

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DIACEREIN TABLETS BY RP-HPLC

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ABSTRACT: This paper describes the analytical method suitable for validation of Diacerein by reversed Phase high performance liquid chromatography (RP-HPLC) method. 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 mobile phases were comprised of A and B of Acetonitrile and Buffer pH-[3.5]. Validation experiments were performed to demonstrate system suitability, specificity, precision, linearity and range, accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 25-150 µg/ml. The method showed good recoveries (80.30% - 118.14%) and the relative standard deviations of intra and inter-day assay were $\pm$ 0.6030 and result were 101.26% respectively. The method can be used for quality control assay of Diacerein.

KEYWORDS: RP-HPLC, Diacerein, Analytical method, Quality control, validation.

INTRODUCTION

Diacerein (DCR) [4, 5-Bis (acetyloxy)-9, 10-dihydro-9, 10-dioxo-2-anthracenecarboxylic acid. Diacerein is directly inhibits IL-1 Synthesis and release in vitro and down modulate IL-1 induced activities and have been shown to posses disease modifying effect in experimental models of Osteoarthritis and in human subject with finger joint and knee Osteoarthritis. Hence, by inhibiting IL-1 Diacerein retards all pathological process initiating in Osteoarthritis (OA) ^[1]

Diacerein inhibit the synthesis of resorptive enzyme and reduces Osteoarthritis differentiation/survival in Osteoarthritis subchondral bone: a possible mechanism for a protective effect against subchondral bone remodeling ^[2].

Chondroprotective ^[3] agents (CPA) such as Glucosamine, Curcumin and Diacerein represent potential remedies for the management of Osteoarthritis and several studies have been performed on their effects in-vitro and in-vivo.

During the analytical of the molecular biochemical consequences of Chondroprotective ^[4] action, real-time quantitative RT-PCR (q PCR) is the method choice for monitoring alteration of gene expression pattern in chondrocytes.

There are many analytical method developments available for the analysis of Diacerein tablets using spectroscopic analysis ^[5] measures the interaction of the materials with the electromagnetic radiation. Amongst the most powerful techniques available to the analyst for the separation of these mixtures, a group of highly efficient methods which are collectively called as chromatography ^[6-7] and general chromatography methods ^[8] are requires that a solute undergo distribution between two phases.

HPLC is the form of liquid chromatography to quantify and analyze mixture of chemical compounds ^[9] and in the reverse phase chromatography method ^[10-11] is the retention by interaction of the stationary phase non-polar hydrocarbon chain with non-polar parts of the sample molecule using the Columns ^[12] are guard,

derivatizing, capillary and preparatory columns and UV detectors ^[13]

Pharmaceutical validations among these methods undergo the world "Validation" means "Assessment" of validity or action of providing effectiveness ^[14-15], Australian GMP validation ^[16-17] and validation as per ICH guidelines ^[18].

MATERIALS AND METHOD

Apparatus:

The analysis was performed by using the analytical balance Shimadzu Libror, pH meter Control Dynamics, the HPLC used is of Water Alliance 2695 with PDA UV detector. Column used in HPLC is Zorbax CN with a flow rate of 1.0 ml/min (Gradient). The mobile phase consists of A & B with mixture of Acetonitrile and the Buffer pH-[3.5] at different proportions A & B which are degassed in a sonicator for about 10 minutes the injection volume is 20 μ L and the ultra violet detection was at 254 nm.

Reagents and solutions:

Pure sample of Diacerein USP of 272.3mg and other reagents such as acetonitrile and water used were of HPLC and milli-q grade. All other chemicals like glacial acetic acid used were of AR grade. Optimized chromatographic conditions are listed in table -1.

Standard solution preparation:

Accurately weigh about 25mg of Diacerein and transfer it into a 1000ml volumetric flask. Add 10ml of Dimethyl acetamide and sonicate to dissolve. Make up to the mark with the mobile phase and mix. Dilute 5.0ml of this solution to 25ml with mobile phase and mix.

Sample preparation:

Weigh accurately about 272.3mg of Diacerein sample and transfer it into a 1000ml volumetric flask. Add 10ml of dimethyl acetamide and sonicate to dissolve. Make up to the mark with the mobile phase and mix. Dilute 5.0ml of this solution was diluted to 25ml with mobile phase and mix.

Linearity and Range:

The Linearity of detector response is established by plotting a graph to concentration versus area of Diacerein standard and determining the correlation coefficient. A series of solution of Diacerein standard solution in the concentration ranging from about 25 to 150 μ g/ml level of the target concentration (10mcg/ml of Diacerein) were prepared and injected into the HPLC system.

Accuracy:

Accuracy for the assay of Diacerein tablets is determined by applying the method in triplicate

samples of mixture of placebo to which known amount of Diacerein standard is added at different levels (80%, 100% and 120%). The sample were filtered through 0.45 μ m membrane filter and injected into the chromatographic system.

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as %RSD. The %RSD was found to be 0.6030%.

Robustness:

The Robustness of the analytical method was studied by analysis of multiple wavelengths with 252nm and 256nm. The Robustness expressed as %RSD was found to be 0.398 and 0.421 in this results are showed in table-2.

RESULTS AND DISCUSSION

Diacerein standard having concentration 10 μ g/ml was scanned in UV- region between 200-400 nm. λ max of Diacerein was found to be at 254 nm. The Diacerein peak in the sample was identified by comparing with the Diacerein standard and the Retention time was found to be around 15 minutes.

The estimation of Diacerein tablets was carried out by RP-HPLC using Mobile phase having a composition of Acetonitrile and Buffer, 53 volumes of Acetonitrile and 47 volumes of Methanol. The ratio pH was found to be 3.5. Then finally filtered using 0.45 μ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was C₁₈ Zorbax CN. Flow rate of Mobile phase was 1.0 ml/min, System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates were 5618.301, and tailing factor – 1.437.

The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method shows that RSD for Method Repeatability was 0.6030% which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 25 to 150 μ g/ml of target concentration for Diacerein standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) =0.9999, which shows that the method is capable of producing good response in UV-detector.

The Accuracy limit is described by the percentage recovery should be in the range of 80.30% to 118.14%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

The Robustness of the analytical method was studied by analysis of multiple wavelengths with

252nm and 256nm are analyses. The Robustness expressed as %RSD was found to be 0.398 and 0.421 this results are showed in table-2.

The Ruggedness of the analytical method was studied by analysis of % Recovery using water 2685

instrument with two different analysts. The ruggedness expressed as % Recovery was found e 102.51% and 100.031% are showed in table-2.

Table – 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Water Alliance 2695 with UV detector)
Column	Zorbax CN
Mobile Phase*	Acetonitrile and Buffer × mixture A & B
Flow rate	1.0 ml/min
Detection	UV at 254 nm
Injection volume	20µl
Temperature column	26°C ± 2°C

Table – 2: System suitability parameters

Parameter	Diacerein
Calibration range (µg/ml)	25-150
Theoretical plates	5618.301
Resolution	-
Tailing factor	1.437
Correlation Coefficient(r^2)	0.9999
LOD (µg/ml)	3.952
LOQ (µg/ml)	11.97
%RSD of Robustness Wavelength (252nm) (256nm)	0.398 0.421
% Recovery of ruggedness Analyst-1 Analyst-2	102.51 100.031

Table – 3: Analysis of formulation and recovery studies

Drug	Label claim (mg)	Estimation		% Recovery	
		mg/tablet	% label claim	Amount added (mg)	% Recovery
Diacerein	50	279.2	100.48	50.24	80.30%.
Diacerein	50	276.3	101.9	50.95	99.24%
Diacerein	50	277.3	101.86	50.9	118.14%

CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Diacerein is done by reverse phase HPLC. The mobile phase consists of buffer (47 volumes of Acetonitrile and 53 volumes of Methanol. The ratio pH was found to be [3.5]. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using UV detector set at 254nm. The solutions are chromatographed at the constant flow rate of 1.0 ml/min. The Retention time for Diacerein was around 5.616 minutes. Linearity range for Diacerein is 25 to150 µg/ml.

The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 50µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The percentage recovery 99.30% to 100.90% for Diacerein.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precise, and Linear, Proportional i.e. it follows Lambert-Beer’s law. The method was found to have a suitable application in routine laboratory analysis with a high degree of accuracy and precision.

Figure 1: CHROMATOGRAM OF STADARD FOR DIACEREIN

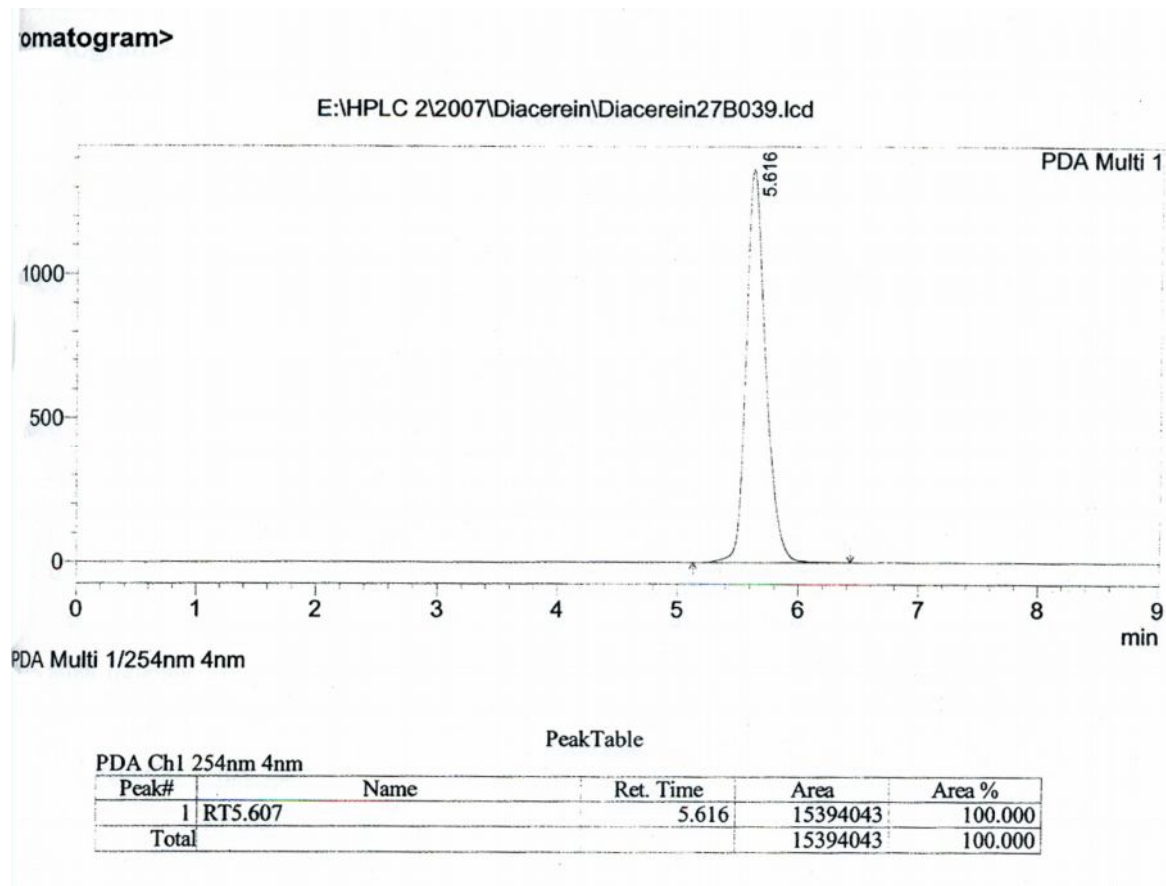
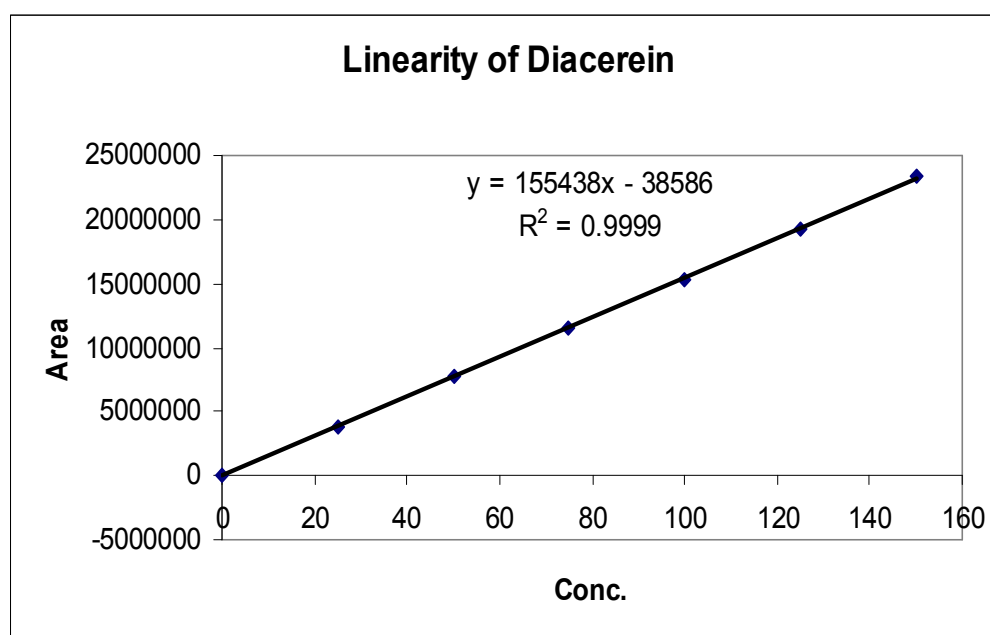


Figure-2: LINEARITY OF DIACEREIN

Regression analysis of the calibration curve for Diacerein showed a linear relationship between the concentration and peak area with correlation

coefficients higher than 0.999 in all the curves assayed.

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