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Comparison of Active Ingredient Consistency of Some Brand and Generically Available Glucosamine Products in Iranian Pharmacies

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Abstract

Glucosamine is used by patients with osteoarthritis and rheumatoid arthritis. Wide variety of simple or complex Glucosamine products are offered with great different prices in Iranian pharmacies. Glucosamine is a small molecule therefore its absorption and bioavailability after oral administration is nearly complete and irrelevant to its formulation. The aim of this report is to consider Glucosamine content of such products and have a comparison to discuss if generics are as effective as brand ones.

A total of 15 products containing Glucosamine were assessed to measure Glucosamine content, at first derivatization was performed by adding phenylisothiocyanate. Then phenylthiourea derivative of Glucosamine was determined by spectrophotometer at 240 nm. To ensure non-interference of other substances, Glucosamine content of complex products also were determined with high-performance liquid chromatography (HPLC), C18 column and mobile phase phosphate buffer/acetonitrile (90/10 1 ml/min) with UV detector at 240 nm.

Glucosamine compared with amount had been mentioned in label was ranging from 93.22% to 125.14%. Almost 85% of products had active ingredient more than amount claimed on label. Comparison of HPLC analysis with spectrophotometric data indicated acceptable selectivity of spectrophotometric method for Glucosamine determination in two sample products and those containing Glucosamine plus other active ingredients such as chondroitin and methylsulfonylmethane (MSM).

One way analysis of variance (ANOVA) performed for comparison of means achieved after several Glucosamine determinations in one brand. In the basis of our study and appropriate bioavailability of Glucosamine, generic products of that also would be acceptable despite lower prices.

Keywords: Glucosamine; Chondroitin; Spectrophotometry; HPLC; Active ingredient

Introduction

Lately Glucosamine preparations are widely used as a complementary drug [1,2]. It is used even as an essential drug to enhance the treatment of rheumatoid arthritis and osteoarthritis [3,4]. Furthermore, athletes and body builders also in some cases use Glucosamine and some individuals use it for prevention of arthritis [5,6]. Glucosamine preparations are economically important among complimentary products because of their long duration of treatment and expensive cost [7].

Complementary drugs and dietary supplements are suspected to illegally manufacture as counterfeit drugs [8,9]. Glucosamine products in Iran pharmacies are presented in various prices. Different content ranges which were revealed in previous studies determined these products in Iran and other countries [10-14]. Even in a study by Adebowal et al. in university of Maryland percent of label claim less than 10% for chondroitin and less than 40% for Glucosamine were reported [15]. Thus quantitative control of nutritional products makes an important role concerning health and financial aspects [16,17].

Glucosamine is among products with more than 80% bioavailability after oral administration and there are a lot of researches have been indicated its oral absorption is not mainly related to its formulation [18,19]. Hence, in this study the active ingredient of Glucosamine contents in different brands and generic products were determined and compared.

Materials and Methods

Apparatus

Spectrophotometer UV/VIS (CECIL, England)

HPLC LC6A (Shimadzu, Japan)

Reagents

Standard: D-Glucosamine sulphate from SciNcelab, Inc. Glucosamine sulphate 2KCl from Darupakhsh, Iran

Derivazation reagent and solvents: Water (double distilled), acetonitrile (HPLC grade), methanol (HPLC grade), sodium acetate, anhydrous (ACS reagent grade), diethyl ether phosphoric acid and phenylisothiocyanate (PITC), 99% all were purchased from E. Merck (Darmstadt, Germany).

Glucosamine products

A total of 15 Glucosamine products presented in Iran pharmacies were chosen randomly and numbered so that the laboratories testing would be blinded (Table 1).

Product	Serving size	Glucosamine content per serving (mg)	Other active ingredients amount per serving	Country of origin
Product 1	1 tablet	Glucosamine sulfate (2KCl) 500 mg	-	Iran
Product 2	1 tablet	Glucosamine sulfate (2KCl) 500 mg	-	Iran
Product 3	1 capsule	Glucosamine sulfate (2KCl) 500 mg	-	USA
Product 4	1 capsule	Glucosamine sulfate 500 mg	Chondroitin sulfate (bovine) 400 mg	Canada
Product 5	1 capsule	Glucosamine sulfate 500 mg	Chondroitin sulfate 400 mg	Canada
Product 6	1 capsule	Glucosamine sulfate 500 mg	-	Canada
Product 7	1 tablet	Glucosamine sulfate (NaCl) 500 mg	Chondroitin sulfate 400 mg	Iran
Product 8	1 capsule	Glucosamine sulfate (2KCl) 500 mg	-	India
Product 9	1 tablet	Glucosamine sulfate (2KCl) 1500 mg	-	India
Product 10	1 capsule	Glucosamine sulfate 500 mg	Chondroitin sulfate 400 mg	Canada
Product 11	1 tablet	Glucosamine sulfate (sodium free) 666.7 mg	MSM 333.3 mg, Silica 33.3 mg, Vitamin C 100mg	Canada
Product 12	3 tablet	Glucosamine sulfate 1500 mg	MSM 900 mg, chondroitin sulfate (bovine) 750 mg	USA
Product 13	2 capsule	Glucosamine sulfate (KCl) 500 mg	Chondroitin sulfate 400 mg, Vitamin C 200 mg, Manganese 2 mg, sodium 30 mg	USA
Product 14	1 capsule	Glucosamine sulfate (2KCl) 500 mg	-	USA
Product 15	2 caplsule	Glucosamine HCl 1500 mg	Vitamin C 60 mg,D 100 IU,E 30 IU, calcium 250 mg, zinc 6 mg, Boron citrate 10 mg	USA

Table 1: Description of Glucosamine containing samples.

Methods

Spectrophotometry: As the method approved by Gaonkar P et al. [20], ten tablets or capsules from each product were weighed and powdered. Equal to 100 mg of glucosamine salt were dissolved in sodium acetate aqueous solution (0.1 M). Then the solution was filtered and diluted to 100 ml. After 24 h phenylthiourea derivatives which were made by adding 0.4 ml PITC along with 6 ml methanol to 4 ml of solution were prepared. This solution was diluted to 25 ml with 60% aqueous methanol and after heating for about 20 min in boiling water, unreacted PITC was extracted via diethyl ether. Five milliliters of aqueous layer was taken and made up to the volume 50 ml with water.

Standard solution was prepared by the same procedure and calibrated in a range of concentration. Absorbance was detected at 240 nm and Glucosamine content was calculated.

HPLC: Samples of phenylthiourea derivative of Glucosamine prepared for spectrophotometric measurement were injected to HPLC with following conditions:

Column C18: 25 cm × 4.6 mm

Mobile phase: Phosphate buffer: acetonitrile (10:90)

Flow rate: 1 ml per min

UV detection at 240 nm [15]

Calculation: Percent of the label claim were estimated by dividing average of the measured amount (mg) into label claim (mg) and multiplied by 100. For those products consist of Glucosamine salt dissimilar to standard, Glucosamine amount was estimated by multiplying the ratio w/p, where w=molecular weight of Glucosamine salt available in certain product, P=molecular weight of Glucosamine compound was used as standard.

As the Glucosamine free-base form is not stable, there are numerous stable compounds in markets (such as Glucosamine sulphate 2KCl, Glucosamine sulphate NaCl, Glucosamine HCl). Free Glucosamine content was a good parameter to compare various products that could be gain by multiplication of the ratio (molecular weight of Glucosamine/molecular weight of Glucosamine compound used as standard). It gives a truthful view for consumers about the amount that printed on the labels based on various salt forms [21].

For instance depending on the molecular weight of formula 1 g of Glucosamine. HCl possess 0.83 g of Glucosamine free base, whereas there is only 0.59 g of Glucosamine free base in 2 Glucosamine free base H_2SO_4.2KCl.

From the fact that could notice the deviation from label value in our study products with same salt of Glucosamine (Glucosamine sulphate) was chosen except number 15. Also Glucosamine sulphate was used as standard and a true comparison of label claimed percent achieved. SPSS version of 17 was used for data analysis (one way ANOVA). And to evaluate contrast between groups, post Hoc LSD and Bonferroni were employed [22].

Results

Percent of active ingredient

The mean of active ingredient in 15 Glucosamine products determined by spectrophotometric method was indicated in Table 2. The lowest is about 93.22% ± 8.72% (of label claim) related to sample 4 and the highest expressed in sample 1, which is 125.14% ± 5.86% (of label claim). Only 2 products had less active ingredient than label claim and whereas the other products had about the range or further. That's remarkable all 4 generic products had more active ingredient than label claim (Table 2).

P value	%GLN by HPLC method	RSD (%)	%GLN by spectrophotometric method	N	Product
0.911	126	5.86	125.14	3	1
0.201	134.8	7.97	117.51	3	2
0.871	101	5.48	102.07	4	3
0.9	91.77	8.72	93.22	3	4
0.072	133.31	3.43	119.27	3	5
-	-	2.3	105.36	3	6
		6.39	114.89	3	7
-	-	5.09	121.92	3	8
0.108	104.86	2.09	98.65	2	9
0.143	124.67	1.82	114.49	2	10
0.565	92.39	11.09	101.15	3	11
		15.97	108.84	3	12
0.125	98.85	1.56	103.22	3	13
0.951	108.7	22.55	106.9	3	14
0.772	115.12	3.61	116.77	2	15
N=number of samples of each product; %GLN=Glucosamine active ingredient percent than label claim; RSD=relative standard deviation					

Table 2: Glucosamine active ingredient (% label claim) of 15 products measured by spectrophotometric and HPLC method.

In this study we used the method described by Gaonkar et al. [20] for determination of Glucosamine in products containing only Glucosamine. Some products of our study contain Glucosamine in combination with other substances such as chondroitin. Hence some products also were determined by HPLC method. Figure 1 depicts a chromatogram of Glucosamine prepared as standard for determination of Glucosamine by spectrophotometric method indicating two separate peaks for each sample (Figure 1).

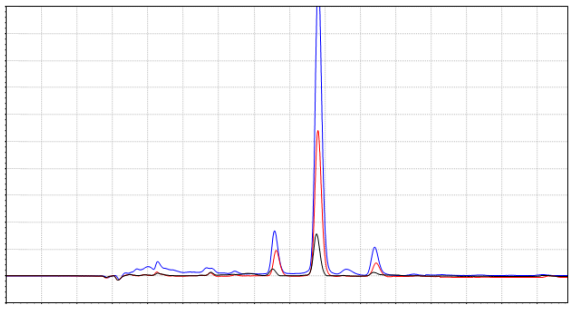

Figure 1: Concentrations of 50 mg/ml, 100 mg/ml and 150 mg/ml of Glucosamine hydrochloride determined by HPLC.

Comparing percent of label claim of HPLC with spectrophotometric method indicating accuracy of spectrophotometric method for Glucosamine determination in products contain both Glucosamine and chondroitin or other substances. Statistical analysis of data also confirms this point.

	N	Average %GLN	Sig.
Brand	11	107.86 ± 2.78	0.158
Generic	4	115.72 ± 4.08	
N=number of samples of each product. %GLN=Glucosamine active ingredient percent than label claim			

Table 3: Comparison of average percent active ingredients of generic and brand products.

Comparison active ingredients of generic and brand products

This study involved 4 generic and 11 brand products that some of them contained other substances such as chondroitin sulphate, MSM, vitamin C other than Glucosamine. Comparison of outcomes didn't indicate significant difference (p-value>0.05) between generic and brand products in spite of large difference in their prices (Table 3).

Comparison of spectrophotometry and HPLC methods for Glucosamine determination is simple than Glucosamine plus chondroitin products. In the basis of our information there wasn't any report for determination of Glucosamine in complex products by spectrophotometric method.

In literature review of our study spectrophotometric method has been just applied for products only containing Glucosamine, probably because of concerning interferences with other substances such as chondroitin [20].

As chondroitin plus products innovatively evaluated with spectrophotometry in our study, for assurance of not interference of chondroitin sulfate and other substances, Glucosamine content of chondroitin plus products were also measured by HPLC and confirms the accuracy of spectrophotometric results (Table 4).

	N	%GLN	Sig.
Chondroitin plus	5	109.02 ± 10.63	0.79
Simple product	10	110.43 ± 9.27	
N=number of samples of each product.			
%GLN=Glucosamine active ingredient percent than label claim			

Table 4: Comparison of average percent active ingredients of Glucosamine and Glucosamine plus chondroitin products.

Discussion

In this study, Glucosamine contents of 15 products contain different salts of Glucosamine as a single drug or in combination with chondroitin, MSM, etc., were studied using spectrophotometry and HPLC methods. Eight of Fifteen products contained Glucosamine in the range 90% to 110% in comparison with the label claim. The lowest percent of Glucosamine determined was 93.22% and the highest was 125.14%. Among Glucosamine products involved in our study only 2 products showed lower amount of active ingredient than label claim.

In a study performed by Asamoah [23] on 9 different products contain sulphate and hydrochloride salts of Glucosamine present in Japan, results showed Glucosamine content in the range of 97.4% to 104.4% in comparison with the amount claimed on labels. Also dissolution and weight uniformity tests were done for these 9 products and different consequences was obtained. Asamoah also compared titration method of Glucosamine determination with HPLC method, and the results indicate different values for these methods (for example 100.4 *vs* 83.9 for product 5) in spite of not significant statistical difference between two methods. In our study Glucosamine contents of some products containing other ingredients were also determined with HPLC. Differences were presented between spectrophotometric and HPLC data (the largest odds were 117.51 for spectrophotometry and 134.8 for HPLC). Furthermore in our study as observed in Japanese study, there wasn't any significant statistical differences between spectrophotometric and HPLC data [23].

Sullivan and Sherma [21] also determined Glucosamine content of 9 different capsule and tablet supplement containing different salt of Glucosamine by a HPTLC method. As observed in our study, percent of Glucosamine in various products were different and even contrast (68.3% to 136.7%) of active ingredient was also observed in different samples of one product [21].

According to final FDA rules, GMP for dietary supplement were published that ensures production quality, contamination and labels accuracy [10,24]. But deviation from label value had been demonstrated different ranges among several studies that determined Glucosamine products even as sulphate form [11-14]. In addition Glucosamine and chondroitin content of commercial products, as products evaluated in this study wasn't relevant to their prices [14,25,26].

In this study more products assessed were at the range 90% to 110% of the amount printed in their labels, this is the acceptable value for pharmaceutical dosage forms. However there isn't specified content range for nutritional supplements in USP [27].

One way analysis of variance (ANOVA) performed for comparison of means and showed a significant (P value=0.007) variation between samples. Post Hoc (LSD) analysis of spectrophotometric data indicated meaningful deviation of samples 1, 4, 5 and 8 which express contents of these products were out of average range comparing with other products involved in our study.

Since LSD mode achieves the lowest p-value, it's not adjusted for multiple comparing. So for correcting multiple tests, we employed Bonfrroni correction and significant deviation was not observed; that indicated all products involved in our study containing acceptable amount of Glucosamine [22]. Glucosamine bioavailability studies after oral administration of Glucosamine sulfate products performed by Persiani and coworkers with doses of 1500 mg also found are in correlation with in-vitro studies [28].

Judging from aforesaid consequences, there wasn't meaningful contrast between generic and brand products (Table 3), besides Glucosamine absorption in gastrointestinal tract is approximately complete and there are many reports indicating more than 90% to 98% oral absorption for different Glucosamine formulation [29,30]. Therefore generic products would be effective as well as brand ones in spite of lower prices; however performing further analysis such as disintegration and dissolution test could certainly confirm this consequence [27,28].

Data analysis of Glucosamine contents in chondroitin plus and Glucosamine simple products weren't show significant differences (Table 4). Thus our results confirm spectrophotometry is applicable for Glucosamine determination in products containing other substances such as chondroitin and MSM. It would be a cost-effective way than using HPLC or other method for assessing Glucosamine content of complex products.

References

1. Kennedy J (2005) Herb and Supplement Use in the US Adult Population. Clin Ther 27: 1845-1858.
2. Kongtharvonskul J, Anothaisintawee T, McEvoy M, Attia J, Woratanarat P, et al. (2015) Efficacy and safety of Glucosamine, diacerein, and NSAIDs in osteoarthritis knee: a systematic review and network meta-analysis, Eur J Med Res 20: 24.
3. Chan KOW, Ng GYF (2011) A review on the effects of Glucosamine for knee osteoarthritis based on human and animal studies. Hong Kong Physiother J 29: 42-45.
4. Renapurkard DK, Mathur S, Jagdishwerrao KL (2010) Evaluation of efficacy and safety of Diacerein in osteoarthritis of knee joint. Int J Pharm Bio 1: 1-12.
5. Chiusaroli R, Piepoli T, Zanelli T, Ballanti P, Lanza M, et al. (2011) Experimental pharmacology of Glucosamine sulfate. Int J Rheumatol 2011: 939265.
6. Imagawa K, de Andrés MC, Hashimoto K, Pitt D, Itoi E, et al. (2011) The epigenetic effect of Glucosamine and a nuclear factor-kappa B (NF-kB) inhibitor on primary human chondrocytes--implications for osteoarthritis. Biochem Biophys Res Commun 405: 362-367.
7. Bansal H, Bansal A (2012) The potential for structural modification hence benefit in osteoarthritis of knee by mineral supplementation-Pilot study. Int J Pharm Bio Sci 3: 736-749.
8. Deisingh AK (2005) Pharmaceutical counterfeiting. Analyst 130: 271-279.
9. Pincock S (2003) WHO tries to tackle problem of counterfeit medicines in Asia. BMJ 327: 1126.
10. Kirkham SG, Samarasinghe RK (2009) Review article: Glucosamine. J Orthop Surg 17: 72-76.
11. Zhou J, Waszkuc T, Felicia M (2005) Determination of Glucosamine in Raw materials and Dietary Supplements Containing Glucosamine Sulfate and/or Glucosamine Hydrochloride by HPLC with FMOC-Su Derivatization: Collaborative Study. J AOAC Int 88: 1048-1058.
12. Russell AS, Aghazadeh-Habashi A, Jamali F (2002) Active ingredient consistency of commercially available Glucosamine sulfate products. J Rheumatol 29: 2407-2409.
13. Oke S, Aghazadeh-Habashi A, Weese JS, Jamali F (2006) Evaluation of Glucosamine levels in commercial equine oral supplements for joints. Equine Vet J 38: 93-95.
14. Amanlou M (2008) Investigation on Counterfeit Glucosamine and Chondroitin Products in Iranian Pharmaceutical Markets. Iran J Pharm Res 7: 123-129.
15. Adebowale A, Cox D, Liang Zh, Eddington N (2000) Analysis of Glucosamine and Chondroitin Sulfate Content in Marketed Products and the Caco-2 Permeability of Chondroitin Sulfate Raw Materials. J Am Nutraceutical Assoc 2: 32-39.
16. http://www.who.int/medicines/services/counterfeit/RomeDeclaration.pdf
17. http://www.who.int/intellectualproperty/documents/thereport/enpublichealthreport.pdf
18. Aghazadeh-Habashi A, Sattari S, Pasutto F, Jamali F (2002) Single dose pharmacokinetics and bioavailability of Glucosamine in the rat. J Pharm Pharm Sci 5: 181-184.
19. Adebowale A, Du J, Liang Z, Leslie JL, Eddington ND (2002) The bioavailability and pharmacokinetics of Glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. Biopharm Drug Dispos 23: 217-225.

20. Gaonkar P, Khanvilkar V, Shettigar R, Gadgoli C (2006) Spectrophotometric method for determination of Glucosamine in tablets. Indian J Pharm Sci 68: 83-88.
21. Sullivan C, Sherma J (2005) Development and validation of an HPTLC-densitometry method for assay of Glucosamine of different forms in dietary supplement tablets and capsules. Acta Chromatographica 15: 119-130.
22. Landau S, Everitt BS (2004) A Handbook of Statistical Analysis using SPSS, by Chapman & Hall, CRC Press LLC, London, UK.
23. Asamoah R (2008) Determination of Glucosamine in drug formulation, Kumasi, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
24. http://www.fda.gov/
25. Hadad GM, Abdel-Salam RA, Emara S (2012) Determination of Glucosamine and Carisoprodol in Pharmaceutical Formulations by LC with Pre-Column Derivatization and UV Detection. J Chromatogr Sci 50: 307-315.
26. Ramey DW, Eddington N, Thonar E, Lee M (2002) An analysis of Glucosamine and chondroitin sulfate content in oral joint supplement products. J Equine Vet Sci 22: 125-127.
27. USP Convention (2006) United States Pharmacopeia. 29th edn. Rockville, MD, USA.
28. Setnikar I, Rovati LC (2001) Absorption, distribution, metabolism and excretion of Glucosamine sulfate: a review. Arzneimittelforschung 51: 699-725.
29. Persiani S, Roda E, Rovati LC, Ocatelli ML, Giacovelli G, et al. (2005) Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline Glucosamine sulfate in man. Osteoarthritis Cartilage 13: 1041-1049.
30. Akarasereenont P, Chatsiricharoenku S, Pongnarin P (2012) Bioequivalence Study of 1,500 mg Glucosamine Sulfate in Thai Healthy Volunteers. J Bioequiv Availab 4: 91-95.