CURRENT

Category : Patient Care
Title : Procedure for estimation of Metformin in human blood plasma by High Performance Liquid Chromatography (HPLC).
SOP No. and Version : TDM 18/01
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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1. Purpose:

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

2. Scope:

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

3. Responsibilities:

The Head of the department is responsible for the medical care and welfare of all patients pertaining to TDM of Metformin. The task of performing estimation of Metformin 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

6. Detailed instructions

1. Preparation of standard and calibrator

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

10 mg of pure powder of Metformin + 10 mL of distilled water

b. Preparation of stock Internal standard (IS): Phenytoin (PHT)

10mg of pure powder **(Phenytoin)** + 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 (10mM):

- **SOLUTION (I)**

  Weigh 10mM 1.360g of Potassium Dihydrogen Phosphate and dissolve in 500 mL of distilled water.

- **SOLUTION (II)**

  Weigh 10mM 2.883g of Sodium Lauryl Sulphate and dissolve in 500 mL of distilled water.

- Adjust the pH of the buffer to 5.2

d. Preparation of Mobile phase

Buffer: Acetonitrile 660:340

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

e. Preparation of Plasma standard

Concentration	Plasma	Metformin	Final volume
20µg/mL (III)	980 µL	20µL of 1mg/ml Stock	1000 µL
10µg/mL (II))	500 µL	500µL of 20 µg/ml Stock	1000 µL
5µg/mL (I)	500 µL	500µL of 10 µg/ml Stock	500 µL

2. Extraction procedure

100µL of plasma **(blank/standards/quality control/patients sample)** in 2mL Eppendorf tube.

⇩

Add 50µL of internal standard (Working standard II) to it, Vortex mix thoroughly for 2 min

⇩

Add 300_µL of Acetonitrile and Vortex mix thoroughly for 2 min -

⇩

Centrifuge at 6000 rpm for 10 minutes.

⇩

Inject 30µL of supernatant into HPLC auto Sampler.

3. HPLC Conditions

a. Injection volume: 30µL

b. Flow rate: 1.3 mL/min.

c. Wavelength: 233 nm (UV detector)

d. Column Oven temperature: 25°C

e. Run Time: 15.00 min

f. Retention times for MET- 12.0-12.8 min, I.S – 7.7– 8.0 min approximately.

g. HPLC Column: Supelco C18 column (250mm × 4.6mm x 5µ).

4. Abbreviations:

a. **HPLC** = High Performance Liquid Chromatography

b. **I.S.** = Internal Standard

c. **MET** = Metformin

d. **Std** = Standard

e. **PHT** = Phenytoin