



Determination of Mycophenolic Acid in human plasma by liquid chromatography-tandem mass spectrometry for bioequivalence study in Indian subjects

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Introduction

Mycophenolic acid is an immunosuppressive agent used in therapy following solid organ transplantation. Mycophenolic acid is important because of its selective effects on the immune system. It prevents the proliferation of T-cells, lymphocytes, and the formation of antibodies from B-cells. It also may inhibit recruitment of leukocytes to inflammatory sites.

A simple, sensitive, selective and rapid high performance liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and validated for the quantification of Mycophenolic acid, using desloratadine as internal standard (IS). Mycophenolic acid was extracted by protein precipitation from 300µL human plasma. The chromatographic separation was achieved on Symmetry® C18 (150mm x 4.6mm, 5µm) analytical column under isocratic conditions, using 10 mM ammonium formate and acetonitrile (25:75, v/v) at a flow-rate of 1.2mL/min, 75% flow splitting.

The parent → product ion transitions for Mycophenolic acid (m/z 321.00→303.00) and IS (m/z 311.00→259.10) were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) and positive ion mode. The linearity of the method for Mycophenolic acid was ascertained in the range of 40-20000ng/mL with the analysis time of 2.2min. The method was fully validated as per USFDA guidelines. The developed method was applied to study the bioequivalence of Mycophenolic acid in human

Method

Major Equipment Involved

Equipment	Make	Model
HPLC	Shimadzu	LC-20 Series
LC/MS/MS	Applied Biosystems	API 3000

Liquid chromatographic conditions

Column	Symmetry® C18(150mmX4.6mm), 5µm particle size
Mobile Phase	10 mM ammonium formate (pH:3.0) : Acetonitrile (25:75 v/v)
Flow	1.2mL/minute, 75% flow splitting
Injection Volume	15µL
Run Time	2.2 minutes

Mass spectrometric conditions

	Mycophenolic Acid	Desloratadine
Parent (Da):	321.00	311.00
Daughter (Da)	303.00	259.10
Dwell (ms):	200	200
DP (V):	34	34
FP (V):	126	260
CE (eV):	14	30

Extraction Procedure

>Add 1mL of ISTD dilution (prepared in acetonitrile) to 300µL of sample, vortex for about 2 minutes.

>Centrifuge the samples at 4000 rpm, at 10°C for 10 minutes.

>Transfer 0.5mL of supernatant into the pre-labeled tubes containing 0.5mL of 10mM ammonium formate solution, vortex to mix.

>Transfer appropriate volume of samples into pre-labeled autosampler vials, and inject 15µL by using HPLC-MS/MS.

Results & Discussion

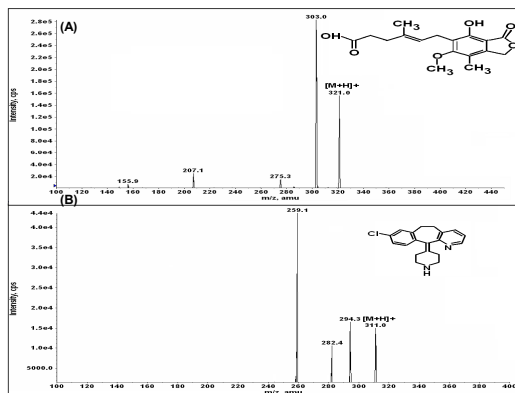


Fig.1- shows the fragmentation pattern of (A) Mycophenolic acid & (B) Desloratadine.

Ion suppression

No ion suppression or enhancement was found at the retention time of analyte in presence of matrix ions through post column infusion of neat solution of analyte.

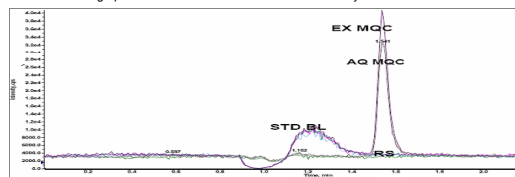


Fig.2- shows the post column infusion spectra of Mycophenolic acid

Specificity

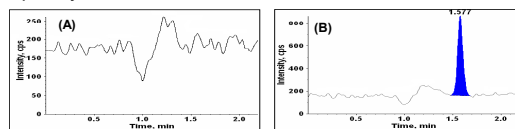


Fig.3- shows the representative chromatogram of (A) Blank & (B) LLOQ for analyte.

Intra-batch and inter-batch precision and accuracy for mycophenolic acid

QC ID	Nominal conc. (ng/mL)	Intra-batch			Inter-batch				
		n	Mean conc. observed (ng/mL) ^a	% CV	% Accuracy	n	Mean conc. observed (ng/mL) ^b	% CV	% Accuracy
HQC	16173.1	6	16919.7	5.9	104.6	30	16466.4	5.9	101.8
MQC	1164.5	6	1033.8	3.6	88.8	30	1066	6.9	91.5
LQC	116.4	6	103.1	5	88.5	30	106.5	7.2	91.5
LLOQ	40.8	6	36.8	4	90.3	30	38	6.1	93.2

CV, coefficient of variance; n, total number of observations.

^a Mean of 6 replicates at each concentration.

^b Mean of 6 replicates for five precision and accuracy batches.

Linearity

The method was validated over the range of 40ng/mL -20000ng/mL with excellent linearity $r^2 > 0.998$.

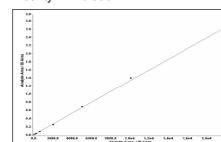


Fig. 4- Representative linearity curve for mycophenolic acid

Stability, Selectivity & Recovery Parameters

Bench Top Stability	Up to 6 hours
Wet Extract Stability	Up to 38 hours at 5±3°C
Freeze Thaw Stability	3 Cycles (-20±5°C & -70±8°C)
Long Term Stability in Human Plasma	102 Days (-20±5°C & -70±8°C)
Specificity	No significant interference
Recovery	76 %

Application of Method for Bioequivalence Study

The validated method was successfully used to quantify the mycophenolic acid concentration in the human plasma samples after the administration of a single 250 mg oral dose of mycophenolate mofetil to 72 healthy volunteers.

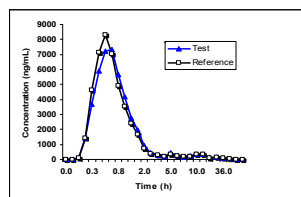


Fig.4- Shows the time-concentration profile of mycophenolic acid in human volunteers

Conclusion

>The bioanalytical methodology for mycophenolic acid is highly specific, rugged and rapid for therapeutic drug monitoring.

>The method involved a simple and specific sample preparation by protein precipitation followed by isocratic chromatographic separation in 2.20 min.

>The overall analysis time is promising compared to other reported procedures for mycophenolic acid.

>The established LLOQ and a wide linear dynamic range is adequate to conduct a pharmacokinetic study with 250mg or higher dose formulations of mycophenolic acid in healthy human volunteers.

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