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Bioavailability of Two Sublingual Formulations of Nitroglycerin 0.6 mg :A Randomized, Open-label, Single-dose, Two period, Crossover, Comparison in Healthy, Indian, Adult Volunteers.

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Abstract

Background and Objective: The aim of manufacturing sublingual tablet is to have rapid absorption, to avoid first pass metabolism and to minimize food effect. The investigational product is a stabilized sublingual compressed Nitroglycerin tablet and it is indicated for the acute relief of an attack or acute prophylaxis of angina pectoris due to coronary artery disease. It is a real challenge for the pharmaceutical scientist to establish bioequivalence for the sublingual formulation due to it's rapid absorption and elimination. The objective of the study is to determine the relative bioavailability of two different formulations of Nitroglycerin sublingual tablets 0.6 mg.

Method: The relative bioavailability and pharmacokinetic profile of a test formulation, Nitroglycerin sublingual tablets 0.6 mg, manufactured in India and reference formulation, Nitrostat® (Nitroglycerin sublingual tablets 0.6 mg)], manufactured in USA were compared in healthy, Indian, adult, volunteers. Subjects' were asked to rinse the mouth with little quantity of water to ensure adequate oral hydration. A single oral dose of the assigned formulation was placed under the tongue of the subject and it was allowed to dissolve for about 60 seconds. Blood samples were collected at scheduled time points and plasma concentrations of Nitroglycerin, 1,2-Dinitroglycerin and 1,3-Dinitroglycerin were analyzed by LC-MS/MS method. The obtained plasma concentrations have been employed for pharmacokinetic analysis by using WinNonlin® software (version: 5.3) and statistical analysis by using SAS® statistical software (version: 9.2 SAS Institute Inc, USA).

Results: Individual disposition kinetic parameters of maximum plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC_{0-t}), area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), time taken to reach maximum concentration (t_{max}), rate elimination constant (K_{el}), Lower limit on time for values to be included in the calculation of K_{el} (K_{el_Lower}), Upper limit on time for values to be included in the calculation of K_{el} (K_{el_Upper}) and half-life ($t_{1/2}$) were calculated by non-compartmental analysis using WinNonlin® software (version: 5.2) and the results are given below:

Nitroglycerin: 774.1525 and 683.6791 pg/mL (C_{max}), 2882.7634 and 2465.1623 pg.min/mL (AUC_{0-t}), 4002.8263 and 3573.2699 pg.min/mL ($AUC_{0-\infty}$), 3.00 (2.00-45.00) and 3.00 (2.00-06.00) min (t_{max}) and 2.9891 ± 3.9791 and 2.4508 ± 0.9324 min ($t_{1/2}$) for test formulation and reference formulation respectively.

*1,2-Dinitroglycerin:*3861.9074 and 3744.9395 pg/mL (C_{max}), 175173.35 and 167756.80 pg.min/mL (AUC_{0-t}), 181369.23 and 174427.37 pg.min/mL ($AUC_{0-\infty}$), 15.00 (6.00-30.00) and 15.00 (3.00-30.00) min (t_{max}) and 46.4994 ± 10.0110 and 48.6231 ± 11.0217 min ($t_{1/2}$) for test formulation and reference formulation respectively.

*1,3-Dinitroglycerin:*1109.5633 and 1004.0984 pg/mL (C_{max}), 46704.284 and 42105.885 pg.min/mL (AUC_{0-t}), 52121.816 and 46935.971 pg.min/mL ($AUC_{0-\infty}$), 20.00 (10.00-30.00) and 15.00 (5.00-30.00) min (t_{max}) and 38.7232 ± 10.4202 and 38.1957 ± 8.1567 min ($t_{1/2}$) for test formulation and reference formulation respectively.

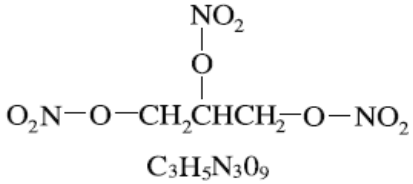
Conclusion: Since Nitroglycerin can not be quantified reliably due to its rapid absorption and elimination, 1,2-Dinitroglycerin and 1,3-Dinitroglycerin were employed for the assessment of bioequivalence. The same was witnessed in this study for Nitroglycerin.

The 90% confidence interval of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were evaluated and were found to be (95.12% to 111.8%), (98.68% to 110.49%) and (98.58% to 109.68%) for 1,2-Dinitroglycerin and (101.91% to 119.82%) (104.33% to 117.93%) and (106.23% to 116.09%) for 1,3-Dinitroglycerin respectively.

Key Words: Nitroglycerin, Sublingual tablets, Bioequivalence, Bioavailability

INTRODUCTION:

Nitroglycerin is 1,2,3-propanetriol trinitrate, an organic nitrate whose structural formula is:



$C_3H_5N_3O_9$

Molecular weight is 227.09. The organic nitrates are vasodilators, active on both arteries and veins.

The principal pharmacological action of nitroglycerin is relaxation of vascular smooth muscle. Although venous effects predominate, nitroglycerin produces, in a dose-related manner, dilation of both arterial and venous beds. Dilation of post capillary vessels, including large veins, remotes peripheral pooling of blood, decreases venous return to the heart, and reduces left ventricular end-diastolic pressure (preload). Nitroglycerin also produces arteriolar relaxation, thereby reducing peripheral vascular resistance and arterial pressure (afterload), and dilates large epicardial coronary arteries; however, the extent to which this latter effect contributes to the relief of exertional angina is unclear. Therapeutic doses of nitroglycerin may reduce systolic, diastolic, and mean arterial blood pressure. Effective coronary perfusion pressure is usually maintained, but can be compromised if blood pressure falls excessively or increased heart rate

decreases diastolic filling time. Elevated central venous and pulmonary capillary wedge pressures and pulmonary and systemic vascular resistance are also reduced by nitroglycerin therapy.

Heart rate is usually slightly increased, presumably due to a compensatory response to the fall in blood pressure. Cardiac index may be increased, decreased, or unchanged. Myocardial oxygen consumption or demand (as measured by the pressure-rate product, tension-time index, and stroke-work index) is decreased and a more favorable supply-demand ratio can be achieved. Patients with elevated left ventricular filling pressures and increased systemic vascular resistance in association with a depressed cardiac index are likely to experience an improvement in cardiac index. In contrast, when filling pressures and cardiac index are normal, cardiac index may be slightly reduced following nitroglycerin administration.

This study was performed with an aim to evaluate the relative bioavailability of test formulation**,** Nitroglycerin sublingual tablets 0.6 mg, manufactured in India and reference formulation, Nitrostat® (Nitroglycerin sublingual tablets 0.6 mg)], manufactured in USA in healthy, Indian, adult volunteers.

INVESTIGATIONAL PRODUCTS:

Nitroglycerin sublingual tablets 0.6 mg, Nitroglycerin sublingual tablets 0.6 mg, manufactured in India and reference formulation, Nitrostat® (Nitroglycerin sublingual tablets 0.6 mg)], manufactured in USA was used as reference formulation.

DESIGN:

This was an open labeled, randomized, two treatment, two-sequence, two period, crossover study.

Subjects:

Eighty healthy, Indian, nonsmoking adult male volunteers (mean age ± SD, 27.71 ± 5.91; range 19-42 years; BMI 22.17 ± 2.02 kg/m^2) were enrolled in the study. Of the 80 enrolled subjects, 72 subjects completed the study. All had normal renal and hepatic function. Subjects were enrolled in the study after normal findings from physical examination, laboratory investigations (including hematological, biochemical tests, serology and urine examination).

Exclusion criteria were any known hypersensitivity to Nitroglycerin, or any major illness in the last three months, ongoing chronic medical illness, renal or liver impairment, abuse of drugs within 3 months (Opioids, Cocaine, Barbiturates), alcohol addiction, patients with cholestasis, myasthenia, hepatic encephalopathy, history or current gastro-intestinal diseases influencing drug absorption and subjects suffering with gastritis, peptic/duodenal ulcer and Zollinger –elision syndrome.

Drug administration:

On dosing day of period I and II, volunteers were dosed with Nitroglycerin sublingual tablets 0.6 mg as per the randomization schedule. Subjects' were asked to rinse the mouth with little quantity of water to ensure adequate oral hydration. A single oral dose of the assigned formulation was placed under the tongue of the subject and it was allowed to dissolve for about 60 seconds.

There was 07 days washout period between two successive dosing days. The volunteers were ambulatory throughout the study but were prohibited from strenuous physical activity, smoking, alcohol and stimulating beverages containing xanthine derivatives (tea, coffee and soft drinks containing caffeine).

Blood sampling:

Blood samples of 6 ml were collected in K_2EDTA vacutainers through an indwelling intravenous cannula placed in a forearm vein before dosing (00.00) and after dosing at 01.00, 02.00, 03.00, 04.00, 05.00, 06.00, 08.00, 10.00, 12.00, 14.00, 15.00, 20.00, 25.00, 30.00, 45.00, 60.00, 75.00, 120.00, 180.00 and 240.00 minutes. Samples collected were kept in a thermo-insulated box containing ice packs until centrifugation. Blood samples were centrifuged at 4000 RPM for 8 minutes at 04°C and the plasma samples were transferred into pre-labeled polypropylene tubes in to double aliquots. After aliquots are prepared, the samples were immediately shock frozen in an ethanol/dry ice bath at –70º ± 10°C. After 5 minutes, the frozen plasma tubes was transferred and stored in a freezer at a temperature below –70º ± 10ºC until analysis.

Analysis of Nitroglycerin, 1,2-Dinitroglycerin and 1,3-Dinitroglycerin concentration in human plasma:

A sensitive and selective LC-MS/MS method was used to estimate Nitroglycerin, 1,2- Dinitroglycerin and 1,3-Dinitroglycerin in human K_2EDTA plasma over the concentration range 44.0240 to 7998.1140 pg/mL, 55.5040 to 10083.8000 pg/mL and 55.5040 to 10083.8000 pg/mL respectively. Nitroglycerin and its metabolites were selectively isolated from 500 µL plasma by extraction technique and estimation was done by mass spectrometric detection using suitable column.

Pharmacokinetic and Statistical analysis:

The various pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, t_{max}, K_{el}, $t_{1/2}$, K_{el_Lower}, K_{el_upper}) were calculated using WinNonlin® software (version: 5.3). Statistical analysis was performed on pharmacokinetic data of subjects by using SAS® statistical software (version: 9.2 SAS Institute Inc, USA).

RESULTS:

Nitroglycerin sublingual tablet 0.6 mg, manufactured in India) or Nitrostat® (Nitroglycerin sublingual tablet) 0.6 mg, manufactured in USA were administered to healthy, Indian, adult, male volunteers (mean age ± SD**,** 27.71 ± 5.91; range 19-42; BMI 22.17 ± 2.02 kg/m^2). The primary pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ and secondary pharmacokinetic parameters K_{el}, t_{max} $t_{½}$, K_{el_Lower} and K_{el_upper} were calculated using WinNonlin® software (version: 5.3).

Since Nitroglycerin can not be quantified reliably due to its rapid absorption and elimination, 1,2-Dinitroglycerin and 1,3-Dinitroglycerin were employed for the assessment of bioequivalence. However nitroglycerin data presented here for information purpose only.

The mean plasma concentration parameters of test formulation and reference formulation are shown in the graph (Figure1-3).

The mean, standard deviation, minimum, median, maximum and geometric mean were calculated for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, t_{max}, $t_{½}$, K_{el}, K_{el_Lower} and K_{el_Upper}. The calculated pharmacokinetic parameters are presented in the tables given below:

Table 1: Mean pharmacokinetic parameters of Test formulation-T and Reference formulation –R (Nitroglycerin)

Pharmacokinetic parameters	*Geometric mean*	
	Test formulation	*Reference formulation*
C_{max} (pg/mL)	774.1525	683.6791
AUC_{0-t} (pg.min /mL)	2882.7634	2465.1623
$AUC_{0-\infty}$ (pg.min /mL)	4002.8263	3573.2699
*t_{max} (min)	3.00 (2.00-45.00)	3.00 (2.00-6.00)
$t_{½}$ (min)	2.9891 ± 3.9791	2.4508 ± 0.9324
K_{el} (min^{-1})	0.3055 ± 0.1006	0.3147 ± 0.0987
$K_{el\_Lower\ (min)}$	5.0000 ± 2.3895	5.2424 ± 2.1843
$K_{el\_upper\ (min)}$	12.3492 ± 5.3011	11.1364 ± 3.1226

Table 2: Mean pharmacokinetic parameters of Test formulation-T and Reference formulation –R (1,2-Dinitroglycerin)

Pharmacokinetic parameters	*Geometric mean*	
	Test formulation	*Reference formulation*
C_{max} (pg/mL)	3861.9074	3744.9395
AUC_{0-t} (pg.min /mL)	175173.35	167756.80
$AUC_{0-\infty}$ (pg.min /mL)	181369.23	174427.37
*t_{max} (min)	15.00 (6.00-30.00)	15.00 (3.00-30.00)
$t_{½}$ (min)	46.4994 ± 10.0110	48.6231 ± 11.0217
K_{el} (min^{-1})	0.0156 ± 0.0032	0.0150 ± 0.0035
$K_{el\_Lower\ (min)}$	69.1806 ± 35.5525	66.0278 ± 33.8139
$K_{el\_upper\ (min)}$	217.5000 ± 32.5338	217.5000 ± 30.9361

Table 3: Mean pharmacokinetic parameters of Test formulation-T and Reference formulation –R (1,3-Dinitroglycerin)

Pharmacokinetic parameters	*Geometric mean*	
	Test formulation	*Reference formulation*
C_{max} (pg/mL)	1109.5633	1004.0984
AUC_{0-t} (pg.min /mL)	46704.284	42105.885
$AUC_{0-\infty}$ (pg.min /mL)	52121.816	46935.971
*t_{max} (min)	20.00 (10.00-30.00)	15.00 (5.00-30.00)
$t_{½}$ (min)	38.7232 ± 10.4202	38.1957 ± 8.1567
K_{el} (min^{-1})	0.0190 ± 0.0045	0.0190 ± 0.0044
$K_{el\_Lower\ (min)}$	48.5278 ± 16.2463	46.0417 ± 16.4646
$K_{el\_upper\ (min)}$	131.2500 ± 32.5095	125.4167 ± 20.8068

*Expressed in terms of median

Tables 4-6 shows the 90% confidence interval of ratio of test formulation and reference formulation (T/R) of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

Table 4: Statistical Results of Test formulation-T versus Reference formulation-R for Nitroglycerin

Pharmacokinetic Parameter	*Ratio %*	*90% Confidence Intervals*
C_{max} (pg/mL)	113.23%	87.68% to 146.24%
AUC_{0-t} (pg.min/mL)	116.94%	84.62% to 161.61%
$AUC_{0-\infty}$ (pg. min/mL)	112.02%	91.21% to 137.58%

Table 5: Statistical Results of Test formulation-T versus Reference formulation-R for 1,2-Dinitroglycerin

Pharmacokinetic Parameter	*Ratio %*	*90% Confidence Intervals*
C_{max} (pg/mL)	103.12%	95.12% to 111.80%
AUC_{0-t} (pg.min/mL)	104.42%	98.68% to 110.49%
$AUC_{0-\infty}$ (pg. min/mL)	103.98%	98.58% to 109.68%

Table 6: Statistical Results of Test formulation-T versus Reference formulation-R for 1,3-Dinitroglycerin

Pharmacokinetic Parameter	*Ratio %*	*90% Confidence Intervals*
C_{max} (pg/mL)	110.50%	101.91% to 119.82%
AUC_{0-t} (pg.min/mL)	110.92%	104.33% to 117.93%
$AUC_{0-\infty}$ (pg. min/mL)	111.05%	106.23% to 116.09%

DISCUSSION AND CONCLUSION:

BA/BE studies provide important information which ensure safety and effectiveness of medicines to patients and practitioners in addition to evaluate the relative bioavailability.

The 90% confidence interval of the least square mean of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for 1,2-Dinitro glycerin were (95.12% to 111.8%), (98.68% to 110.49%), (98.58% to 109.68%) and for 1,3-Dinitroglycerin were (101.91% to 119.82%), (104.33% to 117.93%),(106.23% to 116.09%) respectively, was within the limit of 80.00% and 125.00%.

Based on the evaluation, test formulation (Nitroglycerin sublingual tablets 0.6 mg) manufactured in India and reference formulation NITRO-DUR® (Nitroglycerin sublingual tablets 0.6 mg) manufactured by Parke-Davis, division of Pfizer Inc., NY, USA are found to be bioequivalent in 72 healthy, Indian, adult volunteers.

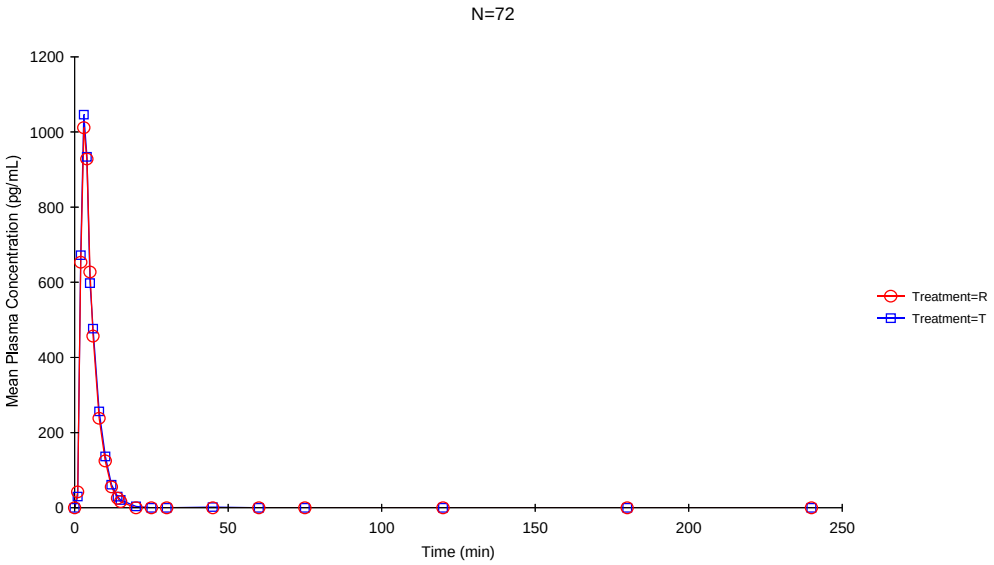


Figure 1: Linear plot of mean plasma Nitroglycerin concentrations versus time

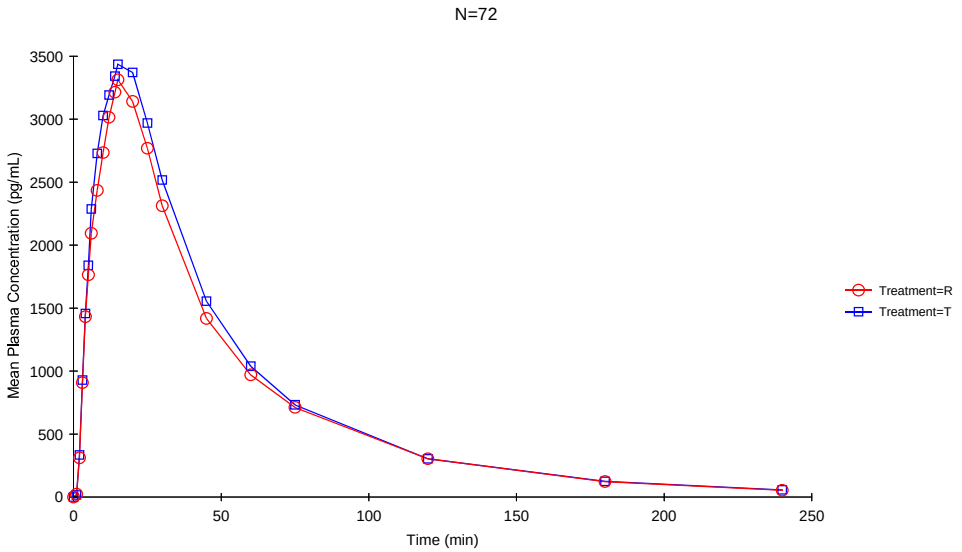


Figure 2: Linear plot of mean plasma 1,2-Dinitroglycerin concentrations versus time

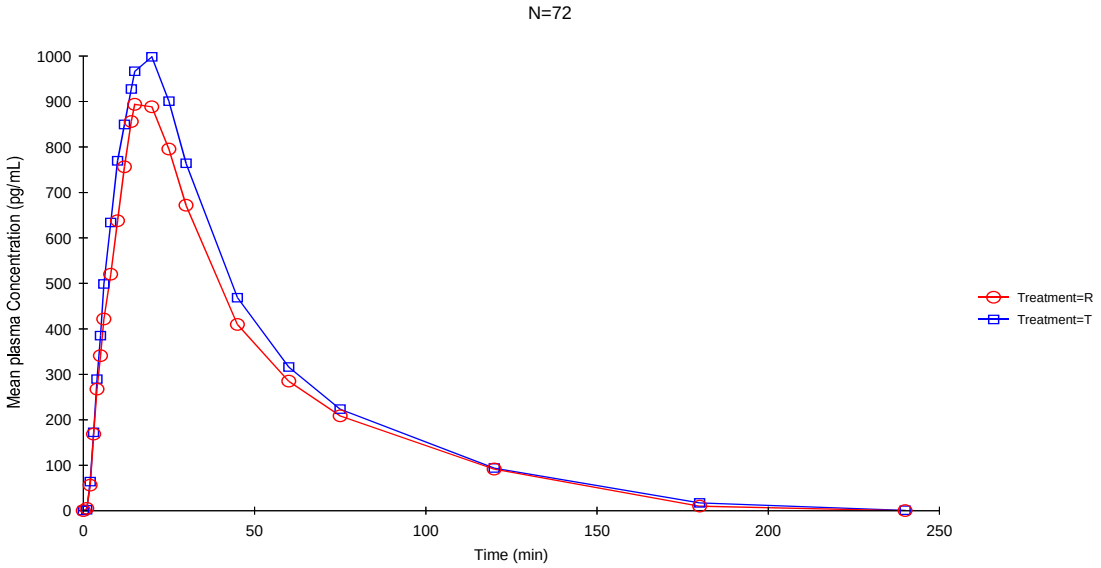


Figure 3: Linear plot of mean plasma 1,3-Dinitroglycerin concentrations versus time

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