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A Direct LC/MS/MS Method to Determine the Amount of Ciclopirox Penetrated Across Human Nail Plate in *In Vitro* Penetration Studies

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Objectives

- Develop and validate a novel direct high throughput method for the determination of ciclopirox by LC/MS/MS
- Analyze ciclopirox *in vitro* nail penetration samples using the validated method

Introduction

Due to severe chelating effect caused by the N-hydroxypyridone group of ciclopirox, there is no published direct HPLC or LC/MS/MS method for determination of ciclopirox in any *in vitro* or *in vivo* matrix. Instead, the time consuming pre-column derivatization method^(1,2) has been adapted for indirect analysis of ciclopirox.

After overcoming the chelating problem by using K₂EDTA coated tubes, we successfully developed and validated a direct, sensitive and high throughput LC/MS/MS method to determine the amount of ciclopirox that penetrated across the nail plate during *in vitro* nail penetration studies. The method was validated over a concentration range of 8 – 256 ng/mL. The validated method was successfully used to analyze ciclopirox formulation and *in vitro* nail penetration samples.

Method

- To a K₂ EDTA tube, 4 mL of internal standard (IS) solution (chloridazon, 100 ng/mL in acetonitrile : 20% NH₄OH, 3:1, v/v) was added and the tube was vortexed.
- To a 96-deep well plate, 100 µL aliquot of STD, QC and experimental samples were added to the designated wells followed by the addition of 100 µL of IS solution from the K₂ EDTA tubes.
- The plate was vortexed briefly and 2 µL from each well were injected for LC/MS/MS analysis.

LC/MS/MS Methods

- Instrument: API4000 QTRAP w/ Shimadzu LC-20AD system
- LC Conditions (+): Gradient, H₂O, 0.1% FA, ACN, 0.1% FA
- Column: Atlantis T3, 2.1X50 mm, 5µ (Waters)
- Ionization mode: ESI (+)
- MRM Transition:
 - ciclopirox: m/z 208.0 → 135.8
 - chloridazon (IS): m/z 221.8 → 77.0

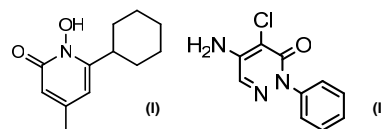
Nail Penetration Study

The nail penetration study was conducted at Cetero Research (Fargo, ND, USA). The *in vitro* Franz human skin finite dose model was used for this study. The human cadaver finger nails were mounted on a modified chamber. A finite dose (12.5 µL/cm²) of Penlac® (Ciclopirox Solution), 8% was applied to the outer surface of the nail daily for a total of 14 days and drug absorption was measured by monitoring its rate of appearance in the reservoir solution (double de-ionized water, ddH₂O) bathing the inner surface of the nail.

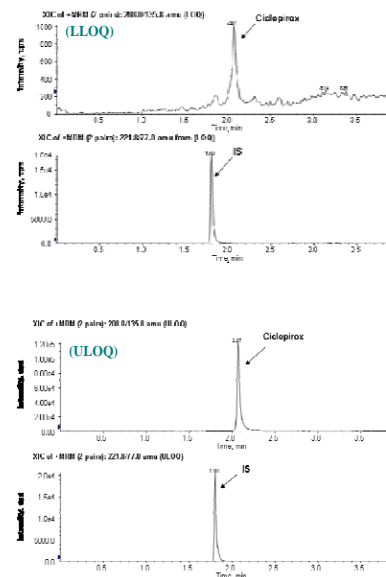
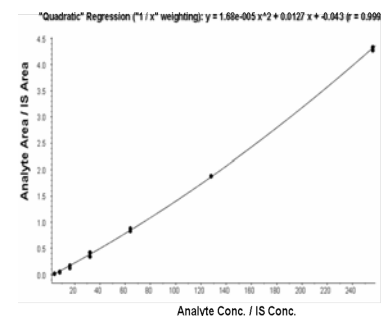
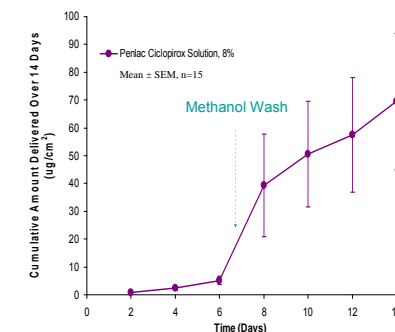
At 48 hour intervals, the receptor solution is removed in its entirety, replaced with fresh receptor solution, and an aliquot (approximate 1.5 mL) was frozen immediately for subsequent analysis.

Table 1. Precision and Accuracy of QC Samples

Assay type	Nominal concentration	n	Mean calculated concentration	Precision (% CV)	Accuracy (% nominal)
Intra-assay	8.00	6	8.82	4.56	110
	40.0	6	42.6	7.42	106
	200	6	214	2.70	107
Inter-assay	8.00	21	9.01	6.57	113
	40.0	21	40.5	10.6	101
	200	21	213	8.26	106

Figure 1. Ciclopirox (I) and Chloridazon (II)


Sources of the structures: Sigma-Aldrich website

Figure 2. Representative LC/MS/MS Chromatograms

Table 2. A Representative Calibration Curve

Figure 3. Cumulative Amount After 14 Days of Daily Dosing


Conclusions

- A direct sensitive, rapid and high throughput LC/MS/MS method for the determination of ciclopirox was developed and validated.
- The *in vitro* skin penetration samples were successfully analyzed.