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# Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets

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ABSTRACT

A stability indicating UPLC method was developed and validated for the simultaneous determination of atorvastatin, fenofibrate and their impurities in tablets. The chromatographic separation was performed on acquity UPLC™ BEH C18 column (1.7 μm, 2.1 mm × 100 mm) using gradient elution of acetonitrile and ammonium acetate buffer (pH 4.7; 0.01 M) at flow rate of 0.5 ml/min. UV detection was performed at 247 nm. Total run time was 3 min within which main compounds and six other known and major unknown impurities were separated. Stability indicating capability was established by forced degradation experiments and separation of known degradation products. The method was validated for accuracy, repeatability, reproducibility and robustness. Linearity, LOD and LOQ was established for atorvastatin, fenofibrate and their known impurities.



## 1. Introduction

Atorvastatin (ATO) calcium, chemically [R-(R*,R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is a synthetic lipid-lowering agent. ATO is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis [1]. Atorvastatin calcium is an organic acid with a p$K_a$ of 4.46 and is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. It is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol. Lactone form of ATO (A-LCT), chemically, 5-(4-fluorophenyl)-2-(1-methyl-ethyl)-N,4-diphenyl-1-[2-[(2R,4R)-tetrahydro-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1H-pyrrole-3-carboxamide, is the major degradation product of ATO. Chemical structures of ATO and A-LCT are shown in Fig. 1(A) and (B), respectively.

Fenofibrate (FEN), chemically 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid 1-methylethyl ester, is a lipid-regulating agent. It is a white solid with melting point of 79–82 °C and is insoluble in water [2]. FEN is official in USP [3], and BP [4]. The USP monograph of FEN [3] mentions related compound A (FEN-A), compound B (FEN-B) and compound C (FEN-C) as its impurities. Chemically, FEN-A is (4-chlorophenyl)(4-hydroxyphenyl)methanone, FEN-B is 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid (Fenofibric acid) and FEN-C is 1-methylethyl-2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy-2-methyl propanoate. Chemical structures of FEN, FEN-A, FEN-B and FEN-C are given in Fig. 1(C), (D), (E) and (F), respectively. FEN is metabolized to the active substance fenofibric acid [5].

The ATO + FEN combination has a highly beneficial effect on all lipid parameters and plasma fibrinogen in patients with type 2 diabetes and combined hyperlipidemia. It improved patients' coronary artery disease risk status significantly more than each drug alone [6]. Atorvastatin is more effective in reduction of total cholesterol level whereas fenofibrate is more efficient in reduction of triglycerides. The combination had beneficial effects on oxidative stress and on vascular reactivity in hyperlipidemia [7].

ATO and its pharmaceutical formulation with FEN is not official in any pharmacopoeia yet. Several analytical methods such as HPLC [8–16], spectrophotometry [17] and HPTLC [18] are reported for determination of ATO in bulk drug, formulations and biological matrices. Literature indicates spectrophotometry [19], square-wave voltammetry [20], HPLC [21–24] methods for determination of fenofibrate in pharmaceutical formulations, drug

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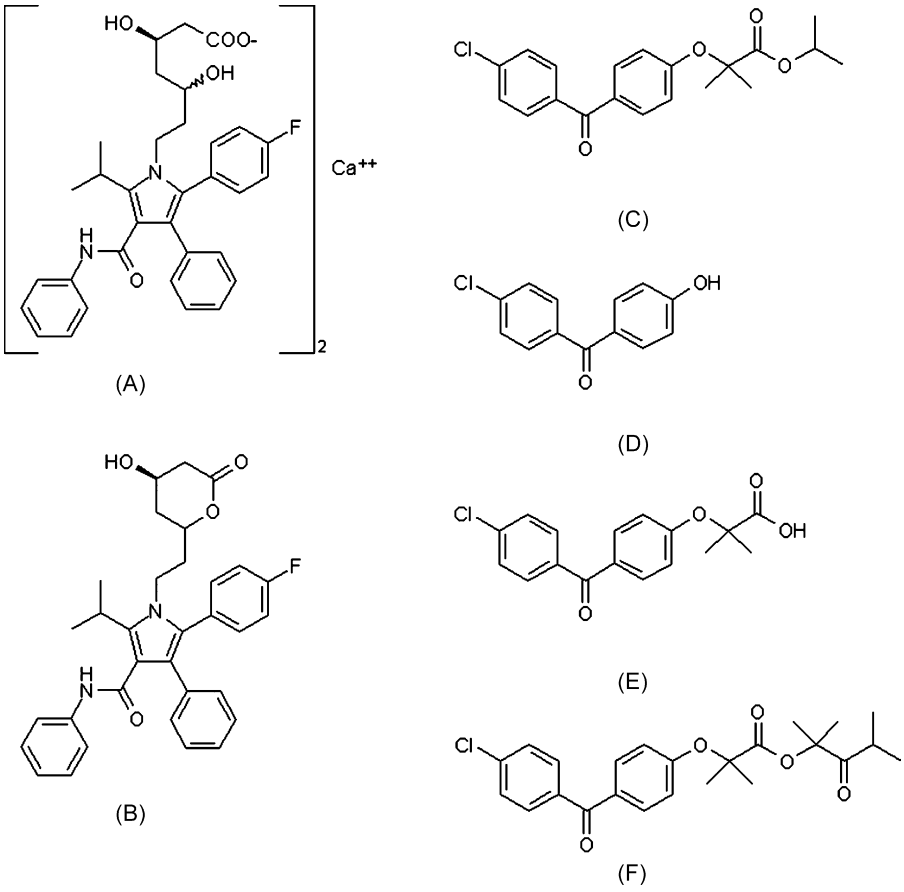


**Fig. 1.** Chemical structures of (A) ATO, (B) A-LCT, (C) FEN, (D) FEN-A, (E) FEN-B, and (F) FEN-C.

substance and biological matrices. Derivative ratio spectrophotometry and chemometric calibrations method is reported for simultaneous resolution of ATO and FEN [25]. Nowadays, the determination of impurities is one of the main and difficult tasks for pharmaceutical analysts during the development of separation methods, especially if more and more impurities are required to be determined. It should be taken into consideration and FEN + ATO should be monitored together with their degradation compounds, preferably in single chromatographic run.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about ninefold decrease in analysis time as compared to the conventional HPLC system using 5 μm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3 μm particle size analytical columns without compromise on overall separation [26,27].

The purpose of this study was to develop a stability indicating method for the simultaneous determination of six compounds (ATO, FEN, FEN-A, FEN-B, FEN-C and A-LCT) in tablets. UPLC technique was chosen because of its above-mentioned advantages. The proposed method was able to separate these compounds as well as two other unknown degradation products within 3 min. Thereafter, this method was validated as per ICH guidelines [28] and successfully applied for separation and quantification of all compounds of interest in the pharmaceutical formulation—atorvastatin and fenofibrate tablets.

## 2. Experimental

### 2.1. Chemicals and reagents

Reference standards of FEN, ATO calcium and A-LCT were kindly gifted by Indoco Remedies (Mumbai, India) with declared purity of 99.5%, 99.0% and 95%, respectively. USP reference standards of FEN-A, FEN-B and FEN-C were also gift samples from them. All the standards were used as received.

Acetonitrile for HPLC was obtained from J.T. Baker (NJ, USA), ammonium acetate was obtained from Merck (Mumbai, India) and ExcelaR grade glacial acetic acid was supplied by Qualigens fine chemicals (Mumbai, India). The 0.45 μm nylon filter used to filter sample preparation was mdi SY25NN which was manufactured by advanced Microdevices (P) Ltd. (Ambala, India). Tablet formulation (Fibator, Sun Pharmaceuticals, India) containing 10 mg of ATO and 160 mg of FEN was procured from local market in Mumbai, India.

### 2.2. Buffer preparation

Solution of ammonium acetate (0.01 M) was prepared by dissolving about 0.77 g of ammonium acetate in one litre of water for HPLC. The pH of this solution was adjusted to 4.70 with acetic acid. The buffer preparation was found stable with respect to pH and visual clarity for 48 h.

### 2.3. Chromatographic system

Analyses were performed on Acquity UPLC™ system (Waters, Milford, USA), consisting of a binary solvent manager, sample man-

**Table 1**
Gradient program for elution of ATO, FEN and impurities

| Time (min) | Flow rate (ml/min) | % A, acetate buffer (pH 4.7; 0.01 M) | % B, acetonitrile |
|---|---|---|---|
| Initial | 0.500 | 50.0 | 50.0 |
| 1.00 | 0.500 | 30.0 | 70.0 |
| 1.40 | 0.500 | 15.0 | 85.0 |
| 2.00 | 0.500 | 15.0 | 85.0 |
| 2.20 | 0.500 | 50.0 | 50.0 |
| 3.00 | 0.500 | 50.0 | 50.0 |

ager and PDA detector. The detector was set at sampling rate of 20 points/s and filter time constant of 0.2 s. System control, data collection and data processing were accomplished using Waters Empower™ chromatography data software. The analytical column was 100 mm × 2.1 mm acquity UPLC™ BEH C18, 1.7 μm particle size (Waters, Milford, USA). The separation of ATO, FEN and impurities was achieved by gradient elution using acetonitrile and acetate buffer (pH 4.70; 0.01 M). The finally selected and optimized conditions were as follows: injection volume 1 μl, gradient elution (Table 1), at a flow rate of 0.5 ml/min at ambient temperature, detection wavelength 247 nm. Under these conditions, the backpressure in the system was about 10,500 psi.

### 2.4. Assay standard solution preparation

Standard solution was prepared by dissolving standard substances in methanol to obtain solution containing 25 μg/ml of ATO and 400 μg/ml of FEN.

### 2.5. Impurity standard solution preparation

Impurity standard solution was prepared by dissolving impurity standard substances in methanol to obtain solution containing 0.4 μg/ml of each of Fen-A, Fen-B, Fen-C and 0.125 μg/ml of A-LCT.

### 2.6. Sample preparation

Twenty tablets were crushed to fine powder. An accurately weighed portion of the powder equivalent to 12.5 mg of ATO and 200 mg of FEN was taken in 50 ml volumetric flask. About 30 ml of methanol was added to this flask and sonicated in an ultrasonic bath for 3 min. This solution was then diluted to the mark with diluent and mixed. It was then filtered through 0.45 μm nylon filter and filtrate was collected after discarding first few millilitres. Five millilitres of the filtrate was transferred to 50 ml volumetric flask, diluted to volume with methanol and mixed.

### 2.7. Method validation

#### 2.7.1. System suitability

System suitability parameters were measured so as to verify the system performance. System precision was determined on six replicate injections of standard preparations. All important characteristics including capacity factor, peak resolution, and theoretical plate number were measured.

#### 2.7.2. Specificity

Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed method. The powdered sample of tablets was exposed to acidic (1N HCl, 50 °C, 60 min), alkaline (1N NaOH, 50 °C, 60 min), strong oxidizing (30% $H_2O_2$, 50 °C, 60 min), thermal (60 °C, 1 d), and photolytic (254 nm, 1 d) degradation conditions. Also, standards of ATO and FEN were exposed to above stress conditions, individually and in combination with each other to identify source of degradation peaks. All the exposed tablet samples and standards were then analyzed by the proposed method.

#### 2.7.3. Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ of ATO, FEN and impurities were determined by using signal to noise approach as defined in International Conference on Harmonization (ICH) guideline [28]. Increasingly dilute solution of each drug and impurity was injected into the chromatograph and signal to noise (S/N) ratio was calculated at each concentration.

#### 2.7.4. Linearity

Linearity was demonstrated from 50% to 150% of standard concentration using minimum six calibration levels (50%, 60%, 80%, 100%, 120% and 150%) for both the compounds and their impurity standards. The method of linear regression was used for data evaluation. Peak area of standard compounds was plotted against respective concentrations. Linearity was described by equation and correlation coefficient was determined as well.

#### 2.7.5. Precision

Precision was investigated using sample preparation procedure for six real samples of commercial brand (Fibator, Sun Pharma, India) of tablets and analyzing by proposed method. Intermediate precision was studied using different column, and performing the analysis on different day.

#### 2.7.6. Accuracy

To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique. Three different levels (50%, 100% and 150%) of standards were added to pre-analyzed tablet samples in triplicate. The percentage recoveries of ATO, FEN and impurities at each level and each replicate were determined. The mean of percentage recoveries ($n = 9$) and the relative standard deviation was calculated.

#### 2.7.7. Robustness

The robustness as a measure of method capacity to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in pH of buffer (±0.2 units), change in column temperature (30 °C) and change in flow rate (±5%).

#### 2.7.8. Stability of sample preparation

Stability of sample solution was established by storage of sample solution at ambient temperature for 24 h. Sample solution was re-analyzed after 24 h and assay/impurities were determined and compared against fresh sample.

## 3. Results and discussion

### 3.1. Method development and optimization

The main criteria for development of successful UPLC method for determination of ATO, FEN and impurities in tablets were: the method should be able to determine assay and impurities of both drugs in single run and should be accurate, reproducible, robust, stability indicating, free of interference from degradation products/impurities and straightforward enough for routine use in quality control laboratory.

The USP [3] monograph for FEN states a RP-HPLC method using C18 column for its assay and related substances. The mobile phase in this method is acetonitrile–buffer (pH 2.5) (70:30, v/v). Same method is described in BP [4]. The separation of all compounds was

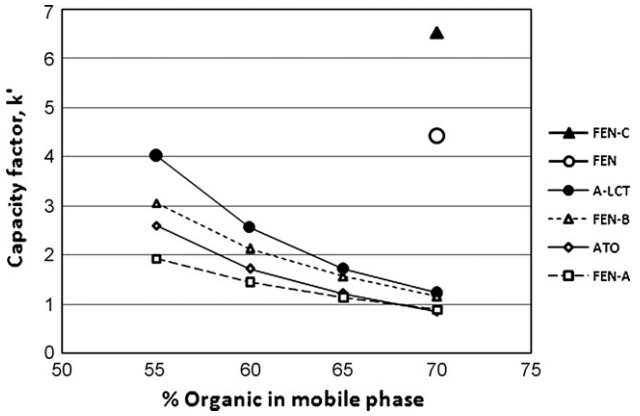


**Fig. 2.** Effect of change in composition of mobile phase on retention of compounds.

studied using this composition on UPLC column and system. ATO and FEN-A were found co-eluting and FEN-B and A-LCT were found co-eluting next to them. FEN and FEN-C were relatively retained for longer time and were well separated from each other. Thus, the critical separation to achieve was between ATO, FEN-A, A-LCT and FEN-B. To develop the stability indicating method, first the retention behavior of these four compounds with change in percentage of organic solvent (acetonitrile) and with change in pH of buffer was studied on C18 UPLC column. While assessing the effect of change of proportion of organic solvent in mobile phase, the pH of buffer was set to 2.0 and while assessing the effect of pH of buffer, the mobile phase composition was buffer–acetonitrile (47:53, v/v). Capacity factors of all compounds were plotted against percentage of organic solvent in mobile phase (Fig. 2) and against pH of buffer preparation (Fig. 3), respectively.

Fig. 2 indicates that the separation between four compounds enhances with decrease in percentage organic in mobile phase. Also Fig. 3 indicates that retention of ATO and Fen-B is pH dependant as they are acidic substances (Fig. 1). Other compounds did not show pH dependency on their retention. The pH/retention profile of FEN and FEN-C was also studied in mobile phase composition of buffer–acetonitrile (30:70, v/v) and their retention was also found pH independent. As seen from Fig. 3, the critical separation between Fen-A, Fen-B, ATO and A-LCT could be achieved in the pH range of 3.5–4.0 or beyond pH 4.5. It was also noted that Fen-B elutes very close to void volume when pH is above 5.5. For faster elution of FEN and Fen-C, and to ensure that all possible non-polar degradation products are eluted, it was decided to use a gradient run.

Thus, in the first step the pH range of 3.5–4.0 was selected and a gradient run was optimized using formate buffer (pH 3.8; 0.01 M).

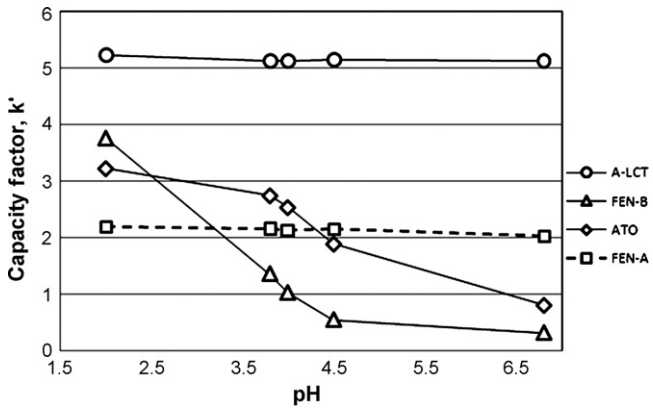


**Fig. 3.** Effect of change in pH of buffer on retention of compounds.

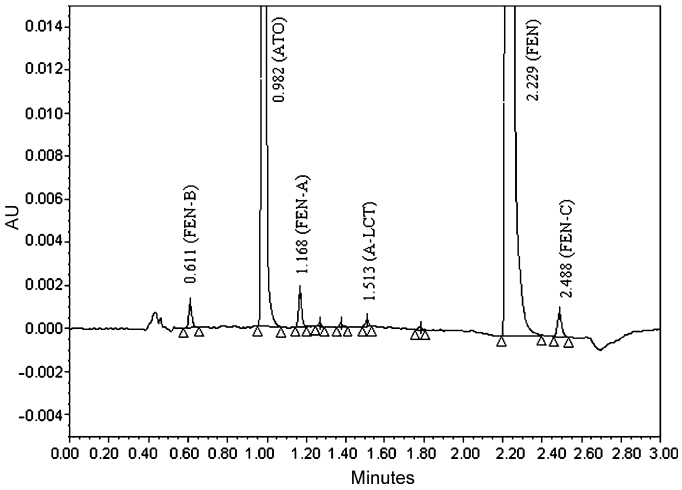


**Fig. 4.** Typical chromatogram showing separation of individual compounds in a solution containing 25 μg/ml of ATO, 400 μg/ml of FEN, 0.4 μg/ml of each of FEN-A, FEN-B, FEN-C and 0.125 μg/ml of A-LCT chromatographed on acquity UPLC™ BEH C18 column (1.7 μm, 2.1 mm × 100 mm) in gradient elution using acetate buffer (pH 4.7; 0.01 M) and acetonitrile as mobile phase and measured at 247 nm.

The satisfactory separation, where resolution was greater than 2.0 was achieved between ATO, Fen-A and Fen-B. But this separation was not robust with respect to pH. At upper pH (+0.20 units), resolution between ATO and Fen-A was decreased whereas at lower pH (−0.20 units) resolution between Fen-A and Fen-B was decreased to below 2.0.

Hence the pH range of 4.5–5.5 was explored for the critical separation of ATO, Fen-A and Fen-B. Above pH 5.0 the retention of Fen-B was not satisfactory ($k' < 0.5$). Any effort to retain Fen-B by increasing initial aqueous composition in gradient run resulted in higher retention of other components as well, thus resulting in higher run time. The buffer pH 4.7 was found most appropriate for robust resolution of all the components of interest in minimum run time. The final gradient run was chosen with regards to the peak resolutions and analysis time as well. The gradient program is given in Table 1. The flow rate of 0.5 ml/min was optimized with regard to the backpressure and analysis time as well.

ATO, FEN and their impurities were well resolved in reasonable time of about 3 min. A typical chromatogram showing separation of ATO, FEN and impurities is shown in Fig. 4. Since the concentration of ATO in sample preparation was 16 times lower than FEN, the wavelength of ATO maximum (247 nm) was used to have good detection of ATO and impurities. At this wavelength, FEN and impurities also have good response and thus simultaneous determination of assay and impurities of both the drugs was possible in single run.

### 3.2. Analytical parameters and validation

After satisfactory development of method it was subjected to method validation as per ICH guideline [28]. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (accuracy, precision, linearity, robustness and stability indicating capability).

#### 3.2.1. System suitability

The percentage R.S.D. of area count of six replicate injections was below 2.0%. The results of system precision are presented in Tables 2 and 3. Low values of R.S.D. of replicate injections indicate that the system is precise. Results of other system suitability

**Table 2**
Method validation results for assay of individual compound

| Parameter | ATO | FEN |
|---|---|---|
| System precision[a] (%R.S.D.) | 0.52 | 0.60 |
| Tailing factor | 1.15 | 1.18 |
| Repeatability[b] (%assay) | 99.9 | 100.4 |
| Repeatability[c] (%R.S.D.) | 0.5 | 0.4 |
| Intermediate precision[b] (%assay) | 100.3 | 100.1 |
| Intermediate precision[c] (%R.S.D.) | 0.6 | 0.7 |
| Linearity[d] (correlation coefficient) | 0.9992 | 0.9998 |
| Linearity[d] (equation) | $Y = 3104x + 385$ | $Y = 2205x + 9758$ |
| LOQ (μg/ml) | 0.003 | 0.04 |
| Accuracy[e] (%R.S.D.) | 0.8 | 0.8 |
| Accuracy[e] (%recovery) | 100.4 | 100.0 |
| Selectivity[f] | No interference | No interference |
| Stability—24 h (%)[g] | 99.9 | 100.1 |

[a] Determined on five replicate injections.
[b] Average of six determinations.
[c] Determined on six values.
[d] Six levels, from 50% to 150% of standard concentration.
[e] Determined at three levels (50%, 100% and 150%) with triplicate determination at each level.
[f] Demonstrated by forced degradation and separation of known degradation products.
[g] Correlation with freshly prepared sample (%).

parameters such as capacity factor, resolution and theoretical plates are presented in Table 4. As seen from this data, the acceptable system suitability parameters would be: relative standard deviation of replicate injections is not more than 2.0%, capacity factor for Fen-B is not less than 0.4 and resolution between ATO and Fen-A is not less than 2.0.

### 3.2.2. Specificity

The results of forced degradation study are given in Table 5. ATO was found highly sensitive to acid hydrolysis. The assay value was dropped to 41.7% and degradation peaks were observed in the chromatogram. Major degradation products of acid hydrolysis were A-LCT and an unknown impurity with area of 3.2% and 49.3%, respectively. Similarly, FEN was found sensitive to alkali hydrolysis yielding Fen-B and an unknown impurity as major degradation products. The area percent of Fen-B was 6.8% and of unknown impurity was 14.2% with respect to FEN. Chromatograms of acid and base degraded tablet samples are presented in Figs. 5 and 6, respectively. The peak labeled as DP1 is unknown degradation product of ATO whereas DP2 is unknown degradation product of FEN. Source of these degradation products in tablet sample was ascertained from chromatogram of standards of ATO and FEN which were simultaneously degraded under same conditions. Further, spectra of unknown degradation products in tablet sample

**Table 3**
Precision, accuracy, selectivity and stability results for impurities

| Parameter | FEN-A | FEN-B | FEN-C | A-LCT |
|---|---|---|---|---|
| System precision[a] (%R.S.D.) | 1.2 | 1.5 | 1.0 | 1.9 |
| Repeatability[b] (%impurity) | N.D. | N.D. | N.D. | 0.31 |
| Repeatability[c] (%R.S.D.) | N.A. | N.A. | N.A. | 5.8 |
| Intermediate precision[b] (%impurity) | N.D. | N.D. | N.D. | 0.29 |
| Intermediate precision[c] (%R.S.D.) | N.A. | N.A. | N.A. | 2.4 |
| Accuracy[d] (%recovery) | 100.9 | 100.5 | 98.3 | 101.0 |
| Accuracy[d] (%R.S.D.) | 1.7 | 2.2 | 0.5 | 1.2 |
| Selectivity[e] | No interference | No interference | No interference | No interference |
| Sample stability | | | | |
| % in fresh sample | N.D. | N.D. | N.D. | 0.29 |
| % after 24 h | N.A. | N.A. | N.A. | 0.31 |

N.D.: not detected; N.A.: not applicable.
[a] Determined on six replicate injections.
[b] Average of six determinations.
[c] Determined on six values.
[d] Determined at three levels (50%, 100% and 150%) with triplicate determination at each level.
[e] Demonstrated by spiking known impurities into sample.

**Table 4**
System suitability parameters and robustness

| System suitability parameter | Robustness parameter | FEN-B | ATO | FEN-A | A-LCT | FEN | FEN-C |
|---|---|---|---|---|---|---|---|
| Capacity factor ($k'$) | No change (repeatability) | 0.52 | 1.45 | 1.90 | 2.77 | 4.54 | 5.18 |
| | pH of buffer (+0.2 units) | 0.45 | 1.31 | 1.91 | 2.77 | 4.55 | 5.19 |
| | pH of buffer (−0.2 units) | 0.67 | 1.64 | 1.90 | 2.77 | 4.54 | 5.17 |
| | Column temperature 30 °C | 0.52 | 1.43 | 1.86 | 2.73 | 4.47 | 5.05 |
| | Flow (+5%) | 0.53 | 1.47 | 1.93 | 2.83 | 4.67 | 5.30 |
| | Flow (−5%) | 0.52 | 1.43 | 1.89 | 2.72 | 4.44 | 5.07 |
| Resolution factor ($R$) | No change (repeatability) | – | 12.91 | 6.37 | 12.16 | 23.87 | 7.08 |
| | pH of buffer (+0.2 units) | – | 12.65 | 7.81 | 11.22 | 23.92 | 7.31 |
| | pH of buffer (−0.2 units) | – | 13.97 | 3.67 | 13.41 | 26.15 | 7.12 |
| | Column temperature 30 °C | – | 13.17 | 6.01 | 12.71 | 24.60 | 6.91 |
| | Flow (+5%) | – | 13.08 | 6.18 | 12.48 | 24.62 | 6.93 |
| | Flow (−5%) | – | 13.25 | 6.70 | 12.08 | 23.37 | 7.18 |
| Column efficiency ($N$) | No change (repeatability) | 7305 | 20,454 | 26,151 | 48,051 | 81,733 | 63,063 |
| | pH of buffer (+0.2 units) | 8007 | 18,139 | 20,913 | 48,491 | 83,220 | 69,091 |
| | pH of buffer (−0.2 units) | 9575 | 24,209 | 26,228 | 70,986 | 83,263 | 63,636 |
| | Column temperature 30 °C | 7894 | 20,838 | 25,102 | 52,732 | 87,105 | 67,687 |
| | Flow (+5%) | 7605 | 19,691 | 24,898 | 48,588 | 84,320 | 62,839 |
| | Flow (−5%) | 8122 | 21,722 | 28,482 | 49,072 | 78,375 | 64,367 |

**Table 5**
Forced degradation data

| Degradation condition | ATO | | FEN | |
|---|---|---|---|---|
| | %Assay | Major degradation products[a] | %Assay | Major degradation products[a] |
| No degradation (control) | 100.8 | – | 100.9 | – |
| Acid hydrolysis (1N HCl, 50 °C, 60 min) | 41.7 | A-LCT (3.2%) and an unknown impurity (49.3%) | 94.1 | Fen-B (0.1%) |
| Alkali hydrolysis (1N NaOH, 50 °C, 60 min) | 99.1 | – | 78.4 | Fen-B (6.8%) and an unknown impurity (14.2%) |
| Oxidation (30% $H_2O_2$, 50 °C, 60 min) | 100.0 | – | 100.3 | – |
| Thermal (60 °C, 1 d) | 101.8 | – | 99.7 | – |
| Photolytic (UV at 254 nm, 1 d) | 100.7 | – | 100.1 | – |

[a] Numbers in parentheses represent percentage area against respective standard.

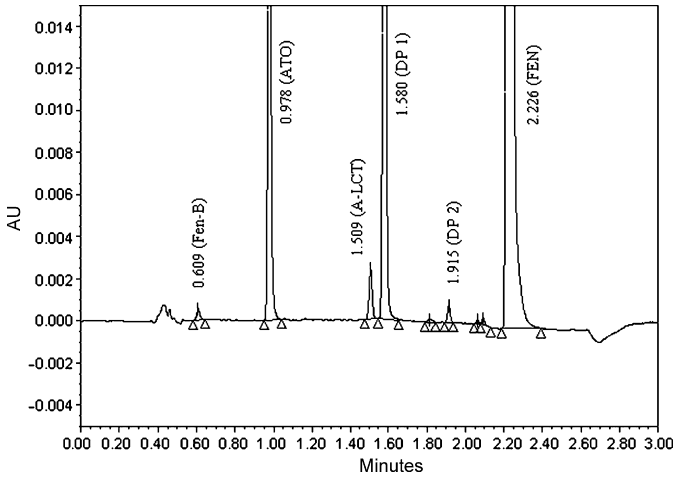


**Fig. 5.** Chromatogram of acid degraded tablet sample, chromatographed on acquity UPLC™ BEH C18 column (1.7 μm, 2.1 mm × 100 mm) in gradient elution using acetate buffer (pH 4.7; 0.01 M) and acetonitrile as mobile phase and measured at 247 nm. DP 1 and DP 2 are unknown degradation products of ATO and FEN, respectively.

were similar to that of unknown degradation products of individual standards eluting at respective retention time. Also spectra of known impurities in degraded tablet sample were similar to its respective impurity standard substance, indicating that there was no co-elution of unknown degradation peak at retention times of respective known impurities. ATO and FEN were stable in oxidation, heat and light degradation. Peaks due to ATO and FEN were investigated for spectral purity in the chromatogram of all exposed samples and standards and found spectrally pure. The max plot of chromatograms of degradation samples was also checked to ensure that no degradation peak is missed due to use of wavelength of 247 nm.

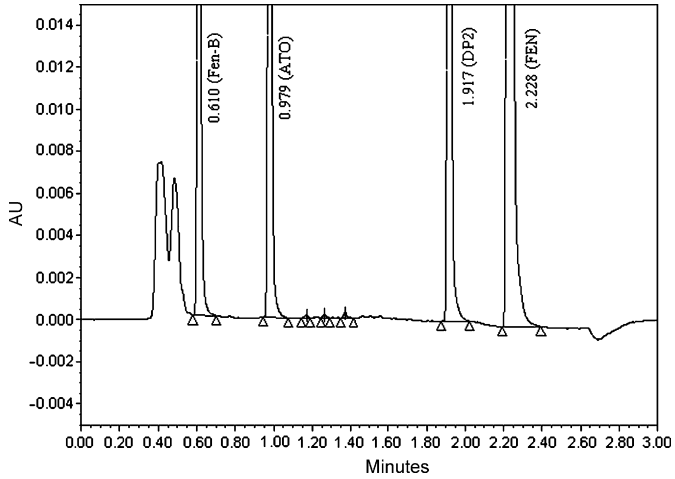


**Fig. 6.** Chromatogram of alkali degraded tablet sample, chromatographed on acquity UPLC™ BEH C18 column (1.7 μm, 2.1 mm × 100 mm) in gradient elution using acetate buffer (pH 4.7; 0.01 M) and acetonitrile as mobile phase and measured at 247 nm. DP 2 is unknown degradation product of FEN.

### 3.2.3. LOD and LOQ

The concentration (in μg/ml) with signal to noise ratio of at least 3 was taken as LOD and concentration with signal to noise ratio of at least 10 was taken as LOQ, which meets the criteria defined by ICH guidance [28]. The LOD and LOQ results of impurities are presented in Table 6 and LOQ results of ATO and FEN are presented in Table 2.

### 3.2.4. Linearity

The response was found linear from 50% to 150% of standard concentration. For all compounds the correlation coefficient was greater than 0.999. Correlation coefficients and linearity equations of main compounds and impurities are presented in Tables 2 and 6, respectively.

### 3.2.5. Precision

The average % assay ($n = 6$) of ATO was 99.9% and of FEN was 100.4% with %R.S.D. of 0.5% and 0.4%, respectively. Results are shown in Table 2 along with intermediate precision data. Low values of R.S.D., indicates that the method is precise. Impurities of FEN were not detected in any of the samples. The average of A-LCT in six samples was 0.31%. The precision result of impurities is shown in Table 3.

### 3.2.6. Accuracy

The amount recovered was within ±2% of amount added, which indicates that the method is accurate and also there is no interference due to excipients present in tablets. The results of recoveries for assay are shown in Table 2 and for impurities are shown in Table 3.

### 3.2.7. Robustness

No significant effect was observed on system suitability parameters such as capacity factor, resolution and theoretical plates of respective components, when small but deliberate changes were made to chromatographic conditions. The results are presented in Table 4 along with system suitability parameters of normal methodology. Thus, the method was found to be robust with respect to variability in above conditions.

### 3.2.8. Stability of sample solution

Sample solution did not show any appreciable change in assay and impurities value when stored at ambient temperature up to 24 h. Assay results are presented in Table 2 and impurities results are presented in Table 3.

**Table 6**
LOD, LOQ and linearity results for impurities

| Parameter | FEN-A | FEN-B | FEN-C | A-LCT |
|---|---|---|---|---|
| LOD (μg/ml)[a] | 0.03 | 0.07 | 0.07 | 0.02 |
| Signal to noise ratio at LOD[b] | 4.1 | 3.3 | 3.6 | 3.1 |
| LOQ (μg/ml)[a] | 0.1 | 0.2 | 0.2 | 0.07 |
| Signal to noise ratio at LOQ[c] | 12.3 | 10.6 | 11.3 | 11.7 |
| Linearity range (μg/ml) | 0.102–0.646 | 0.207–0.662 | 0.224–0.716 | 0.069–0.222 |
| Linearity[d] (correlation coefficient) | 0.9993 | 0.9997 | 0.9994 | 0.9998 |
| Linearity[d] (equation) | $y = 3685x + 37.47$ | $y = 2345x + 83.30$ | $y = 3075x + 10.54$ | $y = 4163x + 1.757$ |

[a] Based on signal to noise (S/N) ratio.
[b] Acceptance criteria, S/N > 3.
[c] Acceptance criteria, S/N > 10.
[d] Determined on six levels.

## 4. Conclusion

A novel UPLC method was successfully developed and validated for simultaneous determination of ATO, FEN and impurities. The total run time was 3 min, within which both the drugs and their degradation products were separated. Method validation results have proved the method to be selective, precise, accurate, robust and stability indicating. Sample solution stability was established for determination of assay as well as impurities. This method can be successfully applied for the routine analysis as well as stability study. Also it can be utilized for determination of content uniformity and dissolution profiling of this product, where sample load is higher and high throughput is essential for faster delivery of results. Overall, the method provides high throughput solution for determination of ATO, FEN and their degradation products in tablets with excellent selectivity, precision and accuracy.

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