample-2	100.00
Sample-3	99.80
Sample-4	99.90
Sample-5	100.19
Sample-6	100.21
Mean	100.04
Standard Deviation	0.169
%RSD	0.17

Table 4. Compiled % of assay value method precision and intermediate precision

Set No.	Method Precision (% assay)	Intermediate Precision (% assay)
1	100.57	100.15
2	100.19	100.00
3	99.80	99.80
4	100.00	99.90
5	100.19	100.19
6	100.78	100.21
Average	100.26	100.04
% RSD	0.36	0.17
	Overall Mean	100.15
	Cumulative % RSD	0.29

In order to detect the precision at different level-1 50%, level-3 100% and level-5 150% sample were prepared and take six absorbance each level, For detect linearity level-50%, level-2 75%, level-3 100%, level-4 125% and level-5 150% sample were prepared and two absorbance were taken at each level in spectrophotometric analysis. Percentage RSD for six samples at level-1 50%, level-3 100% and level-5 150% levels 0.68, 0.17, and 0.22 respectively. It is not more than 2.0. Hence, precision at different levels of the method is established. For linearity plot concentration vs. absorbance at level-1 50%, level-2 75%, level-3 100%, and level-4 125%, level-5 150% is shown in Fig. 1. Squared correlation coefficient was 1.00. It should not be less than 0.99. Squared correlation coefficient value is well within the limit. Hence, the test for linearity passes

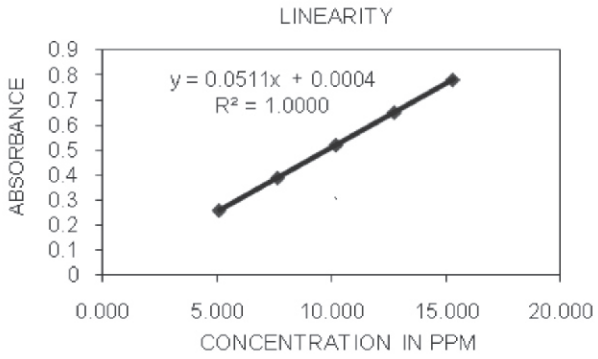


Fig. 1: Linearity plot of atorvastatin

In order to detect the accuracy at by spiking known quantities of drug (at level of 50%, 100% of and 150% of test concentration) to prepared three sample each level and determine amount recovered (mg) in each level with respect of standard atorvastatin (10 ppm) solution. The mean percentage recovery 99.59, 99.78 and 99.80 respectively showed in Table 5. It was between 98 and 102. All the results are well within the acceptance criteria and results indicate that the method is accurate and precise.

Table 5. Accuracy determination

% Recovery level		Placebo Added (mg)	Amount Standard Added (mg)	Amount standard Added With respect to potency(mg)	Amount Recovered (mg)	% Recovery
50	1	55.04	49.55	49.88	49.89	100.02
	2	55.01	50.00	49.98	49.99	99.98
	3	55.09	50.05	49.45	49.44	98.78
100	1	54.81	99.10	99.89	99.90	100.08
	2	55.28	100.00	99.99	99.98	99.98
	3	54.97	100.10	99.98	99.99	99.89
150	1	55.25	148.65	149.25	149.20	100.36
	2	55.11	150.10	149.89	149.90	99.86
	3	55.02	150.00	149.98	148.98	99.32

The range was derived from the data of linearity, precision (at different level) and accuracy study. The results observed for linearity, precision and accuracy study are within the limit, in the specified concentration range. Hence, range of the analyte method is established from 50% to 150 % (50% of lower strength and 150% of higher strength) of test concentration.

In order to detect the stability of analytical solution prepare standard and test solution and stored at room temperature. Absorbance was taken of these solutions at regular 2 h intervals for 12 h. The % difference of absorbance for standard and test preparation was calculated. The % absorbance difference was not more than $\pm$ 2.0 with that of initial. Since % difference of response from initial is less than $\pm$ 2.0 till 12 h. Solution stability was established up to 12 h at room temperature for standard and test preparation as shown in Table 6.

Table 6. Stability of standard and sample preparation

Time (h)	Absorbance (standard)	% Difference	Absorbance (sample)	% Difference
Initial	0.523	NA	0.525	NA
2	0.52	0.57	0.523	0.38
4	0.525	0.38	0.521	0.76
6	0.522	0.19	0.523	0.38

Time (h)	Absorbance (standard)	% Difference	Absorbance (sample)	% Difference
8	0.521	0.38	0.524	0.19
10	0.52	0.57	0.522	0.57
12	0.525	0.38	0.521	0.76

In order to detect the robustness prepare standard and test solution. Absorbance was taken at 247 nm, 248 nm and 249 nm in UV-Spectrophotometer and calculates the % assay value of these samples at each wavelength. System suitability should pass. Assay value should be not more than ±2% from mean value of method precision. The results are well within the acceptance criteria and the study proved the robustness of the analytical method against the deliberate changes. In order to detect the robustness prepare standard and test solution. Absorbance was taken at 247 nm, 248 nm and 249 nm in UV-Spectrophotometer and the % assay value of these samples were calculated at each wavelength. System suitability should pass. Assay value should be not more than ± 2 % from mean value of method precision. The results are well within the acceptance criteria and the study proved the robustness of the analytical method against the deliberate changes. Percentage RSD for five standard solutions for different parameter showed in Table 7. It was not more than 2.0. The result is well within the acceptance criteria and the study concludes the suitability of analytical system for the analysis.

Table 7. Robustness data for system suitability

Sl. No.	Sample (% assay)	247nm	248nm	249nm
1	Sample 1	99.8	100.38	99.6
2	Sample 2	99.61	99.42	100
Mean		99.705	99.9	99.8
± 2% from mean assay value of method Precision		0.45	0.25	0.35

Conclusion

The developed method was validated in terms of accuracy, linearity and precision, good linear relationship was observed for atorvastatin in the concentration ranges of 5-15 g/ml. The correlation coefficient for atorvastatin was found to be 1.00. Specificity showed that there is no interference placebo atorvastatin. Recovery of atorvastatin is lies 98.78%-100.36%.The percentage RSD for precision is <2 which confirms that method is sufficiently precise. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control for atorvastatin.

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