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# INFLUENCE OF SAMPLE SIZE ON THE RESULTS OF BIOEQUIVALENCE STUDIES

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**Abstract**

Great number of drugs coming from different manufactures is available on the market.

The biocquivalence studies give substantial evidence if these drugs, given in same doses and under similar conditions, have similar bioavailability. Studies of bioequivalence are performed on healthy young volunteers in crossover designs and artificially controlled environment to minimize factors, other than the drug, which can affect bioavailability. They usually include 24 healthy volonteers with about 20 blood analyzes giving a total of 500.

This kind of research is of big importance for the determination of pharmacokinetic drug characteristics but is very expensive, especially in small countries.

Considering the importance of cost decrement we set the hypothesis that bioequivalence studies can be performed on smaller number of subjects. This hypothesis is confirmed by the results of our analysis (6) included in cross-over study can be an adequate number.



## Introduction

A fundamental hypothesis of clinical pharmacokinetics is that a relationship exists between the pharmacological or toxic response to a drug, and the accessible concentration of the drug. This hypothesis has been documented for many drugs, although it is apparent for some drugs that no clear of simple relationship has been found between pharmacologic efect and concentration in plasma (Goodman&Gilman's,1996).

Several alternative and equivalent representations of drug disposition can be used to describe the relationship between dose and concentration, and can be modified to account for the passage of time. In

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the early days of pharmacokinetics, emphasis was placed on half-life to decribe the time course of drug concentration. Now we usually think of clearence and volume of distribution as the essential parameters of pharmacokinetic processes because of close mapping of these parameters to identifiable functional and structural features of the body (Holford, 1992).

Various physiological and morbidity variables that dictate adjustment of dosage in individual patients often do so as a result of modification of pharmacokinetic parameters. Three most important parameters are drug clearence, volume of drug distribution and its bioavailability. These factors coupled with dosage, determine the concentration of a drug at its sites of action and hence the intensity of its effects as a function of time.

When the bioavailability of different preparations, salts of forms of a drug are compared at the same molar dose, under similar experimental conditions, and are found to be the same, the drugs are said to be bioequivalent. Bioequivalence of two drugs means that the rate und extent of absorption are extremely similar, the amount of each preparation eaching bloodstream does not significantly differ, and the preparations are chemically equivalent (Spilker, 1991).

Pharmacokinetic parameters used to establish bioequivalence are the peak plasma concentration achieved ($C_{max}$), the time to achieve this peak concentration ($T_{max}$), and the area under the blood (or plasma) time-concentration ($AUC_{o-}$). Ideally the two curves should be super imposable, so one may conclude that the two drugs are bioequivalent, but in practice the curves may differ. According to FDA, the product is considered bioequivalent if blood levels of two drugs agree within 20%. The FDA also has a princliple that 75% of patients should have plasma levels that are between 75% and 125% of the reference standard (Spilker, 1991).

The most important factors, which can affect bioavailability of a medicine, are: age of patient, food ingested, genetic history physiological capacity of the liver to metabolise, disease, interactions with other medicines, kidney function. To minimise these factors, studies of bioequivalence should be performed on healthy young volunteers in crossover designs, so each patient receives both tretaments, and in artificially controlled environment.

Minimum number of 12 evaluated subjects, should be included in any bioequivalence study (CDER, 2001). Generally, this type of pharmacokinetic researches usually include 24 healthy volunteers. However, these researches are very expensive, and our intention is to demonstrate that the same results can be obtained with smaller number of included subjects. This can be a way to decrease the pharmacokinetic research expense.

In order to obtain reliable results, the bioanalytical methods used to determine active principle and/or its biotransformation product in any suitable matrix should meet requirements of specificity, accuracy, sensitivita and precision.

The knowledge of the active substance quality is also very important. In case that products are prepared according to GMP rules, pharmacokinetic profiles are predictable.

The goal of this meta-analysis is to show that the same results from pharmacokinetic studies could be obtained from 24, 12 or 6 healthy volunteers.

## Methods

### *Study design*

In order to prove no significant difference between the pharmacokinetic results compiled from diferently sized groups of subjects we compared data from 3 different bioequivalence studies. Statistical comparison has been made between the compiled results from 24 subject groups, 12 subject groups and 6 subject groups. The groups of 6 and 12 subjects were randomly assigned from the original groups (groups of 23, 24,26 subjects).

### *Inclusion criteria*

Bioequivalence studies to be done according to GCP.
In these studies we included minimum 24 or 12 subjects.

### *Analyzed studies*

Three different bioequivalence studies were included in this analysis: bioequivalence study of sulpiride, bioequivalence study of lisinopril and bioequivalence study of norfloxacin.

### *Randomisation assignment*

A subject number used in the randomisation schedule was assigned to each subject included in the study.

### *Statistical data analysis*

Statistical analysis included data compiled from tabulated pharmacokinetic parameters with summary statistics of the individual study. Statistical analysis was done by statistical programs: Pharma/PCS Version 2.03, Pharmacologic Calculation System; Microsoft Excel 2002; Sigma Stat for Windows Version 2.03, The following paameters were included in the analysis: Cmax, tmax, Ka, Ke, AUC and AUC. Bioequivalence was conformed by Westlake and Hauck tests.

## Results and Discussion

*BIOEQUIVALENCE OF TWO SULPIRIDE-BASED PREPARATIONS*

Study of sulpiride was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of three 50 mg-capsules (150 mg of sulpiride in total).

**Table 1:** *Mean values of sulpiride in serum*

| Sampling time (h) | DRUG A capsules | DRUG A capsules | Comparative drug capsules | Comparative drug capsules |
|---|---|---|---|---|
| | 26 subjects group I | 12 subjects group II | 26 subjects group III | 12 subjects group IV |
| T (h) | Mean concentration (ng/mL) | Mean concentration (ng/mL) | Mean concentration (ng/mL) | Mean concentration (ng/mL) |
| 0.0 | 1.70± 02.46 | 2.94± 2.18 | 2.28±02.17 | 1.57±02.34 |
| 0.5 | 10.48± 8.67 | 8.25±13.44 | 11.13±14.18 | 10.95±09.55 |
| 0.75 | 39.62± 34.64 | 29.22±20.04 | 40.61±33.20 | 36.40±29.36 |
| 1 | 70.90± 48.30 | 64.37±36.48 | 72.66±51.96 | 67.94±37.32 |
| 1.25 | 105.96± 57.87 | 101.06±59.15 | 112.48±81.50 | 105.70±56.60 |
| 1.5 | 123.53± 69.48 | 137.99±71.47 | 135.64±86.34 | 126.63±79.66 |
| 1.75 | 153.88± 79.39 | 147.75±64.95 | 140.15±68.73 | 155.29±94.99 |
| 2.0 | 168.65± 79.56 | 168.02±73.79 | 160.42±72.33 | 163.80±72.23 |
| 2.5 | 186.08± 82.88 | 206.73±75.44 | 193.05±77.28 | 188.31±66.12 |
| 3.0 | 214.92±118.27 | 254.12±87.82 | 227.48±92.64 | 192.32±79.05 |
| 4.0 | 203.53± 81.08 | 225.65±64.98 | 206.62±76.54 | 198.00±68.78 |
| 6.0 | 134.91± 53.09 | 166.24±36.97 | 146.62±45.18 | 135.35±52.45 |
| 8.0 | 102.80± 44.50 | 124.74 ±16.85 | 113.96±26.76 | 99.99±38.99 |
| 12.0 | 70.04± 22.52 | 81.36±16.29 | 74.49±20.27 | 74.74±24.84 |
| 24.0 | 37.62± 11.31 | 45.80±20.73 | 42.03±15.68 | 37.84±11.28 |
| 36.0 | 22.95± 07.01 | 21.08±06.00 | 21.52±05.83 | 21.87±06.35 |
| Statistical significance (t-test) | n.s. | | | |
| 95 percent C.I. for difference of means group I/III : -54.423 to 47.727<br>95 percent C.I. for difference of means group I/II : -62.850 to 45.631<br>95 percent C.I. for difference of means group II/IV : -42.863 to 63.940<br>95 percent C.I. for difference of means group III/IV : -44.906 to 55.461 | | | | |

Subjects, aged from 18 to 48 years were screened for inclusion into the trial. In order to acomplish that 24 evaluated male subjects complete the

study, 26 subjects were included in the study. Sampling for the pharmacokinetic evaluation was performed according to the following schedule:pre-dose, 30 minutes, 45 minute, 2 hours, 2 hours nad 30 minutes, 3, 4, 6, 8, 12, 24, 36 hours post dose. The following parameters were calculated from the serum concentration: $C_{max}$,$t_{max}$, $\alpha$, $\beta$, $t_{1/2}$, KA, KE, AUC and $AUC_{\infty}$.

Statistical analyses did not detect any difference in mean plasma concentrations at any blood collection time point between two products when the sample size was 24 or 12 subjects (Table 1.).

The satistical analysis of non-tranformed data is shown in Table 2. The mean and standard deviation of both formulations in 24 and 12 subject groups were almost same. The t-test was also performed, showing no statistical difference.

**Table 2:** *Values of pharmacokinetic parameters detected in 26 and 12 subjects*

| **Pharmacokinetic parameters** | **DRUG A** capsules | **Comparative drug** capsules | **Statistical significance 12/6 subjects (t-test)** |
|---|---|---|---|
| | **26 subjects** | **26 subjects** | |
| **$C_{max}$ (ng/mL)** | 240±110 | 258±94 | |
| **$T_{max}$ (h)** | 3.0±0.7 | 3.1±0.7 | |
| **$K_e$** | 0.042±0.017 | 0.048±0.016 | |
| **$K_a$** | 0.343±0.150 | 0.347±0.144 | |
| **$AUC_{(0-\infty)}$ (ng/mL h)** | 3278±977 | 3225±813 | |
| **Statistical significance (t-test)** | n.s. | | |
| | **DRUG A** capsules | **Comparative drug** capsules | |
| **Pharmacokinetic parameters** | **12 subjects** | **12 subjects** | **n.s.** |
| **$C_{max}$ (ng/mL)** | 235±78.988 | 284±77.338 | |
| **$T_{max}$ (h)** | 2.9±0.85 | 3.0±0.86 | |
| **$K_e$** | 0.045±0.019 | 0.053±0.015 | |
| **$K_a$** | 0.329±0.171 | 0.338±0.1 | |
| **$AUC_{(0-\infty)}$ (ng/mL h)** | 3261±1140 | 3267±813 | |
| **Statistical significance (t-test)** | n.s. | | |

Mean resistance time and mean absorption time following the calculation of AUMC by trapezoid rule are shown in Table 3 and Table 4.

**Table 3:** *Values of AUMC calculated by trapezoid method*

| Sampling time | DRUG A capsules | | Comparative drug capsules | |
|---|---|---|---|---|
| | 26 subjects | 12 subjects | 26 subjects | 12 subjects |
| t (h) | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ |
| 0.0 | 1.310 | 1031 | 1.391 | 1.368 |
| 0.5 | 4.369 | 4.156 | 4.503 | 3.933 |
| 0.75 | 12.577 | 14.511 | 12.890 | 11.429 |
| 1 | 25.419 | 37.476 | 26.658 | 24.008 |
| 1.25 | 39.719 | 77.554 | 43.008 | 38.648 |
| 1.5 | 56.826 | 133.420 | 56.091 | 55.405 |
| 1.75 | 75.827 | 204.815 | 70.763 | 71.923 |
| 2.0 | 200.632 | 418.123 | 200.872 | 199.598 |
| 2.5 | 277.498 | 737.924 | 291.272 | 261.941 |
| 3.0 | 729.466 | 1570.411 | 754.467 | 684.000 |
| 4.0 | 1623.642 | 3470.463 | 1706.230 | 1604.100 |
| 6.0 | 1631.922 | 5465.851 | 1791.434 | 1612.036 |
| 8.0 | 3334.464 | 9414.531 | 3611.200 | 3392.000 |
| 12.0 | 10486.512 | 21869.307 | 1145.200 | 10830.672 |
| 24.0 | 10375.776 | 33019.587 | 10740.000 | 10174.248 |
| 36.0 | 42628.000 | 27110.500 | 26900.000 | 25712.359 |
| $AUMC_{0\to 36}$ (ng/mL)h$^2$ | 71503.959 | 60130.087 | 57665.979 | 54677.668 |
| $AUMC_{0\to\infty}$ (ng/mL)h$^2$ | 72050.482 | 70671.587 | 58114.312 | 65615.168 |

**Table 4:** *Mean absorption and mean resistance time in different sample sized groups*

| | DRUG A | DRUG A | Comparative drug | Comparative drug |
|---|---|---|---|---|
| | 26 subjects | 12 subjects | 26 subjects | 12 subjects |
| MRT (h) | 21.98 | 21.67 | 18.01 | 20.08 |
| MAT (h) | -1.82 | - 0.54 | -2.82 | - 1.21 |

Bioequivalence of drug A and comparative drug evaluated in 26 and 12 subjects is confirmed by Hauck test, Table 5.

**Table 5:** *Hauck test of bioequivalency*

| Sample size | Hauck test |
|---|---|
| 26 subjects | N-1=25<br>T = 3.154602 E-02<br>δ = 4.561751 E-03<br>p = 1.18047 E-04 |
| 12 subjects | N-1=11<br>T = .355591<br>δ= 2.956155 E-03<br>p = 8.257926 E-04 |

*BIOEQUIVALENCE OF TWO LISINOPRIL-BASED PREPARATIONS*

Study of lisinopril was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of 20 mg-tablet. 12 healthy subjects, aged from 24 to 35 years, were included in the study. Sampling for the pharamacokinetic evaluation was performed according to the following schedule: 0,5, 1, 1,5, 2, 3, 4, 6, 8, 12 and 24 hours post dose.

**Table 6:** *Mean values of lisinopril in serum*

| Sampling time (h) | DRUG B tablets | DRUG B tablets | Comparative drug tablets | Comparative drug tablets |
|---|---|---|---|---|
| | 12 subjects | 6 subjects | 12 subjects | 6 subjects |
| t (h) | Mean concentration (ng/mL) | Mean concentration (ng/mL) | Mean concentration (ng/mL) | Mean concentration (ng/mL) |
| 0.5 | 0.95± 0.33 | 1.11± 0.34 | 1.08± 0.31 | 1.07± 0.30 |
| 1.0 | 3.70± 1.76 | 4.39± 2.13 | 3.62± 1.56 | 3.63± 1.95 |
| 1.5 | 9.32± 5.06 | 11.28± 5.99 | 8.30± 5.49 | 9.46± 7.55 |
| 2.0 | 17.51± 8.06 | 20.30±10.24 | 15.62± 8.51 | 17.01± 1.33 |
| 3.0 | 37.19±13,.10 | 41.95±16.21 | 34.28±12.03 | 36.32±13.59 |
| 4.0 | 56.14±16.58 | 64.13±17.84 | 49.48±14.30 | 53.08±15.65 |
| 6.0 | 68.77±23.40 | 80.71±26.47 | 69.35±15.71 | 74.71±17.50 |
| 8.0 | 65.28±22.25 | 78.24±22.48 | 65.21±20.80 | 10.59±27.21 |
| 12.0 | 51.03±20.20 | 60.67±20.71 | 53.84±21.31 | 60.96±24.16 |
| 24.0 | 15.14±10.00 | 21.27±11.28 | 15.82±10.32 | 1.07± 0.30 |
| Statistical significance (t-test) | n.s. | | | |
| 95 percent confidence interval for difference of means I/II: -32.707 to 20.907<br>95 percent confidence interval for difference of means III/IV: -30.024 to 20.284<br>95 percent confidence interval for difference of means II/IV: -38.932 to 15.702<br>95 percent confidence interval for difference of means I/III: -23.755 to 25.445 | | | | |

Serum concentrations time profile (means ± SD) following intake of single oral dose is shown in Table 6. The serum concentration time curve was very similar after administration of the two preparations in different size groups.

The mean values (± SD ) of pharmacokinetic parameters for the detected evaluation of bioequivalence are shown in Tabele 7. There are no differences of statistical significance.

**Tabele 7:** *Values of pharmacokinetic parameters detected in 12 and 6 subjects*

| **Pharmacokinetic parameters** | **DRUG B** tablets | **Comparative drug** tablets | **Statistical significance 12/6 subjects (t-test)** |
|---|---|---|---|
| | **12 subjects** | **12 subjects** | |
| $C_{max}$ (ng/mL) | 70±23 | 71±18 | |
| $T_{max}$ (h) | 6.5±0.9 | 6.3±0.78 | |
| $K_e$ | 0.1±0.02 | 0.17±0.13 | |
| $K_a$ | 0.36±0.06 | 0.31±0.09 | |
| $AUC_{(0-\infty)}$ (ng/mL h) | 1190±498 | 1188±534 | |
| **Statistical significance (t-test)** | n.s. | | |
| | **DRUG B** tablets | **Comparative drug** tablets | n.s. |
| **Pharmacokinetic parameters** | **6 subjects** | **6 subjects** | |
| $C_{max}$ (ng/mL) | 82 ±25 | 79±20 | |
| $T_{max}$ (h) | 6.7±1 | 6.6±1.0 | |
| $K_e$ | 0.09±0.01 | 0.21±0.17 | |
| $K_a$ | 0.35±0.06 | 0.33±0.08 | |
| $AUC_{(0-\infty)}$ (ng/mL.h) | 1451±570 | 1470±623 | |
| **Statistical significance (t-test)** | n.s. | | |

Values of AUMC, MRT and MAT are shown in Table 8 and Table 9.

**Table 8**: *Values of AUMC calculated by trapezoid method*

| Sampling time | DRUG B tablets | | Comparative drug tablets | |
|---|---|---|---|---|
| | 12 subjects | 6 subjects | 12 subjects | 6 subjects |
| t (h) | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ | AUMC (ng/mL)h$^2$ |
| 0:5 | 1.045 | 1.17 | 1.039 | 1.04 |
| 1.0 | 4.422 | 5.261 | 4.016 | 4.447 |
| 1.5 | 12.253 | 14.379 | 10.923 | 12.046 |
| 2.0 | 73.284 | 83.231 | 67.044 | 71.488 |
| 3 | 168.027 | 191.185 | 150.384 | 160.641 |
| 4 | 637.08 | 740.79 | 614.004 | 660.61 |
| 6 | 934.8 | 1110.198 | 937.684 | 1028.558 |
| 8 | 2268.96 | 1707.8 | 2335.328 | 2623.6 |
| 12 | 5852.16 | 7430.328 | 6153.984 | 7302.528 |
| 24 | 5204.36 | 8722.05 | 2886.469 | 2804.97 |
| $AUMC_{0\to 24}$ (ng/mL)h$^2$ | 15156.391 | 21006.392 | 13160.875 | 14669.929 |
| $AUMC_{0\to\infty}$ (ng/mL)h$^2$ | 16700.268 | 23841.992 | 13746.69 | 15140.439 |

**Table 9:** *Mean absorption time and mean resistance time in different sample sized groups*

| | DRUG B tablets | DRUG B tablets | Comparative drug tablets | Comparative drug tablets |
|---|---|---|---|---|
| | 12 subjects | 6 subjects | 12 subjects | 6 subjects |
| MRT (h) | 14.03 | 16.43 | 11.57 | 10.3 |
| MAT (h) | 3.9 | 4.9 | 5.5 | 5.5 |

Bioequivalence of the products in differently sized groups is confirmed by Hauck and Westlake test, Table 10.

**Table 10:** *Test of bioquivalencies*

| Sample size | Hauck test | Westlake test |
|---|---|---|
| 12 subjects | N - 1 = 11<br>T = 8.168178 E-02<br>δ = 8.462206 E-03<br>p = 5.856753 E-04 | 2.397265 E-22.<br>6.082929E +24<br>Kan=3.948 |
| 6 subjects | N - 1 = 5<br>T = 0.2145618<br>δ = 5.280028 E-03<br>p = 9.949803 E-04 | 1,329071 E-33, 1.701412 E +38<br>Kan=4.893 |

Study of norfloxacin was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of 400 mg tablet. 23 healthy subjects were included in the study. Sampling for the pharmacokinetic evaluation was performed according to the following schedule: 0,5, 1, 1,25, 1,5, 1,75, 2, 2,25, 2,5, 3, 3,5,4, 6, 8, 10 and 12 hours post dose.

Serum concentrations time profile (means ±SD) following the intake of a single oral dose is shown in Table 11. The mean both preparation values, after their administrations in 24 and 12 subject groups, are similar.

**Table 11:** *Mean values of norfloxacin in serum*

| **Sampling time** | **DRUG C** tablets | **DRUG C** tablets | **Comparative drug** tablets | **Comparative drug** tablets |
|---|---|---|---|---|
| | **23 subjects** | **12 subjects** | **23 subjects** | **12 subjects** |
| **t (h)** | **Mean concentration (ng/mL)** | **Mean concentration (ng/mL)** | **Mean concentration (ng/mL)** | **Mean concentration (ng/mL)** |
| 0.50 | 187±180 | 145±105 | 362±366 | 270±229 |
| 1.00 | 528±331 | 546±329 | 686±519 | 572±375 |
| 1.25 | 733±442 | 890±468 | 829±550 | 815±505 |
| 1.50 | 886±551 | 1094±539 | 852±493 | 845±465 |
| 1.75 | 970±618 | 1258±630 | 886±484 | 957±480 |
| 2.00 | 985±599 | 1267±606 | 877±473 | 955±462 |
| 2.25 | 936±541 | 1192±505 | 863±492 | 958±492 |
| 2.50 | 924±492 | 1131±429 | 851±451 | 908±404 |
| 3.00 | 697±368 | 861±331 | 640±297 | 672±263 |
| 3.50 | 549±285 | 636±242 | 554±269 | 597±267 |
| 4.00 | 477±243 | 571±223 | 465±209 | 481±191 |
| 6.00 | 324±171 | 376±155 | 307±149 | 320±142 |
| 8.00 | 226±131 | 266±143 | 213±109 | 225±109 |
| 10.00 | 162±109 | 197±120 | 159± 90 | 164±89 |
| 12.00 | 123± 92 | 157±85 | 104± 87 | 122±86 |
| **Statistical significance (t-test)** | n.s. | | | |
| 95 percent confidence interval for difference of means I/II: -404.705 to 154.038<br>95 percent confidence interval for difference of means I/III: -223.797 to 231.664<br>95 percent confidence interval for difference of means II/IV: -161.178 to 391.311<br>95 percent confidence interval for difference of means III/IV: -238.083 to 209.683 | | | | |

The mean values (±SD ) of pharmacokinetic parameters for the evaluation of bioequivalence are shown in Table 12. There are no differences of statistical significance. The calculated AUMC, MRT and MAT values are shown in Table 14 and Table 13.

**Table 12:** *Values of pharmacokinetic paameters detected in 23 and 12 subjects*

| **Pharmacokinetic parameters** | **DRUG C** tablets | **Comparative drug** tablets | **Statistical significance 12/6 subjects (t-test)** |
|---|---|---|---|
| | **23 subjects** | **23 subjects** | |
| $C_{max}$ (ng/mL) | 1179±453 | 1204±453 | |
| $T_{max}$ (h) | 1.8±0.5 | 1.8±0.6 | |
| $K_e$ | 0.212±0.056 | 0.215±0.033 | |
| $K_a$ | 3.002±1.712 | 3.261±2.206 | |
| $AUC_{(0-\infty)}$ (ng/mL h) | 6244±3539 | 5399±2290 | |
| **Statistical significance (t-test)** | **n.s.** | | |
| | **DRUG C** tablets | **Comparative drug** tablets | |
| **Pharmacokinetic parameters** | **12 subjects** | **12 subjects** | **n.s.** |
| $C_{max}$ (ng/mL) | 1267±585 | 1202±329 | |
| $T_{max}$ (h) | 1.9±0.3 | 1.9±0.1 | |
| $K_e$ | 0.212±0.068 | 0.212±0.039 | |
| $K_a$ | 2.824±1.308 | 3.724±2.215 | |
| $AUC_{(0-\infty)}$ (ng/mL h) | 6941±3666 | 5219±2331 | |
| **Statistical significance (t-test)** | **n.s.** | | |

Bioequivalence of drug C and comparative drug, evaluated on 23 and 12 subjects was conformed by Hauck and Westlake test, Table 15.

**Table 13:** *Values of AUMC calculated by trapezoid method*

| Sampling time | DRUG C tablets | | Comparative drug tablets | |
|---|---|---|---|---|
| | 23 subjects | 12 subjects | 23 subjects | 12 subjects |
| t (h) | AUMC $(ng/mL)h^2$ | AUMC $(ng/mL)h^2$ | AUMC $(ng/mL)h^2$ | AUMC $(ng/mL)h^2$ |
| 0.50 | 155 | 155 | 217 | 177 |
| 1.00 | 181 | 207 | 215 | 199 |
| 1.25 | 281 | 344 | 289 | 286 |
| 1.50 | 370 | 480 | 354 | 368 |
| 1.75 | 458 | 592 | 413 | 448 |
| 2.00 | 510 | 317 | 462 | 508 |
| 2.25 | 552 | 689 | 509 | 553 |
| 2.50 | 1100 | 707 | 1012 | 1071 |
| 3.00 | 1003 | 1202 | 965 | 1026 |
| 3.50 | 957 | 1128 | 950 | 1003 |
| 4.00 | 3852 | 4540 | 3702 | 3844 |
| 6.00 | 3752 | 4384 | 3546 | 3720 |
| 8.00 | 3428 | 4098 | 3294 | 3440 |
| 10.00 | 3096 | 3854 | 2838 | 3104 |
| 12.00 | 9696 | 11377 | 8066 | 9678 |
| $AUMC_{0\to12}$ $(ng/mL)h^2$ | 32159 | 34075 | 26830 | 29427 |
| $AUMC_{0\to\infty}$ $(ng/mL)h^2$ | 32159 | 37153 | 26830 | 32200 |

**Table14:** *Mean absorption time and mean resistance time*

| Parameter | DRUG C tablets | Comparative drug tablets | DRUG C tablets | Comparative drug tablets |
|---|---|---|---|---|
| | 23 subjects | 12 subjects | 23 subjects | 12 subjects |
| MRT (h) | 5.149 | 5.352 | 4.968 | 6.168 |
| MAT (h) | 0.433 | 0.947 | 0.317 | 1.482 |

**Table 15:** *Test of bioquivalencies*

| Sample size | Hauck test | Westlake test |
|---|---|---|
| 23 subject | N-1=22<br>T = 4.469213E-02<br>δ = 9.98002E-04<br>p = 2.658665E-04 | 0, 5.393475 E + 37<br>Kan = 0.433 |
| 12 subjects | N-1=11<br>T = 0.7673818<br>δ = 5.867081 E-04<br>p = 0.658665 E-04 | 8.710414 E-39,<br>1.701412E + 38<br>Kan = 0.947 |

## Conclusion

On the basis of the above-presented statistical evaluation it is concluded that there are no significant differences in the bioequivalence final results between differently sized sample groups. Pharmacokinetic parameters in bioequivalence studies, when performed on healthy young volunteers and in previously determined conditions, are mostly affected by physic-chemical characteristitcs of the medicine, so significant differences in individual pharmacokinetics can not be expected. This hypothesis is confirmed by the results of our analysis. Considering that the number of dropouts determines sample size, the number of includede subjects has to be adjusted to the circumstances. In the situation when we have a good patient compliance and when severe adverse effects are not expected, the minimal sample size of 12 subjects (approved by FDA) is adequate. Our study results show that even a smaller number of subjects (6) included in cross-over study represents an adequate number. This hypothesis should be considered and explored further more for the reasons of importance of pharmacokinetics investigation costs decrement.


## Apstrakt

Na tržištu su dostupni mnogi lijekovi istog sastava proizvedeni od strane različitih proizvodžača. Studije bioekvivalence daju osnovne informacije o tome da li takvi lijekovi, primijenjeni u jednakoj dozi i u sličnim uvjetima imaju približno jednaku bioraspoloživost u organizmu. Da bi se minimizirali faktori koji nisu od strane lijeka, a utiču na njegovu bioraspoloživost, studije bioekvivalence se sprovode sa zdravim dobrovoljcima u *crossover* dizajnu i u kontroliranim uvjetima. U studije se po pravilu uključuju 24 zdrava dobrovoljca za koje je potrebno pojedinačno izvršiti dvadesetak analiza krvi ili ukupno oko 500.

Ovakva ispitivanja su važna za procjenu farmakokinetičkih karakteristika lijekova, ali su i izuzetno skupa, pogotovo u malim zemljama.

Na osnovu temeljnih odredbi bioraspoloživosti, a u cilju smanjenja (za naše uvjete veoma značajnog aspekta) cijene ovakvih istraživanja, postavili smo hipotezu da se studije bioekvivalence mogu sprovesti i sa manjim brojem ispitanika.

Ova teza je potvrdjena rezultatima naše analize. Rezltati su pokazali da čak i manji broj ispitanika (6), u *crossover* dizajnu studije, može biti adekvatan za sigurne studije bioekvivalence.

## Literature

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