CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7320042

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

METHOD DEVELOPMENT AND VALIDATION FOR FENOFIBRATE BY RP-HPLC METHOD

[1]Kanchanapalli Madhuri, [2]Shaheen begum
SSJ College of pharmacy, Gandipet, Hyderabad,Telangana, India.

Article Received: August 2022 **Accepted:** September 2022 **Published:** October 2022

Abstract:
Develop a simple isocratic reverse phase high performance liquid chromatography (RPHPLC) method and validate for the determination of Fenofibrate in bulk and pharmaceutical dosage forms. RPHPLC quantification was carried out using Zorbax C-18 column (5µm, 150cm 4.6mm, ID) with a mobile phase comprising phosphate buffer (pH 3.0) : Acetonitrile in the ratio of 30:70 (% v/v) at a flow rate of 1.0 ml/min. The detection was carried out using a diode array detector at 285 nm. The % of Assay was found to be within the limits of 99-101. The % Recovery for each level around 99.5. The retention time was found to be 19.268 min and produced a linear response in the concentration range of 1-500 µg/mL (R2~0.999). The % RSD was found to be below 2%. The LOD and LOQ were found to be 0.228µg/ml and 0.764µg/ml respectively. Validation of the method was performed for precision, accuracy, linearity, ruggedness, specificity and sensitivity to conform to the ICH guidelines for validation of an analytical method

Keywords: *Fenofibrate, RP-HPLC*

Corresponding author:
Mrs Shaheen begum,
Assistant professor, Dept. of pharmaceutical chemistry
SSJ College of pharmacy, Gandipet, Hyderabad
Telangana, India. E-mail: madhurikanchanapalli22@gmail.com

QR code

Please cite this article in press Shaheen begum et al, ***Method Development And Validation For Fenofibrate By RP-HPLC Method.,*** *Indo Am. J. P. Sci, 2022; 09(10).*

INTRODUCTION:

Fenofibrate is indicated as adjunctive therapy to diet to reduce elevated LDL-C, Total-C, Triglycerides, and Apo B, and to increase HDL-C adults with primary hypercholesterolemia or mixed dyslipidemia.[1] Fenofibrate is also indicated to treat adults with severe hypertriglyceridemia. Fenofibrate activates peroxisome proliferator activated receptor alpha (PPARα), increasing lipolysis, activating lipoprotein lipase, and reducing apoprotein C-III. PPARα is a nuclear receptor and its activation alters lipid, glucose, and amino acid homeostasis. IUPAC name is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoic acid. Molecular formula $C_{20}H_{21}ClO_4$. Molecular Weight is 360.8.

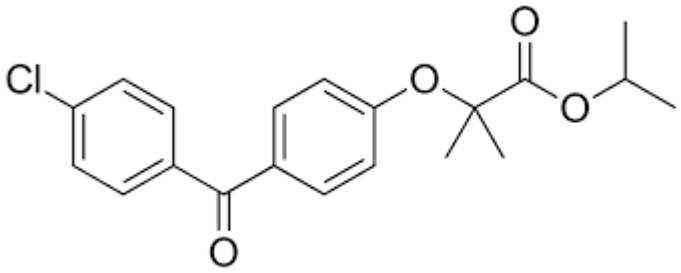

Figure 1: Structure of Fenofibrate

Literature survey reveals that the fenofibrate in both single and simultaneous with other drugs can be estimated by HPLC in biological fluids [2-3], RPHPLC in pharmaceutical dosage forms [4-8], UPLC [9] and spectrophotometric method [10] for the estimation of Choline fenofibrate in pharmaceutical dosage forms. The objective of this study is to develop a simple, fast, economical, selective, accurate, precise and sensitive RP-HPLC method for the determination of Choline fenofibrate in bulk and its pharmaceutical dosage forms suitable for routine quality control analysis.

MATERIALS AND METHODS:

Chemicals and Reagents: Fenofibrate is gift samples obtained from Chandra labs, Hyderabad.. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 285 nm with column Zorbax C-18 column (5µm, 150cm 4.6mm, ID), dimensions at Ambient temperature. The optimized mobile phase consists of phosphate buffer (pH 3.0) : Acetonitrile in the ratio of 30:70 (% v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

The buffer solution was prepared by dissolving accurately weighed 0.900 g of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate in sufficient water to produce 1000 ml. The pH was adjusted to 3.0 using phosphoric acid and sonicated to dissolve.

Preparation of mobile phase:

The Mobile Phase was prepared by mixing HPLC grade acetonitrile (ACN) was mixed with the buffer in a ratio of 30: 70 v/v. It was then sonicated for about 30 min and filtered through 0.45-micron membrane filter which was used for analysis of Fenofibrate.

Diluant Preparation:

Mobile phase is used as Diluant.

Preparation of the individual standard preparation:

Standard stock solution of Fenofibrate (1mg/ ml) was prepared in mobile phase dissolving 25 mg of the drug in a 25 ml clean, dry standard volumetric flask. The solution was kept in an ultrasonic bath to dissolve. The volume is made up to the mark with the mobile phase and mixed well. From the standard stock solution further dilution was made to get working standard solution with concentration 100 µg/ml of Fenofibrate. This working standard solution was analyzed using the HPLC conditions mentioned above.

Preparation of Sample Solution :(Tablet)

The samples were prepared by finely powdering 20 tablets of each batch using mortar and pestle. Sample equivalent to 200 mg of Fenofibrate was weighed in 50mL

volumetric flask. To it, 20 ml of mobile phase was added and kept in an ultrasonic bath for 20 minutes. Then the volume was made up to the mark with the mobile phase and mixed well. 20 ml of this solution was centrifuged at 2500 rpm for 20 minutes. This solution was further diluted to get 50 µg/ml of Fenofibrate and was used for the analysis

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for peaks are measured and the %Assay are calculated by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Zorbax C-18 column (5µm, 150cm 4.6mm, ID) , the mobile phase of composition phosphate buffer (pH 3.0) : Acetonitrile in the ratio of 30:70 (% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Fenofibrate in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: Fenofibrate working standard solutions were prepared across the range of the analytical method with a minimum of 5 concentrations that are within the specified range (1-500 µg/ml) low level (1 µg/ml) and higher level (500µg/ml) for 5 replicating injections were taken and calculated the %RSD. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies:

The accuracy was determined by help of recovery study. The recovery method carried out at three level 80%, 100%,120%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Fenofibrate and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies:

The system precision of the test method was performed by injecting 5 replicate determination of standard preparation injections were injected and the % RSD was calculated. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The results are shown in table 6.

Robustness: Robustness of assay method was carried out with variation of flow rate. Standard preparation was prepared and performed analysis as per test method and evaluated the system suitability parameters. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

$LOD = 3.3\sigma/S$ and

$LOQ = 10\ \sigma/S$, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

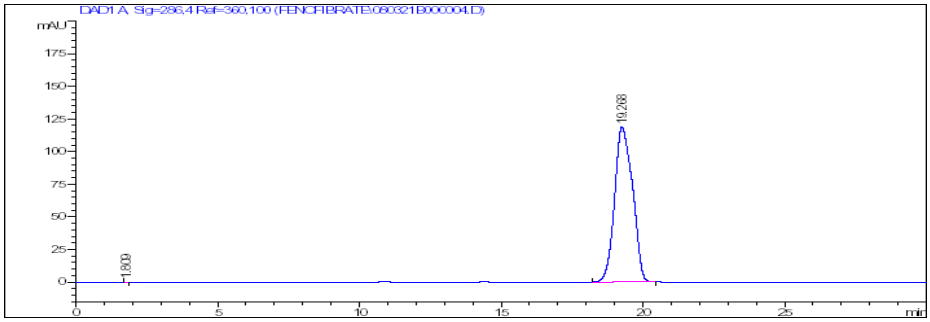

Figure 2: Standard chromatogram

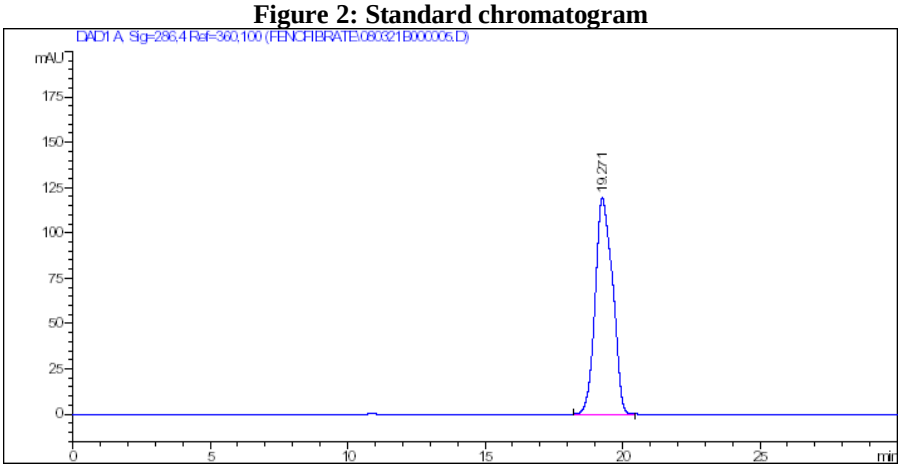

Figure 3: Sample chromatogram

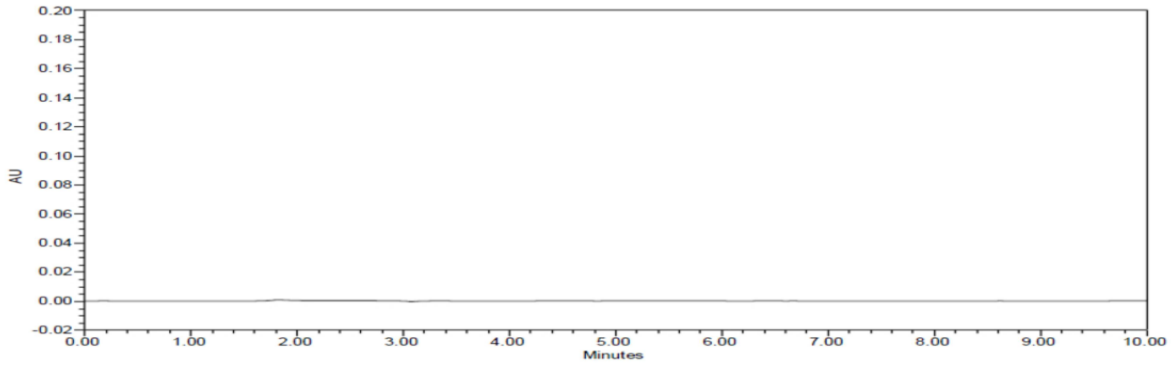

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Formulations tablets	Labeled amount in mg.	Amount recovered in mg Mean ± S.D (n=6)	%CV	% Assay
Tricor (Abbott	200	199.5 ± 0.10	± 0.042	99.7
Supralip	200	199.62 ± 0.67	± 0.136	99.8

Table 2: Assay results for Fenofibrate

Formulations tablets	Labeled amount in mg.	Amount recovered in mg Mean ± S.D (n=6)	%CV	% Assay
Tricor (Abbott	200	199.5 ± 0.10	± 0.042	99.7
Supralip	200	199.62 ± 0.67	± 0.136	99.8

Table 3: Linearity results of Fenofibrate

Concentration (µg/ml)	Peak area Inj. 1	Peak area Inj. 2	Mean Peak area
1	60221	60324	60272.5
5	301109	302257	301683
10	624363	622478	623420.5
50	2932784	2933605	2933195
100	5946475	5965468	5955972
200	11876813	11881977	11879409
250	14636813	14647110	14641962
500	28968923	29013004	29013004

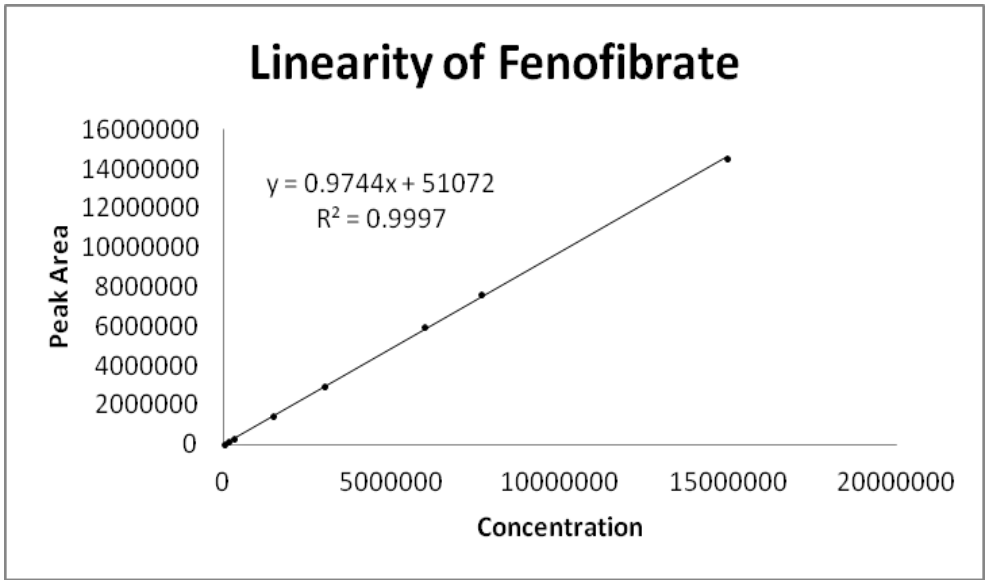

Figure 5: Linearity graph for Fenofibrate

Table 4: Showing accuracy results for Fenofibrate

Sample ID	Concentration (µg/ml) pure drug	Formulation	%Recovery of pure drug	Statistical Analysis	
S1 : 80 %	120	150	99.97	Mean	99.88
S2 : 80 %	120	150	99.81	SD	0.085
S3 : 80 %	120	150	99.84	% RSD	0.085
S4 : 100 %	150	150	99.75	Mean	99.34
S5 : 100 %	150	150	99.50	SD	0.5316
S6 : 100 %	150	150	98.73	% RSD	0.5351
S7 : 120 %	180	150	99.72	Mean	99.73
S8 : 120 %	180	150	99.91	SD	0.215
S9 : 120 %	180	150	99.58	% RSD	0.2156

Table 5: Precision results for Fenofibrate

Concentration	Method precision	Injection 1	Injection 2	Average
100 µg/ml	Sample-1	5946475	5965468	5955971.5
	Sample-2	5889564	5869987	5879775.5
	Sample-3	5948627	5695814	5822220.5
	Sample-4	5981670	5980014	5980842
	Sample-5	5967634	5671233	5819433.5
	Sample-6	5794033	5765242	5779637.5
Statistical analysis			Mean	5872980
			SD	80900.625
			%RSD	1.376

Table 6. Ruggedness results of Fenofibrate

Variables	RT mean±SD	%RSD	Peak area mean±SD	% RSD
Analyst-I	19.1833 ± 0.2136	1.1139	1355195 ± 1950.6	0.1
Analyst-II	19.2387 ± 0.302	0.698	1372607 ± 3209.61	0.928035

Robustness results

Table 7: Flow variation results for Fenofibrate

Parameters (n=6)	Variables	Statistical analysis		Statistical analysis	
		RT Mean ± SD	%RSD	Peak area Mean ± SD	%RSD
Flow rate (ml/min)	0.9	19.277 ± 0.111	0.57	5829527 ± 19951.66	0.34
	1	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
	1.1	19.238 ± 0.075	0.39	5870184 ± 110345.83	1.87
Mobile phase Composition (Buffer : ACN)	25 : 75	18.781 ± 0.327	1.74	6008220 ± 89604.47	1.49
	30: 70	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
	35 : 65	19.683 ± 0.203	1.03	5946741 ± 69779.83	1.17
Temperature (ºC)	26	19.25 ± 0.204	1.05	5938621 ± 22714.90	0.38
	28	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
	30	19.28 ± 0.150	0.77	5907851 ± 71689.5	1.21
pH	2.8	19.271 ± 0.091	0.47	5856382 ± 20382.56	0.34
	3	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
	3.2	19.275 ± 0.117	0.6	5860687 ± 19782.92	0.34

Table 8: LOD, LOQ of Fenofibrate

Drug	LOD	LOQ
Fenofibrate	0.228	0.764

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Fenofibrate in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Fenofibrate in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Balfour JA, McTavish D, Heel RC: Fenofibrate. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in dyslipidaemia. Drugs. 1990 Aug;40(2):260-90. doi: 10.2165/00003495-199040020-00007. (PubMed ID 2226216)
2. Patel H, Rathod R and Dash RP: Simultaneous quantification of rosuvastatin and fenofibric acid by HPLC-UV in rat plasma and its application to pharmacokinetic study. Journal of Liquid Chromatography and Related Technologies 2013; 5:87-92.
3. Zaman MT, Khan SA, Arora A and Ahmad O: Method development and validation of fenofibrate by HPLC using human plasma. Electronic Journal of Biomedicine 2009; 3:41-54.
4. Patel, Archita, Macwana, Parmar, Vishal Patel and Samir: Simultaneous determination of atorvastatin calcium, Ezetimibe, and Fenofibrate in a tablet formulation by HPLC. Journal of AOAC International Ingenta Connect 2012; 95(2):419-423.
5. Jain N, Raghuwanshi R and Deeti Jain: Development and validation of RP-HPLC method for simultaneous estimation of Atorvastatin calcium and Fenofibrate in tablet dosage forms. Indian Journal of Pharmaceutical Sciences 2008; 70(2):263-265.
6. Lacroix PM, Brain A Dawson and Roger: HPLC methods for assay and purity and an NMR method for purity. Journal of Pharmaceutical and Biomedical Analysis 2009; 18(3):42-47.

7. Bhamare PC, Bari SB, Natarajan S, Patil AA and Shirode PT: Development and validation of a precise stability indicating HPLC method for determinations of Metformin hydrochloride and Fenofibrate, in pure form and in pharmaceutical tablets. International Journal of Pharma Tech Research 2011; 3:505-515.
8. Anand Kumar Karunakaran, Vetsa Subhash, Ramu Chinthala and Jayamaryapan Muthuvijayan: Simultaneous estimation of Rosuvastatin calcium and Fenofibrate in bulk and tablet dosage form by UV spectrophotometry and RPHPLC. Stamford Journal of Pharmaceutical Sciences 2011; 4(1):58-64.
9. Kadav and Vora DN: Stability indicating UPLC method was developed and validated for the simultaneous determination of Atorvastatin, Fenofibrate and their impurities in tablets. Journal of Pharmaceutical and Biomedical Analysis 2008; 48(I, 10):120-126.
10. Alaa Gindy, Samy Emara and Mostafa: Spectrophotometric and liquid chromatographic determination of Fenofibrate and Vinpocetine and their hydrolysis products. Elsevier Journal 2005; 60(5):425-438.