# New developments in the assessment of cutaneous bioavailability and bioequivalence of topical dermatological drug products using dermal microdialysis

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- The contents are those of the authors and do not necessarily represent the official views of, nor an endorsement, by FDA/HHS, or the U.S. Government.

# Outline

- **Background**
  - Statement of problem
  - Cutaneous PK assessment methods
  - Skin
- **Microdialysis Optimization**
  - To address factors cause high variabilities
- **BA/BE Assessment**
  - Metronidazole Cream
  - Metronidazole Gel
- **Dermal Disposition Characterization**
  - Lidocaine
  - Prilocaine

# Role of Generic Topical Dermatological Drug Products (TDDPs) in Health Care

- Not enough generic formulations for 80% of the recently approved brand name products
- Based on government accountability office, 57% of topical products experienced an average of 276% increase in their price during 2010-2015
- The rise in the cost of topical dermatological drug products (TDDPs) may be attributed to the barriers involved in the development of TDDPs


- Administration, U.S.F.a.D. *Regulatory Education for Industry: 2019 Complex Generic Drug Product Development Workshop* Sep. 25-26, 2019.
- Li, D.G., C. Joyce, and A. Mostaghimi, *Association Between Market Competition and Prices of Generic Topical Dermatology Drugs.* JAMA dermatology, 2018. 154(12): p. 1441-1446.
- Administration, U.S.F.a.D., *Topical Drug Development Workshop - Evolution of Science and Regulatory Policy 2.* JULY 23 - 24, 2020

# Statement of Problem

- Bioequivalence (BE) for systemically acting drug products: in vivo pharmacokinetic (PK) study
- Bioequivalence in topical dermatological products:
- **Characterization-based BE**
  - ✓ Formulation Samness,Q3 Similarity of the physicochemical properties
  - ✓ In Vitro Release Test studies (IVRT)
  - ✓ In Vitro Permeation Test studies (IVPT)
- **Pharmacodynamics studies (Vasoconstrictor Assay)**
- **Clinical endpoint studies:**
  - ✓ Assessing severe toxicity
  - ✓ Relief of symptoms
  - ▪ Disadvantages: A large number of subjects(≥500-700), lack of sensitivity, time-consuming, and costly

McLeod, C., et al., *Choosing primary endpoints for clinical trials of health care interventions.* Contemporary clinical trials communications, 2019. **16**: p. 100486-100486

Braddy, A.C., et al., Survey of international regulatory bioequivalence recommendations for approval of generic topical dermatological drug products. The AAPS journal, 2015. 17(1): p. 121-133

# Potential Pharmacokinetics-Based Methods to Evaluate BE

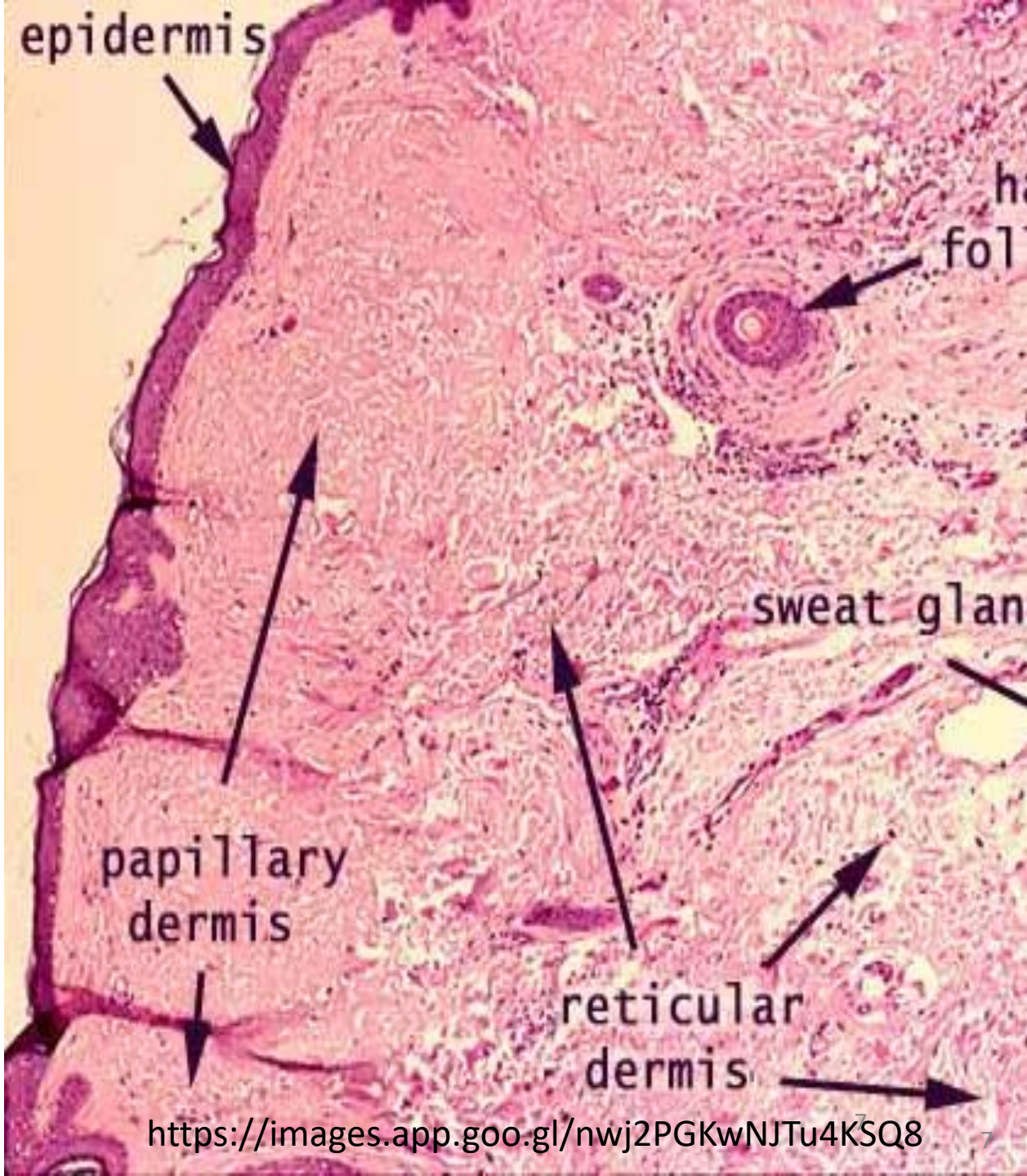


- Pharmacokinetics-based methods that have the potential to provide a standard BE assessment for topical dermatological products are :

  - ✓ *In vivo* skin-stripping
  - ✓ Open flow microperfusion (OFM)
  - ✓ *In vivo* microdialysis (dMD)
  - ✓ In Silico modeling
  - ✓ Raman Imaging

# Skin

- **Epidermis**
  - Categorized into four layers according to keratinocyte morphology and position.
  - Keratinocytes synthesize keratin, a long, threadlike protein with a protective role
  - Outermost layer: stratum corneum (SC), consists of keratinized, dead squamous cells
- ***Dermis***
  - Consists of two indistinct layers, the papillary layer, and the reticular layer. This layer contains fibroblasts, phagocytes, nerve fibers, and touch receptors , lymphatic capillaries and is well vascularized
- **Hypodermis**
  - Connects the skin to the underlying fascia of the bones and muscles, consists of well-vascularized, connective tissue and adipose tissue, which functions as a mode of fat storage




https://images.app.goo.gl/nwj2PGKwNJTu4KSQ8

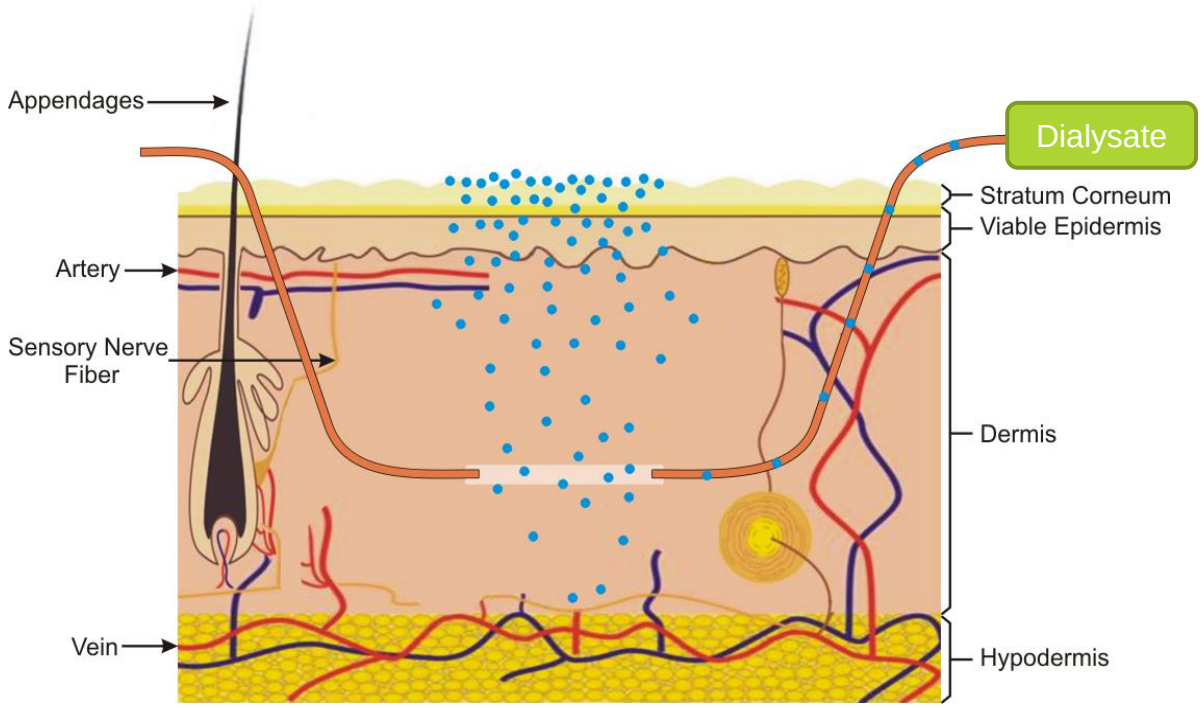
# Skin Metabolism

- Understanding skin metabolism is important when considering drug discovery, safety assessment, and efficacious dose of TDDPs
- Numerous studies identified skin enzyme activity such as cytochromes P450, flavin monooxygenases, glutathione-S-transferases, N-acetyltransferases, and sulfotransferases *( in vitro, ex vivo* and biopsy)
- The viable epidermis is known as the most enzymatically active part of the skin
- The enzymatic activity of dermis is much weaker compared to epidermis
- Cutaneous PK of skin metabolites has not been investigated up to date


- Pyo, S.M. and H.I. Maibach, *Skin Metabolism: Relevance of Skin Enzymes for Rational Drug Design.* Skin Pharmacology and Physiology, 2019. 32(5): p. 283-294.
- Kazem, S., E.C. Linssen, and S. Gibbs, *Skin metabolism phase I and phase II enzymes in native and reconstructed human skin: a short review.* Drug Discov Today, 2019. 24(9): p. 1899-1910.
- Rolsted, K., et al., *Cutaneous in vivo metabolism of topical lidocaine formulation in human skin.* Skin Pharmacol Physiol, 2009. 22(3): p. 124-7.

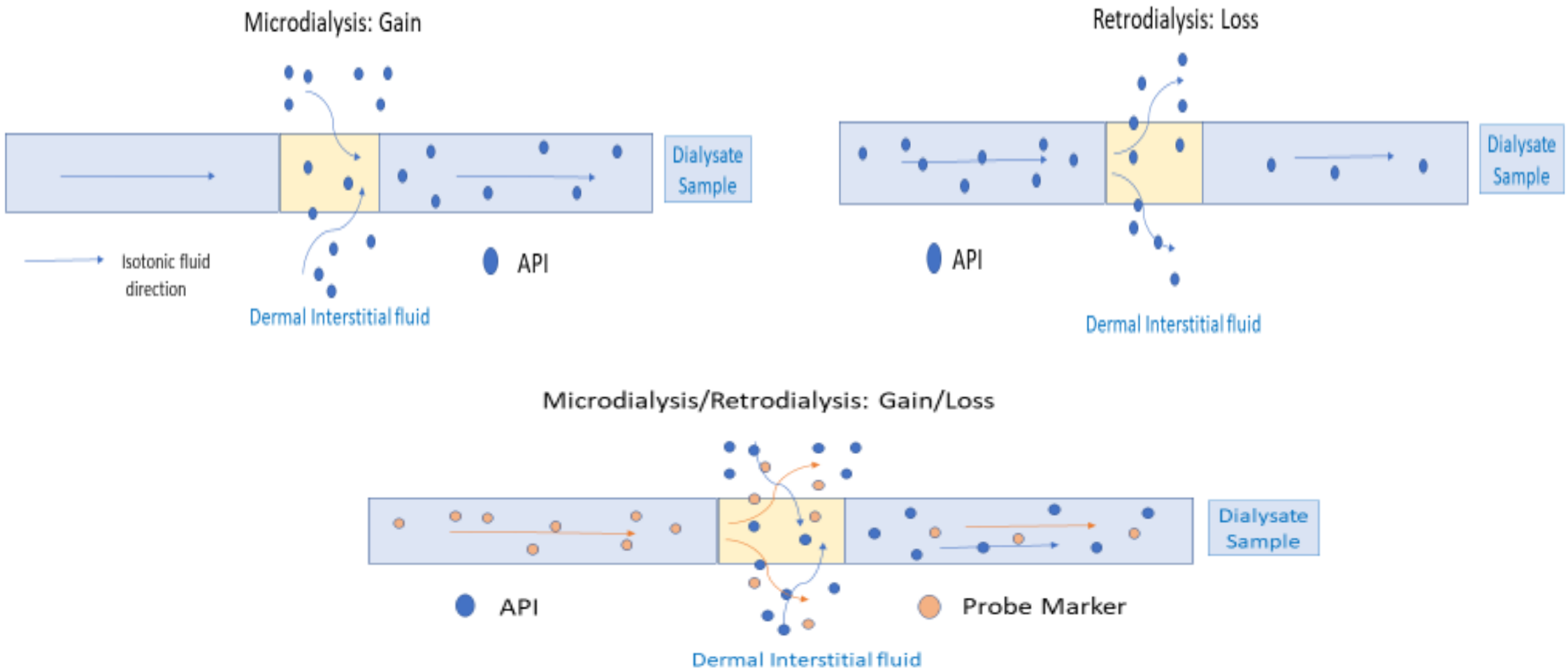
# Dermal Microdialysis (dMD) Principles

- dMD is a technique where a tiny, hollow semi-permeable dialysis membrane with micron-sized pores is inserted into the dermis and perfused with an isotonic fluid to enable continuous sampling of the dermal interstitial fluid (dISF)
- The exchange of drug molecules occurs at the dialysis membrane level of dMD
- Drug molecules with MW smaller than the membrane pores enter or exit the probe according to the concentration gradient between the perfusing fluid and the dISF
- The diffusion process is equal in both directions
- dMD allows measuring the unbound drug molecules, which are responsible for the therapeutic activity of the drug, and accounts as an advantage of dMD



# dMD principles

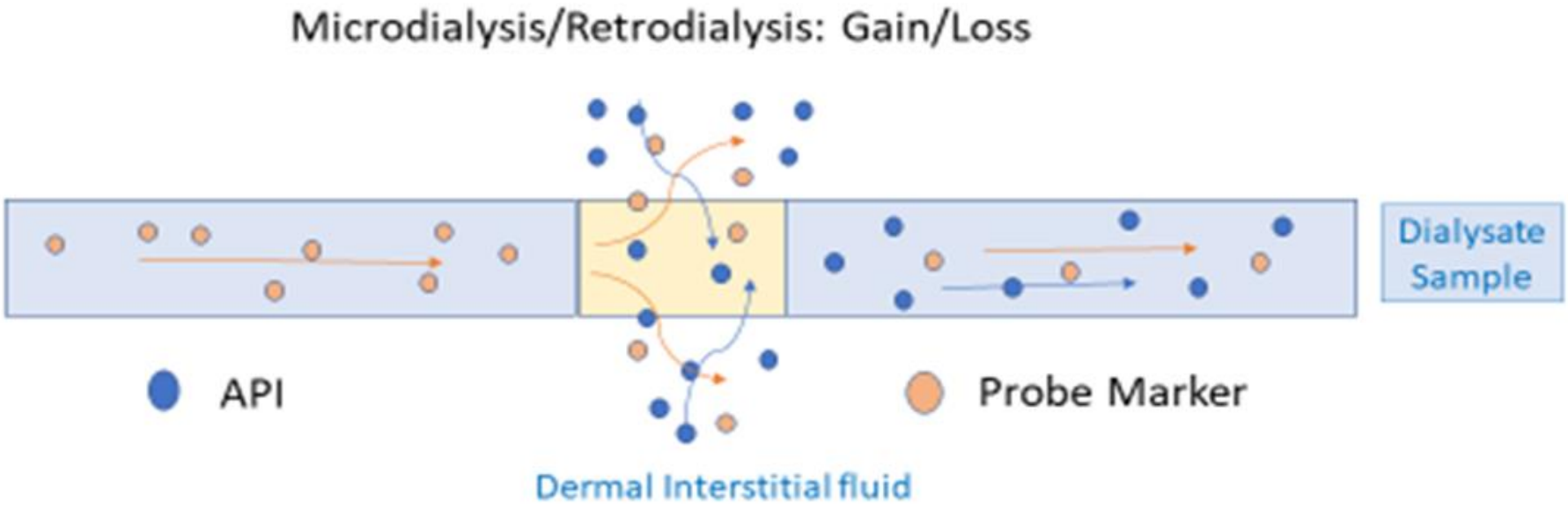




# Advantage of probe marker utilization



- Correction Factor $= \frac{\mathrm{C}\,probe\ marker\ _{\mathrm{Perfusate}} - \mathrm{C}\,probe\ marker\ _{\mathrm{Dialysate}}}{\mathrm{C}\,probe\ marker\ _{\mathrm{Perfusate}}}$
- $dISFconcentration = \frac{\mathrm{C}_{\mathrm{Dialysate}}}{Correction\ Factor}$



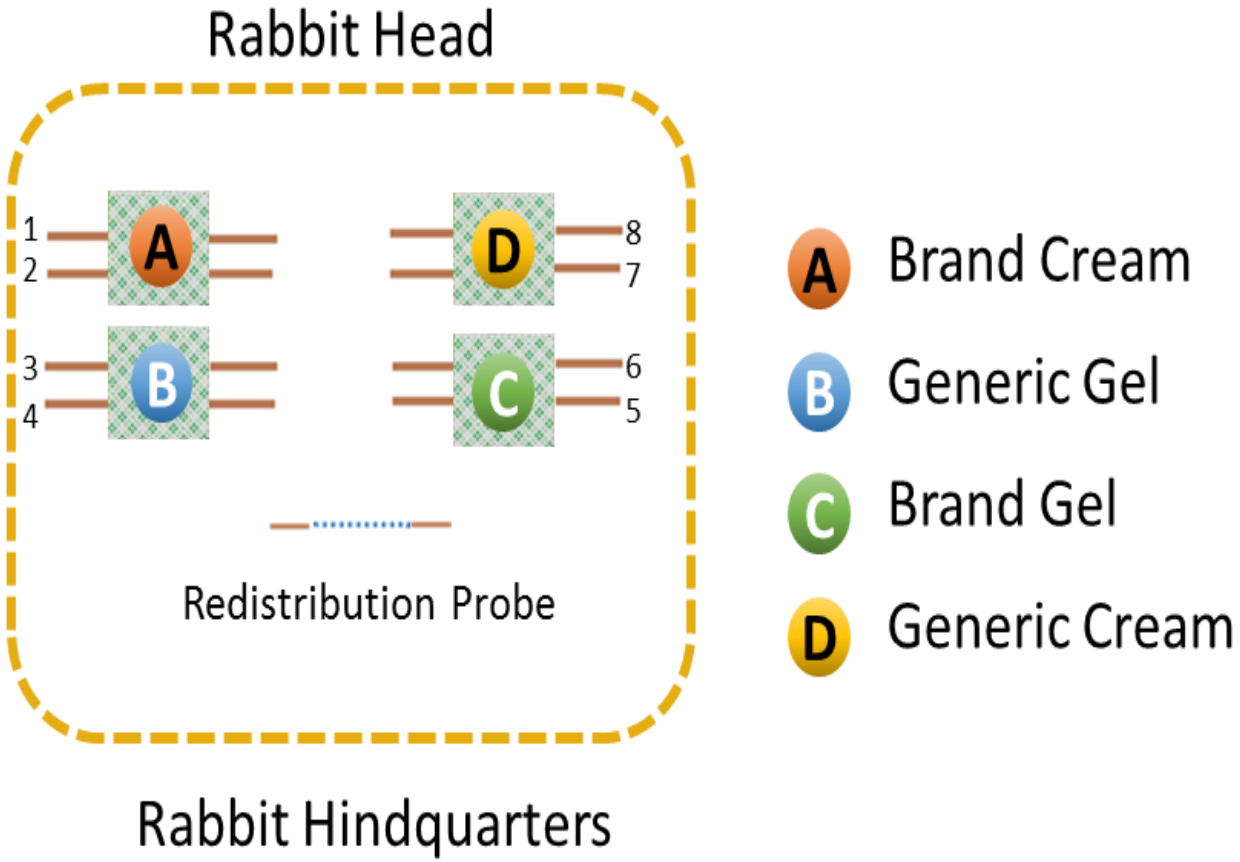
# dMD Technique Optimization



- To adequately characterize cutaneous PK of a topical drug
- Manufacturing optimization: The length of the dialysis membrane is precise
- The use a “probe performance marker”: to calculate actual dermal concentration of the analyte
  - ✓ To assure the consistency of dMD performance for long hours of experiment
- The assessment of probe recovery can be explored as one potential concept to enhance our understanding of the bioavailability of lipophilic, highly protein bound drugs
- Considering several sampling sites and replicates on one subject simultaneously
- Inserting additional probe to assess the possible redistribution of any drug that may have been absorbed systemically

# Metronidazole Topical Products Bioavailability and Bioequivalence Evaluation

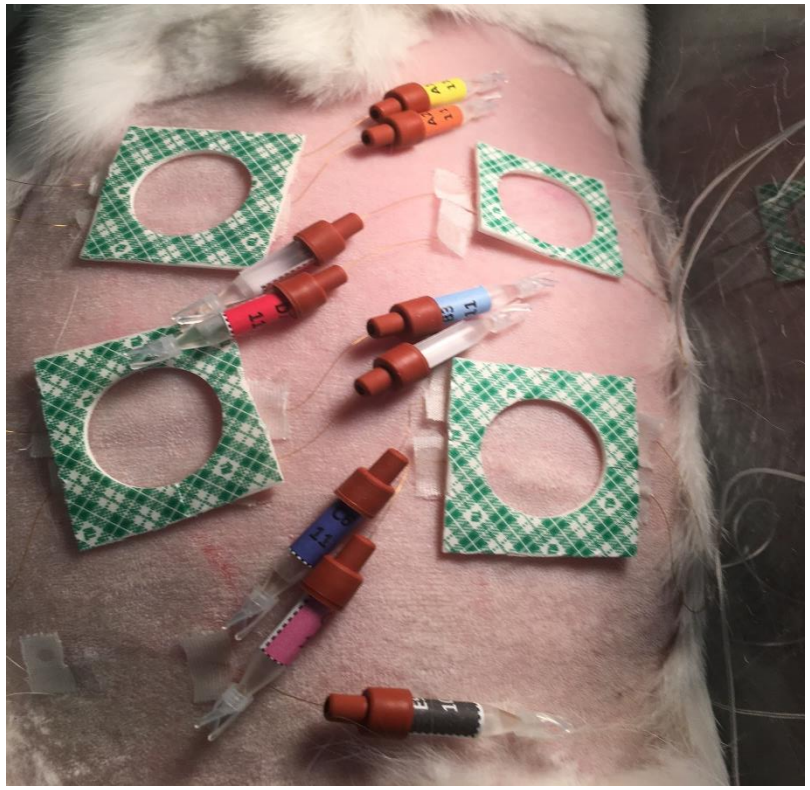
# MTZ *In vivo* Study Design




- MTZ gels
  - Reference (R): MetroGel® 0.75% from Prasco Laboratories
  - Test (T): Metronidazole topical gel, 0.75% from Tolmar
- MTZ creams
  - Reference (R): MetroCream® 0.75% from Galderma Laboratories
  - Test (T): Metronidazole topical cream 0.75% from Fougera Pharmaceuticals
- Product Dose: 10 mg/cm$^2$
- 7 New Zealand Albino Rabbits

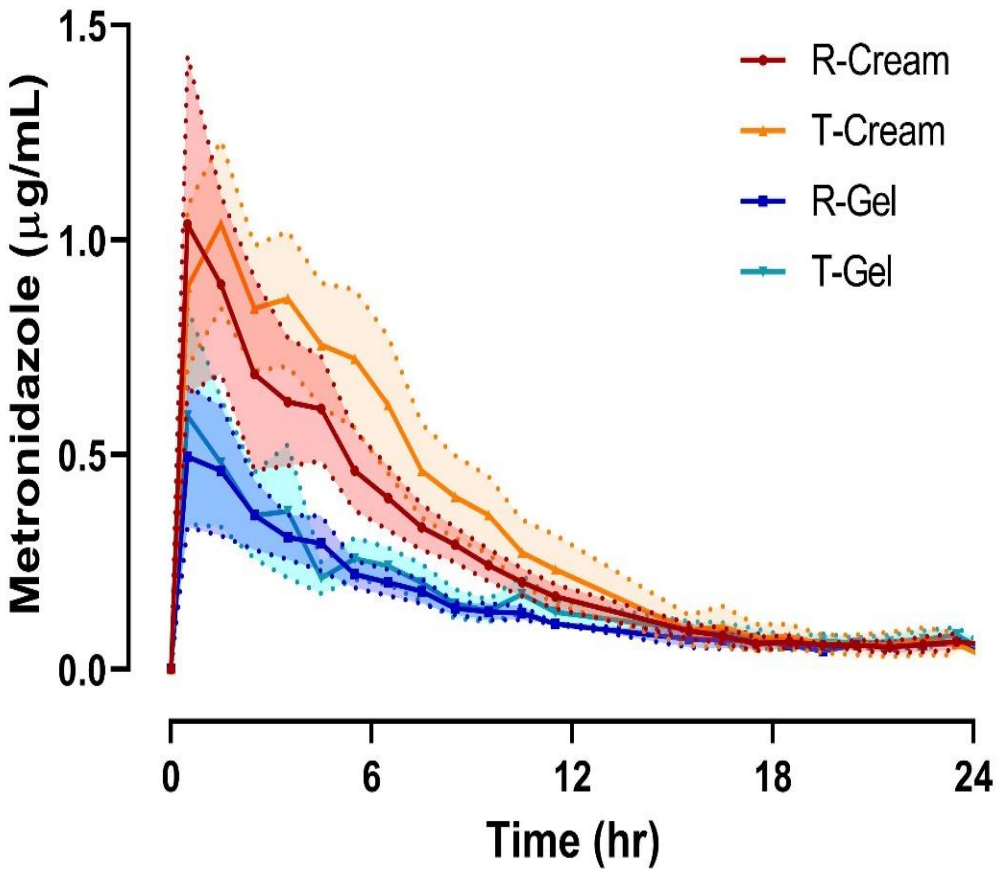
# Study Protocol

- Two probes under the same formulation application site were placed 1 cm apart
- Duration of study: 24 hrs sampling, consists 3hrs rabbit rest
- Samples were collected at 1-hour intervals
- Monitoring redistribution of the drug by the separate probes
- Acetaminophen was used as probe marker (1µg/mL)
- A vapor-meter (TEWL) was used to measure the integrity of the skin at the dosing site before applying TDDP
- After the experiment, the probe depth was measured using ultrasound images



# Bioavailability Results



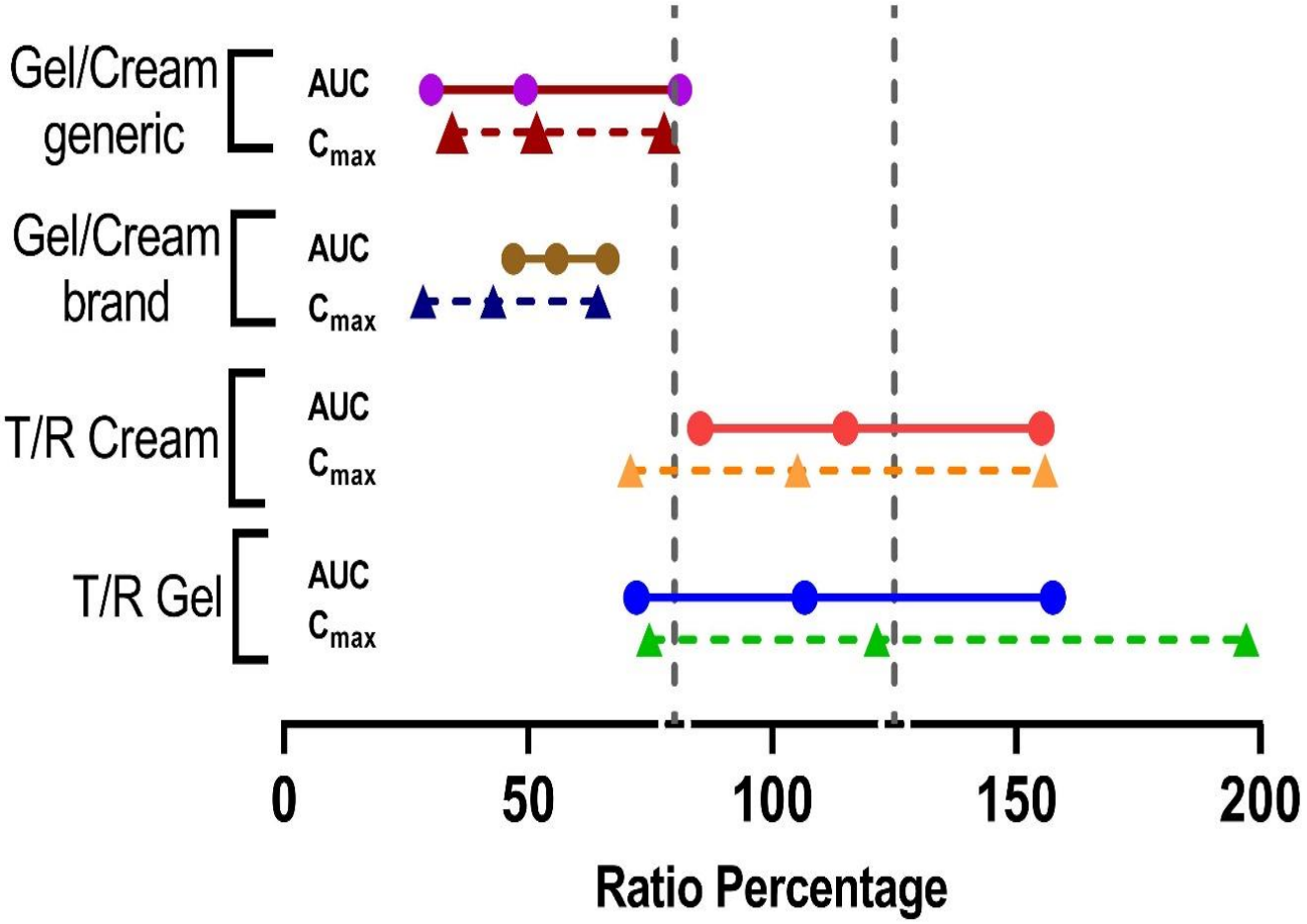



| Formulation | AUCall (µg/mL/hr) | Cmax (µg/mL) | Tmax (hr) | Half-life (hr) |
|---|---|---|---|---|
| R Cream | 5.89 (0.54) | 0.94 (1.22) | 2.5 (0.5-8.5) | 3.85 (0.45) |
| T Cream | 6.77 (0.84) | 0.99 (0.72) | 2.5 (0.5-8.5) | 2.91 (0.32) |
| R Gel | 3.29 (0.57) | 0.40 (1.06) | 1.5 (0.5-10.5) | 5.61 (0.44) |
| T Gel | 3.51 (0.68) | 0.49 (1.15) | 1.5 (0.5-6.5) | 6.43 (0.44) |

T (test), R( reference)

(mean ± SEM, n=7)

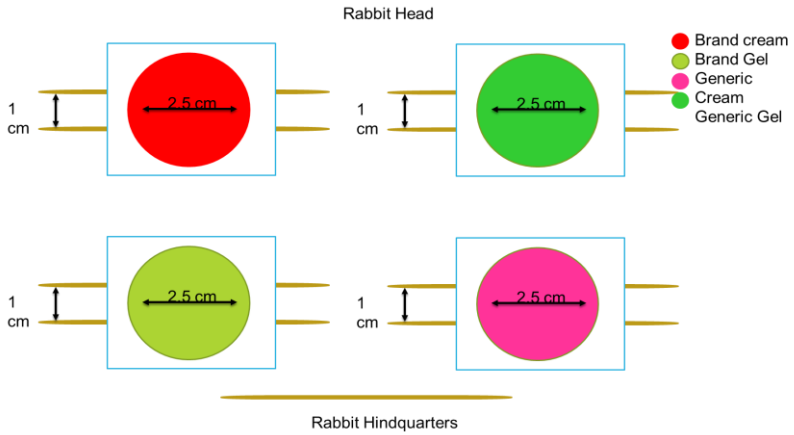
# Bioequivalence Results

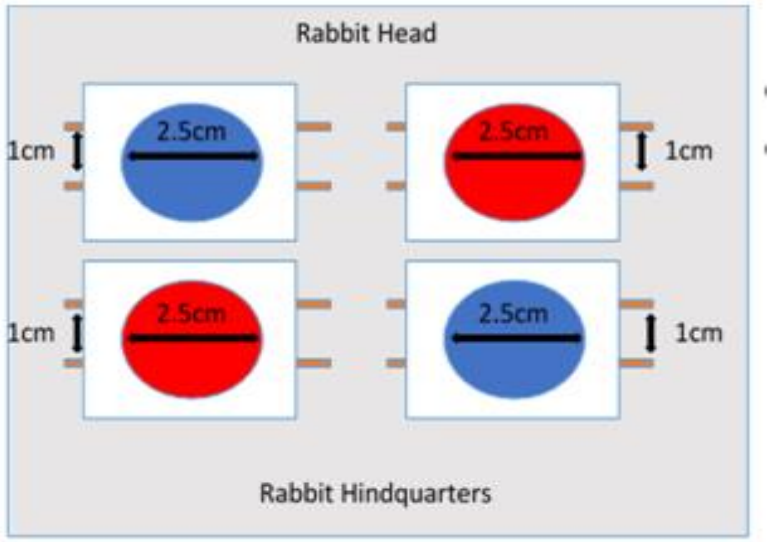



Black lines show 80% and 125% BE limits

# Number of Required Subjects






| Study Design | cPK | Same Study design | Only One dosage form | |
|---|---|---|---|---|
| | | N-rabbits | | |
| **T vs. R gel (RSABE)** | $Ln(AUC_{0-24})$ | 11 | 5 | |
| | $Ln(C_{Max})$ | 10 | 4 | |
| **T vs. R. cream (ABE)** | $Ln(AUC_{0-24})$ | 21 | 10 | |
| | $Ln(C_{Max})$ | 20 | 9 | |

# BE comparison in different sampling duration

| | Parameter | N Rabbit | Geometric mean Ratio % | Lower 90% CI | Upper 90% CI |
|---|---|---|---|---|---|
| **CREAM (T/R)** | $Ln(AUC_{0-24})$ | 7 | 113 | 74 | 155 |
| | $Ln(AUC_{0-12})$ | 7 | 121 | 89 | 166 |
| | $Ln(AUC_{0-5})$ | 7 | 113 | 73 | 177 |
| | $Ln(C_{max})$ | 7 | 111 | 73 | 168 |
| **GEL (T/R)** | $Ln(AUC_{0-24})$ | 7 | 108 | 74 | 158 |
| | $Ln(AUC_{0-12})$ | 7 | 102 | 68 | 152 |
| | $Ln(AUC_{0-5})$ | 7 | 99 | 63 | 156 |
| | $Ln(C_{max})$ | 7 | 115 | 77 | 173 |

# Variability Results



$$CV\% = 100 \times \sqrt{\exp(SD^2) - 1}$$

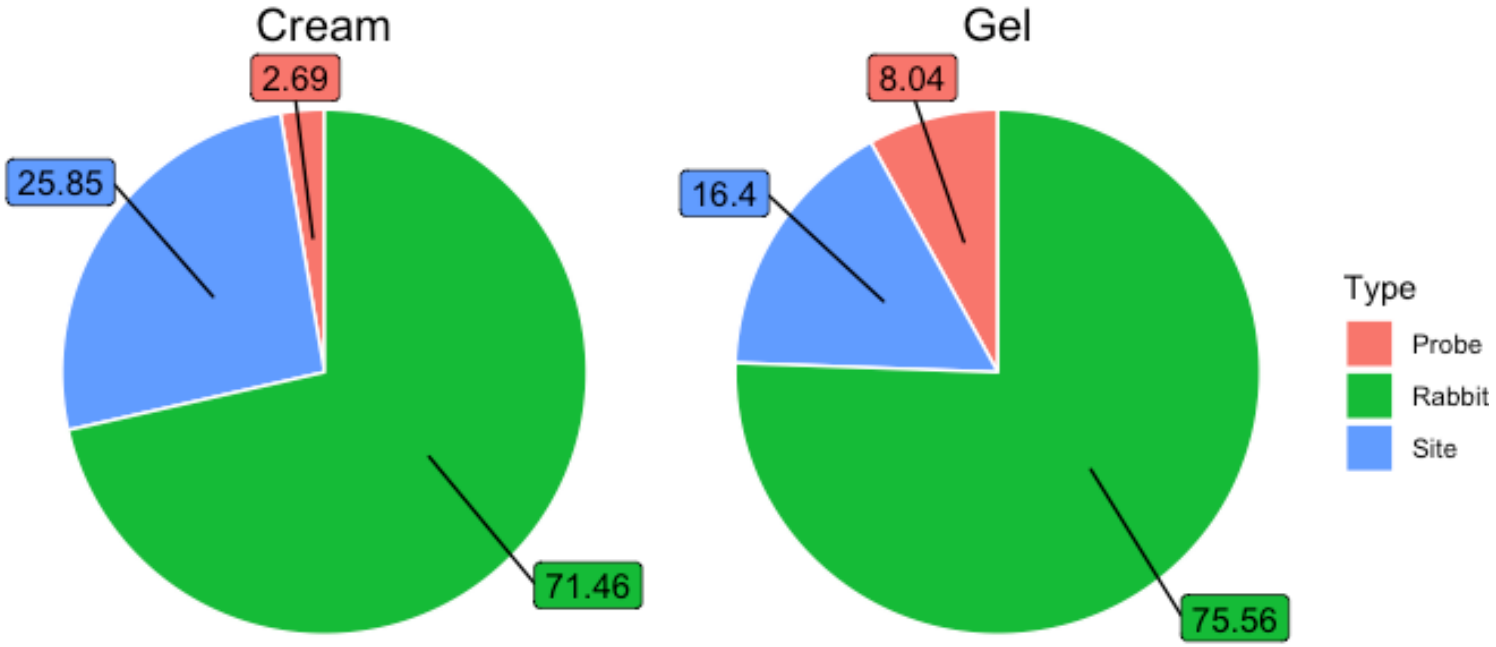
$SD^2$: intra subject variance of formulation or the inter subject variance

| Formulation | CV% Within Sub Ln AUC | CV% Within Sub Ln Cmax |
|---|---|---|
| R Cream | 23.8% | 24.7% |
| T Cream | 27.8% | 28.9% |
| R Gel | 34.2% | 22.4% |
| T Gel | 29.3% | 34.5% |

| Formulation | CV% Inter Sub Ln AUC | CV% Inter Sub Ln Cmax |
|---|---|---|
| T/R Cream | 44.4% | 61.1% |
| T/R Gel | 54.8% | 79.9% |
| Cream/Gel | 28.7% | 50.4% |

# What components contribute to the variability?

- Total CV of log($AUC_{0-24}$) between 44-55%




**Senemar *et al.* (Metronidazole cream)**
Inter-subject variability – 45 %
Intra-subject variability – 24-28 %

Senemar S, et al. (2019) Evaluating the Bioequivalence of Topical Dermatological Drug Products Containing Metronidazole Using Dermal Microdialysis: Preliminary Studies in Rabbits. AAPS 2019

**Benfeldt *et al.* (Lidocaine)**
Inter-subject variability – 61 %
Intra-subject variability – 39 %

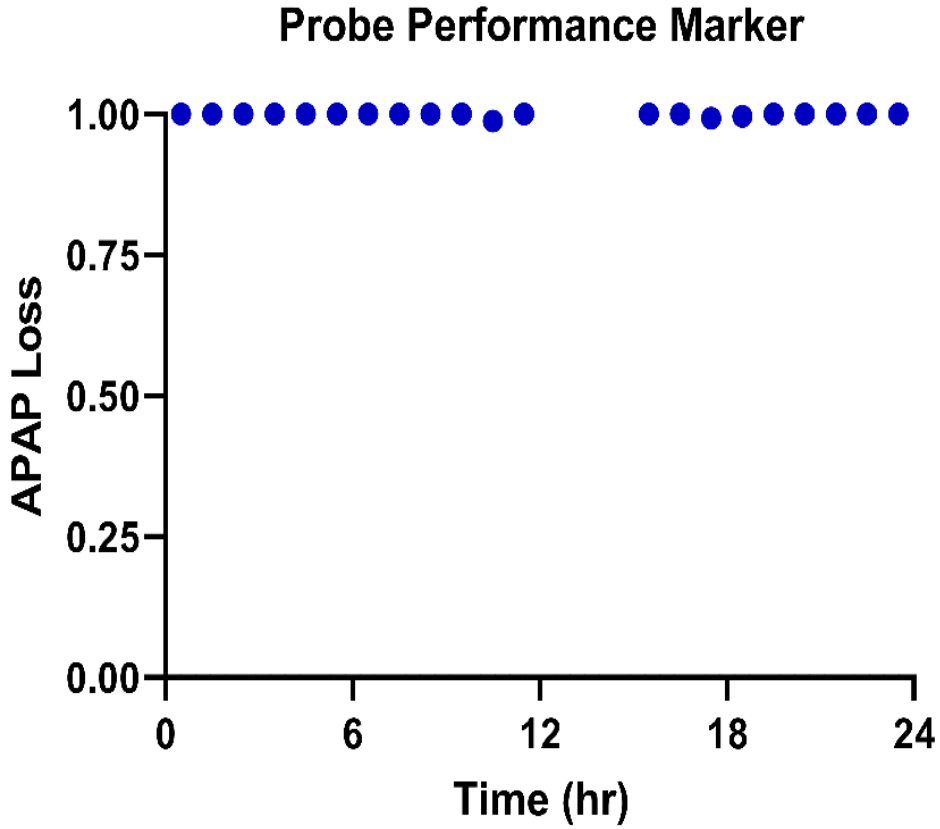
Benfeldt et al., J Invest Dermatol. 2007 Jan;127(1):170-8. Epub 2006 Jul 27

**Ortiz *et al.* (Metronidazole cream)**
Inter-subject variability – 116-223%*
Intra-subject variability – 30-39%*

Ortiz, P. García, et al. *Skin pharmacology and physiology* 24.1 (2011): 44-53.

# Probe Performance Results

- The addition of the probe performance marker improved the quantitative accuracy of dISF calculation
- The probe marker loss in the dialysate was consistent during the 24 hours of sampling (pvalue= 0.40)
- No systemic redistribution was detected, indicating that each probe sampled the MTZ dermis concentrations specific to that site




(mean ± SEM, n=63)

# MTZ BA/BE Conclusions

## Bioavailability Discussion

- ✓ The experiment was sufficiently long to assess an adequate characterization of the cPK for both formulations in test and reference products
- ✓ The addition of the probe performance marker improved the quantitative accuracy of dMD.
- ✓ The within subject variability for cream (24-28%) was lower than the previous attempts (30-39%)

## Bioequivalence Discussion

- ✓ The non-bioequivalence between the two different vehicles, a gel and a cream, was clearly demonstrated
- ✓ MTZ brand and generic were comparable to each other, however, the study was not powered to reach a statistical conclusion of BE
- ✓ the $AUC_{0-12}$ accounted for 82 ± 10% of the $AUC_{0-24}$ , the BE point estimate and 90%CI of $AUC_{0-12}$ were comparable to the ones for $AUC_{0-24}$.

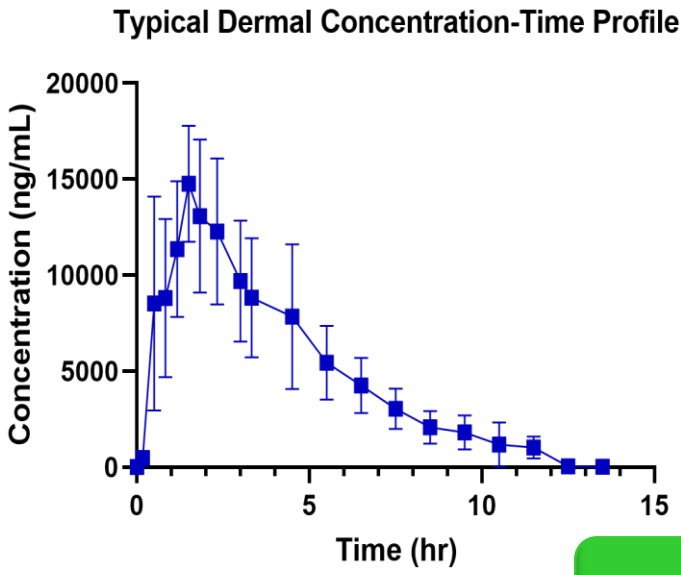

- Garcia Ortiz, P., Hansen, S.H., Shah, V.P., Sonne, J., Benfeldt, E., 2011. Are marketed topical metronidazole creams bioequivalent? Evaluation by in vivo microdialysis sampling and tape stripping methodology. Skin Pharmacol Physiol 24, 44-53.
- Bodenlenz, M., Augustin, T., Birngruber, T., Tiffner, K.I., Boulgaropoulos, B., Schwingenschuh, S., Raney, S.G., Rantou, E., Sinner, F., 2020. Variability of Skin Pharmacokinetic Data: Insights from a Topical Bioequivalence Study Using Dermal Open Flow Microperfusion. Pharm Res 37, 204

# Lidocaine/Prilocaine Dermal Disposition parameters Assessment

# Why we should learn about dermal disposition parameters ?

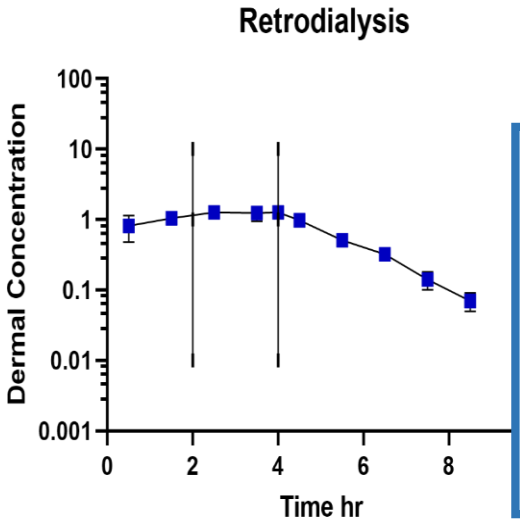
- While the absorption and disposition behavior of systemic administrated drugs are well investigated, our information about the cutaneous pharmacokinetics (cPK) of topical dermatological products (TDDPs) is inadequate
- Estimation of disposition parameters independently of the absorption process will advance the understanding of the TDDP absorption
- If non-linearity in dermal bioavailability is observed, it can be sort out whether non-linearity arises from the absorption process or distribution/elimination from dermis

# Dermal Pharmacokinetics




Absorption

+




Disposition

Distribution

Elimination

Release of Product

Intra Cellular

Inter Cellular

Skin Appendages

Therapeutic Effect

Bound to Proteins

Unbound Drug

Cells

Blood or lymph

Hypodermis ?

Metabolism



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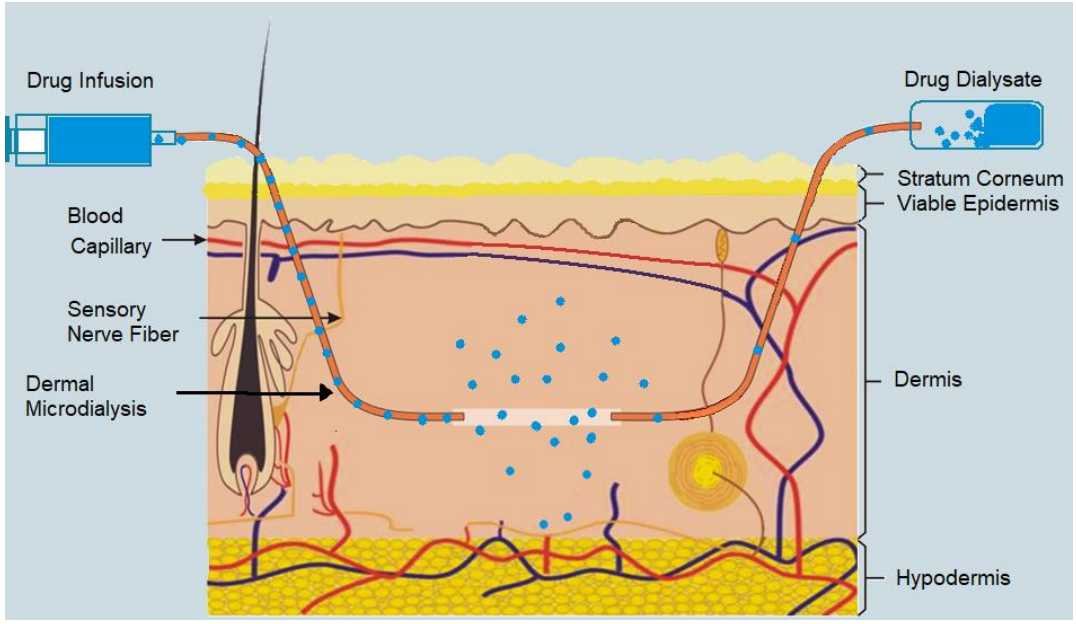
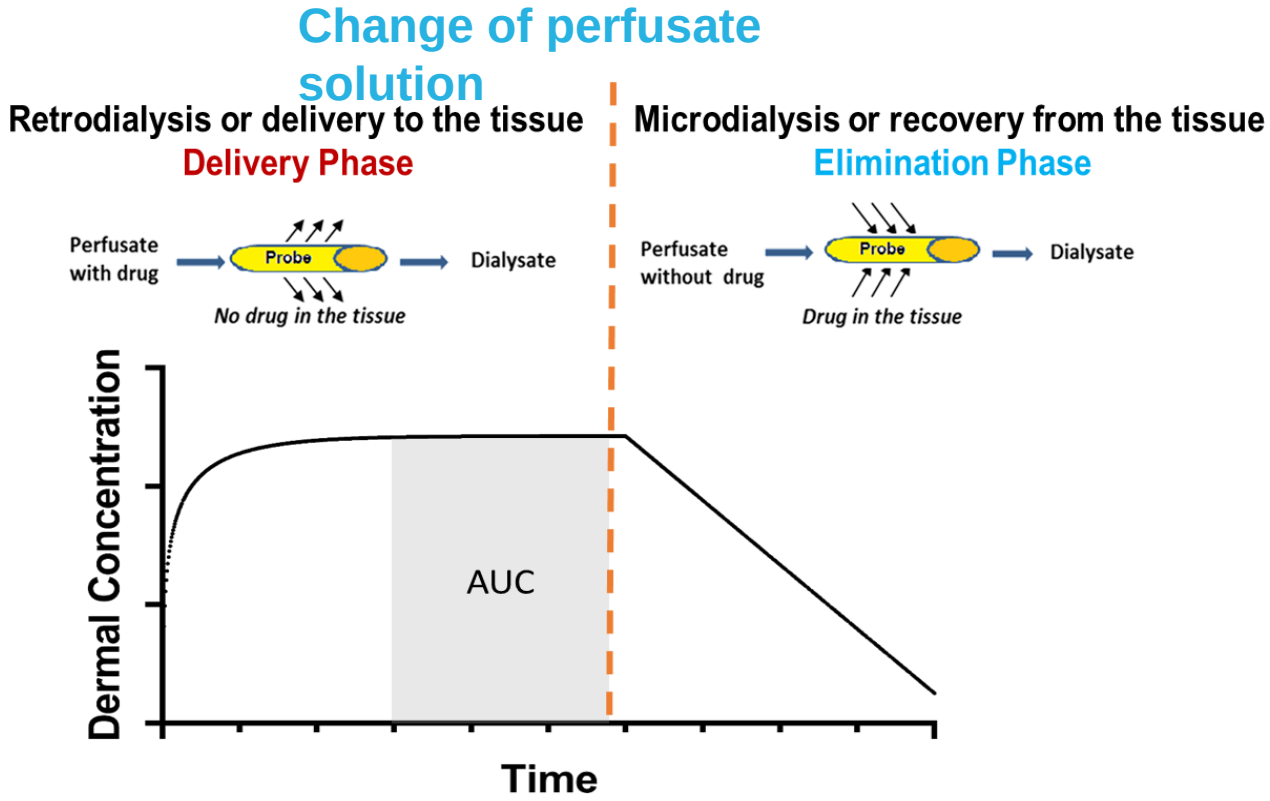
To properly understand the absorption process, it is necessary to characterize the dermal disposition by **delivering the drug directly to the dermis**

# IDEA!

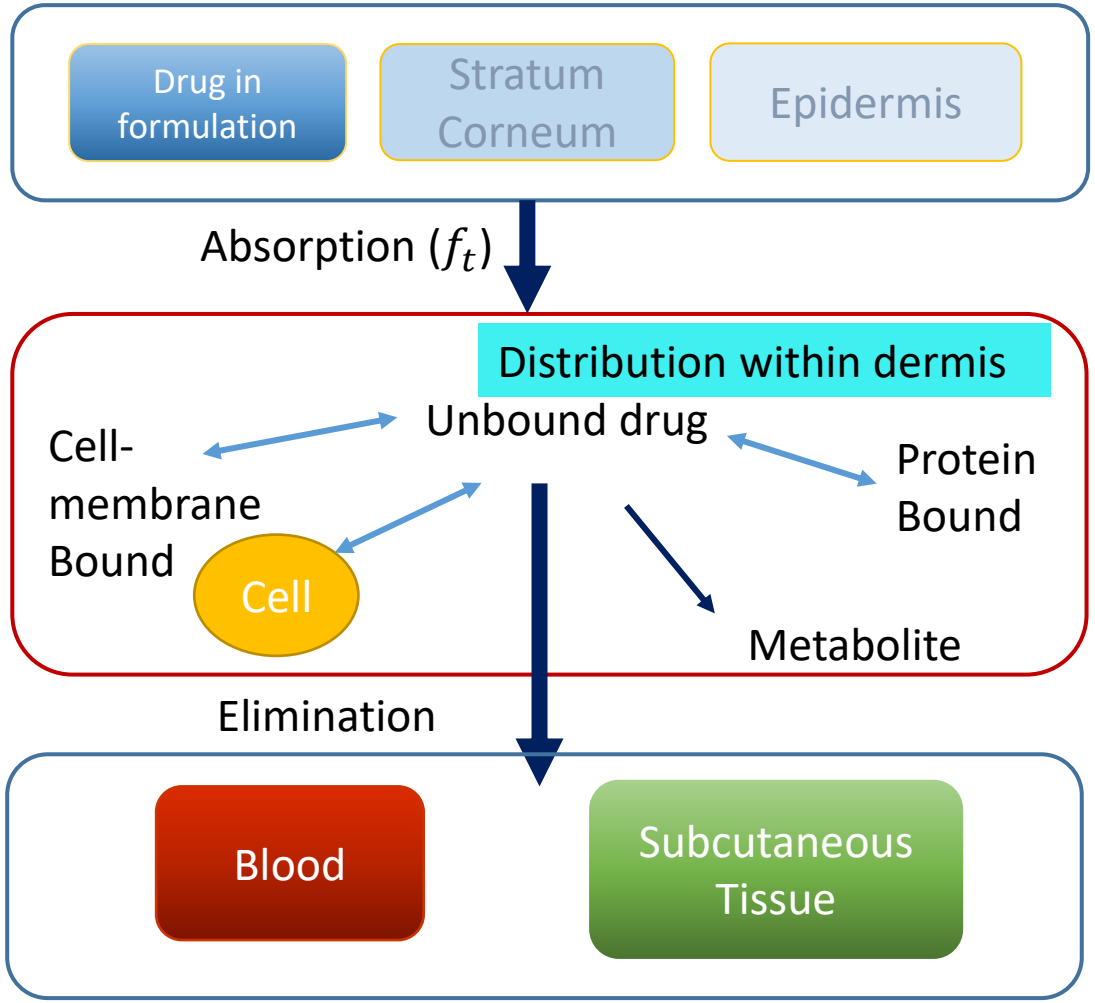
Use the dMD probe to deliver a precise dose directly to dermis: **Dermal Infusion**

## Dermal Infusion



# Why is it Important to estimate the dermal disposition parameters?




## We can estimate:

**Dermal Clearance (dCL)**

**Dermal elimination half-life (dHL)**

**Dermal Volume of Distribution ($dV_d$)**

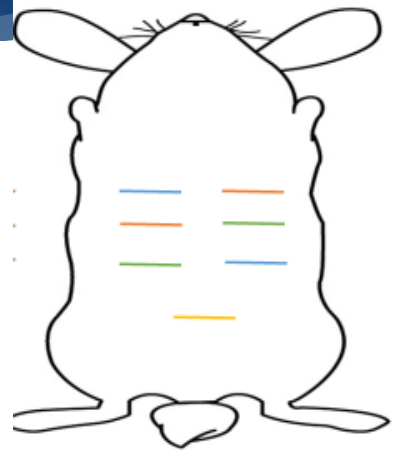
# Dermal Infusion Technique



Deliver API via retrodialysis
- Reach steady state
- Estimate $C_{ss}$, $AUC_{ss}$, and dermal Clearance (dCL)

Switch to microdialysis
- Estimate dermal elimination
- Estimate dermal volume of distribution

Assess variability of disposition parameters across several rabbits



# Equations

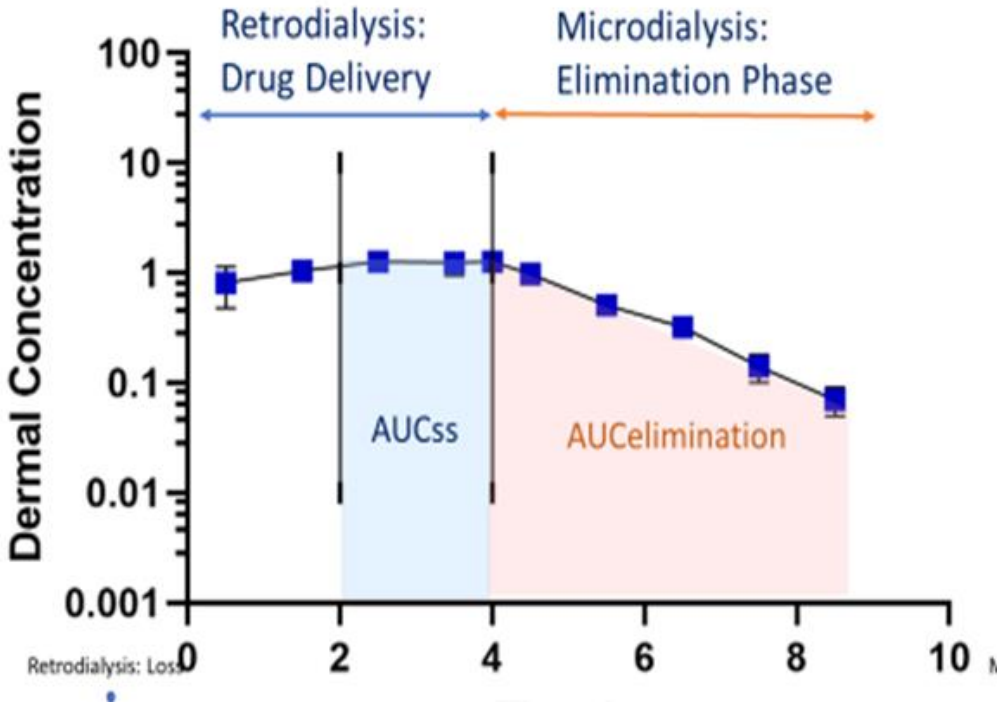


- $Dose_{SS_{(ty-tj)}} = \left(C_{perfusate} - C_{dialysate-ss}\right) \times Volume\ of\ perfusate_{((ty-tj))}$

- $Clearance = Dose\ ss_{(ty-tj)} / AUCss_{(ty-tj)}$
- $Volume\ of\ distribution = \frac{CL}{Ke}$
- dUIR = $\frac{1}{Vd}(e^{-Ket})$

- $\text{Amount}_{\text{end\_infusion}} = Css * Vd$
- $F = \frac{\text{AUC TDDP all} * \text{Amount}_{\text{end\_infusion}}}{\text{AUC}_{\text{elimination}} * \text{Dose topical administrated}} * 100$

*ty:* start of steady state duration
*tj:* end of steady state duration



# Study Design

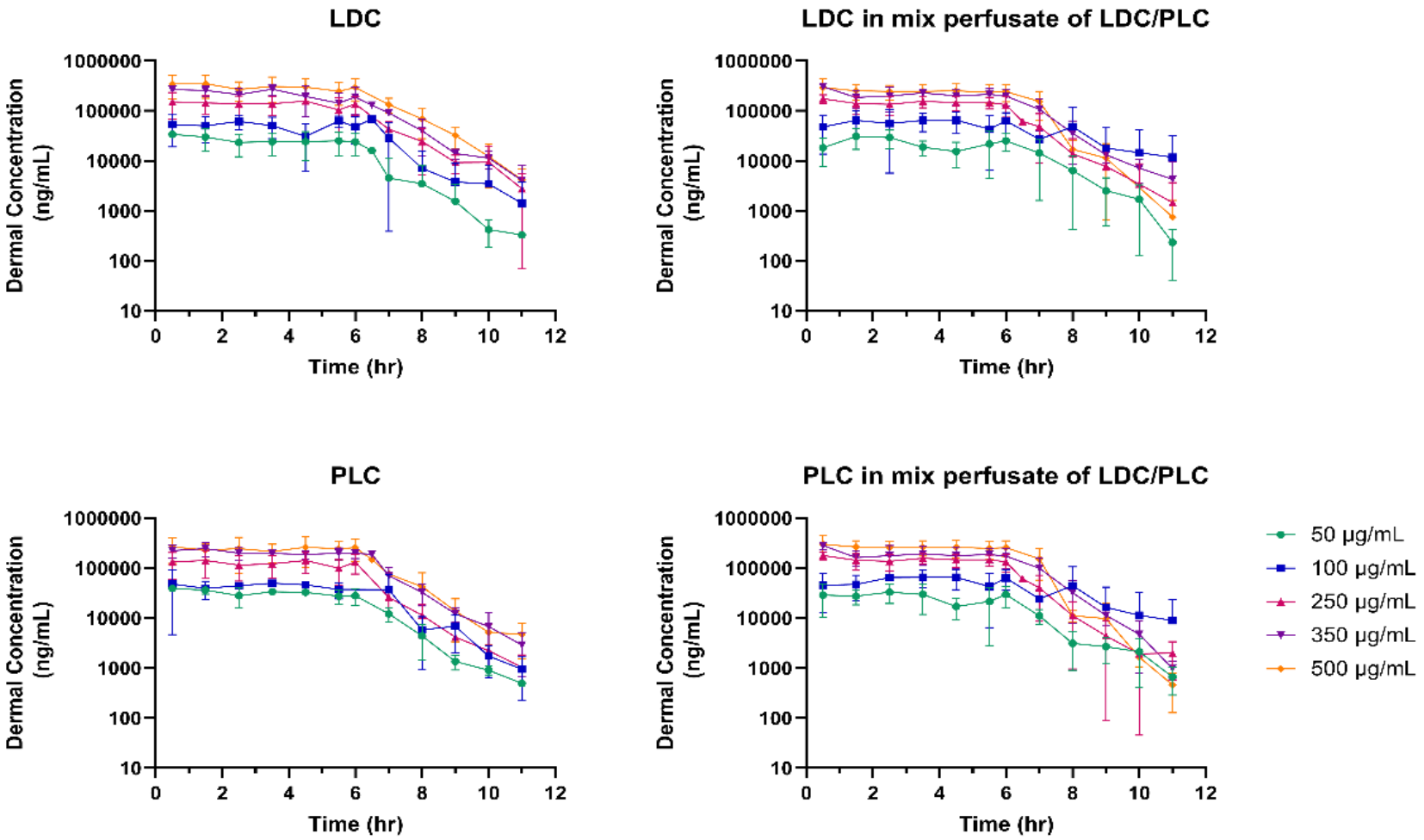
- **Method:**
- Perfuse 15 probes with the LDC, PLC, or LDC/PLC solutions per scheme for six (6) hours; collect samples every hour
- Switch to normal saline containing $D_{10}$-LDC; collect samples every hours for five (5) hours
- 2-3 probes to assess redistribution and lateral perfusion

| LDC µg/mL | PLC µg/mL | LDC+PLC µg/mL |
|---|---|---|
| 50 | 50 | 50 + 50 |
| 100 | 100 | 100 + 100 |
| 250 | 250 | 250 + 250 |
| 350 | 350 | 350 + 350 |
| 500 | 500 | 500 + 500 |

Same range of concentrations as tested in Vitro

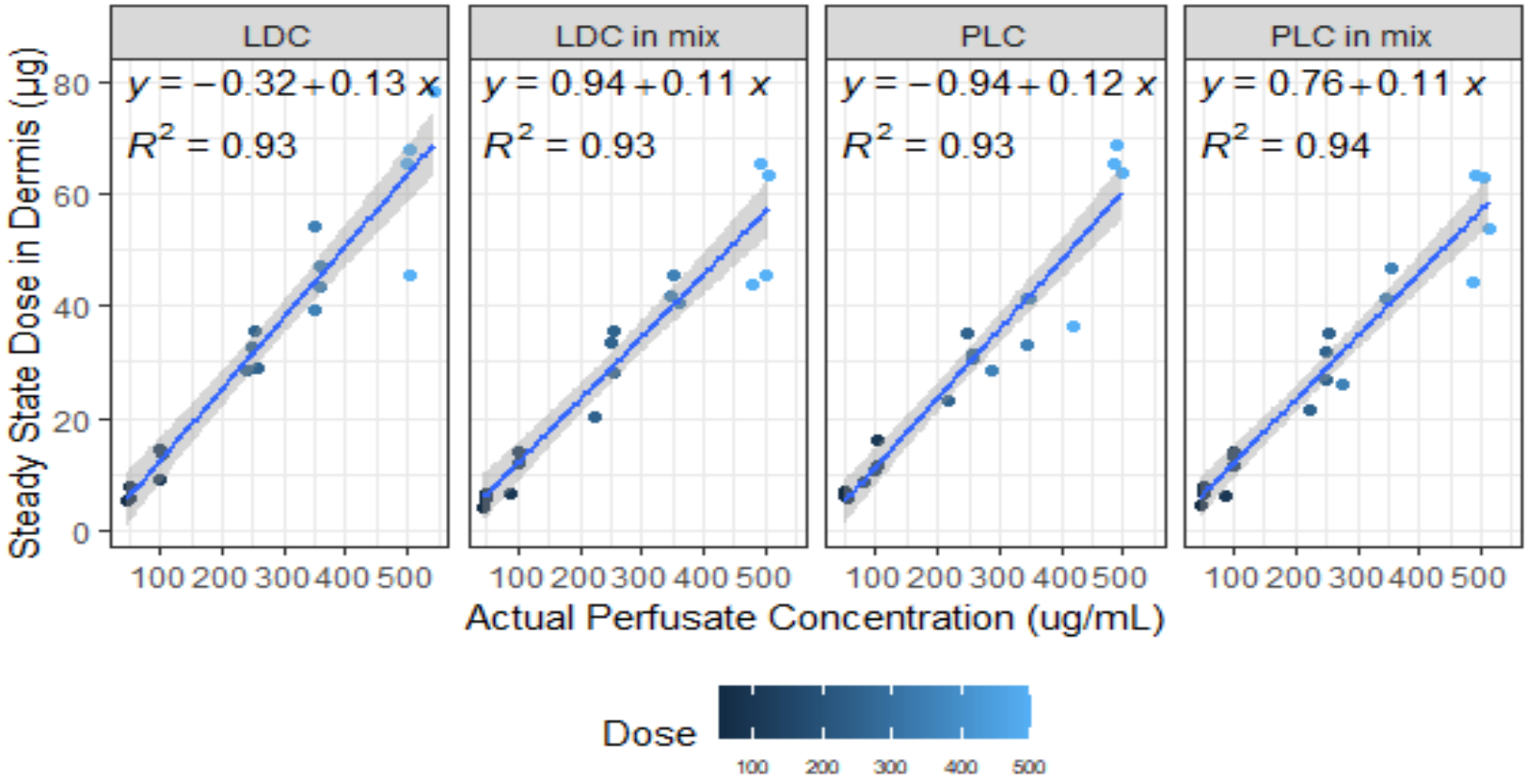
# Dermal Infusion Profiles



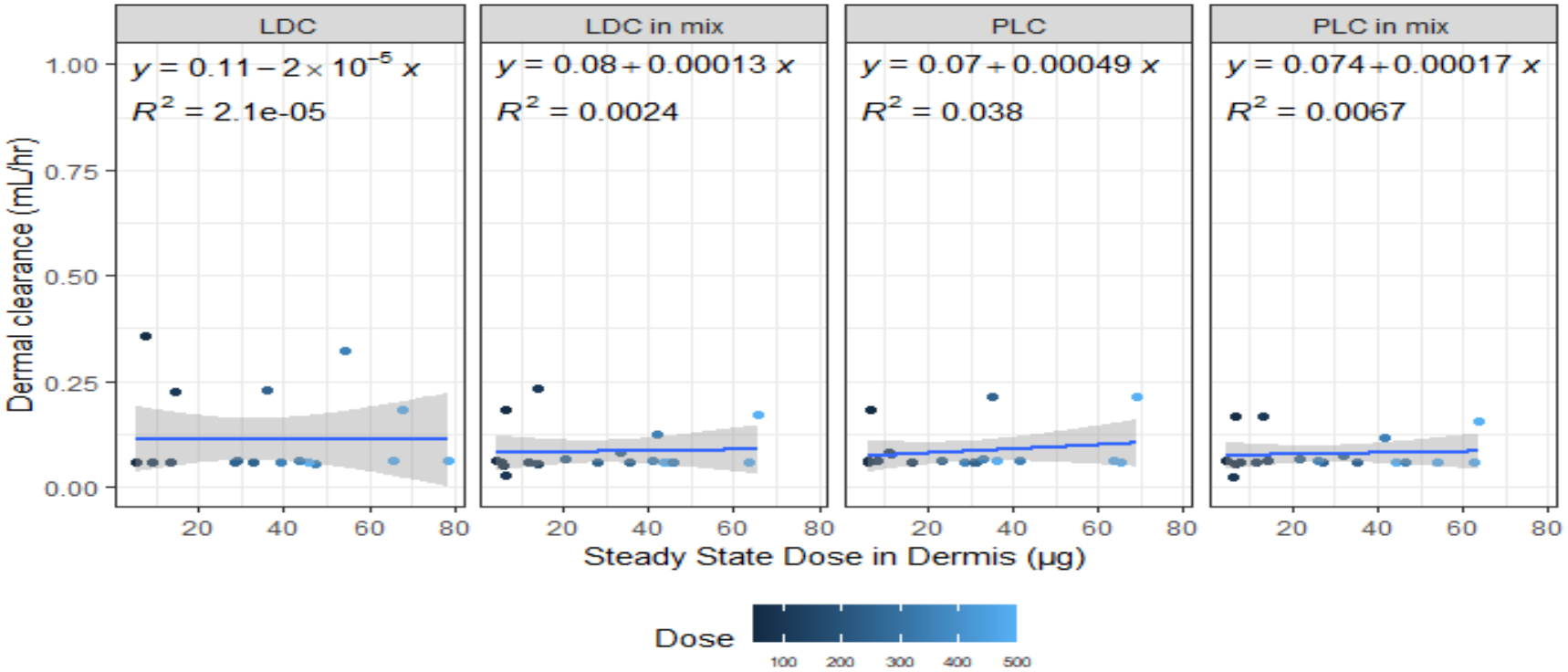



(mean ± SEM, n=4)

# Dermal Dose in steady state phase

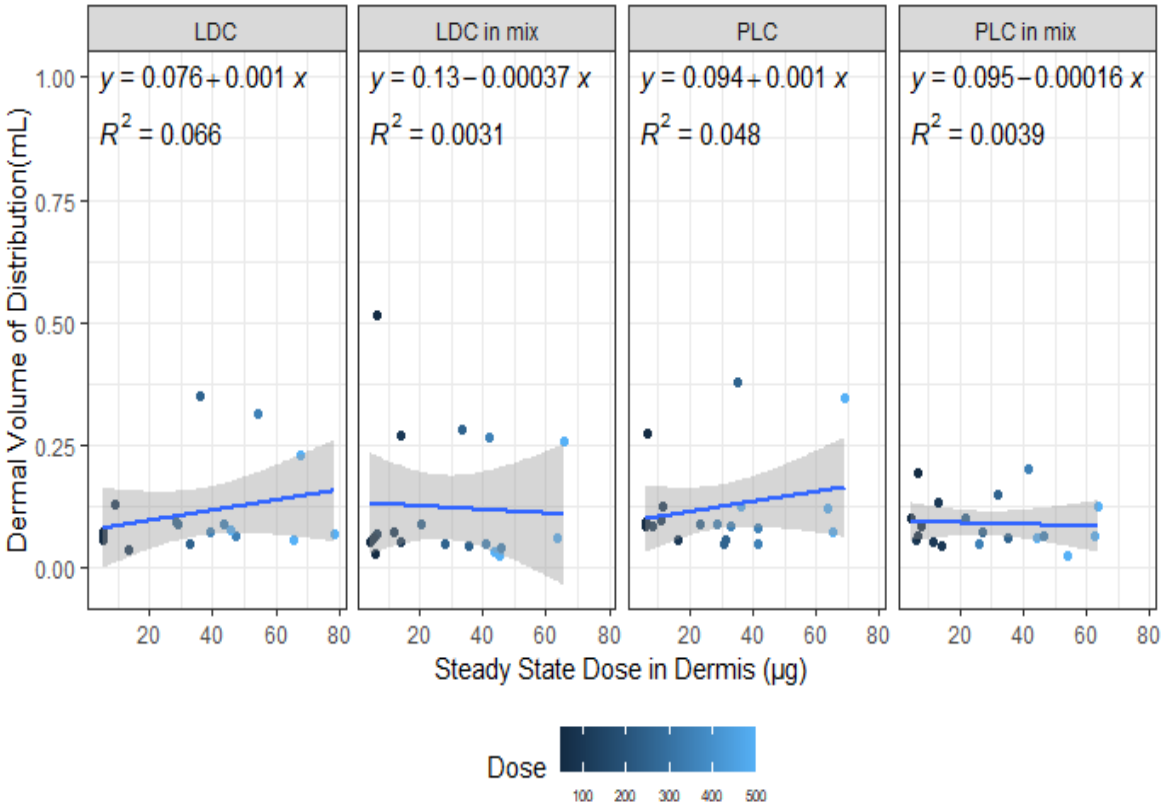


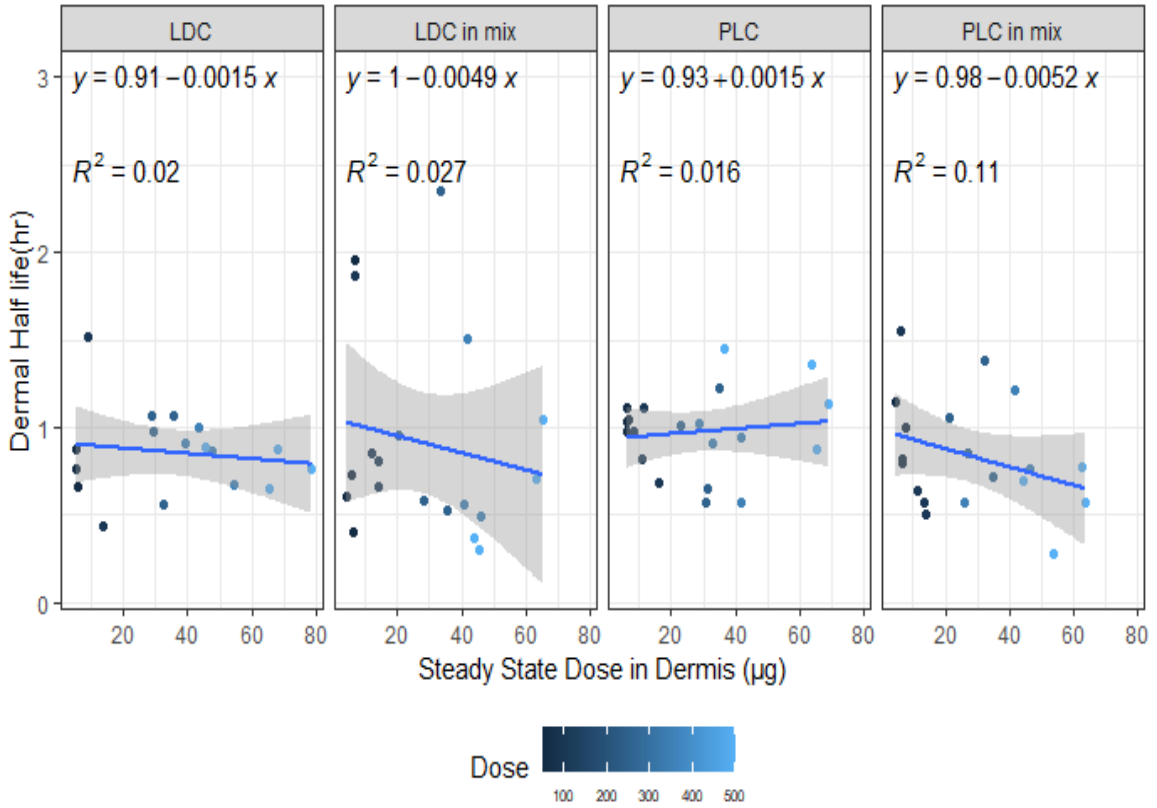
# Dermal Clearance is independent of dermal dose



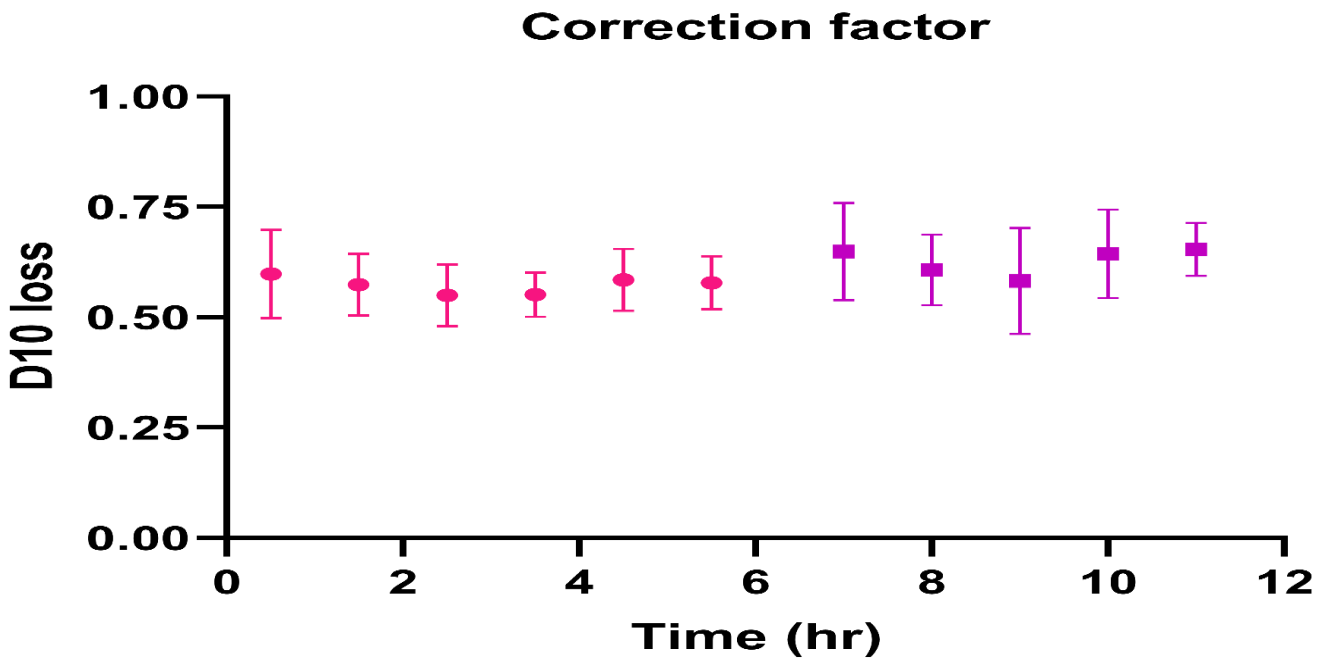

(Anova-tests, R)
Statistical significance was set at $p < 0.05$

# Dermal Volume of Distribution and Dermal Half Life in different dermal doses



# Probe performance




| | |
|---|---|
| Unpaired t test | |
| P value | 0.0060 |

(mean ± SD, n=64)

# Lidocaine and Prilocaine Metabolism



## Liver metabolism

- Lidocaine is transformed through oxidation N-dealkylation by CYP450 2B1, 2B2 in liver to Monoethylglycinexylidide (MEGX)
- MEGX is lidocaine's major systemic metabolite
- Prilocaine is metabolized in both the liver and kidneys by amidases process
- PLC is hydrolyzed to ortho-toluidine (OTE) by Carboxylesterase (CES 1A ,CES 2A)

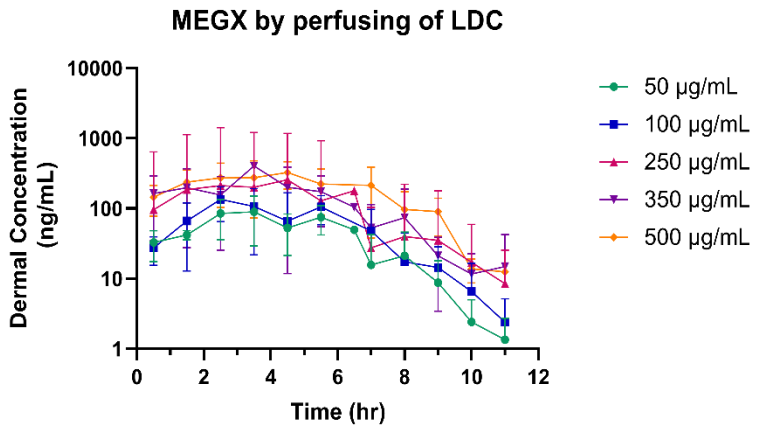
## Skin metabolism

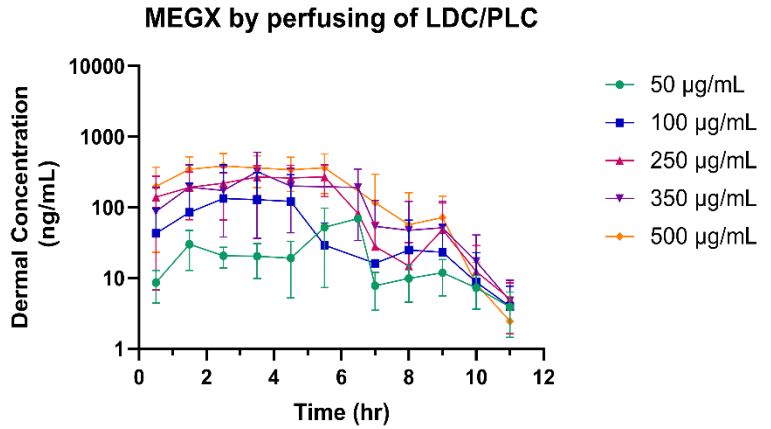
- Cytochrome P450 is the superfamily of enzymes, including CYP 3A4, are expressed in the skin
- CES are expressed in the skin

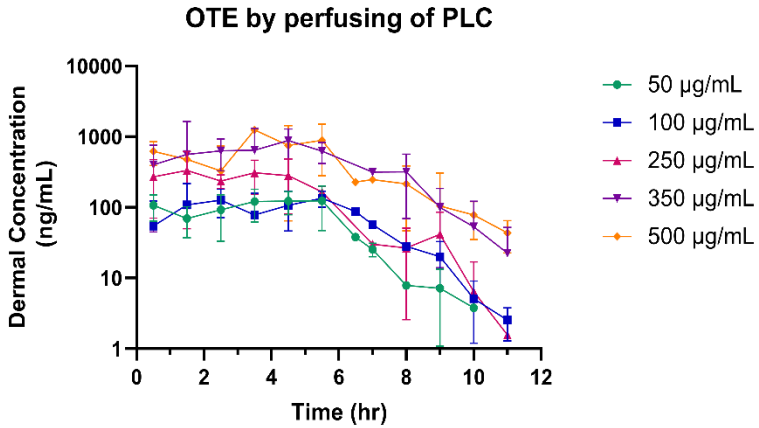

- Kazem, S., E.C. Linssen, and S. Gibbs, *Skin metabolism phase I and phase II enzymes in native and reconstructed human skin: a short review.* Drug Discov Today, 2019. 24(9): p. 1899-1910.
- 35. Rolsted, K., et al., *Evaluation of cytochrome P450 activity in vitro, using dermal and hepatic microsomes from four species and two keratinocyte cell lines in culture.* Arch Dermatol Res, 2008. 300(1): p. 11-8.

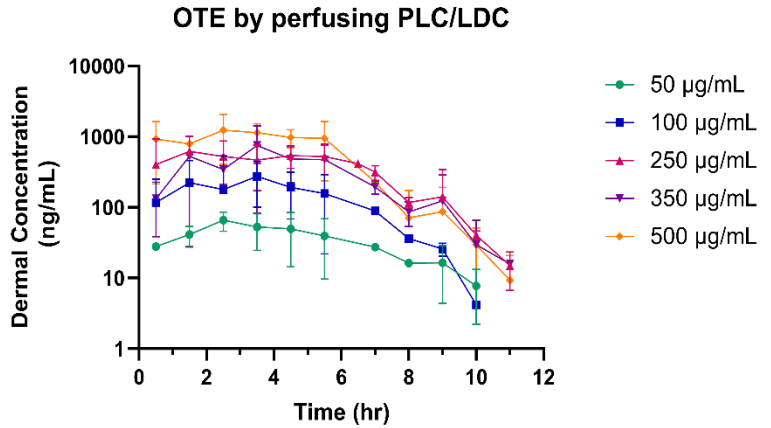
# Dermal Metabolites Profile





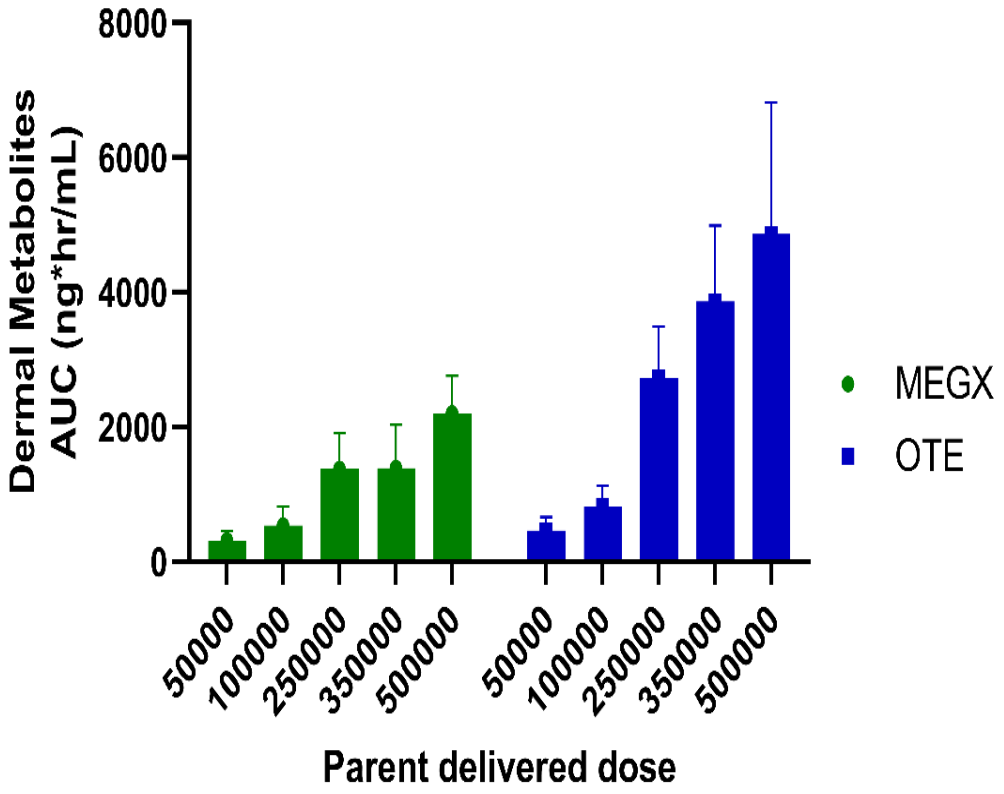




(mean ± SEM, n=4)

# Dermal Metabolites Exposure



# Discussion on Dermal Disposition

- There was a linear relationship between amount of drug in dermis during steady state phase and the dose delivered via dMD to dermis
- Dermal infusion approach demonstrated that the dermal disposition of LDC and PLC is independent of the dose delivered directly to the dermis over a range of therapeutically relevant concentrations
- When administered together, LDC and PLC did not appear to alter each other's dermal disposition.
- Systemic redistribution to skin was negligible.
- This finding may support the application of basic PK principles in the assessment of bioavailability and/or bioequivalence of topical products in animals and humans using dMD technique.

# dMD Developments

- To establish BE between the generic and the corresponding brand formulations and to identify non-bioequivalence between different formulations
- To characterize the cutaneous PK parameters of analytes and dermal metabolites across different doses (study in progress)
- To characterize the disposition parameters for analytes and their dermal metabolites
- To develop an *in vitro – in vivo (IVIVR)* relationship for different formulations

European Journal of Pharmaceutical Sciences 159 (2021) 105741

Contents lists available at ScienceDirect

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**FDA – Office of Generic Drugs (OGD)/ Office of Research and Standards (ORS)**
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# ACKNOWLEDGMENTS

Grants Support - USFDA U01FD005862
USFDA 1U01FD006930


Questions

**Thank You**

# TEWL and Probe Depth Characterization