advances in
dermal models
Introduction

Trends in Dermal Delivery

Drug delivery by the dermal route is increasingly popular for a variety of reasons, including:

• Opportunity to extend pipelines by modifying formulation/route of administration

• Emergence of topical generics and cosmeceuticals

• Consumer benefits, such as improving convenience and flexibility for self-administration, home care, patient and caregiver compliance

• Localized, efficient drug delivery

• Enhanced bioavailability (bypass first-pass metabolism)

An integrated and dynamic experimental approach, incorporating in vitro, ex vivo, and in vivo non-clinical models can be used to evaluate dermal delivery (tissue-based and pharmacokinetic models), compare and optimize formulations, minimize DoE runs, and guide critical investment decisions.

Absorption Systems' unique portfolio of dermal models provides a complete package of predictive testing. We employ a rational and multi-pronged approach which relies on well-validated non-clinical models to bypass clinical studies and/or enhance quality by design (QbD).
In Vitro Dermal Models

In Vitro Release Testing (IVRT)

As drug products continue to increase in complexity, there has been a growing initiative to enhance evaluation by introducing the concepts of quality target product profile (QTPP) and quality by design (QbD). By complementing the traditional paradigm of ‘equivalence by testing,’ the current framework encourages the use of appropriate surrogates to target ‘pharmaceutical equivalence by design.’ One of the fundamental characteristics of topical formulations is release rate. This may be measured using in vitro release testing (IVRT).

In 2012 and 2013, the FDA released a number of unprecedented bioequivalence (BE) guidances for complex products, including the first for a metered-dose inhaler (albuterol sulfate), an ophthalmic emulsion (cyclosporine), and a dry powder inhaler (fluticasone propionate/salmeterol xinafoate).

Of note, the BE recommendation for acyclovir ointment permits an in vitro option in lieu of a clinical endpoint study, assuming the generic formulation and reference listed drug are quantitatively and qualitatively the same (Q1/Q2), the formulations have similar physicochemical properties (viscosity, rheology, etc.), and comparable in vitro release rates. Prior to the acyclovir BE recommendation, the only exceptions to a clinical endpoint study for locally-acting semisolids were the Stoughton-McKenzie vasoconstriction or ‘skin blanching’ assay for corticosteroids and in vivo microdialysis.

<table>
<thead>
<tr>
<th>Active ingredient:</th>
<th>Acyclovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form/Route:</td>
<td>Ointment; Topical</td>
</tr>
<tr>
<td>Recommended study:</td>
<td>2 Options: In Vitro or In Vivo Study</td>
</tr>
</tbody>
</table>

I. In Vitro option:

To qualify for the in vitro option for this drug product pursuant to 21 CFR 320.24 (b)(6), under which "any other approach deemed adequate by FDA to measure bioavailability or establish bioequivalence" may be acceptable for determining the bioavailability or bioequivalence (BE) of a drug product, all of the following criteria must be met:

i. The test and Reference Listed Drug (RLD) formulations are qualitatively and quantitatively the same (Q1/Q2).
ii. Acceptable comparative physicochemical characterization of the test and RLD formulations.
iii. Acceptable comparative in vitro drug release rate tests of acyclovir from the test and RLD formulations.
The FDA stated that there are two key concerns when determining BE for topical dermal products:

1. Are the test and reference formulated similarly such that release characteristics are the same between the two products?
2. Will the amount of drug uptake by the skin be the same or will absorption be affected by differences in formulation and/or manufacturing of the two products?

Furthermore, acyclovir ointment is a unique product whose characteristics distinguish it from other topical semisolids:

- The product does not have a multiphasic vehicle, rather it is a single API in a single-ingredient vehicle (PEG)
- The physicochemical characteristics which have the potential to impact bioavailability are well-established
- Clinical endpoints are difficult given the modest clinical benefit shown for acyclovir

This “characterization-based equivalence” for formulations with the same concentrations of the same inactive ingredients signifies the trend toward a rational, science-driven approach, relying on trusted in vitro methods (not unlike the notion of BCS biowaivers for solid oral dosage forms).
IVRT Methodology

Methodology for IVRT is delineated in the FDA’s “Guidance for Industry: Nonsterile Semisolid Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation” (May 1997), also referred to as “SUPAC-SS.”

The technique, performed in a Franz cell diffusion system with synthetic membrane, was originally designed to compare different lots for the same product manufactured by the same company. Today, IVRT is also used to waive clinical endpoint studies for certain generic products (e.g., acyclovir).

• Number of Samples: Six samples are recommended
• Sample Application: ~300 μg (infinite dosing) of semi-solids/membrane, occluded
• Sampling Time: Suggested: 5 time points: 0.5, 1, 2, 4, and 6 hr
• Ensure unbiased comparison by following a layout or ensure intermixing between test (T) and reference (R)

Two –Stage Design
• Stage 1: Two runs with six cells per run; if low and high ends fall within limits of 75-133.33%, the products pass at the first stage
• Stage 2: Additional four runs; if low and high ends fall within limits of 75-133.33%, the products pass at the second stage
**In Vitro Release Testing (IVRT) Study**

**Study Purpose**
Evaluate the release of test articles from formulations across synthetic membranes in Franz cells

**Deliverables**
- Flux or apparent permeability of the test article and reference compounds
- Percent recovery of the test article
- Comparison of test and reference formulations (if applicable)

**Model Description**
- Unidirectional permeability assessment of test formulation (gel, cream, ointment, patch, or emulsion) across synthetic membranes mounted in Franz cells
- Control compounds run in parallel
- Sampling from receiver compartment at six time points up to 6 hours

**Benefits**
- Compare batch formulations to assess scale-up and post-approval changes
- Waive clinical endpoint studies for certain generic formulations
- Screen formulations before clinical PK or bioequivalence studies
- Absorption Systems provides in-house analytical and formulation services, along with consultative assistance in study design

**At-a-Glance**
- **Type of model**
  - Franz cell
- **Test System**
  - Synthetic Membrane
- **Time**
  - Two to three weeks
**Ex Vivo Dermal Permeability**

*Ex vivo* dermal studies are used to assess the feasibility of transdermal administration, to rank-order compounds in terms of permeability or accumulation, and to optimize formulations.

Such studies are performed with human or porcine skin in Franz cells. Test formulations (gels, creams, ointments, patches, etc.) are applied to the upper (external) surface, and samples are removed at pre-determined time points from the reservoir containing buffer that is in contact with the lower (serosal) surface. Reference compounds are either co-dosed (if the test compound is in solution) or run in parallel (if the test compound is in some other type of formulation) for quality control. The skin is often extracted at the end of the study to quantify accumulation of the test compound.

It is possible to determine accumulation in different layers of the skin by tape stripping or heat separation. Transepidermal water loss (TEWL) may be measured as a proxy for skin dryness. Test formulations may also be incubated with tissue homogenates to assess the metabolic stability of the test article.
Ex Vivo Dermal Permeability Study

Study Purpose
Evaluate the dermal permeability and/or accumulation of test articles

Deliverables
- Flux or apparent permeability of the test article and reference compounds
- Percent recovery of the test article
- Comparison of the absorption potential of test article to that of reference compounds
- Comparison of test and reference formulations (if applicable)
- Accumulation in stratum corneum, epidermis, and dermis (if applicable)
- Demographics of human donor

Model Description
- Unidirectional permeability assessment of test article across frozen dermatomed human cadaver skin (male or female trunk region)
- Skin mounted in Franz cells thermostatically controlled at 37°C
- Test article may be dosed without formulation, or administered as a gel, cream, ointment, patch, or solution
- Suitability experiments may include assessment of chemical stability and/or non-specific binding
- Control compounds (atenolol and testosterone or caffeine) run in parallel
- Sampling from receiver compartment at five time points up to 30 hours
- Optional determination of test article accumulation in skin
- Optional stability assessment in skin homogenate

Benefits
- Screen formulations in human skin before clinical PK or bioequivalence studies
- Rank order compounds in terms of permeability
- Evaluate locally-acting dermal formulations and quantify dermal accumulation
- Absorption Systems provides in-house analytical and formulation services, along with consultative assistance in study design

At-a-Glance

Type of model
- Franz cell

Test System
- Human Skin

Time
- Two to four weeks
Dermal PK and Irritation

In vivo dermal studies are conducted to evaluate the pharmacokinetics, toxicity, and/or efficacy of test compounds and formulations. Studies may be performed in rodents or non-rodents, on intact or compromised skin, and in healthy or diseased animal models. Pharmacokinetic studies determine the local and systemic concentrations of a drug over time. While many dermal products are locally acting, assessment of systemic distribution is also important to rule out exposure to potentially toxic concentrations. This is particularly true in a diseased or wounded model since the condition of the skin may affect the absorption profile of the drug.

Transdermal delivery systems also have the potential to cause irritation at the site of administration since they are in direct contact with the patient’s skin. Dermal irritation of a test article should be evaluated against a placebo formulation and control formulations such as 0.1% sodium lauryl sulfate (SLS, high-irritancy control) and 0.9% saline (low-irritancy control). Draize scoring is the standard method used to quantify observations of the dose site.
### Draize Evaluation of Dermal Reactions

#### Erythema and Eschar Formation (most severely affected area graded)

<table>
<thead>
<tr>
<th>Skin Reactions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Well-defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (beet redness) to slight eschar formation (injuries in depth)</td>
<td>4</td>
</tr>
</tbody>
</table>

#### Edema Formation (most severely affected area graded)

<table>
<thead>
<tr>
<th>Skin Reactions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No edema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight edema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight edema (edges of area well defined by definite raising)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate edema (raising approximately 1 millimeter)</td>
<td>3</td>
</tr>
<tr>
<td>Severe edema (raised more than 1 mm and extending beyond the area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>
Dermal Pharmacokinetics Study

Study Purpose
Evaluate the local and systemic distribution of test articles after dermal application

Deliverables
• Concentration of test article accumulated at the dose site
• Concentration of test article in systemic circulation
• PK parameters using non-compartmental analysis: AUC, C_{max}, T_{max}, and half-life
• Draize scoring (erythema and edema) to evaluate dermal irritation at the dose site

Model Description
• Intact or abraded skin
• Dosing forms: gel, cream, patch, liquid
• Dosing and bandaging procedures are customized for each test article and animal model
• Diabetic, wounded, and infectious models available
• Comparison to intravenous dose to determine bioavailability (optional)

Benefits
• On-site scientists with professional experience with small and large animals
• Dedicated operations manager to facilitate study conduct and minimize turnaround time
• In-house bioanalytical and formulation services
• Consultative assistance in animal model selection

At-a-Glance

Type of model
• Dermal PK

Test System
• Rats
• Minipigs

Time
• 14 to 28 days
Dermal Irritation Study

Study Purpose
Evaluate the irritation caused by test articles after dermal application

Deliverables
- Draize scoring (erythema and edema) of the dose site
- Percent adherence evaluated for patches
- Digital photographs
- Histopathology with standardized scoring system

Model Description
- Four to six dose sites per animal
- Dosing forms: gel, cream, patch, liquid
- Animal’s hair is shaved the day prior to dosing (no depilatories are used)
- Test article is applied directly onto the skin
- Dosing and bandaging procedures are customized for each test article and animal model
- Dose sites are outlined via marker, tattoo, or tincture of benzoin
- Single or repeat dosing regimens available
- Vehicle control dosed in parallel

Benefits
- On-site scientists with professional experience with small and large animals
- Dedicated operations manager to facilitate study conduct and minimize turnaround time
- Bioanalytical capabilities for combined pharmacokinetics (PK) studies
- Consultative assistance in animal model selection

At-a-Glance

Type of model
- Dermal Irritation

Test System
- Rabbits
- Guinea pigs

Time
- 14 to 21 days
Dermal Efficacy Models

When selecting an appropriate animal model for predicting efficacy of a drug, it is important to match the etiology of the disease state as closely as possible to the human condition. However, many human dermatological conditions, such as rosacea, acne, and psoriasis, do not occur in other species, and are therefore difficult to model at the preclinical stage. Instead, indirect approaches are used to address specific physiological processes associated with such disorders, including inflammation, orthokeratosis, angiogenesis, and immunologic response. New treatments under development are often compared to existing products for their efficacy toward these processes.

Animal models for dermal wound healing are much more established and are described in detail in FDA’s “Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds – Developing Products for Treatment” (June 2006). Minipigs are increasingly becoming the species of choice for evaluating wound healing due to their similarity to humans with regard to skin structure and healing properties. A key trait of minipigs is that their skin heals by granulation and re-epithelialization, similar to humans, whereas other species heal by contraction. Wounds can be simulated in animals to match the target clinical state in terms of type (chronic ulcer or burn), depth (full or partial thickness), and size (percent of total body surface area). Procedures for wounding, wound care, and bandaging must be optimized for each test article formulation and animal model. Study design elements such as randomization of wound treatments and timing of exams and observations are also critical to accurately evaluate a test article’s healing properties.
Dermal Wound Healing Model

Study Purpose
Evaluate the efficacy and safety of pharmaceuticals and devices designed to treat epidermal and dermal defects or wounds

Deliverables
• Observations, wound scores, and wound measurements
• Digital photographs
• Histopathological interpretation with representative microscopic images
• Histomorphometry

Model Description
• Full or partial thickness wound creation
• Test article applied by Absorption Systems scientists or Sponsor representatives
• Customized bandaging and wound care
• Clinical examinations at customized time points
• Diabetic and infected wound models available

Benefits
• Positive and negative control groups provide comparative data
• Absorption Systems has significant experience with wound options and bandaging materials to ensure study success
• Consultative assistance in animal model selection

At-a-Glance
Type of model
• Dermal

Test System
• Minipigs
• Rats

Time
• Acute – 3 to 7 days
• Chronic – 28 days
Mouse Tail Psoriasis Model

At-a-Glance

Type of model
- Evaluate test article’s ability to induce orthokeratosis

Test System
- CD-1 mice

Time
- 14 days

Study Purpose
Evaluate the efficacy and safety of test articles designed to treat psoriasis

Deliverables
- Pathology report containing measurements of epidermal thickness and percent of orthokeratotic scales
- Efficacy of formulations evaluated via morphometry and histopathology

Model Description
- Daily topical application of test or control article to the tail for 14 days
  - Comparison of test article treatments to known antipsoriatic products (e.g., retinoic acid)
  - Tails collected and evaluated via histopathology

Benefits
- Mouse tail epidermis keratinization mimics human keratinization in psoriasis
- More accurate classification of the test article’s treatment due to treated and untreated control groups
- Dedicated operations manager to facilitate study conduct and minimize turnaround time
References

1. **Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds — Developing Products for Treatment**  U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH), June 2006


About Absorption Systems

Absorption Systems assists pharmaceutical and medical device companies in identifying and overcoming ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) barriers in the development of drugs and medical devices. The company’s mission is to continually develop innovative research tools that can be used to accurately predict human outcomes or to explain unanticipated human outcomes when they occur. The company’s facilities are strategically located on both US coasts and Panama, and encompass nearly 65,000 sq. ft., servicing hundreds of customers throughout the world. You can trust results based on our senior scientists and support staff’s experience with GLP-compliant processes and international regulatory standards, as well as our AAALAC-accredited and NIH-assured facility. For more information on the company’s comprehensive contract services and applied research programs, please visit absorption.com.

Preclinical Services & Capabilities

Lead Optimization
Physicochemical Properties
Permeability
Stability
Metabolism and Transporters
Binding and Distribution
Formulation Assessment
Bioavailability and Exposure | *rodent, non-rodent*

Candidate Selection
Formulation Development
PK and Biodistribution | *multiple species and dose routes*
Barriers to Bioavailability
*In Situ* and Isolated Organ Perfusion | *rat: brain, liver, intestine*
*Ex Vivo* Tissue Permeability | *intestinal, dermal, ocular, buccal, nasal, vaginal*

IND- and NDA-Enabling
Transporter-Based Drug Interactions
BCS Permeability and Solubility
Classification for Biowaivers
Metabolism-Based Drug Interactions
Metabolite ID and Production
Medical Device Testing
Toxicology

Bioanalysis
Bioanalytical Support from Discovery through Clinical
GLP and Non-GLP