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Stability Indicating RP-UPLC Method For The Simultaneous Estimation Of Rosuvastatin And Fimsartan In Combination

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Abstract: Stability indicating RP-UPLC method has been developed for the simultaneous estimation of ROS and FIM in combination. In RP-UPLC method, chromatographic separation was achieved using C_{18} column (250 mm x 4.6 mm) and Buffer (pH 3.0)-Methanol (60:40) as mobile phase at flow rate of 1.0 ml/min with detection wavelength of 243 nm. The linearity of both ROS and FIM were found in the range of 50-150 µg/ml. Retention time in RP-HPLC method were found to be 4.0 min, 6.5 min for ROS and FIM respectively. The % recovery were found to be 99.58 ± 0.57 for Rosuvastatin and 99.72 ± 0.75 for Fimasartan. The proposed method was validated as per ICH guidelines and successfully applied for the determination of drugs in pharmaceutical formulation. This evaluation supported the method's environmental friendliness regarding solvent usage, chemical substances, energy consumption, and waste generation.

Key words: Rosuvastatin, Fimasartan, Validation, Stability indicating RP-UPLC.

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].

The pharmaceutical preparation of the present disclosure is for the prevention or treatment of cardiovascular diseases, and cardiovascular diseases include hypertension or all the symptoms such as hypertension and complication of the metabolic syndrome patients who comorbidly showing diabetes, obesity, hyperlipidemia, coronary arterial diseases among others, and also include chronic stable angina, vasospastic angina, stroke, myocardial infarction, transient ischemic attack, congestive heart failure, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, diabetic nephropathy, dyslipidemia, cognitive impairments and dementia among others.

Fimasartan and rosuvastatin combination preparation having different acting mechanisms can be used for hypertension treatment but these combination preparation have a problem of affecting disintegration and dissolution of each active ingredient due to effect of interference to each other. That is, fimasartan exhibits decent solubility pattern under comparatively high pH media such as purified water and pH 6.8 dissolution media, but its solubility decreases under low pH media (i.e. pH 1.0 - pH 4.0)

According to the properties of fimasartan explained above, problem of decreasing disintegration and dissolution due to interference between fimasartan and rosuvastatin is raised when preparing combination preparation with rosuvastatin. Especially, decrease of dissolution under low pH media could seriously affect bioavailability at the stomach where initial disintegration and dissolution occurs at the time of oral administration. Under these circumstances, a research on a method of keeping constant disintegration and dissolution rates of fimasartan and rosuvastatin in spite of pH variation in the normal stomach is required. [11]

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 Ultra-Performance Liquid Chromatographic (RP-UPLC) method for the simultaneous

estimation so that can play important role in quantification of ROS and FIM in bulk and its pharmaceutical dosage form [12-21].

Green Analytical Chemistry has earned significant interest and approval due to its origins in green chemistry. Researchers are increasingly adopting Green Analytical Chemistry to reduce environmental impacts and increase the safety of analysts [22, 23]. The GAC concept refers to reducing dangerous chemicals from analytical processes to enhance the environmental friendliness without compromising method performance [24]. Ultra-Performance Liquid Chromatography (UPLC) is the predominant analytical technique employed in pharmaceutical quality control (QC) for the analysis of active pharmaceutical ingredients (APIs) and their impurities in pharmaceutical formulation and biological fluids. This method is more suitable for routine analysis, enabling efficient determination of numerous elements in the pharmaceutical formulation [25].

UPLC used in analytical techniques. It improves three areas – Speed, Resolution and Sensitivity: Speed-1.5min, Pressure-15000psi, Sensitivity-3-5 µl.

UPLC is a rising chromatographic separation technique whose packing materials have smaller particle size lesser than 2.5µm. The technique takes full advantage of chromatographic principles to run separations using columns packed with smaller particle size and higher flow rates. The principle of UPLC is based on Van Deemter Equation which describe the relationship between flow rate and column efficiency or HETP [26,27,28].

$$H = A + B/v + Cv$$

Where, A = Eddy diffusion

B = Longitudinal diffusion

C = Equilibrium mass transfer

v = Flow rate

SIGNIFICANCE:

- To monitor results during stability studies in order to guarantee safety, efficacy and quality.
- To determine if some of the impurities can be removed or minimized by the manufacturing process.
- To provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light.
- It represents also a powerful tool when investigating out-of-trend (OOT)) or out-of-specification (OOS) results in quality control processes.

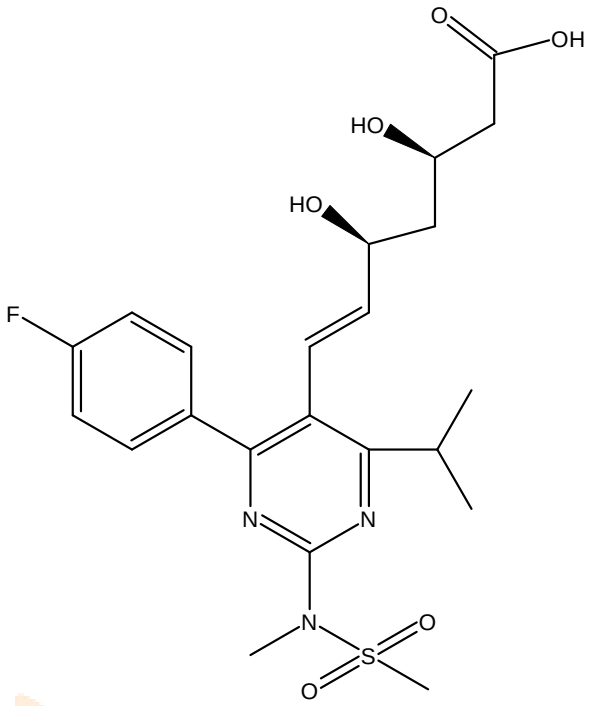


Figure 1. Chemical structure of Rosuvastatin

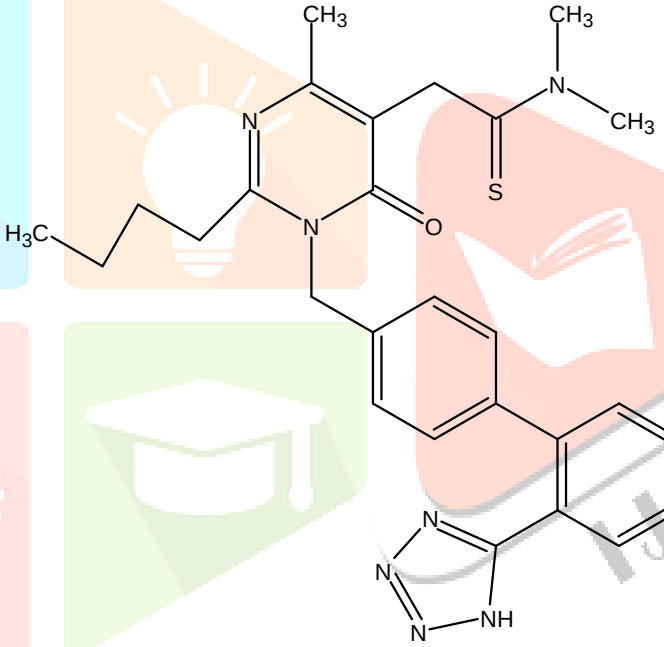


Figure 2. Structure of Fimasartan

This work aims to develop robust and precise analytical methods capable of quantitatively determining the concentrations of FIMA and ROS in Active pharmaceutical ingredient form. These methods offer a cost-effective and environment friendly, making valuable contributions to pharmaceutical research, and facilitating accurate dosage estimation. We conducted a greenness profile assessment utilizing AGREE metrics to evaluate the environmental impact of the newly developed UPLC method. This evaluation confirmed the environmentally friendly nature of the methods, considering aspects such as solvent usage, chemical substances, energy consumption, and waste generation.

The method has several advantages, including rapid analysis, a simple mobile phase, simple sample preparation, and improved sensitivity. It is suitable for analysis of these antihypertensive agents in their ternary formulations in a single isocratic run, in contrast with previous methods. This makes the method suitable for routine analysis in quality-control laboratories.

II.MATERIALS AND METHODS

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

The pharmaceutical dosage form containing 120 mg FIM, 20 mg ROS, Tuvero (**Boryung pharmaceutical, Ltd , South Korea**).

UPLC Instrument

The separation was performed by using C18 column (100 mm × 2.1 mm, 1.7µm) column on Empower software, pump and UV detector. The mobile phase was freshly prepared, filtered and sonicated before use and delivered at a flow rate of 0.4 ml/min and the detector wavelength was set at 243 nm. The injection volume was 10 µl.

Standard solutions preparation

To prepare the standard stock solution for UV spectrophotometry and HPLC, ROS and FIM were each separately dissolved in 50 mL of methanol by adding 10 mg and 60 mg of the respective drug powder to 100 mL volumetric flasks. The solutions were shaken vigorously and made up to 100 mL with methanol. The resulting concentrations were 100 µg/mL for ATOR and 600 µg/mL for FIMA.

Working Std Preparation (Combine Std Preparation):

Take 1ml from ROS stock, 1ml from FIM →10ml with Mobile phase

(mobile phase which used for trials) (ROS-10mcg/ml, FIM-60mcg/ml)

Note: Inject above working std preparation for mobile phase selection

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 20 µg/ml of ROS and 120 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. The wavelength maximums (λmax) was observed at 243 nm.

Analysis of API

Sample Stock Solution
Weight about sample (equivalent to 20mg of ROS/120mg 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-200mcg/ml, FIM-1200mcg/ml)

Working Sample Preparation
Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (ROS-20mcg/ml and FIM-120mcg/ml)

Stability study

Procedure for Stability Study:
Std stock soln of ROS: 20mg→100ml with methanol. (200mcg/ml)
Std stock soln of FIM: 120mg→100ml with methanol (1200mcg/ml)

Sample stock solution:
Weight about sample (equivalent to 20mg of ROS/120mg 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-200mcg/ml, FIM-1200mcg/ml)

Working Std Preparation:
Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (ROS-20mcg/ml and FIM-120mcg/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.

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 8 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.

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.

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.

Photo Degradation Study

ROS 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.

III.RESULTS AND DISCUSSION

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 10-30µg/ml for ROS and 60-180 µg/ml. % RSD of all results were less than 2%.

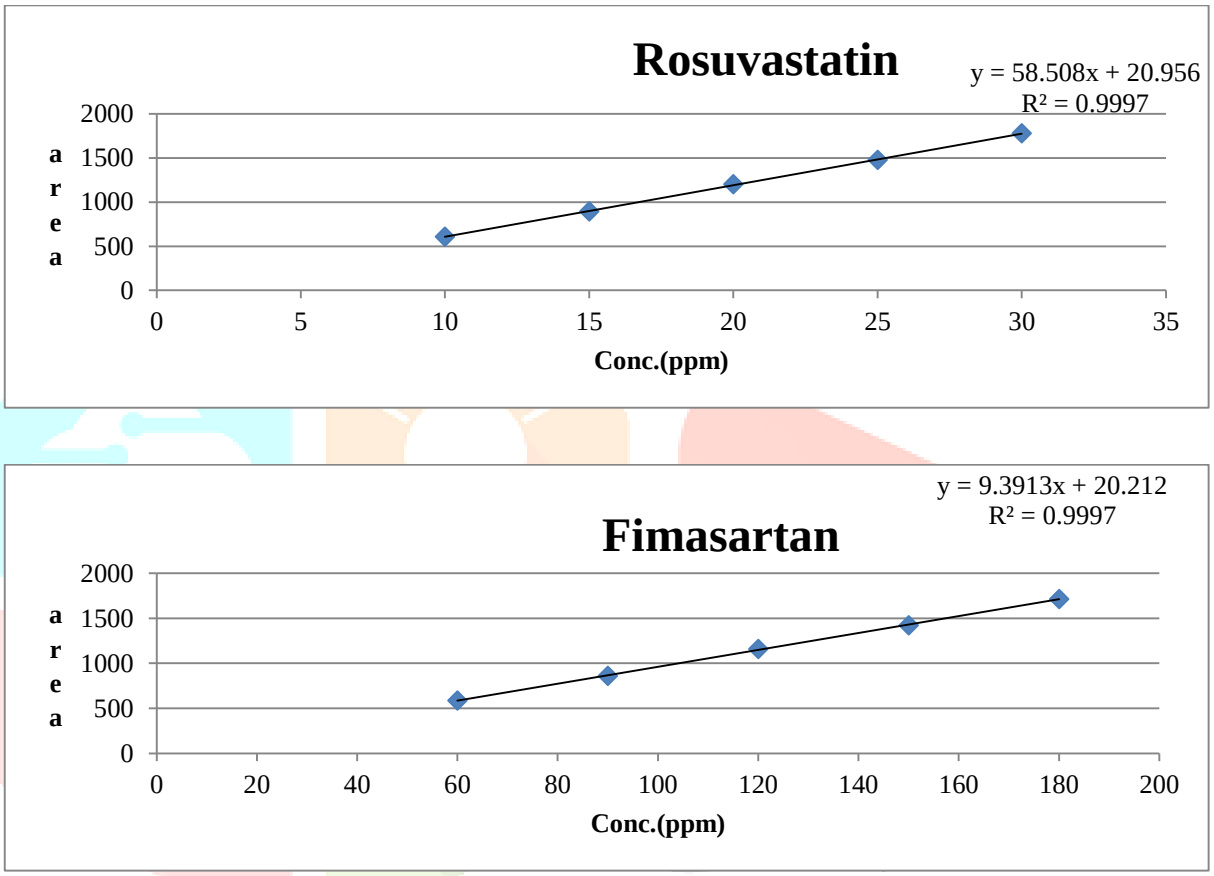


Figure 3. Calibration curve of ROS and FIM in UPLC

Table-1 Linearity data for ROS and FIM in UPLC

Linearity Level (%)		Conc(mcg/ml)		Area	
ROS	**FIM**	**ROS**	**FIM**	**ROS**	**FIM**
50%	50%	10	60	606.967	584.602
75%	75%	15	90	891.77	858.866
100%	100%	20	120	1203.331	1158.93
125%	125%	25	150	1475.882	1421.423
150%	150%	30	180	1777.604	1712.018
correlation coefficient				**0.99976**	**0.99974**

Table-2 Result of LOD and LOQ in RP-UPLC

Parameters	**ROS**	**FIM**
LOD	0.52µg/ml	3.14µg/ml
LOQ	1.59µg/ml	9.53µg/ml

Accuracy:

Table-3 Accuracy study of UPLC method:

Precision	**Conc (µg/ml)**	**(Mean ± SD) (n=3)**	**% RSD**	**Conc (µg/ml)**	**(Mean ± SD) (n=3)**	**% RSD**
Intraday	10	603.473 ± 3.38	0.56	60	584.406± 1.47	0.25
	20	1198.857± 7.35	0.61	120	1157.770± 3.06	0.26
	30	1774.513±5.63	0.31	180	1710.303 ±3.42	0.20
Interday	10	603.961±5.52	0.91	60	603.961±5.52	0.65
	20	1202.629±3.95	0.32	120	1202.629±3.95	0.27
	30	1780.355±10.1	0.56	180	1780.355±10.1	0.37

Precision:

Table-4 Intraday precision of ROS and FIM in UPLC

Drugs	Amount of drugs (µg/ml)	% Of std added	Total amount added	Amount found (µg/ml)	% Recovery (Mean ± SD)	% RSD
ROS	10 (n=3)	80%	8	8.01	100.18 ±1.11	1.11
		100%	10	10.0	100.06± 1.06	1.05
		120%	12	12.04	100.33 ± 0.27	0.27
FIM	60 (n=3)	80%	48	47.98	99.96 ± 1.47	1.47
		100%	60	60.2	100.37 ± 0.74	0.74
		120%	72	72.5	100.78 ± 0.62	0.62

Robustness:

Table-5 Robustness study of UPLC method:

Parameters	Variation	ROS		FIM	
		Mean ± SD (n=3)	%RSD	Mean ± SD (n=3)	%RSD
Flow rate	+0.1 ml/min	1169.036± 2.06	0.17	1124.23± 4.31	0.38
	-0.1 ml/min	1239.472± 4.32	0.34	1195.231± 3.47	0.29
Mobile phase	+2% solvent in mobile phase	1181.263± 4.52	0.38	1137.001± 6.33	0.55
	-2% solvent in mobile phase	1233.572±3.65	0.29	1183.837± 5.14	0.43
Column (Temp)	+5	1188.519±2.48	0.20	1144.8±2.10	0.18
	-5	1203.074±5.63	0.46	1159.221±3.66	0.31

Repeatability:

Table-6 Repeatability study of UPLC method:

ROS		FIM	
Mean ± SD (n=6)	**%RSD**	**Mean ± SD (n=6)**	**%RSD**
1211.395± 16.69	1.37	1166.739± 15.37	1.31

Table-7 System suitability parameters

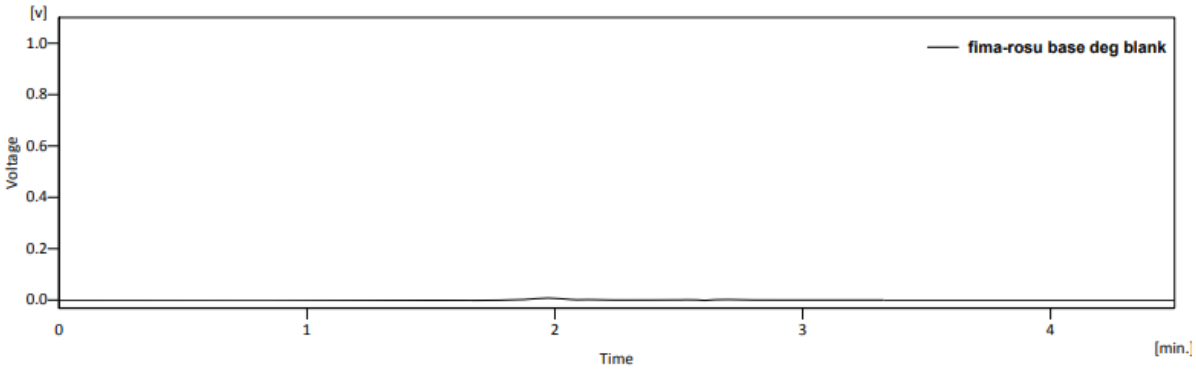
System suitability test parameters	**ROS**	**FIM**
Retention time (min)	2.080	1.567
%RSD	1.37	1.31
Resolution (Rs)	10	
Tailing factor	1.111	1.333
Theoratical plates	21571	19122
System suitability test parameters	**ROS**	**FIM**
Retention time (min)	2.080	1.567
%RSD	1.37	1.31
Resolution (Rs)	10	
Tailing factor	1.111	1.333
Theoratical plates	21571	19122

Table-8 Analysis of Physical mixture

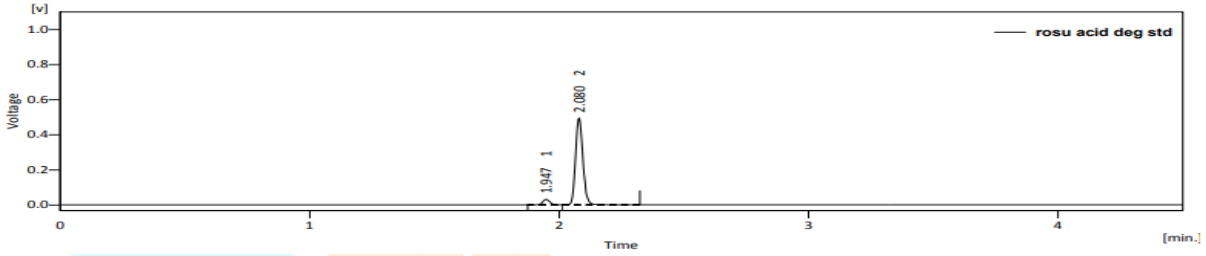
Drugs	**Amount taken**	**%Amount of drug found**	**%RSD**
ROS (n=3)	20 µg/ml	100.9%	0.82
FIM (n=3)	120 µg/ml	98.34%	1.25

Stability study data:

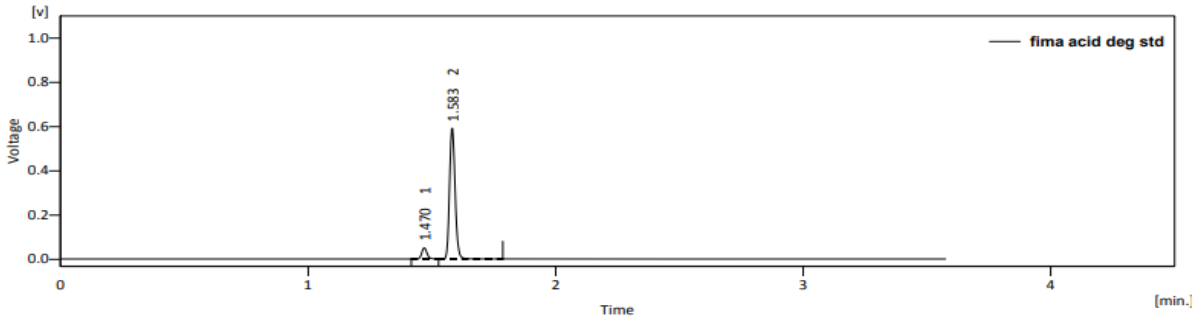
1. **Acid degradation study** (0.1 N HCl, 4 hr)



chromatogram of blank subjected to 0.1N acid degradation

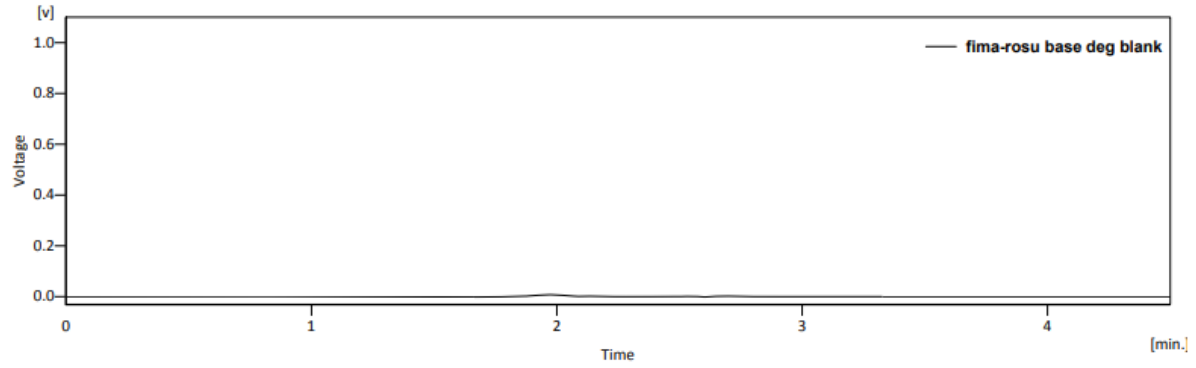


Chromatogram of ROS API subjected to0.1N acidic degradation

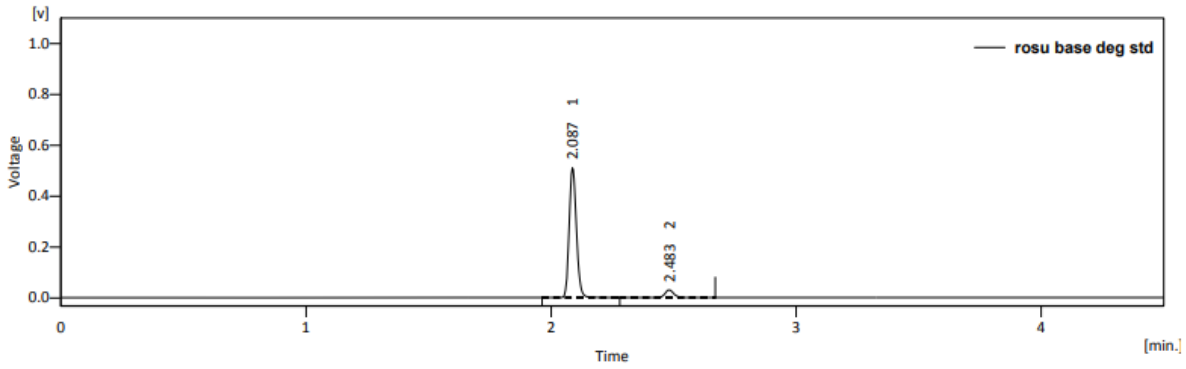


Chromatogram of FIM API subjected to 0.1N acidic degradation

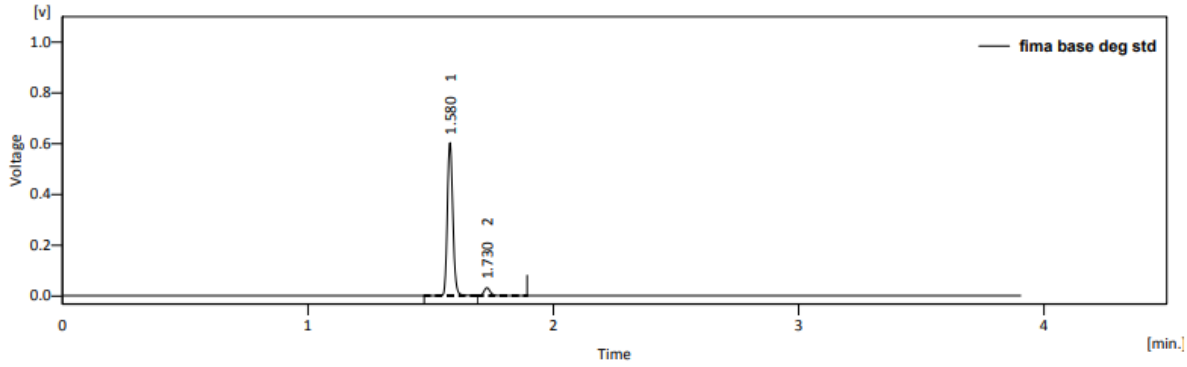
2. **Base Degradation study (0.1 N NaOH, 8 hr)**



Chromatogram of blank subjected to 0.1N Base degradation

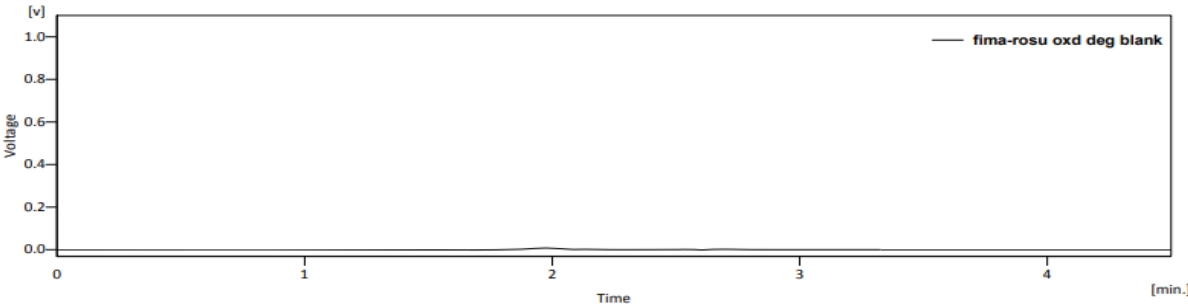


Chromatogram of ROS API subjected to 0.1N Base degradation

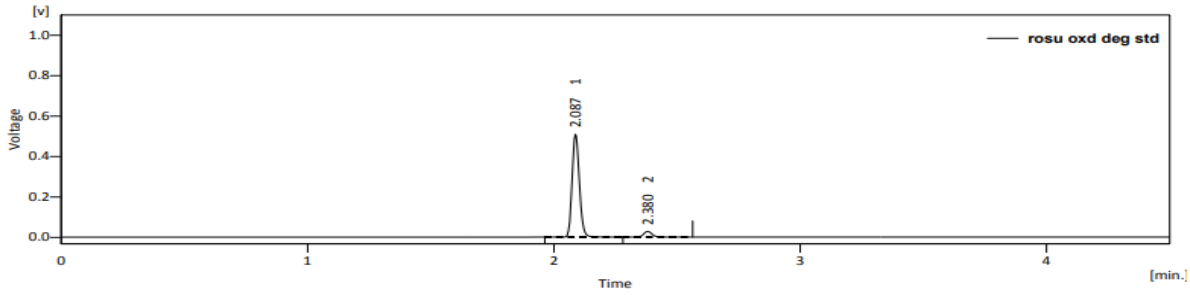


Chromatogram of FIM API subjected to 0.1N basic degradation

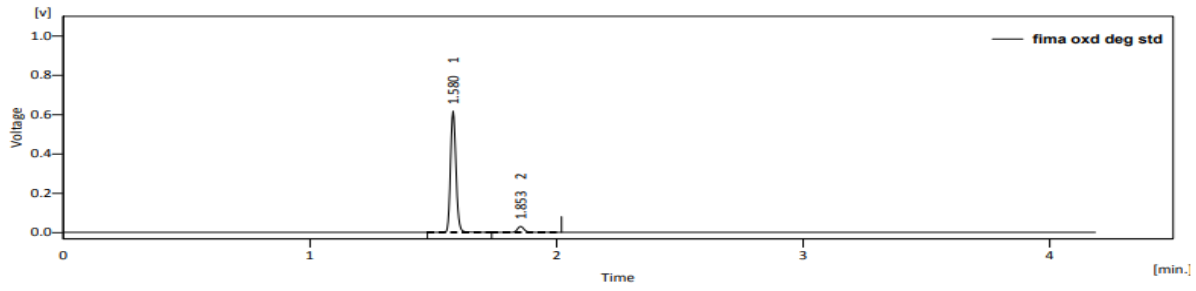
3. Peroxide study (3% H_2O_2 , 4 hr)



Chromatogram of blank subjected to 3% H_2O_2 degradation

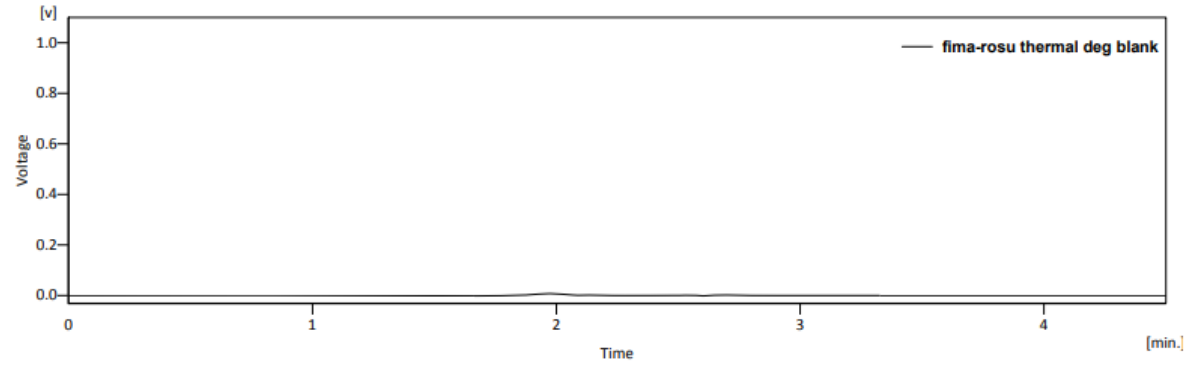


Chromatogram of ROS API subjected to 3% H_2O_2 degradation

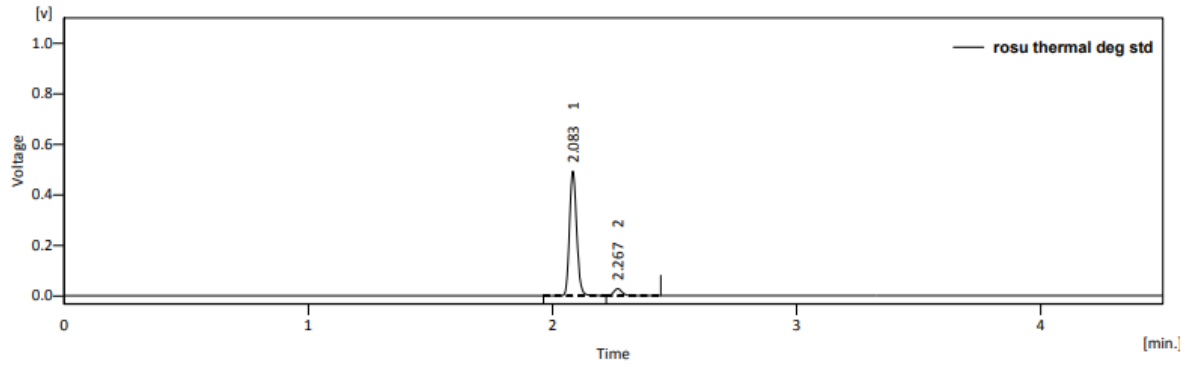


Chromatogram of FIM API subjected to 3% H_2O_2 degradation

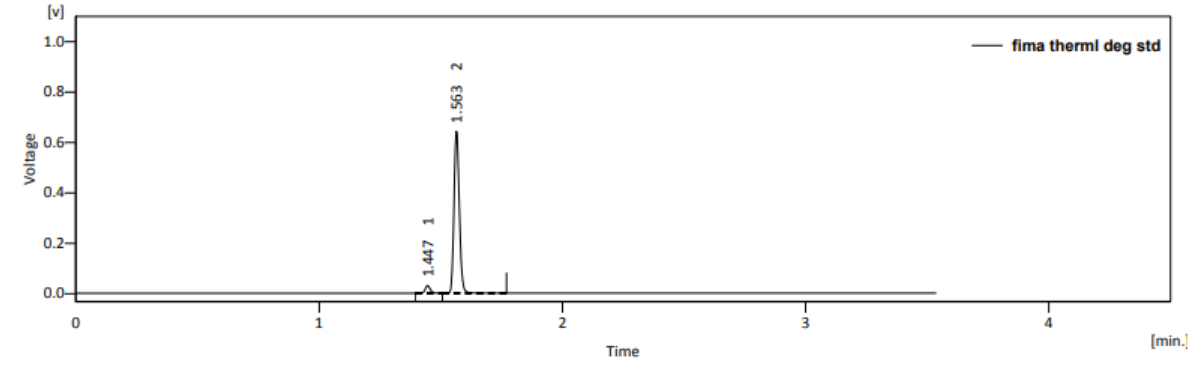
4. Thermal degradation study (72 hours at 105 ºC temperature)



Chromatogram of blank subjected to thermal degradation

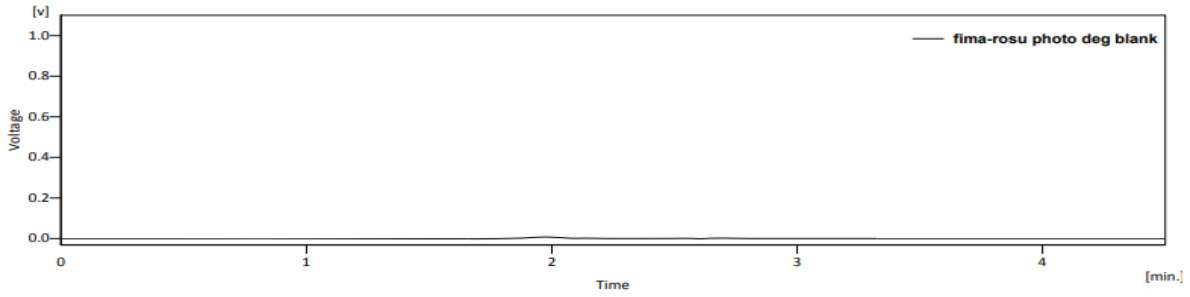


Chromatogram of ROS API subjected to thermal degradation

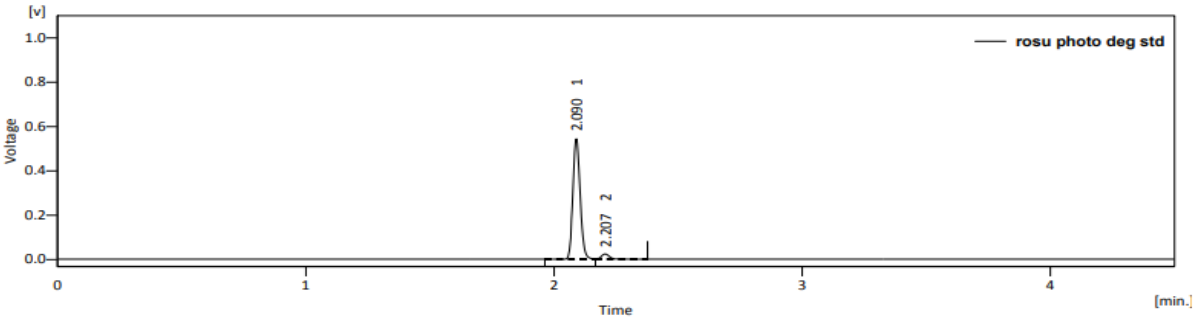


Chromatogram of FIM API subjected to thermal degradation

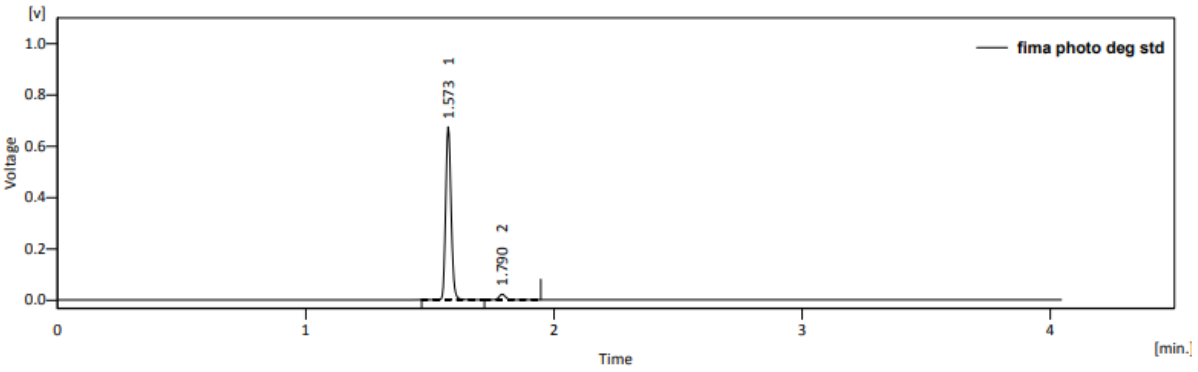
5. Photo degradation study (72 hours at Photo stability chamber)



Chromatogram of blank subjected to Photo degradation



Chromatogram of ROS API subjected to Photo degradation



Chromatogram of FIM API subjected to Photo degradation

Table-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	89.49%	10.5%	87.55%	12.4%
Base degradation	1ml 0.1N NaOH kept for 8 hours	93.47%	6.5%	87.14%	12.8%
Peroxide oxidation stress study	1 ml 3% H_2O_2 kept for 4 hours	92.66%	7.3%	88.01%	11.9%
Thermal stress study	kept at 105^0C 72 hours	97.07%	2.93%	93.67%	6.3%
Photo degradation study	kept at photo stability chamber 72 hours	88.73%	11.2%	90.05%	9.9%

IV.CONCLUSIONS:

Proposed study describes a new stability indicating RP-UPLC method for the estimation 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. The run time is short which enables rapid quantitation of many samples in routine analysis. No interference from the excipients was observed. The results demonstrated that the method would have a great value when applied in quality control and stability studies for this drugs.

So the developed method can be used conveniently for analysis of ROS and FIM in its combined pharmaceutical dosage form. Overall, the stability indicating UPLC method exemplify the potential of green analytical chemistry in reducing the environmental footprint of analytical techniques while maintaining analytical performance. Researchers can contribute to a more sustainable and environmentally conscious scientific community by adopting such green methods.

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VI.**CONFLICT OF INTEREST:**

The authors declare no conflict of interest.

VII.REFERENCES:

1. Tortora GJ, Grabowski SR (2003), "Principles of anatomy and physiology"' 10th edn. *Wiley* New Jersey, pp 758–759.
2. Development and validation of RP-HPLC method for determination of rosuvastatin in bulk and pharmaceutical dosage form, *International journal of pharmaceutical sciences review and research*, (2010), 5:1.
3. Kim JH, Lee JH, Paik SH, Kim JH, Chi YH, " Fimasartan, a novel angioten-sin II receptor antagonist"' *J Arch Pharm Res*, 35(7):1123–1126, 2012.
4. Drug Bank, Rosuvastatin Calcium, 2019. https://www.drugbank. ca/drugs/DB01098 (Accessed 10 July2019).
5. Drug Bank (2019), Fimasartan potassium trihydrate. https:// www.drugbank.ca/drugs/DB09279. Accessed 9 July 2019.
6. Burnham TH, "HMG-CoA reductase inhibitors", In: ed. Drug Facts and Comparisons, Louis: Facts and Comparisons, Inc 2002; 536-542a.
7. Bajaj T, Giwa AO, Rosuvastatin, [Updated 2022 May 29], In: StatPearls [Internet], Treasure Island (FL): *StatPearls Publishing*, 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539883/
8. ICHQ2(R1): Validation of Analytical Procedure, Text & Methodology, International Conference on Harmonization, *IFPMA*, Geneva, Switzerland, 2005.
9. Neal B. Macmahon S, Chapman N, " Effect of ACE inhibitors, calcium antagonists and other blood pressure lowering drugs: results of prospectively designed overviews of randomized trials, Blood pressure lowering treatment trailists Collaborations", Lancet.356 (9246): 1955-64, 2012.
10. Drug Bank (2019), Rosuvastatin Calcium. https://www.drugbank. ca/drugs/DB01098. (Accessed 10 July2019).
11. https://patents.google.com/patent/EP2974719A1/en
12. Tripathi KD, " Essentials of Medical Pharmacology", *Jaypee brothers medical publisher (P)* ltd, New Delhi. 2008-09.
13. ICHQ2B: Validation of Analytical Procedure, Methodology, International Conference on Harmonization, *IFPMA*, Geneva, Switzerland, 2005.

14. ICHQ2(R1): Validation of Analytical Procedure, Text & Methodology, International Conference on Harmonization, *IFPMA*, Geneva, Switzerland, 2005.

15. ICHQ1A (R2), Stability Testing of New Drug Substance and Drug Product, International Conference on Harmonization, *IFPMA*, Geneva, Switzerland, 2003.

16. World Health Organization, "Fact Sheet. The top 10 causes of death."https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. (Accessed March 15, 2020).

17. https://www.who.int/health-topics/hypertension/. (2019)

18. H. W. Moon, A. I. Moon, A. M. Yousaf and H. G. Cho, " Evaluation of stability and simultaneous determination of fimasartan and amlodipine by a HPLC method in combination tablets", *Asian. J. Pharm. Sci*, 9, 123-128, 2014.

19. M. Ashfaq and T. Akhtar, "Simultaneous estimation of rosuvastatin and amlodipine in pharmaceutical formulations using stability indicating HPLC method", *Brasil. J. Pharm. Sci*, 50, 629- 638, 2014.

20. S. R. Potawale, "HPTLC method for simultaneous determination of Rosuvastatin and Fenifibratae in bulk and pharmaceutical formulation", *Int. J. Pharm. Sci*, 6, 323-326, 2014.

21. O'Neil M J, " The Merk Index- an encyclopedia of chemicals and biological, New Jersy: Merk and Co, 2013.

22. PT Anastas, MM, Kirchhoff. Origins, Current Status, and Future Challenges of Green Chemistry, *AccChem Res*, 35 (2002) 686–694. https://doi.org/10.1021/ AR010065M. [27]A Gałuszka, Z Migaszewski, J.

23. The Principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, TrAC - Trends Anal Chem, 50 (2013) 78–84. https://doi.org/10.1016/j.trac.2013.04.010.

24. Mohamed HM, "Green. Environment-friendly", analytical tools give insights in pharmaceuticals and cosmetics analysis, *TrAC Trends Anal Chem*, 66, 176–192, 2015. https://doi.org/10.1016/J.TRAC.2014.11.010.

25. B Nikolin, B Imamovic, S Medanhodzic-Vuk, M. Sober, " High perfomance liquid chromatography in pharmaceutical analyses", *Bosn J Basic Med Sci*, 4 (2004) 5–9. https://doi.org/10.17305/BJBMS.2004.3405.

26. Swartz, M.E, "Ultra performance liquid chromatography (UPLC): An introduction, separation science re-defined", *LCGC Suppl, 8*, 8-14 , 2005.

27. Gita Chawla, Chanda Ranjan, "Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography", *Bentham Open*, ISSN: 1874-8422 — Volume 8, 2021.

28. Ashok Akabari, Pooja Mistry, Sagar Patel, Jasmina Surti, Umang Shah, "Simulteneous estimation of Fimasartan and atorvastatin calcium with greenness assessement using HPLC and uv Spectrophotometric methods", *Green analytical chemistry*, Grreac-D-23-00037.