Development and Validation of Reverse Phase HPLC Method for the Estimation of Fenofibrate in Bulk and Capsule Dosage Form

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Abstract

The aim of the present study was to develop a simple, economical and efficient reverse phase-high performance liquid chromatography (RP-HPLC) method and validate the same for the determination of Fenofibrate in capsule dosage form and the method was developed using Shim Pack C_{18} column (250 x 4.6 mm, 5 µ) with the mobile phase of Acetonitrile and Methanol (60:40 v/v) at a flow rate of 1.0 mL/min. The eluent was detected at 285 nm and the retention time was found to be 3.723 min. The method was validated for system suitability, specificity, linearity, accuracy, precision, robustness, LOD and LOQ in accordance with the ICH $Q_2 R_1$ guidelines. The data of linear regression analysis indicated a good linear response in the concentration range of 10 to 100 µg/mL with correlation co-efficient (R^2) of 0.996. The validated method proves to be useful for the routine analysis of Fenofibrate in bulk & pharmaceutical dosage forms.

Keywords: *RP-HPLC method, Fenofibrate, validation (ICH Q2 R1 guidelines)*

Introduction

Fenofibrate chemically is (FEN), propane-2-yl 2-{4-[(4- chlorophenyl) carbonyl] phenoxy} methyl propanoate (Figure 1). Medicinally it is known as antilipidemic agent. It is effective in decreasing triglyceride levels and increases HDL cholesterol levels. It is used in primary hypercholesterolemia and severe hypertriglyceridemia. The reported technique has the drawbacks such as long runtime and less economical with a high proportion of organic phase as eluent. Hence, an attempt was made to develop a novel RP-HPLC technique which is specific, accurate, precise, and economical for the determination of fenofibrate in capsule and bulk dosage form.

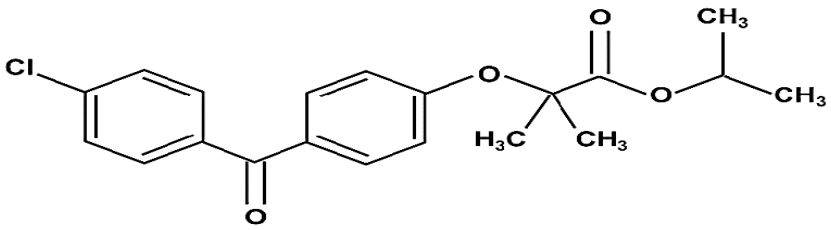

Figure 1: Structure of Fenofibrate

Materials and Method

Fenofibrate bulk drug was procured from Medreich limited, Bangalore as gift sample. Acetonitrile, Methanol (HPLC grade) were obtained from Thermo Fisher Scientific, Mumbai, India. All other chemicals and solvents used for research work were of HPLC grade.

Equipment Description

Shimadzu HPLC system (LC-20 AD) with Lab Solution software was used. The purity determination was performed on Shim Pack C_{18} column (250 x 4.6 mm, 5 µ).

Chromatographic Condition

Fenofibrate (10 µg/mL) was subjected to chromatographic analysis using mobile phases of various strengths employing an injection volume of 20 µL, flow rate of 1 mL/min and detection was carried at 285 nm.

Standard preparation

10 mg of Fenofibrate working standard was accurately weighed and transferred into a 100 mL clean dry volumetric flask and about 70 mL of diluent was added, the volume was made up to the mark with the same solvent and ultrasonicated in water bath for 10 min. From this, appropriate dilutions were prepared using mobile phase to get final concentration of 10 to 100 µg/mL. These standard solutions were analyzed in three replicates using the above-mentioned chromatographic conditions.

Sample preparation: Analysis of Marketed Formulation

To determine the content of Fenofibrate in marketed capsule (label claim Lipicard-200 mg/capsule), 20 capsules were weighed and average weight was calculated. The powder equivalent to the amount of active ingredient present in 10 capsules was transferred into a 100 mL clean dry volumetric flask, 70 mL of diluent was added to it and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent (stock solution). 1 mL of clear solution was transferred to a 10 mL volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter under vacuum filtration before injecting into HPLC system. Peak area of the sample and the amount of Fenofibrate in the sample was calculated. The amount of Fenofibrate per capsule was found.

Method Validation

Optimization of mobile phase

When Fenofibrate was subjected to chromatographic analysis using mobile phases of differing strengths of Acetonitrile: methanol was used as a mobile phase, since it gave good peak shape and acceptable peak parameters. Instrument used was Shimadzu HPLC system (LC-20 AD) and PDA detector with Lab Solution software, Column used was Shim Pack C18 column (250 x 4.6 mm, 5 µ) with Mobile phase Acetonitrile: Methanol (60:40 V/V), Flow rate 1 mL/min at the wavelength 285 nm.

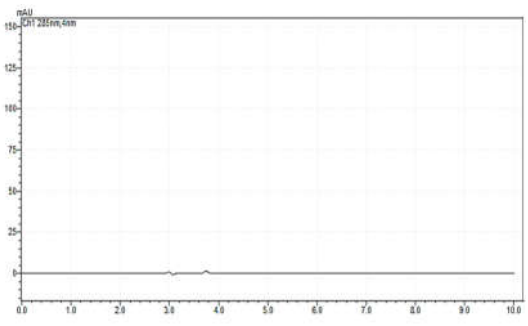

Figure 2: Blank Chromatogram of Fenofibrate

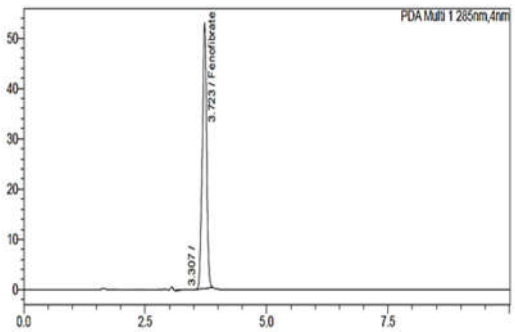

Figure 3: Standard Chromatogram of Fenofibrate

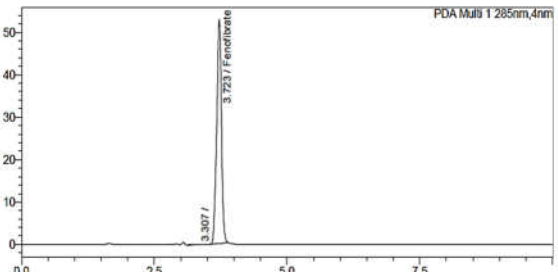

Figure 4: Sample Chromatogram of Fenofibrate

Results and Discussion

1. **System suitability**

Standard solution of Fenofibrate was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the percentage RSD of retention time, tailing factor, number of theoretical plates (NTP) and peak areas from six replicate injections. The results have been tabularized in Table 1.

Injections	Retention time	Peak area	NTP
1	3.723	327376	6187
2	3.723	340376	6134
3	3.733	334831	6117
4	3.723	344861	6175
5	3.733	339552	6199
6	3.733	335899	6222
Average	3.728	337149.2	6172.33
SD	0.005477	5968.4	39.8279
% RSD	0.1448	1.77	0.645

Table 1: System Suitability results of Fenofibrate

2. **Specificity**

The developed technique was specified by correlating the chromatogram of the standard and sample solution. The results have been tabularized in Table 2.

Name	Retention time	Peak area	Tailing factor	NTP
Fenofibrate (Standard)	3.733	2503671	1.067	7464
Fenofibrate (Sample)	3.733	2798050	1.074	8378

Table 2: **Specificity results of Fenofibrate**

3. **Calibration Experiments**

The data obtained in the calibration experiments when subjected to linear-regression analysis showed a linear relationship between peak areas and concentration in the range of 10-100 µg/mL.

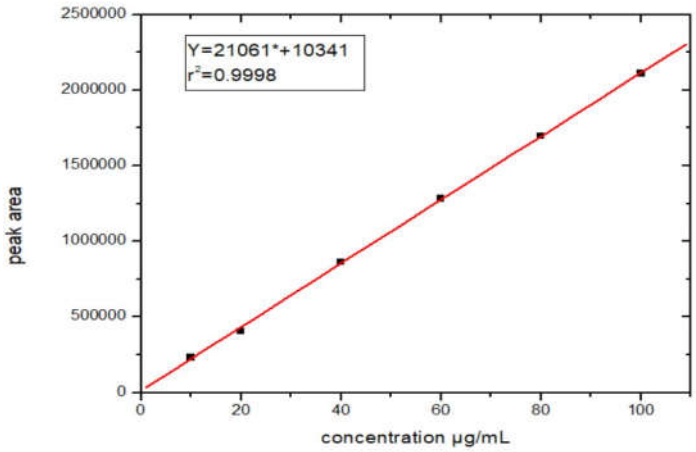

Figure 5: Calibration curve of Fenofibrate

4. Accuracy

To check accuracy of the method, recovery studies were carried out by standard addition method by adding the known amount of standard Fenofibrate to the pre analyzed sample at three different spiked concentration levels i.e., 80%, 100%, 120% of assay concentration and percent recoveries were calculated. The results have been tabularized in Table 3.

Sample No	Spike level	Amount (µg/mL) added	Total conc(µg/mL) added	% Recovery	Mean % Recovery	% RSD
1	80 %	100+80	180	99.65	99.57 %	0.068
		100+80	180	99.55		
		100+80	180	99.52		
2	100 %	100+100	200	99.19	99.48 %	0.251
		100+100	200	99.63		
		100+100	200	99.62		
3	120 %	100+120	220	99.75	99.54 %	0.326
		100+120	220	99.17		
		100+120	220	99.72		

Table 3: Accuracy results of Fenofibrate

5. Precision

Repeatability: The standard solution was injected and analyzed six times on the same day at same time, % RSD of the retention time, peak area and tailing factor were measured for all six injections in HPLC.

Intermediate precision: Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory by calculating the % RSD. The results have been tabularized in Table 4.

Repeatability			
Injections	**Retention time**	**Peak area**	**Tailing factor**

1	3.723	2410666	1.042
2	3.733	2443736	1.05
3	3.723	2467135	1.062
4	3.723	2465295	1.03
5	3.712	2478844	1.053
6	3.712	2503671	1.033
Average	3.712	2461558	1.045
SD	0.00797	31701.73	0.0122
% RSD	0.214	1.28	1.176

Intermediate precision				
	Day 1		**Day 2**	
Injections	**Area**	**Retention time**	**Area**	**Retention time**
1	2410666	3.723	2482346	3.701
2	2443736	3.733	2538040	3.712
3	2467135	3.723	2550879	3.701
4	2465295	3.723	2569371	3.701
5	2478844	3.712	2590233	3.701
6	2503671	3.712	2506958	3.701
Average	2461558	3.712	2539638	3.7028
SD	31701.73	0.00797	39807.47	0.004491
% RSD	1.28	0.214	1.56	0.121

Table 4: Precision Study of Fenofibrate

6. LOD and LOQ

The limits of detection is the lower most sample concentration that can be noticed and limit of quantification is the lowest analyte concentration, evaluated along with adequate accuracy and precision.

Limit of Detection = 3.3σ/S and Limit of Quantification = 10σ/S, where σ is the standard deviation of the regression line, and S is the slope of the calibration plot. The result of LOD and LOQ was found to be 115.3 and 348.87.

7. Robustness

A robustness study of the suggested technique has been assessed by analyzing aliquots with variance in parameters, such as flow rate and mobile phase composition. The effects on retention time and peak area were studied. The results have been tabularized in Table 5.

Flow Rate mL/min				
	0.9 mL/min		**1.1 mL/min**	
Injections	**Area**	**Retention time**	**Area**	**Retention time**

1	3044593	4.107	2588152	3.36
2	3031503	4.117	2598590	3.36
3	3051521	4.107	2610467	3.36
4	3058764	4.107	2613936	3.36
5	3038654	4.107	2627711	3.37
6	3128571	4.107	2650150	3.37
Average	3058934	4.1086	2614834	3.36366
SD	35422.29	0.004082	21948.63	0.00568
% RSD	1.15	0.0993	0.839	0.1688

Mobile phase ratio				
	65:35 v/v		**55:45 v/v**	
Injections	**Area**	**Retention time**	**Area**	**Retention time**
1	2038033	3.712	3011454	3.701
2	2049783	3.712	3070406	3.712
3	2033607	3.701	3015616	3.712
4	2059163	3.723	3092169	3.712
5	2065271	3.712	3084461	3.712
6	2062046	3.712	3078543	3.701
Average	2051317	3.712	3058774	3.708
SD	13144.18	0.006957	35785.36	0.005680
% RSD	0.64	0.1874	1.16	0.1531

Table 5: Accuracy results of Fenofibrate

Conclusion

A novel RP-HPLC method for estimation of Fenofibrate in bulk and formulation was developed and validated successfully. In addition to presided conditions for the development was the major benefits of current research was cost-effectiveness with shorter retention time of 3.723 min than the reported. The analytical procedure was validated as per ICH Q_2R_1 guidelines. The results obtained for all validation parameters, were found within the acceptance criteria. Thus, the proposed method was found to be specific, accurate, linear, precise, robust and can be successfully applied for the routine analysis of Fenofibrate in bulk and pharmaceutical formulation.

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