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A Simple HPLC Method for Quantitation of Diacerein in Tablet Dosage Form

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Abstract

A simple, economic, accurate reverse phase isocratic RP-HPLC method has been developed for the quatitation of diacerein in tablet dosage form. 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.The method has been validated and proved to be accurate, precise, linear, rugged, robust, simple and rapid. The calibration curve was linear in the concentration range 25-150 µg mL^{-1} with coefficient of correlation 0.99942. The percentage recovery of diacerein was found to be 101.60%.The method is useful in the quality for the estimation of diacerein in tablet dosage form.

Keywords:

Diacerein, RP- HPLC, Tablets, Validation

1. Introduction

Diacerein is chemically 4, 5 bis (acetyloxy)-9, 10-dihydro, 9, 10 dioxo-2-antharacene carboxylic acid. It is used in the osteoarthritis, analgesic and NSAIDS [1-4]. In recent years some HPLC method were reported in the bulk drug and biological fluids [5-7] but in case of formulation no method has been reported. The focus of present study was to develop and validate a rapid, stable and economic high performance liquid chromatographic method for the quality control of diacerein in tablet dosage form. The present RP-HPLC method was validated following the ICH guidelines [8, 9].

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2. Experimental

2.1. Chemicals and Reagents

HPLC grade Acetonitrile from Ranboxy Laboratories, Glacial acetic acid from SD Fine Chemicals and Whatman GFC filter were used in the study. Water HPLC grade was obtained from a Milli-QRO water purification system.

2.2. Equipments

The instrument was a Water Alliance 2695 separation module, having water 2996 photodiode array detector in isocratic mode. The system was connected with the help of Millennium 32 software in a computer system for data collection and processing. The analytical column used is Zorbax CN.

2.3. Chromatographic condition

The mobile phase consists of a mixture of ammonium acetate buffer (pH adjusted to 3.5) (53 volumes) and Acetonitrile (47 volumes) was filtered through 0.45µm nylon membrane filter before use. The injection volume was 20 µL with a flow rate 1 mL/min and detection wavelength 254 nm having ambient condition and run time 15 min.

2.4. Standard preparation

About 25 mg sample transferred in to a 100 mL volumetric flask and 10 mL of Dimethyl acetamide was added. The solution was sonicated to dissolve and made up with mobile phase. 5 mL of solution was pipette out into 25 mL standard flask and made up with mobile phase.

2.5. Sample preparation

Weigh accurately about 272.3 mg sample transferred in to a 100 mL volumetric flask and 10 mL of Dimethyl acetamide was added. The solution was sonicated to dissolve and made up with mobile phase. 10 mL of solution was pipette out into 25 mL standard flask and made up with mobile phase.

3. Result and Discussion

3.1. Estimation of Diacerein in tablet dosage form

The HPLC procedure was optimized with a view to develop precise and stable assay method. Diacerein was run in different mobile phase composition and different pH ranges (3.0 to 3.5) of mobile phase with different C_{18} columns (Kromacil 250 mm x 4.6 mm i.d., 5µm), column- Zorbax CN (250 mm x 4.6 mm 5 µm) at ambient temperature (25º and 30º C). The flow rate was also varied from 0.5 mL to 1 mL min^{-1}. The mobile phase consists of and a mixture of ammonium acetate buffer (pH adjusted to 3.5) (47 volumes) and Acetonitrile (53 volumes) was filtered through 0.45 µm nylon membrane filter before use. The column used is zorbax CN.

Twenty tablets were weighed and crushed to fine powder. The powder equivalent to 272.3 mg of Diacerein was taken in a 100 mL volumetric flask and made up with mobile phase. The resultant mixture was filtered through 0.45 µm nylon filter. From this filtrate 10 mL of solution was pipette out into 25 ml standard flask and made up with mobile phase.

The injection volume was 20 µL with a flow rate 1 mL min^{-1} and detection wavelength 254nm having ambient condition and run time 15 min gave sharp and symmetrical peak with retention time 5.51 min for Diacerein. The typical chromatogram of sample solution was shown in Fig.1. The amount present in each tablet of average weight was calculated fom the following formula,

$$\frac{Sample\ area\ x\ Std.\ dilution\ x\ Potency\ x\ Average\ Wt/Tablet}{Std.\ area\ x\ Sample\ Dilution\ x\ 100}$$

The assay procedures were repeated for six times and mean peak area and mean weight of standard drug was calculated. The percentage drug found in formulations, mean, standard deviation was calculated and presented in Table 1. The results of analysis shows that the amount of drug was in good agreement with the label claim of the formulation. The proposed method is simple and do not involves laborious time consuming sample preparation.

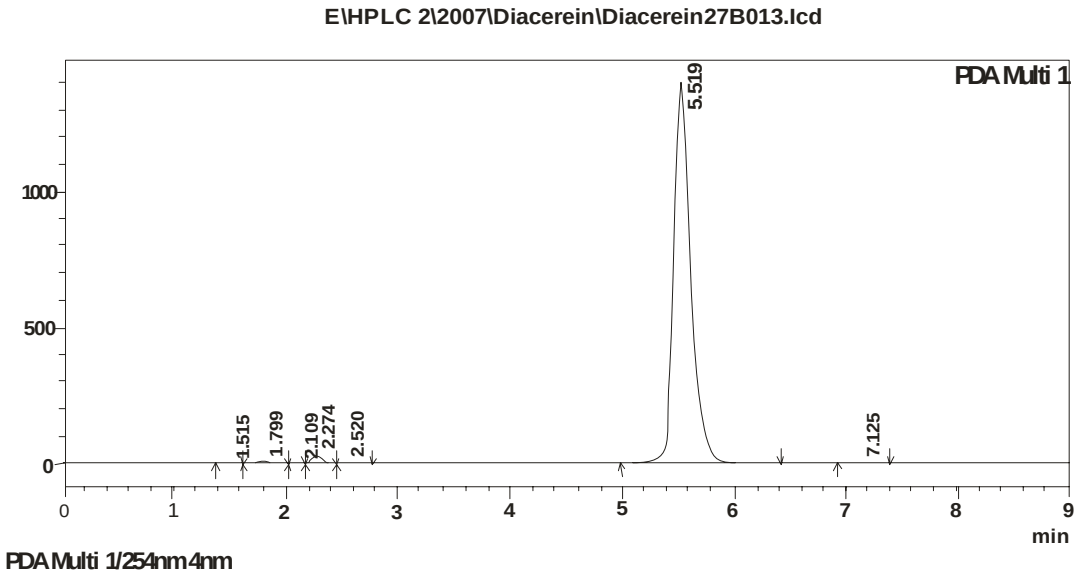

Fig 1. The typical chromatogram of diacerein

Table 1. Estimation of Diacerein in Dosage Forms

Labeled	Amount mg tab^{-1} Found*	%Label Claim (n=6)	Recovery Studies (n=3)			
			Amount added (mg)	Amount recovered (mg)	%Recovery	% RSD
Diacerein 50mg	52.28	102.60±0.2971	40	40.33±0.0665	80.66	0.2858
			50	49.62±0.1102	99.24	0.3845
			60	59.07±0.0568	118.14	0.1667

*Average of six or three determinations, Mean ± Standard Deviation

3.2. Method Validation

The described method has been validated for the assay of diacerein using following parameters.

3.3. Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 80 to 120% of label claim. The

recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 1. The mean recovery is well within the acceptance limit, hence the method is accurate.

3.4. System suitability studies

The system suitability test was carried out on freshly prepared stock solution of Diacerein to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within ± 3% standard deviation range during routine performance of the method.

3.5. LOD and LOQ:

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Diacerein was found to be 3.952 μg mL^{-1}. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 11.97 (μg mL-1) for Diacerein respectively.

Table 2. System Suitability Studies

S.No	Parameters	Diacerein
1	Retention time	5.460
2	USP Tailing factor	1.437
3	Theoretical plate /meter No	5618.30
4	Calibration Range (μg mL^{-1})	25-150
5	Area	15388077
6	LOD (μg mL^{-1})	3.952
7	LOQ (μg mL^{-1})	11.97

3.6. Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 25-150 μg mL^{-1}. 20 μL of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in μg mL^{-1} (X axis). Peak area of linearity range and the parameters were calculated and presented in Table 3 and 4, respectively. The linearity curve of Diacerein was shown in Fig.2.

3.7. Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for diacerein in tablet dosage form.

3.8. Precision

3.8.1. System precision

The system precision of the method was established by six replicate injections of the standard solution containing diacerein. The percentage RSD were calculated and presented in Table 5. From the data obtained, the developed RP-HPLC method was found to be precise.

3.8.2. Method precision

The method precision of the method was established by carrying out the analysis of diacerein (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise. The results obtained were presented in Table 5.

Table 3. Peak area of linearity curve of the HPLC method for the estimation of Diacerein

S.No	Conc. (μg mL^{-1})	Peak area
1.	25	3880825.0
2	50	7747095.0
3	75	4571424.0
4	100	15388392.5
5	125	19314356.5
6	150	23432734.0

Table 4. Analytical performance parameters of linearity curve

S.No	Parameters	Diacerein
1	Linear dynamic range (μg mL^{-1})	25-150
2	Correlation coefficient (r)	0.99942
3	Slope (m)	155438
4	Intercept (c)	-38585.7
5	Curve fitting	99.99

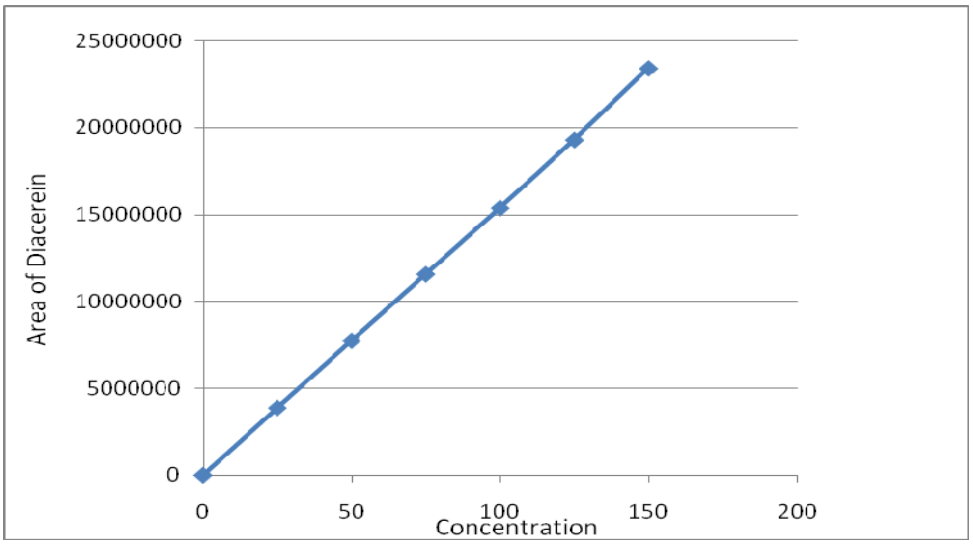

Fig 2. The linearity curve of Diacerein

3.9. Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were up to 24 h at room temperature and refrigerator temperature.

Table 5. Precision studies Of Diacerein In Dosage Forms

Precision	%Assay*	%RSD of Assay(n=6)
System Precision	101.47±0.2034	0.4910
Method Precision	101.56±0.1700	0.4100

*Average of six determinations, mean ± SEM

3.10. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A4T), Water Alliance HPLC 2695 by different operators using different columns of similar type like Kromacil C_{18} column and Zorbax CN column. Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The results of ruggedness were presented in Table 6. The results of robustness were presented in Table 7.

Table 6. Method Reggedness of Diacerein in Dosage Forms

	%Assay* (n=6)	**%RSD of Assay(n=6)**
Day -1 , Analyst-1, Instrument-1&Column-1	101.52 ± 0.1747	0.0.4214
Day -2 , Analyst-2, Instrument-2&Column-2	101.80 ± 0.2417	0.5816

*Average of six determinations, mean ± Standard Deviation

Table 7. Method Robustness of Diacerein in Dosage Forms

Condition	Change	%RSD
Temperature	Normal	0.4210
	-5ºC	0.5806
	+5ºC	0.6329
pH	Normal	0.4864
	-0.2unit	0.4811
	+0.2unit	0.1971
Flow Rate	Normal	0.3830
	-10%	0.4810
	+10%	0.4100
Mobile phase ratio	Normal	0.3628
	-2%	0.4614
	+2%	0.5627
Detection wavelength	Normal	0.3980
	-0.2unit	0.4015
	+0.2unit	0.4200

4. Conclusion

The proposed RP-HPLC method for the estimation of diacerein in tablet dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies.

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