DIFFERENT ANALYTICAL TECHNIQUES FOR QUANTITATIVE ANALYSIS OF ATORVASTATIN CALCIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
Atorvastatin is a member of the drug class known as statins, used for lowering blood cholesterol. Primary uses of atorvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease. Since many years, varied analytical methods were developed in dosage forms and in order to estimate their desired pharmacological action. A few UV spectrometric methods have been reported to estimate the atorvastatin in bulk formulations. Ultimately, different Fluorimetry, visible spectrometric and HPLC methods explained the importance of the methods while estimation atorvastatin in various stability indicating studies as well as in routine drug analysis. This review provides clear and short notes on the various analytical techniques employed for determination of atorvastatin in both bulk and pharmaceutical dosage form.

KEYWORDS: Atorvastatin, dyslipidemia, Fluorimetry, visible spectrometry and HPLC.

INTRODUCTION
Atorvastatin Calcium is chemically described as \( [R-(R^*, R^*)]-2-(4-fluorophenyl)-\beta, \delta \) dihydroxy-5-(1-Methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt trihydrate is An anti hyperlipoproteinemic agent act by inhibiting hmg - co reductase. Many analytical Methods like UV spectroscopy, HPLC were reported For determination of Atorvastatin Calcium alone and combination with other antihypertensive
In the analysis of Atorvastatin, major problem is solubilization of Atorvastatin in most of solvents during analysis. Quantitative estimation of poorly Water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water soluble drugs like Atorvastatin Calcium in tablet dosage forms. Highly sensitive and economic colorimetric method is not Reported for determination of Atorvastatin. Hence in this communication we have reported new UV Spectrophotometric, Fluorimetry, visible spectrometric and HPLC methods.

**ANALYTICAL METHODOLIGIES**

M Lakshmi Surekha et al., developed A simple, precise, rapid, and reproducible RP-hplc method for determination of Atorvastatin in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a Lichrospher C18 (BDS) column (250 X 4.6 mm i.d., particle size 5µm). The mobile phase consisted of Potassium Dihydrogen phosphate buffer ph-3.2 and acetonitrile in the ratio of 50:50 v/v, an isocratic elution at a flow rate of 1ML/min at ambient temperature. The detection was carried out at 246nm using Waters (2487) UV-Visible detector. The retention time of Atorvastatin is found to be 11.93min. The validated method utilized a Shimadzu HPLC system containing LC – 10 A Tvp solvent deliver module and waters 2487 UV-Visible detector.

**Stock and working standard solutions**

Stock standard solution of 1000mg/ ml of AVT was prepared freshly by accurately weighing 25mg of AVT into 25ml volumetric flask. Dissolved and made up to the volume with dihydrogen phosphate buffer (pH-3.2). The solution was further diluted with mobile phase in 10ml volumetric flask to obtain six working standards in the concentration range 100-260 mg/ ml of AVT. Chromatogram was recorded thrice for each individual dilution. All the solutions were prepared in triplicates.

**Assay of sample preparation**

Twenty commercial tablets (labeled concentration 40 mg of AVT) were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25 mg of AVT was quantitatively transfer into a 25 ml volumetric flask with about 20 ml of phosphate buffer pH 3.2. The solution was sonicated for 10 min, brought to the volume with phosphate buffer, mixed well and 1 ml of filtered test solution was transferred into 25 ml volumetric flask and
made up to the volume with mobile phase (40 mg/ml). 1.5ml aliquot solution was transferred into a 10 ml volumetric flask. The theoretical AVT concentration after dilution was 6 mg/ml (100% of AVT). An aliquot of this solution was filtered through a 13mm membrane syringe filter (Pore size 0.2 mm) prior to the injection into the HPLC system. Peak area of AVT was measured for the determinations.

Simionato LD et al., developed a Reversed-Phase Liquid Chromatographic (RP-LC) assay method for the quantitative determination of atorvastatin calcium in the presence of its degradation products. The assay involved an isocratic elution of atorvastatin calcium in a LiChroCARTR 250*4 mm HPLC Cartridge LiChrospherR 100 RP-18 (5 μm) column using a mobile phase consisting of 0.1% acetic acid solution: acetonitrile (45:55, v/v), pH = 3.8. The flow rate was 0.8 mL/min and the analytes monitored at 246 nm. The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (Irvine, CA, United States, Model ISO Chrom. LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Loveland, CO, United States, Series 3395) and a Rheodyne injector (Model 7125).

Preparation of standard solution
An accurately weighed quantity of 25 mg of atorvastatin calcium was placed into a 100 mL volumetric flask, dissolved in 5 mL of methanol and taken to volume with mobile phase. Then, 4 mL were withdrawn in a 100 mL volumetric flask. The volume was made with mobile phase (Conc 20 μg/mL). The solutions were passed through a 0.45 μm nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota, USA).

Sample preparation
Approximately 25 mg of atorvastatin calcium raw material was placed into a 100 mL volumetric flask, dissolved in 5 mL of methanol and taken to volume with mobile phase. Then, 4 mL were withdrawn in a 100 mL volumetric flask. The volume was made with mobile phase (Conc 20 μg/mL). The solutions were passed through a 0.45 μm nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota, USA).

Preparation of Assay Sample
Twenty tablets were weighed and finely powered and an accurately weighed powder sample equivalent to one tablet was transferred to a 50 ml volumetric flask; 10 ml of methanol was
added and the flask was kept in an ultrasonic bath during 5 min. The mixture was then diluted to 50 ml with mobile phase. 1 mL was withdraw in a 10 mL volumetric flask and diluted to volume with mobile phase. The solutions were passed through a 0.45 μm nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota USA).

Kailash P Prajapati et al., developed a simple and rapid UV spectroscopic method for estimation of Atorvastatin calcium in tablet dosage form. During development of analytical method water, phosphate buffer, methanol were tried but drug was found to be soluble in methanol. Standard stock solution was prepared in methanol. Amax was found to be 246nm. UV-Visible double beam spectrophotometer (Jasco V-530).

Preparation of standard stock solution
50 mg of pure Atorvastatin calcium was accurately weighed and transferred to 50ml of volumetric flask. Drug was dissolved in methanol and volume was made up to 50ml. The concentration of drug was 1mg/ml. 2.5ml of this solution was taken in a 25ml volumetric flask and volume was made up to the mark with methanol. Thus Atorvastatin calcium of strength 100 μg /ml was obtained.

Procedure of Atorvastatin calcium tablet
20 Tablets were procured from local market and average weight was determined. The powder equivalent to 50mg of Atorvastatin calcium was weighed accurately and dissolved in 50ml of methanol, shaken for ten minutes and filtered. 2.5ml of this solution was taken in a 25ml volumetric flask and volume was made up to the mark with methanol. Thus Atorvastatin calcium of strength 100 μg /ml was obtained. The solution was diluted in 10 ml volumetric flask with methanol to get a solution of 5,10,15,20,25 μg/ml. Absorbance was measured at 246nm against reagent blank.

Shyni Bernard et al., developed a simple and sensitive visible spectrophotometric method for the determination of Atorvastatin (ATV) in pure form and in tablets using Sulfo-Phospho-Vanillin reagent. The methods is based on the reaction of atorvastatin with sulphuric acid to form carbonium ion, which subsequently react with vanillin phosphate ester and measuring the resulting purple coloured complex at 414 nm. Spectrophotometric analysis was carried out by using Jasco.V 550 UV-visible Spectrophotometer (Jasco Ltd, Japan) with 1cm matched quartz cells.
Preparation of Phospho-Vanillin Reagent
Dissolved 0.6 g of vanillin with 100 ml water in a 100 ml volumetric flask and makeup the volume with water (vanillin reagent). Mixed 35ml of vanillin reagent and 60 ml of concentrated phosphoric acid, with constant stirring add 5.0ml of water and stored in a brown bottle at room temperature.

Preparations of Atorvastatin Standard Solutions
50 mg of pure ATV was dissolved in 50 ml of methanol and stirred for 15 minutes and the final volume was made up to 50 ml with methanol to prepare working concentrations of 1 mg/ml of ATV.

Development of Atorvastatin-Sulfo-Phospho-Vanillin coloured complex
To 1.5 ml of Atorvastatin standard solution, taken in a boiling tube, 2.0 ml of concentrated sulphuric acid was added, mixed the content well, added 5.0 ml of Sulfo- Phospho-Vanillin reagent, placed in boiling water bath for 10min., cooled and transferred to a 25 ml volumetric flask washed the test tube with small volume of methanol and transferred to the flask. The contents were mixed properly and the volume was made up to 25 ml with methanol. The resulting solution had a concentration of 60μg/ml.

Surendra singh inda et al., developed a simple, economic, sensitive, precise and accurate first derivative spectrophotometric method for determination of Atorvastatin Calcium as bulk and in tablet dosage form. The quantitative determination of the drug was carried out using the first derivative values measured at 245.8 nm. A double beam UV-VIS Spectrophotometer (UV CE7400, Cecil, UK) Spectral bandwidth of 1 nm and wavelength accuracy of ±0.5 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (XB120A, Precisa, Switzerland).

Preparation of Standard Stock Solution
Accurately weighed Atorvastatin Calcium (10.0 mg) was transferred to 100 ml volumetric flask, dissolved in about 50 ml of methanol and volume was up-to 100 ml with methanol to obtain stock solution of drug concentration of 100 μg/ml.

Estimation of Atorvastatin Calcium in Tablet dosage form
The powder of 20 Atorvastatin Calcium tablets (label claim 10 mg) of the same batch Were triturated and mixed properly. 531 Accurately weighed 91.1 mg powder (equivalent to 10 mg
of Atorvastatin Calcium) was transferred in 100 ml volumetric flask containing small quantity of reference solvent (methanol). Ultrasonic water bath was used for 20 minutes to complete dissolution. The solution were diluted to volume and filtered through Whatman filter paper no. 40. Further suitable dilutions were made to obtain six replicates of 10 μg/ml solutions. These solutions were analyzed and percent recovery of Atorvastatin Calcium tablet was determined.

CONCLUSION
Many analytical techniques have been developed for the estimation of atorvastatin in dosage form, in bulk form. The techniques include high performance chromatography, UV-Visible spectrometry. Hplc is found to be very effective and sophisticated method for the analysis of the drugs in dosage forms among all the methods. For the analysis of drugs HPLC and UV method is proven to be highly sensitive and accurate.

REFERENCES
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