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HIGH   PERFORMANCE CHROMATOGRAPHIC (GPC) METHOD FOR THE ESTIMATION OF DIACEREIN IN SOLID DOSAGE FORMS

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ABSTRACT: Diacerein is now widely used for the treatment of Osteoarthritis. The mechanism of action differs from the Non-steroidal anti-inflammatory drugs since it is not related to the inhibition of Prostaglandins. Anti-osteoarthritis and cartilage stimulating properties have been demonstrated in vivo and in animal models. Diacerein and Rhein have been shown to inhibit the production of interleukin-I- beta by human monocytes and the effects of the cytokine onchondrocytes in vivo. Diacerein in therapeutic dose inhibit the stimulation of interleukin-1 beta-production and production of nitrous oxide. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor TGF-beta-1 and TGF beta-2 with potential cartilage repairing properties. From the literature, Diacerein is seemed to be a safe and promising drug. This research work has been taken up to develop and validate simple, accurate, sensitive and cost-effective methods for the estimation of Diacerein in dosage forms. A new LC method with UV detection was developed for the quantitative determination of Diacerein in pharmaceutical dosage forms. The chromatographic separation was performed on GPC column with a mobile phase of tetra hydro furan. A flow rate of 1.2ml/minute was used. The resulting chromatogram exhibited a retention time of 5.784 minute. The limit of detection and limit of quantification was found to be 0.5µg/m land 1µg/ml. A linearity range of 1-10µg/ml was established. The correlation coefficient was found to be 1. 007. The results of analysis were treated statically, as per ICH guidelines for validation of analytical procedures and by recovery studies. The results were found to be accurate and not much interferences from capsule excipients. The active pharmaceutical ingredient was extracted from its finished dosage forms using tetrahydrofuran. The percentage recovery was found to be 99.9%w/w.

Key words: Diacerein, Rhein, Gel permeation, HPLC, Linearity, Recovery etc

I.   INTRODUCTION

Diacerein is an anthraquinone derivative that has been used in Osteoarthritis. Osteoarthritis is a common disease in the ageing population and results from a complex interplay of genetic, metabolic, biochemical and biomechanical factors   with secondary local inflammation. The process involves the interaction of degradation and repair of articular cartilage, bone and synovium. The most important cells in the entire process are the chondrocytes[1,2]. Primary Osteoarthritis (OA) which can be either localized or generalized , most often idiopathic, except in rare cases in which a defective gene has been   found to cause a familial form of osteoarthritis[3,4].Diacerein does not   alter renal or platelet cyclooxygenase activity and may therefore be tolerated by patients with prostaglandin dependent renal function[5,6].Diacerein and Rhein have been shown to inhibit the production of inter leukin-1 beta by   human   monocytes and the effects of the cytokine   on chondrocytes *in vivo*[7]. They exert chondroprotective effects in cultured articular cartilage and reduced severity of cartilage, bone and synovial membrane damage in osteoarthritis[8]. Studies inhibit that diacerein does not block the synthesis of prostaglandins, thromboxane of leukotrienes but may actually stimulate prostaglandin synthesis, especially PGF-2 alpha, a prostaglandin with cytoprotective effect on the gastric mucosa[9,10] .Janhavi Rao reported HPLC method of diacerein in capsule dosage forms by isocratic separation was achieved using a perfect target OD-3,250X4.6 mm id columns and using UV detection at254nm.The mobile phase was selected phosphate buffer: acetonitrile(40:60)ratio with pH 4.0.Kannappan N, *at et al* describes validation of Diacerein by High performance liquid chromatography(RP-HPLC) method using Zorbax CN column and the mobile phases were selected Acetonitrile and buffer pH-3.5,None of the

method gives correct values recoveries(approx. 80.30-118.14%w/w) and there it is necessary a method having simple, good precision, lack of errors, relevant values for estimation of Diacerein in solid dosage form.

II. MATERIALS AND METHODS

Chemicals and Reagents

Tetrahydro furan HPLC grade

Diacerein R.S, Obtained from Orbit Life Sciences Pvt Ltd, Mumbai

Commercially available pharmaceutical dosage form of Diacerein 50 mg Capsules, Dycerein- Marketed by Glenmark Pharmaceuticals, Mumbai, INDIA.

Chromatographic system Parameters

Column : Waters Styragel HR-0.5 GPC column

Dimension : 7.8 x300 mm

Stationary Phase : Styrene-divinyl benzene copolymer particles

Pump : Waters 600 series

Injector : Waters 717 plus auto sampler

Flow rate : 1.2 ml/ min

PREPARATION OF STANDARD SOLUTION

Weighed accurately 10 mg of Diacerein R.S and transferred to a 10 ml standard flask, dissolved and made up to the volume with tetrahydrofuran. The solution had concentration of 1 mg/ml (Solution A). From this, 1ml was pipette out, transferred in to a 10 ml standard flask, and made up to the volume with tetra hydro furan. The resulting solution had a concentration of 100μg/ml (solution B). From this solution accurately pipette out0.4,0.6,0.8 in to three 10ml standard flasks and made up to the volume using tetrahydrofuran to get a concentration of 4,6,8μg/ml respectively (Solution C)

Development chromatogram and determination of retention time

After initial stabilization, standard solutions of Diacerein were taken in the micro-syringe and injected 20μl of each solution separately in to the column. The retention time was found to be 5.784 minutes.

Table.1: Determination of Linearity range of Diacerein R.S

SL.NO.	Final concentration (μg/ml)	Peak height	Peak Area
1	4	31575	361368
2	6	43018	491748
3	8	65173	746588

Inference: The calibration curve was found to be linear in the concentration range of 2-10μg/ml.

STATISTICAL EVALUTION OF CALIBRATION PLOT

The data above was used to derive a regression equation of the peak area Y on the concentration X and peak height Y on the concentration X by the principles of least squares and the equation is as follows.

Y=aX +b

Peak area wise

Linear regression Y=100730 +533229

Correlation coefficient was found to be 1.0007

Figure 1. Linearity Plot of Calibration of concentration with Peak Height

Figure.2. Linearity plot of concentration vis Peak Width

Accuracy

The accuracy of an analytical procedure is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample whose true value is known is analysed and measured value should ideally be identical to the true value. Accuracy was determined by carrying out a recovery study and the method was found to be accurate.

Limit of detection (LOD)

The detection, limit of an individual analytical procedure is the smallest level of analyte that gives a measurable response. The limit of detection of Diacerein by proposed method was found to be 0.5μg/m/

Limit of quantification (LOQ)

The limit of quantification of an individual analytical procedure is the smallest concentration of analyte which gives a response that can be accurately quantified. The limit of quantitation of Diacerein by the proposed method was found to be 1µg.ml.

Linearity

The linearity of an individual analytical procedure is a measure of how well a calibration plot of response v/s concentration approximates a straight line.

The calibration curve of Diacerein by the proposed method was found to be linear in the range of 1-10µg/ml

Range of the proposed method was found to be 9µg/ml

ESTIMATION OF DIACEREIN IN DOSAGE FORMS
Details of analysed capsules
Batch No. DJ1245
Mfg. by: Glenmark Pharmaceuticals, Mumbai, INDIA.

Method

The content of 20 capsules was accurately weighed and the average weight calculated. A weight of the powder equivalent to 10 mg was accurately weighed out and transferred to a stoppered flask. Extracted three times successively with 2.5ml of Tetrahydrofuran HPLC grade and filtered through Whatman No. filter paper and the combined extract was then made up to 10 ml with Tetrahydrofuran. The resulting solution had a concentration of 1 mg/ml (Solution A). From the resulting solution, pipetted 1 ml in to another 10ml standard flask and made up to the volume with Tetra hydro furan. The resulting solution had a concentration of 100µg/ml (Solution B). From the solute on B, accurately pipetted out 0.4,0.6,0.8 ml and transferred to three 10 ml standard flask and made up to the volume with Tetrahydrofuran. The final solutions had concentrations of 4,6,8µg/ml respectively.

The chromatogram was generated after injection of 20µl of each solution to the column, under the same chromatographic conditions as mentioned above. The peak arears and peak heights obtained from the chromatogram are tabulated.

Table.2: showing Calibration of Diacerein capsule

SL NO.	CONC µg/ml	Peak area	Peak height	Concentration from calibration curve	Active content/capsule	Average Content/capsules
1	4	363571	32361	3.864	49.9	
2 3	6	484702	43061	5.151	49.9	49.9
	8	747918	65992	7.727	49.8	

CALCULATIONS:

Label Claim   = 50 mg

Average weight of the capsule content = 0.3925 g

Weigh equivalent to 10 mg   = 0.0785 g

Weight taken   = 0.0785g

Average content of Diacerein determined by the proposed method = 49.9 mg

Percentage Label Claim = 49.9X 100

50   = 99.9 %W/W

Standard deviation =0.1529

Coefficient of variation= 0.3079

Standard error   = 0.0882.

III.   RECOVERY STUDIES

The content of twenty capsules was accurately weighed and average weight found out. Finely powdered the contents of the capsules in a glass mortar. A weight equivalent to 5 mg was accurately weighed and transferred in to a stoppered flask, add 5 mg of Diacerein R.S. Extracted three times successively with 2.5 ml of tetrahydrofuran HPLC grade, and filtered through Whatman No.1 filter paper and the combined extract was then made up to 10 ml

with Tetrahydrofuran. The resulting solution had a concentration of 1mg/ml (Solution A). From the resulting solution pipetted 1 ml in to another 10 ml standard flask and made up to the volume with tetrahydro furan. The resulting solution had a concentration of 100µg/ml (Solution B). From the solution B, accurately pipetted out 0.4,0.6,0, 0.8 ml and transferred to three 10 ml standard flask and made up to the volume with tetrahydrofuran. The final solution had a concentration of 4,6,8µg/ml respectively. Chromatogram generated after injection 20µl each of 4,6, and 8µg/ml solution to column, under the same chromatographic conditions were evaluated and the peak area obtained from the chromatogram are tabulated.

Table.3: showing Concentration v/s Peak area of Dy cerein

Serial No.	Concentration (µg/ml)	Peak Area		Percentage recovery
		Standard	**Sample (Dyce rein)**	
1	4	360172	361368	100.3
2	6	492748	491588	99.8
3	8	749588	745895	99.5

CALCULATIONS

Weight equivalent of Diacerein in Capsule powder taken   = 5 mg
Weight of Diacerein R.S taken   = 5 mg
Total amount of Diacerein theoretically   =10 mg
Average value of percentage Recovery   = 99.87%
The following statistical parameters were evaluated
Standard deviation   = 0.3512
Coefficient of variation   = 0.3518
Standard error   = 0.2028

Table.4: Concentration v/s peak area of Neo cerein

Serial No.	Concentration	Peak area Standard	Sample (Neo cerein)	Percentage Recovery
1	4	360571	361368	100.3
2	6	491748	500511	100.1
3	8	749288	744554	99.4

Weight equivalent of Diacerein in capsule powder taken =5 mg
Weight of Diacerein R.S taken   = 5 mg
Total amount Diacerein theoretically   = 10 mg
Average value of percentage Recovery   = 99.9%
The following statistical parameters were evaluated
Standard deviation   = 0.4740
Coefficient of variation   =   0.4744
Standard error   = 0.2726

COMPARISON OF THE PROPOSED METHODS WITH THE PUBLISHED METHOD: -
Diacerein in dosage forms were analysed by the HPLC method as given in published work, and a comparison was done between the proposed method and already published method.
Reagent used
1.Acetnitrile: HPLC grade supplied by Qalinge's Fine Chemicals, Mumbai
2.Phosphate buffer
HPLC variables
Column: Kromasil C-18, 5 µm ,250x 4.6 mm I'd column
Pumps: -Jasco 2080 plus
Injector: Jasco Autosampler 2055
Detector : UV 254 nm
Mobile phase: Phosphate buffer: Acetonitrile (40:60)
Flow rate: 1.5 ml/mn

Retention time: 4.9 minutes

Table.5: Showing Concentration v/s Peak Area

SL. NO.	Conc. Diacerein	Peak area standard	sample	Label claim	Average %
1	4	366726	324419	88.46	
2	6	529342	495547	93.6	91.78
3	8	703613	656331	93.28	

Table.6: Showing concentration v/s Peak height

SL No.	Conc. Diacerein	Peak Height Standard	Sample	Label Claim	Average %
1	4	76483	69900	91.39	
2	6	112509	109726	97.52	94.34
3	8	153247	144215	94.10	

Table.7:
COMPARISON OF THE PROPOSED METHOD WITH THE PUBLISHED METHOD

Method	Content (mg)	% Label claim	% Recovery
HPLC	49.9	99.90	99.85
Published method a) Peak area wise	45.30	91.4	-
b) Peak height wise	45.96	92.2	-

IV. RESULTS AND DISCUSSION

Diacerein is now widely used for the treatment of Osteoarthritis. The mechanism of action defers from the Nonsteroidal anti-inflammatory drugs since it is not related to the inhibition of Prostaglandins. Anti-osteoarthritis and cartilage stimulating properties have been demonstrated *in vivo* and in animal models. Diacerein and Rhein have been shown to inhibit the production of interleukin-I beta by human monocytes and the effects of the cytokine on chondrocytes *in vivo*. Diacerein in therapeutic dose inhibit the stimulation of interleukin-1 beta production and production of nitrous oxide. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor TGF-beta-1 and TGF beta-2 with potential cartilage repairing properties. From the literature, Diacerein is seemed to be a safe and promising drug.

Review of literature suggest that only a few analytical methods have been developed for the estimation of Diacerein in dosage forms. There are no official methods for its quantification. Only One HPLC method with UV detection were published for its estimation 0f Diacerein in capsules.

This research work has been taken up to develop and validate simple, sensitive and cost-effective methods for the estimation of Diacerein in dosage forms. A new LC method with UV detection was developed for the quantitative determination of Diacerein in pharmaceutical dosage forms. The chromatographic separation was performed on GPC column with a mobile phase of tetra hydro furan. A flow rate of 1.2ml/minute was used. The resulting chromatogram exhibited a retention time of 5.784 minute. The limit of detection and limit of quantification was found to be 0.5µg/m land 1µg/ml. A linearity range of 1-10µg/ml was established. The correlation coefficient was found to be 1. 007.The results of analysis were treated statically, as per ICH guidelines for validation of analytical procedures and by recovery studies. The results were found to be accurate and not much interferences from capsule excipients. The active pharmaceutical ingredient was extracted from its finished dosage forms using tetrahydrofuran. The percentage recovery was found to be 99.9%w/w.

Comparison of the proposed method with published method

The proposed method was compared with published HPLC method for analysis of Diacerein in dosage forms using phosphate buffer: Acetonitrile (40:60). The percentage label claim found to be 91.4(Peak area wise).

The proposed method was found to be accurate and reproducible than the published method. Moreover, the proposed HPLC method with UV detection was found to be more sensitive than the published method. This method shows better accuracy, Linearity range of 1-10µg/ml indicates reliability of the method. A low LOD value of 0.5µg

suggest that the method may extended to determine the concentration of Diacerein in body fluids also, after optimization of system parameters and revalidation.

V. CONCLUSION

The efficient development and validation of Analytical methods are critical elements in the development of pharmaceuticals he desirable characteristics for an analytical method include high sensitivity and selectivity, accuracy, precision and robustness.

The HPLC method demonstrated herein are applicable to the estimation of Diacerein in pure as well as in existing dosage forms. In order to ensure that the data generated with each of the above methods are accurate and precise, the experiments have been performed on calibrated equipment using suitable reference standards. To prove and document the reliability of the methods, validation as per ICH guidelines has been carried out to a possible extent.

The proposed HPLC method provides simple, accurate and reproducible quantitative method for routine in vitro tests of Diacerein dosage forms. Finally, as mentioned no pharmacopoeial methods for determination of Diacerein in dosage forms have been reported yet. Hence the proposed method can be considered as simple and specific method for the estimation of Diacerein in pure and dosage forms.

Table.8: REPORT OF COMPARISON BETWEEN THE PROPOSED METHOD

Method Brand used	Content/ Capsule (mg)	% Label claim	Accuracy 100% Level ±SD	Repeatability N=3 % CV	Linearity range	Correlation coefficient	LOD
HPLC Diacerein 50	49.9	99.90	100.20 ±0.4500	-	1-10 μg/ml	1.007	0.5 μg/ml

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