



Mastering IVRT and IVPT: From Technical Complexity to Scientific Confidence

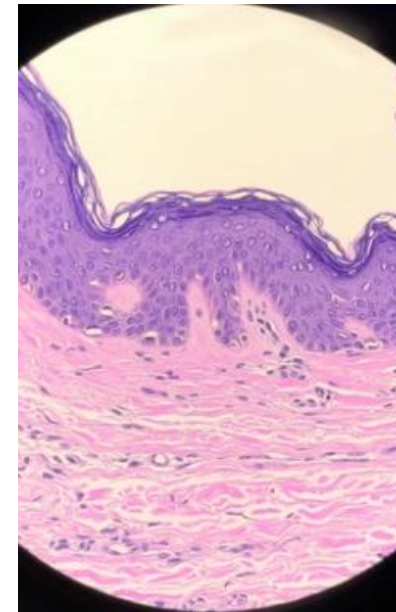
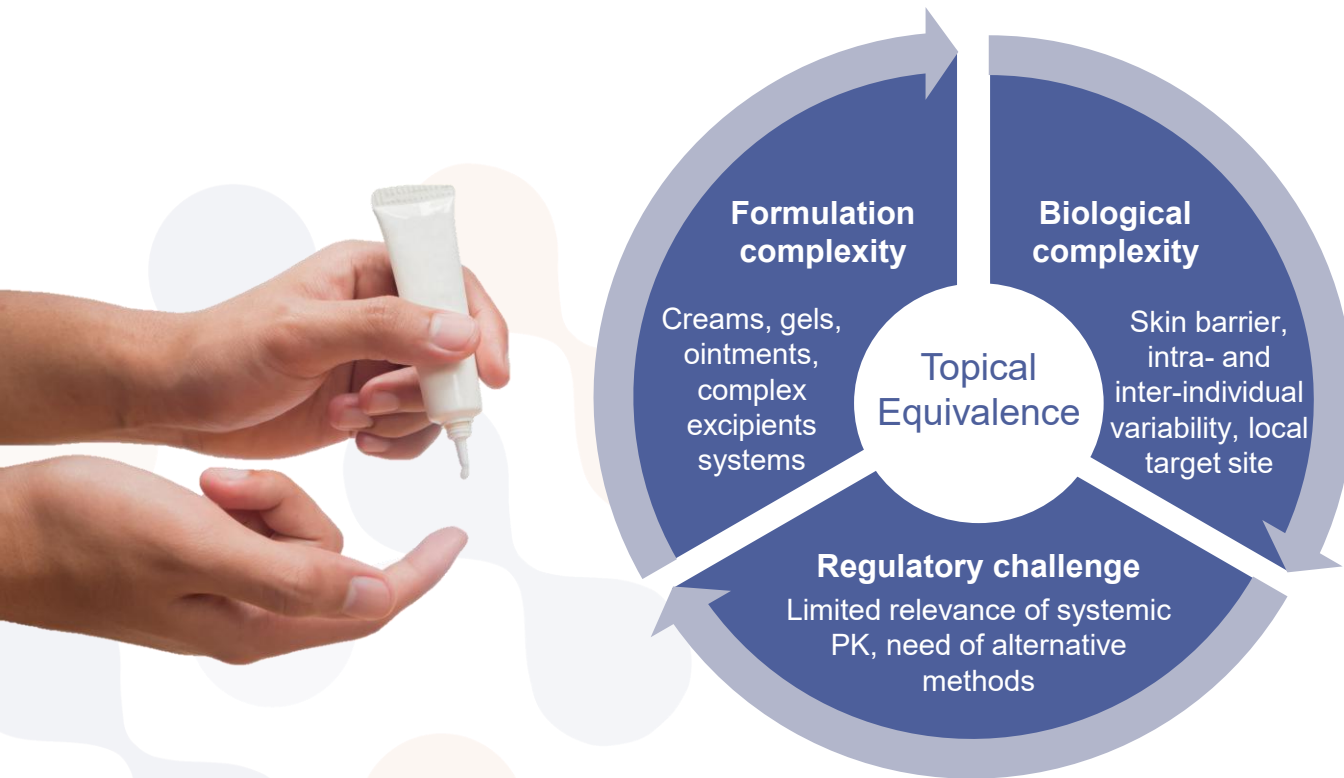
Critical success factors, methodological challenges and practical considerations for robust topical product assessment.

Magali Breisse – Eurofins ADME Bioanalyses

BioBridges, 24-25 sept 2026, Prague



Why topical products performance evaluation is particularly challenging?



Demonstrating equivalence requires a weight-of-evidence approach



9 September 2024
EMA/CHMP/QWP/708282/2018 Corr.1*
Committee for Medicinal Products for Human Use (CHMP)

Guideline on quality and equivalence of locally applied,
locally acting cutaneous products

Q1/Q2
Formulation
similarity

Q3
Physicochemical
characterization

IVRT

IVPT

Clinical
relevance

In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <https://www.regulations.gov>. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Susan Levine at 240-402-7936.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2022
Generic Drugs

In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <https://www.regulations.gov>. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Susan Levine at 240-402-7936.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2022
Generic Drugs

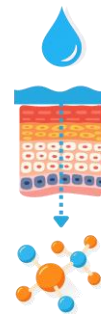
What do IVRT and IVPT actually measure?



How does the drug leave the formulation?



How does the drug cross the skin barrier?



Methods are complementary, not interchangeable.

IVRT: assessing drug release for topical formulations

Topical product in **infinite** occlusive condition in donor compartment



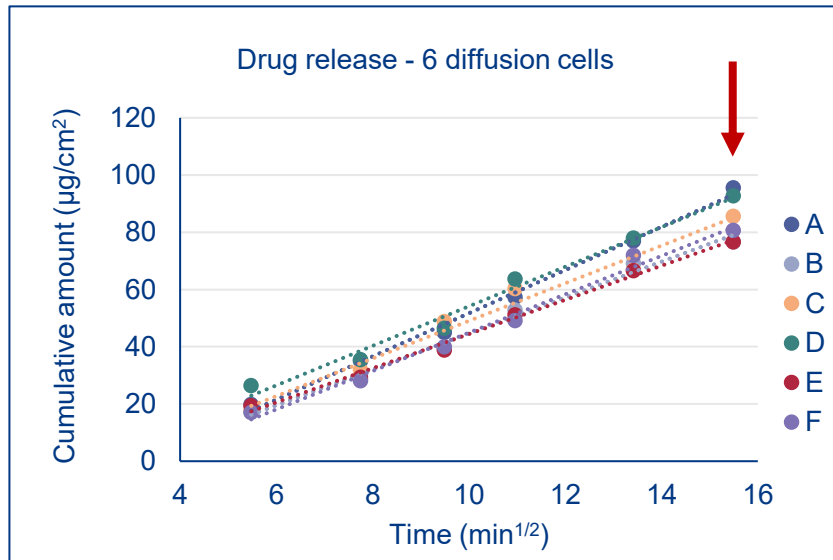
Inert synthetic membrane

Receptor fluid collection through sampling arm

Diffusion cell

Parameters:

- Release rate (slope)
- Cumulative amount at the end of experiment



IVRT: the 4-steps approach

Step 1

Define controlled experimental conditions:

- Membrane type (inertness)
- Receptor fluid composition (solubility)
- Samples collection times

Step 2

Validate the analytical method for receptor fluid samples analysis:

- LC-UV or LC-MSMS
- Chromatographic conditions
- ICH Q2 (R2)
- Accuracy & Precision
- Robustness
- Storage stability

Step 3

Validate the IVRT experimental conditions:

- Accuracy & Precision
- Specificity/selectivity
- Robustness for temperature and stirring
- Discriminative power

Step 4

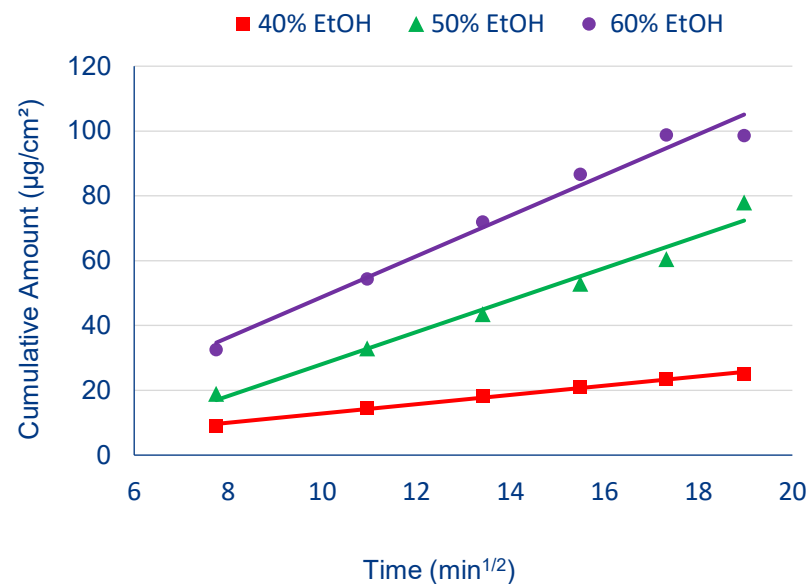
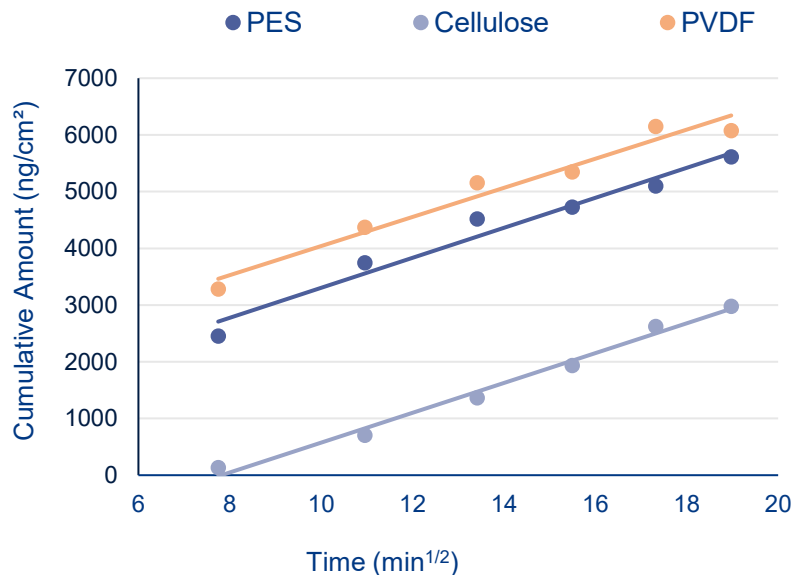
Perform Pivotal IVRT:

- Validated IVRT controlled experimental conditions
- Validated analytical method for samples analysis
- 3 batches of each Test and Reference topical products
- Statistical analysis

IVRT: the 4-steps approach

Step 1: obtain linear release rate and define controlled conditions

- Selection of membrane, receptor fluid, sampling times



IVRT: the 4-steps approach

Step 2: ICH Q2(R2) analytical method validation



INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL
REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES Q2(R2)

Final Version

Adopted on 1 November 2023

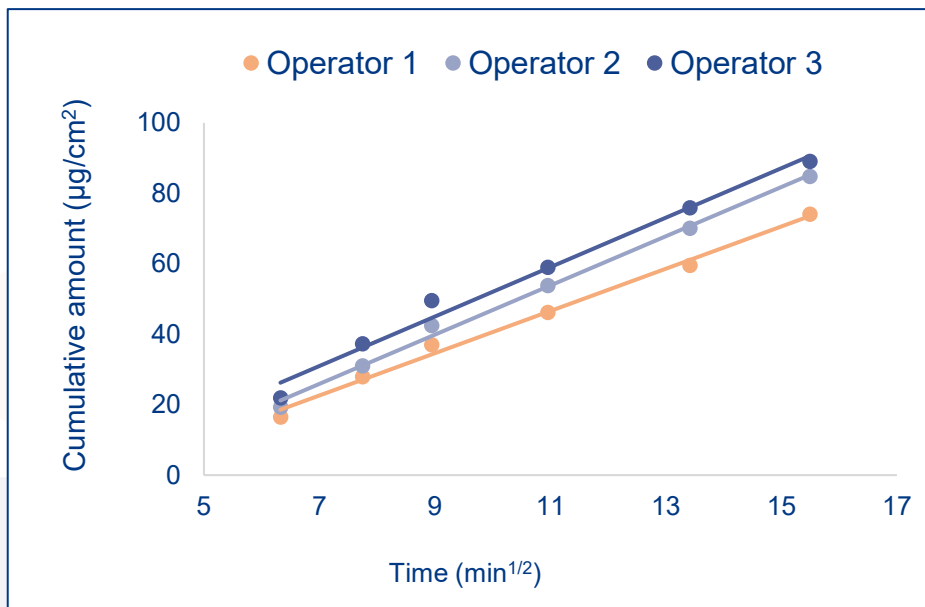
- Demonstrates that the chromatographic analytical method is suitable for quantifying the molecule in the selected receptor fluid

Parameter	Acceptance Criteria
Conformity test (SST)	
System repeatability	%RSD $\leq$ 2.00% for six replicate injections (n=6) of the reference solution (REF) in each run
Tailing factor	$\leq$ 2.00 for the first SST injection in each run
Specificity (n=1 on 1 run)	
Matrix interference	Peak area in blank and placebo RFs $\leq$ 5.00% of the peak area of the LLOQ chromatogram
Linearity : LLOQ to ULOQ n=1 on 3 runs	
Linearity	Deviation $\pm$ 10.00% at the LLOQ and $\pm$ 5.00% at all other concentration levels
Correlation coefficient	$R^2 \geq$ 0.99
Instrument response-to-concentration ratio for each level	%RSD $\leq$ 5.00%
Carry-over (1 run)	Signal $\leq$ 5.00% of the LLOQ
LLOQ prepared n=6 on 1 run	
LLOQ Validation	S/N $>$ 10 Deviation $\pm$ 10.00% %RSD $\leq$ 10.00% (n=6)
Accuracy and Precision : 3 concentrations level tested	
Accuracy	Deviation $\pm$ 5.00% at each level (n=6)
Precision	%RSD $\leq$ 5.00% at each level (n=6)
Intermediate Precision	%RSD $\leq$ 5.00% on calibration curve slopes
Robustness	
Robustness (injections n=6 at 0.5 ULOQ and 1 calibration curve using a different batch of the chromatographic column).	Deviation $\pm$ 5.00% and %RSD $\leq$ 5.00% for 0.5 ULOQ QCs (n=6) For the calibration curve: Deviation $\pm$ 10.00% for LLOQ and $\pm$ 5.00% for other levels $R^2 \geq$ 0.99 %RSD of instrument response-to-concentration ratios $\leq$ 5.0%
Stability	
QC Stability	Mean deviation $\pm$ 5.00% at each concentration level
stability in the conditions of IVRT experimentation (4h-32°C)	
Reinjection reproducibility	
Long term stability	

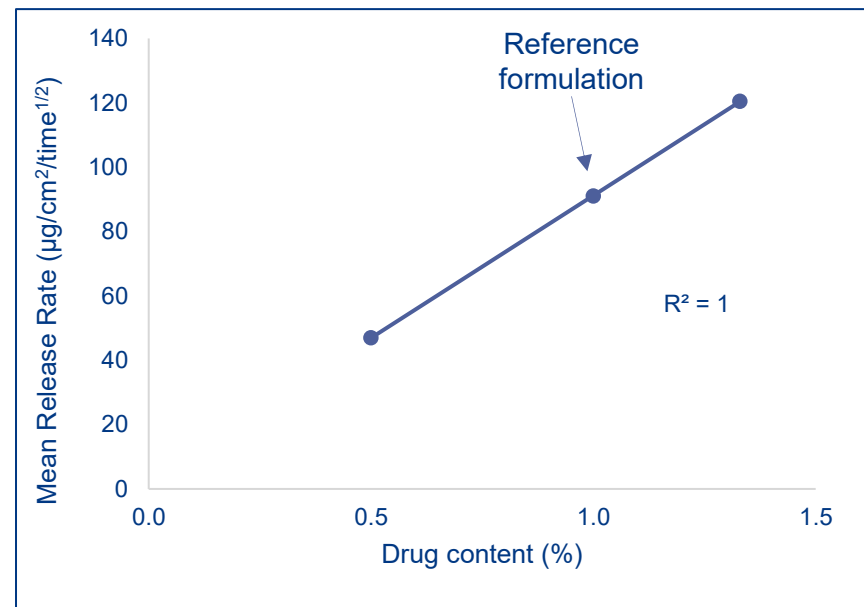
IVRT: the 4-steps approach

Step 3: IVRT method validation

Inter operators repeatability (12 cells each)



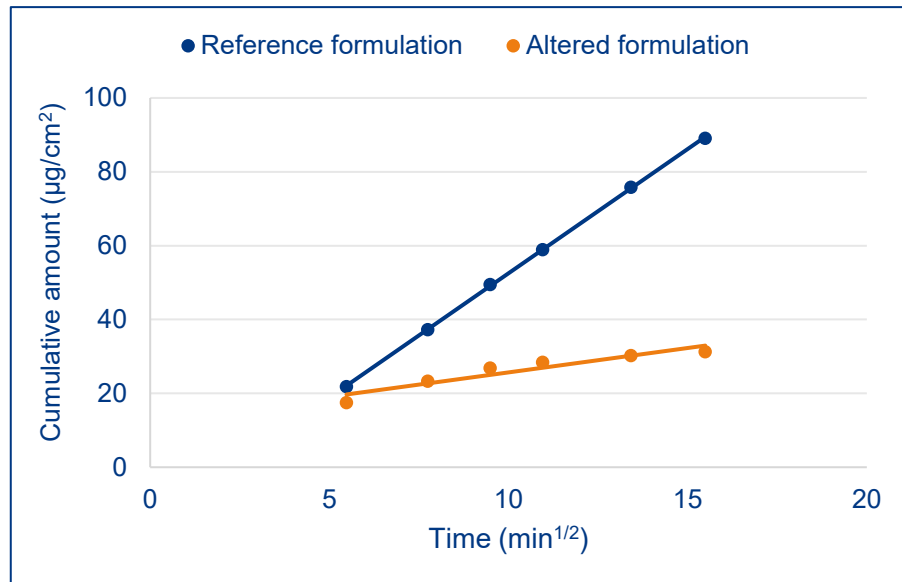
Selectivity/ Specificity (3 formulations, 12 cells each)



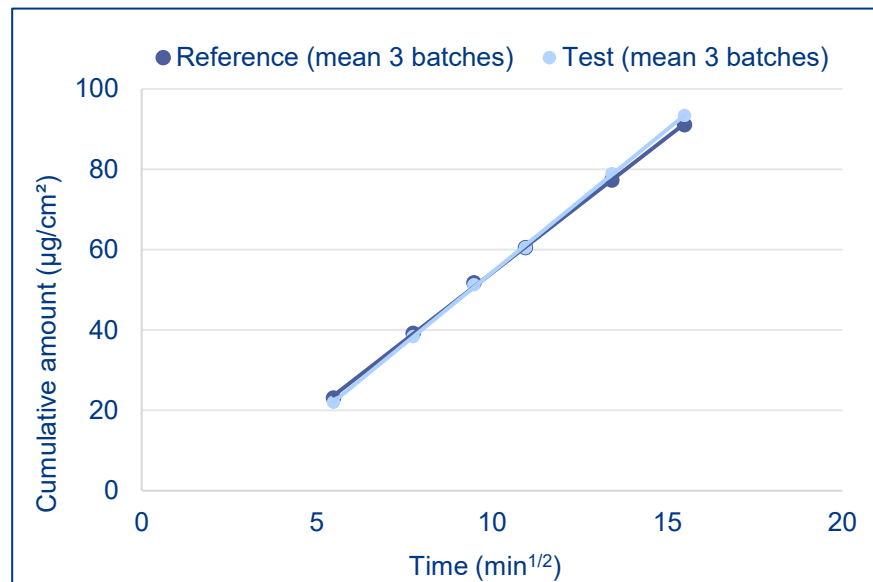
- Demonstrate the relevance of selected IVRT controlled conditions

IVRT: the 4-steps approach

Step 3: Discrimination



Step 4: IVRT Equivalence – 36 cells per formulation



- Formulation discrimination capability and equivalence

IVRT case study

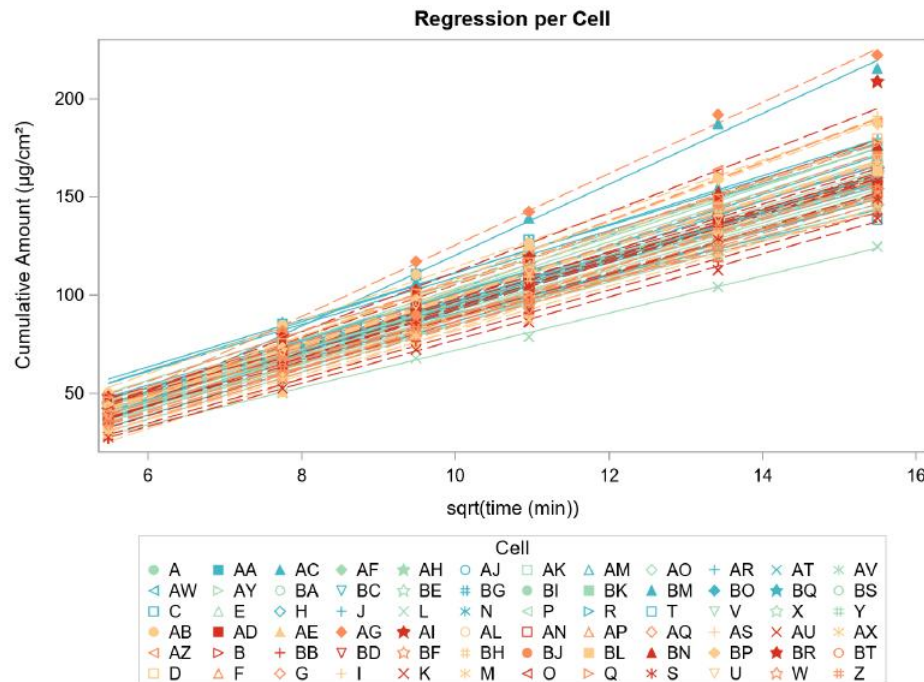
System	Phoenix Hanson
Diffusion cells	Medium size, 15.1 mL with 30 mm-height mixer
Membrane	PTFE, 0.45µm pore size, 25 mm diameter
Receptor fluid	60% ethanol/40% water, degassed
Temperature	32°C
Stirring	400 rpm
Sampling times	30, 60, 90, 120, 180, 240 minutes
Volume	350 µL
Analysis	LC-UV



3 batches of Reference and Test formulations



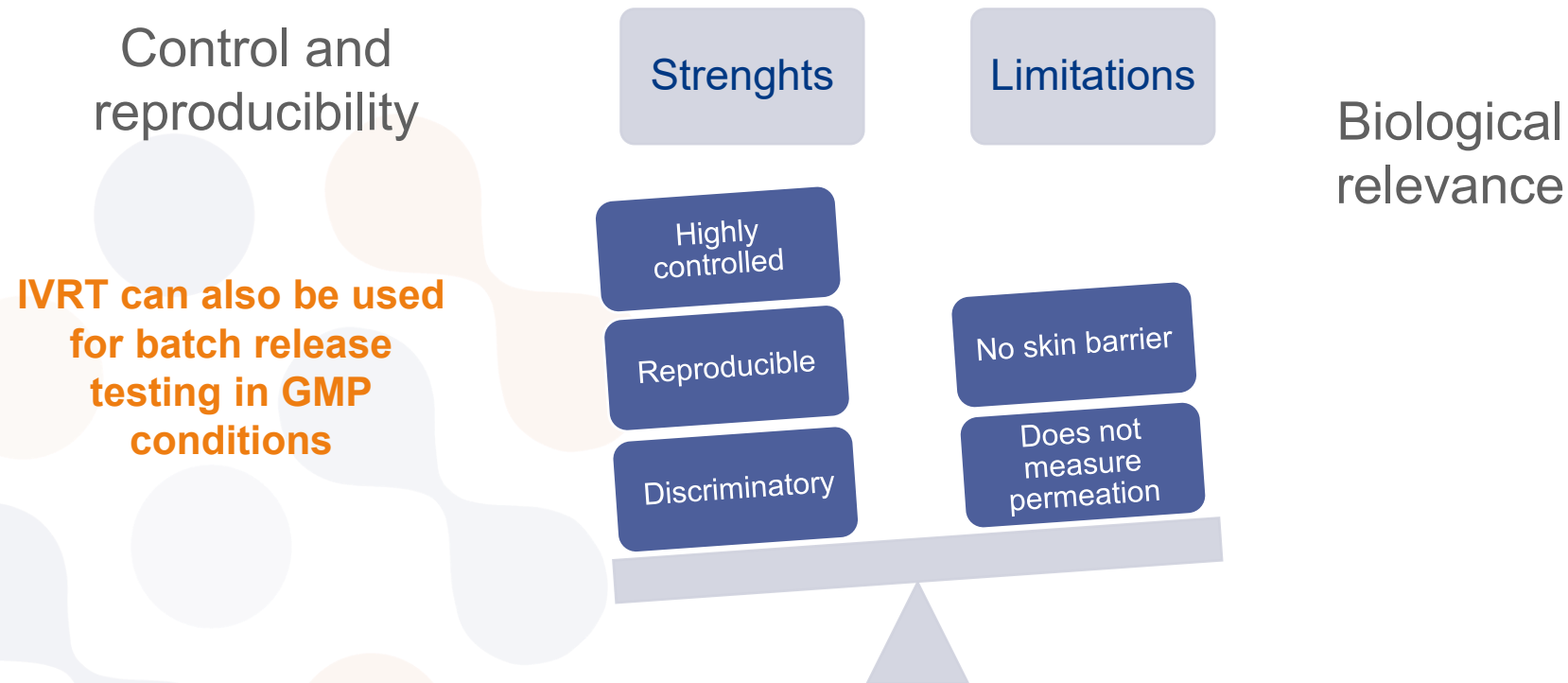
IVRT case study results: statistical analysis to demonstrate equivalence



- 3 batches per Test and Reference formulation
- Batch randomization
- 90% confidence interval calculated for:
 - Drug release rate (slope)
 - End-of-study cumulative amount
- Acceptance range: 90.0% -111.1%
- Acceptance limits may be adapted according to reference product variability

Parameter	Within-batch CV(%) of Reference	Gmean ratio estimate (%)	90% CI lower bound (%)	90% CI upper bound (%)	Conclusion
Slope	10.67	105.26	97.52	113.62	Equivalent
Cumulative amount at 240min	8.64	102.36	95.19	110.07	Equivalent

IVRT: strengths and limitations



IVPT: closer to biological performance, but more complex

Advantages

- Human skin
- Physiological relevance
- Real formulation behavior

More biological relevance

Challenges

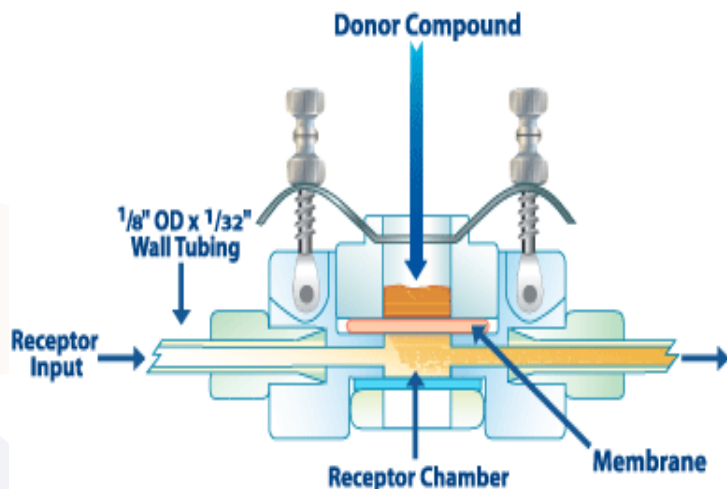
- Skin variability
- Donor variability
- Experimental complexity

More variability



IVPT: assessing drug permeation

Topical product in **finite non-occlusive** condition

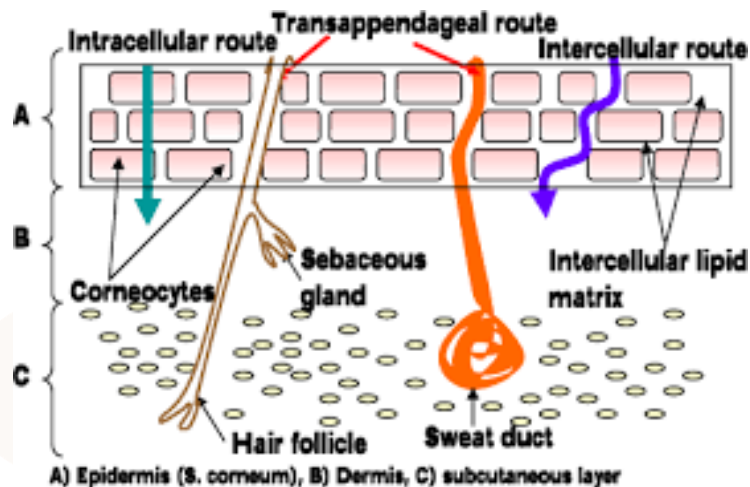


Diffusion cell

Parameters:

- **Cumulative drug permeation in the receptor fluid** at the end of experiment
- **Maximal flux:** speed of drug permeation into the receptor fluid

Automatic collection of receptor fluid during at least 24 hours after application



Human skin barrier complexity and penetration routes

Objective: Reduce inter-donor and inter-cells Variability

- Human skin, from surgery
- Single gender donors
- Restricted donor age range
- Healthy skin samples only
- Single location: abdominal
- Skin size > 200 cm² to perform at least 3-4 cells per donor for each formulation
- Limited storage time before use
- Single freeze-thaw cycle
- No scars, stretch marks or structural defects
- Standardized skin thickness (300-400 µm) following dermatoming
- Barrier integrity assessed for each diffusion cell using TEWL measurement
- Skin surface temperature maintained at 32°C +/- 1°C during the experiment

- **Receptor fluid selection:**
 - Maintain the barrier integrity, physiological pH, no organic solvent
 - No interference with the analytical method
 - Solubility of the drug should be demonstrated
- **Application of formulations**
 - Uniform spreading on the skin surface
 - Accurate and reproducible dosing (double weighing of the applicator, trained operators)
- **Mass balance assessment (requested only by EMA)**
 - Target recovery: $100 \pm 10\%$ of the applied dose
 - Assessed for each diffusion cell
 - Confirms data validity

IVPT: the 4-steps approach

Step 1

Define experimental conditions:

- Amount of formulation applied
- Receptor fluid composition
- Samples collection times
- Mass-balance assessment

Step 2

Validate the analytical method for samples analysis:

- LC-MSMS
- Chromatographic conditions
- ICH M10
- Accuracy & Precision
- Storage stability

Step 3

Perform Pilot IVPT:

- Several donors
- Several replicates
- Sample size determination
- Discriminative power of a Control topical product
- Selection of batches

Several pilot studies may be needed to refine experimental conditions for the pivotal study

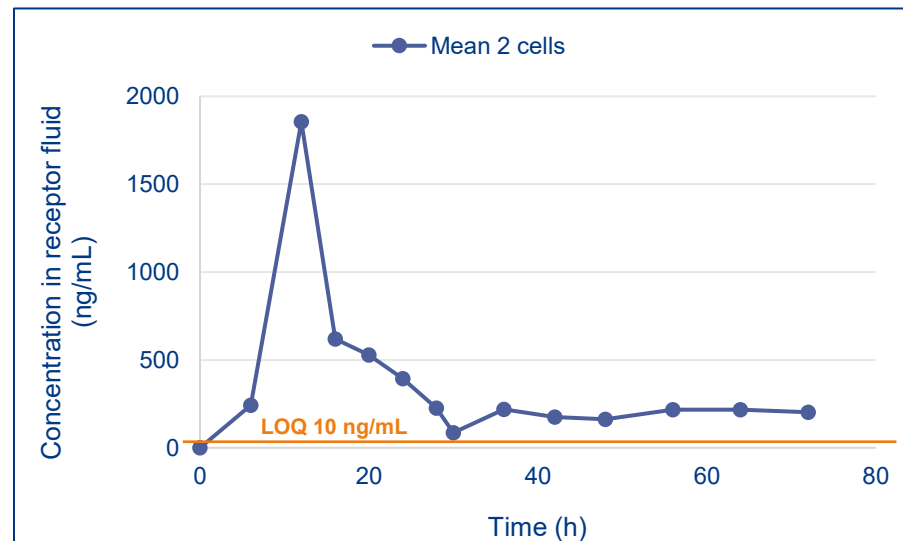
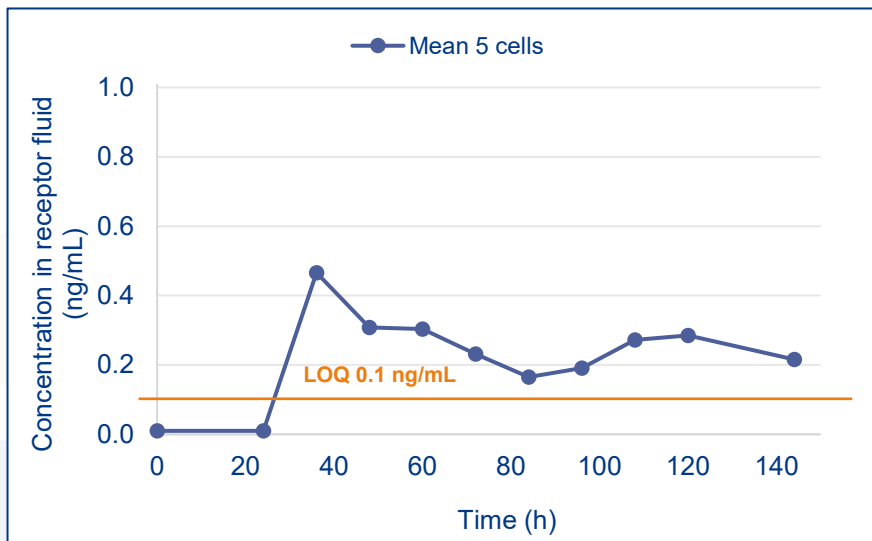
Step 4

Perform Pivotal IVPT:

- At least 12 donors
- At least 2 cells per donor and per formulation
- 1 batch of each Test, Reference (and Control for EMA) formulations
- Statistical analysis

IVPT: the 4-steps approach

Step 1: Is drug permeation sufficient for IVPT assessment ? First evaluation with Reference formulation.

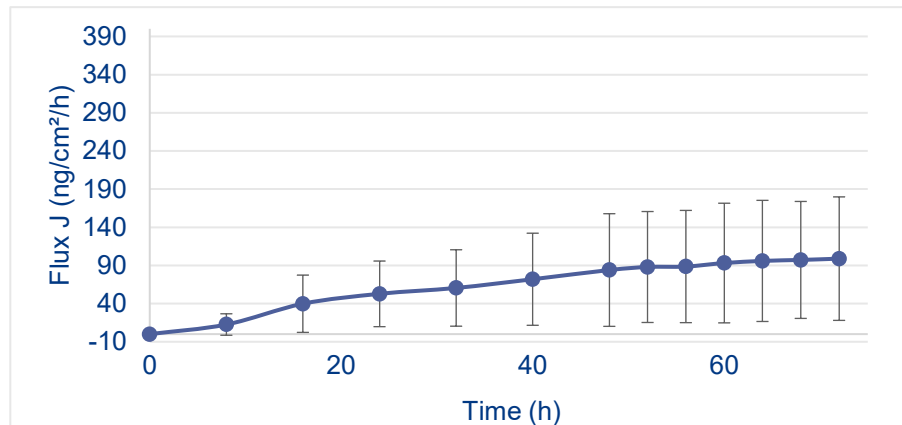
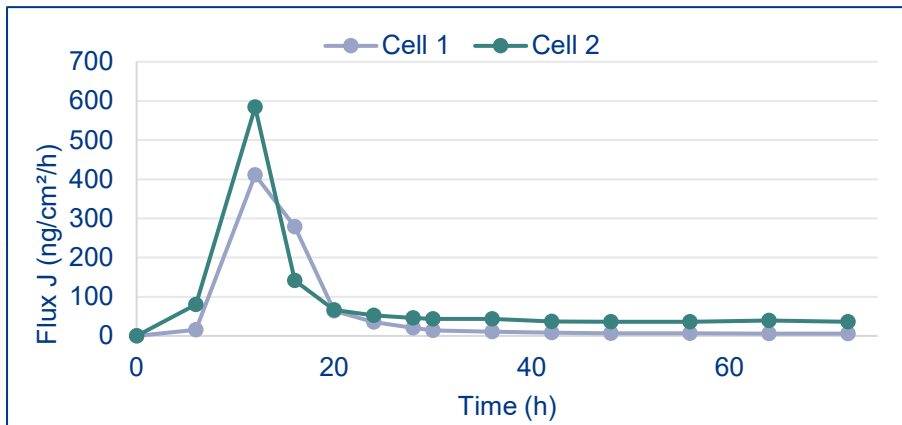


- Insufficient drug concentrations in receptor fluid

- Drug concentrations in receptor fluid OK

IVPT: the 4-steps approach

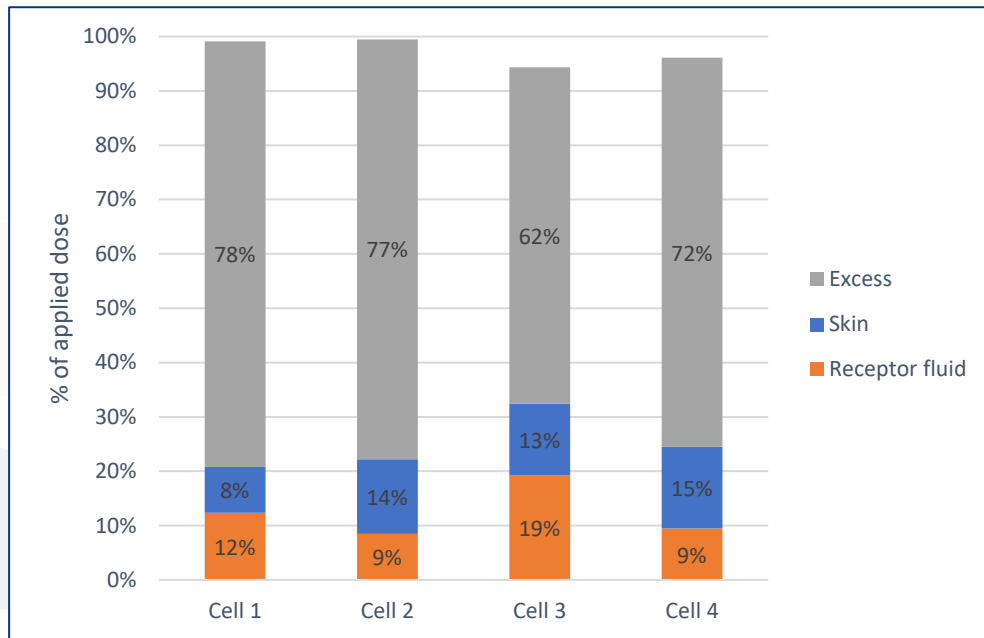
Step 1: Experimental conditions : maximal flux



- Flux profiles may exhibit a peak followed by a decline, or no peak at all, depending on the drug and formulation.
 - Both situations are accepted by the EMA.
 - The kinetic flux profile guides the selection of the experimental duration.

IVPT: the 4-steps approach

Step 1: Mass-balance assessment



Mass balance between 90% and 100% of the applied dose

Required by EMA

Not required by FDA

Step 2: ICH M10

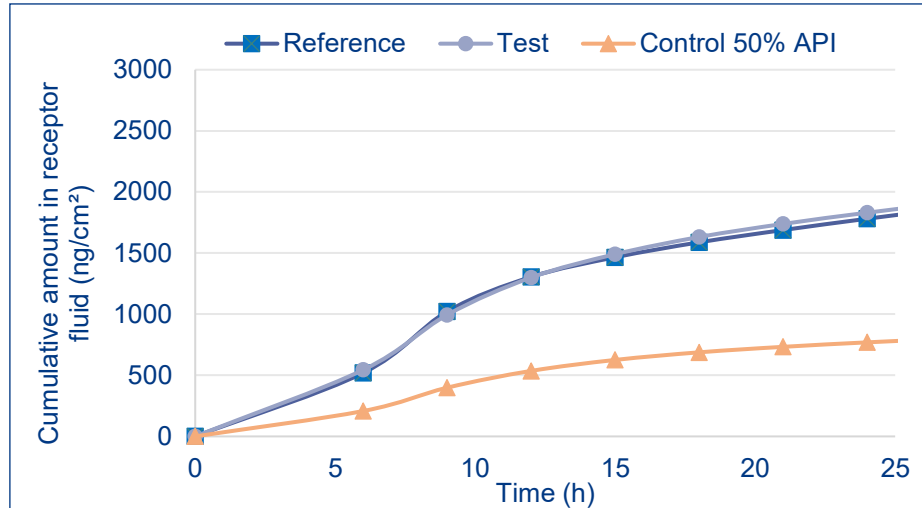
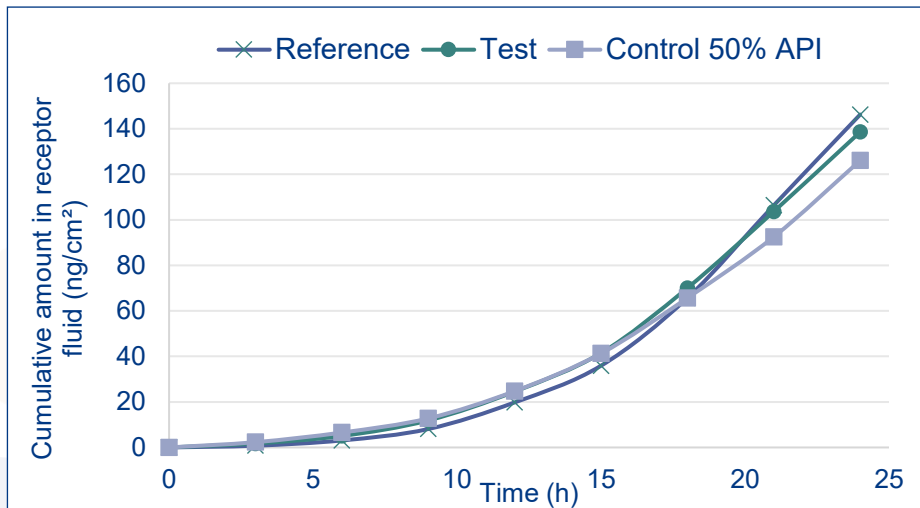
LC-MS/MS Bioanalytical method validation:

- Highly sensitive quantification with low **ng/mL or pg/mL** LOQs
- Full validation across all relevant IVPT matrices: receptor fluid, skin, and wipe-off samples for mass-balance assessment (EMA requirement)
- Assessment of critical validation parameters: linearity, accuracy, precision, specificity, and analyte stability



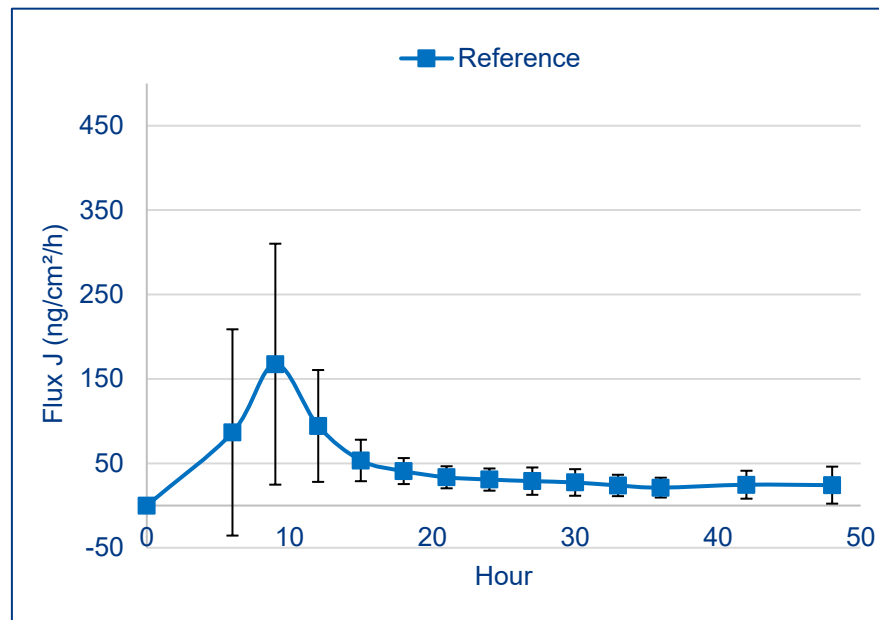
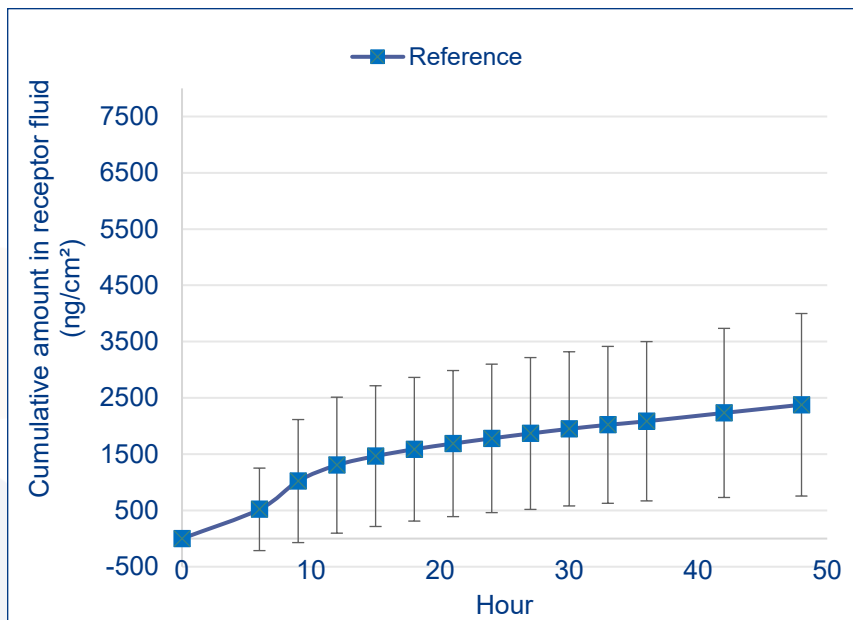
IVPT: the 4-steps approach

Step 3: Pilot study



- A **Control** formulation is essential to demonstrate the discriminatory capability of the IVPT method.
- Low permeation levels make formulation differences more challenging to distinguish.

Step 3: Pilot study – sample size calculation based on Reference group variability for Cumulative amount and Flux



- Low variability enables a reduced number of donors for the pivotal study

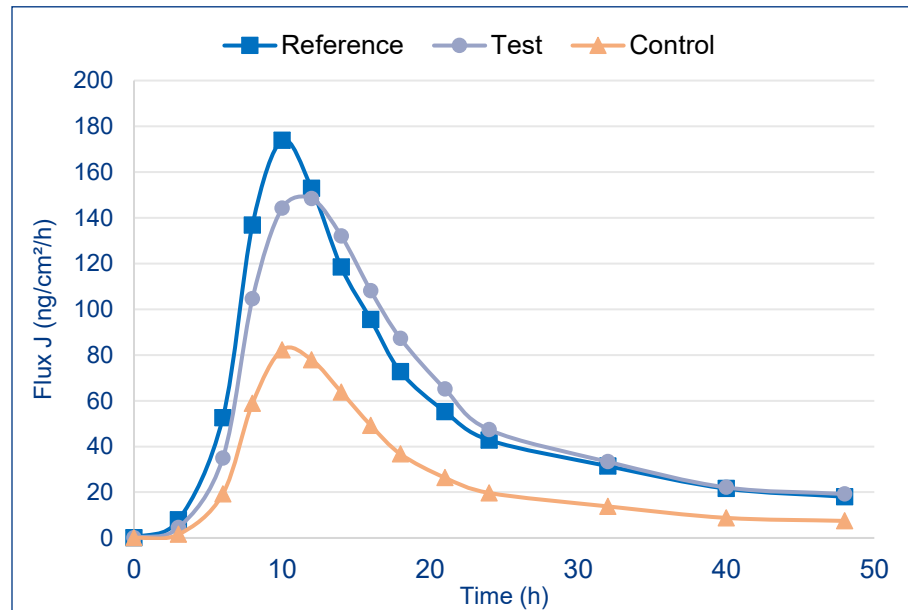
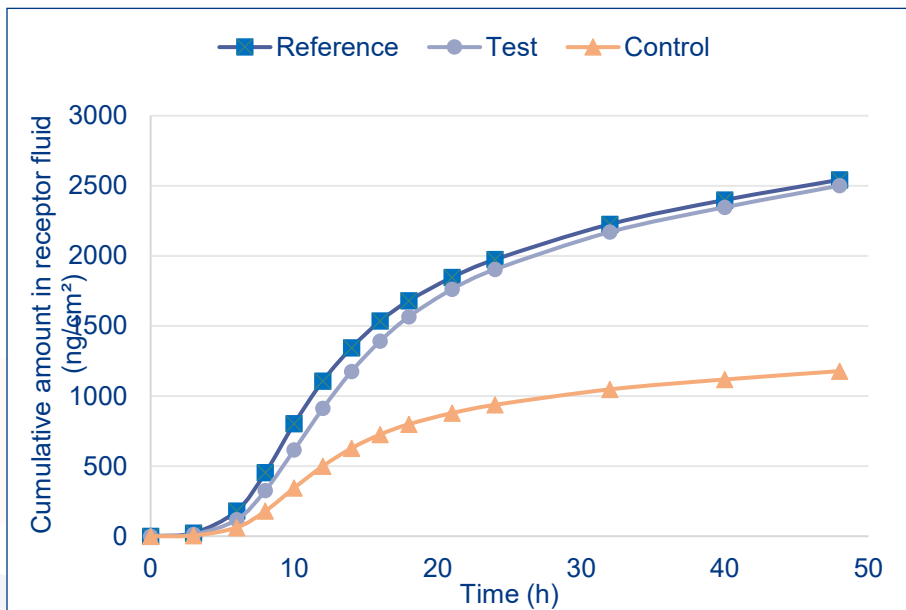
Step 4: Pivotal study

- 2-3 formulations: Reference, Test (and Control for EMA)
- At least 12 donors (more often 15 to 20, depending on reference formulation variability)
- At least 2 cells (3 to 4 preferably) per donor and per formulation



IVPT case study

System	Flow through Bronaugh cells - Application area 1 cm ²
Formulations	1 batch Reference / 1 batch Test / 1 batch Control
Amount applied	10 mg
Donors/cells	18 donors / 2 cells per donor per formulation = 108 cells applied
Skin	Dermatomed 300-400 µm
Temperature/TEWL	32°C +/- 1°C / ≤ 10 g/m ² /h
Receptor fluid	PBS + 0.1% Brij + 0.1% Sodium azide
Receptor fluid flow	1.5 mL/h
Sampling times	0h, 3h, 6h, 8h, 10h, 12h, 14h, 16h, 18h, 21h, 24h, 32h, 40h, and 48h = 1 512 samples analysed
Analysis	LC-MSMS



- **Equivalence statistically demonstrated** for both parameters: cumulative amount and maximal flux
- Control formulation statistically different: **discriminatory power demonstrated**

- **IVPT** provides a **biological relevant assessment** by integrating both formulation properties and the skin barrier
- Donor selection, skin qualification and robust analytical method are essential to **control variability**
- **Pilot studies** play a critical role in optimizing experimental conditions and demonstrating **discriminatory power** before moving forward to pivotal study

Although more complex and variable than IVRT,

IVPT is a relevant and reliable tool to support topical product equivalence

- **IVRT and IVPT answer different scientific questions:**
 - **IVRT** evaluates how the **drug is released from the formulation**
 - **IVPT** evaluates how the **drug permeates through the skin**
- **Methodological rigor is a key to confidence:**
 - Analytical validation, controlled experimental conditions are critical for generating reliable data
 - The quality of the conclusion depends directly on the quality of the study design

IVRT and IVPT together provide complementary information required for topical product equivalence assesement



Founded in 1987
Operations at a single site in the
South of France

In Eurofins Scientific group
since 2005

Specialized in Bioanalysis of Small
and Large Molecules

+20
years

of expertise in
topical products



Dermal &
transdermal
penetration studies
since 2004



Research and
regulatory support for
GLP-compliant
studies



IVRT and IVPT
expertise developed
since 2019



100
persons



2000 m²
facilities



Thank you for your attention

Eurofins ADME BIOANALYSES

75 A, Avenue de Pascalet

France - 30310 Vergèze

Tel +33 (0)4 66 73 17 73

www.eurofinsadmebioanalyses.com

Supporting DMPK from screening to registration