LC-MS/MS analysis of Terbinafine in Digested Diluted Nail Beds

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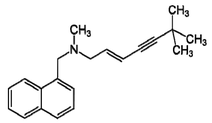
Introduction

Terbinafine hydrochloride is a synthetic allylamine antifungal. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting squalene epoxidase, an enzyme that is part of the fungal cell membrane synthesis pathway. Because terbinafine prevents conversion of squalene to lanosterol, ergosterol cannot be synthesized. Typical bioanalysis of organic molecules are done in biological matrices such as blood, plasma, serum or urine. In the case of Terbinafine, this highly lipophilic molecule tends to accumulate in skin, nails and fatty tissues. This aspect thus requires the use of a more unique matrix. In our case, nail bed was selected for the analysis. Nail bed is the tissue beneath the nail which the nail protects. It contains nerves, lymph and blood vessels. The matrix is responsible for producing cells that become the nail plate. A successful extraction procedure was developed in order to quantitate Terbinafine in digested diluted nail beds.



Method

The extraction procedure consisted of multiple steps. First, digestion of nail beds was done by incubation at 90°C in presence of an appropriate volume of working solution and 5M NaOH. Methanol was used instead of working solution for the preparation of blank samples. Neutralization with an adequate volume of 5M HCl followed by dilution with methanol provided the digested nail bed sample. The second step required another dilution step in which methanol was spiked with the previous digested solution. Diluted digested samples were finally diluted with the internal standard working solution to obtain samples ready for analysis. Chromatography was performed on a reversed phase HPLC using a Zorbax SB-C18 75 x 4.6 mm (3.5 µm) analytical column.

LC-MS/MS Analysis

Chromatographic mode:	Reversed Phase
Analytical Column:	Zorbax SB-C18, 4.6 x 75 mm
Column Temperature:	Room Temperature
Elution mode:	Isocratic
Mobile Phase A:	Milli-Q Type Water/Acetonitrile (35/65), Ammonium Acetate 5mM, Formic Acid 0.5%
Flow Rate:	1.0 mL/minute
Retention Times:	2.25 minutes
Acquisition time:	2.60 minutes
Detector:	AB Sciex API 4000
Source:	TurboIonSpray, Positive mode
Ion Monitored:	292→141 amu

Figure 2. Sample Extraction Procedure

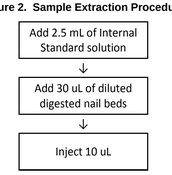
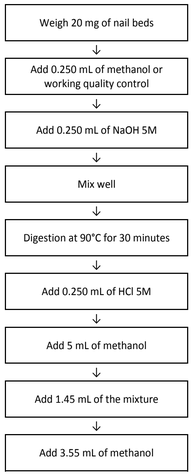


Figure 1. Preparation of Diluted Digested Nail Beds



Results

Calibration curves were prepared in methanol only, in absence of nail bed matrix. Two sets of quality control samples were prepared, one in methanol and one in digested nail beds. Each set was analyzed in order to assess quality of samples prepared either with or without nail bed matrix. The method was validated over the dynamic range of 1-1000 ng/mL. Recovery of analyte in digested nail beds and internal standard were 79% and 108% **(Table 1)**. The matrix effect and the matrix factor **(Tables 2 and 3)** were evaluated and found to have no impact on quantitation. Freeze and thaw, short-term and long-term stability in matrix were evaluated and met acceptance criteria.

Table 1. Recovery of Analytes and Internal Standard

QC Level	% Recovery
Low QC 3.05 ng/mL	78.7
Middle QC 508.99 ng/mL	79.5
High QC 763.48 ng/mL	79.1
Internal Standard	109.2

Table 2. Matrix Effect Evaluation in Digested Nail Beds at Low QC level (3.05 ng/mL)

	Untreated Standard (MFQC1)		Reference solution (RSQC1)				
Donors	Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses	Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
ME01	4123	1002246	4128	987954	1.045	1.009	1.036
ME02	4225	1013110	3883	967353	1.071	1.020	1.050
ME03	4055	1037537	3835	968691	1.028	1.044	0.985
ME04	4153	1036942	3898	977170	1.053	1.044	1.009
ME05	3984	1018746	3825	1020478	1.010	1.025	0.985
ME06	4030	1007912	4100	1039477	1.022	1.014	1.008
		Mean	3944.8	993520.5		Mean	1.0122
						SD(±)	0.02647
						CV(%)	2.62

Table 3. Matrix Effect Evaluation in Digested Nail Beds at High QC level (1017.98 ng/mL)

	Untreated Standard (MFULOQ)		Reference solution (RSULOQ)				
Donors	Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses	Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
ME01	1372311	1031497	1403779	1021779	0.983	0.995	0.987
ME02	1323734	1002577	1354330	1001955	0.948	0.967	0.980
ME03	1427892	1077157	1438858	1060304	1.023	1.039	0.984
ME04	1431198	1050552	1419167	1071900	1.025	1.014	1.011
ME05	1378846	1025108	1389223	1050814	0.987	0.989	0.998
ME06	1470780	1112028	1372932	1011668	1.053	1.073	0.982
		Mean	1396381.5	1036403.3		Mean	0.9903
						SD(±)	0.01194
						CV(%)	1.21

Results

Table 4. Between-Run Accuracy and Precision of Quality Controls in Digested Nail Beds

	Low QC 3.05 ng/mL		Middle QC 508.99 ng/mL		High QC 763.48 ng/mL	
	Conc. Found (ng/mL)	% Bias	Conc. Found (ng/mL)	% Bias	Conc. Found (ng/mL)	% Bias
N	66		66		66	
Mean	3.282	7.6	547.957	7.7	778.43	2.0
SD(±)	0.2375		33.5434		52.715	
CV(%)	7.24		6.12		6.77	

Table 5. Between-Run Accuracy and Precision of Quality Controls in Methanol

	Low QC 3.05 ng/mL		Middle QC 508.99 ng/mL		High QC 763.48 ng/mL	
	Conc. Found (ng/mL)	% Bias	Conc. Found (ng/mL)	% Bias	Conc. Found (ng/mL)	% Bias
N	60		60		60	
Mean	3.202	5.0	537.54	5.6	749.904	-1.8
SD(±)	0.2544		32.691		47.4961	
CV(%)	7.95		6.08		6.33	

Conclusion

Digestion conditions were carefully chosen to achieve the best recovery. This assay was shown to be robust, accurate and precise and was successfully used for study sample analysis.

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