

FAILURE OF STATISTICAL METHODS TO PROVE BIOEQUIVALENCE OF MELOXICAM DRUG PRODUCTS. I. PARAMETRIC METHODS

ANCA VĂTĂȘESCU¹, FLORIN ENACHE², CONSTANTIN MIRCIOIU¹, DALIA SIMONA MIRON¹, ROXANA SANDULOVICI³

¹*Carol Davila University of Medicine & Pharmacy, Bucharest*

²*Institute of Statistics, Romanian Academy, Bucharest*

³*Biopharmacy & Pharmacol Res S.A., Bucharest*

**corresponding author: ancavatasescu@yahoo.com*

Abstract

Establishing the bioequivalence (BE) and interchangeability of two drug products is a problem of building a confidence interval (CI) for the ratio of the means for the main pharmacokinetic parameters of the respective drug products.

The paper presents the case of a BE study concerning two suppositories formulations containing meloxicam. Although the conditions for application of parametric test were not fulfilled following a non-normal distribution of pharmacokinetic parameters of the reference drug, it were applied the "official rules" and it were calculated the 90 % confidence intervals for the maximum concentration (C_{max}) and the area under the curve (AUC). The result of the statistical test was the rejection of BE. But it appeared that the tested drug, at least from the fact that it was less variable, was superior to the reference drug. The fact that plasma levels of the tested drug were greater than plasma levels of the reference drug suggested rather a reduced release of active substance from reference formulation than a super-bioavailability. In this conditions, the rejecting of tested drug was considered both incorrect and unfair since the deficiencies appeared all to the reference drug. Simulations showed that BE could be proved only with a large number of subjects, which was considered a non-ethical approach. Application of "ethical" scaled criteria lead to the conclusion that the products are bioequivalent.

Rezumat

Stabilirea bioechivalenței (BE) și a interschimbabilității a două medicamente este problema construirii de intervale de încredere pentru raportul mediilor parametrilor farmacocinetici ai medicamentelor respective. Lucrarea prezintă cazul unui studiu de bioechivalență privind supozitoare cu meloxicam. Deși condițiile de aplicare a testelor parametrice nu au fost respectate din cauza distribuției non-normale a parametrilor farmacocinetici ai medicamentului de referință, s-au aplicat regulile oficiale și s-au calculat intervalele de încredere 90% pentru concentrația maximă (C_{max}) și aria de sub curbă (AUC). Rezultatul testelor statistice a fost respingerea bioechivalenței. S-a luat însă în considerare și faptul că medicamentul testat a fost superior celui de referință deoarece a fost mult mai puțin variabil. Nivelele plasmatice mai mari ale medicamentului testat față de cele ale medicamentului de referință au sugerat mai curând o eliberare redusă a substanței active din formularea de referință decât o suprabioechivalență. În aceste condiții respingerea medicamentului testat a fost considerată incorectă, deoarece deficiențele aparțineau toate medicamentului de referință. Simulările au arătat că BE ar fi putut fi demonstrată folosind un număr mare de subiecți, dar aceasta a fost considerată a fi o abordare non-etică. Aplicarea unor criterii scalate și etice a dus la concluzia că produsele sunt bioechivalente.

Keywords: meloxicam, bioequivalence, statistical methods.

Introduction

Health services and, particularly the drug supply for the population, entered in profound crisis following the exponential increase of the cost of drugs. The mean expenses associated with research, development and registration of a new drug increased to approximately one billion of dollars.

One method of decreasing the expenses with drugs, applied largely in the entire world, is the support of concurrence in the field of drug production which brings usually up to a tenfold decrease of prices. It is the method of allowing, after twenty years of protection of the innovations, the production of “generic” copies of drugs. The generic differs from the original drug only in what it concerns the inactive excipients and, theoretically and practically, has the same therapeutic effect. Since clinical testing of therapeutic equivalence would require hundreds of thousands subjects [15], all regulatory authorities accept bioequivalence (BE) as surrogate of clinical equivalence.

The scientific base of BE is the axiom that drugs are transported to their active sites in the body through blood. Consequently, if two drugs contain the same active substances which have the same rate and extent of absorption in blood they have the same activity. As measures of rate and absorption, Guidance's (EMA, FDA) [8,9] consider the maximum concentration ($C_{\max}$) and the Area Under the Curve (AUC or $AUC_{0-\infty}$). The United States legislation considers that two drugs, a reference (R) and a tested (T) one are BE if:

$$P\left(0.8 < \frac{\mu_{AUC}^T}{\mu_{AUC}^R} < 1.25\right) \geq 0.9$$

From this point the problem of establishing BE becomes a problem of building a confidence interval (CI) for the ratio of means of main pharmacokinetic parameters. The statistical model for the random variable ($AUC_{0-\infty}$ or $C_{\max}$) is:

$$Y_{ijk} = \mu + S_{ik} + P_j + F_{(j,k)} + C_{(j-1,k)} + e_{ijk}$$

where: μ = general mean, S_{ik} = the effect of the subject number “i” within the sequence number “k”, which, for the sake of testing hypotheses, we must assume to be a normally distributed random variable with mean 0 and variance σ_s^2 , P_j = the effect of the period number “j”, $F_{(j,k)}$ = the direct effect of the drug, $C_{(j-1,k)}$ = the residual effect of the drug,

ε_{ijk} = the random fluctuation which is normally distributed with mean 0 and variance σ_e^2 , and is independent of the S_{ik} .

The assumptions made about S_{ik} and ε_{ijk} imply that the variance of an observation is $\sigma_s^2 + \sigma_e^2$ and that two observations on an individual have covariance σ_s^2 . Observations made on different subjects are independent.

Since it was observed that the Y_{ijk} distribution is a log-normal distribution rather than normal, it was recommended to analyze data after logarithmic transformation.

If even after logarithmic transformation distribution remains far from normal, the use of non-parametric method of building the CI was recommended [9].

New EMEA guidelines [12] allow the enlargement of acceptance interval for highly variable drugs (HVD) but recommend to renounce to non-parametric methods in testing BE. This approach is not usual, since in the case of HVD it is clear that proving the BE would imply BE studies including hundreds of subjects, which is unacceptable from ethical reasons. A large category of drugs become “orphan”, following the unforgivable neglecting of many complex situations appearing in practice.

The present paper is a BE study case concerning suppositories and tablets formulations containing meloxicam where, even though active substance is not highly variable, following a high variability of the reference formulation, application of official rules lead to unethical rejecting of BE.

Materials and methods

Clinical bioequivalence study was standard two-periods, two-sequences study. The study was approved by the National Medicines Agency of Romania. The experiment started with 24 healthy volunteers. Following 6 drop-outs, only 18 evaluable subjects remained in the study.

The bioanalytical assay was performed using a high performance liquid chromatographic method developed and validated [14] in the *Biopharmacy and Pharmacol Laboratory*.

The pharmacokinetic and statistical analysis were performed using the software *Kinetica*.

Results and discussion

Mean plasma level curves are presented in Figure 1. The examination of the graphical representation of mean plasma levels reveals that the tested drug achieves concentrations greater than the reference drug.

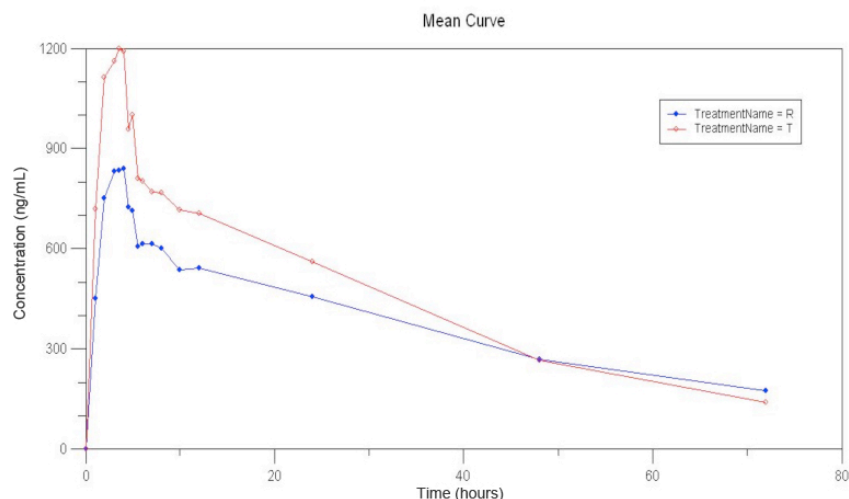


Figure 1

Mean plasma levels (n= 18) of tested and reference drug

A detailed analysis of the primary data – individual profiles, reveals an homogeneous distribution in space of plasma profiles in the case of tested drug and a clear splitting in three classes of reference curves (a subject with abnormally small values, five subjects with high values and the “homogeneous distributed” group), such a partition of curves cannot be considered as a normal situation.

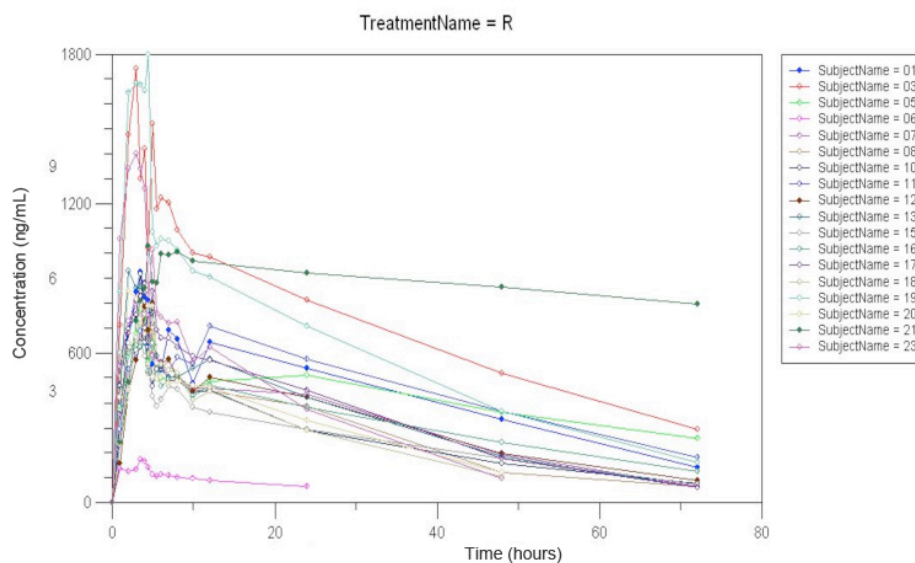
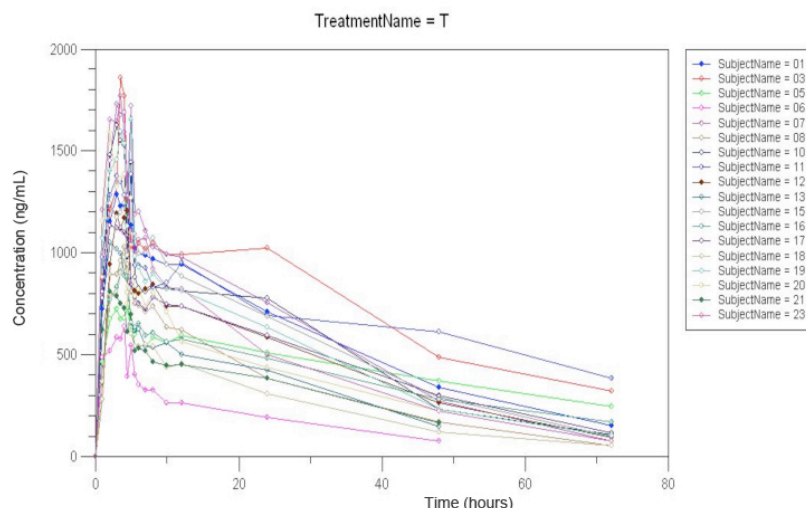


Figure 2

Individual concentration profiles for reference drug

**Figure 3**

Individual concentration profiles for tested drug

The statistical model applied according to the official guidances assumes a normal distribution of the pharmacokinetic parameters of subjects and equality of variabilities of reference and tested groups, and this requirements are clearly not satisfied. Although the conditions for applying the parametric test were not fulfilled, there were applied the “official rules” and there were calculated the 90 % confidence intervals for C_{max} and AUC. The results - Analysis of Variance (ANOVA) and calculus of confidence interval obtained by applying a “validated software” after logarithmic transformation of data are presented below (Table I).

Table I

Latin square design: ANOVA table for C_{max} .

Latin square with neperian logarithm option

SOURCE	D.F	SS	MS	F	P
Period	1	0.181	0.181	3.023	0.1013NS
Subject(Seq)	16	4.710	0.294	4.897	0.0014 ***
Formulation	1	1.227	1.227	20.428	0.0003 ***
Sequence	1	0.013	0.014	0.229	0.6387 NS
Error	16	0.961	0.060		
Total	35	7.09523			

	N	Mean	SD	SEM	GeoMean	Geo SD
Formulation: num = R	18	6.724	0.495	0.117	832	1.64
Formulation: num = T	18	7.093	0.317	0.075	1204	1.37

Root Mean Square Error = 0.245172 ; CV = 0.0354889

BIOEQUIVALENCE TESTS FOR Level R and level T

Reference Confidence Interval: [0.8, 1.25]

90% standard confidence interval (around the ratio:[test form]/[ref form])=[1.25, 1.67]

Cannot conclude equivalence.

Table II
Latin square design: ANOVA table for AUC_{tot}
Latin square with neperian logarithm option

SOURCE	D.F	SS	MS	F	P
Period	1	0.0003	0.0003	0.0011	0.9734 NS
Subject(Seq)	16	10.4047	0.6503	2.1264	0.07096 NS
Formulation	1	0.3202	0.3202	1.0469	0.3214 NS
Sequence	1	0.0100	0.0100	0.0328	0.8586 NS
Error	16	4.8931	0.3058		
Total	35	15.6284			

	N	Mean	SD	SEM	GeoMean	Geo SD
Formulation: num = R	18	10.27	0.8500	0.2003	29012.8	2.340
Formulation: num = T	18	10.46	0.4217	0.0993	35035.2	1.524

Geomean Ratio (Test/Reference) = 1.20

90% standard confidence interval (around the ratio:[test form]/[ref form])= [**0.87, 1.66**]

Cannot conclude equivalence.

It appears that the tested drug, at least from the fact that is less variable, is superior to the reference drug.

Intra and inter-variabilities for suppositories and tablets are presented in Fig. 4. In fact, since the experiments had only two periods, the resulted intra-variabilities estimated by the mean square error (MSE) from ANOVA, represent practically a “pooled” variability between tested and reference drugs.

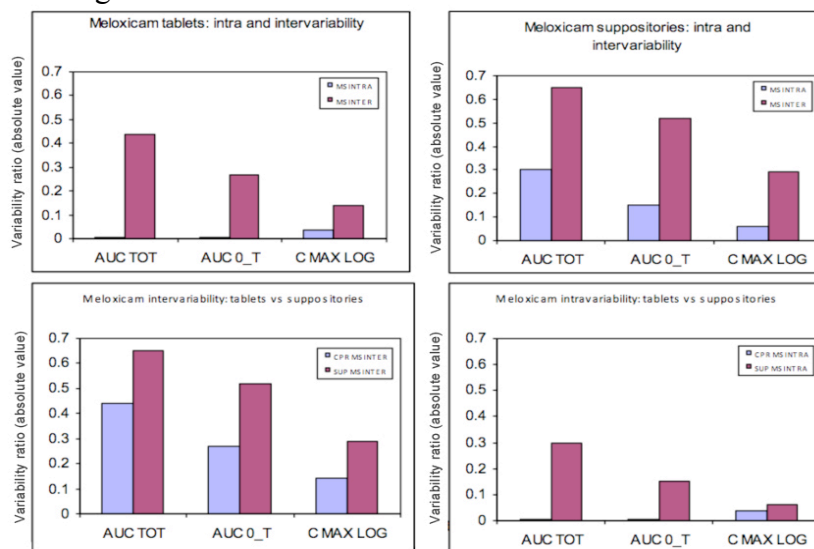


Figure 4

Comparison of intra and inter-variabilities in the studies concerning suppository and tablet formulations (MSINTRA – meloxicam suppositories intravariability, MSINTER – meloxicam suppositories intervariability, CPRMSINTER – meloxicam tablet intervariability, SUPMSINTER – meloxicam suppositories intervariability)

Inter-variabilities are greater than the estimated values, which is an expected result. Also, it can be seen that variabilities are much higher in the case of suppositories.

Intra-variability – which is determinant for BE - in the case of AUC_{tot} is approximately 30% at the limit between variable and “highly variable”. The variability of C_{max} is lower than that of AUC_{tot} , which is an unexpected result.

We cannot compare our data concerning intra-variability with literature data since, unfortunately, the published data is regarding only inter-variability.

In a paper concerning the BE of two tablets formulations with meloxicam, CV is 28% for AUC_{tot} and 16.6% for C_{max} . Our data – 40% for AUC_{tot} and 15% for C_{max} are in the same order, but CV for AUC_{tot} is significantly higher [5,14].

Lower variabilities for C_{max} arise from the fact that meloxicam is eliminated after metabolic transformations and suffers enterohepatic circulation [5,21]. Consequently, C_{max} is less defined. We have rather a plateau of maximum concentration [16,22,23].

The fact that plasma levels of the tested drug are greater than plasma levels of the reference drug raises questions regarding the reference drug. In these conditions, since the result of the test concerning bioequivalence is strongly dependent on the variability σ_e^2 , the rejecting of tested drug is both incorrect and unfair since the deficiencies belong to the reference drug. Decision concerning bioequivalence or non-bioequivalence being based on statistical calculus, a solution is to look for in this respect. Current FDA guidelines allow in such cases the use of alternative non-parametric methods.

Additionally, United States guidance and most of the statisticians recommend criteria scaled with the variance of reference drug for failure of proving BE clearly following reference drug deficiencies [2,3,4,6,10,13,17,18,19,20].

Another solution is, compatible with the proposed regulations, to increase the number of subjects.

The number of subjects required for proving BE with a patient risk α and a potency of the test $1 - \beta$ is given by

$$n \geq (t_{2n-2,\alpha} + t_{2n-2,\beta})^2 \frac{CV^2}{\Delta^2}$$

where CV is the coefficient of intra-individual variation and Δ is the per cent of clinical significant difference. In BE studies, α is taken 0.10, β usually is taken 0.20 and Δ is 20%.

In the case of HVD when the number of subjects is high, ($n \geq 30$) the following formula may be used:

$$n \geq (z_\alpha + z_\beta)^2 \frac{CV^2}{\Delta^2}$$

Since $z_{0.05} = -1.71$ and $z_{0.10} = -1.26$, replacing values in formula results in

$$n \geq \frac{2.97^2}{0.04} CV \cong 225 CV$$

The limit between normal and HVD is considered 30%.

For $CV = 30\%$ the results are: $n \geq 225 \times 0.09 = 20.25$

Since n is the number of subjects in one sequence, for the two sequences the number becomes 40, a high, but still acceptable number.

For $CV = 40\%$ i.e. our case for AUC_{tot} we obtain: $n \geq 225 \times 0.16 = 36$ and $2n = 72$

Considering the number of patients taken into study to be 72, this cannot be accepted due to ethical reasons. It is no more moral to imply suffering and risks to so many people only for respecting a rule which becomes essentially non applicable in such cases.

Since practically a maximum reasonable number in BE studies is 40, if we test the reference *versus* reference we have clearly bioequivalence but the calculus becomes as follows:

$$CI = \left(e^{\ln \bar{X}_R - \ln \bar{X}_R - t_{2n-2, 1-\alpha} \sqrt{MSE} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}, e^{\ln \bar{X}_R - \ln \bar{X}_R + t_{2n-2, 1-\alpha} \sqrt{MSE} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \right) =$$

$$= \left(e^{-t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}, e^{+t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \right)$$

where MSE is the mean square error from the ANOVA calculus. Replacement of MSE by CV is justified in Annex I.

Taking $CV = 40\%$ and $t_{2n-2, \alpha} \cong z_\alpha$ results in:

$$\text{For } 2n=36 \Rightarrow e^{-t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = e^{-1.28 * 0.3 * \sqrt{\frac{1}{18} + \frac{1}{18}}} = 0.88; e^{t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = 1.14 \Rightarrow$$

$$CI = (0.88; 1.14)$$

$$\text{For } 2n=18 \Rightarrow e^{-t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = e^{-1.28 * 0.3 * \sqrt{\frac{1}{9} + \frac{1}{9}}} = 0.83; e^{-t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = 1.20 \Rightarrow$$

$$CI = (0.83; 1.20)$$

i.e. even if we compare the drug with itself the chance to obtain non-bioequivalence is very high.

As it was presented before [1], in the case of highly variable drugs, when we compare two different drugs, we can accept BE if:

- the number of subjects is maximum acceptable from the point of view of the ethical committee;
- the calculated CV is $> 30\%$;
- $\overline{X}_T / \overline{X}_R \in [0.8; 1.25]$.

In our case, if we accept a maximum ratio for $\overline{X}_T / \overline{X}_R = 1.25$,

$$2n=18 \text{ and } CV=40\% \text{ we obtain } e^{\frac{\ln 1.25 + t_{2n-2, 1-\alpha} CV \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}{1}} = 1.77$$

In this conditions it appears that it would be possible to prove BE in our case without an increase of the number of subjects, if we would expected such an important intra-variability.

In the presented study the high variability was connected rather to the suppository base than to meloxicam. Unfortunately the intra-variability of drugs is not known. For the determination of intra-variability, replicated studies are required. In United States many specialists asked [1] FDA to impose as compulsory the publication of such data by innovator companies.

Instead of intravariability estimated from repeated administration of reference drug, it was proposed the use of MSE from two period experiment, which is in fact a pooled intravariability between reference and tested drug.

Conclusions

Starting from both $C_{\max}$ and $AUC_{0-\infty}$ data, official parametric methods for testing bioequivalence indicated that bioequivalence cannot be concluded for the tested and the reference meloxicam suppository formulations.

A global, biopharmaceutical evaluation suggests that, formulations could be bioequivalent but statistical tests, following problems of the reference drug, do not furnish a correct conclusion.

On other hand, the results of the parametric methods were questionable, since the assumption concerning normal distribution of data in the cases of $C_{\max}$ and $AUC_{0-\infty}$ of reference formulation and splitting of data in three classes was not fulfilled.

Simulations proved that application of ethical criteria connected with scaling and limitation of number of subjects prior to performance of the study would lead to the conclusion of bioequivalence.

Annex I

Estimation of variance in the case of logarithmic transformed data.

It was considered that initial data are normally distributed $X \in N(\mu, \sigma)$.

Based on properties of variation operator V we can write $V\left(\frac{X-\mu}{\mu}\right) = \frac{\sigma^2}{\mu^2} = CV^2$

If variation coefficient CV is small, then $\frac{X-\mu}{\mu}$ is small and

$$\ln X - \ln \mu = \ln\left(\frac{X}{\mu}\right) = \ln\left(1 + \frac{X-\mu}{\mu}\right) \cong \frac{X-\mu}{\mu}$$

Consequently, $\ln X \cong \ln \mu + \frac{X-\mu}{\mu}$, $E(\ln X) = \ln \mu$ and $V(\ln X) = CV^2$

It was obtained that, in the case of small CV $\ln X \in N(\ln \mu, CV^2)$

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