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STABILITY INDICATING RP – HPLC METHOD FOR DETERMINATION OF GEMFIBROZIL IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Purpose: To Develop A simple, rapid and accurate and stability indicating RP-HPLC Method for the determination of Gemfibrozil in pure and tablet form. **Method:** Stability indicating RP-HPLC method. **Result:** The method showed a linear response for concentrations in the range of 10-50µg/ml using Methanol: Water solution in the ratio (90:10) as the mobile phase with detection at 274 nm and a flow rate of 0.7 ml/min and retention time 2.287min. The value of correlation coefficient, slope and intercept were, 0.999, 12.389and-3.839, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active Pharmaceutical ingredient with significantly different retention time. **Conclusion:** As the method could effectively separate the drug from its degradation product, it can be employed as a stability indicating one.

KEYWORDS: Gemfibrozil, RP-HPLC, Degradation studies.

INTRODUCTION

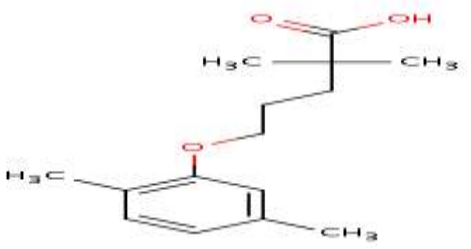

Fig.1 Chemical structure of Gemfibrozil

EXPERIMENTAL

Stability indicating methods have become an important aspect of any analytical method validationandapartofUSFDArequirements.[1] Chemically, Gemfibrzil (GEM) is a dimethylphenoxy derivative 5-(2,5- dimethylphenoxy)-2,2-dimethylpentanoic acid.(fig.-1) . It is a lipid regurating agent used in the treatment of hyperlipidemia similar to clofibrate is used to treat hyperlipoproteinemia and as a second-line therapy for type IIb hypercholesterolemia. Gemfibrozil increases the activity of extrahepatic lipoprotein lipase (LL), thereby increasing lipoprotein triglyceride lipolysis. It does so by activating Peroxisome proliferator-activated receptor-alpha (PPARα) 'transcription factor ligand', a receptor that is involved in metabolism of carbohydrates and fats, as well as adipose tissue differentiation. Chylomicrons are degraded, VLDLs are converted to LDLs, and LDLs are converted to HDL. 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 a hydroxymethyl and a carboxyl metabolite. Approximately seventy percent of the administered human dose is excreted in the urine, mostly as the glucuronide conjugate, with less than 2% excreted as unchanged Gemfibrozil. Half life of the drug was 1.5 hrs and more than 95% of the drug was bound to plasma.

MATERIALS AND METHODS

Gift sample of Gemfibrozil (GEM) was received from flemingo. Commercial formulations LOPID^{TM} containing 300mg of gemfibrozil were purchased from the local market.

INSTRUMENTATION

The analysis of the drug was carried out on a Younglin (S.K) Gradient System UV Detector. Equipped with a Reverse phase (Thermo) C18 Column (4.6mm x 250 mm; 5µm), a SP930D pump, a 20µl injection loop and a UV730D Absorbance detector and running on Autochro-3000 software.

Chromatographic conditions

Mobile phase consist mixture of methanol-water in the ratio of 90:10%v/v (PH was adjusted with 0.05% OPA). The mobile phase was pumped from the solvent reservoir to the column at flow rate0.7ml/min. column temperature was maintained at Ambient. UV Detection performed at 274nm.the mobile phase was degassed by an ultrasonic water bath for

5min.filter through 0.45µ filter under vacuum filtration. The column was equilibrated for at least 30min with mobile phase flowing through the system. Mobile phase used as diluents during the standard and test sample preparation.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 274 nm Wavelength was selected.

Preparation of Standard Solutions

Accurately weigh and transfer 10mg of GEMFIBROZIL working standard into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent To get 1000µg/ml standerd (Stock solution) Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents to get 20µgml solution. Mix well and filter through 0.45µm filter**.** The simple chromatogram are shown in fig no. 2.

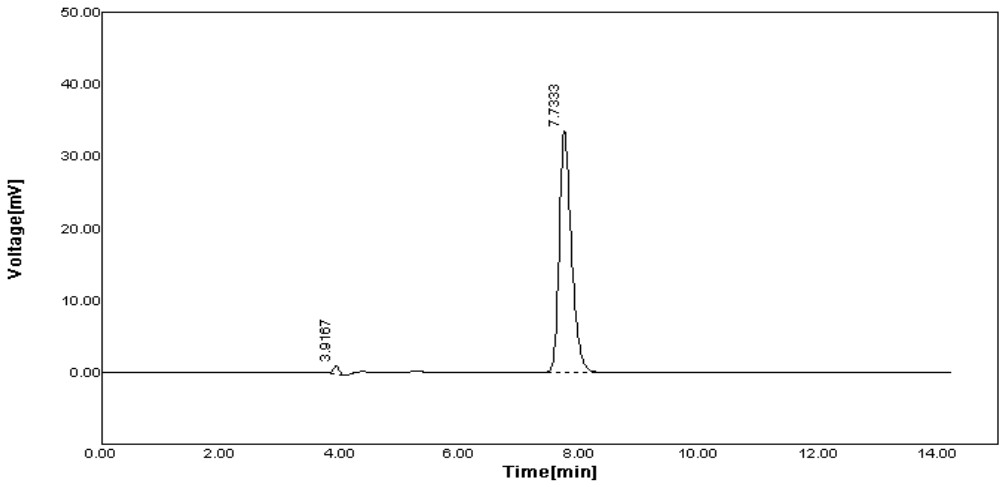

Fig No.2 The Simple Chromatogram of Standard Gemfibrozil.

Preparation of calibration graph

The linearity of response for gemfibrozil assay method was determined by preparing and injecting solutions with concentrations of about 10,20, 30,40,50µg/ml of gemfibrozil. Linearity curve of gemfibrozil shown if fig. no.3.

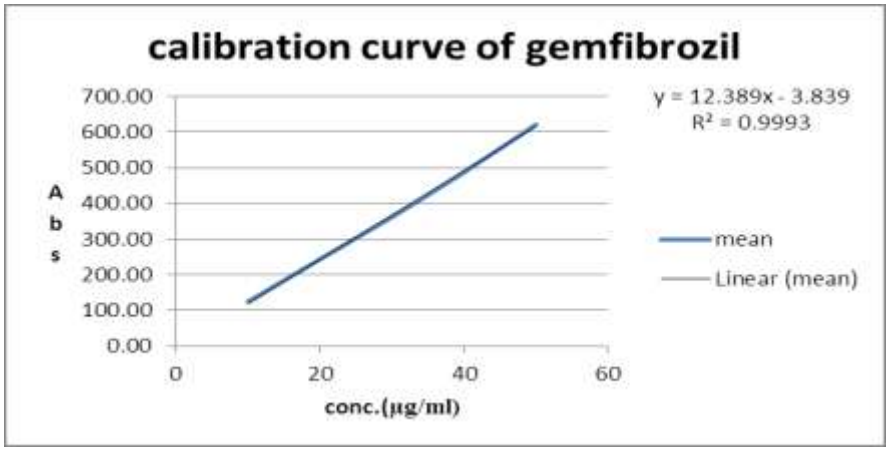

Fig.No.3 Linearity curve of gemfibrozil

Sample Solution Preparation

Weigh 20 gemfibrozil Tablets and calculate the average weight. accurately weigh and transfer the sample equivalent to 10 mg of gemfibrozil into a 10 ml volumetric flask. Add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.(40µg/ml).The simple chromatogram of test gemfibrozil shown in fig. no.4

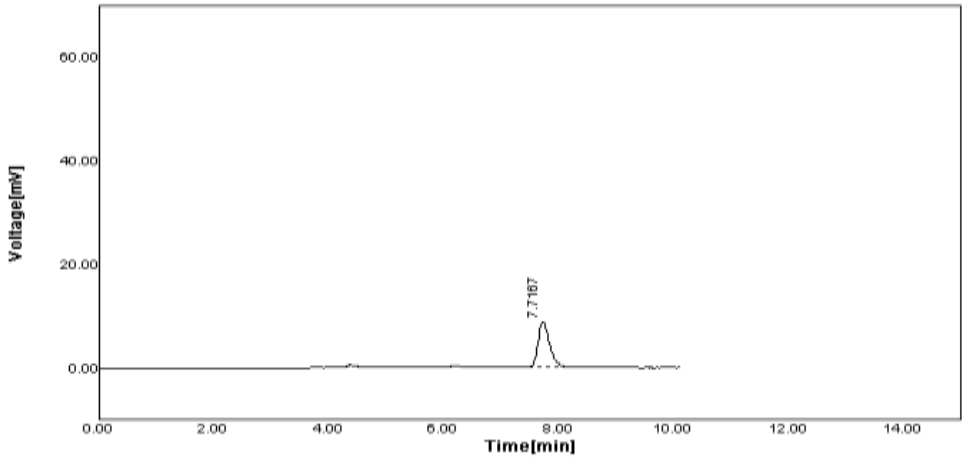

Fig. no.4 Simple Chromatogram of test gemfibrozil

Forced degradation studies

In order to determine whether the analytical method and assay were stability-indicating, GEM tablets and GEM active pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 1N HCl), base hydrolysis (using 0.1N NAOH), oxidative degradation (using 3.0% H2O2) and

thermal treatment (heated at 80°C for two days) to evaluate the ability of the proposed method to separate GEM from its degradation products. GEM at a concentration of 20µg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

Acid Degradation

Forced degradation in acidic media was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 1N HCl to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 1 hr and neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 20µg/ml. chromatograms for acid degradation studies were shown in fig 5.

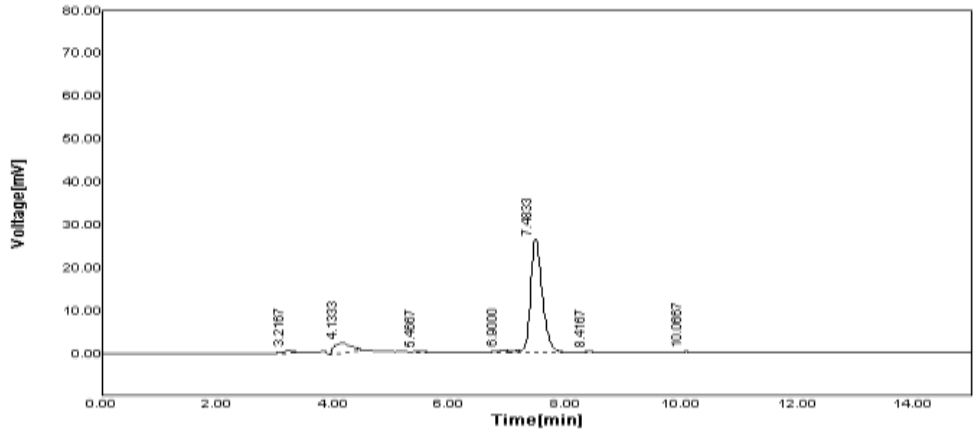

Fig no.5 Chromatogram of Acid Degraded Sample

Base Degradation

Forced degradation in alkaline media was performed using 0.1N NaOH. The representative Chromatograms for alkaline degradation studies were shown in fig-6 respectively.

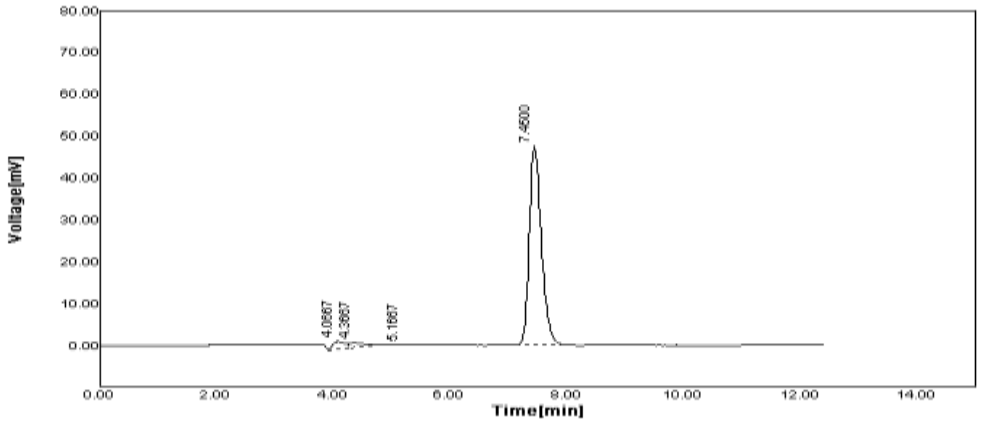

Fig no.6 Chromatogram of Base Degraded sample

Neutral Degradation

Neutral degradation in alkaline media was performed using water. The representative chromatograms for Neutral degradation studies were shown in fig-7 respectively.

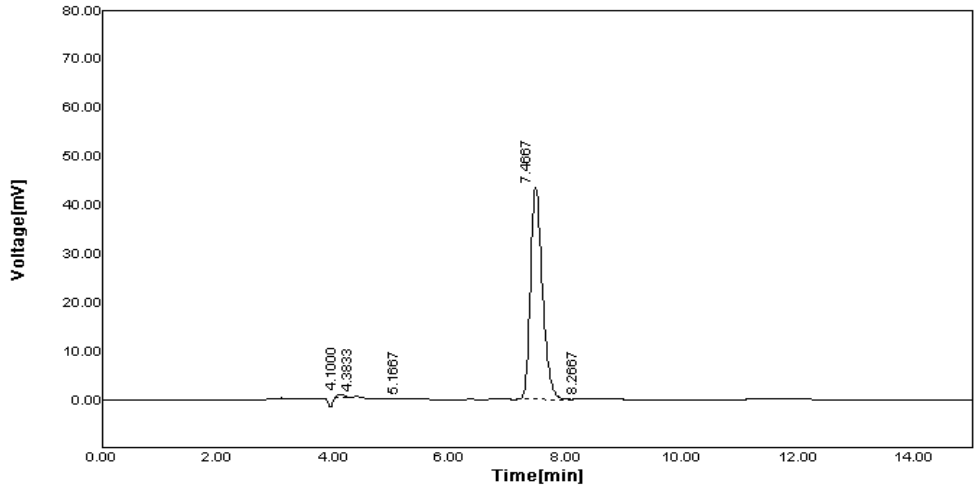

Fig no. 7 Chromatogram of Neutral Degraded sample

Oxidative degradation

Oxidative degradation was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 3%w/v of hydrogen peroxide to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 1hr. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 20µg/ml. The representative Chromatogram was shown in fig-8.

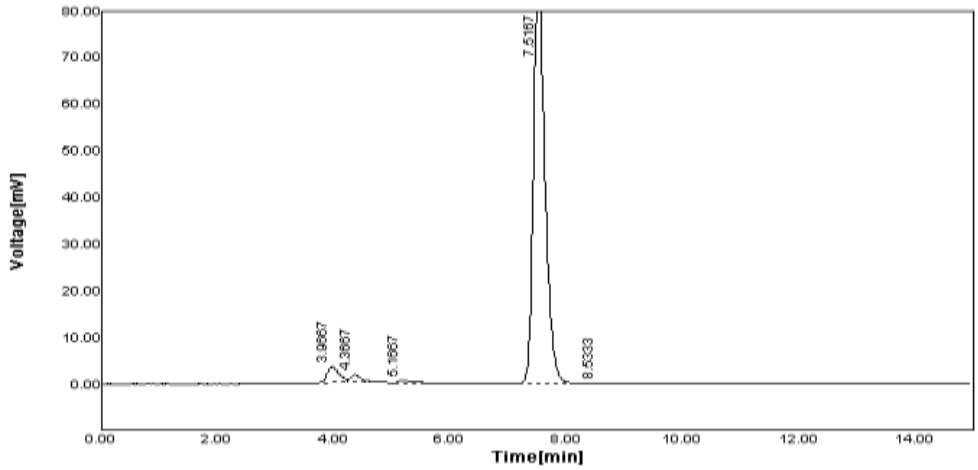

Fig. no. 8 Chromatogram of Oxidative degraded sample

Table No.1 Stress Study of GEMFIBROZIL.

Stress Condition	Time in hr	Mean peak area	% recovery of analyte	Retention time of analyte
Acid hydrolysis (0.1N HCL)	1hr	359.6840	79.42	7.4833
Base hydrolysis (0.5N NAOH)	1hr	663.0801	243.74	7.4500
Oxidation (3%H2o2)	1hr	1158.3657	14.26	7.5167
Neutral	1hr	609.0496	12.96	7.5167

Validation of the Method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery and robustness/ruggedness.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve Shown in fig no.2.The constructed calibration curves were linear over the concentration range of 10– 50µg/ml. Peak areas of GEM was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve Correlation coefficient (n=3) was found to be more than 0.999 with %RSD values were less than 2% across the concentration ranges studied. Typically, the regression equation was: y = 12.38x -3.389 (R^2 = 0.999).

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no.2

Table No.2 Repeatability

Sr. no.	Conc. (µg/ml)	Area	Amt found	% Label Claim
1	20	299.5751	20.04	100.19
2	20	294.6315	19.72	98.58
3	20	298.5757	19.97	99.87
4	20	295.6323	19.78	98.91
5	20	299.7575	20.05	100.25
6	20	298.6464	19.98	99.89
7	20	299.5751	20.04	100.19
8	20	294.6315	19.72	98.58
9	20	298.5757	19.97	99.87
10	20	295.6545	19.78	98.91
	Mean	297.52553	19.90	99.52
	S.D.	1.99	0.13	0.66
	%RSD	0.7145	0.6622	0.6622

Limit of Detection and Limit of Quantitation

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (S) of the calibration plot using the formula LOD = 3.3 σ/S and LOQ =10 σ/S. The limit of quantitation (LOQ) of the present method was found to be 0.72 µg/ml with a resultant %RSD of 0.29% (n = 5). The limit of detection (LOD) was found to be 0.24µg/ml.

Precision

Precision was evaluated in terms of Intraday and Interday precisions. Intraday precision was determined by analyzing sample solutions of GEM from formulations at three levels covering low, medium, and higher concentrations of calibration curve for five times on the same day. Inter day precision was determined by analyzing sample solutions of GEM at three levels covering low, medium, and higher concentrations over a period of seven days (n=5). The peak areas obtained were used to calculate mean and %RSD (relative SD) values.

Table No.3 Precision

INTERDAY				INTRADAY		
CONC µg/ml	**Mean±sd**	**Amt found**	**%Amt found**	**mean±sd**	**Amt Found**	**%Amt Found**
20	244.17±2.64	20.03	100.17	241.97±0.58	19.86	99.28
30	363.30±3.42	29.66	98.85	361.55±1.16	29.51	98.38
40	480.55±1.98	39.13	97.82	482.29±0.58	39.27	98.17

*mean of each 3 reading

Accuracy

Accuracy data for the assay following the determination of the compound of interest is summarized in Table-4.Accuracy was determined by interpolation of replicate (n=5) peak areas of three accuracy standards of different concentration, from a calibration curve that had been prepared as previously described. In each case, the percent relevant error and accuracy was calculated in Table no.4. Accuracy data (n=5)

Table No.4 Accuracy

CONC µg/ml	AREA	AMT FOUND	AMT RECOVERD	% RECOVERY	MEAN RECOVERY
80%	213.1812	17.53	3.68	102.22%	102.88%
100%	237.5596	19.50	4.15	103.94%	
120%	274.7461	22.58	4.51	102.50%	

Specificity

The results of stress testing studies in addition to that of monitoring standard solutions of the drug in the presence of their impurities indicated a high degree of specificity of this

method. The degradation product(s) of the parent compound was found to be similar for both the tablets and API powder. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating.

Ruggedness/Robustness

As recommended in the ICH Guidelines, a robustness assessment was performed during the development of the analytical procedure. The ruggedness of the method is assessed by comparison of the intra- and inter-day assay results that has been performed by two analysts. The %RSD values for intra- and inter-day assays of GEM

tablets performed in the same laboratory by two analysts did not exceed 1.8%, indicating the ruggedness of the method. In addition, the robustness of the method was investigated under a variety of conditions including changes of flow rate, wavelength, mobile phase composition and column temperature. The degree of reproducibility of the results obtained as a result of small deliberate a variations in the method parameters has proven that the method is robust and the data was summarized in Table no. 5.

Table No. 5 Ruggedness/Robustness

Parameters	**Conc.**	**Amount of gemfibrozil detected(mean ±SD)**	**%RSD**
Mobile phase composition-(91:9)	8	388.09±389.79	1.24
Mobile phase composition-(89:11)	8	444.23±0.01	0.15
Wavelength change273nm	8	447.30±0.04	0.01
Wavelength Change 275nm	8	5.4.63 ± 0.70	0.14
Flow rate change(0.6ml)	8	442.63± 0.71	0.16
Flow rate change(0.8ml)	8	297.79 ± 0.97	0.97

Table no.6 System suitability parameter

SR.NO.	**Parameters**	**Result**
1	Range(µg/ml)	10-50µg/ml
2	Detection wavelength	274nm
3	Mean R^2 value	0.999
4	Slop(m)	12.38
5	Intercepts(c)	3.839
6	Run Time (min.)	15
7	Retention Time (min.)	7.7167
8	Theoretical plate (N)	8505.5
9	Tailing Factor	1.3672
10	LOD	0.24
11	LOQ	0.72

Assay

The validated method was applied to the determination of Gemfibrozil GEM in commercially available LOPID^{TM} tablets. Figure 2 and Figure 4 illustrates two typical HPLC chromatograms obtained from Gemfibrozil GEM standard solution and from the assay of LOPID^{TM} tablets respectively. The results of the assay (n = 9) undertaken yielded 100.98% (%RSD = 0.13%) of label claim for GEM. The observed concentration of LBT HCL was found to be 40.38±0.52µg/ml (mean±SD). The mean retention time of gemfibrozil. was 6.33 min. The results of the assay indicate that the method is selective for the analysis of Gemfibrozil GEM without interference from the excipients used to formulate and produce these tablets.

Table no.7 Label Claim

Sample	Label Claimed	% Label Claim* ± SD	% RSD
LOPID^{TM}	Gemfibrozil 300mg	100.98 ± 0.13	0.13

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Gemfibrozil preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Methanol and water were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol : Water 90:10(v/v). The retention time of Gemfibrozil was found to be 6.333 min, respectively. The RSD values for accuracy and precision studies obtained were less than 2%which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 6. The average recovery was found to be 100.49% for gemfibrozil indicating that the proposed method is highly accurate. The LOD and LOQ were found to be 0.72µg/ml and 0.24µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. GEM were found to be relatively stable following photolysis and Thermal degradation. Considerable degradation was observed for both in oxidation, acid and base hydrolysis. The validated method was applied to the determination of GEM in commercially available LOPID^{TM} tablets. The results of the assay indicate that the method

is selective for the analysis of Gemfibrozil GEM without interference from the excipients used to formulate and produce these tablets.

CONCLUSIONS

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of Gemfibrozil in API and tablet dosage forms. The results of stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate the analyte from their degradation products, related substances; excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

ACKNOWLEDGEMENTS

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