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Validated spectrophotometric determination of Fenofibrate in formulation

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ABSTRACT

The aim of present work is to develop and validate spectrophotometric methods for the determination of fenofibrate, an anti-hyperlipidemic, fibric acid derivative in pharmaceutical formulation. Methanol was used as a solvent throughout the study. Quantitative determination of fenofibrate in pharmaceutical formulation was carried out by UV-spectrophotometric method using the absorbtivity values at 287.5 nm and by comparison with standard (method 1a and 1b) and the first order derivative absorbance values at 249.2 nm were utilized for estimation of drug (method 2). The method showed high specificity in the presence of formulation excipients and good linearity in the concentration range of 0-60 µg/mL. Percent recovery values at 287.5 nm were 100.3% while it was 100.18% at in 1st order derivative spectrophotometry at 249.2 nm (n=3). SD values showed that both spectrophotometric methods were reproducible. The intra and interday precision data demonstrated that method is precise.

Key words: Fenofibrate, capsules, tablets, Derivative spectrophotometry.

INTRODUCTION

Fenofibrate, chemically is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanoic acid 1-methyl ethyl ester[1]. It is official in BP[2]. Literature survey revealed that HPTLC[3] and HPLC[4] method has been reported for fenofibrate alone in pharmaceutical formulation. Also HPLC method has been reported for determination of fenofibrate in human serum[5-6] and urine[7]. The present study describes the development and validation of a simple, specific, accurate and precise UV-spectrophotometric method for determination of fenofibrate in pharmaceutical dosage forms.

MATERIALS AND METHODS

Experimental

Fenofibrate working standard was a gift sample from USV Ltd. The Shimadzu UV-VISIBLE spectrophotometer (model UV-1700) was employed for all spectrophotometric measurements. UV-spectra of reference and test solutions were recorded in 1cm quartz cell over the range of 200-400nm.

Reagents and chemicals

Methanol of AR grade purity was procured from local supplier. The commercially available marketed capsule brand containing fenofibrate 200mg in each capsule (Lipicard-200, USV Ltd.) and tablet brand containing fenofibrate 160mg in each tablet (Fenolip , Cipla Ltd.) had been used for estimation.

Preparation of standard stock solution

An accurately weighed quantity of fenofibrate was transferred into a 50 mL volumetric flask, diluted up to the mark with methanol to get a standard stock solution of 0.5 mg/mL. Aliquot portions of standard stock solution was appropriately diluted to get the concentration of 10µg/mL and scanned in the range 400-200 nm. The zero order spectrum and its first order derivative spectrum were recorded and is shown in Figure 1 and 2 repectively.

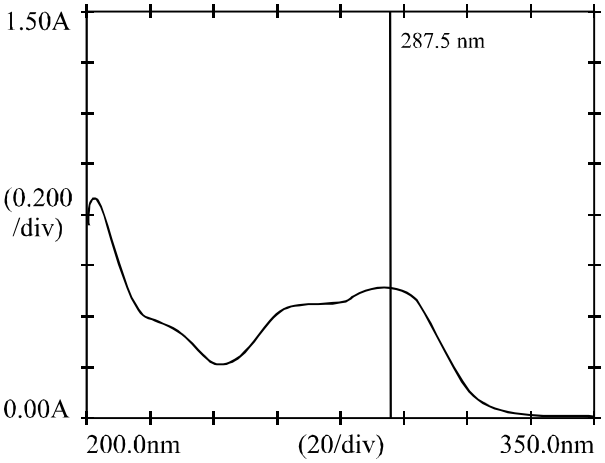

Figure1: Zero order spectra of fenofibrate

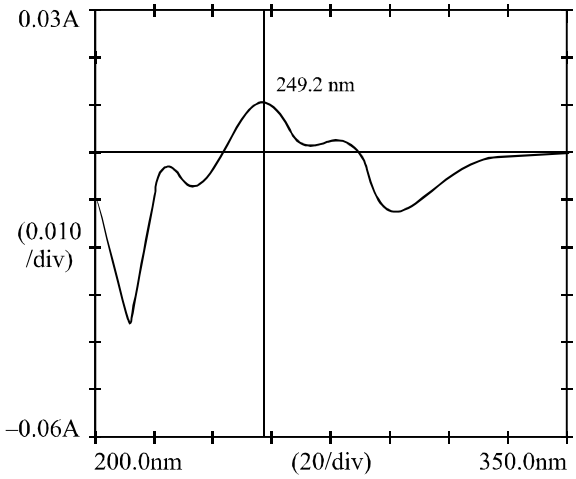

Figure2: First order derivative spectra

Construction of calibration curve

Ten milliliters of this stock solution was further diluted to 50 mL with methanol to give working standard solution (A) of 100µg/mL. Different aliquots of working standard solution (A) was pipetted to prepare a series of concentration from 10-60 µg/mL and zero order absorbance values at 287.5 nm and the first order derivative absorbance values at 249.2 nm (n=3) were read. The calibration curve was constructed by plotting concentration vs. absorbance of fenofibrate from above readings. The drug was found to obey Beer's Law in the concentration range of 10-60 µg/mL for both spectrophotometric methods **Table I**.

Table I: The Statistical Data of Calibration Curve

Parameters	Zero order (at λmax)	First order
Analytical wavelength (nm)	287.5	249.2
Linearity Range (µg/mL)	10-60	10-60
Regression Equation	y = 0.044x + 0.05	y = 0.027x + 0.038
Correlation coefficient	0.9994	0.9999
Detection limit (µg/mL)	0.096	0.096

Assay

An accurately weighed quantity of powder equivalent to about 25mg fenofibrate was transferred to 50 mL volumetric flask, dissolved in methanol, shaken for 15 min and diluted up to the mark

with methanol. The solution was then filtered through Whatmann filter paper (no. 41). Aliquots of stock were appropriately diluted to get concentration of 30µg/mL of fenofibrate(on labeled claim basis). The absorbance of the working solution was read at the selected wavelengths and the amount of fenofibrate was calculated by comparison with the standard and by taking A(1%,1cm) as 468.13±0.938 at 287.5nm (method 1a and 1b) and by derivative spectroscopy at 249.2nm (method 2). Results of estimation are shown in **Table II**.

Table II: Results of estimation of fenofibrate in marketed formulation

Drug	Labeled claim (mg)	Method 1a and 1b		Method 2
		Comparison with standard	By A(1%,1cm)	Derivative spectroscopy
		287.5nm	287.5nm	249.2nm
		% of labeled claim*		% of labeled* claim
Formulation1	25.0	1. 99.13 2. 99.54 3. 99.31 4. 99.21 5. 99.56	1. 98.50 2. 98.86 3. 98.70 4. 98.54 5. 98.89	99.48 100.02 98.83 98.89 98.88
Mean ±SD,CV		99.35±0.19, 0.191	98.70±0.177, 0.179	99.22±0.52, 0.524
Formulation 2	25.0	1. 99.76 2. 99.73 3. 99.57 4. 99.75 5. 99.85	1. 98.74 2. 98.68 3. 98.53 4. 98.71 5. 98.82	100.64 99.65 99.69 100.23 99.77
Mean ±SD,CV		99.73±0.101, 0.101	98.69±0.099, 0.10	99.99±0.48, 0.48

*Mean of 5 observations, Mean ± standard deviation, coefficient of variance

Validation

Analytical method validation was performed as per USP[8] guidelines. The method was validated in terms of accuracy, precision, ruggedness, robustness, specificity, linearity and range and limit of detection.

Accuracy

Accuracy of the method was ascertained by performing recovery studies using standard addition method. The accuracy of the proposed method was examined by recovery studies performed by standard addition method. To a preanalysed sample pure drug was added at four different levels viz. 5mg, 10mg, 15mg, 20mg to a capsule/ tablet powder equivalent to 20mg of fenofibrate. The contents in the flask were appropriately diluted with methanol and zero order absorbance at 287.5 nm and first order derivative absorbance values at 249.2 nm were read and the amount of total drug was calculated and the amount of pure drug recovered was calculated using following formula, Percent recovery= (T-A/S)* 100. Results of recovery studies are shown in **Table III**.

The results are shown in **Table III** indicate excellent recoveries ranging from 99% to 101%. The results indicate that there was no interference from the excipients

Precision

Precision of the analytical method is expressed as SD or RSD of series of measurement by replicate estimation of drugs by proposed method. The percent SD were found to be ±0.19 and

±0.17 at 287.5 nm by comparison with standard and by A (1% 1cm) respectively and ±0.52 by derivative spectroscopy at 249.2 nm for formulation 1. Similarly, the percent SD for formulation 2 were found to be ±0.101 & ±0.099 at 287.5 nm by comparison with standard and by A (1% 1cm) and ±0.48 by derivative spectroscopy at 249.2 nm respectively.

Table III: Result of recovery study

Formulation	Amount of pure drug added (mg)	**287.5nm**	**249.2nm**
		% Recovery	% Recovery
Formulation 1	5.1	101.9	99.41
	10.1	99.90	99.30
	15.1	100.26	101.61
	19.9	99.14	100.43
	*Mean ± SD	100.30 ±1.16	100.18 ±1.07
Formulation 2	5.3	101.88	100.37
	9.5	98.73	97.26
	14.8	99.86	101.41
	20.3	100.64	99.01
	*Mean ± SD	100.27 ±1.001	99.51±1.79

*Mean ± standard deviation

Intermediate Precision

The intermediate precision was evaluated by the intraday (within day) and interday (between days) study. The results of estimation by proposed methods are shown in **Table IV.**

Robustness and ruggedness

Robustness of the proposed method was evaluated deliberately substituting ethanol as solvent. Ruggedness of the proposed method was carried out for three different analysts. The result did not show any considerable statistical difference suggesting that the method developed was robust and rugged. Results are shown in **Table IV**.

Table 4: Results of ruggedness and robustness study

* Mean of three observations

Parameters	Mean of % of labeled claim* ± SD		
	Method 1a and 1b		Method 2
	Comparison with standard	By A(1%,1cm)	Derivative spectroscopy
	287.5 nm	287.5 nm	249.2 nm
Different Analyst	99.69 ± 0.023	99.41 ± 0.04	99.46 ± 0.153
Interday	99.24 ± 0.39	98.97 ± 0.38	98.55 ± 0.409
Intraday	99.62 ± 0.08	99.35 ± 0.07	98.70 ± 0.301
Robustness study	97.04 ± 1.29	98.29 ± 1.315	98.62 ± 0.73

Specificity

It is the ability of an analytical method to assess unequivocally the analyte of interest in the presence of components that may be expected to be present such as impurities, degradation products and matrix components. Assay of fenofibrate was carried out successively by keeping the sample for 24hrs under following different conditions.

1. At 50^0C after addition of 1mL 0.1N NaOH
2. At 50^0C after addition of 1mL 0.1N HCl
3. At 50^0C after addition of 1mL 3% H_2O_2
4. At 60^0C
5. At different humidity condition i.e. 75% and 58%

Dilutions of all these solutions were made as per marketed sample. Results of estimation are shown in **Table V.**

Table 5: Result of specificity studies

condition	% labeled claim		
	Method 1a and 1b		Method 2
	Comparison with standard	By A(1%,1cm)	Derivative spectroscopy
	287.5nm	287.5nm	249.2nm
0.1N NaOH	98.82	98.71	98.82
0.1NHCl	98.01	97.90	97.50
60^0C	97.38	97.29	97.18
3% H_2O_2	98.45	98.32	99.24
Humidity (75%)	98.57	97.89	99.80
Humidity (58%)	98.79	98.49	97.90

Linearity and range

Accurately weighed quantities of capsule powder equivalent to 80, 90, 100, 110 and 120% of label claim of fenofibrate were taken and dilutions were made as per the experimental procedure. The graphs of concentration vs absorbance were plotted and were found to be linear and the coefficient of correlation at 287.5nm and 249.2nm was found to be 0.9984 and 0.9983 respectively.

RESULTS AND DISCUSSION

In the present study, quantitative determinations of fenofibrate in capsule and tablet formulations were carried out by zero order uv-spectrophotometric method and a derivative uv-spectrophotometric method. Beer's law was obeyed in the concentration range of 10-60 µg/mL and correlation coefficient for zero order spectra and for first order derivative spectrum was found to be 0.9994 and 0.9999 respectively **(Table I)**. Results of estimation in marketed formulations and recovery results in formulation 1and 2 shows that method is accurate and precise **(Table II and III)**. The proposed methods were validated as per USP guidelines. The intraday SD was found to be 0.39 and 0.38 for method 1 and 0.409 for method 2. Also interday SD was found to be 0.08 and 0.07 for method 1 and 0.301 for method 2 respectively. Lower values of intraday and interday variation in the analysis indicate that method is precise. SD for robustness and ruggdness study were found to be well below the limits for method 1 and 2 **(Table IV)**. Result of specificity study are shown in **Table V** which indicate that % of labeled claim in all the stress conditions were found to be different than the untreated sample, indicating susceptibility of drug to various stress conditions. Also it was observed that in alkaline condition the spectral pattern shows drastic change as compared to standard hence we expect that drug was degraded as shown in **Figure 3**, though the percent label estimated was nearly 100% could be due to absorbance shown by the degraded product at the wavelength of estimation.

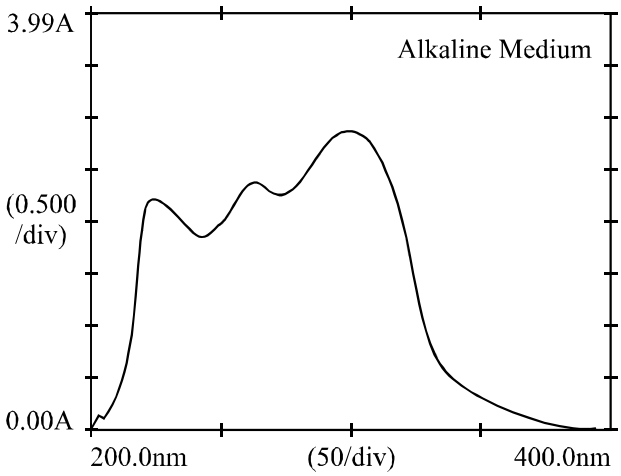

Figure 3: UV spectra of fenofibrate under alkaline stress

Hence, we conclude that the proposed methods are quite reliable, accurate and precise for the quantitative estimation of fenofibrate in marketed formulation and can be adopted for routine analysis of the drug.

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