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DEVELOPMENT, VALIDATION AND ESTIMATION OF ATORVASTATIN CALCIUM BULK AND IN ITS PHARMACEUTICAL FORMULATION BY SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, accurate, precise, specific and highly sensitive spectrophotometric method developed for the determination of atorvastatin calcium in bulk drug and Pharmaceutical formulation. The optimum conditions for the analysis of the drug were established. The λ max of the atorvastatin calcium was found to be 244 nm in 40% methanol.. The method shows high sensitivity with linearity 5 to 25µg/ml. The lower limit of detection and the limit of quantification was found to be 0.2405µg/ml and 0.7289µg/ml respectively. All the calibration curves shows a linear relationship between the absorbance and concentration and coefficient correlation was 0.999. The regression of the curve was $Y = 0.039x+0.016$. The percentage recovery value was higher than 100 %, indicates the accuracy of the method and absenceof interference of the excipients present in the formulation. The proposed method will be suitable for the analysis of atorvastatin calcium in bulk and pharmaceutical formulation.

INTRODUCTION

Atorvastatin calcium is an antihyperlipidimic agent and is chemically known as [R-(R*, R*)]-2-(4-flourophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(Phenylamino)carbonyl]-1H-Pyrrole-1-heptenoic acid, calcium salt (2:1) trihydrate. It acts by inhibiting the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-co A) reductase.This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. [1] [2].It has been demonstrated to be efficacious in reducing both cholesterol and triglycerides. [3] The chemical structure of Atorvastatin calcium is shown in Fig 1. It's molecular formula is is C66H68CaF2N4O10 and its molecular weight is 1209.42 Literature survey reveled that various analytical methods such as uv-spectrophotometric[4]-[7],extractive spectrophotometry [8] , simulataneous uv methods [9]-[13],HPLC simulataneous with other drugs [14] –[23], GC-MS [24] , LC-MS [25] ,LC- electrospray tandem mass spectrometry [26]-[28] and HPTLC [29] methods have been reported for estimation of Atorvastatin calcium from its formulations and biological fluids. However very few methods were reported for quantitation of atrovastatin in tablet dosage forms in the literature. The present study illustrates a simple,accurate and economical spectrophotometric methods for estimation of Atorvastatin calcium in bulk and tablet formulation and it can be utilized for routine quality Control Laboratories.

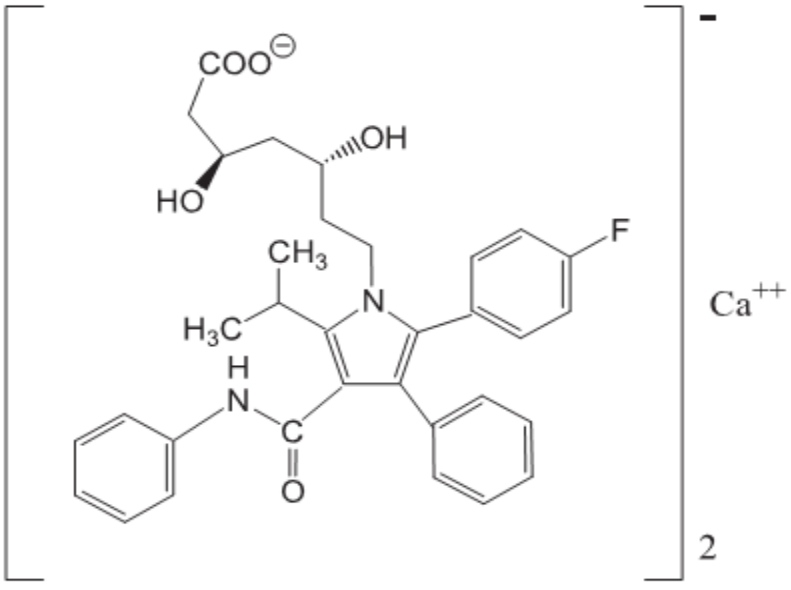

Figure 1: Structure of Atorvastain calcium

MATERIAL AND METHODS

Instrument

UV-Visible SpectrophotometerT60 (model), Analytical technologies Limited, connected to the digital system loaded with UVWin software ver.5.1.1 have an wavelength accuracy of ±5.0nm with quartz cells of 1cm path length.

Reagents and Materials

Working standards of pharmaceutical grade Atorvastatin calcium were procured locally and other chemicals used were of AR grade and purchased from SD fine chemicals, Mumbai.

Solubility of drug

10mg Atorvastatin calcium of was weighed and solubility of this sample was checked in water, methanol and phosphate buffer. The drug was found to be soluble in methanol.finally we select 40%methanol as solvent.

Preparation of standard stock solution

10 mg of pure Atorvastatin calcium was accurately weighed and transferred to 100ml of volumetric flask and then diluted up to 100ml by using Methanol: water (40:60) to produce a concentration of 100 μg ml^{-1} which is the standard stock solution.

Selection of wavelength

In order to ascertain the wavelength of maximum absorption (λmax) of the drug, different solutions of the drugs (5 μg/ml and 25 μg/ml) in 40% methanol were scanned using spectrophotometer within the wavelength region of 200 – 400 nm against 40% methanol as blank. The resulting spectra (5 μg/ml)shown in Fig-2 and the absorption curve showed characteristic absorption maxima at 244 nm for Atorvastatin calcium.

Calibration standards

From the standard stock solution of Atorvastatin calcium, different concentrations were prepared respectively in the range of 5-25μg/ml and measured absorbance at 244nm.The calibration curves were plotted (Figure-3) and data presented in Table 1.

VALIDATION PARAMETERS

Linearity-

Linear correlation was obtained between absorbance and concentration of Atorvastatin calcium in range of 5-25μg/ml .Data of regression analysis was summarized in Table 2.

Accuracy (Recovery)

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (20mg) and the amount of pure drug was varied that is 16mg, 20mg and 24mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery. (Table 3).

Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as %RSD .For this, 10 µg/ml concentration solution was measured three times in day and same was measured in next three days. The %RSD was calculated.(Table 4)

Lod &Loq

LOD (k = 3.3) and LOQ (k = 10) of the method were established according to ICH definitions. LOD and LOQ of method are reported in Table 2. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

LOD = 3.3 S/M; LOQ = 10 S/M

Where S is the standard deviation of the absorbance of the sample and M is the slope of calibrations curve.

ASSAY OF ATORVASTATIN CALCIUM TABLETS

20 Tablets were procured from local market and average weight was determined. The powder equivalent weight of Atorvastatin calcium was weighed accurately and transferred to a 100ml volumetric flask. About 40ml of methanol was added and sonicated for 5 min for complete dissolution of drugs, the volume was made upto the mark with the distilled water and then the above solution was filtered through Whatmann filter paper. Now 2.5ml of the filtrate is transferred to a 50 ml volumetric flask and then the volume was made upto the mark with the 40% methanol. After suitable dilution, the absorbance of final sample was recorded against the blank at 244 nm. All determinations were conducted in triplicate and result was indicated by % recovery given in Table 5.

Figure-2 Absorption spectrum of atorvastatin calcium

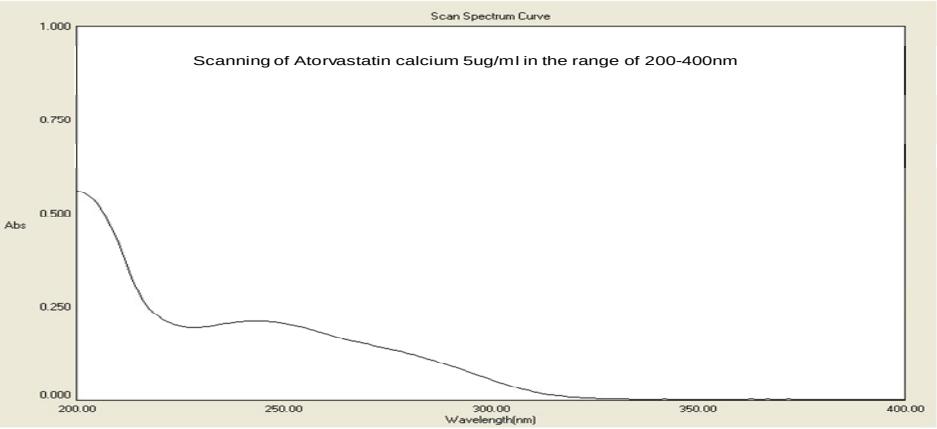

Figure-3 Calibration curve of atorvastatin calcium

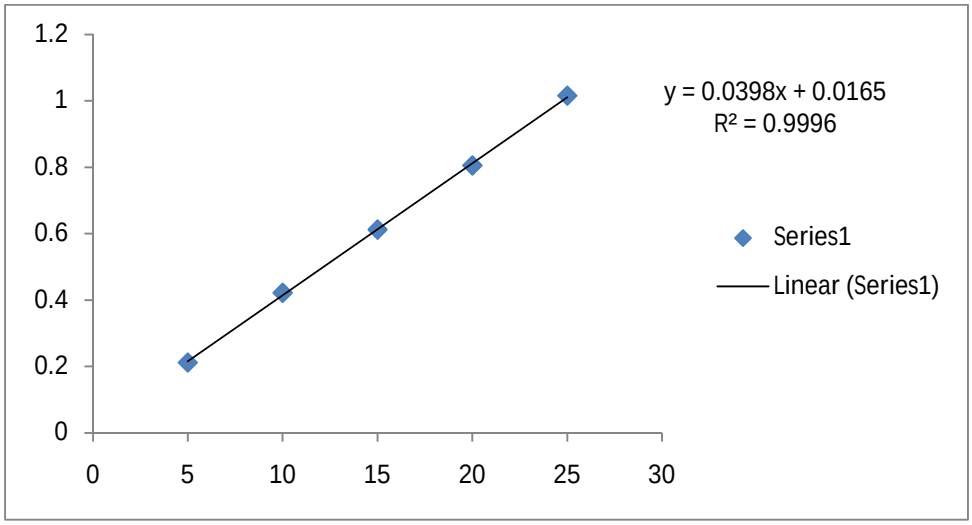

Table-3 Calibration data of the developed method

(Each value is result of nine separate determinations)

S.no	Concentration(μg ml^{-1})	Absorbance at 263nm±(SD)	%RSD
1	5	0.212±0.0028	1.304
2	10	0.422±0.00522	1.223
3	15	0.612±0.00871	1.397
4	20	0.805±0.0064	0.799
5	25	1.015±0.0094	0.927

S.D: Standard deviation, %RSD: Relative standard deviation

Table-2 Optical Characteristics and Regression Equation of Atorvastatin calcium

Y* = ax+b, where 'x' is concentration in µg/ml and Y is absorbance10^{-3}

Parameters	Values
Λmax	244 nm
Beer's law limit (µg/ml)	5-25
Regression equation (y=mx+c)	Y=0.039x+0.016
Slope (m)	0.039
Intercept(c) 0.0233	0.016
Correlation coefficient (r2)	0.999
Limit of detection (LOD) (µg/ml)	0.2405
Limit of quantification (LOQ) (µg/ml)	0.7289

Table 3 Accuracy data of the developed method

(Each value is result of three separate determinations)

Level of Addition (%)	Formulation (µg/ml)	Addition of pure Drug (µg/ml)	% Recovery of pure drug	Recovery(%)± S.D.
80	20	16	100.46	
100	20	20	101.25	100.68 ±0.49
120	20	24	100.33	

Table 4 Precision data of the developed method

(Each value is result of nine separate determinations)

Concentration	Intraday			Interday		
10(µg/ml)	Mean Absorbance	S.D	%RSD	Mean Absorbance	S.D	%RSD
	0.4253	0.00273	0.643	0.4242	0.0030	0.7244

Table 5 Assay Amount of Atorvastatin calcium in tablets

(Each value is average of three determinations ± standard deviation)

Sample	Labelled amount (mg)	Amount found	% Recovery
Atrovastatin calcium	20 mg/Tab	20.142	100.71±0.033

RESULTS AND DISCUSSION

The λ max of the Atorvastatin calcium was found to be 244 nm. From the optical characteristics (Table 2) of the proposed method, it was found that Atorvastatin calcium obeys linearity within the concentration range of 5 to 25µg/ml and coefficient correlation

was found to be 0.999. The regression of the curve was Y = 0.039*x*+0.016.The detection and quantization limits as LOD (k=3.3) and LOQ (k=10) were calculated and these were found to be 0.2405 µg/ml and 0.7289µg/ml respectively. The precision (measurements of intraday and interday) results showed (Table 2) good reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicated that method is highly precised. The percentage recovery value (Table 3) which was higher than 100 %, indicates the accuracy of the method and absence of interference of the excipients present in the formulation. The proposed method was also applied for the assay of Atorvastatin calcium in tablet formulation (in triplicate) and the results as tabulated in Table 5. The results obtained were good agreement with the label claims.

CONCLUSION

Simple UV spectrophotometric method was developed for the determination of atorvastatin calcium in bulk. To the best of our knowledge, the present study is the first report for the purpose. The present method achieved satisfactory percentage recovery and therefore it can be concluded that use of this method can be very economical and save analysis time and money. The proposed method is accurate and precise for the determination of atorvastatin calcium. Hence, it can be employed for routine analysis in of atorvastatin calcium in bulk, pharmaceutical formulations in Quality Control Laboratories.

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