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Short communication

Isolation and structural elucidation of two impurities from a diacerein bulk drug

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ABSTRACT

Two impurities were found in the crude sample of diacerein. The level of these impurities 1.14% and 1.24% were detected by isocratic reverse-phase high performance liquid chromatography (HPLC). The molecular weights of the impurities were determined by liquid chromatography–mass spectroscopy (LC–MS) analysis. These impurities were isolated from crude sample of diacerein by reverse-phase preparative liquid chromatography. These impurities were characterized as 5-acetoxy-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Impurity-1) and 4-acetoxy-5-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Impurity-2) respectively. Structural elucidation of both the impurities were carried out by ^{1}H NMR, ^{13}C NMR, DEPT, 1D NOESY, MS and IR spectroscopy.

1. Introduction

Diacerein is chemically known as 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid, it is an anthraquinone derivative that has been used in the treatment of osteoarthritis [1–8]. Diacerein is also used to treat and prevent vascular diseases [9]. Diacerein can be readily synthesized in few steps from the naturally occurring glucopyranoside aloin [10,11] (Fig. 1). As per the regulatory requirements the impurity profile study has to be carried out for final product [12]. There is no extensive literature found on the impurity profile of diacerein, however the literature indicates a single publication on the stability indicating HPLC method for the determination of diacerein in bulk drug substances [13]. Giannellini et al. has reported three degradation products formed during acid hydrolysis. One was rhein and two were monoacetylated product with molecular weight 326. The aim of the present work was to study impurities present in crude sample of diacerein.

The present study describes identification, isolation and characterization of the process related impurities in diacerein formed in the manufacturing process of diacerein used by Emcure pharmaceutical Ltd., India.

2. Experimental

2.1. Samples and chemicals

The investigated sample of crude diacerein was synthesized in Emcure pharmaceutical Ltd., Pimpri Pune, India. Reagent used for analysis were acetic acid (Fluka and AR grade), acetonitrile (HPLC grade), dichloromethane (AR grade), dimethyl sulphoxide (AR grade), Milli-Q grade water, deuterated dimethyl sulphoxide was purchased from Merck, USA.

2.2. High performance liquid chromatography (HPLC)

A shimadzu 2010 CHT Liquid chromatograph equipped with UV detector and LC Solution 1.21 data handling system was used. The analysis was carried out on Purosphere star RP-18e, 250 mm × 4.6 mm, 5 μm column. 0.10% (v/v) glacial acetic acid in purified water was mixed with acetonitrile in the ratio of 53–47 used as mobile phase. UV detection was carried out at 254 nm and flow rate was kept at 0.8 ml/min. This analytical method was able to detect all the process related substances (impurities) with good resolution.

2.3. Liquid chromatography–mass spectroscopy (LC–MS)

The LC–MS analysis was carried out on Purosphere star RP-18e, 250 mm × 4.6 mm, 5 μm column. 0.10% (v/v) glacial acetic acid in purified water was mixed with Acetonitrile in the ratio of 53–47

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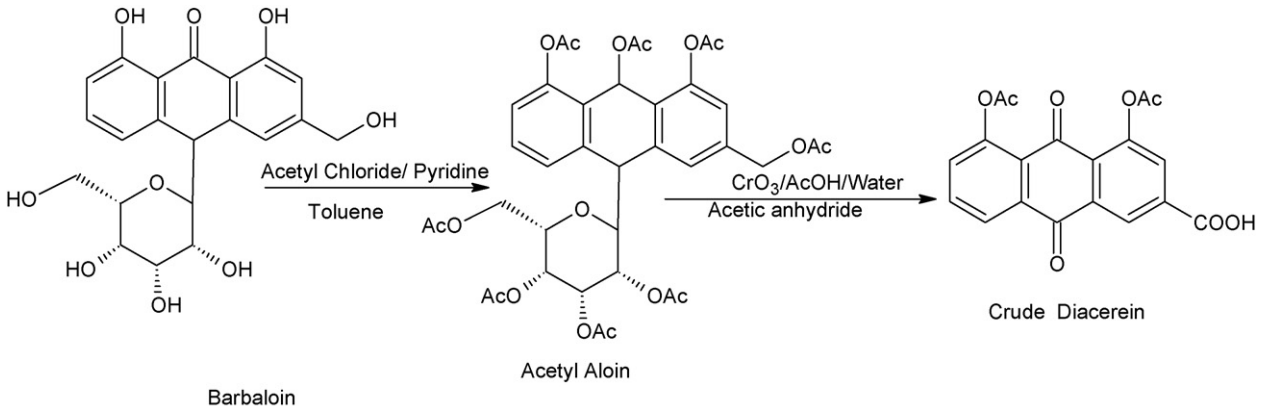

Fig. 1. Synthetic route for the preparation of diacerein.

used as mobile phase. UV detection was carried out at 254 nm and flow rate was kept at 0.8 ml/min. The mass spectrum of the impurities were carried out on a triple quadrupole mass spectrometer (MDS Sciex model API 2000). The analysis was performed in the Negative (−ve) ion mode with electron spray ionization (ESI) technique interface with the following conditions, declustering potential at 40 V, entrance potential at 10 V, focusing potential at 325 V, curtain gas 20 l/min, ion spray voltage 4500 V and temperature 450 °C.

2.4. Preparative liquid chromatography

A waters auto purification system equipped with binary gradient module (Waters 2545), system fluidics organizer (waters SFO), photodiode array detector (Waters 2998) and a sample manager (Waters 2767) with Mass lynx data handling system. Waters Symmetry C-18 (30 mm × 100 mm) preparative column packed with 10 μm was employed for the isolation of impurities. Mobile phase-A consists of 0.5% (v/v) acetic acid in water and Mobile phase-B consists of acetonitrile. Flow rate was kept at 30 ml/min and UV detection was carried out at 254 nm. The gradient programme was as follows. Time (min)/A (v/v):B (v/v), $T_{0.01}$/65:35, $T_{20.00}$/65:35, $T_{23.00}$/10:90, $T_{27.00}$/10:90, $T_{28.00}$/65:35, $T_{30.00}$/65:35.

2.5. NMR spectroscopy

The NMR experiment were performed on Varian NMR spectrometer at 400 MHz. The ^{1}H Chemical shift values were reported on the δ scale in ppm, relative to TMS ($\delta = 0$) as internal standard, ^{13}C NMR spectra (Proton decoupled), DEPT spectra and 1D NOESY was also recorded.

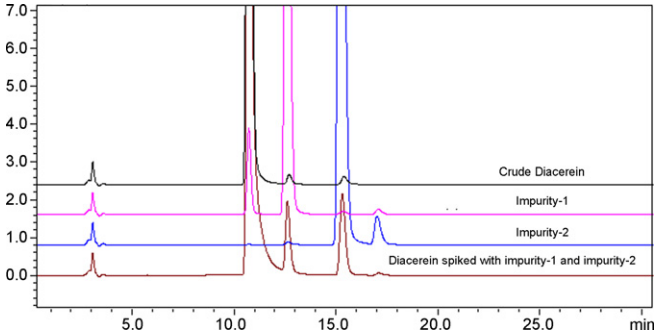

Fig. 2. An overlay analytical HPLC chromatogram of laboratory batch diacerein, isolated impurity-1, isolated impurity-2 and diacerein spiked with isolated impurity-1 and impurity-2.

2.6. FT-IR spectroscopy

The FT-IR spectra of diacerein, impurity-1 and impurity-2 were recorded on Shimadzu FTIR-8400S by using KBr.

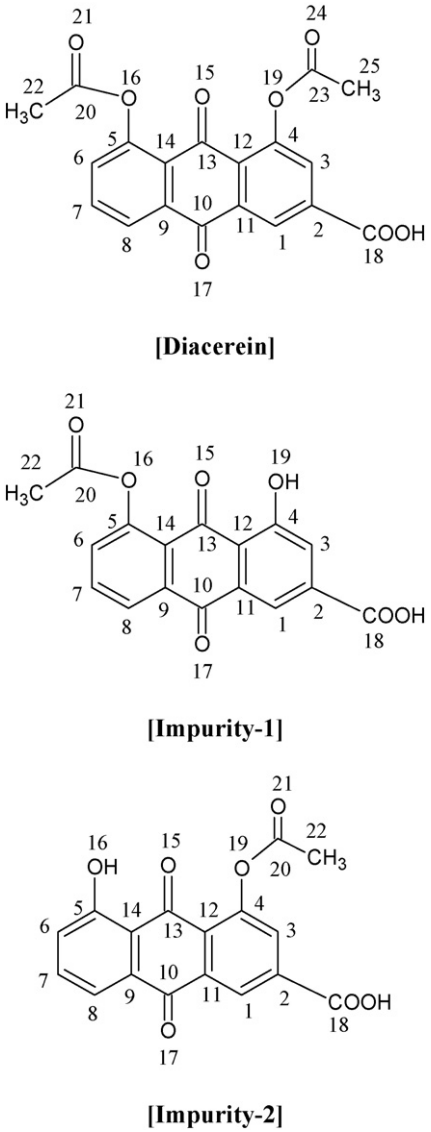

Fig. 3. Chemical structures of diacerein, impurity-1 and impurity-2.

Table 1
^{1}H, ^{13}C NMR and DEPT data of diacerein, impurity-1 and impurity-2.

Position[a]	Diacerein				Impurity-1				Impurity-2			
	^{1}H	δ (ppm) (Hz)	^{13}C	DEPT	^{1}H	δ (ppm) (Hz)	^{13}C	DEPT	^{1}H	δ (ppm) (Hz)	^{13}C	DEPT
1	1H	8.52 (d, *J* = 3Hz)	125.24	CH	1H	7.76 (d, *J* = 1.2Hz)	118.50	CH	1H	8.58 (d, *J* = 1.2Hz)	125.24	CH
2	–	–	136.64	Q	–	–	137.78	Q			137.09	Q
3	1H	8.01 (d, *J* = 3Hz)	135.76	CH	1H	8.13 (d, *J* = 1.2Hz)	125.47	CH	1H	8.07 (d, *J* = 1.2Hz)	124.63	CH
4	–	–	149.99	Q	–	–	161.14	Q	1H	–	150.31	Q
5	–	–	149.78	Q	–	–	150.14	Q	–	–	161.45	Q
6	1H	7.62 (d, *J* = 6Hz)	130.89	CH	1H	7.68 (d, *J* = 7.6Hz)	130.78	CH	1H	7.42 (d, *J* = 8.0Hz)	130.32	CH
7	1H	7.93 (t, *J* = 6Hz)	130.43	CH	1H	8.00 (t, *J* = 7.6Hz)	136.43	CH	1H	7.83 (d, *J* = 8.0Hz)	137.27	CH
8	1H	8.12 (d, *J* = 6Hz)	125.18	CH	1H	8.18 (d, *J* = 7.6Hz)	124.18	CH	1H	7.74 (d, *J* = 7.2Hz)	119.07	CH
9	–	–	134.33	Q	–	–	134.78	Q	–	–	135.16	Q
10	–	–	181.07	Q	–	–	180.94	Q	–	–	180.66	Q
11	–	–	134.78	Q	–	–	133.21	Q	–	–	132.52	Q
12	–	–	128.20	Q	–	–	119.09	Q	–	–	127.03	Q
13	–	–	180.51	Q	–	–	187.02	Q	–	–	186.78	Q
14	–	–	125.54	Q	–	–	124.41	Q	–	–	116.54	Q
15	–	–	–	–	–	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	1H	12.20 (s)	–	–
17	–	–	–	–	–	–	–	–	–	–	–	–
18	–	–	165.28	Q	–	–	165.48	Q	–	–	164.98	Q
19					1H	12.23 (s)	–	–	–	–	–	–
20	–	–	169.26	Q			169.23	Q	–	–	169.11	Q
21	–	–	–	–	–	–	–	–	–	–	–	–
22	3H	2.38 (s)	21.03	CH_3	3H	2.43 (s)	21.01	CH_3	3H	2.43 (s)	20.92	CH_3
23	–	–	169.30	Q	–	–	–	–	–	–	–	–
24	–	–	–	–	–	–	–	–	–	–	–	–
25	3H	2.38 (s)	21.03	CH_3	–	–	–	–	–	–	–	–

s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; J, coupling constant; Q, quaternary carbon.
[a] Refer structures for numbering (Fig. 3).

2.7. Isolation of impurities

For isolation of the impurities, 40 mg/ml solution of crude diacerein was prepared in dimethyl suphoxide and 950 μl solution was injected through auto injector. Elution was carried out by using the condition mentioned in Section 2.4. Initially one pilot run was carried out for simulisation of the auto fraction collector parameter. Collected fractions were also monitored for the chromatographic purity. In the similar way 40 injections were made. All the fractions from 40 injections of >90% chromatographic purity of impurity-1 were mixed together. Similarly all the fractions from 40 injections of >90% chromatographic purity of impurity-2 were mixed together. The mixed fractions of impurities were concentrated on rotavapour to remove the solvents and extracted with dichloromethane. Dichloromethane was evaporated on rotavapour. Finally 40 mg of impurity-1 and 50 mg of impurity-2 was obtained.

3. Results and discussion

Analytical chromatogram of laboratory batch diacerein, isolated Impurity-1, isolated impurity-2 and diacerein spiked with impurity-1 and impurity-2 have been overlaid and attached (Fig. 2). Chemical structures of diacerein, impurity-1 and impurity-2 are shown in Fig. 3.

The LC–MS studies indicated that both the impurities are having the same molecular weight MW = 326. The molecular weight 326 indicates that it is a monoacylated 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-1 shows the molecular ion peak (MH^-) 325.1. It indicates that the molecular weight of the impurity-1 is 326. The molecular weight of 326 confirms that instead of two acetyl groups in diacerein, there is only one acetyl group present in the impurity-1. The fragment ion 281.2 confirms the presence of carboxylic group. The fragment ion 238.9 also confirms the presence of acetyl group in the structure. Same observations were obtained in LC–MS for imputiy-2. Further confirmation of the position of acetoxy group in both the impurities was carried out by ^{1}H NMR spectra, ^{13}C NMR (proton decoupled) spectra, DEPT 135, 1D NOESY. Finally impurity-1 was assigned as 5-acetoxy-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid and impurity-2 was assigned as 4-acetoxy-5-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid.

Table 2
1D NOESY and IR spectra interpretation of impurity-1 and impurity-2.

Sr. no.	Compound	Proton irradiated (position[a])	Enhanced proton (position[a])
1D NOESY			
1.	Impurity-1	2.43 ppm (22)	6
		8.18 ppm (8)	7
		7.68 ppm (6)	7 and 22
2.	Impurity-2	2.43 ppm (22)	3 and 16
		8.07 ppm (3)	22 and 1
		8.58 ppm (1)	3
Sr. no.	Compound	IR (KBr)	
IR spectra			
1.	Impurity-1	2800–3200 (−OH stretching vibration), 1770 (C=O stretch in ester), 1701, 1680 (C=O stretching vibrations in α, β unsaturated ketones), 1643, 1593, 1572, 1483 (aryl C=C stretching vibrations), 1246, 1211, 1199, 1157 (C–H in plane bending vibrations), 771, 772 (C–H in plane bending vibrations), 1269 (C–O–C stretching vibrations)	
2.	Impurity-2	2800–3200 (−OH stretching vibration), 1770 (C=O stretch in ester), 1691, 1680 (C=O stretching vibrations in α, β unsaturated ketones), 1639, 11,608, 1577, 1458 (aryl C=C stretching vibrations), 1247, 1224, 1193, 1155 (C–H in plane bending vibrations), 759 (C–H in plane bending vibrations), 1278 (C–O–C stretching vibrations)	

[a] Refer structures for numbering (Fig. 3).

The ^{1}H and ^{13}C NMR chemical shift values of diacerein, impurity-1 and impurity-2 are given in the Table 1. The 1D NOESY and IR results are given in the Table 2.

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