UV Spectrophotometric Determination of Fenofibric Acid By Using Hydrotropy

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ABSTRACT

Several techniques are used to increase the aqueous solubility of poorly water soluble drugs. Hydrotropic solubilisation technique is one of them. A simple, efficient and new spectrophotometric method has been developed for spectroscopic estimation of fenofibric acid in bulk and tablet dosage forms by using mixed hydrotropic technique. Method A involves the determination of fenofibric acid by standard absorbance method at 299 nm. Method B and Method C involves the determination of fenofibric acid by first derivative spectrophotometry and second derivative spectrophotometry respectively. The normal spectrum was derivatized to first and second order derivative spectrum. The Beer's concentration was found to be taken 5-30 µg/mL; Method D involves the determination of fenofibric acid by area under curve in the range of 275-316 nm and validated as per ICH guidelines. In present study, 2M urea, and 1M sodium citrate solution were used as mixed hydrotropic agents to enhance the aqueous solubility of the poorly water soluble drug fenofibric acid. It does not interfere in the absorption of drug. Proposed method was validated as per ICH guidelines. The results of validation parameters also indicated that proposed method was found to be accurate, precise, reproducible, sensitive and suitable for routine quality control analysis for estimation of fenofibric acid in bulk and tablet dosage form.

KEYWORDS: Hydrotropy**,** Fenofibric acid (FFA); Beer's law; Standard absorbance method; Derivative spectrophotometry; Area under curve (AUC).

INTRODUCTION:

In the pharmaceutical analysis and formulation development fields, it is most often required to increase the aqueous solubility of poorly water-soluble drugs. Most of the newly developed drug molecules are lipophilic in nature and poor solubility is one of the frequent problems encountered. It is well known that drug efficacy can be severely limited by poor aqueous solubility. It is also known that the side effects of some drugs are the result of their poor solubility. The ability to increase aqueous solubility can thus be a valuable aid to increasing efficacy and/or reducing side effects for certain drugs. Various organic solvents like methanol, chloroform, alcohol, acetone, dimethyl formamide, and benzene have been employed for the solubilisation of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution, and error in analysis due to volatility [1-2].

There are various approaches for solubilisation of poorly water soluble drugs. "Hydrotropy" is a solubilisation technique in which addition of large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilising insoluble compounds. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilisation. Review of literature shows that a large number of poorly water-soluble drugs like frusemide, cefixime, salicylic acid, ketoprofen, tinidazole, aceclofenac, using hydrotropic solubilising agents [3-9]. Concentrated aqueous solutions of a large number of hydrotropic agents like sodium benzoate, sodium salicylate, urea, sodium ascorbate, niacin amide and sodium citrate have been employed to enhance aqueous solubility of various poorly water-soluble drugs.

In the present work, hydrotropic solubilisation technique has been applied for method development and validation study of fenofibric acid; it is an antihyperlipidemic belonging to the class of fibric acid derivatives widely used in lipid regulating (dyslipidemia) pharmaceutical formulations, alone or in combination with other drugs. It reduces cholesterol levels in patients with risk of cardiovascular disease; it reduces both LDL, VLDL and triglycerides levels, as well as increases HDL levels. Fenofibric acid is the active metabolite of fenofibrate. It increases Apo lipoprotein A-I–Mediated High-Density Lipoprotein Biogenesis by enhancing transcription of ATP-Binding cassette transporter A1 gene in a liver X receptor–dependent manner. It is chemically Known as 2-[4'-(p-Chlorobenzoyl) phenoxy]-2-methylpropionic acid. Fenofibric acid has been successfully used as a single drug or in association with other drugs in the treatment of hyperlipidemia. It is an almost white, odourless, tasteless compound with a molecular weight of 318.75, and the molecular formula $C_{17}H_{15}ClO_4$ (Fig.1).Fenofibric acid is not an official drug [10].

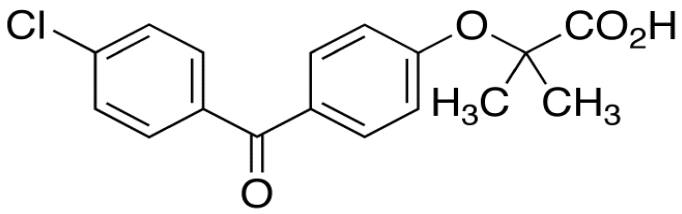

Fig. 1: Fenofibric acid

Literature survey reveals no validated method was found for its quantitative determination in bulk and pharmaceutical dosage forms.

Objective:

The objective of this work was to develop sensitive and efficient analytical methods for quantitative determination of fenofibric acid in bulk forms. In this study, efforts were made to develop a simple, easy, safe and economic UV spectrophotometric method using hydrotropic solubilisation technique for the determination of fenofibric acid in the bulk form. The hydrotropic agent (urea and sodium citrate) used is cost efficient, safe, and non-harmful to environment; hence it can be employed for routine analysis of fenofibric acid in bulk forms.

MATERIALS AND METHODS:

Material and Reagents

Urea, Sodium citrate was purchased from Merck, Mumbai. Distilled water was used throughout the experiment. Fenofibric acid was obtained as a gift sample from well reputed pharma company. Fenofibric acid 35 mg (Fenocor tablets) was purchased from Indian market. The determinations were carried out at room temperature. All absorption spectra were measured by using Shimadzu UV-1650PC (UV-Visible) spectrophotometer with a scanning speed of 200nm/min and band width of 2.0 nm, equipped with matched quartz cells.

Mixed Hydrotropic Solvent

Fenofibric acid being a poor water soluble drug 2M urea and 1M sodium citrate were employed as solubilising agent.

Preparation of 2M urea hydrotropic solvent:

Fenofibric acid is a poor water soluble drug. The 2M urea solution was prepared by accurately weighing 6g in to a 50ml volumetric flask and the volume was made up to 50ml with distilled water.

Preparation of 1M sodium citrate hydrotropic solvent:

The 1M sodium citrate solution was prepared by accurately weighing 12.9g in to a 50ml volumetric flask and the volume was made up to 50ml with distilled water.

Preliminary solubility studies of drug

Solubility of fenofibric acid was determined at 27 ±1°C. An excess amount of drug was added to screw capped 30 ml glass vials each containing different aqueous system viz. distilled water, buffer of different pH (8, 8.5 and 9), 2 M Urea, 1 M sodium citrate solution, 1M sodium citrate and 1 M sodium salicylate. Each vials were shake well 30 minutes and heated 15 minutes. These solutions were allowed to equilibrate for the next 1 hour and then filtered. The filtrates were diluted suitably and analysed spectrophotometrically against respective solvent blank within UV range of 200-400 nm. It was found that the fenofibric acid containing 2M urea and 1M sodium citrate showed maximum absorption.

Preparation of standard stock solution

A standard stock solution of the analyte was prepared by dissolving an aliquot quantity of standard transferred to a 100mL standard flask containing 5ml each of 2M urea, and 1M sodium citrate as mixed hydrotropic solubilising agent and warmed for 15 minutes; dissolved the content completely, final volume was made up with distilled water to produce a concentration of 1mg/mL. The stock solution was further suitably diluted with distilled water to give a varied concentration ranging from 5-30 µg/mL.

Preparation of sample stock solution

Twenty tablets were accurately weighed and powdered. Powder equivalent to 100mg of fenofibric acid was weighed and transferred to 100mL volumetric flask. The powder was then shaken with 5ml each of mixture of 2M urea, and 1M sodium citrate as mixed hydrotropic solubilising agent and heated for 15 minutes; dissolved the content completely and the final volume was made up with distilled water to produce a concentration of 1mg/mL. The solution was then filtered through a Whatmann filter paper. The first few ml of the filtrate was discarded. The remaining filterate was diluted with distilled water to get the required concentration.

ASSAY PROCEDURE

Method A – Standard absorbance method

Aliquots of standard solution of fenofibric acid were suitably diluted to give varying concentrations ranging from 5-30 µg/mL and the solutions were scanned in the range of 200 -400nm using distilled water as blank. Graph was plotted by taking concentrations on X-axis and absorbance's on Y-axis. It was found that fenofibric acid exhibited an intense maximum absorbance at about 299 nm and obeyed Beer's law in the range of 5-30µg/mL. The absorbance obtained for the sample was then interpolated on the calibration graph (Fig.1) and the concentration of fenofibric acid in the sample was then determined. The overlain fundamental spectra are shown in (Fig.2).

Method B – First derivative spectroscopy

The standard stock solution of fenofibric acid was suitably diluted to give varying concentrations ranging from 5-30 µg/mL. The solutions were scanned in the range of 200- 400nm and the primary absorption spectrum was recorded .The primary spectrum was then derivatized to the first order using derivative mode. The amplitude of the negative peak maximum at the zero crossing of the first order curve was measured in mm at 299 nm. A calibration graph was obtained by plotting concentration versus amplitude. The sample solution was suitably diluted to get the required concentration and the absorbance was recorded. The amplitude obtained for the sample was then interpolated on the calibration graph and the concentration of fenofibric acid in the sample was then determined (Fig.5). The linearity graph and the overlain spectra for this method are shown in (Fig 6).

Method C – Second derivative spectroscopy

The primary spectrum obtained for the above was then derivatized to the second order. The amplitude of the negative peak maximum was measured in mm at 299 nm. The respective linearity graph and the overlain spectra are shown in Fig 7&8.

Method D – Area under curve

The standard stock solution of fenofibric acid was suitably diluted to give varying concentrations ranging from 5-30 µg/mL. The solutions were scanned in the range of 200-400nm. The area under the curve between 275-316 nm was measured by using the inbuilt software. The inbuilt software calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated .The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The AUC spectrum is shown in Fig 9&10.

Recovery studies

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery is calculated. The results indicate that there is no interference of other ingredients present in the formulation.

RESULTS AND DISCUSSION

The present paper describes application of hydrotropic solubilisation technique for the estimation of fenofibric acid (FFA) in bulk and tablet dosage form by Standard absorbance method, First order derivative, second order derivative, and area under the curve. Solubility studies indicated that aqueous solubility of fenofibric acid was enhanced in optimized mixed hydrotropic mixture i.e. 2M urea, and 1M sodium citrate as compared to solubility in other economic solvents such as distilled water. It was observed that fenofibric acid remained stable in optimized hydrotropic solvent mixture for 36 hours. Hydrotropic solvent mixture does not interfere in the absorption of fenofibric acid (λmax-299 nm).

Quantitation of fenofibric acid was done by using UV spectrophotometry. Fenofibric acid exhibits an absorption maximum at 299 nm. The Beer's concentrations for all the four methods were found to be lie between 5-30 µg/mL. The correlation coefficient for the all four proposed methods was found to be 0.9996 which shows good linearity between concentration and absorbance. The percentage recovery for all the four method obtained was 99.30% to 100.99% indicating the accuracy of the method. The results of the analysis of formulation show that the proposed methods are in good agreement with the labelled amount of the drug. The regression characteristics like slope, intercept, and correlation co-efficient (r), obtained from different concentrations were calculated and the results are summarized. All the four proposed methods are simple precise accurate and reproducible and could be used for routine analysis.

CONCLUSION

Simple, economic, fast and non-toxic UV spectrophotometric methods have been developed for the estimation of fenofibric acid in bulk and tablet dosage form by absorbance maxima and, first order derivative spectroscopy, second order derivative spectroscopy, and area under the curve method using mixed hydrotropic as solubilisation technique. Hydrotropy method excludes the usage of costlier, toxic and volatile organic solvents by using economic and non-toxic hydrotropic solvents. Here hydrotropic solvent mixture 2M urea and1M

sodium citrate enhanced the aqueous solubility of fenofibric acid. The methods also validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. The results of these Validation parameters indicated that the present UV spectrophotometric methods were found to be linear, precise, accurate and sensitive; and can be used for routine quality control analysis of fenofibric acid in bulk and tablet dosage form.

ACKNOWLEDGEMENT

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Figure 1: Calibration curve of fenofibric acid (Standard absorbance method)

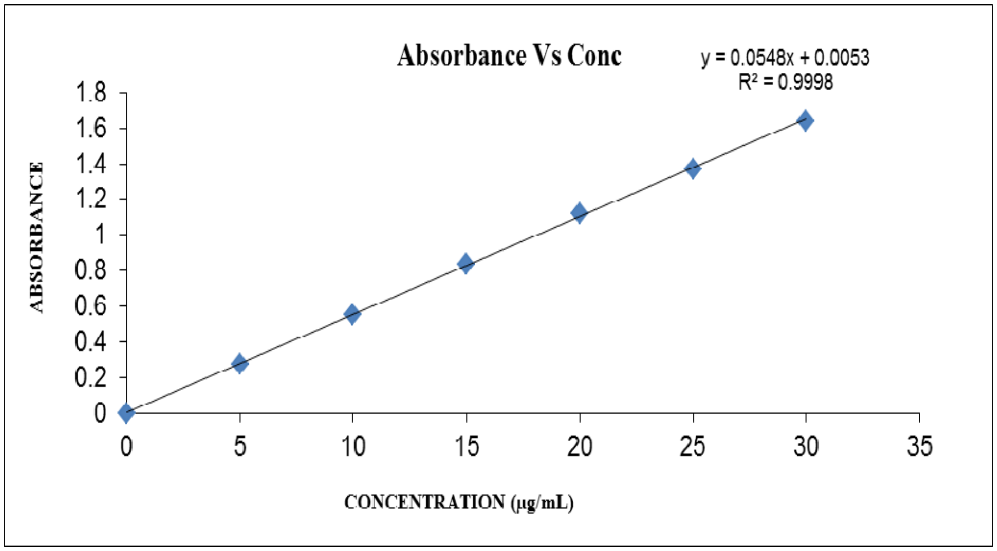

Figure 2: Over lain spectrum of fenofibric acid (Standard absorbance method)

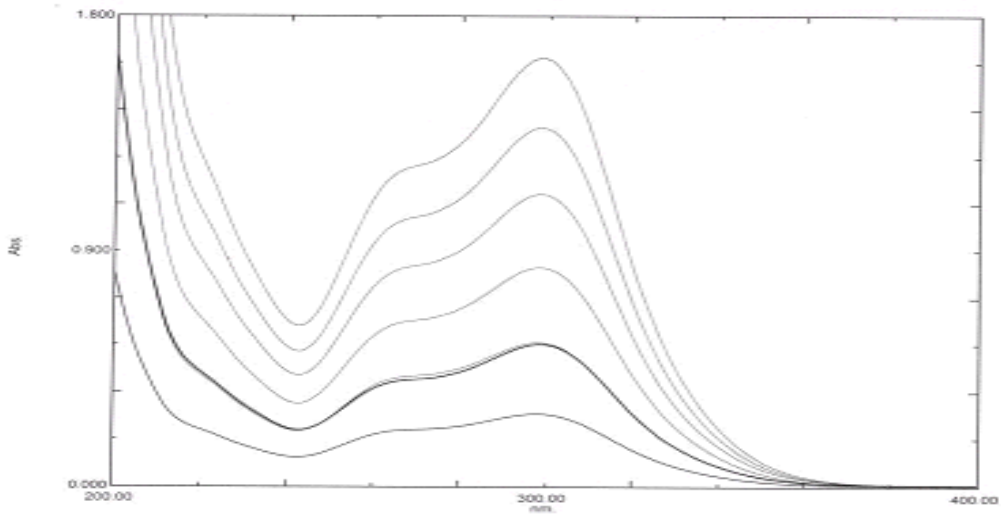

Figure 3: Fenofibric acid standard

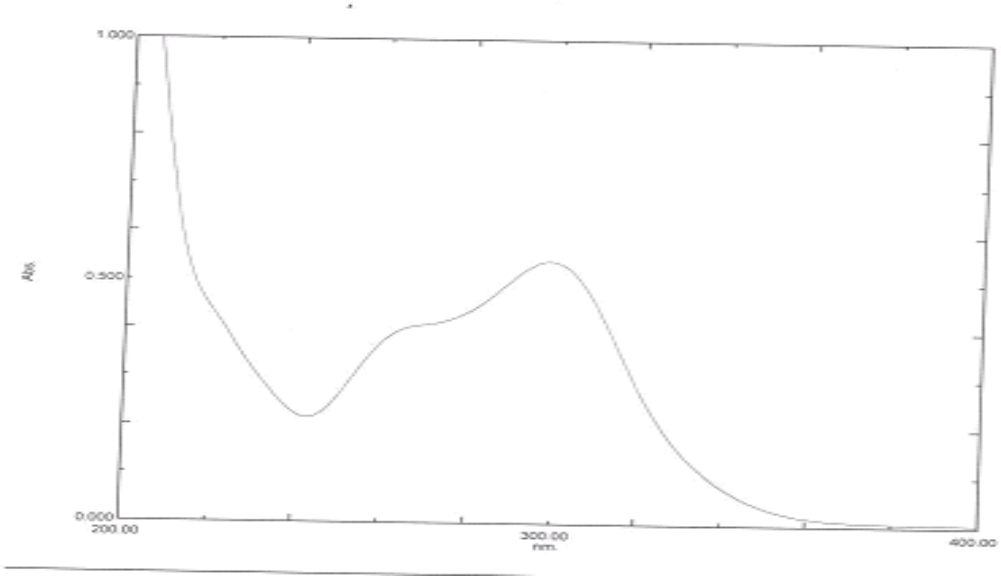

Figure 4: Fenofibric acid sample

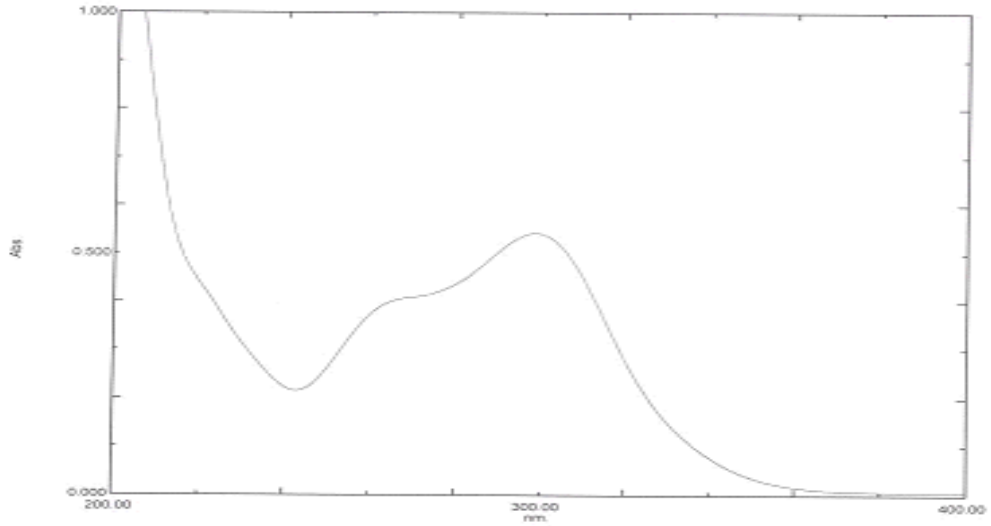

Figure 5: Calibration graph of fenofibric acid (First derivative spectroscopy)

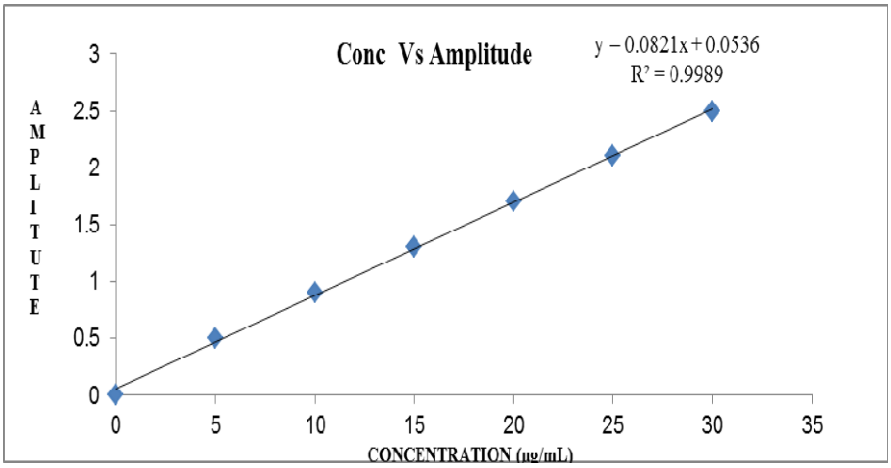

Figure 6: Over lain spectrum of fenofibric acid (First derivative spectroscopy)

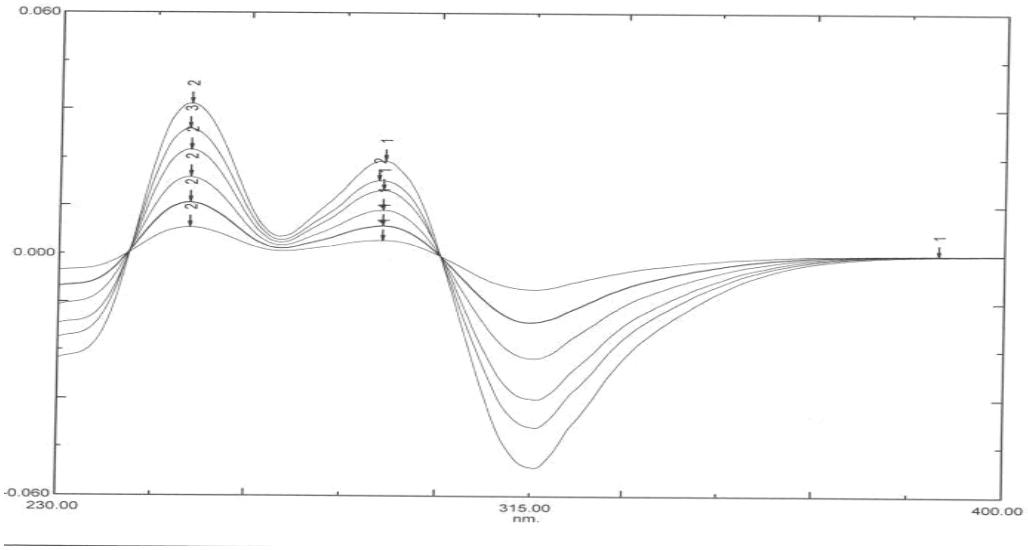

Figure 7: Calibration curve of fenofibric acid (Second derivative spectroscopy)

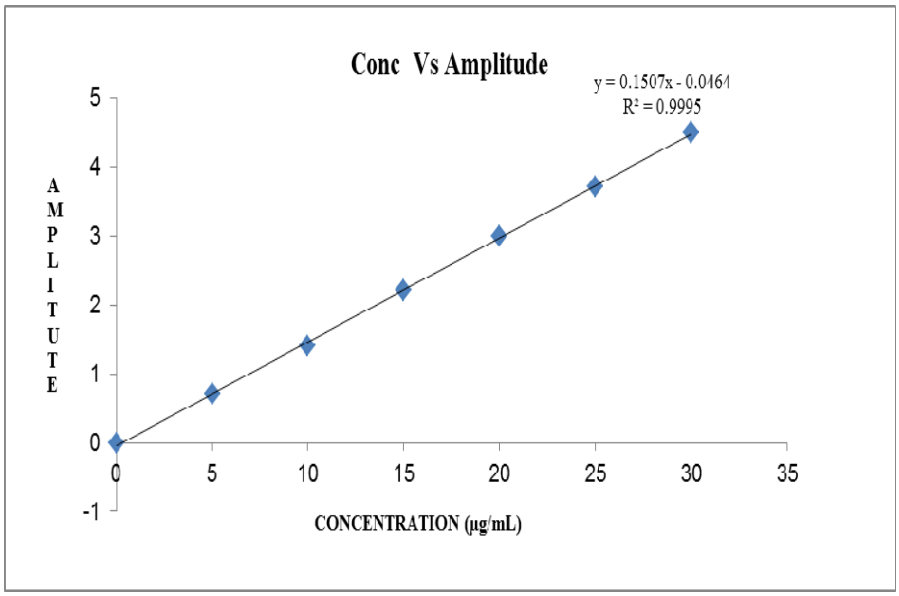

Figure 8: Over lain spectrum of fenofibric acid (Second derivative spectroscopy)

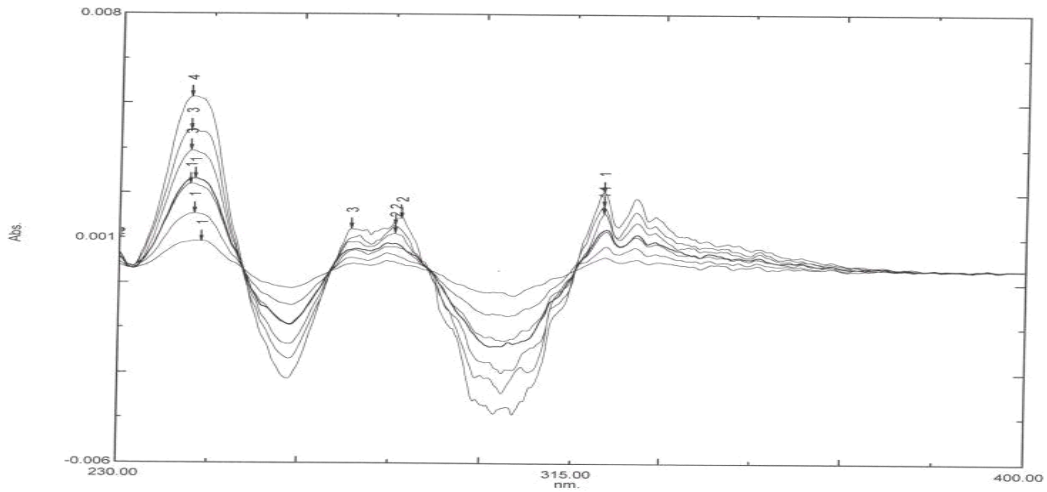

Figure 9: Fenofibric acid sample (Area under curve)

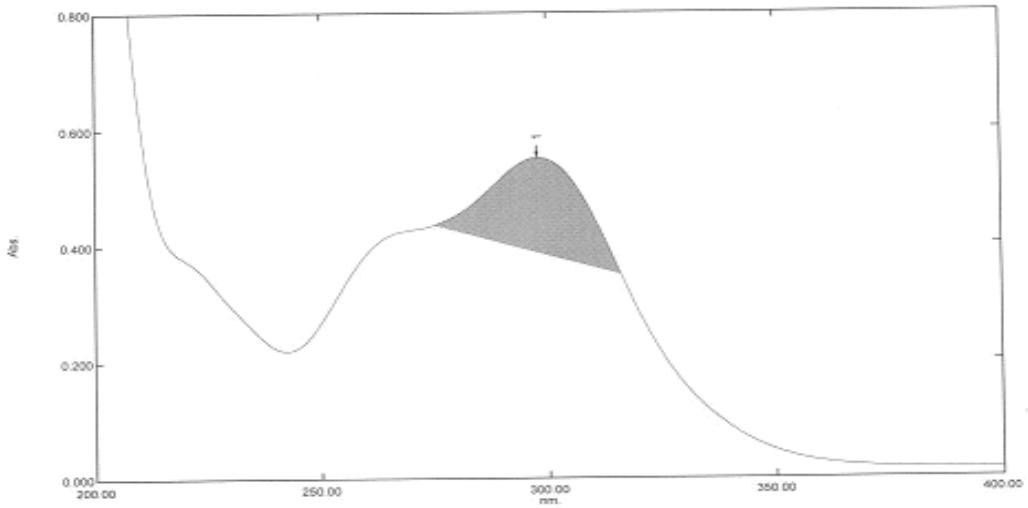

Figure 10: Calibration graph of fenofibric acid (Area under curve)

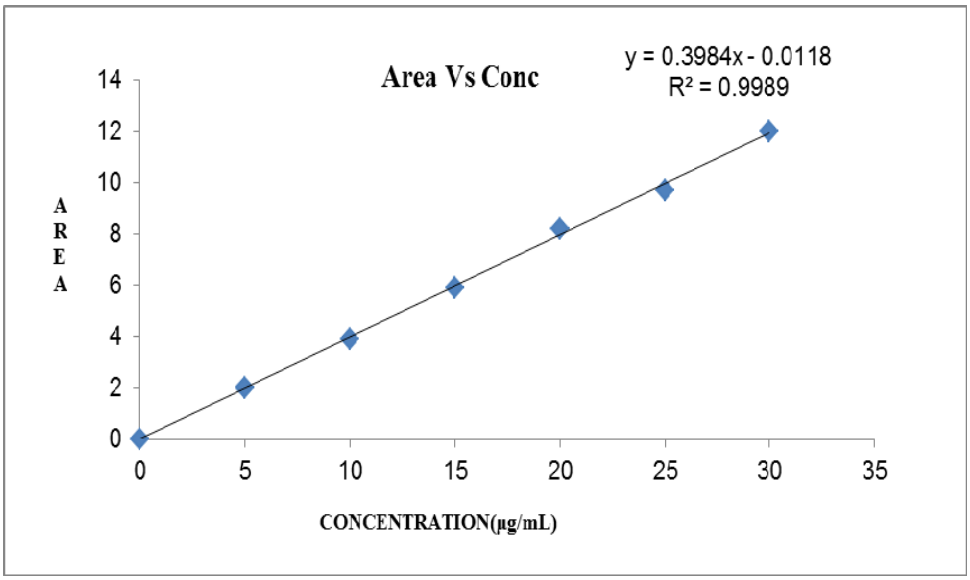

Table-1: Result of analysis of tablet formulation:

Method	Drug	Label Claim (mg)	Amount present in mg	% Purity, Mean ± S.D	% R.S.D**.
Method A	Fenofibric acid	35 mg	35.20	100.58 ± 0.080208	0.227842
Method B			35.31	100.88 ± 0.319739	0.905607
Method C			35.26	100.73± 0.262742	0.745226
Method D			35.35	100.99 ± 0.201080	0.568881

****** Each value is the mean of 3 determinations.

Table-2: Recovery result of fenofibric acid

Sl. NO.	Method	Label claim	Amount Drug added (%)	Amount Drug Recovered (%)	% Recovered
1	UV Spectrophotometry	35 mg	20	20.11	100.54
			40	39.72	99.30
			100	100.36	100.36
2	First Derivative		20	19.93	99.64
			40	39.99	99.98
			100	100.18	100.18
3	Second Derivative		20	20.20	100.99
			40	40.08	100.20
			100	100.09	100.09
4	Area Under Curve		20	20.02	100.09
			40	40.17	100.43
			100	100.18	100.18

Table-3: Optical Characteristics for Fenofibric Acid:

Parameters	UV spectro -Photometry	First derivative	Second derivative	Area under curve
λmax (nm)	299	299	299	275-316
Beer's law limits (µg/mL)	5-30	5-30	5-30	5-30
Molar absorptivity(L mol^{-1} cm^{-1})	17268.47	-	-	-
Sandell's sensitivity (µg cm^{-2} /0.001 abs unit)	0.018083	-	-	-
Slope (m)	0.054836	0.082143	0.150174	0.398386
Intercept(c)	0.005321	0.053571	-0.046429	-0.01179
Regression equation(*Y)	0.054X + 0.005	0.082X + 0.053	0.150X - 0.046	0.398X - 0.011
Correlation coefficient (r)	0.999876	0.999433	0.999742	0.999441
Standard deviation	0.080208	0.319739	0.262742	0.201080
Relative standard deviation (%) **	0.227842	0.905607	0.745226	0.568881
Standard error	0.046310	0.184607	0.151699	0.116097

***** (Y=mx+c) ****** Each value is the mean of 3 determinations.