Development and validation of a rapid High Performance Liquid Cromatography method (HPLC) for the determination of triazoles in human plasma.

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BACKGRAUND AND IMPORTANCE

The incidence of invasive fungal infections has increased significantly. Triazoles are the antifungals of choice in cases of pulmonary aspergillosis, but they have a high intra- and interindividual variability in pharmacokinetics and are associated with a large number of interactions, thus requiring analytical techniques that allow therapeutic drug monitoring to ensure the effectiveness and safety of these drugs.

OBJECTIVES

The aim of this study was the development and validation of a high-performance liquid chromatography (HPLC) methods for measuring voriconazole, isavuconazole and posaconazole in human plasma using tioconazole as an internal standard.

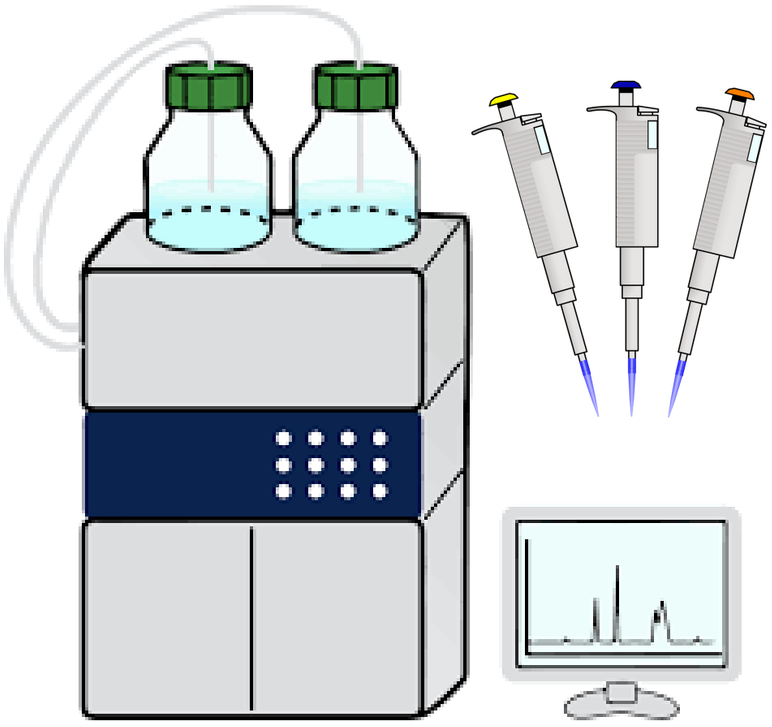
MATERIAL AND METHOD

The system consists of an Agilent® 1260 Infinity chromatograph with an ultraviolet diode array detector (UV-DAD). The column used was a Kinetex F5 4.6x150mm, 5 µm (Phenomenex®, USA). The method was validated according to the Food and Drug Administration (FDA) bioanalytical method validation guidance. The analysis run time for all drugs was 7.5 minutes. The chromatographic conditions are shown in table 1.

Table 1. Chromatographic conditions of methods.

Analyte	Mobile phase	λ (nm)	Calibration range (µg/mL)	Flow (mL/min)	Temperature(ºC)	Injection volume(µL)
Voriconazole	60% BUFFER /40% ACN	254	1-7	1	25	40
Isavuconazole	50% BUFFER /50% ACN	260	0.5-10	1	25	50
Posaconazole	50% BUFFER /50% ACN	260	0.3-1.5	1	25	50

*Buffer: KH2PO4 0.05 M pH=3.5



RESULTS

Table 2. Validation parameters according FDA guidance.

Analyte	Rt (min)	Equation	R^2	Within-day mean (µg/mL) (%VC)			Between-day mean (µg/mL) (%VC)		
				Low	Medium	High	Low	Medium	High
Voriconazole	3.7	y= 0.2363x-0.0083	0.9992	1.56 (3.6)	3.47 (1.3)	6.57 (0.6)	1.56 (4)	3.43 (1.5)	6.51 (0.6)
Isavuconazole	5.8	y= 0.6587x+0.0326	0.0999	1.38 (0.5)	4.82 (0.1)	9.19 (0.1)	1.43 (1.2)	5.14 (0.2)	9.12 (0.6)
Posaconazole	4.1	y= 0.6485+0.0008	0.9989	0.51 (0.8)	1.04 (1.27)	1.39 (0.8)	0.47 (1.23)	0.91 (2.3)	1.29 (1.24)

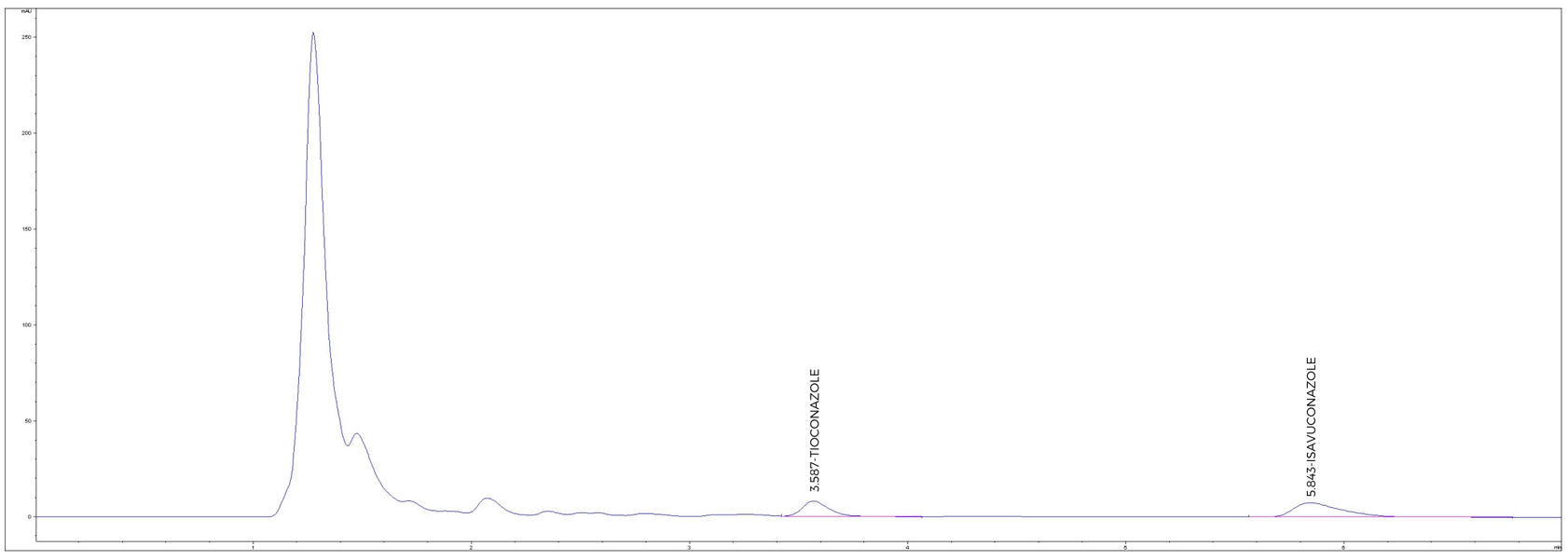


Figure 1: Isavuconazole Chromatogram.

CONSLUSIONS

A method has been validated for the determination of azoles by HPLC in human plasma that will allow TDM to be performed in target patients.