

# Analysis of Glucosamine and Chondroitin Sulfate Content in Marketed Products and the Caco-2 Permeability of Chondroitin Sulfate Raw Materials

Abimbola O. Adebawale, PhD, Donna S. Cox, MS, Zhongming Liang, MS, Natalie D. Eddington, PhD,\*  
Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences,  
School of Pharmacy, University of Maryland, Baltimore, Maryland

## ABSTRACT

**Objective:** The purpose of this report is to evaluate and present the results of analysis of actual contents of several products in the marketplace containing glucosamine and/or chondroitin sulfate and to determine if they significantly deviate from label claim. In addition, the study examined the intestinal transport of several marketed sources of chondroitin sulfate.

**Methods:** A total of fourteen products containing glucosamine hydrochloride or sulfate and eleven products containing chondroitin sulfate were evaluated using a UV-HPLC method. In addition, a total of 32 products containing chondroitin sulfate were tested using a titration method. The permeability of various marketed sources of raw materials of chondroitin sulfate across Caco-2 cell monolayers were assessed. This analysis was an attempt to evaluate whether different suppliers of chondroitin sulfate use different grades of material.

**Results and conclusions:** The amounts of glucosamine and chondroitin found after analysis were significantly different from the label claim in some products, with deviations from label claims ranging from as low as 0% to over 115%. Products with a retail price of less than or equal

to one dollar per 1200 mg of chondroitin sulfate were found to be seriously deficient in meeting label claim (less than 10% of label claim). The permeability of the different molecular weight chondroitin sulfates was found to be significantly different ( $p < 0.05$ ), with the permeability coefficient increasing with decreasing molecular weight. This suggests that molecular weight of chondroitin sulfate could be a possible predictor of permeability.

## INTRODUCTION

Dietary supplements containing glucosamine and/or chondroitin sulfate are numerous and popular. Since the Dietary Supplement Health and Education Act (DSHEA) does not demand the same rigorous requirements for quality manufacturing as pharmaceuticals, many of the dietary supplements marketed may not provide high quality material or meet labeled quantities.

Over the last five years there has been a significant increase in the number of dietary supplements that have been introduced into the market. The glucosamine and chondroitin market has been estimated to be over five hundred million in retail sales between July 1998 and May 1999.<sup>1</sup> Unfortunately the quality of dietary supplements remains in question due to the lack of regulatory provisions that directly evaluate the actual content of active ingredients.<sup>2</sup> Hence consumers have limited ways to judge the quality of the products they are purchasing. Independent analysis on many supplements, including calcium, St. John's Wort, carnitine, Ginkgo biloba, and super oxide dismutase, showed that many marketed brands contained subpotent content, including some with zero active ingredients.<sup>3,4</sup>

---

### \* Correspondence:

Natalie D. Eddington, PhD  
Department of Pharmaceutical Sciences  
School of Pharmacy  
University of Maryland  
20 North Pine Street  
Baltimore, Maryland 21201-1180  
Phone: 410-706-6710 Fax: 410-706-6580  
Email: neddingt@rx.umaryland.edu

Dietary supplements aimed at the management of osteoarthritis, (OA) a common disease that affects more than 40 million Americans,<sup>5</sup> have also proliferated. These supplements generally contain glucosamine, chondroitin sulfate, or a combination thereof. As with other dietary supplements, many products containing either glucosamine or chondroitin sulfate have been reported to not meet label claims.<sup>6,7</sup>

OA is a disease process associated with alteration in the structure and function of synovial joints,<sup>8</sup> resulting from a loss of balance between synthesis and degradation of the macromolecules needed to provide joint tissue with its biomechanical and functional properties.<sup>9</sup> OA usually occurs insidiously, apparently as part of the aging process and without obvious initiating cause (primary or idiopathic OA). The disease progresses in the majority of patients.<sup>10</sup> The typical clinical symptoms are pain, stiffness, and limitation of motion. These factors are reflected in difficulties in performing activities of daily living, eventually altering the patient's quality of life.<sup>11</sup>

Glucosamine, a bioavailable amino sugar when administered as a sulfate or hydrochloride salt, has been shown to help relieve the symptoms of osteoarthritis.<sup>12-17</sup> European research has shown "glucosamine sulfate" to be effective in clinical trials.<sup>12-14</sup> However the radiolabeled pharmacokinetic studies on glucosamine sulfate radiolabeled glucosamine HCL, not sulfate.<sup>17,18</sup> This research also states that the salts of glucosamine sulfate are a prodrug for glucosamine.<sup>19</sup> Glucosamine base is the active component not the salt. Glucosamine is also completely ionized in the stomach, so any salt is likely to be cleaved off, leaving the glucosamine base.<sup>17</sup> Comparative cell culture studies have also shown that glucosamine base, glucosamine HCL, as well as glucosamine sulfate, are equally active.<sup>20,21</sup> The active moiety of any form is the glucosamine molecule, not the salt carrier. To date all the controlled, blinded, published studies in North America with any statistically significant positive outcomes have used glucosamine HCL with the most convincing results in combination with chondroitin sulfate.<sup>15,22-27</sup> The only randomized, placebo-controlled US trial evaluating glucosamine sulfate alone as treatment for osteoarthritis of the knee found that it was no better than placebo in reducing pain.<sup>28</sup> A recent review article from a well known rheumatologist has also stated that "a higher dosage of the sulfate salt rather than the hydrochloride salt is required to provide a 1500 mg dose of glucosamine".<sup>7</sup>

Chondroitin sulfate, a much larger molecule than glucosamine, is a glycosaminoglycan made up of glucuronic acid and galactosamine. When given orally in the pure form of a molecular weight of approximately 16,900 Dalton, it has been shown to be bioavailable and efficacious in decreasing pain and slowing the progression of osteoarthritis in humans.<sup>29-33</sup> The efficacy studies, completed in Europe,

have been evaluated by meta-analysis in the United States.<sup>34,35</sup> Studies completed in the United States have used the same pure low molecular weight chondroitin sulfate in combination with the amino sugar glucosamine with good results.<sup>22-27</sup> It has been noted that results of clinical trials are directly related to the quality of material used.<sup>7</sup>

Pure low molecular weight chondroitin sulfate has been shown to have up to a three month efficacy carry-over effect.<sup>32</sup> This is an interesting point as individuals who take a quality product and switch to an impure product may have three-months of results before symptoms reoccur. This coupled with the standard 20-30% placebo effect in all oral arthritis treatments may lead to a false sense of security in the consumer or prescribing physician. When the name of the product or label makes a claim such as "arthritis cure" or "pain formula" this could possibly potentiate the placebo effect.

Since chondroitin sulfate is a large molecule, bioavailability has been questioned.<sup>36,37</sup> However, recent research has supported absorption after oral administration.<sup>31,38</sup> One factor that may affect absorption is the actual chain length of the molecule. It has been shown that low molecular weight chondroitin sulfate has a superior kinetic profile than high molecular weight.<sup>39</sup> The molecular weight as well as the molecular composition of chondroitin sulfate are dependent upon the species and/or tissue of origin and may be affected by the extraction method.<sup>39</sup>

Referenced studies highlight the current problems with quality control of dietary supplements in general, and specifically products containing glucosamine and/or chondroitin sulfate. The purpose of this report is to evaluate and present the results of an analysis of actual content of several products containing glucosamine and/or chondroitin sulfate and to determine if they significantly deviate from label claim. In addition, as an index of bioavailability, permeability studies were performed using Caco-2 cell monolayers to examine the intestinal transport of several marketed sources of chondroitin sulfate.

Caco-2 cell monolayers are recognized as excellent models of intestinal transport. Caco-2 cells are derived from human colon adenocarcinoma, and have morphological features similar to intestinal epithelia.<sup>40</sup> When grown on semipermeable filters, Caco-2 cells spontaneously differentiate in culture to form a confluent monolayer which both structurally and functionally resembles the small intestinal epithelium. The Caco-2 cell line exhibits a well-differentiated brush border on the apical surface and tight junctions. The monolayer also has bipolar properties of an apical surface as well as a basolateral surface with differentiated and different transport properties. The pharmaceutical industry is increasingly using Caco-2 cells as a model to screen compounds for intestinal absorption and to predict transport routes and even rates of flux before compounds go to preclinical testing. A citation study done through PubMed

showed that since 1979 over 1490 publications dealt with Caco-2 cells, and 527 of those publications have appeared in the last two years, attesting to the rapidly growing use and interest in this model system.

## MATERIALS AND METHODS

A total of 14 products containing glucosamine hydrochloride or sulfate and 11 products containing chondroitin sulfate were evaluated using the UV-HPLC method described below.<sup>16-41</sup> In addition, a total of 32 products containing chondroitin sulfate were tested using the titration method as described in the assay methods section. The products were randomly gathered from the marketplace.

**Chondroitin Sulfate Assay Materials:** 95% chondroitin 4-sulfate (from bovine trachea) was purchased from Bioiberica (Poligono Industrial, Barcelona, Spain). Sodium phosphate monobasic was purchased from Fisher Scientific (Pittsburgh, PA). Sodium phosphate dibasic was purchased from Sigma Chemical Co. (St. Louis, MO). Deionized water was prepared by ultrapure water system Pyrosystem Plus\* (Hydro, Research Triangle Park, NC).

**Glucosamine Assay Materials:** D(+) glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride was purchased from Sigma Chemical Co. Methanol, phenyl isothiocyanate (PITC), sodium dibasic phosphate, and glacial acetic acid were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). All chemicals and solvents were ACS analytical grade or HPLC grade.

**Caco-2 Cell Culture Materials:** The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD). Dulbecco's modified eagle medium (DMEM), Dulbecco's modified phosphate-buffered saline with and without Ca<sup>2+</sup> and Mg<sup>2+</sup> (PBS), nonessential amino acids (NEAA), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%)-EDTA (1mM), and penicillin G-streptomycin sulfate antibiotic mixture were purchased from Gibco Laboratories (Lenexa, KS). T-flasks were obtained from Becton Dickinson Labware (Franklin Lakes, NJ); Transwell clusters, 24 mm in diameter (surface area 4.71 cm<sup>2</sup>) and 0.4- $\mu$ m pores were from Corning Costar (Cambridge, MA). All other chemicals were of analytical grade from Sigma Chemical Co. and Fisher Scientific (Fair Lawn, NJ).

**Preparation of Chondroitin Sulfate Products for Quality Control Testing:** The contents of each capsule or tablet were weighed, transferred to a 100 ml volumetric flask, and made up to volume with 0.1 M phosphate buffer pH 7.0. The resulting solutions (raw materials) or suspensions (capsule and powder) were sonicated for 20-30 minutes and subsequently filtered through a 0.45  $\mu$ m membrane filter. Ten  $\mu$ l of the final solution was assayed by a validated HPLC method.<sup>41</sup>

**Preparation of Samples of Glucosamine Products for Quality Control Testing:** The contents of each capsule or tablet were weighed and transferred quantitatively to a 250 ml volumetric flask and about 150 ml of water added. The mixture was sonicated for 20 minutes and brought to volume with water. A portion of each solution was filtered and 20  $\mu$ l of the filtered solution was derivatized with PITC. Simultaneously, a standard solution of glucosamine hydrochloride was treated in a similar manner. The resulting solutions were assayed by a validated HPLC method.<sup>16</sup>

**Permeability of Chondroitin Sulfate Across Caco-2 Cell Monolayers:** The growth, maturation, and seeding of Caco-2 cells have been previously described.<sup>40</sup> Caco-2 cells with a passage number between 45 and 55 were used in an effort to derive Caco-2 monolayers with consistent morphological and biochemical properties. Caco-2 cells were grown in T-150 flasks at 37°C in an atmosphere of 5% CO<sub>2</sub> using Dulbecco's modified Eagle medium (DMEM) supplemented with 2% L-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin, and 10% fetal bovine serum.

Caco-2 cells grown for 21 days on the transwell filters were washed free of medium and allowed to incubate at 37°C in 1.5 ml Dulbecco's phosphate buffered saline (PBS) in the insert (apical chamber) and 2.5 ml in the basolateral chamber. Bi-directional studies (apical to basolateral and basolateral to apical) were performed, with 1.5 ml (apical) or 2.5 ml (basolateral) fresh PBS containing chondroitin sulfate (0.2 -0.8 x 10<sup>-3</sup> M). At 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 minutes the insert was transferred to fresh solutions (2.6 ml) of PBS in the basolateral chamber. At the end of the experiment (120 minutes), a sample was withdrawn from the donor chamber to evaluate for metabolism and cumulative transport. The incubations were maintained at 37°C and the monolayers were agitated orbitally at 50-60 rpm during the course of the permeability study. The percentage amounts of compound appearing in the apical or basolateral chamber after each sampling interval were added to obtain the cumulative transport. The molecular weights of the raw materials of chondroitin sulfate were determined by using a linear calibration method and the samples were analyzed by a validated HPLC method for chondroitin sulfate.<sup>40</sup>

To determine drug transport from the apical to basolateral chambers, effective permeability coefficients, P<sub>eff</sub> (cm s<sup>-1</sup>), were calculated from the following relationships:

$$P_{\text{eff}} = \frac{V_R \cdot \left(\frac{dC}{dt}\right)_{\text{SS}}}{A \cdot C_o}$$

Where V<sub>R</sub> = volume of the basolateral or apical (receiver) chamber (cm<sup>3</sup>), A = cell monolayer surface area (ie, 4.71

cm<sup>2</sup>), Co = initial donor concentration of solute (mMol ml<sup>-1</sup>), and (dC/dt)<sub>ss</sub> = initial linear portion of a plot of the cumulative receiver concentration of permeant with time, i.e., flux across the monolayer at steady state (mMol ml<sup>-1</sup>s<sup>-1</sup>).

**Chondroitin Sulfate Assay Method from University of Maryland (HPSEC):** A simple, specific, rugged, and precise high-performance size exclusion chromatographic (HPSEC) method was used to quantify chondroitin sulfate in dosage forms and cell culture samples using a Polysep-GFC-P linear column.<sup>41</sup> The samples were solubilized in 0.1M-phosphate buffer and the resulting solutions filtered through a 0.45 $\mu$ m membrane filter. The mobile phase was phosphate buffer (0.1M; pH 7.0) and detection was by ultraviolet absorbance at a wavelength of 207 nm. The intraday and interday precision as indicated by the relative standard deviation was less than 2.83% and 3.53%, respectively.

**Phototrode Titration Method (Bioiberica):** Thirty-two products were also tested by titration (Phototrode) (Bioiberica). This method uses potentiometric titration with photometric indication by titrating with 0.1% solution w/v of N-cetylpyridinium chloride to quantify chondroitin sulfate. The sample preparation and procedure is as follows: weigh accurately 100 mg of sample dried for 2 h at 105°C into a graduated flask. Dissolve in about 30 ml of water, add 10 ml of pH 7.0-phosphate buffer and dilute to the mark with water. Take 5 ml aliquots, bringing these to 30 ml with water and start titration. Adjust the initial transmittance to 70% in the phototrode, adjusting the wavelength at 420 nm. When titration is complete, the sodium chondroitin sulfate percentage is determined. The amount of chondroitin sulfate content is determined by the following equation:

$$\text{Chondroitin Sulfate Sodium Content \%} = \frac{V \times F \times 2000}{P(\text{mg})}$$

Where: V = mL of N-cetylpyridinium chloride used, P = sample weight in mg, 2000 = dilution factor introduced in the titrator as constant CO<sub>2</sub>, and F = N-cetylpyridinium chloride factor against sodium chloride sulfate standard, calculated as sodium chondroitin sulfate assay in mg for 1 ml of N-cetylpyridinium chloride.

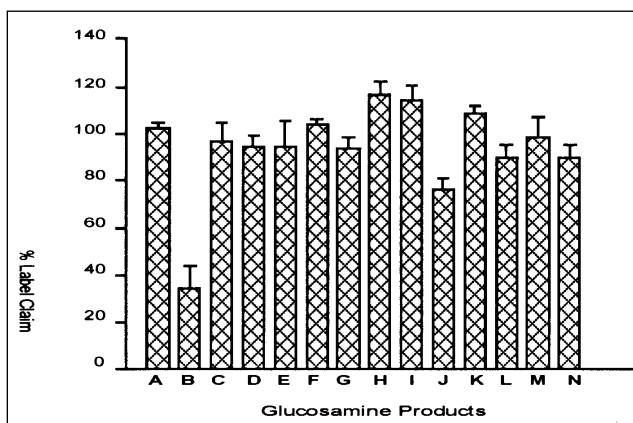
**Glucosamine Hydrochloride Assay Method:** A specific high performance liquid chromatography method was developed to quantitate glucosamine hydrochloride.<sup>16</sup> Reverse phase chromatography using pre-column derivatization with phenyl isothiocyanate, and ultraviolet detection ( $\lambda$ =254nm) was used to quantitate the eluate. The mobile phase consisted of MeOH:H<sub>2</sub>O:CH<sub>3</sub>COOH (10:89.6:0.04) and was pumped at a flow rate of 1.2 ml/min. The precision of the dosage form assay, expressed as the % relative standard deviation (RSD), was <5% at all concentrations. The intraday and interday accuracy, as indicated by the relative error (RE), ranged from -2.54 to 2.70% for glucosamine hydrochloride.

**Data Analysis:** The percent of the label claim for the capsules was calculated as follows:

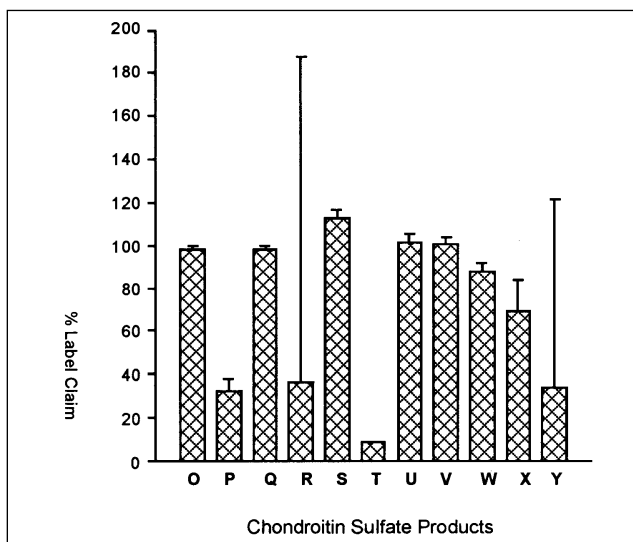
$$\% \text{ Label claim} = \frac{\text{Assayed amount (mg)}}{\text{Labeled amount (mg)}} * 100$$

## RESULTS

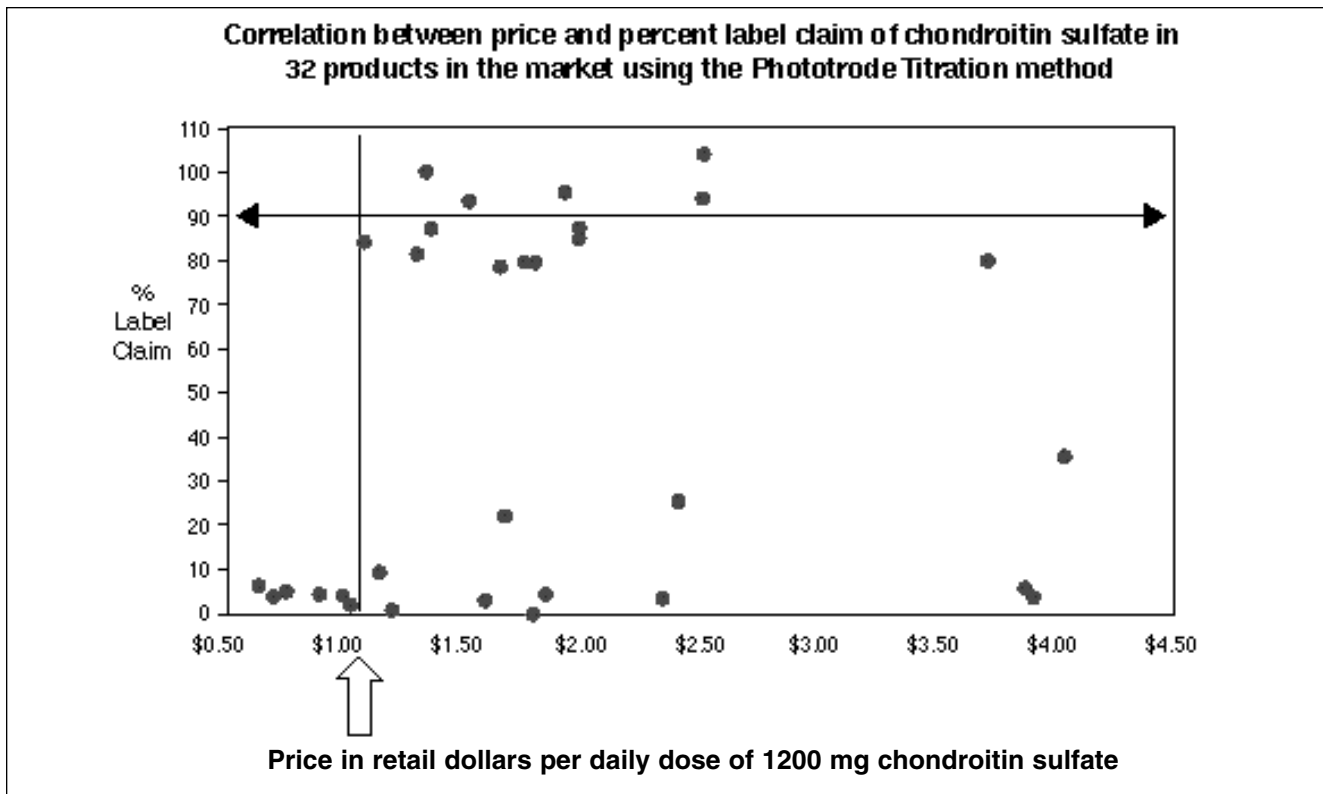
Figure 1 illustrates the percent label claim of 14 products that contained glucosamine hydrochloride or sulfate, some with chondroitin sulfate. As can be seen from the figure, the amount found after analysis was significantly different from the label claim in some products, with deviations from label claims ranging from as low as 25% to over 115%. These results highlight the inconsistencies between label



**Figure 1.** Percent label claim of products containing glucosamine hydrochloride or sulfate as determined by a validated UV-HPLC method with pre-column derivatization (University of Maryland Analysis).<sup>45</sup> The bars indicate the standard error of the mean.



**Figure 2.** Percent label claim of products containing chondroitin sulfate as determined by a validated UV-HPLC method using size exclusion chromatography (University of Maryland Analysis).<sup>45</sup> The bars indicate the standard error of the mean.



**Figure 3.** Relationship between product's label claim and their standard retail price (SP)

claims of dietary supplements and actual content found in the product. Eleven chondroitin sulfate containing products, some also containing glucosamine, were analyzed for content with a validated HPLC method. The results of the label claim testing are presented in Figure 2. The percentage label claims ranged from as low as 33% to as much as 110%. It should be noted that some products (eg, R and Y) displayed significantly large relative standard deviations. This suggests that in addition to these products having percentage label claims of less than 40%, the variability in the amount of chondroitin sulfate found in each capsule also varies significantly.

Figure 3 presents the percent label claim and adjusted retail price of chondroitin sulfate in 32 chondroitin sulfate containing products purchased from pharmacies and health food stores during the period of September 1998 through November 1999 and analyzed by the titration method. A few of these products were labeled to contain chondroitin sulfate alone but the majority was combined with glucosamine. Twenty-six out of 32 products were found to contain less than 90% of the chondroitin sulfate stated on the label with 17 products containing less than 40% of label claim. Only five out of 32 products contained the labeled amount of chondroitin sulfate in the product. This would suggest that in many cases, 84% of brands that were tested are inferior products.

Our initial hypothesis was that the quality of product may be a function of retail price. In a separate analysis of the data contained in Figure 3, the supplement retail purchased prices, not including any consumer rebates, were transformed to reflect a standard retail price (SP) per daily dose of 1200 mg of chondroitin sulfate as reflected on the label. The content-analyzed products and their percentages relative to each product's label claim (PLC) were calculated. Figure 3 is a scatter plot showing that products with a standard retail price of less than or equal to one dollar are seriously deficient in meeting label claim (less than 10% PLC). When the retail price exceeds one dollar, the figure indicates that there are two different clusters of products, with the majority having less than acceptable label claim. Only a few products (5 out of 32) satisfied the industry accepted 10% variation in label claim in levels of chondroitin sulfate. Variation greater than 10% suggests poor quality of raw material or poor manufacturing processes and lack of quality control.

The results of these studies demonstrate that in some instances the amount of the chondroitin sulfate or glucosamine found to be present in the sample product varies significantly from the amount reported on the label. The greatest inconsistencies were obtained in products containing chondroitin sulfate, especially when the daily 1200 mg dose price was less than one dollar. The implications of these results are significant and support the need for regu-

latory intervention of dietary supplements.

In addition to examining the label claims of chondroitin sulfate products, we also examined the permeability of various marketed sources of raw materials of chondroitin sulfate across Caco-2 cell monolayers. This analysis was an attempt to evaluate whether different suppliers of chondroitin sulfate use different grades of material. Studies have shown a very good correlation between drug permeability in the Caco-2 model and intestinal drug absorption in humans.<sup>38</sup> Drugs administered orally have displayed absorption between 50 to 100% when the Papp value has been in the range of 0.20 to 54.5 x 10<sup>-6</sup> cm/sec. However, it should be noted that the molecular weights evaluated were all less than 1,000 Dalton. In order to show that the Caco-2 cells model is acceptable for assessing permeability of polymers, three different MW chondroitin sulfate materials were extracted and purified by the same manufacturer (Bioiberica) to be used specifically for calibration. The permeability of the different molecular weight chondroitin sulfates was found to be significant (p < 0.05), with the permeability coefficient increasing (10.1 x 10<sup>-6</sup> cm/sec, 12.5 x 10<sup>-6</sup> cm/sec, 16.2 x 10<sup>-6</sup> cm/sec) with decreasing molecular weight (16.9, 8.0, and 4.0 x 10<sup>3</sup> Dalton). This suggests that the molecular weight of chondroitin sulfate could be a predictor of permeability.

A comparison of the permeability of chondroitin sulfate from different manufacturers with permeability of known molecular weight chondroitin sulfate, (ie, raw material A) (Bioiberica 95% mol wt = 16,900 Dalton) is presented in Table 1. This reference standard of low molecular weight material has been shown to be efficacious and bioavailable in European and US trials.<sup>22-25, 29-33</sup> These results indicate a low permeability for raw materials F (Papp = 1.03 x 10<sup>-6</sup> cm/sec), E (3.63 x 10<sup>-6</sup>cm/sec), C (7.94 x 10<sup>-6</sup>cm/sec), and B (8.73 x 10<sup>-6</sup>cm/sec) raw materials. The data suggests that the molecular weights of these raw materials are probably > than 16,900. The proposed higher molecular weight chondroitin sulfates (ie, with exceedingly low permeability) suggest that their intestinal permeability and hence absorption would be expected to be significantly less than the low molecular weight chondroitin sulfate (eg, 16,900 dalton). Similar results have been observed in studies examining the permeability of polyethyleneglycol (PEG) of various molecular weights. The permeability of PEG was found to be inversely correlated with molecular weight.<sup>40</sup>

In summary, it would appear that the molecular weight of chondroitin sulfate has a direct influence on its permeability across the gastrointestinal tract, where higher permeability is observed for chondroitin sulfate with lower molecular weight. Further, when comparing Papp values from our chondroitin sulfate studies with values reported in the literature with agents such as dexamethasone (Papp = 12.5 X10<sup>-6</sup> cm/sec) and salicylic acid (Papp = 11.9 x10<sup>-6</sup> cm/sec), it would appear that chondroitin sulfate (of low molecular

Raw Material	Permeability Coefficient (x 10 <sup>-6</sup> ) (n=3) (cm/sec)
A <sup>1</sup>	10.1 (±0.6)
B	8.73 (±9.07)
C	7.94 (± 7.35)
D	0.00 (± 0.00)
E	3.63 (± 2.07)
F	1.03 (± 1.78)

<sup>1</sup>mol Wt = 16,900 dalton (95% Bioiberica)

**Table 1.** Permeability coefficient for transport across Caco-2 cells of chondroitin sulfate from different manufacturers

weight, Papp = 10.1 x 10<sup>-6</sup> cm/sec) should be absorbed after oral administration. Any differences observed experimentally (eg, after clinical or animal bioavailability studies) may be due to molecular weight influences or first pass metabolism prior to systemic circulation. In addition, characteristics other than molecular weight such as flexibility of structure, degree of sulfation, and method of manufacture may also be important for oral absorption.

## DISCUSSION

Glucosamine and chondroitin sulfate when administered in a pure bioavailable form have been shown to be safe and efficacious.<sup>22,23,34,35</sup> Our results suggest that there is a significant deviation between the content of the active ingredients (glucosamine or chondroitin sulfate) and what is stated by the manufacturers on the label. It would appear that this deviation is greater for those products containing chondroitin sulfate. In light of these findings, an interesting question emerges: what can the consumer or health care provider do to obtain a quality product containing pure chondroitin sulfate or glucosamine? A recent review by The Arthritis Foundation provides the following suggestions: (1) consumers should consult with their physician or health care provider concerning these two supplements; (2) healthcare professionals should become knowledgeable about glucosamine and chondroitin sulfate products; (3) consumers should not purchase through the mail or internet unless they know the vendor; and (4) consumers should buy from companies that use USP material (neither chondroitin sulfate nor glucosamine have a monograph as of yet).<sup>6</sup>

Prior to obtaining any supplement containing chon-

droitin sulfate or glucosamine, the consumer should become informed about the manufacturer and the product. The most useful technique recommended by the Arthritis Foundation is to ask the manufacturer for research showing that their brand has been scientifically proven or studied. It is important for manufacturers to evaluate their product, and especially the source of chondroitin sulfate used since not all chondroitin sulfates are identical. Furthermore, consumers should shy away from products that are backed only by testimonials and not scientific research. Products that make overt claims such as regenerates cartilage, renews cartilage, rebuilds cartilage, cures arthritis, or freedom from pain, should be looked at with skepticism as these statements seldom mean that the product has been researched to make these claims.<sup>42</sup> Topical and liquid glucosamine/chondroitin products are also promoted, claiming a higher bioavailability than capsules or tablet dosage forms. To the authors' knowledge, there is no published research to substantiate this claim.

Based on a 1200 mg/day chondroitin sulfate dosage, products that retailed in the pharmacy or health food store for less than \$1.00 per day contained a sub-potent level of CS and did not meet label claims. However, it must be noted that some of the more costly products also did not meet label claims. Therefore, proposed recommendations for consumers are to be wary of inexpensive products and compare the calculated daily price based on 1200 mg of CS/day.

These findings verify the scientific community's skepticism towards nutraceuticals based on the lack of quality control by some manufacturers. These substances are not pharmaceuticals; there is no requirement for pharmaceutical Good Manufacturing Practices to guarantee high quality, batch-to-batch consistency. The use of validated analytical methods for the raw materials and finished products is the only mechanism to verify purity. It is not surprising to know that the Arthritis Foundation has recently recommended that "when a supplement has been studied with good results, find out which brand was used in the study, and buy that".<sup>6</sup>

It is worthy to note that there is great variability in the permeability of the chondroitin sulfate raw materials. Permeability could have a direct effect on efficacy. Therefore, caution is warranted to not extrapolate the results of previously reported experimental and clinical studies to all forms of chondroitin. Some products that tested satisfactory on content could be lacking efficacy because of poor permeability.

In addition to concerns on the content, bioavailability, and effectiveness of dietary supplements, an often-overlooked area is safety. Pure chondroitin sulfate and glucosamine evaluated in published studies is shown to be safe including hemostatic, hematological, and biochemical parameters with minimal side effects.<sup>15,22,23,30,32,33,43</sup> Chondroitin sulfate and glucosamine are extracted from

animal tissues; therefore impurities may cause allergic or other side effects that raise serious concerns.<sup>44</sup> It is unknown if less than pure, non-researched sources have a good safety profile.

The HPLC and titration assay methods used in our study will not detect other compounds that might be present in the product along with chondroitin sulfate or glucosamine. It is possible that some suppliers may dilute chondroitin sulfate with materials that can cause analytical methods to overestimate contents. These compounds could include sugars such as maltose, or other glycosaminoglycans such as dermatan sulfate, keratan sulfate, heparin, or hyaluronic acid. None of these glycosaminoglycans have efficacy in osteoarthritis when given orally.

In summary, this report highlights the problems that consumers encounter when attempting to purchase quality dietary supplements containing glucosamine and chondroitin sulfate. Despite terms like "quality tested" appearing on labels, consumers and healthcare providers have no basis to compare one product against another or to judge the quality of the products they are purchasing or recommending. Over fifty products were tested and a substantial number could not be classified as a "quality product" based on the general agreement of their content with label claim. From our data it is obvious that certain manufacturers of dietary supplements are unwilling to self-regulate their manufacturing practices.

## ACKNOWLEDGEMENT

Partial funding for this study was provided as an unrestricted educational grant from Nutramax Laboratories® Inc., Edgewood, Maryland.

## REFERENCES

1. Hartman Group's Natural Products Census Supplement Report, 1999, Bellevue, Wash.
2. Foley CM, Kratz AM. Nutraceuticals—challenges and opportunities for the new millennium that affect consumers and healthcare professionals who use and recommend nutraceuticals. *JANA* 1999; 2(2): 6-10.
3. Moore T. Messing with Mother Nature. *The Washingtonian* July 1999; 58-115.
4. Beal B. "Evaluation of active enzyme activity in six oral superoxide dismutase products." Paper presented at the 25th annual conference of the Veterinary Orthopedic Society, Snowmass, Colorado, 1998;65.
5. Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998; 41, 778-799.
6. Horstman J. *The Arthritis Foundation's Guide to Alternative Therapies*. The Arthritis Foundation. Atlanta, Georgia:1999;179-180.
7. Deal CL, Moskowitz RW. Nutraceuticals as therapeutic agents in osteoarthritis: the role of glucosamine, chondroitin sulfate, and collagen hydrolysate. *Rheum Dis Clin North Am* May 1999; 25, 379-395.

8. Dieppe P, Brandt KD, Lohmander S, Felson DT. Detecting and measuring disease modification in osteoarthritis: the need for standardized methodology. *J Rheumatol* 1995; 22: 201-203.
9. Lohmander LS, Felson DT. Defining the role of molecular markers to monitor disease, intervention, and cartilage breakdown in osteoarthritis. *J Rheumatol* 1997; 24:782-785.
10. Hochberg MC. Development and progression of osteoarthritis. *J Rheumatol* 1996; 23:1497-1499.
11. Martin DF. Pathomechanics of knee osteoarthritis. *Med Sci Sports Exerc* 1994; 26:1429-1434.
12. Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity of oral glucosamine sulfate in osteoarthritis: a placebo-controlled double-blind investigation. *Clin Ther* 1998; 3:260-272.
13. D'Ambrosio E, Casa B, Bompani R, Scali G, Scali M. Glucosamine sulfate: a controlled clinical investigation in arthrosis. *Pharmacotheurapeutica* 1981; 2(8):504-508.
14. Qiu Xing G, Gao Neng S, Giacobelli G, Rovati L, Setnikar I. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Drug Research* 1998; 48:469-474.
15. Houpt J, McMillan R, Wein C. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. *Journal of Rheumatology* 1999; 26:2423-2430.
16. Liang Z, Leslie J, Adebowale A, Ashraf M, Eddington, ND. Determination of the nutraceutical glucosamine hydrochloride in raw materials, dosage forms, and plasma using pre-column derivatization with ultraviolet HPLC. *J Pharm Biomed Anal* 1999; 20: 807-814.
17. Setnikar I, Giacchetti C, and Zanolo G. Pharmacokinetics of glucosamine. *Drug Res* 1986; 36:729-734.
18. Setnikar I, Giacchetti C, Zanolo G. Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [<sup>14</sup>C] glucosamine to the rat. *Pharmatherapeutica* 1984;3, 8.
19. Setnikar I, Palumbo R, Canali S, Zanolo G. Pharmacokinetics of glucosamine in man. *Drug Res* 1993; 43: 1109-1113.
20. Karzel K, Domenjoz R. Effect of hexosamine derivatives and uronic acid derivatives on glycosaminoglycan metabolism of fibroblast cultures 1970;5:337-345.
21. Blanco FJ, Maneiro E, deToro FJ, et al. "Effect of Different Extracellular Matrix Components on Nitric Oxide Production By Human Osteoarthritic Chondrocytes." Paper presented at the international symposium on regulatory issues, Vienna, Austria, 1999.
22. Das AK, Eitel J, Hammad TA. "Efficacy of a new class of agents (glucosamine hydrochloride and chondroitin sulfate) in the treatment of osteoarthritis of the knee." Paper #180 presented at the 66th annual meeting, American Academy of Orthopedic Surgeons, Anaheim, Calif, February 6, 1999. Accepted for publication in *Osteoarthritis & Cartilage*.
23. Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Mil Med* February 1999; 164:2:85-91.
24. Lippiello L, Woodward J, Karpman R, Hammad TA. Beneficial effect of cartilage disease-modifying agents tested in chondrocyte cultures and a rabbit instability model of osteoarthritis. *Arthritis & Rheum* September 1999; 42(9) (supple S256).
25. Canapp SO, McLaughlin RM, Hoskinson JJ, Roush JK, Butine MD. Scintigraphic evaluation of glucosamine HCl and chondroitin sulfate as treatment for acute synovitis in dogs. *Am J Vet Res* December, 1999; 60(12):1550-1556.
26. Beren J, Hill S, Hammad T, Rose N. The therapeutic effect of glucosamine HCl/chondroitin sulfate combination of type II collagen-induced arthritis in D/A rats. Accepted for publication in the Proceedings of the Society for Experimental Biology and Medicine, 2000.
27. Lippiello L, Hammad T. "Dose response and synergistic effect of glucosamine HCL and chondroitin sulfate on in-vitro proteoglycan synthesis by bovine and human chondrocytes." Paper presented at the 67th annual meeting of the American Academy of Orthopedic Surgeons, Orlando, Florida, 2000.
28. Rindone J., Hiller D., Collacott E, et al. Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. *West J Med* 2000; 172: 91-94.
29. Verbruggen G, Goemaere S, Veys EM. Chondroitin sulfate: S/S/MOAD (structure/disease modifying anti-osteoarthritis drug) in the treatment of finger joint OA. *Osteoarthritis Cartilage* 1998; (supple A): 37-38.
30. Uebelhart D, Thonar EJ, Delmas PD, Chantaine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. *Osteoarthritis Cartilage* 1998; (supple A): 39-46.
31. Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. *Arzneim Forsch* 1995; 45: 918-925.
32. Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the anti-inflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. *J Rheumatol* 1996;23: 1385-1391.
33. Bucsi L, Poor G. Efficacy and tolerability of oral chondroitin sulfate as a symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. *Osteoarthritis Cartilage* May 1998;6(supple A):31-36.
34. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin treatment for osteoarthritis. A systematic quality assessment and meta-analysis. *JAMA* 2000;283,1469-1475.
35. Leeb B, Schweitzer H, Montag K. A meta-analysis of chondroitin sulfate in the treatment of osteoarthritis. *J Rheum* 2000; 27:205-211.
36. Baici A, Horler D, Moser B, Hofer HO, Fehr K, Wagenhauser FJ. Analysis of glycosaminoglycans in human serum after oral administration of chondroitin sulfate. *Rheumatol Int* 1992;12:81-88.
37. Laudi's Response to Baici. Letters to the editor – Bioavailability of oral chondroitin sulfate. *Rheumatol Int* 1993;13: 39-40.
38. Volpi N, Roth M, Uebelhart D. "Quantitative and qualitative evaluation of chondroitin sulfate in normal human plasma." Proceedings of the 12th European League against Rheumatism Congress, Scotland, 289. Abstract.
39. Yamanashi S, Toyoda H, Furuya N, Harada T, Yoshida K, Toida T, Imanari T. Metabolic study on chondroitin sulfates in rabbits. *Yakugaku Zasshi* 1991; 111: 73-76.
40. Artusson P, Ungell A, Lofroth E. Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm Res*. 1993;10:1123-1128.
41. Adebowale E, Liang Z, Ashraf M, Eddington ND. Development and validation of a high performance size exclusion chromatographic method for the quantitation of chondroitin sulfate in raw materials, powder blends and dosage forms. Unpublished data, 1999. Under review *J Pharmaceut Biomed Anal*.
42. Schenck R. Osteoarthritis Treatment and Potential Structure Modification with Nutraceuticals, Special Report. San Antonio, Texas: Dannemiller Memorial Educational Foundation;1999.
43. McNamara PS, et al. Hematologic, hemostatic and biochemical effects in dogs receiving an oral chondroprotective agent for thirty days. *Am J Vet Res* Sept. 1996;57:1390-1394.
44. Hungerford DS. Treating osteoarthritis with chondroprotective agents. *Orthopedics Special Edition* January-April 1998; 39-42.
45. Adebowale E, Liang Z, Eddington ND. Nutraceuticals, a call for quality control of delivery systems: a case study with chondroitin sulfate and glucosamine. *J Nutraceutical Food Marketing* (accepted).