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UV-SPECTROPHOTOMETRIC ASSAY METHOD DEVELOPMENT AND VALIDATION OF GEMFIBROZIL IN BULK AND TABLET FORMULATION

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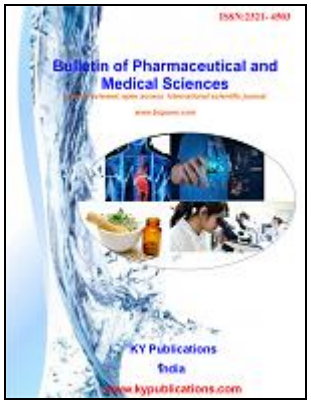

ABSTRACT
A validated simple and sensitive UV-Spectrophotometric assay has been developed and optimized for the determination of gemfibrozil in pharmaceutical formulations. The method is based on the direct measurement of the absorbance of the analyte at 220nm. The present assay was optimized and validated in terms of linearity, repeatability, detection limit, accuracy, and selectivity. Linear calibration curve for the present studied drug was obtained in the range of 5.0-30µg/mL with the detection limit of 2.47µg/mL respectively. The proposed method can be applied successfully for the determination of gemfibrozil in tablet dosage form with a high percentage of recovery, good accuracy and precision, and without measurable interference by the excipients.

KEY WORDS: Gemfibrozil, UV-spectrophotometric assay, ICH validation.

INTRODUCTION:

Gemfibrozil[1-3], 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (**Fig.1**), is a fibric acid anti-lipemic agent used to treat hyperlipoproteinemia and as a second-line therapy for type IIb hypercholesterolemia. It acts to reduce triglyceride levels, reduce VLDL levels, reduce LDL levels (moderately), and increase HDL levels (moderately).it 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. This increase in the synthesis of lipoprotein lipase thereby increases the clearance of triglycerides

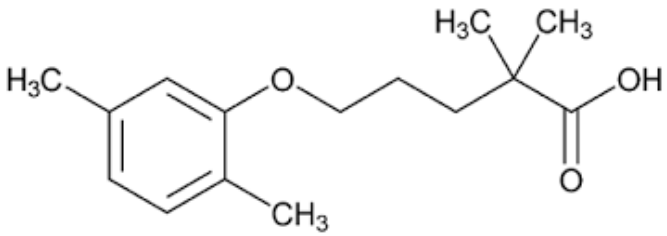

Fig:1 – Molecular Structure of Gemfibrozil

The empirical formula is $C_{15}H_{22}O_3$ and the molecular weight is 250.35. It is available in local pharmacies as capsules for oral administration [Each capsule containing 300mg of gemfibrozil.

Several HPLC methods in various body fluids[4-10] and one UV-spectrophotometric method[11] in pharmaceutical formulations have been reported and published for gemfibrozil assay. This fact prompted the

author to develop a simple, inexpensive UV-spectrophotometric method for the determination of gemfibrozil in pure and in dosage forms. The present research paper describes the development and validation of the UV-spectrophotometric method for the assay of gemfibrozil in pure and from its formulation (tablets) as per ICH validation guidelines using double distilled water as solvent.

MATERIALS AND METHODS

INSTRUMENTATION: UV visible 1601 Shimadzu double beam spectrophotometer was used to measure spectra. The solvent which are used for the assay was water. All weighing experiments were done on Schimadzu Digital Analytical Balance (Japan) and standard glass ware (Borosil Make) was used for preparing of solution.

CHEMICALS AND REAGENTS: Gemfibrozil (99.9% Pure) used was supplied by Dr Reddys Labs, Hyderabad and its formulation (Capsules) in the brand name of Gempar (strength: 300mg of Gemfibrozil) from Cadila labs, India Ltd were purchased from local pharmacy. Double distilled water was used for the preparation of standard and sample solutions without further purification.

DILUENT: Double distilled water was used as diluent in the present assay.

PREPARATION OF STANDARD SOLUTIONS: Accurately weigh 100mg of gemfibrozil test standard and transfer into a 100mL volumetric flask containing 25mL of double distilled water. This was sonicated for about 5 min to dissolve it and the resultant solution was further diluted to 100mL with double distilled water. Working standard solutions in concentration range of 5.0 - 30µg/mL were prepared by transferring aliquot of the above stock solution to a series of different 100mL and diluted to the mark with the same diluent.

PREPARATION OF SAMPLE SOLUTION: For the assay of gemfibrozil in formulations 10 Capsules of gemfibrozil (**Gempar - 300mg** manufactured by Cadila Labs) purchased from local pharmacy. The shells of the prescribed capsules were removed and the powder was weighed. Then powder equivalent to 100mg was transferred into a 100mL clean dry volumetric flask, 70mL of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was latter diluted up to the mark with diluent. Suitable aliquots of this solution was taken and diluted into a series of 10mL volumetric flask with the same diluent up to the mark, to obtain concentrations that obey within the beers law limit for the spectrophotometric measurement of gemfibrozil according to the recommended procedure.

RESULTS & DISCUSSION:

METHOD DEVELOPMENT: Working standard solution (10µg/mL) of gemfibrozil prepared was subjected to scanning between 200 – 400 nm and the absorption maximum was determined and an optimal response was obtained at 220.60nm. This wavelength of 220.60nm was used for the quantification of standard and in dosage forms of gemfibrozil respectively. The absorption spectrum so obtained was shown in **Figure2**.

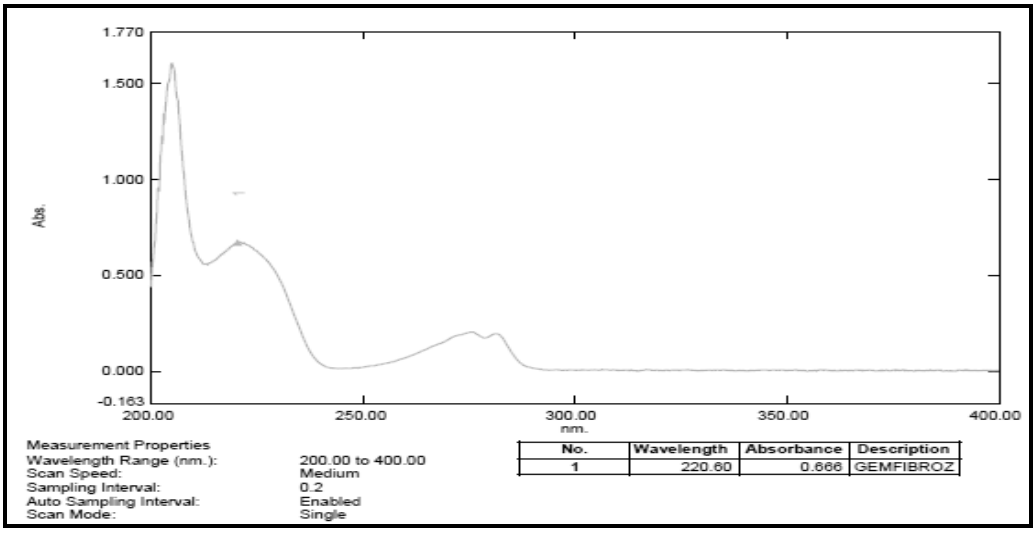

Fig: 2 - A Typical UV Spectra of Gemfibrozil

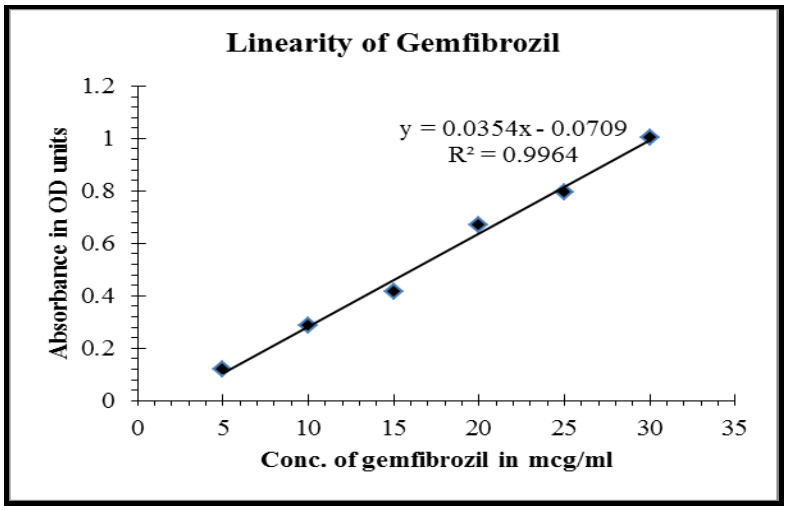

Fig:3 - Linearity Curve of Gemfibrozil

PROCEDURE: Working standard solutions of gemfibrozil in concentration range of 5.0-30µg/mL were placed in an cuvette of UV-spectrophotometer and the absorbance's of the each standard preparation were measured at this fixed wavelength (λ_{max} 220.60nm) and the quantity of gemfibrozil in standard preparation was calculated. The same procedure was carried for gemfibrozil in dosage forms.

METHOD VALIDATION: The developed UV-Spectrophotometric method of gemfibrozil was validated using the following parameters.

a. SPECIFICITY:

i. BLANK INTERFERENCE: The interference of blank at the working wavelength was scanned from 200-800nm and was observed the non-interference of blank at the working wavelength of 220.60nm for gemfibrozil, revealing the specificity of the proposed UV spectrophotometric method for gemfibrozil.

b. LINEARITY: The linearity of the proposed method was made by determining the absorbance of different working concentrations of gemfibrozil in triplicate over a range of 25% (5.0µg/mL) to 150% (30µg/mL). Correspondingly a calibration graph was plotted by plotting the absorbance recorded verses the concentration and was treated by least-squares linear regression analysis. The results of regression analysis i.e, slope, intercept with correlation coefficient more than 0.9999, [**Table.1** & **Figure.3**] indicated the linearity of the proposed method with optimum value of standard error.

LOD and LOQ: The detection and quantization limits were found to be 2.47 and 8.25µg/ml for gemfibrozil and were found to be in sub-microgram level indicating the good sensitivity of the proposed method.

c. PRECISION: The precision of the present proposed method was performed in six replicates of fixed concentration of gemfibrozil and the percentage relative standard deviation (%RSD) was measured. The %RSD of 0.835 tabulated (**Table.2**) was less than 2.0% (Accordance to ICH norms) revealing good precision of the proposed method.

d. ACCURACY: The recovery studies of the proposed method was analyzed in triplicate preparations on composite blend collected from 10capsules of gemfibrozil as per the proposed method at three different levels and the results of percentage recoveries were reported **Table.3.** The percent recovery at each level was ranged from 98.70-99.74% respectively, indicating insignificant interference from the excipients.

e. RUGGEDNESS: The ruggedness of the present proposed method for gemfibrozil was made by the precision study performed on another instrument by another analyst and the results are reported. The %RSD obtained in this study were found to be not more than 2.0% (**Table.4**) making the current method rugged.

f. SOLUTION STABILITY: In this study the absorbance of the same standard and sample solutions of gemfibrozil in triplicate at intervals of 0 hours, 12hours, and 24 hours were recorded and the cumulative %recovery at each interval was determined. The % recoveries tabulated in **Table.5** revealed the stability of the proposed method.

g. ASSAY OF DOSAGE FORMS: Pharmaceutical assay was carried out on available brand of gemfibrozil (**Gempar:** Label claim 300mg) procured from local pharmacy using the developed UV spectrophotometric method and the % assay was calculated and the results were tabulated (**Table.6**). These results revealed that the proposed UV spectrophotometric method can be used for routine quality control analysis for gemfibrozil in its pure and in dosage forms.

TABLE.1. STATISTICAL PARAMETERS FOR UV- SPECTROPHOTOMETRIC DETERMINATION OF GEMFIBROZIL

% LEVEL	CONC	ABSORBANCE
25	5.0	0.121
50	10.0	0.288
75	15.0	0.414
100	20.0	0.669
125	25.0	0.795
150	30.0	1.005
Slope,b		0.0354
Intercept,a		-0.0709
Correlation,r2		0.9964
LOD (µg/mL)		2.47
LOQ (µg/mL)		8.25

TABLE.2: PRECISION STUDIES FOR GEMFIBROZIL

S NO	NAME	ABSORBANCE
1	SOLUTION-1	0.663
2	SOLUTION-2	0.661
3	SOLUTION-3	0.658
4	SOLUTION-4	0.66
5	SOLUTION-5	0.664
6	SOLUTION-6	0.674
AVG*		0.663
STD DEV*		0.0051
% RSD*		0.851

*Average of six determinations considered

TABLE.3: RECOVERY STUDIES FOR GEMFIBROZIL

ACCURACY LEVEL	50%	100%	150%
S NO	ABSORBANCE	ABSORBANCE	ABSORBANCE
INJECTION-1	0.323	0.646	0.975
INJECTION-2	0.325	0.648	0.978
INJECTION-3	0.321	0.643	0.971
AVG*	0.323	0.646	0.975
AMT. RECOVERED*	49.58	98.70	149.60
%RECOVERY*	99.16	98.70	99.74

*Average of three determinations considered

TABLE.4:RUGGEDNESS STUDIES FOR GEMFIBROZIL BY THE PROPOSED METHOD

S NO	NAME	ANALYST -1 ABSORBNACE	ANALYST -2 ABSORBANCE
1	SCAN-1	0.661	0.656
2	SCAN-2	0.66	0.653
3	SCAN-3	0.669	0.659
4	SCAN-4	0.671	0.65
5	SCAN-5	0.673	0.649
6	SCAN-6	0.671	0.659
AVG*		0.668	0.654
STD DEV*		0.006	0.004
% RSD*		0.835	0.667

*Average of six determinations considered

TABLE:5. RESULTS OF STABITY STUDIES OF STANDARD AND SAMPLE SOLUTIONS OF GEMFIBROZIL WITH THE PROPOSED METHOD

TIME INTERVAL (Hrs)	%RECOVERY	
	STANDARD(n=3)	SAMPLE(n=3)
0	99.93	99.88
12	99.97	99.96
24	99.92	99.95

TABLE.6: ASSAY OF GEMFIBROZIL IN MARKET BRANDS

MARKET BRAND OF THE DRUG	TAKEN mg	FOUND* mg	% ASSAY*
GEMPAR	300	299.97	99.94

*Average of three determinations considered

CONCLUSIONS

The proposed method described in this paper is free from rigid experimental conditions and are characterized by wide linear dynamic range with high sensitivity, and employ inexpensive and easily available chemicals. The low detection and quantification limits, simplicity and selectivity make the developed method suitable for quality control in the pharmaceutical industry for routine analysis.

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