Optimization And Validation of UV Analytical Methods for Fenofibrate Using Various Calibration Techniques

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***Abstract*—UV spectrophotometric techniques play a crucial role in pharmaceutical analysis for the estimation of active compounds. This study presents the development and validation of a UV-Visible Spectroscopy method for Fenofibrate using methanol as a solvent. The method adheres to Beer-Lambert's law and ICH guidelines, exhibiting a maximum absorbance at 290 nm. Excellent linearity (R^2 = 0.9994) was observed over the tested concentration range, with precise LOD and LOQ values confirming the method's sensitivity. Additionally, the method demonstrated high precision, accuracy, and robustness, making it a reliable choice for routine pharmaceutical quality control applications.**

***Keywords* — UV spectrophotometry, Fenofibrate, Methanol, Validation, ICH, Pharmaceutical Analysis**

I. INTRODUCTION [1,2,3]

Fenofibrate is official drug Indian Pharmacopoeia. Fenofibrate (FF) is a member of fibrate class with lipid regulating action commonly referred to as fibric acid derivative. Fenofibrate has IUPAC name as -propane-2-yl-2-{4-[4 chlorophenyl)-carbonyl] phenoxy}-2-methyl propanoate. The molecular formula is $C_{20}H_{21}ClO_4$ and molecular weight is 360.83 g/mol. It is used as a lipid regulating agent in the patients which a reat the risk of cardiovascular disorders. Fenofibrate is a member of fibrate class with lipid-regulating action commonly referred to as fibric acid derivative. It is almost white crystalline powder, which is stable under ordinary conditions with melting point 79-82 ^{0}C.

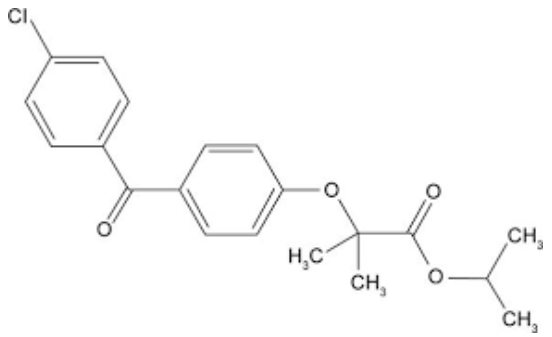

Fig. No. 1

Fenofibrate

It is virtually water insoluble (<0.3 µg/ml) but slightly soluble in alcohol and has relatively high octanol/water partition coefficient (log P 4.6). Fenofibrate is mainly used to reduce the amounts of low-density lipoprotein (LDL) cholesterol, total cholesterol, triglyceride and apolipoprotein B, and increase the amounts of high-density lipoprotein (HDL) cholesterol in the blood. Fenofibrate is a prodrug which is converted rapidly after oral administration through the hydrolysis of the ester bond to fenofibric acid[3-5].

II. MATERIALS AND METHODS

2.1 Instrumentation

The study was performed using a Systronics (India) Limited UV-visible spectrophotometer, Model AU-270, featuring a double beam and double detector arrangement with a 1 cm matched cell. The software used was UV Professional Double Beam. The mobile phase was degassed using an ultrasonicator cleaner, and an electronic balance was used for weighing.

2.2 Chemicals and Reagents

MPS standard was obtained from CTX Lifesciences Pvt. Limited, batch no. 24MS000014, with a manufacturing date of February 2024 and an expiration date of 2029. Analytical-grade reagents were gifted by Ajanta Pharmaceutical PPIC department, Mumbai. Calibrated glassware from Borosil Company was used throughout the analysis.

III. METHODOLOGY

3.1 Selection of Solvent

Methanol was chosen as the solvent due to its superior solubility properties for Fenofibrate.

3.2 Preparation of Standard Stock Solution

A 50 mg quantity of pure Fenofibrate was accurately weighed and dissolved in methanol in a 50 ml volumetric flask to obtain a final concentration of 1000 ppm. A working standard of 100 ppm was prepared from this stock solution using distilled water.

3.3 Determination of Lambda Max

The UV spectrum of MPS was scanned between 200 and 400 nm at a concentration of 100 ppm. The maximum absorbance wavelength was observed at 290 nm.

3.4 Estimation of Fenofibrate [4]

Table No. 1 Absorbance

Concentration	Absorbance
05 PPM	0.138
10 PPM	0.325
15 PPM	0.521
20 PPM	0.697
25 PPM	0.871
TAB 10	0.301

3.4.1 Standard Absorptivity Method

- Prepare standard solutions of Fenofibrate in methanol at concentrations ranging from 10 ppm to 50 ppm.
- Measure the absorbance of each solution at 290 nm using a UV-visible spectrophotometer.
- Calculate the absorptivity (a) using Beer-Lambert's law:

$$A = abc$$

Where:

- A = Absorbance
- a = Molar Absorptivity
- b = Path length (1 cm)
- c = Concentration in ppm

The standard absorptivity method is based on the principle of Beer-Lambert's law, which states that absorbance is directly proportional to concentration by determining the absorptivity coefficient (a), unknown sample concentrations can be estimated accurately. This method is widely used due to its simplicity and ability to provide reliable quantitative results. By using formula, it was noted that the percentage purity of drug by using this method was found 92.61%. (By using Table No. 1)

3.4.2 Single Point Standardization Method

- Prepare a standard solution of Fenofibrate in methanol at a known concentration (e.g., 100 ppm).
- Prepare the test sample solution in methanol.
- Measure the absorbance of both the standard and test sample at 290 nm using a UV-visible spectrophotometer.
- Calculate the concentration of the test sample using the equation:

$$Ctest = (Atest \times Cstd) / Astd$$

Where, Ctest = concentrations of sample

Cstd = concentrations of standard

Atest = absorbance of the sample

Astd = absorbance of the standard

The Single Point Standardization Method is a direct and simple approach for estimating the concentration of an unknown sample by comparing its absorbance with that of a standard solution of known concentration. It is particularly useful for quick and routine analysis where high precision is not required. By using formula, it was noted that the percentage purity of drug by using this method was found 92.61 %. (By using Table No. 1)

3.4.3 Calibration Curve Method

- Prepare standard solutions of Fenofibrate in methanol at concentrations ranging from 10 ppm to 50 ppm.
- Measure the absorbance of each solution at 290 nm using a UV-visible spectrophotometer.
- Plot a calibration curve by graphing absorbance against concentration.
- Determine the equation of the calibration curve using linear regression analysis.
- Use the calibration curve equation to estimate the concentration of unknown samples by interpolating their absorbance values.

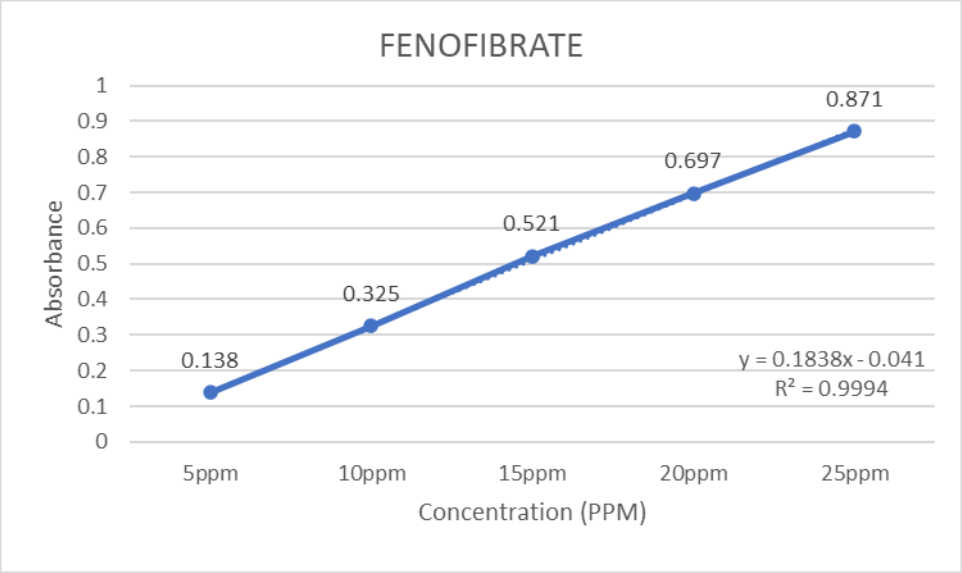

Fig. No. 2 Calibration Curve

The Calibration Curve Method is based on Beer-Lambert's law, where absorbance is directly proportional to concentration. By constructing a standard calibration curve, the concentration of unknown samples can be accurately determined. This method ensures high precision and reliability in quantitative analysis. By using equation of line after calculating by using formula, it was noted that the percentage purity of drug by using this method was found 92.61 %.

IV. METHOD VALIDATION[5]

4.1 Linearity[6-9]

The closeness of the test results obtained by the method to the true value or an accepted reference value. It is usually expressed as a percentage recovery. The regression coefficient (R^2) from the linearity curve was found to be 0.9994, indicating excellent linearity

Table No. 2 Linearity

Concentration	Absorbance	Calculations	
05 PPM	0.138	MEAN	0.4755
10 PPM	0.325	SD	0.518309
15 PPM	0.521	RSD	1.09003
20 PPM	0.697	%RSD	109.003
25 PPM	0.871		
TABLET 10 PPM	0.301		

* Result of RSD & %RSD is Mean of Three Reading

4.2 Precision[10-13]

The degree of repeatability of an analytical method under normal operating conditions. It is divided into:
Intraday and Interday Precision
Results showed minimal variation in absorbance values over different time intervals and on different days, demonstrating the method's precision

Table No. 3 Intraday Precision

Time	Concentration	Absorbance	Calculations	
0 Hrs.	15 PPM	0.521	Mean	0.522
2 Hrs.	15 PPM	0.523	SD	0.002366
4 Hrs.	15 PPM	0.519	RSD	0.004533
8 Hrs.	15 PPM	0.525	%RSD	0.453339

12 Hrs.	15 PPM	0.524		
24 Hrs.	15 PPM	0.52		

Table No. 4 Interday Precision

Time	Concentration	DAY 1	DAY 2	DAY 3
0 Hrs.	15 PPM	0.521	0.52	0.523
2 Hrs.	15 PPM	0.518	0.522	0.525
4 Hrs.	15 PPM	0.519	0.524	0.521
8 Hrs.	15 PPM	0.524	0.526	0.519
12 Hrs.	15 PPM	0.526	0.523	0.524
24 Hrs.	15 PPM	0.523	0.521	0.527
Mean		0.521833	0.522667	0.523167
SD		0.003061	0.001972	0.002609
RSD		0.005865	0.003773	0.004986
%RSD		0.58649	0.377301	0.498645

4.3 Accuracy[13-15]

Accuracy is often assessed at different levels, typically 80%, 100%, and 120% of the target concentration in validation studies. This ensures that the analytical method provides reliable results across a range of concentrations. The accuracy is determined by recovery studies, where known amounts of the analyte are added to the sample, and the percentage recovery is calculated.

Table No.5 Accuracy

Spiked Level (%)	Added Concentration	Absorbance	% Recovery
80	16ppm	0.521	82.92634
		0.526	
		0.523	
100	20 ppm	0.697	100
		0.695	
		0.692	
120	24 ppm	0.871	120
		0.874	
		0.876	

*Result shows mean of 3 readings

%Recovery = mean of reference/mean of stnd*100

4.4 Ruggedness[16-20]

The reproducibility of the method under normal conditions by different analysts, instruments, or laboratories. Two analysts performed the study independently, and % RSD was found to be within acceptable limits

Table No. 6 Ruggedness

Analyst	X	Y
System	Systronics	Shimadzu
Day	Monday	Thursday
Time	Morning	Evening
Concentration	15 PPM	15 PPM
	0.521	0.523
	0.519	0.525
Absorbance	0.525	0.527
Mean	0.521667	0.525
SD	0.002494	0.001633
RSD	0.004782	0.00311
%RSD	0.478167	0.311046

4.5 Robustness[21-23]

The ability of the method to remain unaffected by small but deliberate variations in method parameters

(e.g., pH, temperature, flow rate) and still provide accurate results. The method's robustness was assessed at three wavelengths (287, 290, and 292 nm), showing minimal variation in absorbance values

Table No. 7 Robustness

Wavelength	Concentration	Absorbance	Calculations	
287 nm	15 PPM	0.521	SD	0.002944
287 nm	15 PPM	0.519	RSD	0.00564
287 nm	15 PPM	0.526	%RSD	0.563969
290 nm	15 PPM	0.525	SD	0.00432
290 nm	15 PPM	0.527	RSD	0.008261
290 nm	15 PPM	0.517	%RSD	0.826098
292 nm	15 PPM	0.529	SD	0.001633
292 nm	15 PPM	0.531	RSD	0.003075
292 nm	15 PPM	0.533	%RSD	0.307532

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)[24-27]

LOD is the lowest amount of analyte that can be detected but not necessarily quantified under stated conditions and LOQ is the lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy.

$LOD = \frac{3.3\ X\ \sigma}{S}$ $LOQ = \frac{10\ X\ \sigma}{S}$

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

LOD and LOQ were calculated as 0.0178µg/ml and 0.0503µg/ml, respectively.

5. Estimation of Fenofibrate from Tablet Dosage Form

Weigh and finely grind 20 tablets. Weigh a portion equivalent to 5 mg of Fenofibrate.

Dissolve in 10 mL methanol (Sonicate if needed). Filter the solution to remove excipients. Take 1 mL of the filtrate and dilute to 10 mL (final concentration ~100 ppm). Check the absorbance of this resulting solution at 290 nm. By using formula, it was noted that the percentage purity of drug was found 92.61 %.

Table No. 8 Assay of Marketed Formulation

Concentration	Absorbance at 287 nm
15 ppm	0.521
15 ppm	0.523
15 ppm	0.525
Mean	0.523
SD	0.00163299
RSD	0.00312236
%RSD	0.521

V. DISCUSSION

The ICH recommendations are followed by the established UV spectrophotometric technique for fenofibrate. It exhibits excellent linearity, sensitivity, robustness, accuracy, and precision. The suggested approach offers a productive and economical substitute for standard pharmaceutical analysis.

VI. CONCLUSION

In accordance with ICH requirements, a straightforward, precise, and cost-effective UV-spectrophotometric technique for determining fenofibrate was successfully designed and validated. For routine pharmaceutical quality control, this method provides a dependable solution.

Conflict of Interest

The authors declare no conflicts of interest relevant to this article.

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