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A Formulation Case Study Comparing the Dynamic Gastric Model with Conventional Dissolution Methods

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ABSTRACT

Even in the 21st century, conventional compendial dissolution testing remains a key cornerstone of the drug development process and quality control testing. However, opportunities exist with respect to in vitro technology developments that provide the potential for formulation and analytical scientists to exceed the capabilities of the conventional dissolution test toward a more biorelevant testing regime. This work presents a product development case study in which bioequivalence was observed between an immediate-release (IR) innovator product and a comparative single-layer reference product. Despite this, when the constituent granule of the comparative single-layer reference product was formulated in a bilayer formulation with a nondisintegrating second layer, bioequivalence versus the innovator was not achieved. The use of USP Apparatus 2 dissolution testing failed to predict the bioequivalence failure, and hence an investigation was undertaken to develop a mechanistic understanding of in vivo behavior. Using both USP Apparatus 4 dissolution in the open-loop configuration and the dynamic gastric model (a novel in vitro model designed to mimic the human stomach), an understanding of the dissolution and disintegration properties of the reference product was established. The insights gained using novel technology facilitated the redesign and subsequent improvement in pharmacokinetic parameters of a complex pharmaceutical dosage form.

INTRODUCTION

The correlation of in vitro performance to in vivo behavior is a critically important and cost-effective objective for the drug development process within the pharmaceutical industry (*1*). It is imperative to work toward the development of a mechanistic understanding of the conditions of the gastrointestinal environment and its influence on drug liberation phenomena from the various oral pharmaceutical dosage forms (*2*). While fully characterizing the complexity of the gastrointestinal tract may remain an elusive goal (*3*), understanding the key parameters that can facilitate the prediction of dosage form behavior in vivo may be achieved. A key contributor to developing an understanding is the development of dissolution technologies that are designed to mimic the in vivo environment more closely.

The noncompendial dissolution methods have been detailed in an excellent recent review (*4*). These include multicompartmental models such as the artificial stomach duodenal model (ASD), which has been used to evaluate the effect of gastric emptying on drug dissolution, solubilization, and precipitation in separate duodenal compartment in several studies (*5–8*). In addition, models exist that are designed to simulate GI physical stress forces such as the novel stress dissolution tester (*9*, *10*) or the modified Apparatus 2 by Burke et al. (*11*). Systems that mimic absorption have been described, from the simple partitioning approach using USP apparatus with organic solvents (*12–14*) to the more complex models like the FloVitro Dissolution Testing system provided by Rohm and Haas (*15*). In addition, the human gastric simulator (HGS) is a recent technological advance that has been used to study the gastric digestion of foods (*16*), although this has yet to be applied in the pharmaceutical development space.

The next evolutionary stage of dissolution technology comprises complex systems that are multicompartmental, not only mimicking the hydrodynamics and composition of media but also incorporating mechanical processing, digestion of real food, and gastric emptying. Examples of these systems are the TNO TIM–1 system (*17*) and the dynamic gastric model (DGM). The DGM, developed by the Institute of Food Research in Norwich, UK, is designed to simulate the human gastric compartment of the fundus and antrum (*18*, *19*). It is the first "dynamic" in vitro model that replicates both the complex biochemical conditions and the array of gastric hydrodynamics, critical for the prediction of digestive processes and the bioperformance of pharmaceutical agents and dosage forms. The DGM is gaining increasing utility not only as a general biopharmaceutics tool for the evaluation of dosage form disposition and drug release characteristics, but also for the evaluation of (1) food effect potential, (2) dosage form integrity (especially the propensity for dose dumping), and (3) bespoke drug–alcohol interactions.

This work reports a formulation development case study that utilized noncompendial in combination with traditional in vitro tools. The study has proved useful in gaining insight into the in vivo mechanisms of success and failure of the pharmaceutical product development of a complex multilayer pharmaceutical dosage form.

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MATERIALS AND METHODS

Reagents

Compound A (log $P = 5.7$, $pK_a = 11.82$) was sourced from Merck. It is a BCS Class 2 compound, with low solubility and high permeability as measured by Caco-2, possessing a low solubility at gastric pH (0.04 mg/mL in simulated gastric fluid) and high solubility at duodenal pH (1.5 mg/mL in fasted-state simulated intestinal fluid). The gastric juice compositions were proprietary to the DGM group (PBL/Institute of Food Research, Norwich). Acetonitrile, acetic acid, potassium phosphate, sodium citrate, and sodium acetate were purchased from Sigma Aldrich (Gillingham, UK). Ultrapure water was prepared with a Barnstead NANOpure Diamond purification unit (Barnstead International, Iowa, USA).

Formulation

Compound A was dry granulated together with excipients on an Alexanderwerk WP120 roller compactor (Alexanderwerk, Germany) with 25-mm rolls. Subsequently, bilayer tablets containing compound A were compressed using a Riva Piccola tablet press (Riva GB Ltd., UK) at 2.3 kN tamping force and 30 kN main compression force. A bilayer was used because additional active was present in the other layer. These core tablets were coated with Opadry II (2% w/w of the total tablet weight).

Methods

USP Apparatus

Dissolution testing was conducted in USP Apparatus 1 and 2 using a VK7000 dissolution bath, and samples were taken using a VK8000 autosampler (Varian Inc., Cary, NC, USA) and filtered through a 35-µm full-flow filter. A paddle speed of 75 rpm was used, media volume was 900 mL, and sampling time points were 5, 10, 15, 20, 30, 45, and 60 min with an infinity spin for 30 min at 250 rpm. Dissolution in USP Apparatus 4 was conducted using a Sotax CE7Smart semiautomated system (Sotax AG, Basel, Switzerland) at a flow rate of 4 mL/min. Samples were taken at 5, 10, 15, 20, 30, 45, 60, and 90 min, and then filtered inline by glass wool followed by a 25-mm GF/F disc (Whatman, Maidstone, UK). Samples were then centrifuged at 14,000 rpm for 10 min. All dissolution testing was conducted at 37 °C. All samples were then transferred to HPLC vials for analysis.

Dissolution Media Choice and Rationale

Dissolution in USP apparatus was conducted in 50 mM potassium phosphate pH 6.8, 20 mM sodium citrate pH 4.5, or both. Sodium citrate pH 4.5 was chosen to provide a sink-limited medium and not to represent the gastric fasted environment. SGF would have afforded little drug dissolution, and a disintegration test in the traditional sense would not possess sufficient resolution due to problems presented by the bilayer tablet configuration.

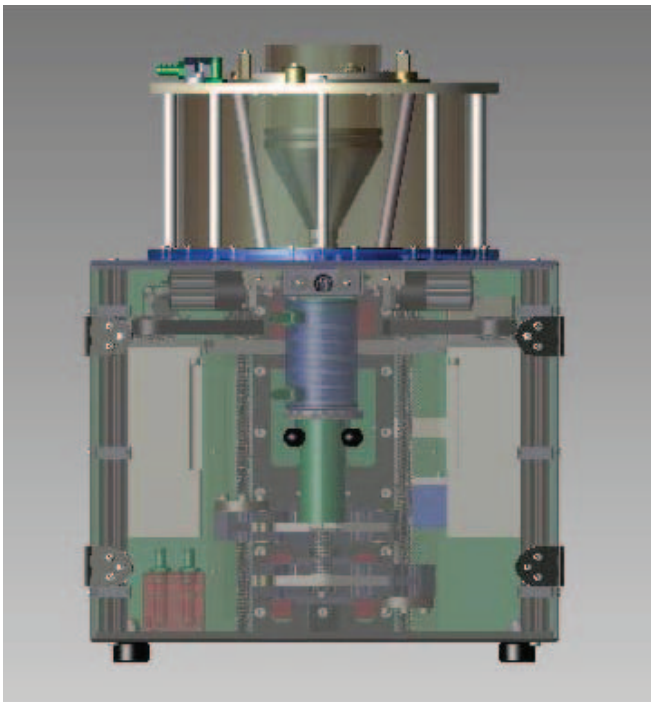

Figure 1. The Dynamic Gastric Model (DGM).

USP Sample Analysis

After filtration, all USP apparatus dissolution samples were analyzed directly by reversed-phase HPLC–UV using an Agilent 1100 equipped with a variable wavelength detector (Agilent Technologies, Stockport, UK), a Phenomenex (Torrance, CA) Onyx C18 column (100 × 4.6 mm) at 45 °C, and an isocratic mobile phase of 55:45 0.1% acetic acid/acetonitrile. Detection was at 244 nm, the injection volume was 10 µL, and the runtime was 2.5 min.

Dynamic Gastric Model (DGM)

In parallel, selected formulations were tested in the DGM (Figure 1) (Plant Bioscience Ltd., Institute of Food Research, Norwich, UK) in a fasted-stomach protocol. The clean DGM "stomach" was brought to 37 °C and primed with 20 mL of gastric secretions (0.01 M hydrochloric acid and salts with gastric enzymes) to simulate the mean residual gastric fluid volume in the stomach. For fasted conditions, 240 mL of ultrapure water at room temperature was slowly poured into the main body of the DGM at the same time as the tablet was dropped in. This simulated taking the formulation with a drink of water on an empty stomach.

For the fasted state, the model was set up to process the gastric contents at a rate of approximately 80 mL/h giving a target value of six gastric samples of 45 mL over a total time of 28 min. All samples collected from the "antrum" were visually inspected for the presence of nondisintegrated tablet, weighed, snap-frozen in liquid nitrogen, and stored prior to analysis.

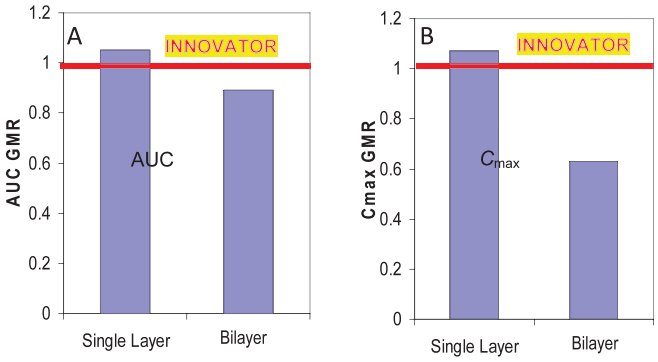

Figure 2. GMR Ratios of (A) AUC and (B) C_{max} of single-layer and bilayer formulations vs innovator formulation.

DGM Sample Analysis

Samples were thawed and equilibrated at room temperature. Each sample was centrifuged, and the supernatant was analyzed by HPLC–UV to measure the portion of drug in solution. The sample was homogenized, and diluent was added to ensure solubilization of any undissolved drug in the gastric samples. This was then analyzed by HPLC–UV to measure the amount of drug that emptied from the DGM as disintegrated particles from each gastric sample. The combined drug concentration was then reported assuming that drug that had entered the duodenum would be rapidly solubilized at the higher pH and hence rapidly absorbed.

In Vivo Studies

Monolayer Study Design

The PK study for all monolayer products was an open-label, randomized, crossover study in 20 healthy, young male and female subjects. In each period, subjects received one of two oral, single-dose treatments in the fasted state.

The order in which subjects received each treatment was randomly assigned according to a computer-generated allocation schedule. There was a minimum of a 7-day washout period between dosing in each period. Plasma samples for compound A were collected pre-dose and at select time points up to 48 h post dose.

Bilayer Study Design

The PK study for all bilayer products was an open-label, randomized, crossover study in 24 healthy, young male and female subjects. In each period, subjects received one of three oral, single-dose treatments in the fasted state.

As per the monolayer clinical study, the order in which subjects received each treatment was randomly assigned according to a computer-generated allocation schedule. There was a minimum of a 7-day washout period between dosing in each period. Plasma samples for compound A were collected pre-dose and at select time points up to 48 h post dose.

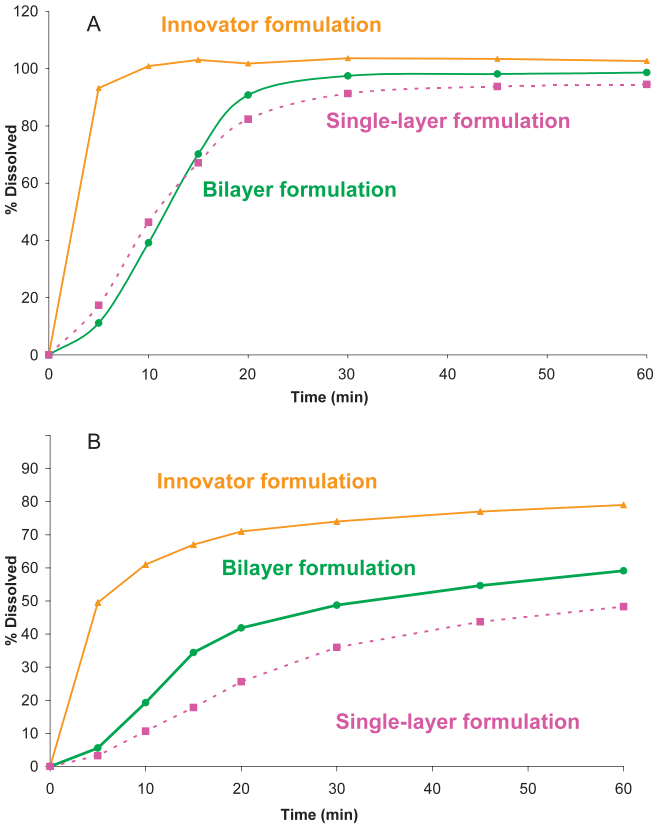

Figure 3. Nonpredictive testing of innovator and single-layer and bilayer reference formulations in (A) pH 6.8 phosphate and (B) pH 4.5 acetate buffers. (Apparatus 2 at 75 rpm, 37 ± 0.5 °C.)

RESULTS AND DISCUSSION

Single-Layer versus Bilayer In Vivo Performance

Initially, an IR formulation of a comparative single-layer reference of compound A and an innovator product of compound A were run in a human clinical study to test for bioequivalence. The reference product was bioequivalent to the innovator product for both AUC and C_{max}. However, when a bilayer comprising the same constituent granule from the reference formulation in the IR layer coupled with a nondisintegrating second layer (to form a bilayer) was run in a human clinical study, bioequivalence was not observed against the innovator product. Human PK data exhibited clear differences in AUC and C_{max} geometric mean ratio (GMR) between reference and innovator, with a reduction in bioperformance in the bilayer formulation compared with the single layer (Figure 2A,B). A large observed decrease in C_{max} was particularly noteworthy.

In Vitro Prediction by Apparatus 2

In contrast, drug release in USP Apparatus 2 using either a dissolution medium with sink conditions (pH 6.8 phosphate buffer, Figure 3A) or sink-limited medium (pH 4.5 acetate buffer, Figure 3B) showed little difference between monolayer and bilayer formulations containing the same IR reference granule used in the in vivo study

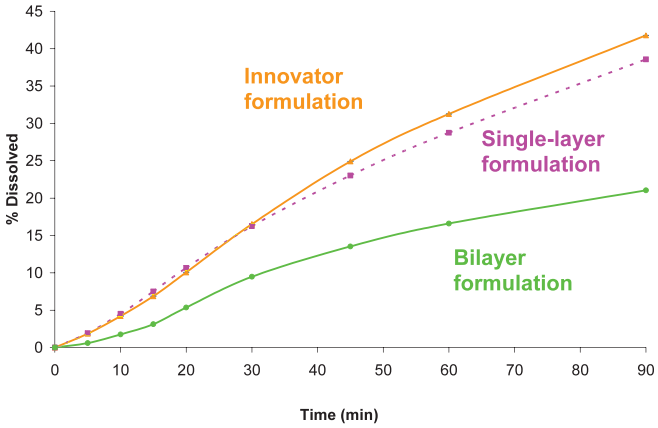

Figure 4. Predictive USP Apparatus 4 testing of the innovator monolayer and single-layer and bilayer reference formulations.

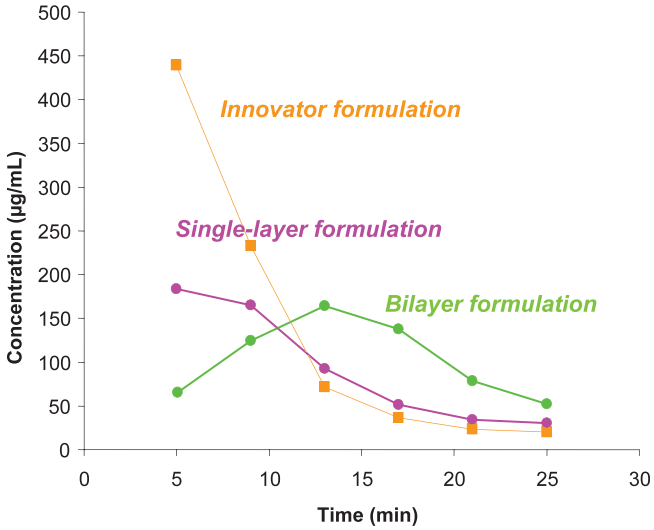

Figure 5. Cumulative drug release in the DGM from the bilayer formulation vs single-layer formulation.

described above. This was despite both being slower than the innovator product, suggesting erroneously little impact of formulation configuration and hence confirming an absence of predictive capability of the standard Apparatus 2 dissolution. To facilitate the screening of new formulation iterations to enhance bioperformance, the development of more predictive in vitro tools was required.

In Vitro Prediction by USP Apparatus 4

USP dissolution Apparatus 4 was evaluated as a predictive in vitro tool to discriminate the differences between dosage form configurations. Interestingly, when the formulations were run in Apparatus 4 (open loop) using a sink-limited medium of pH 4.5 acetate buffer, the formulations appeared in rank order with the existing human PK data (Figure 4). The comparative single-layer reference showed a drug release profile similar to that of the innovator product, while the larger bilayer formulation exhibited markedly slower release. This was encouraging and allowed a certain amount of prediction to utilize as a formulation development aid to screen the next potential clinical formulations. However, it did not discern the necessary mechanistic understanding of the lack of bioperformance in the clinic to give a targeted approach to the development process. When the formulations were tested in the open loop in pH 6.8, the discrimination between formulations was lost due to the higher drug solubility.

It was hypothesized that the dosage form size increase, coupled with the proximity of the IR layer to a nondisintegrating layer, generated a lag in emptying from the fasted-state stomach. This could be caused by material from the IR layer remaining trapped in the bilayer surface. In conjunction with the extremely rapid disintegration of the innovator product, this could afford a sufficient delay to blunt the C_{max}, resulting in a bioperformance issue. The hypothesis validation in animals was not possible because this was a specific case of modeling gastric emptying behavior and passage of a large dosage unit from the antral region of the stomach. Both correlate poorly between different animal species and with humans (*20*). In addition, a human study utilizing gamma scintigraphy, magnetic marker, or other in vivo imaging technology to monitor gastric retention was prohibitively expensive. Therefore, an alternative test system was sought. The DGM system was a viable alternative to test the above scenario. Because of its design, it closely mimics the in vivo environment and processing of the human stomach. It was possible to monitor the gastric emptying profile of the various formulations, information that is not readily achievable with any traditional USP technique.

The results obtained from the DGM were encouraging, and like Apparatus 4, the DGM was able to discriminate the single-layer versus bilayer dosage form configurations. In addition, a similar rank ordering with respect to the single-layer formulation versus bilayer was obtained (Figure 5). In particular, the T_{max} values of compound A during the gastric cycle for the innovator product and single-layer reference product were around the 5-min time point, whereas the bilayer formulation T_{max} was delayed to the third emptying event at 13 min. This afforded clear evidence that a delay in disintegration from the bilayer was hindering the release of compound A from the stomach to the duodenum. This was confirmed through delamination of the bilayer formulation with subsequent dosing of the IR layer in isolation from the nondisintegrating layer. The T_{max} shifted to the earlier 5-min time point. It was also observed that the nondisintegrating dosage layer was retained in the DGM until the housekeeper wave at 25 min or held over to the next fasted cycle. Critically, the emptying from the DGM was dependent on the orientation within the antrum and the rate of size reduction of the nondisintegrating layer.

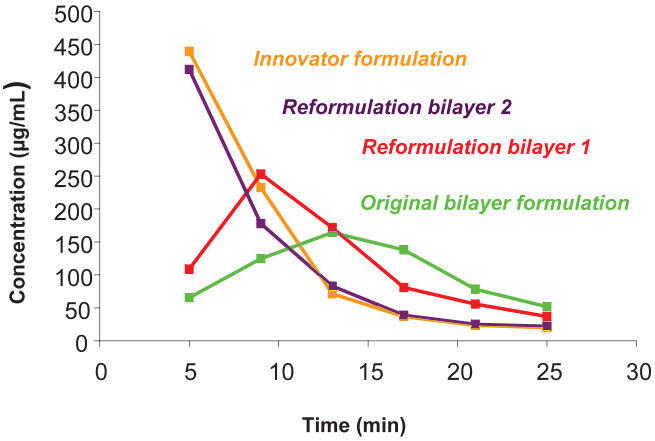

Figure 6. Cumulative drug release in the DGM from the bilayer reformulations compared with innovator reference formulation and original bilayer formulation.

Several factors may have caused the delayed T_{max} between the single layer and bilayer: (1) an increase in dosage form size, (2) a moderately slower disintegration rate compared with that of the innovator, and (3) the trapping of material to the bilayer interface. Of the three, the trapping of material to the bilayer interface was seen as the most important with regard to influencing the bioperformance. If the disintegration from the bilayer interface was delayed sufficiently, the layer containing compound A becomes trapped because of the polymer swelling of the nondisintegrating layer. The drug was poorly soluble at gastric pH and would not readily diffuse through the gel layer of the nondisintegrating layer. This would have the consequence of not being available within the same temporal window as the single-layer reference and innovator products.

The discriminatory performance of these formulations allowed the identification of lead formulation iterations (nominally described as bilayer reformulations 1 and 2). These formulations were taken into human PK studies to further product development (Figure 6). The reformulation efforts were focused on enhancing the separation of the two layers upon contact with aqueous fluids and subsequent rapid disintegration of the constituent granules of the first layer. This was achieved through changes to the filler and disintegrant level in the immediate-release layer. In addition, this allowed determination of the validity of the in vitro DGM screening. Figure 7 shows that the in vivo human data confirmed the value of the predictions made using the PBL–DGM. There was a clear enhancement in the C_{max} and AUC GMR with respect to the innovator product, with bioequivalence being demonstrated for AUC. Although, C_{max} did not pass traditional bioequivalence bounds, the rank ordering of the formulation release rates was mirrored in vivo and supports the value of the formulation development work undertaken.

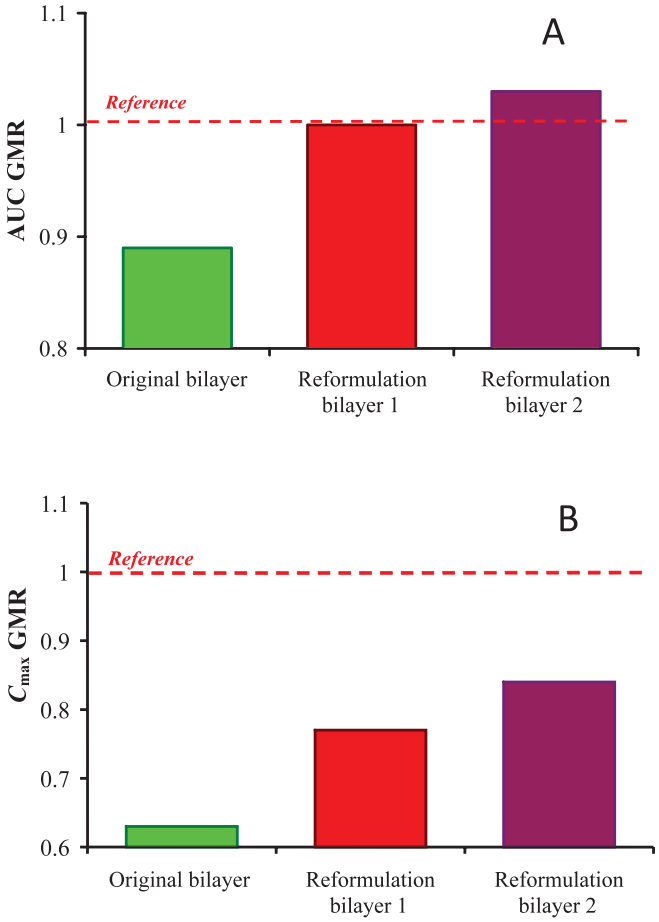

Figure 7. GMR Ratios of original bilayer formulation and bilayer reformulations 1 and 2 vs the innovator monolayer (A) AUC (B) C_{max}.

CONCLUSIONS

This product development case study demonstrates that by decreasing the reliance on conventional USP methods, innovative technology may potentially afford levels of insight and prediction of in vivo performance that are not readily attainable by other techniques. This may be particularly important if pharmaceutical product bioperformance is specifically impacted by the conditions and behavior of the GI tract. In addition, establishing some level of IVIVR is useful as a screen for formulation development, but ultimately it is desirable to have a mechanistic understanding of the in vivo performance that can be afforded by nonconventional methodologies.

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