



Quantitative *in vivo* monitoring of topically applied active pharmaceutical ingredients and penetration enhancers

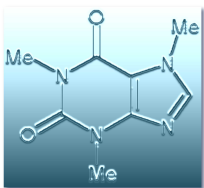


I. INTRODUCTION AND OBJECTIVES

Product formulators in companies active in skin pharmacology work to deliver valuable topically applied active ingredients at the right dose and rate to generate the highest efficacy. It is critical to seek balance among the various requirements, including cost. Typically, the most costly ingredient in a commercially viable formulation is the active pharmaceutical ingredient (API). Immediate cost savings are evident when the overall API content of the pharmaceutical product can be lowered, while its efficacy is maintained. It is vital to apply the most effective measurement technology available to achieve this goal.

Assessment of the formulated product under realistic conditions is usually done either *in vitro* or by invasive sampling of the skin, followed by (wet) chemical analysis. Such tests are often laborious and costly. To solve this problem, River Diagnostics has developed the Model 3510 Skin Composition analyzer. It is fully optimized to measure the spatially resolved chemical composition of the skin *in vivo* in seconds. The method's non-invasiveness allows repeated measurements of the same skin area, which enables monitoring of changes in the skin following a skin treatment. Such changes often include the penetration of components from the formulation into the skin. Therefore, the Model 3510 Skin Composition analyzer is excellently suited as a screening and optimization tool for product development.

In this note, we will highlight this approach by presenting *in vivo* quantitative data on human skin for penetration of the active ingredient caffeine. We will also demonstrate actual penetration enhancement by comparing the results from *in vivo* kinetic monitoring of caffeine penetration in the presence or absence of ethanol.



II. EXPERIMENTAL

Solutions of 1.8 mass % of caffeine in water and in ethanol:water (1:2) were prepared. Volumes of 0.5 ml of the solution were pipetted into a Hill top chamber® and applied to the volar aspect of the lower forearm for 1 or 2 hours. Four volunteers participated in this study and five concentration depth profiles per volunteer were recorded across the stratum corneum.

Quantitative measurements of caffeine content were made for each sampled depth. The caffeine concentration is expressed in mmole of caffeine per gram of keratin. Quantification was based on fitting of the *in vivo* Raman spectra, using a linear superposition of model spectra of caffeine and the main skin components and on the relative Raman intensity response due to caffeine and keratin.

Figure 1 shows a typical Raman spectrum of untreated skin and skin treated with the caffeine in water solution. Raman signals due to the caffeine molecule are labeled and can be observed readily. River Diagnostic's user-friendly data analysis software SkinTools is used to reliably quantify the caffeine content.

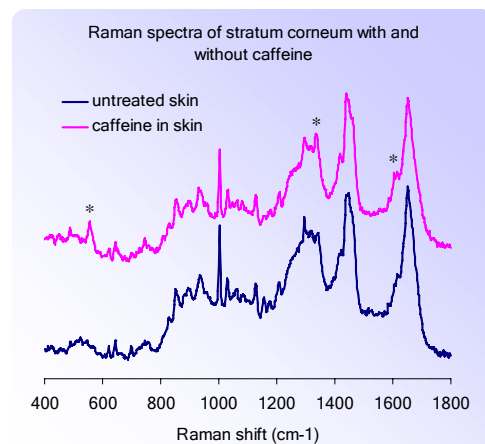


Figure 1. Typical Raman spectra (10 s acquisition time) of the stratum corneum for untreated skin (blue) and skin treated with a caffeine in water solution (purple).



III. RESULTS AND DISCUSSION

The first experiment compares the uptake of caffeine from the ethanol-water solution for different volunteers and two application times. Figure 2 shows the averaged quantitative concentration depth profiles for three volunteers after one hour of application and for one volunteer after two hours of application of the caffeine solution.

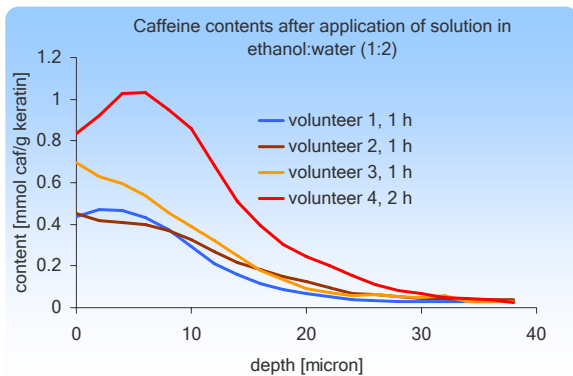


Figure 2. Concentration depth profiles (average of 5 repeats) of caffeine (dissolved in ethanol-water), for three volunteers (1 hour of application) and for one volunteer (2 hours of application).

On the volar aspect of the forearm, the stratum corneum thickness typically lies in the range 12-25 micrometer. In this experiment we sampled a depth range of about 40 micrometer, ensuring coverage of the whole stratum corneum. For the three volunteers, the content of caffeine in the stratum corneum is similar after one hour of application. At a depth of about twenty micrometer, for example, the caffeine content is around 0.1 mmole/gram, corresponding to about 15 mg per gram of keratin. The caffeine content for an application time of 2 hours (volunteer 4), however, is at least two times higher throughout the stratum corneum.

A second experiment was performed (volunteer 2), comparing the caffeine uptake for the solvents water and

ethanol-water. The averaged content depth profiles are shown in Figure 3.

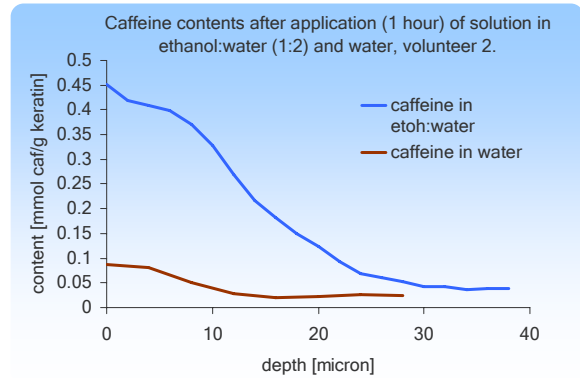


Figure 3. Comparison of concentration depth profiles of caffeine (dissolved in ethanol-water or water), for 1 hour of application.

The caffeine content after application of caffeine in a water solution is significantly lower than for the ethanol-water solution. For the former case, a content of about 0.025 mmole per gram of keratin (about 4 mg/gram) is observed, whereas about four times that much was found for the latter case. This observation illustrates the expected penetration enhancing effect of ethanol.

IV. CONCLUSION

Quantitative concentration depth profiles for the active pharmaceutical ingredient caffeine were measured. For one hour of application of 1.8 mass % caffeine in ethanol-water (1:2), typically 0.1 mmole of caffeine per gram of keratin was observed at a depth of 20 micrometer in the stratum corneum. Ethanol clearly exhibits penetration enhancing properties; without its presence at least three times less caffeine was present in the stratum corneum.

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