

Title: A Highly Specific and Sensitive LC-MS/MS Assay for Itraconazole and Hydroxy-Itraconazole in Human Plasma for Bioequivalence, Bioavailability and Pharmacokinetic Studies

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Introduction

It has been demonstrated that liquid chromatography/tandem mass spectrometry (LC/MS/MS) method offers several advantages over high performance liquid chromatography (HPLC) method including better sensitivity, specificity, and higher throughput. This paper presents a highly specific and sensitive LC-MS/MS method for the simultaneous determination of itraconazole and hydroxyitraconazole (Figure 1) in human plasma, which was achieved by simple protein precipitation, reverse-phase chromatography separation and mass spectrometry detection. This approach eliminated the time-consuming liquid-liquid extraction used in HPLV-UV method, increased the detection limit, and greatly reduced sample processing and instrument acquisition time.

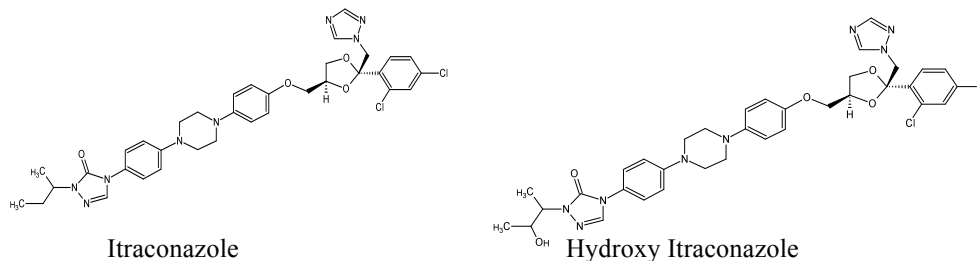


Figure 1. Chemical Structures of Itraconazole and Hydroxy Itraconazole

Method

The analyte was extracted from 100 μ L of plasma samples by methanol protein precipitation. Reverse phase separation was achieved on a Hypersil GOLD AQ column (50 x 4.6 mm, 5 micron) using a Shimadzu HPLC system coupled with an API 4000 mass spectrometer, which was operated in turbo ionspray[®] in positive ion MRM mode. The analytical run time was 4 minutes. The mass transitions (precursor to daughter, m/z) monitored were 705.2/392.2 and 721.2/408.1 for itraconazole and hydroxyl itraconazole. The mass transitions were 710.2/397.3 and 726.2/413.2 for the stable-isotope labeled internal standards itraconazole-d₅ and hydroxyl itraconazole-d₅.

Results

The high throughput LC-MS/MS method was fully validated for accuracy, precision, sensitivity, stability, recovery, and calibration range. Acceptable intra-run and inter-run assay precision ($\leq 6.5\%$) and accuracy (100.0-103.5%) were achieved over a linear range

of 1 to 500 ng/mL for itraconazole and hydroxyl itraconazole in human plasma (Table 1). A cross validation between the LC/MS/MS method and an HPLC-UV method was also successfully performed, with the %difference between the two methods within 5% (Table 2) for bridging PK data between two clinical studies. The method robustness was demonstrated by the incurred sample reproducibility test of 20 clinical study samples, with 100% of the samples meeting criterion (%Difference \leq 20%) for itraconazole and 95% samples for hydroxyitraconazole.

Table 1. Accuracy and Precision of the QC samples for Method Validation

Day ID	N=6	Itraconazole Concentration, ng/mL			Hydroxy Itraconazole Concentration, ng/mL		
		3	40	380	3	40	380
Intraday 1	Mean	3.135	43.119	402.726	3.244	44.771	415.185
	%Nominal	104.5	107.8	106.0	108.1	111.9	109.3
	%CV	1.8	1.5	3.9	1.5	1.1	2.1
Intraday 2	Mean	3.005	39.641	387.796	3.014	39.429	388.840
	%Nominal	100.2	99.1	102.1	100.5	98.6	102.3
	%CV	2.4	2.6	3.7	2.0	2.9	3.8
Intraday 3	Mean	2.861	40.119	389.195	2.915	39.562	382.176
	%Nominal	95.4	100.3	102.4	97.2	98.9	100.6
	%CV	2.1	2.5	2.7	2.8	2.2	2.7
Interday Results	Mean	3.000	40.960	393.239	3.058	41.254	395.400
	%Nominal	100.0	102.4	103.5	101.9	103.1	104.1
	%CV	4.3	4.4	3.7	5.0	6.5	4.6

Table 2. Cross Validation Results between LC-MS/MS and HPLC-UV Methods

Concentration ng/mL	N=6	Itraconazole		%Diff	Hydroxy Itraconazole		%Diff
		LC/UV	LC/MS/MS		LC/UV	LC/MS/MS	
6	Mean	5.36	5.49	2.5	5.61	5.60	-0.3
	%Nom	89.3	91.6		93.6	93.4	
	%CV	1.6	4.2		12.6	2.2	
40	Mean	35.6	37.1	4.1	40.1	38.5	-4.0
	%Nom	89.1	92.8		100.4	96.4	
	%CV	0.6	1.9		4.8	1.5	
380	Mean	339	351	3.6	361	355	-1.8
	%Nom	89.3	92.6		95.2	93.5	
	%CV	1.2	1.7		6.5	1.7	

Conclusion

A simple high throughput LC-MS/MS method has been developed and fully validated for simultaneous determination of itraconazole and hydroxyl itraconazole in human plasma. This method is specific, sensitive, and reproducible and has been successfully used in support of a clinical PK study and also can be used for supporting bioequivalence and bioavailability studies.