Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2010, 2(2): 41-53
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Validated Liquid chromatography tandem mass spectrometric method for quantification of Itraconazole and Hydroxy Itraconazole in human plasma for pharmacokinetic study

Anil. K. Patni*, Tausif Monif*, A. H. Khuroo*, Ashok. K. Tiwary**

**Department of Clinical Pharmacology and Pharmacokinetics, Ranbaxy Research Laboratories, Gurgaon, Haryana, India*
***Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala, India*

Abstract

A simple, sensitive and reproducible high performance liquid chromatography tandem mass spectrometric method was developed and validated for the determination of itraconazole and hydroxy itraconazole in human plasma using itraconazole-d9 and hydroxy itraconazole-d8, respectively, as internal standards. The precursor to product ion transitions of m/z 705.4 →392.5, m/z 716.5 → 402.6, m/z 721.3 → 408.4 and m/z 729.4→ 416.5 were used to detect and quantify itraconazole, hydroxy itraconazole, itraconazole-d9 and hydroxy itraconazole-d8 respectively. Sample preparation was carried out by solid phase extraction using hydrophilic-lypophilic balance cartridges and the processed sample was chromatographed on Chromolith speed rod RP-18e (50 × 4.6 mm) column using 10 mM ammonium formate buffer (pH 4.0): methanol:: 20:80 v/v as the mobile phase. The weighed ($1/x^2$) calibration curves were linear over the range of 0.301 to 151.655 ng/ml and 0.946 to 224.908 ng/ml for itraconazole and hydroxy itraconazole respectively. The RSD of intra-day and inter-day assay was ≤15%. Extraction procedure yielded a recovery of 52.07%, 53.73%, 49.68% and 50.16% for itraconazole, hydroxy itraconazole, itraconazole-d9 and hydroxy itraconazole-d8 respectively. The validated method was successfully employed for establishing pharmacokinetic parameters of two formulations of itraconazole 100 mg capsule in 14 healthy male Indian volunteers.

Keywords: itraconazole, hydroxy itraconazole, liquid chromatography tandem mass spectrometry, human plasma, pharmacokinetic study

---

INTRODUCTION

Itraconazole (R 51 211), (+-)-cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(lH- 1,2,4-triazol- 1-ylmethyl)- 1,3-dioxolan-4-yl] methoxy]phenyl] - 1 - piperazinyl]phenyl] - 2,4 - dihydro - 2 - (1 - methylpropyl)-3H-1,2,4-triazol-3-one (Fig. 1), is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species including dermatophytes, *Malassesia furfur*, *Candida* species, *Aspergillus* species, and *Histoplasma capsulatum* var. *capsulatum* [1]. The mechanism of action of itraconazole relates to its binding of fungal cytochrome P-450 with resultant inhibition of ergosterol synthesis, an essential element of the cell membrane in propagating the growth of fungal and yeast colonies and perturbation of membrane bound enzyme function and membrane permeability [2]. Itraconazole is metabolized via CYP3A4 enzymatic system to form primarily three active metabolites viz. hydroxy itraconazole, keto-itraconazole and N-desalkylitraconazole. Itraconazole and its metabolites are potent inhibitors of CYP3A4 isozyme and have been used as a tool to confirm the drug-drug interaction potential of a number of substrates such as simvastatin, lidocaine, tacrolimus, sirolimus etc [3-5].

There are several clinical situations where the identification and quantification of itraconazole is essential. Estimation of itraconazole and hydroxy itraconazole in plasma facilitates effective treatment of severe fungal infections. Hence, the identification and quantification of itraconazole and hydroxy itraconazole requires a specific and sensitive method that is suitable for routine analysis of biological samples. A number of HPLC (high performance liquid chromatography) methods with UV and fluorometric detection have been reported for the estimation of itraconazole and hydroxy itraconazole in biological matrix to support both preclinical and clinical studies pertaining to itraconazole. However, the HPLC methods are less selective and not reliable [6-9]. The coupling of HPLC to MS with atmospheric pressure ionization leads to a much more specific and sensitive analytical technique. Few papers have been reported for the determination of itraconazole and hydroxy itraconazole in human plasma by LC/MS/MS [10-13]. However, their total analysis time per sample and sample volume requirement often do not suit the requirements for routine plasma sample analysis of itraconazole.

The aim of this work was to exploit the high selectivity and sensitivity of tandem mass spectrometry with an ESI interface for the detection and quantification of itraconazole and hydroxy itraconazole in human plasma. The limitations of HPLC methods were overcome by combining a simple and rapid solid phase extraction procedure with the LC/MS/MS system. The validated method was successfully employed for establishing pharmacokinetic parameters of two formulations (A and B) of itraconazole 100 mg capsule in 14 healthy male Indian volunteers.

MATERIALS AND METHOD

Experimental

Working standard of itraconazole and hydroxy itraconazole were obtained from Chemical Research Division, Ranbaxy Laboratory Limited, Gurgaon, India. Itraconazole-d9 and hydroxy itraconazole-d8 used as internal standard (IS) were purchased from Syncom, Canada. Methanol was purchased from S. D. Fine Chem. Ltd. Mumbai, India whereas ammonium formate and formic acid were purchased from Qualigens, Mumbai, India. Hydrophilic lipophilic balance

solid phase extraction cartridges (Oasis® HLB 1cc 30mg) were purchased from Waters Corporation, Milford, Massachusetts, USA. Milli-Q water from Milli-Q system (Millipore SAS, Molsheim, France) was used for analysis.

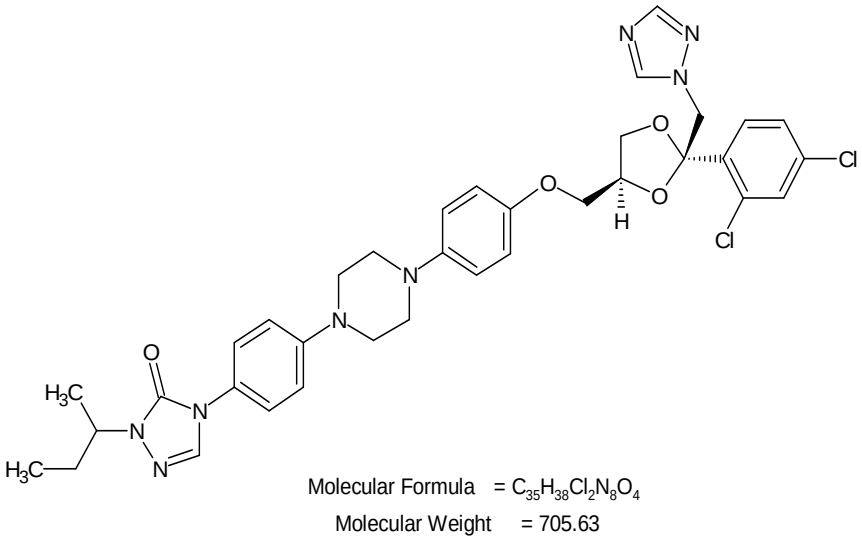

Fig. 1: Chemical structure of Itraconazole

Instrumentation and Operating conditions

Liquid chromatography

The separation was performed on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan), which consisted of a LC-10ADvp pump, a SCL-10Avp system controller (accompanied by an auto sampler), a CTO-10Avp column oven, an FCV-10ALvp low pressure gradient unit, and DGU-14A degasser. The chromatographic separation was carried out in reverse phase conditions using Chromolith Speed Rod RP-18e column (50 mm × 4.6 mm) with an isocratic mobile phase consisting of 10 mM ammonium formate buffer (pH 4.0): methanol (20:80, *v/v*) at a flow rate of 0.6 ml/min. Column oven temperature was set at 35ºC. The autosampler temperature was kept at 5 °C and 25 µl of sample solution was injected with partial loop mode.

Mass spectrometric conditions

API 4000 triple quadrupole tandem mass spectrometer, (Applied Biosystems MDS SCIEX, Toronto, Canada) equipped with an electrospray ionization (ESI) source in positive ionization mode was used for detection. Data processing was performed on Analyst software version 1.4.1 (Applied Biosystems MDS SCIEX Toronto, Canada). Mass spectrometric parameters were optimized using a 10µl continuous infusion of 50 ng/ml each of itraconazole, hydroxy itraconazole and their respective internal standards. Optimized mass spectrometric parameters were, Collision gas: 5 psi, Curtain gas: 20 psi, Gas1 (nebulizer gas): 60 psi, Gas2 (heater gas): 40 psi, Ion spray voltage: 5500 V, Ion source temperature: 500ºC, Declustering potential: 80 V, Collision energy: 50 V, Collision cell Exit Potential: 15 V, Dwell time per transition: 200 ms.

Preparation of Stock and Working Solution

Primary stock solutions of itraconazole, hydroxy itraconazole, itraconazole-d9 and hydroxy

itraconazole-d8 were prepared in acetonitrile: methanol (50:50, *v/v*). Separate stock solutions were used for preparing calibration standards (CC) and quality control samples (QC). CC and QC working solutions for itraconazole and hydroxy itraconazole were prepared by serially diluting the stock solution with methanol: water (50:50, *v/v*) and the final concentrations were corrected for potency, molecular weight and the actual weight transferred. A common working solution of IS containing itraconazole-d9 (4.0 µg/ml) and hydroxy itraconazole-d8 (0.5 µg/ml) was prepared every day by appropriate dilution of the stock solution in methanol: water (50:50, *v/v*). All the solutions were protected from light and stored between 1 to 10°C.

Standard and Quality control sample preparation

Calibration standards were prepared in blank human plasma by spiking 2% *v/v* of the aqueous working solutions for a concentration range of 0.301–151.655 ng/ml and 0.946–224.908 ng/ml for itraconazole and hydroxyl itraconazole, respectively. Similarly, Quality control samples were prepared at lower limit of quantitation (LOQQC) 0.303, 0.946 ng/ml; low level (LQC) 0.801, 2.299 ng/ml; medium level (MQC) 57.184, 85.145ng/ml and at higher limit of quantitation (HQC) 121.669, 181.159 ng/ml, for itraconazole and hydroxy itraconazole, respectively. The spiked samples were stored below -15°C.

Sample preparation

A solid phase extraction technique was used to extract itraconazole, hydroxy itraconazole and their respective internal standards from plasma. The thawed samples were vortexed to ensure complete mixing of contents. 50 µl of IS working solution was mixed with 300 µl aliquot of each plasma sample in polypropylene tubes. 50 µl of 50% ortho phosphoric acid solution (*v/v*) in water was added into these polypropylene tubes and vortexed again to ensure complete mixing for about 30 second. The pre-treated samples were then transferred to solid phase extraction (SPE) cartridges, which had been preconditioned using 1 mL each of methanol and HPLC-grade water at a constant pressure of 15 psi. The plasma loaded cartridges were washed with 1 ml HPLC grade water. The analytes were eluted from the cartridges with 1 ml of methanol twice. Eluents were evaporated to dryness at 15 psi and 50 °C under a stream of dry nitrogen. The residue was reconstituted with 300 µl of mobile phase, transferred into vials and 25 µl was injected into the LC–MS–MS system for analysis.

Method Validation

The method was validated in terms of selectivity, linearity of response, sensitivity, accuracy, precision, recovery, stability (in-injector stability, freeze-thaw stability, bench top stability, long term stability, and blood stability), stock solution stability and matrix effect. Calibration curves were constructed from blank sample (plasma sample processed without IS), a zero sample (plasma sample processed with IS) and eight point calibration standards (lowest and highest standards were used in duplicates). The acceptance criteria for these calibration curves were a correlation coefficient (*r*) of 0.9800 or better. Each back-calculated standard concentration should lie within 15% deviation from nominal value except for the lower limit of quantitation (LLOQ), for which the maximum acceptable deviation was 20% from nominal value. At least 67% of the non-zero standards were required to meet the acceptance criteria including acceptable lower (LLOQ) and upper limit of quantification (ULOQ). The accuracy and precision determination were carried out on three different days with six replicates of low, medium and high QCs.

RESULTS AND DISCUSSION

Selectivity and Sensitivity

The solid phase extraction technique was observed to yield satisfactory results for selectivity and sensitivity for the determination of itraconazole, hydroxy itraconazole and IS in human plasma. The selectivity was determined using six different lots of blank human plasma with K3 ethylenediamine tetra acetic acid (EDTA) as an anticoagulant. Representative chromatograms of extracted blank human plasma (Fig. 2) demonstrated the selectivity and sensitivity of the method. No significant interference from endogenous matrix/impurities was observed at the retention time of the analytes and internal standards.

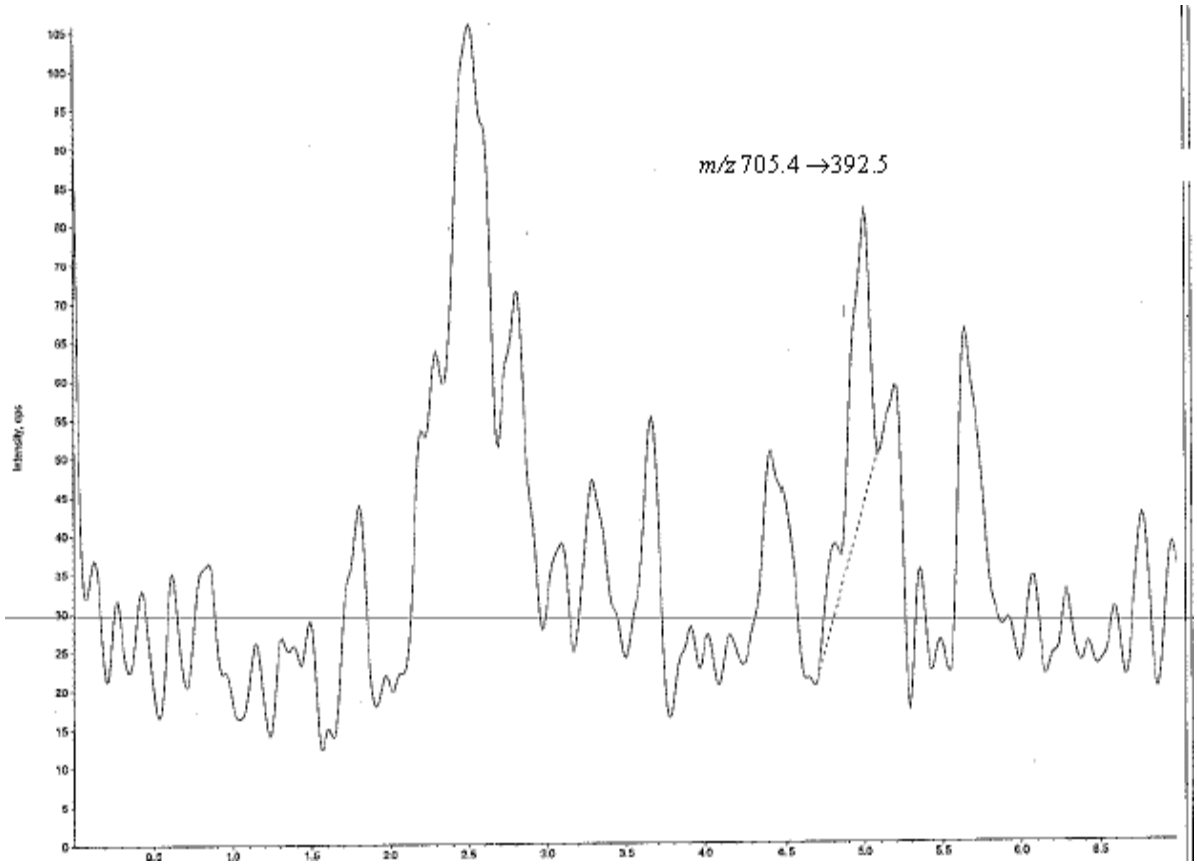

Fig. 2(a): Representative LC-MS/MS chromatogram of extracted blank plasma sample

Linearity

The calibration curves were linear from 0.301 to 151.655 ng/ml for itraconazole and 0.946 to 224.908 ng/ml for hydroxy itraconazole. The limit of quantitation was 0.303 ng/ml for itraconazole and 0.946 ng/ml for hydroxy itraconazole. Each batch of spiked plasma samples included one complete set of calibration curve standards and six replicates of quality control samples at LOQQC, LQC, MQC and HQC levels. Linearity for calibration standards (n = 8) for 3 days was assessed by subjecting the spiked concentrations and the respective peak area ratios to linear regression analysis ($y = mx + c$) with different weighting schemes (1/x, $1/x^2$ and none). The best linear fit and least squares residuals for the calibration curve were achieved with a $1/x^2$ weighing factor. The 'r' values were consistently 0.99 or greater during the course of validation.

The calibration curve data for itraconazole and hydroxy itraconazole is presented in Table 1(a) and Table 1(b).

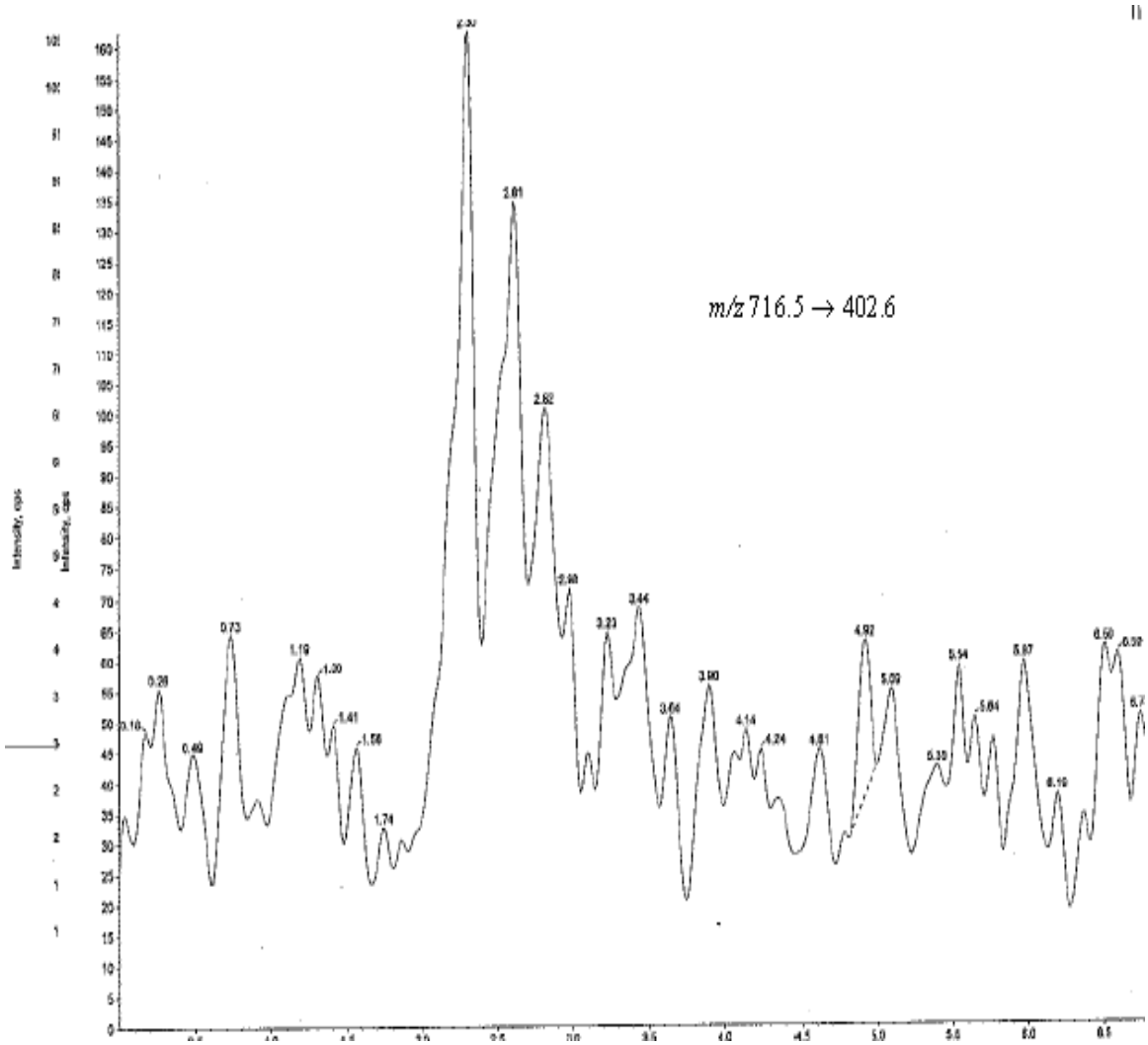

Fig. 2(b): Representative LC-MS/MS chromatogram of extracted blank plasma sample

Back calculated calibration curve concentrations for Itrconazole											
CC-ID	Nominal concentration (ng/ml)								Slope	Intercept	r
	0.301	0.819	1.952	14.786	29.573	59.145	118.291	151.655			
1	0.288	0.933	1.837	14.718	29.908	60.012	113.056	149.304	0.0237	0.00964	0.9977
2	0.301	0.868	1.678	15.126	30.195	56.782	125.804	153.756	0.0234	0.00044	0.9999
3	0.309	0.739	2.078	14.713	29.99	60.698	115.264	151.329	0.0242	0.00197	0.9986
Mean	0.2993	0.8467	1.8643	14.8523	30.0310	59.1640	118.0413	151.4630			
S.D (+/-)	0.01060	0.09874	0.20140	0.23702	0.14783	2.09119	6.81271	2.22902			
C.V. (%)	3.5	11.7	10.8	1.6	0.5	3.5	5.8	1.5			
% Nominal	99.4	103.4	95.5	100.4	101.5	100.0	99.8	99.9			

Table1(a): Calibration curve data for Hydroxy itraconazole

Back calculated calibration curve concentrations for Hydroxy Itraconazole											
CC-ID	Nominal concentration (ng/ml)								Slope	Intercept	r
	0.946	2.376	5.940	16.409	41.023	85.465	170.930	224.908			
1	0.901	2.699	5.793	15.713	40.362	86.112	166.884	227.353	0.00443	0.00047	0.9979
2	0.947	2.437	5.642	15.359	40.735	84.629	174.204	236.145	0.00452	0.00061	0.9991
3	0.972	2.277	5.555	15.911	41.045	89.672	172.358	236.341	0.00464	0.000735	0.9989
Mean	0.9400	2.4710	5.6633	15.6610	40.7140	86.8043	171.1487	233.2797			
S.D (+/-)	0.03601	0.21304	0.12043	0.27965	0.34198	2.59181	3.80690	5.13358			
C.V. (%)	3.8	8.6	2.1	1.8	0.8	3.0	2.2	2.2			
% Nominal	99.4	104.0	95.3	95.4	99.2	101.6	100.1	103.7			

Table 1(b): Calibration curve data for Hydroxy itraconazole

Precision and accuracy

The intraday precision and accuracy were evaluated by conducting replicate analysis of QC samples of itraconazole and hydroxy itraconazole on the same day.

Table 2(a): Intra day precision and accuracy for Itraconazole

Intra day precision and accuracy for Itraconazole				
	LOQ QC	LQC	MQC	HQC
Actual conc. (ng/ml)	0.303	0.801	57.184	121.669
Mean observed conc. (ng/ml) PA1	0.2927	0.7445	57.6180	119.6058
C.V. (%)	7.0	4.7	1.0	1.3
% Nominal	96.6	92.9	100.8	98.3
Mean observed conc. (ng/ml) PA2	0.2700	0.7844	60.6812	120.9815
C.V. (%)	15.3	6.3	2.8	3.0
% Nominal	89.1	97.9	106.1	99.4
Inter day precision and accuracy for Itraconazole				
Actual conc. (ng/ml)	0.303	0.801	57.184	121.669
Mean observed conc. (ng/ml)	0.3273	0.8292	58.3993	122.2022
C.V. (%)	17.6	8.5	1.8	1.6
% Nominal	108.0	103.5	102.1	100.4

Table 2(b): Intra day precision and accuracy for Hydroxy Itraconazole

Intra day precision and accuracy for Hydroxy Itraconazole				
	LOQ QC	LQC	MQC	HQC
Actual conc. (ng/ml)	0.946	2.299	85.145	181.159
Mean observed conc. (ng/ml) PA1	0.989	2.529	80.817	169.934
C.V. (%)	11.3	4.1	2.7	0.7
% Nominal	104.9	110.0	94.9	93.8
Mean observed conc. (ng/ml) PA2	0.993	2.561	84.383	172.350
C.V. (%)	14.1	5.1	2.8	3.2
% Nominal	105.3	111.4	99.1	95.1
Inter day precision and accuracy for Hydroxy Itraconazole				
Actual conc. (ng/ml)	0.946	2.299	85.145	181.159
Mean observed conc. (ng/ml)	0.791	2.284	82.300	171.856
C.V. (%)	18.3	8.0	2.9	1.4
% Nominal	83.9	99.3	96.7	94.9

The run consisted of a calibration curve (consisting of two blank plasma, two blank plasma with internal standard and eight different non-zero concentrations) and six replicates each of LLOQ, low, middle, and high concentration quality control samples. For determining the inter-day

accuracy and precision, analysis of three batches of QC samples was performed on different days. Precision of the assay was measured by the percent coefficient of variation over different concentration levels. The accuracy of the assay was defined as the ratio of absolute value of the calculated mean values of the quality control sample to their respective nominal values, expressed as percentage. Intra and interday precision and accuracy data is summarized in Table 2(a) and Table 2(b).

Recovery

The percentage recovery of itraconazole and hydroxy itraconazole was determined by measuring peak area response of six replicates after extraction of QCs containing low, middle and high drug concentration against the peak area response of six replicates of unextracted QCs containing equivalent concentrations. The recovery of itraconazole at LQC, MQC and HQC levels was 52.8%, 52.5% and 50.9% respectively and the recovery of hydroxy itraconazole at these levels was 50.4%, 53.7% and 57.1%, respectively. Extraction procedure yielded a mean recovery of 52.07% and 53.73% for itraconazole and hydroxy itraconazole respectively. The % CV of recovery across the low, middle and high quality control concentrations was 2.0% for itraconazole and 6.2% for hydroxy itraconazole. The percentage recovery of itraconazole-d9 and hydroxy itraconazole-d8 was determined by measuring the mean peak area response of six replicates of itraconazole-d9 and hydroxy itraconazole-d8 in extracted quality control samples (MQC) against the mean peak area response of six replicates of aqueous IS solution (unextracted) of equal concentration. The recovery of itraconazole-d9 and hydroxy itraconazole-d8 was 49.68% and 50.16% respectively.

Stability Studies

Freeze and Thaw Stability

The stability of spiked plasma samples was determined after three freeze thaw cycle by using four replicate each of stability and comparison quality control samples at low and high QC levels and degradation was determined against freshly spiked calibration curve and quality control samples (comparison samples). Thawing was performed at room temperature. Percent stability observed after three freeze thaw cycle ranged between 100.6-114.5% for itraconazole and 101.3-110.2 % for hydroxy itraconazole.

Bench Top Stability

The bench top stability evaluation involved analysis of four replicate each of stability and comparison quality control samples at low and high QC levels which had been kept at room temperature for a period of 7.50 hours (stability samples). These stability samples were processed along with the freshly spiked calibration curve and quality control samples (comparison samples). Concentration was calculated against the freshly spiked calibration curve. The comparative stability ranged from 99.5-108.4% for itraconazole and 98.5-100.1% for hydroxy itraconazole.

In-Injector Stability

In-injector stability of four replicate quality control samples was determined. The low and high QC samples (stability samples) kept in auto-injector at 5ºC were analyzed after around 48 hours and these samples were run along with the freshly spiked calibration curve and quality control samples (comparison samples). Concentration was calculated against the freshly spiked

calibration curve. The comparative stability ranged from 100.5-109.7% for itraconazole and 101.0-104.8% for hydroxy itraconazole.

Stock Solution Stability (Long-term and Short-term)

Stock solution stability (long term) of itraconazole and itraconazole –d9 was determined for 14 days. The stocks were kept in a refrigerator between 1-10 ºC. After the stability period, fresh stocks were prepared for itraconazole and itraconazole –d9. Samples containing same concentration were prepared from the stock solution and six replicate injections were given. Mean response obtained from the stability stock was compared with the mean response obtained from fresh stock. The percent stability observed for itraconazole was 104.0% and for itraconazole-d9 was 100.1%. Similarly stock solution stability (long term) for 14 days was established for hydroxy itraconazole and hydroxy itraconazole –d8. The percent stability observed for hydroxy itraconazole was 102.0% and for hydroxy itraconazole-d8 was 100.3%. The stability of itraconazole and itraconazole-d9 dilutions at room temperature (short-term) was checked by preparing dilutions of itraconazole and itraconazole-d9 as a mixture and also by preparing separate dilutions of itraconazole and itraconazole-d9. These dilutions were kept at room temperatures for about 17 hours. After 17 hours, the same dilutions were injected along with fresh dilutions of same concentration to check the stability. In case of analyte and internal standard mixture, the area ratio obtained for stability dilution was compared with area ratio obtained from fresh dilutions. The percent stability was 101.0%.

In case of solutions of itraconazole and itraconazole-d9 prepared separately, six injections of fresh and stability dilutions were made. Mean response was compared. The percent stability observed was 111.4% for itraconazole and 110.3 for itraconazole-d9.

To check the stability of hydroxy itraconazole and hydroxy itraconazole-d8 dilutions at room temperature (short-term), their solutions were prepared as a mixture and also separately. These dilutions were kept at room temperatures for about 17 hours. After 17 hours, the same dilutions were injected and compared with injections of fresh dilutions of same concentration. In case of analyte and internal standard mixture, the area ratio obtained for stability dilution was compared with area ratio obtained from fresh dilutions. The percent stability was 99.1%. In case of separate stability dilution of hydroxy itraconazole and hydroxy itraconazole-d8 six injections of fresh and stability dilutions were injected. Mean response was compared. The percent stability observed was 109.2% for hydroxy itraconazole and 110.2 for hydroxy itraconazole-d8.

Long term stability

Long-term stability of spiked plasma samples was determined. The low and high QC samples were stored below –15°C in cold room and below –50°C in deep freezer with Citrate Phosphate Dextrose Adenine (CPDA) and EDTA as anticoagulant for 360 days. These samples were analyzed against freshly spiked calibration curve. The percent stability ranged between 99.1-108.4%. No impact of these anticoagulants on the stability of itraconazole and hydroxy itraconazole was observed after storage for long period.

Matrix Effect

To determine the matrix effect, six lots of plasma (free from analyte and internal standard) were chosen. Concentration equivalent to LQC and HQC was spiked in each lot of plasma. At each

level, samples in duplicate were processed and analyzed for comparison with freshly spiked calibration curve. Precision and accuracy in each plasma lot at LQC and HQC was checked. Precision of 1.3-5.5% and accuracy between 95.5-101.2% was observed for itraconazole. Precision of 1.1-5.2% and accuracy between 94.1-108.3% was observed for hydroxy itraconazole.

Effect of anticoagulant

Different anticoagulants were used like CPDA, K2EDTA and K3EDTA. Effect of anticoagulant was determined by spiking 4 replicate of low and high concentration QC samples with plasma containing CPDA, K2EDTA and K3EDTA as anticoagulant. Calibration curve standards were spiked with plasma containing CPDA as anticoagulant and processed along with all the quality control samples as mentioned above. The mean concentrations were within ± 15% of the nominal concentrations at low and high QC concentration for each anticoagulant. Precision was ≤ 15% at both low and high QC concentration for each anticoagulant. These results suggests that the above mentioned anticoagulants had no effect in the analysis of itraconazole and hydroxy itraconazole in the present validated method and any anticoagulant vacutainer (CPDA, K2EDTA, K3EDTA) could be chosen for clinical sample collection for itraconazole pharmacokinetic study.

Matrix factor

Reference mixtures of internal standard(s) and analyte(s) at concentrations representing 100% extraction of internal standard and analyte at low, middle and high QC concentrations were prepared. These served as 'reference samples'. Twelve aliquots of blank samples were processed without addition of IS upto drying step. Four aliquots each were reconstituted with reference samples at low, middle and high QC concentrations. These samples served as 'matrix samples reconstituted with reference samples'. These samples were injected along with 4 replicates of each reference mixture of internal standard(s) and analyte(s) at low, middle and high QC level (total 24 samples). Matrix factor (MF) at each low, middle and high QC level was calculated by dividing mean peak area ratio of matrix samples reconstituted with reference samples by mean peak area ratio of reference samples. The % C.V. of matrix factor between low, middle and high QC level were found to be ≤ 15% and matrix factor was found to be approximately 1.00 for both itraconazole and hydroxy itraconazole.

CONCLUSION

The above analytical method described is validated for the determination of itraconazole and hydroxy itraconazole over a range of 0.301 to 151.655 ng/ml and 0.946 to 224.908 ng/ml using itraconazole-d9 and hydroxy itraconazole-d8, respectively,as internal standard in human plasma. The method offers significant advantages in terms of lower sample requirement with clean sample preparation using solid phase extraction. The method is fully validated as per the US FDA Guidelines and all the parameters of validation were within the acceptable limits. With simple solid phase extraction procedure, the method can be considered suitable for application to pharmacokinetic studies.

Application

The validated method was successfully employed for clinical sample analysis and establishing pharmacokinetic parameters of two formulations of itraconazole 100 mg capsule in 14 healthy male Indian volunteers using an open label, randomized, two-treatment, two period, single-dose study conducted under fasting condition. The pharmacokinetic parameters obtained are summarized in Table 3(a) and Table 3(b).

Table 3(a) Pharmacokinetic parameters of Itraconazole after administration of 100mg capsule in healthy human subjects

Pharmacokinetic parameters of Itraconazole 100mg capsule in healthy human subjects under fasting condition in Indian population										
	T_{max} (hr)		C_{max} (µg/ml)		AUC_{0-t} (µg.hr/ml)		AUC_{0-inf} (µg.hr/ml)		Half life (hr)	
Formulation	A	B	A	B	A	B	A	B	A	B
Mean	3.107	3.464	99.99	87.55	1128.32	1107.94	1244.0006	1240.2052	23.23	20.73
SD	1.0034	0.9086	65.67	70.60	767.33	913.70	942.99	1088.17	6.57	3.63

Table 3(b) Pharmacokinetic parameters of Hydroxy Itraconazole after administration of 100mg capsule in healthy human subjects

Pharmacokinetic parameters of Hydroxy Itraconazole 100mg capsule in healthy human subjects under fasting condition in Indian population										
	T_{max} (hr)		C_{max} (µg/ml)		AUC_{0-t} (µg.hr/ml)		AUC_{0-inf} (µg.hr/ml)		Half life (hr)	
	A	B	A	B	A	B	A	B	A	B
Mean	3.821	4.214	158.88	140.50	3199.19	3042.37	3385.37	3294.69	11.85	12.14
SD	0.9924	1.281	46.26	63.07	1992.52	2308.85	2320.16	2742.78	4.97	5.44

.

REFERENCES

[1] K. De Beule, G. Van, *Drugs*, **2001**, 61, 27.
[2] M.S. Saag, W.E. Dismukes, *Antimicrob Agents Chemother*, **1988,** 32, 1, 1.
[3] J. Heykants, A. Van Peer, V. Van de Velde, P. Van Rooy, W. Meuldermans, K. Lavrijsen, R. Woestenborghs, J. Van Cutsem, G. Cauwenbergh, *Mycoses*, **1989**, 32, 67.
[4] N.Isoherranen, K.L. Kunze, K.E. Allen, W.L. Nelson, K.E. Thummel, *Drug Metab Dispos*, **2004**, 32, 1121.
[5] I.E. Templeton, K.E. Thummel, E.D. Kharasch, K.L. Kunze, C. Hoffer, W.L. Nelson, N. Isoherranen, *Clin Pharmacol Ther*, **2008**, 83, 77.
[6] P.O. Gubbins, B.J.Gurley, J.Bowman, *J Pharm Biomed Anal*, **1998**, 16, 1005.

[7] C.H. Koks, R.W. Sparidans, G. Lucassen, K.M. Crommentuyn, J.H. Beijnen, *J Chromatogr B Anal Technol Biomed Life Sci*, **2002**, 767, 103.
[8] V. Srivatsan, A.K. Dasgupta, P. Kale, R.R. Datla, D. Soni, M. Patel, R. Patel, C. Mavadhiya, *J Chromatogr A*, **2004,** 1031, 307.
[9] J.W. Wong, U.R. Nisar, K.H. Yuen, *J Chromatogr B Anal Technol Biomed Life Sci*, **2003**, 798, 355.
[10] C. Kousoulos, G. Tsatsou, C. Apostolou, Y. Dotsikas, Y.L. Loukas, *Analytical Bioanalytical Chemistry*, **2006**, 384, 199.
[11] M. Yao, L. Chen, N.R. Srinivas, *J Chromatogr B Anal Technol Biomed Sci App,* **2001**, 752, 9.
[12] M. Vogeser, U Spohrer, X.Schiel, *Clin Chem Lab Med*, **2003**, 41, 915.
[13] 13. A. Carrier, J. Parent, J *Chromatogr B Anal Technol Biomed Sci App,* **2000**, 745, 413.

.

.