RESEARCH ARTICLE

Novel Isocratic RP-HPLC Method Development and Validation of Rosuvastatin and Fenofibrate in Tablets

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ABSTRACT

A novel simple selective, precise, and accurate reverse phase-high performance liquid chromatography (RP-HPLC) isocratic method was developed for the simultaneous estimation of rosuvastatin and fenofibrate in the combined formulation. The drugs rosuvastatin calcium and fenofibrate were separated in presence of fenofibrate related compound A and fenofibrate related compound B. The drugs and related compounds were separated on Kromasil C18 (250 × 4.6, 5 µ) with a reverse phase isocratic elution. 0.01M potassium dihydrogen phosphate adjusted pH 3.0 with dilute phosphoric acid used as a buffer and acetonitrile used as a solvent in the mobile phase with a ratio of 30:70, respectively. The flow rate was 1 mL/min. 242 nm was the detection wavelength. The retention times were about 3.1 minutes for rosuvastatin calcium, 4.7 minutes for fenofibrate related compound A, 5.6 minutes for fenofibrate related compound B, and 21.6 minutes for fenofibrate. The linearity ranges for rosuvastatin calcium and fenofibrate were 50 to 150 and 800 to 2,400 mcg/mL, respectively, with a correlation coefficient of 0.999 for both. The proposed method validated statistically with respect to system suitability, specificity, linearity, and range, precision, accuracy, robustness, and ruggedness. The method was accurate, linear, precise, specific, selective, and rapid suitable for the quantitative estimation of rosuvastatin and fenofibrate in tablets.

Keywords: Fenofibrate, Method development, Related compound, Rosuvastatin, RP-HPLC, Tablets.

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INTRODUCTION

Rosuvastatin calcium, chemically, bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt, fenofibrate, chemically, isopropyl 2-[p-(p-chlorobenzoyl) phenoxy]-2-methylpropanoate. Rosuvastatin calcium and fenofibrate are official in Indian,[1] United States,[2] and European Pharmacopoeia.[3] But the combination of rosuvastatin calcium and fenofibrate is not official in any one of the pharmacopoeia. The tablet combination dosage form in the ratio of 10:160 mg rosuvastatin calcium equivalent to rosuvastatin and fenofibrate, respectively. The average weight of the tablet is about 328 mg. Many formulations are available with many trade names. Many methods have been reported for this combination by HPLC,[4-14] by HPTLC,[15,16] and the combination by both UV and HPLC.[17-19] HPLC method for the determination of fenofibric acid (fenofibrate related compound B) in formulations of fenofibrate[20] from literature reveals no related compounds were separated to estimate the combination of rosuvastatin and fenofibrate. Moreover, as per the United States of Pharmacopoeia (USP), fenofibrate tablets monograph, fenofibrate related compound A, and fenofibrate related compound B used as a system suitability preparation for the assay estimation as in USP.[2] So this present proposed method, the rosuvastatin, and fenofibrate simultaneously estimate in tablet formulation by separating fenofibrate compound A and B. The proposed method validated as per International Council for Harmonization (ICH) guidelines.[21]

MATERIAL AND METHODS

Instrumentation

The separation was carried out on the Shimadzu HPLC system with quaternary gradient and UV and 20 MP PDA detectors, LC solutions software, and Kromasil C18 (250 × 4.6 mm, 5 µm) column.

Chemicals and Reagents

The working standards of rosuvastatin calcium and fenofibrate were provided as gift samples from Bio-Leo Analytical Labs, Hyderabad. The marketed formulation was purchased from a local market. Related compounds fenofibrate was provided as gift samples from JS Labs, Hyderabad. Potassium

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dihydrogen phosphate, ortho-phosphoric acid, water, and acetonitrile of HPLC grade from Rankem.

HPLC Conditions

The mobile phase consisting of buffer and acetonitrile was filtered through 0.45 µm polyvinylidene fluoride (PVDF) membrane filter separately before use, degassed pumped into the column at a flow rate of 1 mL/min. The detection was monitored at 242 nm and the run time was 30 minutes. The injection volume was 20 µL. The column temperature was 30°C, the column was equilibrated for about 30 minutes prior to injection of the drug solutions with the mobile phase.

Preparation of Buffer Solution

Accurately transferred 1.36 grams of potassium dihydrogen phosphate into 1,000 mL water, adjusted a pH 3.0 with dilute ortho-phosphoric acid solution.

Preparation of Mobile Phase

Mixed 30 volumes of the buffer with 70 volumes of gradient grade acetonitrile.

Preparation of Diluent

Acetonitrile and water in the ratio 60:40.

Preparation of System Suitability Solution

Accurately transferred 5 mg each of rosuvastatin calcium, fenofibrate related compound A, fenofibrate related compound B, and 50 mg of fenofibrate into 100 mL volumetric flask dissolve and diluted to volume with diluent. Further diluted to 10 to 100 mL with diluents.

Preparation of Standard Solution

Accurately transferred 10 mg of rosuvastatin calcium and 160 mg of fenofibrate into 100 mL volumetric flask, dissolved and diluted to volume with diluent.

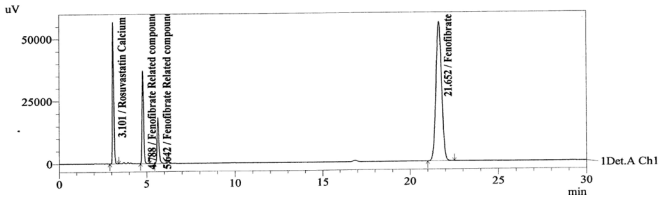

Figure 1: System suitability chromatogram

Preparation of Sample Solution

Accurately transferred sample powder not less than 10 tablets, equivalent to 10 mg of rosuvastatin and 160 mg of fenofibrate into 100 mL of volumetric flask dissolve and dilute with diluent and sonicate for 10 minutes, passed through 0.45 µ PVDF membrane filter.

EXPERIMENTAL

System Suitability Studies

System suitability testing is an integral part of the analytical procedure, the parameters, like resolution, tailing factor, theoretical plates, and area % RSD determined for standard solution and system suitability solution. The system suitability solution chromatogram is shown in Figure 1. The standard solution chromatogram is shown in Figure 2. The results are tabulated in Table 1.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present and to provide an exact content or potency of the analyte in the sample. The test solution monitored on photodiode-array (PDA) is shown in Figure 3.

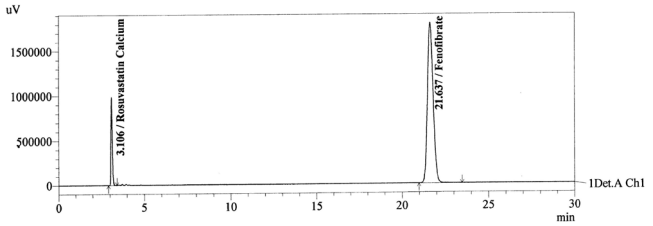

Figure 2: Standard chromatogram

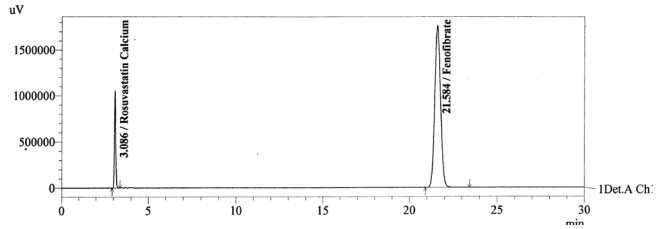

Figure 3: Test chromatogram

Table 1: System suitability results

Parameters	*Rosuvastatin calcium*	*Fenofibrate related compound A*	*Fenofibrate related compound B*	*Fenofibrate*	*Limit*
Retention time (min)	3.101	4.788	5.642	21.652	-
Resolution (R)	-	10.388	4.508	39.99	R > 1.5
Tailing factor (T)	1.28	1.19	1.27	1.01	T < 2
Theoretical plates (N)	6553	12456	11884	21280	N > 2000
Area % RSD	0.14	-	-	0.08	< 2 for n ≥ 5
LOD concentration (mcg/mL)	0.027	0.037	0.06	0.067	-
S/N ratio	67.94	56.99	43.84	35.72	S/N > 3
LOQ concentration (mcg/mL)	0.08	0.11	0.18	0.2	-
S/N ratio	222.68	214.48	167.1	89.84	S/N > 10

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The method accuracy was determined based on recovery experiments. The recovery studies were carried out at three concentration levels with triplicate preparations. The percentage recovery, mean recovery, and standard deviation of the percentage recovery were calculated and tabulated in Tables 2 and 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The % assay was calculated for six different individual preparations of the sample and tabulated in Table 4.

Linearity Range

The analytical procedure linearity is its ability (within a given range) to obtain test results. The results are directly proportional to the concentration (amount) of an analyte in the sample. The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) has been demonstrated that the range of analytical procedure which has a suitable level of precision, accuracy, and linearity.

Linearity was determined triplicate at each level by plotting peak areas for rosuvastatin calcium and fenofibrate against the concentration of solutions. The linearity of rosuvastatin calcium and fenofibrate is determined in the concentration

Table 2: Rosuvastatin calcium equivalent to rosuvastatin accuracy results

Spiked level (%)	*Sample amount added (mcg/mL)*	*Sample amount recovered (mcg/mL)*	*% recovery*	*Mean % recovery*	*% RSD*
50	4.939	4.8641	98.48	98.41	0.09
	4.9451	4.8659	98.4		
	4.9329	4.8607	98.54		
	4.939	4.8597	98.39		
	4.9482	4.8655	98.33		
	4.9421	4.859	98.32		
100	9.878	9.7098	98.3	98.23	0.32
	9.8476	9.7004	98.51		
	9.9085	9.6986	97.88		
150	15	14.7263	98.18	98.26	0.08
	15.7436	15.4846	98.35		
	15.7404	15.476	98.32		
	15.7692	15.4845	98.19		
	15.7564	15.4751	98.21		
	15.7564	15.4884	98.3		

Table 3: Fenofibrate accuracy results

Spiked level (%)	*Sample amount added (mcg/mL)*	*Sample amount recovered (mcg/mL)*	*% recovery*	*Mean % recovery*	*% RSD*
50	79.0244	79.3404	100.4	100.31	0.1
	79.122	79.2921	100.22		
	78.9268	79.2697	100.43		
	79.0244	79.2794	100.32		
	79.1707	79.3156	100.18		
	79.0732	79.309	100.3		
100	158.0488	157.9723	99.95	99.89	0.33
	157.561	157.8475	100.18		
	158.5366	157.806	99.54		
150	240	239.3847	99.74	99.89	0.08
	239.6098	239.519	99.96		
	239.561	239.434	99.95		
	240	239.637	99.85		
	239.8049	239.54	99.89		
	239.8049	239.6746	99.95		

ranges of 50–150 mcg/mL and 800 to 2,400 mcg/mL, respectively. The rosuvastatin calcium regression equation is y = 53391 × - 21748 with a coefficient of correlation (R2) of 0.999. The fenofibrate regression equation is y = 25615x + 72834 with a coefficient of correlation (R2) of 0.999 and shown in Figures 4 and 5. The range of the analytical method determined for rosuvastatin calcium and fenofibrate tabulated in Table 5.

Robustness

The analytical procedure robustness is small, but deliberate variations in method parameters measurement. The capacity of the method to remain unaffected and provides an indication of its reliability when compared to normal usage. Robustness was determined for SST solution and standard solution and the results were tabulated in Table 6.

Ruggedness (Intermediate Precision)

Ruggedness was measured as a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions with a different lot of the column with different analysts and the results were tabulated in Table 7.

Limit of Detection and Quantitation

The detection and quantitation limit of this analytical procedure is the lowest amount of analytes in the sample. These were determined based on the signal-to-noise ratio method and the results were tabulated in Table 1.

RESULTS AND DISCUSSION

System suitability results were given by Table 1 and system suitability parameters are retention time, resolution, tailing, resolution, and plate count were within the acceptable limits. So the system is suitable for the analysis. The test chromatogram extracted on the PDA detector the peak rosuvastatin calcium and fenofibrate purities found > 0.99, respectively, indicates no interference, so the method is specific. Rosuvastatin recovery was found 97.88 to 98.54% and % RSD was 0.09 to 0.32,

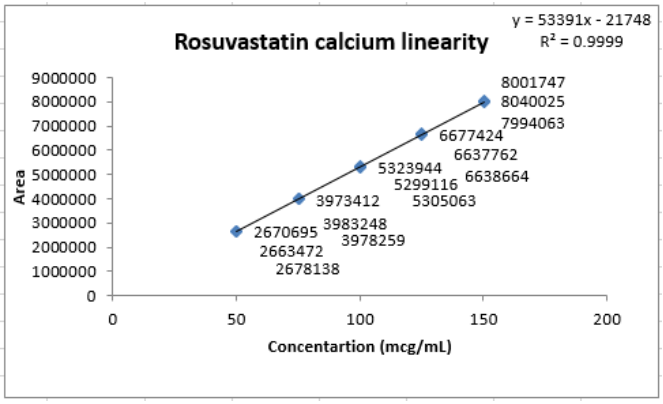

Figure 4: Rosuvastatin calcium calibrationcurve

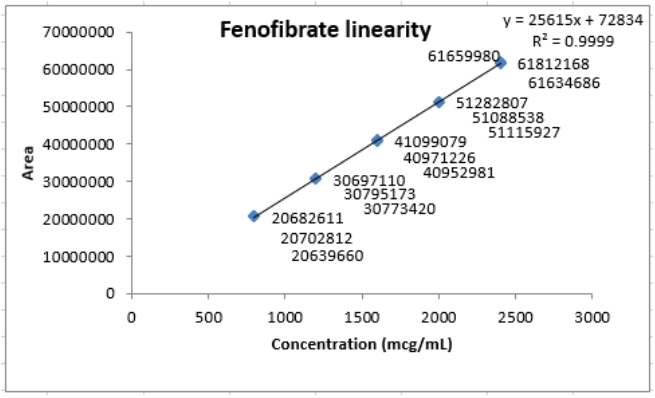

Figure 5: Fenofibrate calibration curve

Table 4: Precision results

S. No.	*% assay (rosuvastatin calcium equivalent to rosuvastatin)*	*% assay (fenofibrate)*
Preparation-1	98.3	99.95
Preparation-2	98.51	100.18
Preparation-3	97.88	99.54
Preparation-4	98.05	99.73
Preparation-5	98.59	100.39
Preparation-6	98.46	99.98
Average assay	98.3	99.96
Std. Dev.	0.28	0.3
% RSD	0.29	0.3

Table 5: Range results

Parameter	*Unit*	*Rosuvastatin calcium*	*Fenofibrate*
Range	mcg/mL	50–150	800–2,400
Linearity	Correlation coefficient (r^2)	0.999	0.999
Accuracy at 50%	% recovery	98.41	100.31
Accuracy at 150%	% recovery	98.26	99.89
Precision at 50%	% RSD	0.09	0.1
Precision at 150%	% RSD	0.08	0.08

Table 6: Robustness results

Parameters	*Rosuvastatin calcium*	*Fenofibrate related compound A*	*Fenofibrate related compound B*	*Fenofibrate*	*Limit*
Resolution (R)					
As such	-	10.388	4.508	39.99	
1.1 mL/min	-	10.014	4.356	38.705	
0.9 mL/min	-	10.858	4.68	40.193	
Column temp. 35°C	-	10.117	4.517	37.824	
Column temp. 25°C	-	10.791	4.884	39.703	R > 1.5
Buffer pH 3.2	-	10.213	3.614	38.456	
Buffer pH 2.8	-	10.244	5.012	37.941	
Organic solvent 63%	-	9.496	3.88	36.114	
Organic solvent 57%	-	11.192	5.058	40.808	
Tailing factor (T)					
As such	1.28	1.19	1.27	1.01	
1.1 mL/min	1.27	1.18	1.23	1	
0.9 mL/min	1.27	1.19	1.23	1	
Column temp. 35°C	1.27	1.16	1.17	0.99	
Column temp. 25°C	1.26	1.16	1.19	1	T < 2
Buffer pH 3.2	1.27	1.16	1.2	0.99	
Buffer pH 2.8	1.27	1.16	1.18	0.99	
Organic solvent 63%	1.28	1.18	1.24	0.99	
Organic solvent 57%	1.25	1.15	1.22	0.99	
Theoretical plates (N)					
As such	6,553	12,456	11,884	21,280	
1.1 mL/min	6,038	11,511	11,113	19,940	
0.9 mL/min	7,122	13,426	12,704	21,144	
Column temp. 35°C	6,547	12,152	12,094	18,846	
Column temp. 25°C	6,666	12,596	12,218	20,392	N > 2000
Buffer pH 3.2	6,503	12,154	11,457	19,348	
Buffer pH 2.8	6,450	12,118	12,159	19,296	
Organic solvent 63%	6,232	11,495	10,899	19,210	
Organic solvent 57%	6,695	12,856	12,057	20,087	
Area %RSD					
As such	0.14	-	-	0.08	
1.1 mL/min	0.02	-	-	0.02	
0.9 mL/min	0.04	-	-	0.04	
Column temp. 35°C	0.04	-	-	0.03	
Column temp. 25°C	0.07	-	-	0.05	< 2 for n ≥ 5
Buffer pH 3.2	0.1	-	-	0.06	
Buffer pH 2.8	0.04	-	-	0.02	
Organic solvent 63%	0.15	-	-	0.17	
Organic solvent 57%	0.08	-	-	0.06	

fenofibrate recovery was found 99.54 to 100.43%, and % RSD was 0.08 to 0.33 indicates the method is accurate 50 to 150% of the target concentration. Six different % assay preparation values of the same homogeneous samples found 97.88 to 98.59% for rosuvastatin and 99.54 to 100.39% for fenofibrate indicates the method is precise. Rosuvastatin calcium linear correlation was found to be y = 53,391x – 21,748, the correlation coefficient was 0.999 and fenofibrate was y = 25,615x + 72,834, and the correlation coefficient was 0.999 indicates the method is linear across the target concentration. The method unaffected due to deliberate changes in flow rate, column oven temperature, buffer pH, organic solvent, and different batch

Table 7: Intermediate precision results

S. No.	*% assay (rosuvastatin calcium equivalent to rosuvastatin)*	*% assay (fenofibrate)*
Preparation-1	100.43	98.72
Preparation-2	100.98	99.39
Preparation-3	101.2	99.59
Preparation-4	100.71	99.06
Preparation-5	100.52	98.86
Preparation-6	100.36	98.63
Average assay	100.7	99.04
Std. Dev.	0.33	0.38
% RSD	0.33	0.38
Cumulative average of precision + intermediate precision	99.5	99.5
Cumulative % RSD of precision + intermediate precision	1.29	0.59

column with different analyst proves the method is robust and rugged. The proposed range 50 to 150% of the target concentration, i.e., 50 to 150 mcg/mL for rosuvastatin calcium and 800 to 2,400 mcg/mL for fenofibrate found linear, accurate, and precise (Table 5). The standard and test solutions were kept at room temperature (< 25°C) and found stable up to 48 hours.

CONCLUSION

A novel, specific, selective, linear, accurate, and precise RP-HPLC isocratic method was developed for the estimation of rosuvastatin calcium equivalent to rosuvastatin and fenofibrate in their pharmaceutical tablet formulation. The proposed method was successfully separated rosuvastatin calcium, fenofibrate, and their related compounds. The proposed method is specific, selective, and stability-indicating power. Hence, the developed method could be adapted to regular quality control analysis and stability analysis.

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REFERENCES

1. Indian Pharmacopoeia. Ed.7th, Vol.II, Government of India, The Indian pharmacopoeia commission, Ghaziabad, 2014,1744-1745,2683-2685.
2. United States of Pharmacopoeia and National Formulary. Vol.I, The united states Pharmacopeial convention, Rockville, 2019,1793-1800, 3916-3921.
3. European Pharmacopoeia, Vol.II, European Directorate for the Quality of Medicines & HealthCare, Council of Europe, France, 2020,2617-2618, 3746-3748.
4. Thukabai S, Uma Maheswara Rao V and Muhammad Rafi Shaik, Development and Validation of RP-HPLC Method For Simultaneous Estimation of Rosuvastatin and Fenofibrate in Bulk and Tablet Dosage Form, International journal of pharmacy 2013; 3(3): 607-612.
5. Ashutosh Kumar S, Manidipa Debnath, Dr. Seshagiri Rao J.V.L.N, Dr. Gowri Sankar D, Development and Validation of a Sensitive RP-HPLC method for Simultaneous Estimation of Rosuvastatin and Fenofibrate in Tablet Dosage form by using PDA Detector in Gradient Mode, Research J.Pharm. and Tech, 2016; 9(5):549-554.
6. Jajam Thriveni, Rambabu R, Venkateswara Rao J and Vidyadhara S, Development And Validation of RP-HPLC Method For Simultaneous Estimation Of Rosuvastatin Calcium And Fenofibrate In Bulk and Pharmaceutical Dosage Forms, International Journal of research in Pharmacy and Chemistry, 2013; 3(2): 208-212.
7. Suresh Kumar GV, Rajendraprasad Y, Development and Validation of Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Fenofibrate in Tablet Dosage Form, International Journal of PharmTech Research, 2010; 2(3):2016-2021.
8. Anuradha Madala, Keerthisikha Palur, A New Stability Indicating Method Development and Validation of RP-HPLC Method For The Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet Dosage Form, Indo American Journal of Pharmaceutical Sciences, 2016; 3(9):953-959.
9. Sumalatha M, Haritha Pavani K, Analytical method development and validation for the simultaneous estimation of Rosuvastatin and Finofibate in tablet dosage form by reverse phase high performance liquid chromatography, International Journal of Research in Pharmacy and biotechnology, 2013; 1(6):850-856.
10. Abhijeet Ladke, Anita Ingle, Pranjali Ranaware and Mrinalini Damle, Development and validation of stability indicating HPLC method for Rosuvastatin calcium and Fenofibrate in combination, Asian Journal of Research in Chemistry, 2012; 5(5):606-610.
11. Nazareth Celina and Bodke Anushka, Development And Validation of A Novel Cleaning Validation and Assay Method For Simultaneous Estimation of Rosuvastatin And Fenofibrate By RP-HPLC, World Journal of Pharmaceutical Research, 2018: 7(3):1454-1465.
12. Moinuddin mohd, Rahaman S.A, ramakrishna Yadav B, Ramakrishna Battu, Development And Validation of A Reverse Phase HPLC Method For Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet Dosage Form, International journal of Pharmacy and Pharmaceutical Sciences, 2012: 4(3):150-154.

13. Madala Anuradha, A New RP-HPLC Method Development and Validation of For The Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet Dosage Form, Indo American Journal of Pharmaceutical Sciences, 2016: 3(8):846-853.
14. Potawale Rani S, Gabhe Satish Y, HPTLC Method For Simultaneous Determination of Rosuvastatin and Fenofibrtae in Bulk and Pharmaceutical Formulation, International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(7): 323-326.
15. Kalyankar Gajanan G, Ghariya Vrunda V, Bodiwala Kunjan B, Lodha Sandesh R, Joshi Shrikant V, Development and validation of HPTLC method for simultaneous estimation of Fenofibrate and Rosuvastatin in tablet dosage form, Journal of Pharmacy and Applied Sciences, 2016, 3(1): 1-7.
16. Usha Rani N, Nagoor Ankani and Bala Bhaskar T, A Review on various HPLC and Spectrophotometric methods for determination of Rosuvastatin Calcium and Fenofibrate in combined dosage forms, Indian Journal of Research in Pharmacy and Biotechnology, 2014, 2(6): 1476-1483.
17. Anandakumar Karunakaran, Vetsa Subhash, Ramu Chinthala, Jayamaryapan Muthuvijayan, Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV-Spectrophotometry and RP-HPLC, Stamfoed Journal of Pharmaceutical Sciences, 2011, 4(1):58-63.
18. Sheetal Sharma, Priya Bhandari, Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV-Spectrophotometry and RP-HPLC, Journal of Pharmacy Research, 2012, 5(4), 2311-2314.
19. Dedhiya Praful P, Chauhan Renu S, Shah Shailesh A, Tailor Pratik M, Determination Of Fenofibric Acid (Related Substance) in Marketed Formulations of Fenofibrate By RP-HPLC, Journal of pharmaceutical sciences and research,2014, 6(10):313-320.
20. ICH Q2 (R1), Validation of analytical procedure: Text and Methodology, November, 2005.