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TECHNICAL NOTE 65158

Quantification of mycophenolic acid in human plasma by liquid chromatography-tandem mass spectrometry for clinical research

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Keywords

Mycophenolic acid, mycophenolate, offline sample preparation, plasma, mass spectrometry

Goal

Development and implementation of a robust, sensitive analytical method for the quantification of mycophenolic acid in human plasma on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer

Application benefits

- Simple offline sample preparation by protein precipitation
- Two-minute injection-to-injection cycle time.

Introduction

An analytical method for clinical research for the quantification of mycophenolic acid in human plasma is reported. Plasma samples are extracted by offline internal standard addition and protein precipitation. Extracted samples are injected onto a Thermo Scientific™ Vanquish™ Flex Binary liquid chromatography (LC) system connected to a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Detection is performed by selected reaction monitoring (SRM) using d3-mycophenolic acid as the internal standard. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass Add-On Set for Mycophenolic acid from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration range, accuracy, and intra-assay precision.

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Experimental

Sample preparation

Reagents included four calibrators (including blank) and three controls from RECIPE, as well as d3-mycophenolic acid as the internal standard for the quantification. 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, 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 1. Total runtime was 2.0 minutes.

Table 1. LC method description

Gradient profile:			
Time (min)	*Flow Rate (mL/min)*	*A (%)*	*B (%)*
0.00	0.6	83	17
0.30	0.6	20	80
0.55	0.6	20	80
0.60	0.6	83	17
2.00	0.6	83	17
Injection volume:	10 µL		
Column temp.:	40 °C		

Mass spectrometry

Analytes and internal standard were detected by SRM on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. A summary of the MS conditions is reported in Table 2. Two SRM transitions were included in the acquisition method for quantification and confirmation, respectively.

Table 2. MS settings

Source type:	Heated electrospray ionization (HESI)
Vaporizer temperature:	350 °C
Capillary temperature:	325 °C
Spray voltage (positive mode):	3500 V
Sheath gas:	50 AU
Sweep gas:	0 AU
Auxiliary gas:	10 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 range, accuracy, and intra-assay precision. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at three different levels provided by RECIPE (MS99113 batch #1486), prepared and analyzed in replicates of five. Intra-assay precision was evaluated on the same run (control samples at three levels, replicates of five) in terms of percentage coefficient of variation (%CV).

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 range covered by the calibrators. Representative chromatograms for the lowest calibrator are reported in Figure 1. A representative calibration curve is reported in Figure 2.

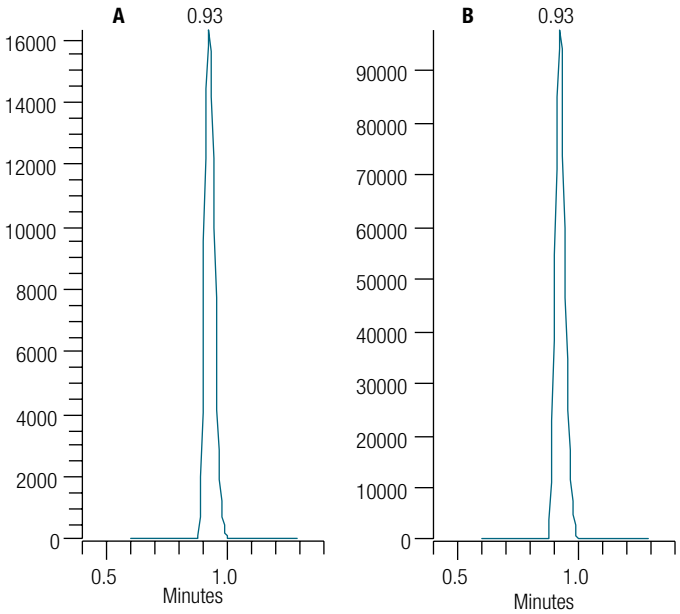

Figure 1. Representative chromatograms of the lowest calibrator for (a) mycophenolic acid and (b) d3-mycophenolic acid

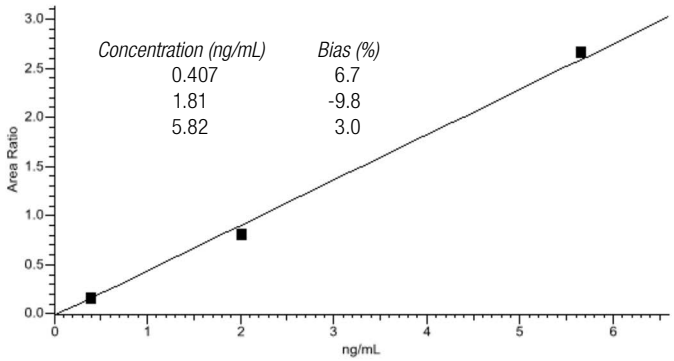

Figure 2. Representative calibration curve for mycophenolic acid

The data demonstrated outstanding accuracy and precision of the method. The percentage bias between nominal and average back-calculated concentration for the control samples was between -0.8% and 1.9%. The %CV for intra-assay precision was below 0.9%. Results are reported in Table 3.

Table 3. Accuracy and intra-assay precision results for control MS99113 batch #1486

Control	Nominal Concentration (ng/mL)	Calculated Concentration (ng/mL)	Average Concentration (ng/mL)	Accuracy (% Bias)	Precision (% CV)
CTRL 1	0.516	0.517 0.509 0.517 0.507 0.509	0.512	-0.8	0.9
CTRL 2	2.45	2.44 2.46 2.47 2.48 2.47	2.46	0.5	0.7
CTRL 3	4.76	4.82 4.87 4.87 4.89 4.79	4.85	1.9	0.9

Conclusions

A robust, sensitive liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of mycophenolic acid in human plasma was implemented. The ClinMass TDM Platform with the ClinMass Add-On Set for Mycophenolic Acid from RECIPE was used. The method was analytically validated on a Vanquish Flex Binary system connected to a TSQ Quantis triple quadrupole mass spectrometer. This method offers 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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