*Bulletin of Pharmaceutical Research* 2012;2(3):159-66
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

# STABILITY-INDICATING UV-VIS SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF ATORVASTATIN CALCIUM AND FENOFIBRATE IN TABLET DOSAGE FORM

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*Received: November 28, 2012 / Revised: December 23, 2012 / Accepted: December 24, 2012*

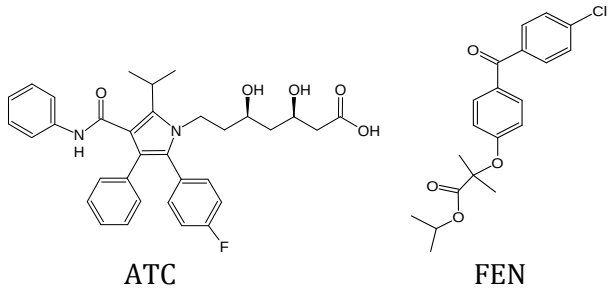
**The present research works discuss the development of a stability-indicating UV spectrophotometric method for the estimation of Atorvastatin calcium (ATC) and Fenofibrate (FEN) in tablet dosages form. The optimum conditions for the analysis of the drug were established. The maximum wavelength ($\lambda_{max}$) was found to be 247 nm for ATC and 287 nm for FEN. The linearity of the proposed method was investigated in the range of 6-16 μg/ml and 2-12 μg/ml for ATC, FEN respectively. Calibration curves showed a linear relationship between the absorbance and concentration. The line equation for ATC (y = 0.041x + 0.043) with $r^2$ of 0.999 and for Fenofibrate (y = 0.054x - 0.003) with $r^2$ of 0.999, was obtained. Validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The LOD was 0.2695 μg/ml, 0.0222 μg/ml for ATC and FEN and the LOQ was 0.8780 μg/ml, 0.222 μg/ml for ATC and FEN respectively. The proposed method may be suitable for the analysis of ATC and FEN in tablet formulation for quality control purpose. The proposed methods were simple, sensitive, precise, accurate, quick and useful for routine quality control. The stability studies of ATC and FEN were conducted and the degradation characteristics were found to be much more prominent in acid hydrolysis in FEN and alkaline hydrolysis in ATC.**

**Key words:** Simultaneous equation, Degradation, Validation, Atorvastatin calcium, Fenofibrate.

## INTRODUCTION

Atorvastatin Calcium (ATC) (**Figure 1**) is calcium salt of (*β*R, 8R)-2-(4-fluorophenyl)-*α*,*δ*-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid trihydrate (IP 2007). ATC is a member of the drug class known as statins, used for lowering blood cholesterol. Atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body.

Fenofibrate (FEN) (**Figure 1**) is 2-[4-(4-chloro benzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester (ChemSpider, 3222) which is a fibric acid derivative that lowers lipid levels by activating PPARα and thus, decreases the risk of heart diseases and prevent strokes.




ATC FEN

**Fig. 1.** Structure of ATC and FEN

Atherosclerotic vascular disease is a condition in which an artery wall thickens as a result of accumulation of fatty materials such as cholesterol. It affects mostly arterial blood vessels. Inflammatory response in walls of arteries is commonly referred to as hardening of

arteries. It is caused by formation of multiple plaques with in arteries. Some of drug combination like Atorvastatin calcium and Fenofibrate has a highly beneficial effect on all lipid parameters. Atorvastatin Calcium is more effective in reduction of cholesterol level whereas Fenofibrate is effective in reduction of triglycerides (Diwan *et al* 2012).

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy. Instability of pharmaceuticals can cause a change in physical, chemical, pharmacological and toxicological properties of the active pharmaceutical ingredients (API), thereby affecting its safety and efficacy. Hence, the pharmacists should take cognizance of various factors such as drug stability, possible degradation products, mechanisms and routes of degradation and potential interactions with excipients utilized in the formulation to ensure the delivery of their therapeutic values to patients. In order to assess the stability of a drug product, one needs an appropriate analytical methodology, so called the stability indicating methods which allow accurate and precise quantitation of the drug, its degradation products and interaction products, if any (Janardhanan *et al* 2011). Forced degradation studies were performed on Atorvastatin calcium and Fenofibrate to prove the stability indicating property of the method. The stress conditions employed for degradation study included light exposure, acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH), and thermal degradation. The duration of time selected for degradation studies was 6 h. The photolytic degradation was performed by exposing the solid drugs to sunlight for 12 h. The concentration of 100 µg/ml of each of Atorvastatin calcium and Fenofibrate were prepared using respective solvents (NaOH, HCl, methanol) separately (ICH Q1A (R2), 2003).

Literature survey reveled that various analytical methods have been reported for estimation of Atorvastatin calcium (ATV) and Fenofibrate (FEB) individually from its formulations and biological fluids (Erturk *et al* 2003; Erk, 2003; Altuntas and Erk, 2004; Gupta *et al* 2010; Salama *et al* 2011). However, no stability-indicating UV spectrophotometric method is reported for the estimation of Atorvastatin calcium (ATC) and Fenofibrate (FEN) in tablet dosages form in the literature.

In continuation of our work on analytical method development of drugs (Singh *et al* 2010; 2011; 2012), the present investigation was directed toward development of a stability-indicating UV spectrophotometric method for the estimation of Atorvastatin calcium and Fenofibrate in tablet dosages form. The objective of the present work is to develop simple, rapid, accurate, specific and economic UV stability indicating method for the estimation of ATC and FEM in bulk and tablet dosages form. The method was further validated as per ICH guidelines (ICH Q2(R1), 2005) for the parameter like precision, accuracy, sensitivity, and linearity. The result of analysis was validated statistically and by recovery studies.

## MATERIAL AND METHODS

### *Samples*

ATC and FEN was kindly provided by Rightaid Laboratories, Hyderabad. The pharmaceutical formulation Mactor F used in this study was procured from local market of Bareilly.

### *Reagents*

Methanol (GR grade), Hydrochloric acid (GR grade), Sodium hydroxide were obtained from Qualingens fine chemicals, Mumbai.

### *Instruments*

UV-Visible double beam spectrophotometer (UV-3200 LAB INDIA) with 1 cm matched quartz cells, Digital balance (K-Roy Electronic), Oven (CLE-101, coslab) and volumetric flask, micropipette were utilized for present work.

### *Preparation of standard stock solution*

An accurately weighed quantity of about 50 mg of ATC was taken in 50 ml volumetric flask and dissolved in sufficient quantity of methanol followed by sonication for 10 min and finally diluted to 50 ml with the same solvent so as to get the concentration of 1000 µg/ml. An accurately weighed quantity of about 50 mg of FEN was taken in 50 ml volumetric flask, dissolved in sufficient quantity of methanol and then sonicated for 15 min and finally diluted up to the mark with same solvent so as to get the concentration of 1000 µg/ml. From this solution, 5 ml was pipetted out in a 50 ml volumetric and volume was made up with methanol and get concentration 100 µg/ml used for making dilution for calibration curve.

### *Determination of $\lambda_{max}$*

The standard solution of ATC and FEN were separately scanned at different concentration in

the range of 200-400 nm and the $\lambda_{max}$ was determined for each drug.

***Preparation of calibration curve***

For each drug, appropriate aliquots were pipetted out from standard stock solution into the series of 10 ml volumetric flask and the volume was made up to the mark with methanol to get concentration of 6-16 $\mu$g/ml of ATC and 2-12 $\mu$g/ml of FEN. Solutions of different concentrations for each drug were analyzed at their respective wavelengths and absorbances were recorded.

***Simultaneous equation method***

Two wavelengths selected for the method (247 nm and 287 nm) that are absorption maximas of ATC and FEN respectively in methanol. Standard stock solution of 100 $\mu$g/ml both the drug was prepared separately in methanol. The stock solution of both drug was further diluted separately with methanol to get series of standard solution of 6-16 $\mu$g/ml for ATC and 2-12 $\mu$g/ml for FEN. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_X = A_1ay_2 - A_2ay_1 / ax_1ay_2 - ax_2ay_1 \quad \text{Eq. 1}$$

$$C_Y = A_1ax_2 - A_2ax_1 / ay_1ax_2 - ay_2ax_1 \quad \text{Eq. 2}$$

where $A_1$ and $A_2$ are absorbances of mixture at 247 nm and 287 nm respectively, $ax_1$ and $ax_2$ are absorptivities of ATC at $\lambda_1$ and $\lambda_2$ respectively and $ay_1$ and $ay_2$ are absorptivities of FEN at $\lambda 1$ and $\lambda 2$ respectively. $C_x$ and $C_y$ are concentrations of ATC and FEN respectively.

***Preparation for tablets assay***

Twenty ATC and FEN tablets (10 mg atorvastatin and 160 mg fenofibrate) were weighed and powdered. A portion equivalent to 160 mg of fenofibrate was weighed into 100 ml clean and dry volumetric flask, About 70 ml of methanol was added and sonicated for 20 min; volume was made upto the mark with methanol, mixed well and filtered through Whatman filter paper No. 41. First few ml of filtrate was discarded and then 5 ml of filtrate was pipetted out and diluted to 50 ml with methanol. Then, the absorbances were recorded at the respective wavelengths.

***Recovery study***

To check the accuracy of the developed method recovery study was carried out as per ICH norms; where to a reanalyzed sample solution, standard solutions of both the drugs were added equivalent to 80, 100 and 120% of its drug content.

**METHOD VALIDATION**

The analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness, and recovery (ICH Q2(R1), 2005).

***Linearity***

Linearity was established by least squares linear regression analysis of the calibration curve.

***Accuracy***

Accuracy was studied by adding two different amounts (corresponding to 80%, 100% and 120% of the test preparation concentrations) of ATR and FEN to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was injected in duplicate.

***Precision***

The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the RSD %. The intermediate (interday) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions.

***LOD and LOQ***

The LOD and LOQ of ATC and FEN were calculated by mathematical equation:

LOD = 3.3 × standard deviation / slope

LOQ = 10 × standard deviation / slope

***Robustness***

Robustness of proposed method was performed by changing UV analyst and remaining conditions (solvent, dilution, UV Spectrophotometer) were same.

**FORCED DEGRADATION STUDIES**

Forced degradation studies were performed on ATC and FEN to prove the stability indicating

property of the method. The stress conditions employed for degradation study included light exposure, acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH), thermal hydrolysis. The duration of time selected for degradation studies was 6 h (ICH Q1A(R2), 2003).

***Acid hydrolysis***

Solutions for acid degradation studies were prepared in methanol (12 *µ*g/ml for ATC and 8 *µ*g/ml for FEN) and added 10 ml 0.1 M hydrochloric acid solution and kept at room temperature (22°C). It was observed that both acid and base hydrolysis were fast reactions for both drugs and almost completed within 3 h of the sample preparation, therefore the samples were analyzed at 247 nm for ATC and 287 nm for FEN after this period of time.

***Base hydrolysis***

Solutions for base degradation studies were prepared in methanol (12 *µ*g/ml for ATC and 8 *µ*g/ml for FEN ) and 100 ml 0.1 M sodium hydroxide add in both dilution and kept at room temperature (22°C) and the resultant solutions analyzed 10 min after preparation at 247 nm for ATC and 287 nm for FEN.

***Photostability studies***

Fifty mg drug was weighed and kept in the sun light for 12 h, after that the solutions for photostability studies were prepared in methanol and the dilutions (12*µ*g/ml - ATC, 8 *µ*g/ml - FEN) were prepared & analyzed in UV spectrophotometer at 247, 287 nm for ATC, FEN.

***Thermal degradation***

Fifty mg drug was weighed and kept in the oven and temperature was maintained at 80°C for 3 h, after that the solutions for photostability studies were prepared in methanol and the dilution (12 *µ*g/ml - ATC and 8 *µ*g/ml - FEN) were prepared and analyzed in UV spectrophotometer.

**RESULTS AND DISCUSSION**

The UV scanning showed spectrum exhibiting $\lambda_{max}$ of 247 nm and 287 nm for ATC and FEN respectively (**Figure 2**).

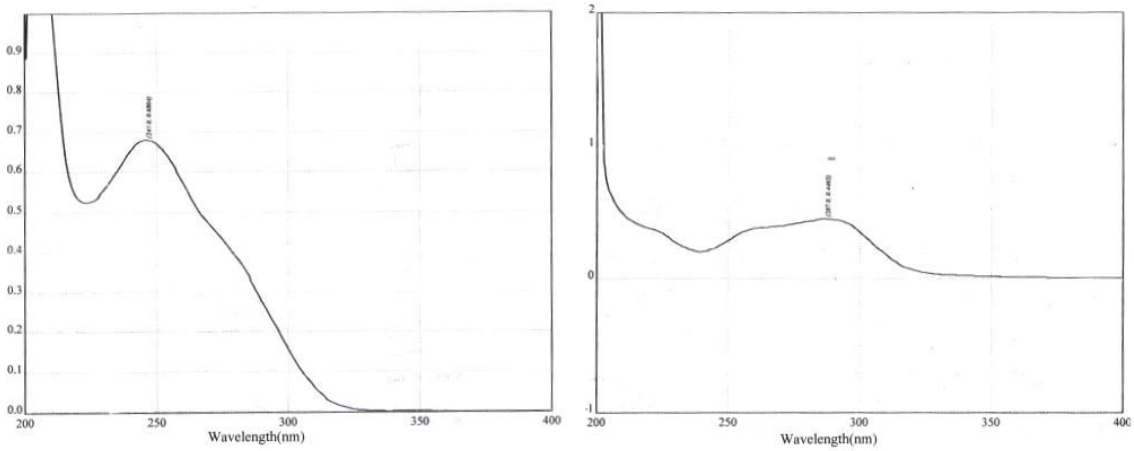



**Fig. 2.** $\lambda_{max}$ of ATC and FEN

The linearity of the proposed method was investigated in the range of 6-16 *µ*g/ml and 2-12 *µ*g/ml for ATC, FEN respectively. Calibration curves showed a linear relationship between the absorbance and concentration. The line equation for ATC (y = 0.041x + 0.043) with $r^2$ of 0.999 and for FEN (y = 0.054x - 0.003) with $r^2$ of 0.999 was obtained (**Table 1, Figure 3**).

**Table 1.** Calibration curve parameters

| S. No. | Parameter | Atorvastatin calcium | Fenofibrate |
|---|---|---|---|
| 1 | Linearity range (*µ*g/ml) | 6-16 *µ*g/ml | 2-12 *µ*g/ml |
| 2 | Correlation coefficient ($r^2$) | 0.999 | 0.999 |
| 3 | Slope | 0.041 | 0.054 |
| 4 | Intercept | 0.043 | 0.003 |

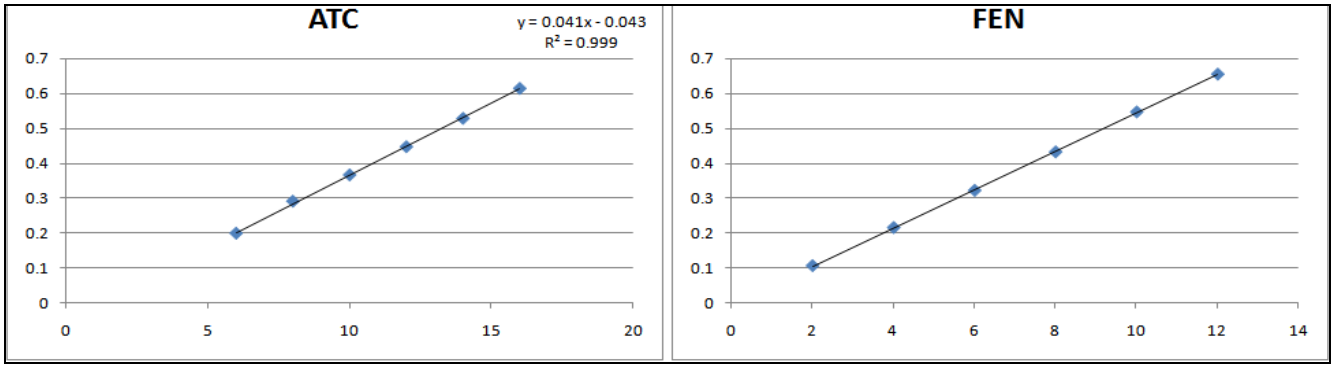



**Fig. 3.** Calibration curve of Atorvastatin and Fenofibrate

The results of tablet assay analysis was found within the prescribed limits and complied with pharmacopoeial standards. The results of tablet analysis are summarized in **Table 2**.

**Table 2.** Analysis of tablet dosage form

| Formulation | Drug | Label claim (mg) | % Label claim (Mean ± SD) |
|---|---|---|---|
| Tablet | ATC | 10 mg | 101.33 ± 0.0018 |
| | FEN | 160 mg | 104.12 ± 0.0034 |

The overlaid spectra of ATC and FEN exhibited $\lambda_{max}$ of 247 nm and 287 nm for ATC and FEN respectively which were quite clearly separated from each other (**Figure 4**).

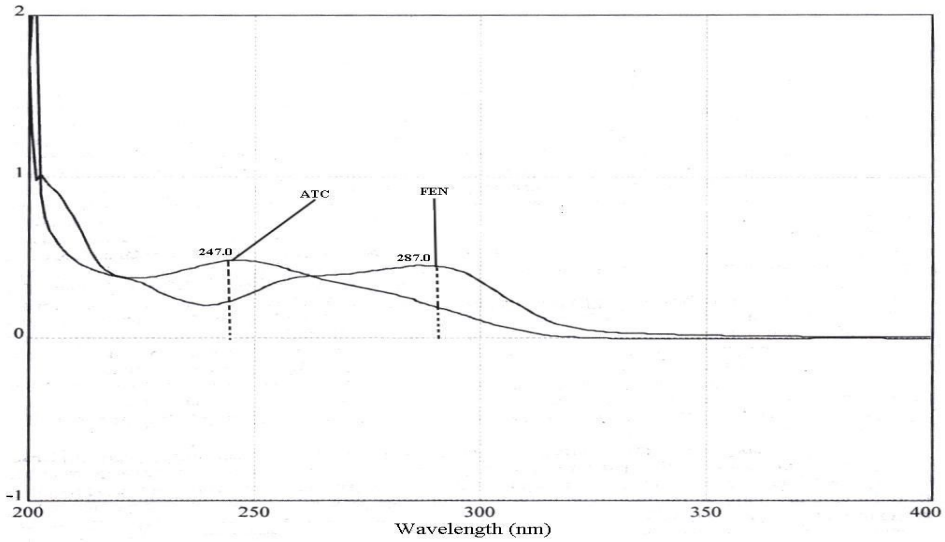



**Fig. 4.** Overlaid spectra of ATC and FEN

The constructed calibration curve was linear over the constructed range for fenofibrate (**Table 3**). The LOD of ATC and FEN were found to be 0.2695*µ*g/ml and 0.0222*µ*g/ml and the LOQ of ATC and FEN were found to be 0.8780 *µ*g/ml and 0.22221 *µ*g/ml. Validation was performed as per ICH guidelines (ICH Q2(R1), 2005) for Linearity, accuracy, precision, LOD and LOQ. The LOD-0.2695 *µ*g/ml, 0.0222 *µ*g/ml for ATC and FEM and the LOQ 0.8780 *µ*g/ml, 0.222 *µ*g/ml for ATC and FEM respectively. The results of method validation parameters are summarized in **Table 4**. The result of forced degradation are compiled in **Table 5.** The stability studies of ATC and FEN were conducted and the degradation characteristics were found to be much more prominent in acid hydrolysis in FEN and alkaline hydrolysis in ATC (**Figure 5, 6**). The specrtrum of photo and thermal degradation studies are evident in **Figure 7, 8.**

**Table 3.** Linearity study of FEN at 287 nm in methanol

| Conc. (μg/ml) | Dilution 1 | Dilution 2 | Dilution 3 | Dilution 4 | Dilution 5 | Dilution 6 | Mean ± SD* |
|---|---|---|---|---|---|---|---|
| 2 | 0.1118 | 0.1072 | 0.1085 | 0.1060 | 0.1058 | 0.1079 | 0.1078 ± 0.0020 |
| 4 | 0.2167 | 0.2185 | 0.2177 | 0.2171 | 0.2161 | 0.2166 | 0.2170 ± 0.00086 |
| 6 | 0.3222 | 0.3234 | 0.3243 | 0.3227 | 0.3241 | 0.3231 | 0.3233 ± 0.00073 |
| 8 | 0.4330 | 0.4324 | 0.4342 | 0.4339 | 0.4333 | 0.4337 | 0.4334 ± 0.00059 |
| 10 | 0.5478 | 0.5490 | 0.5495 | 0.5467 | 0.5497 | 0.5438 | 0.5477 ± 0.0020 |
| 12 | 0.6543 | 0.6556 | 0.6564 | 0.6550 | 0.6555 | 0.6584 | 0.6558 ± 0.001298 |

*SD - Standard deviation

**Table 4.** Validation parameter for ATC and FEN

| S. No. | Parameter (units) | ATC | FEN |
|---|---|---|---|
| 1. | Linearity | 6-16 μg/ml | 2-12 μg/ml |
| 2. | Accuracy (80%) | 99.38 ± 0.0009 | 99.30 ± 0.0014 |
| | Accuracy (100%) | 98.52 ± 0.0013 | 98.69 ±0.0008 |
| | Accuracy (120%) | 98.32 ± 0.0010 | 97.34 ± 0.0029 |
| 3. | Interday Precision (1st day) | 109.87% ± 0.0034* | 99.21% ± 0.00216 |
| | (2nd day) | 109.66% ± 0.0123* | 100.09% ± 0.0014 |
| | (3rd day) | 123.38% ± 0.0013* | 106.10% ± 0.0016 |
| 4. | Intraday Precision 1st h | 109.87% ± 0.0034* | 99.21% ± 0.0026 |
| | 2nd h | 106.22% ± 0.009* | 100.96% ± 0.0001 |
| | 3rd h | 100.44% ± 0.0013* | 99.88% ± 0.002 |
| 5. | LOD | 0.2695 (μg/ml) | 0.0222 (μg/ml) |
| 6. | LOQ | 0.8780 (μg/ml) | 0.2222 (μg/ml) |
| 7. | Robustness | 95.81% ± 0.0007* | 105% ± 0.0008 |

*Standard deviation

**Table 5.** Results of forced degradation study

| S. No. | Condition | Absorbances (λ) | | Mean ± SD* | | Result (% degradation) | |
|---|---|---|---|---|---|---|---|
| | | ATC | FEN | ATC | FEN | ATC | FEN |
| 1. | Acid degradation | 255 nm | No absorbance | 0.2720 ± 0.0021 | 0.4128 ± 0.0009 | 39.12% | 100% |
| 2. | Alkaline degradation | 249 nm | 301 nm | 0.2936 ± 0.0043 | 0.3025 ± 0.0125 | 34.29% | 30.25% |
| 3. | Thermal degradation | 247 nm | 286.5 nm | 0.1993 ± 0.0002 | 0.3645 ± 0.0028 | 55.4% | 100% |
| 4. | Photolytic degradation | 247 nm | 287 nm | 0.3874 ± 0.0109 | 0.6977 ± 0.0022 | 13.3% | 20.0% |

*SD - Standardeviation

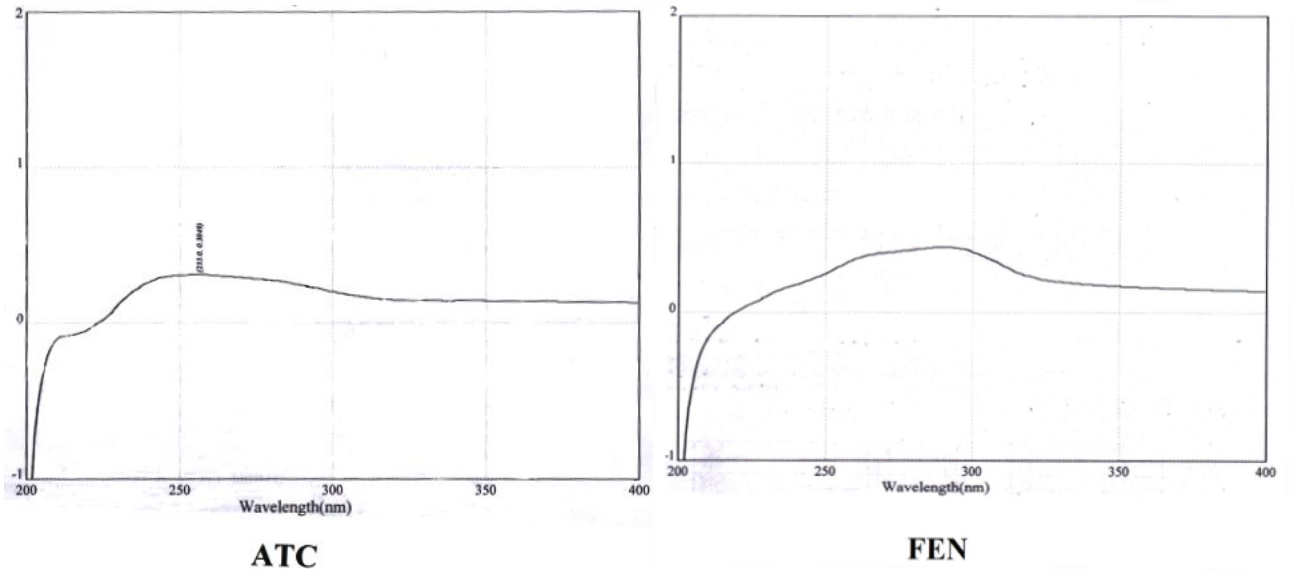



**Fig. 5.** Acid degradation of ATC and FEN

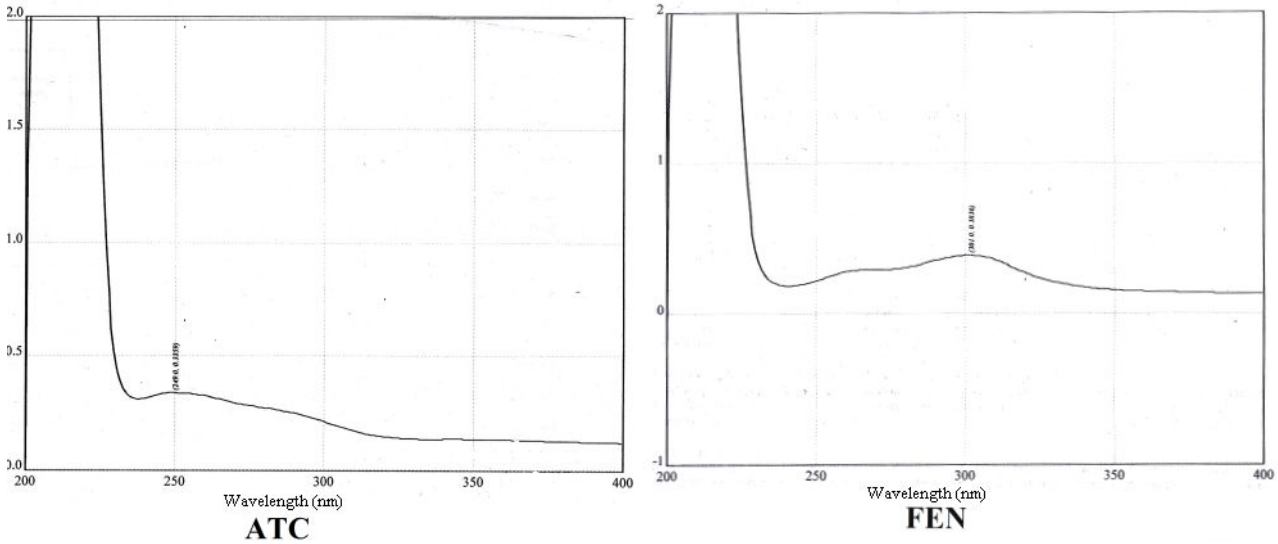



**Fig. 6.** Base degradation of ATC and FEN

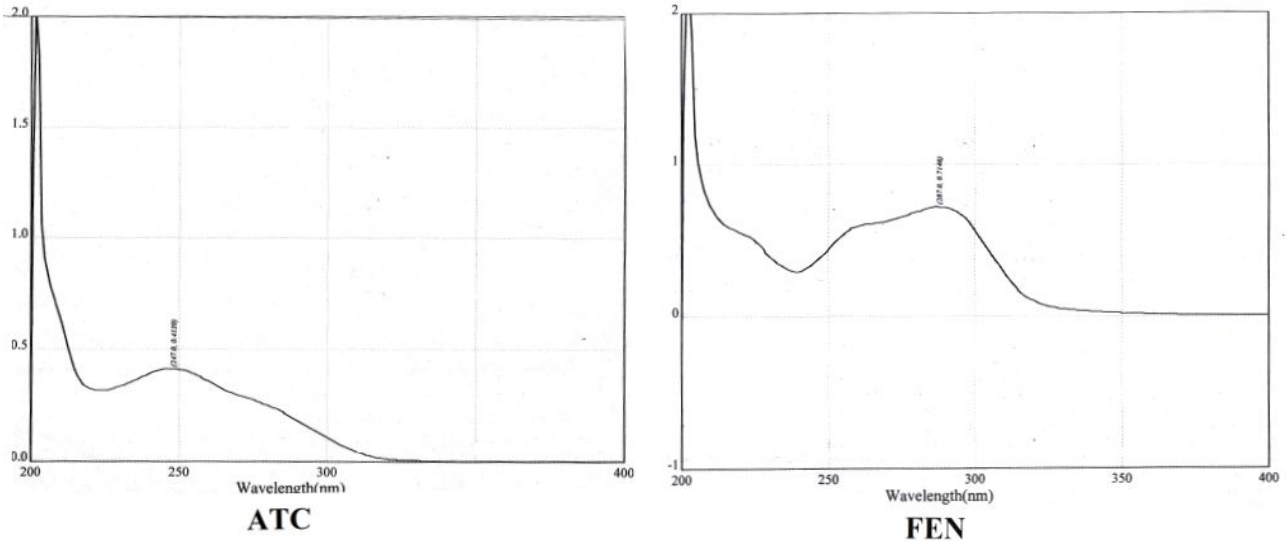



**Fig. 7.** Photostability degradation of ACE and FEN

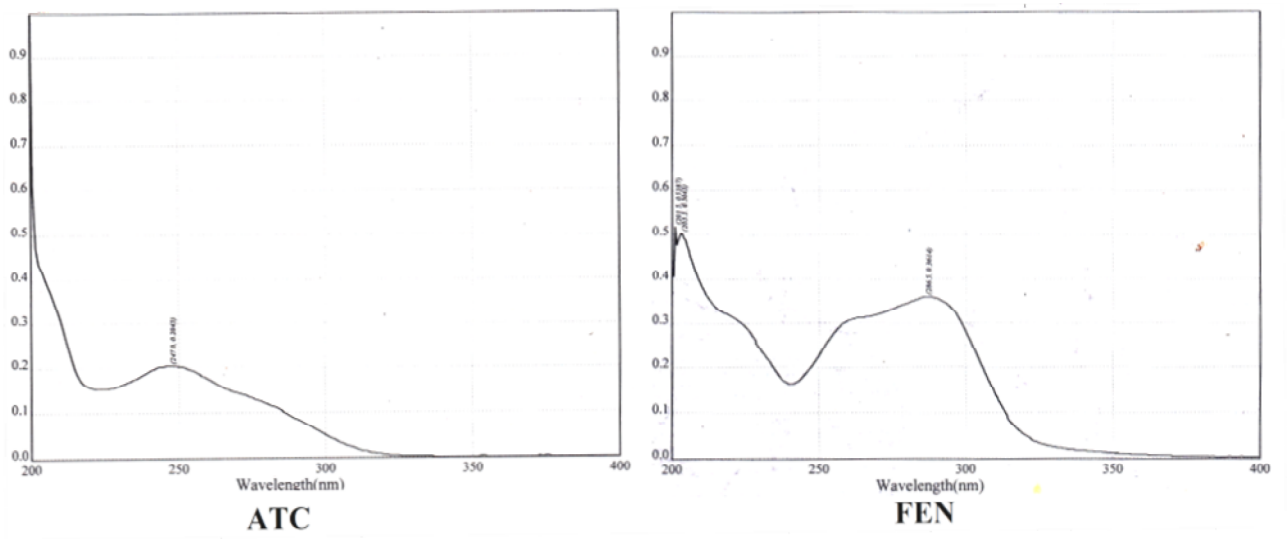



**Fig. 8.** Thermal degradation of ATC and FEN

## CONCLUSION

The proposed method is found to be simple, sensitive and reproducible and hence it can be used in routine analysis for simultaneous determination of stability indicating UV spectrophotometric method for ATC and FEN in bulk as well as in pharmaceutical preparation. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

## ACKNOWLEDGEMENT

Authors are thankful to project guide and management, Invertis Institute of Pharmacy, Invertis University, Bareilly, Uttar Pradesh for providing equipment and facility during entire duration of project work.

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