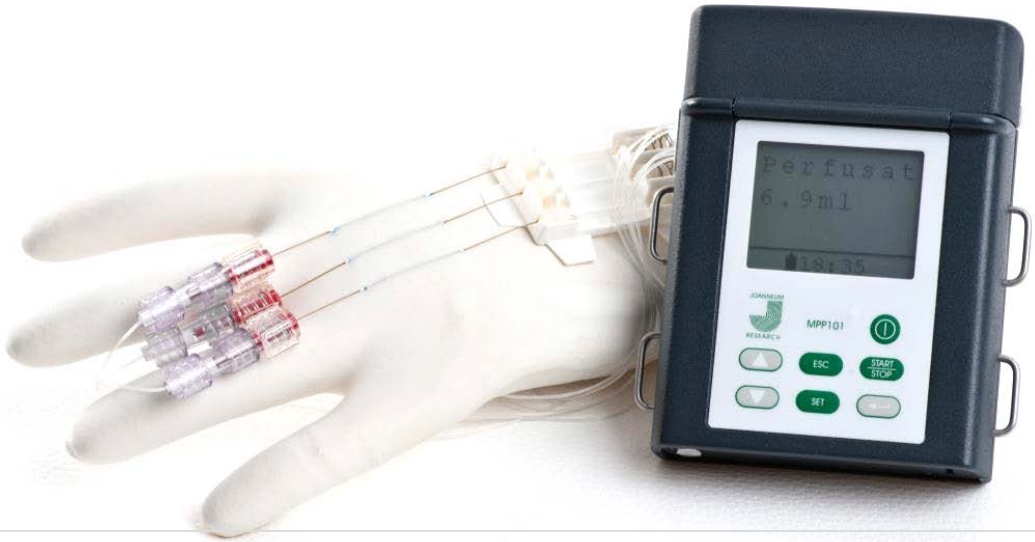
In Vivo Dermal Open Flow Microperfusion: *A Novel Approach to Evaluating Topical Bioavailability and Bioequivalence*



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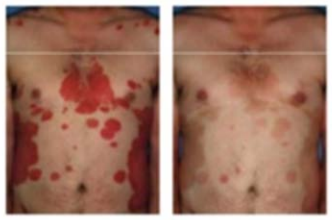
Dermal Open Flow Microperfusion
Vision

FDA approval for topical generic drugs - with some exceptions – requires a

Comparative Clinical Endpoint Bioequivalence Study

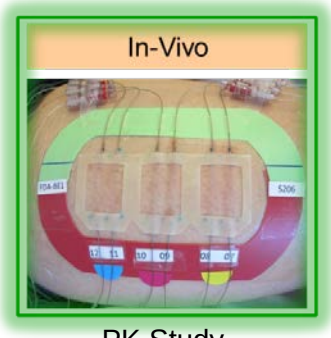
Vision: Using dOFM for PK-based Bioequivalence Studies

In-Vivo



Endpoint Study

Patients
Hundreds to thousands
Several month to years



PK Study

Healthy subjects
20 - 40
Few weeks

Skin PK-based BE approaches

Strengths

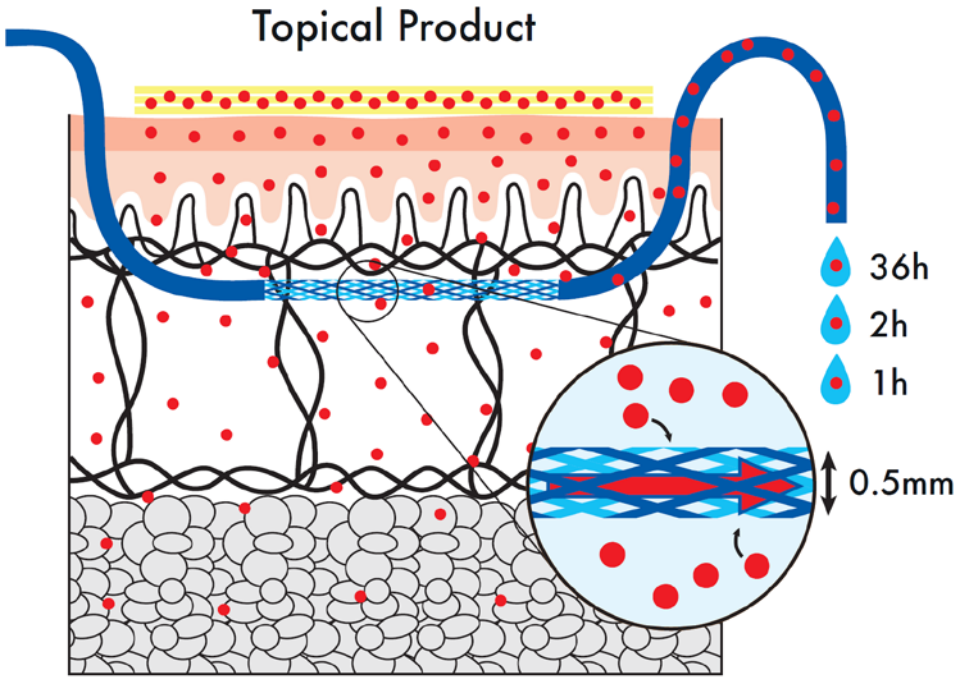
1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the skin.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles by their magnitude.

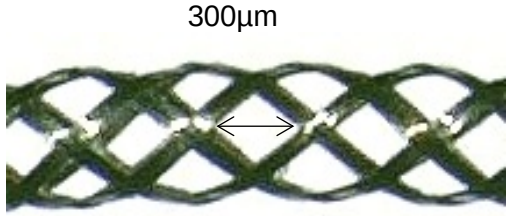
Challenges

1. Existing sampling methods have limitations.
2. Limited sampling time, often < 8 hours.
3. High variability of skin PK data.

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **OFM samples represent diluted but unfiltered interstitial fluid**

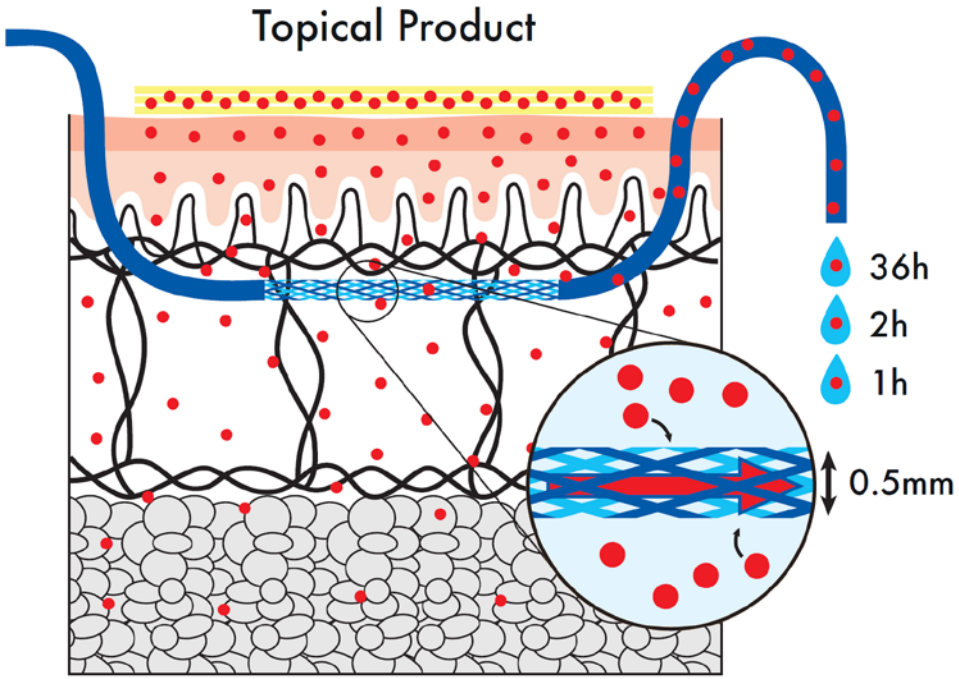




CE-certified for clinical use

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **All drugs are accessible in-vivo in the dermis**



lipophilic substances

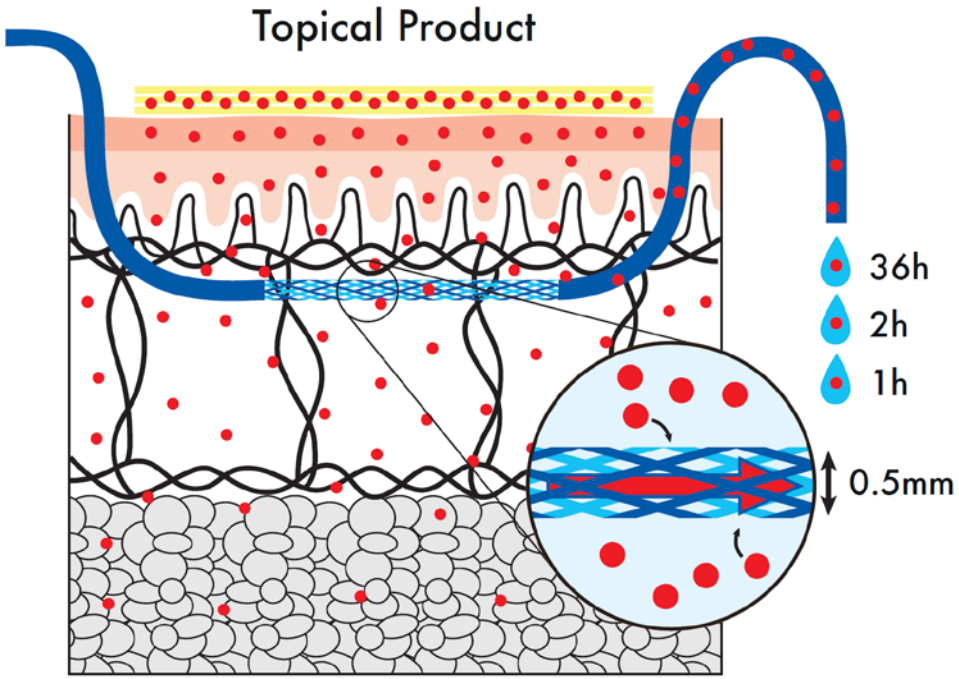
Bodenlenz et al. 2016 (CP-17; logP 3.5)
Holmgaard et al. 2011 (Fentanyl; logP 4.5)

high molecular weight substances (up to cells)

Dragatin et al. 2016
(Quantification of antibodies in skin)
Kolbinger et al. 2016
(Cytokines in the skin in healthy & patients)

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **dOFM shows dose dependent dermal AUC profiles**



Clinical dOFM studies in skin:

Corticoid (topical) – 26 h clinical
Antibody (SC) – 17 h clinical

Skin PK-based BE approaches using dOFM

Strengths

1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the skin.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles by their magnitude.

Challenges

1. Limitations of existing sampling methods

   → no limitation as dOFM samples diluted ISF

2. Limited sampling time, often < 8 hours

   → no limitation as dOFM samples up to 48 hours

3. High variability of skin PK data

   → optimization of dOFM during the project

Clinical Bioavailability
Overall Approach

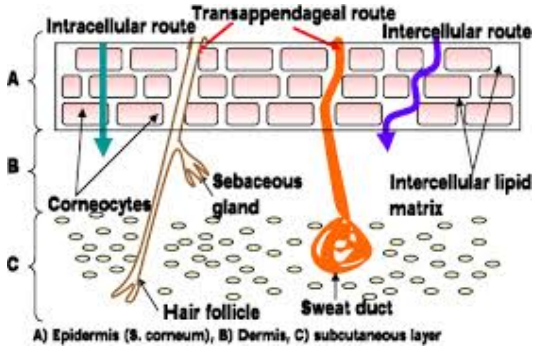
Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

- Head-to-head comparison within one subject to minimize inter-subject effect on BE.

- Use application-triplets with
  - two separate application sites for reference product → for BE
  - one application site for a non-Q1 product → for non-BE
- Healthy subjects with intact skin integrity for best discrimination of formulations.
- Use a drug for which skin PK was never successfully monitored in healthy subjects.

dOFM
Controlled or Monitored Parameters

✓ **Controlling all significantly contributing factors which add data variability - or at least monitoring them.**

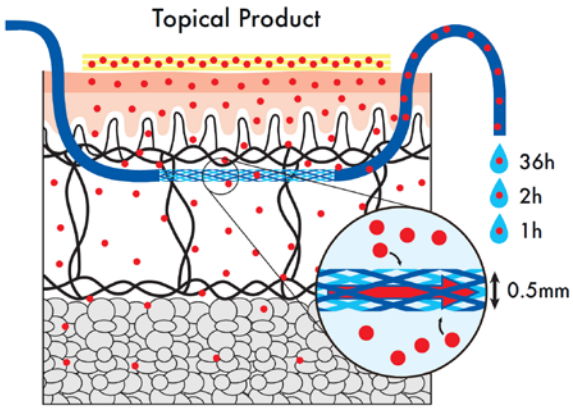


Variations may result from differences in

Hairiness	➔ not controlled
Hair shaving	➔ **subjects are shaved 5 days before dOFM visit**
Sweat duct	➔ not controlled
Skin barrier (stratum corneum) properties	➔ **monitored by TEWL and Impedance**
Skin care products use	➔ **not allowed 5 days before dOFM visit**
Skin condition (e.g. Solarium)	➔ **visual check at screening visit**
Room temperature and humidity	➔ **controlled at 22 ± 1° C ; 40 - 60% rel. humidity**

dOFM
Controlled or Monitored Parameters

✓ **Controlling all significantly contributing factors which add data variability - or at least monitoring them.**



Variations may result from differences in

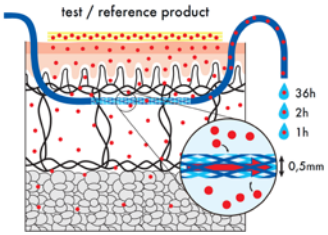
Trauma formation
Application site
Dosage application
Probe depth
Flow rate
Local blood flow
Lateral diffusion and cross-talk
Systemic absorption and cross-talk

Universal Parameters

Drug Dependent Parameters

dOFM
Trauma formation

✓ **Minimized trauma formation by cooling.**



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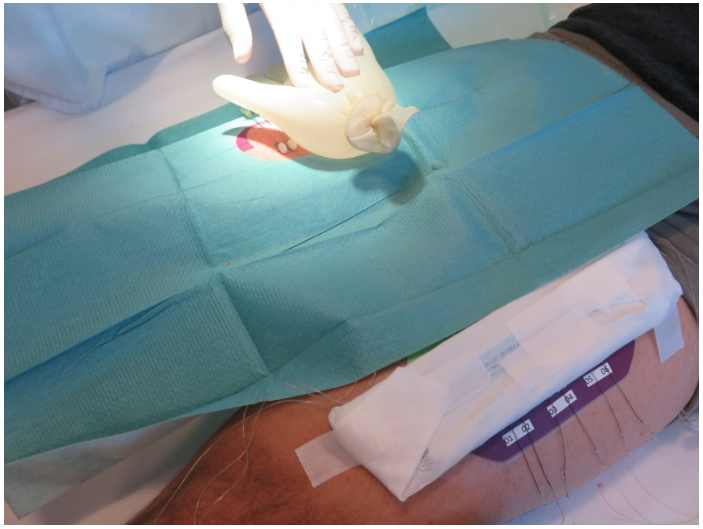
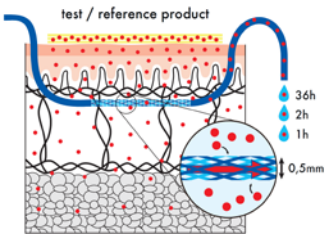
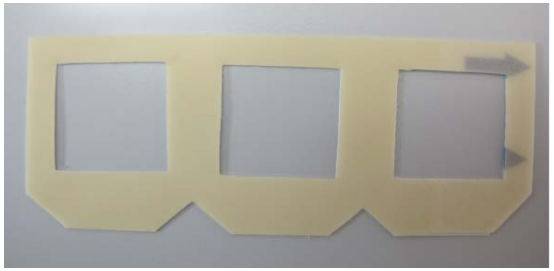


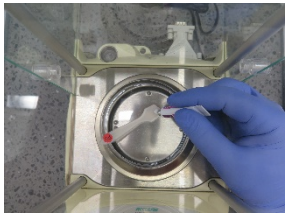
Standardized by cooling after dOFM insertion

dOFM
Drug application

✓ **Homogeneous drug application by using an application template.**







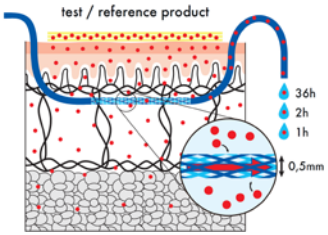
Variations may result from differences in

Trauma formation
Application site
Dosage application
Probe depth
Flow rate
Local blood flow
Lateral diffusion and cross-talk
Systemic absorption and cross-talk

Standardized by use of application template
and
Standardization of application

dOFM
Probe depth

✓ **dOFM probe depth measurement for each probe.**



Variations may result from differences in

Trauma formation
Application site
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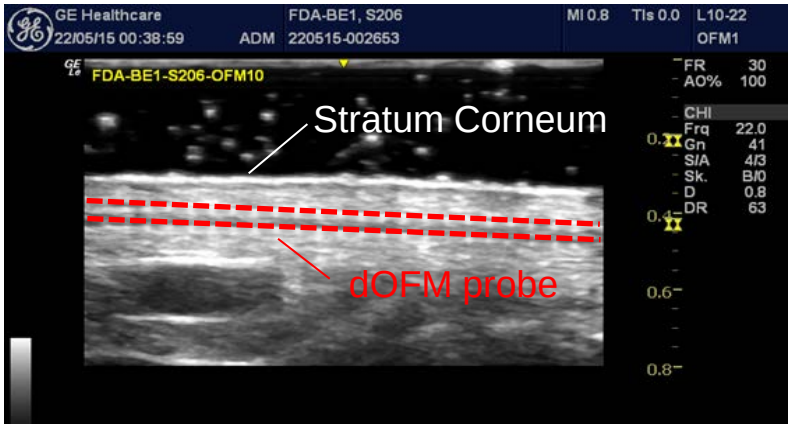
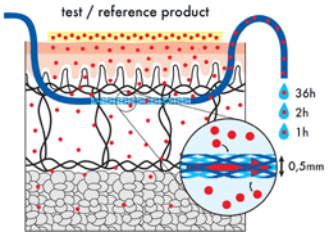


Depth of exchange area measured by ultrasound

dOFM
Flow rate

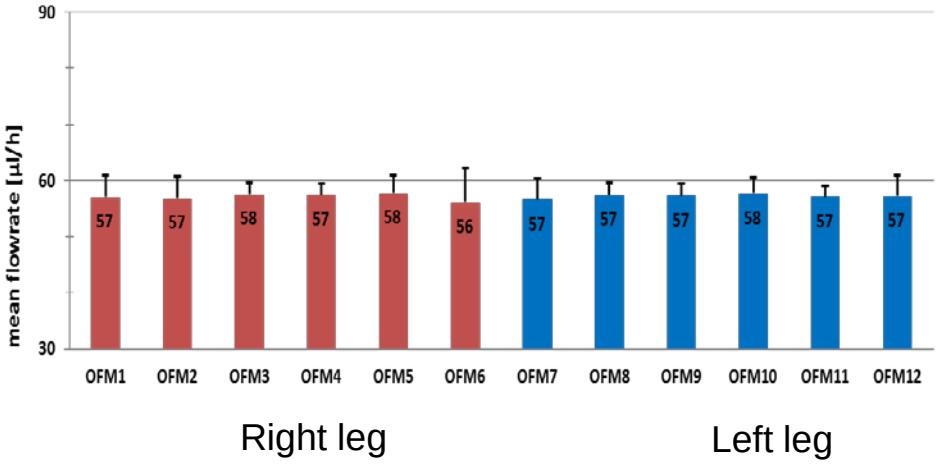
✓ **Stable flow rate of dOFM probes over 36 hours.**



Variations may result from differences in

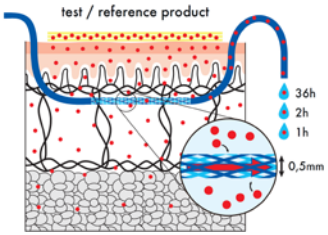
Trauma formation
Application site
Dosage application
Probe depth
Flow rate
Local blood flow
Lateral diffusion and cross-talk
Systemic absorption and cross-talk

Flow rates of all probes in one subject



dOFM
Local blood flow

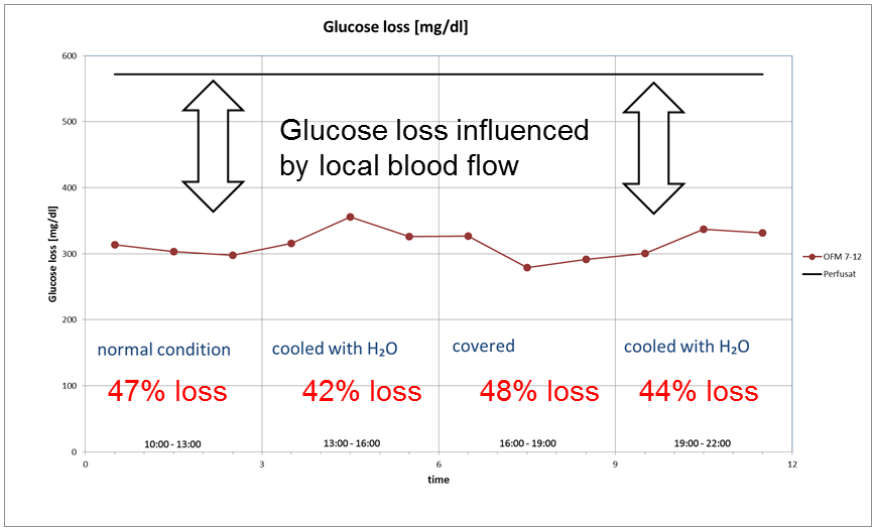
✓ **Monitoring local blood flow by internal standard in OFM perfusate.**



Variations may result from differences in

Trauma formation
Application site
Dosage application
Probe depth
Flow rate
Local blood flow
Lateral diffusion and cross-talk
Systemic absorption and cross-talk

Local blood flow monitoring by loss of glucose from dOFM perfusate

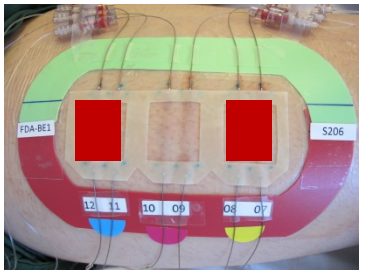


dOFM
Lateral diffusion and cross-talk

✓ **Lateral diffusion for acyclovir is negligible.**

Lateral Diffusion between adjacent application sites

- $R = \frac{|\#dOFM\ Samples\ BLANC\ SITES>LLOD|}{|\#dOFM\ Samples\ US\ ZOVIRAX\ SITES>LLOD|}$
- Definition: no lateral diffusion if $R < 0.05$



US Zovirax
Very high dose
of 50 mg/cm²

Methodology

- results from all 6 subjects of phase 1
- 10.000 bootstrap estimates were computed
- creation of confidence interval for the true population value of the test statistic R
- a one-sided 95% confidence interval was constructed

Results

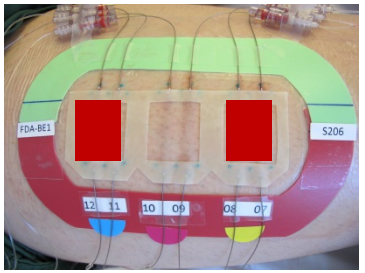
MIN	MEDIAN	P90	P95	P99	MAX
.007633588	0.076336	0.10853	0.11831	0.13492	0.18248

dOFM
Systemic absorption and cross-talk

✓ **No systemic exposure and thus no influence on PK of dOFM site.**

Test for Systemic Exposure

- $R = \frac{|\#Blood\ Samples > LLOD|}{|\#Total\ Blood\ Samples\ |}$
- Definition: no systemic exposure if $R < 0.05$



US Zovirax
Very high dose
of 50 mg/cm²

Methodology

- 6 subjects, 6 application sites
- 10.000 bootstrap estimates were computed
- creation of confidence interval for the true population value of the test statistic R
- a one-sided 95% confidence interval was constructed

Results

MIN	MEDIAN	P90	P95	P99	MAX
0	0.012821	0.025641	0.038462	0.051282	0.064103

dOFM
Quality management systems

✓ **High quality standards are key to reliable skin PK studies.**

GCP

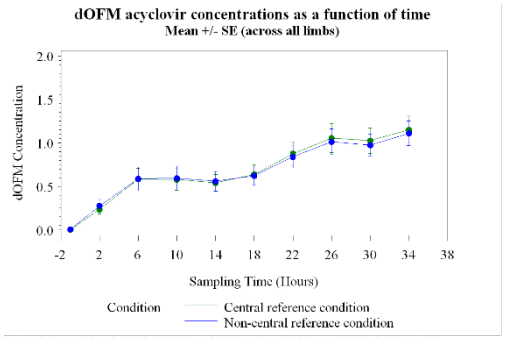
Data Management Plan

eCRFs
SDFs

XLSX → Processing Laboratory Source Data → Mapping Subject ID ↔ OID → CDISC ODM → Import into OpenClinica eCRF → OpenClinica Database

Software Verification and Validation Report
Software Verification and Validation Report
OFMLabData Import Validation Plan
OFMLabData Import Validation Report
OFMLabData Import SPOs

Statistical Analysis Plan

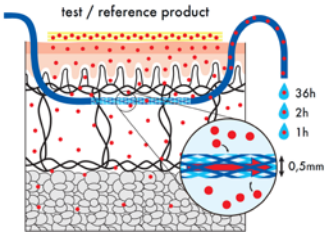


GLP lab

Method Validation Plan
Method validation Report
Method SOPs
Study Analysis Plan

dOFM
Controlled or Monitored Parameters

✓ **Highly controlled set-up has been developed.**



Variations may result from differences in

Trauma formation	➔	**Controlled by cooling**
Application site	➔	**Controlled by application template**
Dosage application	➔	**Controlled by standardization**
Probe depth	➔	**Monitored by ultrasound**
Flow rate	➔	**Monitored by sample weight**
Local blood flow	➔	**Monitored by glucose marker**
Lateral diffusion and cross-talk	➔	**Negligible**
Systemic absorption and cross-talk	➔	**No systemic exposure**

Comparative IVRT study
Investigated drugs

✓ **All 5% acyclovir creams inbestigated.**

- Reference product Zovirax cream 5% (GSK, U.S.) was compared against itself and six test products:
  - Zovirax cream 5% (GSK, Vienna, Austria)
  - Zovirax ointment 5% (GSK, U.S.)
  - Aciclostad 5% (STADA, Austria)
  - Aciclovir 1A Pharma Cream 5% (1A Pharma, Austria)
  - Antiviral cold Sore cream 5% (Boots, UK)
  - Zovirax cold Sore cream 5% (GlaxoSmithKline, Brentford, UK)

- Statistical method:
  Mann-Whitney U test according to
  USP general chapter <1724>

Comparative IVRT study
Apparatus qualification

✓ **IVRT apparatus qualification was passed successfully.**

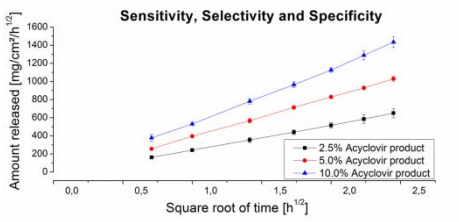
PARAMETER	ACCEPTANCE CRITERIA		RESULTS		
	Intercell Variability (Precision)	Accuracy	Range of variation V	Mean	Pass
Volume of the cells	V ≤0.48 mL[1)]	$\bar{x}_i \in [12 + 0.6\,mL, 12 - 0.6\,mL]$ $for\ 1 \leq i \leq 6$[4)]	0.33 mL	9.77 mL	No
Diameter of the orifice	V ≤0.45 mm[2)]	$\bar{x}_i \in [15 + 0.75\,mm, \quad 15 - 0.75\,mm]$ $for\ 1 \leq i \leq 6$[4)]	0.05 mm	15.01 mm	Yes
Temperature of the receptor medium	-	$\bar{x}_i \in [32 + 1\,°C, \quad 32 - 1\,°C]$ $for\ 1 \leq i \leq 6$	0.23 °C	31.98°C	Yes
Speed of the magnetic stirrer	V ≤ 12 rpm[3)]	$\bar{x}_i \in [600 + 60\,rpm, \quad 600 - 60\,rpm]$ $for\ 1 \leq i \leq 6$[5)]	1.77 rpm	597.98 rpm	Yes
Dispensed sampling volume	-	$\bar{x}_i \in [500 + 15\,\mu L, \quad 500 - 15\,\mu L]$ $for\ 1 \leq i \leq 6$[3)]	10.76 µL	492.40 µL	Yes

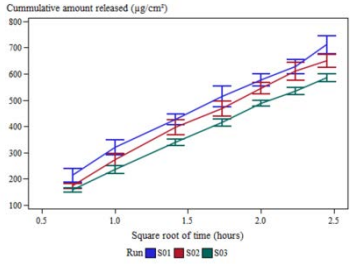
„A Comprehensive Approach to Qualify and Validate the Essential Parameters of an In Vitro Release Test (IVRT) Method for Acyclovir Cream, 5%“ – published online
International Journal of Pharmaceutics – OPEN ACCESS

Comparative IVRT study
IVRT method validation

✓ **IVRT method validation for acyclovir was passed successfully.**

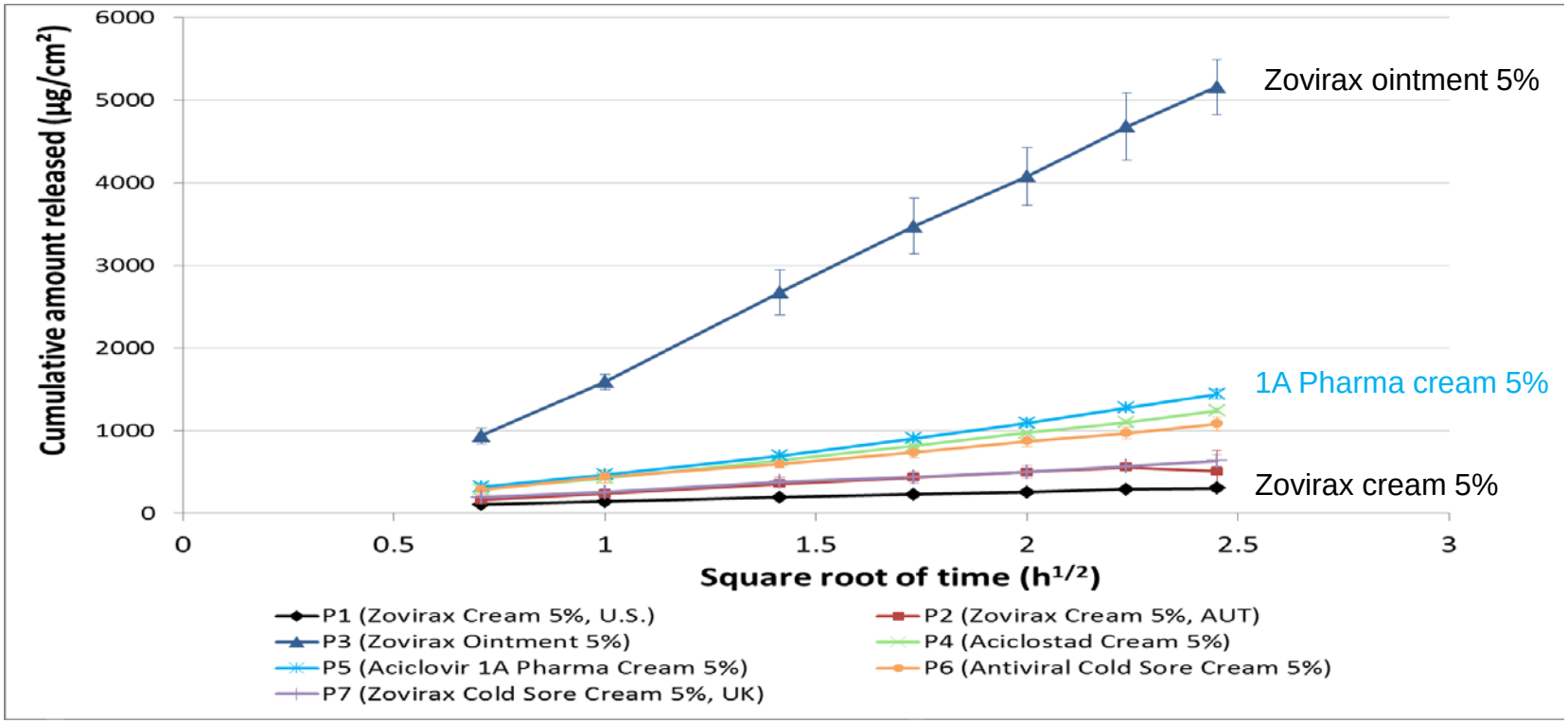
Parameter	Acceptance Criteria	Passed
Membrane Inertness	No acyclovir binding on the membrane: Recovery of 105.5%	✓
Receptor medium solubility	Solubility > 10 times higher than the maximum acyclovir concentration in the receptor medium observed during the IVRT study	✓
Linearity	Lowest R^2 : 0.97, no outlier	✓
Precision and Reproducibility	Inter-run variability 5.8%; intra-run variability 4.4%	✓
Sensitivity	Mean release rate increased with increasing acyclovir concentration	✓
Specificity	Linear regression model (release rate versus product concentration) R^2 =0.943	✓
Selectivity	IVRT method accurately identify in-equivalent and equivalent acyclovir products	✓
Robustness	Release rate for temperature and stirring speed variation deviate < 15%	✓
Recovery	< 10%; no excessive acyclovir depletion	✓





Comparative IVRT study
Results

✓ **IVRT identified different drug release rates.**



dOFM
Clinical Study Details

- ✓ **Test and Reference are both 5% acyclovir creams but NON-Q1**
- ✓ **IVRT: identical release R:R and non identical release T:R**

Zovirax (R) (USA)	Aciclovir-1A (Austria)
Water	Water
Propylene glycol	Propylene glycol
Mineral oil	Viscous Paraffin
White petrolatum	White Vaseline
Cetostearyl alcohol	Cetyl alcohol
SLS	Not disclosed
Poloxamer 407	Not disclosed
Not disclosed	Dimethicone
Not disclosed	Glyceryl Mono Stearate
Not disclosed	Polyoxyethylene stearate

Non-Q1

Equivalence comparison	Computed confidence interval	
	Lower Limit [%]	Upper Limit [%]
Zovirax cream 5% US v. Zovirax cream 5% US	85.7	103.02
Zovirax cream 5% US v. Aciclovir 1A Pharma Cream 5%	16.27	19.60

Acceptance limits: [75%, 133.33%]

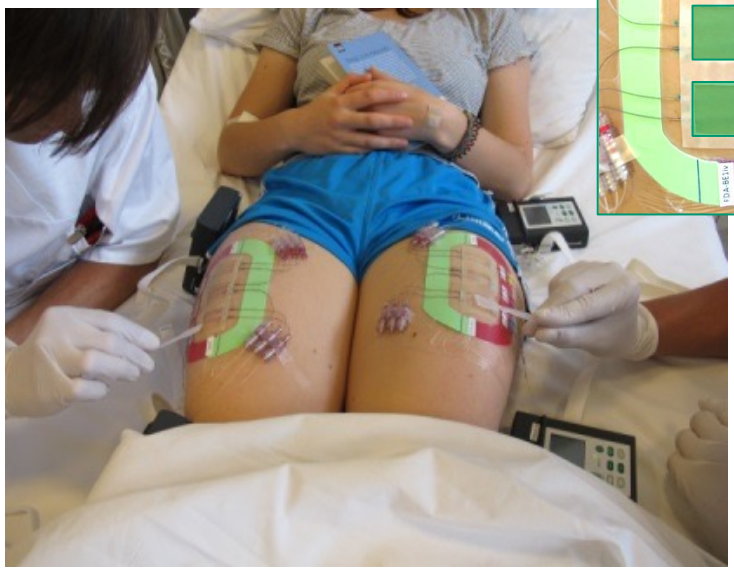
Clinical Bioavailability

Clinical BE Study

Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

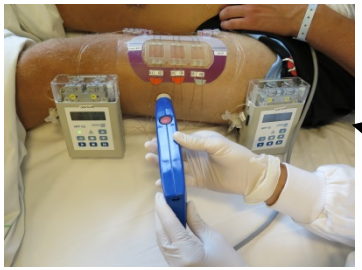
Overview Clinical Studies:

- 20 healthy subjects
- **R**eference: Zovirax® US
- **T**est: Aciclovir-1A Pharma Austria
- 2 application triplets per subject
- 15 mg/cm^2 cream application
- 36 hours dOFM sampling time

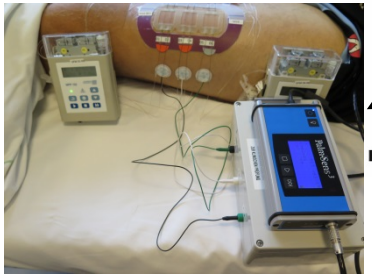


dOFM
Clinical Study Details

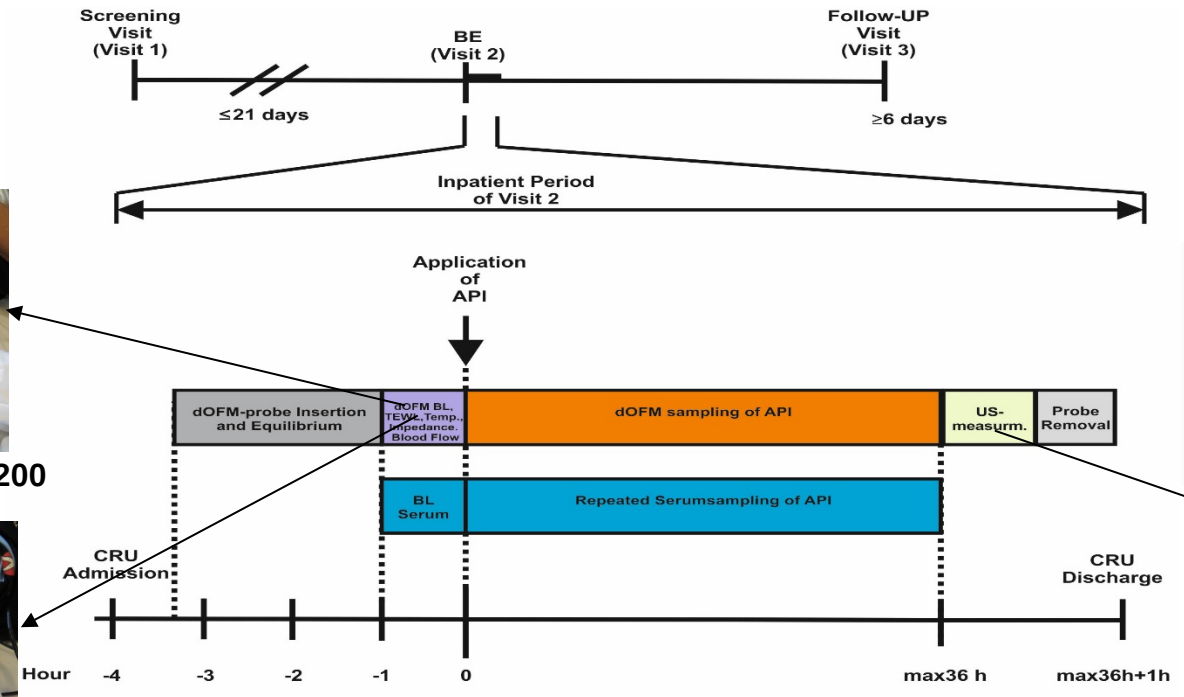
✓ **Highly standardized clinical BE study design.**



TEWL by Aquaflux AF200



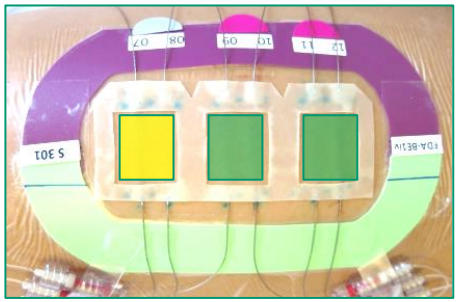
Impedance by JOANNEUM

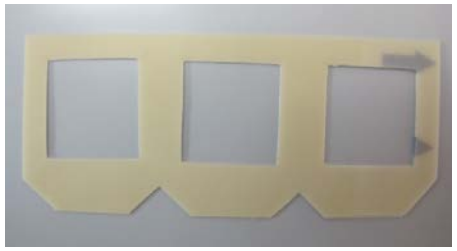


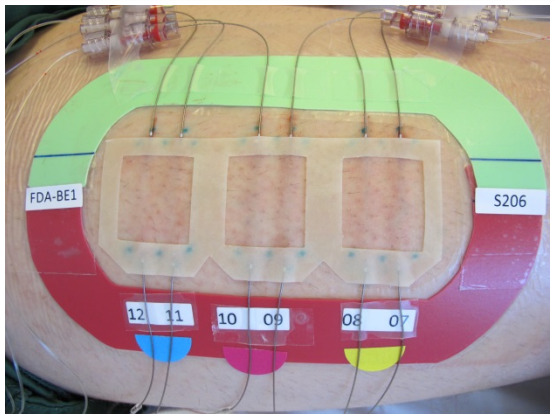
Ultrasound GE-Healthcare

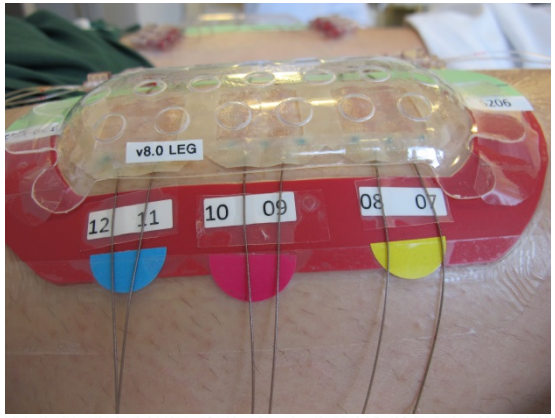
Clinical Bioavailability
Clinical BE Study

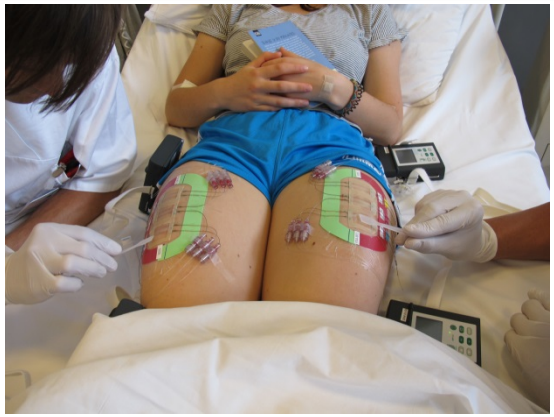
✓ **All procedures are standardized by using templates and SOPs.**

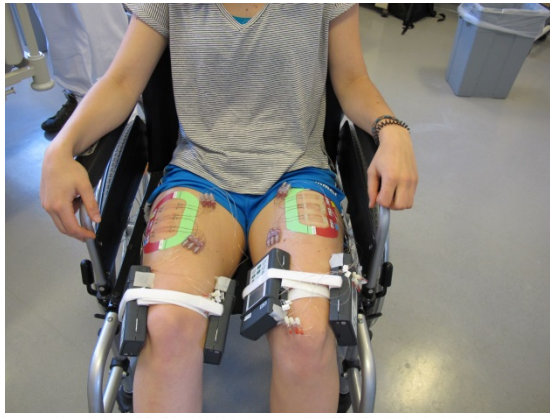


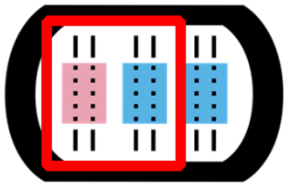






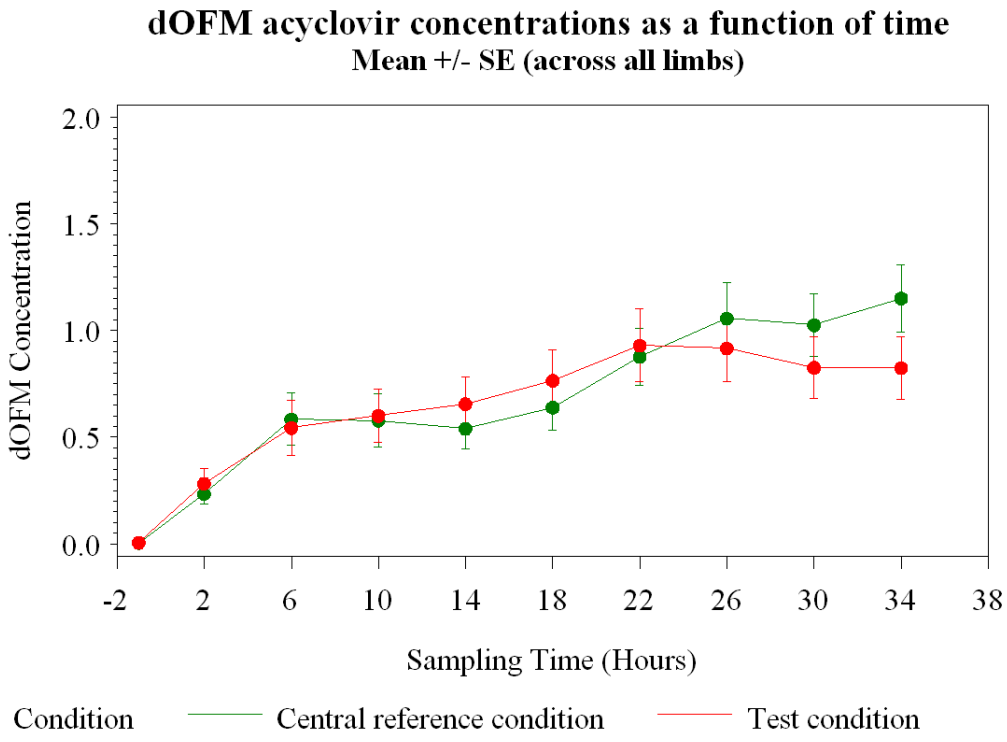




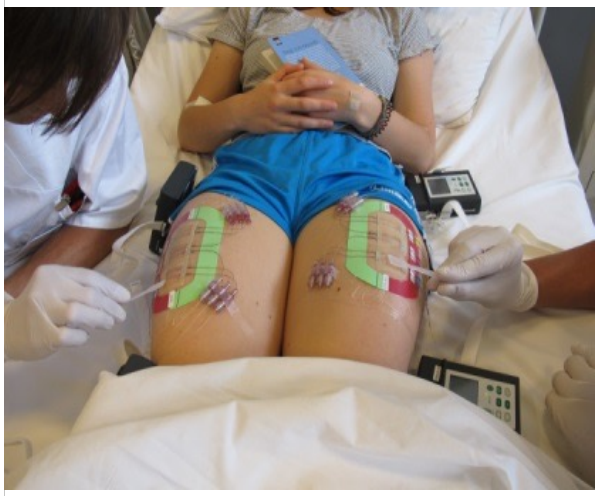


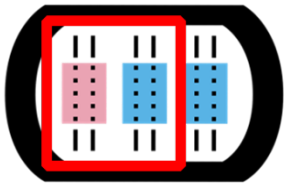
Clinical Bioavailability
Test versus Reference

✓ **Bioavailability: AUC and T_{max} of Aciclovir A1 are highly reproducible**
AUC and T_{max} of Zovirax US are highly reproducible



20 healthy subjects





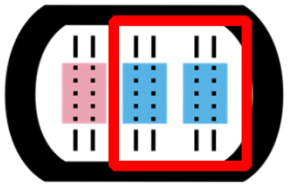
Clinical Bioavailability
Test versus Reference

- ✓ **BA is different for Aciclovir 1A vs Zovirax US based on AUC**
- ✓ **BA is different for Aciclovir 1A vs Zovirax US based on C_{max}**

Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.369 ; 0.050] or [69.1 % ; 105.2 %]	[-0.223 ; 0.223] or [80% ; 125%]	x Failed
log(C_{max})	[-0.498 ; 0.022] or [60.8 % ; 102.2%]		x Failed

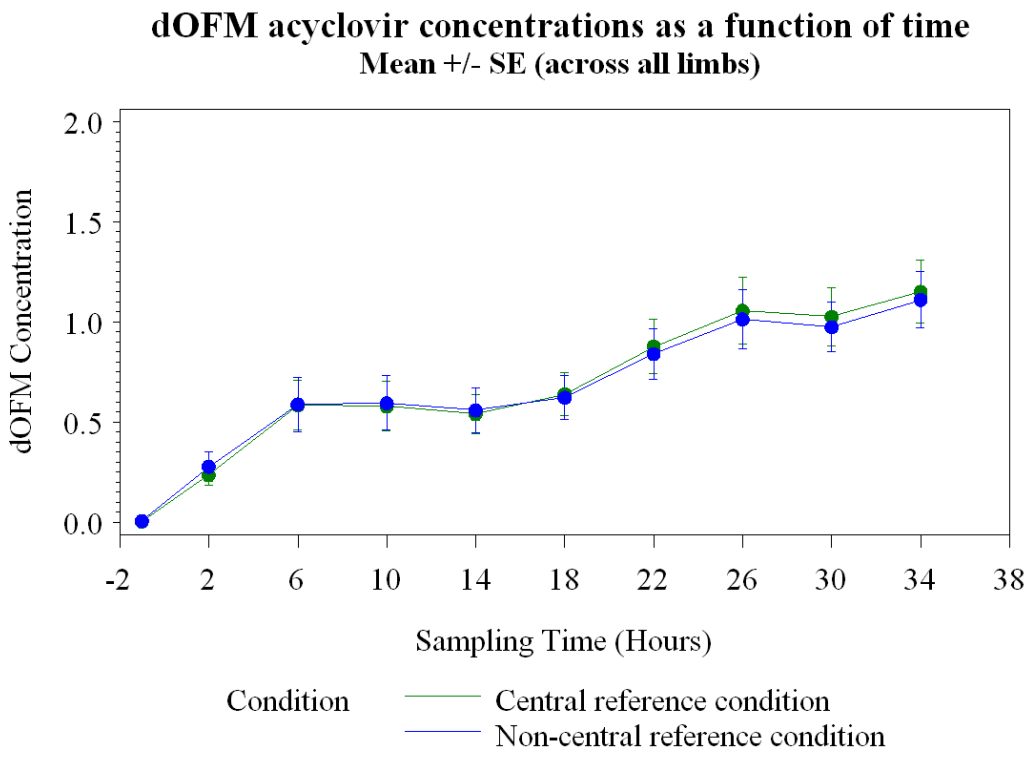
BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between test and reference condition

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

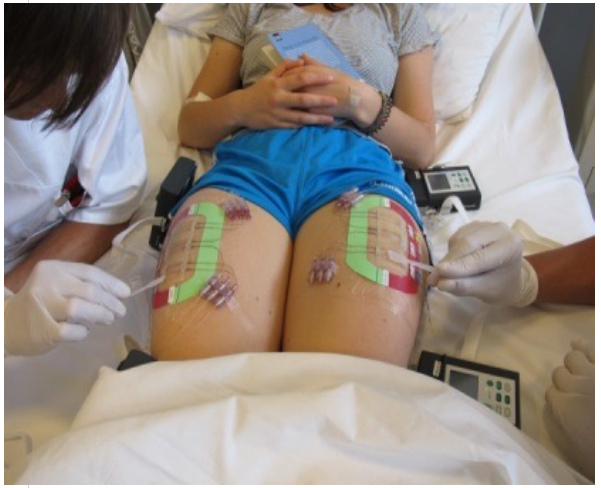


Clinical Bioavailability
Reference versus Reference

✓ **Bioavailability: AUC and C_{max} of Zovirax US are highly reproducible.**



20 healthy subjects



"Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence"

Clin. Pharmacokinet. 8/2016 – OPEN ACCESS

Clinical Bioavailability
Reference versus Reference

- ✓ **Same BA for Zovirax US vs Zovirax US based on AUC**
- ✓ **Same BA for Zovirax US vs Zovirax US based on C_{max}**

Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.148 ; 0.162] or [86.2 % ; 117.5 %]	[-0.223 ; 0.223] or [80% ; 125%]	**passed**
log(C_{max})	[-0.155 ; 0.190] or [85.7 % ; 120.9%]		**passed**

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between the two reference conditions

BA is established if $CI_{90\%}$ falls within the limits of log(0.8) = -0.223 and log(1.25) = 0.223 (cf. FDA Guidance For Industry)

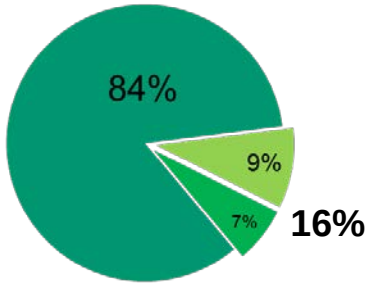
Skin penetration insights
Total variability

✓ **BE study set-up shows low intra-subject variability.**

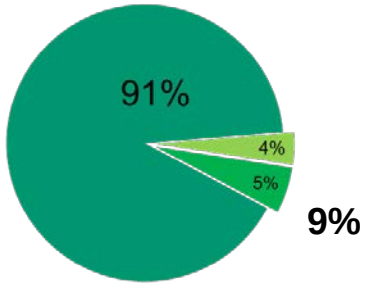
Total $CV_{logAUCacyc}$ was **39% - 44%** (41% Microdialysis Benfeldt et al.)

Components of total CV (ANOVA):

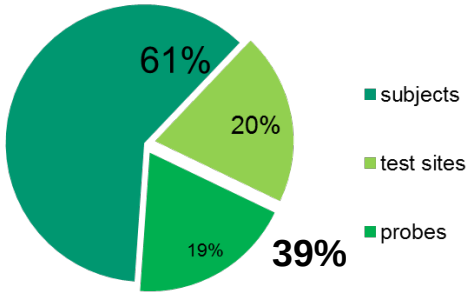
- Inter-subject variability: 84-91% OFM (61% Microdialysis Benfeldt et al.)
- Intra-subject variability: 9-16% OFM (39% Microdialysis Benfeldt et al.)



logAUC Zovirax®



logAUC Aciclovir 1A Pharma



logAUC lidocaine MD (Benfeldt et al.)

Benfeldt et al., J Invest Dermatol. 2007 Jan;127(1):170-8. Epub 2006 Jul 27

Skin penetration insights
Inter- and intra-subject variability

✓ **Skin impedance is a potential screening parameter.**

Inter-subject variability has

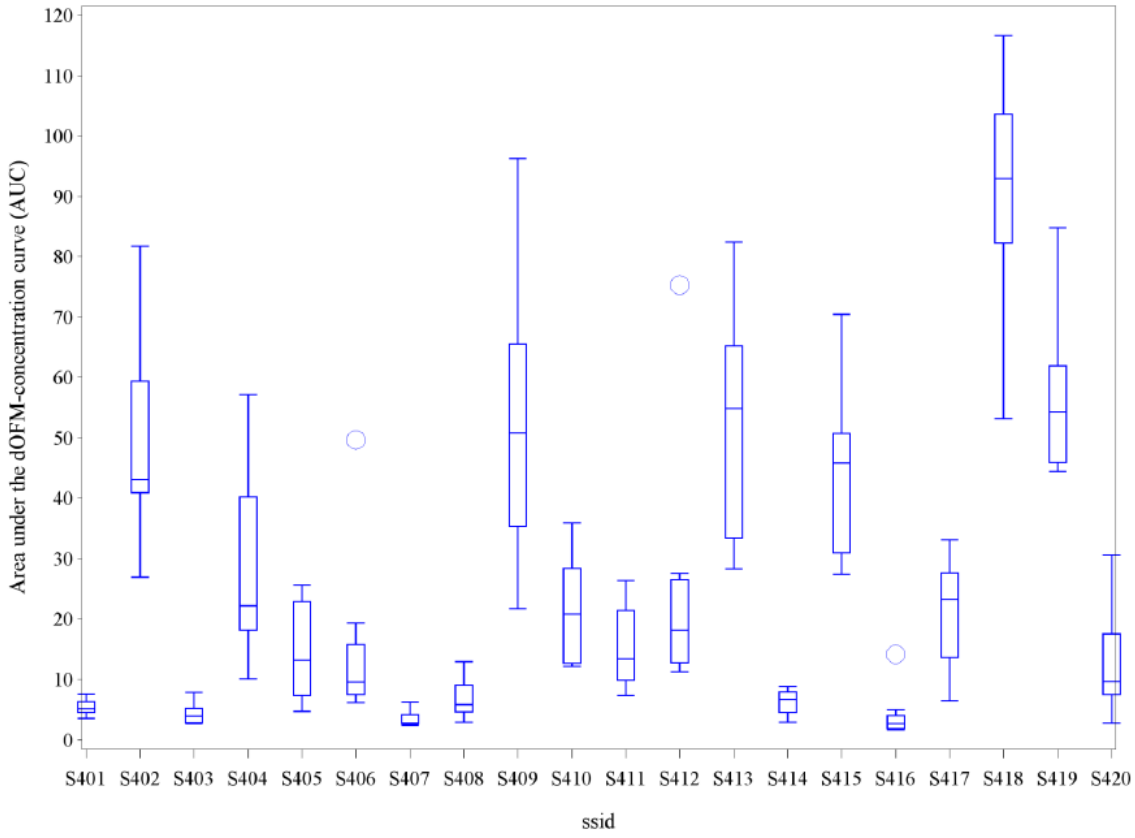
- a strong correlation with skin impedance (Joanneum®) (p=0.69-0.75, p<0.001)
- a weak correlation with TEWL (p=0.29-0.37, n.s)
- **no influence on BE in head-to-head design**

Intra-subject variability has

- a weak correlation with skin temperature (correlation analysis: r=0.25, p<0.05)
- influence on BE in head-to-head design
- **deviations of 100-500% between probes within sites - also published for MD**

Skin penetration insights
Intra-subject distribution

✓ **Is intra-subject variability really due to dOFM?**



Hypothesis:

Local skin shunts (follicles, glands) rather than OFM cause majority of intra-subject variability

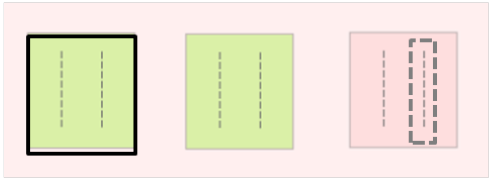
OFM errors ≤ 10% (also for MD, see Kreilgaard et al. 2001)

Skin penetration insights
Skewed skin penetration pattern

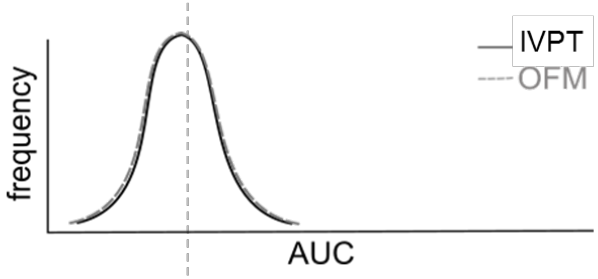
✓ **Skin shunts may lead to skewed distribution**

- Ideal homogenous intact skin

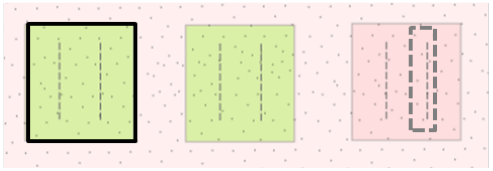
IVPT area (=large)

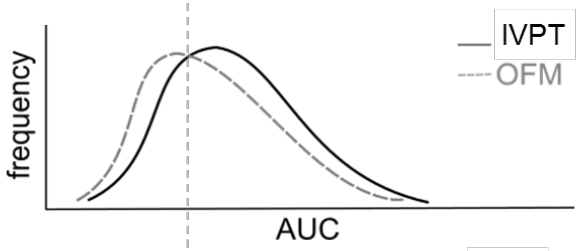


OFM area (=small)



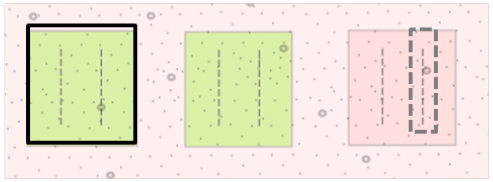
- Small skin impaires

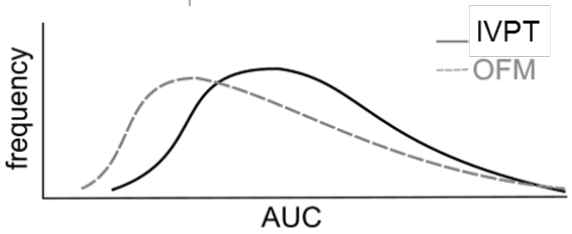




*OFM AUCs would be lower than IVPT

- Large skin impaires





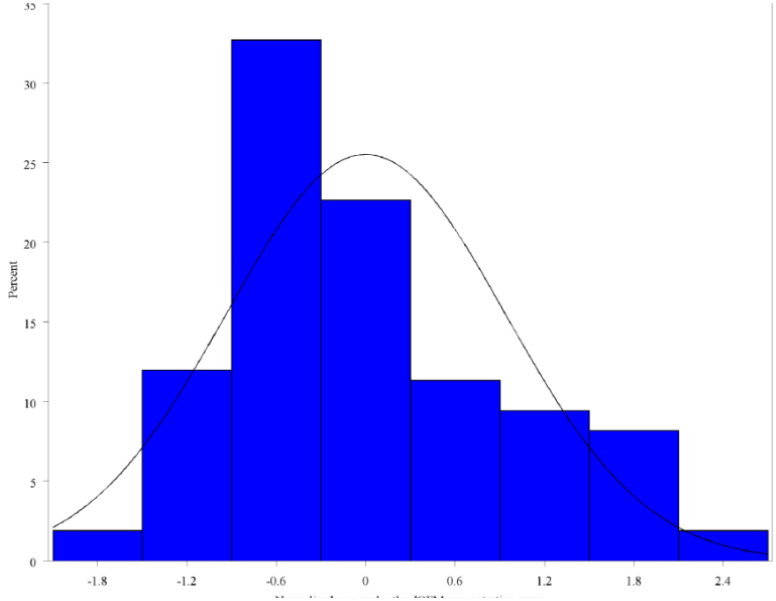
(Particularly) relevant for drug which are bad penetrators.

Reference for follicular penetration of hydrophilic drugs logP<1.9: Frum et al. Eur J Pharm Sci 2007: 280-287

Skin penetration insights
Skewed intra-subject data

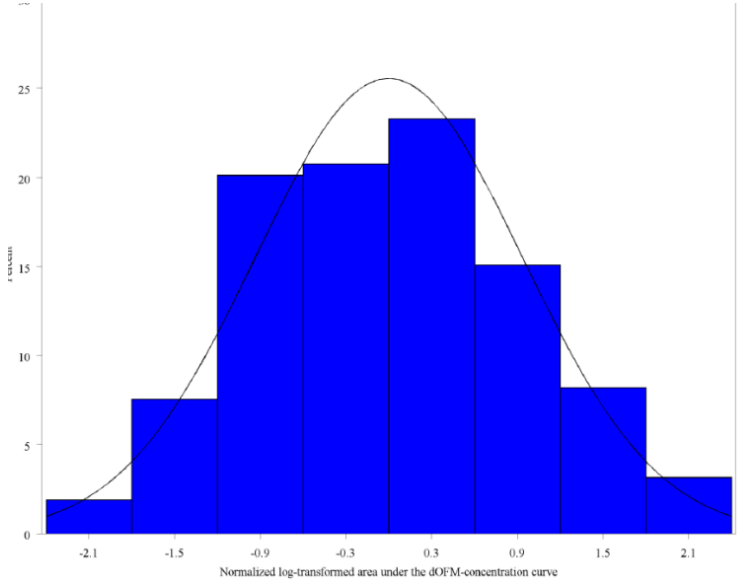
✓ **Acyclovir dOFM AUCs within subjects are log-normal distributed.**

AUCs standarized in each subject by indiv. mean
- non-normal!



Goodness-of-Fit Tests for Normal Distribution				
Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.10521650	Pr > D	<0.010
Cramer-von Mises	W-Sq	0.51013639	Pr > W-Sq	<0.005
Anderson-Darling	A-Sq	2.92818998	Pr > A-Sq	<0.005

logAUCs standarized by indiv. mean in each subject
- normal!



Goodness-of-Fit Tests for Normal Distribution				
Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.04327362	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.04927888	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.32976282	Pr > A-Sq	>0.250

Skin penetration insights
Impact of skewed distribution on BE calculation

✓ **Geometric mean is best for skewed distributed acyclovir data**

Arithm. Mean curve, thereof AUC (published):
BE ✓ - good

Label	Estimate	Standard Error	Df	t-Value	Pr>|t|	alpha	Lower limit	Upper limit
R_2 vs. R_1	**100.7%** *Δ 0.7%*	109.6%	39	0.07	0.9428	0.1	86.2%	117.5% *90% CI width: 31.3%*

Geom. Mean curve, thereof AUC
BE ✓ - better!

Label	Estimate	Standard Error	Df	t-Value	Pr>|t|	alpha	Lower limit	Upper limit
R_2 vs. R_1	**99.7%** *Δ 0.3%*	108.8%	39	-0.03	0.9741	0.1	86.5%	115.0% *90% CI width: 28.5%*

Pharmacokinetics-Based dOFM
Summary

dOFM in-vivo

- is a reproducible, accurate and sensitive method.
- shows very low method-variability.
- reflects in-vivo skin penetration in dermis.
- gives advanced skin penetration insights.

dOFM in-vivo

- can be used to investigate BE on a pharmacokinetic basis.
- could be a useful tool to conduct clinical bioequivalence studies in a low number of healthy subjects.
- is a potential tool to reduce time and costs of clinical bioequivalnce studies.

Clinical Bioavailability
Outlook

Clinical OFM study A: In-Depth Identification of Influencing Factors of Skin Penetration - Moderate Lipopilic/Protein Bound Drugs

- Pilot (n=6): systemic adsorption and cross-talk; lateral diffusion and cross-talk, sample time for C_{max} and ¾ of AUC
- Main study (n=38): investigate BE of (a) RLD to itself, (b) approved generic product to RLD, (c) non-BE product to RLD, (d) BE identify influencing factors

➔ **Optimization of screening and OFM BE study design**

Clinical OFM study B: Standardized BE Study - Highly Protein Bound Drug

- Pilot (n=6): systemic adsorption and cross-talk; lateral diffusion and cross-talk, sample time for C_{max} and ¾ of AUC
- Main study (n=20): investigate BE of (a) RLD to itself, (b) approved generic product to RLD, (c) non-BE product to RLD

➔ **Validate OFM as an universal tool for BE studies for topical drugs**

A big Thanks to…

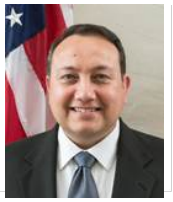
Katrin Tiffner
IVRT and dOFM ex-vivo

Manfred Bodenlenz
Clinical dOFM BE Study

Reingard Raml
Analytics

Thomas Pieber
Clinical PI

Isadore Kanfer
BE Conultant Expert



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Jill Coker
and other….

Thank you for your attention

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