WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 6, Issue 16, 1658-1676. Research Article ISSN 2277–7105

A NEW SEPARATION TECHNIQUE FOR METHOD DEVELOPMENT AND VALIDATION OF ROSUVASTATIN AND MICRONIZED FENOFIBRATE IN IT'S PURE AND PHARMACEUTICAL DOSAGE FORM

Madireddy Mamata[1], Anil Middha[1] and Reddy Sunil*[2]

[1]School of Pharmacy, OPJS University, Rawatsar Kunjla, Near Sankhu Fort, Tehsil. Rajgarh, Distt., Churu (Rajasthan).

[2]SVS Group of Institutions, Institute Of Pharmacy, Warangal, Telangana, India, 506015.

Article Received on
04 Oct. 2017,

Revised on 25 Oct. 2017,
Accepted on 15 Nov. 2017

DOI: 10.20959/wjpr201716-13694

***Corresponding Author**
Dr. Reddy Sunil
SVS Group of Institutions,
Institute of Pharmacy,
Warangal, Telangana, India.
506015.
drsunilsvsjntuh@gmail.com

ABSTRACT

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Rosuvastatin and Micronized Fenofibrate was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Rosuvastatin and Micronized Fenofibrate were found to be from 100-500 µg/ml of Rosuvastatin and 1-5µg/ml of Micronized Fenofibrate. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Rosuvastatin and Micronized Fenofibrate. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: Micronized Fenofibrate, Linear regression, LOD and LOQ, Rosuvastatin.

INTRODUCTION

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. It deals with methods for determining the chemical composition of samples of matter. A **Qualitative Method** yields information about the identity of atomic or molecular species or the functional groups in the sample. A **Quantitative Method**, in contrast, provides numerical information as to the relative amount of one or more of this component. Pharmaceutical analysis especially deals with the identification and quantification of the pure drug and impurities and components of the formulation with the help of analytical tools.

Classification of Analytical Methods

Analytical methods can be separated into classical and instrumental methods. **Classical methods** are also known as **wet chemistry methods**, where,

- Extraction, precipitation, and distillation processes were used for the separation of analytes.
- Qualitative analysis performed by utilizing chemical reactions of analytes with reagents that produce, products that could be identified by their physicochemical properties like boiling or melting points, optical activities, solubilities, refractive indexes and colors.

- Quantitative analysis accomplished by titrimetric or gravimetric methods. In **Gravimetry**, the quantity of the analyte is determined by the mass of product generated in the chemical reaction. In **Titrimetry**, analyte quantity is determined by the volume of a reagent reacted to complete the reaction with anlyte.

❖ **Instrumental methods** by utilizing set of sophisticated equipment that measure the intrinsic physical properties of molecules such as conductivity, light absorption and fluorescence. These techniques can be both qualitative and quantitative in nature. Thus, techniques employed in quantitative analysis are based upon-**Chemical Properties, Electrical Properties, Optical Properties.**

AIM AND OBJECTIVE

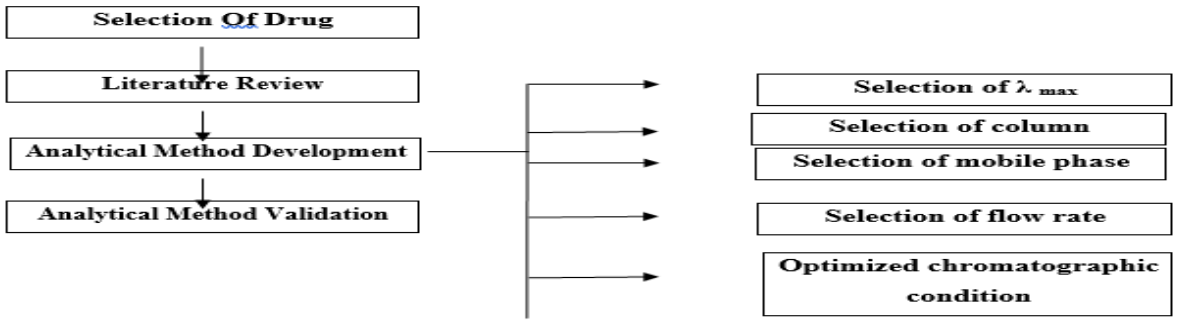
The literature review reveals that very few RP-HPLC methods available for the estimation of Rosuvastatin and Fenofibrate alone and/or in combination with other drugs. Because of the limited availability of analytical methods for the above drugs in combination form it was intend to develop a simple, accurate and more sensitive stability indicating RP-HPLC method by simultaneous estimation.

OBJECTIVE

Development of a HPLC method for analysis of both the drugs. Validation of the method using formulations.

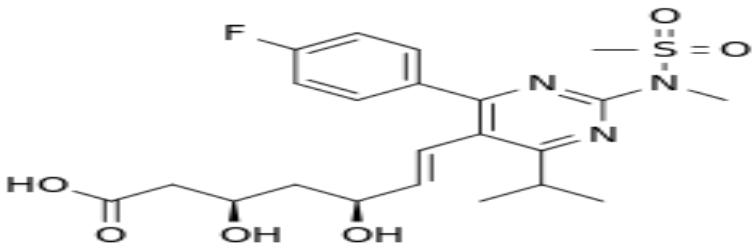
PLAN OF WORK

The work has been carried out in the following steps.



Drug Profile

Rosuvastatin



IUPAC Name: 7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.

Chemical formula: $C_{22}H_{28}FN_3O_6S$

Molecular weight: 481.538, Monoisotopic: 481.168284538

pka : -2.8

Cas No: 287714-41-4

Solubility : Sparingly soluble in water

Category : hypolipidemic agent.

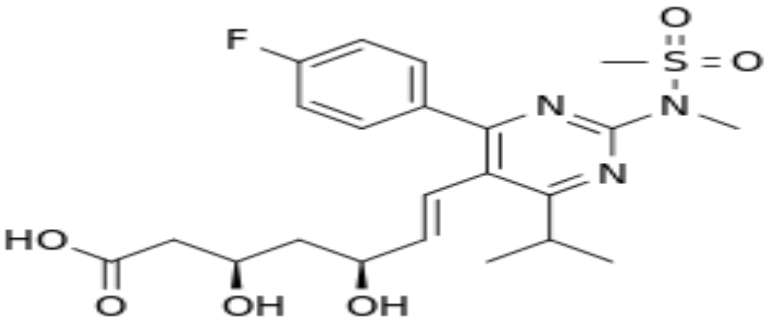
Mechanism of action: Rosuvastatin reduce the cholesterol synthesis by competitively inhibiting the rate determining enzyme of cholesterol bio synthesis, HMG-CoA reductase. It is indicated in obesity, heart failure. Rosuvastatin half life is 19hrs with peak plasma concentration at 4-5hrs of oral administration.

Generic Name: Rosuvastatin.

Brand Name

Astende, Cirantan, Cresadex Rosustatin Rosuvas Rosedex Rosimol.

Fenofibrate



IUPAC Name: propan-2-yl 2-{4-[(4-chlorophenyl)carbonyl]phenoxy}-2-methylpropanoate

Chemical formula: $C_{20}H_{21}ClO_4$

Molecular weight: 360.831, Monoisotopic: 360.112836867.

pka: 4.9.

Cas No: 49562-28-9.

Solubility: Soluble In Water 0.25mg/Ml At 25 °C

Melting Point: 80.5 °C

Category: Hypolipidemic Agents

Mechanism of action

Fenofibrate exerts its therapeutic effects by accelerating the lipolysis process and eliminating triglyceride particles by the activation of lipoprotein lipases. Firates directly activate the peroxisome proliferator activated receptor (PPARa).

Generic Name

Fenofibrate.

Brand Name

Fenogal, Lipanthyl, Lipidil.

MATERIALS AND METHODS

Standards and Samples

Table. List of Standard and Sample details.

S.NO.	NAME	BATCH NO.	MANUFATURER/ SUPPLIER
1.	Rosuvastatin working standard	-	KP labs pvt. Ltd
2.	Fenofibrate working standard	-	KP labs pvt. Ltd

Analytical Method Development

A. Selection of wavelength

The 10µg/ml strength solution of Rosuvastatin and Fenofibrate were prepared in milliQ water and it was scaned by HPLC – PDA detector and by UV-Visible spectrophotometer from 200-400nm. Then 254nm was selected due to the optimal response.

B. Selection of chromatographic condition

The selected drugs Rosuvastatin and Fenofibrate were polar in nature. Due to the polar nature of analytes RP-HPLC was selected for the determination.

C. Initial separation condition

Because of the low viscosity, low back pressure and favourable UV transmittance, the mobile phase selected was milliQ water and HPLC methanol.

Preparation of standard solution: Each of 10mg of rosuvastatin and Fenofibrate were dissolved in 7ml of mobile phase separately in each of 10ml volumetric flasks and the volume made up to the mark. 0.2 and 0.1ml from each standard solution was transferred to another 10ml volumetric flasks and volume made up to the mark by mobile phase to produce 20 µg/ml and 10µg/ml solutions respectively.

Preparation of sample solution: 10 Tablets of rosuvastatin and fenofibrate were powdered and a quantity of powder equivalent to 10 mg of active drugs was dissolved by sonication in a 7ml of mobile phase, then the volume was made up to the mark in a 10ml volumetric flask to produce 1000 µg/ml solution (stock solution). The serial dilution method was utilized to get the solution of desired concentration and they were filtered through 0.45 µm filter before injecting into HPLC system.

Preparation of placebo: Powdered inactive ingredient amount that supposed to be present in 10 tablets was weighed dissolved in 7ml mobile phase by sonication in 10ml volumetric flask and the volume made up to the mark. 0.1 ml of upper clear solution was further diluted in a10ml volumetric flask up to the mark and filtered through 0.45 µm filter.

OPTIMIZED METHOD

Preparation of mobile phase: 6.8 gm of KH_2PO_4 dissolved in Hplc grated water and the pH adjusted to 3 with ortho phosphoric acid in a 1000ml volumetric flask. The mobile phase was prepared by mixing 300ml (30%) of buffer and 700ml (70%) of methanol, then degassed in ultrasonic water bath and was filtered through 0.45 µm filter under vacuum.

Diluent Preparation: Mobile phase was employed as diluent.

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 mins

Test Procedure: Standard, sample and blank preparations in concentration of 20µl were injected into HPLC system and peak responses were calculated.

The developed RP-HPLC method utilized the Agilent C_{18} (4.6 x 150mm), 5µm column. Gradient elution achieved using mobile phase Methanol: Phosphate buffer of pH - 3 [70 : 30, v / v] with flow rate of 0.8 ml / min at 254nm.

Observation: Resolution observed was good between two analytes. Peak asymmetry was not observed. Other impurity interference was not seen and all the results were within the acceptance criteria. Hence the method was well thought-out to be optimized.

Calculation

For Rosuvastatin

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of Rosuvastatin mg/ml.

For Fenofibrate

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of Fenofibrate mg/ml.

The Standard, Sample and Blank Chromatograms for optimized method were shown in **Fig: 9, 10 & 11.**

METHOD VALIDATION

Method validation is aimed to find out the suitability of the developed method for the intended purpose. According to ICH guidelines, the validation parameters are.

1. System suitability 2. Linearity 3. Precision 4. Accuracy 5. Specificity 6. Robustness.

7. Ruggedness 8. Limit of detection 9. Limit of quantification

RESULTS

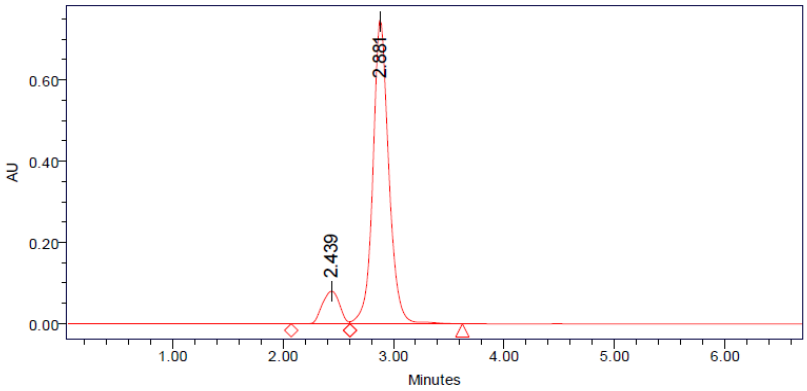


Fig. Chromatogram of Trial 1.

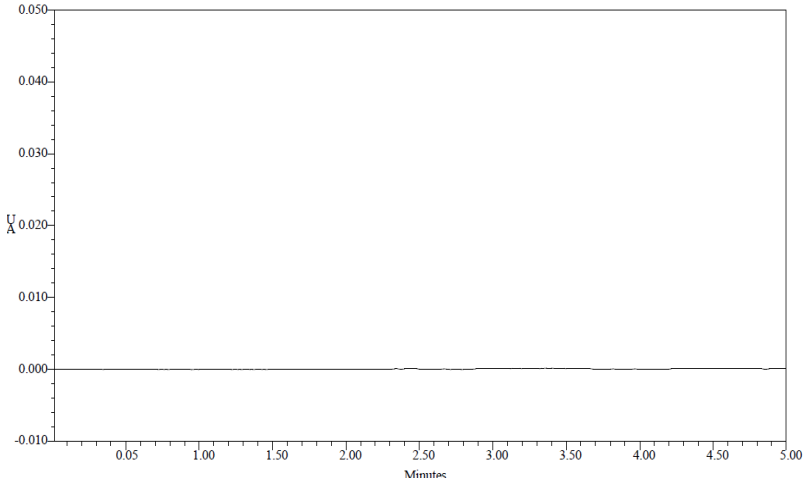


Fig. Chromatogram of Blank.

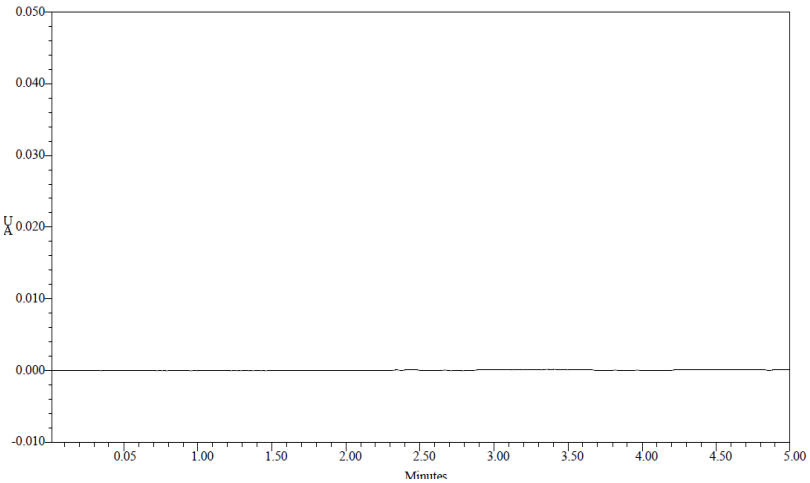


Fig. 12: Chromatogram for Placebo.

METHOD VALIDATION

1. System Suitability

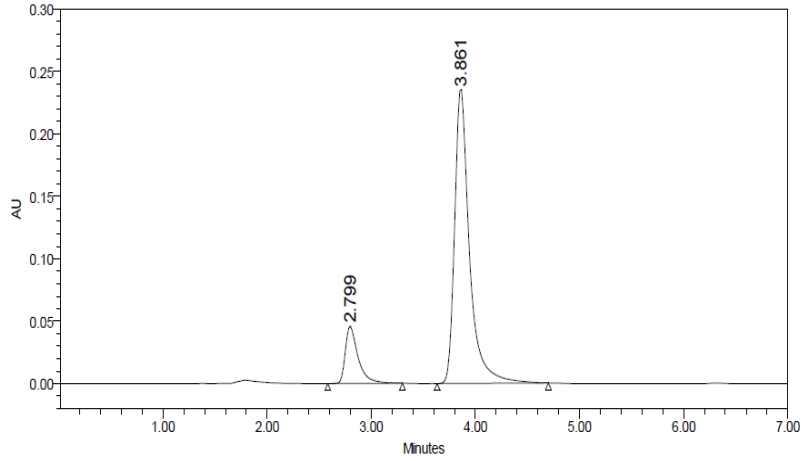


Fig. 13: Chromatograms for System suitability.

Table: Chromatogram values for System suitability

a) 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		

1). Obtained Tailing factor from the standard injection is 1.6

2). Obtained Theoretical Plates from the standard injection is 2496

Assay Results: (Rosuvastatin)

1250595 10 0.2 100 10 99.9 1754.5

---------- x ----------x --------- x -------- x------- x --------- x100 x --------- =99.83%

1251360 10 10 1754.5 0.1100 300

b) Fenofibrate

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			

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Assay Results: (Fenofibrate**).**

928829 10 0.1 10010 99.8 1754.5

---------- x -------- x--------- x ----------x------- x -------- x --------- x100 =98.89%

937364 10 10 1754.5 0.1100 300

2. Linearity

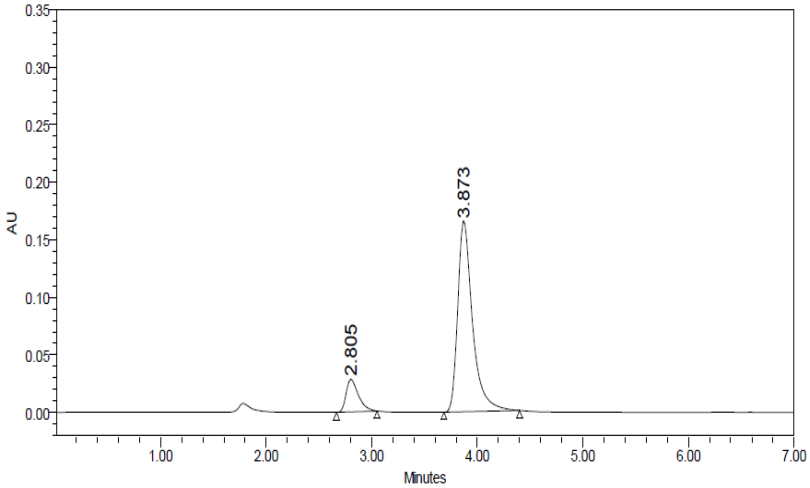


Fig: Linearity level 1 Chromatogram

Table: Linearity results for Fenofibrate

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

PRECISION

A. Repeatability

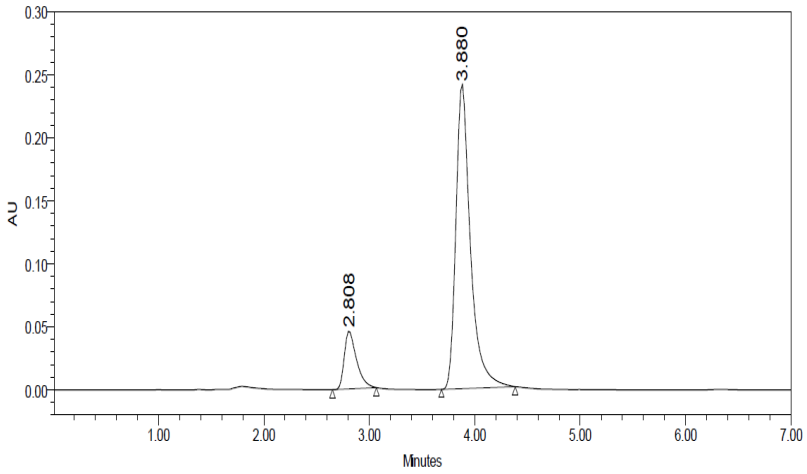


Fig: Sample Chromatograms for Repeatability.

Table: Sample Chromatogram values for Repeatability

a) 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	

b) Fenofibrate

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	

Acceptance Criteria: The % RSD for the area and R_t of five standard injections results should not be More than 2%.

RUGGEDNESS

B) Intermediate precision (Analyst to Analyst variability):

Fig Chromatograms for Intermediate precision

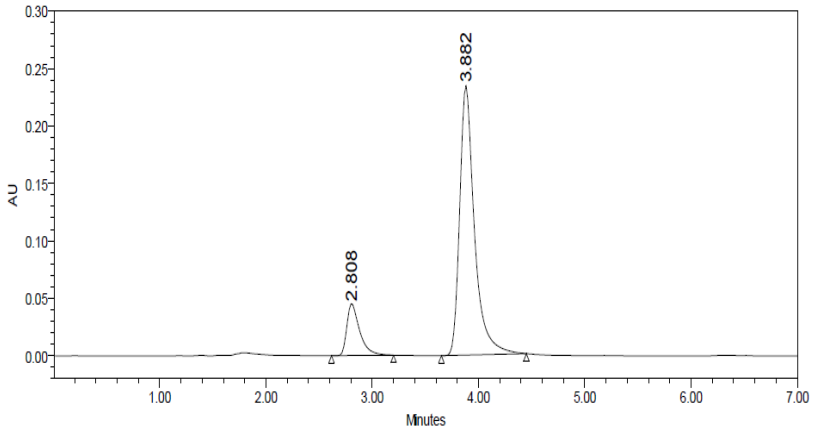


Table. Sample Chromatogram values for intermediate Precision.

a) 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	

b)Fenofibrate

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	

Acceptance Criteria: The % RSD for the areas and Rt'sof five standard injections results should not be more than 2%.

Accuracy

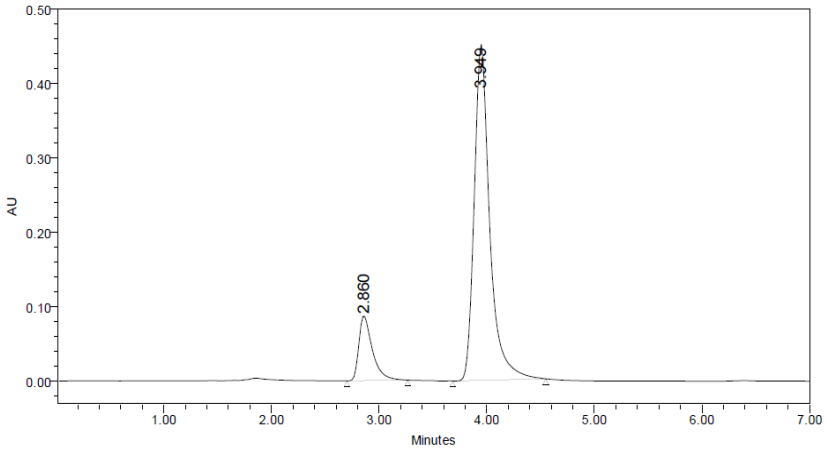


Fig Standard Chromatogram for Accuracy.

Fig Chromatograms for Acc 50%

Table Chromatogram Values For 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%	

Acceptance Criteria

- The % Recovery for each level should be between 98.0 to 102.0%.

Table: Chromatogram Values For Accuracy of Fenofibrate.

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%	

Acceptance Criteria

- The % Recovery for each level should be between 98.0 to 102.0%

Specificity

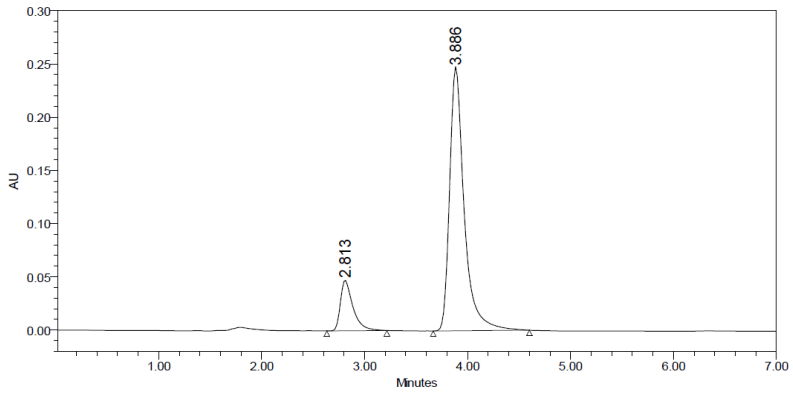


Fig. Standard Chromatogram for Rosuvastatin and Fenofibrate Identification.

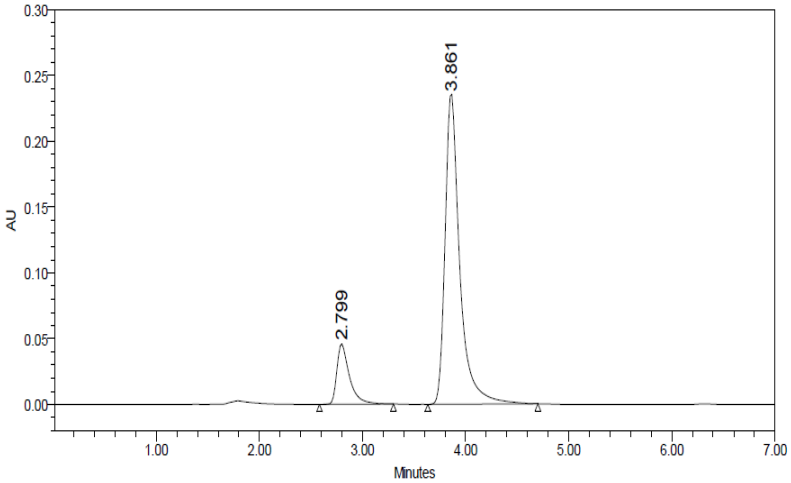


Fig: Sample Chromatogram for Rosuvastatin and Fenofibrate Identification.

ROBUSTNESS

a) Effect of variation in flow rate

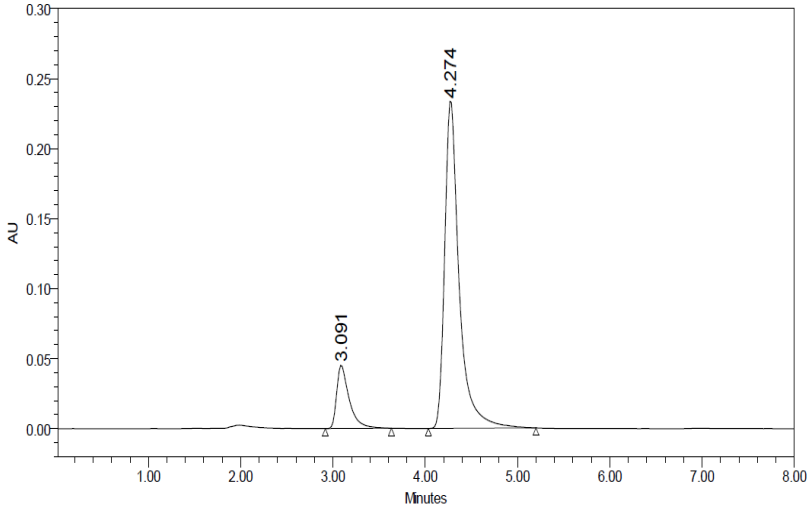


Fig: Chromatogram for less flow rate.

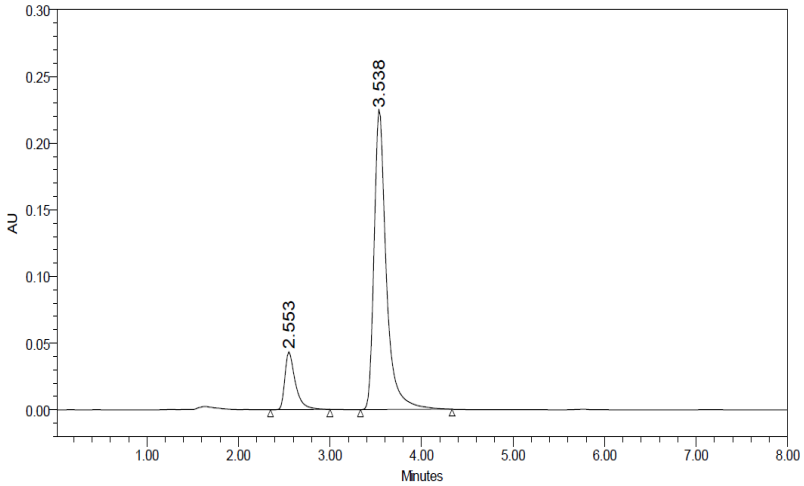


Fig: Chromatograms for more flow rate.

Table: 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: Robstness results forFenofibrate (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

***** Results for actual flow (0.8ml/min) have been considered from Assay standard.

b) Effect of variation in mobile phase composition

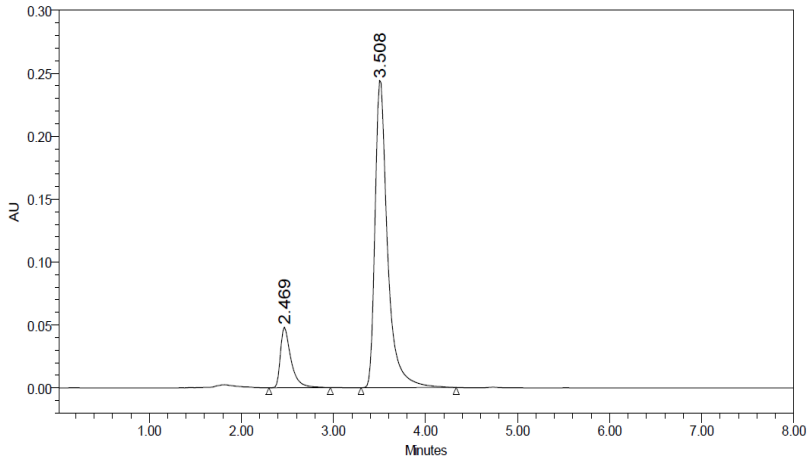


Fig. Chromatograms for more organic mobile phase.

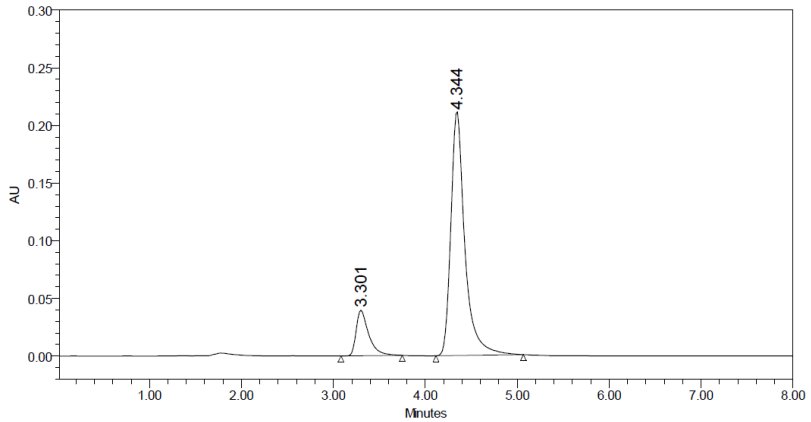


Fig: Chromatogram for Less Organic Mobile Phase.

Table no: Robustness 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 no: Roubstness results for Fenofibrate.

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

***** Results for actual Mobile phase composition (70:30Methanol: Buffer) have been considered from Accuracy standard

Limit of Detection (Lod)

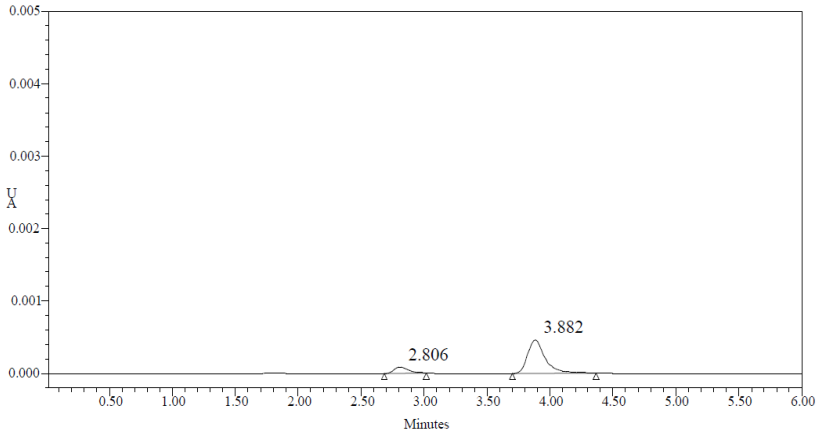


Fig. Chromatogram for Rosuvastatin and Fenofibrate.

Calculation of S/N Ratio for Rosuvastatin

Average Baseline Noise obtained from Blank : 48 µV

Obtained Signal from LOD solution (0.15% of targeted assay concentration) : 146µV

S/N = 146/48 = 3.041.

Acceptance criteria

Values of S/N ratio must be not more than 3.0 for LOD solution.

Calculation of S/N Ratio for Fenofibrate

Average Baseline Noise obtained from Blank : 48µV

Obtained Signal from LOD solution (0.22% of targeted assay concentration) : 148 µV

S/N = 148/48 = 3.08.

Acceptance criteria: S/N Ratio value shall be not more than 3 for LOD solution.

Limit of Quantification (Loq)

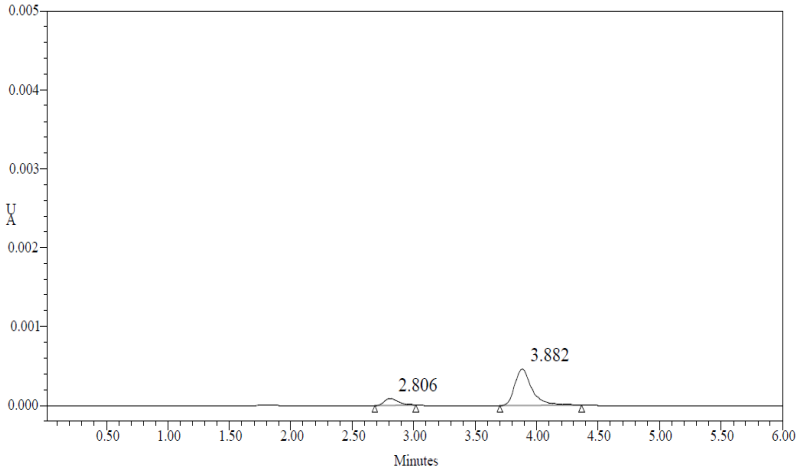


Fig. Chromatogram For Rosuvastatin and Fenofibrate.

Calculation of S/N Ratio for Rosuvastatin

Average Baseline Noise obtained from Blank : 48 µV

Obtained Signal from LOQ solution (0.05% of targeted assay concentration) : 470µV

S/N = 470/48= 9.79

Acceptance Criteria

Value of S/N ratio must be not more than 10 for LOQ solution.

Calculation of S/N Ratio for Fenofibrate

Average Baseline Noise obtained from Blank : 48 µV

Obtained Signal from LOQ solution (0.06% of targeted assay concentration) : 498µV

S/N = 498/48 = 10.37.

Acceptance criteria

S/N Ratio value shall be not more than 10 for LOQ solution.

REFERENCES

1. M. Sumalatha et al, Analytical method development and validation for the simultaneous estimation of Rosuvastatin and Finofibate in tablet dosage form by reverse phase high performance liquid chromatography, Indian Journal of Research in Pharmacy and Biotechnology.
2. Suresh Kumar GV et al, Development and Validation of Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Fenofibrate in Tablet Dosage Form. International Journal of PharmTech Research, 1996; 681(2): 299-306.
3. Anandakumar Karunakaran et al, Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV-Spectrophotometry and RP-HPLC. Stamford journal of pharmaceutical sciences.
4. Devika.G.Set al, A New Improved RP-HPLC Method For Simultaneous Estimation Of Rosuvastatin Calcium And Fenofibrate In Tablets. International Journal of Pharmacy and Pharmaceutical Sciences, 1997; 690: 259-265.
5. Zahi Mohammad Turabi et al, Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Form Research Article, 1997; 691: 145-153.
6. Usha Rani N et al, New Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Sciences and Drug Research, 2014; 6(4): 283-290.
7. Bhavna Patel et al, Development and Validation of Derivative Spectroscopic Method for the Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet. International Journal of Pharma Research & Review, 2011; 54: 168-172.
8. Ezzet, F., Van Vugt, M., Nosten, F., Looareesuwan, S., White, N.J., Antimicrob Agents Chemother, 2000; 44: 697–704.
9. Zeng, M., Lu, Z., Yang, S., Zhang, M., Liao, J., Liu, S., Teng, X., J Chromatogr B., 1996; 681: 299-306.
10. Ashley, E.A., Stepniewska, K., Lindegardh, N., McGready, R., Annerberg, A., Hutagalung, R. et al. Trop Med Int Health, 2007; 12: 201–208.
11. Wahajuddin, Singh, S.P., Jain, G.K., J Chromatogr B., 2009; 877: 1133-1139.
12. Hodel, E.M., Zanolari, B., Mercier, T., Biollaz, J., Keiser, J., Olliaro, P., Genton, B., J Chromatogr B., 2009; 877: 867–886.
13. César, I.C., de Aquino Ribeiro, J.A., de Souza Teixeira, L., Bellorio, K.B., de Abreu,

F.C., Moreira, J.M. et al., J Pharm Biomed Anal, 2011; 54: 114-120.

14. Agarwal, Suraj P; Ali, Asgar, Ahuja, Shipra., A. J. of Chemistry. 2007; 19: 4407-4414.
15. DEVIKA.G.Set al, A NEW IMPROVED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN TABLETS. International Journal of Pharmacy and Pharmaceutical Sciences, 1997; 690: 259-265.
16. Zahi Mohammad Turabi et al, Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Form. Research Article, 1997; 691: 145-153.
17. Usha Rani N et al, New Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Sciences and Drug Research, 2014; 6(4): 283-290.
18. Bhavna Patel et al, Development and Validation of Derivative Spectroscopic Method for the Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet. International Journal of Pharma Research & Review, 2011; 54: 168-172.