J. Biol. Chem. Chron. **2018, 4***(2),* 36-40
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Novel Analytical Method Development and Validation for Estimation of Clinical Important Rosuvastatin in Bulk and Pharmaceutical Dosage Form by UV Spectroscopy Method Using Phosphate Buffer Solubility

Suvarna G. Bhokare[1]* and R.P. Marathe[2]

[1 *] *Government College of Pharmacy (BAMU), Aurangabad - 431001, INDIA*
[2] *Yash Institute of Pharmacy, Aurangabad - 431001, INDIA*

[*] *Correspondence: E-mail: suvarna.bhokare31@gmail.com*

(Received 13 July, 2018; Accepted 21 Aug, 2018; Published 23 Aug, 2018)

ABSTRACT: Biosynthesis of cholesterol is a natural phenomenon and gets completed in lever in 25 steps. Disorder in any of the steps may cause over or under production of cholesterol that may lead ultimately to atherosclerosis, thrombosis or coronary artery disease, depending on disorder. Statins are the class of drugs that inhibit HMG CoA reductase, a rate limiting enzyme, competitively during mevalonate pathway in the synthesis of cholesterol in hepatocytes. Rosuvastatin is a synthetic drug of this class. It is newer drug with 20% bioavailability and 19 hours elimination half-life. Like other statins it principally reduces total cholesterol (Hypercholesterolemia), LDL cholesterol (Hyperlipoproteinemia), triglycerides (Hypertriglyceridemia), lipids (Dyslipidemia) and increases HDL cholesterol (Hypolipoprteinemia) to cure atherosclerosis, thrombosis and coronary artery disease. A rapid, specific and economic UV spectrophotometric method has been developed to determine the rosuvastatin content in bulk and pharmaceutical dosage formulations. At a pre-determined absorption maxima of 246nm in phosphate buffer at pH 7.4, it was proved linear in the range of 10-150µg/ml and exhibited good correlation coefficient (R^2=0.983) and excellent mean recovery (98.12% to 103.54%). This method was successfully applied to the determination of rosuvastatin and validated statistically as per recommended guideline of ICH for recovery studies, linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of rosuvastatin in bulks as well as in the commercial formulations.

Keywords: UV Spectroscopy; Method Development; Validation; Rosuvastatin and Regulatory Requirements for Drug Development.

INTRODUCTION: The first statin to be approved by US FDA was lovastatin, in 1987.[1-3] The benefits of statin therapy in reducing the morbidity and mortality of patients with coronary heart disease were first studied in the Scandinavian heart study published in 1994.[4] Since then, statins have been widely prescribed and, considering, the high prescription frequency, it is crucial to reduce the risk of the severe side-effects that are associated with statins therapy.[5] In 2001, cerivastatin, which was approved by US FDA in 1997, was withdrawn from the market after several fatal cases of myopathy and rhabdomyolysis. The mechanisms underlying these severe side effects are not fully understood; however there is a clear correlation between the risk of developing these side-effects and increased extra-hepatic exposure in humans.[6] Certain single nucleotide polymorphisms (SNP) in the SLCO1B1 gene have been shown to be associated with an increased risk of myopathy particularly at the higher statin doses. However, this correlation was not observed in rats, although it was concluded in the same investigation, that cerivastatin exhibited a higher degree of myotoxicity compared than rosuvastatin and simvastatin.[8,9] The hydrophilic nature of rosuvastatin predicts a low hepatic extraction, however involvement of multiple hepatic transport proteins result in an extensive hepatic distribution. Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme which mediates the conversion of HMGCoA to mevalonate as a part of the biosynthesis of cholesterol in the liver. Reduced intracellular cholesterol causes an up-regulation of low-densitylipoprotein receptors on the cell surface; the increased number of cell receptors further enhances the clearance of cholesterol from the blood. Rosuvastatin was approved by the US Food and Drug administration (FDA) in 2003.[10-12] Apart from rosuvastatin, there are seven statins, namely, atorvas-

tatin, fluvastatin, pravastatin, cerivastatin, pitavastatin, lovastatin and simvastatin. Rosuvastatin is the most potent inhibitor of HMG-CoA reductase of the statins, partly explained by it having the highest number of interaction sites with the enzyme as shown by x-ray crystallography compared to the other statins.[13-18] Analysis is an important component in the formulation development of any drug molecule.[19-26] A suitable and validated method has to be available for the analysis of drug in the bulk, in drug delivery systems, from release dissolution studies and in biological samples.[27-33] If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. The efficient analytical method development and its validation are critical elements in the development of pharmaceuticals.[34-39] An analytical method is selected on the basis of criteria such as accuracy, precision, sensitivity, selectivity, robustness, ruggedness, and the amount of available sample, the amount of analyte in the sample, time, cost, and availability of equipment.[40-43] Thus, present study was undertaken to develop and validate a simple sensitive, accurate, precise and reproducible UV method for rosuvastatin.

MATERIAL AND METHODS:

Instruments: The analysis was performed by using the electronic balance (Model-As22201xs, Rodwag Wagi Electronics, Switzerland), pH meter (Cyber scan), UV-spectrophotometer (UV-1800 Shimadzu, Japan).

Reagents and solutions: Rosuvastatin IP grade was obtained from MSN Laboratories Telangana and reagents for phosphate buffer were used as analytical grade.

Preparation of solvent system:

Phosphate buffer of pH 7.4: 50ml of 0.2M potassium dihydrogen phosphate was placed in 200 ml volumetric flask, 39.1 ml of 0.2M sodium hydroxide was added, water was added to volume and pH adjusted to 7.4 accordingly.

Standard stock solution of rosuvastatin: 100mg of rosuvastatin weighted accurately and transferred into a 100ml volumetric flask containing phosphate buffer. Solution sonicated to dissolve rosuvastatin and cooled at room temperature and mix well. Pipette out 10ml solution and transferred into 100ml volumetric flask resulting solution contained 100µg/ml solution (Stock solution A). Pipette out 10, 20, 30, 40, 50ml solution respectively of standard stock solution A and transferred into a 100ml volumetric flask, mixed well and diluted up to volume with buffer solution. The resulting solution contains 10 to 50µg/ml of rosuvastatin.

Spectral study: The final stock solution scanned in UV spectrophotometer over the range 200-400nm (Figure 1).

Validation of the Method:

Linearity and Range: A series of dilutions were prepared in the concentration range of 10-50µg/ml. Separate calibration curve was plotted between concentration Vs response and slope, intercept and correlation coefficient value (r^2) was determined.

Accuracy: To test accuracy, recovery studies were performed. To a preanalyzed sample solution, a definite quantity of known concentration of standard drug solution was added and then its recovery was studied. Different concentration of pure drug was added to preanalyzed sample, and then the solution was analyzed to determined recovery study.

Precision: A standard stock solution of drug was prepared in same manner. The tests were repeated thrice for all selected concentrations. The intermediate precision was performed by doing repeatability, day-to-day variation, and analyst-to-analyst variation.

RESULTS AND DISCUSSION: The methods discuss in the present work provide a convenient, precise and accurate way for estimation of rosuvastatin in bulk as well as in pharmaceutical dosage form. An absorption maximum of rosuvastatin was selected at 246nm for the analysis. Regression analysis showed linearity over the concentration range of 10-150µg/ml with correlation coefficients of 0.983 (Figure 2).

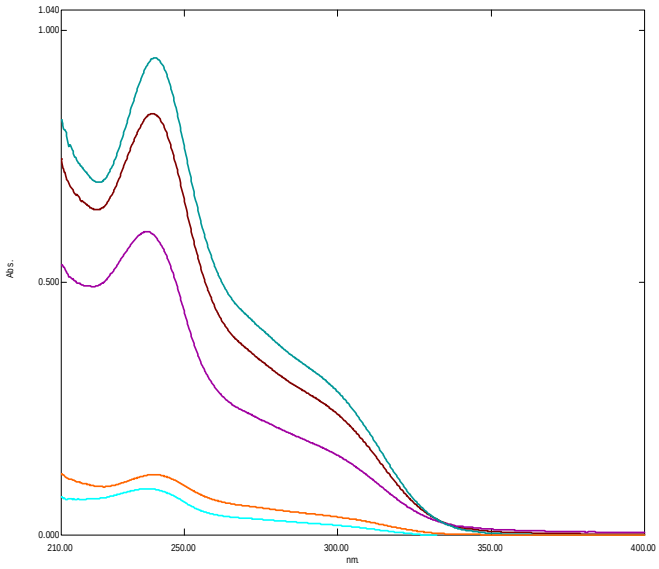

Figure 1: UV Spectra of Rosuvastatin.

The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of present method was ascertained by recovery studies and the results are expressed as percentage recovery. Percentage recovery for rosuvastatin was found within the range of 98.12 % and 103.54% as prescribed by ICH guideline. The assay for rosuvastatin was found to be 102.00±0.85. The % RSD value for rosuvastatin was found to be less than 2%. In this study method development and validation of the rosuvastatin was carried out by UV Spectroscopy and found satisfactory. The result of developed method and validation was given in table 1.

Table 1: Result of method development and validation.

Method	Parameter	Observation	SD*	RSD*
Linearity	Slope and Y-Intercept	0.012x – 0.010	-	-
	R^2	0.983		
Accuracy	% Recovered	98.12 to 103.54	0.4521	0.692
Precision	Repeatability	Method was found repeatable (Validated three times as per ICH guideline)	0.3154	0.821
	Analyst to analyst	Method was found precise when performed by different analyst (Validated three times as per ICH guideline)	0.4581	0.752
	Day to day	Method was found precise when performed three consecutive days (Validated three times as per ICH guideline)	0.5873	0.698
Ruggedness & Robustness		Compiled (Validated three times as per ICH guideline)	0.5214	1.652

**Each value is mean of three replicates*

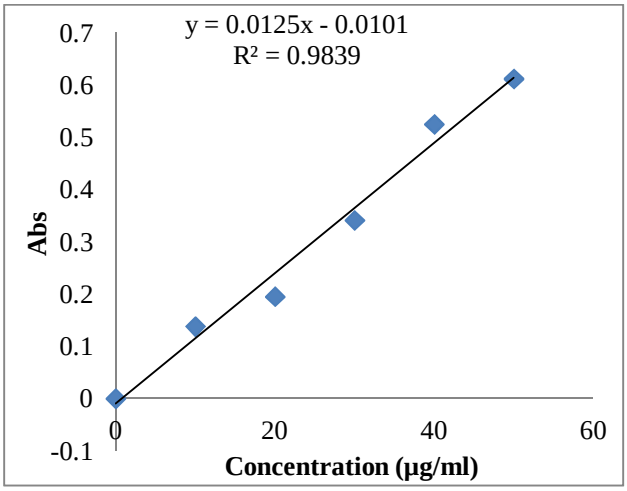

Figure 2: Calibration curve of Rosuvastatin.

CONCLUSION: The analytical method for determination of rosuvastatin has been validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for determination of rosuvastatin in bulk as well as tablet formulation.

REFERENCE

1. Abd-Elsalam, W. H., El-Helaly, S. N., Ahmed, M. A., & Al-Mahallawi, A. M. (2018) Preparation of novel phospholipid-based sonocomplexes for improved intestinal permeability of rosuvastatin: In vitro characterization, dynamic simulation, Caco-2 cell line permeation and in vivo assessment studies, *International Journal of Pharmaceutics*, 548(1), 375-384. https://doi.org/10.1016/j.ijpharm.2018.07.005
2. Al-Kuraishy, H. M., Al-Gareeb, A. I., & Al-Buhadily, A. K. (2018) Rosuvastatin as forthcoming antibiotic or as adjuvant additive agent: In vitro novel antibacterial study, *Journal of Laboratory Physicians*, 10(3), 271-275. https://doi.org/10.4103/JLP.JLP_170_17
3. Husain, I., Akhtar, M., Madaan, T., Abdin, M. Z., Islamuddin, M., & Najmi, A. K. (2018) Rosuvastatin alleviates high-salt and cholesterol diet-induced cognitive impairment in rats via Nrf2-ARE pathway, *Redox Report : Communications in Free Radical Research*, 23(1), 168-179. https://doi.org/10.1080/13510002.2018.1492774
4. Gupta, A. (2018) Systematic approach to novel drug delivery system. A & V Publication India. Ist edition, 58-64.

5. Ishida, K., Ullah, M., Toth, B., Juhasz, V., & Unadkat, J. D. (2018) Transport Kinetics, Selective Inhibition, and Successful Prediction of In Vivo Inhibition of Rat Hepatic Organic Anion Transporting Polypeptides, *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, 46(9), 1251-1258. https://doi.org/10.1124/dmd.118.080770

6. Kim, W., Yoon, Y. E., Shin, S. H., Bae, J. W., Hong, B. K., Hong, S. J., Park, C. G. (2018) Efficacy and Safety of Ezetimibe and Rosuvastatin Combination Therapy Versus Those of Rosuvastatin Monotherapy in Patients With Primary Hypercholesterolemia, *Clinical Therapeutics*, 40(6), 993-1013. https://doi.org/10.1016/j.clinthera.2018.04.015

7. Liu, Y., Chen, T., & Xing, J. (2018). Effect of rosuvastatin on the expression of candidate gene GALNT3 in atherosclerosis. *Experimental and Therapeutic Medicine*, *15*(6), 4880-4884. https://doi.org/10.3892/etm.2018.6008

8. Liu, Z. Q., Yin, H. H., Zhang, X. J., Zhou, R., Wang, Y. M., & Zheng, Y. G. (2018) Improvement of carbonyl reductase activity for the bioproduction of tert-butyl(3R,5S)-6-chloro-3,5-dihydroxyhexanoate, *Bioorganic Chemistry*, 80, 733-740. https://doi.org/10.1016/j.bioorg.2018.07.025

9. Moid, M., Afzal, S., Rahim, N., Ali, T., Iffat, W., Bashir, L., & Naz, S. (2018) High performance liquid chromatographic method validation for determination of rosuvastatin calcium in tablet dosage forms, *Pakistan Journal of Pharmaceutical Sciences*, 31(4(Supplementary)), 1577-1582.

10. Mosepele, M., Molefe-Baikai, O. J., Grinspoon, S. K., & Triant, V. A. (2018) Benefits and Risks of Statin Therapy in the HIV-Infected Population, *Current Infectious Disease Reports*, 20(8), 20-26. https://doi.org/10.1007/s11908-018-0628-7

11. Naumovska, Z., Nestorovska, A. K., Grozdanova, A., Hristova, K., Dimovski, A., Suturkova, L., & Sterjev, Z. (2018). Evaluation of statin utilization in the Republic of Macedonia during 2013-2016, *ClinicoEconomics and Outcomes Research: CEOR*, *10*, 339-347. https://doi.org/10.2147/CEOR.S157842

12. Soko, N. D., Masimirembwa, C., & Dandara, C. (2018) A cost effective RFLP method to genotype Solute carrier organic anion 1B1 (SLCO1B1) c.1929A>C (p.Leu643Phe, rs34671512); a variant with potential effect on rosuvastatin pharmacokinetics. *BMC Research Notes*, *11*(1), 384-388. https://doi.org/10.1186/s13104-018-3469-4

13. Tokuhisa, H., Murai, H., Okabe, Y., Hamaoka, T., Sugimoto, H., Mukai, Y., Takamura, M. (2018) Differential effects of lipophilic and hydrophilic statins on muscle sympathetic nerve activity in heart failure with preserved left ventricular ejection fraction. *Autonomic Neuroscience : Basic & Clinical*, *213*, 8-14. https://doi.org/10.1016/j.autneu.2018.04.006

14. Gupta, A. (2018) Basic concepts in clinical pharmacy practice. A & V Publication India. Ist edition, 78-85.

15. Gupta, A. (2018) Insight of solubility. A & V Publication India. Ist edition, 12-26.

16. Wang, L., Wang, Y., Wang, H., Zhou, X., Wei, X., Xie, Z., Mu, J. (2018) The influence of the intestinal microflora to the efficacy of Rosuvastatin, *Lipids in Health and Disease*, 17(1), 151-158. https://doi.org/10.1186/s12944-018-0801-x

17. Wang, L., Lin, R., Guo, L., & Hong, M. (2018) Rosuvastatin relieves myocardial ischemia/reperfusion injury by upregulating PPARgamma and UCP2, *Molecular Medicine Reports*, *18*(1), 789-798. https://doi.org/10.3892/mmr.2018.9062

18. Zhou, C.-Z., Pan, S.-L., Lin, H., Meng, L.-P., Ji, Z., Chi, J.-F., & Guo, H.-Y. (2018) [Effects of rosuvastatin in homocysteine induced mouse vascular smooth muscle cell dedifferentiation and endoplasmic reticulum stress and its mechanisms], *Zhongguo ying yong sheng li xue za zhi = Zhongguo yingyong shenglixue zazhi Chinese journal of applied physiology*, *34*(1), 43-48. https://doi.org/10.12047/j.cjap.5489.2018.012

19. Gupta, A., Yadav, V., & Rawat, S. (2011) An analytical approach for the determination of amoxicillin and potassium clavulanate in pharmaceutical dosage form. Drug Invention Today, 3(4).

20. Shah, Y., Iqbal, Z., Ahmad, L., Khan, A., Khan, M. I., Nazir, S., & Nasir, F. (2011) Simultaneous determination of rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detection: Method development, validation and optimization of various experimental parameters, *Journal of Chromatography B*, 879(9-10), 557-563.

21. Gomes, F. P., Garcia, P. L., Porto Alves, J. M., Singh, A. K., Kedor-Hackmann, E. R. M. & Miritello Santoro M. I. R. (2009) Development and validation of stability-indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atorvastatin, and rosuvastatin in pharmaceuticals, *Analytical letters*, 42(12), 1784-1804.

22. Rawat, S., Sangali, S., & Gupta, A. (2018) Formulation and Evaluation of Floating Matrix Tab-

lets of Acyclovir using 32 Factorial Design, *Research Journal of Pharmaceutical Dosage Forms and Technology*, 10(1), 01-09.

23. Pasha, M. K., Muzeeb, S., Basha, S. J. S., Shashikumar, D., Mullangi, R., & Srinivas, N. R. (2006) Analysis of five HMG-CoA reductase inhibitors-atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies, *Biomedical Chromatography*, 20(3), 282-293.

24. Gupta, A., Rawat, S., Gandhi, M., & Namdeo, R. (2011) Simultaneous Estimation of Amoxycillin and Potassium Clavulanate in Injection Formulation by Simultaneous Equation Method, *Journal of Pharmacy Research*, 4(4), 1244-1245.

25. ICH, Q2A Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva, October 1994, pg 1-8.

26. Gupta, A., Yadav, V., Yadav, J. S., & Rawat, S. (2011) An analytical approach of doxofylline: a review, *Asian journal of pharmaceutical analysis*, 1(4), 67-70.

27. Trivedi, R. K., Kallem, R. R., Mullangi, R., & Srinivas, N. R. (2005) Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC–MS/MS with electrospray ionization: assay development, validation and application to a clinical study, *Journal of pharmaceutical and biomedical analysis*, 39(3-4), 661-669.

28. Shah, B. B., Patel, B. B., Gohil, K. N., & Patel, P. M. (2012) Difference Spectrophotometric Method Development and Validation For Simultaneous Estimation of Rosuvastatin Calcium and Telmisartan in Bulk and Combined Dosage Form, *International Journal of Research in Pharmacy & Science*, 2(2)15-21.

29. Gupta, A., Rawat, S., & Pandey, A. (2011) Method Development and Photolytic Degradation Study of Doxofylline by RP-HPLC and LC-MS/MS, *Asian J Pharm Anal*, 1, 29-33.

30. Harvey D., Modern Analytical Chemistry, 1st edition, McGraw-Hill Publication, Kingsport-USA, 2000, pg 1-2.

31. Gupta, A., Rajkumar, V. Y., & Rawat, S. (2011) Method development and alkali degradation study of doxofylline by RP-HPLC and LC-MS/MS, *Drug Invention Today*, 3(4), 30-32.

32. Skoog D. A. et al; Principles of Instrumental Analysis, 5th edition, Thomson Books, Delhi, 2001, pg 11-16.

33. Rawat, S., & Gupta, A. (2011) Spectrophotometric method for simultaneous estimation of nimesulide and diclofenac sodium in pharmaceutical dosage forms, *Asian Journal of Pharmaceutical Analysis*, 1(4), 85-87.

34. Pratik P., et al. (2012) Spectrophotometric method Development and Validation for estimation of alpha lipoic acid in tablet dosage form, *Intl J of Pharmaand Pharm sci.*, 5, 519-522.

35. Rawat, S., & Gupta, A. (2011) Regulatory requirements for drug development and approval in United States: a review. Asian J. Pharm. Res, 1(1), 01-06.

36. Reddy, G. V. R., Reddy, B. V., Haque, S. W., Gautam, H. D., Kumar, P., Kumar, A. P., & Park, J. H. (2011) Development and validation of a stability-indicating UPLC method for rosuvastatin and its related impurities in pharmaceutical dosage forms, *Quimica Nova*, 34(2), 250-255.

37. Trivedi, H. K., & Patel, M. C. (2012) Development and validation of a stability-indicating RP-UPLC method for determination of rosuvastatin and related substances in pharmaceutical dosage form, *Scientia pharmaceutica*, 80(2), 393.

38. Rawat, S., & Gupta, A. (2011) Development of novel HPTLC method for estimation of Qurcetine in Ocimum sanctum, *Asian J. Pharm. Tech*, 1(4), 149-151.

39. Chirag et al. (2014) Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms, *Der Pharma Chemica*, 6, 303-311.

40. Gupta, A., Rajkumar, V. Y., & Rawat, S. (2011) Simultaneous estimation of amoxycillin and potassium clavulanate in injection formulation by dual wavelength spectrophotometry, *Drug Invention Today*, 3(4).

41. Sevda, R. R., Ravetkar, A. S., & Shirote, P. J. (2011) UV Spectrophotometric estimation of rosuvastatin calcium and fenofibrate in bulk drug and dosage form using simultaneous equation method, *Int. J. Chem. Tech. Res*, 3, 629-635.

42. Najma, S., Arayne, M. S., & Safila, N. (2011) Validated Method for the Simultaneous Determination of Lisinopril, Pravastatin, Atorvastatin and Rosuvastatin in API, Formulations and Human Serum by RP-HPLC, *Chinese Journal of Chemistry*, 29(6), 1216-1220.

43. Gupta, A., Yadav, V., Yadav, J. S., & Rawat, S. (2011) An analytical approach of doxofylline: a review, *Asian journal of pharmaceutical analysis*, 1(4), 67-70.