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Method Development and Validation of Atorvastatin Calcium Using *$FeCl_3$* by UV-Visible Spectrophotometric Methods

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Abstract: A novel, simple and rapid UV Spectrophotometric determination method for Atorvastatin calcium was successfully developed and validated for the assay of tablets. AUC (Area under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 232 nm and 242 nm. Zero Order Method the solutions were scanned in the range from 400-200 nm and the peaks were observed at 286.5 nm and 317.2 nm. Dual Wavelength Method spectrum of atorvastatin calcium shows identical absorbance at 248.9 nm (λ1) and 258.01 nm.The proposed method is the only method available for Spectrophotometric determination of the drug. It is simple, precise, accurate, sensitive and reproducible and can be used for the routine quality control testing of the marketed formulations.

Key words: Atorvastatin calcium · AUC · Zero Order Method · Dual Wavelength Method · $FeCl_3$

INTRODUCTION

Atorvastatin calcium (βR,αR)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid as the calcium salt belongs to the group of statins. All statins, including atorvastatin reduce the production of cholesterol in the liver by the competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the rate limiting enzyme in the biosynthesis of cholesterol [1]. UV detection at characteristic absorption maxima or different modes of MS detection. Several methods have been reported for quantitative determination of atorvastatin in biological samples [2-4], aqueous samples [5, 6] and tablets [7-9]. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [10-24] using Ferric chloride [25]. The objective of the present work is to develop and validate new analytical methods for determination of Atorvastatin calcium in tablet dosage form.

MATERIAL AND METHODS

Spectral runs were made on a Shimadzu UV Visible spectrophotometer (Model-1700, Japan) was employed with spectral bandwidth of 0.5 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells. Glassware's used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Tablets were purchased from Indian market containing atorvastatin calcium 10mg per tablet. All the solutions were protected for light and were analyzed on the day of preparations. Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug.

Preparation of Working Standard Drug Solution: Standard atorvastatin calcium 10 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol and add 0.5 ml of 5.0 % $FeCl_3$ and heated in a water bath at 65±5°C for 15 minutes, cool and complete volume with ethanol. Measure the absorbance of an orange chelate of atorvastatin calcium with Fe (III) at 395 nm against reagent blank. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 100 µg / ml. From this stock solution, pipette out 50 ml and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a working stock solution containing 50 µg /ml.

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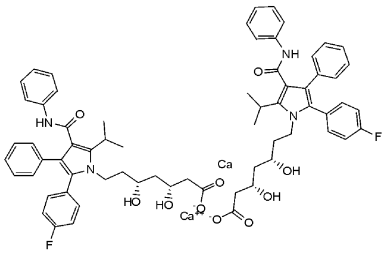

Fig. 1: Atorvastation calcium

Analysis of Marketed Formulations: For the estimation of atorvastatin calcium in tablets formulation by this method, 20 tablets of each brand were weighed and triturate to fine powder. Tablet powder equivalent to 10 mg of atorvastatin calcium weighed and transfer into 100 ml volumetric flask than dissolved with methanol and further diluted with methanol. It was kept for ultrasonication for 30 min; this was filtered through Whatmann filter paper No. 41 to get the solution of 100 µg / ml. The above solution was centrifuged at 2500 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No. 41). From this solution, 50 ml was taken and diluted to 100 ml with methanol to give a solution of 500 µg / ml and used for the estimation of atorvastatin calcium. Various dilutions of the tablet solution were prepared and analyzed.

Determination of Absorption Maxima: The standard solution of atorvastatin calcium (50µg/ mL) was scanned at different concentrations in the range of 200-400nm and the λ max was found to be 264.8 nm against reagent blank.

Area under Curve Method (AUC) [26-27]: In the simultaneous equation using AUC (Area under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 232 nm and 242 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (50µg/ mL) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm and the calibration curve was plotted. All the two method were checked by analyzing the samples with known concentration. As the result obtained were satisfactory, the method was applied for the pharmaceutical formulations. Calibration curves were plotted by taking concentration on x-axis and AUC at 219 to 228 nm or 232 to 242 nm on Y-axis and the regression analysis of calibration curves and absorptivity values (X) of both these drugs. 'X' values were determined as, X= Area under curve of component (from 219 to 228 nm or 232 to 242 nm)/concentration of the component in $\mu g ml^{-1}$. A set of two simultaneous equations framed using these 'X' values as follows,

A1=0.45875C1+ 0.76825C2-(at λ 232.0-242.0 nm)

Zero Order Method [26-27]: The solutions were scanned in the range from 400-200 nm and the peaks were observed at 286.5 nm and 317.2 nm. The wavelength selected for the analysis of the drug was 264.8 nm. The drug followed the Beer's-Lamberts law in the range of 5-50 µg/ml. By using the calibration curve the concentration of the sample solution can be determined.

Dual Wavelength Method [26-27]: The spectrum of atorvastatin calcium shows identical absorbance at 248.9 nm (λ1) and 258.01 nm (λ2) therefore these two wavelengths were selected for the analysis of atorvastatin calcium. All the solutions of were scanned to ensure that the difference between λ_3 and λ_4 is zero.

Calibration Curve: Appropriate volume of aliquots from standard atorvastatin calcium stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 5, 15, 20, 25, 30, 35, 40, 45 and 50 µg / ml. Absorbance spectra of each solution against methanol as blank were measured at 264.8 nm and the graphs of absorbance against concentration was plotted.

Validation of Method Parameters [28]: The aliquots of concentration ranging 5-50µg/mL were prepared in triplicate, but linearity was found to be between 2-10µg/ml concentrations. The linearity was calculated by the least square regression method.

Precision: The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD %. For this, 5µg/ml concentration solution was measured three times in day and same was measured in next three days.

Sensitivity: Molar absorptivity and Sandell's sensitivity for the proposed Spectrophotometric methods were found in the range of 2.433x64 to1.3263x79 Lit $mole^{-1}cm^{-1}$, which shows the high sensitivity of developed methods.

Accuracy (Recovery): The accuracy of the method was evaluated through standard addition method. In this, known amount of standard atorvastatin calcium was added in pre-analyzed sample. This was done for 50 µg/ml in triplicate. The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to pre-analysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 50 µg/ml of atorvastatin calcium.

Robustness: The robustness of the proposed methods was tested by changing parameters such as wavelength range and slit width. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

Ruggedness: Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot (50.0µgml-1) in different laboratories by different analyst using similar operational and environmental conditions. The results are reported in terms of % RSD.

Stability: The standard stock solutions of atorvastatin calcium (Concentration 50µg/ml was subjected to heat treatment on 50°C, 60°c and absorbance were measured. The absorbance for 50°c for 1hr was same while for 60°c, the absorbance was decreasing which was indicative that atorvastatin calcium is stable at 50°c and but at 60°C atorvastatin calcium solutions unstable.

Limit of Detection and Limit of Quantisation: The detection limit and quantisation limit was computed for lower limit of detection and minimum quantity of analyte measured and was found to be satisfactory by proposed spectrophotometric methods.

Statistical Evaluation: The developed methods statistically compared using one way ANOVA and indicate no significant difference between three methods. Hence these methods can be useful in routine analysis of atorvastatin calcium in bulk drug and tablet formulation.

RESULTS AND DISCUSSION

The results of linearity are presented in table 1. The data was statistically validated by means of least square regression method. The detection and quantization limits as LOD and LOQ were calculated and these were found to be 1.07µg/ml and 3.35µg/ml, respectively. The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicated that method is highly précised. The evaluation of accuracy of the method was performed by standard addition method and for this, in 50µg/ml concentration solution, 100% addition was done and it was found to be 99.1% and 100.08%, respectively. This indicated accuracy of proposed method. The proposed method was also applied for the assay of atorvastatin calcium in tablet formulation (in triplicate) and the results as tabulated in Table 3. The results obtained were good agreement with the label claims. These methods were validated as per ICH guidelines. The values of % RSD and correlation of coefficient were satisfactory and result of the recovery study indicates that there is no interference due to excipients present in the formulation. Result of formulation analysis and precision study are summarized in table 1 indicated that method is precise. % Recovery (%RSD) for atorvastatin calcium by Method I, Method II and Method III was found to be in the range of 99.997-101.05 (0.33-1.00) and 99.95-101.7(0.35-1.52) and 100.9-100.86 (0.24-1.11) respectively.

Table 1: Optical Characteristics data of atorvastatin calcium

	atorvastatin calcium					
Parameters	Method I		Method II		Method III	
Working λ (nm) in	232	242	286.5	317.2	248.9	258.01
Beer's law limit (µg/ml)	5-50	5-50	5-50	5-50	5-50	5-50
Absorptive E(1%,1cm)*	266	256	311	318	329	344
Correlation coefficient*	0.9995	0.998	0.9996	0.9990	0.9986	0.9988
Intercept*	0.0022	0.0006	0.0004	0.0016	0.0054	0.0003
Slope*	0.0311	0.0254	0.0376	0.0526	0.0409	0.0301

Method I-, Area under Curve Method (AUC), Method II-Zero Order Method-,Method III-Dual Wavelength Method *Average of six estimation

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	Drug	Label claimmg/tab	Amount found*mg/tab	Label claim(%)	S.D.*	% COV	S.E*.	Amount Added At (%)	% Recovery #
I	ATV	10	9.98	101.01	0.101	0.154	0.253	80	100.63
								100	100.82
								120	99.07
II	ATV	10	9.99	100.35	0.578	1.09	0.465	80	100.01
								100	99.95
								120	99.99
III	ATV	10	10.04	100.31	0.027	1.02	0.440	80	99.54
								100	99.88
								120	100.50

ATV-atorvastatin calcium Method I-, Area under Curve Method (AUC), Method II Zero Order Method-, Method III-Dual Wavelength Method *Average of six estimations. Standard deviation, COV: Coefficient of variation, S.E.: Standard error,*Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery

Table 3: Validation Parameters

				Precision (% COV)			
					Interday*		
Method	Drug	LOD*µg/ml	LOQ*µg/ml	Intraday n=6	First day	Second day	Third day
I	ATV	1.4761	0.876	0.765	0.611	0.543	0.432
II	ATV	2.3887	1.927	1.543	1.109	0.850	0.385
III	ATV	1.694	0.799	1.354	1.115	1.070	0.493

ATV-atorvastatin calcium, Method I-, Area under Curve Method (AUC), Method II Zero Order Method-,Method III-Dual Wavelength Method *Average of six estimationS.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error,

The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The relative standard deviation values are below 2%, indicating the precision of methodology and low standard error values show the accuracy of the method. This method utilizes the active analogue principle that lies at the spectroscopic method [10-25].

CONCLUSION

Simple UV Spectrophotometric method was developed for the simultaneous determination of atorvastatin calcium in bulk and tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in co formulated formulation. The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of atorvastatin calcium. It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

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