International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND FENOFIBRATE BY RP-HPLC METHOD IN MARKETED FORMULATION

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ABSTRACT

A new precise, accurate, reliable validated method has been developed by using reverse phase high performance liquid chromatography (RP-HPLC) for the determination of Metformin and Fenofibrate in pharmaceutical dosage form. Chromatographic separation was carried out using mobile phase buffer (0.02M Potassium dihydrogen phosphate): Acetonitrile: Methanol (40:50:10, PH-5.65 adjusted with Orthophosphoric acid) on Hypersil Silica column (250 x 4.6 mm, 5µ) at a flow rate 1.0ml/min at 30°C. Photodiode array is used as detector and Detection was carried out at 225nm wavelength.The retention times for Metformin and Fenofibrate were 7.436 min and 2.999 min respectively. Both the drugs showed good linearity in the range of 250-750 µg/ml for metformin and 80-240 µg/ml for Fenofibrate. The proposed method has been successfully applied to pharmaceutical formulation and was validated according to ICH guidelines. Method showed good precision with %RSD less than 2%. The percentage recovery for Metformin and Fenofibrate was found between 99.39-100.61% and 100.47-101.43% respectively. And it indicates that the proposed method was accurate and precise.

Key Words: Metformin(MET), Fenofibrate(FEB), RP-HPLC , %RSD.

INTRODUCTION

Metformin is chemically1-carbamimidamido-N,N-dimethylmethanimidamide. It is used in the treatment of diabetes mellitus type-2. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by in the treatment of Hyperlipidaemia. Fenofibrate is a fibric acid derivative. It lowers lipid levels by activating Peroxisome proliferator-activated receptor alpha (PPARα) [3-4] increasing peripheral glucose uptake and utilization. [1-2] Fenofibrate(FEB) is chemically propan-2-yl 2-{4-[(4-chlorophenyl)carbonyl]phenoxy}-2-methylpropanoate.

It is used Combination of metformin and fenofibrate is used in the treatment of simple obesity with non-insulin dependent diabetes.

Literature survey revealed that few analytical techniques are available for estimation of MET alone as well as in combine dosage form such as UV ,HPLC, .[11-12] Similarly few analytical methods are available for estimation of FEB alone and its combination with drugs such as UV and HPLC[5-7].

Very few methods are available for metformin and fenofibrate combination[8-12]. keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Metformin and Fenofibrate which would be highly sensitive having good resolution reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines. [12]

MATERIALS AND METHOD

Equipment: Chromatographic separation was performed on HPLC system - Water's alliance 2695 with 2996 module Photo Diode Array (PDA) detector equipped with a solvent delivery pump, automatic sample injector and column thermostats. Waters Empower2 software was applied for data collecting and processing.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was Potassium dihydrogen ortho phosphate. Reference standards Metformin and Fenofibrate were obtained from Aurobindo Pharma Ltd. FEBMET Tablet of MET (500mg) and FEB (160mg) manufactured by Sun Pharmaceuticals Ltd., and procured from local market.

Preparation of standard solutions: Accurately Weighed and transferred 500mg of Metformin and 160mg of Fenofibrate maleate working Standards into a 500 ml clean dry volumetric flask, add 150ml of diluent , sonicated for 30 minutes and filtered through 0.45µm nylon membrane filter and make up to the final volume with diluents. Dilute 1ml of stock solution in 10 ml of diluent to give 1000ppm of metformin and 320 ppm fenofibrate.(Standard Stock).

Preparation of sample solution: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 250mg of Metformin and 80mg of Fenofibrate was transferred into a 500 mL volumetric flask, 300mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Preparation of buffer: Accurately weighed 2.72gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml of Volumetric flask, about 900ml of milli-Q water was added and sonicated to degas and finally make up the volume with water and 0.6ml of Triethylamine was added. Finally pH is adjusted to 5.65 with dilute orthophosphoric acid solution.

Mobile phase: Buffer, Acetonitrile and Methanol taken in the ratio 40:50:10.

Optimized chromatographic conditions:

Flow rate **:** **1.0**ml/min
Column **:** Hypersil Silica, 250 x 4.6 mm, 5µ.
Detector wave length: 225nm
Column temperature: 30°C
Injection volume **:** 10µL
Run time **:** 10 min
Diluent **:** Water: Acetonitrile (50:50)

METHOD VALIDATION

System suitability test: This parameter was evaluated before each stage of validation. Six replication injections of standard preparation were injected. Symmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Linearity: Solutions were prepared containing 250µg/ml, 375µg/ml, 500µg/ml, 625µg/ml, 750µg/ml, concentrations of Metformin and 80µg/ml, 120µg/ml, 160µg/ml, 200µg/ml, 240µg/ml, concentrations of Fenofibrate which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear- regression analysis.

Accuracy: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analyzed sample preparation. For each concentration, three injections were injected.

Precision: Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness: The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition (buffer: acetonitrile by 5%) and temperature variation of 5°C

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ was calculated from linear curve using formulae
LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope
(Where σ = the standard deviation of the response
and S = Slope of calibration curve).

Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both MET and FEB from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of MET and FEB. The mobile phase containing buffer: Acetonitrile: methanol in proportion of 40:50:10v/v was found ideal to resolve the peak of MET and FEB. Retention time of MET and FEB were 7.436 and 2.999min respectively (Figure 1&2). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 250-750µg/ml for MET and 80-240µg/ml for FEN. The data was shown in Table-2 and Figure-3&4. System suitability parameters were evaluated and results shown in (Table-3), which were within acceptance criteria. The mean percentage recovery for MET and FEB was found to be between 99.39-100.61% and 100.47-101.43% respectively, which are within the limits and hence the method was found to be accurate (Table-4). LOD and LOQ values were 1.21µg/ml and 3.67µg/ml for Metformin and 0.394µg/ml and 1.195µg/ml for Fenofibrate (Table-5). Results of intraday and interday precision were shown in the (Table-6a&6b). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase composition. The result obtained implies method is robust for routine qualitative analysis (Table-7).

CONCLUSION

The proposed RP-HPLC method was validated as per International conference on harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of MET and FEB using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

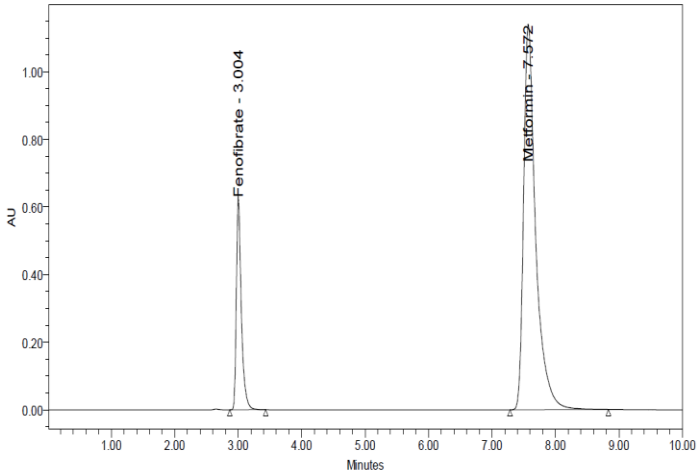

Figure-1: Chromatogram of MET (500µg/ml) and FEB (160µg/ml) standard

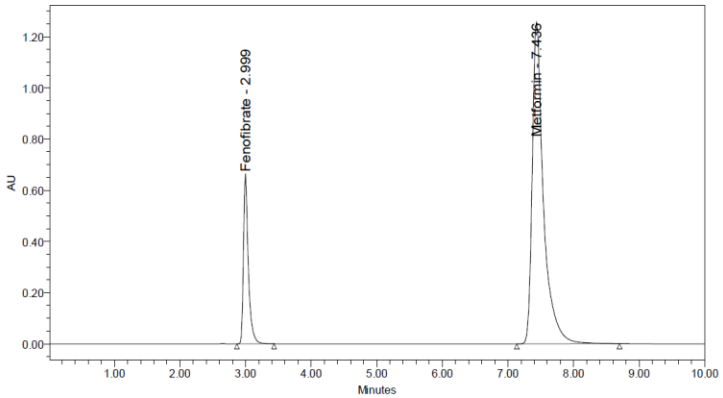

Figure-2: Chromatogram of MET (500µg/ml) and FEB (160µg/ml) sample

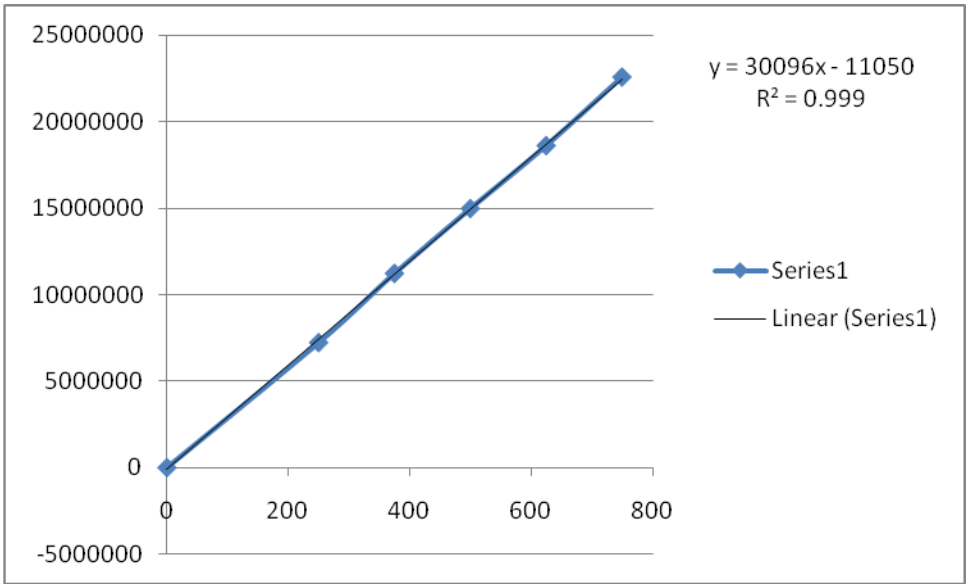

Figure-3: Calibration curve for Metformin

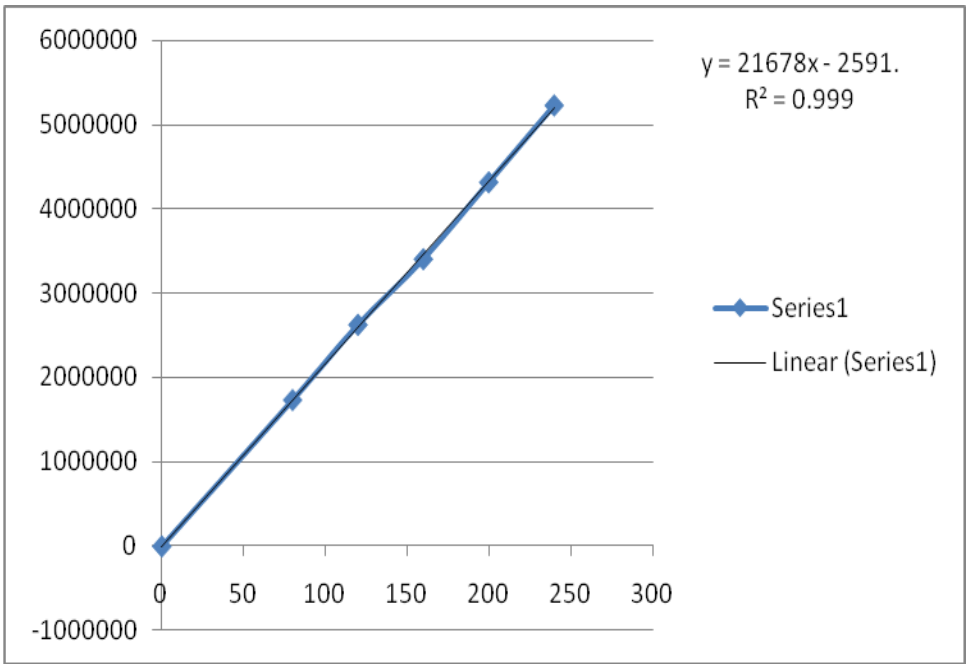

Figure -4: Calibration curve for Fenofibrate

Table -1 Analysis Data of Formulation (FEBMET)

Injection	Label claim(mg)	Assay (%)
MET	500	99.45
FEB	160	99.71

Table – 2: Result of Linearity

S. no	Metformin		Fenofibrate	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	0	0	0	0
2	250	7224702	80	1736339
3	375	11219374	120	2629809
4	500	14965914	160	3408730
5	625	18604613	200	4320220
6	750	22563346	240	5231973

Table-3: System suitability studies

Parameters	Metformin	Fenofibrate	Acceptance criteria
Theoretical plates	9453	8265	Not less than 2000
Tailing factor	1.77	1.53	Not more than 2
%RSD	0.6	0.4	Not more than 2

Table-4: Recovery studies for Metformin and Fenofibrate

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	% RSD
MET	50	250	250.9	100.39	0.57
	100	500	503.05	100.611	0.274
	150	1000	993.97	99.397	0.256
FEB	50	80	80.95	101.194	0.527
	100	160	161.02	100.64	0.25
	150	240	239.28	99.7	0.45

n- Number of replicate injections

Table-5: LOD and LOQ for Metformin and Fenofibrate

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Metformin	1.21	3.67
Fenofibrate	0.394	1.195

Table-6(a): Results of Intraday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
MET	500	16473075	0.3
FEB	160	2612409	0.2

n- Number of replicate injections

Table-6(b): Results of Interday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
MET	500	16618284	1.4
FEB	160	2615656	0.2

Table-7: Results of Robustness study

S. no	Parameter	Condition	Mean Peak area (n=2)		% RSD	
			MET	FEB	MET	FEB
1.	Flow rate	0.9 ml/min	1887628	2945056	0.1	0
		1.1 ml/min	15398696	2400856	0.1	0
2.	Mobile phase	38:52:10 v/v	17131320	2632648	0.1	0
		42:48:10 v/v	17156307	2618372	0.1	0

RSD – relative standard deviation;n- Number of replicate injections

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