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Research Article

# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF DIACEREIN AND ACECLOFENAC AS BULK DRUG AND IN TABLET DOSAGE FORM

**S. P. Gandhi, M. G. Dewani, T. C. Boroleand M. C. Damle***

[1]Department of Pharmaceutical Chemistry, AISSMS College of Pharmacy,Kennedy road, Near RTO, Pune, Maharashtra, India.

***Corresponding Author: mcdamle@rediffmail.com**

**ABSTRACT**

Diacerein is a drug for osteoarthritis and is di-acetylated derivative of rein. Aceclofenac is used as an effective non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic and antipyretic properties. The present study describes degradation of diacerein and aceclofenac under ICH prescribed stress conditions (hydrolysis, oxidation, dry heat, wet heat and photolysis) and establishment of a stability-indicating HPLC assay method. Different degradation peaks were observed for diacerein when it was exposed to alkaline, acid catalysed hydrolysis and photo degradation. For aceclofenac, decrease in peak area was observed. HPLC separation was obtained using HiQSil C18 column with oven tempt of $40^0$ C and mobile phase consisting of Methanol: Mixed phosphate buffer pH 4 (70: 30 % v/v). The quantitation was done at 268 nm as detector wavelength. The method was found to be simple, specific, precise, and stability indicating.

**Keywords:** Diacerein, Aceclofenac, Stability indicating, HPLC and Validation.

## INTRODUCTION

Diacerein 4,5-Bis(acetoxy)-9,10-dihydro-9,10-dioxo-2-anthracenecarboxylic acid is drug for osteoarthritis [1,2]. It is a readily obtained in few synthetic steps from naturally occurring glucopyranoside aloin. It is the di-acetylated derivative of rhein, (4,5-dihydroxy-9,10-dihydro-9,10-dioxo-2-anthracenecarboxylic acid) a molecule with an anthraquinone ring which is actually the active metabolite of diacerein. Diacerein is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentrations of proinflammatory cytokines [3, 4].

Aceclofenac [(2,6-dichlorophenyl)amino] phenylacetoxyacetic acid is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenylacetic 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 spondylatis [5]. Diacerein and aceclofenac is a recent combination available in the market for its synergetic effect in the treatment of different joint disorders.

Literature survey reveals some methods reported for diacerein viz. stability indicating High Performance Liquid Chromatographic (HPLC) methods [6,7] and stability indicating HPTLC method[8]. For aceclofenac literature surveys reveals many papers viz. simple spectrometric methods [9], spectroflurimetric method [10], stability indicating HPLC [11] and HPTLC methods [12]. Some research articles are also available for diacerein and aceclofenac combination as simultaneous UV spectrophotometric methods [13,14], RP-HPLC method [15] and HPTLC method [16].

No reports were found for the stability indicating assay method as per ICH guidelines, for diacerein and aceclofenac in combination by HPLC method. This paper describes a simple, accurate, sensitive and validated stability indicating HPLC method for diacerein and aceclofenac in combination as the bulk drug and in tablet dosage forms.

The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assay should be stability indicating. The aim of the present study accordingly was to establish inherent stability of the diacerein and aceclofenac through stress studies under a variety of ICH recommended test conditions[17] and its validation[18].

## EXPERIMENTAL

### Material and method

Working standard of Diacerein was provided by Creative Healthcare Pvt. Ltd and aceclofenac was provided by Arbro Pharmaceuticals Ltd. New Delhi, India and was used as such without further purification. Methanol, potassium dihydrogen phosphate, disodium hydrogen phosphate, Glacial Acetic acid, Conc. HCl, NaOH and $H_2O_2$ used were of analytical reagent grade.

For stability indicating HPLC method development, Jasco HPLC system consisting Model PU 2080 Plus pump, Rheodyne sample injection port, HiQ SiL $C_{18}$ column, MD 2010 PDA detector and Borwin- PDA software (version 1.5) was used. For photo-degradation studies, Photostability Chamber was used. (Make - Newtronic). All the weighing was done on Shimadzu balance (Model AY-120).

### Selection of Detection Wavelength

Methanolic solution of each drug was scanned over the range of 200-400 nm. It was observed that both the drugs showed considerable absorbance at 268 nm. So, 268 nm was selected as the wavelength for detection [Fig.1].

### Preparation of standard solution

Standard stock solution of Diacerein and aceclofenac were prepared separately by dissolving 25 mg of drug in 25 ml of methanol to get concentration of 1000 mcg/mL. From the standard stock solution, mixed working standard solution was prepared to contain 100 mcg/ml of diacerein and 100 mcg/ml of aceclofenac.

### Preparation of sample solution

Ten tablets (Dycerin-A, Creative Healthcare Pvt Ltd.) each containing 50 mg of diacerein and 100mg of aceclofenac was weighed and powdered. Powder equivalent to 25 mg of diacerein and 50 mg of aceclofenac was transferred to 25ml volumetric flask and was diluted with methanol to 25 ml (1000 mcg/ml of diacerein and 2000mcg/ml of aceclofenac). Further dilutions were made with methanol to get the final concentration of 100 mcg/ml of diacerein and 200 mcg/ml of aceclofenac.

### Stress degradation studies

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and mixed working standard solution subjected to stress conditions. Dry heat and photolytic degradation were carried out in solid state. Then the study was extended to formulation.

### Degradation under alkali catalysed hydrolytic condition

2.5ml of mixed working standard solution was mixed with 2.5ml of 0.01N NaOH. The solution was kept for 5 mins and was neutralized with 0.01N HCl. The resulting solution was diluted with methanol to 25ml and then was injected.

### Degradation under acid catalysed hydrolytic condition

2.5ml of mixed working standard solution was mixed with 2.5ml of 0.1 N HCl. The solution was kept for 15 mins and was neutralized with 0.1 N NaOH. The solution was diluted with methanol to 25ml and then was injected.

**Degradation under neutral hydrolytic condition**

2.5ml of mixed working standard solution was mixed with 2.5ml water. The solution was diluted to 25ml and was refluxed for 3 hrs. The solution was cooled to room temperature and then was injected into the system.

**Degradation under oxidative condition**

2.5ml of mixed working standard solution was mixed with 2.5ml 1% solution of $H_2O_2$. The solution was diluted to 25 ml with methanol and was kept for 30 mins. Then solution was injected into the system.

**Degradation under dry heat**

Dry heat studies were performed by keeping drug sample as mixture of diacerein and aceclofenac in oven ($80^0$ C) for a period of 24 hours. Sample was withdrawn after 24hrs, dissolved in methanol and diluted to get 10 mcg/ml as final conc. and was injected.

**Photo-degradation studies**

Photolytic studies were also carried out by exposure of drug as mixture to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. Sample was weighed, dissolved and diluted get 10 mcg/ml as final conc.

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**Method Validation**

**Linearity and Range**

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration (amount) of analyte in the sample. It was studied by analyzing five concentrations of each drug and process was repeated five times.

**Precision**

Precision of the system was evaluated by analyzing six independent standard preparations and % RSD value was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation.

**Accuracy**

To check accuracy of the method, recovery studies were carried out by addition of standard drug to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

**Limit of detection**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

$$DL = \frac{3.3\,\sigma}{S}$$

Based on the Standard Deviation of the Response and the Slope, detection limit (DL) may be expressed as:

**Limit of quantitation**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the Response and the Slope, The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,

$\sigma$ = the standard deviation of the response

S = the slope of the calibration curve

**Specificity**

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end.

The peak purity was determined on Borwin software.

## RESULTS AND DISSCUSION

**Development of the optimum mobile phase**:
Chromatographic separation studies were carried out on the working standard solution of Diacerein and aceclofenac ($10\mu g/ml$). Initially, trials were carried out using methanol and water by adjusting various pH. After several trials, Methanol: Mixed phosphate buffer pH 4 (70: 30 %v/v) was chosen as the mobile phase, for HPLC, which resulted in good resolution and acceptable peak parameters. Rt were found as Diacerein = 3.4 ± 0.2 mins. and Aceclofenac = 7.1 ± 0.2 mins. [Fig.2]

**Stress Degradation study [Table 1, 2]**
Under alkaline condition, diacerein was completely degraded and showed 5 peaks of degradation products. While no degradation peak was observed for aceclofenac with only 3.23% degradation. [Fig.3] After acid hydrolysis, diacerein showed two peaks of degraded product with 40.34% reduction in peak area. On the other hand aceclofenac showed 50.58% reduction in area with no peak of degradation. [Fig.4] Under the neutral condition, diacerein showed 16.85% degradation and aceclofenac showed 30.81 % degradation. In the oxidative condition, diacerein showed 30.86% degradation and aceclofenac showed 66.85% degradation. After the dry heat or exposing drug to heat condition no degradation peaks were obtained, but some amount of degradation was observed. After the photo degradation study for UV light and Fluorescence light, Diacerein showed 1 peak of degraded product with 43.87% degradation and no peak of degradation was observed for aceclofenac with 39.07% degradation [Fig.5]

**Validation of the developed stability-indicating method [Table 3]**

**Linearity**
The data obtained in the linearity experiment was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 2 - 10 mcg/ml for diacerein and 4-20 mcg/ml for aceclofenac with $r^2$ 0.997 and 0.998 resp.

**Precision**
The developed method was found to be precise as the % RSD value for both interday and intraday were less than 2.

**Accuracy**
Excellent recoveries were obtained at each level of added concentration. The result obtained (n = 3 for each level) indicated the mean recovery between 98% to 102% for both diacerein and aceclofenac.

**Limit of Detection**
The limit of detection as calculated by standard formula as given in ICH guidelines was found to be 0.3292 mcg/ ml for diacerein and 0.04713 mcg/ ml for aceclofenac.

**Limit of Quantitation**
The limit of Quantitation as calculated by standard formula as given in ICH guidelines was found to be 0.9977 mcg/ ml for diacerein and 0.1428 mcg/ ml for aceclofenac.

**Specificity**
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to >995, indicating the non interference of any other peak of degradation product, impurity or matrix.

**Discussion**
The degradation conditions mentioned above were arrived at, after number of initial trials for optimization of extent of degradation. Overall study comprised of stability indicating method development for Diacerein alone and in combination with Aceclofenac. But no considerable difference was observed.

## CONCLUSION

This study presents a simple and validated stability-indicating HPLC method for estimation of Diacerein and Aceclofenac in combination and in the presence of degradation products. The developed method is specific, accurate, precise and reproducible. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products Diacerein and Aceclofenac combination as well as single formulation, as no interference was observed due to excipients or other components present.

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**Table 1: Summary of Stress Degradation Study of Diacerein**

| Sr. No. | Stress Degradation Condition | Percent Degradation % | Rt ( min.) of degradation product |
|---|---|---|---|
| 1 | Initial* | ----- | ------- |
| 2 | Base (0.01 N NaOH, kept for 5 mins) | Degraded completely | D1 = 3.587 |
| | | | D2 = 3.827 |
| | | | D3 = 4.173 |
| | | | D4 = 4.840 |
| | | | D5 = 5.693 |
| 3 | Acid (0.1 N HCl, kept for 15mins) | 40.34 | D1 = 3.987 |
| | | | D2 = 4.533 |
| 4 | Water (reflux, 3 hours) | 16.85 | Not found |
| 5 | $H_2O_2$ 1% (kept for 30 mins) | 30.86 | Not found |
| 6 | Heat dry ($80^0$C,4 hrs.) | 15.78 | Not found |
| 7. | Photo stability [UV, 200 watt hrs/square meter Florescence , 1.2 million Lux. Hrs] | 43.87 | D1 = 4.040 |

**Table 2: Summary of Stress Degradation Study of Aceclofenac**

| Sr. No. | Stress Degradation Condition | Percent Degradation | Rt (mins)of deg. Product |
|---|---|---|---|
| 1 | Initial* | ----- | Not found |
| 2 | Base (0.01 N NaOH, kept for 5 mins) | 3.23 | Not found |
| 3 | Acid (0.1 N HCl, kept for 15mins) | 50.58 | Not found |
| 4 | Water (reflux, 3 hours) | 30.81 | Not found |
| 5 | $H_2O_2$ 1% (kept for 30 mins) | 66.85 | Not found |
| 6 | Heat dry ($80^0$C, 24 hrs) | 20.55 | Not found |
| 7. | Photo stability UV, 200 watt hrs/square meter Fluorescence, 1.2 million Lux. Hrs | 39.07 | Not found |

**Table 3: Validation results**

| S. No. | Validation Parameter | Results | |
|---|---|---|---|
| | | **Diacerein** | **Aceclofenac** |
| 1. | Linearity | Y = 90779x + 24351 | Y = 16143x + 10207 |
| 2. | Range | $R^2$ = 0.997 | $R^2$ = 0.998 |
| 3. | Precision | (%RSD) | (%RSD) |
| | A)Intraday precision | 0.600 | 0.938 |
| | B)Interday precision | 1.011 | 1.003 |
| 4. | Accuracy | % Recovery | % Recovery |
| | 80% | 99.02 % | 99.29% |
| | 100% | 101.59 % | 101.02% |
| | 120% | 98.75 % | 98.97% |
| 5. | LOD | 0.3292 µg/ml | 0.04731 µg/ml |
| 6. | LOQ | 0.9977 µg.ml | 0.1428 µg/ml |
| 7. | Specificity | Specific | Specific |

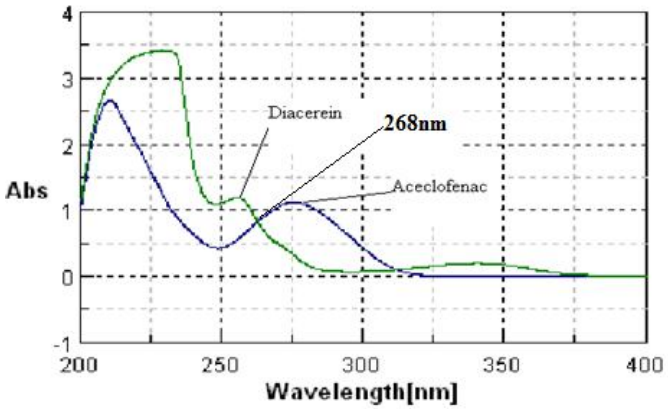


**Fig. 1: Overlain spectra of Diacerein and Aceclofenac**

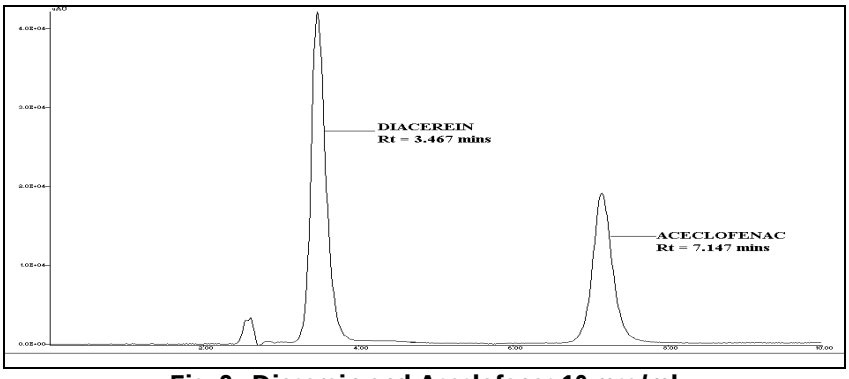


**Fig. 2: Diacerein and Aceclofenac 10 mcg/ml**

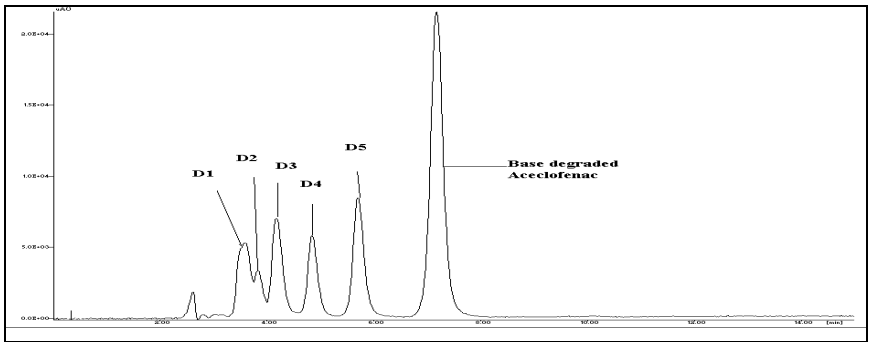


**Fig. 3: Alkaline hydrolysis of Diacerein and aceclofenac in mixture**

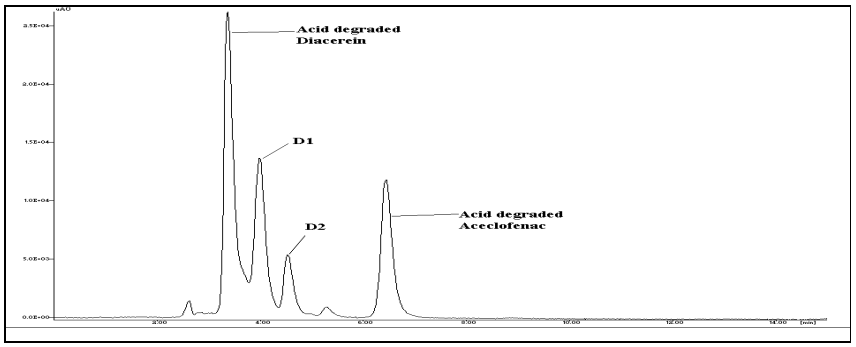


**Fig. 4: Acid Degradation of Diacerein and Aceclofenac in mixture**

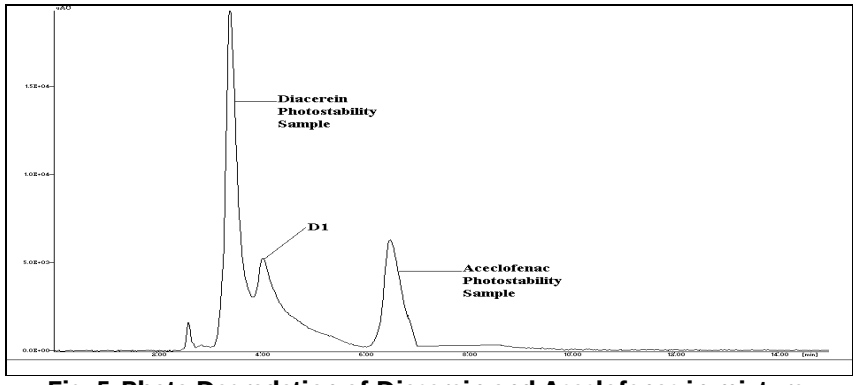


**Fig. 5: Photo Degradation of Diacerein and Aceclofenac in mixture.**

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