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Development and Validation of UV-Vis Spectrophotometric Method for the Estimation of Bezafibrate Parenteral Formulation

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ABSTRACT

Bezafibrate is a fibrate-class antihyperlipidemic medication. For the validation of bezafibrate injection, an efficient, accurate, simple, fast, precise, and reproducible UV-spectrophotometric approach in water was developed. The typical 10 µg/mL solution was scanned between 200 and 400 nm, and the maximum was found to be 228 nm. The developed method's validation parameters were tested in accordance with ICH criteria. The linearity concentration range of 2 - 10 µg/mL demonstrated that it followed Lambert's law and that the developed method was linear with a high correlation coefficient. The proposed approach was tested using the standard addition method, and percent recovery investigations confirmed that it was accurate. For precision investigations, the percent relative standard deviation (RSD) value was determined, indicating that the developed approach was highly exact. Linearity, precision, intraday precision, intermediate precision, accuracy, specificity, limit of detection, and limit of quantification were all validated for the established method. All of these metrics strongly demonstrated that the new method can be utilized for routine validation of bezafibrate injection in pharmaceutical laboratories.

Keywords:Bezafibrate, method development, parenteral, ICH guidelines, UV spectrophotometry

INTRODUCTION

Bezafibrate is an antilipidemic peroxisome proliferator-activated receptor alpha (PPAR) agonist. Bezafibrate lowers triglyceride levels, raises HDL cholesterol levels, and lowers total and low-density lipoprotein (LDL) cholesterol levels [1]. Its chemical name is (2-{*p*-[2-(*p*-chlorobenzamide) ethyl]phenoxy}-2-methylpropanoic acid) [2]. Bezafibrate is only available orally and in both immediate and sustained-release oral dosage forms [3]. Bezafibrate has a very low water solubility, therefore being a BCS class II medication, as a result, its bioavailability upon oral administration is quite low [4]. Drugs delivered orally have limited bioavailability, GI adverse effects, and hepatic first-pass metabolism. The delivery of drugs via the parenteral route is created to address these issues.

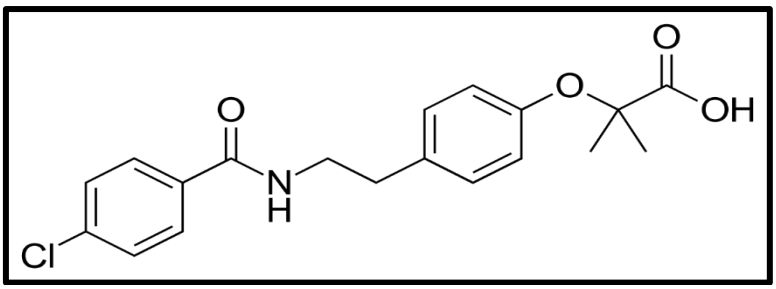

Figure 1. Structure of Bezafibrate

Studies for bezafibrate analysis have included bezafibrate in plasma and urine using HPLC, where bezafibrate in plasma is first extracted with diethyl ether and bezafibrate in urine is analysed directly after being diluted by mobile phase, taking a lot of time and having low accuracy [5].Utilizing the HPLC approach, bezafibrate was administered to human plasma in a tablet dispersion system [6, 7], polarography-based bezafibrate in medicinal formulations [6] HPLC

technique for bezafibrate in rat serum with Ag-Nitrate [8]. Bezafibrate is an oral pharmaceutical formulation that is isolated from other drugs and metabolites using a complex process using the HPLC technique and UV spectrophotometry. A UV spectrophotometric investigation of bezafibrate in the parenteral formulation has not yet been created. In this study, we try to develop a UV spectrophotometric bezafibrate analytical method that is quick, easy, and accurate for parenteral formulation.

MATERIALS AND METHOD

Materials
Bezafibrate was obtained as a gift sample from Sterling Pharmaceuticals. Methanol and distilled water were used.

Instrument
UV-VIS double beam spectrophotometer (UV - 1800 Series, Shimadzu, USA) having the wavelength range of 200nm - 800 nm with two matched quartz cells of 1 cm, Ultrasonic Cleaner (Delmer, India), electronic balance (Electric Mettler Toledo balance, model AL 204) were used.

Preparation of stock and standard solution for the calibration curve
To create a stock solution of bezafibrate for the method development, 10 mg of the medication was dissolved in 10 ml of methanol to produce a final concentration of 1 mg/ml. Following dilutions with distilled water were performed on this stock solution to produce a series of standard solutions with concentrations of 2, 4, 6, 8 and 10 µg/ml.

Preparation of sample
For the preparation of a sample solution, 10.0 mL of the injection formulation of bezafibrate (theoretical content 18 mg) was used to determine the concentration. Distilled water was used to make the proper dilutions, resulting in a final solution that contained 10.0 µg mL^{-1} of free base.

Selection of Wavelength
To determine the absorption maximum (max), the standard solutions' spectrum was run in the 200–400 nm region. Bezafibrate's maximum was discovered at 228 nm. By using distilled water as a blank and measuring the absorbance of the aforementioned dilutions at 228 nm. This study provided a simple, accurate, precise, and reproducible procedure, as demonstrated in Figure 2.

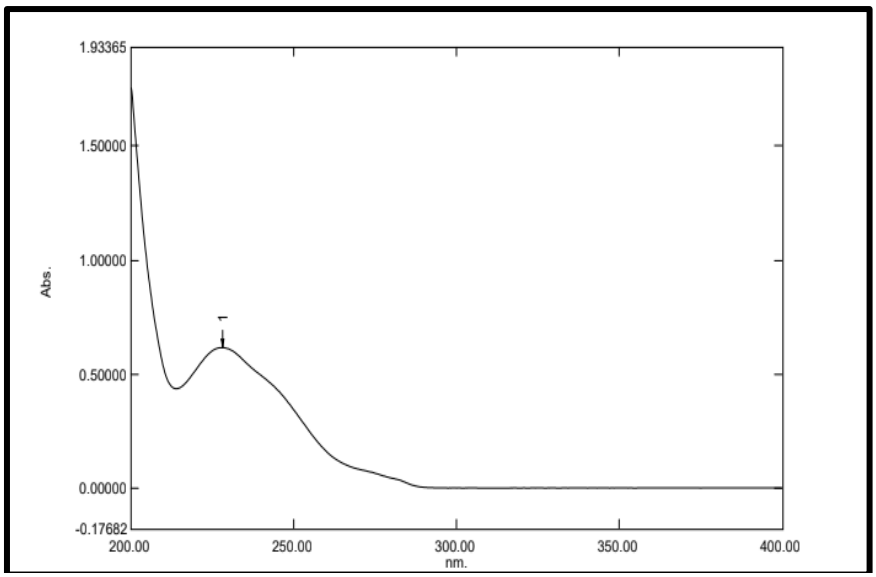

Figure 2. UV spectra of Bezafibrate

VALIDATION OF UV/VISIBLE SPECTROPHOTOMETRIC METHODS

Linearity
To obtain a final concentration of 2, 4, 6, 8 and 10 µg/ml of bezafibrate, five aliquots of the drug solution were taken from the standard stock solution and transferred to a 10 ml volumetric flask. The volume was then filled with distilled water, and the contents of the flask were measured to ascertain the absorbance at the chosen wavelength. At 228 nm, the absorbance of all standard solutions was measured. Concentration vs. absorbance calibration curves were drawn, and correlation coefficient and regression line equations for bezafibrate were calculated.

Table 1. Calibration data of Bezafibrate

Sr. No.	Concentration (µg/ml)	Absorbance
1	2 µg/ml	0.126
2	4 µg/ml	0.26
3	6 µg/ml	0.38
4	8 µg/ml	0.5
5	10 µg/ml	0.618

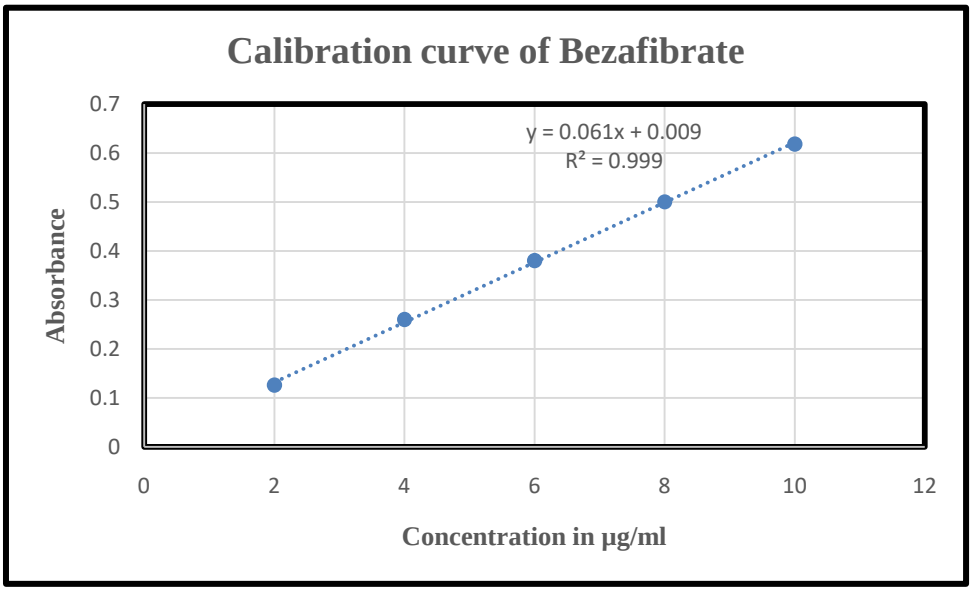

Figure 3: Calibration curve of Bezafibrate

System Precision

By analysing six samples with the same concentration (10 µg/mL) at 228 nm, the precision assessment of the analytical process demonstrates the closeness of the results (Table 2). According to ICH recommendations, the RSD of these samples should not exceed 2%.

Table 2: System Precision

Sr. No.	Concentration	Absorbance	S. D.	% RSD
1	10 µg/ml	0.615	0.00409878	0.686428
2	10 µg/ml	0.618		
3	10 µg/ml	0.607		
4	10 µg/ml	0.612		
5	10 µg/ml	0.616		
6	10 µg/ml	0.61		
		0.613		

Intra-day precision and inter-day precision (intermediate precision)

The intra-day precision was determined by analyzing the samples of Bezafibrate at concentrations of 10.0 µg mL-1. Determinations were performed with three replicates on the same day (Table 3) and inter-day precision was performed by analyzing a series of sample solutions for 3 consecutive days using the proposed UV-spectrophotometric method (Table 4). The precision is expressed as relative standard deviation (RSD) amongst responses. In order to be considered precise, the RSD of the method should be less than 2.0%.

Table 3: Intraday Precision

Sr. No.	Conc.	Abs.	S.D.	% RSD
1	6 µg/ml	0.38	0.002081666	0.5454147
		0.381		
		0.384		
	Mean	0.381666667		
2	8 µg/ml.	0.5	0.005033223	0.9973361
		0.51		
		0.504		
	Mean	0.504666667		
3	10 µg/ml.	0.615	0.004041452	0.6578598
		0.61		
		0.618		
	Mean	0.614333333		

Table 4: Interday Precision

Sr. No.	Conc.	Abs.	S.D.	% RSD
1	6 µg/ml	0.38	0.002081666	0.5421005
		0.388		
		0.384		
	Mean	0.384		
2	8 µg/ml	0.5	0.00305505	0.6077687
		0.502		
		0.506		
	Mean	0.502666667		
3	10 µg/ml	0.615	0.007767453	1.2761424
		0.6		
		0.611		
	Mean	0.608666667		

Accuracy

Through the recovery test, the method's accuracy was assessed. By introducing known concentrations of standard solutions to samples, recovery tests were conducted, and samples were then subjected to analysis using the suggested technique. Standard and sample solutions were divided into aliquots and added to 10 mL volumetric flasks. Distilled water was then added to complete the quantities. Recovery studies were used to evaluate the suggested approaches' accuracy at three different levels, namely 50%, 100%, and 150%. Table 5 reports the findings of recovery research.

Table 5: Accuracy

Drug	Drug amount µg/ml	% Level of addition	Amount added µg/ml	Drug found µg/ml	% Recovery	Average % Recovery
Bezafibrate	10 µg/ml	50	5	4.94	98.8	99.5
		50	5	5.01	100.2	
		50	5	4.96	99.3	
		100	10	9.98	99.8	99.8
		100	10	9.93	99.3	
		100	10	10.02	100.2	
		150	15	14.95	99.7	100
		150	15	15.03	100.2	
		150	15	15.01	100	

Limit of Detection (LOD) and Limit of Quantification (LOQ)

A substance's detection limit is the lowest concentration at which it can be accurately discriminated from its absence with a 1% confidence level. However, the standard's smallest concentration Limit of quantification is a curve that can be quantified with accuracy, precision, and variability (ICH guideline Q2B, 2005).

LOD and LOQ were determined as follows:
LOD = 3.3 × SD/S
LOQ = 10 × SD/S
Where, S = slope of the linearity curve, SD = standard deviation of the y-intercept.

Table 6: Validation Parameters

Absorption maxima	228 nm
Linearity range	2-10 µg/ml
Standard regression equation	y = 0.1224x + 0.0096
Correlation coefficient	0.999
Limit of Detection	0.31 µg/ml
Limit of Quantification	0.94 µg/ml
Beers' law limit	2-10 µg/ml

CONCLUSION

The developed and validated UV-spectrophotometric approach in this work has the benefit of being quick, easy, and reasonably priced while having excellent precision and accuracy for the measurement of bezafibrate in pharmaceutical formulation.

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