EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 December 2023
EMA/CVMP/EWP/133/1999-Rev.1
Committee for Veterinary Medicinal Products (CVMP)

Guideline on the conduct of pharmacokinetic studies in target animal species

Initial guideline adopted by CVMP	8 March 2000
Date of coming into effect	8 September 2000
Draft revised guideline (revision 1) agreed by Efficacy Working Party (EWP-V)	13 September 2017
Adopted by CVMP for release for consultation	9 November 2017
Start of public consultation	20 November 2017
End of consultation (deadline for comments)	31 May 2018
Agreed by EWP-V	October 2023
Adopted by CVMP	7 December 2023
Date for coming into effect	1 July 2024

This guideline replaces the current CVMP guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/99-FINAL).

Keywords	***pharmacokinetics, target animal species, PK/PD, dose finding, veterinary***

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000
An agency of the European Union

Guideline on the conduct of pharmacokinetic studies in target animal species

Table of contents

Executive summary

The objectives of this guideline are to specify the pharmacokinetic factors to be investigated, acknowledging that this will depend on the active substance and its use, and to provide recommendations for the conduct of pharmacokinetic studies with a veterinary medicinal product in the target animal species. The purpose of these studies is ultimately to support the design and interpretation of efficacy and safety studies (pre-clinical and clinical). In addition, general guidance is given on pharmacokinetic-pharmacodynamic modelling and population pharmacokinetics, should applicants opt to pursue these approaches.

1. Introduction (background)

In general, pharmacokinetic studies can be carried out to support clinical efficacy, safety in the target animal, and safety for the consumer, the user and the environment. The principal objectives are to characterise the absorption, distribution, metabolism and excretion of the active substance(s) of the product for which authorisation is sought.

Pharmacokinetic studies, particularly in conjunction with pharmacodynamic data, are important to support effective and safe dosage regimens. Accordingly, a section dedicated to pharmacokinetic-pharmacodynamic modelling approaches has been added to this revised guideline. In addition, pharmacokinetic studies may be used to establish dosage regimens according to various population variables (e.g. age, breed, disease status). To address this, a section on the population pharmacokinetic analyses has been included. Finally, pharmacokinetic studies can also highlight potential drug interactions, either between the active substance(s) and those in concomitantly administered products or between active substances intended for a fixed combination product.

2. Scope

The objective of this document is to provide guidance to applicants on the design, conduct and analysis of pharmacokinetic investigations of a given active substance in a specific pharmaceutical form in the target species. This may be a systemically-acting substance or a locally-acting substance with potentially unintended systemic effects, irrespective of the pharmacological class of the active substance or the animal species in which use of the product is intended. Guidance on studies to investigate consumer safety or studies to assess the pharmacokinetics of substances in biological products is outside the scope of this document. For more information on the pharmacokinetic and residue studies in target species required to address consumer safety, the reader is referred to VICH GL46, GL47 and GL48 on studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals.

The specific methodology to demonstrate bioequivalence between products through pharmacokinetic studies (e.g. in the context of a generic procedure) is not in the scope of the present guideline; it is addressed in the CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000) and in VICH GL52 - Bioequivalence: blood level bioequivalence study.

This guideline only considers general principles and not all points mentioned necessarily apply to each active substance and all target species. Therefore, each study should be planned and designed to take into account the properties and uses of the active substance and the anatomical, physiological and behavioural peculiarities of the species in which the active substance is investigated. For pharmacokinetic studies in fish, it is recommended that the CVMP Guideline on demonstration of target

animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish (EMA/CVMP/EWP/459868/2008) is also consulted.

Moreover, this guideline provides information on the reporting of pharmacokinetic-pharmacodynamic modelling and population pharmacokinetic studies.

3. Legal basis

This document should be read in conjunction with Regulation (EU) 2019/6. Applicants should also refer to relevant European and VICH guidelines, including those listed under 'References'.

In accordance with Annex II of the aforementioned Regulation, all experiments on animals should be conducted taking into account the 3R principles (replacement, reduction and refinement) as laid down in Directive 2010/63/EU on protection of animals used for scientific purposes.

4. Pharmacokinetic factors to be investigated

Depending on the active substance and its use(s), all or some of the following items should be studied: absorption, distribution, metabolism and excretion.

4.1. Absorption

Both the rate and extent to which the active substance or active moiety are available systemically should be determined.

Generally, the rate and extent of absorption can be determined only from plasma/blood concentration-time curve data following extravascular administration. For orally administered products, the systemic exposure is a result of the absorption from the gastro-intestinal tract and of a possible first-pass effect due to metabolism in the liver.

4.1.1. Active substances intended to produce systemic effects

Whatever the route of administration (e.g. oral, intramuscular, subcutaneous, transdermal, inhalational) of the veterinary medicinal product, the rate and extent of absorption of the active substance should be quantified.

Whenever possible, comparison with an intravenous dose should be made, as only intravenous (bolus or infusion) data allow for the evaluation of the absolute bioavailability.

Preferably, a complete pharmacokinetic analysis of the plasma/blood concentration profile should be carried out. This refers particularly to formulations for which a delayed release of the active substance or a prolonged duration of action is claimed. Deviations from this should be justified. As a minimum, the following parameters should be determined for the active substance(s) and/or relevant metabolite(s): concentration at peak (C_{max}), time to reach peak concentration (T_{max}), and area under the concentration/time curve (AUC_{∞} and AUC_t).

The pharmacokinetic studies should be conducted in accordance with the intended use of the product. For orally administered products, it may also be necessary to evaluate the effect of the feeding state (e.g. fed versus fasted) on the rate and extent of absorption of the active substance by means of bioavailability studies.

4.1.2. Active substances not intended to produce systemic effects

Confirming that systemic exposure to the active substance(s) is not of a magnitude at which systemic effects could be elicited may be sufficient to waive further pharmacokinetic investigations. However, a low bioavailability does not necessarily infer an absence of systemic effects since plasma concentrations may still be sufficiently high to produce such effects (e.g. if the administered dose is high). Furthermore, certain active substances can produce systemic effects at very low plasma concentrations (e.g. corticosteroids, antimicrobial agents, ectoparasiticides) or when presented in a particular pharmaceutical form (e.g. intrauterine, intramammary formulations). Therefore, the lower limit of quantification for the analytical method should be justified.

As an alternative to *in vivo* studies, the use of *in vitro*/*in silico* models to demonstrate non-absorption of the active substance(s) should be considered. Models must however be suitably validated and relevant to the species for which the product is intended. *In vitro* testing should preferably involve the final product formulation, unless it can be justified that the excipients and physico-chemical properties of the product will not have a significant impact on (non-)absorption.

4.2. Distribution

Unless otherwise justified, the distribution of the active substance and/or relevant metabolites should be investigated. Distribution of an active substance (and/or its metabolite(s)) in relevant body fluids (e.g. cerebrospinal fluid, synovial fluid, mucus, milk) and tissues should be determined where this may provide supportive information for the clinical efficacy of active substances, or highlight potential safety concerns (e.g. significant distribution of an active substance to renal tissue may highlight potential dose-limiting nephrotoxicity).

Extensive protein binding can significantly increase the variability of drug effects, e.g. as a result of a pathological process. Therefore, the extent of binding of the active substance (and/or relevant metabolites) to proteins should be studied over the anticipated range of drug concentrations in plasma or other relevant biological matrices. This may be obtained from *in vitro* experiments.

The volume of distribution (Vd) should be reported, as a measure of the extent of distribution to tissues, determined indirectly from the plasma drug concentration following intravenous administration. The V_d can be used (together with bioavailability) to calculate the dose corresponding to a desired plasma (unbound) concentration, e.g. a loading dose.

4.3. Metabolism

Unless otherwise justified, the formation of metabolites should be investigated. This should comprise the identification of metabolites present in potentially clinically significant amounts, and also the determination of the major pathways involved in the metabolism of the active substance, in order to establish potential interactions. *In vitro* methods (e.g. microsomal and hepatocyte assays) should be considered first as an option to generate such data, if adequately validated.

If there is an indication that pharmacologically or toxicologically active metabolites are formed, which may contribute to the therapeutic activity and/or adverse effects, then the rate of their formation, distribution and excretion should be investigated in the target species.

4.4. Excretion

The relative contribution of the different routes of excretion of the total substance [active substance + metabolite(s)] should be quantified (e.g. expressed as a percentage of the administered dose). For example, it is useful to know the fraction of the dose subjected to renal and/or hepatic clearance in order to predict the influence of renal and/or hepatic disease on the excretion of the active substance from plasma.

5. Methodology and conditions of study

Studies should generally be conducted according to Good Laboratory Practice (GLP).

In general, pharmacokinetic studies in the target animal should include:

(i) preliminary studies, usually single-dose, investigating the overall pharmacokinetic behaviour of different dose levels and/or formulations. The need for these studies will depend on the availability of existing data (e.g. from literature or laboratory animal studies) relevant for the intended active substance, pharmaceutical form, and target species.

(ii) studies establishing the profile of the final dosing regimen to be used in clinical trials.

The final formulation intended for marketing should normally be used in these studies. Failing this, it should be justified that the test and final formulations can be considered as bioequivalent according to current criteria (see guideline EMA/CVMP/016/2000).

The use of *in vitro*/*in silico* test systems to replace or reduce animal studies should be considered on a case-by-case basis. The validity and reliability of the used test methods should be demonstrated.

5.1. Animals

Studies should be performed in the target species under well-defined and controlled conditions. The breed, age, physiological status (e.g. pregnant, lactating, neutered) and gender should be specified and justified.

Basic pharmacokinetic studies should be carried out under laboratory conditions in healthy animals. The number of animals used should be justified. However, if it is known or suspected, e.g. through peer-reviewed literature, that the pharmacokinetics of the active substance (and/or its metabolites) are likely to be significantly altered by the treated disease or by a common concomitant condition, consideration should be given to investigating pharmacokinetics in diseased animals enrolled in pre-clinical (dose determination and dose confirmation) studies or in clinical trials (see also section 6.2).

If a PK/PD modelling approach, as outlined in section 6.1, has been chosen as a means to select the dosage regimen, the pharmacokinetic data obtained from this(ese) study(ies) (generally conducted in experimental disease models) may be sufficient to satisfy the requirements for pharmacokinetic data.

5.2. Administration

Special attention should be given to the route and method of administration of the veterinary medicinal product, as this may affect the absorption of the active substance.

For administration of the active substance to individual animals, the dose should be expressed on a body weight (mg/kg bw) basis; if the dose is intended to be on a body surface area basis, it should be

expressed both on a body weight and body surface area basis. The procedure used to estimate body surface area should be described.

In case of a solid formulation which cannot be administered precisely on a mg/kg body weight basis (e.g. a tablet), the number of dosing units should be administered as recommended in the SPC, and the actual dose of active substance administered to animals should be calculated based on their individual body weights.

When the product is administered via feed or drinking water, the daily dose of the active substance in mg/kg bw should be calculated, preferably on an individual animal basis. If the exact dose per animal cannot be measured, the dose should be estimated based on the following parameters: number of animals per group, average bodyweight, concentration of active substance in the feed or drinking water, and average feed or drinking water intake. Since the concentration-time profile for an active substance can be affected by the method of administration, administration of such products by oral gavage will only be accepted if suitable for the purpose of the study, e.g. in preliminary studies for the determination of basic pharmacokinetic properties.

5.3. Fixed combinations

When combining substances into a fixed combination product, unintended pharmacokinetic interactions might occur, leading to an altered exposure. Alternatively, an interaction may be the intention of combining substances, e.g. combination with a metabolism inhibitor. In order to evaluate possible pharmacokinetic interactions, the concentration-time profile should be determined for each individual active substance when administered as a mono-substance product and compared to the concentration-time profile of the active substances when administered as the combination product, unless otherwise justified.

The study(ies) should be designed based on the expected behaviour of the substances in combination.

When efficacy and target animal safety of a combination product are intended to be supported by data from single substance products and an absence of interaction is claimed, generally the results of comparative PK study(ies) should be interpreted together with clinical data ultimately showing the absence of clinically significant interactions, in respect of both safety and efficacy. In special cases where such extrapolation from single substance products is intended to be based only on pharmacokinetic data, it is recommended to use bioequivalence testing, in accordance with current criteria (see CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)). Superiority testing is not adequate to conclude on an absence of interaction as a non-significant outcome may relate to a low statistical power.

Where appropriate, the CVMP Guideline on pharmaceutical fixed combination products (EMA/CVMP/83804/2005) should be considered.

5.4. Dosing

In general, the pharmacokinetics of the active substance(s) should be determined at the recommended dosage regimen in the target species.

5.4.1. Dose proportionality

Investigation of dose proportionality in the target species is important to facilitate prediction of the effects of dose adjustments, and this should be investigated during the early phases of product development. These data may be collected in dose determination or PK/PD studies, where sufficient existing PK data are available to support the selection of the tested dose levels.

For an active substance that has not previously been used in a veterinary medicinal product in the target species, pharmacokinetic studies using at least three different dose levels should be performed. The choice of dose levels should be justified. Appropriate statistical tests should be carried out to determine dose proportionality.

For established active substances where a range of therapeutic doses is recommended and dose proportionality is documented in the target species, studies with a single dose level, corresponding to the highest intended therapeutic dose, are generally sufficient. Where there is no dose proportionality or a very steep dose/effect curve, studies using three different dose levels, encompassing the dose range, may be necessary.

For established active substances, pharmacokinetic studies with a single dose level may be sufficient where a single dose level is recommended. The accuracy of dosing should be considered when designing such studies, particularly in cases where there is a solid formulation, e.g. tablet.

5.4.2. Accumulation

If one treatment course requires repeated administration, repeated dose studies should be performed, according to the dosing recommendations. In the case of products intended for long-term continuous use, the duration of such studies should exceed the time required to reach steady-state.

Repeated dose studies should be conducted using the recommended dosage regimen (dose, dosing interval, number of administrations); such studies should give insight into questions such as accumulation kinetics, steady-state levels and induced effects (e.g. altered metabolism rate and altered disposition). Comparison of plasma concentration profiles after administration of the first dose and under steady-state conditions is highly desirable. An appropriate accumulation ratio should be calculated (see Definitions).

Products intended to be administered on a single occasion and containing an active substance with a long half-life (e.g. some antiparasitic VMPs) may cause accumulation if another treatment is necessary. Where such concern exists, repeated-dosing PK data should be provided, taking into account the expected interval of administration, in order to assess accumulation. The rule of thumb that steady-state conditions are reached after approximately 4-5 half-lives (i.e. around 95% of the plateau) should be considered when planning the duration of a repeated-dose study.

Repeated dosing followed by an examination of the washout period may be useful to detect the existence of a slow elimination phase which might not be detected following a single dose.

In general, derivation of pharmacokinetic data relating to repeated dosing from dose confirmation or target animal safety studies may be acceptable.

5.5. Sampling

Suitable biological fluids and tissues, if appropriate, should be selected for pharmacokinetic investigation. Plasma is generally considered to be the most useful biological fluid for such studies.

5.5.1. Blood sampling

Attention should be given to the site of blood collection, sampling procedure, the material used for sampling, blood collecting tubes, anticoagulant and conditions of centrifugation to obtain plasma. The stability of the substance during sampling and under conditions of storage pending analysis should be assessed.

The number of blood samples and the timing of sampling should be appropriate to allow adequate determination of absorption, distribution and elimination phases. With regard to absorption, there should be a sufficient number of samples taken around the anticipated T_{max} to ensure a reliable estimate of peak exposure (C_{max}).

To investigate the distribution and elimination phases, blood samples in the post-absorption phase should be obtained over as long a period of time as is necessary for the purpose of the investigation. At least three samples are needed during the terminal log-linear phase in order to reliably estimate the elimination rate constant and obtain accurate estimation of AUC_{∞}. In addition, unless otherwise justified, AUC_t should equate to at least 80% of AUC_{∞} to achieve a reliable estimate for the extent of exposure.

5.5.2. Other biological fluids and tissues

In some cases, the collection of other biological fluids and/or tissues may be considered appropriate for the determination of pharmacokinetic parameters (e.g. if analytical constraints limit the usefulness of blood samples, urine samples may be used to determine the terminal disposition slope if this is the main route of excretion) or parameters of particular interest (e.g. local distribution to support a claim). The choice of biological fluid and/or tissue should be justified.

Collection of some of these fluids requires special attention (e.g. immediate pH measurement of urine, conditions of storage).

According to Directive 2010/63/EU, 'special attention should be paid to ascertain the absence of pain and discomfort when using a biopsy method'; therefore, repeated biopsies using local anaesthesia are only acceptable in those cases where no other sampling techniques are possible.

5.6. Analytical procedure

Active substance (and its metabolite(s)) concentrations should be determined using appropriate analytical methods. When relevant, the pharmacokinetics of isomers of the active substance should be considered (see CVMP Guideline on investigation of chiral active substances (EMEA/CVMP/128/95)). The omission of pharmacokinetic data for inactive enantiomers is acceptable, provided that their lack of pharmacological and toxicological activity is sufficiently justified.

The use of a chemical assay method is preferred (e.g. HPLC methods). The method and its validation should be accurately described using an internationally accepted format. For validation of the method, other guidelines provide useful information, i.e. ICH guideline M10 on bioanalytical method validation and study sample analysis (EMA/CHMP/ICH/172948/2019) and VICH GL49 on Studies to evaluate the metabolism and residues kinetics of veterinary drugs in human food-producing animals: validation of analytical methods used in residue depletion studies (EMA/CVMP/VICH/463202/2009).

5.7. Pharmacokinetic calculations and interpretation

Pharmacokinetic parameters should generally be calculated using time-concentration data from individual animals, and descriptive statistics should be presented. The individual animal data should be provided.

Appropriate mathematical methods should be used to generate basic parameters (mostly, by means of non-compartmental analysis). With regard to compartmental analysis, the relevance of compartments should be discussed if necessary (e.g. presence of a 'deep' compartment in relation to antimicrobial resistance).

Validated computer programs should be used under specified conditions (regression methods, weighting factor, etc.); program codes and parameters should be provided.

6. Special approaches

Special approaches (e.g. simultaneous modelling of pharmacokinetics and pharmacodynamics, population kinetics) are encouraged, where applicable.

6.1. Pharmacokinetic-pharmacodynamic (PK/PD) modelling

In order to reduce the number of animal studies while potentially improving the accuracy of the dose optimisation process, selection of the dose level and dosing interval by means of a PK/PD modelling approach may be considered. Pharmacokinetic studies may replace standard dose determination studies where pre-existing data include a well-established PK/PD relationship, relevant to the intended formulation and clinical use, and provided that the selected dose level and dosing interval are supported by standard dose confirmation studies. Alternatively, the collection of PK data may be integrated in early pre-clinical efficacy studies, in order to establish the PK/PD relationship.

All aspects of the PK/PD methodology should be justified, based on the latest scientific developments in the concerned field. These include, but are not limited to, the following:

- The number of animals used for the PK/PD study, taking into account expected variability in PK and PD parameters.
- Samples: Samples should be collected from the most relevant biological matrix, in relation to the PK and PD endpoints selected (see below). In most cases, this will be plasma or whole blood but, in some instances, drug concentration in other biological fluids or tissues might be more relevant. The inclusion of a vehicle-treated control group should also be considered for establishing the PD baseline, e.g. if the formulation vehicle is suspected to have a pharmacological effect or if the PD parameter exhibits circadian variation.
- Sample numbers and time-points: The frequency of time-points should allow a detailed description of (a) the rise and decay of drug concentrations, and (b) the onset, duration and cessation of the PD response. To obtain reliable parameter estimates (e.g. E_{max} (maximum effect), EC_{50} (drug concentration that elicits 50% of E_{max})), a study comprising multiple dose levels (including negative control) may be required. In addition, it should be possible to determine if hysteresis (i.e. a temporal delay between drug exposure and PD response) has occurred and, furthermore, if the delay is PK (e.g. slow rate of drug distribution from the plasma to the site of action) or PD (e.g. a cascade of cellular events occurs before the response

is observed) in origin, in order to inform construction of the PK/PD model (e.g. effect compartment model versus indirect response model).

- Endpoint selection: The selected PD endpoint should be reliably linked to the claimed indication and be clearly correlated to the selected PK endpoint. In cases where direct measurement of the clinical endpoint is difficult, the use of biomarkers/surrogate endpoints may be considered, if justified.
- Model selection: The selected PK/PD model, including assumptions and rationale for model components (e.g. temporal changes in baseline, presence/absence of an effect compartment, presence/absence of moderator functions to account for tolerance or drug-induced induction/inhibition of PK processes) should be fully described. The method used for fitting a model to the data, the ability of the model to predict the observed data, and the treatment of outliers and/or missing data should also be provided.
- Interpretation: In addition to discussing the results, the variability observed in the PK and PD parameters should be discussed with regard to the impact on data quality, selection of the PK/PD model and interpretation of the results.

Further guidance on the PK/PD relationship for specific therapeutic drug classes can be found in the following documents:

- CVMP Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/627/2001).
- CVMP Guideline for the conduct of efficacy studies for non-steroidal anti-inflammatory drugs (EMA/CVMP/EWP/1061/2001).

6.2. Population pharmacokinetics (PopPK)

Within a population, the effect of a drug can vary markedly from one individual to another, and therefore between animals used in laboratory-based dose determination studies and those encountered in clinical trials. Sources of this variability may be pharmacokinetic and/or pharmacodynamic in origin, and factors that have been reported to influence pharmacological effect include age, gender, body weight, disease status and genetics. The aforementioned factors may not only alter the mean value of a parameter but also its variance, thereby increasing inter-individual variability. Meanwhile, intra-individual variability may arise through cellular mechanisms such as those related to the development of tolerance or up-regulation of metabolic processes. Further unpredictable variability may occur, e.g. as a result of methodological errors.

Contrary to human medicine, population PK approaches are currently rarely used in the context of the clinical development of veterinary medicinal products. Nevertheless, population pharmacokinetics may be considered as a means to refine dose level and dosing interval using PK data from individuals, which more closely resemble the target population (e.g. if the animals used in laboratory studies have markedly different demographics and/or disease status to those that will be treated in the field) or to support dosing recommendations for specific sub-populations (e.g. with regard to age, breed). In most cases, PopPK data will be generated as part of a clinical trial, though it must be ensured that assessment of efficacy and/or safety endpoints is not compromised. PopPK data will generally be collected in a large number of animals, but a complete profile would not be available from the same animal.

The reasons for collecting PK data in the target population or in subpopulations should be clearly defined, as well as the objectives of the study (e.g. in terms of refinement of the dosing recommendations). The different aspects of study design (number of animals, sampling method and time points, etc.) should be justified accordingly.

The intended modelling approach should be adequately described and justified. This should include a discussion of the underlying assumptions and the inclusion of covariates. All stages of model construction and validation should be presented.

Definitions

Accumulation: The increase in drug exposure that occurs with each additional dose, until steady-state has been reached. It can be measured by the *accumulation ratio*, which is most often calculated as the ratio $AUC_{steady\ state}/AUC_{single\ dose}$, or $Cmax_{steady\ state}/Cmax_{single\ dose}$.

Area under the curve (AUC): Area under the drug concentration versus time curve, which serves as a measure of drug exposure. Several types of AUC can be described:

- AUC_t: While in general this may denote any partial AUC to a given time t, in the context of this guideline, AUC_t is employed as $AUC_{t(last)}$, i.e. it refers to the AUC to the last sampling time associated with quantifiable drug concentrations (see *Lower limit of quantification)*. The last quantifiable drug concentration may occur prior to the last sampling time.
- AUC_∞: AUC_t with the addition of the extrapolated area from the last quantifiable drug concentration to time infinity.

Bioavailability: the fraction of the administered drug amount which reaches the systemic circulation as intact substance.

**Absolute* bioavailability is calculated as the AUC obtained with the intended route of administration, divided by the AUC obtained with the intravenous route (dose-standardised, if necessary):

$$F_{abs} = \frac{AUC_{extravascular}}{AUC_{iv}} * \frac{Dose_{iv}}{Dose_{extravascular}}$$

**Relative* bioavailability is calculated as the AUC obtained with the intended route of administration or formulation, divided by the AUC obtained with a reference route or formulation (dose-standardised, if necessary):

$$F_{rel} = \frac{AUC_{route1}}{AUC_{route2}} * \frac{Dose_{route2}}{Dose_{route1}}$$

Clearance: represents the volume of blood cleared of an active substance per unit of time. It reflects the efficiency of drug elimination.

C_{max}: The observed maximum (or peak) concentration of the active substance or its metabolite(s) in the matrix of interest during a dosing interval.

Covariate: Secondary explanatory variable to the measured clinical variable that likely influences the observed result.

Dose: Amount of active substance(s) to be given to an animal; it is usually expressed in mg/kg bodyweight.

Dose proportionality: For a linear pharmacokinetic system, measures of exposure (e.g. AUC, C_{max}) are proportional to the dose.

Enantiomers: Active substances with a chiral structure, i.e. two forms exist which are non-superimposable mirror images of each other.

Extravascular administration: administration of a drug by any route, except those in which the drug is administered directly into the systemic blood circulation; consequently, this refers to routes where an absorption phase occurs.

Fixed combination: A combination of active substances within a single pharmaceutical form.

Half-life: The period of time required for the concentration of active substance in a compartment (usually, in blood or plasma) to be reduced by one half.

Lower limit of quantification (LLOQ or LOQ): the lower quantifiable concentration of a substance in a given matrix, as determined by the sensitivity of the analytical method.

Pharmacodynamics: the science or study of the activity of an active substance on its biological target and associated physiological or pathological processes, through its interaction with receptors and signalling pathways.

Pharmacokinetics: the science or study of the fate of an active substance in the body, as the result of the following fundamental processes: absorption from the administration site, distribution to the different body compartments, and elimination through metabolization and excretion.

Pharmacokinetic/Pharmacodynamic (PK/PD) modelling: building of a mathematical model for the relationship between a PK parameter (related to the dosing regimen) and a PD endpoint (related to clinical efficacy) with the main purpose to improve the dose optimisation process.

Population pharmacokinetics: the study of the PK profile of an active substance in the final product, and in particular the associated variability, in the target population and under clinical (field) conditions.

Racemic mixture: Mixture composed of equal amounts of left-handed and right-handed enantiomers.

Steady-state: The situation when the amount of drug administered in a given time period is equal to the amount of drug eliminated in that same period.

T_{max}: Time until C_{max} is reached.

Volume of distribution (or *apparent* volume of distribution), V_d: Ratio of the amount of drug in the body (corresponding to the administered dose) to the plasma (blood) concentration following intravenous administration. The larger the V_d, the more likely that the active substance is found in the tissues, while small V_d values indicate that a substance is restricted to plasma or interstitial fluid. The value obtained is theoretical and does not correspond to a real volume.

References

- Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC
- Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes
- ICH guideline M10 on bioanalytical method validation and study sample analysis (EMA/CHMP/ICH/172948/2019)
- CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)
- CVMP Guideline for the conduct of efficacy studies for non-steroidal anti-inflammatory drugs (EMA/CVMP/EWP/1061/2001)
- CVMP Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/627/2001)
- CVMP Guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish (EMA/CVMP/EWP/459868/2008)
- CVMP Guideline on pharmaceutical fixed combination products (EMA/CVMP/83804/2005).
- CVMP Guideline on investigation of chiral active substances (EMEA/CVMP/128/95)
- VICH GL46 – Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: metabolism study to determine the quantity and identify the nature of residues (EMA/CVMP/VICH/463072/2009)
- VICH GL47 - Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: laboratory animals comparative metabolism studies (EMA/CVMP/VICH/463104/2009)
- VICH GL48 - Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods (EMA/CVMP/VICH/463199/2009)
- VICH GL49 - Studies to evaluate the metabolism and residues kinetics of veterinary drugs in human food-producing animals: validation of analytical methods used in residue depletion studies (EMA/CVMP/VICH/463202/2009)
- VICH GL52 - Bioequivalence: blood level bioequivalence study (EMA/CVMP/VICH/751935/2013)
- Good Laboratory Practice (GLP) (see Directive 2004/9/EC and Directive 2004/10/EC)