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Original Article

Investigation on Counterfeit Glucosamine and Chondroitin Products in Iranian Pharmaceutical Markets

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Abstract

The purpose of this report is to present the results of analysis of actual glucosamine and/or chondroitin contents of several such products in the market place and to determine if they significantly deviate from their label claim. A total of fourteen products containing glucosamine sulfate and nine products containing chondroitin sulfate were evaluated. The amounts of glucosamine and chondroitin were found to be significantly different from the label claim in one product, ranging from as low as 59.00% to over 112.14% of the lable claim for glucosamine and 77.69% to over 94.86 % for chondroitin. Retail price of the product did not appear to be related to the quantity of active ingredients. The overall results of this study show that famous brands are better candidates for counterfeiting than expensive ones.

Keywords: Glucosamine; Chondroitin; Counterfeit.

Introduction

Recently, there has been a flood of dietary supplements containing glucosamine and chondroitin sulfate into the marketplace (1). Some brands have been shown to be clinically effective in reducing pain (2-5), and improving mobility in persons with osteoarthritis (6, 7).

Pharmaceutical counterfeiting is a well-recognized worldwide emerging health problem with a particular impact in many countries where drug-regulatory systems are weak and controls on production, distribution and import are insufficient or ineffective (8, 9). The problem of counterfeit and substandard drugs is a global concern and contributes to poor treatment outcomes, wastes the already scarce financial resources, and may cause drug resistance. Counterfeit drugs are usually illegally marketed and most probably illegally manufactured (10, 11).

The scope of illegally marketed drugs is different in undeveloped, developing, and developed countries. In developed countries, counterfeit medicines are the new and the most expensive lifestyle medicines, while in developing countries most counterfeit medicines are those used to treat life-threatening conditions (12-18).

The World Health Organization (WHO) defines a counterfeit medicine as the one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include those with, wrong ingredients, without active ingredients, with incorrect quantity of active ingredients or with fake packaging (11).

Glucosamine and chondroitin containing

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supplements are easily available in many cities of Iran. In this study, we analyzed the glucosamine and chondroitin containing supplements available in Tehran, capital of Iran. Different dosage forms were collected from the market were tested to uncover the rate of counterfeiting in these products.

Experimental

Apparatus

The HPLC system consisted of Waters (Milford, MA, USA) analytical liquid chromatograph equipped with reversed-phase 250 mm x 4.6.mm i.d. 5-µm particles, Nucleosil 100 C_8 (L7) column or Nova-Pak C_{18} steel column (3.9 mm x 150 mm i.d.; 4-µm particles; Waters, MA, U.S.A.), a 510 HPLC pump, WISP 717 plus autosampler,and variable-wavelength 480 UV detector. The data processing system was a multi-channel Chrom and Spec software for chromatography, version 1.5x. The column and the HPLC system were kept in ambient conditions. T90 Titrator (Mettler Toledo) coupled with DP5 Phototrode™ (Mettler Toledo) at wavelength 550 nm was used for automatic titration by 0.1% cetylpyridiniun chloride.

Chemicals, reagents and materials

Fourteen products were evaluated of which five products contained only glucosamine sulfate, seven products had both glucosamine sulfate and chondroitin sulfate,and two products contained glucosamine sulfate, chondroitin sulfate and methylsulfonylmethane (Table 1). Products were randomly gathered from the market during the period of September 2005 through November 2005.

Glucosamine assay materials

D-(+)-glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride and $Na_2HPO_4 \cdot 12H_2O$ were purchased from Sigma (St. Louis, MO). Methanol, acetonitrile, phenyl isothiocyanate (PITC), phosphoric acid (85%) and glacial acetic acid were purchased from E. Merck (Darmstadt, Germany). All chemicals and solvents were ACS analytical grade or HPLC grade. Deionized water was used for the preparation of standards as well as for all measurements.

Chondroitin sulfate assay materials

Chondroitin sulfate and cetylpyridinium chloride were purchased from Vita Prime Laboratories (NY, USA) and Acros Organics (New Jersey, USA) respectively. NaH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4 and K_2HPO_4 were purchased from Sigma (St. Louis, MO). Tween 80 was purchased from E. Merck (Darmstadt, Germany). All chemicals and solvents were ACS analytical grade or HPLC grade.

Glucosamine sample preparation

The contents of each capsule or tablet (n=10) were weighed individually and transferred quantitatively to a 250 ml volumetric flask and about 150 ml of water was added to each of the samples. The mixtures were sonicated for 20 min and brought to volume with water and filtered. For those products containing only glucosamine, 20 µl of the filtered solution was directly injected to chromatograph (19). For those containing chondroitin, 20 µl of the filtered solution was derivatized with PITC befor the injection. The resulting solutions were then assayed by a RP-HPLC method (20, 21). Simultaneously, a standard solution of glucosamine hydrochloride was treated in a similar manner.

Glucosamine assay method

The HPLC method described in the United State Pharmacopeia (USP 28) was used to quantitate glucosamine (19). Briefly, separation was achieved using a Nucleosil 100 C_8 (L7) steel column (250 mm × 4.6.mm i.d. 5-µm particles). The isocratic mobile phase which was pumped consisted of orthophosphoric acid 0.05% and acetonitrile (60:40, v/v) was prepared daily , degassed by passing through a 0.45 µm filter (Millipore, Milford, MA, U.S.A.) and sonicated for 15 min. The injection volume was 20 µl being pumped at a flow rate of 0.6 ml/min, and the wavelength for UV detection was 195 nm.

Previously reported RP-HPLC systems used pre-column derivatization with phenyl isothiocyanate to quantitate glucosamine in products containing glucosamine and

Table 1. Exact labeling and amounts (mg/tablet or mg/capsule) of fourteen glucosamine/chondroitin products.

Code	Active ingredients (mg)	Batch No.	Expiry date	Amounts Per package	Price per dosage form (USD)*	Country of Origin
A	Glucosamine 500	OBO21	01/08	60	0.15	USA
B	Glucosamine 500	OBO23	07/06	100	0.10	Canada
C	Glucosamine 500	03071	10/07	60	0.11	Canada
D	Glucosamine 750	OK10686	01/08	60	0.21	USA
E	Glucosamine 750	927SW	04/09	120	0.19	USA
F	Glucosamine 500 Chondroitin 400	OBO28	01/07	110	0.21	USA
G	Glucosamine 500 Chondroitin 250	OBO233	09/08	60	0.17	USA
H	Glucosamine 500 Chondroitin 400	016	01/08	60	0.21	USA
I	Glucosamine 500 Chondroitin 400	6000506	06/09	100	0.22	USA
J	Glucosamine 500 Chondroitin 400	4405B7	01/09	60	0.26	USA
K	Glucosamine 500 Chondroitin 400	50601122	12/06	60	0.23	USA
L	Glucosamine 500 Chondroitin 400	2181N	02/07	180	0.33	USA
M	Glucosamine 500 Chondroitin 1200 MSM 200	331H9	06/09	180	0.33	U.K
N	Glucosamine 500 Chondroitin 1200 MSM 200	508539	04/09	60	0.26	Canada

* Each USD is equal to 9000 Rials.

chondroitin (20, 21). Separation was performed using a Nova-Pak C_{18} steel column. The isocratic mobile phase which was consisted of MeOH:H_2O:CH_3COOH (10:89.6:0.04; v/v) was prepared daily, degassed by passing through a 0.45 µm filter, and sonicated for 10 min. The injection volume was 25 µ1 being pumped at a flow rate of 1.2 ml/min, and the wavelength for UV detection was 254 nm.

The precision of dosage form assay was evaluated by the relative standard deviation (RSD), which was <5% at all concentrations. The intraday and interday accuracy, as indicated by the relative error (RE), ranged from -1.97 to 2.13% for glucosamine. The percentages of the label claim for the dosage forms were calculated as follows:

% Label claim = [Assayed amount (mg)/ Labeled amount (mg)] × 100

Chondroitin assay method

Standard preparation

A stock solution was prepared at a concentration of 2 mg/ml from which serial dilutions were made to give concentrations of 0.5, 0.8, 1.0, 1.2, and 1.5 mg/ml as standard solutions. To each standard solution 10 ml of phosphate buffer (pH=7.0) was added and the sample was sonicated for 20 min. 5.0 ml samples were put into a 50 ml beaker,and 25 ml of water was added and stirred. Titration was performed as described in next section.

Phototrode titration method

Nine products containing chondroitin were also tested by the same titration method used for standard solution (19, 29). This method uses potentiometric titration with photometric indication by titrating with a 0.1% w/v solution of cetylpyridinium chloride to quantify chondroitin sulfate. The sample preparation and the titration method are as follows: accurately weigh 10 capsules individually and record the weight, ground the contents to fine powder individually, accurately weigh the equivalent of 100 mg chondroitin sulfate and put into a 100 ml volumetric flask, dissolve in 50 ml water, add 10 ml of phosphate buffer (pH 7.0), sonicate for 20 min, and dilute to volume with water. Filter a portion of the solution (about 20 ml) through 0.45 µm membrane filter. Put 5.0 ml of the samples into a 50 ml beaker, add 25 ml of water, and stir. Adjust the initial transmittance to 70% (at 550 nm) in the phototrode. When titration is complete, the chondroitin sulfate percentage is determined. Titrate twice for each sample and report the mean of two. The amount of chondroitin sulfate content is determined by the following equation:

Chondroitin sulfate content (%)= $(V \times F \times 2000)/P$

Where: V=mL of cetylpyridinium chloride used, P=sample weight in mg, 2000=dilution factor introduced in the titrator as constant CO_2, and F=cetylpyridinium chloride factor against sodium chloride sulfate standard, calculated as chondroitin sodium sulfate assay in mg for 1 ml of ceytlpyridinium chloride.

Results and Discussion

According to samples label, fourteen products contained glucosamine, five of which contained only glucosamine (Table 1). The average amount of glucosamine found in the products met what suggested by the label, with content ranging from as low as 94.78±2.85% (mean±SD; n=10) to over 102.85±4.80% (mean±SD; n=10) of label claims. Only one product was far from what was suggested by the label, with a content range from as low as 59.00% to 112.14% of lable claims (84.77±16.90%; mean±SD; n=10) (Figure 1). As can be seen from Figure 1, the amount found from the assay was different from the label claim in one product.

Nine products contained chondroitin sulfate, seven of which contained chondroitin sulfate in combination with glucosamine and two others contained methylsulfonylmethane in addition to glucosamine contained. The average amount of chondroitin sulfate found in the products met what suggested by the label, with content ranging from as low as 96.88±1.55 % (mean±SD; n=10) to 100.46±2.19% (mean±SD; n=10) of label claims. Only one product was far from what was suggested by the label with a content range from as low as 77.69% to 94.86% of lable claims (87.22±5.84%; mean±SD; n=10) (Figure 2). As can be seen from Figure 2, the amount found from the assay was different from the label claim in one product.

These results highlight the inconsistencies between label claims of products and their actual contents. It should be noted that one product

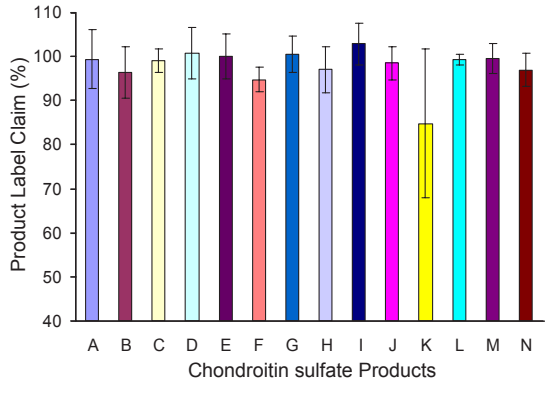

Figure 1. Glucosamine content of the analyzed products based on percent label claimed of products. The bars indicate the standard error of the mean (n=10).

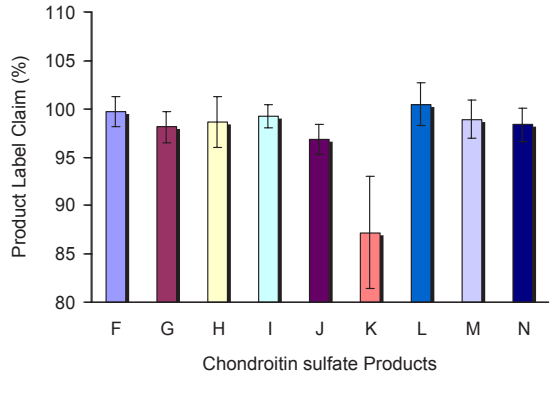

Figure 2. Chondroitin sulfate content of the analyzed products based on percent label claimed of products. The bars indicate the standard error of the mean (n=10).

displayed a significantly large relative standard deviation. This suggests that in addition to having percentages of less than 84.77% for glucosamine and 87.22% for chondroitin with regard to the lable claims, the variability in the amounts of glucosamine and chondroitin sulfate found in each sample was also high (59-112% for glucosamine and 77-94% for chondroitin).

As previously reported, the quality of product might be a function of retail price (22, 23). In order to see if the quality of the products tested might be a function of the retail price, the price of the products tested in this study was calculated on a per gram of ingredient content as reflected on the label. The price of glucosamine varied from a low of 20.89 cents per gram to a high of 66.67 cents per gram. The price of chondroitin sulfate varied from a low of 27.78 cents per gram to a high of 83.33 cents per gram. However, the price of the product did not appear to be related to the quantity of active ingredients (Figure 3 and 4) which was in contrast to other reports (22, 23). Furthermore, one cannot be sure that the most expensive product is also the most pure and effective (22, 23).

Figure 3 presents the percent label claim and adjusted retail price of glucosamine in 14 glucosamine containing products. Only one out of 14 products (7.15%) contained less than 90% of the glucosamine amount stated on the label claim. 13 out of 14 products contained acceptable amounts compared to the labeled amount of glucosamine.

Figure 4 presents the percent label claim and adjusted retail price of chondroitin sulfate in 9 chondroitin sulfate containing products. None of these products was labeled to contain chondroitin sulfate alone but all of them were combined with glucosamine or methylsulfonylmethane. Only one out of nine products was found to contain less than 90% of the chondroitin sulfate amount stated on the label claim. Eight out of nine products containedacceptable amounts comared to the labeled amount of chondroitin sulfate. This would suggest that considerable number of brands that were tested for glucosamine and chondroitin sulfate, are unacceptable products.

In Figures 3 and 4, the supplement retail prices were transformed to reflect a standard retail price (SP) per daily dose of 1500 mg of glucosamine and 1200 mg chondroitin sulfate as reflected on the label.The content percentages of each product's label claim (PLC) were calculated. Statistical analysis revealed that the retail prices of products does not correlate to acceptable percentages of label claims for products containing glucosamine or chondroitin. Only one product (1 out of 14) dissatisfied the industry accepted 10% variation in chondroitin sulfate amount in comparison to the lable claim.

Glucosamine which is currently ranked third behind multivitamins and calcium in the North American supplement category by dollar sales, with retail sales estimated at over $700 million per annum, could be considered as a good candidate for counterfeiting.

This study shows that in some instances, the amount of chondroitin sulfate or glucosamine found in nutraceutical supplement market maybe far from the amount claimed on the label, which

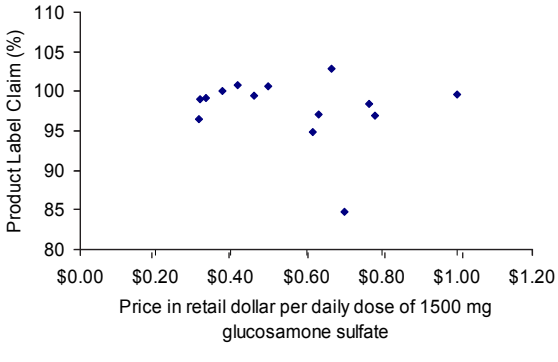

Figure 3. Relationship between glucosamine product's label claim and their standard retail price.

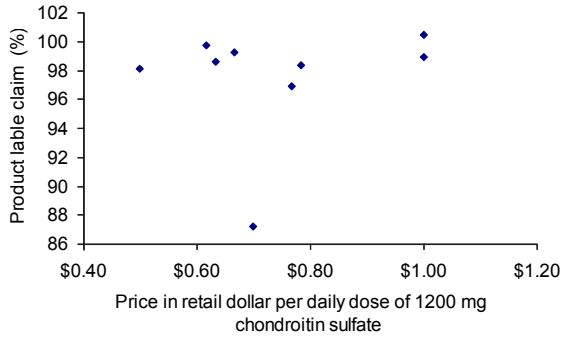

Figure 4. Relationship between chondroitin sulfate product's label claim and their standard retail price.

is in agreement with previous reports from other countries (22-25). The greatest inconsistencies were found in products containing glucosamine. Variations greater than 10% suggests poor quality of raw material or poor manufacturing processes and lack of quality control.However,the results of this study show that famous brands are better candidates for counterfeiting rather than expensive ones. This work was run on a small number of samples and needs further larger-scale surveys to determine the true extent of glucosamine and chondroitin counterfeit in Iran. In addition, other quality attributes like bioavailability, permeability test, disintegration time and dissolution rates of the tablets or capsules have to be studied. These studies will be done in the near future.

These findings validate the overall attitude of skepticism towards the claims of quality control in some of the nutraceutical companies (22, 23).

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