Bioanalytical Method Development and Validation of Atorvastatin in Human Plasma by Using UV -Visibile Spectrophotometry

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Abstract

A novel, safe, rapid and simple Ultra violet spectroscopy (UV) method development and validation at (241 nm) for estimation of Atorvastatin from spiked human plasma. The analyte was extracted with HPLC grade Methanol. A rapid, economical and simple extraction method which was based on liquid–liquid extraction (LLE) for sample preparation and UV detection for quantification of Atorvastatin from spiked human plasma. All the parameters of the analysis were chosen according to ICH [Q2 (R1)] guideline and validated statistically.

Keywords: Atorvastatin, UV Spectrophotometric, Method development; Validation Parameters; ICH [Q2 (R1)], Liquid liquid extraction.

INTRODUCTION

Spectroscopy methods

It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples [9].

Ultraviolet-Visible spectrophotometry

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law.

Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law [10].

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer- Lambert law is expressed as

$A = abc$

Where,

A = absorbance or optical density
a = absorptive or extinction coefficient
b = path length of radiation through sample (cm)
c = concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c. When c is in gm/10 0 ml, then the constant is called Absorbance (1%, 1 cm).

$A = A *1\%/1 \text{ cm}* bc$

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength.

Introduction to Atorvastatin

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- methyl glut aryl coenzyme A (HMGCoA) reductase, the rate limiting enzyme in the biosynthesis of cholesterol [1,2].

Appearance is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, soluble in methanol, slightly soluble in methylene chloride[5].
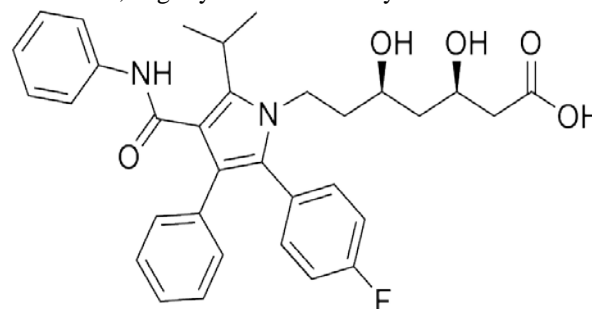

Figure: 1 Structure of Atorvastatin

Mechanism of action:

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase[3]. As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, these results in a subsequent decrease in hepatic cholesterol

levels. Decreased hepatic cholesterol levels stimulates up regulation of hepatic LDL-C receptors which increases hepatic uptake of LDL-C and reduces serum LDL-C concentrations. Atorvastatin, a selective, competitive HMG-CoA reductase inhibitor, is used to lower serum total and LDL cholesterol, apoenzyme B and triglyceride levels while increasing HDL cholesterol. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease and high ratios are associated with higher risk of disease. Increased levels of HDL-C are associated with lower cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, Atorvastatin reduces the risk of cardiovascular morbidity and mortality. Atorvastatin has a unique structure, long half-life and hepatic selectivity, explaining its greater LDL-lowering potency compared to other HMG- CoA[5].

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 plasma and in bulk forms[2].

AIM OF THE WORK
The aim of our work was development of new, stability indicating UV method for determination of Atorvastatin which possess the following advantages when compared to the already existing HPLC methods: simple, cost-effective, using less toxic and more UV-transparent mobile phase composition. The main target for our new developed method is to describe the quantification of Atorvastatin in biological fluids. Thus, it was decided to develop a rapid, economical and simple method which was based on liquid–liquid extraction (LLE) for sample preparation and UV detection for quantification of Atorvastatin from spiked human plasma[6].

EXPERIMENT
Material and Method
Spectrophotometric measurements were made in (ELICO) Double beam SL 210 UV-Visible spectrometer with 0.5 cm quartz cells. Drug Atorvastatin was kindly supplied as a gift sample from laboratory. Blood sample were collected from six sources and the plasma is separated and stored in refrigerator. Other chemicals used for extraction are HPLC grade methanol and ACN, LR grade Ammonium acetate, LR grade Magnesium chloride. Separation of the drug from the plasma was done using centrifuge (REMI R-8C).

Solubility
Solubility of drug 50mg 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.

Selection of wavelength
Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank .Atorvastatin shows λmax at 243. The proposed analytical method is simple, accurate and reproducible (Figure 1).

Preparation of standard drug solution
10 mg of standard drug Atorvastatin was accurately weighed separately and dissolved in 5 ml diluent (methanol), then transferred into a 10 ml volumetric flask, sonicated it for 5 min, finally, volume was made up to the mark with the same solvent to make 1000 µg/ml stock solution. From this 0.1 ml was again diluted to 10 ml to get a concentration of 10 µg/ml solution. It was scanned in UV range [200-400 nm] in 1.0 cm cell against solvent blank. The overlain spectrum of drug was recorded. After the study of spectrum of drug the λmax of the drug Atorvastatin was found to be 243 nm and the absorbance was found to be 0.731.

Extraction
To 3 ml of plasma add 2 ml of 10 ppm of Atorvastatin. It was extracted from plasma sample using liquid-liquid extraction technique. 0.5 ml of ACN was added to plasma sample for protein precipitation. The samples were then vortex mixed for 1min.Then 0.3g of magnesium chloride was added for extraction of Atorvastatin, then vortex-mixed for 2min and centrifuged at 4100 rpm, for 10 min at room temperature. Allow for the phase separation. The clear supernatant layer was separated in a glass test tube and evaporated to complete dryness under the gentle stream of nitrogen at 40°C. After drying, take 1 ml of the extract and measure the absorbance using UV spectrophotometer at 243 nm. The absorbance obtained is 0.731.
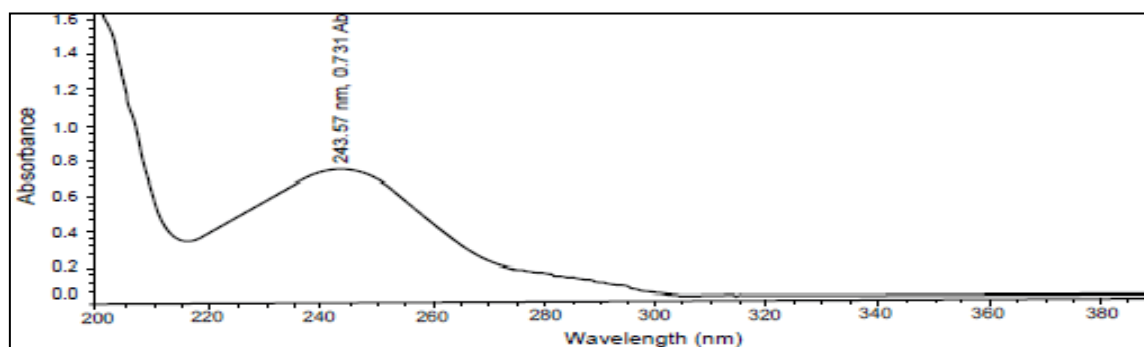

Figure: 2 UV spectrum of Atorvastatin

Table: 1 Evaluation data of accuracy study

Accuracy level	Sample concentration (ppm)	Standard Concentration (ppm)	Drug concentration (ppm)	Amount found (ppm)	%recovery
80%	4	4	8	4.0025	100.6%
100%	4	6	10	6.3021	100.5%
120%	4	8	12	7.9025	98.78%

Method validation

The proposed method was validated for the parameters like linearity, accuracy and robustness as per ICH guideline[7]. Validation is concerned with assuring that a measurement process produces valid measurements. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice[7].

Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments. Solution containing known concentration of atorvastatin was used for this purpose. From the absorbance at selected wave length potency was calculated. The accuracy was assessed from the test results as the percentage of the drug recovered by the assay at 3 levels.

Linearity

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. Linearity of the method was determined by constructing calibration curves by taking . Standard solutions of atorvastatin of different concentrations level (2-16 mcg/ml) were used for this purpose. Each measurement was carried out in six replicates and the absorbance were plotted against the concentrations to obtain the calibration curves and correlation coefficients. Characteristic parameters for regression equation $(y = mx + c)$ of the method were obtained by least squares treatment of the results and these parameters were used to confirm the good linearity of the method .

Robustness

To determine the robustness at +1 nm and -1nm from the fixed wave length. Percent recovery was calculated for both the drug. Analytical methods are generally known as robust if percent recovery is within 98-102%.
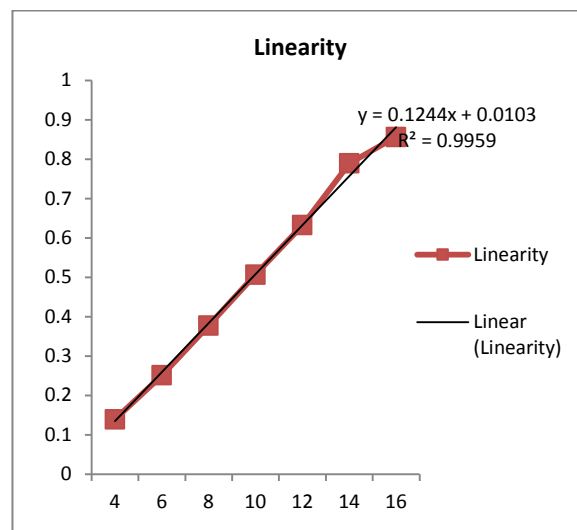

$y = 0.1244x + 0.0103$
$R^2 = 0.9959$

Figure: 3 Evaluations data of linearity

Table: 2 Linearity of Atorvastatin

Concentration in ppm	Absorbance
4	0.1395
6	0.2514
8	0.3778
10	0.5068
12	0.6325
14	0.7895
16	0.8565

Table: 3 Evaluation data for Robustness study.

Sno	242 nm	243 nm	244 nm
1.	0.6492	0.6502	0.6482
2.	0.6397	0.6407	0.6297
3.	0.6392	0.6402	0.6382
Standard deviation	0.0044	0.0045	0.0054
%RSD	0.6846	0.71151	0.8454

CONCLUSION

From this validation study we can conclude that the developed UV method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, accuracy and robustness . The method is versatile for determination of Atorvastatin in blood plasma. Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method. So this method can be used in the quality control department for determination of Atorvastain in human blood plasma.

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