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Designing and performing pharmacokinetic studies for systemically administered drugs in horses

The goal of this editorial is to discuss best-practice design, execution and reporting of a pharmacokinetic (PK) study in horses. Our target readers are clinicians that plan to perform this type of research, either in a field, clinic or research setting but we also hope that this article might help readers of such work to appraise the articles they come across and understand the quality of studies they encounter. Our emphasis will be on appropriate study design and analytical method, drug and drug formulation choice and route of administration, animal choice, sample collection, storage and shipping, and reporting, rather than the PK data-analysis itself.

Study Design

The most common PK study design is to evaluate a single dose administered either in a crossover or parallel fashion. A typical crossover study design compares drug pharmacokinetics in the same horse utilising a 2 treatment, 2 sequence, 2 period design. The advantage of the crossover study design is that, as the same horses are used for both periods, the intra- and inter-animal variability can be evaluated. Therefore, this is usually the preferred study design when comparing the PK of two formulations, routes of administration or effects of feeding/fasting on drug absorption. However, there are several conditions in which a parallel study is preferred, such as young rapidly growing foals where the time between periods would add physiological variability, or for drugs with a long half-life where it may be impractical to wait at least 5 half-

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lives for drug depletion. As the parallel study utilises different horses for each period, these effects are minimised. Parallel studies typically require more animals to find a significant difference in pharmacokinetics. Consulting with a statistician on the type of appropriate study design may be beneficial.

In contrast to a single dose study, multiple dose studies can provide information on drug concentrations at steady state, saturable (non-linear) absorption or elimination, and drug accumulation. Many investigators only collect peak and troughs during multiple dose studies, but while these two points may allow for confirmation of steady-state conditions, these two time points by themselves are insufficient to generate any PK parameters. A better option is to collect multiple samples after the first dose, then peak and trough samples up to the last dosing and then multiple samples after the last dose. This approach will provide enough data to compare the PK parameters after the first and last dose to determine if there was any evidence of non-linear kinetics that could influence drug exposure.

A bioequivalence (BE) study is a specific type of PK study in which a reference drug product is compared to another formulation (test) of the same active drug. The definition of bioequivalence is that the rate and extent of bioavailability of the two drug products are both sufficiently similar that the products' clinical effects and safety would not differ, and therefore the two products can be used interchangeably. The rate is usually represented by the surrogate PK variable, maximum concentration (C_{max}) and the extent is estimated using the area under the time vs. concentration curve (AUC). The standard design for this study is a 2 treatment, 2 sequence, 2 period crossover study. The statistical analysis for determining BE usually uses a two-sided 90% confidence interval for the ratio of the Test/Reference C_{max} and AUC. The acceptance criteria for 90% confidence intervals of these two parameters are 0.80 to 1.25. For non- US Food and Drug Administration (FDA) regulated studies, the confidence interval width and the acceptable ratios are adjustable *a priori*, as justified by differences in the response-effect relationship and safety margin.

The FDA has two study standards for drugs that are being reviewed for approval: Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) (see Recommended References 1-5). The FDA Center for Veterinary Medicine (CVM) and the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) have collaborated on GCP and BE guidelines for animal studies (see Recommended

References). Although most equine research studies are not conducted according to these standards, adherence to at least some of these standards will improve the reliability of the data collected.

There appears to be a general perception that comparative PK studies do not need to be randomised or masked because there is no clinical evaluation and the primary variable is the plasma or serum drug concentration. However, randomisation and masking will improve the data quality by reducing bias. In comparative PK studies where there are physical differences in the drugs or different route of administrations, an unmasked treatment administrator can perform the dosing.

Bioanalytical Methods

The bioanalytical method used is an integral part of the study design and should be determined and ideally validated before the study begins, as the quality of the method determines the validity of the entire study's results. The bioanalytical method will also determine several aspects of the study conduct, e.g. types of tubes to be used and the volume of blood to be collected. A common mistake is not to develop the method prior to conducting the study. This can lead to delays in processing the samples and, depending on the stability of the drug in the matrix, can lead to inaccurate results. Drugs that are being approved by FDA must be measured using validated methods according to the Guidance for Industry (GFI) Bioanalytical Method Validation (see Recommended Reference 6), although most equine research studies report an abbreviated form of method validation. However, it is important to at least measure the precision and accuracy of the method in order to determine the validity of the results. It is also very important to measure free drug for pharmacokinetic/pharmacodynamic (PKPD) antimicrobial studies because the free drug is the active moiety for antimicrobial activity.

A description of the method (free or bound drug) and the validation should be included in the manuscript. A summary of common analytical methods used in equine studies is provided in Table 1. At a minimum, the following information should be reported: the type of methodology (e.g. High Performance Liquid Chromatography), the number and range of concentrations as well as the matrix used for calibration curves, the number and concentrations of the Quality Control (QC) samples, and accuracy and precision of the method. Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Precision measures how close individual measurements are to each other within each run and between

runs. For FDA regulated studies, these values should be less than <15% (20% at the lowest concentrations). However, alternative approaches to validation may be used, and should be described in detail in the manuscript. The accuracy and precision acceptance criteria for the analytical runs should be reported. The limit of quantification (LOQ) and the limit of detection (LOD) should also be defined and reported. If samples were outside of the range of the calibration curve, a description of the methods used to correct drug concentration should be included. There are multiple approaches to estimate samples below the limit of quantification (BLOQ), and the approach used should be described [7]. Additional information from the laboratory running the samples would ideally allow for the assay to be repeatable in other laboratories. This includes providing the type and size of column/guard column used, the mobile phase and any gradient used, the extraction method, and the parameters for drug detection based on the specific detection method used (e.g. wavelength used for UV detection; transitions monitored for mass spectrometry).

Drug choice and routes of administration

Prior to choosing a drug for study, the investigators need to know what formulations are available and whether they are appropriate for administration to horses. Approved, commercially available formulations should be used whenever possible. If the goal of the study is to test a compounded product, the specific formulation and batch should be tested for strength and stability of the active pharmaceutical ingredient (API) by the investigators under the precise storage conditions and for the duration of shelf-life used in the study to ensure that the intended dose is being administered to the animal. Unlike companies manufacturing FDA approved drugs, compounders are not required to have procedures in place, such as release specifications, to ensure batch to batch consistency. Published studies on compounded products from various pharmacies have noted inconsistencies in the strength of the received formulation compared to the label concentration, which can influence multiple pharmacokinetic parameters derived from the data. When using either commercial or compounded formulations, it is best practice to perform the whole study using drug product from the same manufacturing batch.

The route of administration is an important consideration, and each route presents with specific issues. If at all possible, all PK studies should start with intravenous dosing or reference to earlier PK studies where IV dosing was used. Data from an intravenous dose are absolutely necessary to

allow proper identification of many pharmacokinetic characteristics of the drug (e.g. clearance, volume of distribution). Exceptions are drugs which cannot be given IV safely to horses (e.g. doxycycline), or for which an IV formulation cannot be made from available formulations with a reasonable expectation of stability or at a reasonable cost. The lack of IV data will limit the answerable study questions. For IV bolus administration in horses, the jugular vein is the most common site of administration. Administration can be accomplished via direct injection; however for larger volumes, drugs that require slow IV bolus, or difficult patients (e.g. foals), an intravenous catheter is preferred. It is important to remember that this should not be the same catheter through which samples are collected as some drugs, particularly lipophilic drugs, will adhere to the catheter material and cause falsely elevated drug concentrations in samples subsequently collected through that catheter [8]. If it is not possible to use separate catheters, protocols for volume of drug to be discarded and/or number of flush cycles necessary to clear the catheter of drug can be investigated, either through literature searches or *in vitro*, prior to the study. If direct injection is used, it is important to ensure the entire dose is administered in the vein. Partial or total extravascular injection will result in an apparent absorption phase of the drug when samples are analysed. For constant rate infusions, an IV catheter is required, and a fluid pump or syringe pump is recommended to ensure accurate dosing. The pumps should be monitored during the entire treatment interval, as any interruption in drug administration can result in a fluctuation of drug plasma concentrations.

For extravascular parenteral administration such as subcutaneous (SC) or intramuscular (IM) routes, the site of administration and volume per injection site are two important factors to consider. For SC administration, the most common site is in front of the scapula. This route is not typically used in horses, and is often restricted to low volumes of administration (< 15 mL in the authors' experience). For IM administration, the cervical muscles or semimembranosus/semitendinosus muscles are preferred for large volumes. The pectoral muscles are an option for smaller injection volumes. Differences in drug absorption have been noted based on injection site [9], so the site should be clearly reported. The volume per injection site for IM administration is often dictated by an institution's animal care and use committee or a similar governing body. In most cases, volumes should be kept to < 15-20 mL in one site, although redirecting the needle one time is often allowed. Following drug administration, the injection site(s) should be monitored for heat, pain and swelling and markers of muscle injury

may be measured (e.g. creatine kinase) [10]. Horses appear to be more sensitive than cattle with regard to injection site reactions following SC or IM administration, and some drugs used via those routes in cattle are not appropriate for use in horses.

For oral drugs, the investigator must choose whether to administer the drug orally via dose syringe, or intragastrically via nasogastric tube. There are benefits and disadvantages to each route. Dosing via syringe is commonly used in practice and therefore pharmacokinetic results will most closely mimic drug absorption in practice. The drawbacks faced are also those faced with this route of administration in practice, notably, drug loss during administration.

Investigators should ensure there is no feed in the horse's mouth prior to administering the drug and hold the head elevated for a period after drug administration. Some drugs (e.g. opioids) can have significant absorption transmucosally and therefore sampling from the jugular vein following syringe administration can result in falsely elevated drug levels [11]. For these drugs, administration should be performed using a nasogastric tube, or samples should be collected from a distant site, such as the lateral thoracic or cephalic vein. Administration via nasogastric tube ensures the entire volume is delivered directly to the stomach for dissolution and absorption. However, this may overestimate drug absorption in practice, and highly lipophilic drugs may adhere to the plastic of the tube during administration. The volume of diluent administered intragastrically with the drug is often higher than can be used via syringe in practice, and this, in combination with any fluid volume used to flush the tube following drug administration may increase dissolution of the drug and therefore alter absorption rate. Additionally, if the drug is administered intragastrically in the study, but is meant to be used orally in practice, the effects of the drug on oral mucosa, such as mucosal ulceration, as well as avoidance behaviours by the subject due to unpleasant taste or irritation would not be examined.

Animal subjects

Many equine PK studies are conducted in a very small number of horses owing to budgetary or animal use restrictions. Five or 6 horses is generally considered the minimum number of animals for a simple, crossover PK study. These studies can be adequate for pilot data that describe the initial pharmacokinetics of a drug or product that has not previously been studied and can therefore provide data to determine a potential dosage regimen for future studies. For a BE study, the number of animals has a direct impact on the intra- and inter-animal variability, so a larger number of horses is needed. Bioequivalence studies require a sample-size calculation to

185 minimise the risk of no finding (failed study) and therefore these studies cannot be reasonably
186 designed without pilot PK data for each product. As previously mentioned, parallel study designs
187 also require a higher number of animals. It is also recommended to consult with a statistician for
188 a power analysis if enough data are available.

189 Initial pharmacokinetic studies are typically performed on healthy adult horses of similar age,
190 body type, and weight and are usually limited to light horse breeds. This results in a relatively
191 homogenous group of horses and, presumably, homogenous pharmacokinetic parameters. It may
192 not accurately represent clinical patients with the disease being treated, however. If a disease
193 affects a certain subset of animals, then those should be used as animal subjects. For example,
194 foals, ponies, and donkeys may have different pharmacokinetics when compared to adult horses
195 and these animals should be studied separately.

196 The physicochemical properties of a drug may determine the type of horses used in the study.
197 For example, lipophilic drugs may distribute preferentially into fat, so body type as well as
198 weight should be considered when choosing horses for a study using a lipophilic drug.

199 Conversely, horses that are maintained in exercise are likely to have a higher muscle mass,
200 increased blood flow to and from the musculature, and increased cardiac output [12]. This may
201 particularly affect absorption from intramuscular sites. Studies using a heterogeneous population
202 of fit and out of work horses may therefore see a higher variability in pharmacokinetic
203 parameters and be more representative of the population at large. Variability in pharmacokinetic
204 parameters may occur despite an apparently homogenous population. One reason for this is the
205 presence of genetic polymorphisms in drug metabolising enzymes resulting in horses that are
206 poor, extensive or ultra-rapid drug metabolisers [13]. The inter-occasion variability of PK within
207 an individual animal for many species can also be an important component of variability, and can
208 be addressed as part of the study design.

209 *Fed vs Fasted Conditions*

210 Feeding status can affect oral drug absorption in several ways. It can decrease gastric emptying if
211 high fibre feeds (e.g. hay) are fed, alter gastric pH, or bind drug particles making them
212 unavailable for absorption. The overall effect of feeding is typically a decrease in oral
213 absorption. It is therefore common to see equine PK studies in which the horses are fasted for 8-
214 12 hours before oral dose administration. This may increase the bioavailability of a drug, but it
215 does not replicate actual field conditions since horses are rarely fasted and this is impractical for

anything but once daily drug administrations. Therefore, the investigator must decide if the study objective is to maximise the bioavailability of an oral drug or estimate the drug's bioavailability under conditions of use. It is particularly important not to overestimate the oral bioavailability for antimicrobial PKPD studies, because this may lead to ineffective treatment. One aspect that should also be considered is whether or not the horses should be stalled during the entire study, which is required for true fasting. For longer term studies, allowing horses to go out and graze can have a physiological impact on drug bioavailability by improving gut motility.

Statistical Analysis

Descriptive statistics are frequently used for reporting PK parameters. Mean and standard deviation are used for normally distributed data, but median and range are preferred for non-normally distributed data. Because PK studies usually have low numbers of animals, it is not possible to adequately test for normality of distribution of calculated variables. In accordance with the known expectation that several PK variables do not conform to normal distributions, the normality assumption should not be made [14,15]. Other statistical analyses may or may not be included in the study. Differences in pharmacokinetics across routes of administration or between drug formulations may be analysed using appropriate statistical techniques. Power calculations should be used and reported in certain circumstances where statistical comparisons are included, as well as the parameter used for the power calculation (e.g. X number of horses are needed to determine a 20% difference in maximum concentrations between routes of administration). Inclusion of pharmacodynamic effects in the study may also require descriptive or comparative statistics.

Sample Collection

For a new drug or a drug that has not been investigated in horses, deciding when and how long to collect plasma or serum samples can be difficult. If possible, testing the test drug in 1-2 horses can be useful to get an idea of detection times and duration of the absorption and elimination phases. If this is not possible, a general recommendation is to increase the sampling during the predicted absorption phase and around the predicted time to maximum concentration (T_{max}). An inadequate number of samples during the absorption phase of the drug increases the risk of missing the true C_{max} . The duration of sampling is dependent on the half-life of the drug, and a general rule of thumb is to sample for at least 5 half-lives, if an accurate estimation can be made. For example, if the drug is expected to be administered once daily, then the half-life is likely to

be between 8 and 16 hours, and sampling should continue out to 3 or 4 days. For compartmental analysis, 3-4 samples per phase is required.

One of the most common deficiencies in equine PK studies is an inadequate number of samples evaluated, typically due to cost concerns. For some analytical laboratories, the main cost is setting up the assay for each run, so the cost is not directly related to the number of samples. Even if the analytical cost is dependent on the number of samples, considering all of the other costs in conducting the study, it is false economy to minimise the number of samples collected. One option is to collect more samples than will be assayed and keep them frozen, so if there is a problem, there is still an option to re-analyse the data using the reserved samples without repeating the study. Furthermore, with mixed effects hierarchical modelling approaches such as now easily implemented in some software programs, benefit can be gained if analytical costs are constrained by analysing samples from different horses at overlapping times. It is also possible, after analysis of the samples from the first horse, to evaluate the PK profile to improve the sample selection times for further analysis.

It is not helpful to collect blood or plasma samples at time points shorter than five minutes after dosing (intravenously or any other route), because pharmacokinetic modelling assumes a well-mixed central compartment and full mixing of any intravenous dose is not achieved in the horse within the first several minutes post intravenous injection.

Using a catheter for sample collection decreases stress and strain on animals and helps maintain the strict time schedule required for sample collection. The choice of whether or not to catheterise a horse for sample collection depends on multiple factors: the policies of the animal ethics approval committee, the number and timing of samples, the horse's temperament, the location of the vein (jugular or more peripheral vein), the length of the study, and the maintenance of the catheter. It is important to remember that for any non-oral drugs administered to the head (e.g. inhaled drugs) samples collected from the jugular vein will overestimate drug concentrations due to the collateral circulation which drains directly into the jugular. Therefore, sample collection from a peripheral vein such as the cephalic will provide a more accurate measurement of systemic drug exposure. If catheters are used for sample collection, waste blood should be withdrawn and either discarded or aseptically administered back to the animal. Given the large volume of blood available for sampling in horses (and even most foals), it is not necessary to give the blood back unless a large number of samples with a

large volume per samples are to be collected on a given day. The waste blood volume should equal 2-3 times the volume of the catheter and any extension set used in order to decrease the risk of the actual sample being diluted by remaining saline in the line.

Choice of tube for sample collection and storage can also have a significant impact on results. For serum collection, it is safer to use tubes without a clot activator as serum separator tubes may bind some drugs [16]. If these tubes are to be used, in vitro or literature documentation of a lack of binding should be included in the manuscript. If plasma is the sample collected, the choice of anticoagulant should be researched as well. Lithium heparin is a common choice for blood collection tubes. Tubes containing EDTA may chelate some drugs and affect study results. The choice of plastic versus glass for sample collection is also an important decision. Some drugs adhere to either substance and should therefore not be stored or collected in that type of tube. This information may be available on the MSDS of the drug and/or in the literature for the reference standard used for analysis. However, fundamentally this information will be acquired and validated during assay development and validation. This is another reason that pharmacokinetic studies should not be initiated until after the analytical method has been readied.

It is important to avoid contamination of the samples during collection, so the treatment administrator should wash his/her hands after preparing drug for administration and before collecting any blood samples. Alternatively, separate study personnel could administer drug and collect blood samples. The analytical laboratory may need a large volume of drug-free equine plasma or serum to pool as an assay matrix so this should be planned for in advance and included in the Institutional Animal Care and Use committee (IACUC) application. The delay between collection, centrifuging, and freezing should be kept to a minimum, and it is helpful to pre-label tubes to minimise mistakes. Reserve aliquots should be stored in a separate freezer (-20 to -80° C) to decrease the risk of losing all of the samples in case of a freezer failure or shipping error. One set of samples should be shipped and kept frozen during shipment on dry ice. Most major shipping companies have methods to monitor temperature during shipment. Overnight shipment early in the week will help prevent storage errors at the arrival destination.

Pharmacokinetic Analysis

The focus on this editorial is on the design, conduct, and reporting of a PK study rather than the PK analysis itself. We recommend that investigators consult with a pharmacologist to determine

the most appropriate analysis to use depending on the study design. However, at a minimum, the following information should be reported in the manuscript for all analyses:

- The type of model used for the analysis, e.g. compartmental, non-compartmental (NCA), population
- PK software used
- Details of the structural and statistical models used
- Methods to estimate the model's fit
- Method to estimate the slope of the terminal elimination phase
- Method to calculate or estimate the area under the time vs. concentration curve (AUC)
- How missing or below the level of quantification (BLQ) samples were addressed in the analysis
- If the data were log transformed prior to analysis
- If the maximum concentration ($C_{\max}$) and time to maximum concentration ($T_{\max}$) were the observed or extrapolated values

The choice of parameters to report is dependent on the type of analysis and route of administration. At a minimum, the PK parameters listed in Table 2 should be reported for single dose NCA or compartmental models.

Before analysing data to estimate PK parameters, essential assumptions are made. In particular, does the drug appear to behave according to linear stochastic models and is there evidence that the animal is only exposed to the drug once (i.e. no enterohepatic cycling)? If these assumptions are violated, then analysis of the data using routine methods is misleading. It is a common error made by researchers in cases such as this for the data analysis to avoid compartmental modelling and use non-compartmental methods, such as NCA as implemented in the PK software Phoenix WinNonlin (Certa). This is a major error, as the usual methods for calculating PK parameters by non-compartmental methods rely on the same assumptions of linear stochastic models [17].

For multiple dose studies, additional parameters that should be reported are minimum concentration for the dosing interval at steady state ($C_{\min,ss}$), maximum concentration for the

dosing interval at steady state ($C_{\max,ss}$), average concentration for the dosing interval at steady state ($C_{\text{avg},ss}$), accumulation ratio (RA) calculated from $C_{\max}$ or AUC, and dosing interval (τ).

For any PK study, a plot of the time vs. concentration data should be included. In a study with a small sample size, only reporting a plot of the mean time vs. concentration data may not be as informative as the individual data because one horse could substantially bias the mean. The same is true for linear vs. semi-log plots, because a semi-log plot minimises the differences among horses. Therefore, many equine studies are small enough to justify a plot of individual data on a linear scale in addition to a plot of the mean time vs. concentration data either on a linear or semi-log scale (preferred).

Drug Pharmacodynamics

Although a full discussion of pharmacokinetic/pharmacodynamic (PK/PD) modeling and study design is beyond the scope of this study, the authors want to provide the following general information for investigators to consider before designing a PK/PD study in horses.

To determine the clinical relevance of a drug after administration to horses, it is imperative to know something about the plasma concentrations needed to achieve a therapeutic effect. This is rarely known in drugs that have not been previously studied. In some cases, it may be useful to compare concentrations reached in horses to known therapeutic concentrations in other species, although this assumes similar physiology, pathophysiology, receptor concentration, etc. If the PK study is being performed in the target population (e.g. horses with disease), or the drug is meant to be used in healthy horses (e.g. sedatives), select pharmacodynamic parameters can be monitored and correlated to plasma concentrations (PK/PD).

A common form of PK/PD study examines the plasma PK of antimicrobial drugs in horses and relates them to the minimum inhibitory concentrations (MIC) of select bacterial populations.

This information can be useful if the appropriate PK/PD interactions for the drug class are known (i.e. time above MIC, concentration: MIC ratio, etc.). There are many limitations to this form of study, however. The optimum study design for an antimicrobial PK/PD study is one in which samples are collected to determine the susceptibility of common pathogens in the study population. This provides robust data to determine an effective drug concentration for that population. A less optimal study design is to conduct a single dose PK study and then reference the minimum inhibitory concentration for 90% of the isolates (MIC_{90}) established in another

population to speculate that the plasma concentrations would be effective. If the latter approach is chosen, the investigator should describe the population (number of animals, location) and the methods by which the MIC₉₀ was established and then provide a justification as to why the MIC₉₀ from another population is an appropriate target. It is important to note if the susceptibility data were generated in a population of horses in a different country than the study because pathogen susceptibility can differ substantially among countries due to different standards of antimicrobial stewardship.

The culture and sensitivity (C&S) procedures to establish the MIC₅₀ and MIC₉₀ should be described in the manuscript, even if the results are from another study. The MIC₉₀ is the preferred target, although the MIC₅₀ is also reported by microbiology laboratories. The gold standard in the United States is the culture and microbroth dilution procedures established by the Clinical and Laboratory Standards Institute (CLSI). The EU counterpart organisation is the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The major advantage of using the CLSI procedures is to provide uniformity to the results, so that susceptibility can be compared across multiple study populations. The CLSI and EUCAST subcommittees for veterinary antimicrobial susceptibility testing (VAST and VetCAST, respectively) determine antimicrobial breakpoints for bacteria of animal origin (see Recommended References 18-22). The CLSI VET 02 provides *in vitro* susceptibility testing criteria for veterinary antimicrobials and CLSI VET 08 lists the standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. VAST has published a document (VET 09) which provides a comprehensive review of how to accurately interpret susceptibility testing for clinical cases. EUCAST has published a helpful guideline on how to collect and analyse PK data for presentation to VetCAST. Human susceptibility data or clinical breakpoints should not be used unless the investigator provides justification that this is an appropriate PKPD target.

Conclusion

In conclusion, pharmacokinetic studies in horses provide useful information on potential therapeutic options and initial dose estimates for use in equine patients. The usefulness of this data does depend on appropriate study design and analytical method, drug and drug formulation, choice and route of administration, animal choice, sample collection, storage and shipping. All

these aspects should be carefully considered prior to initiating the study. Failure to do so may lead to inaccurate drug concentrations, unreliable data, and an inability to publish study results.

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Table 1: Summary of analytical methods commonly used for drug quantification in horses. This table is not all inclusive.

Method	Advantages	Disadvantages
Liquid chromatography (high performance HPLC or ultra-high	Widely available and less expensive. Good for a wide	Less sensitive or specific than MS for some chemicals.

performance UPLC) with ultraviolet/photodiode array/fluorescence/electrochemical detection	range of chemicals, dependent on the detector used.	Method development is often more involved. Solid phase extraction for sample preparation is often required and sample volumes collected need to be larger.
Liquid chromatography with mass spectrometry (LC-MS or LC-MS/MS)	Highly sensitive and specific. Minimal sample preparation required; protein precipitation methods commonly used. Most useful for substances that are highly potent. Sample volume required for injection very small.	Expensive. Drug must be ionisable. For drugs present in samples at high concentrations, additional dilution steps are required.
Gas chromatography	Useful only for volatile organic compounds; can be coupled with mass spectrometry for increased sensitivity.	Not useful for inorganic, non-volatile compounds without derivatisation
Bioassay	Inexpensive and does not require expensive equipment. Useful for some antimicrobial drugs that undergo minimal metabolism.	Does not differentiate parent compounds and active metabolites; may over or underestimate drug activity in animals with altered metabolism. This journal will not publish bioassay data unless it has been compared to a more specific method.
ELISA/RIA	Rapid detection. Highly	Depending on the drug

	sensitive. Often validated for plasma, serum and urine.	measured, may be less specific. Cross reactivity with other commonly administered drugs should be determined prior to study. Only commercially available for a few compounds in horses.
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Table 2: Compartmental and Non-compartmental PK parameters.

Non-Compartmental Analysis	
C _{max}	Maximum plasma concentration
C ₀ (IV)	Plasma concentration extrapolated to time 0 after IV administration
T _{max}	Time to maximum concentration after extravascular administration
AUC _{LOQ}	Area under the concentration-time curve to the limit of quantification
AUC _{inf}	Area under the concentration-time curve from time zero extrapolated to infinity
AUC _{extrap} %	Percentage of area under the concentration-time curve extrapolated to infinity
Half-life Lambda z	Half-life of the terminal phase
F	Extravascular bioavailability
Vd/F	Volume of distribution after extravascular

	administration
V _d (IV)	Volume of distribution of the terminal phase after IV administration
Cl/F	Total plasma clearance after extravascular administration
CL (IV)	Total plasma clearance after IV administration
AUMC	Area under the first moment curve
MRT	Mean residence time
Compartmental Analysis	
C _{max}	Maximum plasma concentration
C ₀ (IV)	Plasma concentration extrapolated to time 0 after IV administration
T _{max}	Time to maximum concentration after extravascular administration
AUC _{LOQ}	Area under the concentration-time curve to the limit of quantification
AUC _{inf}	Area under the concentration-time curve from time zero extrapolated to infinity
α (model dependent)	Absorption rate constant
k _a (model dependent)	Absorption rate constant
k ₀₁ (model dependent)	Absorption rate constant
k _{el} (model dependent)	Elimination rate constant from the central compartment
β (model dependent)	Elimination rate constant

k_{10} (model dependent)	Elimination rate constant
k_{el} half-life ($t_{1/2}$) (model dependent)	Elimination half-life
$t_{1/2\beta}$ (model dependent)	Elimination half-life
k_{10} half-life (model dependent)	Elimination half-life
V_c (IV)	Volume of distribution of the central compartment
V_d/F	Volume of distribution after extravascular administration
CL/F	Total plasma clearance after extravascular administration

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