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The ICH guidance in practice: Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form

Md. F. Hossain, Subrata Bhadra, Uttom Kumar and Abu S. S. Rouf*

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

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ABSTRACT

This paper describes degradations of aceclofenac (ACF) in tablet dosage form under different International Conference on Harmonization (ICH) prescribed stress conditions (thermal, acid degradation, base degradation, oxidation and photolysis), and establishments of a simple, rapid and precise stability-indicating reversed-phase HPLC assay method. Chromatographic separation of ACF and its major and minor degradation products was successfully achieved on a Nucleosil C_{18} (250 mm length × 4.6 mm i.d., 5 µm particle size) column in an isocratic separation mode with mobile phase consisting of 0.07% of orthophosphoric acid and acetonitrile in the ratio of (68:32, v/v) at pH 7.0±0.05, and the flow rate was maintained at 1.2 ml/min and the effluent was monitored at 275 nm. Peak purity data of ACF was obtained using photodiode array (PDA) detector in the stressed sample chromatograms. The method was validated with respect to linearity, precision, accuracy, selectivity, specificity, and ruggedness to assay ACF in tablets. The results were statistically compared using one way ANOVA. The force degradation studies revealed ACF was stable in absence of light and at or below 80°C, however it was labile in acid, alkali, oxidative stress, in presence of light and at 105°C. Degradation products resulting from stress studies did not interfere with the detection of ACF. The method was found linear over the concentration ranges of 160–240 µg/ml (R^2 = 0.9993). The proposed economic method could be applicable for routine quality control analysis of ACF in pure and tablet dosage form.

Keywords: Aceclofenac, Analysis, Stress degradation, Quality control, Validation

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INTRODUCTION

ACF is chemically 2-{2-[2-(2,6-dichlorophenyl)aminophenyl]-acetyl}oxyacetic acid [1] (Fig. 1). It is white or almost white crystalline powder, practically insoluble in water, freely soluble in acetone and in dimethylformamide, soluble in alcohol and methanol [2]. It has molecular formula of $C_{16}H_{13}Cl_2NO_4$ and molecular weight of 354.2 with a melting point of 149-150°C [1]. It is an NSAID [2] that has been widely used for the treatment of arthritis. It acts by blocking the action of cyclooxygenase, which is involved in the production of various chemicals in the body, some of which are known as prostaglandin [3].

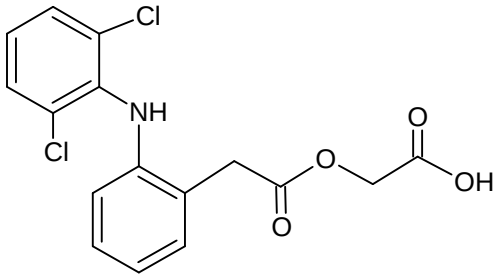

Fig. 1: Chemical structure of ACF

Forced degradation or stress testing of ACF is carried out according to stability test guidelines entitled "Stability Testing of New Drug Substances and Products" issued by ICH for establishing its inherent stability characteristics and for supporting the suitability of the proposed analytical procedure. The aim of stability testing is to prove how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors and is stated that the testing should include the effects of temperature, humidity where appropriate, oxidation, photolysis, and susceptibility to hydrolysis across a wide ranges of pH values. Regulatory agencies recommend the use of stability-indicating methods for the analysis of stability samples. Thus, stress studies are required in order to generate the stressed samples, method development, and its validation [4-7, 27].

Literature survey revealed that various methods have been reported for the estimation of ACF, such as striping voltammetric [8], titrimetric [2, 9], high performance thin layer chromatographic (HPTLC) [10, 11], spectrophotometric [12-18], spectrofluorimetric [18], high-performance liquid chromatographic (HPLC) [1, 3, 19-25] and liquid chromatography–tandem mass spectrometric [26] methods for its determination in laboratory-prepared mixture, pharmaceutical preparations and biological matrices such as human plasma. But there is very few published methods that is extensively focused on stress stability of ACF according to ICH issued stability test guidelines Q1A (R2) [4, 5]. Therefore, the aim of the present work is to establish the inherent stability of ACF through forced degradation studies and to develop a simple, precise, specific, accurate, cost-effective, and validated RP-HPLC method according to ICH guidelines Q2 (R1) [27] for the estimation and routine analysis of ACF in the presence of its degradants in pure form and in pharmaceutical formulations.

MATERIALS AND METHODS

2.1. Reagents and Materials. ACF tablets were prepared from Formulation Laboratory, Department of Pharmaceutical Technology, University of Dhaka, Bangladesh. Each film coated tablet contained aceclofenac (100 mg, based on 100% potency, APIChem, China) as an active ingredient, and microcrystalline cellulose / avicel PH 101 (Mingtai Chemical, Taiwan), croscarmellose sodium (Roswell Inc., USA), polyvinylpyrrolidone (BASF, Germany), colloidal silicon dioxide / aerosil-200 (Degussa, Germany), and magnesium stearate (Novochem GmbH, Germany) as excipients. HPLC grade acetonitrile was procured from Merck, India and reagent grade orthophosphoric acid (Sigma-Aldrich, Switzerland), hydrochloric acid (Merck, India), sodium hydroxide (Scharlu, Spain) and hydrogen peroxide (Scharlu, Spain) were used for analytical purposes. Milli-Q water was used to prepare the mobile phase.

2.2. Preparation of ACF Film Coated Tablets and Placebo Tablets. The immediate release ACF 100 mg tablet was prepared by wet granulation technique. The required amount of the active drug, avicel PH 101 (diluent) and a portion of aerosil-200 (glidant) were sieved through no. 24 sized mesh and then mixed by geometrical order. At the same time, binder solution was prepared by mixing polyvinylpyrrolidone (binder) in sufficient amount of purified water. Then a wet mass was developed by mixing the binder solution to the dry mix and its moisture content was reduced within a predetermined limit by fluid bed drier. Then the lumps were crushed and passed through no. 24 sized mesh to get uniform granules. Rest portion of aerosil-200, croscarmellose sodium (disintegrant) and magnesium stearate (lubricant) were mixed with it passing through no. 40 sized mesh. Then the granules were compressed in single rotary mini compressed machine using "D"-Tooling of punch size 11.1 mm in diameter (round shape), 8 stations. The tablets were then film coated with ethyl cellulose (EC) and hydroxypropyl methylcellulose (HPMC) mixed with the plasticizer polyethylene glycol 6000, coloring material opadry grey, and titanium dioxide as opacifier. Placebo tablets were prepared by following the same order and technology as mentioned above excluding the active drug.

2.3. Characterization of ACF Tablets. The film coated tablets obtained were ash colored, round and biconvex shaped. The average weight of the tablets varied from 365 mg ± 5% [28], thickness from 3.9 ± 0.05 mm and

hardness from 15 ± 2 kp. The disintegration test of both core and coated tablets [29] and the friability test of core tablets [30] were found well within the British Pharmacopoeia (BP) acceptable limit.

2.4. HPLC Instrumentation and Chromatographic Parameters. The chromatographic system used for the investigation was Shimadzu (Kyoto, Japan) prominence integrated with variable wavelength programmable photo diode array (PDA) detector composed of binary pump, degasser, auto injector, and column oven. The chromatographic analysis was performed in an isocratic separation mode on a Nucleosil C_{18}, 250 mm length × 4.6 mm i.d. with 5 µm particle size column. The mobile phase was a homogenous mixture of 0.07% v/v of orthophosphoric acid (OPA) and acetonitrile in the ratio of (68 : 32, v/v) at pH 7.0 ± 0.05, pumped at a flow rate of 1.2 $mLmin^{-1}$. The column temperature was maintained at 30°C, and the detection wavelength was 275 nm. Measurements were made with injection volume 10 µL and the run time was 40 min for each injection of stressed sample. The retention time of unstressed ACF was about 7.0 min and the peak purity was obtained directly from the spectral analysis report.

2.5. Preparation of Mobile Phase. Accurately measured 0.7 mL of orthophosphoric acid was transferred and diluted with distilled water up to 1000 mL. Then 680 mL of this solution was mixed with 320 mL of HPLC grade acetonitrile and mixed well. Finally the resulting solution was filtered using 0.2 µm filter.

2.6. Preparation of Stock Solution of Standard Aceclofenac. A stock solution of ACF was prepared at a concentration of 1 $mgmL^{-1}$ in mobile phase. Accurately weighed 200 mg of ACF was transferred to a 200 mL volumetric flask and about 50 mL of mobile phase was added. Then sonicated for 5 min for complete dissolution of drug. The solution was allowed to cool at room temperature and then the volume was made up to the mark with the same diluting solution.

2.7. Preparation of Assay Sample Solution. For the analysis, not less than 20 tablets were taken and their average weight was calculated. The tablets were then crushed and powdered finely. To prepare assay sample solution, powdered sample equivalent to 100 mg of ACF was weighed and transferred to a clean and dry 100 mL volumetric flask. About 25 mL of mobile phase was added as diluting solution and shaken thoroughly to extract the drug from the excipients and then sonicated for 5 min for complete dissolution of drug. The solution was allowed to cool at room temperature and then the volume was made up to the mark with the same diluting solution. From this solution, 10 mL was transferred to a clean and dry 50 mL volumetric flask and the volume was made up to the mark with the same diluent. The solution was then filtered through Whatman filter paper (No. 42) and then finally filtered through 0.2 µm disk filter. The drug concentration of the resulting sample solution was determined by HPLC using the calibration curve of standard solution. All determinations were conducted in triplicate. To validated the proposed method, the different analytical performance parameters such as system suitability, linearity, accuracy, specificity, precision, sensitivity (limit of detection and limit of quantitation), ruggedness and robustness were determined according to ICH issued analytical method validation guidelines Q2 (R1) [27].

2.8. Acid Degradation Studies. Acid degradation studies of the drug were carried out in 0.1 N hydrochloric acid (HCl). Two sample solutions were prepared, where 10 mL of stock solution was diluted with 10 mL of acid in two separated 50 mL volumetric flask. These solutions were then subjected to heating at 30°C (±1°C) and 80°C (±2°C) for 1 h respectively. Samples were allowed to cool at room temperature and suitably diluted with diluting solution before analysis. At the same time, a blank sample was prepared in a 50 mL volumetric flask by diluting 10 mL of 0.1 N HCl with the diluting solution up to the mark. The samples were then filtered through 0.2 µm disk filter and subjected to HPLC analysis. All determinations were conducted in triplicate.

2.9. Base Degradation Studies. Base degradation studies of the drug were carried out in 0.1 N sodium hydroxide (NaOH). Two sample solutions were prepared, where 10 mL of stock solution was diluted with 10 mL of alkali in two separated 50 mL volumetric flask. These solutions were then subjected to heating at 30°C (±1°C) and 80°C (±2°C) for 1 h respectively. Samples were allowed to cool at room temperature and suitably diluted with diluting solution before analysis. At the same time, a blank sample was prepared in a 50 mL volumetric flask by diluting 10 mL of 0.1 N NaOH with the diluting solution up to the mark. The samples were then filtered through 0.2 µm disk filter and subjected to HPLC analysis. All determinations were conducted in triplicate.

2.10. Oxidative Degradation Studies. Oxidative degradation studies of the drug were carried out in 10% hydrogen peroxide (H_2O_2) solution. Two sample solutions were prepared, where 10 mL of stock solution was diluted with 5 mL of hydrogen peroxide in two separated 50 mL volumetric flask. These solutions were then subjected to heating at 30°C (±1°C) and 80°C (±2°C) for 1 h respectively. Samples were allowed to cool at room temperature and suitably diluted with diluting solution before analysis. At the same time, a blank sample was prepared in a 50 mL

volumetric flask by diluting 5 mL of 10% H_2O_2 with the diluting solution up to the mark. The samples were then filtered through 0.2 µm disk filter and subjected to HPLC analysis. All determinations were conducted in triplicate.

2.11. Photolytic Degradation Studies. For photolytic degradation studies, two nominal standard solutions of ACF (200 µgmL^{-1}) were prepared; one was exposed to light in a photostability chamber for 10 days and the other one was kept in dark for the same period. Samples were withdrawn at different time periods (0, 5, and 10 days) and subjected to HPLC analysis after filtering through 0.2 µm disk filter. All determinations were conducted in triplicate.

2.12. Thermal Degradation Studies. For thermal degradation studies, active ACF was subjected to dry heat in petri-dishes at 60°C (±1°C), 80°C (±2°C), and 105°C (±2°C) for 1 h respectively. Then nominal standard solution of ACF (200 µgmL^{-1}) at three different stressed conditions were prepared by diluting with the mobile phase. The samples were then filtered through 0.2 µm disk filter and subjected to HPLC analysis. All determinations were conducted in triplicate.

2.13. Method Validation

2.13.1. System Suitability. To assess system suitability of the proposed method, the repeatability, theoretical plates, tailing factor, and retention time of six replicate injections of working standard ACF of concentration 200 µgmL^{-1} were used and percentage relative standard deviation (%RSD) values were calculated in each case.

2.13.2. Linearity. The linearity was evaluated by analyzing five working solutions of ACF over the concentration range 160 to 240 µgmL^{-1} corresponding to 80 to 120% of nominal test concentration (200 µgmL^{-1}) for routine analysis of ACF and prepared in triplicate. Three calibration curves were prepared with the following concentrations (160, 180, 200, 220, and 240 µgmL^{-1}). The linearity was evaluated by linear regression analysis, which was then evaluated by the least-square regression analysis. The regression line was calculated as Y = A + BX, where X was the ACF concentration (µgmL^{-1}) and Y was the response (peak area expressed as AU).

2.13.3. Specificity. The specificity of the developed method was determined by placebo analysis. Placebo of ACF tablet formulations containing all the formulation ingredients except ACF was prepared for this study and was treated in the same manner as the test samples. Finally peak purity tool was used to check the peak purity of the test solution.

2.13.4. Accuracy (Recovery Test). Accuracy of the proposed method was studied by recovery experiments for both drug and drug-matrix solution. In case of drug solution, standard solution of ACF, corresponding to 80, 90, 100, 110, and 120% of the nominal analytical concentration of ACF (200 µgmL^{-1}) were compared with reference standard solution of ACF of known purity (200 µgmL^{-1}), and the percent recoveries (mean ± %RSD of three replicates) of ACF in pure form were calculated. In case of drug-matrix solution, recovery test was applied which consist of adding known amount of ACF to the samples' solutions in the beginning of the process. This test was realized by assaying five different drug solutions, three replicate of each, containing 160, 180, 200, 220, and 240 µgmL^{-1} of ACF standard solution added to ACF placebo solution, corresponding to 80, 90, 100, 110, and 120% of the nominal analytical concentration of ACF (200 µgmL^{-1}), and the percent recoveries (mean ± %RSD of three replicates) of ACF in drug-matrix form were calculated.

2.13.5. Precision. To determine the repeatability (intra-day precision) and intermediate precision (inter-day precision) of the method, the ACF solution at nominal standard concentration (200 µgmL^{-1}) and sample solution (200 µgmL^{-1}) were analyzed in six replicates on the same day (intra-day precision) and daily for six times over a period of three days (inter-day precision). The results were expressed as %RSD of the measurements.

2.13.6. Sensitivity. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated in accordance with the 3.3s/m and 10s/m criteria, respectively, according to ICH Q2 (R1) recommendations, where 's' is the standard deviation of the peak area and 'm' is the slope of the calibration curve, determined from linearity investigation.

2.13.7. Ruggedness. Ruggedness of the proposed method was determined by analyzing six assay sample solutions of ACF film coated tablet formulation having concentration of 200 µgmL^{-1} by two analysts in the same laboratory to check the reproducibility of the test result. The percentage recovery and standard deviation were calculated in both cases.

2.13.8. Robustness. The robustness is the ability of method to remain unaffected by small changes in parameters. To determine the robustness of the current method, the pH of the buffer solution was assessed at 6.9 and 7.1 instead of 7.0. The effect of flow rate was studied at 1.1 and 1.3 mLmin^{-1} instead of 1.2 mLmin^{-1}. The effect of column temperature was studied at 25° and 35°C instead of 30°C. The effect of mobile phase composition was assessed at

(0.07% OPA : Acetonitrile = 66 : 34, v/v) and (0.07% OPA : Acetonitrile = 70 : 30, v/v) instead of (0.07% OPA : Acetonitrile = 68 : 32, v/v). The %RSD of robustness testing under these conditions was calculated in all cases.

RESULTS AND DISCUSSION

3.1. Degradation Behavior of ACF. The drug was very susceptible to decompose under hydrolytic stress (acidic and alkaline). It was also shown liability in oxidative stress and exposure to light. The major degradation product was identified as diclofenac through comparison with the standard. But the drug was found stable under dry heat (at or below 80°C) and in the dark. In all cases, chromatographic peak purity data of ACF were obtained from the spectral analysis report. The peak purity value was found to be greater than 99.99%, indicating a homogenous peak of ACF and confirming the absence of other substance in the same retention time (RT).

3.2. Degradation in Acid. The drug gradually decreased with time on heating in 0.1 N HCl for 1 h, forming degradation products at RT of 5.925 at 30°C (±1°C) and at RT of 3.530 and 5.902 at 80°C (±2°C) (Fig. 2b and 2c). The degradation product at RT of 5.91±0.02 was found to be diclofenac, as the standard drug solution of diclofenac gave a peak at the same RT. Acid stressed blank sample also gave a peak at RT of 2.35±0.02 (Fig. 2a). The amount of drug degraded were 22.6% and 91.1% at 30°C (±1°C) and 80°C (±2°C) respectively.

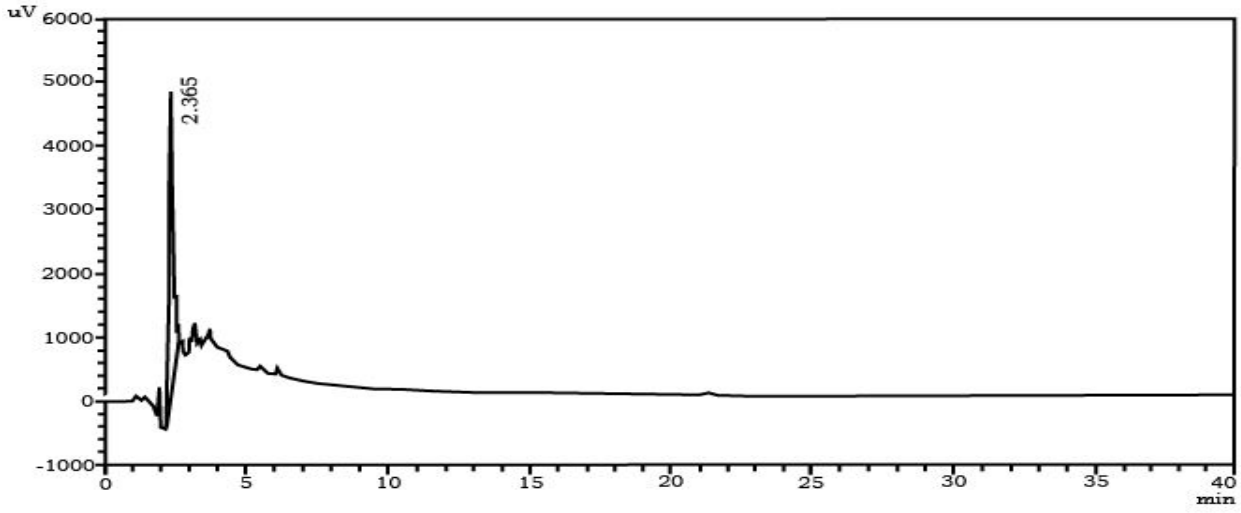

Fig. 2a: Chromatogram of 0.1 N HCl stressed blank sample

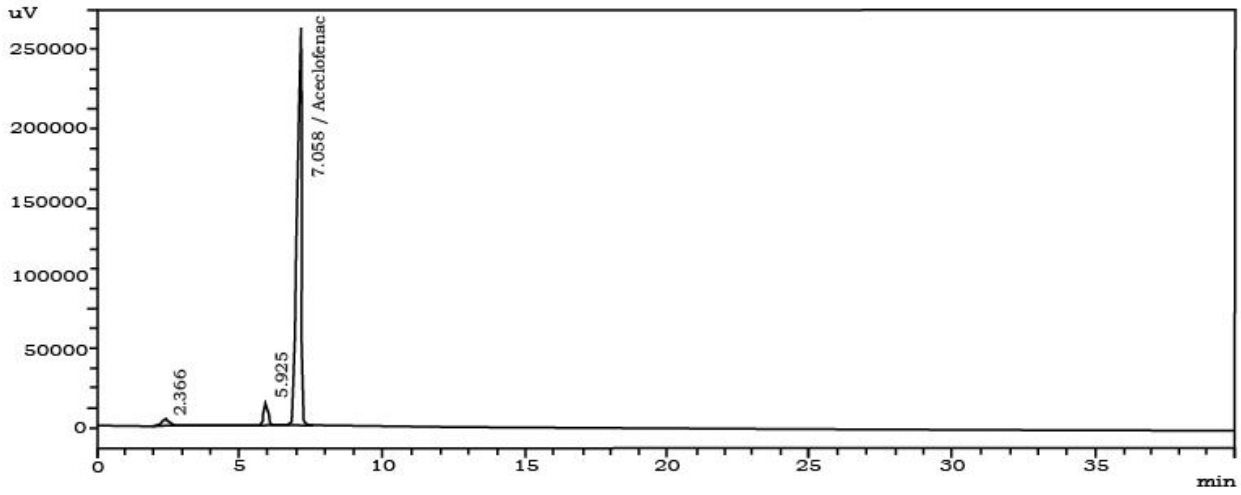

Fig. 2b: Chromatogram of 0.1 N HCl stressed ACF solution at 30°C (±1°C) for 1 h

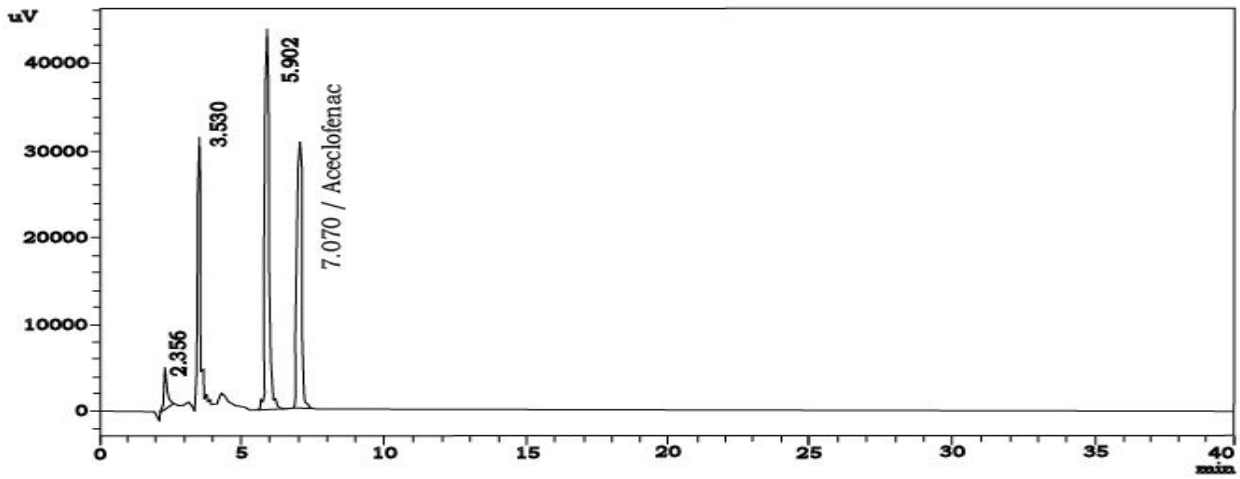

Fig. 2c: Chromatogram of 0.1 N HCl stressed ACF solution at 80°C (±2°C) for 1 h

3.3. Degradation in Alkali. The drug was found to be highly labile to alkaline hydrolysis. The drug drastically decreased with time on heating in 0.1 N NaOH for 1 h. The reaction in 0.1 N NaOH at 80°C (±2°C) was so fast that whole of the drug was degraded in 1 h, forming degradation products at RT of 2.365, and 3.545 (Fig. 3c). Subsequently, studies were performed in 0.1 N NaOH at 30°C (±1°C), forming degradation product at RT of 1.482, 3.698, and 4.684 (Fig. 3b). In both cases, ACF gave peak at RT of 5.49±0.01, whereas the unstressed drug gave peak at RT of 6.97±0.05. The amounts of drug degraded were 70.8% and 99.4% at 30°C (±1°C) and 80°C (±2°C) respectively. Alkaline stressed blank sample also gave a peak at RT of 1.48±0.04 (Fig. 3a). Drug degradation was associated with rise in a major degradation product at RT 4.684±0.02 at 30°C (±1°C) which was found to be diclofenac, as the standard drug solution of diclofenac gave a peak at the same RT. Studied carried out in 0.1 N NaOH at 80°C (±2°C) for 1 h gave no peak at diclofenac zone, almost the entire drug was degraded along with the ACF which was confirmed by performing the same alkaline degradation studies on standard diclofenac solution at 80°C (±2°C) for 1 h.

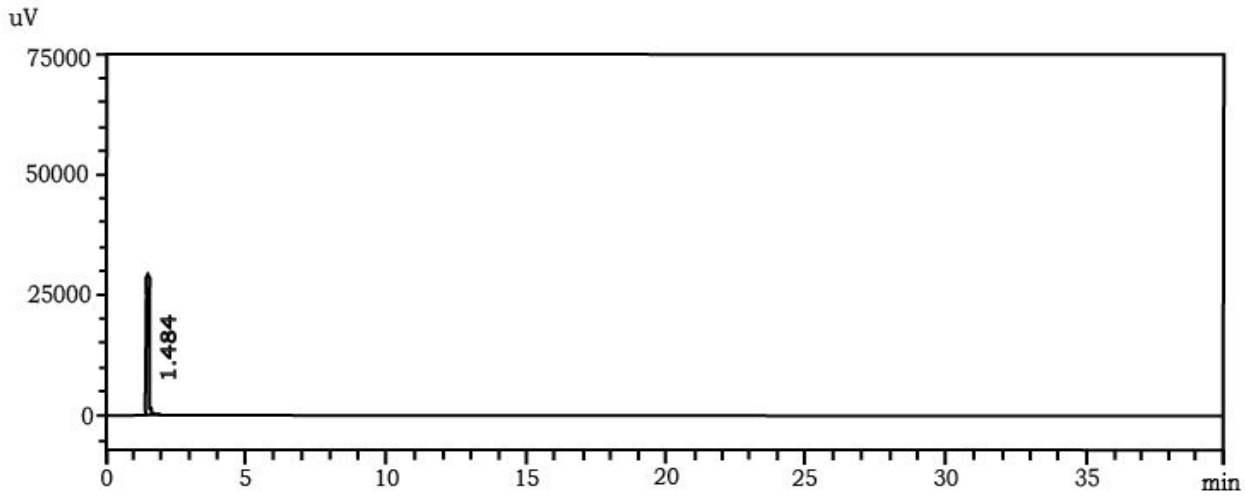

Fig. 3a: Chromatogram of 0.1 N NaOH stressed blank sample

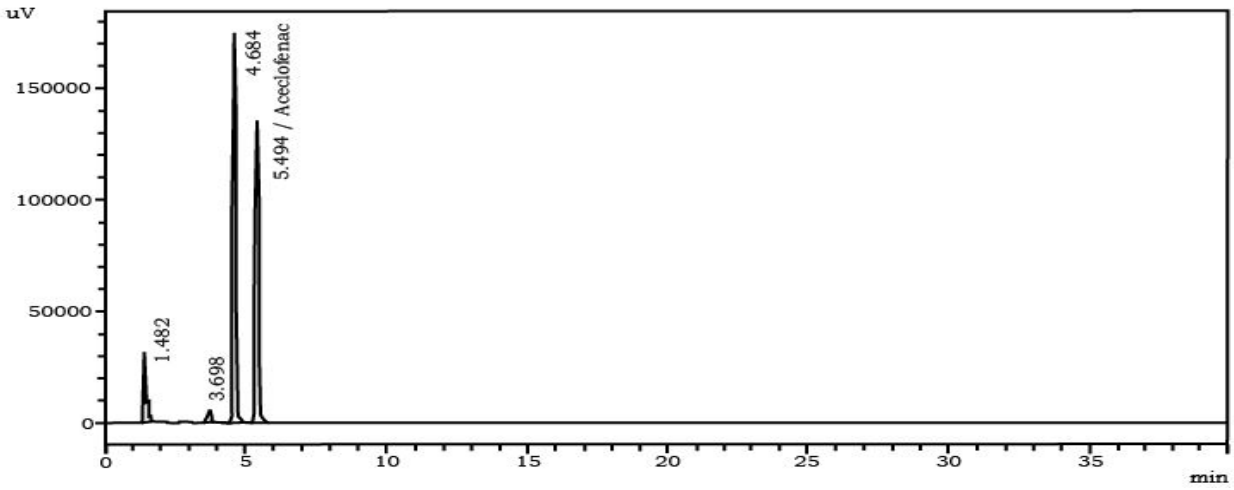

Fig. 3b: Chromatogram of 0.1 N NaOH stressed ACF solution at 30°C (±1°C) for 1 h

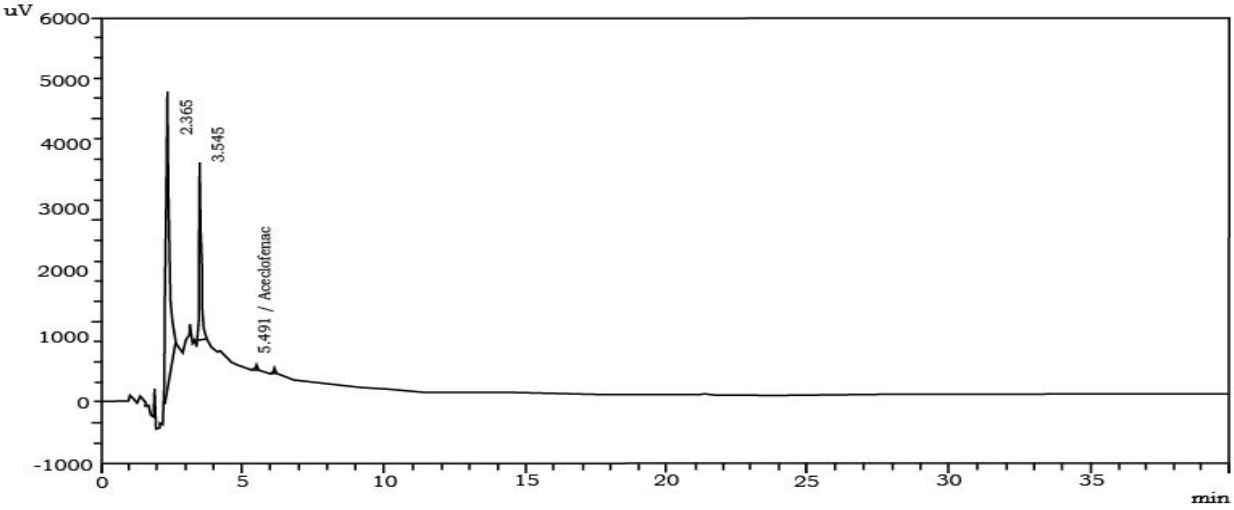

Fig. 3c: Chromatogram of 0.1 N NaOH stressed ACF solution at 80°C (±2°C) for 1 h

3.4. Oxidative Stress. Oxidative degradation studies were carried out in 10% H_2O_2 solution for 1 h, forming several degradation products at RT of 1.932, 2.669, 3.539, 3.659, 3.945, 4.230, and 5.523 at 30°C (±1°C) and at RT of 1.934, 2.678, 3.539, 3.670, 3.945, 4.232, and 5.534 at 80°C (±2°C) (Fig. 4b and 4c). Major degradation peaks observed at RT of 4.23±0.01 and 5.53±0.01 in both cases. ACF gave peak at RT of 6.86±0.005, whereas the unstressed drug gave peak at RT of 6.97±0.05. Peroxide stressed blank sample also gave a peak at RT of 2.65±0.01 (Fig. 4a). The amounts of drug degraded were 25.1% and 29.7% at 30°C (±1°C) and 80°C (±2°C) respectively.

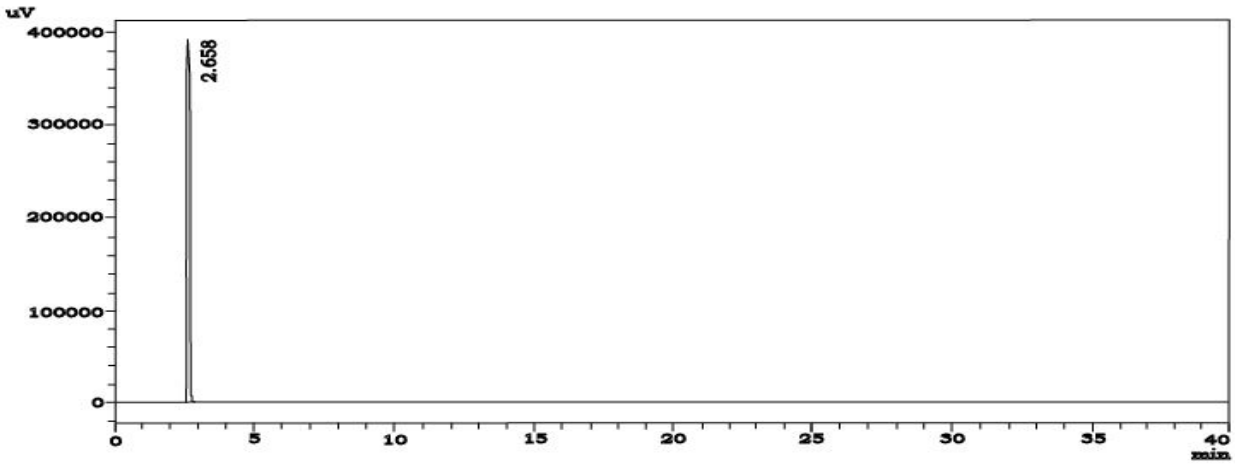

Fig. 4a: Chromatogram of 10% H_2O_2 stressed blank sample

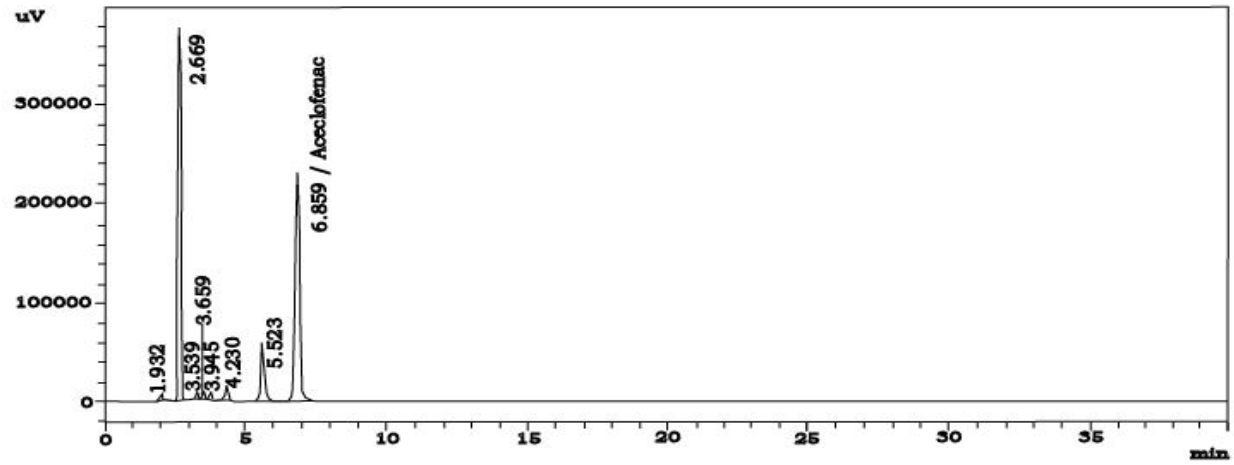

Fig. 4b: Chromatogram of 10% H_2O_2 stressed ACF solution at 30°C (±1°C) for 1 h

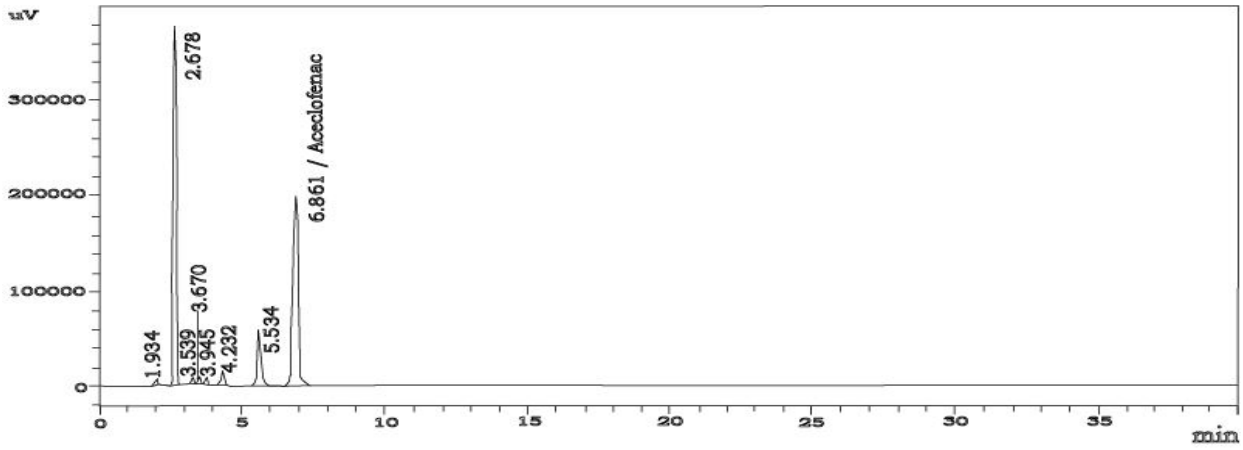

Fig. 4c: Chromatogram of 10% H_2O_2 stressed ACF solution at 80°C (±2°C) for 1 h

3.5. Photolytic Stress. The drug solution was found to be highly labile to light while the rate of degradation in dark was much slower. Under light for 5 days, the drug solution formed several degradation products at RT of 6.382, 9.981, 10.480, and 13.798 (Fig. 5b) and for 10 days, clusters of degradation products along with the previous four were observed at RT of 4.344, 4.837, 5.459, 6.367, 8.057, 9.973, 10.496, and 13.804 (Fig. 5c) which showed a sequential increase in the peak area. On the other hand, drug solution left in dark for 5 days formed degradation products at RT of 10.395, and 13.783 (Fig. 5e) and for 10 days, an addition peak was observed at RT of 6.210 along with the previous two (Fig. 5f), which also showed sequential increase in peak area. Samples withdrawn at 0 day gave no major degradation peak (Fig. 5a and 5d). The amount of drug degraded in presence of light were 20.7% and 22.9% for 5 days and 10 days respectively, and in dark these values were 4.9% and 14.1% respectively.

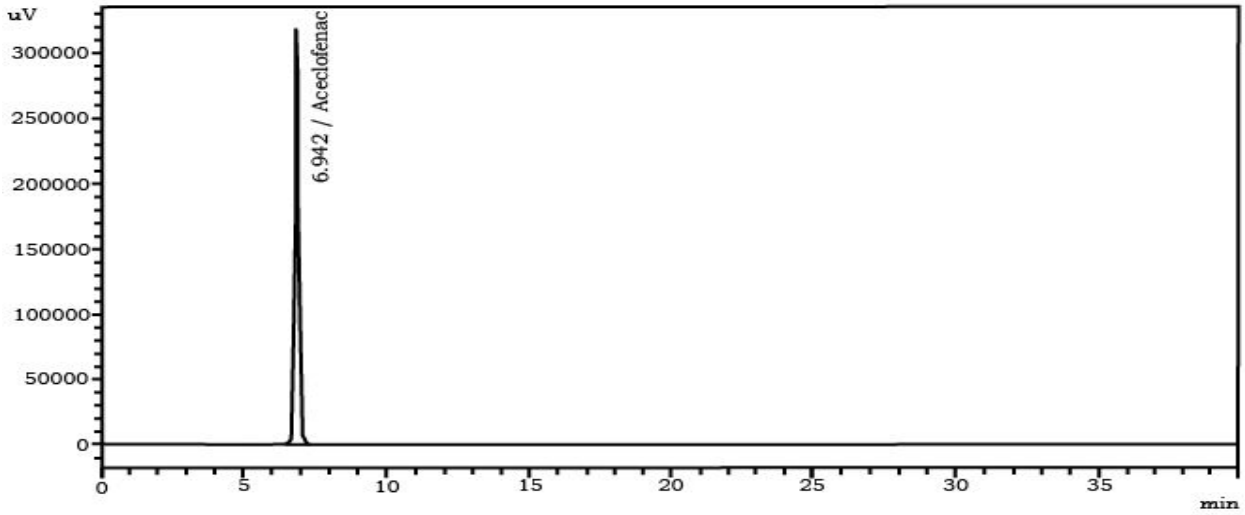

Fig. 5a: Chromatogram of ACF solution in presence of light for 0 day

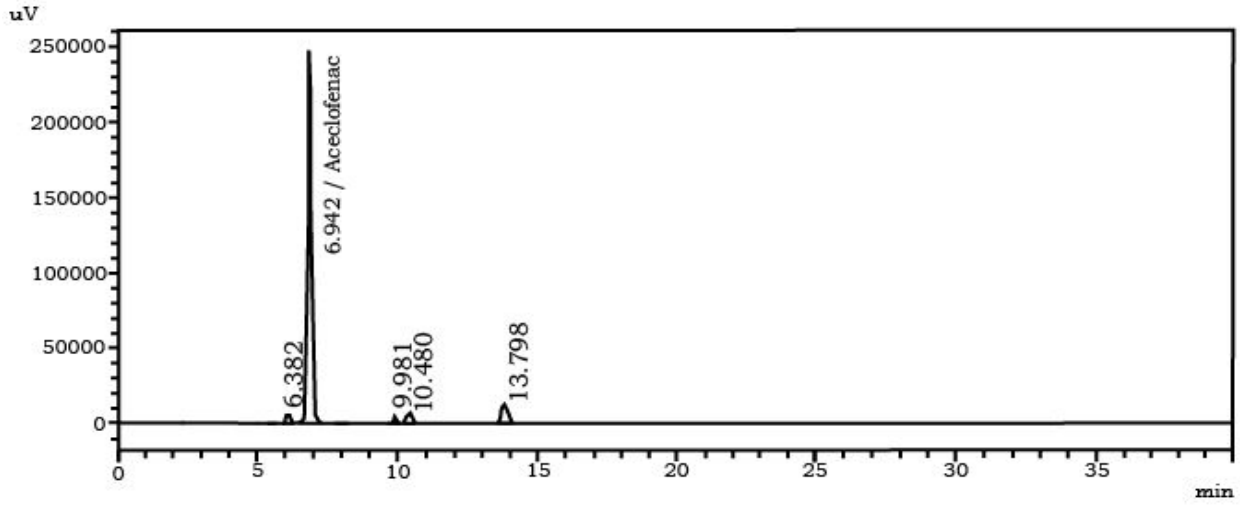

Fig. 5b: Chromatogram of ACF solution in presence of light for 5 days

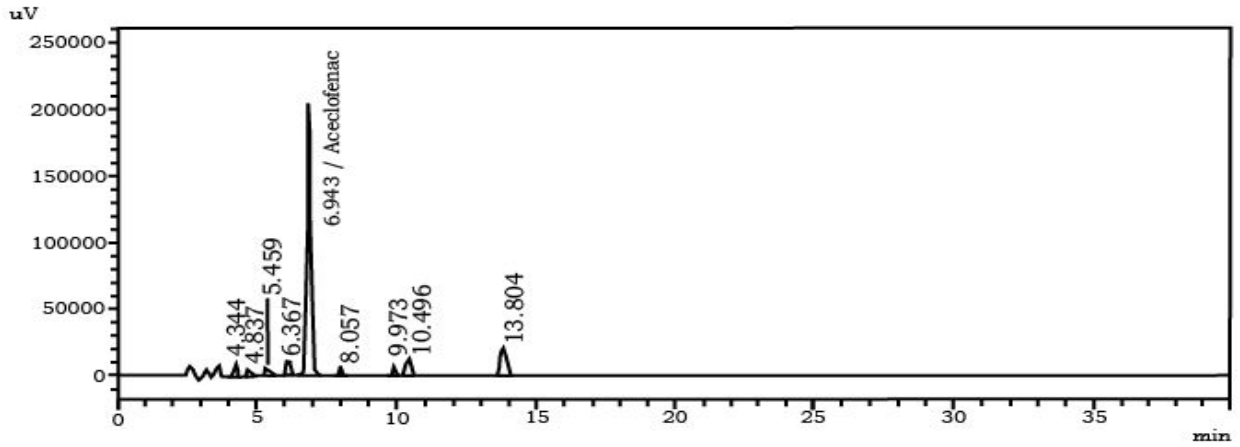

Fig. 5c: Chromatogram of ACF solution in presence of light for 10 days

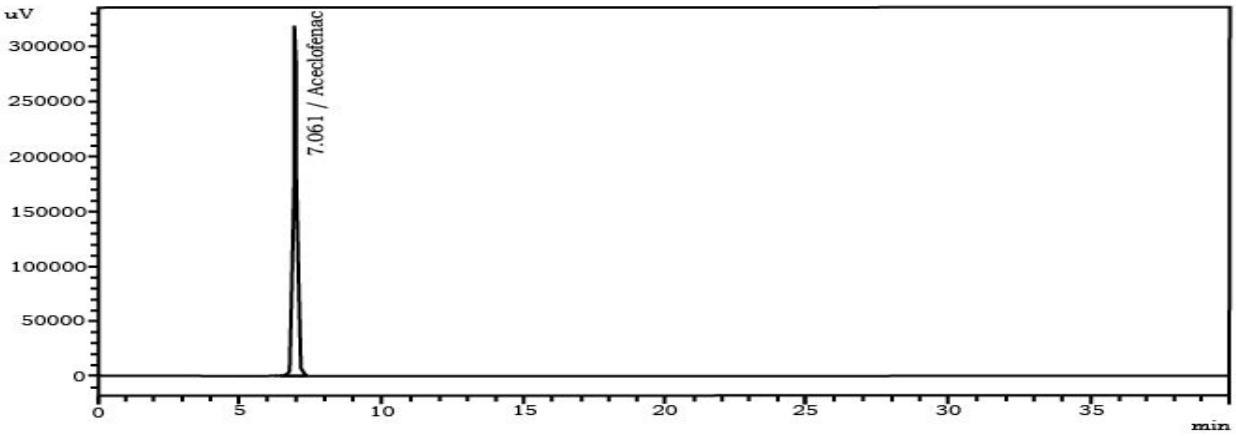

Fig. 5d: Chromatogram of ACF solution kept in dark for 0 day

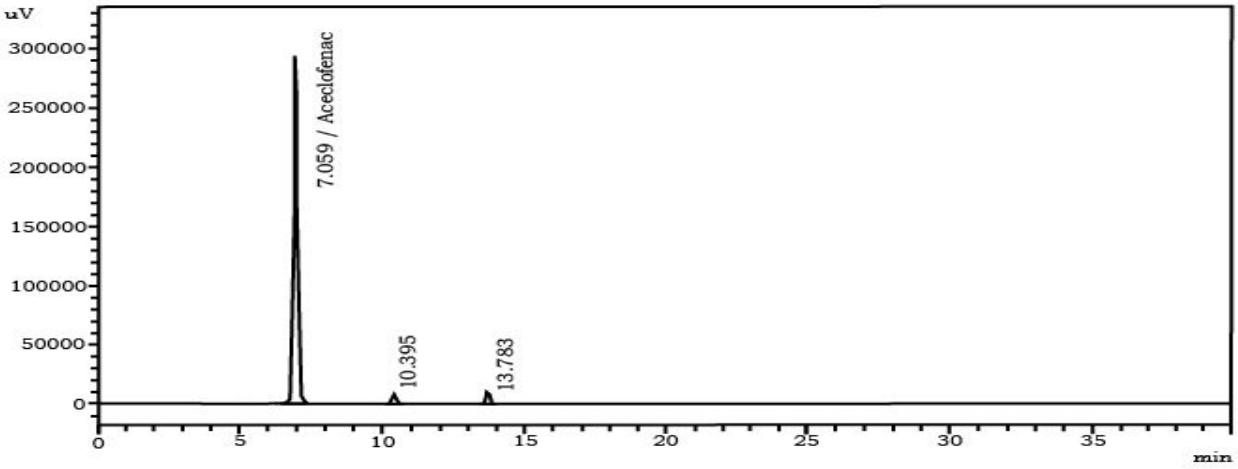

Fig. 5e: Chromatogram of ACF solution kept in dark for 5 days

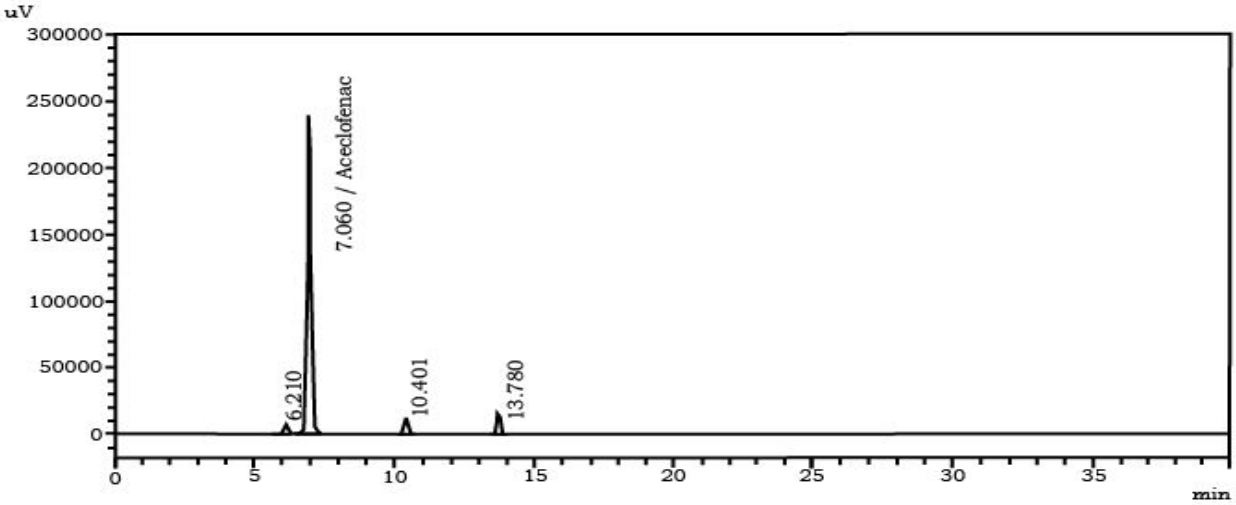

Fig. 5f: Chromatogram of ACF solution kept in dark for 10 days

3.6. Thermal Stress. The drug was found to be stable to the effect of temperature, when the drug powder was exposed to 60°C (±1°C) for 1 h and 80°C (±2°C) for 1 h, forming a minor degradation product at RT of 5.69±0.01 (Fig. 6b and 6c). The amount of drug degraded were 0.1% and 0.2% at 60°C (±1°C) for 1 h and 80°C (±2°C) for 1 h respectively. However the drug drastically decreased with major degradation product at RT of 5.589 at 105°C (±2°C) for 1 h (Fig. 6d) and the amount of drug degraded was 60.7%. It was also observed that RT of ACF gradually decreased as the exposed temperature was increased. On the other hand, thermal stressed blank sample (diluting solution) showed no peak at 80°C (±2°C) for 1 h (Fig. 6a).

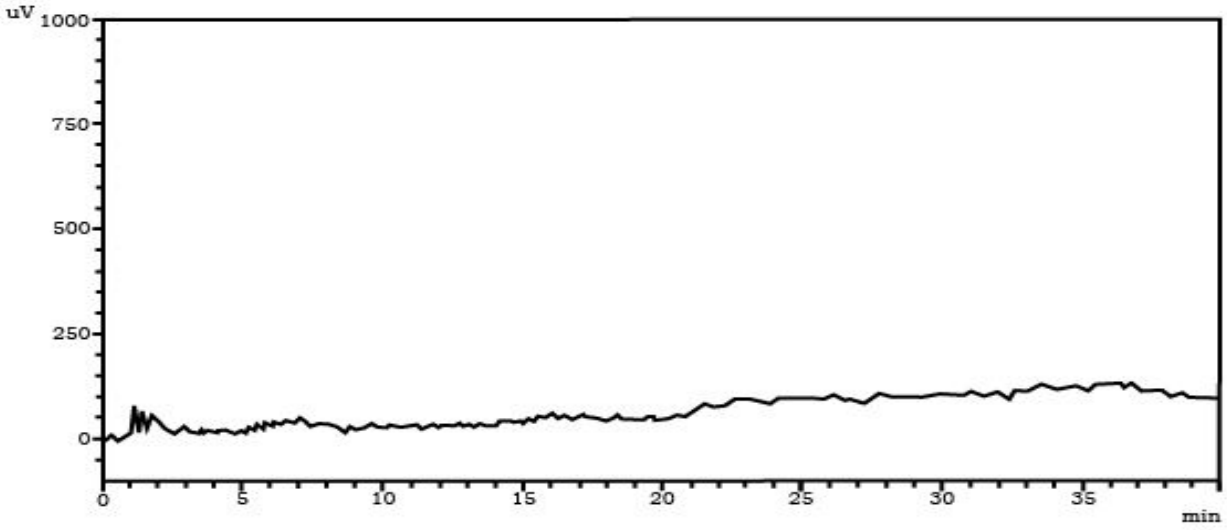

Fig. 6a: Chromatogram of thermal stressed blank sample at 80°C (±2°C) for 1 h

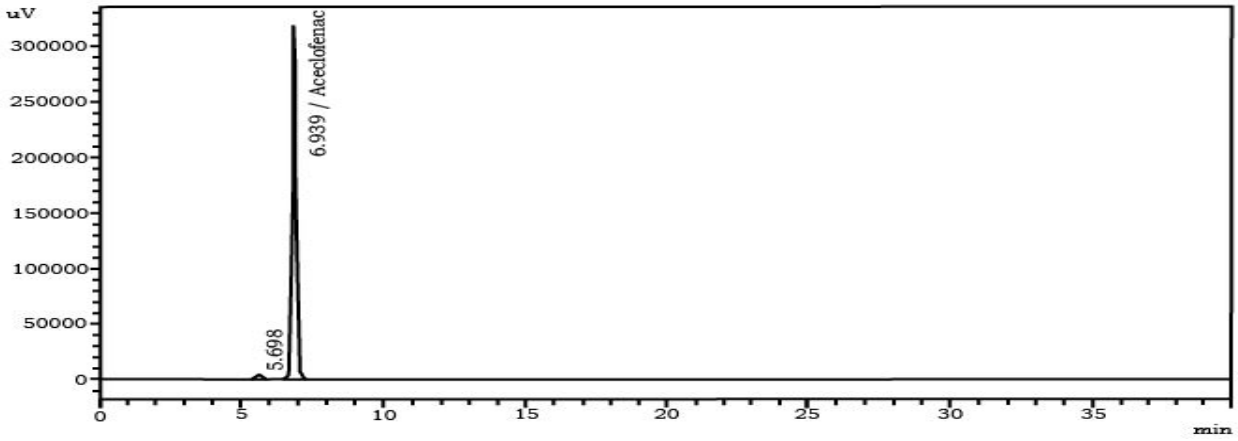

Fig. 6b: Chromatogram of thermal stressed ACF at 60°C (±1°C) for 1 h

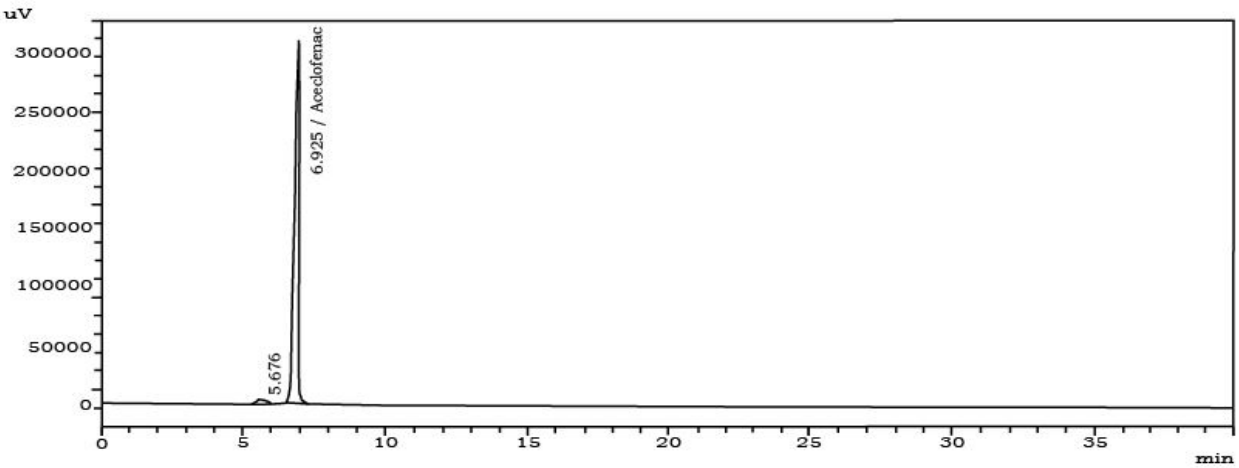

Fig. 6c: Chromatogram of thermal stressed ACF at 80°C (±2°C) for 1 h

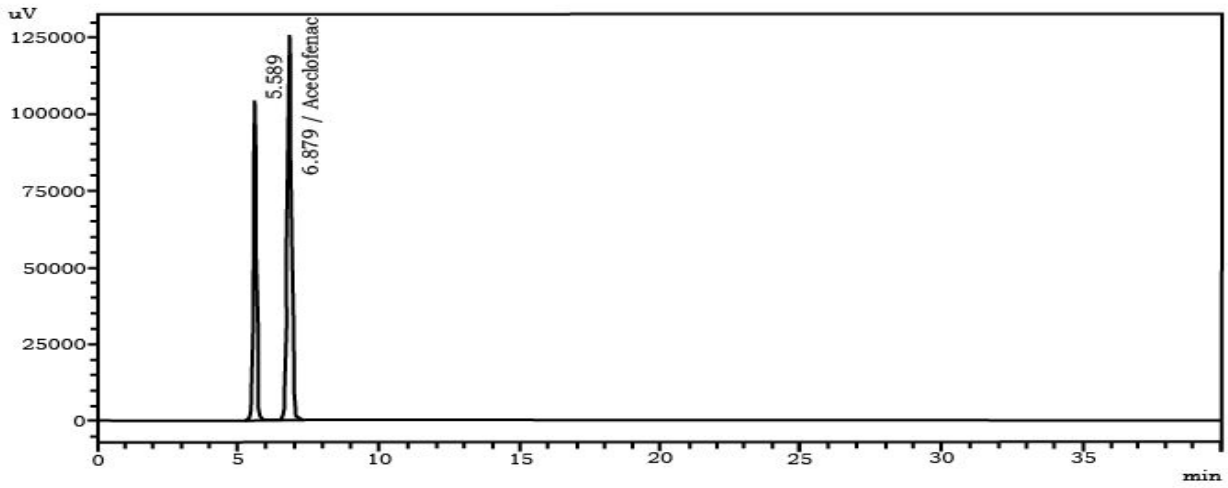

Fig. 6d: Chromatogram of thermal stressed ACF at 105°C (±2°C) for 1 h

3.7. Method Validation

3.7.1. System Suitability. The results (Mean ± %RSD of six replicates) of the chromatographic parameters are plotted in Table 1 indicating the good performance of the system.

Table 1: Chromatographic characteristics of system suitability solution

Parameter	Value (Mean ± %RSD)
Peak area	3217711.2 ± 0.06
Tailing factor	1.109 ± 0.34
Theoretical plate	3132.419 ± 0.33
Retention time	6.9515 ± 0.03

3.7.2. Linearity. The calibration curve was obtained using the linear least squares regression procedure. The representative linear equation was Y = - 493066 + 37370X by plotting peak area (Y) versus the concentration (X) studied from 160 to 240 µgmL^{-1}. The correlation coefficient (R^2) value was 0.9993. The validity of the method was verified by means of the ANOVA and according to it there is no deviation from linearity (P < 0.05).

3.7.3. Specificity. Good resolution was obtained between the drug and the degradation products formed under different stress conditions, indicating good specificity of the method. The HPLC chromatogram (Fig. 7a and 7b) recorded for the mixture of the inactive ingredients revealed no peaks within retention time around 6.95 min, and the peak purity was 99.99%, indicating ACF is clearly separated from the response of any interfering peak(s).

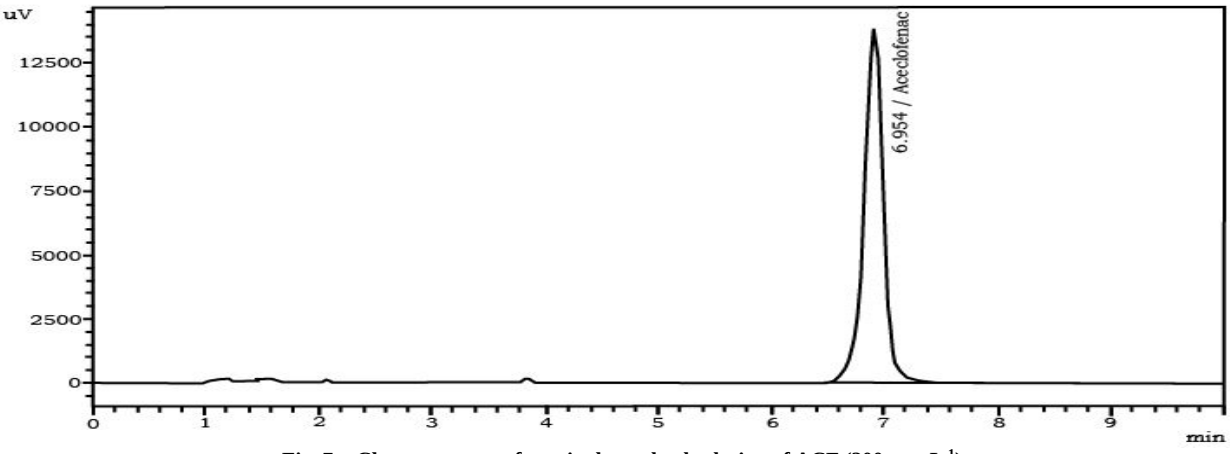

Fig. 7a: Chromatogram of nominal standard solution of ACF (200 µgmL^{-1})

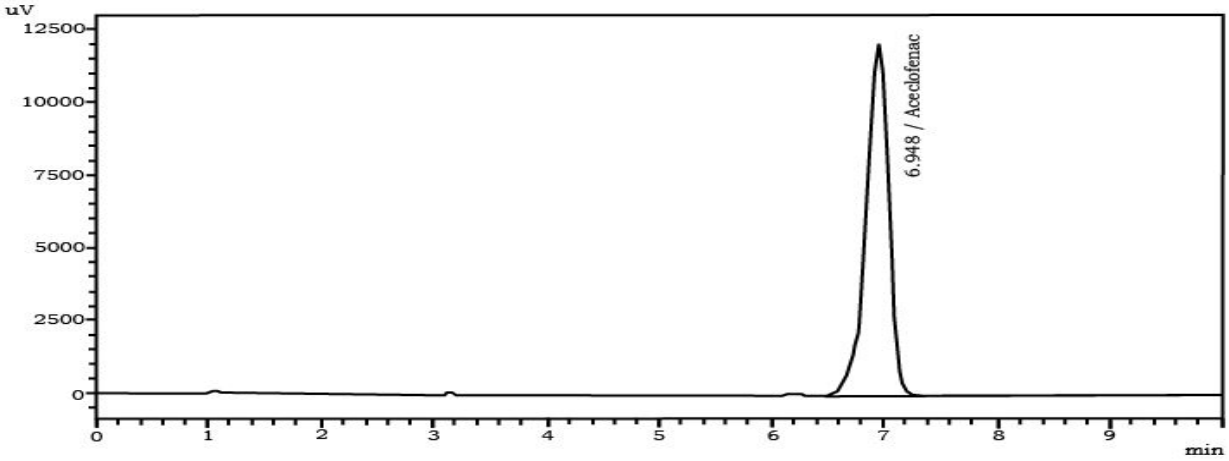

Fig. 7b: Chromatogram of ACF sample (200 µgmL^{-1})

3.7.4. Accuracy. Accuracy of the method was studied by recovery experiments. The overall results of percent recoveries (mean ± %RSD of three replicates) of ACF in pure and drug-matrix solutions are plotted in Table 2, indicating good accuracy of the proposed method. The calculated recovery values of ACF ranged from 99.82% (±0.03%) to 101.08% (±0.003%) in pure and from 99.76% (±0.02%) to 101.03% (±0.02%) in drug-matrix solutions.

Table 2: Accuracy studies of ACF in pure and drug-matrix solutions

	Amount added (µgmL^{-1})	Peak area* (Mean ± %RSD)	Amount recovered* (Mean ± %RSD)	% Recovery* (Mean ± %RSD)
Standard solution	160	2571237±0.03	159.7087±0.03	99.8179±0.03
	180	2895409±0.04	179.8442±0.04	99.9134±0.04
	200	3219719±0.02	199.9882±0.02	99.9941±0.02
	220	3580053±0.003	222.3698±0.003	101.0772±0.003
	240	3884080±0.13	241.2540±0.13	100.5225±0.13
Drug-matrix solution	160	2569612±0.02	159.6077±0.02	99.7584±0.02
	180	2896441±0.04	179.9083±0.04	99.9490±0.04
	200	3220446±0.03	200.0334±0.03	100.0167±0.03
	220	3578379±0.02	222.2659±0.02	101.0299±0.02
	240	3888564±0.05	241.5325±0.05	100.6385±0.05

** Mean and %RSD value of three replicates.*

3.7.5. Precision. The results obtained from repeatability (intra-day) and intermediate (inter-day) precision analyses are listed in Table 3 as mean recovery (%). Table 3 indicates there was no significant differences between assay results of both standard solution and sample solution either within-day or between days, implying that the precision of the proposed method was good (%RSD less than 1%).

Table 3: Summary of intra-day and inter-day precision data for ACF

	Spike level (%)	Intra-day** (Mean ± %RSD)	Inter-day** (Recovery amount ± %RSD)			Inter-day (Mean ± %RSD)
			Day 1	Day 2	Day 3	
Standard solution	100*	3209712±0.3	3196727±0.03	3187974±0.04	3177407±0.05	3187369±0.25
Sample solution	100*	3208481±0.07	3197510±0.04	3188927±0.05	3178772±0.07	3188403±0.25

** Spike level 100% indicates 200 µgmL^{-1} in both standard solution and sample solution.*
*** Mean and %RSD value of six determinations.*

3.7.6. Sensitivity. The limit of detection (LOD) and limit of quantitation (LOQ) of ACF by the proposed method were found 0.05 µgmL^{-1}and 0.2 µgmL^{-1}, respectively. Fig. 8a to 8c shows the sensitivity of the current method.

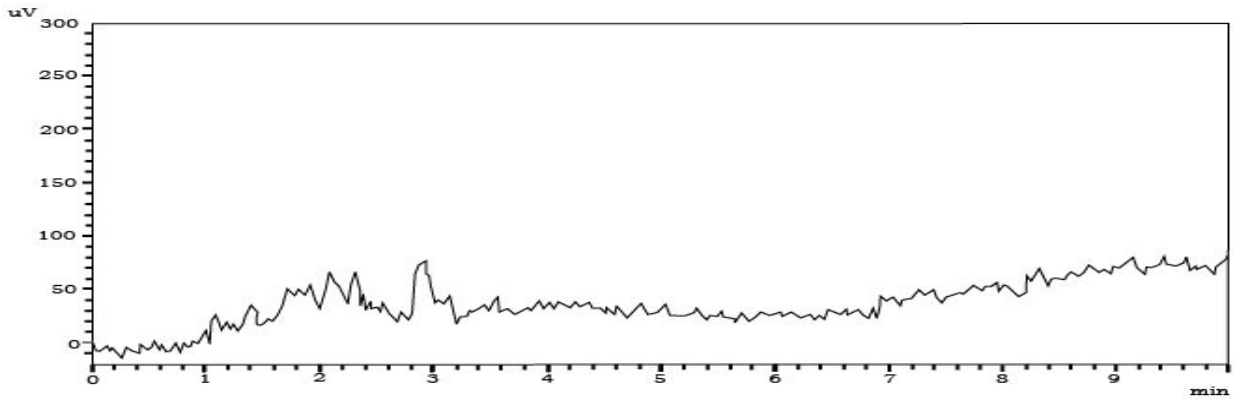

Fig. 8a: Chromatogram of blank

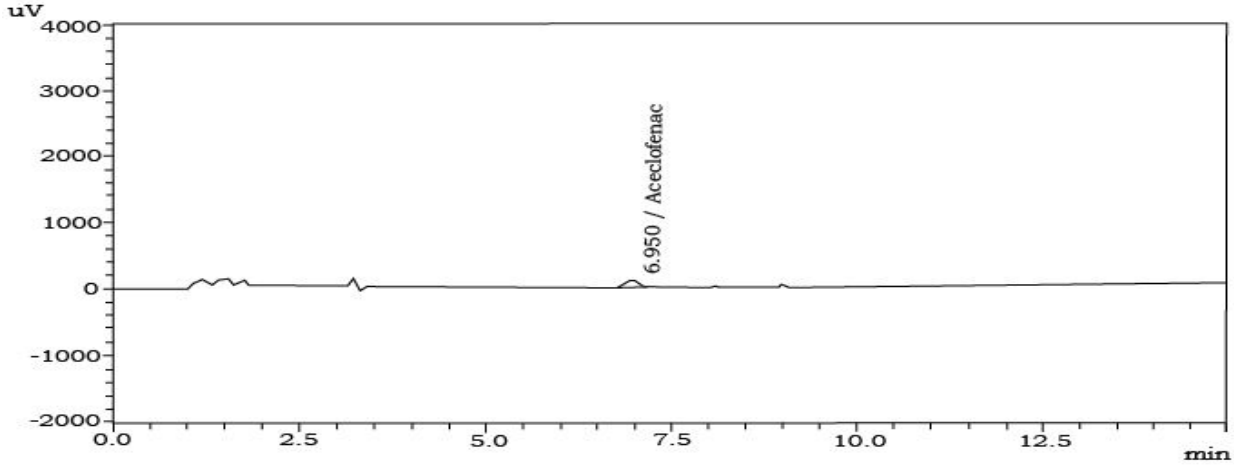

Fig. 8b: Chromatogram of LOD of ACF

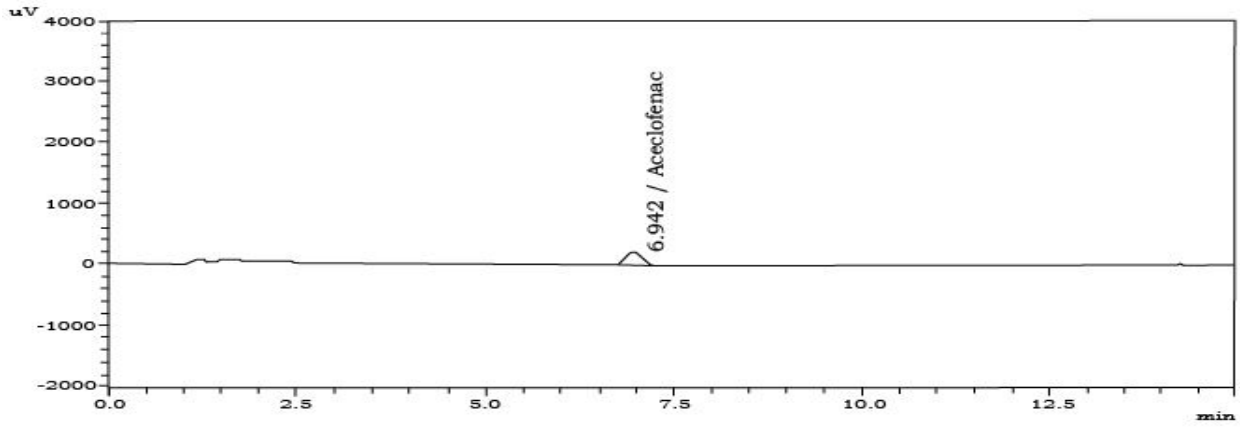

Fig. 8c: Chromatogram of LOQ of ACF

3.7.7. Ruggedness. The results (% of recovery ± standard deviation of six assay samples) are given in Table 4, indicating the ruggedness of the current method.

Table 4: Results of ruggedness study

Sample	Analyst 1		Analyst 2	
	Amount found (mg)	%Recovery ± SD*	Amount found (mg)	%Recovery ± SD*
Aceclofenac 100 mg Tablet	100.45	100.45±0.095	100.267	100.27±0.111

** %Recovery ± standard deviation (SD) of six samples (assay).*

3.7.8. Robustness. The %RSD of robustness study under different altered conditions is given in Table 5, indicating that the current method is robust.

Table 5: Results of robustness study

Parameter	Variations	Amount of ACF added (μgmL^{-1})	Amount of ACF detected (Mean ± SD)*	%RSD
Mobile phase flow rate	1.1 mLmin^{-1}	200	201.33±0.15	0.075
	1.2 mLmin^{-1}	200	201.02±0.04	0.019
	1.3 mLmin^{-1}	200	201.42±0.12	0.061
Mobile phase composition	0.07% OPA:ACN = 66:34	200	200.94±0.06	0.031
	0.07% OPA:ACN = 68:32	200	200.79±0.05	0.027
	0.07% OPA:ACN = 70:30	200	200.93±0.05	0.025
Mobile phase pH	6.9	200	201.09±0.04	0.020
	7.0	200	201.06±0.03	0.015
	7.1	200	201.12±0.04	0.022
Column temperature	25°C	200	200.90±0.02	0.012
	30°C	200	200.85±0.02	0.008
	35°C	200	200.72±0.03	0.017

** Mean ±SD of three replicates.*

CONCLUSION

The studies showed that ACF is a labile molecule in acid and alkali, and also showed lability in dry heat at 105°C, oxidative stress, and exposure to light. However it is stable to dry heat at or below 80°C and in the absence of light. A stability-indicating method was developed, which separates all the degradation products formed under variety of stressed conditions. The developed method proved to be versatile, accurate, precise, specific, selective, and obviously less time consuming. Hence it is recommended for routine quality control and stability-indicating studies on ACF in bulk as well as in tablet formulations.

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