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Revised guideline on the conduct of bioequivalence studies for veterinary medicinal products

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Executive summary

It is the objective of this guideline to specify requirements for the design, conduct, and evaluation of bioequivalence studies for pharmaceutical forms with systemic action. In addition, guidance is given on how *in vitro* data in specific cases may be used to allow bridging of safety and efficacy data.

1. Introduction (background)

Bioequivalence is defined as the 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. When using blood drug concentrations as a surrogate for demonstrating product bioequivalence, there is an underlying assumption that two products having an "equivalent" rate and extent of drug absorption, as measured in the blood, will be therapeutically indistinguishable and therefore interchangeable in a clinical setting. Rate and extent of absorption are typically estimated by C_{max} (peak concentration) and AUC (total exposure over time), respectively, in plasma.

Bioequivalence studies are often part of applications for generic veterinary medicinal products to allow bridging of safety and efficacy data associated with a reference veterinary medicinal product. Other types of applications may also require demonstration of bioequivalence or other comparative pharmacokinetic data (see section 4).

2. 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.

If bioequivalence cannot be demonstrated using pharmacokinetic parameters as endpoints, pharmacodynamic or clinical endpoints may be used, in exceptional circumstances, to demonstrate similar efficacy and safety. However, this situation is outside the scope of this guideline and the reader is referred to therapeutic area specific guidelines where available.

Recommendations for modified release products are given in this guideline as there are specific issues to be addressed for these products.

The scope is limited to chemical entities.

3. Legal basis

This document is intended to provide guidance on the conduct of bioequivalence studies for veterinary medicinal products. It should be read in conjunction with Directive 2001/82/EC and VICH GL52 Bioequivalence: blood level bioequivalence study (EMA/CVMP/VICH/751935/2013). Applicants should also refer to other relevant European and VICH guidelines, including those listed under References.

4. Situations when bioequivalence may be applicable

Bioequivalence data may be pivotal in a number of different situations. In the following text the level of detail differs according to the anticipated need for guidance and some parts, as indicated in the text, are applicable for generic products only.

4.1. Product development prior to the first authorisation of a veterinary medicinal product containing a new chemical entity (NCE) or a known active substance

During development of a product containing an NCE or a known active substance, bioequivalence studies or other comparative pharmacokinetic data may be needed as bridging studies between different formulations e.g. between pivotal and early clinical trial formulations.

For this purpose, bioequivalence within the acceptance criteria as defined in this document might not be needed and study designs other than those presented in this document might be found appropriate. For example, where a tolerance study (systemic tolerance to the active substance) is performed with a different formulation, it will be sufficient to show that the rate and extent of absorption from this formulation are at least as high as that for the formulation intended to be marketed.

4.2. Extensions and variations

Approvals of extensions and variations such as alternative pharmaceutical forms, new dosage strengths, new routes of administration or significant changes to manufacturing or composition which may impact on bioavailability often need the support of bioequivalence studies. Waivers from bioequivalence studies should always be justified.

4.3. Applications according to Directive 2001/82/EC as amended, Article 13(3)

This type of application refers to situations where the strict definition of a 'Generic veterinary medicinal product' as outlined in Directive 2001/82/EC, Article 13(2)(b) is not met. This includes conditions where bioavailability studies cannot be used to demonstrate bioequivalence (for example where the new product is supra-bioavailable) or where there are changes in the active substance(s), therapeutic indications, strength, pharmaceutical form or route of administration of the generic product compared to the reference veterinary medicinal product. In most cases, comparative pharmacokinetic data are needed as part of such applications.

4.4. Product containing a known substance intended to be a generic according to Directive 2001/82/EC, Article 13(2)(b)

In the case of systemically active substances when reference is made to an approved product in terms of efficacy and safety, bioequivalence to this product should be demonstrated. It should be noted that there are several aspects such as palatability, animal owner's compliance, local tolerance and residue concentrations at the injection site that might differ between products and that are not covered by bioequivalence data. The need to document such aspects might differ between applications and is beyond the scope of this guideline. It should be noted that bioequivalence or waivers cannot be used for extrapolation of withdrawal periods between products with a potential to leave local residues (for

example intramuscular and subcutaneous injectables, dermal and transdermal applications). In this case, information on the behaviour of residues at the site of administration needs to be assessed before the withdrawal period is extrapolated. It should also be noted that for formulations (i.e. active substance plus all excipients) that are qualitatively and quantitatively identical, a justification for the absence of residues data would be acceptable.

5. The design and conduct of bioequivalence studies

In the following sections, requirements for the design and conduct of bioequivalence studies are formulated. It is assumed that the applicant is familiar with pharmacokinetic principles underlying bioequivalence studies. The design should be based on a reasonable knowledge of the pharmacokinetics of the active substance and the properties of the formulation in question.

5.1. General requirements

All bioequivalence studies must be conducted in a manner that assures the reliability of the data generated. Bioequivalence studies should be conducted according to the principles of Good Laboratory Practice (GLP) and/or Good Clinical Practice (GCP), as appropriate.

Cross-over, parallel and alternative study designs

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If two formulations are compared, a randomised, two-period, two-sequence single dose crossover design is recommended.

The study design is as follows:

	Sequence A	Sequence B
Period 1	Test	Reference
Period 2	Reference	Test

Note that to eliminate potential confounding by period effects, there need to be two sequences included in the design of a two-period crossover 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.

Under certain circumstances, provided that the study design and the statistical analyses are scientifically sound, alternative well-established designs could be considered such as a parallel design.

A parallel study design may be preferable in the following situations:

- The parent compound and/or its metabolites induce physiological changes in the animal (e.g., liver microsomal enzyme induction, altered blood flow) that can alter the bioavailability of the product administered in the second period of a cross-over study.

- The parent compound and/or metabolites, or the drug product (e.g. flip-flop kinetics) has a terminal half-life so long that a risk is created of residual drug present in the blood at the time of the second-period dosing (i.e. wash-out period is not practical).
- The duration of the washout for the two-period crossover study is so long as to result in significant physiological changes in the study subjects (e.g. fast growing animals).
- The total blood volume of the species precludes the capture of blood concentration-time profiles for more than one period.

For substances with highly variable disposition where it is difficult to show bioequivalence due to high intra-individual variability, different alternative designs have been suggested in the literature (e.g. replicate study design). A replicate study design is an investigation where at least one of the treatments is repeated, using three (partial replication where for example, the reference is replicated in all subjects) or four (full replication, where each subject receives the test and reference products twice) periods. It is recommended to ask for scientific advice if it is estimated that a traditional crossover design would not be feasible without the inclusion of a very high number of animals. A two-stage (sequential) design is also possible, for example when the variability is unknown (see section 5.15). To obtain approvals in multiple regions, a 3-treatment crossover or a multiple reference parallel study design may be considered when performing one study with two different reference products, depending on the products registered in the respective regions.

Regardless of how the study will be conducted, the design should be described *a priori* in the protocol.

Single dose versus multiple dose studies

Regarding single dose versus multiple dose studies, single dose studies are preferred as the potential to detect a difference in rate of absorption is lower if the active substance is accumulated. Multiple dose designs should be justified and could be considered if, for example, problems of the sensitivity of analytical method preclude sufficiently precise plasma concentration measurements after single dose administration or there are saturable elimination processes. Both single and multiple dose studies can be conducted using a crossover study or parallel design. Due to complications associated with studies of very long duration, the use of sequential and replicate study designs are generally not recommended for multiple dose studies.

Prandial state

For the oral route, special attention must be paid to the different factors that may affect absorption of the active substance, such as feeding. 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 pharmacokinetics of the active substance. Feeding may interfere with drug absorption, depending upon the characteristics of the active substance and the formulation. Feeding may also increase the inter- and intra-individual variability in the rate and extent of drug absorption. For these reasons, fasting conditions are recommended in bioequivalence studies for canine and feline immediate-release oral formulations unless the SPC of the reference veterinary medicinal product recommends administration only in the fed state, in which case the bioequivalence study should be conducted accordingly. Fasting should be a minimum of 8 hours prior to dosing and 4 hours after dosing. The rationale for conducting a bioequivalence study under fasting or fed conditions should be provided in the protocol. The protocol should describe the diet and feeding regimen that will be used in the study.

5.2. Special considerations for modified release formulations

When bioequivalence studies are used to bridge efficacy and safety data between formulations designed to modify extent, rate or site of absorption, special consideration is needed. In veterinary medicine, there are numerous different types of modified release formulations. These could be for oral use such as prolonged release tablets for companion animals or intraruminal boluses. Many modified release formulations are topically applied, such as spot-ons and pour-ons which are absorbed through the skin, or they may be prolonged release injectable formulations. In most cases, such products are intended for single dose use. If so, single dose bioequivalence data are normally sufficient to demonstrate similarity between products. For prolonged release formulations intended for repeated dosing where the aim of the modification is to reduce fluctuations during steady state or to reduce the frequency of administration, demonstration of bioequivalence 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, C_{trough} is an important parameter to consider, in addition to C_{max} and AUC. It should be noted that C_{trough} may not be equal to $C_{min,ss}$ in the case of products with a lag time between administration of the formulation and systemic appearance of the active substance. If there is no or negligible accumulation, single dose bioequivalence data are normally also sufficient for prolonged release formulations intended for repeated dosing.

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

For pour-ons and spot-ons the main absorption route is through the skin. However, absorption may also occur from the GI-tract if the animals are licking themselves or each other. When conducting bioequivalence studies with products intended for dermal absorption, issues related to possible oral uptake need to be considered.

5.3. Special considerations for products for use in medicated feeding stuffs or drinking water or milk/milk replacer

Premixes and other pharmaceutical forms for in-feed use may be eligible for a biowaiver (see Appendix I).

Most veterinary medicinal products, excluding suspensions and emulsions, for use in drinking water, milk or milk replacer are likely to be exempted from the demand of *in-vivo* bioequivalence data (see section 7.1 and Appendix I).

In cases where *in-vivo* data cannot be waived, it is recommended to ask for scientific advice regarding the appropriate study design.

5.4. Reference and test product

For Article 13(1) and 13(3) marketing authorisation applications reference must be made to the dossier of a reference veterinary medicinal product for which a marketing authorisation is or has been granted in the European Union on the basis of a complete dossier in accordance with Articles 12 (3), 13a, 13b or 13c of Directive 2001/82/EC, as amended. The product used as the reference veterinary medicinal product in the bioequivalence study should be part of the global marketing authorisation of

the reference veterinary medicinal product (as defined in Article 5(1) the second subparagraph of Directive 2001/82/EC).

For a generic application according to Article 13(1), the test product should be compared with the same pharmaceutical form of a reference veterinary medicinal product (various immediate-release oral pharmaceutical forms shall be considered to be one and the same, Article 13(2)b of Directive 2001/82/EC). In the case of an application under Article 13(3), the test product may be compared with a pharmaceutical form differing from that of the reference veterinary medicinal product. In an application for extension of a concerned veterinary medicinal product which has been initially approved under Article 12(3) of Directive 2001/82/EC and when there are several pharmaceutical forms of this product on the market, the formulation used for the initial approval of the concerned product (and which was used in clinical efficacy and safety studies) should be used as the comparator product, unless otherwise justified.

Batch control results of the test and reference veterinary medicinal products should be reported. Unless otherwise justified, the assayed content of the batch used as the test product should not differ by more than 5% from that of the batch used as the reference veterinary medicinal product determined with the test procedure proposed for routine quality testing of the test product.

The test product used in the study should be representative of the final formulation of the product to be marketed and this should be justified by the applicant.

For example, for oral solid forms for systemic action:

a) The test product should originate from a batch of at least 1/10 of production scale unless otherwise justified.

b) The production of batches used should provide a high level of assurance that the product and process will be feasible on an industrial scale.

c) The characterisation and specification of critical quality attributes of the active substance, such as dissolution, should be established from the test batch, i.e. the clinical batch for which bioequivalence has been demonstrated.

d) Samples of the product from an additional pilot and /or full-scale production batches, submitted to support the application, should be compared with those of the bioequivalence study test batch and should show similar *in vitro* dissolution profiles when employing suitable dissolution test conditions.

   Comparative dissolution profile testing should be undertaken on the first three production batches. In case full-scale production batches are not available at the time of initial marketing authorisation, appropriate post-authorisation commitment should be provided to perform comparative dissolution studies on first three full-scale batches.

   The results should be provided at a Competent Authority's request, or if the dissolution profiles are not similar, together with proposed action to be taken.

For other immediate release pharmaceutical forms for systemic action, justification of the representative nature of the test batch should be similarly established.

The study report should include the reference product name, strength (including assayed content), dosage form, batch number, expiry date, 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.

5.5. Animals

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., C_{max}). 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.

Where the number of animals necessary to demonstrate bioequivalence cannot be precisely estimated, a two-stage approach can be chosen (see section 5.15).

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

The experimental animals should be free of any drug residues prior to the *in vivo* phase of the bioequivalence study. In some cases, the necessary drug-free period may need to exceed that associated with drug residues to account for potential physiological carryover effects that could influence the data generated in the bioequivalence trial.

Animals used in bioequivalence studies should be clinically healthy representatives of the target population. In cross-over design studies the nutritional status of the animals should be well controlled and comparable between treatments and periods if applicable (i.e. fasted or fed in case of oral administration).

In parallel design studies, the treatment groups should be homogeneous and comparable in all known prognostic variables that can affect the pharmacokinetics of the active substance e.g. age, breed, weight, gender nutritional status, level of production (if relevant). This is an essential pre-requisite to giving validity to the study results.

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

5.6. Species to be studied

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).

If bioequivalence is established based on a study where widened acceptance criteria for C_{max} have been accepted (see section 5.15), data cannot be extrapolated to any other species.

5.7. Route of administration

For applications for generic products, the route of administration should always be the same for test and reference veterinary medicinal products. When the generic product is intended for more than one route of administration (e.g. both intramuscular and subcutaneous administration), all different routes should be tested unless justified as biowaivers.

5.8. Strength to be tested

If an application concerns several strengths of the active substance, a bioequivalence study investigating only one strength may be acceptable (see section 7.2). If the strength of the test product differs from that of the reference veterinary medicinal product and this precludes equal doses in the two treatment groups, it is recommended to use different doses and then dose normalise (i.e. to divide AUC and C_{max} with the amount administered) the pharmacokinetic parameters. Prerequisites for dose normalisation are that it was prospectively defined in the protocol and that there is linear pharmacokinetics for the active substance.

Tablets intended to be divided may be divided along their score lines but not into smaller pieces.

The same strength should be administered to all animals throughout the entire study independent of their bodyweight unless the animals differ substantially in body size (see section 5.9).

5.9. Dose to be tested

For bioequivalence studies, do not dose animals according to the assay content of the test and reference batches but rather to the labelled dose.

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. In the same manner, when conducted as part of the development of a product containing a new chemical entity bioequivalence studies should be performed at the highest proposed dose or at any dose within the proposed dose range provided that dose linearity has been demonstrated.

In exceptional cases where a batch of reference product with an assay content differing by less than 5% from the test product cannot be found, the data could be dose normalised. In such cases, the procedure for dose normalisation should be pre-specified and justified by the inclusion of the results from the assay of the test and reference products in the protocol.

For some animal species e.g. the dog, it could be difficult to find animals suitable for investigation of high strength solid pharmaceutical forms. In this case, overdose studies might be considered if tolerated.

A 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 less than proportional increase in AUC with an increase in dose (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.

Most products have a single approved dose adjusted for body weight which is expressed as e.g. mg/kg body weight. Thus, exact dosing can only be achieved for pharmaceutical forms that allow an indefinite number of dose levels (such as an oral suspension). For all solid pharmaceutical forms, the amount to be administered will depend on the different strengths available and the exact dose per kg bodyweight might, therefore, vary somewhat between animals and potentially within animals over time due to change in body weight. To limit the amount of bias introduced due to difficulties regarding dose accuracy the following should be considered:

a) If there are no tolerance concerns, administration of higher or lower doses than the approved dose may be acceptable acknowledging the fact that there might not be suitable strengths available to allow the approved weight-adjusted dose to be administered to all animals included in the study.

b) 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.

c) An attempt should be made to minimise differences in weight between the test animals in order to maintain the same dose across study animals (as applicable).

d) When a solid oral pharmaceutical form is compared to a pharmaceutical form that allows an indefinite number of dose levels, the amount administered should (for both formulations) depend on the options available with the solid form.

Where relevant, doses should be rounded up based on the available strength of the solid oral dosage form, or to the nearest upper division on the dosing equipment.

Care should be taken to ensure that solid oral pharmaceutical forms are not manipulated in a way that could bias the bioequivalence study. In general, all sorts of manipulation such as grinding or filing in order to achieve equal doses should be avoided. Breaking tablets along score lines may be acceptable

if the uniformity of the scored sections can be supported by compliance with the test for subdivision of tablets detailed in the Ph. Eur. monograph for tablets. , but tablets should not be divided into smaller pieces. 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 dose administered to each animal in each period of the study.

5.10. Suprabioavailability

If suprabioavailability is found, i.e. if the test product displays an extent of absorption appreciably larger than the reference veterinary medicinal product following administration of the same dose, the bioequivalence concept could be a useful tool to demonstrate that equivalent AUC and C_{max} are achieved following administration of a lower dose of the test product as compared to the reference veterinary medicinal product. It may then be expected that the two products have similar systemic efficacy and safety although administered at different doses. It should be noted that suprabioavailable products cannot be generics, but rather applications according to Article 13(3) of Directive 2001/82/EC, as amended, or extension applications.

5.11. Analytes to be measured

Parent compound or metabolites

General recommendations

In principle, evaluation of bioequivalence should be based upon measured concentrations of the parent compound. The reason for this is that C_{max} of a parent compound is usually more sensitive to detect differences between formulations in absorption rate than C_{max} of a metabolite.

In general, product bioequivalence will be determined on the basis of the total (free plus protein-bound) concentrations of the active substance.

Inactive pro-drugs

In the context of this guideline, a parent compound can be considered to be an inactive pro-drug if it has no or very low contribution to clinical efficacy. For inactive pro-drugs, demonstration of bioequivalence for the parent compound is recommended and the active metabolite does not need to be measured. However, some pro-drugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstrating bioequivalence for the parent compound. In this situation it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of the parent compound. Applicants should provide a scientific rationale for the compound to be quantified.

Use of metabolite data as surrogate for active parent compound

The use of a metabolite as a surrogate for an active parent compound is not encouraged. This can only be considered if the applicant can adequately justify that the sensitivity of the analytical method for measurement of the parent compound cannot be improved. Due to recent developments in bioanalytical methodology, it is unusual that the parent drug cannot be measured accurately and precisely. Hence, the use of a metabolite as a surrogate for the active parent compound is expected to

be accepted only in exceptional cases. When using metabolite data as a substitute for the active parent drug concentrations, the applicant should present any available data supporting the view that the metabolite exposure will reflect the parent drug.

Enantiomers[1]

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

a) the enantiomers exhibit different pharmacokinetics

b) the enantiomers exhibit differences in pharmacodynamics

c) the exposure (AUC) ratio of enantiomers is modified by a difference in the rate of absorption

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.

Endogenous substances

If the substance being studied is endogenous, the calculation of pharmacokinetic parameters should be performed using baseline correction so that the calculated pharmacokinetic parameters refer to the additional concentrations provided by the treatment.

The method for baseline correction should be specified and justified *a priori* in the study protocol. The recommended method of baseline correction is a subtraction of the mean endogenous concentrations obtained from the pre-dose concentrations estimated at the same time on three consecutive days. If diurnal variations in the concentrations of the endogenous compound are anticipated, profiles characterising this variation may be appropriate In rare cases where substantial increases over baseline endogenous levels are seen, baseline correction may not be needed.

In bioequivalence studies with endogenous substances, it cannot be directly assessed whether carry-over has occurred, so extra care should be taken to ensure that the washout period is of an adequate duration. The length of the washout period should be addressed and justified *a priori* in the protocol. For endogenous substances, the pre-dose (baseline) drug concentrations for the first period should be comparable to the pre-dose concentrations for the second period.

5.12. 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 t_{max} to provide a reliable estimate of peak exposure. For routes of administration other than intravenous injection, the sampling schedule should be planned to avoid C_{max} being the first point of a concentration-time curve. It should also cover the plasma concentration-time curve for long enough to

[1] This section supersedes the chiral guideline in this area while it allows for the use of achiral bioanalytical methods, not only when both enantiomers show linear pharmacokinetics, but also in case of non-linearity.

provide a reliable estimate of the extent of exposure which is achieved if AUC_t is at least 80% of AUC_∞. At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant λ_z (which is needed for a reliable estimate of AUC_∞).

For active substances with a long terminal half-life, relative bioavailability can be adequately estimated using truncated AUC (and in this case AUC will be less than 80% of total systemic exposure) as long as the absorption phase has been completed during the applied sample collection period. In such cases, the duration for which samples are collected should be scientifically justified.

In multiple-dose studies, the pre-dose sample should be taken immediately before dosing and the last sample is recommended to be taken as close as possible to the end of the dosage interval to ensure an accurate determination of AUC_τ. Sampling should also be performed to show that steady state conditions are reached (i.e. trough concentrations during the loading period should be sampled until C_{trough} is stable).

For endogenous substances, the sampling schedule should allow characterisation of the endogenous baseline profile for each animal in each period.

The planned and actual timing of blood sample collections for each individual should be included in the study report.

5.13. Parameters

Actual time of sampling should be used in the estimation of the pharmacokinetic parameters.

In single dose studies AUC_t, AUC_∞, C_{max} and t_{max} should be determined and bioequivalence should be based on AUC_t and C_{max}.

In steady state studies AUC_τ, $C_{max,ss,}$ $C_{trough,}$ and $t_{max,ss}$ should be determined and bioequivalence should be based on AUC_τ, $C_{max,ss}$ and C_{trough}.

Additional parameters that may be relevant to report from studies include λ_z, $t_{1/2}$ and t_{lag}. Parameters may only be dose normalised in special cases (see section 5.8).

Non-compartmental methods should be used for determination of pharmacokinetic parameters in bioequivalence studies. The use of compartmental methods for the estimation of parameters is not acceptable.

The study report should state the method used to derive the PK parameters from the raw data.

5.14. Chemical analysis

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-Rev.1).

The bioanalytical part of bioequivalence trials should be conducted according to the principles of GLP. However, as such studies fall outside the formal scope of GLP, the sites conducting the studies are not required to be certified as part of the GLP compliance certification scheme.

The bioanalytical methods used must be well characterised, fully validated and documented to yield reliable results that can be satisfactorily interpreted.

The lower limit of quantitation should be equal to 1/20 of C_{max} or lower, as pre-dose concentrations should be detectable at 5% of C_{max} or lower (5.15, Reasons for exclusion).

Reanalysis criteria of study samples should be predefined in the study protocol (and/or SOP) before the actual start of the analysis of the samples. Normally reanalysis of study subject samples because of a pharmacokinetic reason is not acceptable. This is especially important for bioequivalence studies, as this may bias the outcome of such a study.

Analysis of samples should be conducted without information on treatment groups.

5.15. Evaluation

In bioequivalence studies, the pharmacokinetic parameters should in general not be dose normalised. However, it may be justified in exceptional cases where a reference batch with an assay content differing by less than 5% from the test product cannot be found (see section 5.9). In such cases, this should be pre-specified in the protocol and justified by the inclusion of the results from the assay of the test and reference veterinary medicinal products in the protocol if relevant.

Dose normalisation could also be accepted in cases where the strengths of the test product differ from those of the reference veterinary medicinal product and this precludes equal doses (see section 5.8).

In rare instances involving bioequivalence trials designed as a parallel study and when the drugs are administered on a mg rather than on a mg/kg basis, between-animal differences in body weight could inflate the magnitude of the residual error to an extent that a prohibitively large increase in subject numbers would be necessary to maintain study power. In these situations, the acceptability of dose normalisation and the corresponding method of data analysis should be discussed with the regulatory authorities.

Animal accountability

Ideally, all treated animals should be included in the statistical analysis.

Reasons for exclusion

Unbiased assessment of the results from randomised studies requires that all animals are observed and treated according to the same rules. These rules should be independent of treatment or outcome. In consequence, the decision to exclude an animal from the statistical analysis must be made before bioanalysis and adequate justification for removal must be provided in the study report.

There are situations that occur with sufficient frequency to require stipulation in the study protocol (e.g. vomiting or expulsion of orally administered formulations from the mouth). The criteria for removal of subject data from analysis due to vomiting (e.g. time interval between drug administration and vomiting and the allowable amount of material lost in the vomiting) should be defined *a priori* in the study protocol as well as the conditions when re-dosing after vomiting is considered to be an option in the study.

It is important that all available data be included in the statistical analysis. If for example, an animal is excluded from the second period in a crossover trial, the data gathered from that animal in the first period should not be excluded from the statistical evaluation. However, for the calculation of confidence intervals only animals which have data for both periods should be included.

To ensure that all potential statistical concerns have been addressed, descriptive statistics with and without data from animals excluded from the bioequivalence evaluation should be provided.

Exclusion of data cannot be accepted on the basis of statistical analysis or for pharmacokinetic reasons alone because it is impossible to distinguish the formulation effects from other effects influencing the pharmacokinetics.

Parameters to be analysed and acceptance criteria

The parameters to be analysed are AUC_t, C_{max} and C_{trough} (if applicable). A statistical evaluation of t_{max} 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 C_{max} and C_{trough} should also generally be within 80% to 125%.

However, as these parameters may exhibit a greater intra-individual variability, 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 C_{max}. In case of breaching of the upper acceptance limit of 125 %, then residue data to confirm the withdrawal period are required (see also section 4.4).

Statistical analysis

The assessment of bioequivalence is based upon 90% confidence intervals for the ratio of the population geometric means (test/reference) for the parameters under consideration. This method is equivalent to two one-sided tests with the null hypothesis of bioequivalence at the 5% significance level.

The pharmacokinetic parameters under consideration should be analysed using ANOVA. The AUC and C_{max} data should be transformed prior to analysis using a logarithmic transformation. A confidence interval for the difference between formulations on the log-transformed scale is obtained from the ANOVA model. This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio on the original scale. A non-parametric analysis is not acceptable.

Natural log (Ln) transformation should be used for BE evaluation because it generally improves our ability to meet the assumptions of the ANOVA. Reasons for this include:

- PK models are multiplicative rather than additive
- Ln transformation stabilises the variances
- BE comparisons are generally expressed as ratios rather than differences

Other types of data transformation will be difficult to interpret.

The precise model to be used for the analysis should be pre-specified in the protocol. The statistical analysis should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. The terms to be used in the ANOVA model are usually sequence, the

animal within a sequence, period and formulation. Fixed effects, rather than random effects, should be used for all terms.

When using a parallel study design, the treatments are generally compared using a one-way ANOVA (i.e., treatment is the sole effect being tested by the statistical model). Accordingly, the residual error (random effect) is the appropriate error for statistically comparing the test and reference products.

Other statistical methods may be appropriate, depending upon study design. The statistical model and randomisation process should be defined *a priori* in the study protocol.

Two-stage (or sequential) design

It is acceptable to use a two-stage (or sequential) approach when attempting to demonstrate bioequivalence. An initial group of animals can be treated and their data analysed. If bioequivalence has not been demonstrated an additional group can be recruited and the results from both groups combined in a final analysis. If this approach is adopted appropriate steps must be taken to preserve the overall type I error of the experiment and the stopping criteria should be clearly defined prior to the study. The analysis of the first stage data should be treated as an interim analysis and both analyses conducted at adjusted significance levels (with the confidence intervals accordingly using an adjusted coverage probability which will be higher than 90%). For example, using 94.12% confidence intervals for both the analysis of stage 1 and the combined data from stage 1 and stage 2 would be acceptable, but there are many acceptable alternatives and the choice of how much alpha to spend at the interim analysis is at the company's discretion. The plan to use a two-stage approach must be pre-specified in the protocol along with the adjusted significance levels to be used for each of the analyses.

When analysing the combined data from the two stages, a term for stage should be included in the ANOVA model.

Presentation of data

All individual concentration data and pharmacokinetic parameters should be listed by formulation together with summary statistics such as geometric mean, median, arithmetic mean, standard deviation, a coefficient of variation, minimum and maximum. Individual plasma concentration/time curves should be presented in linear/linear and log/linear scale. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The number of points of the terminal log-linear phase used to estimate the terminal rate constant (which is needed for a reliable estimate of AUC_{∞}) should be specified.

For the pharmacokinetic parameters that were subject to statistical analysis, the point estimate and 90% confidence interval for the ratio of the test and reference veterinary medicinal products should be presented.

For single dose studies, the percentage of AUC_{∞} that is covered by AUC_t should be reported for each animal in each period.

The ANOVA tables, including the appropriate statistical tests of all effects in the model, should be submitted. For the normal two-period, two sequence crossover design, the presentation should include a 2x2-table that presents for each sequence (in rows) and each period (in columns) means, standard deviations and number of observations for the observations in the respective period of a sequence. In addition, tests for difference and the respective confidence intervals for the treatment effect, the period effect, and the sequence effect should be reported as descriptive data.

The report should be sufficiently detailed to enable the pharmacokinetics and the statistical analysis to be repeated, e.g. data on actual time of blood sampling after dose, drug concentrations and the values of the pharmacokinetic parameters for each animal in each period and the randomisation scheme should be provided.

Drop-out and withdrawal of animals should be fully documented. If available, concentration data and pharmacokinetic parameters from such animals should be presented in the individual listings, but should not be included in the summary statistics.

6. Study report

6.1. Bioequivalence study report

The report of the bioequivalence study should give the complete documentation of its protocol, conduct and evaluation. Although bioequivalence studies are normally conducted to GLP standard, the animal phase of the report should be written in accordance with the structure of VICH GL9.

Names and affiliations of the responsible investigator(s), the site of the study and the period of its execution should be stated. Audit certificate(s), if available, should be included in the report.

The study report should include evidence that the choice of the reference veterinary medicinal product is in accordance with Article 13(1) and Article 13(2) of Directive 2001/82/EC, as amended. This should include the reference veterinary medicinal product name, strength, pharmaceutical form, batch number, manufacturer, expiry date and country of purchase.

The name and composition of the test product(s) used in the study should be provided. The batch size, batch number, manufacturing date and, if possible, the expiry date of the test product should be stated.

Certificates of analysis of reference and test batches used in the study should be included in an appendix to the study report.

Concentration and pharmacokinetic data and statistical analyses should be presented in the level of detail described above (section 5.15, *Presentation of data*).

6.2. Other data to be included in an application

The bioanalytical method should be documented in a pre-study validation report. A bioanalytical report should be provided as well. The bioanalytical report should include a brief description of the bioanalytical method used and the results for all calibration standards and quality control samples.

A representative number of chromatograms or other raw data should be provided covering the whole concentration range for all standard and quality control samples as well as the specimens analysed. This should include all chromatograms from at least 20% of the animals with QC samples and calibration standards of the runs including these animals.

The applicant should submit a signed statement confirming that the test product has the same quantitative composition and is manufactured by the same process as the one submitted for authorisation. A confirmation as to whether the test product is already scaled-up for production should be submitted. Comparative dissolution profiles (see section 7.2) should be provided.

7. Waivers from bioequivalence study requirements for immediate release formulations

7.1. Comparisons between formulations

The formulation and the characteristics of the active substance are factors which may affect the requirements regarding support of data from bioequivalence studies. When the test product contains a different salt, ester, ether, isomer, a mixture of isomers, complex or derivative of an active substance from the reference veterinary medicinal product, bioequivalence should be demonstrated in *in-vivo* bioequivalence studies. However, when the active substance in both test and reference veterinary medicinal products is identical (or the products contain salts with similar properties as defined in Appendix I, section III), *in-vivo* bioequivalence studies may in some situations not be required as described below and in Appendix I.

Studies to compare the rate and extent of absorption between two formulations or products containing identical active substances are generally not required if both products fulfil one or more of the following conditions:

a) The product is to be administered solely as an aqueous intravenous solution containing the same active substance as the currently approved product. However, if any excipients interact with the active substance (e.g. complex formation), or otherwise affect the disposition of the active substance, a bioequivalence study is required unless both products contain the same excipients in very similar quantity and it can be adequately justified that any difference in quantity does not affect the pharmacokinetics of the active substance.

b) For products intended for intramuscular, subcutaneous or systemically acting topical administration, bioequivalence studies are not required in cases when the product is of the same type of solution, contains the same concentration of the active substance and comparable excipients in similar amounts as the reference veterinary medicinal product, if it can be adequately justified that the difference(s) in the excipient(s) and/or their concentration have no influence on the rate and/or extent of absorption of the active substance.

c) If the test product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an approved reference veterinary medicinal product presented as an aqueous oral solution at time of administration, bioequivalence studies may be waived if the excipients contained in it do not affect gastrointestinal transit (e.g. sorbitol, mannitol), absorption (e.g. surfactants or excipients that may affect transport proteins), solubility (e.g. co-solvents) or *in-vivo* stability of the active substance. Any difference(s) in the amount(s) of excipients should be justified by reference to other data; otherwise, an *in-vivo* bioequivalence study will be required. The same requirements for similarities in excipients apply for oral solutions as for biowaivers according to the relevant criteria (see Appendix I, section IV.2).

d) The formulations are identical (identical active substances and excipients as well as physicochemical properties [e.g. identical concentration, dissolution profile, crystalline form, pharmaceutical form and particle size distribution with identical manufacturing process]).

e) The products are classified as biowaivers in accordance with principles underlying the BCS (see Appendix I).

f) The product is intended to be a gas for inhalation at the time of administration.

g) The product is a reformulated product by the original manufacturer that is identical to the original product except for small amounts of colouring agents, flavouring agents, preservatives or other excipients, which are recognised as having no influence on bioavailability.

7.2. Comparisons between strengths

If an application concerns several strengths of the active substance, a bioequivalence study investigating only one strength may be acceptable provided *in vitro* equivalence data are presented for additional strengths. A pre-requisite is that all of the following conditions are fulfilled:

a) the pharmaceutical products are manufactured by the same manufacturing process;

b) the qualitative composition of the different strengths is the same;

c) the composition of the strengths is quantitatively proportional, i.e. the ratio between the amount of each excipient to the amount of active substance(s) is the same for all strengths (for immediate release products, coating components, capsule shell, colour agents and flavours are not required to follow this rule). If there is some deviation from the quantitatively proportional composition, condition c) is still considered fulfilled if conditions i) and ii) **or** i) and iii) below apply to the strength used in the bioequivalence study and the strength(s) for which a waiver is considered:

    i. the amount of the active substance(s) is less than 5 % of the tablet core weight, or of the weight of the capsule content (in the case of capsules),

    ii. the amounts of the different core excipients or capsule content are the same for the concerned strengths and only the amount of active substance is changed,

    iii. the amount of a filler is changed to account for the change in amount of active substance. The amounts of other core excipients or capsule content should be the same for the concerned strengths;

d) appropriate *in vitro* dissolution data should confirm the adequacy of waiving additional *in-vivo* bioequivalence testing.

The criteria above apply also to the situation where there are several strengths of a generic immediate release product to be approved. If one of the strengths is found to be bioequivalent with the reference veterinary medicinal product, *in vitro* data could be sufficient to document bioequivalence for the other strengths of the generic application. The similarity of *in vitro* dissolution should be demonstrated at all conditions within the applied product series, i.e. between additional strengths and the strength(s) (i.e. batch(es)) used for bioequivalence testing.

The conditions regarding proportional composition should be fulfilled for all active substances of fixed combinations. When considering the amount of each active substance in a fixed combination the other

active substance(s) can be considered as excipients. In the case of bilayer tablets, each layer may be considered independently.

At pH values where sink conditions may not be achievable for all strengths *in vitro* dissolution may differ between different strengths. However, the comparison with the respective strength of the reference veterinary medicinal product should then confirm that this finding is active substance rather than formulation related. In addition, the applicant could show similar profiles at the same dose (e.g. as a possibility two tablets of 5 mg versus one tablet of 10 mg could be compared).

General aspects of *in vitro* dissolution experiments are briefly outlined in section 8, including basic requirements for use of the similarity factor (f2-test).

8. Dissolution testing

During the development of a veterinary medicinal product, a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the active substance. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistency and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, in certain instances, a dissolution test can be used to demonstrate bioequivalence. Therefore, dissolution studies can serve several purposes:

a) Testing on product quality

- To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control.
- To be used as a tool in quality control to demonstrate consistency in manufacture.
- To get information on the reference veterinary medicinal product used in bioavailability/bioequivalence studies and pivotal clinical studies.

b) Bioequivalence surrogate inference

- To demonstrate in certain cases similarity between different formulations of an active substance and the reference veterinary medicinal product (biowaivers e.g., variations, formulation changes during development and generic products).
- To investigate batch to batch consistency of the products (test and reference) to be used as a basis for the selection of appropriate batches for the *in-vivo* study.

Unless otherwise justified, the specifications for the *in vitro* dissolution to be used for quality control of the product should be derived from the dissolution profile of the test product batch that was found to be bioequivalent to the reference veterinary medicinal product. In the event that the results of comparative *in vitro* dissolution of the biobatches do not reflect bioequivalence as demonstrated *in-vivo,* the latter prevails. However, possible reasons for the discrepancy should be addressed and justified.

Test methods should be developed which are product-related and based on general and/or specific pharmacopoeial requirements. If those requirements are shown to be unsatisfactory and/or do not reflect the *in-vivo* dissolution (i.e. biorelevance) alternative methods can be considered when it is justified that these are discriminatory and able to differentiate between batches with an acceptable and non-acceptable performance of the product *in-vivo*. Current state-of-the-art information including the

interplay of characteristics derived from the BCS classification and the pharmaceutical form must always be considered.

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. For rapidly dissolving products, where complete dissolution is within 30 minutes, generation of an adequate profile by sampling at 5- or 10-minute intervals may be necessary.

If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pH-range and the excipients are known not to affect bioavailability. A bioequivalence study may in those situations be waived based on similarity of dissolution profiles which are based on discriminatory testing, provided that the other biowaiver criteria in Appendix I are met. The similarity should be justified by dissolution profiles attained at three different buffers spanning the range of possible physiological pH values for the concerned species (e.g. pH 1.2, 4.5 and 7.5).

In contrast, if an active substance is considered to have a limited or low solubility, the rate limiting step for absorption may be pharmaceutical form dissolution. This is also the case when excipients are controlling the release and subsequent dissolution of the active substance. In these cases a variety of test conditions is recommended and adequate sampling should be performed.

If the active substance has been demonstrated to be insoluble in classical dissolution media surfactants may be used in case of comparative dissolution testing between different strengths or variations in composition, manufacture, etc., in the lowest possible concentration where the dissolution test has sufficient discriminative power.

Similarity of dissolution profiles

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.

For modified release products, the advice given in the relevant guidance should be followed.

Dissolution similarity may be determined using the $f2$ statistic as follows:

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

In this equation $f2$ 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 $f2$ statistic is not suitable, then the similarity may be compared using model-independent or model-dependent methods e.g. by statistical multivariate comparison of the parameters of the Weibull function or the percentage dissolved at different time points.

Alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified.

The similarity acceptance limits should be pre-defined and justified and not be greater than a 10% difference. In addition, the dissolution variability variance of the test and reference veterinary medicinal product data should also be similar, however, a lower variability of the test product may be acceptable.

Evidence that the statistical software has been validated should also be provided.

A clear description and explanation of the steps taken in the application of the procedure should be provided, with appropriate summary tables.

Definitions

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

ANOVA: Analysis of variance model

BCS: Biopharmaceutics Classification System, see Appendix I

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.

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

Comparative pharmacokinetic studies: Any study which compares the pharmacokinetics between products that contain the same active substance. A bioequivalence study is an example of a comparative pharmacokinetic study.

Dose: Amount of active substance(s), to be given to an animal; it is often expressed in mg/kg body weight.

Immediate release formulations: Formulations showing a release of the active substance(s) which is not deliberately modified by a special formulation design and/or manufacturing method. In the case of a solid pharmaceutical form, the dissolution profile of the active substance depends essentially on its intrinsic properties.

Modified release formulations: Formulations where the rate and/or place of release of the active substance(s) is different from that of a conventional-release pharmaceutical form administered by the same route. This deliberate modification is achieved by a special formulation design and/or manufacturing method. Modified-release pharmaceutical forms include prolonged-release, delayed-release and pulsatile-release pharmaceutical forms.

Prolonged-release pharmaceutical forms: Prolonged-release pharmaceutical forms are modified-release pharmaceutical forms showing a slower release of the active substance(s) than that of a conventional-release pharmaceutical form administered by the same route. Prolonged-release is achieved by a special formulation design and/or manufacturing method. Prolonged-release pharmaceutical forms include e.g. slow-release intramuscular or subcutaneous injections.

Delayed-release pharmaceutical forms: Delayed-release pharmaceutical forms are modified-release pharmaceutical forms showing a release of the active substance(s) which is delayed. Delayed release is achieved by a special formulation design and/or manufacturing method. Delayed-release pharmaceutical forms include gastro-resistant preparations.

Pulsatile-release pharmaceutical forms: Pulsatile-release pharmaceutical forms are modified-release pharmaceutical forms showing a sequential release of the active substance(s). The sequential release is achieved by a special formulation design and/or manufacturing method. Pulsatile-release pharmaceutical forms include e.g. intraruminal pulse-release devices.

NCE: New chemical entity

Strength: The amount of active substance(s) included in a certain formulation.

Pharmacokinetic parameters

AUC_t:	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;
AUC_{tau}:	AUC during a dosage interval at steady state; mathematically, the quantity equals AUC_∞ of the first dose if there is linear (non-saturable) pharmacokinetics.
C_{max}:	Maximum plasma concentration;
$C_{max,ss}$:	Maximum plasma concentration at steady state;
$C_{min,ss}$:	Minimum plasma concentration at steady state; in the absence of a measurable delay between drug administration and the first appearance of drug in the systemic circulation $C_{min,ss}$ equals C_{trough}.
C_{trough}:	plasma concentration at steady state immediately prior to the administration of a next dose;
t_{max}:	Time until C_{max} is reached;
$t_{max,ss}$:	Time until $C_{max,ss}$ is reached;
t½:	Plasma concentration half-life;
λ_z:	Terminal rate constant;
t_{lag}	Absorption lag time

References (scientific and/or legal)

CHMP guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009)

Guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/EWP/133/99)

Guideline on Fixed Combination Products (EMEA/CVMP/83804/2005)

Guideline for investigations of chiral substances (EMEA/CVMP/128/95)

Guideline on statistical principles for veterinary clinical trials (CVMP/816/00)

Good Clinical Practice (GCP) VICH GL9 (CVMP/VICH/595/1998)

Good Laboratory Practice (GLP) (see Council Directive 88/320/EEC as amended)

Quality of Modified Release Pharmaceutical forms for Veterinary Use (EMEA/CVMP/680/02)

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

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 species differences of relevance 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, extensions of innovator products, variations that require bioequivalence testing, and between early clinical trial products and to-be-marketed products.

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; for details see section III), and
- 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 (see section IV.1), and
- excipients that might affect bioavailability are qualitatively and quantitatively the same. In general, the use of the same excipients in similar amounts is preferred (see section IV.2).

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; for details see Annex section III), and
- 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 (see section IV.1), and
- excipients that might affect bioavailability are qualitatively and quantitatively the same and other excipients are qualitatively the same and quantitatively very similar (see section IV.2).

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

Generally, sound peer-reviewed literature may be acceptable for known compounds to describe the particular characteristics of the active substance required in this biowaiver concept.

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; see sections III.1 and III.2). 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.

It is recommended to ask for scientific advice before applying the BCS approach to products containing pro-drugs.

III.1 Solubility

The pH-solubility profile of the active substance should be determined and discussed. 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. It is strongly recommended to ask for scientific advice well in advance of any such submission to ensure consistency. Replicate determinations at each pH condition may be necessary to achieve an unequivocal solubility classification (e.g. shake-flask method or another justified method). Solution pH should be verified prior and after addition of the active substance to a buffer.

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 (see section 8 of the guideline). *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.

Test and reference veterinary medicinal products should meet requirements as outlined in section 5.4 of the main guideline text. In line with these requirements, it is advisable to investigate more than one single batch of the test and reference veterinary medicinal products.

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; Ph.Eur. buffers recommended)

- Other conditions: no surfactant; in case of gelatin capsules or tablets with gelatin 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 laurilsulfate 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 (as appropriate to the SPC directions) 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.