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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF GEMFIBROZIL IN BULK AND PHARMACEUTICAL FORMULATION BY RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination Gemfibrozil in bulk and pharmaceutical dosage form. The column used was Agilent Zorbax C_8 (150 ×4.6mm, 5µ) in gradient mode, with mobile phase containing Solvent-A: phosphate buffer adjusted the pH-3.0 with orthophosphoric acid Solvent-B: Methanol the flow rate was 1.5 mL/ min and eluents was monitored at 276 nm. The retention time Gemfibrozil was 5.158 min, respectively. The linearity for Gemfibrozil was in the range of 30-450 µg/ml respectively. The recovery of Gemfibrozil was found to be 99.5%, respectively. The proposed method was validated and successfully applied to the estimation of Gemfibrozil in capsule dosage form.

Keywords: Validation, RP-HPLC, Gemfibrozil

INTRODUCTION

Gemfibrozil (5-(2, 5-dimethylphenoxy)-2, 2-dimethyl-pentanoic acid) a fibric acid antilipemic agent similar to clofibrate, is used to treat hyperlipoproteinemia and asa second-line therapy for type IIb hypercholesterolemia. Gemfibrozil increases the activity of extrahepaticlipoprotein lipase (LL), thereby increasing lipoprotein triglyceride lipolysis. It does so by activating Peroxisomeproliferator-activated receptor-alpha (PPARα) transcription factor ligand, a receptor that is involved in metabolism of carbohydrates and fats, as well as adipose tissue differentiation. This is accompanied by a slight increase in secretion of lipids into the bile and ultimately the intestine. Gemfibrozil also inhibits the synthesis and increases the clearance of apolipoprotein B, a carrier molecule for VLDL. Well absorbed from gastrointestinal tract (within 1-2 hours). Gemfibrozil mainly undergoes oxidation of a ring methyl group to successively form ahydroxymethyl and a carboxyl metabolite. Literature survey revealed that only a few analytical methods such as liquid chromatography-high performance liquid chromatography (HPLC) method have been reported. Hence, a new sensitive and efficient HPLC method was developed and validated for the assay of the drug in tablets.The structure of Faropenem is shown in figure 1.

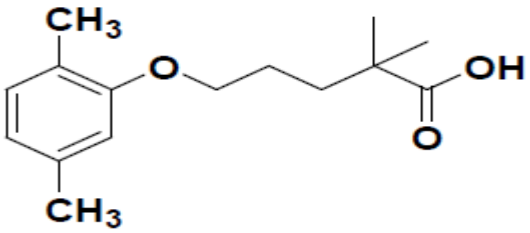

Fig-1. Structure of Gemfibrozil

MATERIALS AND METHODS

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Agilent Zorbax C8 (150.0×4.6mm 5µ) A Bandline sonerex

sonicator was used for enhancing dissolution of the compounds. An Adwa digital pH meter was used for pH adjustment. Analytically pure Gemfibrozil was obtained as gift samples from M/s Blue Cross Ltd., (Mumbai, India) and M/s Mercury Laboratories Ltd., (Vadodara, India), respectively. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid and potassium dihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase.

Preparation of mobile phase and stock solutions: Potassium dihydrogen phosphate was weighed (1.36 g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 3.0 with ortho phosphoric acid. The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1) and used.

Time (Minutes)	Solvent-A (%)	Solvent-B (%)
0.01	45	55
2	55	45
4	50	50
8	40	60
10	60	40

Sample Preparation: For the estimation of Gemfibrozil from the capsules, twenty capsules were taken and their contents were mixed thoroughly. Average weight was calculated. Capsule content or the powder equivalent to 100mg was weighed accurately and transferred into a 100ml volumetric flask, dissolved and dilute up to mark with diluent (1000ppm). Mix well and filter through 0.45µm filter.

Chromatographic conditions: A reverse phase C8 column equilibrated with mobile phase phosphate buffer-methanol adjusted to pH 3.0 was used. Mobile phase flow rate was maintained at 1.5 mL/min and eluents was monitored at 276 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 10 min. Appropriate aliquot of Gemfibrozil stock solutions was taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 30, 75,150,300,450 µg/mL of Gemfibrozil The solution was injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was calculated for Gemfibrozil.

Determination of* Gemfibrozil *dosage form: For the estimation of Gemfibrozil from the capsules, twenty capsules were taken and their contents were mixed thoroughly.

Average weight was calculated. Capsule content or the powder equivalent to 100mg was weighed accurately and transferred into a 100ml volumetric flask, dissolved and dilute up to mark with diluent. Take above solution 30 ml in 100 ml volumetric flask dilute up to mark with diluent (300ppm). Mix well and filter through 0.45µm filter. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated for accuracy, precision, specificity, and robustness.

Accuracy: The accuracy of the method was determined by calculating recovery of Gemfibrozil by the normal method. Known amount of Gemfibrozil was added to a pre quantified sample solution, and the amount of Gemfibrozil was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Precision: The intraday and inter day precision study of Gemfibrozil was carried out by estimating the corresponding responses 6 times on the same day and on different days. The results are reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 6 different concentrations of Gemfibrozil and results are reported in terms of relative standard deviation (%RSD).

Specificity: Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Robustness: Robustness of the method was studied by changing change in the chromatographic parameters: Effect of Variation in column oven temperature.± % 10 and the flow 1.0 and 2.0 ml/min instead of 1.5 ml/min.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on asymmetric factor and peak area obtained for Gemfibrozil. The mobile phase phosphate buffer-methanol(Gradient) adjusted to pH 3.0 using ortho phosphoric acid was found to be satisfactory and gave symmetric peak for Gemfibrozil The retention time for Gemfibrozil was 5.158 min

respectively (Figure 2). The calibration curve for Gemfibrozil was obtained by plotting the peak area of Gemfibrozil versus the concentration of Gemfibrozil over the range of 30-450 µg/mL, and it was found to be linear with $r^2 = 0.999$. The validation parameters are summarized in (Table-1). The recovery Gemfibrozil was found to be 99.5% respectively. The system suitability test parameters are shown in (Table-1). The liquid chromatographic method was applied to the determination of Gemfibrozil in dosage form. The results for Gemfibrozil were comparable with the corresponding labeled amount.

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of Gemfibrozil using simple mobile phase with low buffer concentration compared to the reported method. The method gives short analysis time (<6 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Gemfibrozil in dosage form.

Table 1: Validation parameters and data for proposed method

Validation parameter	Results
Linearity	30-450 µg/mL
Regression coefficient (r^2)	0.999
*Accuracy (% recovery)	99.5%
Precision	
**System Precision (%RSD)	0.46
Method precision (%RSD)	0.90
Assay value (%)	99.46
System suitability parameter	
Tailing factor	1.1
Number of theoretical plates	6794.2

** Replicates of three concentration levels (in three determinations); ** Six repetitive injections of same homogeneous sample*

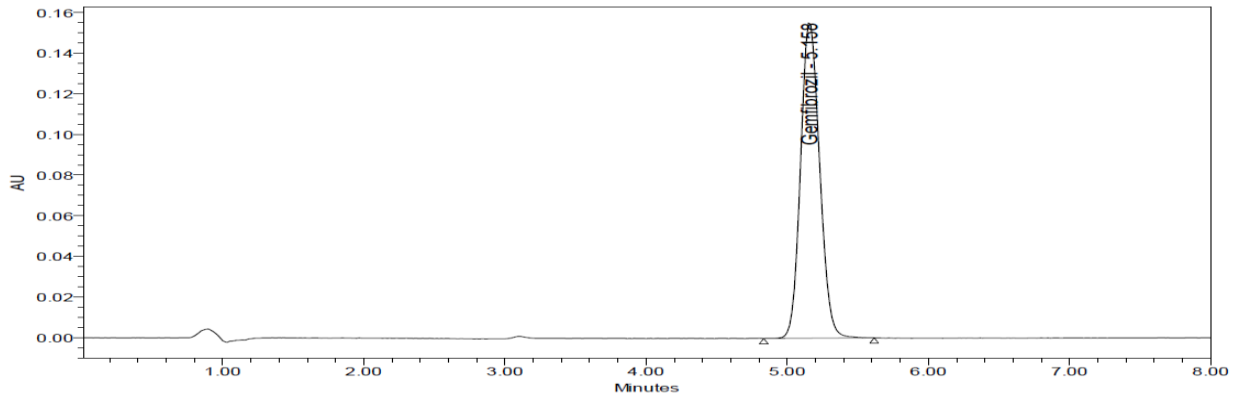

Figure 2: HPLC chromatogram of Gemfibrozil in optimized chromatographic conditions

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