

Application of a Validated Stability-Indicating HPTLC Method for Simultaneous Estimation of Paracetamol and Aceclofenac and their Impurities

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Abstract

A sensitive, accurate, precise and stability indicating HPTLC method has been developed and validated for the simultaneous determination of Paracetamol (PCT), Aceclofenac (ACF) and their impurities namely, 4-amino phenol (AP) and Diclofenac acid (DA), respectively, which are the hydrolytic degradation products and related substances of the drugs. The chromatographic separation was achieved using optimized mobile phase, ethyl acetate: methanol: glacial acetic acid (8.5:1.5:0.25 V/V) on pre-activated silica gel 60 F₂₅₄ TLC plates with ultraviolet detection at 231 nm. Statistical analysis revealed linear relationships in the concentration ranges of 3900–9100, 1200–2800 for PCT, ACF respectively and 100-600 ng/band for AP and DA with correlation coefficient more than 0.99. The chromatographic conditions gave compact spots at R_f value (\pm SD) 0.60 (\pm 0.011), 0.21 (\pm 0.01), 0.83 (\pm 0.01) and 0.08 (\pm 0.015) for PCT, ACF, AP and DA respectively. The method was validated as per ICH guidelines, demonstrating to be accurate and precise within the corresponding linearity range of analytes. Inherent stability of these drugs was studied by exposing drug substances to stress conditions as per ICH guidelines namely oxidative, thermal, photolysis and hydrolytic conditions under different pH. Relevant degradation was found to take place under these conditions. PCT and ACF were found to undergo extensive acidic and basic hydrolysis while ACF was also susceptible to neutral hydrolysis; producing stated impurities as their major degradation products. The developed method resolved the drugs from degradation products generated under stress conditions; with the peak purity values greater than 0.999. Thus, a new simple, accurate, precise, sensitive and economic stability-indicating High Performance Thin Layer Chromatography (HPTLC)/densitometry method has been developed and validated for the simultaneous estimation of the titled analytes and their stated impurities in pharmaceutical dosage form. The high sensitivity and specificity make it valuable quantitative method suitable to be employed in the routine estimation of impurities and in stability studies.

Keywords: Paracetamol; Aceclofenac; HPTLC; Densitometry; Degradation; Related substances; Impurities; Forced degradation; Validation

Introduction

Paracetamol (PCT) is chemically N-(4-hydroxyphenyl) acetamide (Figure 1A). It is widely used as a minor analgesic, which is an alternative to aspirin without the side effects of salicylate on gastric mucosa [1]. 4-amino phenol (AP) (Figure 1C) has been found to be the primary hydrolytic degradation product of PCT. It is also reported impurity and related substance of PCT [2-4].

Aceclofenac (ACF) is chemically 2-[2-[(2,6-dichlorophenyl)amino]phenyl]oxyacetic acid (Figure 1B). It is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenyl acetic acid in the relief of pain and inflammation in rheumatoid arthritis, ankylosing spondylitis and osteoarthritis [5]. It has pronounced anti-inflammatory, analgesic and antipyretic properties. It undergoes extensive degradation in alkaline conditions, yielding its main degradation product as diclofenac acid (DA) (Figure 1D) derived from the phenyl acetic acid which is also related substance [6]. The combination of PCT and ACF is available in the market for its synergetic effect in pain management.

Literature survey reveals that numerous methods have been reported for analysis of PCT such as UV spectrophotometry [7], electro analytical method [8], stability indicating HPLC [9], HPLC applied to related Substances [10] and HPTLC [11] either alone or in combination with other drugs. For ACF methods reported are spectrophotometry [12,13], stability indicating HPLC [14], UPLC [15] and HPTLC [16,17] either alone or in combination with other drugs. It was further revealed that there are analytical methods reported for simultaneous estimation

of PCT and ACF like Spectrophotometry [18], HPLC [19-21], HPTLC [22], stability indicating HPLC [23] while few reported methods like HPLC [24], HPTLC [25] and stability indicating HPTLC [26] involve simultaneous estimation of PCT and ACF combined with one more additional drug. No study so far has been reported that involves studies related to simultaneous estimation of the titled analytes and their structurally related substances i.e., AP and DA that might co-exist as impurities in bulk drug.

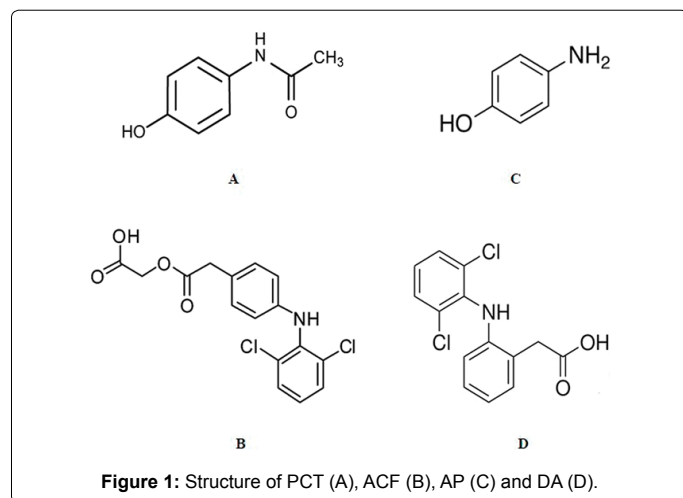
Analytical monitoring of impurities is a key component of the recent guidelines issued by the ICH Q3A [27]. Nowadays, it is mandatory requirements in various pharmacopoeias to know the impurities present in drug substances [28]. The ICH Q1A guideline states that the validated stability indicating testing methods must be employed to monitor the characteristic features of drug substance which are susceptible to change during storage and are likely to affect the quality, safety and/or efficacy of the formulation [29]. Therefore, it was decided to develop and validate stability-indicating simultaneous

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quantification HPTLC method for PCT and ACF and their related substances in bulk drug and in pharmaceutical dosage form. The HPTLC technique was chosen as it has an advantage of handling large number of samples at a time, it is sensitive enough to detect small amount of related substances/impurities, degradation products formed under stress conditions and at the same time it has low maintenance cost, short run time, less mobile phase consumption per sample; hence it can fulfill the demands of a routine analytical technique. It also facilitates automated application of sample and scanning of the plate [30]. Thus, the aim of the present study was to develop an accurate, precise, specific, sensitive, robust and stability indicating HPTLC method for simultaneous determination of PCT and ACF and their stated impurities in bulk drug and pharmaceutical dosage form. The analytes were purposely degraded by forced degradation to check the stability and to develop stability indicating assay method that can resolve degradation products from analytes. The developed analytical method was validated as per ICH guidelines.

Materials and Methods

Chemical and reagents

PCT and ACF standards were kindly provided as a gift sample by Zest Pharma, Indore and Vapi Care Pharma. Pvt. Ltd., Gujarat respectively, its impurities AP (99.52%) and DA (99.75%) were procured from Aarti Drugs Ltd., Thane. The marketed formulation used in this study was Acemiz Plus (Lupin Lab. Ltd., India) procured from the local market, labeled to contain 325 mg of PCT and 100 mg of ACF per tablet. The solvents and chemicals used in the development of method were of AR grade purchased from Merck Pvt. Ltd., Mumbai.

Instrumentation

Camag HPTLC system consisting Linomat V sample applicator, chromatogram development chambers (Twin Trough Chambers 20 cm × 10 cm), pre-coated silica gel 60 F₂₅₄ aluminium plates (20.0 cm × 10.0 cm, 250 μm thickness; Merck, Germany), TLC scanner III and winCATS version 1.4.4 Software, (Sr. No. 1508W015) was used for development of chromatographic separation.

Chromatographic conditions

TLC plates were pre-washed with methanol and activated at 110°C for 10 min prior to application. Suitable volumes of standard, sample and resolution solutions were applied to the HPTLC plates using CAMAG Linomat V sample applicator (Muttentz Switzerland)

equipped with 100 μl syringe (Hamilton, Reno, Nevada, USA) with spraying speed as 150 nl/s. Sample and standard zones were applied to the plates as bands (10 mm from the bottom and 8 mm from the side edges) with band length of 6 mm. The linear ascending chromatographic separation was carried using ethyl acetate: methanol: glacial acetic acid (8.5:1.5:0.25, by v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 90 mm. Before detection, the plates were dried to eliminate mobile phase and separation was achieved within 10 minutes. A common wavelength for the simultaneous determination of all the analytes was selected based on the overlaying spectra (Figure 2). The wavelength at 231 nm was chosen for detection as it resulted in better detection sensitivity for drug substances and their related impurities compared to the well accepted λ_{max} of either drug substance. Densitometric scanning was performed using Camag TLC scanner III in the reflectance/absorbance mode at 231 nm with a slit dimension (4.00 mm × 0.45 mm, Micro), scanning speed 20 mm/s and data resolution 100 μm/step. Data were integrated using winCATS Software.

Preparation of solutions

Standard solution: Standard stock solutions of drug substances were prepared by dissolving 130 mg of PCT and 40 mg of ACF, in 10 ml of methanol separately to obtain a concentration of 13 mg/ml of PCT and 4 mg/ml of ACF (Solution A). An aliquot of 1 ml of solution A of each drug substance was diluted to 10 ml with methanol separately to obtain 1300 μg/ml of PCT and 400 μg/ml of ACF (Solution B). Similarly, the impurity stock solutions were prepared by dissolving 10 mg of AP and DA, in 10 ml of methanol separately to obtain a concentration of 1 mg/ml (Solution C). An aliquot of 1 ml of each impurity solution was further diluted to 1 ml with methanol separately, to obtain 100 μg/ml of each impurity. The ratio of drug substances was fixed, taking into account their proportion in tablet formulation.

Resolution solution: An aliquot of 1 ml of standard solution A of each drug substance and 1 ml of solution C of each impurity was mixed and diluted to 10 ml with methanol to get resolution solution containing 1300 μg/ml PCT, 400 μg/ml ACF, 100 μg/ml of each impurity.

Formulation assay

Twenty tablets were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 65 mg of the PCT (20 mg of ACF) was transferred to a 40 ml methanol, sonicated for about 10 min to ensure complete dissolution of drugs and the volume was made up to 50 ml with methanol, resulting into the concentration of 1300 μg/ml of PCT and 400 μg/ml of ACF. The extract was filtered through Watmann filter paper No 41. An aliquot (5ml) of sample

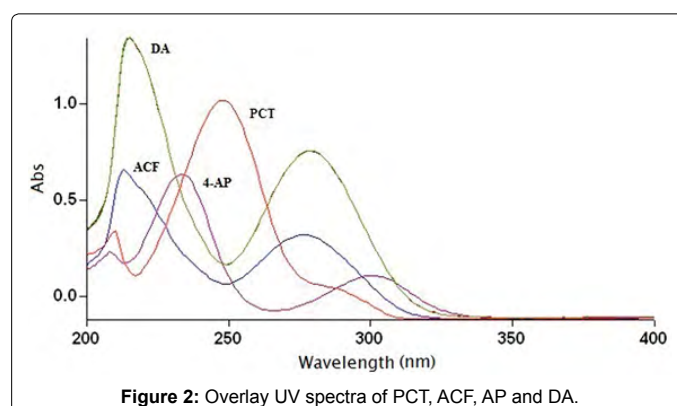


Figure 2: Overlay UV spectra of PCT, ACF, AP and DA.

solution was further diluted to 10 ml with methanol; 10 μ l of the resulting solution (containing 6500 ng/band of PCT and 2000 ng/band of ACF) was applied to TLC plate, developed as per optimized chromatographic conditions and scanned. The amount of each drug present was determined using calibration curves.

Forced degradation studies

The study was intended to ensure the optimum resolution of PCT and ACF and their degradation product peaks. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. The objective of stress study was to generate the degradation products under various forced degradation conditions and to verify that the degradation peaks are well resolved from the analyte peaks by the developed method [29,31]. Each drug substance was subjected to stress degradation, carried out in the dark, to avoid the possible degradation due to light except for photo stability studies. Forced degradation studies were carried out on the bulk drug separately and in combination. It was also applied to tablet formulation to study the presence of additional degradation products that may arise from drug excipient interaction.

Acid, base, oxidative and wet heat degradation

To 1 ml of working standard solution A of each analyte (i.e., 13 mg of PCT and 4 mg of ACF), 5 ml of each stressor i.e., 0.1N HCl / 0.1N NaOH/ 6% H₂O₂/ HPLC grade water was added and refluxed at 80°C for 2 h. The resultant solution was neutralized except for oxidative and wet heat degradation and diluted up to 10 ml with methanol. The suitable volume (5 μ l containing 6500 ng/band of PCT and 2000 ng/band of ACF) was applied to a TLC plate.

Thermal degradation and photo-degradation

For solid state dry heat and photo degradation studies, PCT and ACF 50 mg each was spread as thin film in two separate petri dishes (50 mm diameter) in two sets. The first set of petri dish was heated in an oven at 70°C for 7 days and the second was exposed to UV radiation 200 Watt h/m² for 28 h in the UV chamber [32]. The solution (10 ml) was prepared using 13 mg of PCT and 4 mg of ACF of the exposed powder in methanol and suitable volume (5 μ l) was applied to TLC plate.

Validation

The method was validated for parameters namely, linearity, range, accuracy, precision, sensitivity, specificity and robustness in accordance with ICH guidelines [28,33].

Linearity and range: The calibration curve was obtained in the range of 3900–9100 ng/band and 1200–2800 ng/band for PCT and ACF respectively and for impurities, in the range of 100–600 ng/band; The appropriate volume of resolution solution (3–7 ml) was diluted to 10 ml with methanol to get mixed standards in the stated concentration range and 10 μ l of the resulting solution was applied on TLC plate. The analytes were resolved under optimized chromatographic conditions and the peak area was observed. The whole procedure was repeated thrice starting from weighing of analytes to preparation of resolution solution as described under standard solution preparation. The average of peak area of three readings was plotted against concentration to obtain calibration curve. The slope and intercept were calculated. The linearity of the method was evaluated by linear regression analysis, using least square method, F test and the residual plot of relative response against concentration applied. The value of experimental Fischer ratio was compared against the critical value found in statistical tables, at the

95% confidence level while the trend was observed in residual plot [34].

Accuracy (recovery study): Recovery studies were conducted by the standard addition method where known amounts of the standard substances were spiked to the pre-analyzed sample at 80%, 100% and 120% level. The resulting samples were analyzed independently in triplicate starting from weighing. In short, the quantity of tablet blend equivalent to 32.5 mg of PCT was spiked with 26 mg, 32.5 mg and 39 mg of standard PCT powder separately. These blends were also spiked with 8 mg, 10 mg and 12 mg of standard ACF powder separately. The assay was carried out as per the procedure elaborated under formulation assay. For impurity recovery study, a standard powder blend containing 91 mg PCT and 10 mg AP was spiked with 8 mg, 10 mg and 12 mg of standard AP separately. Likewise, a standard powder blend containing 28 mg ACF and 10 mg DA was spiked with 8 mg, 10 mg and 12 mg of standard AP separately. These mixtures were suitably diluted and analyzed under optimized chromatographic conditions. Thus, the recovery studies were performed in order to find out how much of the drug substance is retrieved during the process of sample preparation from tablet triturate and how much of impurity is retrieved during the process of sample preparation from bulk drug.

Precision: The precision of proposed analytical method was demonstrated by repeatability and intermediate precision studies. Resolution solution containing 6500 ng/band of PCT, 2000 ng/band of ACF and 200 ng/band of each impurity was obtained after independent preparation of each solution starting from weighing were analyzed six times on the same day and six times by different analysts on different days. Briefly, 65 mg of PCT, 20 mg of ACF and 20 mg of each impurity was diluted suitably with methanol to obtain 650 μ g/ml of PCT, 200 μ g/ml of ACF and 20 μ g/ml of each impurity. An aliquot of 10 μ l was applied on TLC plate. Similarly, the assay of tablet triturate was performed six times independently, as per the procedure elaborated under formulation assay on different days. The % RSD value and mean recovery were calculated to demonstrate precision of the method.

Method sensitivity (limit of detection and limit of quantification): The LOD and LOQ of the developed method were calculated as per ICH guidelines. A series of standard preparations containing 3900–9100 ng/band and 1200–2800 ng/band of PCT and ACF respectively and 100–600 ng/band of impurities, were prepared over different levels. Calibration graphs were plotted for the obtained area under the curve of each level against the concentration. The LOD and LOQ were calculated using equations, $LOD=3.3 \times \sigma/S$; $LOQ=10 \times \sigma/S$, respectively where σ is the standard deviation of the response and S is the slope of the calibration curve. Thus, obtained LOD and LOQ values were further confirmed by applying different volumes of stock solution of each analytes in three replicate separately on a TLC plate and % RSD values were calculated.

Specificity: Specificity is the capacity of the method to measure the analyte response in the presence of components that may be expected to be present like impurities, degradants and formulation excipients. Therefore these studies were conducted in two parts, Part-I and Part-II. Part-I, involved demonstration of the ability of the developed method for the separation and resolution of PCT, ACF and their impurities without any interference by analytes and matrix. Peak purity of the analytes was measured to evaluate the specificity of the method. The sample and standard bands were scanned at three distinct levels, i.e., peak start (S), peak apex (M), and peak end (E) positions. The peak purity was determined by winCATS software. Part-II, involved forced degradation of drug substances under various stress conditions, Viz. Oxidative, photolytic, thermal and hydrolytic conditions under

different pH values. Degraded samples were applied on the TLC plates, developed as mentioned previously. Developed densitograms were observed for separation of degraded product and spots were evaluated for peak purity as described before.

Robustness and stability of sample solution: The effect of small but deliberate variations in method parameters like the volume and composition of the mobile phase, time from spotting to development and development to scanning were evaluated in this study. One variable at a time was altered and the effect on the R_f and % recovery was checked. Similarly sample solution was applied at regular time interval for 8 h and the percentage assay was noted to study stability of the sample solution.

Results and Discussion

Development and optimization

For the selection of optimized mobile phase to achieve sufficient resolution of PCT, ACF, their impurities and degradation products several runs were made by using mobile phases containing solvents of varying polarity in different proportion like toluene, chloroform, n-butanol, methanol, glacial acetic acid, formic acid, ethyl acetate, ammonia, acetone. Several different compositions of the above solvents were tried at different concentration levels to achieve optimum resolution between analytes and their impurities. The mobile phase consisting of ethyl acetate: methanol: glacial acetic acid (8.5:1.5:0.25 V/V) was found to give the optimum resolution with sharp well defined peaks at R_f value 0.60, 0.21, 0.83 and 0.08 for PCT, ACF, AP and DA respectively. Densitogram obtained shows no interference between analyte and impurities peak and/or from other constituents originating from the tablet excipients (Figure 3). The peak purity of more than 0.999 indicates that there is not any interference in the separation and estimation of the analytes and the developed method is specific for

drug substances and their impurities (Figure 4).

Validation of the developed stability-indicating method

The developed method was validated as per ICH guidelines. The following parameters were evaluated [28,33].

Linearity and range: The calibration curve was plotted for drug substances and their impurities in the stated range. The linearity was evaluated by regression analysis, F-test and residual plots. The correlation coefficient was found greater than 0.99 for both the drug substances and their impurities. The calculated experimental F value was found less than the critical tabulated F value at 95% confidence level for titled analytes (Table 1). The plot for residuals showed random distribution without any trend/pattern in it (Figure 5). These results proved that an excellent correlation existed between peak areas and concentration of the drugs within the selected concentration range.

Method sensitivity: The low values of LOD and LOQ with % RSD values <10 (Table 1) for drug substances and impurities indicate that the developed method is sensitive enough to detect impurities in the drug substance and to be used as stability indicating and can be applied to dilute sample.

Accuracy: The % mean recoveries for PCT and ACF for each 80%, 100%, 120% level were in the range of 99.61% to 101.1% while for impurities % mean recoveries were found to be in the range of 98% to 100.6%. The overall % RSD was observed to be satisfactory thus, indicates accuracy of the developed method (Table 2).

Precision: The overall % RSD values for repeatability were found not more than 0.79% for drug substances while for impurities it was not more than 1.80%. The overall % RSD values for intermediate precision were found not more than 1.14% for drug substances and it was not

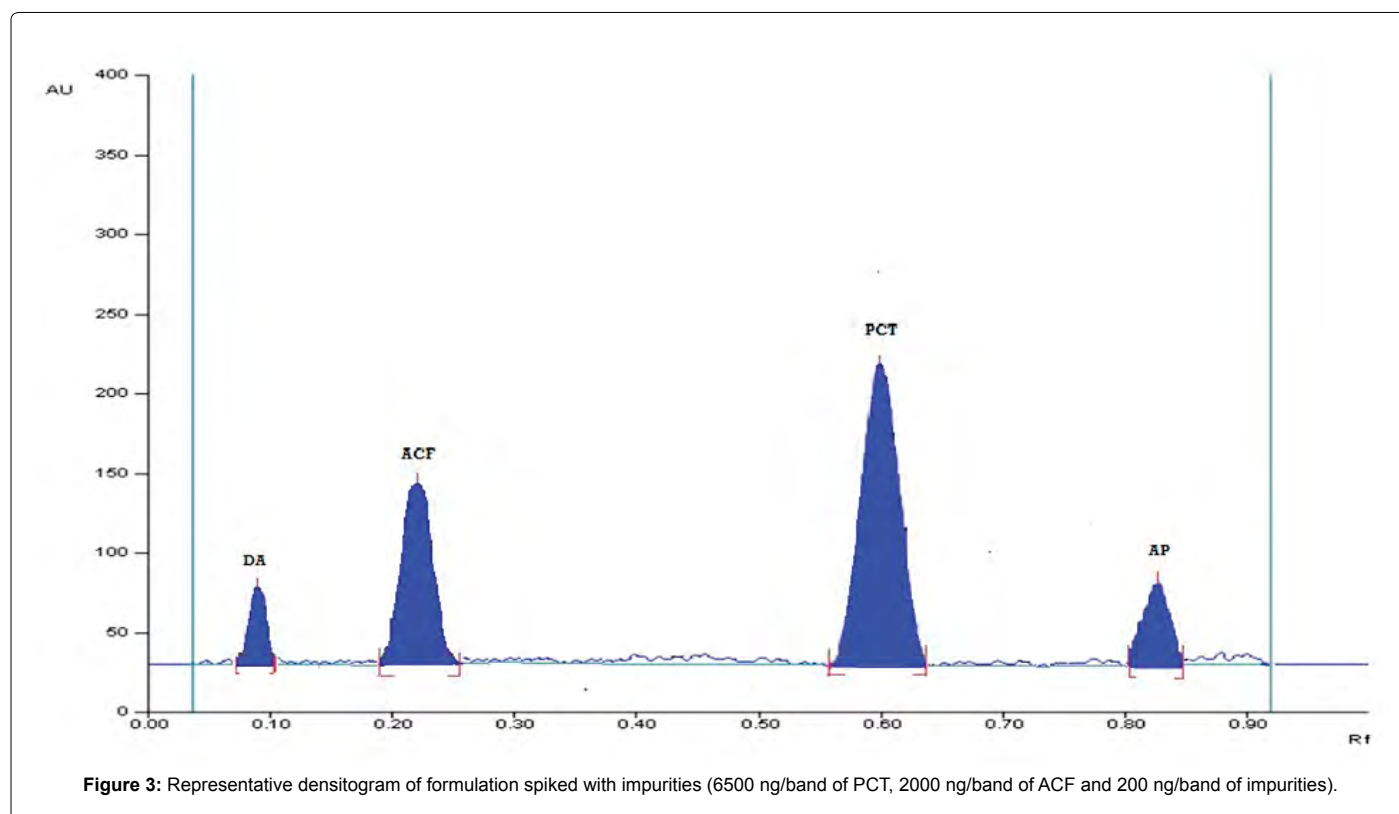


Figure 3: Representative densitogram of formulation spiked with impurities (6500 ng/band of PCT, 2000 ng/band of ACF and 200 ng/band of impurities).

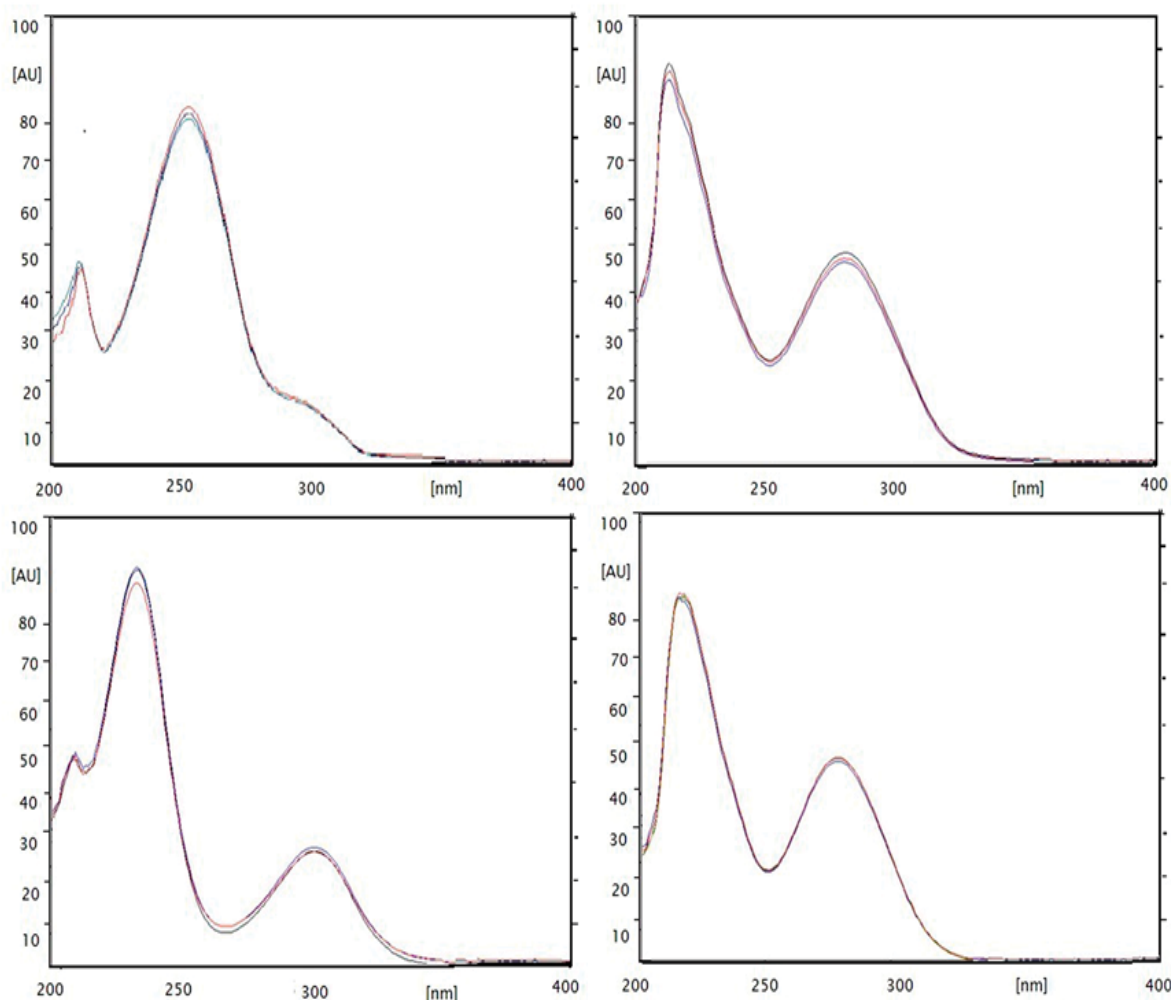


Figure 4: Peak purity spectra of Drug substances: PCT (A), ACF (B) and Impurities: AP (C) DA (D) at 231 nm.

Parameter / Analytes →	PCT	ACF	AP	DA
Concentration range (ng/band)	3900–9100	1200–2800	100–600	100–600
Rf	0.60	0.21	0.83	0.08
Linearity (n=3)				
Regression equation	y=2.5741x+6234.4	y=4.1713x+117.87	y=3.7625x+335.06	y=5.3694x+29.978
Correlation coefficient (R ²)	0.998	0.998	0.998	0.998
Experimental Variance σ ² _e	45296.77	5867.11	1229.83	2011.33
Lack of fit variance σ ² _{Lof}	116239.99	12963.45	3720.36	6197.10
Experimental Fischer variance ratio (F)	2.566	2.209	3.025	3.081
Tabulated Fischer variance ratio (F)	3.259	3.259	3.259	3.259
Mean % Assay (% RSD) (n=6)	100.73 (0.72)	99.92 (0.32)		
Precision (n=6)				
Repeatability Mean % Assay (% RSD)	99.97 (0.79)	100.14 (0.71)	98.92 (1.73)	99.81 (1.80)
Intermediate Mean % Assay (% RSD)	99.45 (1.03)	100.02 (1.14)	99.80 (2.15)	100.23 (2.04)
Method sensitivity (n=3)				
LOD (ng/band) (% RSD)	128.82 (6.17)	122.44 (7.60)	28.14 (8.03)	24.50 (9.05)
LOQ (ng/band) (% RSD)	387.34 (4.86)	371.03 (5.79)	85.29 (6.12)	74.26 (7.64)
Specificity (Typical Peak purity data)				
r(S,M)	0.9997	0.9999	0.9996	0.9998
r(S,E)	0.9996	0.9996	0.9997	0.9996

% RSD=Relative standard deviation; n=Number of repetitions

Table 1: Result of Linearity, range, precision, method sensitivity and specificity study.

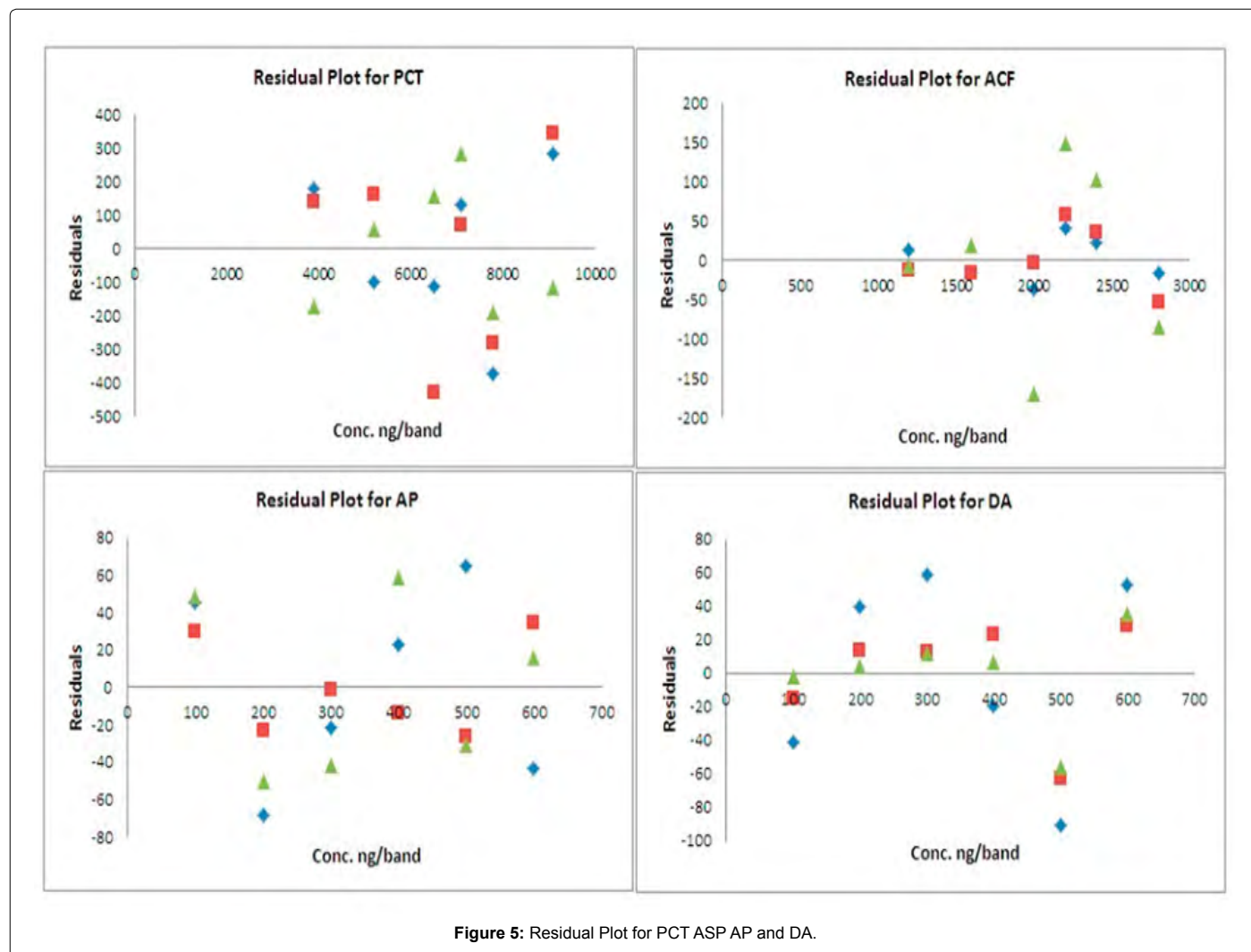


Figure 5: Residual Plot for PCT ASP AP and DA.

Recovery level	PCT		ACF		AP		DA	
	%MR	%RSD	%MR	%RSD	%MR	%RSD	%MR	%RSD
80%	100.77	0.75	99.61	0.4	99.2	1.8	98.76	2.01
100%	100.51	0.92	100.41	0.55	98.49	1.73	100.56	1.52
120%	101.1	0.94	100.92	0.36	100.44	1.95	98.58	1.37

n=Number of repetitions; MR-Mean Recovery

Table 2: Results of Accuracy (Recovery) study by standard-addition method (n=3).

more than 2.15% for impurities. The low % RSD indicates sensitivity and repeatability of the proposed method which proves the proposed method is precise (Table 1).

Robustness: Small deliberate changes in optimized chromatographic conditions did not affect the assay and R_f value significantly for the investigated compounds; low % RSD indicates that the developed method is robust enough to minor changes in the experimental conditions during routine usage (Table 3). The assay values were found to be above 99% and % RSD less than 1.80 indicates stability of sample solution over a period of 8 h.

Specificity: The specificity of the method was verified by peak purity studies. In the specificity part I study, the densitogram showed well resolved peaks without any overlapping (Figures 3 and 4) indicating separation without any interference by each other or formulation

excipients (Table 1). While the specificity part II study, showed peak purity values greater than 0.999 for drug substances and impurities indicating resolution without any interference by degradation products or matrix (Table 4). Thus, the presented HPTLC method in this study is selective for PCT, ACF and their related substances AP and DA which might co-exist as impurities.

Forced degradation studies: The results of forced degradation studies of PCT and ACF using the developed method are summarized in Table 4. Degradation products of both analytes were well resolved from the drug substances under the optimized chromatographic conditions; which were further confirmed by peak purity study. The peak purity r (s,m) and r (s,e) values were above 0.999; indicating homogeneous peaks. Thus developed method is demonstrated to be specific. The percentage degradation of PCT and ACF was calculated by area normalization technique [29]. PCT and ACF were found to undergo considerable degradation under acid hydrolysis (Figure 6A). ACF showed DA as major hydrolytic degradation product with R_f value 0.08 (densitogram 1 about 20.5%) while the PCT showed AP as major hydrolytic degradation product with R_f value 0.82 (densitogram 6 about 9.6%). Both the analytes showed additional negligible degradation peaks. Base induced degradation studies were performed with and

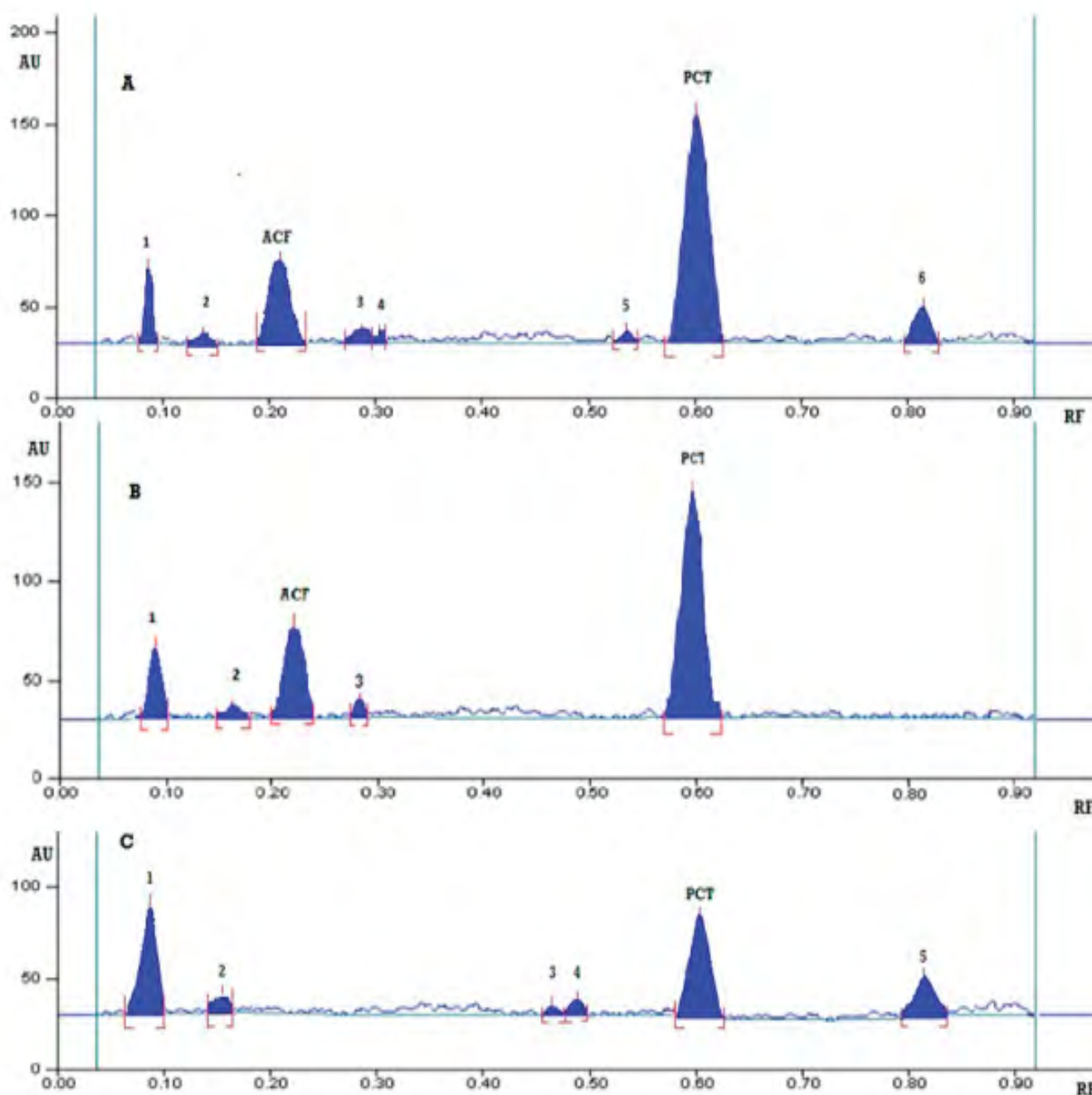


Figure 6: Representative Densitogram showing degradation under A: Acid induced hydrolysis, B: Base induced hydrolysis (without reflux), C: Base induced hydrolysis (with reflux).

without reflux. Under base hydrolysis without reflux, ACF formed DA as the major degradation product with R_f value of 0.08 (densitogram 1, about 28.56%) while PCT did not show any degradation and peak area remained almost constant (Figure 6B). Extensive degradation of both the drug substances was found to occur under base hydrolysis with reflux condition. The ACF was extremely susceptible to reflux condition and found to be completely degraded, producing DA as the major degradation product with R_f value 0.08 (densitogram 1, about 94.28%) and PCT showed AP as major degradation product at R_f value

0.82 (densitogram 5, about 38%) (Figure 6C). Additional negligible degradation peaks were observed. Thermal degradation studies involved wet and dry heat induced degradation. ACF was found to be susceptible to wet degradation and showed additional peak of DA at R_f value 0.08 (densitogram 1, about 20%) while PCT showed negligible degradation with an additional peak at R_f value 0.82 (densitogram 2, about 1.03%) and drug peak area remained almost constant (Figure 7A). The analytes were found to be stable with no additional peaks under dry heat degradation (Figure 7B). PCT and ACF under oxidative and photo degradation produced negligible degradation products

Condition	PCT		ACF		AP		DA	
	%Assay,% RSD	R _f	%Assay , %RSD	R _f	%MR, %RSD	R _f	%MR, %RSD	R _f
Mobile phase Composition (± 0.1ml)	99.51, 0.89	0.69	99.60, 1.26	0.29	99.47, 1.92	0.88	98.92, 1.52	0.15
	100.2, 1.37	0.68	100.54, 1.18	0.28	100.69, 2.36	0.88	99.26, 1.79	0.15
Chamber Saturation Time (± 5min)	100.87, 1.22	0.67	101.18, 0.82	0.29	99.91, 1.93	0.88	100.99, 2.08	0.15
	101.05, 1.00	0.68	101.84, 0.36	0.28	99.73, 2.30	0.89	100.08, 2.13	0.15
Time from spotting to development (± 10 min.)	100.81, 0.91	0.69	100.34, 1.18	0.28	100.27, 1.60	0.88	98.48, 1.82	0.15
	99.33, 0.90	0.70	100.58, 1.09	0.28	99.94, 2.68	0.88	99.39, 2.02	0.15
Time from development To scanning (± 10 min.)	100.31, 1.02	0.69	100.61, 0.97	0.29	99.47, 2.34	0.89	100.82, 1.96	0.15
	101.03, 0.64	0.68	100.06, 1.20	0.28	98.05, 1.72	0.89	99.49, 2.01	0.15

MR-Mean Recovery; n- Number of repetitions

Table 3: Robustness study for the developed method (n=3).

Analyte→ Stress condition ↓	ACF				PCT			
	R _f of Degraded Product	% Degradation	% Recovery	Peak Purity r(S,M), r(S,E)	R _f of Degraded Product	% Degradation	% Recovery	Peak Purity r(S,M), r(S,E)
Acid (0.1 N HCL, reflux 80°C 2 h.)	0.08,0.14,0.29, 0.31	20.5,1.30, 1.41,1.05	71.28	0.9997, 0.9999	0.82, 0.54	9.6,1.14	89.26	0.9997, 0.9998
Base (0.1 N NaOH, R.T. 4 h)	0.08, 0.16, 0.28	28.56, 1.26, 1.81	68.37	0.9997, 0.9996	—	—	97.82	0.9999, 0.9996
Base (0.1 N NaOH, reflux, 80°C, 2 h)	0.08, 0.15	94.28, 5.72	—	0.9996, 0.9998	0.82, 0.46, 0.49	38,1.05, 1.8	59.15	0.9998, 0.9993
Wet Heat (reflux, 80°C, 2 h)	0.08	20.0	80.0	0.9997, 0.9998	0.82	1.03	98.07	0.9999, 0.9989
Dry Heat (80°C, 5 h)	—	—	99.60	0.9997, 0.9995	—	—	100.02	0.9995, 0.9999
Hydrogen peroxide 6% (reflux, 60°C, 2 h)	0.17	1.35	98.65	0.9996, 0.9999	0.30, 0.39, 0.49	1.01, 2.41, 2.10	95.49	0.9997, 0.9995
Photolysis	0.27	1.5	98.5	0.9995, 0.9996	—	—	100.21	0.9996, 0.9994

Table 4: Summary of Forced Degradation Study for ACF and PCT.

(Figure 8A and 8B). Thus, overall, it was found that PCT and ACF were susceptible to acid and base hydrolysis and formed the impurities i.e., AP and DA respectively as their major degradation products.

Conclusion

Monitoring and controlling of impurities assures the quality and safety of a drug. Thus, analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the drug molecule. Therefore an attempt has been made in the proposed research work, to develop stability indicating method for simultaneous estimation of the titled analytes along with their stated impurities. The present research involves quantification of the related substances with vital information about the forced degradation under different stress conditions. The development and validation studies, give satisfactory % mean recovery, lower % RSD and optimum peak purity values; prove that the proposed method can resolve the drug substances, their stated impurities and degradants in

short run time with optimum resolution and desirable sensitivity to be utilized in impurity quantification. The proposed method found robust to minor changes in the experimental conditions and can give accurate and precise results during routine analysis. The method has been successfully applied for the analysis of tablet formulation without any interference from excipients. The stability study reveals that, both drugs are more sensitive towards acid and base hydrolysis producing their related substance as major degradants. The published stability indicating HPLC method for titled analytes has high run time and more LOD and LOQ values indicating lower sensitivity in comparison to the proposed method [23]. Extensive literature revealed that there is stability indicating HPTLC method available for simultaneous estimation of the titled analytes along with one more drug substance, in bulk and pharmaceutical dosage form [26]. Although this method estimate the analytes simultaneously and is sensitive with short run time but is not applicable for simultaneous estimation of stated impurities and hence cannot be utilized in impurity quantification. Thus, the proposed stability indicating HPTLC method is simple, reproducible and economic with the appropriate level of sensitivity. It

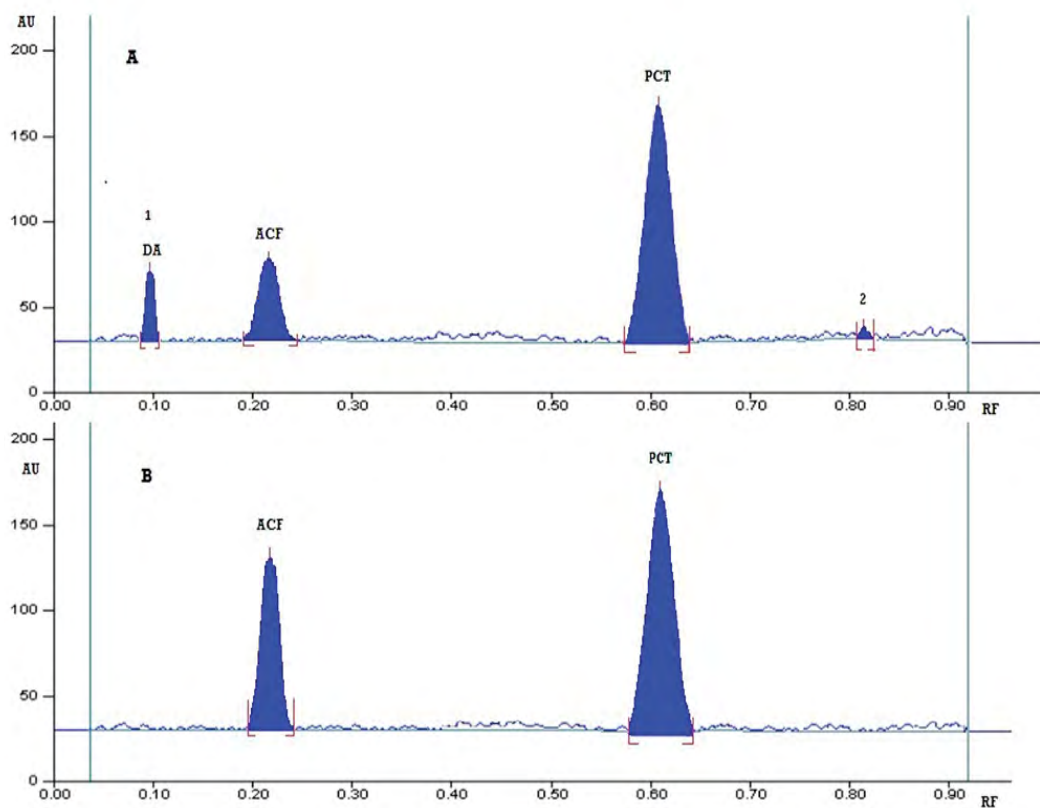


Figure 7: Representative Densitogram showing degradation under thermal condition A: Wet heat B: Dry heat.

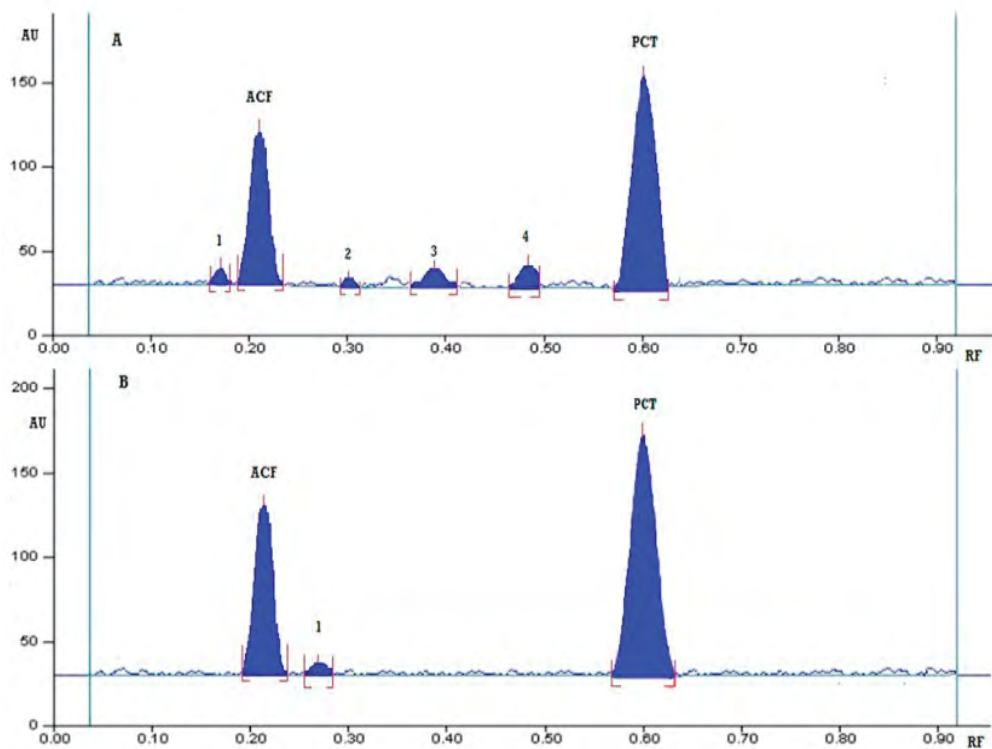


Figure 8: Representative Densitogram showing degradation under A: oxidative degradation, B: photo degradation.

can be utilized for simultaneous determination of PCT, ACF and their stated impurities in impurity estimation. The developed densitometric method is suitable not only for separation and determination of related impurities for monitoring of synthetic reaction but also for stability studies of drug substances and in routine analysis.

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