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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

GUIDELINES FOR THE CONDUCT OF BIOEQUIVALENCE STUDIES FOR VETERINARY MEDICINAL PRODUCTS

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CONDUCT OF BIOEQUIVALENCE STUDIES FOR VETERINARY MEDICINAL PRODUCTS

This note for guidance replaces the previous guidance note in volume 7A (7AE4a, pg.119, 1999).

1. DEFINITIONS

A veterinary medicinal product is a finished dosage form that contains the pharmacologically active substance(s) with or without excipient(s).

Bioequivalence techniques are scientific methods for the comparison of different veterinary medicinal products containing the same active substance, different batches of the same veterinary medicinal products, and, in a broader sense, different routes of administration. Bioequivalence testing aims to demonstrate that two medicinal products produce plasma concentrations similar enough to conclude that the systemic effects of the two products, in respect to efficacy (and possibly safety), are the same. *In vitro* bioequivalence study may be sufficient in certain cases to achieve this aim: *in vitro* tests can establish equivalence based on physical criteria that should be then correlated to pharmacokinetics. Bioequivalence techniques may also be used to compare similar formulations encountered during development of a given product

The first recommended method for demonstrating bioequivalence is based on the determination of the time course of the active substance concentration in blood. Bioequivalence exists between veterinary medicinal products or between routes of administration if, under identical and appropriate experimental conditions, the bioavailability of the active substance only differs within acceptable limits. Limits must be qualified, a priori, according to the aim of the tests. Bioavailability of a veterinary medicinal product is defined by the rate and extent to which the active substance reaches the systemic circulation and becomes available to the site(s) of action. Rate and extent are typically measured by C_{max} (peak concentration) and AUC (Area under the Curve), respectively.

This note for guidance does not cover demonstration of equivalence using other means than bioequivalence. In some cases, when the direct measurement of the rate and extent of absorption is inappropriate, e.g. bioavailability cannot be measured it may be necessary to demonstrate bioequivalence on the basis of a pharmacological end-point study. The pharmacological parameter chosen should be relevant (related to the drug activity).

Bioequivalence studies could preclude the need for further studies, but attention needs to be placed upon the cases where, for example, differences at the injection sites might lead to altered tissue depletion or local tolerance. Sponsors are referred to the appropriate guidelines, which govern the conduct of such studies. For specific aspects concerning chiral substances, the "note for guidance: investigations of chiral substances" should be considered (EMEA/CVMP/128/95 FINAL).

For Fixed Combination Products, the Applicant is referred to the guideline on Fixed Combination Products in regards to requirements for safety and efficacy of new combination products

For statistical aspects, the guideline on “Biostatistical Methodology in clinical trials” should be considered.

2. RATIONALE FOR BIOEQUIVALENCE STUDIES

It is scientifically valid to assume that, if an active substance or therapeutic moiety of a test veterinary medicinal product reaches the systemic circulation with the same rate and extent as the active substance or therapeutic moiety of a reference veterinary medicinal product, the local availability (concentration in tissue) of the active substance or therapeutic moiety will be similar for the test and

reference products. The similarity of availability at the site(s) of action is the basis of concluding therapeutic equivalence of the products.

Changes of excipients in a veterinary medicinal product or in the manufacturing process may have significant effects on bioavailability.

The *in vivo* bioequivalence of a veterinary medicinal product is demonstrated if the rate and extent of absorption, as determined by comparison of measured parameters derived from relevant data (e.g. concentration of the active substance in the blood or pharmacological effects) do not indicate a biologically relevant difference in the rate and extent of absorption from the reference product.

3. NEED AND AIM FOR BIOEQUIVALENCE STUDIES

3.1 Product containing a new substance or a known substance but not intended to be a generic

When changing the specification of the dosage form, its composition or manufacturing process, the new product must be demonstrated to be bioequivalent to the product with which the clinical trials were conducted and to which reference is given in terms of efficacy and/or safety. Concerning changes in the manufacturing process, bioequivalence is only requested if relevant, and *in vitro* studies can be used if justified.

3.2 Product containing a known substance intended to be a generic

If reference is made to an approved product in terms of efficacy and/or safety, bioequivalence to this product must be demonstrated.

3.3 Route of administration

Two routes of administration are bioequivalent when their plasma concentration profiles are the same. In some cases, concentration profiles in another biological fluid may be more appropriate than concentration profiles in plasma.

4. EXEMPTIONS

Omission of bioequivalence studies should be justified. Bioequivalence studies are generally not necessary if the product fulfils one or more of the following conditions:

a) The product is a solution intended solely for intravenous administration and contains the same active substance or therapeutic moiety as an intravenous solution approved for use in the target species which is the subject of the new application;
b) The product is to be parenterally or orally administered as a solution and contains the same active substance(s) and excipients in the same concentrations as a veterinary medicinal product currently approved for use in the target species which is the subject of the new application;
c) The formulations are identical (identical active and inactive substances as well as physicochemical properties (e.g. identical concentration, dissolution profile, crystalline form, dosage form and similar particle size distribution with identical manufacturing process) and bioavailability of the reference formulation has been adequately demonstrated in the target species;
d) The product is an oral dosage form which is not intended to be absorbed (e.g. antacid, radioopaque medium);
e) The product meets all of the following conditions:
   – It is an oral solution, syrup, or other similarly solubilised form;
   – It contains an active substance or therapeutic moiety in the same concentration as a product approved for use in the target species that is the subject of the new application;
   – It contains no inactive substance that can significantly affect the absorption of the active substance or therapeutic moiety.

f) The product is a reformulated product by the original manufacturer that is identical to the original product except for colouring agents, flavouring agent or preservatives, which are recognised as having no influence upon bioavailability.
g) Inhalant volatile anaesthetic solutions.

5. TYPES OF BIOEQUIVALENCE STUDIES

Bioequivalence can be demonstrated *in vivo* or, under specified conditions, *in vitro*.

5.1 In vivo study

In vivo bioequivalence testing must be conducted by using the most accurate, sensitive and reproducible approach. A classification of such approaches is listed below, in descending order of accuracy, sensitivity and reproducibility:

a) *In vivo* testing in target species in which the concentration of the active substance or therapeutic moiety or its representative metabolite(s) in blood, plasma, serum or if justified, other biological fluid or appropriate tissues is measured as a function of time. Reliance on *in vivo* bioequivalence data relies upon an assumption that the measured concentrations of active substance have meaning with respect to the objective of the trial and the intended label claim. It also necessitates that adequate drug concentrations are achieved to allow for the determination of product concentration versus time profile in the blood or other biological fluid.
b) *In vivo* testing in target species in which an appropriate acute pharmacological effect of the active substance or therapeutic moiety or its metabolite(s) is measured as a function of time. This approach can be used when analytical methods are not available. Its use requires demonstration of dose-related response. For veterinary medicinal products intended for local effect, pharmacological end-points can be the most relevant approach for the demonstration of bioequivalence.

5.2 In vitro study

In vitro bioequivalence studies could support *in vivo* bioequivalence in the following cases;

1. In vivo bioequivalence has been demonstrated for the highest dosage strength and in vitro dissolution data is used to support the bioequivalence of the lower dosage strengths for that generic formulation. In these cases, use of in vitro requires that the following conditions are all met:
   - The dosage strengths differ only by the mass of the active substance;
   - The drug is known to be associated with linear pharmacokinetics;
   - The composition of all formulations are qualitatively identical;
   - The ratio between active substances of the test- and reference product are identical;

2. In vitro comparability might be adequate to confirm the comparability of the reference product and its generic product to be administered orally. This applies particularly to immediate release oral dosage forms that are rapidly dissolving and contain drug substances that are both highly soluble and highly permeable.

3. There is a very minor formulation change to an approved product (or a minor pre-approval change to a product that has undergone extensive clinical trials), and it has been determined that the change requires only in vitro confirmation of comparability to the formulation that underwent the original clinical trials.

4. Ensuring batch to batch consistency within a product.

6. THE DESIGN OF SINGLE DOSE *IN VIVO* BIOEQUIVALENCE STUDIES

Where possible, a single dose *in vivo* bioequivalence study comparison of the products or routes of administration to be tested should be conducted with the intended target animal species. For examples of when a multiple dose in vivo bioequivalence study may be necessary, refer to section 7.

6.1 Reference product

The sponsor must justify his choice of reference product. The most appropriate reference standard is the first authorised product with a full dossier. When several approved products exist, but with different labels, claims or species, bioequivalence testing must be conducted with the reference product approved for the same indications as intended for the generic product. When several approved products containing the same active substance exist but at different concentrations, the best choice of reference product will be that with identical concentration.

The reference product should be taken from a current batch of an approved product, which contains the same active substance moiety as the new formulation, new dosage form or new salt. For example, different esters of the same therapeutic moiety are considered to be different products.

For a given product, one formulation may serve as a reference for demonstrating bioequivalence to other formulations that were part of development.

6.2 Reference route

The reference route of administration is the route for which clinical or toxicological trials were made and to which reference is given in terms of efficacy and safety.

6.3 Standards for test drug products and reference product

Both the products to be tested and the reference product will be shown to meet all compendial or other applicable standards of identity, strength, quality and purity.

6.4 Animals

Animals used in bioequivalence studies must be clinically healthy and from a homogeneous group (age, breed, weight, hormonal and nutritional status, level of production, etc.). Where possible it is advisable to restrict the studies to one gender if there is no evidence of interactions between gender and products. When it is difficult to conserve homogeneity of all animals within a study (e.g. the horse) it would be acceptable to use non-homogeneous stock provided that animals in each treatment group were carefully matched for age, weight, gender (if relevant), etc. This should be done by restricted randomisation based on the relevant blocking factor(s).

Selected animals must be from the target population for which the product is intended.

Group size: the appropriate number of animals should be carefully estimated: it depends on several factors including variance of the response, differences in the two formulations and level of rejection of the hypothesis. Crossover design has advantages in terms of power and number of animals needed.

6.5 Trial conditions

Bioequivalence should be conducted under Good Laboratory Practice (GLP).

For the oral route, special attention must be paid to the different factors that are known to affect disposition of the active substance.

Feeding may either enhance or interfere with drug absorption, depending upon the characteristics of the drug and the formulation. Feeding may also increase the inter- and intra- subject variability in the

rate and extent of drug absorption. The rationale for conducting each bioequivalence study under fasting or fed conditions should be provided in the protocol. The protocol should describe the diet and feeding regime that will be used in the study. If the reference product label indicates that the product is limited to administration either in the fed or fasted state, then the bioequivalence study should be conducted accordingly.

6.6 Dose to be tested

In general, when linear pharmacokinetic has not been demonstrated, the dose must be the approved dose. When several doses are approved for the reference product, bioequivalence testing must be conducted with the highest dose.

For a product labelled for multiple claims which may involve different pharmacological actions (e.g. therapeutic versus production claims) multiple bioequivalence studies at different doses are required if linearity has not been demonstrated.

6.7 Collection of samples

According to the type of bioequivalence trial, suitable variables to be sampled are the concentration of the active substance and/or representative metabolites in blood, serum or plasma, or the amount excreted in the urine; another possibility is the measurement of pharmacological effects.

6.8 Experimental design

In general, single dose studies should use a crossover design and, where possible, include a wash-out period of at least ten times the terminal half-life of the active substance or its metabolites. An additional period of time may be required to provide for the disappearance of any pharmacological effects such as microsomal enzyme induction, etc.. The allocation of test subjects to the treatment sequences should be randomised.

The study should be conducted as a two period, two treatment, two sequence crossover design to avoid potential confounding of period and treatment effects. The use of a high-order or replicated crossover design may be employed when there is the risk of carryover effects that could bias the treatment comparison. When a design other than a two treatment, two period, two sequence crossover is intended, it should be justified. For example, a parallel design could be recommended in cases where the wash out period is not compatible with a cross over design (e.g; drugs with long half life or studies performed on growing animals).

In order not to have to repeat studies unnecessarily, the number of test animals must be appropriate to ensure a sufficiently high probability that one is right when rejecting non-equivalence, i.e. when accepting bioequivalence. When *a priori* information exists (generic product), the number of subjects must be estimated and stated in the protocol.

6.9 Sampling Time Considerations

The total number of sampling times necessary to characterise the drug concentration profile will depend upon the curvature of the profile and the magnitude of the variability associated with the concentration values. The sampling times should be chosen so that they allow as far as possible an accurate determination of the peak concentration or the plateau time and the extent of absorption and elimination. To achieve the latter, the sampling times will usually need to extend to at least 3 elimination half-lives beyond T max. However, this duration of sampling may not be feasible with orally administered dosage forms with rapid elimination and long elimination half-lives. In these cases, product bioequivalence can be assessed on the basis of duration of sampling less than 3 times the elimination half-life time. However, the investigator must confirm that the sampling period is of adequate duration to cover the time needed to fully characterise the drug absorption phases of both test and reference products.

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

6.10 Analysis

The analytical methods used in bioequivalence studies must be fully validated to comply with standard criteria of validation as given in the VICH guideline GL1 Validation of analytical procedures: definition and terminology (CVMP/VICH/97/076).

7. THE DESIGN OF MULTIPLE-DOSE *IN VIVO* BIOEQUIVALENCE STUDIES

7.1 Basic principles

In selected circumstances it may be necessary for the test product and the reference product to be compared after repeated administration in order to determine steady-state levels of the active substance or therapeutic moiety.

A multiple dose study is required when:
a) The action of the product is dependent on steady-state concentrations of the investigated substance in the blood, or
b) The active substance shows non-linear and/or time-dependent kinetics.

A multiple dose study may be encouraged when:
a) There is excessive intrasubject variability in bioavailability, or
b) The concentration of the active substance resulting from a single dose is too low for accurate determination by the analytical method.

7.2 Reference product and trial conditions

As previously stated.

7.3 Dose to be tested

When linear pharmacokinetic cannot be assumed , the approved dosage regimen rendering the highest steady state drug concentrations (C_{SS}) (dose, dosing interval, number of administrations) must be selected.

7.4 Achievement of steady-state conditions

Steady-state conditions must be achieved, unless another approach is more appropriate for scientific reasons.

7.5 Collection of samples

Blood and/or urine samples should be taken to establish that steady-state conditions are achieved (e.g. by measuring two or more maximum (C_{max}) or minimum (C_{min}) blood, plasma or serum concentrations or by collecting approximately 10 blood samples (including just prior to administration of next dose) during dosing interval.

7.6 Steady-state parameters

When the steady state is achieved, a more complete characterisation of the blood concentration during the absorption and elimination phases of a single dose must be obtained after administration of the final dose to permit estimation of the total area under the concentration time curve, and to obtain pharmacokinetic parameters of interest. If bioavailability is assessed at steady-state, it may not be necessary to measure an elimination half-life.

7.7 Experimental design

As for single dose studies.

In a multiple dose study the wash-out period chosen should be justified.

In addition, the wash-out period must permit the disappearance of pharmacological effects that may persist after total clearance of the active substance.

8. THE DESIGN OF *IN VITRO* EQUIVALENCE STUDIES FOR ORAL DOSAGE FORMS

8.1 Basic principles

The *in vitro* test must be a validated predictor of the *in vivo* dissolution of the product, i.e. the *in vitro* test conditions has to have been previously related to *in vivo* conditions. An *in vitro* test cannot be used when the mean dissolution time is higher than the mean absorption time. Moreover, the longer is the dissolution time, the more difficult will be the extrapolation between the *in vitro* and *in vivo* conditions. It is therefore not recommended to perform *in vitro* test when the dissolution time is too long.

The equivalence of the two dissolution profiles would not be required in all cases: if the *in vitro* test is used for products administered orally in solid form when the process of dissolution is not the rate limiting step with respect to the rate and extent of absorption, the demonstration that the dissolution times of both products are sufficiently short in comparison to the absorption times could be sufficient.

8.2 Trial conditions

Conditions for performing *in vitro* bioequivalence studies should be clearly defined, e.g. pH, temperature, dissolution medium, stirring...). The use of at least three pH conditions is indicated in order to give some confidence to the extrapolation from the *in vitro* to the *in vivo* conditions. If there is no influence of pH and no studies are needed, this should be justified. The apparatus used for an *in vitro* bioequivalence study should be defined in the European Pharmacopoeia. A validated analytical method should be used to analyse the level of active substance released. Criteria for validation should be those given in the VICH guideline GL1 Validation of analytical procedures: definition and terminology (CVMP/VICH/97/076).

8.3 Collection of experimental units

The experimental units collected for *in vitro* test are tablets, defined quantities of a paste, or a powder in a specified packaging. These units are collected using a sampling plan previously established in the protocol, and based on a randomisation procedure. The plan should take into account the factors retained in the experimental design (e.g. product batches). The sampling procedure should be the same for the reference formulation and for the tested formulation. Wherever possible, the final set of experimental units of each formulation should be representative of the entire population: e.g., the number of batches from which experimental units are sampled for the *in vitro* test should be related to the expected variability between batches.

8.4 Experimental design

The experimental design should take into account the main sources of variation, which are likely to influence the final result: product batch, time of conservation, apparatus used in the test (e.g., vessel in a dissolution test). Precautions to avoid bias must be taken, such as an equal repartition of units of each formulation in each analytical run. When relevant, replicates of measures should be made, in order to take into account the variation inherent to the analytical method.

8.5 Sample size

In case of a study design comparable to the *in vivo* bioequivalence study design is relevant to be used, the sample size should be determined to provide sufficient power in the demonstration of equivalence. The residual error (coefficient of variation) used in the calculation of the sample size should be obtained from pilot studies, or estimated form the variance reproducibility of the analytical method. These points must be documented in the protocol.

9. STATISTICAL ANALYSIS OF BIOEQUIVALENCE TRIALS

9.1 Characteristics to be analysed

Pharmacokinetic parameters derived from individual blood serum or plasma curves must be analysed. To avoid potential bias, pivotal parameter comparisons should be based upon observed rather than fitted data.

9.1.1 Single dose studies

Parameters must be selected a priori (i.e. before the assay) and must be justified with regard to clinical and/or toxicological effects.

The pivotal parameters to demonstrate bioequivalence are the Area Under the concentration/time Curve (AUC) and Cmax (peak concentration). Other variables which may be relevant under some circumstances include Tmax (time to peak concentration), AUMC (Area under the Moment Curve) and MRT (Mean Residence Time) which is based upon AUMC and AUC that are values extrapolated to time infinity. The AUC t-last up to the last measured time (t-last) corresponding to a c-last exceeding the Limit Of Quantification (LOQ) should be calculated using the linear trapezoidal method. If sampling times are spaced during the elimination phase, logarithmic trapezoidal or other methods must be considered. The method of AUC calculation must be qualified; extrapolation should preferably be avoided. In all cases, it is not possible to use the AUC (0-inf) if the extrapolated AUC (between C_{last} and infinity) corresponds to more than 20% of the total AUC. Similar techniques must be used for AUMC and MRT.

The characteristics T_{max} and C_{max} are only meaningful if the maximum concentration is clearly defined and can be determined with accuracy due to suitable sampling times in the region of the maximum.

Observed and/or calculated values for C_{max} and T_{max} should be reported when pharmacokinetic modelling is used. It is recognised that T_{max} is both a function of absorption and elimination rate constants and that the power of the observed T_{max} is usually low because of discrete data. Calculation of T_{max} by a mathematical model or any fitting process is generally a more powerful approach, although modelling can be troublesome. If necessary, robust estimates of C_{max} and T_{max} (e.g. using spline interpolation) may be considered.

The Mean Residence Time (MRT) can be used as a supplementary variable when it is reflecting the Mean absorption time (MAT). MRT can only be used when MRT following IV administration has been determined in the same animals.

9.1.2 Multiple dose study

After reaching steady-state with a constant dosing interval τ, the AUC $(0, \tau)$ measured over the complete dosing interval serves as the characteristic for the extent of absorption. The average steady-state concentration (C_{SS}) where C_{SS} = AUC $(0. \tau)/\tau$ can be used as a characteristic.

The range of fluctuation between the maximum concentration ($C_{ss,\ max}$) and minimum concentration ($C_{ss,\ min}$) may be considered.

9.1.3 In vitro bioequivalence studies

Parameters must be selected *a priori* and must be justified with regard to the correlation with the pharmacokinetic. It could be sufficient to discuss the relation between the dissolution time and the absorption rate for the products compared (i.e. when the process of dissolution is not the rate limiting step with respect to the rate and extent of absorption). *In vitro* bioequivalence could be then demonstrated by comparison of dissolution profiles after fitting to a mathematical model or by comparison of parameters like 50% dissolution time and 90% dissolution time and total amount dissolved and AUC. Statistical analysis could be comparable to the analysis used in the case of an *in vivo* bioequivalence study. However, the predetermined equivalence interval should be carefully justified. It should be kept in mind that exemption of *in vivo* studies are only possible when results of *in vitro* studies could lead to the deduction of similar pharmacokinetic behaviour between the two products compared.

9.2 Criteria for bioequivalence determination (bioequivalence interval)

The criteria must be selected before the beginning of the experiment and qualified in the protocol. The bioequivalence interval must be justified with regard to pharmacological or expected clinical effects.

The decision that two or more products are bioequivalent should take into account not only the statistical significance of numerical values but also the medicinal significance of differences and intra- and intersubject variability. For certain products, greater variance in bioavailability can be tolerated because of the intended therapeutic use or because the product does not require careful patient dosage regimen.

Confidence intervals should be routinely used to interpret bioequivalence data.

For AUC, the general rule is that 90% confidence interval for the ratio of the two treatment means should be entirely contained within the limits (80%-125%). However, for compounds with a large safety margin or a large efficacy window, differences exceeding the limits can be tolerated. On the other hand, for compounds with steep dose-response curves, a 20% difference may be unacceptable.

For Cmax, the generally acceptable limits for the 90% Confidence interval are 80% to 125%. However, as the parameter may exhibit a greater variation and is strongly dependant on the sampling scheme, limits of 70% to 143% could be acceptable, when based on clinical evidence and when pre-specified in the protocol**.**

For time dependant parameters as T_{max}, (when their uses are relevant), an absolute interval of variation must be selected recognising that a ± 20% variation for a T_{max} of 10 min does not have the same meaning as a ± 20% variation for a T_{max} of 120 min. The bioequivalence range for this parameter should be carefully defined and justified.

For *in vitro* equivalence testing, the *a priori* equivalence ranges must be justified, on the basis of known relations of the in vitro criteria with pharmacokinetic or pharmacodynamic effects, when these data are available; For criteria such as the time to reach a specified level of dissolution, an absolute equivalence range is preferred, with limits set *a priori* by the sponsor.

9.3 Data analysis

Data analysis has to be given in detail. An analysis of variance (including formulation, period, sequence, animal-nested-in-sequence and, where appropriate, gender and gender-by-formulation effects) is necessary to estimate the error variance that will then be used in the calculation of the confidence interval. For AUC and C_{max}, before performing the analysis of variance, the log

transformation of data is recommended. For observed time dependent parameters, this transformation is not applicable, and it could be better to use a non-parametric approach. To conclude about bioequivalence, the upper and lower limits of the confidence interval calculated with the estimated error variance found in the ANOVA tables should be compared to the predetermined limits, i.e. 0.8-1.25 or 0.7-1.43 for log transformed data or 0.8-1.2 or 0.7-1.3 for untransformed data.

If a sequence effect has been detected, the first period of the cross over design should be analysed as a parallel design.

When several criteria are used in the demonstration of bioequivalence (general case), the final conclusion in favour of bioequivalence is taken only if the null hypothesis of non equivalence is rejected for all relevant parameters.

Other appropriate validated statistical analysing techniques could also be used.

9.4 Report

The study report and data handling should be made in accordance with the VICH GCP guideline.