Commentary

Demonstrating Comparative *In Vitro* Bioequivalence for Animal Drug Products Through Chemistry and Manufacturing Controls and Physicochemical Characterization: A Proposal

Marilyn N. Martinez^{1,2} and Raafat Fahmy¹

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Abstract. The assessment of *in vivo* bioequivalence (BE) of nonsystemically absorbed drug products has been a longstanding challenge facing drug manufacturers and regulators of human or animal health products. Typically, in situations where blood level BE studies are not feasible, clinical endpoint BE trials have provided the only option for generating interproduct comparisons. Given the imprecision and logistic challenges associated with these studies, there has been an effort to identify alternative pathways that can reliably ensure the equivalence of product performance and quality. This commentary provides a proposal for an *in vitro* approach for evaluating the *in vivo* BE of veterinary drug products that are either nonsystemically absorbed or that act both locally and systemically but where the local site of action is proximal to the absorption window. The assumption underlying this approach is that equivalence in product physicochemical attributes and *in vitro* product performance translates to equivalence in product *in vivo* behavior. For sponsors with a right of reference to underlying safety and effectiveness data, this approach could be used to support pre and post-approval changes. When comparing a generic test product to the pioneer (reference listed new animal drug, RLNAD) product, a demonstration of sameness across a battery of *in vitro* test procedures could be used to confirm that the test and RLNAD products are bioequivalent.

KEY WORDS: bioequivalence; critical quality attributes; *in vitro* dissolution; veterinary drug products.

INTRODUCTION

Blood level bioequivalence (BE) trials remain the gold standard for comparing two formulations that are systemically absorbed and which act at sites reached through the blood. However, there are circumstances when the generation of blood level BE data may not be feasible or appropriate because the active moiety(s) is not systemically absorbed prior to reaching its site of action. Up to now, these situations typically necessitated that sponsors of animal drug products conduct clinical endpoint BE trials, the execution and interpretation of which can be very challenging (1). For example, the poor statistical power associated with equivalence trials can necessitate the use of hundreds of animals in groups receiving the reference listed new animal drug product (RLNAD), the test formulation, or a placebo. The need for

large study cohorts reflects the inherent variability in exposure-response relationships and the difficulty in powering a study to detect small differences in product performance. In this discussion, exposure refers to the peak or extent of local drug concentration over time as a function of the administered dose. The challenge associated with the latter is further complicated by the labeled dose at which these tests are performed, often at or approaching the plateau of the exposure-response relationship. There is also the question of whether one or more studies may be necessary to cover all of the indications associated with the approved RLNAD.

Another difficulty associated with reliance on clinical endpoint BE trials are that outcomes can be dependent upon study conditions. When this occurs, the demonstration of equivalence under one set of conditions may not translate to equivalence in other situations. For example, if two products are compared at the plateau of the exposure-response relationship, there could be substantial differences in *in vivo* drug exposure yet negligible difference in the measured therapeutic effect. However, these differences in drug exposure may ultimately influence a clinical outcome if there is a shift in the exposure-response relationship (e.g., a shift in pathogen susceptibility). To illustrate, consider two products, a test formulation (T) and the RLNAD product (R) (Fig. 1). In this example, T is associated with an exposure of six arbitrary units while product R was associated with an

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¹USFDA Center for Veterinary Medicine, Office of New Animal Drug Evaluation, 7500 Standish Place, HFV-100, Rockville, Maryland 20855, USA.

²To whom correspondence should be addressed. (e-mail: marilyn.martinez@fda.hhs.gov)

exposure of 10 arbitrary units. Based upon the hypothetical example, the ratio in the percent success associated with the administration of T vs R was 100/100 in the presence of the “wild-type” pathogen, T and R. However, under conditions of reduced pathogen susceptibility, the ratio of T/R percent success would be 50/92 (ratio=0.54). Thus, conclusions of product comparability would change from that of equivalence (T/R effectiveness ratio of 1.0 when tested in the “wild-type” pathogen population to one of inequivalence) (T/R effectiveness ratio of 0.54 when tested in the less susceptible parasite population).

There clearly exists an unmet need for alternative BE approaches for nonsystemically absorbed veterinary drug products. In this commentary, we wish to present our proposal for such an approach, basing our suggestions upon an appreciation of the critical quality attributes (CQAs) that influence *in vivo* product performance and upon the ability to use *in vitro* release test methods as a tool for evaluating the rate and extent to which drug is released from an investigational drug product. A CQA is a physical, chemical, biological, or microbiological property associated with the drug substance, excipients, intermediates (in-process materials), and the drug product.

Grounded upon a basis of product understanding, a battery of *in vitro* tests and equivalence criteria can be developed to define the range of acceptable deviations in the physicochemical characteristics within which we can be assured of consistent *in vivo* product performance. Considering the range of animal species and dosage forms, it would be best to avoid efforts to establish a hard and fast rule on what constitutes an acceptable deviation. For example, an acceptable range of test product particle size distribution will depend upon the particle size distribution characteristics of the RLNAD product. For pH, an acceptable deviation could be defined by the pH of the GI tract of the target animal species. Deviation in viscosity could be a function of whether the product is administered into a large fluid environment (e.g., intramammary or rumen) *versus* topical (where deviations might be constrained within a narrow range, depending upon

the rate-limiting step influencing drug diffusion, such as the matrix *versus* the biological membrane). Once this characterization of product CQAs is established, an appropriate set of conditions for evaluating *in vitro* drug release can be identified. Comparability of product *in vitro* release characteristics would need to be demonstrated across a range of test conditions deemed to be biologically relevant (as discussed later in this commentary). The hope is that this commentary will set the stage for future discussions of the strengths and challenges associated with implementation of this approach.

Examples of the types of products for which an *in vitro* BE approach could potentially be applied include some orally administered products (e.g., type A medicated articles, topical formulations, e.g., emulsions, ointments, creams, suspensions), and intramammary infusions. In cases where the drug exerts both systemic and local effects, the blood level data could provide information on product comparability as it pertains to the systemic effects (i.e., that component whose effects depend upon delivery of the drug from blood to a remote site of action), and the *in vitro* release information could be used to ensure equivalence with respect to the drug's local actions.

This proposal for using alternative *in vitro* approaches to establish product BE is consistent with human drug regulations. The Federal Food, Drug, and Cosmetic Act (FD&C Act) for human drugs at section 505(j)(8)(A)(ii) indicates that “For a drug that is not intended to be absorbed into the bloodstream, the secretary may assess bioavailability by scientifically valid measurements to reflect the rate and extent to which the active ingredient or therapeutic ingredient becomes available at the site of drug action”. With respect to animal drug products, section 512(c)(2)(H)(ii)(III) allows for alternate methods when it is inappropriate or impractical to measure the drug in animal fluids.

AN OVERVIEW OF THE *IN VITRO* APPROACH FOR PRODUCT COMPARISONS

Due to the physiological, pharmacokinetic, and biopharmaceutical complexities associated with systemically absorbed drug products, we currently are focusing our discussion on the *in vitro* BE of immediate release non-systemically absorbed veterinary dosage forms. However, this does not exclude its potential applicability to drugs with both systemic and local effects when the effect site is proximal to the site of absorption and when blood level BE cannot be adequately linked to the equivalence of drug product therapeutic effects. It is also critical to note that this alternative approach should not be construed as a means for obtaining a biowaiver (e.g., CVM Guidance for Industry #171 (2)). In this regard, Guidance #171 pertains solely for type A medicated articles that are “fully soluble” under conditions in the GI tract of the intended target animal species. The current proposal imposes no such limitation.

When applied to generic drug formulations, the use of the *in vitro* BE approach would be predicated on the demonstration of Q1/Q2/Q3 sameness. These parameters are defined as follows (3):

- Q1=components: the test and RLNAD products contain the same active and inactive ingredients.

Fig. 1. Example of potential challenges of evaluating product BE through the use of a clinical endpoint BE trial. This figure showcases the impact of pathogen susceptibility on the ability to identify product inequivalence (T test product, R RLNAD, WT wild-type microorganism, Less pathogen with reduced susceptibility)

- Q2=quantitative similarity of composition: typically, Q2 implies that the test and RLNAD products contain the same amounts of active and inactive ingredients. To ensure pharmaceutical and therapeutic equivalence, sameness has frequently been described as test product Q2 values deviating within $\pm 5\%$ relative to that of the RLNAD. However, we acknowledge that for sponsors with right of reference to underlying safety and effectiveness data, potential modifications in this strict limitation may be appropriate (see below).
- Q3=comparability of product physical and chemical characteristics.

Examples of factors that can influence the product performance attributes of products such as topical ointments and gels are summarized in Table I (3).

It is recognized that in the absence of “right of reference” to the original formulation, reverse engineering can help generic drug sponsors identify the composition and components of the innovator formulation. For companies with “right of reference”, Q1 and Q2 comparisons would not pose such a challenge and “minor” deviations may be acceptable based upon a comparison of *in vitro* drug release and Q3 comparability. For generic drug sponsors, Q3 comparability can be demonstrated by testing lots of the generic and the RLNAD product formulations. For innovators,

comparability can be based upon Q3 comparison of the new formulation to the information contained within the original product dossier.

As part of this *in vitro* BE proposal, we suggest that the quality of the excipients used in the drug product formulation, as well as that of the container/closure systems, would be expected to meet the USP standards (where available and appropriate). Otherwise, suitable acceptance criteria would need to be established for the non-USP excipients. Even with Q1/Q2 sameness, special attention needs to be directed toward the grade of the excipient because different grades of excipient can have a significant impact on drug product CQAs. It is important to be aware that different vendors or grades may contain different impurities that can influence *in vivo* product performance.

Because of accessibility to the proprietary data upon which product safety and effectiveness was determined, the criteria for assessing “sameness” can be modified when we are dealing with *in vitro* BE evaluations to support formulation modifications of the RLNAD product. It is also of value to note that with respect to innovator modifications in product formulations and/or method manufacture, a justification for negligible *in vivo* relevance of these changes can be obtained through an understanding of product CQAs and design space (e.g., refer to <http://www.fda.gov/downloads/drugs/guidances/ucm073507.pdf>).

Table I. Points to be Considered in the Formulation and Design of Ointments and Gels (Based upon Information in Chang *et al.* (3))

Parameters	Specific factors	Considerations
Physicochemical properties of drug product	Particle size, viscosity, pH, strength, release profile, <i>in vitro</i> permeation rate, homogeneity, etc.	A comparison of the physical and chemical properties of the RLNAD and the generic products is needed to determine the CQAs integral to a determination of Q3 comparability.
Chemical stability	Consistency of the chemical properties over time	Products maintain assay values within the specification throughout its shelf-life. Impurity level should remain close to 0% throughout the shelf-life period. Excessive product degradations can compromise its safety and effectiveness.
Physical stability	Time-associated consistency of product physical properties such as excipient melting point (liquid, low melting point, or high melting excipient)	The physical properties of the drug product should not vary during the shelf-life period. Potential problems that need to be avoided include separation of phases, syneresis, lack of homogeneity of drug in the dosage form, or changes in pH, specific gravity, or viscosity.
Manufacturability and scalability	Process equipment and process parameters, such as agitation rate, mixing time, and temperature.	Appropriate process equipment and process parameters need to be identified. Based on the past scale-up experience of the same type of formulation and process as well as engineering principles, the commercial size scale up, and equipment changes should be justified.
Excipients	Use of compendial (USP) vs. non-compendial material Excipient compatibility	Compendial excipients usually are preferred; non-compendial materials are acceptable with justifications. Excipient compatibility study using a binary mixture is desired to ensure drug product stability prior to product development. It is important to note that different vendors or grades may interact differently or may contain impurities that can trigger drug degradation.
Container closure system	In cases of generic products, the container closure system should be as close as possible to that of the innovator.	Since the container closure system can influence product stability, stability studies should be conducted using the final container closure.

PHYSICOCHEMICAL CHARACTERIZATION

The test product would be compared to the physicochemical characteristics of the RLNAD (4–6). Because the issue being considered is product BE, reliance solely upon USP specifications is not adequate. Examples of tests that may be used to compare the test product *versus* RLNAD products include (but are not limited to) the following:

- Particle size distribution: the relative amount, typically by mass, of particles present according to size. Particle size distribution information can help predict formulation behaviors such as viscosity, flocculation or aggregation, coagulation, rate of settling, separation or creaming, and the rate of diffusion.
- Zeta potential: the zeta potential is a key indicator of the stability of a suspension. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion.
- Specific gravity: the ratio of the density of a substance to the density (mass of the same unit volume) of a reference substance (generally water for liquids)
- Distribution of the API in the dosage form
- pH
- Rate of settlement (suspensions, emulsions, and micro-emulsions)
- Assay: a laboratory method for testing the amount of a particular substance within a mixture
- Moisture content: provides information about texture since increasing levels of moisture provide water mobility and lower the glass transition temperature
- Stability: reflects the chemical and physical integrity of the dosage unit during the shelf life. The monograph specification of identity, strength, and purity apply throughout the shelf life of the product.
- Oscillatory strain (for gels and lotions): a valuable tool for understanding the structural and dynamic properties of the elastic-like and viscous-like systems.

IN VITRO DISSOLUTION TEST PROCEDURES

If the physicochemical characterization and interproduct comparison has been deemed acceptable, BE can be further evaluated through an interproduct comparison of *in vitro* dissolution profiles. A confirmation of product sameness necessitates that the two products perform comparably, regardless of the *in vitro* conditions under which the profiles are generated.

The dissolution rate (DR) of any product is a function of the available surface area of the drug product (A), the diffusion coefficient (D) of the drug (i.e., its ability to move from the undissolved portion of the API to the surrounding dissolution medium), the effective boundary layer thickness (h , which is the water that physically surrounds the undissolved API), the saturation concentration of the API under the conditions of the dissolution test (C_s), the amount of drug already dissolved (X_d), and the volume of fluid (V) within which the dissolution must occur (7).

These interrelationships are described by the Noyes-Whitney equation:

$$DR = \frac{A \times D}{h} \times \left(C_s - \frac{X_d}{V} \right)$$

For any given apparatus, the shape of the dissolution profiles can be modified by adjusting the agitation speed, the shape of the vessel, temperature, and the composition and volume of the dissolution medium (e.g., refer to (8)). Although the paddle (USP apparatus II) or basket (USP apparatus I) would be employed for most animal drug products, there are situations for which alternative *in vitro* dissolution methods may need to be considered. What constitutes an appropriate dissolution method can vary as a function of drug and dosage form (9). For example, for those products administered directly on the skin, appropriate *in vitro* test methods may include the flow-through cell diffusion apparatus, Franz cell, and paddle over disk, and rotating cylinder (10).

The nature of the dissolution medium can have a substantial impact on the characteristics of the *in vitro* dissolution profile (7,11,12). For this reason, a critical component of the *in vitro* BE approach is the comparability of product performance under a wide range of *in vitro* test conditions. The dissolution media should reflect the range of *in vivo* conditions to which the dosage form will be exposed, thereby allowing the *in vitro* dissolution tests to be used as a prognostic tool for evaluating drug release and dissolution and the ability of the drug to remain in solution once dissolved. When conducted in this manner, these tests can be used to forecast the solubility and dissolution of the drug in the GI tract (13). Relevant physiological considerations include pH, buffer capacity, ionic strength and osmolarity, surface tension, *in vivo* micelle formation, and the hydrodynamics at the site of release. For example, when applied to a monogastric species such as the dog or cat, types of media that may be considered appropriate include the following:

- 1) 0.1 N HCL
- 2) Simulated gastric fluid with surfactants
- 3) Acetate buffer, pH 4.5
- 4) Phosphate buffer, pH 6.8
- 5) Fed simulated small intestinal fluid
- 6) Fasted simulated small intestinal fluid

Surfactants (generally bile salts, but may include sodium lauryl sulfate—SLS) and salts can be used to control osmotic pressure and to buffer against solvation-induced changes in pH. Examples of simulated biological fluid media (based upon human physiology) for a variety of body compartments (including but not limited to the GI tract) have been compiled by Marques *et al.* (14). For intramammary products, alternative media may need to be considered (15).

Given the substantial interspecies differences in the composition and properties of GI fluids (16,17) and the specific challenges associated with the potential sites of administration, the composition of the dissolution media will need to be tailored in a manner that corresponds to each specific situation. Through the use of multiple media, the goal is to provide a range of conditions such that discrimination in

drug release characteristics can be achieved. Through an understanding of the *in vivo* fluid composition and hydrodynamics of the system, the assumption (to be discussed in an open forum) is that we can obtain *in vitro* sink conditions that mimic the rate-limiting factors governing *in vivo* drug release.

Within the context of our proposal, to ensure that the *in vitro* comparisons are not biased by the performance of single RLNAD product batch, multiple RLNAD product batches would need to be characterized (along with multiple runs of twelve replication per run generated with the test product). Ultimately, to confirm comparability of the release of the test and RLNAD product profiles, profile similarity across the diverse test conditions selected would be confirmed *via* statistical methods. It is frequently observed that when due to the large within and between lot variability associated with the *in vitro* dissolution of dosage forms such as medicated articles, the criteria associated with the application of the similarity factor (F2) (18) cannot be met. In these situations (and particularly when considering the combined effects of within and between lot variability), we would wish to obtain information on the spread of the individual dissolution values (e.g., multiple lot or within lot across bags of the type A-medicated article and within dissolution runs) of the test as compared to that observed for the RLNAD product formulation. A potential approach that may be applicable in this situation is the tolerance interval (where the tolerance interval is a statistical method for defining the likelihood that a fixed proportion of the population is being covered with some prescribed level of confidence). For example, the 90% tolerance limit defining the *in vitro* release with 90% confidence could be evaluated based upon the individual observations generated for the RLNAD (e.g., 5 lots×12 replications per lot) and comparability confirmed by

all observations for the test product (e.g., 3 runs, 12 replications per run) being contained within the bounds described for the RLNAD dissolution for each of the *in vitro* release profiles.

For innovators, when linked to *in vivo* data, these *in vitro* test procedures can also be used to define the CQAs of a product and accordingly, the product design space (13).

CONCLUSION

The objective of this commentary is to provide a proposal that can set the stage for future discussions of an *in vitro* BE approach for the evaluation of veterinary drug products that are not eligible for a biowaiver, and is either not systemically absorbed or exerts a portion of its effect at a location proximal to the site of absorption.

While *in vivo* blood level studies remain the gold standard for comparing *in vivo* product performance, blood level studies cannot be used to ensure product comparability when the drug is not systemically absorbed. In these situations, the *in vitro* BE approach provides a promising alternative mechanism for generating interproduct comparisons. The overall approach is described in the flow diagram below (Fig. 2).

In addition to its greater prognostic capabilities (as compared to that of clinical endpoint BE trials), the *in vitro* BE approach is highly efficient. Through a comprehensive series of product comparisons from the perspective of drug physicochemical characteristics, product CQAs and *in vitro* dissolution, this *in vitro* BE approach can provide a highly precise and accurate method for evaluating *in vivo* product comparability. As a result, the ability to apply this alternative approach for defining the equivalence of nonsystemically

Fig. 2. Thought map describing the proposed *in vitro* dissolution BE approach for evaluating nonsystemically absorbed generic new animal drug products or revised formulations of the RLNAD

absorbed, low solubility drugs would be of benefit to drug sponsors, regulators, and most importantly, the end users.

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