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Stability indicating HPTLC Method Development and Validation for Estimation of Glucosamine and Diacerein as Bulk Drug and in Drug Formulation by Derivatization

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Abstract : The objective of the method was to develop and validate a sensitive stability indicating high performance thin layer chromatographic (HPTLC) method for simultaneous estimation of glucosamine and diacerein. The chromatographic separation was done on aluminum plates precoated with silica gel $60F_{254}$ using n-butanol: Water: Glacial Acetic Acid (7:1.5:1.5 v/v/v) as mobile phase. Developed plates were scanned at 254 nm for spot of diacerein followed by derivatization with ninhydrin reagent by heating at 110^0C for 5 min in a preheated oven and scanned at 366 nm for spot of glucosamine. The retention factor for glucosamine and diacerein were found to be 0.23 and 0.81, respectively. Validation of the proposed method was carried out according to International Conference on Harmonization (ICH) guidelines. The current method demonstrates good linearity with correlation coefficients values 0.9996 and 0.9995 for glucosamine and diacerein, respectively. The method was validated for different parameters like precision, recovery and robustness and the values obtained were within ICH limits. For forced degradation studies the drugs were subjected to oxidation, acid and base hydrolysis, dry heat and UV light as per ICH guidelines. Forced degradation studies indicated the suitability of the method for stability studies. Since the method effectively separates the drug from its degradation products it could be used as stability indicating method for analysis of individual drugs and the combined dosage form.
Keywords : Glucosamine, diacerein, HPTLC, stability indicating, Validation.

Introduction

Glucosamine (2-amino-2-deoxy-D-glucose) (Fig. 1-A) is an amino sugar and a prominent precursor involved in the biosynthesis of glycosylated proteins and lipids, acting as a preferred substrate for the biosynthesis of glycosaminoglycan chains, and subsequently, for the production of aggrecan and other proteoglycans of cartilage. Glucosamine is believed to be effective in easing osteoarthritis pain, rehabilitating cartilage, renewing synovial fluid and repairing joints that have been damaged from osteoarthritis. Hence, glucosamine is often prescribed for the treatment of osteoarthritis.[1-4]

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Diacerein1, 8-Diacetoxy-3-carboxyanthraquinoneis a di-acetylated derivative of rhein, (Fig. 1-B) a molecule with ananthraquinone ring. Diacerein is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentration range of pro inflammatory cytokines. In contrast to NSAIDS, diacerein does not inhibit synthesis of prostaglandin and thus it is free form gastrodeodenal toxicity. It also involved in prevention of loss of hydroxyproline and proteoglycans in joint cartilage.[5-9]

Literature survey reveals that several methods based on HPLC chromatographic assay, stabilityassay methods and UV Spectrophotometric methods for glucosamine[10-15] and fordiacerein[16-18] were reported for their individual estimation. Various HPLC and UV spectrophotometric methods are also reported for estimation of the titled analytes in combination with other drugs[19-23]. Literature survey also indicate that there is no stability indicating method available for estimation of the titled analytes; whereas stability indicating method is required for stability studies as per ICH guidelines.[24-29]HPTLCoffer several advantages and is widely employed method for the identification and quantification of pharmaceuticals currently.Advantages offered by HPTLC include short analysis time, reliable and accurate results, low maintenance cost, low mobile phase consumption per sample, multiple or repeated scanning of chromatograms. System configuration is explored for simultaneous analysis of many samples from same or different projects, thus many analysts can use the system simultaneously; there is no need of prior treatment for solvents. Currently HPTLC testsare included in pharmacopoeia due to its high sensitivity and its utility; thus the applications of HPTLC technique are getting wide acceptance.[30-33]The present study describes a simple and validated HPTLC method for the simultaneous estimation of glucosamine and diacerein in presence of their degradation products formed under the applied stress conditions. The validated HPTLC method demonstrates no interferences of degradation products with the assay of active drug components and can be used for estimation of these components in routine analysis as well as stability studies.

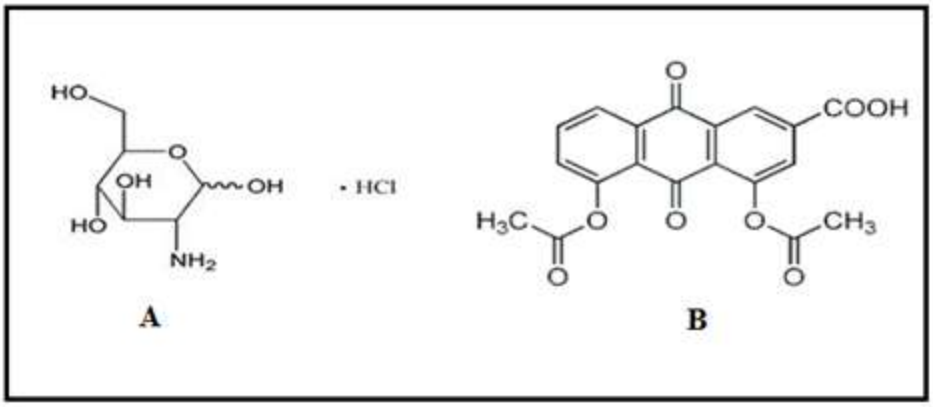

Figure.1: Structure of Drugs, A-Glucosamine and B-Diacerein

Experimental

Solvents and chemicals

Standard drug glucosamine was purchased from Radha Enterprises, Shivajinagar, Pune and Diacerein was supplied as gift sample by Glenmark Pharmaceuticals, Ltd., Kurkumbh, India. All other chemicals such as butanol, acetic acid used in the study was of analytical grade and purchased from Merck Pvt. Ltd., Mumbai. The pharmaceutical dosage form used in this study was Durajoint GM tablets (Abbott Healthcare) each containing glucosamine750 mg and diacerein50 mg was procured from local market.

HPTLC Instrumentation and optimization of chromatographic conditions

The stationery phase used were of TLC plates pre-washed with methanol and activated at 110 ^{0}C for 15 min. Suitable volumes of sample and standard solutions were applied on precoated silica gel aluminium HPTLC plate 60F_{254} (20 cm × 10 cmwith 250 µm thickness; E. Merck, Darmstadt, Germany) in the formof bands of 6

mm width with a Hamilton syringe (100 µl) using a Camag Linomat V (Switzerland) sample applicator. The slit dimension was kept at 5mm × 0.45 mm and 10 mm/s scanning speed was employed. HPTLC plate was then developed, with20 ml mobile phase consisting of n-butanol: Water: Glacial Acetic Acid (7:1.5:1.5v/v/v).Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The chamber saturation time for mobile phase was 25 min atroom temperature. Densitometric scanning was performed using Camag TLC scanner III with winCATS software version 1.4.4.

Detection wavelength and Derivatization method

After chromatographic development the plate wasdried under air current and the bands were scanned over the range of 200-600 nm with ascan speed of 10 mm/s at 254 nm for identification of spot. The same plate was then derivatized. Derivatizing reagent ninhydrin(0.2gm in 100 ml ethanol) was sprayedon the plate by using sprayer. The plate was then heated at 110 °C for 5 min. in a pre-heated oven. Densitometric scanning was performed within 20 min after derivatization process and the bands were again scanned over the range of 200-600 nm with as can speed of 10 mm/s. It was observed that diacerein showed spot before derivatization at 254 nm and glucosamine showed spot only after derivatization at 366 nm. Therefore diacerein was always analysed by scanning the band at 254 nm after plate development. For estimation of the glucosamine, platewas subjected to derivatization process with ninhydrin and scanned at 366 nm.

Preparation of standard stock solutions

Standard stock solutions of pure compounds were prepared separately by dissolving 100mg each of glucosamine and diacerein in 100 ml methanol to get concentration of 1000µg/ml. These stock solutions were sonicated for 15 min and used for further studies. A series of solutions containing mixture of drugs were prepared by transferring appropriate aliquots from standard stock solutions and diluting to volume with methanol. Seven working standard stock solutions containing glucosamine and diacerein from 150 –1050and 10 –120µg/ml, respectively were prepared. The concentrations were fixed, taking into account the proportion in which glucosamine and diacerein are present in tablet formulation used for the study.

Preparation of sample solutions

Twenty tablets were weighed and average weight was determined. An accurately weighed powder sample equivalent to 10 mg of diacerein(150 mg of glucosamine)was transferred to a 100 ml volumetric flask containing50 ml of methanol, flask was sonicated for about 20 min and the volume was made with methanol and the resultant solution was filtered through Whatman filter paper no 41. Working sample solutions were freshly prepared by diluting suitable volumes of the sample stock solution with methanol.

Method Validation

The developed method was validated as per the International Conference on Harmonization(ICH) guidelines[24-26] by evaluating the parameters like linearity, accuracy, precision, robustness, limit of detection, limit of quantification and specificity.

Linearity

Linear regression data over the range of150-1050 ng/spot for glucosamine and 10-120 ng/spot for diacerein were spotted on a TLC plate and were evaluated. The analytes were resolved under optimized chromatographic conditions. Linearity of diacerein was carried out before derivatization and for glucosamine itwas carried out after derivatization. Peak area versus concentration was subjected to least square linear regression analysis and the intercept, slope and correlation coefficient for the calibration were determined. The whole procedure was repeated thrice starting from weighing of analytes to preparation of the standard solution. A good linear relationship between response (peak area) and concentration was obtained. Also, the residual plots of relative response against concentration were plotted, and observed for trending.

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

The limits of detection and limits of quantification of the developed method were calculated using regression equation. It was calculated from the standard deviation of the y-intercepts and slope of the calibration

curves of glucosamine and diacerein using the formulae as LOD=3.3x σ/S and LOQ =10x σ/S, where σ is standard deviation and S is slope of calibration plot.

Precision studies

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. For the precision both repeatability and intermediate precision of the method for drug was checked. For repeatability the peak area of sample band was measured repeatedly (n=6) and the % relative standard deviation (% RSD) was calculated. For intraday precision, the peak area of sample at three different concentrations (450, 600 and 750ng/spot, and 40, 60 and 80ng/spot for glucosamine and diacerein, respectively) were measured for three times (n=3) on the same day. For interday precision same concentrations were applied on plate; plate was developed and scanned on three different days and the % relative standard deviation (% RSD) was calculated.

Accuracy

The accuracy was determined by standard addition method. The studies were performed by spiking three different known quantity of pure standard drug into the sample solution. The sample was spiked with standard at levels 80%, 100% and 120% of test concentration. The quantities of formulation used for spiking at three levels were 600ng/band for glucosamine and 40ng/band for diacerein. The resulting spiked sample solutions were assayed in triplicate and the results were compared with the expected results; % RSD (relative standard deviation) and the mean recovery were calculated.

Assay:

The drug content obtained from standard and the sample was found to be comparable with no interference from the excipients commonly present. For the study 20 tablets were weighed and average weight was calculated. Working sample solutions were prepared by diluting suitable volumes of the sample stock solution with methanol in the conc. range of 50ng/band of diacerein and 750ng/band of glucosamine. The resultant solutions were applied in triplicate to the HPTLC plate. The area of the bands was calculated before derivatization for diacerein at 254 nm and after derivatization for glucosamine at 366 nm.

Robustness studies

Robustness of the method was ascertained by deliberately altering the chromatographic conditions. The effect of small, deliberate variation of the analytical conditions on the peak areas and retention factor of the analytes were examined. Parameters such as change in mobile phase composition (Butanol ±0.1ml), amount of mobile phase (±5%), chamber saturation time (±5 min),time from spotting to chromatography (±5 min), time from chromatography to derivatisation(±5 min) and time from derivatisation to scanning (±5 min)were studied. The effect of these changes on both the R_f values and peak areas were studied by calculating% RSD for each parameter. Robustness of the method was evaluated at concentration level of 600ng/band and 40ng/band of glucosamine and diacerein, respectively.

Specificity

The specificity of the method was evaluated by separating the peaks of both tablet and API. The chromatogram was observed for any unresolved excipient peaks as well as for any coelution with analyte peaks. The $R_f$values of glucosamine and diacerein in the sample was confirmed by comparing it with standard spots. Stress degraded samples of glucosamine and diacerein were analysed to check separation of analyte peak form stress degraded peaks. The chromatograms of sample and stress degraded solutions under study were evaluated for peak purity.

Band Stability

Two-dimensional chromatography using the same solvent system was used to find out any decomposition occurring during spotting and development processes.The time when the sample is left to stand on plate prior to chromatographic development can influence the stability of separated spots and are required to be investigated for validation.

Colour stability:

The time the plate is left to stand after derivatization can influence the colour stability of the analyte bands and are required to be investigated. After derivatisation glucosamine bands were scanned at 366 nm at different time interval after derivatization and area of bands recorded for 5, 10, 20, 30 and 60 min and % RSD was calculated.

Solution (Method) stability:

Stability of solution was studied after 0, 1, 2, 4 hours storage at room temperature. The solutions were applied on TLC plate, plate were developed and scanned before and after derivatisation at 254 nm and 366 nm for diacerein and glucosamine, respectively. The stability of the solutions was determined by comparing peak areas of the developed band at each time point against freshly prepared standard solution and the percentage relative standard deviation was calculated.

Forced degradation study [27-29]

Forced degradation study of glucosamine and diacerein was carried out under acidic, alkaline, neutral, oxidative, photolytic and thermal stress conditions. The objective of stress study was to generate the degradation products under various stress conditions and to verify that the degradation peaks are well resolved from the main peaks by developed method. The stock solutions were prepared separately which contains 100 µg/ml of glucosamine and diacerein each. These solutions were subjected to stress degradation as per the procedures described in following section. The resulting solutions were applied on TLC plates and the chromatograms were run under the conditions described above. The stress degradation studies were performed on the bulk drugs separately and in combined formulation.

Acid and base degradation

For acid / base stress degradation studies,2 ml of working solution was transferred to 10ml volumetric flask, to it 2 ml of degradant (0.01 N HCl/ 0.01 N NaOH) was added and allowed to stand for 20 min at room temperature. The samples were neutralized and diluted up to 10 ml. The resultant solutions were applied on TLC plate and scanned at 254 nm for diacerein, chromatogram was acquired and peak area recorded. Similarly for glucosamine 2 ml of working solution was transferred to 10 ml volumetric flask, to it 2 ml of degradant (0.1 N HCl/ 0.1 N NaOH) was added and allowed to stand for 2 hours at room temperature. The samples were neutralized and diluted up to 10 ml. The resultant solutions were applied on TLC plate; plate was scanned at 254 nm for diacerein and the chromatogram was recorded. For glucosamine the plate was derivatised and scanned at 366 nm and the chromatograms were recorded.

Oxidation

To 2 ml of each working standard solution 2 ml of 3 %v/v of hydrogen peroxide was added and allowed to stand for 2 hours at room temperature. The resultant solutions of diacerein and glucosamine were suitably diluted and applied on TLC plate.

Thermal Degradation and Dry Heat Degradation

Stress testing under neutral conditions was studied by refluxing 2 ml of stock solution in water for 2hours at 60ºC.The resultant solution was suitably diluted and applied on TLC plate and analysed.For dry heat degradation 50 mg of both the drugs were transferred separately to petri plate and was exposed to dry heat at 80 °C for 4 hours. The resultant solution was suitably diluted and applied on TLC plate.

The samples exposed to acid, base, oxidation, thermal and dry heat degradation study were applied as band on TLC plates, plates were developed and scanned at 254 nm before derivatisation for diacerein and at 366nm after derivatisation for glucosamine. The determination of % of analyte degradation, peak area and a $R_f$values of analytes and degraded products were recorded.

Results and Discussion

HPTLC method optimization

Based on literature survey various neat solvents were used to study the chromatographic behavior both the analytes separately. Various mobile phases containing different ratios of n-hexane, butanol, ethanol, methanol, glacial acetic acid, ethyl acetate, acetone and water were tried which gives poorly resolved peaks and no separation of drugs. Based on these experiments ternary mobile phase containing n-Butanol: Water: Glacial Acetic Acid (6:2:2 v/v/v) was initially selected as mobile phase. In this mobile phase peaks were well resolved but the retention factor of one drug was more than 0.88.Furthervarious trials were carried out by changing the composition of the solvents. Finally, the mobile phase consisting of n-butanol: Water: Glacial Acetic Acid (7:1.5:1.5 v/v/v) was selected which gave well resolved peaks without tailing and fronting and the retention factors were in limit for both the analytes. One of the separated compounds was colorless, and scanned over the range of 200-600 nm. This compound gives a compact spot at 254 nm with retention factor 0.81. Other compound does not respond to UV radiation and do not fluoresce. Various mobile phases were used to resolve the issue but none of these shown the spot. At last derivatization technique was used. Several derivatizing agents viz. 10% sulphuric acid, anisaldehyde, 5% sulphuric acid and ninhydrin reagent were used to resolve the problem. By using anisaldehyde and ninhydrin solution the second spot was identified at 366 nm. The most promising and reproducible results with chromophore stability of more than 8hours was shown by ninhydrin reagent; and hence the same was selected for derivatization. The ninhydrin solution was prepared by dissolving 0.20 gm ninhydrin in ethanol in 100 ml volumetric flask and final volume was adjusted with ethanol. Densitometric scanning was performed within 10 min after derivatisation process using Camag TLC scanner III with win CATS software version 1.4.4.Thebands were scanned over the range of 200-600 nm with scan speed of 10 mm/s. It was observed that second drug showed absorbance at 366 nm and the retention factor was found to be 0.23 after derivatisation[Fig.2].

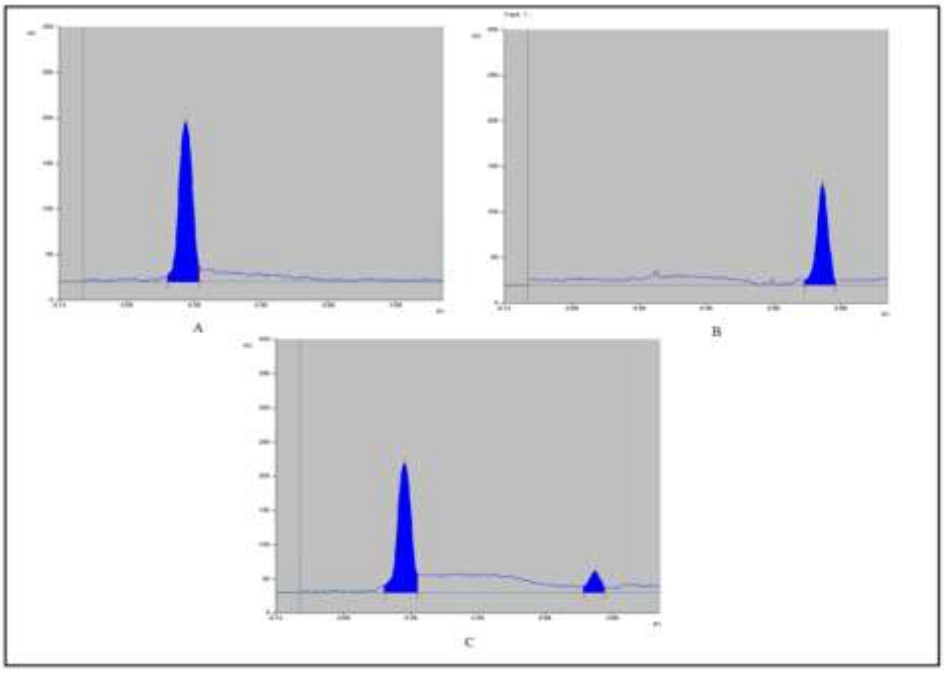

Figure 2:A- Chromatogram of Glucosamine after derivatization (R_f: 0.23 at 366 nm), B-Chromatogram of Diacerein before derivatization (R_f: 0.81 at 254 nm), C-Chromatogram of Glucosamine and diacerein after derivatization at 366 nm.

HPTLC method validation

Linearity, limit of detection and limit of quantitation

Linear regression data for glucosamine and diacerein was computed and a correlation coefficient was obtained. Linear regression data for the calibration plots (n = 3) are listed in [Table 1]. The results were found to be linear over the range of 150-1050ng/spot for glucosamine and 10-120 ng/spot for diacerein. The correlation coefficients values were 0.9996and 0.9995 for glucosamine and diacerein, respectively which shows excellent linearity between the concentration of analytes and chromatographic response. From the above data, values of standard deviation and the slope were calculated for both analytes. Further method sensitivity was determined form the calculated values of LOD and LOQ by using equation as described in procedure section.The LOD and LOQ values were found to be 33.02, 100.07 ng/spot for glucosamine and 4.65, 14.09 ng/spot for diacerein, respectively. To further confirm linearity, F test was applied where the value of experimental Fischer ratio was compared against the critical value found in statistical tables for both analytes; experimental F ratio values were found to be less than the tabulated value at the 95% confidence level[34]. Furthermore the residual plots of relative response against concentration were plotted for glucosamine and diacerein as presented in Figure 3, where residual plots show no trending.

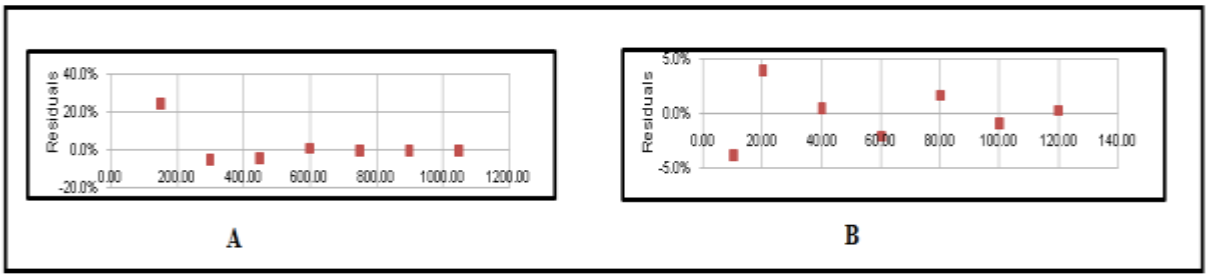

Figure 3: Residual plot of A) Glucosamine; B)Diacerein.

Table1: Summary of linear regression and method sensitivity study(n=3)

Parameter	Glucosamine	Diacerein
Linearity Range	150-1050 ng/spot	10-120 ng/spot
Linear regression equation	y=34.91x-3306	y= 51.22x+922.7
Slope	34.91	51.22
Intercept	3306	922.7
Correlation coefficient	0.9996	0.9995
Limit of detection (LOD)	33.02	4.65
Limit of quantitation (LOQ)	100.07	14.09

Precision

Precision of study was expressed in terms of relative standard deviation (%RSD) of the peak area.It was determined by repeatability and intermediate precision. Study shows that the repeatability, intra- and inter-day variation of the results for glucosamine and diacerein were within the acceptable range. The coefficients of variation for both the inter-day and intraday precision of the method was found to be less than 1 for both drugs indicating a good precision. The summary of intermediate precision study of both the analytes is presented in Table 2.

Table 2: Results of the precision study (n=3)

Drug	Glucosamine			Diacerein		
Concentration (ng/band)	450	600	750	40	60	80
Intraday (% RSD)	0.36	0.49	0.58	0.41	0.72	0.46
Inter day (% RSD)	0.52	0.45	0.47	0.63	0.81	0.38

Accuracy

The recovery study was carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The results of percentage recovery of glucosamine and diacerein at all the three levels were found to be satisfactory. The % recovery was found in the range of 98.23% -99.98% for glucosamine and for diacerein between 99.74% -100.12% [Table 3].

Table3: Recovery studies (n=6)

Drug	Recovery level (%)	Amount (ng/band)		% Recovery	% RSD
		Base level*	Spiked		
Glucosamine	80	600	480	99.23	0.66
	100	600	600	99.98	0.53
	120	600	720	98.93	0.48
Diacerein	80	40	32	100.12	0.84
	100	40	40	99.91	0.46
	120	40	48	99.74	0.68

Analysis of marketed formulation

Marketed formulation was analyzed for the drug content and specificity. The %of drugs was found to be in the range of 99.89 % and 100.20 % for glucosamine and diacerein. There was no interference of the excipients present in the marketed formulations and the low % RSD value indicated the suitability of this method for routine analysis of glucosamine and diacerein in pharmaceutical dosage form [Table 4].The chromatogram of glucosamine and diacerein in tablet dosage form was shown in figure4.

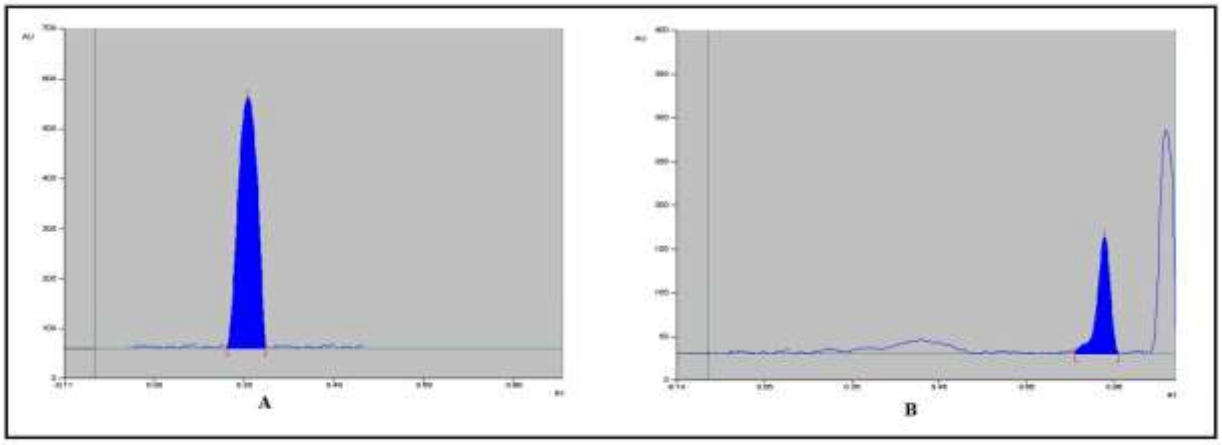

Figure 4: Chromatogram of A) Glucosamine after derivatization at 366 nmB) Diacerein before derivatization at 254 nm in tablet dosage form.

Table 4: Assay results of the pharmaceutical dosage form (n= 6)

Amount of drug present (mg per tablet)	% Assay	% RSD
Glucosamine 750mg	99.89	0.56
Diacerein 50mg	100.20	0.49

Robustness studies

Robustness of the proposed method was evaluated by deliberate alterations of the analytical parameters. The study indicated that areas of peaks of interest and retention factor remained unaffected by small changes in the parameters. Percentage (%) RSD of the test results for the selected parameters at different condition was calculated and found within the ICH limit (% RSDNMT 2%), indicating that the method is sufficiently robust to analyze glucosamine and diacerein in pharmaceutical dosage form, Table 5 indicates robustness of the method.

Table 5: Results of robustness study (n=6)

Parameter	**Parameter Level**	**Glucosamine**		**Diacerein**	
		% Assay, %RSD	R_f value	% Assay, %RSD	R_f value
Volume of mobile phase	20 ±1 ml	99.45, 0.57	0.25	99.67,0.83	0.82
Time of Chamber saturation	20± 5 min	100.2, 0.48	0.23	100.3,0.23	0.81
Composition of mobile phase (Butanol volume)	7±0.1ml	99.85,1.2	0.24	99.58,0.85	0.82
Time from chromatography to Scanning at 254 nm	15±5 min	----	----	100,0.89	0.81
Time from Scanning to derivatisationat 366 nm	15±5 min	100.2,0.98	0.23	----	-----
Time from derivatization to scanning at 366 nm	10±5min	100.37,0.86	0.23	----	----

Specificity

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form. The chromatograms were recorded for blank and the formulations and compared with that of standard, where no additional peaks were observed. Good correlation was also obtained between standard and sample chromatogram of glucosamine and diacerein. The results of the stress degradation study show adequate resolution of the degraded product peaks form both analyte peaks which confirm the specificity of the method [Fig. 4].Under the optimized chromatographic conditions; degradation products of both analytes were well resolved which was further confirmed by peak purity study [Table 6]. Peak purity r (s, m) and r (s, e) values were above 0.99; indicating homogeneous peaks which confirms the method was specific.

Solution (Method) stability:

Stability of solution as described in method development was studied. The solution was assayed after 1, 2, 4; 6 hr. and the results were compared. The R_f value of 0.23 for glucosamine and 0.81 for diacerein shows no significant difference. The peak area of glucosamine and diacerein were found within the acceptable limit. The % RSD was found to be less than 2.0 indicating no significant degradation within the indicated period.

Forced degradation study

Forced degradation study of glucosamine and diacerein under different stress conditions was carried out as described in procedure section. The main peaks of the pure drugs were well resolved from their degraded products. The chromatograms obtained after degradation under various conditions are shown in the Figure5. From the chromatograms it was clear that glucosamine was stable under the applied stress conditions whereas diacerein shows maximum degradation in alkaline hydrolysis. The degradation of diacerein in acidic condition was 27.7% and in basic condition the degradation was 35.74% which suggest that the drug is very sensitive to basic condition. The degradation under oxidative stress and thermal stress was 10.16% and 5.3% respectively. The amount of drug recovered after degradation studies, the R_f value of degradation products and peak purity test data is presented in Table 6.

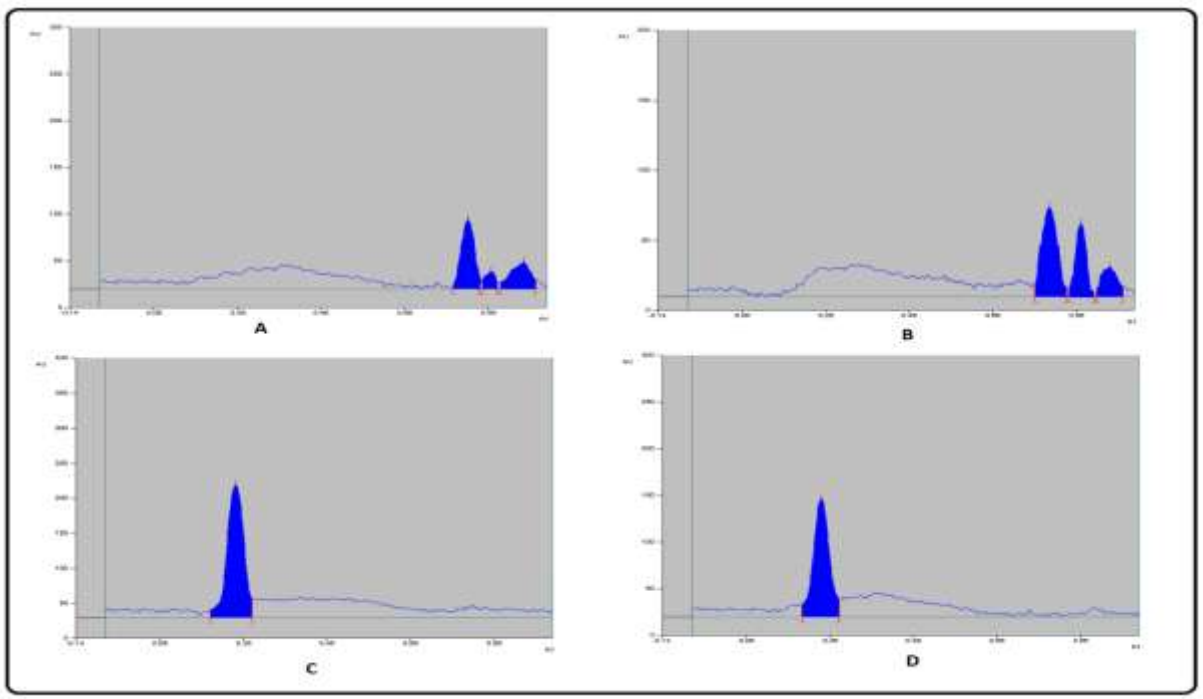

Figure 5: Forced degradation study Densitogram A) Acid degradation of Diacerein B)Base degradation of Diacerein and C)Acid degradation of Glucosamine D) base degradation of Glucosamine.

Table 6: Results of forced degradation study

Stress Conditions	Glucosamine			Stress Conditions	Diacerein		
	% Assay	R_f value of degraded products	Peak Purity r(S,M), r(M,E)		% Assay	R_f value of degraded products	Peak Purity r(S,M), r(M,E)
Acid 0.1 N HCl,2 hrs at RT	98.56	Not detected	0.9972, 0.9969	Acid 0.01 N HCl,20 min at RT	72.3	0.86, 0.92	0.9968, 0.9975
Base 0.1 N NaOH, 2hrs RT;	93.63	Not detected	0.9936, 0.9952	Base 0.01 N, NaOH 20 min at RT	64.26	0.86,0.92	0.9948, 0.9981
H_2O_2 3 % w/v,2 hrs R.T	98.62	Not detected	0.9974, 0.9986	H_2O_2 3 % w/v,2 hrs R.T	89.84	0.88	0.9971, 0.9984
Wet (Thermal)heat 60ºC), 2 hrs	98.32	Not detected	0.9988, 0.9951	Wet (Thermal)heat 60ºC), 2 hrs	94.63	0.88,0.92	0.9963, 0.9928
Dry Heat, 4.0 hrs(80ºC)	98.85	Not detected	0.9973, 0.9972	Dry Heat, 4.0 hrs(80ºC)	97.25	Not detected	0.9978, 0.9983

Conclusion

A validated stability-indicating HPTLC method for the estimation of glucosamine and diacereinin bulk drug and in formulation has been developed. Stress degradation in acidic, alkaline, neutral, oxidative, photolytic and thermal conditions was performed. The method was capable of accurately estimating glucosamine and

diacerein simultaneously in the presence of all possible degradation products. The procedure has provided solution to the TLC chromatographic problem by using derivatization of non UV absorbing analyte. The advantage of the present HPTLC method over reported methods is that the technique offers fast, low-cost analysis without compromising accuracy, specificity and sensitivity. The standard deviations, % RSD for the method are low, indicating a high degree of precision. Hence, it can be concluded that the developed HPTLC method is accurate, precise, robust, selective and stability indicating nd can be employed successfully in the estimation of glucosamine and diacerein in bulk drug and in pharmaceutical formulation.

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