Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (4): 1705-1712

(International Peer Reviewed Journal)

A New Validated RP-HPLC Method For The Estimation of Diacerein In Pharmaceutical Dosage Form

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Accepted on 17th July 2014

ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of Diacerein in tablet. Chromatographic separation of Diacerein was performed by using a Chromosil C_{18} column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of Methanol : Water 80:20 (v/v) at a flow rate of 0.5mL min^{-1} and UV detection wave length at 250nm and 20µL sample was injected. The retention time for Diacerein was 8.29min. The percentage RSD for precision and accuracy of the method was found to be 0.399%. Results of recovery studies are shown range 99.00-101.45%. The limit of detection for Diacerein was found to be 0.06. The recovery was calculated by standard addition method. The proposed method was found to be simple, sensitive and reproducible for the analysis of Diacerein.

Keywords: Diacerein, RP-HPLC, UV detection, Reproducible, Sensitive.

INTRODUCTION

Diacerein, also known as diacetylrhein, is a (4, 5- diacetoxy-9, 10-dihydro 9, 10 di-oxo-2 anthracene carboxylic acid) is a new anti-inflammatory, analgesic and antipyretic drug used in the treatment of osteoarthritis. A 2005 Cochrane review found diacerein to be slightly, but significantly, more effective than placebo in diacerein has a small effect in improving pain and slowing the progress of osteoarthritis (in the hip)[1].

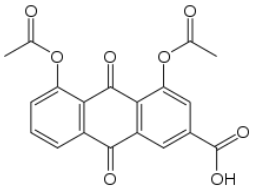

Fig: 1. Diacerein

It has a novel mode of action that differentiates it from NSAIDs and other conventional form of drug therapy[2]. In addition to effect on macrophage migration and phagocytosis, it also inhibits superoxide production, chemotaxis and phagocytic activity of neutrophils[3,4]. However, diacerein lacks cyclo oxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis [5,6]. Therefore, it has been considered as a slow-acting antiarthritic drug not belonging to the NSAIDs that may interfere with the pathological course of osteoarthritis [7]. The most common side effects of diacerein treatment are gastrointestinal, such as diarrhea[1,8]. M. Jagadeeswaran et al **[9]** has developed a simple Rp-Hplc method for the simultaneous determination of diacerein and aceclofenac in tablets. Chromatographic separations of the two drugs were analyzed on a Phenomenex C18 column (250 × 4.60 mm, 5 µ). The mobile phase constituted of 0.01 M potassium dihydrogen phosphate and acetonitrile 60:40 (*v/v*) and pH adjusted to 4.5 using glacial acetic acid was delivered at the flow rate 2.0 mL min-1. Detection was performed at 280 nm. Sarika Narade et al., [10] has been developed that Diacerein shows maximum absorbance at 258.5 nm with molar absorptivity of 4.2258×10^4 L mol $^{-1}$ cm^{-1}. Beer's law was obeyed in the concentration range of 1-10 µg mL^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02 µg/ml and 0.07 µg mL^{-1}, respectively. N. Kannappan et al[11] described that the method utilized RP-HPLC (Water Alliance 2695 with PDA UV detector) model, a column Zorbax CN, the Analytical balance Shimadzu Libror and a pH meter Control Dynamics. The method showed good recoveries (80.30% - 118.14%) and the relative standard deviations of intra and inter-day assay were ± 0.6030 and result were 101.26% respectively. Janhavi Rao et al., [12] has developed that an isocratic separation was achieved using a perfectsil target ODS-3, 250×4.6 mm i.d., 5 µm particle size columns with a flow rate of 1 ml/min and using a UV detector to monitor the eluate at 254 nm. The mobile phase consisted of phosphate buffer : acetonitrile (40:60, v/v) with pH 4.0 adjusted with phosphoric acid. The method was linear over the concentration range of 1-10 µg/ml (r^2 = 0.9996) with a limit of detection and quantitation of 0.01 and 0.05 µg/ml respectively. **Sarika Narade et al[13]** has developed that the linearity for both diacerein and aceclofenac was in the range of 1-10 µg/ml and 5-40 µg/ml respectively. Keddal G. Lalitha et al**[14]** described that the quantitation was carried out using Zorbax CN column. The mobile phase was ammonium acetate buffer (pH adjusted to 3.5) : Acetonitrile [53:47]. The LOD and LOQ are found to be 3.952 µg mL-1 and 11.97 µg mL^{-1} respectively. The flow rate was 1mL/min with UV detection at 254 nm. R. Siva kumar et al., [15] has been described a RP-HPLC method for the simultaneous estimation of Aceclofenac and Diacerein in tablet dosage forms using C column (Phenomenex, 250 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase consisted of 0.02 M phosphate buffer: acetonitrile with 5 mL of 0.4% triethylamine in ration of (35:65 v/v) and adjusted to pH 4. The detection wavelength was carried out at 254 nm. The recoveries of aceclofenac and Diacerein were found to be in the range of 99.23-100.98% and 99.45-100.61% respectively. The validation of method was carried out using ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. Useni Reddy Mallu et al.,[16] described that the method was proven to be linear over a Glucosamine concentration range of 84 to 504µg mL^{-1} with a mean correlation coefficient of 0.9999 and a Diacerein concentration range of 5.6 to 33.6µg mL^{-1} with a mean correlation coefficient of 0.9998.

MATERIALS AND METHODS

Instrumentation: Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Chromosil C18 column (250 mm × 4.6 mm, 5µm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

Chemicals and Solvents: The reference sample of Diacerein (API) was obtained from Cipla, Mumbai. The Formulation DYCERIN (Diacerein) was procured from the local market. Methanol, Water used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

The mobile phase: A mixture of Methanol : Water in the ratio of 80:20 v/v was prepared and used as mobile phase.
Standard solution of the drug: For analysis 100 ppm standard solution was prepared, required concentrations were obtained from 100 ppm solution by appropriate dilution.
Sample (Capsule) solution: The formulation tablets of Diacerien (DYCERIN - 50 mg) were crushed to give finely powdered material. From the Powder prepared a 3 ppm solution with mobile phase and then filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

Method Development: For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.
Detection wavelength: The spectrum of 10ppm solution of the Diacerein in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Diacerein were showed maximum absorbance at 250nm.
Choice of stationary phase: Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and peak shapes were obtained on chromosil C18 (250 mm x 4.6 mm, 5µm) column.
Selection of the mobile phase: In order to get sharp peak, low tailing factor and base line separation of the separation of the components, a number of experiments were carried out by varying the composition of various solvents and flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and Acetonitrile with or without different buffers indifferent combinations were tested as mobile phases on a Chromosil C18 column. A mixture of Methanol : Water in the ratio of 80:20 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.
Flow rate: Flow rates of the mobile phase were changed from 0.5 – 1.5 mL min^{-1} for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 0.5 mL min^{-1} flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions: Chromatographic conditions as optimized above were shown in table 1. These optimized conditions were followed for the determination of Diacerein in bulk samples and in its Formulations. The chromatogram of standard (3ppm) shown in figure 2.

Table 1: Optimized chromatographic conditions for estimation Diacerein

Mobile phase	Methanol :Water 80:20 v/v
Pump mode	Isocratic
Mobile phase pH	7.5
Diluent	Mobile phase
Column	chromosil C18 column (250 mm x 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	250 nm
Injection Volume	20 µl
Flow rate	0.5 mL/min
Run time	10 min
Retention Time	8.29 min

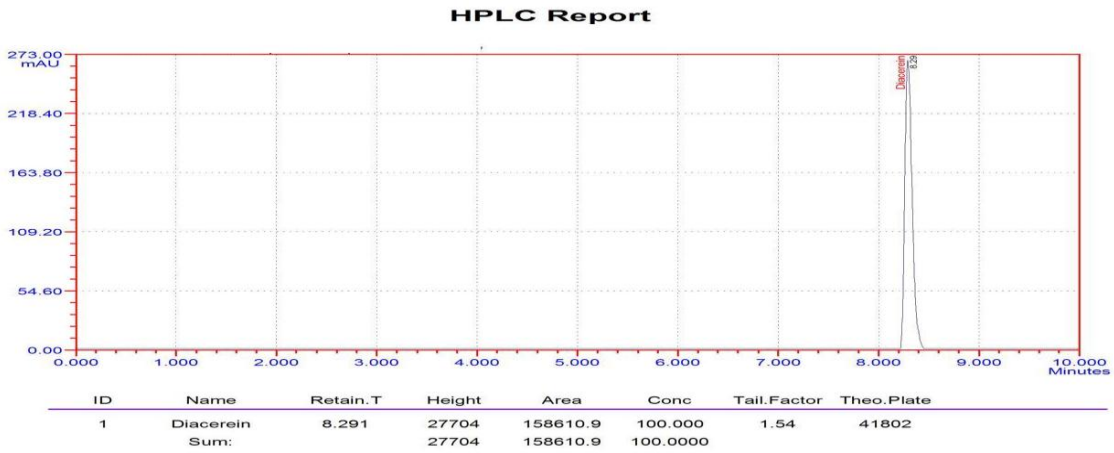

Figure 2.

Validation of the Proposed Method: The proposed method was validated [25-33] as per ICH guidelines [25]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity: The specificity of method was performed by comparing the chromatograms of blank, standard and sample (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in table 2.

Table 2: Specificity study

NAME OF THE SOLUTION	Retention Time in Min
Blank	NO PEAKS
Diacerien (Standerd)	8.29
Diacerien (Sample)	8.5

Linearity: Linearity was performed by preparing mixed standard solutions of Diacerein at different concentration levels including working concentration mentioned in experimental condition i.e. 3ppm. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 250 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in table 3.

Table 3

Level	Concentration ofDiacerien In ppm	Mean peak area
Level -1	1	54378.9
Level -2	2	104301.4
Level -3	3	158610.9
Level -4	4	209028.0
Level -5	5	261072.4
Range: 1-5ppm	Slope Intercept Correlation coefficient	51811.36 2044.24 0.999

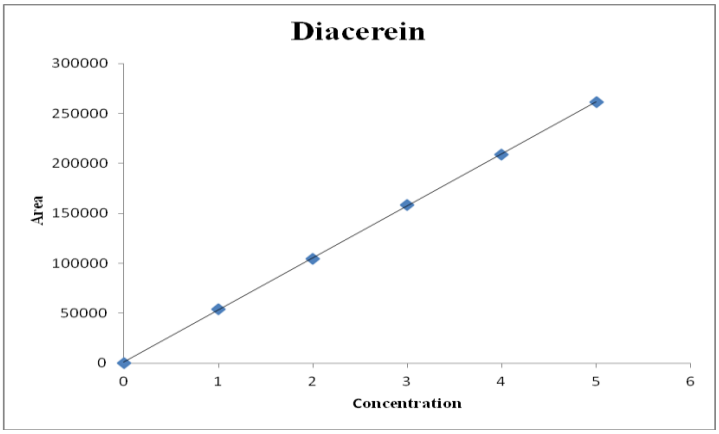

Fig 3. On X axis concentration of sample, On Y axis peak area response

Precision: Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision and inter day precision six replicate standard solutions (5ppm) of Diacerein were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.916, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in table 4. For inter day the percent relative standard deviation (% RSD) was calculated and it was found to be 0.399, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in table 5

Table 4

SAMPLE	CONC (PPM)	INJECTION NO	PEAKS AREA	R.S.D(Acceptance criteria ≤ 2.0%)
Diacerien	5	1	49266.99	0.916
		2	47785.14	
		3	49185.61	
		4	48990.49	
		5	49161.94	
		6	48140.86	

Table 5

SAMPLE	CONC (PPM)	INJECTION	PEAKS AREA	R.S.D Acceptance criteria ≤ 2.0%)
Diacerien	5	1	48327.10	0.399
		2	48368.06	
		3	47640.57	
		4	48268.82	
		5	47869.36	
		6	48218.26	

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level of 2ppm. The solutions were analyzed in triplicate at

each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table 6 Satisfactory recoveries ranging from 99.0 to 102.0 were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 6

Level	Amount of Diacerien spiked (ppm)	Amount of Diacerien recovered(ppm)	% Recovery	%RSD
50 %	3	3.01	100.3	0.678
	3	2.97	99.00	
	3	2.98	99.33	
100%	4	3.96	99.00	0.901
	4	4.01	100.25	
	4	4.03	100.75	
150%	5	4.96	99.2	0.759
	5	4.97	99.4	
	5	5.03	100.6	
			Mean % of recovery 99.75	Mean RSD = 0.779

Robustness: The robustness study was performed by slight modification in flow rate of Mobile phase, pH of the buffer and composition of the mobile phase. Diacerein at 4 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in table 7.

Table 7.

Condition	Mean area	% assay	% difference
Unaltered	209028.0	100.0	0.0
Flow rate at 0.4 mL/min	209441.6	100.19	0.19
Flow rate at 0.6mL/min	208145.3	99.57	0.43
Mobile phase: MEOH: Water			
75% 25%	209047.2	100.009	0.009
85% 15%	208941.5	99.95	0.05
pH of mobile phase at 7.3	207922.5	99.47	0.53
pH of mobile phase at 7.7	208842.1	99.91	0.09

System suitability: System suitability was studied under each validation parameters by injecting six replicates of the standard solution 2 ppm). The results obtained were within acceptable limits (Tailing factor ≤ 2 and Theoretical plates ≥ 2000) and are represented in table 8. Thus, the system meets suitable criteria.

Table 8.

Parameter	Tailing factor	Theoretical plates
Specificity study	1.54	41802
Linearity study	1.34	69829
Precision study	1.46	47785

Limit of detection and Limit of quantification: Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest Concentration that can be quantified reliably with a specified level of accuracy and Precision. For this sample was dissolved by using Mobile Phase and injected until peak was disappeared. After 15ng/ml dilution, Peak was not clearly observed. So it confirms that 0.06ppm is limit of detection and 0.18ppm dilution is Limit of quantification. For this study six replicates of the analyte at lowest concentration were Measured and quantified. The LOD and LOQ of Diacerein are given in table 9.

Table 9

parameter	Measured volume
Limit of Quantification	0.18ppm
Limit of Detection	0.06ppm

APPLICATIONS

Formulation: For assay 20 Diacerein (DICERIN - 50mg) tablets were weigh and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Diacerein in to a 10mL volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 3 ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Diacerein was measured for the determination.

Discussion on the Result: The drug Diacerein is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of water and methanol was selected as mobile phase. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in table 8 Thus, the system meets suitable criteria. The linearity range of Diacerein with coefficient of correlation r= 0.9999, Intercept 2044.24, Slope 51811.36 were found.. The data of regressing analysis of the calibration curves are shown in table 8. Percentage relative standard deviation (%RSD) was found for Intraday-0.916, Interdat-0.399) to be less than 2% for within a day and day to day variations, which proves that method is precise. To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Results of recovery studies are shown range 99.00-101.45%. The proposed method has been applied to the assay of commercial tablets (DYCERIN - 50 mg) containing Diacerein.

CONCLUSIONS

Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of Diacerein in multi component pharmaceutical preparation. The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Diacerein in bulk as well as in pharmaceutical preparations.

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