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Short communication

A stability-indicating HPLC method for the determination of glucosamine in pharmaceutical formulations

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Abstract

A stability-indicating high performance liquid chromatographic (HPLC) method was developed for the assay of glucosamine in bulk forms and solid dosage formulations. The HPLC separation was achieved on a Phenomenex Luna amino column (150 mm × 4.6 mm, i.d., 5 μm particle size) using a mobile phase of acetonitrile–phosphate buffer (75:25, v/v, pH 7.50) at a flow rate of 1.5 ml min^{-1} and UV detection at 195 nm. The method was validated for specificity, linearity, solution stability, accuracy, precision, limit of detection, and limit of quantitation. The detector response for glucosamine hydrochloride was linear over the selected concentration range from 1.88 to 5.62 mg ml^{-1} with a correlation coefficient 0.9998. The accuracy was between 98.9 and 100.5%. The precision (R.S.D.) amongst six sample preparations was 1.1%. The limit of detection and the limit of quantitation are 0.037 and 0.149 mg ml^{-1}, respectively. The sample and standard solutions were stable for 1 week. The method was successfully used for analysis of active-excipient compatibility samples used for development of a solid dosage formulation in our laboratory and subsequent stability studies. The method was also used for the analysis of glucosamine in several commercially available solid dosage forms.

Keywords: Glucosamine; HPLC; Stability-indicating; Validation; Amino column

1. Introduction

An estimated 21 million adults in the USA live with osteoarthritis or degenerative joint disease—one of the most common type of arthritis [1]. This type of disease is commonly treated with prescribed non-steroidal anti-inflammatory agents (NSAIDs). Alternatively, it can also be managed with some dieatary supplements such as glucosamine and chondroitin preparations. Glucosamine is a natural amine sugar extracted from the chitin in the sea shrimp and crab shell. Researches have shown that glucosamine in combination with chondroitin sulfate can build blocks for cartilage, up-regulate chondrocyte and reduce the extent of cartilage degradation [2,3]. The study on the bioavailability and pharmacokinetics of glucosamine hydrochloride and chondroitin sulfate after single and multiple doses to beagle dogs by Adebowale et al. [4] suggests that glucosamine and chondroitin are bioavailable after oral dosing.

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A number of clinical trials have demonstrated that glucosamine and chontroitin sulfate are efficacious in the management of OA in humans and animals [5–7]. The first multicenter clinical trial funded by National Institutes of Health in the US to test the effects of glucosamine and chondrotin sulfate used separately or in combination for treatment of osteoarthritis of the knee is under progress.

Several high performance liquid chromatographic (HPLC) methods have been published for the direct analysis of glucosamine bulk materials and formulations in pharmaceutical products [8,9]. However, initial attempts at using these methods did not achieve the separation of force degraded sample desired in a stability-indicating HPLC assay. Other published HPLC methods involving an initial derivatization step were available, and very sensitive, but these assays were not studied because the extra derivatization step was not favored for routine analysis and the commonly-found problem of higher than desired R.S.D. values for derivatization methods [10,11]. The purpose of this work was to develop and validate a simple and stability-indicating HPLC method for glucosamine bulk materials and pharmaceutical formulations using an amino column for chromatographic separation followed by UV detection at 195 nm.

2. Experimental

2.1. Materials

D-(+)-Glucosamine HCl (99.9% purity, Fig. 1) used as a standard was purchased from Sigma Chemical (St. Louis, Missouri, USA). The glucosamine HCl bulk material was purchased from Ferro Pfanstiel Inc. (Maukegan, IL, USA). The water and acetonitrile used were HPLC grade, and the potassium phosphate dibasic (KH_2PO_4), 28% NH_4OH, 5.0 M NaOH, 5.0 M HCl, H_3PO_4, and 30% H_2O_2 used were purchased from Sigma Chemical Co. with analytical reagent grade or better. Sample preparation solvent was made by combining 500 ml acetonitrile and 500 ml water. Phosphate buffer was made by dissolving 7.0 ± 0.1 g KH_2PO4 in 2 l water, adding 0.5 ml NH_4OH, and adjusting the pH to 7.50 ± 0.05 with H_3PO_4. An in-house formulation (tablet) coded as A1 and placebo as P1 were used for the validation of method accuracy, precision, and specificity. Each tablet contains a declared amount of 375.0 mg of glucosamine hydrochloride. In addition, six commercially available solid dosage forms containing glucosamine analysed using this method were coded as C1–C6.

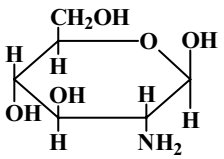

Fig. 1. Chemical structure of glucosamine.

2.2. Instrumentation

Two brands of HPLC systems were used, a Waters Alliance 2690D system equipped with a 2487 dual λ absorbance detector, a column oven and a quaternary pump system, and an Agilent 1100 system equipped with a quaternary pump, an autosampler, a thermostatted column heater and a DAD detector. Single wavelength data analysis was done using Peak Pro software version 8.4b. Diode array data analysis was performed using Agilent Chemstation software version A.08.04. As appropriate, DryLab 2000 version 3.0.09 chromatography method development software (Waters, Milford, MA, USA) was used in data analysis.

2.3. Chromatographic conditions

The column used was a 150 mm × 4.6 mm, 5 μm Phenomenex Luna amino column. The mobile phase was 75:25 (v/v) acetonitrile/phosphate buffer (0.020 M KH_2PO4 adjusted to pH 7.5 with H_3PO_4). The flow rate was 1.5 ml min^{-1}, the wavelength was at 195 nm, the injection volume was 10 μl, the column temperature was thermostatted at 35 °C, the run time was 15 min, and quantitation was performed using peak area counts.

2.4. Standard preparation

An accurately weighed amount (approximately 375 mg) of glucosamine hydrochloride standard was transferred into a 100 ml volumetric flask, approximately 50 ml of the sample preparation solvent was

added, shaken for 5 min, and brought to volume with the sample preparation solvent, and mixed well. This was the working standard with a concentration of approximately 3.75 mg ml^{-1}.

2.5. Assay sample preparation

Twenty tablets were weighed, and the average tablet weight was determined. The tablets were ground to a homogenous powder. A portion of the powder corresponding to the average tablet weight was weighed, and transferred into a 100 ml volumetric flask. About 50 ml sample preparation solvent was added and shaken for a minimum of 30 min to dissolve, and brought to volume and mixed well. The sample was filtered into an HPLC vial using a 25 mm, 0.45 μm nylon syringe filter.

3. Results and discussion

3.1. Development and optimisation of the HPLC method

Early method development highlighted limitations placed on the chromatography due to the physico-chemical properties of glucosamine. Glucosamine UV absorbance is too weak for quantitation above 205 nm, so the mobile phase composition was limited to acetonitrile as the organic solvent and phosphate as the pH buffer. C_{18}, C_8, and phenyl columns were found to be very weak retentive for glucosamine with or without ion pairing. A critical point was that the chloride anion was not separated from glucosamine using reversed-phase chromatography. Cyano, diol, strong cation exchange, and porous graphitic carbon columns were tried and showed some potential for retaining glucosamine but were clearly inferior to amino column in this respect. Thus, further method development was limited to an acetonitrile/phosphate buffer mobile phase on an amino column at low UV wavelengths, pH, temperature, %organic, buffer strength, and flow rate.

Temperature, buffer strength, flow rate, and UV wavelength were found to be less critical and were addressed first in method development. An 150 mm × 4.6 mm, 5 μm Phenomemnex Luna amino column with a 75:25 (v/v) acetonitrile/pH 7.5 buffer was used to evaluate temperature (30, 35, and 40 °C), buffer strength (5, 10, and 20 mM), flow rate (0.5, 1.0, and 2.0 ml min^{-1}), and UV wavelength (191, 195, 200 and 205 nm). The sample used was a glucosamine HCl standard stored at room temperature in pH 11 buffer for 1 day to obtain 10–20% degradation of glucosamine, then neutralised to pH 7 before use. The flow rate had little impact on the separation, and was set at 1.5 ml min^{-1}. Increasing the temperature shortened retention times for glucosamine and its degradants, with minor selectivity changes, and 35 °C was chosen because of improvements in column efficiency compared to 30 °C. Increasing the buffer strength increased glucosamine retention relative to the degradants, with 20 mM buffer being chosen as optimal. The UV absorbance for glucosamine was highest at 195 nm, and was chosen, although several degradants had increased absorbance at 205 nm.

The pH and ratio of acetonitrile to buffer were optimised with the set conditions at 35 °C temperature, 195 nm wavelength, 20 mM (aqueous) buffer strength, and 1.5 ml min^{-1} flow rate. There were two pH and ratio of acetonitrile/buffer ranges where the glucosamine retained with the targeted 6–20 min retention time; around pH 3.5 and 10–50% acetonitrile, where glucosamine showed reversed-phase retention and eluted earlier with increased percent acetonitrile, and around pH 7.0 and 55–75% acetonitrile, where glucosamine showed normal phase retention and eluted later with increased percent acetonitrile. However, compared to the low-pH region, the high-pH region separated glucosamine with the degradants better, and appeared to give better absorbance for the degradants, and so was further studied. The pH (6.5, 7.0, and 7.5) and percent acetonitrile (60, 65, and 75) were varied, and retention time data on glucosamine and its degradants were entered into DryLab for analysis. The optimal conditions were finalized to those listed in the Section 2. Fig. 2 shows typical HPLC chromatograms of glucosamine standard and glucosamine in a pharmaceutical formulation.

3.2. System suitability

A system suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability/calibration standard and one injection of a check standard were

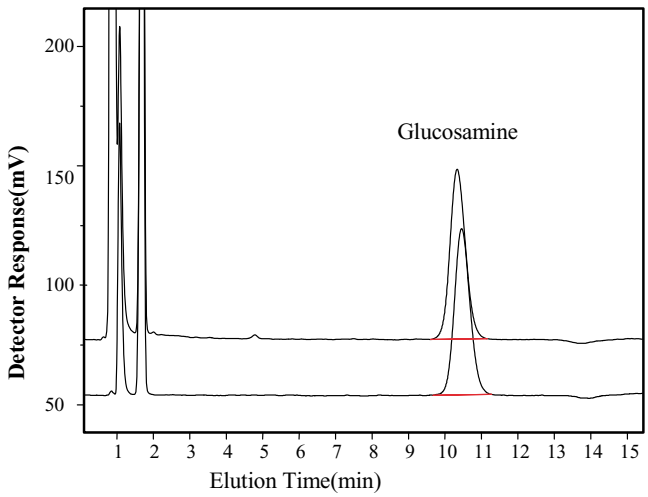

Fig. 2. Typical HPLC chromatograms of glucosamine standard (bottom) and glucosamine in a pharmaceutical formulation (top).

made. Area relative standard deviation, tailing factor, and efficiency for the five suitability injections were determined. The check standard was quantified against the average of the five suitability injections. For all sample analyses, the tailing factor was $\leq$2.0, efficiency $\geq$1500, %R.S.D. $\leq$2.0%, and 100.0 $\pm$ 2.0% check standard recovery.

3.3. Linearity

A set of five standards at the following concentrations were prepared: 1.88, 2.81, 3.75, 4.69, and 5.62 mg ml^{-1} glucosamine HCl. This set ranges from 50 to 150% of the nominal assay concentration of 3.75 mg/ml glucosamine HCl. Each of the six standards was analyzed in triplicate. Table 1 shows the results. The calibration curve was constructed by plotting the peak area against the concentration using linear regression analysis. It showed that the slope was 555.74 with a *y*-intercept of 5.1598 and a correlation coefficient of 0.9998, indicating an excellent linearity. At the range, the percent relative standard deviations of the peak areas of three replicate injections were found to be between 0.3 and 1.3%.

3.4. Specificity/forced degradation studies

The forced degradation study was conducted by subjecting standard, placebo, and tablet formulation samples to heat, light, oxidation, acid, and base degradation. The samples were appropriately neutralised and analysed using the method. Single wavelength data at 195 nm was collected following the method. Additional photodiode array data was collected for the purposes of the peak purity evaluation. Thermal degradation was induced by storing the samples at 60 $\pm$ 2°C for a period of 14 days. Photodegradation was induced by exposing the samples in an open Petri dish to the

Table 1
Linearity results

%Nominal (assay)	Concentration (mg ml^{-1})	Peak area	Mean peak area	%R.S.D.
50	1.877	1043.35 1044.53 1039.27	1042.38	0.3
75	2.815	1557.34 1575.31 1597.16	1576.60	1.3
100	3.754	2056.68 2070.62 2083.27	2070.19	0.6
125	4.692	2568.73 2600.04 2613.12	2593.96	0.9
150	5.631	3087.71 3105.52 3128.77	3107.33	0.7

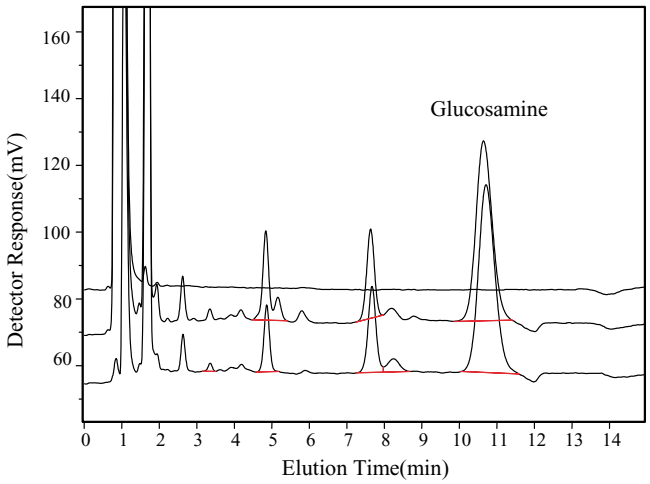

Fig. 3. Chromatograms of base-degraded placebo (top), glucosamine hydrochloride standard (middle), and formulation A1(bottom).

light equivalent to 1.2 million lux hours with an integrated near UV energy of not less than 200 W h^{-2} meter. Oxidative degradation was induced by storing the samples at room temperature in 3.0% hydrogen peroxide for a period of 7 days. The specificity experiments showed that the only condition under which glucosamine clearly degraded was the base degradation condition. Fig. 3 shows chromatograms of base stressed standard, placebo, and an in-house formulation. Baseline resolution between glucosamine and degradation product peaks was achieved. Diode array detection peak homogeneity tests showed that no peak interfered with the glucosamine peak. The negative peak eluting right after glucosamine is a system peak from the sulfuric acid used to neutralize the base, and would not be seen in an actual sample.

3.5. Accuracy/recovery

The accuracy was demonstrated by preparing placebo samples of A1 that were additionally spiked to approximately 75, 100, and 125% of the theoretical glucosamine HCl concentration level in sample A1. The spiked placebo samples at the 75, 100, and 125% level were prepared in triplicate, six replicates, and in triplicate, respectively, using glucosamine HCl in the solid form for spiking the placebo. The results in Table 2 shows that the average recovery at each level was within 100.0 ± 2.0% and the %R.S.D. at each level was ≤2.0%.

3.6. Precision

The repeatability of the method was demonstrated by preparing and analyzing six sample replicates from a homogenous composite blend of 20 tablets. The results in Table 3 shows that the average recovery was

Table 2
Accuracy results

Level (%)	Sample	Glucosamine HCl, %recovery
75	1	99.9
	2	100.1
	3	101.5
	Mean	100.5
	%R.S.D.	0.9
100	1	101.4
	2	100.5
	3	101.4
	4	99.2
	5	98.5
	6	98.3
	Mean	99.9
	%R.S.D.	1.4
125	1	100.1
	2	98.4
	3	98.2
	Mean	98.9
	%R.S.D.	1.0

Table 3
Precision results

Sample	Glucosamine HCl (mg per tablet)	Glucosamine HCl, %target
1	377.5	100.7
2	379.2	101.1
3	385.6	102.8
4	378.7	101.0
5	378.4	100.9
6	372.3	99.3
Mean	378.6	101.0
%RSD	1.1	NA

within 100.0 ± 3.0% of label claim (375.0 mg per tablet), and the %R.S.D. was 1.1%, indicating excellent precision.

3.7. Solution stability

The stability of glucosamine in standard and sample preparation was evaluated. The solutions were stored at ambient temperature and tested at intervals of 0, 2, 4, and 7 days. The responses for the aged solutions were evaluated using a freshly prepared standard. The results in Table 4 shows that sample and standard solutions retained a potency of 100.0 ± 2.0% as compared with the fresh solution over a time of 1 week.

3.8. Limit of detection and limit of quantitation

The LOD and LOQ were measured as the concentrations corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values for glucosamine were found to be 37.0 and 149.0 μg/ml, respectively.

Table 4
Solution stability results

Interval	Glucosamine in standard solution %peak area relative to initial	Glucosamine in sample solution %peak area relative to initial
Day 0 (initial)	100.0	100.0
Day 1	101.2	101.4
Day 2	102.0	100.3
Day 5	102.7	100.2
Day 7	101.4	100.6

3.9. Application to solid dosage forms

The developed method was applied for the determination of glucosamine content in six marketed products (C1–C6). Products C1–C4 contain chondroitin sulfate besides glucosamine hydrochloride. Products C5 and C6 contain single active, glucosamine sulfate and glucosamine hydrochloride, respectively. Products C1, C2, and C4 are in tablet forms. Products C3, C5, and C6 are in capsule forms. The method was also used for analyzing the stability samples of formulation A1. The samples were stored at 40 °C/75RH, pulled periodically and analyzed for the active content. The chromatograms from stability samples and commercial products showed the well separation of glucosamine peak with other degradants or components. The purity of the glucosamine peak was checked by PDA and found to be 100%, demonstrating that no interference existed between glucosamine and degradants or other components. The results showed the method was suitable for stability-indicating analysis and assay for commercial products
C1–C6.

4. Conclusion

A simple HPLC method using an amino column was developed for the analysis of glucosamine in solid dosage formulations. This method was also successfully used for the analysis of glucosamine in various different marketed formulations. Since the forced degradation and stability studies of the in-house formulation showed no interference with the glucosamine peak, the method is specific and stability-indicating. The method is also accurate and precise. Hence, the method is recommended for routine quality control analysis.

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