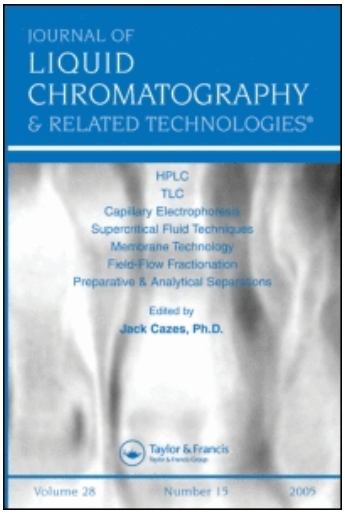

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF GLUCOSAMINE IN NUTRITIONAL SUPPLEMENTS BY REVERSED-PHASE ION-PAIRING HPLC

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Online publication date: 11 July 2000

J. LIQ. CHROM. & REL. TECHNOL., 23(18), 2861–2871 (2000)

DETERMINATION OF GLUCOSAMINE IN NUTRITIONAL SUPPLEMENTS BY REVERSED-PHASE ION-PAIRING HPLC

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ABSTRACT

A reverse-phase high performance liquid chromatography (HPLC) method for the determination of glucosamine in nutritional supplements was developed and validated based on USP guidelines. Separations were performed using a MetaChem Inertsil ODS-3 column with an isocratic mobile phase consisting of 10% Methanol, 90% 0.005M octanesulfonate, pH = 2.1. Glucosamine was detected using a refractive index (RI) detector. The octanesulfonate ion pairing agent allowed for retention on a C-18 column.

The method proved highly reliable with respect to standard performance characteristics. The method allows for convenient quality control in the growing nutritional supplement market.

INTRODUCTION

Glucosamine is an aminosugar and functions as the raw building blocks of glycosaminoglycans such as hyaluronic acid. The mechanism of action, although not completely understood, seems to be related to the activation of chondrocytes to secrete glycosaminoglycans.[1,2] There are two main salts of glucosamine available, the hydrochloride and sulfate forms, with the hydrochloride form delivering more glucosamine relative to the weight of the salt.[3]

Glucosamine salts are considered a prodrug for glucosamine, as the salt component is completely ionized in the stomach, leaving the glucosamine base.[4,5] Comparative cell culture studies have demonstrated that glucosamine base, glucosamine HCL, and glucosamine sulfate are equally active.[6,7]

Pharmacokinetic studies with radiolabled glucosamine have indicated that glucosamine is well absorbed.[4,5,8] These radiolabled bioavalibility studies on glucosamine have been supported by other work.[9]

European trials have shown glucosamine to be effective in reducing the pain associated with osteoarthritis; however, North American trials have had conflicting results.[10-13] To date, all the controlled blinded published trials in the USA with statistically significant positive outcomes, have used glucosamine HCL in combination with low molecular weight chondroitin sulfate.[1,14-17] The same combination has also been shown to be synergistic in stimulating chondrocytes and prevent the progression of osteoarthritis.[1,2]

Since glucosamine especially in combination with chondroitin sulfate has been shown to be clinically effective in reducing pain associated with osteoarthritis, many glucosamine supplements have appeared on the market.[18] Osteoarthritis is a progressive disease that affects over 40 million Americans and the estimated yearly retail sales of glucosamine/chondroitin sulfate supplements is over five hundred million dollars.[19,20] These compounds are classified as dietary supplements; the label claim and, thus, presumed efficacy varies greatly between marketed brands.[21,22]

The inconsistencies between products has caused much confusion in the general public with many groups demanding quality assurance, standardization, and clinical validation of marketed products.[23] In fact, "The Arthritis Foundation's Guide to Alternative Therapies" recommends that consumers purchase the brand that was used, with good results, in clinical trials.[24]

The purpose of this research was to develop and validate an analytical method for assaying glucosamine salts in nutritional supplements, which would be useful for monitoring product content in a quality control environment. While many liquid chromatography methods have been described to separate and quantitate glucosamine,[9,25-33] these methods are primarily geared towards trace level detection in biological samples such as plasma and blood. Sample preparation described in these methods is generally more sophisticated than common quality control laboratories are accustomed to.

These methods often include derivatization[9,26-28,32] steps to allow for retention on C18 reverse phase columns. Moreover, derivatization with a chromophore such as phenylisothiocyanate allow for ultraviolet (UV) detection. Ion exchange methods[25,30,31,33] with pulsed amperometric, conductivity, and refractive

index detection, as well as, post-column labeling for fluorometric detection are well documented, although these methods are not easily suited for routine assays.

However, the method described uses sample preparation which allows for quick and inexpensive quality control assaying.

EXPERIMENTAL

Apparatus

HPLC analysis was performed using a Varian Model 9010 Solvent Delivery System equipped with a Varian Model 9090 Autosampler and an Erma Optical Works ERC-7510 Refractive Index Detector set at 40°C. Glucosamine was separated on a MetaChem Technologies Inertsil ODS-3 column, 250 x 4.6 mm, maintained at 30°C by a MetaChem Technologies MetaTherm column heater.

Samples and standards were introduced using a 50 µL injection size. ChromPerfect Chromatography Software, by Justice Software provided data acquisition and manipulation. A summary of the analysis conditions is shown in Table 1.

Robustness testing was performed using a second system. A Varian Model 9012 Solvent Delivery System with a Rainin Model AI-200 Autosampler and an Erma Optical Works ERC-7510 Refractive Index Detector were used with an Alltech Alltima C18 column, 250 x 4.6 mm while other conditions were held constant.

Reagents and Chemicals

D-glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride, reference grade, was from Pfanstiehl, Waukegan, Illinois. Octanesulfonate, sodium salt

Table 1

Conditions

Mobile Phase	10% Methanol, 90% 0.005M octanesulfonate, pH = 2.1
Flow Rate	1.0 mL/min
Injections	50 µL partial-loop
Column	MetaChem Inertsil ODS-3, 250 x 4.6mm, 30°C
Detector	Refractive Index at 40°C

was obtained through Regis Technologies, Inc., Morton Grove, Illinois. Nutritional supplement samples, Cosamin®DS, were obtained through Nutramax Laboratories® Edgewood, MD.

HPLC grade water was prepared from a Millipore Milli-Q water purification system. All other materials were obtained from commercial sources.

Preparation of Mobile Phase

Approximately 1.1 grams of the sodium salt of octanesulfonate was dissolved in 1000 mL HPLC grade water. The solution was adjusted to pH 2.1 using o-phosphoric acid. The mobile phase was prepared by mixing 900 mL of this solution with 100 mL methanol.

Preparation of Standards for Analysis

Glucosamine HCl is hygroscopic and was stored in a vacuum dessicator. Approximately a 1 gram portion was accurately weighed into a 100 mL volumetric flask and diluted to volume with mobile phase. This stock solution was used to prepare standard solutions of approximately 0.05, 0.08, 0.10, 0.15, and 0.20% (w/w) glucosamine HCl in mobile phase.

Preparation of Nutritional Supplement Samples for Analysis

An amount of sample theoretically equivalent to approximately 100 mg glucosamine HCl was weighed into a 100 mL volumetric flask and diluted to volume with mobile phase. Samples were sonicated for approximately 20 minutes.

Often, some of the nutritional supplement matrix did not dissolve in the mobile phase. Samples were then filtered using 0.45 micron PTFE filters.

RESULTS

The method was validated using 99% D-glucosamine hydrochloride reference standard and a finished product. The finished product, Cosamin®DS contains manganese ascorbate, magnesium stearate, and low molecular weight chondroitin sulfate, in addition to glucosamine hydrochloride.

The validation of the method followed USP guidelines. The data appears in Table 2 and shows the performance of the method to meet all customary criteria.

Table 2

Validation Data

Analytical Parameter	Measurement
Accuracy	98.4% Recovery
Precision	2.13% RSD of 12 Samples
Linearity	> 0.999 Correlation (R^2)
Range	52 to 210% of Target
Specificity	Interferences < 1% of Target
Robustness	97.5% of First Analysis

A placebo formulation containing all ingredients except glucosamine HCl was obtained through Nutramax Laboratories®. To determine method linearity, range, and accuracy, ten (10) samples of the placebo formulation were prepared with spikes of glucosamine hydrochloride in the range of 52% to 210% of label claim. These were analyzed, along with two replicates of the placebo formulation, according to the method.

Over the entire range tested, glucosamine hydrochloride recoveries ranged from 94.4% to 99.9% with an average recovery of 98.4%. The correlation coefficient (R^2) of these measurements was greater than 0.999.

Method precision was determined by analyzing twelve (12) replicate samples of Cosamin® DS according to the method. A typical chromatogram of Cosamin® DS is shown in Figure 1. Preliminary experiments showed unacceptable precision when standards and samples were prepared in water. This is apparently due to the sensitivity of the RI detector to changes in the solvent composition.

A comparison of the two preparations is shown in Figure 2. Results ranged from 484 to 521 mg/g glucosamine hydrochloride, with an average of 506 mg/g and 2.13% RSD. The Cosamin® DS capsules weigh about 1 gram, which shows the measurements to be 96-104% of label claim (500 mg) with an average of 101% of label claim. Analyzing formulation blanks and monitoring the intensity around the retention time of glucosamine hydrochloride was the measure of method specificity.

Additionally, photodiode array data were collected for formulation blanks and glucosamine hydrochloride standards. Neither showed any indication of UV-absorbing (200-360 nm) species in the region of the glucosamine hydrochloride peak.

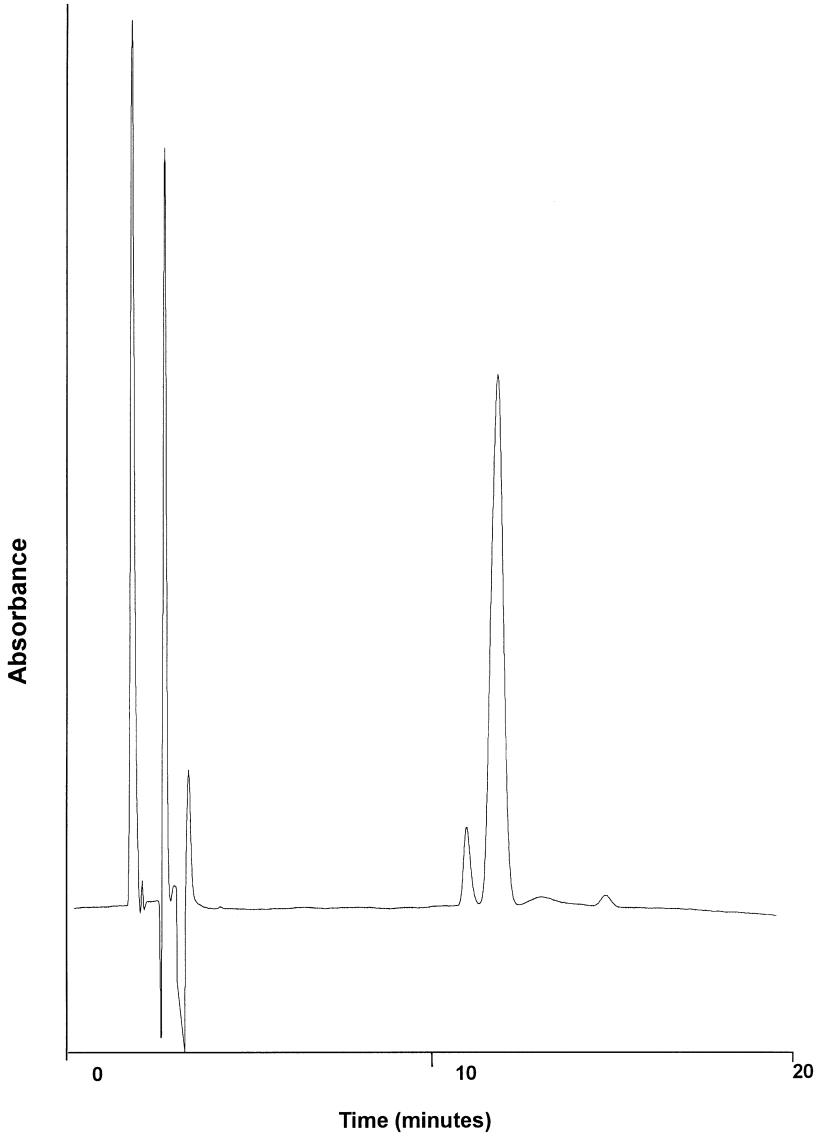

Figure 1. Typical chromatogram of Cosamin DS®.

Figure 2. Chromatogram of Cosamin DS® prepared in water instead of mobile phase.

Robustness of the method was determined by analyzing samples on a different day using an Alltech Alltima C18 column with a second HPLC instrument. The results were an average of 97.5% of the first determination.

DISCUSSION

Glucosamine, shown in Figure 3, poses several difficulties to the HPLC development chemist. It is a small, polar molecule which has poor retention characteristics in reverse phase liquid chromatography. Furthermore, glucosamine has virtually no UV absorbance which makes UV detection impossible without derivitization.

The method described here, utilizes an anionic pairing reagent in the mobile phase to interact with the positively charged glucosamine molecule, which allows for separation on a C18 RPC column. Furthermore, a refractive index (RI) detector was used in this method, thus avoiding the need for chromaphore modification of the glucosamine.

The implementation of an ion pair reagent and RI detection provides the benefit of a method suitable for a routine testing environment.

Most methods in the literature for analyzing glucosamine salts have used pre-column preparation steps in order to modify the separation or detection. These methods can be difficult and time consuming, making them impractical for a quality control laboratory.

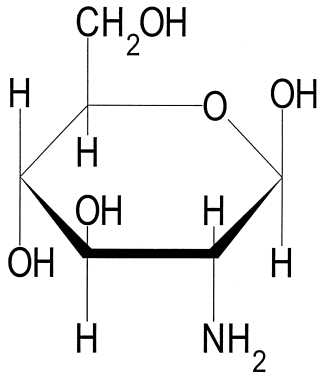

Figure 3. Chemical structure of glucosamine.

While the method described is simple, there is a potential for interference from other aminosugars such as galactosamine and mannosamine. Preliminary investigations have indicated that it is feasible to separate these three aminosugars using ion pair chromatography, although the current method has not been optimized for that separation.

With the possibility of additional government regulation of the nutritional supplement industry, validated quality control methods for assaying nutritional supplement actives will be required.

ACKNOWLEDGMENTS

This work was supported by Nutramax Laboratories®, Incorporated. Furthermore, we thank Jeff Niemeyer for his insightful discussions.

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Received May 1, 2000 Author's Revisions June 28, 2000
Accepted May 27, 2000 Manuscript 5304