Original Article

Stress degradation studies and development of a validated stability-indicating-assay-method for determination of diacerein in presence of degradation products

Abstract

Background: To understand the degradation behavior of diacerein and to develop a simple, rapid, sensitive, and validated RP-HPLC method for the determination of diacerein, in the presence of its degradation products. **Materials and Methods:** An accurate, sensitive, precise, rapid, and isocratic reversed-phase *high-performance liquid chromatography* (RP-HPLC) method, equipped with a photo-diode array (PDA) detector for analysis of diacerein in the bulk drug has been developed and validated. The best separation was achieved on a 250 mm × 4.6 mm i.d., 5-µm particle, RP C18 column with 50 : 50 (v/v) of water (pH adjusted to 2.9 with orthophosphoric acid) : acetonitrile as the mobile phase, at a flow rate of 1.0 ml/minute. The detection wavelength was set at 257 nm. **Results:** The response was a linear function of concentration over the range of 0.50 – 20 µg/ml (r = 0.999) and the limits of detection and quantitation were 0.1 µg/ml and 0.50 µg/ml, respectively. The method was validated in accordance with the *International Conference on Harmonization* (ICH) guidelines. The drug was subjected to oxidative, hydrolytic, photolytic, and thermal stress. The drug decomposed under alkaline hydrolytic stress conditions and also on thermal degradation and photolysis. It was stable on acid hydrolysis and oxidation. The degradation products produced as a result of this stress did not interfere with the detection of diacerein, and the assay could thus be regarded as stability-indicating. **Conclusion:** The method was suitable for application in the analysis of formulations of diacerein in quality-control laboratories, because it was simple and rapid, with good accuracy and precision.

Key words: Accurate, diacerein, photo-diode array detector, stability-indicating

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INTRODUCTION

Quality control has become a stringent aspect of pharmaceutical manufacture to minimize batch-to-batch variation and ensure quality. Today, stability is the main and most significant quality requirement for a pharmaceutical product. Stable preparations have a direct emphasis on the quality of the product, assuring its precise delivery. Also the shelf life period of the drug formulation is dependant on the analytical studies at normal and stressed conditions. The ICH drug stability testing guideline Q1A (R2) emphasizes that the analysis of samples of active pharmaceutical ingredients, which are subjected to stress conditions, should be carried out, to establish their inherent stability characteristics, thereby leading to identification of the degradation products through the use of validated stability-indicating analytical methods. Stability-indicating-assay-methods (SIAMs) are specific ones, which evaluate the drug in the presence of its degradation products, excipients, and additives.[1]

Diacerein also known as diacetylrhein is chemically 4,5-diacetyloxy-9,10-dioxo-anthracene-2- carboxylic acid [Figure 1]. It is a yellow anhydrous powder that is practically insoluble in water, soluble in dimethyl sulfoxide and N,N-dimethylacetamide, and slightly soluble in methanol.[2] The drug is used widely

used in the treatment of osteoarthritis. Diacerein is reported to act as an interleukin-1 inhibitor. It directly inhibits IL-1 synthesis and release *in vitro* and down modulates IL-1-induced activities. Also, it has been shown to possess a disease-modifying effect in experimental models of osteoarthritis and in human subjects with finger, joint, and knee osteoarthritis. Diacerein is extensively converted *in vivo* to several hydroxylated metabolites via cytochrome P-450 (CYP) oxidative metabolism.[3-10]

In literature, the analytical methods reported include, the HPLC method for determination of diacerein in bulk drug and pharmaceutical dosage forms, using the UV detector.[11] A further literature survey also revealed that there was no stability-indicating assay method for the drug, employing the ICH-suggested approach. Therefore, the objective of the present study was to understand the degradation behavior of diacerein and to develop a simple, rapid, sensitive, and validated RP-HPLC method for the determination of diacerein, in the presence of its degradation products. Hence, an isocratic RP-HPLC method with a photo diode array detector was successfully developed and validated, in accordance with the requirements of the ICH guidelines.[12-13]

EXPERIMENTAL

Reagents and materials

Diacerein working standard (98% pure) was procured from Umedica Laboratories Pvt. Ltd. HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Orthophosphoric acid used for adjusting the pH of the mobile phase was of AR grade (S. D. Fine Chemicals). The deionized and ultra-pure water used in all experiments was obtained from the Milli-Q System (Millipore).

Instrumentation and chromatographic conditions

Chromatography was performed with the Shimadzu HPLC equipment, comprising of an LC-8A VP pump, a Shimadzu SCL-10A VP system controller, a Rheodyne injector fitted with a 20-µL loop, and a Shimadzu SPD-M10A VP photo diode array detector. The data was recorded and evaluated using the Class VP 5.032 software as the data integrator. Compounds were separated at room temperature (25 ± 2°C) on a 250 mm × 4.6 mm i.d., 5-µm particle size, (Waters) RP-Spherisorb C18 reversed phase column, with 50 : 50 (*v*/*v*) of water (pH adjusted to 2.9 with orthophosphoric acid) : acetonitrile as the mobile phase, at a flow rate of 1.0 ml/minute. The injection volume was 20 µl. The mobile phase was filtered through a 0.45 µm filter paper and sonicated before use. The detection wavelength was set at 257 nm. The pH of the mobile phase was checked on a pH / ion analyzer (Lab India PHAN, India). Refluxing of the drug in hydrolytic conditions was carried out in a round bottom flask-condenser assembly. The Mettler Toledo (MT5) analytical balance was used for weighing.

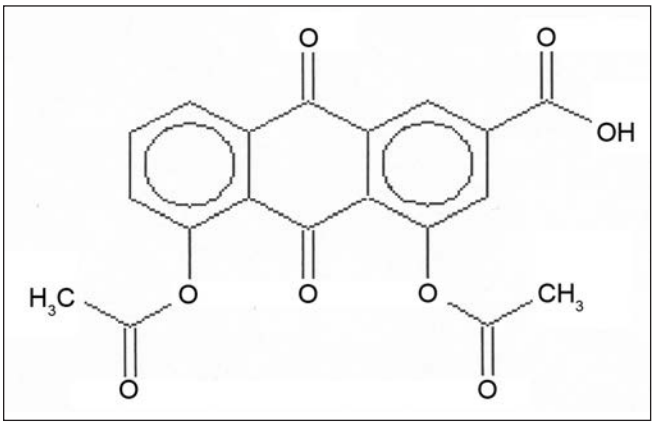

Figure 1: Structure of diacerein

Standard solution preparation

Ten milligrams of working standard of diacerein was accurately weighed and dissolved in 10 ml of methanol to give a stock solution of 1 mg/ml. Furthermore, standard solutions were made by diluting the stock solution with the mobile phase to give solutions in the concentration range of 0.50 µg/ml to 20.00 µg/ml.

Stress degradation studies

Acid hydrolysis

Acid-induced, forced degradation was performed by adding an aliquot of stock solution (1 mg/ml) of diacerein to 10 ml each of methanol and 0.1 M HCl and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room temperature, neutralized to pH 7 by the addition of 0.1 M NaOH, and diluted to 100 ml with the mobile phase so as to get a final concentration of 10 µg/ml.

Alkaline hydrolysis

Forced degradation in alkaline media was performed by adding an aliquot of stock solution (1 mg/ml) of diacerein to 10 ml each of methanol and 0.1 M NaOH, and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room

temperature, neutralized to pH 7 by addition of 0.1 M HCl, and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 μg/ml.

Oxidative degradation

To study the effect of oxidizing conditions, an aliquot of stock solution (1 mg/ml) of diacerein was added to 10 ml of 30% H_2O_2 solution and the mixture was refluxed at 60°C for approximately six hours. The solution was left to reach room temperature and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 μg/ml.

Thermal degradation

To study the effect of temperature, approximately 50 mg diacerein was stored at 100°C in a hot air oven for 24 hours. It was then dissolved in 10 ml of methanol and the volume was adjusted to 50 ml with the mobile phase. The above solution was further diluted with the mobile phase, to give a solution of final concentration equivalent to 10 μg/ml of diacerein.

Photolysis

To study the effect of UV light, approximately 50 mg of diacerein was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 24 hours, and then dissolved in 10 ml of methanol. The volume was made up by the mobile phase in a 50 ml volumetric flask, and then 1 ml of stock solution was further diluted with the mobile phase to give a solution of final concentration equivalent to 10 μg/ml of diacerein.

Twenty microliters of the resulting solution was injected into the HPLC system and the chromatograms were recorded. The stability samples were analyzed using a PDA detector to determine the peak purity.

Validation of the method

Linearity and range

A stock solution of the drug was prepared at a strength of 1 mg/ml. It was diluted to prepare solutions containing 0.50 – 20.00 μg/ml of the drug. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 μl).

Precision

Twelve injections, of three different concentrations (1.5, 10, and 17 μg/ml), were made on the same day and the values of relative standard deviation (% R.S.D.) were calculated to determine the intra-day precision. These studies were also repeated on different days to determine the inter-day precision.

Accuracy

Accuracy was evaluated for the known concentrations (1.5, 10, and 17 μg/ml) of the drug. The recovery of the added drug was determined.

Specificity and selectivity

The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks.

LOD and LOQ

The LOD and LOQ were determined at signal-to-noise ratios of 3 : 1 and 10 : 1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness

Robustness of the method was investigated by varying the chromatographic conditions, such as, change of flow rate (± 10%), organic content in the mobile phase (± 2%), wavelength of detection (± 5%), and pH of the buffer in the mobile phase (± 0.2%). Robustness of the developed method was indicated by the overall % RSD between the data, at each variable condition.

Solution stability

The solution stability was carried out by storing standard solutions of diacerein in tightly capped volumetric flasks at -20^{0}C for seven days. These solutions were assayed after seven days against fresh samples.

RESULTS AND DISCUSSION

Degradation behavior

High-performance liquid chromatography studies on diacerein, under different stress conditions, suggested the following degradation behavior [Table 1]. The representative degradation chromatograms of the degradation are shown in Figures 2a-f. The drug was comparatively stable to acid hydrolysis and oxidative hydrolysis [Figures 2c and e]. There was severe decomposition of the drug on alkaline hydrolysis, followed by decomposition under thermal degradation and photolysis [Figures 2d, f and g]. The percent degradation was calculated by the formula:

Table 1: Percent degradation of diacerein and retention time of the degradation products

Sr. No.	Conditions	Retention time of drug / degradation products (Min)	Peak area of drug	Percent degradation of drug (n = 5)
1	Untreated stock solution (10 µg/ml)	5.21	6438127.50	-
2	Acid hydrolysis	5.57, 8.33	5825476.00	9.52
3	Base hydrolysis	5.22, 6.61, 7.14, 8.55, 14.24, 25.39	3086344.81	52.06
4	Oxidation	5.21, 8.25	5982790.52	7.07
5	Thermal degradation	5.60, 8.53	3432351.56	46.69
6	Photolytic degradation	5.61, 8.48, 14.23	5124187.00	20.41

Table 2: Results from regression analysis and system suitability of diacerein

Description	Values
Retention time (min)	5.20
Linear range (µg/ml)	0.50 – 20
Limit of detection (LOD) (µg/ml)	0.10
Limit of quantification (LOQ) (µg/ml)	0.50
Slope (*m*)	224398.42
Intercept (*c*)	152531.40
Standard deviation	2671.15
% RSD	1.190
Correlation coefficient (*r*)	0.999
Tailing factor	1.19
Theoretical plates	6053.96

Table 3: Precision and recovery data

Actual concentration (µg/ml)	Precision		% Recovery	
	Measured Concentration (µg/ml) ± S.D.; % R.S.D.			
	Intra-day	Inter-day	Intra-day	Inter-day
1.50	1.51 ± 0.05; 2.03	1.50 ± 0.03; 2.19	100.66	100.40
10	9.99 ± 0.10; 1.01	9.98 ± 0.09; 0.99	99.97	99.89
17	17.06 ± 0.30; 1.69	17.02 ± 0.26; 1.56	100.36	100.09

$$\% \text{ degradation} = \frac{\text{Peak area of untreated stock solution} - \text{peak area of treated stock solution}}{\text{Peak area of untreated stock solution}} \times 100$$

Establishment of a stability-indicating method

The stability-indicating ability of the method was thus established. Maximum degradation of diacerein was observed in alkaline medium, followed by decomposition under thermal degradation and photolysis. The drug was stable to acid hydrolysis and oxidative conditions. Percent degradation of diacerein under all stressed conditions and retention time of the degradation products are included in Table 1. Separation of diacerein from its degradation products has been performed on an RP C18 column. Initially water and acetonitrile in various proportions were tried. However, good resolution between peaks was not obtained. Different ratios of orthophosphoric acid in water (pH 2.9) and acetonitrile were tried. Increasing the acetonitrile ratio was accompanied by a decrease in retention time of the different components; however, separation was still achieved. In order to ensure complete separation and high resolution, the chosen ratio was 50 : 50 (% *v*/*v*). Simultaneous monitoring with the PDA detector was carried out at a range of wavelength between 200 – 400 nm. Detection was carried out at 257 nm, where maximum sensitivity was observed. The specificity of the method is illustrated in all the chromatograms of the degradation studies.

Validation of the method

The method was validated with respect to parameters like linearity, precision, accuracy, specificity, and robustness.

The LOD and LOQ concentrations were found to be 0.10 and 0.50 µg/ml. Results from the regression analysis with system-suitability data are listed in Table 2. Results of precision and accuracy are listed in

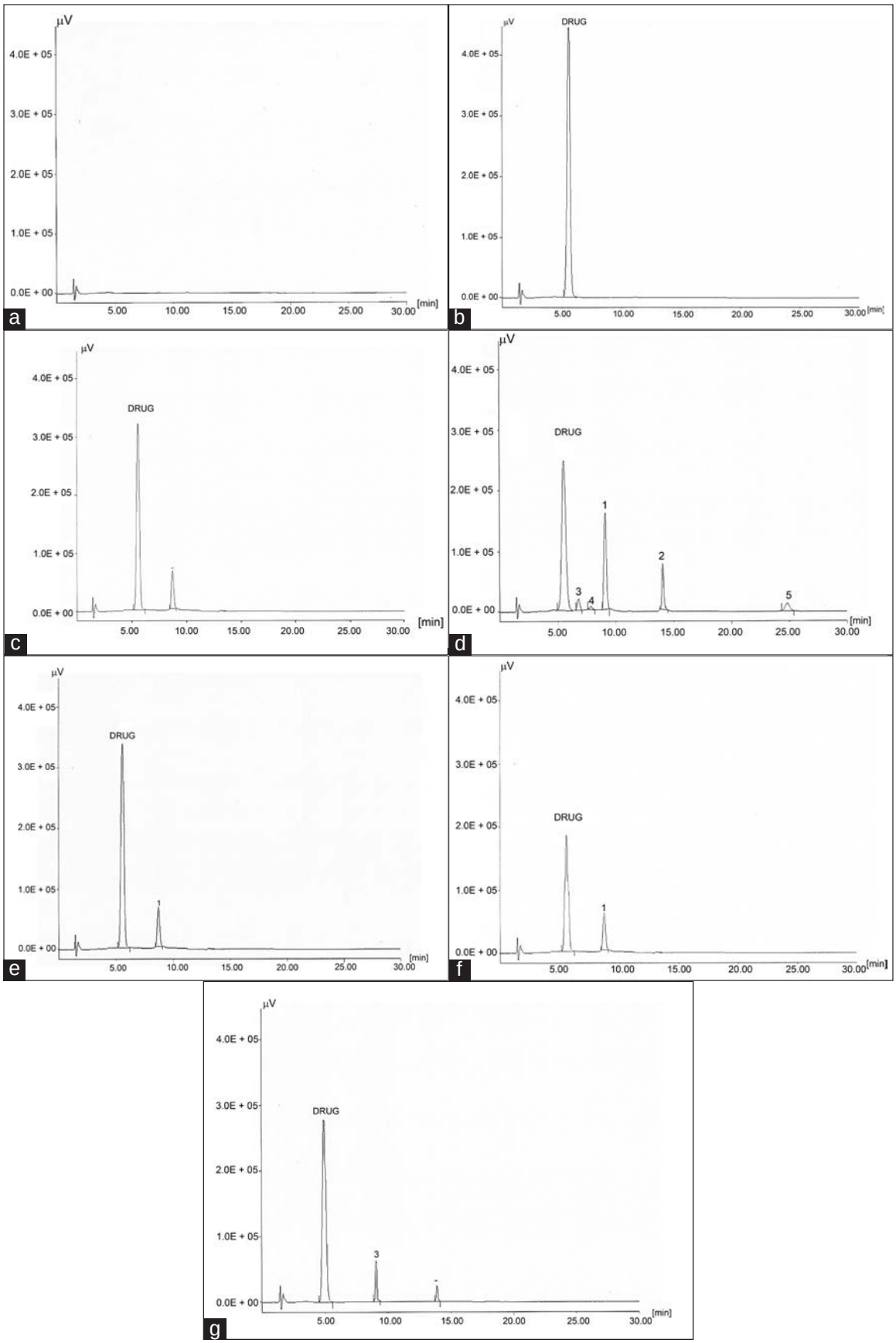

Figure 2: Representative chromatograms of diacerein for the stability method (a) Blank (b) Untreated stock solution, (c) Acid hydrolysis, (d) Base hydrolysis, (e) Oxidation, (f) Thermal degradation, (g) Photolytic degradation

Table 3. Influences of small changes in chromatographic conditions, such as, change in flow rate (± 10%), organic content in the mobile phase (± 2%), wavelength of detection (± 5%), and pH of buffer in the mobile phase (± 0.2%) were studied, to determine the robustness of the method. The RSD was < 2% in all cases. The RSD values of the assay of diacerein during solution stability experiments were < 2%. No significant changes were observed during solution stability. The solution stability data confirms that the sample solutions were stable for at least seven days.

CONCLUSIONS

The study concludes that diacerein is most labile to alkaline hydrolysis followed by thermal degradation and photolysis. It is stable to acid hydrolysis and oxidative stress conditions. The proposed method is sensitive, precise, accurate, and stability-indicating, resolving all the degradation products from the drug. Thus, the proposed method can have its application in the determination of diacerein in bulk drug, pharmaceutical formulation, as well as in the presence of all its degradation products. The ICH guidelines have been followed throughout the study for method validation and stress testing, and thus the proposed method has wide industrial applicability.

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