Volume 2, Issue 3, 2023, Page 435-446

International Journal of Pharma Research and Technology

Development and Validation of UV-Spectroscopy and UHPLC Method for Gemfibrozil in Bulk Drug and Pharmaceutical Dosage Form

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Abstract

The present study attempts to validate the approach by UV-Spectroscopy at a wavelength of 273nm. The UHPLC separation was performed using reversed phase chromatography on a C18 column (100mmX4.6mm, 2.5m) in gradient mode at a flow rate of 1.0 mL min. At a wavelength of 273nm, diode array detection was performed using a mobile phase composed of a 50:50 (v/v) mixture of methanol and water (0.1% acetic acid).The technique for Gemfibrozil was linear in the concentration range of 5 - 25 g/ml, with a correlation coefficient of 0.9991. For Gemfibrozil, the limit of detection was 0.1058 g/ml. The limit of quantification for Gemfibrozil was 0.3208 g/ml. The analyte recovers at a rate of 98%-102% on average. The approach was found to be accurate, precise, specific, linear calibration curve, and resilient for both pure and pharmaceutical dosage forms. The method was validated in terms of linearity, recovery, accuracy, specificity, LOD/LOQ values, and solution stability, and it was effectively employed for Gemfibrozil determination. For Gemfibrozil, a simple, precise, accurate, and fast ultra high-performance liquid chromatographic (UHPLC) method has been designed and validated.

Keywords: Gemfibrozil; Method development; Validation; UHPLC; UV Spectroscopy

Introduction

Gemfibrozil, often known as "lopid" in the pharmacy, is a fibrate medicine licenced by the FDA that is structurally an amphipathic carboxylic acid molecule.[1] Gemfibrozil was successfully launched to the market in 1976 as a hypolipidemic medication with a significant ability to lower plasma triglyceride levels.[2]

Gemfibrozil [GEM] is a 5-[2, 5 dimethylphenoxy]-2, 2-dimethylpentanoic acid. The medication is used to treat hyperlipidemia. Gemfibrozil is a lipid-regulating drug that lowers serum triglycerides and very low density lipoprotein [VLDL] cholesterol while increasing high density lipoprotein [HDL] cholesterol.[3] Gemfibrozil stimulates extrahepatic lipoprotein lipase (LL) activity, resulting in increased lipoprotein triglyceride lipolysis. It accomplishes this by activating the transcription factor ligand

of Peroxisome proliferator-activated receptor-alpha (PPAR), a receptor implicated in glucose and fat metabolism as well as adipose tissue development. This is accompanied by an increase in lipid production into the bile and, eventually, the intestine. Apolipoprotein B, a transport molecule for VLDL, is also inhibited and cleared by gemfibrozil.[4]

For gemfibrozil test, many HPLC methods in various body fluids.[5-11] and one UV-spectrophotometric approach in pharmaceutical formulations.[12] have been reported and published. This fact prompted the author to develop a simple, inexpensive UV-spectrophotometric method for the determination of gemfibrozil. The current study also details the development and validation of a UV-spectrophotometric method for the assessment of gemfibrozil in pure form and in its formulation (tablets) utilising distilled water as a solvent, in accordance with ICH validation requirements.

Ultra High Performance Liquid Chromatography

Ultra-high performance liquid chromatography (UHPLC) refers to liquid chromatography separations that use columns that contain particles smaller than the 2.5-5 m sizes commonly used in high-performance liquid chromatography (HPLC).[13] UHPLC operates under the same assumption as HPLC,[14] with the controlling concept being that as column packing particle size decreases, efficiency and consequently resolution accretion.[15] Separations with smaller particles provide improved efficiency per unit time,[13, 14] however efficiency cannot be minimised at higher mobile phase flow rates or linear velocities.[16] Following attribute, smaller particles, faster speeds, and higher peak resolution can be absolute to new boundaries.[17]

The primary purpose of this research was to create a selective and time-efficient method for ultra-high performance liquid chromatography of Gemfibrozil in dose form. In addition, the proposed method is proved to be useful in routine drug determination in tablet formulation.[3] A review of the literature found that just a few analytical procedures, such as liquid chromatography-high performance liquid chromatography (HPLC), have been documented. As a result, for the assay of the medication in tablets, a new sensitive and efficient UHPLC method was devised and validated. The structure of Faropenem is shown in figure 1.

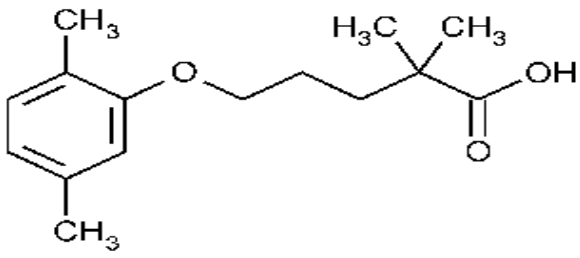

Figure No.1: Structure of Gemfibrozil

Material and Methods

Instrumentation

Using Agilent Tech's Gradient System with Auto Injector and Chemstation 10.1 software, the drug was analysed. Equipped with an Agilent C18 (100mm X 4.6mm, 2.5m), a 20l injection loop, and DAD (Diode Array Detectors). Other equipment includes the VSI pH metre (VSI 1-B), WENSARTM High Resolution Balance, and Analytical Technologies Limited UV-Spectrophotometer.

Reagents and Chemicals

We received analytically pure gemfibrozil as free samples from RSITC Jalgaon. The solutions were made using methanol, water, acetonitrile, and deionized water. The tablet formulation [Lopid 600mg, Pfizer] that was acquired from the local market contained the 600 mg of Gemfibrozil that was specified on the label.

Chromatographic Conditions

The mobile phase is a 50:50 combination of methanol and water with 0.1% acetic acid (PH was adjusted to 0.3). With a run period of 15 minutes, a 20-L mobile phase sample was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min.The temperature of the columns was held constant. At 273nm, UV detection is carried out. An ultrasonic water bath was used to degas the mobile phase for 5 minutes. Filter through a 0.45 filter while using a hoover. With mobile phase running through the system, the column was equilibrated for at least 30 minutes. During the preparation of the standard and test samples, mobile phase was utilised as diluents.

Selection of Detection Wavelength

The UV detector was selected because it is dependable and simple to configure at a fixed wavelength. A fixed concentration of the analyte was examined at several wavelengths between 200 and 400 nm. The chosen wavelength of 273 nm was determined by the analyte's reaction.

Preparation of Standard Solutions

For spectrophtomeric method, to make a stock solution with a concentration of 10 g/ml, accurately weigh and transfer 5 mg of Gemfibrozil working standard into a 10 ml volumetric flask as close to dilution with Methanol that has been prepared in full. After 15 minutes of sonication to dissolve the stock solution, take 0.5 ml and transfer it to a 10 ml volumetric flask while adding Methanol to the required volume.

For chromatographic method, accurately weigh and transfer 5mg of Gemfibrozil working standard into a 10 ml volumetric flask add about 10 ml of diluent (methanol) and sonicate to dissolve it completely to get 500µg/ml standard (Stock solution), further pipette 0.1-0.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents to get 5, 10,15,20,25 µg/ml solution. Filter through a 0.45-m filter after thoroughly mixing.

Preparation of Calibration Graph

For spectrophotometric method, different concentrations in the range of 2–10 g/ml for Gemfibrozil were produced from the standard stock solution of the drug and measured at 273 nm. The data from the regression equation shown in the table below were used to plot the calibration curves. For chromatographic method, the stationary phase and mobile phase were allowed to equilibrate until a steady baseline was attained. Pipette 5 mg of Gemfibrozil into a 10 ml volumetric flask, then mix it with mobile phase after taking it out of the

freshly made standard stock solution. From it, 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the solution were pipetted out into a 10 ml volumetric flask, and the volume was then brought up to 10 ml with mobile phase to obtain the final concentration of 5, 10, 15, 20, and 25 g/ml of Gemfibrozil. Samples were injected, and peaks were measured at 273 nm, as seen in the graph below plotting drug concentration versus peak area.

Determination of Gemfibrozil Dosage Form

The powder from 20 tablets weighed 14.46 gms to determine the amount of gemfibrozil included in commercially available tablets, and the average weight of the powder was calculated to be 723 gms. The powder from the triturated tablet was weighed at 6.025 mg. Using 10 mL of methanol, the drug was extracted from the powder. It was sonicated for 15 minutes in order to achieve thorough extraction. Following that, mobile phase was used to dilute 0.1 mL of supernatant up to 10 mL. After injecting the resultant solution into the UHPLC, the drug peak area was observed.

System Suitability Parameters for UHPLC

The system suitability test is used to determine whether the chromatographic system's resolution and repeatability are sufficient for conducting the analysis. Data were gathered from two replicate injections of the reference solution during the test.

Validation Parameters for UV & UHPLC Method

Linearity: The capacity of an analytical method to yield test findings that are proportional to the concentration of analyte in samples within a specified range, either directly or through a well stated mathematical transformation, is known as linearity.

Accuracy: The degree to which test findings acquired using an analytical method are near to the actual value is the method's accuracy. By analysing known additional amounts of analyte, accuracy is sometimes reported as a percentage of recovery. By using the approach on analysed samples that have known amounts of analyte added, one can assess the accuracy of an analytical method. The percentage of analyte recovered by the assay is used to calculate accuracy from test results.

Precision: When the procedure is routinely used on numerous Samplings of a homogenous sample, the precision of an analytical method is the level of agreement among individual test findings. Standard deviation or relative standard deviation are typically used to describe the precision of an analytical process. In addition, one-way ANOVA was used to compare the results, and the F-test was used to calculate the within-day mean square and between-day mean square.

The variations of results between days as well as variations of outcomes within the same day were examined. By examining gemfibrozil three times in one day, intraday precision was found. Three days of daily drug analysis were used to evaluate the precision between days.

Robustness: A method is said to be robust if it can withstand minor, intentional changes to the parameters. Small but intentional changes were made to the optimised technique parameters in order to test the resilience of the suggested approach. Study was conducted on the impact of variations in mobile phase composition and

flow rate, wavelength on retention time, and tailing factor of drug peak.

Limit of Detection: The lowest detectable limit is known as the LOD. The limit of detection (LOD) may be expressed as follows depending on the response's standard deviation and slope: LOD = 3.3 X Avd.SD/Slope

Where, SD = Standard deviation of Y intercept, S = Slope

Limit of Quantitation: The LOQ is the lowest concentration at which a quantitative measurement is possible. Using the slope and the S.D. deviation of the response,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

Where, SD = Standard deviation Y intercept

S = Slope

Results

In the standard of Gemfibrozil theoretical plates were found above 2000 i.e. for Gemfibrozil 7866 at minimum retention time 3.517. (Fig no.1)

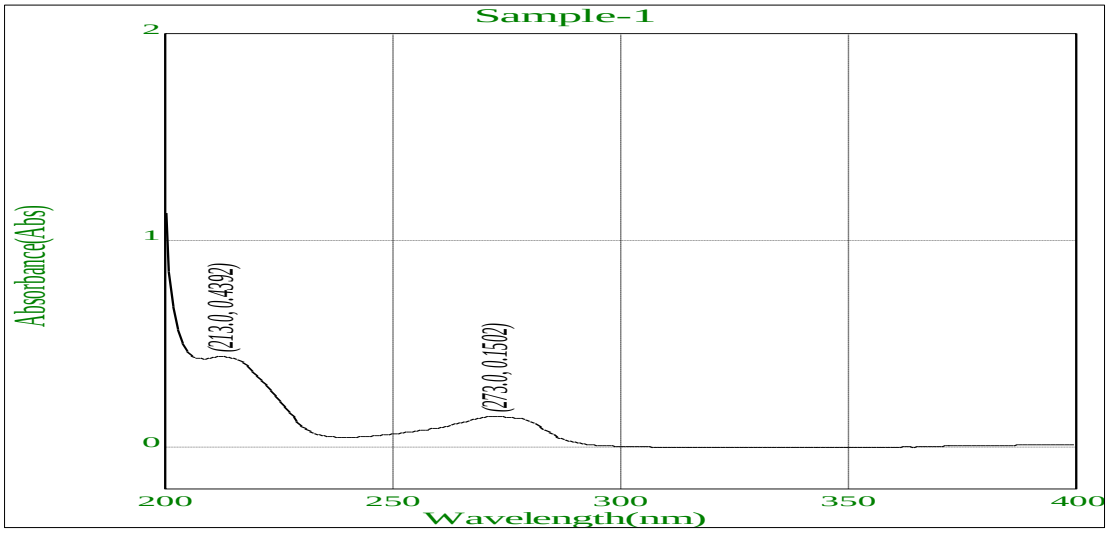

Figure No.2: UV Spectrum of Gemfibrozil

From multiple trials ,it has been observed that, using mobile phase of meoh+0.1% acetic acid (50:50 % v/v),PH 3.0,273 nm, flow rate 1 ml gave adequate detention time at 3.562 min with good peak shape (Theoretical plates: Gemfibrozil 7698)

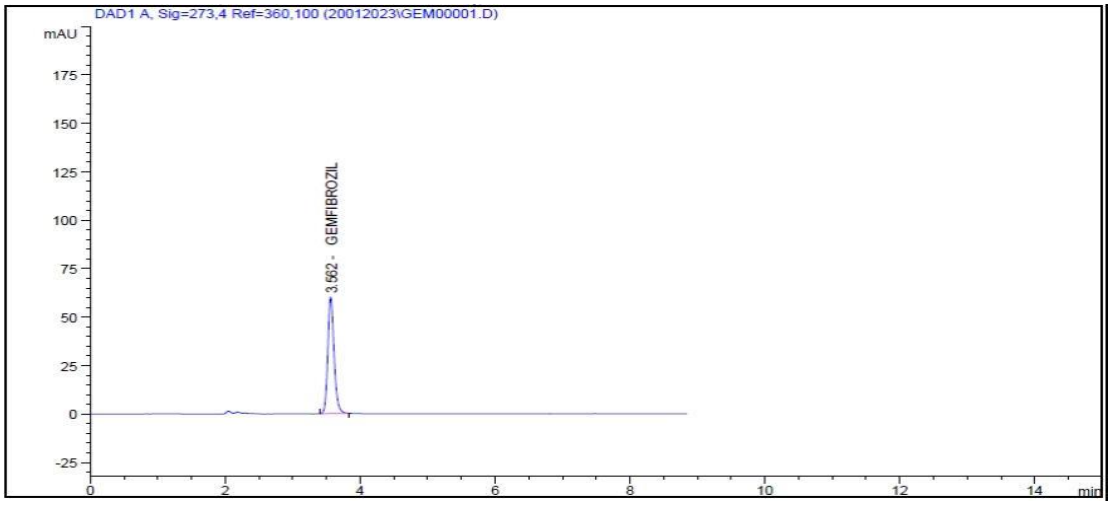

Figure No. 3: Chromatogram of final trial of Gemfibrozil

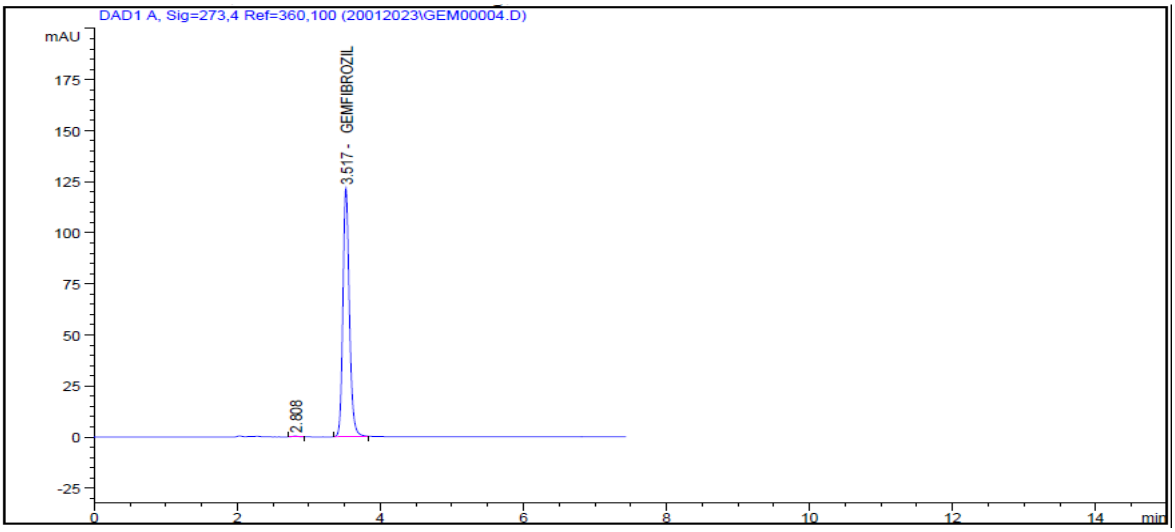

Figure No.4: Chromatogram of standard Gemfibrozil

Calibration Experiment

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 2-10 µg/mL for Gemfibrozil depict the calibration data of Gemfibrozil. The linear equation for Gemfibrozil was y = 0.050X+0.001 where x is the concentration is area of peak. The correlation coefficient was 0.999. The calibration curve of Gemfibrozil is depicted in fig no.5

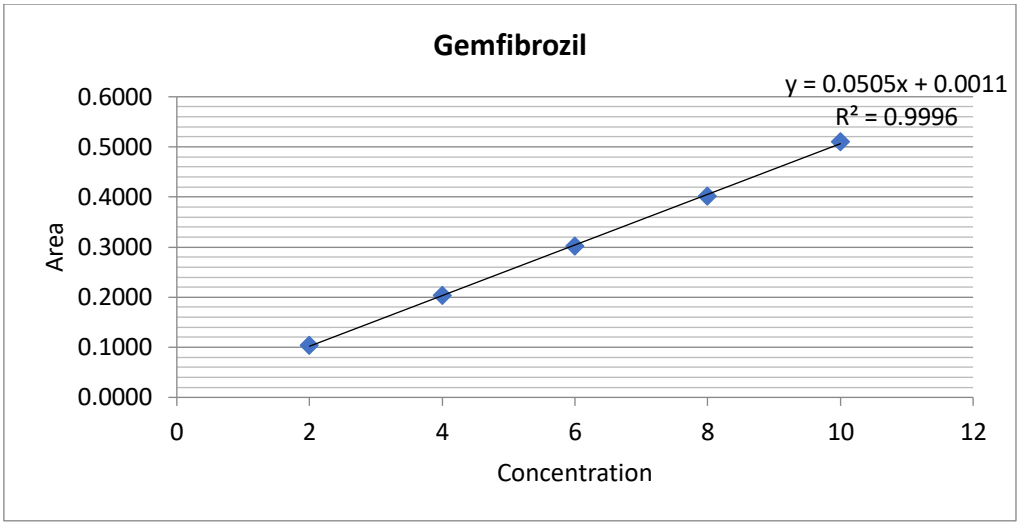

Figure No.5: Calibration curve of Gemfibrozil for (UV method)

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 5-25µg/mL for Gemfibrozil depict the calibration data of Gemfibrozil. The respective linear equation for Gemfibrozil was Y= 73.83x+14.22 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Gemfibrozil is depicted in fig no.6

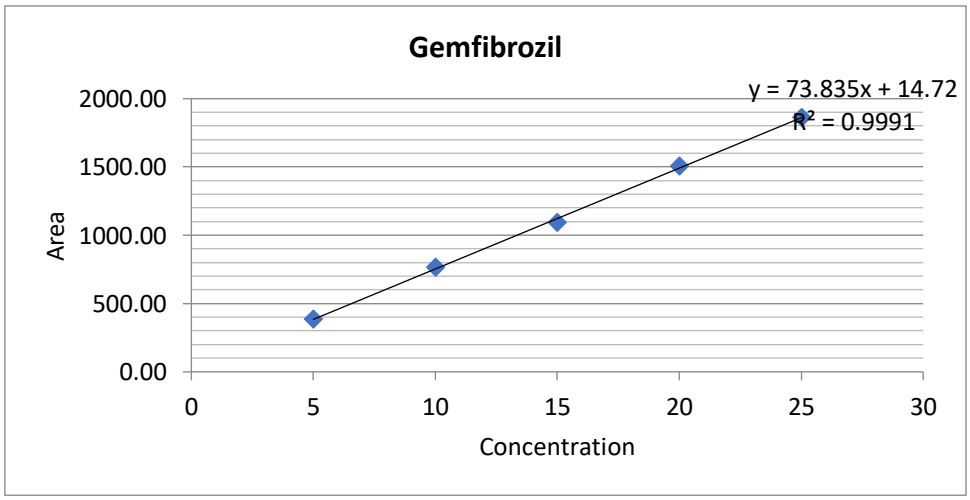

Figure No.6: Calibration curve of Gemfibrozil (UHPLC Method)

Linearity

From Gemfibrozilstandard stock solution, di fferent working standard solution (5-25µg/ml) were prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using mixed volume loop injector chromate grams were recorded. The area for each concentration was recorded (table1

Table No.1: Linearity data for Gemfibrozil

Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
	1	2			
5	386.39	384.124	385.26	1.60	0.42
10	767.7985	764.742	766.27	2.16	0.28
15	1095.703	1089.09	1092.40	4.68	0.43
20	1505.462	1506.59	1506.03	0.80	0.05
25	1859.421	1863.10	1861.26	2.61	0.14
Equation		Y= 73.83x+14.22			
R^2		0.999			

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed marketed solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed in table below (Table No.2)

Table No.2: Result of Recovery data for Gemfibrozil

Drug	Level (%)	Mean % Recovery	S D	% RSD
GEM	80%	100.89	0.67	0.67
	100%	100.10	0.32	0.32
	120%	100.38	0.13	0.13

3. System Suitability Parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Gemfibrozil system suitability parameters were studied. The result shown in below (Table No.3)

Table No.3: Repeatability studies on UHPLC for Gemfibrozil

Sr. No.	Concentration of Gemfibrozil (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	25	1857.599	24.98	99.93
2	25	1860.769		
Mean			1859.18	
SD			2.24	
%RSD			0.12	

4. Precision

The method was established by analyzing various replicates standards of Gemfibrozil. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday &interday is shown on (Table No.4) respectively.

Table No.4: Result of Intraday and Interday Precision studies for Gemfibrozil

Conc^{n} (µg/ml)	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	757.30±1.03	100.58	0.3587	761.89±0.52	101.20	0.07
15	1111.93±3.58	99.07	0.3224	1117.39±1.63	99.57	0.15
20	1508±0.80	101.19	0.0533	1511.39±1.64	101.36	0.11

5. Robustness

The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by (±1ml/min^{-1}**)**, and wavelength change (±1 ml/min^{-1}**)** of optimized chromatographic condition. The results of robustness studies are shown in (Table No.5).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table No.5: Result of Robustness Study of Gemfibrozil

Parameters	Conc.	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(49+51)	10	770.0±0.83	0.11
Mobile phase composition-(51+49)	10	767.45±2.06	0.27
Wavelength change272nm	10	753.7±2.38	0.32
Wavelength Change 274 nm	10	1241.41±0.60	0.05
Flow rate change(0.9ml)	10	847.40±1.56	0.18
Flow rate change(1.1ml)	10	694.10 ± 1.45	0.21

6. Limit of Detection (LOD)

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD= 3.3XAvd.SD/Slope
=3.3X2.37/73.83
=0.1058

Where, SD = Standard deviation of Y intercept, S = Slope

The LOD of Gemfibrozil was found to be 0.1058 (µg/mL) analytical methods that concluded.

7. Limit of Quantification (LOQ)

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

=10 X 2.37 / 73.83

= 0.3208

Where, SD = Standard deviation Y intercept S = Slope

The LOQ of Gemfibrozil was found to be 0.3208 (µg/mL)

Analysis of Marketed Formulation:

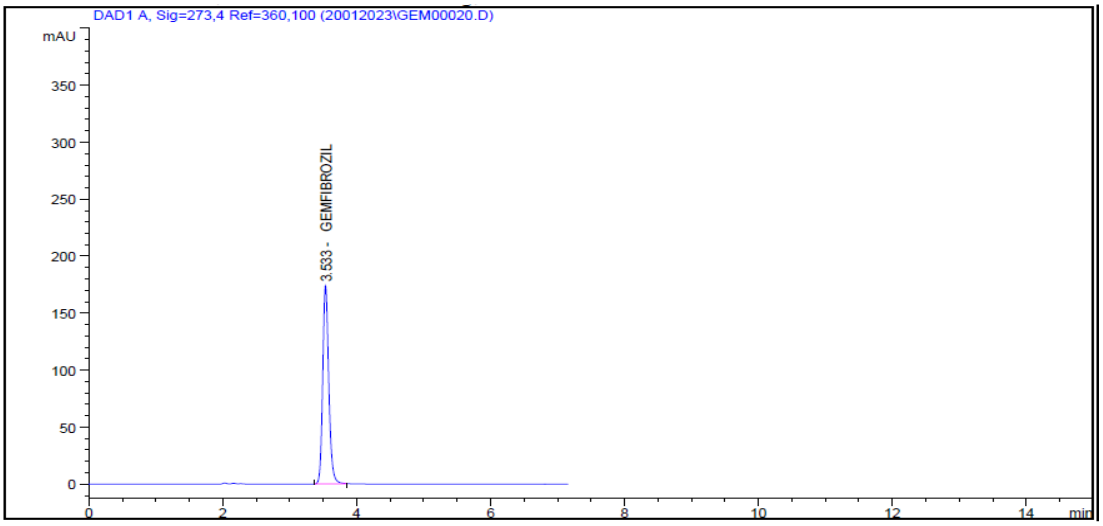

Figure No 7.Chromatogram for Marketed Formulation

Table No.6: Analysis of Marketed Formulation

Assay	Drug	Label Claimed	Amt. Found	%Lable Claim	SD	%RSD
UHPLC Method	GEM	20	20.19	100.99	0.070	0.348
		20	20.29	101.49	0.352	0.348
UV Method	GEM	6	6.002	100.03	0.001	0.024
		6	6.004	100.07	0.024	0.024

Analysis of marketed formulation were also %label claim was found to be 98-102% satisfactory are concluded**.**

Discussion

There are various analytical procedures that estimated Gemfibrozil by various combinations, untill today, except UHPLC. In order to evaluate the safety and purity of Gemfibrozil in both its bulk drug and pharmaceutical dose form, the goal of this effort was to create and validate a more reliable approach. The determination of Gemfibrozil in bulk and dosage form was done using the ultra high performance liquid chromatographic technique. The wavelength of gemfibrozil in water was discovered to be 273 nm when standard solutions were scanned in the 200-400 nm range against 10

ml of methanol and volume made with water as the reference system (fig.1). In chromatographic conditions, it was discovered that the mobile phase of methanol and 0.1% acetic acid (Gradient) adjusted to pH 3.0 was excellent and produced a symmetric peak for the drug gemfibrozil. The duration of retention for gemfibrozil was 3.562 minutes. When the data from the calibration trials were put through a linear regression analysis, it was discovered that there was a linear relationship between peak areas and concentrations in the 5–25 g/mL range (fig.5), and R^2= 0.9991 confirmed this. The recovery investigations for Gemfibrozil were conducted at 80%, 100%, and 120%, and the recovery was discovered to be between 98% and 102% (table no. 2). The analyte recovered for sample solutions ranged from 99.57 to 101.36%, as shown by the recovery studies for three days reported in table no. 4, indicating the accuracy and precision of the analytical method. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis of Gemfibrozil in bulk drug as well as in formulations.

Conclusion

According to ICH criteria, the devised approach was validated. The linearity, precision, range, and robustness all fell within the bounds set forth by the ICH recommendations. The procedure was therefore determined to be straightforward, accurate, precise, cost-effective, and repeatable.

Acknowledgement

I would like to express my sincere gratitude to P. S. G. V. P. M's College of Pharmacy for providing all the necessary requirements.

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