Egyptian guideline for conducting bioequivalence studies for generic Veterinary medicinal products

I. Introduction

This document is intended to provide guidance for the design and analysis of bioequivalence studies.
Two products are considered to be bioequivalent when they are equally bioavailable; that is, equal in the rate and extent to which the active ingredient(s) or therapeutic ingredient(s) is (are) absorbed and become(s) available at the site(s) of drug action.
Bioequivalence studies are used in a variety of situations, most often a part of a generic veterinary medicinal products application. A bioequivalence study may also be part of other types of applications.
Bioequivalence studies should be conducted in accordance with good laboratory practice (GLP) regulations.

II. Scope

The aim of this guideline is to provide guidance regarding study design, conduct and evaluation of bioequivalence studies for pharmaceutical forms with systemic action and *in vitro* dissolution tests.
In addition, recommendations are given on when *in vivo* studies are mandatory and when *in vitro* data are likely to be sufficient.

III. General Considerations

A. Selection of Reference Product for Bioequivalence Testing

The proposed generic product should be tested against the original pioneer product that contains the same API.
The original pioneer product must be authorized by one of the stringent regulatory authorities approved by the technical committee of drug control.
If the original pioneer product is no longer marketed, but remains eligible to be copied, then the first approved and available generic copy of the pioneer should be used as the reference product for bioequivalence testing against the proposed new generic product.
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 test product.
The study report should include the reference product name, strength (including assayed content), dosage form, batch number, expiry date (when available), and country of purchase. The test product

name, strength (including assayed content), dosage form, composition, batch size, batch number, manufacturing date, and expiry date (where available) should be provided.

B. Criteria for Waiver of *in vivo* Bioequivalence Study

The requirement for the *in vivo* bioequivalence study may be waived for certain generic products.
Categories of products which may be eligible for waivers include, but are not limited to, the following:
1- Parenteral solutions intended for injection by the intravenous, subcutaneous, or intramuscular routes of administration.
2- Oral solutions or other solubilized forms.
3- Topically applied solutions intended for local therapeutic effects. Other topically applied dosage forms intended for local therapeutic effects for non-food animals only.
4. Inhalant volatile anaesthetic solutions.
In general, the generic product being considered for a waiver contains the same active and inactive ingredients in the same dosage form and concentration and has the same pH and physico-chemical characteristics as an approved pioneer product.
However, bioequivalence waivers for non-food animal topical products with certain differences in the inactive ingredients of the pioneer and generic products would be considered.

C. Selection of Blood Level, Pharmacologic End-point, or Clinical End-point Study

In vivo bioequivalence may be determined by one of several direct or indirect methods.
Selection of the method depends upon the purpose of the study, the analytical method available, and the nature of the drug product.
Bioequivalence testing should be conducted using the most appropriate method available for the specific use of the product.
The preferred hierarchy of bioequivalence studies (in descending order of sensitivity) is the blood level study, pharmacologic end-point study, and clinical end-point study.
When absorption of the drug is sufficient to measure drug concentration directly in the blood (or other appropriate biological fluids or tissues) and systemic absorption is relevant to the drug action, then a blood (or other biological fluid or tissue) level bioequivalence study should be conducted.
The blood level study is generally preferred above all others as the most sensitive measure of bioequivalence. The applicant should provide justification for choosing either a pharmacologic or clinical end-point study over a blood-level (or other biological fluids or tissues) study.

When the measurement of the rate and extent of absorption of the drug in biological fluids cannot be achieved or is unrelated to drug action, a pharmacologic end-point (i.e., drug induced physiologic change which is related to the approved indications for use) study may be conducted.

Lastly, in order of preference, if drug concentrations in blood (or fluids or tissues) are not measurable or are inappropriate, and there are no appropriate pharmacologic effects that can be monitored, then a clinical end-point study may be conducted, comparing the test (generic) product to the reference (pioneer) product and a placebo (or negative) control.

D. Species Selection

A bioequivalence study generally should be conducted for each species for which the pioneer product is approved on the label, with the exception of "minor" species.

The test animals should be of the target species. Where a product is intended for more than one species, bioequivalence studies should normally be performed in each target animal species.

Extrapolation of results from a major species in which bioequivalence has been established to minor species could be acceptable if justified based on scientific information to demonstrate similarity in the anatomy and physiology (such as pH in the gastrointestinal tract, gastric volume and gastrointestinal tract transit time in the case of oral formulations, injection site anatomy and physiology in the case of injectable formulations etc.) and taking into account properties of the active substance (e.g. solubility/ permeability) and formulation (e.g. dissolution rate of a tablet).

E. Dose Selection

The bioequivalence study should generally be conducted at the highest labelled (e.g. mg/kg) dose approved for the reference product. By using the highest approved dose, significant formulation differences are more easily detected in most cases. However, if it can be substantiated that the reference product exhibits linear pharmacokinetics across the entire dose range, then any approved dose may be used if a scientific justification is provided as to why the highest dose cannot be used.

Bioequivalence study conducted at a higher than approved dose may also be appropriate when a multiple of the highest approved dose is needed to achieve measurable blood levels. In general, the maximum dose would be limited to 3x the highest dose approved for the reference product. The reference product should have an adequate margin of safety at the higher than approved dose level and should exhibit linear pharmacokinetics (i.e. there are no saturable absorption or elimination processes). In this case, a scientific justification should accompany the choice of the dose.

For reference products with nonlinear kinetics across the therapeutic range, the following should be considered:

• When there is evidence indicating that the product absorption may be limited by saturable absorption processes, this can lead to two formulations appearing to be bioequivalent when administered at the highest labelled dose but fail to be bioequivalent when administered at lower approved doses. To avoid this situation, use of a dose that is less than the highest approved dose is preferable. In this case, a scientific justification should accompany the choice of the dose (showing that the dose is within the linear range);

• if there is nonlinearity over the therapeutic range due to low solubility, then BE should be established at both the highest labelled dose and at the lowest labelled dose (or a dose in the linear range), i.e. in this situation, two BE studies may be needed.

In crossover studies, the same total dose should be administered to each animal in all study periods. The use of dose adjustments in those rare situations where large weight changes are anticipated (e.g., studies conducted in rapidly growing animals where there is a risk of differences in drug absorption, distribution, metabolism, or elimination in period 1 vs 2 that could bias the within-subject comparison) will need to be considered on a case-by-case basis.

Solid oral dosage forms should not be manipulated in a way that could bias the study, e.g., by grinding or filing to achieve equal doses. Breaking tablets along score lines may be acceptable if the uniformity of the scored sections can be supported by pharmaceutical/manufacturing data (e.g., content uniformity of the halves). For reference products, in the absence of manufacturing or pharmaceutical data, the information included in the product labelling can be used as a guide for allowable tablet manipulation.

The study report should include the labelled dose administered to each animal in each period of the study.

F. Multiple Strengths of Solid Oral Dosage Forms

One *in vivo* bioequivalence study with highest strength product may suffice if the multiple strength products have the same ratio of active to inactive ingredients and are otherwise identical in formulation. *In vitro* dissolution testing should be conducted, to compare each strength of the generic product to the corresponding strength of the reference product.

G. Manufacturing of Pilot Batch ("Biobatch")

A pilot batch or "biobatch" should be the source of the test product used in bioequivalence studies

IV. BLOOD LEVEL STUDIES

Blood level bioequivalence studies compare a test (generic) product to a reference (pioneer) product using parameters derived from the concentrations of the drug moiety and/or its metabolites, as a function of time, in whole blood, plasma, serum (or in other appropriate biological fluids or tissues). This approach is particularly applicable to dosage forms intended to deliver the active drug ingredient(s) to the systemic circulation (e.g., injectable drugs and most oral dosage forms). Generally, the study should encompass the absorption, distribution, and depletion (elimination) phases of the drug concentration vs time profiles.

A. Assay Consideration

The analytical methods used in bioequivalence studies must comply with standard criteria of validation as given in the CHMP Guideline on bioanalytical method validation *(EMEA/CHMP/EWP/192217/2009).* The analysis should be conducted according to the principles of GLP. Analysis of samples should be conducted without information on treatment groups.

The following aspects should be addressed in assessing method performance:

1.Concentration Range and Linearity

The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. For linear relationships, a standard curve should be defined by at least 5 concentrations. If the concentration response function is non-linear, additional points would be necessary to define the non-linear portions of the curve. Extrapolation beyond a standard curve is not acceptable.

2.Limit of Detection (LOD)

The standard deviation of the background signal and LOD should be determined. The LOD is estimated as the response value calculated by adding 3 times the standard deviation of the background response to the average background response.

3.Limit of Quantitation (LOQ)

The initial determination of LOQ should involve the addition of 10 times the standard deviation of the background response to the average background response. The second step in determining LOQ is assessing the precision (reproducibility) and accuracy (recovery) of the method at the LOQ. The LOQ will generally be the lowest concentration on the standard curve that can be quantified with acceptable accuracy and precision (see items 5 and 6 below).

4.Specificity

The absence of matrix interferences should be demonstrated by the analysis of 6 independent sources of control matrix. The effect of environmental, physiological, or procedural variables on the matrix should be assessed. Each independent control matrix will be used to produce a standard curve, which will be compared to a standard curve produced under chemically defined conditions. The comparison of curves should exhibit parallelism and superimposability within the limits of analytical variation established for the chemically defined standard curve.

5.Accuracy (Recovery)

This parameter should be evaluated using at least 3 known concentrations of analyte freshly spiked in control matrix, one being at a point 2 standard deviations above the LOQ, one in the middle of the range of the standard curve ("mid-range") and one at a point 2 standard deviations below the upper quantitative limit of the standard curve. The accuracy of the method, based upon the mean value of 6 replicate injections, at each concentration level, should be within 80-120% of the nominal concentration at each level (high, mid-range, and LOQ).

6.Precision

This parameter should be evaluated using at least 3 known concentrations of analyte freshly spiked in control matrix, at the same points used for determination of accuracy. The coefficient of variation (CV) of 6 replicates should be ± 10% for concentrations at or above 0.1 ppm (0.1 µ g/mL). A CV of ± 20% is acceptable for concentrations below 0.1 ppm.

7.Analyte Stability

Stability of the analyte in the biological matrix under the conditions of the experiment (including any period for which samples are stored before analyses) should be established. It is recommended that the stability be determined with incurred analyte in the matrix of dosed animals in addition to, or instead of, control matrix spiked with pure analyte. Also, the influence of 3 freeze-thaw cycles at 2 concentrations should be determined.

Stability samples at 3 concentrations should be stored with the study samples and analyzed through the period of time in which study samples are analyzed. These analyses will establish whether or not analyte levels have decreased during the time of analysis.

8.Analytical System Stability

To assure that the analytical system remains stable over the time course of the assay, the reproducibility of the standard curve should be monitored during the assay. A minimal design would be to run analytical standards at the beginning and at the end of the analytical run.

9.Quality Control (QC) Samples

The purpose of QC samples is to assure that the complete analytical method, sample preparation, extraction, clean-up, and instrumental analysis perform according to acceptable criteria. The stability of the drug in the text matrix for the QC samples should be known and any tendency for the drug to bind to tissue or serum components over time should also be known.

Drug free control matrix, e.g., tissue, serum, etc. that is freshly spiked known quantities of test drug, should be analyzed contemporaneously with test samples, evenly dispersed throughout each analytical run. This can be met by the determination of accuracy and precision of each analytical run.

10.Replicate and Repeat Analyses

Single rather than replicate analyses are recommended, unless the reproducibility and/or accuracy of the method are borderline. Criteria for repeat analyses should be determined prior to running the study and recorded in the method SOP.

B. Experimental Design Considerations

1.Dosing by Labelled Concentration

The potency of the pioneer and generic products should be assayed prior to conducting the bioequivalence study. The potency of the pioneer and generic lots should differ by no more than ±5% for dosage form products.

The animals should be dosed according to the labelled concentration or strength of the product, rather than the assayed potency of the individual batch (i.e., the dose should not be corrected for the assayed potency of the product). The bioequivalence data or derived parameters should not be normalized to account for any potency differences between the pioneer and generic product lots.

2.Single Dose Vs Multiple Dose Studies

A single dose study at the highest approved dose will generally be adequate for the demonstration of bioequivalence. A single dose study at a higher than approved dose may be appropriate for certain drugs (refer to the section on Dose Selection).

In most situations, a single dose BE study is recommended for both immediate- and modified release drug products because single dose studies are generally the more sensitive approach for assessing differences in the release of the API from the drug product into the systemic circulation.

A multiple dose study may be appropriate when there are concerns regarding poorly predictable drug accumulation, (e.g. a drug with nonlinear kinetics) or a drug with a narrow therapeutic window.

For extended-release formulations intended for repeated dosing, demonstration of BE should be based on multiple dose studies if there is accumulation between doses (i.e., if there will be at least a 2-fold increase in drug concentrations at steady state as compared to that observed after a single dose). In such cases, the C_{trough} could be an important parameter to consider, in addition to C_{max} and the AUC. It should be noted that C_{trough} may not be equal to C_{min} in the case of products with a lag time. If there is no or negligible accumulation, single dose BE data could also be sufficient for extended-release formulations intended for repeated dosing.

Both single and multiple dose studies can be conducted using a crossover study or parallel design.

3.Subject Characteristic

Ordinarily, studies should be conducted with healthy animals' representative of the species, class, gender, and physiological maturity for which the drug is approved. The bioequivalence study may be conducted with a single gender for which the pioneer product is approved, unless there is a known interaction of formulation with gender.

An attempt should be made to restrict the weight of the test animals to a narrow range in order to maintain the same total dose across study subjects.

The animals should not receive any medication prior to testing for a period of two weeks or more, depending upon the biological half-life of the ancillary drug.

For parallel design studies, the animal's/treatment groups should be homogeneous and comparable in all known and prognostic variables that can affect the PK of the API, e.g. age, body weight, gender, nutrition, physiological state, and level of production (if relevant).

Animals should be randomized and an equal number of animals should be assigned to each sequence (crossover design) or each treatment (parallel study design).

A complete description of the above information should be included in the study report.

4. Prandial State:

For all species prandial state and exact timing of feeding should be consistent with animal welfare (e.g., ruminants would not be fasted) and the PK of the API.

For canine and feline drug products administered via the oral route, studies should be conducted in fasted animals unless the approval for the reference product recommends administration in the fed state only, in which case the study should be conducted accordingly. Fasting should be a minimum of 8 hours prior to dosing and 4 hours after dosing.

For orally administered modified release formulations intended for non-ruminants, BE normally needs to be established under both fed and fasted conditions unless adequately justified.

The study protocol and study report should contain the rationale for conducting the BE study under fed or fasted conditions and should describe the diet and feeding regimen.

5. Sampling Time Considerations

A sufficient number of samples to adequately describe the plasma concentration-time profile should be collected. The sampling schedule should include frequent sampling around the predicted tmax to provide a reliable estimate of peak exposure.

The total number of sampling times necessary to characterize the blood level profiles will depend upon the curvature of the profiles and the magnitude of variability associated with the bioavailability data (including pharmacokinetic variability, assay error and interproduct differences in absorption kinetics).

6. Subject Number

The number of test animals must be appropriate for statistical analyses and should be carefully estimated and justified in the protocol. The sample size for a bioequivalence study should be based upon the number of subjects needed to achieve bioequivalence for the pharmacokinetics parameter anticipated to have the greatest magnitude of variability and/or difference in treatment means (e.g. Cmax).

It should be noted that for a stud y to be internationally acceptable, a minimum 12 evaluable animals per treatment is necessary. For a crossover trial, this implies that the minimum number of subjects per sequent (n) = 6 (and therefore, the total number of study animals in a two-period, two-sequence crossover study, N, should be equal to or greater than 12). For a parallel study design, there should be no less than 12 evaluable subjects per treatment group (and thus the total number of animals enrolled in the BE trial would be equal to or greater than 24).

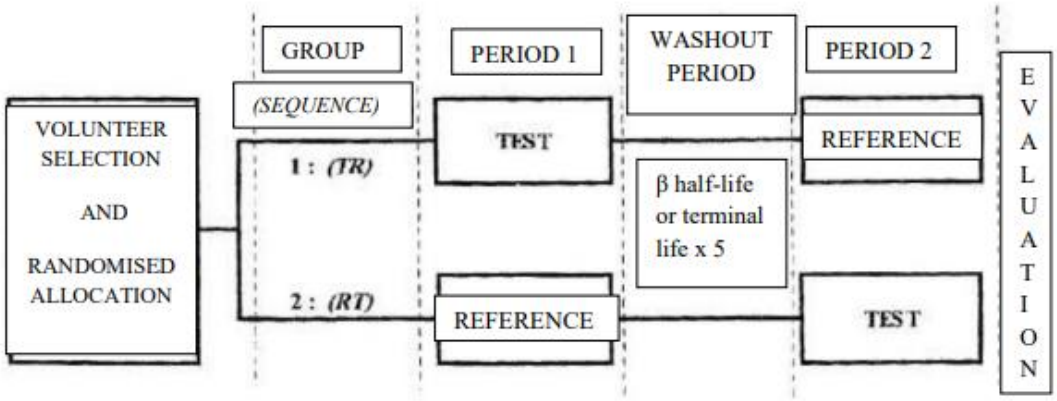
When the risk of subject loss is a concern, the applicant may elect to design the study to include additional animals. In this situation, if animals are removed as the study progresses (due to vomiting or dosing errors or death/injury) the additional animals placed on study may allow appropriate statistical power to be maintained.

7. Cross-over, parallel and alternative study designs

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).

A two-period cross-over design:

- Generally, a two-sequence (TR/RT), two-period (Period 1/Period 2), two treatments, 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.

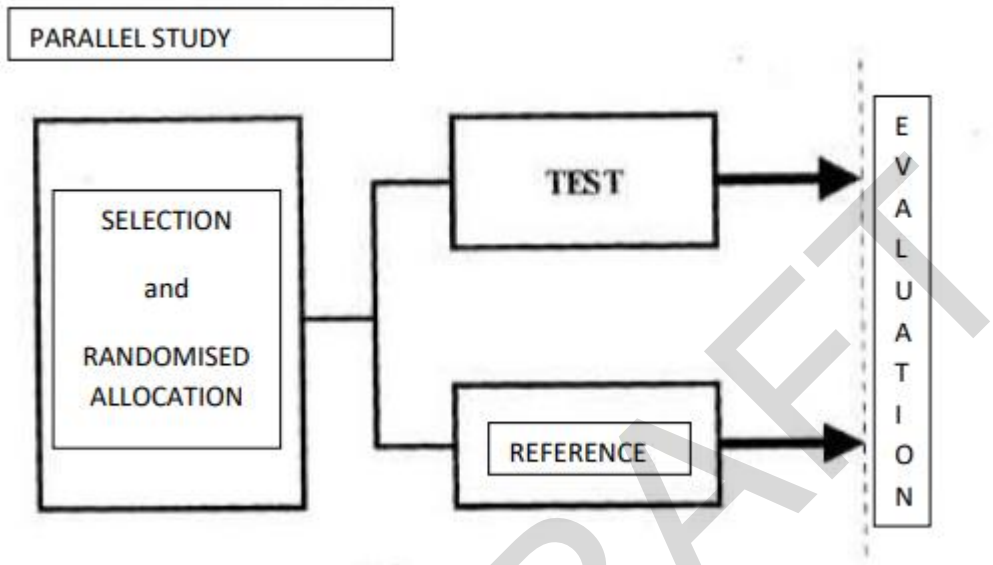


- Duration of Washout Time for Cross-over Study

The treatment periods should be separated by a sufficiently long wash-out period to ensure that concentrations of the active substances are below the lower limit of quantification of the bioanalytical method in all animals at the beginning of the second period and that no physiological effects, such as metabolic enzyme induction, remain from the first period. Normally, at least 5 terminal half-lives are necessary to achieve this

A one-period parallel design

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.



A one-period parallel design may be preferable in the following situations:

a. The drug induces physiological changes in the animal (e.g., liver microsomal enzyme induction) which persist after total drug clearance and alter the bioavailability of the product administered in the second period.

b. The drug has a very long terminal elimination half-life, creating a risk of residual drug present in the animal at the time of the second period dosing.

c. The duration of the washout time for the two-period cross-over study is so long as to result in significant maturational changes in the study subjects.

Other designs, such as the two-period design with four treatment sequences (Test/Test, Reference/Reference, Test/Reference and Reference/Test) or the extended period design may be appropriate depending on the circumstances.

8. Parameters to be analysed and acceptance criteria

The parameters to be analysed are AUCt, Cmax and Ctrough (if applicable). A statistical evaluation of tmax is not required. For AUC, the ratio of the two-treatment means should be entirely contained within the limits 80% to 125%. The acceptance criteria for Cmax and Ctrough should also generally be within 80% to 125%.

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, a maximal widening of the limits to 70% to 143% could in rare cases be acceptable if it has been prospectively defined in the protocol together with a justification from efficacy and safety perspectives.Valid data would be, for example, data on PK/PD relationships for efficacy and safety which demonstrate that the proposed wider range does not affect efficacy and safety in a clinically significant way. If PK/PD data are not available, persuasive clinical data may still be used for the same purpose. With regard to antimicrobials and antiparasitic products, risks for resistance development should also be considered when defining acceptance criteria. Post hoc justifications of wider acceptance criteria are not acceptable for any parameter.

If bioequivalence data are used to substantiate an extrapolation of a withdrawal period between formulations, the 90% confidence interval for the ratio should be below the 125% acceptance limit for both AUC and Cmax. In case of breaching of the upper acceptance limit of 125 %, then residue data to confirm the withdrawal period are required

9. Route of Administration Selection

Unless otherwise justified when conducting an *in vivo* BE study:

- The same route and site of administration should be used for the test and reference products.
- Separate BE studies should be submitted for each route of administration approved for the reference product.

10. Analyte to be measured

In principle, BE evaluations should be based upon measured concentrations of the parent compound because the Cmax of a parent compound is usually more sensitive to differences between product absorption rates as compared to the Cmax of a metabolite. In general, product BE will be determined on the basis of total (free plus protein bound) concentrations of the API.

a-Pro-drugs

BE demonstration should be based upon the parent compound unless the parent compound is a pro-drug and that pro-drug is associated with negligible blood concentrations. In cases where there are negligible systemic concentrations of the pro-drug, the active metabolite (the compound formed upon absorption of the pro-drug) should be measured. Applicant should provide scientific rationale for the compound to be quantified.

b-Enantiomers

Under most situations, use of an achiral assay will suffice for the assessment of product bioequivalence. However, the use of an enantiomer-specific analytical method will be necessary when all of the following conditions are met:

-The enantiomers exhibit different PK.

-The AUC ratio of the enantiomers is modified by a difference in their respective rates of absorption.

-The enantiomers have different pharmacodynamic characteristics.

If all three conditions are met, chiral (stereospecific) analytical methods will be needed. In addition, chiral methods may be necessary when the test or reference products include the use of a stereospecific (chiral) excipient that can selectively alter the absorption of one or both enantiomers. It may also be needed when a drug is a single enantiomer that undergoes *in vivo* chiral conversion.

V. PHARMACOLOGIC END-POINT STUDIES

Where the direct measurement of the rate and extent of absorption of the new animal drug in biological fluids is inappropriate or impractical, the evaluation of a pharmacologic end-point related to the labelled indications for use will be acceptable. Typically, the design of a pharmacologic end-point study should follow the same general considerations as the blood level studies. However, specifics such as the

number of subjects or sampling times will depend on the pharmacologic end-point monitored. The parameters to be measured will also depend upon the pharmacologic end-points and may differ from those used in blood level studies.

IV. CLINICAL END-POINT STUDIES

If measurement of the drug or its metabolites in blood, biological fluids or tissues is inappropriate or impractical, and there are no appropriate pharmacologic end-points to monitor (e.g., most production drugs and some coccidiostats and anthelmintics), then well-controlled clinical end-point studies are acceptable for the demonstration of bioequivalence. Generally, a parallel group design with three treatment groups should be used. The groups should be a placebo (or negative) control, a positive control (reference/pioneer product) and the test (generic) product. The purpose of the placebo (or negative) control is to confirm the sensitivity or validity of the study. Dosage(s) approved for the pioneer product should be used in the study. Dosage(s) should reflect consideration for experimental sensitivity and relevance to the common use of the pioneer product.

IIV Dissolution Studies:

In evaluating dissolution data relevant to target animal efficacy and safety, consideration is to be given to the dissolution test method and the statistical analysis (such as the similarity factor) of the test results. Sampling time points should be sufficient to obtain meaningful dissolution profiles, and at least every 15 minutes. More frequent sampling during the period of greatest change in the dissolution profile is recommended.

Dissolution profile similarity testing and any conclusions drawn from the results (e.g. justification for a biowaiver) can be considered valid only if the dissolution profile has been satisfactorily characterised using a sufficient number of time points.

*Where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar based on a single time point.

*In case more than 85% is not dissolved at 15 minutes but within 30 minutes, at least three-time points are required: the first time point before 15 minutes, the second one at 15 minutes and the third time point when the release is close to 85%. In these cases, mathematical evaluation such as calculation of similarity factor f2 (see below) may be required to demonstrate comparable dissolution.

*In case more than 85% is not dissolved within 30 minutes, more than three time points may be required.

Dissolution similarity may be determined using the *f*2 statistic as follows:

$$f_2 = 50 \cdot \log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}[R(t)-T(t)]^2}{n}}}\right]$$

In this equation *f*2 is the similarity factor, n is the number of time points, R(t) is the mean percent drug dissolved of e.g. a reference veterinary medicinal product, and T(t) is the mean percent substance dissolved of e.g. a test product.

The evaluation of the similarity factor is based on the following conditions:

• A minimum of three-time points (zero excluded).

• The time points should be the same for the two formulations

• Twelve individual values for every time point for each formulation.

• Not more than one mean value of > 85% dissolved for any of the formulations.

• The relative standard deviation or coefficient of variation of any product should be less than 20% for the first point and less than 10% from second to last time point.

An f2 value between 50 and 100 suggests that the two dissolution profiles are similar.

When the *f*2 statistic is not suitable, then the similarity may be compared using model-independent or model-dependent methods.

Alternative methods to the *f*2 statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified

Appendix I: BCS-Based Biowaivers

I. Introduction

The BCS (Biopharmaceutics Classification System) based biowaiver approach is intended to reduce the requirements for *in vivo* bioequivalence studies, i.e. it may represent a surrogate for *in vivo* bioequivalence. *In vivo* bioequivalence studies may be exempted if an assumption of equivalence in

in vivo performance can be justified by satisfactory *in vitro* data.

The concept is applicable to solid and semi-solid immediate release pharmaceutical products for oral administration and systemic action having the same pharmaceutical form.

As per BCS, the active substances can be classified as follows:

• Class I - High Permeability, High Solubility;

• Class II - High Permeability, Low Solubility;

• Class III - Low Permeability, High Solubility;

• Class IV - Low Permeability, Low Solubility.

The BCS based approach is mainly based on human data and very few studies to validate this system have been conducted in animals. However, the principles behind the BCS based approach could still be effectively applied in veterinary medicine, if possible, differences of relevance between species are considered. Compared to its application in human medicine, a larger variety of GI-tract pH values has to be considered as well as a variety of gastric/intestinal fluid volumes and transit times. Therefore, the approach presented below represents a summary of requirements to fulfil any "worst case scenario" specific to target (sub)-species. Of note is that in order to apply the BCS system to animals, the solubility classification has been modified in comparison to that used in humans.

The application of BCS-based biowaiver is restricted to highly soluble active substances with known absorption in target animals. Specific guidance is provided for biowaivers for BCS Class I substances (high solubility, high permeability) and for Class III substances (high solubility, low permeability). The classification is species specific.

The principles may be used to establish bioequivalence in applications for generic medicinal products and variations that require bioequivalence testing.

II. Summary Requirements

BCS-based biowaivers are applicable for an immediate release formulation if:

• The active substance has been proven to exhibit high solubility and complete absorption (BCS Class I)

• Very rapid (more than 85% within 15 minutes) *in vitro* dissolution characteristics of the test and reference veterinary medicinal product have been demonstrated considering specific requirements

• Excipients that might affect bioavailability are qualitatively and quantitatively the same. In general, the use of the same excipients in similar amounts is preferred.

BCS-based biowaivers could potentially also be applicable for an immediate release formulation if:

• The active substance has been proven to exhibit high solubility and limited absorption (BCS Class III)

• Very rapid (more than 85% within 15 minutes) *in vitro* dissolution characteristics of the test and reference veterinary medicinal product have been demonstrated considering specific requirements.

• Excipients that might affect bioavailability are qualitatively and quantitatively the same and other excipients are qualitatively the same and quantitatively very similar.

Generally, BCS Class III biowaivers can only be granted on a case-by-case basis and when justified by the appropriate supporting data, validated in the (sub)-species concerned. Moreover, the risks of an inappropriate biowaiver decision should be more critically reviewed (e.g. site-specific absorption, the risk for transport protein interactions at the absorption site, excipient composition and therapeutic risks) for products containing BCS class III compared to BCS class I substances. If there are insufficient data available on such aspects for a certain target animal species, biowaivers cannot be granted.

Notably, for species where there are considerable differences between subgroups within the species (e.g. ruminant and pre-ruminant cattle), special consideration is needed to cover all the categories/subspecies of animals.

III Active Substance

A biowaiver may be applicable when the active substance(s) in the test and reference veterinary medicinal products are identical. A biowaiver may also be applicable if test and reference veterinary medicinal products contain different salts provided that both belong to BCS-class I (high solubility and complete absorption).

A biowaiver is not applicable when the test product contains a different ester, ether, isomer, mixture of isomers, complex or derivative of an active substance from that of the reference veterinary medicinal product, since these differences may lead to different bioavailabilities not deducible by means of experiments used in the BCS-based biowaiver concept.

III.1 Solubility

Since gastric and intestinal fluid volumes differ markedly across animal species, the solubility classification in the context of this guideline is different to the classification applied in human medicine. In order to be eligible for a veterinary biowaiver, an amount of the active substance equivalent to twice the highest dose for the maximum anticipated bodyweight for the target species should be soluble in a specified volume of an aqueous solution. This specified volume should be justified by reference to the physiology and gastric fluid volume for the (sub)-species. Solubility should be demonstrated at the relevant body temperature, and within the range of possible physiological pH values for the (sub)species, and it requires the investigation in at least three buffers spanning this range, and in addition at the PKa, if it is within the specified pH range.

III.2 Absorption

An active substance is considered to have complete absorption when the extent of absorption has been determined to be ≥ 85 % in comparison to an intravenous reference dose. Complete absorption is generally related to high permeability. Where relevant data are missing in the target animal (sub)species, the active substance will not be considered to have complete absorption.

IV. Veterinary Medicinal Product

IV.1 *In vitro* Dissolution

IV.1.1 General aspects

Investigations relating to the medicinal product should ensure immediate release properties and prove similarity between the investigative products, i.e. test and reference veterinary medicinal product should have a similar *in vitro* dissolution considering physiologically relevant experimental pH conditions.

In vitro dissolution should be investigated within the physiological pH range relevant for the target animal (sub)-species. Additional investigations may be required at pH values in which the active substance has minimum solubility. The use of any surfactant is not acceptable.

Comparative *in vitro* dissolution experiments should follow current compendial standards. Hence, thorough description of experimental settings and analytical methods including validation data should be provided. It is recommended to use 12 units of the product for each experiment to enable statistical evaluation. Usual experimental conditions are e.g.:

• apparatus: paddle or basket;

• volume of dissolution medium: 900 ml or less;

• temperature of the dissolution medium: 37±1 °C;

• agitation: paddle apparatus - usually 50 rpm;

• basket apparatus - usually 100 rpm

• sampling schedule: e.g. 10, 15, 20, 30 and 45 min;

• buffer: e.g. pH 1-1.2 (usually 0.1 N HCl or Simulated Gastric Fluid (SGF) without enzymes), 4.5 and 7.5 (or Simulated Intestinal Fluid (SIF) without enzymes); (pH should be ensured throughout the experiment)

• other conditions: no surfactant; in case of gelatine capsules or tablets with gelatine coatings the use of enzymes may be acceptable.

Complete documentation of *in vitro* dissolution experiments is required including a study protocol, batch information on the test and reference batches, detailed experimental conditions, validation of experimental methods, individual and mean results and respective summary statistics.

IV.1.2 Evaluation of *in vitro* dissolution results

Veterinary medicinal products are considered to be 'very rapidly' dissolving when more than 85% of the labelled amount is dissolved within 15 minutes. In cases where this is ensured for the test and reference veterinary medicinal products, the similarity of dissolution profiles may be accepted as demonstrated without any mathematical calculation. Generally, comparison at 15 minutes is considered to be an acceptable indicator that complete dissolution is reached before gastric emptying.

However, the selection of another appropriate time point can be justified by the provision of relevant data demonstrating that the selected time point is shorter than the gastric emptying time under fed/fasting conditions for the target (sub)species.

IV.2 Excipients

Although the impact of excipients in immediate release formulations on the bioavailability of highly soluble and completely absorbable active substances (i.e. BCS-Class I) is considered rather unlikely it cannot be completely excluded. Therefore, even in the case of Class I substances it is advisable to use similar amounts of the same excipients in the composition of the test product to those used in the reference veterinary medicinal product.

If a biowaiver is applied for a BCS-class III active substance, excipients have to be qualitatively the same and quantitatively very similar in order to exclude different effects on membrane transporters.

As a general rule, for both BCS-class I and III active substances, well-established excipients in usual amounts should be employed and possible interactions affecting bioavailability and/or solubility characteristics should be considered and discussed.

A description of the function of the excipients is required with a justification of whether the amount of each excipient is within the normal range.

Excipients that might affect bioavailability, e.g. sorbitol, mannitol, sodium laury sulfate or other surfactants, should be identified as well as their possible impact on

- gastrointestinal motility;
- susceptibility to interactions with the active substance (e.g. complexation);
- drug permeability;
- interaction with membrane transporters.

Excipients that might affect bioavailability should be qualitatively and quantitatively the same in the test product and the reference veterinary medicinal product.

V. Fixed Combinations

BCS-based biowaivers are applicable for immediate release fixed combination products if all active substances in the combination belong to BCS-Class I or III and the excipients fulfil the requirements outlined in section IV.2. Otherwise, *in vivo* bioequivalence testing is required.

VI. Biowaivers for pharmaceutical forms for use in medicated feeding stuffs or drinking water, milk or milk replacer

VI.1 Biowaiver for pharmaceutical forms for in-feed use

These products may be treated as immediate release formulations and can be regarded as eligible for a biowaiver if they contain substances that belong to BCS Class I or III. Feed constituents may affect the bioavailability of the active substances administered with feed. However, it is believed that this should not be a factor in considering a biowaiver request since the variability in feed constituents between the test and reference veterinary medicinal products should not be greater than the natural variations that can occur in the final feed to which the animal will be exposed, whether that feed contains the test product or the reference veterinary medicinal product. Accordingly, a product for in-feed use which contains insoluble constituents as excipients could also be eligible for a biowaiver provided the active substance fulfils the BCS criteria.

VI.2 Biowaiver for soluble pharmaceutical forms for in drinking water or milk use

The conceptual basis for granting biowaivers for these soluble pharmaceutical forms is that once a medicinal product is presented in a solution prior to administration, the product's formulation will usually not influence the bioavailability of the active substance. This is because, from a mechanistic perspective, it is believed that the rate-limiting step in systemic drug absorption will be: a) the rate of gastric transit; and b) the permeability of the active substance across the gastrointestinal mucosal membranes. Both of these variables are here formulation-independent. The only exceptions are when the formulation contains substances other than the active substance that could cause a direct pharmacologic effect in the target animal (sub)-species (e.g. altered gastrointestinal transit time, membrane permeability, or drug metabolism), or when there is inactivation of the active substance by, for example, a chelating agent.

For products to be administered in milk or milk replacer, data to demonstrate solubility and stability in milk and/or milk replacer should be provided. In order to be exempt from *in vivo* studies, the active substance must be demonstrated to be highly soluble in the aqueous milk fraction.

IIIV Glossary:

Bioavailability: The fraction of an administered dose that reaches the systemic circulation as intact substance.

Bioequivalence: Absence of a difference (within predefined acceptance criteria) in the bioavailability of the active pharmaceutical ingredient (API) or its metabolite(s) at the site of action when administered at the same molar dose under similar conditions in an appropriately designed study.
The Biopharmaceutics Classification System (BCS): Is a system to differentiate drugs on the basis of their solubility and permeability, see Appendix I.

Biowaiver: The possibility of waiving *in vivo* bioequivalence studies.

Acceptance criteria: The upper and lower limits (boundary) of the 90% confidence interval that is used to define product bioequivalence.

Pharmacokinetics (PK): The study of the absorption, distribution, metabolism, and excretion of an API and/or its metabolite(s).

Linear pharmacokinetics: When the concentration of the API or its metabolite(s) in the blood increases proportionally with the increasing dose, and the rate of elimination is proportional to the concentration, the drug is said to exhibit linear pharmacokinetics. The clearance and volume of distribution of these drugs are dose-independent.

Nonlinear pharmacokinetics: As opposed to linear pharmacokinetics, the concentration of the API or metabolites in the blood does not increase proportionally with the increasing dose. The clearance and volume of distribution of these may vary depending on the administered dose. Nonlinearity may be associated with any component of the absorption, distribution, and/or elimination processes.

Test product：The drug product used for BE comparison to the reference product.

Assay content: The amount of the analyte in a sample

Enantiomer：a pair of chiral isomers (stereoisomers) that are direct, nonsuperimposable mirror images of each other. Enantiospecificity in pharmacokinetics can arise because of enantioselectivity in one or more of the processes of drug absorption, distribution, metabolism and excretion.

Cmax: The maximum (or peak) concentration of API or its metabolite(s) in blood.

Cmin: The minimum concentration of the API or its metabolites in the blood at steady state.
In the absence of a measurable delay between drug administration and the first appearance of drug in the systemic circulation Cmin equals Ctrough.

Ctrough: The concentration of API or its metabolite(s) in blood at steady state immediately prior to the administration of a next dose.

Area under the curve (AUC): Area under the plasma drug concentration versus time curve, which serves as a measure of drug exposure.

AUCt: Area under the plasma concentration curve from administration to last observed concentration at time t.

AUC∞: Area under the plasma concentration curve extrapolated to infinite time.

AUCtau: AUC during a dosage interval at steady state; mathematically, the quantity equals AUC∞ of the first dose if there is linear (non-saturable) pharmacokinetics.

Tmax: Time to the Cmax.

References:

- Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000-Rev.4*)

- VICH GL52 Bioequivalence: blood level bioequivalence study (EMA/CVMP/VICH/751935/2013)

- CVM GFI #35 Bioequivalence Guidance (FDA)

- Guide for Conducting Bioequivalence Studies for Veterinary Medicines (World organization for Animal Health)