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Development and Validation of Stability Indicating RP-HPLC Method For the Simultaneous Estimation of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein in Tablet Dosage Form

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Glucosamine sulphate, Methyl sulfonyl methane, Diacerein, RP-HPLC, stability indicating and validation.

ABSTRACT: Purpose: The objective of the proposed method is to develop an economical, accurate, precise, sensitive, reproducible, selective and validated RP-HPLC for simultaneous estimation of Glucosamine sulphate (Glu), Methyl Sulfonyl Methane (MSM) and Diacerein (Dia) in combined dosage form. Method: Mobile phase containing water and acetonitrile in the ratio of 50:50v/v was pumped through the Zodiasil C_{18} 150×4.6mm,3.5µ column. The flow rate was 0.8ml/min and temperature was maintained at 30°C. The eluents were observed at a wavelength of 210nm using PDA detector. Results: The retention time of Glu, MSM, and Dia were found to be 2.323, 2.842 and 3.453 respectively. The percentage RSD of intraday precision were found to be 0.5 for Glu, 0.2 for MSM and 0.7 for Dia. The percentage RSD of interday precision was found to be 0.7 for Glu, 0.8 for MSM, and 0.9 for Dia. Percentage recovery was found to be 100.30% for Glu, 99.65% for MSM and 100.13% for and Dia. Conclusion: The developed method was simple, precise, accurate, linear, rapid, economical and degradation studies revealed that the method has the ability to separate degradation products in pharmaceutical dosage forms so it can be adopted in regular quality control test for the simultaneous estimation of Glu, MSM, and Dia in tablet dosage form.

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INTRODUCTION

Glucosamine sulphate (**Fig 1a**), chemically known as bis(3R,4R,5S)-3-amino-6-hydroxymethyl) oxane-2,4,5-triol is present in the shells of shellfish, animal bones and bone marrow [1-2]. It is a synthetic corticosteroid drug and used as a therapy for osteoarthritis.

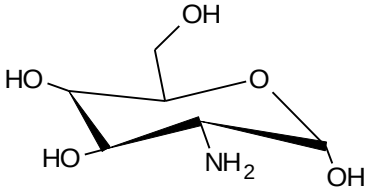
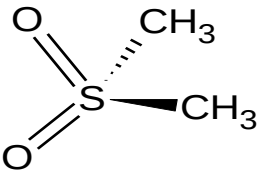


Fig. 1 a: Glucosamine



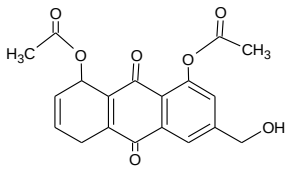


Fig. 1 b: Methyl sulfonyl methane c: Diacerein

Methyl Sulfonyl Methane (**Fig 1b**), is chemically known as dimethyl sulfone and sulfonyl methane, has been proven to have anti-inflammatory and antioxidant mechanisms in which human neutrophils are artificially stimulated to produce oxidative compounds, including hydrogen peroxide, superoxide, and hypochlorous acid [3-4].

Diacerein (**Fig 1c**), chemically known as 4,5- diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid also known as diacetyl rhein is a slow acting medicine of the class anthraquinone used to treat joint diseases such as osteoarthritis. Diacerein works by blocking the actions of interleukin-1 beta, a protein involved in inflammation and destruction of cartilage that play -a role in the development of symptoms of degenerative joint disease [5-6].

The literature survey revealed that few GC [8], RP-HPLC [9-17], UV [18-19] methods are available for individual estimation of Glu and Dia and in combination with other drugs. There is only a single RP-HPLC method available for simultaneous estimation of these three drugs in tablet dosage form. However, some of these methods have a few drawbacks like complexity in the composition of the mobile phase, higher retention time, higher amount of buffer that can affect column performance and lack of stability studies. Therefore an attempt was made to develop and validate a stability indicating RP-HPLC method for simultaneous estimation of Glucosamine sulfate, Methylsulfonylmethane, and Diacerein in tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

The standard drug samples of Glu, MSM, and Dia were obtained as gift samples from Spectrum Pharma Research Solutions Pvt. Ltd., Hyderabad. Pharmaceutical dosage form of combination TOVOK, manufactured by Intas Pharmaceuticals Ltd. was purchased from the local pharmacy store. Water and acetonitrile of HPLC grade, triethyl amine, potassium dihydrogen ortho phosphate and orthophosphoric acid of AR (Analytical Reagent) grade were obtained from Ranchem chemicals.

Instrumentation

Analysis was performed on Waters HPLC 2695 series system equipped with quaternary pumps, PDA detector, an autosampler and zodiasil C_{18} column (150×4.6mm, 3.5µm) compartment with Empower 2 software, UV double beam spectrophotometer (UVwin5, lab India), Ultrasonic bath (BVK enterprises, India) Digital weighing balance (Denver company TR-203) and nylon syringe filter (Millipore, India) were used.

Chromatographic Conditions

The chromatographic separation with good retention time was achieved on zodiasil C_{18}, 150×4.6mm, 3.5µm column. Mobile phase composed of water and acetonitrile in the ratio of 50:50 v/v with a flow rate of 0.8ml/min and run time of 6min. The detection of three drugs was observed at a wavelength of 210nm using PDA detector. The injection volume was 10µl and the temperature was maintained at 30°C.

Preparation of standard stock solution

An accurately weighed amount of 187.5mg of Glu, 62.5 mg of MSM and 12.5 mg of Dia was transferred into three 25ml volumetric flasks separately. 10ml of diluent was added and sonicated for 15 minutes. The final volume was made up to mark with diluent and labeled as standard stock solution 1, 2, and 3 respectively.

Preparation of standard working solution (100% solution)

From the above solution, serial dilutions were made with diluent to obtain a concentration of 750µg/ml of Glu, 250 µg/ml of MSM, and 50 µg/ml of Dia solution.

Preparation of sample stock solution

20 tablets were accurately weighed and powdered. The average weight of each tablet was calculated and weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask. 25ml of diluent was added and sonicated for 50 minutes and made up to final volume with diluent and filtered.

Preparation of sample working solutions (100% solution)

From the above filtered solution, serial dilutions were made with diluent to obtain a concentration of 750µg/ml of Glu, 250 µg/ml of MSM, and 50 µg/ml of Dia solution.

RESULTS AND DISCUSSION

Method Validation

System suitability parameters

Sample solution and six replicate injections were injected from freshly prepared standard solutions of Glu, MSM and Dia. Each solution was analyzed for their peak area, theoretical plates, resolution, and tailing factor. The optimized chromatogram was shown in **Fig. 2** and all the system suitability parameters were represented in **Table 1.**

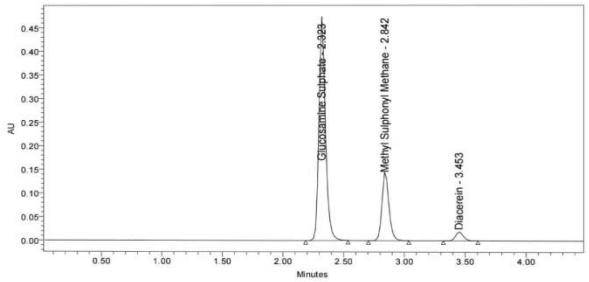


Fig. 2: Optimized chromatogram.

Specificity

The specificity of the method is the ability to identify the interference of excipients with that of analyte peaks. The results were shown in **Fig 3, 4, & 5**.

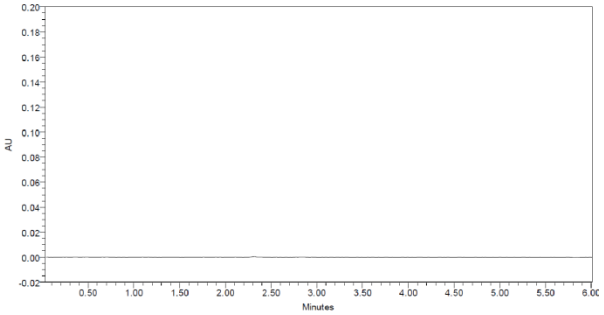


Fig. 3: Blank chromatogram for specificity.

Table 1: System Suitability Parameters

S.no	Glucosamine sulphate			Methyl sulfonyl methane			Diacerein		
Injection	***RT(min)**	****TP**	**Tailing**	**RT(min)**	**TP**	**Tailing**	**RT(min)**	**TP**	**Tailing**
1	2.320	6612	1.11	2.841	7760	1.04	3.447	9288	1.08
2	2.321	6651	1.09	2.842	7854	1.03	3.449	9010	1.03
3	2.321	6639	1.08	2.844	7927	1.02	3.450	9118	1.01
4	2.322	6147	1.08	2.847	7673	1.04	3.454	9645	1.08
5	2.323	6204	1.12	2.848	7907	1.03	3.453	9128	1.02
6	2.323	6366	1.12	2.850	7578	1.03	3.457	8531	1.00

*RT = Retention time, , **TP = Theoretical Plates.

Table 2: Accuracy results for Glu, MSM and Dia.

Drug Name	Concentration	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
Glucosamine sulphate	50	375	373.42	99.58	100.30%
		375	378.57	100.95	
		375	376.80	10048	
	100	750	751.81	100.24	
		750	749.12	99.88	
		750	752.61	100.35	
	150	1125	1133.04	100.71	
		1125	1120.7	99.62	
		1125	1134.77	100.87	
Methyl sulfonyl methane	50	125	124.31	99.45	100.30%
		125	126.14	100.91	
		125	126.15	100.92	
	100	250	250.63	100.25	
		250	250.69	100.28	
		250	249.77	99.91	
	150	375	377.11	100.56	
		375	373.68	99.65	
		375	372.25	99.27	
Diacerein	50	25	25.10	100.38	99.87%
		25	24.84	99.36	
		25	24.98	99.93	
	100	50	49.62	99.25	
		50	49.82	99.63	
		50	50.00	100.00	
	150	75	74.28	99.04	
		75	75.45	100.60	
		75	75.50	100.66	

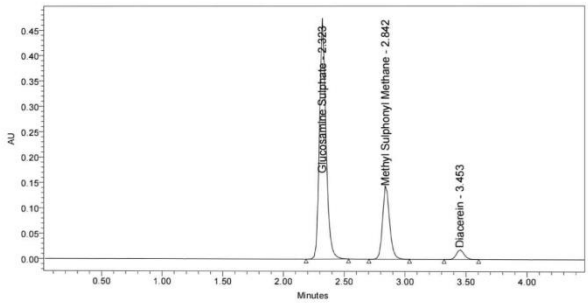


Fig 4: Standard chromatogram for specifity.

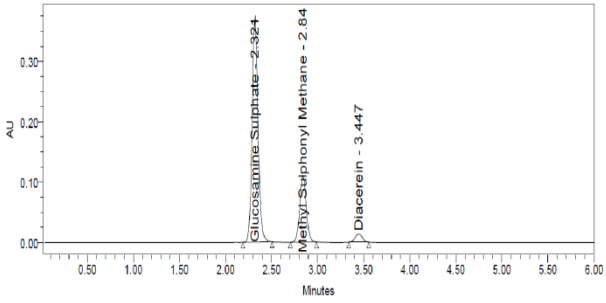


Fig. 5: Sample Chromatogram for Specificity

Accuracy

The accuracy of the method was determined by carrying out recovery studies. The recovery studies were performed at three concentration levels, i.e. 50%, 100%, and 150%. Each concentration level was repeated for three times. The percentage recovery and standard deviation were calculated. The accuracy results were represented in **Table 2.**

Precision

Precision was performed by repeatability (intraday) and intermediate precision (Interday) studies. Repeatability was studied by preparing a minimum of six determinations of 100% of the test concentration and analyzed as per method. Intermediate precision of the method was performed by carrying out six independent assays of test sample preparation on different days under the same experimental conditions and the percentage RSD of assays were calculated.

Linearity

Six linear concentrations of Glu (187.5-1125µg/ml), MSM (62.5-375µg/ml) and Dia (12.5-75µg/ml) were injected in a triplicate. Regression equations obtained for Glu was y = 4561.x + 5792, MSM was y = 4653.x + 30459 and Dia was y = 2935.x + 526.7. Correlation coefficients of three drugs were found to be 0.999.The linearity plots were shown in **Fig. 6, 7, & 8**.

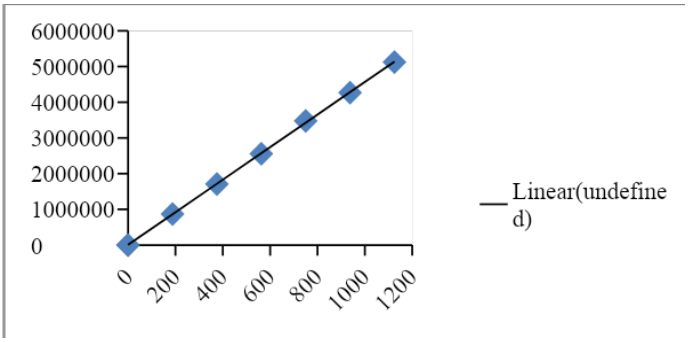


Fig. 6: Linearity plot of Glu

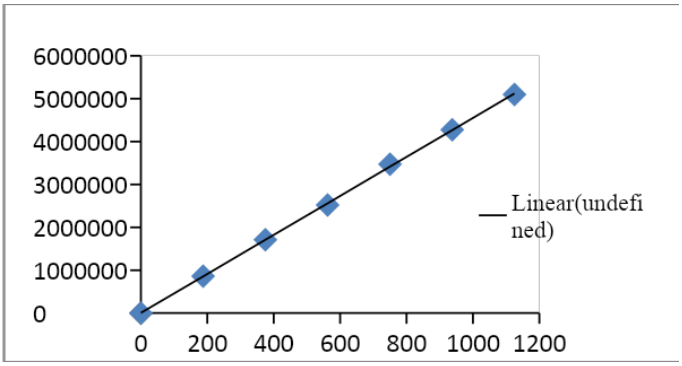


Fig. 7: Linearity plot of MSM.

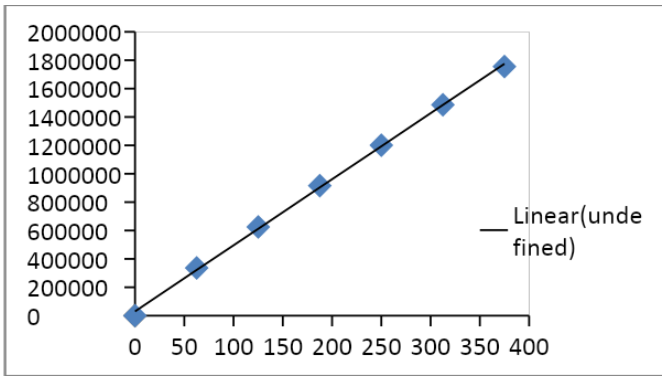


Fig. 8: Linearity plot of Dia.

Limit of Detection and Limit of Quantification (LOD&LOQ)

To determine LOD and LOQ series of solutions were injected, and the signal-to-noise ratio for each injection was calculated.

Robustness

Robustness of the method was determined by making small deliberate changes in the chromatographic conditions like change in flow rate ±0.1ml/min (0.7 and 0.9 ml/min), mobile phase±5% (45A:55B and 55A:45B) and temperature variation ±5°C (25°C and 35°C). The samples were injected in duplicate manner and percentage RSD was calculated.

Forced Degradation Studies

The stability studies were determined by applying the physical stress like acid, base, peroxide, thermal, water and light to the product. Results of forced degradation studies were represented in **Table 3.**

Acid degradation

Acid degradation studies were determined by mixing 1ml of standard stock solution with 1ml of 2N HCl and refluxed for 30mins at 60ºC. Then the solution was allowed to cool and 5ml of 2N NaOH solution was added. Then the final volume was made up to mark with diluent. The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM, and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Base degradation

Base degradation studies were determined by mixing 1ml of standard stock solution with 1ml of 2N NaOH and refluxed for 30 minutes at 60ºC. Then the Then the solution was allowed to cool and 5ml of 2N HCl solution was added. Then the final volume was made up to mark with diluent. The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Oxidative degradation

Oxidative degradation studies were determined by mixing 1ml of standard stock solution with 1ml of 20% H_2O_2 solution and refluxed for 30 minutes at 60ºC.The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry heat degradation (Thermal)

Thermal degradation studies were carried out by accurately weighing the amount of tablet powder quantitatively equivalent to 187.5mg of Glu, 62.5mg of MSM and 12.5mg of Dia and transferred to a clean and dry Petri dish, spread it throughout the plate and placed in an oven for 150ºC for 1hr. The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM, and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photolytic degradation (UV)

Photo stability degradation studies were determined by exposing 7500µg/ml of Glu, 2500µg/ml of MSM and 500µg/ml of Dia solutions to UV light by keeping the beaker in Photo stability chamber for 1day or 200watthours/m^2. The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral degradation (water)

Neutral degradation studies were carried out by refluxing the drug in water for 6 h at a temperature of 60ºC.The resultant solution was diluted to obtain 750µg/ml of Glu, 250µg/ml of MSM, and 50µg/ml of Dia.10µl of above solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

The method has been developed after performing several trails by changing columns (Agilent C_{18} (4.6 x 250mm, 5µm), Agilent C_{18} (4.6 x 150mm, 5µm) and Zodiasil C_{18} (4.6 x 150mm, 3.5µm)), run time (6 minutes, 8minutes and 10 minutes) mobile phase ratios (water: acetonitrile), temperature at 30°C and injection volume (10µl/ml,5µl/ml. The wavelength selected was 210nm. The peaks were eluted with good shape and satisfied system suitability parameters. The retention times of Glucosamine sulphate (Glu), Methyl Sulfonyl Methane (MSM), and Diacerein (Dia) were found to be 2.323min, 2.842min and 3.453min respectively in optimized method. The precision of the method based on %RSD were found to be 0.7 for Glu, 0.8 for MSM and 0.9 for Dia. The %RSD was within the limits (<2), thus the method was precise for Glu, MSM and Dia. Linearity range was obtained by regression equations y= 4561.x+5792 for Glu, y=4653.x+30459 for MSM and y=2935.x+526.7 for Dia(y= mx+c). The accuracy was measured by calculating %recovery and was found to be 100.30% for Glu, 100.13% for MSM and 100.11% for Dia. LOD, LOQ values obtained from the regression lines of Glu, MSM and Dia were 1.28, 0.68, 0.31 and 3.87, 2.07, 0.95 respectively. Retention time and run time were decreased for Glu, MSM and Dia than the earlier reported methods [9]. In all the stress conditions of forced degradation studies, there was no significant degradation of the drug substance was observed and in each condition the purity threshold value was found to be greater than the purity angle value and no purity flags were observed. This implies that there was no interference of degradants with that of analyte peaks and the developed method was stable. The developed method was more economical than the available methods (Sandya Rani et al, 2018) [9] and can be used for simultaneous estimation of Glucosamine sulphate, Methyl Sulfonyl Methane and Diacerein in combined dosage form. The future plans of the studies will involve accelerating the stress conditions like treating the drugs with high strength acid and alkali to get degradants and establishing the structures of degradants using hyphenated analytical techniques which further provides better quality control for pharmaceutical industries.

Table 3: Results of Force Degradation studies for Glu, MSM and Dia.

Degraded Condition	Glu			MSM			Dia		
	%dg*	A**	B***	%dg*	A	B	%dg	A	B
Acid	4.71	0.140	0.290	4.47	4.47	0.117	7.97	0.299	0.506
Alkali	7.44	0.111	0.285	5.40	5.40	0.179	6.62	0.266	0.481
Oxidation	2.92	0.188	0.308	3.78	3.78	0.126	3.74	0.246	0.481
Thermal	2.45	0.166	0.306	2.97	2.97	0.116	2.92	0.273	0.479
UV	1.5	0.152	0.291	1.79	1.79	0.117	2.00	0.268	0.276
Water	0.24	0.160	0.315	0.57	0.57	0.118	0.93	0.260	0.486

%dg* = percentage Drug Degraded**, A**** = Purity Angle**, B***** = Purity Threshold.

Table 4: Summary of results for Glu, MSM and Dia.

Parameters	Glucosamine sulphate	Methyl Sulfonyl Methane	Diacerein
Linearity(Regression coefficient)	0.999	0.999	0.999
Assay (% mean assay)	99.46%	99.65%	100.24%
System precision(%RSD)	0.5	0.2	0.7
Method precision(%RSD)	0.7	0.8	0.9
Accuracy % recovery	100.30%	100.13%	99.87%
LOD	1.28µg/ml	0.68µg/ml	0.31µg/ml
LOQ	3.87µg/ml	2.07µg/ml	0.95µg/ml
Robustness (%RSD)	0.55	0.4	0.81

CONCLUSION

The developed HPLC method was found to be simple, accurate, precise for simultaneous estimation of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein in tablet dosage form. Degradation studies revealed that the developed method was a stability indicating method. As retention times and run time were decreased than the reported methods. The developed method was simple and economical that can be adopted in regular quality control tests for the simultaneous estimation of Glucosamine Sulphate, Methyl Sulfonyl Methane and Diacerein in tablet dosage forms.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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DATA AVAILABILITY

Not declared.

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