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Stability-Indicating UPLC Method for the Estimation of Fenofibrate (Micronized) Capsules

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Abstract: This article presented a stability indicating "Ultra Performance Liquid Chromatography" (UPLC) method for the analysis of Fenofibrate (Micronized) capsules. The developed method was found to be accurate, precise and specific while validated on different parameters. This proved its suitability for routine quality control analysis of Fenofibrate (Micronized) capsules. The analytical procedure showed linearity over the concentration range of 50% to 150% (33.5760 µg/ml to 100.7290 µg/ml). Repeatability study indicated %R.S.D of 0.2 for Fenofibrate, while the average recovery rate was found to be 99.5%, falling within the acceptable range of 98-102%. Robustness testing confirmed how robust the technique towards concentration, mobile phase, pH, oven column temperature and volumetric flow, etc. This innovative UPLC method, developed in accordance with ICH guidelines, offers a cost-effective and practical approach for the estimation of Fenofibrate (Micronized) capsules. The developed method can be used for routine analysis of Fenofibrate in bulk as well as in pharmaceutical preparations.

Keywords: Fenofibrate, Ultra Performance Liquid Chromatography, ICH, Micronized, Capsules

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Introduction

Fenofibrate belongs to the class known as anti-lipemic; this drug helps to eliminate cholesterol. It stimulates lipolysis and increases the activation of lipoprotein lipase, thus reducing apoprotein C-III. It starts formation of peroxisomes that contain hydrogen peroxide, hydroxyl radicals and reactive oxygen species which are responsible for the degradation of lipid. Fenofibrate is also used to prevent pancreatitis caused by excessively high triglyceride levels when combined with an appropriate diet (Chapman, 1987; Miller and Spence, 1998; Qi *et al.*, 2000). Fenofibrate is a drug, which should be used along with a diet designed to reduce levels of cholesterol. Fenofibrate lowered triglycerides and VLDL cholesterol along with total cholesterol. This drug

generally prescribed along with low fat diet and exercise; to reduce undesirable lipid levels in serum. Thus in this way Fenofibrate helps to prevent atherosclerosis, which is considered responsible factor of conditions like angina, strokes and heart attacks, etc. (Balfour *et al.*, 1990; Miller and Spence, 1998; Qi *et al.*, 2000).

The qualitative and quantitative estimation of pharmaceutical products or pure drugs can be performed with precision using modern analytical technique like UPLC. The particle size used in this technique is much smaller than other liquid chromatographic techniques. Therefore UPLC offers advantages of higher sensitivity, higher resolution, and shorter analysis times and less tailing, etc (International Conference Harmony, 2005; Branch, 2005; Bhavyasri *et al.*, 2019). Considering therapeutic significance of Fenofibrate this research focuses on developing and validating a reliable UPLC method for the estimation of Fenofibrate in micronized capsule formulation.

Materials and Methods

The drug (Fenofibrate) was procured from manufacturer as gift sample and its identification was done through FTIR analysis. Analytical grade solvents and reagents were procured from Merck Pvt. Ltd.

Characterization and Identification:

Physico-chemical characterization was performed to check colour, odour, melting point and solubility of drug. Identification of Fenofibrate (Micronized) was done using FTIR analysis.

Chromatographic Conditions:

The analysis was performed using an Acquity UPLC BEH C_{18} column (50 × 2.1 mm, 1.7 µm) with a flow rate of 0.4 ml/min. The oven temperature was maintained at 30°C, while the sample cooler temperature was set to 25°C. The injection volume was 2 µl, and the detection wavelength (λ) was 285 nm. The total run time for the analysis was 5 min.

Preparation of Mobile phase, Sample and Standard Solutions:

Preparation of Buffer pH 3:

The buffer solution was prepared by dissolving 130 mg of monobasic potassium phosphate in 1 L of water and adjusting the pH to 3.0 ± 0.05 using diluted phosphoric acid.

Preparation of Mobile phase:

The mobile phase consisted of a mixture of buffer (pH 3.0) and methanol in 20:80 ratio; which was also used as a diluent.

Preparation of Standard Solution:

Accurately weighed drug was transferred to a 50 ml volumetric flask, little amount of diluent was added to dissolve the drug, mixed well and volume was made up to the mark with same solvent.

Preparation of Sample Solution:

Capsules were weighed, powdered and transferred into a 250 ml volumetric flask. Solvent (175mL) was added to dissolve the content. This solution was filtered through 0.22 µm syringe and filtrate was used for further analysis.

Validation

The validation of the developed UPLC method was conducted in accordance with the guidelines developed for the respective parameter as summarized below (Breaux2003; Sharma *et al.*, 2018; Swartz and Krull, 2018; Sahu *et al.*, 2018):

Specificity:

The specificity study was carried out to confirm that the analytical method used is specific to the identification and quantification of the target drug without interference from other substances.

Linearity:

Fenofibrate was dissolved in various concentrations, which were then injected to the UPLC column. The concentration plotted against peak area produced a calibration curve. Linearity was expressed in terms of the correlation coefficient.

Table 1: Physico-chemical Characterization of Fenofibrate (Micronized)

S. No.	Characteristic	Observation
1	Colour	White or almost white, crystalline powder
2	Odour	Odourless
3	Melting point range	Between 79.0°C to 82.0°C
4	Solubility	Soluble in methylene chloride

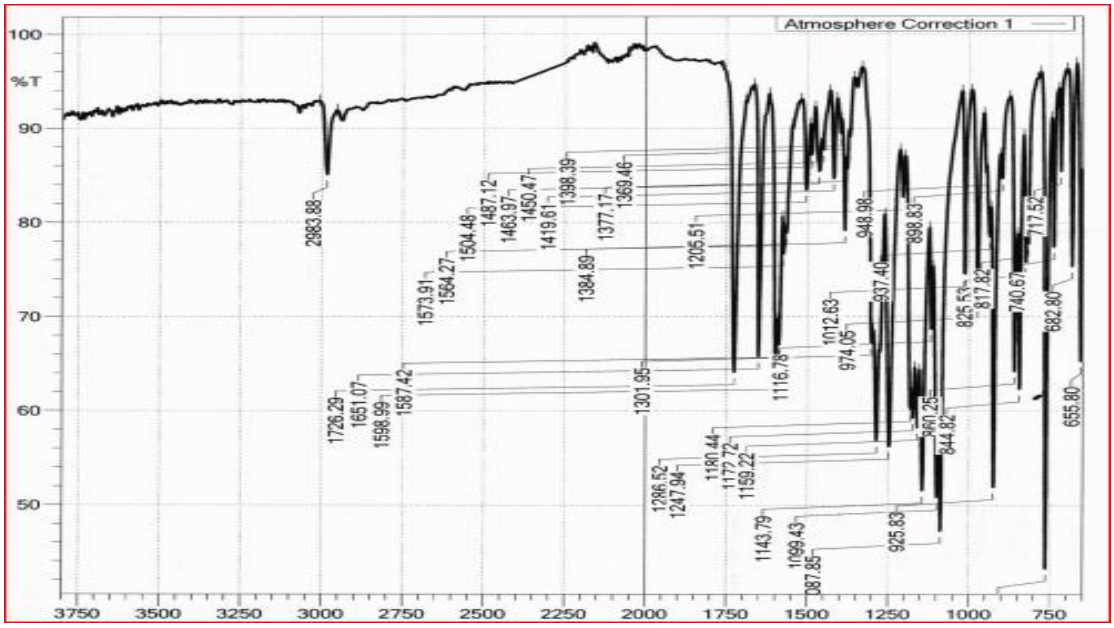

Fig. 1: IR Spectrum of Fenofibrate (Micronized).

Accuracy:

Per cent recoveries for Fenofibrate at LOQ, 100%, and 150% were tested in triplicate at every level.

Precision:

Six independent samples (spiked and unspiked) were compared with a qualified reference standard and inter-day precision was determined. The %RSD of the assay was calculated.

Robustness:

Robustness of the method was demonstrated by the intentional slight change in the chromatographic conditions like alteration in mobile phase ratio, flow rate, column oven temperature, and the pH of the buffer showing that the method was not affected by such changes.

Results and Discussion

This study achieved optimum efficiency while used specified column and solvent system. Fenofibrate was successfully estimated in capsule formulation using developed UPLC method.

Characterization and Identification:

Physico-chemical characterization was performed for Fenofibrate (Micronized) and result of same is depicted in Table 1. The identification of drug was done using FTIR analysis and spectrum of same is presented in Figure 1.

Based on the FTIR spectrum of the standard and sample, the identification of Fenofibrate (Micronized) was established. The characteristic absorption bands observed in the sample included N-O stretching at 1296 cm^{-1} (within the expected range of 1372-1290 cm^{-1}), ester (C-O) at 1129 cm^{-1}, imine (C=N) at 1694 cm^{-1} and C-H stretching at 2955 cm^{-1}. These spectral data confirmed the presence of Fenofibrate in the sample.

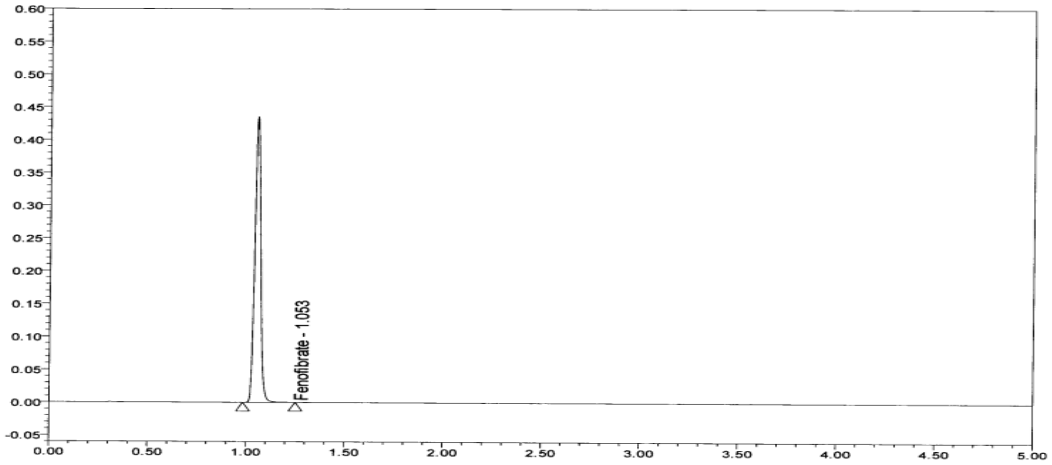

Fig. 2: Chromatogram of Drug in Formulation.

Chromatographic Analysis:

The optimization of chromatographic conditions involved refining the mobile phase parameters to achieve optimal separation and detection. The final optimized chromatographic conditions were established using an Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm) with a flow rate of 0.4 ml/min. The oven temperature was maintained at 30°C, while the sample cooler temperature was set at 25°C. The injection volume was 2 µl, and detection was carried out at a wavelength of 285 nm. The mobile phase consisted of buffer and methanol in 20:80 ratio. These conditions ensured precise and reproducible chromatographic performance with adequate peak shape (Fig. 2) and higher column efficiency.

Method of Validation:

The validation of the developed UPLC method was conducted according to specified standard guideline for different parameter as mentioned below:

Specificity:

Specificity was performed by check interference between the chromatogram of placebo solution, standard and sample solutions, the results of same are presented in Figures 3-5.

The specificity study observed no interference at the retention time of Fenofibrate peak in the chromatograms obtained from the placebo solution. The data indicates that the method is specific for its intended use.

Linearity:

The Linearity study was performed within the concentration range from 50% to 150 % (33.5760 to 100.7290 µg/ml) of the working concentration of method. The results are presented in Table 2; which meets the acceptance criteria. The analytical procedure was found to be linear within the specified concentration range for Fenofibrate.

System Precision:

The system precision was assessed from five replicate injections of standard solution from the same vial. The standard solution was prepared and analyzed as per the analytical procedure. The % RSD was calculated as mentioned in Table 3 to evaluate the system precision.

Repeatability study showed % R.S.D of Fenofibrate within the acceptable range, therefore, it can be inferred that the analytical methodology exhibited favourable level of precision in terms of repeatability.

Method Precision:

Method precision was established by performing

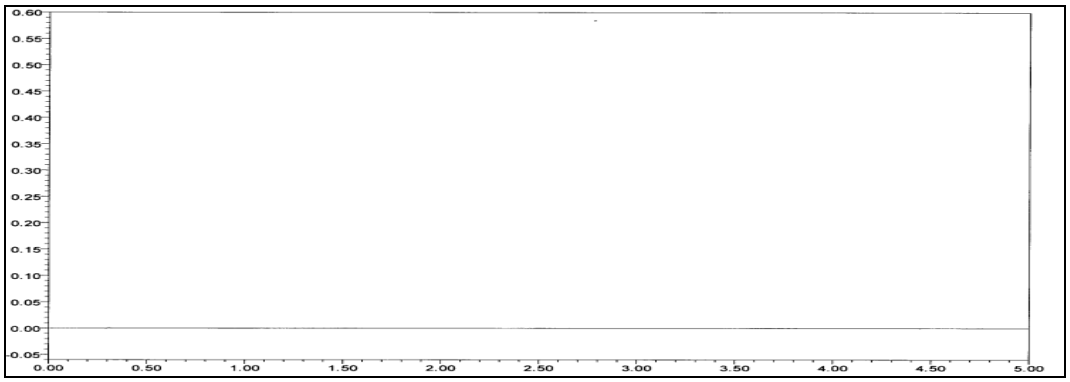

Fig. 3: Chromatogram of Placebo solution.

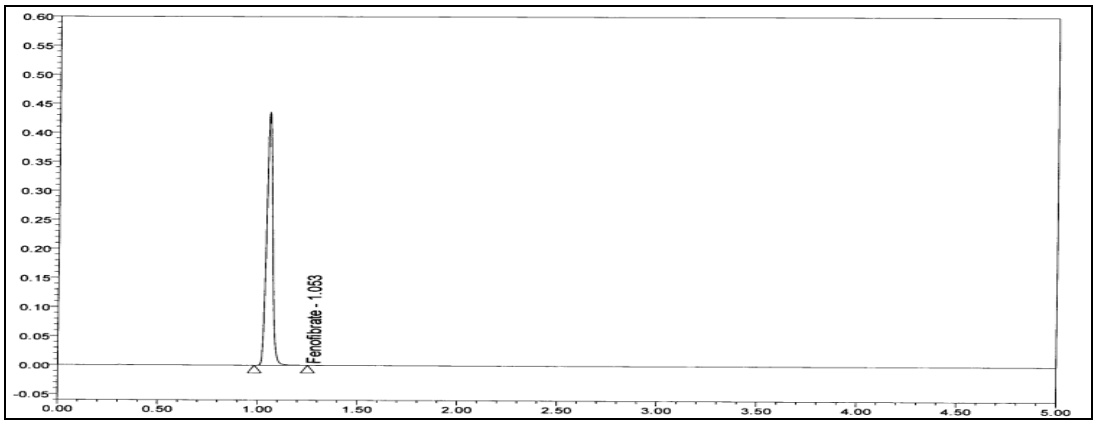

Fig. 4: Chromatogram of Standard Solution.

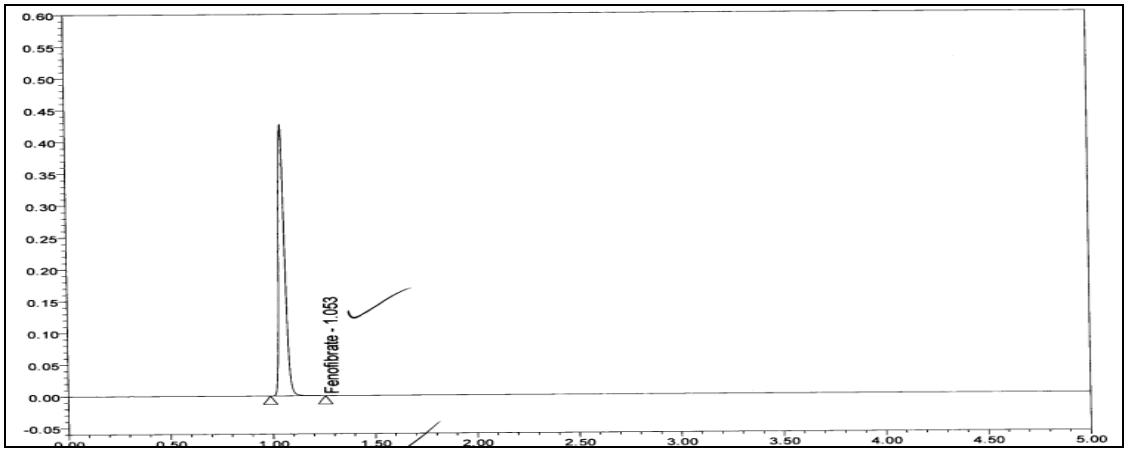

Fig. 5: Chromatogram of Sample Solution.

assay test on six individual capsules of Fenofibrate (Micronized) capsules as per the analytical procedure. The result of assay was summarized in Table 4.

This study observed no significant difference in % assay results, additionally mean % RSD was also found under the range 0.2 %. These findings indicated that the developed analytical procedure was precise for its intended use.

Accuracy:

The recovery samples were prepared in triplicate by spiking Fenofibrate into placebo at 50 %, 100 % and 150 % with respect to working concentration of assay method. The results are presented in Table 5.

Accuracy study determined 99.5 % of average recovery which was found within accepted range

Table 2: Linearity data for drug sample

Linearity Level	Concentration (µg/ml)	Area
50 %	33.5760	449549
80 %	53.7220	720573
100 %	67.1520	906566
120 %	80.5830	1077676
150 %	100.7290	1355824
Correlation Coefficient	0.999	
Slope	13467.9225	
Y-Intercept	-2365.7191	

Table 3: Data of System Precision

Injection	Peak Area
1	905782
2	904295
3	900537
4	903375
5	903030
Mean	903404
% RSD	0.2

Table 4: Data of Method Precision

S. No.	Assay Results (%)
1	98.3
2	98.2
3	97.7
4	98.3
5	98.1
6	98.2
Mean	98.1
% RSD	0.2

Table 5: Results of Accuracy Study

Recovery Level	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean % Recovery	% RSD
50 % Set-1	33.576	33.546	99.9	99.6	0.4
50 % Set-2	33.656	33.345	99.1		
50 % Set-3	33.546	33.482	99.8		
100% Set-1	67.552	67.167	99.4	99.7	0.3
100% Set-2	67.672	67.638	99.9		
100% Set-3	67.572	67.431	99.8		
150% Set-1	100.599	100.038	99.4	99.7	0.3
150% Set-2	100.529	100.425	99.9		
150% Set-3	100.309	99.959	99.7		

Table 6: Results of Robustness Study

Change in Parameters	Value	Mean Results (%)	% Difference
Actual	As Such	98.1	N/A
Flow rate (± 10 %)	0.36 ml/ min	98.3	0.2
	0.44 ml/ min	98.3	0.2
Column Oven Temperature (±5 °C)	Temperature 25°C	98.2	0.1
	Temperature 35°C	98.3	0.2
Mobile Phase Composition	+ 6 ml Buffer	98.0	0.1
	- 6 ml Buffer	98.2	0.1
Buffer pH	+ 0.2 pH	98.3	0.2
	-0.2 pH	98.4	0.3

of mean percentage recovery (98-102%). The values fall within this range, which indicated that the analytical procedure was accurate for its intended use.

Robustness:

Robustness study was performed, by analyzing the standard and test solutions at different conditions. The results obtained with altered conditions were compared against results obtained under normal chromatographic conditions. Robustness was performed by the changes in the different chromatographic condition with respect to normal condition and data are shown in Table 6.

The results were found within the limits under the acceptance criteria during robustness study. The robustness results indicated that the test method was robust enough and not get changed by slight modification in flow rate, column oven temperature, mobile phase composition and buffer pH.

Conclusion

The developed UPLC method for the estimation of Fenofibrate (Micronized) capsules was validated according to standard guidelines. The robustness studies proved that minor changes in the chromatographic conditions did not influence the

outcome of the experiment much, thus validating the stability of the method. The method was found to be specific, linear, accurate, and precise, thus considered reliable for routine quality control analysis. The UPLC method provides an economical, efficient and reproducible analytical tool for the estimation of Fenofibrate in bulk as well as pharmaceutical formulations.

Ethical Statement

Not applicable to this study as no animals were used.

Author Contributions

Each author has equally contributed in planning the study, performing the analysis, writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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