**RESEARCH ARTICLE**

**BIOAVAILABILITY STUDY OF NICARDIPINE LIQUISOLID COMPACT TABLETS IN RABBITS AFTER ORAL ADMINISTRATION.**

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**Abstract**

Aim of the present research work is to conduct bio availability study of nicardipine liquisolid compact tablet in rabbit and compare with plain nicardipine drug. Study is conducted by using Randomized Balanced Incomplete Block Design (BIBD) method. Total 8 healthy rabbits were selected with weight of 2.5 kg to 3 kg. Rabbits were labeled by numbers. Each rabbits receiving both formulations after proper wash out period (7 days). Blood samples were collected from marginal ear vein at pre determined time intervals up to 24 Hrs. then blood samples were analyzed by validated high performance liquid chromatography method. Liquisolid compact exhibit c\text{max} at 212 ng/ml, t\text{max} at 1.63 Hr, AUC(0-t) at 1349 ng.min/ml, AUC(0-\infty) at 1403 ng.min/ml and t\text{1/2} at 1.25 hr. AUC and maximum plasma concentration of the liquisolid compact is higher than pure nicardipine drug it indicates liquisolid compacts produce more bioavailability than nicardipine powder.

**Introduction:**
Nicardipine is used in the treatment of hypertension (Pompano R et al., 2004; Catarina M et al., 2002). It is calcium channel blocking agent. Nicardipine hydrochloride is a [2-(Benzyl (methyl) amino) ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine.3,5-dicarboxylate hydrochloride] (Graham D et al., 1985). Nicardipine have low bio availability due to its low aqueous solubility. So many methods are present to increase solubility of the drug (David et., 2012; Yoshida et al., 2012; Zhang et al., 2012; Sheth et al., 2012). Solubility of nicardipine was increases 10 folds when nicardipine was complexation with carboxylic acid buffer system (Maurin et al., 1994). In the present research work we are using liquid solid compact technique to enhance solubility of nicardipine. To carry out this research work Tween 80 is used as solvent and Avicel and aerosil were used as carrier and as coating material and cross povidone used as super disintegrating agent.

**Material and Methods:**
**Materials:** Nicardipine was gifted by Natco laboratories Hyderabad, tween80, avicel pH 102 and aerosil were purchased from E.Mecrk (India), Crospovidone, Methanol for HPLC grade were purchased from SD fine chemicals (India).

**Preparation of nicardipine liquisolid compact tablets:** 20 mg of nicardipine is solubilised in 200 mg of tween 80. Then to this liquid drug add 512 mg of Avicel as carrier material. Avicel absorb the total liquid drug and turned as...
wet mass. 52 mg of aerosil is added to the above wet mass and mix it continuously until it produce dried powder. Then 64 mg of cross povidone is added and finally talc and magnesium stearate is added. And mix all the ingredients gently. From this blend we are taken dose according dose calculation for rabbit.

**Dose calculation for rabbit:**
Animal dose calculations were based on BSA as per the following formula.

\[
\text{HED (mg/kg)} = \frac{\text{animal dose (mg/kg)}}{(\text{animal km/human km})}
\]

**Human equivalent dose (mg/Kg)** = 20mg/60kg= 0.33mg/Kg

Km factor for rabbit = 12
Km factor for human = 37
Animal dose (mg/kg) =? 
0.33 = Animal dose X 12/37
Animal dose= 1.01mg/kg
Animal dose for rabbit weighing 2.5 kg = 2.525 mg of nicardipine

**Apparatus and chromatographic condition:** A model of waters alliance 2695 XE separation module with a UV-detector and an online degasses was mixed and empowers chromatography software to be used in prediction of samples. Chromolith TM Performance RP-C18 (50mm×4.6mm, 5μ) column is used. Mobile phase consist mixture of methanol and water at the ratio of 13:87 v/v was delivered at the rate of 1.0 ml/min. The injection volume was 10μL.

**Construction of standard calibration curve and quality control samples:** Stock solutions of nicardipine were prepared by dissolving in methanol (1 mcg/ml). Then again nicardipine solution were diluted with methanol to produce 1, 2, 4, 8, 16, 32, 64 and 128 ng/ml internal standard solution di ethyl stelbesterol solution further diluted with methanol to produce 1 mg/ml. 1 ml of rabbit plasma is spiked with 25 μL of drug solutions to get 25, 50, 100, 200, 400, 800, 1600 and 3200 ng/ml solutions. In the above concentrated solution 25 μL of internal standard solution was added. From this solution 10μL solution is injected to HPLC. Quality control samples were prepared at concentration 100 ng/ml, 200 ng/ml and 400 ng/ml of nicardipine in blank plasma (Rupender rupali et al., 2014; Leandro tasso et al., 2008)

**Administration of the dose and blood sample collection:** 8 healthy rabbits were selected with the average weight of 2.5 kg to 3 kg. Two study periods are conducted on each rabbit. Between two study periods one wash out period is maintained i.e., 7 days. Equivalent to 2.5 mg of the nicardipine is taken from liquisolid compact powder and it is dispersed in 0.25% carboxy methyl cellulose. Then drug solution administered through oral feeding tube (Nanda gopal anitha et al., 2014). Then at predetermined time intervals blood samples were collected up to 24 hrs from marginal ear vein at 0.0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 6.00, 12.00 and 24.00 Hrs. blood samples were collected in heparin contain test tubes. Then separation of plasma done by centrifugation process at 5000 rpm for 5min and stored under frozen condition till the analysis was performed.

**Sample preparation:** 0.5 ml plasma sample is transferred to 2 ml of test tube then 10 mcg/ml of di ethyl stelbesterol was added and centrifuged then supernatant was removed. 200 μL of methanol was added and mix well then centrifuge for 5 min for separation of phases then evaporate at room temperature. Then again 200 μL of mobile phase was added then 10μL solution is injected to HPLC.

**Method validation:** linearity was determined (Meiling et al. 2006) by preparing 3 sets of samples at the concentration of 25, 50, 100, 200, 400, 800, 1600 and 3200 ng/ml. inter day precission was evaluated on 100 ng/ml, 200 ng/ml and 400 ng/ml samples on 3 different days. Intraday precision (Ashesh bhandary et al., 2013) was evaluated on five sets of 100 ng/ml 200ng/ml and 400 ng/ml samples on the same day. QC recovery was conducted on three set on 100 ng/ml, 200 ng/ml and 400 ng/ml.

**Data analysis:** Pharmaco kinetic parameters were calculated by Wagner nelson method. Cmax, Tmax, t1/2, k0, AUC(0-t), AUC(0-∞) were evaluated in individual rabbit.
Result and discussion:-

**Linearity:** linearity was determined on 3 sets of samples concentration of 25 ng/ml, 50 ng/ml, 100 ng/ml, 200 ng/ml, 400 ng/ml, 800 ng/ml, 1600 ng/ml and 3200 ng/ml were used to draw calibration curve. Calibration curve give linear regression equation $y = 0.009x - 0.144$ and the correlation co efficient value $r^2$ was 0.999 (Figure 1)

![Standrad calibration curve of nicardipine in rabbit plasma](image)

**Figure 1:** linear calibration curve of nicardipine in rabbit plasma

**Precission:** the inter day and intraday precision were expressed in % relative standard deviation (Onkar jagtap wt al., 2011). Relative standard deviation for inter day precision were from 0.1 to 0.21 (table no 1) and intraday precision were from 0.11 to 0.22 (table no 2). All the RSD values less than 2 it indicted values are within the acceptable limit.

**Table no 1:**- Inter day precision

| Concentration (ng/ml) | Inter day precision |
|-----------------------|---------------------|
|                       | 100 ng/ml           | 200 ng/ml           | 400 ng/ml           |
| Day 1                 | 20779               | 41095               | 102334              |
| Day 2                 | 20861               | 41124               | 102125              |
| Day 3                 | 20790               | 41281               | 102241              |
| Mean                  | 20810               | 41166               | 102233              |
| SD                    | 44.51               | 100.07              | 104.71              |
| % Rsd                 | 0.21                | 0.24                | 0.10                |

**Table no 2:**- Intraday precision:

| Concentration (ng/ml) | Intraday precision data |
|-----------------------|-------------------------|
|                       | 100 ng/ml               | 200 ng/ml               | 400 ng/ml               |
| Area 1                | 20850                   | 41058                   | 102124                   |
| Area 2                | 20905                   | 41085                   | 102284                   |
| Area 3                | 20875                   | 41125                   | 102115                   |
| Area 4                | 20921                   | 41278                   | 102294                   |
| Area 5                | 20890                   | 41064                   | 102354                   |
| Mean                  | 20888.2                 | 41122                   | 102234.2                 |
| SD                    | 27.36                   | 91.07                   | 108.12                   |
| % Rsd                 | 0.13                    | 0.22                    | 0.11                     |

**QC recovery:** recovery of qe sample were from 99 to 105% (table. 3)
Table no 3:- QC recovery

| Concentration (ng/ml) | % Recovery |
|-----------------------|------------|
| 100                   | 99         |
| 200                   | 105        |
| 400                   | 102        |

Pharmacokinetic of nicardipine:-

The individual plasma concentrations of test and reference products in each subject were given in Table 4 and 5. Individual pharmacokinetics parameters of test and reference product in each subject was given in table 6 and 7. The plots of comparative mean plasma concentrations of test and reference products in Rabbit were shown in figure 2.

The mean peak plasma concentration of test (T) formulation $C_{\text{max}}$ 212.25 ng/ml was gradually reached in 1.63 hr. In case of conventional reference formulation (R) the $C_{\text{max}}$ was 125.25ng/ml. It takes time 1.8 hr. The $C_{\text{max}}$ of the Test formulation (T) was higher when compared with Reference (R) formulation. The lower in $T_{\text{max}}$ of test formulation (1.63) was clearly indicating the drug shown quicker on set action when compared to reference product. The $AUC_{0-t}$ of the reference (R) was found to be 1017.65ng.min/ml. The increase in $AUC_{0-t}$ was observed in the test (T) formulation, which was around 1348.95ng.min/ml. This clearly indicates the drug shown higher bio-availability than reference formulation.

Decrease in elimination rate constant ($K_e$) from 0.59 hr$^{-1}$ (Test) to 0.23 hr$^{-1}$ (reference) indicates the reference formulation shown slow release rate of the drug in the body. Test formulation fastly releases the drug in the body.

Half life of test product (1.25 hr) is lower compare to reference product (3.06 hr) it indicates test product fastly absorbed and shows faster action.

Table no 4:- Individual concentrations of Nicardipine after administration of Test product in each subject

| Subject | Study period | Time in Hrs | Plasma concentration (ng) of optimized nicardipine liquisolid compact tablet (Test) |
|---------|--------------|-------------|----------------------------------------------------------------------------------|
|         |              | 0 | 0.25 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 6 | 12 | 24 |
| 1001    | 1            |   | 30   | 60  | 115| 170| 227| 190| 85  | 64 | 45 | 34 |
| 1002    | 2            |   | 80   | 110 | 152| 210| 171| 121| 72  | 57 | 49 | 27 |
| 1003    | 1            |   | 60   | 86  | 145| 162| 195| 114| 98  | 65 | 41 | 37 |
| 1004    | 2            |   | 74   | 104 | 161| 227| 137| 111| 99  | 67 | 33 | 21 |
| 1005    | 1            |   | 54   | 69  | 148| 178| 139| 114| 91  | 66 | 37 | 29 |
| 1006    | 1            |   | 57   | 76  | 137| 219| 164| 130| 78  | 61 | 42 | 33 |
| 1007    | 1            |   | 65   | 115 | 159| 221| 147| 114| 68  | 51 | 42 | 31 |
| 1008    | 2            |   | 71   | 95  | 161| 221| 135| 112| 72  | 61 | 44 | 34 |
| N       |              |   | 8    | 8   | 8  | 8  | 8  | 8  | 8   | 8  | 8  | 8  |
| Mean    |              |   | 61.38| 89.38| 147.25| 201.00| 164.38| 125.75| 82.88| 61.50| 41.63| 30.75 |
| SD      |              |   | 15.45| 19.99| 15.54| 26.44| 32.65| 26.70| 12.19| 5.35 | 4.90 | 5.04 |
| Min     |              |   | 30   | 60  | 115| 162| 135| 111| 68  | 51 | 33 | 21 |
| Median  |              |   | 62.5 | 90.5| 150| 214.5| 155.5| 114 | 81.5| 62.5| 42  | 32  |
| Max     |              |   | 80   | 115 | 161| 227| 227| 190| 99  | 67 | 49 | 37 |
### Table 5: Individual concentrations of Nicardipine after administration of Reference product in each subject

| Subject | Study period | Time in Hrs | 0 | 0.25 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 6 | 12 | 24 |
|---------|--------------|-------------|---|------|-----|---|-----|---|-----|---|----|----|----|
| 1001    | 2            | 0           | 21 | 41   | 85  | 112|124  | 94 |84   |54 |37 |21 |
| 1002    | 1            | 0           | 15 | 35   | 75  | 111|118  | 105|74   |55 |37 |22 |
| 1003    | 2            | 0           | 18 | 31   | 71  | 117|121  | 105|94   |47 |31 |19 |
| 1004    | 1            | 0           | 16 | 29   | 84  | 114|131  | 117|91   |41 |27 |20 |
| 1005    | 2            | 0           | 18 | 36   | 91  | 128|116  | 102|87   |52 |34 |18 |
| 1006    | 1            | 0           | 11 | 39   | 82  | 117|127  | 112|89   |47 |31 |23 |
| 1007    | 2            | 0           | 27 | 48   | 79  | 119|109  | 98 |83   |45 |29 |19 |
| 1008    | 1            | 0           | 22 | 37   | 89  | 109|137  | 121|93   |48 |32 |24 |

**Plasma concentration (ng) of nicardipine conventional tablet (Reference)**

| N       | Mean | SD | Min | Median | Max |
|---------|------|----|-----|--------|-----|
| 8       | 18.5 | 37 | 82  | 115.87 | 8   |
| 0.8     | 0.48 | 0.7 | 0.69 | 0.48   | 0.7 |
| 0.18    | 0.37 | 0.18 | 0.29 | 0.37   | 0.18 |
| 0.5     | 0.83 | 0.7 | 0.74 | 0.74   | 0.7 |
| 0.5     | 0.83 | 0.7 | 0.74 | 0.74   | 0.7 |
| 0.5     | 0.83 | 0.7 | 0.74 | 0.74   | 0.7 |
| 0.5     | 0.83 | 0.7 | 0.74 | 0.74   | 0.7 |
| 0.5     | 0.83 | 0.7 | 0.74 | 0.74   | 0.7 |

### Table 7: Pharmacokinetic data of Test product-T in each subject

**Pharmacokinetic data of optimized nicardipine liquidosolus compact tablets (Test)**

| Treatment | Subject | Tmax (hr) | Cmax (ng/ml) | AUC(0-4) | AUC(0-∞) | K_el | t1/2 |
|-----------|---------|-----------|--------------|----------|----------|------|------|
| Test      | 1       | 2         | 227          | 1426.75  | 1461.35  | 0.98 | 0.7  |
|           | 2       | 1.5       | 210          | 1373.75  | 1412.77  | 0.69 | 1    |
|           | 3       | 2         | 195          | 1410.25  | 1464.01  | 0.68 | 1    |
|           | 4       | 1.5       | 227          | 1273.25  | 1323.13  | 0.42 | 1.64 |
|           | 5       | 1.5       | 178          | 1292.12  | 1350.74  | 0.49 | 1.4  |
|           | 6       | 1.5       | 219          | 1354.75  | 1425.75  | 0.46 | 1.49 |
|           | 7       | 1.5       | 221          | 1292.37  | 1353.33  | 0.5  | 1.36 |
|           | 8       | 1.5       | 221          | 1368.37  | 1436.37  | 0.5  | 1.37 |

| N         | Mean   | SD  |
|-----------|--------|-----|
| 8         | 1.63   | 0.23|
| 8         | 212.25 | 17.36|
| 8         | 134.95 | 57.32|
| 8         | 1403.39| 53.99|

### Table 8: Pharmacokinetic data of Reference product-R in each subject

**Pharmacokinetic data of conventional nicardipine tablets (Reference)**

| Treatment | Subject | Tmax (hr) | Cmax (ng/ml) | AUC(0-t) | AUC(0-∞) | K_el | t1/2 |
|-----------|---------|-----------|--------------|----------|----------|------|------|
| Reference | 1       | 2         | 124          | 1077.12  | 1170.46  | 0.22 | 3.08 |
|           | 2       | 2         | 118          | 1063.37  | 1157.59  | 0.23 | 2.96 |
|           | 3       | 2         | 121          | 992.12   | 1059.09  | 0.28 | 2.44 |
|           | 4       | 2         | 131          | 944.62   | 1033.08  | 0.22 | 3.06 |
|           | 5       | 1.5       | 128          | 1036.75  | 1128.16  | 0.19 | 3.51 |
|           | 6       | 2         | 127          | 1020.62  | 1112.1   | 0.25 | 2.75 |
|           | 7       | 1.5       | 119          | 950      | 1039.28  | 0.21 | 3.25 |
|           | 8       | 2         | 134          | 1056.62  | 1174.19  | 0.2 | 3.39 |

| N         | Mean   | SD  |
|-----------|--------|-----|
| 8         | 1.88   | 0.23|
| 8         | 125.25 | 5.75|
| 8         | 1017.65| 50.82|
| 8         | 1109.24| 58.41|
| 8         | 0.23   | 0.03|
| 8         | 3.06   | 0.35|
Mean plasma concentration in rabbit

Figure 2: Mean plasma concentration of test and reference formulation

Figure 3: HPLC spectra of blank rabbit serum
Figure 4: HPLC spectra of nicardipine

Figure 5: HPLC spectra of Di ethyl stelbesterol
Conclusion:
From the above study it was concluded that, the test product Nicardipine Liquid compacts was increases bioavailability with reference product of Nicardipine marketed formulation.

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