

# CURRENT

Category : Patient Care  
Title : Procedure for estimation of Voriconazole in human blood plasma by High Performance Liquid Chromatography (HPLC).

SOP No. and Version : TDM 13/01

Date first effective: 1<sup>st</sup> January 2025

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Department of Clinical Pharmacology, 1st Floor, New MS Building,  
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### 1. Purpose:

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

### 2. Scope:

This SOP is limited to estimation of Voriconazole in  $\mu\text{g/ml}$  in human blood 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 Voriconazole. The task of performing estimation of Voriconazole 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: Waste management.
- SOP No.TDM01/01: Collection and separation blood plasma for TDM
- SOP No.TDM05/01:Operation of High Performance Liquid Chromatography

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6. Detailed instructions

1. Preparation of standard and calibrator

a. Preparation of Stock Standard (Voriconazole 1mg/mL) :(10mg)

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

b. Preparation of stock Internal standard (IS): Carbamazepine (CBZ)

10mg of pure powder (Carbamazepine) IS + 10mL of Methanol-stock IS

a. Working standard I: 1mL of stock IS + 9mL of Methanol

b. Working standard II: 1 mL of working standard I + 9 mL of Methanol

c. Preparation of Mobile Phase buffer (0.05M):

Weigh 3.854g of Ammonium acetate ( $C_2H_7NO_2$ ) and dissolve in 1000 mL of distilled water

d. Preparation of Plasma standard

	5 $\mu$ g/mL ( I)	10 $\mu$ g/mL ( II)	20 $\mu$ g/mL ( III)
Blank plasma	500 $\mu$ L	500 $\mu$ L	980 $\mu$ L
(Voriconazole 1mg/mL)	-----	-----	20 $\mu$ L mix well and transfer to
20 $\mu$ g/mL	500 $\mu$ L	500 $\mu$ L	

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### 3. Preparation of Mobile phase

- a. Take Ammonium acetate ( $C_2H_7NO_2$ ) buffer (0.05M): Acetonitrile: Methanol; in the ratio of 40:16:44.
- b. Filter the mobile phase through 0.22-micron filter and sonicate for 15 minutes.

### 4. HPLC Conditions

- a. Injecting volume: 25 $\mu$ L
- b. Flow rate: 1.0 mL/min.
- c. Wavelength: 255 nm (UV detector)
- d. Column Oven temperature: 40°C
- e. Run Time: 08.00 min (approximately)
- f. Retention times for VORI- 5.0-6.0 min, I.S – 4.0– 5.0 min approximately.
- g. HPLC Column: Supelco C18 column (250 $\times$ 4.6mm, 5 $\mu$ ).

### 5. Abbreviations:

- a. **HPLC** = High Performance Liquid Chromatography
- b. **I.S.** = Internal Standard
- c. **VORI** = Voriconazole
- d. **Std** = Standard
- e. **CBZ** = Carbamazepine