Raman-assessed microstructure, dermatopharmacokinetics & bioequivalence of doxepin topical products

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Introduction

The development of robust methods for quantification of cutaneous pharmacokinetics of an active pharmaceutical ingredient (API) can be used as an alternative approach to clinical trials to assess topical bioavailability and bioequivalence. Raman-based methods offer a potential non-invasive solution, provided that the technique is applicable to a wide range of topical drugs and the signal from the API is not impacted by the presence of the formulation components. In this work, confocal Raman spectroscopy was used to assess the bioequivalence of commercial doxepin (DOX) creams (reference listed drug (RLD) and generic) vs. one, purposefully laboratory-made non-equivalent solution formulation. Additionally, stimulated Raman scattering (SRS) was used to visualise the microstructure of the commercial products themselves, and to study metamorphosis.

Formulation microstructure visualized with SRS microscopy

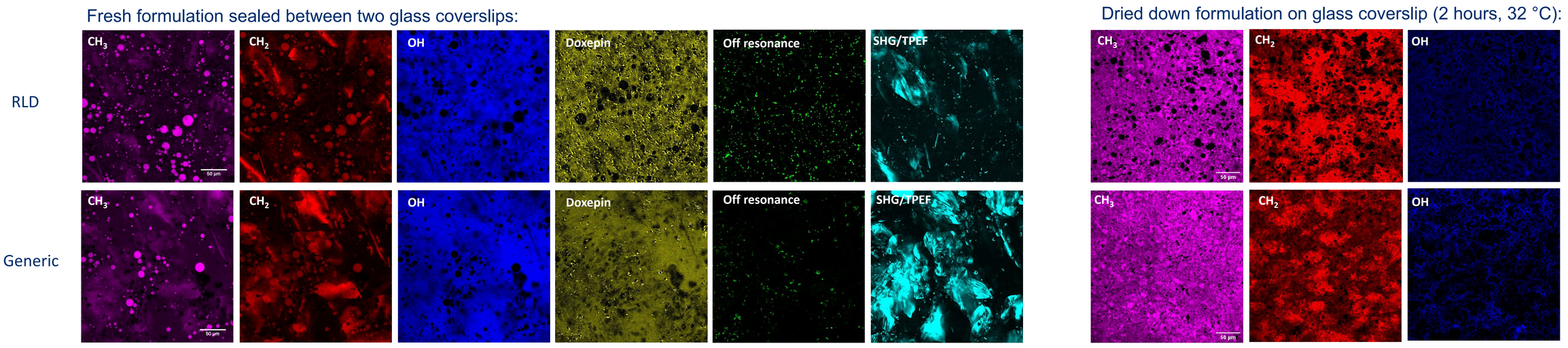

Figure 1: SRS is an effective method to reveal the microstructure & ingredient co-localisation. Bright off resonance signals were attributed to titanium oxide (TiO_2) particles. These primarily impact the DOX signal in the fingerprint region, which has much poorer signal to noise than the high wavenumber region. Upon drying, the TiO_2 became sufficiently concentrated to mask the DOX signal (images not shown).

Dermatopharmacokinetics & bioequivalence

The formulations were applied on porcine skin for 6 or 12 h (uptake phase) followed by 2 and 4h clearance phases to generate typical absorption – elimination profiles.
Due to the overlap of the DOX signal at 1605 cm^{-1} and dodecanol signal at 1448 cm^{-1} with those from endogenous skin species, an unmixing strategy was devised, based on the relative ratios of the two aforementioned signals to phenylalanine in untreated skin. These two ratios in untreated skin were constant with depth and across the three different pigs:

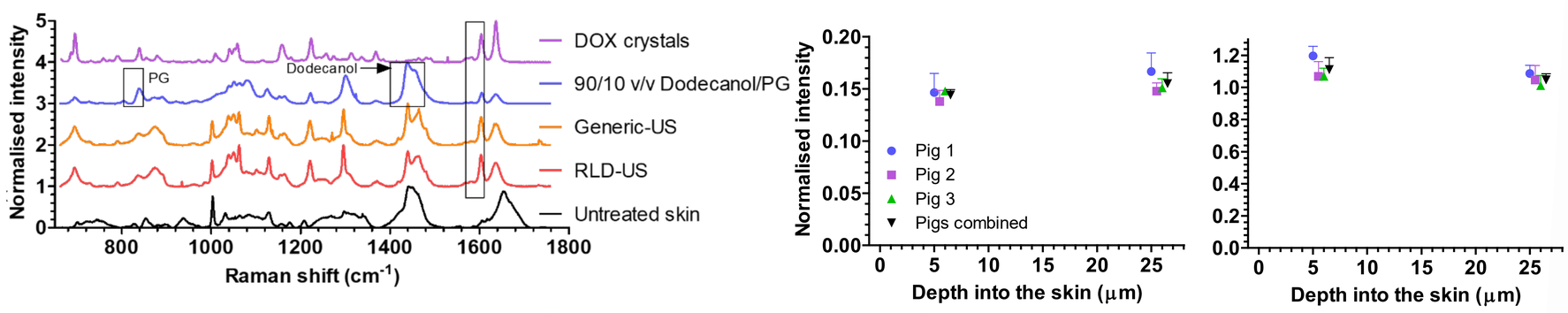

Figure 2: Signal of endogenous species at 1605 cm^{-1}/phenylalanine (left) and at 1448 cm^{-1}/phenylalanine (right) ratios as function of depth for untreated skin from 3 different pigs (n = 3 per pig) and their average. Data are mean ± SD; some data have been shifted on the x-axis for visualisation.

Knowing the intensity of phenylalanine in treated skin and the two ratios in untreated skin, it is possible to calculate the intensity of endogenous species at 1605 and 1448 cm^{-1} in treated skin and subtract them from the recorded maximum intensity at these two frequencies, to deduce the intensities of unmixed signals of interest:

$$I_{dox,treated\ \ skin,1605cm^{-1}} = I_{total,treated\ \ skin,1605cm^{-1}} - \left(\frac{I_{end.,untreated\ \ skin,1605cm^{-1}}}{I_{phenylalanine\ ,untreated\ \ skin,1003cm^{-1}}}\ x\ I_{phenylalanine\ ,treated\ \ skin,1003cm^{-1}}\right)$$

Nominal depth into skin (µm)	Formulation			
	RLD-US-R1	RLD-US-R2	Generic-US	90/10 v/v Dodecanol/PG
5	1.14 ± 0.29	1.15 ± 0.03	1.17 ± 0.08	4.15 ± 1.48
25	0.42 ± 0.21	0.46 ± 0.18	0.42 ± 0.09	1.68 ± 0.91

Table 1: Area under the concentration vs time curve (AUCt) values post-application at different skin depths. Data are the mean value of the AUCt calculated based on the average normalised intensities at each time and depth (n = 3) in each pig (n = 3 pigs) ± SD.

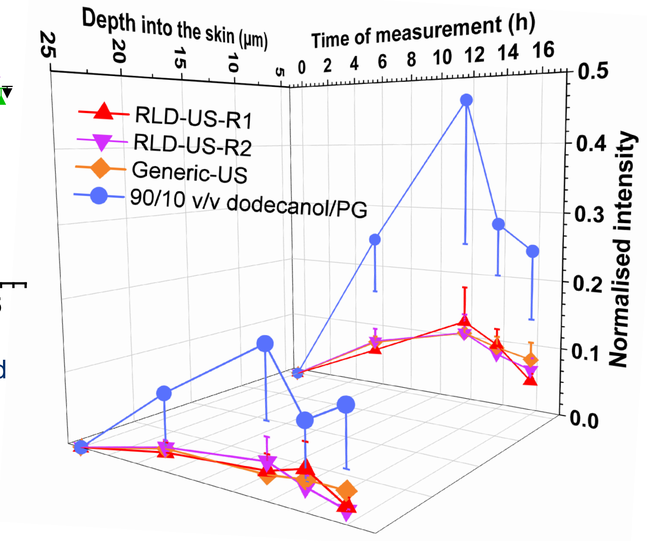

Figure 3: DOX maximum Raman intensities, normalised by phenylalanine, as functions of time of measurement and depth into the skin for the creams and solution studied (n=3 per pig). The RLD was run in duplicate to evaluate reproducibility of the method, R1 and R2). Data are mean ± SD.

Conclusions

- SRS can facilitate visualization of formulation microstructure and ingredient co-localisation, including changes during metamorphosis. However, highly absorbing particles such as TiO_2 cause strong parasitic contributions, so advanced SRS signal detection approaches are required to isolate and remove these contributions when studying formulations that contain them.
- It was demonstrated that the dermatopharmacokinetic profiles of the commercial products were similar, while that of the laboratory-made non-equivalent solution was not. In addition to the API, it was also possible to spectroscopically track the disposition of two functional excipients, providing further insight into the overall product performance.

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