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Bioanalysis Supporting *In-Vitro* Permeation Tests:

Alternative to Tick-Box Assay Validations

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Agenda

1 What are we validating for?

2 Bioanalytical Validation Guidelines for IVPT

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What are we validating for?

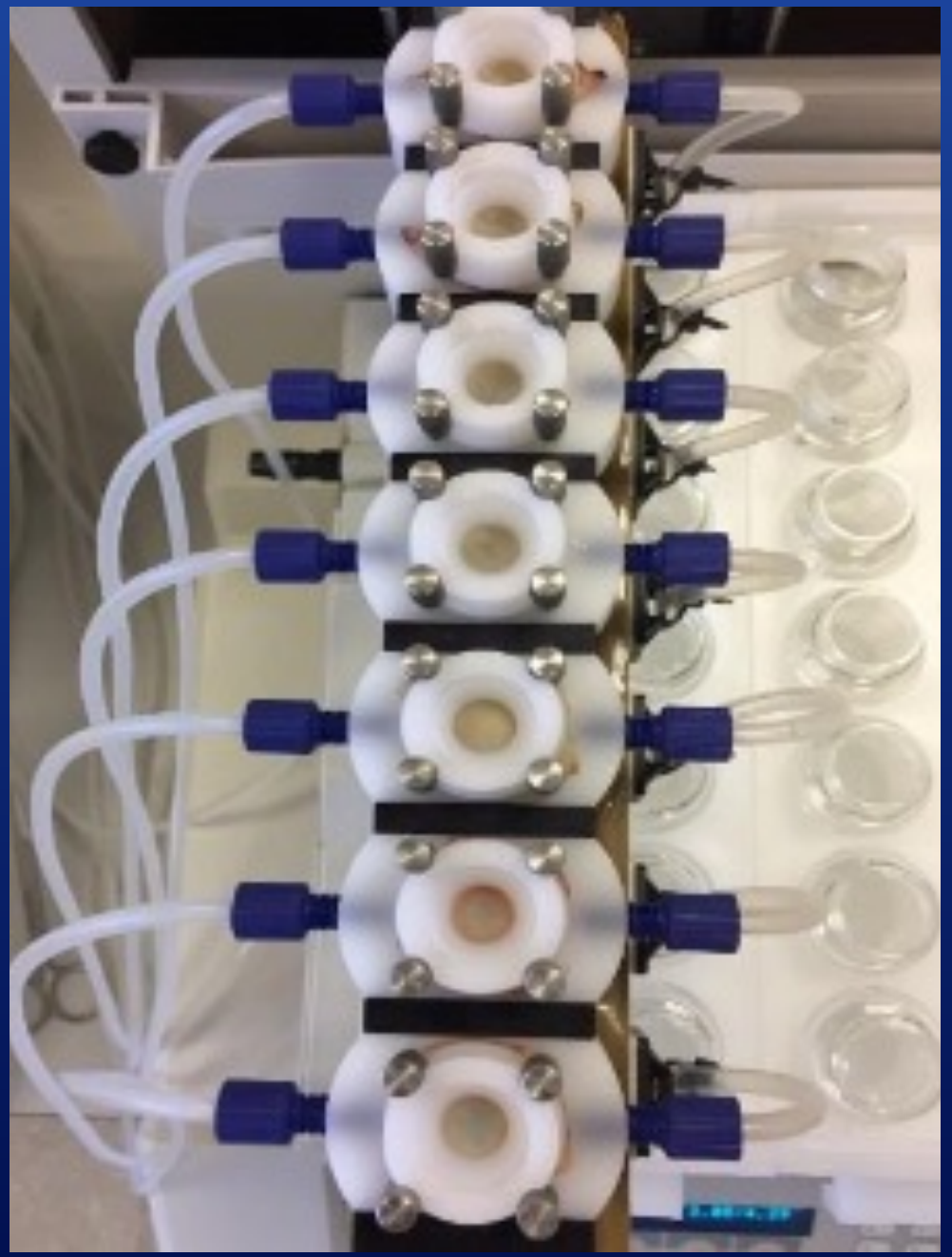
In-Vitro Permeation Testing (IVPT) studies are used as a replacement for in-vivo models over many industries such as pharmaceutical, chemical, animal health and cosmetic

IVPT studies provide information on the behavior of a test substance, when it is applied to the surface of the skin

Main objective is to apply test item to the skin and determine rate of absorption through the various layers of skin into the circulatory system. Each study should also aim to simulate the conditions that the product would be used in real life situations

Prior to application, the skin is clamped to a cell and a buffer solution below the skin (receptor fluid) is used as a proxy to imitate the blood flow

The design of an IVPT study will differ significantly based on the endpoint of the study and thus, a variety of matrices may require analysis



What are we validating for?

Examples:-

Hair Dye:- applied to skin and left for nominal time, later washed off with soap solution and dried with tissue

Potential matrices to analyse:-

- Receptor fluid, epidermis, dermis, unexposed skin, tape strips (stratum corneum), skin wash, tissue swabs and formulation

Dermal Patch:- applied to skin and left for nominal time. No need to wash off application

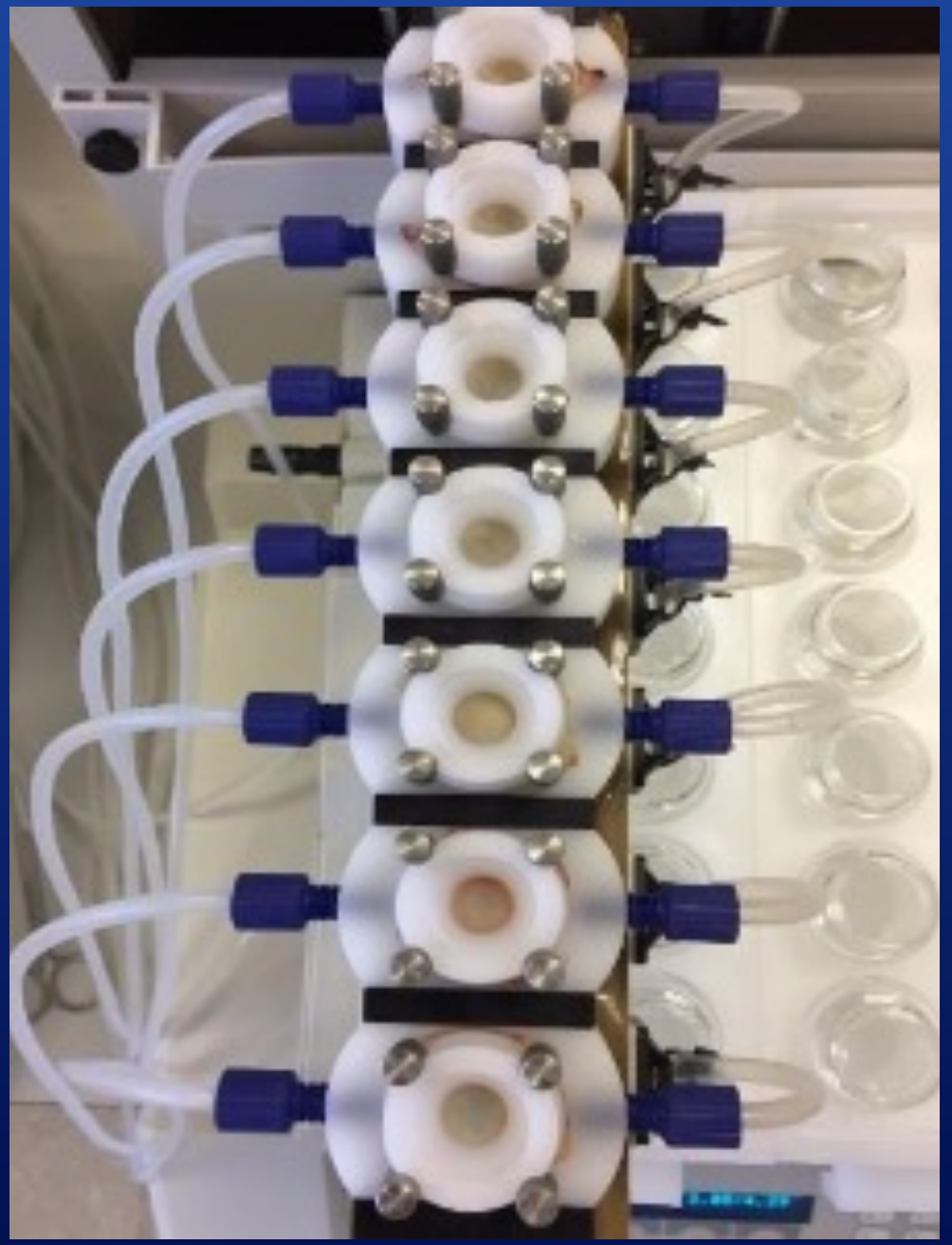
Potential matrices to analyse:-

- Receptor fluid, epidermis, dermis, unexposed skin, tape strips (stratum corneum), patch

However, the *In-Vitro* regulatory guidelines for each industry have different priorities, which in turn determine the matrices to be analysed

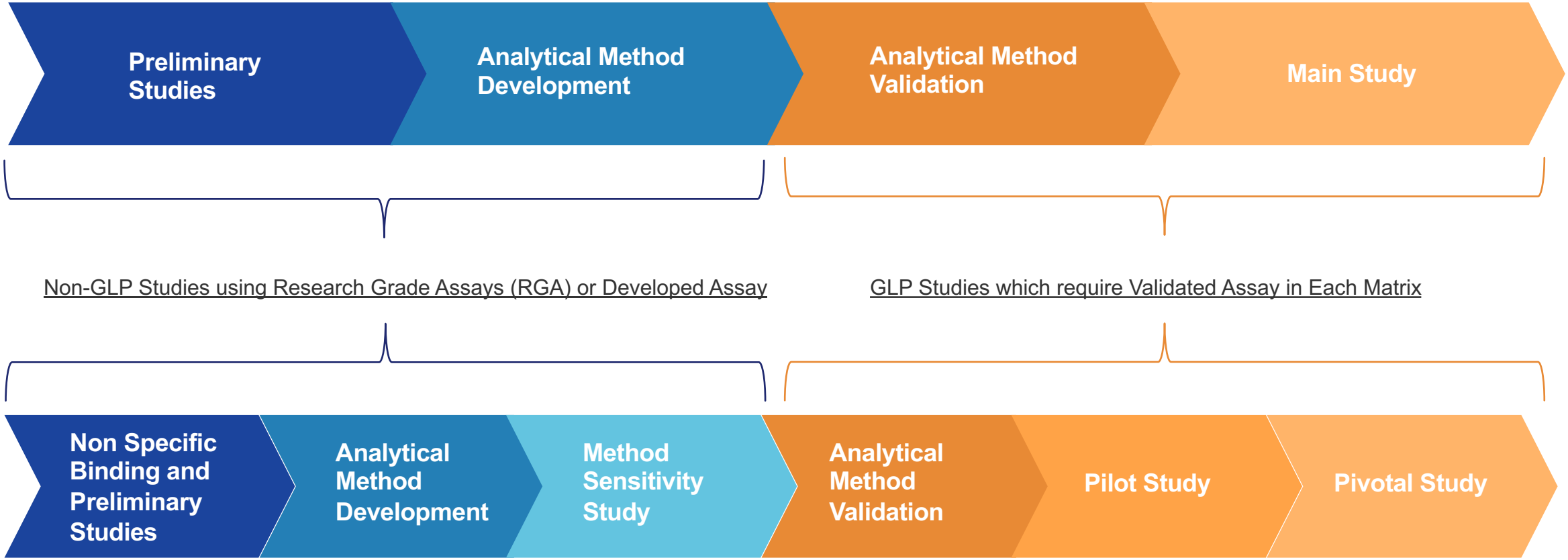
With so many variables, having one validation guideline to support all endpoints is challenging. Therefore, having a validation strategy is essential

A question to start with is “when to validate?”…



Typical *In-Vitro* Product Development

Safety Assessment Studies



Bioequivalence Studies

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Industries for *In-Vitro*

Regulatory Guidelines

Typically, skin absorption studies adhere to the overarching OECD Guideline for Testing of Chemicals, Guideline 428 – “Skin Absorption: In Vitro Method”.

Adapt the study design to meet Good Laboratory Practice (GLP) requirements as well as regulatory guidelines across various industries such as:

Pharmaceuticals

- FDA (2022). Draft Guidance: In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs. Guidance for Industry.
- EMA (2018). Draft guideline on quality and equivalence of topical products

Consumer Products

- SCCS (2021). Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation, 11th Revision

Agrochemical Products

- EFSA (2017) Guidance on Dermal Absorption (EFSA Journal, 2017, 15 (6): 4873

How do each of these guidelines differ?

Points to consider in validations

FDA

The FDA focuses on the absorbed component “receptor fluid”

Endpoints for In-Vitro Study are

- Rate of absorption
- Total amount permeated (AMT)
- Jmax = maximum flux at the peak of the drug flux profile

EMA

EMA requires analysis of extra matrices to obtain a **mass balance**

Therefore, recovery of test material from all matrices is vital

Consumer

SCCS guidance dictates that all matrices are retained for analysis to achieve a **mass balance** (100 ± 15% of applied dose)

Each *In-Vitro* study design differs based on the endpoint

Therefore, having a “One validation fits all” approach is challenging

Bioanalytical Validation Guidelines

As it stands there are no validation guidelines to support *In-Vitro* Permeation Testing studies with the exception of bioequivalence studies (in Draft):-

- Plaza et al. (2021). Support for Regulatory Assessment of Percutaneous Absorption of Rectronecine-type Pyrrolizidine Alkaloids through Human Skin. Planta Med 88(2): 144-151

SANCO/SANTE guidelines are available but can be analytically restricting for supporting an IVPT endpoint

Therefore, ICH M10 bioanalytical guidelines are a good place to start

Although ICH M10 guidelines are designed with an *In-Vivo* endpoint in mind, most experiments in these guidelines are applicable to prove a robust accurate method to validations supporting IVPT

IVPT studies differ greatly depending on their endpoint and therefore, the experiments included in a bioanalytical validation should revolve around these endpoints

Validation Strategy

Triage validation requirements!

With all the various designs and regulatory guidelines from an *In-Vitro* perspective, what's the best strategy to validate and ensure you have a robust and accurate method?

First triage point:-

- Safety Assessment
- Bioequivalence

This will assess analytical strategy

Second triage point:-

- FDA
- EMA or SCCS

This will determine the strategy for the IVPT study and therefore the matrices required for validation

Validation Experiments

FDA –Bioequivalence

Typically, only a validation in receptor fluid is required:-

- Same validation experiments required as if matrix was plasma

However, based on the type of compound and previous preliminary work, a full mass balance may be required for FDA compliant studies

EMA & SCCS (SA)

Validation Matrices:-

- Receptor Fluid
- Solvent used for extracting compounds from skin, tape and tissue
- Skin Wash
- Formulation

Main point of consideration is a recovery experiment:-

- Aim for recovery from skin, tape and tissue of 100 ± 15% to support mass balance endpoint

EMA Bioequivalence

Validation Matrices:-

- Receptor Fluid
- Solvent used for extracting compounds from skin, tape and tissue
- Skin Wash
- Formulation

Main points to consider for a full mass balance bioequivalence validation:-

- Recovery within 100 ± 15%
- Alternative stability experiment
- Formulation stability in solvent experiment

Validation Experiments

FDA - Bioequivalence

- Solution Stability
- Linearity
- Accuracy & Precision
- Selectivity
- Carryover
- Matrix Effects & Recovery
- Storage stability
- Freeze/Thaw Stability
- Autosampler stability
- Solubility in Receptor Fluid (RF)

EMA & SCCS - Safety

- Solution Stability
- Linearity (in RF and solvent)
- Accuracy & Precision (in RF and solvent)
- Recovery in each matrix
- Selectivity
- Carryover
- Matrix Effects (Skin only)
- Storage stability (RF & solvent)
- Freeze/Thaw Stability (RF & Solvent)
- Autosampler stability
- Recovery from formulation
- Solubility in Receptor Fluid

EMA - Bioequivalence

Same validation experiments required as EMA & SCCS (safety assessment)

Stability in skin, tape and tissue is performed using blank extract from these matrices instead of pure solvent

Stability of formulation extracts are also assessed once recovery has been proven to be within acceptance

Reduced complexity in stability experiments for safety studies compared to bioequivalence studies. This approach reduces validation length and significantly lowers cost whilst still meeting the requirements of the safety endpoint.

Validation Experiments

Remember your endpoint!

- Remember the regulatory guidelines for the IVPT study
- Matrices may differ study to study based on the *In-Vitro* design
- Adapt ICH-M10 *In-Vivo* Bioanalytical Validation Experiments to support the *In-Vitro* endpoint
- Do not over engineer the validation to meet all aspects of the ICH-M10 guidelines
- If the validation suggests mass balance is not achievable – assess the impact and consider if the endpoint can still be achieved

Conclusion

- Currently no guidelines for bioanalytical method validation supporting IVPT studies and therefore bespoke validations are required
- Triage studies based on endpoint and *In-Vitro* regulations for the study type
- Ensure validation experiments are based on the endpoint of the study rather than the “tick-box” approach
- Validated method limitations should be kept in mind when interpretating any analytical data obtained for any IVPT studies
- Same approach can be applied for validations supporting other In-Vitro endpoints such IVRT *(In-Vitro* Release Testing)

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