Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 113 – 118

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DIACEREIN IN CAPSULES

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A simple, sensitive, rapid, accurate and precise spectrophotometric method has been developed for the estimation of diacerein in bulk and pharmaceutical dosage forms. Diacerein shows maximum absorbance at 258.5 nm with molar absorptivity of 4.2258×10^4l/mol/cm. Beer's law was obeyed in the concentration range of 1-10 µg/ml. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02 µg/ml and 0.07 µg/ml, respectively. The results of recovery studies (98.68-101.11%) indicated that proposed method is accurate and precise for the determination of diacerein in capsules.

(Received December 2, 2009; accepted February 23, 2010)

Keywords: Diacerein, UV spectroscopy, Validation, Capsules

1. Introduction

Diacerein, chemically, 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Fig. 1) is a chondroprotective agent intended for the treatment of osteoarthritis [1, 2]. Diacerein is the di-acetylated derivative of rhein, a molecule with an anthraquinone ring which is actually the active metabolite of diacerein [3]. Diacerein is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentrations of proinflammatory cytokines [4, 5]. In addition to effect on macrophage migration and phagocytosis, it also inhibits superoxide production, chemotaxis and phagocytic activity of neutrophils [6, 7]. However, diacerein lacks cyclooxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis [8, 9]. Therefore, it has been considered as a slow-acting anti-arthritic drug not belonging to the NSAIDs that may interfere with the pathological course of osteoarthritis [3].

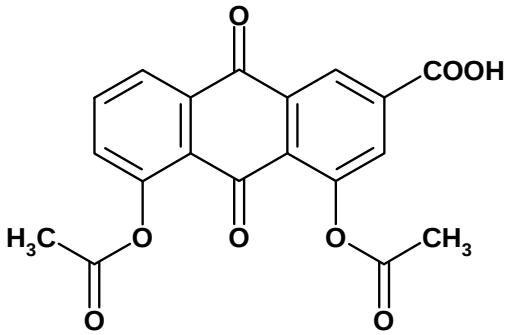

Fig. 1 Chemical structure of diacerein

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Literature survey revealed that, two stability indicating HPLC methods have been reported for the quantitative estimation of diacerein in bulk drugs [10] and in capsule dosage forms [11]. Two impurities from diacerein bulk drug have been isolated and structurally elucidated by HPLC and LC-MS methods, respectively [12]. Diacerein has been also found to be estimated by chemiluminescence technique in pharmaceutical dosage forms [13]. However, no UV spectrophotometric method is available for the quantitative determination of diacerein in its pharmaceutical dosage forms.

The objective of the present work was to develop simple, rapid, accurate and specific UV spectrophotometric method for the estimation of diacerein in pharmaceutical dosage forms. The developed method for the analysis of diacerein was validated with respect to stability, linearity, sensitivity, precision, accuracy, specificity, robustness and ruggedness [14-17].

The limit of detection (LOD) and limit of quantification (LOQ) were also determined. The results of analysis were validated statistically and by recovery studies. This method of estimation of diacerein was found to be simple, precise and accurate.

2. Experimental

2.1. Materials

Diacerein was obtained as a gift sample from Glenmark Pharmaceuticals Ltd., Mumbai, India. Diacerein capsules were procured from local pharmacy. All the reagents were of analytical grade. Glass double distilled water was used throughout the experiment. A Shimadzu UV/VIS 1700 spectrophotometer with 1 cm matched quartz cells were used for the estimation.

2.2. Standard Preparation

An accurately weighed 5 mg of diacerein was dissolved in 10 ml of dimethyl formamide (DMF) in a 50 ml volumetric flask and the volume was adjusted up to the mark with distilled water to obtain a stock solution of 100 µg/ml. The solution was filtered through Whatman filter paper No. 41. Aliquots of 0.1 to1 ml portions of standard solution were transferred to a series of 10 ml volumetric flasks and volume in each flask were adjusted to 10 ml with distilled water to obtain a concentration of range of 1-10 µg/ml. One of the solutions was scanned in UV range using DMF: distilled water (1:4) as a blank and λ_{max} was found to be 258.5 nm. The absorbance of solutions was measured at 258.5 nm against blank and calibration curve of diacerein was constructed.

2.3. Sample preparation

Twenty capsules of diacerein were emptied and powder was weighed. Amount equivalent to 5 mg was transferred to 50 ml volumetric flask, dissolved in 10 ml of DMF and made up the volume with distilled water to obtain a concentration of 100 µg/ml. The solution was filtered through Whatman filter paper No. 41 and filtrate was diluted to obtain concentration in between linearity range. The absorbance of sample solution was measured and amount of diacerein was determined by referring to the calibration curve. Recovery studies were carried out at 50, 100 and 150% level by adding a known quantity of pure drug to the preanalyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

3. Results and discussion

3.1. Optical characterization and precision

The results of optical characterization and precision of the proposed method for estimation of diacerein are presented in Table 1. The proposed method of determination of diacerein showed molar absorptivity of 4.2258×10^4 l/mol/cm and Sandell's sensitivity 0.008709 mcg/Sq.cm/0.001-absorbance units. Diacerein exhibits its maximum absorption at 258.5 nm (Fig. 2) and obeyed

Beer's law in the range of 1-10 µg/ml (Fig. 3). Linear regression of absorbance on concentration gave equation $y = 0.11483x + 0.014333$ with a correlation coefficient of 0.9998. Relative standard deviation of 0.0021 was observed for analysis of 6 replicate samples, indicating developed method is precise.

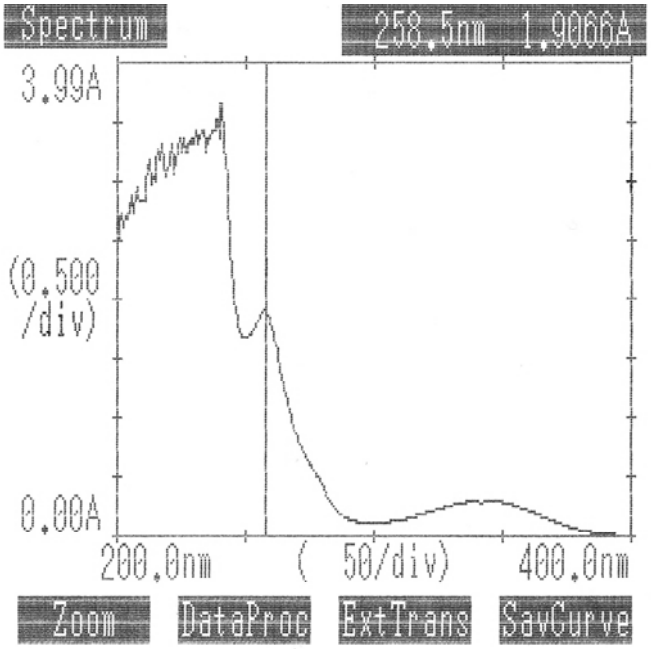

Fig. 2 λ_{max} of diacerein in DMF: distilled water (1:4)

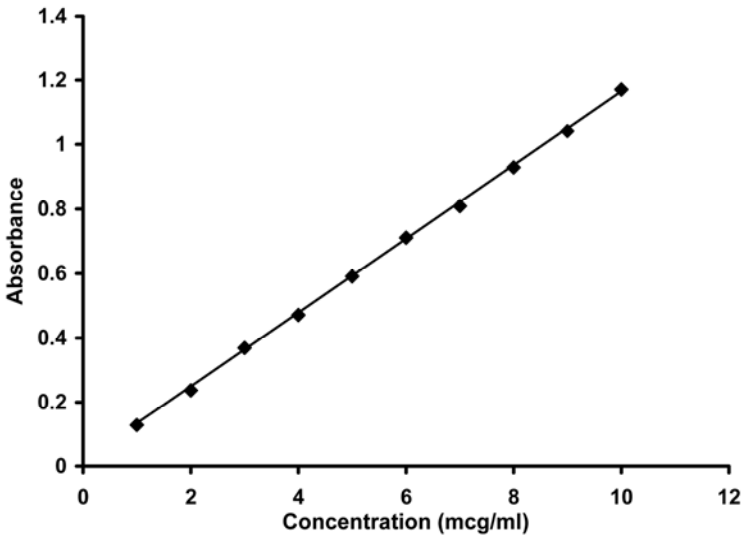

Fig. 3 Calibration curve of diacerein in DMF: distilled water (1:4) at 258.5 nm

Table 1 Validation parameters for standard diacerein

Parameter	**Value**
λ_{max} (nm)	258.5
Beer's range (µg/ml)	1-10
Molar absorptivity (l/mol/cm)	4.2258×10^4
Sandell's sensitivity (µg/cm^2/0.001AU)	0.008709
Correlation coefficient (r^2)	0.9998
Regression equation	$y = 0.11483x + 0.014333$
Intercept (a)	0.014333
Slope (b)	0.11483
Limit of detection (LOD µg/ml)	0.02
Limit of quantification (LOQ µg/ml)	0.07
Precision (% RSD)*	0.0021

* Indicates mean of six determinations (n=6); RSD: Relative standard deviation

3.2. Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by Eqs. LOD = $\frac{3.3\delta}{s}$ (1) and LOQ = $\frac{10\delta}{s}$ (2), respectively, where δ is the standard deviation of blank and s is slope of calibration [18].

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02 µg/ml and 0.07 µg/ml respectively (Table 1) indicating proposed UV method is highly sensitive.

3.3. Analysis in capsule formulations, accuracy and reproducibility

The proposed method has successfully estimated the amount of diacerein in the range of 98.56-99.14% in all tested formulations (brands). The accuracy and specificity of the proposed method was checked by recovery experiments (Table 2). The percentage recovery values for Hilin, Dycerin and Cartishine were found to be 98.68%, 98.20% and 101.11% respectively (Table 2). The high recoveries with low % RSD values indicated that the method had a good accuracy and specificity, as there was no interference from the excipients present in formulations.

Intra-day precision and accuracy were determined by analyzing replicate samples of different concentrations, prepared on same day. Inter-day variability was evaluated by analyzing two concentrations on three different days. The % RSD values reported in Table 3 shows an acceptable intra-day and inter-day variation of diacerein for the proposed method indicating accuracy and reproducibility of the assays.

Table 2 Results of analysis of formulations and recovery studies

Formulations (Brand)	**Label Claim mg**	**% Estimated***	**%RSD**	**% Recovery***	**%RSD**
Hilin	50	98.82	0.97	98.68	1.11
Dycerin	50	99.14	0.87	98.20	0.78
Cartishine	50	98.56	0.64	101.11	1.06

RSD: Relative standard deviation; (n=6).

Table 3 % RSD values for repeatability, intra- day, inter-day variation and ruggedness

Formulations (Brands)	**Parameters**				
	Repeatability	**Precision**		**Ruggedness**	
		Intra-day	**Inter-day**	**Analyst 1**	**Analyst 2**
Hilin	1.12	0.77	1.32	0.47	1.02
Dycerin	0.89	0.96	0.98	0.38	0.57
Cartishine	1.22	0.67	1.41	0.62	0.82

RSD: Relative standard deviation; (n=3).

3.4. Ruggedness and robustness

Ruggedness of the proposed methods was determined by analyzing diacerein by different analysts, using identical operational and environmental conditions. The % RSD values were found to be less than 2% (Table 3).

Robustness of the proposed method was tested by minor changes on the selected wavelength. No significant difference was found in the absorbance of samples. Therefore, the proposed method was considered as robust.

4. Conclusions

The developed method was found to be sensitive, accurate, precise and reproducible and can be used for the routine quality control analysis of diacerein in bulk drugs and formulations.

Acknowledgements

We are grateful to Glenmark Pharmaceuticals Ltd., Mumbai, India, for providing gift sample of drug for research work. We are thankful to Principal, Govt. College of Pharmacy, Karad for providing laboratory facility and constant encouragement.

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