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Eco-Friendly Method Development and Validation for the Estimation of Fenofibrate and Atorvastatin in Marketed Formulation

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ABSTRACT

This study focuses on the development and validation of an eco-friendly analytical method for the simultaneous estimation of Fenofibrate (FNF) and Atorvastatin (ATV) in a marketed formulation. The stability of both drugs was evaluated using a mixed hydrotropic solution of 2M Ammonium Acetate and 2M Sodium Citrate (1:1). This work focuses on the development and validation of an eco-friendly method for the simultaneous estimation of Fenofibrate and Atorvastatin in a marketed formulation. The stability of both drugs in a mixed hydrotropic solution was confirmed, and the method exhibited good linearity, recovery, and precision. The validated method was successfully applied to analyze a tablet formulation, demonstrating its applicability in routine quality control. The use of environmentally friendly practices in analytical methods contributes to sustainable pharmaceutical analysis.

Keywords: Eco-friendly method, Fenofibrate, Atorvastatin, Hydrotropic solution, Validation, Marketed formulation, Simultaneous estimation

1. INTRODUCTION

In recent years, the pharmaceutical industry has been increasingly focusing on adopting eco-friendly practices in various stages of drug development, including analytical methods for drug estimation.[1] Green chemistry principles aim to reduce the environmental impact of chemical processes, and their application in pharmaceutical analysis aligns with the global sustainability goals.[2] This study explores the development and validation of an eco-friendly method for the simultaneous estimation of Fenofibrate and Atorvastatin in a commercially available formulation.[3] Green chemistry involves the design of products and processes that minimize the use and generation of hazardous substances.[4]

In the context of pharmaceutical analysis, the choice of solvents, reagents, and methodologies plays a crucial role in reducing the environmental footprint of analytical procedures.[5] Utilizing green chemistry principles in method development contributes to sustainable practices without compromising the accuracy and precision of analytical results.[6] Fenofibrate and Atorvastatin are commonly prescribed medications for managing dyslipidemia and cardiovascular diseases.[7]

The need for accurate and eco-friendly methods for their estimation is paramount, considering the widespread use of formulations containing these active pharmaceutical ingredients.[8] The primary objective of this study is to develop and validate an eco-friendly analytical method for the simultaneous estimation of Fenofibrate and Atorvastatin in a marketed pharmaceutical formulation. The method aims to use environmentally benign solvents and optimized conditions without compromising the analytical performance.

2. MATERIALS AND METHODS

FNF and ATV standard were obtained from Alembic Pharmaceutical (Baddi). Ammonium Acetate and Sodium Citrate (A.R Grade; Qualigens) and RO water used for the study.

2.1 Instrumentation

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

2.2 Determination of Solubility Enhancement by UV VIS. Spectroscopy

The observed substantial solubility enhancement of Fenofibrate (FNF) and Atorvastatin (ATV) in a 2M Ammonium Acetate: 2M Sodium Citrate mixtures (1:1) signify the potential utility of this solvent system for improving the dissolution characteristics of these drugs. The fold increase in solubility, 22 times for FNF and 25 times for ATV, suggests a remarkable improvement compared to other solvents or conditions.

The combination of Ammonium Acetate and Sodium Citrate is known to act as a hydrotropic system, enhancing the aqueous solubility of poorly water-soluble drugs. The positive outcomes observed in this study align with the hydrotropic solubilization strategy, where these salts aid in overcoming the intrinsic poor solubility of FNF and ATV. The interaction between the drug molecules and the hydrotropic agents appears to result in a substantial increase in solubility.

2.3 Establishment of Stability Profile

Stability of both drugs was observed by dissolving FNF and ATV in 2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution used as solvent. Solution of FNF and ATV was prepared in the conc. of 5µg/ml and 40µg/ml respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution.

2.4 Linearity Range and Calibration Graph

2.4.1 Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80`mL mixed hydrotropic solution containing 2M Ammonium Acetate: 2M Sod. Citrate (1:1) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made upto 100 mlwith mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

2.4.2 Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution Aof FNF and ATV and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml (Stock-B).

2.4.3 Preparation of Working Standard Solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for FNF.

1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively for ATV.

2.4.4 Selection of wavelength for Linearity

Solutions of 5µg/ml of FNF and 40µg/ml ATV were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of FNF and ATV was observed at 282.0 nm and 244.0 nm, respectively. FNF and ATV showed linearity in the concentration range of 2-10µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

2.5 Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 5µg/ml of FNF and 40µg/ml of ATV were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. FNF showed an absorbance peak at 282.0nm, whereas ATV at 244.0nm. The overlain spectra also showed is absorptive points at 273.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drugs can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 282.0 nm and 244.0 nm that are λ_{max} of FNF and ATV respectively. The absorbances were measured at the selected wavelengths and absorptivity's ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C\,GLP = \frac{A1ay2 - A2ay2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(1)$$

$$C\,PGZ = \frac{A1ax2 - A2ax2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(2)$$

Where, A_1 and A_2 are absorbances of mixture at 282.0 nm and 244.0 nm respectively, ax_1 and ax_2 are absorptivity's of FNF at λ_1 (282.0 i.e. λ_{max} of FNF) and λ_2 (244.0 i.e. λ_{max} of ATV) respectively and ay_1 and ay_2 are absorptivity's of ATV at λ_1 and λ_2 respectively. C_{ATV} and C_{FNF} are concentrations of FNF and ATV respectively. The overlain spectra of both the drugs in 2:30 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the FNF and ATV.

2.5 Validation of Simultaneous Equation Method [8]

2.5.1 Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

2.5.2 Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Fenofibrate and Atorvastatin to prenasalised tablet solutions. The resulting solutions were then re-analyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample.Thisrecoveryanalysiswasrepeated at 3 replicates of 5 concentrations levels.

2.5.3 Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables 6.12-6.13.

2.5.4 Analysis of Tablet Sample

TwentymarketedtabletsofFenofibrate and Atorvastatin wereweighedandgroundtoafinepowder; amount equal to 5mg of FNF was taken in 10 ml volumetric flask. The ATV present in this amount of tablet powder was 30mg. Then 8mlof2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. Aftersonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

3. RESULTS AND DISCUSSION

Simultaneous estimation of Fenofibrate (FNF) and Atorvastatin (ATV) using mixed hydrotropic solubilizing agents.The developed methods were found to be linear. The linearity study results for Fenofibrate (FNF) and Atorvastatin (ATV) using the designated analytical method are presented in Table 1. The working λ_{max} values, representing the wavelengths at which maximum absorbance occurs, were found to be 282 nm for FNF and 244 nm for ATV.

The Beer's law limits, which define the linear concentration range, were determined as 5-25 µg/ml for FNF and 10-50 µg/ml for ATV. The high correlation coefficients (r^2) of 0.999 and 0.997 for both FNF and ATV respectively indicate a robust linear relationship between concentration and response. Additionally, the calculated slopes (m) and intercepts (c) further confirm the method's sensitivity and accuracy.

The method demonstrates its suitability for the quantitative analysis of Fenofibrate and Atorvastatin, providing essential information for their concentration determination in pharmaceutical formulations. The specified wavelengths contribute to precise measurements, enhancing the reliability of the method in pharmaceutical quality control and research applications.

The recovery studies conducted on the marketed formulations of Fenofibrate and Atorvastatin are summarized in Table 2. At various recovery levels, namely 80%, 100%, and 120%, the mean percentages of recovery along with standard deviations were calculated. For FNF, the recovery percentages were 97.45±0.525 at 80%, 98.17±0.904 at 100%, and 98.93±0.500 at 120%. Similarly, for ATV, the recovery percentages were 98.95±0.354 at 80%, 99.07±0.489 at 100%, and 98.93±0.632 at 120%.

These results indicate a high level of accuracy and precision in the recovery of FNF and ATV from the marketed formulations. The close agreement between the observed and expected concentrations at different recovery levels demonstrates the reliability and effectiveness of the developed analytical

method. The low standard deviations suggest good precision and reproducibility of the method, making it suitable for the quantification of FNF and ATV in commercial formulations. These findings contribute to the robustness and reliability of the proposed analytical method for quality control purposes in the pharmaceutical industry.

Table 3 presents the results of the validation parameters, including precision, for the developed method for Fenofibrate and Atorvastatin. The precision was evaluated through various aspects, namely repeatability, day-to-day precision, analyst-to-analyst variation, and reproducibility.

For repeatability, the mean precision for FNF was found to be 97.607±0.055, and for ATV, it was 98.684±0.055. In the context of day-to-day precision, the mean values were 97.897±0.055 for FNF and 99.592±0.015 for ATV. Analyst-to-analyst variation showed mean precision values of 98.455±0.055 for FNF and 99.066±0.041 for ATV. Lastly, the reproducibility of the method resulted in mean precision values of 97.864±0.055 for FNF and 99.504±0.114 for ATV.

The obtained results demonstrate the robustness and reliability of the developed analytical method for the quantification of FNF and ATV. The low standard deviations across various precision parameters indicate consistent and accurate performance, making the method suitable for routine analysis and quality control in pharmaceutical applications.

The analysis of the tablet formulation of Fenofibrate and Atorvastatin is presented in Table 4, providing information on the label claim, the amount found, and the percentage of label claim, along with standard deviations and the percentage relative standard deviation (% RSD) for each parameter.

For Fenofibrate, the label claim was 20 mg, and the amount found was 19.747 mg, resulting in a percentage label claim of 98.74%. The standard deviation (S.D.) was recorded as 0.115, with a % RSD of 0.236. These values indicate a close agreement between the labeled and found amounts of Fenofibrate, suggesting the accuracy and precision of the analytical method in quantifying this component in the tablet formulation.

Similarly, for Atorvastatin with a label claim of 160 mg, the amount found was 159.65mg, achieving a percentage label claim of 99.78%. The standard deviation was lower at 0.195, and the % RSD was 0.285. These results indicate a high level of accuracy and precision in determining the Atorvastatin content in the tablet formulation. The low standard deviations and % RSD values in both cases suggest that the developed method is reliable and reproducible for the quantitative analysis of FNF and ATV in tablet formulations.

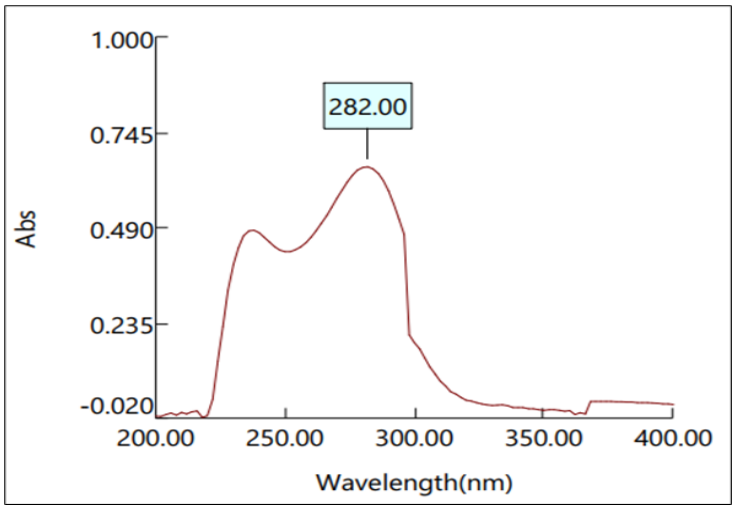

Figure 1: Determination of λ_{max} of FNF

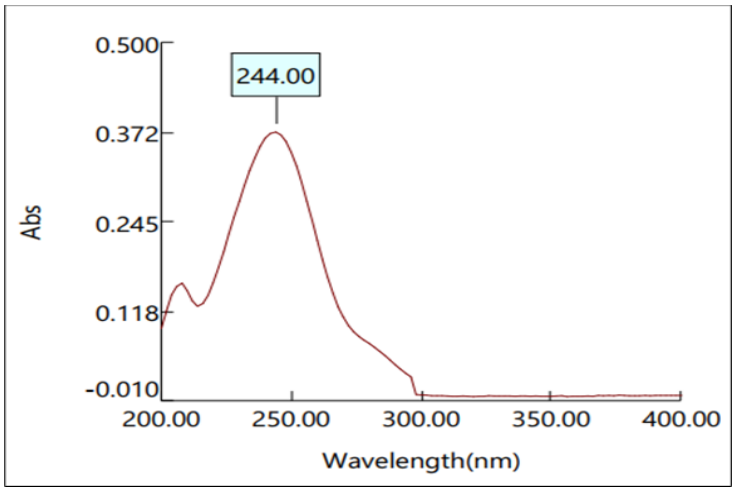

Figure 2: Linearity of ATV

Table 1: Results of Linearity of Fenofibrate (FNF) and Atorvastatin (ATV)

Parameter	**Method**	
	FNF	ATV
Working λ_{max}	282.0 nm	244.0 nm
Beer's law limit (µg/ml)	5-25	10-50
Correlation Coefficient (r^2) *	0.999	0.997
Slope (m)*	0.057	0.03
Intercept (c)*	0.001	0.039

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	**% Recovery (Mean±SD) ***	
	FNF	ATV
80	97.45±0.525	98.95±0.354
100	98.17±0.904	99.07±0.489
120	98.93±0.500	98.93±0.632

*Average of three determination

Table 3: Results of validation (Mean±SD) *

Parameter		**Method**	
		FNF	ATV
Precision*	Repeatability	97.607±0.055	98.684±0.055
	Day-to-Day	97.897±0.055	99.592±0.015
	Analyst-to-Analyst	98.455±0.055	99.066±0.041
	Reproducibility	97.864±0.055	99.504±0.114

*Average of five determination

Table 4: Analysis of Tablet Formulation of FNF and ATV

Drug	**Label claim (mg)**	**Amount found (mg)**	**Label claim (%)**	**S.D.**	**% RSD**
FNF	20	19.747	98.74	0.115	0.236
ATV	160	159.65	99.78	0.195	0.285

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