

## SKIN ABSORPTION OF CAFFEINE IN PORCINE EAR SKIN IN VITRO

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*In vitro* skin absorption methods are excellent models for the investigation of compounds diffusion into the skin (penetration into the stratum corneum, epidermis, and dermis) or across the skin to a fluid reservoir (mimicking systemic circulation). The OECD test guideline 428 provides information to conduct *in vitro* skin absorption assays as a screening tool to compare the delivery of active pharmaceutical ingredients into and across the skin from different formulations. The guideline also allows the investigation of skin absorption in different species, including humans. To demonstrate the performance and reliability of the skin permeation protocols and systems used by our group, a skin permeation test was performed using a reference substance according to OECD, caffeine (n = 6). The test was carried out using full-thickness porcine ear skin in a static Franz diffusion cell, through the application of 10  $\mu\text{L}/\text{cm}^2$  doses of the test solution (4 mg/mL of water caffeine solution). After 1, 2, 3, 4, 6, 8, 10, 22, and 24h, 0.5 mL aliquots were removed from the receptor chamber. To complete the skin permeation test, all components of the test system were analyzed by a previously validated LC-MS method: samples were taken from the (i) receptor chamber; (ii) donor chamber, and (iii) the skin surface fractionated into stratum corneum and dermis + epidermis. Through this assay, it was plotted a graphic of the accumulative amount of caffeine absorbed per unit skin area *versus* time. The maximum absorption rate (1262 ng/cm<sup>2</sup>/h) and time to the maximum rate (6 hours) were determined. Furthermore, the amount of caffeine retained in the skin fractions, accumulated in the receptor fluid, and the total recovery were determined. These results showed an excellent correlation with published literature data, demonstrating the performance and reliability of the method and protocols adopted by our scientific team.