CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1205445

Available online at: http://www.iajps.com Research Article

VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PRAVASTATIN AND FENOFIBRATE IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:

A novel simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable, cost effective and accurate RP-HPLC (Reverse Phase High Performance Liquid Chromatography) method has been developed for the simultaneous estimation of Pravastatin and Fenofibrate in Pharmaceutical dosage forms. The chromatographic separation was achieved on Shimadzu (LC 20 AT VP) Agilent 1200 series HPLC using Inertsil sustain column, C_{18} (250×4.6mm× 5µm) maintained at ambient temperature with mobile phase consisting of a mixture of Phosphate buffer (KH_2PO_4) pH 4.5 Methanol: Acetonitrile (40:20:40v/v/v), with detection of 249 nm, flow rate 1.0 ml/min, load volume 20 µl and a run time of 6 min. The UV detection was performed at 229 nm. Buffer was prepared with KH_2PO_4 and adjusted pH to 4.5 with Ortho-Phosphoric Acid. The retention time and mean recoveries obtained for Pravastatin was 2.190 min and 99.43%, for Fenofibrate was 3.710 min and 99.44% respectively. Linearity response was established over the concentration range of 12-28 µg/ml for Pravastatin and 87-203µg/ml for Fenofibrate. The correlation coefficient for Pravastatin and Fenofibrate was 0.9990 and 0.9993 respectively. The recovery studies ascertained the accuracy of proposed method and the results were validated as per ICH guidelines. This novel method can be used for the routine quality control of both drugs in combination in tablet dosage form

Key words: *Pravastatin, Fenofibrate, RP-HPLC, correlation coefficient, Validation*

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Please cite this article in press Ganesh Akula., ***Validated RP-HPLC Method for the Simultaneous Estimation of Pravastatin and Fenofibrate in Pharmaceutical Dosage Forms,*** *Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

Pravastatin [1], (3R, 5R)-7-[(1S, 2S, 6S, 8S, 8aR)-6-hydroxy-2-methyl-8-{[(2S)-2- methylbutanoyl] oxy}-1,2,6,7,8,8a-hexa hydro naphthalen-1-yl]-3,5-dihydroxy heptanoic acid (**Figure 1**), is a cholesterol-lowering agent that belongs to a class of medications known as statins. It was derived from microbial transformation of mevastatin, the first statin discovered. It is a ring-opened dihydroxy acid with a 6'-hydroxyl group that does not require in vivo activation. Pravastatin is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase.

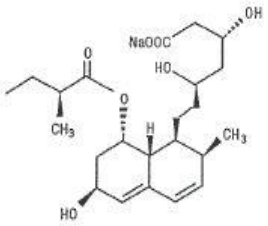

Fig-1: Pravastatin

Fenofibrate [2], Propan-2-yl-2-{4-[(4-chloro phenyl) carbonyl] phenoxy}-2-methylpropanoate (**Figure 2**), exerts its therapeutic effects through activation of peroxisome proliferator activated receptor a (PPARa). An anti lipidemic agent reduces both cholesterol and triglycerides in the blood.

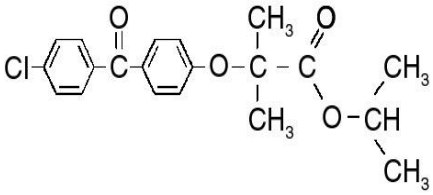

Fig-2: Fenofibrate

Various methods were reported for determination of Pravastatin and Fenofibrate in literature and determined by using HPLC methods [3]. However, a few analytical methods were also reported for the simultaneous determination of Pravastatin and Fenofibrate in a mixture by UV spectrophotometry [4] and stability indicating RP-HPLC. An extensive review of the literature did not revealed any simple economical HPLC method for simultaneous determination of both the drugs. Therefore, attempts were made to develop and validate simple, precise, and sensitive, reverse phase high performance liquid chromatographic method for simultaneous determination of both drugs in pharmaceutical formulations.

MATERIALS AND METHODS:

Instruments: The HPLC system consisted of Shimadzu (LC 20 AT VP) system equipped with Agilent 1200 series, Inertsil Sustain column,C18(250x4.6 ID), Nicolet evolution 100 UV-visible detector and Citizen, Digital Ultrasonic Cleaner were used. Peak areas were integrated using spinchrome CFR software program.

Drugs & Reagents: Pravastatin & Fenofibrate bulk drugs were procured from the Chandra labs, Hyderabad and formulated products were obtained from local pharmacy. Methanol, Acetonitrile and milli-Q water were used as HPLC grade. Sodium acetate, Potassium phosphate, Ammonium acetate & Triethylamine are analytical Grade from Merck was used throughout analysis.

Determination of working wavelength: In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are inter convertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution: 10 mg of Pravastatin/Fenofibrate was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Mobile Phase preparation: A mixture of 40 volumes of Ammonium acetate Buffer pH 4.5, 20 volumes of Methanol and 40volumes of Acetonitrile. The mobile phase was sonicated for 10min to remove gases.

Preparation of Ammonium Acetate Buffer (30mM): 4.08 gms of KH_2PO_4 was weighed and dissolved in 1000ml of water and volume was made up to 1000ml with water. Adjust the pH to 4.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

METHOD DEVELOPMENT [5]:

Optimized Chromatographic Method:

Preparation of mixed standard stock solution: weigh accurately 20 mg of Pravastatin and 145 mg of Fenofibrate in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase from above stock solution 20 µg/ml of Pravastatin and 145 µg/ml of Fenofibrate is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

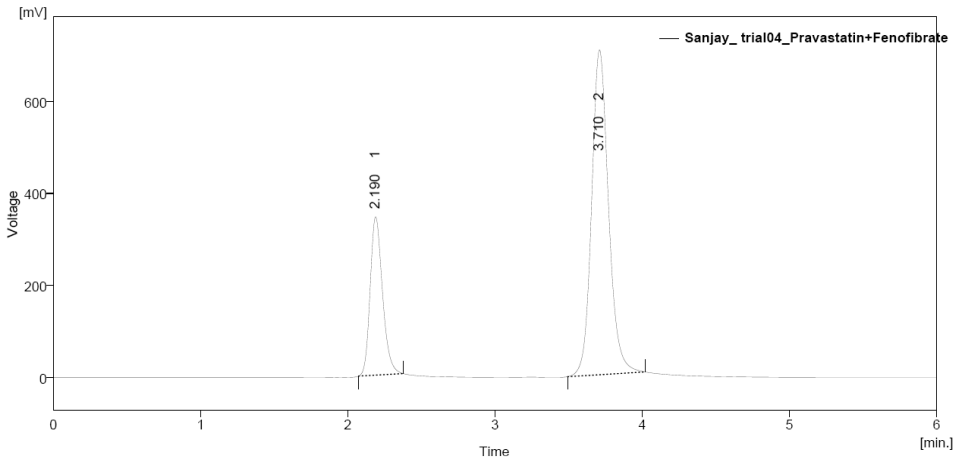

Fig. 3: Chromatogram of Pravastatin and Fenofibrate by using mobile phase

The peak Asymmetry factor was less than 2 for both Pravastatin and Fenofibrate and the efficiency also good, and the retention time was also satisfactory for both the drugs. The details are given in the table-1 and figure-3.

Table 1: Optimized chromatographic conditions

Mobile phase	Phosphate buffer (KH_2PO_4): Acetonitrile: Methanol (40:40:20)
pH	4.5
Column	INERTSIL SUSTAIN column (250×4.6mm× 5µm)
Flow rate	1.0 ml/min
Column temperature	Room temperature (20-25°C)
Sample temperature	Room temperature (20-25°C)
Wavelength	249nm
Injection volume	20 µl
Run time	6 min
Retention time	About 2.190 min for Pravastatin and 3.710 min for Fenofibrate

RESULTS:

The wavelength of maximum absorption (λ max) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 4 and the absorption curve shows characteristic absorption maxima at 213 nm for Pravastatin, 290nm for Fenofibrate and 249nm for the combination.

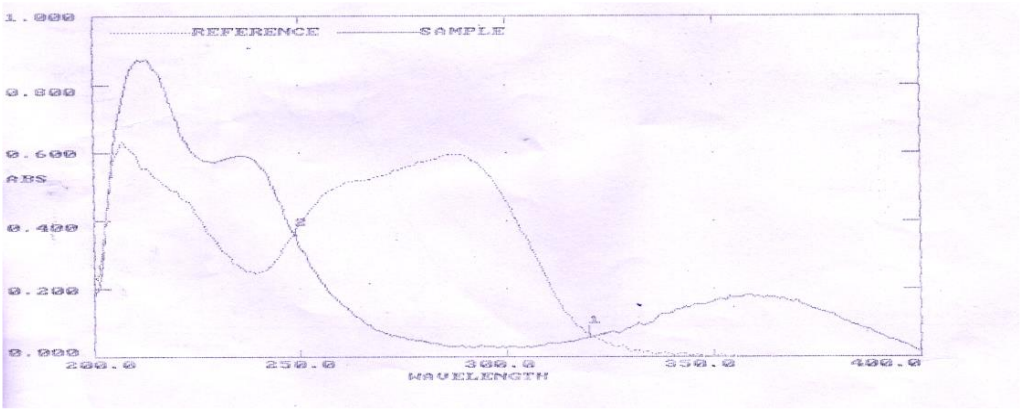

Fig.4: UV-VIS spectrum of Pravastatin and Fenofibrate

Assay:

Preparation of Standard and Sample solutions: weigh accurately 20mg of Pravastatin and 145 mg of Fenofibrate dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 20 µg/ml of Pravastatin and 145 µg/ml of Fenofibrate was made by adding 1 ml of stock solution to 10 ml of mobile phase.The amount of Pravastatin and Fenofibrate present in the taken dosage form was found to be 99.34 % and 99.44 % respectively. Assay results were shown in Table No.2.

Table 2: Assay Results

Injections	Pravastatin		Fenofibrate	
	Standard area	Sample area	Standard area	Sample area
Injection-1	2098.671	2127.173	5764.513	5735.754
Injection-2	2117.772	2099.315	5647.887	5752.216
Injection-3	2086.371	2080.644	5780.212	5696.842
Injection-4	2108.269	2150.86	5803.414	5829.83
Injection-5	2090.36	2075.523	5731.065	5772.773
Average area	2100.289	2106.703	5745.418	5757.483
Tablet average weight (mg)	350.32		350.32	
Standard weight (mg)	20.01		145.21	
Sample weight (mg)	350.56		350.56	
Label amount (mg)	20		145	
Std. Purity	99.2		99.3	
Amount found in mg	19.89		144.19	
Assay(%purity)	99.43		99.44	

VALIDATION [6]:

System suitability: The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The % RSD for the retention times and peak area of Pravastatin and Fenofibrate were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Table-3: Results for system suitability

Injection	PRAVASTATIN				FENOFIBRATE			
	Retention time (min)	Peak area	Theoretical plates	Tailing factor (TF)	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.243	2051.334	3020	1.455	3.733	5645.897	5076	1.253
2	2.239	2034.811	3010	1.456	3.725	5650.988	5070	1.258
3	2.234	2046.910	3017	1.453	3.723	5646.67	5071	1.250
4	2.232	2042.704	3014	1.456	3.722	5609.908	5075	1.252
5	2.236	2045.444	3012	1.457	3.723	5668.048	5075	1.243
6	2.233	2068.826	3013	1.451	3.725	5699.087	5079	1.251
Mean	2.2362	2048.338	-	-	3.725	5653.433	-	-
SD	0.0042	11.436	-	-	0.004	29.329	-	-
%RSD	0.19	0.56	-	-	0.11	0.52	-	-

Specificity by Direct comparison method: There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form. Standard solution and tablet sample solution were prepared as per the guidelines.

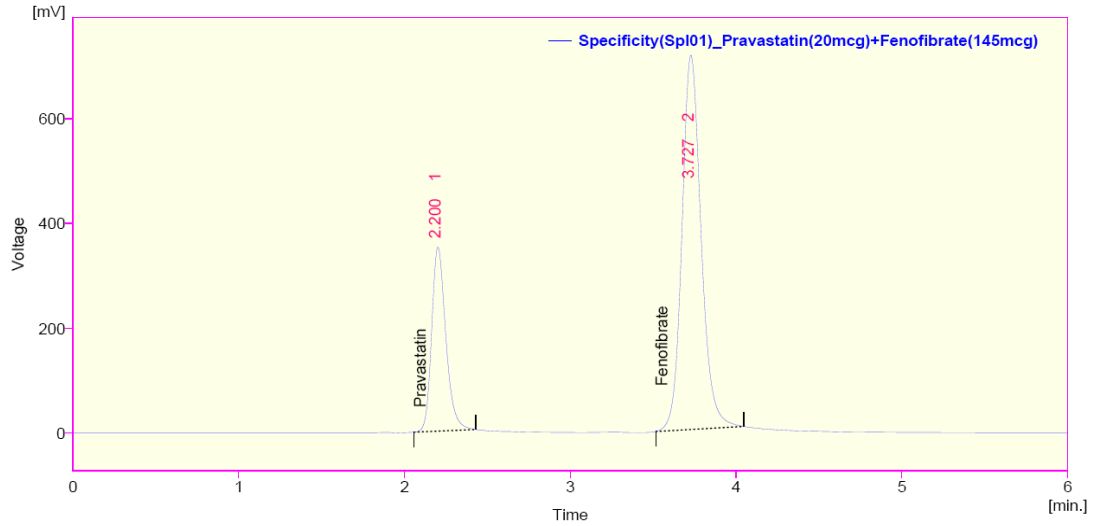

Fig. 5: Chromatogram for specificity of Pravastatin and Fenofibrate sample

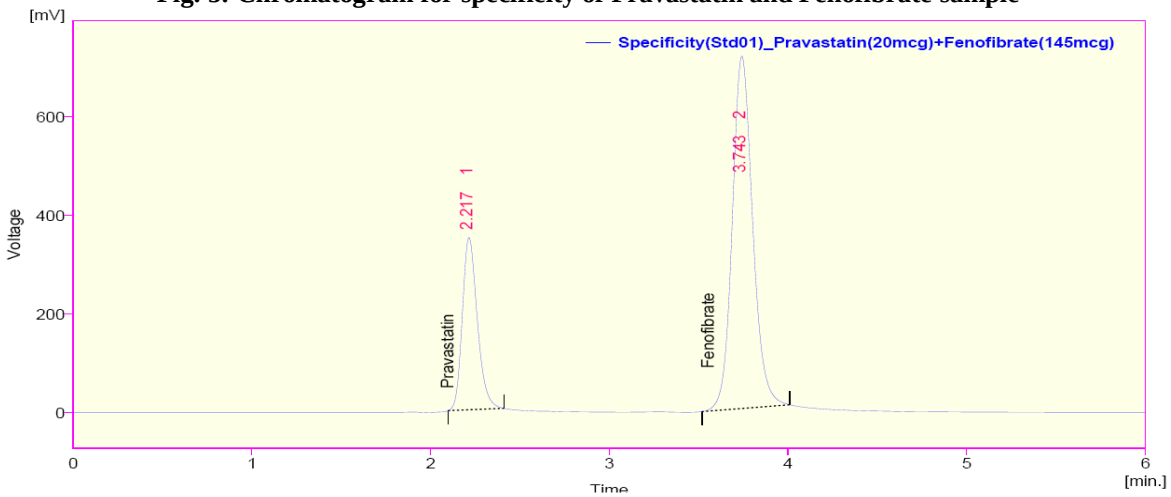

Fig. 6: Chromatogram for Specificity of Pravastatin and Fenofibrate standard

Linearity:

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Pravastatin and Fenofibrate is 0.9990 and 0.9993 respectively. The relationship between the concentration and area of Pravastatin and Fenofibrate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Table 4: Results of linearity

S.No.	PRAVASTATIN		FENOFIBRATE	
	Conc.(µg/ml)	Area	Conc.(µg/ml)	Area
1	12	1284.855	87	3655.286
2	16	1677.9	116	4600.036
3	20	2079.471	145	5702.059
4	24	2407.74	174	6601.370
5	28	2796.682	203	7635.418

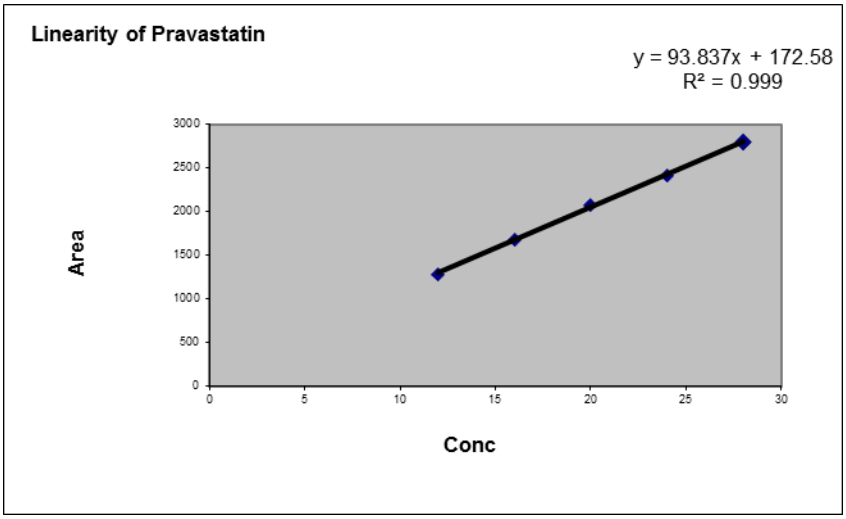

Fig. 7: Linearity graph of Pravastatin

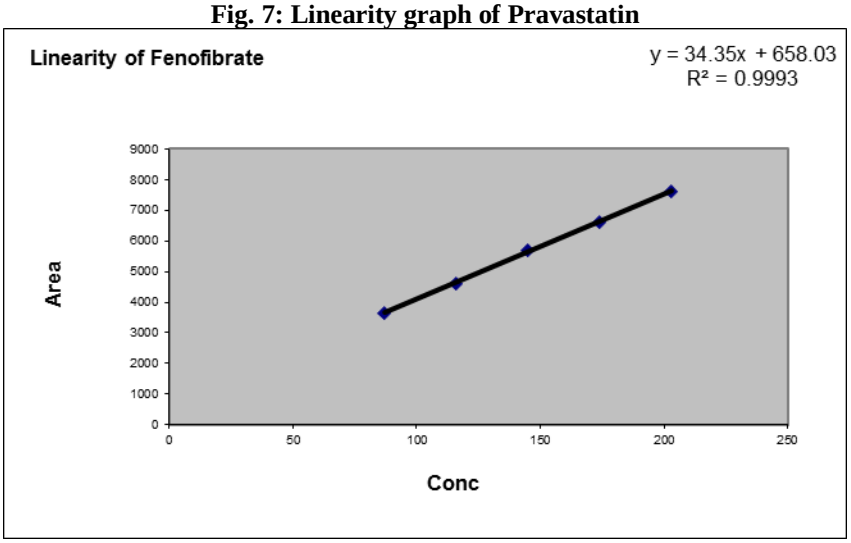

Fig. 8: Linearity graph of Fenofibrate

Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. The percentage mean recovery of Pravastatin and Fenofibrate is 101.59% and 100.18% respectively.

Table 5: Recovery results for pravastatin

Recovery level	Accuracy of PRAVASTATIN					
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	Average % Recovery
80%	100	2129.471	2126.932	20.28	101.41	101.59%
	100	2128.957				
	100	2122.368				
100%	120	2547.740	2556.357	24.59	101.44	
	120	2547.505				
	120	2573.825				
120%	140	2787.828	2797.531	27.89	101.92	
	140	2814.451				
	140	2790.313				

Table 6: Recovery results for Fenofibrate

Recovery level	Accuracy of FENOFIBRATE					
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	Average % Recovery
80%	4	5782.059	5784.542	145.87	100.60	100.18%
	4	5798.059				
	4	5773.507				
100%	4.8	6951.370	6968.091	174.74	100.43	
	4.8	6955.657				
	4.8	6997.247				
120%	5.6	7624.494	7663.912	202.01	99.51	
	5.6	7654.945				
	5.6	7712.298				

Precision

Method precision: Prepared samples as per test method and injected 6 times in to the column. Results were shown in Table-7.

Table 7: Results for Method precision of Pravastatin and Fenofibrate

PRAVASTATIN		
S.No.	**Rt**	**Area**
1	2.213	2001.034
2	2.190	2003.811
3	2.210	2062.991
4	2.203	2052.703
5	2.21	2039.124
6	2.203	2058.026
Avg.	2.2048	2036.282
SD	0.0083	27.425
%RSD	0.38	1.35

FENOFIBRATE		
S.No.	**Rt**	**Area**
1	3.740	5647.876
2	3.710	5639.718
3	3.733	5692.9
4	3.727	5709.739
5	3.740	5765.096
6	3.733	5740.309
Avg	3.731	5699.273
SD	0.011	49.710
%RSD	0.30	0.87

Limit of Detection: The LOD for this method was found to be 0.26 µg/ml & area 21.01 for Pravastatin and 5.21 µg/ml & area 152.70 for Fenofibrate.

Limit of Quantification: The LOQ for this method was found to be 0.79 µg/ml & area 63.66 for Pravastatin and 15.77µg/ml & area 462.73 for Fenofibrate

Robustness: To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like flow rate and wavelength . System suitability parameters were compared with that of method precision. Results were shown in Table 8.

Table 8: Result of Robustness study

Parameter	PRAVASTATIN		FENOFIBRATE	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow rate				
0.8 ml/min	2.930	1.444	4.907	1.308
1.0 ml/min	2.213	1.362	3.712	1.214
1.2 ml/min	1.780	1.368	2.980	1.185
Wavelength				
247 nm	2.223	1.364	3.710	1.219
249 nm	2.213	1.360	3.711	1.211
251 nm	2.203	1.409	3.707	1.219

Table 9: Results for Ruggedness

Analyst	Pravastatin % Assay	Fenofibrate % Assay
Analyst -1	99.783	99.54
Analyst -2	99.60	98.67
% RSD	0.032%	0.035%

Ruggedness:

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. The %RSD between two analysts, assay values not greater than 2.0%, hence the method was rugged.

DISCUSSION:

A simple and selective LC method is described for the determination of Pravastatin and Fenofibrate tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Phosphate buffer (KH_2PO_4) pH 4.5 Methanol: Acetonitrile (40:20:40v/v/v), with detection of 249 nm. Linearity was observed in the range 12-28 µg /ml for Pravastatin (r^2 =0.9990) and 87-203µg /ml for Fenofibrate (r^2 =0.9993) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION:

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Pravastatin and Fenofibrate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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