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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF ATORVASTATIN CALCIUM IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Atorvastatin Calcium in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Atorvastatin in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, very slightly soluble in water and slightly soluble in ethanol. So, the analytical grade methanol is used as a diluent in method. The melting point of Atorvastatin was found to be 174-175˚C (uncorrected). It showed absorption maxima 272 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 15µg/ml (PPM). The linearity was observed between 5-25 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.32% and for Ruggedness is 0.46%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Atorvastatin in tablet dosage form. The method could be considered for the determination of Atorvastatin in quality control laboratories.

KEYWORDS: Atorvastatin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Atorvastatin was first synthesized in 1985 by Dr. Bruce Roth and approved by the FDA in 1996. It is a penta substituted pyrrole 3 formed by two contrasting moieties with an achiral heterocyclic core unit and a 3,5-dihydroxypentanoyl side chain identical to its parent compound. Atorvastatin (Lipitor®), is a lipid-lowering drug included in the statin class of medications.[1] By inhibiting the endogenous production of cholesterol in the liver, statins lower abnormal cholesterol and lipid levels, and ultimately reduce the risk of cardiovascular disease. More specifically, statin medications competitively inhibit the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase, 8 which catalyzes the conversion of HMG-CoA to mevalonic acid.[2] This conversion is a critical metabolic reaction involved in the production of several compounds involved in lipid metabolism and transport, including cholesterol, low-density lipoprotein (LDL) (sometimes referred to as "bad cholesterol"), and very-low-density lipoprotein (VLDL).[3] Prescribing statins is considered standard practice for patients following any cardiovascular event, and for people who are at moderate to high risk of developing cardiovascular disease. The evidence supporting statin use, coupled with minimal side effects and long term benefits, has resulted in wide use of this medication in North America.[4]

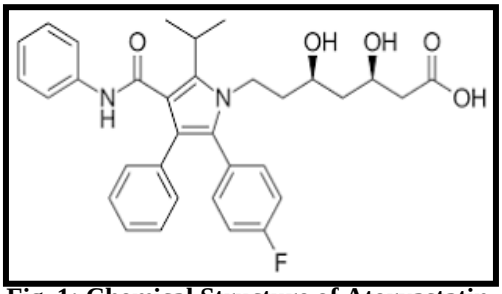

Fig. 1: Chemical Structure of Atorvastatin.

Literature survey revealed that a limited number of Spectrophotometric method found in combination with atorvastatin[5], RP-HPLC[7] methods were reported for the assay of Atorvastatin alone and in combination with other drugs. Also some method reported for Quantitative determination of Atorvastatin in Human urine, CSF, Human Plasma and rat tissue.[6,8,9] Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Atorvastatin alone in tablet dosage form.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Atorvastatin was obtained as a gift sample. Atorvastatin tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method Development

A. Determination of λ max (15 PPM)[11, 12]

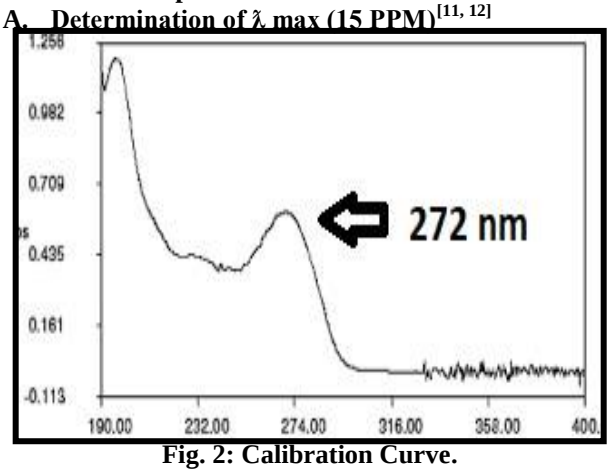

Fig. 2: Calibration Curve.

50 mg weighed amount of Atorvastatin was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1.5 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Atorvastatin in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 1.5 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 15 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[11,12]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Atorvastatin was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1.5 ml of the filtered solution and diluted up to 100 ml with analytical grade methanol. The absorbance was measured at 272 nm. The absorbance was recorded.

Table 1: Absorbance of Dosage Form.

Cipla Pharma LTD (80 mg) Tablets.		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.6584
3	Sample	0.6515

Table 2: Dosage Form Specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Cipla Pharma LTD (80 mg)	05/2021	07/2025	GPH 2145	0.1254	98.95

E. Method of validation[10,12,13]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 5 - 25 PPM at 272 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	5 PPM	0.2284
2	10 PPM	0.4212
3	15 PPM	0.6564
4	20 PPM	0.8654
5	25 PPM	1.0895
Correlation coefficient		0.9993 ~ 0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade methanol and measuring the absorbance at 272nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6552
2	Sample Solution 2	0.6554
3	Sample Solution 3	0.6511
4	Sample Solution 4	0.6557
5	Sample Solution 5	0.6525
MEAN		0.6540
SD		0.0021
% RSD		0.3147

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6519	0.6523	99.07	0.4553
		0.6526			
2	Analyst 2	0.6545	0.6565	99.71	
		0.6584			

RESULTS

1. Solubility of Atorvastatin

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Analytical grade Methanol	Freely Soluble
2	Water	Very Slightly soluble
3	Ethanol	Slightly soluble

2. Melting point of Atorvastatin

The melting point of Atorvastatin was found to be 174-175˚C (uncorrected).

3. Results for linearity for assay method of Atorvastatin

The linearity of method was determined at concentration level ranging from 5 to 25 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9993 ~ 0.999.**

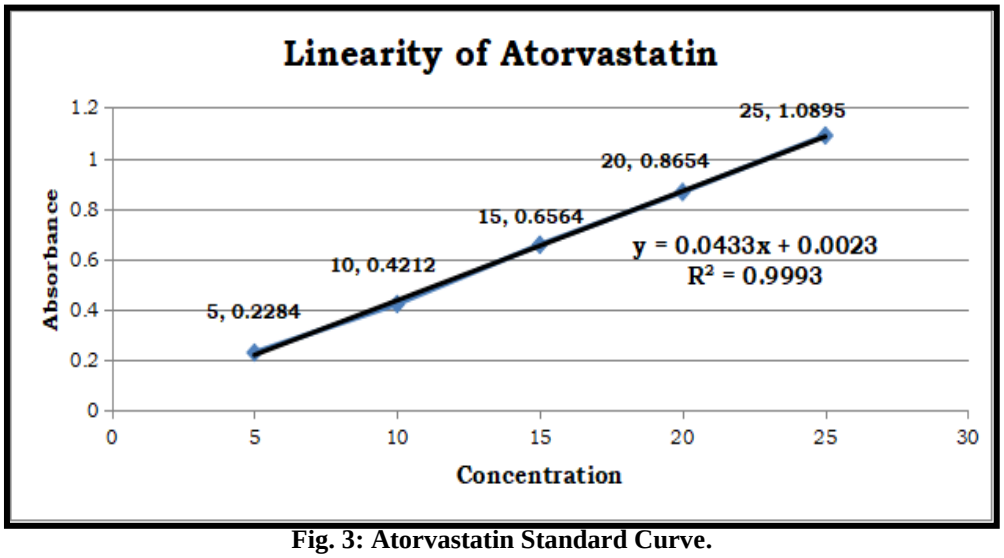

Fig. 3: Atorvastatin Standard Curve.

4. Results for accuracy for assay method of Atorvastatin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Atorvastatin

The % RSD for different sample of precision was found to be 0.3147 ~ 0.32 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Atorvastatin

The %RSD for different sample of ruggedness was found to be 0.4553 ~ 0.46 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Atorvastatin calcium in tablet form has been developed. From the spectrum of Atorvastatin, it was found that the maximum absorbance was 272 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 5-25 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Atorvastatin in solid dosage forms. Hence, the method could be considered for the determination of Atorvastatin in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. MS - Mass Spectroscopy
6. LC - Liquid Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml - Microgram per milliliter
15. Avg - Average
16. Wt - Weight
17. g - gm
18. HMG-CO A - Hydroxymethylglutaryl- Coenzyme A
19. LDL - Low Density Lipoprotein
20. VLDL - Very Low Density Lipoprotein

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