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A Validated Reversed-Phase HPLC Analytical Method for the Analysis of Fenofibrate in Bulk Drug and Tablet Dosage Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: A accurate, precise, and stability-indicating Reversed-Phase HPLC technique has been established for the estimation of fenofibrate in tablet formulation.
Study Design: Experimental study.
Place and Duration of Study: Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur-440033, Maharashtra, India between June 2019 and March 2020.
Methodology: The chromatographic separation was attained on RP Princeton column (C18) (250 mm x 4.6 mm, 5 µ) with mobile solvent system as a mixture of water (pH 3.0 along o-phosphoric acid) and acetonitrile in the proportion (40:60) v/v, flow rate 1.0 ml per minute, at 240 nm. The retention time of fenofibrate was 3.905 minutes.
Results: The method demonstrated linearity in the concentration range of 87-232 µg/ml with a coefficient of correlation (r^2) of 0.9994. The % RSD was <2% and percentage recovery was found to be 99.13-100.74%. The assay of marketed tablet formulations was found to be 99.98%.

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Conclusion: The developed and validated technique as per ICH rules for specificity, accuracy, precision, linearity, and system suitability. Reverse Phase-HPLC technique was utilized to the market formulation.

Keywords: Fenofibrate; reverse phase-HPLC; validation.

1. INTRODUCTION

Fenofibrate (FEN), propane-2-yl 2-{4-[(4-chlorophenyl) carbonyl] phenoxy} methyl propanoate [1] (Fig. 1). Medicinally it is known as antilipemic agents. It is effective in decreasing triglyceride levels and increases HDL cholesterol levels. It is used in primary hypercholesterolemia and severe hypertriglyceridemia [2]. The literature survey shows that HPLC should be technique mentioned for the estimation of fenofibrate in individual or in combined tablet dosage formulation [3-16], the reported technique has the drawbacks long runtime and less economical with a high proportion of organic phase. Hence, an attempt was made to develop RP-HPLC which is specific, accurate, precise, and, economical technique for the determination of fenofibrate in combined tablet and bulk dosage form [17-19].

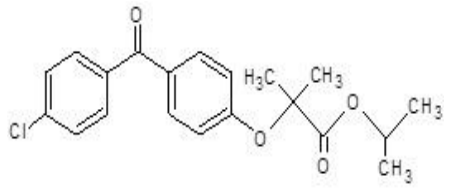

Fig. 1. Structure of Fenofibrate

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical grade Fenofibrate were procured from pharmaceutical company Cadila Pharmaceuticals Limited., Ahmedabad, Zyrova F-10 a tablet marketed formulation.

Acetonitrile, methanol, o-phosphoric acid of analytical grade was used.

2.2 Instrumentation

Shimadzu HPLC system and PDA detector with Lab Solution software were used.

2.3 Optimized Chromatographic Conditions

Chromatographic isolation was attained on a RP column Princeton C-18 (5 µ, 250 mm × 4.6 mm) at ambient temperature using a mobile phase containing of a mixture of buffer (3.0 pH, with o-phosphoric acid) & ACN in the proportion of (40:60) v/v, flow rate 1.0 ml per minute, at 240 nm. The pH of the solvent system at 3.0, Injection volume 10 µl (Table 1). A typical chromatograph of a mixture of standard and sample fenofibrate is summarized by Fig. 2 and Fig. 3 respectively.

2.4 Procedure for Preparation of Standard Solution of Fenofibrate

Precisely weighed 14.5 mg of fenofibrate was conveyed to a 10.0-milliliter volumetric flask and soften in 5-milliliter of diluent. The quantity was made up to 10.0-milliliter with diluent. 1.0 milliliter, the resulting solution was pipetted in a 10.0-milliliter flask and up to 10.0-milliliter with diluent to add a solution of concentration 145 µg per milliliter of fenofibrate.

2.5 Preparation of Sample Solution of Fenofibrate

20 tablets were weighed and finely powdered and a precisely weighed quantity of powder equal to 14.5 mg of fenofibrate was conveyed into a 10.0-milliliter volumetric flask. The powder was added with 5-milliliter of methanol. The resulting solution was dilute up to the level with diluent and filtered over filter paper (Whatman Grade-I). One milliliter filtrate was conveyed into a 10-milliliter flask and the quantity was made up to the level with diluent to provide a sample solution consist of 14.5 µg per milliliter of fenofibrate. Six replicate tablet solutions consist of 14.5 µg per milliliter of fenofibrate solutions were prepared similarly.

2.6 Assay Procedure

After equilibration of stationary phase, 3 replicate injections of each of sample solutions were made solely and chromatograms were reported. By using peak zone of a quantity of drug existing in the average weight of tablet as percent labeled claim was calculated by using formula given below.

$$\%\ Assay = \frac{A_{sam} \times C_{std} \times \mathrm{DF} \times \mathrm{Avg.\,Wt.}}{A_{std} \times \mathrm{Wt.\,taken} \times \mathrm{LC}}$$

Where,

A_{sam} = Area of Sample taken
A_{std} = Area of Standard taken
C_{std} = Concentration of standard, µg/ml
DF = Dilution Factor
Avg. Wt. = Average weight of tablets
Wt. taken = Weight of tablet powder taken
LC = Labeled Claim

Table 1. Optimized chromatographic condition

Chromatographic condition	
Mobile phase	Water (3 pH with o-phosphoric aci):ACN (40:60) v/v
Flow rate	1.0 ml/min.
Column	Princeton C-18 column (5 µ, 250 mm × 4.6 mm)
Detector wavelength	240 nm
Column temperature	30 °C
Injection volume	10 µl
Runtime	20 minutes
Diluent	Acetonitrile:Water (50:50)
Retention time (RT)	3.905 minutes for Fenofibrate peak

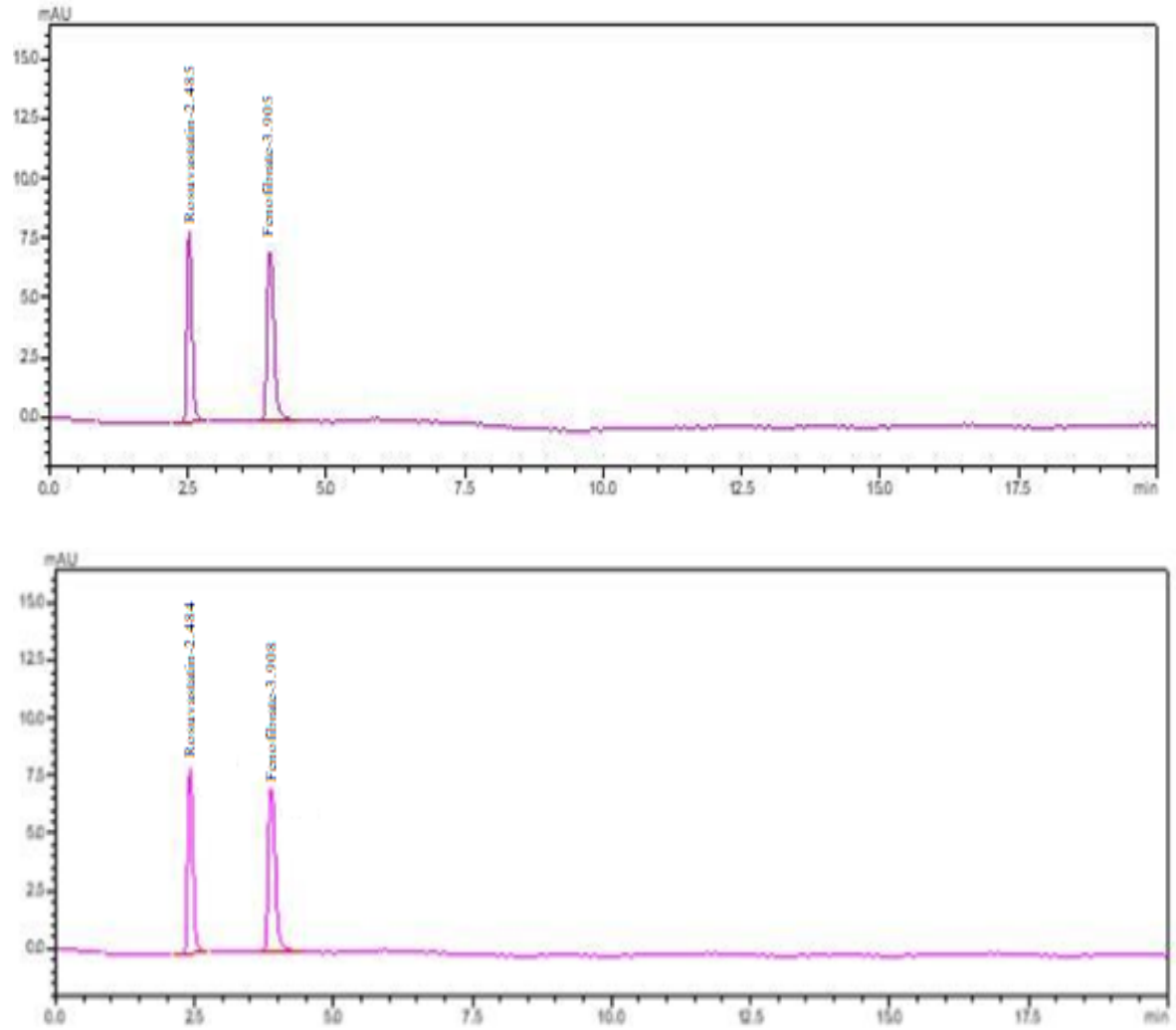

Fig. 3. A typical Chromatograph of sample Fenofibrate

3. METHOD VALIDATION

3.1 Specificity

The developed technique was specified by correlating the chromatograph of the standard and sample solution (Table 2).

3.2 LOD and LOQ

The limits of detection are the lowermost sample concentration that can be noticed and limits of quantification is the lowest analyte concentration, evaluated along adequate accuracy and precision. LOQ and LOD were settled, under ICH guidelines, by the purpose of the equations 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.

3.3 Linearity

Linearity test solutions of fenofibrate were arranged by diluting the stock solution at concentration levels of 116-174 µg/ml. Linearity was settled by the least-squares linear regression analysis obtained. Peak areas versus linear regression analysis and corresponding concentrations were achieved on the resulting curves. The linear curve of fenofibrate was shown in Fig.4.

3.4 Precision

The measurement of the precision an area of 6 qualified working standards for fenofibrate calculating the % RSD. The assay technique precision was estimated by operating six independent assays of test samples of fenofibrate across qualified working standards and considering the %RSD. The intermediate precision of the technique was also proved using different analysts and different days.

3.5 Accuracy

The analytical accuracy operation suggests the adjacency of covenant midway the value, which is confirmed either as an ideal correct value or a received mention value. It was computed at 3 different levels (80%, 100%, and 120%) of the label claim (Table 4).

3.6 Robustness

To specify the robustness study of the validated chromatographic technique, the chromatographic conditions were consciously variation and the resolution for fenofibrate was estimated. To survey the outcome of wavelength on the assessment, and the wavelength variation by ± 2 nm, i.e., 238 and 242 nm from the original wavelength, 240 nm. To survey the outcome of flow rate on the assessment, the flow rate was varied by ± 0.1 millimeters per minute i.e., 0.9 and 1.1 millimeters per minute from the certain flow rate, 1.0 millimeters per minute (Table 5).

Table 2. System suitability results

Parameter	Fenofibrate
Theoretical Plate	4997
Retention Time	3.905
Tailing factor	1.45
% RSD	0.4

Table 3. Linearity results

Parameter	Fenofibrate
Concentration Range (µg/ml)	87-232
Slope (m)	415.83
Intercept	557.22
Coefficient correlation (r^2)	0.9994

Table 4. Recovery results

Compound	Spiked conc. (%)	Amount conc. taken (µg/ml)	Amount conc. found (µg/ml)	Percentage Recovery
Fenofibrate	80	116	116.51	100.44
	100	145	143.74	99.13
	120	174	172.55	99.16

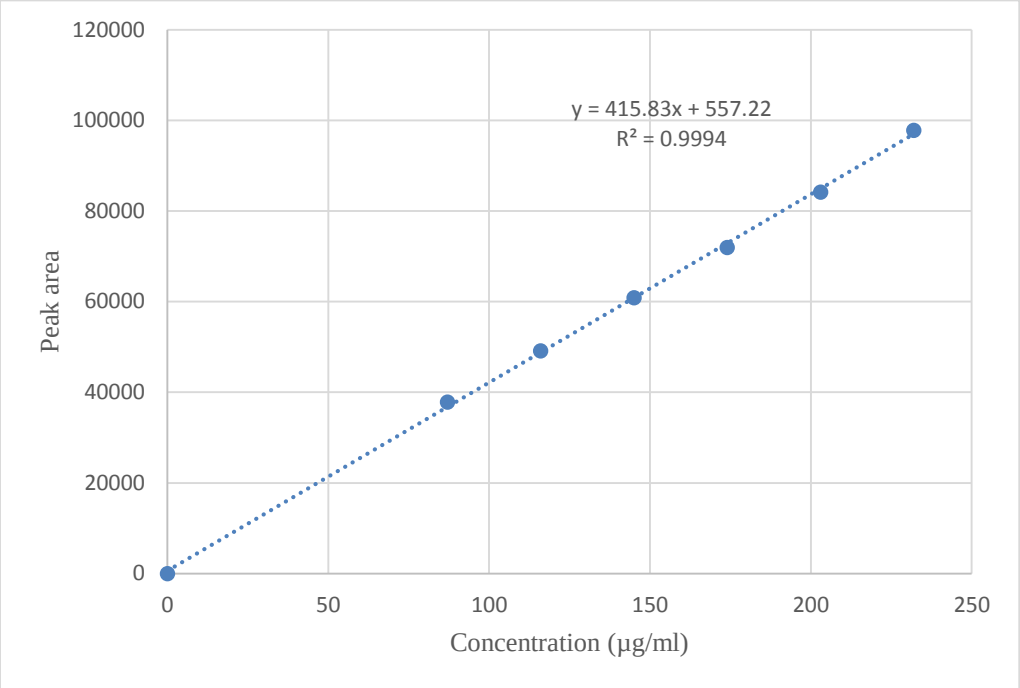

Fig. 4. Linearity curve for fenofibrate

Table 5. Robustness results

Condition		Fenofibrate	
		Amount estimated [%]	**RSD [%]**
Change in wavelength (240±2 nm)	**238 nm**	100.16	0.0900
	242 nm	100.08	0.1417
Change in flow rate (1.0±0.1 ml/min)	**0.9 ml/min**	99.37	0.2068
	1.1 ml/min	99.56	0.2009

** Each value is a mean of three observations*

3.7 Stability of Solution

The stability of the sample was noticed for 24 hours. % Relative standard deviation of 0.9 indicates the stability of the technique for 24 hours. Thus, the technique was found to be specific

4. RESULTS AND DISCUSSION

4.1 High Performance Liquid Chromatography Development and Optimization

Initially, pure drugs solution was chromatographed using a solvent system containing a combination of buffer (3.0 pH, with o-phosphoric acid) & acetonitrile in the proportion of (40:60) v/v, flow rate 1.0 ml/min gives well-resolved peaks of drugs, at 240 nm. The retention time 3.905 minutes.

4.2 Validation of the Technique

4.2.1 Limit of Quantification and Limit of Detection

The Limit of Quantification and Limit of Detection of fenofibrate were 70.08 and 23.12 respectively.

4.2.2 Linearity

Linearity by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 116-174 µg/ml for fenofibrate. Peak areas were plotted against the linear regression analysis and respective concentrations performed on the resulting curves. The equation for the calibration plots of fenofibrate was Y= 415.83x + 557.22, correlation coefficient 0.9994.

Table 6. Summary

Parameter	Fenofibrate
Calibration range (µg/ml)	87-232
Optimized wavelength (nm)	240
Retention Time	3.905
Regression equation (Y)	Y= 415.83x + 557.22
Slope	415.583
Intercept	557.22
Coefficient correlation (r^2)	0.9994
Precision (% RSD)	
Intraday	0.4
Interday	0.8
% Assay	100.15
LOD (µg/ml)	23.12
LOQ (µg/ml)	70.08

% RSD: Percentage relative standard deviation

4.2.3 Precision

The results of interday precision and intraday precision were 0.8 and 0.4 for fenofibrate. The % RSD of method, system, and intermediate precision results within ±2.0%, indicate that the technique was precise.

4.2.4 Accuracy

The percentage of recoveries was 100.15± 0.5016% for fenofibrate.

4.2.5 Robustness

To estimate the robustness of the developed technique, the chromatographic conditions were deliberately altered, and determined. To study the effect of wavelength flow rate on the estimation (Table 6).

4.2.6 Analysis of Fenofibrate from marketed tablets

Percentage analysis of the marketed formulation was erect to be 99.98 for fenofibrate.

Hence, an attempt was made to develop RP-HPLC which is short runtime and high economical with a less proportion of organic phase, specific, accurate, precise, and, economical technique for the determination of fenofibrate in combined tablet and bulk dosage form

5. CONCLUSION

The technique enables simple, rapid, accurate, precise, specific, economical, and sensitive estimation of fenofibrate in combined tablet and bulk dosage formulations. The technique therefore, utilized for regular estimation of fenofibrate in tablet and bulk dosage form.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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