



## DEVELOPMENT OF VALIDATED STABILITY INDICATING HPTLC METHOD FOR ACECLOFENAC IN BULK DRUG AND FORMULATIONS

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### ABSTRACT

A sensitive, selective, precise and stability-indicating high performance thin layer chromatography method for analysis of Aceclofenac both as a bulk drug and in formulations was developed and validated. The method employed thin layer chromatography aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of Toluene- Ethyl Acetate - Glacial Acetic Acid (6:4:0.02%V/V). This system was found to give compact spots for Aceclofenac ( $R_f$  value of  $0.26 \pm 0.02$ ). Aceclofenac was subjected to acid degradation. Also, the degraded product was well resolved from the pure drug with significantly different  $R_f$  value ( $0.47 \pm 0.02$ ). The method was validated for linearity, precision, limit of detection, limit of quantitation, accuracy, specificity and sensitivity as per ICH guidelines. Linearity was found to be in the range of 0.5-4 $\mu$ g/spot with a correlation coefficient of 0.9906. The slope and intercept values were found to be 2.046 and 22.65 respectively. The limits of detection and quantitation were found to be 100 and 500ng/spot respectively. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one.

**Keywords:** Aceclofenac, HPTLC, Stress degradation, Method validation.

### INTRODUCTION

Aceclofenac (ACE), [2-[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid, is a non-steroidal anti-inflammatory (NSAID) drug<sup>1</sup>. It is a white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in alcohol. Aceclofenac has higher anti-inflammatory action than conventional NSAIDs. It is a cytokine inhibitor. Aceclofenac works by blocking the action of a substance in the body called cyclo-oxygenase. It has low potential for causing gastroduodenal mucosal damage in humans than diclofenac sodium<sup>2</sup>.

Literature survey reported<sup>3,4</sup> accelerated stability testing of Aceclofenac by UV spectrophotometric method. Consequently, the implementation of an analytic methodology to determine Aceclofenac in pharmaceutical dosage form in presence of its degradation products is a pending challenge of the pharmaceutical analysis. To our knowledge, no article related to the stability indicating high-performance thin-layer chromatography (HPTLC) determination of Aceclofenac in pharmaceutical dosage forms has ever been mentioned in literature. The international conference on harmonization (ICH) guideline entitled *Stability Testing of New Drug Substances and Products* requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. The aim of the present work is to develop an

accurate, specific, repeatable and stability-indicating HPTLC method for the determination of Aceclofenac in presence of its degradation products through stress studies and related impurities for assessment of purity of bulk drug and formulations. The proposed method was validated as per ICH guidelines and its updated international convention.

### MATERIALS AND METHODS

An analytically pure gift sample of Aceclofenac from Lessac Research, Pondicherry, India, was used as working standard. Tablets (Lametec) 100 mg were obtained from the market (manufactured by Torrent pharmaceuticals, India). Toluene of LR grade, ethyl acetate and glacial acetic acid of AR grade (S.D.Fine Chemicals Ltd.) to prepare the mobile phase were used without further purification. Hydrochloric acid (Himedia Laboratories Pvt. Ltd, India), Hydrogen peroxide and Sodium hydroxide (Rankem Laboratories, India) were used.

#### Instrumentation

Camag (Muttens, Switzerland) Linomat V applicator, a Camag twin trough TLC chamber, a Camag TLC scanner 3, Camag Wincats software and Hamilton (Reno, Nevada, USA) Syringe (100 $\mu$ L). Electronic balance (Make SHIMADZU Model AY-120) was used for weighing purpose.

#### Selection of Detection Wavelength

Wavelength selected was 282nm. At 282nm, Aceclofenac show high absorbance.



### Preparation of Standard stock solution

Stock solution of Aceclofenac (1000 µg/mL) was prepared in methanol. Sample was applied on pre-coated silica gel G plate. This plate was developed in development chamber using selected mobile phase.

### Chromatographic conditions

Solution of Aceclofenac was applied to a precoated silica gel 60F<sub>254</sub> HPTLC plate by means of Linomat 5 sample applicator equipped with a 100 µL syringe and operated with settings of band width, 6 mm; slit dimension, 5×0.45 mm. The plate was developed in a twin- trough chamber previously saturated for 20 minutes with the mobile phase, toluene, ethyl acetate and glacial acetic acid (6:4:0.2% v/v/v), for a distance of 8 cm. The bands were air dried and were scanned with the scanner 3 at 282 nm using the deuterium source.

### Stress degradation studies<sup>5</sup>

#### Preparation of acid induced degradation products

10mg of Aceclofenac was weighed accurately and transferred into three different 10ml standard flasks. To this 5ml of 1N, 0.5N, 0.1N Hydrochloric acid was added separately and made up to 10ml with methanol. This solution was refluxed for 30 minutes at 80°C. The samples were analysed by HPTLC.

#### Preparation of base induced degradation products

10mg of Aceclofenac was weighed accurately and transferred into three different 10ml standard flasks. To this 5ml of 1N, 0.1 N, 0.01N NaOH was added separately and made upto 10ml with methanol. This solution was refluxed for 30 minutes at 80°C. The samples were analysed by HPTLC for any degradation.

#### Preparation of hydrogen peroxide induced degradation products

10mg of Aceclofenac was dissolved in methanol. To this 5ml of 30% H<sub>2</sub>O<sub>2</sub> was added and made upto 10ml with methanol and kept for 24 hours at room temperature. The solution was then analyzed for degradation using HPTLC.

#### Preparation of thermal degradation products

10mg of Aceclofenac was weighed and transferred to a petridish. It was then placed in hot air oven at 80°C for 8 hours. Then the drug was dissolved in methanol and made up to the 10ml with methanol. The solution was analysed for degradation using HPTLC.

#### Preparation of photo chemical degradation products

The study was carried out by exposing the drug to sunlight for about 8 hours. The drug solution was prepared using methanol and was analysed by HPTLC.

### Analysis of marketed formulation

20 tablets, each containing 100mg of Aceclofenac were weighed, average weight was calculated. Quantity

equivalent to 10mg was transferred to 10ml volumetric flask, extracted with methanol, finally made up to volume with the same. This solution was filtered through Whatmann filter paper and suitable aliquots of formulation solution were prepared and the solution was analyzed using the developed method.

### Validation of the HPTLC method<sup>6-7</sup>

#### Linearity

Aliquots of acid stressed sample of Aceclofenac solution (500-4000ng/spot) were applied on a precoated silica gel 60 F<sub>254</sub> aluminium sheet. It was developed using the mobile phase of toluene, ethyl acetate and glacial acetic acid (6:4:0.02%v/v). The TLC plate was dried and chromatograms were recorded at 282nm. The peak area was plotted against drug concentration. The linear graph showed a good linear relationship over a concentration range of 0.5-4µg/spot for Aceclofenac. The slope, intercept and correlation coefficient values were found to be 2.046, 22.65 and 0.9906.

#### Specificity

The specificity of the HPTLC method was determined by complete separation of Aceclofenac in the presence of its degradation products. The mobile phase toluene, ethyl acetate and glacial acetic acid (6: 4: 0.02% v/v) designed for the method resolved for Aceclofenac along with its degradation products very efficiently in 0.1 N HCl.

#### Sensitivity

The sensitivity of measurement of Aceclofenac by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and the lowest concentration detected under the chromatographic conditions as the Limit of Detection (LOD). The LOQ and LOD were calculated by the use of the equation  $LOD = 3 \times N/B$  and  $LOQ = 10 \times N/B$ , where N is standard deviation of the peak areas of the drugs taken as a measure of noise, and B is the slope of the corresponding calibration curve.

#### Precision

Precision studies were performed using standard solutions containing Aceclofenac. Intraday precision was found out by carrying out the analysis of the standard drug at two different concentrations in the linearity range of drug for three times on the same day. Each concentration was applied in triplicate and % RSD was calculated. Interday precision was found out by carrying out the analysis of the standard drug at two different concentrations in the linearity range of drugs for three days and % RSD was calculated.

#### Recovery studies

Recovery studies of the degraded sample were carried out for the accuracy parameter. For that, to 10 mg of stressed sample, 5mg and 10mg of pure drug were added and shaken well, aliquots were spotted six times on a precoated silica gel 60 F<sub>254</sub> Aluminium sheets.



**Stability**

Stability of Aceclofenac on chromatographic plate was studied at different time intervals and peak areas were compared with the peak area of freshly scanned plate. A 10 % reduction in peak area was considered as loss of stability.

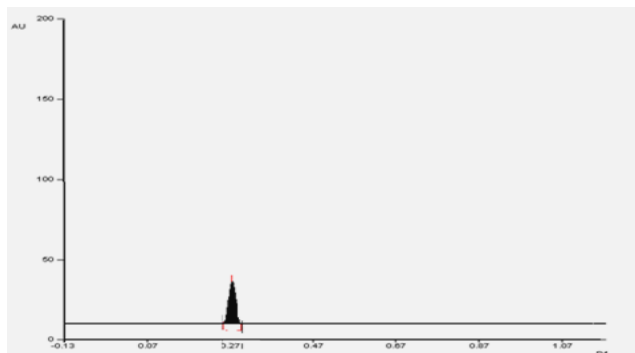
**RESULTS AND DISCUSSION**

**Development of the optimum mobile phase**

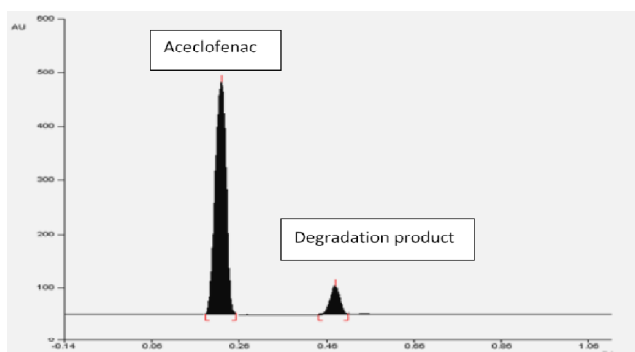
TLC procedure was optimized with a view to develop a stability-indicating assay method. The drug reference standards were spotted on the TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve Aceclofenac. Best suited mobile phase was found to be Toluene, Ethyl Acetate and Glacial Acetic Acid (6:4:0.2% v/v/v), for a distance of 8 cm. Developed mobile phase resulted in resolution for Aceclofenac with Rf 0.26 ± 0.02. Well defined spots were obtained when the chamber was saturated with the mobile phase at room temperature. The representative densitogram is given in Figure1.

**Validation of the developed stability-indicating method**

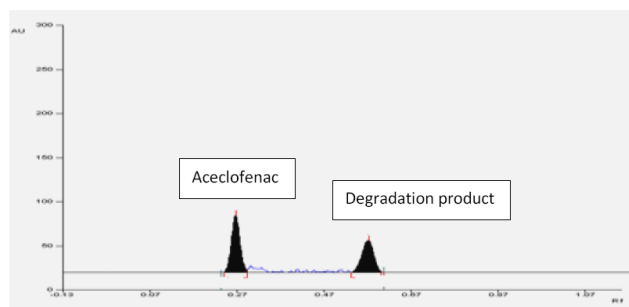
The response for the drug was found to be linear in the concentration range (500-4000ng/spot). The RSD value for precision study was found to be not more than 2%, thus confirming precision of the method. The accuracy of the method was determined at 50 and 100% level. The Limit of Detection was found to be 100 ng/band and Limit of Quantitation was found to be 500ng/band. The developed plate was found to be stable for about 24 hrs for Aceclofenac.



**Figure 1:** Representative Densitogram of Aceclofenac at 282nm.



**Figure 2:** Representative Densitogram showing degradation of Aceclofenac under acidic condition.



**Figure 3:** Representative Densitogram showing degradation of Aceclofenac formulation under acidic condition.

**Table 1:** Validation Summary

Validation parameters	Aceclofenac
Linearity	0.9906
Precision	NMT 2%
Accuracy	
50 %	99.8
100 %	100.5
LOD	100 ng
LOQ	500 ng
Specificity	Specific

**Degradation observed**

Major degradation of Aceclofenac was observed under acidic hydrolysis condition. Aceclofenac was completely degraded under alkaline conditions. No degradation was found for Hydrogen peroxide, Dry heat and Photolytic conditions.

**Table 2:** Analysis of formulation

Drug Brands	Amount (mg/tablet)		% Label claim	% RSD
	Labeled	Estimated		
ACEBLOC	100	101.4	101.4	0.9870
HIFENAC	100	100.25	100.25	0.9951
TOROXX-A	100	99.82	99.82	0.9850

**CONCLUSION**

The proposed HPTLC method provides a simple, accurate, reproducible and stability- indicating method for the determination of Aceclofenac in pharmaceutical tablets and in the presence of its acidic degradation products. The developed method was applied to assay the formulations. It was found that Aceclofenac was rapidly degraded in alkaline medium, while it is more stable in acidic medium. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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