CURRENT

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SOP No. and Version: TDM-16/01
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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Author: **Swati More** 30/Dec/24
Lab. Analyst

Dr. Bhaskar Krishnamurthy
Lab. In-charge

30/DEC/2024
Dr. Bhaskar Krishnamurthy
Assistant Professor,
Department of Clinical Pharmacology,
Seth GSMC and KEMH,Mumbai -400 012.

Signature with date:

Reviewer: **Dr. Mahesh Belhekar**
Associate Professor

Signature with date: 30/Dec/2024
Dr. Mahesh N. Belhekar
Associate Professor
Department of Clinical Pharmacology
New MS Building, First Floor,
Seth GS Medical College and KEM Hospital
Acharya Donde Marg, Parel,
Mumbai - 400 012. India

Approved by: **Dr. Nithya Gogtay**
Professor and Head

Signature with date:

Dr. Nithya Gogtay
Professor & Head
Department of Clinical Pharmacology
1st Floor, MS Building,
Seth GS Medical College & KEM Hospital
Parel, Mumbai - 400 012.
30/12/24

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1. Table of Contents

1. Purpose:

This SOP describes the technique for qualitative and quantitative estimation of Hydroxyurea in human Plasma by High Performance Liquid Chromatography (HPLC).

2. Scope:

This SOP is limited to estimation of Hydroxyurea in human 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 Hydroxyurea. The task of performing estimation of Hydroxyurea will be delegated to trained personnel (laboratory technicians) who will perform this function.

4. Applicable rules, regulations and guidelines

- ICMR Good Clinical Laboratory Practices Guidelines 2008 (http://icmr.nic.in/guidelines/GCLP.pdf)

5. Reference to other applicable SOPs

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

6. Detailed instructions

1. Preparation of standard and calibrator

a. Preparation of Stock Standard (Hydroxyurea 1mg/mL)

10 mg of pure powder of Hydroxyurea + 10 mL of Distilled water

b. Preparation of stock Internal standard (Carbamazepine 1mg/mL)

10mg of pure powder **Carbamazepine** + 10mL of Methanol

a. Working standard IS (25 ug/ml): 25µL of stock IS + 975µL of Methanol

c. Preparation of Stock (Xanthydrol 1mg/mL) :(10mg)

10 mg of pure powder of Xanthydrol + 10 mL Methanol

a. Working stock (100 ug/ml): 100µL of stock IS + 900µL of Methanol

d. Preparation of Stock Standard (Hydrochloric acid 1mg/mL) :(10mg)

10 mg of Hydrochloric acid + 10 mL Distilled Water

a. Working stock (100 ug/ml): 100µL of stock IS + 900µL of Distilled water

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

Weigh 1.54165 gm of Ammonium acetate ($C_2H_7NO_2$) and dissolve in 1000 mL of distilled water

2. Preparation of plasma standards:

Concentration µg/mL	20 (I)	10 (II)	5 (III)
Blank plasma	980µL	500µL	500µL
HU Stock 1mg/mL	20µL mix well	-----	---
		500µL stock(I)	500µL stock (II)

3. Extraction procedure

200µL of Plasma **(blank plasma/standards/quality control/patients' sample)** in 2mL Eppendorf tube.

⇩

Add 50µL of Internal standard (Working standard 25 ug/ml)

⇩

Add 600µL of Methanol

⇩

Add 100µL of Xanthydrol (Working standard 100 ug/ml)

⇩

Add 50µL of HCl

⇩

The mixture and vortex 2 sec

⇩

Leave at room temperature protected from light for 5 min

⇩

Centrifuge the suspension at 8000 rpm for 10 mins

⇩

Directly Inject Supernant into HPLC for analysis.

4. Preparation of Mobile phase

a. Take Ammonium acetete ($C_2H_7NO_2$) buffer (20mM): Acetonitrile; in the ratio of 700: 300 for 1000mL.

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

5. HPLC Conditions

a. Injecting volume: 25µL

b. Flow rate: 1.0mL/min.

c. Wavelength: 240 nm (UV detector)

d. Run Time: 25.00 min (approximately)

e. Retention times for HU- 8.1- min, I.S 9.1 – min approximately.

f. HPLC Column: Pico. Tag for Free Amino acid Analysis column (3.9×300mm,).

6. Abbreviations:

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

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

c. **HU** = Hydroxyurea

d. **Std** = Standard

e. **CBZ** = Carbamazepine

f. **Xanth**= Xanthydrol

g. **HCl** = Hydrochloric acid