Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ROSUVASTATIN CALCIUM

D. Balanagamani ^{1*}, K. Deepthi ², K. Lakshmi Sivasubramanian ¹

¹Department of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy, Chittoor, India

²Department of Pharmaceutical Analysis, Bharat Institute of Technology, Hyderabad, India

*Corresponding Author Email: mani.pharmsmiley@gmail.com

Article Received on: 20/11/16 Revised on: 08/12/16 Approved for publication: 15/12/16

DOI: 10.7897/2230-8407.0712157

ABSTRACT

A stability indicating linear, accurate, specific and sensitive reverse phase - HPLC method has been developed along with validation parameters for the determination of Rosuvastatin calcium (RSV) in pharmaceutical dosage form. The chromatographic separation was performed by using symmetric C₁₈ column (75*4.6mm, 3.5µm particle size). Mobile phase was used in the ratios (60:40) of 0.1% of formic acid in water and acetonitrile (ACN). Flow rate was set at 1ml/min at room temperature with detecting wavelength at 242nm by using chemstation software. Retention time of RSV was found to be about 3.6min. The linearity was performed by using concentration ranges from 10-60 µg/ml with a correlation coefficient value of 0.9999. The percentage recovery was found to be 100.77 – 106.75. Limit of detection and Limit of Quantification were performed by using a method of signal by noise (S/N) ratio and was found to be 0.2, 0.5 µg/ml respectively. The developed analytical method has been validated and the values were lies within the acceptance limit according to ICH guidelines. Degradation studies were performed by subjecting to different stress conditions like acidic, alkaline, thermal (60, 80, 100), oxidative degradation. Based on the percentage of degradation, the samples were subjected to LC-MS for determining the mass elucidation of degraded compounds.

Keywords: Rosuvastatin calcium, RP-HPLC, Degradation studies, LC-MS.

INTRODUCTION

Rosuvastatin (RSV) Calcium, 7-[4-(4-fluorophenyl)-6-(-1-methylethyl)-2-[(methyl sulfonyl) amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptanoic acid calcium, which is a highly effective HMG-COA Reductase Inhibitor. It is widely used in the treatment of hyperlipidemia¹ and related conditions to prevent cardiovascular diseases. Literature survey reveals that few methods were reported for the determination of Rosuvastatin Calcium in the pharmaceutical formulations by using HPLC¹⁻⁶, UPLC⁷, Spectrophotometry⁸. In the present work we focused on the development and validation of optimum chromatographic conditions for the determination of RSV in pharmaceutical dosage forms along with structural elucidation of degraded products formed under different stress conditions can be applied successfully to quality control purposes.

MATERIALS AND METHODS

Reagents and Chemicals

Pure sample of RSV was gifted by DR'ILS, Hyderabad Central University (HCU), Gachibowli, Hyderabad. All Chemicals used were HPLC grade. Acetonitrile, Formic acid, Analytical grade Hydrochloric acid, Sodium hydroxide and 30% Hydrogen Peroxide solution (v/v) were obtained from Merck limited, Mumbai, India. Rosuvas 10 was obtained from a local pharmacy which is manufactured by Ranbaxy laboratories. High purity De ionised water was obtained from a Milli-Q (Millipore, Milford, MA, USA) purification system.

Equipment and Chromatographic conditions

The chromatographic system used to perform the development and validation of proposed method was an Isocratic HPLC system of Agilent Technologies with 1200 series pump and DAD detector with chemstation software, symmetric C₁₈ column (75*4.6mm, 3.5µm). Separation was achieved by using a mobile phase consist of 0.1% Formic acid in water –Acetonitrile (60:40 v/v) solution at a flow of 1.0ml/min. The eluent was monitored using PDA detector at a wavelength 242nm. The column was maintained at room temperature and 10µl sample was injected for each determination. The mobile phase was filtered prior to use through 0.45µm filter.

Preparation of Stock, Standard and Test solution

Stock solution (1000µg/ml) of RSV reference standard was prepared by transferring 5 mg, accurately weighed into 5ml volumetric flask and mobile phase was added after sonicated for 10 min. The solution was diluted by using mobile phase. Standard solution (100µg/ml) was prepared by diluting 5ml of stock solution to 50 ml with mobile phase.

To prepare stock solution (1000 µg/ml) for assay, 20 tablets were weighed and powdered. An aliquot of powder equivalent to the weight of 10 tablets was accurately weighed and transferred to 5ml volumetric flask, water and acetonitrile (80:20 v/v) were added with sonication for 10 min at each addition separately. Standard solution (100µg/ml) for assay was prepared by diluting 5ml stock solution to 50 ml using same diluent with sonication at each addition. This solution was filtered through 0.45 µm nylon syringe filter. To prepare test solution (30 µg/ml)

for assay, 1.5 ml of standard solution was diluted to 5ml with diluent.

Method Validation⁹

In the developed method validation parameters were performed with accuracy, precision, linearity. The method specificity was evaluated to ensure that there was no interference with placebo components.

Linearity

Seven solutions were prepared containing 5, 10, 20, 30, 40, 50, 60 µg/ml RSV concentrations. Each duplicate solution was injected. Linearity was evaluated by linear-regression analysis.

Precision¹⁰

System precision was evaluated by analyzing the standard solution for five times and repeatability (method precision) was performed by assaying 6 sets of samples on the same day (intra-day precision).

Accuracy¹⁰

Accuracy was assessed by the determination of the recovery of method at three different concentrations (equivalent to 80, 100, 120% of test solution) by addition of known amount of standard and placebo preparation. For each concentration three replicates were prepared and injected.

System suitability

The suitability of chromatographic system was tested before each stage of validation with 5 replicates of standard solution (30µg/ml). System suitability parameters like asymmetry, number of theoretical plates and tailing factor were determined.

Forced degradation studies¹¹

To perform the forced degradation studies stock solution (1000 µg/ml) was prepared by taking 10 mg RSV and subjected to acidic, alkaline, oxidative, thermal, photolytic conditions. For acidic degradation, stock solution was mixed with 0.1N HCl at RT for 24 hrs. For alkaline degradation, stock solution was treated with 0.1N NaOH at RT for 24 hrs. For oxidative degradation¹², stock solution was mixed with H₂O₂ (30%v/v) for 24 hrs at RT. For thermal degradation, stock solution was exposed at 60°C, 80°C, 100°C for 2hrs. For photolytic degradation, stock solution was exposed to UV light for 1 hr. Blank solution also subjected to the same conditions. The resulting solutions were left to return to the RT and diluted with mobile phase to get 50µg/ml concentration. The stressed samples were measured by PDA detector. Based on the percentage of degradation, these samples were further subjected to LC-MS¹³ for mass elucidation.

RESULTS AND DISCUSSION

In this work, analytical HPLC method was used for determination of degraded products which was developed and validated. The basic chromatographic conditions were designed to be simple and easy to use was selected after testing different methods and different compositions of mobile phase. The symmetry C₁₈ column was used because of its high resolving capacity and low back pressure. For mobile phase selection,

preliminary trials were done by using different compositions of water and acetonitrile, 0.1 % formic acid was mixed with water for better peak shape. The proportion of mobile phase components were optimized to reduce the retention time and to get good peak resolution of RSV from the degraded products. A Detection wavelength of 242 nms was selected after scanning from 190 -370 nms by using PDA detector which results in good linearity and good resolution. The Retention time (R_t) for RSV was found to be 3.6 minutes as shown in Figure 1. The drug substance was easily extracted from the dosage form by using water and acetonitrile (80:20v/v).

After development of analytical method, it was validated according to ICH guidelines. This furnished evidence of the method was suitable for its intended purpose. The specificity of the method was determined by checking interference with drug and placebo components. The specificity of the method was evaluated by checking the peak purity of analyte peak during the forced degradation study.

Linearity was determined by plotting a calibration curve of RSV concentration against peak area. Linearity was good in the concentration range 5-60 µg/ml. The regression equation was $y = 11.24x + 6.849$, where x is the concentration in µg/ml and y is the peak area in absorbance units the correlation coefficient was 0.9999. The data of regression analysis and calibration curve was shown in [Table 1, Figure 2].

For system precision [(n=3)*6] R.S.D was found to be 0.25%, 1.2%, 1.7% respectively on the same day (intra-day). For Accuracy [(n=3)*3] determination of percentage recovery was performed by internal addition method (80%, 100%, 120% levels) and were found to be 102.15%, 100.77%, 106.75% respectively. The results were obtained within the limits and were shown in [Table 2]. LOD and LOQ were performed by using signal by noise (S/N) ratio method and resulted as 3.4, 11.1 respectively. The Number of theoretical plates (n), Asymmetric factor (A_s), Retention time were shown in [Table 3]. System suitability parameters (n=3) were performed and precise results were obtained for all validation parameters without significant variation as per ICH guidelines were shown in [Table 4].

Forced degradation studies were performed by subjecting to different stress conditions like acidic, alkaline, oxidative, thermal (60°C, 80°C, 100°C) and photolytic conditions. Major degradation was observed in acidic conditions (up to 40%) with 5 degraded samples at R_t values 0.653, 3.889, 5.770, 7.789, 8.261 with percentage areas 1.6%, 13.5%, 2.6%, 3%, 16.7% respectively. The chromatograms obtained for different degraded products were shown in [Figure 3].

The drug was degraded approximately 29% under thermal degradation at 80°C with 4 degraded samples at R_t values 3.854, 7.7, 8.19 with percentage areas 8%, 3%, 16.5%. Negligible degradation was observed when subjected to other degradation conditions. Based on the percentage degradation the samples were further subjected to LC-MS to elucidate mass determination.

The same LC conditions were used for further analysis in LC-MS and reports for acid and thermal degradation at 80°C were shown in [Figure 4 & 5] respectively. From the LC-MS reports, degraded products were observed in acidic and thermal (80°C) conditions and their m/z ratio's were found to be 50.10, 60.30, 480.30 and 464.30.

Figure 1: Chromatogram for method development of RSV

Table 1: Linearity data

S.no	Parameters	RSV	Acceptance criteria
1.	Linearity range	5-60	-
2.	Correlation coefficient(r^2)	0.9999	>0.996
3.	Slope (m)	11.35	-
4.	Intercept	10.26	-

Figure 2: Calibration curve showing linearity of RSV

Table 2: Accuracy study data

Accuracy level	No.	Area count	Mean value of peak area	Mean % Recovery
80%	1	613.6	610.1	102.15%
	2	604.8		
	3	611.9		
100%	1	671.9	672.96	100.77%
	2	673.3		
	3	673.7		
120%	1	762	762.9	106.75%
	2	763.5		
	3	763.4		

Table 3: LOD and LOQ Data

Validation parameter	Time range (min.)		RT (min.)	N	A_s	S/N
	From	To				
LOD	5.00	6.00	3.766	7857	0.92	3.4
LOQ	5.00	6.00	3.761	6101	0.86	11.1

Table 4: System Suitability Data

S.No	Parameters	Results
1.	Width at half height	0.115
2.	5 sigma	0.292
3.	Tangent	0.202
4.	Tailing	0.283
5.	USP Tailing	1.273
6.	Symmetry	0.758

Figure 2: Chromatograms of forced degradation studies: Chromatographic data after subjected to a)Acidic b)Alkaline c)Oxidative d)Thermal (at 60°C) e)Thermal (at 80°C) f) Photolytic (UV) degradation

Figure 3: m/z ratios of acid degradation samples

Figure 4: m/z ratios of Thermally (80 °C) degraded sample

Table 5: mass to charge ratios for Degraded compounds

S.No	Degraded product	m/z ratio
1.	A-I & T-III	480.30
2.	A-II & T-I	464.30
3.	A-III	50.10
4.	T-II	60.30

ACKNOWLEDGEMENT

Authors are thankful to Dr. Reddy's Institute of life sciences (DR'ILS), HCU, Gachibowli, Hyderabad for providing facilities to execute the research work. It gives a great pleasure to acknowledge my immense respect and depth of gratitude to Dr. Srinivas Oruganti, Head of CPRI (*Center for Process and Research Innovation*) at DR'ILS, Hyderabad Central University, Gachibowli, Hyderabad.

REFERENCES

- Godavariya vijay.D, Prajapati pintub B, Marolia Bhavin P, Shah sailesh A. Development and Validation of RP – HPLC method for the simultaneous estimation of Rosuvastatin Calcium and Aspirin in marketed formulation, *Int. Res. J. Pharm.* 2012;3(8),173 – 175.
- Dipali Tajane, Amol M.Raurale, Pradeep D. Bharande, Anil N.Mali, Amol V.Gadkari, Vishal R.Bhosale. Development and validation of a RP-HPLC-PDA method for simultaneous determination of Rosuvastatin calcium and Amlodipine besylate in pharmaceutical dosage form, *Journal of Chemical and Pharmaceutical Research* 2012; 4(5),2789-2794.
- Aruna Jyothi, Buchi N. Nalluri. Development and Validation of Rapid HPLC - PDA Method for the Simultaneous estimation of Rosuvastatin Calcium and Telmisartan in Bulk and Dosage Forms, *Journal of Pharmacy Research* 2012;5(8),3994-3997.
- Chiragsinh solanki, nishitkumar patel. Development and validation of RP-HPLC method for simultaneous estimation of Rosuvastatin calcium and aspirin in capsule dosage form, *International Journal of Pharma and Bio Sciences* 2012 July; 3(3),577 – 585.
- P.S.R.Ch.N.P. Varma D, A. Lakshmana Rao, S.C. Dinda. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe in combined tablet dosage form, *Rasayan Journal of chemistry* 2012,July-September;5 (3),269-279 .
- Safwan Ashour, Soufafa Omar. Validated High-Performance Liquid Chromatographic Method for the Estimation of Rosuvastatin Calcium in Bulk and Pharmaceutical Formulations, *International Journal of Biomedical Science* 2011;7(4),283-288.
- Harshal Kanubhai TRIVEDI, Mukesh C. PATEL. Development and Validation of a Stability-Indicating RP-UPLC Method for Determination of Rosuvastatin and Related Substances in Pharmaceutical Dosage Form, *Scientia Pharmaceutica* 2012;80,393–406.
- Anandakumar Karunakaran, Vetsa Subhash, Ramu Chinthala, Jayamaryapan Muthuvijayan. Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV- Spectrophotometry and RP-HPLC, *Stamford Journal of Pharmaceutical Sciences* 2011;4(1),58-63.
- ICH - Validation of analytical procedures text and methodology, *International conference on harmonization, IFPMA, Geneva*, 1996.
- Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT, Shah AK. A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets, *Indian Journal of Pharmaceutical Sciences* 2010;72,592-598.
- ICH - guidelines Q1A (R2), *Stability Testing of New Drug Substances and Products (revision 2)*, November 2003.
- Nadia M. Mostafa, Amr M. Badawey, Nesrine T. Lamie, Abd El-Aziz B, Abd El-Aleem. Stability-indicating methods for the determination of rosuvastatin calcium in the presence of its oxidative degradation products, *International Journal of Pharmaceutical and Biomedical Sciences* 2012;3(4),193-202.
- Dujuan Zhang, Jing Zhang, Xiaoyan Liu, Chunmin Wei, Rui Zhang, Haojing Song, Han Yao, Guiyan Yuan, Benjie Wang, Ruichen Guo. Validated LC-MS/MS Method for the Determination of Rosuvastatin in Human Plasma:

- Application to a Bioequivalence Study in Chinese Volunteers, Scientific Research journal of Pharmacology & Pharmacy 2011;2,341-346.
14. Anuradha K. Gajjar , Vishal D. Shah. Development and Validation of a Stability-Indicating Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe from Their Combination Dosage Forms. Eurasian Journal of Analytical Chemistry 2010;5(3),265-283.
15. Smita Sharmaa, M. C. Sharma, D. V. Kohlic, S. C. Chaturvedi. Micellar Liquid Chromatographic Method Development for Determination of Rosuvastatin Calcium and Ezetimibe in Pharmaceutical Combination Dosage Form. Der Pharma Chemica 2010; 2(1),371-377.
16. M.A. Ambasana, H.O.Kaila, R.S.Thakkar, H.T.Saravaia, A.K.Shah. Validation of an analytical method for assay of Magnesium Valproate by Gas Chromatography, International Journal of Chem Tech Research 2011;3(1),342-347.

Cite this article as:

D. Balanagamani, K. Deepthi, K. Lakshmi Sivasubramanian. Development and validation of stability indicating RP-HPLC method for Rosuvastatin calcium. Int. Res. J. Pharm. 2016;7(12):118-123 <http://dx.doi.org/10.7897/2230-8407.0712157>

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

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.