

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of Antimycotics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

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User Benefits

- ◆ Full solution provided by Shimadzu and RECIPE®
- ◆ Fully automated sample preparation
- ◆ Verified method for RECIPE® ClinMass® TDM Kit System for Antimycotics in Serum / Plasma

Introduction

Antimycotics (antifungal drugs, antifungals) are a class of drugs that are used for the treatment and prophylaxis of fungal infections. The spectrum of antimycotics for clinical use has expanded in the recent years and includes several classes of compounds, such as azole (triazole, imidazole), echinocandine, polyene and pyrimidine antifungals^[1].

For all compounds therapeutic drug monitoring (TDM) is recommended to monitor the variability of pharmacokinetics of antifungal agents in patients with life-threatening fungal infections.

RECIPE's fully validated analytical method provides the quantification of 8 Antimycotics and metabolites (Table 2) for TDM using LC-MS/MS.

By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards from the ClinMass® TDM Kit System for Antimycotics followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis. Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 5.45 min. Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

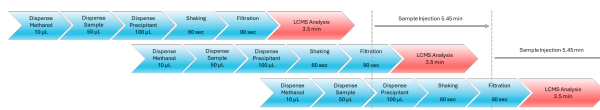


Fig. 2 Scheme fully automated sample preparation and analysis



Fig. 1 CLAM LCMS TQ

Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

Eight Antimycotics in serum were verified using the ClinMass® TDM Platform (order no. MS9000) in combination with the ClinMass® Add-on Set for Antimycotics in Serum / Plasma (order no. MS9600) (RECIPE®, Germany).

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060	
Ionization	: Electrospray Ionization (ESI), positive	
Interface Voltage	: 3 kV	
Heating Gas	: 10 L/min	
DL Temp.	: 250 °C	
Interface Temp.	: 300 °C	
Nebulizing Gas	: 3 L/min	
Drying Gas	: 5 L/min	
Heat Block	: 250 °C	
CID	: 270 kPa	
UHPLC	: Nexera X3	
Column Oven	: 40 °C	
Injection Volume	: 0.2 µL	
Flow rate	: 0.6 mL/min	
Time Programme	: Binary gradient	
Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
Initial	100	0
0.05	100	0
0.10	70	30
2.10	40	60
2.20	2	98
2.40	2	98
2.41	100	0
3.50	100	0

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

Analyte / IS	Quantifier MRM		Dwell Time msec	Q1 Pre Bias V	CE V	Q3 Pre Bias V
	Precursor [m/z]	Product [m/z]				
5-Fluorocytosine	130.30	58.000	15	-15	-31	-22
Fluconazole	307.00	238.05	15	-19	-30	-19
Hydroxy-Itraconazole	721.10	408.00	50	-28	-38	-27
Isavuconazole	438.10	224.05	15	-27	-40	-20
Itraconazole	705.10	392.05	50	-28	-38	-19
Ketoconazole	531.00	489.00	20	-26	-32	-23
Posaconazole	701.20	614.15	15	-28	-35	-22
Voriconazole	350.00	281.00	15	-21	-30	-11
¹³C,¹⁵N₂-5-Fluorocytosine	133.30	115.05	20	-14	-23	-22
d₄-Fluconazole	311.00	242.15	20	-19	-30	-19
d₅-Hydroxy-Itraconazole	726.10	413.10	20	-20	-36	-30
¹³C,d₄-Isavuconazole	443.10	224.15	20	-27	-40	-20
d₅-Itraconazole	710.10	397.30	50	-26	-34	-23
d₈-Ketoconazole	539.00	497.20	20	-26	-30	-26
d₄-Posaconazole	705.20	618.25	20	-26	-36	-32
d₃-Voriconazole	353.00	284.10	15	-21	-30	-11
Qualifier MRM						
5-Fluorocytosine	130.30	112.95	15	-14	-21	-21
Fluconazole	307.00	220.05	15	-19	-35	-23
Hydroxy-Itraconazole	721.10	392.05	15	-20	-38	-28
Isavuconazole	438.10	369.00	15	-15	-30	-26
Itraconazole	705.10	432.10	20	-28	-34	-30
Ketoconazole	531.00	244.00	20	-26	-34	-25
Posaconazole	701.20	344.05	15	-28	-45	-24
Voriconazole	350.00	127.10	15	-22	-10	-15

■ Results

The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 3.

To determine the precision two different levels of QC samples were prepared in 8-fold and analyzed in a single analysis sequence. The intraassay precision for each level is summarized in Table 4.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of the ClinCal® Serum Calibrator Set lyophil. for Antimycotics (order no. MS9613, RECIPE®, Germany) were prepared in 3-fold and analyzed in a single analysis sequence. The results for linearity evaluation and for the LLOQ are summarized in Table 5.

Table 3 Trueness of measurement

Analyte	Sample	Target value [mg/L]	Measured value [mg/L] Mean (n = 4)	CV [%]	Bias [%]
5-Fluorocytosine	Control Sample Level I	21.2	19.0	3.7	-10.5
	Control Sample Level II	49.8	45.6	6.4	-8.4
Fluconazole	Control Sample Level I	2.75	2.62	1.0	-4.6
	Control Sample Level II	6.40	6.23	6.5	-2.6
Isavuconazole	Control Sample Level I	2.05	1.88	5.1	-8.1
	Control Sample Level II	4.74	4.48	4.9	-5.5
Itraconazole	Control Sample Level I	0.614	0.639	4.4	4.1
	Control Sample Level II	1.48	1.56	5.0	5.3
Ketoconazole	Control Sample Level I	1.72	1.61	2.1	-6.5
	Control Sample Level II	3.97	3.79	5.9	-4.6
OH-Itraconazole	Control Sample Level I	0.659	0.675	5.9	2.5
	Control Sample Level II	1.59	1.61	7.3	1.5
Posaconazole	Control Sample Level I	1.00	0.97	1.8	-3.1
	Control Sample Level II	2.31	2.29	5.0	-0.7
Voriconazole	Control Sample Level I	1.12	1.10	5.1	-1.7
	Control Sample Level II	2.66	2.59	4.3	-2.7

Table 4 Intraassay results [CV%]

Analyte	Sample	Measured value [mg/L] Mean (n=8)	CV [%]
5-Fluorocytosine	Control Sample Level I	19.2	1.3
	Control Sample Level II	45.7	3.2
Fluconazole	Control Sample Level I	2.62	2.9
	Control Sample Level II	6.24	3.0
Isavuconazole	Control Sample Level I	1.89	2.1
	Control Sample Level II	4.53	3.6
Itraconazole	Control Sample Level I	0.653	2.0
	Control Sample Level II	1.61	1.9
Ketoconazole	Control Sample Level I	1.64	1.7
	Control Sample Level II	3.97	2.7
OH-Itraconazole	Control Sample Level I	0.690	2.6
	Control Sample Level II	1.63	2.0
Posaconazole	Control Sample Level I	1.02	3.4
	Control Sample Level II	2.45	2.3
Voriconazole	Control Sample Level I	1.05	2.1
	Control Sample Level II	2.53	3.0

Table 5 Linearity evaluation, including LLOQ / LOD

Analyte	Linear Range [mg/L]	R ²	LLOQ [mg/L]	LOD [mg/L]
5-Fluorocytosine	0.262 – 222	0.9984	0.262	0.0873
Fluconazole	0.167 – 28.2	0.9987	0.167	0.0557
Isavuconazole	0.115 – 20.4	0.9989	0.115	0.0383
Itraconazole	0.0650 – 5.96	0.9989	0.0650	0.0217
Ketoconazole	0.100 – 17.7	0.9993	0.100	0.0333
OH-Itraconazole	0.0455 – 7.82	0.9997	0.0455	0.0152
Posaconazole	0.116 – 10.8	0.9992	0.116	0.0387
Voriconazole	0.130 – 11.7	0.9984	0.130	0.0433

■ Conclusion

The ClinMass® TDM Kit System for Antimycotics in Serum / Plasma (order no. MS9000 and MS9600) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 8 analytes: 5-fluorocytosine, fluconazole, isavuconazole, itraconazole, ketoconazole, hydroxy-itraconazole, posaconazole and voriconazole passed the acceptance criteria for accuracy (trueness, precision) and linearity.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® TDM Kit System, Antimycotics in Serum / Plasma, RECIPE® Chemicals + Instruments GmbH



Shimadzu Corporation
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SHIMADZU Europa GmbH,
www.shimadzu.eu

The analysis method described is intended solely to illustrate the potential application opportunities.

In the case of a potential clinical application, follow the instructions on the ClinMass® TDM Kit System.

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