TFC038

Liquid chromatographic method for assay of lipoic acid in skin samples, an important tool for *in vitro* and *in vivo* skin delivery studies

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Introduction: Lipoic acid (PM 206.32) is an organosulfur compound, which has a strong sulfhydryl antioxidant capable of modulating the redox status of cells and interacting with other antioxidants. Lipoic acid's antioxidative potential mediates the protective action against skin disorders, including inflammatory response and depigmenting effect. **Objective:** the aim of this studied was to develop and validate a simple and rapid analytical procedure for the measurement of lipoic acid (LA) level in skin samples after *in vitro* permeation studies. **Methods:** Lipoic acid was quantified by RP-HPLC (Shimadzu system – Kyoto, Japan) using two C 18 column in line (10 cm length, 5 µm, 4 mm idLiChrospher; Merck, Darmstadt, Germany), and UV detection at 340 nm. The isocratic mobile phase was phosphate solution pH 2.5: Acetonitrile 50:50 (v/v) and the flow rate was 0.8 mL/min. Accuracy and Precision intra and inter-assay were performed and the both detection and quantification limit was available. The selectivity of the method was confirmed by individual analysis of interfering samples (acetonitrile neat, mobile phase, solution of skin homogenate and adhesives tapes). LA recovery from skin samples was determined by the ratio of the amount of drug extracted from the spiked samples to the amount of drug added (n=3). Exact 40 µL of LA solution (1 mg/mL) was applied on the skin (1.77cm^2) and added 6 mL of acetonitrile. Samples were shaken for 1 min followed by tissue cutter Ultra Turrax for 1 min and maintained in an ultrasound bath during 15 min. The homogenate was centrifugated for 5 min at 2500 rpm and an aliquot of 20 µL of the samples was assayed by HPLC. **Results:** The LA retention time was about 6 min and the assay was linear in the concentration range of 1.0 to 8.0 µg/mL. The linear correlation coefficient for the calibration curve (r^2) was greater than 0.99 and y = 75.112x. The lower limit of detection and quantification were 1µg/mL and both intra and inter assay variabilities were below 10%. The lower limit of detection was the concentration at which signal-to-noise ratio (S/N) is three and lower limit of quantification was determined based on the parameters of the analytical curves, considering r^2= 0.99. They were determined from their respective chromatograms through software. Extraction of LA from skin samples showed recovery higher than 80.0%. The results obtained demonstrated that LA can be quantitatively extracted from porcine skin samples and can be determined by HPLC in a simple, linear, precise, accurate and reproducible assay. **Conclusion**: The method was considered appropriate for the assay of LA in skin samples and can be adopted to assess *in vitro* permeation and retention of LA in the development of topical or transdermal delivery systems.

Key words: lipoic acid, RP-HPLC method, validation, skin samples.

Financial support: Capes, CNPq