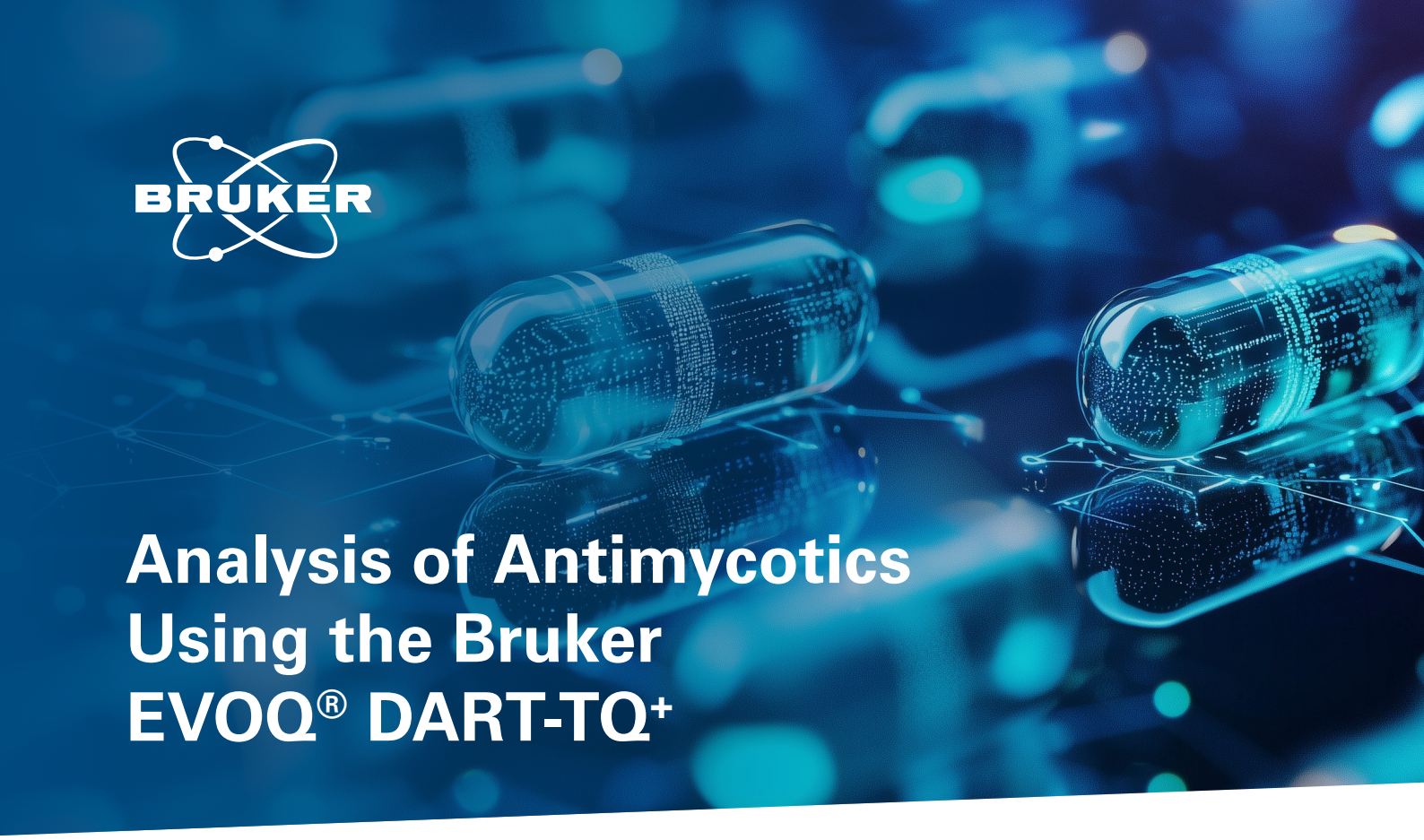

Analysis of Antimycotics Using the Bruker EVOQ® DART-TQ+

This study presents the results obtained for the analysis of 8 antimycotics in serum with the ClinMass® TDM Platform MS9000 and the ClinMass® Add-on Set MS9600 (RECIPE Chemicals + Instruments GmbH, Munich).

Keywords:
Therapeutic drug monitoring (TDM); TQ

Introduction

Impact of fungal infections on immunocompromised patients

Fungal infections, predominantly aspergillosis and candidiasis, can cause morbidity or even mortality, particularly in immunocompromised patients. Although current estimates of fungal disease incidence and mortality are imprecise, attributable mortality rates suggest an annual incidence of 6.5 million invasive fungal infections and 3.8 million deaths, of which about 2.5 million were directly attributable [1]. Patients with acute leukemia undergoing myelosuppressive chemotherapy and allogeneic stem cell transplant recipients are at particularly high risk.

Antimycotic treatment of fungal infections

Fungal infections can be treated or prevented with antimycotics (antifungal drugs, antifungals), which include several classes of compounds, such as azoles (triazole, imidazole), echinocandine, polyene and pyrimidine antifungals. Azole compounds such as voriconazole, posaconazole, itraconazole and its main metabolite hydroxy-itraconazole are widely used for the management of fungal infections as a result of their broad-spectrum antifungal activity. However, all triazole agents can greatly affect serum concentrations of other drugs (e.g. immunosuppressants). Triazole antifungals are subject to induction or inhibition of metabolism, thus co-medication with interacting compounds is a common rationale for performing therapeutic drug monitoring (TDM). As the large intra- and interindividual variability of pharmacokinetics of antimycotics can be affected by many different factors, it is important to monitor their blood concentration in patients with life-threatening fungal infections.

Rafaela Martin; Bruker Daltonics GmbH & Co. KG, Bremen, Germany

Experimental

Analysis was performed on an EVOQ® DART-TQ+ system coupled to an Elute UHPLC using the ClinMass® TDM Platform MS9000 (RECIPE), which included the mobile phase, autosampler washing solution, and precipitation reagent, as well as the HPLC column with prefilter. The ClinMass® Add-on Set MS9600 (RECIPE) contained all analyte-specific components for the determination of 8 different antimycotics.

Prior to analysis with liquid chromatography-mass spectrometry (LC-MS/MS), a short sample preparation was carried out in order to remove the sample matrix and to spike the samples with internal standards.
100 µL precipitation reagent containing the isotopically labelled internal standards were added to 50 µL serum samples and vortexed for 30 seconds. After centrifugation for 5 minutes at 10,000 x g, 50 µL supernatant were transferred to HPLC vials and diluted with 450 µL Diluting Solution D for analysis.

Measurement of the analytes was carried out in Multiple Reaction Monitoring (MRM) Mode.

This analytical method enables a robust and reliable quantitation in complex biological matrices by use of 8 different isotope labelled internal standards (Table 3).

Table 1. HPLC conditions

LC System	Bruker Elute UHPLC with column oven
Column	ClinMass® TDM MS9030 with Prefilter MS9032 RECIPE
Eluent A	Provided by ClinMass® MS9000 Kit RECIPE
Eluent B	Provided by ClinMass® MS9000 Kit RECIPE
Gradient	

	Time (min)	Flow (mL/min)	%A	%B
1	0.00	0.600	100.0	0.0
2	0.05	0.600	100.0	0.0
3	0.10	0.600	70.0	30.0
4	2.10	0.600	40.0	60.0
5	2.20	0.600	2.0	98.0
6	2.40	0.600	2.0	98.0
7	2.41	0.600	100.0	0.0
8	3.50	0.600	100.0	0.0

Temperature	40°C
Injection volume	2 µL (partial loop fill)
Wash solution 1	Mobile Phase A
Wash solution 2	Autosampler washing solution MS9005 RECIPE

Table 2. Mass Spectrometry conditions

MS system	Bruker EVOQ® DART TQ+
Ionization	VIP HESI (Vacuum Insulated Probe Heated Electrospray Ionization)
Polarity	Positive
Spray voltage	5000 V (+)
Cone gas	20 psi at 350°C
Probe gas	40 psi at 450°C
Nebulization gas	40 psi
Exhaust gas	Active exhaust, venturi effect with air
Acquisition mode	MRM (multiple reaction monitoring)
Scan time	Automatically calculated by software
Detector voltage	EDR (Extended Dynamic Range)

Table 3. Retention times, MRM transitions and internal standards

Analyte	Retention time [min]	Precursor ion	Product ion 1	CE 1 [V]	Product ion 2	CE 2 [V]
5-Fluorocytosin	0.41	130.2	58.0	28.6	113.0	15.8
5-Fluorocytosin-13C,15N2	0.41	133.1	115.0	15.8		
Fluconazol	0.86	307.1	238.0	9.9	220.0	12.8
Fluconazol-D4	0.86	310.9	242.1	9.9		
Hydroxy-Itraconazol	2.24	721.4	408.1	38.5	392.0	28.6
Hydroxy-Itraconazol-D5	2.24	726.2	413.2	38.5		
Isavuconazol	2.38	438.1	224.0	28.6	369.0	28.6
Isavuconazol-13C,D4	2.38	443.1	224.0	28.6		
Itraconazol	2.75	705.2	392.2	37.5	432.2	28.6
Itraconazol-D5	2.75	710.2	397.2	37.5		
Ketoconazol	2.03	531.1	489.0	23.7	243.9	30.6
Ketoconazol-D8	2.03	539.1	497.1	23.7		
Posaconazol	2.2	701.3	614.3	34.5	344.1	44.4
Posaconazol-D4	2.2	705.3	618.3	34.5		
Voriconazol	1.46	350.1	281.0	10.9	127.0	35.5
Voriconazol-D3	1.46	353.0	284.0	10.9		

Results & discussion

Eight antimycotic compounds (Table 3) were analyzed in just 3.5 minutes with effective chromatographic separation of the analytes (Figure 1).
The linearity of calibration was excellent at R^2 0.9991-1.0000 (Table 4). There was also a high level of precision for quality control samples with 4 consecutive injections resulting in RSD of 0.6-3.6%. Accuracy of the QC samples was also very good, with a mean recovery of most QC samples at 97-104% and some at 108-115% (Table 5). The values for precision and accuracy are well within the range required by common guidelines for quantitative results.

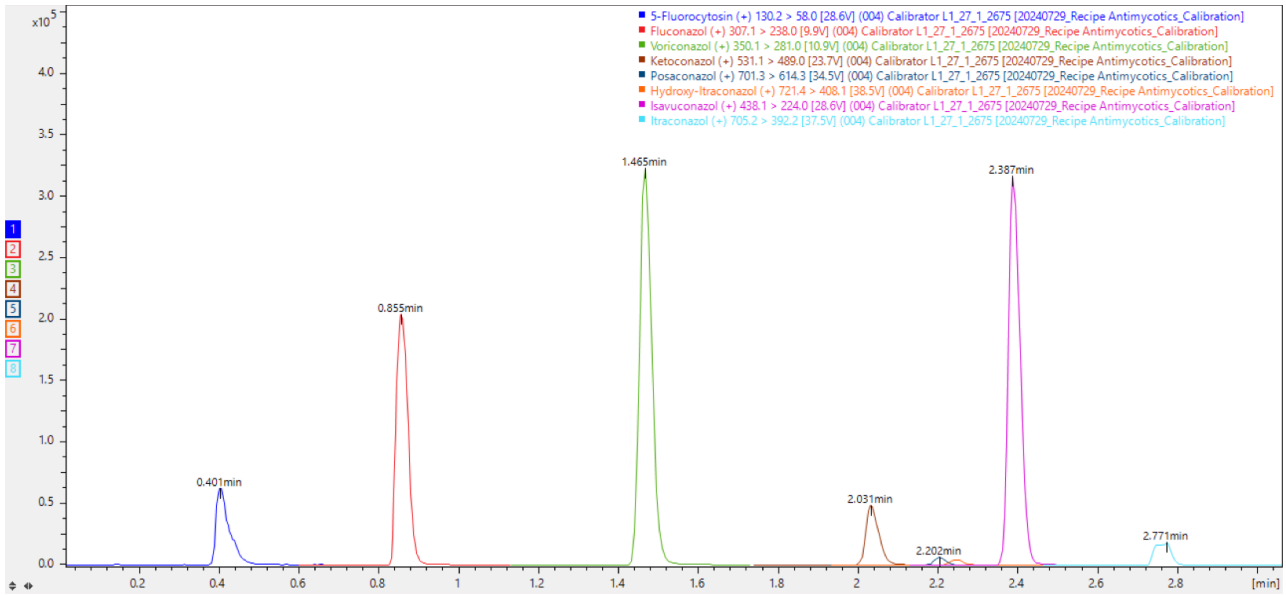

Figure 1 Chromatogram of lowest calibrator.

Table 4. Calibration results summary: RF = response factor = relative area/quantity expected.

Analyte	Internal Standard	Calibration Range [mg/L]	R^2	RSD RF
5-Fluorocytosin	5-Fluorocytosin-13C,15N2	5.23 – 111	0.9999	1.46
Fluconazol	Fluconazol-D4	0.669 – 14.1	1.0000	0.73
Hydroxy-Itraconazol	Hydroxy-Itraconazol-D5	0.182 – 3.91	0.9994	6.98
Isavuconazol	Isavuconazol-13C,D4	0.458 – 10.2	0.9996	2.23
Itraconazol	Itraconazol-D5	0.130 – 2.98	1.0000	4.10
Ketoconazol	Ketoconazol-D8	0.401 – 8.84	0.9994	3.11
Posaconazol	Posaconazol-D4	0.231 – 5.42	0.9991	3.31
Voriconazol	Voriconazol-D3	0.259 – 5.85	0.9991	6.91

Table 5. Quantitation of Quality Control samples (4 consecutive injections).

Sample	ClinChek – Control, Level I				ClinChek – Control, Level II			
Analyte	**Specified Value [µg/L]**	**Actual Value, mean [µg/L]**	**Mean Recovery [%]**	**RSD [%]**	**Specified Value [µg/L]**	**Actual Value, mean [µg/L]**	**Mean Recovery [%]**	**RSD [%]**
5-Fluorocytosin	21.1	21.7	103	1.2	50.3	49.3	97.9	1.3
Fluconazol	2.70	2.70	99.8	1.2	6.14	6.09	99.1	0.7
Hydroxy-Itraconazol	0.729	0.795	109	1.0	1.72	1.69	98.4	3.6
Isavuconazol	1.99	2.00	100	0.8	4.59	4.56	99.4	1.0
Itraconazol	0.592	0.641	108	1.1	1.41	1.46	104	0.6
Ketoconazol	1.73	1.72	99.4	1.6	3.92	3.89	99.2	1.7
Posaconazol	0.922	1.06	115	2.4	2.25	2.40	107	3.0
Voriconazol	1.15	1.14	99.4	0.8	2.67	2.60	97.3	1.2

Conclusions

The Bruker Elute UHPLC coupled to the EVOQ® DART-TQ+ generates quick and reliable LC-MS/MS quantitative results using the ClinMass® TDM kit system. Low sample requirements (50 µL serum), easy preparation, and short run time (3.5 minutes) support high LC-MS/MS sample throughput. Linearity of response, sensitivity, precision, and accuracy of the 8 antimycotic compounds in serum analyzed by the EVOQ DART-TQ+ were outstanding.

Acknowledgements

The authors acknowledge RECIPE Chemicals + Instruments GmbH (Munich, Germany) for providing the ClinMass® TDM Platform MS9000 and the ClinMass® Add-on Set MS9600.

References

[1] Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis. 2024 Jul;24(7):e428-e438. doi: 10.1016/S1473-3099(23)00692-8. Epub 2024 Jan 12. PMID: 38224705.

[2] Scott BL. Allogeneic stem cell transplantation for high-risk acute leukemia and maintenance therapy: no time to waste. Blood Adv. 2020 Jul 14;4(13):3200-3204. doi: 10.1182/bloodadvances.2019000388. PMID: 32663296; PMCID: PMC7362353.

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

marketing.bams.emea@bruker.com - www.bruker.com

linkedin.com/company/brukerappliedmassspec

Learn more about the product on our website: