Executive Summary: *In Vitro* Test Methodologies
To Characterize Bioavailability-Enhancing Formulations
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Lonza
Pharma & Biotech

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INTRODUCTION

Some of the most important technologies available to formulators are those that enhance bioavailability, given the large percentage of low-solubility compounds in today's pharmaceutical pipelines. Some estimate that the percentage of compounds in Class II and IV of the Biopharmaceutical Classification System (BCS) is as high as 75%, making bioavailability enhancement key to the advancement of many new medicines.

Formulators have many choices in their toolkit when it comes to bioavailability enhancement, so the tough call becomes which one to use for a given compound. Choices include (1) those that alter the form or particle size of the solid-state compound (such as solid amorphous dispersions, nanoparticles, and nanocrystals; (2) those that create new crystalline compounds (such as cocrystals and salts); and (3) those that rely on solvation or complexation (such as surfactants, cosolvents, and lipids).

Clearly, accurate comparison of formulation approaches early in the development process is crucial to avoid wasting valuable active pharmaceutical ingredient (API), time, and money. Methods are needed to pinpoint which formulation is best relative to other options and to ensure *in vitro* or *in silico* results accurately track with *in vivo* performance.

To address this need, Lonza has developed a full complement of tools that accurately predict the performance of bioavailability-enhanced formulations. With careful consideration of test methodology and design, Lonza's scientists have bridged the gap between every compound being a "research project" to ensuring an efficient science-based testing platform is available to evaluate formulations.

TESTING PLATFORM FOR BIOAVAILABILITY-ENHANCED FORMULATIONS

Understanding Your Compound

Lonza's methodology begins with a thorough characterization of the API, considering physical and biological barriers to drug absorption. Key properties include the ratio of melt temperature to glass-transition temperature (T_m/T_g), lipophilicity, polar surface area, food effects, solubilities in various solvents, intestinal permeability, metabolic pathways, pKa, and dose. These characterization tests provide key data for subsequent *in silico* and *in vitro* tests and dictate which tests make sense to pursue.

Using Modeling and Mapping

In silico tools are important in assessing why the target API is poorly absorbed. These can be simple, such as predictions based on basic compound properties, or involve the use of dimensionless numbers such as dose, dissolution, and permeation numbers.

Technology maps—based on the characterization data—are employed to verify that the bioavailability enhancement approach being considered is appropriate given the compound's physicochemical properties. These maps, which leverage Lonza's extensive *in vivo* datasets from previous work with thousands of compounds, indicate the typical limits of applicability for bioavailability-enhancing technologies.[1]

Selecting the Right *In Vitro* Tests

Lonza's platform-based approach provides a scientific methodology for the selection of the appropriate *in vitro* tests from the wide range of possibilities available. This involves a three-step process: (1) predicting the *in vivo* problem statement, based on whether precipitation, dissolution rate, or solubility/permeability is thought to be the rate-limiting factor to absorption; (2) selecting the right test apparatus; and (3) choosing the appropriate test parameters. *In vitro* tests can be used to measure a wide range of factors, including dissolution rate, amorphous solubility, precipitation risk (and rate), polymer selection, drug/polymer interactions, emptying rate, maximum apparent drug concentration, and speciation, which accounts for the contribution of micelles, colloids, and particles to unstirred water-layer diffusion and dissolution rate.

The combined data from these tests can provide an accurate picture of formulation performance, information on which factors are limiting bioavailability, and accurate predictions of performance *in vivo*. Below, we illustrate how this platform has been used in recent case studies.

[1] To learn more, please see the Lonza white paper titled "Technology Selection for Bioavailability Enhancement."

CASE STUDIES

Intraconazole Dispersions

In this case study, we used *in silico* and *in vitro* testing to evaluate the performance of amorphous spray-dried dispersions (SDDs) made from itraconazole, a BCS Class II compound. Two 25:75 SDDs were prepared with itraconazole and hydroxypropyl methylcellulose acetate succinate (HPMCAS), using either HPMCAS-L (Affinisol 716HP) or HPMCAS-H (Affinisol 126HP) as a dispersion polymer. The former formulation is termed the "hydrophilic SDD" and the latter the "hydrophobic SDD." For comparison, we also tested a commercial Sporanox® spray-layered dispersion.

We considered dimensionless numbers—dose number, dissolution number, and permeation number—to predict the impact of these factors on performance. The compound's high lipophilicity and neutral charge state at intestinal pH drives low solubility but high lipid membrane permeability, so we expected to see aqueous boundary layer (ABL)-limited flux *in vitro*. *In silico* analysis showed that solubility-permeability was expected to be the dominant limitation *in vivo*.

For the *in vitro* testing, we used a material-sparing membrane flux test that can be used to assess solubility-permeability-limited absorption with three different media: blank phosphate buffer solution (PBS), 6.7 mM simulated intestinal fluid (SIF), and 27 mM SIF. The test design is illustrated in Figure 1 and the results of these in tests are shown in Figure 2.

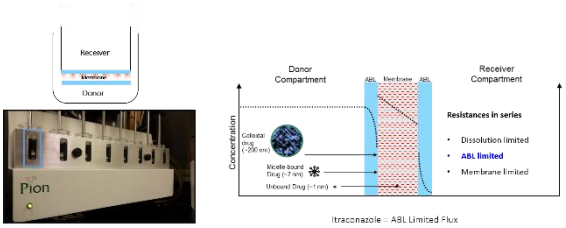
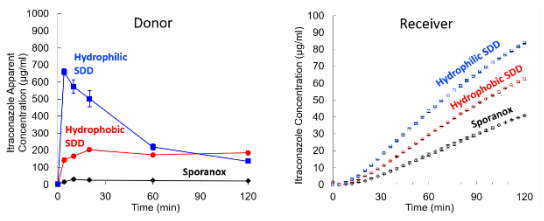


Figure 1

In vitro membrane flux test



No.	Formulation	Dispersion polymer	Flux (µg/min/cm²)	Colloid (µg/ml)
■	25% ITZ/75% HPMCAS SDD	AFFINISOL 716HP	1.18	602
●	25% ITZ/75% HPMCAS SDD	AFFINISOL 126HP	0.85	150
◆	Sporanox® spray layered dispersion	HPMC	0.53	0

Figure 2

In vitro membrane test results

As the figure shows, the hydrophilic SDD had the highest flux. A steady-state diffusion model was developed that supported the *in vitro* measurements. This model successfully predicted that the calculated flux of the hydrophilic SDD was highest.

In vivo testing in rats verified the results of the *in vitro* and *in silico* predictions, as shown in Figure 3. The case study

- identified unique drug speciation from itraconazole SDDs compared to the commercial Sporanox formulation,
- evaluated contributions of these species to *in vitro* flux based on ABL-limited diffusion,
- described contributions of drug species mathematically, and
- demonstrated the impact *in vivo*, showing that absorption rate trended with *in vitro* flux.

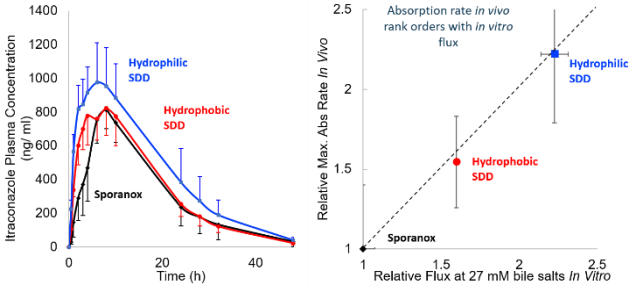


Figure 3

In vivo test results in a rat model

This case study provides just one example of how a suite of tools can be used to compare formulations, develop deep understanding of the science behind performance (such as speciation and forces limiting absorption), and successfully predict relative *in vivo* performance. Use of Lonza's platform-based methods gives formulators the freedom to focus on the most-promising approaches early in the development process.

Belinostat Dispersions

In this case study, we combined *in silico* and *in vitro* tests to forecast and understand the *in vivo* performance of amorphous solid dispersions (ASDs) made with belinostat, a low-solubility API. This understanding can be challenging considering the dynamic behavior around supersaturation, solubilization, and precipitation of ASDs, a particularly effective bioavailability enhancement technology for low-solubility APIs.

For these tests, we prepared three amorphous solid dispersions (25% w/w belinostat with either HPMCAS-M, PVP VA64 or PVP K30). The goal of this exercise was to maximize oral absorption from an amorphous formulation of belinostat.

For *in vitro* testing, we used an amorphous solubility assay combined with fiber optic UV probes (shown in Figure 4) as well as a gastric to intestinal transfer test to monitor ASD bioperformance. *In silico* tests consisted of absorption modeling using GastroPlus v9.7 software. *In vitro* amorphous solubility and formulation dissolution data, *in vivo* clearance terms established from intravenous data reported in the literature, and physicochemical properties calculated using ADMET predictor v9.0 were used as inputs to the model. *In vivo* tests were conducted in fasting beagle dogs at a 50 mg dose. The results of the tests are shown in Figure 5.

As the figure shows, the *in vitro* tests were predictive in determining *in vivo* performance of belinostat dispersions particularly with respect to dissolution rate and extent as well as capturing the impacts of polymer type on the belinostat amorphous solubility. *In silico* modeling suggested that the key driver for absorption was dissolution in the stomach prior to transit into the proximal intestine, suggesting the PVP K30 dispersion should perform the best *in vivo*, which was confirmed from the dog study results.

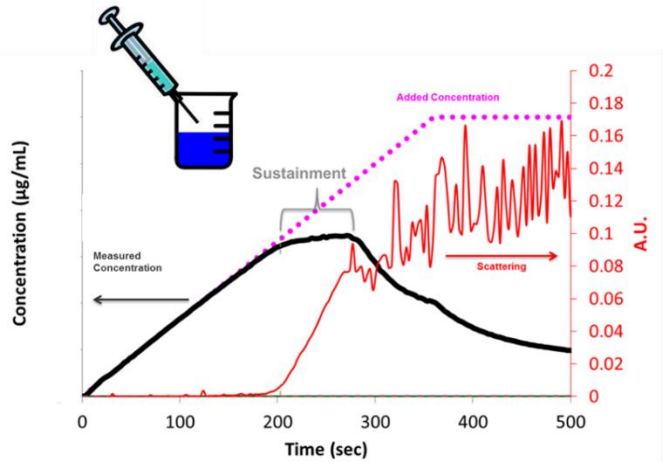


Figure 4

Schematic of the amorphous solubility assay

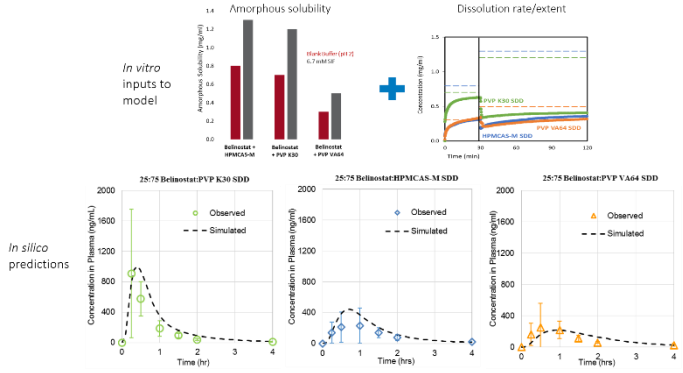


Figure 5

In vitro and in vivo test results, including comparison with in silico predictions

The results of this case study highlight the utility of combining *in vitro* testing and *in silico* modeling to forecast and understand *in vivo* performance of bioavailability-enhancing formulations, resulting in a successful *in vivo* test for belinostat. The key driver for *in vivo* performance was determined to be dissolution in the stomach prior to transit into the proximal small intestine, as evidenced by the *in vitro* data and *in silico* predictions.

ABOUT LONZA

At Lonza, we combine technological innovation with world class manufacturing and process excellence. Together, these enable our customers to deliver their discoveries in the healthcare, preservation, and protection sectors.

We are a preferred global partner to the pharmaceutical, biotech, and specialty ingredients markets. We work to prevent illness and promote a healthier world by enabling our customers to deliver innovative medicines that help treat or even cure a wide range of diseases. Founded in 1897 in the Swiss Alps, Lonza today operates in 120 sites and offices in more than 35 countries. With approximately 15,500 full-time employees, we are built from high-performing teams and of individual employees who make a meaningful difference to our own business, as well as the communities in which we operate. Find out more at https://pharma.lonza.com.