Quantitative Determination of Terbinafine in Nail Hydrolysate Using LC/MS/MS

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Introduction
Terbinafine has strong antifungal activity and is indicated for the treatment of fungal infections of the skin and nails associated with dermatophytes. TMI-358 is a formulation implant containing terbinafine hydrochloride and polyethylene glycol. Once the TMI-358 had been injected into the nail bed, it is anticipated that terbinafine is released locally to the nail matrix and bed. This local delivery approach allows for administration of a much lower and systemic exposure to terbinafine than is currently provided via oral therapy. The quantitation of the terbinafine in human nail has required the development of a validated method.

No published article was found for LC/MS/MS analysis of terbinafine in human nail. Human nail clippings were hydrolyzed in 5N sodium hydroxide. A robust and reliable method has been established for the measurement of terbinafine in human nail hydrolysate by LC/MS/MS in the concentration range of 0.500 to 60.0 ng/mL. This validated method is suitable for determination of terbinafine in human nail clippings.

Method
The hydrolysate of nail clippings in 5N sodium hydroxide was extracted along with the internal standard, terbinafine-d7, using liquid-liquid extraction. After extraction, the residue was chromatographed using reversed phase HPLC on an Aquasil C18, 3 μm (30 x 2.1 mm) column. Terbinafine and terbinafine-d7, were detected by monitoring the precursor and product ions (m/z 292→241 for terbinafine and m/z 299→148 for terbinafine-d7) using an Applied Biosystems API3000 or API4000 LCMS/MS. The quantification was based on a calibration graph established by a weighted linear regression (1/x2) of the peak area ratio (analyte/internal standard) versus the concentration of the analyte. The lower limit of quantitation was 0.500 ng/mL.

Results
The method was fully validated over a range of 0.500 to 60.0 ng/mL with weighed linear regression (1/x). The correlation coefficients for first three validation batches were 0.997 or better (Figure 2).

Liquid Chromatography
Shimadzu 10ADvp HPLC system
Column: Aquasil C18 (30 x 2.1 mm) 3 μm
Mobile Phase A: ACN/10 mM Ammonium acetate (pH 5.8) 20/80, v/v
Mobile Phase B: Acetonitrile
Gradient Program: Flow rate (0.30 mL/min)
Time (min) 0.01 1.50 2.00 2.01 5.00 % of B 65.0 85.0 85.0 65.0 stop

Mass Spectrometry
Applied Biosystems/MDI-Sciex API 3000 or API 4000
Polarity: positive
Scan type: Multiple Reaction Monitoring
Terbinafine: m/z 292→141 m/z
Terbinafine-d7 (I.S.): m/z 299→148 m/z

Extraction
Aliquot 0.10 mL of human nail hydrolysate
Add 0.75 mL DI-water
Add 0.10 mL of IS solution (10 ng/mL terbinafine-d7), and 4.00 mL of extraction solvent (50:50 MTBE/acetone)
Vortex and centrifuge
Flash-freeze the aqueous layer and transfer the organic supernatant to a glass tube.
Evaporate to dryness using a TurboVap.
Reconstitute 0.25 mL 50/50 ACN/10 mM Ammonium Acetate, pH 5.8

Validation results obtained in this study indicate that a robust and reliable method has been established for the measurement of terbinafine in human nail hydrolysate by LC/MS/MS in the concentration range of 0.500 to 60.0 ng/mL. This validated method is suitable for determination of terbinafine in the human nail clippings.