

# CURRENT

**Category** : Patient Care  
**Title** : Procedure for estimation of LTG, PBT, PHT and CBZ in human plasma by High Performance Liquid Chromatography (HPLC).  
**SOP No. and Version:** TDM06/02

**Date first effective:** 1<sup>st</sup> January 2025 **Review date:** 31<sup>st</sup> 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*  
Swati More  
Lab. Analyst

**Dr. Bhaskar Krishnamurthy**  
Lab. In-charge

Signature with date:

*BK*  
30/DEC/2024

**Reviewer:**

*Belhekar*  
30/12/2024  
**Dr. Mahesh Belhekar**  
Associate Professor

**Dr. Bhaskar Krishnamurthy**  
Assistant Professor,  
Department of Clinical Pharmacology,  
Seth GSMC and KEMH, Mumbai -400 012.

Signature with date:

**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**

*NG*  
30/12/24  
Professor & Head  
Department of Clinical Pharmacology  
1<sup>st</sup> Floor, MS Building,  
Seth GS Medical College & KEM Hospital,  
Parel, Mumbai - 400 012.

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### **1. Purpose:**

This SOP describes the technique for the qualitative and quantitative estimation of Lamotrigine, Phenobarbitone, Phenytoin and Carbamazepine in human plasma by High Performance Liquid Chromatography (HPLC).

### **2. Scope:**

This SOP is limited to estimation of LTG, PBT, PHT and CBZ in  $\mu\text{g/ml}$  of in human plasma by High Performance Liquid Chromatography (HPLC).

### **3. Responsibilities:**

The Head of the department is responsible for the medical care and welfare of all patients pertaining to TDM of LTG, PBT, PHT and CBZ. The task of performing estimation of PBT, PHT, LTG and CBZ will be delegated to trained personnel 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: Biomedical waste management.
- SOP No. TDM01/02: Collection and separation blood plasma for TDM
- SOP No. TDM05/02: Operation of High-Performance Liquid Chromatography

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## 6. Detailed instructions

### 1. Preparation of standards and calibrators

#### Preparation of Stock Standards

#### i. Preparation of Stock Standard (Lamotrigine-LTG) 1mg/ml:

10 mg of LTG in 10 ml of Methanol, shake it well

#### ii. Preparation of Stock Standard (Phenobarbital-PBT) 1mg/ml:

10 mg of PBT in 10 ml of Methanol, shake it well.

#### iii. Preparation of Stock Standard (Phenytoin-PHT) 1mg/ml:

10 mg of PHT in 10 ml of Methanol, shake it well.

#### iv. Preparation of Stock Standard (Carbamazepine-CBZ) 1mg/ml:

10 mg of CBZ in 10 ml of Methanol, shake it well.

#### v. Preparation of Stock Internal Standard (10-Methoxy carbamazepine) 1mg/ml(I):

10 mg of S.I.S in 10 ml of Methanol, shake it well.

#### vi. Preparation of stock Internal Standard (10-Methoxy carbamazepine) 100µg/ml (II):

1ml of Stock (I) I.S in 9 ml of Methanol, shake it well. (II)

#### vii. Preparation of working Internal Standard (I.S-III) 10µg/ml.:

1ml of Stock (II) I.S in 9 ml of Methanol, shake it well. (III)

#### 1.1. Preparation of Plasma Stock Standards:

	PBT	LTG	PHT	CBZ
Blank Plasma	450µl	450µl	450µl	450µl
	50µl PBT stock(1mg/ml)	50µl LTG stock(1mg/ml)	50µl PHT stock(1mg/ml)	50µl CBZ stock(1mg/ml)

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**1.2 Preparation of Plasma Stock Standards:**

	2µg/ml	4µg/ml	8µg/ml	10µg/ml
<b>Blank Plasma</b>	400µl	300µl	100µl	600µl
				100µl PBT,LTG,PHT, and CBZ stock standard Mix well and transfer to
<b>10µg/ml(stock)</b>	100µl	200µl	400µl	

**Preparation of buffer for Mobile Phase:**

0.25g Di. Sodium hydrogen phosphate + 0.1504g of Potassium hydrogen Phosphate. Dissolve in 500ml distilled water.

**1. Extraction procedure**

Take 100µl of blank Plasma/Standards/Quality control sample in clean stoppered round bottom tube



Spike 25µl (10-Methoxy Carbamazepine 10µg/ml) of Internal standard



To it add 200µl of extraction buffer (pH-3.9). Precipitation takes place



The drug is extracted in 5.0ml of ethyl acetate



The tubes are centrifuged at 2500 rpm for 20minutes



The supernatant obtained is evaporated to dryness under gentle flow of nitrogen at ambient temperature



During analysis by High Performance Liquid Chromatographic method reconstitute the sample with 100µl of methanol and inject 25µl

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## 2. Preparation of Mobile phase

- a. Take Buffer: Methanol: Acetonitrile in the ratio of **11:4:4** according to the number of injections
- b. Filter the mobile phase through **0.22-micron** filter and sonicate for 15 minutes

## 3. HPLC Conditions

- a. Injecting volume: 10 $\mu$ l
- b. Flow rate: 1.1 ml/min.
- c. Wavelength: 220 nm
- d. HPLC Column: C18 column
- e. Run Time: 20 min (approximately)
- f. Retention times for LTG- 3.0-3.5 min, PBT- 4.0-4.5 min, PHT- 9.5 – 10.5, CBZ – 11.00 – 12.00 and I.S.-14.00-16.00 min approximately.

## 4. Abbreviations:

- a. **CBZ** = Carbamazepine
- b. **HPLC** = High Performance Liquid Chromatography
- c. **I.S.** = Internal Standard
- d. **LTG** = Lamotrigine
- e. **PBT** = Phenobarbital
- f. **PHT** = Phenytoin
- g. **Std** = Standard