Research Article

ASSESSMENT OF BIOAVAILABILITY OF GLUCOSAMINE HYDROCHLORIDE MR 1500 mg TABLETS IN HEALTHY HUMAN SUBJECTS

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ABSTRACT

Glucosamine is an amino sugar and dietary supplement, used in the treatment of osteoarthritis, rheumatoid arthritis, knee pain, back pain and glaucoma. To compare the Bioavailability and characterize the pharmacokinetic profile of test product with respect to the Reference product in normal, Healthy, adult, human subjects under fasting conditions. This was a single oral dose, randomized, open label, balanced, two treatment, two period, two sequence, two way cross over study. The comparative bioavailability study was conducted under fasting condition with a washout period of 7days. The test and reference products were received according to a randomization schedule. A total of 19 blood samples (5ml each) were collected from each subject in each phase, centrifuged and separated plasma and collected in pre-labeled polypropylene tubes and stored in a freezer at a temperature below -20°C until analysis. The samples were analyzed by LC-MS/MS method and calculated $C_{max,}$ $T_{max,}$ AUC_{0-t} and AUC $_{total.}$ Six normal, healthy, adult human subjects were participated in the fasting study. The arithmetic mean (SD) of C_{max} for the Test and Reference products were 1577.61 (141.44) and 2574.49. (373.97) ng/mL, T_{max} for the Test and Reference products were 5.8333 (0.408) and 2 hours, AUC_{0-t} for the Test and Reference products were 19143.3 (1487.309) and 8241.33 (1070.524)ng.h/mL and AUC_{total} for the Test and Reference products were 19445.98 (1559.535) and 8309.117 (1088.345)ng.h/mL. No clinically significant adverse events were reported during the study and calculated the % difference of AUC for test and reference products. The above study reveals that, the test product showed improved bioavailability of 56.95% when compared to immediate release reference product.

Keywords: Bioavailability, Fasting study, Glucosamine HCl, Human subjects, Two way cross over.

INTRODUCTION

Glucosamine is a natural substance found in chitin, mucoproteins and muco polysaccharides and is involved in the manufacture of glycosaminoglycan, which forms cartilage tissue in the body; glucosamine is also present in tendons and ligaments. Glucosamine must be synthesized by the body, but the ability to do this declines with age. Glucosamine and its salts have therefore been advocated in the treatment of rheumatic disorders including osteoarthritis. Glucosamine may be isolated from chitin or prepared synthetically; glucosamine sulfate and hydro iodide have also been used. Glucosamine is chemically 2-amino-2-deoxy-D-glucopyranose hydrochloride (D-Glucose, 2-amino-2-Deoxy-hydrochloride. Glucosamine and its salts are widely available as licensed products or so-called 'health supplements' used for the management of osteoarthritis.[1-3]

MATERIALS AND METHODS

Inclusion criteria

Six non-smokers healthy adult, human subjects between 18 and 45 years of age, having a Body Mass Index (BMI) between 18.5 and 30.0kg/m^2 and body weight not less than 50 kg were selected living in India. Healthy individuals are evaluated by personal history and medical history. Vital parameters such as Blood Pressure, Pulse rate, body temperature should be within the acceptable limit. The Negative HIV 1 and 2 antibodies, Hepatitis B surface antigen, Hepatitis C antibody, Syphilis tests and alcohol breath analysis.[4]

Exclusion criteria

Subjects were excluded from the study if subjects having the history of diabetes mellitus, tuberculosis, hypertension, cardiac failure, GIT problems, respiratory tract infections, renal failure are judged to be clinically significant and the subjects having the history of smoking nine or more cigarettes or beedies per day and/or inability to withhold smoking or consumption of tobacco containing products during the study. The subjects having the history of difficulty in swallowing, Consumption of grapefruit, habituation to coffee, tea or other xanthene containing products.[5, 6]

Investigational medicinal products

Two Glucosamine hydrochloride oral formulations were evaluated. The Test product-A, Glucosamine hydrochloride MR 1500mg tablet and The Reference product-B (Bioglan) was commercially available formulation of Glucosamine hydrochloride IR 1500mg tablets.

Study design

The study was designed as a single oral dose, randomized, balanced, open label, two treatment, two period, two sequence, two way cross over study. The detailed study

protocol and all related documents were approved by independent ethical committee before conducting the study. The study was conducted as per the ICH guidelines, Schedule Y of Drugs & Cosmetics Act 1940 and E6 'Guideline for Good Clinical Practice. The study was conducted under fasting condition with a washout period of 7days. The order of receiving the test and reference product for each subject was according to a randomization schedule. The blood samples were collected through IC (indwelling cannula) placed in a forearm/arm vein of the subjects. A total of 19 blood samples (5ml each) were collected from each subject in each phase of study i.e. pre dose (0.0) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 24.0, 36.0 and 48.0 hours after dosing. The first 17 samples were collected through indwelling cannula placed in a forearm and the remaining samples were collected through direct venous puncture. After collection of blood samples, these were immediately transferred into pre-labeled sample collection tubes containing sodium heparin as the anticoagulant and there were kept in wet ice bath. The samples were centrifuged within 30 minutes of sample collection at 4000 rpm for 10 minutes at 4±2°C to separate the plasma and separated plasma was transferred to pre-labeled polypropylene tubes, and stored in a freezer at a temperature below -20°C until analysis.[7-9.]

Sample analysis

The validated LC-MS/MS method was used to analyze the plasma samples by using various reagents such as ammonium acetate buffer, Acetonitrile (HPLC grade), D-Glucosamine hydrochloride, Deionized water and all other reagents used were of analytical grade. The Glucosamine concentrations in human plasma were measured by 8-point calibration curve. Calibration curve standards were generated using calibration samples obtained from Glucosamine free plasma with Glucosamine at concentrations ranging from 10ng/ml (the LLOQ of the method) to 1500 ng/ml (the HLOQ of the method). The calibration curve standards were prepared by spiking screened human blank plasma (Glucosamine free) with the appropriate spiking solutions that were prepared from stock dilution of Glucosamine. The stock solution of Glucosamine was prepared in 10mM ammonium acetate buffer, pH 7.5 and stored at -20°C until use.[10]

Pharmacokinetic analysis

The basic pharmacokinetic parameters like C_{max}, T_{max}, AUC_{0-t} and AUC_{total} are required for the comparison of bioavailability of test and reference products. The pharmacokinetic parameters were analyzed in each subject using a non compartmental model of Kinetica 2000 for each period.

Table 1: Individual and Mean plasma concentrations of test product in each subject

Time (h)	Subject Number						Mean	SD
	01	02	03	04	05	06		
	Concentration (ng/mL)							
0	0	0	0	0	0	0	0	0
0.5	325.25	362.12	385.17	340.28	312.94	375.15	350.15	28.624
1.0	627.36	702.71	689.01	617.85	648.21	669.74	659.15	33.874
1.5	741.42	820.75	769.08	824.91	792.74	901.74	808.44	55.525
2.0	790.25	821.89	850.17	937.89	927	993.48	886.78	78.082
2.5	889.59	921.17	975	1047.01	1067.2	1086.34	997.72	81.460
3.0	1021.94	1051.36	1089.74	1201	1189.7	1228.73	1130.41	86.988
3.5	1189.48	1159.02	1296.34	1297.62	1287.52	1347.29	1262.88	72.434
4.0	1284.35	1218.0	1375.3	1369.21	1408.3	1496.8	1358.66	97.203
5.0	1329.11	1289.12	1474.89	1589.78	1565.9	1621.51	1478.39	140.477
6.0	1463.35	1358.09	1637.12	1401.8	1689.21	1728.09	1546.28	158.053
8.0	1189.65	1061.78	1369.7	1208.01	1321.07	1439.27	1264.91	137.623
10.0	1021.85	963.72	1148.72	928.9	961.28	1028.3	1008.80	78.489
12.0	934.82	891.74	921.78	792.31	746.12	845.7	855.412	74.901
16.0	612.81	528.91	712.91	569.41	499.71	603.89	587.94	74.970
20.0	262.18	267.43	409.42	318.21	238.41	307.02	300.45	61.052
24.0	52.38	75.23	128.1	108.9	83.14	99.21	91.16	26.706
36.0	0	29.89	45.98	36.17	33.78	45.89	31.95	16.956
48.0	0	0	0	0	0	0	0	0

Table 2: Individual and Mean plasma concentrations of Reference product in each subject

Time (h)	Subject Number						Mean	SD
	01	02	03	04	05	06		
	Concentration (ng/mL)							
0	0	0	0	0	0	0	0	0
0.5	890.24	724.80	1020.30	927.89	889.34	942.32	899.15	97.92
1.0	1836.00	1624.10	1873.52	1442.64	1733.60	1882.12	1732.00	172.49
1.5	2461.24	2089.10	2206.75	1568.02	2188.14	2102.34	2102.60	294.19
2.0	2836.75	2136.97	2853.87	2057.31	2845.16	2716.89	2574.49	373.97
2.5	2245.98	1869.41	2367.36	1639.31	2298.40	2503.74	2154.03	329.56
3.0	1823.78	1589.74	1987.19	1236.92	1632.43	2130.80	1733.48	318.69
3.5	1532.71	1309.15	1487.93	986.71	1198.41	1458.71	1328.94	208.90
4.0	1237.49	999.78	1147.46	782.31	897.56	1101.47	1027.68	168.36
5.0	701.06	519.53	752.69	501.84	498.83	699.73	612.28	117.40
6.0	340.24	246.63	218.63	258.78	218.34	326.59	268.20	53.10
8.0	147.20	106.95	109.55	111.45	130.18	171.58	129.48	25.76
10.0	39.78	28.30	46.20	21.20	20.90	40.89	32.88	10.86
12.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: Pharmacokinetic Means of Test and Reference products (n=6)

Parameters	C_{max} (ng/mL)	T_{max} (Hours)	AUC_{0-t} (ng.h/mL)	AUC_{total} (ng.h/mL)
Test Product				
Mean	1577.61	5.8333	19143.3	19445.98
SD	141.44	0.408	1487.309	1559.535
% CV	8.97	8.965	7.769	8.019
Reference Product				
Mean	2574.49	2	8241.33	8309.117
SD	373.97	0	1070.524	1088.345
% CV	14.52	0	12.989	13.098

RESULTS AND DISCUSSION

Demographic Data

Six normal, healthy, adult human subjects were allocated to each study. All subjects were participated in the fasting study. The mean age (SD) was 29.33 (5.89) years and the mean BMI (SD) was 21.92 (2.42) kg/m^2.

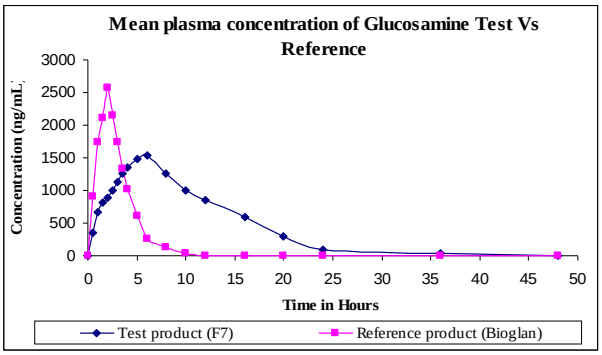

Figure 1: Mean plasma concentration of Glucosamine Test and Reference

Pharmacokinetic parameters

The drug-plasma concentrations obtained from the bioavailability study for Test and Reference products were analyzed in each subject by LC-MS/MS method and calculated the basic Pharmacokinetic parameters such as C_{max}, $T_{max,}$ AUC_{0-t} and AUC_{total}. The arithmetic mean (SD) of C_{max} for the Test and Reference products were 1577.61 (141.44) and 2574.49. (373.97) ng/mL, T_{max} for the Test and Reference products were 5.8333 (0.408) and 2 hours, AUC_{0-t} for the Test and Reference products were 19143.3 (1487.309) and 8241.33 (1070.524)ng.h/mL and AUC_{total} for the Test and Reference products were 19445.98 (1559.535) and 8309.117 (1088.345) ng.h/mL. The Bioavailability was determined by calculating % of AUC for Test and Reference products and the Test product showed 56.95% improved Bioavailability over Reference product. The individual plasma and mean plasma concentrations of Test and Reference products in each subject were given in table 1-2. The Pharmacokinetic

means of Test and Reference products were given in table 3 and comparison of mean plasma concentrations of test and reference graph was shown in figure 1. The comparison of Bioavailability for Test and Reference products by AUC% was given in table 4.

Table 4: Comparison of Bioavailability for Test and Reference products

Parameter	Observation
AUC_{test} - AUC_{ref}	10901.97
AUC_{test} - AUC_{ref} / AUC_{test}	0.569493
% of AUC	56.95

CONCLUSION

The above study reveals that, the test product showed improved bioavailability of 56.95% when compared to immediate release reference product. From these results it concluded that Glucosamine hydrochloride is a good candidate for development of modified release formulations.

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