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CONFERENCE

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TITLE

Quantification of Itraconazole and Its Metabolite Hydroxyitraconazole in Human Plasma by LC-MS/MS

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ABSTRACT

Novel Aspect: A simple, rapid and reliable method for itraconazole and hydroxyitraconazole in human plasma using liquid-liquid extraction and LC-MS/MS

Introduction

Itraconazole is an anti-fungal drug and derives its medicinal properties by inhibiting the fungal enzymes that produce ergosterol, an important component of the fungal cell wall. Itraconazole is extensively metabolized by CYP3A4 to give side-chain hydroxylation product of hydroxyitraconazole as a major metabolite, which also posses anti-fungal properties. In our current study, we report the method for the determination of itraconazole and hydroxyitraconazole in human plasma employing liquid-liquid extraction and LC-tandem MS. Our data shows that the method reported here is rugged, enables short turn-around times and is highly specific.

Methods

Itraconazole and hydroxyitraconazole and deuterated internal standards were extracted from human plasma samples by liquid-liquid extraction using MTBE:ethyl acetate at 50:50(v:v) as extraction solvent. The supernatant was transferred into plastic disposable cultural tubes and evaporated to dryness in a 40°C bath under nitrogen stream. Then samples were reconstituted and chromatographed on a Synergi 4µ Polar-RP column (50 x 2 mm) using gradient mobile phase. The retention times were ~1.25 and ~1.5 min for hydroxyitraconazole and itraconazole. The analytes were detected and quantified by MRM on a Sciex API4000 tandem mass spectrometer under positive turboionspray mode.

Preliminary results

The assay was validated over concentration ranges of 1-500 ng/mL for itraconazole and 2-1000 ng/mL for hydroxyitraconazole. The intra-and inter-day precision (%CV) were found to be 0.7-7.2% and 1.3-5.9% respectively for itraconazole, and 0.8-6.9% and 1.9-7.2% respectively for hydroxyitraconazole. The intra- and inter-day accuracy (%RE relative error from the nominal) was determined as 3.7 to 9.1% and 4.1 to 8.4% for itraconazole and -2.8 to 10.2% and 1.8 to 8.9% for hydroxyitaconazole, respectively. The results indicate the method to be sensitive, selective, accurate, and reproducible. Itraconazole and hydroxyitraconazole were found stable in human plasma during room temperature bench-top storage, freeze-thaw-cycles, and freezer storage. This method provides a useful tool in quantification of itraconazole and hydroxyitraconazole in pharmacokinetic and bioequivalent studies.