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Short communication

# A validated HPLC stability-indicating method for the determination of diacerhein in bulk drug substance

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**Abstract**

A novel stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for the assay of diacerhein in bulk forms. Diacerhein was found to degrade in alkaline and acidic conditions and also under oxidative stress. The drug was stable to dry heat and in presence of light. Resolution of drug, its potential impurities and degradation products were achieved on a RP18 (endcapped) column utilizing 0.1 M phosphoric acid and methanol (40:60, v/v) as eluent at the detection wavelength of 254 nm. The validation studies were carried out fulfilling International Conference on Harmonisation (ICH) requirements. The procedure was found to be specific, linear, precise (including intermediate precision), accurate and robust.


*Keywords:* Diacerhein; Rhein; Aloe-emodin; HPLC; Stability-indicating; Quality control

## 1. Introduction

Diacerhein (**1**) and its active metabolite rhein (**2**) [1] (Fig. 1) are anthraquinone compounds that ameliorate the course of osteoarthritis [2–8]. Diacerhein is a slow acting symptomatic treatment of osteoarthritis, which has demonstrated efficacy on functional manifestations of osteoarthritis and on the structural component. In a recent report [9], two mechanisms of action have been validated: in vitro inhibition of interleukin-1 (IL-1) synthesis, the main cytokine involved in cartilage destruction, and activity on the synthesis of proteoglycans and hyaluronic acid, the principal component of cartilage. Moreover, Cruz and Pastrak have related the use of rhein or diacerhein to treat and prevent vascular diseases [10].

Diacerhein is readily obtained in few synthetic steps from the naturally occurring glucopyranoside aloin [11,12].

In spite of its longstanding commercial distribution as oral tablets, methods are not reported for the determination of diacerhein. Therefore, a study was carried out to develop a high-performance liquid chromatographic (HPLC) method for the quality control of diacerhein bulk drug substance. Moreover, a stress testing on the drug substance was carried out, as required by the revised parent drug stability test guideline [13] issued by International Conference on Harmonisation (ICH), for supporting the suitability of the proposed analytical procedure.

Accordingly, a simple, rapid stability-indicating method was validated for routine quality control analysis.

The determination was accomplished by conventional DAD-UV detection. Identification of the degradation products was confirmed by liquid chromatography–mass spectrometry (LC–MS) analyses.

## 2. Experimental

### 2.1. Materials

Diacerhein raw material (batch 70882), its reference standard and related substances, rhein and aloe-emodin, of

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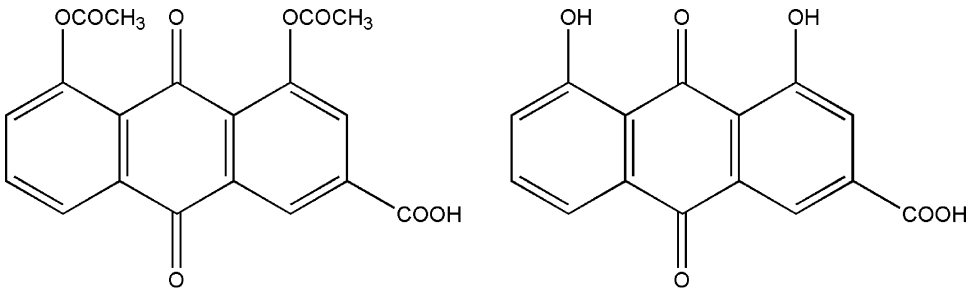


**1** **2**

Fig. 1. Chemical structures of diacerhein (**1**) and rhein (**2**).

pharmaceutical purity grade, were kindly provided by SIMS (Reggello, Firenze, Italy). All chemicals and reagents were of analytical grade and used as obtained. Water, deionised by inverse osmosis, was further purified by means of a Milli-Q Plus water purification system (Millipore, Massachusset, USA).

### 2.2. Apparatus

For HPLC, a Perkin-Elmer LC200 Series apparatus consisting of a quaternary pump with autosampler, a diode array UV–vis detector and a Peltier column oven was employed, under the control of Totalchrom 6.1 data handling software (Perkin-Elmer, Shelton, Connecticut, USA).

Confirmation of the degradation products identities was obtained by using a LC–MS system composed by a two pumps Varian Prostar with autosampler coupled with ESI–MS Varian 1200 L triple quadrupole (Varian, California, USA). The acquisition was performed, in negative ion mode, recording between 150 and 700 range.

Weighing at 0.01 mg level was performed with a Mettler XS 105 DU (Mettler-Toledo GmbH, Greifensee, Switzerland).

### 2.3. Chromatographic conditions

HPLC measurements were carried out using a reversed-phase Luna C18(2), 150 mm × 4.6 mm, 5 μm particle size column (Phenomenex Inc., California, USA) operated at 40 °C isocratically at 1.0 mL $min^{-1}$ with a mobile phase of 0.1 M phosphoric acid and methanol (40:60, v/v); detection: UV absorbance at 254 nm; injection volume 20 μL.

Chromatographic separation for LC–MS measurements were performed using a reversed-phase Luna C8, 50 mm × 2.0 mm, 3 μm particle size column (Phenomenex Inc., California, USA) operated at 0.2 mL $min^{-1}$ with a mobile phase A: 0.05% formic acid and B: 0.05% formic acid in acetonitrile, gradient A:B (90:10) to A:B (10:90) in 25 min.

### 2.4. Standard and sample preparations

A diacerhein standard solution, containing 203.0 μg $mL^{-1}$, was prepared in a 100-mL volumetric flask by dissolving 20.30 mg of pure diacerhein in 10 mL of *N*,*N*-dimethylacetamide and then diluting to volume with methanol.

For the linearity study five levels of concentration within the range 80–120% of the standard concentration (200 μg $mL^{-1}$) were prepared. Each concentration level was prepared individually dissolving 16.24, 18.27, 20.30, 22.33 and 24.36 mg of diacerhein standard in 10 mL of *N*,*N*-dimethylacetamide and then diluting to 100 mL with methanol.

For the recovery procedure a sample solution of diacerhein was obtained dissolving 10.20 mg of batch 70882 in 5 mL of *N*,*N*-dimethylacetamide and then diluting to 25 mL with methanol. In three volumetric flasks, each containing 5 mL of the above sample solution, 0.5, 1.0 and 1.5 mL of diacerhein standard solution were added and diluted to 10 mL with methanol.

### 2.5. Specificity procedure

For the specificity study, to determine the absence of interference on behalf of the related substances, a sample was used mixing equal volumes of two solutions (solution A and solution B), obtained as follows. Solution A: 20.00 mg of diacerhein dissolved in 2 mL of *N*,*N*-dimethylacetamide were poured in 50 mL MeOH·$H_2O$ (50:50, v/v), refluxed for 1 h and kept at room temperature overnight; solution B: 10.20 mg of rhein and 11.1 mg of aloe-emodin were dissolved with 5 mL of DMA and diluted to 100 mL with methanol; 5 mL of this solution were diluted with MeOH·$H_2O$ (50:50, v/v) in a 50 mL volumetric flask. The injection volume was 20 μL.

### 2.6. Degradation studies

Drug at a concentration of 203.0 μg $mL^{-1}$ was used in all degradation studies. The samples corresponding to placebo and raw product were subjected to stress conditions in 1N NaOH and 1N HCl at room temperature and 37 °C for 3 h. Diacerhein drug powder samples were subjected to the effect of temperature (105 °C) and UV light for 24 h. The samples were also submitted to an oxidation treatment performed in 5% $H_2O_2$ at room temperature for 3 h.

After the stress assays, the samples were analyzed in the above reported chromatographic conditions.

## 3. Results and discussion

### 3.1. Forced degradation studies

HPLC and LC–MS studies performed on the stressed samples of diacerhein suggested the following consideration. The main degradation reaction, detectable by the above-considered techniques in the most stressed conditions, was an hydrolytic pathway leading to the formation of rhein (**2**).

In the base stressed samples at room temperature and at 37 °C, the degradation proceeded very fast giving rhein in stoichiometric amount.

On the other hand, in acid stressed samples, it was observed that 40% of the drug at room temperature and 85–90% at 37 °C degraded giving rise to rhein and two unknown by-products observed at retention times 8.5 and 12.2 min. LC–MS studies indicated that the hydrolysis proceeded on the two phenolic positions at the same rate giving two products both monoacetylated with MW = 326.

These two deacetylated compounds represented the final products formed in equal proportion in the neutral condition described for solution A in the specificity procedure.

The drug was found to be stable to the effect of temperature and UV light, while 73.5% of diacerhein was recovered after oxidative stress and no degradation products were detected.

### 3.2. Specificity

Selectivity was assessed by comparing the HPLC traces of standard and stressed diacerhein with impurities samples; complete separation of the compounds was obtained as illustrated in Fig. 2.

The identities of the impurities were confirmed by MS spectra. Moreover, Totalchrom software afforded automatic calculation of the peaks purity by comparing the UV spectra, in the 220–400 nm range, on the upslope and downslope of the peak of each sample. Hence, this HPLC method is perfectly able to detect and accurately measure diacerhein in the presence of its related impurities and degradation products.

### 3.3. System suitability

A system suitability test was performed to evaluate the chromatographic parameters (capacity factor, number of theoretical plates, asymmetry of the peaks and resolution between two consecutive peaks) before the validation runs. Three replicate injections of the standard solution and three injections of the solution prepared for the specificity procedure were used.

Efficiency and tailing factor at 5% height of the main peak were determined giving the following data: $N = 4326.6$, tailing factor = 1.1. The resolution obtained between diacerhein and the related impurities was always >2.0.

Table 1
Day-to-day calibration parameters

| Day* | Slope | Intercept | $R$ | Day** | Slope | Intercept | $R$ |
|---|---|---|---|---|---|---|---|
| 1 | 940741 | 1000000 | 0.9964 | 1 | 884895 | 476201 | 0.9908 |
| 2 | 964956 | 2000000 | 0.9949 | 2 | 911657 | 261826 | 0.9999 |
| 3 | 889845 | 476201 | 0.9908 | 3 | 889110 | 221452 | 0.9994 |
| Mean | 930197 | 719012 | 0.9982 | Mean | 895220 | 145276 | 0.9982 |

* Same set of standards.
** Different sets of standards.

### 3.4. Linearity

Five-points calibration curves were obtained in a concentration range from 162.40 to 243.60 μg $mL^{-1}$ for diacerhein; three independent determinations were performed at each concentration. The response for the drug was linear and the calibration equation was $y = 930{,}187x + 719{,}012$ ($n = 15$) with $R^2 = 0.9982$, S.E. = 146,282, slope S.D. = 22,787, intercept S.D. = 467,188.

To study the variability of the calibration parameters the curves were obtained in three consecutive days (same set of standards) and in different days (different sets of standards). The parameters of the calibrations are reported in Table 1.

### 3.5. Accuracy and precision

The accuracy and the precision of the proposed method were obtained with analyses of the standard reference material (over the range of 80–120% of the amount corresponding to the mid point of the curve) performed during the same day and consecutive days (six replicates at three levels per day). The values are shown in Table 2.

The recovery, evaluated with the standard addition procedure at three concentrations in triplicate, is reported in Table 3.

For the intermediate precision, a study was carried out by three analysts working on different days ($n = 18$ number of analyses per day; six replicates at three levels). The results are reported in Table 4.

The instrumental precision was determined at three levels (six replicates) giving good R.S.D. values for retention time, partition, number of theoretical plates and tailing factor (Table 5).

In all these cases, the R.S.D. values obtained were far below the percentage limit set for the precision study of the instrumental system, thus showing that the equipment and the method used were highly repetitive.

### 3.6. Robustness

To evaluate the robustness of the method, a Licrosphere RP18 (endcapped) column, 250 mm × 4.6 mm i.d., 5 μm particle size, (Merck, Darmstadt, Germany) was considered. Operating in the above conditions or with minimal variation

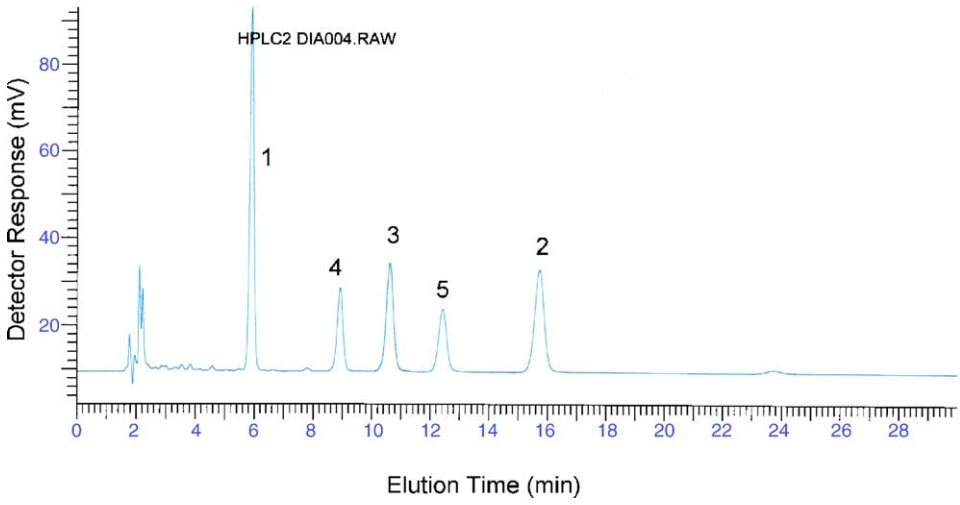


Fig. 2. Typical HPLC chromatogram of specificity procedure solution: diacerhein (1), rhein (2), aloe-emodin (3), monoacetilrhein I (4) and monoacetilrhein II (5).

Table 2
Inter and intra-day, over three consecutive days, precision and accuracy from the calibration curve validation experiments

| Calculated ($\mu g\,mL^{-1}$) | Measured ($\mu g\,mL^{-1}$) ($n=3$), R.S.D.% | Mean ($\mu g\,mL^{-1}$) | Bias (%) | R.S.D. (%) |
|---|---|---|---|---|
| 162.4 | 159.4, 2.41; 162.5, 0.23; 158.9, 2.42 | 160.3 | −1.3 | 1.22 |
| 203.0 | 202.3, 2.17; 203.1, 2.59; 201.2, 2.20 | 202.2 | −0.4 | 0.47 |
| 243.6 | 241.6, 0.24; 242.1, 2.57; 239.1, 2.55 | 240.9 | −1.1 | 0.67 |

Table 3
Precision and recovery of theoretical spike in diacerhein batch 70882

| Calculated concentration ($\mu g\,mL^{-1}$) | Measured concentration ($\mu g\,mL^{-1}$) (mean ($n=3$)) | Bias (%) | R.S.D. (%) |
|---|---|---|---|
| 214.1 | 215.5 | +0.7 | 1.01 |
| 224.3 | 224.2 | −0.1 | 1.99 |
| 234.6 | 236.9 | −1.0 | 0.12 |

Table 4
Analysis of variances for investigation of the investigation of intermediate precision

| Level | Analyst 1 | | | Analyst 2 | | | Analyst 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 80% | 100% | 120% | 80% | 100% | 120% | 80% | 100% | 120% |
| Mean | 14560648 | 18086514 | 21700471 | 14603910 | 1581247 | 21429465 | 14325624 | 18362534 | 21346729 |
| R.S.D.% | 0.20 | 0.10 | 0.10 | 0.12 | 0.14 | 0.13 | 0.25 | 0.04 | 0.23 |
| Overall mean | | | | 14496722 | | | 18149293 | | 21492221 |
| Repeatability R.S.D.% | | | | 2.17 | | | 2.59 | | 2.2 |
| Intermediate precision R.S.D.% | | | | 2.02 | | | 2.34 | | 2.08 |

Table 5
Instrumental precision

| Level | 80% | | | 100% | | | 120% | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean ($n=6$) | ±S.D. | R.S.D. (%) | Mean ($n=6$) | ±S.D. | R.S.D. (%) | Mean ($n=6$) | ±S.D. | R.S.D. (%) |
| $T_R$ | 6.03 | 0.03 | 0.55 | 6.03 | 0.15 | 2.54 | 5.91 | 0.05 | 0.92 |
| $k'$ | 601.6 | 3.25 | 0.54 | 601.7 | 15.43 | 2.57 | 589 | 5.53 | 0.94 |
| $N$ | 4351 | 79.92 | 1.84 | 4200 | 98.21 | 2.34 | 3952 | 108.81 | 2.75 |
| Tailing | 1.09 | 0.02 | 1.83 | 1.07 | 0.02 | 1.57 | 1.15 | 0.03 | 2.54 |

Table 6
Accuracy for robustness

| Calculated ($\mu g\,mL^{-1}$) | Measured ($\mu g\,mL^{-1}$) $n = 3$ | R.S.D. (%) | Bias (%) |
|---|---|---|---|
| 164.8 | 165.6 | 2.73 | +0.48 |
| 206.0 | 208.1 | 1.13 | +1.02 |
| 247.2 | 252.6 | 0.95 | +2.02 |

of the mobile phase percent composition, the peak relative retention times remained unchanged. The analysis data demonstrated that suitability parameters were unaffected by the length and/or the brand of the column, giving tailing factor 1.1, theoretical plate number >4000 and capacity factor >500.

Accuracy data carried out on three levels (Table 6) confirmed the consistency of the method.

## 4. Conclusion

A new analytical method has been set up to be routinely applied to determine diacerhein in bulk drug substance. The specificity procedure and forced degradation studies revealed that the potential impurities aloe-emodin and/or rhein and degradation products do not interfere with the determination of diacerhein. The HPLC procedure has been evaluated over the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. It has been proved that it was selective, linear between 80 and 120% of the work concentration (200 $\mu g\,mL^{-1}$), precise, accurate and robust with respect to the column parameters.

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