Open Access
Full Length Article

ORIGNAL ARTICLE

HPLC Method Development and Validation of Atorvastatin Calcium in Bulk and Tablet Dossage Form

Samina Alam[1], Saima Saleem[1],Safila Naveed[1], Huma Dilshad[1], Fatima Qamar[1], Tanweer Alam[2], Halima Sadia[1], Sidrah Khan[1],Mariam Karim[1], Amanullah Khan[3]

[1] Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan.
[2] Drug Regulatory Authority, Government of Pakistan
[3] Drug Testing laboratory ,Quetta, Health Department, Baluchistan

Keywords: *atorvastatin calcium, API and pharmaceutical formulation, method development, HPLC*

Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscripts

Article info.
Received: Mar 15, 2018
Accepted: Mar 26, 2018

Funding Source: *Nil*
Conflict of Interest: *Nil*
Cite this article: *Alam S, Saleem S, Naveed S, Dilshad H, Qamar F, Alam T, Sadia H,Khan S,Khan A.* HPLC method development and validation of atorvastatin calcium in bulk and tablet dossage form. *RADS J. pharm. pharm. sci. 2018;6(1):83-87.*

Address of Correspondence
Author: *drsaima1981@gmail.com*

ABSTRACT

Objective: The present work was aimed at the development of a simple, precise, rapid and selective RP-HPLC method for the estimation of Atorvastatin as API in both- bulk and pharmaceutical formulation.

Methods: The method was carried out on HPLC a C18 column (25 cm x 4.6 mm) with mobile phase consisting of methanol:water:acetonitrile: orthophosphoric acid (85:10:4, 1 v/v) and pH was adjusted to 3.2 with flow rate of 1.8 ml per min.Detection was carried out at 247 nm. Besides, all parameters were found to be under required limits including limits of detection and limits of quantification.Further,the method was developed and validated; linearity, accuracy, precision, ruggedness and robustness, through an efficient HPLC technique in accordance with the ICH validation guidelines.

Results: The results of all validated parameters - Linearity(r^2= 0.999), Accuracy (101.5%) ,Precision, Ruggedness, Robustness, LOD (0.0008µg/ml) and LOQ (0.0002µg/ml) were found to be within required limits.

Conclusion: As per above discussion the developed method is in accordance with ICH guidelines and can be easily used for Bulk and pharmaceutical formulation testing.

INTRODUCTION

Hyperlipidemia is the most common form of Dyslipidemia found in wide group of people all over the world[1].It is amongst the major risk factor for CHD than other prevalent conditions-hypertension and diabetes mellitus[2].The 3-hydroxy-3-methyl-glutaryl coenzymes are used for its management -popularly known as statins[3, 4]. Statins including Atorvastatin,as first line agents tend to lower serum low-density lipoprotein cholesterol (LDL-C) concentrations[5, 6]. The Atorvastatin is a synthetic HMG-CoA reductase inhibitor reducing triglycerides levels via inhibiting endogenous cholesterolsynthesis[7-10].Atorvastatin Tablets is administered orally at 10- 80 mg.The structural formula is given below[16].

In literature, various methods are available for determination of atorvastatin by using HPTLC method [11], capillary electrophoresis method

[12] RP-HPLC method and High Performance Liquid Chromatography (HPLC) method as well[13, 14] Simple UV Spectroscopic method have also been used for the evaluation [15]of atorvastatin in Human plasma and Urine but hardly for both bulk and pharmaceutical dosage forms.

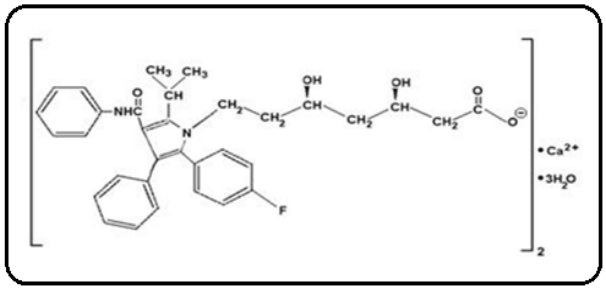

Figure 1: Structure of atorvastatin

Experimental

Atorvastatin calcium was obtained as a gift from Bosch pharmaceutical (Pvt) Ltd. All other reagents and solvents used were of analytical grade and purchased from Daejung reagent chemicals, Korea which included HPLC methanol , acetonitrile, orthophosphoric acid. The Instruments included weighing Balance Mettler Toledo AB-20 4S, Ultrasonic water Bath, Magnetic stirrer, Whatman filter and HPLC LC-20 with software version LC 2.0 and UV Visible detector. The column was specified as C18, 250 x 4.6mm, 5 micron(Shimadzu SPD-20 A, Japan). The flow rate was set at 1.8 ml per minute with 20microliters of injection volume.

RESULTS AND DISCUSSION

Optimization of HPLC Method

Method development were performed on HPLC-Model named Shimadzu-LC-20 AT.The model was equipped with UV–visible detector model SPD-10A(V).Morover,it was connected by CBM-102 communication from Bus Module Shimadzu to corei3 machine. LC-20 Version 1:62 was used for obtaining the required chromatograms. HPLC Column with defined dimensions of C18, 250 mm, 4.6 mm, i.e. 5 µm. Ambient temperature (25 ±2^{o}C) was maintained during the study. A mixture of methanol: water: acetonitrile: orthophosphoric acid (85:10:4, 1 v/v) were used as mobile phase and pH was adjusted to 3.2 using orthophosphoric acid. 1.8 ml per min flow rate was maintained and 20 µL was injection volume. Samples were prepared in methanol and all solutions such as mobile phase, test, standard and sample solutions were sonicated for 30 minutes by WUC-A02H on-line degasser which was filtered through 0.45-micronfilter before use. UV detection was performed at the wavelength 247 nm for atorvastatin calcium.

METHOD VALIDATION

System suitability

UV spectra of atorvastatin calcium standard in respective solvent of methanol are shown in figure, indicating maximum absorbance of the drug at 247 nm respectively.

Table 1: System suitability parameters

Formulation and standard	Ret. Time	Peak Area	Resolution	Theoretical Plates (N)
Standard	2.19	420656	1.655	4178.7
FT-01	2.19	426856	1.723	4670.9

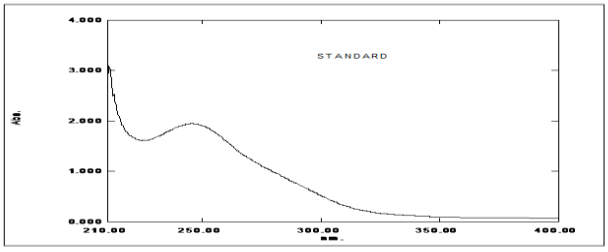

Figure 2: UV spectra in methanol for selection of suitable wavelength

Specificity and Linearity

The Linearity of the proposed process was assessed at various concentrations. For statistical analysis, Linear Correlation coefficient, intercept and slope values were calculated.

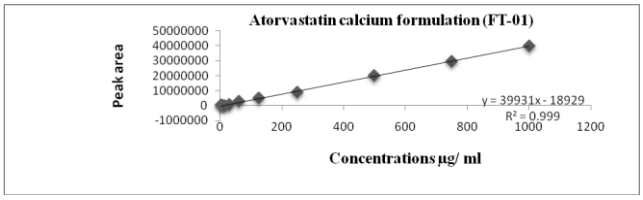

Figure 3 : HPLC calibration Curves for atorvastatin calcium formulations (FT-01)

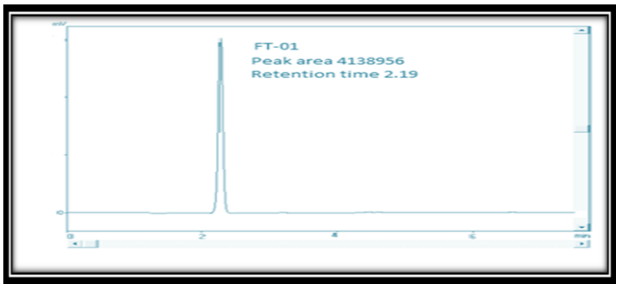

Figure 4: Representative HPLC chromatogram of FT-01

Accuracy and Precision

The proposed method was also checked for accuracy which was calculated at different concentration levels of 80%, 100% and 120% ,done through spiking known quantities of the atorvastatin calcium. The solutions were injected three times to HPLC system and percent recovery was calculated at every time. Whereas,for knowing the precision of the method, solutions were injected six times at each level to HPLC system on two different non successive days in each case and percent RSD and conc. found was calculated.

Table 2: % Recovery by HPLC

% Recovery	Standard	FT-01
80%	99.16	100.99
100%	100.0	101.07
120%	99.91	101.71

Table 3: Inter and Intraday Precision Evaluation of Atorvastatin

Conc.	%RSD Interday		%RSD Intraday	
	Atorvastatin calcium	FT-01	Atorvastatin calcium	FT -01
3.96	0.0027	0.0029	0.0026	0.0028
7.8	0.2141	0.0202	0.2141	0.0202
15.25	0.2174	0.001	0.2173	0.01
31.25	0.0825	0.0006	0.0825	0.0006
62.5	0.0003	0.002	0.0003	0.002
125	0.0001	0.0012	0.0001	0.0011
250	0.0001	0.0011	0.0001	0.0011
500	0.0002	0.0003	0.0001	0.0003
750	0.001	0.0002	0.0002	0.0002
1000	0.002	0.0012	0.0002	0.0012

Table 4: REGRESSION CORELATION, LOD AND LOD

DRUGS	Regression equations	R^2	Accuracy (%)	LOD (µg/ml)	LOQ (µg/ml)
Standard	y = 37560x – 36807	R^2= 0.998	100.0 %	0.00182	0.00055
FT-01	y = 39931x – 18929	R^2= 0.999	101.5%	0.0008	0.0002

Table 5: Robustness of the method

STANDARD				FT-01			
Parameters	**Level**	**Resolution**	**Theoretical plates(N)**	**Parameters**	**Level**	**Resolution**	**Theoretical plates (N)**
p H of mobile phase							
3.0	-0.2	1.654	4176.2	3.0	-0.2	1.720	4669.2
3.2	0	1.655	4178.7	3.2	0	1.723	4670.9
3.4	+0.2	1.656	4179.2	3.4	+0.2	1.726	4671.3
Flow rate (ml/min)							
1.6	-0.2	1.652	4176.3	1.6	-0.2	1.725	4669.3
1.8	0	1.653	4178.7	1.8	0	1.726	4671.2
2.0	+0.2	1.654	4175.2	2.0	+0.2	1.724	4672.3
Wavelength (nm)							
245	-0.2	1.652	4177.6	245	-0.2	1.722	4668.5
247	0	1.655	4178.7	247	0	1.723	4670.8
249	+0.2	1.658	4175.4	249	+0.2	1.720	4672.5

Limit of detection and quantification

LOD of our presented method was determined using method formula LOD = 3.3 SD/ slope. Similarly,The LOQ - the least level of the drug that is exactly measured and it was calculated by the formula as ten times the noise level LOQ =10 o/S, where o is used for the standard deviation of the least standard concentration and S is taken as the slope of the standard curve.

Robustness

Robustness was performed through making slight changes in the mobile phase-percentages of water, methanol and acetonitrile.The flow rate, wave length and pH were also subjected to minor variations.That's why five frequent samples were injected under little variations of

every parameter. Parameters were changed to +/- 0.2 % in flow rate whereas pH was adjusted with +/- 0.2 % and +/- 2% wave length from its optimum condition.

Ruggedness

Our proposed method was determined for ruggedness by performance of same procedure by two different operators on two consecutive days. The lab was Research, Department of Pharmaceutics, Faculty of Pharmacy, and Jinnah University for women Karachi. All parameters such as Peak area, Peak height, and theoretical plates of the column, Retention time and resolution were compared.

CONCLUSION

The research work led to the method development of the immediate release formulation of atorvastatin calciumwith two different super disintegrant, which will provide the drug release in 30 minutes to achieve the desired therapeutic outcomes. Thus, it is an attempt to design an effective and economical formulation for improved patient compliance. All the validation parameters designed for the drug system holds promise to further in-vivo studies and commercialization.

REFERENCES

1. *Laslett LJ, Alagona Jr P, Clark III BA, Drozda Jr JP, Saldivar F, Wilson SR, Poe C, Hart M. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. Journal of the American College of Cardiology. 2012 Dec 25;60(25):S1-49.*
2. *Assmann G, Schulte H. The Prospective Cardiovascular Münster (PROCAM) study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. American heart journal. 1988 Dec 1;116(6):1713-24.*
3. *Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001 May 11;292(5519):1160-4.*
4. *Athyros, V., et al., The effect of statins versus untreated dyslipidaemia on renal function in patients with coronary heart disease. A subgroup analysis of the Greek atorvastatin and coronary heart disease evaluation (GREACE) study. Journal of clinical pathology, 2004. 57(7): p. 728-734.*
5. *Kim, J.-S., et al., Physicochemical properties and oral bioavailability of amorphous atorvastatin hemi-calcium using spray-drying and SAS process. International Journal of Pharmaceutics, 2008. 359(1): p. 211-219.*
6. *Shah, D., et al., RP-HPLC determination of atorvastatin calcium and amlodipine besylate combination in tablets. Indian journal of pharmaceutical sciences, 2006. 68(6): p. 796.*
7. *Kim, M.-S., et al., Preparation, characterization and in vivo evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. European journal of pharmaceutics and biopharmaceutics, 2008. 69(2): p. 454-465.*
8. *Anwar, M., et al., Enhanced bioavailability of nano-sized chitosan–atorvastatin conjugate after oral administration to rats. European Journal of Pharmaceutical Sciences, 2011. 44(3): p. 241-249.*
9. *Mukharya, A., et al., Stable and Bio-equivalent Formulation of HMG-CoA reductase inhibitor: Atorvastatin Calcium. International Journal of Pharmaceutical Sciences Letters, 2011. 2(1): p. 12-20.*
10. *Sievens-Figueroa, L., et al., Preparation and characterization of hydroxypropyl methyl cellulose films containing stable BCS Class II drug nanoparticles for pharmaceutical applications. International journal of pharmaceutics, 2012. 423(2): p. 496-508.*
11. *Aiyalu, R. and K. Mani, HPTLC method development, validation, and stress degradation studies for atorvastatin and ezetimibe in multicomponent tablet dosage form. Medicinal Chemistry Research, 2012. 21(7): p. 1297-1301.*
12. *Attimarad, M., Capillary Electrophoresis Method Development for Simultaneous Determination of Atorvastatin and Ezetimibe from Solid Dosage Form. Journal of Young Pharmacists, 2017. 9(1): p. 120.*
13. *Ertürk, S., et al., An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. Journal of pharmaceutical and biomedical analysis, 2003. 33(5): p. 1017-1023.*
14. *Ashutosh Kumar, S., M. Debnath, and J. Seshagiri Rao, New Validated RP-HPLC Analytical Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in Bulk Samples as Well in Tablet Dosage Forms by Using PDA Detector. Current drug discovery technologies, 2014. 11(4): p. 259-270.*
15. *Naveed, S., Simple UV spectrophotometric assay of Atorvastatin API formulation and their comparative study. Global Journal of Medical Research, 2014.*
16. *Razavi B, Abdelmelek SB, Song W, O'Shea KE, Cooper WJ. Photochemical fate of atorvastatin (lipitor) in simulated natural waters. Water research. 2011 Jan 1;45(2):625-31.*