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DEVELOPMENT AND VALIDATION OF REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY METHOD FOR ESTIMATION OF FENOFIBRATE IN TABLET DOSAGE FORM PREPARED USING CRYSTALLO-CO-AGGLOMERATES OF THE DRUG

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ABSTRACT

The aim of the present study was to develop a simple isocratic reverse phase-high performance liquid chromatography (RP-HPLC) method and validate for the determination of fenofibrate in tablet dosage forms. RP-HPLC method was developed using Hi Q Sil C_{18} (250 cm × 4.6 mm, 5 µm) and mobile phase comprising 1 mM ammonium acetate buffer: Acetonitrile (10:90 v/v) at a flow rate of 1.0 mL/min. The detection was carried out at 290 nm. The retention time was found to be 6.15 ± 0.03 min. Validation of the method was performed for precision, accuracy, linearity, robustness, specificity and sensitivity to conform to the International Conference on Harmonization (ICH) guidelines. The data of linear regression analysis indicated a good linear response in the concentration range of 5 µg/mL–30 µg/mL with correlation co-efficient (R^2) of 0.997. The developed method was found to be simple, sensitive, accurate and repeatable for assay of tablets of fenofibrate prepared using crystallo-co-agglomerates of the drug.

Keywords: Fenofibrate, Isocratic HPLC, RP-HPLC, Validation

INTRODUCTION

Fenofibrate, 1-methylethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate, is used as antihyperlipidemic drug. Fenofibrate activates lipoprotein lipase, which reduces triglycerides and increases high density lipoprotein cholesterol. It exerts a variable but generally modest low density lipoprotein cholesterol-lowering effect (Gupta *et al.* 2010) (Figure 1).

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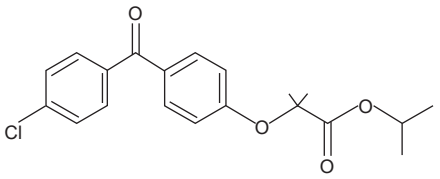

Figure 1: Structure of fenofibrate.

Literature survey revealed that, high performance liquid chromatography (HPLC) (Sahoo, Sahu and Patro 2014), stability indicating HPLC method (Rao *et al*. 2013), UV spectrophotometric (Salama *et al*. 2011) has been reported for fenofibrate estimation alone in pharmaceutical formulation. Also HPLC method has been reported for determination of fenofibrate in human serum (Manish Kumar *et al*. 2011; Zaman *et al*. 2009). Few HPLC methods are published for simultaneous estimation of fenofibrate along with atorvastatin (Deepan *et al.* 2011), rosuvastatin (Thriveni *et al*. 2013; Suresh Kumar and Rajendra Prasad 2010) and ezetimibe (Choudhari and Nikalje 2010). The present study describes the development and validation of a simple, specific, accurate and precise RP-HPLC method for determination of fenofibrate in tablet dosage forms, validated according to the International Conference on Harmonization (ICH) guidelines (ICH 2005).

METHODS

Reagents and Chemicals

Fenofibrate was a kind gift from Lupin Ltd. Polyethylene glycol (PEG 6000), was purchased from Loba Chemie (Mumbai, India). Acetonitrile (HPLC grade) and dichloromethane (DCM) were purchased from Merck Specialities Ltd. HPLC grade water was collected at college using ELGA water purification system. Talc, sodium lauryl sulphate (SLS), starch, PVP K-30, lactose used were purchased from Loba Chemie (Mumbai, India).

Preparation of Optimised Crystallo-Co-Agglomerates (Kadam, Mahadik and Paradkar 1997)

In a crystallisation vessel, fenofibrate was dissolved in required amount of acetone (good solvent) to make saturated solution. This was added to aqueous solution of PEG 6000 (bad solvent), with stirring using a mechanical stirrer (Remi Motors, Mumbai) for 15 min, following which dichloromethane (DCM) was added slowly which acted as bridging liquid. The temperature of the crystallisation system was maintained below 5°C using sodium chloride ice mixture. The stirring was continued to obtain agglomerates, which were then filtered and dried overnight at room temperature.

Preparation of Tablets of Optimised Crystallo-Co-Agglomerates (Patil *et al*. 2011; Dongare, Bhalekar and Gandhi 2017)

All the materials are shown in the formula (Table 1). The material was then mixed by geometric mixing technique. Mixing was continued for about 30 min until a homogenous powder blend was obtained. Lactose was used as diluent, PVP K-30 was used as dry

binder, SLS was used as dispersing agent, talc was used as lubricant and starch as disintegrant. Tablets were prepared by direct compression method using standard 10.5 mm concave punches on rotary tablet compression machine (Rimek Mini Press II MT). All the product and process variables like mixing time and hardness, were kept constant and within permissible limits.

Table 1: Formulae for preparation of tablets.

No.	Ingredient	Amount (mg)
1	Fenofibrate crytalllo-co-agglomerates	80
2	Starch	4.5
3	SLS	3
4	PVP K-30	12
5	Talc	3
6	Lactose	Quantum satis (QS)

Note: #Total weight of the tablets was kept 150 mg.

Selection of Mobile Phase and Chromatographic Conditions

Chromatographic separation studies were carried out on the working standard solution of fenofibrate 10 µg/mL. Initially, trials were carried out using methanol and acetonitrile in various proportions along with buffer of varying pH, to obtain the desired system suitability parameters. Reverse phase (RP)-HPLC method was developed on Jasco HPLC system equipped with UV/VIS detector using Hi Q Sil C18 (250 cm × 4.6 mm, 5 µm) and mobile phase comprising 1 mM ammonium acetate buffer: Acetonitrile (10:90 v/v) at a flow rate of 1.0 mL/min, which gave good resolution and acceptable peak parameters. (Figure 2). The detection was carried out at 290 nm. The retention time was found to be 6.15 ± 0.03 min. The method was validated as per ICH guidelines (ICH 2005).

Preparation of 1 mM Ammonium Acetate Buffer and Mobile Phase (Indian Pharmacopoeia 2007)

Ammonium acetate buffer (1 mM) was prepared by dissolving 7.71 mg of ammonium acetate in 80 mL of HPLC grade water, 0.05 mL acetic acid was added and volume made up to 100 mL with HPLC grade water. Mobile phase was prepared by mixing acetonitrile and 1 mM ammonium acetate buffer in the ratio of 90:10 v/v. It was then filtered through 0.45 µm membrane filter paper using filtration assembly and then sonicated on ultrasonic water bath for 15 min.

Preparation of Standard Stock Solution

Standard stock solution of fenofibrate was prepared by dissolving 10 mg of fenofibrate in 10 mL of acetonitrile to get concentration of 1,000 µg/mL (A). From the standard stock solution, working standard solution was prepared containing 100 µg/mL of fenofibrate in acetonitrile. From this further dilution was made in acetonitrile to get final 10 µg/mL concentration.

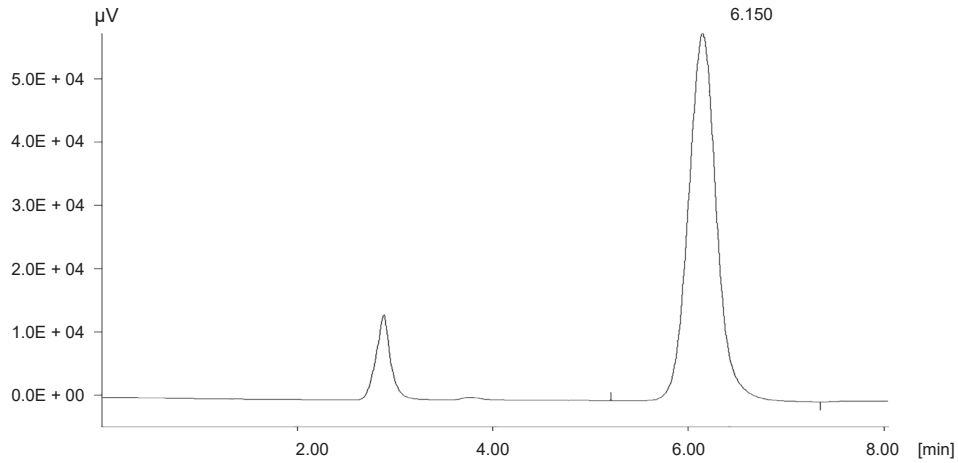

Figure 2: Representative chromatograph of fenofibrate (10 μg/mL) (RT 6.15 ± 0.03 min). The peak at 2.85 is solvent peak.

Preparation of Sample Solution

Twenty tablets each containing 80 mg of fenofibrate were weighed and crushed to powder. Powder equivalent to 10 mg of fenofibrate was transferred to 10 mL volumetric flask and was diluted with acetonitrile, sonicated for 10 min and volume made to 10 mL with acetonitrile. Solution was filtered and further dilutions were made with acetonitrile to get the final concentration of 10 μg/mL of fenofibrate.

Determination of λ_{max} of Fenofibrate by UV Spectroscopy

Fenofibrate 10 mg was accurately weighed and dissolved in 10 mL of methanol to obtain a concentration of 1 mg/mL. The solution was then suitably diluted with distilled water to get a final concentration of 10 μg/mL. UV spectrum was recorded on Jasco double beam UV-Vis spectrophotometer (Model V550) over wavelength range 200 nm–400 nm. The λ_{max} was found to be 290 nm (Figure 3).

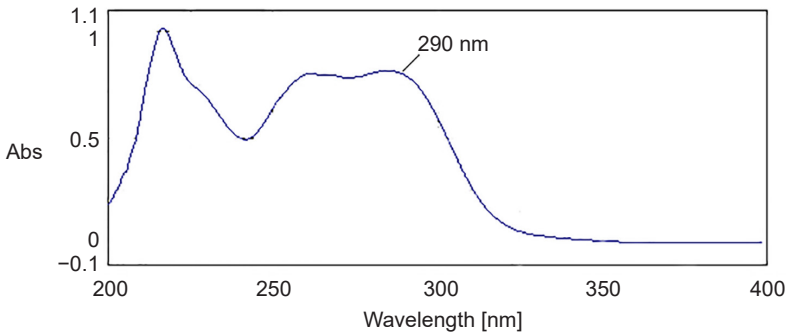

Figure 3: UV spectra of fenofibrate

RESULTS

Validation of Analytical Method (ICH 2005)

Linearity *(ICH 2005)*

From the standard stock solution (1,000 µg/mL), solution was prepared containing 100 µg/mL of fenofibrate with acetonitrile. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analysing five replicates over the concentration range of 5–30 µg/mL. The equation of calibration curve was found to be $y = 126255x - 35592$ with $r^2 = 0.997$. The results obtained are shown in Table 2.

Table 2: Linearity of fenofibrate.

Replicates	Concentrations of fenofibrate					
	5 µg/mL	**10 µg/mL**	**15 µg/mL**	**20 µg/mL**	**25 µg/mL**	**30 µg/mL**
			Peak area			
1	571367.8	1196436.0	1979121.8	2454209.0	3072397.0	3769700.0
2	560655.5	1162149.0	1928560.0	2391534.0	2961390.0	3729161.0
3	586211.9	1160268.0	1990811.0	2380938.0	2942197.0	3643038.0
4	579245.0	115131.0	2003144.6	2454143.8	3022452.0	3797519.0
5	580241.2	1190735.0	1964916.0	2441704.0	3009879.0	3654640.0
Mean	575544.3	1180943.0	1973310.6	2424505.6	3001663.0	3718811.0
SD	9857.4	18151.5	28736.8	35502.7	51626.7	68468.1
RSD (%)	1.712	1.537	1.456	1.46	1.71	1.84

Limit of Detection and Limit of Quantification (ICH 2005)

Limit of detection (LOD) and limit of quantification (LOQ) are calculated from the formula:

$$LOD = 3.3\ \sigma/S \text{ and } LOQ = 10\ \sigma/S$$

Where,
σ = standard deviation of *y* intercept
S = slope of the calibration curve

The LOD and LOQ were found to be 0.14 µg/mL and 0.45 µg/mL, respectively.

Precision (ICH 2005)

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies, 3 × 3 replicates of fenofibrate 10 µg/mL, 20 µg/mL and 25 µg/mL were analysed in a day and percentage relative standard deviation (RSD) was calculated.

For the inter-day variation studies, three different concentrations were analysed on three consecutive days and percentage RSD were calculated. The results obtained were found to be within limits (less than 2% RSD). The results obtained for intra-day and inter-day variations are shown in Tables 3 and 4.

Table 3: Inter-day precision of fenofibrate.

Replicates	Concentration (µg/mL)		
	10	**20**	**25**
1	1165243.5	2346354.2	3013652.1
2	1195324.6	2415642.6	3025479.2
3	1173986.3	2339546.0	2993146.4
Mean	1178184.8	2367180.9	3010759.2
SD	15473.8	42106.9	16359.4
RSD (%)	1.313	1.778	0.543

Table 4: Intra-day precision of fenofibrate.

Replicates	Concentration (µg/mL)		
	10	**20**	**25**
1	1175487.3	2435976.3	2986571.1
2	1165897.0	2395687.1	2965324.9
3	1145678.3	2401597.8	3004213.2
Mean	1162354.2	2411087.1	2985369.7
SD	15217.0	21756.4	19472.0
RSD (%)	1.309	0.902	0.652

Specificity (ICH 2005)

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Assay (ICH 2005)

Tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded for each drug. Concentration and % purity was determined from linear equation. The results obtained are shown in Table 5.

Table 5: Assay of fenofibrate tablets.

Replicates	Peak area	Amount recovered (µg/mL)	Recovery (%)
1	1210805	9.872	98.720
2	1207011	9.842	98.420
3	1206077	9.834	98.350
4	1197074	9.763	97.633
5	1183358	9.654	96.550
6	1208848	9.856	98.565
Mean	1202196	9.804	98.039
SD	10370.11	0.082	0.821
RSD (%)	0.862598	0.840	0.838

Accuracy (ICH 2005)

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablets sample solution, at three different levels around 50%, 100% and 150%. Basic concentration of sample solution chosen was 10 µg/mL of fenofibrate. Percentages of recovery was determined from linearity equation. The results obtained are shown in Table 6.

Table 6: Recovery studies of fenofibrate.

Level	Sample	Standard	Area	Amount recovered	Recovery (%)	RSD (%)
50%	10	5	1911099	15.41	102.79	1.51
			1878130	15.15	101.05	
			1853378	14.96	99.74	
	Mean		1880869	15.17	101.19	
100%	10	10	2460681	19.77	98.85	0.20
			2451241	19.69	98.48	
			2453196	19.71	98.56	
	Mean		2455039	19.72	98.63	
150%	10	15	3066535	24.57	98.28	1.44
			3096392	24.80	99.22	
			3156123	25.27	101.11	
	Mean		3106350	24.88	99.54	

Robustness (ICH 2005)

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, flow rate were altered and the effect on the area were noted. The results obtained are shown in Table 7.

Table 7: Robustness study.

	Detection wavelength ± 2 nm			**Flow rate ± 0.05 mL/min**		
Fenofibrate	288	290	292	0.95	1.00	1.05
RSD (%) values	0.53	0.96	1.26	0.78	0.96	1.33

DISCUSSION

The developed, method was found to be simple, sensitive, accurate and precise for assay of tablets of fenofibrate prepared using crystallo-co-agglomerates of the drug. Validation of the method was performed for precision, accuracy, linearity, robustness, specificity and sensitivity to conform to the ICH guidelines for validation of an analytical method. One paper is available in literature for RP-HPLC method (Sahoo, Sahu and Patro 2014). In work the retention time was found to be 19.26 min while in present work is was found to be 6.15, thus, the present method is time saving. The summary of all validation parameters is given in Table 8.

Table 8: Summary of validation study by HPLC method.

No.	**Validation parameter**	**Results**
1	Linearity	y = 126255x − 35592 r^2 = 0.9971
2	Range	5–30 µg/mL
3	Precision	RSD (%)
	Intra-day precision	0.95
	Inter-day precision	1.21
4	Limit of detection	2.62 µg/mL
5	Limit of quantitation	7.96 µg/mL
6	Assay	98.03
7	Accuracy	99.79
8	Robustness	Robust
9	Specificity	Specific

CONCLUSION

The developed RP-HPLC method was found to be simple, precise, accurate, linear, robust, specific and sensitive, and can be successfully used for estimation of fenofibrate in tablet dosage form in routine analysis.

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