UNIVERSITY OF BATH

Measuring drug concentration in the skin *in vivo*: techniques and challenges

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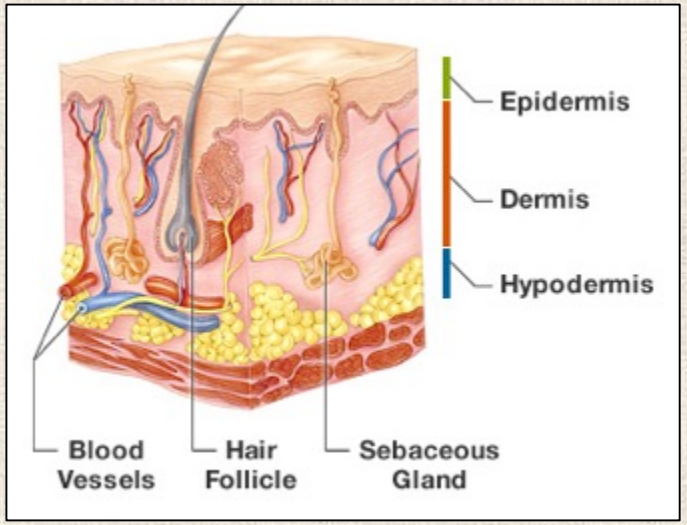
AAPS Webinar on Topical Dosage Forms and Formulations

September 13, 2019

Measuring drug concentration in skin *in vivo*

Drug concentration in skin is logically related to that at site of action

- ***stratum corneum***
- ***epidermis (especially basal cell layer)***
- ***dermis***
- ***appendages (e.g., pilosebaceous unit)***
- *subcutaneous tissue*

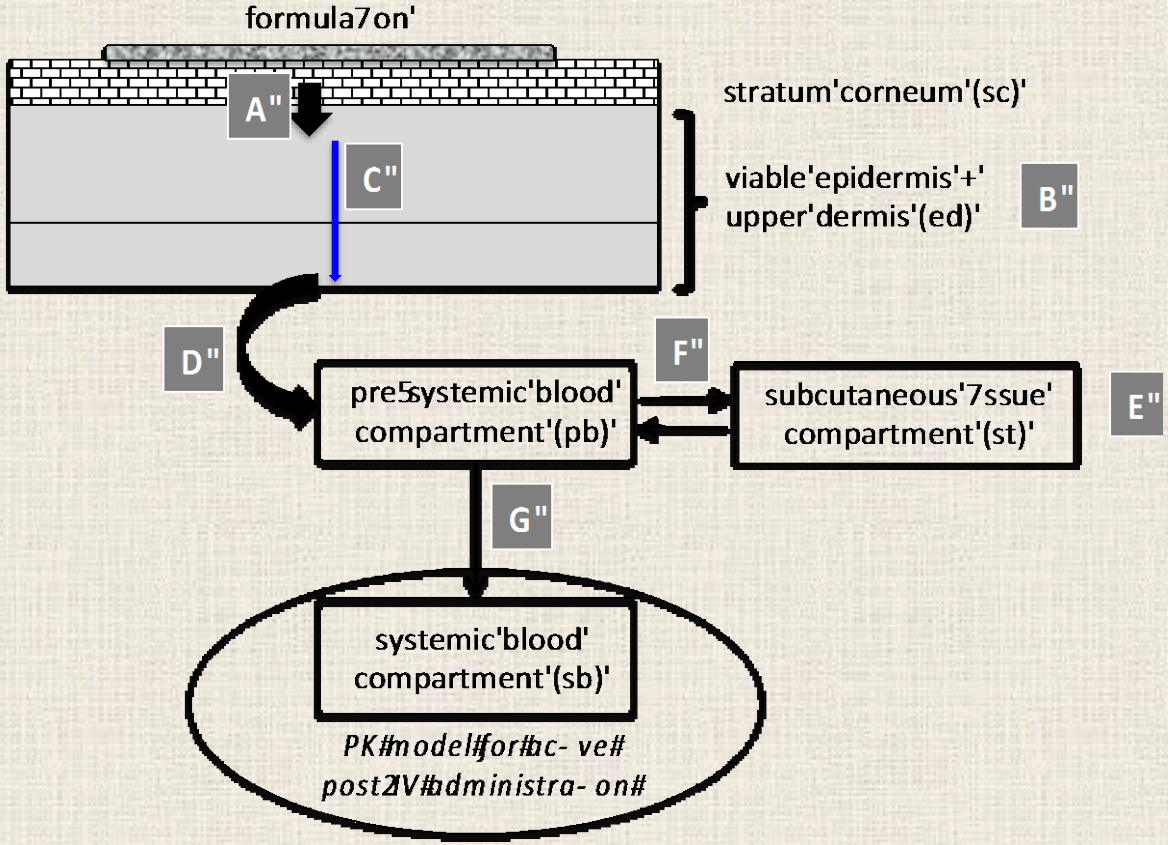


Measurement site is close or at the site of disease

- ***contrast oral drug administration and bioavailability assessment***

Key questions concern where to measure, healthy vs. diseased skin, use of surrogate compartments, complementarity of methods, absolute vs. relative measures?

Measuring drug concentration in skin *in vivo*



A. Input function of active from SC into viable skin.

B. Viable epidermis and upper dermis combined.

C. Passive diffusion from SC to dermal microcirculation.

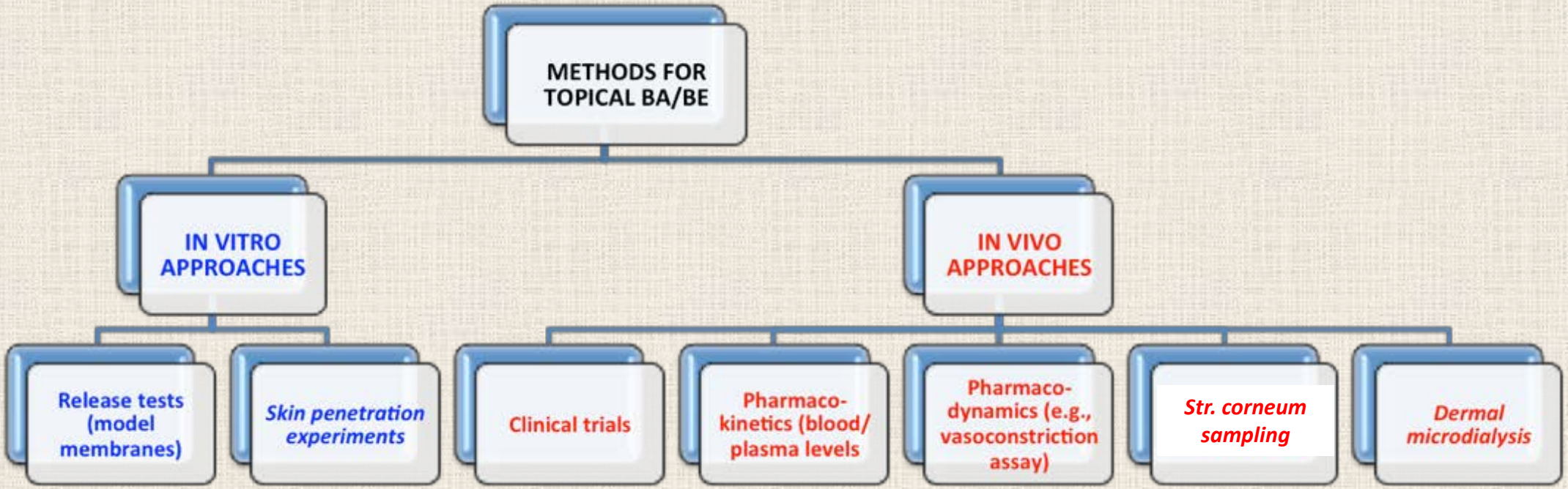
D. Skin “clearance” (blood flow, ‘extraction coefficient’).

E. Subcutaneous tissue compartment.

F. Extent of distribution into subcutaneous tissue.

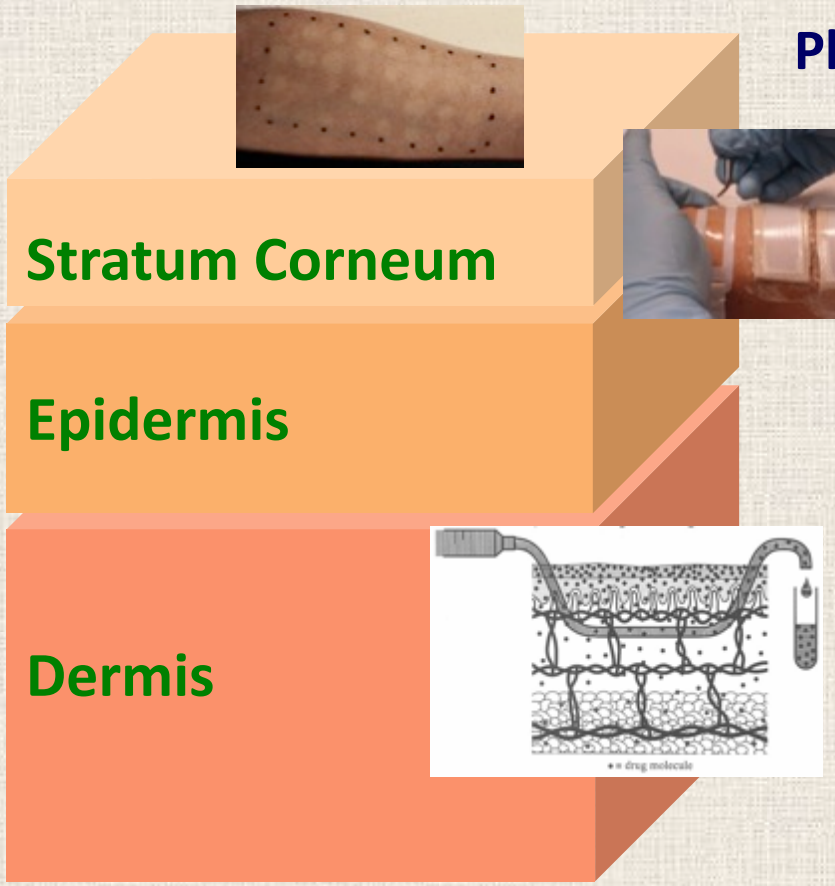
G. ‘Elimination’ of active from skin into blood.

Current methods to measure topical bioavailability/bioequivalence (BA/BE)



- **Clinical trials obviously essential for NCEs, but represent a blunt tool (insensitive and expensive) for BE assessment.**
- **Mathematical modelling/simulation and spectroscopic/imaging methods represent complementary tools under evaluation and development**

Assessing skin bioavailability *in vivo* in man



Pharmacodynamic assay

SC sampling, IR/Raman spectroscopy/imaging

Suction blister x

Microdialysis/oFM

Biopsy x

Blood levels

Assessing skin bioavailability *in vivo* in man

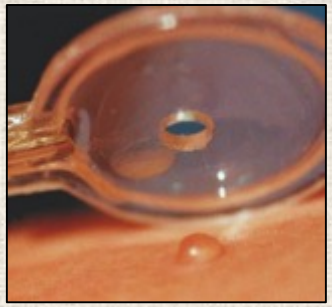
Suction blister and biopsy approaches are very invasive.

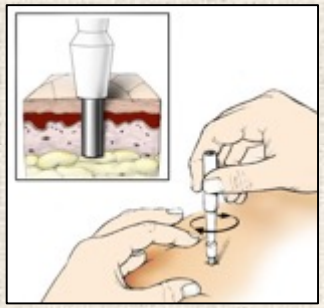
Vasoconstriction assay (PD) is limited to a single drug class (corticosteroids).

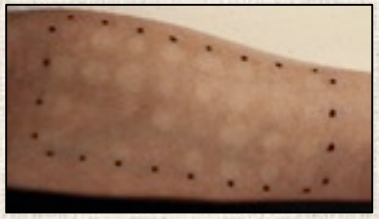
- FDA guidance for many years… would this be acceptable if introduced today?

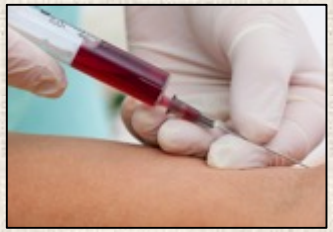
Blood levels often dismissed as (a) too low to be measurable, and (b) not relevant to drug concentration profile in skin; however...

- greatly enhanced analytical capabilities now available (LC-MS^n)
- generic lidocaine patch has been approved by FDA based on systemic PK









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Suction blister and biopsy approaches are very invasive.

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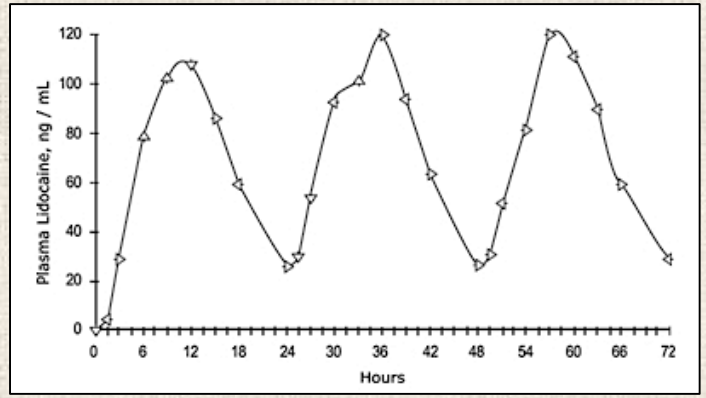
- FDA guidance for many years… would this be acceptable if introduced today?

Blood levels often dismissed as (a) too low to measure, and (b) irrelevant to drug concentration in skin; however...

- **greatly enhanced analytical capabilities now available (LC-MS^n)**
- **generic lidocaine patch has been approved by FDA based on systemic PK**

Is this a precedent for other dermatological drugs?

If blood levels from 'test' and 'reference' products match, how can those in skin be different?



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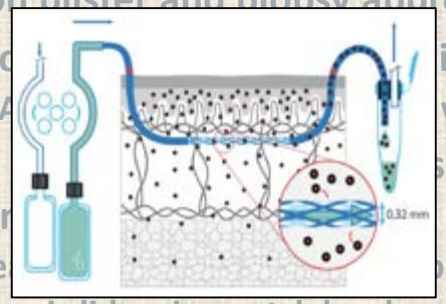
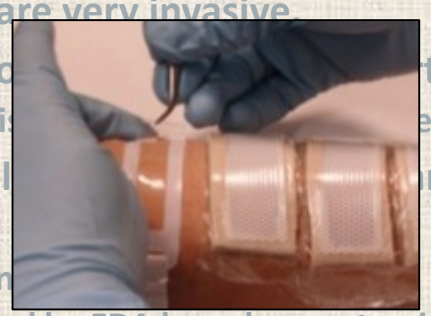




Microdialysis appears to offer most relevant information, but technically demanding.

- recent results from dermal open-flow microperfusion are impressive

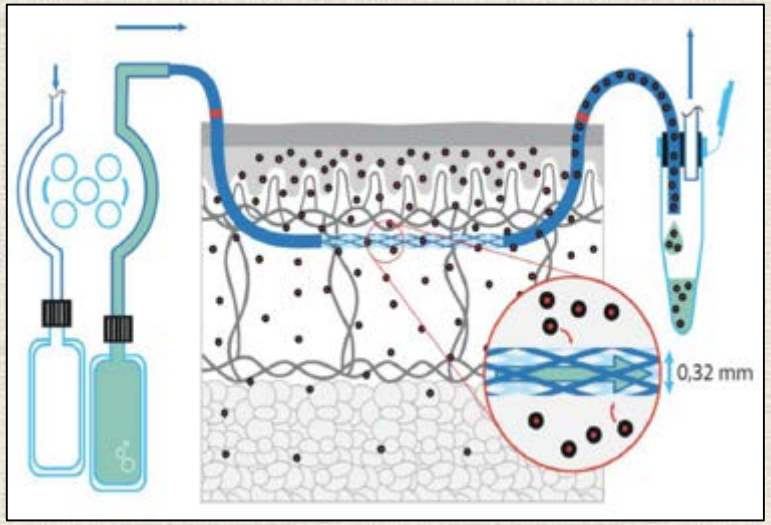
Stratum corneum sampling has a chequered history at FDA; however...

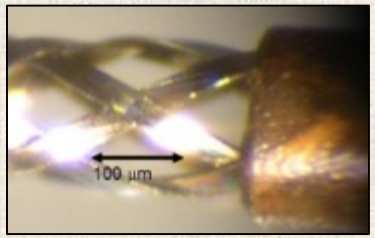
- improved protocol yields high-quality data that correlates with drug input to deeper skin

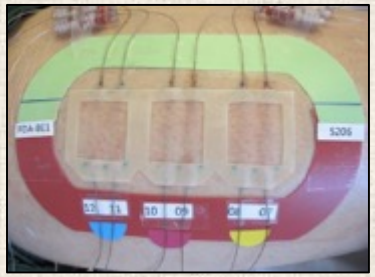
Non-invasive spectroscopic/imaging methodology shows promise, but...

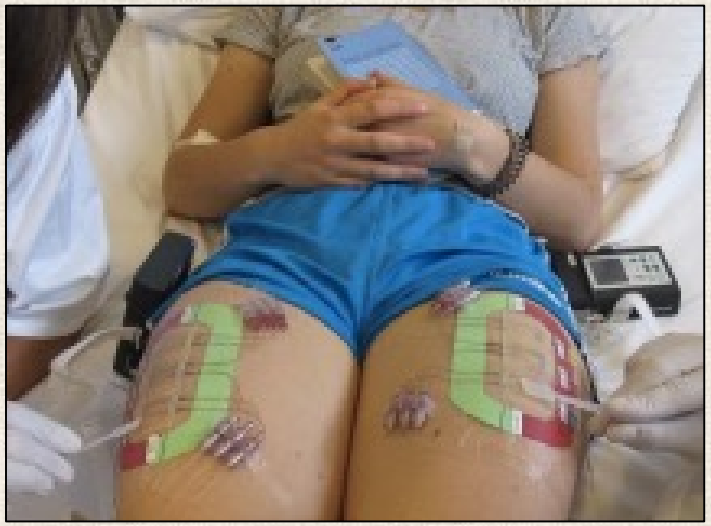
- currently, semi-quantitative with calibration, attenuation and sensitivity issues to be addressed

Dermal open-flow microperfusion









oFM samples are diluted but unfiltered interstitial fluid (i.e., all drugs accessible).

oFM procedures are highly standardised; limited mobility of volunteers is possible.

Stable flow rates achieved and *in vivo* sampling in the dermis is possible for up to 48 hrs.

Controls all important contributory factors to data variability.

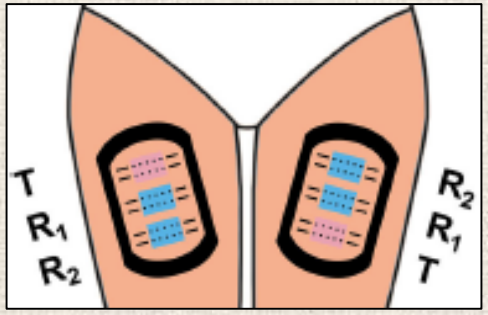
- monitors those which cannot be controlled (e.g., probe depth)

Courtesy of Dr. Frank Sinner, Joanneum Research, Austria

Dermal open-flow microperfusion

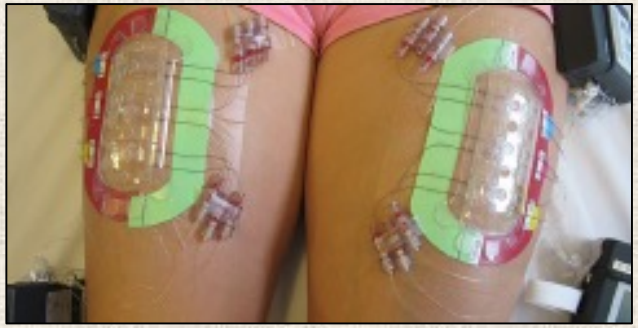
Clinical study in 20 volunteers comparing 2 topical acyclovir (5%) creams.

- **Zovirax (US), the reference formulation**
- **1A Pharma cream (Austria), the test formulation**



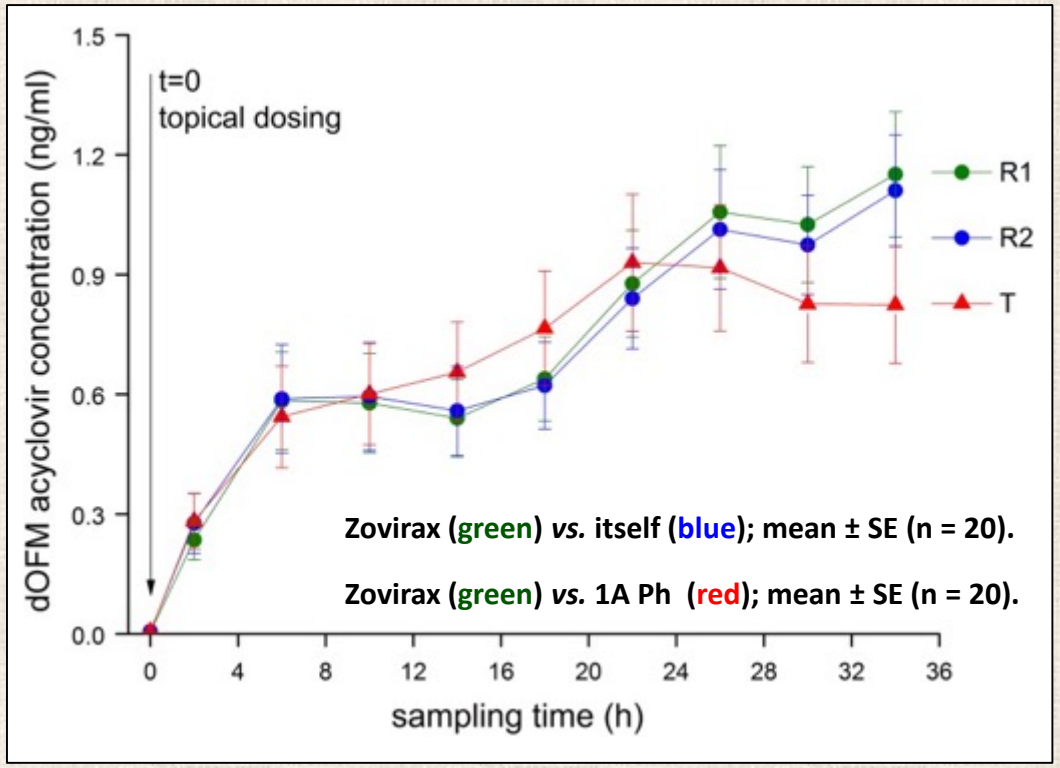
Open flow microperfusion data collected for 36 hours permitting:

- **Zovirax to be compared with itself (reference R1 *vs.* reference R2 "control")**
- **Zovirax to be compared with 1A Pharma (reference R1 *vs.* test T)**



M. Bodenlenz *et al.*, *Clin. Pharmacokin.* 56 (2017) 91-98.

Dermal open-flow microperfusion



Clinical study (n = 20) comparing two topical acyclovir 5% creams.

Open flow microperfusion data collected for 36 hours permitting:

- **Zovirax compared with itself**
- **Zovirax compared with 1A Pharma**

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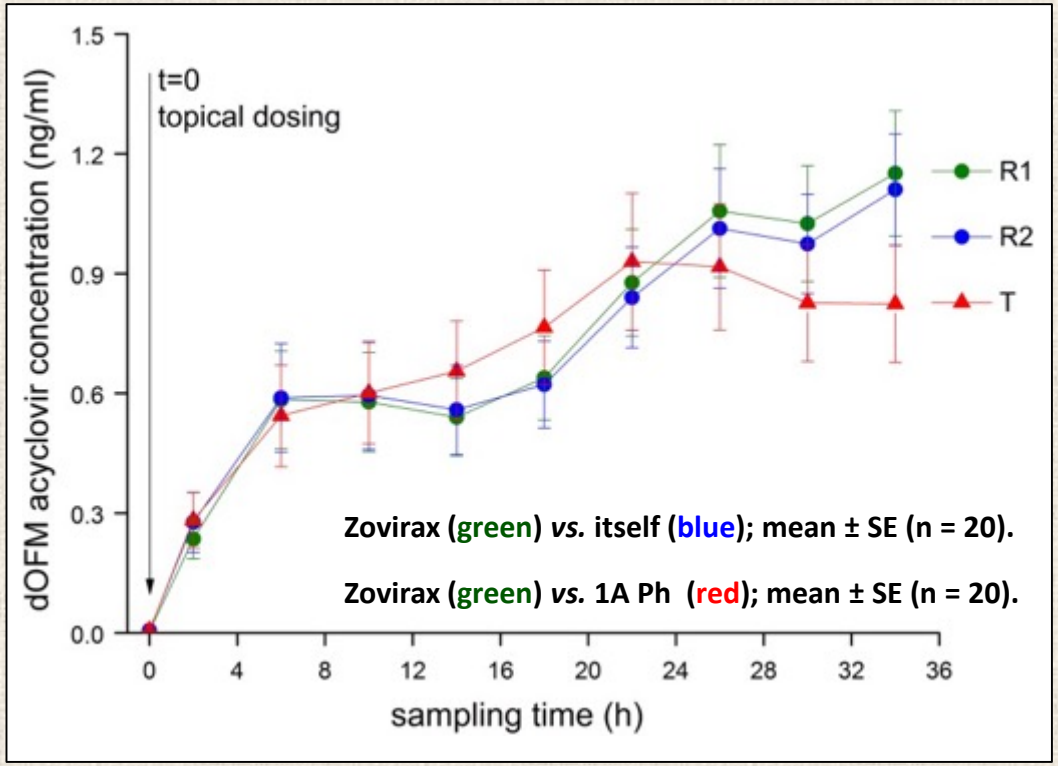
Dermal open-flow microperfusion



Exceptionally high quality data.

Excellent repeatability shown with "reference *vs.* reference" comparison.

C_{max} and $AUC_{0\text{-}36\ hr}$ suggest inequivalence between products.

- are these the appropriate metrics?

- what is the appropriate duration of such a study for this drug?

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Stratum corneum (SC) sampling *in vivo*

aka 'skin tape-stripping', 'dermatopharmacokinetics', DPK

A brief history…

1983 – Rougier *et al.*, the beginning?

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- simplify the method (fewer analyses and decreased variability)

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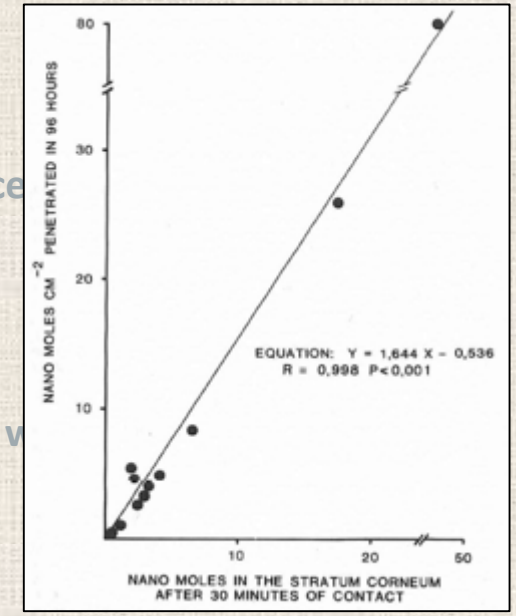
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 81:275–278, 1983

Vol. 81, No. 3
Printed in U.S.A.

In Vivo Correlation Between Stratum Corneum Reservoir Function and Percutaneous Absorption

ANDRE ROUGIER, DIDIER DUPUIS, CLAIRE LOTTE, ROLAND ROGUET, AND HANS SCHAEFER

Département de Biologie, Laboratoires de Recherche Fondamentale de l'Oreal (AR, DD, CL, RR), Aulnay sous Bois, and Centre International de Recherches Dermatologiques (HS), Valbonne, France



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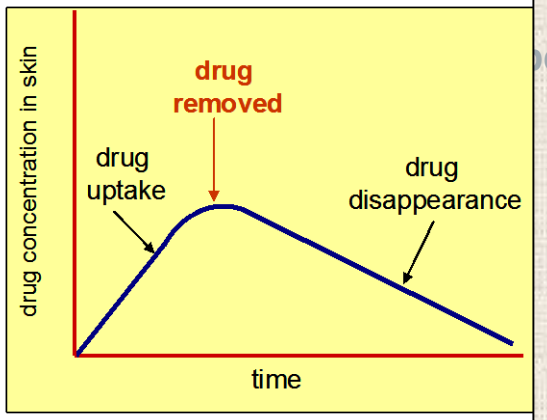
2003 – FDA sponsors ecific goals:

- simplify the metho
- decrease/eliminat

* **Stratum corneum sampling *in* vivo to replace clinical trials (primarily for bioequivalence).**

- **determination of drug in stratum corneum versus time curves for topical actives**
- ***analogous to plasma drug concentration vs. time profiles after systemic administration***

Assumption: Drug amount versus time profile in SC is a valid reflection of that in the epidermis and/or dermis.



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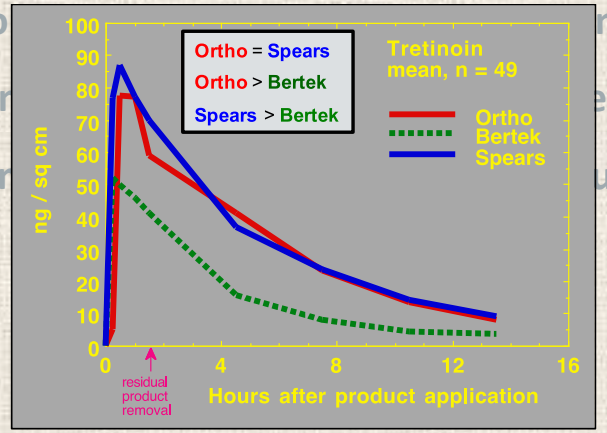

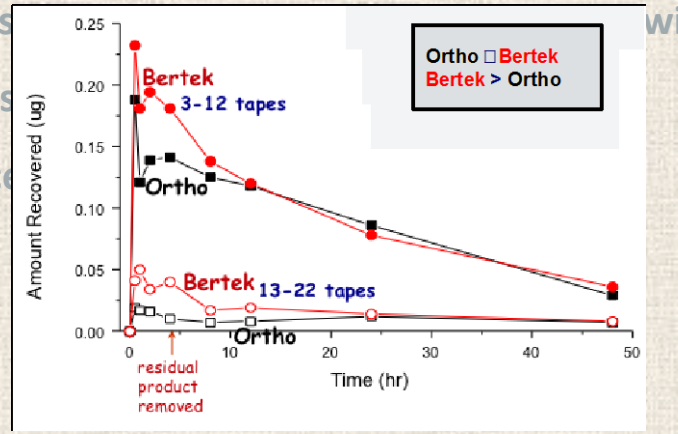

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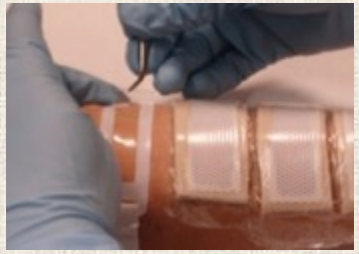
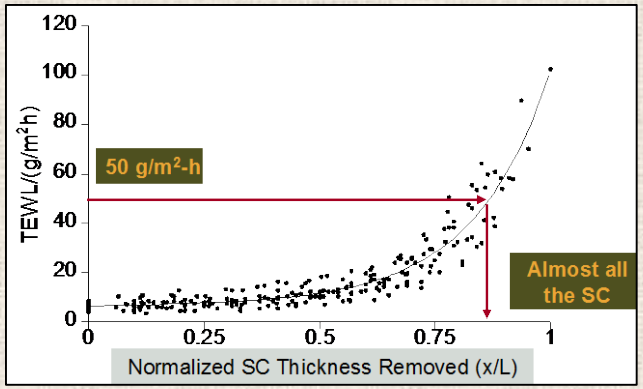
Stratum corneum sampling *in vivo* – improvements!

- Despite inconsistency in pivotal study, methodology did discriminate between products.
- Obvious advantages:
  - *in vivo*, in humans
  - permits comparisons within subjects
  - minimally invasive



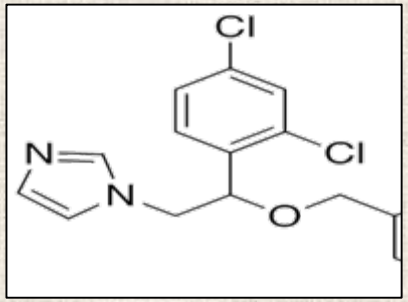
- Stripped area < drug product application area (control both).
- Simpler method: 1 uptake time, 1 clearance time, duplicate at each time.
- Improve skin surface cleaning procedure (alcohol swab).
- Reduce variability by improving drug collection.
  - collect most of stratum corneum – TEWL
  - removes issues related to tape choice/pressure used
  - assess drug on all tapes (none discarded)



Kalia et al. *Pharm. Res.* 17: 1148-1150 (2000)

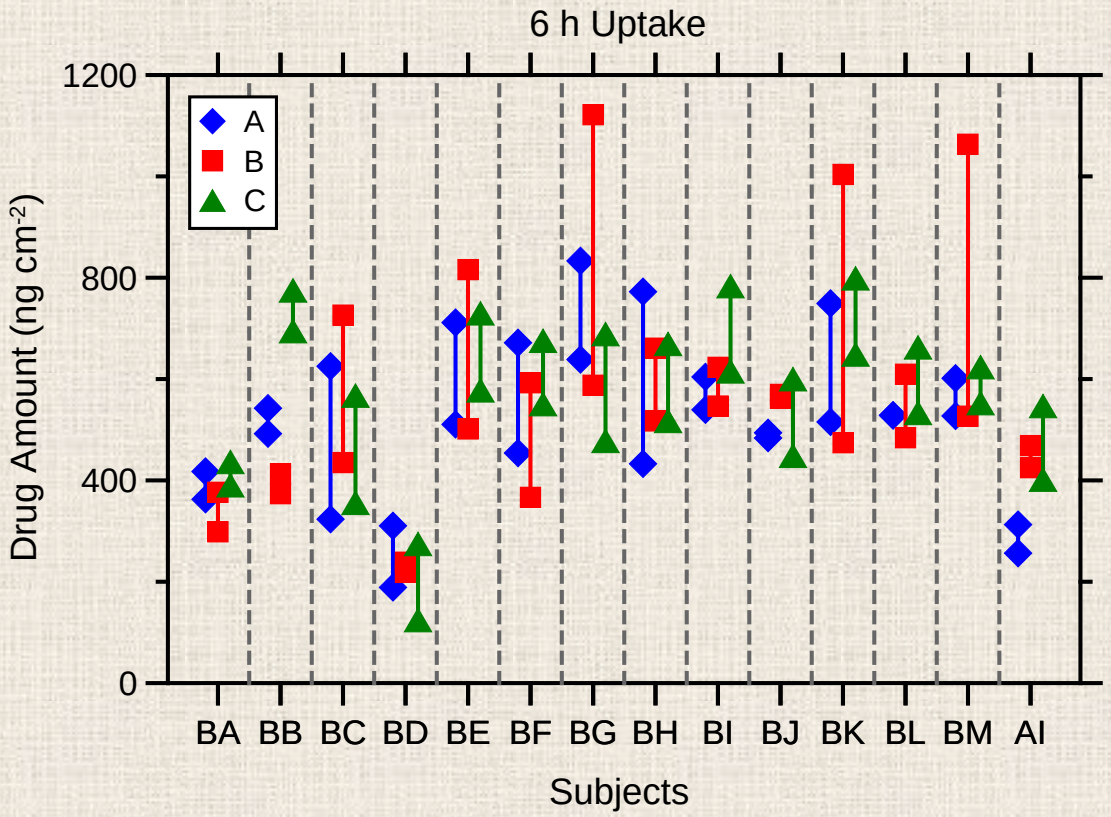
“Improved” protocol developed for FDA

- Econazole nitrate cream (1%): 2 generics to reference-listed drug (RLD)
- 4 treatment sites per product (12 sites total)
  - duplicate determinations at 2 times
  - 1 uptake time (6 hr) & 1 clearance time (17 hr); convenience
- Unabsorbed drug removed using isopropyl alcohol wipes
- Determined *all* drug in SC by removing most of SC
  - removed SC until TEWL was 8-fold greater than pre-stripping value
  - at least 12 tape strips, but not more than 30 (for Scotch book-tape used here)
  - tape stripping area < drug application area (control both areas)
- BE of uptake and clearance were assessed separately
- Tape strips analyzed in groups to optimize analytical sensitivity
- Compare within each subject and then across subjects



N'Dri-Stempfer *et al.*, Pharm. Res., 26, 316-328 (2009)

Econazole uptake into SC



- Drug uptake from 3 clinically BE formulations measured in duplicate (n = 14).
- A = Clay Park. B = Ortho (RLD). C = Taro.
- Duplication of measurements improved results.

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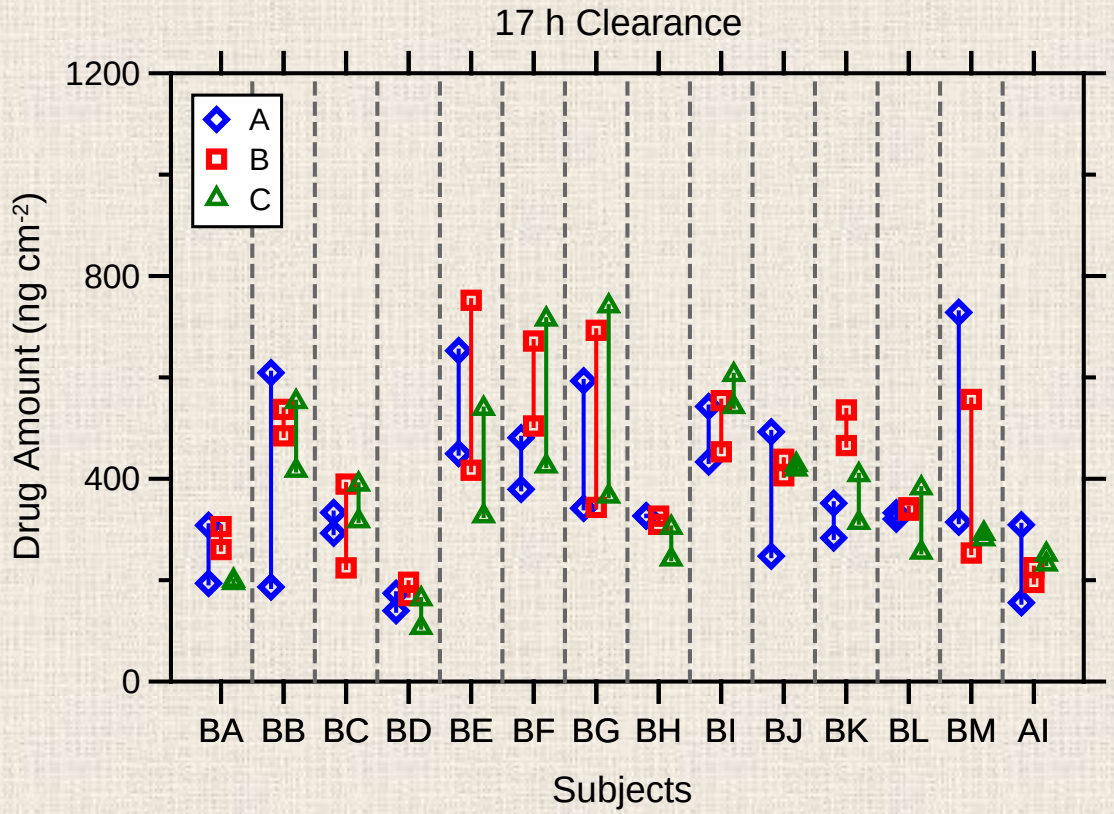
Econazole clearance into SC



- **Drug clearance of 3 clinically BE formulations measured in duplicate (n = 14).**
- **A = Clay Park. B = Ortho (RLD). C = Taro.**

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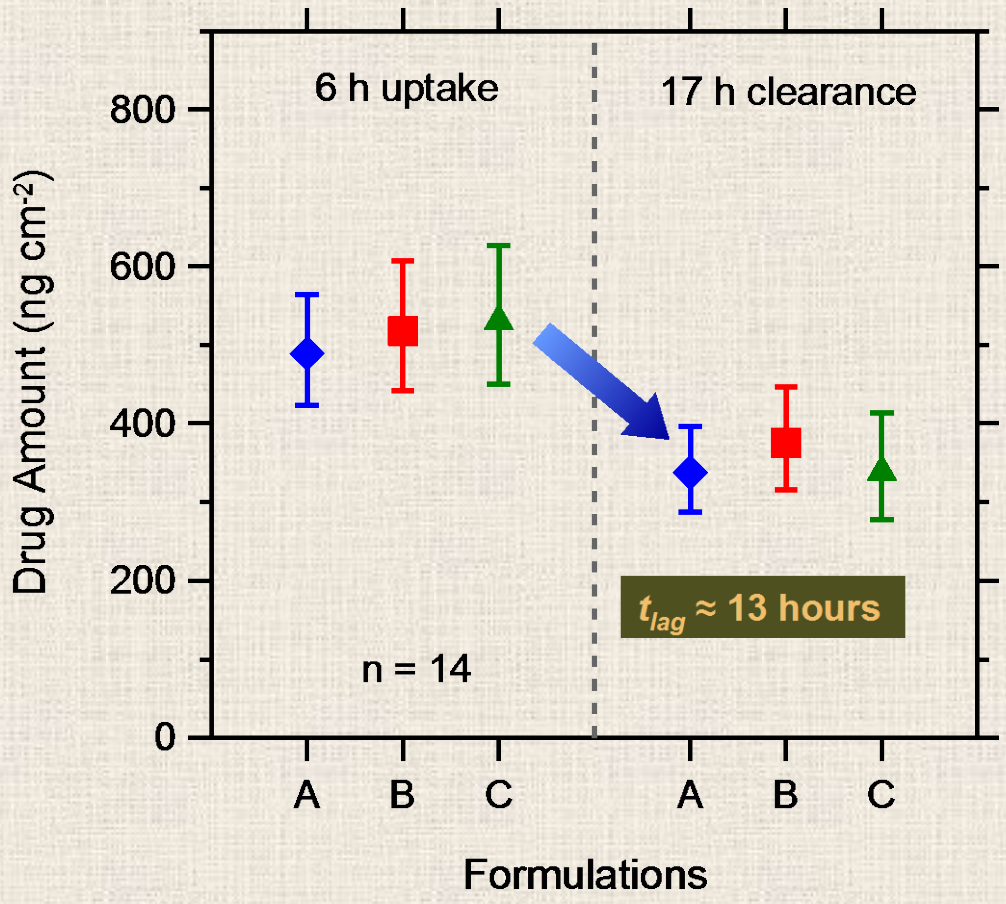
Econazole: average drug amounts in SC



A = Clay Park

B = Ortho (RLD)

C = Taro

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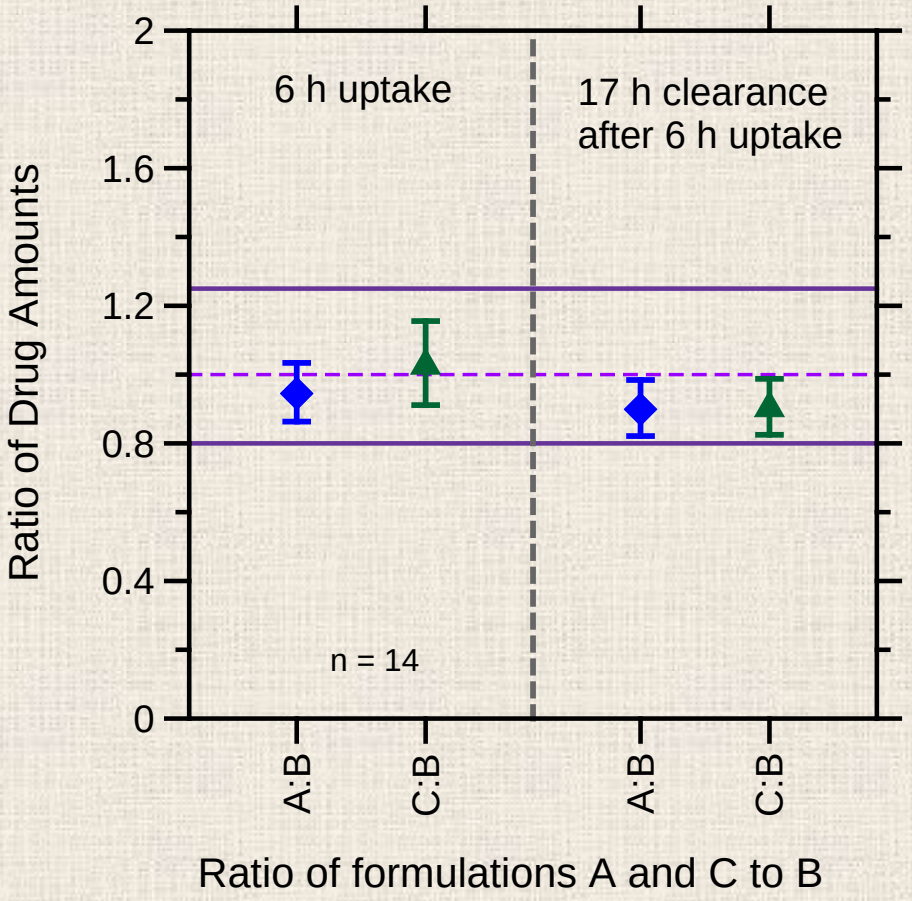
Econazole: assessment of bioequivalence (BE)



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., 26, 316-328 (2009).

Stratum corneum sampling *in vivo*

Facile method, "obvious" for drugs acting on or in stratum corneum

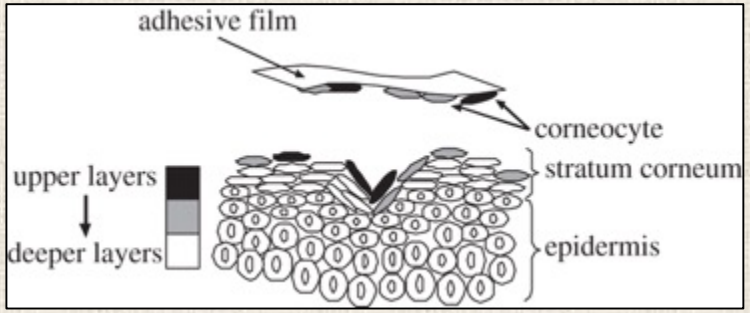
Improved approach is much more robust than original

Direct application of approach on diseased skin is unlikely, but…

- this is true of the vasoconstriction assay for corticosteroids

Correlation with clinical outcome requires further validation

- potential complementarity with IVPT, microdialysis, etc.
- relevance for targets deeper in the skin???
- selection of optimal metrics???



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- simplify the method (fewer analyses and decreased variability)

- decrease/eliminate lab-to-lab differences (reduce sensitivity to different operators)

- despite results, FDA concerns remain about value of method when target is not the SC

2013 - FDA sponsors research to assess *in vitro* – *in vivo* correlations…

Stratum corneum (SC) sampling *in vivo*

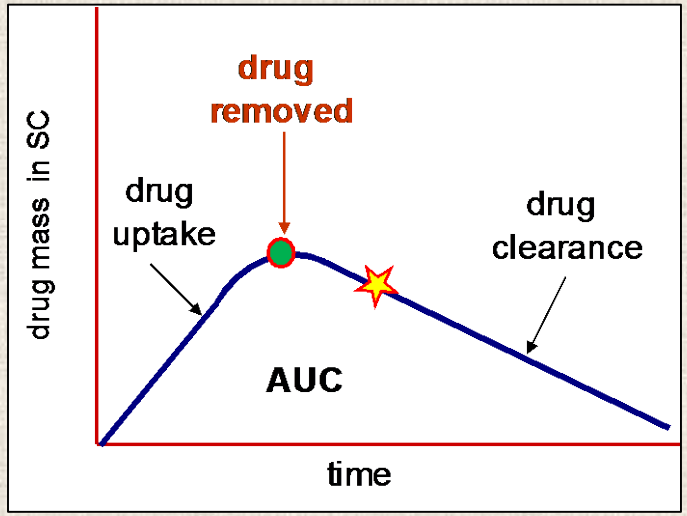
what if stratum corneum is not the target?

Translational *in vivo* methodology for *in vitro* correlation

- drug/formulation specific for IVIVC
- simpler than PK; feasible when plasma levels too low
- simpler than open flow microperfusion/microdialysis

Measures drug delivery rate from SC

- measure mass of drug in SC after period of clearance (✸)
- compare to mass of drug in SC at end of uptake (●)

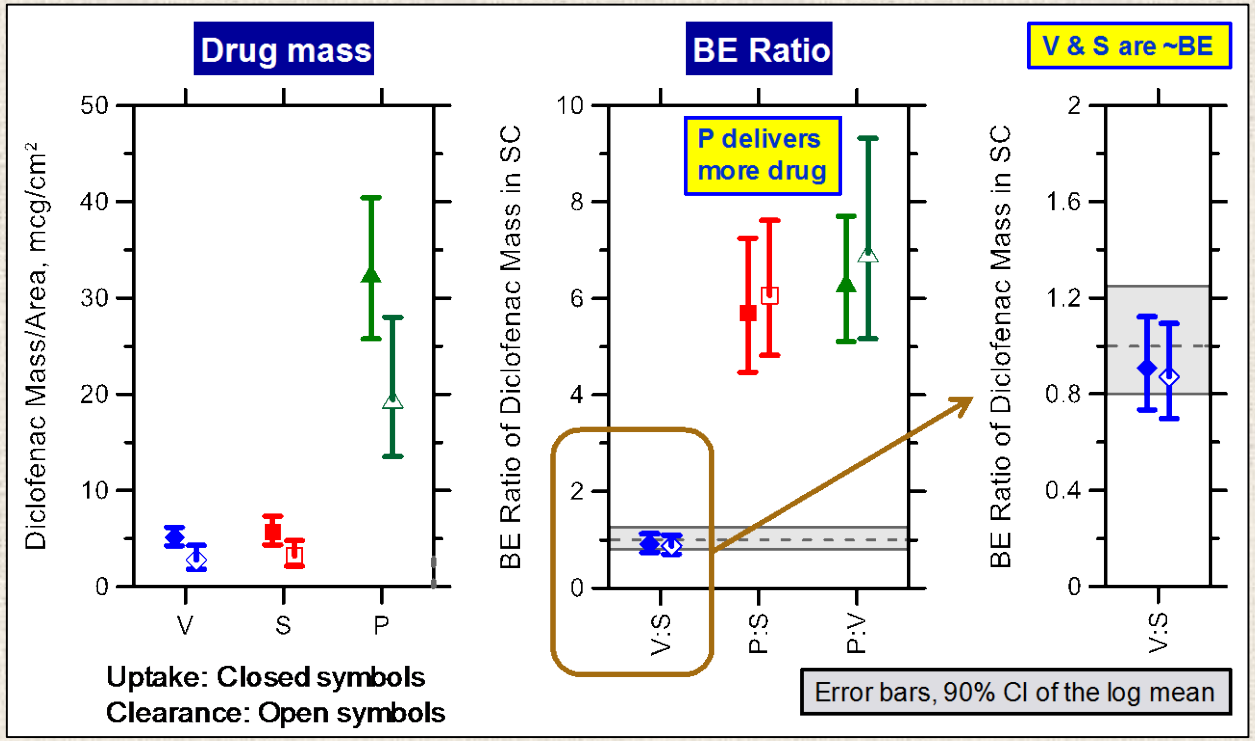


Calculate the average flux from the SC to deeper tissues:

$$\text{Average Flux} = \frac{\left(M_{Up} - M_{Clear}\right)/A}{t_{Clear} - t_{Up}}$$

Stratum corneum (SC) sampling *in vivo*

Diclofenac: target = subcutaneous tissue

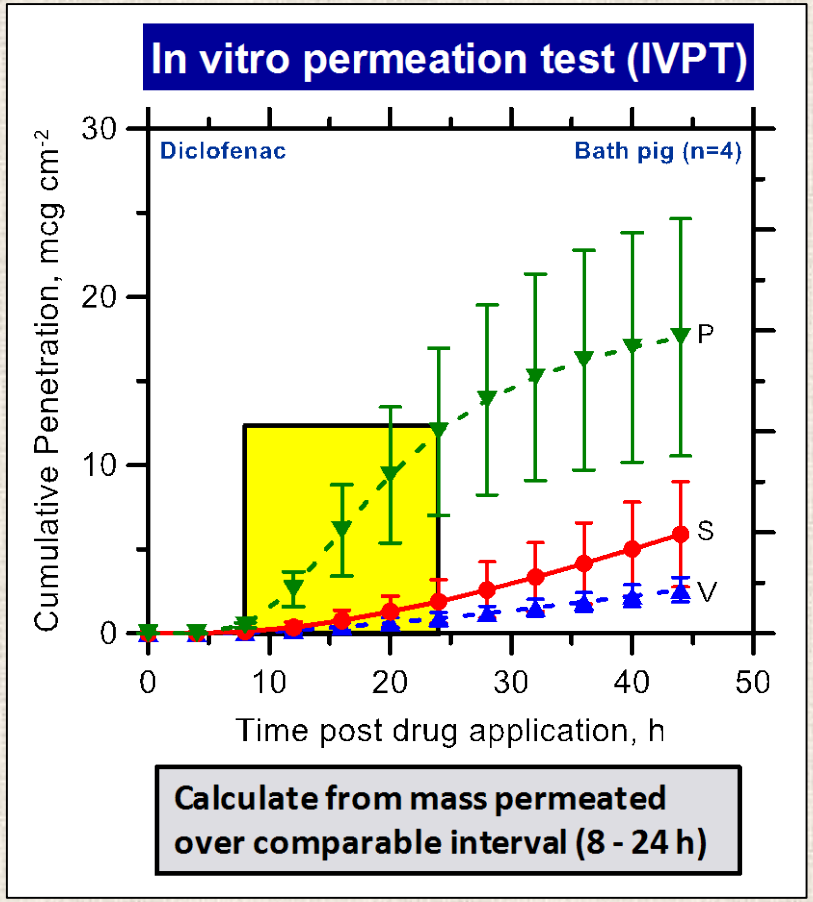


- Protocol identical to that used for econazole (n = 14)
- 3 formulations: Solaraze, Penssaid, Voltaren
- One uptake time – 6 hrs. One clearance time – 17 hrs

Cordery SF *et al. Int J Pharm.* 529 (2017) 55-64.

Stratum corneum (SC) sampling *in vivo*

Diclofenac: compare in vitro and in vivo delivery rates to skin



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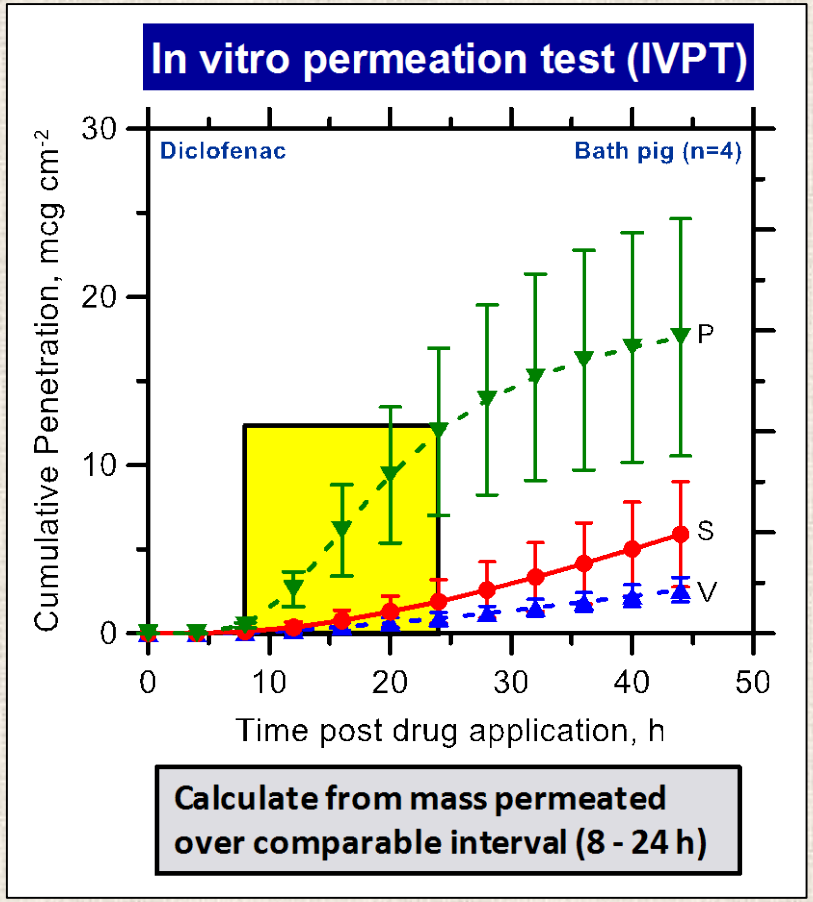
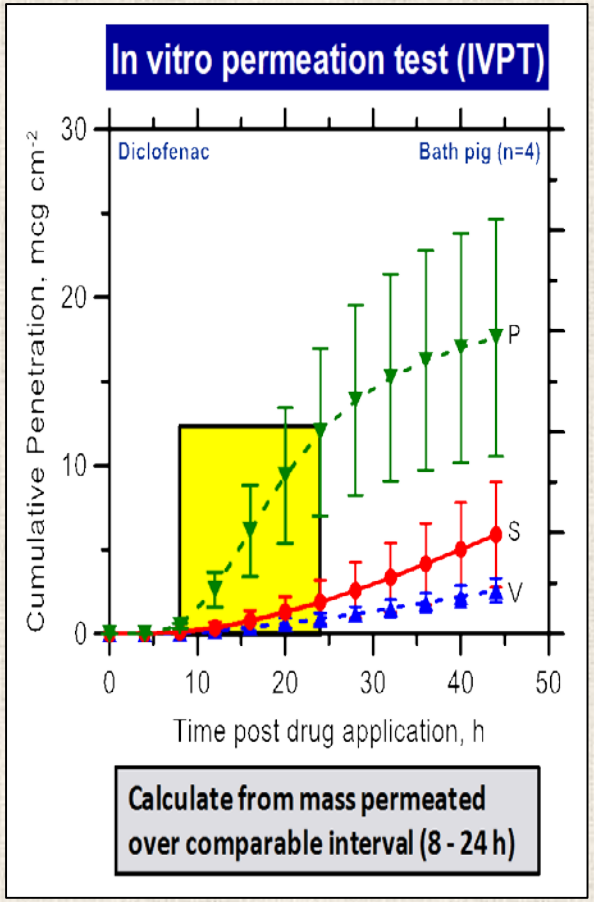




$$\text{Average Flux} = \frac{\left(M_{Up} - M_{Clear}\right)/A}{t_{Clear} - t_{Up}}$$

Cordery SF *et al. Int J Pharm.* 529 (2017) 55-64.

Stratum corneum (SC) sampling *in vivo*

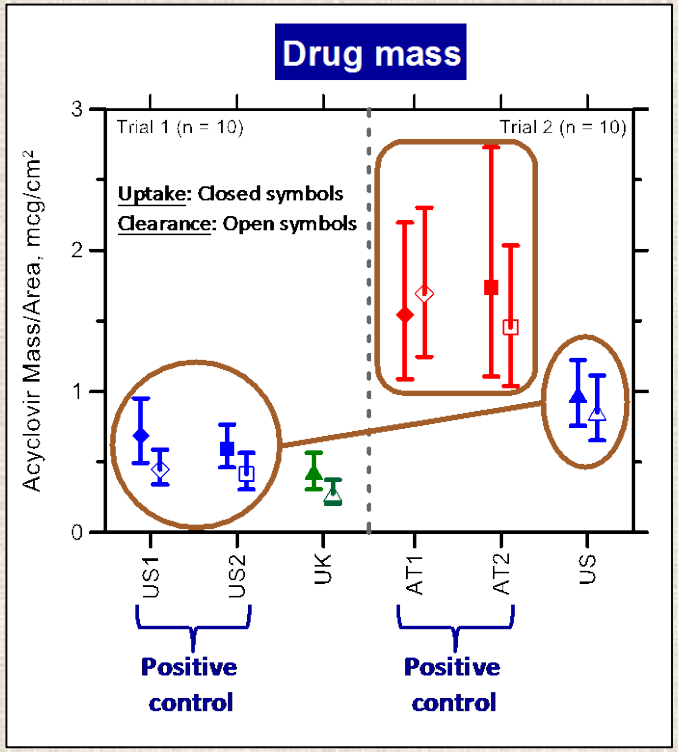
Acyclovir: target = epidermis

3 acyclovir creams (5%) compared in two trials (n = 10)

One uptake time – 6 hrs. One clearance time – 17 hrs

Trial 1: US Zovirax (R1 and R2) *vs.* UK Zovirax (T)

Trial 2: 1A Pharma (R1 and R2) *vs.* US Zovirax (T)



Pensado A *et al.*, unpublished

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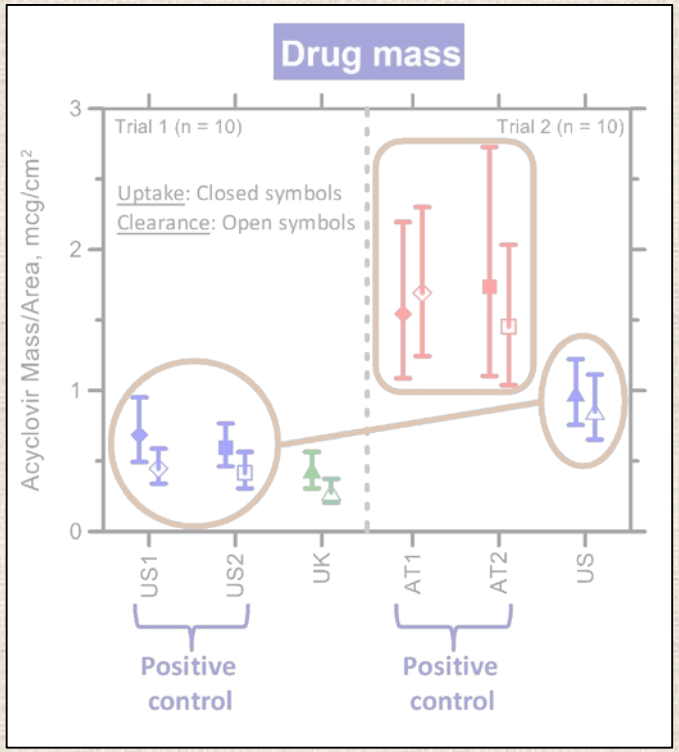
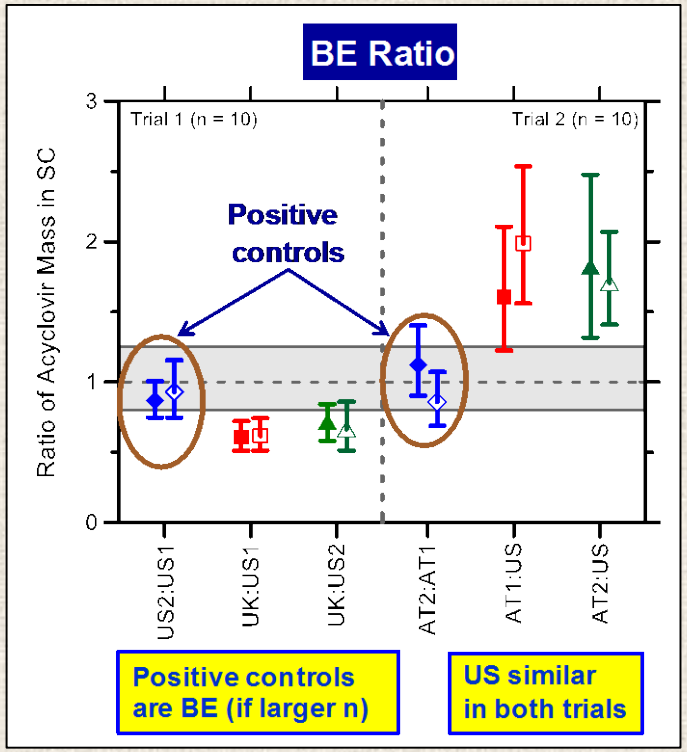
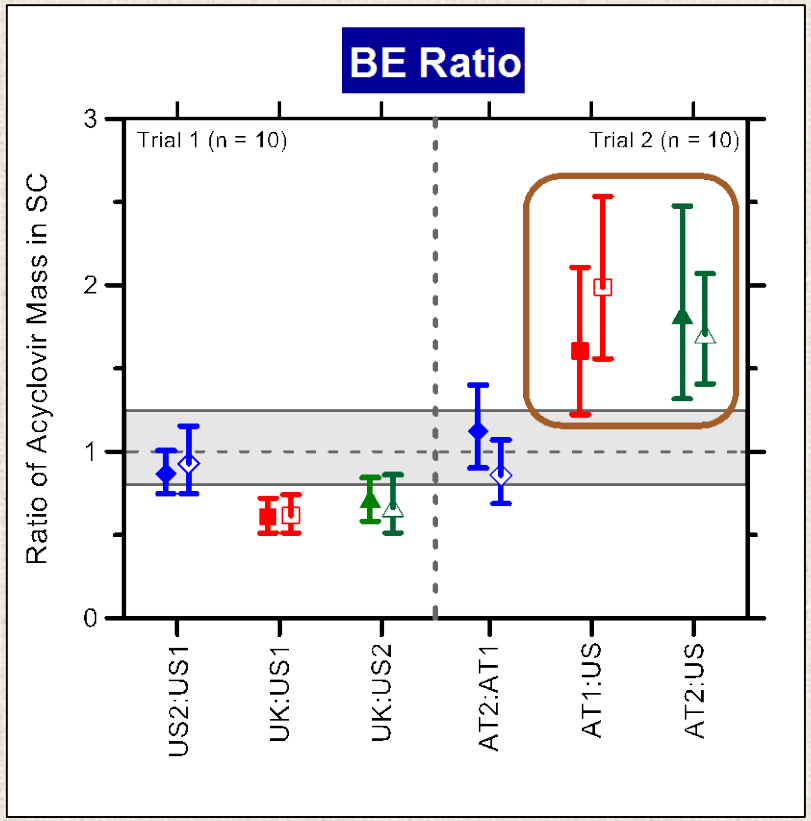




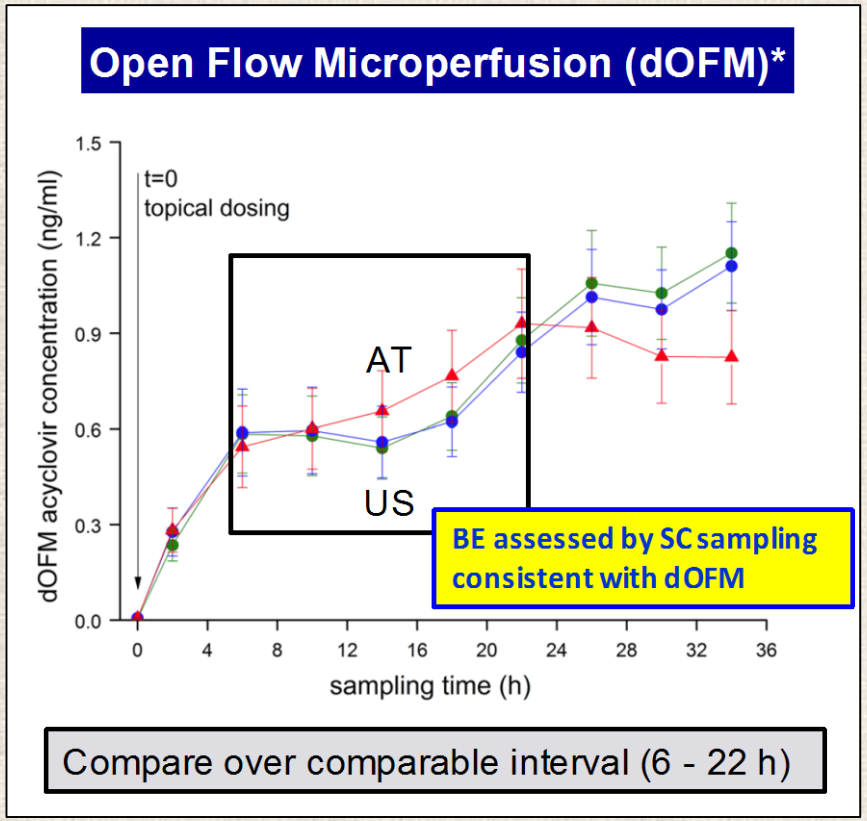
Pensado A *et al.*, unpublished

Stratum corneum (SC) sampling *in vivo*

Acyclovir: comparison with dermal oFM



Pensado A *et al.*, unpublished



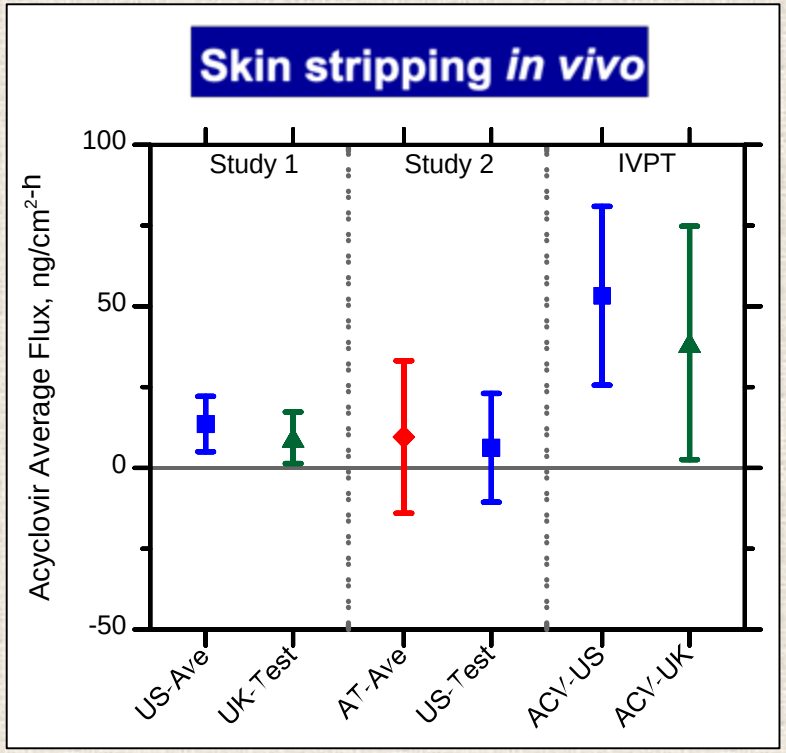
M. Bodenlenz *et al.*, *Clin. Pharmacokin.* 56 (2017) 91-98.

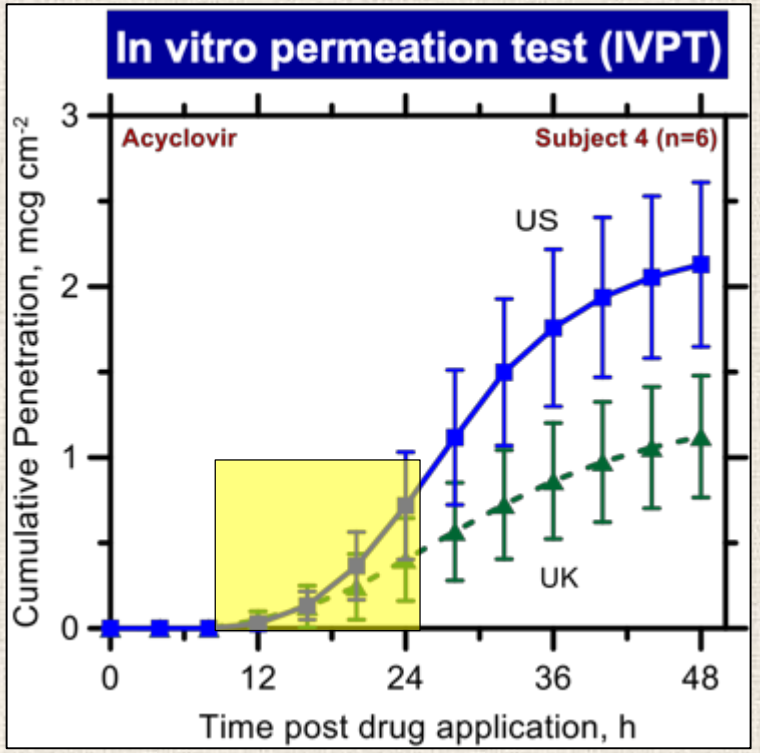
Stratum corneum (SC) sampling *in vivo*

Acyclovir: comparison with in vitro skin permeation

Flux from skin stripping similar for US, UK & AT

Flux from IVPT for US & UK also similar





Average flux from mass permeated over comparable interval (8 - 24 h)

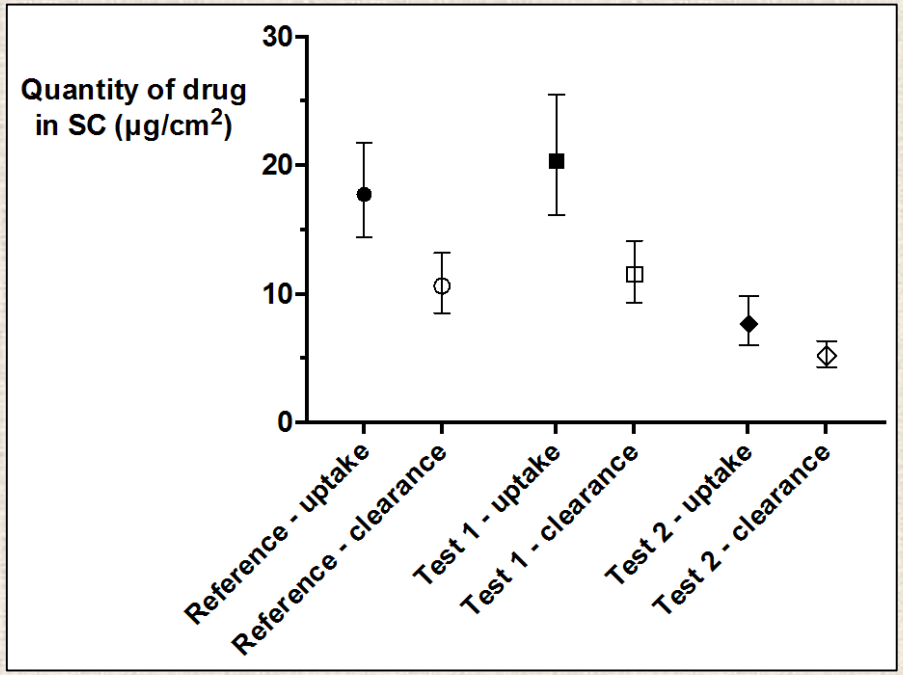
Pensado A *et al.*, unpublished

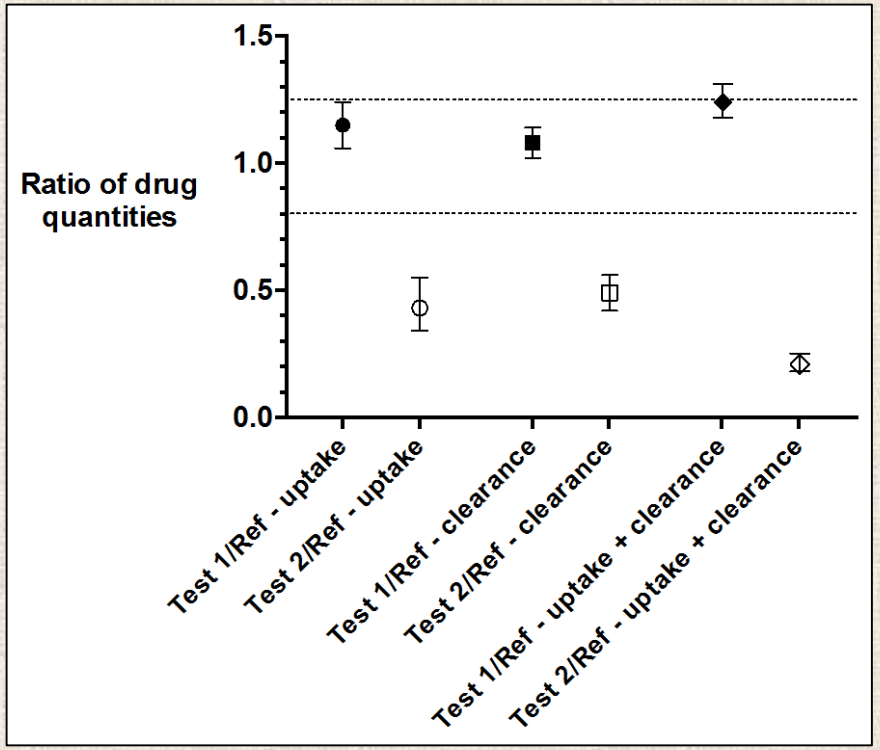
Stratum corneum (SC) sampling *in vivo*

Metronidazole: target = viable skin

Protocol identical to that used for econazole and diclofenac (n = 14)

3 formulations: Rozex® and two extemporaneous formulations. One uptake time – 6 hr. One clearance time – 6 hr

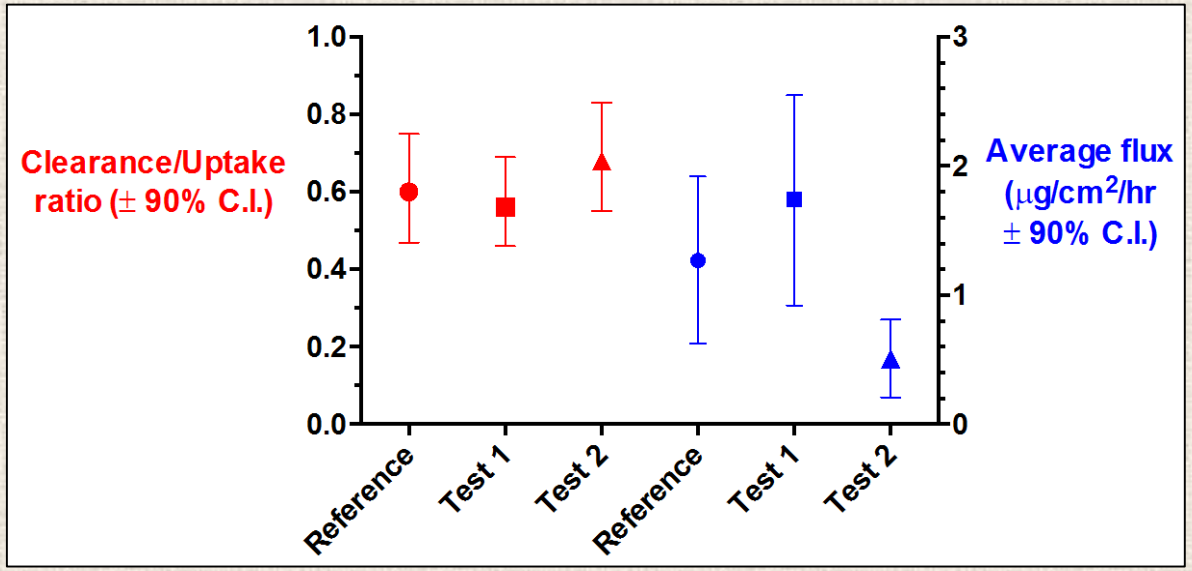




Pedon de Araujo *et al. Int J Pharm.* 541 (2018) 167-172.

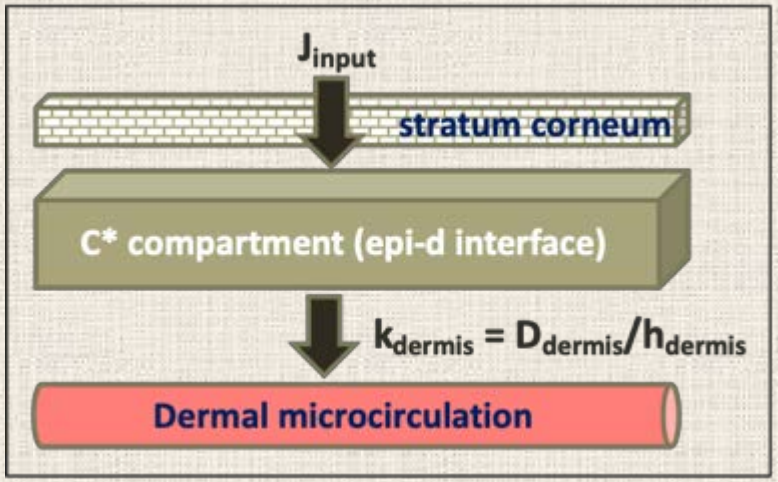
Stratum corneum (SC) sampling *in vivo*

Metronidazole: target = viable skin



Pedon de Araujo *et al. Int J Pharm.* 541 (2018) 167-172.

Drug concentration (C*) at the target site?



Higuchi C* concept, at steady-state:

$J_{input} = k_{dermis} \times C^*$

$C^* = J_{input}/k_{dermis} = J_{input}/(D_{dermis}/h_{dermis})$

Stratum corneum (SC) sampling *in vivo*

Summary

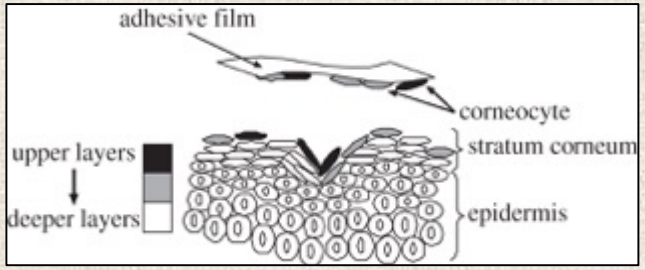
Measured in humans *in vivo*.

Improved SC sampling protocol demonstrated to be robust and reliable across laboratories and operators.

- demonstrated now for 4 drugs, 3 formulations/drug, 3 laboratories, 5 operators
- technically accessible and economical method

Complementary to other surrogate assessment methods.

- IVPT, open flow microperfusion/microdialysis, plasma PK
- obvious value for drugs acting on or in the stratum corneum
- added value for drugs acting deeper in the skin
- benchmark for (Raman) spectroscopic approach?

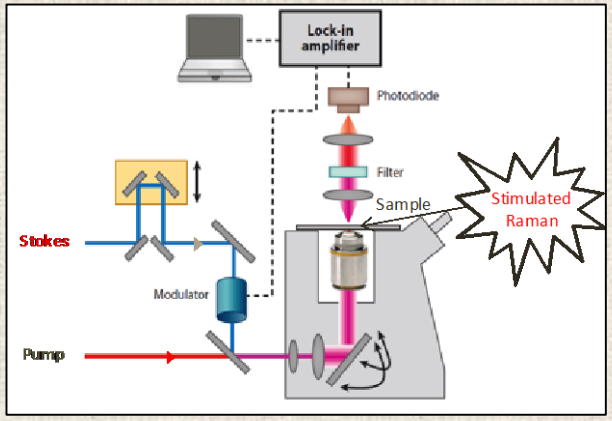


Potential to assess clinically-relevant topical bioavailability (BA).

- formulation effects on skin barrier function after repeat dosing

(Raman) Spectroscopy and imaging

- Open-flow microperfusion, while offering high-quality and highly relevant information about BA/BE *in vivo* is technically demanding.
- SC sampling *in vivo* is simpler but, even with an improved method, involves significant sample handling and analytical chemistry.
- An ideal approach would be non-invasive, simple to use and allow real-time data collection/analysis.
- Relatively recently, Raman spectroscopy has been investigated as a potential solution to this issue.
- Two formats have been examined, in particular:
  - confocal Raman spectroscopy *in vivo*
  - coherent Raman scattering and stimulated Raman scattering, primarily *in vitro*, offer novel imaging capability of significant value

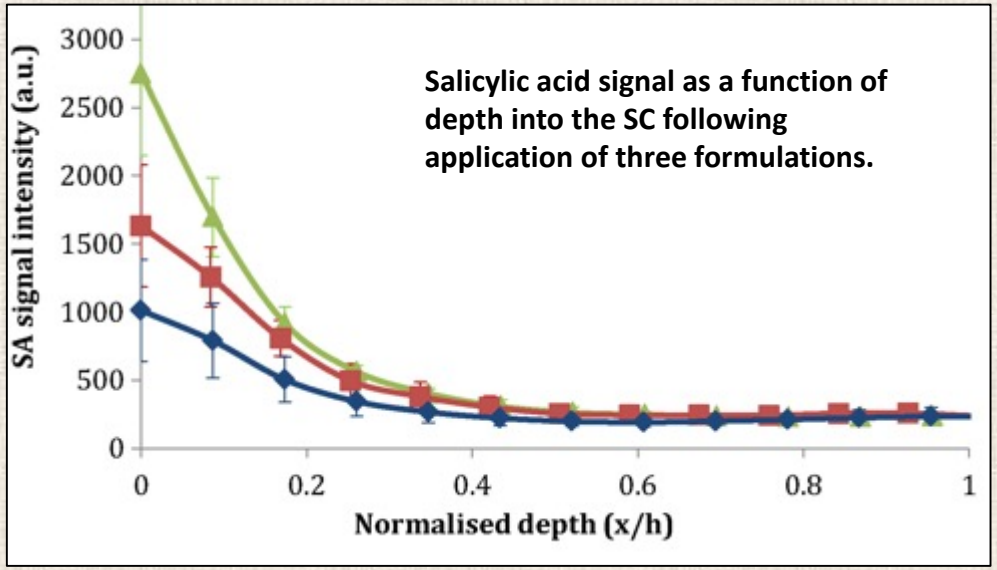


Confocal Raman spectroscopy *in vivo*

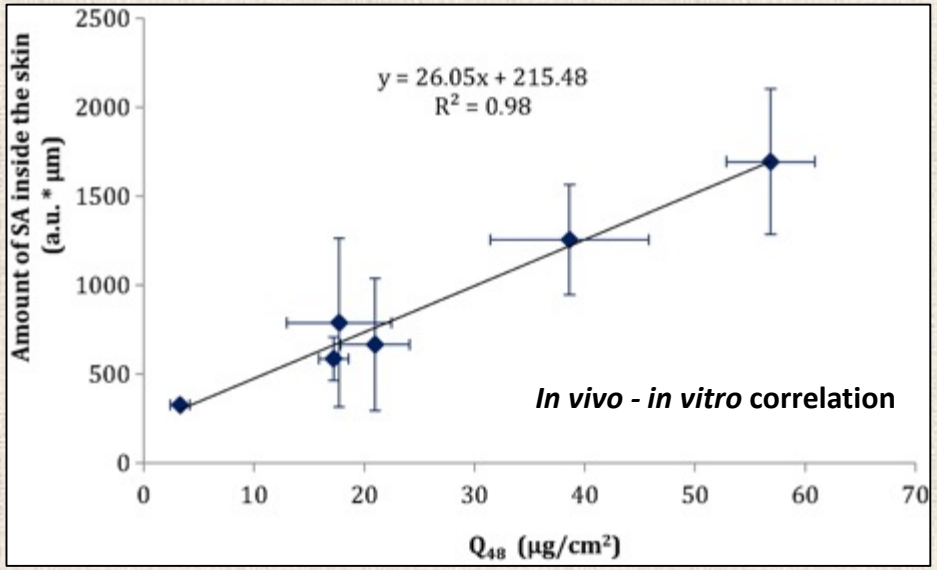
Skin absorption of salicylic acid in humans *in vivo* and across excised tissue *in vitro*.

Results from confocal Raman spectroscopy and *in vitro* skin penetration tests compared.

- **Confocal Raman microspectrometer (Model 3510 Skin Composition Analyzer, River Diagnostics, The Netherlands) was used**
- **data acquired using RiverIcon® software Version 2.5.2 and analysed using Skin Tools® software Version 2.0 (River Diagnostics)**



Salicylic acid signal as a function of depth into the SC following application of three formulations.



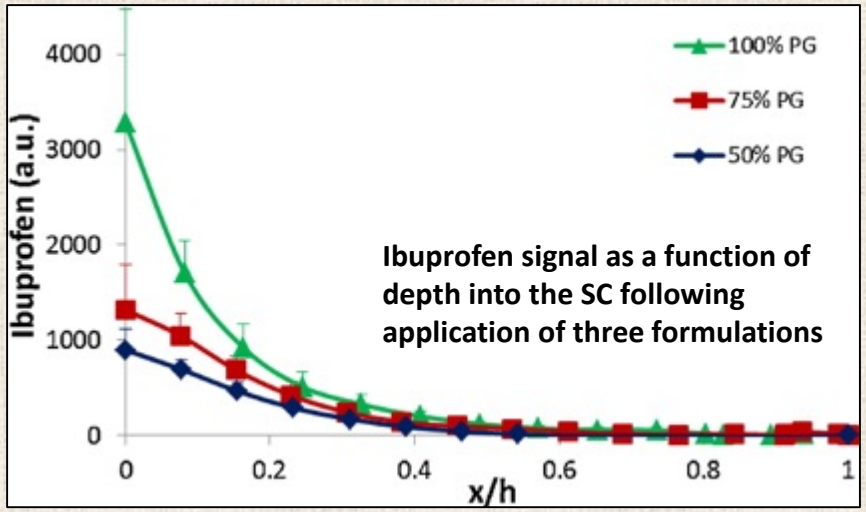
***In vivo - in vitro* correlation**

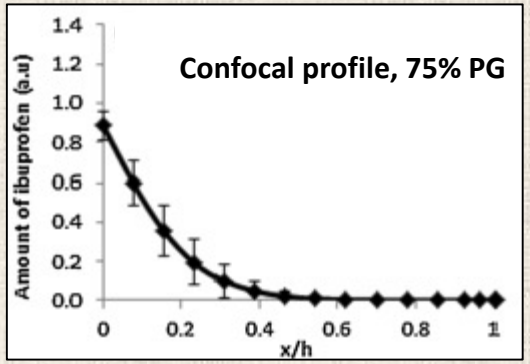
Mateus, R. *et al. Int. J. Pharmaceut.* 475 (2014) 471-474.

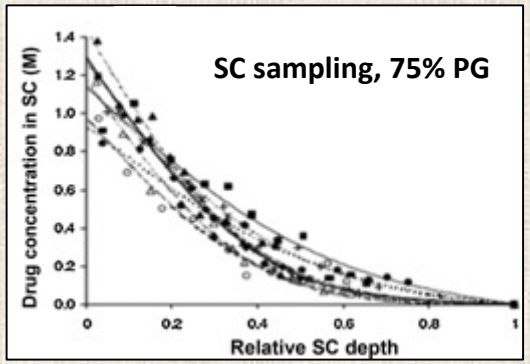
Confocal Raman spectroscopy *in vivo*

Skin absorption of ibuprofen in humans *in vivo*.

Results from confocal Raman spectroscopy and stratum corneum sampling compared.



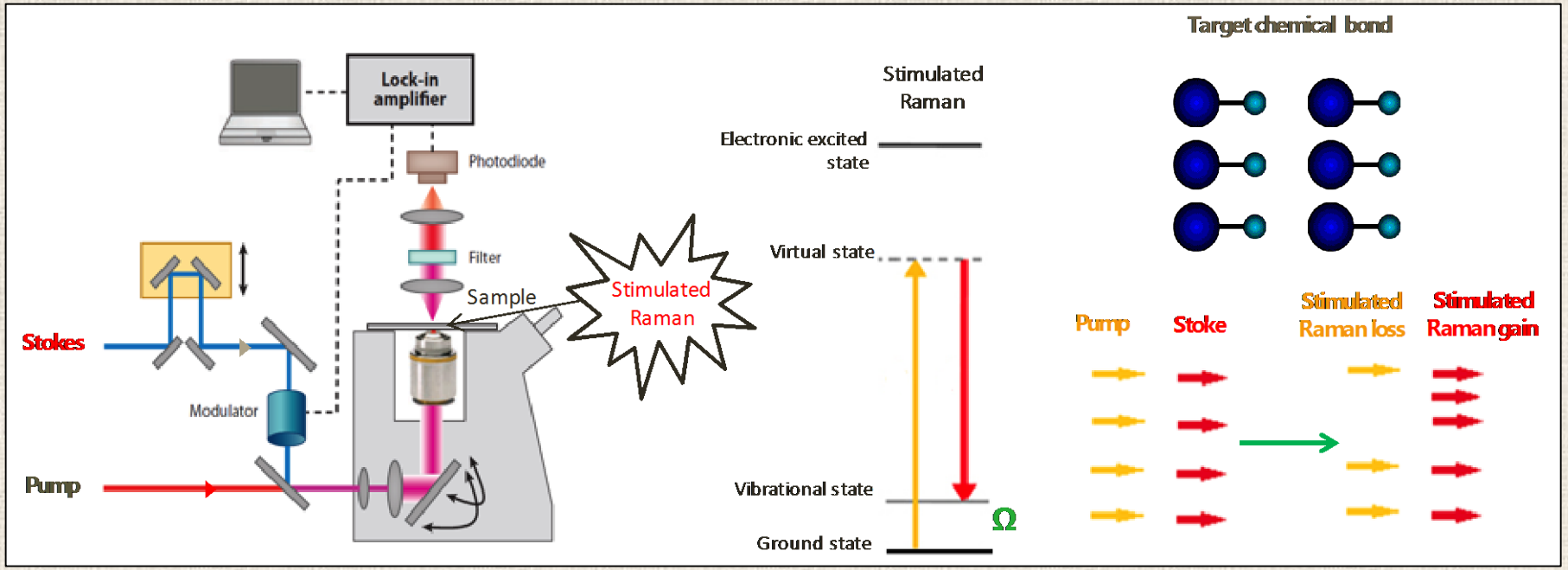




Mateus, R. *et al., Int. J. Pharmaceut.* 444 (2013) 106-108.

Herkenne, C. *et al., J. Invest. Dermatol.* 127 (2007) 887–894.

Coherent and stimulated Raman scattering

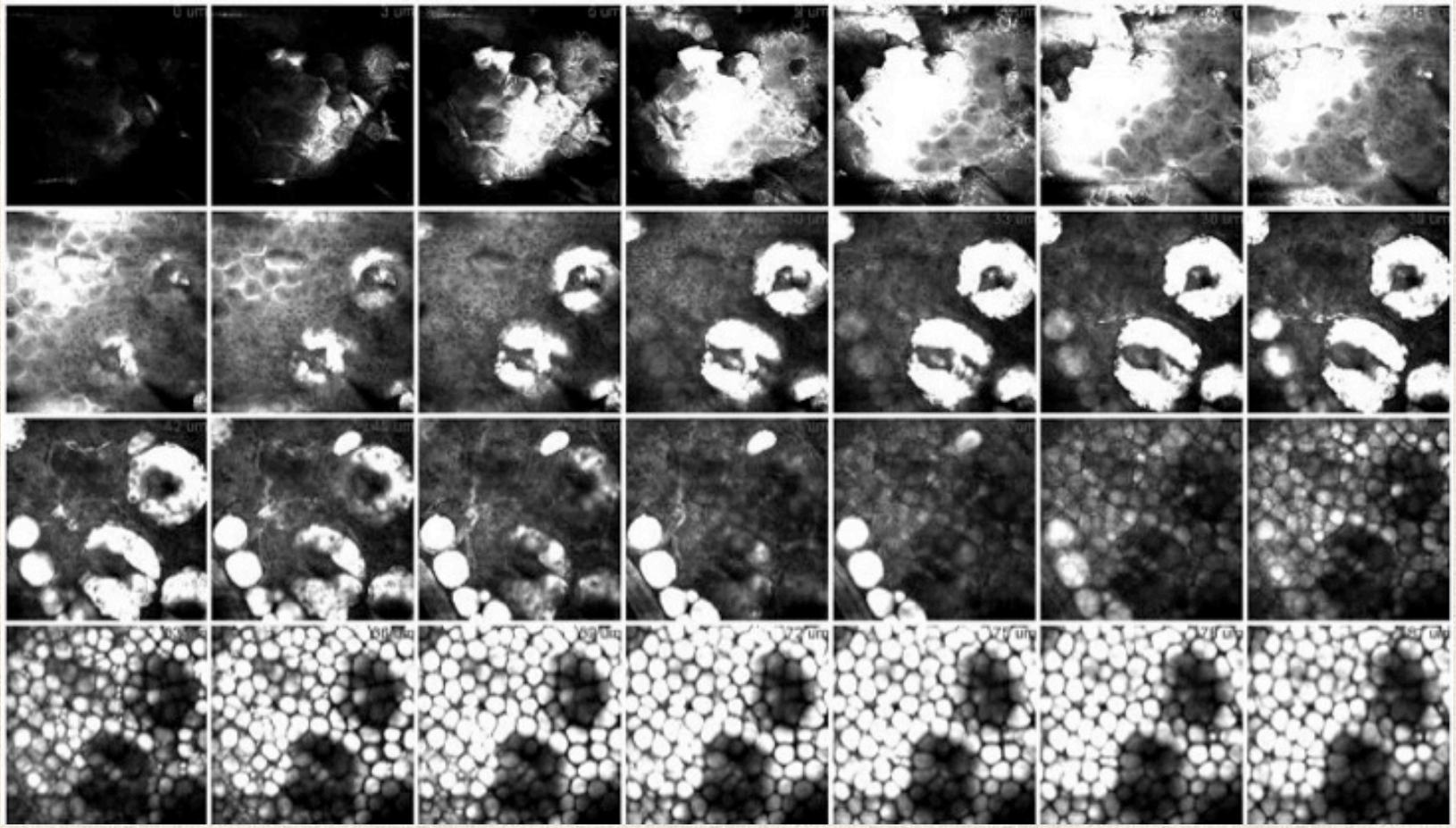


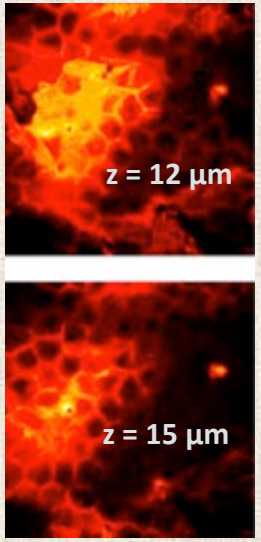
- Images the specific chemical bond of interest
- Stimulated excitation of coherent molecular vibration: $\omega_{pump} - \omega_{Stokes} = \omega_{vib}$
- SRS signal is linearly proportional to concentration of target molecule
- Information on penetration depth and pathways of multiple components of a formulation

A Zumbusch, GR Holtom, XS Xie, Phys Rev Lett 82, 4142-4145 (1999)

Coherent and stimulated Raman scattering

CH_2 images of mouse ear skin at 2846 cm^{-1}

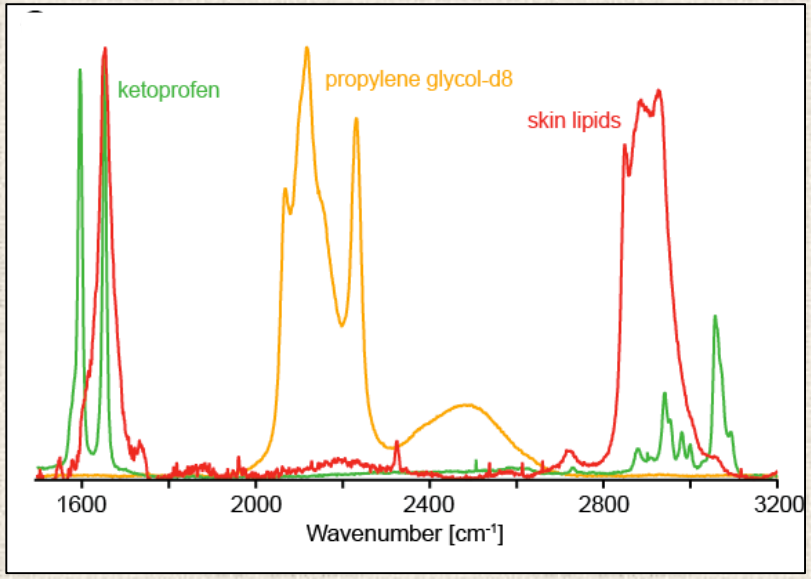


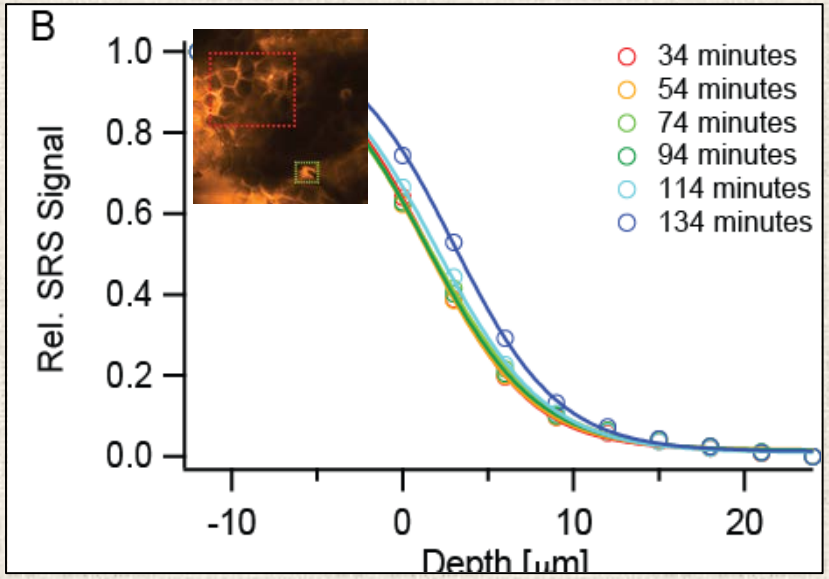


BG Saar, LR Contreras-Rojas, XS Xie, RH Guy,
Molecular Pharmaceutics 8, 969-975 (2011)

Coherent and stimulated Raman scattering

imaging skin penetration and vehicle 'metamorphosis'





SRS contrast is based on spontaneous Raman spectra, which are used to determine optimal excitation wavelengths: 1599 cm^{-1}, 2120 cm^{-1} and 2845 cm^{-1} report on ketoprofen, deuterated PG and skin lipids, respectively.

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

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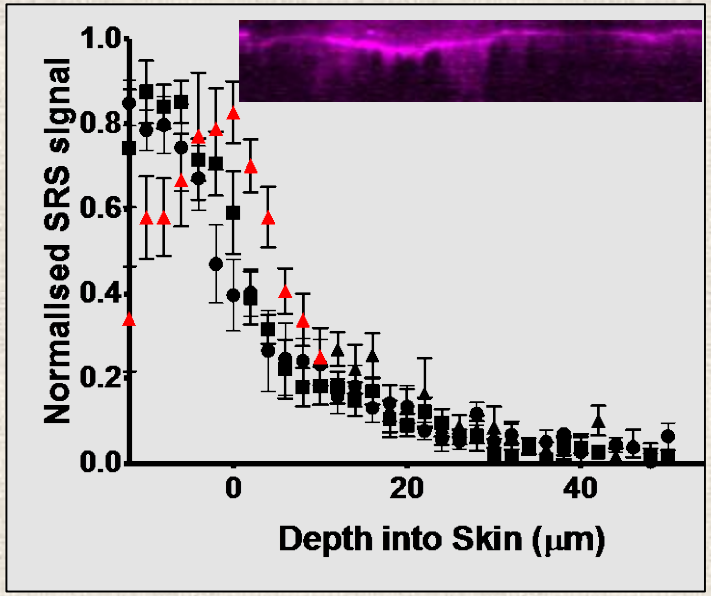

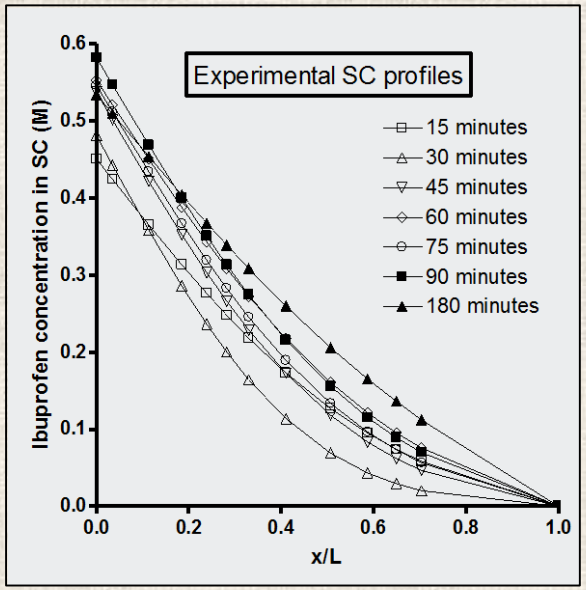

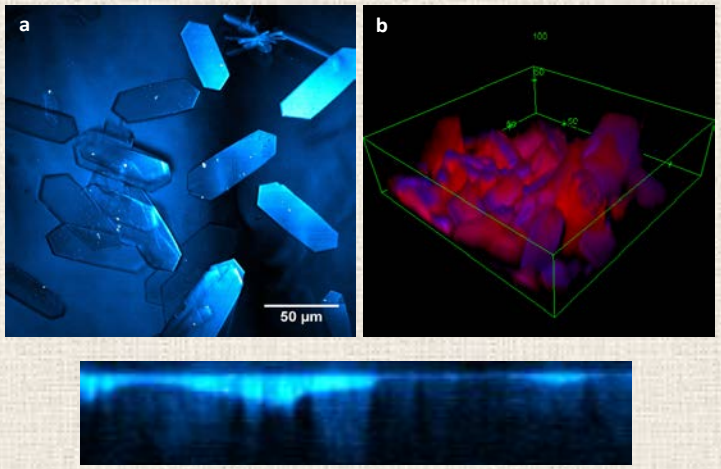

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011).

C Herkenne et al., *J. Invest. Dermatol.* 127 (2007) 887–894.

Coherent and stimulated Raman scattering

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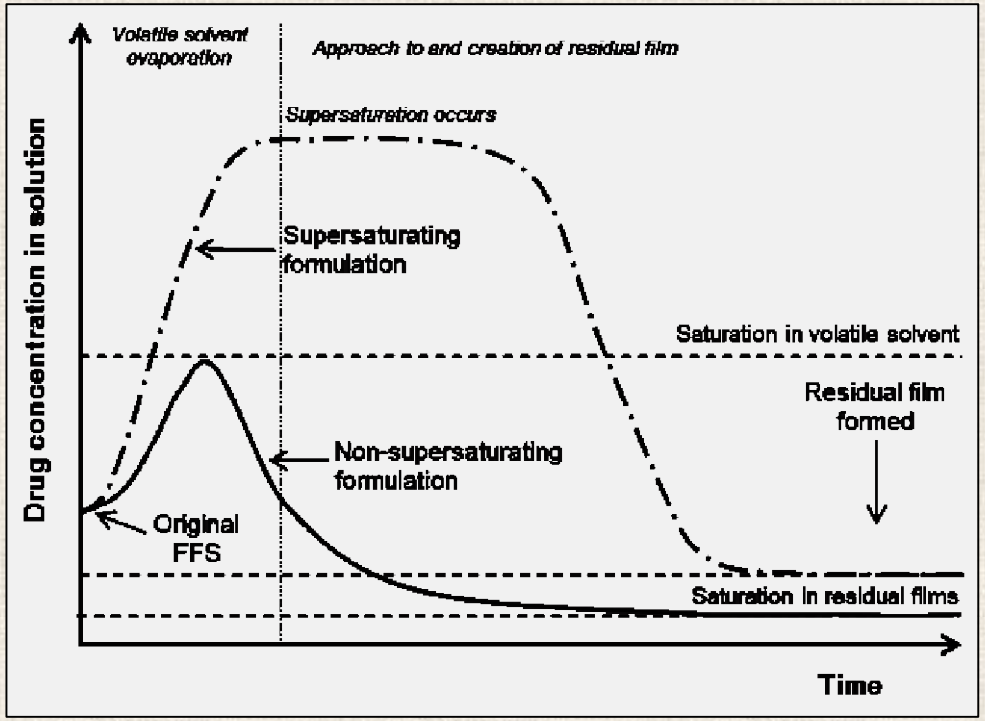
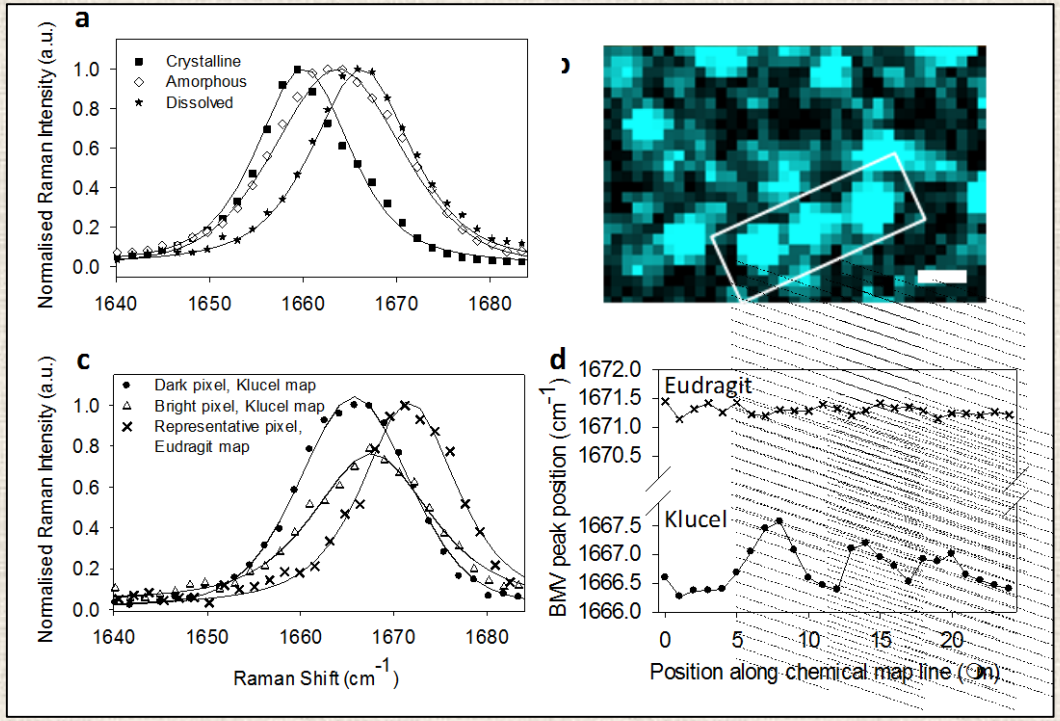




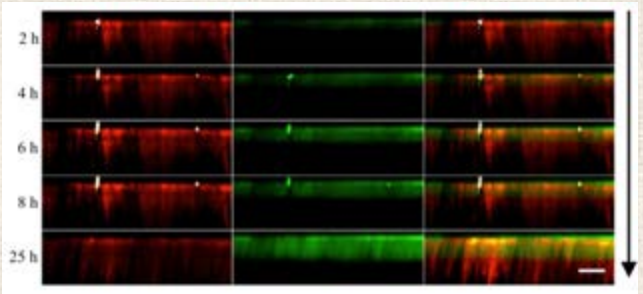
BMV appears "dissolved" in the acrylate polymer, but in the solid state in the hydrophilic, Klucel FFS

Skin uptake of BMV is greatest from acrylate FFS with MCT

H. Garvie-Cook, K. Frederiksen *et al.*
J. Control. Release 212, 103-112 (2015)

(Raman) Spectroscopy and imaging

- Confocal Raman imaging has the unique advantage of a non-invasive *in vivo* technique.
- CRS and SRS have mostly been limited to *ex vivo* study, although *in vivo* proof-of-concept has been demonstrated.
- Drug and excipient disposition can be followed simultaneously in real-time.
- Both approaches are limited by three important factors:
  - inherent insensitivity of Raman spectroscopy
  - requirement of a distinct Raman signal (e.g., vibration) from molecule of interest
  - attenuation of signal with increasing skin depth due to scattering/absorption
- Raman signals 'easily' detected from stratum corneum; from deeper, 'viable' skin is a work-in-progress.
  - imaging of appendageal structures (e.g., sweat glands) has been demonstrated
- Correlation between Raman and stratum corneum sampling established.



Conclusions

- Assessment and optimisation of topical bioavailability *in vivo* remains an important goal.
- Techniques available all have clear advantages, but none is without limitation:
  - dermal open-flow microperfusion
  - stratum corneum sampling (*aka* tape-stripping)
  - Raman spectroscopy and imaging
- Understanding of local pharmacokinetics in skin requires improved (quantitative) measurements.
  - how can drug concentration in the target skin 'compartment' be found?
- Regulatory objective of *in vitro* surrogate methodology demands *in vivo* validation.

I've got you under my skin", written by Cole Porter in 1936, and a Frank Sinatra classic."

Acknowledgements

- Drs. Annette Bunge, Audra Stinchcomb, Leila Leal, Begoña Delgado-Charro, Tom Franz, Sam Raney, Priyanka Ghosh, Wing Chiu, Sarah Cordery, Andrea Pensado, Berthe N'Dri-Stempfer, William Navidi, Natalie Belsey, Hazel Garvie-Cook, Kit Frederiksen, Karsten Petersson

- U.S. Department of Health & Human Services, Food & Drug Administration (award numbers: D3921303, 1-U01-FD004947 and 1-U01-FD006533). *The views expressed in this presentation do not reflect the official policies of the U.S. Food & Drug Administration or the U.S. Department of Health & Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.*

- CAPES Foundation, Ministry of Education, Brazil (Chamada de Projetos MEC/MCTI/CAPES/CNPq/FAPs, Nº 09/2014).

- GSK-Stiefel and Leo Pharma A/S