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SOP No. : TDM 11/01
Date first effective: 1st January 2024 Review date: 31st December 2024
Department of Clinical Pharmacology, 1st Floor, New MS Building,
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Author: *Swati More*
Swati More Lab-Technician Dr. Bhaskar Krishnamurthy Lab- In-charge

Signature with date:

BK
30/DEC/2024

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

Reviewer: *Beth*
Dr. Mahesh Belhekar Associate Professor

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

Signature with date:

N
30/DEC/24
Dr. Nithya Gogtay
Professor & Head
Department of Clinical Pharmacology
1st Floor, MS Building,
Seth GS Medical College & KEM Hospital,
Parel, Mumbai - 400 012.

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1. Purpose:

This SOP describes the technique for qualitative and quantitative estimation of vancomycin in human plasma by Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS).

2. Scope:

This SOP is limited to find out the concentration in $\mu\text{g/ml}$ of vancomycin in human plasma by Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS).

3. Responsibilities:

The head of the department is responsible for the medical care and welfare of all patients under her/his care. 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.24/01: Waste management.

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6. Detailed instructions:

A. Chemicals and materials:

- i. Pure Powder of Vancomycin
- ii. Pure powder of Phenacetin (IS)
- iii. Miscellaneous: Methanol (HPLC Grade-Merck make), Ammonia Solution (HPLC grade), Ammonium formate (GR Grade-Merck make), Formic Acid (HPLC Grade-Merck make), Purified water.
- iv. LC column : C18, 150 mm x 4.6 mm, 5 micron particle size
- v. Cartridges :
- vi. Glassware: Eppendorf vials (2.0mL), Glass Test Tubes (10mL), Conical Flasks (100, 250, 500, 1000 mL), Measuring Cylinders (100mL, 500mL), falcon tubes, volumetric flasks (10mL).

B. Equipments: High Performance Liquid Chromatography (Shimadzu), Mass Spectrometry (API 2000), Cooling Centrifuge 15,000 X g (Biofuge), Ultra sonicator machine (Imeco), Vortex machine(Spinage), Auto pipettes-eppendorf (10-100 μ L, 100-1000 μ L), Centrifuge(Remi-R23), Manifold Solid Phase Extraction (Varian).

C. Preparation of solutions, mobile phase and calibration standards:

a. Solutions:

- i. **Preparation of stock 5mM ammonium formate buffer, pH 3.0:** - 0.3153 gm of ammonium formate dissolved and diluted up to 1000 ml of distilled water and adjusted pH 3.0 with formic acid solution.
- ii. **Preparation of 5% ammonia solution (Elution solution) in methanol:** - 5 mL of ammonia solution (25% ammonia) diluted up to 100 mL with methanol.

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- iii. **Preparation of 2% formic acid (Acid solution) solution:**
2 mL of formic acid solution diluted up to 100 mL with purified water.
- iv. **Preparation of 1: 1 Methanol: Purified water solution: -**
50 mL of Methanol diluted up to 100 mL with purified water
- v. **Preparation of system suitability solution:** 975 uL of mobile phase + 25 uL of 1mg/mL of stock (1mg/mL)
- vi. **Mobile phase:** Acetonitrile: Ammonium formate buffer (60:40)

b. Calibration standards (Vancomycin):

- i. **Preparation of aqueous stock standard solution (1 mg/mL) of vancomycin:** 10 mg of vancomycin hydrochloride dissolve and diluted up to 10 mL with 1:1 methanol: purified water solution and vortexes for 2 mins.
- ii. **Preparation of aqueous standard solution (100 µg/mL) of vancomycin:** 1 mL of stock solution (1 mg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- iii. **Preparation of aqueous standard solution (75 µg/mL) of vancomycin:** 750 µL of stock solution (1 mg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- iv. **Preparation of aqueous standard solution (50 µg/mL) of vancomycin:** 5 mL of stock solution (100 µg/mL) diluted

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- up to 10 mL with 90% methanol solution (0.1% formic acid)
- v. **Preparation of aqueous standard solution (25 µg/ml) of vancomycin:** 5 mL of stock solution (50 µg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- vi. **Preparation of aqueous standard solution (10 µg/ml) (Tuning solution II) of vancomycin :** 1 mL of stock solution (100 µg/mL) diluted up to 10 ml with 90% methanol solution (0.1% formic acid)
- vii. **Preparation of aqueous standard solution of vancomycin (Tuning solution I):** 1 mL of stock solution (10 µg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- viii. **Preparation of aqueous standard solution (0.5 µg/mL) of vancomycin:** 5 mL of stock solution (1 µg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- ix. **Preparation of aqueous stock standard solution (0.25 µg/ml) of vancomycin:** 5 mL of stock solution (0.5 µg/mL) diluted up to 10 mL with 90% methanol solution (0.1% formic acid)
- c. **Preparation of Internal Standard (Phenacetin):**
- i. **Preparation of stock aqueous internal standard (1 mg/mL):** 10 mg of phenacetin dissolve and diluted up to 10 ml of methanol (100%) and vortexes for 2 mins.

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- ii. **Preparation of stock aqueous internal standard (100 µg/mL):** 1 mL of stock solution (1 mg/mL) diluted up to 10 ml with methanol (100%)
 - iii. **Preparation of working internal standard (10 µg/mL):** 1 ml of stock solution (100 µg/mL) diluted up to 10 ml with methanol (100%)
 - iv. **Preparation of tuning solution (1 µg/mL):** 1 mL of stock solution (10 µg/mL) diluted up to 10 ml with 90% methanol (0.1% formic acid).
- d. Preparation of plasma Standard (vancomycin):**
- i. **Preparation of stock plasma standard (100 µg/mL) of vancomycin:** 1 mL of aqueous stock solution (1 mg/mL) of vancomycin diluted with 9.0 mL of blank plasma
 - ii. **Preparation of plasma standard (75µg/mL):** 750 µL of stock plasma standard (100 µg/mL) of vancomycin diluted with 250 µL of blank plasma.
 - iii. **Preparation of plasma standard (50 µg/mL):** 2.0 mL of stock plasma standard (100 µg/mL) of vancomycin diluted with 2.0 mL of blank plasma.
 - iv. **Preparation of plasma standard (25 µg/mL):** 1ml of stock plasma standard (50 µg/mL) of vancomycin diluted with 1.0 mL of blank plasma.

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- v. **Preparation of plasma standard (10 µg/mL):** 300 µL of stock plasma standard (100 µg/mL) of vancomycin diluted with 2700 µL of blank plasma.
- vi. **Preparation of plasma standard (5µg/mL):** 500 µL of plasma standard (10µg/mL) of vancomycin diluted with 500 µL of blank plasma.
- vii. **Preparation of plasma standard (1 µg/mL):** 300 µL of stock plasma standard (10µg/mL) of vancomycin diluted with 2700 µL of blank plasma.
- viii. **Preparation of plasma standard (0.5µg/mL):** 1000 µL of plasma standard (1 µg/mL) of vancomycin diluted with 1000 µL of blank plasma.
- ix. **Preparation of plasma standard (0.250µg/mL):** 500 µL of plasma standard (0.5µg/mL) of vancomycin diluted with 500 µL of blank plasma.

e. Preparation of quality control samples:

- i. **Preparation of lower limit of quantification (LLOQ) sample (0.5 µg/mL):** 1000 µL of plasma standard (1 µg/mL) of vancomycin diluted with 1000 µL of blank plasma.
- ii. **Preparation of Middle Quality Control (MQC) sample (25 µg/ml):** 1.5 mL of stock plasma standard (50 µg/mL) of vancomycin diluted with 1.5 mL of blank plasma.
- iii. **Preparation of High-Quality Control (HQC) sample (75 µg/ml):** 2250 µL of stock plasma standard (100 µg/mL) of vancomycin diluted with 750 µL of blank plasma.

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f. Extraction procedure

Plasma standard preparation (Pretreatment): 500 μ L of plasma standard + 100 μ L of (10 μ g/mL) working internal standard. Vortex for 30 seconds and adds 500 μ L of acid solution. Then vortex for 15 seconds.

Solid phase extraction: MCX (Waters) 1cc, 30 mg cartridges

Conditioning: 1 mL of methanol



Equilibrating: 1 mL of 2% acid solution



Load entire sample



Washing 1: 1 mL of acid solution

Washing 2: 1 mL of methanol



Elution: 1 mL of elution solution (5% ammonia in methanol solution)



Evaporate at 50°C and reconstitute with 0.5 mL of mobile phase, vortex for 30 seconds.

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g. LCMS condition:

LC parameters

- i. Injecting volume: 10 μ L
- ii. Flow rate: 0.3 mL/min
- iii. Column: C18, 150 mm x 4.6 mm, 5-micron particle size
- iv. Auto sampler temperature: 25 $^{\circ}$ C
- v. Column oven temperature: 30 $^{\circ}$ C
- vi. Total run time: 10 mins

MS parameters:

Peak name	Q1 mass	Q3 mass	Time (msec)	ID	DP	FP	EP	CE	CXP
Vancomycin	725.0	144.00	200	Vanco	15	350	8	25	5
IS	180.10	110.10	200	IS	23	350	8	30	5

Scan type: MRM

Polarity: Positive

Curtain gas: 30 PSI

CAD: 10 PSI

Ion Spray voltage: 5500 v

Heater temperature: 425 $^{\circ}$ C

GS1 : 30

GS2: 50

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1. Abbreviations :

- i. **LCMS** = Liquid Chromatography Mass Spectrometry
- ii. **QC** = Quality Control
- iii. **LLOQ** : Lower Limit of Quantification
- iv. **MQC** : Middle Quality Control
- v. **HQC** : High Quality Control
- vi. **I.S.** = Internal Standard
- vii. **DP** = Declustering Potential
- viii. **FP** = Focusing Potential
- ix. **EP** = Entrance Potential
- x. **CE** = Collision Energy
- xi. **CXP** = Collision Exit Potential