

Government of Nepal
Ministry of Health and Population
Department of Drug Administration
National Medicines Laboratory
Quality and Method Validation Section
Analytical profile of Cholecalciferol Drops

Analytical Profile No.: Chole D 078/079/AP 102

Cholecalciferol Drops contains not less than 90.0% and not more than 150.0% of the stated amount of Cholecalciferol.

1. Identification:

In the Assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

2. Wt/ml: As per manufacturer's specification

3. pH: As per manufacturer's specification

4. Microbial Enumeration Test: As per IP latest edition

5. Absence of specified Microorganism: As per IP latest edition

6. Uniformity of Content:

Determine by liquid chromatography, as described in the Assay, using the following solution as the test solution.

Test Solution: Weigh a quantity of sample about 5.5 gm in 50 ml volumetric flask. Add about 30 ml of methanol, sonicate, cool at room temperature and make up the volume to 50 ml with same solvent. Dilute 5 ml of this solution to 20 ml with same solvent.

7. Assay: *Determine by liquid chromatography*

[Note: Use low actinic glassware (amber color) throughout the procedures]

7.1 Test Solution: Weigh a quantity of sample about 5.5 gm in 50 ml volumetric flask. Add about 30 ml of methanol, sonicate, cool at room temperature and make up the volume to 50 ml with same solvent. Dilute 5 ml of this solution to 20 ml with same solvent.

7.2 Reference solution: A 0.00005% solution of Cholecalciferol WS in methanol.

7.3 Chromatographic system:

- **Column:** C18, (10 x 4.6 mm), 5 μ particle size

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- **Flow rate:** 1.5 ml/min
- **Wavelength:** 264 nm
- **Injection volume:** 50 μ l
- **Detector:** UV
- **Column temperature:** Ambient
- **Mobile Phase:** A mixture of 3 volumes of Water and 97 volumes of Methanol

7.4 Procedure: Inject the reference solution five times. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0%. Inject the test solution. Measure the peak responses. Calculate the content of cholecalciferol in drops.

8. Other tests: As per pharmacopoeial requirement.