In Vitro Data Analysis Issues: IVPT Analyses and Other Challenges

Streamlining Generic Drug Development by Matching Reference Product Composition and Performance, In Vitro and In Vivo

Session 1 In Vitro-Based BE Approaches

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Priyanka Ghosh, PhD

Pharmacologist

Division of Therapeutic Performance, Office of Research and Standards

Office of Generic Drugs |CDER | US FDA

Disclaimer

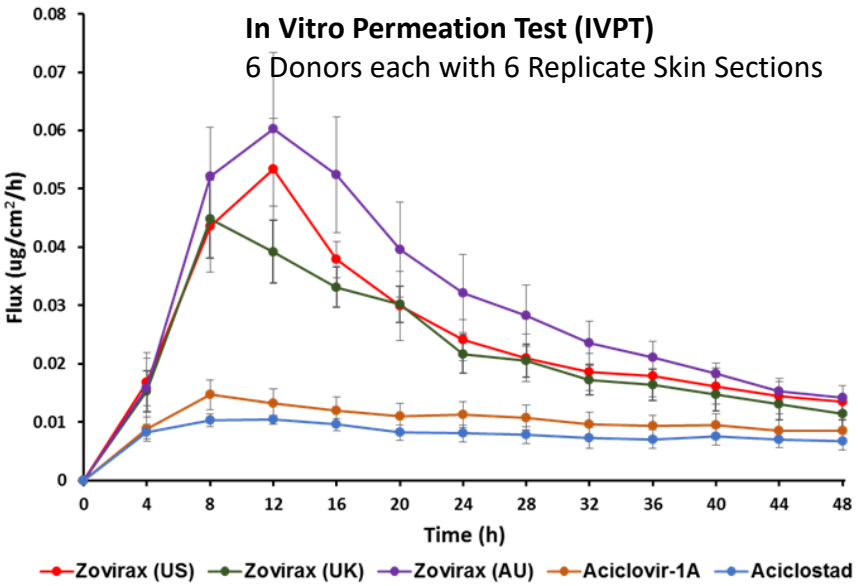
- This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

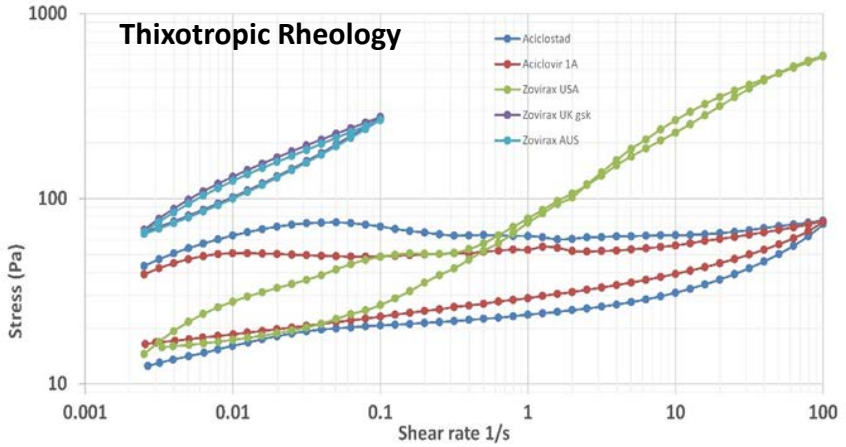
In Vitro Based BE Approaches

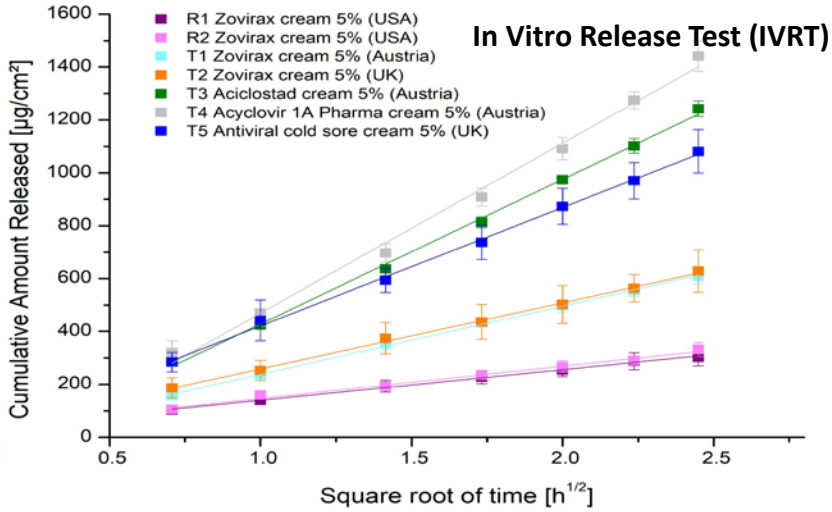
- **A <u>Modular</u> Framework for In Vitro BE Evaluation**
  - **Q1/Q2** sameness of inactive ingredient components and quantitative composition
  - **Q3 (Physical & Structural Characterization)** as relevant to the nature of the product
  - **IVRT** (In Vitro Release Test) and
  - **IVPT** (In Vitro Permeation Test) or another bio-relevant assay may be recommended based on the complexity of the arrangement of matter in the drug product
- **A <u>Scalable</u> Framework for BE Evaluation**
  - **In Vivo** systemic PK studies may be appropriate
  - **In Silico** computational modeling may be useful

Correlation of Quality and Performance

	Zovirax (USA)	Zovirax (UK)	Zovirax (Austria)	Aciclostad (Austria)	Aciclovir-1A (Austria)
	Water	Water	Purified water	Water	Water
	Propylene glycol	Propylene glycol	Propylene glycol	Propylene glycol	Propylene glycol
	Mineral oil	Liquid Paraffin	Liquid Paraffin	Liquid Paraffin	Viscous Paraffin
	White petrolatum	White soft paraffin	White Vaseline	White Vaseline	White Vaseline
	Cetostearyl alcohol	Cetostearyl alcohol	Cetostearyl alcohol	Cetyl alcohol	Cetyl alcohol
	SLS	SLS	SLS	[redacted]	[redacted]
	Poloxamer 407	Poloxamer 407	Poloxamer 407		
	[redacted]	Dimethicone 20	Dimethicone 20	Dimethicone	Dimethicone
		Arlacel 165	Glyceryl Mono Stearate	Glyceryl Mono Stearate	Glyceryl Mono Stearate
		Arlacel 165	Polyoxyethylene stearate	Macrogol stearate	Polyoxyethylene stearate
Density (g/cc)	1.02	1.02	1.02	1.02	1.01
Content Uniformity (%)	97.9 ± 0.7	99.6 ± 1.4	100 ± 2.2	99.7 ± 1.7	98.3 ± 2.6
Polymorphic Form	2,3 hydrate	2,3 hydrate	2,3 hydrate	2,3 hydrate	2,3 hydrate
Crystilline Habit	Rectangular	Rectangular	Rectangular	Ovoid	Ovoid
Particle size (d50) (µm)	3.8	2.5	3.4	6.8	6
pH	7.74	7.96	7.54	4.58	6.05
Work of Adhesion	59	81	60	17	18
Drug in Aq (mg/g)	0.49	0.64	0.49	0.37	0.26
Drying Rate (T-30%)	>12h	~8h	~7h	<1h	<1h
Water Activity	0.75	0.73	0.74	0.95	0.95



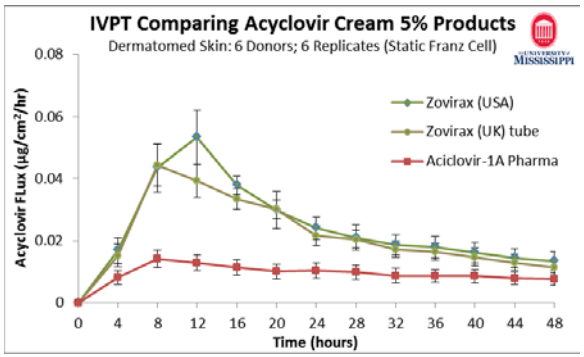


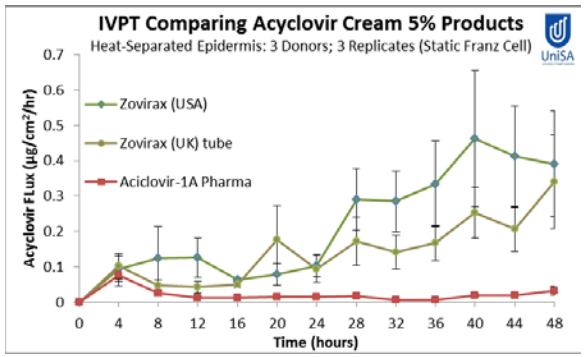


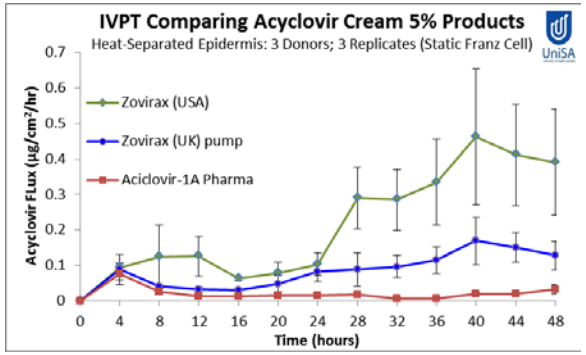
Data provided courtesy of Prof. Narasimha Murthy & Dr. Frank Sinner

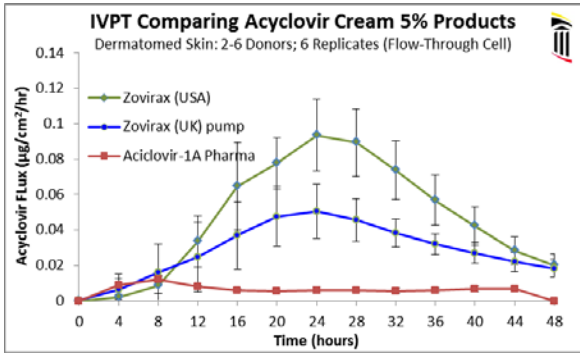
Influence of Quality on Performance

- Influence of Dose Dispensing on Bioavailability





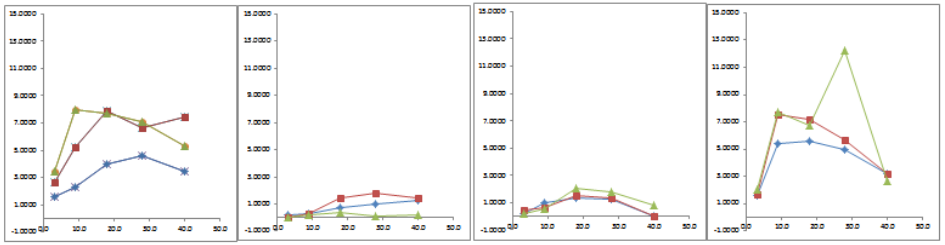




Data provided courtesy of Prof. Narasimha Murthy, Dr. Audra Stinchcomb & Dr. Michael Roberts

Typical Intra-Donor Variability

RLD Product
Lot# "**1**" (redacted)



RLD Product
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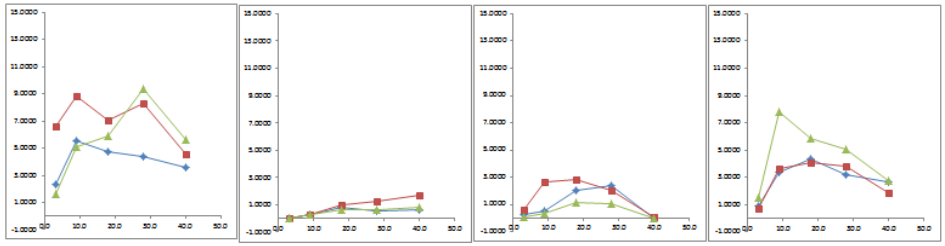
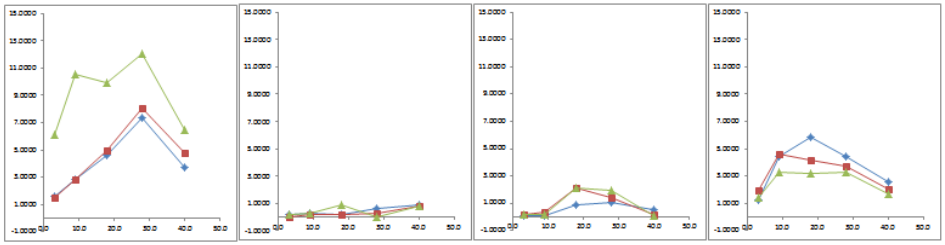
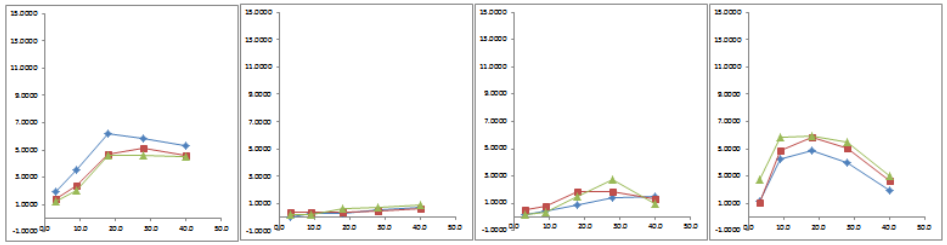


RLD Product
Lot# "**3**" (redacted)

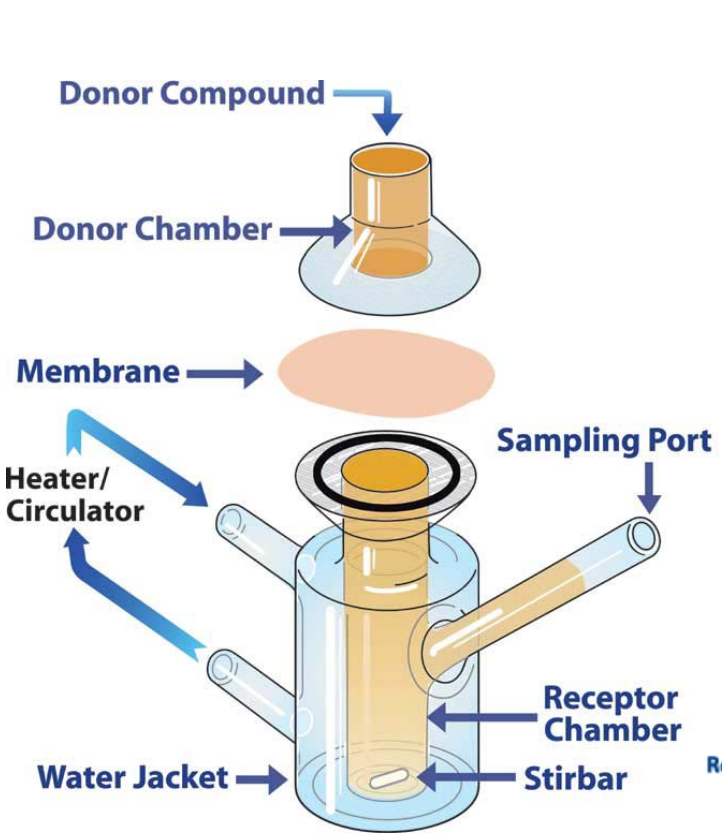


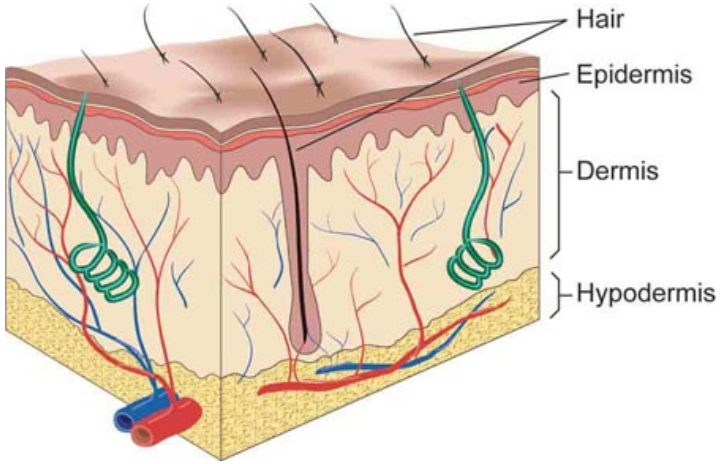
Test Product

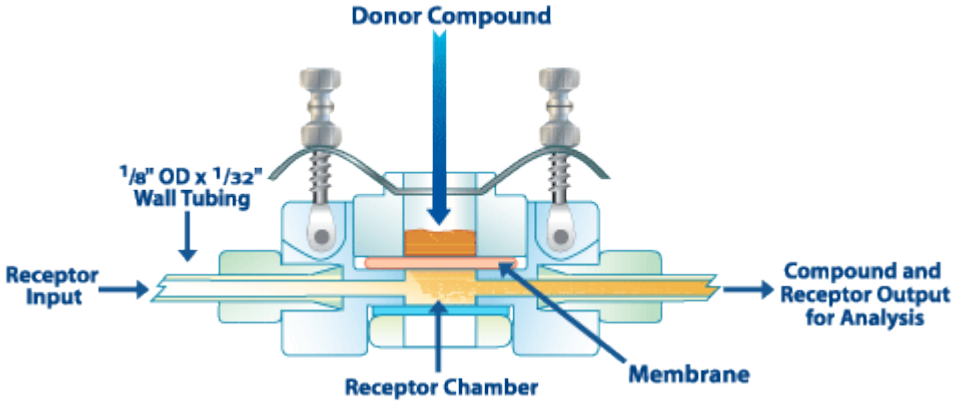


In Vitro Cutaneous Pharmacokinetics

- IVPT (In Vitro Permeation Test)

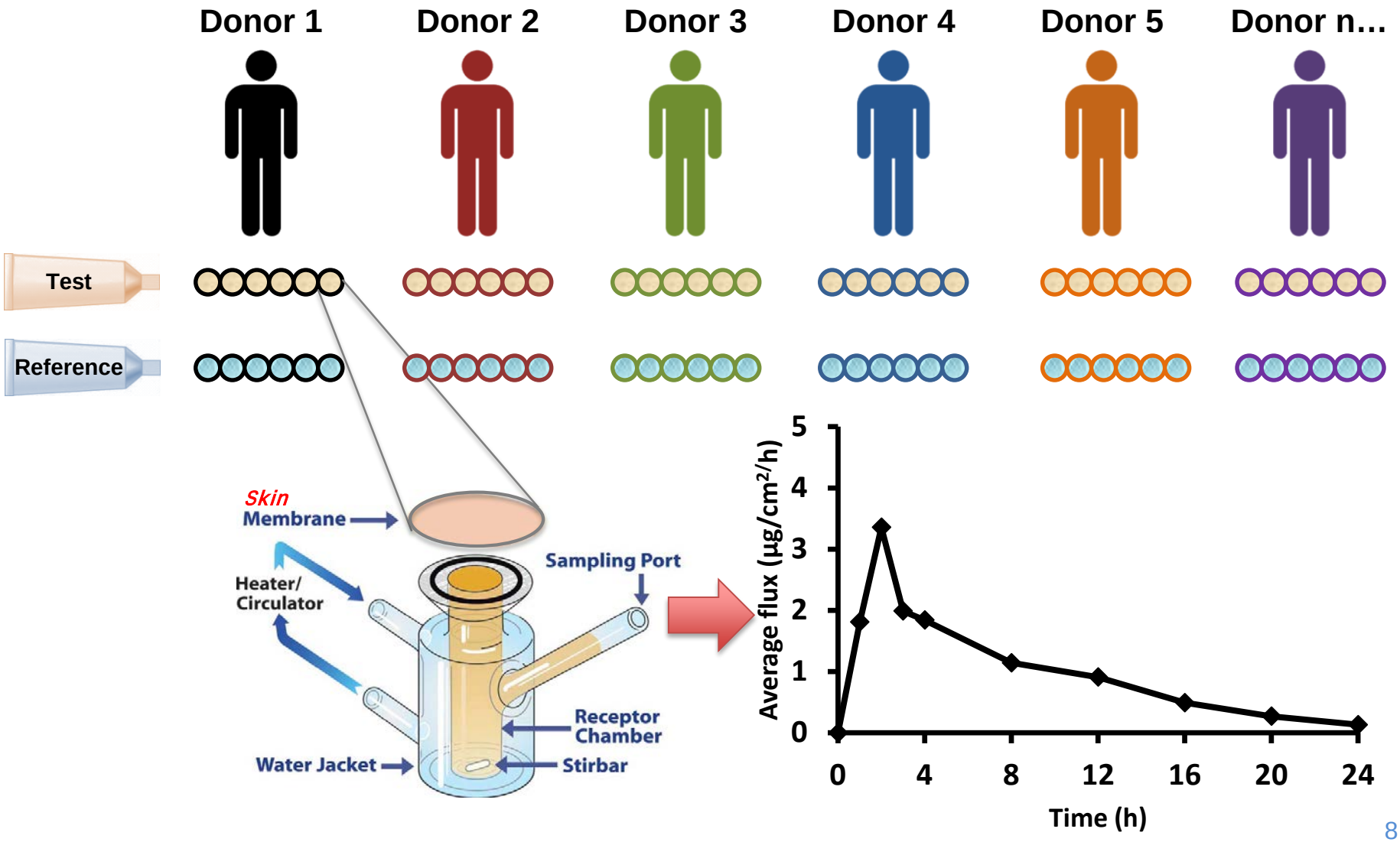




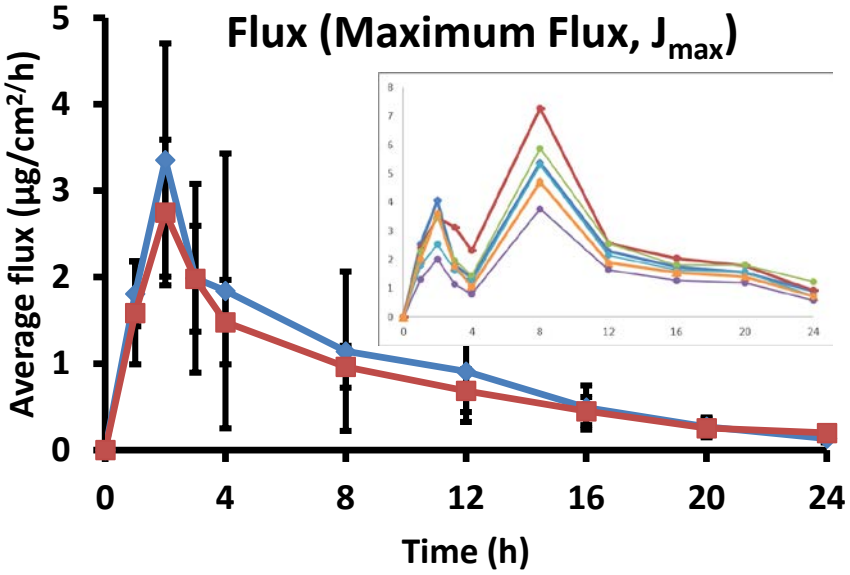


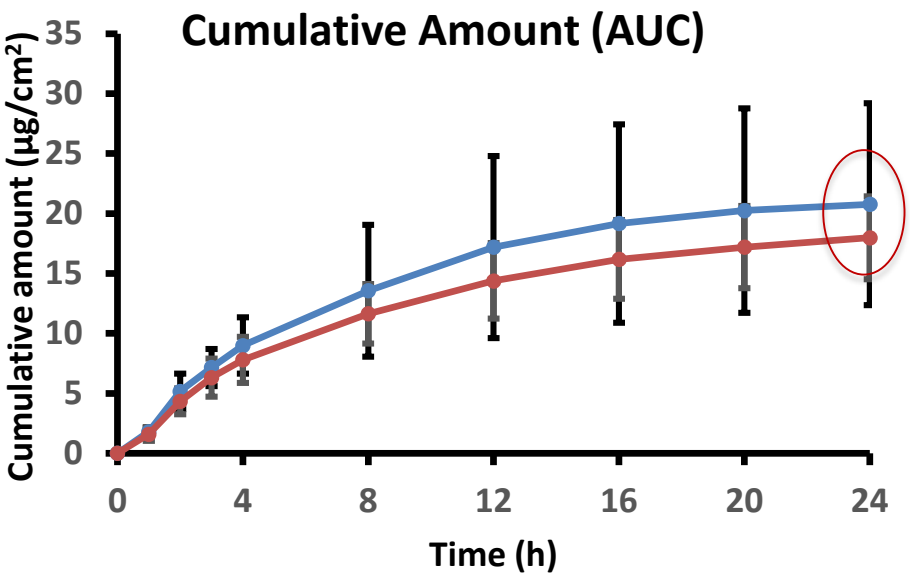
Cell images courtesy of PermeGear

IVPT Study Design



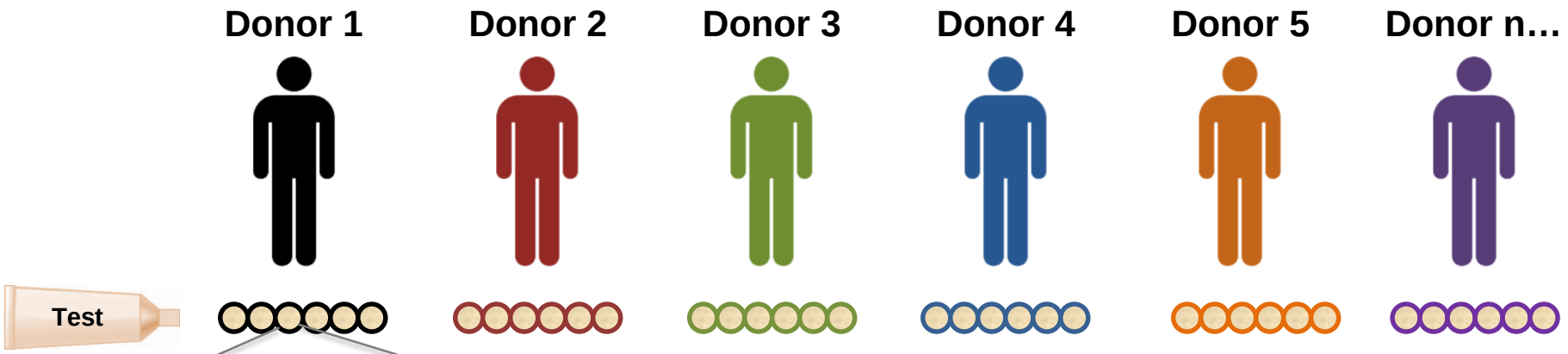
Deriving Pharmacokinetic Parameters

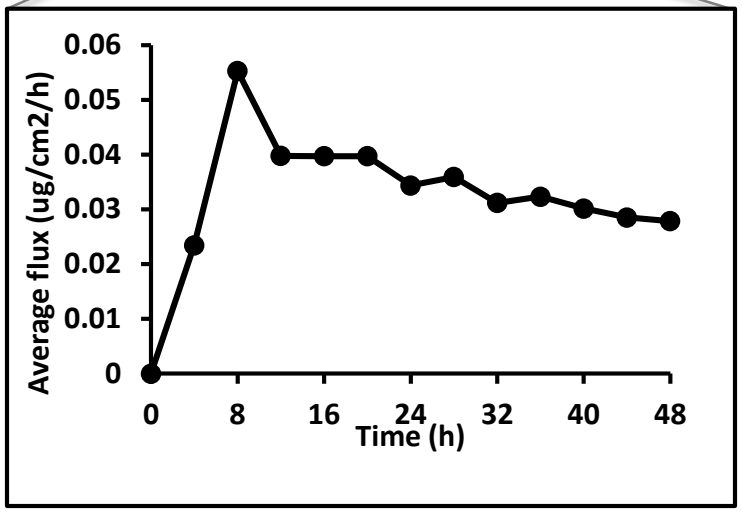




Data from 1 donor, represented as mean ± std. deviation

Data Analysis





$\text{Log}_e\ (J_{max})$

OR

$\text{Log}_e\ (AUC)$

Test:

$$T_{11}, T_{12}, \ldots, T_{1r}$$
$$T_{21}, T_{22}, \ldots, T_{2r}$$
$$\vdots$$
$$T_{n1}, T_{n2}, \ldots, T_{nr}$$

Data Analysis

Test:

21,22,23
31,32,33
⋮
41,42,43

Reference:

27,28,29
41,42,43
⋮
53,54,55

Derivation of Point Estimate

For each donor $I_j = \frac{1}{r}\sum_{i=1}^{r}(T_{ij} - R_{ij})$

$I_j = \frac{(21-27)+(22-28)+(23-29)}{3} = (-6)$ (for donor 1)

Average across all donors $\bar{I}_{\cdot} = \frac{1}{n}\sum_{j=1}^{n} I_j$

$\bar{I} = \frac{(-6)+(-10)+(-12)}{3} = (-9.33)$

Data Analysis

Test:

21,22, 23

31,32, 33

⋮

41,42, 43

Reference:

27,28, 29

41,42, 43

⋮

53,54, 55

Estimation of Inter-donor Variability

$$S_I^2 = \frac{1}{(n-1)} \sum_{j=1}^{n} (I_j - \bar{I}_.)^2$$

I_j = =(-6) (for donor 1)

I_j = =(-10) (for donor 2)

I_j = =(-12) (for donor 3)

I = =(-9.33) (for donor 1)

$$S_i^2 = \frac{1}{3-1}((-6+9.33)^2 + (-10+9.33)^2 + (-12+9.33)^2)$$

Data Analysis

Estimate of Within-reference Variability

$$S_{WR}^2 = \frac{\sum_{j=1}^{n}\sum_{i=1}^{r}(R_{ij} - \overline{R}_{.J})^2}{(r-1)n}$$

$$R_j = \frac{27+28+29}{3} = 28\ (for\ donor\ 1)$$

Reference:

27,28, 29

41,42, 43

⋮

53,54, 55

$$S_{wr}^2 = \frac{((27-28)^2 + (28-28)^2 + (29-28)^2 + \cdots)}{(3-1)*3}$$

$$S_{wr}^2 = 1$$

$\overline{R}_{.J}$ is the average across all r replicates for donor J of R

Mixed Scaled Criterion

$$S_{WR} \leq 0.294$$

Average Bioequivalence (ABE)

$$\bar{I} \pm t_{(n-1),\alpha/2} * \sqrt{\frac{S_I^2}{n}}$$

Two one-sided tests (TOST)

T and R are deemed bioequivalent if the confidence interval is within the following limits $[0.8, 1.25]$

$$S_{WR} > 0.294$$

Scaled ABE (SABE)

$$H_0: \frac{(\mu_T - \mu_R)^2}{\sigma_{WR}^2} > \theta$$

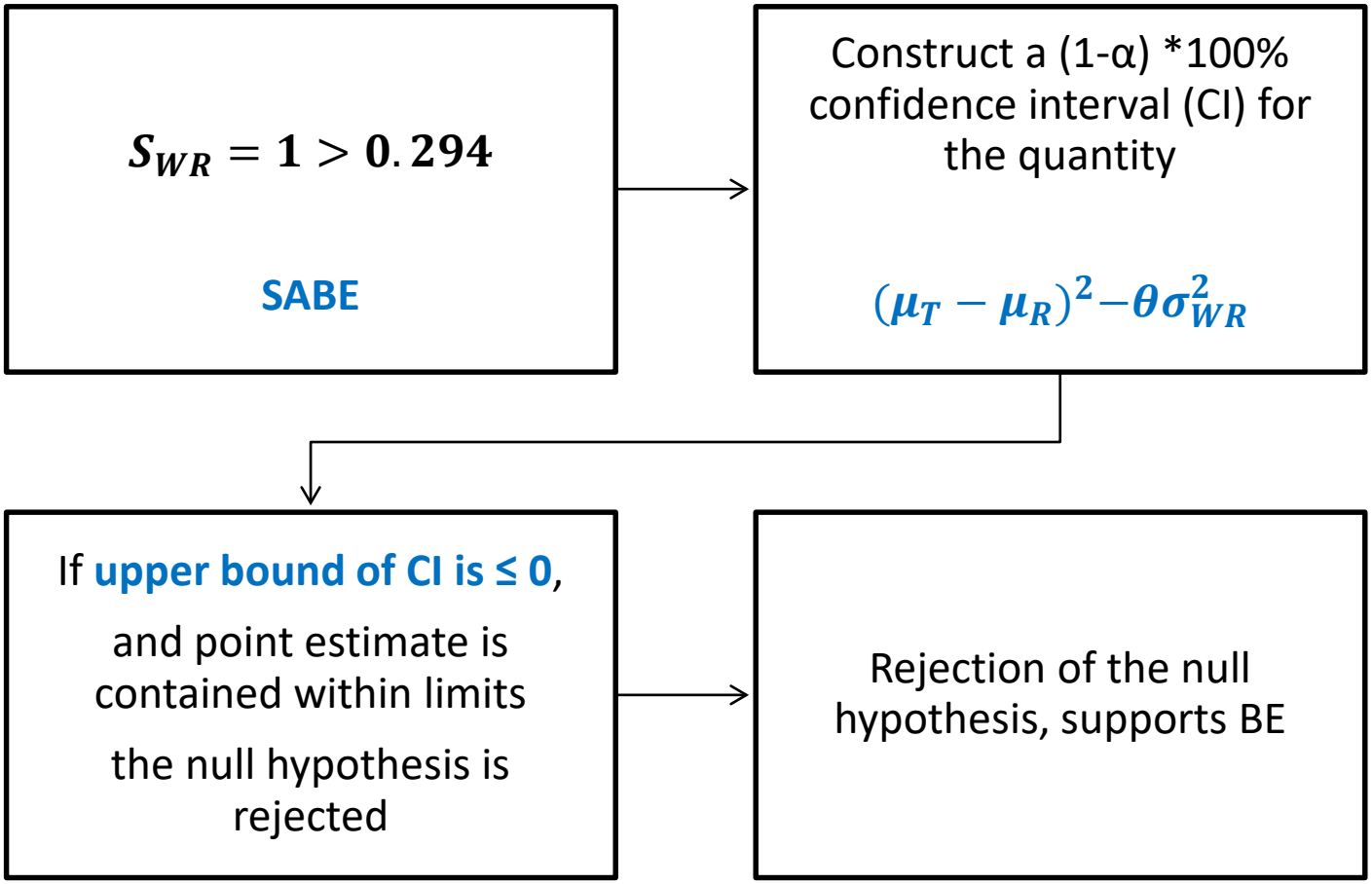
$$H_a: \frac{(\mu_T - \mu_R)^2}{\sigma_{WR}^2} \leq \theta$$

Where $\theta = \frac{(\ln(1.25))^2}{(0.25)^2} = 0.7966$

T and R are deemed bioequivalent if the null hypothesis is rejected. Rejection of the null hypothesis is supported if a double criterion is satisfied:

1. The upper 95% of the scaled confidence interval is ≤ 0 and
2. The point estimate is contained within the limits [0.8, 1.25].

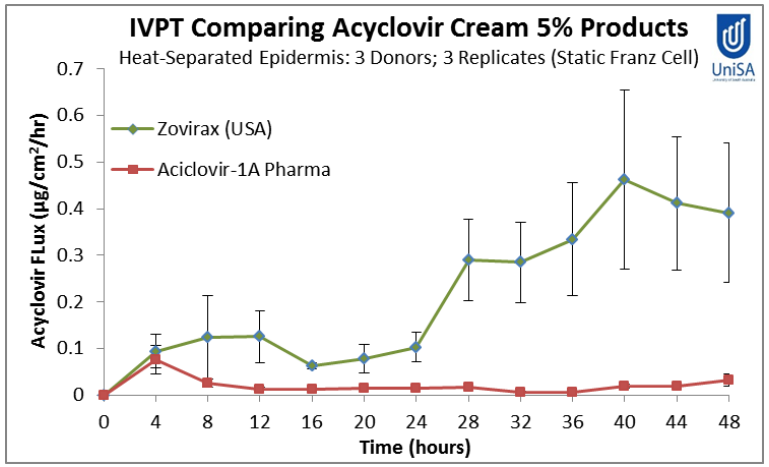
Mixed Scaled Criterion

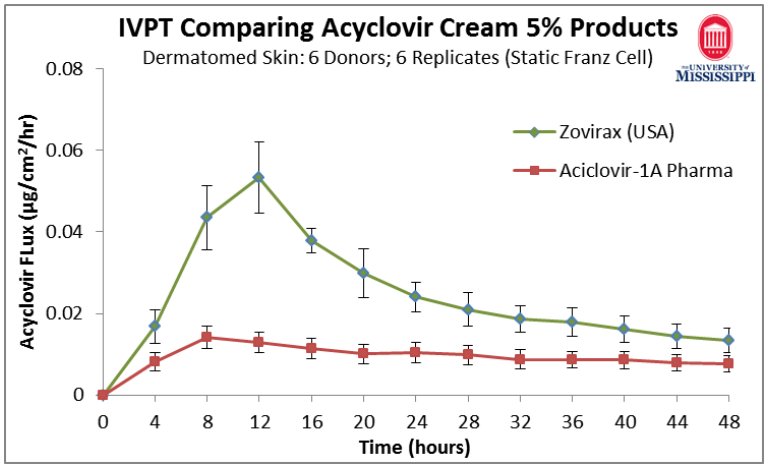


In the example: The upper bound of the CI was >0 so we fail to reject the null hypothesis. Therefore, BE of T and R is not supported.

IVPT Statistical Analysis

- **Negative Controls** for BE: Aciclovir-1A® vs. Zovirax® US





Aciclovir-1A® (T) vs. **Zovirax® US (R)**

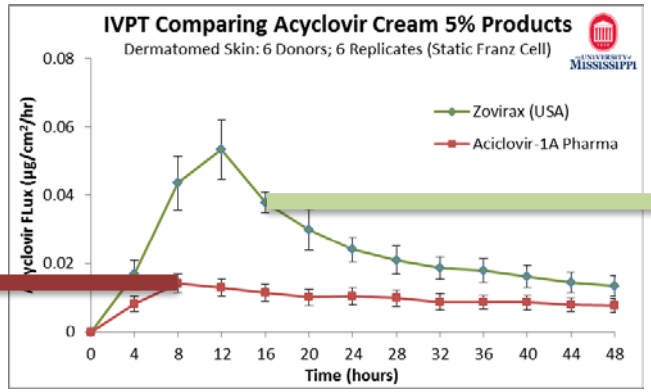
IVPT PK Endpoint	Maximum Flux **(Jmax)**	Total Bioavailability **(AUC)**
Point Estimate	0.172	0.104
$S_{Within\ Reference}$	0.521	0.551
SABE [0.80, 1.25]	4.433 (**Non-BE**)	7.236 (**Non-BE**)
N for [0.80, 1.25] with 3 Replicates	6	8

Aciclovir-1A® (T) vs. **Zovirax® US (R)**

IVPT PK Endpoint	Maximum Flux **(Jmax)**	Total Bioavailability **(AUC)**
Point Estimate	0.290	0.366
$S_{Within\ Reference}$	0.575	0.419
SABE [0.80, 1.25]	2.383 (**Non-BE**)	1.884 (**Non-BE**)
N for [0.80, 1.25] with 6 Replicates	8	20

IVPT Statistical Analysis

- **Positive Controls** for BE: Aciclovir-1A® and Zovirax® US



Comparison to Self by dividing up 6 replicates

Comparison to Self by dividing up 6 replicates

Aciclovir-1A® (T) vs. **Aciclovir-1A® (R)**

IVPT PK Endpoint	Maximum Flux **(Jmax)**	Total Bioavailability **(AUC)**
Point Estimate	0.983	0.958
$S_{Within\ Reference}$	0.303	0.318
SABE [0.80, 1.25]	-0.026 (**BE**)	-0.041 (**BE**)
N for [0.80, 1.25] with 4 Replicates	26+	15
N for [0.80, 1.25] with 3 Replicates	26+	15

Zovirax® US (T) vs. **Zovirax® US (R)**

IVPT PK Endpoint	Maximum Flux **(Jmax)**	Total Bioavailability **(AUC)**
Point Estimate	0.962	1.101
$S_{Within\ Reference}$	0.697	0.469
SABE [0.80, 1.25]	-0.214 (**BE**)	-0.020 (**BE**)
N for [0.80, 1.25] with 4 Replicates	12+	14
N for [0.80, 1.25] with 3 Replicates	14	15+

Conclusions

- IVPT is used for the assessment of bioavailability for complex drug products
- The parallel, single-dose, multiple-replicate per treatment group study design is recommended based on an understanding of the inherent variability associated with permeability of molecules across human skin
- The statistical method used for data analysis is based on the mixed scaled criterion used by CDER for Highly Variable Drugs (HVD)
- The SABE has been adapted to analyze cutaneous pharmacokinetic data
- The SABE approach can be adequately powered for establishing BE using 6-36 donors depending on variability associated with PK parameters

References

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- Schuirmann, Donald J. "A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability." *Journal of pharmacokinetics and biopharmaceutics* 15, no. 6 (1987): 657-680

- FDA Draft Guidance for Industry on Progesterone oral capsules http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm209294.pdf

- Davit, Barbara M., Mei-Ling Chen, Dale P. Conner, Sam H. Haidar, Stephanie Kim, Christina H. Lee, Robert A. Lionberger et al. "Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products by the US Food and Drug Administration." *The AAPS journal* 14, no. 4 (2012): 915-924

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- Michael Roberts, PhD

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- Audra Stinchcomb, PhD

GDUFA Award U01FD00**4946**

- Frank Sinner, PhD


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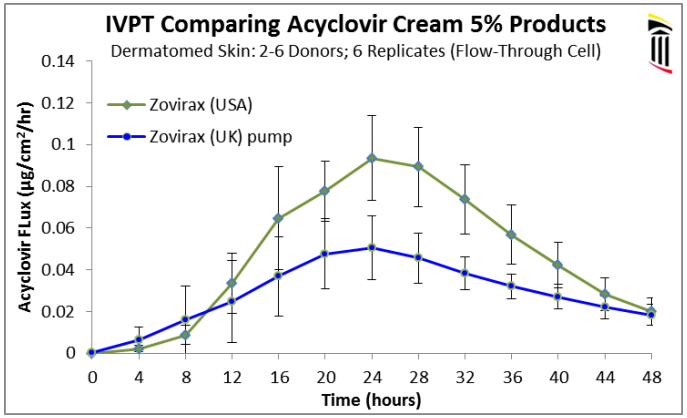
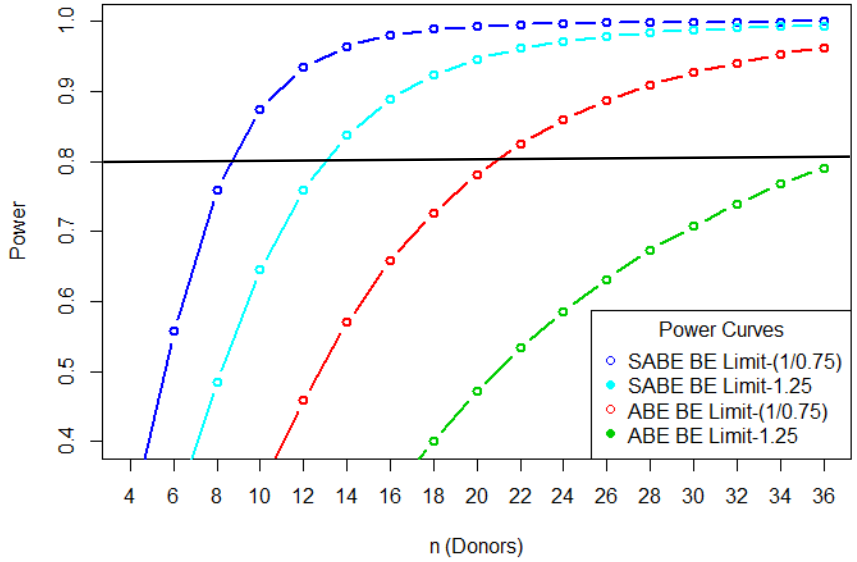
IVPT Bioequivalence Limits

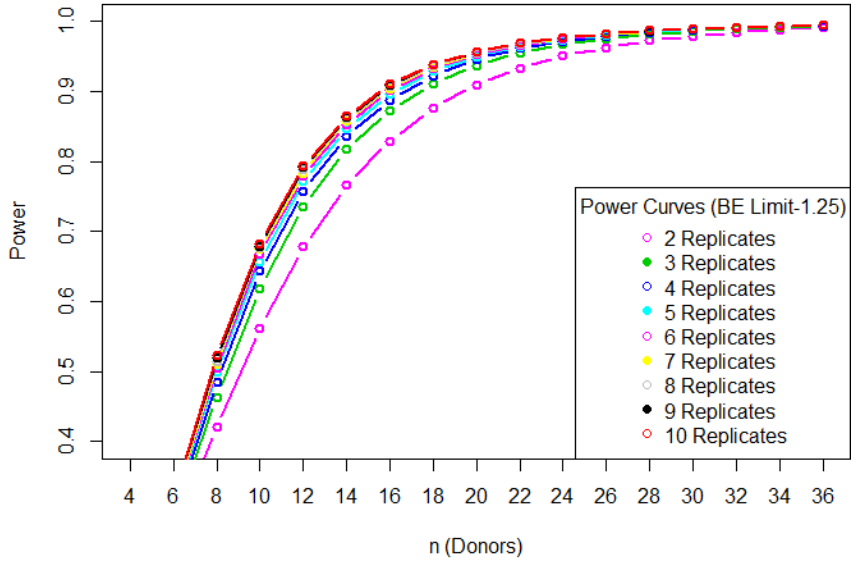
- **Bioequivalence Limits, Study Power and Study Size**



UK-US Jmax



UK-US Jmax

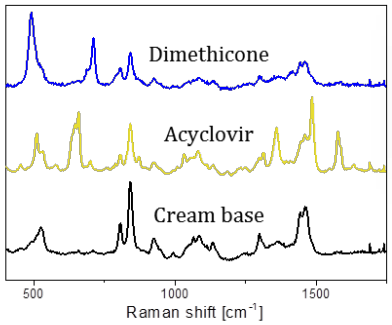


IVPT Method Validation

- Apparatus Qualification
- **Membrane (Skin) Qualification**
- **Receptor Solution Qualification**
- **Receptor Solution Sampling Qualification**
- IVPT Receptor Solution Sample Analytical Method Validation
- Environmental Control
- **Pilot Study**
- Permeation Profile and Range
- Precision and Reproducibility
- Recovery, Mass Balance & Dose Depletion
- **Sensitivity and Selectivity**
- Robustness

Influence of Dispensing Stress on Q3

- Influence of Dose Dispensing on Product Quality



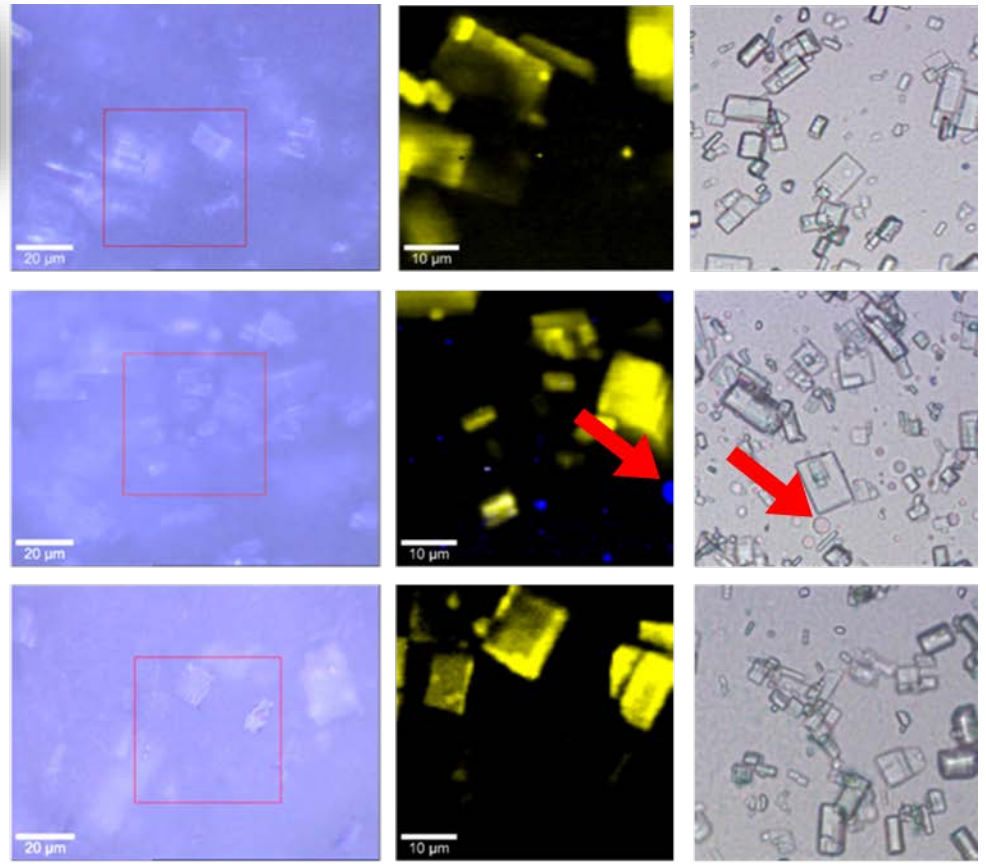
Zovirax® UK
Tube

University of South Australia

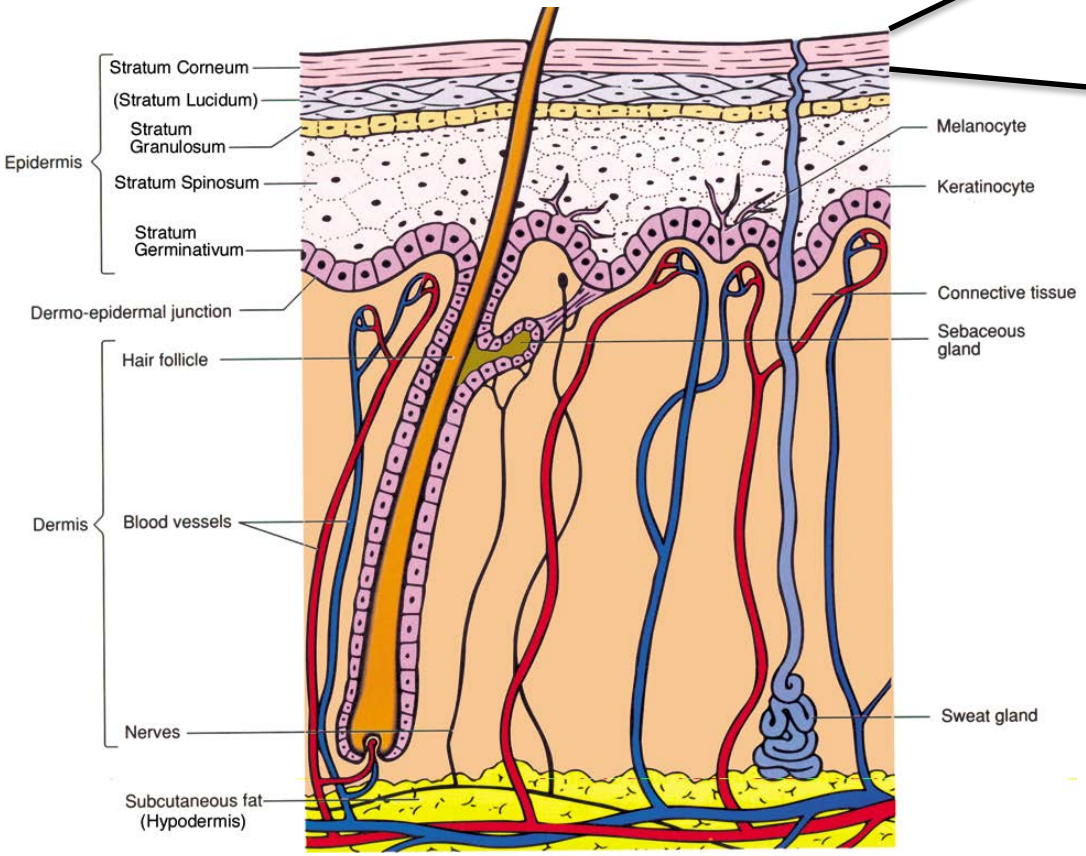
UNIVERSITÄT DES SAARLANDES

Zovirax® UK
Pump

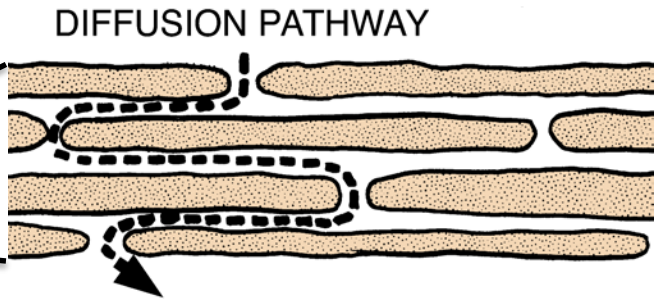
Zovirax® UK
Pump
(from inside container)



Human Skin Structure



Adapted from Cerio and Archer, 1998



Drawing adapted from Odland, 1971.