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# An Evaluation of Relative Bioavailability of Nitroglycerin Transdermal Infusion System 0.6 mg/hr in Healthy Volunteers

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**Abstract**

**Background and Objective:** The Nitroglycerin transdermal system is a flat unit designed to provide continuous controlled release of Nitroglycerin through intact skin. The objective of the study is to determine the comparative bioavailability of two difference formulation of transdermal infusion system 0.6mg/hr.

**Method:** The relative bioavailability and pharmacokinetic profile of a test formulation (Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$) manufactured in India and reference formulation NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$) were compared in healthy human male volunteers. A single transdermal patch of either test or reference formulation was applied to the antero-lateral part of the thorax specifically to the chest of each subject halfway between the nipple and mid-clavicular line in sitting posture at ambient temperature under fasting conditions with a washout period of 04 days. A hypo-allergic tape was applied over the patch and surrounding skin to maintain patch adhesion, immediately after the patch application. Blood samples were collected at scheduled time points and plasma concentrations of Nitroglycerin were analyzed by LC-MS/MS method. The obtained plasma concentrations have been employed for pharmacokinetic analysis by using WinNonlin® software (version: 5.2) and statistical analysis by using SAS® statistical software (version: 9.1.3 SAS Institute Inc, USA).

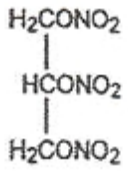
**Results:** Individual disposition kinetic parameters of maximum plasma concentration ($C_{max}$), area under the plasma concentration-time curve ($AUC_{0-t}$), area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), time taken to reach maximum concentration ($t_{max}$), rate elimination constant ($K_{el}$) and half-life ($t_{1/2}$) were calculated by non-compartmental analysis using WinNonlin® software (version: 5.2) and their results are found to be ($C_{max}$) 586.6085 and 634.7250 pg/mL, ($AUC_{0-t}$) 3442.5059 and 3675.3902 pg.hr/mL, ($AUC_{0-\infty}$) 4088.4743 and 4480.8912 pg.hr/mL, ($t_{max}$) 11.00 (1.00 - 14.50) and 5.50 (1.00 – 14.50) hr and ($t_{1/2}$) 2.35 ± 3.01 and 4.95 ± 8.38 hr for test and reference formulation respectively.

**Conclusion**: The 90% confidence interval of $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were evaluated and were found to be (68.38 % to 124.90 %), (73.08 % to 120.04 %) and (65.71 % to 126.70 %) respectively.

**Key Words**: Nitroglycerin, Transdermal infusion, Transdermal patch, Bioequivalence, Bioavailability

## INTRODUCTION:

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




and whose molecular weight is 227.09. The organic nitrates are vasodilators, active on both arteries and veins. It is a flat unit designed to provide continuous controlled release of Nitroglycerin through intact skin. The rate of release of Nitroglycerin is linearity dependent upon the area of the applied system, each $cm^2$ of applied system delivers approximately 0.03 mg of Nitroglycerin per hour. Thus, the 7-, 14- and 21-$cm^2$ systems deliver approximately 0.2, 0.4 and 0.6 mg of Nitroglycerin per hour, respectively. The remainder of the Nitroglycerin in each system serves as a reservoir and is not delivered in normal use. After 12 hours, for example, each system has delivered approximately 6% of its original content of Nitroglycerin.

The nitroglycerin transdermal system comprises 3-layers, 1) a transparenmt outer backing layer composed of a composite plastic film and is printed with the name of the drug and strength. 2) Nitroglycerin in acrylic-based polymer adhesive with a cross-linking agent. 3) a protective white, translucent peelable liner which covers the second layer and must be removed prior to use. Each system is sealed in a foil-lined pouch. The 7-, 14- and 21– $cm^2$ systems contain 37.3 mg, 74.6 mg and 111.9 mg of Nitroglycerin, respectively. The inactive ingredients are polyester film, silicone and acrylic adhesive with a cross-linking agent.

Cross section of the system:

| |
|---|
| **OUTER BACKING**(Impermeable) |
| **SECOND LAYER**(Nitroglycerin in adhesive) |
| **PROTECTIVE PEELABLE LINER**(release liner) |

The principal pharmacological action of Nitroglycerin injection is relaxation of vascular smooth muscle and consequent dilatation of peripheral arteries and veins, especially the latter. Dilatation of the veins promotes peripheral pooling of blood and decreases venous return to the heart, thereby reducing left ventricular end-diastolic pressure and pulmonary capillary wedge pressure (preload). Arteriolar relaxation reduces systemic vascular resistance, systolic arterial pressure, and mean arterial pressure (afterload). Dilatation of the

coronary arteries also occurs. The relative importance of preload reduction, afterload reduction, and coronary dilatation remains undefined. Dosing regimens for most chronically used drugs are designed to provide plasma concentrations that are continuously greater than a minimally effective concentration. This strategy is inappropriate for organic nitrates. Several well-controlled clinical trials have used exercise testing to assess the anti-anginal efficacy of continuously-delivered nitrates. In the large majority of these trials, active agents were indistinguishable from placebo after 24 hours (or less) of continuous therapy. Attempts to overcome nitrate tolerance by dose escalation, even to doses far in excess of those used acutely, have consistently failed. Only after nitrates have been absent from the body for several hours has their anti-anginal efficacy been restored.

This study was performed with an aim to evaluate the relative bioavailability of test formulation [Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$)] and reference formulation [NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$)] in healthy volunteers.

**Study drugs:**

Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$) manufactured in India was used as test formulation. NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$) manufactured by Key Pharmaceuticals, NJ, USA was used as reference formulation.

**Study design:**

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

**Subjects:**

Fourteen healthy, nonsmoking adult male volunteers (mean age ± SD, 26.86 ± 4.66; range 20-33 years; BMI 22.35 ± 1.90 kg/$m^2$) were enrolled in the study. All the 14 enrolled subjects were 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, volunteers with high or low BP and pulse rate below 60/minutes or above 100/minutes.

The volunteers were asked to abstain from taking any medication (including any prescribed drugs) throughout the study period.

**PATCH APPLICATION AND APPLICATION PROCEDURE:**

On dosing day of period I and II volunteers were applied with the transdermal patch of Nitroglycerin.

**Step 1.** The patch was applied on a non-hairy, clean and dry area at the application site which is free of cuts, scratches, tattoos, scars and abrasions. The patch was not applied on a skin that is very oily, sunburned and irritated skin site. Clipping of hair was done on the application site and complete shaving was avoided. Care was taken to ensure that creams, lotions, powders or other tropical formulations were not applied on the application site before patch application.

**Step 2.** Before 60 minutes prior to patch application, the application site was wiped gently with a warm water washcloth and then lightly patted dry with a soft towel. Care was taken to ensure that the skin area was completed dried for patch application.

**Step 3.** The patch was not placed in area where the patch would be rubbed by tightly fitted clothing.

**Step 4.** The packages were opened before five minutes of usage but the unit was not removed from the pouch until it was being used. The pouch was opened along one side without damaging the system.

**Step 5.** Unit was removed from the pouch.

**Figure 1**

**Step 6.** The sticky side of the unit was placed facing the application site by bending the unit backward and then the release liner was removed from the unit. Care was taken to ensure that hands were not exposed to the adhesive surface of the unit.

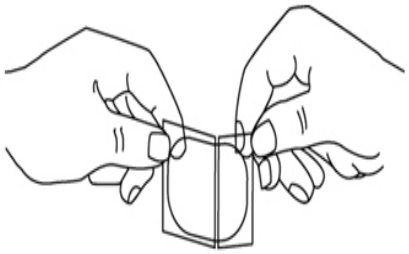


**Figure 2**

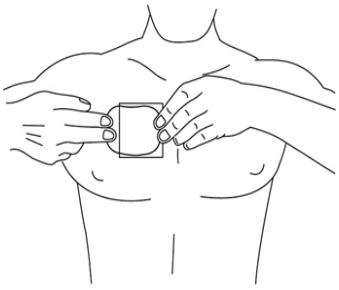


**Figure 3**

**Step 7.** The sticky side of the unit was applied on the application site. The release liner was removed and the unit was pressed onto the skin using moderate pressure. Care was taken to ensure that the unit was stuck well to the skin especially around the edges.

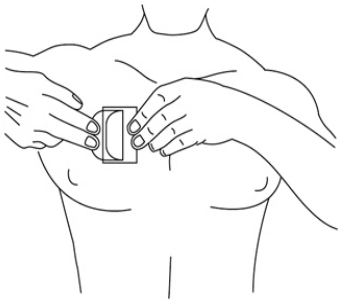


**Figure 4**

**Step 8.** The patch application was performed by pressing the patch firmly and holding on for 30 seconds with the palm of the hand to ensure that the edges of the patch are firmly adhered.

**Step 9.** A hypo-allergic tape was applied over the patch and surrounding skin to maintain patch adhesion, immediately after the patch application.

In period II, the site opposite of that used in the first period was used for application. Patch adhesion was monitored at 01.00, 02.00, 03.00, 04.00, 05.00, 06.00, 07.00, 08.00, 10.00, 12.00, 13.00 and 14.00 hrs (prior to the removal of patch).

**Patch removal:**

After patch removal, it was adhered with a new oversized release liner and stored in an oversized pouch and any adhesive residue left on the application site was gently removed using alcohol swabs and allowed to dry in air. The alcohol swabs used were stored in a separate pouch. The old release liner was stored in the original pouch. All these pouches were stored in a container and analysed for residual nitroglycerin amount. Assessment of skin irritation was performed at 30 minutes and 24 hours after removal of patch application and any adhesive residue left on the site was rated as none, light, medium or heavy.

There was 04 days washout period between two dosing days. They received standard lunch after 4 hrs, snacks after 9 hrs and dinner after 13 hrs post dose. 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). Volunteers were monitored constantly throughout the study period by a medical doctor.

**Blood sampling:**

Blood samples of 6 ml were collected in pre-chilled Lithium-heparin plastic vacutainers through an indwelling intravenous cannula placed in a forearm vein before patch application and at 01.00, 02.00, 03.00, 04.00, 05.00, 06.00, 07.00, 08.00, 09.00, 10.00, 11.00, 12.00, 13.00, 14.00 (prior to removal of patch application), 14.50 and 15.00 hours after removal of patch application. Samples collected were kept in a thermo-insulated box containing ice packs until centrifugation. Blood samples were centrifuged at 4000 ± 50 RPM for 8 minutes at 02°C to 04°C. Immediately after centrifugation, the accurately measured 1235 µL plasma were transferred into pre-labeled polypropylene tubes containing 65µL of 50mM silver nitrate solution in duplicates and the resulting mixture was vortexed for about 10-20 second for complete mixing. After aliquots are prepared, the plasma samples were immediately shock frozen in an ice bath containing a mixture of methanol and dry ice and stored at temperature below -50$^{o}$C until analysis.

**Analysis of Nitroglycerin concentration in human plasma:**

The plasma samples were analyzed by Liquid Chromatography - Mass Spectrometry/Mass Spectrometry method. Nitroglycerin was selectively isolated from 1000 µL plasma by extraction procedure and separation of analyte and internal standard were achieved by a suitable column and estimation was done by mass spectrometric detection. The lower limit of quantification was 26.1600 pg/mL during analysis.

**Pharmacokinetic and Statistical analysis:**

The various pharmacokinetic parameters ($C_{max,}$ $AUC_{0-t,}$ $AUC_{0-\infty,}$ $t_{max,}$ $K_{el,}$ $t_{1/2}$) were calculated using WinNonlin® software (version: 5.2). Statistical analysis was performed on pharmacokinetic data of subjects by using SAS® statistical software (version: 9.1.3 SAS Institute Inc, USA).

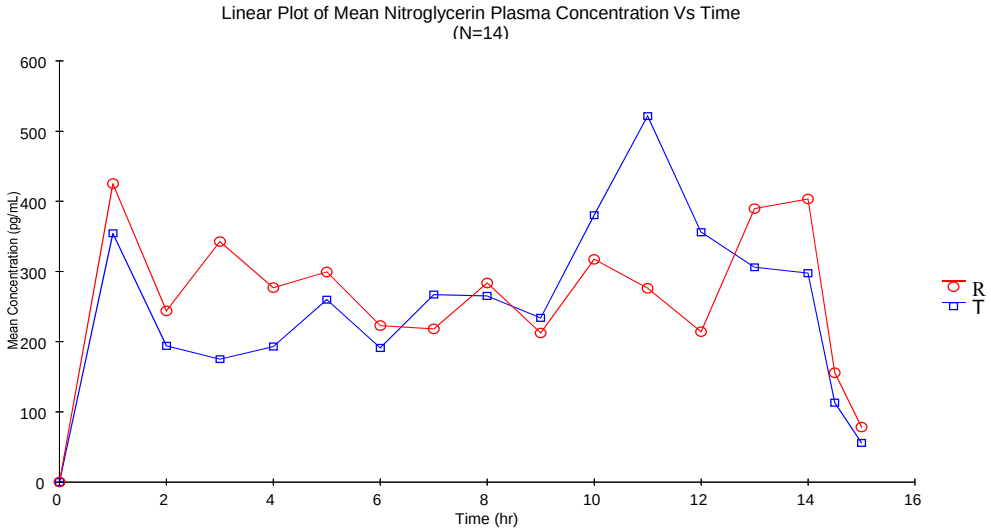



**Figure 5**: Mean Plasma Concentrations of Test formulation (Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$) and Reference formulation [NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$)] in 14 healthy volunteers.

**RESULTS:**

Transdermal patch of Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$) (manufactured in India) or NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$) (Key Pharmaceuticals, NJ, USA) were applied to 14 healthy, adult, male volunteers (mean age ± SD**,** 26.86 ± 4.66; range 20-33; BMI 22.35 ± 1.90 $kg/m^2$). The primary pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ and secondary pharmacokinetic parameters $K_{el}$, $t_{max}$ and $t_{½}$ were calculated using WinNonlin® software (version: 5.2) The mean plasma concentration time profiles of two formulations are shown in the graph. (Figure 5)

The mean of $C_{max}$ was 586.6085 pg/mL (%CV 10.07) for test formulation and 634.7250 pg/mL (% CV 7.20) for reference formulation. For $t_{max,}$ the median values were found to be 11.00 (1.00-14.50) hr for test formulation and 5.50 (1.00 – 14.50) hr for reference formulation. For $AUC_{0-t,}$ the values obtained were 3442.5059 pg* hr /mL (% CV 6.74) and 3675.3902 pg* hr /mL (% CV 5.69) for for test and reference formulation respectively.

The mean $AUC_{0-\infty}$ values were found to be 4088.4743 pg* hr /mL (% CV 6.60) for test formulation and 4480.8912 pg* hr /mL (%CV 2.56) for reference formulation.

Half life of test formulation was found to be 2.35 ± 3.01 hr and reference formulation was found to be 4.95 ± 8.38 hr. $K_{el}$ was 0.72 ± 0.74 $(hrs)^{-1}$ for test formulation and 0.78 ± 0.66 $(hrs)^{-1}$ for reference formulation.

**Table 1:** Mean pharmacokinetic parameters of Nitroglycerin Transdermal Infusion System

| **Test formulation (N=14)** | | |
|---|---|---|
| **Pharmacokinetic parameters** | **Geometric mean** | **CV %)** |
| $C_{max}$ (pg/mL) | 586.6085 | 10.07 |
| $AUC_{0-t}$ (pg* hr /mL) | 3442.5059 | 6.74 |
| $AUC_{0-\infty}$ (pg* hr /mL) | 4088.4743 | 6.60 |
| *$t_{max}$ (hr) | 11.00 (1.00 – 14.50) | N/AP |
| $t_{½}$ (hr) | 2.35 ± 3.01 | N/AP |
| $K_{el}$ $(hr^{-1})$ | 0.72 ± 0.74 | N/AP |
| **Reference formulation (N=14)** | | |
| **Pharmacokinetic parameters** | **Geometric mean** | **CV %)** |
| $C_{max}$ (pg/mL) | 634.7250 | 7.20 |
| $AUC_{0-t}$ (pg* hr /mL) | 3675.3902 | 5.69 |
| $AUC_{0-\infty}$ (pg* hr /mL) | 4480.8912 | 2.56 |
| *$t_{max}$ (hr) | 5.50 (1.00 – 14.50) | N/AP |
| $t_{½}$ (hr) | 4.95 ± 8.38 | N/AP |
| $K_{el}$ $(hr^{-1})$ | 0.78 ± 0.66 | N/AP |

*Expressed in terms of median; N/AP – Not applicable

Table 2 shows the 90% confidence interval of ratio of test and reference (T/R) formulations of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max.}$ Ratio of Least square Means (T/R) percent was found to be 92.42 % for $C_{max,}$ 93.66 % for $AUC_{0-t}$ and 91.24 % for $AUC_{0-\infty.}$ The study revealed that at a 90% confidence interval of $C_{max,}$ $AUC_{0-t}$ and $AUC_{0-\infty}$ were found to be within the range of 68.38 % to 124.90%, 73.08 % to 120.04% and 65.71% to 126.70 % respectively. All of these

values are not within the bioequivalence accepted range of 80.00% - 125.00%.

**Table 2:** 90% confidence interval for different pharmacokinetic parameters from log data for assessment of bioequivalence.

| Pharmacokinetic Parameter | Ratio % | 90% Confidence Intervals |
|---|---|---|
| $C_{max}$ (pg/mL) | 92.42 % | 68.38 % to 124.90 % |
| $AUC_{0-t}$ (pg.hr/mL) | 93.66 % | 73.08 % to 120.04 % |
| $AUC_{0-\infty}$ (pg.hr/mL) | 91.24 % | 65.71 % to 126.70 % |

## 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.

Based on the evaluation, test formulation (Nitroglycerin transdermal infusion system 0.6 mg/hr (34 $cm^2$) manufactured in India and reference formulation NITRO-DUR® (Nitroglycerin transdermal infusion system 0.6 mg/hr) (30 $cm^2$) are found to be bioinequivalent in 14 healthy human male volunteers.

## ACKNOWLEDGEMENT:

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