



**DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD
FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND TIZANIDINE
HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM**

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ABSTRACT

A simple, accurate, precise and selective stability- indicating high performance thin layer chromatographic method has been developed and validated for simultaneous estimation of Aceclofenac and Tizanidine hydrochloride in combined tablet formulation. Chromatographic separation of both the drugs was achieved by using Toluene: Ethyl acetate: Methanol: Triethylamine (5: 3: 1: 0.5, v/v/v/v) as mobile phase with UV detection at 277 nm. The retention factors for Aceclofenac and Tizanidine hydrochloride were found to be 0.30 ± 0.005 and 0.73 ± 0.008 , respectively. Stability of both drugs was accessed by subjecting the drugs to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. The developed method was validated in terms of linearity, accuracy, precision and robustness as per ICH guidelines. Linear response was observed in the concentration range of $1000\text{-}3500 \text{ ng band}^{-1}$ for Aceclofenac and $20\text{-}120 \text{ ng band}^{-1}$ for Tizanidine hydrochloride. The method has been applied successfully for the analysis of drugs in their combined tablet dosage form. The % assay (Mean \pm S.D.) was found to be 99.95 ± 0.3 for Aceclofenac and 100.95 ± 0.8 for Tizanidine hydrochloride. The developed method can be used for the analysis of these drugs without any interference from the excipients and can be successfully used to estimate the amount of drugs in the formulations by easily available low cost materials.

KEYWORDS: Aceclofenac, Tizanidine, HPTLC, Forced degradation, Tablet dosage form.

INTRODUCTION

Aceclofenac (ACF), chemically, 2-(2-(2-(2, 6-dichloroanilino) phenyl) acetyl) oxy-acetic acid is a non-steroidal anti-inflammatory drug used in various pain conditions such as rheumatoid arthritis and osteoarthritis.^[1] Tizanidine hydrochloride (TZH), 5-chloro-N-(4, 5-dihydro-1H-imidazole-2-yl)-2, 1, 3 benzothiadiazol-4-amine is a centrally acting muscle relaxant and an alpha-2-adrenergic agonist that acts mainly at the level of spinal cord.^[2]

Extensive literature review revealed that different analytical methods such as UV spectrophotometry^[3-6], high performance liquid chromatography (HPLC)^[7,8] and high performance thin layer chromatography (HPTLC)^[9] has been reported in the literature for ACF determination in pharmaceutical formulations either as single drug or in combination with other drugs. Analytical methods reported for TZH includes UV^[10-12], HPLC^[13-16] and HPTLC^[17,18] either as single drug or in combination with other drugs. RP-HPLC method of analysis for simultaneous determination of ACF and TZH in pharmaceutical formulation has been also reported.^[19]

To the best of our Knowledge no reports were found for stability indicating high performance thin layer chromatography (HPTLC) method for simultaneous estimation of ACF and TZH in solid dosage form. This paper describes development and validation of simple, precise, accurate stability indicating HPTLC method for determination of Aceclofenac and Tizanidine Hydrochloride in accordance with International Conference on Harmonization Guidelines.^[20-21]

MATERIALS AND METHODS

Chemical and reagents

Pharmaceutical grade working standards ACF and TZH were supplied as gift samples from Geno Pharmaceuticals Ltd. (Mumbai, India). The pharmaceutical dosage form used in this study was Zerodol-MR tablets labeled to contain 100 mg of ACF and 2 mg of TZH were procured from the local market. Toluene, Methanol, Ethyl acetate and Triethylamine (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

In order to achieve the separation of both drugs, Merck TLC plates pre-coated with silica gel 60 F254 (10 cm × 10 cm with 250 µm layer thickness) were utilized as stationary phase. Samples were spotted on the plate as a band with 5 mm width using CAMAG Linomat 5 sample applicator (Switzerland) provided with Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) with the use of toluene: ethyl acetate: methanol: Triethylamine (5: 3: 1: 0.5, v/v/v/v) as mobile phase. Prior to development, the chamber was saturated with mobile phase for a period of 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner III at 277 nm for all developments operated by winCATS software version 1.4.2. Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as radiation source.

Preparation of standard stock solutions

Standard stock solution of ACF was prepared by dissolving 10 mg of drug in 10 mL of methanol to get solution of concentration 1000 µg mL⁻¹. For TZH, 2 mg of drug was dissolved in 10 mL of methanol to obtain the solution having concentration 20 µg mL⁻¹.

Selection of detection wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 277 nm. So, 277 nm was selected as the wavelength for detection.

Tablet formulation analysis

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 100 mg of ACF (2 mg TZH) was transferred to a 10 mL volumetric flask containing about 6 mL of methanol. The contents were sonicated for 15 min and filtered and volume was made upto the mark with methanol to have final concentration 1000 µg mL⁻¹ for ACF and 20 µg mL⁻¹ for TZH. Different volumes (1.5 µL of ACF and 2 µL of TZH) were applied by overspotting on TLC plate to obtain final sample concentration of 1500 ng band⁻¹ for ACF and 40 ng band⁻¹ for TZH. Peak areas of the bands were measured at 277 nm and the amount of each drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for

the analysis of homogenous sample. The % drug content after analysis of tablet formulation was found to be 99.95 and 100.95 for ACF and TZH, respectively.

Stress degradation studies of bulk drugs

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method.

The stability studies were performed by subjecting the bulk drugs individually to the physical stress (acid, base, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 1000 µg mL⁻¹ of ACF and 20 µg mL⁻¹ of TZH in methanol. The hydrolytic studies were carried out by mixing the drug solutions of ACF and TZH with 0.1 N HCl and 0.1 N NaOH and were kept separately at room temperature for 4 h and 2 h, respectively. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 3000 ng band⁻¹ and 100 ng band⁻¹ of ACF and TZH, respectively. Neutral hydrolysis study was performed by treating the drugs separately with water and the resulting solutions were kept at room temperature for 3 h. The oxidative degradation was carried out in 3% H₂O₂ and the sample was diluted with methanol to obtain solution having concentration 3000 ng band⁻¹ and 100 ng band⁻¹ of ACF and TZH, respectively. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 60°C for a period of 2 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square meter⁻¹ for 3 d. Thermal and photolytic samples were diluted with methanol to get the concentration of 1000 ng band⁻¹ and 20 ng band⁻¹ ACF and TZH, respectively.

RESULTS AND DISCUSSION**Method optimization**

The major objective in developing this stability indicating HPTLC method is to achieve the resolution of ACF and TZH and its degradation products. The separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Toluene: Ethyl acetate: Methanol: Triethylamine (5: 3: 1: 0.5, v/v/v/v) as mobile phase and detection was carried out at 277 nm. The retention factors were found to be 0.30±0.03 and 0.73±0.02 for ACF and TZH, respectively. Representative densitogram of mixed standard solution of ACF and TZH is shown in Figure 1.

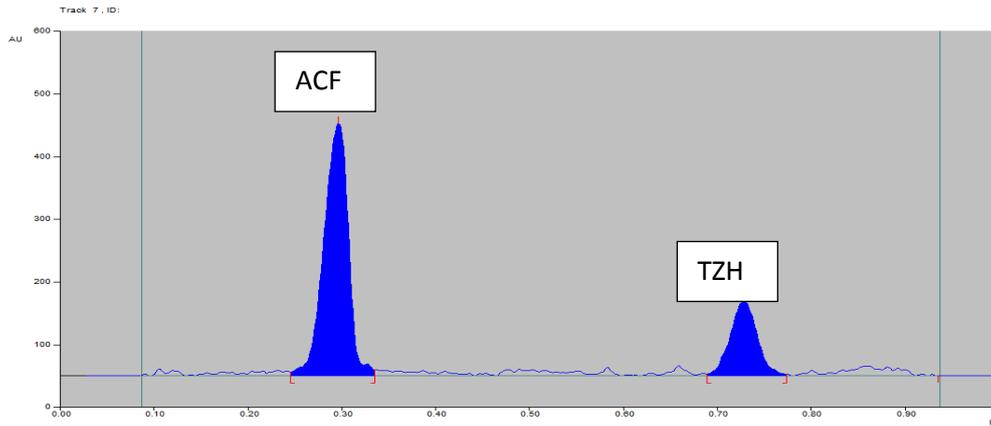


Fig. 1: Representative densitogram of mixed standard solution of ACF (Rf 0.30 ± 0.03) and TZH (Rf 0.73 ± 0.02) Forced degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative, thermal and photolytic stress conditions. Marked degradation was observed without appearance of degradation peaks but there was reduction in the peak

areas of both drugs after degradation. Figures 2 and 3 shows the densitograms of acid hydrolysis and oxidative degradation, while Figures 4 and 5 shows the densitograms of thermal and photolytic degradation, respectively.

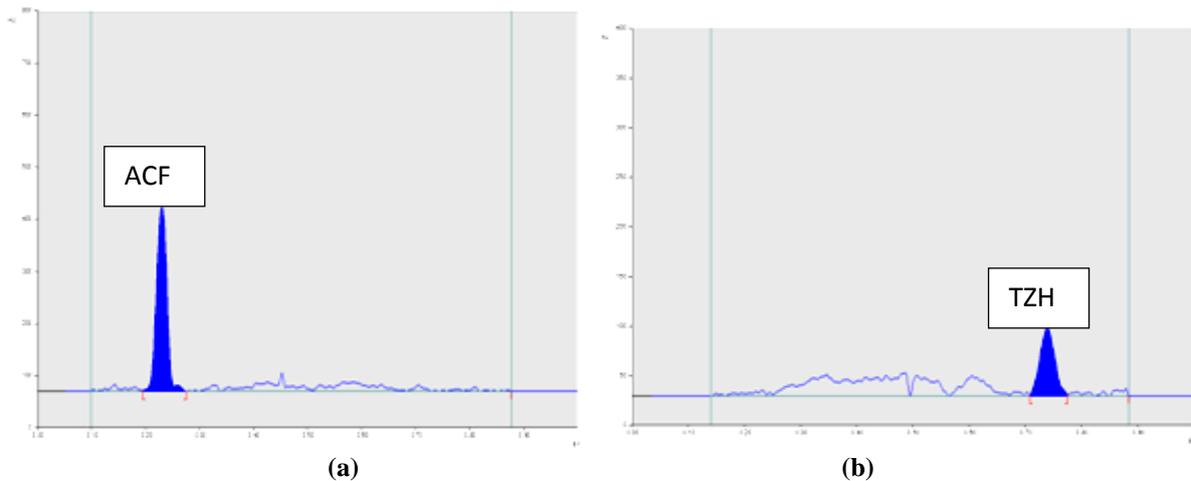


Fig. 2: Representative densitogram of acid treated (a) ACF and (b) TZH (0.1 N HCl, Kept at RT for 4 h)

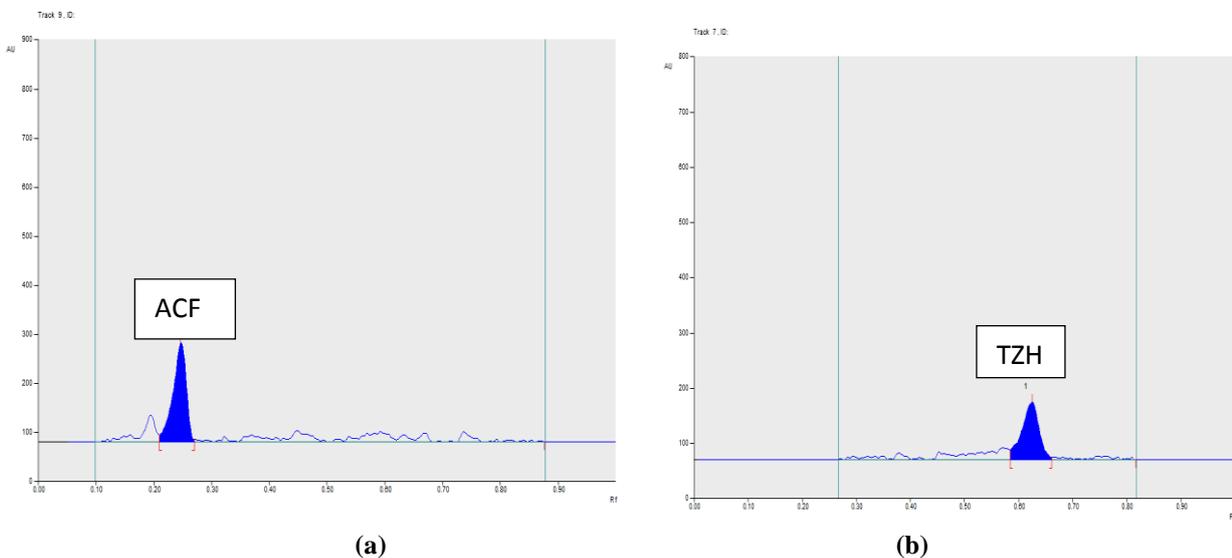


Fig. 3: Representative densitogram of (a) ACF and (b) TZH after peroxide treatment 3 % H₂O₂, Kept at RT for 2 h)

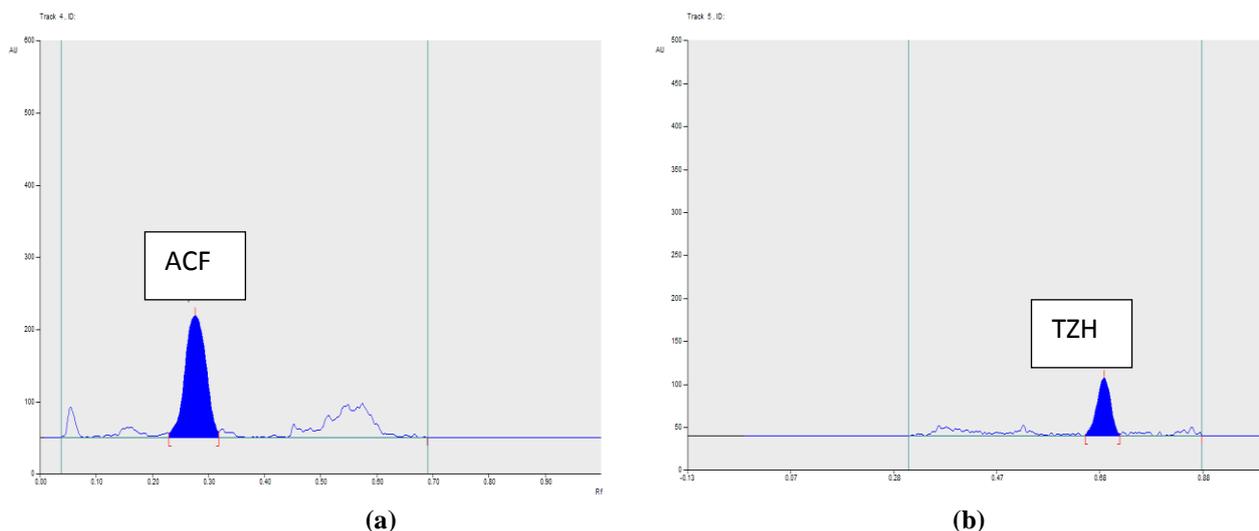


Fig. 4: Densitogram for (a) ACF and (b) TZH after thermal stress (60°C, 2 h)

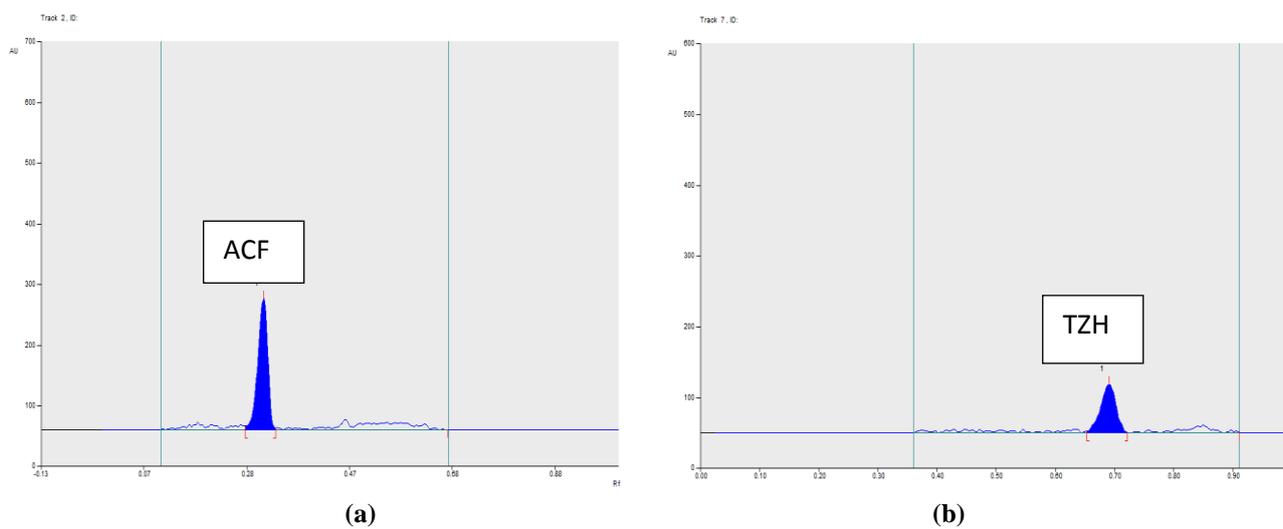


Fig. 5: Densitogram of (a) ACF and (b) TZH after photolysis

Peak purity results greater than 991 indicate that peaks for both drugs are homogeneous in all stress conditions tested. The unaffected assay of tablet formulation

confirmed the stability indicating power of the method. The findings of degradation studies are represented in Table 1.

Table 1: Data of forced degradation studies of ACF and TZH

Stress conditions/ duration	ACF		TZH	
	% Assay of active substance	% degradation	% Assay of active substance	% degradation
Acid/0.1 N HCl/ Kept at RT for 4 h	70.77	29.23	76.58	23.42
Alkali /1 N NaOH/ Kept at RT for 2 h	74.98	25.02	79.64	20.36
Oxidative /3 % H ₂ O ₂ / at RT/ Kept at RT for 2 h	65.19	34.81	77.58	22.42
Dry heat/ 60°C/ 2 h	70.73	29.27	72.13	27.87
Photolysis	76.97	23.03	78.54	21.46
Neutral/ H ₂ O/3 h	88.89	11.11	87.89	12.11

Method Validation

The method was validated with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines

Preparation of Calibration Curve

Aliquots of 1, 2, 3, 4, 5 and 6 μL of the standard stock solutions of ACF and TZH ($1000 \text{ ng } \mu\text{L}^{-1}$ and $20 \text{ ng } \mu\text{L}^{-1}$) were applied by overspotting on TLC plate to obtain the concentration in the range $1000\text{-}3500 \text{ ng band}^{-1}$ for ACF and $20\text{-}120 \text{ ng band}^{-1}$ for TZH, respectively. Straight-line

calibration graphs were obtained in the concentration range indicated above. The values of correlation coefficient close to unity also proved linearity of developed method.

Precision

Set of three different concentrations in three replicates of standard solutions of ACF and TZH were prepared. All the solutions were analyzed on the same day in order to

record any intra-day variations in the results. Intra-day variation, as R.S.D. (%), was found to be in the range of 0.30 to 0.92 for ACF and 1.08 to 1.28 for TZH. For Inter-day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Inter-day variation, as R.S.D. (%) was found to be in the range of 0.20 to 1.40 for ACF and 0.19 to 0.65 for TZH. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Table 2: Intra-day precision

Drug	Spotted concentration (ng band ⁻¹)	Mean area	S.D.	% R.S.D.*
ACF	1000	2253.43	6.98	0.30
	1500	3689.53	2.40	0.06
	2000	4589.33	42.57	0.92
TZH	20	828.6	8.96	1.08
	40	1040.2	13.37	1.28
	60	1166.6	13.74	1.17

*Average of three determinations.

Table 3: Inter-day precision

Drug	Spotted concentration (ng band ⁻¹)	Mean area	S.D.	% R.S.D.*
ACF	1000	2444.2	35.30	1.40
	1500	3562.4	9.05	0.25
	2000	4532.6	9.07	0.20
TZH	20	829.31	4.35	0.52
	40	1038.33	2.05	0.19
	60	1156.93	7.61	0.65

*Average of three determinations.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated as $3.3 \sigma/S$ and $10 \sigma/S$, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 226.59 ng band⁻¹ and 686.65 ng band⁻¹ for ACF and 8.36 ng band⁻¹ and 15.51 ng band⁻¹ for TZH, respectively.

sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 1500 ng band⁻¹ for ACF and 40 ng band⁻¹ for TZH from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drugs in tablet dosage form. The results obtained are shown in Table 4.

Recovery studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to pre-analyzed

Table 4: Recovery studies of ACF and TZH

Drug	Amount taken (ng band ⁻¹)	Amount added (ng band ⁻¹)	Total amount found (ng band ⁻¹)	% Recovery	% R.S.D.*
ACF	1500	1200	2719.6	100.78	1.06
	1500	1500	3010.6	100.35	0.93
	1500	1800	3371.0	99.17	0.43
TZH	40	32	72.20	100.33	0.64
	40	40	79.77	99.72	0.43
	40	48	87.72	99.73	0.49

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were

found to be more than 991, indicating the no interference of any other peak of the degradation product, impurity or matrix.

Robustness studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, time from development to scanning was altered and the effect on the area of the drug was noted. When deliberate variations were made to the method conditions, there were no marked changes in chromatographic behavior, indicating the method is robust.

CONCLUSION

Stability indicating HPTLC method has been developed and validated for the simultaneous determination of ACF and TZH as the bulk drugs and in combined tablet dosage form. The developed method is simple, precise, selective and accurate can be used for quantitative analysis of ACF and TZH in the pharmaceutical dosage form as well as for routine analysis in quality control laboratories.

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