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Simultaneous Determination of Glucosamine and Diacerein in Pharmaceutical Dosage Form by RP-HPLC

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S. Pulla reddy[1] and C Rambabu*

*[1,]*Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India*

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ABSTRACT

The chromatographic analysis was performed on Agilent, Zebra C_{18} reversed phase column with mobile phase consisting Ammonium acetate: acetonitrile in the ratio 60:40% v/v, at a flow rate of 1.0 mL/min and eluents monitored at 267nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per International Conference on Harmonization (ICH) guidelines. The retention times of glucosamine and diacerein were 2.710 and 3.203min. respectively. The calibration curves of peak area versus concentration, which was linear from 3000-9000 µg/mL for glucosamine and 100-300µg/mL for diacerein, having regression coefficients (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of glucosamine and diacerein in tablets. The proposed method is simple, economical, accurate and precise, and could be successfully employed in routine quality control for the simultaneous estimation of glucosamine and diacerein in tablets.

INTRODUCTION

Glucosamine [1-9] [**FIG.1.01.A**] is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. It is absorbed rapidly from the intestine and transported to the connective tissues and helps in the restoration of damaged joint tissue in osteoarthritis. It has been used for osteoarthritis, back pain, joint pain and glaucoma, by itself or in combination with chondroitin sulfate, diacerein. It is one of the most commonly used supplementary medicines as non-vitamin, non-mineral and natural product. It may decrease catabolic activity by inhibiting the synthesis of proteolysis enzymes and other substances that contribute to damage of the cartilage matrix. Glucosamine is required for the synthesis of glycoprotein, glycolipids and glycosaminoglycans (mucopolysaccharides).

Diacerein [10] [**FIG.1.01.B**], also known as diacetylrhein, is a (4, 5- diacetoxy-9, 10-dihydro9, 10 di-oxo-2 anthracene carboxylic acid) is a new anti-inflammatory, analgesic and antipyretic drug used in the treatment of osteoarthritis. It has a novel mode of action that differentiates it from NSAIDs and other conventional form of drug therapy. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor (TGF) - beta 1and TGF-beta 2, with, potential cartilage repairing properties. Diacerein does not alter renal or platelet cyclo-oxygenase. In addition to effect on macrophage migration and phagocytosis, it also inhibits superoxide production, chemotaxis and phagocytic activity of neutrophils. However, Diacerein lacks cyclooxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis.

Glucosamine [11-15] and Diacerein [16-23] have reported methods by chemical and instrumental methods in pure drug, pharmaceutical dosage forms and in biological samples either in single or in combined forms.

The objectives of this study were, therefore, to develop a simpler, economic, rapid, precise, isocratic, and accurate RP-HPLC method with good sensitivity for quantitative analysis of glucosamine and diacerein in pharmaceutical dosage forms and to validate the method in accordance with International Conference on Harmonization (ICH) guidelines [30]. The direct use of the mobile phase for dilution of the formulations for quantitative analysis would minimize errors that might occur during tedious extraction procedures.

EXPERIMENTAL

i) Chemicals and Reagents:

HPLC grade acetonitrile, analytical reagent grade Ammonium acetate and phosphoric acid were purchased from Merck, Darmstadt, Germany. High pure water was prepared by using Millipore Milli-Q plus water purification system.

ii) Mobile Phase:

The mobile phase selected was Ammonium acetate buffer (pH 3.5): acetonitrile in the ratio of 60:40(v/v), and before analysis mobile phase was degassed.

iii) Standard preparation:

100 mg of Glucosamine sulphate and Diacerein and were accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 70 ml of diluents (mobile phase) was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1.0mg/ml glucosamine and diacerin and filtered through 0.45 µm nylon filter. (stock solution). From the above stock solutions different aliquots was transferred into 10ml volumetric flask and diluted up to the mark by the diluent to achieve a concentration of 3000-9000µg/ml and of 100-300µg/ml of µg/ml glucosamine and diacerin respectively.

iv) Sample Preparation:

20 Tablets were weighed and triturated in glass mortar. The quantity of powder equivalent to 100mg of active ingredient present in 20 tablets was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.8 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 mm nylon filter before injecting into HPLC system.

HPLC Instrumentation and Chromatographic Conditions:

Chromatography was performed with Shimadzu HPLC equipment comprising an LC-10A VP quaternary pump, a variable-wavelength programmable UV–visible detector, an SPD-10AVP column oven, and an SCL10AVP system controller. A Rheodyne injector fitted with a 20-µL loop was also used and data were recorded and evaluated by use of Class-VP 5.032 software. Glucosamine and diacerein was separated, at ambient temperature (30°C) on a 250 mm × 4.6 mm i.d., 5-µm particle, Agilent, Zorbax C_{18} reversed phase column with Ammonium acetate buffer (3.5): acetonitrile in the ratio of 60:40(v/v) as mobile phase at a flow rate of 1.0 $mL.min^{-1}$. Before use the mobile phase was filtered through a 0.45µm Nylon filter. UV detection was performed at 267nm.

RESULTS AND DISCUSSION

HPLC METHOD DEVELOPMENT

To optimize the chromatographic conditions, different combinations of Ammonium acetate buffer (pH 3.5): acetonitrile (40:60, 50:50 and 60:40). Ammonium acetate: acetonitrile in the ratio (60:40) was preferred because it resulted in a greater response to glucosamine and diacerein. The composition and flow rate of the mobile phase were changed to optimize the separation conditions. Decreasing the organic modifier content resulted in a decrease in the retention time (RT) of the drug. The effect of the flow rate was studied in the range 0.8 to 1.2 $mL.min^{-1}$. High acetonitrile content and flow rate resulted in prolonged analysis time. A low acetonitrile concentration was therefore used at a flow rate of 1.0 $mL.min^{-1}$, keeping in mind the possibility that potential minor degradation products could appear after stress studies and might co elute with the drug because of the reduced RT if the flow rate was increased. High flow rates also reduce the life time of both column and pump. Under these conditions, the analyte peak was well-defined and free from tailing (**Fig.1.02.C**).The retention time (RT) of glucosamine and diacerein were 2.710 and 3.203min respectively. Other advantages of this mobile phase included its low cost and simplicity. The short retention time achieved implied that many samples can be run using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis.

METHOD VALIDATION

The developed RP-HPLC method is validated in accordance with ICH guidelines for assay of glucosamine and diacerein using the following Parameters.

A. SPECIFICITY

1. BLANK AND PLACEBO INTERFERENCE

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig.1.02.A)** showed no peaks at the retention time of glucosamine and diacerein peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of glucosamine and diacerein in tablets. Similarly chromatogram of placebo solution **(Fig.1.02.B)** showed no peaks at the retention time of glucosamine and diacerein peak. This indicates that the placebo used in sample preparation do not interfere in estimation of glucosamine and diacerein in their formulations.

B. LINEARITY OF DETECTOR RESPONSE

The standard curve was obtained in the concentration range of 3000-9000µg/ml for glucosamine and 100-300µg/mL for diacerein. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were plotted and calculated and are given in **Fig.1.03.A & Table:1.02.A** for glucosamine **Fig.1.03.B & Table:1.02.B** and for diacerein demonstrating the linearity of the proposed method. The LOD value for glucosamine and diacerein were found to be 2.939µg/mL and 2.985µg/mL, respectively and the LOQ value 9.79µg/mL and 9.95µg/mL and are reported in **Table:1.02.A&B** respectively.

C. PRECISION

The method precision study for six sample preparations in marketed samples showed a **%** RSD of 0.5% and glucosamine and diacerin respectively revealing high precision of the proposed RP-HPLC method (**Table.2.04)**

D. ACCURACY

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of glucosamine and diacerin, analyzed as per the proposed method. The percentage recoveries was found to be %100 with an overall %RSD of 0.5 for glucosamine and the percentage recoveries with found in the range of 100 with an overall %RSD of 0.2 for diacerin. From the data reported in **Table: 1.04.A&B** reported that the developed RP-HPLC method was found to be accurate for glucosamine and diacerin assay.

E. ROBUSTNESS STUDIES

The robustness study of the developed assay method for glucosamine and diacerein were established in all variance conditions. Assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence, the analytical method would be concluded as robust.

F. ANALYSIS OF MARKETED FORMULATION

Analysis of marketed tablets ((T-MINIC Tab, Novartis) was carried out using optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for glucosamine and diacerein was found to be 99.98 and 99.96, respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 105%. The results are given in **Table.1.05**.

CONCLUSION

A simple, rapid, sensitive and economical RP-HPLC method has been developed for the estimation of Glucosamine and Diacerin single and also in combined dosage forms. The credibility of the proposed method has been established by validation as per the ICH guidelines. The results of validation were in good agreement with acceptable limits. Therefore the method has proven to be accurate, precise, linear, specific and robust. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine quality control analysis of Glucosamine and Diacerin in pure and also in combined dosage forms.

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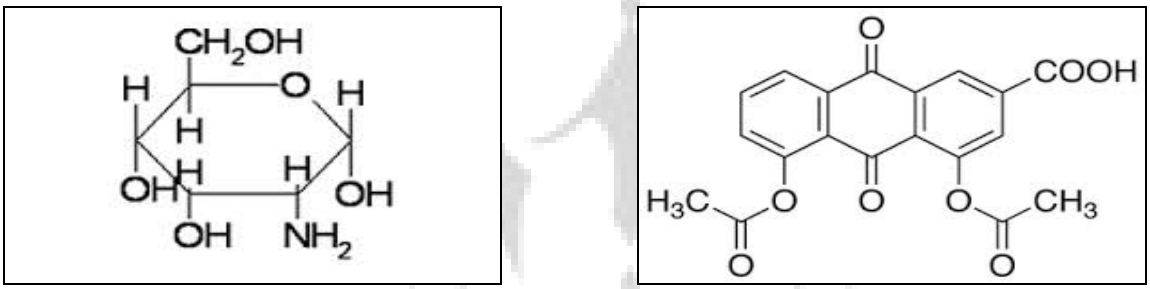


GLUCOSAMINE (A) **DIACEREIN (B)**

Fig.1.01.A&B. Structure of Glucosamine and Diacerein

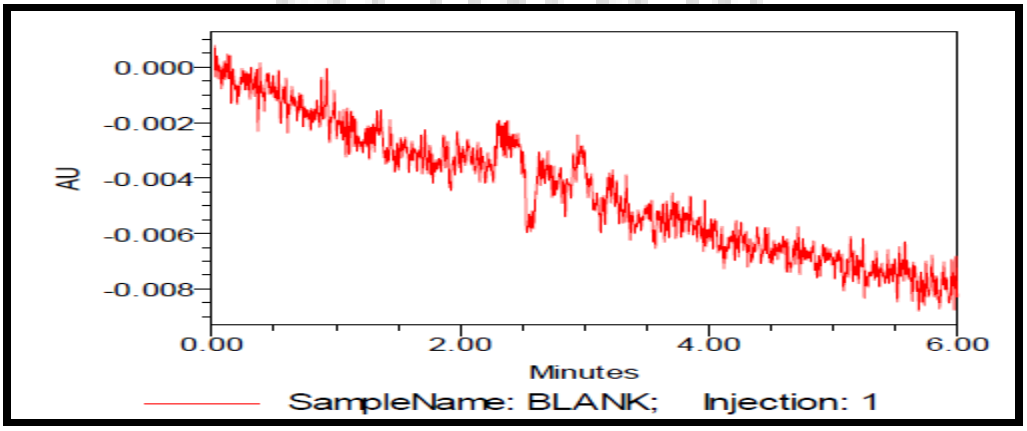


Fig: 1.02.A - Typical HPLC Chromatogram Showing the No Interference of Blank for Glucosamine And Diacerein

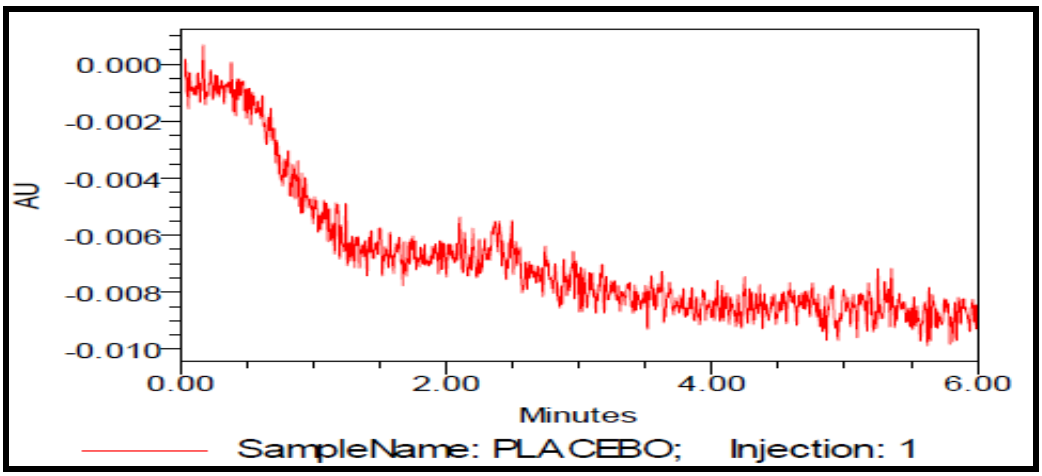


Fig: 1.02.B - A Typical HPLC Chromatogram Showing the No Interference of Placebo for Glucosamine and Diacerein

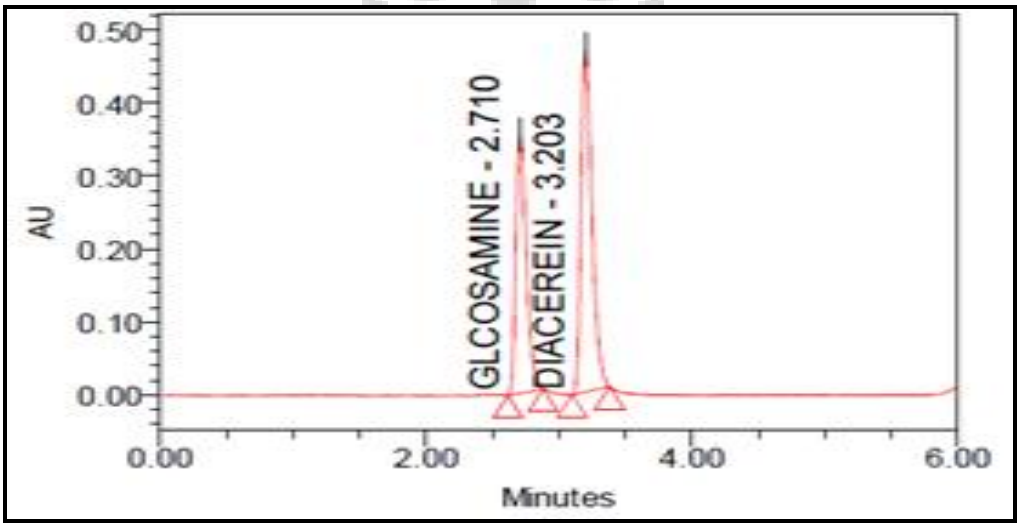
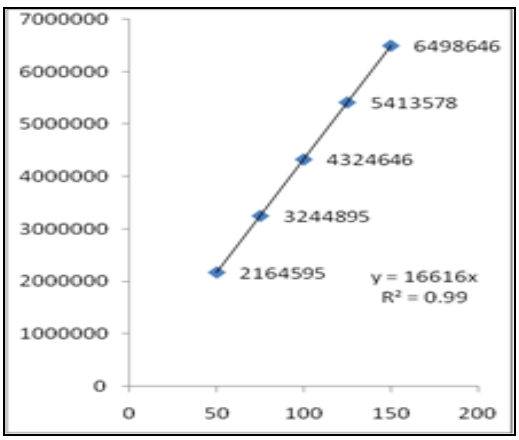


Fig: 1.02.C – Validated HPLC Chromatogram of Glucosamine and Diacerein



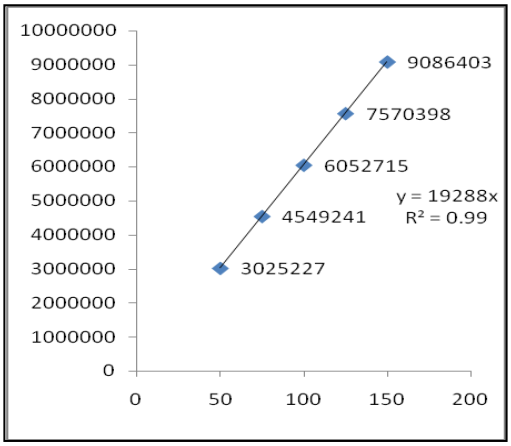


Fig: 1.03. A&B. Linearity Curve Of Glucosamine (A) and Diacerein (B)

Table.1.01: System Suitability Parameters of Glucosamine and Diacerein

NAME OF THE COMPOUND	RETENTION TIME	THEORETICAL PLATES	TAILING FACTOR	USP RESOLUTION
GLUCOSAMINE	3.158	8719	1.334	-
DIACEREIN(B)	5.916	18971	1.230	17.235

1.02. A. Linearity Data Results of Glucosamine

LINEARITY STUDY FOR GLUCOSAMINE		
% LEVEL (APPROX.)	CONC. µg/ml	AREA
50	3000	2164595
75	4500.00	3244895
100	6000.00	4324646
125	7500	5413578
150	9000	6498646
Slope		166160
RSQ(r2)		0.999
LOD		2.939
LOQ		9.796

1.02. B. Linearity Data Results of Diacerein

LINEARITY STUDY FOR DIACEREIN		
% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	100	3025227
75	150	4549241
100	200	6052715
125	250	7570398
150	300	9086403
Slope		19288
RSQ(r2)		0.9999
LLD (µg/ml)		2.985
LLQ (µg/ml)		9.95

Table.1.03: Method Precision (Inter and Intraday) Studies for Glucosamine and Diacerein by the Proposed Method

S. No.	Injection	GLUCOSAMINE	DIACEREIN
1	Injection 1	6497705	9085117
2	Injection 2	6491526	9088537
3	Injection 3	6498412	9087330
4	Injection 4	6490109	9087407
5	Injection 5	6491976	9082988
6	Injection 6	6497498	9084818
Average		6494538	9086033
Standard Deviation		3716.218	2070.14
% RSD		0.0572	0.022

*Average of six determinations

Table: 1.04.A: Recovery Studies For Glucosamine and Diacerein by the Proposed Method

GLUCOSAMINE						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	936.23	2163710	2973.016	2971.62	100	
50%	936.23	2168278	2973.016	2977.90	100	
50%	936.23	2162390	2973.016	2969.81	100	100
50%	936.23	2168849	2973.016	2978.68	100	
50%	936.23	2166938	2973.016	2976.06	100	
50%	936.23	2165082	2973.016	2973.51	100	
100%	1872.45	4322438	5946.000	5936.41	100	
100%	1872.45	4322030	5946.000	5935.85	100	100
100%	1872.45	4322240	5946.000	5936.13	100	
150%	2808.70	6497705	8919.079	8923.90	100	
150%	2808.70	6491526	8919.079	8915.42	100	
150%	2808.70	6498412	8919.079	8924.87	100	100
150%	2808.70	6490109	8919.079	8913.47	100	
150%	2808.70	6491976	8919.079	8916.03	100	
150%	2808.70	6497498	8919.079	8923.62	100	

Table: 1.04.B: Recovery Studies For Glucosamine and Diacerein by the Proposed Method

DIACEREIN						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	445.65	3022767	99.701	99.52	100	
50%	445.65	3021937	99.701	99.49	100	
50%	445.65	3023067	99.701	99.53	100	100
50%	445.65	3024800	99.701	99.59	100	
50%	445.65	3021039	99.701	99.46	100	
50%	445.65	3021832	99.701	99.49	100	
100%	891.30	6054176.00	199.400	199.33	100	
100%	891.30	6058487.00	199.400	199.47	100	100
100%	891.30	6053897.00	199.400	199.32	100	
150%	1337.00	9085117	299.103	299.12	100	
150%	1337.00	9088537	299.103	299.23	100	
150%	1337.00	9087330	299.103	299.19	100	100
150%	1337.00	9087407	299.103	299.19	100	
150%	1337.00	9082988	299.103	299.05	100	
150%	1337.00	9084818	299.103	299.11	100	

Table.1.05: Analysis of Marketed Tablets

DRUG	LABALCLAIM	QUANTITYFOUND*	%RSD	%ASSAY
GLUCOSAMINE	500mg	499.92	0.013	99.98
DIACEREIN	50mg	49.98	0.085	99.96

*Average of six determinations