IN-VITRO PERMEATION STUDIES
QMX stepwise approach

Qualimetrix is a customer-driven CRO that employs the Six Sigma philosophy in order to design and implement optimized processes with the aim of transforming customer inputs and requirements into “customer value”. As such, the first and probably the most critical factor for a successful project is its proper definition in terms of both customer and technical requirements.

To this end, a comprehensive study request form is provided to the customer with the following objectives:

- The definition of the type and scope of the study
- The provision of critical product information
- The determination of the most suitable, expedient and cost-effective approach

Locally applied products – Advantages and Therapeutic Equivalence establishment

Topical products are exemplified by medicines for cutaneous use; but in broadest scope, they are locally applied, locally acting products. They can be applied to any of the diverse external surfaces of the body that may present a physiological barrier to drug absorption e.g. skin, eye, ear.

Apart from topical products, transdermal patches, containing one or more active substances, intended for systemic absorption, are designed to provide a controlled delivery of the active substance(s) through the skin, principally by diffusion, resulting in a defined rate and extent of systemic delivery of active substance. Transdermal and topical drug delivery have the following advantages:
However:

The bioavailability of the active substance at the site of action from topical products is known to be affected mainly by:

- The active substance’s physicochemical properties
- The topical formulation design
- The manufacturing process

To this end, small changes in formulation, dosage form, administration or manufacturing process may significantly influence the efficacy and/or safety and this presents challenges to the prediction of therapeutic equivalence.

In assessing generic formulations, regulatory agencies require the demonstration of bioequivalence (BE) to a reference drug product. The US Food and Drug Administration (FDA) guidelines note that, taken together with the confirmation of pharmaceutical equivalence, establishing BE allows for a regulatory conclusion of
therapeutic equivalence.

For the majority of topical drug products comparative clinical endpoint studies are used to demonstrate BE to the RLD. The use of clinical endpoints to determine BE of topical products, although providing a direct assessment in patients that is reassuring to clinicians, is associated with a number of challenges such as the ones presented in Figure 1 below:

**Figure 1: Challenges of Clinical endpoint studies**

- Formulation differences might not be detected efficiently
- High variability and low sensitivity that make such studies less reliable and less efficient
- The number of patients enrolled can be quite large
- The high cost
- Their invasive nature

It is evident from the above that there is a clear need for BE studies using alternate approaches which are faster, less expensive, more reproducible and sensitive to differences in locally applied products. This need for suitable surrogates seems to be “embraced”, despite the skepticism, by the regulatory authorities (i.e. FDA, EMA) as reflected by recent guidance documents.

**EMA Concept Paper on the development of a guideline on quality and equivalence of topical products**

According to the above document, an extension of the waiver of the need to provide therapeutic equivalence data in the case of solutions, is possible:
The extended concept of pharmaceutical equivalence practically refers to the provision of comparative quality data with the relevant reference medicinal product. When comparing a generic drug product with the reference product, the following classifications are used:

- **Q1** means qualitative similarity between generic and reference products with respect to composition of the individual ingredients;
- **Q2** represents quantitative similarity of each ingredient;
- **Q3** products encompass Q1 and Q2 requirements but also have structural similarity to the reference, with the same arrangement of matter and state of aggregation of the product.

According to the concept paper, the additional measures of equivalence currently available include *in vitro* drug release through an *artificial membrane* and/or *human skin membrane* to determine the rate and extent of drug release or permeation, tape stripping to determine dermatopharmacokinetics and possibly microdialysis.

**FDA’s position on bioequivalence of topical products**

The FDA recognizes the need to find more sensitive and efficient surrogate approaches to demonstrate BE for topical products. In the “Critical Path Initiatives”, the FDA identifies the *in vitro* diffusion study combined with rheological testing to demonstrate bioequivalence of qualitatively and quantitatively (Q1, Q2) equivalent drug products.

Furthermore, the FDA has issued a draft guidance and ANDA approval has been granted utilizing *in vitro* characterization to establish pharmaceutical equivalence of acyclovir ointment and grant a waiver for a clinical end-point bioequivalence study. A summary of this product-specific guidance is provided in the following table.
Study Requirements

**Formulation Q1 / Q2 sameness**

The test and reference products are qualitatively and quantitatively the same.

**Q3 Similarity**

The physicochemical properties of the test and reference products are similar.

**In Vitro Release Test (IVRT) studies**

The test and reference products have an equivalent rate of acyclovir release.

**In Vitro Permeation Test (IVPT) studies**

The rate and extent of acyclovir permeation through excised human skin from the test and reference products are comparable.

### Table 1: Acyclovir FDA Guidance Recommendations

<table>
<thead>
<tr>
<th>Study</th>
<th>Requirements</th>
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<tbody>
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### In Vitro Release (IVR) Studies

According to the FDA’s SUPAC-SS guidance, an *in-vitro* release rate can reflect the combined effect of several physical and chemical parameters, including solubility and particle size of the active ingredient and rheological properties of the dosage form. To this end, IVR testing is a useful test to assess product “sameness” under certain scale-up and post approval changes for semisolid products.

Qualimetrix performs IVR testing for semisolid preparations (i.e. creams, ointments, lotions and gels) according to the provisions of USP General Chapter <1724> Semisolid Drug Products – Performance Tests. The Diffusion cell (Fig.2) is a reliable and reproducible means of measuring drug release from semisolid dosage forms.
IVR Process Description

The process involves the application of a thick layer of the semisolid product in the donor chamber which is placed in contact with a medium in a reservoir (i.e. receptor chamber). The latter acts as a receptor when the drug substance diffuses through the formulation, across an inert, highly permeable support membrane, and into the reservoir. Samples are then withdrawn from the receptor chamber at predefined time intervals. For each cell, the amount of drug released (μg/cm²) at each sampling time is determined and the cumulative amount released plotted versus √t. The slope of the resulting line is a measure of the rate of drug release.

The product performance test employs the Mann-Whitney U test to calculate the 90% confidence interval for the ratio of the slopes between the test and reference batches.

IVR Method Development

The first and probably the most critical step for setting up a suitable IVR test is method development. The following key points / parameters are taken into account and optimized during project “kick-off”.
Figure 3: IVR Method Development Parameters

- **Receptor Medium Selection**
  - Sink conditions maintained throughout the experiment - "diffusional sink"
  - No interaction between medium / membrane / formulation

- **Synthetic Membrane Selection**
  - No interaction with the medium
  - No binding with the drug substance
  - Minimum diffusional resistance

- **Number of Samples**
  - A minimum of six cells

- **Temperature**
  - $32 \pm 0.5°C$ ($37 \pm 1°C$ for vaginal preparations)

- **Sampling**
  - At least 5 sampling time points over a period of 4 - 6 hours
IVR Method Validation

It is of paramount importance that the apparatus, methodology and study conditions utilized in the IVR study are appropriately validated, qualified, verified and/or justified. Detailed protocols and well-controlled study procedures are developed for each project to ensure the precise control of dosing, sampling, and other IVRT study variables or potential sources of experimental bias. The validation scheme of each IVR test method incorporates the following qualifications and controls, performed using validated sample analytical procedures:

**Figure 4: IVR Qualification / Validation scheme**

### Qualification

- **Apparatus qualification**
  - (e.g. cell diffusional area, receptor compartment volume, temperature, etc.)

- **Membrane qualification**
  - (e.g. absence of drug substance binding to the membrane)

- **Receptor medium qualification**
  - (i.e. saturation solubility study)

### Validation

- **Analytical Method Validation**
  - (according to ICH Q2)

- **Linearity and Range**
  - ($r^2 \geq 0.90$ across the range of sampling times)

- **Precision and Reproducibility**
  - ($%CV \leq 15\%$)

- **Mass balance and dose depletion**

- **Discrimination Sensitivity and Selectivity**

- **Robustness**
IVR Method Validation

**Figure 5: IVR Test Applications**

- Assessment of product "sameness" under certain scale-up and post-approval changes
- Optimization of product performance (i.e. release profile) during formulation development
- Assessment of product stability / Batch-to-batch uniformity QC test
- Initial screening of the *in-vivo* performance of lead candidates prior to proceeding with clinical end-point / *in-vitro* permeation studies

**In vitro Permeation (IVP) Studies**

Clinical end point studies for the assessment of “bioequivalence” of locally applied products are often characterized by high variability and low sensitivity that make such studies less reliable and less efficient. Furthermore, they are also cumbersome, invasive, time-consuming and expensive. To this end, *in vitro* drug absorption into and across excised human skin mounted on diffusion cells can serve as a powerful and sensitive tool.

**IVP Test principle**

The test formulation is applied to the surface of a tissue (e.g. skin, cornea) sample separating the two chambers of a diffusion cell. The formulation remains on the tissue for a specified time under specified conditions. The receptor fluid is sampled at time points throughout the experiment and analysed for the test chemical and/or metabolites.
When metabolically active systems are used, metabolites of the test chemical will be analysed by appropriate methods. At the end of the experiment the distribution of the test chemical and its metabolites are quantified, when appropriate.

Using appropriate conditions, which are described in the study protocol, the absorption of a test substance during a given time period is measured by analysis of the receptor fluid and the treated tissue. Analysis of the other components (material remaining in the donor chamber, applicator, and tissue layers) allows for further data evaluation, including total test substance disposition and percentage recovery.

**IVP Study project management process**

In vitro permeation studies are carefully designed according to the client’s requirements, the purpose of the study and the provisions of the following guidelines:

- EMA/CHMP/QWP/608924/2014, Guideline on quality of transdermal patches Annex 1: In-vitro permeation studies

All studies are performed under GMP / GLP environment according to an approved by the client written protocol that clearly indicates the objectives and the methods to be employed. The general step-wise approach followed for each IVP study is schematically presented in Figure 7. A brief description of each stage is given in the following figure.
Following the submission of sponsor’s request for conducting an *in-vitro* permeation study, a preliminary project assessment is performed by the CRO. Based on the technical aspects / method complexity and the resources required for the study, a quotation is issued and sent to the client.

**Review of request**

Before proceeding with the development of an analytical method suitable for its intended purpose, it is crucial to establish the specificity of the candidate receptor media. This step is established during the next process stage which is Analytical Method Validation. However, it is of paramount importance to proceed with a preliminary evaluation of the candidate receptor media in terms of specificity.

**Analytical Method Development / Specificity**

The first and most critical step is the development of an analytical method that will be suitable for its intended purpose. The latter is established during the next process stage which is Analytical Method Validation. However, it is of paramount importance to proceed with a preliminary evaluation of the candidate receptor media in terms of specificity.

**Analytical Method Validation Study**

The *in-vitro* permeation method should be suitably discriminating while the analytical methods for determining the content of the test substance in the receptor fluid should be validated according to ICH Q2(R1). The analytical method is validated in terms of: Selectivity, Linearity, Accuracy, Precision, LOQ/LOD and the Stability of the test substance in the receptor medium.

**Receptor Medium Suitability / Solubility Study**

The solubility of the target analyte in the receptor medium should not be a rate-determining step in the *in vitro* permeation experiment. This condition is experimentally substantiated by the demonstration that the analyte's solubility in the receptor medium is at least an order of magnitude higher than the maximum concentration expected during the *in vitro* permeation experiment.

**In-vitro permeation pilot study**

In cases where the purpose of the *in-vitro* permeation study is the pharmacokinetic comparison (i.e. comparison of the rate and extent of *in vitro* permeation) between a test and a reference product, a pilot study should preferably be performed in order to estimate the number of donors required for the pivotal study and optimize the sampling scheme.

**In-vitro permeation pivotal study**

The purpose of a pivotal study is to compare the rate and extent of *in vitro* permeation between a reference and a test formulation in order to support submissions claiming equivalence. Its design (sampling times, number of time points, number of donors / Lots, etc.) highly depends on the outcome of the pilot study.
Review of Request

Analytical Method Development / Specificity of candidate receptor media

Analytical Method Validation Study

Receptor Medium Suitability / Solubility Study

Product Development / Screening study

Product Development / Screening study of Pharmacokinetic study?

Pilot Study Protocol and Report

Pivotal Study Protocol and Report

Pharmacokinetic study

In-vitro permeation pilot study

In-vitro permeation study based on a protocol meeting the sponsor’s requirements and purpose of the study

Figure 7: In Vitro Permeation Study Process Flow Chart
NOTE:
According to the process flow chart presented, the step following analytical method development refers specifically to Analytical Method Validation. This is due to: a) the fact that the other two components of Method Validation, namely, the discriminating power and mass balance, may not always be required for screening studies and b) the fact that the evaluation of both components can be performed only at the stage of an actual in-vitro permeation experiment.

The IVP method validation components that are addressed during the pilot and pivotal study are depicted in the following figure.

**Figure 8: IVP Method Validation Components**

- **Discriminating Power Evaluation**
  - The method should be suitably discriminating in order to be able to detect changes in the pharmacokinetics of the test substance as a function of differences in its delivery.
  - Flux profiles obtained with presentations containing different amounts of the test substance are compared in order to demonstrate that consistently higher flux profiles are obtained for the formulation with the higher content in response to the increased dose amount.
  - The method should be able to discriminate between batches with respect to critical parameters that are known to have an impact on the bioavailability of the product.

- **Mass Balance**
  - All components of the test system are analyzed by means of the validated analytical method and the recovery determined.
  - An adequate mean recovery is in the range of 100 ± 10%. Under certain circumstances (e.g. volatile substance that has to be trapped in a filter) recovery boundaries 100 ± 20% may be acceptable.

- **Method Precision and Reproducibility**
  - The in-vitro permeation study flux and cumulative permeation results are presented in the final study report for each diffusion cell and time point, with summary statistics to describe the intra-donor average, standard deviation, and %CV among replicates as well as the inter-donor average, standard error, and %CV.
Tissues from human or animal sources can be used. Although viable tissue is preferred, non-viable tissue can also be used provided that its integrity can be demonstrated. Either epidermal membranes (enzymically, heat or chemically separated) or split thickness skin (typically 200-400 μm thick) prepared with a dermatome, are acceptable.

Other tissue models, based in artificial membranes, provided by MatTek Corporation, can also be employed. These models include but are not limited to the following examples:

Also known generically as a Reconstructed Human Epidermis (RHE), EpiDerm is a ready-to-use, highly differentiated 3D tissue model consisting of normal, human-derived epidermal keratinocytes (NHEK) cultured on specially prepared tissue culture inserts.

EpiDerm™

EpiOcular™

EpiOral™

MatTek’s EpiOral tissues consist of normal, human-derived epithelial cells. The tissues, which are cultured on specially prepared cell culture inserts using serum free medium, attain levels of differentiation on the cutting edge of in vitro cell culture technology. Morphologically, the tissue models closely parallel native buccal human tissues.

EpiOral™

EpiIntestinal is a highly differentiated 3D tissue model that closely recapitulates the physiology, tissue structure, and function of the epithelium of the small intestine.

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Tissue Integrity Tests
Any physical deterioration in the tissue preparations (e.g. due to time at ambient temperature or hydration, pretreatment) may result in an overestimate of permeability. Pre-study evaluation is always performed in order to ensure that damaged tissue will be eliminated before performing the test. The methods employed are the following:

✔ checking that trans-epidermal water loss (TEWL) from the stratum corneum is in the normal range for the skin type,
✔ measuring the penetration characteristics of a reference material

Tissue viability / Maintenance of Metabolic Activity
The biotransformation of the compound in skin prior to systemic absorption may be significant for some compounds. In cases where the tissue's metabolic activity is within the scope of the study (i.e., there is a requirement for the identification of metabolites) the maintenance of the tissue's viability is demonstrated at the end of the experiment (e.g. by monitoring the conversion of MTT - a water-soluble tetrazolium dye - to the insoluble purple metabolite, formazan).

IVP test Applications
The major advantage of *in vitro* studies is the possibility for controlling the conditions of the experiment and therefore changes in permeation should only arise from changes in the formulation and / or the tissue employed. The IVP test is a valuable measure of product quality and performance and may reflect the thermodynamic activity of the active substance in the product. To this end, it can serve as a valuable tool for the following applications:

- Preclinical development / Screening and selection of formulation trials
- Surrogate for bioequivalence studies when same qualitative (Q1), quantitative (Q2) composition and arrangement of matter (Q3) have been established
- Change control / Evaluation of the impact of formulation, manufacturing process changes in the permeation profile
Connecting Ideas