

# Validation of a Chiral HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of the Enantiomers of Ketoconazole in Human and Dog Plasma

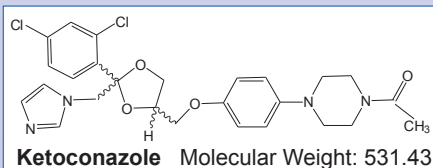
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## Overview

- **Purpose** - Develop and validate a chiral HPLC/MS/MS method to determine concentrations of the enantiomers of Ketoconazole in plasma
- **Methods** – 96 well-plate SPE extraction and Chiral HPLC/APCI/MS/MS (API3000)
- **Results** – Range from 5 -1,000 ng/mL with accuracies and precision better than 15% using Chiral HPLC/MS/MS

## Introduction

Ketoconazole is an azole antifungal drug used for the treatment of fungal infections. However, ketoconazole is not widely used due to its liver toxicity. Ketoconazole is a chiral drug and is currently administered as the racemic mixture. Recent studies have shown that the enantiomers have different *in vitro* activity; therefore, the stereochemistry of ketoconazole could modulate its biological effects. Previous HPLC assays quantified ketoconazole as the racemate and limits of quantitation >12 ng/mL were achieved. While some non-bioanalytical assays were able to resolve the two ketoconazole enantiomers, these assays had long HPLC run times (>30 minutes). Here we report on an accurate and precise validated LC/MS/MS assay for the determination of the enantiomers of ketoconazole from human and dog plasma.



## Methods

### Extraction

- Alkalinize plasma sample
- SPE using C8 cartridges
- Elute with methanol containing 1% formic acid, then evaporate
- Reconstitute in 3:7 Hexane:Ethanol

### HPLC USING CHIRAL SEPARATION

- Isocratic HPLC at 65:35 Ethanol:Hexane for 10 minutes.
- Flow rate = 0.25 mL/minute
- Chiralpak AD-H 150X2.1mm (Chiral Technologies, Inc.)
- Forty µL injections

### Mass Spectrometry

- Sciex API3000 operating in MRM mode
- APCI
- Positive ion mode
- MRM transitions for Ketoconazole (sum multiple ions for quantitation)
  - 531 → 82
  - 531 → 244
  - 531 → 490

**Table 1.** Standard Curve and QC Results for the HPLC/MS/MS Analysis of Ketoconazole Enantiomers from Human Plasma.

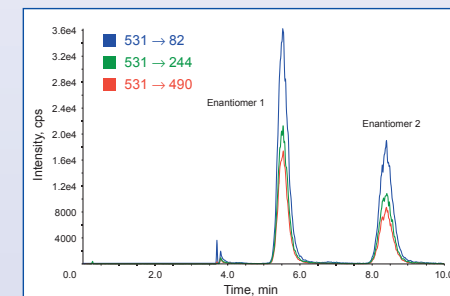
Correlation Coefficient Enantiomer 1: 0.9967  
Correlation Coefficient Enantiomer 2: 0.9974

Curve Conc. (ng/mL)	Enantiomer 1		Enantiomer 2	
	Calculated Conc. (ng/mL)	Accuracy (%)	Calculated Conc. (ng/mL)	Accuracy (%)
5.00	4.76	95.1	4.89	97.7
5.00	5.56	111	5.38	108
15.0	13.8	91.7	13.6	90.7
15.0	13.8	91.7	14.4	96.3
50.0	45.2	90.3	45.3	90.5
50.0	48.9	97.8	48.8	97.5
100	100	100	98.9	98.9
100	104	104	105	105
800	788	98.5	775	96.9
800	846	106	834	104
1000	1070	107	1080	108
1000	1070	107	1070	107
15.0 QC	15.3	102	15.6	104
15.0 QC	15.2	101	15.5	103
100 QC	101	101	103	103
100 QC	105	105	105	105
800 QC	836	105	825	103
800 QC	842	105	835	104

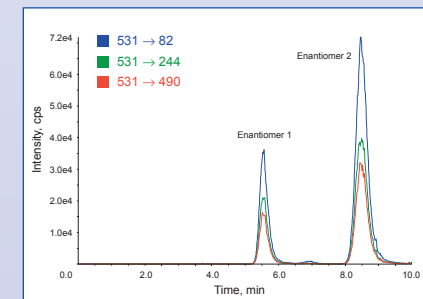
**Table 2.** Short-Term Stability Results for the HPLC/MS/MS Analysis of Ketoconazole Enantiomers from Human and Dog Plasma.

	Freeze Thaw Stability (% Difference from T0)	Benchtop Stability (Hours)	Extract Stability (Hours)
<b>Enantiomer 1</b>			
Human	±15%	> 6	> 26
Dog	±15%	> 6	> 50
<b>Enantiomer 2</b>			
Human	±15%	> 6	> 26
Dog	±15%	> 6	> 50

HPLC/MS/MS Chromatogram from the Analysis of a 100 ng/mL Standard of the Enantiomers of Ketoconazole Extracted from Dog Plasma



HPLC/MS/MS Chromatogram from the Analysis of the Enantiomers of Ketoconazole Extracted from a Dog Plasma Sample 2 Hours Post Dose



## Conclusions

- Developed and validated a chiral HPLC/MS/MS method to quantify the enantiomers of Ketoconazole from human and dog plasma
- Method supports PK studies for enantiomers of Ketoconazole in toxicokinetic and clinical trial studies