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

Ying Li[1], Gene Ray[1], Yansheng Liu[1], Dari Dadgar[1], Charles Ting[2]
[1]KCAS, Shawnee, Kansas; [2]Talima Therapeutics, Inc. San Carlos, California 94070

## 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 fold, 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 approved 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.

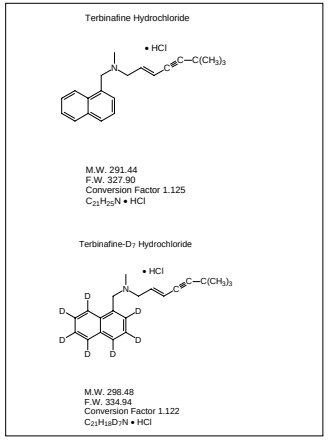



**Figure 1**. Structure of Terbinafine and Terbinafine-$D_7$

## Method

The hydrolysate of nail clippings in 5N sodium hydroxide was extracted along with the internal standard, terbinafine-$d_7$, using liquid-liquid extraction. After extraction, the residue was chromatographed using reversed phase HPLC on an Aquasil C18, 3 µ (30 x 2.1 mm) column. Terbinafine and terbinafine-$d_7$ were detected by monitoring the precursor and product ions (m/z 292→241 for terbinafine and m/z 299→148 for terbinafine-$d_7$) using an Applied Biosystems API3000 or API4000 LC/MS/MS. The quantification was based on a calibration graph established by a weighted linear regression ($1/x^2$) of the peak area ratio (analyte/internal standard) versus the concentration of the analyte. The lower limit of quantitation was 0.500 ng/mL.

### Liquid Chromatography

Shimadzu 10ADvp HPLC system
Column : Aquasil C18 (30 x 2.1 mm) 3µm
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 Biosystem/MDS-Sciex API 3000 or API 4000
Polarity : positive
Scan type : Multiple Reaction Monitoring
Terbinafine : 292 → 141 m/z
Terbinafine-$d_7$(I.S.) : 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-$d_7$)
and 4.00 mL of extraction solvent (50/50 MTBE/pentane)
↓
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

## Results

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

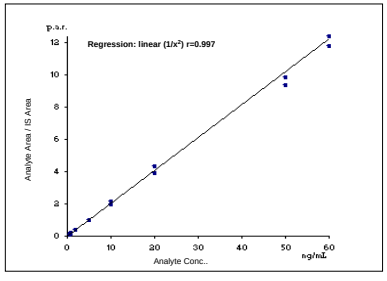



**Figure 2**. Calibration curve 0.500 to 60.0 ng/mL for Terbinafine in human nail hydrolysate

**Figures 3 through 5** show typical chromatograms of extracted lower and upper limits of quantitation (LLOQ 0.500 ng/mL; ULOQ 60.0 ng/mL), and an extracted blank matrix. At the LLOQ, the signal-to-noise ratio was greater than 20. No interference, or carryover was observed in the chromatogram of blank matrix.

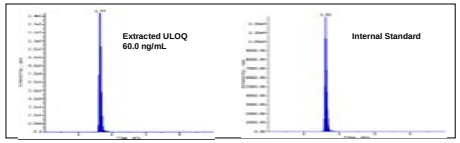



**Figure 3**. Chromatogram of an extracted ULOQ sample (60.0 ng/mL).

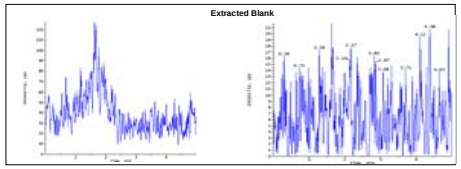



**Figure 4**. Chromatogram of an extracted blank sample

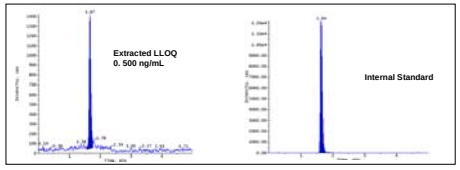



**Figure 5**. Chromatogram of an extracted LLOQ sample (0.50 ng/mL).

**Table 1** summarizes results of inter-batch, intra-batch validation batches. All concentration levels demonstrate excellent precision and accuracy.

| Intra-batch precision and accuracy | | 0.500 ng/mL (LLOQ) | 1.50 ng/mL (Low) | 15.0 ng/mL (Mid) | 40.0 ng/mL (High) |
|---|---|---|---|---|---|
| **Batch 1** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.531 | 1.51 | 16.0 | 40.8 |
| | **cv (%)** | 5.8 | 4.0 | 2.0 | 4.1 |
| | **bias (%)** | 6.3 | 0.6 | 6.3 | 2.0 |
| **Batch 2** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.537 | 1.67 | 15.9 | 42.6 |
| | **cv (%)** | 2.2 | 4.9 | 2.4 | 1.7 |
| | **bias (%)** | 7.4 | 11.4 | 6.0 | 6.4 |
| **Batch 3** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.50 | 1.64 | 15.5 | 43.0 |
| | **cv (%)** | 2.3 | 0.9 | 3.0 | 3.3 |
| | **bias (%)** | 0.0 | 9.6 | 3.2 | 7.5 |
| **Inter-batch Precision and accuracy** | | | | | |
| **n** | | 18 | 18 | 18 | 18 |
| **Mean** | | 0.523 | 1.61 | 15.8 | 42.1 |
| **Precision (cv %)** | | 4.8 | 5.7 | 2.7 | 3.8 |
| **Accuracy (bias %)** | | 1.6 | 7.2 | 5.2 | 5.3 |

Stability results for terbinafine are listed in **Table 2**. Selectivity was tested by determining precision of low-level QCs (1.50 ng/mL) in six different lots of blank matrix (**Table 3**). No interference or matrix effects were observed.

**Table 2**. Summary of Stability Results for Terbinafine in nail hydrolysate.

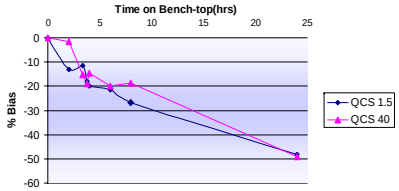
| | |
|---|---|
| Matrix stability | |
| Freeze-thaw stability | 4 cycles |
| Bench-top stability* | 3.33 hours |
| Long-term stability | 76 days |
| Extracted Matrix stability | |
| Autosampler stability at room temperature | 46 hours |
| Refrigerated extract stability | 74 hours |
| Individual sample reinjection at room temp. | 5 hours |
| Whole batch reinjection Integrity | 65.5 hours |
| Solution stability | |
| Primary solution stability at 4 °C ** | 109 days |

* It was found that terbinafine in nail hydrolysate is not stable at room temperature. Figure 6 showed the bench top stability trend.
** In the solution of MeOH

**Table 3**. Matrix Effect for Terbinafine in Human Nail Hydrolysate.

| | Avg drug area | Avg IS area | Avg area ratio |
|---|---|---|---|
| **ME 1** | 18108 | 71666 | 0.2526651 |
| **ME 2** | 16170 | 65856 | 0.2455401 |
| **ME 3** | 19089 | 67494 | 0.2828202 |
| **ME 4** | 18777 | 71694 | 0.2618990 |
| **ME 5** | 17790 | 65225 | 0.2727398 |
| **ME 6** | 20962 | 76328 | 0.2746256 |
| **mean** | **18482** | **69710** | **0.265048** |
| **n** | **6** | **6** | **6** |
| **SD** | **1585.4** | **4276.5** | **0.014217** |
| **%CV** | **8.6** | **6.1** | **5.4** |

**Figure 6**. Bench Top Stability Trend for Terbinafine in Human Nail Hydrolysate.




It was noticed that terbinafine in nail hydrolysate is only stable for up to 3.33 hrs. The nail hydrolysate was aliquotted to multiple containers after hydrolysis. Each time an unthawed aliquot is used for analysis to minimize the bench top time.

## Conclusion

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.