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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF GEMFIBROZIL IN BULK AND TABLET DOSAGE FORM

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Abstract: A novel, straightforward, precise, accurate, and cost-effective UV-spectrophotometric method has been developed for the quantitative estimation of Gemfibrozil in both bulk and tablet dosage forms. The mobile phase comprised a mixture of methanol and distilled water in a ratio of 10:90%v/v at pH 5.0. The method exhibited linearity in the concentration range of 5µg/ml to 25µg/ml, with regression coefficients (r^2) of 1 for Absorption Maxima and 0.9998 for AUC (Area under Curve). The accuracy of the absorption maxima method was determined to be 98.61%, while the AUC method yielded an accuracy of 99.48%. The limits of detection (LOD) and quantification (LOQ) were found to be 0.6237µg/ml and 1.89µg/ml, respectively, for the absorption maxima method, and 0.5908µg/ml and 1.79µg/ml, respectively, for the AUC method. These low LOD and LOQ values indicate the high sensitivity of the developed method. Statistical validation was performed to assess the linearity, accuracy, precision, and selectivity of the method, in accordance with the International Conference on Harmonization (ICH) guidelines. The percent relative standard deviation (%RSD) obtained from all studies fell within the acceptable limits of less than 2%. Therefore, the method was successfully validated, demonstrating precision, accuracy, robustness, low LOD and LOQ values, and satisfactory recovery. This validated method can be reliably employed for further analysis of Gemfibrozil in pharmaceutical formulations, ensuring quality control and compliance with regulatory standards.

Index Terms **- Gemfibrozil, UV Vis Spectrophotometer, Absorption maxima, AUC, ICH, %RSD**

1. INTRODUCTION

Fibrates are a class of medications commonly prescribed for the treatment of lipid disorders. They work by targeting the peroxisome proliferator-activated receptor-alpha (PPAR-α) in the liver and adipose tissue, leading to a reduction in triglyceride levels and an increase in HDL cholesterol levels. Fibrates are particularly effective in managing hypertriglyceridemia, where lifestyle modifications have been insufficient. They may also be used in combination with other lipid-lowering drugs to address mixed dyslipidemia. While fibrates can be beneficial in improving lipid profiles, they may also have side effects such as gastrointestinal symptoms, myopathy, and liver function abnormalities. Close monitoring is necessary to ensure patient safety, especially in those with renal or hepatic impairment. Overall, fibrates serve as an important therapeutic option for managing lipid disorders and reducing the risk of cardiovascular complications.

Gemfibrozil is a medication that belongs to the class of fibrates. It is primarily used to treat high levels of triglycerides (a type of fat) in the blood, along with other lipid abnormalities.

Gemfibrozil, sold under the brand name Lopid among others, is a medication used to treat abnormal blood lipid levels. It is generally less preferred than statins.Use is recommended together with dietary changes and exercise. It is unclear if it changes the risk of heart disease.It is taken by mouth.

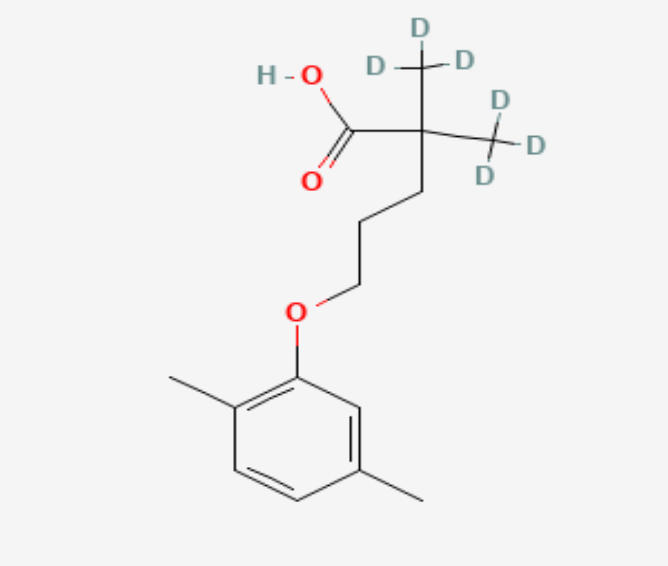

Figure no. 1: Chemical structure of Gemfibrozil

- **IUPAC name:-** 5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic acid
- **Formula:-**C15H22O3
- **Trade names:-** Lopid, Jezil, others
- **Routes of Administration:-** By mouth
- **Molar mass:-** 250.338 g·mol−1
- **Bioavailability:-**Close to 100%
- **Proteinbinding:-**95%
- **Metabolism:-**Liver (CYP3A4)
- **Eliminationhalf-life:-**1.5 hours
- **Excretion** :- Kidney 94%, Feces 6%

Side effects

Stomach upset, stomach/abdominal pain, or unusual taste may occur. If any of these effects last or get worse, tell your doctor or pharmacist promptly.

This drug may rarely cause muscle problems (which can rarely lead to a very serious condition called rhabdomyolysis).: muscle pain/tenderness/weakness (especially with fever or unusual tiredness), signs of kidney problems (such as change in the amount of urine).

1.1 Analytical chemistry

Analytical chemistry studies and uses instruments and methods to separate, identify, and quantify matter. In practice, separation, identification or quantification may constitute the entire analysis or be combined with another method. Separation isolates analytes. Qualitative analysis identifies analytes while quantitative analysis determines the numerical amount or concentration. Analytical chemistry consists of classical, wet chemical methods and modern, instrumental methods. Classical qualitative methods use separations such as precipitation, extraction, and distillation. Identification may be based on differences in colour, odour, melting point, boiling point, solubility.

Analytical chemistry is also focused on improvements in experimental design, chemo metrics, and the creation of new measurement tools. Analytical chemistry has broad applications to medicine, science, and engineering, radioactivity or reactivity. Classical quantitative analysis uses mass or volume changes to quantify amount. Instrumental methods may be used to separate samples using chromatography, electrophoresis or field flow fractionation. Then qualitative and quantitative analysis can be performed, often with the same instrument and may use light interaction, heat interaction, electric fields or magnetic fields. Often the same instrument can separate, identify and quantify an analyte.

1.2 Pharmaceutical Analysis

The purpose of Pharmaceutical Analysis is to identify substances, purify them, separate them, quantify them, determine the molecular structures of chemical compounds that make up pharmaceuticals, and determine how these compounds are combined to make up a pharmaceutical product. Specifically, it relates to the analysis of raw materials and pharmaceutical formulations, entails the determination of ingredients, impurities, excipients, and uniformity, solubility, and dissolution rate to identify active components, contaminants, and impurities. Depending on the dosage form and the compound, the sample may be singular or combination. The substance utilized for pharmaceutical purposes is animals, plants, microbes, minerals, and a wide variety of synthetic chemicals. This study focuses mainly on assessing the quantities and quality of drugs and their impurities in the development process. In the discipline of pharmaceutical development, this domain addresses needs related to drug safety, manufacturing efficiency, lowering development expenses, and providing market information on evidence-based information.

Following are the three methods by which a pharmaceutical sample can be analyzed. The manual method of analysis: Taste, smell, texture, colour, and appearance are some of the organoleptic qualities that the senses can perceive. Chemical method of analysis: There are various methods available for analyzing the samples as well as titrations and microbiological tests (antibiotics). This method of analysis falls under the chemical approach as reagents are chained together in chain reactions. Instrumental analysis method: A sample can be evaluated using multiple instrumental techniques such as UV spectroscopy, HPLC, fluorescence, atomic absorption spectroscopy, polarography, gravimetry, NMR, infrared spectroscopy, etc.

It is possible to categorize pharmaceutical analysis into two types:

1.2.1 Qualitative analysis: The number one priority of any scientist is to identify the sample; qualitative analysis involves determining what kind of chemical compounds are present, such as elucidating a molecule's structure and determining its molecular weight when present in samples.

1.2.2 Quantitative analysis: Quantitative or quantitative evaluations of the component quantities, weights, or concentrations in the sample are also done.

1.3 UV- Vis Spectroscopy

UV–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. Being relatively inexpensive and easily implemented, this methodology is widely used in diverse applied and fundamental applications. The only requirement is that the sample absorb in the UV-Vis region, i.e. be a chromophore. Absorption spectroscopy is complementary to fluorescence spectroscopy. Parameters of interest, besides the wavelength of measurement, are absorbance (A) or transmittance (%T) or reflectance (%R), and its change with time

Photometry- To study the measurement of intensity of light.

Colorimetry- To study the colour of specific wavelength of visible light (400 -750 nm).

Spectrophotometry- To study of colour of a very narrow range of wavelength in UV (10-200nm), visible (400-750nm) and IR (2.5-100µm). Concentration of a biochemical compound can be determined. Used to estimate the compounds in a complex mixture.Though the instruments different the basic principles of both are however the same.

1.3.1 Principle

Beer's Law:-The amount of light absorbed is proportional to the concentration of the absorbingsubstance.

Lambert's Law:-The amount of light absorbed is proportional to the thickness (length) of the absorbing material.

1.3.2 Instrumentation

A. Light Source

- Tungsten filament lamps and Hydrogen-Deuterium lamps are the most widely used and suitable light sources as they cover the whole UV region.
- Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

B. Monochromator

- Monochromators generally are composed of prisms and slits.
- Most of the spectrophotometers are double beam spectrophotometers.
- The radiation emitted from the primary source is dispersed with the help of rotating prisms.
- The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelengths to pass through the slits for recording purposes.
- The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

C. Sample and reference cells

- One of the two divided beams is passed through the sample solution and the second beam is passé through the reference solution.
- Both sample and reference solution is contained in the cells.
- These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

D. Detector

- Generally, two photocells serve the purpose of the detector in UV spectroscopy.
- One of the photocells receives the beam from the sample cell and the second detector receives the beam from the reference.
- The intensity of the radiation from the reference cell is stronger than the beam of the sample cell. This results in the generation of pulsating or alternating currents in the photocells.

E. Amplifier

- The alternating current generated in the photocells is transferred to the amplifier.
- The amplifier is coupled to a small servo meter.
- Generally, the current generated in the photocells is of very low intensity, the main purpose of the amplifier is to amplify the signals many times so we can get clear and recordable signals.

F. Recording devices

- Most of the time amplifier is coupled to a pen recorder which is connected to the computer.
- The computer stores all the data generated and produces the spectrum of the desired compound.

1.3.3 Applications of UV Spectroscopy

A. Detection of Impurities:-

- It is one of the best methods for the determination of impurities in organic molecules.
- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.
- By also measuring the absorbance at a specific wavelength, the impurities can be detected.

B. Structural elucidation of organic compounds:-

- It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of heteroatom's.
- UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation.
- UV absorption spectroscopy can characterize those types of compounds that absorb UV radiation thus used in the qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.
- This technique is used to detect the presence or absence of a functional group in the compound. The absence of a band at a particular wavelength is regarded as evidence for the absence of particular group.
- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.
- Many drugs are either in the form of raw material or in the form of the formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at a specific wavelength.
- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
- UV spectrophotometer may be used as a detector for HPLC.

2. MATERIAL AND METHODS

2.1. Materials

A. Chemicals:-

I. Methanol - K. R. Chemicals, India Research Lab Fine Chem IndustryMumbai 400 002, Batch no.1001120219

II. Distilled water- K. R. Chemicals, India Research Lab Fine Chem IndustryMumbai 400 002, Batch no.6551604211

B. Glasswares :-

i. Round bottom flask - borosilicate glass
ii. Test-tube. - borosilicate glass
iii. Breaker. - borosilicate glass
iv. Conical flask - borosilicate glass
v. Volumetric flask - borosilicate glass
vi. Measuring cylinder - borosilicate glass
vii. Pipette - borosilicate glass

C. Instrument:-

i. UV-VIS Spectrophotometer:-UV-PROFESSIONAL Analysis Software, Model no. LT-2201.
ii. Sonicator:- ultrasonic electronic instrument
iii. Weigh Balance:- WENSAR HighResolutionBalance

2.2 Methods

A. Area under curve:-

In the field of pharmacokinetics, the area under the curve (AUC) is the definite integral of the concentration of a drug in blood plasma as a function of time (this can be done using liquid chromatography–mass spectrometry). In practice, the drug concentration is measured at certain discrete points in time and the trapezoidal rule is used to estimate AUC.

B. Absorption Maxima:-

Wavelength of maximum absorption (Lambda Max) the extent to which a sample absorbs light depends upon the wavelength of light. The wavelength at which a substance shows maximum absorbance is called absorption maximum or lambda max the value of lambda max is important for several reasons.

1) This wavelength is characteristic of each compound

2) It provides information on the electronic structure of the analyte

3) It ensures highest sensitivity and minimizes deviation from Beers Law

3. RESULT AND DISCUSSION

A. Linearity:-

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 5-25µg/ml for Gemfibrozil in triplicate into the UV-VIS system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Table No. 1: Linearity of data of gemfibrozil

Sr. No.	Concentration in µg/ml	Absorbance at 272 nm	Area Under Curve (AUC)
1	5	0.1038	1.7746
2	10	0.2042	3.1253
3	15	0.3032	4.6877
4	20	0.4001	6.2318
5	25	0.5098	7.6204

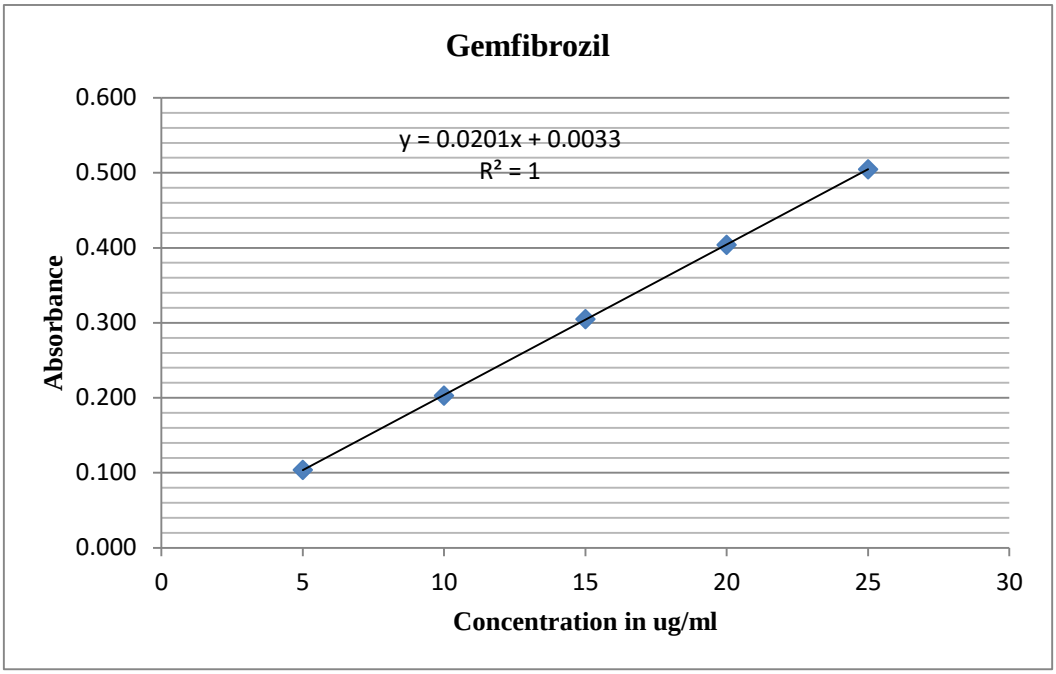

Figure No. 2: Calibration of Curve of Gemfibrozil.

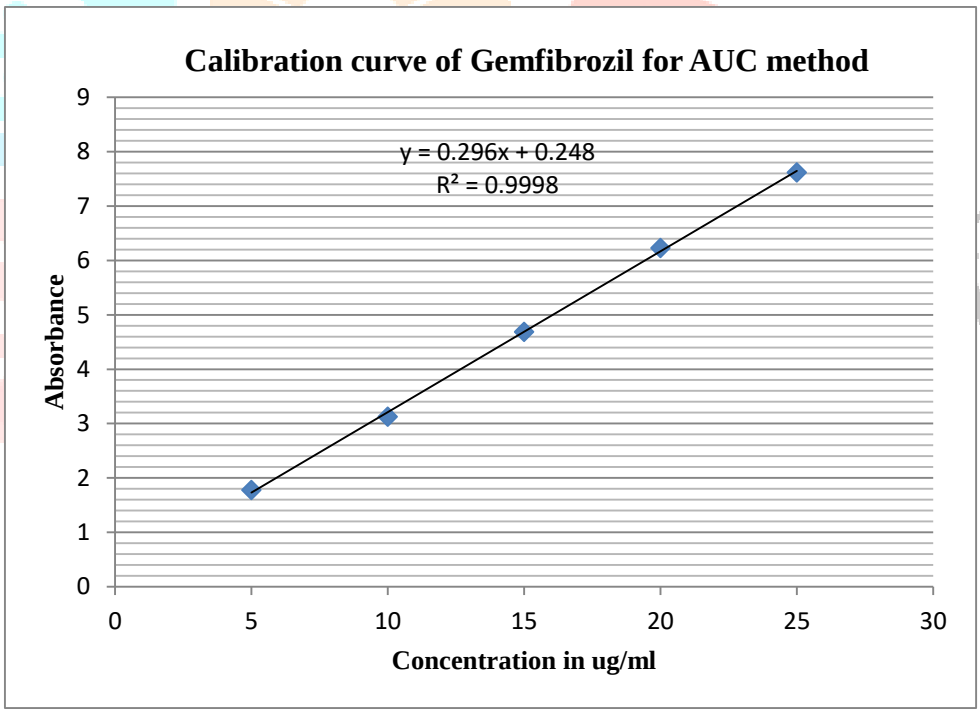

Figure No. 3: Calibration curve of Gemfibrozil for AUC method

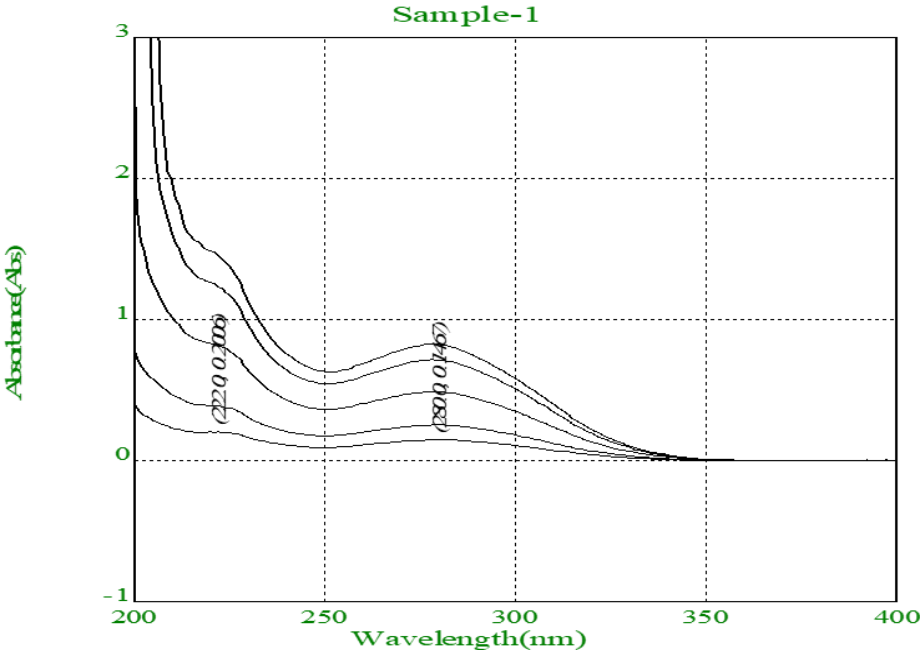

Figure No. 4: Overlay spectra of Gemfibrozil

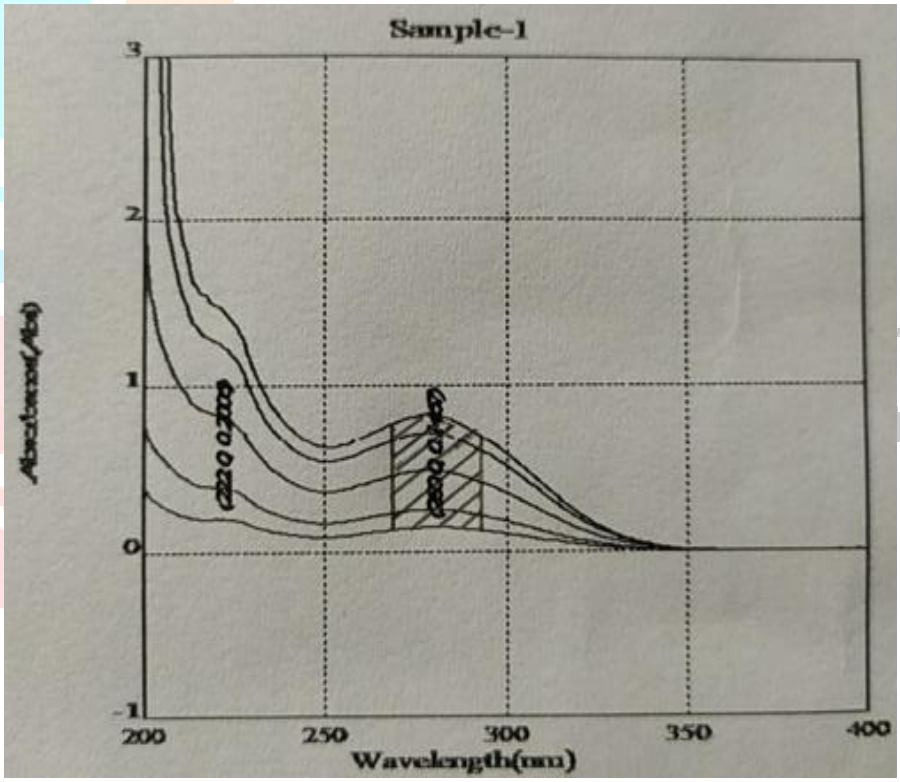

Figure No. 5: Overlay spectra of Gemfibrozil for AUC method

B. Precision:-

Precision of the method was verified by repeatability and intermediate precision studies. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of %RSD (relative standard deviation). Repeatability studies were performed by analyses of concentrations 20µg/ml of Gemfibrozil for UV-VIS on the same day. Intermediate precision of the method was checked by repeating these studies on two different days.

Table No. 2: Precision of data of gemfibrozil.

Sr. No.	Concentration	Intraday		Interday	
		Abs	AUC	Abs	AUC
1	10	0.2021	0.8838	0.2032	0.8913
2	15	0.3095	1.6965	0.3075	1.7453
3	20	0.4004	6.1784	0.4002	6.2632
4	SD	0.011	0.053	0.08	0.021
5	%RSD	0.5229	0.7352	0.2518	0.2432

C. Accuracy:-

For both UV-VIS method recovery studies was carried out by spiking known amount of standard drug corresponding to 80%, 100% and 120% w/w of label claim had been added to marketed drug sample (Standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

Table no. 3: Accuracy data of Gemfibrozil

Drug taken	Amt added	Abs. maxima	Amount found	% Amount Found	SD	%RSD
80%						
10	8	0.3591	17.91	97.56	0.07	0.42
10	8	0.3612	17.81	98.87	0.07	0.94
100%						
10	10	0.4011	19.90	99.05	0.02	0.09
10	10	0.4005	19.88	98.75	0.02	0.18
120%						
10	12	0.4371	21.70	98.63	0.03	0.13
10	12	0.4378	21.74	98.81	0.03	0.24

Table no. 4: Accuracy data of AUC of Gemfibrozil

Drug taken	Amt added	AUC	Amount found	% Amount Found	SD	%RSD
80%						
10	8	5.6363	17.95	99.72	0.09	0.50
10	8	5.6474	17.99	99.91	0.09	0.96
100%						
10	10	6.2632	19.94	99.70	0.04	0.11
10	10	6.2745	19.98	99.90	0.04	0.17
120%						
10	12	6.8598	21.53	97.80	0.05	0.15
10	12	6.8786	21.99	99.91	0.05	0.21

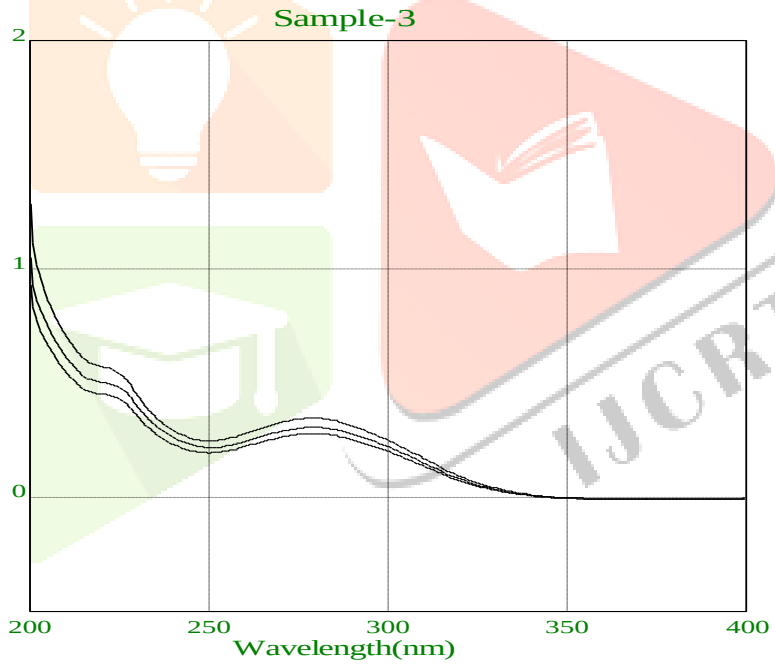

Figure No. 6: Overlay spectra of Gemfibrozil for Accuracy

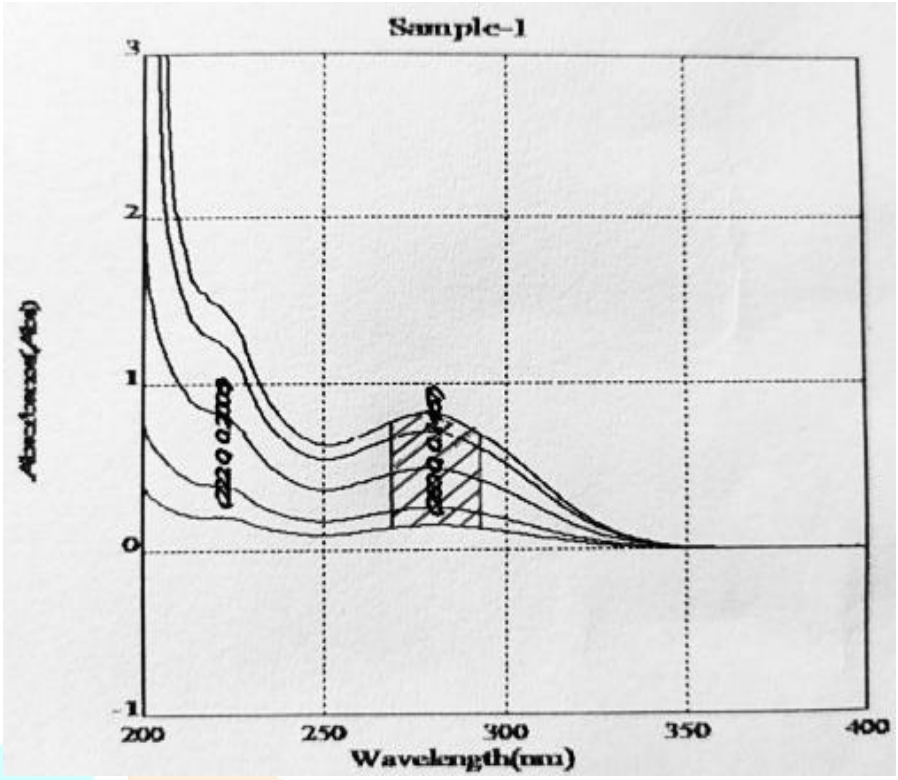

Figure No. 7: Overlay spectra of Gemfibrozil for AUC method

D. Repeatability:-

Implementing the procedure under spectrophotometric condition of experimental section, the homogeneous mixture of 20µg/ml of each selected analytes was injected six times with similar procedure within a same day. The% RSD was calculated and found it is less than 2%.

Table no. 5: Repeatability data of gemfibrozil.

Sr. No.	Concentration	Absorbance	Amount found	AUC	SD	%RSD
1	20	0.4001	19.86	6.2465	0.18	0.90
2	20	0.4012	19.91	6.2342		
3	20	0.4011	19.90	6.1986		
4	20	0.4015	19.92	6.1785		
5	20	0.4010	19.50	6.2213		

E. Ruggedness:-

Table no. 6: Ruggedness data of Gemfibrozil

Sr. No.	Concentration	Analyte	Amount found	% Amount found
1	20	0.4011	19.91	99.55

F. Tablet Assay:-

Twenty tablet of (Gemfibrozil 600mg tablet, LOPID, Pfizer Pvt. Ltd.) were weighed accurately and powdered weigh equivalent to 30 mg of Gemfibrozil was weighed accurately and transferred to 100.0 ml volumetric flask to get 300µg/ml. The solution was filtered through filter paper no. 41; 3.0 ml of this filtrate was further diluted to 10 ml Distilled Water. From this solution, 5ml pipette out and diluted to 10ml to get the final concentration of 15µg/ml of Gemfibrozil. The solution was scanned in the range of 200-400 nm against blank. Absorbance's were recorded at wavelength 272nm. The concentration of drug was then calculated**.**

Table no. 7: Tablet Assay data of Gemfibrozil.

Sr. No.	Concentration in µg/ml	Absorbance	Amt found	AUC	Amt Found	% Amt found	SD	%RSD
1	15	0.3051	15.10	1.7427	15.07	100.70, 100.47	0.35	0.37

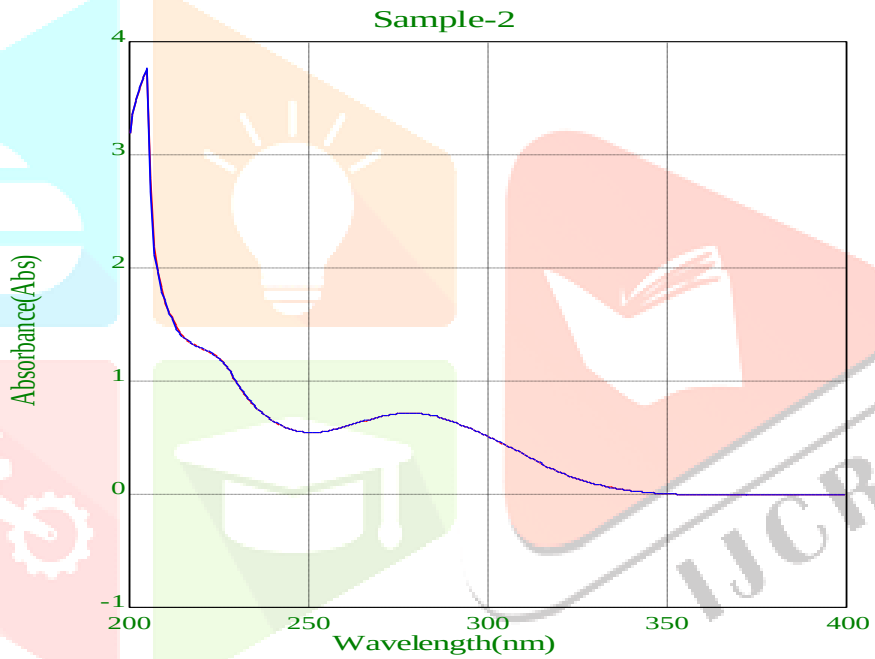

Figure No. 8: Spectrum of tablet assay for Gemfibrozil

G. LOD and LOQ

The LOD and LOQ were found to be 0.6237µg/ml and 1.89µg/ml for absorption maxima, 0.5908µg/ml and 1.79µg/ml for AUC respectively, these low values of LOD and LOQ confirmed that the method is sensitive.

Table no. 7: LOD and LOQ data of Gemfibrozil

Parameters	Absorption maxima	AUC
LOD (µg/ml)	0.6237	0.5908
LOQ (µg/ml)	1.89	1.79

4. CONCLUSION

Analytical Technologies Limited UV-Visible Spectrophotometer Model SPECTRO UV 2080 was used to develop accurate and precise method employing Methanol: Water (10:90 % v/v) as a solvent. The methods that have been developed for the estimation of gemfibrozil in bulk and dosage form are Absorption maxima and Area under Curve method. The method involves absorbance measurement at 272 nm (λ_{max} of gemfibrozil). The method followed Beer's law in the range of 5-25µg/ml for both methods at selected wavelengths. Correlation coefficient was found in the range of 1 to 0.9998 for the methods. The proposed methods were first applied to bulk powder and results indicated that Gemfibrozil could be estimated accurately and precisely by these methods. The developed methods were then employed for the analysis of marketed formulation. The developed methods were validated as per ICH guidelines.

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