Method development for the evaluation of *in vitro* skin permeation of clascoterone from clascoterone topical cream, 1%

Yang Yang[1], Jiang Wang[1], Apipa Wanasathop[1], Ahmed Zidan[1], Mengmeng Niu[2], Priyanka Ghosh[2], Patrick Faustino[1], Muhammad Ashraf[1], and Xiaoming Xu[1]

[1] Division of Product Quality Research, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA
[2] Division of Therapeutic Performance I, Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, Food and Drug Administration, Spring Silver, Maryland, USA

Purpose

Winlevi® (clascoterone) topical cream, 1% was approved by the FDA in August 2020 for the treatment of acne vulgaris. Clascoterone (CLA) is not stable in physiological solutions and can be hydrolyzed to cortexolone (COR) via cortexolone 21-propionate (COR-21) at body temperature (Figure 1), posing an analytical challenge. This study aims to develop an analytical method for the evaluation of CLA and COR following an *in vitro* skin permeation test (IVPT) using Winlevi® cream, 1%.

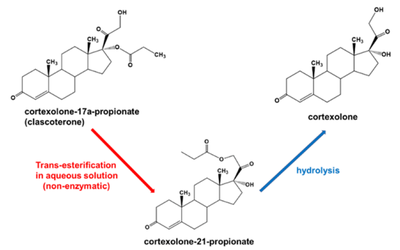

Figure 1. CLA (cortexolone-17α-propionate) can be quickly hydrolyzed by the skin and plasma esterase into COR via conversion to COR-21 as the intermediate. (Ref: Med. Chem. Commun., 2014, 5, 904.)

Methods

Simultaneous quantitation of CLA and COR in IVPT samples was performed using a Sciex Exion ultra-high performance liquid chromatography (UHPLC) system coupled to a Qtrap 6500+ tandem mass spectrometer equipped with a Turbo Ion Spray source (UHPLC-MS/MS, Sciex, Framingham, MA). Analytes and internal standard (IS) were separated on a C18 UPLC column (The LC separation was achieved with a C18 column with advanced sub-2µm core-shell particles). The mobile phase consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile/methanol was used at 0.8 mL/min with a 2-min gradient program. The mass spectrometer was operated in the positive mode with multiple reaction monitoring. Nitrogen was used as the nebulizer, heater and curtain gas as well as the collision gas. Analyst® Software was used for data acquisition and processing. The analytical method was validated according to FDA Guidance for Industry: *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022).

IVPT was carried out using in-line flow-through cells (PermeGear, Inc., Hellertown, PA). The test was performed using dermatomed skin samples (n=3), obtained from each of four donors, with the average skin thickness of 250 µm. The receptor solution was phosphate buffered saline (PBS) containing 5% (w/v) of bovine serum albumin (BSA) as solubilizer. A finite dose (10 mg/cm^2) of cream was applied to the skin maintained at 32.0˚C. IVPT samples were collected every 2 hours for the first 24 h and every 4 hours from 24 h till 48 h. CLA and COR were then extracted from IVPT samples using a protein precipitation method with methanol at a 1:9 (v/v) ratio. After centrifugation, the supernatant (400 µL) was mixed with 300 µL of H_2O containing 0.3% of formic acid. Samples were then loaded into the LC-MS/MS autosampler with controlled temperature (5°C) and analyzed immediately. After the analysis, samples were stored in the -20°C freezer. To evaluate the stability of CLA and COR in the receptor solution, a stock solution of CLA or COR with nominal concentration of 0.5, 1.00, 500 and 800 ng/mL was spiked into blank receptor solution and extracted right away. Extracted samples were stored at -20°C for one week and analyzed using LC-MS/MS.

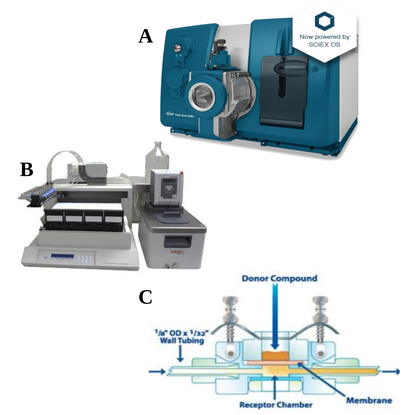

Figure 2. (A) Qtrap 6500+ tandem mass spectrometer (Sciex, Framingham, MA), (B) In-line flow-through diffusion system (PermeGear, Inc., Hellertown, PA), (C) **S**chematic diagram of the flow-through diffusion cell.

Results

Table 1. Mass spectrometry ion source parameters for CLA, COR and IS (d7-cortexolone) on SciEx 6500+ Mass Spectrometer.

Spray Type	Electrospray
Ionization mode	Positive
Source Temp. (°C)	550
Curtain Gas	25
Charged aerosol detection (CAD)	12
Ionization voltage (IS)	5500
Gas 1 (GS1)	50
Gas 2 (GS2)	70

Table 2. Data acquisition parameters for CLA, COR, and IS (d7-cortexolone) on the Sciex 6500+ Mass Spectrometer (Positive mode)

	CLA	COR	IS
Precursor ion m/z	403.2	347.2	354.2
Product ion m/z	329.2	109.2	113.2
Dwell time (ms)	50	50	50
Declustering potential (DP)	75	90	90
Entrance potential (EP)	12	10	10
Collison Energy (CE)	18	31	35
Collision cell exit potential (CXP)	12	11	11

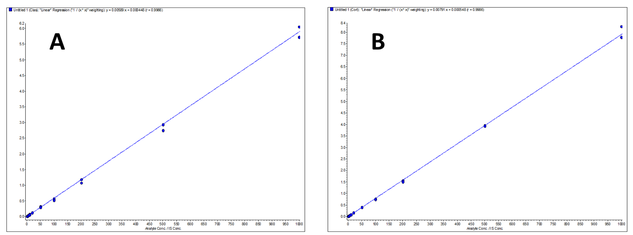

Figure 3. Calibration curves of (A) CLA and (B) COR were linear over the validated range of 0.5-1000 ng/mL. Correlation coefficients were > 0.99. The analytical method was determined to be accurate and precise at all quality control levels following the FDA Guidance for Industry: *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022).

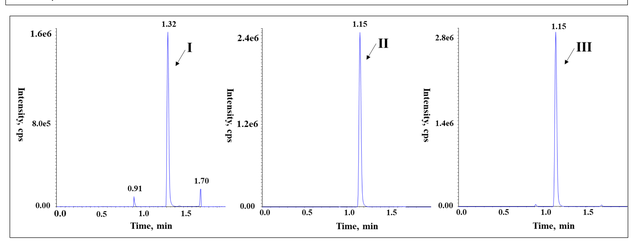

Figure 4. Representative chromatogram of the system suitability sample: I, CLA; II, COR; III, IS

Table 3. Stability of CLA in IVPT receptor solution at -20°C (1 week) (n=6 repeats in LC-MS/MS)

Nominal concentration (ng/mL)	0.50	1.00	500	800
1	0.45	1.02	493	746
2	0.50	1.00	492	741
3	0.56	1.00	480	744
4	0.52	0.87	479	740
5	0.48	0.94	475	744
6	0.51	1.03	479	732
Average	0.50	0.98	483	741
Standard deviation	0.04	0.06	7.56	5.0
Accuracy	100.5%	98%	97%	92.6%
Precision	7.73%	6.29%	1.57%	0.67%

Table 4. Stability of COR in IVPT receptor solution at -20°C (1 week) (n=6 repeats in LC-MS/MS)

Nominal concentration (ng/mL)	0.50	1.00	500	800
1	0.52	1.04	492	784
2	0.55	1.06	500	783
3	0.53	0.98	496	790
4	0.48	1.04	497	795
5	0.51	0.99	496	792
6	0.49	0.99	492	775
Average	0.52	1.01	496	787
Standard deviation	0.03	0.04	3.08	7.3
Accuracy	103.1%	101%	99%	98.3%
Precision	5.04%	3.55%	0.62%	0.93%

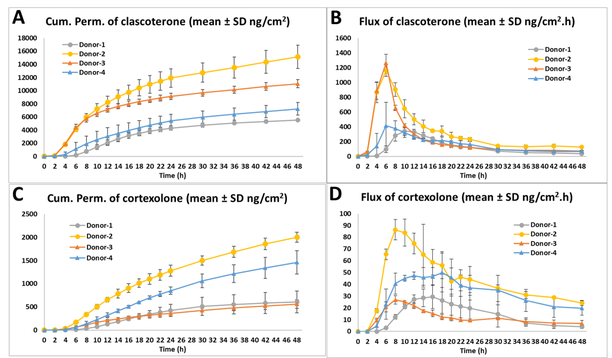

Figure 5. IVPT profiles of CLA and COR. A) cumulative permeation of CLA; B) flux of CLA ; C) cumulative permeation of COR; and D) flux of COR. Data are presented as mean ± SD. N=3 for all four donors.

Conclusions

An UHPLC-MS/MS method was successfully developed for quantifying the skin permeation of CLA and COR from Winlevi®. The data enhanced our understanding of permeation of CLA and its metabolites following topical application of the cream.

Disclaimer: This poster reflects the views of the authors and should not be construed to represent the views or policies of the Food and Drug Administration.

FDA U.S. FOOD & DRUG ADMINISTRATION

www.fda.gov