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UV-Spectrophotometric estimation of Diacerein in pharmaceutical formulation

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ABSTRACT

Three simple, precise and economical UV methods have been developed for the estimation of Diacerein in Pharmaceutical Formulations. Diacerein has the maximum absorbance at 256.2 nm in zero order spectra formed the basis for method A while first order derivative spectrum showed peak at 250.0 nm when n = 1 for method b and method C applied was Area under Curve (AUC) in wavelength range of 262.0-250.0 nm.. Method A utilises A1%, 1cm value at λmax for its analysis. Calibration curve (Regression equation) was used for method B and C for analysis of Diacerein respectively. Drug was found to obey the Beer-Lambert's law in the concentration range of 5-30µg/mL in all three proposed methods. Results of the analysis were validated statistically and by recovery studies. Results were found to be satisfactory and can be adopted for routine analysis of the drug.

Keywords: Diacerein, UV spectroscopy, Derivative spectroscopy and Area under Curve.

INTRODUCTION

Diacerein [1,2] also known as diacetylrhein is a drug used in the treatment of osteoarthritis. Chemically it is 4, 5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid. It is slow acting symptomatic treatment of osteoarthritis by inhibiting interleukin-1, which has demonstrated efficacy on functional manifeststation of osteoarthritis and on the structural component. Literature survey reveals HPLC stability indicating method for determination of diacerein in bulk substance [3,4]. A direct spectrophotometric determination of diacerein in capsule [5], in which diacerein has showed absorption maxima at 502.0 nm in 0.1 N sodium hydroxide. Hence an

attempt has been made to develop new UV methods for its estimation in pharmaceutical formulation with good accuracy, simplicity, precision and economy.

EXPERIMENTAL SECTION

Materials and Methods

A Shimadzu UV 1700 series Spectrophotometer was used with 1 cm matched quartz cells.

Preparation of Standard Solution

An accurately weighed quantity of diacerein (~25mg) was dissolved in appropriate quantity of N,N-dimethylacetamide (DMA) and transferred in a 50.0 mL volumetric flask, then volume was made up with methanol up to the mark to get standard stock solution of concentration 500µg/mL. A standard stock solution was further diluted with methanol to get final solution of concentration 20µg/mL.

Spectral Scanning and Wavelength Selection

The standard solution of diacerein (20µg/mL) was scanned in the range of 400-200nm (Method A) in 1.0 cm cell against solvent blank and spectra was recorded, the absorbance maximum was observed at 256.2.0 nm (**Figure 1**). The First derivative spectra at n = 1 (method B) showed a sharp peak at 250.0 nm (**Figure 2**). The absorbance difference calculated at n=1 (dA/dλ) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solutions. The calibration curve of dA/dλ against concentration of the drug showed linearity, which are used for the estimation of the drug. The AUC (Area under Curve) method (method C) involves the calculation of integrated value of absorbance with respect to the wavelength λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis was selected by entering the wavelength range over which the area has to be calculated. The wavelength range from 262.0- 250.0 nm was selected which showed good linearity between area under curve and concentration.

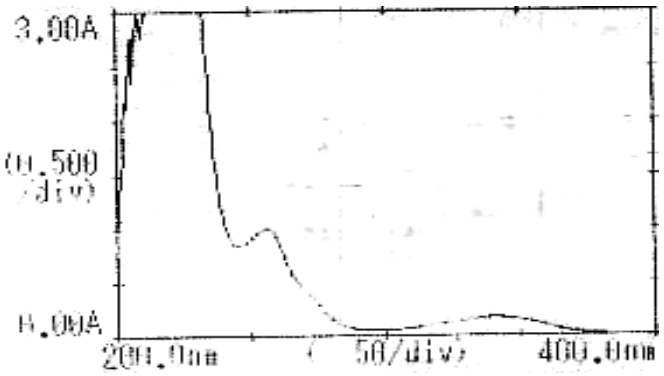

Figure 1 Spectrum of Diacerein

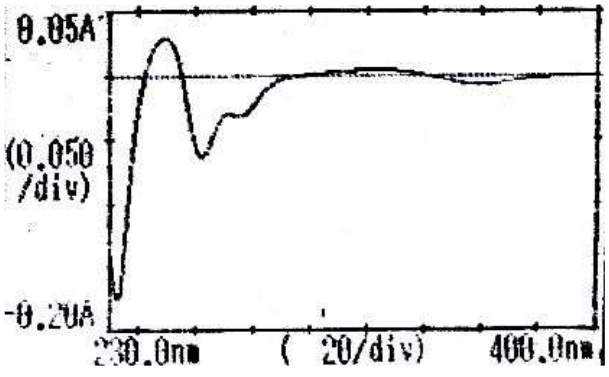

Figure 2 Derivative first order Spectrum

Construction of Calibration Curve

Different concentration of the standard drug solution (5-30µg/mL) were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm. Calibration curves were plotted as

absorbance vs concentration (method A), differential absorbance vs concentration (method B) and area under curve against concentration (Method C). The optical characteristics are recorded in **Table I**

Table I: Optical Characteristics

Parameters	Method A	Method B	Method C
λ_{Max} (nm) / wavelength range	256.0	250.0	262– 250.0
Beer-Lambert's range (µg/mL)	5-30	5-30	5-30
Correlation coefficient (r)	0.9999	0.9998	0.9999

Assay

An accurately weighed quantity of tablet powder equivalent to about 25 mg diacerein was transferred to 50.0 mL volumetric flask. To it appropriate quantitiy of DMA and methanol was added, sonicated for 15 min and diluted up to the mark with methanol. The contents of the flask was then filtered through Whatmann filter paper (no. 41) & was further diluted with methanol to get final solution of concentration 20µg/mL (on label claim basis). The absorbance of the resultant solution was read and the amount of diacerein was calculated by taking A (1%, 1cm) at 256.2.0 nm (method A), by first order derivative absorbance at 250.0 nm (method B) and by Area under Curve (AUC) (method C). Calculation was done by using following formulae for method A and for method B and C were done by using calibration curve and regression equation.

Method A

$$\% \text{ of labelled Claim} = \frac{\text{Absorbance} \times \text{Dilution factor} \times \text{Avg wt}}{A\ (1\%, 1cm) \times \text{wt.taken} \times \text{Label claim}} \times 100$$

The results of estimation of diacerein are shown in **Table II**

Table II: Results of estimation in tablet formulation

Method	Tablet Formulation	% Label Claim (±)S. D.*	C.V.*
A	D1	100.16 ±0.86	0.86
	D2	100.97 ±0.42	0.41
B	D1	100.88 ±0.17	0.17
	D2	100.59 ±0.05	0.05
C	D1	99.22 ±0.42	0.42
	D2	101.55 ±0.23	0.22

Where, A is zero order derivative spectrum method, B is first order derivative method with n = 1, C is the AUC method , D1 and D2 are two different brands of tablet formulations.

** The results are the mean of five readings (n = 5).*

Validation [6,7]

All these methods were validated according to ICH guidelines for accuracy, Precision, Specificity, linearity and range and Ruggedness.

i**) Accuracy (Recovery Studies)**

Accuracy was ascertained on the basis of recovery studies by standard addition method. Recovery studies were carried out at four different levels by adding the pure drug (5.04, 10.08, 15.12 and 20.16 mg) to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated. The recoveries of the drug were observed to be very close to 100 % representing the accuracy of the method and also show that excipients have no interference in the estimation. Results are recorded in **Table III**.

Table III: Recovery Study Data

Statistical Parameter	Method A	Method B	Method C
Mean	99.15	99.55	98.98
±SD	0.78	1.38	0.45
CV	0.79	1.38	0.45

Where,A is zero order derivative spectrum method with n = 0, B is first order derivative method with n = 1, C is the AUC method. The results are the mean of triplicate at each level of recovery

ii) Precision

Precision of analytical method is expressed in terms of SD, % RSD of series of measurements. Study was carried out by replicate analysis of homogeneous samples of tablet powder. Results were recorded in **Table II**.

iii) Intraday Precision and Inter-day precision

An accurately weighed quantity of tablet powder equivalent to about 25 mg was diluted to get the final concentration 10 µg/mL on label claim basis. The absorbencies and area of the solutions were taken at an interval of 3h within the day at selected wavelengths for intraday study for all the three methods. Similarly the same solution was measured on 1^{th}, 4^{th}, 7^{th} and 14^{th} day. Percent label claim was calculated. The results are recorded in **Table IV**.

Table IV: Summary of validation parameters

Parameters	Mean Percent Label Claim		
	Method I	**Method II**	**Method III**
Intraday Precision (n=3)	101.73	97.13	96.43
Amount found	0.26	1.94	0.62
% RSD			
Interday precision (n=4)			
Amount found	101.11	99.96	100.42
% RSD	1.25	4.02	1.22
Analyst to Analyst (n = 3)			
Amount found	100.24	100.51	101.31
% RSD	0.113	0.110	0.301
Linearity and Range			
Correlation coefficient	0.9975	0 .9925	0.9979

iv) Linearity and Range

Accurately weighed quantity of tablet powder equivalent to 80, 90, 100, 110, 120% of label claim of diacerein were taken and dilutions were made as described under assay. The absorbance and area of the resulting solutions were measured at selected wavelengths respectively against blank. The graphs of concentration Vs absorbance were plotted and were found to be linear.

v) Ruggedness

The studies were carried out for analyst to analyst variation. The tablet samples were analyzed by proposed method by three different analysts and results were recorded in **Table IV**.

vi) Stability studies:

The forced degradation studies of diacerein were carried out for the following conditions:
-Acidic hydrolysis (0.1N HCL) -Alkaline hydrolysis (0.1N NaOH)
-H_2O_2 (6%) -Humidity (75%) -60ºC for 24 hr -UV exposure

After the specified period of exposure the samples were diluted as described under assay procedure and the percent label claim was analysed by all the proposed methods. The results of estimation are shown in **Table V**

Table V: Results of estimation for stability studies

Parameters	Mean Percent Label Claim		
	Method I	Method II	Method III
NaOH (0.1 N)	52.29	---	61.18
HCL (0.1 N)	78.46	---	79.30
H_2O_2 (6%)	84.83	83.51	86.01
Humidity (75%)	100.49	97.39	100.56
60ºC for 24 hr	98.21	97.23	100.24
UV exposure	93.67	94.35	96.15

RESULTS AND DISCUSSION

All the methods A, B and C for the estimation of diacerein in tablet dosage form were found to be accurate, simple and reproducible. The drug followed the Beer's- Lambert's law in concentration range of 5-30 µg/mL for all three methods and correlation coefficient was 0.9999, 0.9999 and 0.9999 for Method A, B, and C respectively. The recovery studies were close to 100% (**Table III**). The results of validation studies suggest that the drug in solution was not stable for more than 2 hours as suggested from intraday and interday studies indicated, by large CV (**Table IV**). Also, the stability studies suggest that the drug is not stable under hydrolytic conditions and Uv exposure (photo-degradation) as indicated by difference the percent label claim as compared to normal by all proposed methods, indicating the degradation (**Table V**). The estimation of drug from formulation was found to be satisfactory and was capable of estimation in presence of its degradation.

CONCLUSION

From the above studies it can be concluded that the UV spectrophotometric methods for quantitative estimation of Diacerein in marketed formulation are quite reliable, accurate and precise and can be adopted for routine analysis of the drug.

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