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RP-HPLC method development and validation for the simultaneous estimation of glucosamine, methyl Sulfonyl methane and Diacerein in tablet Dosage form

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Abstract
A simple, accurate, precise method was developed for the simultaneous estimation of the Glucosamine Sulphate (GLU), Methyl Sulfonyl Methane (MET) and Diacerein (DIA) in solid dosage form. Chromatogram was run through Symmetry C18 150x4.6mm, 5µ. Mobile phase containing 0.01N KH_2PO_4 buffer (pH-3.5) and Acetonitrile in the ratio of 60:40 v/v was pumped through column at a flow rate of 0.7ml/min. Temperature was maintained at 30 ℃. Optimized wavelength for Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein was found to be 210.0 nm. Retention time of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein were found to be 2.121 min, 2.609 min and 3.576 min respectively. % RSD of system precision for Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein. Were and found to be 0.6, 0.4 and 0.8 respectively. % RSD of method precision for Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein. Were and found to be 0.2, 0.3 and 0.6 respectively. % recovery was obtained as 100.01%, 100.10% and 100.59% for Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein Respectively. LOD, LOQ values are obtained from regression equations of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein were 2.23 µg, 0.51 µg, 0.04 µg and 6.77 µg, 1.54 µg, 0.13 µg respectively. Regression equation of Glucosamine Sulphate was y = 11709x – 11390, Methyl Sulfonyl Methane was y = 3178x – 6144 and of Diacerein was y = 10040x - 1209.6.

Keywords: Glucosamine sulphate, methyl Sulfonyl methane, Diacerein, RP-HPLC

Introduction
Chemically Glucosamine (GLU) was an (3R, 4R, 5S, 6R)-3-amino-6-(hydroxymethyl) oxane-2, 4, 5-triol. Molecular weight and molecular formula of GLU were 179.172 g/ mole and $C_6H_{13}NO_5$ respectively. Glucosamine is a precursor of glycosylated proteins and lipids. Oral glucosamine is commonly used for the treatment of osteoarthritis. Since glucosamine is a precursor for Glycosaminoglycans, and Glycosaminoglycans are a major component of joint cartilage, supplemental glucosamine may help to rebuild cartilage and treat arthritis. Its use as a therapy for osteoarthritis. Structure of the GLU was shown in figure 1 (B) [1].
Chemically Methyl Sulfonyl Methane (MET) was methane sulfonyl methane or Dimethyl sulfone Methyl sulfone. Molecular weight and molecular formula of MSM were 94.13 g/mole and $C_2H_6O_2S$ respectively. MET is an organic sulfur compound belonging to a class of chemicals known as sulfones. Structure of the MET was shown in figure 1 (C) [2].
Chemically Diacerein (DIA) was an 4, 5-bis (Acetyloxy)-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid. Molecular weight and molecular formula of DIA were 368.294 g/mole and $C_{19}H_{12}O_8$ respectively. Diacerein is a prodrug which is metabolized to rhein. Diacerein active metabolite rhein, Rhein reduces cartilage destruction by decreasing expression of matrix metalloproteinase (MMP)-1 and -3 as well as up regulating tissue inhibitor of matrix metalloproteinase which serve to reduce the activity of several MMPs. The anti-inflammatory action of rhein reduces the level of interleukin-1beta activity which plays a large role in reduction of extracellular matrix production, MMP activity, and continued inflammation. Rhein reduces abnormal osteoblast synthetic activity through an unknown mechanism. Structure of the DIA was shown in figure 1 (A) [3, 4].

Literature survey reveals there are several methods to estimated thee drugs in single or in combination of two drugs [5-9]. but there is only very few HPLC methods are available for simultaneous estimation of GLU, MET and DIA, so the scope of developing and alidating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

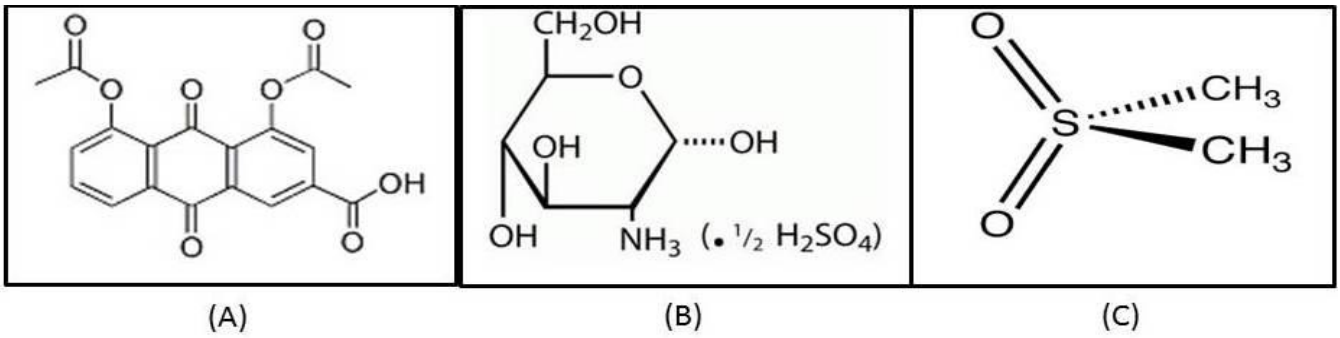

Fig 1: Structure of (A) Diacerein (B) Glucosamine (C) Methyl Sulfonyl Methane

Materials and Methods

Reagents and Chemicals

The active pharmaceutical ingredient samples of Glucosamine, Methyl sulfonyl methane and Diacerein were obtained from Spectra Pharma Pvt. Ltd., Hyderabad. All the chemicals and solvents used were HPLC grade. The tablet pharmaceutical dosage of combination of these drugs was purchased from local pharmacy.

Instrumentation

Waters HPLC (2695 series) with quaternary pumps, Photo Diode array detector and auto sampler integrated with empower software-2 was used for separation of these drugs.

Chromatographic conditions

The Symmetry C18 150 x 4.6mm, 5m.) 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 (60:40%v/v) mobile phase for the investigation with a flow rate of a 0.7ml/min. The temperature was maintained at 30^0 C. The injection volume was 10µl and the UV detection was achieved at 210nm.

Preparation of potassium Dihydrogen Ortho phosphate buffer (pH:3.0)

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Mixture of 600 ml of 0.01N KH_2PO_4 buffer (pH-3.5) and 400 ml of Acetonitrile in the ration of 60:40 v/v were mixed and degased in ultrasonic water bath for 15 minutes and filtered through 0.45 µ filter paper. Mobile phase was used as a diluent.

Preparation of mixture Standard stock solution (GLU 750 mg/ml, MET 250 mg/ml and DIA 50 mg/ml)

Accurately weighed 187.5mg of GLU, 62.5 mg of MET and 12.5mg of DIA and transferred into three 25ml volumetric flasks separately. 10ml of Diluent was added to each flask and sonicated for 20 mins. Each flask was made up with diluent up to the mark. Pipette out 1ml from each stock solution taken into a 10ml volumetric flask and made up with diluent. The concentrations of 750 mg/ml for GLU, 250 mg/ml for MET and 50 mg/ml were achieved respectively.

Preparation of Sample (Tablet) stock solutions

20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet (GLU 750 mg, MET 250 mg & DIA 50mg) was transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents. (GLU 750 mg/ml, MET 250 mg/ml and DIA 50 mg/ml).

Optimized chromatographic conditions

Column:	Symmetry C18 (150 x 4.6 mm, 5µ.)
Mobile phase:	0.01 N KH_2PO_4 buffer (pH-3.0): Acetonitrile (60:40 v/v)
Flow rate:	0.7 ml/min
Wavelength:	210.0 nm
Temperature:	30 ^{0}C
Injection Volume:	10.0µl

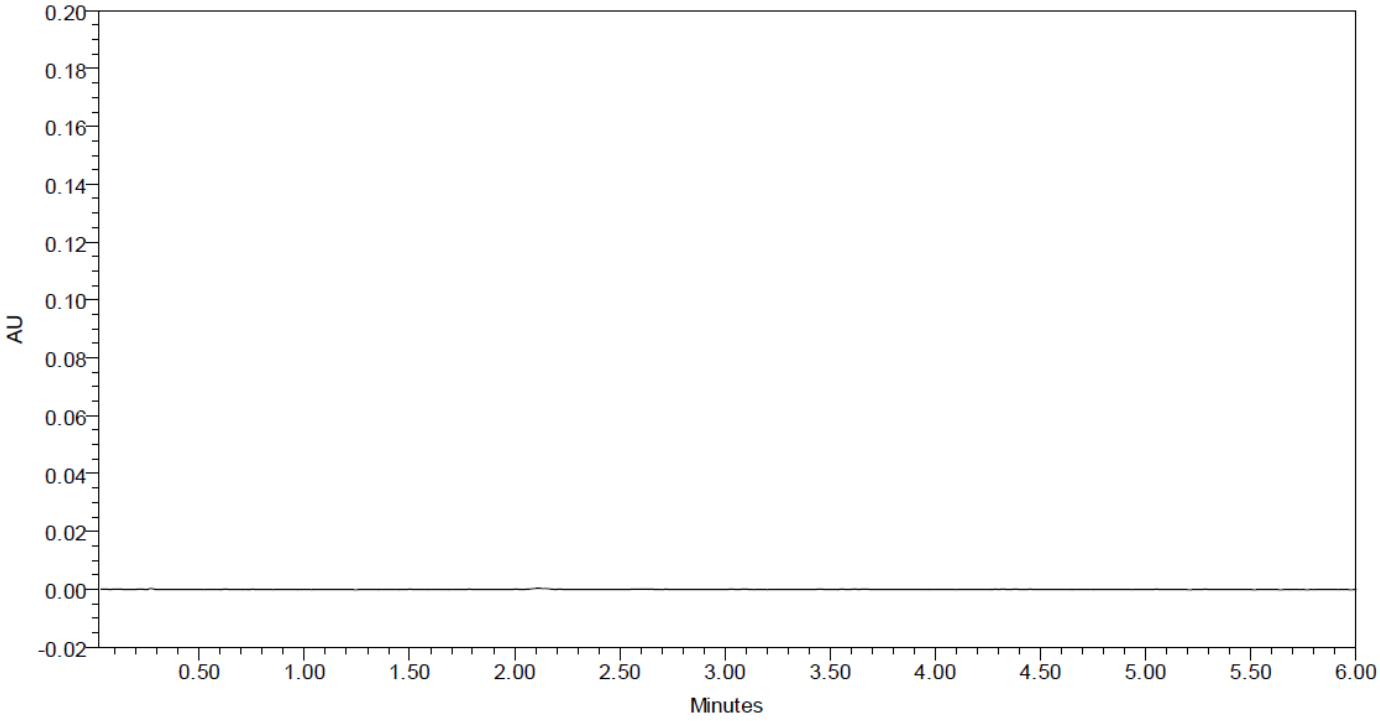

Fig 2: Blank Chromatogram

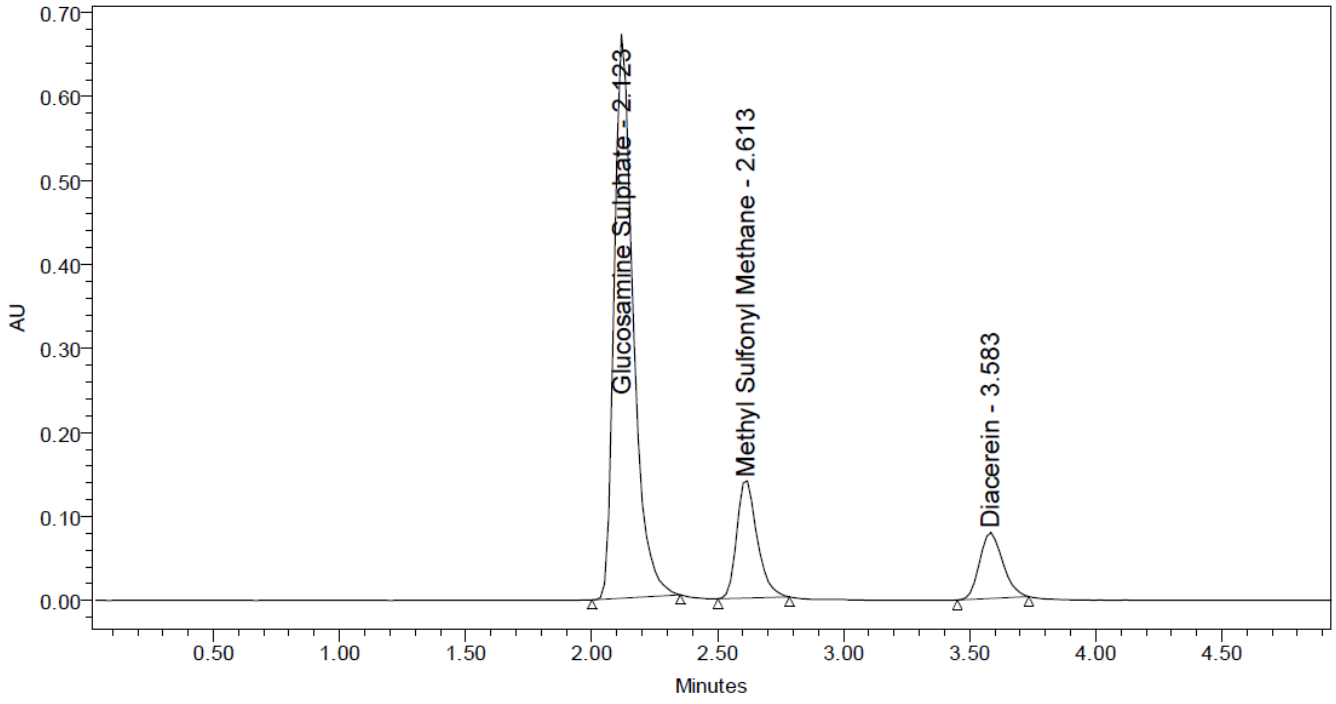

Fig 3: Chromatogram of standard mixture of GLU, MET & DIA

	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Glucosamine Sulphate	2.123	3504362	3718.1		1.4
2	Methyl Sulfonyl Methane	2.613	788719	5319.4	3.4	1.3
3	Diacerein	3.583	499061	6906.8	5.9	1.1

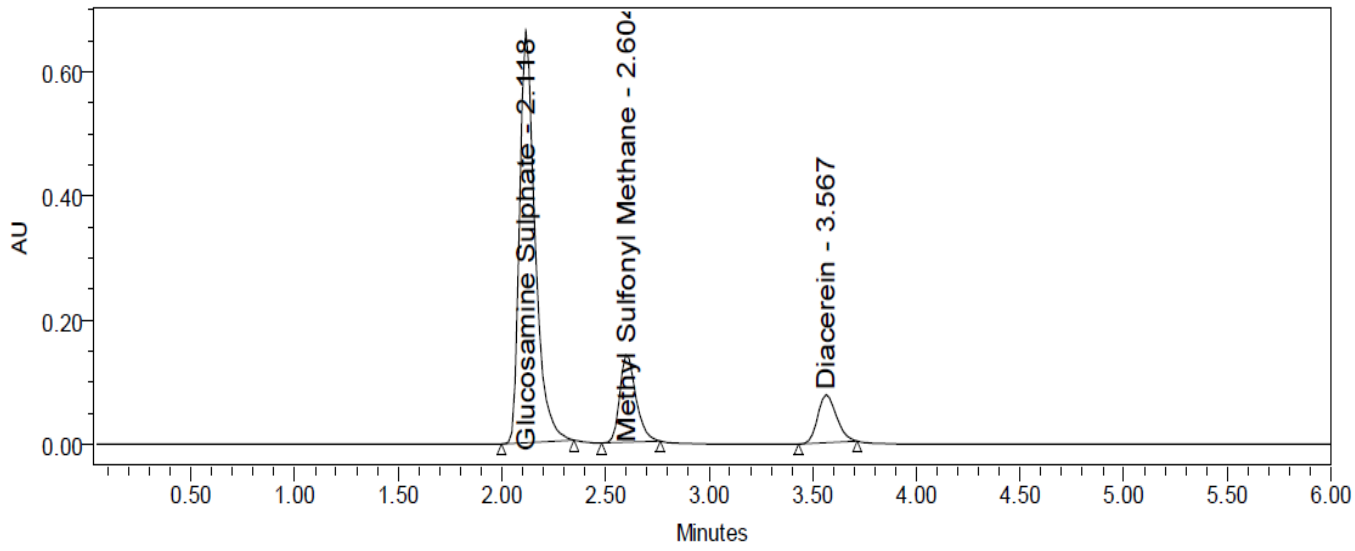

Fig 4: Chromatogram of sample mixture of GLU, MET & DIA

Validation

The above optimized chromatographic method has been validated for the assay of GLU, MET and DIA using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of GLU, MET and DIA drug mixtures (187.5-937.5 µg/ml of GLU, 625-375 µg/ml of MET and 12.5-75 µg/ml of DIA respectively). Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of GLU, MET and DIA 750 µg/ml, 250 µg/ml and 50 µg/ml respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of drug (of GLU, MET and DIA750 µg/ml, 250 µg/ml and 50 µg/ml respectively). The % RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of GLU, MET & DIA. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

Result & Discussion

The regression equation for GLU was found to be y = 11709x – 11390 (slope, intercept and correlation coefficient were found to be 11709, -11390 and 0.999 respectively) and linear over beer's range of 187.5-937.5 µg/ml. The regression equation for MET was found to be y = 3178x – 6144 (slope, intercept and correlation coefficient were found to be 3178, -6144 and 0.999 respectively) and linear over beer's range of 62.5-375 µg/ml. The regression equation for DIA was found to be y = 10040x - 1209.6 (slope, intercept and correlation coefficient were found to be 10040, 1209.6 and 0.999 respectively) and linear over beer's range of 12.5- 75 µg/ml. Linearity graph of GLU, MET & DIA were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The percentage of content of GLU, MET and DIA in tablet dosage form was 100.01±0.157 %, 100.10±0.29 % and 100.59±0.65 % respectively. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. The % RSD of precision and ruggedness of GLU were found to be 0.6 and 0.8 respectively; for MET were 0.4 and 0.7 and for DIA 0.8 & 1.0 respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug GLU, MET and DIA and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.99±0.73, 99.86±1.31 and 99.49±0.36%w/w for 50%, 100% and 150% respectively for GLU. The mean percentage recoveries were found to be 100.05±0.32, 99.20±0.17 and 99.82±1.02%w/w for 50%, 100% and 150% respectively for MET. The mean percentage recoveries were found to be 99.82±0.51, 100.33±0.76%w/w and 99.89±0.63 for 50%, 100% and 150% respectively for DIA. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for GLU, MET and DIA was found to be 2.23 µg, 0.51µg and 0.04 µg respectively. LOQ for GLU, MET and DIA was found to be 6.77 µg, 1.54 µg and 0.13 µg respectively. Summary of all the validation parameter shown in table 4.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Degradation data shown in table 3.

Conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein in Tablet dosage form was developed and the proposed method as suitable for routine analysis of GLU, MET and DIA.

Table 1: Linearity data of standard mixture of GLU, MET and DIA

S. NO	Glucosamine		Methyl Sulfonyl Methane		Diacerein	
	Conc. (µg/ml)	Mean peak area	Conc. (µg/ml)	Mean peak area	Conc. (µg/ml)	Mean peak area
1	187.5	875675	62.5	190446	12.5	120492
2	375	1722965	125	387018	25	250469
3	562.5	2646085	187.5	600555	37.5	380417
4	750	3486327	250	787160	50	503755
5	937.5	4384993	312.5	983512	62.5	621364
Slope		4683.732		3178.038		10040.24
Intercept		-11390.4		-6144		-1209.6
Correlation coefficient		0.999		0.999		0.999

Table 2: System precision data of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein

S. No	Peak Area of Glucosamine Sulphate	Peak Area of Methyl Sulfonyl Methane	Peak Area of Diacerein
1.	3514285	784923	509311
2.	3489254	787111	508837
3.	3498289	784144	512737
4.	3484689	790222	500724
5.	3535053	788921	509624
6.	3524816	781512	506089
Mean	3506420	786139	507887
S.D	20213.0	3231.8	4099.1
% RSD	0.6	0.4	0.8

Table 3: Degradation Data of GLU, MET and DIA

S. NO	Degradation Condition	Glucosamine			Methyl Sulfoyl methane			Diacerein		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	9.53	0.258	0.429	6.07	0.236	0.855	7.86	0.27	0.324
2	Alkali	6.49	0.254	0.434	4.26	0.261	0.858	5.97	0.265	0.328
3	Oxidation	3.38	0.207	0.379	3.81	0.788	1.072	3.56	0.247	0.319
4	Thermal	2.64	0.24	0.412	2.17	0.218	0.895	2.4	0.25	0.326
5	UV	1.74	0.236	0.239	1.16	0.357	0.841	1.96	0.252	0.327
6	Water	0.68	0.239	0.431	0.58	0.304	0.847	0.92	0.257	0.321

Table 4: Summary of validation data of GLU, MET and DIA

Validation	Parameters	Glucosamine Sulphate	Methyl Sulfonyl Methane	Diacerein
Linearity	**Range (µg/ml)**	187.5-1125µg/ml	62.5-375 µg/ml	12.5-75µg/ml
	Regression coefficient	0.999	0.999	0.999
	Slope(m)	4683	3178	10043
	Intercept(c)	-11390	-6144	-1209
Assay	Mean % content	100.01%	100.10%	100.59%
Specificity		Specific	Specific	Specific
System precision	% RSD	0.6	0.4	0.8
Method precision	% RSD	0.2	0.3	0.6
Accuracy % recovery	% Recovery	99.79%	99.69%	100.02%
LOD		2.2323µg	0.51µg	0.04µg
LOQ		6.77µg	1.54µg	0.13µg

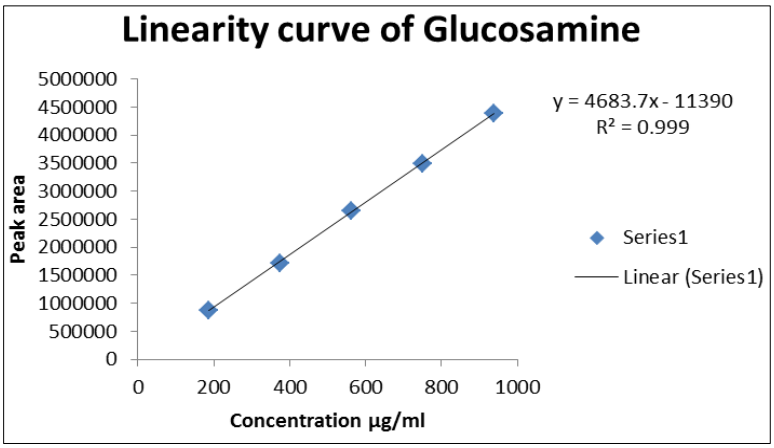

Fig 5: Linearity curve of Glucosamine

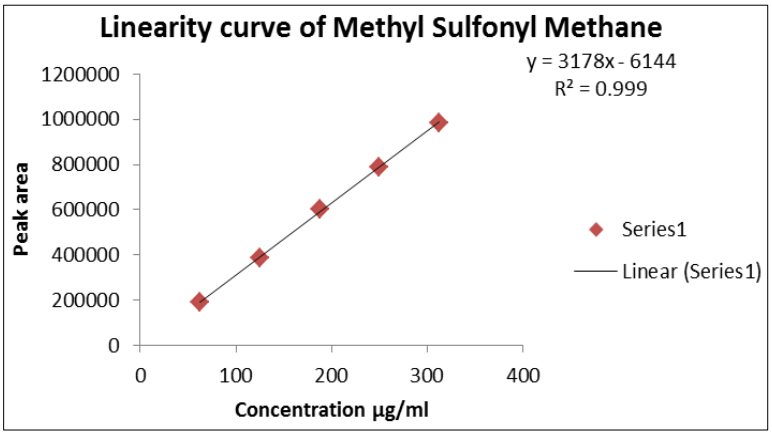

Fig 6: Linearity curve of Methyl Sulfonyl Methane

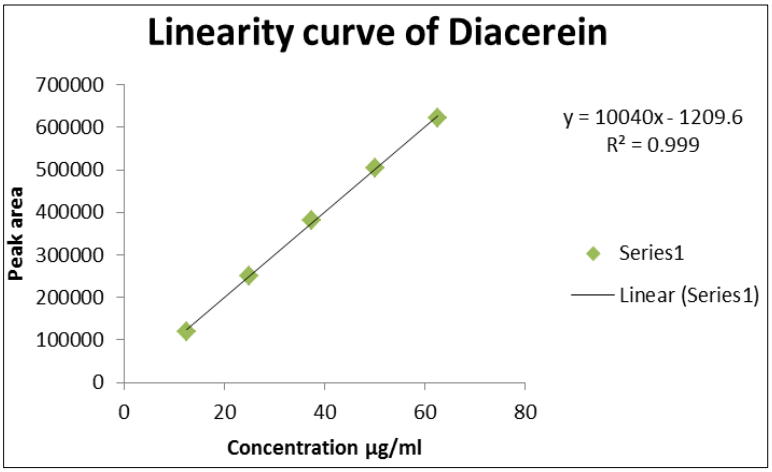

Fig 7: Linearity curve of Diacerein

References

1. Glucosamine monograph (Online) available on URL: https://www.drugbank.ca/drugs/DB01296
2. Methyl sulfonyl methane monograph (Online) available on URL: https://www.drugbank.ca/drugs/DB14090
3. Diacerein monograph (Online) available on URL: https://www.drugbank.ca/drugs/DB161994
4. Diacerein monograph (Online) available on URL: https://pubchem.ncbi.nlm.nih.gov/compound/Dimethyl-sulfone#section=13C-NMR-Spectra)
5. Useni Reddy Mallu, Hussain Reddy K, Varaprasad Bobbarala, Somasekhar Penumajji. HPLC Method Development for Glucosamine Sulphate and Diacerein Formulation, Journal of Pharmacy Research. 2010; 3(2):361-363.
6. Jatupon Kongtharvonskul, Thunyarat Anothaisintawee. Efficacy and safety of glucosamine, diacerein, and NSAIDs in osteoarthritis knee: a systematic review and network meta-analysis, European Journal of Medical Research, 2015, 24.
7. Praneeth Kumar A, Sunil Kumar Chaitanya P, Rohini Reddy G, Umadevi SK. Method development & validation for simultaneous estimation of diacerin and glucosamine sulphate by RP-HPLC in bulk and tablet dosage form, Indo American Journal of Pharmaceutical Research, 2014, 4(03).
8. Pulla Reddy S, Rambabu C. Simultaneous Determination of Glucosamine and Diacerein in Pharmaceutical Dosage Form by RP-HPLC, IJPPR International journal Pharmacy & Pharmaceutical Research, 2015, 2(2).
9. Sravanthi S, Padmalatha H, Ravi Kumar V. RP-HPLC method development and validation for simultaneous estimation of diacerein and glucosamine sulphate in tablet dosage form, world journal of pharmacy and pharmaceutical sciences. 2017; 6(12):1623-1641.
10. Sandya Rani J, Devanna N. Analytical Method Development and alidation of Simultaneous Estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane By RP-HPLC In Pharmaceutical Tablet Dosage Forms. IOSR Journal of Applied Chemistry. 2018; 2(2):47-54.