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IVPT study – partial/full volume replacement techniques

In Vitro Permeation Testing (IVPT) has become a primary scientific tool to compare the rate and extent of drug permeation from test and reference topical products across human skin, enabling sensitive bioequivalence (BE) assessments without large clinical endpoint studies. Recent guidance from the U.S. FDA (2022 draft) and the EMA (final guideline adopted Sept 2024; effective Apr 2, 2025) formalises method development, validation, and pivotal study execution, and it clarifies how IVPT data may support therapeutic equivalence and ANDA/MAA strategies.

The affordability of generic topical drug products is enabled by scientifically rigorous and regulatory-efficient pathways that support bioequivalence (BE) demonstrations without requiring extensive/expensive clinical trials.
For locally acting dermatological products, the *In Vitro* Permeation Test (IVPT) has emerged as a pivotal tool in characterising cutaneous pharmacokinetics, offering a sensitive and reproducible method to compare the rate and extent of drug permeation between a Test Generic Product and its Reference Listed Drug (RLD).

The FDA's draft guidance on IVPT studies outlines a comprehensive framework for method development, validation/pilot, and pivotal study execution.

Key elements include:

- **Use of human skin membranes** (e.g., dermatomed or heat-separated epidermis) with verified barrier integrity.
- **Finite dose application** under controlled conditions using vertical diffusion cells.
- **Validated receptor solutions** that maintain sink conditions without compromising skin integrity.
- **Statistical modelling approaches**, such as scaled average bioequivalence, to assess BE with high sensitivity and optimising sample size requirements.

IVPT studies are particularly valuable for semi-solid and liquid-based dosage forms (e.g., creams, gels, lotions), where traditional clinical endpoint studies may lack sensitivity and reproducibility. When properly designed, developed and validated, IVPT can serve as primary evidence of bioequivalence, supporting ANDA approval.

Regulatory agencies are increasingly recognising the value of IVPT in the evaluation of advanced and non-traditional dosage forms. The adoption of IVPT in regulatory frameworks is largely attributed to its ability to generate comprehensive data on the rate and extent of drug permeation through biological membrane. By simulating the drug release (IVRT) and permeation behaviour under controlled laboratory conditions, IVPT facilitates a deeper understanding of formulation characteristics (Q3). It supports critical decision-making during both early-stage development and regulatory submission, ensuring that products meet predefined standards of quality, efficacy, and consistency.

BACKGROUND & RATIONALE

Topical dermatological products often act locally with minimal systemic exposure, making clinical endpoint studies insensitive and variable. IVPT, using human skin in diffusion cells under finite dose conditions, provides highly discriminating performance metrics (e.g., flux, lag time, cumulative mass permeated) and can support BE decisions when designed and analysed per guidance and PSGs.

Foundational principles are also aligned with OECD Test Guideline 428 and the OECD Guidance Document for Skin Absorption, which set expectations for ex vivo human (or other mammalian) skin use, temperature control, receptor medium selection, and mass balance accounting—practices broadly adopted across pharmaceutical, cosmetic, and chemical safety communities.

Partial vs. Full Volume Replacement (Sampling strategies)
In Vitro Permeation Testing (IVPT) can be conducted using two primary methodological approaches: partial volume replacement and full volume replacement. Each technique presents distinct operational characteristics, advantages, and limitations that must be carefully considered during method development.

- **Partial Volume Replacement** involves the periodic removal and replenishment of a portion of the receptor medium. This approach is often preferred when maintaining sink conditions is critical, and it allows for continuous sampling without significantly disturbing the system equilibrium. However, it may introduce variability in concentration gradients and requires precise handling to ensure consistency.
- **Full Volume Replacement,** on the other hand, entails the complete replacement of the receptor medium at defined intervals. This method ensures uniformity in sampling and minimises the risk of cumulative drug concentration effects. Nevertheless, it may be more resource-intensive and could potentially disrupt the permeation dynamics if not executed with care.

In this white paper, we present a comprehensive evaluation of partial volume replacement technique, focusing on their applicability to specific product types and regulatory expectations.

We also detail the development of customised IVPT protocols tailored to the needs of the product sponsor, along with a summary of the experimental results and key findings derived from the testing.

In Vitro Permeation Testing Using Partial Volume Replacement (PVR) emphasises the importance of maintaining sink conditions, especially in scenarios where accurate flux measurement is vital for product development, bioequivalence testing, or safety assessments. By minimising fluid disruption and simplifying receptor fluid management, PVR enhances reproducibility and data reliability. This white paper explores not only the methodology but also includes a practical case study to demonstrate real-world implementation.

This approach not only ensures therapeutic equivalence but also significantly reduces development timelines and costs, thereby enhancing patient access to affordable, high-quality generic dermatological therapies.

EXECUTIVE SUMMARY

In vitro permeation testing (IVPT) is a pivotal tool in the pharmaceutical, cosmetic, and dermal drug delivery industries for assessing the rate and extent of compound absorption through biological membranes. A common challenge in IVPT is the need to maintain sink conditions while preserving membrane integrity and minimising experimental variability. The partial volume replacement technique offers a refined approach to manage these parameters during permeation studies. This white paper outlines the principles, methodology, advantages, limitations, and regulatory landscape surrounding IVPT with partial volume replacement.

METHODOLOGY

In Vitro Permeation Testing (IVPT) involves studying the diffusion of a compound (e.g. active ingredient) across a biological membrane such as human cadaver skin. The goal is to simulate and predict in vivo absorption characteristics, especially for topically or dermally applied formulations.

Partial volume replacement (PVR) is a sampling strategy where only a portion of the receptor fluid is removed at specific time intervals and replacing with fresh medium. This approach contrasts with complete fluid replacement (CFR) and aims to reduce disturbances to the system, better maintain steady-state conditions, and ensure cumulative permeation profiles are accurate.

The occurrence of negative flux is a misconception and can be ruled out, as it is likely caused by partial volume replacement. Additionally, the formation of air bubbles during sample removal and subsequent buffer replacement in the cell may contribute to this observation.

EXPERIMENTAL SETUP

- **Diffusion cells:** Typically, Franz diffusion cells (vertical) are used.
- **Membrane:** Human cadaver skin.
- **Receptor fluid:** Chosen to mimic physiological conditions and maintain sink condition.
- **Sampling:** At predetermined time intervals, a small portion (commonly 500–750 µL +/-5%) of the receptor fluid is withdrawn and replaced with an equal volume of fresh receptor medium.

PARAMETERS TO CONSIDER FOR IVPT METHOD OPTIMISATION

Skin selection	Male/ female donor age >18, no stretch, wrinkles, no tattoos
Dose selection	Application range of 2-15 mg /cm2
Selection of receiving media	Physiological condition with antimicrobial agent
Selection of equipment related parameters	Cell size and Surface area to be optimised, temperature 32±1°C is appropriate, sampling frequency to suit flux profile with minimum of 8 non-zero sampling points across with 3 time points at elimination phase.
Selection of range of quantification	Strength of formulation, dose and cell volume, target concentration to be calculated
Dose depletion and mass balance	cumulative amount of the active substance permeated with overall recovery of the active substance of 90-110%
Discriminating capability of method	Change in composition / Change in excipients to be demonstrated
IVPT sensitivity	Ability of the method to detect changes with different dose amounts
IVPT selectivity	Ability of the method to discriminate
Sensitive detection method	Selection of specific and sensitive method

KEY PARAMETERS

Cumulative Amount Permeated (Q_n): Calculated by summing the drug amount collected at each time point and adjusting for dilution.

Correction Formula:

$$Q_n = \Sigma(C_i \times V_s) + C_n \times (V_r - n \times V_s)$$

Where:

C_i: concentration at time i

V_s: sample volume

V_r: total receptor volume

Flux and Permeability Coefficient: Derived from the linear portion of the cumulative amount-time curve.

Applications	
Topical drug product evaluation	Bioequivalence testing (clinical biowaiver study), formulation development
Transdermal system design	Evaluation of drug release profiles
Cosmetic and dermatological products	Testing for safety and efficacy purposes
Toxicological risk assessment	Assessing dermal exposure for chemicals or actives

Advantages and limitations for consideration in case of Partial Volume Replacement (PVR)	
Advantages	**Limitations**
Maintains sink conditions	Dilution correction complexity
Minimises system disruption	Analytical sensitivity
Improved accuracy	Membrane integrity
Suitable for long-term studies	Evaporation control

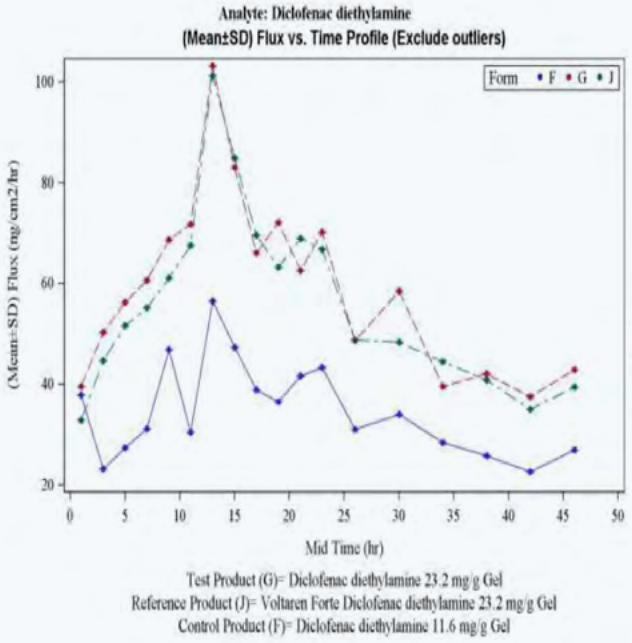
CASE STUDY: APPLICATION OF PVR IN A TOPICAL GEL FORMULATION

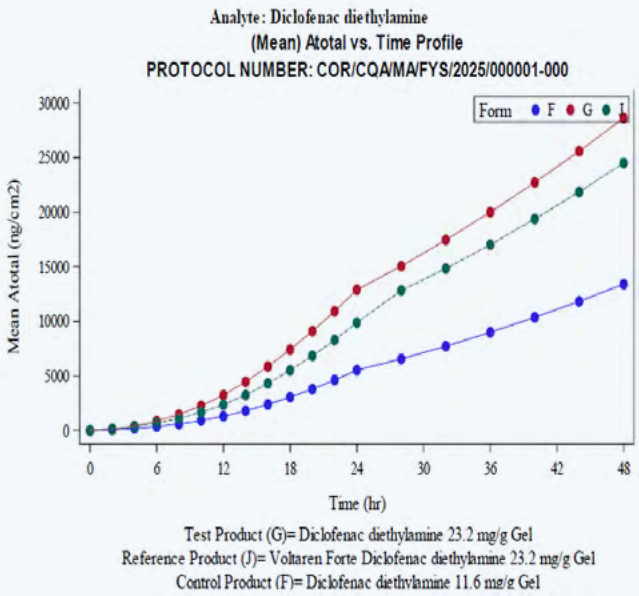
A topical diclofenac diethyl amine gel formulation was evaluated using *in vitro* permeation testing with partial volume replacement.

Franz diffusion cells were employed using dermatomed human skin mounted between the donor and receptor chambers. Receptor fluid consisted of phosphate-buffered saline with anti-microbial agent to ensure solubility, stability and maintain sink conditions.

Sampling was performed at Predose 0hours,2,4,6,8,10,12,14, 16,18,20,22,24,28,32,36,40,44,48 Hours, with 700 µL withdrawn and immediately replaced at each interval. The cumulative amount permeated was calculated using a dilution correction formula. Data revealed a steady-state flux achieved with acceptable permeability coefficient.

This study demonstrates the effectiveness of partial volume replacement in maintaining membrane integrity and accurate mass balance, yielding reproducible permeation profiles suitable for regulatory submission and formulation optimisation.





CONCLUSION

Partial volume replacement in *in vitro* permeation testing represents a refined and reliable method for generating accurate permeation profiles while preserving experimental integrity. Its adoption is especially relevant in regulated environments where precision and reproducibility are paramount. With growing demand for non-animal testing alternatives and precise skin absorption data, the PVR technique in IVPT continues to gain prominence across industries.

Our IVPT platform provides a scientifically rigorous, regulatorily aligned, and industry-proven pathway for topical product development, offering sponsors a compliant alternative to clinical endpoint studies by leveraging validated methodologies fully consistent with the latest FDA IVPT draft guidance (2022) and the EMA's 2025 Guideline on Quality & Equivalence of Locally Applied, Locally Acting Cutaneous Products; by partnering with our laboratory, customers gain access to expertly qualified human skin panels with stringent barrier integrity testing, optimised PVR/FVR sampling strategies tailored to drug solubility and permeation kinetics, advanced diffusion cell infrastructure with precise temperature control, high-sensitivity analytical methods that ensure accurate flux and cumulative permeation calculations, and robust mass-balance practices harmonised with OECD expectations, collectively reducing program risk, improving data reliability, and accelerating the path to regulatory acceptance, thereby ensuring that each IVPT study we deliver not only meets but anticipates agency expectations and positions your product for successful submission and competitive market entry.

REGULATORY GUIDANCE AND COMPLIANCE

FDA Guidance: Recognises IVPT for bioequivalence in topical generic drug submissions (e.g., 2016 Draft Guidance).
EMA and OECD: Endorse IVPT methodologies for safety and efficacy assessments.
ICH Guidelines: Recommend robust, validated methods for product development and batch consistency.

References

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3. Franz, T.J. "Percutaneous Absorption: On the Relevance of In Vitro Data." Journal of Investigative Dermatology, 1975.
4. EMA. "Guideline on Quality and Equivalence of Topical Products," 2018.

About Recipharm

Recipharm is a leading contract development and manufacturing organisation (CDMO) headquartered in Stockholm, Sweden. We operate development and manufacturing facilities in France, Germany, India, Italy, Portugal, Spain, Sweden and the US and are continuing to grow and expand our offering for our customers. We are supporting pharmaceutical companies with our full service offering, taking products from early development through to commercial production. For over 30 years, we have partnered with our clients throughout the entire product lifecycle, providing pharmaceutical expertise and managing complexity, time and time again. We conduct our business as we always have and continue to deliver value for money with each customer's needs firmly at the heart of all that we do.

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