APPLICATION NOTE

Predicting Bioequivalence During Generic Drug Formulation

Small formulation changes can impact bioequivalence, but simultaneous dissolution-permeation testing can reveal the true absorption potential.

Pion's Rainbow R6™, external USPII Bath, stirring unit, and MacroFLUX™ vessels.

Generic drugs play a critical role in expanding patient access to safe, effective, and affordable therapies. By design, they contain the same active pharmaceutical ingredient (API) as the brand-name reference product and are required to demonstrate equivalent clinical efficacy and safety when administered to patients under the conditions of use specified in the labeling.

Although a generic small molecule drug must contain the same API as the reference product, the overall formulation does not need to be identical. Manufacturers can select different excipients to improve manufacturability, stability, or availability of the product, as long as those choices do not alter efficacy and safety. This flexibility allows developers to change the formulation, but it also underscores the need to evaluate how excipients may influence the dissolution and absorption characteristics of the API, and ultimately, whether there is *in vivo* bioequivalence.

The U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and other regulatory agencies mandate bioequivalence studies as a prerequisite for approving generic drugs. These evaluations are used to confirm the efficacy and safety of the generic formulation, facilitate the approval process, and support patient access to cost-effective treatments.

Bioequivalence is defined in 21 CFR 314.3(b) as the "*absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.*" [1]

The process to confirm the bioequivalence of various generic drug formulations with the reference product typically includes *in vitro* dissolution studies, which are

particularly critical for poorly-water soluble APIs, prior to any clinical trials. These screening studies aim to predict the *in vivo* bioavailability of formulations that have been altered to improve critical drug characteristics and identify optimized formulation options. While human clinical trials for bioequivalence are the ultimate test, they are extremely costly and time-consuming, hence the need for more informative *in vitro* approaches.

Limitations of Conventional Dissolution Tests to Predict Bioequivalence

While dissolution tests described by the United States Pharmacopeia (USP) offer an approach for analyzing the final dosage form of a drug for QC purposes, the *in vitro* results often show poor correlation with *in vivo* data.[2] One reason this discrepancy occurs is that, in the body, dissolution and absorption happen in sequence, whereas separate *in vitro* methods are typically used to study these properties. This approach can lead to results that do not accurately reflect real-world biology.

When developing formulations for poorly water-soluble APIs, it is also essential to account for the relationship between solubility and permeability. Many solubilizing excipients, such as surfactants, cosolvents, and cyclodextrins, affect not only how readily the drug dissolves but also how it passes through biological membranes, influencing overall absorption.[3] For example, excipients used to enhance dissolution may also modify the API's solubility in a way that hinders absorption (such as binding or micellar entrapment).[4]

Simultaneous Measure of Dissolution and Absorption

The Pion BioFLUX™ and MacroFLUX™ systems are designed to simulate drug absorption by measuring the movement of compounds across a biomimetic lipid-coated artificial membrane, which separates a donor compartment (containing the sample) and an acceptor compartment which represents the blood (Figure 1). This design allows for continuous, real-time measurement of drug concentration on either side of the membrane, enabling detailed assessment of drug dissolution, permeation, and the effects of different formulation strategies and excipients.

This application note describes the use of BioFLUX™ and MacroFLUX™ systems for the simultaneous, *in vitro* evaluation of dissolution and absorption of generic versions of telmisartan and itraconazole. By enabling simultaneous measurements, these flux-based systems offer the potential to improve *in vitro-in vivo* correlation (IVIVC), which can help generic drug developers improve the outcome of bioequivalence studies. When applied to generic drug development from excipient selection through final testing of dosage forms, promising formulations can be more rapidly identified, while risk is reduced, late-stage surprises are minimized, and *in vivo* bioequivalence is more often achieved.

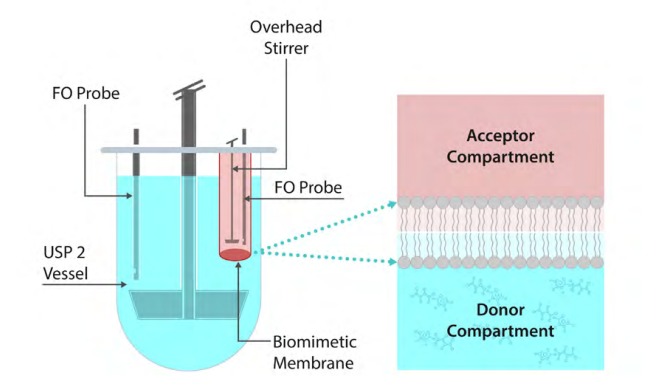

Figure 1. Schematic of MacroFLUX™ system which is designed for simultaneous measurement of dissolution and absorption. Includes two FO (fiber optic) probes.

In Vitro Dissolution and Absorption of Generic Telmisartan Formulations

Reference and four generic formulations of telmisartan, a poorly water-soluble antihypertensive drug, were evaluated using the MacroFLUX™ system to determine the effect of different formulation additives on dissolution and absorption.[3] Table 1 provides the qualitative composition of the reference drug (Micardis) and four generic formulations. The *in vitro* results were then compared to *in vivo* bioequivalence study results published by the developer of the generic products.

The results of this study demonstrated that the *in vitro* MacroFLUX™ test was sufficiently sensitive to identify even small differences in and among the reference formulation and generic formulations that resulted from the incorporation of different excipients. By changing from sorbitol to mannitol in the formulation, the flux through the membrane was reduced by approximately 10%; changing the salt forming agent and caused a reduction in permeation of approximately 20% compared to the brand formulation. This significant difference aligns with published *in vivo* results. The use of lactose monohydrate in one of the formulations also led to an

Table 1. Composition of marketed telmisartan formulations.

Micardis	Generic A	Generic B	Generic C	Generic D
Povidon	Povidon K90	Povidon K30	Povidon	Povidon
Meglumine	Meglumine	Meglumine	Croscarmellose sodium	Meglumine
NaOH	NaOH	NaOH	KOH	NaOH
Sorbitol	Sorbitol	Lactose monohydrate	Mannitol	Mannitol
Magnesium stearate	Magnesium stearate	Magnesium stearate	Magnesium stearate	Magnesium stearate
	HPMC 3c	Sorbitol		

approximate reduction in permeation of 10%. The results show that by using different excipients, the dissolution of telmisartan was not altered significantly, but the permeation through the membrane was significantly changed.

Bioequivalence studies are pivotal in evaluating the pharmacokinetic parameters of the test and reference formulations to show that they fall within acceptable limits. Key parameters include maximum plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC), and the time to reach maximum plasma concentration (T_{max}).[5] Two products are deemed bioequivalent if the 90% confidence intervals of the geometric mean generic/innovator (test/reference) C_{max} and the AUC ratios fall within the bioequivalence limits of 80-125%.[6]

As shown in Figure 2, of the four telmisartan generics that were evaluated using the MacroFLUX™ system, three were confirmed to be bioequivalent and one was slightly out of the acceptance range. This formulation was also on the low limit of acceptance in the *in vivo* study, and the acceptance limit range was extended to 75-133% with the regulatory authority, allowing the bioequivalence claim to be based on the wider interval.

In Vitro Dissolution and Absorption of Generic Itraconazole Formulations

The telmisartan study demonstrated good bioequivalence among formulations and highlighted one that was on the low limit of the range. The following study illustrates an example in which the same approach can be discriminative and show non-bioequivalence.

Itraconazole is a biopharmaceutics classification system (BCS) class II antifungal drug. It is a very lipophilic weak base with extremely low aqueous solubility and is marketed in three formulations (Table 2).

The first marketed formulation was the Sporanox® capsule, which was among the first drug products to be manufactured as an amorphous solid dispersion. The formulation required a fed-dosing condition, which presented a disadvantage for typical patients taking the drug. Given this challenge, Sporanox® solution was developed as a follow-up formulation to ensure suitable absorption in a fasted condition. Human data showed

	Flux Ratio (%)[a]	Lower 90% CI in %	Upper 90% CI in %
Reference Product	**100**	**0**	**0**
Generic A	100.1	2.8	2.9
Generic B	91.4	4.1	4.4
Generic C	81.6	2.4	2.6
Generic D	92.1	6.4	7.3

[a] flux of Generic / flux of Reference

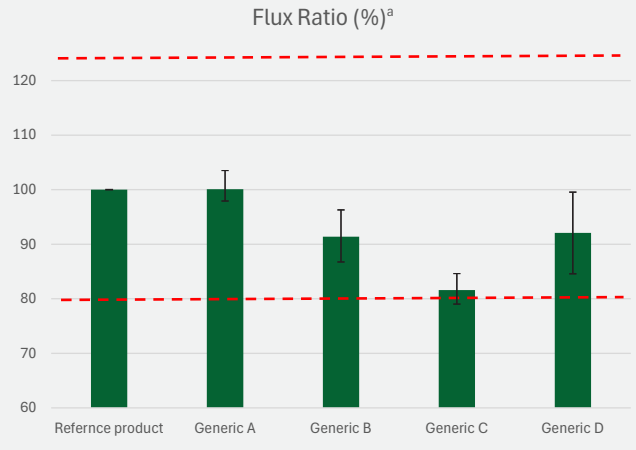

Figure 2. Bioequivalence is demonstrated here as the Flux Ratio % = acceptor profile of the formulation / acceptor profile of the reference x 100, where CI = the Confidence Interval. Bioequivalence limits were set to 80-125% of the Reference Product.

Table 2. Marketed formulations of Itraconazole.

Formulation	Description
Sporonox® capsules 100 mg	• First commercial solid formulation of itraconazole • Contains the API in amorphous form dispersed within hydroxypropylmethylcellulose (HPMC) on sugar spheres • To be taken after a full meal
Sporonox® oral solution 10 mg/ml	• Marketed five years after the capsules • Has higher bioavailability in the fasted state than in the fed state • 400 mg/mL of (2-hydroxypropyl)-β-cyclodextrin
SUBA-Itraconazole capsules 50 mg	• Novel amorphous solid dispersion • Contains ITRA in a pH-dependent polymer matrix of hydroxypropylmethylcellulose-pthalate (HPMC-P) • Designed to enhance dissolution and intestinal absorption

a significant difference between the bioavailability of the solution and capsule forms; as such, these dosage forms are not bioequivalent and cannot be used interchangeably, as the solution formulation provides about three times higher bioavailability. SUBA-Itraconazole was marketed claiming no food effect and therapeutic equivalence to the Sporanox® capsule with half dose.[8]

Borbas *et al*, performed simultaneous dissolution-absorption measurements using the Pion BioFLUX™ system to predict the bioequivalence of the marketed formulations of itraconazole and compared results to *in vivo* results published by the United States Food and Drug Administration (FDA).[9]

In this publication, predicted fraction-absorbed ratios for SUBA-Itraconazole and the reference product, Sporanox capsules, under fasted and fed conditions were compared. Results are shown in Figure 3 along with the *in vivo* bioequivalence study findings. Bioequivalence in the fed state was predicted accurately, while fasted state performance of SUBA-ITRA was overestimated, likely due to the dose difference between the formulations.

Since the Sporanox solution was recommended to be taken before meals and the solid formulation should be taken after a meal, Sporanox solution in the fasted state was compared to the Sporanox capsule in the fed state (Figure 4). The *in vitro* predictions showed superior

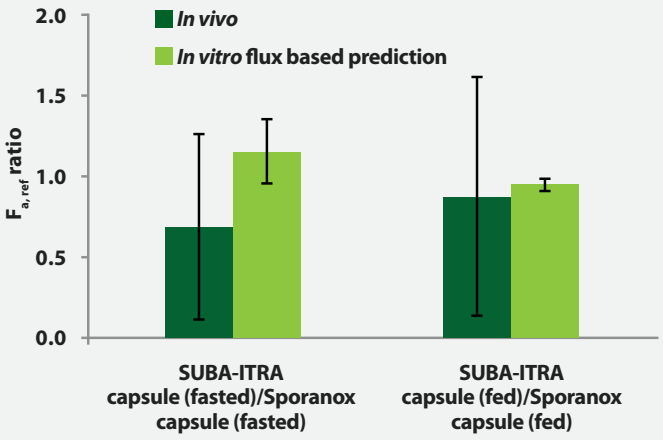

Figure 3. Predicted fraction absorbed ratios for the comparison of SUBA-Itraconozole (test) and Sporanox (reference) capsules in fasted and fed conditions and in vivo data from the bioequivalence study results.

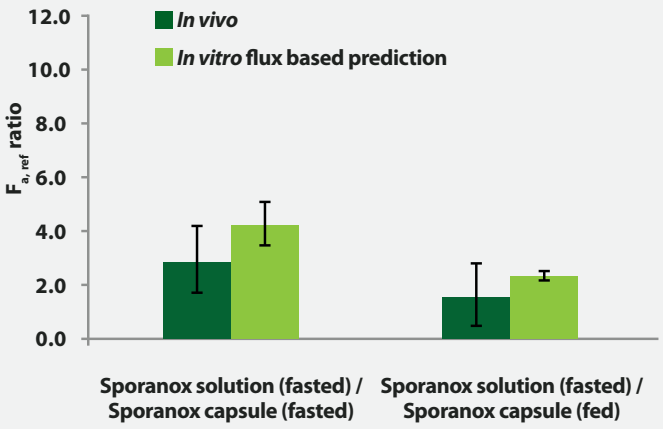

Figure 4. Predicted fraction absorbed ratios for the comparison between Sporanox solution and Sporanox capsule in fasted and fed conditions and in vivo data.

bioavailability of itraconazole from the Sporanox solution, which agreed well with the *in vivo* results. Since the bioavailability of itraconazole was greater from Sporanox solution, the solution and the capsule are not considered to be bioequivalent products.

Conclusion

During generic drug formulation development, it is essential to understand how different excipients affect both API dissolution and absorption. While the effect

on dissolution is relatively straightforward to determine using USP dissolution tests, determination of the rate of absorption is more challenging and less predictive using conventional methods like $CaCo_2$ cells.

The findings from the itraconazole and telmisartan studies underscore the limitations of relying solely on traditional USP dissolution methods and highlight the enhanced predictive value of simultaneously measuring dissolution and absorption. Because this approach captures how excipients and formulation strategies influence both drug release and membrane permeation, it offers a more realistic view of how a product is likely to perform *in vivo*. The ability of the MacroFLUX™ system to mirror clinical outcomes has been demonstrated through strong agreement between *in vitro* dissolution-permeation data and the corresponding *in vivo* bioequivalence study results.

Critically, applying this simultaneous *in vitro* assessment before initiating *in vivo* bioequivalence studies provides a powerful decision-making tool. By identifying formulations that are unlikely to achieve adequate absorption, developers can avoid advancing high-risk candidates into costly and time-consuming human studies. This not only reduces overall development timelines and resource expenditure but also enables teams to focus efforts on formulations with the greatest likelihood of *in vivo* success.

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