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Research Article

Development and Validation of RP-HPLC Method For Simultaneous Determination Of Rosuvastatin And Clopidogrel In Tablet Dosage Form

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Abstract

The aimed of research the method development and validation by RP-HPLC method of the Rosuvastatin calcium and Clopidogrel bisulphate. The method is a simple, accurate, specific, precise, reproducible and sensitive. The λ max of ROSU and CLOP was found to be 240nm. Coefficient correlation 0.999, Beer's Law limit 50-150 µg/ml, from the four trial of different concentration of mobile phase was selected Methanol:Water 80:20 v/v, pH 3.0 at 240nm, flow rate 1ml/min, sample inlet 20 µL, C 18 Prontosil, %RSD of ROSU 1.017 and CLOP 0.173, theoretical plates ROSU 7797.53 and ROSU 8257.53, Retention time ROSU 3.483min and CLOP 4.983min, Tailing factor ROSU 1.1787 and CLOP 1.074, limits 2 NMT, Accuracy ROSU 0.37 %RSD, Recovery 99.59% and CLOP 0.18 %RSD, recovery 100.41% was show good efficacy and results. The methods indicate Future scope in analysis quality control of the estimation of ROSU and CLOP for routine drug quality analysis investigation.

Key words: RP HPLC, Rosuvastatin calcium, Clopidogrel bisulphate, Simultanious estimation, validation.

INTRODUCTION

Rosuvastatin calcium (ROSU) is chemically 7-[4-(4-Fluorophenyl)-6-(1-methylethyl)-2-(methyl-methyl sulfonyl-amino)-pyrimidin-5-yl]-3,5-dihydroxy-hept-6-enoic acid calcium (**Fig 1a**). It is in a group of drug called hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors, or statins. ROSU reduces levels of low density lipoprotein and triglycerides in blood, when increases level of high density liproprotein in management of hyperlipidaemias[1]. If we manufacture nanoparticle attached to UV scattering substance like ZnO and TiO_2 and specifically target these nanoparticles to skin cells with sunscreen on nanoscale[2].

Clopidogrel bisulphate (CLOP) is chemically methyl (2S)-2-(2-chlorophenyl)-2-(4H, 5H, 6H, 7H-thieno[3,2 c]pyridine-5-yl) acetate sulfate (**Fig 1b**). It is a USP-NF enlist drug and a new thienopyridine derivative. CLOP is an anti-platelet agent, which directly inhibit the binding of adenosine diphosphate (ADP) to its platelet receptor and blocks the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, so inhibiting platelet aggregation[3]. In the last few decades, the application of nanomaterials in the area of biology and medicine has revolutionized the field of drug delivery, theranostics, imaging, diagnosis, wound healing and medical devices with miscellaneous properties[4].

Rosuvastatin calcium is determined alone also by UV-method[5] and RP-HPLC method[6]. The gel stimulates cell growth and enhances the restoration of damaged skin[7].Clopidogrel was also estimated using UV-method, derivative spectroscopy, HPLC, HPTLC and LCMS/MS[8]. The chewing sticks have been widely used in the Indian subcontinent, Middle East and Africa since ancient time period[9].

The aim of current research to developed and validated UV spectrophotometry and RP HPLC for

simultaneous estimation of rosuvastatin calcium and clopdogrel bisulphate. The accuracy, precision, %RSD and recovery study was indicated the reproducibility.

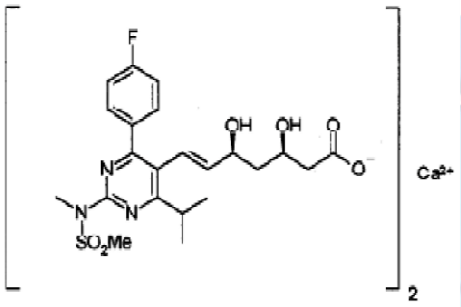

Fig 1a: Chemical structure of Rosuvastatin calcium

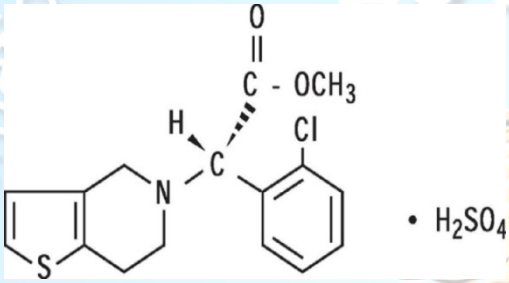

Fig 1b: Chemical structure of Clopidogrel bisulphate

MATERIALS AND METHOD

Chemicals and Reagents

Water (HPLC grade), Methonol (HPLC grade), OPA. All reagents and chemicals were used of HPLC grade.

Pure Sample

Table 1: Pure drug information

Drugs	Supplier	Quantity	Purity
Rosuvastatin calcium	Zim Laboratory	10.0 g	99.98% w/w
Clopidogrel bisulphate	Zim Laboratory	10.0 g	99.99% w/w

The drugs used for the present investigation were donated as gift samples.

Marketed formulation available

Table 2: Marketed formulation information

Brand Name	Mfg By	Content	Quantity
Rosloy CV 5mg/75mg Tab	Lloyed Healthcare Pvt Ltd	Rosuvastatin calcium	5 mg
		Clopidogrel Bisulphate	75 mg

The marketed formulation was purchased from local market.

Instrumentation

Table 3: Required Instruments information

Name of Equipment	Make	Model
UV-Visible Spectrophotometer	Thermo Electron	Double beam carry-07 Bio
HPLC	Water	996 PDA Detector 600E EMPOWER Software with Autosampler
pH Meter	Systronics	pH meter 335
Balance	Citizen	CY 104 (Micro Analytical Balance)
Column	Prontosil (5µm)	C18 [4.6 x 250 mm(id)]

EXPERIMENTAL

Preparation of Calibration Curve[10]

Accurately weighed ROSU and CLOP separately about 10 mg and dissolved in methanol with volume made up to 10ml mark to obtain 1000 µg/ml. the stock standard solutions was diluted further with concentration range 50-150 µg/ml.(**Fig 2a** and

Fig 2b)

HPLC Method[11]

Mobile phase

From the various trial (**Fig 3a,3b,3c,3d**) , mobile phase containing, methanol: water with different concentration at pH 3.0 with column C 18 was selected since it gives sharp reproducible retention time for ROSU and CLOP (**Fig 3d**), chromatographic condition also determined. (**Table 5**)

Standard solution

Accurately weighed separately about 5 mg ROSU and 75mg CLOP was dissolved into separate volumetric flask in methanol and volume was made up to mark 10 ml by same to obtain 500 µg/ml ROSU and 7500µg/ml stock solution.

Pipette out 1 ml from standard stock solutions and diluted it with 10ml methanol to obtain 50µg/ml ROSU and 750µg/ml CLOP.

From the stock solution were dilutions were made in the concentration 50, 80, 100, 120, 150 µg/ml of ROSU and CLOP. A 20µL of each sample was injected and chromatogram was recorded at 240nm. This above concentration range was found to be linear and obeys Beer's law.

Analysis of marketed preparation[12]

The twenty tablet of Rosloy CV 5mg/75mg Tab (Label claim: Rosuvastain calcium 5mg and Clopidogrel bisulphate 75mg) was weight to equivalent to label claim. Finely powder and prepare the solutions of ROSU and CLOP separately in 100 ml of volumetric flasks. Add 50ml methanol and disperse the powder completely. Then sonicated for 10min to it added 50ml of diluents to make up volume and sonicate 5min to dissolve with intermittent shaking. Allow the solutions to cool at room temperature. The stock of 1000µg/ml was prepared. From this stock solution pipette out 1ml supernatant liquid in 10ml volumetric flask and make up the volume with diluents to make 100ppm. To produce the 50µg/ml ROSU and 750µg/ml CLOP.

Method Validation[13-14]

The method was developed and validated according to ICH guidelines.

Linearity

Calibration graphs constructed by plotting peak area v/s concentration of ROSU and CLOP, the regression equation were calculated. The calibration range 50-150 µg/ml for both the drugs. Aliquots 20µL of each solution injected under the operating chromatographic condition. (**Table 6**)

System suitability test

System suitability is a pharmacopoeial requirement and used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from 5 replicate injections of standard solutions. (**Table 7**)

Accuracy

The accuracy of the methods was established by recovery studies of ROSU (**Table 8**) and CLOP (**Table 9**)

Precision

The intra-day and inter-day precision of the proposed method was determined by analyzed the solution of ROSU and CLOP on the same and different days. (**Fig 5**) (**Table 10**)

Limit of detection and limit of quantitation (LOD &LOQ)

The LOD and LOQ of ROSU and CLOP were determined by calculating signal to noise (S/N) ratio, according to International Conference on Hormonization guidelines.

Robustness

The robustness of the method was evaluated by assaying the sample solution after slight but deliberate changes in the analytical condition inject into HPLC system at -10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min).

The flow rate 1ml/min, 0.9ml/min and 1.1ml/min. (**Table 11**). Organic changes ie -10% and +10% Methanol also studied. (**Table 12**)

RESULTS AND DISCUSSION

λ max determination: The λ max of ROSU and CLOP was found to be 240 nm.

Calibration curve

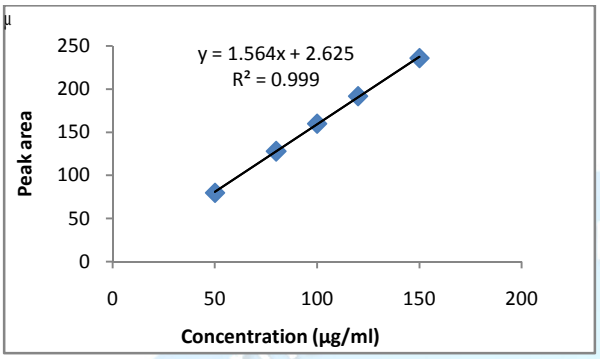

Fig 2a: Calibration curve of ROSU

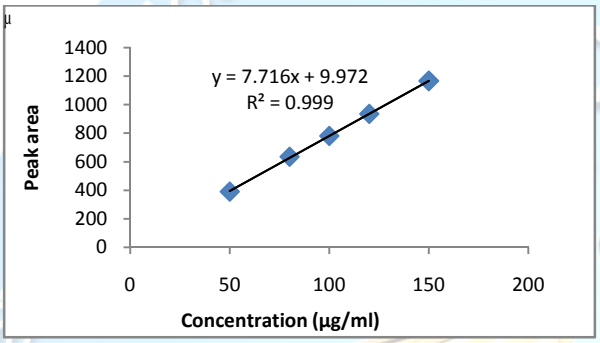

Fig 2b: Calibration curve of CLOP

HPLC Method

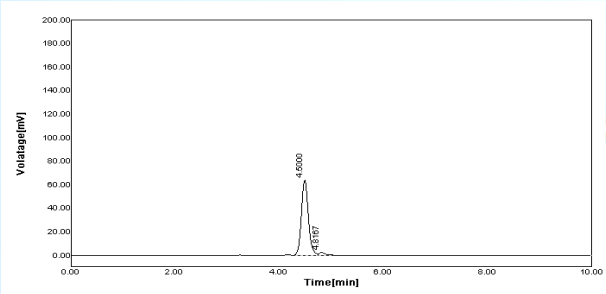

Fig 3a: Trial 1: chromatogram of ROSU and CLOP with mobile phase Methanol: water (100:00 v/v) 1ml/min at 270nm.

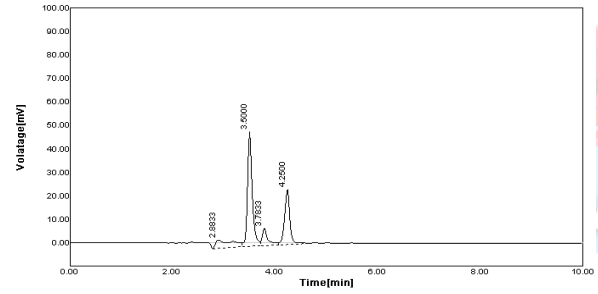

Fig 3b: Trial 2: chromatogram of ROSU and CLOP with mobile phase Methanol: Water (80:20 v/v) pH 3.0

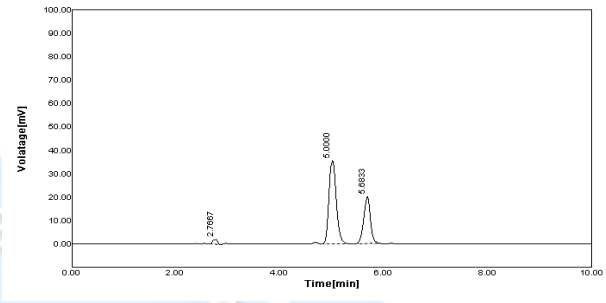

Fig 3c: Trial 3: chromatogram of ROSU and CLOP with mobile phase Methanol: Water (70:30 v/v) pH 3.0

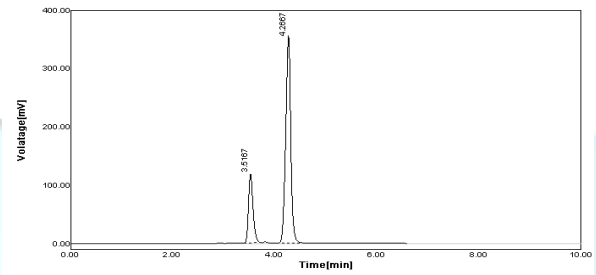

Fig 3d: Trial 4: Chromatogram of ROSU and CLOP with mobile phase Methanol: water (80:20 v/v) pH 3.0. The summary of trials is shown in Table-4.

From the above four trials (**Fig 3a, 3b, 3c, 3d**) and (**Table 4**) the selection of mobile phase done and chromatographic condition established.

Table 4: Observations for trial of different concentration of mobile phase

Trial	Mobile phase	Peak characteristics
1	Methanol:Water (100:00 v/v) 1ml/min at 270nm	Peaks are not separated. (**Fig 3a**)
2	Methanol:Water (80:20 v/v) [pH 3.0] 1ml/min at 270nm	Peaks were separated but asymmetry was out range. (**Fig 3b**)
3	Methanol;Water (70:30 v/v) [pH 3.0] 1ml/min at 240nm	Peaks were separated but resolution are out of range. (**Fig 3c**)
4	Methanol:Water (80:20 v/v) [pH 3.0] 1ml/min at 240nm	All parameters are in range as per USP. (**Fig 3d**)

Chromatographic conditions

Table 5: Chromatographic condition

Column	Prontosil [4.6 x 250mm (id)]
Particle size packing	10 µm
Stationaray phase	C 18 Prontosil (5µm)
Mobile phase	Methanol:Water (80:20 v/v) pH 3.0
Detection wavelength	240nm
Flow rate	1ml/min
Run time	08 min
Temperature	Ambient
Sample size	20 µL
Diluent	Methanol

Table 6: Linearity observation

\Parameters	ROSU	CLOP
λmax, nm	240	240
Beer's Law limit (µg/ml)	50-150	50-150
Regression equation (Y*)	Y=1.564x+2.625	Y=7.716+9.972
Correlation coefficient (r^2)	0.999	0.999
Slope (b)	1.564	7.716
Intercept (a)	2.62	9.97

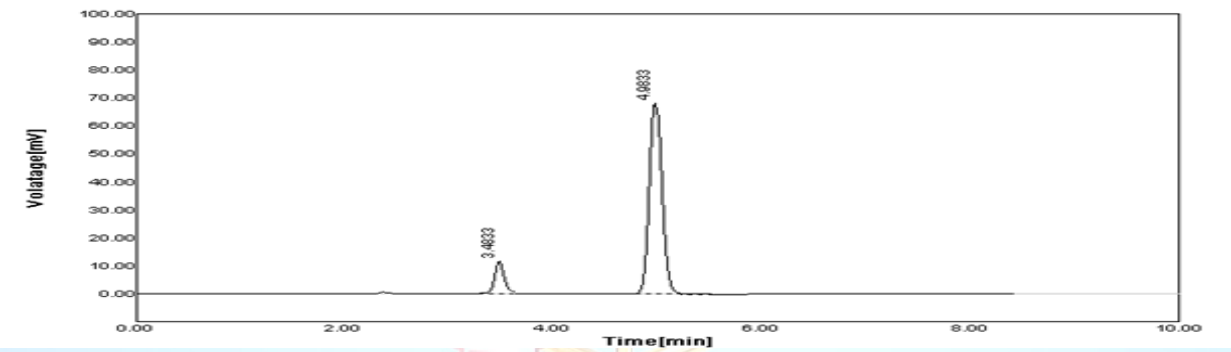

Fig 4: Separation of two drug in selected mobile phase showing Retention time ROSU 3.483 min and CLOP 4.983 min.

Table 7: Observation of system suitability

Sr.No	Peak area		Retention Time		Asymmetry		No of theoretical Plates		Resolution
	ROSU	CLOP	ROSU	CLOP	ROSU	CLOP	ROSU	CLOP	
1	75.0602	408.35	3.483	4.983	0.752	0.956	7849.1	8338.7	6.62
2	74.6202	408.85	3.450	4.950	0.763	0.954	7700.2	8142.8	6.42
3	76.1110	409.75	3.483	5.083	0.742	0.971	7843.3	8290.0	6.40
4	72.0124	406.82	3.466	5.066	0.766	0.956	7794.1	8067.3	6.42
5	74.1202	408.15	3.450	4.950	0.757	0.978	7681.5	8800.2	6.40
Mean	75.26	408.98	3.460	4.994	0.752	0.960	7797.53	8257.53	6.48
S.D	0.765	0.709	0.017	0.076	0.010	0.009	84.34	101.99	0.121
%R.S.D	1.017	0.173	0.513	1.537	1.39	0.967	1.081	1.235	1.877

Table 8: Accuracy of ROSU

	Rosuvastatin calcium					
	Peak Area	**Amount Taken(mg)**	**Amount recovered (mg)**	**% Recovery**	**Average recovery**	**% RSD**
80%	120.10	1.6	3.63	98.61	100.66	0.27
	120.59	1.6	3.65	99.63		
	120.32	1.6	3.64	99.06		
100%	132.490	02	4.04	99.30	99.28	0.38
	133.44	02	4.07	100.86		
	133.120	02	4.06	100.34		
120%	145.13	2.4	4.46	100.10	99.44	0.46
	146.020	2.4	4.49	101.32		
	146.270	2.4	4.50	101.67		
Mean					**99.59**	**0.37**

Table 9: Accuracy of CLOP

Recovery level	**Clopidogrel bisulphate**					
	Area	**Amount Taken(mg)**	**Amount recovered (mg)**	**% Recovery**	**Average recovery**	**% RSD**
80%	601.21	24	53.95	99.54	99.41	0.22
	602.780	24	54.13	99.32		
	600.890	24	53.91	99.38		
100%	654.810	30	60.36	100.21	100.49	0.13
	655.090	30	60.47	100.56		
	656.090	30	60.51	100.72		
120%	708.510	36	66.36	101.46	101.33	0.20
	707.160	36	66.62	100.91		
	709.290	36	66.88	101.62		
Mean					**100.41**	**0.18**

Table 10: Observation of Precision

Sr. No.	**Parameter**	**Observations**		**Limits**
		ROSU	**CLOP**	
1	The % RSD of peak area response for three replicate injections of standard	1.017	0.173	NMT 2.0
2	Theoretical plates	7797.53	8257.53	NLT 2000
3	Tailing factor	1.1787	1.074	NMT 2.0

Table 11: Observation of changing in flow rate

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for three replicate injections	ROSU	1.017	0.82	0.75	NMT 2.0
		CLOP	0.173	0.20	0.05	
2	Theoretical plates	ROSU	7797.53	6038.7	4556.9	NLT 2000
		CLOP	8257.53	5965.7	5328.9	
3	Tailing factor	ROSU	1.28	1.91	1.10	NMT 2.0
		CLOP	1.06	0.950	1.00	
4	Retention Time (Min)	ROSU	3.483	3.85	2.86	
		CLOP	4.983	5.46	4.13	

Table 12: Observaation of changes in organic composition (-10% and +10% Methanol)

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for three replicate injections	ROSU	1.017	0.655	0.046	NMT 2.0
		CLOP	0.173	0.021	0.030	
2	Theoretical plates	ROSU	7797.53	4896	6347.6	NLT 2000
		CLOP	8257.53	8060	9386.6	
3	Tailing factor	ROSU	1.28	1.166	1.08	NMT 2.0
		CLOP	1.06	1.062	0.88	
4	Retention Time (Min)	ROSU	3.483	3.46	3.38	
		CLOP	4.983	5.08	4.80	

CONCLUSION

The research indicate that UV spectrophotomerty and RP-HPLC method to be simple, accurate, specific, precise, reproducible, and sensitive. No interference of additives, matrix and good recovery and environmently friendly method. 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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