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Research Article

NEW VALIDATED ISOCRATIC RP-HPLC METHOD FOR ASSAY OF FENOFIBRATE

DILLIP KUMAR SAHOO*[1], PRAFULLA KUMAR SAHU[2], CHANDRA SEKHAR PATRO[2]

[1]Department of Pharmaceutical Analysis, Avanthi Institute of Pharmaceutical Sciences, Cherukupally, Bhogapuram, Vizianagaram, Andhra Pradesh, 531162.[2]Department of Pharmaceutical Analysis, Raghu College of Pharmacy, Dakamarri, Bheemunipatnam, Visakhapatnam, Andhra Pradesh, 531162. Email: sahoo4@rediffmail.com

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ABSTRACT

Objective: 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.

Methods: 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 286 nm.

Results: The retention time was found to be 19.268 min and produced a linear response in the concentration range of 1-500 µg/mL (R^2~0.999). The % RSD was found to be below 2%. The LOD and LOQ were found to be 0.229µg/ml and 0.765µg/ml respectively.

Conclusion: 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, isocratic mode, RPHPLC, diode array detection.

INTRODUCTION

Fenofibrate is a lipid regulating drug. It is used for reducing the serum cholesterol and triglycerides concentration. Literature survey revealed that few analytical methods are reported for the determination of fenofibrate in various samples. Krishna R. Gupta et al [1] described spectrophotometric methods for the determination of fenofibrate. Few HPLC methods are reportedfor the estimation of fenofibrate in human plasma [2] described a HPLC method for the estimation of Fenofibrate in human plasma. An HPLC-MS/MS method was used for identifying the probable cause of analytical interference because of fenofibrate medication taken by the patients by Meikle AW et al [3].A HPLC method was established by LI Jia-yu et al [4] for the determination of fenofibrate in the soft capsules. Few HPLC methods are published for simultaneous estimation of Fenofibrate along with Rosuvastatin [5], Atorvastatin [6,7] and Ezetimibe [8]. A densitometric TLC method for simultaneous analysis of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms has been validated by Atul A. Shirkhedkar et al [9]. Spectrophotometric and HPLC methods are presented by El-Gindy et al [10] for the determination of fenofibrate, vinpocetine and their hydrolysis products. HPLC methods for drug content and HPLC and NMR methods for related compounds in fenofibrate raw materials were developed by Pauline M. Lacroix et al [11].The objective of this study is to develop a simple, very fast, selective, accurate, precise and sensitive isocratic RP-HPLC method for the determination of Fenofibrate in bulk and Pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation

The LC system, used for method development and method validation was on a Waters- 2695, with an auto injector, and waters 2696 PDA detector. The output signal was monitored and integrated using Empower software. Zorbax Eclipse XDB-C_{18} 250mm x 4.6 mm, 5 µm column was used.

Preparation of solutions

Preparation of mobile phase

Buffer was prepared by dissolving 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. 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.

Preparation of standard solution

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.

Method development

Method Optimization

The wavelength for the analysis of Fenofibrate was selected from the UV spectrum. A wavelength of 286 nm was selected for the analysis. For selection of mobile phase, various mobile phase compositions were observed for efficient elution and good resolution. Various compositions like 20:80, 30:70 and 50:50 of buffer: ACN mixtures were tried at different flow rates. The buffer: ACN (30: 70 v/v) was found to be the optimum composition for efficient elution of analyte with a flow rate of 1ml/min at ambient temperature.

Method Validation

The validation parameters like linearity, sensitivity, accuracy, precision, robustness and specificity of the assay and the recovery were studied according to the US Food and Drug Administration (FDA) guidance for the validation of analytical methods. Calibration curves were prepared by assaying standard sample solutions ranging from 1 to 500µg/ml. The linearity of each method matched calibration curve was determined by plotting the peak area (y) versus the concentration (x) of Fenofibrate.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined on the basis of response and slope of the regression equation. The precision of the method was ascertained separately from the areas under the curve obtained by actual determination of eight replicates of a fixed amount of drug and the percent relative standard deviations were calculated. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak

areas for a set of drug solutions on three different days. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples of Fenofibrate within the linearity ranges were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated.

To study the robustness of the method, the test solutions were injected with deliberate variations in method parameters like flow rate, temperature, pH and mobile phase composition. For the ruggedness study, the prepared test solution as per the test method was analyzed by different analysts on a different instrument using the same column.

Assay of formulation

The samples were prepared by finely powdering 20 tablets of the 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. The peak area of the sample solutions were compared with the standard graph and % assay was calculated by the following formula.

$$\%\ Assay = \frac{\text{Sample area} \times \text{Standard weight} \times \text{Average weight of 20 tablets}}{\text{Standard area} \times \text{Sample weight} \times \text{Label claim}}$$

RESULTS AND DISCUSSION

Chromatographic conditions

The λ_{max} of Fenofibrate was found to be 286nm. The mobile phase Acetonitrile: Phosphate buffer (pH- 3.0) of 70:30%v/v at a flow rate of 1.0 ml/ min gave peaks with good resolution for Fenofibrate are eluted at retention time around 19.268 min and with symmetric peak shape.

Method Validation

Linearity

When a series of dilutions were analyzed, the concentration range of 1 to 500µg/ml was found to give a straight line. The linearity study is given in Table 1, Fig. 1 and the typical chromatogram of the pure drug is given in Fig. 2.

Table 1: Linearity study

Concentration	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

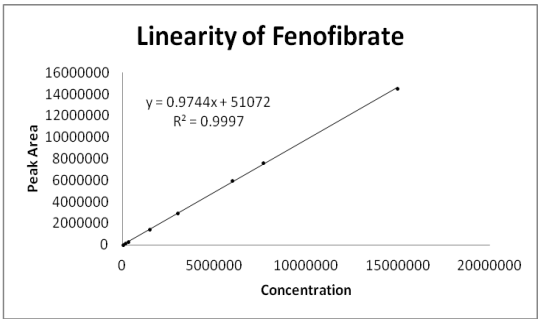

Fig. 1: Linearity study of Fenofibrate

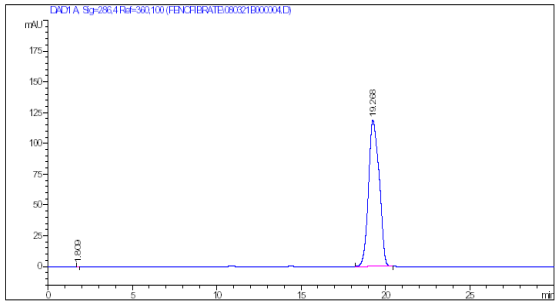

Fig. 2: Typical chromatogram of Fenofibrate pure drug (50µg/ml)

Assay of formulation

The amounts of drug present in two different brands tablet dosage forms were calculated. The standard deviations (SD) were 0.10, 0.67 and the percent relative standard deviations (%RSD) were found to be 0.042, 0.136 respectively for the two brands used. A typical chromatogram of Fenofibrate in formulation was shown in Fig. 3 and the assay results are given in Table 2.

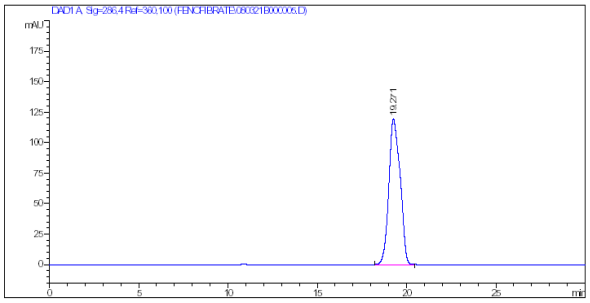

Fig. 3: Typical chromatogram of Fenofibrate in formulation (50µg/ml)

Table 2: Assay of formulations

Formulations tablets	Labeled amount in mg.	Amount recovered in mg Mean ± S.D (n=6)	%CV	% Assay
Tricor (Abbott Labs)	200	199.5 ± 0.10	± 0.042	99.8
Supralip (Solvay Ph.)	200	199.62 ± 0.67	± 0.136	99.9

Precision and Accuracy

The method was found to be precise and the SD and %RSD of the area under curves were calculated to be 80900.625 and 1.377 respectively and presented in the Table 3. The accuracy of the method was determined by recovery studies and percentage recovery values were calculated. The accuracy results were shown in Table 4.

Table 3: Precision study

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	5872980.08
			SD	80900.625
			%RSD	1.377

Table 4: Accuracy study

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis	
	Pure drug	Formulation	pure drug		
S_1 : 80 %	120	150	99.98	Mean	99.88
S_2 : 80 %	120	150	99.82	SD	0.085
S_3 : 80 %	120	150	99.85	% RSD	0.085
S_4 : 100 %	150	150	99.76	Mean	99.34
S_5 : 100 %	150	150	99.51	SD	0.5316
S_6 : 100 %	150	150	98.74	% RSD	0.5351
S_7 : 120 %	180	150	99.73	Mean	99.73
S_8 : 120 %	180	150	99.94	SD	0.215
S_9 : 120 %	180	150	99.51	% RSD	0.2156

Limit of Detection and Quantification

The parameters LOD and LOQ for this method were found to be 0.229µg/ml and 0.765µg/ml respectively.

Robustness

To study the robustness of the method, the test solutions were injected with deliberate variations in method parameters like flow rate, temperature, pH and mobile phase composition. The reliability of the method is shown by the robustness study result given in Table 5.

Table 5: Robustness study (100µg/ml)

Parameters (n=6)	Variables	Statistical analysis		Statistical analysis	
		RT Mean ± SD	%RSD	Peak area Mean ± SD	%RSD
Flow rate	0.9	19.277 ± 0.111	0.57	5829527 ± 19951.66	0.34
(ml/min)	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	25 : 75	18.781 ± 0.327	1.74	6008220 ± 89604.47	1.49
Composition	30: 70	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
(Buffer : ACN)	35 : 65	19.683 ± 0.203	1.03	5946741 ± 69779.83	1.17
Temperature	26	19.25 ± 0.204	1.05	5938621 ± 22714.90	0.38
(ºC)	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
	3.0	19.268 ± 0.061	0.31	5872980 ± 19100.62	0.32
	3.2	19.275 ± 0.117	0.60	5860687 ± 19782.92	0.34

Ruggedness

The prepared test solutions as per the test method were analyzed by a different analyst on a different instrument using the same column. The ruggedness study result is given in Table 6.

Table 6: Ruggedness study (100µg/ml)

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	0.697	1372607 ± 3209.61	0.928035

System Suitability testing:

System suitability test of the HPLC method gave good relative retention time (α = 3.6), column capacity (K'= 2.8), and tailing factor (T = 1.1). The system suitability parameters are given in Table 7.

Table 7: System Suitability

Parameters	Obtained value	Reference value
Relative retention (α)	3.6	>1
Tailing factor (T)	1.1	<1.5–2 or <2
Capacity factor (K')	2.8	1–10 acceptable
Theoretical plates (N)	8260	>2000

CONCLUSION

A simple isocratic reverse phase high performace liquid chromatography (RPHPLC) method was developed for determination of fenofibrate in bulk and pharmaceutical dosage form. 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. This method, which can be run in 20 minutes, may be suitable for analysis in Quality control units of Pharmaceutical industries.

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