

CURRENT

Category : Patient Care
Title : Procedure for estimation of Primaquine and Carboxyprimaquine in human plasma by High Performance Liquid Chromatography (HPLC).
SOP No. and Version: TDM 09/01
Date first effective: 1st January 2024 Review date: 31st December 2024
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 Primaquine and carboxyprimaquine in human plasma by High Performance Liquid Chromatography (HPLC).

2. Scope:

This SOP is limited to estimation of primaquine and carboxyprimaquine 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 primaquine and carboxyprimaquine. The task of performing estimation of Primaquine and carboxyprimaquine 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.TDM01/02: 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 (Primaquine and carboxyprimaquine 1mg/ml):(10mg)

10 mg of pure powder of Primaquine + 10 mL of Methanol

10 mg of pure powder of Carboxyprimaquine + 10mL of Methanol

b. Preparation of stock Internal standard (IS): Phenacetin (PHE)

10mg of pure powder (Phenacetin) IS + 10ml of Methanol-stock IS

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

b. Working standard II: 5 mL of working standard I + 5 mL of Distilled water

c. Working standard III: 5 mL of working standard II + 5 mL of Distilled water

d. Preparation of Mobile Phase Reagent buffer (Aqueous Mixture) Step-1

a. Aqueous Mixture: Add 0.35%v/v/triethylamine and 0.3%v/v trifluoro acetic in Distilled water 400ml. Shake well and sonicate for 5 min

b. Isocratic mobile phase composition: (Aqueous mixture: Acetonitrile: Methanol) 66%: 22%: 12%.

For Preparation of 400 ml Aqueous solution and Add 1400 μ L of Triethylamine and 1200 μ L of Trifluoro acetic acid in 400mL Distilled Water after adding both the reagent shake well and sonicate for 5 min

e. Mobile Phase Preparation

a. 330:110:60 (Aqueous mixture: Acetonitrile: Methanol) & filter the mobile phase through 0.22-micron filter and sonicate for 15 minutes.

f. Preparation of Extraction Reagent

0.1%v/v Perchloric Acid :25mL of Perchloric acid in 25mL distilled water

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2. Preparation of plasma Mother stock standards:

Serial no.	Final concentration µg/mL	Primaquine	Carboxy Primaquine	Methanol	Total volume of mixture
1	200	200µL(1mg/mL)	500µl(400µg/mL)	300 µL	1000 µL
2	100	100µl(1mg/mL)	250µl(400µg/mL)	650 µL	1000 µL
3	50	50µl (1mg/mL)	125µl(400µg/mL)	825µL	1000 µL
4	40	40µl (1mg/mL)	100µl(400µg/mL)	860 µL	1000 µL
5	20	200µl (100µg/mL of mixture 2)		800 µL	1000 µL
6	10	100µL (100µg/mL of mixture 2)		900 µL	1000 µL
7	5	500µL (10µg/mL of mixture 6)		500 µL	1000 MI

3. Preparation of plasma Sub stock standards:

Serial no.	Final concentration µg/MI	Mixture of Primaquine and carboxyprimaquine	Methanol	Total volume of mixture
1	20	100	900	1000µl
2	10	100	900	1000µl
3	5	100	900	1000µl
4	4	100	900	1000µl
5	2	100	900	1000µl
6	1	100	900	1000µl
7	0.5	100	900	1000µl

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- d. Add 2.5mL of ethyl acetate and shake well manually 100 times and centrifuge at 3500 rpm for 15 mins.
- e. Remove 2ml upper organic layer rich in analytes in V- shaped glass tube.
- f. Keep the tubes in nitrogen gas evaporator at 45°C for 15 mins.
- g. Reconstitute the residue with 50 µL of methanol and vortex for 30 seconds.
- h. Inject into HPLC for analysis.

7. HPLC Conditions

- a. Injecting volume: 25 µL
- b. Flow rate: 1.0 mL/min.
- c. Wavelength: 254 nm (UV detector)
- d. Column oven temperature: Ambient
- e. Run Time: 15.00 min (approximately)
- f. Retention times for PQ- 3.0- 4.0 min, CPQ- 6.0-7.0 min, I.S – 4.0– 5.0 min approximately.
- g. HPLC Column: Supelco C18 column (150×4.6mm, 5µ).

8. Abbreviations:

- a. **HPLC** = High Performance Liquid Chromatography
- b. **I.S.** = Internal Standard
- c. **PQ** = Primaquine
- d. **CPQ** = Carboxyprimaquine
- e. **Std** = Standard
- f. **PHE** = Phenacetin