Bioanalytical methods for the determination of itraconazole and hydroxyitraconazole: overview from clinical pharmacology, pharmacokinetic, pharmacodynamic and metabolism perspectives

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ABSTRACT: Itraconazole represents an important therapeutic option for the treatment of fungal infections. Itraconazole undergoes rapid metabolism to form hydroxyitraconazole, which also contributes to the anti-fungal activity exhibited by the parent compound. Since both itraconazole and hydroxyitraconazole are effective inhibitors of cytochrome P450 (CYP) 3A4 and p-glycoprotein (pgp)-mediated efflux transporters, they have the potential to elicit drug–drug interaction with a number of CYP3A4 and/or pgp substrates. This review focuses on providing comprehensive details on the bioanalytical methods available for the quantitation of both itraconazole and hydroxyitraconazole. Additionally, it provides an overview of the clinical pharmacology (several case studies of drug–drug interactions), pharmacokinetics, pharmacodynamics and metabolism related aspects of itraconazole. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: itraconazole; hydroxyitraconazole; bioanalytical, pharmacokinetics; pharmacodynamics; anti-fungal; cytochrome P450; CYP3A4; p-glycoprotein

Introduction

Itraconazole, a classical member of the triazole class, is an important drug in our arsenal to treat fungal infections because it exhibits broad-spectrum anti-fungal activity (De Beule and Van Gestel, 2001; Poirier and Cheymol, 1998; Van Cutsem, 1989). The mechanism of action for its antifungal activity is believed to involve efficient inhibition of the fungal cytochrome P450 (CYP) enzyme known as 14-α-demethylase by itraconazole. This inhibition results in the blockade of the synthesis of ergosterol, an essential element of the cell membrane in propagating the growth of fungal and yeast colonies (Saag and Dismukes, 1988).

Interestingly, the metabolism of itraconazole, via the CYP3A4 enzymatic system, in the gastrointestinal tract and liver results in the formation of a hydroxylated metabolite known as hydroxyitraconazole (Heykants et al., 1989; Isoherranen et al., 2004). Since hydroxyitraconazole is an active metabolite, which is formed not only rapidly but exists in a much larger proportion in the systemic circulation as compared to itraconazole, its contribution is considered very vital for the claimed broad spectrum activity of the drug (De Beule and Van Gestel, 2001; Van Cutsem, 1989).

Itraconazole and its various metabolites (Figure 1), including hydroxyitraconazole, have been confirmed to be potent inhibitors of CYP3A4 isozyme (Templeton et al., 2008) and have been used as a tool to confirm the drug–drug interaction potential of a number of substrates such as imidafenacin, repaglinide, gefitinib, pioglitazone, aripiprazole, oxatadine, etizolam, bromazepam, simvastatin, prednisolone, methylprednisolone, cyclosporine, tacrolimus, sirolimus, loperamide, cimetidine, haloperidol, pero-spirome, nateglinide, 3-keto-desogestrel, telithromycin, brotizolam, lidocaine, rosuvastatin, budesonide and fexofenadine (Tateishi et al., 2008; Ohno et al., 2007, 2008; Kanayama et al., 2007; Saad et al., 2006; Niemi et al., 2005, 2006; Park et al., 2004, 2006; Masui et al., 2006; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005; Korhonen et al., 2004, 2006; Masui et al., 2005; Shi et al., 2005;}
et al., 2005; Kajosaari et al., 2005; Swaisland et al., 2005; Jaakkola et al., 2005; Kubo et al., 2005; Goto et al., 2005; Karyekar et al., 2004; Osaani et al., 2004; Ishigam et al., 2002; Ishigam et al., 2001; Lebrun-Vignes et al., 2001). A recent review documented the likely drug interactions that may occur while patients are being treated with the three major classes of antimicrobial agents: (a) macrolides and ketolide group (erythromycin, telithromycin, clarithromycin, etc.); (b) quinolones class (ciprofloxacin, moxifloxacin, levofloxacin, etc.); and (c) azole group (ketoconazole, itraconazole, fluconazole, voriconazole, etc.) (Shakeri-Nejad and Stahmann, 2006). The comprehensive review of Lipp (2008) brought into perspective the variable drug levels and the need for therapeutic drug monitoring during antifungal treatment using itraconazole, voriconazole or posaconazole. Recently, in another review the importance of pharmacodynamic aspects of antifungal treatment including the evaluation of plasma/serum concentration vs efficacy data has been captured eloquently for four antifungal drugs including itraconazole (Smith and Andes, 2008) Additionally, since CYP3A4-mediated metabolism is very prevalent among several xenobiotics, a general frame for prediction of CYP3A4-based drug–drug interaction has been proposed using the exposure increase (i.e. AUC) as a key parameter—in this report itraconazole along with other CYP3A4 inhibitors was used in the data evaluation for prediction (Ohno et al., 2007).

**Scope**

This review is intended to deliver the following: (a) to provide up to date information of bioanalytical methods available for the quantitation of both itraconazole and hydroxyitraconazole including assay conditions, parameters validated and succinct remarks for the tabulated assay; (b) to provide select case studies regarding the clinical pharmacological attribute of itraconazole/hydroxyitraconazole of causing CYP3A4/pgp inhibition—imedafenasin, cimetidine, fexofenadine enantiomers, brotizolam, and budesonide; (c) to provide some newer insights in the clinical pharmacokinetics, metabolism-related (discussion on stereochimistry) and pharmacodynamic-related attributes of itraconazole/hydroxyitraconazole; and (d) to provide some recent developments towards stereoselective separation of itraconazole and hydroxyitraconazole.

**Clinical Pharmacology-related Case Studies of Select Substrates**

**Case Study 1: Imidafenacin (Ohno et al., 2008)**

Imidafenacin, under development for the treatment of overactive bladder, is known to undergo metabolism by CYP3A4 and phase II conjugation (via UGT1A4) leading to the formation of major metabolites, namely M-2 (oxidative metabolite of imedafenasin) and N-glucuronide conjugate of imedafenasin, respectively. Based on the above considerations, a clinical study was designed to evaluate the pharmacokinetics of imedafenasin in the presence of itraconazole, a CYP3A4 inhibitor.

The study was carried out under steady-state conditions of itraconazole. Two single doses of imedafenasin were administered, one prior to itraconazole dosing (control) and one co-administered with itraconazole on day 8 of dosing. Both imedafenasin and M-2 plasma concentration levels were monitored for both treatments administered in the study. The pharmacokinetic data suggested that itraconazole enhanced both the rate of absorption (Cmax was increased by almost 32%) and the extent of absorption (AUC0–inf was increased by almost 78%) of imedafenasin and effectively reduced the apparent oral clearance values (57 L/h for control vs 32 L/h for itraconazole treated group). Although M-2 levels were quantified, the pharmacokinetic parameters were not reported in this study. A cursory evaluation of the plasma time concentration vs time plots suggested a diminished formation of the M-2 metabolite from imedafenasin as a result of inhibition of CYP3A4 enzyme by itraconazole. It was noted that co-administration of itraconazole could lead to an increased exposure of imedafenasin; however, this interaction was classified as a weak one.

**Case Study 2: Cimetidine (Karyekar et al., 2004)**

Cimetidine, which belongs to the H2-receptor antagonist class, is widely used for the management and treatment of gastrointestinal ulcers and heartburns. A large portion of the administered dose of the drug (~80%) was excreted via the renal route. The renal excretion rate was 3–4 fold greater than glomerular filtration rate and therefore there was a suggestion of the involvement of p-glycoprotein (pgp) efflux pumps for its efflux transport since cimetidine is an organic cation. Interestingly, itraconazole in addition to being an effective CYP3A4 inhibitor has been shown to inhibit pgp in both experimental models and in clinical studies with pgp substrates such as digoxin and quinidine. Based on the...
above facts a clinical study was designed to evaluate the effects of itraconazole on the pgp efflux-mediated renal elimination of cimetidine.

The study was conducted as an open label with two treatments in a sequential design: on day 1, cimetidine was administered intravenously (loading bolus dose for 1 min + followed by a maintenance infusion over 4 h) to achieve an average plasma concentration of approximately 0.7 μg/mL. On days 2–4, subjects were given oral doses of itraconazole. On day 5, intravenous dosing of cimetidine as described before was co-administered with the oral dosing of itraconazole. On both days 1 and 5, iothalamate was administered (per a standard protocol) for the continuous monitoring of glomerular filtration rate.

The pharmacokinetic results confirmed the inhibitory effect of itraconazole on the pgp-mediated renal excretion pathway of cimetidine. The total plasma clearance for cimetidine was reduced by almost 26% by itraconazole (655 mL/min on day 1 vs 485 mL/min on day 5). The sole contributor for the reduced plasma clearance of cimetidine was the renal tubular transport pathway blocked by itraconazole, which accounted for a reduction of almost 30% (day 1 value of 410 mL/min vs day 5 value of 310 mL/min). The measurement of glomerular filtration rate using iothalamate confirmed that there was no alteration in the glomerular filtration rate between days 1 and 5 and therefore the only explanation of the reduced clearance observed for cimetidine on day 5 was attributable for the blockade of the pgp-mediated renal secretion of cimetidine by itraconazole.

Case Study 3: Fexofenadine Enantiomers (Tateishi et al., 2008)

Fexofenadine, comprising of R and S enantiomers, is an anti-allergy drug. R/S fexofenadine is a known substrate for efflux transporters such as pgp, human organic anion-transporting polypeptide (OATP) 1A2 and OATP2B1. A recent report has suggested the possibility of stereoselectivity of fexofenadine in plasma levels favouring the R-enantiomer due to chiral discrimination imposed by the pgp transport pathway. Although itraconazole can effectively inhibit the pgp transporter-mediated pathway, pharmacokinetic data on its influence on racemic substrates such as fexofenadine have not been reported. Therefore, a clinical study was undertaken to evaluate how itraconazole could modulate the stereoselective pharmacokinetics of R/S-fexofenadine by the blockade of pgp transporters.

The study design followed a two-way, double-blinded, randomized crossover with a 2 week washout period in which a single dose of fexofenadine and/or a matching placebo was administered in healthy subjects. The employment of stereoselective assay procedure enabled the computation of the pharmacokinetic parameters for the two enantiomers of fexofenadine. Additionally, the pharmacokinetics of both itraconazole and hydroxyitraconazole was gathered as part of the investigation. The pharmacokinetic data confirmed the inhibitory role played by itraconazole on the pgp-mediated transport of the enantiomers of fexofenadine. The plasma concentrations of both enantiomers increased by the co-administered itraconazole (the exposure value was increased by a factor of 2.5–to 3-fold). While the elimination half-life values were largely not affected by the co-administered itraconazole, it appeared to decrease the volume of distribution of both enantiomers along with the observed reduced clearance of the two enantiomers. Therefore, it supported the theory that disposition of fexofenadine was under the control of pgp-mediated pathways, which could be influenced by an inhibitor of the pgp transporter. Interestingly, itraconazole’s inhibition of pgp transporter resulted in an altered enantioselectivity in the plasma R to S ratio of the enantiomers of fexofenadine (R/S ratio of 1.6 to 2.1 in the placebo phase was altered to 1.2 to 1.6), suggesting that itraconazole may possibly mute or reverse the observed enantioselectivity. However, at the doses studied in this clinical study, itraconazole did not completely eliminate the stereoselectivity in the pgp-mediated transport of fexofenadine. Overall, the interesting pharmacokinetic interaction data obtained in this study confirmed the role of pgp in the stereoselective disposition of fexofenadine. The modulation of pgp transport was made possible by the inhibitor, itraconazole, such that it muted the stereoselectivity observed in the pgp transport by favouring the transport of the S-enantiomer of fexofenadine. Some interesting revelations from the urine data collected in this study included: (a) greater urinary excretion of S-fexofenadine than R-fexofenadine; and (b) the influence of pgp inhibition on the urinary excretion rates and/or stereoselectivity in the process of renal transport, suggesting that further studies would be needed to understand the underlying mechanism(s).

Case Study 4: Brotizolam (Osanai et al., 2004)

Brotizolam, a triazolothienodiazepine derivative, is extensively used as a short-acting hypnotic agent. Brotizolam has been reported to have oral bioavailability of about 65% and it manifests both rapid and complete absorption following oral dosing. Interestingly, it has been reported to be completely metabolized to two active metabolites: (a) α-hydroxybrotizolam (equi-potency to brotizolam); and (b) 6-hydroxybrotizolam (lesser potency than brotizolam). Since the oxidative metabolic pathway has been catalyzed by CYP3A4 enzyme, potential for a drug–drug interaction existed by compound(s) that can inhibit CYP3A4 enzyme. Therefore, given the importance of this class of hypnotic agents, consideration was given to probe the in vivo effects on the pharmacokinetic disposition and pharmacodynamic attributes of brotizolam by the inhibition of CYP3A4 using itraconazole.

It was a double-blind, randomized, crossover study with two treatment phases separated by a six-week washout period. The subjects were dosed once daily for up to 4 days with either itraconazole or a matching placebo; on day 4, they received a single dose of brotizolam. Blood samples were collected for appropriate pharmacokinetic assessments. The pharmacodynamic battery of assessments included digit symbol substitution tests (DSST), visual analog scales and UKU side-effect rating scale (to measure sleepiness).

The co-administration of itraconazole produced a significant pharmacokinetic interaction such that the oral clearance of brotizolam was decreased by 5-fold as evidenced by almost 4- to 5-fold increased exposure value. The elimination half-life value of brotizolam was significantly prolonged (4.5 h with placebo vs 23 h with itraconazole). While the peak concentration appeared to be not affected, the time for attainment of peak concentration was longer after itraconazole treatment (0.75 h with placebo vs 3.2 h with itraconazole). The increased exposure to brotizolam in the presence of itraconazole was reflected in the pharmacodynamic scores computed for brotizolam. The psychomotor activity appeared to be more depressed and the evidence of counterclockwise hysteresis loops in several of the psychomotor scores showed severe impairment due to excessive of drug concentrations in the system.
Inhaled budesonide is effective for the management of asthma. Because lungs are not involved in the biotransformation of budesonide, the entire drug load reaching the lungs is completely bioavailable for systemic circulation. The inhaled bioavailability of budesonide reaches 20–30%, while its oral bioavailability is very poor (~10%), presumably due to excessive metabolism via the CYP3A4 enzyme. This study was carried out with the intent to evaluate the effects of itraconazole on the pharmacokinetic disposition and pharmacodynamic activity of an inhaled budesonide.

The study followed a double-blind, randomized, crossover design with a 4-week washout period between the two treatment phases. The subjects were randomized to either itraconazole or a matching placebo dosing schedule for 5 days (once a day dosing); on the fifth day budesonide was administered via the inhaled route (the administration was using an appropriate inhalation device and per a defined protocol). The blood samples collected at various time points ensured the measurement of pharmacokinetic parameters for budesonide and at the same time (i.e. coinciding with budesonide measurements) the plasma concentration of cortisol was also estimated.

The plasma levels of inhaled budesonide were drastically increased by itraconazole. This was reflected in both the rate and extent of absorption of budesonide. The total exposure for budesonide increased by about 4-fold following itraconazole dosing and the terminal half-life of 1.6 h (placebo) was prolonged to 6.2 h (itraconazole). As to be expected, there was almost 1:1 correlation of the observed pharmacokinetic effects with those of the pharmacodynamics. Therefore, the suppression of cortisol production was enhanced by the presence of itraconazole. Interestingly, it was shown that itraconazole by itself had no effect on the cortisol production and therefore the observed effect on cortisol production was only due to budesonide when the inhaled drug was coadministered with itraconazole.

Pharmacokinetics and Disposition

Intravenous Pharmacokinetics in Children and Adolescents (Abdel-Rahman et al., 2007)

In this interesting study, children aged between 3 and 17 years were given a single dose of itraconazole formulated using hydroxypropyl-beta-cyclodextrin (HP-β-CD). The investigation aimed to obtain a pharmacokinetic characterization of itraconazole and its metabolite in children who were at risk of fungal infections. Accordingly, the plasma profiles of itraconazole, hydroxyitraconazole and HP-β-CD were recorded in the study. The peak concentrations of itraconazole, hydroxyitraconazole and HP-β-CD were 1015 ng/mL, 293 ng/mL and 329 μg/mL, respectively, while the corresponding exposure data (i.e. AUC0–24h) were 4922 ng h/mL, 3811 ng h/mL and 641 μg h/mL. It was found that age difference did not influence the total body clearance of itraconazole nor the formation of hydroxyitraconazole. However, weak associations were noted between age and volume of distribution for itraconazole as well as age and rate of elimination. Based on the study, it was recommended that itraconazole could be dosed in adolescents and children of pediatric age group in a weight-normalized fashion without any safety and/or tolerability issues.

Population Pharmacokinetic Model for Prophylaxis Treatment of Febrile Neutropenia (Kanbayashi et al., 2008)

In this interesting work clinical pharmacokinetic data were collected from patients undergoing prophylaxis treatment for febrile neutropenia with an oral dose of 200 mg/day of itraconazole solution. The computed population parameters for itraconazole were 12.7 L/h, 333 L and 1.72 h⁻¹ for clearance, volume of distribution and absorption rate constants, respectively. The authors noted that the reported population PK in patients undergoing treatment for febrile neutropenia were different from that in the normal healthy population. Such data justified the development of a population pharmacokinetic model for itraconazole in the patient population. Accordingly, a trough concentration of >250 ng/mL was necessary for an effective prophylactic treatment of itraconazole in febrile neutropenia and it was observed that in almost 95% of the patients treated with a 200 mg/day dose of itraconazole there was the possibility of achieving the desired trough concentration of itraconazole.

Metabolism Related

Moity Responsible for CYP3A4 Inhibition—Preclinical Investigation (Quinney et al., 2008)

Quinney et al. (2008) have performed an elegant experiment in a rat model to evaluate the hepatic availability (Fh) of itraconazole—two aspects relating to time-dependency and concentration-dependency were evaluated after oral and duodenal itraconazole administration at two concentrations. Simultaneous pharmacokinetic samples were collected from multiple sites, namely: aorta, portal vein and hepatic vein. In this experiment, there appeared to be time lag in the complete inhibition of CYP by itraconazole (gradual increase from an initial Fh value of 0.2 to 1 was noted). On contrary, there appeared to be no time lag in the CYP inhibition by hydroxyitraconazole. As soon as hydroxyitraconazole metabolite entered the liver, the CYP inhibition appeared to be almost complete. Additionally, the presence of duodenal administration of itraconazole confirmed the intestinal formation of the hydroxy metabolite. Based on the plot of Fh vs liver input concentration of hydroxyitraconazole, a K value of approximately 38 no was estimated for CYP inhibition. Overall, this work confirmed that formation of hydroxyitraconazole was the key for a complete CYP inhibition and it was solely responsible for controlling the time course of hepatic CYP inhibition.

Itraconazole vs Metabolites—Contribution for CYP3A4 Inhibition: Clinical Investigation (Templeton et al., 2008)

In a clinical study, Templeton et al. (2008) investigated the contribution of itraconazole alone vs itraconazole plus all its metabolites for CYP3A4 inhibitory drug–drug interactions mediated by the oral dosing of itraconazole. The metabolites that were considered for this clinical investigation were: hydroxyitraconazole, keto-itraconazole and N-desalkylitraconazole. The daily oral dosing protocol of itraconazole lasted for 7 days and the clinical pharmacokinetic samples gathered in this analysis were analyzed for the concentrations of itraconazole and the three metabolites mentioned above. Using the liver microsomal inhibition rate constants and the steady-state free fraction (i.e. unbound) plasma concentrations, the authors predicted approximately 3.9-fold decrease in the hepatic intrinsic clearance of a standard CYP3A4
enzymatic substrate, which took into account the parent compound and the metabolites. Because of the availability of individual concentration data and the in vitro microsomal inhibition rate constants, it was unequivocally confirmed that the metabolite data were needed to correlate the in vitro and in vivo CYP3A4 inhibition as the exposure data of the parent alone was not adequate.

**In Vitro Metabolism and in Vivo Metabolic Disposition Work with a Focus on Stereoselectivity**

Although initial work using capillary electrophoresis technique suggested that itraconazole's metabolism is subjected to stereoselectivity as evidenced by the data obtained from a patient sample and confirmation obtained from an in vitro investigation (Breadmore and Thomann, 2003), it was only some years later that a full understanding on the stereoselective aspects of itraconazole's metabolism was determined. Kunze et al. (2006) have recently reported the metabolism of itraconazole in both in vitro and in vivo systems, with special emphasis on the stereoselectivity aspects, since itraconazole has multiple chiral centers and the drug used in therapy comprises a mixture of four stereoisomers.

**In Vitro Metabolism—Focus on Stereoselectivity (Kunze et al., 2006)**

The formation of hydroxyitraconazole was specific to a pair of stereoisomers, (2R,4S)-itraconazole (individual stereoisomers were 2R,4S,2'R and 2R,4S,2'S). The diastereomeric pair representing 2S,4R-itraconazole (i.e. 2S,4R,2'R and 2S,4R,2'S) did not form the hydroxymetabolite and additionally it was observed that depletion of this diastereomeric pair did not occur during the incubations. Interestingly, under identical experimental condition, the formation hydroxyitraconazole from (2R,4S,2'S) itraconazole was 5 times greater compared with the (2R,4S,2'R) itraconazole. Additionally, both 2R,4S,2'S and 2R,4S,2'R stereoisomers of itraconazole that led to the formation of hydroxymetabolite were found to involve in sequential metabolism to form the corresponding keto-itraconazole metabolite. Essentially, all four stereoisomers (2R,4S) of hydroxyitraconazole led to the formation of the keto-itraconazole metabolites. The two keto-itraconazole stereoisomers formed from the four hydroxyitraconazole stereoisomers carried the configurations 2R,4S,2'R and 2R,4S,2'S. Finally, keto-itraconazole was sequentially metabolized to N-desalkylitraconazole which carried the configuration of 2R,4S only.

**In Vivo Stereoselective Disposition and Pharmacokinetics (Kunze et al., 2008)**

Because of the difficulty of the separation of 2R,4S,2'R-itraconazole and 2R,4S,2'S-itraconazole via chromatography, the analytical data for these two pairs of stereoisomers were summed up together. The pharmacokinetic estimates for these two pairs of stereoisomers were calculated from the summed data of the isomers and reported in the study. In contrast the stereoisomeric pairs of 2S,4R,2'R-itraconazole and 2S,4R,2'S-itraconazole were chromatographically separable and interestingly were found to be superimposable and also reported to have identical pharmacokinetic properties. Overall author justified and reported the stereoselective pharmacokinetic parameters of the stereoisomeric pairs instead of each individual stereoisomer of itraconazole in this study. Accordingly, a profound stereoselectivity was noted between the 2S,4R-itraconazole and 2R,4S-itraconazole stereoisomeric pairs such that it favoured the 2S,4R-itraconazole on both day 1 and at steady state (day 7). The profound stereoselectivity that was observed on day 1 (almost 8-fold difference in exposure) appeared to reduce on day 7 (approximately only 2.5 fold difference in exposure was noted). Regardless of stereoselectivity changes between days 1 and 7, the accumulation of both stereoisomeric pairs was observed (2R,4S-itraconazole appeared to accumulate to a much higher proportion than the 2S,4R-itraconazole). Interestingly, the elimination half-life values for the two itraconazole stereoisomeric pairs were 2-fold different on both day 1 and day 7 (day 1 and day 7 values were 19 and 25 h for 2S,4R-itraconazole and 9 and 10 h for 2R,4S-itraconazole). The plasma samples from a single subject in this study were considered for the analysis of stereoselectivity of the metabolite(s) of itraconazole, namely hydroxyitraconazole, keto-itraconazole and the N-desalkylitraconazole. Both keto- and N-desalkyl circulating metabolites in plasma were found to have 2R,4S configuration. Since the hydroxyitraconazole stereoselectivity is complicated because of the likelihood of the formation of 8 stereoisomers, the determination of absolute configuration for the circulating metabolite was difficult; however, the in vivo formation of a specific isomer, namely of the configuration 2R,4S,2'S,3'S-hydroxyitraconazole was ruled out because this isomer could be isolated chromatographically from other stereoisomers of hydroxyitraconazole.

**Comparative Data on CYP Activities of Itraconazole vs Other Antifungal Drugs (Niwa et al., 2005)**

The CYP-related activity of five drugs, namely itraconazole, fluconazole, miconazole, voriconazole and micafungin, was investigated. The evaluation of CYP2C9 oxidative pathway was performed using tolbutamide as a substrate and CYP2C19 oxidative pathway was performed using mephenytoin as a substrate. The rank order of the various anti-fungal agents in terms of increasing potency (IC50 value) was as follows: >10 μM (itraconazole), 12.3 μM (fluconazole), 8.7 μM (voriconazole) and 0.33 μM (miconazole), for the S-mephenytoin 4'-hydroxylation and 30.3 μM (fluconazole), >10 μM (itraconazole), 8.4 μM (voriconazole) and 2 μM (miconazole) for the tolbutamide hydroxylation. Therefore, it appeared that miconazole was the strongest CYP2C9 and CYP2C19 inhibitor. It was also important to note that, despite high concentrations tested, micofungin did not catalyze CYP2C9- or CYP2C19-based hydroxylation reactions. Additionally, all the compounds were found to exhibit no evidence of mechanism-based CYP inhibition.

**Pharmacodynamics-related**

Espinel-Ingroff et al. (2008) compared the in vitro activities of voriconazole, itraconazole and amphotericin B against 590 molds obtained from 323 patients undergoing a phase 3 clinical evaluation for voriconazole. It was not surprising to see that Aspergillus specis dominated the isolates (~69%) and a distant second was the Scedosporium species (11.5%). The MIC90 values of itraconazole and voriconazole were the same for all Asperigillus species (0.5 mg/mL). Interestingly, voriconazole exhibited resistance pattern somewhat higher than that of itraconazole. However, a number of mold isolates appeared to be resistant to
amphotericin B. The isolates of Aspergillus that showed resistance development to itraconazole required higher MICs (range 1–16 mg/mL).

Lacroix and de Chavin (2008) have compared the antifungal activity of several drugs specially targeted at the clinical isolates of scytalidium dimidiatum and scytalidium hyalinum. The MIC ranges for itraconazole, posaconazole, voriconazole, terbinafine, caspofungin and amphotericin B were 0.03–16, 0.03–0.5, 0.06–2, 0.06–8 and 0.06–1. As evidenced by the data, there appeared to be a number of clinical isolates that may have developed resistance for itraconazole, in particular.

Morace et al. (2007) have evaluated the activities of itraconazole along with voriconazole and amphotericin B against various filamentous mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better fungal drugs exhibited good activity against the mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better fungal drugs exhibited good activity against the mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better fungal drugs exhibited good activity against the mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better fungal drugs exhibited good activity against the mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better fungal drugs exhibited good activity against the mold isolates. It was found that, although all antifungal drugs exhibited good activity against the mold isolates, numerically itraconazole appeared to have lower and better funga

Morace et al. (2007) have evaluated the activities of itraconazole on the lipoproteins in men undergoing treatment of serious fungal infection, onychomycosis. The treatment in this study lasted for two one-week cycles with a daily dose of 400 mg itraconazole. During the itraconazole treatment period there was a reduction in both total cholesterol (12%; p < 0.001) and LDL-C (17%; p < 0.001). At the same time, it increased the HDL-C by approximately 21% (p < 0.001). Interestingly, the computed ratio of LDL-C/HDL (a measure of atherogenic risk) decreased by 30%. The authors confirmed an increase in apoA-1 levels following the treatment with itraconazole.

Bioanalytical-related

Table 1 provides critical and comprehensive information on various aspects of the published methods inclusive of the validation parameters for the quantitative determination of itraconazole and its hydroxy metabolite. As noted in Table 1, various HPLC or liquid chromatographic–mass spectrometric assays have been used to support both preclinical and clinical studies pertaining to itraconazole.

Stereoselective Analysis Applicable to Plasma Samples

Breadmore and Thomann (2003) used capillary electrophoresis as an analytical tool for the successful separation of the stereoisomers of itraconazole and its major metabolite, hydroxyitraconazole. The method was fully optimized with regard to several important parameters, namely chiral selector selection (1% w/v sulfated β-cyclodextrin was found optimal), choice of organic solvent (33–36% v/v methanol appeared to give the best selectivity), other co-solvents to aid the solubility of itraconazole (i.e. the usefulness of PEG 4000 was confirmed more with methanol solvent than with ethanol solvent and PEG 4000 addition was important to separate the steroisomeric pairs at nearly baseline levels) and further tweaking of sensitivity (the addition of methanol in the reconstitution solvent appeared to improve the signals and a concentration of 90% v/v of methanol provided the best trade-off between high volatility vs loss in sensitivity). Overall, the method developed by Breadmore and Thomann (2003) was capable of separating two stereoisomeric pairs of itraconazole and four stereoisomers of hydroxyitraconazole for the first time and paved the way for identification of stereoselectivity in the metabolism of itraconazole.

Kunze et al. (2006) developed a HPLC method coupled with mass spectrometry (LC/MS) to analyze the stereoisomeric plasma levels of itraconazole and to determine the absolute configuration of the metabolites formed from itraconazole. The separation of stereoisomeric pairs of itraconazole was achieved on a Chiralpak AS-RH analytical column with a flow-rate of 0.2 mL/min. The mobile phase comprising of initially of 30% acetonitrile–70% aqueous 5 m ammonium acetate was run in a gradient up to 80% acetonitrile during the course of the chromatographic separation. The ions monitored as [MH+] included: m/z 705 (itraconazole), m/z 721 (hydroxyitraconazole), m/z 719 (keto-itraconazole) and m/z 649 (N-desalkylitraconazole). Only two of the four stereoisomers of itraconazole (25,4R,2′S- and 25,4R,2′R-itraconazole) were separated using this method. Since this was not possible to separate the eight available stereoisomers of hydroxyitraconazole, a partial separation of some of the stereoisomers of hydroxyitraconazole was possible by this method. The absolute configuration assignments for both keto-itraconazole and N-desalkylitraconazole peaks were based on the assumption of the rigid stereochemistry of the dioxolane ring; accordingly, (25,4R) stereoisomers eluted followed by (2R,4S)-stereoisomers.

Conclusions

Itraconazole and its metabolite hydroxyitraconazole are important drugs in our arsenal for treatment of fungal infections. Interestingly, the clinical pharmacology attributes of itraconazole and hydroxyitraconazole need to be considered when CYP3A4 and/or pgp substrates are co-administered with itraconazole because they can cause potent inhibition of CYP3A4 enzyme and/or pgp transport leading to a potential drug–drug interaction. The increased use of itraconazole as a mechanistic tool to probe and to understand the altered pharmacokinetics of CYP3A4/pgp substrates has been observed. The availability of numerous bioanalytical procedures would provide opportunities for therapeutic drug monitoring and/or evaluating the pharmacokinetics of itraconazole and hydroxyitraconazole in mechanistic, preclinical and clinical studies. The latest developments in the field, which involved probing of the stereochemistry aspects in the metabolism and clinical pharmacokinetics of itraconazole and hydroxyitraconazole, should provide further impetus for additional research in the field.

References


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<tr>
<td>Bharathi et al. (2008)</td>
<td>Itraconazole and hydroxyitraconazole; IS, fluconazole</td>
<td>LC–MS/MS—detection in positive ion mode for both ITZ and OH-ITZ using a MDS-Sciex API-4000 mass spectrometer, equipped with a Turbomass™ interface at 400°C. MRM monitoring the transition of the m/z 705.3 → 393.2 for ITZ, 721.4 → 408.3 for OH-ITZ, and 307.0 → 220.1 for IS.</td>
<td>Regression type—linear using weighting factor (1/x^2). Calibration range—0.5–263.63 and 0.49–255.88 ng/mL for ITZ and OH-ITZ, respectively. Accuracy—the percentage accuracy calculated was within 94.17–105.02% for ITZ and OH-ITZ, respectively. Precision—intra-day precision values were 0.98–4.68% for ITZ and 1.06–8.81% for OH-ITZ, respectively; while inter-day precision values were 3.86–6.80% for ITZ and 2.14–11.5% for OH-ITZ, respectively. Selectivity—no interfering peaks from endogenous compounds are observed at the retention times of analytes and IS in the human control plasma. Stability—predicted concentrations for each analyte at LQC and HQC samples deviated within ±15% of the nominal concentrations in a battery of stability tests, viz. in-injector (18 h), bench-top (10 h), repeated three freeze–thaw cycles and at −80 ± 10°C for at least for 15 days. The method can be useful for bioequivalence studies with desired precision and accuracy.</td>
<td>Method can be used to determine ITZ and OH-ITZ simultaneously, offers the highest sensitivity (0.5 ng/mL) using a simple SPE extraction procedure and commercially available IS. The LLOQ reported by us is 4-fold and 8-fold lower than the earlier reported LLOQ for ITZ and OH-ITZ, respectively. The method can be useful for bioequivalence studies with desired precision and accuracy.</td>
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<td>Analyte(s) and solvent</td>
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<td>Kousoulos et al. (2006)</td>
<td>Itraconazole and hydroxyitraconazole; IS, R51012</td>
<td>LC-MS/MS—turbo ion spray of the mass spectrometer was operated in the positive ionization mode MRM monitoring—m/z 705.3 → 392.1 for ITZ, m/z 721.3 → 408.2 for OH-ITZ and m/z 733.3 → 460.3 for the IS (R51012). Extraction—mixture of acetonitrile (ACN) and methyl t-butyl ether (MTBE) as the organic solvent. Chromatography—on a YMCPack ODS-A (C_{18}) (Schermbeck, Germany) analytical column (50 × 4.0 mm i.d), which was thermostatically controlled at 50°C. Mobile phase—the isocratic HPLC elution mobile phase was composed of 80% acetonitrile and 20% 10 mM ammonium acetate (v/v).</td>
<td>Regression type—linear regression using 1/x² weighting Calibration range—2–500 ng/mL for ITZ and 4–1000 ng/mL for OH-ITZ. Detection limit—2 ng/mL for ITZ and 4 ng/mL for OH-ITZ. Accuracy—intra-day accuracy was within 101.5–108.3%, and 85.1–97.9% for ITZ and OH-ITZ, respectively. Inter-day accuracy was within 100.6–105.6% and 96.4–102.5% for ITZ and OH-ITZ, respectively. Precision—intraday precision values were 1.42–12.73 and 6.54–9.88% for ITZ and OH-ITZ, respectively; while inter-day precision values were 1.42–10.40 and 2.47–13.29% for ITZ and OH-ITZ, respectively. Stability—predicted concentrations for each analyte at samples deviated within &lt;10% of the nominal concentrations in a battery of stability tests, auto-sampler, repeated three freeze/thaw cycles and at −20°C for at least for 30 days.</td>
<td>A semi-automated 96-well LLE, LC/MS/MS method for the simultaneous determination of ITZ and OH-ITZ in human plasma. The application of two liquid-handling robotic workstations greatly simplified the extraction process. Small quantity of human plasma used for analysis. Moreover, the mixture of ACN/MTBE was used as the extraction solvent, aside from eliminating the formation of the irregular emulsion observed when only MTBE was employed. The proposed method was applied to a bioequivalence study of ITZ, and it allowed the study to be completed in just 4 days.</td>
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<td>Redmann and Charles (2006)</td>
<td>Itraconazole and hydroxyitraconazole; IS, R51012</td>
<td>HPLC—protein precipitation acetonitrile, followed by 10 μL zinc sulfate solution (20% w/v) Chromatography—the column was a C_{18} Symmetry™ (3.9 mm i.d. × 150 mm) maintained at 30°C. The flow-rate was 1 mL/min Mobile phase—the mobile phase of methanol (75% v/v and water (25% v/v) was used for isocratic elution Retention times for ITZ, OH-ITZ and the IS were 4.7, 8.3 and 12.5 min, respectively</td>
<td>Regression type—linear regression using 1/c² weighting Calibration range—50–2000 ng/mL (R² &gt; 0.99). The lower limit of quantification (LLOQ) was set to 75 ng/mL. Accuracy—inaccuracy was −7.1 to −14.7% for ITZ and −0.1 to −9.7% for OH-ITZ. Precision—intraday and interday imprecision (CV%) was 4.8–17.3 and 6.3–16.6% for ITZ, and 4.6–17.9 and 7.02–18.4% for OH-ITZ. Stability—N/A. Absolute recovery—87.1–96.6% over a concentration range 75–1750 ng/mL for ITZ and 87.9–96.7% for OH-ITZ.</td>
<td>Method was applied for samples collected from 23 children for measurement of ITZ and OH-ITZ in plasma samples drawn from pediatric cystic fibrosis patients, who were prescribed itraconazole for treatment of allergic bronchopulmonary aspergillosis.</td>
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<td>Srivatsan et al. (2004)</td>
<td>Itraconazole and hydroxyitraconazole</td>
<td><strong>HPLC</strong>—fluorescence detection, <strong>Extraction</strong>—LLE: using a hexane–dichloromethane (70:30) mixture, after addition of loratidine as an internal standard (IS) <strong>Chromatography</strong>—a reversed-phase C18 column (250 × 4.6 mm) employing fluorescence detection (excitation, 264 nm; emission, 380 nm) <strong>Mobile phase</strong>—the mobile phase consisted of 0.01% triethylamine solution adjusted to pH 2.8 with orthophosphoric acid–acetonitrile (46:54)–isopropanol (90:10, v/v) at a flow-rate of 1.0 mL/min. Retention times for ITZ, OH-ITZ and IS were observed at 6.2, 8.2 and 19.1 min, respectively.</td>
<td><strong>Regression type</strong>—linear regression using $1/c^2$ ($c$ = concentration) weighting <strong>Calibration range</strong>—5–500 ng/mL ($r^2 &gt; 0.98$) <strong>LOQ</strong>—5 ng/mL for both analytes <strong>Accuracy</strong>—intra-day accuracies were −4.8–8.2 and −4.6–4.8% for ITZ and OH-ITZ, respectively; inter-day accuracies were −5.2–10.7 and 0.8–10.1% for ITZ and OH-ITZ, respectively <strong>Precision</strong>—intra-day precision values were 5.2–11.4 and 3.4–13.9% for ITZ and OH-ITZ respectively; while inter-day precision values were 5.8–12.1% and 9.4–14.7% CV or ITZ and OH-ITZ respectively <strong>Recovery</strong>—more than 70% for drug, metabolite and internal standard <strong>Stability</strong>—no significant loss of ITZ and OH-ITZ concentrations from studies of storing sample in autosampler for 50 h at 4°C, freeze/thaw after three cycles, kept on bench-top after 3 h at room temperature and long-term storage below −20°C after 237 days</td>
<td>The applicability of this method to pharmacokinetic studies was established after successful application to 35 subjects in a bioavailability study.</td>
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<td>Wong et al. (2003)</td>
<td>Itraconazole and hydroxyitraconazole</td>
<td><strong>HPLC</strong>—fluorescence detection at an excitation wavelength of 260 nm and emission wavelength of 365 nm <strong>Extraction</strong>—extracted from alcalinized plasma samples using a 3:2 (v/v) mixture of 2,2,4-trimethylpentane and dichloromethane <strong>Chromatography</strong>—a Genesis CN column (4 μm, 250 × 4.6 mm) with guard column was used for the chromatographic separation at room temperature <strong>Mobile phase</strong>—the mobile phase consisted of acetonitrile and 0.02 M potassium dihydrogen phosphate (50:50, v/v) and the mixture was adjusted to pH 3.0 with 5 M hydrochloric acid and delivered at a flow-rate of 0.9 mL/min Retention times for ITZ, OH-ITZ and IS were observed at 9.7, 6.7 and 10.8 min, respectively.</td>
<td><strong>Regression type</strong>—linear calibration range <strong>Calibration range</strong>—2.8–720 ng/mL for ITZ and 5.6–720 ng/mL for OH-ITZ ($r^2 = 0.999$) <strong>Sensitivity</strong>—LLOQ was 3 and 6 ng/mL for ITZ and OH-ITZ, respectively <strong>Accuracy</strong>—intra-day accuracies were −8.7–8.9 and −7.1–5.2% for ITZ and OH-ITZ, respectively; inter-day accuracies were 4.9–14.3% and −7.1–7.9% for ITZ and OH-ITZ, respectively <strong>Precision</strong>—within-day and between-day precision was &lt;12% CV for both analytes <strong>Recovery</strong>—&gt;85 for both analytes <strong>Stability</strong>—the itraconazole and hydroxyitraconazole in plasma samples were found to be stable for at least 3 months when stored frozen at −20°C, The methanolic standard solutions for both drugs were also stable for at least 6 months when stored at 4°C</td>
<td>The HPLC method was sensitive, specific and suitable to be used for determination of plasma ITZ and OH-ITZ in pharmacokinetic/bioavailability studies.</td>
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<td>Vogeser et al.</td>
<td>Itraconazole and hydroxyitraconazole; IS, R51012</td>
<td>LC-MS/MS—electrospray atmospheric pressure ionization in the positive mode was used MRM monitoring—m/z 705 → 392 for ITZ, m/z 721 → 408 for OH-ITZ and m/z 733 → 460 for the IS (R51012) Extraction—plasma (50 μL) was precipitated with acetonitrile followed by on-line solid-phase extraction with a Waters Oasis HLB column (25 μm, 1.0 × 50 mm). The elution for column wash was using water–methanol (95:5 v/v) at a flow-rate of 3 mL/min. After 1 min, the extraction column was switched in a back-flush mode to analytical column for HPLC separation and MS detection Chromatography—Li-Crospher C18 end-capped column (125 × 4 mm; 5 μm) Mobile phase—acetonitrile–2 mM ammonium acetate (80:20, v/v) delivered at a flow-rate of 0.85 mL/min Retention times for ITZ, OH-ITZ and IS were observed at 3.6, 2.9 and 3.9 min, respectively</td>
<td>Regression type—Linear Calibration range—10–10,000 ng/mL for ITZ and OH-ITZ (r &gt; 0.999) Sensitivity—LOQ was 10 ng/mL for ITZ and OH-ITZ. Accuracy—N/A Precision—&lt;5.2%CV for both analytes Recovery: &gt;90 for analytes and IS Stability—N/A</td>
<td>The method proved rugged, enables short turn-around times and is highly specific. Since there is growing evidence for the importance of therapeutic drug monitoring of itraconazole in the prophylaxis and treatment of invasive fungal infections, the method is of interest for a large number of tertiary care hospital laboratories</td>
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<td>Koks <em>et al.</em> (2002)</td>
<td>Itraconazole and hydroxyitraconazole; IS, R051012</td>
<td><strong>HPLC fluorescence</strong>—detection set at 265 and 363 nm for excitation and emission</td>
<td><strong>Regression type</strong>—linear fit with weight</td>
<td>The method was successfully employed to delineate the PK profile of ITZ and OH-ITZ in an HIV-infected patient. It demonstrated as a selective, and sensitive HPLC assay</td>
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**Extraction**—SPE; Supelclean™ LC-SCX cartridges conditioned using 1 mL methanol and 2 mL of 1.1% (v/v) perchloric acid in water (pH 1.5). A 0.5 mL aliquot of plasma sample with internal standard was loaded to the cartridge and washed with 0.01 M hydrochloric acid in methanol, 1.0 mL of 0.1 M phosphate buffer (pH 6.0) and 1.0 mL of distilled water, elution of the analytes was performed with 1.0 mL methanol **Chromatography**—Symmetry C\(_18\) column (100 × 4.6 mm, \(d_p = 3.5\) μm, average pore diameter = 10 nm), protected by a Symmetry C\(_18\) pre-column (20 × 3.8 mm, \(d_p = 5\) μm) **Mobile phase**—55% (v/v) of 30 mM acetonitrile and 45% (v/v) of 0.1% (w/v) triethylamine in water; the aqueous phase was adjusted to pH 3.0 using 85% (w/v) phosphoric acid. Flow-rate was 1.0 mL/min. Retention times for ITZ, OH-ITZ and IS were 3.9, 10.3 and 15.5 min, respectively

**Calibration range**—10–1000 ng/mL

**Accuracy**—ranged from 104 to 113% for ITZ and from 91 to 103% for OH-ITZ.

**Precision**—intra-assay precisions were <9% for ITZ and <8% for OH-ITZ.

**Sensitivity**—LLQ (lower limit of quantification) was 10 ng/mL. Absolute recovery—85% for ITZ and 86% for OH-ITZ between 77.4 to 83.2%. Stability—ITZ and OH-ITZ are stable for 4 h at ambient and 1 h at 56°C in plasma, for three days at ambient in exacted sample and 24 h at ambient for the final samples.
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<td>Yao et al. (2001)</td>
<td>Itraconazole; IS, nefazadone</td>
<td>LC-MS—detection by electrospray ionization in positive mode. All conditions such as curtain gas, ion source gas, collision gas ion spray voltage and turbo gas voltage were operated under optimal conditions SIM monitoring—itraconazole, m/z 705.2 for ITZ; m/z 470.4 for IS Extraction—plasma was precipitated with acetonitrile. Chromatography—BDS hypersil 3 μm ODS column (50 × 2 mm) coupled with a guard column. Column was maintained at room temperature Mobile phase—isocratic solvent comprising the mobile phase, acetonitrile, and 10 mM ammonium formate (pH 4, adjust by formic acid; 60:40, v/v). Flow-rate was 0.3 mL/min. Retention times for ITZ and IS were observed at 2.48 and 1.36 min, respectively</td>
<td>Regression type—linear regression using 1/x weighting Calibration range—4–1000 ng/mL ($r^2 = 0.994$) Accuracy—the mean predicted concentrations of the quality control (QC) samples deviated by less than 10% from the corresponding nominal values Precision—the intra-assay and inter-assay precision of the assay were within 8% relative standard deviation Absolute recovery—96.1% for ITZ over a concentration range of 4–1000 ng/mL concentration Stability—stable in autosampler at room temperature for at least 24 h</td>
<td>A sensitive, selective, accurate and precise HPLC procedure with single ion monitoring by single quadrupole mass spectrometer was developed. The method was successfully applied to a PK study in rat.</td>
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<td>Carrier and Parent (2000)</td>
<td>Itraconazole and hydroxyitraconazole; IS, miconazole</td>
<td>LC-MS—detection by electrospray ionization in positive mode. All conditions such as electrospray capillary (3.5 kV), drying and nebulizing gas and fragmenter voltage (140 V), were operated under optimal conditions. Dwell time was set for 100 ms for each analyte SIM monitoring—itraconazole, m/z 705.2 for ITZ; m/z 721.2 for OH-ITZ and m/z 417.0 for IS Extraction—LLE; 0.5 mL of dog plasma with addition of IS and phosphate buffer (pH 8.5) was extracted by 4 mL of methyl-tert-butyl ether. Organic phase was separated and evaporated to dryness under nitrogen Chromatography—analytical column was Zorbx SB-C18 (3 × 0.46 cm i.d.) with a 3.5 μm particle size; analytical column was kept at 25°C Mobile phase—consisted of acetonitrile–10 mM ammonium acetate, pH 3.5 (60:40, v/v). Isocratic elution approach was used Flow-rate was 1 mL/min. Retention times for OH-ITZ, ITZ and IS were 0.8, 1.9 and 2.8 min, respectively</td>
<td>Regression type—least-squares linear regression Calibration range—2.5–2000 ng/mL ($r &gt; 0.99$) for both analytes Accuracy—intra-day accuracies deviated within 15% of nominal values; inter-day accuracies and precision deviated within &lt;13% for ITZ and &lt;9.5% for OH-ITZ Precision—between-day precision values were 10.5% RSD for ITZ and 11.9% for OH-ITZ Extraction recovery—at two concentrations (100 and 800 ng/mL) the observed mean recovery was 70% for ITZ and OH-ITZ for OH-ITZ. The mean recovery for IS was 80% Stability—both analytes were stable in extracted and reconstituted samples at ambient temperature up to 8 h</td>
<td>A fast and sensitive LC–MS method for determining ITZ and OH-ITZ levels in dog plasma samples was developed. The method provides complete analysis of the two drugs within 3.5 min. The method was suitable for the determination of itraconazole and hydroxyitraconazole concentrations in dog PK studies.</td>
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Bioanalytical methods for the determination of itraconazole and hydroxyitraconazole

Gubbins et al. (1998) Itraconazole and hydroxyitraconazole; IS, sapraconazole

HPLC-UV—column effluent was monitored by ultraviolet absorbance detection (263 nm) Extraction—250 μL of human serum was precipitated with 1 mL acetonitrile. The supernate was transferred to clean tubes and the organic solvent evaporated to dryness at 60°C under a stream of nitrogen Chromatography—C₁₈ base-deactivated (250 × 4.6 mm i.d., 5 μm particle size) Alltech Alltima® column Mobile phase—the mobile phase consisted of acetonitrile, 0.05 M phosphate buffer (pH 6.7), and methanol (47:45:8 v/v). Flow-rate was 1.0 mL/min Retention times for OH-ITZ, SAP and ITZ were approximately 9.5, 11.0 and 21.0 min, respectively

Calibration range—25–1500 ng/mL and for both analytes with \( r^2 > 0.999 \) Accuracy—intra-day accuracies deviated within 12% of nominal values; inter-day accuracies and precision deviated within <14% of nominal values Precision—within-day precisions were <11.7% for ITZ and <10% for OH-ITZ; between-day precision values were <14% for ITZ and 10% for OH-IT. Stability—not reported

The described method has been used to monitor both ITZ and OH-ITZ serum concentrations in healthy volunteers, bone marrow transplant recipients, and patients suffering from blastomycosi

Cox et al. (1997) Itraconazole and hydroxyitraconazole; IS, R51012

HPLC—detection by fluorescence at excitation 260 nm and emission 365 nm Extraction—protein precipitation with methanol Chromatography—RCM C₁₈ column (8 × 100 mm, 4 μm particle size) Mobile phase—use water-acetonitrile-diethylamine (42:58:0.05, v/v) at a pH 2.45. Flow-rate 1.0 mL/min. Retention times for OH-ITZ, ITZ and IS were 7.4, 14.0 and 18.5 min, respectively

Calibration range—10–1000 ng/mL with \( r^2 > 0.997 \) for ITZ and \( r^2 > 0.994 \) Detection limit—5 ng/mL Accuracy—within-day accuracy was <10% for ITZ and between-batch accuracy was <9.4% of nominal values for OH-ITZ Precision—within-batch precision was <1.8% RSD for ITZ and <4.2% for OH-ITZ, while between-batch precision was 10% RSD Absolute recovery—>82% for ITZ and >83% for OH-ITZ at concentrations ranging from 10 to 1000 ng/mL

The method allows for rapid and efficient clean-up of small plasma and serum or tissue samples using HPLC analysis

Cociglio, et al. (1997) Itraconazole and hydroxyitraconazole; IS, R51012

HPLC—detection by UV Extraction—to 1 mL of blank sample were successively added 1 mL of spiking extraction solution and an excess (about 0.5 g) of solid potassium chloride Chromatography—Lichrocart cartridge (250 × 4 mm i.d.) filled with LiChrospher RP8 (5 μm) Mobile phase—the isocratic mobile phase was acetonitrile–water (55:45, v/v) at a flow-rate of 1.5 mL/min; the detection wavelength was 263 nm, and the analysis was performed at room temperature

Calibration range—0.06–5 mg/L Detection limit—30 ng/mL Accuracy—within-day accuracy was <10% for ITZ and between-batch accuracy was <9.4% of nominal values for OH-ITZ Precision—within-batch precision was <1.8% RSD for ITZ and <4.2% for OH-ITZ, while between-batch precision was 10% RSD Absolute recovery—~130% for ITZ, OH-ITZ and IS

The use of a spreadsheet program aided the development and validation of the assay for ITZ and OH-ITZ in serum samples. The proposed assay was used for therapeutic drug monitoring of ITZ and OH-ITZ

ACN, acetonitrile; CV, coefficient of variation; OH-ITZ, hydroxyitraconazole; HIV, human immunodeficiency virus; HQC, high-quality control sample; i.d., internal diameter; IS, internal standard; ITZ, itraconazole; LLE, liquid–liquid extraction; LLOQ, lower limit of quantitation; LQC, low quality control sample; MRM, multiple-reaction monitoring; MTBE, methyl tert-butyl ether; m/z, mass-to-charge ratio; N/A, not applicable; PK, pharmacokinetics; RSD, relative standard deviation; SAP, sapraconazole; SPE, solid-phase extraction.


Quinney SK, Galinsky RE, Jivamapa-Semsa VA, Chen Y, Hamman MA, Hall SD, Akamagbo-Njama RA and Ita. Hydroxyitraconazole, formed during intestinal first-pass metabolism of itraconazole, controls the time course of hepatic CYP3A inhibition and the bioavailability of itraconazole in rats. Drug Metabolism and Disposition 2008; 36: 1097–1101.

Raaska K, Niemi M, Neuvonen M, Neuvonen PJ and Kivisto KT. Plasma...
concentrations of inhaled budesonide and its effects on plasma cortisol are increased by the cytochrome P4503A4 inhibitor itraconazole. Clinical Pharmacology and Therapeutics 2002; 72: 362–369.