Simultaneous Quantification of Triazoles in Plasma by HPLC with Bond Elut Plexa SPE

Application Note

Clinical Research

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Introduction

The pharmacokinetics of systemic antifungal triazoles are characterized by a large number of intra- and inter-individual variations that can cause a great deal of drug interaction. Therefore, the measurement of triazole plasma levels is necessary. Until recently no method existed for the simultaneous detection and quantification of antifungal triazoles. The goal of this research was to develop a fast and sensitive method to analyze five systemic antifungals (fluconazole, voriconazole, ketoconazole, posaconazole, and itraconazole) and triazole's metabolite hydroxy-itraconazole, in human plasma for preclinical and biopharma studies. This application note describes analysis of triazoles using automated solid phase extraction (SPE) and HPLC analysis with UV detection.

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Materials and Methods

Sample preparation

The SPE process was automated on an ASPEC XLi system using Agilent Bond Elut Plexa cartridges.

1. Add 300 µL plasma to 750 µL internal standard linezolide (3 µg/mL), pH 2.
2. Condition Bond Elut Plexa sorbent with 1 mL MeOH and 1 mL H_2O.
3. Apply 1 mL sample.
4. Wash with 1 mL 1% NH_4OH, 1 mL H_2O:MeOH (70:30).
5. Elute with 1 mL MeOH.
6. Evaporate with N_2 at 50 °C.
7. Redilute in 80 µL H_2O:MeOH (50:50).

Analysis was conducted as recommended by the US Federal Drug Administration (FDA) Bioanalytical Method Validation [1].

Conditions

Column:	Phenyl C6, 4.6 × 150 mm		
Sample prep:	Agilent Bond Elut Plexa, 30 mg (p/n 12109301)		
Eluent:	A: Na_2HPO_4 buffer, pH 7 B: acetonitrile		
Injection volume:	25 µL		
Gradient:	Time (min)	B%	Flow rate (mL/min)
	0	25	1.0
	4	25	1.0
	5	55	1.1
	11	55	1.1
	12	80	1.3
	14	80	1.3
	15	25	1.0
	19	25	1.0
Detector:	UV, 210 nm, 12 nm bandwidth for fluconazole; 260 nm, 12 nm bandwidth for the other analytes		

Results and Discussion

The developed analysis, which meets the criteria of the FDA validation, is reliable and reproducible and can be used for quantitative measurement of analytes in biological matrixes. Figures 1 to 6 show the calibration curve, quality control, blank plasma, and chromatograms of plasma samples treated with the triazoles. Table 1 reveals the validation data accuracy, relative standard deviation, and concentration observed during daily sampling of these antifungal compounds. The analysis time was less than 15 minutes.

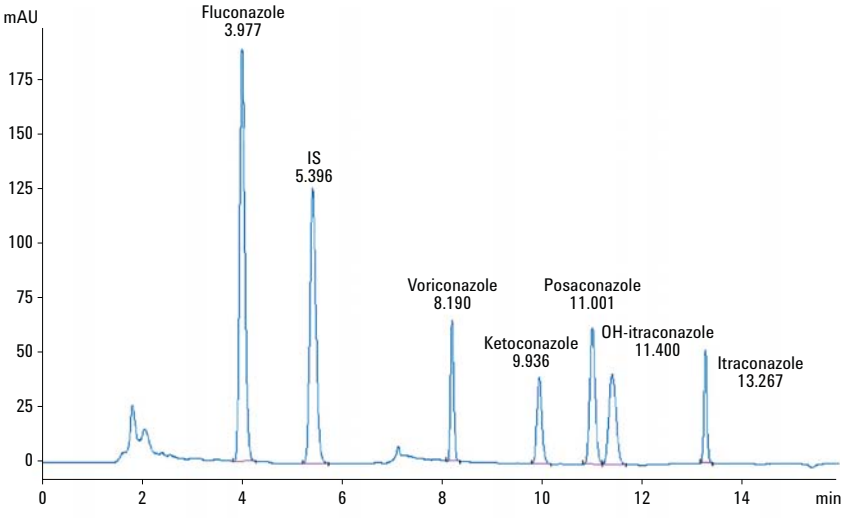

Figure 1. Chromatogram for quality control.

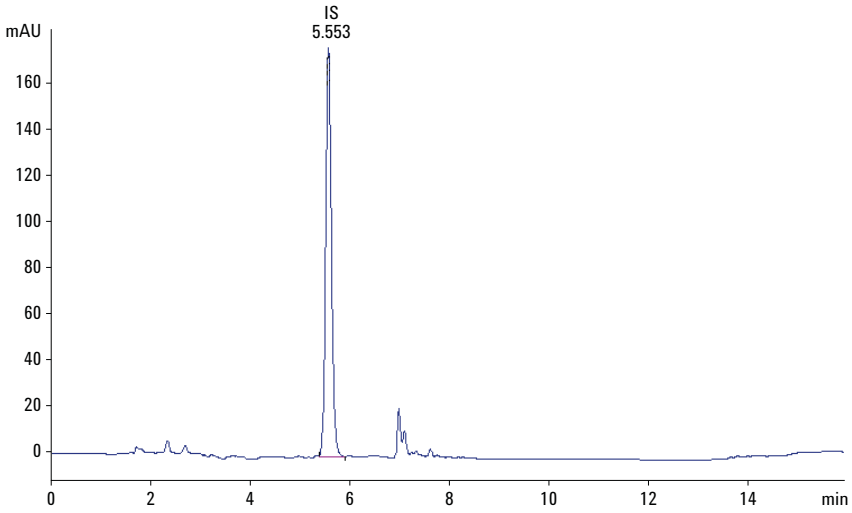

Figure 2. Chromatogram of a blank plasma sample after SPE extraction with Agilent Bond Elut Plexa.

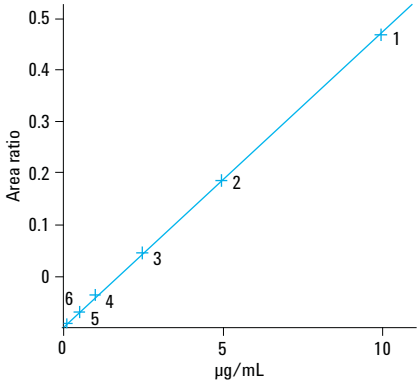

Figure 3. Calibration curve for voriconazole (R^2 = 0.9998). 1 = 10 µg/mL, 2 = 5 µg/mL, 3 = 1 µg/mL, 4 = 0.5 µg/mL, 5 = 0.25 µg/mL, 6 = 0.1 µg/mL.

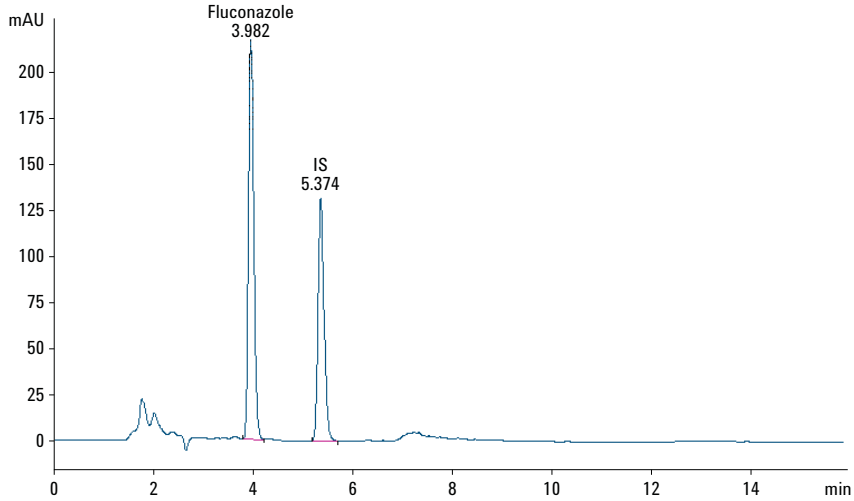

Figure 4. Chromatogram of a plasma sample with fluconazole.

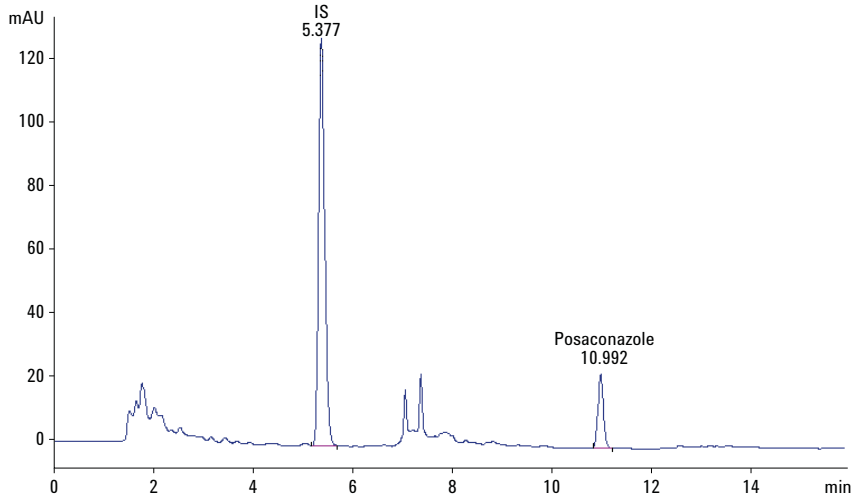

Figure 5. Chromatogram of a plasma sample with prosaconazole.

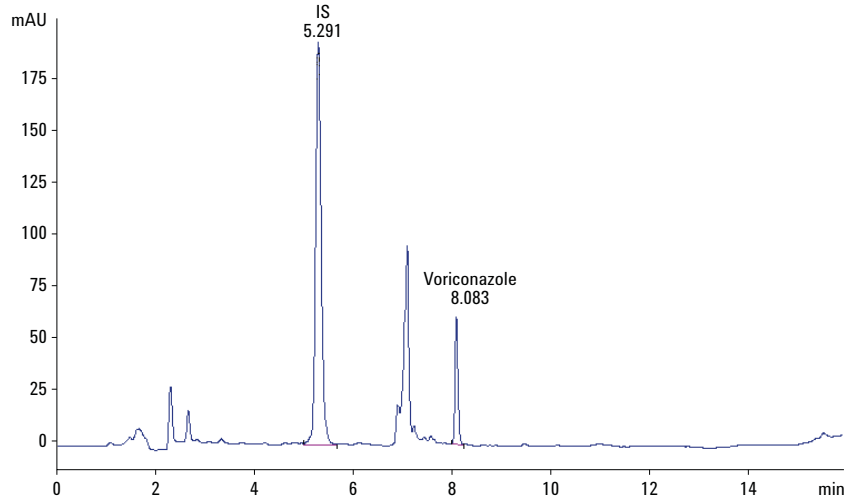

Figure 6. Chromatogram of a plasma sample with voriconazole.

Conclusions

Selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites are critical for the successful conduct of preclinical and biopharmaceutical studies. The fast, specific, and sensitive method shown in this application note is ideal for clinical research applications into the optimization of systemic antifungals. Automation of the method saves valuable time by using the same analytical conditions for all antifungal determinations.

Reference

1. US Department of Health and Human Serices Food and Drug Administration. Guidance for Industry. Bioanalytical Method Validation, (May 2001).

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Table 1. Daily validation data from the analysis of five antifungal triazoles and the metabolite itraconazole (n = 6).

Triazole	Concentration (µg/mL)	Accuracy (%)	RSD (%)
Fluconazole			
0.15	0.161 ± 0.011	107.3	6.83
3.75	3.670 ± 0.021	97.9	0.57
15.00	15.270 ± 0.253	101.8	1.66
Voriconazole			
0.15	0.143 ± 0.012	95.3	8.39
1.50	1.510 ± 0.004	100.5	0.24
7.50	7.350 ± 0.107	98.0	1.46
Posaconazole			
0.15	0.147 ± 0.014	98.0	9.52
1.50	1.540 ± 0.123	104.0	7.97
7.50	7.250 ± 0.406	96.7	5.61
Ketoconazole			
0.15	0.162 ± 0.010	108.0	6.25
1.50	1.450 ± 0.088	96.7	6.07
7.50	7.460 ± 0.309	99.5	4.14
Hydroxy-itraconazole			
0.30	0.311 ± 0.034	110.3	10.27
1.50	1.540 ± 0.141	102.7	9.16
7.50	7.590 ± 0.445	101.2	5.86
Itraconazole			
0.30	0.285 ± 0.029	95.0	10.18
1.50	1.570 ± 0.132	104.7	8.41
7.50	7.400 ± 0.512	98.7	6.92

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