Development and Validation of Stability Indicating HPTLC Method for Determination of Diacerein and Aceclofenac as Bulk Drug and in Tablet Dosage Form

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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 HPTLC assay method. Different degradation peaks were observed for diacerein when it was exposed to alkaline, and acid catalysed hydrolysis. For aceclofenac, decrease in peak area was observed with single peak of degradation product after oxidation. For HPTLC, RP-18 F_{254s} pre-coated plates, and mobile phase consisting of methanol: water 7: 3 v/v was used to achieve separation. Quantitation was done at 268 nm. The method was validated as per ICH Q2 R1 guidelines and results were in limit. The method was found to be simple, specific, precise, and stability indicating.

Keywords: Diacerein, Aceclofenac, Stability indicating, HPTLC, 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. 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 spondylitis⁵. 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 method⁸. For aceclofenac literature surveys reveals many papers *viz.* simple spectrometric methods⁹, spectrofluorimetric method¹⁰, stability indicating HPLC¹¹, and HPTLC methods¹².

Some research articles are also available for diacerein and aceclofenac combination as simultaneous UV spectrophotometric methods^{13,14}, RP-HPLC method¹⁵, and HPTLC method¹⁶.

No reports were found for the stability indicating assay method as per ICH guidelines, for diacerein and aceclofenac in combination by HPTLC method. This paper describes a simple, accurate, sensitive, and validated stability indicating HPTLC method for diacerein and aceclofenac in combination as the bulk drug and in tablet dosage forms as per ICH guidelines^{17,18}.

Experimental

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, Conc. HCl, NaOH and H₂O₂ used were of analytical reagent grade.

For stability indicating HPTLC method development, Camag HPTLC system consisting of Linomat-5 applicator, Camag TLC Scanner 3, and WinCATS software V 1.4.2 were used. For photo-degradation studies, Photostability Chamber was used. (Make - Newtronic) All the weighing was done on Shimadzu balance (Model AY-120).

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 100 mg of aceclofenac was weighed and powdered. Powder equivalent to 25 mg of diacerein and 50 mg of aceclofenac was transferred to 25 mL volumetric flask and was diluted with methanol to 25 mL (1000 mcg/mL of diacerein and 2000 mcg/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.

For alkali hydrolysis, 2.5 mL of mixed working standard solution of diacerein was mixed with 2.5 mL of 0.01 N NaOH. The solution was diluted to 25 mL with methanol and kept for 5 min. For acid degradation, 2.5 mL of mixed working standard solution of diacerein was mixed with 2.5 mL of 0.1 N HCl. The solution was diluted to 25 mL with methanol and kept for 15 min. For neutral hydrolysis, 5 mL of mixed working standard solution of Diacerein was mixed with 5 mL water. The solution was diluted to 50 mL and was reflux for 3 h. The solution was cooled to room temperature and again volume was made to 50 mL if required. For oxidation study, 2.5 mL of mixed working standard solution of diacerein was mixed with 2.5 mL 1% solution of H_2O_2 . The solution was diluted to 25 mL with methanol and was kept for 30 min. Dry heat studies were performed by keeping drug sample (mixture of diacerein and aceclofenac) in oven at 80°C for a period of 24 h. Samples were withdrawn after 24 h, dissolved in methanol and diluted to get $10\text{ }\mu\text{g/mL}$ as final conc.. 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. Samples were weighed, dissolved and diluted get $10\text{ }\mu\text{g/mL}$.

Method Validation

Method was validated for all the parameters as per ICH guidelines. Linearity was studied by analyzing five concentrations of each drug and process was repeated five times. Precision of the system was evaluated by analyzing six independent standard preparations and % RSD value was calculated to determine any intra-day and inter-day variation. 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.

The detection limit (DL) and the quantitation limit (DL) were calculated based on the Standard Deviation of the lowest concentration (σ) and the Slope (S). $\text{DL} = 3.3\text{ }\sigma/\text{s}$ and $\text{QL} = 10\text{ }\sigma/\text{s}$. The specificity of the method was ascertained by peak purity profiling studies and Robustness was also studied.

Results and Discussion

Chromatographic Conditions

$10\text{ }\mu\text{g/mL}$ of mixed solution was prepared from mixed working solution ($100\text{ }\mu\text{g/mL}$) $10\text{ }\mu\text{L}$ (100 ng/spot) of this solution was applied on TLC plate. The development chamber was saturated with mobile phase methanol: water 7: 3v/v for 15 min. The spotted plate was placed in the saturated chamber and developed up to 90 mm distance. The plate was dried and was scanned over 90 mm distance at 268 nm. The retention factor was found to be: Diacerein = 0.71 ± 0.03 Aceclofenac = 0.53 ± 0.02 (Figure 1).

Stress Degradation Study

After alkaline degradation, Diacerein was completely degraded and showed 5 peaks (D1-D5) of degradation products. While no degradation peak was observed for aceclofenac with 88.95% recovery. [Figure 2] After acid hydrolysis, 62.48% Diacerein was recovered with 3 peaks (D1-D3) of degraded product while 41.75% Aceclofenac was recovered with no peak of degraded product [Figure 3]. After neutral hydrolysis, 81.10% Diacerein was recovered and 66.06% Aceclofenac was recovered. After oxidation, 74.24% Diacerein was recovered while 29.84% Aceclofenac was recovered with 1 peak (d-1) of degraded product [Figure 4]. After dry heat, 87.13% Diacerein was recovered with no peak of degraded product while 84.27% Aceclofenac was recovered with no peak of degraded product. After photo-degradation, 57.92% Diacerein was recovered while 58.93% Aceclofenac.

Figure 1. A- MeOH, B- Mixture (100 ng/band of both).

Figure 2. A- Blank NaOH, B- Mixture (500 ng/band) treated with NaOH.

Figure 3. A- Blank HCl, B- Mixture (500 ng/band) treated with HCl.

Figure 4. A- Blank H₂O₂, B- Mixture (500ng/band) treated with H₂O₂.

Validation [Table 1]

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 20 - 100 ng/band for diacerein and 40-200 ng/band for aceclofenac with r^2 0.998 for both the drugs. The developed method was found to be precise as the % RSD value for both interday and intraday were less than 2. The result obtained for recovery studies at each level ($n = 3$ for each level) indicated the mean recovery between 98% to 102% for both diacerein and aceclofenac. The limit of detection as calculated was found to be 5.354 ng/band for diacerein and 4.051 ng/band for aceclofenac. The limit of Quantitation was found to be 16.241 ng/b and for diacerein and 12.277 ng/band for aceclofenac. The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to >0.995 , indicating the non interference of any other peak of degradation product or impurity. Robustness was calculated as %RSD and was less than 2%.

Table 1. Validation results.

S. No.	Validation Parameter	Results	
		Diacerein	Aceclofenac
1.	Linearity	$Y = 30.51x + 444.9$	$Y = 15.85x + 412$
2.	Range	$R^2 = 0.998$	$R^2 = 0.998$
3.	Precision	(%RSD)	
	A) Intraday precision	0.76	0.108
	A) Interday precision	1.414	1.392
4.	Accuracy	% Recovery	
	80%	100.45	99.19
	100%	101.29	98.98
	120%	100.83	101.41
5.	LOD	5.354 ng/band	4.051 ng/band
6.	LOQ	16.241 ng/band	12.277 ng/band
7.	Specificity	Specific	Specific
8.	Robustness	Robust	Robust

Conclusion

This study presents a simple and validated stability-indicating HPTLC 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.

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