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Development and validation of RP-HPLC method for simultaneous estimation of rosuvastatin and bisoprolol fumarate in bulk and formulations

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***Abstract*---**A simple, accurate and precise RP-HPLC method was developed for simultaneous estimation of Rosuvastatin and Bisoprolol fumarate in bulk and in formulation. Separation was achieved using and C18 column and Methanol: Phosphate buffer (pH 3.5) at a ratio of 45:55% V/V, flow rate was maintained at 1.0 ml. min and 245 nm was used as determination wavelength. System suitability parameters shows values within specified limits. Developed method was validated as per ICH guidelines and all the parameters were found to be with in the specified limits as per regulatory guidelines. Linearity was found to be 4-20 and 8-40 mcg/ml for Rosuvastatin and Bisoprolol fumarate respectively. Results of accuracy was found to be with in 98-102% which shows accuracy of the developed method.

***Keywords*---**RP-HPLC, Rosuvastatin, Bisoprolol Fumarate, Validation.

Introduction

Rosuvastatin is an antihypertensive agent with a chemical formula C27H28N2O7, chemically it is 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en-1-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[1]. Bisoprolol is also an anti-hypertensives agent with a chemical formula of $C_{18}H_{31}NO_4$, chemically it is 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy] propan-2-ol[2]. Bisoprolol was analyzed alone or in combination with other drugs using, RP-HPLC [3-8], UV-Spectroscopic[9-11], voltametric [12], UPLC[13], methods. Literature survey

revealed analytical methods like, RP- HPLC[14-16], LC-MS-MS [16-17] UPLC [18] for analysis of Rosuvastatin individually and in combination with other drugs. Similarly,
There is only one RP-HPLC method reported for simultaneous estimation of these drugs in combination, but the method had a drawback of long run time. To reduce the run time of the method a new method was developed with a short run time.

Experimental Method

Preparation of working and standard solutions

An amount equivalent to 10.0 and 10 mg of Bisoprolol fumarate and Rosuvastatin were weighed separately and transferred to 100.0 mL standard volumetric flask. Added 50.0 mL of methanol sonicated for 3.0 min and then made up to the volume with same solvent to get 0.10 mg $mL^{-1}$for both drugs respectively. This solution was further diluted to get desired concentration range of 2 to 40 mcg/ml.

Preparation of Sample Tablets

We have prepared in house production of sample tablets in a ratio of 1: 2 for the two drugs. For preparation of tablets, we have used Magnalium stearate, Lactose and Sodium starch Glycolate as excipients based on previously available literature. Weight of each tablet was set at 150 mg and each tablet contain 10 mg of Bisoprolol Fumarate and 20 mg of Rosuvastatin respectively. These tablets were prepared with direct compression technique after continuous blending to get uniform drug content. Tablet evaluation tests were performed, and further tablets were used for determination of drug content.

Preparation of Tablet Sample

The twenty tablets were weighed, and then average weight was determined and finely grounded. The weight of the powdered tablet equivalent to 5.0 mg of Bisoprolol Fumarate and 10 mg of Rosuvastatin were transferred into a 100 mL standard volumetric flask. Added 50 mL of methanol sonicated for 10 min and diluted to 100 mL with the same solvent. The resulting solution has 50 μg mL^{-1} of Bisoprolol Fumarate and 100 μg mL^{-1} of Rosuvastatin. The resulting solutions were sonicated for 15 min and then filtered through Whatmann filter paper No:41. Insoluble excipients were separated out. One mL of the above filtered solution was suitably diluted to get desired concentration of sample solution.

Preparation of Buffer

A 6.80 gm of Potassium dihydrogen orthophosphate was dissolved in sufficient water (HPLC grade) with aid of Sonicator and then made up to 1000.0 mL to get 0.1 M solution. Then ortho phosphoric acid was used to adjust the pH to < 5.

Preparation of Mobile phase

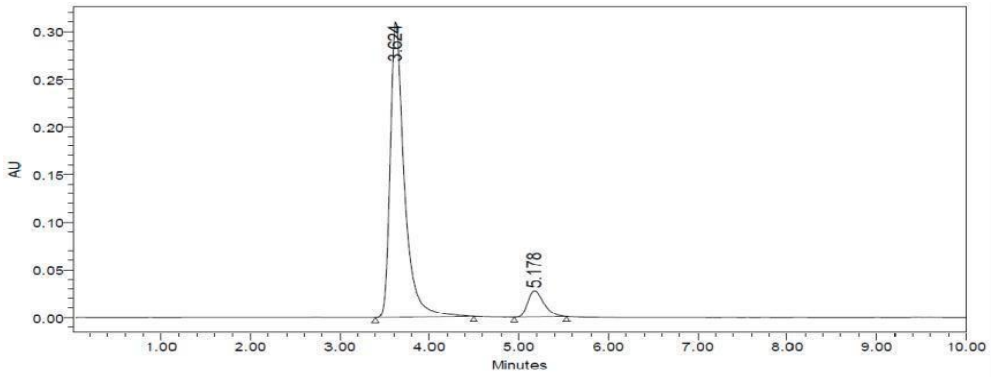
Prepared by mixing 55 volumes of 10 mM of phosphate buffer (potassium

dihydrogen phosphate) with 45 volumes of methanol and pH was adjusted to 2.6 with ortho phosphoric acid.

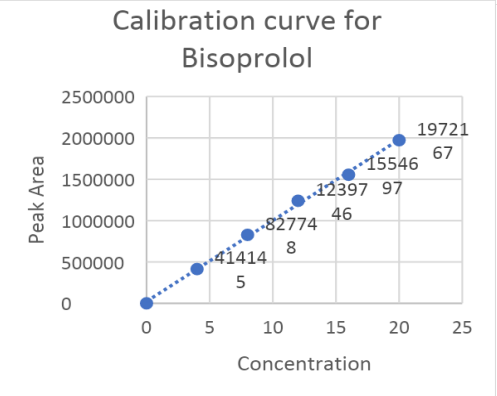
Results and Discussion

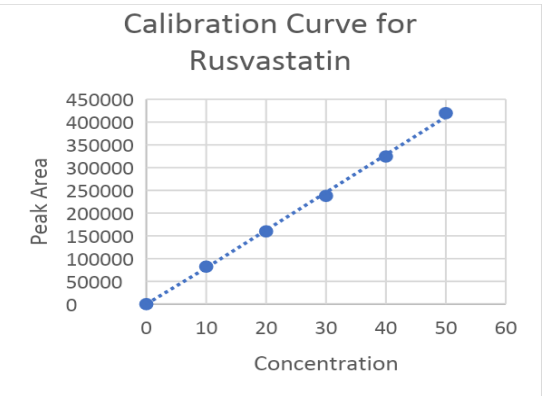
The optimized method in HPLC were carried out by using flow rate 1.0 mL/min, pH adjusted to 3.5 and methanol in the ratio of 55:45 %(v/v) was used, the time required for analysis is less than 6.0 min on C18 analytical column, UV-PDA detection wavelength at 220 nm and 10 µl of injection volume, which gave a retention time for the last compound as 5.17 min.

Validation and Application



The developed method was validated for linearity, range, accuracy, and precision, limit of detection (LOD), limit of quantitation (LOQ), specificity, robustness, and ruggedness parameters, in accordance with ICH Q2 (R1) guidelines. The Linearity was established at the concentration range of 4 -20 and 8 - 40 µg mL^{-} for Bisoprolol fumarate and Rosuvastatin respectively. The standard stock solution was diluted with mobile phase to get 4, 8, 12, 16 and 20 µg mL^{-1} of Bisoprolol fumarate and 8, 16, 24, 32 and 40 µg mL^{-1} for Rosuvastatin. Each concentration was analyzed in 3 replicates. Peak areas (y) of Bisoprolol fumarate and Rosuvastatin were plotted versus their respective concentrations (x) and linear regression analysis performed on the resultant calibration curves. LOD and LOQ of the method is 1.017 and 2.76 µg mL^{-1} for determining BIS and 3.38 and 9.19 µg mL^{-1} for RUS.





Parameters	Bisoprolol fumarate	Rosuvastatin
Calibration range (µg mL^{-1})	4 - 20	8 - 40
Correlation coefficient (r)	0.998	0.998
Slope (S)	97818	8293
Intercept	23239	-3297
LOD (µg mL^{-1})	1.017	2.76
LOQ (µg mL^{-1})	3.387	9.19

Accuracy of the method was determined by performing the recovery experiment at 50, 100 and 150 % of the expected assay value of the drugs in the commercial tablet dosage form. To the preanalyzed sample, standard API's were added 12 (50 %), 16 (100%) and 24 µg mL $^{-1}$ (150%) level for Bisoprolol fumarate and 24 (50%), 32 (100%) and 40 µg mL $^{-1}$ (150%) for Rosuvastatin. These solutions were then analyzed by the proposed method in 3 replicates. The % mean recovery at each level for 50, 100 and 150 % level in studies were found to be in the range of 99.1, 100.7, and 100.8 for RUS and 99, 100.4 and 99.5 for BIS determined. The recoveries of Bisoprolol fumarate and Rosuvastatin at each level were found to lie well within the acceptable criteria of bias 0.601– 0.991 (±2%).

% Level Amount Present (µg mL^{-1})	Amount Added (µg mL^{-1})		Amount Recovered (µg mL^{-1})		% Recovered* ± S.D (% RSD)	
	BIS	CLI	BIS	CLI	BIS	CLI
50	12	24	11.9	23.77	99.17 ± 0.932	99.04 ± 0.832
					(0.912)	(0.822)
100	16	32	16.11	32.12	100.69 ± 1.034	100.38 ± 1.022
					(0.991)	(0.973)
150	20	40	20.16	39.82	100.80 ± 0.838	99.55 ± 0.662
					(0.785)	(0.601)

Precision was determined by studying the repeatability and intermediate precision. The intra-day and inter-day assay precision was studied at 10 and 20 µg mL^{-1} for Bisoprolol fumarate and Rosuvastatin respectively, (n = 3) and precision was confirmed with 99.4 and 99.8 for Inter day and 99.97 and 100. 3

for inter day precision studies, both the studies % RSD values were in the range of 0.79 to 1.4 was well within the target criterion of the results was less than 2%, which indicates the method is precise.

Table Results of repeatability and intermediate precision

Precision Interday					
Drug Name	Label Claim mg tab^{-1}	Amount found	% Recovery	Mean % recovery ± S.D	% RSD
Bisoprolol fumarate	10	9.92	99.20	99.40 ± 0.9165	0.9220
		9.86	98.60		
		10.04	100.40		
Rosuvastatin	20	19.78	98.90	99.83 ± 0.9504	0.9520
		19.96	99.80		
		20.16	100.80		
Precision Intraday					
Bisoprolol fumarate	10	9.88	98.80	99.97 ± 0.7967	0.7969
		9.96	99.60		
		10.03	100.30		
		10.12	101.20		
		10.01	100.10		
		9.98	99.80		
Rosuvastatin	20	19.79	98.95	100.26 ± 1.4857	1.4819
		19.82	99.10		
		20.37	101.85		
		19.74	98.70		
		20.26	101.30		
		20.33	101.65		

The specificity of the method was evaluated after analyzing the placebo containing Bisoprolol fumarate (6 µg mL^{-1}) and Rosuvastatin (12 µg mL^{-1}) at the concentration mentioned in parenthesis. The selectivity of the method is depicted by the sharp well resolved peaks for Bisoprolol fumarate and Rosuvastatin. While the specificity of the method was checked by comparing the chromatograms obtained from the runs of standard, sample and the corresponding placebo. The retention time obtained for the standard and sample solutions were identical and no ghost peaks were found. This confirmed the specificity of the method.

Table Robustness data for Rosuvastatin

Parameters	Peak Area	T Plates	Asymmetry
Flow Rate			
Flow Rate	82340	4376	1.13
0.8 mL/min.	82312	4322	1.19

	82367	4401	1.14
Flow Rate	82672	4216	1.29
1.2mL/min.	82713	4198	1.27
	82558	4205	1.3
Wavelength			
Wavelength	81890	4264	1.19
258nm	81995	4244	1.22
	82054	4252	1.23
Mobile phase			
Mobile phase	82423	4256	1.26
58-42	82189	4199	1.13
	82835	4192	1.11
Mobile phase	82087	4270	1.18
62-38	82178	4268	1.15
	82251	4278	1.17

Table Robustness data for Bisoprolol fumarate

Parameters	Peak Area	T Plates	Asymmetry
Flow Rate			
Flow Rate	414154	5213	1.65
0.8 mL/min.	411234	5261	1.63
	412315	5234	1.77
Flow Rate	414123	5234	1.63
1.2mL/min.	412525	5223	1.89
	414267	5243	1.56
Wavelength			
Wavelength	414209	5211	1.64
258nm	414201	5231	1.76
	410945	5234	1.79
Mobile phase			
Mobile phase	414205	5234	1.77
58-42	414133	5223	1.63
	414219	5212	1.62
Mobile phase	414127	5213	1.73
62-38	414205	5233	1.56
	414153	5231	1.69

Assay (content estimation) was performed to determine the purity of Bisoprolol fumarate and Rosuvastatin in tablet formulation. The nominal concentration from calibration curve was selected and quantification of Bisoprolol fumarate and Rosuvastatin were performed. The tablet formulation manufactured in house was used for analysis and the percentage purity of analytes present in formulation were found to be in the range from 98.87 to 99.97 %. The % RSD values were found to be 0.650 and 1.118 for Bisoprolol fumarate and Rosuvastatin respectively.

Table Results of assay of the formulation

Drug Name	Label Claim mg tab^{-1}	Amount found	% Recovery	Mean % recovery ± S.D	% RSD
Bisoprolol fumarate	10	9.86	98.6	98.87 ± 0.6429	0.6503
		9.84	98.4		
		9.96	99.6		
Rosuvastatin	20	19.89	99.45	99.97 ± 1.1184	1.1188
		19.84	99.2		
		20.25	101.25		

Conclusion

The developed method was found to be accurate, Precise and economical .This method has been evaluated for linearity, precision, accuracy and selectivity, and has proved to be convenient and effective for the quality control of Bisoprolol fumarate and Rosuvastatin in raw material and its formulations.

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