CURRENT

Category : Patient Care
Title : Procedure for estimation of Valproate (VAL) in human plasma by High Performance Liquid Chromatography (HPLC).
SOP No. and Version : TDM 04/02
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.

Category: Patient Care

Title: Procedure for estimation of Valproate (VAL) in human blood plasma by High Performance Liquid Chromatography (HPLC).

SOP No.: TDM 04/02 **Total pages:** 06

Date first effective: 1st January 2025 **Next Review date:** 31st December 2025

Version: 02

Author: **Swati More**, **Lab. Analyst** — 30/Dec/24 — **Dr. Bhaskar Krishnamurthy**, **Lab In-charge** — 30/DEC/2024

Signature with date:

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

Reviewer: **Dr. Mahesh Belhekar**, **Associate Professor** — 30/Dec/2024

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** — 30/12/24

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.

1. Table of Contents

1. Purpose:

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

2. Scope:

This SOP is limited to the estimation of Valproate 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 Valproate. The task of performing estimation of Valproate 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 of 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 (Valproic acid 1mg/mL) :(0.010g)

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

b. Preparation of extraction buffer:

0.952 g of potassium dihydrogen phosphate + 1.87 g of disodium hydrogen phosphate, dissolve in 50 mL of distilled water

c. Preparation of extracting reagent:

0.5g of 4-bromophenacyl bromide + 0.0125g of Dicyclohexane-18-crown-6, dissolve in 50mL of acetonitrile

d. Preparation of stock Internal standard (IS): (Nonanoic acid)

16.67µL **(Nonanoic acid)** of IS + 10mL of acetonitrile-stock IS

e. Preparation of Working Internal standard (IS):

Working standard I	1mL of stock IS	9 mL of Acetonitrile
Working standard II	1 mL of working standard I	9 mL of Acetonitrile
Working standard III	5 mL of working standard II	5 mL of Acetonitrile

e. Preparation of Mobile Phase buffer (0.003M):

a. Weigh 0.1021g of KH2PO4 and dissolve in 250 mL of distilled water

Preparation of Valproic acid plasma standards:

	25µg/mL (I)	**50µg/mL (II)**	**100µg/mL(III)**
Blank Plasma	375 µL	100µL	450 µL
valproic acid 1 mg/mL			50µL mix well transfer to
	125µL **(100µg/mL)**	100µL(**100µg/mL**)	

d. Extraction procedure

Add 0.1 mL of plasma **(blank/std/quality control/sample)**

⇩

0.025 mL of extracting buffer pipette in a clean dry 'U' shaped stoppered test tube

⇩

Add 0.25mL of internal standard to it, Mix thoroughly for 2 min.

⇩

Centrifuge at 2000 to 2500 rpm for 15mins

⇩

Transfer 200µL of supernatant in clean 'V' shaped test tube.

Add 50 µL of extracting solution

⇩

Esterify the content at 70°c for 15 minutes in water bath

⇩

Cool the tubes to room temperature.

⇩

And inject HPLC 50 µL

2. Preparation of Mobile phase

a. Take buffer: Acetonitrile in the ratio of 1:4

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

3. HPLC Conditions

a. Injecting volume: 50µL

b. Flow rate: 1.4 mL/min.

c. Wavelength: 254 nm

d. Run Time: 9.00 min (approximately)

e. Retention times for VAL- 5.0-6.0 min, I.S – 7.5 – 8.5 min approximately.

f. HPLC Column: C18 column

4. Abbreviations:

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

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

c. **VAL** = Valproate/Valproic Acid

d. **Std** = Standard