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A Validated Stability-Indicating Method for the Estimation of Aceclofenac in Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

The aim of the research study was the development and validation of a simple, rapid, accurate and precise reversed-phase high performance liquid chromatography (RP-HPLC) stability-indicating method for the determination of aceclofenac in bulk and pharmaceutical dosage forms. The RP-HPLC studies was performed on the instrument Jasco HPLC system with Jasco UV 2010 photo diode array detector, ODS C18 RP-column (Intersile 250 mm × 4.6 mm; i.d. 10 µm), Rheodyne injection syringe with 20µL loop volume and windows based chrompass software was used for separation. The isocratic elution was performed using the mobile phase ratio of methanol: water (65:35 v/v) and UV detection wavelength at 263 nm. The overall run time of the analysis was 20 minutes and the flow rate was 1.0 mL/min. The RP-HPLC method developed for analysis of aceclofenac was validated as per the ICH guidelines with respect to specificity, selectivity, linearity, accuracy, precision and robustness. The linearity for developed method was observed in the concentration range of 5-50 µg/mL with the correlation coefficient (r^2) of 0.9992. The percentage accuracy of aceclofenac ranged from 99.40 to 100.79%. The relative standard deviation for inter-day precision was lower than 2.0%. The assay of aceclofenac was determined in tablet dosage form was found to be within limits. Aceclofenac was subjected to stress conditions such as neutral, acidic, alkaline, oxidation, and photolysis degradations as per ICH guidelines. The results of degradation studies revealed that the drug degraded a maximum (32.68%) in acidic conditions followed by alkaline conditions (15.05%). The drug was found to be resistant towards neutral, acidic and photolytic degradation conditions.

Keywords: Aceclofenac, RP-HPLC, Validation, Stability-indicating studies, ICH guidelines.

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INTRODUCTION

Forced degradation studies (stress testing) are very important tool in pharmaceutical research and development of stable formulation.[1] In forced degradation process, the conditions such as light, heat, humidity and oxidation are accelerated individually or in combination with automated stress to accelerate the degradation of the molecule by physical or chemical means. As per the International Committee for Harmonization (ICH) guidelines, the stability of the molecule, different degradative pathways and validation of the developed stability procedures are studied using forced decomposition studies. The details of drug molecules that undergoes degradation and the different products that are formed with respect to time changes under the impact of different environmental parameters and understanding of stability data are well explained using the Food and Drug Administration (FDA) and ICH guidelines.[2-8]

Non steroidal anti-inflammatory drugs (NSAIDs) are being extensively used for their anti-inflammatory, analgesic and anti-pyretic activities. Aceclofenac (ACF, Figure 1) is relatively a new NSAID belongs to phenyl acetic acid group. It is a potent COX-II blocker and inhibits the synthesis of prostaglandin E2. The anti-inflammatory properties are comparable to other NSAIDs like diclofenac.[9,10] Aceclofenac (CAS 89796-99-6) chemically [(2-{2,6-dichloro phenyl)amino}phenylacetooxyacetic acid], is a crystalline powder with a molecular formula $C_{16}H_{13}Cl_2NO_4$ and molecular weight of 354.19 g/mol. ACF acts by inhibiting the production of inflammatory mediators including prostaglandin E2 (PGE2), tumor necrosis factors (TNF) and different interleukins (IL1- β, IL-2). ACF is also reported to possess chondroprotective effects by the synthesis of glycosaminoglycan.[12-15]

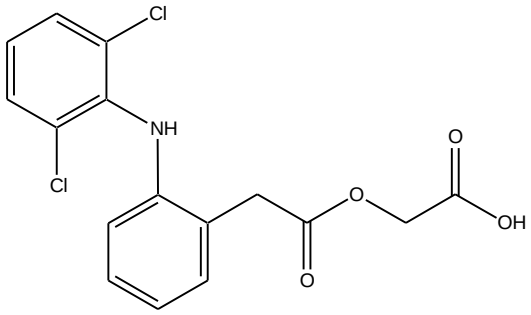

Figure 1: Chemical Structure of Aceclofenac (ACF)

Through literature survey on the analysis of aceclofenac indicated that it can be analyzed in bulk or combination by various analytical techniques like titrimetric,[16] colourimetric,[17] spectroflurimetric,[17] densitometric,[18] HPLC,[19-22] HPTLC,[23] spectrophotometric[24-26] and stripping voltametric..[27] From the literature reports, it is profound that the RP-HPLC stability indicating methods are mainly for the simultaneous analysis of aceclofenac in pharmaceutical dosage forms.[19-22, 28-33]

The aim of the present study was designed to develop and validate a simple, precise and rapid RP-HPLC method for the quantitative determination of aceclofenac. The analytical method developed was validated as suggested by ICH guidelines[4, 34, 35]

MATERIALS AND METHOD

Reagents and Chemicals

Methanol and water used were of HPLC grade (Fisher Scientific, UK). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were obtained from Scharlau, Spain. Aceclofenac (ACF) standard (purity 100%) was gifted by Julphar Gulf Pharmaceuticals, Ras Al Khaimah, UAE. All the chemicals procured were of analytical grade and used as received

HPLC APPARATUS AND CONDITIONS

The RP-HPLC studies was performed on the instrument using Jasco HPLC system with Jasco UV 2010 photo diode array (PDA) detector, ODS C-18 RP-column (Intersile 250mm × 4.6mm; id 10 µm). Rheodyne injection syringe with 20 µl loop volume and windows based chrompass software was used for separation. The isocratic elution was performed using the mobile phase methanol: water (65:35 v/v) and UV detection at 263 nm. The overall run time of the analysis was 20 minutes and the flow rate was set to 1.0 mL/min.

METHOD DEVELOPMENT

Mobile Phase Employed:

The mobile phase was prepared by mixing methanol: water (65:35 v/v). The solution was filtered using 0.45µm nylon filter paper and sonicated for about 10 minutes.

Preparation of Standard Stock Solutions of ACF:

Aceclofenac Stock Solution:

The stock solution of aceclofenac was prepared by weighing accurately a quantity of ACF (100 mg), transferred in 100 mL volumetric flask, and dissolved in methanol (50 mL) with the help of sonicator (Ultrasonic cleaner, model OU-72SPL) for about 10 minutes. Finally, the volume was adjusted to 100 mL mark using HPLC methanol to get ACF standard stock solution (1 mg/mL).

Aceclofenac working Standard Solution:

The working standard solution of ACF was prepared by pipetting out 5 mL of the above stock solution into a 50 mL volumetric flask and diluted upto the 50 mL mark with mobile phase to get the concentration of 100 µg/mL. The solution was mixed well and filtered through 0.45µm nylon filter paper. The working standard solution (10 µg/mL) was further prepared by taking 1.0 mL of 100µg/mL in 10 mL volumetric flask and diluted up to 10 mL with methanol. The solution was filtered through 0.45µm nylon filter paper. Aliquots of the suitable ACF working standard

solutions was transferred into a series of 10 mL volumetric flasks so that the final concentration was in the range of 5-50 µg/mL.

ANALYTICAL METHOD VALIDATION:

The RP-HPLC analytical method development for ACF per ICH Guidelines (Q2A(R1) method validation has been performed for the parameters such as specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability.

System suitability:

The system suitability was assessed by six replicate analyses of aceclofenac at a concentration of 10 µg/ml. The acceptance criterion was ± 2% for the percent relative standard deviation (% RSD) for the peak area and retention times for aceclofenac. The method was evaluated by analyzing the repeatability, retention time, peak area, capacity factor, tailing factor, theoretical plates of the column.[34-35] The method was found to be precise and specific. The standard chromatogram of ACF is shown in Figures 3A-3B and the results of analysis are summarized in Table 1.

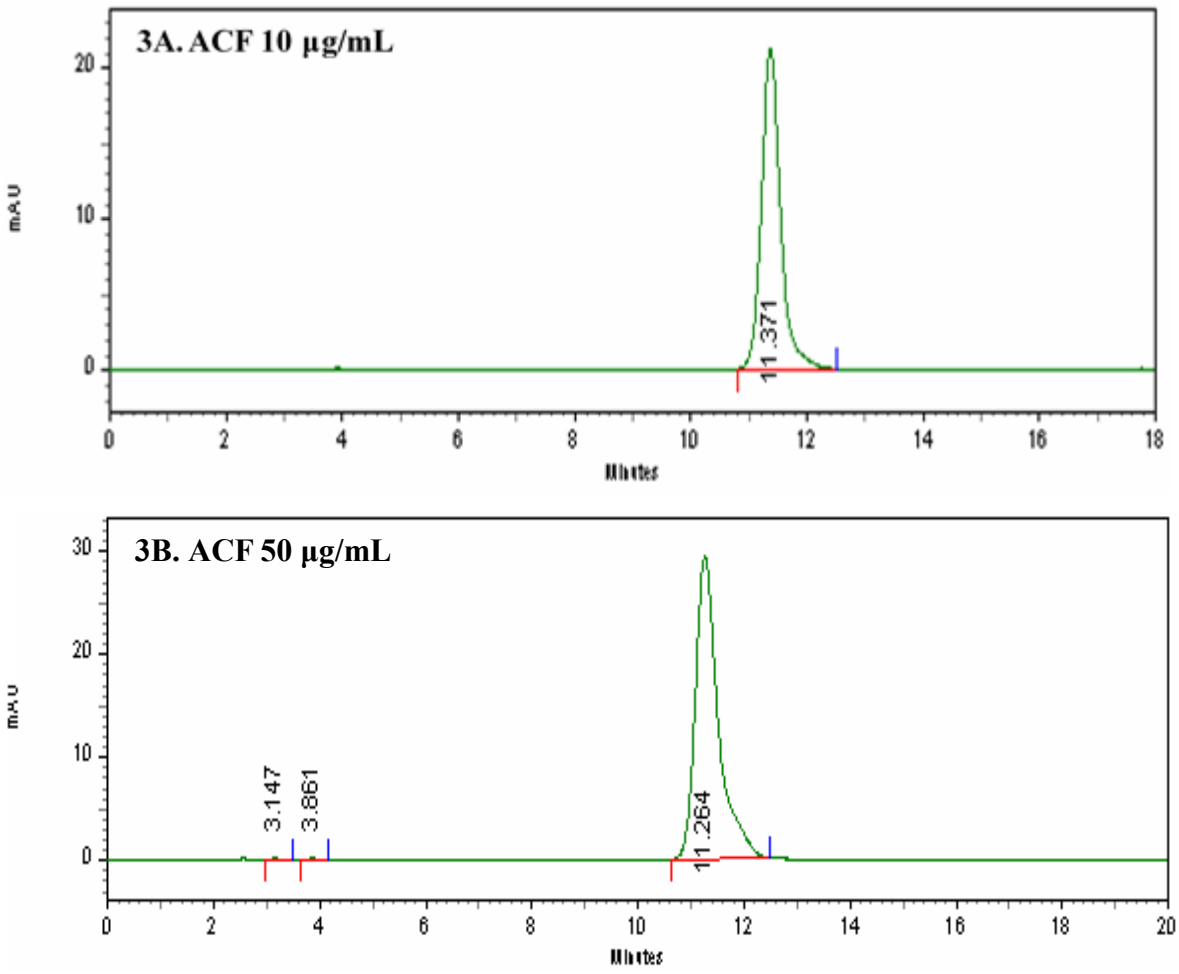

Figure 3: Typical chromatograms of Aceclofenac pure drug: 3A (10 µg/mL);

Linearity

Aliquots of the standard solution of ACF was transferred into series of six 10 mL volumetric flasks (appropriately labeled). The volume of working standard solutions was adjusted to the mark (10 mL) with HPLC grade methanol to get the working concentrations of 5-50 µg/mL. Evaluation of ACF was performed with PDA detector at 263 nm. The calibration or linearity curve for the proposed RP-HPLC method plotted is shown in Figure 2 and the data for linear regression studies is displayed in Table 2.

Sensitivity

The sensitivity studies of ACF was determined in terms of Limit of quantification (LOQ) and Limit of detection (LOD) as per the USP guidelines.[36] The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of aceclofenac using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD was found to be 0.534 µg/mL and LOQ was found to be 0.259 µg/mL

Precision

Precision mainly communicates the variations or reproducibility of the analytical data. It was determined by repeatability (intraday precision) and intermediate precision (interday precision) of standard and sample solutions aceclofenac. Precision was determined in six replicates of aceclofenac solution in the concentration range 5-50 µg/mL on the same day (intra-day precision) and daily for six times over a period of three days (interday precision). The results were expressed as % RSD of the measurements.

Intra-day precision

The intra-day precision studies was performed using six replicate injections of standard solutions of aceclofenac in the concentration (5-50 µg/mL) were injected into the HPLC system at different time intervals within a day. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Inter-day precision

In the inter-day studies, six injections of standard solutions of aceclofenac in the concentration (5-50 µg/mL) were injected into the RP-HPLC system at different time intervals over a period of three days. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Accuracy

Accuracy of the method was determined by calculating recoveries of drug by method of standard addition. Known amount of standard drug of aceclofenac corresponding to 50%, 100% and 150% of the label claim was added to prequantified sample solution and the amounts of drug were estimated by measuring peak areas and the results of the study is represented in the Table 4.

Robustness

The robustness as a measure of method capacity to remain unaffected by slight deliberate changes in chromatographic conditions. The chromatographic parameters selected were the effect of methanol in the mobile phase composition (63 and 67%), flow rate (0.8 and 1.2 mL/min) and wavelength (261 and 265 nm). Only one parameter was changed while the others were kept constant. Results of the study are summarized in Table 5.

Analysis of Marketed Formulations

Two different brands of aceclofenac tablets (Avenac, Acefenac; label claim 100 mg) were used to determine the drug content. Twenty tablets from respective marketed tablets of aceclofenac were accurately weighed and their average weight was calculated and finely powdered using mortar pestle. An aliquot of powder equivalent to the weight of 100 mg was accurately weighed and transferred into a 100 mL volumetric flask and dissolved completely using HPLC methanol. The resulting solution was sonicated for 10 min to complete the dissolution of ACF; the solution was filtered using 0.45µm membrane filter paper. The concentration of the assay sample solution was 1000 µg/ml. The working standard solution of 10 µg/mL was prepared by diluting above stock solution up to 10ml with methanol. These solutions were filtered through a 0.45 µm membrane filter and subjected to chromatographic analysis in triplicate. The drug peak area was referred to linear regression equation to get the sample concentration and nominal % of label claim. Chromatograms are shown in the figures: 3B-3C and the percent recovery data is summarized in table 6.

Forced degradation solutions

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[4, 34-35] Stability of aceclofenac was determined by subjecting it to oxidative, alkaline, acidic, neutral, and photolytic conditions in order to accelerate conditions auspicious for degradation. The stress solutions of ACF at concentration of 10 µg/mL were prepared from stock solution of 1 mg/mL using methanol and subjected to heating (40°C). Standard stress solutions ACF was filtered through 0.45 µm membrane filter paper and injected in to HPLC at regular time intervals. The HPLC chromatograms of the degradation studies are shown in Figures 5A-E and percent drug degraded is displayed in the table 7.

Neutral degradation

Aceclofenac (ACF) sample (10 µg/mL) was treated with methanol for about 30 min in a thermostat maintained at temperature of 40 ºC. Later cooled to room temperature and diluted with methanol, filtered through 0.45 µm membrane filter paper and injected into HPLC system. 20 µl of ACF sample solutions were injected into the HPLC system and the chromatogram recorded is presented in Figure 5A.

Acidic degradation

Acid degradation studies of ACF (10 µg/mL) was performed by treating ACF solution with 0.1 N hydrochloric acid (0.1N HCl) for about 30 min in thermostat maintained at 40 ºC. Later cooled to room temperature neutralized with 0.1N NaOH and diluted with methanol, filtered through 0.45 µm membrane filter paper and injected into the HPLC system. The HPLC chromatogram recorded is presented as Figure 5B.

Alkaline degradation

Alkaline degradation studies of ACF (10 µg/mL) was performed by treating ACF solution with 0.1 N sodium hydroxide for about 30 min in a thermostat maintained at 40 ºC. Later cooled to room temperature neutralized with 0.1N HCl, diluted with methanol and filtered through 0.45 µm membrane filter paper before injecting into the HPLC system. The HPLC chromatogram recorded is shown in **Figure 5C**.

Oxidative degradation

Oxidative degradation of ACF (10 µg/mL) was performed by treating ACF solution with 3 % H_2O_2 for 30 min in a thermostat maintained at 40 ºC. Later cooled to room temperature, diluted with methanol and filtered through 0.45 µm membrane filter paper before injecting into the HPLC system. The HPLC chromatogram recorded is shown in Figure 5D.

Photolytic degradation:

ACF was exposed to direct sunlight for 7 days. Stock solution of ACF (1 mg/mL) was prepared using the standard procedure described above. The solution obtained was further diluted with methanol to obtain a concentration of 10 µg/mL and 20µL was injected into the HPLC system. The HPLC chromatogram recorded is shown in Figure 5E.

RESULTS AND DISCUSSION

Method Development

The present experimental work was aimed to develop a new simple and rapid RP-HPLC method for determining aceclofenac and its degradations products. Different mobile phase compositions were tested during the development of the analytical method. The mobile phase that was chosen

to achieve maximum separation and sensitivity was the mixture consisting of methanol and water (65:35, v/v). A flow rate of 1.0 ml/min gave an optimal signal to noise ratio and the retention time of ACF (10 µg/mL) was observed to be 11.275 minutes and the detection wavelength of 263 nm. Several preliminary chromatographic runs were performed to investigate the suitability for drug content estimation and cost because of the increasing importance of rapid economic analysis in pharmaceutical analysis to increase the throughput.

System suitability

This test was performed by collection of data from a standard solution containing 10 µg/ml of ACF that was injected six times of standard resolution solution. The parameters measured were tailing factor, capacity factor, theoretical plates, and retention time. % RSD for tailing factor was 1.091 ± 1.31, the capacity factor was more than 2 (3.358 ± 0.513) and the theoretical plates were more than 2000 (2354.38 ± 0.48). The average of retention time was 11.275 minutes and peak area was 53583.8 ± 0.536. The results (Mean ± % RSD of six replicates) of the chromatographic parameters are shown in Table 1. Typical chromatograms of aceclofenac pure drug 10 and 50 µg/mL is shown in the Figures 3A and 3B, respectively. The method was found to be precise and specific.

Table 1: Chromatographic characteristics of system suitability study of Aceclofenac (ACF)

Sl. No	Parameters	Value (Mean ± %RSD)*
1	Retention time	11.275 min
2	Peak area	53583.8 ± 0.536
3	Tailing factor	1.091 ± 1.31
4	Theoretical plates	2354.38 ± 0.48
5	Capacity factor	2.858 ± 0.743

* Mean and % RSD of six samples of ACF

Method Validation

HPLC method was validated according to the International Conference on Harmonization Guidelines.[34-35] The method was validated with respect to parameters including linearity, limit of detection (LOD), and limit of quantitation (LOQ), recovery, precision, accuracy, robustness, and specificity.

Linearity

Linearity response of ACF was observed in the concentration range 5 - 50 µg/mL. The calibration curve for ACF is generated with concentration against peak area (Figure 2). The linear regression data values are displayed in Table 2. The regression equation for the calibration curve was found to be y = 2030.8x + 31838 and the correlation coefficient (r^2) of 0.9992 was obtained. Good linearity was observed between the peak area and analyte concentration.

Table 2: Parameters of regression analysis data for aceclofenac (ACF)

	Statistical Parameters	Values
1	Concentration range	5-50 µg/mL
2	Regression equation	2030.8x + 31838
3	Correlation coefficient	0.9992

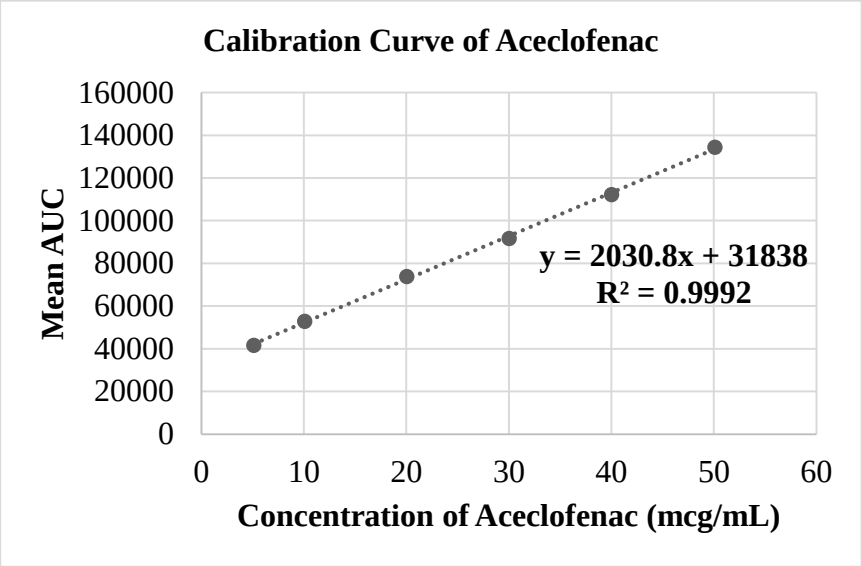

Figure 2: **Calibration curve for Aceclofenac (concentration range 5-50 µg/mL)**

Precision

Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (interday). The precision of the analytical method was estimated by performing six independent determinations of the standard aceclofenac solutions of six different concentrations (5-50 µg/mL) and calculating RSD (%). For day 1 (one) precision studies, the RSD (%) values for the six samples of ACF was observed in the range of 0.55 - 0.79 while for day 3 (three) precision studies the RSD (%) range was 0.57 - 0.73. This shows that precision of the method is satisfactory as % relative standard deviation (% RSD) is not more than 2.0%. The results of the precision studies are illustrated in Table 3.

Table 3: Results of intraday and interday precision studies for Aceclofenac (ACF)

Concentration (µg/mL)	Day 1		Day 3	
	* Peak Area (Mean ± SD)	%RSD	* Peak Area (Mean ± SD)	% RSD
5.031	36680 ± 143.53	0.79	37644 ± 193.57	0.71
10.05	53583 ± 258.27	0.55	53146 ± 276.27	0.61
20.01	73124 ± 442.66	0.72	73786 ± 472.37	0.73
30.02	92499 ± 697.55	0.69	92275 ± 653.55	0.62
40.03	112910 ± 866.25	0.59	116522 ± 879.25	0.62
50.05	136764 ± 955.08	0.55	135489 ± 996.08	0.57

* Mean and % RSD of six samples of aceclofenac

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection and limit of quantitation for ACF was calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. By the analysis of samples with known concentrations of analyte and establishing the minimum level at which the analyte can be reliably detected. The LOD was found to be 0.534 µg/mL and LOQ was found to be 0.259 µg/mL. The results indicate that this method is sensitive.

Accuracy:

To prove the accuracy of the proposed RP-HPLC method, recovery studies were accomplished by standard addition method at three different concentration levels (50%, 100% and 150%) summarized in table 4. Percent RSD for ACF was found to be in the range 0.566 -0.71 and the percentage recovery was 99.40-100.79 %. The results of the recovery test indicate that the RP-HPLC method is very accurate.

Table 4: Results of accuracy studies for Aceclofenac (ACF)

Amount added (µg/mL)	*Mean Peak area for ACF ± SD	% RSD	*Amount recovered(µg/mL)	% Recovery
5.03	37531.76 ± 145.74	0.71	5.07	100.79
10.02	53146.98 ± 256.07	0.68	10.04	99.40
15.03	64587.29 ± 289.36	0.566	15.05	100.13

* Mean and % RSD of six samples of aceclofenac

Robustness

Robustness of the analytical method was determined by consistency of the peak height and peak shape with the deliberate small changes in the experimental conditions. Under all the deliberately altered chromatographic conditions (flow rate, mobile phase and wavelength), peaks were adequately resolved and elution orders remained unchanged which indicate that the developed method for ACF is robust. The results are summarized in Table 5.

Table 5: Results of Robustness studies for aceclofenac (ACF)

Condition	Modification	*Mean Peak area for ACF ± SD	%RSD
Mobile phase composition	63:37	52683 ± 268.27	0.56
[Water, methanol (65: 35 v/v)]	67:33	53466 ± 238.43	0.69
Flow rate	0.8 mL	53673 ± 248.13	0.65
(1mL/min)	1.2 mL	53867 ± 287.13	0.52
Wavelength 263 nm	261 nm	52879 ± 235.08	0.58
	265 nm	53217 ± 233.87	0.61

* Mean and % RSD of six samples of aceclofenac

Analysis of Marketed Formulations of Aceclofenac

In the present study, the proposed RP-HPLC validated method was applied for the quantification of two different brands of aceclofenac tablets (Avenac, Acefenac; label claim 100 mg). The results of the ACF assay is shown in Table 6 and HPLC chromatograms for the representative assay samples is shown in Figures 4A-4B. The percentage recovery of the ACF drug is observed in the range 99.95 - 101.02. The ACF assay results indicate that the validated method was sensitive and specific for the quantitative analysis of aceclofenac in the marketed formulation.

Table 6: Determination of Aceclofenac (ACF) in tablet dosage form

	Tablet brand names	Label claim (mg)	Amount recovered (mg)	% Recovery
1	Avenac	100	102.02	101.02
2	Acefenac	100	99.95	99.95

* Mean of six samples of aceclofenac

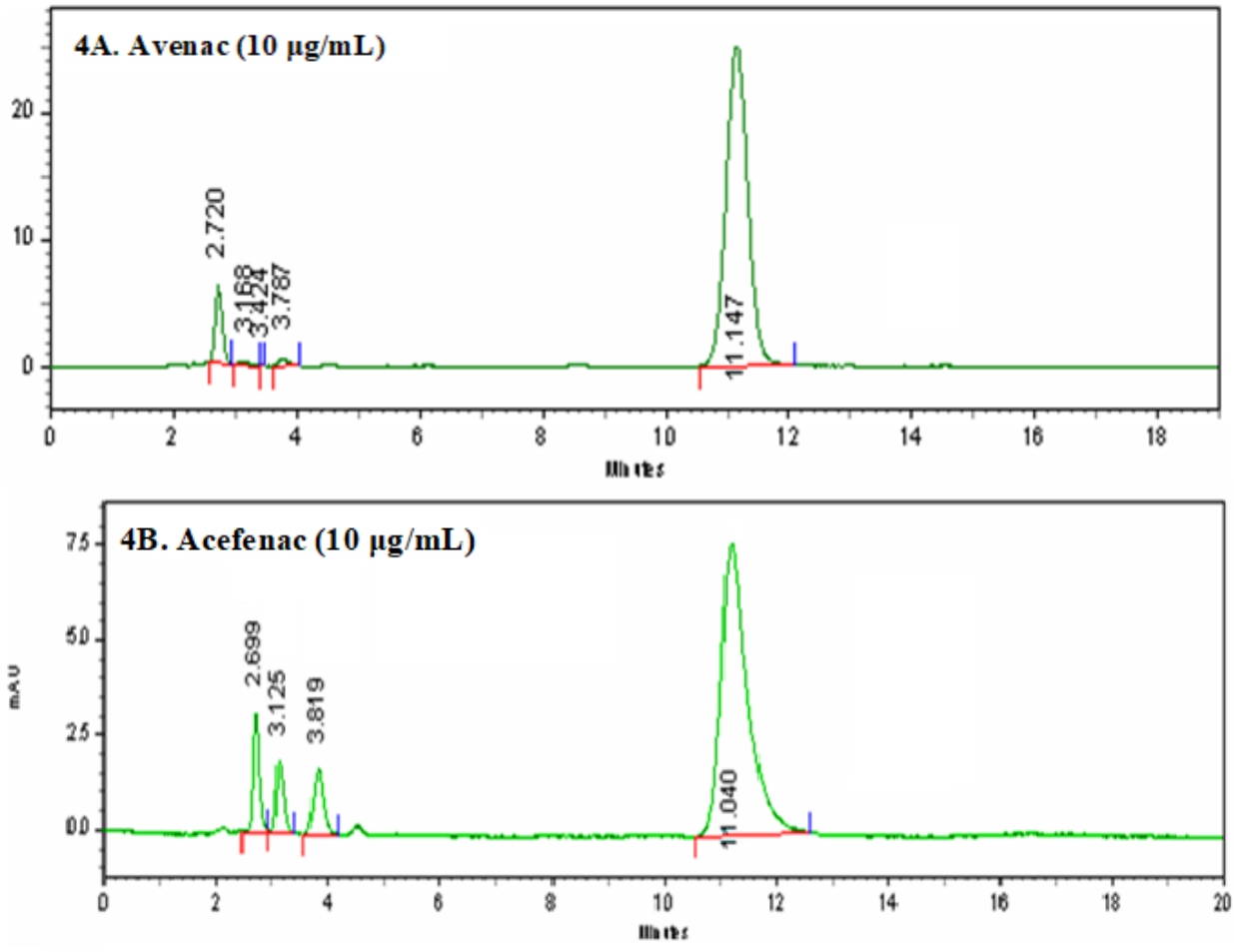

Figure 4: Typical HPLC chromatograms for the formulations of Aceclofenac (10 µg/mL): 4A. Avenac; 4B. Acefenac.

Forced Degradation Studies

In order to evaluate the stability indicating properties of the developed RP-HPLC method, forced degradation studies were carried out in accordance with ICH guidelines.[4, 34-35] The stability of ACF was determined by exposing the pure sample to neutral, acidic, alkaline, oxidative, and photolytic conditions in order to accelerate conditions favorable to degradation. The results and typical chromatograms is displayed in the Table 7 and figures 5A-E, respectively.

From the results of the degradation studies, notable percentage of degradations for aceclofenac was observed during acid hydrolysis (32.68 %) and alkaline hydrolysis (15.05 %) degradation peak was observed around 14 minutes. However, the sample of aceclofenac was found to be stable towards neutral hydrolysis, oxidative and photolytic degradation conditions and also there was no detectable degradation peak(s). The samples in presence of neutral, oxidative and photolytic stress

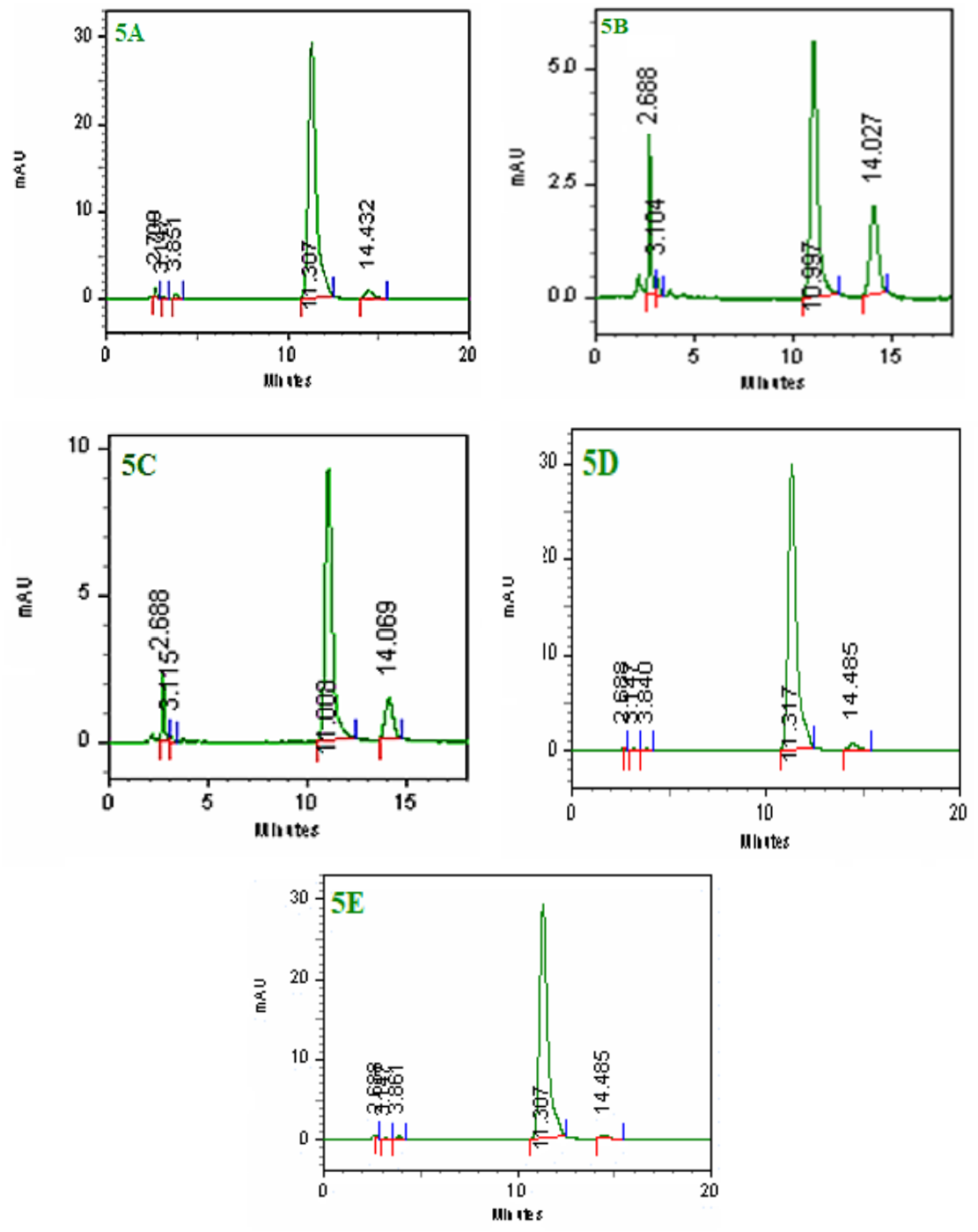

Figure 5: **HPLC Chromatograms showing the degradation of aceclofenac (10 µg/mL) 5A**: Neutral degradation**; 5B**: Acid hydrolysis (0.1N hydrochloric acid for 30 min 40º C); **5C**: Alkaline hydrolysis (0.1N sodium hydroxide for 30 min 40ºC); **5D:** Oxidative degradation (3% H_2O_2 for 30 min in a thermostat maintained at 40ºC)**; 5E**: Photolytic degradation.

Table 7: Results of Stress degradation studies for Aceclofenac

Sl. No	Stress condition	*Mean Peak area for ACF ± SD	%Drug recovered	% Drug degraded
1	Neutral	52476 ± 238.27	98.73	1.27

2	Acidic	53278 ± 241.34	67.32	32.68
3	Alkaline	52987 ± 232.45	84.95	15.05
4	Oxidative	53465 ± 246.15	98.87	1.13
5	Photolytic	52984 ± 259.54	99.12	0.88

* Mean and standard deviation (SD) of six samples of aceclofenac

degradation conditions displayed the degradation percent for ACF as 1.27, 1.13 and 0.88, respectively. The present stability-indicating method for the determination of ACF in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with the ACF peak, indicating the selectivity of the method. The proposed stability-indicating HPLC method was validated as per ICH guidelines. The % RSD in precision, accuracy and robustness studies was found to be less than 2 indicating that the proposed method is precise, accurate and robust. The method was found to be specific, as the drug peak elution did not interfere with any degradants during the forced degradation studies.

CONCLUSION

The present RP-HPLC method developed for the determination of aceclofenac was found to be reliable, simple, accurate, sensitive and precise. The RP-HPLC method was validated as per ICH guidelines and may be applied for the determination of aceclofenac in tablet dosage forms. The good validation criteria of the proposed method allow its use in quality control laboratories as an alternative to the official methods. The results of 0forced degradation studies proved that the method is specific for the analyte and free from the interference of blank and unknown degradation products. In addition, the results indicate the suitability of the method for neutral, acid, base, oxidation, and sunlight degradation studies. The method is suitable for the analysis of stability samples and the routine analysis of aceclofenac in tablets.

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