Characterizing In Vitro Bioavailability of Acyclovir and Metronidazole Topical Products, and In Vitro – In Vivo Correlation Results with Transdermal Systems

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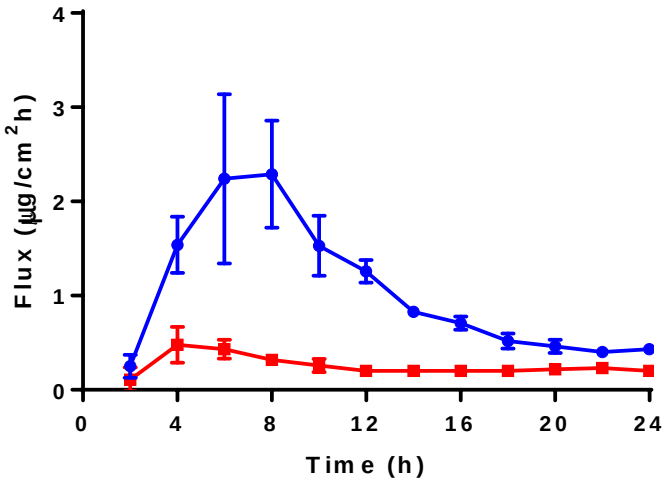
Topical Dose Administration Techniques

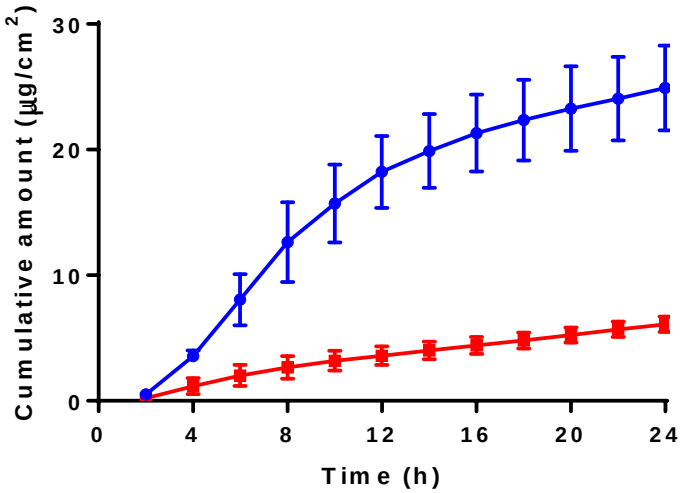
- Highly variable among labs, researchers, and patients
  - Methods of dispensing formulation
  - Duration of rubbing
  - Force used for rubbing
  - Loss of formulation during rubbing

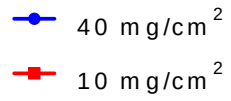
- Need a reproducible, clinically-relevant, and practical technique for IVPT

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

IVPT Results Variability
Importance of Dose Application – Voltaren® gel example
Dose Test and Reference Products the Same





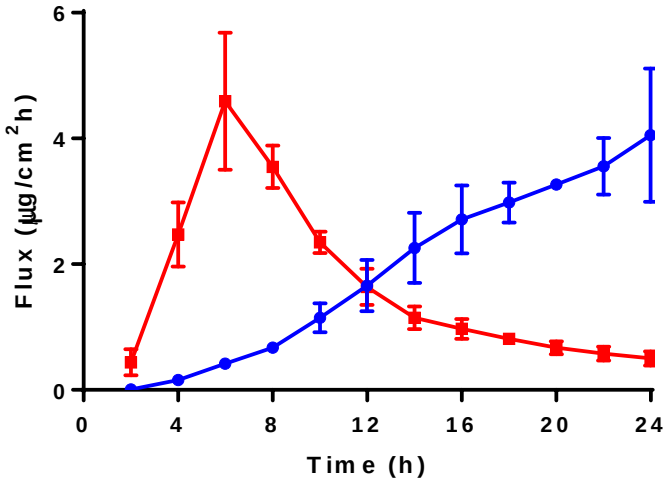


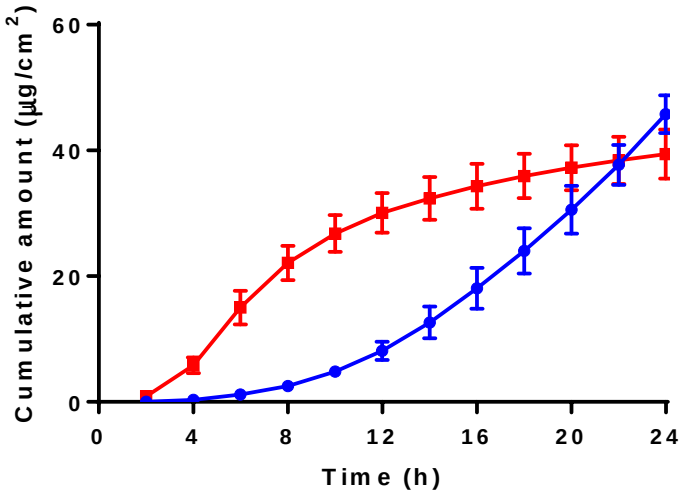
Mean ± SD (n=3)
Yucatan Miniature
Pig Skin

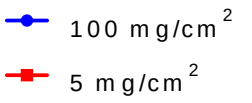
	J_{max} ± SD (µg/cm²/h)	T_{max} (h)	Cumulative Amount ± SD (µg/cm²)
40 mg/cm²	2.29 ± 0.57	8	24.91 ± 3.38
10 mg/cm²	0.48 ± 0.19	4	6.10 ± 0.61

HPLC vial rubbing application technique

IVPT Results Variability
Importance of Dose Application – Pennsaid® 2%
Dose Test and Reference Products the Same







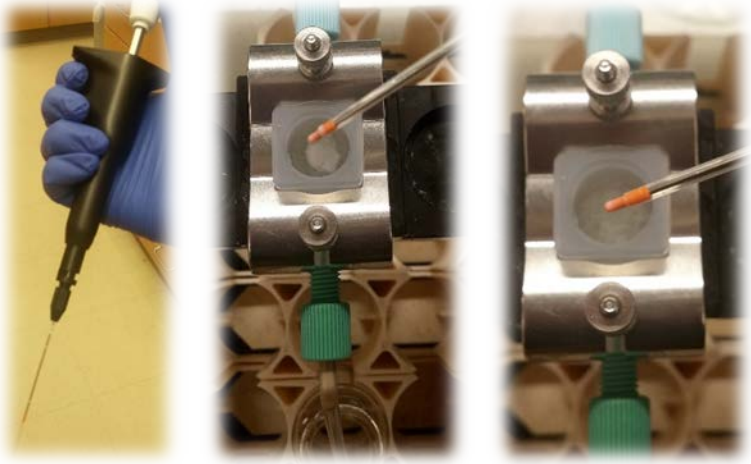
Mean ± SD (n=3-4)
Yucatan Miniature
Pig Skin

	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

HPLC vial rubbing application technique

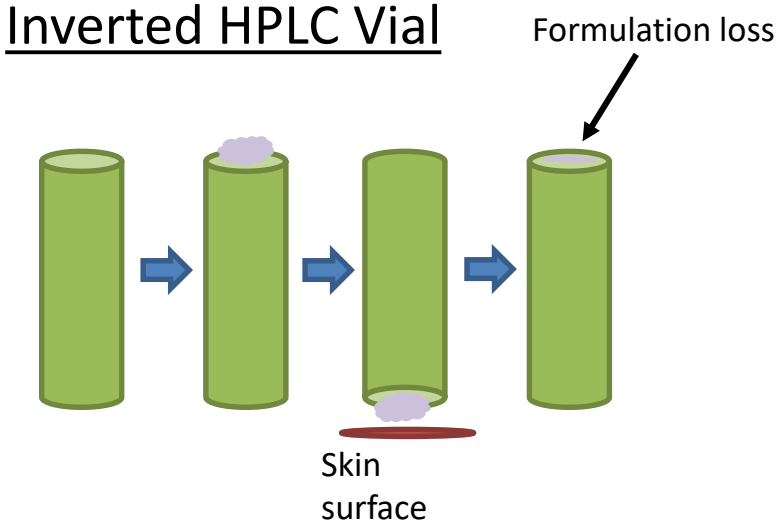
Dose Administration Techniques

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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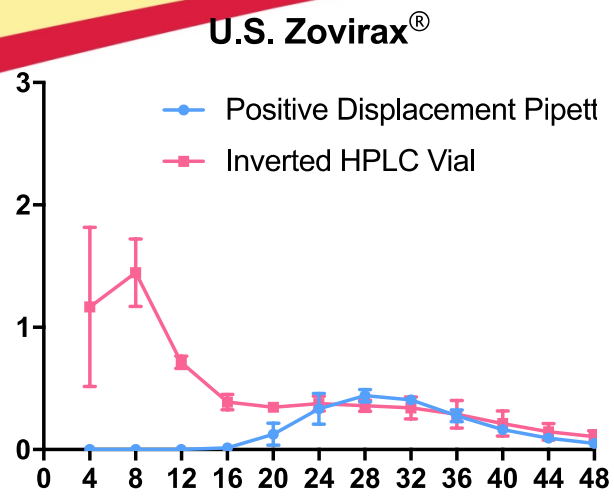
Dose Administration Techniques

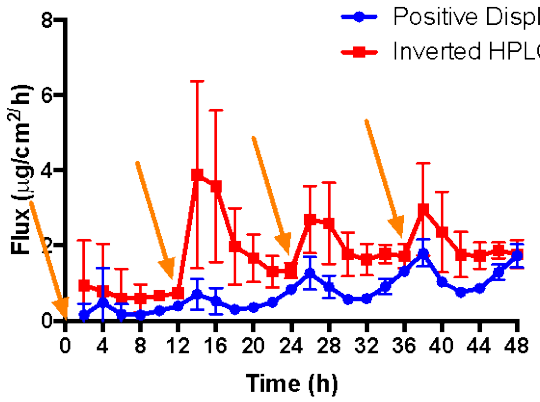


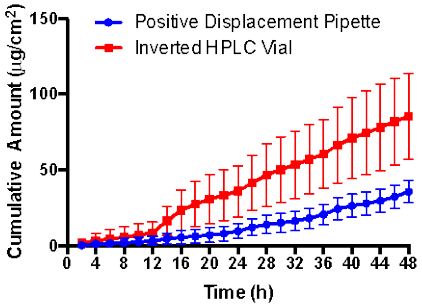
/cm²)

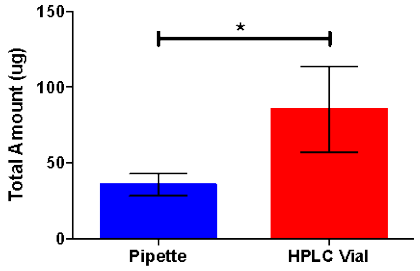
Ex vivo human skin
Mean ± SD (n=3-4 for each technique)

Preliminary: Dose Administration Techniques

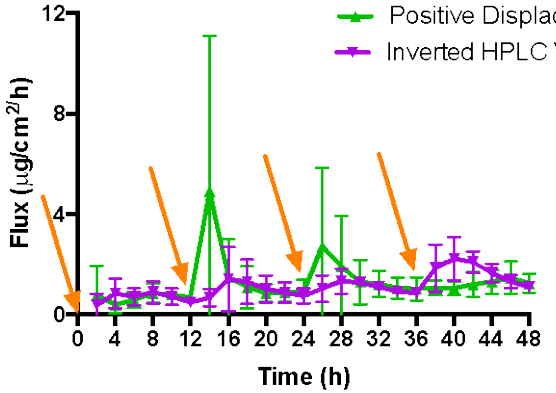
Pennsaid® 2% (more viscous)

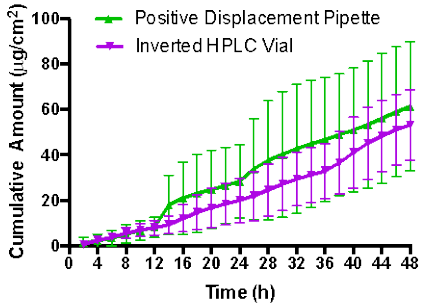


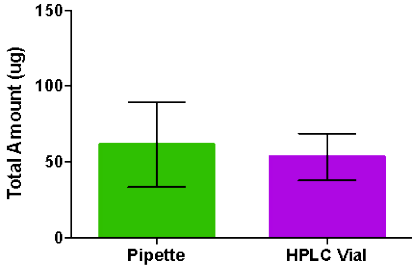




Pennsaid® 1.5%



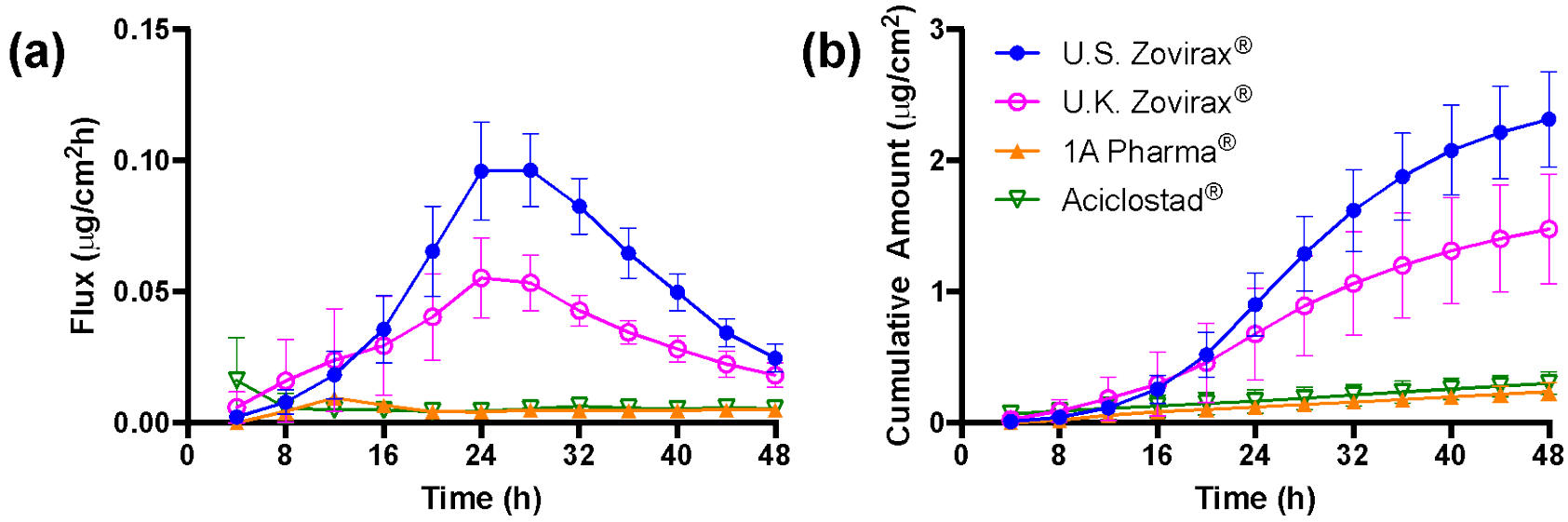




Orange Arrow: dosing (~5 mg/cm^2 of formulation)

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

Four Acyclovir Cream Products

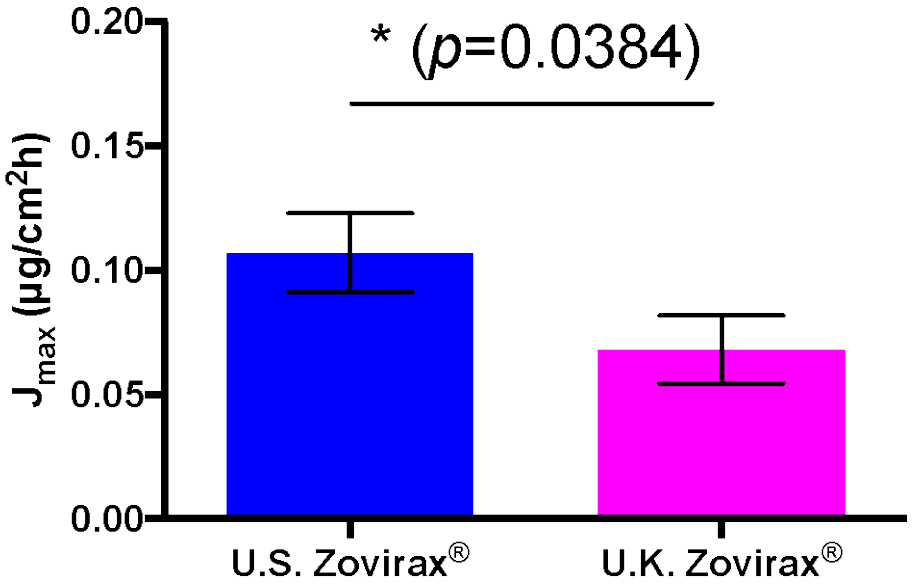


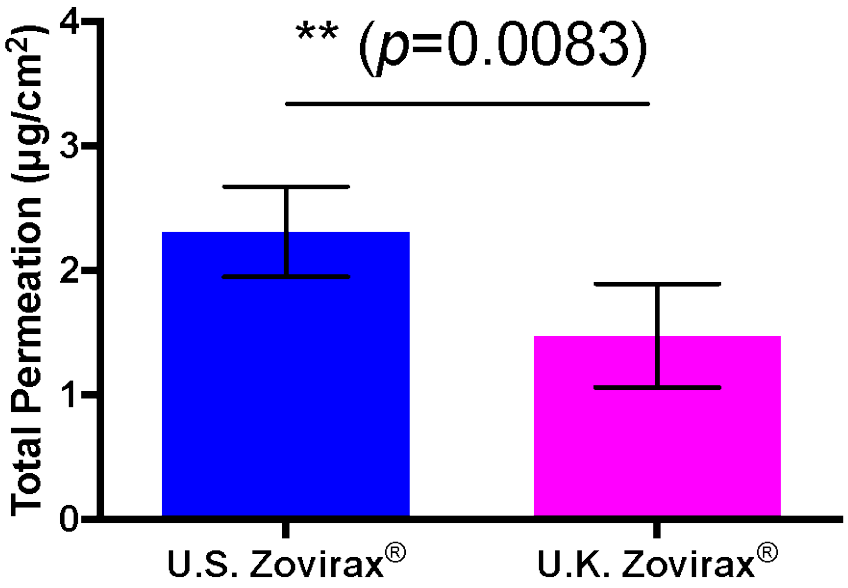
(Mean ± SEM, n= 6 donors with 4-7 replicates per donor for Zovirax® creams and n = 2 donors with 3-4 replicates per donor for non-Zovirax® creams)

****The IVPT method was able to discriminate the Reference and Test acyclovir products, based on Jmax and the total amount of acyclovir permeated over 48 h**

Positive displacement pipette application

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

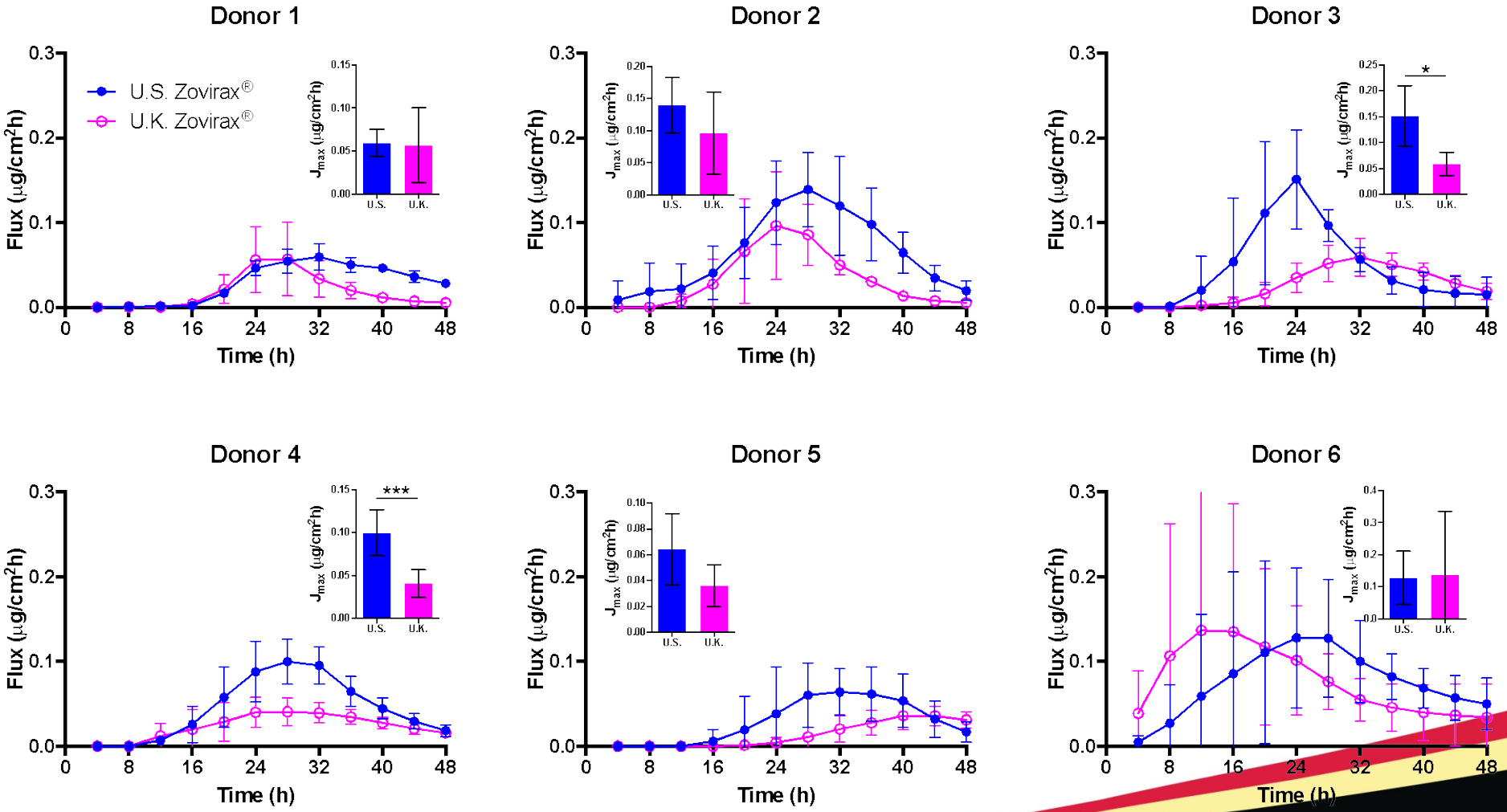




Positive displacement pipette application

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

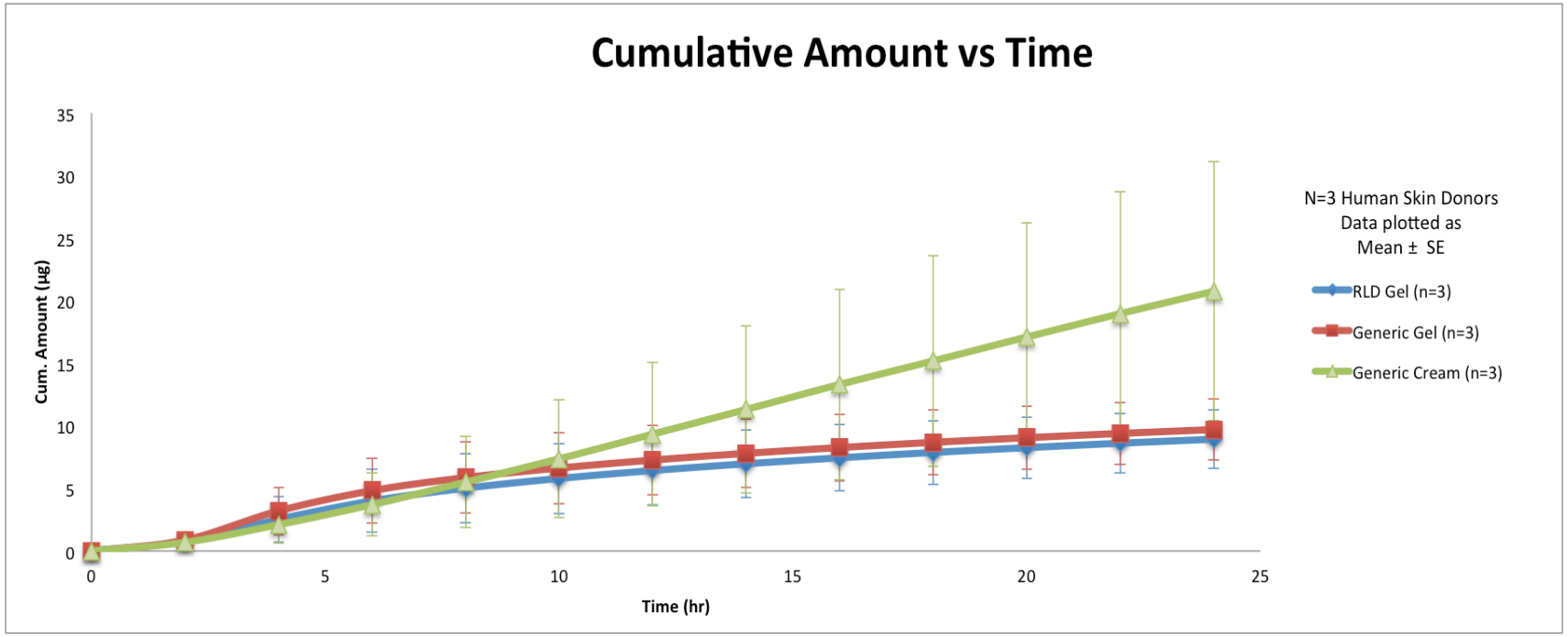
U.S. vs. U.K. Zovirax® creams per donor



Positive displacement pipette application

Metronidazole RLD Gel & Generic vs. Generic Cream

Product Name	Cumulative Cutaneous Absorption (µg)
RLD Gel (n=3)	8.93 ± 2.33
Generic Gel (n=3)	9.70 ± 2.42
Generic Cream (n=3)	21.0 ± 10.32

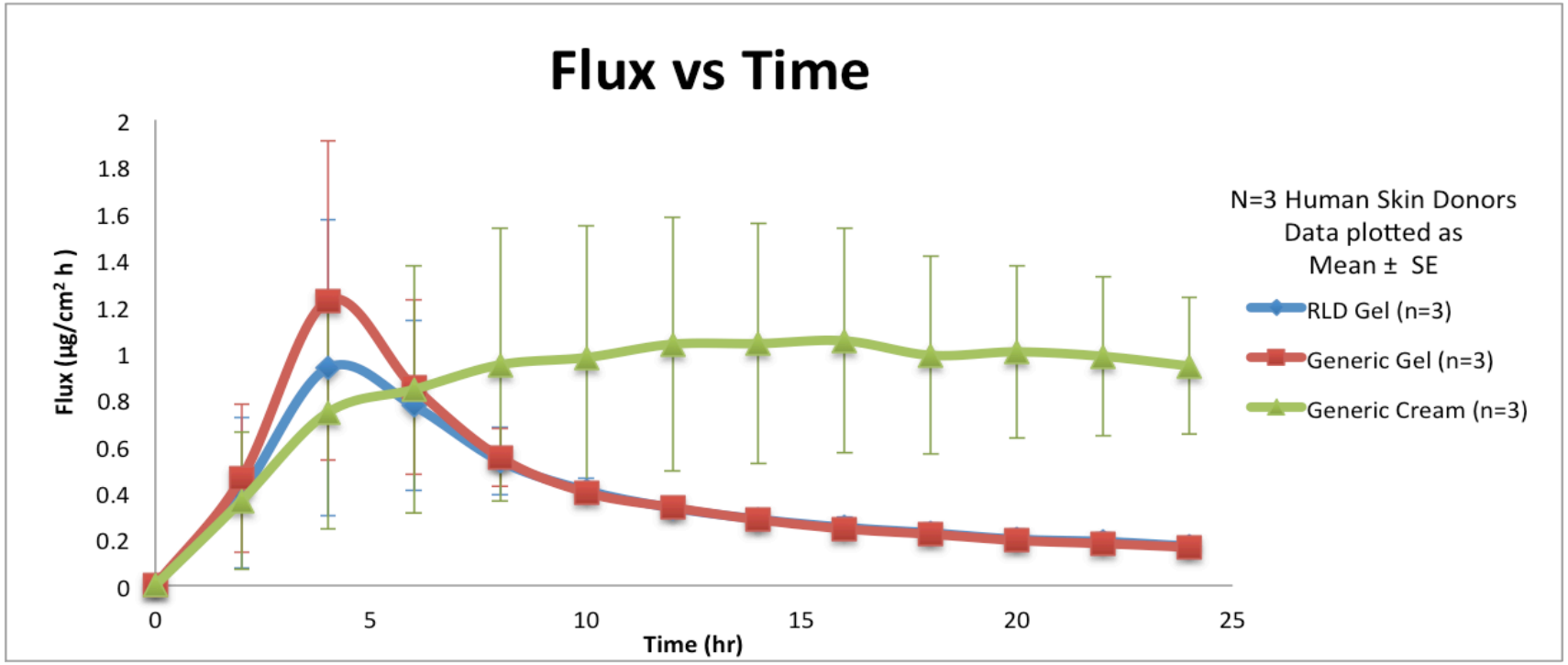


Cumulative absorption from RLD gel, generic metronidazole gel and generic metronidazole cream over 24-h study duration.

Dosing Technique: Inverted HPLC vial
Target dose: 10 mg/cm^2
Flow rate: 1.0 mL/h
Skin surface temperature: 32 ± 2°C (circulating water bath)
Receiver solution: Isotonic phosphate buffer (pH 7.4 ± 0.1)
Skin: human abdominal skin from three donors with four replicate skin sections per donor per product

Metronidazole RLD Gel & Generic vs. Generic Cream

Product	Maximum Flux ($\mu g/cm^2/h$)
RLD Gel (n=3)	0.93 ± 0.63
Generic Gel (n=3)	1.22 ± 0.69
Generic Cream (n=3)	Observed at ≥ 12 h



Flux profile from RLD gel, generic metronidazole gel and generic metronidazole cream over 24-h study duration.

Conclusion: Metronidazole IVPT results

- IVPT studies may have utility to help support an evaluation of bioequivalence for topical drug products
  - RLD and generic gels
    - Positive controls for bioequivalence relative to each other
    - Had a similar rate and extent of metronidazole delivery
    - Discriminated the cutaneous bioavailability from the cream as being different from that for both gels
  - Generic cream
    - Negative control for bioequivalence relative to the reference gel
    - Distinct rate and extent of metronidazole delivery with respect to both gels
- Consistent with the expectation that differences in physical and structural critical quality attributes between topical semisolid drug products (e.g., between a gel and a cream) can alter the bioavailability of metronidazole

Qingzhao Zhang PhD Candidate, AAPS Poster 2017, Human PK Study Pending

Can the in vitro permeation test (IVPT) predict the performance of TDS (patch) and heat effects on drug delivery and absorption in vivo?

Model Drugs: **Nicotine** & Fentanyl

I. Evaluation of the influence of transient heat (1 h) on the release and permeation of drug from TDS using the *in vitro* permeation test (IVPT)

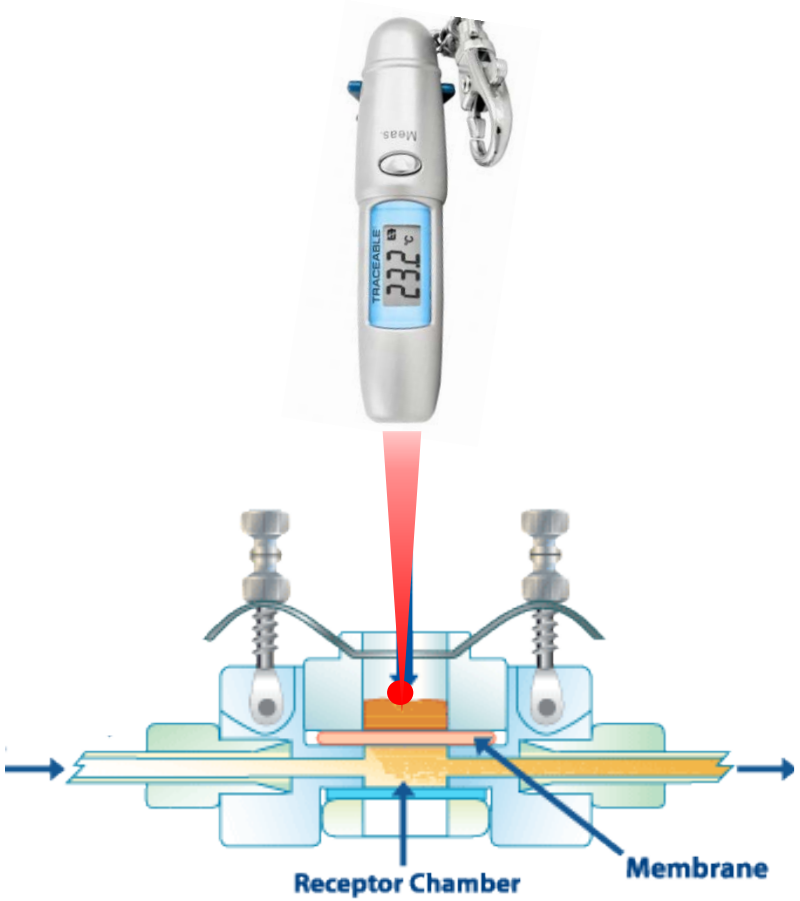
II. Evaluation of the influence of transient heat (1 h) on the TDS pharmacokinetics *in vivo* by conducting PK studies in human subjects

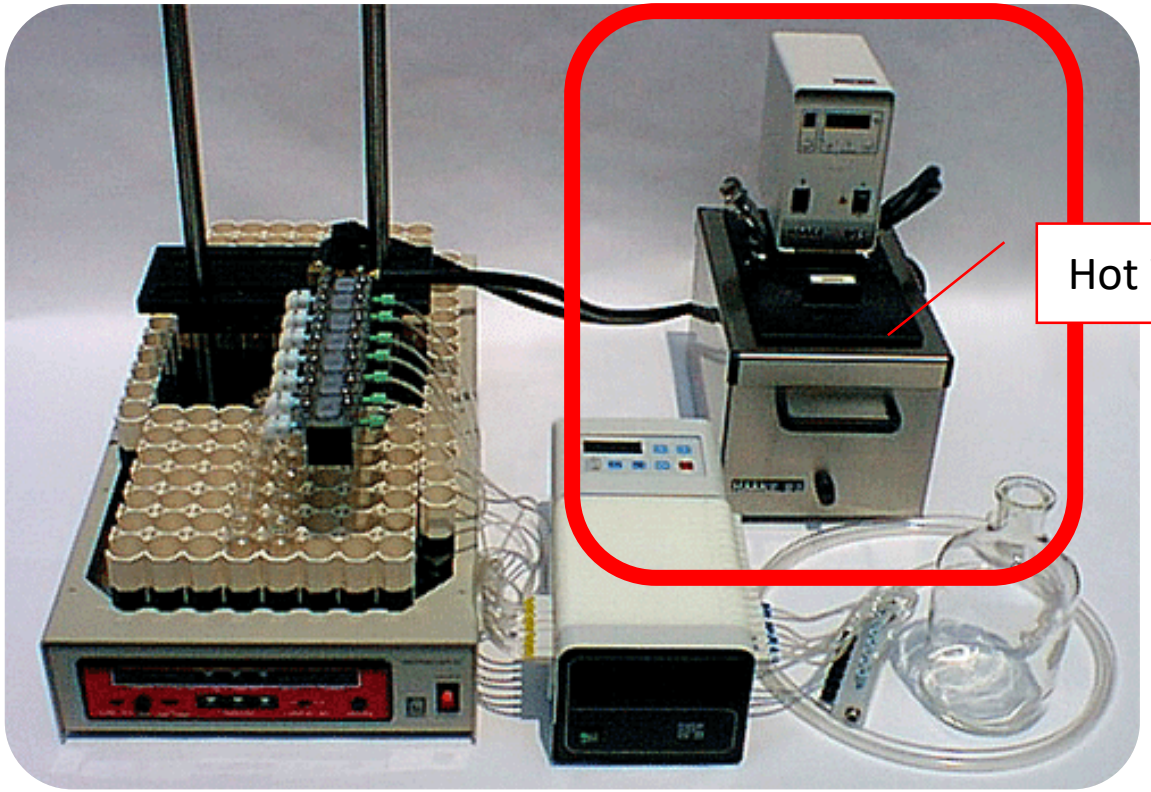
III. Evaluation of preliminary *in vitro* and *in vivo* correlations (IVIVC) of TDS

***This TDS project is informative for topical drug product evaluation since many provide quantifiable blood levels of drug**

Temperature Monitoring & Heat Application *In Vitro*

Infrared Thermometer





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

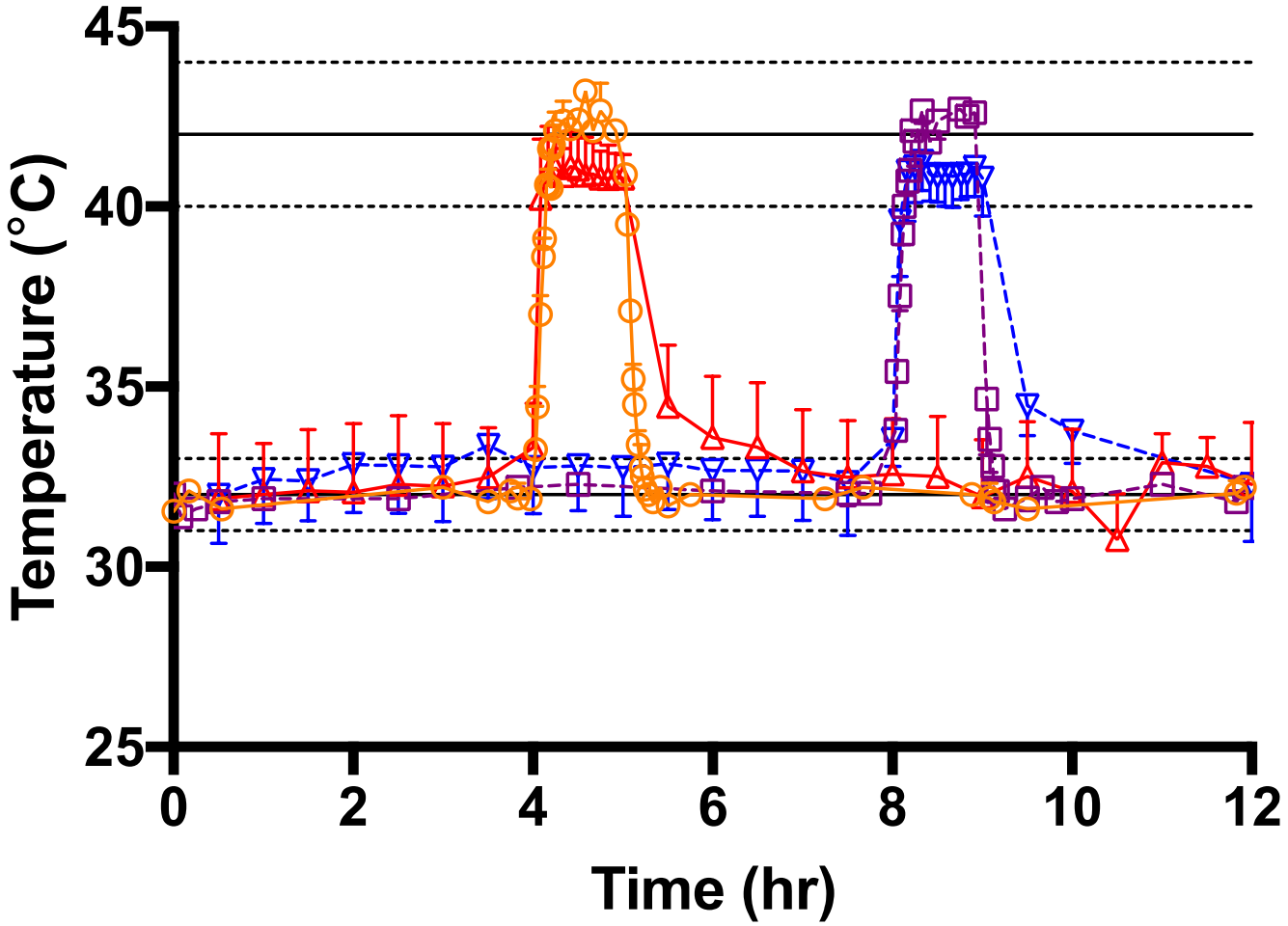
Temperature Monitoring & Heat Application *In Vivo*

- Kevlar sleeve with an opening to expose TDS, while protecting skin outside the dosing area
- Thermometer probe adjacent to TDS

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

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

Temperature: *In Vitro* & *In Vivo*



Early Heat - In Vitro

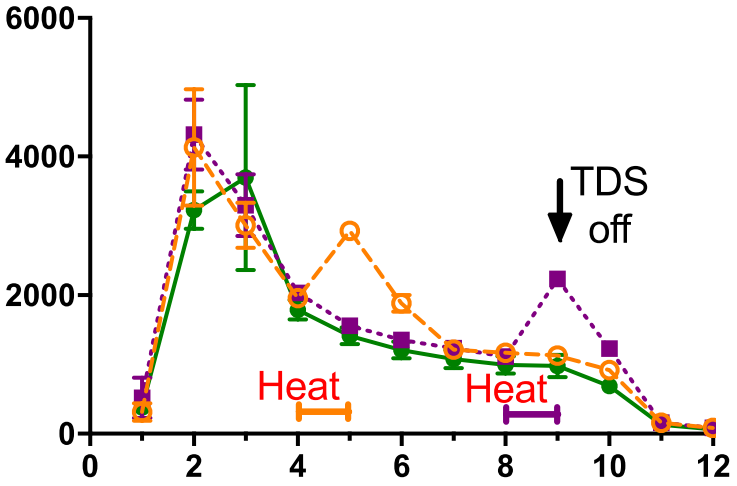
Late Heat - In Vitro

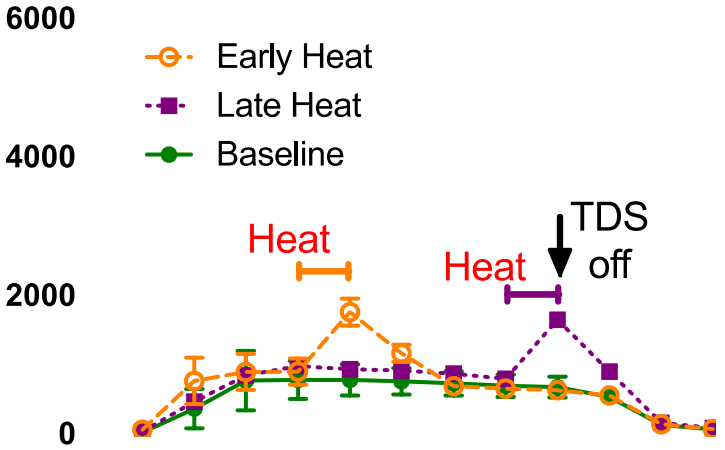
Early Heat - In Vivo

Late Heat - In Vivo

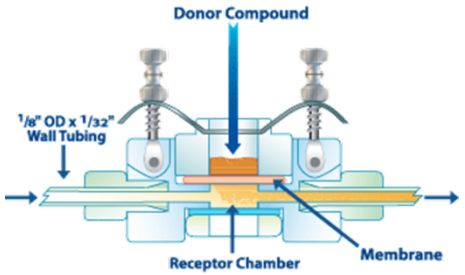
(42 ± 2°C)

IVPT Results



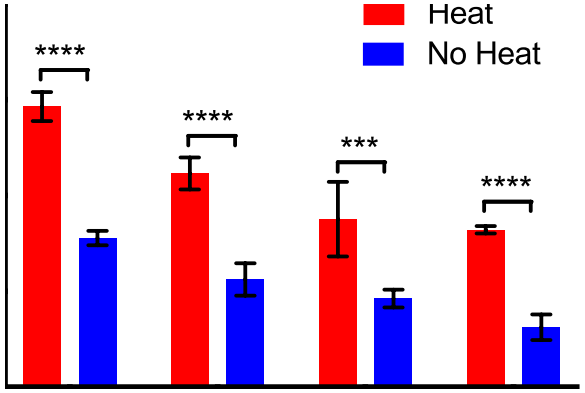


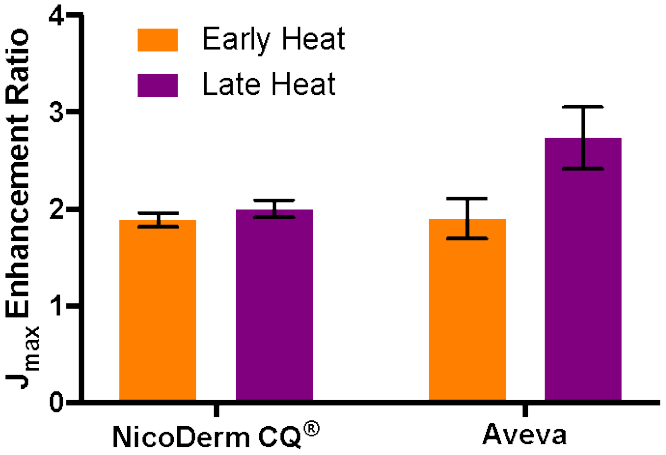
Human Skin Data

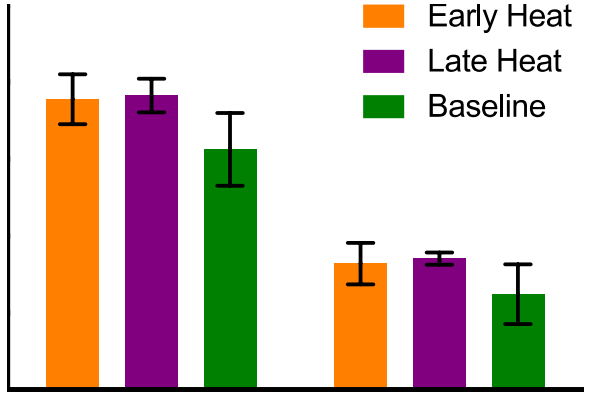


Mean ± SEM from 4 donors for Early Heat and Late Heat, 2 donors for Baseline with n=4 per donor

Flux+ = Flux value multiplied by TDS size to account for the whole TDS

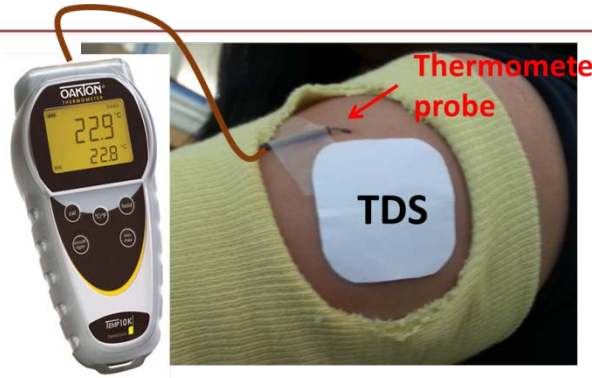






Two-way ANOVA followed by Bonferroni's post-hoc multiple comparisons

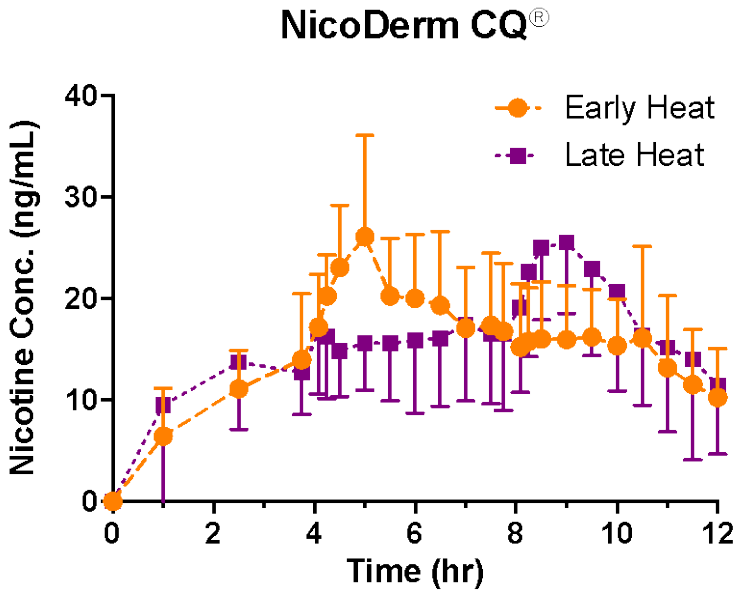
In Vivo Results

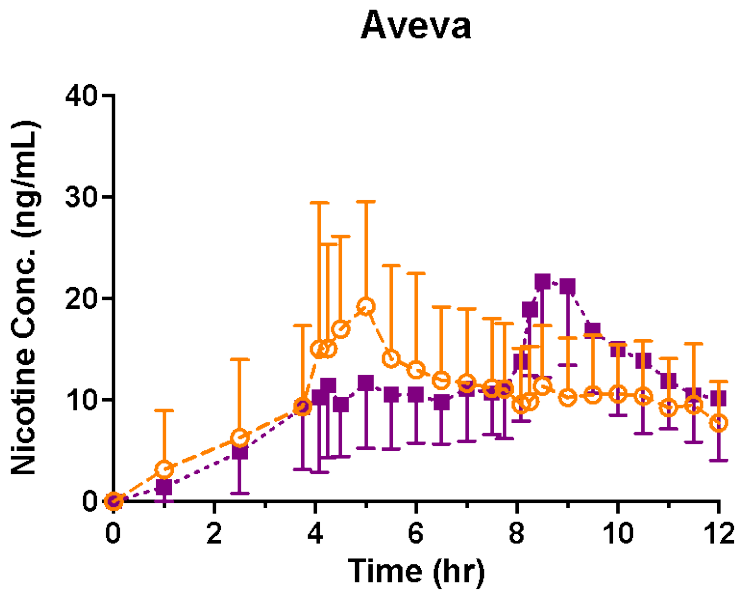


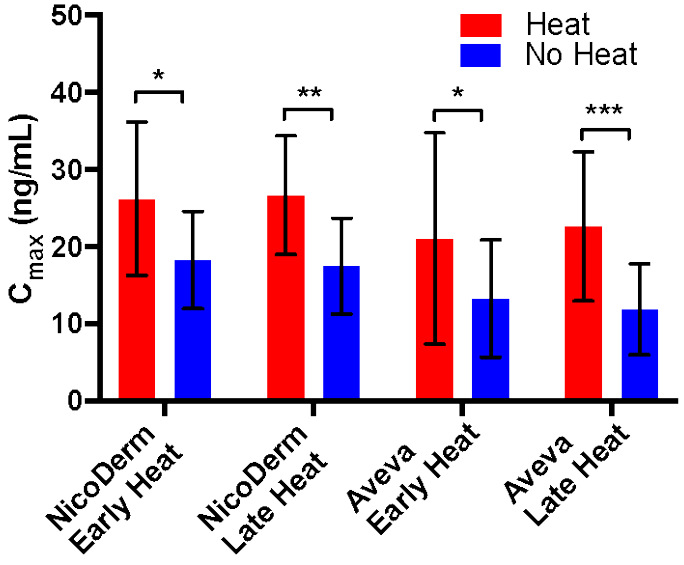
Mean ± SD
from 10 human subjects

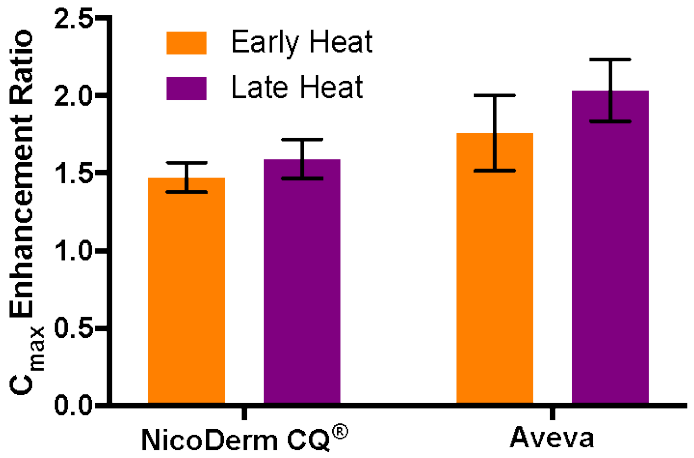
Smokers

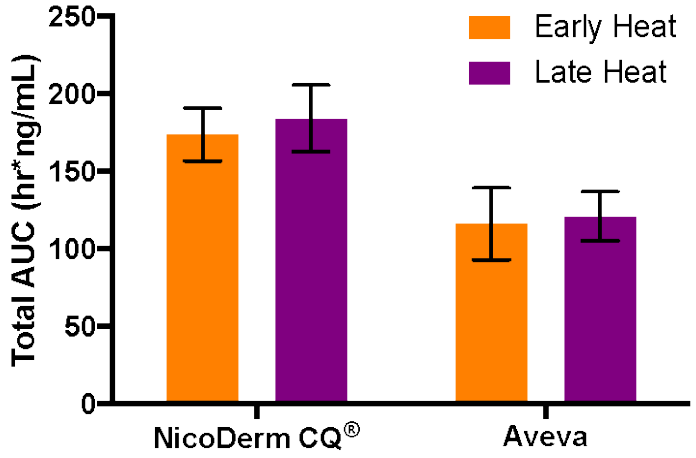
Patch off 9h











Two-way ANOVA followed by Bonferroni's post-hoc multiple comparisons

Conclusions – Nicotine

- Early vs. Late Heat effect comparable both *in vitro* and *in vivo*
- Heat effect on two differently formulated TDS comparable both *in vitro* and *in vivo*
- *In vitro* and *in vivo* heat effect ratios were comparable
- Strong preliminary IVIVCs (IVIVRs) between IVPT and clinical human PK studies under the matched study designs

IVIVC

- Definition by the U.S. FDA

  "a predictive mathematical model describing the relationship between an *in-vitro* property of a dosage form and an *in-vivo* response"

- Level A: a point-to-point correlation between *in vitro* and *in vivo* profiles
- Level B: comparison between *in vitro* dissolution time and *in vivo* residence time
- Level C: a single point correlation between *in vitro* and *in vivo* parameters (e.g. J_{max} vs. C_{max})

Level A is most informative and useful

Approach I Level A

Eq. 1 prediction while TDS was worn:

$$C_s = \frac{F \times R_{in} \times H_i}{CL_{IV}} \times \left(1 - e^{-k_1 t}\right)$$

Eq. 2 prediction after TDS removal:

$$C_s = C_0 \times e^{-k_2 t}$$

Or may need 2 or 3 compartment model
Depending on drug and available data

C_s: Predicted in vivo serum concentration

F: Absolute bioavailability for TDS $\quad F = \frac{AUC_{0-\infty,TDS} \times Dose_{IV}}{AUC_{0-\infty,IV} \times Dose_{TDS}}$

R_{in}: Rate of input (mean flux during steady-state in IVPT experiments)

H_i : In vitro heat effect coefficient (composite heat effect during and after heat exposure); ratio of flux values with heat and without heat

CL: Total body clearance obtained from literature/product package information

k: Elimination rate constant obtained from literature/product package information

(k_1: after IV dose; k_2: after TDS dose) k_1 is a derived PK parameter from the two fundamental PK parameters (Cl and V). k_1=Cl/V. k_1 is a re-parameterization of Cl and V

F X Rin is used to mimic an IV dose and as a result Cliv is used. Therefore Kiv (Cliv/V)

t: Time after administration of TDS for Eq.1 and time after removal of TDS for Eq. 2

C_0: Initial concentration after TDS removal

Approach II and III

1. Reconstruct baseline (without heat) profile by combining non-heat portion from two study designs

2. Deconvolute in vivo baseline conc. vs time profile using Phoenix®

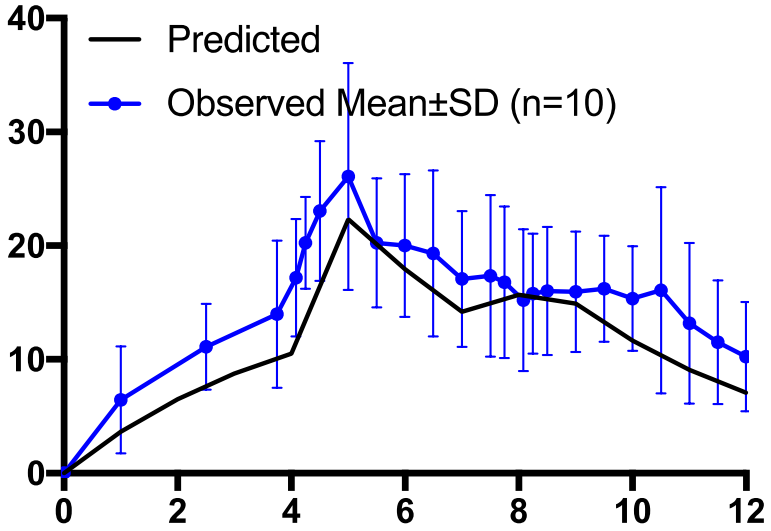
3. Construct IVIVC model by plotting fraction permeated in vitro vs. fraction absorbed in vivo

4. Predict in vivo fraction absorbed using the IVIVC model and IVPT data

5. Convolute the predicted in vivo fraction absorbed data using Phoenix® to obtain conc. vs. time profile

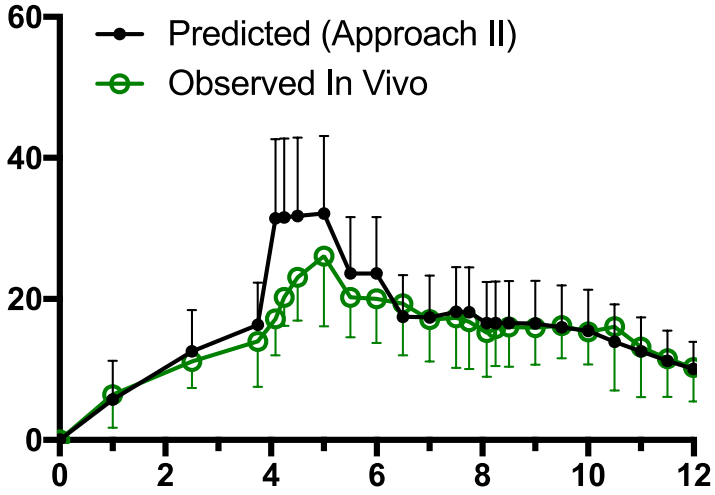
6. Apply **in vitro** heat effect coefficient, H_i (**Approach II**) or **in vivo** heat effect coefficient, H_{ii} (**Approach III**) to the predicted in vivo profile

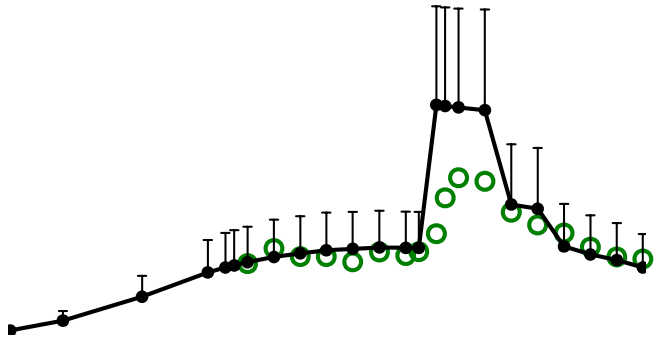
Approach I



Approach II

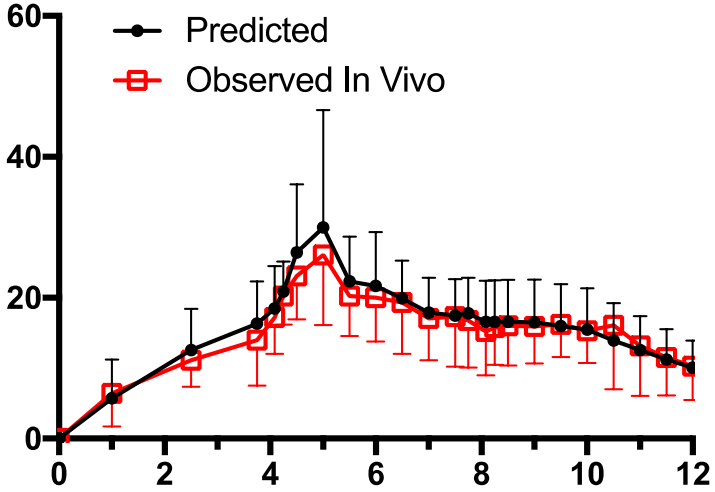
in vitro heat effect coefficient, Hi

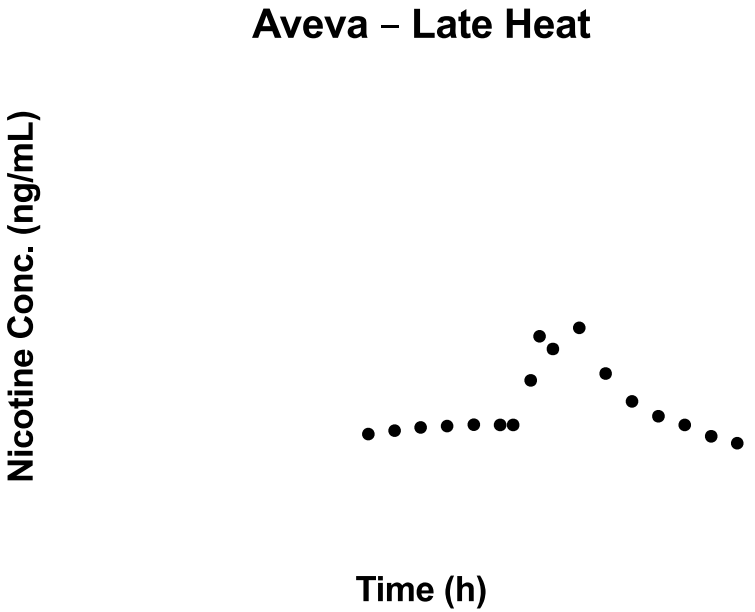




Approach III

in vivo heat effect coefficient, Hii



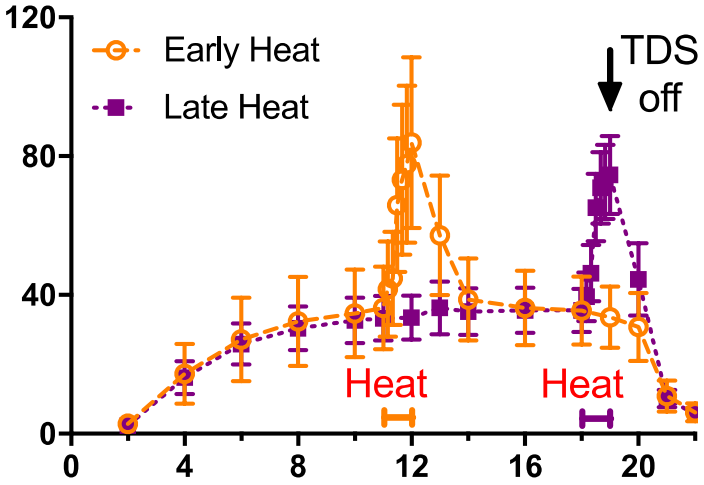


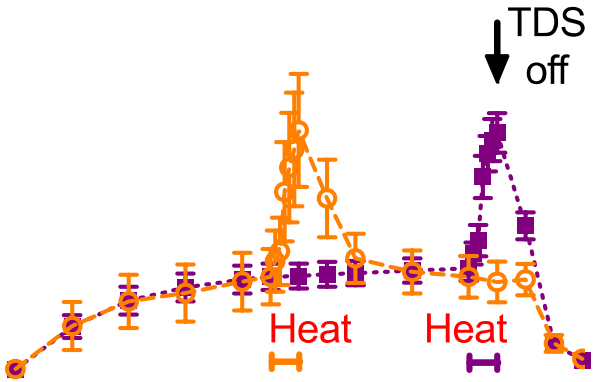
% Prediction Error

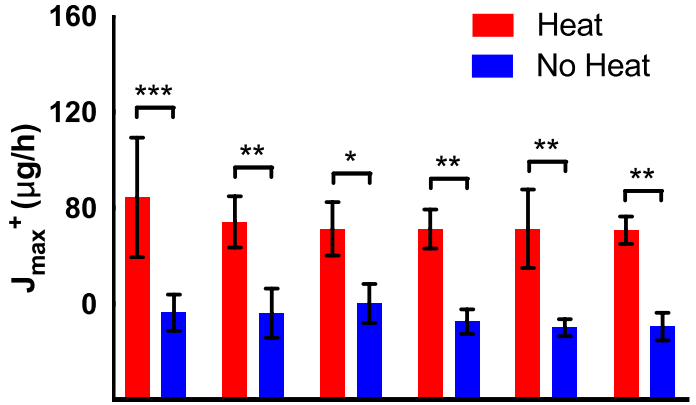
Nicotine TDS	NicoDerm CQ®		Aveva	
	Early Heat	Late Heat	Early Heat	Late Heat
Approach I				
Total AUC	20.3	12.9	7.5	5.0
C_{max}	14.4	16.6	9.8	13.5
Approach II				
Total AUC	10.3	5.0	1.5	13.3
C_{max}	23.3	30.2	3.5	47.5
Approach III				
Total AUC	5.1	1.2	1.1	4.5
C_{max}	15.0	5.8	8.9	17.7

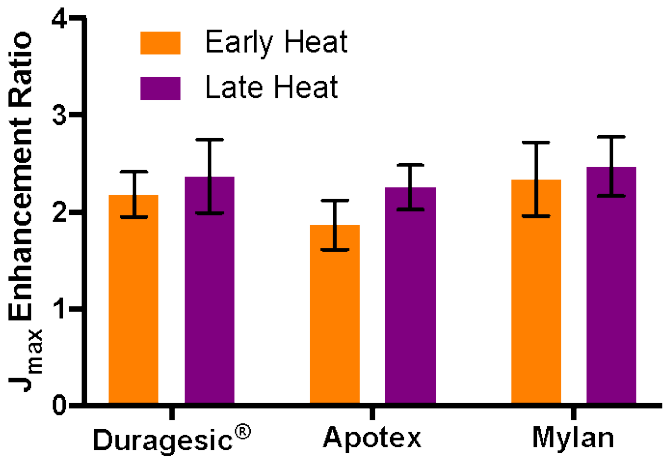
Fentanyl IVPT Results

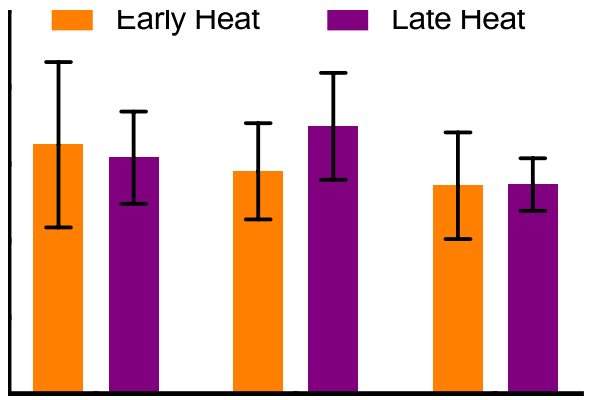
Mean ± SEM from 4 donors
with n=4 per each donor











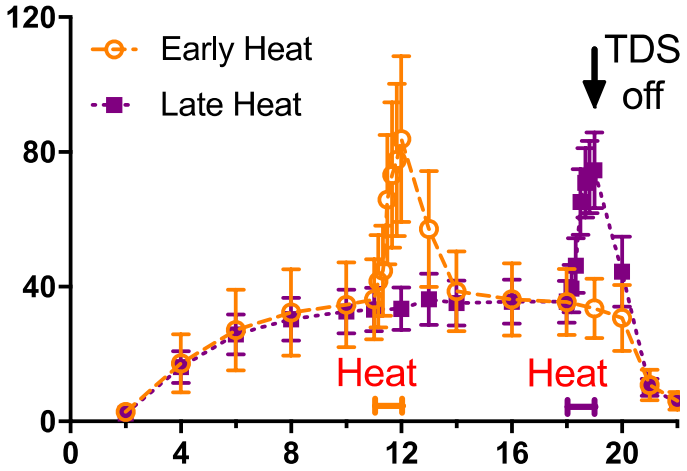
Flux+ = Flux value multiplied by TDS size to account for the whole TDS

Two-way ANOVA followed by Bonferroni's post-hoc multiple comparisons

Fentanyl Results

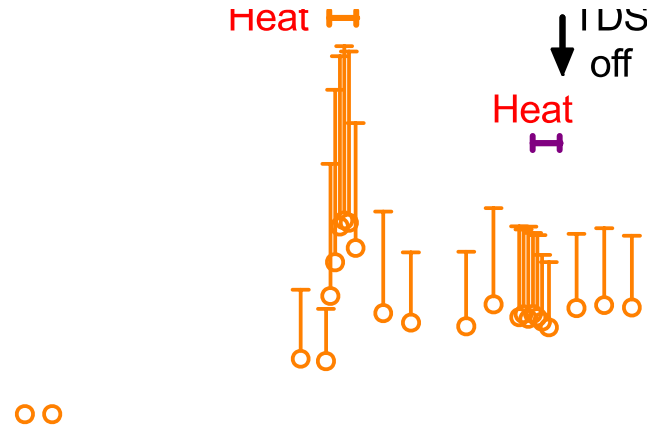
In Vitro

Mean ± SEM from 4 donors with n=4 per donor (Human Skin)



In Vivo

Mean ± SD from 10 Healthy Adults



Flux+ = Flux value multiplied by TDS size to account for the whole TDS

Clearance Value of Fentanyl

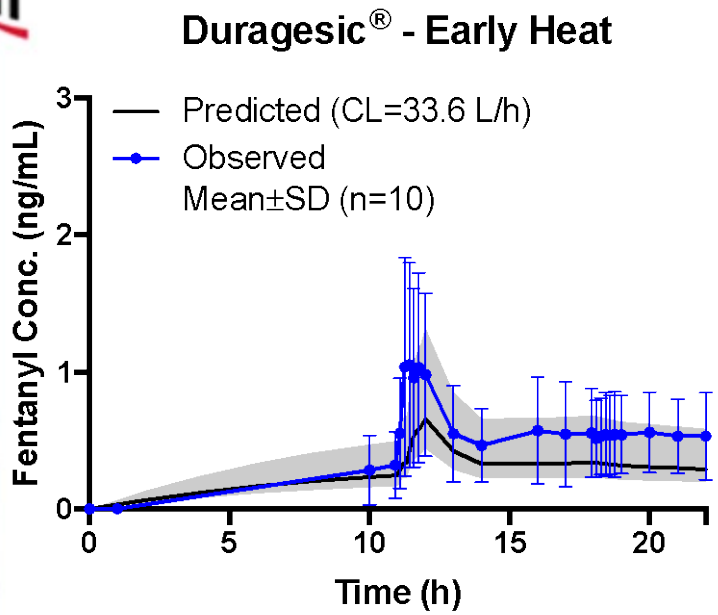
Reference	Subject #	Condition	CL_{IV} (L/h)	# of comp for PK Analysis
Ariano et al. J Clin Pharmacol 2001	18	Healthy	128	1
Bower et al. Br J Anaesth 1982	7	Healthy	92	2
Bentley et al. Anesth Analg 1982	5	Surgical	59	3
McClain et al. Clin Pharmacol Ther 1980	5	Healthy	57	3
Varvel et al. Anesthesiology 1989[1]	8	Surgical	46	3
Shibutani et al. Anesthesiology 2004	16	Surgical	43	3
Haberer et al. Br J Anaesth 1982	13	Surgical	42	2
Scott et al. J Pharmaol Exp Ther 1986	15	Healthy	34	2
Hengstmann et al. Br J Anaesth 1980	5	Surgical	26	2
Schleimer et al. Clin Pharmacol Ther 1978	6	Surgical	12	3
Fung et al. J Clin Pharmacol 1980	9	Healthy	10	3
Univ. of Maryland, Baltimore (ongoing)	14	Healthy	11	2

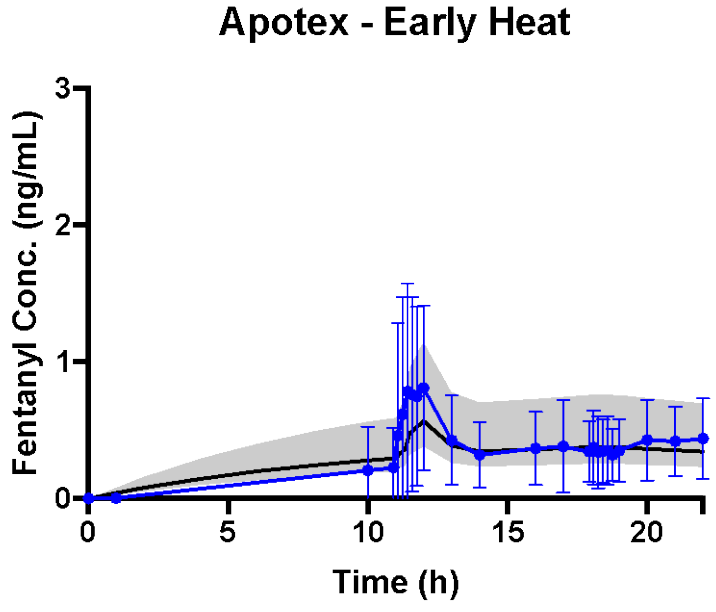
Weighted Mean CL_{IV} from Healthy subjects with PK value obtained from 2 or 3 compartmental analysis = **33.6 L/h**

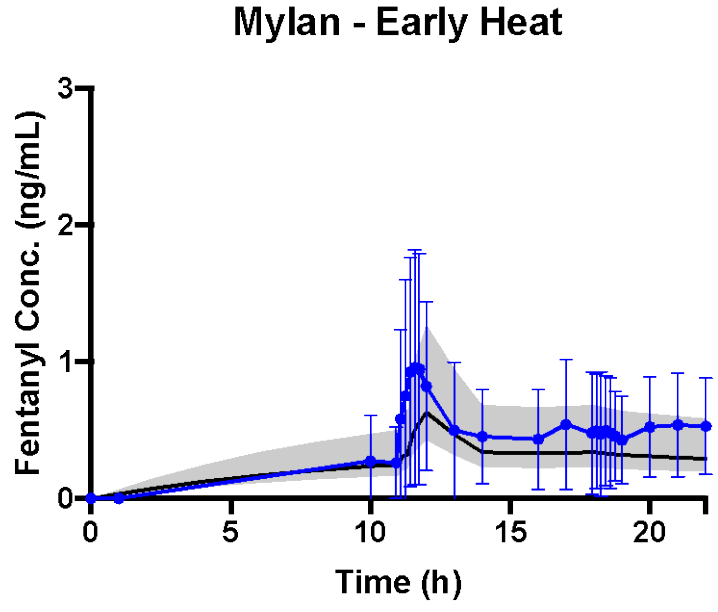
[1] Source of IV PK parameters reported in Duragesic® Package Insert

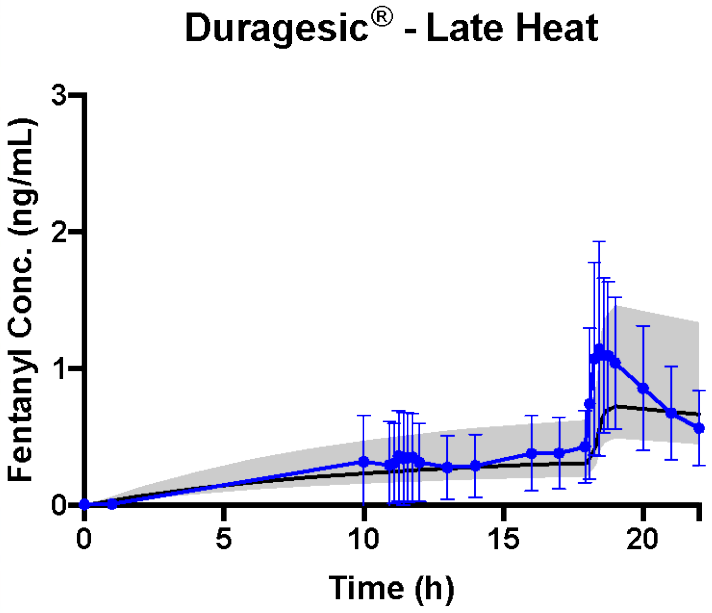
Approach I

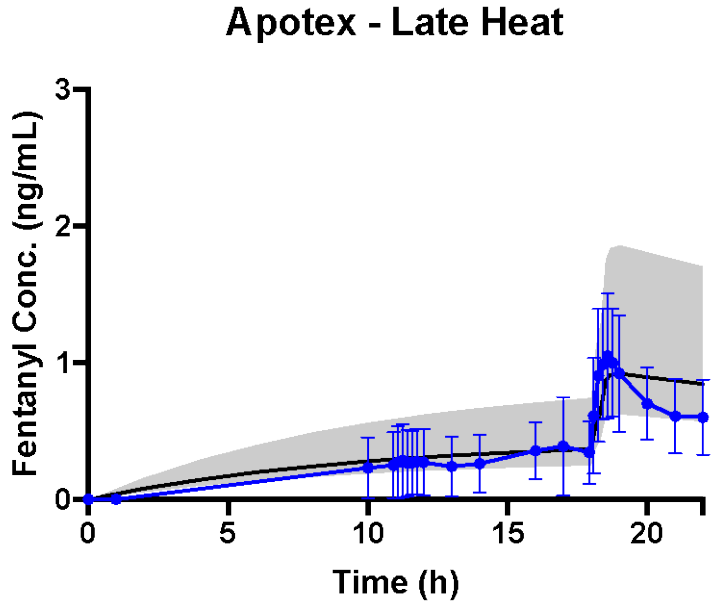
Grey shade represents prediction range when inter-subject variability of CL = 50%

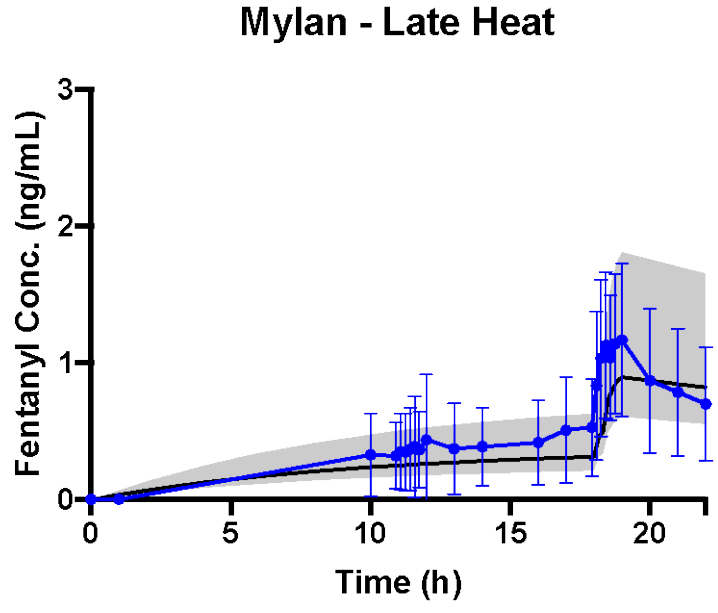






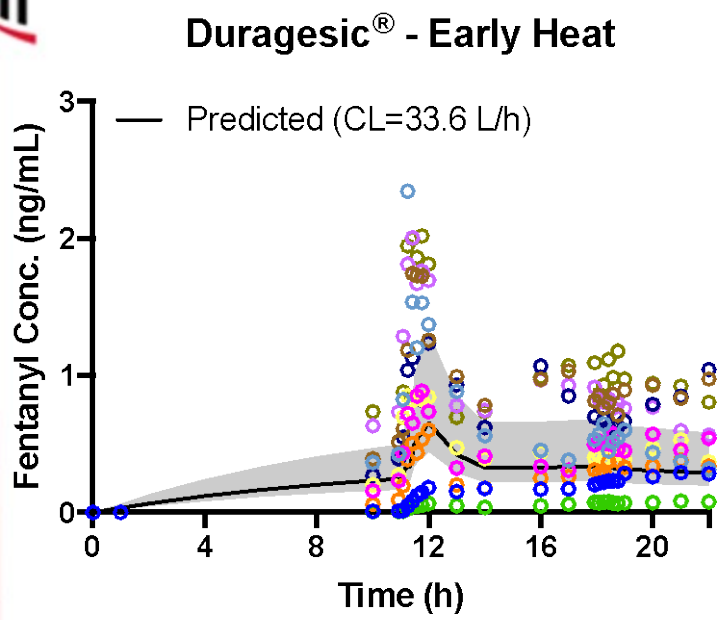


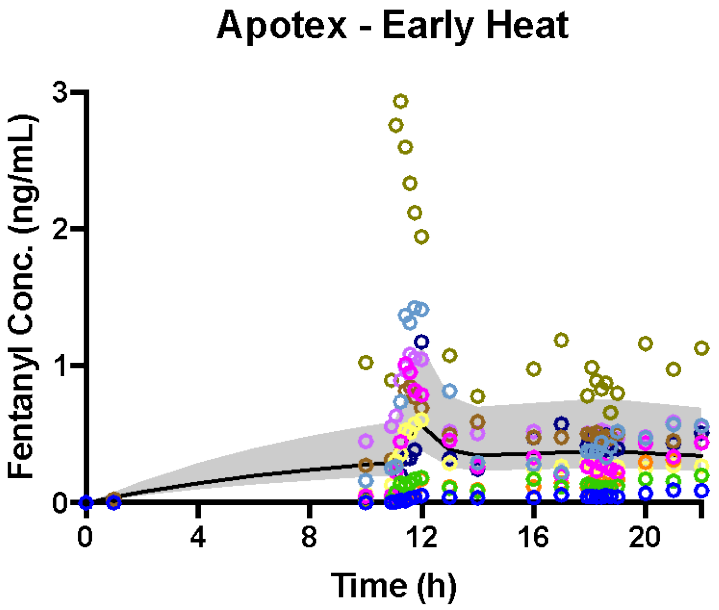


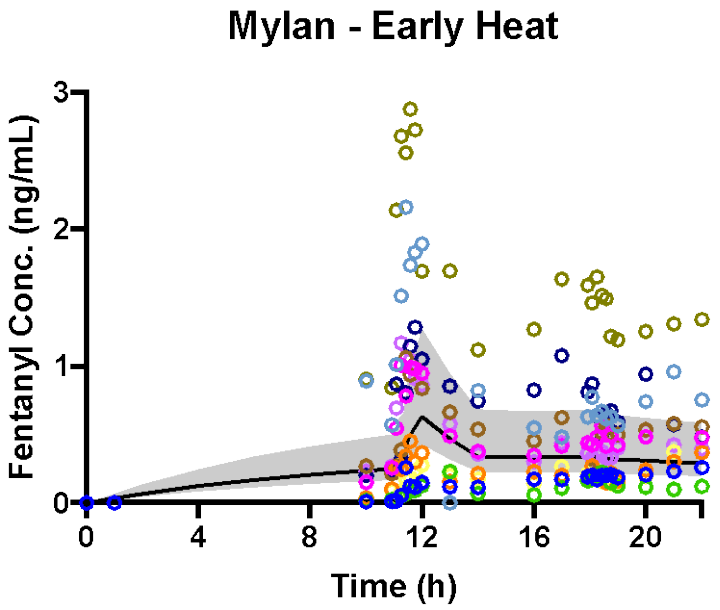


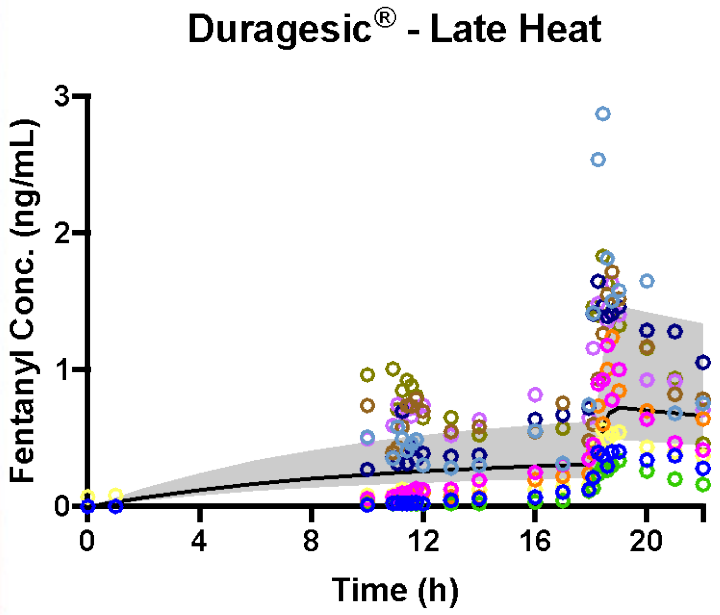
Approach I

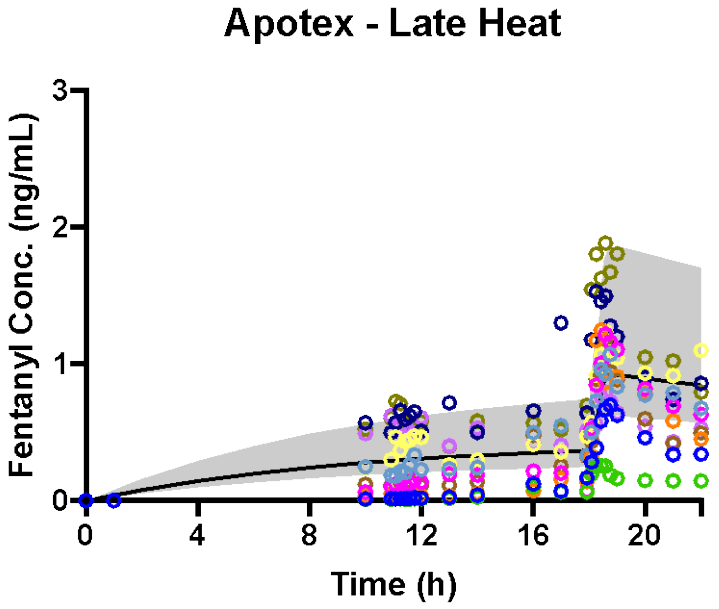
Grey shade represents prediction range when inter-subject variability of CL = 50%

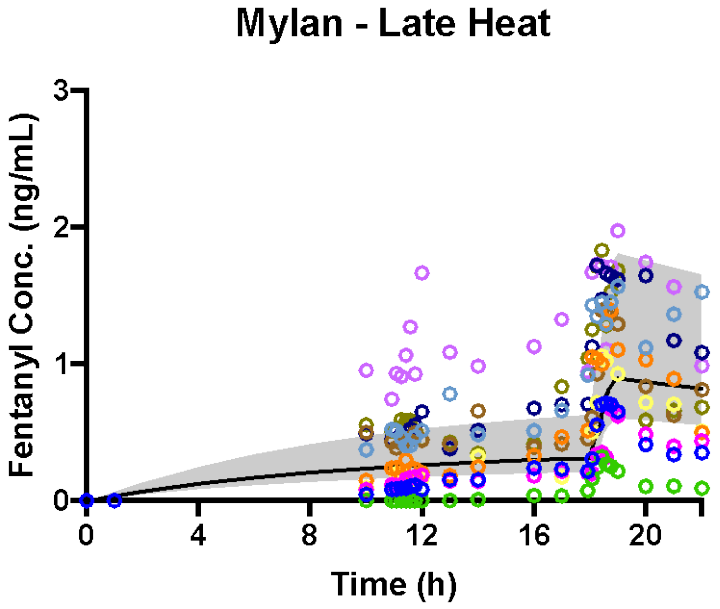






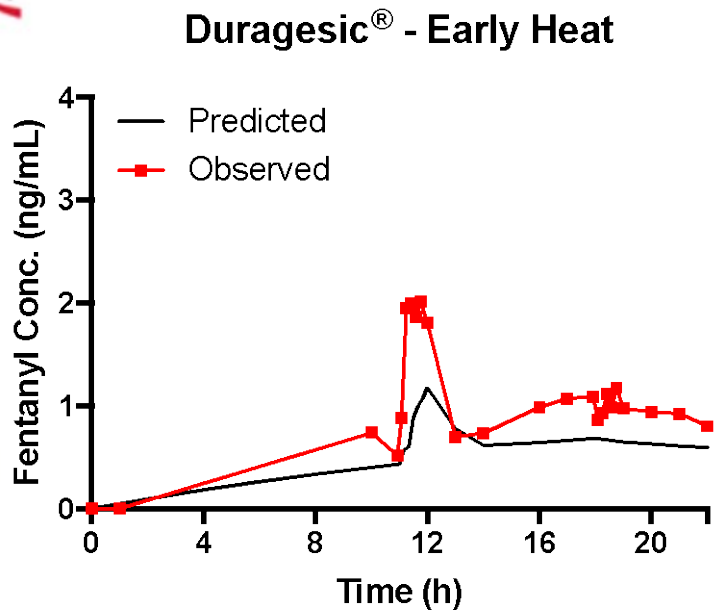


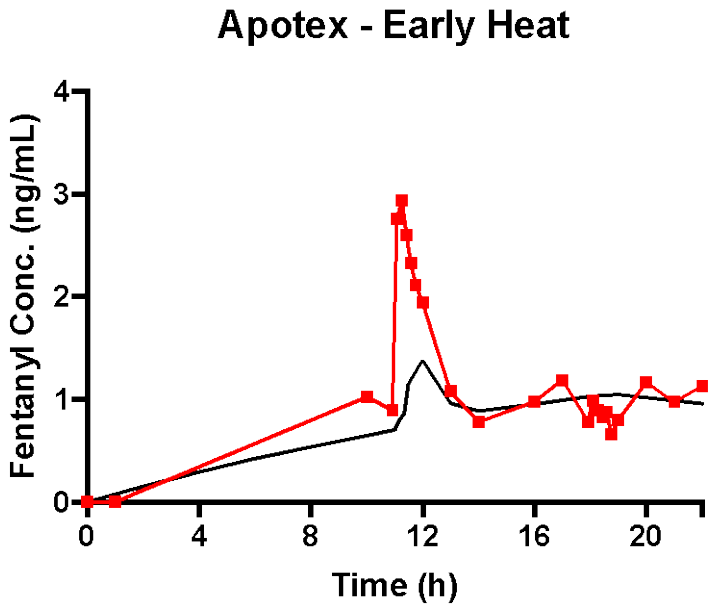


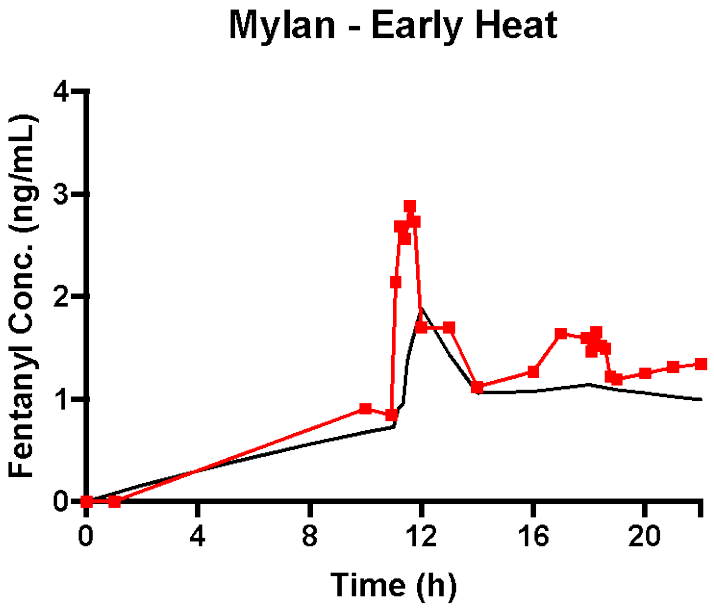


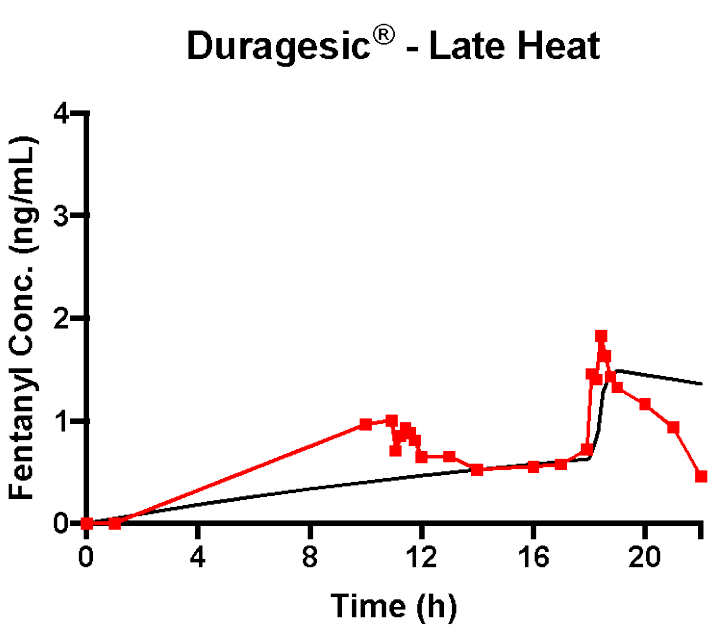
Approach I

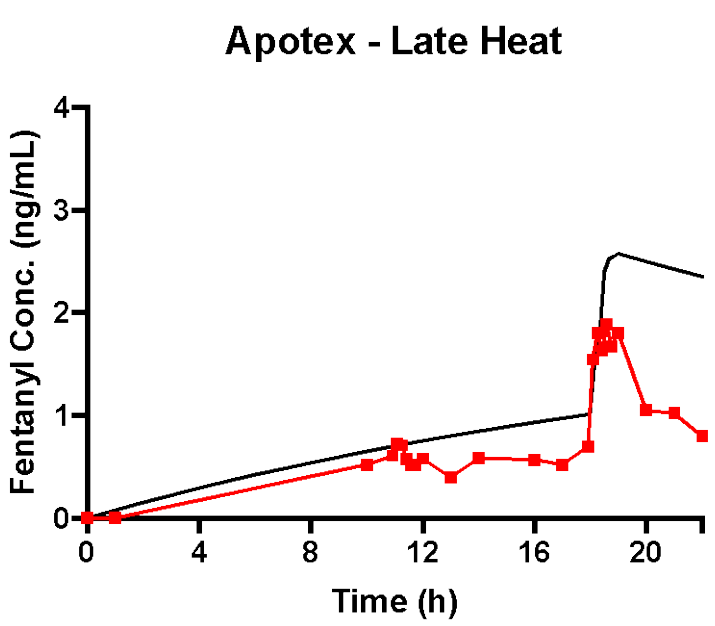
Subject TDF 024: Predicted using the subject's own F, CL_{IV} and k values

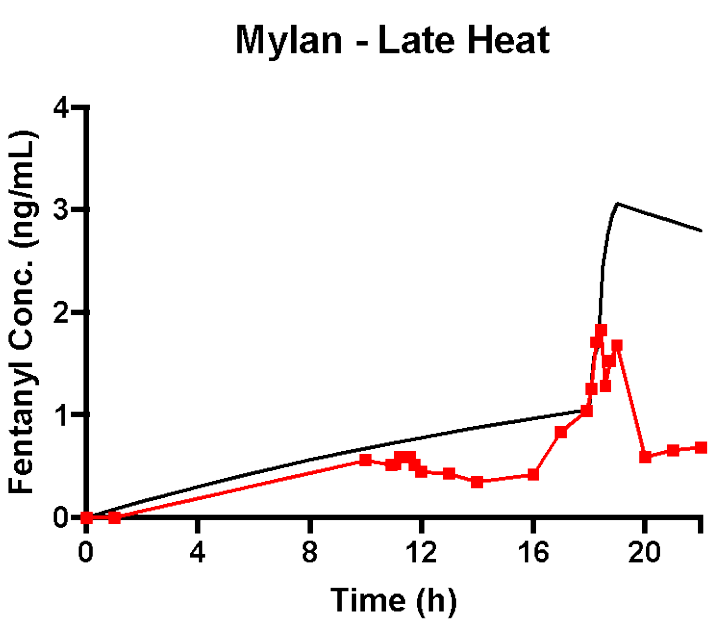




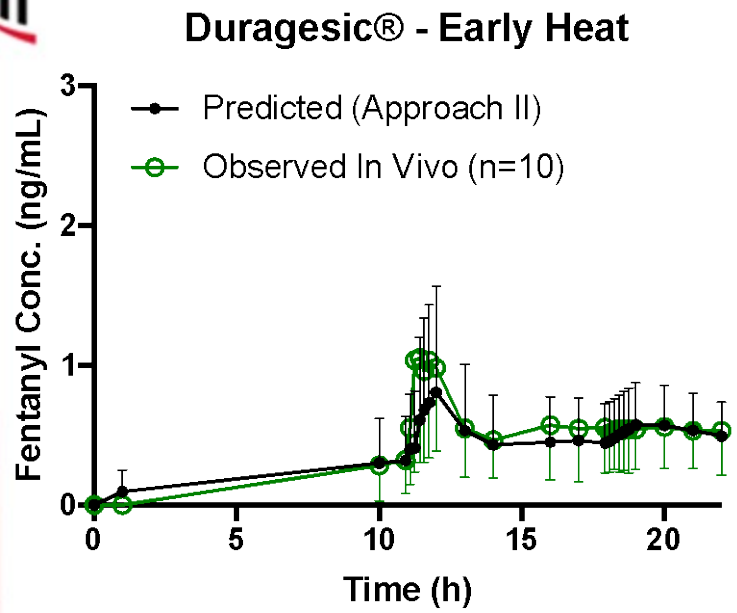


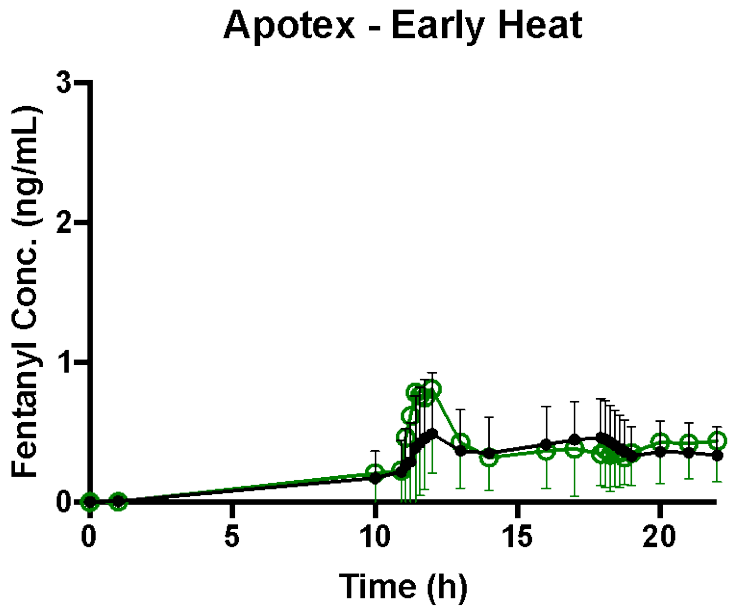


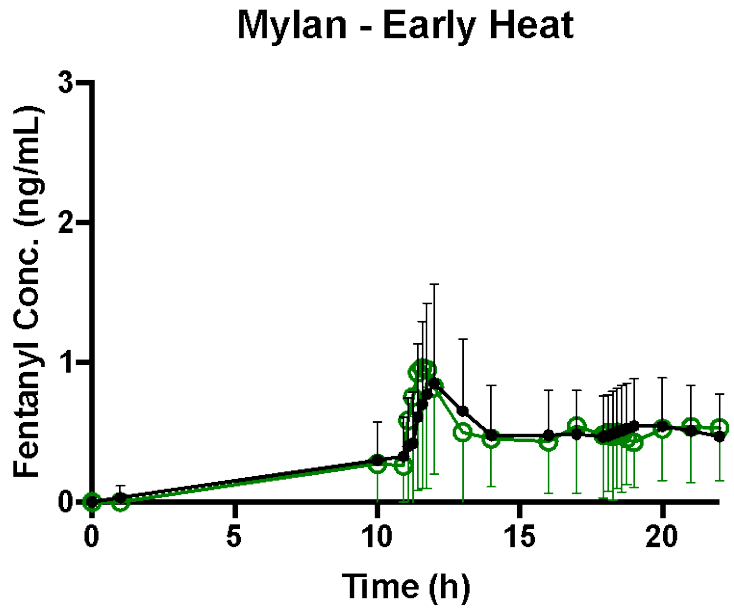


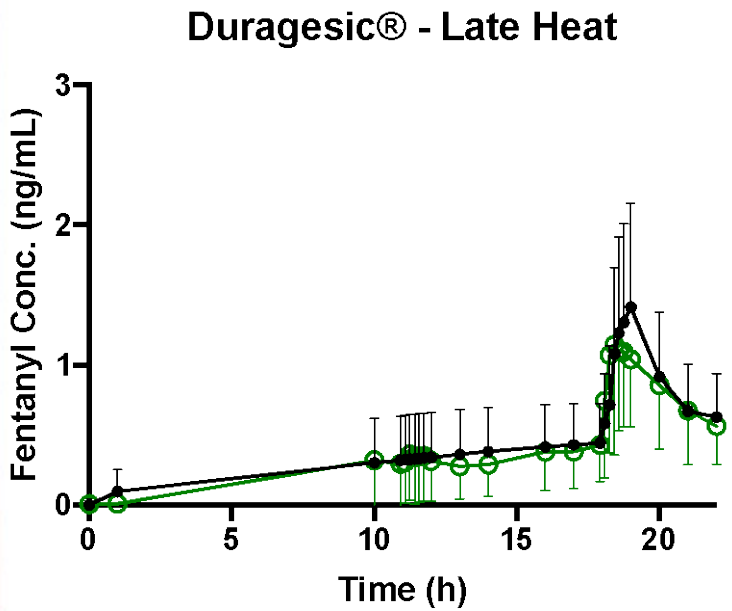


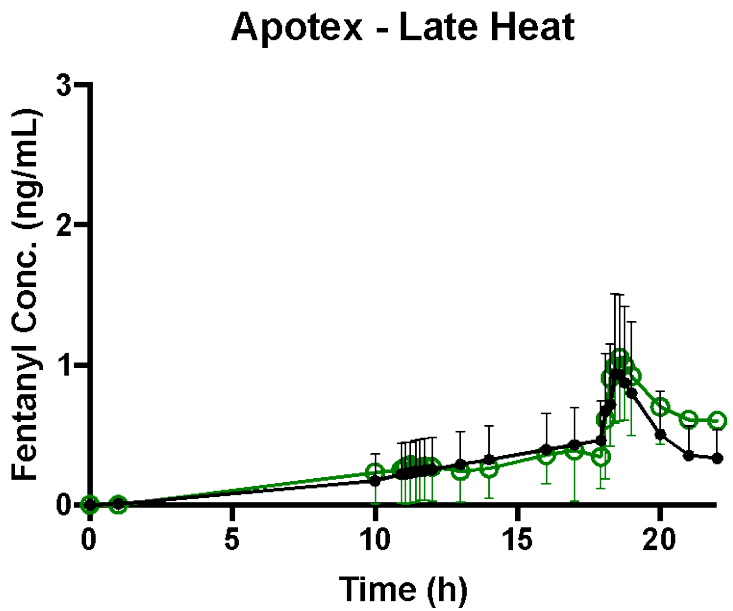
Approach II

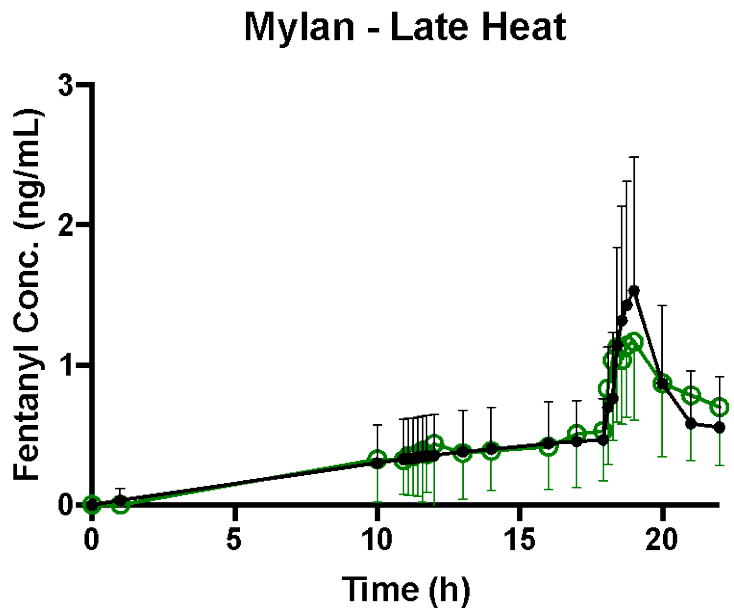

Duragesic® - Early Heat
Predicted (Approach II)
Observed In Vivo (n=10)
Fentanyl Conc. (ng/mL)
Time (h)


Apotex - Early Heat
Fentanyl Conc. (ng/mL)
Time (h)

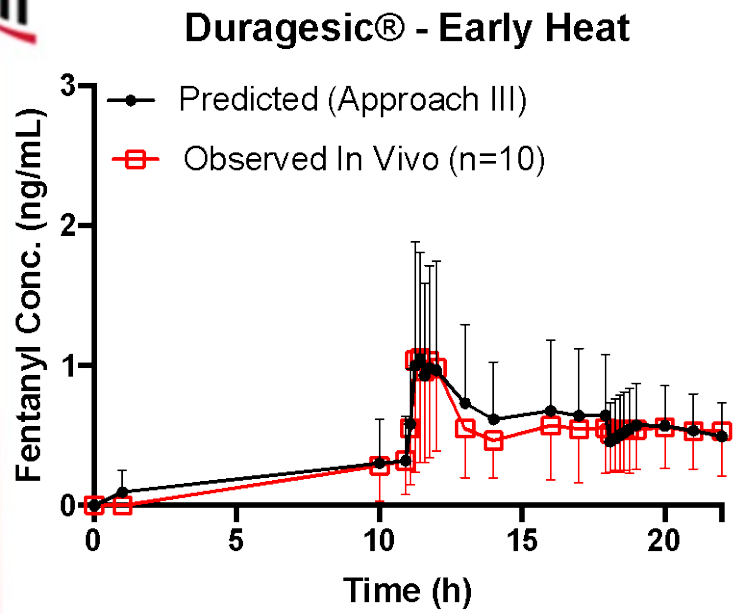

Mylan - Early Heat
Fentanyl Conc. (ng/mL)
Time (h)

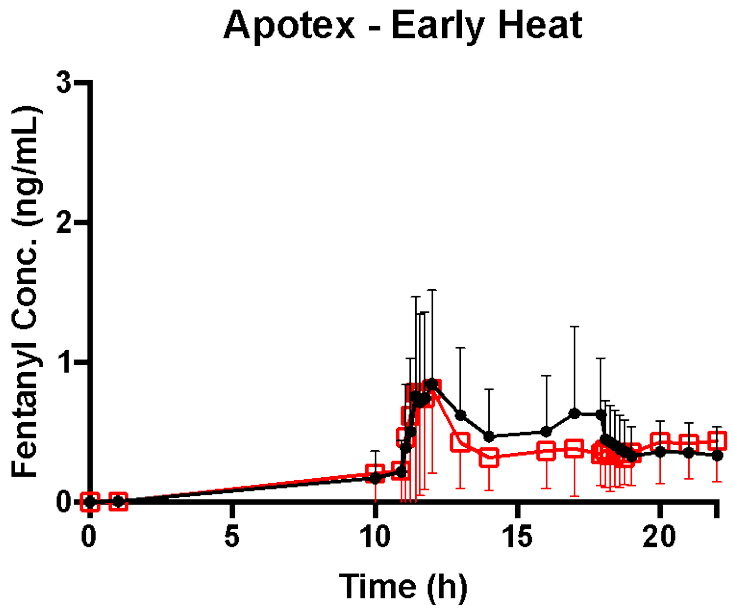

Duragesic® - Late Heat
Fentanyl Conc. (ng/mL)
Time (h)

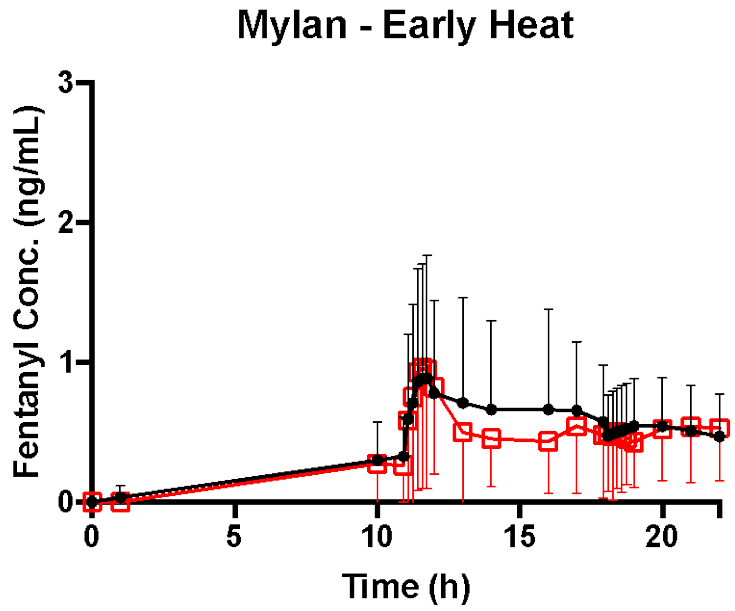

Apotex - Late Heat
Fentanyl Conc. (ng/mL)
Time (h)

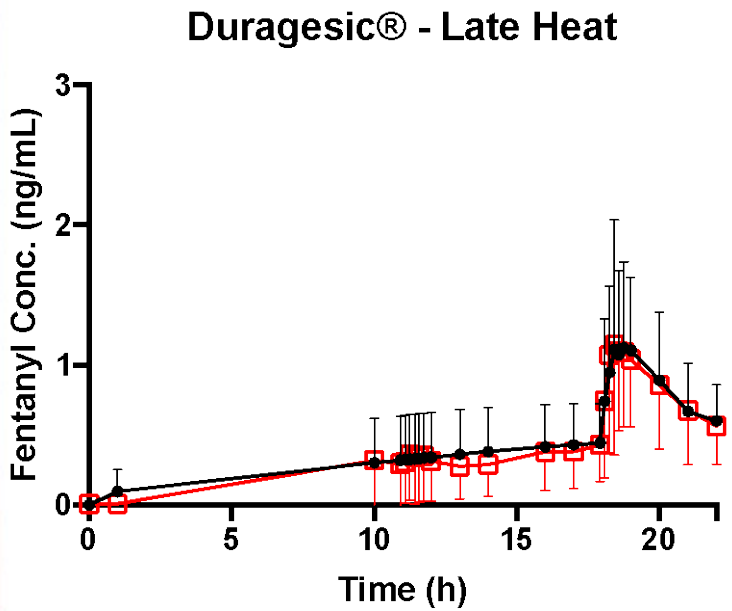

Mylan - Late Heat
Fentanyl Conc. (ng/mL)
Time (h)

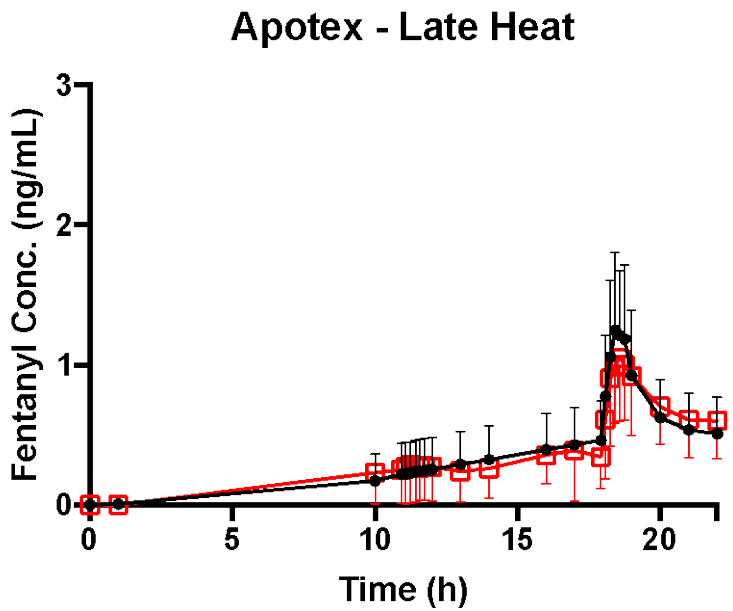
Approach III

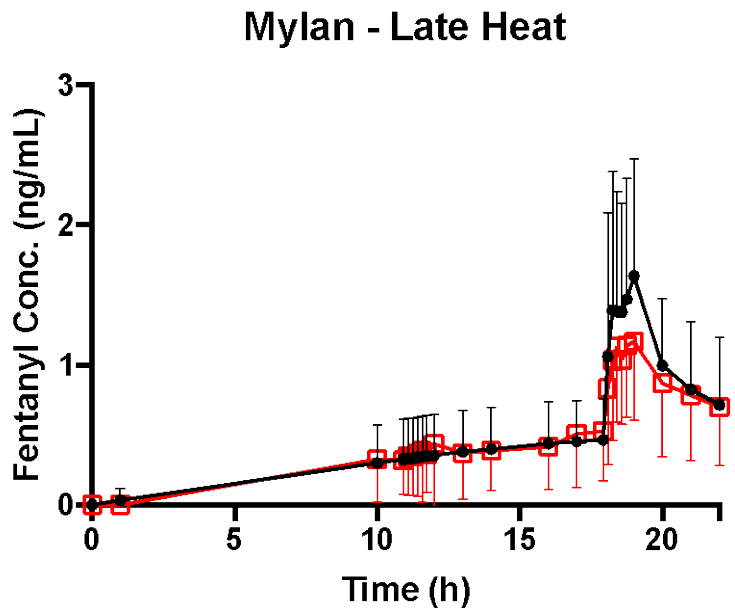












% Prediction Error

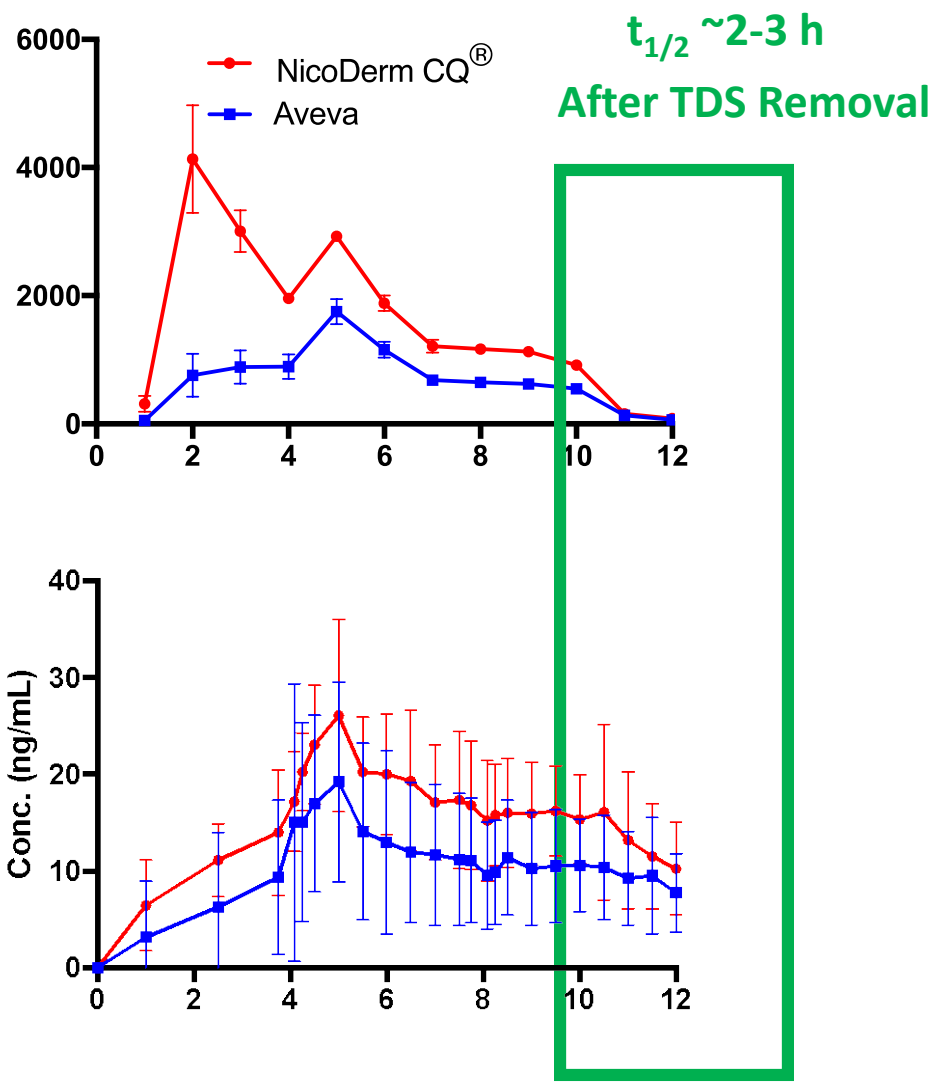
Fentanyl TDS	Duragesic®		Apotex		Mylan	
	Early Heat	Late Heat	Early Heat	Late Heat	Early Heat	Late Heat
Approach I						
Total AUC	31.7	17.5	4.0	19.3	24.3	18.4
C_{max}	37.7	36.8	29.8	12.4	34.1	23.2
Approach II						
Total AUC	3.3	13.1	10.2	11.8	5.1	0.6
C_{max}	23.4	23.6	39.6	11.2	11.4	31.5
Approach III						
Total AUC	15.2	10.1	11.9	0.8	18.1	8.3
C_{max}	0.5	2.3	4.4	18.7	7.7	40.5

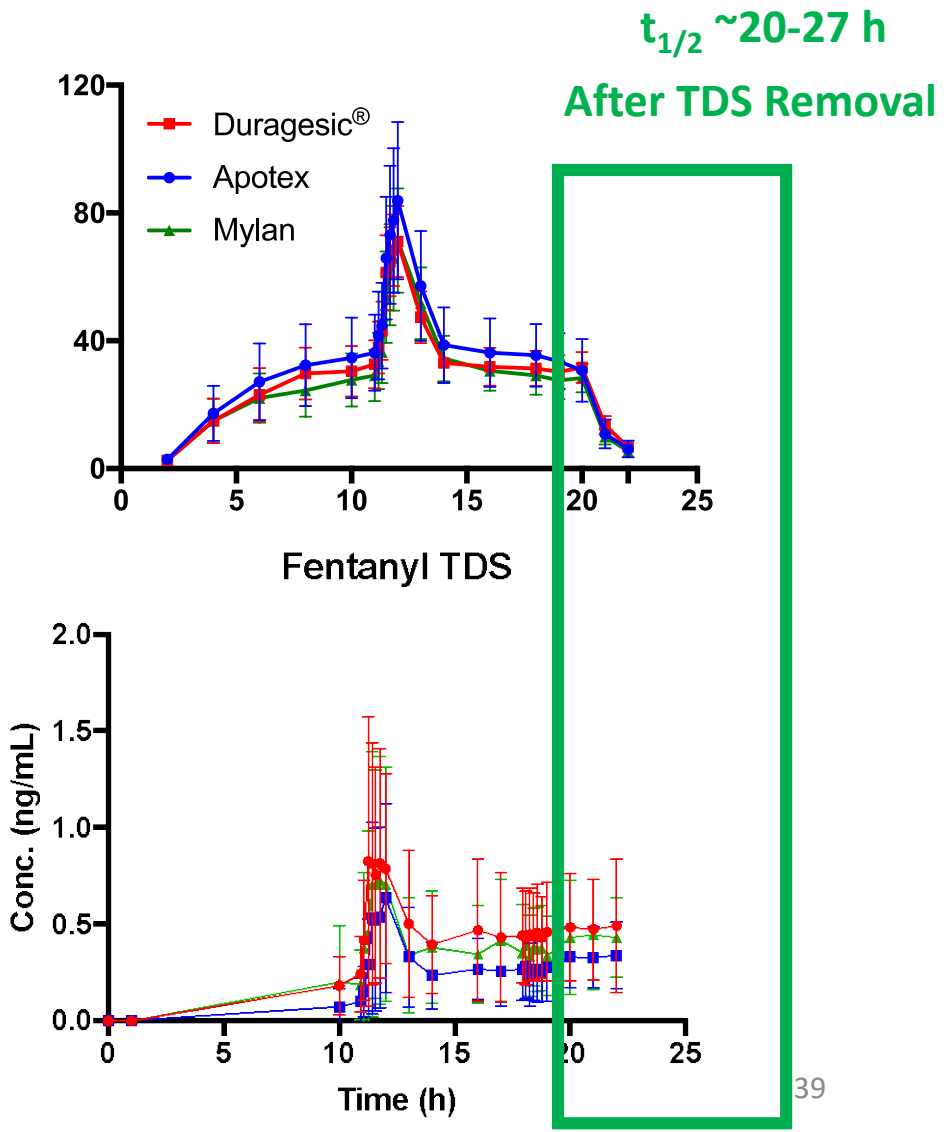
Conclusions – Fentanyl

- Early vs. Late Heat effect comparable both *in vitro* and *in vivo*
- Heat effect on three differently formulated TDS comparable both *in vitro* and *in vivo*
- However, *in vivo* heat effect seemed to be higher compared to the *in vitro* heat effect
- Preliminary IVIVCs between IVPT and clinical human PK studies under the matched study designs
  ⇒ Not as predictive compared to nicotine…

Why??

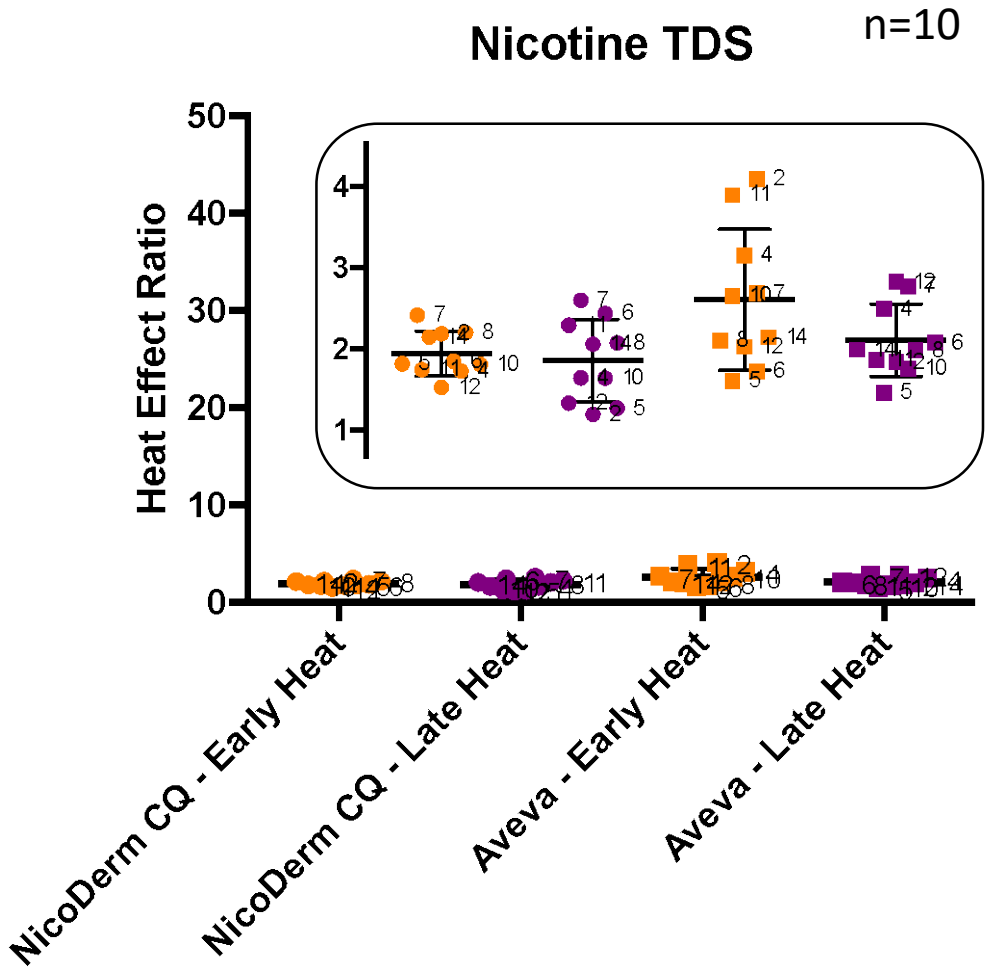
1. Lipophilicity of Fentanyl

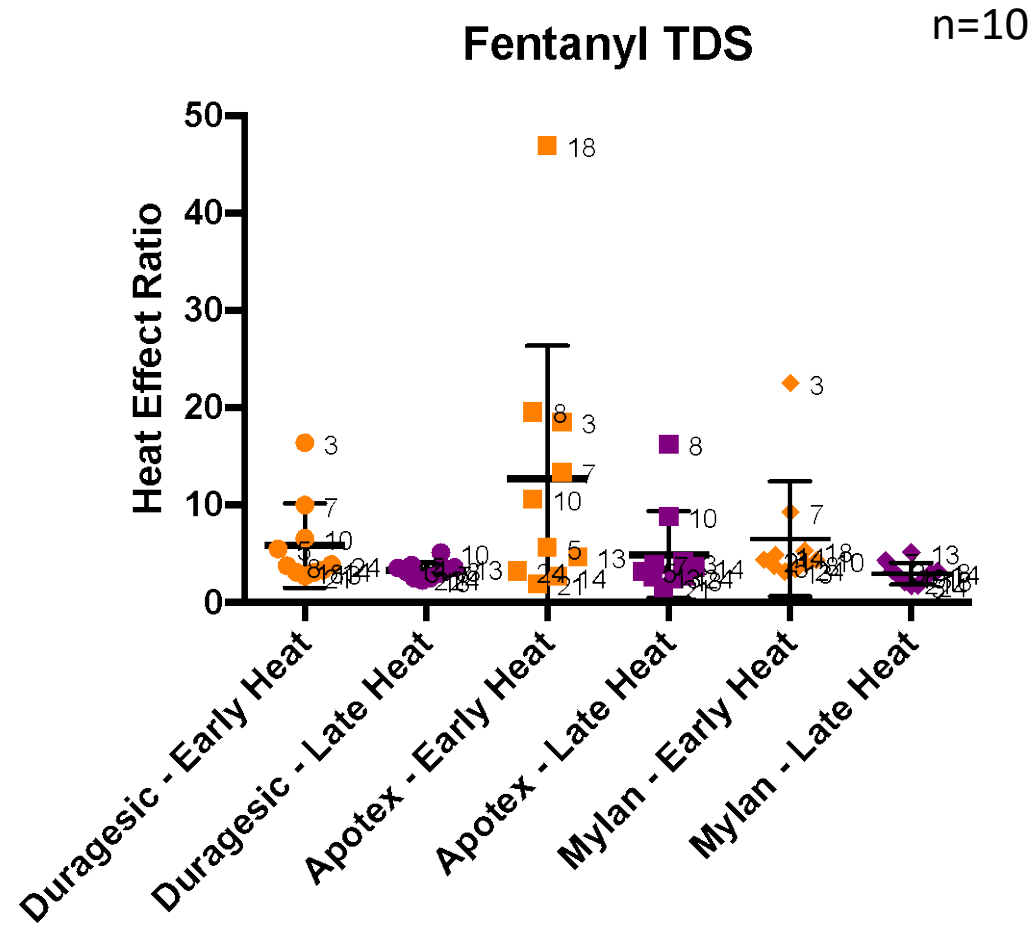




2. High Inter-subject Variability of Fentanyl

Heat Effect Ratio was determined by the ratio of the C_{max} during the 3h window and the concentration immediately before heat application





Conclusions - IVIVC

- Three approaches were evaluated to demonstrate a preliminary Level A IVIVC (IVIVR) for TDS
- Good preliminary IVIVC demonstrated for nicotine TDS, including heat effect
- Weaker preliminary IVIVC found for fentanyl TDS
  - Limitation of mimicking drug reservoir in skin layers, microcirculation and subcutaneous tissue *in vitro*
  - High inter-subject variability for fentanyl (+ Lack of reliable PK parameters)

Take Home Messages

- An *in vitro* heat effect study may be able to predict the *in vivo* heat effect for some drugs, following an IVIVC validation
- For certain drugs, an *in vivo* heat factor may need to be determined
- Heat effects are drug molecule and formulation excipient dependent---Diclofenac formulation data not shown
- Patches are not the only topical products affected by heat

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Acknowledgments

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Dr. Wilbur Chen
Dr. Jeff Fink
Melissa Billington
UMB GCRC nurses
Clinical Study Participants

Past & Current Lab Members

Contributors to the work presented:

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- Soo Hyeon Shin (Fentanyl, nicotine, acyclovir, diclofenac)
- Sherin Thomas (Lidocaine, buprenorphine, diclofenac)
- Dana Hammell (Lab Manager and Document Control)
- Dani Fox (Clinical Coordinator)
- Sagar Shukla (Lidocaine)
- Paige Zambrana (Sunscreens & glucose monitoring)
- Qingzhao Zhang (Metronidazole & rivastigmine)
- Past: Juliana Quarterman
- Dr. Inas Abdallah

U.S. FDA

- Dr. Sam Raney
- Dr. Priyanka Ghosh
- Dr. Caroline Strasinger

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- U01FD004955
- U01FD004275 (NIPTE-MD-2015)

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Back-Up

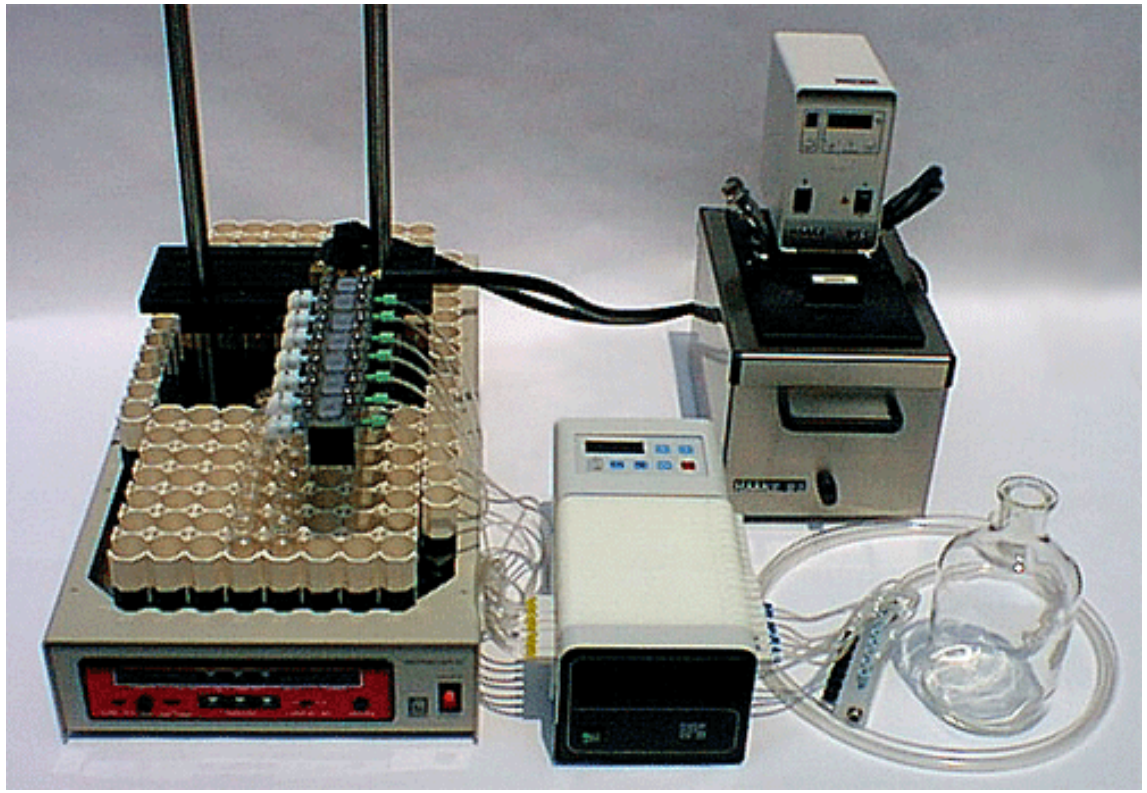
Methods: Skin Preparation

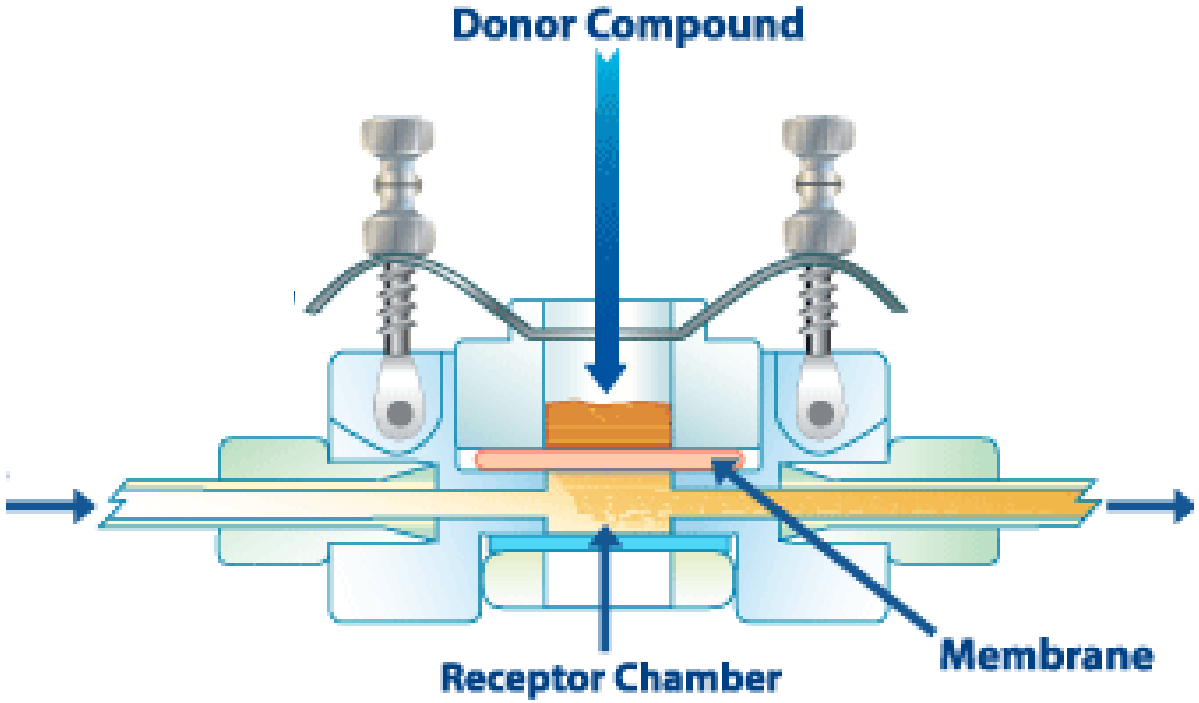
- Fresh human skin samples obtained post abdominoplasty surgery
- Dermatomed to ~250 microns
- Frozen until the day of experiment

Image obtained from the Stinchcomb Lab's SOP

IVPT Setup

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

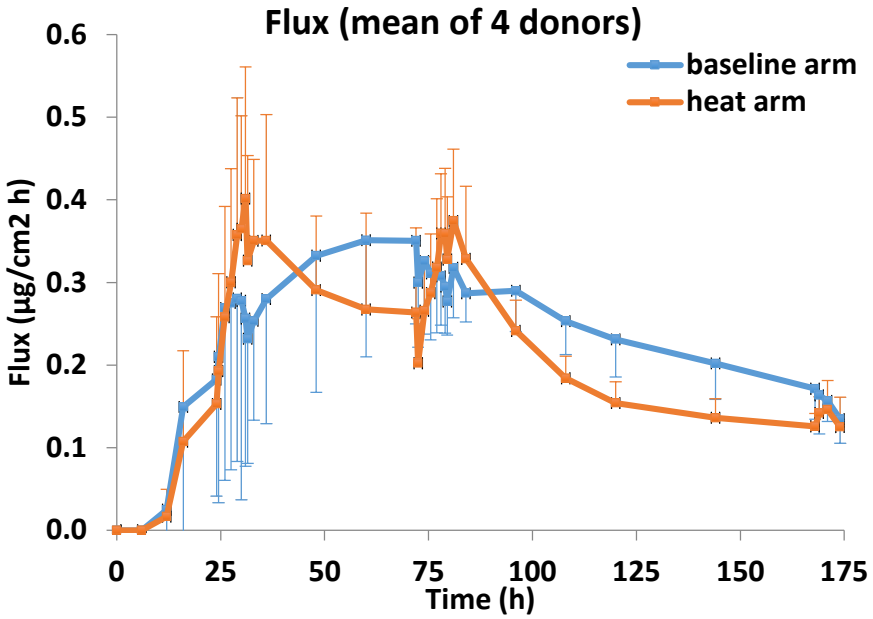




Images from www.ibric.org and www.permegear.com

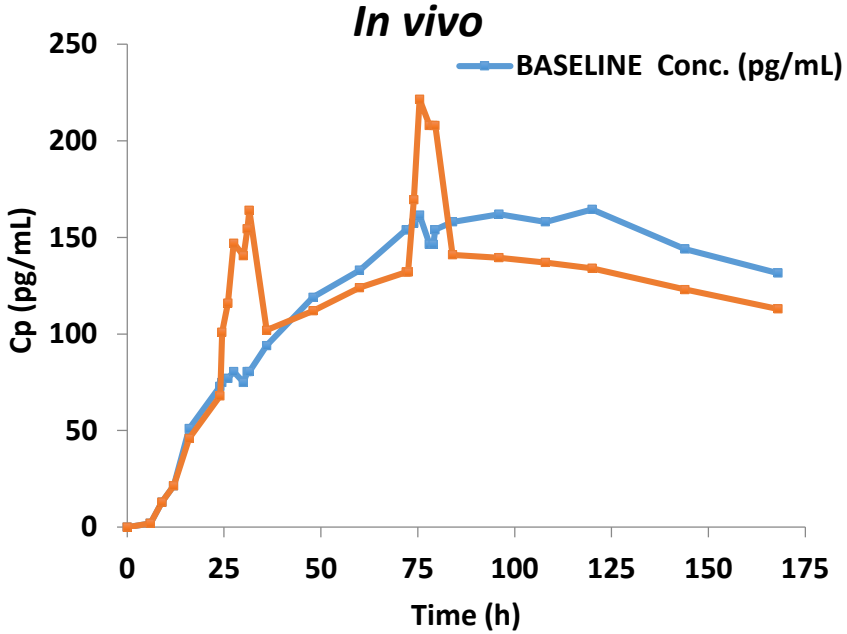
Buprenorphine Patch

Mean (± SD) *in vitro* flux n=4 donors



Mean *in vivo* concentration n=19/20 subjects

(values from graph grabbing software for graph taken from *Clinical Pharmacology and Biopharmaceutics Review* document for Butrans® available at Drugs@FDA.)



Human skin donor 4 (n=4 replicates per arm)

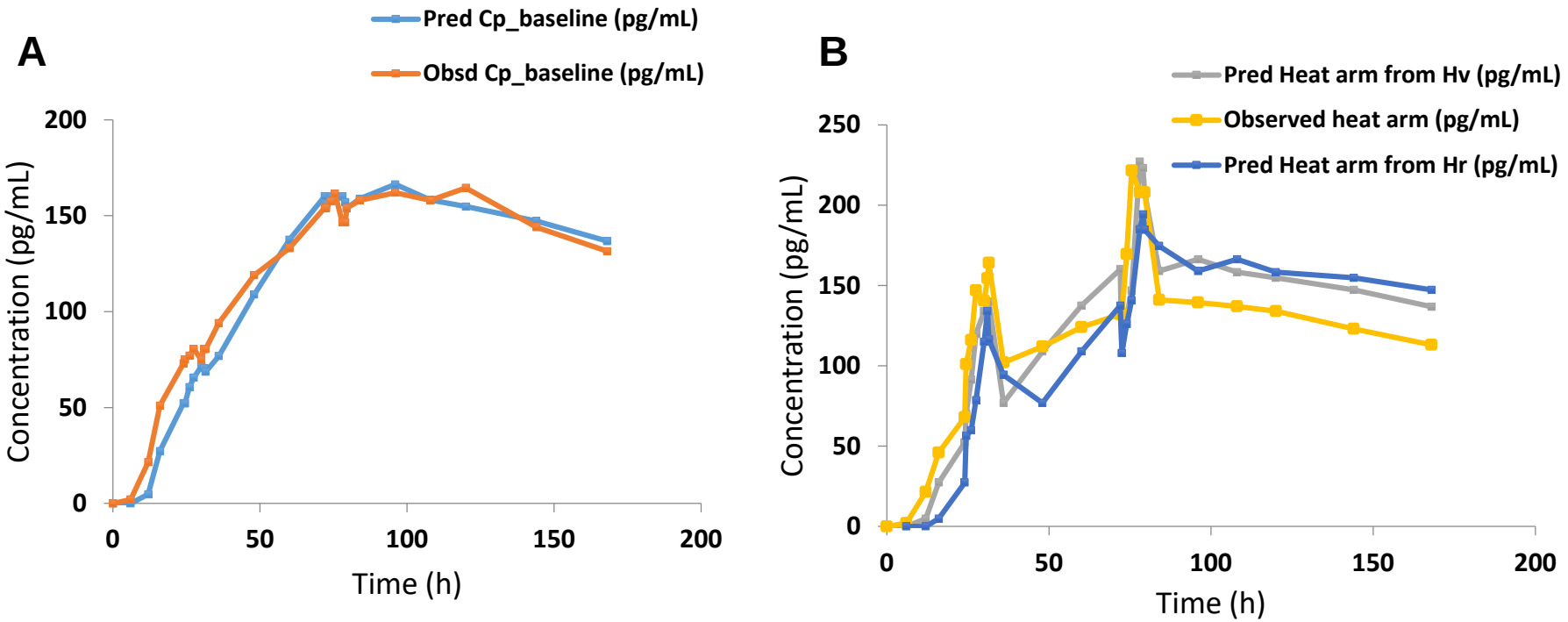
Heat Arm:

Early heat-heat applied from 24 to 31 h (every 2 h with 30 min gap)

Late heat- heat applied from 72 to 79 h (every 2 h with 30 min gap)

Patch off at 168 h

Buprenorphine Patch

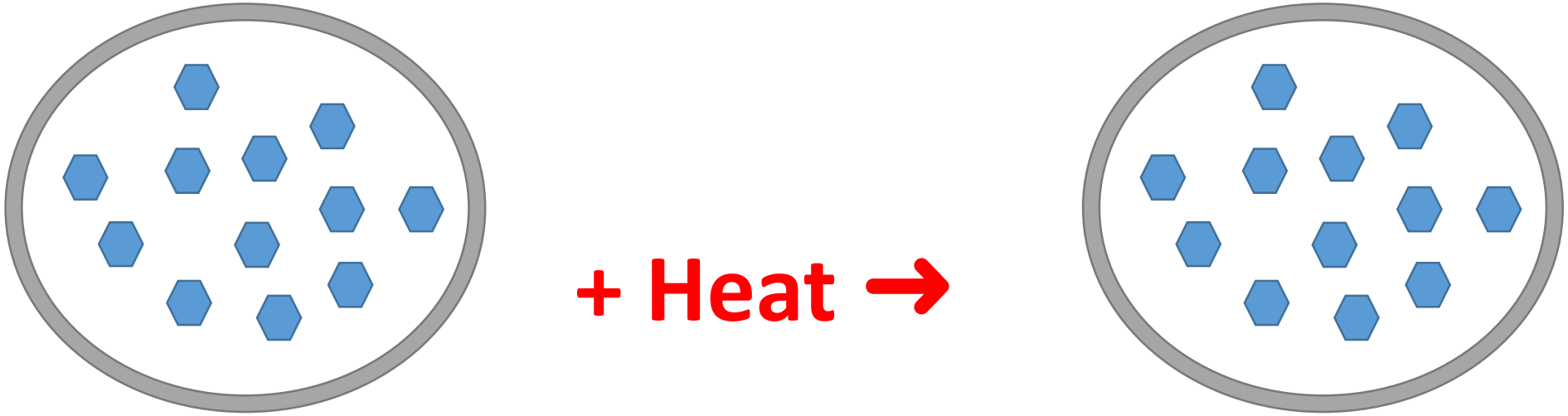


Plot for observed and predicted concentration versus time profiles for baseline arm (**A**) and heat arm (**B**)

Hv = *in vivo* heat factor **Hr = *in vitro* heat factor**

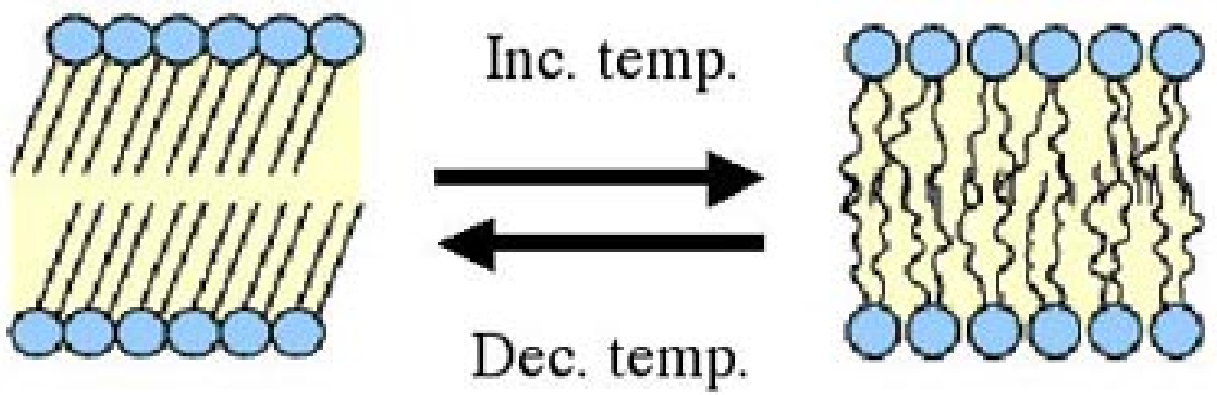
Influence of Heat on Percutaneous Absorption

1) ↑ Diffusivity of Drug from its Vehicle



Influence of Heat on Percutaneous Absorption

2) ↑ Fluidity of Stratum Corneum Lipids



Very regular,
Ordered structure

Less tightly packed,
Hydrocarbon tails
Disordered.

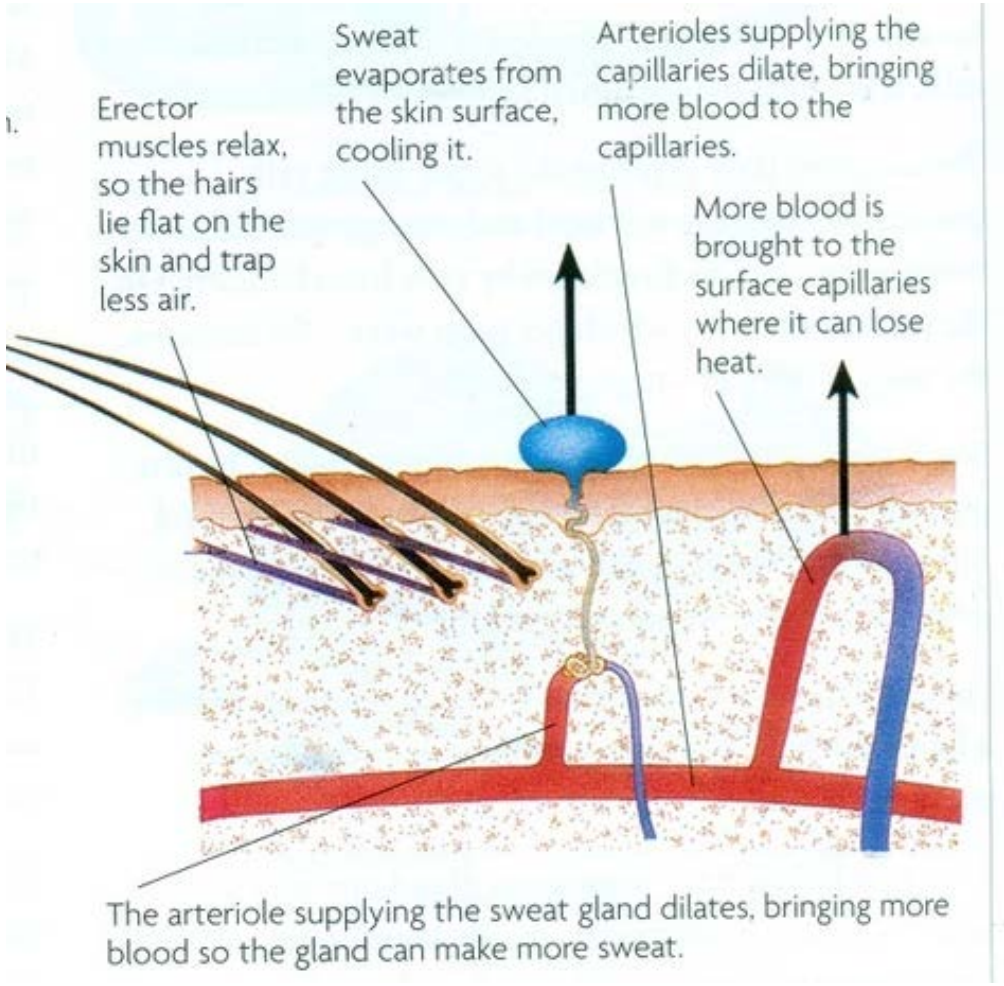
https://biochemistry3rst.wordpress.com/tag/phosphodiate/

Influence of Heat on Percutaneous Absorption

3) ↑ Cutaneous Vasodilation

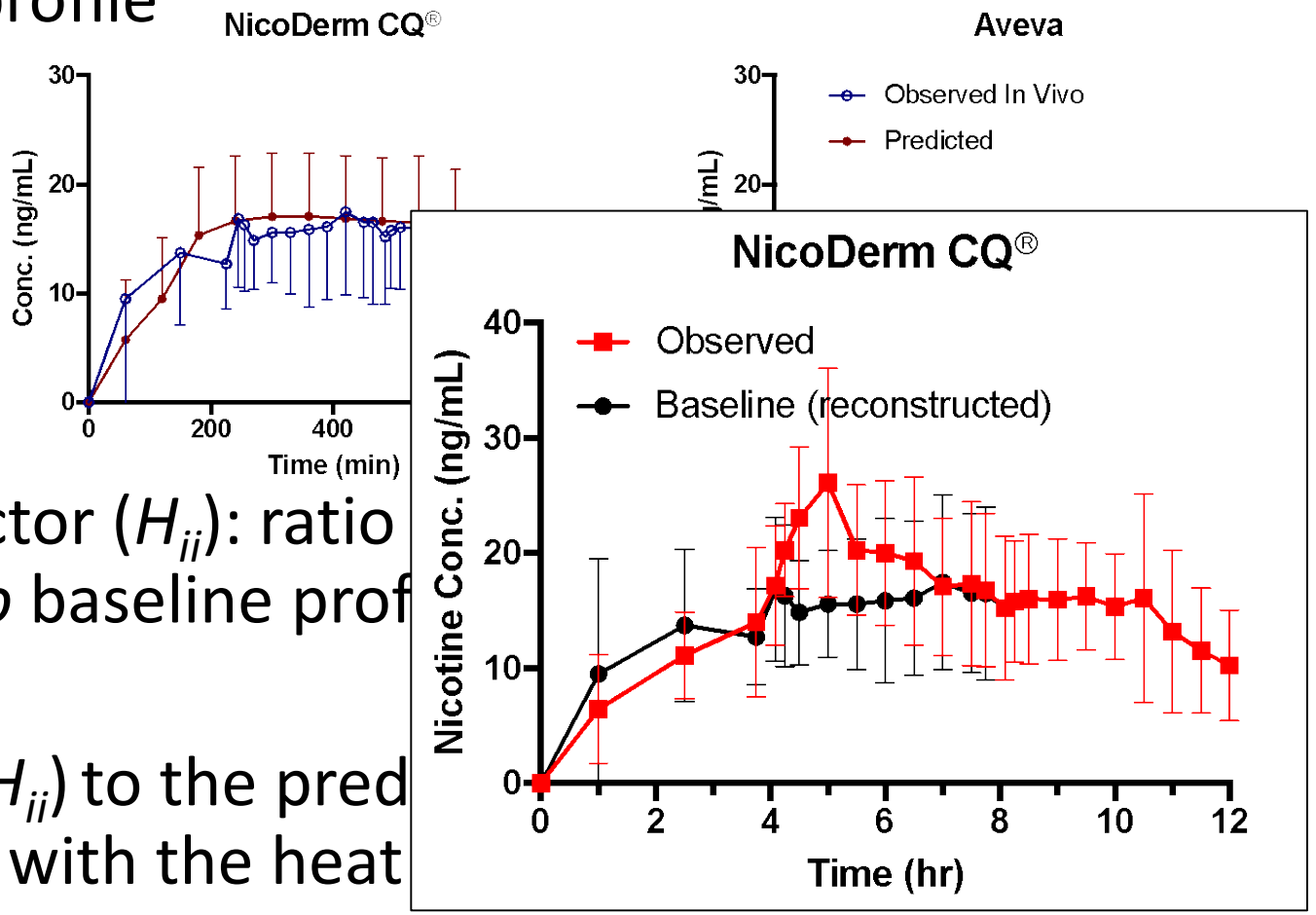
Body temperature regulation

When the body is too hot



IVIVC: Level A (Approach II & III)

6) Convolute the predicted fraction of drug absorption vs time profile to obtain conc. vs time profile



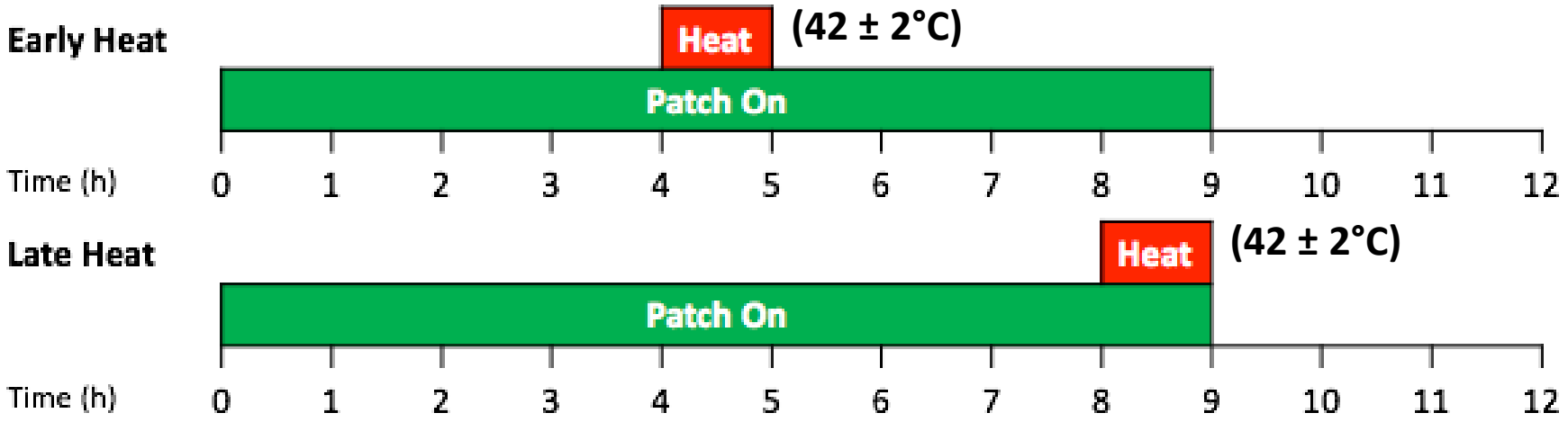
7) Calculate *in vivo* heat factor (H_{ii}): ratio
the reconstructed *in vivo* baseline prof
exposure

8) Apply heat factor (H_i or H_{ii}) to the pred
complete the prediction with the heat

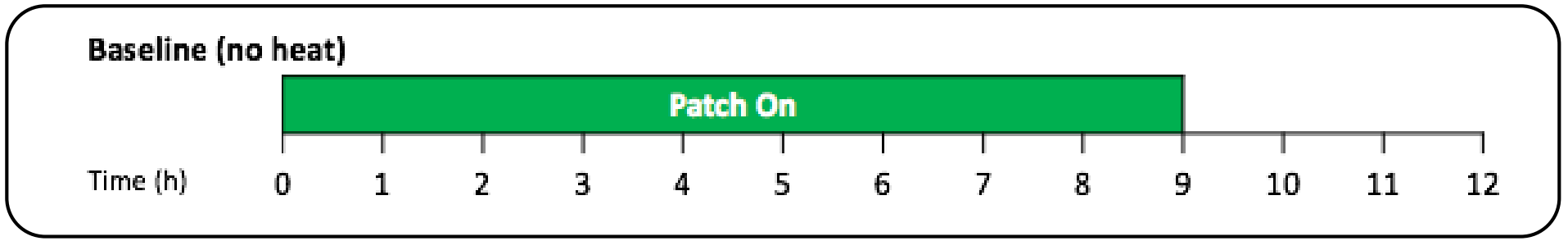
Nicotine TDS, 14 mg/24 h

	NicoDerm CQ®	Aveva
TDS size (cm^2)	15.75	20.12
Drug content (mg)	Not available	Not available
Rate/Area ($\mu g/h/cm^2$)	37	29
Adhesive	Polyisobutylene	Acrylate/Silicone
Other Inactive ingredients	Ethylene vinyl acetate-copolymer, high density polyethylene between clear polyester backing	Polyester

Study Designs



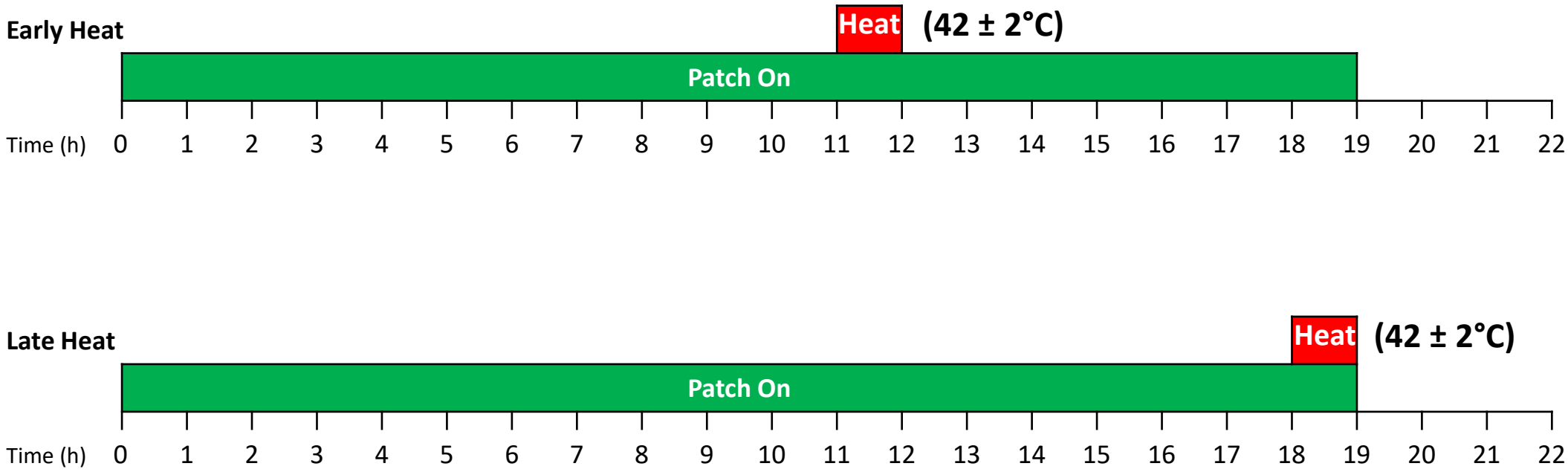
In Vitro Only



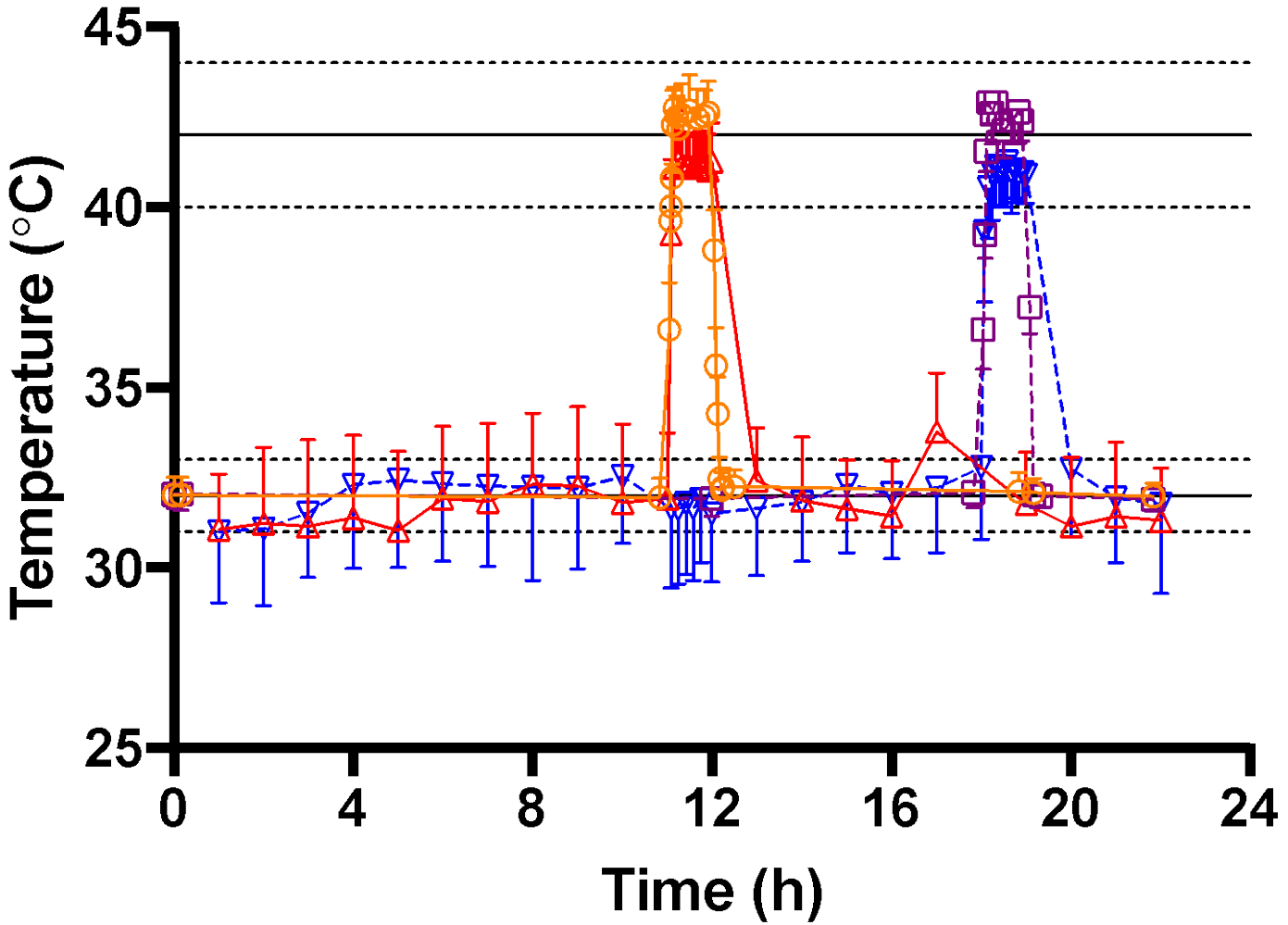
Fentanyl TDS, 25 µg/h

	Duragesic®	Apotex	Mylan
Drug Load (mg)	4.20	2.76	2.55
Size (cm^2)	10.50	10.70	6.25
Thickness (µm)	110	200	190
Adhesive	Polyacrylate	Polyisobutene	Silicone
Other Inactive Ingredients	Polyester/ ethyl vinyl acetate backing film, copovidone	Isopropyl myristate, octyldodecanol, polybutene, polyethylene/ aluminum/ polyester film backing	Dimethicone NF, polyolefin film backing
Appearance			

Study Designs



Temperature: *In Vitro* & *In Vivo*



Early Heat - In Vitro

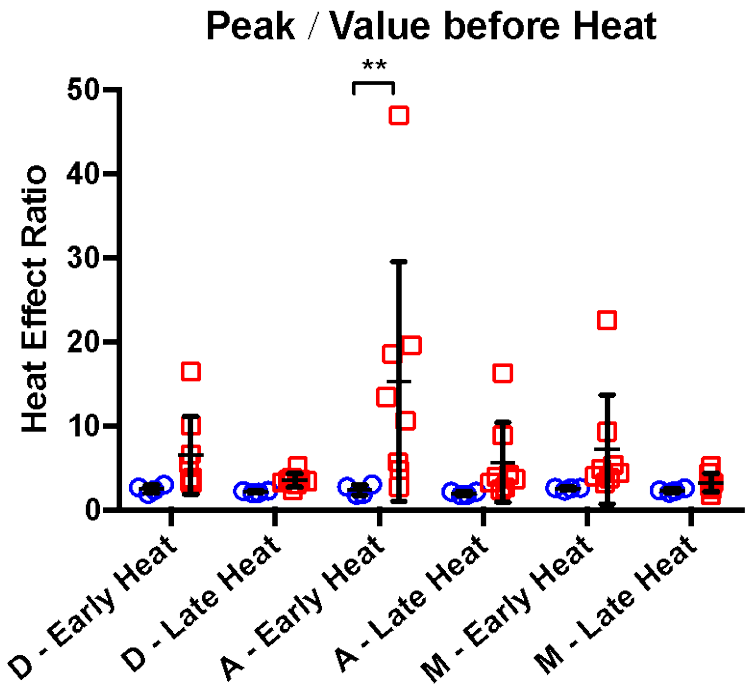
Late Heat - In Vitro

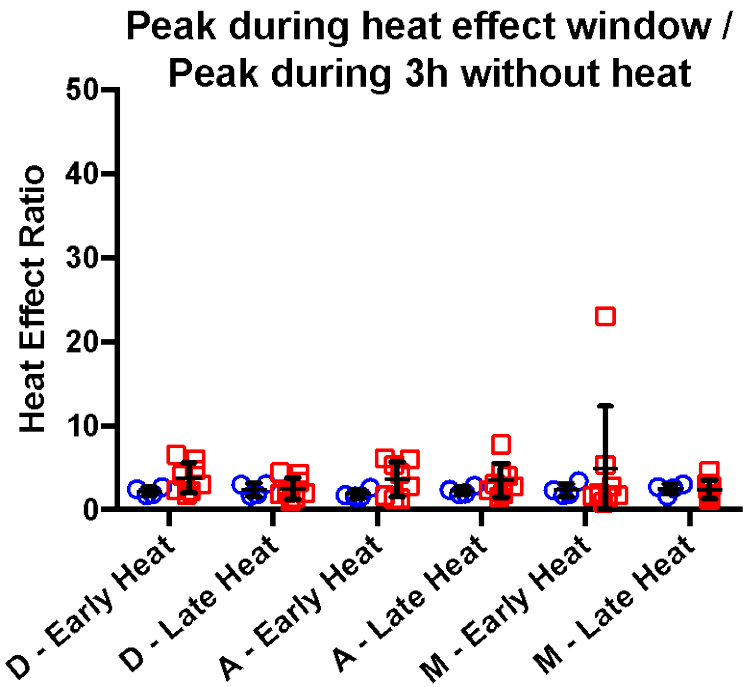
Early Heat - In Vivo

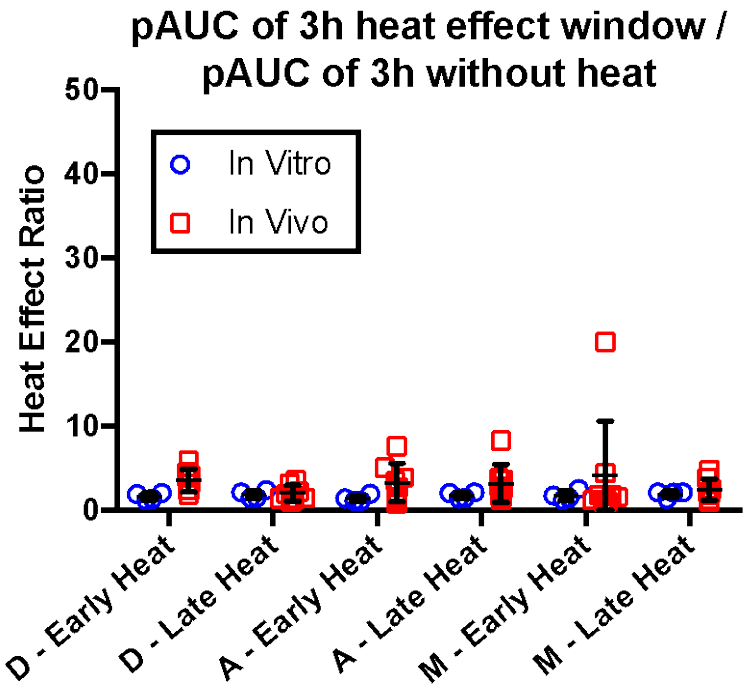
Late Heat - In Vivo

(42 ± 2°C)

IVIVC: Heat Effects





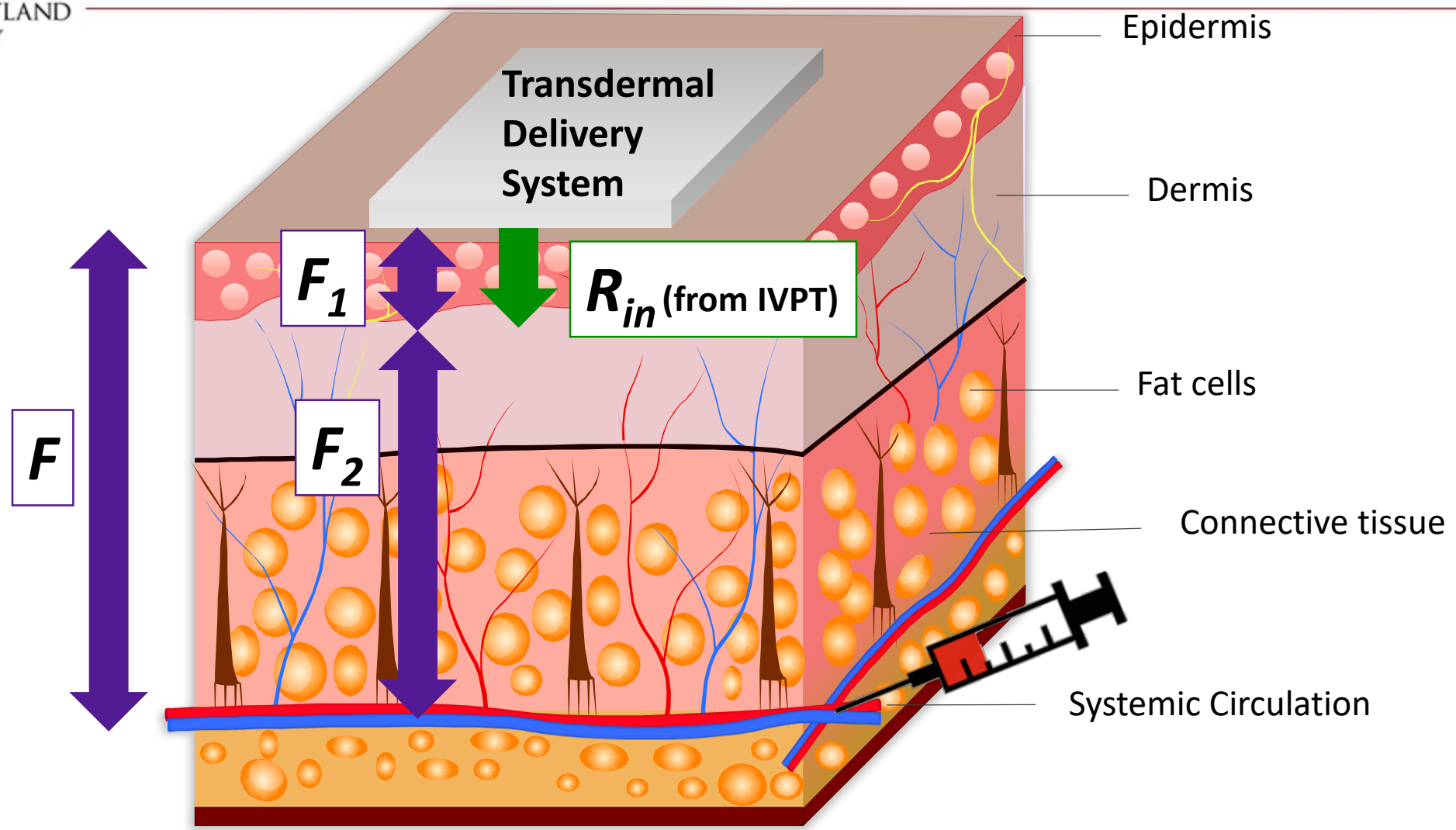


D: Duragesic®
A: Apotex
M: Mylan

In vivo heat effect is greater than *in vitro*, with higher variability

(Two-way ANOVA followed by Bonferroni's post-hoc multiple pair comparisons)

- *In vitro* data from 4 donors with n=4 replicates per donor
- *In vivo* data from 10 subjects



R_{in}, obtained from IVPT, does not fully capture F, which accounts for skin metabolism and skin depot effect in skin layers. In addition, the small fraction of F accounted for in R_{in} (F_1) has limitations in capturing *in vivo F* since R_{in} is coming from the *in vitro* system. Thus using F in *Equation 1* gives the best possible prediction of drug concentration in systemic circulation.

Diclofenac

	Patch	Solution	1% Gel	3% Gel
Inactive ingredients	Adhesive in aqueous base containing sodium polyacrylate, sodium carboxymethylcellulose	DMSO, ethanol, purified water, propylene glycol, hydroxypropyl cellulose	Carbomer homopolymer Type C, cocoyl caprylcaprate, fragrance, isopropyl alcohol, mineral oil, polyoxyl 20 cetostearyl ether, propylene glycol, purified water, strong ammonia solution	Hyaluronate sodium, benzyl alcohol, polyethylene glycol monomethyl ether, purified water
Dose applied (Equivalent amount of diclofenac)	- (878 mg/cm^2)	5 mg/cm^2 (approx. 100 µg/cm^2)	10 mg/cm^2 (approx. 100 µg/cm^2)	20 mg/cm^2 (approx. 300 µg/cm^2)

Diclofenac

Formulation	Heat Enhancement Ratio (Heat/No Heat)		##*p* value (Heat vs No Heat)	
	J_{max}	Cum. Amt.	J_{max}	Cum. Amt.
Patch	2.3	5.0	0.034	0.104
Solution	4.0	5.0	0.006	0.002
1% Gel	2.6	3.0	0.001	<0.001
3% Gel	1.0	0.87	0.961	0.883