

CURRENT

Category : Patient Care
Title : Procedure for estimation of Mycophenolic Acid (MPA) in human blood plasma by High Performance Liquid Chromatography (HPLC).
SOP No. and Version : TDM 19/01
Date first effective: 1st January 2025 **Review date:** 31st December 2025
**Department of Clinical Pharmacology, 1st Floor, New MS Building,
Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.**

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TDR/HR/02

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1. Purpose:

This SOP describes the technique for quantitative estimation of Mycophenolic Acid in human blood plasma by High Performance Liquid Chromatography (HPLC).

2. Scope:

This SOP is limited to estimation of Mycophenolic Acid in human blood plasma by High Performance Liquid Chromatography (HPLC) in the Department of Clinical Pharmacology, Seth GSMC and KEMH, Mumbai.

3. Responsibilities:

The Head of the department is responsible for the medical care and welfare of all patients pertaining to TDM of Mycophenolic Acid. The task of performing estimation of Mycophenolic Acid will be delegated to trained personnel (laboratory analysts) who will perform this function.

4. Applicable rules, regulations and guidelines

- ICMR Good Clinical Laboratory Practices Guidelines 2021 (<http://icmr.nic.in/guidelines/GCLP.pdf>)

5. Reference to other applicable SOPs

- SOP No.24/02: Waste management.
- SOP No.TDM01/01: Collection and separation blood plasma for TDM
- SOP No.TDM05/01:Operation of High Performance Liquid Chromatography

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6. Detailed instructions

1. Preparation of standard and calibrator

a. Preparation of Stock Standard (Mycophenolic Acid 1mg/mL) :(10mg)

10 mg of pure powder of Mycophenolic Acid + 10 mL of Methanol

b. Preparation of stock Internal standard (IS): Carbamazepine (CBZ)

10mg of pure powder (Carbamazepine) + 10mL of Methanol-stock

a. Working standard I: 1mL of stock IS + 9mL of Methanol

b. Working standard II: 1 mL of working standard I + 9 mL of Methanol

c. Preparation of Mobile Phase buffer (20mM):

➤ BUFFER SOLUTION (I)

Weigh 10mM 3.609g of Potassium Dihydrogen Phosphate and dissolve in 500 mL of distilled water.

➤ BUFFER SOLUTION (II)

Weigh 10mM 1,7418g of Dipotassium Hydrogen Phosphate and dissolve in 500 mL of distilled water.

➤ Adjust the pH of the buffer to 3.5 using

➤ Mobile Phase Composition:

Buffer: Acetonitrile

49 : 51

d. Filter the mobile phase through 0.22-micron filter and sonicate for 15 minutes.

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2.0. Preparation of Plasma standard

Concentration	Plasma	Mycophenolic Acid	Final volume
20 µg/mL	980 µL	20µL of 1mg/ml Stock	1000 µL
10 µg/mL	500 µL	500µL of 10 µg/ml Stock	1000 µL
5 µg/mL	500 µL	500µL of 20 µg/ml Stock	500 µL

2. Extraction procedure

300µL of plasma (blank/standards/quality control/patients sample) in
2mL Eppendorf tube.



Add 40µL of internal standard (Working standard II) to it, Vortex mix
thoroughly for 30 seconds.



Add 400µL of Acetonitrile and Vortex mix thoroughly for 1 min



Centrifuge at 13000 rpm for 10 minutes.



Inject 20 µL of Supernatant into HPLC for analysis.

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3. HPLC Conditions

- a. Injection volume: 25 μ L
- b. Flow rate: 1.2 mL/min.
- c. Wavelength: 215 nm (UV detector)
- d. Column Oven temperature: 25 $^{\circ}$ C
- e. Run Time: 10.0 min
- f. Retention times for MPA- 5.0-6.0 min, I.S – 9.0– 10.0 min approximately.

HPLC Column: Supelco C18 column (250mm \times 4.6 mm \times 5 μ)

4. Abbreviations:

- a. HPLC = High Performance Liquid Chromatography
- b. I.S. = Internal Standard
- c. MPA = Mycophenolic Acid
- d. Std = Standard
- e. CBZ = Carbamazepine