

# CURRENT

Category : Patient Care  
Title : Procedure for operation of High Performance Liquid Chromatography (HPLC) machine.

SOP No. and Version: TDM 03/02

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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**1. Table of Contents**

No.	Contents	Page No.
1	Purpose	3
2	Scope	3
3	Responsibility	3
4	Applicable rules/guidelines	3
5	Reference to other applicable SOPs	3
6	Detailed instructions	4
7	Abbreviations	5



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#### 6. Detailed instructions

1. Filter the solvents ((Distilled water, Acetonitrile: Distilled Water (70:30) or Methanol: Distilled on the rack in TDM assay lab) and mobile phase used for HPLC through 0.22-micron membrane filter
2. Sonicate all filtered solvents for 15 minutes in the sonicator
3. Switch on HPLC
4. Fix the column of the respective assay to HPLC (Direction of column should be in position from pump to detector).
5. Purge the HPLC pump with distilled water at 3 ml/min flow rate for 3 to 5 minutes to remove the air bubbles
6. Wash HPLC column with filtered and sonicated distilled water for 30 minutes at 1 ml/min flow rate.
7. Wash the HPLC column with filtered and sonicated Acetonitrile: Distilled Water (70:30) for VAL, VORI, VAN and LEV assay and Methanol: Distilled Water (70:30) for PBT, PHT, CBZ and LTG assay for 30 minutes at 1 ml/min flow rate
8. Prepare mobile phase as per the given in SOP of respective drug assay.
9. Filter the mobile phase and sonicate for 15 minutes
10. Saturate the column with mobile phase for 10 minutes at flow rate 1ml/min
11. Change the flow rate as per assay and keep for 10 minutes for stabilization
12. After stabilization commence with the injections according to assay protocol
13. After completion stop the pump and switch off the detector
14. Repeat steps 5 and 6
15. Switch off the HPLC



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### 1. Purpose:

This SOP describes the technique for the qualitative and quantitative estimation of drugs in human plasma using High Performance Liquid Chromatography (HPLC) machine.

### 2. Scope:

This SOP is limited to the technique used for Therapeutic drug monitoring (TDM) of drugs in human plasma by High Performance Liquid Chromatography (HPLC) machine.

### 3. Responsibilities:

The Lab technician and Lab In charge are responsible for the operation and maintenance of HPLC machine for TDM.

### 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.02/02: Estimation of PBT, PHT and CBZ
- SOP No. 03/02: Estimation of VAL
- SOP No. 04/02: Estimation of LTG
- SOP No. 07/02: Estimation of Li
- SOP No. 08/02: Estimation of LEV
- SOP No. 09/01: Estimation of PQ & CPQ
- SOP No. 10/01: Estimation of VAN
- SOP No. 11/01: Estimation of VORI



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## **7. Abbreviations**

1. HPLC-High Performance Liquid Chromatography
2. CBZ - Carbamazepine
3. LTG - Lamotrigine
4. PBT- Phenobarbitone
5. PHT- Phenytoin
6. VAL - Valproate
7. LEV – Levetiracetam
8. PQ- Primaquine
9. CPQ- Carboxyprimaquine
10. VAN- Vancomycin
11. VORI- Voriconazole