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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DIACEREIN AND GLUCOSAMINESAMINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, Rapid, Specific and accurate reverse phase high performance liquid chromatography method was develop for simultaneous estimation of Diacerein and Glucosamine in pharmaceutical dosage form. A chromatographic condition was performed on ODS (C18) (4.6×250mm, 5µ) column, with mobile phase is Triethylamine pH4: Methanol: Acetonitrile in the ratio of 50:10:40 % (v/v), at the flow rate 1ml/min at detection wavelength of 250nm. The retention times of Diacerein and Glucosamine were found to be 2.242 and 3.678mins respectively with a run time of 6min, theoretical levels for Diacerein and Glucosamine were 8596 and 4547 respectively. As per ICH guidelines the method was validated for linearity, accuracy, and precision, limit of detection and limit of quantification, and ruggedness, robustness. Linearity of Glucosamine was found in the range of 75-375µg/ml and that for DIA was found to be 5-25µg/ml. The LOD values for Diacerein and Glucosamine were 19.3µg/ml and 1.05µg/ml respectively. The LOQ values for Diacerein and Glucosamine were 58.6µg/ml and 3.19µg/ml respectively.

KEYWORDS: Glucosamine, Diacerein, PDA detection Tablet dosage forms.

INTRODUCTION

Diacerein[1] is chemically 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate (fig.1)

Diacerein (INN), also known as **diacetylrhein**, is a slow-acting medicine of the class anthraquinone used to treat joint diseases such as osteoarthritis (swelling and pain in the joints).[1] It works by inhibiting interleukin-1 beta. A 2005 Cochrane review found diacerein to be slightly, but significantly, more effective than placebo in improving pain and slowing the progress of osteoarthritis in the hip and knee Solubility - Slightly soluble in water and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

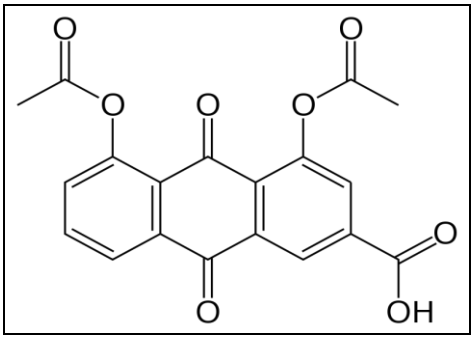

Fig 1: Chemical Structure of Diacerein

Glucosamine: chemical name

(3R,4R,5S)-3-Amino-6-(hydroxymethyl)oxane-2,4,5-triol (fig 2). Used to treat a Anti osteoarthritis and Glucosamine is an amino sugar produced from the shells of chitin (shellfish) and a key component of cartilage. Glucosamine works to sti-mulate joint function and repair. It has been proven effective in numerous scientific trials for easing osteoarthritis pain, aiding in the rehabilitation of cartilage, renewing synovial fluid, and repairing joints that have been damaged from osteoarthritis.[1] As stated by the companies of the product tested for analysis, glucosamine provides the building blocks for construc-ting cartilage and enhances lubrication.

Solubility – Soluble in Acetonitrile, practically insoluble in water**.**

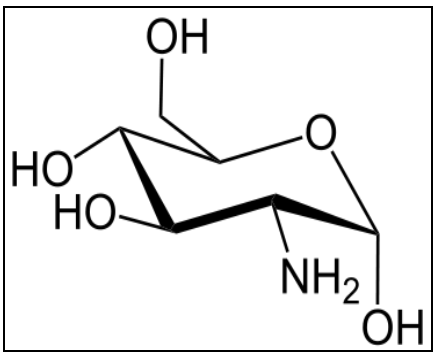

Fig 2: Chemical structure of Glucosamine

From the literature survey it was found that many methods are available for determination of Diacerein and Glucosamine individually and few methods in combination with other drugs. However, no stability indicating HPLC has been reported for simultaneous determination of Diacerein and Glucosamine in combination.

In the proposed study an attempt will be made to develop a stability indicating HPLC method for simultaneous estimation of Diacerein and Glucosamine in pharmaceutical formulation (tablet).

Solvents and Reagents

Pharmaceutical grade of Diacerein, and Glucosamine were kindly supplied as gift samples by Suralabs pvt ltd Dilshuknagar Hyderabad India, certified to contain > 99% (w/w) on dried basis. Commercially available **Oscicare plus** Tablets (**Systopic laboratory pvt. ltd**India) tablets claimed to contain 50 and 375 mg Diacerein and Glucosamine have been utilized in the present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals,Hyderabad, India.

Chromatographic system and conditions

The HPLC system (Analytical Technologies Gujarat, India) consisted of pump.The Analytical column a cosmosil packed column $5C_{18}$ MS-II(250mm x 4.6 i.d.)was operated at ambient temperature (20 $\pm$ 1^{o}c) . Isocratic elution with Acetonitrile: methanol:phosphate buffer (20:50:30 v/v pH 4.0)was used at flow rate at 1.0 ml/min . Column (250×4.6 i.d). The mobile phase. Before analysis the mobile phase was filtered through a 0.2 µm membrane and degassed by ultrasonification. Detection was monitored at 250 nm and injection volume was 20 µl. All the experiments were performed at ambient temperature.

Standard solutions and calibration graphs for chromatographic measurement

Stock standard solutions were prepared by dissolving separately 5 mg of Diacerein and Glucosamine in 10 ml mobile phase (1000 µg/ml). The standard calibration solutions were prepared by appropriate dilution of the stock solution with methanol to reach a concentration range of 75-375 µg/ml for Diacerein and 5-25 µg/ml for Glucosamine. 20 µl injections were made for each concentration and chromatographed under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation

Twenty tablets contents were accurately weighed, their mean weight was determined and they were mixed and finely powdered. A portion equivalent to about one tablet was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml mobile phase, sonicated for 15 min and diluted to 100 ml with mobile phase. The resulting solution was centrifuged at 100 rpm for 15 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µ filter (Millipore, Milford, MA). The original stock solution was further diluted to get sample solution of drug concentration of 75-375 µg/ml for Diacerein and 5-25 µg/ml Diacerein. A 20 µl volume of sample solution was injected into HPLC, six times. The peak areas for the drugs were measured at 250 nm and amounts of Diacerein and Glucosamine were determined using the related linear regression equations.

Method validation

The developed method was validated according to the ICH guidelines,[18-19] The system suitability was evaluated by six replicate analyses of Diacerein and Glucosamine mixture at a concentration of 50 µg/ml Glucosamine and 25 µg/ml Diacerein. The acceptance criteria were a R.S.D. of peak areas and retention times less than 2%, Theoretical plate numbers (N) at least 2500 for each peak and tailing factors (T) less than 1% for Diacerein and Glucosamine Standard calibration curves were prepared in the mobile phase with six concentrations ranging from 5-25 µg/ml for Diacerein and 50-375 µg/ml for Glucosamine into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments were carried out at three levels 50, 100 and 150%. Known concentrations of commercial tablets were spiked with known amounts of Diacerein and Glucosamine At each level of the amount six determinations were performed and the results obtained were compared with expected results. Recovery for pharmaceutical formulations should be within the range 100±5%. The percent R.S.D. of individual measurements was also determined. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for 2 consecutive days. Three different concentrations of Diacerein and Glucosamine were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision).. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of percent RSD.

All chromatgrams were examined to determine if compounds of interest co-eluted with each other or with any additional excipients peaks. Marketed formulations were analyzed to determine the specificity of the optimized method in the presence of common tablet excipients. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ were calculated using 3.3σ/s and 10σ/s formulae, respectively, where, σ is the standard deviation of the peak areas and *s* is the slope of the corresponding calibration curve. To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, percentage of buffer in the mobile phase, and pH of mobile phase.

RESULTS AND DISCUSSION

During the optimization of HPLC method, columns (cosmosil packed column 5c-18 ms-II 250 mm × 4.6 i.d), two organic solvents (acetonitrile,methanol and phosphate buffer), two buffers (acetate and phosphate) at two different pH values (3 and 4) were tested. Initially methanol:water, acetonitrile:water, acetonitrile: phosphate buffer, methanol:phosphate buffer were tried in different ratios at pH 3 and 4. Diacerein eluted with the tried mobile phases, but Glucosamine was retained. Then, with acetonitrile:methanol: phosphate buffer all the two drugs eluted. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients. Other criteria, *viz*. time required for analysis, appropriate k range (1<k<10) for eluted peaks, assay sensitivity, solvent noise were also considered. Finally a mobile phase consisting of a mixture of Triethylamine: methanol: Acetonitrile in the ratio of 50:10:40 %(V/V) at a flow rate of 1 ml/minute and detection wavelength of 250 nm. The retention times of the drugs were found to be 2.242 and 3.678 min respectively. gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C18 column, the retention times for Diacerein and Glucosamine were observed to be 2.2 and 3.6 min. respectively. Total time of analysis was less than 10 min. The chromatogram at 250 nm showed a complete resolution of all peaks.

Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability test. All critical parameters tested met the acceptance criteria on all days. As shown in the chromatogram, all three analytes are eluted by forming symmetrical single peaks well separated from the solvent front.

Excellent linearity was obtained for all the two drugs in the range of 5-25Diacerein and 75-375 Glucosamine. The correlation coefficients (r^2) were found to be greater than 0.999 (n=6)

in all instances. The results of calibration studies are summarized in Table 1.The proposed method afforded high recoveries for Diacerein and Glucosamine tablet. Results obtained from recovery studies presented in Table 2, indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in tablet. Precision of the analytical method was found to be reliable based on % RSD (< 2%) corresponding to the peak areas and retention times. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the two drugs.

The chromatograms were checked for the appearance of any extra peaks. It was observed that single peak for Diacerein (R_t±SD, 4.915±0.01) and Glucosamine (R_t±SD, 8.056±0.01) were obtained under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with the standard and % purity calculated was found to be within the limits. These results demonstrate the specificity of the method

Table 1 Linearity Parameters for the Simultaneous Estimation of Glucosamine (*N*=6)

Parameters	Diacerein	Glucosamine
λ_{max} (nm)	250	236
Beers law limit (µg/ml)	5-25	75 -375
Correlation coefficient (r)	0.9994	0.9993
Regression equation (y=mx+c)	y = 30375x + 3568	y = 17100x + 14963
Slope (m)	30375	17100
Intercept (c)	3568	14963
LOD (µg/ml)	1.05	19.3
LOQ (µg/ml)	3.19	58.06

Table 2 Accuracy Results of Glucosamine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1919977	112.5	111.4	99	99
100%	3883331	225	226	100	
150%	5756264	337.5	335.7	99	

Table 3 Accuracy Results of Diacerein

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	229437.3	7.5	7.43	99	99
100%	458725.7	15	14.9	99	
150%	686406	22.5	22.4	99	

The accuracy studies were shown as % recovery for Diacerein and Glucosamine at 50%, 100%,150% ,the limits of recovery should be in range of 98-102% the limits obtained for Diacerein and Glucosamine were found to be within the limits. Hence the method was found to be accurate. The accuracy studies shows % recovery of the Glucosamine 99% and Diacerein 99%. The limits of % recovery of drugs were 98-102% and from the above results it's indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical formation do not interfere in the proposed method. The chromatograms of shown in **Figs 3** and results were shown **Tables 4.**

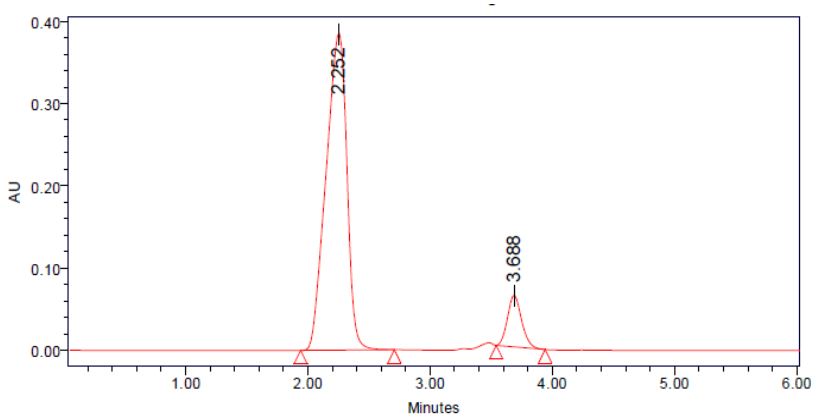

Fig.3 Model Chromatogram for Diacerein and Glucosaine

TABLE -5. System Suitability Parameters for the Optimized Chromatogram by RP – HPLC.

PARAMETERS	Glucosamine	Diacerein
Tailing factor	1.10	1.18
Asymmetrical factor	1.10	1.18
Theoretical plates	8596	11187
Capacity factor	6.697	14.377
Theoretical plate per unit Length	206.19	242.05
Resolution	Between Diacerein and Glucosamine 4.04	

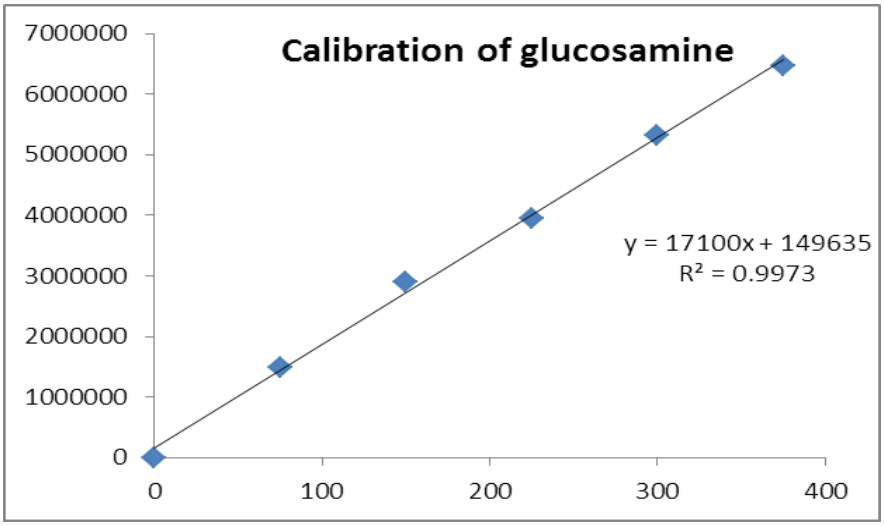

Fig 4: Calibration Curve Of Glucosamine

Table 6. Linearity Results of Glucosamine

S.No.	Concentration µg/ml	Average Peak Area
1	75	1490079
2	150	2908897
3	225	3941453
4	300	5325341
5	375	6470088
	Correlation Coefficient	0.997

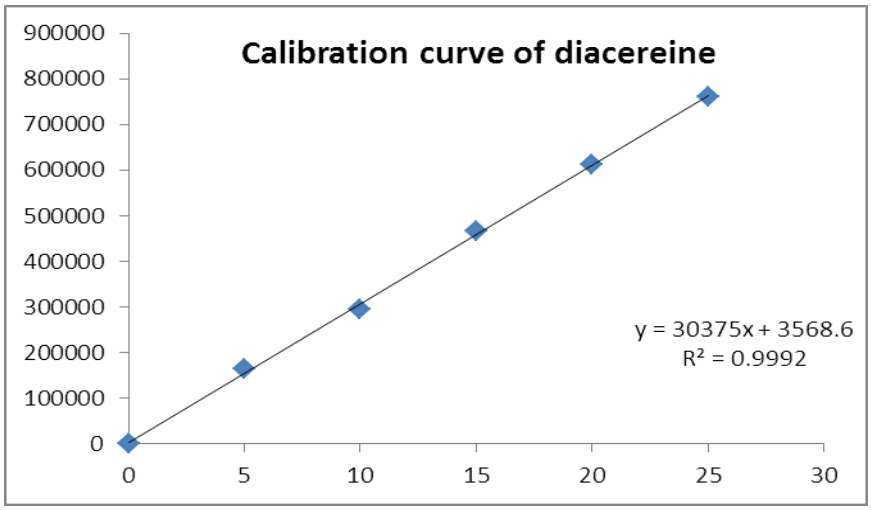

Fig 5: Calibration Curve Of Diacerein

Table 7. Linearity Results of Diacerein

S.No.	Concentration µg/ml	Average Peak Area
1	5	165120
2	10	295241
3	15	465667
4	20	613522
5	25	760009
	Correlation coefficient	0.999

Table 8.Optimized Method Parameters

PARAMETERS	CONDITIONS
Mobile phase	Triethylamine pH4: Methanol: Acetonitrile
Column	ODS C18 (4.6×250mm) 5µ
Detector	PDA Detector
Wave length	250 nm
Flow rate	1ml /min
Volume of injection	10µl
Column temperature	30ºC

From the above experiment it was found that Diacerein and Glucosamine can effectively be analyzed and separated by using RP-HPLC method with triethylamine: methanol: Acetonitrile in the ratio of 50:10:40%(V/V) at a flow rate of 1 ml/minute and detection wavelength of 250 nm. The LOD and LOQ were found to be 0.1379 µg/ml and 0.4180 µg/ml for Glucosamine and 0.0677 µg/ml and 0.2051 µg/ml for Diacerein. In all deliberately varied conditions, the SD of retention times of Diacerein and Glucosamine were found to be well within the acceptable limit. The tailing factor for all the two peaks was found to be < 1.5 (Table 9). The validated method was used in the analysis of marketed conventional tablet Amlopres with a label claim: 750 mg Glucosamine and 50 mg Diacerein per tablet.. The results for the drugs assay show a good agreement with the label claims.

SUMMARY AND CONCLUSION

RP-HPLC method was developed for simultaneous estimation of Diacerein and Glucosamine in pharmaceutical dosage form. Chromatographic separation was performed on symmetry ODS C18 (4.6×250mm) 5µ column, with mobile phase comprising of mixture of Triethylamine pH4: Methanol: Acetonitrile in the ratio of 50:10:40% **(**v/v), at the flow rate 1ml/min. The detection was carried out at 250nm.

Table 9 Summary for RP-HPLC Method

Parameters	Acceptance criteria	Results obtained
System suitability	Theoretical Plates- NLT 2000	GLUCO- 8596 DIA-454
	Tailing factor - NMT 2	GLUCO-0.92 DIA-1.23
	Retention time	GLUCO-2.242 DIA-3.678
Precision	% RSD of GLUCO-NLT 2 % RSD of DIA-NLT 2	GLUCO-0.8 DIA-0.5
ID Precision	% RSD of GLUCO-NLT 2 % RSD of DIA-NLT 2	**DAY-1** GLUCO-0.3 DIA-0.5 **DAY-2** GLUCO-0.9 DIA-0.7
Linearity	Correlation coefficient NLT 0.999	GLUCO-0.997 DIA-0.999
Accuracy	Percentage Recovery 98-102%	GLUCO-99 DIA-99
Limit of Detection		GLUCO-19.3µg/ml DIA- 1.05 µg/ml
Limit of quantitation		GLUCO-58.6 µg/ml DIA- 3.19µg/ml

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Diacerein and Glucosamine in tablet dosage form. It was also proved to be convenient and effective for the determination of Diacerein and Glucosamine in the bulk and combined dosage form.

It inferred the method found to be simple, accurate, precise and linear; The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy, precision.

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