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GUIDE FOR CONDUCTING BIOEQUIVALENCE STUDIES FOR VETERINARY MEDICINES

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CONDUCTING BIOEQUIVALENCE STUDIES FOR VETERINARY MEDICINES

1. INTRODUCTION

In order to deliver an optimal therapeutic action, an active ingredient must be released at the site of action at an effective concentration during the intended period of time. To reliably predict the therapeutic effect of a drug, the performance of the dosing form containing the active ingredient must be suitably characterised.

Several therapeutic failures observed in the past associated with differences in bioavailability indicate the need to evaluate the performance of dosing forms in transporting the active ingredient to the systemic circulation, and from there to the site of action. Hence, the bioavailability of the active ingredient of a pharmaceutical product must be known and reproducible. If it is assumed that, in a given subject, a specific plasma concentration profile relative to time will result in essentially similar concentrations at the site of action as another drug and, therefore, will have an essentially similar effect, pharmacokinetic information can be used instead of therapeutic results to establish equivalence: bioequivalence.

In practice, evidence of bioequivalence generally constitutes the most suitable proof to support therapeutic equivalence between medicinal products. Therefore, reasonable evidence must be furnished in order to establish that the product studied is equivalent in therapeutic terms to the reference product.

It should be noted that current background information concludes that bioequivalence studies are generally not suitable to support a restriction period for use prior to slaughter, milking, or collection of eggs or honey. Residue depletion studies are very rarely covered adequately by a bioequivalence study, as is the case with the limit of quantification of the method, study duration, and statistical analysis of results. When these conditions are not met, studies will be required in addition to bioequivalence studies to confirm residue depletion in pharmacological medicines destined for food producing species.

The objective of this technical guide is to establish requirements for the design, execution and evaluation of bioequivalence studies. Addendum I envisages the possibility of using supplementary *in vitro* studies to demonstrate therapeutic equivalence.

This guideline, as every guideline, is not intended for establishing a mandatory or specific regulatory requirement. It is a tool created to allow, under the decision of the register owner and in consensus with the regulatory authorities, the use of this kind of tests to assure therapeutic equivalence, avoiding the unnecessary animal sacrifices.

2. DEFINITIONS

2.1 Pharmaceutical equivalent:

Two medicinal products are pharmaceutical equivalents when they contain the same quantity of the same active ingredient with the same salt or ester in the same pharmaceutical form, are destined for administration by the same route, and meet identical or comparable quality standards. However,

pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in excipients and/or in the manufacturing process may generate faster or slower dissolution or absorption, which may lead to differences in product behaviour (WHO).

2.2 Pharmaceutical alternative:

Two products are pharmaceutical alternatives when they contain the same molar quantity of the same active principle, but differ in terms of their pharmaceutical form (eg: capsule vs. pill) and/or chemical form (eg: different salts or esters). Pharmaceutical alternatives deliver the same active principle through the same route of administration, but are not pharmaceutical equivalents. They may, or may not, be bioequivalent to, or therapeutic equivalents of, the reference product.

2.3 Bioavailability:

Bioavailability refers to the rate and degree at which an active substance or its active ingredient is released from a pharmaceutical form and becomes available to the general circulation to exert an effect.

The bioavailability of a veterinary medicine is defined by the speed and magnitude at which the active substance reaches the systemic circulation and becomes available at the site or sites of action. The speed of absorption is measured in terms of the maximum plasma concentration obtained (Cmax), the time to reach maximum concentration (Tmax) and the area under the curve (AUC).

In most cases, substances have been developed to exhibit a systemic therapeutic effect. Therefore, a more practical definition can be provided to reflect that the substance in the general circulation is undergoing a dynamic exchange with the substance at the site of action.

It may be useful to distinguish between the "absolute bioavailability" of a given dosing form – as compared with the 100% bioavailability obtainable from the administration of an IV solution of the same drug (ex: oral versus IV solution), and the "relative bioavailability" – as compared with another form administrated via an extravascular route (ex: pills versus oral solution).

2.4 Bioequivalence:

Two medicinal products are bioequivalent when they are pharmaceutical equivalents or pharmaceutical alternatives to one another, and when their bioavailability (amount of active principle absorbed and speed of absorption) following administration at the same molar dose is similar to the extent that their effects in terms of efficacy and safety in the target species are essentially the same (and not necessarily similar in terms of safety in humans or for the environment). These products should be suitably labelled and manufactured in compliance with the prevailing Good Manufacturing Practices (CAMEVET GMP or WHO GMP).

Bioequivalence is said to exist between veterinary medicines when: following administration of the same molar dose using the same route of administration, under standardised experimental conditions, the speed of absorption and quantity of active substance absorbed differ only within pre-established limits.

The active substances to be compared must have similar physical and chemical properties, i.e. dissolution profile, crystalline form and particle size. In the case of active principles presented in a racemic mixture, these must display the same proportion of isomers.

2.5 Therapeutic equivalence:

A medicinal product is only considered a therapeutic equivalent of another medicinal product when they are **pharmaceutical equivalents or alternatives,** and it has the same quality and displays the same efficacy and safety - through *in vivo* or *in vitro* studies – as the reference product, whose efficacy and safety have already been established.

2.6 Reference product

A reference product is one whose quality, efficacy and safety have been established, assessed and approved by the competent health authority of the country where bioequivalence test is submitted to endorse a new register, a new administration way or a formulation change.

3. SCOPE AND OBJECTIVES OF BIOEQUIVALENCE STUDIES

Bioequivalence studies are valid scientific methods used to compare:

3.1 A significant change in formulation that may affect the bioavailability of the active principle. *When a change is made to the composition of a pharmaceutical form, these studies may be used to show that the new product is bioequivalent to the product used to carry out the clinical trials.*

3.2 Different routes of administration for the same product. *A product with a sole qualitative and quantitative formula may be applied using different routes of administration. Two routes of administration are bioequivalent when their plasma concentration profiles are similar within pre-established limits.*

3.3 Different veterinary medicinal products that are pharmaceutical equivalents. To avoid cruel and unnecessary safety and/or efficacy studies when bioequivalence can be demonstrated with another approved product for which these studies have already been carried out. *Ex: new product vs. reference product. When comparative reference is made to an approved product in terms of efficacy and/or safety, bioequivalence with that product must be demonstrated (where the regulatory authority accepts bioequivalence as a tool for product registration).*

Although certain *in vitro* equivalence studies exist which are sufficient in some cases to fulfil this objective, these studies most often apply to solid pharmaceutical forms (ex: pills). These studies are presented in Addendum I.

4. CASES THAT DO NOT REQUIRE *IN VIVO* BIOEQUIVALENCE STUDIES

Generally, *in vivo* bioequivalence studies are not required when a product meets one or more of the following conditions:

a) The product is manufactured as a solution for administration only via the **intravenous** route and contains the same active substance as a previously approved product for use via the same route in the same target species that is the subject matter of the new application.

b) The product is an oral dosage form designed not to be absorbed (ex: antacid, radiopaque medium).

c) The product meets all of the following conditions:

- It is an **oral solution**, syrup or other similar rapid-release and high-absorption solubilised form, or a solid pharmaceutical form whose rapid dissolution has been demonstrated previously, and which contains one or more highly soluble and high-absorption active principles (BCS - Biopharmaceutics Classification System).
- Contains an active substance in the same molar dose as the reference product.
- Has been shown not to contain inactive substances that could significantly affect the absorption of the active substance.

d) The product has been reformulated by the original manufacturer and is identical to the reference product except for colouring, flavouring and/or conserving agents, which have been shown not to have an effect on bioavailability.

e) Inhalation volatile anaesthetic solutions that contain the same active principle at the same dose.

f) Topical solutions indicated for obtaining local therapeutic effects. Other topical pharmaceutical forms for local use only in animals not destined for human consumption.

The fact that *in vivo* bioequivalence trials are not carried out does not imply that *in vitro* trials are not conducted.

5. SAFETY CONSIDERATIONS RELATING TO FOOD FOR HUMAN CONSUMPTION

In general, the fact that two formulations have been shown to be bioequivalent does not guarantee that they both need the same withdrawal period. Small variations in absorption at very low concentrations could produce significant differences in the elimination slope, which is used to determine the withdrawal period.

Consequently, a product may only be exempted from the requirement to submit a withdrawal period determination study when:

a. The method used to quantify the active ingredient in plasma has a quantification limit equivalent to or below half the MRL,

b. At least two determinations have been carried out at time points subsequent to the restriction period (withdrawal period) of the original product.

c. It is shown that there are no significant differences between the results obtained for the two products in these determinations.

In any other case, the submission of a bioequivalence study will not constitute an exemption from the requirement to conduct a withdrawal period determination study.

6. CRITERIA FOR EVALUATING BIOEQUIVALENCE STUDIES FOR PRODUCTS THAT CONTAIN HIGH VARIABILITY OR NARROW THERAPEUTIC INTERVAL PHARMACEUTICALS

In specific cases where the active principle of the product analysed offers a narrow therapeutic interval (NTI), in other words, where small variations in plasma levels can cause serious therapeutic failures (sub-therapeutic concentrations) or serious adverse reactions (supra-therapeutic concentrations), it is necessary to assess the need to narrow the bioequivalence acceptance intervals, for example: establishing a smaller AUC acceptance interval, generally of 90-110%. This would require clinical justification, because the dose-response curve displays a sharp slope, indicating that small changes in plasma concentrations generate significant variations in clinical results (ex: cyclosporine). This requires narrower acceptance limits in order to guarantee safety in the use of these drugs.

In the case of drugs with high intra-individual variability, i.e. with significant/major variability (CV ≥ 30%) in terms of the quantity and/or speed of absorption in a given individual, a broader interval could be accepted, but would require scientific justification based on safety and efficacy considerations. It should be noted that, for drugs with high variability in the Cmax parameter, it is recommended to plan a larger sampling quantity close to Tmax, in order to suitably characterize both the speed and quantity of absorption.

In both cases, therapeutic equivalence must be demonstrated through comparative *in vivo* studies.

7. DESIGN OF SINGLE DOSE BIOEQUIVALENCE STUDIES

Whenever possible, the products or routes of administration to be evaluated in the target animal species must be compared with a single dose *in vivo* bioequivalence study. Section 7 provides examples of situations where a multiple dose *in vivo* bioequivalence study may be necessary.

7.1 Reference product

Whenever bioquivalence tests are used to endorse the register of a new product, proposed as therapeutic equivalent of other one, the most suitable reference product is the first authorised product with a complete dossier. When there are several approved products with different labels, applications or target species, a bioequivalence study must be carried out with the reference product that has obtained approval for the same indications as those of the problem (or test) product.

The reference product must be taken from a valid batch of a product that has been approved in the country where registration of the drug is sought, which contains the same active substance as the new formulation, new dosing form or salt. For example, different esters of the same therapeutic entity are considered different products.

For a given product, a formulation can serve as a reference to show its bioequivalence with other formulations that formed part of the development process.

Reference Products or Comparators will be proposed by the study sponsor upon approval of the protocol, and defined by the health authority of the corresponding country.

7.2 Reference route of administration

The reference route of administration is the one used during clinical or toxicological trials, and the one used as a reference in terms of efficacy and safety.

7.3 Standards for test and reference pharmacological products

Both the test product and the reference product must be shown to meet all the standards included in compendia or other applicable standards relating to identity, concentration, quality and purity, and must comply with all the requirements of the Good Manufacturing Practices (CAMEVET GMP or WHO GMP).

7.4 Animals

Animals used in bioequivalence studies must be clinically healthy and form a homogeneous group (in terms of age, breed, weight, hormonal and nutritional status, production level, etc.). Wherever possible, it is recommended to restrict studies to the same sex when there is no evidence of interaction between sex and products. When it is difficult to maintain the homogeneity of all the animals included in a study (ex: horses), it will be acceptable to use non-homogenous cattle provided that the animals in each treatment group have been matched by age, weight, sex (where relevant), etc. This must be done using restricted randomization based on the relevant blocking factor(s).

The animals selected must belong to the target population for which the product is intended.

Group size: the appropriate number of animals must be estimated carefully and will depend on several factors, including variation in response, differences in the two formulations and level of rejection of the hypothesis. The cross-over study design offers advantages in terms of potency and number of animals required. It is recommended to use a minimum of 6 animals per group for cross-over study design, and 12 animals per group for the parallel group trial design.

7.5 Conditions of the trial

Bioequivalence must be carried out in compliance with the requirements of Good Clinical Practices (GCP) and Good Laboratory Practices (GLP).

For products administrated via the oral route, special attention must be paid to the different factors known to affect the arrangement of the active substance. The administration of food can improve or interfere with the absorption of the drug, depending on the characteristics of the drug and the formulation. Feed intake can also increase inter- or intra-subject drug absorption speed and magnitude variability. The protocol must include justification for conducting a bioequivalence study with unfed or fed subjects. The protocol must describe the diet and eating schedule to be followed during the study. For all species, the prandial state and exact time of feeding must be in line with animal welfare considerations (for example, ruminants must not be subjected to fasting), and with the pharmacokinetics of the active principle. Studies concerning drugs for canines and felines for oral administration must be carried out using unfed animals, unless the reference product label indicates that the product must be administrated only after being fed. Animals must have remain unfed for 8 hours before being dosed and 4 hours after the administration of the drug. For prolonged release oral medication indicated for non-ruminants, bioequivalence studies must be carried out in fed and unfed state, unless another condition is duly justified. The protocol must contain the rationale for carrying out the bioequivalence study in unfed or fed animals, and must describe the diet and feeding schedule.

If the reference product label indicates that the product must be administrated only to unfed animals or fed animals, the bioequivalence study must be carried out following the same indications relating to feed.

7.6 Dose to be tested

The approved dose must be used, and must be effective.

When several doses have been approved for the reference product, the bioequivalence test must be carried out using the highest dose.

7.7 Sampling

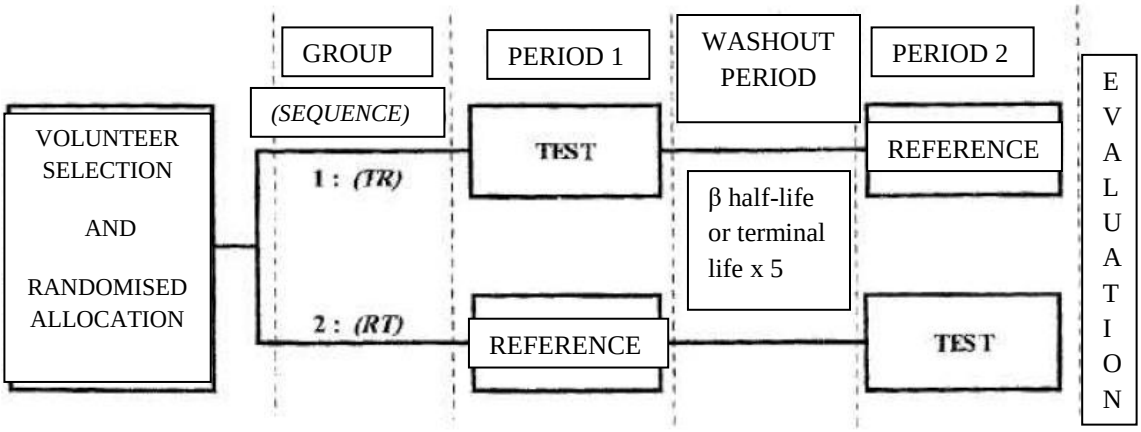
The concentrations of active principle and/or its active metabolites can be determined in biological samples such as blood, serum, plasma and other biological fluids (ex: milk, urine).

Sampling must be carried out so as to suitably measure Cmax and AUC. Measurements must include at least 2 points before Cmax, 2 to 3 points around Cmax, and 3 to 4 points during the active principle elimination phase.

7.8 Experimental design

The design of bioequivalence studies must seek to reduce to the greatest extent possible any variability not associated with the formulations studied - test (T) and reference (R). Generally, a two-sequence (TR/RT), two-period (Period 1/Period 2), two treatment, balanced, non-replicated, randomised cross-over design is used for bioequivalence studies, with a single dose in each period. All animals included in the study (equal number in each sequence) must receive the two treatments - T and R. This design avoids possible confusion between treatment effects and period.

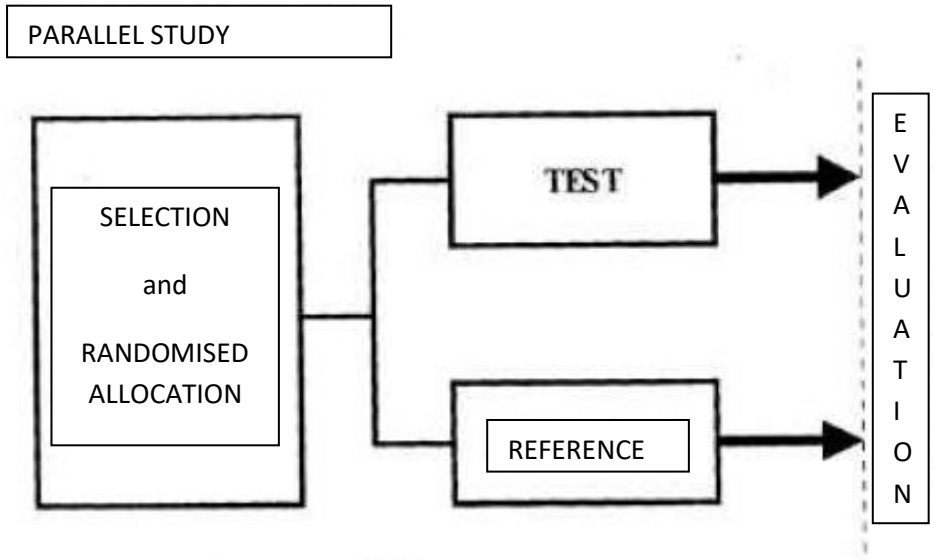
The time elapsed between the administration of each dose of T or R formulation is called the washout period, and must be sufficiently long to ensure that no concentration of the active principle administered in the first period is detected at the time of the second administration, or that any concentration detected is sufficiently low to have no pharmacokinetic impact on the new administration. The classical cross-over design is illustrated in the figure below.



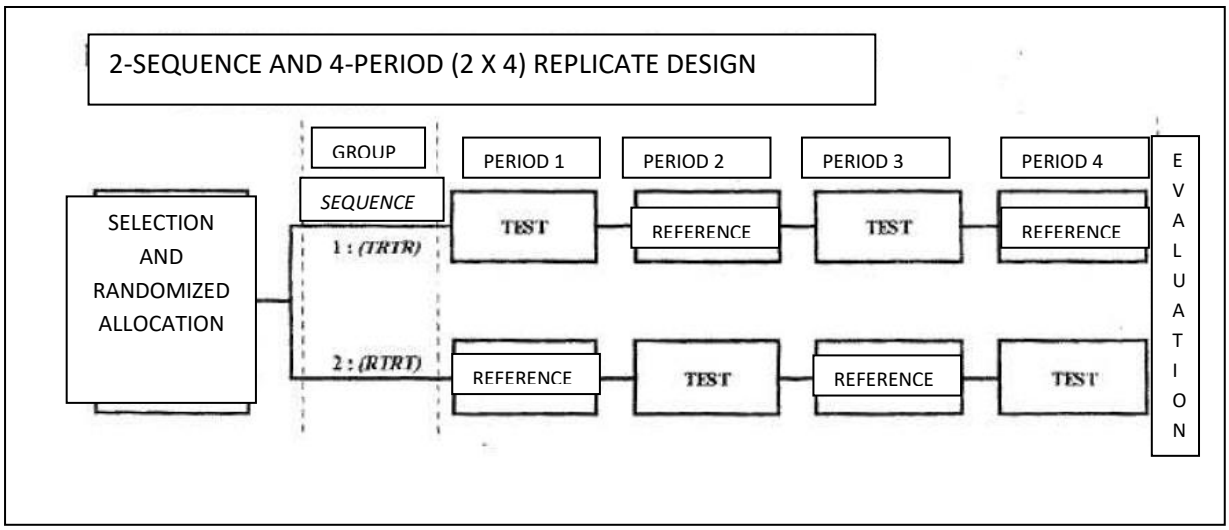
The washout period must be similar in all the animals, and its duration must be at least ten times the elimination half-life of the active substance or its metabolites. An additional period of time may be

required to ensure the disappearance of any pharmacological effect, such as the induction of microsomal enzymes.

If the washout period is not compatible with a classical cross-over design, as is the case with drugs with an extended half-life, or when studies must be carried out in growing animals, a parallel design may be used comprising two groups with an identical number of animals (group 1 and group 2), where one group receives only one dose of a different product from the one assigned to the other group. The parallel design is illustrated in the figure below.



When formulations contain an active principle with high pharmacokinetic variability (CV ≥ 30%), and a short elimination half-life, a possible model is a two-sequence, four-period replicate study design, where: Sequence 1: TRRT, and Sequence 2: TRRT. Figure 4 of the annex illustrates the two-sequence, four-period replicate design.



7.9 Sample size

The number of animals needed to carry out a bioequivalence study is determined according to the level of significance established, the difference expected to be detected, the expected potency of the trial, and the variation error associated with the primary characteristic to be studied expressed as intra-individual CV. The value of the intra-individual CV can be obtained from the results of a pilot study, the results of studies carried out previously, or from data contained in publications.

The number of animals must be calculated using appropriate methods and must not be less than 6 animals per group for a cross-over study design and no less than 12 animals per group for a parallel study design.

The method for calculating the number of animals for a multiplicative model (natural log-transformed data) is presented in equation 1 of the annex. This method of calculation allows the estimation of the number of individuals for a classical cross-over design based on several CV values, values for the ratio of the geometric means (μ_T/μ_R) of the pharmacokinetic parameters used, and of the potency expected from the statistical method (1-β). For a parallel design, this value must be multiplied by 2.

The statistical test for showing bioequivalence must display a potency of no less than 80%, with a risk to the consumer of 5% (α risk; 0.05) and a risk for the pharmaceutical industry of 20% (β risk; 0.20). Since potency is estimated as 1-β, the risk for the pharmaceutical industry can be reduced by increasing the potency of the statistical test. This is achieved by increasing the number of animals included in the study. Table 1 of the annex presents the number of individuals needed to carry out a bioequivalence study for various potency values of the statistical test, different CV values, and different ratios of the geometrical means of the fundamental pharmacokinetic parameters.

Study sponsors must select a suitable number of subjects taking into account possible losses or withdrawals from the study. Since the replacement of animals during the study can hinder the model and the statistical analysis, it is generally recommended not to replace losses. Therefore, it is recommendable to recruit a greater number of animals than required for the study based on the sample size calculation.

7.10 Considerations relating to sampling time

Sampling times must be selected in order to describe, to the extent possible, the active principle plasma concentration profile and allow an accurate determination of Tmax and Cmax.

To maximize sampling time efficiency, a pilot study may be necessary to help identify the shape of the concentration/time curve and the probable variability in concentration values.

7.11 Analysis

The analytical methods used in bioequivalence studies must be fully validated in order to meet the standard validation criteria set forth in the Guidance for Industry, Bioanalytical Method Validation, FDA, Guidance for bioanalytical method of validation, EMEA, or the CAMEVET Guide for validation of residue studies.

8. DESIGN OF MULTIPLE DOSE BIOEQUIVALENCE STUDIES

8.1 Basic Principles

In some cases, it is necessary to compare the Test product with the Reference product after repeated administration in order to determine plasma concentrations during the stationary equilibrium state. This

may be the case with very potent active principles that cause pharmaceutical effects at very low plasma concentrations that are below the resolution of the analytical technique. This occurs very occasionally thanks to new developments in analytical techniques.

A multiple dose study is required in the following cases:

a) When the product´s action depends on the active ingredient plasma concentrations in the stationary state.

b) When the active principle displays non-linear and/or time-dependent kinetics.

c) When the concentration of the active substance following a single dose is too low to be determined accurately using the analytical method.

d) For prolonged-release pharmaceutical forms with a tendency to accumulation.

8.2 Reference product and experimental conditions

As stipulated previously.

8.3 Dose

Dose selection for Test and Reference products will be defined as set forth in point 5.6.

8.4 Frequency of administration

The frequency of administration that results in the highest concentrations of the drug in stationary state (Css) must be selected. This can be determined through a pilot study.

8.5 Sampling

Samples must be taken to establish that stationary equilibrium conditions have been achieved (ex: by measuring two or more maximum (Cmax) or minimum (Cmin) blood, plasma or serum concentrations, or by collecting approximately 10 blood samples (including immediately prior to administration of the following dose) during the dosing interval.

Blood samples must be taken with sufficient frequency to suitably assess Cmax, AUC, Cmin and other parameters. Experimental sampling points must include a pre-dosing sample, at least 1 or 2 time points before Cmax, 2 sampling points close to Cmax, and 3 to 4 sampling points during the elimination phase.

8.6 Experimental design

Bioavailability can be determined in a state of stationary equilibrium without requiring a washout period between the administration of the Test and Reference formulations.

This type of trial comprises a single group of animals, and the two formulations - Test and Reference – are administrated to each animal using a pre-established interval between doses, until the stationary equilibrium state is reached (E_{ss}).

The number of doses required to reach E_{ss} is given by the time established as the interval between doses (τ) and the elimination half-life of the formulation. It is accepted that E_{ss} is reached when the pre-established doses have been administrated during a period of time equivalent to 4-5 times the value of the formulation´s elimination half-life. Under these conditions, the AUC estimated based on administration carried out after reaching E_{ss} ($AUC_{R,SS\ 0\text{-}\tau}$) is equal to the one that would be estimated following administration of a single dose of the formulation ($AUC_{R\ 0\text{-}\infty}$). Following the administration of the last dose of Reference formulation, the Test formulation begins to be dosed at the pre-established intervals during the time required to reach a new E_{ss}. Once this condition is reached, the AUC obtained following the administration of the last dose of Test formulation ($AUC_{T,SS\ 0\text{-}\tau}$) is estimated. An illustration of the experimental design for demonstrating bioequivalence through the administration of multiple doses is provided in Figure 5 of the annex.

9. STATISTICAL ANALYSIS OF BIOEQUIVALENCE TRIALS

9.1 Pharmacokinetic parameters to be analysed

The pharmacokinetic parameters derived from the concentration curves of the active principle in the biological matrix used for making the determination must be analysed. To avoid any possible bias, the calculation of the fundamental parameters must be based on the experimental data observed, avoiding the use of data estimated using any mathematical procedure. Exceptionally, the use of data estimated through pharmacokinetic modelling, interpolation or other procedures must be suitably justified for their inclusion in the bioequivalence study, and calculation methods must be defined previously in the study protocol.

There are numerous situations in which the data obtained for a given animal during a drug bioequivalence study may be eliminated in full or in part. Technical justification must be provided in the study for this type of elimination.

The repetition of certain circumstances may require these to be stipulated in the study protocol. For example: loss of dose administered due to regurgitation by the animal. In these cases, criteria for data elimination must be specified previously in the study protocol. Additionally, data elimination must be evaluated in these cases, taking into account factors such as:

- Acceptable time elapsed between drug administration and regurgitation event.
- The amount of material lost (food with drug) is considered relevant to the study.

Additionally, if an animal is re-dosed following a loss event, criteria for re-dosing must be clearly established in the study protocol.

Lastly, it is important to include all available data in the statistical analysis.

9.2 Single dose studies

In single dose studies, the essential parameters for demonstrating bioequivalence are: area under the plasma drug concentration-time curve (AUC) and maximum plasma concentration observed (C_{max}).

The AUC value must be calculated using plasma concentration data observed using the linear trapezoidal method.

The AUC value may only be used in the study if the estimated AUC from zero time to the time of the last plasma concentration measurement was observed (AUC_{0-tz}) is equal to or greater than 80% of the AUC extrapolated to infinity ($AUC_{0-\infty}$).

The values for C_{max} observed will only be useful for estimating bioequivalence if they are clearly defined and have been determined with relative accuracy. This is achieved through appropriate sampling times in the region of maximum probability of appearance of the peak plasma concentration, determined based on a pilot study or from data available in literature.

Other complementary parameters can be calculated and included in the study to provide additional information on the pharmacokinetic behaviour of the products to be tested, such as time at which the maximum plasma concentration is observed (T_{max}), area under the first moment curve (AUMC), mean residence time (MRT), and apparent elimination half-life ($t_{1/2el}$).

T_{max} is derived from the speed of absorption and elimination constants. This value is useful when the same considerations are applied for C_{max}. However, T_{max} is less robust than C_{max} because it quantifies a discrete variable (sampling times) whose values were pre-established in the experimental design. Consequently, it is included in the group of complementary parameters.

The AUMC is a pharmacokinetic parameter with no direct interpretation, however its calculation is mandatory in order to estimate the MRT, and therefore, its values may be included in the study.

MRT may be used as a complementary variable when it reflects mean absorption time (MAT). MRT can only be used when it has been determined following IV administration in the same animals.

If the design requires biological matrices other than plasma, justification must be provided for the parameters selected.

9.3 Multiple dose studies

The essential pharmacokinetic parameter for the determination of bioequivalence in multiple dose studies is the area under the curve in a state of equilibrium between administrations ($AUC_{0-\tau}$).

Average concentration in stable state (estimated as $AUC_{0-\tau}$ relative to the interval between administrations (τ) ($AUC_{0-\tau}/\tau$)), and the fluctuation range between maximum concentration and minimum concentration observed once a state of stationary equilibrium has been reached (C_{max} - C_{min}), may be considered as supplementary parameters.

9.4 Criteria for determining bioequivalence (bioequivalence interval)

Criteria must be selected prior to the commencement of the experiment and described in the protocol. The bioequivalence interval must be justified in relation to the expected clinical or pharmacological effects.

To establish that two formulations are bioequivalent, the 90% confidence interval must be established (IC90%) as a ratio of the geometric means (μ_T/μ_R) for AUC and Cmax, and must be shown to fall within an interval whose lower and upper limits are 0.80 and 1.25.

In specific cases where the active principle of the test product has a narrow therapeutic interval, as is the case with compounds with sharp dose-response curves (with large variations in small time intervals), the limits should be narrower.

To demonstrate bioequivalence between products under research whose active principles have a broad therapeutic interval, limits may be extended to 0.7 -1.43. This is very frequent in the case of C_{max}. The use of these extended limits must be based on clinical evidence and specified in the protocol.

9.5 Data analysis

Detailed data analysis must be furnished. A variance analysis must be carried out (including formulation, period, sequence, sequentially nested animals, and, where applicable, effect of sex per formulation) to estimate the variation risk, which will be used subsequently to calculate the confidence interval. For AUC and C_{max}, before conducting a variance analysis, log transformation of data is recommended. Transformation does not apply to time-dependent parameters observed; in this case, a non-parametric approach may be better. To conclude the bioequivalence analysis, the upper and lower limits of the confidence interval -calculated with the estimated variance error- must be compared. These are contained in the Analysis of Variance tables (ANOVA), with the predetermined limits, i.e. 0.8 to 1.25 or 0.7 to 1.43 for log-transformed data, or 0.8 to 1.2 or 0.7 to 1.3 for untransformed data.

If an effect is detected in the sequence, the first period of the cross-over design must be analysed as a parallel design.

When several criteria are used to demonstrate bioequivalence (which is generally the case), the final conclusion in favour of bioequivalence is only reached if the null hypothesis of non-equivalence is rejected for all the relevant parameters.

Other validated and duly justified statistical analysis techniques may also be used.

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ANNEX TO THE GUIDE FOR CONDUCTING BIOEQUIVALENCE STUDIES FOR VETERINARY MEDICINES: GRAPHS AND TABLES.

Figure 1. Diagram showing the rationale for studies to demonstrate bioequivalence between a Reference formulation and a Test formulation. If the two formulations are pharmaceutical equivalents, and have a similar bioavailability (active principle speed of absorption and quantity absorbed) following administration at the same molar dose within pre-established limits, it is assumed that their effects in terms of efficacy and safety are the same.

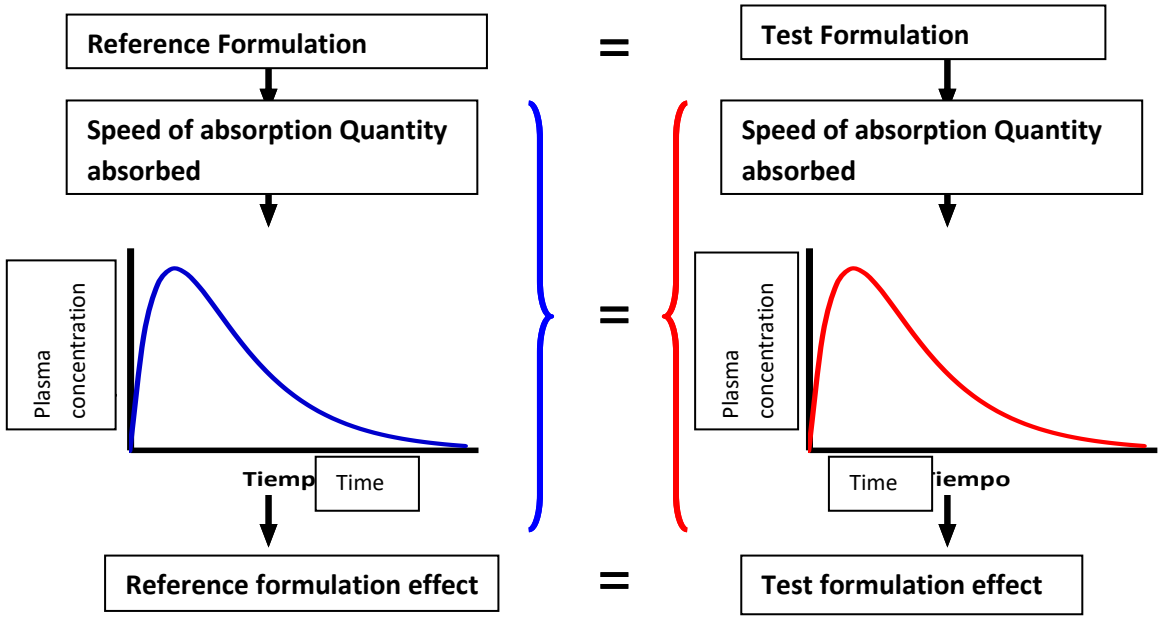


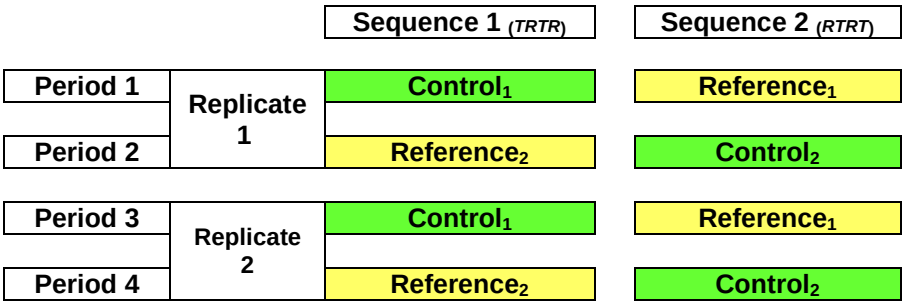
Figure 2. Diagram illustrating the two-sequence (TR/RT), two period (Period 1/Period 2), two treatment (Reference and Test) randomised, non-replicate, balanced, experimental cross-over study design with a single dose in each period.

	Sequence 1 *(TR)*	Sequence 2 *(RT)*
Period 1	$Control_1$	$Reference_1$
Period 2	$Reference_2$	$Control_2$

Figure 3. Diagram illustrating a parallel experimental design. This design comprises two groups (group 1 and group 2), each with the same number of animals, where one group receives a single dose of a different product from the one assigned to the other group.

Group 1	Group 2
Control	Reference

Figure 4. Diagram illustrating of a two-sequence, four period, replicate experimental design.



Equation 1. Algorithm proposed by D. Hauschke & coll. (1992) to estimate the number of individuals needed to carry out an average bioequivalence study.

$$If \; 1 < \mu_T/\mu_R < \Theta_S, \; then \; N \geq \left[t_{2N-2}^{1-\alpha} + t_{2N-2}^{1-\beta}\right]^2 \left[\frac{CV}{ln\Theta_S - ln(\mu_T/\mu_R)}\right]^2$$

$$If \; \Theta_I < \mu_T/\mu_R < 1, \; then \; N \geq \left[t_{2N-2}^{1-\alpha} + t_{2N-2}^{1-\beta}\right]^2 \left[\frac{CV}{ln\Theta_I - ln(\mu_T/\mu_R)}\right]^2$$

where, μ_R y μ_T are the geometric means of the pharmacokinetic parameters for the Reference and Test formulations, respectively, $ln\Theta_S$ and $ln\Theta_I$ are the natural logarithms of the upper and lower limits to demonstrate bioequivalence, CV is the inter-individual variation coefficient, t is the statistical value of the unilateral test for t, α (0.05) and β (0.20) is the consumer risk (5%) and the pharmaceutical risk (20%), $2N$-2 is the degree of freedom for a classical cross-over experimental design - in a parallel design this value must be replaced by N-1.

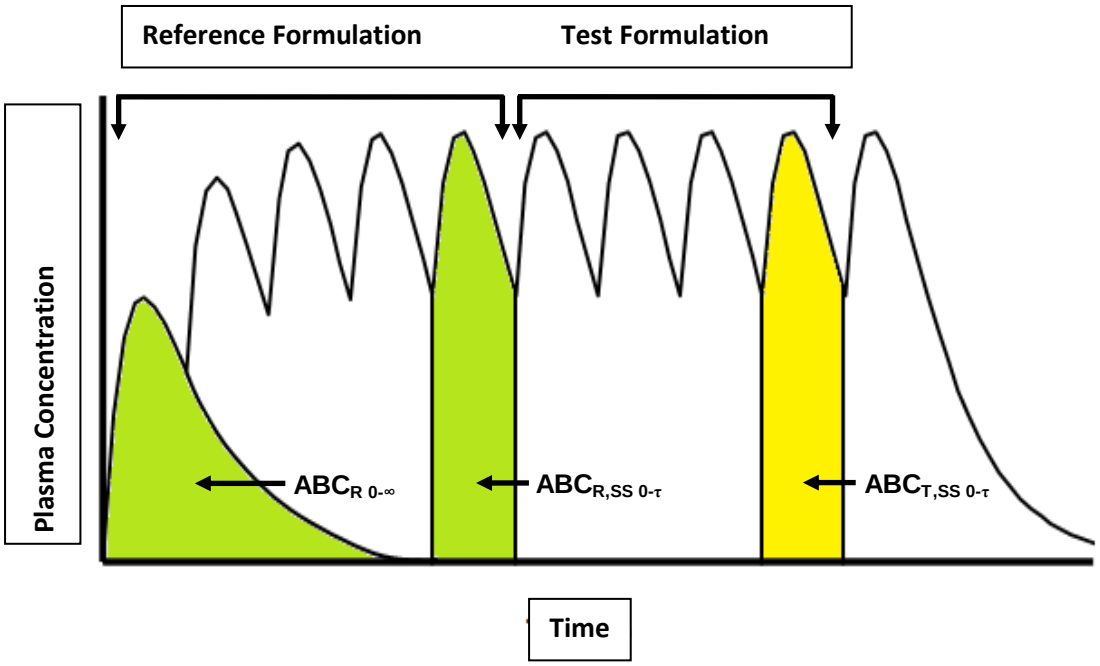
Table 1. Sample sizes (number of individuals) for obtaining 70%, 80% and 90% statistical potency, and various values of inter-individual variation coefficients (CV%) when a multiplicative model is applied to demonstrate bioequivalence, where; $\alpha = 0.05$ (5%), $\Theta_I = 0.8$ and $\Theta_S = 1.25$. Non integers have been rounded off to the next highest figure and are presented in italics.

CV (%)	Potency (%)	μ_T/μ_R 0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20
5.0	70	*10*	6	4	4	4	4	6	16
7.5		16	6	6	4	6	6	10	34
10.0		*28*	*10*	6	6	6	8	16	58
12.5		42	*14*	8	8	8	*12*	*24*	*90*
15.0		*60*	*18*	10	*10*	10	*16*	32	128
17.5		*80*	22	12	*12*	12	*20*	*44*	172
20.0		102	*30*	*16*	*14*	*16*	*26*	*56*	*224*
22.5		128	*36*	*20*	16	*20*	30	*70*	*282*
25.0		*158*	*44*	*24*	20	22	*38*	84	344
27.5		*190*	*52*	*28*	*24*	26	44	*102*	414
30.0		*224*	60	*32*	*28*	*32*	52	*120*	*490*
5.0	80	*12*	6	4	4	4	6	*8*	*22*
7.5		*22*	*8*	6	6	6	*8*	12	44
10.0		*36*	*12*	*8*	6	*8*	10	20	76
12.5		54	16	*10*	8	*10*	14	30	*118*
15.0		*78*	22	12	10	12	*20*	*42*	*168*
17.5		*104*	*30*	*16*	*14*	*16*	*26*	56	226
20.0		134	*38*	*20*	16	18	32	*72*	*294*
22.5		168	46	*24*	*20*	*24*	*40*	90	368
25.0		206	56	28	*24*	*28*	48	110	452
27.5		*248*	*68*	*34*	*28*	*34*	*58*	132	*544*
30.0		292	*80*	*40*	32	38	*68*	*156*	*642*
5.0	90	14	6	4	4	4	6	8	28
7.5		28	*10*	6	6	6	8	16	60
10.0		48	14	8	8	8	*14*	26	104
12.5		74	*22*	*12*	*10*	*12*	18	40	*162*
15.0		106	*30*	*16*	12	*16*	*26*	*58*	*232*
17.5		142	*40*	20	*16*	*20*	34	76	312
20.0		*186*	50	*26*	*20*	24	*44*	*100*	*406*
22.5		232	*64*	*32*	*24*	30	54	124	*510*
25.0		284	*78*	*38*	28	36	*66*	*152*	*626*
27.5		342	92	44	34	*44*	78	*182*	*752*
30.0		*404*	108	52	*40*	*52*	92	214	888

Table 2. Sample sizes (number of individuals) for obtaining statistical potency of 70%, 80% and 90% and various values of inter-individual variation coefficients (CV%) when a multiplicative model is applied to demonstrate bioequivalence, where; $\alpha = 0.05$ (5%), $\Theta_I = 0.7$ and $\Theta_S = 1.43$. Non integers have been rounded off to the next highest figure and are presented in italics.

CV	Potency	μ_T/μ_R												
(%)	(%)	0.75	0.80	0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20	1.25	1.30	1.35
15.0	70	46	14	8	6	6	6	6	6	8	10	14	26	68
20.0		80	24	12	8	8	8	8	8	10	14	24	44	118
25.0		122	34	18	12	10	10	10	12	14	22	34	66	180
30.0		172	48	24	16	12	12	12	14	20	30	48	94	256
35.0		230	64	32	20	16	16	16	20	26	38	64	124	342
40.0		296	80	40	26	20	20	20	24	32	48	80	160	438
45.0		366	100	48	30	24	24	24	28	40	60	100	198	544
50.0		444	120	58	36	30	28	30	34	48	72	120	238	658
55.0		524	142	68	42	34	32	34	40	56	84	142	282	780
60.0		610	164	80	50	40	38	40	46	64	98	164	328	906
15.0	80	60	18	10	8	6	6	6	8	8	12	18	34	88
20.0		104	30	16	10	8	8	8	10	12	18	30	56	154
25.0		160	44	22	14	12	10	12	14	18	28	44	86	236
30.0		226	62	30	20	16	14	16	18	26	38	62	122	336
35.0		302	82	40	26	20	18	20	24	32	50	82	162	448
40.0		388	106	52	32	24	22	24	30	42	62	106	208	576
45.0		482	130	62	38	30	28	30	36	50	78	130	258	714
50.0		582	158	76	46	36	32	34	44	62	94	158	312	864
55.0		688	186	90	54	42	38	40	50	72	110	186	370	1022
60.0		802	216	104	62	48	44	46	58	84	128	216	430	1190
15.0	90	82	24	12	8	8	6	8	8	10	16	24	46	122
20.0		144	40	20	14	10	10	10	12	16	24	40	78	212
25.0		220	60	30	18	14	12	14	18	24	36	60	120	326
30.0		312	86	42	26	18	18	18	24	34	50	86	168	464
35.0		418	114	54	34	24	22	24	32	44	68	114	224	620
40.0		536	144	70	42	30	28	30	40	56	86	144	288	796
45.0		666	180	86	52	38	34	38	48	70	106	180	358	988
50.0		806	216	104	62	46	40	44	58	84	128	216	432	1196
55.0		954	256	122	74	52	48	52	68	98	152	256	512	1416
60.0		1108	298	142	86	62	54	60	80	114	176	298	594	1646

Figure 5. Experimental design for demonstrating bioequivalence through stationary equilibrium conditions.



where $ABC_{R\ 0-\infty}$ is the area under the curve (AUC) for the reference product if administrated as a single dose, $ABC_{R,SS\ 0-\tau}$ and $ABC_{T,SS\ 0-\tau}$ are the areas under the curve (AUC) for the Reference and Test products estimated during the intervals between administrations (0-τ), after having reached stationary equilibrium state in each case.

Bibliography:

D. Hauschke & coll. (1992). Sample size determination for bioequivalence assessment using a multiplicative model. *J. Pharmacokin. Biopharm.* 20:557-561.

Diletti E, Hauschke D, Steinijans VW. (1992) Sample size determination for bioequivalence assessment by means of confidence intervals. Int J Clin Pharmacol Ther Toxicol; (30), Supplement N°1. pp S51-58.

ADDENDUM to the Bioequivalence Guide

Contents

1. ALTERNATE STUDIES

In vitro equivalence studies may support bioequivalence in the following cases:

1. Where bioequivalence has been demonstrated for a given formulation, information relating to *in vitro* dissolution can be used to support the equivalence of lower concentrations of that generic formulation. In this case, when the *in vitro* method is used, all the conditions listed below must be met:
   - Dose concentrations must differ only in terms of active substance concentration.
   - The drug is known to be associated with linear pharmacokinetics.
   - The composition of the formulations is qualitatively identical.
   - The proportion of active principle to excipient for the different dosing forms is essentially the same or, where the content of active principle is very low, the proportion between excipients is the same.
   - The new formulations are manufactured by the same laboratory, at the same manufacturing site and using the same procedures.

2. When an insignificant change has been made to the formulation of an approved product (or prior to obtaining approval for a product that has been submitted to extensive clinical testing) and it has been determined that the change only requires confirmation of *in vitro* equivalence with the formulation that was submitted to the original clinical trials.

3. To ensure consistency between different batches of a given product.

2. DESIGN OF *IN VITRO* EQUIVALENCE STUDIES FOR ORAL DOSE FORMS

A medicinal product for oral administration comprises one or more pharmacological substances, and excipients, in specified proportions. The type of excipients and method used to manufacture the final product are selected based on the contents, physical and chemical properties, and bulk properties of the drug, and its absorption properties. Taken as a whole, these properties determine the dissolution characteristics of each product.

During the development of these medicinal products, a dissolution test is used as a tool to identify factors relating to formulation that influence bioavailability and may have a crucial impact on it. As

soon as the composition and manufacturing process of a drug have been defined, a dissolution test is carried out as part of the quality control conducted on scaling batches and production batches to ensure consistency between batches and verify that the dissolution profiles are similar to those of the laboratory batches. Additionally, the dissolution test may be used to support the bioavailability of a new pharmacological product, the bioequivalence of an essentially similar product, or its variations.

Consequently, dissolution studies may be useful for various purposes:

- Quality assurance
    - To obtain information on test batches used in bioavailability/bioequivalence studies and in clinical trials to support product specifications.
    - As a tool for demonstrating consistency in manufacturing.
    - To obtain information on reference products used in bioavailability/bioequivalence studies and in clinical trials.
- Indirect inference of bioequivalence:
    - To demonstrate similarity between the different formulations of an active substance and the reference medicinal product. Different formulations refer to variations of a given formulation or to new formulations, including essentially similar products.
    - To gather information on the consistency between product batches (test and reference) that will be used as a basis for selecting appropriate batches for the *in vivo* study.

The test methodology must comply with the requirements of the applicable pharmacopeia, unless these requirements have been shown to be unsatisfactory. The use of alternate methods may be considered when justification is provided to show that these are discriminatory and capable of differentiating between acceptable and unacceptable performance of product batches *in vivo*.

If an active ingredient is considered to be highly soluble, it can be reasonably expected not to cause bioavailability problems if, additionally, the dosing system is rapidly dissolved within the expected physiological pH range following product administration. In these situations, it may not be necessary to carry out a bioequivalence study based on the background information available and the similar dissolution profiles, which are based on discrimination tests that are compliant with 85% dissolution in 15 - 30 minutes[1]. Similarity must be justified by dissolution profiles that cover at least three different time points, using three different buffers (normally with a pH range of 1-6.8; when needed a pH range of 1-8 may be used).

3. STUDY DESIGN FOR ORAL DOSE FORMS

3.1 Basic principles

The *in vitro* test must be a validated prediction factor for the *in vivo* dissolution of the product, i.e. the *in vitro* test conditions must previously have related to *in vivo* conditions. *In vitro* testing cannot be used

when the mean dissolution time is greater than the mean absorption time. Also, the longer the dissolution time, the harder it will be to establish *in vitro* to *in vivo* extrapolation. Therefore, it is advised not to carry out *in vitro* testing when dissolution time is very prolonged.

3.2 Experimental conditions

The conditions for carrying out *in vitro* equivalence studies must be clearly defined (ex: pH, temperature, dissolution medium, stirring, etc.). The use of at least three pH conditions is indicated to provide a degree of assurance for the extrapolation of *in vitro* to *in vivo* conditions. When studies aside from pH are not considered necessary, this must be justified. The specifications of equipment used for an *in vitro* equivalence study must be defined by international reference agencies. A validated analytical method must be used to analyse the level of active substance released.

3.3 Sampling

Samples used for *in vitro* trials comprise pills, defined quantities of powder or paste in a specified container. Sampling is carried out in line with a pre-established plan contained in the protocol and based on a randomisation procedure. This plan must envisage the factors included in the experimental design (ex: product batches). The same sampling procedure must be used for the reference formulation and the test formulation. Whenever possible, the final group of samples for each formulation must be representative of the total population; ex: the number of batches sampled for the *in vitro* test must be related to the expected variability between batches.

3.4 Experimental design

The experimental design must take into account the main sources of variation that will probably have an impact on the final result: product batch, storage time, equipment used for the test (ex: a container in a dissolution test). Precautions must be taken to avoid any bias, such as the even distribution of units of each formulation in each analytical test. Where applicable, replicate determinations must be made to take into account the variability inherent to the analytical method.

3.4.1 Sample size

When it is relevant to use a design similar to the *in vivo* bioequivalence study, the sample size must be determined in order to deliver sufficient potency to demonstrate equivalence. The variation coefficient used to calculate the sample size must be obtained from pilot studies or be estimated based on the variability of the analytical method. These aspects must be documented in the protocol.

3.4.2 Statistical analysis for *in vitro* dissolution studies

Parameters must be selected beforehand and must be justified in terms of their correlation to pharmacokinetics. It may suffice to discuss the relationship between dissolution time and rate of absorption for the products compared (when the dissolution process is not a limiting factor for speed and magnitude of absorption). *In vitro* equivalence may be demonstrated through a comparison of the dissolution profiles after their adjustment to a mathematical model, or through comparison of parameters such as 50% dissolution time, 90% dissolution time, and area under the curve (AUC). The statistical analysis may be similar to the one used in a bioequivalence study. However, the predetermined equivalence interval must be justified carefully. It should be noted that

exemption from carrying out *in vivo* studies only applies when the results of *in vitro* studies allow a similar pharmacokinetic behaviour to be inferred for the two products compared.

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[1] Pharmaceutical Research. Vol 15, No. 1, 1998 – Review "Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms" Jennifer Dressman, Gordon Amidon, Christos Reppas and Vinod Shah.