Development and evaluation of a liquid chromatography tandem mass spectrometry method for the quantitation of isavuconazole in human serum samples

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Introduction

Therapeutic drug monitoring of isavuconazole, which is a novel broad spectrum antimycoticum against invasive fungal infections, ensures an effective exposure of the drug and minimizes the risk of toxicity (1). The aim of the present study was to evaluate a widely applicable LCMS method eligible for the use in clinical routine for the quantification of isavuconaole.

Methods

The method was performed on a Voyager TSQ Quantum triple quadrupole instrument equipped with an Ultimate 3000 chromatography system (Thermo Fisher Scientific, San Jose, California, USA). Chromeleon Xpress software for device management and LCQuanTM 2.7 for data processing were used. Isavuconazole and its deuterated isotope were kindly provided by Basilea (Basel, Switzerland). Calibrators and controls were prepared by spiking drug free plasma from healthy donors with stock solutions. In brief, 20 µL serum samples (calibrators or controls) were deproteinized by adding of icecold 100 µL of methanol containing the internal standard (isavuconazole-d4, 1000 ng/mL). After vortexing and centrifugation at 24,000 g (5 min), the clear supernatant was 10 fold diluted with 5% ACN. Ten µL were loaded on a trapping column (POROS™ R1 20, 2.1 x 30 mm, Thermo Fisher) with mobile phase 1 (5:95 v/v ACN/water). After a short washing period the analytes were transferred and separated on a Luna 5µm Phenyl – Hexyl column 100A 50 x 2.1mm (Phenomenex™ Aschaffenburg, Germany) with a linear gradient of mobile phase 2 (0.1% formic acid in MS grade water) and mobile phase 3 (0.1%formic acid in MS grade ACN). Isavuconazole and internal standard were monitored in a positive multiple reaction monitoring mode using characteristic precursor–product ion transitions: m/z 438.1→214.9 (438.1→368.9 as a second qualifier) and m/z 442.1→218.9 (442.1→372.9 as a second qualifier), respectively.

Figure 2: Chromatogram

Representative chromatogram of a patient with 3.63 µmol/L isavuconazole. Every peak has over twenty measurement points. Peaks are shown without smoothing. A: Isavuconazole: m/z 438.1→214.9 as the quantifier and 438.1→368.9 as a second qualifier. B: Internal standard isavuconazole d4: m/z 442.1→218.9 as the quantifier and 442.1→372.9 as a second qualifier.

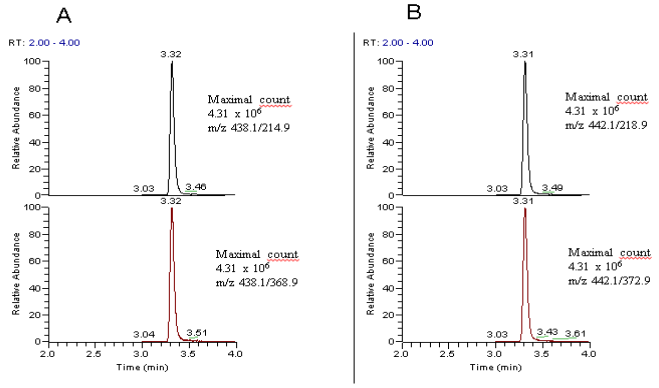
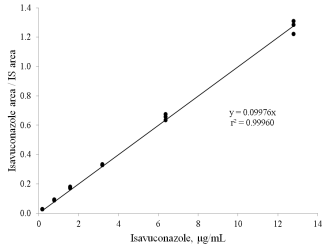


Figure 3: Calibration Curve and stability study

Calibration curve of isavuconazole:
y =0.0997 x, r² = 0.998. IS, internal standard



Stability measurements of isavuconazole at room temperature (RT)

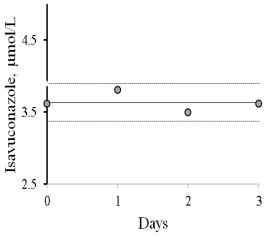
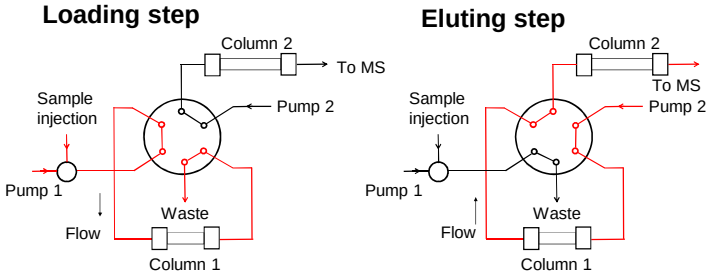


Figure 1: Chromatographic procedure



At initial time, 10 µL of the prepared sample is injected onto column 1. Immediately, the flow rate quickly increases to 2 mL/min to wash out interfering matrix components, with the end of column 1 shifting to waste. In the meantime, column 2 is equilibrating with mobile phase 2. After the washing period of column 1, the valve is switched to the second position. The analytes are then eluted in the back flush mode from the pre-column to the analytical column and are subsequently detected on a Voyager TSQ Quantum triple quadrupole instrument (Thermo Instruments, San Jose, USA).

Table 1: Method characteristics

Working range, µg/mL	**0.2 – 12.8**		
Calibration curve			
Slope	**0.0997**		
Intercept	**0.000**		
Correlation r^2	**0.9998**		
Intra-day precision n=6			
Mean µmol/L	**Control low**	**Control medium**	**Control high**
Standard deviation, SD µmol/L	**2.85**	**4.78**	**6.62**
Coefficient of variation, CV , %	**0.09**	**0.07**	**0.12**
	2.96	**1.40**	**1.78**
Inter-day precision n= 20			
Mean, µmol/L	**2.89**	**4.79**	**6.59**
SD, µmol/L	**0.09**	**0.09**	**0.10**
Coefficient of variation, CV, %	**3.01**	**1.81**	**1.53**
Recovery (mean extraction efficiency), % Internal standard 102.3 %	**93.9-102.7**		
Limit of quantification (LOQ)			
µmol/L	**0.10**		
SD, µmol/L	**0.006**		
Coefficient of variation , %	**5.7**		
Stability in the matrix tested	**Three days**		

Results

The method characteristics are shown in Table 1. Calibration curve was linear throughout the selected ranges. A chromatogram of a patient is shown in Fig. 2. The correlation coefficient (r^2) always exceeded 0.99 (Tab 1, Fig. 3). The lower limit of quantification (LLOQ) was 0.1 µmol/L. Within-day CVs were 2.9 % (2.9 µg/mL) and 1.5 % (6.6 µg/mL), and between-day CVs were 3.1% (2.9 µg/mL) and 1.8 % (6.6 µg/mL). On the lower limit of quantification (0.1 µg/mL) the coefficient of variation (CV) was <10%. Recovery and stability studies demonstrated at all levels satisfied results.

Summary

The method evaluated here is a reliable and robust diagnostic tool for the therapeutic drug monitoring of isavuconazole in daily clinical routine.

LITERATUR: (1) Jenks JD, Mehta SR, Hoenigl M. Broad spectrum triazoles for invasive mould infections in adults: Which drug and when?. Med Mycol. 2019: 57; S168-S178