Title: Study of in vitro percutaneous permeation of caffeine through the

stratum corneum and hair follicle.

Student: Yasmin Charik Hassoun

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Supervisor/s: Dra. Maria Victòria Sanz Nebot

Departament of Chemical Engineering and Analytical Chemistry

Dra. Maria Luisa Coderch Negra

IQAC - CSIC

In vitro percutaneous absorption assay is normally used to determine drug permeation through the different skin layers. However, the hair follicle is considered as well as an interesting pathway for a drug penetration. In this work, new methodology called Differential Stripping will be optimized in order to study the follicle penetration of caffeine.

First, an analytical methodology has been optimized and validated to determine the caffeine content using a previously established HPLC-DAD method.

A caffeine solution was prepared at 1% concentration in ethanol/water (1:1) and it applied on porcine skin surface to perform the permeation study. The skin biopsies were placed on Franz vertical diffusion cells. After the exposure time, the different layers of the skin were separated (stratum corneum, epidermis, dermis, hypodermis and receptor fluid). In the case of stratum corneum, two methods were performed: conventional tape stripping and differential stripping.

After the analysis, the results were obtained and interpreted. The average total of caffeine recovered from all components of skin represented an accepted overall recovery of  $102.2 \pm 3.5\%$  in both methodologies. Results obtained for caffeine penetration with stratum corneum conventional tape stripping and differential stripping were compared to evaluate the two mechanisms of drug penetration. Results showed that a  $4.67 \pm 0.77\%$  of caffeine penetrated by following pathway of the hair follicle.