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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DIACEREIN BULK AND PHARMACEUTICAL DOSAGE FORMULATION

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ABSTRACT

A simple, selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of Diacerein in bulk and in tablet dosage form has been developed and validated. Sample was resolved on a Agillent TC C18 (150mm X 4.6 mm i.d., particle size 5µ) column. The mobile phase consisted of Buffer and Acetonitrile were mixed in the ratio of 25:75 V/V and sonicated to degas was delivered at a flow rate of 1.2 ml/min at ambient temperature and the retention time was about 4 minutes. Studies were performed on an HPLC system equipped with a UV/Visible detector at 257nm. The method is specific to Diacerein and able to resolve the drug peak from formulation excipients. The calibration curve was linear over the concentration range of 1-10 µg/ml (R=0.9996).The results of analysis of formulation was found to be 99.35±0.5205. The lower limits detection for Diacerein was found to be 0.001µg/ml and the quantification limit was about 0.0466µg/ml. The proposed method is applicable to routine analysis of Diacerein in bulk and in tablet dosage form.

Keywords: Diacerein, RP-HPLC, Validation, Pharmaceutical dosage form.

INTRODUCTION

Chemically Diacerein[1] (DIA) is known as 4, 5-Bis(acetyloxy)-9, 10-dioxo-2-anthracene carboxylic acid (Fig.1). Diacerein is a novel anti-inflammatory drug and used for the treatment of not only Osteoarthritis but also used for rheumatoid arthritis when used in combination[2,3]. Diacerein is a di-acetylated derivative of rhein, a molecule with an anthraquinone ring which is actually the active metabolite of Diacerein[4]. DIA is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentration range of proinflammatory cytokines[5,6]. However DIA lacks cyclooxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis. Therefore it has been considered as a slow-acting antiarthritic drug.

Analytical methods play a vital role in new drug development, preformulation and formulation studies, stability studies, quality control testing and quality assurance programmes. So analysts are always in search of developing rapid and accurate new methods of analysis that are able to exist in routine analytical work. Diacerein is not official in IP, USP and BP. The review of literature reveals that only few chromatographic methods have been reported for the estimation of Diacerein like LCMS, LCNMR MS, capillary gas chromatography,

HPLC[7, 8]. There is a need for developing newer methods in HPLC for developing a simple and economic method and so we proceeded with HPLC and validated as per the ICH guidelines. The present analytical work comprises of simple, precise, rapid, sensitive and accurate methods for the estimation of Diacerein bulk and dosage form.

EXPERIMENTAL

Instrumental specification

HPLC - AGILLENT-Model NO.1120 LC Compact System Consisting of Agillent TC-C18 ODS column output signal was monitored and integrated using Lab monitor diagnosis and EZ-chrome.

MATERIALS AND METHODS

Diacerein working Standard was obtained from SAIN Medicaments Pvt, Ltd, Hyderabad and commercial dosage forms containing the studied drug were obtained from local market. All the reagents used were analytical reagents, solvents were of HPLC grade.

Buffer Preparation

Dissolve 0.57ml of Acetic Acid in 1000ml of HPLC water and 0.205gm of Sodium acetate in 250ml of water then take fresh beaker add 847ml of 0.01M of Acetic acid and 153ml of 0.01M Sodium acetate, Filter through 0.45µm membrane filter and degas.

Mobile Phase

Buffer and Acetonitirile were mixed in the ratio of 50:50 V/V and sonicated to degas.

Preparation of Standard Solution

Stock solution of Diacerein sample was prepared by dissolving accurately weighed 10 mg of drug initially in Tetrahydrafuran in 10 ml volumetric flask and further dilutions were made by taking 0.1 ml of stock solution and made up with methanol and degassed by ultra bath sonicator for 30 min. Finally passed through 0.45 µm filter (The final concentration is 10µg/ml).

Preparation of sample stock solution

Diacerein 5 tablets were accurately weighed and powdered. Amount equivalent to 0.3074 gm of Diacerein from the powdered formulation were accurately weighed and dissolved in Tetrahydrafuran and future dilution of 10µg/ml were made by taking 0.1ml from stock solution and made with methanol in 10 ml of volumetric flask and degassed by ultra bath sonicator for 30 min. Finally passed through 0.45 µm filter (The final concentration is 10µg/ml).

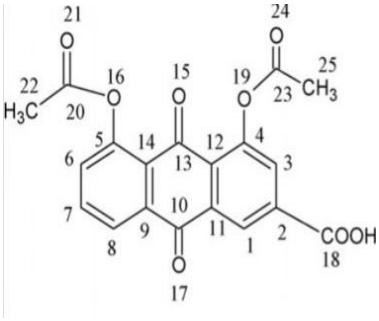

Fig. 1: Chemical Structure of Diacerein (DIA)

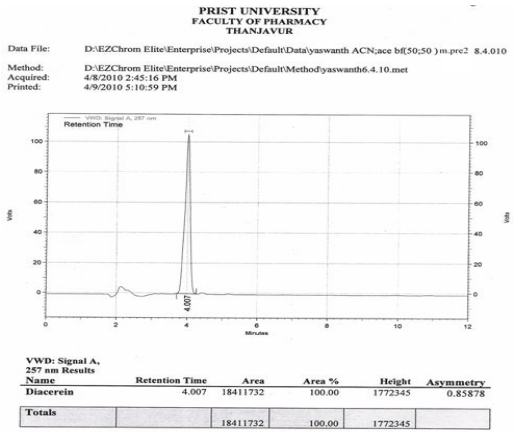

Fig. 2: RP-HPLC data of Diacerein

RESULTS

METHOD VALIDATION

The described method has been validated for the assay of Diacerein using following parameter[9-11].

Accuracy (Recovery): Demonstrated the accuracy of the test method by preparing recovery samples (i. e. spiking formulation with known quantities of API.) at the level of 50 %, 100 %, and 150 % of target concentration. Prepare the recovery sample in triplicate in each level. The sample was prepared as like

assay method. The results were tabulated in Table 1.

Method Precision: Prepared and analyzed six replicate sample preparations as per method. And calculate the % of assay value. The results were tabulated in Table 2.

System Precision: Prepared and analyzed six replicate sample preparations as per method. And calculate the %RSD of area of response. The results were tabulated in Table 3.

Ruggedness: The ruggedness of an analytical method was determined by analysis of aliquots from homogenous lots by different analyst using operational and environmental conditions that may differ but were still within the specified parameters of the assay. The degree of reproducibility of the test results was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions to obtain a measure of the ruggedness of the analytical method.

Inter day precision: Prepared and analyzed three replicate for day-1 and day-2 sample preparations as per method. And calculate the %RSD of assay level. The results were tabulated in Table 4.

Robustness: The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameters of the assay by changing physical parameters like flow rate mobile phase and pH. HPLC system was set with small but deliberate change in method as mentioned below and their effect on system suitability test and the values were given in Table 5.

Specificity: Prepare blank, diluted standard preparation and sample preparation as per method.20µl of the above mentioned solution was injected into HPLC system. Values were tabulated in Table 6

Linearity: The linearity of an analytical method is to elite test results that are directly, of by well defined mathematical transformation, proportional to the concentration of analyte in sample within a given working range. The results were tabulated in Table 7

Limit of detection and quantification (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the caliberation curve of the analyte.

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the caliberation curve of the analyte.

DISCUSSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 257nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area (figure 2). The column used for study on Diacerein, Zorabax C18, 250, 5µm was chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.2ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of 50:50 Acetate buffer: Acetonitrile was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Diluent 1-Tetarhydrofuron, Diluent 2 methanol was selected because of maximum extraction, sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 12min because analyze gave peak around 4.0 and also to reduce the total run time.

The percent recovery was found to be 98.96-101.77 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. In specificity study all degradant impurity and excipient peaks were separated from the analyte peak. Detection limit was found to be 0.001µg/ml. Linearity study was, correlation coefficient and curve fitting was found to be 10µg/ml. The analytical method was found linearity over the range of 2-10µg/ml of the

target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was all satisfactory.

CONCLUSION

A simple, rapid and reproducible HPLC method was developed and validated for the estimation of Diacerein in tablet dosage form. Agilent TC-C18 ODS column, in gradient mode with mobile phase containing Acetronitrile and Acetate buffer pH4.0 (50:50v/v) was used. The flow rate was 1.2ml/min and the analyte was monitored at 257 nm. The retention time for Diacerein was 4.00 minutes.

The system was validated for system suitability, precision, accuracy, linearity, ruggedness and robustness. The system suitability parameter were within the limit, hence it was concluded that the system was suitable to perform the assay. Linearity was obtained in the concentration range of 2µg/ml to 10µg/ml with correlation coefficient of 0.999. The percentage recovery of Diacerein was found to be in the range of 98% -102%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in flow rate, Mobile phase composition separately and analysis being performed by different day with different columns respectively. Therefore it was concluded that the proposed method can be used for routine analysis of Diacerein in its tablet dosage form.

Table 1: Recovery of Diacerein

Level	µg/ml recovered	µg/ml added	Recovery%	Mean%	%RSD
Level-1 50%	4.99mcg	5µg	99.84	100.00	0.8129
	4.96mcg	5µg	99.29		
	5.04mcg	5µg	100.89		
Level-2 100%	9.98mcg	10µg	99.80	100.1	1.4439
	9.89mcg	10µg	98.96		
	101.77mcg	100µg	101.77		
Level-3 150%	15.08mcg	15µg	100.57	100.1	0.6030
	15.05mcg	15µg	100.34		
	14.91mcg	15µg	99.43		

Table 2: Method Precision

Sample Preparation Number	Diacerein Assay %
1	**99.39**
2	99.03
3	99.42
4	99.59
5	99.28
6	100.43
Mean	99.35
%RSD	0.5205

Table 3: System Precision

Standard Preparation Number	Standard Response
1	18516904
2	18638271
3	18724032
4	19637412
5	18918043
6	18421791
Mean	112856453
%R.S.D	1.4751

Table 4: Ruggedness

S. No.	Assay % of Diacerein			
	Day-1	Day-2	Analyst-1	Analyst-2
1	97.98	97.04	100.44	10.69
2	98.88	100.67	97.05	100.87
3	100.24	100.24	97.37	99.17
Mean	99.03	99.67	98.43	100.2
%RSD	1.1488	2.0	1.73	0.9334

Table 5: Robustness

Test	Flow rate 1.4ml/min	Flow rate 1ml/min	Mobile phase Composition ACN:Buffer (52:48)	Mobile phase Composition ACN:Buffer (48:52)	pH 4.2	pH.3.8
Mean standard area	3322789	31179637	15588070	15089173	18882689	18361019
%RSD	0.67	1.3091	1.6580	0.23	0.13	0.58

Table 6: Specificity

S. No.	Sample Name	Area	Rt
1	Blank	----------	--------
2	Formulation	18479518	4.001
3	Standard	18678942	4.007

Table 7: Linearity of Diacerein

S. No.	Concentration (µg/ml)	Area of Diacerein
1	2	3695903
2	4	7412806
3	6	11087709
4	8	14583612
5	10	184563484
Correlation coefficient (r)		0.999
Slope		480876.03
y-intercept		0.2346 X

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