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

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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

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
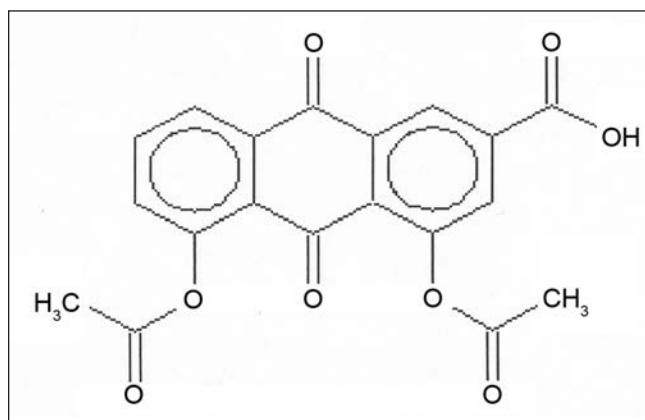

Figure 1: Structure of diacerein

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.

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