INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT AND VALIDATION OF UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND EZETIMIBE IN PHARMACEUTICAL FORMULATION

Sharma Amit Kumar*, Dharamsi Abhay
Department of Pharmaceutical Analysis, Tirumala College of Pharmacy,Tirumala Nagar, Bardipur(V), Dichpally(M), Nizamabad, India

Article Received on: 05/01/12 Revised on: 20/02/12 Approved for publication: 20/03/12

*E-mail: rohitrx@gmail.com

ABSTRACT
A simple, rapid and accurate UPLC method was developed and validated for estimation of atorvastatin and ezetimibe in combined dosage forms. With the objective of reducing analysis time and maintaining good efficiency in the area of fast chromatographic separations the UPLC has proven to be one of the most promising developments in the area of fast chromatographic separations. In this work a isocratic reverse phase chromatographic method was developed using UPLC for the estimation of atorvastatin and ezetimibe in pharmaceutical formulations. The chromatographic separation of atorvastatin and ezetimibe was achieved as waters acquity BEH C_{18},50*2.1mm,1.7um column within a short runtime of 8min by using pioglitazone as IS. The method was validated according to the regulatory guidelines with respect to precision, accuracy and linearity.

KEYWORDS: UPLC, Atorvastatin, Ezetimibe, Validation.

INTRODUCTION

Atorvastatin (Fig-I) is chemically[R-(R*, R*0]-2-(4-Fluorophenyl)-β, γ-dehydroxy-5-(1-mehtylethyl)-3-phenyl-4-[9phenylamino)-carbonyl]-1H-pyrrole-1-heptanoic acid. It competitively inhibits (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early rate limiting step in cholesterol biosynthesis, HMG-CoA reductase inhibitors increase HDL cholesterol and decrease LDL cholesterol (LDL-C), VLDL cholesterol and plasma triglycerides.Ezetimibe (Fig-I) is chemically 1,-(4-fluorophenyl)-3-[3-(4-fluorophenyl)-3-hydroxy-propyl]-4-(4-hydroxy phenyl) - azetidin-2-one. Ezetimibe localizes at the brush border of the small intestine where it inhibits the absorption of cholesterol, decreasing the delivery of intestinal cholesterol to the liver[1-7].

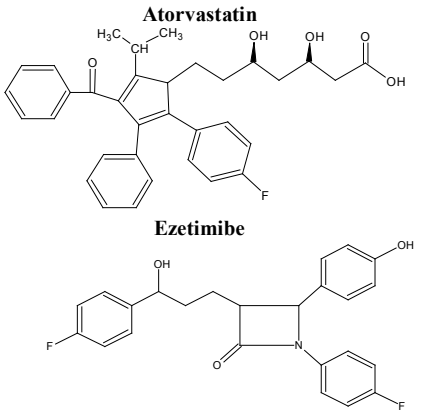

Figure1. The structure of Atorvastatin and Ezetimibe

MATERIALS AND METHODS

Apparatus

UPLC waters acquity system equipped with binary solvent delivery pump an auto sampler and tunable PDA detector, water acquity BEH 50*2.1mm, 1.7um, C_{18} column.

Materials: Atorvastatin standards were obtained from Zydus Medica laboratories, Ahmedabad (INDIA).Ezetimibe were obtained from MSN laboratories, Hyderabad (INDIA).

Chromatographic Conditions: A chromatographic system UPLC consisting of waters acquity system equipped with binary solvent delivery pump an auto sampler and tunable PDA detector, water acquity BEH 50*2.1mm,1.7um, C18. The instrumental settings were a flow of 1ml/min. The injection volume was 0.8ul.The detection wavelength was 248nm for all three analytes.The peak purity was checked with the photodiode array detector.

Mobile Phase: The mobile phase consisted of water and acetonitrile in the ratio of 60:40(v/v). the pH of the mobile phase was adjusted to 6.5ml of orthophosphoric acid in the double distilled water. The mobile phase was mixed and filtered through a nylon filter and degassed[8-22].

Preparation of Standard Solution

10 mg of Atorvastatin was taken in a 10 ml standard flask. To this 2 ml of methanol was added for dissolving the drug. Sonicate it for one min. To get a clear solution and make up the volume to 10 ml with mobile phase (Stock solution A).

10 mg of Ezetimibe was taken in a 10 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and make up the volume to 10 ml with mobile phase (Stock solution B).

The internal standard solution was prepared by taking 10 mg of pioglitazone in a 10 ml standard flask. It is dissolved by adding 3 ml of a mixture of 0.1 % Orthophosphoric acid and Acetonitrile in the ration of 1:1. Sonicate for few minutes to get a clear solution and make up the final volume to 10 ml with mobile phase.

The final standard solution was prepared in such a way that each standard flask contains 1.5, 3, 4.5, 6 and 7.5 µg of Atorvastatin and Ezetimibe and 5µg of pioglitazone (IS).

Preparation of Formulation Solutions

Twenty tablets each containing 10 mg of Atorvastatin and Ezetimibe were weighed and finely powdered. From the

powdered tablet, a quantity of powder equivalent to 10 mg was weighed. This was then extracted with 25 ml each of 25 ml of mixture of Acetonitrile and water (1:1 v/v). This was then filtered and diluted to 100 µg/ml of Atorvastatin and Ezetimibe. From this, 0.45 ml of Atorvastatin and Ezetimibe were drawn and mixed with 0.5 ml of internal standard so that these solutions when diluted to 10 ml with mobile phase contains 4.5 µg/ml of Atorvastatin and Ezetimibe and 5 µg/ml of internal standard (Table-1).

Method or Recording of chromatogram

With the optimized chromatographic conditions mentioned above, a steady baseline for about 20 min. was recorded. After the stabilization of the baseline for about 30 min., the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas was satisfactory and finally 0.8 µl of the standard solution of the individual samples were injected and the chromatograms were recorded. The typical chromatograms of the sample solutions were also recorded and shown in Fig. 3. Successive aliquots of 0.8µl of mixed standard solutions were injected and the chromatograms were recorded.

This procedure was repeated using the sample solution. The peak areas were noted and the response factors of the standard and sample solution peaks were calculated. The elution order of mixture was found as Atorvastatin (retention time 3.42), Pioglitazone (retention time 4.27) and Ezetimibe (retention time 6.89).

Calibration curve solutions: The calibration curve solution containing 1.5,3,4.5,6 and7.5 ug/ml of Atorvastatin and Ezetimibe and 5ug/ml of pioglitazone in each calibration level was prepared.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Our objective of chromatographic method development was reducing analysis time,maintaining good efficiency, retention time below 8 minutes, Along with resolution between Atorvastatin,Ezetimibe and internal standard (Pioglitazone).

The chromatographic separation was achieved using water acquity BEH 50*2 .1mm, 1.7um, C_{18}column. The chromatographic method was optimized by changing the composition of mobile phase and pH of the mobile phase.

Validation of the method:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in Table 2.

The precision of the method was determined by studying repeatability and reproducibility. The response factor of drug peaks and percentage relative standard deviation were calculated and presented in Table 3&4. The results revealed that the method developed is reproducible.

The standard drug solutions in varying concentrations ranging from 50 to 150 % of the targeted level of the assay concentration containing internal standard were examined by the assay procedure. The linearity and range for both the drugs was found to be from 1.5 to 7.5 µg/ml.

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of Atorvastatin and Ezetimibe were found to be 0.996 and 0.999 respectively. The calibration curves were plotted using response factor Vs concentration of the standard solutions (Fig 5&6). The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use.

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). LOD of Atorvastatin, Ezetimibe and Pioglitazone were found to be 20, 20 and 50 ng/ml. the LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Atorvastatin, Ezetimibe and Pioglitazone were found to be 100, 100 and 500 ng/ml.

The resolution, capacity factor, theoretical plates/meter, peak symmetry was calculated for the standard solutions and is presented in Table 5& 6. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

SUMMARY AND CONCLUSION

The developed UPLC method for the determination of atorvastatin and ezetimibe was found to be capable of giving faster retention time maintaining good resolution. The method was completely validated showing satisfactory data for all the parameters used. This method exhibited an excellent performance in terms of sensitivity amnd speed hence can be used for routine analysis of commercially available drugs.

REFERENCES

1. David C. Lee, Michael Webb, Pharmaceutical Analysis, Pg No: 1, 32, 44.
2. P.D. Sethi, Quantitative Analysis of Pharmaceutical Applications, 1997, 3rd Edition, Pg. No: 23-65.
3. Satinder Ahuja, Chromatography and Separation Science, Volume 4 of the Separation Science and Technology Series, Pg. No: 153-156.
4. Douglas Skoog A., James. J, and Leary, Principles of Instrumental Analysis, 5th edition, Pg. No: 3-4.
5. Lloyd R. Snyder., Joseph J. Kirkland., Jopseph l. Glajch., Practical HPLC Method Development, 2nd edition, Pg. No: 3-4, 234-242, 351-352, 25-27, 42, 653-656.
6. Chung Chow Chan, Y.C. Lee, Herman Lam, Xue – Ming Zhang, Analytical Method Validation and Instrument Performance Verification, Pg. No: 35-45.
7. George. Lunn, HPLC methods for recently approved pharmaceuticals, Pg. No: 64-65, 245-246.
8. ICH Topic Q2A, "Validation of Analytical Procedures": Methodology, 6th Nov 1996, www.ich.org.
9. ICH Stability Testing: Photo stability Testing of New Drug Substances and Products, ICH, Geneva, Switzerland, Nov. 1996, www.ich.org.
10. Hawk, G.GL and Kingston, H.M. (1988) Laboratory Robotics and Trace Analysis in quantitative Trace Analysis of Biological Materials, Elsevier.
11. Desty, D.H. (1989) LC-GC, 4(5), 32-40.
12. Ettre, L.S. (2000) Chromatographia, 51(1/2), 7.
13. Isenhour, T.L. (1985) J. Chem. Information and Computing, 25, 292.
14. Stockwell, P.B and Corns, W.T (1996) Automatic Chemical Analysis, Taylor and Francis, London.
15. Lynch, T. (1999) VAST, Chromatography and Separation Technology, 8, 4.
16. R. Sistla, V.S.S.K. Tata, Y.V. Kashyap, D. Chandrasekar and P.V. Diwan, Journal of Pharmaceutical and Biomedical Analysis, Vol. 39, Issue 3-4, Sept. 2005, Pg. No: 517-522.
17. T. Vetrichelvan, K. Ramakrishna and K. Anandakumar, Scientific Abstracts, 2006, Pg. No: 81.
18. A. Raj, K.E.V. Nagoji, M.E.B. Rao, S. Kopuri, P.K..Singh and G. Madhavi, Scientific Abstracts, 2006, Pg. No: 293.
19. Nilesh Jain. M.R. Khan and Deepti Jain, Scientific Abstracts, 2006, Pg. No: 75.

20. B.V.V. Ravi Kumar, P.K.Singh, K.E.V. Nagoji, M.E.B. Rao, A. Raj and G. Madhavi, Scientific Abstracts, 2006, Pg. No: 289.
21. Lamba H.S, Manuvesh, rathour Arvind, Jitender Singh, Scientific Abstracts, 2006, Pg. No: 297.
22. Lamba H.S, Manuvesh Sangwan, Varun Arora, Arvind Rathour, Scientific Abstracts, 2006, Pg. No: 300.

Table 1: Analysis of Formulation

Drug	Label Claim (mg/tablet)	Estimated Amount (mg/tablet)	% Label claim	SD
Atorvastatin	10	9.86	98.6	0.05
Ezetimibe	10	10.16	101.6	0.01

Table 2: Accuracy (Recovery Studies)

S. No	Name of the Drug	Label Claim (mg/tablet)	Amount added 100%	Amount recovered 100%	% Recovery	RSD
1.	Atorvastatin	10mg	10	9.28	92.8	0.013
2.	Ezetimibe	10mg	10	9.87	98.7	0.011

Table 3: Intra day Studies

Rf of Atorvastatin	Mean	SD	RSD	Rf of Ezetimibe	Mean	SD	RSD
1.027 1.023 1.013 1.042 1.034	1.027	0.0106	1.061	1.402 1.401 1391 1.403 1.406	1.4000	0.00568	0.405

Table 4: Interday Studies

Day	Rf of Atorvastatin	Mean	SD	RSD	Rf of Ezetimibe	Mean	SD	RSD
Day 1	1.023 1.016 1.036 1.053 1.011	1.0278	0.015	1.47	1.392 1.413 1.405 1.401 1.399	1.402	0.0077	0.549
Day 2	1.021 1.022 1.034 1.018 1.012	1.027	0.010	0.991	1.406 1.421 1.403 1.386 1.412	1.403	0.0105	0.748
Day 3	1.036 1.028 1.043 1.018 1.009	1.0268	0.013	1.32	1.412 1.403 1.394 1.408 1.401	1.403	0.0068	0.484

Table 5: Linearity and Range in HPLC

Internal Standard Peak area (5µg/ml)	Atorvastatin			Ezetimibe		
	Concentration (µg/ml)	Peak area	Response factor	Concentration (µg/ml)	Peak area	Response factor
490905	1.5	504733	1.028	1.5	687828	1.40
490118	3	1031468	2.104	3	1017319	2.07
525537	4.5	1566001	2.97	4.5	1445075	2.75
515926	6	2083000	4.037	6	1748045	3.38
514355	7.5	2575820	5.007	7.5	2068693	4.02

Table 6: System suitability studies in HPLC

Parameters	Atorvastatin	Ezetimibe	Pioglitazone
Theoretical plates / meter	12126	22692	19234
Resolution	3.32		3.41
Capacity factor	0.2750	0.557	1.56
LOD (μg /ml)	0.02	0.02	0.05
LOQ (μg /ml)	0.06	0.06	0.15

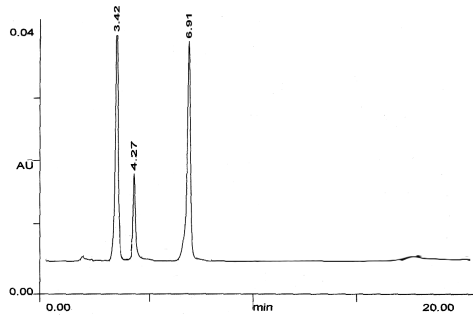

Fig-2 Chromatogram of Recovery Studies

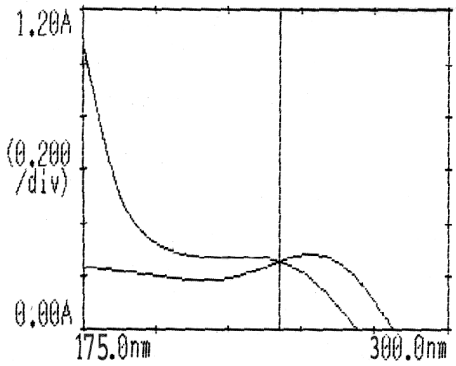

Fig-4 Overlain spectra of Atorvastatin and Amlodipine

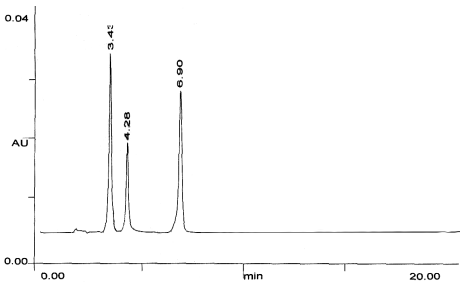

Fig-3 Chromatogram of sample solution of Atorvastatin and Ezetimibe

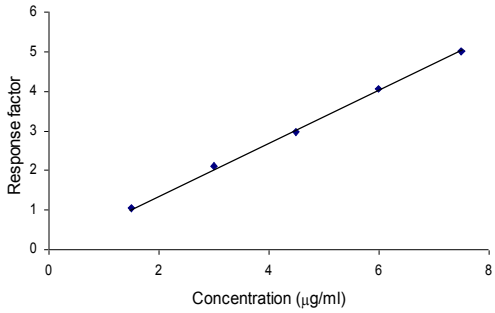

Fig-5 Calibration curve of Atorvastatin

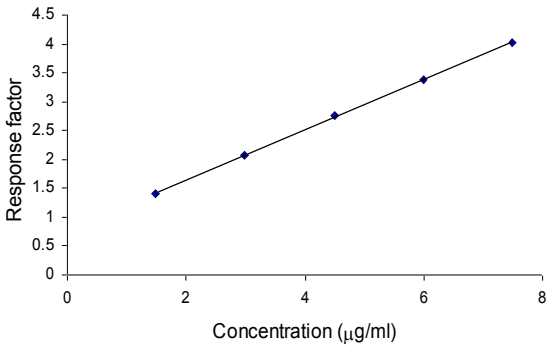

Fig-6 Calibration curve of Ezetimibe

Source of support: Nil, Conflict of interest: None Declared