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

Analytical profile of Tetracycline Hydrochloride Bolus [Veterinary]

Analytical Profile No.: Tetra 080/81/AP 162

Tetracycline Hydrochloride Bolus contain not less than 90.0% and not more than 110.0% of the stated amount of Tetracycline 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 Water

Speed: 75 rpm

Time: 60 minutes

2.3 Test Solution: After completion of the test withdraw a specimen from the dissolution medium and filter. Reject the first few ml of the filtrate. Dilute 1 ml of the filtrate to 50 ml with dissolution medium.

2.4 Reference Solution: Weigh 55.0 mg of Tetracycline Hydrochloride WS accurately and transfer in 100 ml of a completely dried volumetric flask. Add 70 ml of water and sonicate to dissolve and make up the volume with the same and mix. Dilute 1 ml of the solution to 50 ml with water.

2.5 Procedure: Measure the absorbance of both the standard and test solutions at 276 nm. Calculate the percent release of Tetracycline Hydrochloride.

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

3. Assay: *Determine by liquid chromatography*

3.1 Test solution: Weigh the content of 10 bolus and calculate the average weight. Weigh the powder equivalent to 50 mg of Tetracycline Hydrochloride in 100 ml of dry volumetric flask, add 50 ml of 0.01M methanolic hydrochloric acid, and sonicate for 10 minutes to dissolve. Cool the sample solution to room temperature and make up the volume with the same solvent, mix, and filter the solution through 0.25-micron filter paper.

3.2 Reference solution: Weigh accurately about 50.0 mg of Tetracycline Hydrochloride WS and transfer to a 100 ml completely dried volumetric flask. Dissolve in 50 ml of 0.01M methanolic hydrochloric acid with the aid of ultrasound and make up the volume with the same solvent. Then, filter through a 0.25-micron filter paper.

3.3 Chromatographic system:

Column: C8 (4.6mmX 250-mm, 5µm)

Wavelength: 280 nm

Injection volume: 20 µl

Flow Rate: 1.5 ml/minute

Column Temperature: 40ºC

Mobile Phase: A mixture of 68 volumes of 0.1 M ammonium oxalate, 27 volumes of dimethylformamide, and 5 volumes of 0.2 M dibasic ammonium phosphate, the pH of the mixture is adjusted if necessary to 7.6 to 7.7 with 3M ammonia or 3 M phosphoric acid.

Diluent: 0.01 M Methanolic Hydrochloric acid

3.4 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 Tetracycline Hydrochloride.

3.5 Acceptance criteria: NLT 90.0% and NMT 110.0% of the stated amount.

4. Other tests: As per Pharmacopoeial requirements.