Simultaneous Analysis of Antifungals in Plasma by SPE with Agilent Bond Elut Plexa and an Agilent 1260 Infinity LC/MS/MS

Application Note

BioPharma

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Abstract

Multicompound analysis of six systemic antifungal drugs in human plasma is effectively achieved using an Agilent LC/MS/MS system composed of an Agilent Bond Elut Plexa polymeric sorbent, an Agilent Pursuit XRs$^{Ultra\ 2.8}$ Diphenyl column, and an Agilent 1260 Infinity LC Triple Quadrupole LC/MS. Agilent Bond Elut Plexa delivered extremely clean samples, which was demonstrated by detection down to pg levels. All compounds showed near 100% recovery and a single digit % RSD. Superb linearity was achieved in the calibration curves with $R^2 \geq 0.995$ for all compounds.

Introduction

Systemic antifungal medications are designed to treat fungal infections in the human body. Many single-compound analyses have been reported; however, multicompound analyses with low detection limits have limited availability and have not yet been established [1-4]. The Agilent high quality, mono-disperse polymeric solid phase extraction (SPE) plate, Bond Elut Plexa, delivers clean samples to achieve extremely low-picogram (pg) detection limits of multi-antifungal drugs in human plasma. This application note presents good recovery, linearity, and precision data on antifungal drugs in human plasma.

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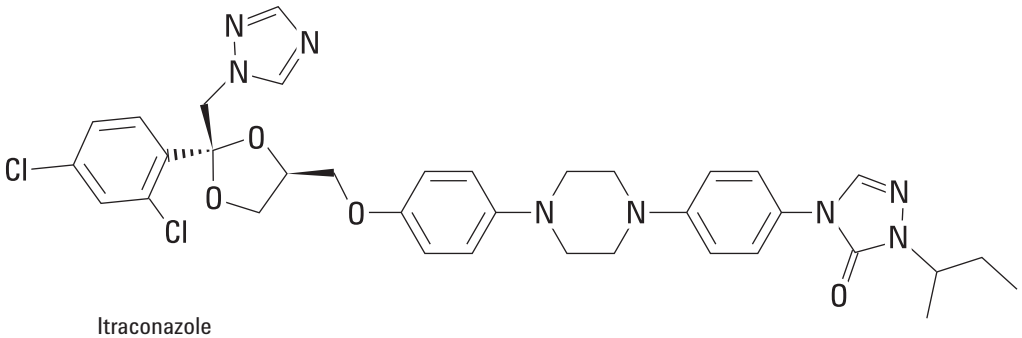
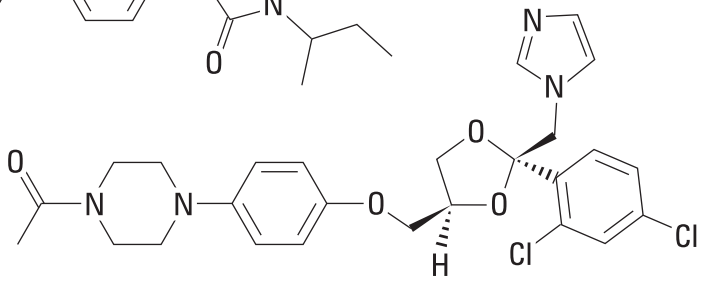
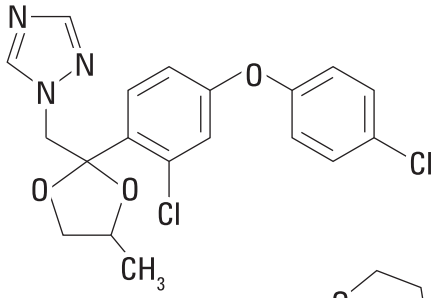
Figure 1 shows examples of systemic antifungal drugs.



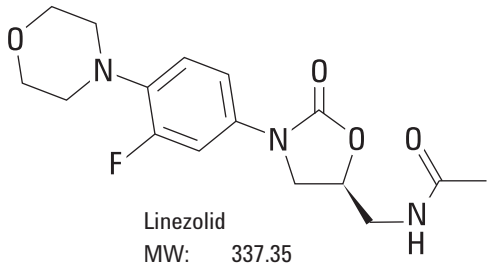
Itraconazole
MW: 306.27
log P: 5.66



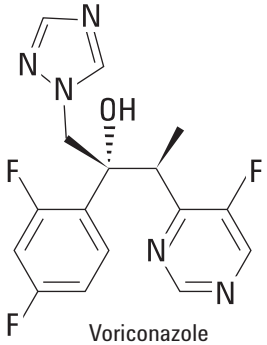
Ketoconazole
MW: 531.43
log P: 4.35



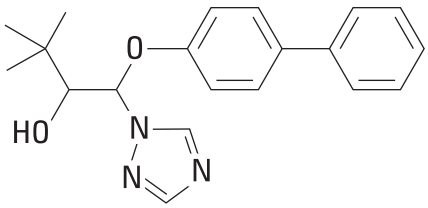
Difenoconazole
MW: 406.26
log P: 4.30



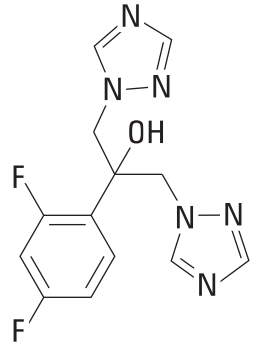
Linezolid
MW: 337.35
log P: 0.90



Voriconazole
MW: 349.31
log P: 1.80



Bitertanol
MW: 337.42
log P: 4.16



Fluconazole
MW: 306.27
log P: 0.40

Figure 1. Structures of some systemic antifungal drugs.

Materials and Methods

SPE reagents and solutions

SPE sorbent:	Agilent Bond Elut Plexa 96-well plate (10 mg) (p/n A4969010)
Sample:	100 µL human plasma spiked with antifungals*
Ammonia:	2% aqueous ammonia add 1 mL of NH_4OH to 50 mL of water
Methanol:	reagent grade or higher, 10% MeOH add 10 mL of MeOH to 90 mL of water

SPE method

1.	Pretreatment	Dilute with 300 µL 2% aqueous ammonia
2.	Condition	1. 500 µL MeOH 2. 500 µL H_2O
3.	Load	400 µL diluted sample from pretreatment (100 µL of actual plasma)
4.	Wash	500 µL 10% MeOH
5.	Elute	Twice with 250 µL MeOH
6.	Evaporate/ reconstitute	Evaporate under gentle air stream at room temperature and reconstitute in 100 µL 30% MeOH

*Difenoconazole, internal standard (IS), was spiked at 50 ng/mL in all samples

Calibration samples were prepared from 0.05 to 100 ng/mL in human plasma at 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL. For itraconazole, linezolid, and betertanol, a concentration range of 0.5 to 100 ng/mL was used due to detection limits.

LC/MS conditions

Column:	Agilent Pursuit XRs$^{Ultra\ 2.8}$ Diphenyl, 2.0 x 50 mm (p/n A7521050X020)	
Instrument:	Agilent 1260 Infinity Triple Quadrupole LC/MS	
Mobile phase A:	0.1% formic acid in H_2O	
Mobile phase B:	MeOH	
Flow rate:	0.4 mL/min	
Injection volume:	5 µL	
Gradient:		
Time :	(min)	% B
	0	30
	2	85
	4	95
	5	95
	5.1	30
	7.0	30
Temperature:	ambient, sample and column	
Ion source:	ESI+ with Agilent Jet Stream Technology (JST) enhanced electrospray source	
Gas temperature:	300 °C	
Gas flow:	13 L/min	
Nebulizer:	45 psi	
Sheath gas temperature:	350 °C	
Sheath gas flow:	12 L/min	
Capillary:	4,000 V	

Samples:

Antifungal	log P	MS/MS transition	Collision energy	Fragmentor
Fluconazole	0.40	307.1 → 238.1	12	92
Ketoconazole	4.35	531.2 → 82.2	50	164
Itraconazole	5.66	705.3 → 392.2	36	200
Linezolid	0.90	338.2 → 148.1	44	128
Voriconazole	1.80	350.1 → 281.1	12	92
Bitertanol	4.16	338.2 → 70.1	4	128
Difenoconazole (IS)	4.30	406.1 → 251.0	24	128

Results and Discussion

Figure 2 shows all compounds were baseline-separated chromatographically with good retention using the Agilent Pursuit XRs$^{Ultra\ 2.8}$ Diphenyl column. Excellent detection limits were achieved for most compounds with the Agilent 1260 Infinity Triple Quadrupole LC/MS, down to the 0.05 ng/mL level in human plasma.

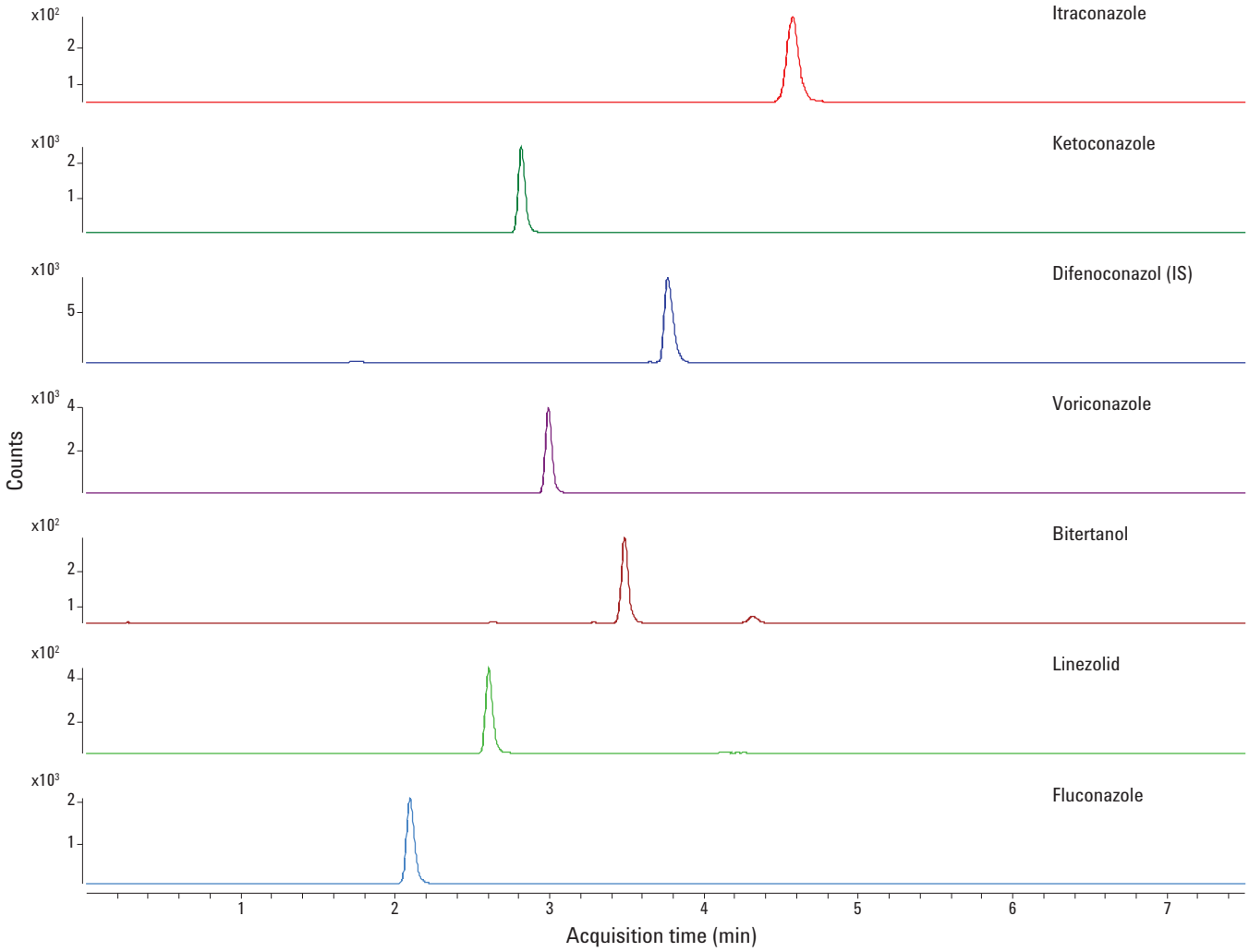


Figure 2. MS chromatogram for antifungal drugs at 10 ng/mL spiked in human plasma (IS was spiked at 50 ng/mL).

All compounds showed great linearity with $R^2 \geq 0.995$ over the entire calibration range (Figure 3).

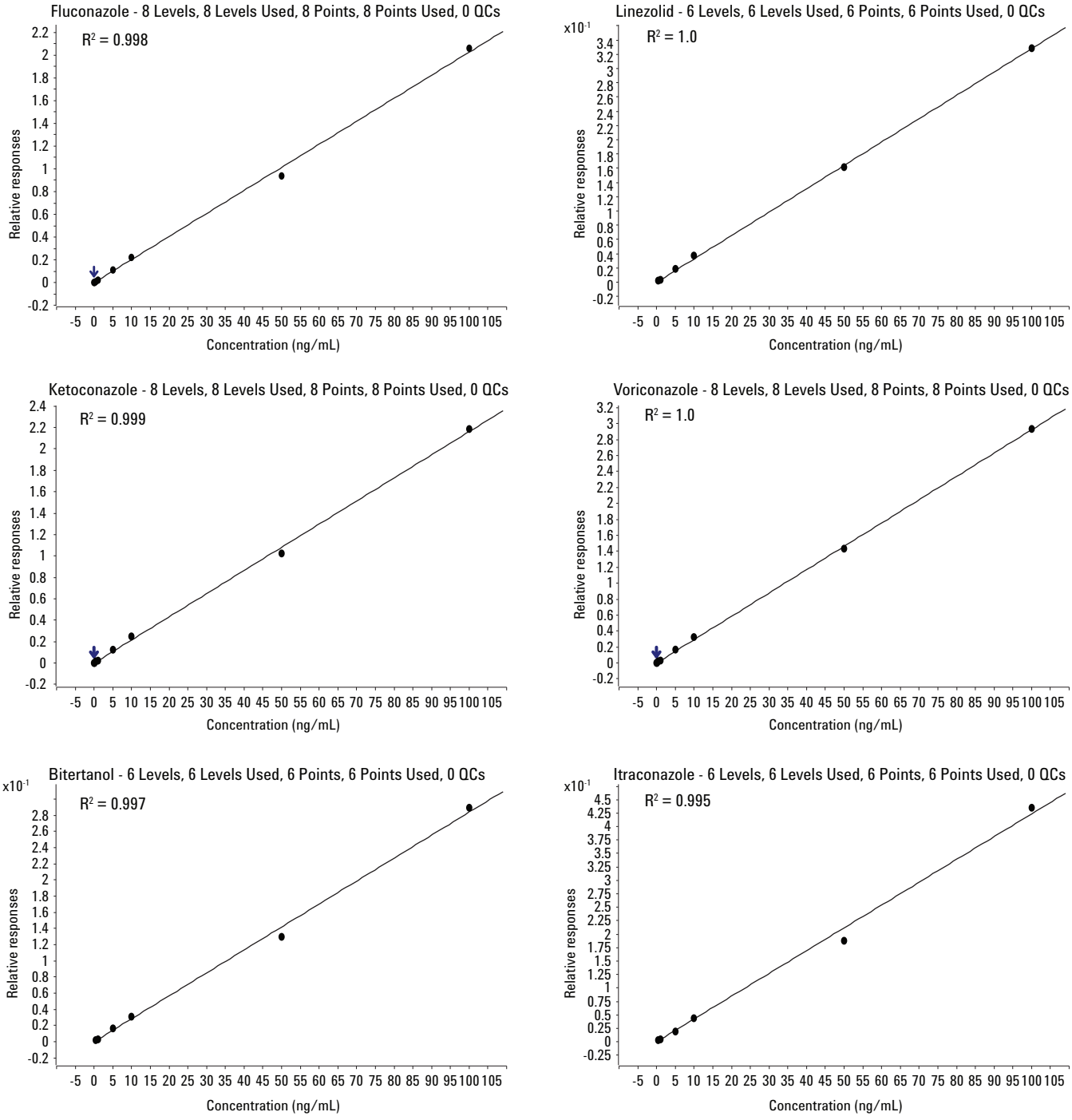


Figure 3. Calibration curves for antifungals in human plasma from 0.05 to 100 ng/mL (itraconazole, linezolid, and bitertanol use a 0.5 to 100 ng/mL range due to LOD).

A recovery experiment was performed with n = 6 samples for four different concentration levels: 1, 5, 50, and 100 ng/mL spiked in human plasma. Most of the compounds showed excellent recovery and % RSD values for all concentration levels.

	LOD (ng/mL)	LOQ (ng/mL)	1 ng/mL		5 ng/mL		50 ng/mL		100 ng/mL		Correlation coefficient R^2
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	
Fluconazole	0.05	0.05	104	1.7	97.0	3.8	100	1.4	105	2.1	0.998
Ketoconazole	0.05	0.1	101	1.6	101	2.6	104	3.4	106	4.5	0.999
Itraconazole	0.5	0.5	93.4	5.1	97.8	4.7	88.6	4.1	85.3	5.2	0.995
Linezolid	0.5	1	103	1.9	103	2.5	102	2.5	110	2.2	1.0
Voriconazole	0.05	0.1	96.8	2.1	94.4	4.4	91.9	8.2	93.4	8.8	1.0
Bitertanol	0.5	1	102	5.9	99.5	3.9	107	1.4	106	3.5	0.997

Conclusion

A reproducible and accurate analytical method was developed for several antifungal drugs in human plasma. Agilent Bond Elut Plexa delivered clean samples, which was demonstrated by the detection of pg levels of antifungals. All compounds showed close to 100% recovery and a single digit % RSD with n = 6 samples. Superb linearity was achieved in the calibration curves with $R^2 \geq 0.995$ for all compounds.

References

1. K. Y. Beste, O. Burkhardt, and V. Kaever. (2011) "Rapid HPLC-MS/MS method for simultaneous quantitation of four routinely administered triazole antifungals in human plasma". *Clinica Chimica Acta*, 413, 240 – 245.
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Printed in the USA
February 19, 2013
5990-9729EN

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