

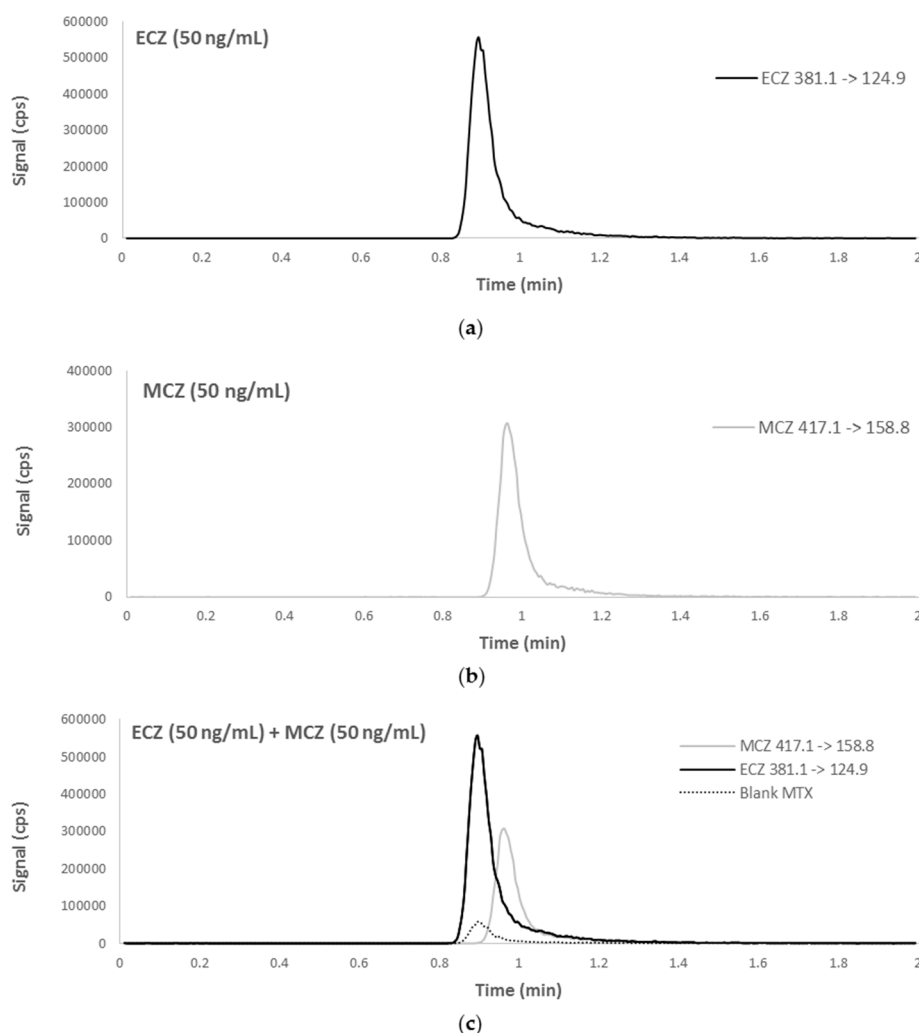
# Supplementary Materials: Cutaneous Biodistribution: A High-Resolution Methodology to Assess Bioequivalence in Topical Skin Delivery

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## Validation of the UHPLC-MS/MS Method

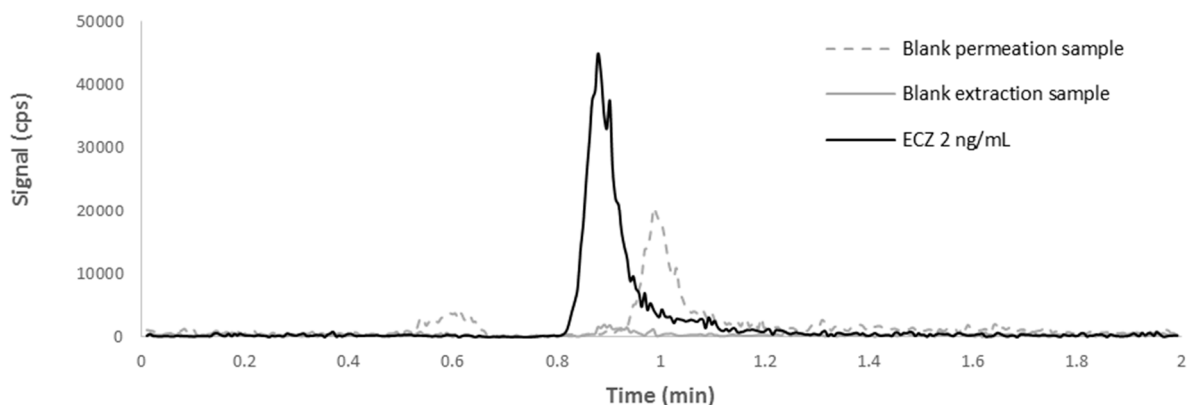
### *Specificity in Skin Matrix*

Figure S1 shows the MRM trace of (a) ECZ standard alone, (b) MCZ standard alone and (c) a mixture of both compounds.



**Figure S1.** Respective MRM traces of (a) ECZ standard alone (b) MCZ standard alone and (c) mixture of both compounds.

Figure S2 presents the chromatogram obtained for an ECZ standard at a concentration of 2 ng/mL, a blank skin extraction sample and a blank permeation sample. No ECZ was found in the permeation sample or in the skin sample. The method was considered as specific for ECZ quantification in skin samples.



**Figure S2.** MRM monitoring for ECZ in skin sample (381.1 → 124.9 transition) chromatogram of ECZ standard (2 ng/mL), blank extraction sample and blank permeation sample.

### Linearity

Calibration curves were constructed by plotting the ratio of ECZ and MCZ peak area against the ratio of ECZ and MCZ nominal concentration. A good linear fit was found in the concentration range of 1–100 ng/mL. Correlation coefficients for all calibration curves were found to be superior to 0.99.

### Limit of detection and limit of quantification

The lowest concentration of ECZ detected (LOD) and lowest limit of ECZ quantified (LOQ) were found to be 0.7 ng/mL and 2.0 ng/mL, respectively.

### Precision and accuracy

Intra- and inter-day precision and accuracy was assessed using 2, 5, 10, 20, 50, 100 ng/mL ECZ solutions in acetonitrile/water (80/20) skin extract. Table S1 shows a summary of intra-day accuracy and precision and Table S2 shows a summary of inter-day accuracy and precision for the method.

**Table 1.** Intra-day precision and accuracy for quantification of ECZ in skin sample (values are given as mean ± SD).

[ECZ] Theo (ng/mL)	Intra-Day 1			Intra-Day 2			Intra-Day 3		
	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)
2	2.4 ± 0.3	11.2	119.6	2.1 ± 0.0	1.2	107.1	2.1 ± 0.1	5.1	105.5
5	5.5 ± 0.2	3.6	111.0	5.5 ± 0.3	4.7	110.7	5.6 ± 0.1	2.0	111.6
10	10.8 ± 0.3	2.5	108.1	11.4 ± 0.3	2.9	114.2	10.9 ± 0.0	0.2	109.5
20	21.5 ± 0.2	2.5	107.6	21.2 ± 0.1	0.4	106.1	20.8 ± 0.3	1.6	103.9
50	53.5 ± 0.6	1.1	107.0	53.9 ± 0.8	1.5	107.9	51.5 ± 1.0	2.0	103.0
100	109.5 ± 0.4	0.4	109.5	107.3 ± 2.0	1.9	107.3	102.8 ± 1.4	1.4	102.8

**Table 2.** Inter-day precision and accuracy for quantification of ECZ in skin sample (values are given as mean ± SD).

[ECZ] Theo (ng/mL)	Intra-Day 1			Intra-Day 2			Intra-Day 3		
	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)	[ECZ] Meas (ng/mL)	RSD (%)	Recovery (%)
2	2.1 ± 0.0	2.0	103.9	2.2 ± 0.2	10.6	112.0	2.3 ± 0.2	9.6	116.3
5	5.4 ± 0.1	2.0	104.1	5.6 ± 0.1	1.4	107.6	5.7 ± 0.1	1.0	109.9
10	10.8 ± 0.3	2.5	108.3	11.1 ± 0.4	3.4	111.0	11.2 ± 0.4	3.4	112.4
20	21.1 ± 0.7	3.3	105.6	21.2 ± 0.3	1.3	105.8	21.2 ± 0.2	0.7	106.2
50	52.6 ± 2.1	4.1	105.3	52.8 ± 0.9	1.6	105.6	53.5 ± 1.0	1.9	107.0
100	106.3 ± 4.4	4.1	106.3	107.0 ± 3.4	3.2	107.0	106.2 ± 3.1	2.9	106.2

The mean recovery observed for intra-day measurement on day 1 was from 107.0 to 119.6% (RSD 0.4–11.2%), on day 2 it was from 106.1 to 114.2% (RSD 0.4–4.7%) and on day 3 from 102.8 to 111.6% (RSD 0.2–5.1%). The mean recovery observed for inter-day 1 analysis was between 103.9 and 108.3% (RSD 2.0–4.1%), for inter-day 2 it was between 105.6 and 112.0% (RSD 1.3–10.6%), for inter-day 3 it was between 106.2 and 116.3% (RSD 0.7–9.6%).

The method was considered as accurate and precise as all measured values were within the acceptance limits of the ICH guidelines Validation of Analytical Procedure Q2(R1) (2005).

## Abbreviations

Table S3 shows the abbreviations used in the article.

**Table 3.** Table of abbreviations.

AUC	area under the curve
BE	bioequivalence
CI	confidence interval
C <sub>max</sub>	peak drug concentration in the blood
ECZ	econazole
EMA	European Medicines Agency
ICH	International Council for Harmonisation
IVPT	<i>in vitro</i> permeation test
IVRT	<i>in vitro</i> release test
LOD	limit of detection
LOQ	limit of quantification
MCZ	miconazole
MRM	multiple reaction monitoring
MS	mass spectrometry
OECD	Organisation for Economic Co-operation and Development
RMP	reference medicinal product
RSD	relative standard deviation
SC	<i>stratum corneum</i>
T <sub>max</sub>	time at C <sub>max</sub>
UHPLC	ultra-high pressure liquid chromatography