Journal of Medicinal Chemistry and Drug Discovery

Available online at http://www.jmcdd.com
November-December, 2015, Vol. 4, pp 14-37
ISSN: 2347-9027

Research Article

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF DIACERERIN IN RAT PLASMA

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ABSTRACT

Diacerein is 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-anthetharnoic acid diacetate, used for osteoarthritis. This study was designed to develop and validate high performance liquid chromatography method of diacerein in rat plasma. The samples were analyzed by using HiQ sil C_{18} (4.6×250 mm, 5 micron) columns, using 0.1 M Sodium dihydrogen orthophosphate: Methanol (45:55 v/v) as a mobile phase. The method showed linearity (r^2=0.993) over a concentration range of (0.2-2.5 µg/ml).The method showed good mean recovery (88.35%) for diacerein. The method was found to be accurate, precise, linear, specific, sensitive and stable.

KEY WORDS: Diacerein, HPLC, Rat plasma, Analytical method, Validation.

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INTRODUCTION

Diacerein is 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-anthetharnoic acid diacetate. Diacerein contains not less than 98.0% and not more than 101.0% of $C_{19}H_{12}O_8$, calculated on the anhydrous basis**.** Category of diacerein is antirheumatic[1]. It directly inhibits IL-1 synthesis and release in vitro and down modulates IL-1 induced activities and have been shown to possess disease modifying effect in experimental models of osteoarthritis and in human subjects with finger joint and knee osteoarthritis. IL-1 plays a fundamental role in osteoarthritis pathophysiology and cartilage destruction. IL-1 also promotes expression of inducible nitric oxide synthase, increase release of prostaglandin E2, IL-6, IL-8 in human osteoarthritis chondrocytes, which promote joint degradation. Hence, by inhibiting IL-1 diacerein retards all pathological prepossess initiated in OA. Diacerein also inhibits IL-1 induced expression of cartilage degrading enzymes. It also enhances expression of TGF BETA-1 and TGF BETA 2 thus favoring matrix synthesis and turnover in articular chondrocytes, thereby accounting for disease modifying property of diacerein. It also inhibits superoxide production, chemotaxis and phagocytic activity of neutrophils in addition to effect on macrophage migration and phagocytosis. In contrast to NSAIDS diacerein does not inhibit synthesis of prostaglandins; hence no gastroduednal toxicity has been observed with diacerein. It is also demonstrated to be involved in prevention of loss of hydroxiproline and proteoglycans in the joint cartilage, an effect not observed with conventional NSAIDS or COX-2 inhibitors[2]. Oral diacerein undergoes complete deaccetylation to its active metabolite rhein. The apparent availability of rhein, as assed by urinary data, was 35%.The maximum plasma concentration (C_{max}) of rhein was 3.2 mm/lit, 2.2hrs after administration of single oral dose of diacerein 50mg.Area under curve (AUC) was 21.3mg/lit/hr, apparent volume of distribution was 13.2 liter, terminal elimination half-life ($t_{1/2}$) was 4.3hrs, apparent total plasma clearance was1.6lit/hrs and renal clearance was (Clr) was 0.13lit/hr. Rehin is metabolized to glucourono-and sulpho-conjugates[3].

Several spectroscopic and chromatographic methods are available in literature to determine concentration of Diacerein, individually or in combination with other drugs or metabolites include reverse phase HPLC with UV[4-11], UV[12-19], liquid chromatography-tandem mass spectroscopy[20-21], RP-HPLC[22], LC-MS[23] fluorescence detection[24-26] , HPTLC[27].

The present article describes a simple HPLC method for estimation of Diacerein and validation of the method as per the guidelines of ICH[28].

EXPERIMENTAL

Reagents and chemicals

Diacerein (Elder pharma, Mumbai, India), Methanol HPLC grade, Double distilled water, Trichloroacetic acid AR grade, Sodium dihydrogen o-phosphate AR grade.

Selection of mobile phase

Different mobile phases like methanol and 0.1 M potassium dihydrogen o-phosphate (pH-6), acetonitrile and 0.1 M sodium dihydrogen o-phosphate were tried in different ratio order to find the best conditions for the separation of Diacerein (DCN) and Piroxicam (PIR). After several trials 0.1M Sodium dihydrogen o-phosphate: Methanol in ratio of (45:55 v/v) was chosen as the mobile phase for analysis in which best results were obtained.

Preparation of mobile phase

Solution of 0.1 M sodium dihydrogen o-phosphate was prepared by dissolving 3.9 g of sodium dihydrogen o-phosphate in 250 ml of doubled distilled water. Mobile phase was prepared by mixing 0.1 M sodium dihydrogen o-phosphate with Methanol in 45:55 v/v proportions, filtered through 0.45-µ-membrane filter paper and then sonicated in sonicator bath for 15 min.

Preparation of standard stock solutions of Diacerein (500µg/ml, 50µg/ml, 5 µg/ml)

5 mg of Diacerein was dissolved in 1ml of methanol and then diluted with mobile phase to final volume of 10 ml in volumetric flask to get concentration 500 µg/ml (stock I). 1 ml stock I solution of Diacerein diluted to 10 ml with mobile phase in another volumetric flask to get concentration 50 µg/ml (stock II). 1 ml stock II solution of Diacerein diluted to 10 ml with mobile phase in another volumetric flask to get concentration 5 µg/ml (stock III).

Preparation of intermediate stock solution by using stock solutions of Diacerein (500 µg/ml and 50 µg/ml, 5 µg/ml) for Plasma Calibration Curve

Using a calibrated pipette 1.6 ml of (stock III) solution were pipette out into 10.0 ml volumetric flasks and made up the volume with mobile phase to get final concentration 0.8 µg/ml. Pipette out 0.4, 0.8, 1.6, 2.4, 3.2 ml of (stock II)

solution in separate volumetric flasks and then diluted to 10 ml with mobile phase to get concentrations 2, 4, 8, 12, 16 µg/ml. Pipette out 0.4 ml of (stock I) solution in volumetric flask and then diluted to 10 ml with mobile phase to get concentration 20 µg/ml.

Preparation of ISTD stock solution of Piroxicam (500 µg/ml)

5 mg of Piroxicam was dissolved in 1 ml of methanol and then diluted with mobile phase to final volume of 10 ml in volumetric flask to get concentration of 500 µg/ml.

Using a calibrated pipette, 0.8 ml of ISTD stock solution (500 µg/ml) was pipette into a 10.0 ml volumetric flask and made up the volume with the mobile phase to get concentration of 40 µg/ml.

Preparation of Plasma sample solution

To 0.5 ml of rat plasma, 50 µl of an I.S. solution (Piroxicam, 40 µg/ml), 0.5 ml of stock solution of DCN (Concentrations: 0.8, 2, 4, 8, 12, 16, 20 µg/ml), 0.5 ml methanol and 0.5 ml of trichloroacetic acid (10 % w/v) were added to a glass tubes. Each sample was vortex mixed for 5 min and centrifuged (5000 rpm for 10 min). After centrifugation 50 µl aliquots of supernatant of each concentration were injected into the HPLC system(table 1).

Selection of analytical wavelength

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200-400 nm and the spectra were overlain.

Summary of chromatographic parameters selected:

a) Column : HiQ sil C_{18} (4.6×250 mm, 5 micron)

b) Mobile phase : 0.1 M Sodium dihydrogen orthophosphate: Methanol (45:55 v/v).

c) Flow rate : 1.00 ml/min

d) Detection Wavelength : 257 nm

e) Sample injector : 50 µl loop

f) Temperature : Ambient

g) Internal standard : Piroxicam

Method Validation

Selectivity/Specificity

Selectivity is the ability of an analytical method to differentiate and quantify the analytes in the presence of other components in the sample. The selectivity of the method was evaluated by analyzing 6 replicates of plasma samples spiked at LLOQ (Lower Limit of Quantification - 0.2 µg/ml).

Linearity

Linearity was tested for the range of concentrations 0.2-5 µg/ml. Each standard in three replicates were analyzed and peak areas were recorded. The response factors were plotted against the corresponding concentrations to obtain the calibration graphs.

Accuracy

The accuracy of the assay was calculated as the absolute value of the ratio of the calculated mean values of the quality control samples to their respective nominal values, expressed as percentage. Accuracy should be measured using minimum five determinations per three concentrations (0.5, 2, 4 µg/ml). 50-µl aliquots of supernatant of each concentration were injected in to the HPLC system.

Precision

The precision of this method was evaluated by the % CV at different concentration levels corresponding to LQC, MQC and HQC during the course of validation.

A] Inter Day Precision (Reproducibility)

The reproducibility (inter-assay precision) was evaluated in three replicates for three different concentrations of DCN (0.5, 2, 4 µg/ml) on three consecutive days (fresh samples were prepared every day). The results, expressed as mean amounts of drug found in plasma and summarized in the table 6.

B] Intra Day Precision (Repeatability)

The repeatability (intra-assay precision) of the method was evaluated in five replicates on the same day for three different concentrations of DCN (1, 3, 4 µg/ml). The results, expressed as mean amount of drug found in plasma and summarized in the table 7.

Recovery

The % mean recoveries were determined by measuring the responses of the extracted plasma quality control samples against unextracted quality control samples at HQC, MQC and LQC levels. Recovery from human plasma samples was evaluated in triplicate for each three concentrations of DCN (0.5, 2, 4 µg/ml). 50-µl aliquots of supernatant of each concentration were injected in to the HPLC system.

Stability

Drug stability in a biological fluid was a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage conditions.

I) Freeze and Thaw stability

Freeze thaw stability of the spiked quality control samples was determined after three freeze thaw cycles stored at -5 °C ± 0 °C. Comparing them against the freshly spiked quality control samples assessed stability.

II) Bench top stability

Bench top stability of the spiked quality control samples was determined for a period of 5 hours 30 min stored at room temperature. Comparing them against the freshly spiked quality control samples assessed stability.

III) Stock solution stability

Stock solution stability of the HQC and LQC was determined for a period of 5 hours 30 min stored at room temperature. Comparing them against the freshly weighed stock solution assessed for stability.

RESULTS AND DISCUSSION

Selection of analytical wavelength

It was observed that both drugs showed considerable absorbance at 257 nm and No endogenous interferences are noted at the retention time of the drugs as shown in figure 1.

Method Validation

Selectivity/Specificity

The precision and accuracy for at LLOQ level are found to be 3.79 % (as % CV) and 112.86 % (as % recovery), respectively. The results are summarized in table 2. No endogenous interferences are noted at the retention time of the drugs as shown in figure 3 and 4.

Linearity

All the three calibration curves analyzed during the course of validation were found to be linear for the standards concentration ranging from 0.2-5 µg/ml and best fitted by a linear equation y = mx + c, the coefficient of determination for plane diacerein (R^2) is 0.997 and plasma containing diacerein (R^2) is 0.993. An averaged calibration curves are shown in figure 5 and 6

Accuracy

The % mean accuracy of calculated concentrations for all quality control samples at LQC, MQC and HQC concentration levels are ranged from 86.79 % to 100.92 %, which is within acceptance limit 85.00 - 115.00 %.

Precision

A] Inter Day Precision (Reproducibility)

The % CV of calculated concentrations for all quality control samples of LQC, MQC and HQC concentration levels are ranged from 1.99 to 7.17 %, which is within the acceptance limit of 15.00 % as shown in table 6.

B] Intra Day Precision (Repeatability)

The % CV of calculated concentrations for all quality control samples at LQC, MQC and HQC concentration levels are ranged from 3.25 to 5.27 %, which is within acceptance limit 15.00 % as shown in table 7.

Recovery

The % mean recovery for diacerein at HQC, MQC and LQC levels are found to be 87.05 %, 91.27 % and 86.72 % respectively. Over all % CV at all QC levels is 2.87 %, which is within the acceptance limit of 15.00 % and % over all mean recovery is 88.35 %, which is within the acceptance limit of 20.00 %. The results are summarized in the table 8.

Stability

I) Freeze and Thaw stability

The % mean stability for HQC (4 µg/ml) and LQC (0.5 µg/ml) are found to be 86.60 % and 87.14 % respectively, which is within the acceptance limit of 85.00 - 115.00 %. The results are summarized in the table 9.

II) Bench top stability

The % mean stability for HQC (4 µg/ml) and LQC (0.5 µg/ml) are found to be 88.67 % and 90.92 % respectively, which is within the acceptance limit of 85.00 - 115.00 %. The results are summarized in the table 10.

III) Stock solution stability

The % mean stability for HQC (4 µg/ml) and LQC (0.5 µg/ml) are found to be 94.11 % and 96.54 % respectively, which is within the acceptance limit of 90.00 - 110.00 %. The results are summarized in the table 11.

CONCLUSION

This study presents a simple and validated HPLC method for estimation of Diacerein from rat plasma.

REFERENCES

1. Indian Pharmacopoeia (2014), Vol-II, 7th ed. Indian Pharmacopoeia Commission, Ghaziabad, pp 1543-1544.

2. Mahajan A, Singh K, Tandon V, Kumar S, Kumar H.(2006). Diacerein: A New Symptomatic Slow Acting Drug for Osteoarthritis. JK Science, 2006, 8 (3), 173-175.

3. Medhi B , Singh PK, Prakash A , Sen R, Wadhwa S.(2007). Diacerein: A New Disease Modulating Agent in Osteoarthritis. Indian Journal of Pharmaceutical and Medical Research, 18 (2), 48-52.

4. Yi L, Jian-Ping G, Xu X, Lixinus D.(2006). Simultaneous determination of baicalin, rhein and berberine in rat plasma by column-switching high-performance liquid chromatography. Journal of Chromatography B, 838(1), 50-55.

5. Zhu W, Wang XM, Zhang L, Li XY, Wang BX. (2005). Pharmacokinetic of Rhein in Healthy Male Volunteers Following Oral and Retention Enema Administration of Rhubarb Extract: A Single Dose Study. American journal of Chinese Medicine,33(6),839.

6. Zhu W, Zhang L, Wang XM, Wang BX , Li XY. (2005). The pharmacokinetics of rhein in 12 healthy volunteers after oral administration of rhubarb extract. Zhongguo Zhong Yao Za Zhi., 30(18), 1458-61.

7. Lee JH, Kim JM, Kim C., (2003). Pharmacokinetic analysis of rhein in Rheum undulatum L. Journal of Ethno pharmacology. 84(1), 5-9.

8. Cameron B, Philips M, Fenerty C. (1988).Milk transfer of rhein in the rhesus monkey. Pharmacology B, 36(1), 221-5.

9. Takizawa Y, Morota T, Takeda S, Aburada M. (2003). Pharmacokinetics of Rhein from Onpi-to, an Oriental Herbal Medicine, in Rats. Biological and Pharmaceutical Bulletin. 26(5), 13–617.

10. Tang WF, Huang X, Yu Q, Qin F, Wan MH, Wang YG, Liang MZ. (2007). Determination and pharmacokinetic comparison of rhein in rats after oral dosed with Da-Cheng-Qi decoction and Xiao-Cheng-Qi decoction. Biomedical Chromatography. 21(11), 1186-1190.

11. Ojha A, Rathod R, Padh H. (2009). Simultaneous HPLC-UV determination of rhein and aceclofenac in human plasma. Journal of chromatography B, 877, 1145-1148.

12.Abirami G, Anandakumar K , Velmurugan R.(2012).Development And Validation of UV-Spectroscopy Method For The Determination of Diacerein Hydrochloride in Pharmaceutical Formulation. Journal of Pharmacy Research, 5(4), 1949-1951.

13. Selvam P, Mahalingam U, Sridharan D, Thenmozhi A. (2011). A Simple UV Spectrophotometric Determination of Diacerein In Pure and In Pharmaceutical Dosage Form, Asian Journal of Biochemical and Pharmaceutical Research,1,(3),124-126.

14. Bhoir S, Dhole S, Kulkarni N, Sangole P, Thorat S, Bhoite D. (2012) Novel and validated spectrophotometric estimation of diacerein in bulk and capsule formulation using mixed hydrotropic solubilisation approach, International Journal of Pharmacy and Pharmaceutical Sciences, 4(4), 501-504.

15. Chitlange S, Pawbake G, Mulla A, Wankhede S.(2010). Simultaneous Spectrophotometric Estimation of Diacerein and Aceclofenac in tablet dosage form. Der Pharma Chemica, 2(1), 335-341.

16. Gupta K, Samrit V, Thakur V , Hemke A.(2010) UV Spectrophotometric estimation of Diacerein in pharmaceutical formulation. Journal of Chemical and Pharmaceutical Research, 2(3),467-472.

17. Nyola N , Kalra N. (Oct-Dec.2010). Spectrophotometric determination of Diacerein in bulk and pharmaceutical formulation. International Journal of Pharma and Bio Sciences, 1(4), 202-207.

18. Pandey R, Patil P, Patil M, Deshmukh P, .Bari S(Jan-June 2012). Quantitative estimation of Diacerein in bulk and capsule formulation using mixed hydrotropic solubilisation by UV spectroscopy and first order derivative method using area under curve method.Pharmaceutical Methods, 3 (1),4-8

19. Sreejith K, Premalatha K.(Jul –Sep 2011). Novel Spectrophotometric Methods for Estimation of Diacerein from Formulations. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2 (3), 992-999.

20. Zhu C, Zheng Z, Chen Z, Liang X. (2002). Determination of Rhein in plasma of rat by HPLC/MS. Zhong Yao Cai , 25(9), 646.

21. Layek B, Kumar T , Trivedi R , Mullangi R , Srinivas N.(2008). Development and validation of a sensitive LC-MS/MS method with electro spray ionization for quantitation of rhein inhuman plasma: application to a pharmacokinetic study. Biomedical chromatography, 22(6), 616-624

22. Kannappan N, Madhukar A, Srinivasan R, Srinivas R. (Jan-Mar-2010).Analytical method development and validation of Diacerein tablets by RP-HPLC. International journal of Chem.Tech Res.2 (1), 143-148.

23. Shirwaikar, A, Devi S, Premalatha K, Sreejith K.(Oct-Dec 2012). Determination of Diacerein in Rabbit Plasma by Liquid Chromatography - Mass Spectroscopy and Its Application to Bioavailability Study. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3(4), 1738-1744.

24. Yan D, Ma Y. (2007) . Simultaneous quantification of five anthraquinones in rat plasma by high-performance liquid chromatography with fluorescence detection.Biomedical Chromatography, 21(5), 502-7.

25.Krumbiegel G, Schulz H. (1993). Rhein and Aloe-Emodin Kinetics from Senna Laxatives in Man. Pharmacology, 47, 120-124.

26. Yan D, Yueming Ma. (2007) Simultaneous quantification of five anthraquinones in rat plasma by HPLC with fluorescence detection. Biomedical chromatography, 21(5), 502-507.

27. Gandhi S, Dewani M, Borole T, Damle M (2012) .Development and Validation of Stability Indicating HPTLC Method for Determination of Diacerein and Aceclofenac as Bulk Drug and in Tablet Dosage Form. E-Journal of Chemistry, 9(4), 2023-2028.

28.http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf

TABLE:

Table 1: Plasma sample preparation

Rat Plasma	Stock solution of DCN (0.5 ml)	Stock solution of PIR (50 µl)	MeOH	TCA (10% w/v)	Conc. of DCN (µg/ml)	Conc. of PIR (µg/ml)
0.5 ml	Mobile phase	Mobile phase	0.5 ml	0.5 ml	Blank	Blank
0.5 ml	0.8 µg/ml	40 µg/ml	0.5 ml	0.5 ml	0.2	1
0.5 ml	2 µg/ml	40 µg/ml	0.5 ml	0.5 ml	0.5	1
0.5 ml	4 µg/ml	40 µg/ml	0.5 ml	0.5 ml	1	1
0.5 ml	8 µg/ml	40 µg/ml	0.5 ml	0.5 ml	2	1
0.5 ml	12 µg/ml	40 µg/ml	0.5 ml	0.5 ml	3	1
0.5 ml	16 µg/ml	40 µg/ml	0.5 ml	0.5 ml	4	1
0.5 ml	20 µg/ml	40 µg/ml	0.5 ml	0.5 ml	5	1

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Table 2: Results for Selectivity

Replicate No.	Nominal Conc. (LLOQ) (0.2 µg/ml)
	Calculated Conc. (µg/ml)
1	0.22
2	0.24
3	0.23
4	0.22
5	0.23
6	0.22
Mean	**0.23**
SD	**0.00857**
% CV	**3.79**
% Mean Accuracy	**112.86**
Acceptance Criteria: At least 67 % (4 out of 6) sample should be within 80.00-120.00 %. The % Mean accuracy should be within 80.00-120.00 %. The % CV should be ≤ 20.00 %.	

Table 3: Observation table for Linearity of Diacerein plain sample

Concentration (µg/ml) (n=3)	Response Factor*
0.2	0.3658
0.5	0.4794
1	0.9389
2	1.9627
3	2.8720
4	3.5388
5	4.5492

*Average of three determinations

Table 4: Observation table for Linearity of Diacerein in plasma sample

Concentration (µg/ml) (n=3)	Response Factor*
0.2	0.3921
0.5	0.5642
1	1.0006
2	2.0891
3	3.1698
4	4.1216
5	4.6742

*Average of three determinations

Table 5: Results for Accuracy

Replicate No.	LQC	MQC	HQC
	Nominal concentration (µg/ml)		
	0.5	2	4
	Calculated Concentration (µg/ml)		
1	0.41	2.30	3.31
2	0.40	1.93	4.01
3	0.47	2.06	3.73
4	0.46	1.93	3.96
5	0.42	1.86	3.35
Mean	**0.43**	**2.02**	**3.67**
SD	**0.0330**	**0.1737**	**0.3297**
%CV	**7.61**	**8.61**	**8.98**
% Mean Accuracy	**86.79**	**100.92**	**91.84**
Acceptance Criteria: The % Mean Accuracy for HQC, MQC, and LQC sample should be within 85.00-115.00 %.			

Table 6: Observation table for Inter Day Precision

A	DAY 1	DAY 2	DAY 3
	Nominal concentration 0.5 µg/ml (LQC)		
	Calculated concentration (µg/ml)		
1	0.435	0.449	0.451
2	0.442	0.434	0.435
3	0.425	0.433	0.430
Mean	**0.434**	**0.439**	**0.439**
SD	**0.0086**	**0.0091**	**0.0112**
% CV	**1.99**	**2.08**	**2.55**
B	**Nominal concentration 2 µg/ml (MQC)**		
	Calculated concentrations (µg/ml)		
1	2.047	1.934	1.916
2	1.846	1.927	2.157
3	1.979	1.799	2.046
Mean	**1.957**	**1.887**	**2.039**
SD	**0.1022**	**0.0754**	**0.1204**
% CV	**5.22**	**3.99**	**5.90**
C	**Nominal concentration 4 µg/ml (HQC)**		
	Calculated concentrations (µg/ml)		
1	3.860	3.889	3.428
2	3.680	3.693	3.548
3	3.838	3.679	3.927
Mean	**3.793**	**3.754**	**3.634**
SD	**0.0981**	**0.1177**	**0.2605**
% CV	**2.59**	**3.14**	**7.17**
Acceptance Criteria: The % CV for LQC, MQC and HQC samples should be within 15.00 %.			

Table 7: Observation table for Intra Day Precision

Replicate No.	Nominal concentrations		
	(LQC) (1 µg/ml)	(MQC) (3 µg/ml)	(HQC) (4 µg/ml)
	Calculated concentrations (µg/ml)		
1	0.930	2.986	3.868
2	0.812	3.094	4.097
3	0.867	3.303	4.072
4	0.886	3.108	3.809
5	0.916	3.119	3.900
Mean	**0.88**	**3.12**	**3.95**
SD	**0.0465**	**0.1141**	**0.1282**
% CV	**5.27**	**3.66**	**3.25**

Acceptance Criteria:
The % CV for HQC, MQC, and LQC samples should be within15.00 %.

Table 8: Observation table for Recovery

Replicate No.	LQC (0.5 µg/ml)		MQC (2 µg/ml)		HQC (4 µg/ml)	
	Plane sample	Plasma sample	Plane sample	Plasma sample	Plane sample	Plasma sample
	Calculated concentrations (µg/ml)					
1	0.490	0.394	1.992	1.913	4.461	3.904
2	0.496	0.432	2.126	1.864	4.486	3.815
3	0.458	0.426	2.056	1.858	4.228	3.750
Mean	0.481	0.417	2.058	1.878	4.392	3.823
SD	0.0203	0.0207	0.0673	0.0302	0.1426	0.0776
% CV	4.21	4.97	3.27	1.61	3.25	2.03
% Mean Recovery	86.72		91.27		87.05	
% Overall Mean Recovery	88.35					
Overall SD	2.54					
Overall % CV	2.87					
Acceptance Criteria: The % CV of recovery at each QC levels should be ≤ 15.00 %. The overall mean recovery for all QC levels should be ≤ 20.00 %.						

Table 9: Results for Freeze and Thaw stability Study

Replicate No.	Nominal concentrations			
	LQC (0.5 µg/ml)		HQC (4 µg/ml)	
	Comparison Sample	Stability Sample	Comparison sample	Stability sample
	Calculated concentrations (µg/ml)			
1	0.382	0.342	3.920	3.587
2	0.428	0.341	3.894	3.262
3	0.391	0.363	4.047	3.423
Mean	0.400	0.349	3.954	3.424
SD	0.024	0.012	0.082	0.163
% CV	6.08	3.54	2.07	4.75

% Mean stability	87.14	86.60
Acceptance Criteria: The % CV for LQC and HQC should be sample should be ≤ 15.00. The % mean stability of LQC and HQC sample should be within 85.00-115.00 %.		

Table 10: Results for Bench top Stability Study

Replicate No.	Nominal concentrations			
	LQC (0.5 µg/ml)		HQC (4 µg/ml)	
	Comparison sample	Stability Sample	Comparison sample	Stability Sample
	Calculated concentrations (µg/ml)			
1	0.422	0.370	3.890	3.388
2	0.402	0.386	3.664	3.386
3	0.406	0.362	4.154	3.607
Mean	**0.410**	**0.373**	**3.903**	**3.460**
SD	**0.010**	**0.012**	**0.246**	**0.127**
% CV	**2.52**	**3.21**	**6.288**	**3.67**
% Mean Stability	**90.92**		**88.67**	
Acceptance Criteria: The % CV for LQC and HQC should be sample should be ≤ 15.00. The % mean stability of LQC and HQC sample should be within 85.00-115.00 %.				

Table 11: Results for Stock solution Stability Study

Replicate No.	Nominal concentrations			
	LQC (0.5 µg/ml)		HQC (4 µg/ml)	
	Comparison sample	Stability sample	Comparison sample	Stability sample
	Calculated concentrations (µg/ml)			
1	0.410	0.410	3.904	3.861
2	0.397	0.389	3.957	3.778
3	0.400	0.367	4.104	3.621
Mean	**0.402**	**0.388**	**3.988**	**3.753**
SD	**0.0071**	**0.0214**	**0.1033**	**0.1222**
% CV	**1.76**	**5.50**	**2.59**	**3.26**
% Mean stability	**96.54**		**94.11**	
Acceptance Criteria: The % mean solution stability for drug should be within range 90.00-110.00 %.				

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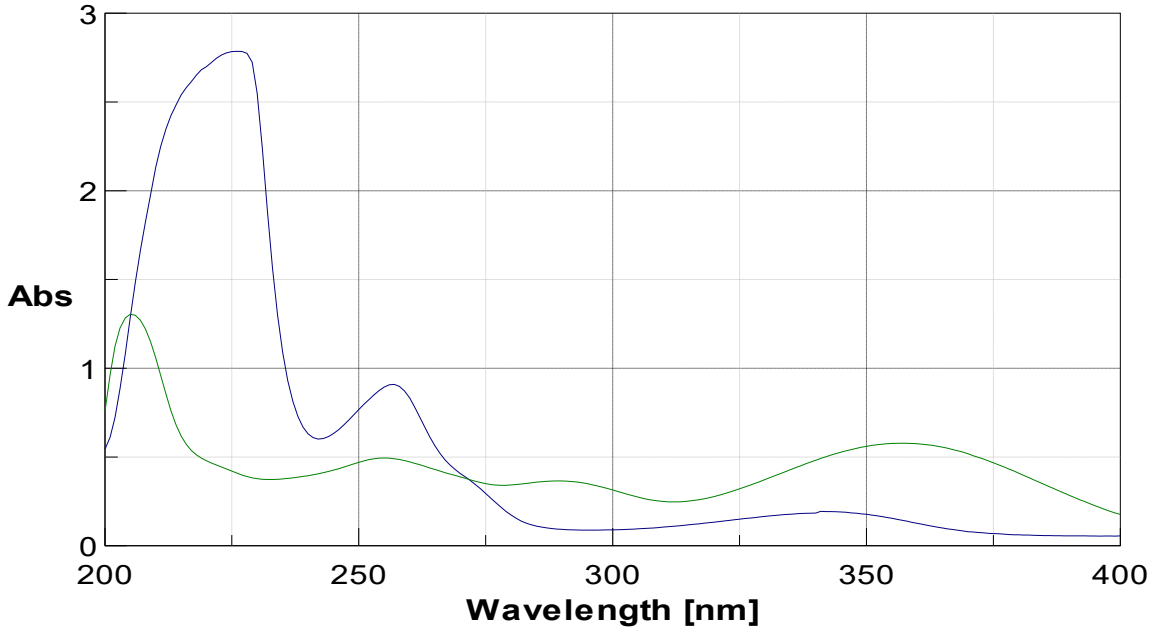

Figure 1: Overlain spectra of Diacerein (10 µg/ml) and Piroxicam (10 µg/ml)

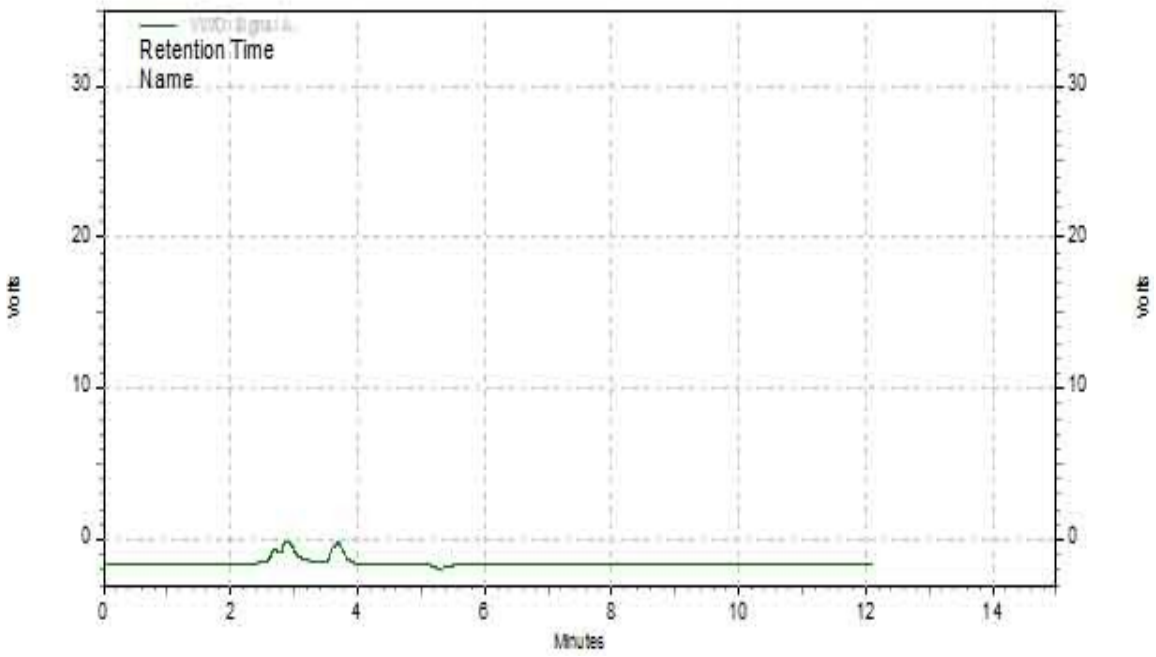

Figure 2: Chromatograph of Blank Plasma

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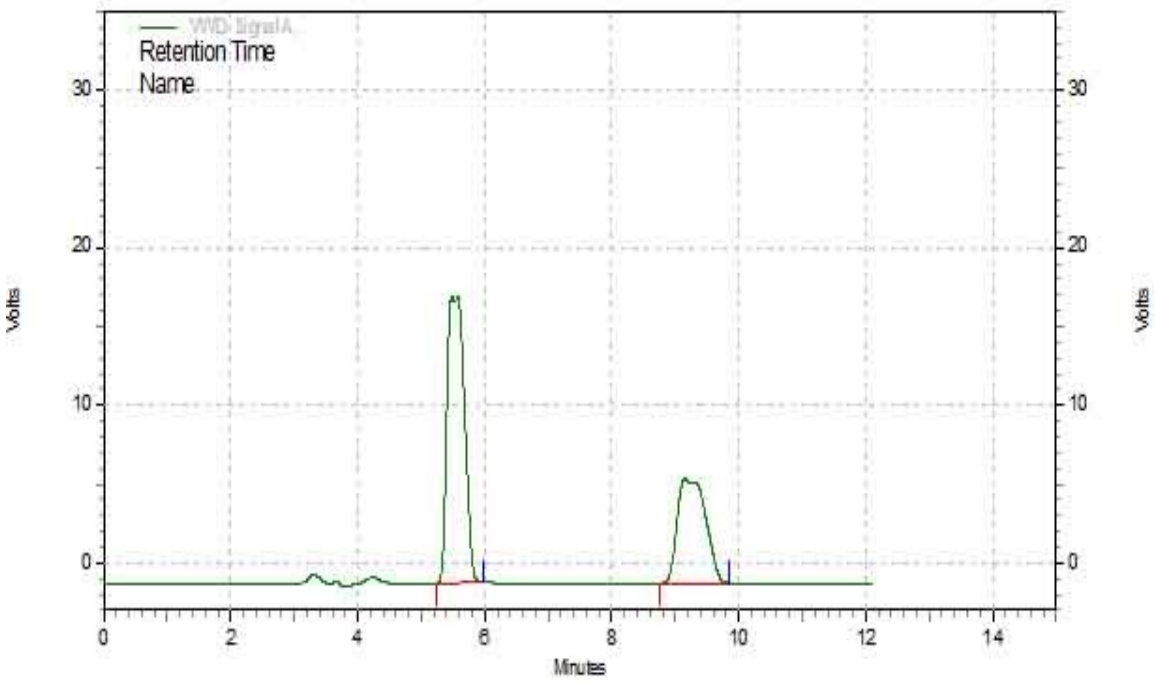

Figure 3: Chromatograph of Standard Piroxicam (t_R-5.480) and Diacerein (t_R-9.150)

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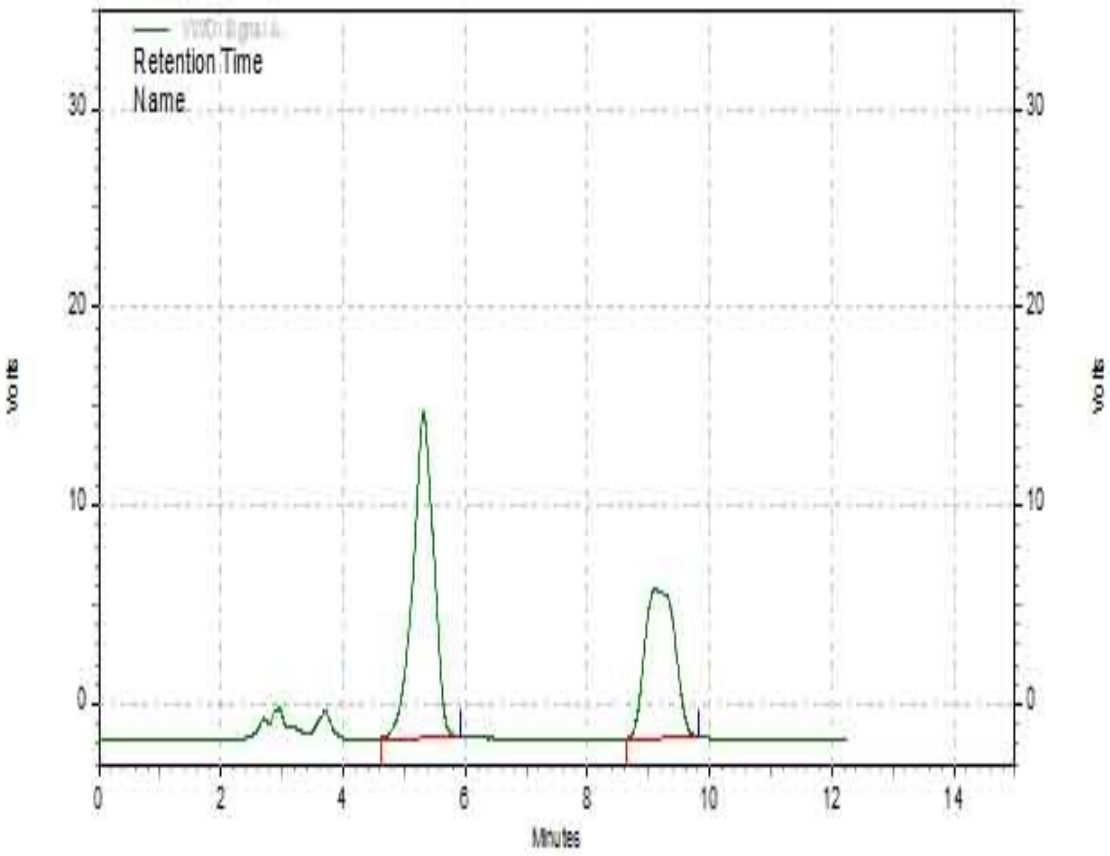

Figure 4: Chromatograph of Plasma spiked with Piroxicam (t_R-5.472) and Diacerein (t_R-9.015)

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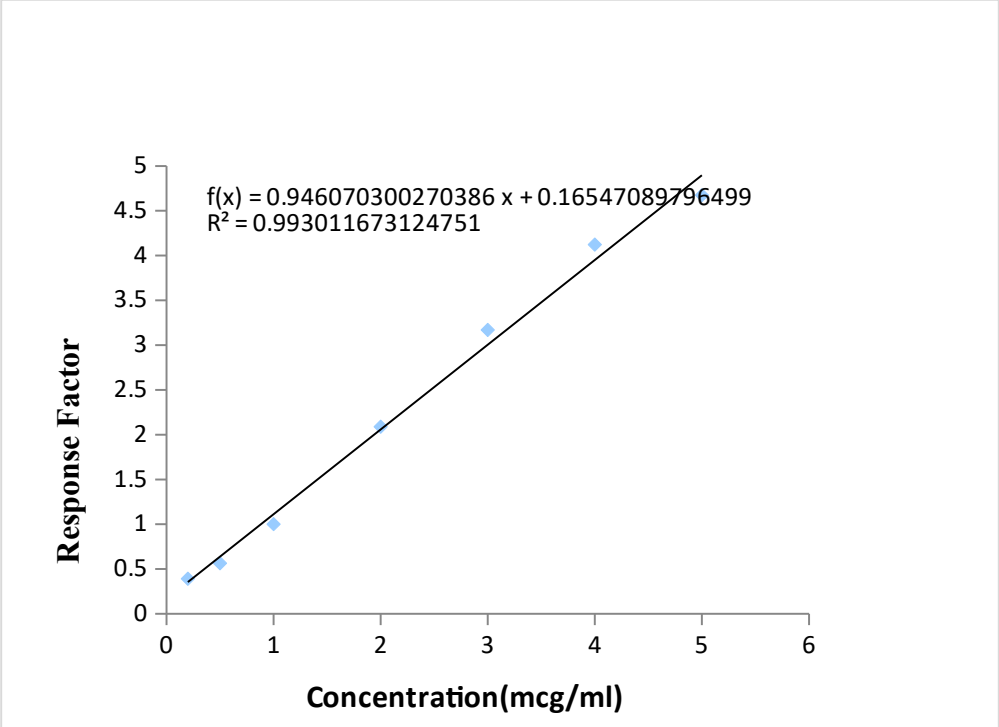

Figure 5: Calibration curve plain Diacerein sample

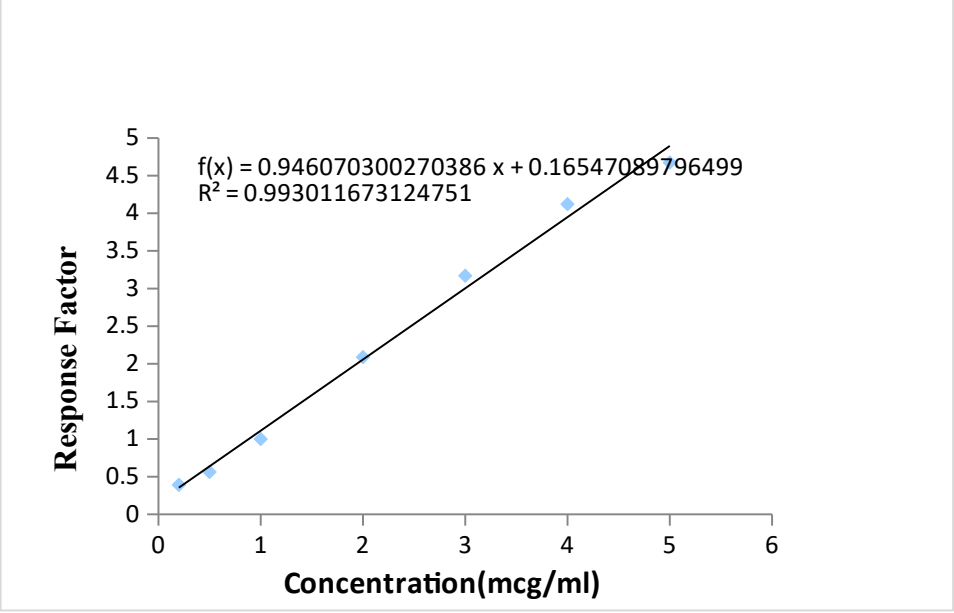

Figure 6: Calibration curve Diacerein in plasma sample