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

Analytical Profile of Oxytetracycline Hydrochloride Bolus

Analytical Profile No.: Oxytetra 081/082/AP 164

Oxytetracycline Hydrochloride Bolus contains not less than 90.0% and not more than 110.0% of the stated amount of Oxytetracycline Hydrochloride.

Usual Strength: 500 mg

1. Identification:

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

2. Dissolution: *Determine by UV Spectrophotometry*

2.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 900 ml 0.1 M Hydrochloric Acid

Speed and Time: 100 rpm and 45 minutes

Withdraw a suitable volume of medium & filter.

2.2 Test Solution: Dilute 2 ml of the filtrate to 50 ml with dissolution medium.

2.3 Procedure: Measure the absorbance of the sample solution at the maximum at about 353 nm. Calculate the content of Oxytetracycline Hydrochloride in the medium taking 282 as the specific absorbance at 353 nm.

2.4 Limit: NLT 80% (Q) of the stated amount.

3. Assay: Determine by liquid chromatography

3.1 Diluent: 0.01 M Hydrochloric acid

3.2 Test solution: Weigh the content of 20 bolus and calculate the average weight. Weigh the powder equivalent to 40 mg of Oxytetracycline Hydrochloride in a 50 ml dry volumetric flask, add 30 ml of 0.01 M Hydrochloric acid, and sonicate for 10 minutes to dissolve. Cool the sample solution to room temperature, make up the volume with the same solvent, and mix. Dilute 5 ml of the resulting solution to 50 ml with the same solvent, and mix.

3.3 Reference solution: Weigh accurately about 40.0 mg of Oxytetracycline Hydrochloride WS and transfer to a 50 ml completely dried volumetric flask. Dissolve in 30 ml of 0.01 M hydrochloric acid with the aid of ultrasound and make up the volume with the same solvent. Dilute 5 ml of the resulting solution to 50 ml with the same solvent and mix.

3.4 Chromatographic system:

Column: C8 (4.6 mm X 150 mm, 5 µm)

Wavelength: 254 nm

Injection volume: 10 µl

Flow Rate: 1.3 ml/minute

Column Temperature: 30° C

Mobile Phase A: A 0.05% v/v solution of trifluoroacetic acid in HPLC water

Mobile Phase B: A mixture of 80 volumes of acetonitrile, 15 volumes of methanol, and 5 volumes of tetrahydrofuran

Gradient Programming

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0	90	10
5	90	10
20	65	35
25	90	10
30	90	10

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

4. Other tests: As per Pharmacopoeial requirements.