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Development of Validated Stability Indicating HPTLC Method and Its Application to the Assay of Formulation and Accelerated Stability Studies of Aceclofenac

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ABSTRACT

A simple validated high performance thin layer chromatographic method was developed for the determination of Aceclofenac in presence of its degradant. Separation of Aceclofenac from the degradant could be achieved using aluminium backed silica gel 60 F_{254} plate with toluene: ethyl acetate: glacial acetic acid, (6:4:0.02v/v) as mobile phase. Densitometry analysis was carried out at 282 nm. The method showed high sensitivity with good linearity over the concentration range of 0.5 – 4 µg/spot. The method was successfully applied to the analysis of pharmaceutical formulation containing Aceclofenac with excellent recovery. The LOD and LOQ were found to be 0.1 and 0.5 µg/spot. Aceclofenac was subjected to hydrolytic, oxidative, thermal and photolytic degradation. It was found that the drug was highly susceptible to acid hydrolysis. Kinetic investigation of the drug followed pseudo-first order reaction. From the Arrhenius plot the activation energy was found to be 13.19 kcal/mole. Statistical analysis revealed that the developed method is accurate and reliable. Hence it can be used for routine quality control analysis of Aceclofenac in tablet formulation.

Keywords: Aceclofenac; HPTLC; degradation kinetics.

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INTRODUCTION

Aceclofenac[1] [[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy acetic acid] is a white or crystalline powder, soluble in alcohol and acetone, sparingly soluble in water. Aceclofenac is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenyl acetic acid with pronounced anti-inflammatory, analgesic and antipyretic properties. It has good tolerability profile in variety of painful conditions like rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Literature survey reveals simple UV spectrophotometric[2,3], HPLC and HPTLC methods[4-8] reported for Aceclofenac in plasma as well as formulations. However, high performance thin layer chromatographic technique was found for Aceclofenac determination in bulk and formulations. The International Conference on Harmonization (ICH) guideline entitled "*Stability testing of new drug substances and products*" requires testing to be conducted to assess the inherent stability of the active substances[9]. An ideal stability indicating method is one that quantifies the drug and resolves its degradation product. Unlike HPLC, HPTLC technique has several advantages[10] which include possibility of analysis of several samples with small amount of mobile phase. This reduces analysis time and cost per analysis. The objective of this work was to develop stability indicating HPTLC method for determination of Aceclofenac in the presence of its degradation products and application to accelerated stability studies.

MATERIALS AND METHODS

Materials

Aceclofenac powder was kindly supplied from Lessac Research, Pondicherry. It purification was checked in our laboratory according to the reported method of analysis and found to be 99.18 - 100.10%. Tablets were purchased from the local market. Each tablet is claimed to contain 100mg of Aceclofenac. All chemicals used were of analytical grade. Hydrchloric acid,sodium hydroxide, hydrogen peroxide, toluene, methanol, ethyl acetate, and glacial acetic acid were obtained from S.D. Fine Chemicals Ltd., India. Pre-coated plates**:** Silica gel 60 GF_{254} on Aluminium sheets were procured from Merck, Germany.

Instrumentation and Chromatographic conditions

Shimadzu digital electronic balance, Camag HPTLC system (with TLC scanner, Wincats sofware and Linomat -V as application device), hot air oven, sonicator. Chromatography was performed on 10 × 10 cm aluminium backed plates coated with 0.2 mm layers of silica gel 60 GF_{254} (Merck, Germany). Samples were applied to the plates as bands 6 mm wide and 14 mm apart by use of a Camag (Muttenz, Switzerland) Linomat V applicator fitted with a Camag

microliter syringe. The rate of sample application was constant at 0.5 - 4 µg/ spot. Linear ascending developments of the plates to a distance of 80 mm was performed with toluene: ethyl acetate: glacial acetic acid (6:4:0.02%v/v) as mobile phase in a twin trough glass chamber previously saturated with mobile phase vapour for 20 min at room temperature. After development the plate was scanned 282 nm by means of a Camag TLC scanner in absorbance mode, using the deuterium lamp. The slit dimensions were 5 × 0.45 mm and the scanning speed was 20 mm/s.

Forced degradation of Aceclofenac

Preparation of acid induced degradation of Aceclofenac:

10mg of Aceclofenac was weighed accurately and transferred into three different 10ml standard flasks. To this 5ml of 1N, 0.5N, 0.1N HCl was added separately and made up to 10ml with methanol. This solution was refluxed for 30 minutes at 80^0C. The samples were analyzed by HPTLC.

Preparation of base induced degradation of Aceclofenac:

10mg of Aceclofenac was weighed accurately and transferred into three different 10ml standard flasks. To this 5ml of 1N, 0.5N, 0.1N NaOH was added separately and made up to 10ml with methanol. This solution was refluxed for 30 minutes at 80^0C. The samples were analyzed by HPTLC.

Preparation of H_2O_2 induced degradation of Aceclofenac:

10mg of Aceclofenac was dissolve in methanol. To this 5ml of 30% H_2O_2 was added and made upto 10ml with methanol and kept for 24 hrs at room temperature. The solution was analyzed by HPTLC.

Preparation of thermal induced degradation of Aceclofenac:

10mg of Aceclofenac was weighed and transferred to a petridish. It was then placed in hot air oven at 80^0C for 8 hrs then dissolved and made upto the 10 ml with methanol. The solution was analysed by HPTLC.

Preparation of photo chemical induced degradation of Aceclofenac:

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

Method validation[11]

Linearity

Aliquots of acid stressed sample of Aceclofenac solution were applied on a pre-coated silica gel 60 F_{254} aluminum sheet. It was developed using the mobile phase toluene: ethyl acetate: glacial

acetic acid (6:4:0.02%v/v). The TLC plate was dried and chromatograms were recorded at 282 nm. The peak area was plotted against drug concentration.

Precision

In accordance with ICH recommendations precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability of sample application was determined as intra-day variation whereas intermediate precision was determined by measuring inter-day variation for triplicate analysis of Aceclofenac at two different concentrations (5 and 7µg/spot).

Limit of detection and limit of quantification

The LOD and LOQ of the drug were determined by applying progressively lower concentration of standard solution on the plate, dried, developed and scanned. The lowest concentration at which the peak is detected is called the 'limit of detection (S/N=3).

Recovery

Recovery studies of the degraded sample were carried out for the accuracy parameter. For that 10 mg standard Aceclofenac was added to the degraded sample of 10 mg solution. From this, 0.5 ml was diluted to 10ml and applied on the pre-coated TLC plate. From this 50 and 100% recovery were calculated.

Assay of formulation:

Twenty tablets, each containing 100mg of Aceclofenac were weighed, average weight was calculated for all the three brands selected. 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 solutions were analyzed using the developed method.

Kinetic studies[12]

For studying the order of reaction

10mg of Aceclofenac was dissolved in methanol in a 10ml volumetric flask, and 5ml of 0.1N hydrochloric acid was added and made up to the volume with methanol. This solution was then transferred into a clean round bottom flask and refluxed at 80^0C for half an hour. The degraded solution and standard solution of Aceclofenac were applied on HPTLC plates. Plates were placed in chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: glacial acetic acid (6:4:0.2%v/v) and then air dried. Chromatograms were recorded. The concentration of Aceclofenac was calculated from the regression equation. Log % of Aceclofenac remaining against time.

For studying the effect of hydrochloric acid concentration on the reaction rate

Into a series of 10ml volumetric flasks, 10mg of Aceclofenac was dissolved in methanol and 5ml of 0.01, 0.05 and 0.1N hydrochloric acid was added and made up to the volume with methanol. These solutions were transferred into a clean round bottom flask and refluxed at 80^0C for half an hour. The degraded solution and standard solution of Aceclofenac were applied on HPTLC plate. The plate was placed in a chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: glacial acetic acid (6:4:0.02%v/v) and then dried in the air. The chromatograms were recorded. The log of % of Aceclofenac remaining was plotted against time for different normalities of hydrochloric acid and the rate constant and half-life were calculated.

For studying the effect of temperature on the reaction rate

10mg of Aceclofenac was taken in three different 10ml volumetric flasks, dissolved in methanol and 5ml of 0.01, 0.05 and0.1N hydrochloric acid was added separately and the volume was made up to the mark with methanol. These solutions were transferred into clean dried round bottom flasks and then refluxed at 60°C, 70°C, 80°C and 90°C for half an hour. The stressed sample solutions and standard solution of Aceclofenac were applied on HPTLC plate. The plate was placed in a chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: glacial acetic acid (6:4:0.02%v/v) and then air dried. The chromatogram was recorded. The log of % of Aceclofenac remaining was plotted against time at different temperatures. The Arrhenius plot was also constructed for the effect of temperature on the rate of hydrolysis. The effect of temperature was studied by conducting the reaction at different temperatures using different concentrations of the acidic solution.

RESULTS AND DISCUSSION

Chromatographic conditions

A stability indicating HPTLC method for the analysis of Aceclofenac in bulk drug and formulations were developed. The solvent system that was fixed for HPTLC includes toluene: ethyl acetate: glacial acetic acid (6:4:0.02%v/v) which resulted in dense, compact spot with R_f value 0.26±0.02. The study was carried out using silica gel 60 GF_{254} aluminum sheet and Aceclofenac was detected at 282 nm.

Forced degradation studies

ICH guidelines recommend 10-20% degradation for establishing stability indicating nature of the assay method while Singh and Bakshi, in their article on stress testing suggested a target

degradation of 20-80%[13]. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 10-80%, this could not be achieved in some cases even after exposure for prolonged duration (12 hours). Figure 1 show normal chromatogram of standard Aceclofenac while figure 2 & 3 show the chromatogram of forced degraded samples. The degradation study indicated that Aceclofenac was susceptible to alkaline hydrolysis more than acid and neutral hydrolysis and was stable to H_2O_2, thermal and direct sunlight. Specificity of the method for Aceclofenac in presence of its degradant was demonstrated by the absence of co-eluting peaks with the main peaks.

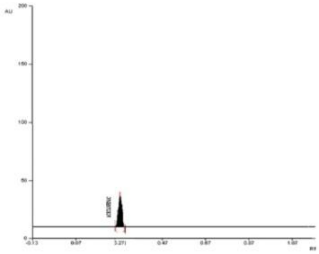

Figure 1: Chromatogram of standard Aceclofenac

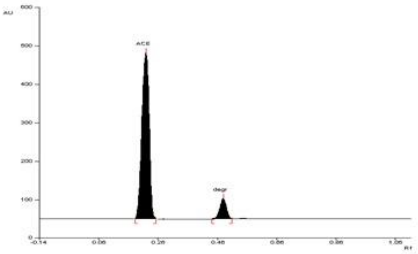

Figure 2: Chromatogram of acid degraded Aceclofenac

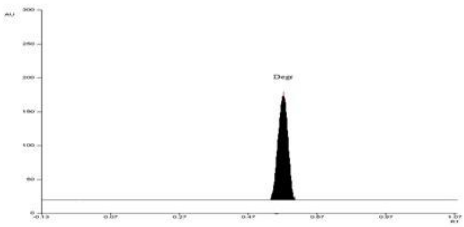

Figure 3:Chromatogram of base degraded Aceclofenac

The chromatogram of acid degraded sample of Aceclofenac showed two peaks one at R_f 0.26 ± 0.02 and other additional peak at R_f 0.47 ± 0.02. The peak at R_f 0.26 ± 0.02 corresponds to Aceclofenac and peak at R_f 0.47 ± 0.02 was that of degradant. The chromatogram of base degraded sample of Aceclofenac showed only a single peak which corresponds to that of the degradant (R_f 0.47 ± 0.02). Here a complete degradation of Aceclofenac was observed figure 3. The chromatogram of hydrogen peroxide, photo and thermal degradation, stress sample of Aceclofenac showed only a single peak corresponds to Aceclofenac (R_f 0.26 ± 0.02). Peak purity

of Aceclofenac as well as degradation product was found to be 1, which confirms the specificity of the method.

Validation

The described method has been validated for linearity, LOD, LOQ, accuracy and precision[11]. The standard solutions for linearity were prepared at eight different concentration levels. The calibration curve for Aceclofenac was found to be linear over the range of 0.5-4 µg/spot, in presence of degradant. The slope, intercept and correlation coefficient values were found to be 2.046, 22.65 and 0.9906, respectively. The low % RSD values of within a day, day to day variations, repeatability of measurement and application for Aceclofenac revealed that the proposed method is precise (Table 1). Accuracy of the method was carried out by recovery studies using standard addition method at two different concentration levels and the results are presented in table 2.

Table 1: Results of precision studies

Drug	Concentration (µg/spot)		Precision	%RSD*
Aceclofenac	5	7	Intra-day	0.8593, 0.2774
			Inter-day	0.8689, 0.8730
			Repeatability of measurement	0.7824, 0.2410
			Repeatability of application	0.5035, 0.2179

*mean of six determination

Table 2: Results of recovery study

Level	% Recovery	% RSD*
50%	99.8	0.1348
100%	100.5	0.2749

*mean of six determination

Assay of Aceclofenac from its tablet dosage forms

The assay results of Aceclofenac in tablet dosage forms were comparable with the values of labeled claim. The results presented in table 3 indicate the suitability of the method for routine analysis of Aceclofenac from its tablet dosage form.

Table 3: Results of assay of marked formulations

Brands	Amount(mg/tab)		%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

*mean of six determination

Kinetic of the degradation

Linear relationship was obtained by plotting the log concentrations of the Aceclofenac remaining against time (Figure 4). K value was obtained by graphical method and it was found to be 0.6080 min^{-1}**.** The effect of different parameters on the rate of degradation of Aceclofenac was carried out. At each temperature, the rate constant and half-life were calculated. The log of the rate constant was plotted against the reciprocal of the temperature in Kelvin units (Arrhenius plot - Figure 5) to demonstrate the effect of temperature on the rate constant. Also, the energy of activation was determined by calculating the rate constants from the equation: $Log\ k_2/k_1 = E_a/2.303R\ (T_2-T_1)/\ T_2T_1$. Where, k1 and k_2 - Rate constants at two different temperatures, Ea - Energy of Activation, R - Gas constant (1.987 kcal $degree^{-1}mole^{-1}$), T_1 and T_2 - Temperature at 60^0C and 90^0C**.** The calculated "E_a" was found to be 13.19 kcal/mol. Another factor studied was the effect of strength of HCl on the degradation rate of Aceclofenac. The rate of hydrolysis was found to increase with increasing concentration of hydrochloric acid, but the effect was less compared to temperature effect.

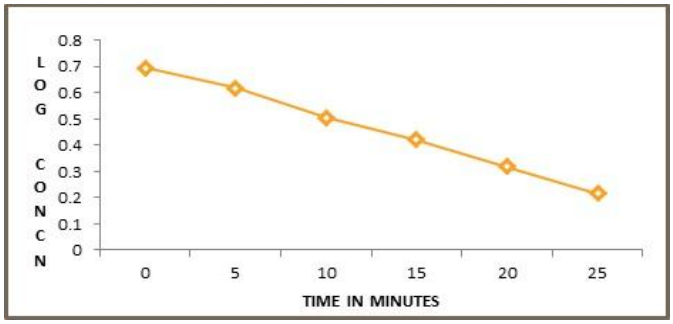

Figure 4: First order plot of the hydrolysis of Aceclofenac with 0.1 N HCl at 80^0C

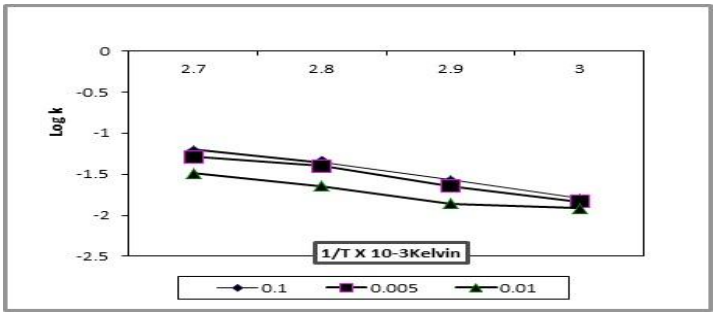

Figure 5: Arrhenius plot for the hydrolysis of Aceclofenac with 0.1, 0.05, 0.01N HCl

CONCLUSION

A sensitive, selective, precise and stability indicating HPTLC method was developed and validated for analysis of Aceclofenac both as bulk drug and in formulation. The method was also applied to accelerated stability studies of Aceclofenac. From the study it was observed that

temperature had a major effect over the acid hydrolysis of Aceclofenac compared with the varying strength of hydrochloric acid. Further, this work can be extended for the determination of shelf life of formulations containing Aceclofenac.

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