Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

Direct spectrophotometric determination of diacerhein in capsules

S. H. M. Borgmann, L. M. Parcianello, M. Z. Arend, S. G. Cardoso

Received November 19, 2006, accepted December 19, 2006

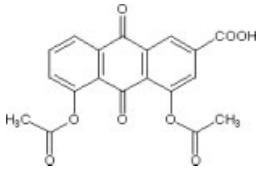
Prof. Dr. Simone Gonçalves Cardoso, Departamento de Farmácia Industrial – Prédio 26, Sala 1105, Universidade Federal de Santa Maria, Campus, Santa Maria – RS – Brazil – CEP 97.105-900
simonegc@ccs.ufsm.br

Pharmazie 62: 483–485 (2007) *doi: 10.1691/ph.2007.7.6263*

A simple, rapid and precise ultraviolet spectrophotometric method using 0.1 N sodium hydroxide has been developed and validated for the assay of diacerhein. The drug can be estimated at 277 nm (ultraviolet region, UV) as well as at 502 nm (visible region, VIS). The absorbances were linearly correlated with concentration in the 6.0–24 $\mu g \cdot mL^{-1}$ range ($r = 0.9999$) and 10–34 $\mu g \cdot mL^{-1}$ range ($r = 0.9998$), respectively at 277 and at 502 nm. The relative standard deviation values for intra ($n = 6$) and inter-day ($n = 3$) precision were $< 2\%$. Recoveries ranged between 99.0 and 101.7%. The method has been successfully applied to the drug assay in capsules. It was also found that the excipients in the commercial capsules did not interfere with the method. Statistical analysis showed no significant difference between the results obtained at two wavelengths (277 nm or 502 nm).

1. Introduction

Diacerhein (DAR) is an oral agent that has been developed specifically for the treatment of osteoarthritis (Lequesne 1994; Spencer and Wild 1997). No official methods are available for DAR in bulk or in its pharmaceutical forms and there is only one reported method for its determination in bulk form by high-performance liquid chromatography (HPLC) with UV detector (Giannelinni et al. 2005). Chromatographic techniques are time consuming, costly and require expertise, while spectrophotometric methods are easy to use and robust methods for the quantitation of drugs in formulations where there is no interference with excipients (Watson 1999). Thus, the aim of the present work was to develop and to validate a simple and reproducible spectrophotometric procedure for the quantitative determination of DAR in capsules. The proposed method can be applied routinely because it does not require high cost reagents and equipment.



2. Investigations, results and discussion

For media optimization various diluents and solvents, like 0.1 N HCl, 0.1 N NaOH, methanol, ethanol and acetonitrile, were evaluated. Due to better solubility of the drug in 0.1 N NaOH, this diluent was chosen. The absorption spectrum of DAR in 0.1 N NaOH shows a strong absorbance peak at 241 nm and other two peaks at 277 and 502 nm (Fig.). The absorption peaks at 277 and 502 nm were selected for analytical studies. According to Giannelinni et al. (2005) in alkaline conditions (1 N NaOH), at room temperature, DAR was found to degrade rapidly to its active metabolite rhein, in stoichiometric amount. Then, we can propose that rhein is probably the species measured. No interference with excipients were observed with the proposed methods. The optimal characteristics of the method, such as Beer's law limit, apparent molar absorptivity, correlation coefficient, slope, intercept, and con-

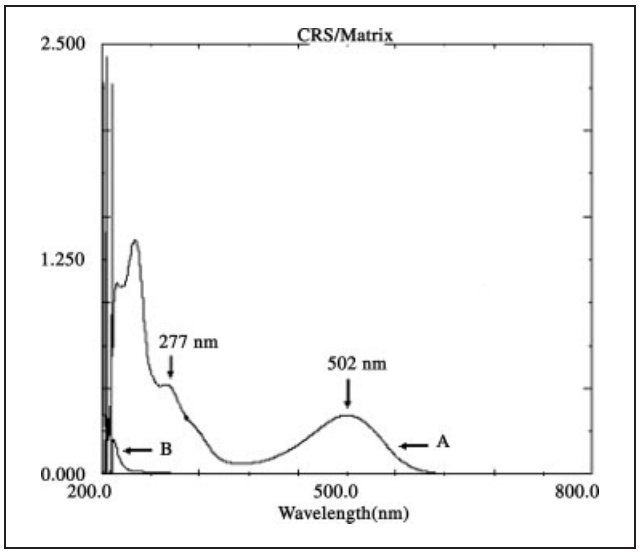


Fig.: Absorption spectrum in 0.1 N NaOH: (A) diacerhein chemical reference substance at 15 μg/mL; (B) capsules excipients

Table 1: Results of the regression analysis of diacerhein by spectrophotometric method

Parameters	277 nm	502 nm
Concentration range ($\mu g \cdot mL^{-1}$)	6–24	10–34
Apparent molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	12570.16	9483.63
Slope ± standard error[a]	0.0009 ± 0.0003	0.0079 ± 0.0027
Intercept ± standard error[a]	0.0341 ± 0.0002	0.0260 ± 0.0005
Correlation coefficient (r)[a]	0.9999	0.9999
Linear regression[b]	212626 (4.6)	28278 (4.6)
Linearity deviation[b]	1.325 (2.96)	0.407 (2.96)

[a] Data obtained from three calibration curves
[b] Figures in parentheses corresponding critical values for F at P = 0.05

fidence limits are presented in Table 1. The correlations coefficients were >0.999, indicating a linear relation between absorbance and concentration of drug. The linearity data were validated by the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($P < 0.05$). The experimental results obtained for the determination of DAR in capsules are shown in Table 2 and the % RSD values for the intra-day and inter-day were less than 2%, indicating a satisfactory precision (Table 2). Statistical analysis showed no significant difference between the results obtained at 277 nm and 502 nm ($P < 0.01$) for all evaluated products. Good recoveries results were obtained (Table 3), and no significant differences were observed between the amount of DAR added and the amount found, which indicated the accuracy of the method. It was shown that the variation of the ionic force of the diluent from 0.1 N to 1 N as well as changes of the wavelength from 277 nm to 282 or ± 5 nm from 502 nm did not have any effects on the analytical results (Table 4). However, the change of ionic force of NaOH from 0.1 N to 0.01 N or change of the wavelength from 277 nm to 272 nm, had a significant effect on the results.

Table 2: Intra and inter-day assay variations of diacerhein by spectrophotometric method

	Intra-day[a]		Inter-day[b]	
	277 nm			
Product	% ± s.e.m.	% RSD	% ± s.e.m.	% RSD
A	99.4 ± 0.52	1.3	99.2 ± 0.25	1.1
B	97.8 ± 0.57	1.4	97.8 ± 0.35	1.2
C	99.4 ± 0.41	1.0	99.3 ± 0.35	1.0
	502 nm			
A	97.8 ± 0.18	0.5	97.9 ± 0.14	0.6
B	98.7 ± 0.63	0.1	99.5 ± 0.37	1.1
C	98.0 ± 0.64	0.1	99.7 ± 0.23	0.6

[a] average of six determinations
[b] average of three determinations
s.e.m. = standard error of the mean

Table 3: Recovery test of diacerhein capsules by standard addition method

λ (nm)	Amount of reference ($\mu g \cdot mL^{-1}$)		%	
	Added	Recovered	Recovery	Average
277	4.0	3.97	99.0	
	7.0	7.03	100.2	99.4
	10.0	9.92	99.0	
502	6.0	6.12	101.7	
	10.0	10.02	99.9	100.4
	14.0	13.95	99.4	

Table 4: Robustness test of spectrophotometric method

λ (nm)		Ionic force of NaOH		
		0.01 N[a]	0.1 N[a]	1 N[a]
277	% ± s.e.m.	103.0 ± 0.2	98.6 ± 0.1	98.1 ± 0.4
	% RSD	0.3	0.2	0.6
502	% ± s.e.m.	101.2 ± 0.2	98.9 ± 0.2	96.8 ± 0.3
	% RSD	0.2	0.3	0.4
		Change of wavelengths		
		272	277	282
	% ± s.e.m.	103.6 ± 0.7	98.6 ± 0.1	98.9 ± 0.1
	% RSD	1.0	0.2	0.1
		497 nm[a]	502 nm[a]	507 nm[a]
	% ± s.e.m.	99.1 ± 0.4	98.9 ± 0.2	99.6 ± 0.3
	% RSD	0.5	0.3	0.5

[a] mean of two determinations
s.e.m. = standard error of the mean

3. Experimental

3.1. Chemicals

Diacerhein chemical reference substance (CRS) (assigned purity, 99.8%) was obtained from DEG (Brazil). Capsules (Product A) and compounded capsules (Products B and C) were purchased at the local market and were claimed to contain 50 mg DAR each. Sodium hydroxide analytical grade was obtained from Merck (Germany).

3.2. Equipment

A double-beam UV-VIS spectrophotometer (Shimadzu, Japan) model UV – 1601 PC, with a fixed slit width (2 nm) and a 10 mm quartz cell was used to obtain spectrum and absorbance measurements.

3.3. Sample preparation

Twenty capsules were weighed to obtain the average weight. An amount of the powdered sample equivalent to 12.5 mg of DAR was transferred to a 100 mL volumetric flask, about 70 ml of 0.1 N NaOH were added and the flask was sonicated for 20 min, followed by addition of 0.1 N NaOH to volume (final concentration of 125 $\mu g \cdot mL^{-1}$). This solution was filtered through a quantitative paper filter (Schleicher & Schuell) and further dilution was made with the same solvent in order to give final concentrations of 15 $\mu g \cdot mL^{-1}$ and 22 $\mu g \cdot mL^{-1}$ to be measured at 277 nm and 502 nm, respectively.

3.4. Method validation

The method was validated by the determination of the following operational characteristics: specificity, linearity, precision, accuracy and robustness (USP 29 2006, ICH Q2R1, 2005).

3.4.1. Specificity

The influence of commonly used capsule excipients was investigated before the determination of the drug in dosage forms.

3.4.2. Linearity

For measurement at 277 nm, aliquots of DAR CRS stock solution ($100\ \mu g \cdot mL^{-1}$ in 0.1 N N OH), ranging from 1.5 to 6.0 ml, were transferred to 25 ml volumetric flasks and the volumes were made up with 0.1 N NaOH to obtain solutions at concentrations of 6, 9, 12, 15, 18, 21 and $24\ \mu g \cdot mL^{-1}$. In a similar way, for measurement at 502 nm, aliquots of stock solution ranging from 2.5 to 8.5 ml were transferred to a 25 ml volumetric flasks and the volumes were made up with 0.1 N NaOH to obtain solutions at concentrations of 10, 14, 18, 22, 26, 30 and $34\ \mu g \cdot mL^{-1}$. The curves were prepared in three different days. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

3.4.3. Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as a R.S.D.% of a series of measurements. Repeatability was calculated by assaying six samples of the 100% standard concentration (15 or 22 $\mu g \cdot ml^{-1}$). The analyses were repeated on three different days in order to evaluate the intermediate precision.

3.4.4 Accuracy

The accuracy was determined by % recovery of known amounts of DAR substance reference added to the samples at beginning of the process. All solutions were prepared and assayed in triplicate. The percentage recovery of added DAR substance reference was calculated using the equation proposed by A.O.A.C. (1990).

3.4.5 Robustness

The samples were assayed under different conditions, such as different ionic force of the diluent (0.01 N NaOH and 1 N NaOH) and at different wavelengths (272 ± 5 nm and 502 ± 5 nm).

Acknowledgement: This work was supported by CAPES-Brazil and FAPERGS.

References

Giannellini V, Salvatore F, Bartolucci G, Coran SA, Bambagiotti-Alberti M (2005) A validated HPLC-stability-indicating method for the determination of diacerhein in bulk drug substance. J Pharm Biom Analysis 39: 776–780.

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005), ICH Steering Committee, Validation of Analytical Procedures: Text and Methodology Q2 (R1)

Lequesne M (1994) Symptomatic slow acting drugs in osteoarthritis: a novel therapeutic concept? Rev. Rhum (Engl Ed) 61: 69–73.

Official Methods of Analytical Chemists of A.O.A.C. (1990) 15th ed., XVII.

Spencer CM, Wild MI (1997) Diacerhein. Drugs 53: 987–106.

The United States Pharmacopoeia (USP) 29 ed. Rokville, United States Convention, 2006.

Watson DG (1999) Pharmaceutical Analysis – A textbook for pharmacy students and pharmaceutical chemists, Churchill Livingstone.