ANALYTICAL METHOD VALIDATION PROTOCOL FOR LEVOCETIRIZINE DIHYDROCHLORIDE TABLETS USP 5 MG

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1.0 PRE-APPROVAL:

This review page is the first page of the Protocol for Analytical Method Validation for Assay of Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg by HPLC and is a record of document approval. Signatures below indicate that this document has been reviewed and approved by a representative of various departments and ensures all relevant sections meet requirements.

APPROVED BY:

QC Manager	
MANAGER QA	

ANALYTICAL METHOD VALIDATION PROTOCOL FOR LEVOCETIRIZINE DIHYDROCHLORIDE TABLETS USP 5 mg

2.0 Objective:

The aim of the validation of the Analytical method, for Assay of Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg by HPLC, as per the ICH Guidelines, is to ensure that a selected analytical procedure will give reproducible and reliable results.

3.0 Scope:

This document covers the method for Assay of Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg by HPLC. Instrument, location and analyst are used to validate the analytical method as per ICH guidelines.

4.0 Responsibilities:

S.No.	Department	Designation	Responsibility
1.	Quality Control	QC Executive	Preparation of Protocol and to carry out QC test Procedures
2.	Quality Control	Manager QC	Implementation and supervision of protocol.
3.	Quality Assurance	Manager QA	Approval of protocol and review data with QC

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5.0 Product Profile:

Name of active material	Levocetirizine Dihydrochloride
Specifications	USP
Label claim	5 mg/ Tablet

6.0 Equipment/Material needed:

For Testing (Chemicals)	**Testing equipment**
Levocetirizine Dihydrochloride RS	HPLC
Acetonitrile	Weighing balance
1 M sulfuric acid	4.6-mm × 25-cm; 5-µm packing L3
Chlorobenzhydryl Piperazine RS	Ultrasonic bath
	Filtration Assembly
	Glass apparatus

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7.0 Analytical method to be used:

Solution A: 1 M sulfuric acid and water (5.7: 94.3)
Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)
System suitability solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS and 0.2 µg/mL of USP Chlorobenzhydryl Piperazine RS in Mobile phase
Standard solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase
Sample solution: Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Chromatographic system
(See Chromatography ⟨621⟩, System Suitability.)
Mode: LC
Detector: UV 230 nm
Columns
Guard: 4-mm × 0.3-cm; 5-µm packing L3
Analytical: 4.6-mm × 25-cm; 5-µm packing L3
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: System suitability solution
[NOTE—See Table 1 for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine
Tailing factor: NMT 1.5 for levocetirizine
Relative standard deviation: NMT 1.0% for levocetirizine
Analysis
Samples: Standard solution and Sample solution Calculate the percentage of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = Peak response of Levocetirizine from the Sample solution
r_s = Peak response of Levocetirizine from the Standard solution
C_S = Concentration of USP Levocetirizine Dihydrochloride RS in the Standard solution (mg/mL)
C_u =Nominal concentration of Levocetirizine Dihydrochloride in the Sample solution (mg/mL)

Limit: NLT 95.0% to NMT 105.0%

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8.0 The following analytical performance parameters are to be carried out.

8.1 Specificity

8.2 Precision

a. Instrument Precision

b. Method Precision

c. Intermediate precision (Ruggedness)

8.3 Accuracy

8.4 Linearity and Range

8.5 Solution Stability

8.6 Robustness

ANALYTICAL METHOD VALIDATION PROTOCOL FOR LEVOCETIRIZINE DIHYDROCHLORIDE TABLETS USP 5 mg

System Suitability:

Preparation of standard solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Procedure: Separately inject equal volume of about 20µl of standard preparation six times into the equilibrated HPLC chromatographic system, record the chromatograms and measure the response of the major peaks.

➢**Acceptance Criteria:**

% RSD should not be more than 2.0 % for area and RT. Theoretical plate should be NLT 1500, Tailing Factor should be NMT 1.5.

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8.1 Specificity:

Preparation:

a) Placebo preparation:

Transfer 50 mg of placebo, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

b) Preparation of standard solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase.

c) Sample preparation :

Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

- **Acceptance Criteria :**

Placebo, Blank, sample and standard solution should not interfere in the main peak of the Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg.

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8.2 Precision:

a) Instrument Precision :

Preparation of standard solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

- **Acceptance Criteria:**

% RSD should not be more than 2.0 % for area and RT, Theoretical plate should be NLT 1500, Tailing Factor should be NMT 1.5.

b) Method Precision :

Sample solution:

Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

- **Acceptance Criteria :**

%RSD for %Assay should not be more than 2.0%

Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg should be within limits (NLT 95.0% to NMT 105.0%).

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c. Intermediate precision: Ruggedness

Intermediate precision expresses within laboratories variations: different days different analysts, different equipment, etc.

Here, two difference day two difference analyst perform as per methodology described in section 7.0.

Preparation of standard solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase.

Sample solutions:

Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Acceptance Criteria:

- %RSD should not be more than 2.0 % for individual and for both the analysts for assay.
- Levocetirizine Dihydrochloride content in tablet should be within limits NLT 95.0% to NMT 105.0%
- Difference of average assay between two analysts should not be more than 2.0

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8.3 Accuracy

Preparation of standard solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase.

Test Preparation:

80% Level:

Transfer a number of Tablets (NLT 10), equivalent to 40 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

100% Level:

Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

120% Level:

Transfer a number of Tablets (NLT 10), equivalent to 60 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Procedure :

Separately inject equal volume (about 20µl) of the Standard preparation and three replicates of 80%, 100%, 120% level of Test preparations, and measure the responses of standard solution, three replicates of 80%, 100%, 120% level of Test preparations.

- **Acceptance Criteria :**

% RSD for % Recovery should not be more than 2.0 % for area.
% Recovery should be between 98.0% to 102.0% for individual and for all level.

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8.4 Linearity :

Prepare sample solution concentration 50%, 70%, 100%, 130% & 150% as per given below table.

Sample solution : Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Sample stock solution concentration	Further dilute	Diluted up to (ml)	Concentration (%)
0.25 mg/ml	2.5 ml	25 ml	50
0.25 mg/ml	3.5 ml	25 ml	70
0.25 mg/ml	5.0 ml	25 ml	100
0.25 mg/ml	6.5 ml	25 ml	130
0.25 mg/ml	7.5 ml	25 ml	150

Procedure: Separately inject equal volume (about 20µl) of the Standard preparation and Sample preparation of 50%, 70% 100%, 130%, and 150% level of standard solutions, record the chromatograms, and measure the responses for the analyte peaks.

- **Acceptance Criteria:** Correlation coefficient should not be less than 0.999

Range:

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Lower Concentration (50%)

Transfer a number of Tablets (NLT 10), equivalent to 25 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the

contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Upper Concentration 150%:

Transfer a number of Tablets (NLT 10), equivalent to 75 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

8.5 Solution Stability:

Procedure: Inject the 1st precision sample preparation once after every 12 hours interval up to 24 hours into the chromatographic condition, record and calculate the % assay. This assay value compare with initial assay value which is determined in the precision test.

- **Acceptance Criteria:**

% Assay value difference should not be more than 2.0 %.

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8.6 Roboustness:

Preparation of standard stock solution

0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in Mobile phase

Sample solution:

Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of Solution A, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

- **Acceptance Criteria:**

Percentage assay difference for each sample should be within 2.0 %

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9.0 Acceptance criteria :

S.No.	Parameter		Limit
1.	System Suitability		➢%RSD should not be more than 2.0% for area and RT. ➢Theoretical plate should be NLT 1500. ➢Tailing Factor should be NMT 1.5
2.	Specificity		Placebo and Blank should not interfere in the main peak of the Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets USP 5 mg.
3.	Precision	Instrument precision	Instrument: ➢%RSD should not be more than 2.0% for area and RT. ➢Theoretical plate should be NLT 1500 ➢Tailing Factor should be NMT 1.5
		Method precision	Method: ➢%RSD for %Assay should not be more than 2.0 % ➢ Levocetirizine Dihydrochloride content in Tablet should be within limits NLT 95.0% to NMT 105.0%
4.	Accuracy/ Recovery		➢%RSD for %Recovery should not be more than 2.0 %. ➢% Recovery should be between 98.0% to 102.0 % for individual and for all level.
5.	Linearity and Range		➢Correlation coefficient should not be less than 0.999
6.	Solution Stability		➢%Assay difference should not be more than 2.0 %
7.	Ruggedness		➢%RSD should not be more than 2.0 % for individual and for both the analysts for assay. ➢ Levocetirizine Dihydrochloride content in Tablet should be within limits NLT 95.0% to NMT 105.0% ➢Difference of average assay between two analysts should not be more than 2.0
8.	Robustness		Percentage assay difference for each sample should be within 2.0 %

ANALYTICAL METHOD VALIDATION PROTOCOL FOR LEVOCETIRIZINE DIHYDROCHLORIDE TABLETS USP 5 mg

10.0 Approval

Prepared by:	Approved by:	Authorised by:
Executive – QC	Manager – QC	Manager – QA