dx.doi.org/10.14227/DT240117P6

Characterization of the In Vitro Drug Exchange Profile of a Modified-Release Parenteral Solution for Veterinary Use

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ABSTRACT

The release or exchange profile of an active pharmaceutical ingredient (API) from its carrier or formulation matrix is an important characteristic of a drug product, especially for that of a long-acting or modified-release formulation. The ability to measure this phenomenon in vitro assures that the product is manufactured using the intended quality of raw materials, including API and excipients, and that control of the overall compounding process is maintained. Developing and validating an in vitro drug exchange (IVDE) method that is capable of reproducibly demonstrating quality required addressing some fundamental challenges. Primarily, a suitable medium was identified to extract the API over a reasonable amount of time to generate a release profile, while also ensuring the API does not degrade in this medium during the exchange interval and brief storage prior to being assayed. Secondarily, a system and processes that can be used in a quality control (QC) laboratory setting to enable extraction, sampling, and quantitative measurement of the API exchanged from one medium (phase) into another was designed. The final challenge was the ability to differentiate high-quality drug product from others containing the same API in what could be a lesser quality, or perhaps even a completely different formulation. To meet the third objective, the method development strategy included comparison of the release profiles of the developmental product against numerous other formulations. These included a commercial product containing the same API in a different matrix, lab-scale batches containing the same API and excipients at variable concentrations, lab-scale batches containing similar or related excipients, and lab-scale batches containing degraded excipients. Statistical criteria were set to demonstrate that the method was capable of discriminating the IVDE profiles of the developmental formulation from all others. In addition, the same criteria were set for QC testing at release and real-time stability intervals to assure long-term quality of the drug product.

KEYWORDS: Exchange medium; exchange profile; exchange rate; in vitro drug exchange; in vitro release; modified-release parenteral; release profile; release rate; sink conditions; tolerance interval.

INTRODUCTION

In 2001, a collaborative group of scientists from industry and academia and regulatory agents attended a workshop in Washington, DC, cosponsored by the American Association of Pharmaceutical Scientists (AAPS), the U.S. Food and Drug Administration (FDA), and the United States Pharmacopoeia (USP) to assess the status of formulation processes, manufacturing, and the testing of controlled- and sustained-release parenteral drug products (*1*). The title of this workshop was "Assuring Quality and Performance of Sustained and Controlled Release Parenterals," which led to recommendations for future workshops aimed at addressing topics that were deemed important for further research and evaluation. Many of the same attendees and sponsors reconvened in 2003 in Basel, Switzerland, for a workshop of the same title, this time cosponsored by the European Federation for Pharmaceutical Sciences (EUFEPS), European Agency for the Evaluation of Medicinal Products (EMEA), the European Pharmacopoeia (EP), as well as the original sponsors, the AAPS, FDA, and USP (*2*). At this workshop, many of the proposed requirements for testing the IVDE or release characteristics were discussed, including suitable testing methods, instrumentation, and the reasoning to support specifications and other criteria for testing conventional and modern specialty (e.g., liposomal, microspheres, biopolymers), injectable, and modified-release formulations.

A select group of publications that focus on the concept of characterizing the in vitro release of an active pharmaceutical ingredient (API) from parenteral

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formulations either contributed to or followed up on the topics covered at these workshops. Many explore the concepts (*3–5*), instrumentation (*6–11*), or processes (*12–15*) used to characterize the drug product for the purpose of setting QC specifications. Those that are directly comparable to the efforts presented here are limited due to the physicochemical characteristics of the contents of the developmental formulation. The API of the drug product studied here is very lipophilic, with a log P ≈ 6, and the bulk solvent comprises processed natural oils, which are also lipophilic or hydrophobic. Thus, applying information from these publications directly to the needs of this lipophilic API and drug product matrix proved challenging. However, some helpful guidance was obtained for this purpose.

In June of 2016, the FDA Center for Veterinary Medicine (FDA–CVM) released the Guidance for Industry #238 titled "Modified Release Veterinary Parenteral Dosage Forms: Development, Evaluation, and Establishment of Specifications" (*16*). This document provides the current opinion of the regulatory agency in general terms for designing and validating analytical test methods that are capable of measuring the IVDE or drug release characteristics of API from injectable drug products. With the benefit of this guidance, an investigator can then incorporate the critical aspects as recommended, such as what material to test during development and the necessary criteria for setting appropriate meaningful specifications to confirm the quality and essential characteristics of modified-release drug products are met at release and maintained throughout the product shelf life.

The guidance itself is applicable to the Chemistry, Manufacturing, and Controls (CMC) dossier section for approval of modified-release formulations. Testing used to characterize these types of drug products shall be suitable to determine the IVDE characteristics at release and on stability, as well to assess potential process changes over time. It is recommended that drug product from as early in development as possible be used to establish IVDE specifications, especially batches used to evaluate target animal safety and effectiveness. The criteria proposed for this type of method development include an assessment of the effects of the identity and quality of excipients used in modified-release formulations. This includes the ability of the method to discriminate the IVDE characteristics of a quality product from a product that is produced using incorrect or degraded excipients or an alternative manufacturing process. The discriminatory power of such a method is enhanced by setting appropriate specifications at multiple time points from the beginning, middle, and end of the drug exchange period, using a quality product under controlled conditions. Of prime importance is working within a system that is not limited by solubility or the capacity of the acceptor medium to accommodate a steady increase in concentration of the analyte to greater than eighty percent exchanged.

The body of work presented here describes much of the effort to develop and validate an IVDE method for use in the QC laboratory for batch release and at stability intervals. The terms in vitro drug exchange and release may be used interchangeably throughout the document and are assumed to be descriptive of the same phenomenon. Method development required three phases of studies. First, exchange medium screening was pursued to assure that sink conditions would be met and that API stability would be kept throughout the exchange, sampling, and assay period. During this phase, approximately twenty combinations of emulsifiers, surfactants, and acids in aqueous and organic based media were screened. Second, once a suitable medium was identified, both USP dissolution Apparatus 2 with the Distek topical drug cell and Apparatus 4 with the Sotax dialysis cell (*7, 10, 11*) were compared directly. Apparatus 2 with the Distek topical drug cell was determined to be best suited for this product and application. Conditions were then optimized in phase three to ensure sample preparation, exchange medium contents, and Apparatus 2 setup and settings were capable of discriminating the exchange profiles of the developmental formulation from numerous alternative formulations.

The discriminatory power of this IVDE method includes several criteria to increase confidence that the method can differentiate quality product from product that is formulated or processed improperly. The challenge to settle on a method that enables one to distinguish numerous types of products from a single quality product is one of degree. While conditions might be set to create a high level of differentiation from one alternative formulation, the same conditions might not enable differentiation from another single or class of alternative formulations. Thus, the degree of discrimination or differentiation varies depending on the comparator formulation. In fact, this occurred during this set of investigations and is part of the outcome that will be discussed.

MATERIALS AND METHODS

Developmental and Alternative Batches

The drug product characterized by the method described here is a proprietary developmental parenteral formulation intended for veterinary use. The API is present at a concentration of 10% (100 mg/mL) in a low-viscosity oily matrix. The remaining excipients in this formulation are blended by conventional means to create a solution that completely dissolves the API. The overall appearance and character is one of a clear, oily liquid with low viscosity to enable facile syringeability for loading and dispensing the injectable drug product. Stability is enhanced with the use of an antimicrobial preservative that also serves as a cosolvent. In combination with an emulsifier, the drug product is classified as a modified-release formulation for veterinary use. A generic description of the formulation in functional terms is summarized in Table 1. The contents of several alternative formulations containing the same API compounded using similar, related, but chemically different excipients, will also not be disclosed. All batches were compounded and blended using USP–NF and/or Ph. Eur. grade excipients.

Table 1. Formulation (Functional) Summary

Material	Function	Quantity (% w/v)
API	Active pharmaceutical ingredient	10.0
Aromatic alcohol	Preservative/cosolvent	7.0
Fatty acid–conjugated sorbitan	Emulsifier	4.0
Polar solvent	Filtration aid	0.5
Low viscosity (10 mPa•s) oily lipid	Bulk solvent	q.s. to volume

Exchange Medium

The exchange medium consisted of 55% propylene glycol, 35% isopropyl alcohol, and 10% purified (18 MΩ) water. The volume of exchange medium was 500 mL per sample kettle.

Distek Topical Drug Dissolution Cell

The Distek topical drug dissolution cell fitted with a 25-mm Strat-M membrane (Millipore) was used to retain the sample separate from the bulk volume of the exchange medium once it was immersed in the medium during the exchange process. The sample cell was carefully assembled, filled, and sealed stepwise with 300 µL of drug product and approximately 200 µL of exchange medium with the conditioned Strat-M membrane oriented with the shiny surface facing the exchange medium. Prior to assembly, the Strat-M Membrane was conditioned by soaking in exchange medium for fifteen minutes.

Exchange Apparatus

The exchange apparatus was the conventional USP dissolution Apparatus 2 with the paddle rotation rate set at 125 rpm. The exchange temperature was equilibrated and maintained at 37 °C throughout, with sample collection (without replacement) occurring at 2, 4, 6, 8, 14, 24, 36, and 48 h. Not all time points appear in Figures 1–5.

HPLC System

The samples collected at the specified time intervals were measured for API content by HPLC using a previously validated method. The chromatographic system comprised a Waters Nova-Pak analytical column (C18, 150 × 3.9 mm, 4 µm) and a Brownlee guard column (RP–18, 15 × 3.2 mm, 7 µm) using a mobile phase of acetonitrile/water (70/30) containing ammonium acetate adjusted to pH 6.0 at a flow rate of 2.5 mL/min. The columns were maintained at 50 ± 1 °C with UV absorption of the analyte measured at 242 nm. Data are presented as percentage API exchanged as a function of time.

Data Processing

All statistics were evaluated using SAS JMP software to set the specifications that discriminate properly formulated and manufactured product from product that is not. Specifications were determined from tolerance intervals at 95% confidence and a 99% data distribution. Although samples taken from numerous time intervals from 2 to 48 h, product release specifications were set for four time points (2, 8, 24, and 36 h). In general, achieving greater than 80% exchange is a check on sample preparation and system setup by demonstrating sink conditions exist so that API exchange is not limited by solubility. The 85% exchange limit set at 36 h was set for API exchange characteristics of this specific drug product (Table 2). All figures were produced using OriginPro software by OriginLab Corporation.

Table 2. IVDE Release and Stability Specifications

Sampling Interval	Specification
2 h	$18\% \le Q_1 \le 32\%$,
8 h	$42\% \le Q_2 \le 85\%$,
24 h	$Q_3 \ge 75\%$
36 h	$Q_4 \ge 85\%$

RESULTS

The IVDE profiles presented for the developmental drug product were all derived from the same data. The data

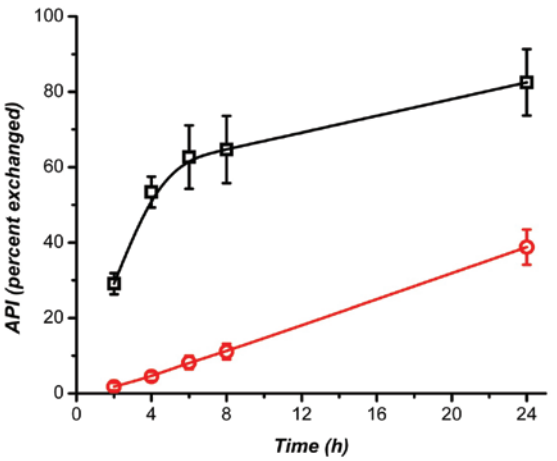

Figure 1. IVDE profiles comparing the exchange characteristics of the developmental formulation (—, □) with an entirely different formulation (—, ○).

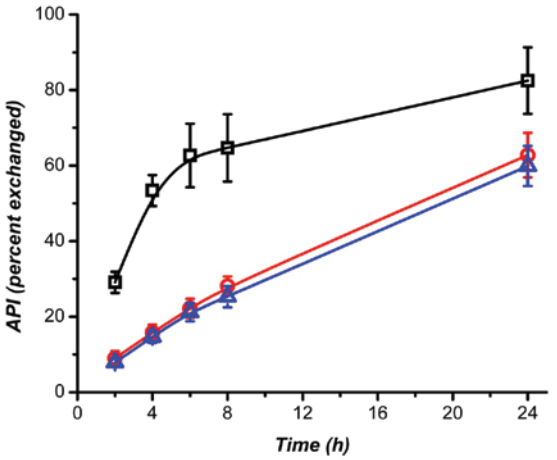

Figure 2. IVDE profiles comparing the exchange characteristics of the developmental formulation (—, □) with two alternative formulations (—, ○ and —, △) compounded with different bulk solvents that are of the same class as the solvent used in the developmental drug product.

set comprises the average of 36 individual profiles ± the standard error of the mean (SEM). The IVDE profiles presented for the alternative formulations in Figures 1–5 are derived from data sets that comprise the average of six individual profiles plus/minus the SEM. The initial comparison (Figure 1) demonstrates the ability of the method to discriminate the developmental formulation from a formulation containing the same API but an entirely different blend of excipients and manufacturing process.

Figure 2 shows a comparison between the developmental formulation and two formulations blended using different bulk solvents of a related class. This confirms that the

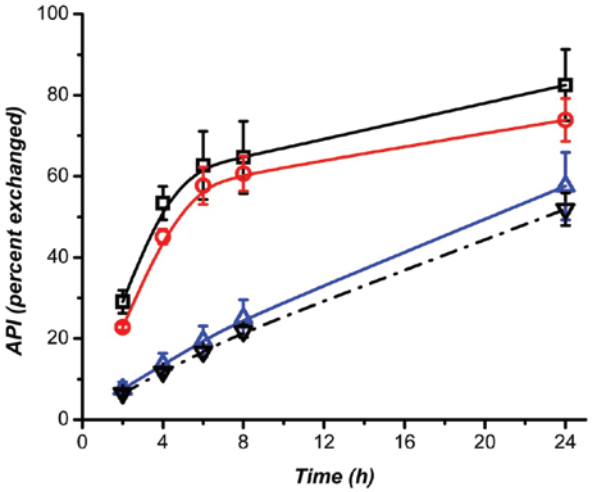

Figure 3. IVDE profiles comparing the exchange characteristics of the developmental formulation (—, □) with three alternative formulations containing a different emulsifier (—, ○), a different solvent and emulsifier at 1% w/v (—, △), and a different solvent and emulsifier at 21% w/v (–●–, ▽).

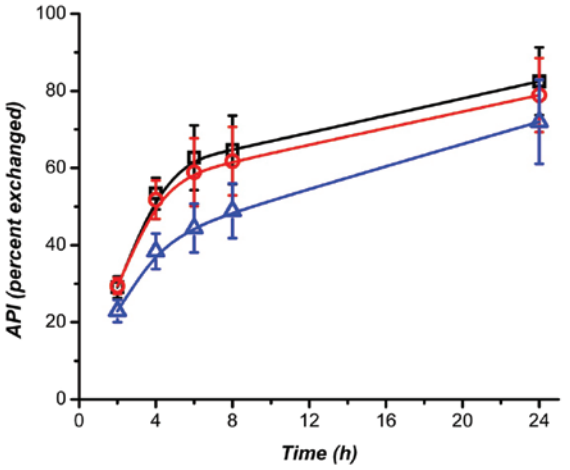

Figure 4. IVDE profiles comparing the exchange characteristics of the developmental formulation (—, □) with two alternative formulations containing (simulated) degraded bulk solvent at 10% w/w (—, ○) and 25% w/w (—, △).

method is capable of discriminating the exchange characteristics of the API contained in similar but not identical alternative solutions made from low-viscosity, oily lipids.

Figure 3 compares the IVDE profiles of the developmental formulation with three other formulations. The alternative formulations differ in their excipient content. In the first case, the only difference is the emulsifier. In the second and third formulations, the emulsifier was different from that of the developmental formulation, as was the bulk solvent, which was identical to Solvent 2 from Figure 2.

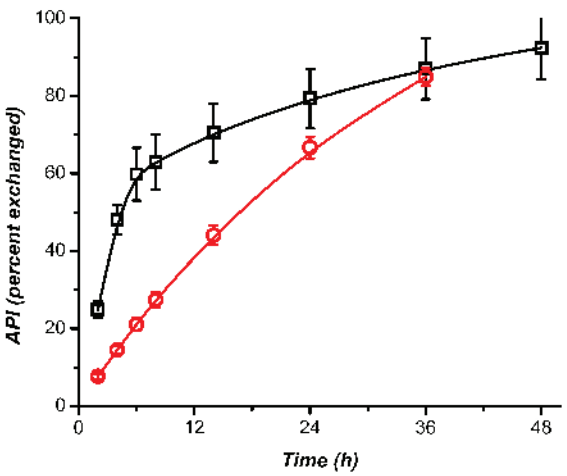

Figure 5. IVDE profiles comparing the exchange characteristics of the developmental formulation (—, □) with an alternative formulation having differential PK characteristics (—, ○).

Degraded excipients were used to compound two alternative batches for the comparison with the developmental formulation illustrated in Figure 4. In the first case, the batch was compounded with 10% (simulated) degraded bulk solvent, and the second batch contained 25% (simulated) degraded solvent.

In Figure 5, the IVDE profile of the developmental formulation is compared directly with an alternative formulation that contained both a different bulk solvent and emulsifier. The alternative batch was also used in a pharmacokinetics (PK) study in the target species and demonstrated significantly different PK characteristics.

Data were collected during the method validation phase of the investigation. Thirty-six individual profiles were used to calculate the specification range by assessing a 95% confidence interval for 99% of the data. Specification ranges were set from these data at the 2- and 8-h intervals, and the minimum exchange limits for the 24- and 36-h intervals. The specifications are found in Table 2.

CONCLUSION

The data describe many of the steps taken during the development of an IVDE method to characterize the exchange profile, and to set specifications accordingly, for a modified-release injectable solution. While in principle the concept of characterizing in vitro drug release appears straightforward, doing so for an oily solution does present significant challenges. First, the need to keep the product separate from the exchange medium with use of a barrier to eliminate mixing, and to limit a burst transfer, is essential. Additional complications arise due to the nature of the formulation being a nonaqueous oily liquid, which creates the potential for incompatibilities with the containment assembly parts, the barrier membrane, or both. Because the bulk solution and analyte are both extremely hydrophobic, the requirements for the exchange medium to accommodate these conditions to establish a driving force for transfer of the API across the membrane, while also being nondestructive to the assembly and the membrane itself, have to be met. In combination, these factors and others were evaluated empirically to assure the method was able to achieve an acceptable level of variability.

In addition, the developmental IVDE profile presents the average of 36 profiles, ±SEM from six separate trial sessions that includes known bias incorporated into the test sessions. These biases were intended to impose batch-to-batch, analyst-to-analyst, day-to-day, membrane-to-membrane, and instrument-to-instrument variability on the method, which is presumably manifest as increased error in comparison to the error of the data sets from the alternative formulations that comprise six profiles ±SEM from one batch, one analyst, one session, one membrane lot, and one instrument. This is assumed to be the reason the alternative formulation data sets are presented with significantly less error than the developmental formulation data set.

The initial evaluation compared the IVDE profile of the developmental formulation and a commercial drug product containing the same API from a completely different, unrelated manufacturing process and formulation matrix (Figure 1). This was considered the proof-of-concept comparison in this series of studies. In other words, if the method could successfully differentiate between these two products, the discriminatory power of the method to differentiate products that are more alike should also be achievable. However, if the method were incapable of discriminating these two formulations, it would be unrealistic to expect any degree of differentiation among batches that were only subtly different. The differences in the release profiles of Figure 1 illustrate with a high degree of certainty that the method is capable of differentiating quality product from product made using a completely different manufacturing process, which is among the proposed expectations of FDA–CVM from Guidance #238 (*16*).

The bulk solvent of the developmental formulation is described as a low-viscosity (10 mPa·s) oily lipid. Two different but related chemicals were used as bulk solvents to compound two alternative formulations,

keeping all other excipients the same. The comparison illustrated in Figure 2 demonstrates the IVDE profiles of the alternative formulations were vastly different from the developmental formulation, although all other testing would meet specifications, even the API assay. From our perspective, this met CVM's criterion of altering the formulation with excipients that would delay the rate of release of the API.

Figure 3 displays three comparisons. The first comparison is one between the developmental formulation and one that contains an alternative emulsifier. While the initial rate of exchange is similar, yet delayed, the time to achieve greater than eighty percent exchange is significantly extended. The other two comparisons are from batches that were made with one of the alternate bulk solvents from Figure 2 as well as the alternative emulsifier. The combination of the two changes greatly diminishes the exchange rate of these formulations. Altogether, the IVDE profile comparisons of these formulations meet CVM's criterion to assess the effect of changes to the emulsifier content on the release rate.

FDA–CVM recommends assessing the effects of degraded excipients. An approach was taken to meet this expectation while not actually degrading the excipients, but rather by simulating the degradation of the bulk solvent. The bulk solvent used in this drug product comprises three distinct chemical entities that are covalently bonded into one, more complex molecule. The pure bulk solvent containing equimolar ratios of the three distinct constituents totaling 10% and 25% (w/w) was used to compound two alternative formulations. The assumption was that by combining the three well-characterized, miscible, pure chemicals, more control would be achieved than with the use of chemically or thermally degraded excipients. The overall effect would be to simulate the degradation of the bulk solvent with the use of a known percentage of the individual principal components. In Figure 4, both alternative profiles are compared with the profile of the developmental formulation. In this comparison, the 10% simulated degraded formulation appears similar, whereas the 25% simulated degraded formulation can be differentiated.

These results provide an opportunity to explain a necessary point. This method was optimized to discriminate a number of different alternative formulations. During the optimization process, several conditions were explored that provided different rates of exchange for the developmental product. So, it has been speculated that if the aim were to optimize several different methods to discriminate between each and every alternative formulation individually, that aim would also have been met by using different exchange media, membranes, sample preparation, and so forth. However, the aim was to develop and validate a single robust method for QC purposes that at the same time would discriminate the exchange profile of the developmental formulation from exchange profiles of numerous formulations. Although there are varying degrees of discrimination, this objective has been met with the efforts described here, especially in selecting formulations that are most different from the developmental product.

The exchange profiles of Figure 5 illustrate the most meaningful comparison between the developmental formulation and an alternative formulation that had different PK characteristics. This confirms the ability of the method to discriminate the in vitro release profile of the developmental formulation from an alternative formulation that was previously demonstrated to have differential pharmacokinetic properties as well. In other words, this method serves as an in vitro indicator of drug product quality that differentiates product that is effective from product that is not.

The specifications for the IVDE method for this developmental formulation are based on tolerance interval calculations at 95% confidence, using 99% proportion of the method validation data set. The data set consisted of a total of 36 drug exchange profiles from the method validation study—three method precision experiments and three intermediate precision experiments (six samples per experiment). The challenge incorporated batch-to-batch, analyst-to-analyst, day-to-day, membrane-to-membrane, and instrument-to-instrument variation. A standard confidence of 95% was chosen; 99% of the data set was chosen to use nearly all of the limited data currently available and to fully factor the method variance into the specifications.

CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

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