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Article

Development and validation of a HILIC-HPLC-ELSD method for simultaneous determination of glucosamine hydrochloride and chondroitin sulfate in dietary supplements

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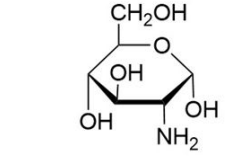
The objective of the present study is to develop and validate a simple, selective and accurate hydrophilic interaction liquid chromatography – a high performance liquid chromatography incorporating an evaporative light scattering detector (HILIC-HPLC-ELSD) method for simultaneously determining glucosamine hydrochloride and chondroitin sulfate in dietary supplements. The chromatographic separation was carried out on a ZIC-HILIC column (150 mm x 4.6 mm x 5µm) in isocratic system mode with a mobile phase of acetonitrile, 30 mM ammonium formate and water (77:20:3, v/v/v) at pH 4.5, a column temperature of 35°C, a flow rate of 1 $mL.min^{-1}$, and an injection volume of 5 µL. An evaporative light scattering (ELS) detector was used. Effective separation was achieved by means of analyte resolution of more than 1.5 with an analysis run time of approximately 20 minutes. The linearity of glucosamine hydrochloride and chondroitin sulfate ranged from 0.4 to 2.5 $mg.mL^{-1}$. The limits of the detection and quantification of glucosamine hydrochloride were 20 and 80 $mg.mL^{-1}$ respectively, while for chondroitin sulfate they were 80 and 400 $mg.mL^{-1}$. All validation parameters satisfied the acceptance criteria in accordance with International Conference on Harmonisation (ICH) guidelines. The method was successfully applied to the assay of commercial dietary supplement samples.

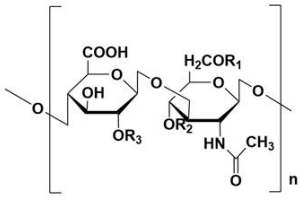
Keywords: Glucosamine hydrochloride. Chondroitin sulfate. HPLC-HILIC-ELSD. Method development. Dietary supplements.

INTRODUCTION

Glucosamine hydrochloride goes by the chemical name of (3R,4R,5S)-3-amino-6-(hydroxymethyl)oxane-2,4,5-triol hydrochloride. Glucosamine constitutes an important precursor in the biosynthesis of numerous connective tissues (Huskisson, 2008; Kirkho, Samarasighe, 2009). Chondroitin 6 sulphate (3-acetamido-2,5-dihydroxy-6-sulfooxyoxan-4-yl) oxy-3,4,5-trihydroxyoxane-2-carboxylic acid is a glycosaminoglycan composed of repeating disaccharide units containing N-acetylgalactosamine and a glucuronic acid (Sugara *et al*., 2003; Raman, Sasisekharan, Sasisekharan, 2005). Glucosamine hydrochloride and chondroitin sulfate (Figure 1) are widely employed either separately or in combination as dietary supplements in the treatment of osteoarthritis.



Glucosamine



Chondroitin sulfate

FIGURE 1 - The chemical structure of glucosamine and chondroitin sulfate.

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The chromatographic separation and detection of glucosamine hydrochloride (pKa=8.23, log P=-2.175) and chondroitin sulfate (pKa = -1.9 (strongest acid), pKa = -3.7 (strongest basic), log P = -6.2) remains a challenge due to the lack of UV absorption and their high polarities (Megantara, Mutakin, Levita, 2016, ChemAxon, 2020). Therefore, the common reverse-phase HPLC with UV detection is infrequently applied for routine analytical purposes.

Several reports on the analytical method for simultaneous determination of glucosamine and chondroitin sulfate have been produced. These include electrophoresis with UV detection (Vaclavikova, Kvasnicka, 2013), ion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex Corporation, 2008), HPLC with a UV detector (Gatti *et al*., 2008), HPLC with a fluorescence detector featuring pre-derivatization (Harmita, Jatmika, Nugraha, 2017), and HPLC with a refractive index (RI) incorporating pre-derivatization (Jahangir, Khalid, Ahmad, 2015). For HPAEC-PAD, the epimerization and degradation of glucosamine and chondroitin sulfate may occur when NaOH aqueous solution is employed as the mobile phase (Vaclavikova, Kvasnicka, 2013). HPLC, coupled with ultraviolet detection, is widely used in the analysis of pharmaceutical drugs and dietary supplements. However, the major problem associated with the detection of glucosamine and chondroitin sulfate is that it does not encompass UV chromophores. Therefore, pre-derivatization of glucosamine and chondroitin sulfate requires the addition of UV chromophores prior to analysis (El-Saharty, Bary, 2002; Gatti *et al*., 2008; Harmita, Jatmika, Nugraha, 2017; Shao *et al*., 2004; Tyler *et al*., 2007). Moreover, derivatization methods suffer from the drawback of being both labourious and time-consuming.

The hydrophilic interaction liquid chromatography (HILIC) method has several advantages in terms of analyzing polar compounds compared to the reversed-phase mode (Ikegami *et al*., 2008). For example, the HILIC method is less susceptible to generating peak deformations in the sample matrix. Conversely, polar analytes potentially suffer from retention time shifts and poor peak shape in reversed-phase methods. Furthermore, polar analytes can be more strongly retained by the HILIC method compared to its reversed-phase counterpart. The HILIC method produces a superior response to that of the reversed-phase method in evaporative light scattering detectors (ELSDs). In the HILIC mobile phase, most solvents are polar and volatile, resulting in greater sensitivity due to the evaporation process in the ELSDs (Lifford *et al*., 2009; Mitchell *et al*., 2009; Vervoort, Doemen, Torok, 2008).

ELSDs are usually selected as an alternative form of detector when the compounds of interest lack a UV chromophore and gradient compatibility is required (Lafosse, Herbreteau, 2002; Mitchell *et al*., 2009; Nogueira *et al*., 2005; Vervoort, Doemen, Torok, 2008). An ELSD is a semi-universal detector employed to detect analytes by means of light scattering after mobile phase nebulization and evaporation have been completed enabling it to detect analytes with low volatility in evaporation processes (Lafosse, Herbreteau, 2002). Compared to a refractive index detector (RID), an ELSD has a lower detection limit for carbohydrate group compounds (Mitchell *et al*., 2009; Vervoort, Doemen, Torok, 2008). Chhavi *et al.* (2019) developed a HILIC technique coupled with a corona charged aerosol detector for quantification of glucosamine in dietary supplements that is both simple and selective. However, a method of simultaneous determination of glucosamine hydrochloride and chondroitin sulfate in dietary supplements has yet to be developed.

In terms of the background to the study, this research was carried out to develop the HPLC-ELSD with a HILIC separation mode for the simultaneous determination of glucosamine hydrochoride and chondroitin sulfate in dietary supplements. The method developed was validated in accordance with the International Conference on Harmonisation (ICH), (2005) guidelines in terms of selectivity, linearity and range, precision and accuracy as well as limit of detection (LOD) and limit of quantification (LOQ). Its application was demonstrated by the analysis of glucosamine hydrochloride and chondroitin sulfate contents of marketed dietary supplement samples.

MATERIAL AND METHODS

Chemicals

Reference Standards of glucosamine hydrochloride and chondroitin sulfate sodium were purchased from

USP (Rockville, US), while ammonium formate was obtained from Sigma-Aldrich (USA). Acetonitrile HPLC grade was procured from Mallinckrodt and Water HPLC grade, hydrochoride acid and formic acid from Merck, Darmstadt Germany. The products used as samples were sourced from local pharmacies in Surabaya, Indonesia.

Instrumentation and analytical conditions

Chromatography was performed on Agilent 1100 series HPLC connected with ELSD Agilent 380, while separation of a zwitterion hydrophilic interaction liquid chromatography (ZIC-HILIC) column with a 200 Å pore size (SeQuant ZIC-HILIC, 150 mm x 4.6 mmx 5 µm, Merck Millipore, VIC, Australia) was achieved.

Separation was effected by means of an isocratic system, with the mobile phase composition of acetonitrile: water: 30 mM ammonium formate (77:3:20, v/v/v), pH 4.5, a column temperature of 35°C, a flow rate of 1 $mL.min^{-1}$, and an injection volume of 5 µL. ELSD was employed as a detector at nebulization and evaporation temperatures of 50°C and 80°C respectively, with a flow rate of nitrogen of 1.10 standard liter per minutes (SLM).

Development of the HPLC method

A HILIC-HPLC-ELSD method was developed to achieve the optimum simultaneous separation of glucosamine hydrochloride and chondroitin sulfate by injecting the mixed standard solution of these two chemicals into HPLC-ELSD. For method development, the various compositions of acetonitrile, ammonium formate and pH buffer were evaluated and the effects of chromatographic parameter investigated. ZIC-HILIC column was used to separate glucosamine hydrochloride and chondroitin sulfate in the matrix sample. The nebulization temperature and evaporation of ELSD were also analyzed.

Preparation of standard and sample solutions

Preparation of standard solutions

Standard stock solutions, including the standard of glucosamine hydrochloride and chondroitin sulfate sodium (5.0 $mg.mL^{-1}$) were prepared by dissolving 50.0 mg of standard (99%) in a water pro HPLC (v/v) contained in a 10.0 mL measuring flask. The volume was subsequently made up with pro HPLC water to obtain a mixed standard solution of glucosamine hydrochloride (2.5 $mg.mL^{-1}$) and chondroitin sulfate (2.5 $mg.mL^{-1}$).

Sample preparation

The samples used in this study consisted of caplets containing glucosamine hydrochloride and chondroitin sulfate purchased from a local pharmacy in Surabaya Indonesia. Table I lists the label content of those dietary supplements. For validation studies, a supplement containing glucosamine hydrochloride and chondroitin sulfate and the sample matrix (placebo) was obtained from PT Interbat Indonesia.

Twenty tablets containing glucosamine hydrochloride and chondroitin sulfate were obtained and accurately weighed before being ground into a homogeneous powder. For the HPLC method, 300 mg of the HPLC grade powder was weighed and diluted with 50.0 mL of water. The solution was sonicated for 15 minutes before being filtered through 0.45 µm filter membranes. A maximum of six replications of each sample were prepared.

TABLE I - Labeled content for each type of dietary supplement

Sample	Formulation	mg per serving		Other ingredients
		Glucosamine hydrochloride	Chondroitin sulfate	
A	caplet	500 mg	400 mg	Ascorbic acid 50 mg, vitamin E 50 mg, Mn 2.5 mg, Mg 20 mg, selenium 50 mcg, Zn 5 mg
B	caplet	500 mg	400 mg	Ascorbic acid 50 mg, Mn 0.5 mg, Mg 10 mg, Zn 5 mg
C	caplet	500 mg	400 mg	Ascorbic acid 50 mg, Mn 50 mg, Mg 10 mg, Zn 5 mg

Prevalidation

Stability tests

Stability tests of glucosamine hydrochloride and chondroitin sulfate were conducted at room temperature and at 4°C for seven days. The mixed standard solutions containing 0.6 $mg.mL^{-1}$ of glucosamine hydrochloride and 0.4 $mg.mL^{-1}$ of chondroitin sulfate were prepared and stored at room temperature and at 4°C. An aliquot (1 mL) was withdrawn at 0 (immediately after preparation) and on days 1, 2, 4 and 7 post-storage. The analysis involved comparing the parts of each solution with the newly produced solution (zero condition). The chromatogram result was calculated using a two-way ANOVA test statistical analysis.

System suitability tests

System suitability was investigated using the theoretical plate number (N), the retention time of glucosamine hydrochloride and chondroitin sulfate (R_t) and the resolution factor (Rs) for glucosamine hydrochloride and chondroitin sulfate peaks. A system suitability test was performed by injecting the mixed standards solution six times. 0.6 $mg.mL^{-1}$ of standard solution was filtered using a 0.45 µm nylon membrane and 5 µL injected into the HPLC system. The percentage of RSD retention time and the p area were subsequently calculated.

Validation of the analytical method

The developed method was validated in accordance with ICH guidelines (ICH, 2005).

Selectivity method

The selectivity method showed that the ability of the analytical method was selective for glucosamine hydrochloride and chondroitin sulfate and that there were no interference with the compound's retention time. The selectivity in this study was demonstrated by the injecting of blank solvent, the use of placebos, mixed standard solutions, placebos spiked with standard solution, and sample solutions. Placebos in tablet form which contained excipients were employed. The excipients included mycrocristaline selulose, titanium dioxide, zinc sulfate, magnesium sulfate, mangane sulfate, ascorbic acid and vitamin E. All precisely weighed components containing 0.6 $mg.mL^{-1}$ glucosamine hydrochloride and chondroitin sulfate were transferred into 10 ml volumetic flasks. Prepared solutions were filtered through a 0.45 µm membrane filter and analyzed in HPLC under specified conditions. The selectivity test parameter met requirements provided that the value of Rs was ≥1.5 (Yuwono, Indrayanto, 2005).

Linearity and limits of detection and quantification

The linearity test was carried out by preparing six solutions of different standard concentrations ranging from 0.4 to 2.5 $mg.mL^{-1}$. A series of standard solutions were injected after being filtered through a 0.45 µm nylon membrane to HPLC system. Linear regression analysis was undertaken using the concentration of standard solution versus peak area. From the chromatogram obtained, a regression line equation was produced from the concentration data and the detector response in order to obtain the regression line equation of $Y = bX + a$.

The limits of detection and quantification were determined based on a signal-to-noise (S/N) ratio by establishing the minimum concentration at which glucosamine hydrochloride and chondroitin sulfate could be reliably detected and quantified. The limit of detection (LOD) and limit of quantification (LOQ), on the basis of response at a signal-to-noise ratio (S/N), were 3 and 10 respectively.

Precision test

A precision or repetition test was performed to determine repeatability by adding a mixed standard solution to the placebo and conducting a minimum of six replications. Measurements relating to retention times and peak areas were used to assess repeatability, while the RSD value was determined in keeping with RSD precision requirements of <2%. (AOAC International, 2016).

Accuracy

In this study, the spiked placebo recovery method was used by adding three different concentrations; 80%, 100% and 120% standard solutions to the placebo mixture. The analysis was conducted using three replications. The accuracy requirement was assessed on the basis of a recovery percentage between 92% and 105% (AOAC International, 2009).

RESULTS AND DISCUSSION

Method development

In order to obtain the optimum separation of glucosamine and chondroitin sulfate, various compositions of acetonitrile, buffer composition and pH were tried. Satisfactory separation was achieved when the mobile phase composition included 77% acetonitrile. Acetonitrile is recommended as the preferred organic solvent for HILIC because of its lower hydrogen binding ability and its considerable volatility (Vervoort, Doemen, Torok, 2008). The effect of different compositions of acetonitrile on separation, retention time, the peak area of glucosamine hydrochloride and chondroitin sulfate was assessed. The results indicated that improving water percentage in the mobile phase would reduce the retention time, while the resolution would decrease. In a normal phase of chromatography, water forms pseudo-stationary layers during the polar stationary phase of the ZIC-HILIC column (Mitchell *et al.*, 2009). The strong eluent in HILIC is water and its presence should be a minimum of 2% of the total volume in the mobile phase (Lifford *et al.*, 2009; Mitchell *et al.*, 2009; Vervoort, Doemen, Torok, 2008).

Glucosamine and chondroitin sulfate are polars highly susceptible to immobilization in water-rich pseudo-stationary layers. The elution rate of immobilized glucosamine and chondroitin sulfate depends on the hydrophilic nature of the mobile phase.

The effect of ammonium formate on the separation of glucosamine hydrochloride and chondroitin sulfate in its various compositions was investigated with 30 mM of the substance being selected for analysis. The increase in ammonium formate went undetected since its occurrence could have been due to the sulfonate group-generated repulsive forces in the terminal end of the column's stationary phase being insufficient for the early elution of sulfate ions. This was due to their interaction with positively charged ammonium ion supplied by the buffer (Chhavi *et al.*, 2019). The effect of the pH was observed by changing the pH buffer. Satisfactory separation was achieved when the pH buffer was 4.5. The temperature of nebulization and evaporation of the ELSD was set at 50°C and 80°C respectively. The flow

rate was 1.0 mL.min^{-1} and the volume injection was 5 µL. The chromatogram of the mixture standard solution of glucosamine hydrochloride and chondroitin sulfate under selected conditions is shown in Figure 2.

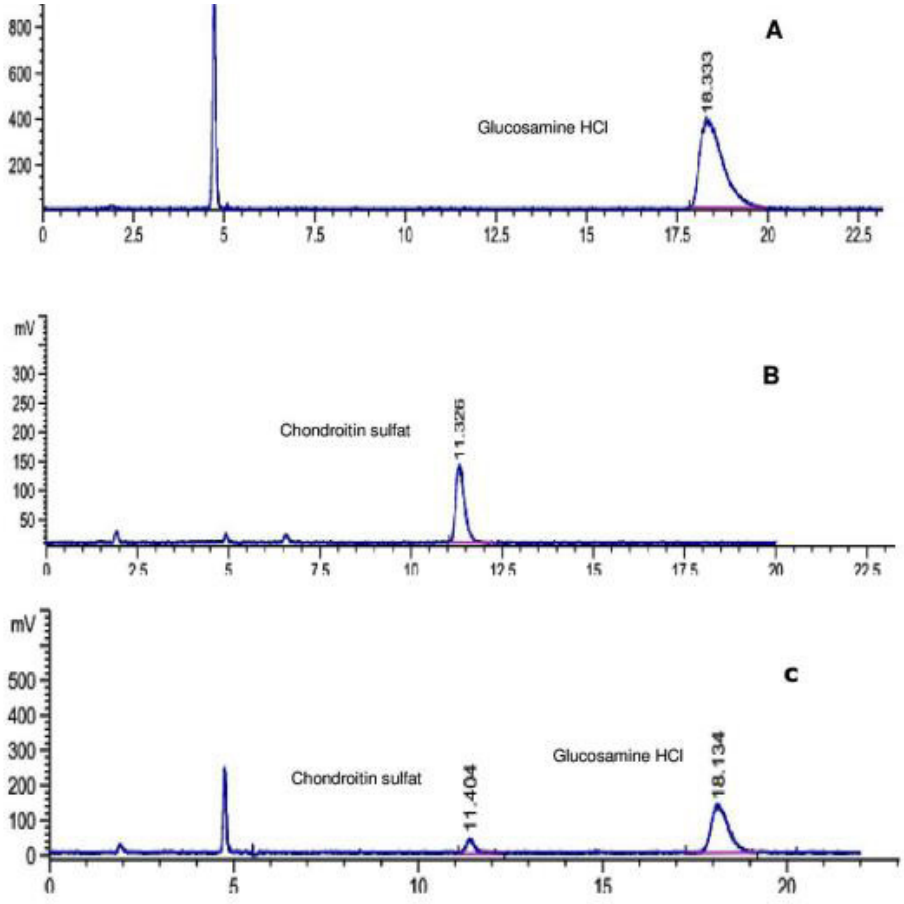

FIGURE 2 - Chromatogram of (A) standard of glucosamine hydrochloride; (B) standard of chondroitin sulfate; (C) standard mixture of glucosamine hydrochloride and chondroitin sulfate using a HILIC-HPLCELSD with mobile phase of acetonitrile:water:30 mM ammonium format (77:3:20, v/v/v), pH 4.5, ELS detector, injection volume 5 µL.

The ultra-performance liquid chromatoghraphy-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) (Zheng *et al*., 2017) and the use of a corona charged aerosol detector (CAD) (Chhavi *et al*., 2019) have been developed for determination of glucosamine in dietary supplements by means of a HILIC column. However, the simultaneous determination glucosamine and chondroitin sulfate have not been described. While a mass spectrometry detector provides sensitive results, this instrument is expensive and requires specialist operational knowledge of instruments. CAD and ELSD show comparable sensitively in HILIC and the detection of analyte does not depend on a UV chromophore. Compared to CAD, ELSD offers the advantages that glucosamine hydrochloride and chondroitin sulfate can be selectively separated from other ingredients by means of a small volume injection, thereby avoiding problems associated with detection and column overload.

Pre-validation

Stability test

The retention time and peak area assay results (standard mixture of 0.6 mg.mL^{-1} glucosamine hydrochloride and 0.4 mg.mL^{-1} chondroitin sulfat) on days 1, 2, 4 and 7 were relative to the standard solution at 0 hour. The concentrations of glucosamine hydrochloride and chondroitin sulfate at 0

hour were considered to be 100%. The results of the stability standards solution iare listed in Table II.

In this study, all samples were prepared simultaneously and retained for analysis in the HPLC auto sampler. The runtime was of 22 minutes' duration while the time required for analysis of the first to the last samples was approximately 16 hours. Consequently, changes in the concentration occurred whether or not degradation of the auto sampler took place, resulting in the need for a stability test to be conducted.

TABLE II - The results of stability glucosamine and chondroitin sulfate standards solution

Standard solution	**Area Glucosamine hydrochloride**					
	Storage at Room temperature (n=3)			**Storage at 4°C (n=3)**		
	Mean	RSD	Compare to initial, %	Mean	RSD	Compare to initial, %
Initial	2103.5	0.56		2103.5	0.56	
Day 1	2116.1	1.44	100.6	2104.3	0.42	100.0
Day 2	2121.3	1.88	100.8	2100.2	1.25	99.8
Day 4	1785.8	0.27	84.9	2016.1	1.33	95.8
Day 7	1615.0	0.00	76.3	1765.4	1.91	83.9
Area Chondroitin Sulfate						
Standard solution	Storage at Room temperature (n=3)			Storage at 4°C (n=3)		
	Mean	RSD	Compare to initial, %	Mean	RSD	Compare to initial, %
Initial	359.2	1.88		359.2	1.88	
Day 1	359.9	1.19	100.2	358.4	1.61	99.8
Day 2	358.2	1.81	99.7	359.0	1.84	99.9
Day 4	321.2	0.67	89.4	345.3	0.17	96.1
Day 7	214.2	0.03	59.8	285.4	1.33	79.5

*Result were compared using an ANOVA, storage and day represented no significantly different values between day 1 and day 2

System suitability test

A system suitability test was performed by injecting 5 µL of standard solution into the HPLC system. Tailing factors were 0.7 for glucosamine hydrochloride and 0.8 for chondroitin sulfate, while the theoretical plates (N) were 7,267 for glucosamine hydrochloride and 18,389 for chondroitin sulfate. The suitability test system results satisfied the required percentage of RSD < 2%. The percentages of RSD for retention time and peak area were 0.26% and 1.39% for glucosamine hydrochloride and 0.43% and 0.62% for chondroitin sulfate.

Validation

Selectivity tests

Selectivity was tested by comparing the retention time and resolution of the glucosamine hydrochloride and chondroitin sulfate peaks. The retention times for glucosamine and chondroitin sulfate were 18.13 minutes and 11.28 minutes respectively. The resolution between glucosamine and chondroitin sulfate was 9.69. Therefore, the resolution between peaks met the ICH recommended requirements (R>1.5). This HPLC method successfully

separated glucosamine hydrochloride and chondroitin sulfate from other components. Figure 3 contains the type of chromatogram in which the placebo was added to the mixture standard and the sample, indicating good separation and the absence of peak interference from the placebo with the analyte peak.

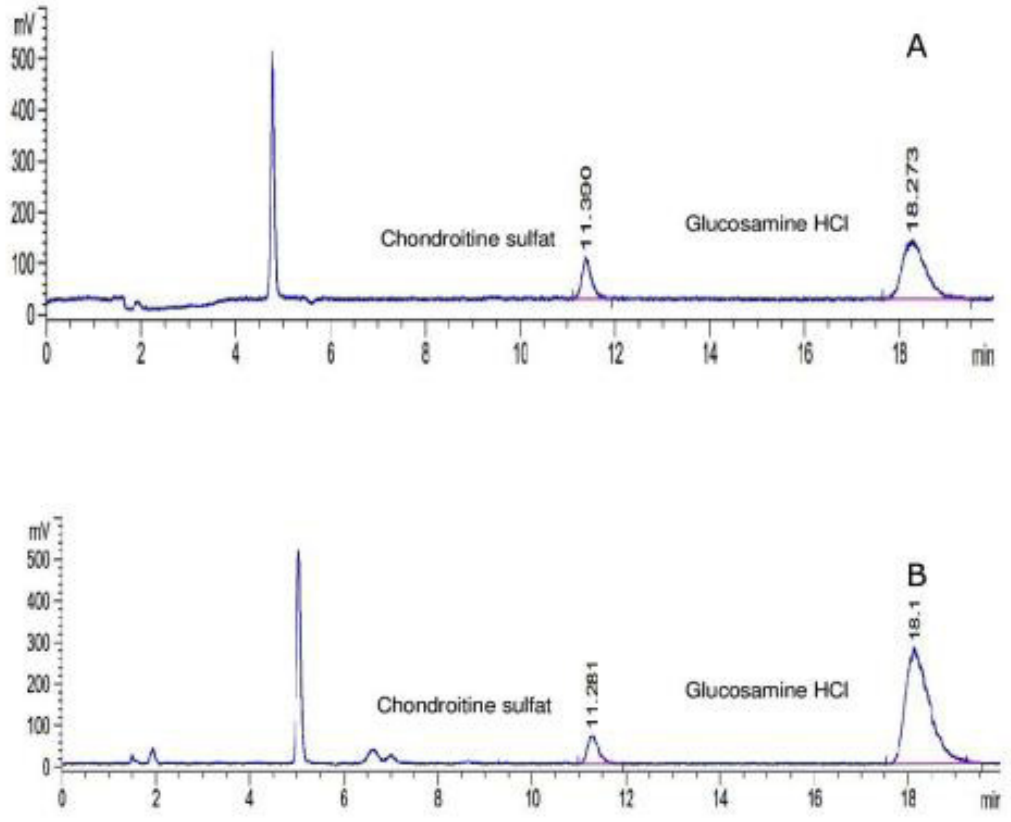

FIGURE 3 - Chromatogram of (A) matrix spiked of standard glucosamine hydrochloride and chondroitin sulfate, (B) sample using a HILIC-HPLC-ELSD with mobile phase of acetonitrile:water:30 mM ammonium format (77:3:20, v/v/v), pH 4.5, ELS Detector, injection volume 5 µL.

Linearity and range

Linearity was carried out at six different concentration levels between 0.4 $mg.mL^{-1}$ and 2.5 $mg.mL^{-1}$ for glucosamine hydrochloride and chondroitin sulfate. The peak areas are summarized in Table III according to their respective concentration levels. The results of the linier regression equation (y= ax + b) and the coefficient correlation (r) are shown in Figure 4.

TABLE III - Linearity data of glucosamine and chondroitin sulfate

Standard solution	Concentration range mg.mL^{-1}	Linear correlation	r	R^2	Vxo
Glucosamine HCl	0.4 – 2.5	Y = 6.65x – 1793.4	0.999	0.998	3.0
Chondroitin sulfate	0.4 – 2.5	Y =0.64x + 106.9	0.999	0.998	2.6

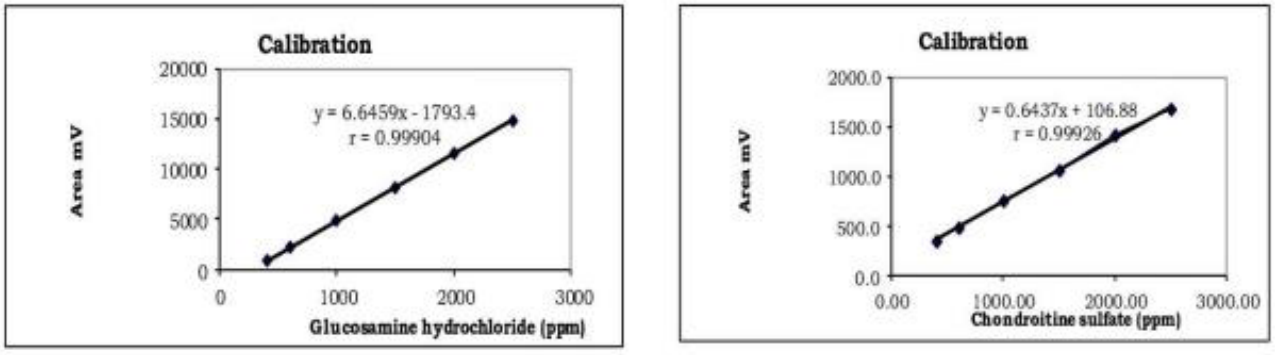

FIGURE 4 - Linearity Curve glucosamine hydrochloride and chondroitin sulfate.

Accuracy

The determination of accuracy was undertaken by adding the matrix sample (placebo) to the standard. The percentage of glucosamine hydrochloride and chondroitin sulfate standard recovery (Table IV) showed the recovery of glucosamine hydrochloride and chondroitin sulfate to be 100.6-101.0 % and 97.65-102.34 % respectively.

TABLE IV - Accuracy of glucosamine hydrochloride and chondroitin sulfate based on spike recovery

Standard Addition	Glucosamine HCl				Mean ± RSD (%)	Chondroitin sulfate			
	Replication	Amount known	Amount obtained	Recovery (%)		Amount known	Amount obtained	Recovery (%)	Mean ±RSD (%)
80%	1	800	806.90	100.86	100.6 ± 0.60	640	623.97	97.49	97.65 ± 0.18
	2	800	799.65	99.97		640	613.95	95.93	
	3	800	807.74	100.97		640	624.73	97.61	
100%	1	1000	1015.89	101.59	101.04 ± 0.60	800	829.21	103.65	102.34 ± 1.20
	2	1000	1010.87	101.09		800	817.43	102.18	
	3	1000	1004.45	100.44		800	809.55	101.19	
120%	1	1200	1207.79	100.65	100.46 ± 0.20	960	962.50	100.26	101.37 ± 1.00
	2	1200	1203.48	100.29		960	981.48	100.24	
	3	1200	1205.21	100.43		960	975.53	101.62	

Precision test

A precision or repetition test was performed to determine repeatability by adding a standard solution of 100% concentration to a placebo and repeating a minimum of six replications (Table V). The average values of glucosamine and chondroitin sulfate inter-day precision were 1007.2 $\mu g.mL^{-1}$ and 817.1 $\mu g.mL^{-1}$ with RSD 0.5% and 1.4%, and those of glucosamine and chondroitin sulfate intra-day precision were 1010.3 $\mu g.mL^{-1}$ and 820.4 $\mu g.mL^{-1}$ with RSD 0.3% and 1.6%. These values met the RSD regulation limit of <2%. Table V contains a summary of the method validation study results.

TABLE V - Mean inter-day and intra-day precision of glucosamine hydrochloride and chondroitin sulfate

Inter-day precision								
	Glucosamine HCl				**Chondroitin sulfate**			
Replication	Concentration ($\mu g.mL^{-1}$)	Mean ($\mu g.mL^{-1}$)	SD	% RSD	Concentration ($\mu g.mL^{-1}$)	Mean ($\mu g.mL^{-1}$)	SD	% RSD
1	1015.9	1007.2	5.1	0.5	829.2	817.1	11.8	1.4
2	1010.9				821.5			
3	1004.5				827.1			
4	1005.8				817.4			
5	1002.5				809.6			
6	1003.8				797.8			
Intra-Day precision								
	Glucosamine HCl				Chondroitin sulfate			
Replication	Concentration ($\mu g.mL^{-1}$)	Mean ($\mu g.mL^{-1}$)	SD	% RSD	Concentration ($\mu g.mL^{-1}$)	Mean ($\mu g.mL^{-1}$)	SD	% RSD
1	1015.3	1010.3	3.3	0.3	833.4	820.4	13.4	1.6
2	1010.9				797.7			
3	1008.9				810.8			
4	1005.4				826.4			
5	1011.9				827.3			
6	1009.2				827.2			

Limit of detection (LOD) and limit of quantification (LOQ)

The detection and quantification limits were determined on the basis of a signal-to-noise (S/N) ratio by establishing the minimum concentration at which glucosamine hydrochloride and chondroitin sulfate could be reliably detected and quantified. A S/N ratio test was performed by comparing the peak height of the sample with that of the blank sample. On the basis of the response at a signal-to-noise ratio (S/N), the limit of detection (LOD) and limit of quantification (LOQ) were 3 and 10 respectively. The LOD of the method was found to be 20 $\mu g.mL^{-1}$ (glucosamine hydrochloride) and 80 $\mu g.mL^{-1}$ (chondroitin sulfate), while the LOQ of the method for glucosamine hydrochloride and chondroitin sulfate was 80 $\mu g.mL^{-1}$ and 400 $\mu g.mL^{-1}$ respectively.

Determination of samples

The development method was used to analyze three different supplements containing glucosamine hydrochloride and chondroitin sulfate. The maximum percentage loss of tablet content was < 0.02%. The results of the determination of the average glucosamine and chondroitin sulfate levels are shown in Table VI.

TABLE VI - Labeled and average amount of glucosamine hydrochloride and chondroitin sulfate observed in commercial. glucosamine hydrochloride

No	Sample	Concentration Mean (mg/ tablet) ± RSD (%), n=6	Claimed amount (mg)	%	Acceptance criteria USP 43
1	A	461.92 ± 0.15	500	92.38	90 - 120 %
2	B	480.32 ± 0.32	500	96.18	
3	C	483.24 ± 0.07	500	96.65	
Chondroitin sulfate					
No	Sample	Concentration Mean (mg/ tablet) ± RSD (%), n=6	Claimed amount (mg)	%	Acceptance criteria USP 43
1	A	405.15 ± 1.04	400	101.29	90 - 110 %
2	B	176.63 ± 1.30	400	44.16	
3	C	458.85 ± 0.90	400	114.71	

CONCLUSION

The proposed method of HILIC-HPLC-ELSD is simple, selective and accurate. The statistical analysis of the method validation study proves that the method is repeatable, selective and accurate with regard to the simultaneous analysis of glucosamine hydrochloride and chondroitin sulfate. This method can be employed to simultaneously determine the glucosamine hydrochloride and chondroitin sulfate content of samples in commercially available dietary supplements.

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