



## Bioanalytical method development and validation of fenofibrate

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Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 30<sup>th</sup> April 2018, revised 18<sup>th</sup> November 2018, accepted 2<sup>nd</sup> February 2019

### Abstract

A selective, rapid, sensitive and Simple HPLC method development and validation was performed for estimating amount of fenofibrate in plasma (human). Ethyl acetate is used for the extraction of drug. At 295nm in U.V. detector on 5 $\mu$ m intensil using 60:40 v/v 20 mm ammonium acetate buffers with initial pH, C18 column (4.6x250mmx5 $\mu$ m), ACN as MP at a flow rate of 1 micro liter/minute chromatographic peaks was separated. From plasma, chromatograms showing excellent resolution and no interference. R.T of Fenofibrate and Nevirapine (internal standard) were approximately 6.6 $\pm$ 0.05 minutes, 5.2 $\pm$ 0.03 min respectively. Above 62% the average recovery from human plasma was determined. According to the concentration range of 0.3 to 20 $\mu$ g/milliliter with coefficient of correlation ( $r^2$ ) 0.9983 this method was linear.

**Keywords:** Fenofibrate, bioanalytical method, LLE, HPLC.

### Introduction

Fenofibrate is a hypolipidemic drug can reduce increased plasma concentration levels of TG'S, LDL and total cholesterol<sup>1-2</sup>. A simple HPLC and UV-Spectrophotometric techniques estimating fenofibrate in bulk drugs, HPLC and TLC-densitometry, TLC-Video densitometry and expensive UPLC-LC-MS/MS methods were developed and validated for predicting the Drug in samples such as plasma, urine, and serum in biological system<sup>3-6</sup>. So far to our present knowledge there is no economical process for quantitative determination of fenofibrate<sup>6-9</sup>.

The work also includes the method development and validation. The formulated approach was originated heaven reliable, exceptive, and also faster as well as genuine than another recorded bio analytical methods<sup>10</sup>.

### Materials and methods

Fenofibrate as well as nevirapine were received from CIPLA Pharmaceuticals, India. Ethyle acetate, methyl alcohol obtained from Qualikems vadora, India. Ammonium acetate was bought from Qualikems, vadora, India. Human plasma absence of drug obtained from kakathiya blood blank hanamkonda.

**Requirements for Chromatography:** Wavelength detected as set at 295nm. The HPLC system comprises of dual  $\lambda$  Absorbance UV detector with Alliance waters 2695, through Shim-pack C18 column (4.6x150mmx5 $\mu$ m) Separation were proceeded using ammonium acetate 60:40v/v as buffer, with flow rate of 1 ml/min through nylon milli pore (0.2 $\mu$ m) membrane filter ACN as MP the mobile phase was filtered, bought through pall life sciences, to be from gas bathsonicator is used. At room temperature 25<sup>o</sup>C chromatography was carried out and column temperature is maintained at 32<sup>o</sup>C.

**Standard solutions preparation:** Standard preparations of fenofibrate (0.5micro gram/micro liter), Nevirapine (1micro gram/micro liter) obtained from methyl alcohol. After in 60% acetonitrile dilutions were prepared. With fenofibrate stock solution by spiked in fresh human plasma to following concentrations of 0.3, 0.6, 1.2, 2.5, 5.0,10, and 20 $\mu$ g/ml freshly for the calibration standards.

**EQA standards:** Lowest, Median and highest QA standards were predisposed by perforating current human plasma mixing with fenofibrate to form solution containing 1, 8, also 16 $\mu$ g/ml correspondingly. They hold at -20<sup>o</sup>C until approach was analyzed.

**Preparation of samples:** To 0.5ml of plasma, 0.05ml of nevirapine (50 $\mu$ g/ml) was combined and mixed. Fenofibrate was separated along with 3ml of ethyl ethanoate and go for 15minutes at a centrifugation rate of 2000 rotations per minute on a cooling centrifuge at 4<sup>o</sup>C. Biological aspect dried and isolated with lyophilizer. 250 $\mu$ l from MP was combined to the residue and relevant test samples infused over column.

**Validation: Study on specificity:** Solution under optimized chromatographic conditions, consisting 0.3 $\mu$ g/ml placed into column to find out the impurities separated from fenofibrate from the human plasma.

**Linearity:** Huge calibration range noticed for concentrations (0.3 $\mu$ g/ml to 20 $\mu$ g/ml) by plotting concentrations versus area under peaks of fenofibrate to nevirapine results were calculated.

**Recovery studies:** Percentage mean of the recovery studies are noticed by calculating results of QC samples of extracted plasma at High quality control, Medium quality control, Lower quality control with Q.C of unextracted samples at High, Middle, Lower quality controls.

**Quantitation limit:** The validation parameter QI was estimated as follows; under observed chromatographic conditions, plasma in absence of drug was extracted and injected with ten consecutive times. After the determination of noise levels, QI for fenofibrate was recorded. (Noise to Signal ratio=10).

**Accuracy and precision:** By analyzing quality control standards (1, 8 and 16µg/ml) accuracy and precision of intraday was determined and LLOQ (0.15µg/micro liters) Quality control standards was done randomly five times a day. By analyzing quality control standards (1, 8 and 16µg/micro liters) interday accuracy and precision was noticed followed by five different days for the study of LLOQC standards (0.3µg/ml).

**Studies on stability:** Overtime to time by measuring the change in concentration of control sample the stability of fenofibrate was determined. In eppendorff tubes at -20<sup>o</sup>c plasma control samples were stored. Plasma quality controls stability was

tested, by placing them and storing them to three freeze thaw cycles at room temperature.

### Results and discussion

Test sample showing fine peaks for drug and internal standard, under employed chromatographic conditions. R.T of neveripine was 5.2±0.03 minutes and R.T of fenofibrate (6.6±0.05 min and (Figure-1). Method was carried out for various validation parameters according to USFDA guidelines, such as accuracy and precision, linearity, specificity and stability.

In this method, peaks at reaction time of drug and internal standard are absent which shows the specificity (Figure-2).

The percentage mean recovery for fenofibrate in Low quality, Medium and High quality controls were 62.7%, 64.2% and 65.3% respectively (Table-1).

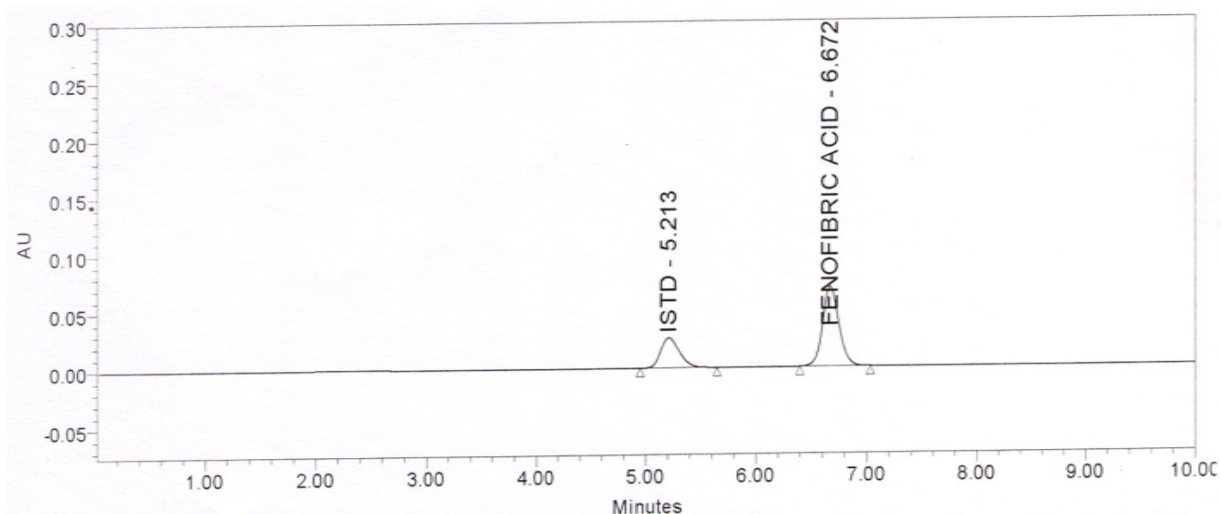


Figure-1: Chromatogram showing retention times of Fenofibrate (5µg/ml) and neveripine (5µg/ml).

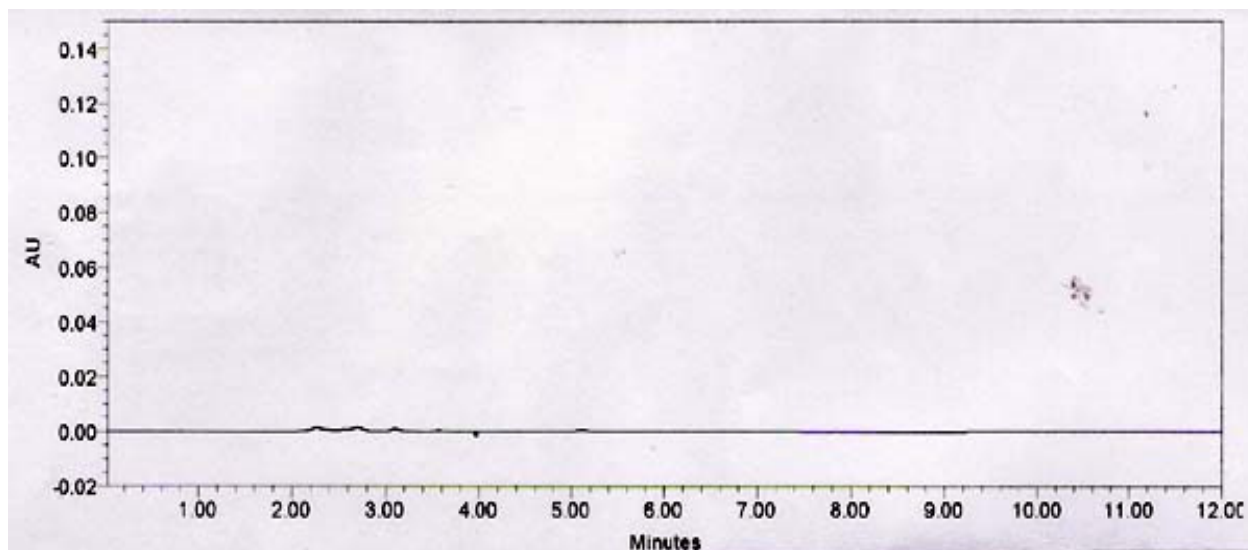
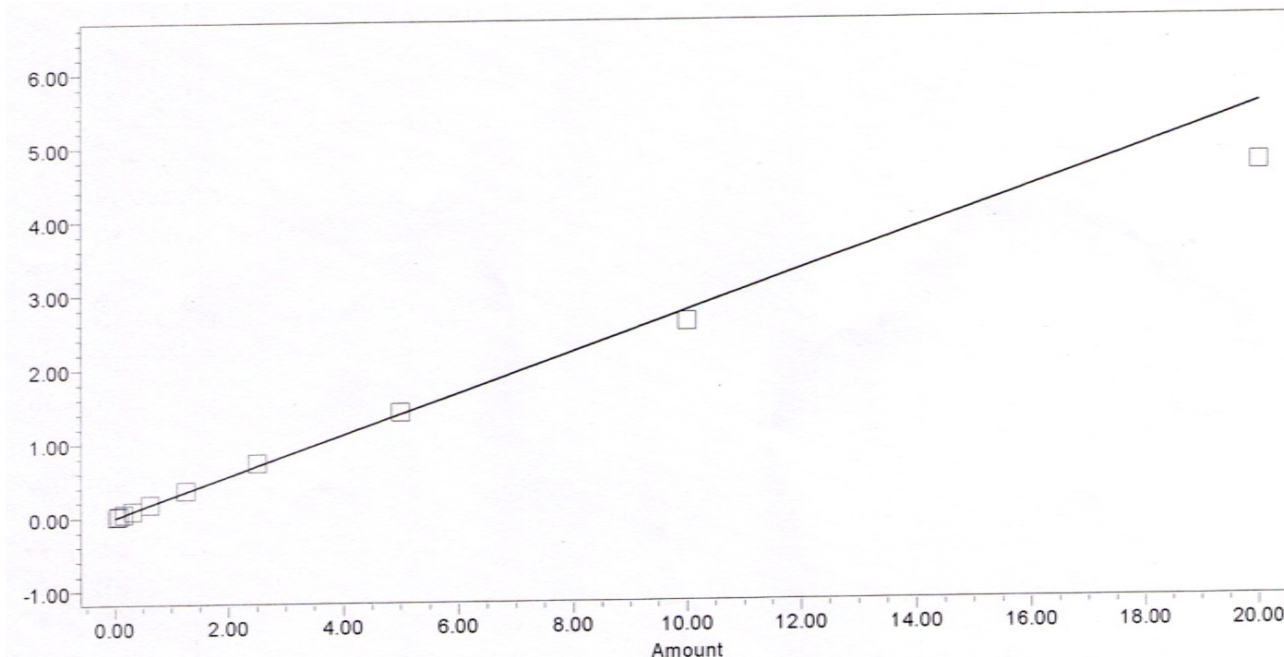


Figure-2: Chromatogram showing blank plasma sample showing with no interference.



**Figure-3:** Spiked concentrations (0.3 to 20µg/milli liters) were plotted against peak area ratio Vs Concentration with eight point calibration used for quantification by linear regression. Analysis equation  $Y=5.32e-0.05x-4.82e-0.003$  is the straight line equation with  $(r^2) = 0.9979$ .

**Table-1:** Recovery-Fenofibrate.

ID	LQC			MQC			HQC		
	Unextracted (area ratio)	Extracted (area ratio)	% Recovery	Unextracted (area ratio)	Extracted (area ratio)	% Recovery	Unextracted (area ratio)	Extracted (area ratio)	% Recovery
1	0.099	0.062	62.63	0.867	0.554	63.90	1.573	1.048	66.62
2	0.098	0.063	64.29	0.867	0.556	64.13	1.572	1.057	67.24
3	0.098	0.064	65.31	0.867	0.549	63.32	1.574	0.993	63.09
4	0.098	0.058	59.18	0.866	0.569	65.70	1.572	0.992	63.10
5	0.098	0.061	62.24	0.867	0.574	66.21	1.569	1.082	68.96
6	0.099	0.062	62.63	0.867	0.542	62.51	1.566	0.992	63.35
Mean	0.098	0.062	62.712	0.867	0.557	64.296	1.571	1.027	65.394
±SD	0.001	0.002	2.092	0.000	0.012	1.410	0.003	0.040	2.546
%CV	0.53	3.35	3.34	0.05	2.17	2.19	0.19	3.89	3.89

The interday and intraday results for quality control samples regarding Accuracy and precision were noticed respectively as 103.12 to 107.3 and 0.25 to 4.85%. The range of acceptable limits of accuracy, precision were found to be (85 to 115%), (15%) respectively (Table-2). The limit of quantitation noticed

as 0.3µg/ml. Inter day accuracy (102.3%), precision (2.85%) are noticed respectively from the above concentration, within a range of limits, accuracy (80 to 120%) and precision is (20%) (Table-3).

**Table-2:** Quality Control Standards of Accuracy and Precision.

Intraday	QC	Lower	Medium	High	Interday	QC	Lower	Medium	High
	conc. (µg/mL)	1	8	16		conc. (µg/mL)	1	8	16
	1	1.062	8.527	17.05		1	1.049	8.176	16.495
	2	1.076	8.447	17.09		2	1.055	8.177	16.481
	3	1.068	8.975	17.075		3	1.05	8.184	16.514
	4	1.073	8.046	17.069		4	1.055	8.181	16.493
	5	1.073	9.131	17.087		5	1.049	8.191	16.508
	6	1.072	8.99	17.066		6	1.053	8.19	16.508
	Mean	1.061	8.588	16.920		Mean	1.052	8.183	16.500
	± SD	0.003	0.416	0.015		± SD	0.003	0.006	0.012
	% CV	0.25	4.85	0.09		% CV	0.27	0.08	0.07
	% Accuracy	106.06	107.35	105.75		% Accuracy	105.18	102.29	103.12

**Table-3:** Accuracy and Precision data.

Quality control	Lower limit QC
Con:mcg/ml	0.3125
1	0.307
2	0.323
3	0.319
4	0.317
5	0.332
AVG	0.3196
±Standard deviation	0.009
%RSD	2.85
% Accuracy	102.3

By using freshly thawed quality control samples Stability was measured by matching against the test samples. For LQC and HQC the percentage mean stability was found to be 97.6 and 109.6%, with a limit of acceptance of 115 to 85%. The Fenofibrate QC plasma samples are showing good stability for one month atleast (Table-4).

**Table-4:** Stability Data.

Freeze – thaw III Cycles		
Quality control	Lower	Higher
conc.(µg/mL)	1	16
1	0.975	17.473
2	0.983	17.516
3	0.983	17.657
4	0.956	17.48
5	0.982	17.473
6	0.98	17.628
Mean	0.977	17.538
± SD	0.010	0.083
% CV	1.07	0.47
% Accuracy	97.65	109.61

Fenofibrate is soluble in methyl alcohol, so the standard solutions were prepared in methyl alcohol. In the mobile phase the proportion of ACN was upgraded up to 60% and MP prepared with buffer ammonium acetate (pH: 4) up to 40%. Not

to effect the reaction time the concentration of ACN and pH was slightly increased and decreased by 2%. Based on liquid-liquid extraction technique the extraction of fenofibrate was done. For recovery studies various solvent systems were utilized. With ethyl acetate the maximum recovery was obtained. For selection as internal standard five drugs were attempted. Under the optimized chromatographic conditions when tried with other drugs the reaction time of fenofibrate was overlapped.

## Conclusion

Estimating fenofibrate quantitatively from plasma the analytical procedure established and validated which is rapid, specific, accurate, precise, simple, sensitive. Hence this procedure was appropriate in finding fenofibrate from biological samples (humans).

## Acknowledgement

My sincere thanks to professors of BIPS, Narsampet, for encouraging and supporting my project work. I also thankful to my and colleagues for their intense help regarding my project work.

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