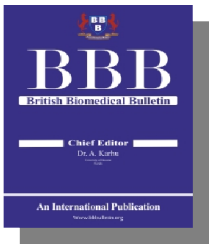
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN



Original

Validated UV-Visible Spectrophotometric Method for the Estimation of Atorvastatin in Pure and Pharmaceutical Dosage Form Using Methyl Orange Reagent

G. Manasa*, K. Vanitha Prakash and P. Vandana

Department of Pharmaceutical Sciences, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Hyderabad-500 075, Inida

A R T I C L E I N F O

Received 12 May 2014
Received in revised form 04 June 2014
Accepted 10 June 2014

Keywords:
Atorvastatin,
Methyl Orange,
UV-Visible spectrophotometry,
Molar absorptivity.

Corresponding author: Department of Pharmaceutical Sciences, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Hyderabad-500 075, India.
E-mail address:prakash.karnam@gmail.com

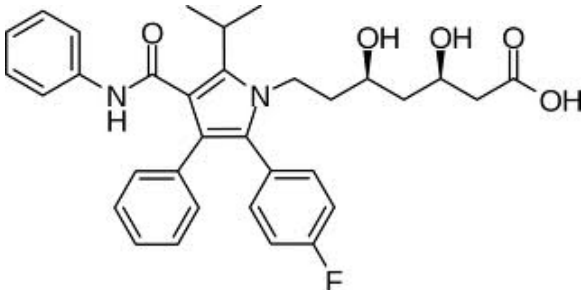
A B S T R A C T

A simple UV-Visible spectrophotometric method has been developed for the determination of Atorvastatin in its pure form as well as pharmaceutical dosage form using Methyl Orange reagent. The method is based on the measurement of absorbance of Atorvastatin in methanol at 410 nm. The Beer's law is obeyed over the linear range 50-300µg /ml of Atorvastatin. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the method was tested by analyzing the drug in its pharmaceutical preparations. Good recoveries were also obtained. Assay for the tablet preparation was performed using UV-Visible spectrophotometric method and the results were found to be within acceptable limits.

Introduction

Atorvastatin (3R, 5R) -7-[2-(4-fluorophenyl) -3-phenyl-4-(phenylcarbamoyl) -5-(propan-2-yl) -1H-pyrrol-1-yl] -3,5-dihydroxyheptanoic acid belongs to the group of stations which acts by reducing 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[1,2].

Obesity has become one of the life threatening disease in both developed as well as in some parts of developing countries of the world for which proper treatment has to be taken to prevent the risk of some of the life threatening diseases like atherosclerosis, etc. Thus, there is a need to develop a more simple rapid accurate precise method to estimate Atorvastatin (An anticholesteremic drug) in bulk and pharmaceutical dosage forms.



A survey of literature reveals[3-10] that the reported methods for Atorvastatin calcium include Spectrophotometry, HPLC, first derivative method, degradation monitoring method, but no spectrophotometric method was reported for Atorvastatin using Methyl Orange. Hence, a simple UV-Visible spectrophotometric method using methyl orange reagent has been developed for the assay of Atorvastatin in its bulk form as well as pharmaceutical dosage form (tablet).

Materials and Methods

Materials

Elico double beam UV-Visible spectrophotometer SL-164 with 1cm matched quartz cells was used for the electronic spectral measurements. Atorvastatin and all the other chemicals used were analytical reagent grade (AR grade). Atorfit*-10 (10 mg Atorvastatin) were manufactured by Ajanta Pharma Limited, Mumbai and purchased.

Method

Identification and Assay by Extractive spectrophotometric method.

Preparation of Standard Stock Solution

An accurately weighed quantity of 100 mg Atorvastatin was transferred into 100 ml volumetric flask with methanol. The volume was made up to mark with methanol. Aliquots of this standard stock solution were transferred to 10 ml volumetric flask (in different concentrations) and to this 2 ml Methyl Orange (in 0.1 M HCl) and 2 ml of Acetate buffer was added. Then the solutions are made up to the mark with Chloroform and kept aside for a few minutes for the formation of yellow complex and scanned over the visible range of 400-800 nm. The Absorption spectrum of different concentrations of Atorvastatin was plotted and the wavelength 410 nm was selected for analysis at which drug showed maximum absorbance **(Table 1, Fig 1).**

Procedure

For calibration curve (study of Beer's-Lambert's law)

From standard stock solution 0.5 ml to 3 ml were pipetted out and transferred to 10 ml standard flasks and then 2 ml of Acetate buffer (pH 3), 2 ml of Methyl orange in 0.1 M HCl was added to each

flask and then the volume is made up with chloroform and kept as such for a few minutes to form a yellow complex and scanned at 410 nm **(Table 2, Fig 2).** Absorbance was plotted against the concentration and the calibration graph was recorded.

Estimation of Atorvastatin in tablet formulation sample

Ten tablets were weighed accurately and powdered. Powder equivalent to 10 mg (label claim -10 mg) was taken and transferred to 100 ml volumetric flask and dissolved in methanol and diluted further to get final concentration 100 µg/ml of Atorvastatin. From the above solution 1.5 ml to 2.5 ml were pipetted out and transferred to a 10 ml standard flask and then 2ml Methyl orange in 0.1M HCl, 2 ml acetate buffer was added to each flask and the volume is made up with chloroform and kept as such for a few minutes to form yellow complex and scanned at 410 nm.

Method validation

Validation is a process of establishing documented evidence, which provide a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics[11]. The validation for UV-Visible method development was performed using parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of detection (LOD), Limit of quantification (LOQ) and Sensitivity.

Linearity

Various aliquots were prepared from standard stock solution ranging from 50-300 µg/ml. The samples were scanned in UV-Visible spectrophotometer at a wavelength of 410nm against the reagent blank. It was found that the selected drug shows linearity.

Accuracy

Accuracy is the closeness of agreement between the value, which are accepted either as a conventional true value or an accepted reference value and the value found. Accuracy is represented and determined using recovery studies by spiking standard drug solutions of Atorvastatin to pre analyzed samples **(Table 3).**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of a homogenous sample under the prescribed conditions.

The Precision of the method was evaluated by carrying out the six independent test samples of Atorvastatin. The percent relative standard deviation (%RSD) obtained was found to be good **(Table 4).**

Robustness and ruggedness

The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters”.

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions.

Robustness of the method were determined by carrying out the analysis under different temperature conditions and the Ruggedness was performed by change in parameter such as change in analyst. The results of Robustness and Ruggedness were found to be within limits **(Table 5).**

Limit of Detection

The LOD for any analytical procedure, the point at which analysis is just

feasible. The Detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantification as an exact value **(Table 5).** It was calculated using the formula involving standard deviation of response and the slope of the calibration curve.

$$LOD = \frac{3\sigma}{m}$$

Limit of Quantification

The limit of Quantification or concentration at which quantitative results can be reported with a high degree of confidence. It was calculated using the formula involving standard deviation of response and the slope of the calibration curve **(Table 5).**

$$LOQ = \frac{10\sigma}{m}$$

Sensitivity

Molar absorptivity and Sandell's sensitivity of the proposed method were found to within the limit **(Table 5)** which shows the high sensitivity of developing methods.

Results and Discussions

See table 1 to 5 and figure 1&2.

Conclusion

A Simple, rapid, sensitive and selective spectrophotometric method have been proposed for the analysis of Atorvastatin in pure form and in tablets. The proposed method utilized Methyl orange as Chromogenic reagent for the determination of Atorvastatin based on the formation of the ion-pair complex with the drug. From the Sandell's sensitivity and LOD it is clear that the method developed is sensitive. Moreover the proposed method is inexpensive and chemicals are easily available. Hence, the proposed method can readily be adopted by pharmaceutical quality control laboratory for routine analysis.

Acknowledgement

We are very thankful for SSJ College of Pharmacy, Vattinagulapally, Gandipet, Hyderabad for gratis sample of Atorvastatin and also for providing sophisticated equipment and other facilities to complete this work successfully.

References

1. The Merck Index, XIII edition, 2001, Merck Research Laboratories.
2. Sean C Sweetman, Martindale-The Complete Drug Reference, 34th edition.
3. Kailash P Prajapati*, A. Bhandari, *Indo Global Journal of Pharmaceutical Sciences*, 2011; 1(4): 294-299, ISSN 2249 – 1023.
4. Onkar S. Havelel, Shweta S. Havelel 2*, *International Journal of Pharmacy and Pharmaceutical Science Research* 2011; 1(2): 75-79, ISSN: 2249 – 0337.
5. K. Rajesh[1*], R.Rajalakshmi[1], S. Vijayaraj[1] and T. Sreelakshmi[1], *Asian Journal of Research in Chemistry*, Volume 04, Issue 08, August, 2011, ISSN 0974-4169.
6. S. Sharma and M. C. Sharma, *American-Eurasian Journal of Toxicological Sciences* 3 (2): 105-110, 2011, ISSN 2079-2050.
7. V.Juyal1, M. Chaudary1, P. Kumar1*, G. Gnanarajan, P. K. Yadav, *Journal of Pharmacy Research*, Vol.1. Issue 2. Oct-December 2008.
8. T. Deepanet.al., *European Journal of Applied Sciences* 3 (2): 35-39, 2011. ISSN 2079-2077.
9. Surendra Singh Inda*, Sanjay Sharma, Vishnu das, Bharat Kumar, Anil Bhandari, *International Journal of Pharmacy and Pharmaceutical Sciences* ISSN- 0975-1491 Vol 5, Issue 3, 2013.
10. Nalini Kanta School*, Madhusmita Sahu2, P. Praneeth3, P. S. K. Manjeera4, K. Lavanya5, *Journal of Pharmaceutical and*

Biomedical Sciences 2013 August; 33(33): 1551-1557.
11. ICH, Q2 (R1) validation of analytical procedures; text and methodology. *International Conference on Harmonisation*; Nov,1996.

Table 1. Absorption spectrum

Wavelength (λ) nm	Absorbance
400	0.3015
402	0.3910
404	0.4612
405	0.5142
407	0.5618
410	0.6368
412	0.5932
414	0.5103
416	0.3945
418	0.2423
420	0.1027

Table 2. Calibration curve for atorvastatin

Concentration (µg/ml)	Absorbance
50	0.1562
100	0.2535
150	0.4532
200	0.5726
250	0.7152
300	0.8674

Table 3. Accuracy

Tablet formulation	Labelled amount (mg)	Amount obtained (mg)* by a proposed method	**% recovery by the proposed method
1	10	9.65	96.5
2	10	9.89	98.9
3	10	9.85	98.5

*Average of three determinations. ** After spiking the sample.

Table 4. Precision

Concentration (µg/ml)	Absorbance	Statistical analysis
150	0.452	Mean = 0.452 Standard deviation = 0.0008 %RSD = 0.177
150	0.453	
150	0.451	
150	0.453	
150	0.452	
150	0.453	

Table 5. Optimal characteristics of atorvastatin

Parameter	Method proposed
λ_{max}	410 nm
Beers law limit	50-300 µg/ml
Molar absorptivity ($L.mol^{-1}cm^{-1}$)	1.416×10^3
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.394
Regression equation (y) slope (m) intercept (c)	0.24 0.078
Correlation coefficient	0.946
Precision (% relative standard deviation)	0.177
Standard error of estimate	0.013
Limit of detection (µg/ml)	0.01
Limit of quantification (µg/ml)	0.03

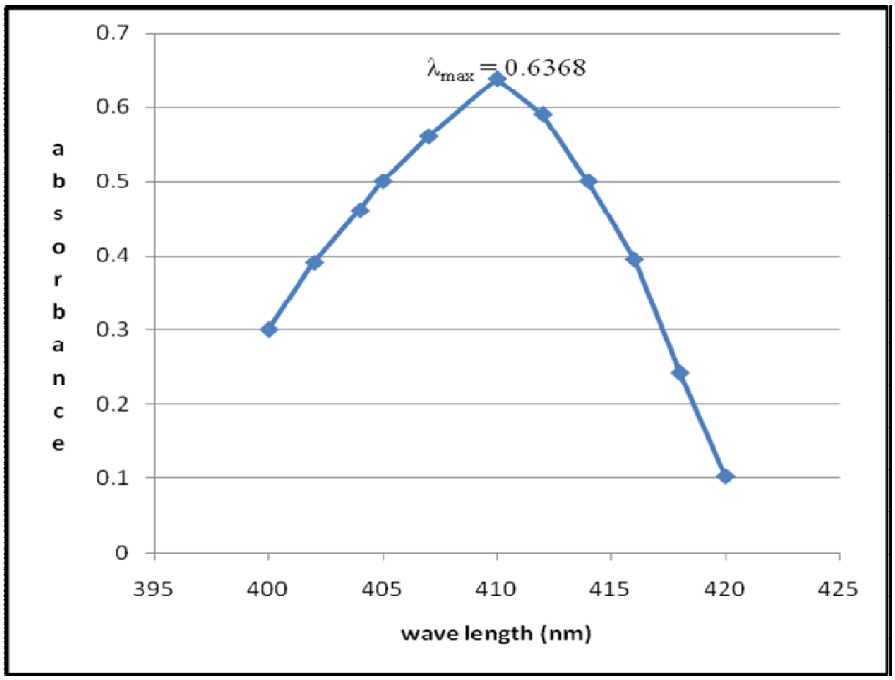


Figure 1. Absorption spectrum

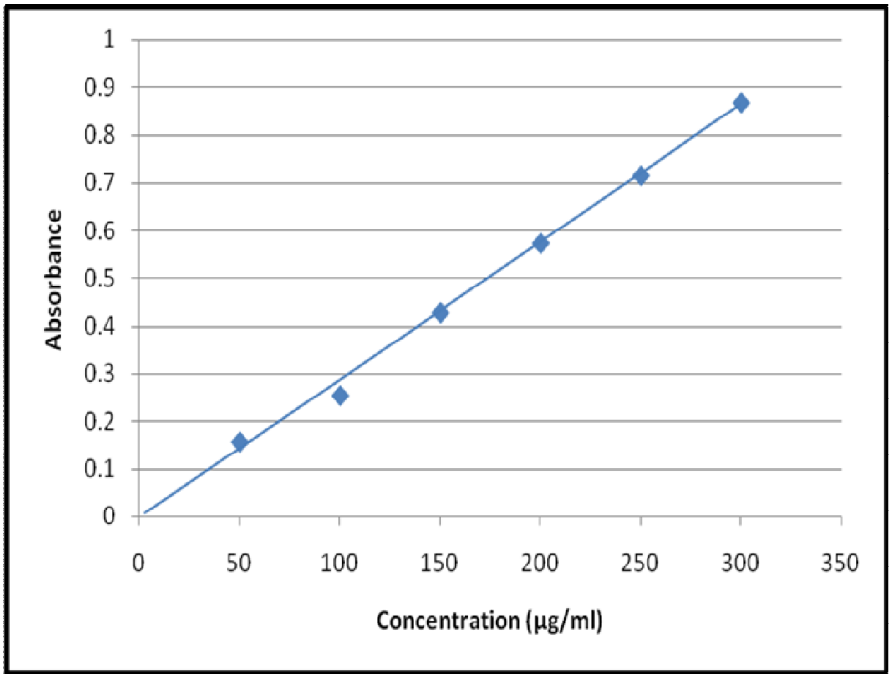


Figure 2. Calibration curve