CURRENT

Category

: Patient Care

Title

: Procedure for estimation of Aspirin in human serum by

Spectrophotometer

SOP No. and Version: TDM 15/01

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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Author:

Lab. Analyst

Dr. Bhaskar Krishnamurthy

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Dr. Bhaskar Krishnamurthy

B1~16

Lab. In charge

Signature with date:

Reviewer:

Dr. Mahesh Belhekar Associate Professor Dr. Mahesh N. Belhekar

Signature with date:

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: Professor & Head

Dr. Nithya Gogtay

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 Aspirin in human Serum by Spectrophotometer

2. Scope:

This SOP is limited to estimation of aspirin in $\mu g/mL$ in human Serum by Spectrophotometer

3. Responsibilities:

The Head of the department is responsible for the medical care and welfare of all patients pertaining to TDM of aspirin The task of performing estimation of Spectrophotometer will be delegated to trained personnel (laboratory technician) 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/serum for TDM

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6. Detailed instructions

- 1. Preparation of standard and calibrator
- a. Preparation of Stock Standard (Aspirin 1mg/mL): 10 g of pure powder of Aspirin + 10 mL of 1:1 distilled water: methanol

> Preparation of plasma

add $100~\mu\text{L}$ Powder of clean test tube and add whole blood mix well after 5-10 min centrifuge 3500 rpm 10 min and collect plasma and transfer to clean tube

> Preparation of Reagent & Chemical Solvent

a. Ferric Mercuric reagent-

- 10g of mercuric chloride dissolved in small amount of distilled water-Beaker 1
- 10g of ferric nitrate dissolved in 30 ml of 1N hydrochloride acid (HCl) -
- Mix both the beakers (Beaker 1 & Beaker 2) in 250mL volumetric flask and make up to the mark by distilled water.

2. Preparation of plasma standards:

Plasma Standard	10μg/mL	20μg/mL	40μg/mL	100μg/mL
Blank Plasma	500μL	500μL	600μL	1800μL
Aspirin 1mg/mL				200µl mix well and transfer to
100μg/mL(stock)	500μL	500μL	400μL	

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3. Extraction procedure

 $250\mu L$ of plasma standards/QC/ Sample

Heated for 5 minutes in water bath (45°C)

Cool the tube in running water

1.25mL of Ferric mercuric reagent is added to each of the standard test tube and mix well

Vortex for 1 minute

Centrifuge at 4500 rpm for 5 minutes

The supernatant solution is collected and absorbance (540nm) is measured

- 4. Spectrophotometer Conditions
- a. Wavelength: 540 nm
- 5. Abbreviations:
- a. SPECTO= Spectrophotometer

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