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Analytical Method Development and Validation of Simultaneous Estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane By RP-HPLC In Pharmaceutical Tablet Dosage Forms

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ABSTRACT: *A simple, Precise, Rapid, Specific and accurate reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane in drug product. A chromatographic method was scrutinized on Ascentis express C18 (100×4.6mm, 2.7µ) column and a mobile phase consists of Acetonitrile: Potassium dihydrogen orthophosphate and one drop of triethyl amine in every 100ml of buffer solution pH :3.0 (50:50%v/v). The flow rate was 1.0 ml/min with UV detection at 210nm. The retention time (R_t) of Diacerein, Glucosamine and Methyl Sulfonyl Methane were found to be 2.586, 3.182 and 4.469min respectively. As per ICH guidelines the developed method was validated in terms of specificity, Linearity, Accuracy, Precision, LOD, LOQ and robustness. All the parameters were found to be with in the limit. The linearity for Diacerein was in the range of 2.5 to 15 ppm, Glucosamine was in the range of 37.5 to 225 ppm and Methyl Sulfonyl Methane was in the range of 12.5 to 75 ppm. HPLC method was simple, Accurate, Precise and suitable for analysis of marketed tablet formulation containing Diacerein, Glucosamine and Methyl Sulfonyl Methane.*

Keywords: *Diacerein, Glucosamine, Methyl Sulfonyl Methane,RP-HPLC, Validation.*

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I. Introduction

Diacerein (Fig.1) is also known as diacetyl Rhein, is a slow acting medicine of the class anthraquinone used to treat joint diseases, such as Osteoarthritis. It works by inhibiting interleukin-1 beta. Diacerein works by blocking the actions of interleukin-1 beta a protein involved in the inflammation and destruction of cartilage that play a role in the development of symptoms of degenerative joint diseases. The IUPAC name of Diacerein is 4,5- diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid. Molecular formula $C_{19}H_{12}O_8$ andmolecular weight 368.294 g/mol.

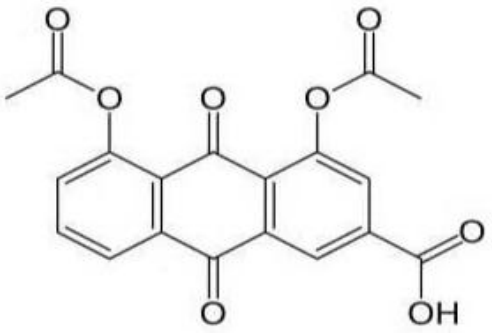

Fig. 1. Structure of Diacerein

Glucosamine (Fig.2) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine works to stimulate joint function and repair. It has been proven effecting in numerous scientific trails for easing osteoarthritis pain, aiding in the rehabilitation of cartilage, renewing synovial fluid and repairing joints that have been damaged from osteoarthritis. The IUPAC name of Glucosamine is (3R,4R,5S)-3-amino-6-(hydroxymethyl) oxane-2,4,5-triol. Molecular formula $C_6H_{13}NO_5$ and molecular weight 179.172 g/mol.

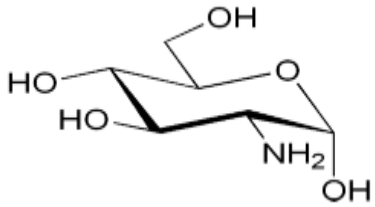

Fig. 2. Structure of Glucosamine

Methyl Sulfonyl Methane (Fig.3) is an organosulfur compound with the formula $(CH_3)_2SO_2$. MSM is used for the treatment on both animals and humans. This is used particularly for treatment of oxidative stress and osteoarthritis. It is used to protect muscles from damage by reducing the amount of oxidative stress damage incurred through exercise. The IUPAC name of methyl sulfonyl methane is dimethyl sulfone, Sulfonyl bis methane. Molecular formula $C_2H_6O_2S$ and molecular weight 94.13 g/mol.

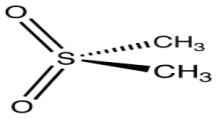

Fig. 3. Structure of Methyl Sulfonyl Methane

From the literature survey it was concluded that only a few methods are available. For individual estimation of each drug, several methods are available in the literature. And also, several methods are available for the estimation of two drugs at a time. But there is no method is available for the estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane in pharmaceutical dosage forms.

II. Materials and methods

Reagents and Chemicals:

The pharmaceutical drug samples of Diacerein, Glucosamine and Methyl sulfonyl methane were obtained from Geno pharma Pvt. Ltd., Goa. All the chemicals and solvents were used as HPLC grade. The pharmaceutical dosage form Ostovit DM was purchased from local pharmacy.

Instrumentation:

HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Ascentis C_{18} (100×4.6mm,2.7µ) column. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions:

TheAscentis C_{18} (100×4.6mm,2.7µ) column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of buffer solution (pH3.0) and Acetonitrile was taken in the ratio of (50:50%v/v) mobile phase for the investigation with a flow rate of a 1.0ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 200nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0):

Accurately weighed 1.36 gms of potassium dihydrogen ortho phosphate in a 1000 ml of volumetric flask and add about 900 ml of milli-Q water and degas to sonicate and finally make up to the volume with water. Then added 1ml of triethyl amine and pH was adjusted to 3.0 with dilute orthophosphoric acid solution.

Preparation of Mobile Phase:

Mixture of above buffer solution 500 ml and 500 ml of Acetonitrile were mixed (50:50 v/v) and degassed in ultrasonic water bath for 10 min and filtered through 0.45µ filter paper under vacuum filtration.

Diluent Preparation:

The diluent was optimized as mixture of water and acetonitrile (50: 50 v/v).

Preparation of the standard solution:

Accurately weighed and transferred 5 mg, 75 mg and 25 mg of Diacerein, Glucosamine and Methyl Sulfonyl Methane working standards were taken in to a 50 ml clean dry volumetric flask and added 25 ml of diluent. Sonicated for 30 min andmade up to mark with the diluent. From this solutions 1 ml was pipetted out in to a 10 ml volumetric flask and then made up to the mark with the diluent to get a mixed standard solution. The concentration of 10 ppm for Diacerein, 150 ppm for Glucosamine and 50 ppm for Methyl Sulfonyl Methane were achieved respectively.

Preparation of the sample solution:

20 tablets were accurately weighed and powdered in glass mortar. Then the weight equivalent to one tablet (Diacerein 50 mg, Glucosamine 750 mg and Methyl Sulfonyl Methane 250 mg) was transferred in to a 500 ml clean dry volumetric flask. For this 60 ml of diluent was added to it and was shaken by mechanical stirrer, sonicated for 30 min by shaking at intervals of 5 min each and was made up to the final volume with diluent and allowed to stand. Further 1 ml of solution was pipette out in to a 10 ml volumetric flask and made up to the volume with diluent and the solution was filtered through 0.45µm filter before injection in to HPLC system.

III. Results and Discussion

Method Development:

Initially, the method trails were carried out with volatile and phosphate buffers, methanol and acetonitrile by using isocratic and gradient mode. The method was analyzed with different stationary phases like C18, Amino, C4, C8 and cayano. In addition, the mobile phase such as ammonium acetate, formic acid, acetic acid and phosphate buffers respectively. Finally, the chromatographic separation was achieved on a Ascentis C_{18} (100×4.6mm,2.7µ) column, solution-A wasPotassium dihydrogen orthophosphate and one drop of triethyl amine in every 100 ml of buffer solution pH :3.0 with ortho phosphoric acid and solution-B was acetonitrile (solution-A: solution-B, 50:50 v/v). The flow rate was 1.0 ml/min, column oven temperature was 30^0c andthe injection volume was 10µl. The total run time of analysis was less than 10 minutes.

Method Validation:

Specificity:

The developed method was checked specificity by injecting blank, standard and sample solutions. There was no interference of the diluent at the retention time of Diacerein, Glucosamine and Methyl Sulfonyl Methane.The retention time of Diacerein, Glucosamine and Methyl Sulfonyl Methane were found to be 2.586, 3.182 and 4.469 minutes, respectively. Specificity chromatograms shown in fig. 4 to 6.

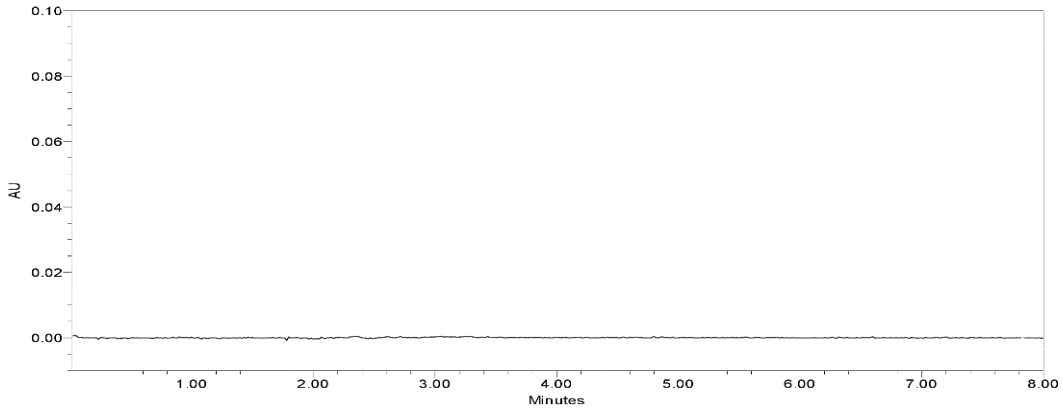

Fig. 4: Blank chromatogram for specificity.

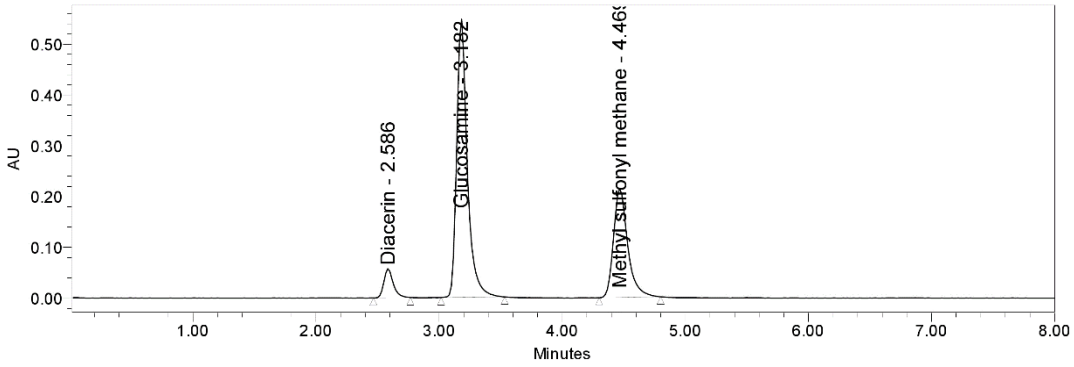

Fig. 5: Standardchromatogram for specificity.

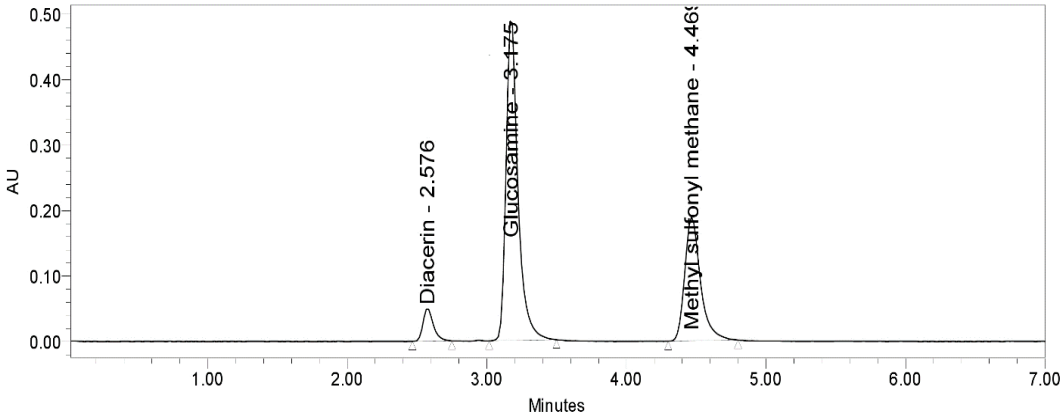

Fig. 6: Sample (marketed formulation)chromatogram for specificity.

System suitability:

The system suitability of the developed method where theoretical plates, tailing factor and resolution were within the acceptance criteria, which was shown in table-1.

Table. 1. System suitability parameters.

Name of the drug	Retention time (min.)	Area	USP Resolution	USP tailing	USP plate count
Diacerein	2.586	297746	---	1.30	6028
Glucosamine	3.182	3312177	4.1	1.44	7681
Methyl Sulfonyl Methane	4.469	1710221	7.2	1.36	8090

Linearity:

The calibration curve was constructed by plotting concentration vs peak area. It was found that there exists a linear relationship in the concentration range of 2.5 to 15 ppm for Diacerein with 0.9997 as the value of correlation coefficient, for Glucosamine the linearity in the range of 35.5 to 225 ppm with 0.9999 as the value of correlation coefficient and for Methyl Sulfonyl Methane the linearity in the range of 12.5 to 75 ppm with 0.9998 as the value of correlation coefficient.The linearity values were shown in table-2 and calibration curve shown in Fig. 7 to 9.

Table-2: Linearity study of Diacerein, Glucosamine and Methyl Sulfonyl Methane.

Diacerein				Glucosamine				Methyl Sulfonyl Methane			
Linearity range (ppm)	Correlation co-efficient	Slope	Y-intercept	Linearity range (ppm)	Correlation co-efficient	Slope	Y-intercept	Linearity range (ppm)	Correlation co-efficient	Slope	Y-intercept
2.5	0.9997	28958	476	37.5	0.9999	21702	1528	12.5	0.9998	34190	3439
5				75				25			
7.5				112.5				37.5			
10				150				50			
12.5				187.5				62.6			
15				225				75			

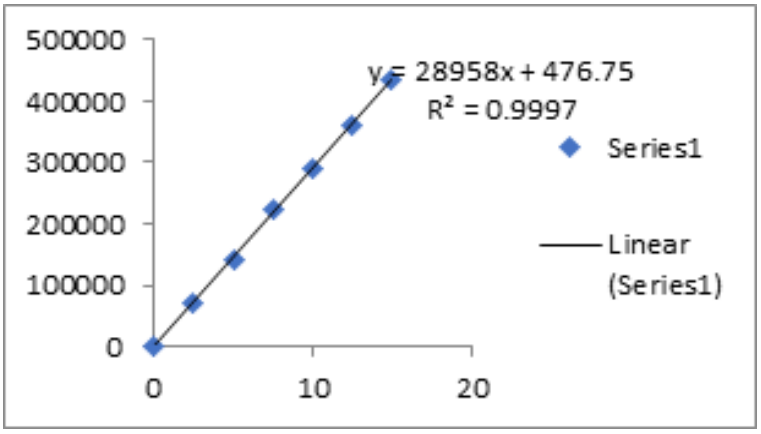

Fig. 7: Linearity plot forDiacerein

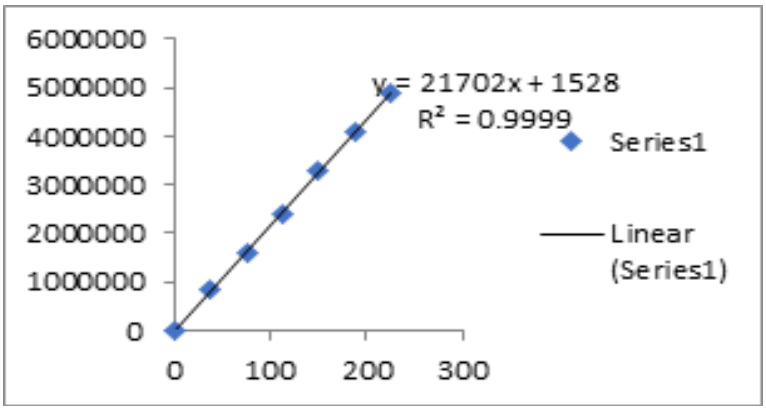

Fig. 8: Linearity plot forGlucosamine

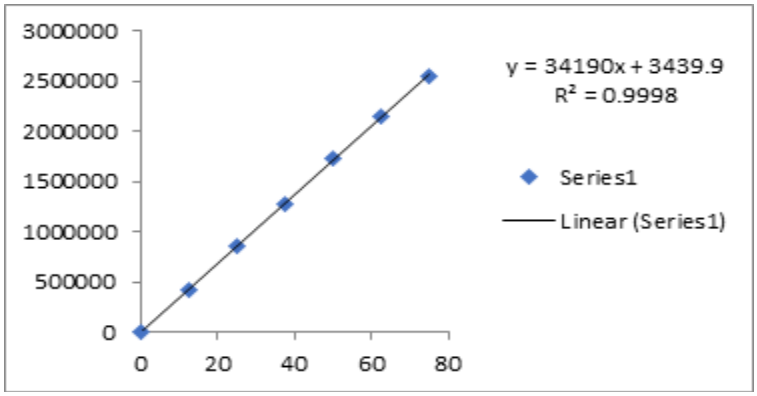

Fig. 9: Linearity plot forMethyl Sulfonyl Methane

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the linearity curve method using slope and standard deviation of intercepts of calibration curve. LOD and LOQ values were shown in table-3.

Table-3:LOD and LOQ values for Diacerein, Glucosamine and Methyl Sulfonyl Methane.

Drug name	LOD (µg/ml)	LOQ (µg/ml)
Diacerein	0.02	0.05
Glucosamine	0.01	0.04
Methyl Sulfonyl Methane	0.02	0.07

Precision:

In the system precision study %RSD was found to be less than 2%,for Diacerein 0.9%, Glucosamine 0.9% and Methyl Sulfonyl Methane 0.5%. Each system precision indicates that the system has good reproducibility. In the method precision study %RSD was found to be less than 1 %,for Diacerein 0.8%,

Glucosamine 0.8% and methyl Sulfonyl Methane 0.4 %. Which indicates that the method has good reproducibility. In the intermediate system precision study % RSD was found to be less than 2%,for Diacerein 1.8%, Glucosamine 1.5% and Methyl Sulfonyl Methane 1.7%. Which indicates that the system has good reproducibility.In the intermediateprecision study %RSD was found to be less than 1 %,for Diacerein 0.6 %, Glucosamine 0.5 % and methyl Sulfonyl Methane 0.3 %. Precision study values were shown in table-4.

Table-4: Precision study forDiacerein (Dia), Glucosamine(Glu) and Methyl Sulfonyl Methane (MSM)

Injection no.	System precision			Method precision			Intermediate precision		
	Dia	Glu	MSM	Dia	Glu	MSM	Dia	Glu	MSM
1	289739	3259151	1721646	294740	3263394	1727386	272498	2501584	1520279
2	290708	3273485	1726634	292242	3279296	1720728	280478	2576838	1523322
3	291909	3328163	1728402	297746	3312177	1710221	269941	2507248	1569306
4	294709	3320710	1725667	291912	3323249	1723583	280777	2570352	1571558
5	290373	3271567	1716422	291974	3286801	1731052	271483	2514215	1521808
6	296472	3258938	1703180	294550	3262350	1727686	279342	2584602	1566951
Average	292318	3285336	1720325	293861	3287878	1723443	275753	2542473	1545537
S. D.	2689.5	30977.4	9428.8	2294.4	25169.4	7398.1	4960.9	38585.7	26058.2
% RSD	0.9	0.9	0.5	0.8	0.8	0.4	1.8	1.5	1.7

Accuracy:

The recovery studies by the standard addition method were performed and recovery study performed for Diacerein, Glucosamine and Methyl Sulfonyl Methane at 50%, 100% and 150% level. The % recovery found for Diacerein 99.8%, Glucosamine 100.3% and methyl sulfonyl Methane 99.6% and recovery values shown in table-5. The results which indicates that the method was accurate.

Table-5: Accuracy study for Diacerein, Glucosamine and Methyl Sulfonyl Methane

Drug name	Concentration (%)	Amount added (ppm)	Amount found (ppm)	Recovery (%)	Mean Recovery (%)
Diacerein	50	5	5.02	99.6	99.8
		5	4.96		
		5	4.96		
	100	10	10.11	100.4	
		10	10.02		
		10	10.02		
	150	15	14.92	99.5	
		15	14.97		
		15	14.91		
Glucosamine	50	75	75.43	100.3	100.3
		75	74.8		
		75	75.4		
	100	150	150.7	100.5	
		150	149.5		
		150	152.0		
	150	225	225.8	100.2	
		225	225.8		
		225	224.9		
Methyl Sulfonyl Methane	50	25	24.81	99.5	99.6
		25	25.05		
		25	24.78		
	100	50	49.65	99.5	
		50	49.94		
		50	49.63		
	150	75	74.59	99.7	
		75	75.23		
		75	74.48		

Robustness:

The robustness study evaluated for newly developed method, the small deliberate changes in developed method such as flow rates (±0.2), change in mobile phase composition by changing the organic ratio by 10% and change in temperature (±2) and all the studies were within the acceptance limits and indicates that the method was robust.The results were shown in table-6-8.

Table-6: Robustness study for changes in flow rate

S.No.	Change in flow rate (ml/min)	Diacerein			Glucosamine			Methyl Sulfonyl Methane		
		USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution
1.	Less	5678	1.33	---	7421	1.49	4.9	7867	1.42	8.1
2.	*Actual	6028	1.30	---	7681	1.44	4.1	8090	1.36	7.2
3.	More	6291	1.24	---	7987	1.39	3.4	8349	1.31	6.3

Table-7: Robustness study for changes in mobile phase

S.No.	Change in organic mobile phase	Diacerein			Glucosamine			Methyl Sulfonyl Methane		
		USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution
1.	Less	5867	1.32	---	7511	1.48	4.3	7910	1.37	8.1
2.	*Actual	6028	1.30	---	7681	1.44	4.1	8090	1.36	7.2
3.	More	6361	1.27	---	7877	1.41	3.9	8246	1.33	6.3

Table-8: Robustness study for changes in temperature

S.No.	Change in temperature (^{0}C)	Diacerein			Glucosamine			Methyl Sulfonyl Methane		
		USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution	USP plate count	USP tailing	Resolution
1.	Less	6198	1.28	---	7912	1.41	3.9	8298	1.39	6.9
2.	*Actual	6028	1.30	---	7681	1.44	4.1	8090	1.36	7.2
3.	More	5879	1.33	---	7496	1.47	4.3	7897	1.33	7.9

IV. Conclusion

The newly developed isocratic RP-HPLC method was found to simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane in drug product (combined tablet dosage form). The method was validated as per ICH guidelines. The sample recovery in the formulation was good in agreement with respect their label claims and this is suggested no-interference excipients in the estimation. Hence, this method can be easily adopted for routine quality control and stability analysis for estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane in active pharmaceuticalingredient and drug product (Combined tablet dosage form).

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