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VALIDATION OF AN LC METHOD TO DETERMINE COUMESTROL IN PORCINE EAR SKIN LAYERS FROM NANOEMULSIONS

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*Mestrando – Início: 2009/1

Introduction: Coumestrol (COU) is a naturally occurring plant estrogen found mainly in soybeans – *Glicine max* (Fabaceae). COU has recently received special attention due to its beneficial effects in preventing skin aging, especially as regards antioxidant and estrogenic properties.[1,2] The design of topical nanoemulsions containing COU is under development in our research group due to the low water solubility of this bioflavonoid.

Objective: The present study aims to validate an analytical method to determine COU in porcine ear skin layers from topical nanoemulsions.

Materials and Methods: Nanoemulsions composed of medium chain triglycerides, egg-lecithin, polysorbate 80 water and COU (at 1mg/mL) were obtained by means of a spontaneous emulsification procedure[3]. To estimate the amount of retained COU in skin layers, an LC method was validated according to ICH[4]. The analyses were performed at room temperature on a reversed-phase C18 column using a mobile phase consisting of methanol/water (70:30, v/v) and trifluoracetic acid 0.1% at 0.8 mL min^{-1}. The detection was carried out on a UV detector at 343nm. The linearity was evaluated in the presence of skin layer extracts, within the range of 0.06 – 4.0 µg/mL. The specificity was assessed through the comparison of the COU peak retention time in the presence of skin layer extracts. The precision of the method was assessed considering repeatability and intermediate precision at three concentration levels (0.6, 3.0, and 3.6 µg/mL) and at the LLOQ (estimated as 0.06 µg/mL) on three different days. To determine recovery, epidermis and dermis samples were spiked with a methanol solution of COU. After 1h, to allow COU penetration and solvent evaporation, methanol (5 mL) was used to extract COU from samples. The samples were shaken and maintained in an ultrasound bath for 30min. Samples were then filtered through a 0.45µm pore size membrane filter. Accuracy was calculated as the correlation between both the measured and the theoretical value, and was expressed as the relative error of measurement (%).

Results and Discussion: The method was specific since no interference of formulation and skin components could be observed in the COU retention time at 343 nm. The response for the COU was linear and the calibration equations showed excellent determination coefficients ($r^2 > 0.99$), which proved to be significant for the method ($P < 0.05$). The LLOQ was set at 0.06 µg/mL, indicating a satisfactory sensitivity of the proposed method for retention studies. The precision test showed R.S.D. values of lower than 8.92% in all experiments. The difference between nominal and found concentrations of the standards demonstrated that the assay was accurate for its application. The recovery of COU could be observed at between 87.83% and 100.77%.

Conclusions: The proposed LC method was specific, linear, accurate and precise to determine COU in porcine ear skin layers. Further studies are now in progress in order to evaluate the skin permeation/retention profile of COU in porcine ear skin using Franz diffusion cells.

References
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