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Topical Bioequivalence: Performance Evaluation In Vivo and In Vitro by Skin Stripping and IVPT

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F6 Pharma

Outline

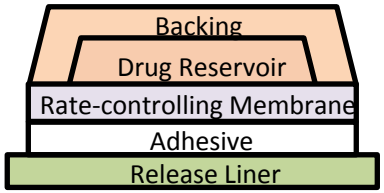
IVIVC

- Influence of Heat on TDS *in vitro (IVPT)*
- Influence of Heat on TDS *in vivo (humans)*

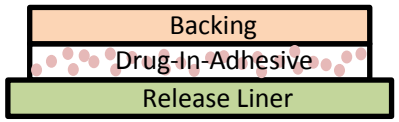
Methods to Evaluate BA for Topical Drug Products

- Tape-stripping
  - (Bunge, Guy, Delgado-Charro)
- IVPT (In Vitro Permeation Tests)

Transdermal Delivery Systems (TDS)



Reservoir Type



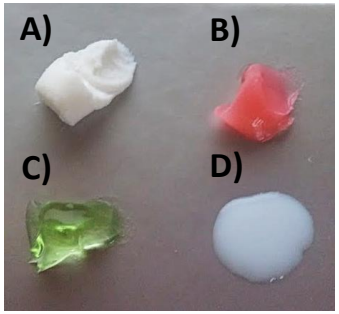
Matrix Type

- Therapy can be interrupted
- Low drug efficiency

- Systemic absorption is intended
- Blood levels ≈ Efficacy
- Occluded applications
- Highly reproducible application techniques
- Sustained and constant delivery

- BA: based on PK endpoint (C_{max}, $t_{max,}$ AUC, etc)

Topical Drug Products (locally-acting)



A) Cream

B) Ointment

C) Gel

D) Lotion

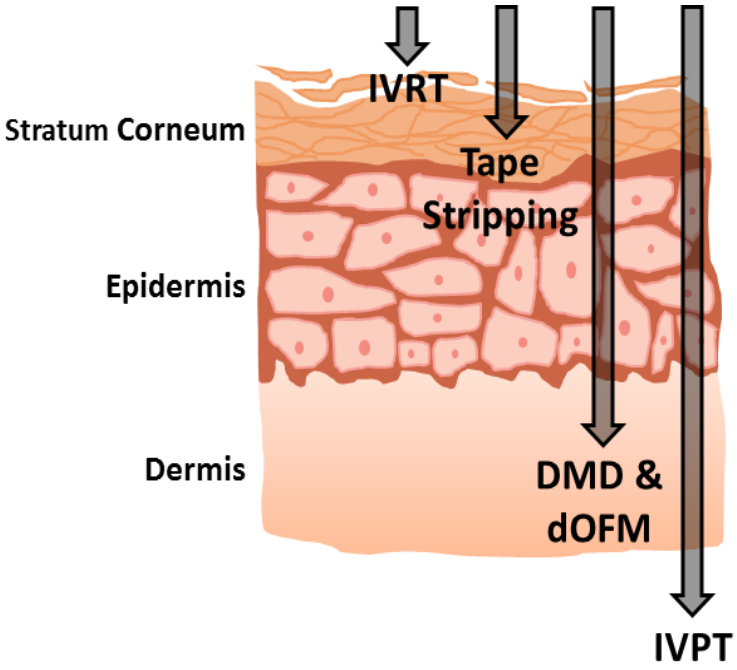
- Therapy can be interrupted
- Low drug efficiency

- Systemic Absorption is NOT desirable
- Local tissue levels ≈ Efficacy
- Open applications
- Highly individualized application techniques
- Short-acting

- No straightforward BA evaluation method

Flynn G.L. (2002). Cutaneous and Transdermal Delivery – Processes and Systems of Delivery. In *Modern Pharmaceutics* (pp. 187-235). New York, NY: Marcel Dekker, Inc.

Methods to Determine Bioavailability (BA)



- IVRT (in vitro release test)
- Tape-stripping
- DMD (dermal microdialysis) & dOFM (dermal open flow microperfusion)
- IVPT (in vitro permeation test)

+ VCA (Vasoconstriction Assay)

+ Clinical Studies

Question

Among so many methodologies, which one is considered the best?

The likely answer may be a combination of the different tests, depending on the drug, product, dosing frequency, tissue target, etc.

➪ A **Clinical Trial** is the only approval route for generic transdermal & topical products

※ Except VCA for glucocorticoids

and

Acyclovir Draft Guidance

Active ingredient: Acyclovir

- **Form/Route:** Ointment; Topical
- **Recommended study: 2 Options: *In Vitro* or *In Vivo* Study**
- **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.
- **II. In Vivo option:**
- Type of study: BE Study with Clinical Endpoint Design: Randomized, double-blind, parallel, placebo-controlled in vivo

http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm296733.pdf

Problems/Limitations of Clinical Studies

- Clinical trials are time-consuming and costly in general

For **Topical** Drug Products:

- Comparative clinical endpoint trials are relatively insensitive
- PK-based clinical trials
  - Amount of drug in blood is very small and difficult to quantify
  - Drug levels in blood can potentially be irrelevant to therapeutic activity at the site of action

→ Slows development of generic drug products

→ Burdens ($$$) healthcare system and patients

Objective

- Identify surrogate method(s) which closely simulate the complex mechanism of drug permeation through skin layers and drug retention within skin layers *in vivo* for selected transdermal and topical drug products

Hypothesis

- IVPT and/or other surrogate methods can predict the performance of transdermal and topical drug products *in vivo*

Positive Outcomes

- Examine IVPT and other surrogate methods for their relevance in developing IVIVC
- Develop IVIVC models which can predict the *in vivo* performance of transdermal and topical drug products

Selected TDS

Nicotine TDS

Fentanyl TDS

	NicoDerm CQ®	Aveva	Duragesic®	Mylan	Apotex
Patch size (cm^2)	15.75	20.12	10.5	6.25	10.7
Drug content (mg)	Not available	Not available	4.2	2.55	2.76
Rate/Area ($\mu g/h/cm^2$)	37	29	2.4	4.0	2.3
Inactive ingredients	Ethylene vinyl acetate-copolymer, polyisobutylene and high density polyethylene between clear polyester backing	Acrylate adhesive, polyester, silicone adhesive	Polyester/ethyl vinyl acetate backing film, polyacrylate adhesive	Dimethicone NF, silicone adhesive, polyolefin film backing	Isopropoyl myristate, octyldodecanol, polybutene, polyisobutene adhesive

Skin Preparation

- Fresh human skin samples obtained post abdominoplasty surgery
- Dermatomed to ~250 microns
- Frozen until the day of experiment

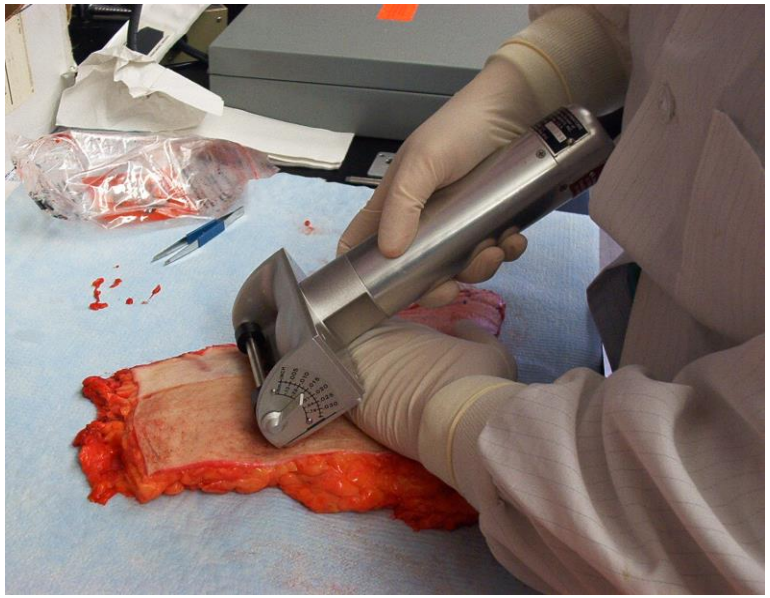
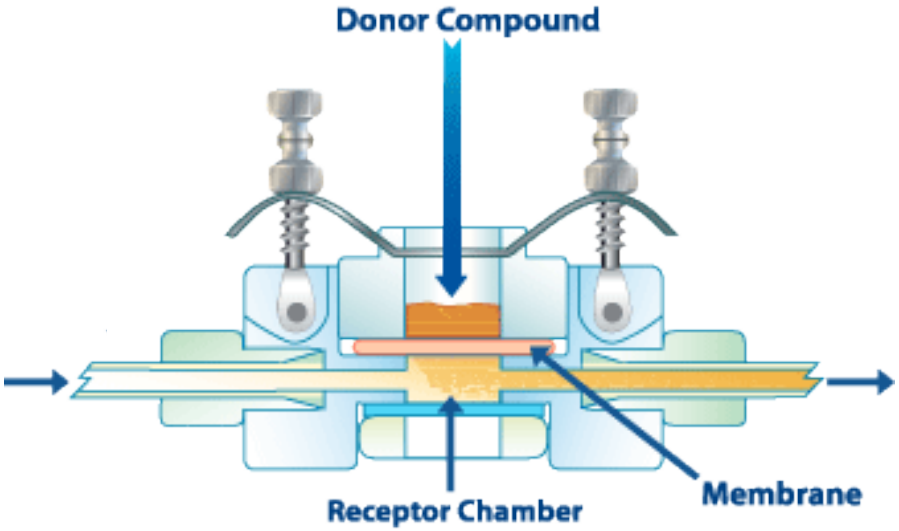
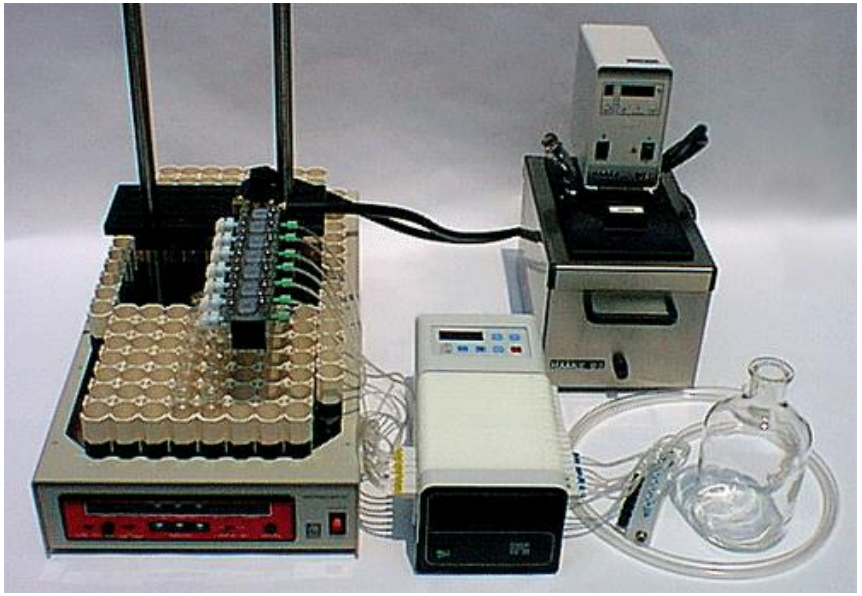


Image obtained from the Stinchcomb Lab's SOP

IVPT Setup

- In-line flow-through diffusion system
- Permeation area of 0.95 cm^2

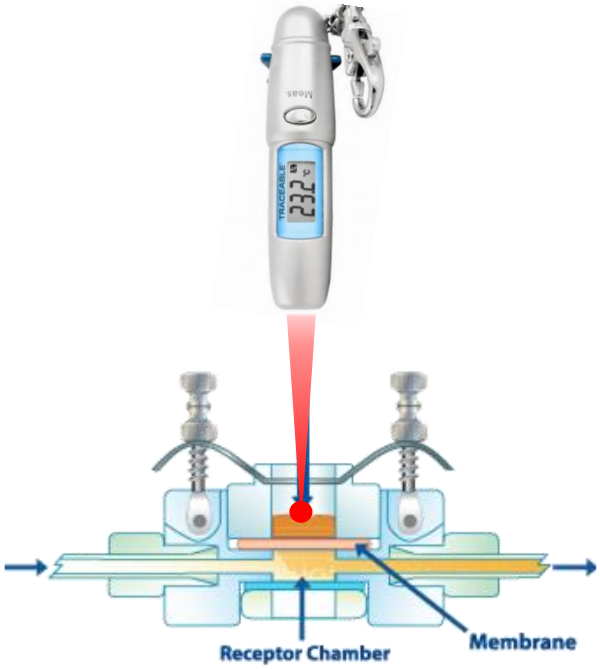


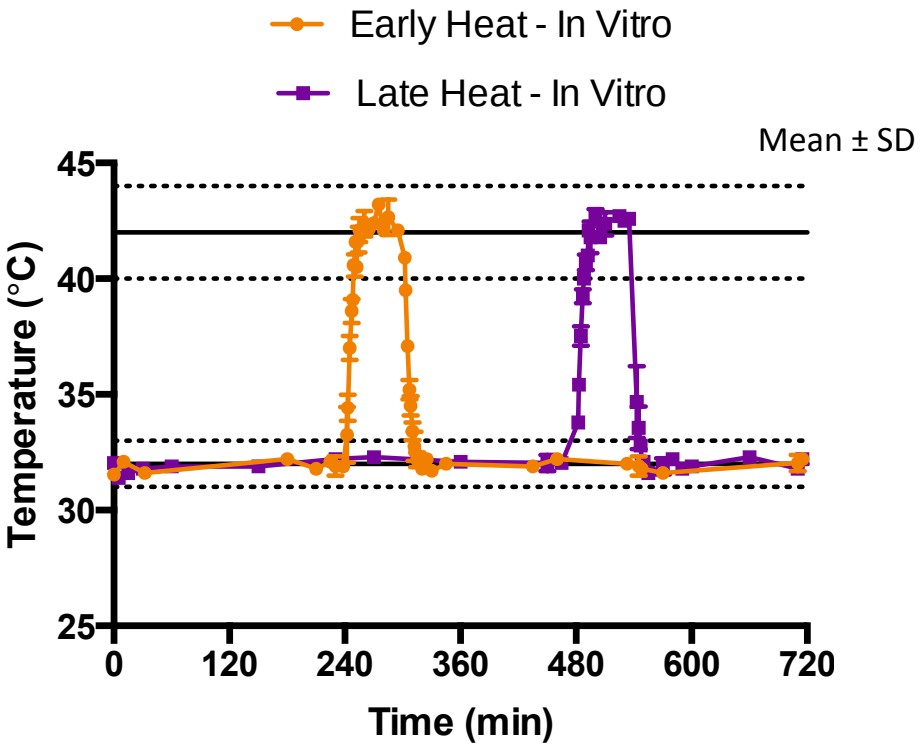


Images from www.ibric.org and www.permegear.com

Temperature Monitoring

- Infrared Thermometer





Images from https://traceable.com/products/thermometers/4480.html and www.permegear.com

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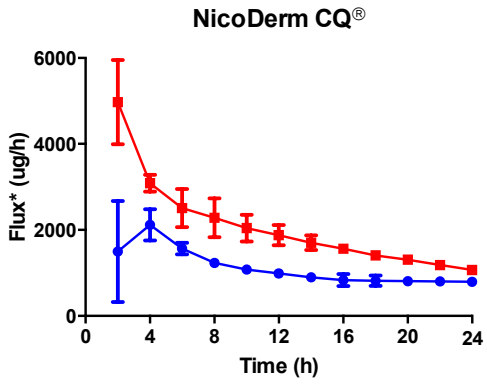
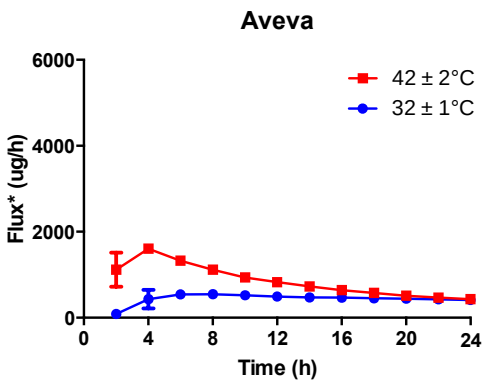
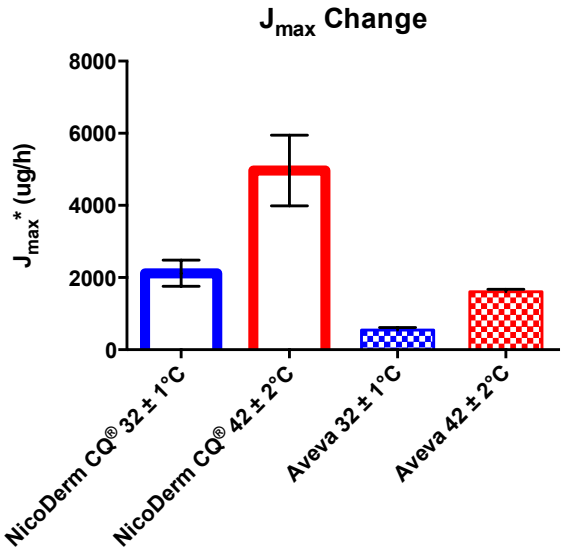
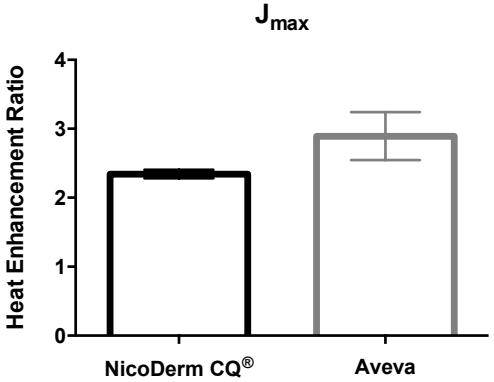
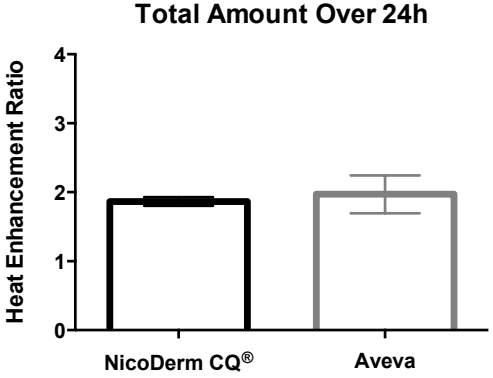
IVPT Continuous Heat Effect









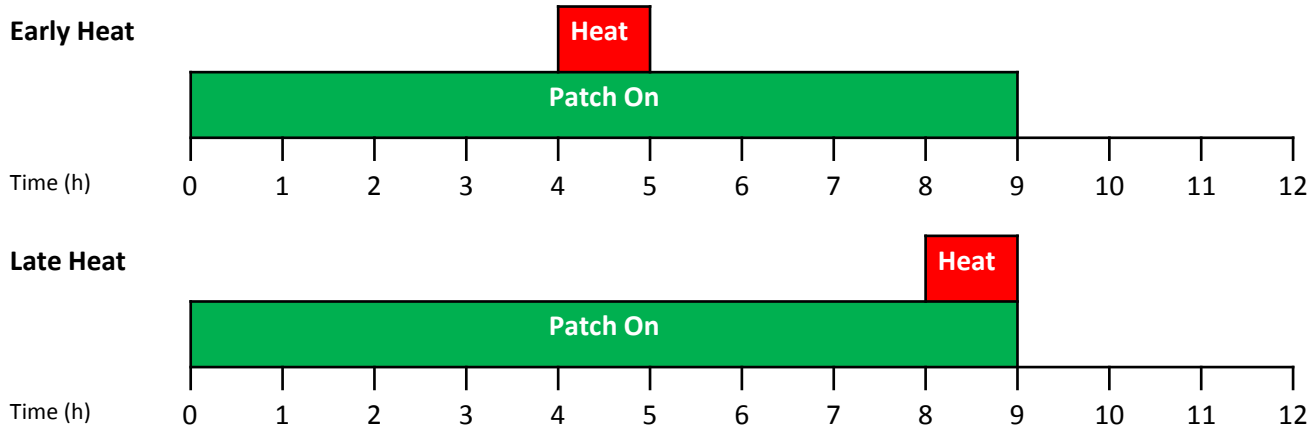


Human Skin Data

Mean ± SD from 2 donors with n=4 per each donor

Clinical Study Designs – Nicotine

- A four-way crossover PK study in 10 adult smokers (two nicotine TDS)



- Residual amount of nicotine in TDS was analyzed

- Temperature of skin surface was monitored throughout the study

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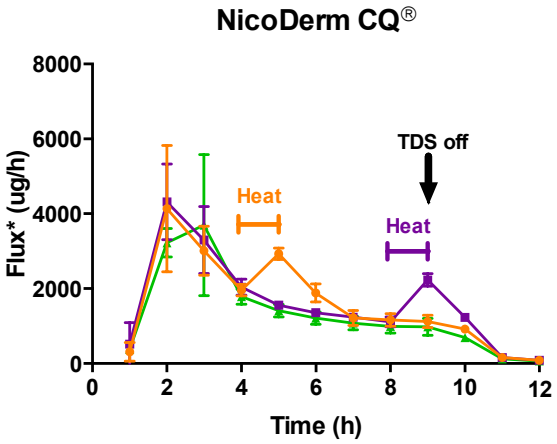
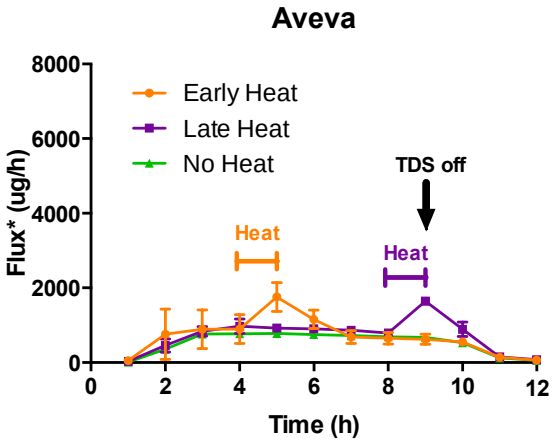
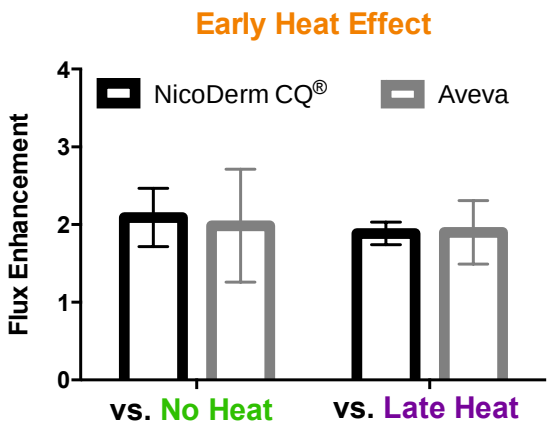
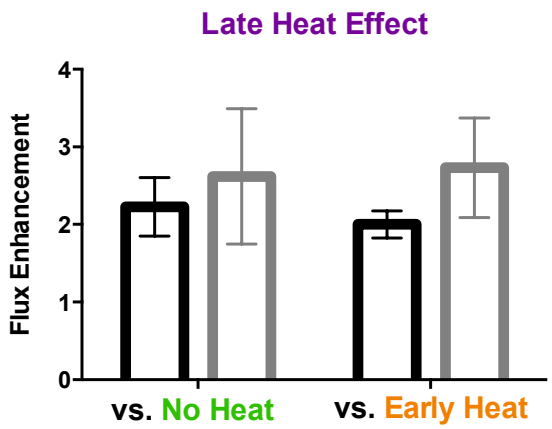
Preliminary: IVPT Temporary (1h) Heat Effect





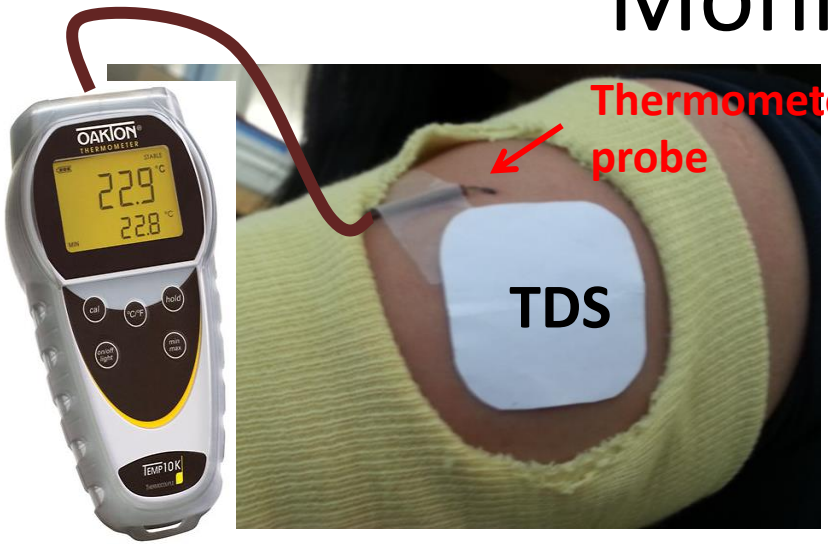




Human Skin Data

Mean ± SD from 4 donors for Heat and 2 donors for No Heat with n=4 per each donor

Heat application and Temperature Monitoring

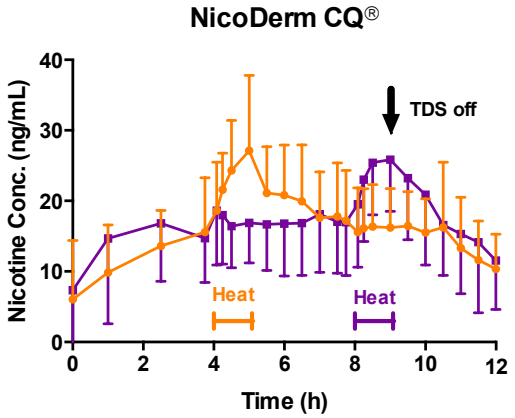


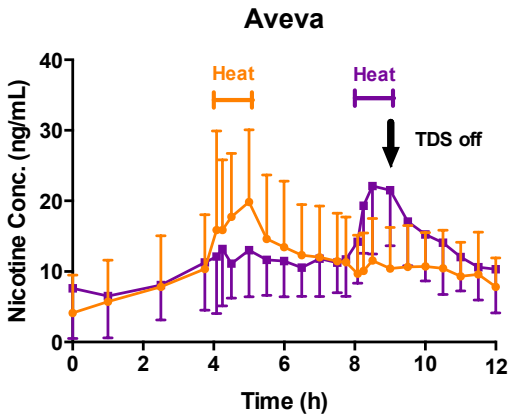
- Kevlar sleeve with an opening to expose TDS, while protecting skin from other areas
- Thermometer probe adjacent to TDS

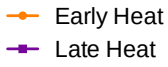
- Pre-heated heating pad
- ACE™ Bandage to ensure good contact between TDS and heating pad

Image from http://static.coleparmer.com/large_images/91427_10_5.jpg

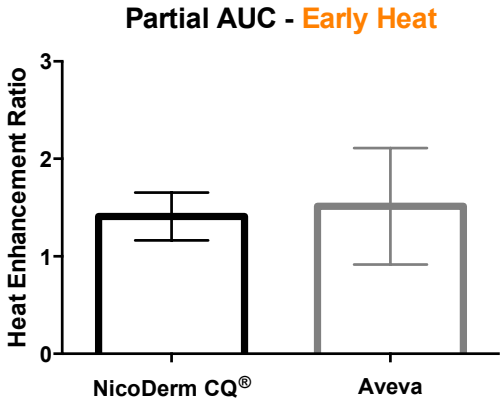
Nicotine PK profiles

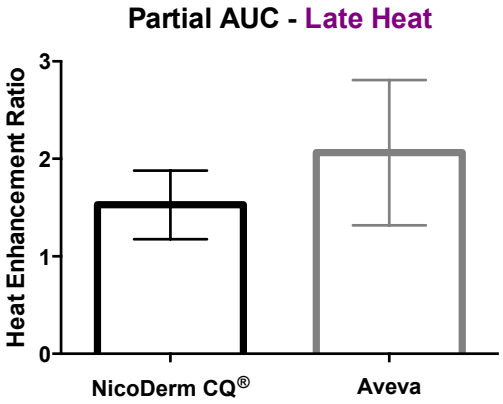






Mean ± SD from 10 Subjects





- Serum samples analyzed by S. Thomas
- LC-MS/MS method developed by I. Abdallah

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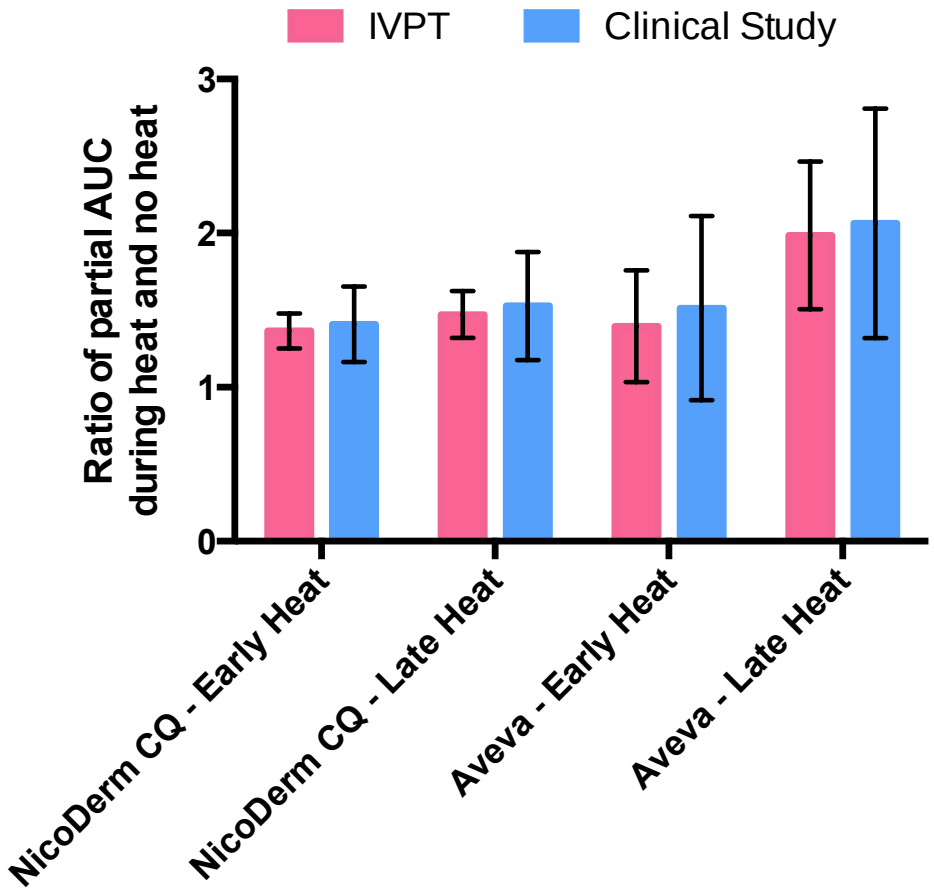
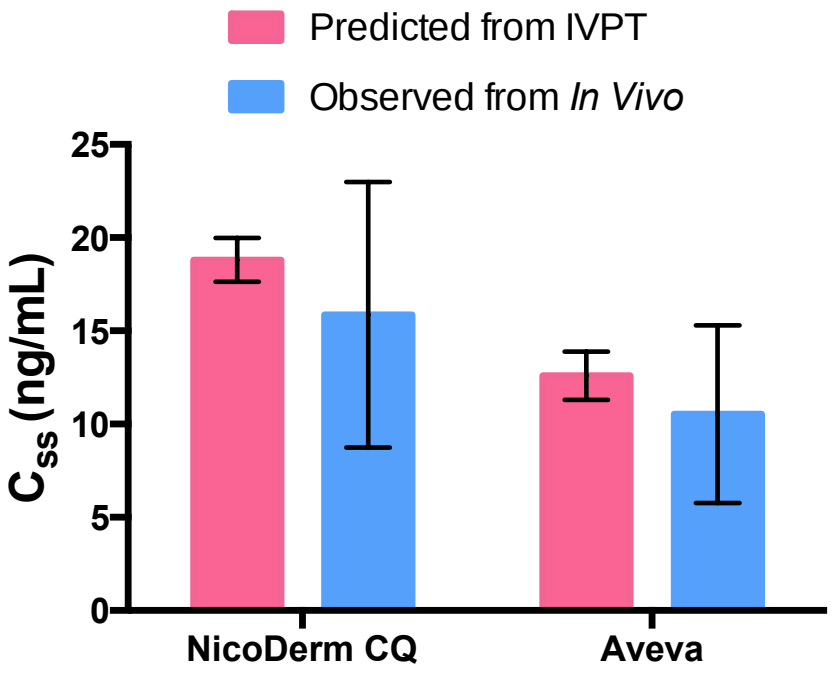
IVIVC – Heat Effect on Nicotine TDS



- $p > 0.05$ between IVPT and clinical study results
- IVPT can predict heat effect on TDS *in vivo*

IVIVC – Absence of Heat



- At steady-state, $R_{in} = R_{out}$
- R_{in} (ng/hr) = J (ng/cm^2/hr) x Area (cm^2)
- $R_{in} = CL \times C_{ss}$
- CL = 72000 mL/h

- $p > 0.05$ between predicted and observed C_{ss}
- IVPT can predict the performance of TDS *in vivo*

Evaluation of the relative bioavailability of topical drug products by various surrogate methods and development of IVIVC

Hypothesis: Well-designed and optimized surrogate method(s) can be used to predict bioavailability and performance of topical drug products *in vivo.*

Approach

1) IVPT experiments will be done with a focus of investigating effects of different experimental conditions and techniques involved in IVPT

- Dose amount selection
- Dose administration techniques & rubbing effect
- Multiple-dosing designs

2) Other surrogate methods which evaluate the drug retention within skin layers will be investigated and performed

Biosensors

Infrared Spectroscopy

DPK—Tape stripping

3) Obtained data through experiments, literature, and collaborators will be compared to determine which method(s) best predict the performance of topical drug products *in vivo*

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Dermatopharmacokinetics (DPK) Tape-stripping

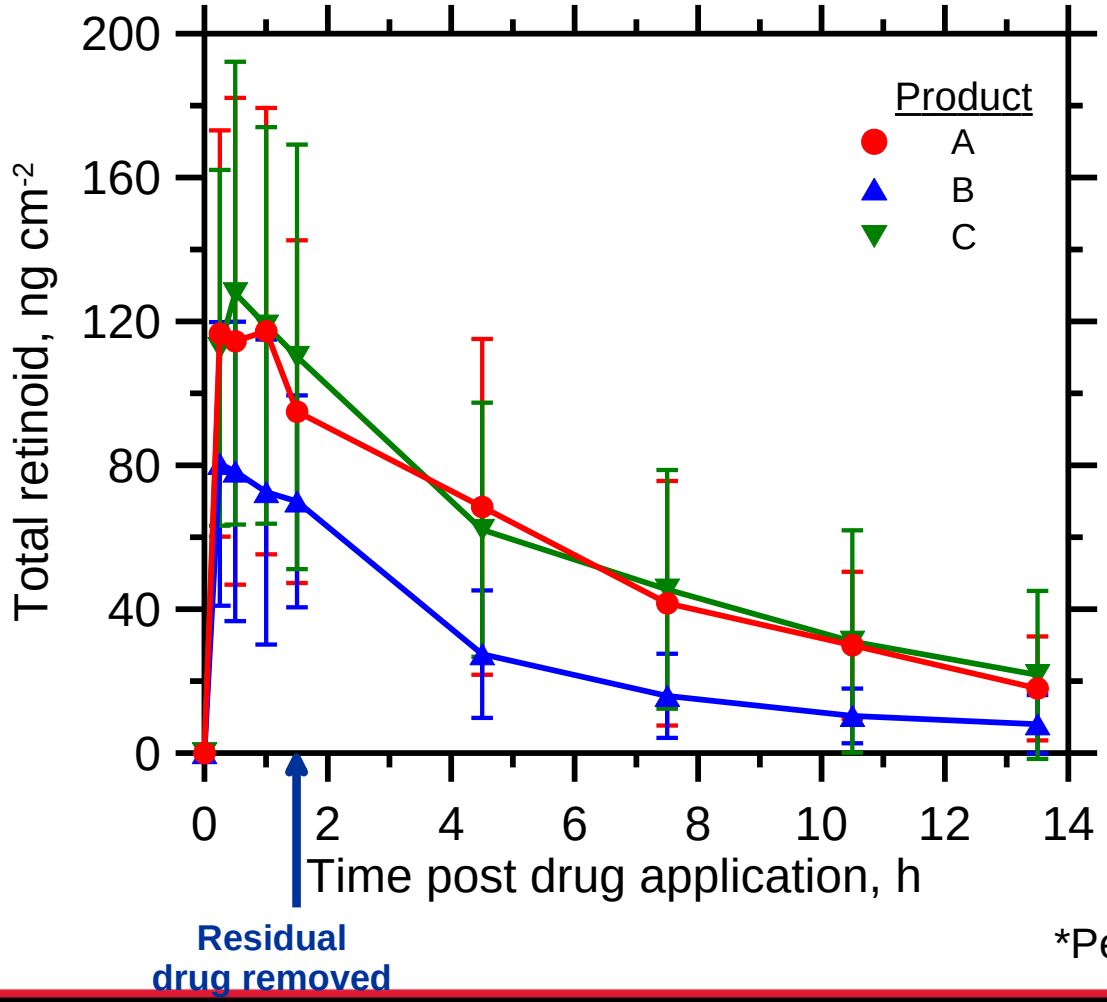
Dr. Annette Bunge, CO School of Mines

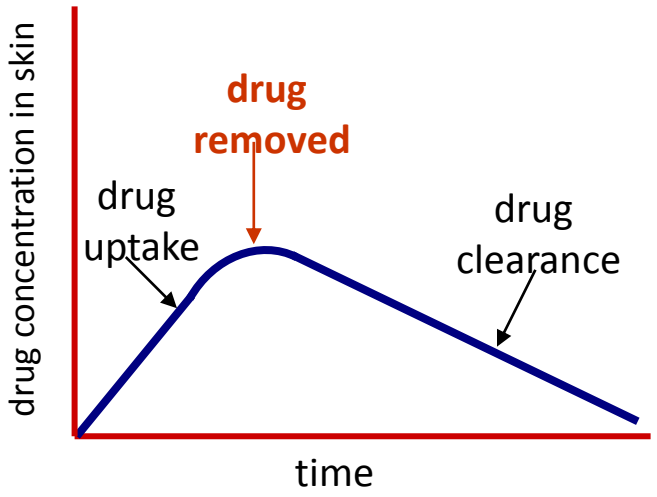
Univ. of Bath--Dr. Richard Guy

Dr. Begoña Delgado-Charro

Assess BE using DPK: *Tretinoin gel 0.025%**

Comparing Products B and C to Product A (RLD)





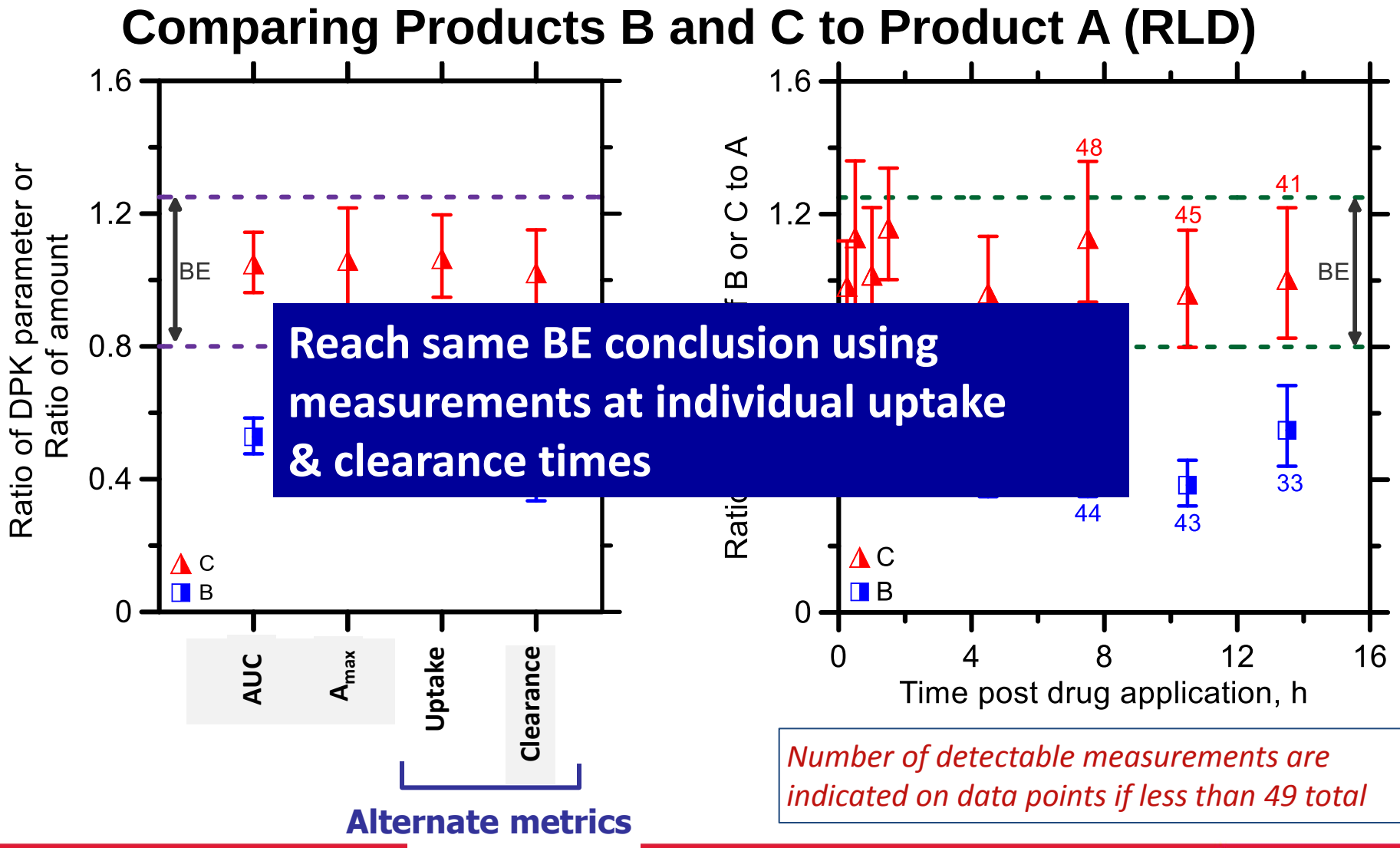
Mimic oral BE assesment

Compare AUC & A_{max}

*Pershing et al., J Am Acad Dermatol 2003

*Data from Pershing; N'Dri-Stempfer *et al.*, Pharm Res, 2008

Assess BE using DPK: *Tretinoin gel 0.025%**

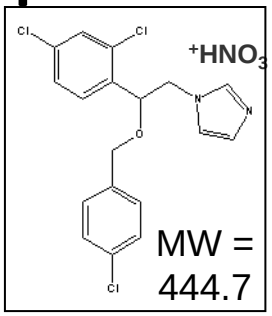


Number of detectable measurements are indicated on data points if less than 49 total

Improved protocol developed for FDA

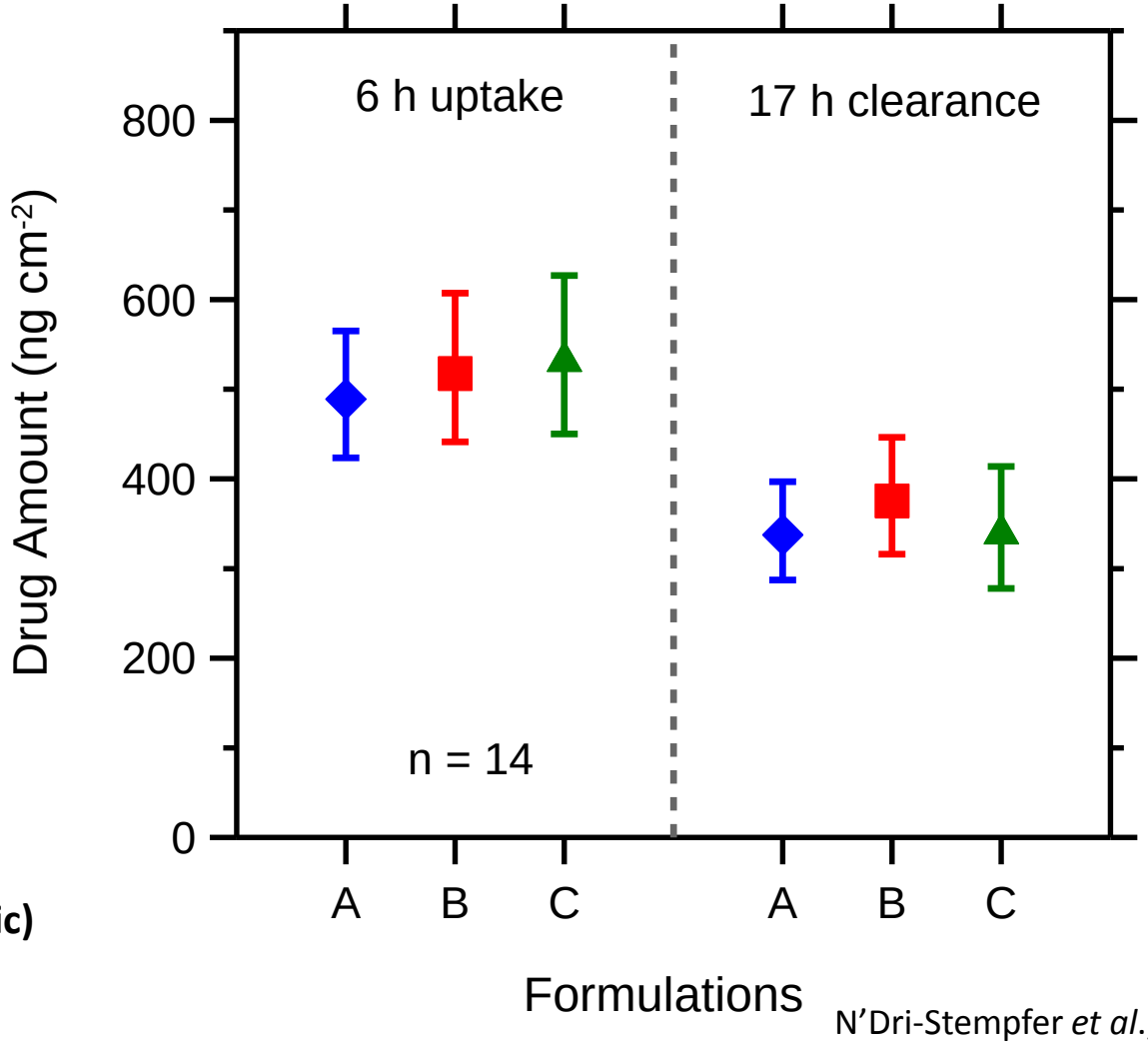
- 4 treatment sites / product
  - 1 uptake time & 1 clearance time
  - Duplicate determinations at each time
- Remove unabsorbed drug using isopropyl alcohol wipes
- Total drug amount = Drug from all tapes (no tapes discarded)
- Determine ~*all* drug in SC by removing nearly all of the SC
  - Remove SC until TEWL > 8 x (TEWL before stripping)
  - At least 12 tape strips, but not more than 30 tape strips
  - Tape stripping area < drug application area (control both areas)
- Assess BE of uptake and clearance separately
- Analyze tape strips in groups to optimize analytical sensitivity
- Compare within each subject and then across subjects

Demonstrating the improved protocol



- Econazole nitrate 1% cream
  - Antifungal – SC is target site
- Compare 2 generic products to RLD
  - Both products Q1 and Q2 equivalent
- 6 h uptake time & 17 h clearance time
  - Chosen based on pilot study results, and
  - Convenience for subjects and operator

Econazole in SC: *Average drug amounts*



A = Clay-Park (Generic)

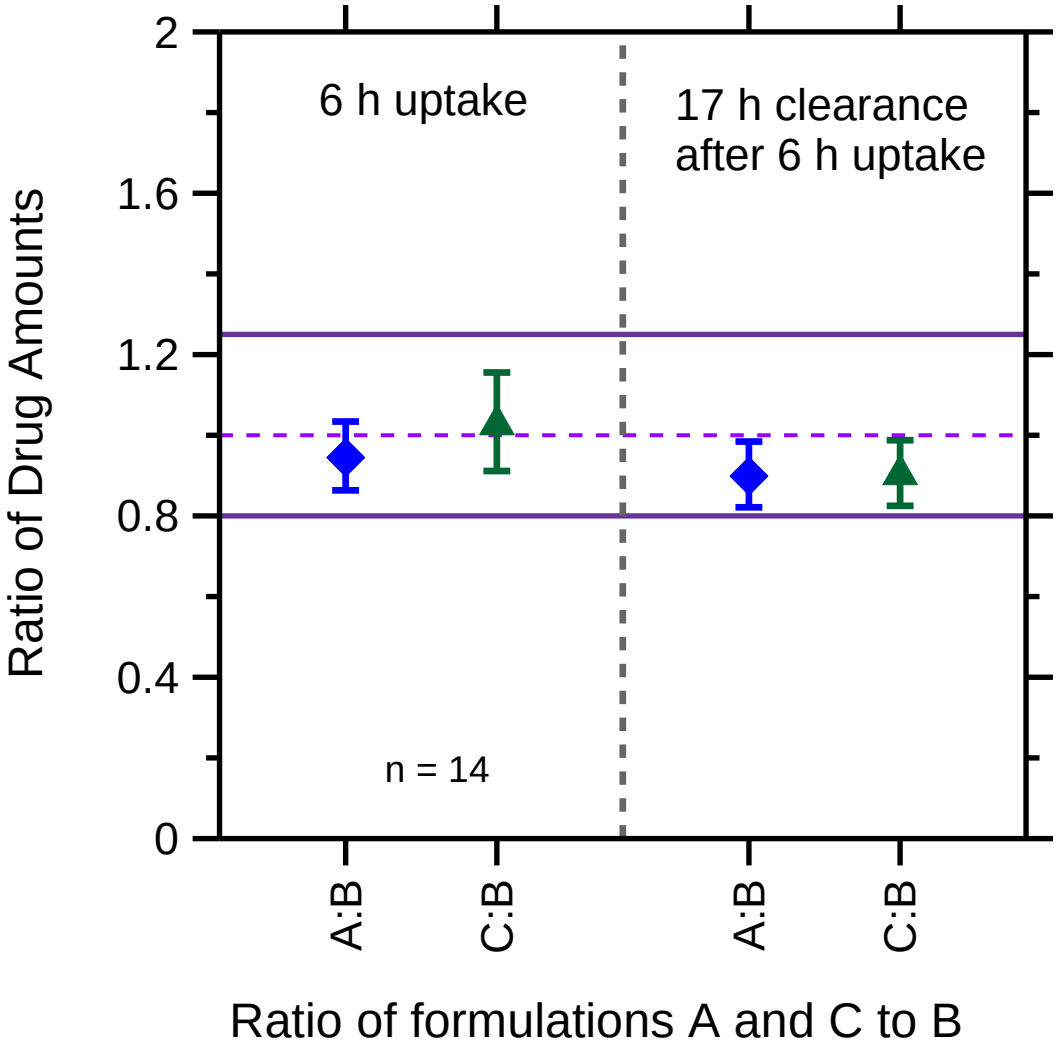
B = Ortho (RLD)

C = Taro (Generic)

N'Dri-Stempfer *et al.*, Pharm Res, 2009

Econazole in SC: *BE assessment*

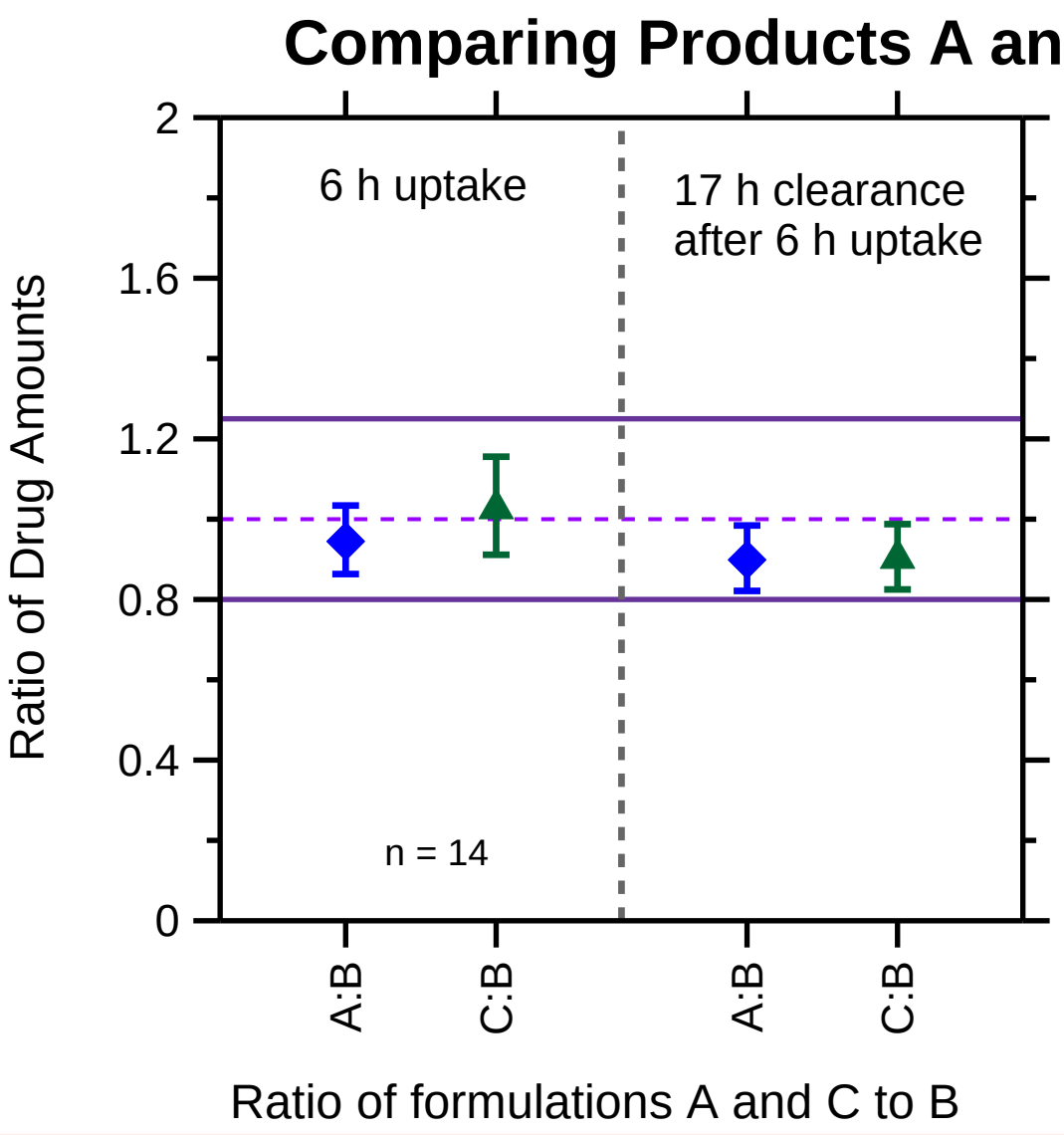
Comparing Products A and C to Product B



- Both A and C were conclusively BE with B after uptake and clearance, evaluated separately.

N'Dri-Stempfer *et al.*, Pharm Res, 2009

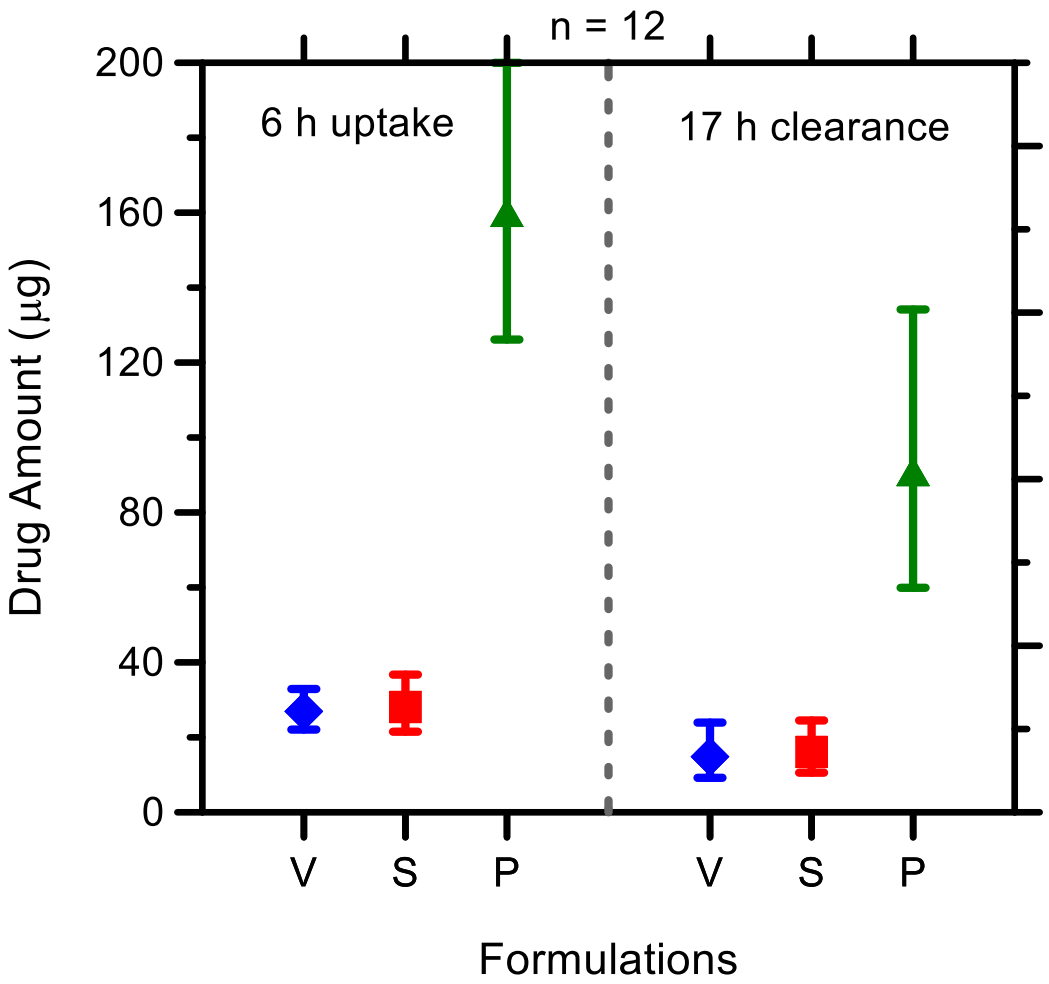
Econazole in SC: *BE assessment*



- Both A and C were conclusively BE with B after uptake and clearance, evaluated separately.
- Only **168 sites** (3 products in 14 subjects with replicates for uptake & clearance = 3 x 14 x 2 x 2)
- Compare with **1176 sites** in tretinoin gel study (3 products in 49 subjects with 8 sites/product = 3 x 49 x 8)

N'Dri-Stempfer *et al.*, Pharm Res, 2009

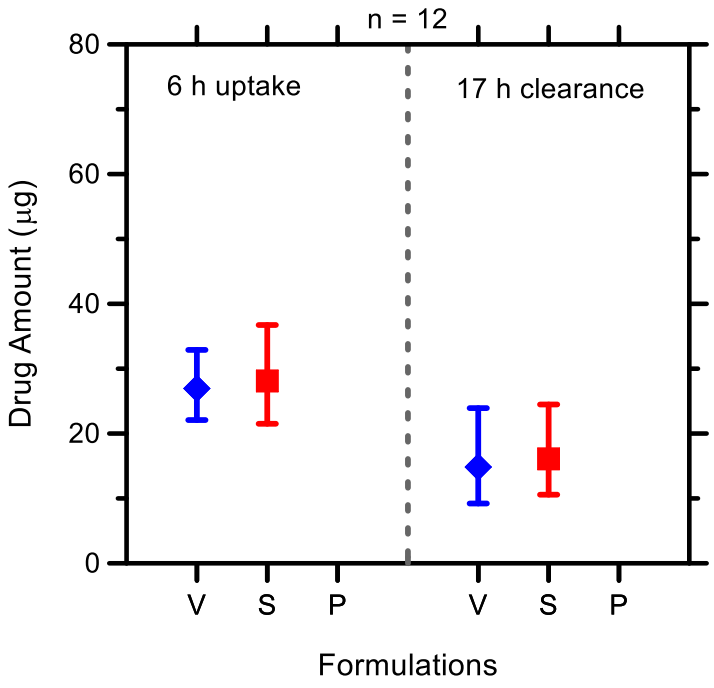
Diclofenac: *Average drug amounts in SC*



V = Voltaren

S = Solaraze

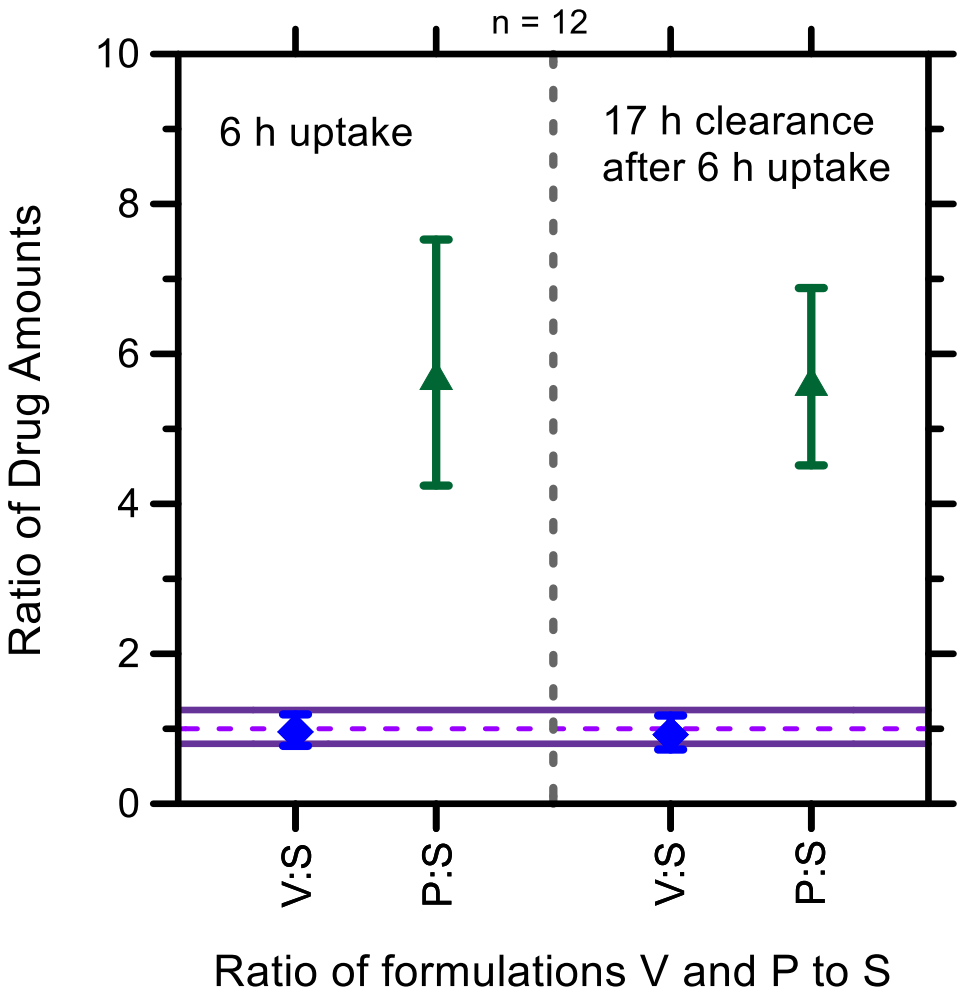
P = Pennsaid



Error bars, 90% CI of the log mean

Diclofenac: *BE ratio of drug amounts in SC*

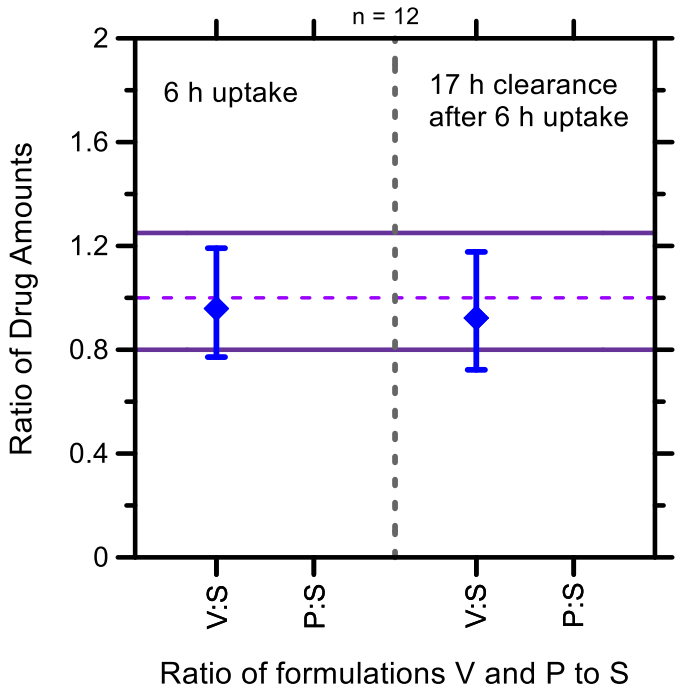
Comparing Products V and P to Product S



V = Voltaren

S = Solaraze

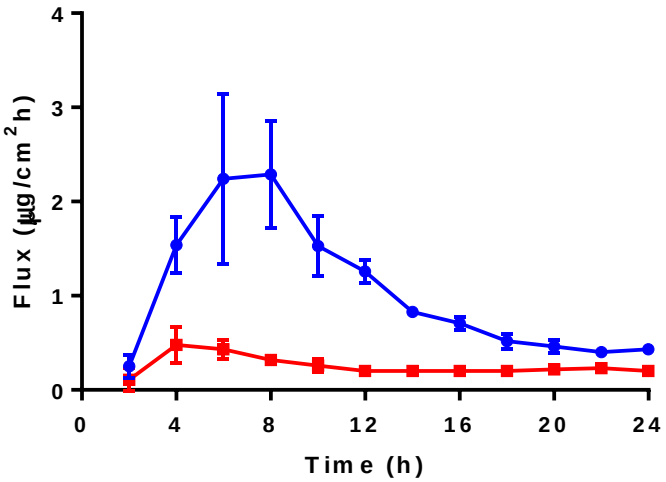
P = Pennsaid

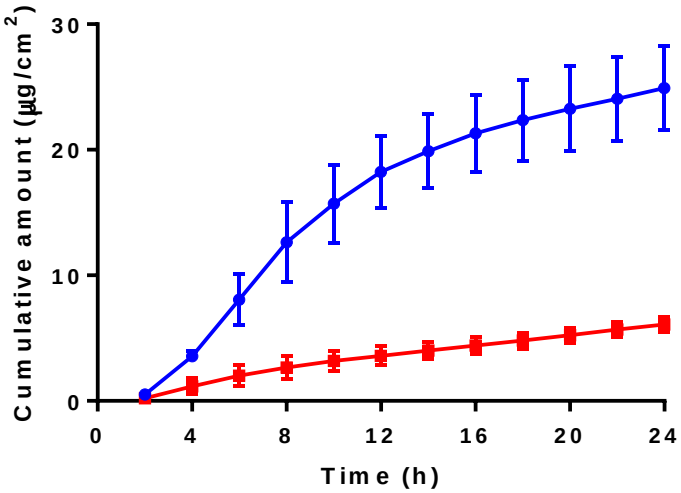


Error bars, 90% CI of the log mean

IVPT

Importance of Dose – Voltaren® gel

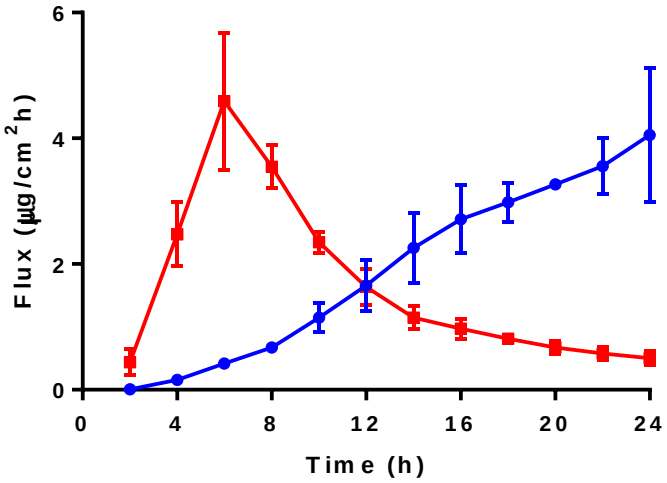


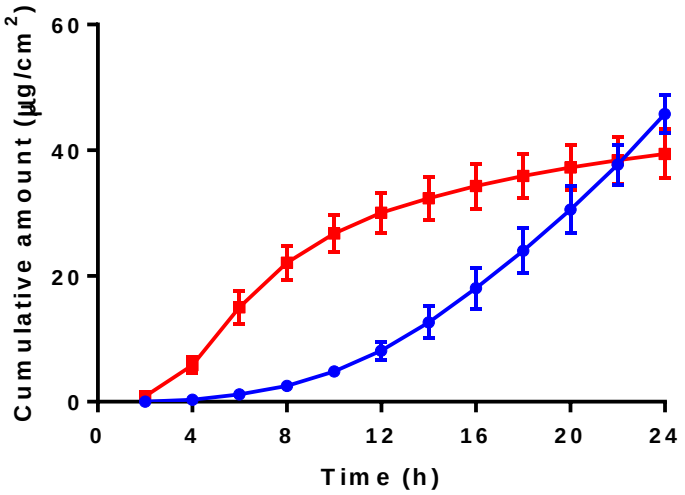


Mean ± SD (n=3)
Yucatan Miniature Pig Skin

	J_{max} ± SD (µg/cm²/h)	T_{max} (h)	Cumulative Amount ± SD (µg/cm²)
40 mg/cm²	2.29 ± 0.57	8	24.91 ± 3.38
10 mg/cm²	0.48 ± 0.19	2	6.10 ± 0.61

Importance of Dose – Pennsaid® 2%





Mean ± SD (n=3-4)
Yucatan Miniature Pig Skin

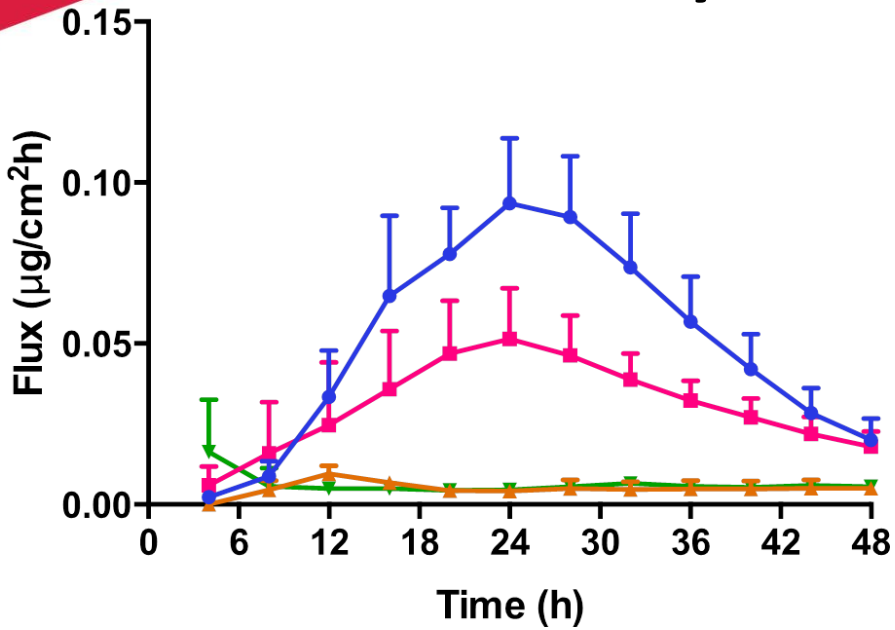
	J_{max} ± SD (µg/cm²/h)	T_{max} (h)	Cumulative Amount ± SD (µg/cm²)
100 mg/cm²	4.05 ± 1.06	24	45.79 ± 3.00
5 mg/cm²	4.59 ± 1.09	6	39.43 ± 3.90

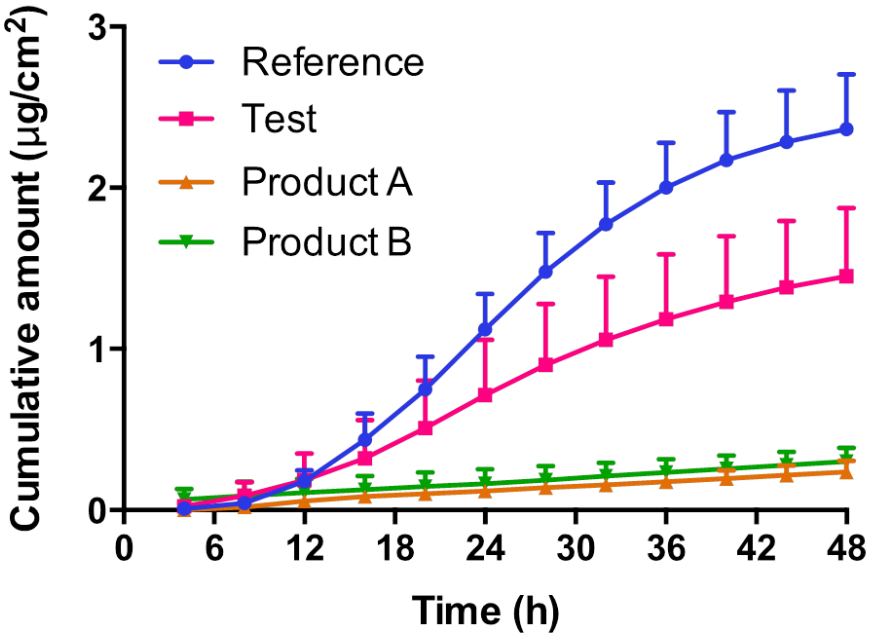
Dose Administration Techniques

- Highly variable among labs, researchers, and patients
  - Methods of dispensing formulation
  - Duration of rubbing
  - Force used for rubbing
  - Loss of formulation during rubbing
- Need a reproducible and clinically-relevant technique

Image from http://www.telegraph.co.uk/expat/expatlife/10441983/Pale-and-interesting.html

Four Acyclovir Cream Products





(Mean ± SE, n= 6 donors with 4-7 replicates per donor for Reference and Test products and n = 2 donors with 3-4 replicates per donor for Products A and B)

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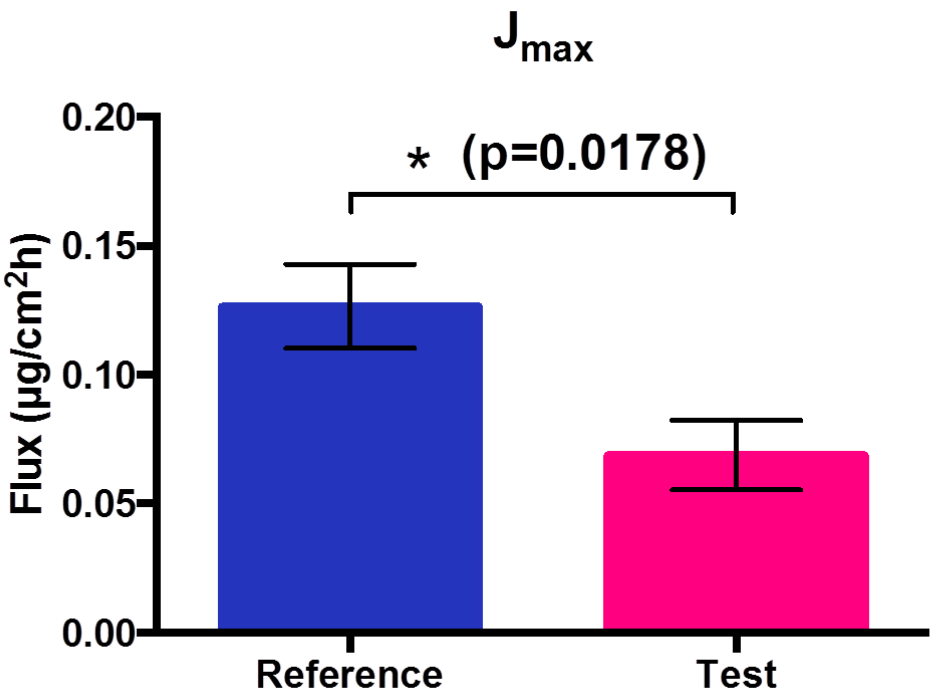
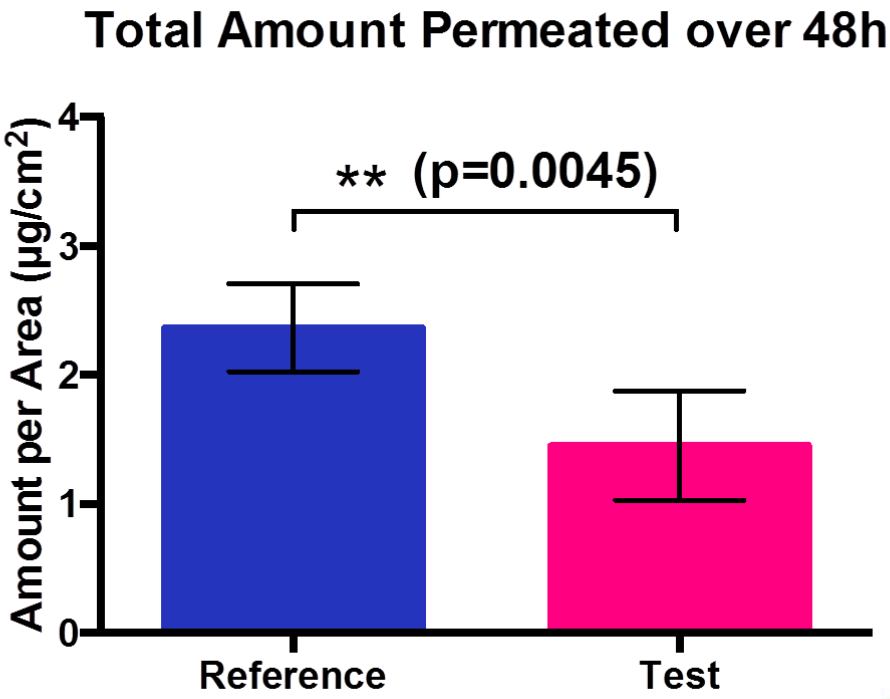
J_{max} and the total amount of acyclovir permeated over 48h between Reference and Test

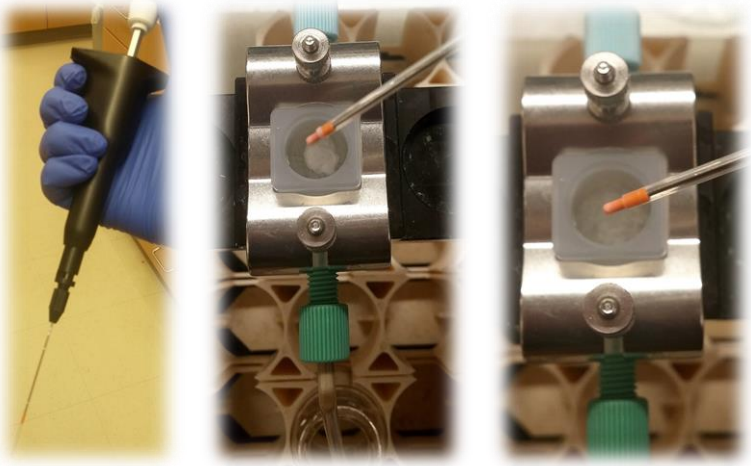




Comparisons of products(Mean ± SE, n= 6 donors with 4-7 replicates per donor)

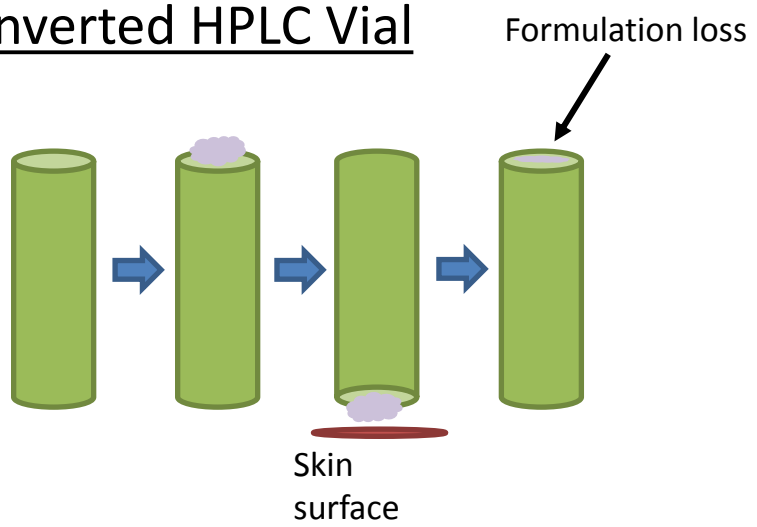
Dose Administration Techniques

Positive Displacement Pipette



- Quick, convenient, low variability
- Minimal formulation loss
- Lack of rubbing effect

Inverted HPLC Vial



- Time-consuming, more variability
- Some formulation loss
- Simulates clinically-relevant rubbing effect

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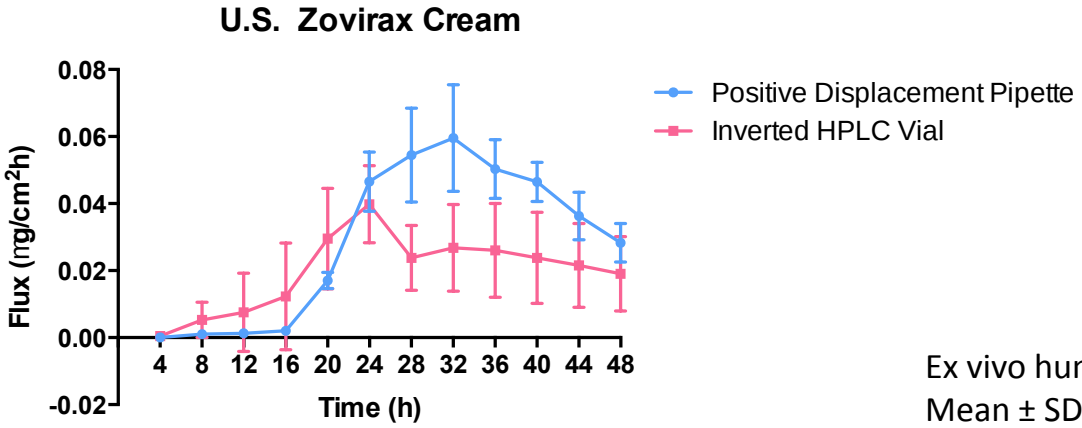
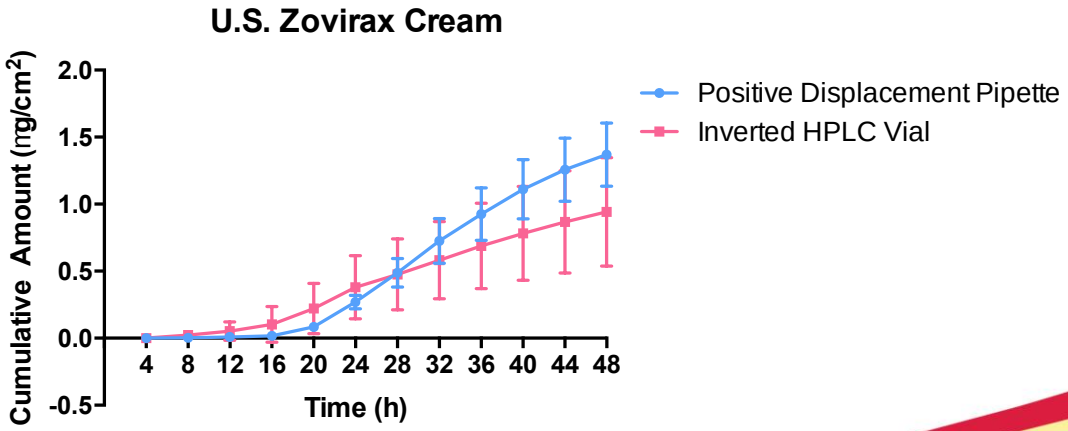
Dose Administration Techniques





Ex vivo human skin
Mean ± SD (n=4 for each technique)

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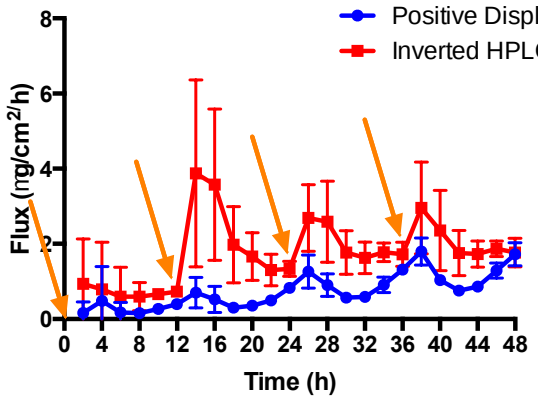
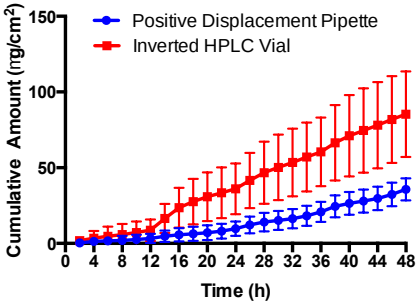
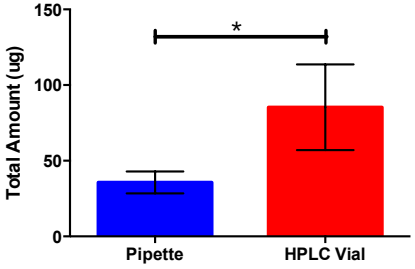
Preliminary: Dose Administration Techniques

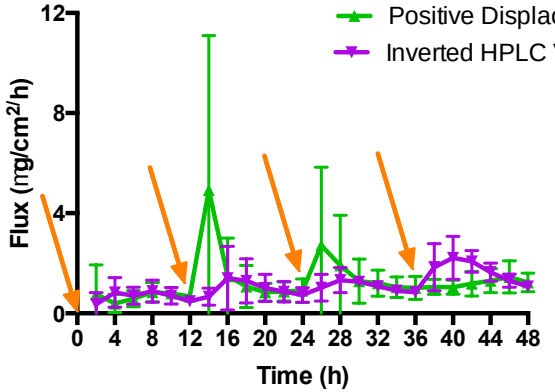
Pennsaid® 2% (more viscous)

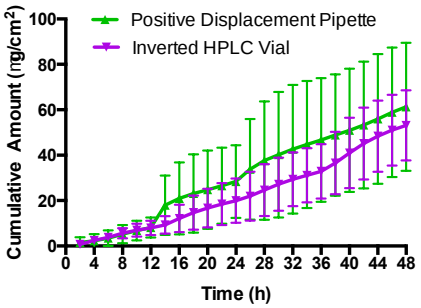


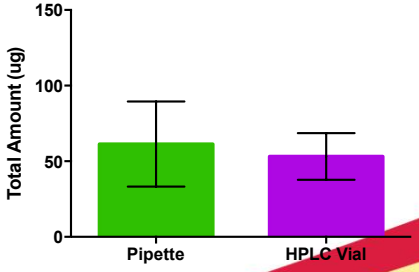




Pennsaid® 1.5%







Orange Arrow: dosing (~5 mg/cm^2 of formulation)

Mean ± SD (n=3-4)
Yucatan Miniature Pig Skin

Conclusions

- Limitations of clinical studies for topical drug products highlight the needs for developing surrogate methods to evaluate BA
- The IVPT method was able to discriminate the Reference and Test acyclovir products, based on J_{max} and the total amount of acyclovir permeated over 48h
- In order for surrogate methods to be recognized by regulatory agencies, they need to be able to produce data that is reliable, low in variability and relevant to clinical settings
- Each method will have its own challenges to overcome
  - Needs to be addressed in order to evaluate IVIVC

The views expressed in this presentation do not reflect the official policies of the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

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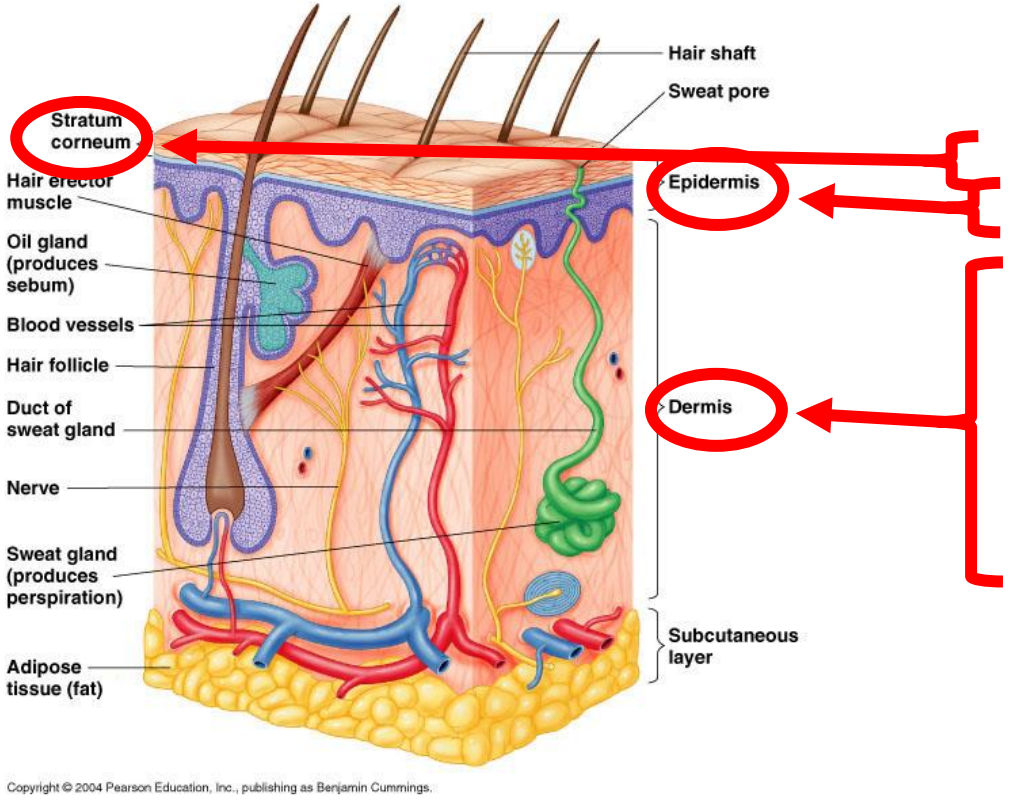
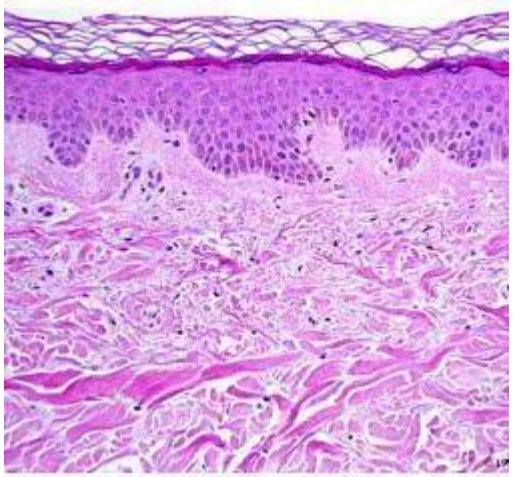
- 1U01FD004955-01

Back Up

Skin Structure

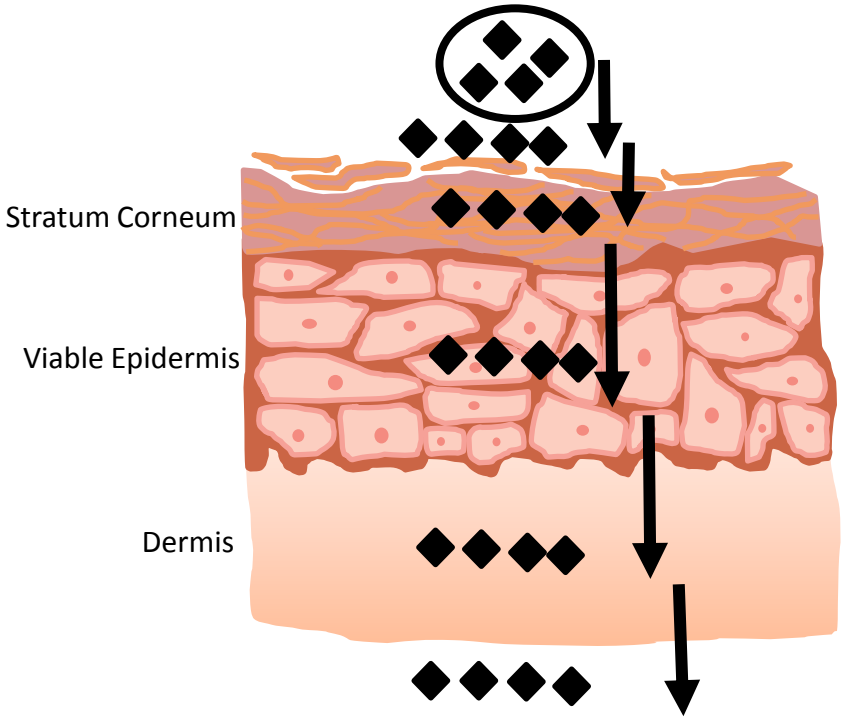




Images from http://classes.midlandstech.edu/carterp/courses/bio225/chap21/ss1.htm and http://www.scienceprog.com/skin-structure/

Percutaneous Absorption (Transepidermal route)

Release of drug from vehicle → Penetration through skin barriers → Activation of pharmacological response



- **Dissolution of drug in vehicle**
- **Passive diffusion of drug out of its vehicle to skin surface**
- **Drug partition into SC**
- **Drug diffusion through SC**
- **Drug partition into viable epidermis**
- **Drug diffusion through viable epidermis**
- **Drug partition into dermis**
- **Drug diffusion through dermis**
- **Drug partition into blood capillary**
- **Systemic uptake**

Factors Affecting Percutaneous Absorption

Drug

- M.W. < 500 Dalton
- Suitable log $P_{oil/water}$
  - High log P (very lipophilic) -> too much retention in the skin
  - Low log P (very hydrophilic) -> difficult to cross the SC
- Unionized molecules cross SC faster

Vehicle/Formulation

(Inactive Ingredients)

- Partition coefficient, $k_{membrane/vehicle}$
- pH

Skin

- Hydration level
- Age
- Gender
- Race
- Species
- Disease state

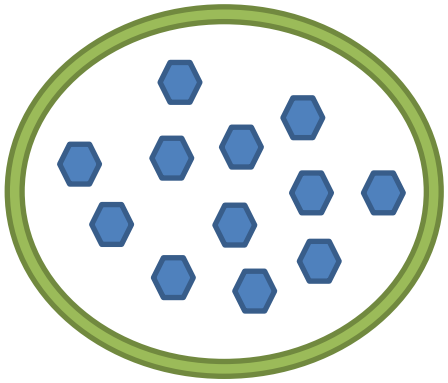
Environmental factors

- Humidity
- Occlusion
- Heat (high temperature)

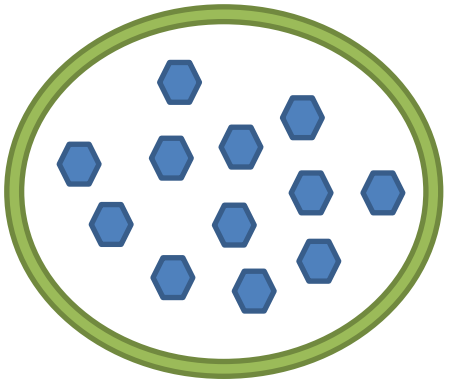
Flynn G.L. (2002). Cutaneous and Transdermal Delivery – Processes and Systems of Delivery. In *Modern Pharmaceutics* (pp. 187-235).
Barry B.W. (2007). Transdermal Drug Delivery. In *Aulton's Pharmaceutics: The Design and Manufacture of Medicines* (pp. 565-597).

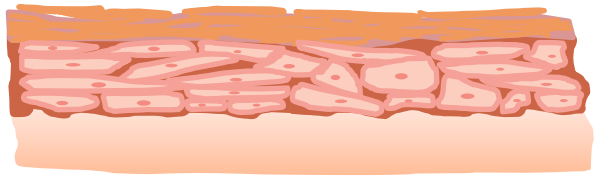
Influence of Heat on Percutaneous Absorption

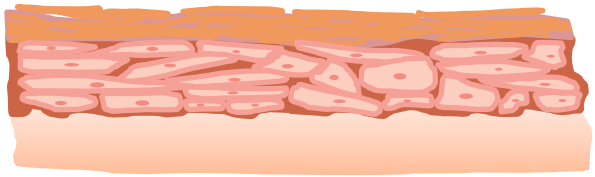
1) ↑ Diffusivity of Drug from its Vehicle



+ Heat ➜

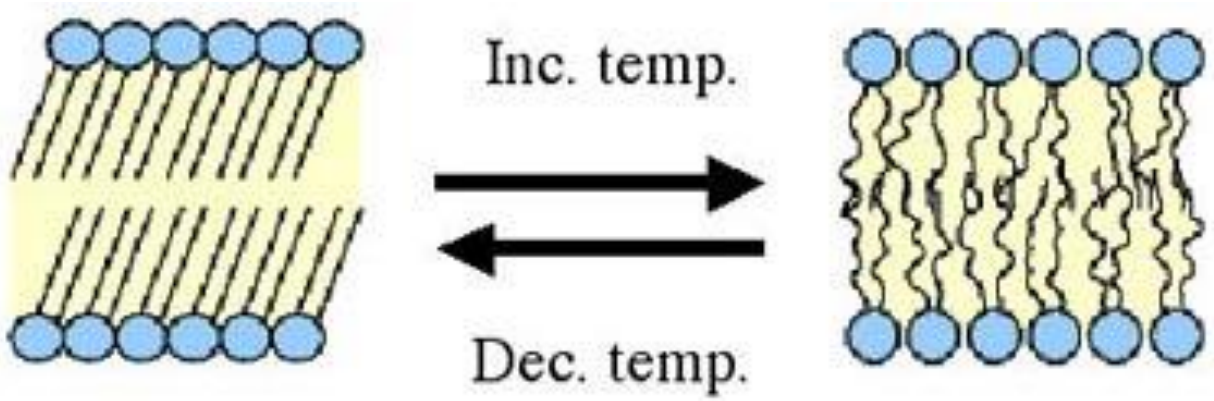






Influence of Heat on Percutaneous Absorption

2) ↑ Fluidity of Stratum Corneum Lipids



Very regular,
Ordered structure

Less tightly packed,
Hydrocarbon tails
Disordered.

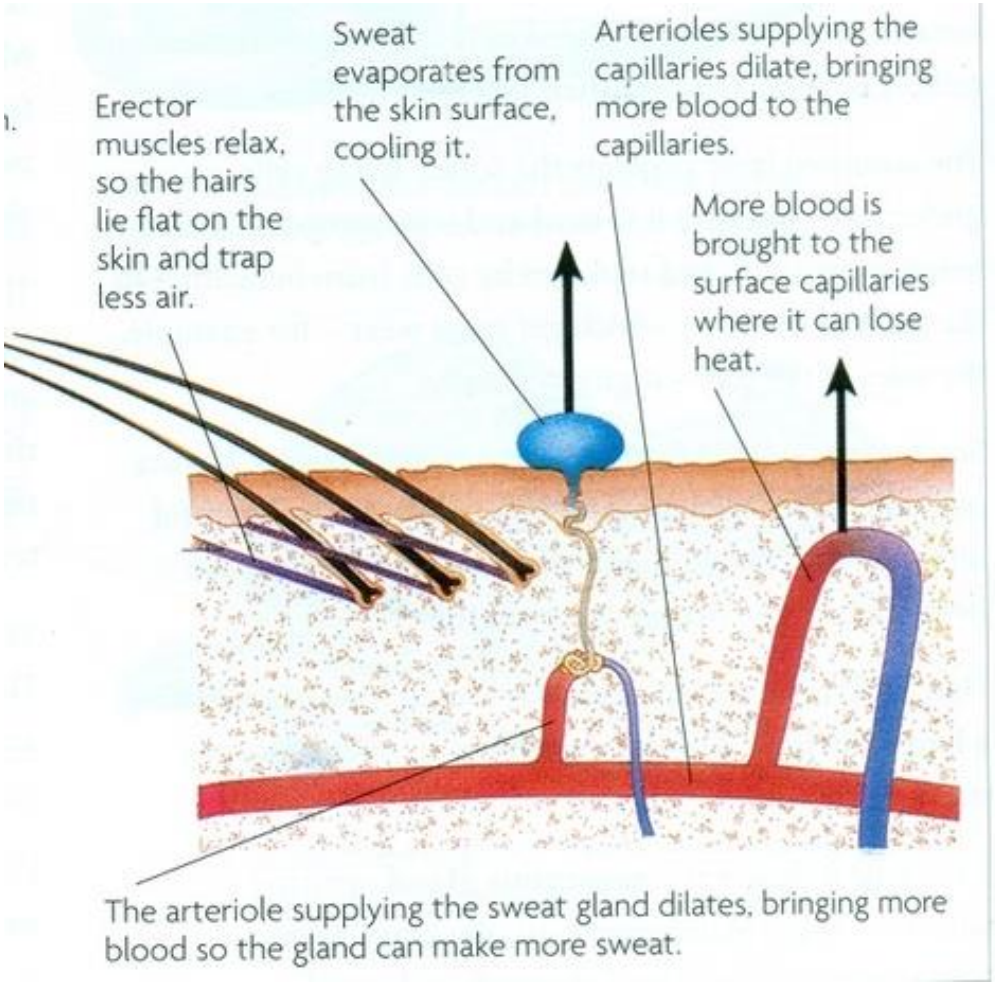
https://biochemistry3rst.wordpress.com/tag/phosphodiate/

Influence of Heat on Percutaneous Absorption

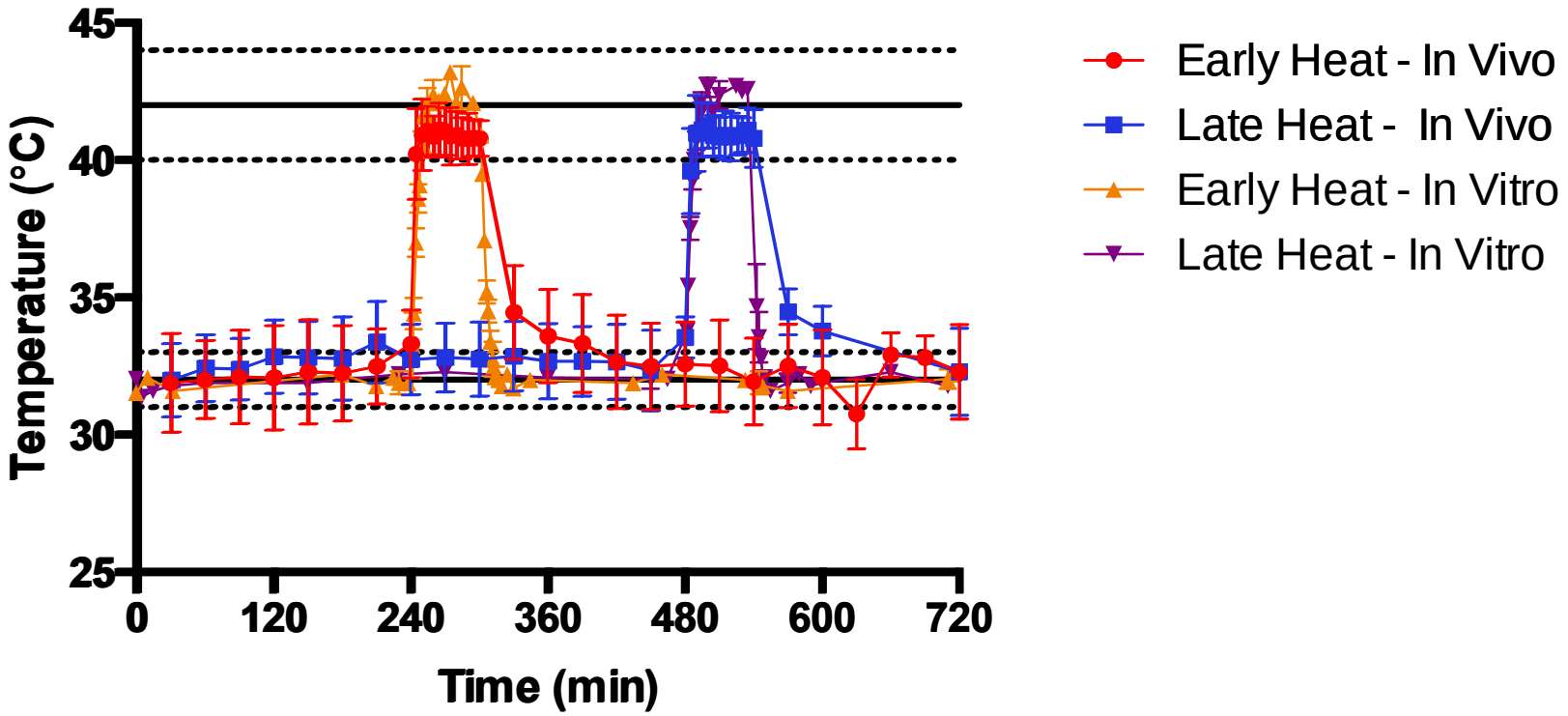
3) ↑ Cutaneous Vasodilation

Body temperature regulation

When the body is too hot



Temperature Monitoring



Residual Patch Analysis

- Objective: to investigate whether residual patch analysis can be a potential surrogate method for predicting the extent of drug absorption from TDS
- Extraction solvent, volume of extraction solvent, and the duration of extraction needs to be tested and optimized for each TDS
- For nicotine TDS, the total drug content is unknown:

Therefore, unused patch was extracted using the selected extraction method

Amount extracted from unused patch − Amount extracted after IVPT = Amount expected to be delivered

$$\frac{\text{Amount remaining after IVPT}}{\text{Amount extracted from unused patch}} \times 100 = \%\ \text{drug remaining}$$

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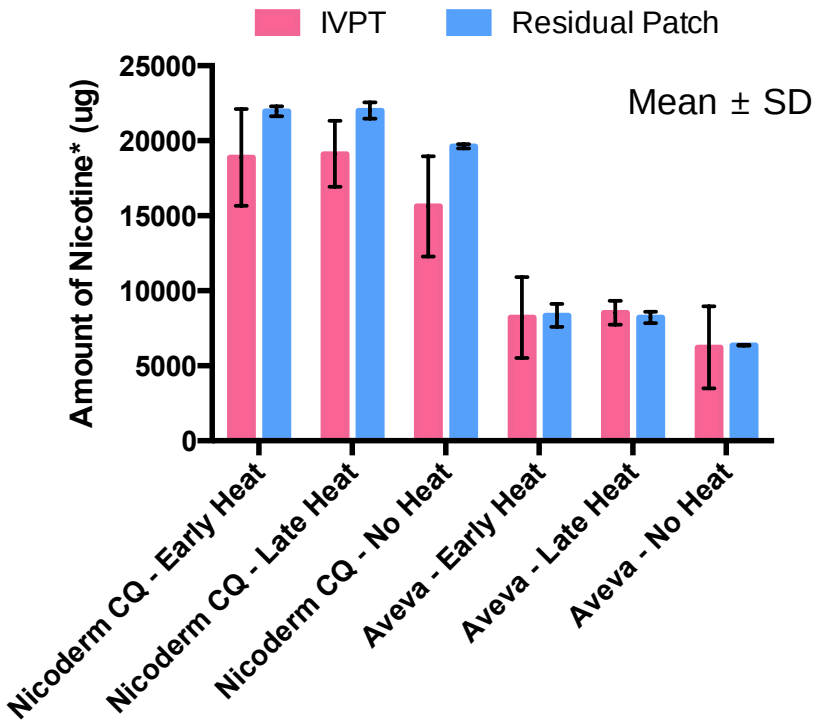
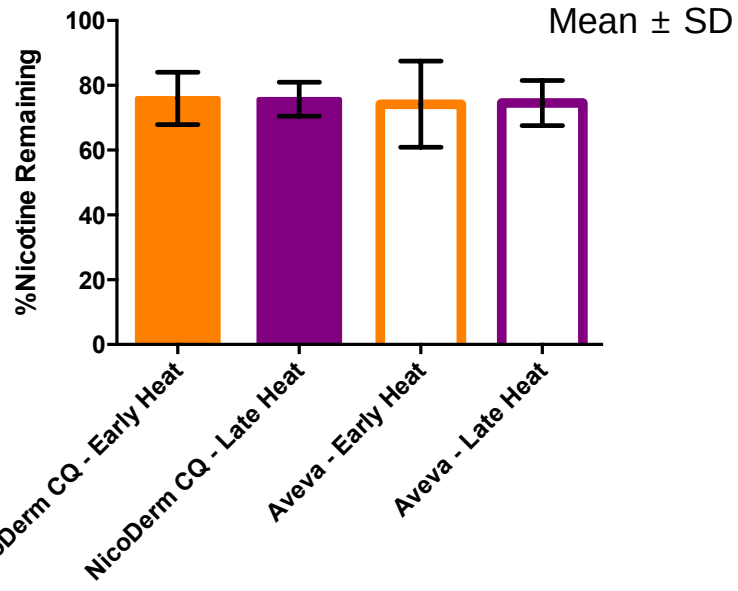
Nicotine Residual TDS Extraction



p > 0.05 for all treatment groups between IVPT and Residual Patch Analysis Data



p > 0.05 between early vs. late heat
⇒ paralleled the results from IVPT

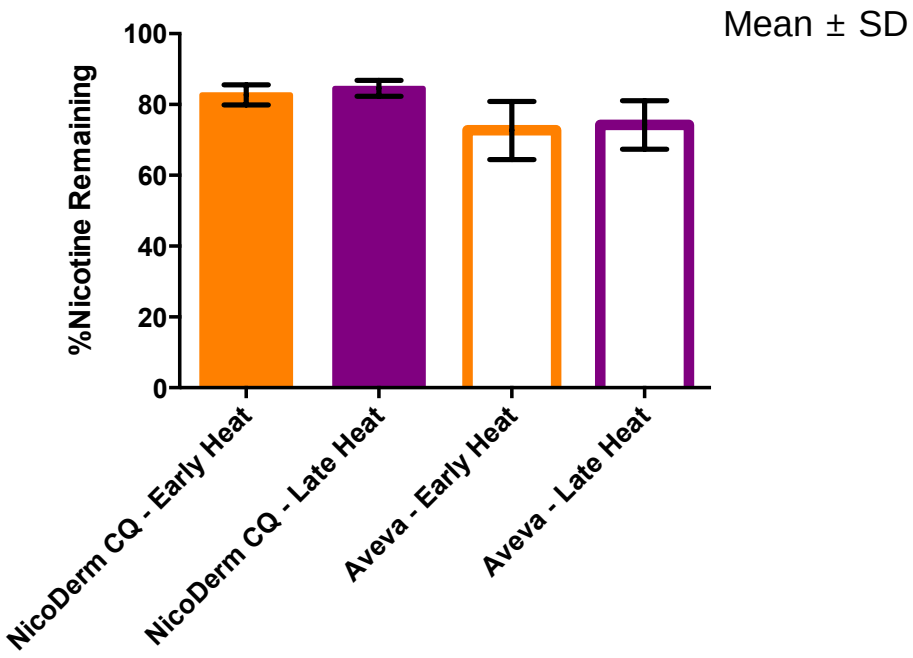
Evaluation of the relative bioavailability of nicotine and fentanyl TDS under the influence of heat in human subjects and development of IVIVC

Hypothesis: TDS with different formulations behave differently under the influence of heat *in vivo*, which can be predicted by the *in vitro* permeation tests.

Approaches:

1) A crossover pharmacokinetic clinical study, with study designs mimicking the *in vitro* experimental designs
   - Sample analysis by a validated LC-MS/MS method
2) Analysis of residual drug content in patch after patch removal from clinical study
   - Sample analysis by a validated HPLC method
3) Evaluate relationships between *in vitro* and *in vivo* data
4) Develop IVIVC models in which IVPT data can predict the performance of TDS *in vivo*

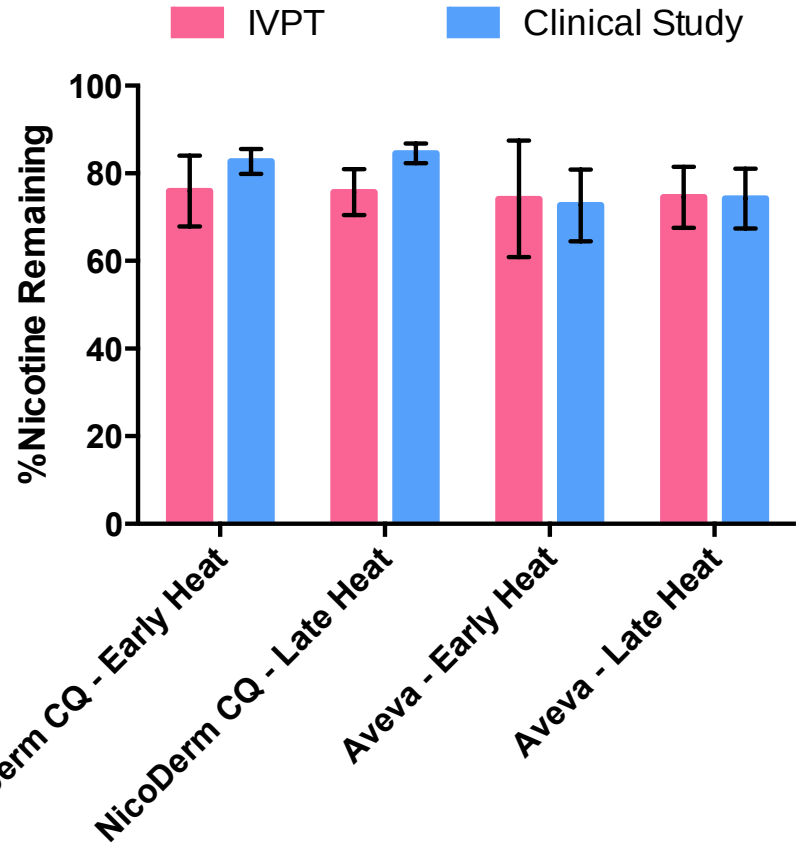
Nicotine Residual TDS Extraction



$p > 0.05$ between early vs. late heat

⇒ paralleled the results from *in vivo* PK and IVPT

Preliminary: IVIVC – Residual TDS Analysis



- $p > 0.05$ between IVPT and clinical study results

Dermatopharmacokinetics(DPK, tape-stripping)

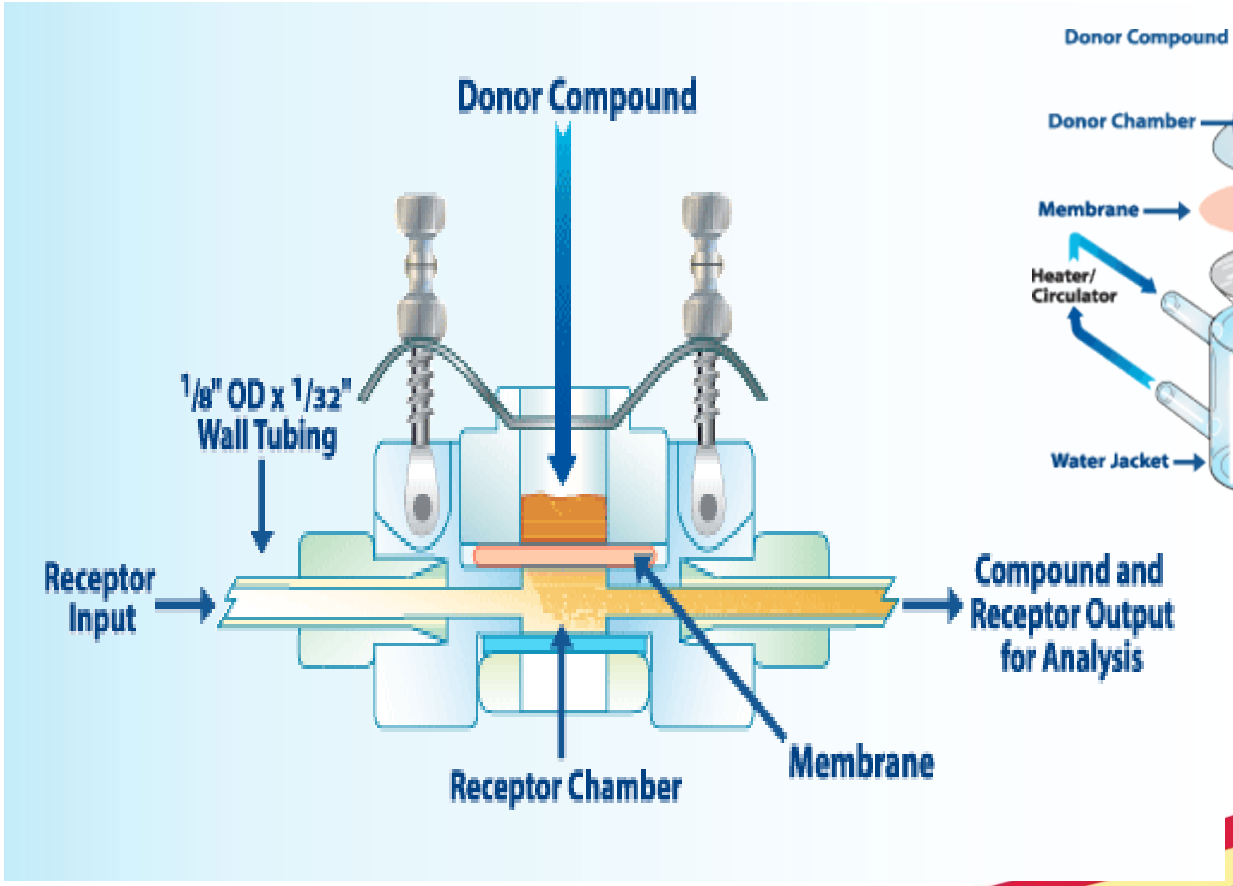
- Measures amount in SC measured in time after application and cleaning
- Analysis of PK parameters: AUC (area under amount in SC versus time curve), T_{max}, C_{max}
  - e.g., Pershing & Franz tretinoin studies (FDA guidance 1998-2002)
  - Complicated and same BE answer is achievable with a simpler 1-uptake and 1-clearance analysis (Bunge and Guy et al.)
  - Navidi, W, Hutchinson, A, N'Dri-Stempfer, B and Bunge, A (2008). Determining bioequivalence of topical dermatological drug products by tape-stripping. *J Pharmacokin Pharmacodyn*, 35:337-348
  - N'Dri-Stempfer, B, Navidi, WC, Guy, RH and Bunge, AL (2008). Optimizing metrics for the assessment of bioequivalence between topical drug products. *Pharm Res*, 25:1621-1630
  - Nicoli S, Bunge AL, Delgado-Charro MB, Guy RH. Dermatopharmacokinetics: factors influencing drug clearance from the stratum corneum. Pharm Res. 2009; 26: 865-71
  - N'Dri-Stempfer, B, Navidi, WC, Guy, RH and Bunge, AL (2009). Improved bioequivalence assessment of topical dermatological drug products using dermatopharmacokinetics. *Pharm Res*, 26:316-328

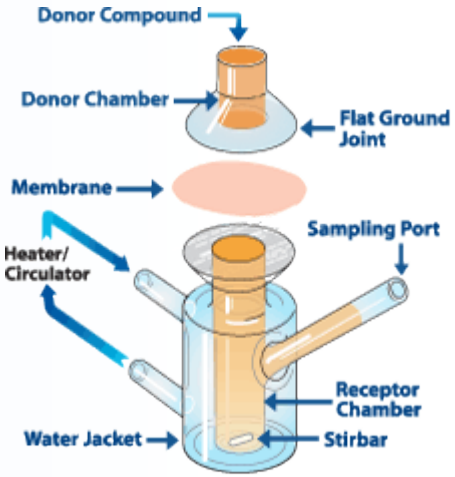
Dermatopharmacokinetics (DPK, tape-stripping)

- four improvements made by Bunge and Guy et al. to the original DPK methodology
  - improved cleaning of excess drug from each test site at the end of the uptake period
  - determination and inclusion of drug from the first two tape strips in the reported total amount taken up into the SC
  - an increase in the number of tape strips collected combined with a method to ensure reliable collection of nearly all the SC
  - improved control of the tape strip sampling area within the drug application area (to avoid edge effects)

In Vitro Skin Permeation Study (IVPT)

Automated
In-Line
Flow Through
System





Standard
Franz cell

www.permegear.com

http://www.permegear.com/inline.htm

Historical IVIVC for Bioequivalence

- Previous examples of IVIVC*
  - IVPT compared with total absorption after 1 application in humans
  - Studied same drug products with same methodology (harmonization)
  - Measured the same metric (usually total % absorbed)
  - *In vivo* and *in vitro* results were the same
  - Relatively robust set of data demonstrates that *in vitro* measurements are good representations of the *in vivo* system
  - Rate and extent are coupled in the total % absorbed (i.e., rate and extent are not determined separately, 1 time point)
- Total % absorbed is not typically measured by other *in vivo* methods; for example:
  - Pharmacokinetic (i.e., blood levels)
  - DPK
  - We will be incorporating this metric with DPK and PK

*Lehman, PA, Raney, SG and Franz, TJ (2011). Percutaneous absorption in man: In vitro-in vivo correlation. *Skin Pharmacol Physiol*, 24:224-230.

*Franz, TJ, Lehman, PA and Raney, SG (2009). Use of excised human skin to assess the bioequivalence of topical products. *Skin Pharmacol Physiol*, 22:276

*also Chapter 9 in Transdermal and Topical Drug Delivery, Benson ed., Lehman et al. 2012