Confocal Raman Spectroscopic Assessment of the Topical Bioavailability of Metronidazole: Comparison of laboratory-made formulations and approved drug products

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Background

- Unambiguous Raman spectroscopic analysis (RSA) of a chemical in the skin - (free of background noise and signal attenuation) – has been achieved, and real-time confocal RSA following topical application of a formulation can provide a measure of the chemical's "input kinetics" into the viable epidermis ex vivo [1].
- The present work aims to build on this foundation and apply RSA to the assessment of topical bioavailability.

Objectives

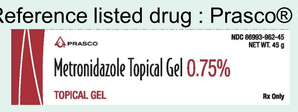
- To demonstrate that RSA can characterise the epidermal bioavailability of a topically applied drug and correctly distinguish formulations that are expected to be bioequivalent from those that are not.

Methods

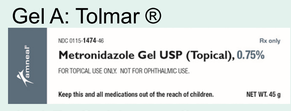
Formulations

Fully saturated metronidazole (MTZ) solutions in 90:10 and 30:70 v/v water/propylene glycol (PG), and 0.75% w/w MTZ gels

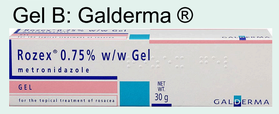
Reference listed drug : Prasco® Gel



Gel A: Tolmar ®

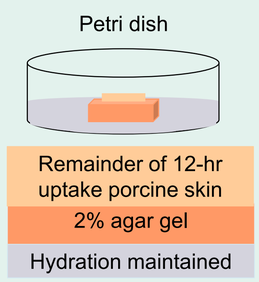


Gel B: Galderma ®



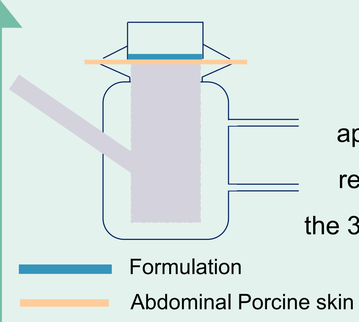
***Ex vivo* Drug Clearance**

Drug clearance was assessed by RSA in sub-samples collected 2 and 4 hr later (i.e., at 14 and 16 hr after the initial application) placed on the shown setup.



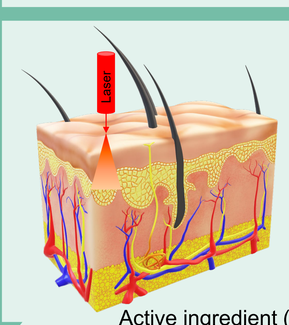
***Ex vivo* Drug Uptake**

Assessed after 6- and 12-hr applications (without occlusion) in 4 replicate skin samples from each of the 3 animals. Uptake was determined by RSA in sub-sections samples.



Raman Spectroscopy

RSA signals were detected as a function of depth and normalized to account for signal attenuation with depth as before [1].



Results – Active ingredient (MTZ)

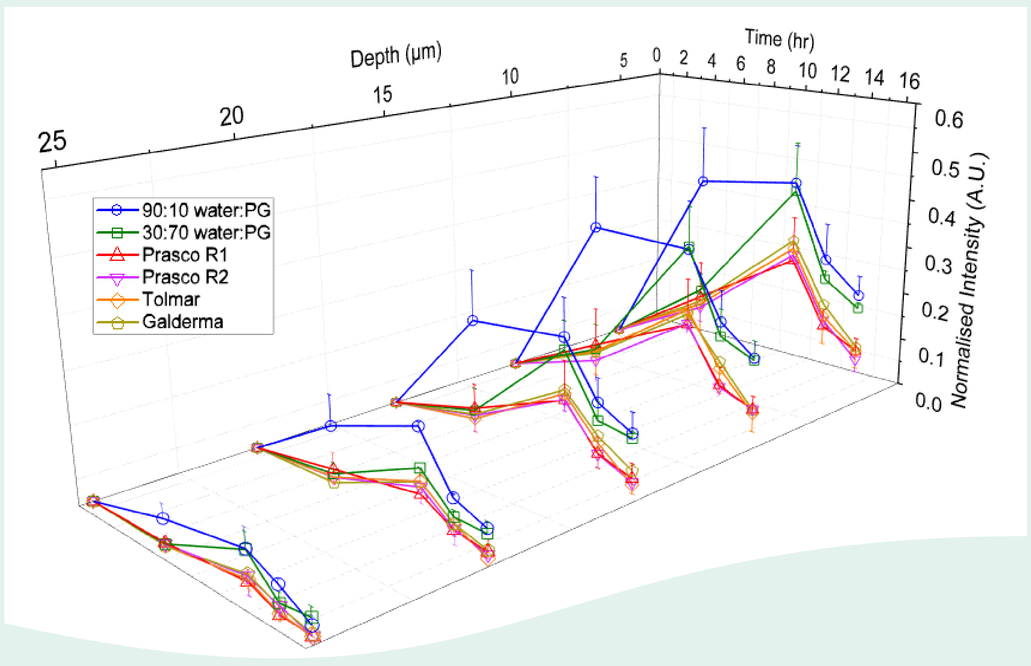


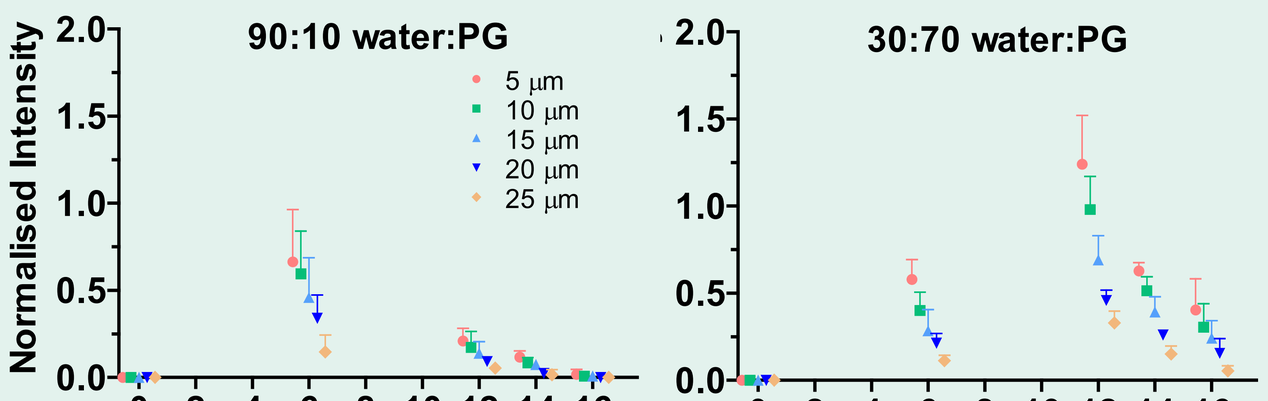
Figure 1: *Normalised MTZ Raman signal intensities, as functions of depth and time (6- and 12-hr uptake, and 2 and 4 hr of clearance after 12-hr uptake, plotted at 14 and 16 hr), after application of three gels and two laboratory-made (solution) formulations. Experiments with the reference gel were duplicated to provide an internal control. Mean ± SD (n = 12)*

- The Raman-deduced disposition of MTZ from the gels appeared to be consistent as a function of time and depth into the skin – both for the within-gel comparison of the reference product, and comparison across the three gel products (Figure 1).
- In contrast, the composition of the two solutions clearly influenced the skin pharmacokinetics of MTZ (Figures 1 and 2).

Results – Inactive ingredient (PG)

Figure 2*: The normalised PG spectroscopic signals at the designated depths following application of the solution formulations plotted at 6- and 12-hr uptake, and at 14 and 16 hr for 2 and 4 hr of clearance after 12-hr uptake; PG signals were not detectable post-application of the gels. Mean ± SD (n = 12)*

- A possible explanation for the observed differences in MTZ disposition when applied as solutions with different water/PG ratios is suggested by the results in Figure 2 - the maximum amount of PG in the skin is smaller for the formulation with less PG, which correlates with smaller uptake of MTZ.
- Specifically, the rapid water evaporation/metamorphosis of the 90:10 water/PG MTZ solution results in the visual appearance of drug crystals on the skin surface (i.e., precipitation of MTZ), which led to the observed drug bioavailability.



Conclusions

It has been demonstrated that RSA can characterise, at least in part, the epidermal pharmacokinetic profile of a topically applied drug. It is now possible to undertake further analysis of the observations presented – including the application of spectral unmixing methods to improve the quality and precision of the confocal Raman data - to extract appropriate metrics to quantify the topical bioavailability of MTZ and to determine bioequivalence (or not) between the products assessed.

Reference: [1] P Zarmpi et al., AAPS PharmSci 360 annual meeting, USA, 2021: https://www.eventscribe.net/2021/PharmSci360/

Acknowledgements

This research is supported by the U.S. Department of Health & Human Services, Food & Drug Administration (1-U01-FD006533 and 1-U01-FD004947). The views expressed in this poster do not reflect the official policies of the U.S. FDA or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the U.S. Government.