TECHNICAL NOTE 66061

Quantification of mycophenolic acid in human plasma using an Orbitrap Exploris 120 high-resolution mass spectrometer for clinical research

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Application benefits

- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- High-resolution mass spectrometry for improved selectivity
- Robust, sensitive hardware enables increased confidence in data

Goal

Implementation of an analytical method for the quantification of mycophenolic acid in human plasma on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer

Introduction

Therapeutic drug monitoring (TDM) research involving mycophenolic acid (MPA), an immunosuppressive agent commonly used for organ transplant and autoimmune support, can be effectively performed using liquid chromatography coupled to high-resolution mass spectrometry (LC-MS). Alternative technologies, such as immunoassays, often produce results influenced by cross-reactivity, thus leading laboratories to adopt accurate, sensitive, and selective analytical methods, such as LC-MS. The availability of convenient and easy to use drug-specific kits for TDM research further supports the accuracy of and preference for the LC-MS method.

An LC high-resolution accurate-mass (HRAM) mass spectrometry method for the quantification of MPA in human plasma is reported here. Plasma samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system. Detection was performed on an Orbitrap Exploris 120 mass spectrometer with heated electrospray ionization (HESI) using a full scan acquisition mode. Method performance

Table 1. Molecular formula and expected mass/charge for mycophenolic acid and d_3-mycophenolic acid

Analyte	Molecular formula	Expected mass (*m/z*)	Internal standard	Molecular formula	Expected mass (*m/z*)
Mycophenolic acid	$C_{17}H_{20}O_6$	321.1333	d_3-mycophenolic acid	$C_{17}H_{17}D_3O_6$	324.1521

was evaluated using the ClinMass® TDM Platform with the ClinMass Add-On Set for Mycophenolic Acid in Serum/Plasma from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration range, lower limit of quantification (LLOQ), carryover, accuracy, and intra- and inter-assay precision.

Experimental

Target analytes

d_3-MPA was used as the internal standard. The molecular formula and expected mass/charge for the analyte and internal standard are detailed in Table 1.

Sample preparation

Four calibrators (MS99113 batch #1059), including a blank, from RECIPE covering a concentration range of 0.391–7.4 mg/L were used together with two controls (MS99183 batch #1417). Concentration ranges covered by the kit, LLOQ, and retention time are included in Table 2. Samples of 50 µL of plasma were protein precipitated using 100 µL of precipitating solution containing the internal standard. Precipitated samples were vortex-mixed and centrifuged. Finally, 50 µL of the supernatant were transferred to a clean plate or vial and diluted with 450 µL of Diluting Solution D (MS9022) prior to injection.

Liquid chromatography

A Vanquish Flex Binary UHPLC system was used for chromatographic separation utilizing mobile phases and an analytical column provided by RECIPE. Details of the analytical method are reported in Table 3. Total runtime was 1.9 minutes.

Mass spectrometry

Analyte and internal standard were detected by Full Scan combined with data-dependent MS^2 ($ddMS^2$) acquisition mode using an Orbitrap Exploris 120 mass spectrometer with a HESI source operated in positive ionization mode. Full scan mode was used for quantification, and a $ddMS^2$ experiment was used for confirmation based on ion ratio. A summary of the MS conditions is reported in Table 4.

Table 2. Concentration ranges covered by the kit, LLOQ, and retention time

Analyte	Concentration range (mg/L)	LLOQ (mg/L)	Retention time (min)
Mycophenolic acid	0.391–7.40	0.020	0.8

Table 3. LC method description

Time (min)	Flow rate (mL/min)	B (%)
0.00	0.5	17
0.30	0.5	80
0.55	0.5	80
0.60	0.5	17
1.90	0.5	17

Table 4. MS parameters

Ion source parameters	
Source type	Heated Electrospray Source Ionization (H-ESI)
Spray voltage – Positive (V)	3,500
Sheath gas (Arb)	50
Aux gas (Arb)	10
Sweep gas (Arb)	0
Ion transfer tube temp. (°C)	325
Vaporizer temp (°C)	350
Settings	
Mild trapping	No
Internal mass calibration	RunStart EASY-IC™
Data acquisition mode	Full Scan – $ddMS^2$
Full scan parameters	
Resolution (at *m/z* 200)	60,000
Scan range (*m/z*)	300–550
RF lens (%)	70
AGC target	Standard
Maximum injection time mode	Auto
Polarity	Positive
$ddMS^2$ scan parameters	
Isolation window (*m/z*)	2
Collision energy type	Normalized
HCD collision energy (%)	30
Resolution (at *m/z* 200)	15,000
Scan range mode	Auto

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration range, LLOQ, carryover, accuracy, trueness of measurements, and intra- and inter-assay precision for both analytes.

LLOQ was determined by performing a serial dilution of the lowest calibrator down to 20-fold using blank matrix; a full set of calibrators (four levels), diluted calibrators (four levels), and controls (two levels) were extracted in replicates of five (n=5), injected in a single batch and all used for the linear interpolation. The LLOQ was set as the lowest level that could be determined with a CV < 20%.

Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected just after it.

Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the quality control samples provided by RECIPE at two different levels prepared and analyzed in replicates of five on three different days.

Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five. Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 5.1 software.

Results and discussion

A linear response with 1/x weighting was used not only in the calibration range covered by the calibrators but also down to an LLOQ of 0.02 mg/L. The percentage bias between nominal and back-calculated concentration was always within ±15% for all the calibrators (±20% for the lowest calibrator) in all the runs. Representative chromatograms at the LLOQ for analyte and internal standard together with a representative calibration curve in the concentration range covered by the kit (three calibrators) are depicted in Figure 1.

No carryover was observed, with no signal detected in the blank injected after the highest calibrator.

The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -2.9% and 1.0% (Table 5).

Table 5. Analytical accuracy results for control MS99183 batch #1417

Analyte	Control	Nominal concentration (mg/L)	Average calculated concentration (mg/L)	Bias (%)
Mycophenolic acid	Level I	0.491	0.491	-0.1
	Level II	2.35	2.28	-2.9
	Level III	4.59	4.63	1.0

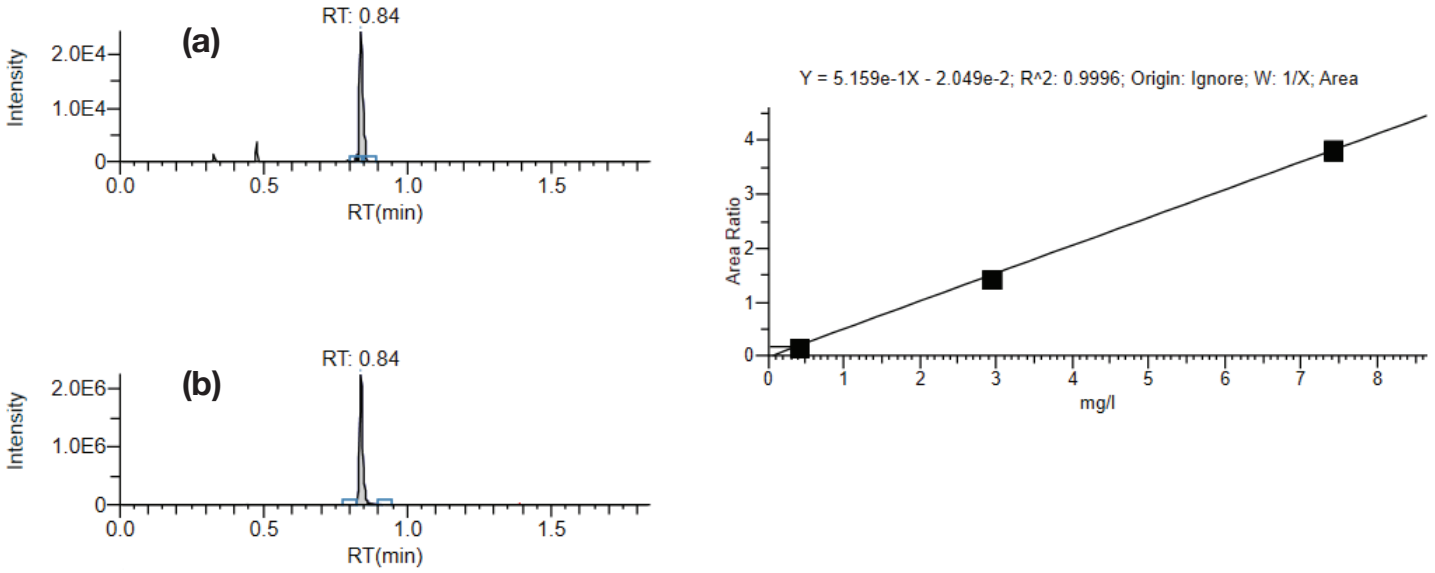

Figure 1. Representative chromatograms of the lower limitation of quantification (left) and calibration curve (right) for (a) mycophenolic acid and (b) d_3-mycophenolic acid

Table 6. Analytical intra- and inter-assay precision results for control MS99183 batch #1417

Analyte	Control	Intra-assay						Inter-assay	
		Day 1		Day 2		Day 3			
		Average calculated concentration (mg/L)	CV (%)	Average calculated concentration (mg/L)	CV (%)	Average calculated concentration (mg/L)	CV (%)	Average calculated concentration (mg/L)	CV (%)
Mycophenolic acid	Level I	0.492	2.8	0.488	3.1	0.492	3.1	0.491	0.5
	Level II	2.19	11.8	2.32	3.7	2.33	2.4	2.28	3.4
	Level III	4.71	8.9	4.64	5.9	4.56	3.4	4.63	1.6

A good reproducibility was also obtained, with a maximum %CV of 11.8% and 3.4% for intra- and inter-assay precision, respectively (Table 6).

Conclusion

A robust, reproducible, and sensitive liquid chromatography–high-resolution Orbitrap mass spectrometry method for clinical research for the quantification of MPA in human plasma was implemented. The ClinMass TDM Platform with the ClinMass Add-On Set for Mycophenolic Acid in Serum/Plasma from RECIPE contains all required analyte-specific components, while ensuring an adequate chromatographic separation from MPA glucuronide (MPAG), which eliminates false positives caused by in-source fragmentation of MPAG. The method was analytically validated on a Vanquish Flex Binary UHPLC system connected to an Orbitrap Exploris 120 mass spectrometer with a HESI probe using a Full Scan acquisition mode. The method described here offers quick and simple offline protein precipitation with concomitant internal standard addition. This method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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