Stimulated Raman scattering (SRS) microscopy and deep learning: A novel pharmacokinetic approach for evaluation of topical bioequivalence

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HARVARD
MEDICAL SCHOOL

Disclaimer

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Topical Drug

- Applying medication to the skin or mucous membranes.

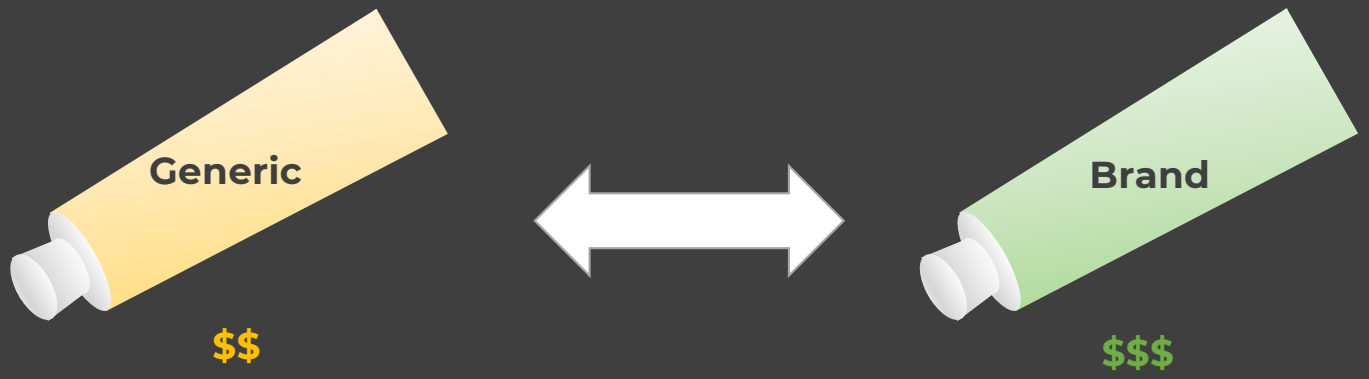
- Drug Formulation:
  Active pharmaceutical ingredients(API) , permeation enhancers, preservatives, emulsifiers, etc.

- API-Tazarotene:
  For the topical treatment of of numerous skin conditions including acne vulgaris and psoriasis.

Image from: HTTPS://WWW.OUTSOURCING-PHARMA.COM/ARTICLE/2019/08/21/PROJECTED-GROWTH-FOR-THE-TOPICAL-DRUG-DELIVERY-MARKET

Image from: https://www.almirall.us/home/prescription-medicines

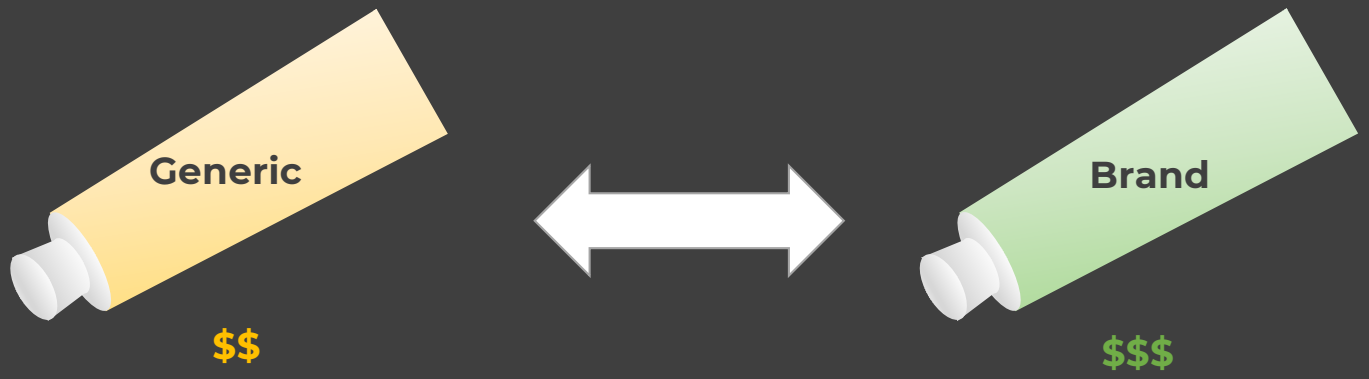
Bioequivalence(BE)



A generic drug is a medication created to be the same as an already marketed brand-name drug in dosage form, safety, strength, route of administration, quality, performance characteristics, and intended use[1].

[1] https://www.fda.gov/drugs/generic-drugs/what-approval-process-generic-drugs

Bioequivalence(BE)



- The generic medicine is bioequivalent to the brand-name medicine.
- Two products are considered to be bioequivalent when they are equal in the rate and extent to which the active pharmaceutical ingredient (API) becomes available at the site(s) of drug action[2].

[2] https://www.fda.gov/animal-veterinary/abbreviated-new-animal-drug-applications/bioequivalence

Bioequivalence(BE)

- Tazarotene topical cream
  - FDA recommended studies[3] :

    One option is to have characterization tests and in vitro bioequivalence studies, including the in vitro release test (IVRT) showing an equivalent rate of tazarotene release, and the in vitro permeation test (IVPT) showing an equivalent total cumulative amount and maximum flux.

- Pharmacokinetic (PK) approach
  - Maximum flux indicated by maximum concentration C_{max}
  - Cumulative amount indicated by area under the curve (AUC)
  - Release and permeation rate indicated by t_{max}

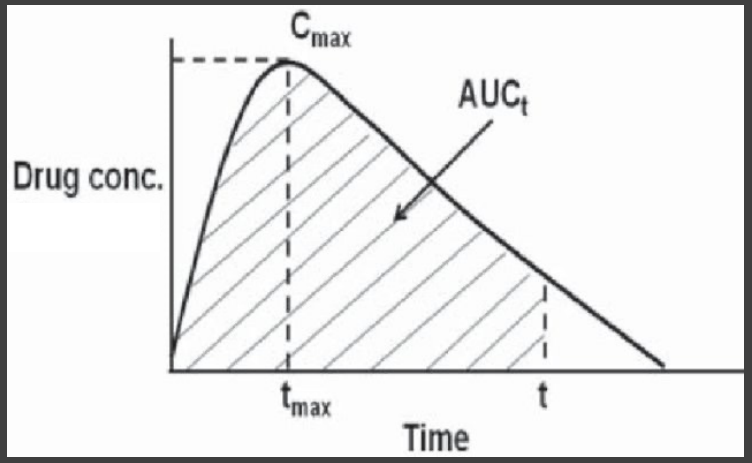


Image from: Setiawati, Arini. (2011). The importance of bioequivalence study: Focus on clopidogrel. Medical Journal of Indonesia. 20. 149. 10.13181/mji.v20i2.445.

[3] Draft Guidance on Tazarotene, FDA, Unique Agency Identifier: PSG_021184-Cre-0.1P. Recommended Jun 2011; Revised Feb 2019, Oct 2022

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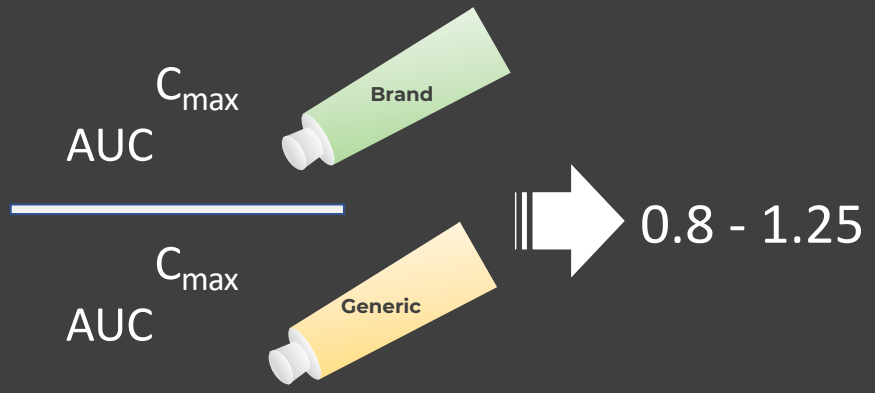
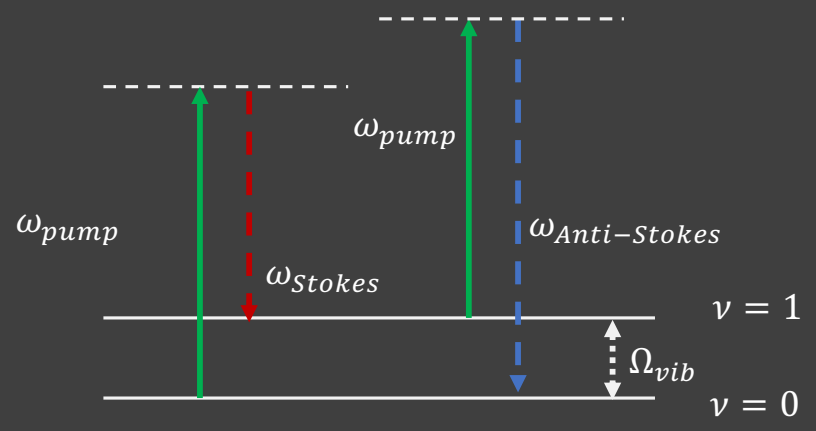


[3] Draft Guidance on Tazarotene, FDA, Unique Agency Identifier: PSG_021184-Cre-0.1P. Recommended Jun 2011; Revised Feb 2019, Oct 2022

Spontaneous Raman Scattering

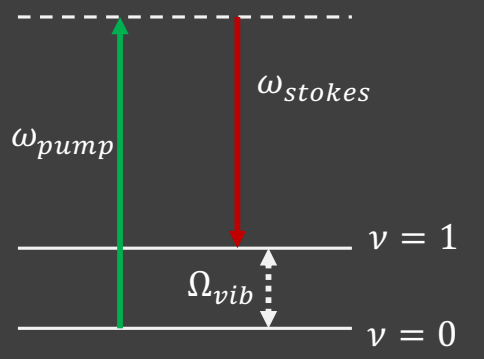
- Spontaneous Raman Imaging utilizes a laser to excite a molecular vibrational mode and measures inelastically scattered light.



- ✓ Intrinsic molecular feature
- ✓ Non-invasive
- ✓ Cellular-level resolution
- ✓ Signal is linear with the molecular concentration
- ❑ Low signal intensity
- ❑ Long integration time for images (seconds a pixel, hours an image)
- ❑ Tissue autofluorescence interference

Stimulated Raman Scattering

- Stimulated Raman Scattering (SRS) utilizes two synchronized lasers (pump and stokes beams) to excite a resonant molecular vibrational mode



- ✓ Intrinsic molecular feature
- ✓ Non-invasive
- ✓ Cellular-level resolution
- ✓ Signal is linear with the molecular concentration
- ✓ > 1000 times faster imaging speed
- ✓ Video-rate time resolution
- ✓ Avoid non-resonant background

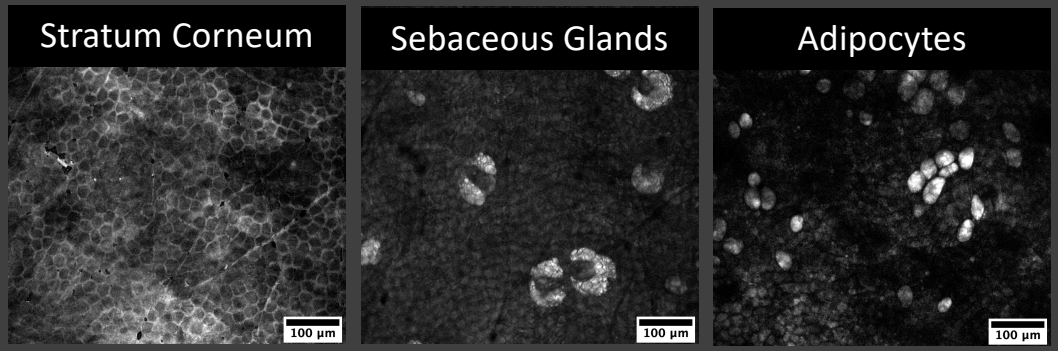
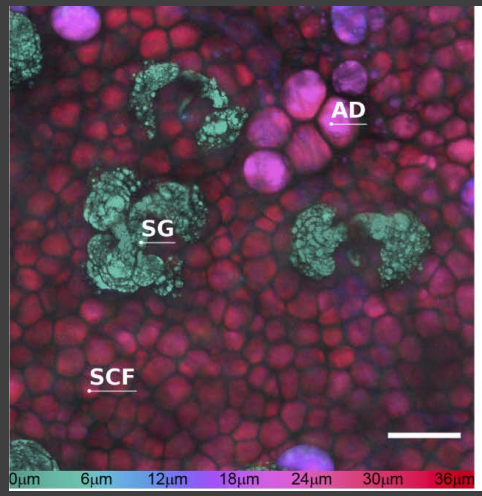
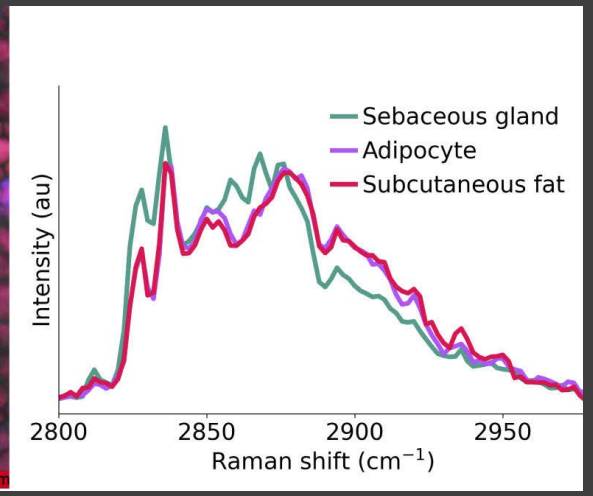


Image from: Kuzma, B.A. et al. *Journal of Visualized Experiments* (2021)

Sparse Spectral Sampling Stimulated Raman Scattering – S⁴RS

- Wide tunable range covering fingerprint(700-1800 cm^{-1}), silent (2000-2400 cm^{-1}), and high wavenumber (2700-3300 cm^{-1}) windows of the Raman spectrum.
- Wavenumber tuning rapidly (<5 ms)
- Applications for topical drug study:
  - Skin structures
  - Topical drug compositions

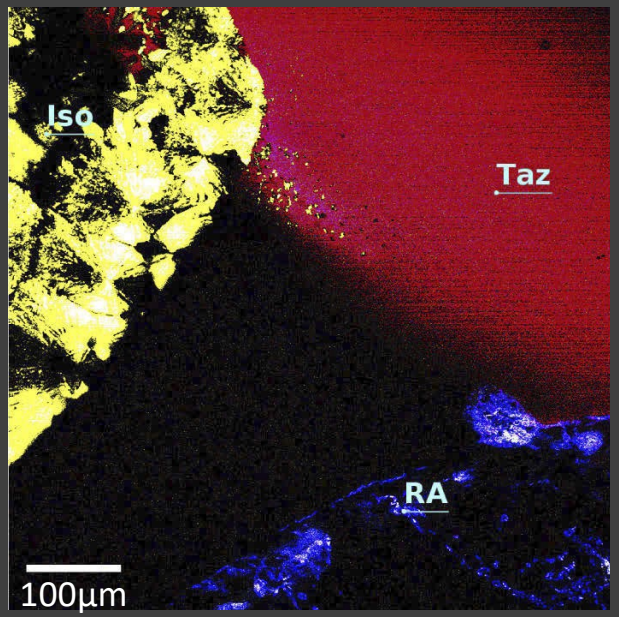


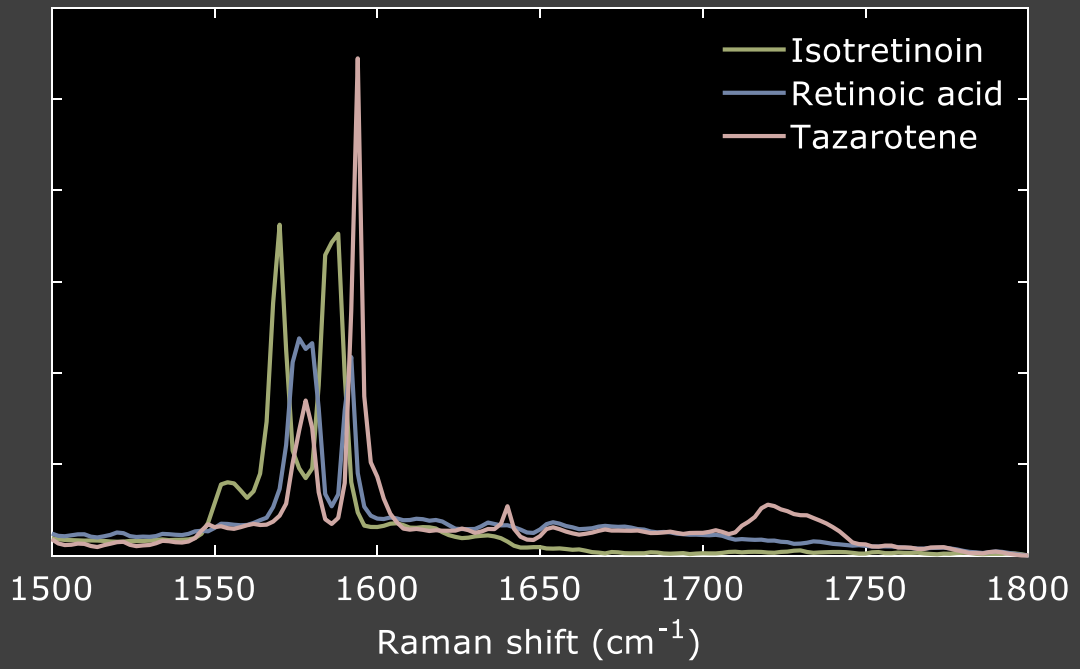


Pence, J.P. et al. Multi-window sparse spectral sampling stimulated Raman scattering microscopy. *Biomedical Optics Express* (2021)

S⁴RS for Drug Differentiation

3 APIs, fingerprint region.



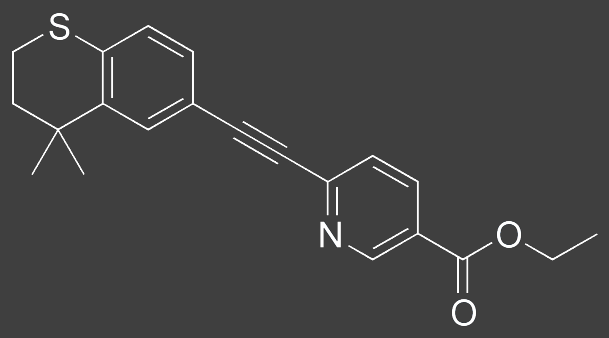


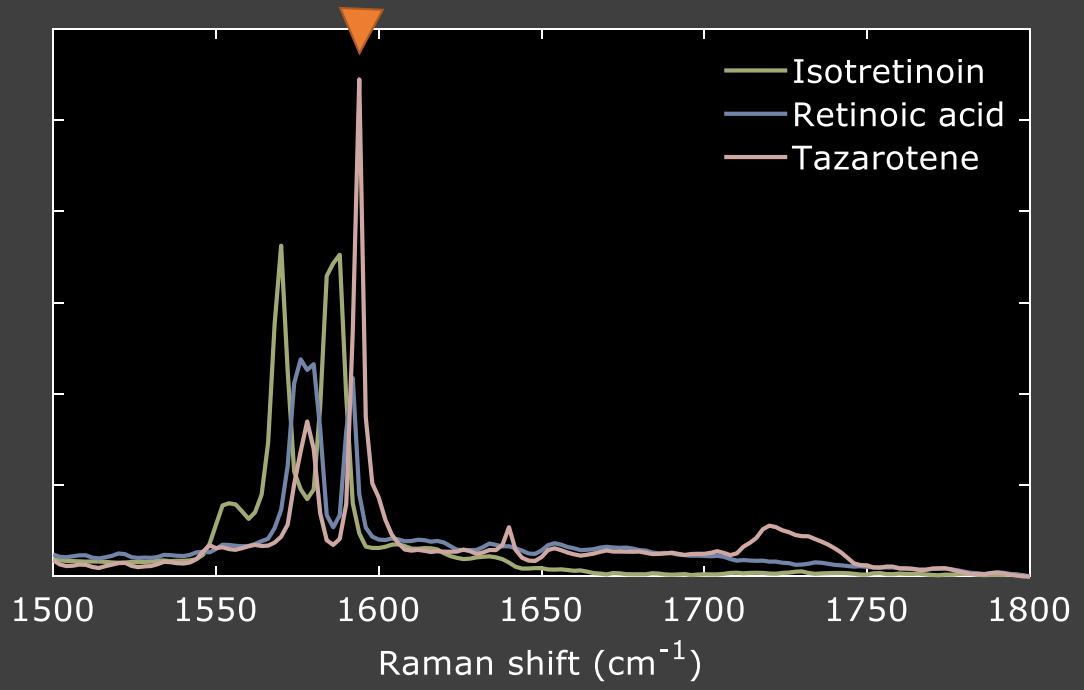
Retinoids imaged using fingerprint vibrational bands. (A) Isotretinoin (cis-13-retinoic acid, yellow, 1568 cm^{-1}), retinoic acid (blue , 1582 cm^{-1}), tazarotene (red , 1594 cm^{-1}) dissolved in DCM and deposited as films within a single 20X FOV. (B) Average spectra extracted from SRS imaging stack for each component.

Pence, J.P. et al. Multi-window sparse spectral sampling stimulated Raman scattering microscopy. *Biomedical Optics Express* (2021)

SRS for Evaluation of Topical BE

- Tazarotene
- 3rd Generation Retinoid for the treatment of numerous skin conditions including **acne vulgaris and psoriasis**
- SRS tune to wavenumber ~1594 cm^{-1} , attributed to delocalized vinyl stretch





Experiment Methods

Treatment groups:

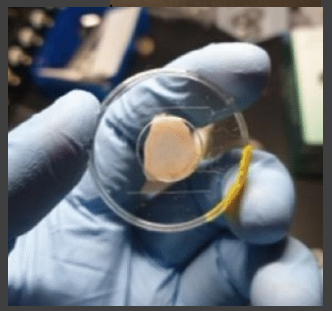
- **Reference product (R1)**: Tazorac® Almirall, LLC;
  Dosage form: cream;
- **Generic product**: Taro Pharmaceuticals U.S.A., Inc;
  Dosage form: cream;
- **Reference product (R2)**: Same as reference product
  (Provides a measure of inter-experimental variability)
- **Alternative formulation**: Tazorac®;
  Dosage form: gel;
- **Alternative formulation (PEG solution):** Taz in PEG-200 (0.1 % w/w)

Experiment Methods

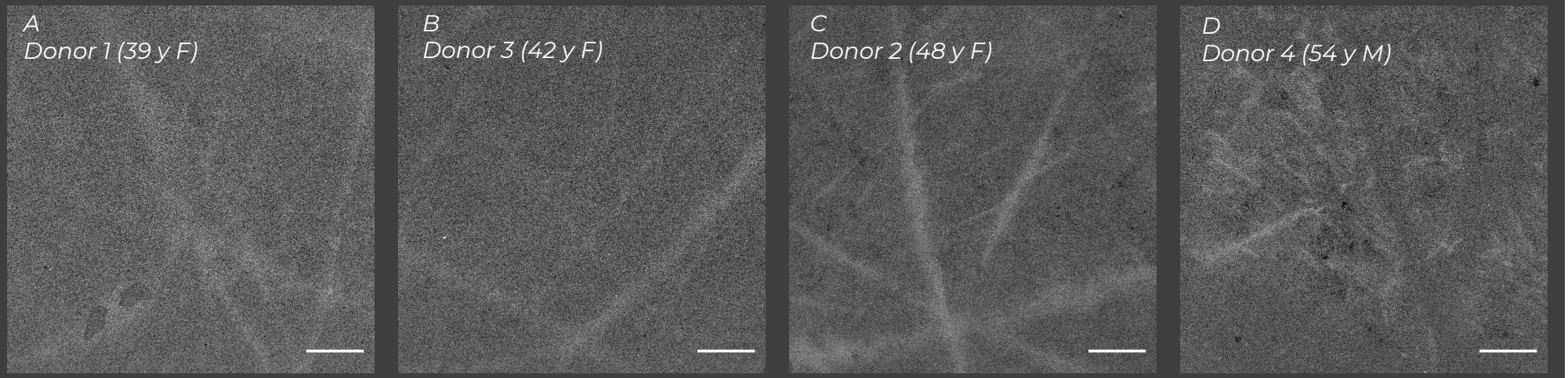
API & drug concentration in formulations	Tazarotene 0.1% (w/w)
Skin donors	**Donor 1**: 39 years old; Female; Abdomen **Donor 2**: 48 years old; Female; Abdomen **Donor 3**: 42 years old; Female; Abdomen **Donor 4**: 54 years old; Male; Abdomen
Skin preparation	Full-thickness – Subcutaneous fat trimmed to allow SRS signal detection in the forward direction
Source of skin procured	Massachusetts General Hospital; Cooperative Human Tissue Network
Number of skin samples & regions of interest (ROIs)	4 samples per formulation; 4 ROIs per skin sample (1024 x 1024 pixel)
Depth stack	Step size: 8 µm; number of slices: 9; final depth at 64 µm
Time per cycle (8 ROIs – pair of formulations)	~25 min
Study duration	~6.5 hours of imaging (15 cycles)
Skin uptake conditions	Finite dose (5 µL); Occlusive; 32°C

Experiment Methods

1. The frozen skin was thawed at room temperature for ~5 min.
2. Rinsed with phosphate buffered saline (PBS) and then patted dry with a cotton bud.
3. Applied 5µL of the drug product onto the skin
4. Rubbed the formulation on the surface of skin using plunger tip
5. Flipped over and placed in the glass bottom petri-dish
6. Found a field of view
7. Imaged the sample by SRS tuned to CH_2 band (2870 cm^{-1}) of the lipid structure of skin
8. Imaged the sample by SRS tuned to delocalized C=C stretching vibration (1590 cm^{-1}) of the Tazarotene backbone
9. Repeat the imaging cycles until ~6.5 hours



Estimation of Epidermal Thickness

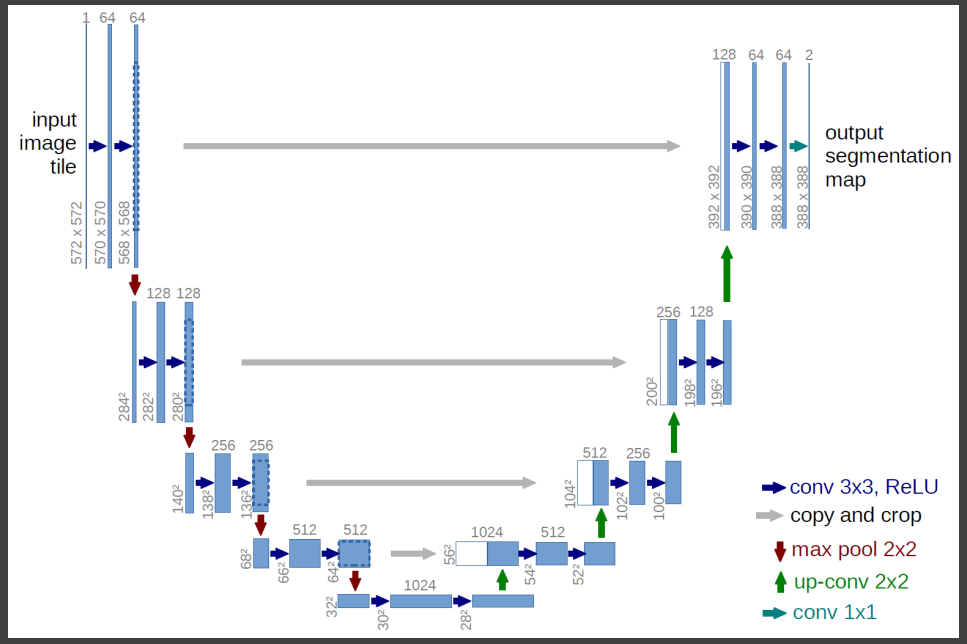


Depth stack of untreated (blank) human skin, obtained by SRS microscopy *ex vivo*. Using a step size of 1 µm to a final depth of 72 µm. (A-D) Gray-scale images of skin samples by tuning to the skin lipid CH_2 stretching frequency (2,870 cm^{-1}). Scale bar: 100 µm. Apparent *stratum corneum* thickness estimated as 18.7 ± 3.2, µm, 17 ± 2.6 µm, 16 ± 1 µm, 20 ± 1 µm for Donors 1, 2, 3 and 4, respectively.

Upper skin layers:
0- 16 µm

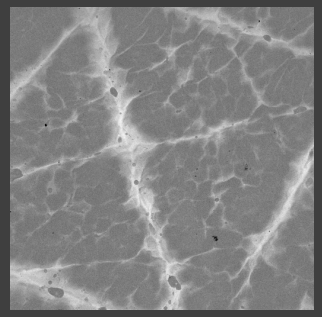
Deep Learning for Segmentation

- Use a U-Net Convolutional Neural Network (CNN) along with a server-based python pipeline

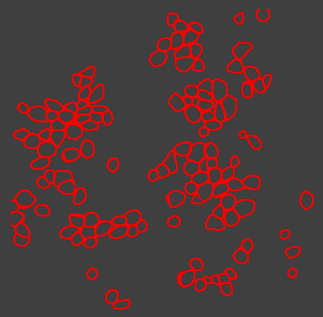


Schematic of the U-Net training and validation process

Original training image set

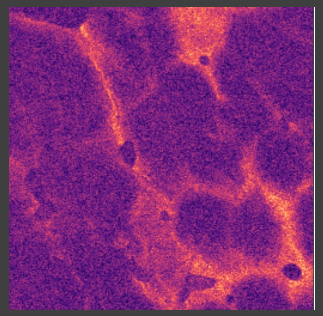


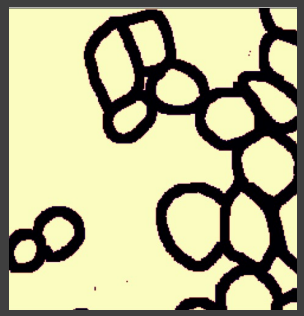
Example image



Hand-drawn annotations

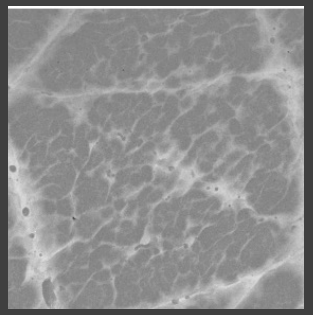
Data augmentation



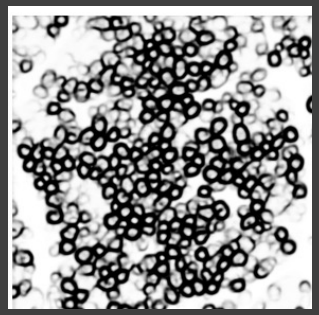


U-Net training on Image/ Annotations pairs

Validation on test images

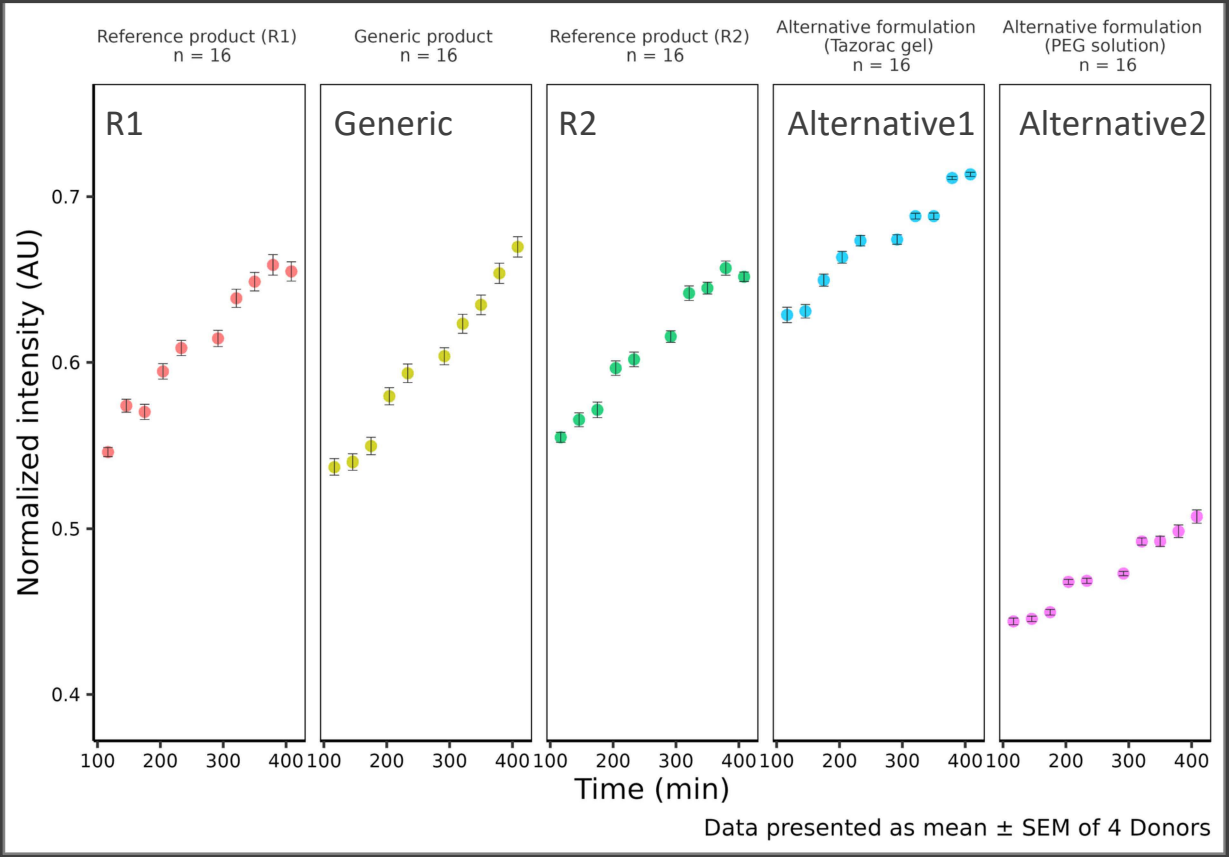


Example test image



Machine generated outcome

Mean concentration profiles for all donors

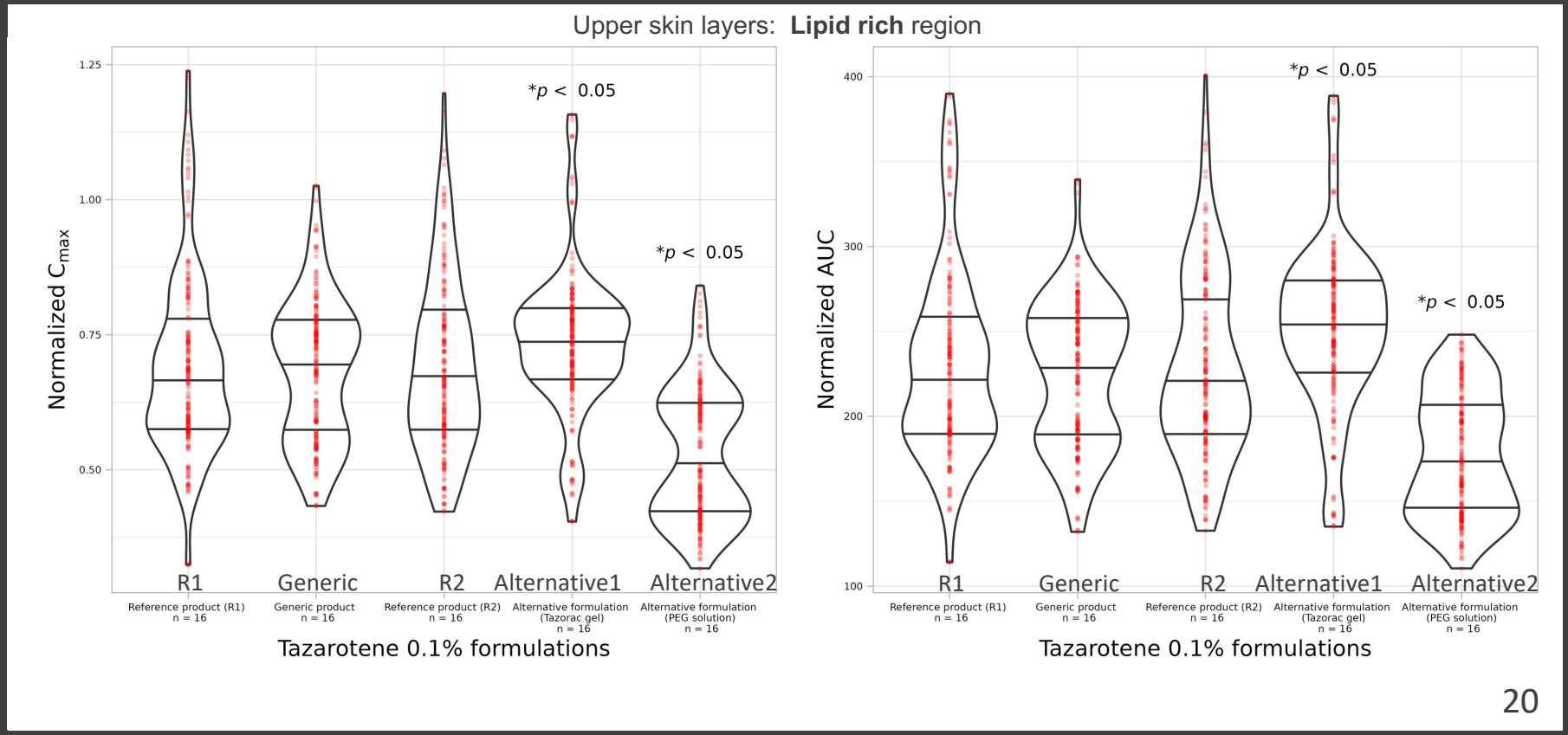


- Upper skin layers
  Lipid rich region

Concentration profiles of Tazarotene (AU) across the skin estimated by SRS microscopy for various formulations following finite dose application *ex vivo*. Reference product (R1): Tazorac® cream; Generic product: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution. Upper skin layers: 0-16µm.

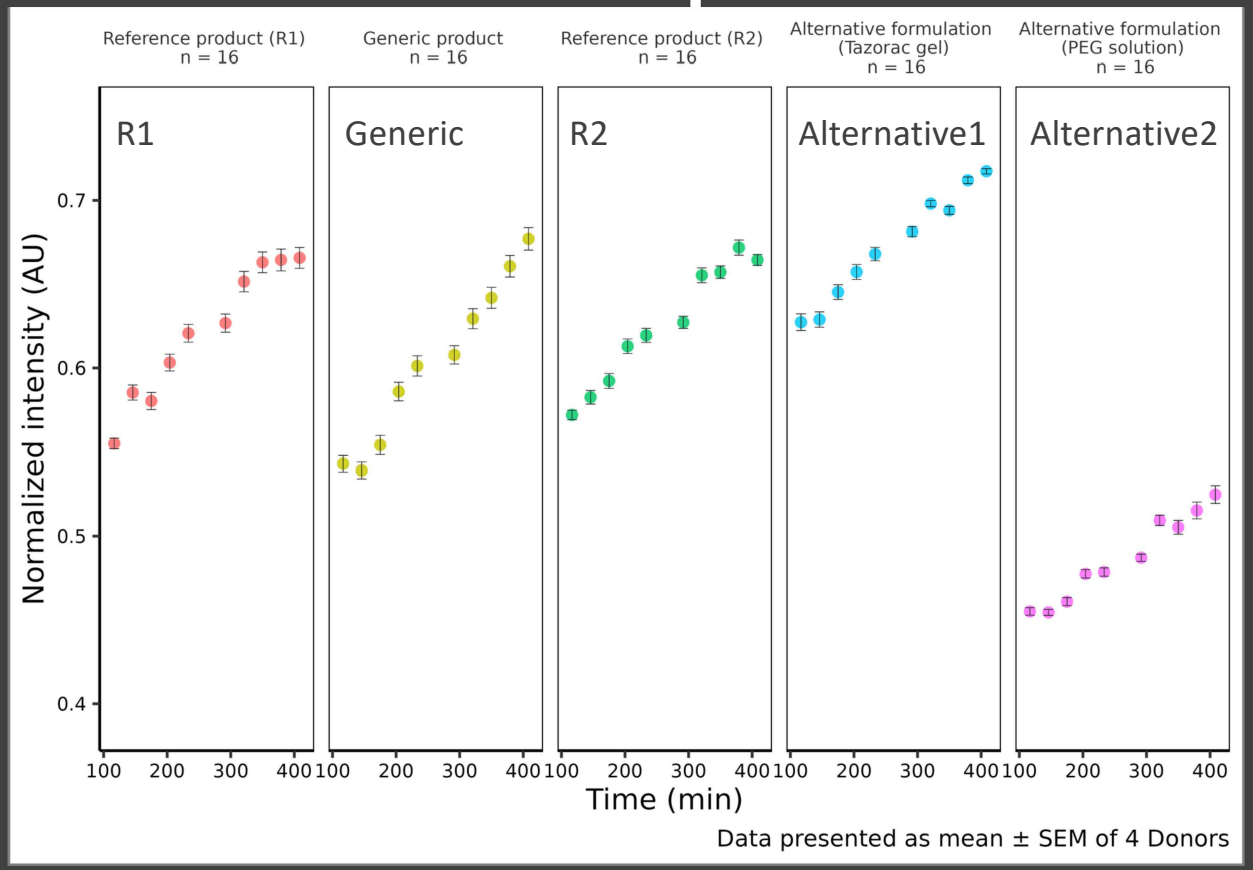
(Mean±SEM of 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate)

Pharmacokinetic Parameters: Lipid-Rich



Estimated cutaneous pharmacokinetic parameters following finite dose application of Tazarotene-containing formulations to human skin *ex vivo*. Reference product (R1): Tazorac® cream; Generic: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution. Statistical significance determined by Kruskal-Wallis Test and Dunn test for multiple pairwise comparisons. The family-wise error rate was controlled using Dunn's Bonferroni adjustment. Upper skin layers: 0-16µm.

Mean concentration profiles for all donors



- Upper skin layers
  Lipid poor region

Concentration profiles of Tazarotene (AU) across the skin estimated by SRS microscopy for various formulations following finite dose application *ex vivo*. Reference product (R1): Tazorac® cream; Generic product: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution. Upper skin layers: 0-16µm.

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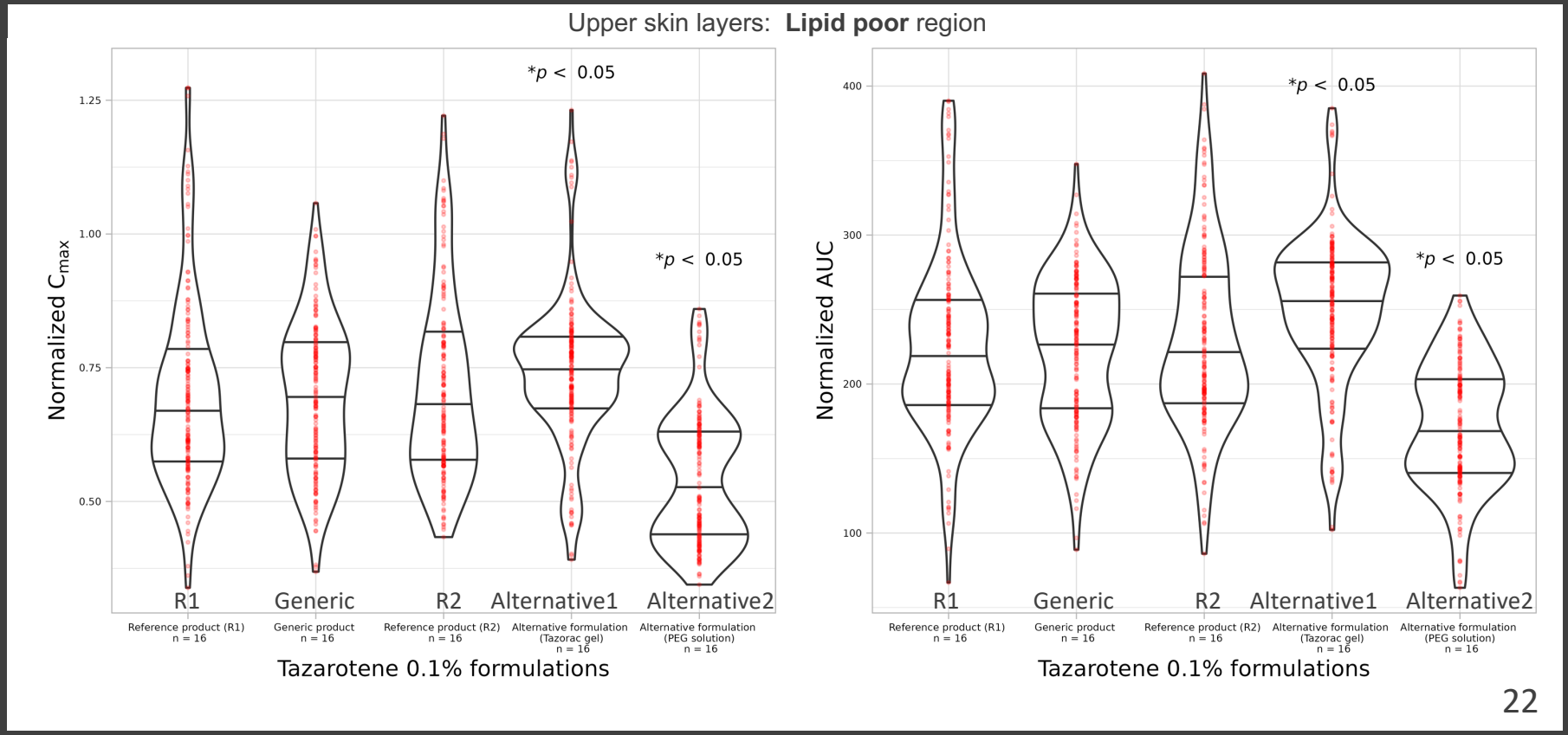
Pharmacokinetic Parameters: Lipid-Poor



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Estimated cutaneous pharmacokinetic parameters following finite dose application of Tazarotene-containing formulations to human skin *ex vivo*. Reference product (R1): Tazorac® cream; Generic: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution. Statistical significance determined by Kruskal-Wallis Test and Dunn test for multiple pairwise comparisons. The family-wise error rate was controlled using Dunn's Bonferroni adjustment. Upper skin layers: 0-16µm.

Sample data for BE analysis

Estimated C_{max} and AUC values for **Reference product (R1): Tazorac® cream.** Data from SC intercellular uptake values for all ROIs and all experiments for each donor (n=16)

Donor reference	Mean of C_{max} values	Mean of AUC values	Mean of log-transformed C_{max} values	Mean of log-transformed AUC values
Abd 39 F	0.83	15526.02	-0.21	9.62
Abd 42y F	0.60	11650.93	-0.52	9.33
Abd 48y F	0.75	14203.60	-0.35	9.49
Abd 54y M	0.64	13004.83	-0.45	9.47
Mean(x)	0.70	13596.34	-0.38	9. 48
SEM	0.01	103.77	0.01	0.01
90% CI [LL, UL]	[0.69, 0.71]	[13424.83, 13767.86]	[-0.40, -0.37]	[9.47, 9.49]

Estimated C_{max} and AUC values for **Generic product: Taro cream** Data from SC intercellular uptake values for all ROIs and all experiments for each donor (n=16)

Donor reference	Mean of C_{max} values	Mean of AUC values	Mean of log-transformed C_{max} values	Mean of log-transformed AUC values
Abd 39y F	0.73	13951.92	-0.33	9.50
Abd 42y F	0.54	10316.19	-0.62	9.23
Abd 48y F	0.76	14319.14	-0.28	9.55
Abd 54y M	0.74	14907.85	-0.31	9.61
Mean(x)	0.69	13373.78	-0.39	9.47
SEM	0.01	130.09	0.01	0.17
90% CI [LL, UL]	[0.68, 0.70]	[13158.75, 13588.80]	[-0.41, 0.37]	[9.27, 9.67]

Difference of the means of the log-transformed PK metrics (Reference product (R1) – Generic product)

Donor number	Difference of the means of the log-transformed C_{max} values (Reference product (R1) – Generic product)	Difference of the means of the log-transformed AUC values (Reference product (R1) – Generic product)
Abd 39y F	0.12	0.12
Abd 42y F	0.10	0.10
Abd 48y F	-0.07	-0.06
Abd 54y M	-0.14	-0.14
Mean(x)	0.03	0.01
SEM	0.06	0.06
90% CI [LL, UL]	[-0.15, 0.15]	[-0.14, 0.15]

Mean values of both metrics are found between the ln(0.8) and ln(1.25) limits (i.e., -0.22, 0.22), suggesting bioequivalence

Reference product (R1): Tazorac® cream; Generic product: Taro Pharmaceuticals U.S.A (cream)., Inc

Data from SC intercellular uptake values

Sample data for BE analysis

Estimated C_{max} and AUC values for **Reference product (R1): Tazorac® cream.** Data from SC intercellular uptake values for all ROIs and all experiments for each donor (n=16)

Donor reference	Mean of C_{max} values	Mean of AUC values	Mean of log-transformed C_{max} values	Mean of log-transformed AUC values
Abd 39 F	0.83	15526.02	-0.21	9.62
Abd 42y F	0.60	11650.93	-0.52	9.33
Abd 48y F	0.75	14203.60	-0.35	9.49
Abd 54y M	0.64	13004.83	-0.45	9.47
Mean(x)	0.70	13596.34	-0.38	9. 48
SEM	0.01	103.77	0.01	0.01
90% CI [LL, UL]	[0.69, 0.71]	[13424.83, 13767.86]	[-0.40, -0.37]	[9.47, 9.49]

Estimated C_{max} and AUC values for **Alternative formulation: PEG-200 solution.** Data from SC intercellular uptake values for all ROIs and all experiments for each donor (n=16)

Donor reference	Mean of C_{max} values	Mean of AUC values	Mean of log-transformed C_{max} values	Mean of log-transformed AUC values
Abd 39y F	0.64	10568.73	-0.47	9.20
Abd 42y F	0.51	10268.61	-0.69	9.19
Abd 48y F	0.50	10032.91	-0.71	9.20
Abd 54y M	0.52	10100.11	-0.67	9.20
Mean(x)	0.54	10242.59	-0.63	9.20
SEM	0.01	14.97	0.01	0.0002
90% CI [LL, UL]	[0.54 0.55]	[10217.84, 10267.34]	[-0.65,-0.62]	[9.1986 9.1994]

Difference of the means of the log-transformed PK metrics (Reference product (R1) – Alternative formulation (PEG-200)

Donor number	Difference of the means of the log-transformed C_{max} values (Reference product (R1) – Alternative formulation (PEG-200))	Difference of the means of the log-transformed AUC values (Reference product (R1) – Alternative formulation (PEG-200))
Abd 39y F	0.26	0.42
Abd 42y F	0.17	0.14
Abd 48y F	0.36	0.29
Abd 54y M	0.22	0.27
Mean(x)	0.25	0.28
SEM	0.04	0.06
90% CI [LL, UL]	[0.16, 0.35]	[0.15, 0.42]

Reference product (R1): Tazorac® cream; Alternative formulation (PEG-200)

Mean values of both metrics are found outside the ln(0.8) and ln(1.25) limits (i.e., -0.22, 0.22), suggesting the two products are not bioequivalent

Data from SC intercellular uptake values

Conclusions and Next Steps

- SRS imaging is capable of quantifying the permeation of APIs within the epidermis
- SRS images can be processed to extract concentration-time profiles and the PK parameters T_{max}, C_{max}, and AUC
- Preliminary analysis suggests that SRS can assess bioavailability and bioequivalence of APIs in different topical formulations
- Upcoming Sparse Spectral Sampling SRS (S^4RS) methods will enable SRS bioequivalence experiments in a wide range of topical products

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