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TECHNICAL NOTE 65149

Quantification of antimycotics in human plasma or serum by liquid chromatography-tandem mass spectrometry for clinical research

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Keywords

Antimycotics, offline sample preparation, plasma, serum, mass spectrometry

Goal

Implementation of an analytical method for the quantification of eight antimycotics in human plasma or serum on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer.

Application benefits

- Simple offline sample preparation by protein precipitation
- Eight antimycotics in a single quantitative method

Introduction

An analytical method for clinical research, quantifying eight antimycotics in human plasma or serum, is reported. Analysis includes 5-fluorocytosine, fluconazole, hydroxyitraconazole, isavuconazole, itraconazole, ketoconazole, posaconazole, and voriconazole. Plasma or serum samples are extracted by offline internal standard addition and protein precipitation. Extracted samples are injected onto a Thermo Scientific™ Vanquish™ Flex Binary system connected to a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer with heated electrospray ionization. Detection is performed by selected reaction monitoring (SRM) using an isotopically labeled internal standard for each target analyte. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass Add-On Set for Antimycotics from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, accuracy, and intra- and inter-assay precision for each analyte.

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Experimental

Target analytes

The analytes and corresponding concentration ranges covered by the calibrators used are reported in Table 1.

Table 1. Concentration ranges covered by calibrators.

Analyte	Concentration (µg/mL)
5-Fluorocytosine	5.12–117
Fluconazole	0.565–12.6
Hydroxyitraconazole	0.147–3.27
Isavuconazole	0.482–10.7
Itraconazole	0.122–2.67
Ketoconazole	0.406–8.34
Posaconazole	0.21–4.61
Voriconazole	0.247–5.52

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE, as well as eight isotopically labeled internal standards for the quantification. Samples of 50 µL of plasma or serum were protein precipitated using 100 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged, and the supernatant was transferred to a clean plate or vial.

Liquid chromatography

Chromatographic separation was achieved using mobile phases and analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 3.7 minutes.

Table 2. Liquid chromatographic method description.

Gradient profile:			
Time (min)	*Flow Rate (mL/min)*	*A (%)*	*B (%)*
0.00	0.65	100	0
0.10	0.65	70	30
2.10	0.65	40	60
2.20	0.65	2	98
2.40	0.65	2	98
2.41	0.65	100	0
3.70	0.65	100	0
Injection volume:	4 µL		
Column temp.:	40 °C		

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization operated in polarity switching mode. A summary of the MS conditions is reported in Table 3. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively.

Table 3. MS settings.

Source type:	Heated electrospray ionization (HESI)
Vaporizer temperature:	400 °C
Capillary temperature:	350 °C
Spray voltage (positive/negative):	3500 V
Sheath gas:	50 AU
Sweep gas:	0 AU
Auxiliary gas:	15 AU
Data acquisition mode:	Selected-reaction monitoring (SRM)
Collision gas pressure:	1.5 mTorr
Cycle time:	0.300 s
Q1 mass resolution (FWMH):	0.7
Q3 mass resolution (FWMH):	0.7

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, accuracy, and intra- and inter-assay precision for each analyte. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at two different levels provided by RECIPE (MS9682 batch #1367), prepared and analyzed in replicates of five on three different days. Intra-assay precision was evaluated for each day on the same set of runs (control samples at two levels, replicates of five each day, three days) in terms of percentage coefficient of variation (%CV). Inter-assay precision was evaluated on the same controls including all the 15 replicates of the three days.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

The method proved to be linear in the calibration ranges covered by the calibrators. Representative chromatograms for the lowest calibrator for 5-fluorocytosine, voriconazole, and their internal standards are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

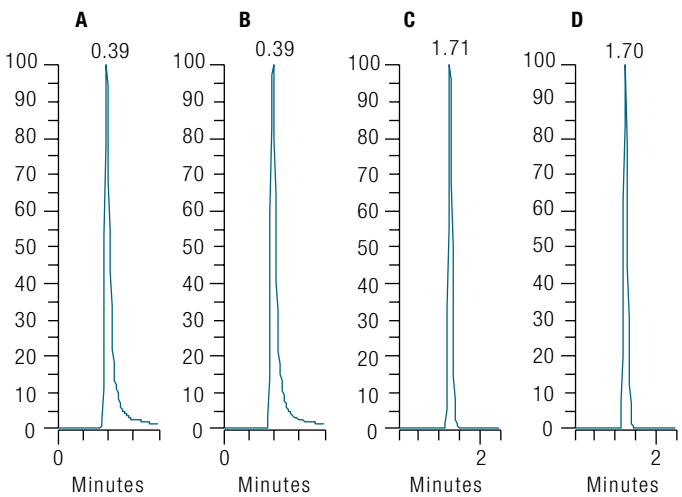

Figure 1. Representative chromatograms of the lowest calibrator for (A) 5-fluorocytosine, (B) 13C,15N2-fluorocytosine, (C) voriconazole, and (D) d3-voriconazole.

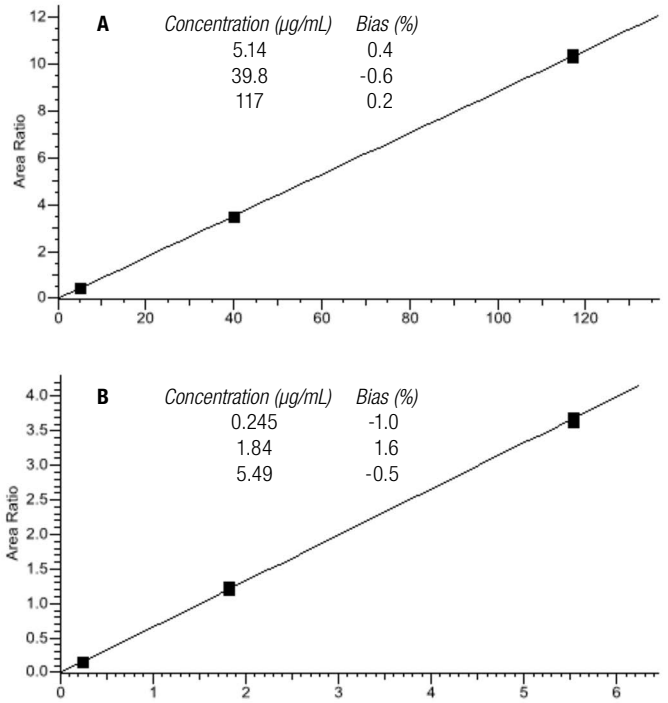

Figure 2. Representative calibration curves for (A) 5-fluorocytosine and (B) voriconazole - day 2.

The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -8.9% and 1.6%. Results are reported in Table 4.

The %CV for intra-assay precision was always below 12.7% for all the analytes (Table 5). The maximum %CV for inter-assay precision including all the analytes was 9.4% (Table 6).

Table 4. Analytical accuracy results for control MS9682 batch #1367.

Analyte	Control 1			Control 2		
	Nominal Concentration (µg/mL)	Average Calculated Concentration (µg/mL)	Bias (%)	Nominal Concentration (µg/mL)	Average Calculated Concentration (µg/mL)	Bias (%)
5-Fluorocytosine	21.9	21.01	-4.0	50.9	48.2	-5.3
Fluconazole	2.29	2.21	-3.6	5.40	5.12	-5.2
Hydroxyitraconazole	0.594	0.604	1.6	1.42	1.29	-8.9
Isavuconazole	1.92	1.90	-1.1	4.55	4.39	-3.5
Itraconazole	0.480	0.474	-1.3	1.15	1.07	-7.1
Ketoconazole	1.63	1.59	-2.3	3.68	3.52	-4.4
Posaconazole	0.855	0.835	-2.3	2.00	1.96	-2.0
Voriconazole	1.00	0.970	-3.0	2.37	2.27	-4.2

Table 5. Intra-assay precision results for control MS9682 batch #1367.

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)
5-Fluorocytosine	22.3	3.9	19.6	1.8	21.2	2.0	52.1	2.1	47.0	1.3	45.5	2.1
Fluconazole	2.40	2.4	2.11	1.7	2.11	2.1	5.59	2.3	5.02	0.9	4.75	1.3
Hydroxyitraconazole	0.629	4.8	0.603	5.3	0.580	7.1	1.42	5.0	1.22	3.5	1.24	5.2
Isavuconazole	2.08	3.4	1.80	1.9	1.82	2.2	4.85	2.7	4.30	1.1	4.01	1.9
Itraconazole	0.505	5.6	0.448	4.4	0.468	6.1	1.12	5.0	1.06	1.3	1.02	2.0
Ketoconazole	1.63	4.6	1.61	4.3	1.54	6.4	3.83	6.0	3.56	3.7	3.16	1.0
Posaconazole	0.842	12.7	0.858	9.7	0.800	4.1	2.04	9.2	1.89	6.1	1.95	8.0
Voriconazole	1.04	2.6	0.933	3.0	0.938	1.1	2.43	2.2	2.22	0.9	2.17	1.1

Table 6. Inter-assay precision results for control MS9682 batch #1367.

Analyte	Control 1		Control 2	
	Average Calculated Concentration (µg/mL)	CV (%)	Average Calculated Concentration (µg/mL)	CV (%)
5-Fluorocytosine	21.0	6.1	48.2	6.4
Fluconazole	2.21	6.6	5.12	7.2
Hydroxyitraconazole	0.604	6.3	1.29	8.3
Isavuconazole	1.90	7.3	4.39	8.4
Itraconazole	0.474	7.2	1.07	5.2
Ketoconazole	1.59	5.5	3.52	9.1
Posaconazole	0.835	9.4	1.96	8.0
Voriconazole	0.970	5.7	2.27	5.4

Conclusions

A liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of eight antimycotics in human plasma or serum was implemented. The ClinMass TDM Platform with the ClinMass Add-On Set for Antimycotics from RECIPE was used. The method was analytically evaluated on a Vanquish Flex Binary system connected to a TSQ Quantis triple quadrupole mass spectrometer. The method offers the quick and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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