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STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF FENOFIBRATE IN THE BULK DRUG AND IN A PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A novel stability-indicating RP-HPLC method has been developed and validated for quantitative analysis of fenofibrate in the bulk drug and in a pharmaceutical dosage form. Use of a 250 mm × 4.6 mm, 5-µm particle, C18 column with 95:05%v/v acetonitrile and acetate buffer pH 5.0 as isocratic mobile phase enabled separation of the drug from its degradation products. UV detection was performed at 268 nm. The method was validated for linearity, accuracy (recovery), precision, specificity, and robustness. The linearity of the method was satisfactory over the range 10–50 µgmL^{-1}(correlation coefficient 0.9997). The limits of detection and quantification were 0.011 and 0.043 µg mL^{-1}, respectively. Recovery of fenofibrate from the pharmaceutical dosage form ranged from 100.6 to 104.1%. Fenofibrate was subjected to stress conditions (hydrolysis (acid, base), oxidation, photolysis, and thermal degradation) and the samples were analyzed by this method. The substance was unstable in basic conditions. The drug was stable under the other stress conditions investigated. The degradation products were well resolved from main peak. The forced degradation study prove the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of fenofibrate as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Keywords: *Fenofibrate; RP-HPLC; Forced degradation; Method validation.*

INTRODUCTION

Fenofibrate 1-Methylethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate is the lipid lowering agent. Active metabolites of fenofibrate, fenofibric acid lowers plasma triglycerides by inhibiting triglyceride synthesis, resulting in the reduction of VLDL. Also, stimulates the catabolism of VLDL[1]. It reduces serum uric acid levels by increasing the urinary excretion of uric acid. Fenofibrate is official in USP[2] and BP[3].

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, light, and enables storage conditions, retest periods and shelf life to be recommended[4,5]. The two main aspects of study of the stability of a drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies. Assay of a drug product in a stability test sample must be performed with stability-indicating method, as recommended by the international Conference on Harmonization (ICH)[6]. The objective of this work was to develop a simple, precise and rapid analytical LC procedure which would serve as stability-indicating method for analysis of a dosage form of fenofibrate. A literature survey revealed that some HPLC methods have been reported for analysis of fenofibrate and its hydrolyzed metabolites in human plasma and urine [7-9]. Several HPLC and NMR methods have been developed to measure the purity of fenofibrate pure and combination with other drugs [10, 11]. None of the reported procedures enables analysis of the fenofibrate in pharmaceutical dosage forms in the presence of their degradation products. This manuscript describes the development and validation, in accordance with ICH guidelines [12], of a rapid, economical, precise and accurate stability-indicating isocratic reversed phase HPLC method for analysis of fenofibrate in the presence of its degradation products. This paper mainly deals with the forced degradation of fenofibrate under the stress conditions such as acidic and basic hydrolysis, oxidation, heat, and light and validation of the method for accurate quantification of fenofibrate in the bulk drug and solid dosage form.

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EXPERIMENTAL

Chemicals and Solutions

Fenofibrate bulk drug (purity 99.9%) was obtained from Plethico Pharma, Indore (M.P.) and Tablet finolip (200 mg) were obtained from Cipla, Goa, India. Glacial acetic acid and acetonitrile (HPLC grade), anhydrous sodium acetate, sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were obtained from Merck Fine Chemicals Mumbai, India. Double HPLC grade water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade.

Standard stock solution (1 mg mL^{-1}) of fenofibrate was prepared by dissolving the working standard in 80% methanol and diluting with the same solvent. Standard calibration solutions (10–50 µg mL^{-1}) for assessment of linearity were prepared from this stock solution by dilution with methanol.

Chromatography

HPLC was performed with LC- 10ATVP Shimadzu equipped with binary solvent manager, multiple wavelengths UV-PDA detectors operated at 268 nm. HPLC column incorporated Solvent delivery model LC-10ATVP. Compounds were separated on a HPLC column is Inertsil (250mm x 4.60mm), partical size 5µ and have guard column Phenomenex security (universal fit). The HPLC was connected to personal computer with Class-LC10/M10A software. The chromatographic separation was performed using the isocratic mobile phase consisted of acetonitrile and acetate buffer pH 5.0 in the ratio of 95:05%v/v and was delivered at a flow rate of 0. 5 ml min^{-1}. Before use it was filtered through a 0.45-µm Nylon filter and degassed in an ultrasonic bath. The injection volume was 20µL and detection was carried out using a UV-PDA detector at 268 nm. Peak homogeneity was expressed as peak purity and was obtained directly from the spectral analysis report obtained by use of the LC10/M10A software.

Analysis of Dosage Forms

Twenty tablets were weighed and their mean weight was determined. Weight equivalent to 50mg of fenofibrate was dissolved in 50ml of diluent and then sonicated for 30 min. and filtered through watmann paper no. 41. The filtrate was appropriately diluted to get concentration of 20, 30, 40µg/ml and analyzed.

Forced Degradation Study

To study the effect of acid, accurately weighed about 100 mg fenofibrate WS was dissolved in 80 ml methanol (HPLC) and volume was made upto 100 ml with 5N HCl to gets a concentration of 1000 µg mL^{-1} in 1N 80 % methanolic HCl and kept on water bath at 60°C for 300min. Aliquots of above solution was neutralized with 1 N NaOH and diluted with diluent to get 25 µg mL^{-1} solution. The sample solution was analyzed and chromatogram was recorded.

To study the effect of alkali, accurately weighed 100 mg of fenofibrate WS was dissolved in 80 ml methanol and volume made up to 100 ml with 1N sodium hydroxide to achieve the solution of 1000 µg mL^{-1} in 0.2 N methanolic NaOH. The above mixture was kept on a boiling water bath (temp. 55°C-60°C) for 180 min. and aliquot were withdrawn time to time, to check degradation.

To study the effect of oxidizing conditions, accurately weighed about 100mg fenofibrate WS was dissolved in 80 ml methanol (HPLC) and volume was made up to 100ml with 50% hydrogen peroxide to achieve a solution of 1000 µg mL^{-1} in 10 % hydrogen peroxide and kept on water bath at 70°C for 180min. Aliquots of above solution was neutralized with 0.2 N NaOH/0.2N HCl and diluted with diluent to get 25µg mL^{-1} solution. The sample solution was analyzed and chromatogram was recorded.

To study the effect of temperature, accurately weighed about 1.0 g fenofibrate WS was kept at 50°C in oven for 3 days. Sample equivalent to 10 mg of drug was withdrawn after every 24 hour and diluted as per the procedure. Aliquots of 25µg mL^{-1} concentration were prepared and chromatogram was recorded,

To study the effect of UV light, accurately weighed about 1.0 g fenofibrate WS was exposed to short and long wavelength UV light (222 and 366 nm, respectively) for 48 h, Sample equivalent to 10 mg of drug was withdrawn after every 24 hour and diluted as per the procedure. Aliquots of 25 µg mL^{-1} concentration were prepared and chromatogram was recorded.

METHOD VALIDATION

The method was validated for specificity, linearity, limits of detection (LOD) and quantification (LOQ), system suitability, precision, accuracy, robustness and stability in accordance with ICH guidelines. To assess specificity, peak purity was determined by use of the photodiode-array detector.

To test linearity, test solutions of fenofibrate were prepared at six concentrations (10–50µg mL^{-1}. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration. Linearity was checked over the same concentration range on three consecutive days. RSD (%) of the slope and Y-intercept of the calibration plot were also calculated.

The limits of detection (LOD) and quantification (LOQ) for fenofibrate were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration.

Precision, as RSD (%) was determined by measuring the concentration of drug in the tablets six times. Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory.

The accuracy of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100, and 120% of the label claim of 200 mg fenofibrate per tablet) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot. The robustness of the method was assessed by deliberate alteration of the experimental conditions and determining the effect on resolution of fenofibrate from the main product obtained by degradation under basic conditions. The change was made in the ratio of mobile phase, Instead of acetonitrile: acetate buffer pH 5.0 (95:05%V/V), acetonitrile: acetate buffer pH 5.0 (82:18%V/V) was used as a mobile phase. During these tests all other conditions were held constant at the optimum values.

The stability of fenofibrate and sample solutions (at ambient temperature) were tested by analysis after 24, 48, and 72 h, comparison of the results with those obtained from freshly prepared standard solutions, and calculation of RSD.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The primary objective in developing this stability-indicating HPLC method was to achieve resolution between fenofibrate and its degradation products. To achieve this, Shimadzu-LC-10AT$_{VP}$ with SPD-M10A$_{VP}$ detector and C_{18} column was employed for envisaged work. Combination of acetonitrile: acetate buffer (95:05) as mobile phase was attempted for quantitation of fenofibrate with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 268nm as detection wavelength. Linearity was found 0-50 μg mL^{-1} at 10.5 + 0.5 min with correlation coefficient r^2 = 0.9997 having equation as: AUC = 106209 Conc. + 46454.1. The column temperature was 25°C. The tailing factor for fenofibrate was <2 and retention times were approximately 10.5 + 0.5 min for main peak and less than 10 min for the degradation products. This low total run time resulted in high productivity and low cost of analysis as per sample.

Forced Degradation Study

Singh and Bakshi [13] suggested target degradation of 20–80% when establishing the stability-indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for conditions other than exposure to acid, base and oxidising agent, even after long exposure.

Peak purity test results confirmed the fenofibrate peak was homogeneous under all the stress conditions tested. The mass balance of fenofibrate in stress samples was close to 100% and, moreover, assay of unaffected fenofibrate in the tablets confirmed the stability-indicating nature of the method. The results from forced degradation studies are summarised in Table 1.

Table 1: *Results from analysis of samples from the forced degradation study, showing Percentage degradation and peak purity of fenofibrate*

Stress conditions and duration	Degradation (%)	Peak purity*
Acidic/80 % methanolic 1N, 2N, 5N HCl /60°C/6 h	0	99.911
Basic/80% methanolic 0.2N NaOH /55 °C /3 h	87.46	99.931
Oxidising/30% H_2O_2/60°C/6 h	0	99.946
Thermal/80°C/48 h	0	99.912
UV light/222 nm/48 h	0	99.928
UV light/366 nm/48 h	0	99.971

**Peak purity values in the range of 99–100 indicate a homogeneous peak*

Chromatographic peak-purity data were obtained from the spectral analysis report; peak purity greater than 99 is indicative of a homogeneous peak. The peak purity for fenofibrate from degradation studies was in the range 99.9–100.0, indicating homogeneous peaks and thus establishing the specificity of the method. Chromatograms from the solutions obtained after degradation under acidic, basis and oxidising conditions are shown in Fig 1-3 respectively. No peaks co-eluted with the fenofibrate peak, suggesting the method enabled specific analysis of fenofibrate in the presence of its degradation products.

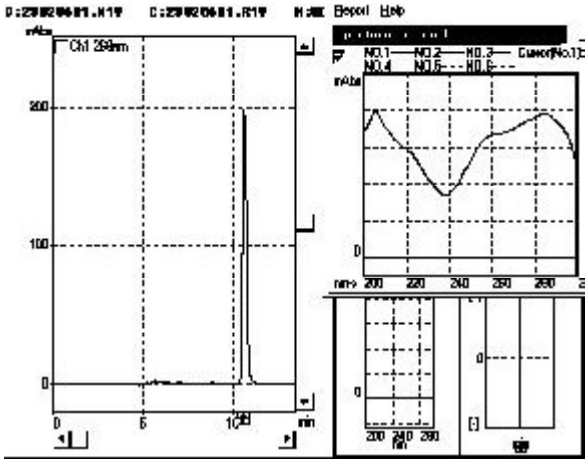

Fig.1: *Typical chromatogram obtained after degradation of fenofibrate under acidic conditions sidewise UV spectra of fenofibrate*

METHOD VALIDATION

Peak purity was >99.9% for drug substance and drug degradation products at 268 nm, which shows the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks. LOD and LOQ for fenofibrate were 0.011 and 0.043 μg mL^{-1}, respectively, for 20 μL injection volume. Results from

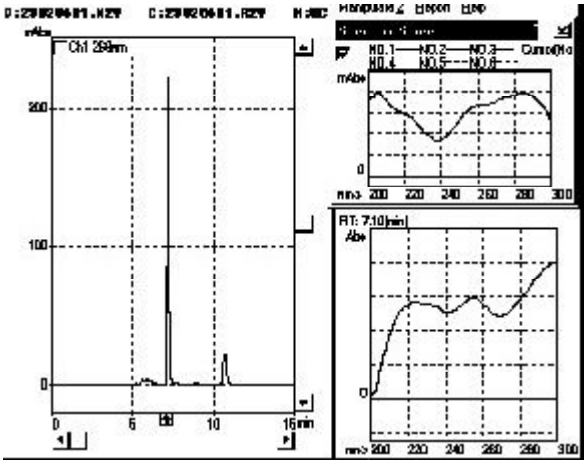

Fig.2: *Typical chromatogram obtained after degradation of fenofibrate under basic condition, sidewise UV spectra of fenofibrate (Upper) and spectra of degradent (lower)*

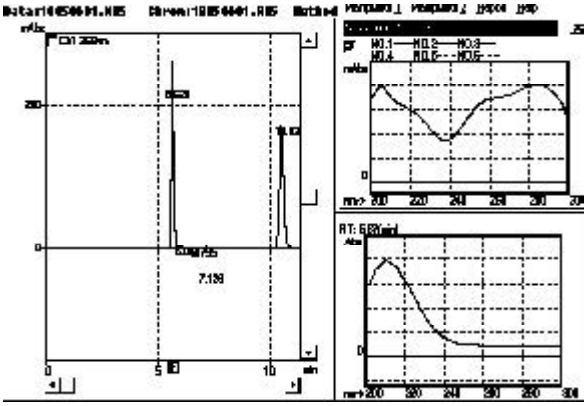

Fig.3: *Typical chromatogram obtained after degradation of fenofibrate under oxidising conditions sidewise UV spectra of fenofibrate(Upper) and spectra of H_2O_2 (lower)*

regression analysis are listed in Table 2, with system-suitability data. When precision was determined by six fold analysis of drug tablets, the RSD of fenofibrate peak area was less then 2%, indicating that the method is reliable. Results from assessment of precision are listed in Table 3. Results obtained from determination of recovery are listed in Table 4 and Results from robustness testing are shown in Table 5.

CONCLUSION

The method developed for quantitative analysis of fenofibrate is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. In other words it can be mentioned that the method developed can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The method was completely validated and satisfactory results were obtained for all the characteristics tested. The method is stability-indicating and can be used to assess the stability of fenofibrate in the bulk drug.

The method can be conveniently used for routine analysis of fenofibrate as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Table 2: *Results from regression analysis and system-suitability data*

Parameter	Fenofibrate
Retention time (min)*	10.5 ± 0.5 min
Tailing factor *	1.24
Theoretical plates *	14127
Linear range (µg mL^{-1})	0-50
Limit of Quantification (µg mL^{-1})	0.043
Limit of detection (µg mL^{-1})	0.011
Linear equation	106209 Conc.+ 46454.1
Slope	106209
Intercept	46454.1
Correlation coefficient	0.9997
S.D. of Slope	972.4
%R.S.D. of Slope	0.091
S.D. of Intercept	14988
%R.S.D. of Intercept	0.322

**Mean of six readings*

Table 3. *Result of precision of test method of fenofibrate*

Std. Conc. (µg/ml)	Repeatability	Intermediate Precision	
		Day To Day	Analyst To Analyst
10	101.06	103.9	99.00
20	102.70	100.9	99.26
30	101.00	99.5	100.00
40	100.50	100.5	100.19
50	100.70	100.2	99.80
Mean	101.2	101	99.65
S.D.	0.8731	1.70	0.503
%R.S.D.	0.86	1.68	0.5

Table 4: *Recovery of fenofibrate.*

Level of addition	Std. drug sol. added (µg/ml)	% Mean recovered
80	10	104.8
100	20	101.1
120	30	100.7

Table 5: *Results from robustness testing.*

Parameter	Result
% Mean*	100.5
S.D.	0.916
%R.S.D.	0.91

**mean obtained at three concentrations and three replicate*

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