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STABILITY INDICATING HPLC ASSAY METHOD FOR DIACEREIN AND ACECLOFENAC IN TABLETS

Krishna R Gupta*, Varun E. Samrit, Anuradha H Mahapatra, Sudhir G.Wadodkar

Department of Pharmaceutical Chemistry, S.K.B. College of Pharmacy, RTM Nagpur University, New Kamptee-441002, Nagpur (MS) India

ABSTRACT

The present work describes the development of stability indicating assay method for Diacerein and Aceclofenac in their combined dosage form that would provide helpful information to the manufacturers. Stress studies were conducted on the drug substance and product under the ICH prescribed stress conditions viz. hydrolysis, oxidation, humidity, photolysis, thermal stress. The separation of the drug from its degradation products, trials were made by taking acetonitrile: water, acetonitrile: phosphate buffer, acetonitrile: phosphoric acid in various blends. Separation was achieved using C-18 column and a mobile phase comprising of Acetonitrile: Phosphoric acid 0.1 M (61: 39) at a flow rate of 1.5 mL/min. The detection wavelength was 260 nm. The drug showed sufficient decomposition under alkaline hydrolysis (0.05 N NaOH), acidic hydrolysis (0.05 N HCl), neutral hydrolysis (distilled water), and oxidative hydrolysis (6% H_2O_2). The drug was found to be moderately sensitive to humidity studies (75 % RH), photochemical studies (UV 254 nm), and thermal studies (60^0C). Recovery studies were also carried out for both the drugs and the mean percent recovery were found to be 100.69 for diacerein and 99.15 for aceclofenac. Mean percent estimation in marketed formulation gave 99.63% for diacerein and 100.04% for aceclofenac. The above method was validated for accuracy, precision, ruggedness, limit of detection, limit of quantitation and was found to be satisfactory for routine analysis of diacerein and aceclofenac in their combined dosage form in the presence of their degradation products.

Key words: Aceclofenac, Diacerein, Stress Degradation, Stability Indicating, Validation

*Corresponding Author Email: krg1903@gmail.com

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INTRODUCTION

Diacerein (DIAC) is chemically[1] 4,5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid; (Figure 1) known as diacetylrhein, used in the treatment of osteoarthritis. It acts by inhibiting interleukin-1 synthesis. Diacerein is a low molecular weight compound which rapidly metabolizes in the body to active metabolite, Rhein. Diacerein also inhibits IL-1 induced expression of cartilage degrading enzymes. Aceclofenac[2] (ACEL) ([2-(2-6-dichlorophenyl) amino] phenyl acetoxy acetic acid; (Figure 2) belongs to the class of non-steroidal anti inflammatory drugs (NSAIDs). It has pronounced anti-inflammatory, antipyretic, antirheumatoid and analgesic effect and an improved gastro-intestinal tolerance. It is well absorbed after oral administration and circulates mainly as unchanged drug. This drug is also more than 99% bound to plasma proteins.

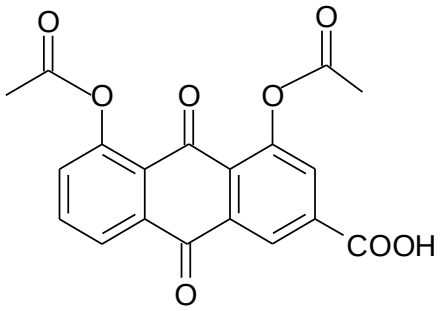

Figure 1: Structure of Diacerein

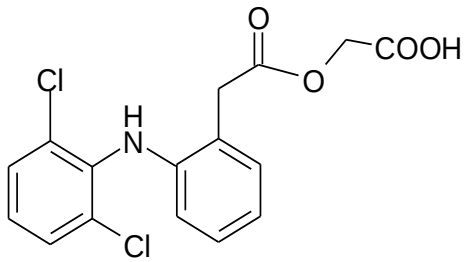

Figure 2: Structure of Aceclofenac

In recent years pharmaceutical preparations containing both these drugs have been available commercially. Literature revealed various methods, A HPLC-UV determination of rhein and aceclofenac[3,4], A validated HPLC stability indicating method for the determination of diacerein in bulk substance[5], Direct spectrophotometric determination of diacerein in capsules[6], Stability indicating methods for the determination of aceclofenac[7], Simultaneous spectrophotometric estimation of aceclofenac and paracetamol in tablet dosage form[8], Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form[9] and Simultaneous determination of aceclofenac and diclofenac in human plasma by narrow bore HPLC using column-switching[10].

Many methods for estimation of DIAC and ACEL individually have been reported in the literature. A comprehensive literature survey revealed the lack of suitable stability indicating assay method for the determination of these two drugs in pharmaceutical dosage forms. Therefore, the aim of the present work is the development simple, sensitive, precise, accurate

stability indicating HPLC method for estimation of DIAC and ACEL in their combined dosage form.

MATERIAL AND METHODS

Chemicals and Reagents

All experiments were performed with pharmaceutical grade DIAC and ACEL and analytical grade reagents. HPLC grade solvents and Doubled distilled water were used for analysis. Solvents were filtered through 0.45 µ cellulose membrane filters. All dilutions were performed in standard volumetric flasks.

Instrumentation

Stability studies were carried out using Thermolab Stability Chamber and Thermolab Photostability and Humidity Chamber. The HPLC System Analysis was performed with a Shimadzu (Japan) 1100 series equipped with an LC-10 AD VP binary solvent-delivery module, an SPD-10A UV–visible detector and Spinchrome software for data handling using C-18 column. Samples were injected through a Rheodyne injector valve model 7125 with 20-µL sample loop.

Chromatographic Conditions

The chromatographic separation was carried out on a Phenomenex ODS analytical column (250 mm × 4.6 mm i.d. 5-µm particles) under reversed-phase partition chromatographic conditions. Before analysis the mobile phase was degassed by use of a sonicator (PCI analytics, INDIA) and filtered through a 0.45 µm filter (Millipore, Bangalore, India). Sample solutions were also filtered through a 0.45µm filter. The system was equilibrated before each injection.

HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

a) Preparation of Standard Solution

Diacerein stock solution was prepared in 50 mL volumetric flask, dissolved in 5 mL dimethylacetamide and volume was made up to the final mark with methanol to get the final concentration 500 µg/mL. Similarly, aceclofenac stock solution was prepared in 50 mL

volumetric flask, dissolved in 5 mL dimethylacetamide and volume was made up to the final mark with methanol to get the final of final concentration to 1000 µg/mL.

b) Selection of Detection Wavelength

Mixed standard solution was prepared by appropriately diluting the above stock solution to make the final concentration of 20µg/mL and 40µg/mL for DIAC and ACEL respectively. The mixed standard solution was scanned over the range 400 to 230 nm and detection wavelength was selected as 260 nm for both the drugs (Figure 3).

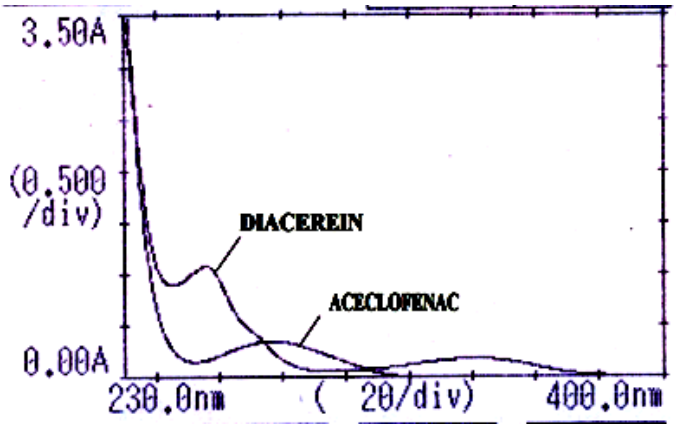

Figure 3: Overlain UV spectrum of Diacerein and Aceclofenac

c) Selection of Mobile Phase

Five different mobile phases were set and 20 µL of mix standard solution was injected. The mobile phase Acetonitrile and Phosphoric acid (61:39) was selected as it gave well resolved sharp peaks with reasonable retention time for both the drugs. The analysis was carried out using HPLC Shimadzu1100 series binary gradient system, Phenomenex ODS 5µ C18 column (250 X 4.6 mm), flow rate 1.5 mL/min.

d) Study of System Suitability Parameters

Five replicate injections of mix standard solution (20µL) volume were injected and chromatograms were recorded for peak response. The results showed that the resolution, retention time, asymmetry and theoretical plate/meter were found to be within limits.

e) Study of Linearity (Calibration Curve)

Aliquots of mix standard solution were taken in the range 1.0 mL to 5.0 mL and diluted with mobile phase. The linearity range of both the drug was studied and the correlation coefficient was found to be satisfactory.

STRESS DEGRADATION STUDIES[11,12]

A) Solution State Analysis

Hydrolysis Studies under Reflux Condition (Alkaline, Acidic, Neutral and Oxidative)

Procedure for standard drugs

Accurately weighed quantities of DIAC and ACEL in the ratio of 1:2 were transferred to two different round bottom flasks. To it 25 mL of 0.05 N NaOH (for alkaline) was added and refluxed for three hours. At the end of three hours 5 mL DMA was added and dilutions were made so as to get the final concentration of DIAC 40 µg/mL and ACEL 80 µg/mL. 20 µL volumes from these solutions were injected and chromatographed separately. The overlain chromatogram is shown in Fig. 5. Similar procedure was adopted with 0.05 M HCl for acidic, distilled water for neutral and 6% H_2O_2 for oxidative hydrolysis respectively.

Procedure for sample

Tablet powder equivalent to (~25 mg) DIAC were transferred to series of six different round bottom flasks. To it 25 mL of 0.05 N NaOH (for alkaline) was added and refluxed for 30, 60, 90, 120 and 150 and 180 mins. To it 5 mL DMA were added and the contents were sonicated for 20 mins and filtered separately. The solutions were further diluted so as to get the final concentration of DIAC 40 µg/mL and ACEL 80 µg/mL (on labeled claim basis) and chromatographed. Similar procedure was adopted with 0.05 M HCl for acidic, Distilled Water for neutral and 6% H_2O_2 oxidative hydrolysis respectively.

The amounts of DIAC and ACEL were estimated by comparing the peak area of sample with that of standard (untreated) and the results were calculated by using following formula no.1

$$\% \text{ Label Claim} = \frac{\text{Au x Wstd x Avg. wt}}{\text{As x Wtab x L . C.}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, Au = Peak area of sample, As = Peak area of Standard, Wstd = Wt (mg) of DIAC or ACEL, Wtab = Wt of tablet powder, Avg. wt =Average weight of tablet, LC= Label Claim (mg/tablet)

B) **Solid State Analysis**

i) Humidity Studies (75 % RH)

Tablet powder was subjected to 75 % RH. Tablet powder equivalent to 25mg DIAC was weighed and further diluted as stated above to get the desired concentration on 0^{th}, 3^{rd} and 7^{th} day. The solutions were injected and chromatograms were recorded.

ii) Photochemical Studies (UV 254 nm)

Tablet powder was kept in UV chamber at 254 nm and was weighed equivalent to 25 mg DIAC and further diluted to get the desired concentration on 0^{th}, 3^{rd} and 7^{th} day. The solutions were injected and chromatograms were recorded.

iii) Thermal Studies (60°C)

Tablet powder was kept in an oven at 60°C and was weighed equivalent to 25 mg of DIAC and further diluted to get final concentration on 0^{th}, 3^{rd} and 7^{th} day. The solution was injected and chromatograms were recorded.

Assay

Twenty tablets were weighed and average weight was calculated. The tablet was triturated thoroughly and mixed. An accurately weighed quantity equivalent to 25 mg of DIAC was taken. A 5 ml DMA was added and dilutions were made with the mobile phase, sonicated and filtered. Aliquots of this solution were diluted to get the final concentration of DIAC 20 µg/mL and ACEL 40 µg/mL on label claim basis. Five such samples were prepared and injected separately and after equilibration of stationary phase and chromatograms were recorded. The contents of DIAC and ACEL in each sample were calculated by comparing the peak area of sample with that of standard using formula no 1. The results are shown in Table 1

Table 1 Results of Estimation in Marketed Formulation

Drug	% Label Claim*	±SD	CV
DIAC	99.63	0.95	0.95
ACEL	100.04	0.99	1.00

*Mean of five observations

Recovery Studies

This is carried out by standard addition method. An accurately weighed quantity equivalent to 20.0 mg of DIAC was taken in four different flasks. To each flask standard DIAC and ACEL was added at four different levels. Final dilutions were injected and chromatographed. The chromatograms were recorded and peak areas were noted and results were calculated by using formula as below

$$\% \text{ Recovery} = \frac{\text{Total Drug Estimated} - \text{Amt .Contributed}}{\text{Amt .of Pure Drug added}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Method Validation

The developed chromatographic method was validated using ICH guidelines before implementation. Validation parameters performed include linearity, limit of detection and quantitation, specificity, robustness, accuracy and repeatability.

Accuracy The accuracy of the method was performed by adding known amounts of DIAC and ACEL to the marketed formulation at four levels i.e. 2.7, 5.0, 7.9, 9.7 mg and 5.5, 9.5, 14.6, 20.1 mg respectively and analyzed by proposed method (Table 2).

Table 2 Results of Recovery Study

Amount. of Pure drug added (mg)		Amount Recovered (mg)		% Recovery	
DIAC	**ACEL**	**DIAC**	**ACEL**	**DIAC**	**ACEL**
2.7	5.5	2.71	5. 40	100.74	98.27
5.0	9.5	5.09	9.38	101.93	98.80
7.9	14.6	8.03	14.70	101.69	100.04
9.7	20.1	9.54	19.99	98.42	99.50
			Avg.	100.69	99.15
			±SD	1.60	0.77
			CV	1.60	0.78

Precision

To check the precision of the proposed method was analyzed by within-day and between days. The within-day precision was determined by calculating the relative standard deviation of three replicate analyses of samples on the same day. The between day precision was determined by calculating the relative standard deviation of the results from the same samples analyzed for period of fifteen days intermittently

Ruggedness

It was done by studying analyst to analyst variation using proposed method (Table 3).

Table 3 Results of validation Study

Parameters	Percent Label Claim	
	DIAC	**ACEL**
Analyst to Analyst (n=2)		
Mean, CV	99.70, 0.26	100.37, 0.15
Intraday Study (n=4)		
Mean, CV	97.46, 0.61	101.18, 0.69
Inter-day Precision (n=4)		
Mean, CV	92.52, 5.47	94.41, 5.18

RESULTS AND DISCUSSION

Development and optimization of the method

During development of the method, number of mobile phases and stationery phases were attempted to elute both the components simultaneously. Method development was started with acetonitrile: water, acetonitrile: phosphate buffer, acetonitrile: phosphoric acid used in different proportions and with different pH. At the composition of Acetonitrile: Phosphoric acid 0.1 M (61: 39), the elution of both the components occurred with good resolution between the two peaks giving resolution of 7.05, assymetric factor less than 1.5 and column efficiency more than 2000 measured as theoretical plates. Upon application of the developed method, well separated

peaks were obtained for both DIAC and ACEL. The representative chromatogram of mix standard solution is given in Figure 4**.**

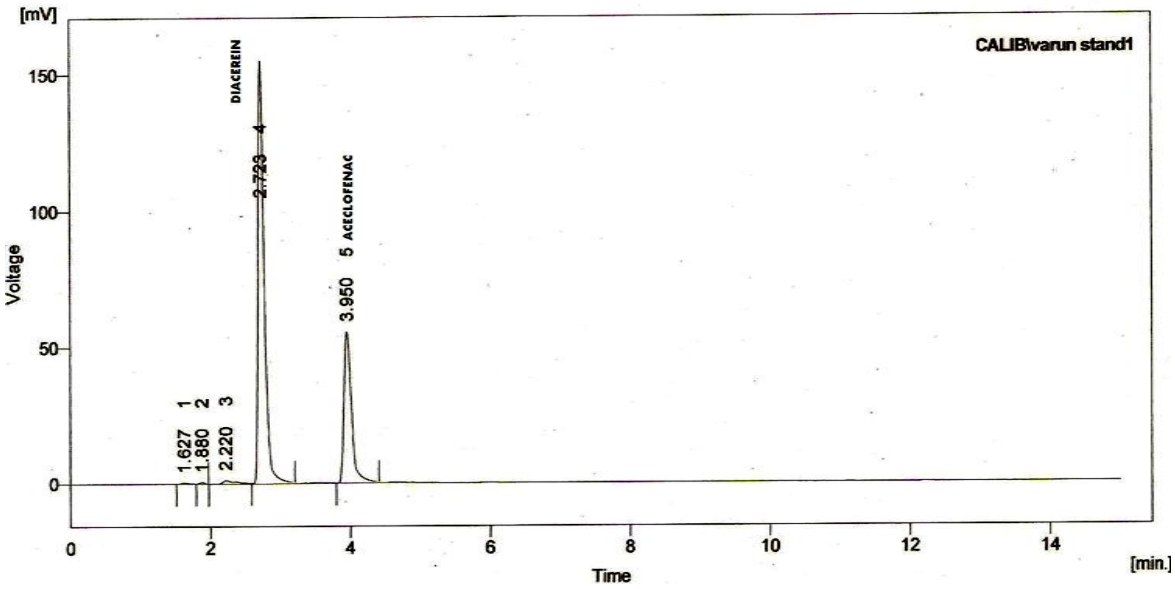

Figure 4: Chromatogram of mixed standard solution

HPLC studies on stressed solutions

Diacerein and Aceclofenac were found to be susceptible to all hydrolytic conditions where both the drugs were found to be degraded to about 50-80%, subsequently the studies were done at lower temperatures viz, 60°C and room temperature for alkaline and acidic hyrolysis. On Thermal, UV and Humidity (75%RH) stress where both the drugs showed a degradation of about 15-20% on exposure for a period of 7 days. Table 4 indicates the percent degradation of DIAC and ACEL remained under various stress conditions. The degradation products and drugs carry the numerical notations in accordance with the sequence in which the peaks appeared from left to right on the HPLC chromatogram.

Table 4 Results of Solution State Analysis (Hydrolysis Studies)

Sr. No	Time sample	% Drug Undegraded (Hydrolysis)							
		Alkali		Acid		Neutral		Oxidative	
		DIAC	ACEL	DIAC	ACEL	DIAC	ACEL	DIAC	ACEL
1.	30	63.19	72.34	21.22	86.75	25.50	93.90	25.20	101.90
2.	60	62.75	72.05	20.05	75.41	20.90	73.02	24.58	77.80
3.	90	62.32	71.55	17.89	55.70	19.42	61.09	23.84	75.03
4.	120	61.23	66.48	16.71	50.68	18.13	51.15	19.83	61.76
5.	150	60.44	60.50	16.20	41.42	16.21	50.67	19.18	48.40
6.	180	55.31	50.01	15.54	30.90	15.93	49.48	16.51	46.33
7.	**Std** (after 180 min)	53.76	59.57	12.99	31.42	8.45	32.73	10.81	25.32

HPLC studies of the stressed samples showed the following degradation behavior:

Hydrolytic conditions

The drugs showed more than 50% degradation in 0.05N HCl reflux, 0.05N NaOH refluxed for 3h, 60°C and at room temperature.

i) Alkaline degradation

Percent degradation of sample DIAC was found to be around 45-50% while ACEL showed degradation from 50-80% at different temperature of alkaline exposure. Generation of four additional peaks was seen at higher temperature. The degradation products carry the numerical notations in accordance with the sequence in which the peaks appeared from left to right on the HPLC chromatogram Peak 1 and 2 shows gradual increase while peaks 3 and 4 shows gradual decrease over a period of 3h at reflux temperature (Figure 5a) Peaks 1 and 2 were not seen while peaks 3 and 4 were stable upto 10h at 60°C (Figure 5b) and for 24h at room temperature (Figure 5c). No such generation of additional peaks were seen in standard drugs under similar stress (Figure 5d).

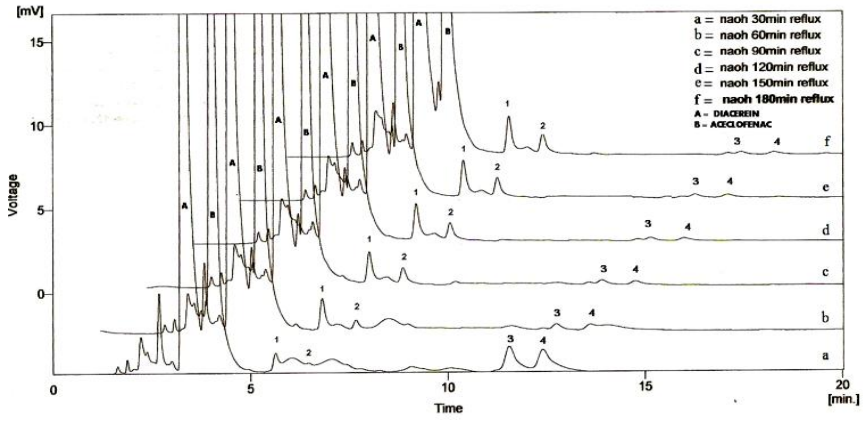

Figure 5a: Overlain chromatograms of sample under alkali hydrolysis (Reflux)

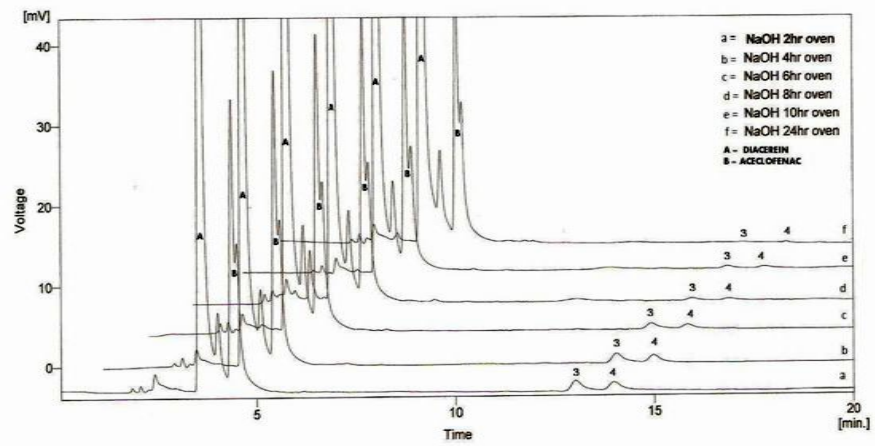

Figure 5b: Overlain chromatograms of sample under alkali hydrolysis (60°C)

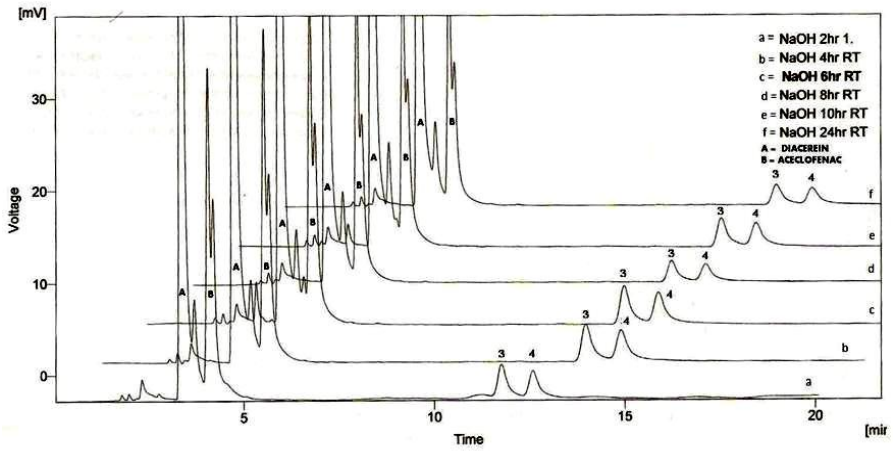

Figure 5c: Overlain chromatograms of sample under alkali hydrolysis (RT)

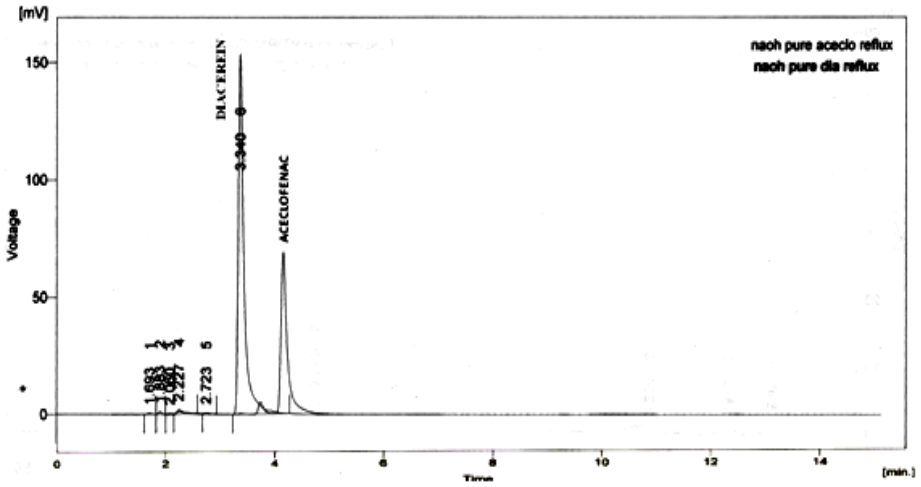

Figure 5d: Overlain chromatograms of standard drug under Alkali hydrolysis

ii) Acid degradation

Percent degradation of sample DIAC was found to be around 85% and ACEL around 70%. The chromatogram showed formation of three additional peaks of DIAC while ACEL showed gradual increase in two peaks over a period of 3h at reflux temperature (Figure 6a). Similar observations were seen with standard drugs (Figure 6b). The rate of hydrolysis for DIAC and ACEL was more in acid as compared to that of alkali or water.

iii) Neutral degradation

Percent degradation of sample DIAC was found to be around 85% and ACEL 50%. The chromatogram showed generation of two additional peaks of DIAC and ACEL over a period of 3h at reflux temperature (Figure 7a). The standard DIAC showed similar behavior but standard ACEL shows presence of only one peak (Figure 7b).

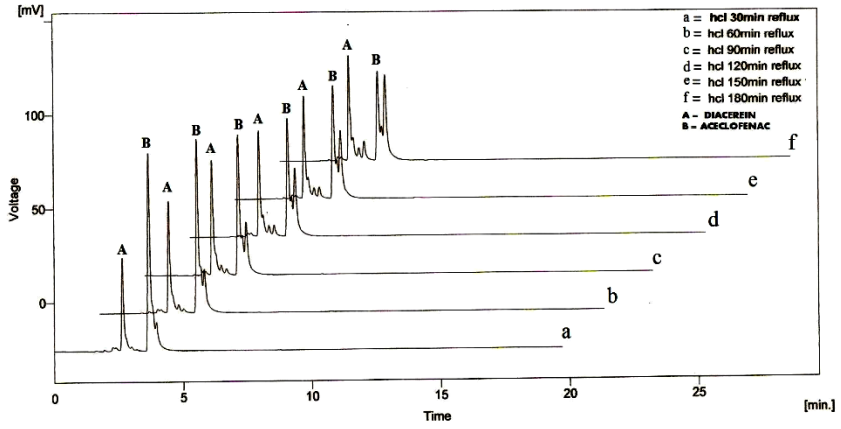

Figure 6a: Overlain chromatograms of sample under acid hydrolysis

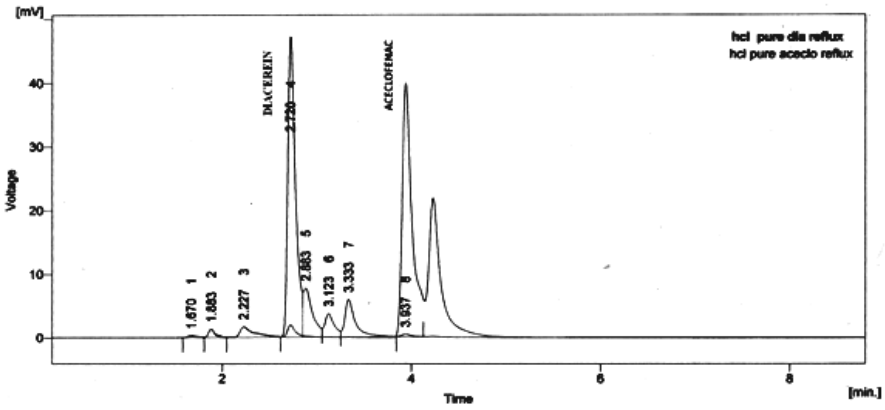

Figure 6b: Overlain chromatograms of standard drug under Acid hydrolysis

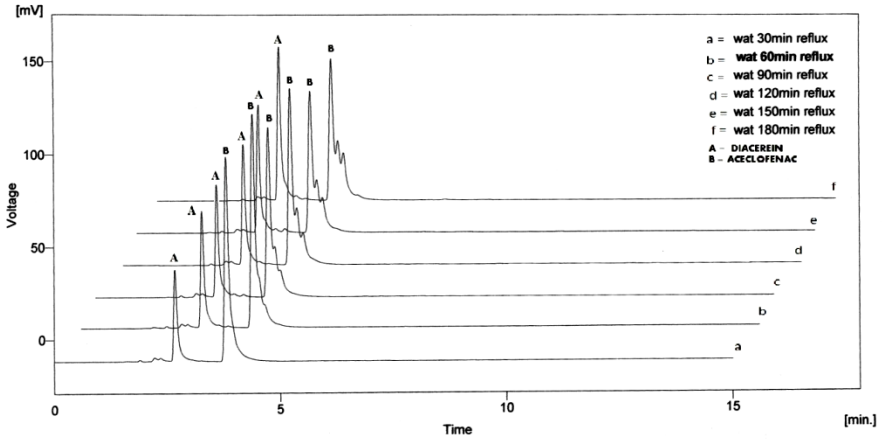

Figure 7a: Overlain chromatograms of sample under Neutral hydrolysis

iv)Oxidative hydrolysis

Percent degradation of sample DIAC was found to be around 84% and ACEL 54%. The chromatogram showed generation of two additional peaks for DIAC and three additional peaks for ACEL over a period of 3h at reflux temperature (Figure 8a). The standard DIAC showed similar behavior but standard ACEL shows presence of only one peak (Figure 8b).

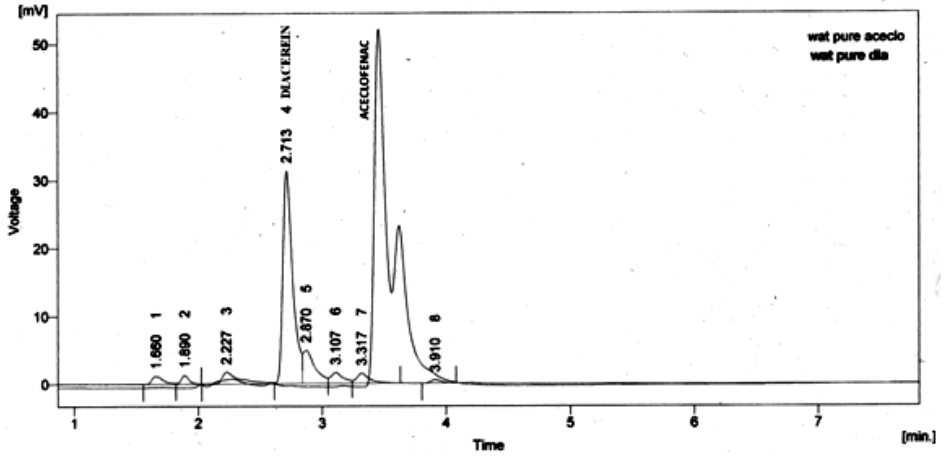

Figure 7b: Overlain chromatograms of standard drug under Neutral hydrolysis

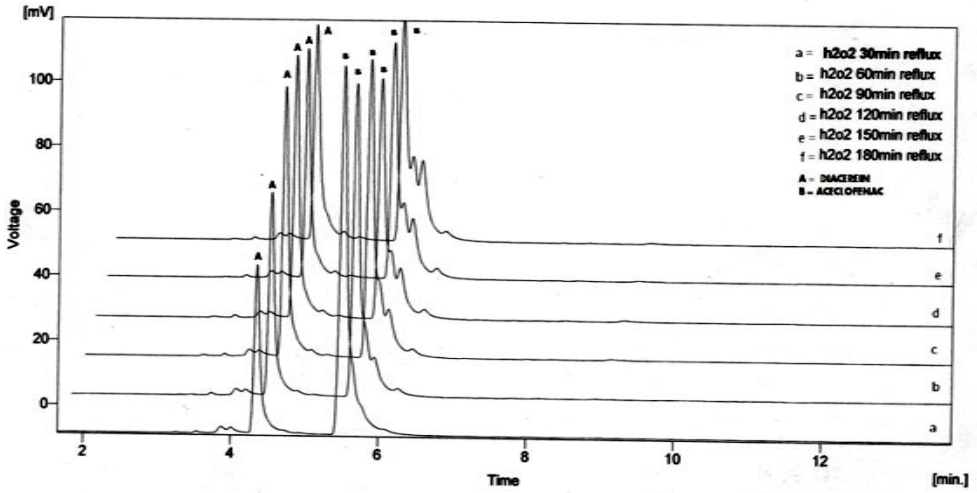

Figure 8a: Overlain chromatograms of sample under Oxidative hydrolysis

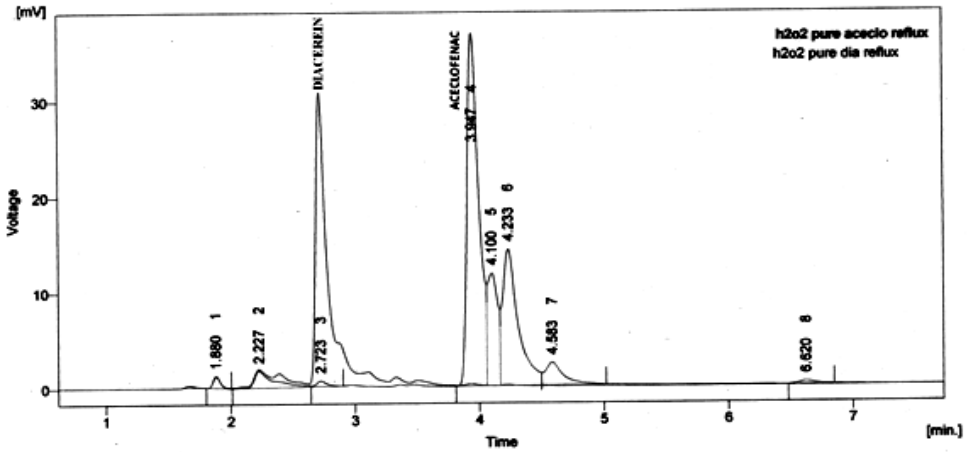

Figure 8b: Overlain chromatograms of standard drug under oxidation hydrolysis

The results of solution state analysis are shown in Table 4**.** The retention times (t_r) and %area of degraded products of DIAC and ACEL are shown in Table 5a and Table 5b respectively.

Table 5a Summary of degradation analysis at reflux temperature for Diacerein

Stress condition	Degradation products					
	Retention time (t_r)			% Area		
	I	II	III	I	II	III
Alkaline reflux (180 min)	-	-	-	-	-	-
Acidic reflux (180 min)	2.84	3.07	3.27	-	6.1	1.3
Neutral reflux (180 min)	3.07	3.28	-	3.3	0.7	-
Oxidation reflux (180 min)	3.08	3.29	-	-	5.6	-

Table 5b Summary of degradation analysis at reflux temperature for Aceclofenac

Stress condition	Degradation products					
	Retention time (t_r)			% Area		
	I	II	III	I	II	III
Alkaline reflux (180 min)	11.55	12.40	-	1.7	1.8	-
Acidic reflux (180 min)	3.95	4.08	-	7.2	29.1	-
Neutral reflux (180 min)	3.99	4.11	-	12.9	17.1	-
Oxidation reflux (180 min)	4.01	4.13	4.46	10.6	15.5	2.5

Solid state studies

Humidity studies (75 % RH)

The chromatogram of sample showed no generation of additional peaks for a period of 7days though DIAC was degraded to extent of 10.5% and ACEL about 7% (Figure 9).

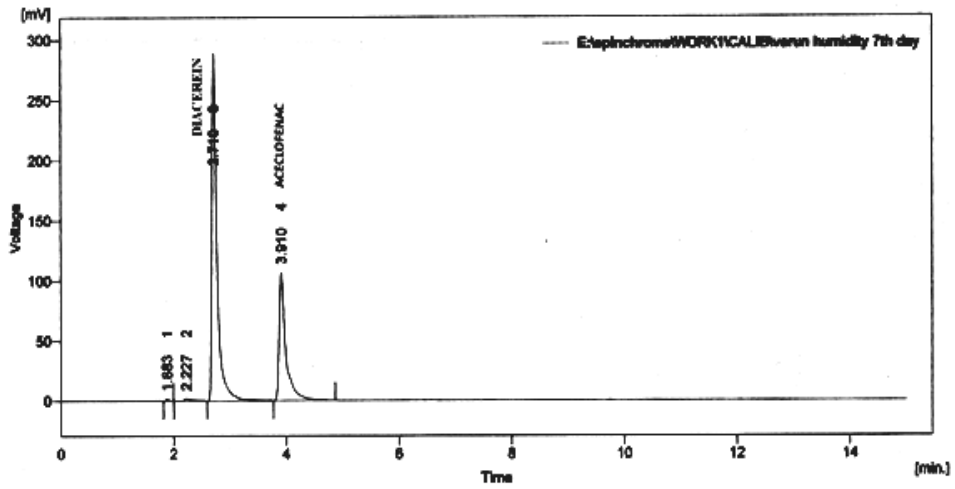

Figure 9: Chromatogram of sample under humidity study (7th day)

Photolytic studies (UV chamber 254nm)

Both the drugs showed about 15% degradation but no prominent degradation peaks could be seen in the chromatogram on 7th day (Figure 10)

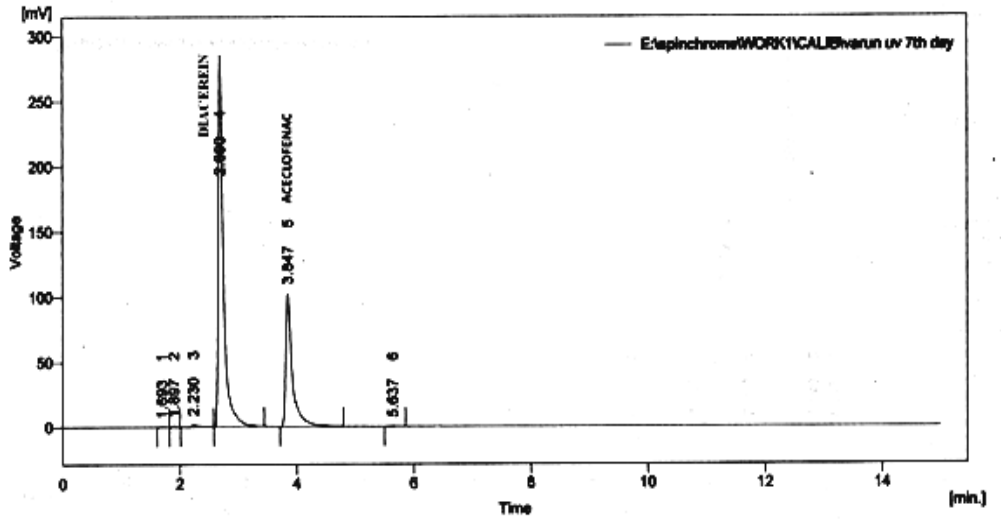

Figure 10: Chromatogram of sample under UV study (7th day)

Thermal studies (60°C)

Both the drugs showed about 14% degradation but no prominent degradation peaks could be seen in the chromatogram on 7th day (Figure 11).

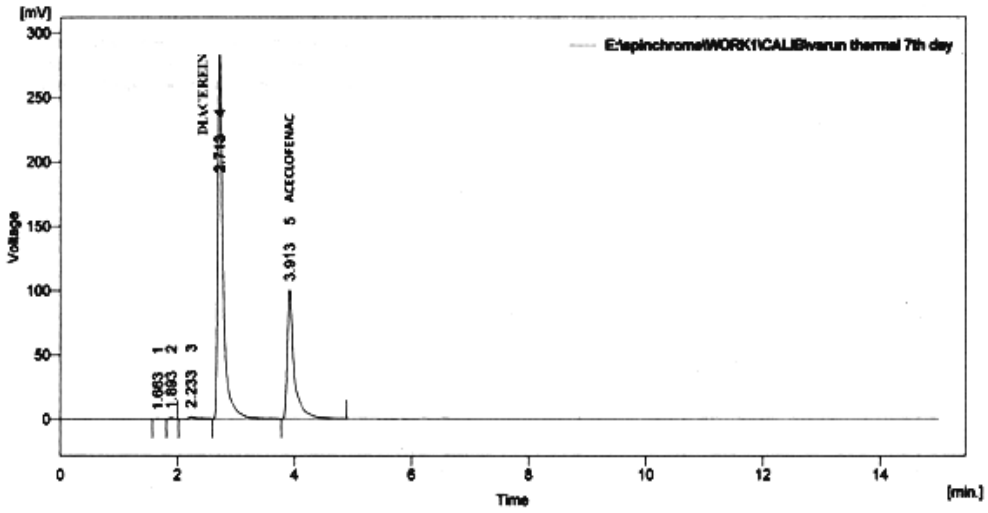

Figure11: Chromatogram of sample under thermal study (7th day)

Singh and Bakshi, in their article on stress testing[18], suggested a target degradation of 20-80% for the establishing stability indicating nature of the assay method, as even inter-mediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20-80%, this could not be achieved in some cases even after exposure for prolonged duration. DIAC and ACEL showed extensive degradation in acidic & alkaline hydrolysis, oxide and neutral degradation conditions while they were found to be stable in nearly all solid stress conditions. The results of solid state analysis are shown in Table 6

Table 6 Results under Solid State Analysis

Sr.No.	Time Interval (Days)	% Drug Undegraded					
		Humidity Studies		Photochemical Studies		Thermal Studies	
		DIAC	ACEL	DIAC	ACEL	DIAC	ACEL
1.	0	102.08	101.09	102.08	101.09	102.08	100.69
2.	3	101.82	97.54	101.33	99.36	101.93	101.03
3.	7	89.55	93.02	85.25	85.39	87.60	86.71

Method validation

The recovery range (accuracy) for DIAC and ACEL were found to be in the range 98.42 to 101.69% and 98.27 to 100.04. The relative standard deviation ranged from 0.77 to 1.60%. Precision was ascertained by replicate analysis of homogenous samples of tablet powder. Assay precision was expressed as the relative standard deviation (RSD, %), found to be ≤ 2.0% for both the drugs. Intra-day precision was determined and %RSD was found to be within limits; inter-day precision were determined by replicate analysis of the solutions on different days. The %RSD was found to be more, as the solution analysed on 11th day showed the presence of additional peaks at t_r_3.11 and 4.50 (Peak **5** for DIAC and Peak **7** for ACEL as they appear in chromatogram, Figure 12).

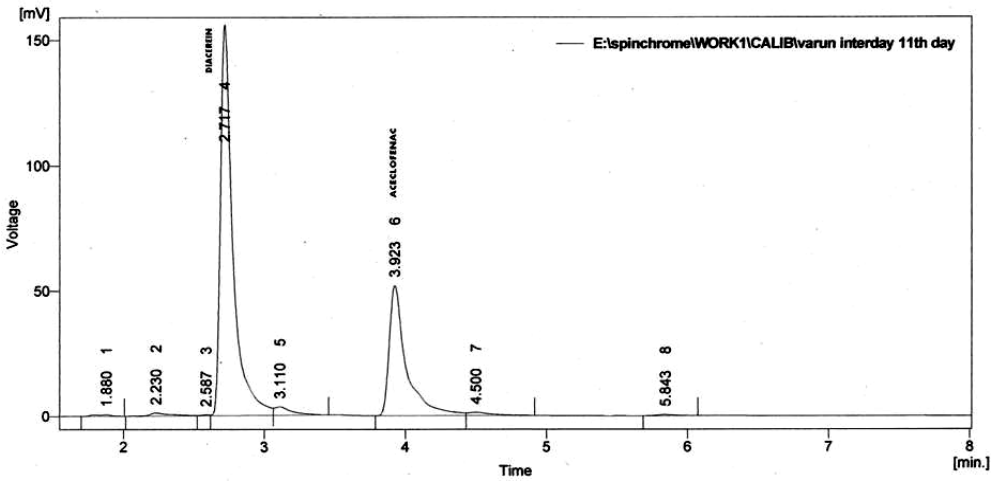

Figure 12: Chromatogram of sample under Inter day precision study (11th day)

LOD and LOQ

Limit of Detection for DIAC and ACEL were found to be 0.0308µg/mL and 0.1385µg/mL respectively. Limit of quantitation for DIAC and ACEL were found to be 0.0935µg/mL and 0.4199µg/mL respectively.

CONCLUSION

From above stress degradation studies it was observed that both the drug showed degradation to a large extent during hydrolysis. In solid state analysis the drugs were degraded to some extent

depending upon time and found to be within limit. The proposed method is capable of analyzing in presence of the degradation products. The proposed method was found to be simple, specific, linear and rugged and can be used for routine quality control.

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