CURRENT

Category : Patient Care
Title : Procedure for estimation of Vancomycin in human serum by High Performance Liquid Chromatography (HPLC).
SOP No. and Version: TDM010/01
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. Purpose:

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

2. Scope:

This SOP is limited to estimation of Vancomycin in µg/ml in human serum 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 Vancomycin. The task of performing estimation of Vancomycin 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 standard and calibrator

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

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

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

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

c. Preparation of Working standard (IS): [Caffeine (CAF)]

Working standard I	1mL of stock IS	9mL of Distilled water
Working standard II	5 ml of working standard (I)	5 mL of Distilled water
Working standard III	5 ml of working standard (II)	5 mL of Distilled water.

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

Weigh 5.7515g of Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and dissolve in 1000 mL of distilled water

2. Preparation of serum standards:

Concentration	Plasma	Vancomycin	Final volume
20 µg/mL	980 µL	20µL of 1mg/ml Stock	1000 µL
10 µg/mL	500 µL	500µL of 10 µg/ml Stock	1000 µL
5 µg/mL	500 µL	500µL of 20 µg/ml Stock	500 µL

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3. Extraction procedure

500µL of serum **(blank/standards/quality control/patients' sample)** in 2mL Eppendorf tube.

⇩

Add 30µL of internal standard (Working standard III) to it, Vortex mix thoroughly for 30 seconds.

⇩

Add 500µL of Isopropanol: Acetonitrile (1:1) diluent and Vortex mix thoroughly for 2 min.

⇩

Keep the Eppendorf tubes in ice tub for 10 minutes to facilitate protein precipitation.

⇩

Remove from ice tub after 10 minutes and shake 100 times manually and again vortex for 2 minutes.

⇩

Centrifuge the suspension at 15000 rpm for 15mins at 4°C.

⇩

Transfer the Supernatant with Pasteur pipette to another 7mL glass test tubes.

⇩

Add 3.5mL methylene chloride to the supernatant and vortex for 2 minutes. Again, centrifuge at 2500 rpm for 20 minutes

⇩

Remove the 100µL of supernatant and add transfer into HPLC vials. Inject into HPLC for analysis.

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4. Preparation of Mobile phase

a. Take Ammonium dihydrogen phosphate **($NH_4H_2PO_4$)** buffer (50mM): Acetonitrile; in the ratio of 90:10 and adjust the PH to 2.2 with orthophosphoric acid and 100µL formic acid for 1000mL.

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

5. HPLC Conditions

a. Injecting volume: 25µL

b. Flow rate: 0.90 mL/min.

c. Wavelength: 205 nm (UV detector)

d. Run Time: 20.00 min (approximately)

e. Retention times for VAN- 10.0-11.0 min, I.S – 11.0– 12.0 min approximately.

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

6. Abbreviations:

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

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

c. **VAN** = Vancomycin

d. **Std** = Standard

e. **CAF** = Caffeine

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