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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Bezafibrate in Tablet Dosage Form

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ABSTRACT:

A stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of Bezafibrate, a drug used in the treatment of Hyperlipidemia. The desired chromatographic separation was achieved on a ZORBAX XDB C-18 (150*4.6)5 μ column, using isocratic elution at a 228 nm detector wavelength. The optimized mobile phase consisted of a ACN: WATER (60:40v/v, pH 2.8 adjusted with 10% *o*-phosphoric acid) as solvent. The flow rate was 1.5 mL/min and the retention time of Bezafibrate was 5.55 min. The linearity for Bezafibrate was in the range of 5-40 μ g/mL. Recovery for Bezafibrate was calculated & stability-indicating capability was established by forced degradation experiments. The developed RP-HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines. This validated method was applied for the estimation of Bezafibrate in commercially available tablets.

KEYWORDS: Bezafibrate, method validation, forced degradation, RP-HPLC, Hyperlipidemia

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INTRODUCTION:

Bezafibrate (figure-1) is a Lipid lowering agent used in the treatment of Hyperlipidemia¹⁻⁸. Bezafibrate was approved for the treatment of Hyperlipidemia as 200 mg and 400 mg tablets for oral administration. Few methods have been reported for the quantitative determination of Bezafibrate in tablet formulations by high performance liquid chromatography (HPLC) and spectrophotometric procedures. There is no official and reported stability indicating RP-HPLC method was found for estimation of Bezafibrate in tablet dosage form. The proposed assay is able to separate Bezafibrate from tablet ingredients and from unknown degradation products within 10 minutes. This assay was validated according to the International Conference on Harmonization (ICH) guidelines.⁸⁻¹²

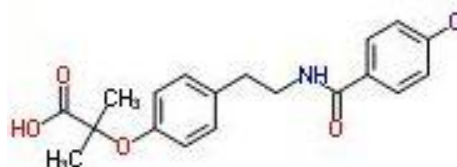


Figure-1 structure of bezafibrate

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Materials and Methods

Materials and reagents

Analytical pure Bezafibrate was procured from AHPL & HPLC grade methanol, Acetonitrile, water (Merck Laboratory, India) were used for the preparation of the

mobile phase. Bezalip-400 tablet (containing 400 mg Bezafibrate per tablet) was purchased from local pharmacy.

Chromatographic conditions

Chromatographic separations was achieved by using an thermo scientific product HPLC system comprising a pump, auto sampler, thermo stated column compartment, uv detector. The column compartment temperature to 40 °C; 20 µL of sample was injected into the HPLC system.⁸⁻¹⁶ Separations were performed on the reversed-phase column (ZORBAX XDB C-18 (150*4.6)5µ. The isocratic mobile phase consisted ACN: WATER (60:40, v/v, pH 2.8 adjusted with o-phosphoric acid) the mobile phase was delivered at a flow rate of 1.5 mL/minute (min). Eluate was monitored at 228 nm.

❖ Preparation of Standard stock solution

Take 10 mg of bezafibrate standard in 10 ml volumetric flask and make up the volume with methanol (1000µg/ml). Pipette out 1 ml of this solution in 10 ml volumetric flask and make the volume with methanol and sonicate it for 15 minute (100µg/ml) which was used as working standard solution.

❖ Preparation of test solution bezafibrate (10 µg/ml):

An accurately weighed tablet powder equivalent to 100 mg of bezafibrate was transferred in to 100 ml volumetric flask. To this 70 ml of methanol was added and sonicated for 15 min. Volume was made up to the mark with methanol further take 1 ml of this solution in 100 ml volumetric flask and make the volume with methanol then solution was filtered through whatman filter paper no.41. Then the sample solution was filtered through 0.45 µm cellulose acetate filter paper (0.45 µm).

Forced degradation study¹⁷⁻¹⁹

Control samples were used in each condition. A minimum 10% degradation of the initial concentration remaining was considered to indicate a significant loss in terms of stability.

❖ Acid degradation

Accurately measured 1 ml of bezafibrate (10 µg/ml) was transferred into 10 ml volumetric flask and add 1 ml of 0.1N HCl solution and heated for 2 hours at 70° C for acid hydrolysis. Then neutralize with 0.1N NaOH and Filtered through 0.45 µm membrane filter paper and volume was made up to the mark with methanol Injected in to HPLC system.

❖ Base degradation

Accurately measured 1 ml of bezafibrate (10 µg/ml) was transferred into 10 ml volumetric flask and add 1 ml of 0.1N NaOH solution and heated for 2 hours at 70° C for Base acid hydrolysis. Then neutralize with 0.1N HCl and Filtered through 0.45 µm membrane filter paper and and volume was made up to the mark with methanol and injected in to HPLC system.

❖ Oxidative hydrolysis:

Accurately measured 1 ml of bezafibrate (100 µg/ml) was transferred in to10 ml volumetric flask and add 1 ml with 3% H₂O₂ heated for 2 hours at 70° C for Oxidative hydrolysis and volume was made up to the mark with methanol. Filtered through 0.45 µm membrane filter paper and injected into HPLC system.

❖ Thermal Degradation

For dry heat degradation study, powder of bezafibrate was spread over petri dish and exposed to dry heat (80°C) for 2 hour in an oven then from that powder an accurately weighed quantity of 100 mg bezafibrate was transferred to 100 ml volumetric flask and dissolved in 30 ml of methanol. The flask was sonicated for 5 min and volume was made up to mark with methanol to get 100 µg/ml of bezafibrate. Further dilution was carried out by diluting 1 ml of solution in 10 ml volumetric flask with mobile phase to get 10 µg/ml of bezafibrate.

• METHOD VALIDATION²⁰⁻²²

Calibration curve of standard of bezafibrate

A calibration curve was plotted over a concentration range of 5-40 µg/ml for bezafibrate. Accurately measured working standard stock solution of bezafibrate (0.5,1, 1.5, 2, 3, and 4 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask made up to the mark with methanol to get concentration range of 5-40 µg/ml for bezafibrate. The resulting solution was injected into the column and the peak area obtained at 5.55 minute at flow rate 1.5 ml/min were measured at 228 nm for bezafibrate. Calibration curve was constructed by plotting peak area versus concentration at 228 nm.

A. Linearity

The linear response of bezafibrate was determined by analyzing six independent levels of the calibration curve in the range of 5-40 µg/ml for bezafibrate.

B. Precision

It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

1) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of method was performed by preparing the test solution of bezafibrate (10µg/ml) for six times from tablet dosage form and analyzed as per the proposed method. Percentage relative standard deviation (RSD) should be less than 2%.

2) Intermediate Precision

It expresses within laboratory variations as on different days analysis or equipment within the laboratory

a. Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision for HPLC method was determined for three concentration of Bezafibrate solution for the three times on the same day.

b. Inter-day precision

Variation of results amongst days called Inter-day precision. The Inter-day precision for HPLC method was determined for Bezafibrate for three days.

Accuracy

Accuracy of the method was confirmed by recovery study from tablet sample solution at 3 level of standard Addition (50%, 100%, and 150%) of targeted solution (10µg/ml Bezafibrate) in triplicate.

D. Limit of Detection & Limit of Quantification

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions.

Limit of detection can be calculated using following equation as per ICH guidelines

$$LOD = \frac{3.3 \sigma}{S}$$

Where, σ =Standard deviation of the y intercept
S = Slope of the calibration curve

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guideline.

$$LOQ = \frac{10 \sigma}{S}$$

Where, σ = Standard deviation of the y intercept
S = Slope of the calibration curve

E. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was studied by changing the flow rate; change in wavelength, change in pH, and composition of mobile phase and change in response of drugs were studied.

F. Application of proposed Method to the pharmaceutical dosage form

The prepared sample solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.5 ml/min. From the peak area obtained in the chromatogram, the amounts of the drug was calculated.

Results and Discussion:

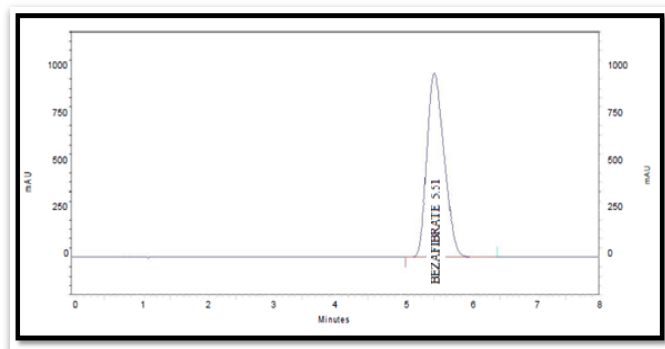


Figure-2 Chromatogram of Std Solution Of Bezafibrate (10 µg/ml)

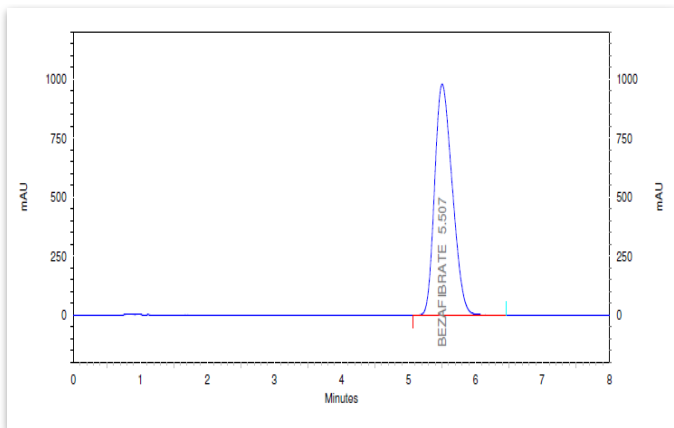


Figure-3 Chromatogram of test Solution of Bezafibrate (10 µg/ml)

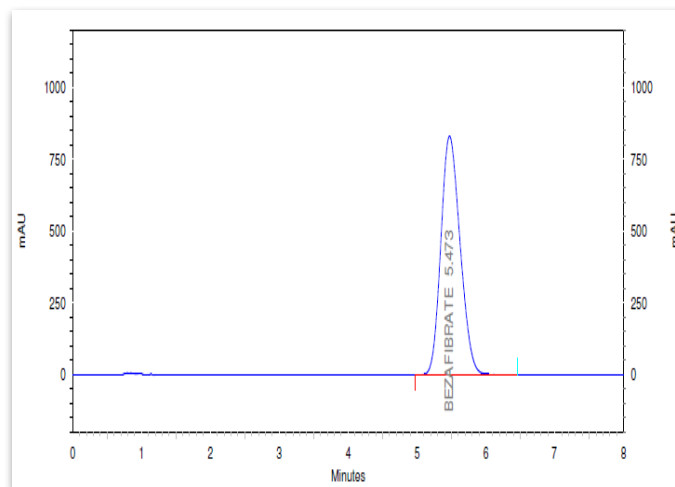


Figure-5 Chromatogram of Bezafibrate under base Degradation

Forced degradation study

Forced degradation studies were performed to demonstrate selectivity and stability-indicating capability of the proposed RP-HPLC method

Result of Forced degradation study of Bezafibrate

Table No.-1 Result of Stability study of Bezafibrate

Sr No.	Condition of forced degradation	%Degradation of Bezafibrate
1	0.1 N HCL, 2 hr , 70 °C	15.71
2	0.1 N NaOH , 2 hr , 70 °C	13.23
3	3% H ₂ O ₂ , 2 hr , 70 °C	16.11
4	Thermal,80° C, 2 hours	23.84

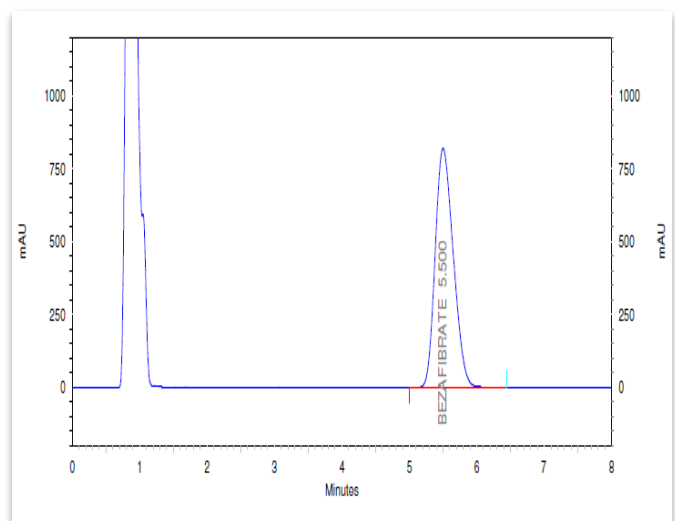


Figure-6 Chromatogram Of Bezafibrate Under H₂O₂ Degradation

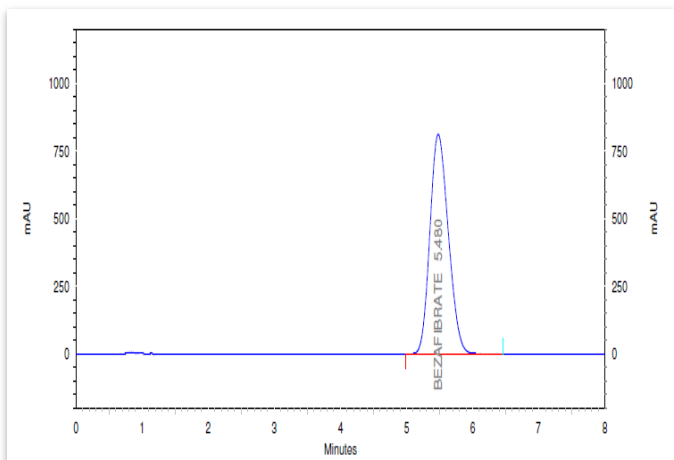


Figure-4 Chromatogram Of Bezafibrate Under Acid Degradation

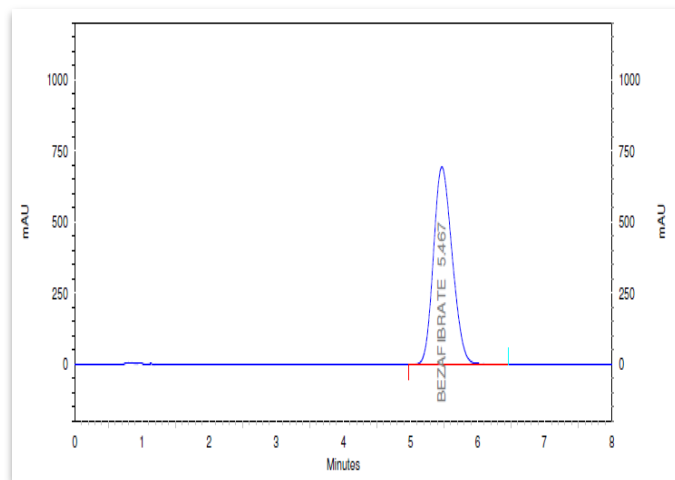
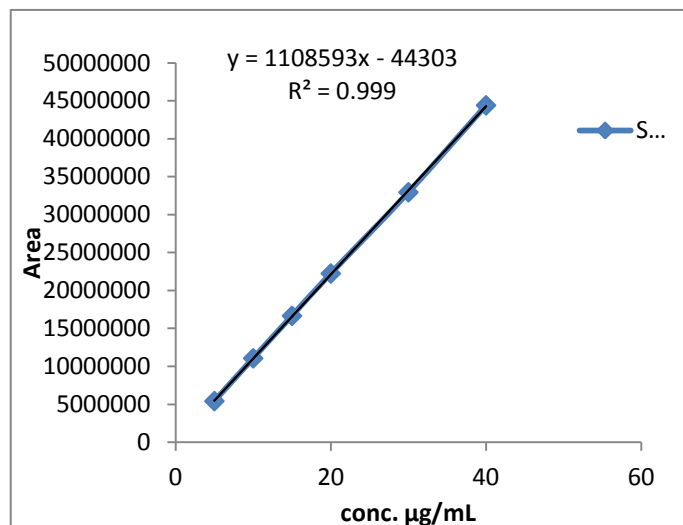


Figure-7 Chromatogram Of Bezafibrate Under Thermal Degradation

Validation of the method

Linearity

The linearity was determined by preparing standard solutions at 6 different concentrations levels ranging from 5 to 40 µg/mL. The regression equation of calibration curves was obtained as $y = 1108593x - 44303$ with a correlation coefficient of 0.9999.



Result of validation parameter

Table No.-2 Result of validation parameter

Parameter	BEZAFIBRATE	
Wavelength for estimation	228 nm	
Linearity range (µg/ml)	5-40 µg/ml	
Regression equation	$Y = 1108593X - 44303$	
Correlation coefficient (r^2)	0.999	
Retention time (min.)	5.55	
Accuracy (n=3)	98.71 ± 0.66	
Precision		
Repeatability (n=6)	% Found \pm SD	97.03 ± 0.81412096
	% RSD	0.83901426
Intraday Precision (n=3)	% Found \pm SD	97.92546 ± 0.848982
	% RSD	0.848982
Interday Precision (n=3)	% Found \pm SD	99.5412 ± 0.548087
	% RSD	0.54985
Assay (n=6)	99.74 ± 0.75	
%RSD	0.756061	
LOD (µg/ml)	0.39	
LOQ (µg/ml)	1.18	

Application of the method to dosage forms

The present method is applied to the estimation of bezafibrate in their commercially available tablets. The % recovery for bezafibrate was found to be 99.74 ± 0.75 (mean value \pm standard deviation) of three determinations, which was comparable to the corresponding labeled amounts.

Conclusion

A rapid, RP-HPLC method was successfully developed for the determination of bezafibrate in the pharmaceutical tablets. The developed method is selective, precise, accurate, and linear. Forced degradation data proved that the method is specific for the analytes and free from the interference of blank and unknown degradation products. The run time (5.5 min) enables rapid determination of the tablet dosage form. Also, the results indicate the suitability of the method for acid, base, oxidation, and sunlight degradation studies. The method is suitable for the analysis of stability samples and the routine analysis of bezafibrate in tablets.

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