

Development and Validation of a Dissolution Method with Spectrophotometric Analysis for Diacerhein Capsules

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Abstract

The aim of this work was to develop and validate a dissolution test for diacerhein in capsules using spectrophotometric method. The dissolution established conditions were: 900 mL of sodium phosphate buffer pH 7.0 with 0.75 % of sodium lauryl sulphate as dissolution medium, using a basket apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 258 nm. The method was validated to meet requirements for a global regulatory filing. The validation included specificity, linearity, precision and accuracy. In addition, filter suitability and drug stability in medium were demonstrated. The comparison of the obtained dissolution profiles of capsules, obtained from three different brands (denominate product A, B and C) of 50 mg diacerhein, was performed and the results showed no significative difference among the products.

Keywords

Dissolution • Validation • Diacerhein capsules

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Introduction

Diacerhein (DAR, Figure 1) an oral agent described as 4,5-Bis(acetyloxy)-9,10-dioxo-2-anthracenecarboxylic acid, is a low molecular weight heterocyclic compound [1]. After absorption, the drug is metabolized to its active metabolite rhein [2, 3]. DAR and rhein are anthraquinone compounds designated to treatment of osteoarthritis (OA) [4, 5]. DAR has been demonstrating efficacy on functional manifestations of OA and on the structural component, and has been classified as symptomatic slow-acting drug [6, 7]. The mechanism of action of DAR is different from those described for classic nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. DAR has no inhibitory effect on phospholipase A₂, cyclooxygenase, or 5-lipoxygenase *in vitro*. However, it is able to stimulate prostaglandin E₂ synthesis in human chondrocyte cultures [8, 9] and to inhibit the effects of IL-1 on chondrocytes [10, 11]. Drug absorption from a dosage form after oral administration depends on the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilisation under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance [12, 13]. The dissolution test is a very important tool in drug development and quality control. At the present time there are no official monographs for DAR raw material and capsules and no dissolution test has been described in literature for this drug. Few methods have been reported for DAR determination in bulk [14] and capsules [15]. Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official monographs [13]. For this reason, there is a crescent numbers of works describing the development of dissolution test for citalopram, fexofenadine, cetirizine, amlodipine, ritonavir, bisoprolol and valdecoxib [16–22]. The present paper describes the development and validation of dissolution test for quality control of DAR in capsules. The best dissolution conditions were used to evaluate the

dissolutions profile of three different brands, including manufactured and compounded capsules.

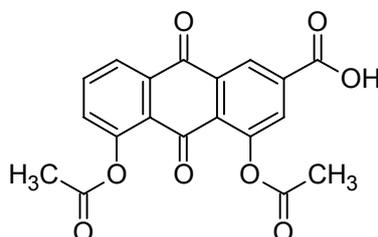


Fig. 1. Chemical structure of diacerhein

Experimental

Materials

Diacerhein chemical reference substance (CRS) (assigned purity, 99.8%) was obtained from DEG (Brazil). Capsules and compounded capsules were purchased at the local market and were claimed to contain 50 mg DAR each. One batch of manufactured capsules (A), and two batches of compounded capsules (B and C) were purchased from the market. All reagents and solvents used were analytical grade. Ultra-pure water was obtained from a Labconco Water Purification Unit (Missouri, USA). Sodium acetate and sodium phosphate buffer solutions were prepared according to USP Pharmacopoeia [23].

Instrumentation

Dissolution test was performed in a Nova Etica dissolution test system, model 299 multi-bath (n=6), in accordance to USP Pharmacopoeia [23] general method. The mediums were vacuum degassed under house vacuum and were maintained at $37.0 \pm 0.5^\circ\text{C}$ by using a thermostatic bath. A double-beam UV-Vis spectrophotometer (Shimadzu, Japan) model UV – 1601 PC, with a fixed slit width (2 nm) using 1.0 cm quartz cells was used for all absorbance measurements. The Field-Lab Schott potentiometer was used to determine the pH of all solutions.

Dissolution test conditions

DAR solubility was determined in 900 mL of 0.1M HCl, sodium acetate buffer pH 5.5 and sodium phosphate buffer pH 7.0, using an amount of the drug equivalent a three times of the dose in the pharmaceutical formulation. Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products [24–26], using basket (USP Apparatus 1) at 75 and 100 rpm (Table 1). Sampling aliquots of 5.0 mL were withdrawn at 0, 5, 10, 15, 30 and 60 minutes, and replaced with an equal volume of the fresh medium to maintain a constant total volume. After the end of each test time, samples aliquots were filtered, diluted in dissolution medium, when necessary, and quantified. The assay of the three tested products was performed using previously validated spectrophotometric method [15], and the contents results were used to calculate the percentage release on each time of dissolution profile. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the *in vitro* dissolution data ($n=12$). Dissolution Efficiency (DE) of profiles was calculated from the area under the curve at time t_i (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time [27] DAR stability in dissolution medium was evaluated at $37.0 \pm 0.5^\circ\text{C}$ for 2 hours. The filtration procedure of DAR CRS and samples (capsules dissolved in dissolution medium, $n=3$) was evaluated using: a) quantitative filter (Schleicher & Schuell, Germany); b) $0.45 \mu\text{m}$ cellulose acetate membrane filter (Phenomenex), and c) quantitative filter together with cellulose acetate membrane filter. The absorbances of filtered and unfiltered (centrifuged) solutions (at concentration $5.5 \mu\text{g/ mL}$ in dissolution medium) were evaluated.

Tab. 1. Evaluated conditions and dissolution efficiency (DE)

Condition	DE
Phosphate buffer pH 7.0 / 75 rpm	43.7
Phosphate buffer pH 7.0 / 100 rpm	45.0
Phosphate buffer pH 7.0 / 100 rpm / 0.1% SLS	67.0
Phosphate buffer pH 7.0 / 100 rpm / 0.25% SLS	66.5
Phosphate buffer pH 7.0 / 100 rpm / 0.5% SLS	77.5
Phosphate buffer pH 7.0 / 100 rpm / 0.75% SLS	87.1
Phosphate buffer pH 7.0 / 75 rpm / 0.75% SLS	84.7

SLS = sodium lauryl sulphate

Method validation

The UV spectrophotometric method used to analyze the DAR samples in phosphate buffer pH 7.0 with 0.75 % of sodium lauryl sulphate (SLS) dissolution medium was validated for specificity, linearity, precision and accuracy, according to USP Pharmacopoeia [23] and ICH guideline [28]. All absorbances were determined at 258 nm.

Results and discussion

Development of dissolution test conditions

Dissolution is an official test used by pharmacopoeias for drug evaluation release of solid and semisolid dosage forms, and it is routinely used in Quality Control (QC) and Research & Development (R&D). The purpose of *in vitro* dissolution studies in QC is batch to batch consistency and detection of manufacturing deviation while in R&D the focus is to provide some predictive estimate of the drug release in respect to the *in vivo* performance of a drug product. For QC, an over-discriminatory test might be suitable to detect even small production deviations. However, for prediction of the *in vivo* performance of drug product a dissolution test should be sensitive and reliable [29]. The accomplishment

of dissolution profiles is recommended as support in the development and optimization of drug formulation as well as in the establishment of *in vitro/in vivo* correlation. When dissolution test is not defined in the monograph of the dosage form, or if the monograph is not available, comparison of drug dissolution profiles is recommended on three different dissolution media, in the pH range of 1–7.5 [24]. The selection of a dissolution medium may be based on the solubility data and dosage range of the drug product [24, 30]. Hydrochloric acid, acetate buffer, phosphate buffer and purified water are typical mediums used to dissolution test [24], and these mediums were evaluated. DAR was insoluble in acid mediums (0.1N HCl and acetate buffer pH 5.5) and showed a low solubility in sodium phosphate buffer pH 7.0. For poor soluble drugs, a percentage of surfactant can be used to enhance drug solubility [24, 31]. Then, different concentrations of SLS (0.1, 0.25, 0.5 and 0.75%) were added to sodium phosphate buffer pH 7.0 medium to improve DAR solubility. The profiles achieved with product A, at stirring rate of 100 rpm, are show in Figure 2. It was observed that more than 80% of drug was dissolved at 15 minutes in phosphate buffer pH 7.0 with 0.75% SLS. In the other conditions the % drug dissolved was < 65%. The influence of rotation speed was evaluated and the results are show in Figure 3. The analysis of variance showed no significant difference between the results obtained at 75 and 100 rpm ($p < 0.05$). However, it was observed that drug release percent (Figure 3) and the DE (Table 1) were higher at 100 rpm. The DE acquired in phosphate buffer pH 7.0 with 0.75 % SLS at 100 rpm was greater than other tested conditions. According to USP [23], as a general rule, basket apparatus is used for dissolution test of capsules, and it was selected for DAR dissolution test. Based in these results, the selected conditions for dissolution test of DAR in capsules were: 900 mL of sodium phosphate buffer pH 7.0 with 0.75% of SLS, using basket apparatus at stirring rate of 100 rpm. In these conditions, DAR was stable for 2 hours (variation less than 2%). Filtration of the dissolution samples is usually to prevent undissolved drug particles from entering the analytical sample and further dissolving. It removes insoluble excipients that may otherwise cause high background or turbidity [24]. The quantitative and 0.45

μm cellulose acetate filters showed recoveries between 98–102%. However, due to low cost of quantitative filter when compared to 0.45 μm cellulose acetate membrane filter, the first filter was chosen. Typical acceptance criteria for the amount of drug dissolved are in the range of 70 – 80 % dissolved (23). In the present study, the % dissolved for all products was > 90% in 30 minutes (Figure 4), and the suggested acceptance criteria could be 85% in 30 minutes.

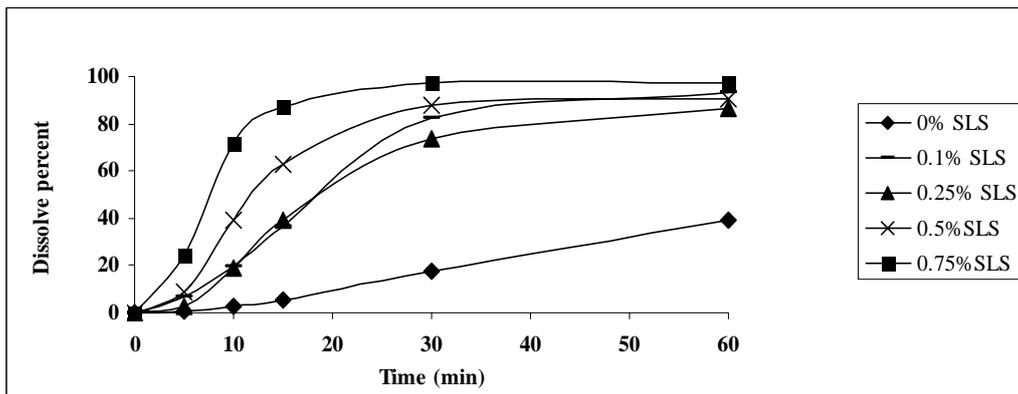


Fig. 2. Dissolution profiles of diacerhein capsules in sodium phosphate buffer pH 7.0 with different concentration of sodium lauryl sulfate (SLS) using basket at stirring rate of 100 rpm.

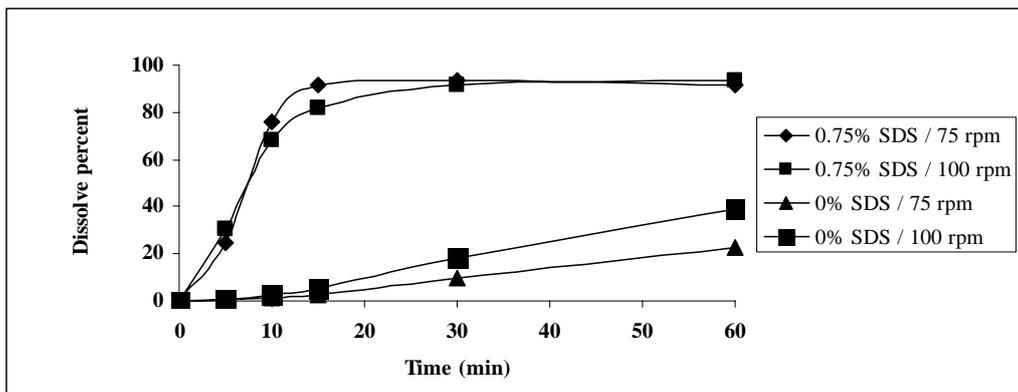


Fig. 3. Dissolution profile of diacerhein capsules in sodium phosphate buffer pH 7.0 with 0 and 0.75% of sodium lauryl sulfate (SLS) using basket at stirring rate of 75 and 100 rpm.

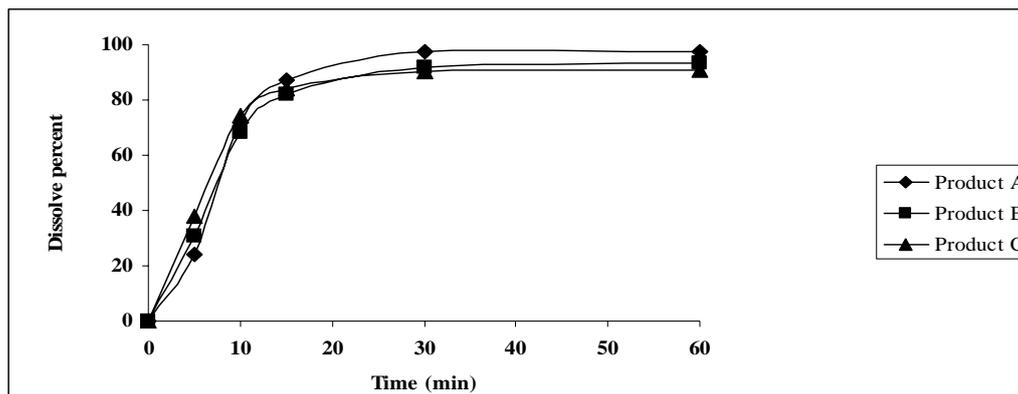


Fig. 4. Dissolution profile of products A, B and C in sodium phosphate buffer pH7.0 with 0.75% of sodium lauryl sulfate using basket at 100 rpm.

Method validation

UV/VIS spectrophotometry and high performance liquid chromatography are the most analytical methods used for quantifying drug release in dissolution tests [24, 32]. The UV spectrophotometric method may be used if drug has a UV chromophore and no UV interferences due excipients and/or capsules shells used in the formulation are observed [33]. This method has the advantage of very rapid time of analysis and the relative low cost for the routine quality control.

Specificity

Specificity was examined by analyzing a solution of a placebo, which consisted of all the excipients and shell capsules without the drug. The excipients were lactose and magnesium stearate. Their concentrations were determined based in Handbook of Pharmaceutical Excipients [34] and calculated for a medium weight of content. The absorption spectrum of DAR in dissolution medium shows an absorbance peak at 258 nm (Figure 5A). At this wavelength, no interferences from the shell capsules (Figure 5B) or the placebo (Figure 5C) were observed.

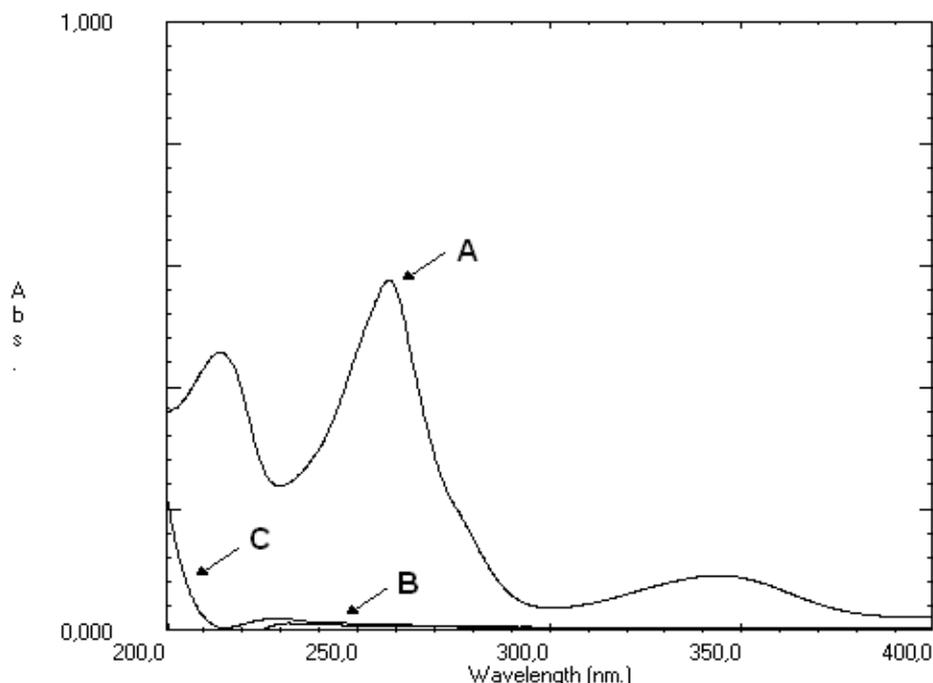


Fig. 5. Absorption spectrum of Diacerhein: (A) CRS DAR, (B) shell capsules and (C) Placebo

Linearity

The linearity of DAR response was evaluated from 0.27 – 6.6 $\mu\text{g/mL}$ range and showed good correlation coefficient (0.999). To assess the linearity, three standard curves of DAR were constructed, plotting concentration ($\mu\text{g/mL}$) *versus* absorption. Linear regression was performed and the obtained equation was $y = 0.1017 x$ (standard error: ± 0.0010) + 0.0022 (standard error: ± 0.0008). The calibration data were validate by means of ANOVA that demonstrated significant linear regression (f calculated = 10,622.46 > f critical = 4.6; $P = 5\%$) and no significant deviation from linearity (f calculated = 0.304 < f critical = 2.96; $P = 5\%$).

Precision

The precision of the method was determined by measuring the repeatability (intra-day precision) and the intermediate precision (inter-day precision), both expressed as RSD (%). Capsules (n=6) of each product (A, B and C) were subjected to dissolution test conditions (900 mL of dissolution medium pre-heated at $37^\circ\text{C} \pm 0.5$, basket with stirring rate of 100 rpm, 30 minutes) in the same day

(intra-day precision) and in two different days (inter-days precision). All the data (Table 2) are within the acceptance criteria of 5% (24). The DAR release found on the two days (Table 2) were equivalent for all products ($P < 0.05$).

Tab. 2. Precision of the method and dissolution efficiency (DE).

Product	Mean \pm RSD			ANOVA ($p=0.5$)	DE
	1st* Day	2nd*Day	Inter-Day**		
A	94.0 \pm 4.1	97.5 \pm 2.3	95.3 \pm 3.9	1.81	86.1
B	93.0 \pm 3.9	90.3 \pm 1.9	91.7 \pm 3.3	2.59	85.3
C	91.5 \pm 4.0	90.3 \pm 2.1	91.1 \pm 3.2	1.02	88.1

* n=6, ** n=2

Accuracy

The accuracy was evaluated applying the proposed method to the analysis of the in-house mixture of the tablet excipients with known amounts of the DAR CRS, corresponding to the concentrations of 20, 100 and 120%, which were subjected to dissolution test conditions described above. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix. The percent recoveries obtained (Table 3) were considered acceptable [24].

Tab. 3. Accuracy results for diacerhein (% recovery)

Amount of reference ($\mu\text{g/mL}$)			%	
Added	Recovered	Recovery	Mean	RSD
1.11	1.09	98.3	97.4	1.4
5.55	5.31	95.8		
6.66	6.53	98.1		

Conclusions

The dissolution test developed and validated for DAR capsules was considered satisfactory. The conditions that allowed the dissolution determination were 900 mL of sodium phosphate buffer pH 7.0 with 0.75% SLS at 37.0 ± 0.5 °C, basket apparatus, 100 rpm stirring speed and filtration with quantitative filter. In these conditions, the DAR stability was guaranteed. The % drug delivery was higher than 90% in 30 minutes for all evaluated products. The analysis of variance of the DE values showed that the dissolution profiles among the products A, B and C were similar ($p < 0.05$). The spectrophotometric method was validated and showed to be specific, linear, precise and accurate.

Acknowledgments

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