



A VALIDATED LC METHOD FOR DETERMINATION OF THE ENANTIOMERIC PURITY OF ATORVASTATIN IN BULK DRUG AND DOSAGE FORMS

M. Vishnu murthy^{1,2}, Katkam Srinivas¹, N.Ramesh kumar¹, K.Mukkanti²

¹Analytical Research and Development, Integrated product development operations, Dr. Reddy's Laboratories Ltd., Bachupally, Qutubullapur, R. R. Dist. 500 072 Andhra Pradesh, India.

²Department of Chemistry, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-5000 072, India.

Email: vishnumm@drreddy's.com

ABSTRACT

A simple, rapid isocratic chiral HPLC method has been developed for the separation of (S, S)-Atorvastatin from (R, R)-Atorvastatin and quantitative determination of (S, S)-Atorvastatin enantiomer in bulk drug and pharmaceutical dosage forms. Normal phase chromatographic separation was achieved on chiral stationary phase, Chiral pak AD-H (250 mm × 4.6 mm ID) column at 30°C temperature. Flow rate was kept at 1.0 mLmin⁻¹. The elution time was ~ 8.0 min and the resolution (R_s) between the enantiomers is greater than 2.5. Interestingly (S, S) - form of Atorvastatin peak was eluted prior to the (R, R) - form of Atorvastatin. The limit of detection (LOD) and limit of quantification (LOQ) for the (S, S) Atorvastatin were 0.18µg mL⁻¹ and 0.60µg mL⁻¹ respectively, for a 10µL load of the sample. The method was validated in terms of linearity, precision and accuracy and satisfactory results were obtained. Robustness studies were also performed. The sample solution stability of Atorvastatin was determined and the compound was found to be stable up to 48 h.

Keywords: Chiral HPLC method, quantitative determination, atorvastatin

INTRODUCTION

Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effects of enantiomers of the existing drug molecules to have a detailed impurity profile. The determination of amounts of different enantiomeric forms in pharmaceuticals is essential in this connection and high performance liquid chromatographic method (HPLC)¹ is generally opted for this purpose. The Atorvastatin Calcium is chemically [R--(R*, R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrole - 1-heptanoic acid calcium, which is a reductase inhibitor of the enzyme 3-hydroxy-3-methylglutarate-coenzyme A (HMG-CoA) and therefore is a useful anti-hyperlipoproteinemic agent. It has been proven to be a highly effective medication for the treatment of disorders such as hyperlipidemia and hypercholesterolemia which are conditions that are known risk factors for arteriosclerosis and coronary heart disease. The empirical formula of atorvastatin calcium is (C₃₃H₃₄ FN₂O₅)₂Ca and its molecular weight is 1155.4. Structural formula of the both the enantiomers were shown in (Fig -1).

Enantiomers of the racemic drugs often differ in pharmacokinetic behaviour or pharmacological action². The development of analytical methods for the quantitative analysis of chiral compounds are extremely challenging due to the fact that enantiomers possess virtually identical properties³. Although many analytical techniques such as capillary electrophoresis (CE), Super fluid liquid chromatography (SFC) can be employed to attain this, the most widely used is liquid chromatography (LC) employing a chiral stationary phase (CSP)^{4,6}.

Even though corresponding S-isomer being controlled in starting stages of Atorvastatin synthesis, it is quite important to monitor the level of other isomer in finished product. In literature rapid solid phase

extraction for determination of Atorvastatin and metabolites in human plasma by LC-tandem MS was reported⁷. So far, to our present knowledge no enantioselective LC methods were reported in the literature for the enantiomeric purity of Atorvastatin. Hence, we herewith are reporting a stereo selective, rapid isocratic HPLC method to separate and quantitate (S, S) -Atorvastatin from (R, R) -of Atorvastatin. This method was validated in compliance with ICH guidelines⁸⁻⁹ in terms of linearity, precision, accuracy, specificity and robustness.

EXPERIMENTAL

Chemicals

HPLC grade n-hexane and ethanol were procured from Merck, India. Trifluoro acetic acid was purchased from Aldrich. (S, S) and (R, R) isomers of Atorvastatin calcium and Reference standard of Atorvastatin calcium were obtained from process development laboratory of Dr. Reddy's Laboratories Ltd., API, IPDO, Hyderabad, India.

Chromatographic Conditions

A Waters Model Alliance 2690 separation module equipped with an auto sampler and waters 2996-photodiode array UV detector (Waters Corporation, Milford, USA) was used for the analysis. The data was recorded using Waters Empower software. The separations and quantification were performed on chiral pak-AD-H column (250 x 4.6 mm, Make: Diacel Chemical Industries Ltd. Japan).

Sample and Standard Preparation

Individual stock solutions of (R, R) and (S, S) isomers of Atorvastatin 1000 $\mu\text{g mL}^{-1}$ were prepared by dissolving an appropriate amount of the substances in the methanol: ethanol in the ratio (1:1) (v/v). Working solutions were also prepared in the same diluent.

System suitability solution

Prepared a solution containing 1000 $\mu\text{g mL}^{-1}$ of (R, R)-Atorvastatin and 1.5 $\mu\text{g mL}^{-1}$ of (S, S) – Atorvastatin in 10mL volumetric flask.

RESULTS AND DISCUSSION

Method Development and optimization of chromatographic conditions

The method development strategies adopted using chiral pack AD-H column involves different experiments based on nature and structure of compound. These trials include the addition of acid additives for acidic compounds and base additives for basic compounds to the mobile phase. The design of mobile phase consists of a combination of alkane and polar alcohols based on normal or polar interactive modes. Initiated the screening analysis with the above combination of experiments to derive best suitable column and mobile phase conditions.

Initially, experiments were carried out by using chiral columns in the reverse phase for the separation of (R, R) and (S, S) isomers of Atorvastatin, but it was not achieved. Later several experiments were carried out in the normal phase using various chiral columns. While using the chromatographic conditions like chiral cel-OJ, chiral cel-OD columns, flow rate 0.8 mLmin⁻¹ and mobile phase of n-hexane, 2-propanol (85:15) (v/v) mixture, it has been observed that the (R, R) and (S, S) isomers of Atorvastatin were eluted as broad peaks with longer retention times. Inadequate separation was observed on these chiral stationary phases (CSP). However an improvement in peak shapes was observed when Chiralpak AD-H column, with same mobile phase and same flow rate are used, but still, there are certain constraints like longer retention times and improper separation (R_s less than 1) between enantiomers were observed. The next attempt was made on this amylose based CSP, wherein ethanol was used as a polar organic modifier in place of 2-Propanol, which would provide considerable separation (R_s less than 1.5) between the isomers. Due to the presence of carboxylic acid functional group in Atorvastatin, 0.1% of Trifluoro acetic acid was introduced to the above mobile phase i.e. n-hexane: ethanol (85:15)(v/v). Addition of trifluoro acetic acid has improved the peak shapes by reducing the widths of analyte peaks and consequently the resolution was enhanced between the isomers. The additive ratio was further increased to 0.3% to control the peak shape which resulted in no significant improvement. In addition to this base line noise was increased drastically. Further trials were continued on the same CSP by changing additives viz acetic acid and

formic acid. Despite these two additives, trifluoro acetic acid is the only acid, which gave comparatively good peak shape as well as good resolution between the analyte peaks. In view of possible interference study, attempts were also made by adjusting the mobile phase flow rate to separate the process related impurities and excipients from (S, S) Atorvastatin.

In the present optimized method, the typical retention times of (S, S) Atorvastatin and (R, R) isomers of Atorvastatin are 6.6 min and 7.6 min respectively (Fig.2). The peak purity of (S, S) Atorvastatin is found to be homogeneous in all spiked samples of stability studies. The resolution (R_s) between the two enantiomers was about 2.8. Diluent methanol: ethanol (1:1) (v/v) was used as blank and there was no interference of the blank and of excipients with (S, S) and (R, R) isomers of Atorvastatin. The developed method is found to be selective from process related impurities.

Finally, the resolution was found to be more than two for the separation of Atorvastatin (S, S) and (R, R) isomers with the mobile phase consisting of n-Hexane, ethanol and 0.1% trifluoro acetic acid in the ratio of 85 : 15 : 0.1 (v/v/v). The elution was monitored at wavelength $\lambda = 246$ nm. Then, the same conditions were maintained for the determination of (S, S) - Atorvastatin in (R, R) Atorvastatin.

Method Validation

The method was validated in accordance with ICH guidelines [8-9].

Limit of Detection and Quantification

The limit of detection (LOD) represents the concentration of analyte that would yield an S/N (signal to noise) ratio of 2.0. LOD for (S, S) -Atorvastatin was found to be $0.18 \mu\text{g mL}^{-1}$. The limit of quantification (LOQ) represents the concentration of analyte that would yield an S/N ratio of 10. LOQ for (S, S) - Atorvastatin calcium was found as $0.60 \mu\text{g mL}^{-1}$. The % RSD value of precision at LOQ level is 4.1. The percent recovery values at LOQ (95 to 101) were obtained in acceptable range as per ICH guidelines.

Linearity and Range

Linearity test solutions of (S, S) -Atorvastatin ranging from 0.6 and $3.0 \mu\text{g mL}^{-1}$. i.e LOQ to 150% (0.06 % (LOQ), 0.075, 0.1125, 0.15 and 0.225%) of the permitted maximum level were prepared by diluting the standard stock solution of (S, S) -Atorvastatin. A five point calibration curve was drawn between the peak areas of (S, S) -Atorvastatin versus its concentration. The slope, intercept and correlation coefficient were derived from linear least-square regression analysis. The results revealed that an excellent correlation existed between the peak area and concentration of the analyte. The data is presented in Table-1. Linearity was checked for the (S, S) -Atorvastatin over the same concentration ranges on three consecutive days. The % RSD of the slopes and y-intercepts of the calibration plots for the (S, S) - Atorvastatin was 3.2 and 4.5, respectively. These results are indicative of excellent correlation between peak area and concentration. The range of the method for the (S, S) -Atorvastatin was found from 0.06 % (LOQ) to 0.225% of the analyte concentration ($1000 \mu\text{g mL}^{-1}$)

Precision

The precision was evaluated by calculating the relative standard deviation of six replicate determinations using the same solution containing (S, S) -Atorvastatin at three levels viz. 0.75, 1.5 and $2.25 \mu\text{g mL}^{-1}$ in presence of $1000 \mu\text{g mL}^{-1}$ (R, R) -Atorvastatin and the percentage relative standard deviation values of injection repeatability for (S, S) -Atorvastatin were found to be 1.5, 1.6 and 1.1 respectively which is in the acceptable range as per the ICH guidelines. Intra day and inter day precision was also performed and compared with initial data. The results are given in Table-1.

Accuracy

Standard addition and recovery experiments were conducted to determine accuracy of the method for quantification of (S, S) -Atorvastatin in bulk drug samples and dosage forms.

The accuracy of method was evaluated by assaying freshly prepared solutions in triplicate at four concentration levels of 50%, 75%, 100% and 150%. The percent recovery values are in the acceptable range as per ICH guidelines. The data is presented in Table-1.

Robustness

To assess robustness of method, the experimental conditions were deliberately altered and the resolution between the enantiomers was checked. To study the effect of flow rate, the flow was changed from 1.0 to 0.9 and 1.1 mLmin⁻¹. The effect of column temperature was studied at 28 and 32°C. The effect of mobile phase composition on resolution was assessed by changing the ethanol by ±1%. The effect of changing the additive, trifluoro acetic acid was also studied. In the varied chromatographic conditions viz. flow rate, column temperature, mobile phase composition and additive concentration, the resolution between the peaks of (S, S) and (R, R) -Atorvastatin was found to be more than 2.0 illustrating the robustness of the method.

Solution stability and mobile phase stability

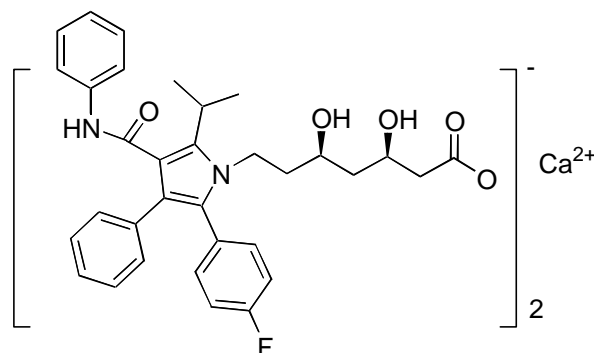
No significant change was observed in the content of (S, S) Atorvastatin during solution stability and mobile phase stability experiments when performed using chiral method. The solution stability and mobile phase stability experiments data confirm that the sample solutions and mobile phase were stable up to 48 hours.

CONCLUSION

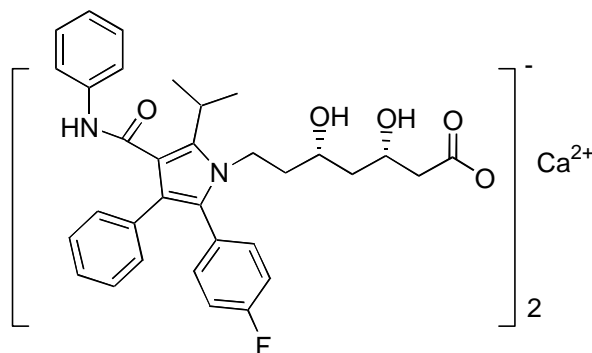
An isocratic, stereo selective, chiral liquid chromatographic method developed for the enantiomeric separation and quantitative determination of (S, S) Atorvastatin content is found to be precise, accurate and specific in bulk active substance and finished dosage forms. The method is fully validated as per ICH guidelines, showing satisfactory results for all method validation parameters tested. Hence, the method is and can be used for the routine analysis of plant batches of Atorvastatin calcium in quality control laboratories of bulk samples and tablet samples of Atorvastatin.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd., IPDO, Hyderabad for supporting this work. We would also like to thank colleagues in product delivery team Mr. P. K. Seshu Kumar (Analytical Research and Development) and Mr.S.Rajeswar Reddy (Research and Development) for their co-operation in carrying out this work.



[R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrole-1-heptanoic acid, calcium (R, R) - Atorvastatin



[S-(S*,S*)]-2-(4-fluorophenyl)- β,δ -dihydroxy-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrole-1-heptanoic acid, calcium (S, S) - Atorvastatin

REFERENCES

- Lloyd R. Snyder , Joseph J.Kirkland , Joseph L. Glajch, Practical HPLC Method Development, 2nd Edition.
- C.G. Shajwalla, Marcel Dekker Inc., New York, **141**,421-426 (2004)
- T.E. Beesley, R.P.W. Scott, Chiral Chromatography. Wiley, New York, 23-26 (1998).
- G. Subramanian , A Practical approach to chiral separations by liquid Chromatography.VCH Publishers, Wienheim(1994)
- H.Y. Aboul-Enein, I.W. Wainer, The impact of stereo chemistry in drug development and use. Wiley, New York(1997)
- S. Allenmark, Chromatographic enantioseparation: methods and applications, 2nd edn. Ellis Horwood, New York(1991).
- M.Hermann, H.Christensen, J.L.E.Reubasaet; Determination of Atorvastatin and metabolites in human plasma with solid-phase extraction followed by LC-tandem MS; *Anal Bioanal Chem* ,**382**,1242-1249(2005)
- Draft ICH guidelines on Validation of Analytical Procedures , Definitions and terminology. Federal Register, **Vol. 60**, IFPMA, Switzerland 11260 (1995).
- Validation of Compendial methods, The United States Pharmacopeia, 30th. edn.USP30(2007).

Fig.-1:Chemical structures of (S, S)-Atorvastatin calcium and (R, R)-Atorvastatin calcium

Table-1: Linearity, accuracy and precision data for (S, S)-Atorvastatin

R ²	Linearity (LOQ to 150%)	Accuracy % Mean recovery				Precision (n = 6) % RSD			
	Regression equation	LOQ	50%	100%	150%	LOQ	50%	100%	150%
0.9997	y = 155.1x - 0.5455	97.2	98.9	99.1	98.6	4.1	1.5	1.6	1.1
	Intraday Precision(n = 6) : Different column						2.1	1.7	1.2
	Intraday Precision(n = 6) : Different System						1.2	1.5	0.8
	Intraday Precision(n = 6) : Different Analyst						1.8	1.3	0.8
	Inter day Precision(n = 6)						1.1	1.8	0.5

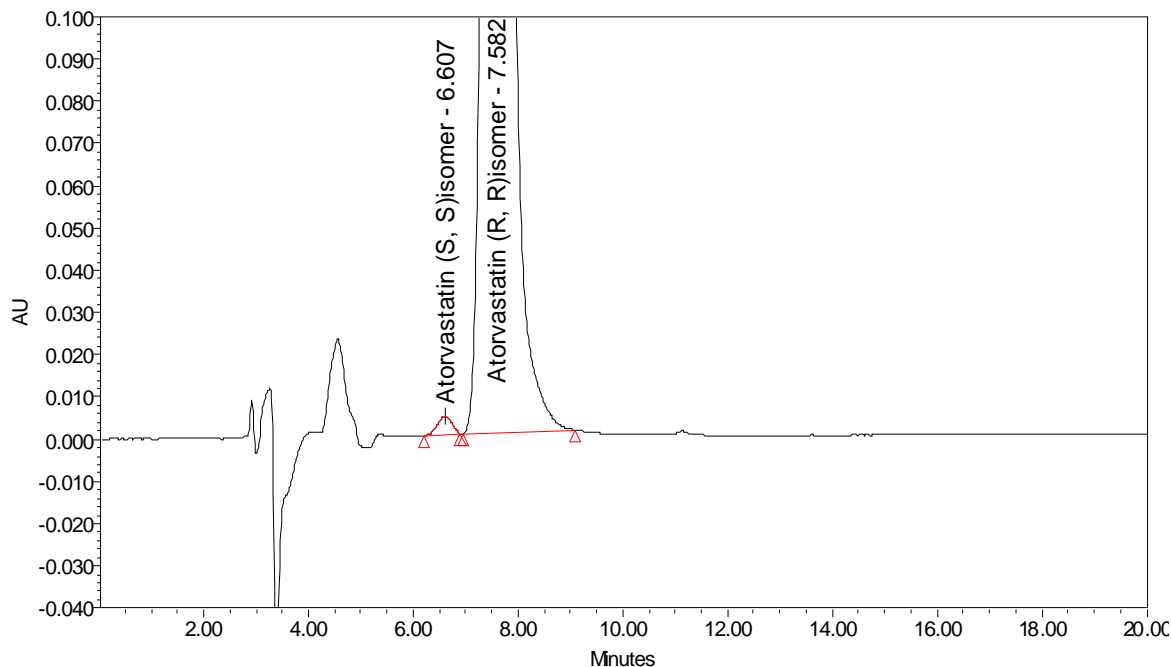


Fig. -2: Typical HPLC chromatograms of System suitability solution

(Received: 25 September 2009

Accepted: 25 October 2009

RJC-459)



[http:// www.rasayanjournal.com](http://www.rasayanjournal.com)

Be a Proud Life Member of RJC

Life Membership for Individuals: Rs.8000/- for Indians and USD 1000 for others.

Life Membership for Institutional: Rs.10000/- for Indians and USD 1500 for others.

BENEFITS OF LIFEMEMBERSHIP:

1. You will receive the journal and all its special issues regularly life long.
2. You will receive all other future publications (Proceedings, Edited Books, Monographs etc.) published by RJC on 50% discount.
3. If you are a LIFE MEMBER, you need not to pay subscription fee every time for publication of your paper in RJC.
4. You'll be a Reviewer for RJC manuscripts of your Field Interest and we'll publish your name in our journal.
5. You will be exempted from Registration Fee of any National or International future events (i.e. workshop, seminars, Conferences etc.) organized by RJC.
6. You may be elected as Editorial Member of RJC (Note: It'll depend upon your publication and scientific achievements).
7. You'll have a very personalized gift from RJC with Complements.

For being a **Life Membership**, just mail to editor-in-Chief with your detailed Resume.