Category : Patient Care
Title : Procedure for estimation of Levetiracetam in human plasma by High Performance Liquid Chromatography (HPLC).
SOP No. and Version: TDM08/02
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. Table of Contents

1. Purpose:

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

2. Scope:

This SOP is limited to estimation of Levetiracetam in µg/mL 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 Levetiracetam. The task of performing estimation of Levetiracetam 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

6. Detailed instructions

1. Preparation of standard and calibrator

a. Preparation of Stock Standard (Levetiracetam acid 1mg/mL) :(0.010g)

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

b. Preparation of stock Internal standard (IS): (Caffeine)

10mg of pure powder **(Caffeine)** IS + 10mL of Distilled water-stock IS

c. Preparation of stock Standard: (25 µg/ml)

a. 25µL of 1 mg/mL stock solution + 950 µL of Distillated Water

e. Preparation of Mobile Phase buffer (50mM):

Weigh 6.804g of Potassium dihydrogen phosphate (KH_2PO_4) and dissolve in 1000 mL of distilled water

2. Preparation of plasma standards:

	25µL of stock (I)	**50µL of stock(II)**	**100µLofstock(III)**
Blank Plasma	500µL	500µL	900µL
(Levetiracetam acid 1mg/mL)	---	----	100µL mix well and transfer to
	500µL of **(50µL of stock)**	500µL of **(100µL of stock)**	

3. Extraction procedure

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

⇩

Add 25µL of internal standard (Working standard III) to it, Vortex mix thoroughly for 1 min.

⇩

Add 200µL of Dimethyl sulfoxide (DMSO) and Vortex mix thoroughly for 1 min.

⇩

Centrifuge at 8000 rpm for 15mins

⇩

Remove 100µL of supernatant and add transfer into HPLC vials.

⇩

25 µL Inject into HPLC for analysis.

4. Preparation of Mobile phase

a. Take Potassium dihydrogen phosphate (KH_2PO_4) buffer (50Mm): Acetonitrile in the ratio of 97:03

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

5. HPLC Conditions

a. Injecting volume: 20µL

b. Flow rate: 1.5 mL/min.

c. Wavelength: 205 nm

d. Run Time: 10.00 min (approximately)

e. Retention times for LEVE- 6.0-7.0 min, I.S – 8.0– 9.0 min approximately.

f. HPLC Column: C18 column (250×4.6mm, 5µ).

6. Abbreviations:

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

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

c. **LEV** = Levetiracetam

d. **Std** = Standard