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Development and validation of green chemistry HPLC-RI method for the simultaneous estimation of glucosamine and chondroitin sulfate from drug products

Abstract

Design and development of analytical methods with environment friendly reagents and solvents is the need of the hour for labs engaged in analytical services. Every year thousands of chemical and pharmaceutical laboratories worldwide are generating tons of toxic chemical wastage causing environmental pollution thereby endangering the human existence. The objective of this study was to develop and validate a sensitive, stability-indicating, accurate and precise green chemistry RP-HPLC method with RI detector for the simultaneous quantitation of glucosamine and chondroitin sulfate from various pharmaceutical dosage forms. Complete separation of both the actives was achieved in isocratic mode by using Hypersil BDS Phenyl (250 x 4.6 mm, 5µm) HPLC column. Purified water as a diluent and phosphoric acid buffer pH 2.5 was used as a mobile phase at a flow rate of 0.2 mL/min. The column temperature was maintained at 40°C. Both the molecules being non-chromophoric in nature, refractive index (RI) detector was used for detection.

The proposed method being environment friendly does not require organic solvents, gradient elution and complex derivatization unlike the reported methods. The developed method is successfully validated as per ICH guidelines. The method is stability indicating, sensitive and economical. Hence, it can be successfully used for the routine analysis of commercial batches of these combination products.

Keywords: glucosamine, chondroitin sulfate, HPLC-RI detector, green chemistry, development, validation

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Abbreviations: RP-HPLC, reverse-phase high-performance liquid chromatography; RI Refractive index detector; UV ultra-violet; BDS base deactivated silane; ICH International conference on harmonization; USP United States Pharmacopeia; LOD limit of detection; LOQ, limit of quantitation; STDEV, standard deviation; RSD, relative standard deviation; mg milligram; °C degree centigrade; RH, relative humidity; m2 meter square; N normality; PPM parts per million

Background

It is the right time for the chemical and pharmaceutical industries to adopt green chemistry practices like usage of environment friendly chemicals, reagents and solvents during synthesis and analysis of pharmaceutical products to reduce the risk to environment and their exposure to human beings. This environment friendly chemistry approach is known by many names such as green chemistry,[1–3] environmentally benign chemistry, clean chemistry, atom economy[4] benign by design chemistry etc.[5] Unfortunately, industries and scientists worldwide are giving more attention on the disposal of generated toxic waste to reduce the pollution rather than adopting an environmental friendly green chemistry approach to prevent the pollution. In the present article, we have successfully developed a sensitive green chemistry analytical method for the simultaneous estimation of glucosamine and chondroitin sulfate from various pharmaceutical dosage forms by using simple RP-HPLC method with RI detector.

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar in which hydroxyl group is replaced by an amine group. It is one of the most abundant monosaccharides[6] found within the cartilage of joints in humans. It is body's natural shock-absorbent and a joint lubricant. It is available in salt form such as glucosamine sulfate or hydrochloride. The IUPAC name of glucosamine (sulfate) is 3-amino-6-(hydroxy methyl) oxane-2, 4, 5-triol; sulfuric acid (Figure 1a). Chondroitin sulfate ($C_{13}H_21NO_{15}S$) is normally found in human and animal cartilage around joints in the body. It is usually manufactured from animal sources, such as shark and cow cartilage. The IUPAC name of chondroitin sulfate is (2S,3S,4S,5R,6R)-6-[(2R,3R,4R,5R,6R)-3-acetamido-2,5-dihydroxy-6-sulfooxyoxan-4-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid (Figure 1b). Both these molecules have been used individually or together with other ingredients for the preparation of various pharmaceutical anti-inflammatory medications such as tablets, capsules, creams, gels and solutions etc. for the treatment of osteoarthritis.[7,8]

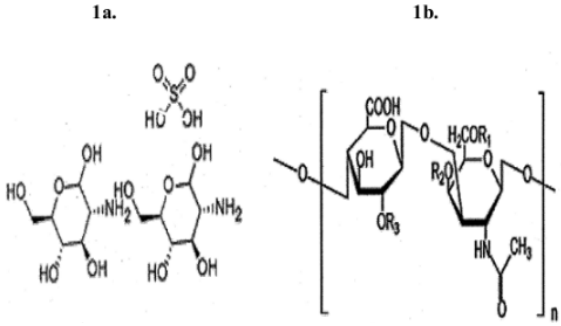

Figure 1 Chemical structures of glucosamine and chondroitin sulfate.

Due to the lack of a chromophore, glucosamine is not detected by using the UV-visible detector. During the literature review, we found

that few authors have derivatized glucosamine by using fluorogenic reagents like ortho-phthalaldehyde (OPA), phenylisothiocyanate, and 9-fluorenylmethoxycarbonyl chloride, to form fluorescent compounds which is detected by HPLC coupled with a fluorescence detector.[9,10] The other reported method for glucosamine estimation include reverse phase ion-pairing HPLC-RI.[11] The reported methods for chondroitin sulfate include titration with cetylpyridinium chloride[12] and size exclusion chromatography[13] both the methods are non-specific and cannot distinguish between chondroitin sulfate and related glycosaminoglycan's (GAGs). The reported methods for combination products include ion exchange chromatography with a fluorescence detector after enzymatic digestion and HPLC with UV detector after enzymatic hydrolysis,[14] isotachophoresis and ionic analyte separation using electrophoresis with UV detection[15] HPAE-PAD anion-exchange chromatography using a CarboPac PA20 column,[16] RP-HPLC with DAD detector after 8 h hydrolysis,[17] and RP- HPLC with a fluorescence detector.[18,19] All the reported methods are quite complex, time consuming and need organic solvents and toxic reagents. The reported RP- HPLC methods with UV detector[20–22] lack in sensitivity, resolution and peak symmetry. Due to the lacks of chromophores in glucosamine and wide molecular weight variation of chondroitin sulfate polymers with strong ionic nature the qualitative and quantitative analysis of glucosamine and chondroitin sulfate is extremely challenging. Because of the unavailability of peak symmetry, resolution, precise, accurate and sensitive analytical methodology, the commercially available products of these molecules showed significant variation in the assay results [23,24] with the reported methods. Hence, in this article an attempt has been made to develop a simple, economical, sensitive and accurate environment friendly analytical method for the simultaneous determination of both the compounds from tablet and cream dosage forms.

Methods

Materials and chemicals

Milli Q (Make- Merck Millipore, Germany) HPLC grade water was used throughout the study for the preparation of mobile phase, standard and samples. AR grade ortho-phosphoric acid (Make-Rankem, India) was used for mobile phase pH adjustment. 0.45 µm nylon syringe filters (Make-MDI Membrane Technologies, India) were used for sample filtration. AR grade sodium hydroxide (Make-Rankem, India), AR grade hydrochloric acid (Make-Rankem, India) and AR grade hydrogen peroxide (Make-Rankem, India) was used for forced degradation study. Glucosamine sulfate (potency 98.0%) and Chondroitin sulfate sodium (potency 97.5%) standard (Make-Sigma Aldrich, India) was used. Tablets manufactured by Sava Healthcare Ltd and cream manufactured by Celest Pharma Labs Pvt. Ltd, India were used for the study.

Equipment

Alliance e2695 separation module with RI detector (Make – Waters, USA) was used for HPLC method development and validation studies. The HPLC instrument consisted of a quaternary gradient pump, an online degasser, an auto-sampler and a thermostatically controlled column compartment. The chromatographic data was recorded by using an Intel Xeon CPU E3-1225v5 3.30GHz computer system installed with Waters Empower® 3 software for the analytical data processing.

Chromatographic conditions

Mobile phase was prepared by adjusting the HPLC grade water to pH 2.5 with ortho-phosphoric acid. The solution was sonicated and filtered through a 0.45 µm filter before use. Hypersil, BDS Phenyl, 250 x 4.6 mm, 5 µm particle size HPLC column was used. The analysis was performed at 0.2 mL flow rate at 40°C column temperature. The detection was performed by using RI detector at internal detection temperature 40°C and detector sensitivity 128. Before the analysis, all ports, injector loop and detector were purged with HPLC grade water. The HPLC system with column and detector was equilibrated with mobile phase for 4 hours for smooth baseline.

Preparation of mix standard solution

A 500 µg/mL glucosamine and 400 µg/mL chondroitin sulfate mix standard solution was prepared in HPLC grade water.

Preparation of sample solution

Glucosamine and chondroitin sulfate tablets and cream formulations were used for the method development and quantitation studies.

Tablet and cream sample equivalent to 500 mg glucosamine and 400 mg chondroitin sulfate was accurately weighed and dissolved in 500 mL water with sonication and intermediate shaking for 30 minutes for tablet and 10 min vortex for cream formulation. The solution was filtered and 10 mL was further diluted to 20 mL with diluent.

HPLC method development and optimization

The present work was aimed for developing a simple, rapid, economical and green chemistry RP-HPLC assay method for glucosamine and chondroitin sulfate combination from different dosage forms. The RP-HPLC with universal RI detector assay method was successfully developed on Hypersil BDS, phenyl column in isocratic elution mode with good resolution. The developed method does not require any organic solvent and toxic reagents.

Due to the absence of chromophore in glucosamine and high molecular weight of chondroitin sulfate, the detection and proper separation of analytes was difficult on HPLC with ultra-violet (UV) detector. RI detector was chosen for the study since it is universal and affordable as compared to fluorescence, mass spectroscopy (MS) and evaporative light scattering (ELSD) detectors. The sensitivity of the RI detector depends on the difference in the refractive index of the mobile phase and the analyte. To improve the detection sensitivity, the HPLC chromatographic conditions were optimized. Both, glucosamine and chondroitin sulfate are freely soluble in water; hence water was used as a diluent. Initially method development trials were initiated with water as a mobile phase and then by using low refractive index solvents such as methanol and acetonitrile in different ratio to achieve good detection and peak resolution. Best resolution with steady baseline without background noise was obtained by adjusting the water pH to 2.5 with ortho-phosphoric acid. Other organic solvents were not considered due to its toxicity towards environment. Different HPLC columns having C18, C8 and Phenyl packing were evaluated for proper resolution and peak shape. C18 and C8 columns, being more hydrophobic as compared to phenyl column, showed poor peak shape. Best resolution for both the analytes was observed with Hypersil BDS, phenyl (250 x 4.6 mm, 5 mm) column. Water was found to be the most suitable diluent during the recovery study. The diluent, placebo, standard and sample solution chromatograms are reported in Fig. 2 a, b, c and d respectively.

System suitability

The system suitability test was performed by injecting five replicates of the mix standard solution. The relative standard deviation

(RSD) for peak area and retention time was found to be less than 2.0%, tailing factor of both the peaks was found to be less than 1.5. Theoretical plates for glucosamine and chondroitin sulfate were 7000 and 1000 respectively. The system suitability results are reported in Table 1.

Calculations

Equal volume of both the standard and sample solutions were injected in to the HPLC system and area under the curve for each analyte peak was recorded. The amount of glucosamine and chondroitin sulfate in % was calculated.

Analytical method validation

Validation of the analytical method was successfully done according to the International Conference on Harmonization (ICH) guidelines[25–27] for the tablet dosage form. The method was validated for specificity (selectivity and forced degradation study), sensitivity (LOD and LOQ determination), linearity and range, precision, accuracy, filter interference study, stability of analytical solutions and robustness.

Specificity

Selectivity

Selectivity study is carried out to prove the ability of a method to assess unequivocally the analyte in the presence of components which may be expected to be present in sample. To prove the selectivity of method diluent, placebo, standard solution and sample solution were prepared as per the described method and injected in to the HPLC system for to check the any interference from diluent and placebo at retention time of both actives.

Forced degradation

To prove the stability-indicating nature of the developed analytical method, the forced degradation study was performed on placebo and sample through different stress conditions like thermal, photo, humidity, acid, base and oxidative degradation. For thermal degradation placebo and sample was kept at 60°C for 2 Days, for photolytic degradation placebo and sample was kept in photolytic chamber at 1.2 million lux hours and 200 watt hours/m^2, for humidity degradation placebo and sample was kept at 40°C/75% RH for 7 days. For acid degradation, 50 mL of 0.1 N hydrochloric was added in the placebo and sample and kept at room temperature for 5 hours and the sample was neutralized with the 0.1N NaOH solution. For basic degradation, 50 mL of 0.1 N NaOH solution was added in the placebo and sample and kept at room temperature for 5 hours, the sample was neutralized with 0.1N HCl solution. For oxidative degradation, 50 mL of 3% hydrogen peroxide was added to the placebo and sample and kept at room temperature for 24 hours. All degraded samples are prepared as per described method and prepared samples are analyzed.

Sensitivity (LOD and LOQ determination)

The sensitivity of the analytical method was estimated in terms of LOD and LOQ which was defined based on signal to noise ratio of 3:1 and 10:1 respectively and calculated from the calibration line preparing the solutions at 2.5-50 µg/mL concentration levels for both the actives. The lowest concentration that could be quantitatively determined with acceptable accuracy and precision was considered as LOQ.

Linearity and range

The linearity study was performed at 9 different concentration levels and each level injected in triplicate. The calibration curve was constructed by plotting concentration on X-axis and average peak area on Y-axis. Regression equation and value of co-relation coefficient (r) was calculated using linear regression analysis. The developed method was checked for upper and lower amount of both the analytes in the sample for which it has been demonstrated that the method has suitable level of precision, linearity and accuracy.

Precision

The precision of an analytical process defines the goodness of agreement among the series of measurements acquired from many identical samples. As per ICH validation guidelines, system precision, intra day (method precision), and inter day (intermediate precision) were evaluated.

System precision

The standard solution was injected in six replicates as described in analytical method and the % RSD was determined. The % RSD for peak area of both the analytes for six identical injections of standard solutions was set at not more than 2.0%.

Intra day (method precision)

The method precision was carried out by preparing six samples of a single batch. The % assay of the six samples was calculated. The precision of the method was examined by calculating the % RSD of the results.

Inter day (intermediate precision)

Intermediate precision expresses ability of method to produce reliable results under laboratory conditions, viz., different days, analysts, system, and column. Six samples were prepared as per the test procedure by using the same batch of formulation and injected. The % assays of these samples were examined and the ruggedness of the method was estimated by calculating the % RSD of the results.

Accuracy

The accuracy of an analytical method reflects the closeness of agreement between the values that is acceptable either as a conventional true or an accepted reference value, and the value observed by the technique. The accuracy of the method was evaluated by spiking both the actives in placebo at four different concentration levels (LOQ, 50, 100, and 150%) and analyzed as per the described method. The mean % recovery for the analytes at each concentration level should be in the range of 98- 102% and % RSD of % recovery for the analytes at every level must not be greater than 2.0% as per ICH guidelines.

Filter interference study

The filter interference study was performed to evaluate the suitability of filter for sample preparation. Performed on 0.45 µ Nylon (mdi) filters by discarding the 0 mL, 2mL and 5 mL sample through filter against the centrifuged sample at 5000 rpm.

Stability of analytical solutions

The standard solution and sample solution was prepared on day zero of experiment and stored at room temperature. The solution was analyzed on subsequent days for 2 days. The standard solution used was prepared freshly for the investigation and the assay results were calculated for sample solution to evaluate the stability sample of solution. The solutions are considered stable, if the cumulative % RSD of the stored sample and standard solution is not more than 2.0.

Robustness

Robustness of an analytical method measures the capacity to stay unaffected by minor changes in the method parameters. This study was performed by introducing small variations in flow rate (0.2 ±0.02 mL/min), column oven temperature (40 ±5°C), and mobile phase pH (2.5 ±0.2) to evaluate the methods' capacity to remain unaffected by small changes in chromatographic parameters.

Results

Specificity (Selectivity and Forced degradation study)

During the selectivity study diluent and placebo solution did not show any interfering peaks at the retention time of chondroitin sulfate (about 10min) and glucosamine (about 20min). The representative overlay HPLC chromatogram for diluent, placebo and sample solution is shown in Figure 2&3.

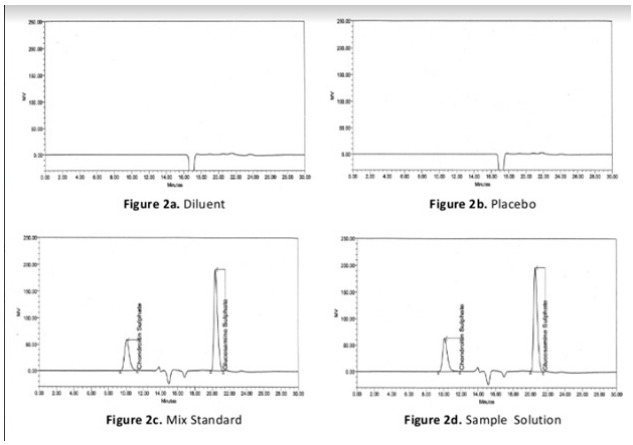

Figure 2a. Diluent **Figure 2b.** Placebo

Figure 2c. Mix Standard **Figure 2d.** Sample Solution

Figure 2 Diluent, placebo, mix standard and sample solution chromatograms as per described method.

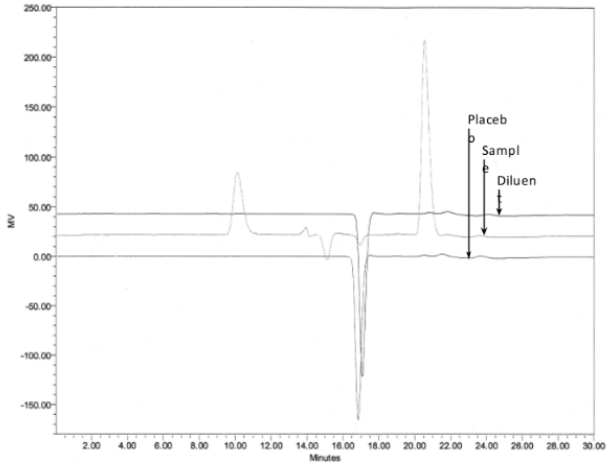

Figure 3 Overlay chromatograms of diluent, placebo and sample solution as per described method.

The stress conditions and absolute % degradation in the assay of both the actives were determined against the control sample (Table 1). The results obtained during forced degradation study are reported in Table 2.

Sensitivity (LOD and LOQ determination)

Calibration line from 2.5-50 µg/mL for both the actives was plotted. The statistically obtained LOD and LOQ for glucosamine and chondroitin sulfate were found to be 1.8 µg/mL and 3.2 µg/mL and 5.49 µg/mL and 9.62 µg/mL respectively. The method was capable to calculate 5 µg/mL concentrations of both the actives with acceptable accuracy and precision. Based on recovery of quantification at LOQ level (5 µg/mL), the average % recovery for glucosamine was 100.1% with 1.04% precision RSD and 98.7% with 0.73% precision RSD for chondroitin sulfate. The signal to noise ratio at LOQ level (5 µg/mL) concentration level for glucosamine and chondroitin sulfate were found to be 74.0 and 22.0 respectively, hence the 5 µg/mL was LOQ for both the actives.

Linearity

The linearity of calibration curve was evaluated using linear regression analysis. For glucosamine regression equation was: y = 10775x + 26618 and for chondroitin sulfate: y = 5845x – 50082. The observed co-relation coefficient (r) was 1.0000 for both actives. The linearity of the method was observed over the concentration range of 5-770 µg/mL with r value 1.000 for glucosamine and 5–700 µg/mL with r value 1.000 for chondroitin sulfate under the experimental conditions. For linearity study refers the Figure 4.

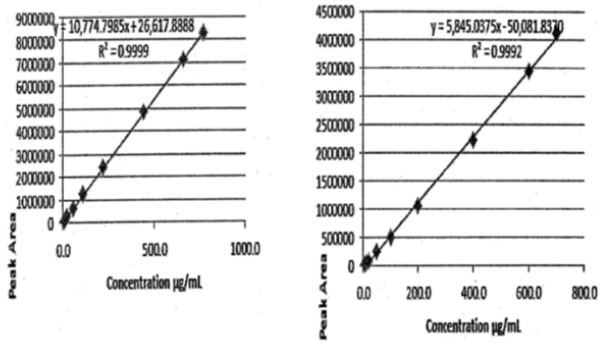

Figure 4a. Linearity curve for Glucosamine **Figure 4b.** Linearity curve for Chondroitin Sulfate

Figure 4 Linearity curve for glucosamine and chondroitin sulfate as per described method.

Precision

The intra day and inter day precision study was performed on six samples on same day (intra-day precision) and different days (inter-day precision). The obtained overall % RSD for glucosamine was 1.38 and for chondroitin sulfate was 0.78%. The results are reported in Table 3.

Accuracy (recovery)

The percent recovery for glucosamine and chondroitin sulfate was found to be 98.7 to 101.9% and 98.2 to 100.4 respectively. The accuracy (recovery) study results are reported in Table 4.

Filter interference study

The observed absolute % difference in the area of sample between the centrifuged and filtered solution was found to be less than 1.5 for both the actives. The filter interference study results are reported in Table 5.

Stability of analytical solutions

No significant change in assay result was observed up to 2 days for both standard and sample solutions. The results are reported in Table 6.

Citation: Kulkarni S, Sawant A, Zinjad P, et al. Development and validation of green chemistry HPLC-RI method for the simultaneous estimation of glucosamine and chondroitin sulfate from drug products. *J Appl Biotechnol Bioeng.* 2022;9(3):67–74. DOI: 10.15406/jabb.2022.09.00287

Table 1 System suitability study results

Inj. No.	Chondroitin sulfate			Glucosamine		
	Area	Tailing factor	Theoretical plates	Area	Tailing factor	Theoretical plates
1	2289404	1.4	1296	5185076	1.3	7522
2	2233613	1.5	1276	5266294	1.3	7530
3	2235308	1.4	1284	5259680	1.3	7515
4	2249476	1.4	1276	5267904	1.3	7515
5	2260177	1.4	1278	5258161	1.3	7532
Average	2253596			5247423		
STDEV	22783.04			35100.83		
% RSD	1.01			0.67		

Table 2 Forced degradation study results

S.N.	Sample / Degradant	Degradation condition	Glucosamine %	Chondroitin %
	Control Sample	Not applicable	102.4	100
			% absolute degradation in assay	
1	Thermal degradation	60°C for 2 Days	4.4	8.7
2	Photolytic degradation	1.2 million lux hours and 200 watt hours/m2	5.1	6.7
3	Humidity degradation	40°C/75% RH for 7 days	19.7	12
4	Acid hydrolysis	0.1N HCl for 5 hours at room temperature	0.3	5
5	Alkali hydrolysis	0.1N NaOH for 5 hours at room temperature	1.1	4.4
6	Oxidative degradation	3% H2O2 for 24 hours at room temperature	3.8	5.7

Table 3 Intra-day and inter-day precision study results

Sample	Glucosamine % Assay		Chondroitin sulfate % Assay	
	Intra day	Inter day	Intra day	Inter day
1	102.1	100.2	99.8	99.7
2	102.5	100.8	99.5	99
3	102.3	101	98.9	100.8
4	102.9	99.3	101	100.3
5	103.1	101.2	100.7	101.2
6	101.7	104.3	100.1	100.9
Overall Average	101.8		100.2	
Overall STDEV	1.37		0.78	
Overall % RSD	1.35		0.78	

Table 4 Recovery study at various concentration levels

		Glucosamine				Chondroitin sulfate			
Levels	S. N.	Added (µg/mL)	Recover (µg/mL)	(%) Recovery	% RSD	Added (µg/mL)	Recover (µg/mL)	(%) Recovery	% RSD
LOQ	1	5.16	5.15	99.8	1.04	4.1	4.03	98.3	0.73
	2	5.11	5.21	101.9		4	3.93	98.3	
	3	5.21	5.21	100.1		3.94	3.87	98.2	
	4	5.17	5.11	98.7		4.02	4.01	99.8	
	5	5.15	5.16	100.2		4.07	4	98.3	
	6	5.19	5.18	99.8		3.98	3.96	99.5	
50%	1	258.94	262.53	101.4	0.52	200.2	198.41	99.1	0.57
	2	259.73	261.24	100.6		198.2	194.97	98.4	
	3	262.53	266.66	101.6		199.2	198.26	99.5	
100%	1	509.87	516.48	101.3	0.15	400.45	401.75	100.3	0.26
	2	510.51	516.2	101.1		400.71	402.19	100.4	
	3	510.29	517.5	101.4		400.18	399.68	99.9	
150%	1	764.35	776.84	101.6	0.1	602.78	601.81	99.8	0.21
	2	765.11	776.5	101.5		600.42	600.81	100.1	
	3	765.94	776.88	101.4		603.45	601.39	99.7	

Citation: Kulkarni S, Sawant A, Zinjad P, et al. Development and validation of green chemistry HPLC-RI method for the simultaneous estimation of glucosamine and chondroitin sulfate from drug products. *J Appl Biotechnol Bioeng.* 2022;9(3):67–74. DOI: 10.15406/jabb.2022.09.00287

Table 5 Filter Interference study results

Sample Solution	Chondroitin sulfate	Glucosamine
	Absolute % Difference in Area	Absolute % Difference in Area
Centrifuged	NA	NA
0 mL discarded	1.14	1.24
2 mL discarded	0.19	0.04
5 mL discarded	0.16	0.11

Table 6 Solution stability study results

Solution Stability data for Standard solution						
Time point	% Assay for Chondroitin sulfate	Cumulative Average		STDEV	% RSD	
Day-0(Initial)	100	NA		NA	NA	
Day-1	101.7	100.9		1.2	1.19	
Day-2	100.3	100.7		0.91	0.9	
Time point	% Assay for Glucosamine	Cumulative Average	STDEV			% RSD
Day-0(Initial)	100	NA	NA			NA
Day-1	98.5	99.3	1.06			1.07
Day-2	101.5	100	1.5			1.5
Solution Stability data for Sample solution						
Time point	% Assay for Chondroitin sulfate	Cumulative Average	STDEV			% RSD
Day-0(Initial)	97.8	NA	NA			NA
Day-1	99.2	98.5	0.99			1.01
Day-2	96.2	97.7	1.5			1.54
Time point	% Assay for Glucosamine	Cumulative Average		STDEV		% RSD
Day-0(Initial)	102.1	NA		NA		NA
Day-1	100.2	101.2		1.34		1.32
Day-2	103.9	102.1		1.85		1.81

Table 7 Comparative evaluation of the existing analytical methods with the developed method

S.N.	Reported methodology	Any Organic solvents, chemicals used	For Glucosamine	For Chondroitin sulfate	Method reference
1	RP-HPLC-UV	Acetonitrile, Sodium Octane sulfonate	LOD – Not reported	LOD – Not reported	Zeng-yuan NIU, 2006[21]
			LOQ – Not reported	LOQ – Not reported	
			Linearity range - 10 - 1500 µg/mL	Linearity range- 10 – 750 µg/mL	
2	HPLC-RI	Methanol, Octane sulfonate	LOD – Not reported	Not reported	Way WK, 2007,[11]
			LOQ – Not reported		
			Linearity range - Not reported		
3	LC-UV-DAD	Methanol, Sodium acetate, Hydrochloric acid, alkaline media	LOD – Not reported	LOD – Not reported	Gatti R, 2010,[17]
			LOQ – 60 pmol	LOQ – 60 pmol	
			Linearity range - Not reported	Linearity range - Not reported	
4	HPLC-UV	Acetonitrile, Ammonium dihydrogen phosphate	LOD – Not reported	Not reported	Jin P, 2011[22]
			LOQ – Not reported		
			Linearity range – 1000 – 3000 µg/mL		
5	HPLC-UV	Acetonitrile, Octane sulfonic acid, Triethyl amine, Ortho-phosphoric acid	LOD – Not reported	LOD – Not reported	Nagarajan P, 2013, [20].
			LOQ – Not reported	LOQ – Not reported	
			Linearity range – 800 – 1200 µg/mL	Linearity range – 650 – 950 µg/mL	

Citation: Kulkarni S, Sawant A, Zinjad P, et al. Development and validation of green chemistry HPLC-RI method for the simultaneous estimation of glucosamine and chondroitin sulfate from drug products. *J Appl Biotechnol Bioeng.* 2022;9(3):67–74. DOI: 10.15406/jabb.2022.09.00287

Table Continued...

S.N.	Reported methodology	Any Organic solvents, chemicals used	For Glucosamine	For Chondroitin sulfate	Method reference
6	HPLC-Fluorescence	Acetonitrile, Tetrahydrofuran, Sodium hydroxide	LOD – 5.51 µg/mL	LOD – 154.31 µg/mL	Harmita H, 2017, [18].
			LOQ – 18.38µg/mL	LOQ – 516.02 µg/mL	
			Linearity range – 5 - 80µg/mL	Linearity range – 100 – 1000 µg/mL	
7	RP-HPLC-UV visible by derivatization for only glucosamine	Acetonitrile, Methanol, Acetic acid, Hydrochloric acid, Sodium acetate, Sodium borate, Derivatizing reagents etc.	LOD – Not reported	USP reports Titrimetric method for Chondroitin	USP 43.
			LOQ – Not reported		
			Linearity range – 500 - 1200µg/mL		
8	RP-HPLC-RI	Ortho-phosphoric acid	LOD – 1.8µg/mL	LOD – 3.2µg/mL	Present study data.
			LOQ – 5.0µg/mL	LOQ – 5.0µg/mL	
			Linearity range – 5 - 770µg/mL	Linearity range – 5 – 700µg/mL	

Robustness

% RSD of peak area, tailing factor, theoretical plates and retention time for both the actives were monitored during the study and found within acceptance limit of 2%.

Discussion

In this paper, we have successfully attempted and developed green chemistry simultaneous RP-HPLC with RI detector methodology for efficient resolution of glucosamine and chondroitin sulfate from each other and from their degradation peaks in tablet and cream dosage forms. While chondroitin sulfate eluted at about 10 minute and glucosamine eluted at about 20 minute under the described HPLC conditions. The purpose of this study included establishing a green chemistry method which capable of separating and evaluating glucosamine and chondroitin sulfate efficiently in the shortest feasible run time with reasonable accuracy and reliability. The green chemistry analytical methodology approach was successfully implemented for the RP-HPLC method development. From the literature review, it is clear that in reported methods stress study data is not available, the LOD and LOQ values are either not reported or found more than the developed method. Table 7 summarizes comparative evaluation of the existing analytical methods with the developed method (Table 7 is here). A forced degradation study was performed by applying various stress conditions to the samples to evaluate stability-indicating nature of the developed method. During the forced degradation study, we observed that degradation of both glucosamine and chondroitin sulfate was more significant in humidity condition as compared to other degradation conditions. The developed method was validated as per ICH guidelines, the validated method was found to be linear, precise, accurate, specific, robust, and timesaving with without usage of organic solvents.

Conclusion

An attempt has been made in this article to develop an analytical method based on the green chemistry fundamentals. The proposed method does not require any organic solvent, gradient elution and derivatization. The method is sensitive at low levels (5 µg/mL) for both the actives. The developed method is successfully validated as per the ICH Q2(R1) validation guidelines for various parameters. Hence, the developed method can be successfully used for the simultaneous estimation of both the compounds from tablets and creams dosage form during the commercial manufacturing.

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Conflicts of interest

The authors state that there is no conflict of interest.

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