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Development and Validation for Simultaneous Estimation of Rosuvastatin Calcium and Clopidogrel Bisulfate in Pharmaceutical Dosage Form by Reverse Phase-High Performance Liquid Chromatography

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Abstract

Aim: The present work was focused on the development and validation of reversed phase high performance liquid chromatography (RP-HPLC) method which is simple, rapid, precise, accurate, sensitive, and economical for the quantification of rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet formulation. **Methods:** The separation was attained on reversed phase Princeton (C18) column with dimensions (250×4.6 mm, 5µ) employing buffer which is mixture of water (pH 3.0, adjusted with ortho phosphoric acid) and methanol in the ratio (20:80) v/v as mobile phase, at flow rate 1.0 ml/min and detection was carried out at wavelength 240nm. The retention time under optimized condition of rosuvastatin calcium and clopidogrel bisulfate was found to be 2.844 min and 4.388 min respectively. **Results:** The linearity of the method was demonstrated in the concentration range of 6-16 µg/ml and 45-120 µg/ml for rosuvastatin calcium and clopidogrel bisulfate with a correlation coefficient (r^2) of 0.9999 and 0.9996 respectively. The percentage relative standard deviation was <2% and percentage recovery was found to be 100.12-101.37% and 99.72-101.09% for rosuvastatin calcium and clopidogrel bisulfate respectively. Assay of marketed tablet formulations was found to be 98.99% and 99.92% respectively. **Conclusion:** The developed RP-HPLC method was found to be simple, specific, sensitive, rapid, linear, accurate, precise and economical and could be used for regular quality control of rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet formulations.

Keywords
Rosuvastatin calcium, Clopidogrel bisulfate, RP-HPLC, Validation.

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INTRODUCTION

Rosuvastatin calcium (RSV), is chemically (3R, 5R) -7- [4- (4-Fluorophenyl)-2 [methyl (methylsulfonyl) amino] - 6 - propan-2-ylpyrimidin - 5-yl] -3, 5-dihydroxyhept -6enoic acid (Fig. 1). It is HMG-CoA reductase inhibitor used in the treatment of hypertension, abnormal lipid. Clopidogrel bisulfate (CLO), is chemically Methyl 2-(2- Chlorophenyl) -2-(6, 7-dihydro thieno [3, 2-C] Pyridine-5(4H)-yl) Acetate sulphate (Fig. 2). It is an Anti-platelet agent as an ADP receptor blocker mainly to treat patients with acute coronary syndrome, myocardial infarction (MI), peripheral vascular disease and some stroke (Ischemic type) patients [1-4]. An extensive literature

survey revealed that several HPLC methods were reported for the estimation of rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet formulation [5-19]. The proposed method is simple, accurate, reproducible, economical and suitable for routine determination of rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet formulations. The method was validated in compliance with ICH guidelines [20-22].

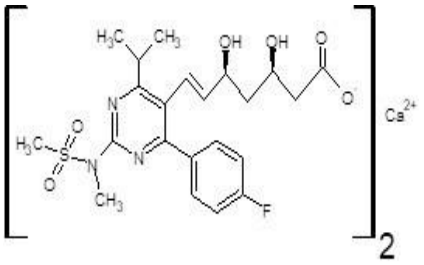

Figure 1: Chemical structure of rosuvastatin calcium (RSV)

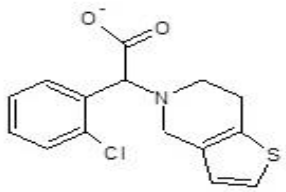

Figure 2: Chemical structure of clopidogrel bisulfate (CLO)

MATERIALS AND METHODS

Chemicals and Reagents:

Pharmaceutical grade Rosuvastatin calcium and Clopidogrel bisulfate were procured as a gift samples from Cadila Pharmaceuticals Ltd., Ahmedabad (India), Rosmi-CV a tablet formulation, obtained commercially. Methanol, ortho-phosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide 30% of analytical grade were used throughout the work.

Instrumentation:

The HPLC instrument used was SHIMADZU LC-6AD system equipped with a photodiode array detector PDA FRC-10A.

Selection of Solvents:

On the basis of solubility study methanol was selected as the solvent for dissolving RSV and CLO.

Chromatographic Conditions:

Chromatographic separation was achieved on a reverse phase Princeton (C18) column with dimensions (250×4.6 mm, 5µ) at ambient temperature using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with ortho phosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. Detection was carried out at 240nm. The mobile phase system after preparation was filtered through a membrane filter (0.22µm) and sonicated for 10 minutes. The pH of the mobile phase was set at 3.0, Injection volume used for assay studies was 10µl. The optimized chromatographic condition is shown in Table 1.

Table 1: Optimized chromatographic condition

Chromatographic condition	
Mobile phase	Water (pH adjusted to 3.0 with ortho phosphoric acid): Methanol (20:80) v/v
Flow rate	1.0 ml/min.
Column	Princeton C18 (250×4.6mm, 5µ)
Detector wavelength	240nm
Column temperature	30°C
Injection volume	10 µl
Runtime	20 min.
Diluent	Methanol
Retention time	About 2.844 min. for Rosuvastatin calcium peak and 4.388 min. for Clopidogrel bisulfate peak

Preparation of standard solution of RSV and CLO:

For RSV, an accurately weighed 1.0 mg of RSV was transferred to 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 10µg/ml of RSV.

For CLO, an accurately weighed 7.5 mg of CLO was transferred to 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 75µg/ml of CLO.

Preparation of working solution of RSV and CLO:
For the working mixed standard solution, an accurately weighed 1.0 mg of RSV and 7.5 mg of CLO were transferred to 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 10µg/ml and 75µg/ml of RSV and CLO respectively.

Preparation of sample solution of RSV and CLO:
Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 1.0 mg of RSV and 7.5 mg of CLO was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of methanol was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of filtrate was transferred to a 10 ml volumetric flask and then volume was made up to the mark with methanol to furnish a sample solution containing 10µg/ml of RSV and 75µg/ml of CLO. Six replicate of tablet powder equivalent to 1.0 mg of RSV and 7.5 mg of CLO were transferred into six 10.0 ml volumetric flask and homogenous sample solutions were prepared in a similar manner.

Method Validation:
The developed method was validated in accordance with ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness.

Accuracy:
The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value, and the value found. The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e., 80, 100 and 120% of the label claim. Standard addition and recovery experiments were conducted to determine the accuracy of RSV and CLO for the quantification of drug in the samples.

Precision:
The system precision was evaluated by measuring area of six qualified working standard for RSV and CLO and calculating the percentage of relative standard deviation (RSD). The assay method precision was evaluated by conducting six independent assays of test samples of RSV and CLO against qualified working standards and calculating the percentage of relative standard deviation (RSD). The intermediate precision of the method was also verified using different analysts and different days.

Linearity:
Linearity test solutions of RSV and CLO were prepared at concentration levels of 6-16µg/ml and 45-120µg/ml respectively. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by least-squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of rosuvastatin calcium and clopidogrel bisulfate were shown in Fig. 3 and Fig. 4, respectively.

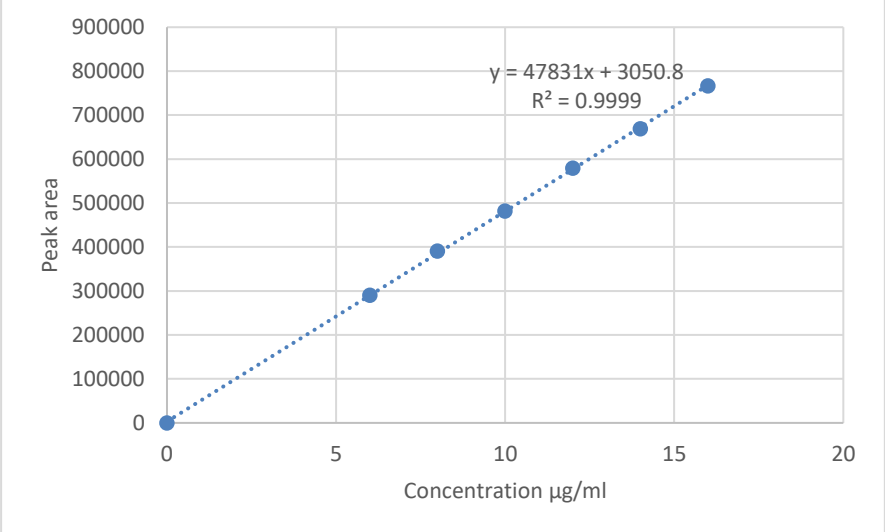

Figure 3: Linear curve of rosuvastatin calcium (RSV)

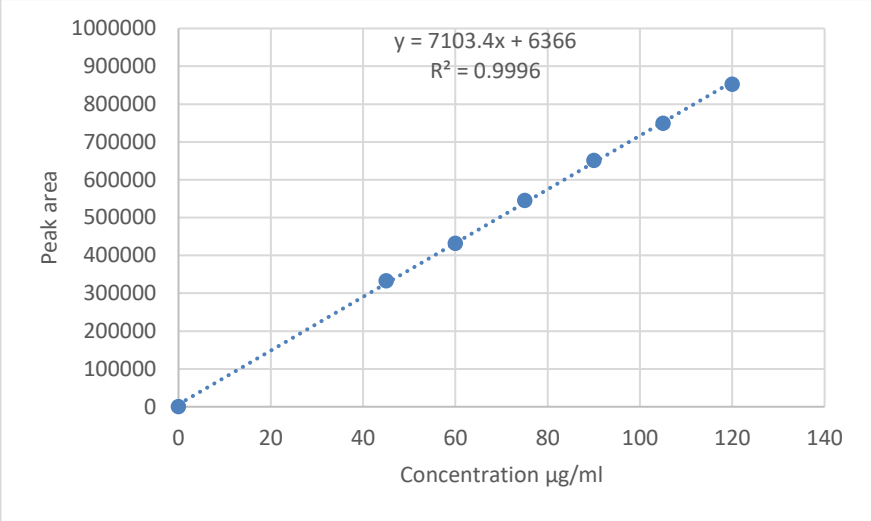

Figure 4: Linear curve clopidogrel bisulfate (CLO)

Specificity:

Specificity of the developed method was established by comparing the chromatograph of standard and sample. It was found that there was no interference due to excipients and impurities at the retention time of the drug.

Limit of detection (LOD) and Limit of quantitation (LOQ):

The LOD is the lowest analyte concentration that can be detected. LOQ is the lowest analyte concentration that can be quantified with acceptable accuracy and precision. The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of calibration plot. LOD and LOQ were established, in accordance with ICH definitions, by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot.

Robustness:

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between RSV and CLO was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by ± 2 nm, i.e., 238 and 242 nm from the actual wavelength, 240 nm. To study the effect of flow rate on estimation, flow rate was altered by ± 0.1 ml/min i.e., 0.9 and 1.1 ml/min from the actual flow rate, 1.0 ml/min.

RESULTS AND DISCUSSION

HPLC method development and optimization:

Initially, pure drugs solution was chromatographed using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with ortho phosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. gives well resolved peaks of drugs as well. Detection was carried out at 240nm. The retention time under optimized condition of rosuvastatin calcium and clopidogrel bisulfate was found to be 2.844 min & 4.388 min respectively. The total run time of chromatogram was about 20 min. A typical chromatograph of mixture of standard and sample of RSV and CLO is summarized by Fig. 5 and Fig. 6 respectively.

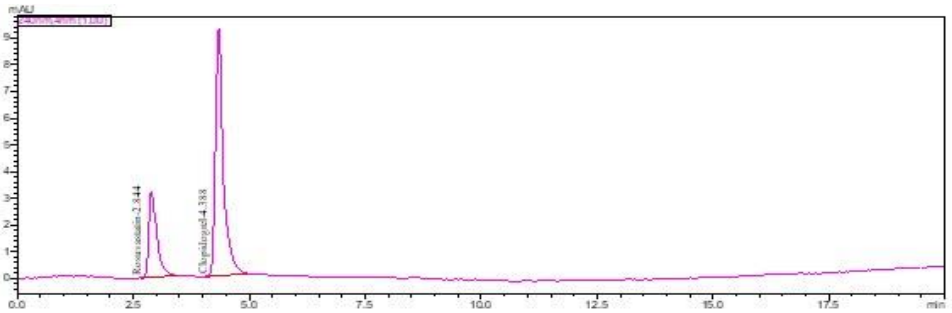

Figure 5: A typical chromatograph of mixture of standard RSV and CLO

Rosuvastatin calcium (RSV) and Clopidogrel bisulfate (CLO) with Rt of 2.844 min. and 4.388 min. respectively.

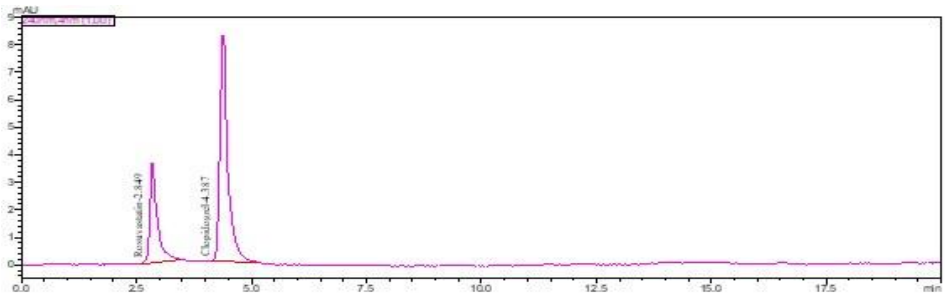

Figure 6: A typical chromatograph of mixture of sample RSV and CLO
Rosuvastatin calcium (RSV) and Clopidogrel bisulfate (CLO) with Rt of 2.849 min. and 4.387 min. respectively.

Validation of the method:

System Suitability:

Suitability of the system was demonstrated by assessing various parameters. It was established by injecting six replicate injections of the standard solution. Theoretical plates were found to be 2816 and 3811, tailing factor of 1.60 and 1.54, and %RSD of peak area was 0.9 and 0.8 for RSV and CLO respectively (Table 2). All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Table 2: System suitability results

Parameter	RSV	CLO
Theoretical Plate	2816	3811
Retention Time	2.844	4.388
Tailing Factor	1.60	1.54
% RSD	0.9	0.8

Rt: Retention time, %RSD: Percentage relative standard deviation

Table 5: Robustness results

Condition	RSV		CLO	
	Amount estimated [%]	RSD [%]	Amount estimated [%]	RSD [%]
Change in wavelength 238 nm	97.28	0.5698	100.16	0.0900
(240±2 nm) 242 nm	98.21	0.5733	100.08	0.1417
Change in flow rate 0.9 ml/min	99.55	0.6381	99.37	0.2068
(1.0±0.1 ml/min) 1.1 ml/min	99.24	0.5078	99.56	0.2009

%RSD: Percentage relative standard deviation

Table 6: Summary of validation parameter

Parameter	RSV	CLO
Calibration range (µg/ml)	6-16	45-120
Optimized wavelength (nm)	240	240
Retention Time	2.844	4.388
Regression equation (Y)	Y = 47831x+3050.8	Y = 7103.4x+6366
Slope	47831	7103.4
Intercept	3050.8	6366
Coefficient correlation (r^2)	0.9999	0.9996
Precision (% RSD)		
Intraday	0.6	0.8
Interday	0.9	0.4
% Assay	98.99	99.92
LOD (µg/ml)	0.65	6.90
LOQ (µg/ml)	1.98	20.91

%RSD: Percentage relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

Linearity

Linearity was established by least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range 6-16 µg/ml for RSV and 45-120 µg/ml for CLO. Peak areas were plotted against the respective

concentrations and linear regression analysis performed on the resulting curves. Equation for the calibration plots of RSV was Y=47831x+3050.8. Equation for the calibration plots of CLO was Y=7103.4x+6366, Correlation coefficient for RSV and CLO was 0.9999 and 0.9996, respectively. The results of linearity were shown in Table 3.

Table 3: Linearity results

Parameter	RSV	CLO
Concentration Range (µg/ml)	6-16	45-120
Slope (m)	47831	7103.4
Tailing factor	3050.8	6366
Coefficient correlation (r^2)	0.99999	0.9996

Table 4: Recovery results

Drug (%)	Spiked level	Amount taken (µg/ml)	Amount found (µg/ml)	%Recovery
RSV	80	8	8.11	101.37
	100	10	10.01	100.12
	120	12	12.05	100.41
CLO	80	60	59.83	99.72
	100	75	75.81	101.09
	120	90	90.75	100.83

%Recovery: Percentage recovery

Accuracy

The percentage recoveries were 99.66-100.37% and 99.72-101.09% for RSV and CLO, respectively. The %RSD value was found to be less than 2%. The results of recovery are shown in Table 4.

Precision

The result of intraday for RSV and CLO was 0.9 and 0.8 respectively. The result of interday for RSV and CLO was 0.6 and 0.4, respectively. The percentage RSD of system, method and intermediate precision study was well within ± 2.0%, indicate that the method was precise.

LOD and LOQ

The LOD of RSV and CLO was 0.65 and 6.90 µg/ml. respectively. The LOQ of RSV and CLO was 1.98 and 20.91 µg/ml. respectively.

Robustness:

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between RSV and CLO was evaluated. To study the effect of wavelength and effect of flow rate on the estimation. The results of robustness are shown in Table 5.

Table 5: Robustness results

Condition		RSV		CLO	
		Amount estimated [%]	RSD [%]	Amount estimated [%]	RSD [%]
Change in wavelength (240±2 nm)	**238 nm**	97.28	0.5698	100.16	0.0900
	242 nm	98.21	0.5733	100.08	0.1417
Change in flow rate (1.0±0.1 ml/min)	**0.9 l/min**	99.55	0.6381	99.37	0.2068
	1.1 l/min	99.24	0.5078	99.56	0.2009

%RSD: Percentage relative standard deviation

Analysis of rosuvastatin calcium and clopidogrel bisulfate from marketed tablets

Percentage assay of tablet formulation was found to be 98.99% and 99.92% for RSV and CLO respectively. % RSD 0.9 and 0.8 for RSV and CLO respectively, indicates the stability of the method for 24 h. The results of various validation parameter are summarized in Table 6. Hence, the method was found to be specific.

Table 6: Summary of validation parameter

Parameter	RSV	CLO
Calibration range (µg/ml)	6-16	45-120
Optimized wavelength (nm)	240	240
Retention Time	2.844	4.388
Regression equation (Y)	Y = 47831x+3050.8	Y = 7103.4x+6366
Slope	47831	7103.4
Intercept	3050.8	6366
Coefficient correlation (r^2)	0.9999	0.9996
Precision (% RSD)		
Intraday	0.6	0.8
Interday	0.9	0.4
% Assay	98.99	99.92
LOD (µg/ml)	0.65	6.90
LOQ (µg/ml)	1.98	20.91

%RSD: Percentage relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical and sensitive analysis of rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet dosage form. This method was validated as per ICH guidelines. The method can therefore be used for routine quality-control analysis rosuvastatin calcium and clopidogrel bisulfate in bulk and tablet dosage form.

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REFERENCES

1. https://en.wikipedia.org/wiki/rosuvastatin
2. https://en.wikipedia.org/wiki/clopidogrel
3. The United States Pharmacopeia 29; National Formulary 24, U. S. Pharmacopeial Convention, 796, (2007).
4. The United States Pharmacopeia 29; National Formulary 24, U. S. Pharmacopeial Convention, 1280, (2007)
5. Rajput P., Shah D.B., Maheshwari D.G., A review on chromatographic method for estimation of Rosuvastatin calcium. Int J Res Pharmacy Pharm Sci, 3(1): 28-31, (2018).
6. Thammera R.K., Shitut N.R., Pasikanti K.K., Menon V.C.A., Venkata V.P.K., Mullangi R., Determination of Rosuvastatin in rat plasma by HPLC and its application to pharmacokinetic studies. Biomed Chromatogr, 20(9): 881-887, (2006).
7. SirishaMulukuri N.V., Srinivasarao T., Raveendra B.G., New RP-HPLC method development and validation for the estimation of rosuvastatin calcium in bulk drugs and formulations. J Pharm Res, 11: 257-60, (2017).
8. Rajput S.J., George R.K., Ruikar D.B., Chemometric Simultaneous Estimation of Clopidogrel bisulfate and Aspirin from combined dosage form. Indian J Pharm Sci, 70: 450-454, (2008).
9. Gosula V.R.R., Bobba V.R., Syed W.H., Haum D.G., Poonam K., Development and validation of a stability indicating UPLC method for rosuvastatin and its related impurities in pharmaceutical dosage forms. Quim Nova, 34: 250-255, (2011).
10. Singh S.S., Sharma K., Patel H., Jain M., Shah H., Gupta S., Estimation of Rosuvastatin in Human plasma by HPLC Tandem Mass Spectroscopic method and its application to Bioequivalence study. J Braz Chem Soc, 16(5): 944-950, (2005).
11. Chaudhari P.B., Pawar P.D., Narkhede K.P., Stability indicating spectrophotometric method for determination and validation of Clopidogrel bisulfate in tablet dosage form. Int J Res Ayur Pharm, 1: 418-423, (2010)
12. Savani P., Chauhan S., Jain V., Raj H., Patel S., Development and Validation of Analytical Method for Clopidogrel Bisulphate and Irbesartan by Simultaneous Equation Spectroscopic Method. Asian Journal of Pharmaceutical Analysis, DOI: 10.5958/2231-5675.2016.00015.6
13. Sheth A., Patel K.N., Ramlingam B., Shah N., Simultaneous Estimation of Rosuvastatin Calcium and Clopidogrel Bisulphate From Bulk and Commercial Products Using A Validated RP-HPLC Techniques. International Research Journal of Pharmacy, 3(11): 154-157, (2012)
14. Bhagat D., Mannur V., Mastiholimath V., Development and Validation of RP-HPLC Method for the Estimation of Clopidogrel Bisulphate. Malaysian Journal of Analytical Sciences, 17(3): 387-393, (2013)
15. Sajjanwar R., Bhaskaran S., Kakati K., Jha S.K., A Validated Reverse Phase-HPLC Method for The Simultaneous Estimation of Clopidogrel Bisulfate and Rivaroxaban in Pharmaceutical Application. Journal

of Applied Pharmaceutical Research, 3(3): 09-16, (2015)
16. Devkare P.N., Jain H.K., Development and Validation of RP-HPLC Method for Simultaneous Estimation of S (-) Amlodipine Besylate and Clopidogrel Bisulphate in Tablet Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3): 770-775, (2013)
17. Pisal P., Nigade G., Kale A., Pawar S., Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Determination of Aspirin, Rosuvastatin, Clopidogrel in Bulk and Pharmaceutical Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences, 10(10): 51-56, (2018)
18. Porwal P.K., Ahmad R.A., Chhajed S.S., Chatpalliwar V.A., Liquid Chromatographic Method for Simultaneous Quantitation of Clopidogrel, Aspirin and Atorvastatin in Rat Plasma and Its Application to the Pharmacokinetic Study. Journal of Chromatographic Science, 53:1155-1162, (2015)
19. Masud A.A., Begum I., Development and Validation of A RP-HPLC Method for Simultaneous Estimation of Aspirine and Clopidogrel in Combined Tablet Dosage Form. International Journal of Pharmaceutical Sciences and Research, 7(11): 4443-4448, (2016)
20. ICH, Q2A, Harmonized Tripartite Guideline, Test on Validation of Analytical Procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March, (1994).
21. ICH, Q2B, Harmonized Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March (1996).
22. ICH Guidance on Analytical Method Validation, in: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, and September, (2002).