Application Note

SIMULTANEOUS DISSOLUTION-PERMEABILITY STUDIES

A Strategy for De-risking Formulation Changes

Introduction

In the current landscape of pharmaceutical development there is tremendous pressure to get to phase I clinical trials in the shortest time frame and to minimize expense. Often this results in additional formulation/process development required after phase I in order to support later stage clinical trials and scalability for commercial manufacturing. For the sponsor, the required changes to the formulation or process also carry the potential risk of changes in drug release and absorption which ultimately can impact bioavailability.

To date, assessment of potential differences in bioavailability has typically been evaluated in-vitro using standard compendial dissolution procedures. These methods may indicate differences in drug release and/or solubility, but do not address the potential changes, or lack thereof, to drug absorption. Because of this, many projects suffer delays trying to deal with the shortcomings of the phase I formulation or process to avoid costly additional clinical trials to de-risk changes in the pharmacokinetics between phase I and phase II/III clinical trials.

To help address the gap between dissolution and drug absorption, Logan Instruments has pioneered a groundbreaking dissolution/permeability system which allows simultaneous quantitation of drug release and diffusion. This innovative technology enables *in-vitro* comparison of different formulations, providing a valuable tool for pharmaceutical researchers to assess bioequivalence and de-risk formulation/process changes. Unlike traditional methods relying on complex modeling, direct *in-vitro* permeation comparison has proven more predictive of *in-vivo* outcomes. This simplifies the assessment of formulation performance. Historically, *in-vitro* permeability testing focused on predicting bioavailability or pharmacokinetics, requiring extensive testing, and sophisticated *in-silico* modeling. In contrast, direct *in-vitro* permeation comparison of formulations containing BCS Class II, III, and IV compounds has shown that relative *in-vitro* differences translate more reliably to *in-vivo* outcomes. In essence, if two formulations exhibit similar flux and permeability *in-vitro*, it's reasonable to expect similar *in-vivo* performance; provided that active transport and/or efflux mechanisms are not at play.

Principle Behind the Assay

The Permetro assay for permeability measurements is based on the passive diffusion of a compound through an artificial lipid bi-layer membrane (PermeaPad®) which has been shown to have similar permeability to the intestinal epithelium. The permeable membrane divides the donor and

receiver sides of a two compartment cell. Water cannot cross the membrane so the fluids in each compartment do not mix. The donor compartment is connected to the dissolution vessel which continuously circulates the donor media with dissolved drug parallel to the membrane surface. The receiver cell is filled with a suitable sink media and stirred continuously at 37°C ± 0.5. Unbound-dissolved drug passively diffuses across the lipid membrane relative to the concentration gradient and intrinsic permeability of the sample. At each time point aliquots can be collected from either/both the donor (d) and receiver (r) compartments; the flux (J(t)) and permeability coefficients (Pe) are calculated from the relative sample concentrations (c) in both compartments. The volume (v) of each compartment is used to calculate the total drug release and/or diffusion and at time (t).

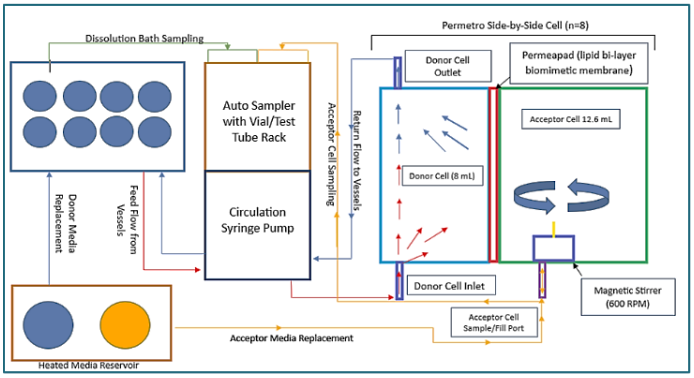

Figure 1: Permetro Operational Diagram

eq. 1: $\%D = [(C_d \times V_d) + \sum_{i-1}^{n-1}(Cd_i \times Vd_i)] \times \frac{100}{LC}$

eq. 2: $J(t) = [(C_r \times V_r) + \sum_{i-1}^{n-1}(Cr_i \times Vr_i)] \times \frac{1}{At}$

eq. 3: $Pe = \frac{J(t)}{Cd-Cr}$ → *where Cd >> Ca* → $Pe \approx \frac{J(t)}{Cd}$

Study Design

This study aimed to compare phase II/III clinical formulations of a Ref-1's redox function blocker indicated for treating multiple cancers. These formulations were prepared with different compositions and processes to enhance scalability compared with the phase I clinical formulation. The sponsor also sought to assess potential bioequivalence between the free acid form of the API used in phase I and a more stable calcium salt form for use phase II/III trials. The objectives included evaluating API solubility, assessing drug release from IR tablet formulations, and comparing permeability to estimate the relative risk of changes in bioavailability associated with a change in formulation and/or API form.

Materials and Methods

Instruments

- USP Dissolution Bath – Apparatus II (Vankel)
- Permetro Diffusion Cell/Pump system (Logan Inst.)
- SCR-DL Auto Sampler (Logan Inst.)
- 2695 HPLC/UV (Water Corp.)

Materials

- PermeaPad® 35 mm Lipid Barrier
- Sodium Dodecyl Sulfate 2% w/v Solution (receiver sink media)
- 0.1N Hydrochloric Acid (dissolution/donor media)
- HPLC Mobile Phase: 60:40, Acetonitrile:Water with 0.1% Acetic Acid
- HPLC Column: Waters Symmetry C18, 150 x 4.6 mm 3.5 µm

Experimental Procedure

Prior to dissolution/permeability testing the saturation solubility and compatibility of the free acid and calcium salt forms of the API in the donor and receiver media was established. These data also aided in analysis of the permeability data as differences in solubility can impact passive diffusion. The assay of each formulation under test was confirmed by HPLC to be within 95.0-105.0% of the target (120 mg) dose. The dissolution vessels were filled with 900 mL of 0.1N HCl and equilibrated to 37°C ± 0.5 for 30 minutes. Replacement donor (0.1N HCl) and receiver (2% SLS) media were also equilibrated to 37°C ±0.5. The permeation cells were assembled with the PermeaPad® barriers. Then the system was primed to fill the donor recirculation loop and receiver cell. Immediately after initializing the run, one tablet was added to each dissolution vessel (n=6), and drug release/permeation was monitored for 7 hours.

The flux was calculated as the total drug permeating into the receiver cell over time (equation 2). Dissolution (equation 1) was reported as a percent of drug release for comparison of

the profiles. And the actual micrograms per milliliter at steady state was used for calculation of the permeability coefficient (equation 3).

Measurement Parameter	Instrument Setting
Temperature	37.0 ±0.5 °C
Receiver Temperature	37.0 ±0.5 °C
Dissolution Paddle Speed	75 RPM
Receiver Stirring Speed	600 RPM
Dissolution Volume	900 mL
Receiver Volume	13.5 mL
Dissolution Sample Points	15, 30, 45, 60, 90, 300, 420 minutes
Permeation Sample Point	60, 120, 180, 240, 300, 420 minutes
Analysis	HPLC/UV - 270 nm

Table 1: Dissolution-Permeation Instrument Parameters

Results

Comparison of the dissolution profiles (Figure 1) demonstrated significant differences in the drug release between the phase I clinical formulation and the test formulations of the calcium salt. But both test formulations appeared to be very similar to each other. Which suggests that the API form has the greatest impact on the drug release. As per the FDA guidance for f1/f2 comparison of the dissolution profiles (Table 2) both of the test formulations failed to meet the criteria for equivalence to the phase I clinical formulation.

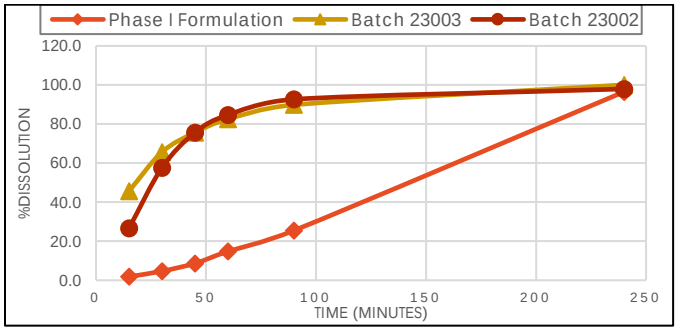

Figure 2: USP dissolution profile comparison in 0.1N HCl

Batch	F1	F2
23002	478.5	9.5
23003	484.1	9.3
Pass/Fail	0-15	50-100

Table 2: f1/f2 analysis of the test formulations to the phase I clinical formulation

However, despite the differences in the rate of drug release, the results for the flux and permeability comparison (Table 3) indicate that there was not a significant difference in the potential rate of drug absorption. The Permeability for the test formulations was compared to the clinical formulation using the Mann-Whitney Rank Sum Test. These data demonstrated that the upper and lower limits of the 90% confidence interval for the permeability of both test batches was with 80-120% of the clinical batch. But because batch 23002 was at the very edge of the upper limit formulation 23003 was concluded to be the best selection for phase II clinical trials.

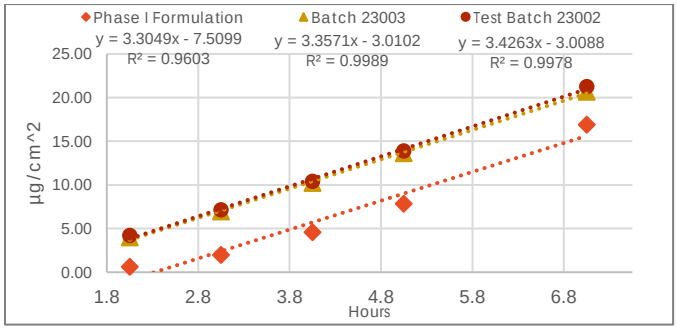

Figure 3: Flux profile comparison

Result	Formulation		
	Phase I	23002	23003
Steady State (µg/mL)	144	131	134
Avg. Flux (µg/mL*cm^2)	3.31	3.43	3.36
Avg. Pe (cm/sec)	1.374	1.570	1.500
Standard Deviation	0.13	0.35	0.10
Avg. %Equiv.	NA	111.53%	109.28%
Upper 90% CI	NA	120.30%	117.60%
Lower 90%CI	NA	95.19%	101.00%

Table 3: Dissolution-Permeation Instrument Parameters

The difference in the conclusion for bioequivalence between the dissolution and permeation results is due to the inherent permeability of the drug substance. In this case, the rate of permeation for the API is significantly slower than the rate of dissolution. The steady state, or saturation solubility (Table 3), was not significantly impacted by the change to the API form. Because of this, the increased rate of drug release did not have a significant impact on drug absorption.

Tips

- ✓ The donor and sink media should be verified to be compatible with the drug substance and the lipid membrane. We have used FaSSIF, FeSSIF, 0.1 N HCl, SLS (up to 2%) and CTAB (up to 1.0%) with good success.
  - ❖ Buffer CTAB between pH 6-7. This helps prevent precipitation. But never leave CTAB on any instrument overnight as this can lead to clogs.
  - ❖ There are proprietary sink medias for flux/permeability you can purchase, but experience has shown no observed a difference in permeability for these media compared to a simple sink media with similar solubility.
- ✓ Install 70 µm full flow filters on the dissolution vessel sample and circulation pump cannulas. This will prevent large particles or capsule pieces from clogging the pumps and/or settling in the donor cell.
- ✓ The donor circulation pump stroke volume is adjustable, but we have found that an 8 mL stroke volume results in the lowest vessel-to-vessel variance.
- ✓ It is good to obtain 1 or two dissolution samples at the middle and end of the permeation run to assess the donor concentration and determine if steady state calculations are appropriate.
- ✓ It is always good to verify the integrity of the lipid membrane at the end of each run. This can be accomplished easily by spiking each dissolution vessel with a polar dye solution (e.g. FD&C Red 40). Circulated this across the donor side for 30-60 minutes and test the receiver side for dye intrusion by UV-Vis. Any dye in the receiver side indicates membrane failure.

Conclusion

Simultaneous evaluation of dissolution and permeation can provide critical information for determining the impact of a formulation or process changes. These data can be used to reduce the risk of changes in bioavailability and provide a valuable bridge between pre-clinical or early phase formulations and more refined later stage formulations. *In-vitro* permeability testing is also a valuable tool for selecting formulations during development.

Based in the permeation data, in conjunction with solubility and the phase I clinical data, it was concluded that the test formulations would demonstrate similar bioavailability to the previous phase I clinical formulations.

References for Further Reading

M. di Cagno et al. (2015): New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drugs. European Journal of Pharmaceutical Sciences 73: 29-34

H.A. Bibi et al. (2015): Permeapad™ for investigation of passive drug permeability: The effect of surfactants, co-solvents and simulated intestinal fluids (FaSSIF and FeSSIF). International Journal of Pharmaceutics 493: 192-197

H.A. Bibi et al. (2017): Simultaneous lipolysis/permeation in vitro model, for the estimation of bioavailability of lipid based drug delivery systems. European Journal of Pharmaceutics and Biopharmaceutics 117: 300-307.

Wu IY, Bala S, Škalko-Basnet N, di Cagno MP (2019): Interpreting non-linear drug diffusion data: Utilizing Korsmeyer-Peppas model to study drug release from liposomes. Eur J Pharm Sci. 138:

Contact Us

For more information about how permeability testing can be used in your development or clinical program, contact us at https://www.corerxpharma.com/contact/ or visit our website at www.corerxpharma.com.

For more information on Permetro and full specifications on the individual components please contact Logan Instrument Corp. at info@loganinstruments.com or visit our website www.loganinstruments.com.

Travis Webb, M.S. Pharm.
Principal Scientist, Product Development
CoreRx Inc.