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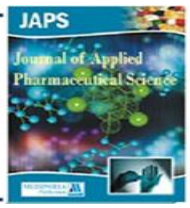

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Spectrophotometric method of estimation of atorvastatin calcium using sulfo-phospho-vanillin reaction

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ABSTRACT

A simple and sensitive visible spectrophotometric method has been developed 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. Under the proposed optimum condition, Beer's law was obeyed at the concentration range of 30-100µg/ml. The result of analysis of tablet was found to be 99.81 %. The good results of recovery studies showed that the co-formulated substances did not interfere with the determination. The method was validated according to ICH guidelines by performing linearity, accuracy, and precision, limits of quantification, limit of detection and selectivity.

Keywords: Atorvastatin, Spectrophotometry ,Tablets,Vanillin.

INTRODUCTION

Lowering the concentration of low-density lipoprotein (LDL) cholesterol and raising high-density lipoprotein (HDL) cholesterol slows the progression of atherosclerosis. The Statins are the drugs of first choice for treating hyper cholesterolaemia. Statins should be considered for all patients, including the elderly, with coronary heart disease and occlusive arterial disease (British National Formulary., 2002). Atorvastatin calcium chemically [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is an inhibitor of HMG –CoA reductase, an enzyme involved in cholesterol biosynthesis (Clarke's Analysis of Drugs and Poisons in Pharmaceuticals.,2004). The chemical structure of Atorvastatin calcium is shown in Figure. 1. The drug has been demonstrated to be efficacious in reducing both cholesterol and triglycerides (Poswar et al., 1996 and Curtis *et al.*, 2002).The effectiveness of atorvastatin in lowering cholesterol is dose-related. Atorvastatin calcium is not official in Indian Pharmacopoeia, British Pharmacopoeia, United States and European Pharmacopoeia.

Literature survey revealed that very few sophisticated analytical methods such as HPLC (Atluntas *et al.,* 2004 and Gowri *et al.,* 2005), GC-MS (McKenney et al., 1998), LC-MS (Black *et al*., 1998), HPLC-Electron spray tandem mass spectrometry (Bullen et al., 1999) and HPTLC (Yadav *et al.,* 2005) have been reported for the estimation of Atorvastatin calcium and its combinations from its formulations and biological fluid. The primary objective of the present investigation was to develop simple, precise and an accurate spectrophotometric method for the estimation of Atorvastatin calcium from its marketed formulations.

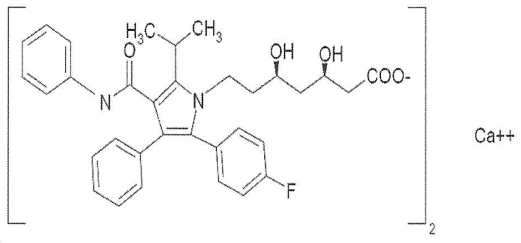

Fig. 1: Structure Of Atorvastatin Calcium.

MATERIALS AND METHODS

Instrument

Spectrophotometric analysis was carried out by using Jasco.V 550 UV-visible Spectrophotometer (Jasco Ltd, Japan) with 1cm matched quartz cells

Reagents and Chemicals

Reference standard of atorvastatin was generous gift from Intas Pharmacetical Industry,Dehradun (India). Vanillin and Phosphoric acid were analytical grade obtained from Himedia Laboratories Mumbai and Methanol A.R and Sulphuric acid was purchased from Merck,Mumbai.

Methods

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(Christopher *et al.,* 1972).

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 (Christopher *et al.,* 1972) 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.

Preparation of the Reagent Blank

The reagent blank was prepared in the same manner as discussed omitting the standard drug solution.

Study of the Spectral Characteristics

The background correction for the instrument (Jasco.V 550) was done from 350-700nm using a reagent blank. The purple coloured drug -reagent complex was then scanned through 350-700nm.The spectrum exhibited absorption maximum at 414nm.(Figure 3).

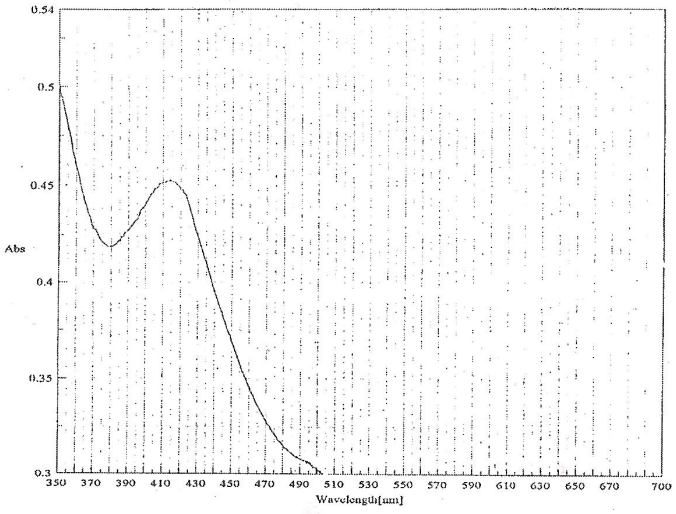

Fig. 3: spectrum of atorvastatin-reagent colour complex.

Beer's Law Plot for Drug-Reagent Complex

From the Atorvastatin standard solution 0.5, 0.75, 1.0, 1.25, 1.5,1.75, 2.0,2.25 and 2.5ml were pipetted out into nine boiling tubes and the drug reagent colour complex was prepared as disussed. The contents were mixed properly and the volume was made up to 25 ml with methanol. The absorbance of the solutions were measured at 414 nm against reagent blank. The Data obtained are given in Table.1 and is graphically represented in (Figure 2).

Table. 1: Data for Beer's Law plot.

S. No:	Volume of ATV stock solution(ml)	Concentration of ATV in final solution (µg/mL)	Absorbance at 414 nm
1.	0.50	20	0.056
2.	0.75	30	0.123
3.	1.0	40	0.224
4.	1.25	50	0.309
5.	1.5	60	0.412
6.	1.75	70	0.524
7.	2.0	80	0.620
8.	2.25	90	0.767
9.	2.0	100	0.879

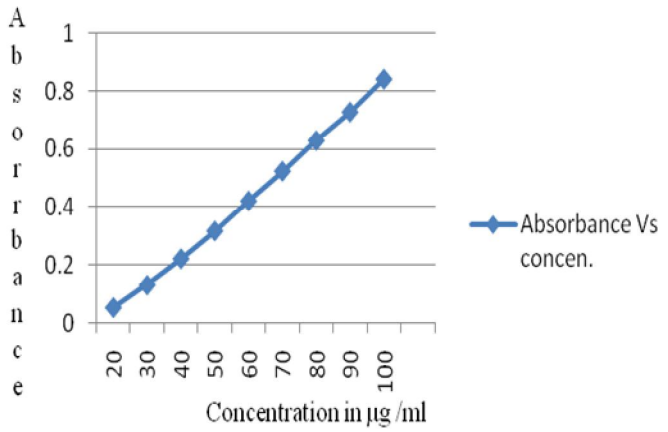

Fig. 2: beer's law plot.

Standardisation of volume of the Reagent

Sulfo-Phospho-Vanillin reagent was prepared and the drug-reagent complex was developed by adding different volumes of reagent and different volumes of sulphuric acid to varying concentrations of the Atorvastatin standard solution. To each tube containing drug volume of 0.75,1.0 and 1.25 ml, added 3.0ml , 5.0ml and 6.0 ml volumes of reagent. The procedure was repeated with 1.0ml and 2.0ml volumes of sulphuric acid. It can be seen that the system obeys Beer's law when the volume of the reagent was 5.0 ml and sulphuric acid volume was 2.0 ml. Hence 5.0 ml of Phospho-Vanillin reagent and 2.0 ml of concentrated sulphuric acid was taken as the optimum volumes for the method.

Stability Profile of Drug-Reagent Complex

The period over which the coloured complex gave a steady absorbance was investigated. Three different concentrations, ie. 30, 40 and 50 mcg/ml were used for the study. The drug-reagent complex was prepared and the absorbance of the solutions were measured at 414 nm at 15 minutes intervals for 1hr against reagent blank. The data obtained are given in Table-3.

Table. 3 : Data for stability profile of the drug-reagent complex.

S. No.	Concentration of Atorvastatin Calcium µg/mL	Absorbance at 414 nm At 15minutes time intervals				
		2min	15min	30min	45min	60 min
1	30	0.123	0.116	0.114	0.113	0.110
2	40	0.223	0.222	0.217	0.218	0.218
3	50	0.310	0.311	0.312	0.308	0.307

Procedure for analysis of tablet formulation

Twenty tablets of Lipitor 10mg (Pfizer) was purchased from local market, accurately weighed and finely powdered. The weight of tablet equivalent to 25 mg was accurately weighed out and extracted using methanol. The volume was finally made up to 25ml with methanol. The resulting solution had a concentration of 1mg/ml (solution A). Accurately pipetted out 1.0 and 1.25ml of solution A in to two boiling tubes, prepared the drug reagent complex as discussed and made up the volume with alcohol. The solutions had final concentration of 40µg/ml and 50 µg/ml respectively. The absorbance of each solution was measured at 414 nm using reagent blank and the amount of drug present in the sample solutions were obtained from the slope and intercept values obtained from the calibration curve (Table 1). The experiments were repeated three times to check its reproducibility. The results of analysis of tablet formulations were recorded in Table 4.

Validation of the proposed Method:

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte (ICH Q2B., 1994).

Linearity and sensitivity

Under optimum conditions, a linear relation was obtained between absorbance and concentration of ATV in the range 30-100µg/ml (Fig. 2). The calibration graph is described by the equation:

$$Y = a + bX$$

where Y = absorbance, a = intercept, b = slope and X == concentration, obtained by the method of least squares. The correlation coefficient, intercept and slope for the calibration data, sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and limit of quantification were summarized in Table 2. The limits of detection (LOD) and limit of quantification (LOQ) were calculated according to the ICH guidelines using the formulas:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s,$$

where 'σ' is the standard deviation of five reagent blank determinations and 's' is the slope of the calibration curve.

Table. 2: Optical Characteristics of coloured complex for the developed method.

Parameters	
Y= a+ bX	y=0.010385X- 0.18754444444
Molar absorptivity	1.0385 x 10^4.L/mol.cm.
Sandell's sensitivity	0.11237 (µg/cm^2/ 0.001/ absorbance unit)
Limit of detection (LOD), µg/ml	1.8
Limit of quantification (LOQ), µg/ml	5.6
Intercept (a)	-0.1875
Slope (b)	0.01038
Regression coefficient (r)	0.99865

*Limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm and l = 1cm.

Precision and accuracy

Intra-day precision and accuracy of the proposed method were evaluated by replicate analysis (n = 5) of calibration standards at three different concentration levels in the same day. Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (Table 5).

Table. 4: Data of analysis of tablets.

Concentration µg/ml	Absorbance * At 414 nm	% Label claim	Active content per tablet (mg)	Mean% Label claim	Standard deviation	Standard Error
40	0.221	99.80	9.980	99.81	0.001549	0.0006324
50	0.309	99.82	9.982		0.001673	0.0006831

*Mean of three determinations.

Table. 5: Evaluation of intra-day and inter-day accuracy and precision (RE: relative error; RSD: relative standard deviation).

ATV taken µg/ml	Intra-day accuracy and precision			Inter-day accuracy and precision		
	ATV found µg/ml	RE,%	RSD,%	ATV found µg/ml	RE,%	RSD,%
40	39.28	1.80	1.80	39.4	1.52	1.38
50	49.7	1.52	1.63	49.7	0.60	1.31
60	59.04	1.60	1.58	58.99	1.60	1.60

Recovery studies

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ATV at three concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed method. The added ATV recovery percentage values ranged between 106.80 and 111.8% with the standard deviation of 1.24-1.48% (Table 6) indicating that the recovery was good and the co-formulated substances did not interfere with the determination.

Table. 6: Data of Recovery studies.

ATV in tablet µg/mL	Pure ATV added (spiked)µg/mL	Total concentration found µg/mL	Pure ATV recovered* (Mean±S.D) (n=3)
39.9	5	45.49	111.80±1.3
39.9	10	50.81	109.10±1.48
39.9	15	55.92	106.8±1.24

*Average of three determinations

RESULTS AND DISCUSSION

The "sulfo-phospho-vanillin" reaction for the determination of total serum lipids was first introduced by Chabrol and Charonnat (Press med.,1937) It has been suggested that the reaction requires a carbon-carbon double bond (Frings *et al*.,1970). The reaction apparently is specific for unsaturated organic compounds. Major function of the sulfuric acid is to hydrolyze the lipid esters. Here, an unsaturated double bond accepts a proton from sulfuric acid (strong acid) to form a highly reactive carbonium ion. The ion is efficiently formed at 100^0C (lower temperatures lead to considerably less reaction),on cooling, the ion so formed is stable for at least several hours. Vanillin reagent is prepared in water, but when it is mixed with concentrated phosphoric acid reacts with the hydroxyl group of vanillin to produce an aromatic phosphate ester before use. The colour reaction takes place in three steps (Figure 4): In the first step the unsaturated compounds react with sulfuric acid to produce a carbonium ion, in second step vanillin reacts with phosphoric acid to produce an aromatic phosphate ester and in third step the carbonium ion reacts with the activated carbonyl group of phospho- vanillin to produce a charged colored complex that is stabilized by resonance and absorbs maximally at about 414 nm(Zoeliner *et al*.,1962). Alcohols with more than two carbon atoms also react and are readily dehydrated by concentrated sulfuric acid to unsaturated compounds which subsequently react as just described (Morrison *et al*., 1962). It is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. The mean percentage label claims of marketed tablets (Table 4) were very close to 100 with low values of standard deviation and standard error which confirms the accuracy of the proposed method. Accuracy and precision of the proposed method were further confirmed by the mean percentage recovery values (106 to 111), which were close to100 with low values of standard deviation (Table.6). The proposed method for the determination of ATV showed molar absorptivity of 1.0385 x 10^4.L/mol.cm. and Sandell's sensitivity of 0.11237 (µg/cm^2/ 0.001/ absorbance unit). Linear regression of absorbance on concentration gave the equation Y=0.010385X- 0.18754444444 with a correlation coefficient r = 0.99865 (Table 2).

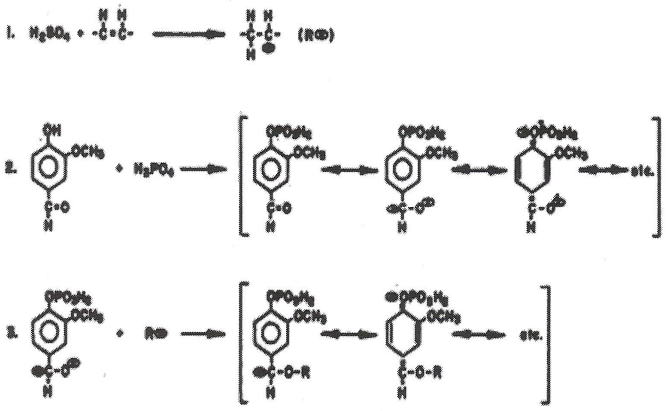

Fig. 4: proposed reaction sequence for sulfo-phospho-vanillin reaction.

CONCLUSION

A new visible spectrophotometric method is developed which is economic, simple, precise, sensitive and rapid and hence can be employed for the routine analysis for the estimation of atorvastatin from marketed formulations.

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