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Original Research Paper

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF ALKALINE DEGRADANT OF DIACEREIN USING LC–MS

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ABSTRACT

Isolation identification and characterization of the structures of degradation products is typically collaborative research involving knowledge of analytical and organic chemistry with spectroscopic information. Stability testing guidelines issued by International Conference on Harmonization (ICH) require the reporting, identification and characterization of degradation products. A simple, selective and reproducible stability-indicating liquid chromatographic method was developed for the determination of Diacerein. Developed method was used to evaluate the Diacerein in bulk drug and its degradation product. The drug was subjected to stress conditions of acid hydrolysis, alkali hydrolysis, oxidation, thermal, and photo degradation according to ICH guidelines. The drug was found to degrade extensively in alkali. The drug and the degraded compounds were separated by isocratic reverse-phase high performance liquid chromatography. An isocratic separation was achieved using a thermosil C18 column (ODS-3, 250×4.6 mm, 5 µm) with a flow rate of 1 ml/min and using a UV detector set at 254 nm. The mobile phase consisted of Phosphate buffer (pH 3.6): acetonitrile (45:55 % v/v). Degradation products resulting from the stress studies did not interfere with the detection of diacerein. Structural elucidation of impurity was carried out by LC–MS, 1H NMR, and IR spectroscopy. The isolated impurity was characterized as 5-acetoxy-4-hydroxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid. An understanding of the different parts of the molecule that are susceptible to degradation can help in the designing of more stable analogs.

Keywords: Stability-indicating method, Forced degradation, Characterization, LC-MS, Diacerein.

INTRODUCTION

Diacerein is chemically 1, 8-diacetoxy-3-carboxy anthraquinone (Figure 1) is a novel anthraquinone derivative that has been used in the treatment of arthritis which selectively inhibits the Interleukin-1 (IL-1).[1] It is semisynthetic anthraquinone derivative extracted from plants. It directly inhibits IL-1 synthesis and release which plays a fundamental role in arthritis. In contrast to NSAIDS, diacerein does not inhibit synthesis of prostaglandin and thus it is free form gastrodeodenal toxicity. It also involved in prevention of loss of hydroxyproline and proteoglycans in joint cartilage.[2] The ICH guideline states that stress testing are required to be performed to elucidate the inherent stability characteristics of the active drug substance and establishing the degradation pathways.[3-5] The identification of impurities and degradants in pharmaceuticals is critically important for reasons of both product efficacy and patient safety. The impurities and degradants may evoke any form of adverse response, either pharmacologic or

toxicologic in patients undergoing medication. A degradants is the simplest unwanted constituent in a pharmaceutical agent. It may be formed by the degradation of the pharmaceutical agent itself or through an interaction or reaction of the active ingredient in a formulation with one of the other constituents in a dosage form. Generally, the level of a degradation product will increase with time. Forced degradation study is a well-established method to identify the possible degradation impurities. Stress testing is an important tool for the prediction of stability-related problems. Separation of all these impurities including degradation impurities in a single analytical HPLC method is a challenging job. ICH guidelines are forcing to monitor and control the level of impurities to the specified limit in drug substances and drug products and hence there is a practical and scientific need to develop a suitable and efficient analytical method for analysis.[6-8] Well-designed stress-testing studies can lead to a thorough understanding of the intrinsic stability characteristics of the drug molecule. Literature survey revealed that there are few chromatographic methods available for force degradation of diacerein and few UV methods are also reported. These methods include UV spectrophotometric,[9-13], HPTLC[14-15] and HPLC[16-20] methods for determination of diacerein alone or in combination with other drugs. Information on isolation and characterization of degradation impurities formed under the stress conditions is lacking. As per the requirements of various regulatory authorities, the impurity profile study of drug substance and drug product involves characterization of impurities using a suitable analytical method in the final product.[21-22] The aim of the present work was to study alkali degraded compound of diacerein and its characterization by LC-MS, Fourier transform-IR, and 1H NMR which will be beneficial in establishing the degradation pathways.

EXPERIMENTAL

Chemical and Reagents

Diacerein pure compound was supplied by Glenmark Pharmaceuticals, Ltd., Kurkumbh, India. All the chemicals used were of HPLC grade. HPLC grade water was obtained by double distillation in the laboratory, filtered through Millipore, Milford filter and was used throughout study.

Selection of Detector and Wavelength

Diacerein standard drug was prepared in acetonitrile and scanned in UV visible spectrometer (UV 630). Diacerein had maximum UV absorbance at 254 nm; hence detection at 254 nm was selected for the method development process.

HPLC Instrumentation and Conditions

The HPLC system consists of a pump (Jasco PU-2080, intelligent HPLC pump) with injecting facility programmed at 20 µl capacity per injection. The detector consists of a UV/ VIS (Jasco UV 2075) set at a wavelength of 254 nm. The chromatographic separation was performed using a thermosil® ODS-3, 250 mm × 4.6 mm, 5µm column. Mobile phase selected was phosphate buffer (pH 3.6): acetonitrile (45**:**55 % v/v) at a flow rate of 1 ml/min [5] .The eluent was monitored at a wave length of 254 nm.

LC–MS Conditions

LC–MS analysis was carried out for the alkali stress sample of diacerein using a Agilent Series liquid chromatography coupled with 6460 Q Trap triple quadruple mass spectrophotometer. The LC–MS analysis was carried out by using column Zorbax C18, 100 mm x 4.6 mm, 5 µm as stationary phase. A combination of acetonitrile used as solvent A and a 0.01 M ammonium formate buffer as solvent B was used as mobile phase in a ratio of 55:45 % v/v. UV detection was carried out at 254 nm and flow rate was adjusted to 0.8 ml/min. The mass spectrum of the impurities was carried out on a triple quadrupole mass spectrometer. The analysis was performed in the Negative (−ve) ion mode with electron spray ionization (ESI) technique.

NMR Spectroscopy

The NMR experiment was performed on NMR spectrometer at 400 MHz on Bruker NMR AV Microbay at Dept. of Chemistry, Savitribai Phule Pune University, Pune. The 1H Chemical shift

values were reported on the scale in ppm, relative to TMS as internal standard.

FT-IR Spectroscopy

The FT- IR spectra of standard diacerein and isolated impurity were recorded on Jasco FTIR 4100 by using KBr pellet technique.

Preparation of Stock and Standard Solutions

A standard stock solution of diacerein was prepared by accurately weighing 100 mg and diluting to 100 ml with acetonitrile. Aliquots of standard stock solutions of diacerein were transferred into 10 ml volumetric flasks and diluted to volume with mobile phase to get the final concentrations.

Forced Degradation Studies

Diacerein pure drug was stressed under various conditions according to ICH guidelines to conduct forced degradation studies.[16-19] From stock solution of 1000 µg/ml aliquots of standard diacerein drug solutions were prepared and were used for forced degradation.

Alkali Degradation Studies

For alkali degradation study, to 2 ml of std. stock solution, 2 ml of 0.01N sodium hydroxide solution was added and was kept for 20 minutes at room temperature. The resultant solution was neutralized and diluted to obtain 20 µg/ml and 20 µL was injected into the system and the chromatograms were recorded.

Isolation of Impurities

For isolation of the alkali degradant of diacerein, 5 ml of 0.1 N NaOH was added to 100 mg of diacerein, dissolved and kept for 48 hrs. Degraded product was separated and filtered through Whatman filter paper and washed with filtered distilled water and dried at 60 ^{o}C for 10 min. 25mg of this degraded compound was dissolved in 25 ml acetonitrile to make the stock solution. The resultant solution was diluted and made up to the volume with mobile phase to obtain 20 µg/ml solution and injected into the system and the chromatograms were recorded.

RESULTS AND DISCUSSION

Stability Studies

In the present study, alkali degraded impurity of diacerein in bulk drug was detected by HPLC. Extensive degradation of diacerein occurred only in alkaline hydrolytic conditions. The degradation products were separated from the standard compound. The drug was found to be more unstable under basic stress conditions when kept for 20 minutes at room temperature. An attempt was made to isolate degradation compound. Diacerein was found to be totally degraded when it was kept for 48 hrs in 0.1 N NaOH. This degraded compound was filtered, washed and dried as described in procedure section. It was dissolved in acetonitrile and diluted with mobile phase to obtain 20 µg/ml solution and injected into the system. The standard drug and the degraded compound were separated by isocratic reverse-phase high performance liquid chromatography. The chromatograms were recorded. Diacerein standard and the major degradation product of alkali hydrolysis of diacerein was found at retention time of 2.9 min and 3.6 min, respectively (Figure 2 a, b, c) and (Figure 3). The Alkali major degradation product was then characterized by chromatographic and spectroscopic studies. Based on the molecular weight and the fragmentation pattern, the presence of degradation product was confirmed and the structure was proposed.

Structural Elucidation of Impurity-1

The LC–MS studies confirm the presence of degradation product depending on the fragmentation pattern. The fragmentation pattern (Figure 4) clearly indicates that there is one possible structure of degradation product formed under alkali condition. The LC–MS studies indicated that the impurity is having the molecular weight MW=326. The molecular weight 326 indicates that it is a mono-acylated diacerein. The position of the acylation was confirmed by NMR studies. The LC–MS was conducted in Negative (−ve) ion Electron spray ionization (ESI). Impurity shows the molecular ion peak (MH−) 326.01. It indicates that the molecular weight of the impurity is 326. The molecular weight of 326 confirms that instead of two acetyl groups in diacerein, the impurity

contains only one acetyl group. The fragment ion 282.01 confirms the presence of carboxylic group. Further confirmation of the position of acetoxy group in impurity was carried out by 1H NMR spectra and IR spectra.

CONCLUSION

This research paper describes the identification, isolation and structural elucidation of alkali degraded product of diacerein. The major unknown alkali degradation product was isolated and was characterized by using chromatographic and spectroscopic techniques namely LC-MS, NMR and IR. Finally from the chromatographic and spectroscopic combined data impurity was assigned as 5-acetoxy-4- hydroxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid. The information presented in this research article could be very useful for quality monitoring of bulk samples and also employed to check the quality of drug during stability studies. The LC method used for the study was found to be simple, selective, precise, accurate and robust hence; this method can be used for routine testing as well as stability analysis of diacerein and its degradation product in drug substance and drug products. Determining the structures of the major degradation products can reveal whether a known carcinogen or toxic compound is or might possibly be formed or not. It can also be useful for preclinical discovery efforts during structure-activity relationship investigations. The development of a stable formulation is also aided by an understanding of the reactive parts of the drug molecule. Hence, it can be concluded that structure elucidation of degradation impurities can be helpful in formulation development to comply with the regulatory requirements for impurity limits. Finally from the chromatographic and spectroscopic data impurity-1 was assigned as 5-acetoxy-4- hydroxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid. The structures of diacerein and the isolated impurity are shown in Figure 4 and Figure 5 respectively.

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Table1: ^{1}H NMR and IR Data

Isolated Impurity	
^{1}H NMR chemical shift values, in δ ppm	12.05 (s, 1H, COOH), 7.36-8.14 (m, 5H, aromatic), 2.51(s, 3H, OCOCH3) ppm, 3.64 (s OH)
IR spectra	3410 (−OH stretching vibration aromatic), 3061 (O-H stretch, COOH), 3028 (C-H aromatic), 1698 (C=O stretch, carbonyl), 1760 (C=0 ester),1651 (C=O stretch, COOH), 1476 (C=C stretch, aromatic)

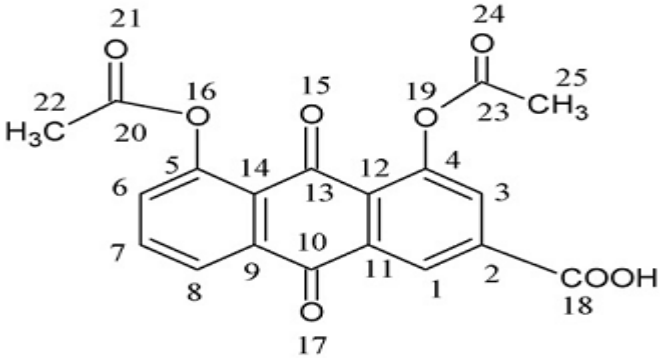

Figure 1: Diacerein standard drug

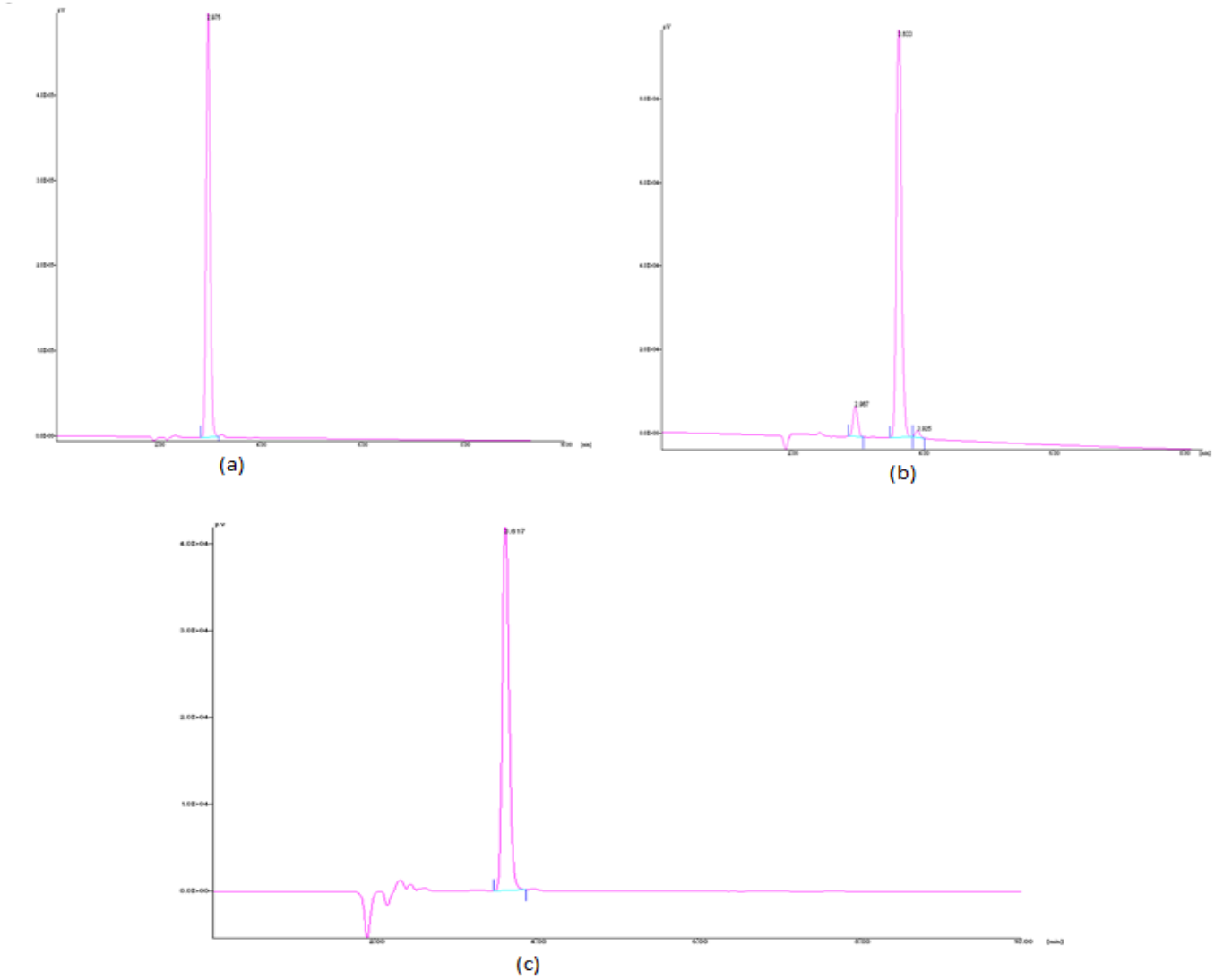

Figure 2: (a) Diacerein standard eluted at a t_R 2.9 min, (b.) Degradation compound t_R 3.6 min, (c.) Isolated compound of alkali major degradation of diacerein eluted at t_R 3.6 min.

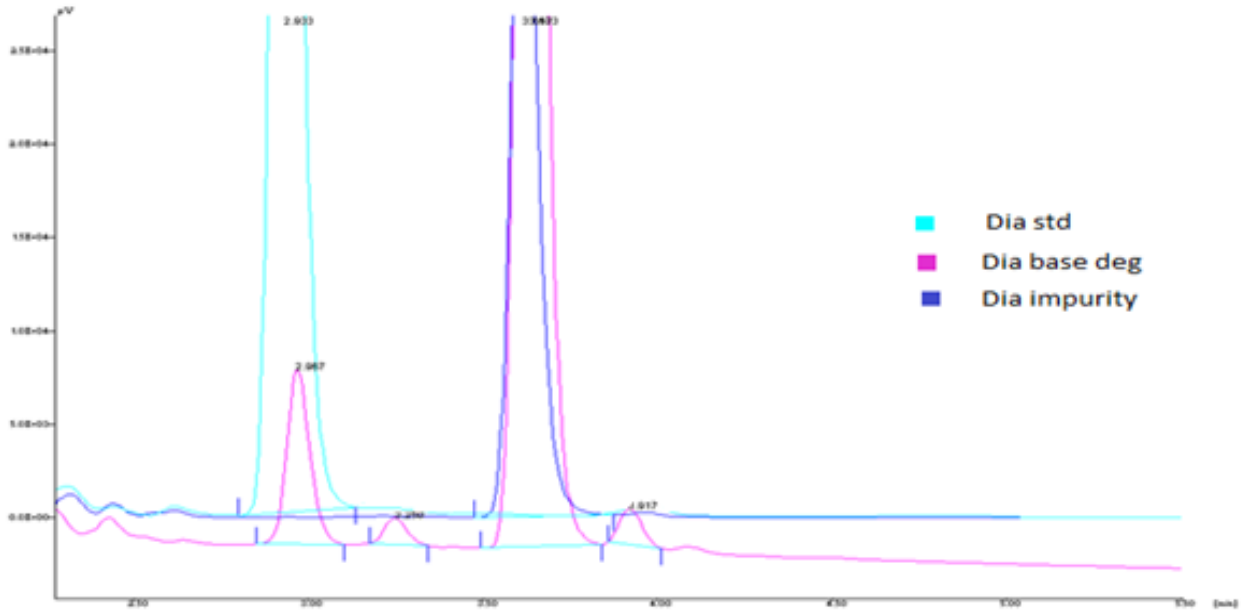

Figure 3: Overlay Chromatogram of std diacerein drug, alkali degradation in solution and isolated compound

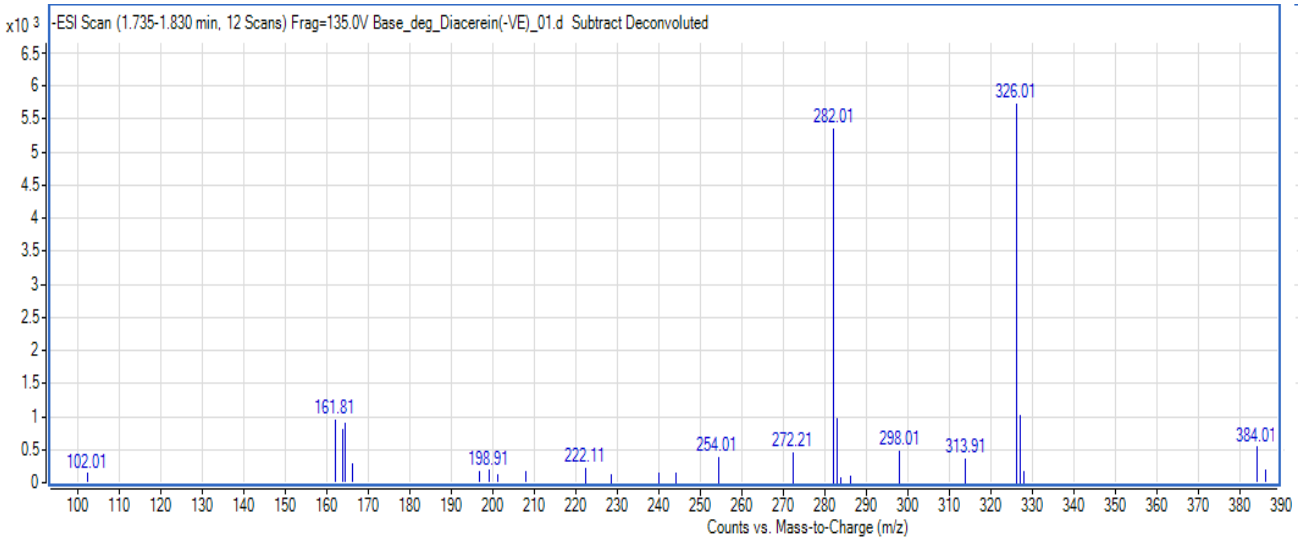

Figure 4: LC -MS of alkali degraded isolated product

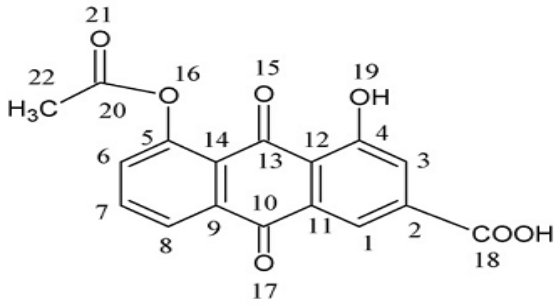

Figure 5: Isolated impurity: 5-acetoxy-4- hydroxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid.

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