In Vitro Permeation Study of Acyclovir Cream 5% Using the Phoenix Diffusion System to Compare for Full Media Replacement and Partial Media Replacement ; i.e., Aliquot Sampling Technique

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Summary

The US Food and Drug Administration (FDA) recommends full media replacement at each sampling interval when performing in vitro permeation test (IVPT) studies of acyclovir cream due to "apparent negative flux." Full replacement requires extensive analyst intervention or complicated automation, and often the use of LC-MS to detect such low concentrations, all of which can be costly and complicated. Choosing to use an aliquot sampling method for IVPT can be simpler and can allow for the use of less-expensive LC equipment, as the concentration of analyte remains high enough during the study to allow accurate quantitation using HPLC UV detection while maintaining solubility sink conditions throughout the extended run.

This research study demonstrates that aliquot sampling is suitable for IVPT studies of acyclovir cream when using a modern diffusion apparatus; this is likely due to diffusion cell design innovations which ensure receptor chamber and arm stem homogeneity while eliminating "apparent negative flux," a phenomenon that industry hasn't been able to explain. Although this study used acyclovir as the test ingredient, partial media replacement can be used with other compounds such as steroidal or antifungal compounds.

Introduction

The widespread availability of generic versions of many pharmaceutical drug products in the United States has had a profound economic and social impact.[1] The affordability of generic drug products is a direct consequence of highly efficient scientific and regulatory approaches utilized to develop most generic drug products, which typically rely upon a comparative pharmacokinetic (PK) study to evaluate whether the rate and extent to which the drug becomes available at or near the site(s) of action is the same for the generic product and the reference listed drug (RLD) product. This approach to evaluating comparative bioavailability (BA) is among the most accurate, sensitive, and reproducible ways to demonstrate bioequivalence (BE) for a generic drug product.[2]

For the drugs that are intended to be delivered to the systemic circulation, as is the case for many oral dosage forms, this approach involves taking blood samples at multiple time points following dose administration and measuring the concentration of the drug (or a related analyte) in the plasma or serum, which is a relatively straightforward process. However, for locally acting drugs, like those in a topical dermatological drug product, evaluating the concentration

Image 1: Phoenix RDS Dry Heat Diffusion System

of the drug at or near the site of action in the skin is more challenging. As a result, the development of generic versions of topical drug products has instead typically relied upon comparative clinical endpoint BE studies, which are less accurate, sensitive, and reproducible than PK studies and considerably more costly and time consuming. Therefore, the expensive and inefficient development pathways to which most generic topical drug products have been relegated represented a barrier to entry for generic drug developers and negatively impacted their availability and their affordability.

The notable exceptions among topical dermatological drug products have been those that contain corticosteroid (glucocorticoid) drugs, for which a more efficient in vivo pharmacodynamic (PD) vasoconstrictor study has been recommended by the U.S. FDA for the demonstration of BE. As a result, although these corticosteroid creams, ointments, and other topical dosage forms represents a small portion of topical dermatological drug products overall, these RLD products have multiple generic versions available. The hypothesis of this work was that if similarly efficient cutaneous PK/BE methodologies could be developed, these methods could facilitate the greater availability of affordable generic topical dermatological drug products.

The feasibility of existing in vitro and in vivo cutaneous PK methodologies has been considered during recent decades, with each methodology offering different strengths and limitations. Amongst these methodologies, one of the most promising is the IVPT methodology, which has been shown to correlate well with in vivo results. Notably, IVPT studies have specifically shown promise to correlate with and be predictive of in vivo assessment of BE.

The goal of this work is to evaluate an IVPT method for the BA of acyclovir cream with complete receptor solution replacement[3] compared to partial receptor solution replacement.

The study was conducted under the supervision of an experienced professor by a well-trained researcher at the Vivekanand Education Society's College of Pharmacy, Chembur East, Mumbai, India location. Hanson's Phoenix DB-6 system was installed with IQ, OQ, and PQ performed prior to study initiation by Teledyne Hanson's service engineer based in Mumbai.

Material and Methods

Materials: The following reagent and materials were used in the study:

Sr. No.	Name of Reagent	Supplier	Lot Number	Expiry Date
1.	Orthophosphoric acid	Loba Chemie	H30287220	Dec. 2026
2.	Potassium dihydrogen phosphate	Sisco Research Laboratory	1515595	Feb. 2023
3.	Methanol	Rankem	R367L21	Nov. 2026
4.	Potassium chloride	Loba Chemie	A278111903	Feb. 2024
5.	Disodium hydroigen phosphate	Merck	QB5Q650227	Feb. 2023
6.	Sodium chloride	High Purity Laboratory Chemicals	V051057L5LA22	Jan. 2023
7.	Sodium Azide	High Purity Laboratory Chemicals	C0660343KLLH20	Mar. 2023
8.	HPLC grade Water	Rankem	R283B22	Feb. 2023
9.	Hydrochloric acid	Loba Chemie	L247181712	Nov. 2023
10.	Sodium Dihydrogen Orthophosphate	Chemdyes	13472-35	Mar. 2029
11.	Zovirax Cream	GSK, USA	B133569	Feb. 2024
12.	Acyclovir Reference Standard	Sigma Aldrich	R283B22	Feb. 2023

The following instruments were used in the study:

Sr. No.	Instrument Name	Make and Model
1.	Analytical Balance	MAB 220
2.	Deep Freezer	Vestforst
3.	Franz Diffusion Cells	Phoenix DB-6
4.	Infrared Thermometer	Vandelay xiTix
5.	HPLC	Jasco BS 4000-1
6.	Micrometer Screw Gauge	Aerospace
7.	Micro Pipette	Jsil
8.	pH Meter	DBK digital pH meter
9.	Sonicator	PCl analytics

Skin was procured from National Burn Center, Navi Mumbai, Maharashtra, India:

Skin Donor	Donor ID	Age	Race	Sex	Anatomical region of the excised skin	Skin Thickness
Donor1	104/21-22/NBC	77	Asian	F	Legs, Thighs	0.35-0.4 mm
Donor2	103/21-22/NBC	98	Asian	F	Legs, Thighs, Back	0.3-0.35 mm

Skin Preparation: Skin Harvesting Procedure:

Phase 1: Skin is harvested with a dermatome and stored in 50% glycerol with antibiotics.

Phase 2: Transferring each strip from 50% glycerol to 85% glycerol containing antibiotics.

Phase 3: Skin is stored at 4-8°C in 85% glycerol after phase 2 (21Days).

On the day of analysis, skin was thawed, washed, and rinsed with PBS solution (receptor medium), cut to 25 mm diameter circles as per the required number of pieces using a Neiko 02614A heavy-duty hollow hole punch set. Each piece was observed visually under a magnifying glass for any damage or inappropriateness for use in an integrity confirmation. Furthermore, skin thickness was measured with a calibrated micro screw gauge for each skin section. After the thickness measurement, the skin was mounted in the donor compartment of a 12 mL Franz diffusion cell.

In Vitro Permeation Test

A Teledyne Hanson diffusion system (Phoenix DB-6, manual system) was used for IVPT experiments. 12 mL diffusion cells with a permeation area of 1.0 cm^2 were utilized. The receptor solution was PBS buffer (pH 7.4) with 0.1% sodium azide, stirred at 400 RPM. The dermatomed piece of skin was mounted in the diffusion cell with its epidermis facing the donor compartment (n = 3). A single dose of 26.55 mg/cm^2 of acyclovir cream (1.37 mg of acyclovir per cm^2) was applied using a positive displacement pipette. After the cream was dispensed on top of the skin surface, the exposed polytetrafluoroethylene tip of the positive displacement pipette was used to gently spread the formulation over the entire permeation area of the skin. The dose application area was left open to the air without any occlusion for the entire duration of the experiment. The IVPT experiments were performed for 72 h with continuous sampling time points. The two parameters, J_{max} and the cumulative total permeation corresponding to the AUC of the acyclovir permeation profile, were chosen as two key parameters. The resulting receptor solution samples were analyzed by high-performance liquid chromatography (HPLC).

HPLC Analysis of Samples

The IVPT samples were analyzed on an HPLC system consisting of Jasco LC-4000 series units with PDA detectors and data acquisition with ChromNav software, version 1. A partially validated HPLC method was used to quantify the IVPT samples. A BakerBond 5µ C18 (250 x 4.6 mm) column was used to elute acyclovir. The mobile phase used was 5:95 (v/v) methanol: 50 mM sodium phosphate, pH adjusted to 6.0 with phosphoric acid. The flow rate was 1.0 mL/min. The acyclovir peaks were detected at 254 nm. The injection volume for IVPT samples was 100 µL. The lower limit of quantification was 0.025 µg/mL for IVPT diffusion samples.

In Vitro Permeation Test (IVPT) Results:

When acyclovir cream was screened and compared by conducting IVPT test experiments using skin obtained from two donors (n = 3), the permeation of acyclovir through the skin from the Zovirax cream was measured using two different sampling techniques; i.e., (1) with partial sample withdrawal and (2) with complete sample withdrawal.

Figure 1 and **Figure 2** represent the IVPT data using partial sampling at each time interval. Intra-donor variability was observed, but it was only one third that of the total replacement control (% RSD < 5.0). The overall results from the two donors indicate that the AUC (0-72 h) for the acyclovir cream is 102 $\mu g/cm^2$ and 64.4 $\mu g/cm^2$, respectively. The J_{max} for the acyclovir cream were 2.8 $\mu g/cm^2/h$ and 3.9 $\mu g/cm^2/h$. The T_{max} was 24 h for both of the donors.

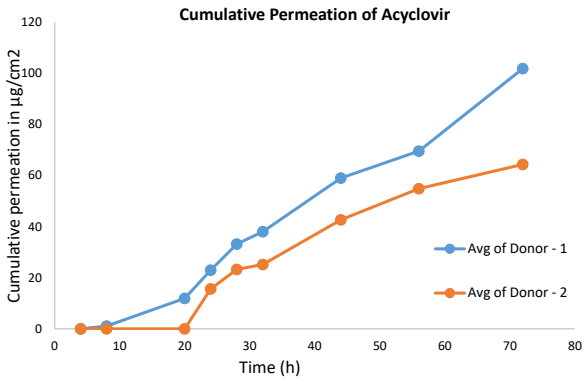

Figure 1: Cumulative Permeation of Acyclovir (Partial Replacement)

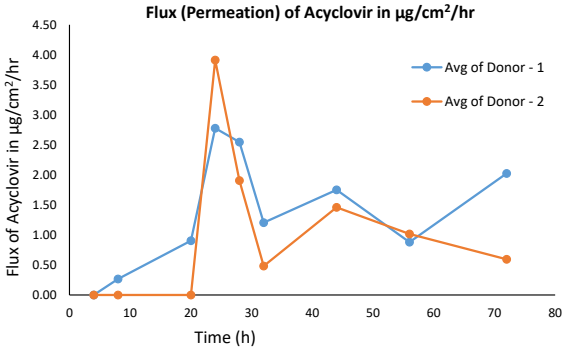

Figure 2: Permeation Profile of Acyclovir (Partial Replacement)

Figures 3 and **4** represent the IVPT data using complete media replacement sampling at each time interval. Intra donor variability was observed but intra donor variability was consistently less (% RSD <15.0). The overall results from the two donors indicate that the AUC (0-72 h) for the acyclovir cream is 10.9 µg/cm^2 and 4.8 µg/cm^2 respectively. The J_{max} for the acyclovir cream were 0.4 µg/cm^2/h and 0.12 µg/cm^2/h. The T_{max} was 72 h for both donors. In this study, high variability was observed in complete replacement because of the extremely low API concentration in the receptor chamber — which is expected at early time points in IVPT studies — and further amplified when replacing the full chamber at each time point, due to the lower limit of quantitation (LLOQ) of the HPLC vs. an LC-MS. Conversely, partial replacement allows the increase of API concentration in the receptor solution over time, while still maintaining proper sink conditions, making a traditional HPLC system the analytical technique of choice because the instrument is being run within a detection range that allows for more consistent results.

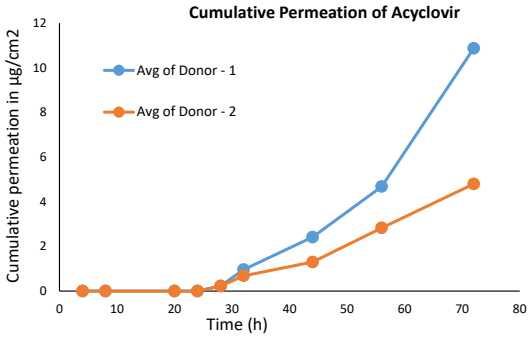

Figure 3: Cumulative Permeation of Acyclovir (Complete Replacement)

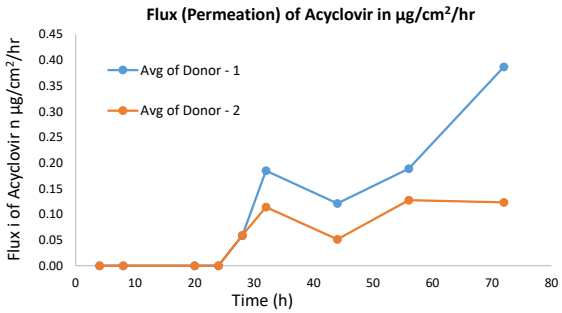

Figure 4: Permeation Profile of Acyclovir (Complete Replacement)

Results – Summary Table 1

Sampling Type	Donor	AUC (µg/cm^2)	Jmax (µg/cm^2)	Tmax (h)
Partial Replacement	Donor1	102.0	2.8	24
	Donor2	64.4	3.9	24
Complete Replacement	Donor1	10.9	0.4	72
	Donor2	4.8	0.12	72

Conclusion

These results demonstrate that an IVPT study can be performed using both sampling procedures. From the data, it is evident that using partial volume replacement produced less variability than complete replacement. Lag time of up to 24 h was observed in the complete replacement method, while lag time was only 4 h in the partial replacement study when using the Teledyne Phoenix apparatus and a traditional HPLC analytical method. This 24 h lag time was due to the complete replacement and hence acyclovir concentration was below LLOQ for HPLC analysis. In the partial replacement study, the observed value of J_{max} is higher because the concentration of active ingredient gradually increases throughout the study period, while in the case of complete replacement, the observed value of J_{max} is very low. So, it is recommended to use LC-MS for the quantification when complete replacement sampling procedure is used. The LC-MS is an expensive instrument to use and maintain in any organization, and also needs special environmental conditions for routine maintenance. Therefore, we recommend performing the IVPT study using a partial replacement sampling method with the Teledyne Phoenix diffusion apparatus and an analytical HPLC analytical method; i.e., the aliquot sampling procedure, instead of performing a compete replacement of receptor media at each time point.

Resources

1. Oh, L., Yi, S., Zhang, D. *et al*. In Vitro Skin Permeation Methodology for Over-The-Counter Topical Dermatologic Products. *Ther Innov Regul Sci* 54, 693–700 (2020). https://doi.org/10.1007/s43441-019-00104-3
2. Shin, S., Rantou, E., Raney, S.G. *et al*. Cutaneous Pharmacokinetics of Acyclovir Cream 5% Products: Evaluating Bioequivalence with an In Vitro Permeation Test and an Adaptation of Scaled Average Bioequivalence. *Pharm Res* 37, 210 (2020). https://doi.org/10.1007/s11095-020-02821-z
3. U.S. Food and Drug Administration. Draft Guidance on Acyclovir. Recommended Dec 2014, Revised Dec 2016. https://www.accessdata.fda.gov/drugsatfda_docs/psg/Acyclovir_topical%20cream_RLD%2021478_RV12-16.pdf

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