ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DIACEREIN AND GLUCOSAMINE IN BULK AND TABLET DOSAGE FORMS BY RP-HPLC

Karri Sravya *, P.V. Madhavi Latha

Department of Pharmacy, Viswanadha Institute of Pharmaceutical Sciences, Affiliated to JNTUK, Visakhapatnam - 530009, A.P, INDIA

*Corresponding author E-mail: sravyakish@gmail.com

ARTICLE INFO

Key Words

Diacerein , Glucosamine , RP-HPLC

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of Diacerein and Glucosamine in Tablet dosage form. Chromatogram was run through Ascentis Express- C_{18}, BDS column (150 x 4.6mm, 5µ) column. Mobile phase containing Acetonitrile: Potassium Di-hydrogen phosphate Buffer taken in the proportions 50:50v/v was pumped through column at flow rate of 1.0ml/min. Temperature was kept ambient. Optimised wavelength selected was 210nm. Retention time of Diacerein and Glucosamine were observed to be 2.586min and 3.182min. %RSD of the Diacerein and Glucosamine were and observed to be 0.90 and 0.90 respectively. %Recovery was obtained as 99.89% for Diacerein and 100.35% for Glucosamine respectively. LOD, LOQ values obtained from regression equations of Diacerein and Glucosamine were 0.01, 0.06 and 0.04, 0.19 respectively. Regression equation of Diacerein is y = 28958x + 476.75, and y = 21702x + 1528 of Glucosamine . Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular quality control test in industries.

INTRODUCTION

Diacerein is a symptomatic slow acting drug in Osteoarthritis (SYSADOA) with anti-inflammatory, anti-catabolic and pro-anabolic properties on cartilage and synovial membrane. Glucosamine plays a vital role in building cartilage and also used as a supplement to treat arthritis and osteoarthritis. The combination of Diacerein and Glucosamine are commonly used in the treatment of Symptomatic mild to moderate knee Osteoarthritis to relieve joint pain and delay joint destruction and cartilage loss. [1-2]

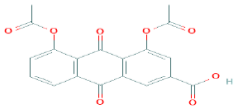
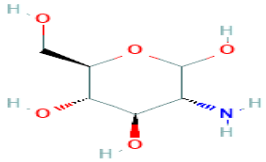


Fig-1: structure of Diacerein

Fig-2: structure of Glucosamine



MATERIALS and METHODS

Preparation of buffer:

Preparation of phosphate buffer: Accurately weighed 1.36g of potassium di-hydrogen ortho phosphate was taken into a 1000 mL of volumetric flask, and add about 900ml of distilled water. The flask was shaken until the particles get dissolved, made up to the mark with water and then add 1ml of triethylamine.

© Journal of Global Trends in Pharmaceutical Sciences

The pH was adjusted to 3.0 with dilute ortho phosphoric acid solution.

Preparation of mobile phase: Accurately measured 500ml 0f (50%) of HPLC Acetonitrile and 500ml of phosphate buffer (50%) were mixed and degassed in a digital ultra sonicator for 25min and then filtered through 0.45 microns filter under vacuum filtration.

Sample solution:

Preparation of sample solution: By taking an average weight of 5 tablets, which areallowed to crush in a motor by using pestle from which, accurately weight equivalent to 1 tablet was transferred into the 500ml clean dried volumetric flask. To this 30ml of diluent was added and sonicated for 25min, which is again make up to mark with same diluent. From that above solution, 1ml was pipette and transferred into 10ml volumetric flask which is again diluted with diluents upto the mark. The final concentrations of Diacerein and Glucosamine sample were found to be 10µg/ml and 150µg/ml respectively.

Standard solution:

Preparation of standard solution: 5mg of Diacerein and 75mg of Glucosamine API Standards were accurately weighed and is transferred into a neat and dry volumetric flask of 50ml. About 30ml of diluent was added and allow for to sonicate to remove the complete air bubbles formed in it, which is again make up to mark with same diluent. Form the stock solution 1ml was pipetted out and transferred into 10ml volumetric flask which is again diluted with diluent upto the mark to get 10µg/ml Diacerein and 150µg/ml Glucosamine. **(3)**

Procedure: Sample solutions (10µL) in duplicates were injected and the peak responses were measured. % assay were calculated for Diacerein and Glucosamine.

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability, Intermediate precision, limit of detection, limit of Quantification, Robustness.

Method development: Method development was performed by changing various chromatographic conditions like mobile phase ratios, buffers, and flow rates.

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: To establish the linearity of the method, serial dilutions were prepared to obtain the mixture of Diacerein and Glucosamine ranging from 2.5ppm to 15ppm and 37.5ppm to 225ppm level all the solutions were filtered through a 0.45µm Millipore filters. The final solution was injected in duplicate manner keeping the injection volume 10µl. Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of Diacerein and Glucosamine was shown in figure no.5 and 6 . The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear. They were tabulated in table 1. **(4)**

ACCURACY: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 & 3. **(5)**

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Diacerein and Glucosamine were injected at intermediate precision. The %RSD was calculated and results were reported and table no. 4 and 5. **(6)**

© Journal of Global Trends in Pharmaceutical Sciences

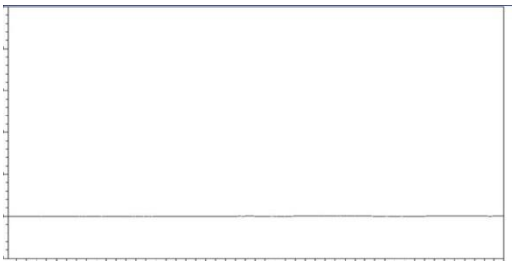


Fig-3: Chromatogram showing blank

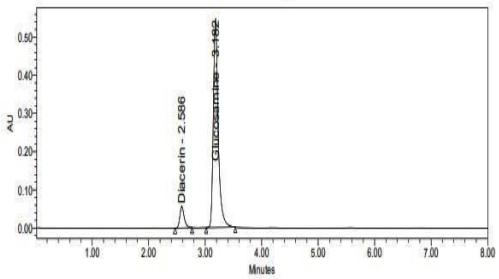


Fig-4: Chromatogram showing optimised condition

Table 1: Linearity results for Diacerein and Glucosamine

S.No	Concentration of Diacerein (µg/mL)	Peak area	Concentration of Glucosamine (µg/mL)	Peak area
1	2.5	71893	37.5	837506
2	5.0	143185	75	1617978
3	7.5	223801	112.5	2408772
4	10.0	289209	150	3270930
5	12.5	361822	187.5	4087965
6	15.0	433729	225	4877642

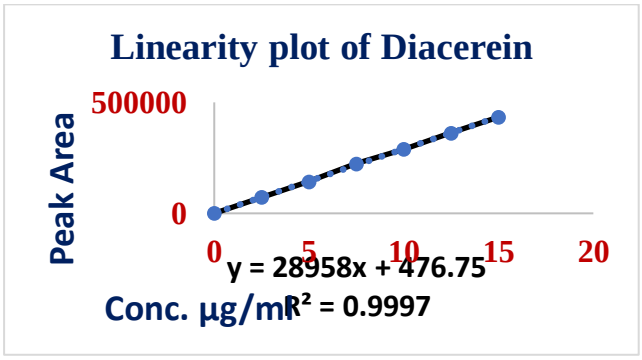


Fig-5: showing calibration curve of Diacerein

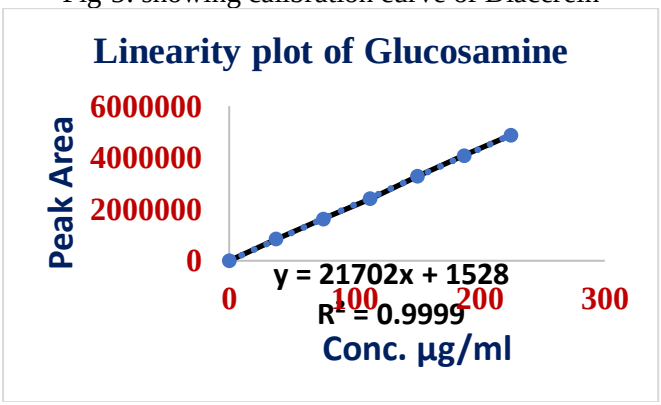


Fig-6: showing calibration curve of Glucosamine

© Journal of Global Trends in Pharmaceutical Sciences

Table 2: Accuracy data for Diacerein
Table 3: Accuracy data for Glucosamine

Concentration of sample taken (µg/ml)	% of spiked level	Amount added(µg)	Amount found(µg)	% Recovery	Statistical Analysis of % Recovery
10µg/ml	50% Injection 1	5	5.02	100.4	MEAN = 99.65 %RSD = 0.65
	50% Injection 2		4.96	99.28	
	50% Injection 3		4.96	99.28	
	100 % Injection 1	10	10.11	101.09	MEAN = 100.48 %RSD = 0.52
	100 % Injection 2		10.02	100.15	
	100% Injection 3		10.03	100.21	
	150% Injection 1	15	14.92	99.44	MEAN = 99.55 %RSD = 0.25
	150% Injection 2		14.97	99.83	
	150% Injection 3		14.91	99.38	

Concentration of sample taken (µg/ml)	% of spiked level	Amount added(µg)	Amount found(µg)	% Recovery	Statistical Analysis of % Recovery
150µg/ml	50% Injection 1	75	75.43	100.58	MEAN = 100.3 %RSD = 0.48
	50% Injection 2		74.80	99.74	
	50% Injection 3		75.40	100.58	
	100 % Injection 1	150	150.70	100.52	MEAN = 100.5 %RSD = 0.81
	100 % Injection 2		149.50	99.73	
	100% Injection 3		152.0	101.35	
	150% Injection 1	225	225.8	100.40	MEAN = 100.25 %RSD = 0.23
	150% Injection 2		225.8	100.36	
	150% Injection 3		224.9	99.99	

© Journal of Global Trends in Pharmaceutical Sciences

Table-4: Intermediate precision data for Diacerein

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	2.573	289739	5946	6068	1.29
2	2.574	290708	6138	5914	1.30
3	2.576	291909	6427	6028	1.30
4	2.576	294709	6891	6006	1.30
5	2.583	290373	6914	5430	1.25
6	2.591	296472	6935	6022	1.28
Mean		292318			
SD		2689.5			
% RSD		0.9			

Table-5: Intermediate precision results for Glucosamine

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	3.170	3259151	55238	7221	1.41
2	3.170	3273485	56814	7285	1.40
3	2.173	3328163	56928	7681	1.44
4	3.175	3320710	56936	7667	1.45
5	3.179	3271567	56982	7142	1.42
6	3.215	3258938	56991	7344	1.40
Mean		3285336			
SD		30977.4			
% RSD		0.9			

Table-6: LOD and LOQ data for Diacerein and Glucosamine

Drug	Average slope	Average intercept	Standard deviation of the intercept	Regression coefficient (R^2)	LOD (µg/ml)	LOQ (µg/ml)
Diacerein	28958	476.75	132.16	0.9997	0.01	0.04
Glucosamine	21702	1528	416.28	0.9999	0.06	0.19

Table-7: Robustness data for Diacerein

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	226035	1.33		289739	1.29		30474731	1.24

Table-8: Robustness data for Glucosamine

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	2477440	1.49		3259151	1.41		2856527	1.39

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6 . [7]

© Journal of Global Trends in Pharmaceutical Sciences

ROBUSTNESS: The small deliberate changes in method like flow rate was made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.8ml/min), flow plus (1.2ml/min), temperature ambient was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.7 and 8 . **(8)**

CONCLUSION:

A simple, fast, accurate and specific RP-HPLC method has been developed for the simultaneous quantification of Diacerein and Glucosamine in bulk and tablet dosage form by studying different parameters. The maximum absorbance was found to be at 210nm for Diacerein and 210nm for Glucosamine. The common wavelength observed was 210nm and the peaks purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Ascentis Express – C18, BDS column and resulted good peak shape. 30℃ temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50 (Acetonitrile :Potassium dihydrogen phosphate buffer) was fixed due to good symmetrical peaks and good resolution. So, this mobile phase was used for the proposed study.

Acknowledgement: The author expresses sincere thanks to Aurobindo Pharmaceuticals pvt.Ltd. For providing facilities and greatest support to carry out the research work.

REFERENCES:

1. Martel-Pelletier J, Pelletier JP. Effects of diacerein at the molecular level in the osteoarthritis disease process. Therapeutic advances in musculoskeletal disease. (2010) ; 2(2): 95-104.
2. Reginster JY, Neuprez A, Lecart MP, Sarlet N, Bruyere O. Role of glucosamine in the treatment for osteoarthritis. Rheumatology international. (2012) ; 32(10): 2959-2967.
3. Nyola N, Jeyabalan GS, Kalra N, Parveen G, Choudhary S. Development and validation of a RP-HPLC method for simultaneous estimation of diacerein and aceclofenac in pharmaceutical dosage form. Research and reviews: journal of pharmaceutical analysis. (2012) ; 1(1): 1-8.
4. Raghava Raju TV, Kumar SR, Srinivas A, Kumar NA, Rao IM, Rao NS. Development and Validation of a Stability-Indicating RP-HPLC method for the Estimation of Diacerein Impurities in API and Pharmaceutical Formulation. Journal of Liquid Chromatography & amp; Related Technologies. (2014) ; 37(4): 498-515.
5. Rani JS, Devanna N. Analytical Method Development and Validation of Simultaneous Estimation of Diacerein, Glucosamine and Methyl Sulfonyl Methane By RP-HPLC In Pharmaceutical Tablet Dosage Forms. IOSR J. Appl. Chemistry. (2018) ; 2: 47-54.
6. Pullareddy S, Rambabu C. Simultaneous determination of Glucosamine and Diacerein in pharmaceutical dosage form by RPHPLC. International Journal of Pharmacy and Pharmaceutical Research. (2015) ; 2(2): 139-151.
7. Sriveena T, Srividya A, Ajitha A, Rao VU. Rp-hplc method development and validation for simultaneous estimation of diacerein and glucosaminesamine in bulk and pharmaceutical dosage form. World Journal of Pharmaceutical Research. (2015) ; 4(8): 2349-2360.
8. Reddy M, Reddy KH, Bobbarala V, Penumajji S. HPLC Method development for glucosamine sulphate and diacerein formulation. J Pharma Res. (2010); 3(2): 361-36.

© Journal of Global Trends in Pharmaceutical Sciences