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Stability-indicating hptlc method for estimation of diacerein in pharmaceutical dosage form

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ABSTRACT

The present work describes a stability-indicating HPTLC method for analysis of diacerein in bulk and pharmaceutical dosage form. Precoated silica gel 60 F_{254} plate was used as stationary phase. The separation was carried out using toluene: isopropyl alcohol: ammonia (4.6:4.6:0.8 %v/v/v) as mobile phase. The densitometric scanning was carried out at 258 nm. The Rf value for the drug was found to be 0.30±0.01. The linearity was obtained in the range 100-350ng/band (r^2 = 0.9909). The method was validated as per ICH guidelines. Diacerein was subjected to forced degradation by acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drug with significantly different Rf values.

Keywords: Diacerein, HPTLC, Validation, Stability Studies.

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INTRODUCTION

Diacerein is chemically 4, 5-diacetoxy-9-10-dioxo-9-10-dihydroanthracene-2-carboxylic acid [1]. Diacerein and its derivatives are known to possess antiarthhtic and moderate anti-inflammatory, antipyretic and analgesic activity and have a good safety profile. Diacerein alone or in combination with other drugs is reported to be estimated by physico-chemical and structural characterization [2], isolation and structural elucidation [3], spectrophotometry [4, 5, 6], HPLC [7], flow injection chemiluminiscence [8], LC-MS/MS [9] and HPTLC [10].

The present work describes a new method for determination of diacerein in capsules using HPTLC-densitometry. The method is simple, requires less time for routine analysis of bulk and marketed formulation.

MATERIALS AND METHODS

Materials

Diacerein was supplied as a gift sample by Glenmark pharmaceuticals Ltd, Mumbai. All chemicals and reagents used were of HPLC/AR grade.

Instrumentation and chromatographic conditions

The standard solution ranging from 100-350ng/band was applied on precoated silica gel 60 F_{254} plate in the form of bands with 100 µl sample syringe using automatic sample applicator LINOMAT V. It was developed in a twin trough glass chamber which was already saturated for 30 min. with the mobile phase. The mobile phase consisted of toluene: isopropyl alcohol: ammonia (4.6:4.6:0.8 %v/v/v). After development, plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved bands of drug were scanned at 258 nm with Camag TLC scanner III densitometer controlled by WINCAT's software version 4.

Standard solutions and calibration graphs

Stock solution was prepared in 50 ml volumetric flask by dissolving 50 mg of diacerein in 5 ml of dimethyl sulfoxide, drug solution was diluted to the mark with the methanol (1000µg/ml). 1 ml of stock solution was further diluted to 10 ml with methanol to get stock solution of 100ng/µl. The standard solutions were applied to reach a concentration range 100-350ng/band for diacerein. The plate was developed using previously described mobile phase and well resolved band of drug were scanned at 258 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

Analysis of marketed formulation

Twenty capsules were weighed, finely powdered and powder equivalent to 50 mg diacerein was transferred into 50 ml volumetric flask, dissolved in 5 ml of dimethylsulfoxide to this sufficient quantity of methanol was added and sonicated for 30 min. The volume was then made up to the mark using same solvent. The solution was filtered through Whatman paper No. 41. From the filtrate 1 ml was further diluted to 10ml with methanol to get sample stock solution of diacerein 100ng/µl. Sample solution were applied six times on TLC plate to give spot concentration 150ng/band of diacerein. The plate was developed in the previously described chromatographic conditions. The peak area of the spots was measured at 258 nm and concentrations in the samples were determined using multilevel calibration.

Method validation

The method was validated in compliance with ICH guidelines [11].

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RESULT AND DISCUSSION

Optimization of procedures

Different proportions of toluene: isopropyl alcohol: ammonia were tried while mobile phase selection. Ultimately toluene: isopropyl alcohol: ammonia (4.6:4.6:0.8 %v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of diacerein was obtained as shown in fig 1. Peak was symmetrical in nature and no tailing was observed when plates were scanned at 258 nm.

Linearity

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 100-350ng /band. (r^2 = 0.9909). The standard calibration curve is given in fig 2 and standard calibration data for diacerein is given in Table No.1.

Table No 1: Linear regression data for calibration curves

Detection Wavelength (nm)	258
Beer's Law Limit (ng/band)	100-350
Regression equation	y = 19.66x + 588.97
Correlation Coefficient (r^2)	0.9909
Intercept (c) ± SD	588.97 ± 58.15
Slope (m) ± SD	19.66 ± 0.38
Limit of detection (ng)	9.71
Limit of quantitation (ng)	29.55

Analysis of the marketed formulation

The spot at Rf 0.30 was observed in the densitogram of the drug samples extracted from capsules. There was no interference from the excipients commonly present in the capsules. The diacerein content was found to be close to 100% and the results are summarized in Table No 2. The low %RSD value indicated the suitability of this method for routine analysis.

Table No 2: Results of marketed formulation analysis

Marketed formulation	Label claim (mg)	Area* of densitogram (150 ng/band)	Amt. of drug estimated (mg) ± S.D*	% Mean amount estimated*± S.D*
Dycerin (Glenmark pharmaceuticals Ltd.)	50	3485.10	50.11 ±0.01	100.53 ±0.59

*Average of six determination

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table No 3.

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Table No 3: Statistical evaluation of precision of developed method (n = 6)

Drug - Diacerein	Repeatability*	Precision	
		Intraday*	Interday*
Conc.(ng/band)	150	150	150
Mean area ± SD	3486.20 ± 3.07	3479.12 ± 1.78	3481.23 ± 0.45
% Content ± SD	100.74± 0.03	100.23± 0.03	100.49 ± 0.008
RSD (%)	0.03	0.03	0.008
S.E.	0.0173	0.0173	0.0046

*Average of six determinations

Recovery studies

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard diacerein were added to pre-analyzed samples and were subjected to the proposed HPTLC method. Results of recovery studies are shown in Table No 4.

Table No 4: Result of recovery studies

Level of recovery (%)	Amount taken (ng/band)	Amt of std added (ng/band)	Total amt recovered (ng/band)	% Recovery*	SD	S.E.	% COV
80	150	120	270.95	100.35	0.39	0.2254	0.38
100	150	150	301.10	100.36	0.29	0.1676	0.28
120	150	180	330.67	100.21	0.19	0.1098	0.18

*Average of three determinations

Robustness

The robustness of the method was determined by variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 10, 20, 30 min). One factor at a time was changed at a concentration level of 150 ng/band of diacerein, to study the effect on the peak area of the drugs. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that method is robust. Results are shown in Table No 5.

Table No.5: Results of robustness studies

A: Chromatographic Changes (% of toluene in mobile phase)

% change in mobile phase	Rf	Peak area
+2%	0.31	3490.13
0%	0.30	3482.87
-2%	0.32	3489.77
Mean*± S.D.	0.31±0.01	3487.59 ±4.09

*Average of three determinations

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B: Chromatographic Changes (chamber saturation)

Chamber saturation (Time in min.)	Rf	Peak area
33	0.33	3486.30
30	0.30	3484.23
27	0.28	3479.10
Mean*± S.D.	0.30 ±0.03	3483.21 ±3.70

*Average of three determinations

C: Chromatographic Changes (development distance)

development distance (mm)	Rf	Peak area
85	0.32	3488.77
80	0.30	3482.77
75	0.29	3484.83
Mean*± S.D.	0.30 ±0.01	3485.46 ±3.04

*Average of three determinations

D: Chromatographic Changes (Time from application to development)

Time from application to development	Rf	Peak area
0	0.30	3483.73
10min	0.31	3478.40
20min	0.33	3472.77
30min	0.32	3470.43
Mean*± S.D.	0.31 ±0.01	3476.33 ±5.9

*Average of three determinations

E: Chromatographic Changes (Time from development to scanning)

Time from development to scanning	Rf	Peak area
0	0.31	3473.07
10min	0.30	3481.60
20min	0.30	3478.27
30min	0.29	3486.38
Mean*± S.D.	0.30 ±0.008	3479.83 ±5.6

*Average of three determinations

Stability-indicating property [12]

HPTLC studies of the samples obtained during the stress testing of diacerein under different conditions using toluene: isopropyl alcohol: ammonia (4.6:4.6:0.8 %v/v/v) as the mobile phase shows different degradation peaks as shown in figures 4-7. The amount of drug recovered after degradation studies and the Rf of degradation products are given in Table No 6.

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Table No 6: Results of forced degradation studies

Stress condition	Time (hours)	% Assay of active substance	R_f values of degradation products
Acid hydrolysis (0.1 M HCl)	24	68.11	0.13
Base hydrolysis (0.1 NaOH)	24	71.15	0.15, 0.16
Oxidation (3% H_2O_2)	24	53.65	0.18, 0.35,0.63,
Thermal degradation (50^0C)	24	40.92	0.66

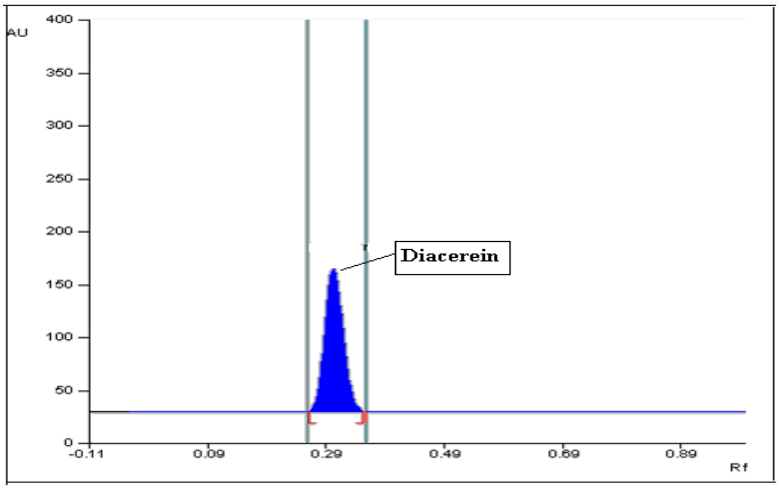

Figure No. 1: Densitogram of diacerein

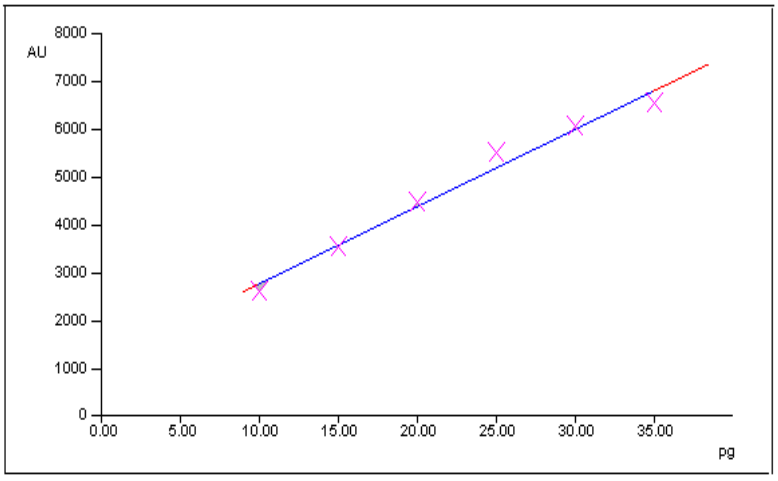

Figure No 2: Calibration curve of diacerein

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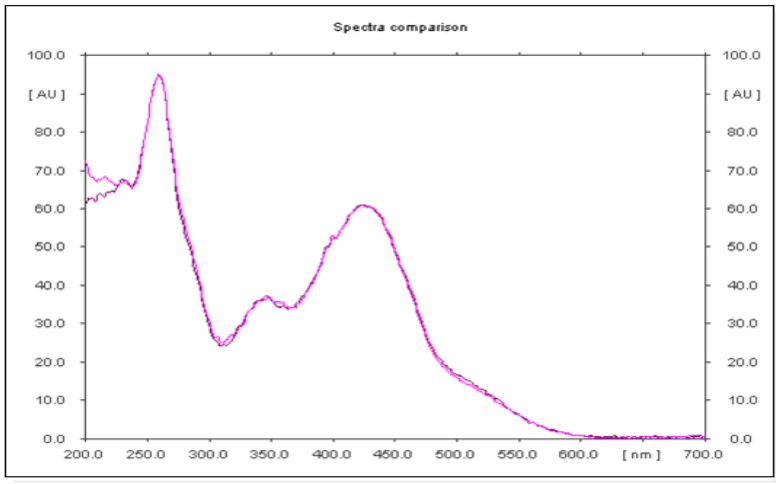

Figure No.3: Spectrum of diacerein standard and sample measured from 200 to 700 nm.

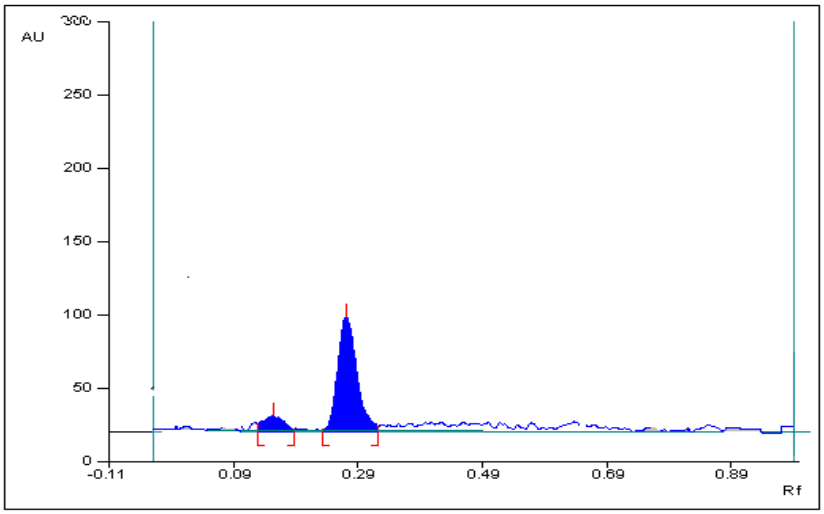

Figure No 4: Acid degradation

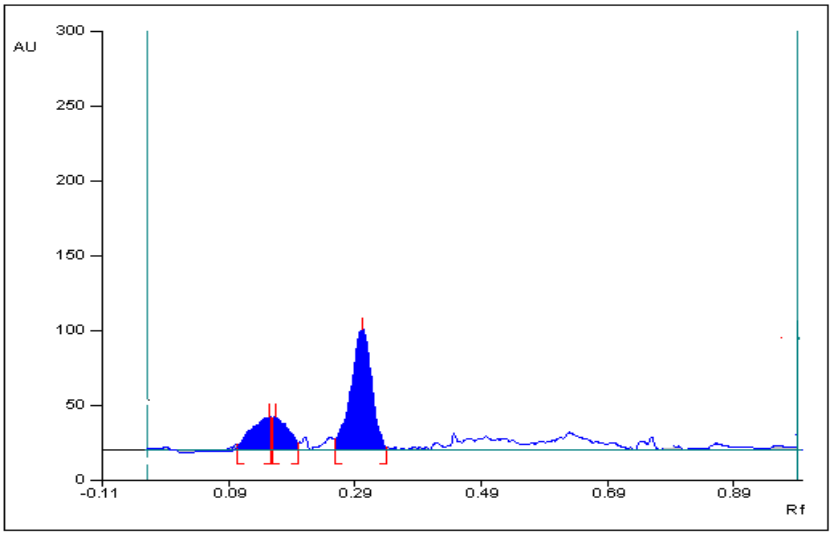

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Figure No 5: Alkali degradation

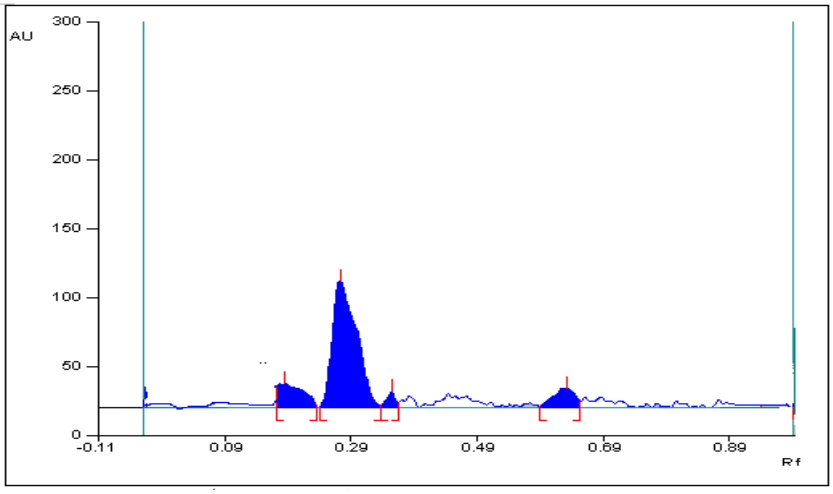

Figure No 6: H_2O_2 degradation

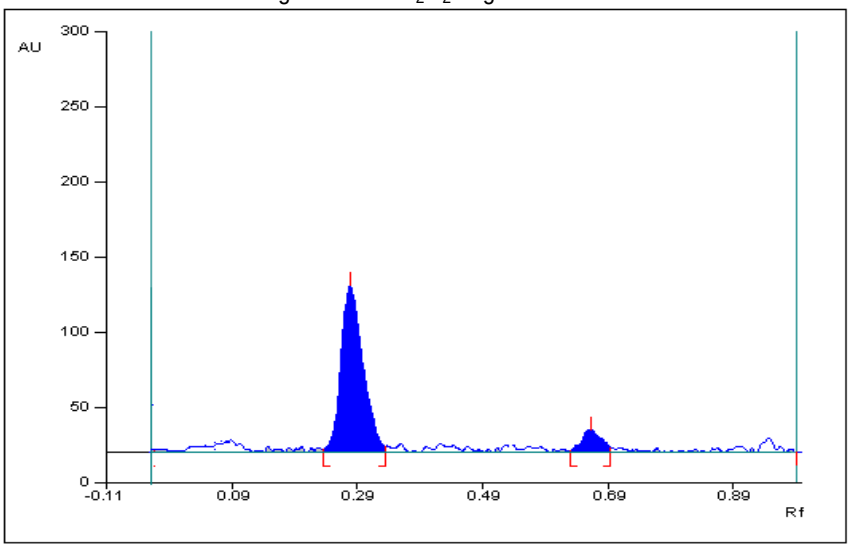

Figure No 7: Heat degradation

Acid-induced degradation

1 ml of 0.1N hydrochloric acid was added to drug solution and it is diluted with methanol to get the final concentration of 100ng/µl of drug. This solution was allowed to stand for 24 hrs. The drug was degraded in acidic condition and shows different degradation products at Rf 0.13 as shown in Figure No 4.

Base-induced degradation

1 ml of 0.1N sodium hydroxide was added to drug solution and it is diluted with methanol to get the final concentration of 100ng/µl of drug. This solution was allowed to stand for 24 hrs. The drug was degraded in alkaline condition and shows different degradation products at Rf 0.15, 0.16 as shown in Figure No 5.

Hydrogen peroxide-induced degradation

1 ml of a 3% hydrogen peroxide solution was added to drug solution and it is diluted with methanol to get the final concentration of 100ng/µl of drug. This solution was allowed to stand for 24 hrs. The drug was degraded

in hydrogen peroxide (3%) at room temperature and shows different degradation products at Rf 0.18, 0.35, 0.63 as shown in Figure No 6.

Heat degradation

A drug solution containing 100ng/µl of drug was exposed to 50°C for 24 hrs. The drug when subjected to heat was degraded and degradation products appeared at Rf 0.66 as shown in Figure No 7.

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of the stress studies indicated the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise, selective and can be employed successfully for the estimation of diacerein in capsule formulation.

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