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Research Article

Development And Validation Of UV-HPLC Method for the Estimation Of Diacerein In Bulk And Tablet Dosage Form

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ABSTRACT

Objective: Objective of the present analytical research work was to develop and validate Spectrophotometric method and High Performance Liquid Chromatographic method (HPLC Method) for the Diacerein bulk and tablet dosage form

Methods: A spectrophotometric method and a HPLC method have been developed and validated for estimation of Diacerein in bulk. **Method A (UV SPECTROMETRY Method):** Methanol was used for the preparation of stock and working standard solutions of the drugs. 400-200nm UV range was used to scanned standard solutions of drugs using UV spectrophotometer. The λ_{max} of Diacerein was found to be 248 nm.

Method B (HPLC Method): The HPLC Method for Diacerein was developed using Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm), as stationary particle, isocratic mode. Methanol : Water (80:20v/v) pH3as mobile phase. Mobile phase was maintained at a flow rate of 1 ml/min and detection was carried out at 248 nm. Both the methods were validated in accordance with ICH guidelines **Results:** Diacerein was found to be linear in the concentration range of 5-25 µg/ml for spectrophotometric and HPLC method. Retention time was found to be 4.3 min for Diacerein. **Interpretation and Conclusion:** Results of validation study were found to be satisfactory. So, the methods can be successfully applied for the routine analysis of Diacerein.

INTRODUCTION

Diacetylane (INN), also known as diacetylrain, is a slow-acting anthraquinone class used to treat joint disorders such as osteoarthritis (swelling and pain in joints). It works by inhibiting interleukin-1 beta. The latest Cochrane review in 2014 found that diacerein had a slight beneficial effect on pain.

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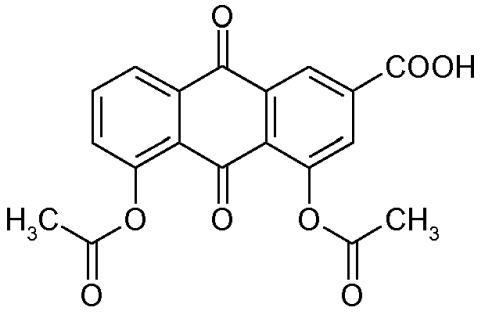

Figure 1: Structure of Diacerein

By chemically Diacerein is (*S*)-2-(2-Oxopyrrolidin-1-yl)butanamide with molecular formula and weight of C19H12O8 and 368.297 g/mole respectively. This research strategy follows the ICH approval rules. This study aims to promote a rapid and compelling new technique for ensuring diacerein in the dough structure, as indicated by the ICH Q2 R1 rules.

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure samples of Diacerein were kindly supplied by Invochem labratories, Water (HPLC Grade), Acetonitrile and MeOH (AR grade) Merck specialities private limited, Mumbai

Instrument Used

Electronic Weighing Balance (Shimadzu AY-248), Ultrasonicator (Wenser pvt ltd PGB-100), Cellulose Acetate Filter, 0.45 µm (Nylon 66), HPLC System (Analytical Technologies),UV VIS Spectrophotometer (Shimadzu UV-1800)

1. Spectrophotometric Method

1.1 Development of Spectrophotometric Method

Selection of Solvent

Diacerein solution (1000 µg / ml) was prepared with various solvents such as ACN, methanol and water. These solutions were scanned in the UV visible range (200 nm to 800 nm) to determine absorption intensity and absorption wavelength.

Preparation of Standard Stock Solution

Standard stock solution was prepared.

Selection of Wavelength Range

Of the stock solution, 0.1 ml of diacerein was transferred to a 10 ml volumetric flask and the volume was adjusted according to the label with MeOH to a concentration of 10 µg / ml. The solution was scanned in the UV 200-400 nm range.

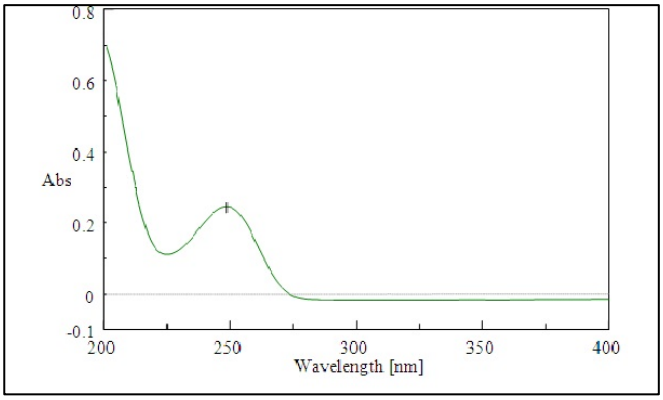

Fig 2. UV spectra of Diacerein

Preparation for Calibration Curve

Calibration curve were prepared and graph was plotted.

Analysis of Tablets

For commercial preparation analysis, twenty tablets were weighed, averaged, and ground to a fine powder. The exact weight of the powder, corresponding to 10 mg of diacerol, was transferred to a 10 ml volumetric flask with 5 ml of methanol, shaken manually for 10 minutes, the volume adjusted to the same solvent and filtered through Whatman filter paper. The adsorption of the sample solution was at a wavelength of 248 nm.

1.2. Validation of Spectrophotometric Method

Linearity and Range

The linearity of the analytical method for diacerein was determined by studying standard calibration curves. The range of the analytical method was decided from the interval between the upper and lower level of the calibration curves by plotting the logarithmic curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 50%, 100%, 150%. Standard concentration of 5,10 and 15 µg/ml was added into

10 µg/ml of tablet concentration. The % recovery was then calculated by using formula

% Recovery = A – B/ C,

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analysed basis

C = Amount of Pure drug added

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the 5,10,15 µg/ml of Diacerein for three times in the same day.

Inter-day Precision

Inter-day precision was determined by measuring the 5,10,15 µg/ml of Diacerein for three consecutive days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOD = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Ruggedness

The robustness of the method was verified by two different analysts maintaining the same environmental and experimental conditions. An appropriate concentration of 15 and 25 µg / ml diacerein was tested and the concentration determined. This procedure was repeated three times.

2. Chromatographic Method

2.1. Development of Chromatographic method

Description

The sample of Diacerein was observed for its color and texture.

Solubility

The sample of Diacerein was taken in test tubes and observed for solubility in various solvents like alcohol and water.

Selection of Mobile Phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey, Methanol and water were selected as a first choice.

Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of Diacerein, the solution of the same in the MeOH were scanned separately by UV–Visible spectrophotometer in the range of 190-400 nm and the spectrum were recorded.

Preparation of Mobile Phase

***Mobile Phase A*:** AR grade Methanol (80%)

***Mobile Phase B*:** AR grade water (20%)

All the solvents was degassed in sonicator for 15 min

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 10 mg of Diacerein in 10 ml methanol that gives concentration of 1000 g/ml of Diacerein and labeled as Standard stock Diacerein.

Preparation of Calibration Curve

Analysis of tablets

Determine the diacerein content in conventional tablets; The twenty tablets were weighed, their average weight was determined and they were finely fed and transferred 10.0 mg of diacerein powder equivalent to a 10 ml volumetric flask containing 5 ml of methanol, sonicated for 30 min and diluted to 1000 ml with methanol. The resulting solution was filtered using a 0.22 µm filter and 15 µg / ml was injected into the system. The amount of diacerein was determined. The test procedure was repeated six times and calculated using the following equation.

Ct= Rt × Cs / Rs

Where, Ct and Cs = Concentration of Sample and Standard Solution, respectively.

Rt and Rs = Peak Area for Sample and Standard Solution, respectively.

2.2 Validation of HPLC Method

Linearity

The linearity of the analytical method for diacerein was determined by studying standard calibration curves. The range of the analytical method was decided from the interval between the upper and lower level of the calibration curves by plotting the logarithmic curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 50%, 100%, 150%. Standard concentration of 5,10, and 15 µg/ml was added into 10 µg/ml of tablet concentration. The % Recoveries was calculated by applying regression equation.

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the standard solutions of Diacerein (5,15,25 µg/ml) and at three different time intervals on same day.

Inter-day Precision

Inter-day precision was determined by analyzing the combined standard solution of Diacerein (5,15,25 µg/ml) on three consecutive days. The results were reported in terms of % RSD.

Limit of Detection and Limit of Quantitation

The limit of detection and the limit of quantification were determined as a function of the standard deviation of the intersections and of the calibration curves and the average slope of the calibration curves.

Robustness

Standard solution of Diacerein (15 µg/ml)) were used and analyzed at different flow rate (0.7,0.8,0.9 ml/min) and wavelength (246,248,250 nm).

Ruggedness

The robustness of the method was verified by two different analysts maintaining the same environmental and experimental conditions. An appropriate concentration of 15 µg / ml diacerein was tested and the concentration determined. This procedure was repeated three times.

System Suitability

A diacerein standard solution (15 µg / ml) was prepared and analyzed. Chromatograms for different parameters such as tailing factor, resolution and theoretical plates were studied to see whether or not they meet the recommended limit.

RESULT AND DISCUSSION

1. UV-Visible Spectrophotometric Methods

Linearity study

Standard solution having concentration range of 5-25 µg/ml of Diacerein was prepared. Absorbances of these solutions were recorded at 248 nm. Calibration curve was plotted, absorbance *vs* concentration.

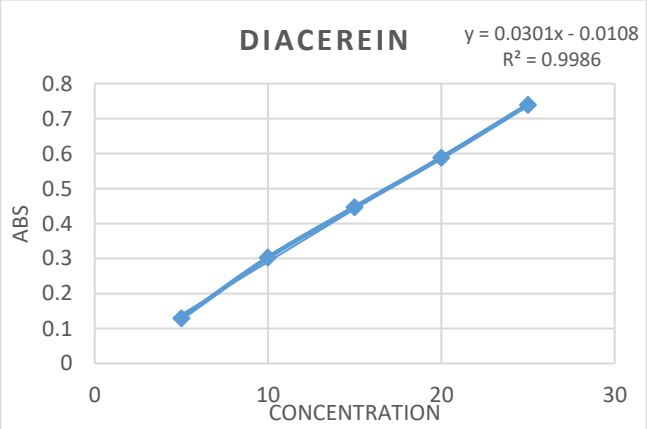

Fig 3. Calibration curve by UV

Table 1. Data of calibration curve by UV

Sr. No.	Conc. (µg/mL)	Absorbance
1	5	0.1289
2	10	0.3026
3	15	0.4469
4	20	0.5879
5	25	0.7395

Table 2. linear regression analysis by UV

Sr.No	Parameters	Zero Order spectrophotometric method
1	λmax (nm)	248
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 0.0301x - 0.0108
4	Slope[m]	0.0301
5	Intercept [c]	-0.0108
6	Correlation coefficient [r2]	0.9986
7	Limit of detection (LOD) (µg/mL)	0.0548
8	Limit of quantitation (LOQ) (µg/mL)	0.1661

Validation Parameters

Method validation was performed according to ICH guidelines. The precision of the method was determined at the 50%, 100% and 150% level by the standard addition method and the percent recovery of diacerein was found to be in the range of 99.72-99.83%. The precision of the method was determined by the% RSD of intraday precision, interday precision. It was found to be less LOD and LOQ of diacerein was 0.0719 and 0.218 µg / ml, respectively.

Table 4. Result of Accuracy study

Level of addition	% Mean recovery*	SD	% RSD
50%	99.72	1.3805	1.384342
100%	99.72	0.8505	0.85296
150%	99.83	0.2786	0.279023

Table 5A. Result of intraday precision

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1279	0.12873333	0.00085	0.66066
2	5	0.1296			
3	5	0.1287			
4	15	0.4463	0.44576667	0.001102	0.247106
5	15	0.4458			
6	15	0.4452			
7	25	0.7426	0.73733333	0.004903	0.665018
8	25	0.7365			
9	25	0.7329			

Table 5B. Result of interday precision

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1284	0.1274	0.001	0.78492936
2	5	0.1264			
3	5	0.1274			
4	15	0.4465	0.44603333	0.00056862	0.12748466
5	15	0.4454			
6	15	0.4462			
7	25	0.7402	0.73933333	0.00090185	0.12198151
8	25	0.7394			
9	25	0.7384			

Table 6A. result of robustness study

Parameters	Change In Wavelength(±2 nm)			
	Wavelength (246nm)		Wavelength (250nm)	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7394	0.4459	0.7386
SD	0.0007	0.0006	0.0001	0.0016
% RSD	0.1917	0.0988	0.4818	0.2634

Table 6B. result of robustness study

Parameters	Change In Solvent			
	Water		0.1N NaOH	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7364	0.4487	0.7365
SD	0.00079	0.0015	0.0006	0.0008
% RSD	0.290	0.3614	0.2086	0.2091

Table 7. result of ruggedness study

Parameters	Change In Analyst			
	Analyst I		Analyst II	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7368	0.4485	0.7402
SD	0.0006	0.0008	0.0011	0.0006
% RSD	0.2807	0.2076	0.5467	0.1460

2. Chromatographic Method

Selection of Analytical Wavelength

Mobile phase diacerein standard solutions (10 µg / ml) were scanned in the UV region of 190-400 nm and overlapping spectra were recorded. Diacerein drugs were observed to show absorbance at 248 nm. So the detection wavelength used was 248 nm.

Table No. 8: Optimized Parameters

Mobile phase	Methanol : Water (80:20v/v) pH3
Selection of column	Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm)
Injection volume	20 µL
Flow rate	0.8 ml/min
Column temperature	Room Temperature
Detection wavelength	248nm
Retention time	4.3 min

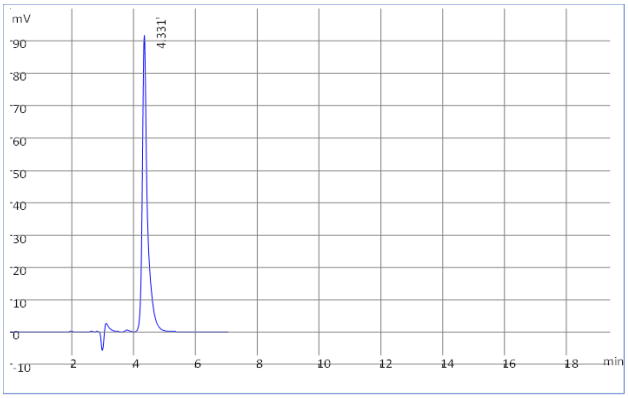

Figure 4. Typical chromatograph of Diacerein by HPLC at Optimized condition

Linearity Study

Diacerein was found to be linear in the concentration range of 5-25 µg/ml.

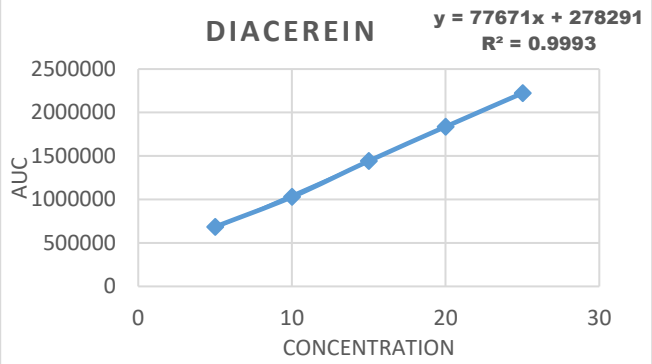

Fig 5. calibration curve by HPLC

Table 9. Result of calibration curve

Sr. No.	Conc. (µg/ml)	Area
1	5	685547
2	10	1028449
3	15	1442605
4	20	1837217
5	25	2222926

Table 10. Linear regression analysis

Sr.No	Parameters	Values
1	λmax (nm)	248
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 77671x +
4	Slope[m]	77671
5	Intercept [c]	278291
6	Correlation coefficient [r^2]	0.9993
7	Limit of detection (LOD) (µg/mL)	0.072
8	Limit of quantitation (LOQ) (µg/mL)	0.221

Validation Parameters

This method was validated according to the ICH guidelines. The percentage of recoveries of diacerein was found in the range of 99.97 to 100.4%. The precision of the method was determined by the% RSD found between the intraday precision and the interday precision. The LOD and LOQ of diacerein were found to be 0.072 and 0.221 µg / ml, respectively. For the robustness study, the effect of the change in wavelength and flow rate (± 0.1 ml / min) on the mean peak area,% RSD and% assay was studied. The percentage of RSD of each peak in all variables was found to be less than 2%.

Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit.

Table 11. Result of Accuracy by HPLC

Level of addition	% Mean recovery*	SD	% RSD
50%	100.3	0.5669	0.565153
100%	100.4	0.512	0.509871
150%	99.97	0.0339	0.033924

Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2.

Table 12A. Result of intraday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	685547	686768.667	1717.151	0.250033
2	5	686027			
3	5	688732			
4	15	1442605	1442417.67	3268.147	0.226574
5	15	1443950			
6	15	1440698			
7	25	2222926	2227928.67	9711.893	0.435916
8	25	2221738			
9	25	2239122			

Table 12B Result of Interday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	685519	687394.667	2008.02747	0.29212148
2	5	689513			
3	5	687152			
4	15	1459633	1447040.33	11794.4692	0.81507536
5	15	1445236			
6	15	1436252			
7	25	2235980	2234477	12258.7996	0.54862053
8	25	2221536			
9	25	2245915			

Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions.

Table 13. Result of robustness study

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.7	1442605	1442417.67	1634.07	0.11329
2		0.8	1443950			
3		0.9	1440698			
1	Change in Wavelength (nm)	246	1459633	1447040.33	11794.5	0.81508
2		248	1445236			
3		250	1436252			

Ruggedness

Ruggedness was studied by different analyst.

Table 14. Result of Ruggedness

Sr.No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	1445600	1446497.333	6754.85	0.4669
			1453656			
			1440236			
2	Analyst-II	30	1462531	1445908.667	12218.91	1.0525
			1442536			
			1432659			

% Assay of Marketed formulation

Formulation	Area of Standard	Area of degraded Sample	% Assay
Diawell 50	1442605	1440359	99.84

Specificity

Excipients and impurities were not interacting with the standard drug, hence method is specific.

Table No.23: Data for specificity study

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
5	10	15	684152	683396.667	748.107835	0.10946905
5	10	15	683382			
5	10	15	682656			
10	10	20	1015694	1024601	8453.94151	0.82509596
10	10	20	1025595			
10	10	20	1032514			
15	10	25	1459263	1449270.33	8754.90242	0.60409036
15	10	25	1445600			
15	10	25	1442948			

Table 15. Result of system Suitability

Sr. No.	conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	15	4.32	8487	1.25
2	15	4.31	8552	1.24
3	15	4.39	8462	1.25
4	15	4.28	8359	1.23
5	15	4.29	8252	1.24
6	15	4.35	8539	1.25
Mean		4.323333333	8441.83333	1.2433333
SD		0.040824829	115.691688	0.008165
%RSD		0.944290572	1.37045691	0.6566997

CONCLUSION

In the present investigation, the UV spectrophotometric method developed and validated turned out to be a simple, inexpensive and fast method. HPLC was found to be more accurate, precise, robust and robust for the determination of diacerein. The excipients usually present in the pharmaceutical formulation did not interfere with the determination of diacerein. This method is also beneficial to the formulation and development department. These methods are

always useful for analysis, purity testing, and testing. Time and chemical consumption is less compared to other tedious methods. This is a new concept for method development validation and method transfer in pharmaceutical companies. The results and statistical parameters demonstrate that the proposed UV and HPLC spectrophotometric method is simple, fast, specific, accurate and precise.

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No conflict of interest.

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