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Der Pharmacia Lettre, 2013, 5 (3):90-98
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Stability-indicating absorption ratio method for estimation of atorvastatin calcium and fenofibrate in tablet dosage form by using UV-Vis spectrophotomer

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ABSTRACT

Development of a Stability-indicating UV spectrophotometric method by absorption ratio method for the estimation of Atorvastatin calcium (ATC) and Fenofibrate (FEN) in tablet dosages form. In absorption ratio method absorbance measurement of sample at 262nm (isoabsorptive point, λ_1) and 287nm, λ_2. The absorbance ratio method was developed using methanol as solvent. The linearity of the proposed method was investigated in the range of 6-16 µg/ml and 2-12 µg/ml for ATC and FEN respectively. Calibration curves show 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 ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The LOD-0.2695 µg/ml, 0.0222 µg/ml for ATC and FEN and the LOQ-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 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 word: Absorption ratio method, degradation product, validation, atorvastatin calcium, fenofibrate.

INTRODUCTION

Atorvastatin Calcium (ATC) (**Fig. 1**) is calcium salt of (β*R*, 8*R*)-2-(4-fluorophenyl)-α,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(Phenylamino) carbonyl]-1*H*-pyrrole-1-heptanoic acid trihydrate[1]. ATC is a member of the drug class known as statins, used for lowering blood cholesterol. ATC is a HMG CoA reductase inhibitor. Fenofibrate (FEN) (**Fig. 1**) is 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester, it is antilipemic agent[2] which reduces cholesterol and triglycerides in blood thus decreases the risk of heart diseases and prevent strokes.

Atherosclerotic Vascular disease is a condition in which an artery wall thickness as a result of accumulation of fatty materials such as cholesterol. It affects mostly arterial blood vessels, inflammatory response in walls of arteries 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 where as Fenofibrate in reduction of triglycerides.[3]

Forced degradation studies

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 [4]. 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 includes light exposure, acid hydrolysis (0.1 N HCL), base hydrolysis (0.1N NAOH), and thermal degradation. The duration of time selected for degradation studies was 6 hours. The photolytic degradation was performed by exposing the solid drugs to sunlight for 12 hours. The concentration of 100 µg/ml of each of Atorvastatin calcium and Fenofibrate were prepared using respective solvents (NAOH, HCL, methanol) separately [5].

The objective of the present work is to develop simple, rapid, accurate, specific and economic UV Stability indicating method for the estimation of ATC & FEM in bulk and tablet dosages form. The method was further validated as per ICH guidelines [6] for the parameter like precision, accuracy, sensitivity, and linearity. The result of analysis was validated statistically and by recovery studies.

MATERIALS AND METHODS

Samples

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

Reagents

Methanol was used of analytical grade. All other reagents used were of analytical grade for the forced degradation studies. Hydrochloric acid (GR grade), Sodium hydroxide, from Qualingens fine chemical Mumbai.

Instruments

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

PREPARATION OF STANDARD STOCK SOLUTION

Accurately weighed quantity of about 50 mg of ATC was taken in 50 ml volumetric flask dissolved in sufficient quantity of methanol then sonicate for 10 min and 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 then sonicate for 15 min and diluted up to the mark with same solvent so as to get the concentration of 1000 µg/ml. and this solution 5 ml pipette out in 50 ml volumetric and volume was make up with methanol and get concentration 100 µg/ml used for making dilution for calibration curve.

Determination of isoabsorptive point

The standard solution of ATC and FEN were separately scanned at different concentration in the range of 200-400 nm and the λ_{max} was determined. The overlain spectrum for both drugs was run. The isoabsorptive point (where both the drugs show equal absorbance) was obtained from the overlain spectra of ACE and FEN. The overlain spectra showing isoabsorptive point at 262 nm. (**Fig. 2**)

Preparation of calibration curve

For each drug appropriate aliquots were pipette 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 µg/ml of ATC and 2-12

µg/ml of FEN (**Fig. 3**). Solutions of different concentrations for each drug were analysed at their respective wavelengths and absorbances were recorded. (**Table. 1**)

METHOD DEVELOPMENT

Absorption ratio method

Two wavelength selected for the method are 247 nm and 287nm that are absorption maximas of ATC and FEN respectively in methanol, and the isoabsorptive point the λ_{max} is 262 nm. (Fig. 2) Standard stock solution of 100µg/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 µg/ml for ATC and 2-12 µg/ml for FEN The absorbances were measured at the selected wavelengths and absorptivities 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. Two equations are constructed as described for the method of simultaneous equation. Their treatment is somewhat different, however, and uses the relationship $a_{x1} = a_{y1}$ at λ1. Assume b = 1cm

$A_1 = a_{x1}C_x + a_{x1}C_y$ ..... (1)
Let $Q_X = a_{x2} / a_{x1,}$ $Q_Y = a_{y2}/ a_{y1,}$ and $Q_M = A_2 / A_1$
$C_x = (Q_M - Q_Y) A_1 / (Q_X - Q_Y) a_{x1}$

Above equation gives the concentration of X in terms of absorbance ratios, the absorbance of mixture and the absorptivities of the compounds at the isoabsorptive wavelength.

Preparation of tablet for 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, added about 70 ml of methanol and sonicated for 20 minutes and volume made upto the mark with methanol. Mixed well and filtered through Whatman filter paper No. 41. First few ml filtrate discarded and then 5 ml of filtrate pipette out and diluted to 50 ml with methanol. Then the absorbances were recorded at the respective wavelengths. (**Table. 2**)

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 all the two drugs are added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study. (**Table. 3**)

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 Q2R1 2003).

Linearity

Linearity is established by least squares linear regression analysis of the calibration curve. The constructed calibration curve is linear over the constructed range 6-16 µg/ml (**Table. 4a**), 2-12 µg/ml for ATC, FEN. (**Table. 4b**)

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 (**Table. 5**)

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. (**Table. 5**)

LOD and LOQ

The LOD and LOQ of ATC and FEN are calculated by Mathematical equation.

LOD= 3.3×standard deviation ÷ slope
LOQ=10×standard deviation ÷ slope

The LOD of ATC & FEN were found to be 0.2695 µg/ml and 0.0222 µg/ml and the LOQ of ATC and FEN were found to be 0.8780µg /ml and 0.22221µg/ml (**Table. 5**)

Robustness
Robustness of proposed method was perform by changing UV analyst and remaining condition (solvent, dilution, UV Spectrophotometer) is same. (**Table. 5**)

FORCE DEGRADATION
Forced degradation studies are performed on ATC and FEN to prove the stability indicating property of the method. The stress conditions employed for degradation study include light exposure, acid hydrolysis (0.1 N HCL), base hydrolysis (0.1N NAOH), thermal hydrolysis, The duration of time selected for degradation studies was 6 hours (ICH QA (R2). (**Table. 6**)

Acid hydrolysis
Solutions for acid degradation studies are prepared in methanol (12 µg/ml for ATC and 8 µg/ml for FEN) and add 10 ml 0.1 M hydrochloric acid solution and kept at room temperature (22 °C). It observed that both acid and base hydrolysis was a fast reaction for both drugs and almost completed within 3hrs of the sample preparation, therefore the samples are analyzed at 247 nm for ATC and 287 nm for FEN after this period of time. (**Fig. 4)**

Base hydrolysis
Solutions for base degradation studies are prepared in methanol (12 µg/ml for ATC and 8 µg/ml for FEN) and 100ml 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. (**Fig. 5**)

Photostability studies
Weigh 50 mg drug and kept in the sun light for 12 hrs after that the Solutions for Photostability studies are prepared in methanol and the dilution (12 µg/ml for ATC and 8 µg/ml for FEN) were prepared and analysed in UV Spectrophotometer at 247 nm for ATC and 287 nm for FEN. **(Fig. 6**)

Thermal degradation
Weigh 50 mg drug and kept in the oven and temperature are maintain 80 ºC for 3 hrs after that the Solutions for Photostability studies are prepared in methanol and the dilution (12 µg/ml for ATC and 8 µg/ml for FEN) were prepared and analysed in UV Spectrophotometer. (**Fig. 7**)

Statistical analysis
Means, standard deviation (SD), relative standard deviation (RSD) and linear regression analysis were calculated using Microsoft Excel 2007

RESULTS AND DISCUSSION

Many pharmaceutical compounds undergo degradation during storage or even during the different processes of their manufacture. Several chemical or physical factors can lead to the degradation of drugs. Hydrolysis and oxidation are the most famous chemical degradation routes of drugs. The main classes of drugs that are subject to degradation are esters, amides and lactams. Ester hydrolysis is frequently base catalysed, which makes the reaction rapid and irreversible.

The overlain spectra of ATC and FEN exhibit λ_1 262 nm (isoabsorptive point) and $\lambda_2$287 nm respectively. The present research works discuss the development of a Stability-indicating UV spectrophotometric method for the estimation of ATC and FEN in tablet dosages form. The optimum conditions for the analysis of the drug were established.

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 show 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**. Validation was performed as ICH guidelines (Q2R1) for Linearity, accuracy, precision, LOD and LOQ. The LOD-0.2695 µg/ml, 0.0222µg/ml for ATC and FEM and the LOQ-0.8780 µg/ml, 0.222 µg/ml for ATC and FEM respectively and the result of forced degradation is denoted on **Table. 6**, 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.

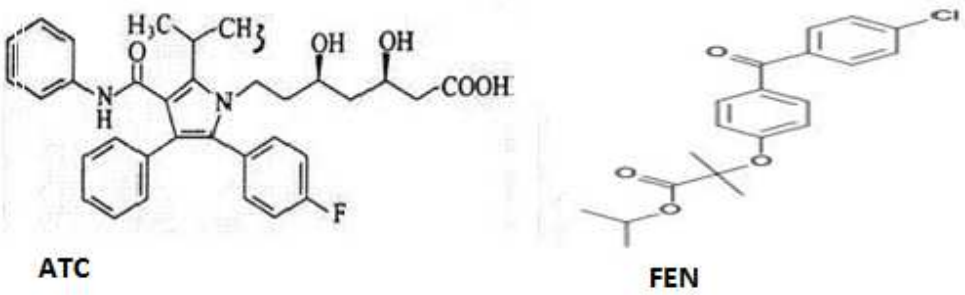

Fig. 1 Structure of ATC & FEN

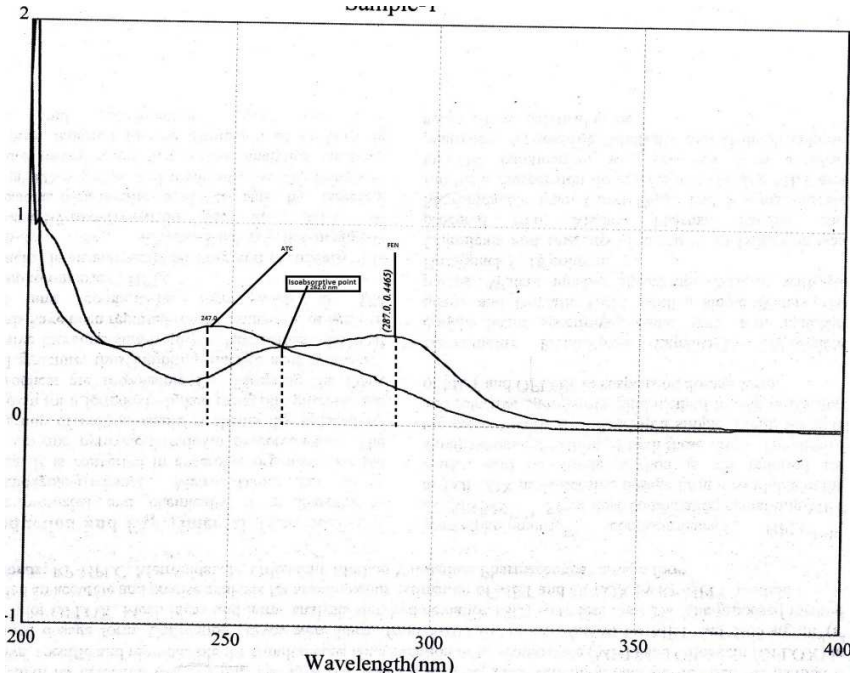

Fig. 2 Isoabsorptive point of ATC & FEN

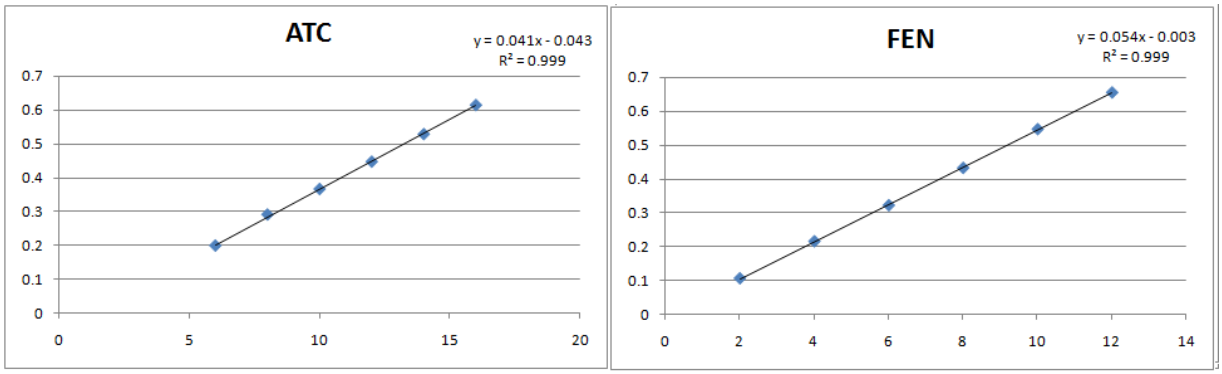

Fig. 3 Calibration curve of ATC and FEN

Fig. 3 calibration curve of ATC & FEN

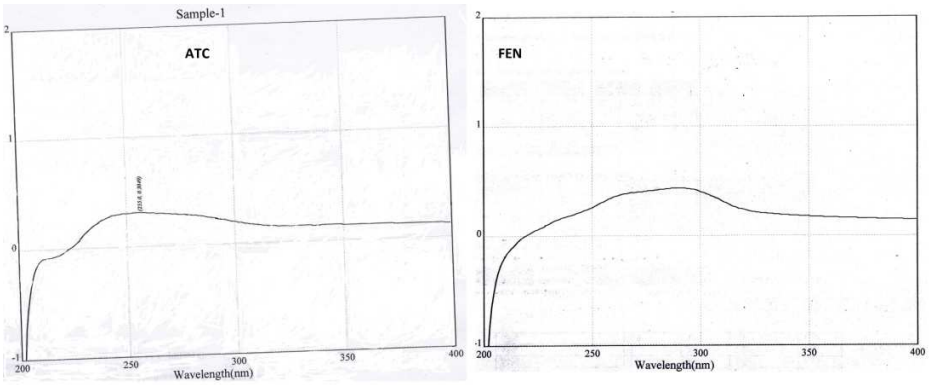

Fig. 4 Acid degradation of ATC and FEN

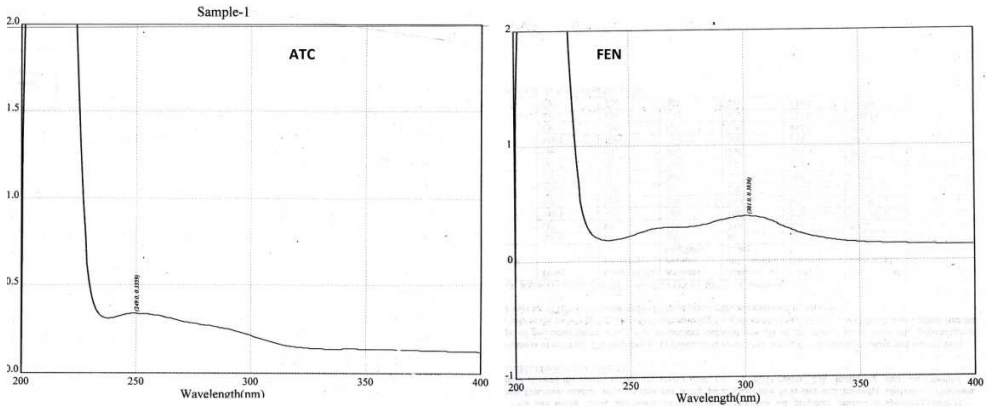

Fig. 5 Base degradation of ATC and FEN

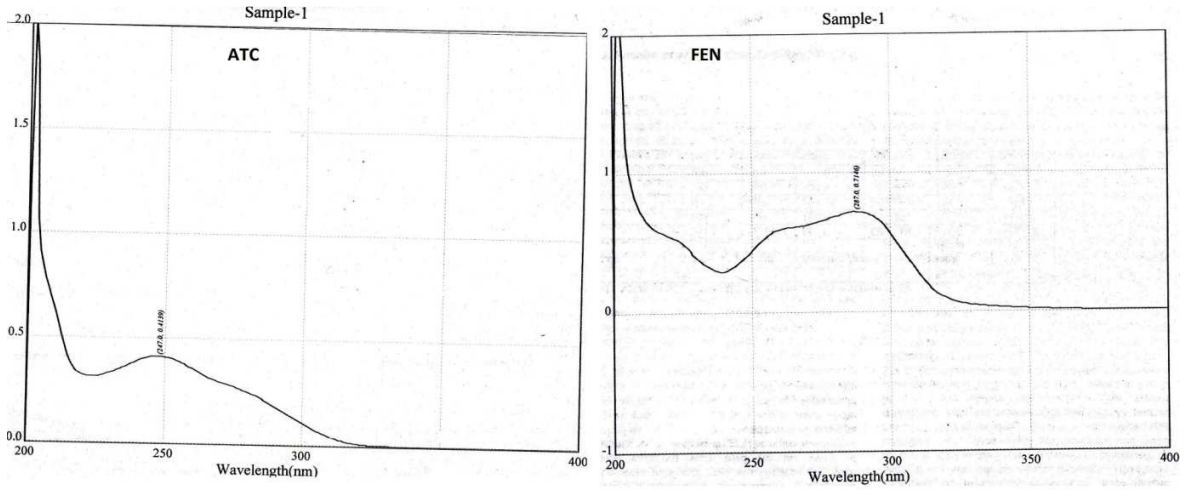

Fig. 6 Photostability degradation of ACE and FEN

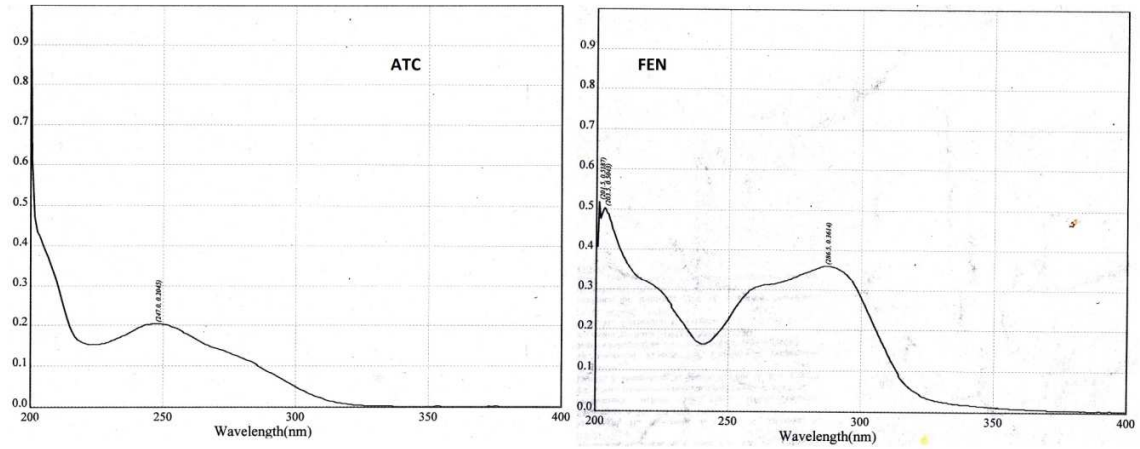

Fig. 7 Thermal degradation of ACE and FEN

Table 1: UV analysis (Calibration curve)

S.NO.	Parameter (units)	Atorvastatin calcium	Fenofibrate
1.	Linearity range (µg/ml)	6-16 µg/ml	2-12µg/ml
2.	Correlation coefficient (r^2)	0.999	0.999
3.	Slope	0.041	0.054
4.	Intercept	0.043	0.003

Table 2: Analysis of tablet dosage form

Formulation	Drug	Label claim (mg)	% Label claim (Mean ± SD)
Tablet	ATC	10 mg	100.21 ± 0.0018
	FEN	160 mg	101.12 ± 0.0024

Table 3: Result of statistical validation of recovery

% added	Drug	Mean%± SD	%RSD
80%	ACE	100.12 ± 0.0019	0.0022
	FEN	99.80±0.0004	0.0033
100%	ACE	99.62±0.0013	0.0046
	FEN	100.09±0.0009	0.0046
120%	ACE	99.22±0.0020	0.0033
	FEN	99.54±0.0019	0.0042

Table 4a: Linearity study of ATC in methanol

Con(µg/ml)	Dilution (1)	Dilution (2)	Dilution (3)	Dilution (4)	Dilution (5)	Dilution (6)	Mean ± SD*
6	0.1950	0.1928	0.1963	0.2019	0.2023	0.2045	0.1988 ± 0.0042
8	0.2909	0.2889	0.2904	0.2862	0.2878	0.2998	0.2906 ± 0.0043
10	0.3642	0.3624	0.3643	0.3706	0.3694	0.3659	0.3661 ±0.0029
12	0.4477	0.4448	0.4454	0.4480	0.4479	0.4470	0.4468 ± 0.0012
14	0.5279	0.5306	0.53281	0.5265	0.5286	0.5286	0.5283 ± 0.0012
16	0.6320	0.6135	0.6079	0.6080	0.6103	0.6129	0.6141 ± 0.0082

SD - Standard deviation*

Table 4b: Linearity study of FEN in methanol

Con (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 5: 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%)	100.12 ± 0.0019	99.80±0.0004
	Accuracy (100%)	99.62±0.0013	100.09±0.0009
	Accuracy (120%)	99.22±0.0020	99.54±0.0019
3.	Interday precision (1^{st} day)	104.84% ± 0.0024*	99.21% ± 0.00216
	(2^{nd} day)	107.54% ± 0.0143*	100.09% ± 0.0014
	(3^{rd} day)	110.41% ± 0.0043*	106.10% ± 0.0016
4.	Intraday precision 1^{st} hrs	107.87% ± 0.0024*	99.21% ± 0.0026
	2^{nd} hrs	108.22% ± 0.0019*	100.96% ± 0.0001
	3^{rd} hrs	105.44% ± 0.0018*	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	98.25% ± 0.0010*	102.50% ± 0.0015

** Standard deviation*

Table 6: Forced degradation study (ATC & FEN)

S.NO.	Condition	Absorbances (λ)		Mean ± SD*		Result (% degradation)	
		ATC	FEN	ATC	FEN	ATC	FEN
1.	Acid degradation	255 nm	No absorbane	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*

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence it can be used in routine analysis for 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. The relative standard deviation (RSD) for all parameters was found to be less than one, which indicate that the validity of method are also within the limit so the proposed method can be used for routine quantative analysis of both the drug.

Acknowledgement
I thankful to the project guide and management, Invertis institute of pharmacy, Invertis University Bareilly to providing the equipment and facility for entire duration of project work. and also thankful to Rightaid Laboratories, Hyderabad for providing API of ATC and FEN as a gift sample.

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