Mass balance method validation

The mass balance method for both the washing and extraction procedure was validated using 5 µL of 2 % (w/w) solution of EA in PG. The mass balance method validation experiments were conducted according to procedures described for permeation studies; however, the receptor compartment of the cells was left empty. The formulations were left on the skin for 5h. Subsequently, the skin surface was washed once with 1 mL of methanol, and five times with 1 mL of water:methanol (50:50). For skin extraction, 1 mL of water:methanol (50:50) was used and samples were taken at 5h and 24h. All samples were analysed by the HPLC method.

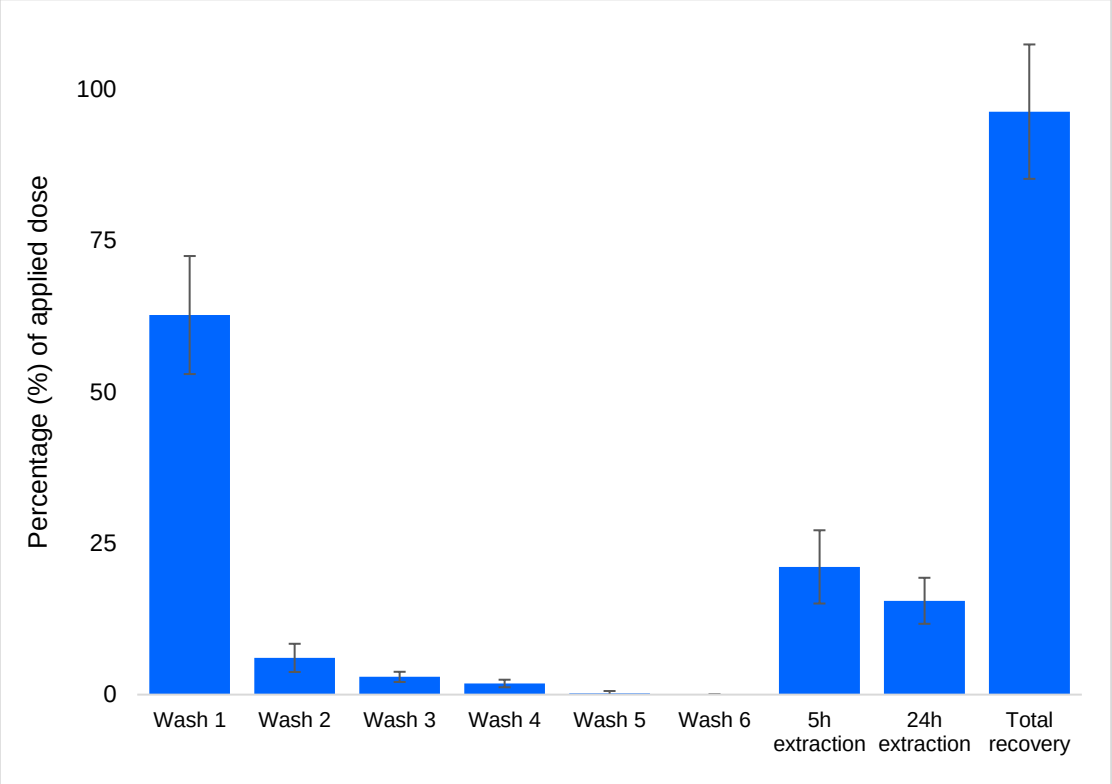

Figure S1 – Percentage recovery of the applied 3-O-ethyl l-ascorbic acid following mass balance procedures (n = 3; mean ± SD).

Most of the applied EA was recovered from the first wash, which showed a ten-fold difference from the second wash. About 0.2 % of the applied dose was recovered from the fifth wash, while no EA was detected from the sixth wash. With regards to extraction of EA from inside the skin, similar percentages were recovered when the skin was extracted for 5h (21.1 %) and 24h (15.5 %, $p > 0.05$). The total recovery of EA was 96.3 %.