Method Development and Validation of Atorvastatin Calcium by UV-Visible Spectroscopy.

Bhoskar Vishal*[1], Shinde Punam[2] ,Kokate Ranjeet[3], Bagal Usha[4], Kalmulkar Ankita[5]

*[1] Assistant Professor, Department of Pharmaceutical Chemistry, Saraswati Institute of Pharmacy Kurtadi, Hingoli. India-431701.

E-Mail:vbhoskar1@gmail.com

[2]Assistant Professor, Department of Pharmacology, Saraswati Institute of Pharmacy, Kurtadi, Hingoli.

India-431701.

E-Mail:punamshinde7887@gmail.com

[3]Associate Professor, Department of Pharmaceutical Chemistry, Saraswati Institute of Pharmacy, Kurtadi, Hingoli. India-431701

E-Mail:kokateranjeet21@gmail.com

[4]Lecturer, Department of Pharmacology, Saraswati Institute of Pharmacy, Kurtadi Hingoli.

India-431701

E-Mail:ushabagal9307@gmail.com

[5]Lecturer, Department of Pharmaceutics, Saraswati Institute of Pharmacy, Kurtadi Hingoli.

India-431701

E-Mail:kalmulkarankita@gmail.com

ABSTRACT

The objective of this research is to describe the optimization, validation, and application of spectrophotometric techniques for determination of Atorvastatin Calcium in their pharmaceutical formulation (tablets). In this paper simple, rapid, accurate and sensitive spectrophotometric methods have been developed and validated. This method is a direct spectrophotometric analytical method depends on dissolve of Atorvastatin calcium in diluted in water and methanol in ratio of (90:10). The maximum absorption wavelength for determination of ATR drug was found to be 241 nanometer (nm), for Beer's law was obeyed in the concentration range from 4 to 32 µg/ml for UV- Spectrophotometric analysis method.

Keywords-*UV Spectroscopy, Atorvastatin calcium, Method Development, Solvent and Validation.*

INTRODUCTION:

Development of simple and reproducible analytical methods for estimation of drugs is very important part of quality control and assurance. Chemically Atorvastin is [R-(R*R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate.Atorvastatin is a selective, competitive inhibitor of the 3-hydroxy methyl glutaryl coenzyme A

(HMG-CoA) reductase enzyme that is involved in the conversion of HMG-CoA to mevalonate (a precursor of sterols, including cholesterol). A reduction of intracellular cholesterol levels promotes an expression of LDL (low-density lipoprotein) receptors on the hepatocyte surface, resulting in an increased extraction of LDL from the blood. As an additional cholesterol-lowering mechanism, HMGCoA reductase inhibitors also decrease the blood concentrations of VLDLs (very low-density lipoproteins) by inhibiting their synthesis and promoting their catabolism. Atorvastatin calcium also inhibits the cholesterol synthesis in the liver and increases the hepatic LDL receptors on the cell surface to enhance the uptake and catabolism of LDL. The drug also reduces the LDL production and the number of LDL particles. The structure is shown in fig.1

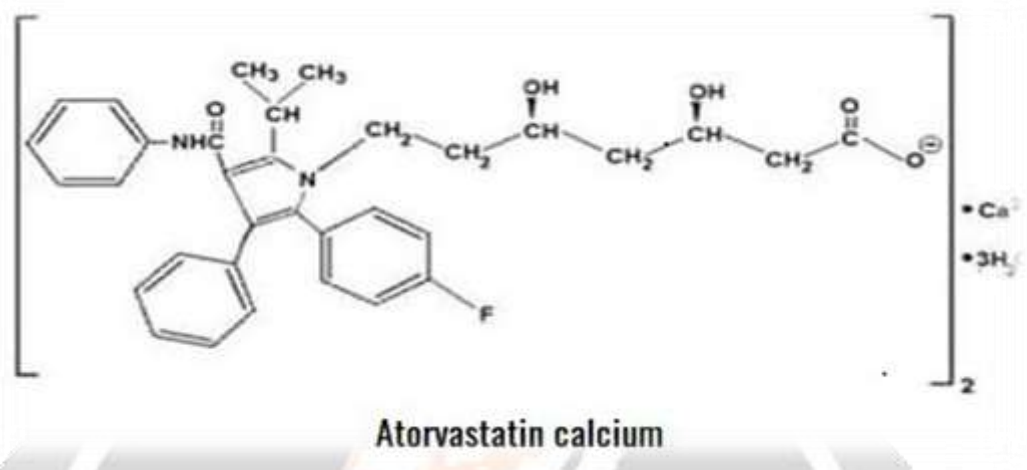

Fig-1: Structure of Atorvastatin calcium

MATERIAL AND METHODS

Apparatus and software

UV-Visible spectrophotometer (UV-1800 Shimadzu Double Beam Spectrophotometer) computer loaded with Shimadzu UV Probe 2.33 software was used for all the spectrophotometric measurements. The spectral bandwidth was 1nm and the scanning speed was very fast. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm.

Reagents and materials

Atorvastin Calcium (99.5% purity) was received as gift samples from Aurobindo Pharma Ltd..A.R grade Methanol (Merck Index), Pharmaceutical formulation tablets (label claim 10 mg ATR) was used in UV analysis.

Preparation of stock solutions:

Preparation of stock solution- Standard stock solution of Atorvastatin Calcium was prepared by dissolving accurately weighed 10mg of Atorvastatin Calcium in water and methanol in ratio of 90:10in 10ml volumetric flask to give a concentration of 1mg/ml. which is the standard stock solution.

Determination of Maximum Absorbance (max)

From the above stock solution 0.1ml was pipette out into 10ml volumetric flask and dilution was made with water to obtain concentration 10µg/ml. The samples was then scanned in UV spectrophotometer from a range of

200-400nm against water and methanol in ratio of 90:10 as blank and the wavelength corresponding to maximum absorbance in water and methanol was found at 241nm.

Results of analysis of commercial tablet formulation

An accurately weighed quantity of tablet powder equivalent to 10mg of Atrovastin calcium was transferred to 10 ml volumetric flask shaken with water and methanol in ratio of 90:10 to get stock solution of 1000 µg/ml..Aliquot portion were further diluted to get concentration of 10 µg/ml Atrovastin calcium. The absorbance of the final solution was read at selected wavelength. The values of % recovery are found to be very close to each other as well as to the label value of commercial pharmaceutical formulation, which shows that the method is applicable.

Table-1: Analysis of tablet formulation

Sr. No.	Taken concentration (ug/ml)	Absorbance	Find concentration (ug/ml)
1	10	0.244	9.9854
2	10	0.241	9.8982
3	10	0.238	9.8110

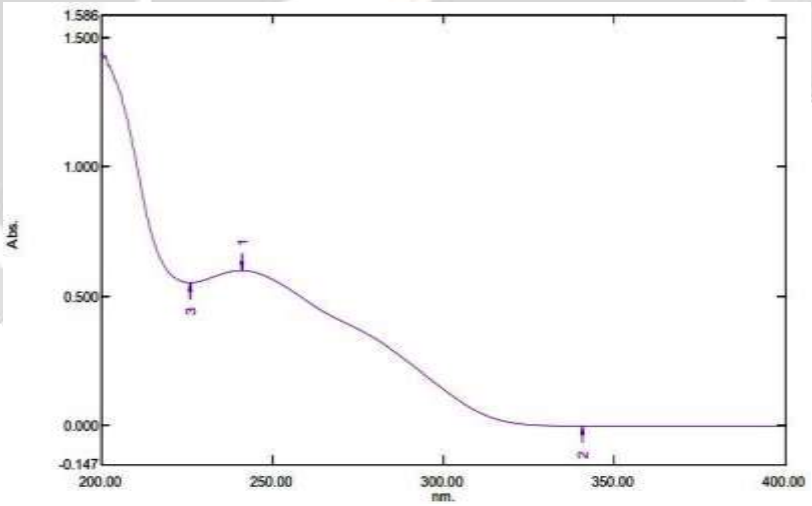

Fig-.2: UV Spectrum of Atorvastatin Calcium

Selection of analytical concentration ranges:

From the standard stock solution of ATR, appropriate aliquots were pipette out in to 10ml volumetric flask and dilutions were made with water and methanol in ratio of 90:10 to obtain working standard solutions of concentrations from 4-32µg/ml.Absorbance for these solutions were measured at 241nm.

METHOD VALIDATION

Linearity and range

For the preparation of standard calibration curve, concentration of 4-32µg were prepared by pipetting out 0.4, 0.8, 1.2, 1.6, 2, 2.4, 2.8, 3.2ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with water and methanol in ratio of 90:10.The absorbance of each solution was measured at 241nm against water and methanol in ratio of 90:10 as blank. Calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis. The curve showed linearity in the range of 4-32µg/ml with correlation coefficient 0.9991.

Table-02: Linearity Data

Concentration(µg/ml)	Absorbance
4	0.129
8	0.272
12	0.429
16	0.567
20	0.669
24	0.823
28	0.963
32	1.101

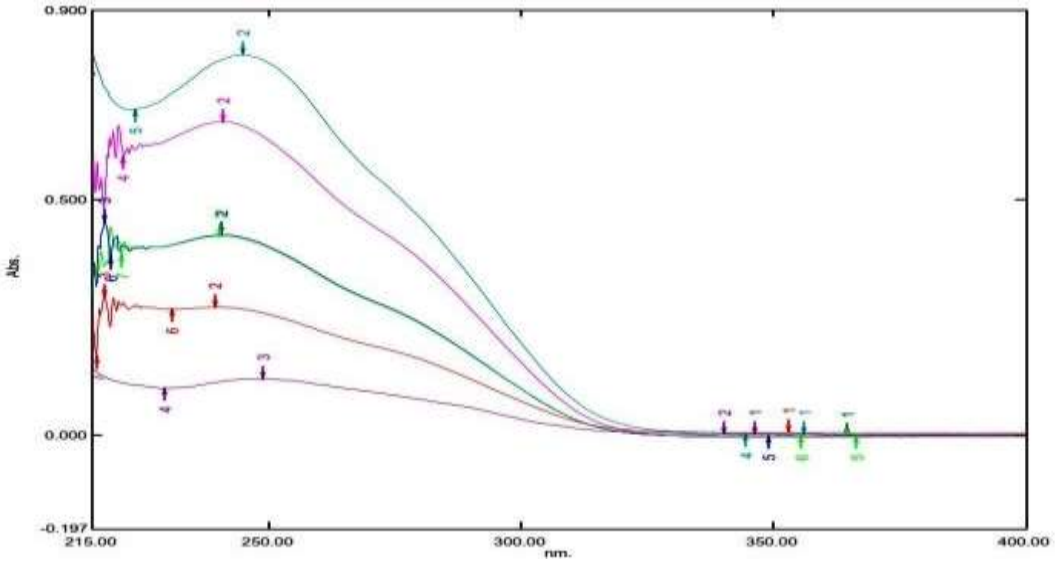

Fig-03: Overall spectrum of Atorvastin calcium

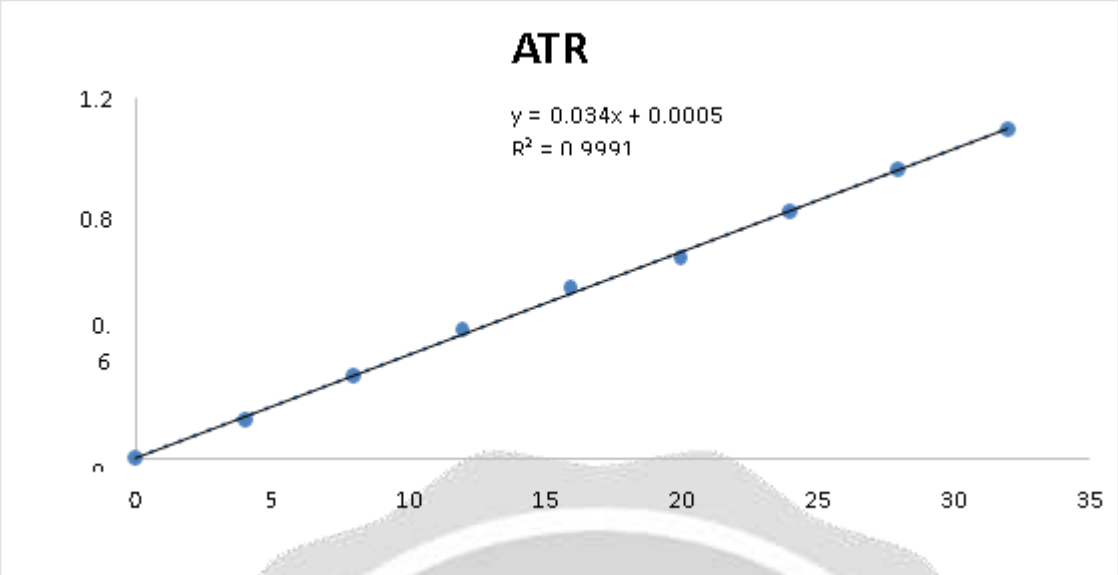

Fig-.4: Calibration curve Atorvastin Calcium

Table-.03: Optical and regression characteristics.

Sr. No	Parameters	Atorvastatine Calcium
1	Slope	0.0344X
2	Intercept	0.0005
3	Correlation coefficient	0.9991
4	Linearity range (µg/ml)	4-32 µg/ml

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing three replicates of same concentration of the sample and the absorbance was measured. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2. The results of intraday and interday precision studies are shown in Table.4

Table-04: Precision data

Sr. No.	Interval of Time	Concentration (µg/ml)	Absorbance	%Purity
I	Intra-day	10	0.245	100.00
II		10	0.246	100.36
III		10	0.246	100.36
I	Inter-day	10	0.245	100.00
II		10	0.247	100.72
III		10	0.247	100.72

Table-05: Statistical validation of intra-day precision data

Mean	SD	%RSD
100.24	0.2078	0.2073

*** Indicates average of three determinations**

Table-06: Statistical validation of inter-day precision data

Mean*	SD	%RSD
100.48	0.4156	0.4137

*** Indicates average of three determinations**

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100% and 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in Table No.7.

Table-07: Recovery study data.

Level ofRecovery	Amount present (mg)	Added concentration (mg)	Amount recovered (mg)	% Recovery
80%	10	08	7.36	96.44
	10	08	7.96	99.77
	10	08	8.03	100.16
100%	10	10	9.95	99.75
	10	10	10.12	100.6
	10	10	9.85	99.25
	10	12	11.97	99.86

120%	10	12	11.99	99.95
	10	12	12.01	100.04

Table-08: Statistical validation of recovery study data

Level of Recovery	% Mean recovery	SD	% RSD
80%	98.79	1.9795	1.9802
100%	99.86	0.6825	0.6834
120%	99.95	0.09	0.0900

LOD and LOQ

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD was determined using the following equation LOQ-10s/m, LOD-3.3s/m where s is the standard deviation of the response and m is the slope of the related calibration curve.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD. The results are shown in Table No.9

Table-9: Ruggedness data

Sr. No.	Analyst	Concentration (µg/ml)	Absorbance	%Purity
I	1st	10	0.244	99.63
II		10	0.246	100.36
III		10	0.244	99.63
I	2nd	10	0.245	100.00
II		10	0.245	100.00
III		10	0.244	99.63

Table-10: Statistical validation of Ruggedness data (Analyst 1st)

Mean*	SD	%RSD
99.87	0.4214	0.422

Table-11: Statistical validation of Ruggedness data (Analyst 2nd)

Mean*	SD	%RSD
99.87	0.4214	0.422

Robustness

Analysis was carried out at two different ratio concentrations, Methanol: warer; 05:9.5 ratios determine the robustness of the method and the respective absorbance was measured. The results were indicated as %RSD.

Table-12: Robustness data

Sr. No.	Ratio of	Concentration (µg/ml)	Absorbance	%Purity
I	Methanol:water 0.5:9.5	10	0.242	100.36
II		10	0.241	100.00
III		10	0.244	99.63

Table-13: Statistical validation of Robustness data (Methanol:Water; 0.5:9.5)

Mean*	SD	%RSD
99.99	0.365011	0.365024

*** Indicates average of three determinations.**

RESULTS AND DISCUSSION

The solubility of Atorvastin Calcium studied and Methanol-water (0.5:9.5) is selected as a solvent. For calibration curve method Atorvastin Calcium showed wavelength maxima at 241 nm. The drug follows Beer-Lambert's law over the concentration range of 4-32 µg/ml with a correlation coefficient of 0.9991. The present study of proposed method showed precision in terms of the repeatability and, reproducibility is found to be not more than 2%. The recovery results are in the range of 98 to 102%. Hence, the results of the analysis are validated as per ICH guidelines. Quantitative determination of Atorvastin Calcium in API and tablet dosage form by employed the method, the assay values found 100.60%.

Table-14 : Optical parameters for UV-Spectrophotomtric Method.

Parameters	Result.
Working Wavelength(nm)	241nm
Linearity Range(µg/ml)	4-32 µg/ml
Limit of Detection (µg/ml)	0.860 µg/ml
Limit of Quantification (µg/ml)	1.73 µg/ml
Y= mx+c	0.0344x+0.0005
Slope ± S.D.	0.0344x

Intercept ±S.D.	0.0005
Regression Coefficient ±S.D.	0.9991
Specificity	Specific

CONCLUSION:

The newly developed method of Atorvastin Calcium is simple, precise, and validate in terms of linearity, precision, accuracy, reproducibility. Therefore, the developed spectroscopic method used for routine estimation of Atorvastin Calcium in bulk &tablet dosage form.

ACKNOWLEDGEMENT

Authors are thankful to Aurbindo Pharma Ltd Hyderabad, Telangana, for providing us the drug sample. Also thankful to research guide Mr. Mohammad Zameeruddin HOD Pharmaceutical Chemistry, SSS Indira College of Pharmacy Vishnupuri Nanded for providing necessary Instruments facilities, also Management and Principal of Saraswati Institute of Pharmacy, Kurtadi Hingoli for providing research platform and support.

REFERENCE

1. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's Textbook of Quantitative Analysis.5th ed. New York: Longman Scientific and Technical, 1991:217-235.
   Chatwal GR. Sham KA. Instrumental methods of chemical analysis. 5th Ed. Himalaya Publication 2010:2.107-2.184.
2. Skoog DA, West DM. Principle of Instrumental Analysis. Stanford University. Saunders college publication, London 1980:2-3.
3. Chatwal GR, Anand SK. Instrumental Methods of chemical Analysis 5TH edition. Himalaya Publication House Mumbai 2002: 2.567-2.585.
4. Bai. G. Wang, Y. Armenante, Velocity profiles and shear strain rate variability in the USP Dissolution Testing Apparatus 2 at Different Impeller Agitation Speeds, International Journal of Pharmaceutics,2011: 403 (1-2), Pages 1–14
5. United States Pharmacopeia 34/National Formulary 29: 2011.
6. The United states Pharmacopoeia 32,NF27, United States Pharmacopoeia Convention ,Inc, Rockville, Annual Asian Edition, 2010: 263
7. Saeed Qureshi, Drug Dissolution Testing: Selecting a Dissolution Medium for Solid Oral Products, American Pharmaceutical Review, 1-5.
8. Chafetz L: Stability-indicating assay methods for drugs and their dosage forms. Journal of Pharmaceutical Sciences 1971, 60(3): 335-345
9. ICH Q1A (R2): Stability Testing of New Drug Substances and Products. Step 4 version 6 Feb 2003, ICH: 01-17.
10. ICH Q1B: Stability testing, Photo stability testing of new drug substances and products. Step 4 version 6 Nov 1996, ICH: 01-08.
11. Hirani J, Rathod D, Vadalia K. Orally disintegrating tablets. Tropical Journal of Pharmaceutical Research 2009;8(2):161-172.
12. Olmez SS, Vural I. Advantages and quality control of disintegrating tablets. FABAD Journal of Pharmaceutical Sciences 2009;34 (1):167-172.
13. Bangale S.G, Shinde GV, Rathinaraj BS. New generation of orodispersible tablets: recent advances and future prospects. International Journal of Advances in Pharmaceutical Sciences2011;2(3):17-28.
14. Kumar P, Ahmad Y (2012) Stability Indicating Method Development for Simultaneous Determination of Ezetimibe and Atorvastatin in Dosage form by UV &RP-HPLC. Der Pharmacia Sinica 3:413-421.
15. Ahmadi F, Asaadi N (2013) Performance Evaluation of a Novel Potentiometric Membrane Sensor for Determination of Atorvastatin in Pharmaceutical Preparations. Iranian J Pharma Res 12:645-657.
16. O'Neil MJ, Smith M, Heckelman PE (2001) The Merck Index (13th edn) Merck and Co. Inc., New Jersey, pp:3489.
17. Doshi N, Sheth A, Patel T, Dave JB, Patel CN (2010) Spectrophotometric Absorption Factor Method Development and Validation for Estimation of Rosuvastatin Calcium and Telmisartan in Solid Dosage Form. J Chem Pharma Res 2:15-24.

18. Rajitha B, Rao S, Kumar TV (2015) Spectrophotometric Determination of Drugs and Pharmaceuticals Using Cerium (IV) as oxidant and Amaranth dye as Analytical Reagent. J Appl Chem 8:15-23.