International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Analytical Method Development and Validation for the Simultaneous Estimation of Rosuvastatin and Clopidogrel by RP-HPLC Method

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ABSTRACT

A new method was established for simultaneous estimation of Rosuvastatin and Clopidogrel by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Rosuvastatin and Clopidogrel by using: Agilent C_{18} (4.6 x 150mm, 5µm) , 5□m column in gradient mode using mobile phase composition of Methanol: Phosphate buffer P^H 4(60:40) with flow rate of 0.8ml/min at 254nm. detection wavelength was 254nm. The instrument used was WATERS, software: Empower, 2695 separation module. 2996 PDA detector., Empower-software version-2. The retention times were found to be 2.972 mins and 3.548 mins. The % purity of Rosuvastatin and Clopidogrel was found to be 101.27% and 99.97% respectively. The system suitability parameters for Rosuvastatin and Clopidogrel such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Rosuvastatin and Clopidogrel was found in concentrationof 20 µg/ml and 20 µg/ml respectively coefficient (r^2) was found to be 0.99932 and 0.99916 % recovery was found to be 0.23793 and 0.5232 %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable.LOD value was 3.041 and 9.79, and LOQ value was 3.08 and 10.37 respectively.

Keywords: Agilent C18, Rosuvastatin and Clopidogrel, RP-HPLC

ARTICLE HISTORY: Received 31 February 2020, **Accepted** 31 May 2020, **Available Online** 27 July 2020

Citation: Gampa Vijaya Kumar, *et al.* Analytical Method Development and Validation for The Simultaneous Estimation of Rosuvastatin and Clopidogrel by RP-HPLC Method, 8(7), 2020: 150-154.

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1. Introduction

Rosuvastatin, sold under the trade name Crestor among others, is a statin medication, used to prevent cardiovascular disease in those at high risk and treat abnormal lipids. It is recommended to be used together with dietary changes, exercise, and weight loss. It is taken by mouth.

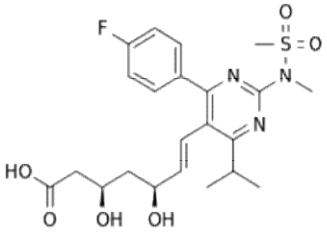

Fig 1: Chemical structure of Rosuvastatin

Clopidogrel, sold under the trade name Plavix among others, is an antiplatelet medication used to reduce the risk of heart disease and stroke in those at high risk. Common side effects include headache, nausea, easy bruising, itching, and heartburn. More severe side effects include bleeding and thrombotic thrombocytopenic purpura.

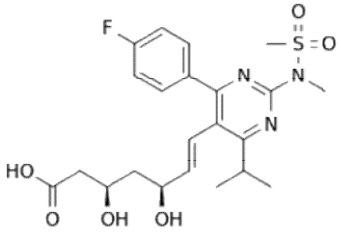

Fig 2: Chemical structure of Clopidogrel

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Rosuvastatin and Clopidogrel, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4, Sodium perchlorate.

Optimized Chromatographic conditions:

Flow rate : 0.8 ml per min
Column :Agilent C_{18} (4.6 x 150mm, 5µm)
Detector wavelength :254nm
Column oven : Ambient
Injection volume :10µl
Run time : 10 min
Mobile phase :Methanol(60%) Phosphate buffer (40%)

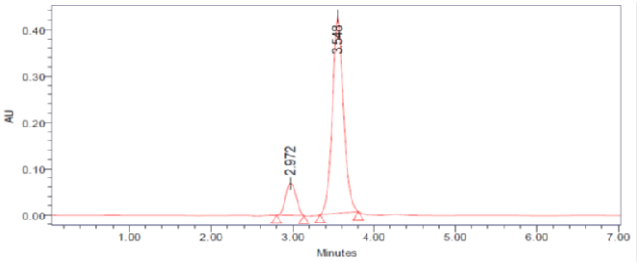

Fig 3: Optimized Chromatogram

Preparation of sample solution:

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Rosuvastatin and Clopidogrel was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicate for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution).

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: System suitabilityresults for Rosuvastatin

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	2.799	1250763	2487	1.62
2	2.799	1247867	2489	1.58
3	2.813	1255849	2496	1.64
Mean		1251360		
SD		3850.679		
% RSD		0.30722		

Table 2: System suitability results for Rosuvastatin & Clopidogrel

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	3.861	940627	2281	1.51	3.04
2	3.863	931161	2244	1.47	3.09
3	3.886	940306	2261	1.47	3.05
Mean		937364.7			
SD		5374.93			
% RSD		0.573409			

Table 3: Linearity results for Rosuvastatin

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	839286
2	II	40 ppm	1067774
3	III	60 ppm	1246474
4	IV	80 ppm	1439994
5	V	100 ppm	1639065
Correlation Coefficient			0.99932

Table 4: Linearity results for Clopidogrel

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	626221
2	II	15 ppm	778750
3	III	20 ppm	931447
4	IV	25 ppm	1070162
5	V	30 ppm	1196060
Correlation Coefficient			0.99916

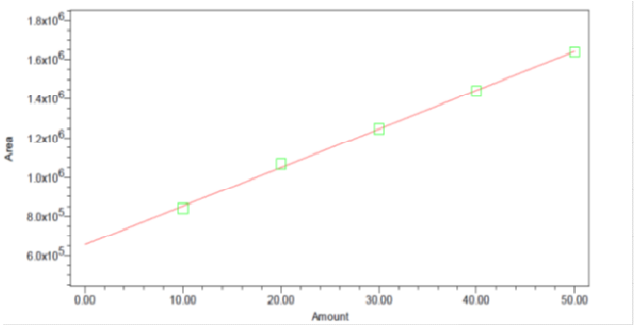

Fig 3: Calibration curve of Rosuvastatin

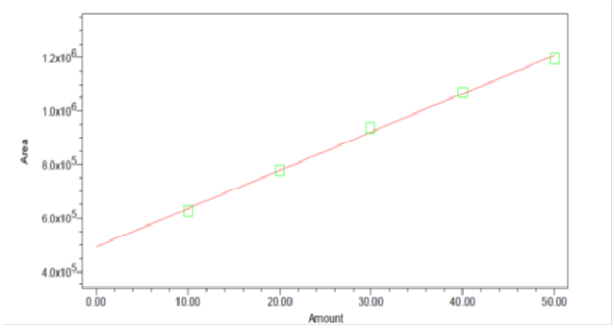

Fig 4: Calibration curve of Clopidogrel

Table 5: Repeatability results of Rosuvastatin

Injection No	Peak Area	R_t
1	1247256	2.808
2	1248579	2.807
3	1243273	2.804
4	1243262	2.806
5	1249574	2.805
Avg	1246389	
SD	2965.62	
% RSD	0.23793	

Table 6: Repeatability results of Clopidogrel

Injection No	Peak Area	R_t
1	935035	3.880

2	929353	3.882
3	930459	3.881
4	932389	3.878
5	922057	3.882
Avg	929858.6	
SD	4865.16	
% RSD	0.5232	

Table 7: Intermediate precision results of Rosuvastatin

Injection No	Peak Area	R_t
1	1231404	2.808
2	1233196	2.806
3	1231008	2.805
4	1238575	2.807
5	1232407	2.804
Mean	1233318	
SD	3061.06	
%RSD	0.2481	

Table 8: Intermediate precision results of Clopidogrel

Injection No	Peak Area	R_t
1	912412	3.882
2	913062	3.880
3	909642	3.801
4	916881	3.882
5	914005	3.880
Mean	913200.4	
SD	2621.886	
% RSD	0.287	

Table 9: Accuracy of Rosuvastatin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 10: Accuracy of Clopidogrel

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

Table 11: Robustness results for Rosuvastatin (flow rate)

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.6	2511	1.6
2	0.8	2547	1.5
3	1.0	2484	1.6

Table 12: Robstness results for Clopidogrel (flow rate)

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.6	2279	1.4
2	0.8	2195	1.4
3	1.0	2185	1.4

Table 13: Roubstness results for Rosuvastatin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3249	1.6
2	*Actual	3245	1.6
3	5 % more	3829	1. 6

Table 14: Roubstness results for Clopidogrel

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2249	1.4
2	*Actual	2245	1.4
3	5 % more	2829	1.4

4. Conclusion

The research indicate that UV spectrophotomerty and RP-HPLC method to be simple, accurate, precise, reproducible, and sensitive.This implies that proposed UV and HPLC method can be used for routine quality control analysis of ROSU and CLOP in combination pharmaceutical dosage form.

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