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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE COMBINATION OF ROSUVASTATIN AND FIMASARTAN IN SYNTHETIC MIXTURE

[1]Chavda Nirma, [2]Dr. Suresh Kumar

[1]Associate Professor, [2]Professor and HOD
[1]Pharmaceutical Chemistry,
[1]B. Pharmacy College, Rampura, India

Abstract: Stability indicating RP-HPLC method has been developed for the simultaneous estimation of ROS and FIM in bulk and its pharmaceutical dosage form. In RP-HPLC method, chromatographic separation was achieved using a C_{18} column (250 mm x 4.6 mm) and Buffer (pH 3.0)-Methanol (60:40) as mobile phase at a flow rate of 1.0 ml/min with detection wavelength of 243 nm. The linearity of ROS was found in the range of 5-15 µg/ml and FIM 30-90 µg/ml. Retention time in RP-HPLC method was found to be 3.9 min and 6.1 min for FIM and ROS respectively. The % recovery was found to be 100.17 ± 7.67 for Rosuvastatin and 100.1± 6.14 for Fimasartan. The proposed method was validated as per ICH guidelines and successfully applied for the determination of drugs in pharmaceutical formulation.

Keywords: Rosuvastatin, Fimasartan, Validation, Stability indicating RP-HPLC

I. INTRODUCTION

Hypertension is a sustained increase in blood pressure ≥ 140/90 mm Hg, a indicator where the risk of hypertension-related cardiovascular disorder is more enough to merit medical observation.[1] Rosuvastatin calcium (ROS) which is (3R, 5S, 6E)-7-(4-(4-fluorophenyl)- 6-(1-methylethyl)-2-(ethyl(methylsulfonyl)amino)-5-pyrimidinyl)-3,5-dihydroxy-6-heptenoic acid. Fimasartan potassium trihydrate which is chemically2- (2-butyl-4-methyl-6-oxo-1-{[2′-(1H-1,2,3,4tetrazol-5-yl)- [1,1′-biphenyl]-4-yl]methyl}-1,6-dihydropyrimidin-5-yl)- N,N-dimethylethanethioamide. Rosuvastatin calcium is an HMG Co A reductase inhibitor and Fimasartan is an angiotensin II receptor antagonist. [2,3] Both drugs used in combination to treat hypertension [4-5] · The mechanism of action of rosuvastatin is blocking 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase.[6] This enzyme is the rate-limiting step in cholesterol synthesis and decreases the production of mevalonic acid from HMG-CoA. Moreover, this results in a rise of low-density lipoprotein receptors on hepatocyte membranes and stimulation of low-density lipoprotein catabolism. HMG-CoA reductase inhibitors also lower levels of high sensitivity C-reactive protein (CRP). They also have pleiotropic properties, involving inhibition of platelet aggregation, anticoagulant effects, reduced inflammation at the site of a coronary plaque, and enhanced endothelial function. [7]. In blocking the AT1 receptor, Fimasartan blocks vasoconstriction and supports vasodilation. At the kidney and adrenal gland, AT1 blockage and inhibition of aldosterone formation rise the excretion of water and salt by the kidneys, which lowers overall blood volume.[8] At the heart, AT1 blockage lowers contractility and the stimulatory effects of the sympathetic nervous system.[9] Collectively, fimasartain helps to a reduction in blood pressure and relieves hypertensive symptoms. ARBs such as fimasartan have also been shown to be protective against stroke, myocardial infarction, and heart failure.[10] Literature survey reveals that Rosuvastatin can be estimated by spectrophotometric, Reverse Phase High- Performance Liquid Chromatography (RP-HPLC) and High Performance Thin Layer Chromatography (HPTLC) methods

either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for Fimasartan includes spectrophotometric HPLC and HPTLC either as a single drug or in combination with other drugs. Literature survey reveals that not a single stability indicating RP-HPLC method of analysis has yet been reported for simultaneous analysis of Rosuvastatin and Fimasartan. The objective of the present investigations was to develop a rapid, accurate, economical and validated Reverse-Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation so that can play important role in quantification of ROS and FIM in bulk and its pharmaceutical dosage form [11-20]

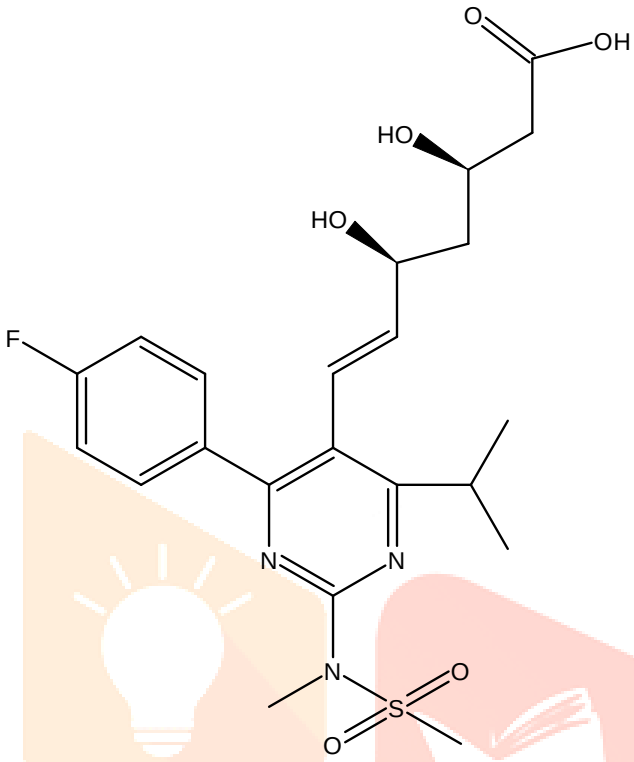

Figure 1: Chemical structure of Rosuvastatin

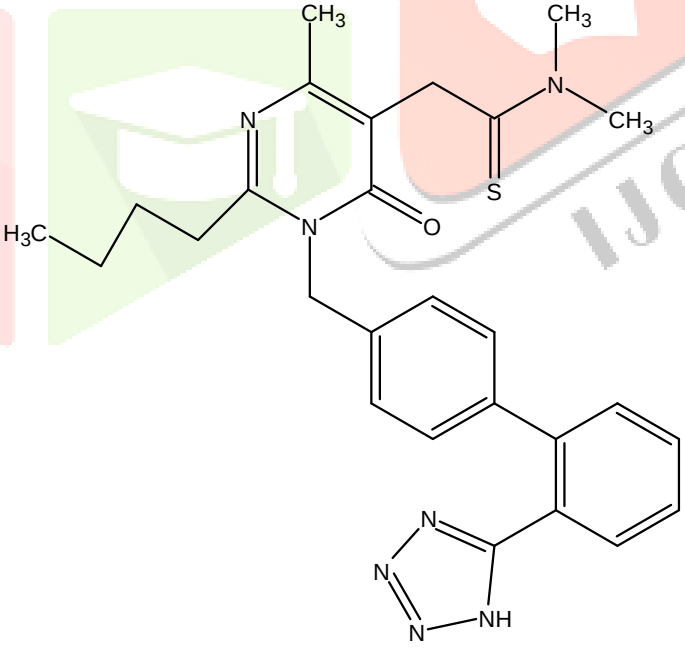

Figure 2: Structure of Fimasartan

II. MATERIALS AND METHODS

2.1 Chemicals and Materials

Pharmaceutical grade of Rosuvastatin (ROS) and Fimasartan (FIM) were kindly supplied as a gratis sample by Montage Laboratories Pvt Ltd and Mackur Laboratories. All solvents and chemicals used were of analytical grade or HPLC grade purchased from Merck and Aquarch. Methanol and Acetonitrile were used of HPLC Grade (Merck, Mumbai, India) and Potassium Dihydrogen Phosphate and Acetic Acid used was of AR Grade (Spectrochem, India). All the other chemicals used were also of AR, LR and HPLC grade (Merck, India).

Sr no.	Materials	Sources
1	Rosuvastatin	Montage Laboratories Pvt Ltd
2	Fimasartan	Mackur Laboratories
3	Chemicals/ Reagents: Acetonitrile and Methanol Potassium Dihydrogen Phosphate, Acetic Acid	 Merck, India Spectrochem, India

HPLC Instrument

The separation was performed by using C_{18} column (250 mm × 4.6 mm, 5 µm) column on alchrome A2000 Chromatographic software,pump and UV detector. The mobile phase was freshly prepared, filtered and sonicated before use and delivered at a flow rate of 1.0 ml/min and the detector wavelength was set at 243 nm. The injection volume was 20 µl.

2.2 Preparation of standard stock solution

Standard stock solution was prepared 100 µg/ml for ROS and 600 µg/ml for FIM by using mobile phase. From the standard stock solution the range of 5 µg/ml to 15 µg/ml for ROS and 30 µg/ml to 90 µg/ml for FIM were prepared.

2.3 Selection of analytical wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives optimum response at a single wavelength for both drugs that are to be detected. In the present study, drug solutions of 10 µg/ml of ROS and 60 µg/ml of FIM were prepared separately in Methanol and scanned in the range of 200-400 nm to determine the optimum wavelength of detection.

2.4 Analysis of API

Sample Stock Solution

Weight about sample (equivalent to 10mg of ROS/60mg of FIM) into a 100ml volumetric flask. Add 60ml methanol and put this volumetric on water bath at 60°C for 15 minutes then allow to cool at room temperature. Shake for 15 minutes. Make up volume with methanol up to 100ml. Filter this solution with Whatman filter paper no-1. (ROS-100mcg/ml, FIM-600mcg/ml)

Working Sample Preparation

Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (ROS-10mcg/ml and FIM-60mcg/ml)

2.5 Stability study

Procedure for Stability Study:

Standard stock solution:

Weight about sample (equivalent to 10mg of ROS/60mg of FIM) into a 100ml volumetric flask. Make up volume with methanol up to 100ml. Filter this solution with Whatman filter paper no-1. (ROS-100mcg/ml, FIM-600mcg/ml)**.**

Sample stock solution:

Weight about sample (equivalent to 10mg of ROS/60mg of FIM) into a 100ml volumetric flask. Add 60ml methanol and put this volumetric on water bath at 60°C for 15 minutes then allow to cool at room temperature. Shake for 15 minutes. Make up volume with methanol up to 100ml. Filter this solution with Whatman filter paper no-1. (ROS-100mcg/ml, FIM-600mcg/ml)

Working Std Preparation:

Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (ROS-10mcg/ml and FIM-60mcg/ml)

Acid Hydrolysis Study

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 1 ml of 0.1 N HCl was added in both and kept for 4 hours at room temperature. Then 1 ml of 0.1 N NaOH was added to neutralize it and volume was made up to mark with mobile phase mixed well and injected.

Degradation of Sample:

1ml from sample stock solution and 1ml 0.1N HCl kept for 4 hours and then neutralize with 1ml 0.1N NaOH to stop the degradation further. Now make up volume with mobile phase

Base Hydrolysis study

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 1 ml of 0.1 N NaOH was added to both and kept for 4 hours at room temperature. Then 1 ml of 0.1 N HCl was added to neutralize it and volume was made up to mark with mobile phase mixed well and injected.

Degradation of Sample:

1ml from sample stock solution and 1ml 0.1N NaOH kept for 8 hours and then neutralize with 1ml 0.1N HCL to stop the degradation further. Now make up volume with mobile phase

Peroxide Oxidation Study

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 1 ml of 3% H_2O_2 was added to both and kept for 4 hours at room temperature. Then volume was made up to mark with mobile phase mixed well and injected.

Degradation of Sample:

1ml from sample stock solution and 3% H_2O_2 both kept for 4 hours. Now make up volume with mobile phase.

Thermal Stress Study

ROS and FIM std degradation:

1gm ROS and FIM both powder kept at 105^0C 72 hours. After 72 hours, weigh 25 mg of ROS and FIM powder and dissolve both in methanol in 100ml volumetric flask. Pipette out 1ml stock solution into 10ml volumetric flask and make up the volume with mobile phase.

Degradation of Sample:

1gm powder kept at 105^0C 72 hours. After 72 hours, weigh 25 mg of powder and dissolve in methanol in 100ml volumetric flask. Pipette out 1ml stock solution into 10ml volumetric flask and make up the volume with mobile phase.

Photo Degradation Study

ROS and FIM std degradation:

1gm ROS or FIM powder kept at photo stability chamber 72 hours. After 72 hours, weigh 25 mg of ROS or FIM powder and dissolve in methanol in 100ml volumetric flask. Pipette out 1ml stock solution into 10ml volumetric flask and make up the volume with mobile phase.

Degradation of Sample:

1gm powder kept at photo stability 72 hours. After 72 hours, weigh 25 mg of powder and dissolve in methanol in 100ml volumetric flask. Pipette out 1ml stock solution into 10ml volumetric flask and make up the volume with mobile phase.

III. RESULTS

Linearity and Range:

The linearity study was carried out for both drugs at different concentration levels. The linearity of ROS and FIM was in the range of 5-15 µg/ml for ROS and 30-90 µg/ml. % RSD of all results were less than 2%.

Table 3.1: Linearity data for ROS and FIM in HPLC

Linearity Level (%)		**Conc(mcg/ml)**		**Area**	
ROS	FIM	ROS	FIM	ROS	FIM
50%	50%	5	30	2192.656	910.026
75%	75%	7.5	45	3288.963	1364.656
100%	100%	10	60	4379.209	1832.298
125%	125%	12.5	75	5463.107	2266.38
150%	150%	15	90	6565.49	2723.858
correlation coefficient				0.9999	0.9999

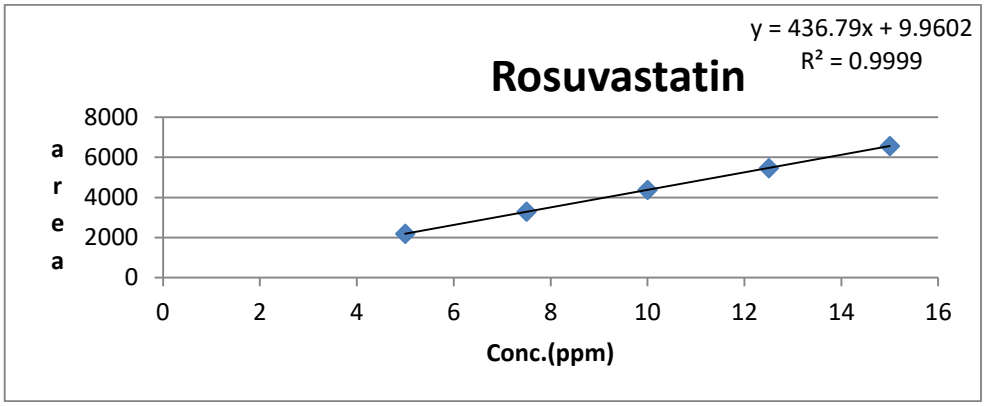

Figure 3: Calibration curve of ROS in HPLC

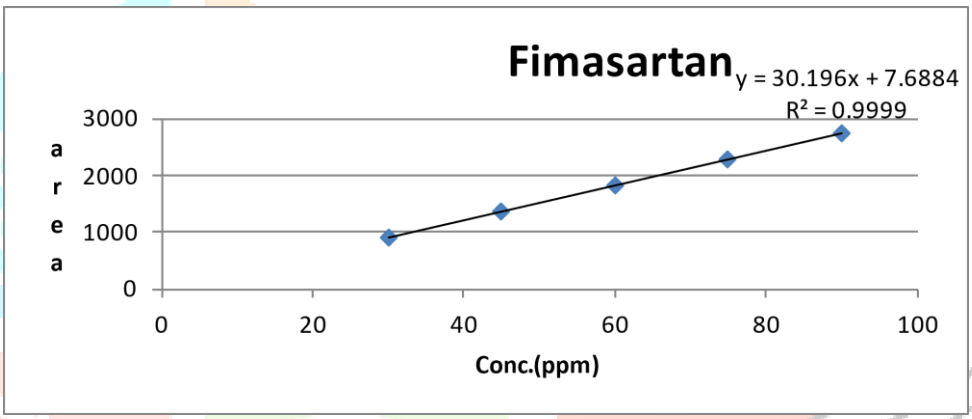

Figure 4: Calibration curve of FIM in HPLC

Table 3.2: Result of LOD and LOQ in RP-HPLC

Parameters	ROS	FIM
S.D. of the Y-intercepts of the 5 calibration curves	4.88778	8.5509
Mean slope of the 5 calibration curves	436.792	30.195
LOD	0.036	0.93
LOQ	0.11	2.83

Accuracy:

Table 3.3: Accuracy study of HPLC method:

Drugs	Amount of drugs (µg/ml)	% Of std added	Total amount added	Amount found (µg/ml)	% Recovery (Mean ± SD)	% RSD
ROS	5	80%	4	3.93	99.27 ±0.77	0.77
		100%	5	4.96	100.79± 0.72	0.71
		120%	6	5.96	100.47 ± 0.62	0.61
FIM	30	80%	24	23.7	99.20 ± 0.75	0.75
		100%	30	30.1	100.72 ± 0.69	0.68
		120%	36	36.1	100.38 ± 0.59	0.59

Precision:

Table 3.4: Intraday precision of ROS and FIM in HPLC

	ROS			FIM		
Precision	**Conc (µg/ml)**	**(Mean ± SD)**	**% RSD**	**Conc (µg/ml)**	**(Mean ± SD)**	**% RSD**
Intraday	5	2214.102 ± 15.6	0.70	30	918.813± 6.49	0.70
	10	4434.007± 22.53	0.50	60	1839.472± 9.09	0.49
	15	6565.675±19.10	0.29	90	2723.579 ±7.43	0.27
Interday	5	2191.191±7.54	0.34	30	909.4983±3.08	0.33
	10	4389.781±14.9	0.33	60	1821.264±6.23	0.34
	15	6550.159±22.9	0.35	90	2717.467±9.12	0.33

Robustness:

Table 3.5: Robustness study of HPLC method:

Parameters	**Variation**	**ROS**		**FIM**	
		Mean ± SD	**%RSD**	**Mean ± SD**	**%RSD**
Flow rate	+0.2 ml/min	4297.834± 7.09	0.16	1784.412± 3.01	0.16
	-0.2 ml/min	4548.772± 9.53	0.20	1886.573± 4.65	0.24
Mobile phase	+2% solvent in mobile phase	4445.898± 14.07	0.31	1844.57± 5.73	0.31
	-2% solvent in mobile phase	4400.843±15.02	0.34	1826.159± 5.95	0.32
Ph	+0.2pH	4296.115±11.26	0.26	1782.054±4.35	0.24
	-0.2pH	4267.27±11.82	0.27	1770.299±4.73	0.26

Repeatability:

Table 3.6: Repeatability study of HPLC method:

ROS		**FIM**	
Mean ± SD	**%RSD**	**Mean ± SD**	**%RSD**
4399.60016± 49.43	1.12	1825.612± 20.56	1.12

Table 3.7: System suitability parameters

System suitability test parameters	**ROS**	**FIM**
Retention time (min)	3.930	6.150
%RSD	1.12	1.12
Resolution (R_S)	-	
Tailing factor	1.346	1.350
Theoratical plates	7071	7250

Table 3.8: Analysis of Physical mixture

Drugs	Amount taken	%Amount of drug found	%RSD
ROS	100 µg/ml	99.89%	0.51
FIM	600 µg/ml	101.56%	0.55

Table 3.9: Summary of stress degradation condition

Stress type	Stress conditions	Rosuvastatin		Fimasartan	
		% Assay	% Degradation	% Assay	% Degradation
Acid Degradation	1ml 0.1N HCl kept for 4 hours	74.85	25%	86.70%	13.3%
Base degradation	1ml 0.1N NaOH kept for 8 hours	81.57%	18.4%	80.64%	19.3%
Peroxide oxidation stress study	1 ml 3% H_2O_2 kept for 4 hours	75.38%	24.6%	86.86%	13.1%
Thermal stress study	kept at 105^0C 72 hours	81.42%	18.5%	73.77%	26.2%
Photo degradation study	kept at photo stability chamber 72 hours	86.68%	13.3%	86.82%	13.1%

IV. CONCLUSIONS:

Proposed study describes a new stability indicating RP-HPLC method for the estimation for the combination of ROS and FIM in combination using simple mobile phase. The method gives good resolution between the compounds along with its degradation products with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise and stability indicating. So the developed method can be used conveniently for analysis of the combination for ROS and FIM in its synthetic mixture.

V. ACKNOWLEDGEMENT:

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VI. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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