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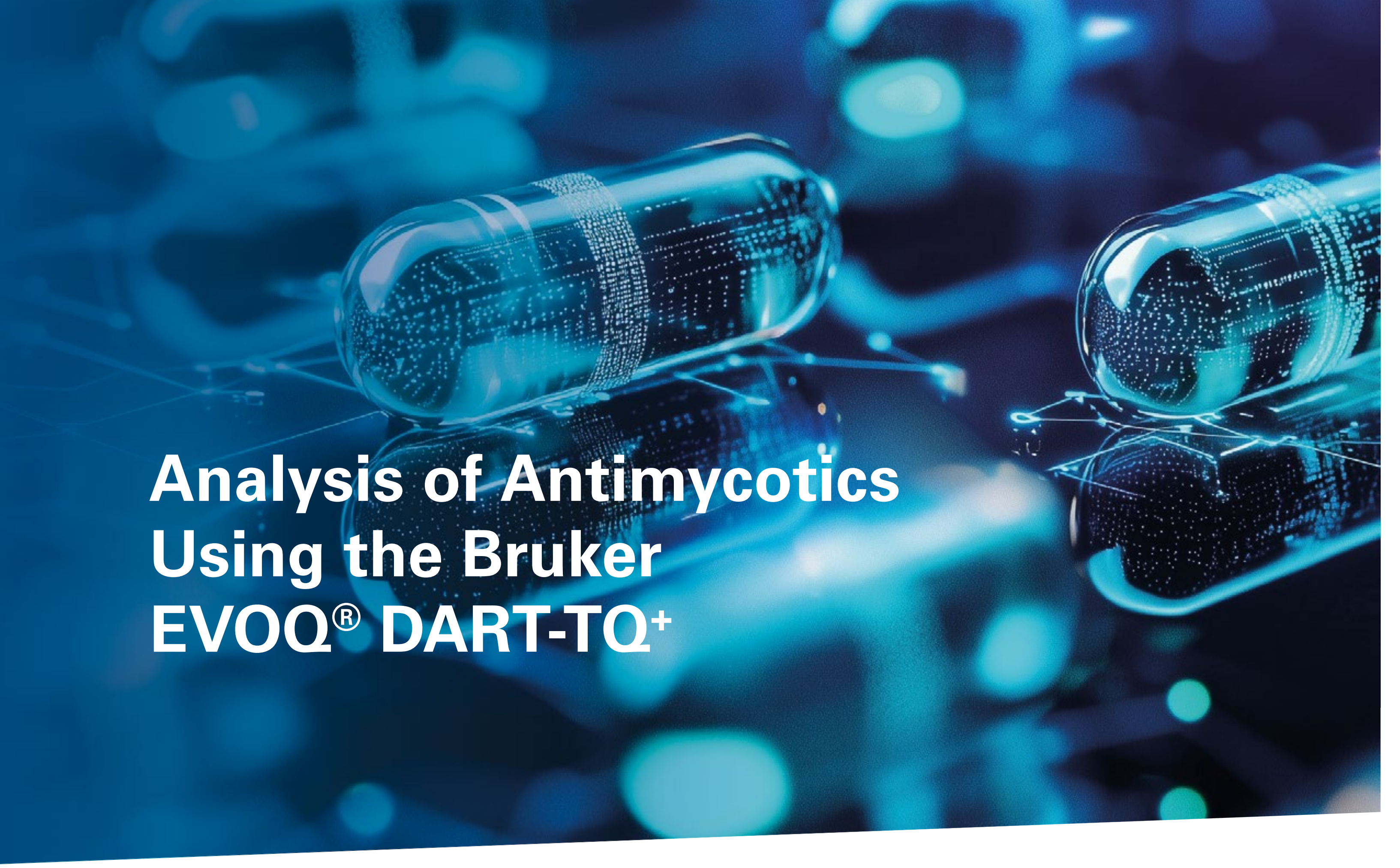

Analysis of Antimycotics Using the Bruker EVOQ® DART-TQ⁺

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 inter-individual 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.

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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.

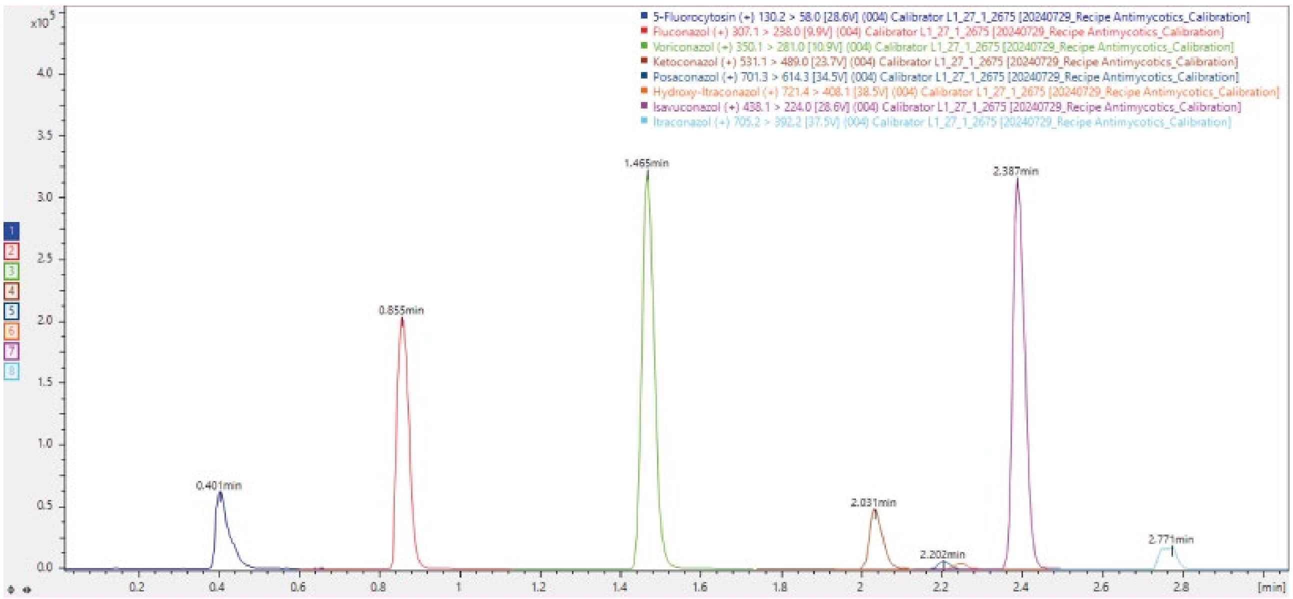


Figure 1 Chromatogram of lowest calibrator.

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

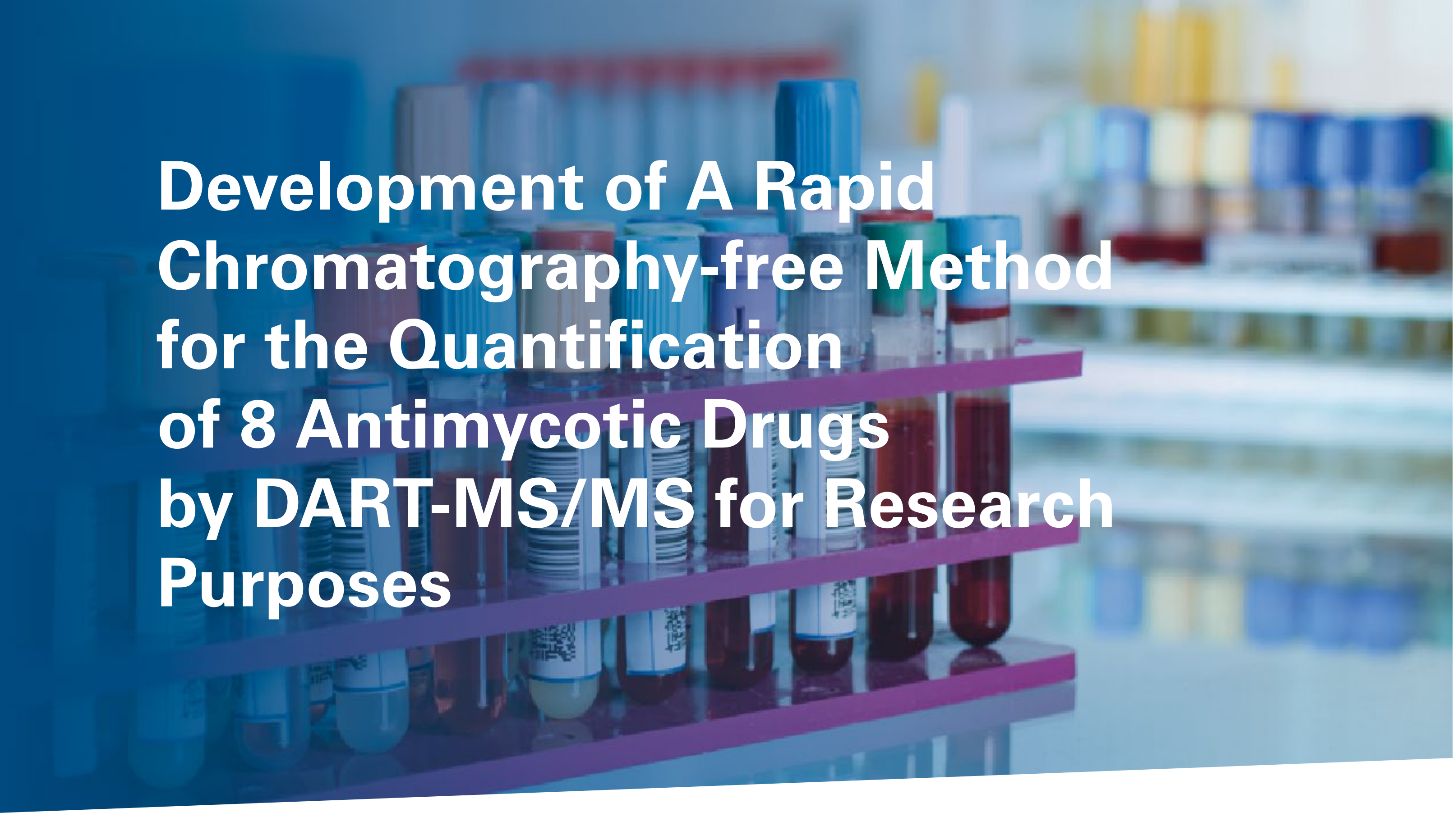
The authors acknowledge RECIPE Chemicals + Instruments GmbH (Munich, Germany) for providing the ClinMass® TDM Platform MS9000 and the ClinMass® Add-on Set MS9600.

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.

Development of A Rapid Chromatography-free Method for the Quantification of 8 Antimycotic Drugs by DART-MS/MS for Research Purposes



Abstract

This study demonstrates that DART-MS/MS has the potential to provide a chromatography-free approach for rapidly quantifying antimycotics in serum.

A maximized throughput can be achieved with an analysis time of 26 sec/sample or 96 samples in less than 42 min.

Keywords:
Antimycotics, DART, Triple Quad, Chromatography-free

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Introduction

Many drugs require optimal blood levels to function properly. Therefore, the quantitative determination of drug concentrations is a crucial measure to ensure effective and safe medication. Investigations using conventional LC-MS/MS analysis take ~5 minutes per run which means when blanks, calibrators, QCs, and patient samples need to be analyzed, the number of analyses can build up to several hours of turnaround time for a result. The study aims to demonstrate that rapid, quantitative analysis of eight common antimycotic drugs in serum using DART (Direct Analysis in Real Time) is, in principle, feasible.

DART employs a helium-driven corona discharge to ionize compounds. The analysis was performed using the EVOQ® DART-TQ+ (Bruker Daltonics) triple quadrupole mass spectrometer with a fully integrated DART ion source. The single-point-of-entry software enables automated data acquisition and processing to empower a most efficient workflow.

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Experimental

Figure 1. Chromatography-Free Workflow

A new extraction method was developed with a focus on optimization for DART-MS/MS analysis. This easily automated extraction process used labelled internal standards for each compound. Liquid-liquid extraction (LLE) involved mixing 50 µL of serum or plasma sample, 20 µL of the internal standard solution and 100 µL of the organic solvent before centrifugation.

Aliquots of the organic layer from each calibrator, quality control sample and blank were transferred onto a QuickStrip HTS-96 mesh and dried at room temperature for five minutes. The mesh was then loaded onto the EVOQ DART-TQ+ mass spectrometer for analysis (Fig. 1). Mass transitions were evaluated to maximize sensitivity while ensuring that the background would not affect accuracy or reproducibility.

The MRM Builder tool was used to optimize analyte-specific collision energies and mass transitions including confirmatory ions for all eight antimycotics (table 2). DART gas temperatures and grid voltages were tested across the instrument range to identify suitable settings for the entire panel. Cone temperature and cone gas were tuned to maximize ion transmission and keep the instrument clean. JumpShot was selected for the analysis at 4 seconds per spot.

The volume of the sample spotted onto the mesh was evaluated, as this can affect signal intensity and reproducibility depending on the composition of the sample and solvent. Similarly, the DART acquisition mode, acquisition time and MS scan times were optimized to maximize reproducibility (table 1).

Figure 2. EVOQ® DART-TQ+ triple quadrupole mass spectrometer with a fully integrated DART ion source.

Table 1. Analytical Instrument conditions

MS system	Bruker EVOQ® DART-TQ+
Ionization	DART
Polarity	Positive
Cone Temperature	275°C /350°C
Cone pressure	23 psi
DART Temperature	300°C /400°C
Grid Voltage	50V
CID Pressure	2.0 mTorr
Exhaust gas	Active exhaust, venturi effect with air
Acquisition mode	MRM (multiple reaction monitoring)
Scan time	40ms
Detector voltage	EDR (Extended Dynamic Range)

Table 2. MRM Transitions of analytes and isotopically labeled internal standards

Analyte/ISTD	Quantifier MRM		CE
	Precursor [m/z]	Product [m/z]	V
5-Fluorocytosine	130.1	113.1	14.8
Fluconazole	306.9	238.0	7.9
OH-Itraconazole	721.2	408.2	34.5
Isavuconazole	438.1	224.0	10.9
Itraconazole	705.2	392.2	29.6
Ketoconazole	531.1	489.1	26.6
Posaconazole	701.3	614.3	30.6
Voriconazole	350.0	281.0	11.8
5-Fluorocytosine-13C,15N2	133.1	115.1	14.8
Fluconazole-D4	310.9	242.1	7.9
OH-Itraconazole-D5	726.2	413.2	34.5
Isavuconazole-13C,D4	443.1	224.0	10.9
Itraconazole-D5	710.2	397.2	29.6
Ketoconazole-D8	539.1	497.1	26.6
Posaconazole-D4	705.3	618.3	30.6
Posaconazole-D4	353.0	284.0	11.8
	Qualifier MRM		
5-Fluorocytosine	130.1	58.1	31.6
Fluconazole	306.9	121.0	34.5
OH-Itraconazole	721.2	256.2	33.6
Isavuconazole	438.1	369.0	18.8
Itraconazole	705.2	255.7	29.6
Ketoconazole	531.1	244.0	35.5
Posaconazole	701.3	344.1	39.5
Voriconazole	350.0	224.0	11.8

Results

Linearity was evaluated by preparing five replicate calibration series in serum at 12 levels, which covered the relevant concentration ranges. The method demonstrated excellent linearity ($R^2 > 0.995$), with acceptable %CV shown by all compounds at all levels (table 3). The LOD and LOQ values obtained in this study were sufficiently sensitive to measure the relevant concentrations of all eight antimycotic drugs.

Intra- and inter-day precision were determined using matrix-matched quality control samples (QCs) prepared at three concentration levels. The intra-day precision was determined by analysis of 20 replicates per level in a single run while the inter-day precision study was performed over five days, with four time points per day. All results for both precision studies were within acceptable limits (table 4)

Table 3. Results for the linearity test.

Analyte	Fit type and weighting	R^2	Linear range [mg/L]
5-Fluorocytosine	linear, 1/x	0.997	1.05 – 222
Fluconazole	linear, 1/x	0.999	0.134 – 28.2
OH-Itraconazole	linear, 1/x	0.997	0.091 – 3.91
Isavuconazole	linear, 1/x	0.999	0.229 – 10.2
Itraconazole	linear, 1/x	0.995	0.065 – 2.98
Ketoconazole	linear, 1/x	0.999	0.080 – 17.7
Posaconazole	linear, 1/x	0.996	0.046 – 5.42
Voriconazole	linear, 1/x	0.997	0.259 – 5.85

Table 4. Intra and Interday Precision Results.

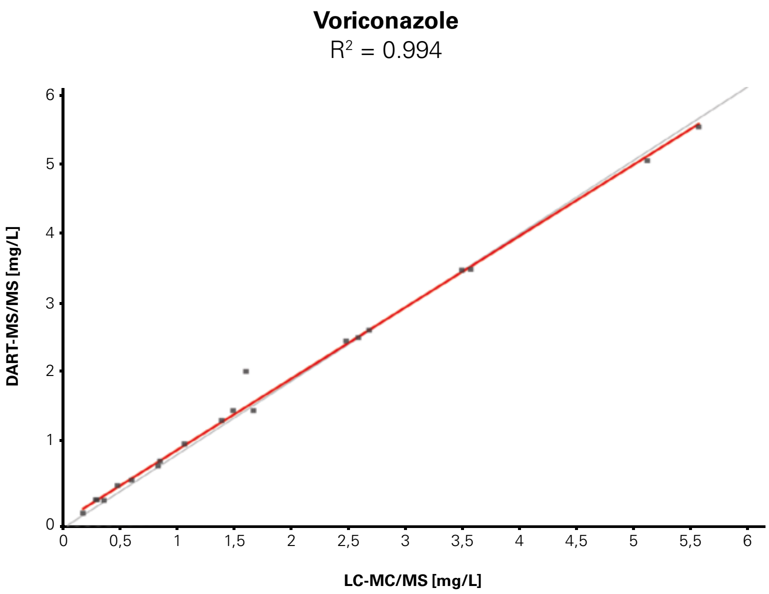
	5-Fluorocytosine			Fluconazole			OH-Itraonazole			Isavuconazole		
	I	II	III	I	II	III	I	II	III	I	II	III
Target Value [mg/L]	5.23	37.8	111	0.669	4.71	14.1	0.182	1.31	3.91	0.458	3.5	10.2
Intra-day precision [CV]	2.6%	1.7%	2.5%	2.9%	2.5%	2.5%	8.1%	3.6%	5.6%	1.9%	1.5%	2.1%
Inter-day precision [CV]	4.7%	6.6%	6.1%	7.5%	2.4%	1.6%	12.9%	6.1%	6.1%	3.0%	2.5%	2.7%

	Itraconazole			Ketoconazole			Posaconazole			Voriconazole		
	I	II	III	I	II	III	I	II	III	I	II	III
Target Value [mg/L]	0.13	0.968	2.98	0.401	2.99	8.84	0.231	1.75	5.42	0.259	2.08	5.85
Intra-day precision [CV]	10.9%	6.6%	4.9%	2.0%	1.7%	2.5%	8.4%	3.6%	6.6%	10.2%	2.7%	4.3%
Inter-day precision [CV]	14.3%	6.9%	6.3%	3.9%	2.2%	2.4%	11.7%	6.9%	7.7%	13.7%	3.1%	2.6%

Stability of extracted samples after spotting on the mesh was verified at room temperature over 7 hours.

A method comparison study was performed by analyzing patient samples using both Bruker DART-MS/MS and a respective commercially available LC-MS/MS kit from RECIPE Chemicals + Instruments GmbH. The newly developed DART method correlated well with the LC-MS/MS measurements ($R^2 > 0.99$) with a slope of nearly one (fig. 2).

No interference was found in this study for 144 substances incl. antiepileptics, neuroleptics, antidepressants, and benzodiazepines. LOD and LOQ values obtained in this study were sufficiently sensitive to measure relevant concentrations for all eight antifungal agents. In this study, matrix effect and extraction efficiency experiments met criteria, confirming the method's reliability in serum and plasma.



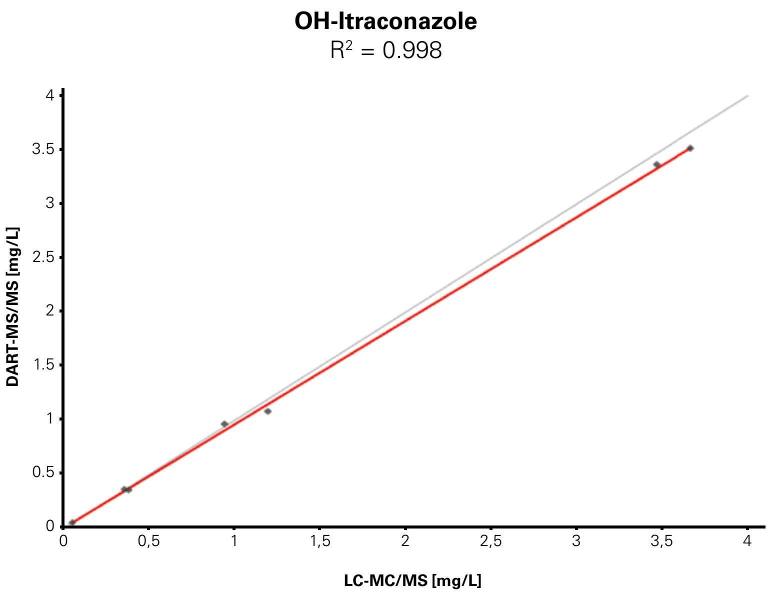
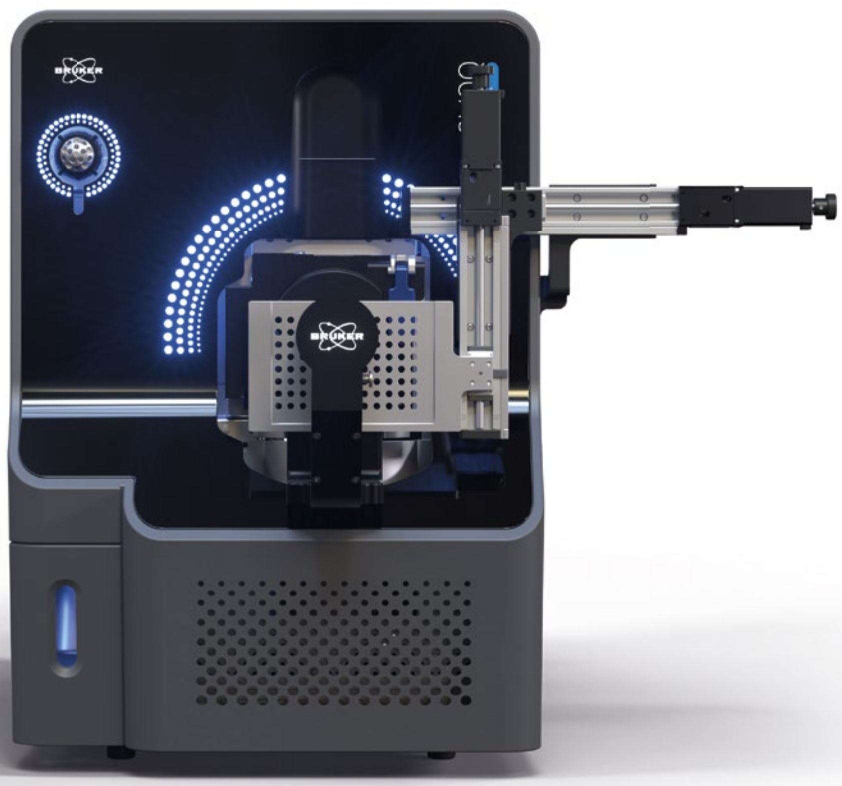


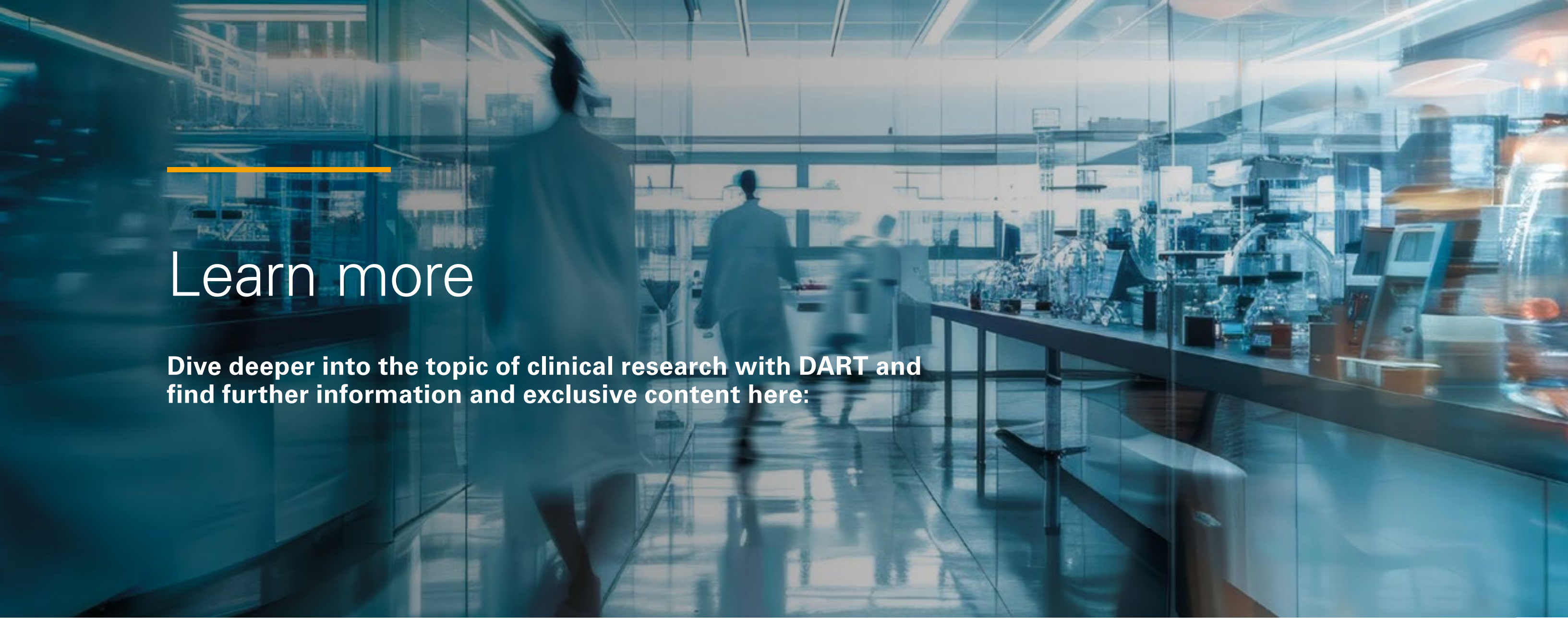
Figure 3. LC-MS/MS Correlation

Conclusions

The data of this study provides initial evidence that the chromatography-free DART-MS/MS method could be suitable as a rapid and reproducible workflow for quantifying eight common antimycotic agents in serum and plasma. The time per sample analysis is reduced 10x from 5 min for LC-MS/MS to <30 sec for DART-MS/MS allowing for fast turnaround of sample results. This clinical research work also shows promise for expansion to further compound classes such as antiepileptics, tricyclic anti-depressants, and antibiotics.

96 samples can be analyzed in less than 42 min. Beyond faster analysis and rapid turn-around-time, minimal solvent and gas usage further reduces operational costs and supports green chemistry.





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