ANALYTICAL METHOD VALIDATION REPORT

LEVOCETIRIZINE DIHYDROCHLORIDE

TABLETS

QUALITY CONTROL DEPARTMENT

This document is an exercise on Analytical Method Validation of the various analytical Methods used in determination of active ingredients in Quality Control Laboratory

CONTRIBUTIONS:

This protocol is a team effort of Quality control Laboratory chemists to achieve the objective of validating the analytical methods carried out to estimate the contents of pharmaceutical products manufactured

Analytical Method Validation Protocol Number			
Validation Frequency	Analytical Methods should be validated during and at the end of development process and after any significant change in analytical method.		
	Designation	**Name of the Person**	**Sign /Date**
Prepared By	Officer QC		
Checked By	Manager QC		
Reviewed By	Manager QA		
Approved By	Operation Head		

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOCETIRIZINE TABLETS 5 MG

What is Validation?

Validation is the evaluating of processes, products or analytical methods to ensure compliance with product or method requirements. One of the most popular definitions of Validation came from the 'US FDA' General Principle of Validation **"Establishing documented evidence which provides a high Degree of assurance that a specific process will consistently produce a product meeting its Predetermined specifications and quality attributes."**

The term Validation & Qualification are often mixed up and there is also some overlap. Equipment Qualification means checking an instrument for compliance with previously defined functional and Performance specifications. For Operational Qualification generic standards and analytical conditions Are used rather than real sample conditions. Validation relates more to the entire but sample specific Process including sample preparation, analysis, and data evaluation.

Validation efforts in the analytical laboratory should be broken down into separate components Addressing the equipment and the analytical methods run on that equipment. After these have been Verified separately they should be checked together to confirm expected performance limits **(System Suitability Testing),** and finally the sample analysis data collected on such a system should be Authenticated with suitable validation checkouts. All methods / equipment that are used to create, Modify, maintain, archive or distribute critical data for cGMP/GLP.

Analytical method should be validated prior to routine use and after changing method parameters. Peoples involved in Validation exercise should be qualified for their jobs. This includes education, training and/or Experience.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Typical analytical performances characteristics that should be considered in the validation of the types of methods are as follows.

- **Accuracy**
- **Precision**
- **Specificity**
- **Detection Limit**
- **Quantitation Limit**
- **Linearity**
- **Range**

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USP 30 in "(1225) Validation of compendial procedures" says Category I (Analytical methods for Quantization of major components of bulk drug substances or active ingredients including Preservative in finished pharmaceutical products) should comply with **Accuracy, Precision, Specificity, Linearity, Robustness, & Range.**

However after discussions with many experts & referring some of the IDAM – APA magazines, we have decided to at least comply with **Accuracy,, Linearity, Precision, Robustness .**

Validation Report:

Once the method has been validated, a validation report should be prepared that includes.

- Objective & scope of the method (applicability, type).
- Summary of the methodology.
- Type of compound & matrix.
- All chemical, reagents, reference standards, detailed instruction on their preparation.
- Method parameters.
- Detailed condition on how the experiments were conducted including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.
- Statistical procedures & representative calculations.
- Representative plots
- Performance data for acceptance limit
- Criteria for revalidation
- Summary & conclusions
- Approval with name, designations, date & signatures of those responsible for the review & approval of the analytical test procedure.

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Validation Report for Levocetirizine Dihydrochloride Tablets

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE: The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

Product Name	Levocetirizine Dihydrochloride Tablets.
Ingredient	Levocetirizine Dihydrochloride.
Label Claim	Each film coated tablet contains::
	Levocetirizine Dihydrochloride ------5.0 mg
Test Method	By Liquid Chromatography.

Levocetirizine Dihydrochloride

Specificity (Diluents Interference):
Placebo Preparation:

A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here used the placebo solution area at 230 nm, Observation Result: Nil

Conclusion for Specificity:
We observed that at wavelength 230 nm there is no significant area for placebo (Diluents) for Levocetirizine assay method. Therefore specificity of the method considered acceptable.

System Accuracy:
The system precision of the above method was carried out by taking area for six times of the sample preparation of exact weight.

Text data collection sheet:

Serial No.	Area of Levocetirizine
1.	3594230
2.	3581528
3.	3562146
4.	3558459
5.	3559771
6.	3564407
Mean	3570090
% RSD	0.406%

Acceptance Criteria: RSD is not more than 2.0%**.**

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Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well Defined mathematical transformation, proportional to the concentration of the analyte in samples Within a given range. Linearity is usually expressed in terms of the variance around the slope of the Regression line calculated according to an established mathematical relationship from test results Obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay: Levocetirizine Dihydrochloride Tablets
(Limit: 90.0 % to 110.0 % of the labeled amount).

Chromatographic Condition:-

- a stainless steel column 25 cm X 4.6 mm, packed with octadecylesilane bonded to porous silica (5um),
- Flow rate 1.0ml per minute,
- spectrophotometer set at 230 nm,
- Injection volume. 20 µl.

Buffer:

A solution containing 0.05M of potassium dihydrogen phosphate.

Mobile Phase: A mixture of 60 volumes of buffer and 40 volumes of Acetonitrile, mix well, adjust pH to 6.0 with 10 per cent w/v of sodium hydroxide. Then degas and filter through with 0.45 µm filter paper.

Standard preparation:

Weigh accurately 25.8mg of Levocetirizine hydrochloride in 50 ml of mobile phase, shake and makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Sample Preparation:

Weigh accurately as required quantities of the sample powdered tablets add 50 ml of mobile phase and sonicate to dissolve, makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Chromatographic system:

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOCETIRIZINE TABLETS 5 MG

Inject the standard and sample solutions. If the tailing factor more than 2.0 and column efficiency is less than 2000 theoretical plates the test is not valid. The relative standard deviation for replicate injection is not more than 2.0 %. Inject the test solution and reference solution.

Procedure:

Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Levocetirizine hydrochloride by the formula given below:-

$$\frac{\text{Sample area X Std. dilution X Potency X 100 X Avg. Wt.}}{\text{Standard area X SPL dilution X 100 X Claim}} = \quad \%$$

Test data collection sheet:

S.No.	Standards	Area of Levocetirizine
1.	Standard-1	3594619
2.	Standard-2	3597757
3.	Standard-3	3599372
4.	Standard-4	3606568
5.	Standard-5	3604707
6.	Standard-6	3589576
7.	Mean	3598700
8.	%RSD	0.176%

Acceptance Criteria: RSD is not more than 2.0%

Samples	Sample Area of Levocetirizine	Mean
Sample-A-01 80%	2818464	2820403
Sample-A-02 80%	2820576	
Sample-A-03 80%	2822168	
Sample-B-01 90%	3177768	3184668
Sample-B-02 90%	3182023	
Sample-B-03 90%	3194213	
Sample-C-01 100%	3567343	3540025
Sample-C-02 100%	3531328	
Sample-C-03 100%	3521405	
Sample-D-01 110%	3878556	3885821
Sample-D-02 110 %	3885741	
Sample-D-03 110 %	3893166	
Sample-E-01 120%	4255842	4237033
Sample- E-01 120%	4228207	
Sample- E-01 120%	4227049	

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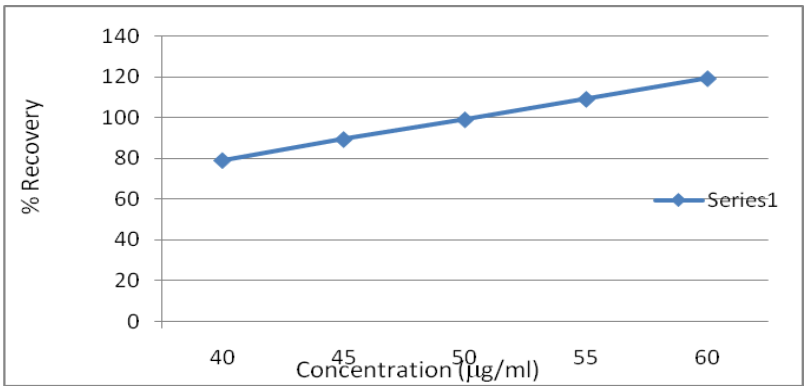
Data Collection:

Concentration (µg/ml)	Concentration in %	Corr. Coefficient	Sample mean area	% Recovery	Corr. Coefficient
40.0	80.0	1.0	2820403	79.24	0.99996
45.0	90.0		3184668	89.56	
50.0	100.0		3540025	99.24	
55.0	110.0		3885821	109.30	
60.0	120.0		4237033	119.42	

From the above results, draw a curve.

Linearity plot for Levocetirizine -

Concentration (µg/ml)	Recovery %
40.0	79.24
45.0	89.56
50.0	99.24
55.0	109.30
60.0	119.42



Linearity plot for Levocetirizine -

Concentration (µg/ml)	Area
40.0	2820403
45.0	3184668
50.0	3540025
55.0	3885821
60.0	4237033

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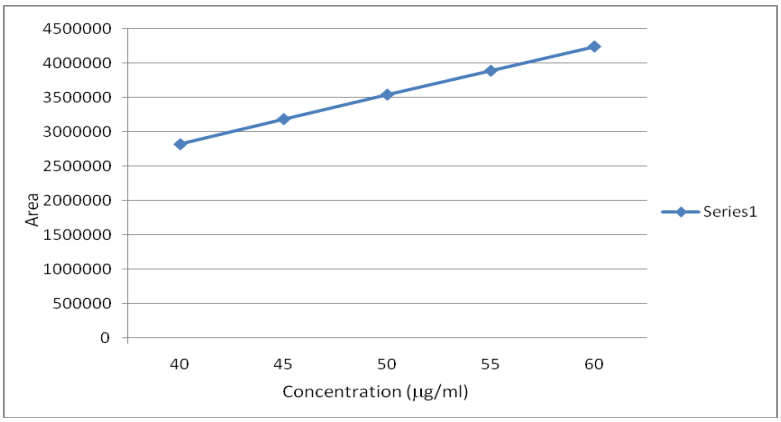

R-squared value ($R^{2)}$

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

y = m x + b

Where m is the slope and b is the intercept.

x = concentration

y = Area Value

Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x

b ⟶ 0.163

Conclusion for Linearity:

The graphical representation & data collected during this exercise proves of Levocetirizine in Levocetirizine tablets for demonstrate linearity in the range of 80% to 120% when determined by Liquid Chromatographic method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

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Precision – Method precision: Levocetirizine Dihydrochloride Tablet
(Limit: 90.0 % to 110.0 % of the labeled amount).

Chromatographic condition:-

- A stainless steel column 25 cm X 4.6 mm, packed with octadecylesilane bonded to porous silica (5um),
- Flow rate 1.0ml per minute,
- spectrophotometer set at 230 nm,
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Buffer: A solution containing 0.05M of potassium dihydrogen phosphate.

Mobile Phase: A mixture of 60 volumes of buffer and 40 volumes of Acetonitrile, mix well, adjust pH to 6.0 with 10 per cent w/v of sodium hydroxide. Then degas and filter through with 0.45 µm filter paper.

Standard preparation:
Weigh accurately 25.8mg of Levocetirizine hydrochloride in 50 ml of mobile phase, shake and makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Sample Preparation:
Weigh accurately as required quantity of the sample powdered tablets eq. to 25mg of Levocetirizine hydrochloride add 50 ml of mobile phase and sonicate to dissolve, makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Chromatographic system: Inject the standard and sample solutions. If the tailing factor more than 2.0 and column efficiency is less than 2000 theoretical plates the test is not valid. The relative standard deviation for replicate injection is not more than 2.0 %. Inject the test solution and reference solution.

Procedure:

Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Levocetirizine hydrochloride by the formula given below:-

$$\frac{\text{Sample area} \times \text{Std. dilution} \times \text{Potency} \times 100 \times \text{Avg. Wt.}}{\text{Standard area} \times \text{SPL dilution} \times 100 \times \text{Claim}} = \quad \%$$

Sample Dilutions:
By ……..:-

(A) Take 825.1mg of the sample and proceed as per above.

(B) Take 821.8mg of the sample and proceed as per above.

(C) Take 822.3mg of the sample and proceed as per above.

(D) Take 828.4mg of the sample and proceed as per above.

(E) Take 831.8mg of the sample and proceed as per above.

(F) Take 828.2mg of the sample and proceed as per above.

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOCETIRIZINE TABLETS 5 MG

Test Data Collection:-

S.No.	Standards	Area of Levocetirizine
1.	Standard-1	3594619
2.	Standard-2	3597757
3.	Standard-3	3599372
4.	Standard-4	3606568
5.	Standard-5	3604707
6.	Standard-6	3589576
7.	Mean	3598700
8.	%RSD	0.176%

Samples		Sample Area Levocetirizine	Mean
Sample A	T1	3521006	3519837
	T2	3518667	
Sample B	T1	3508305	3508982
	T2	3509658	
Sample C	T1	3506924	3506448
	T2	3505973	
Sample D	T1	3546966	3548413
	T2	3549860	
Sample E	T1	3560763	3559260
	T2	3557757	
Sample F	T1	3538791	3539021
	T2	3539251	

Estimated Amount of Levocetirizine:-

- Assay on % of Theory for sample A---- 99.24%
- Assay on % of Theory for sample B---- 99.33%
- Assay on % of Theory for sample C-----99.19%
- Assay on % of Theory for sample D----- 99.64%
- Assay on % of Theory for sample E----- 99.54%
- Assay on % of Theory for sample F----- 99.40%

Table for Six Replicate Assays

Sample Number	Estimated % Amount of Levocetirizine	Mean	% RSD
Sample A	99.24	99.39%	0.175%
Sample B	99.33		
Sample C	99.19		
Sample D	99.64		
Sample E	99.54		
Sample F	99.40		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation).

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Conclusion for precision:
The overall % Relative standard deviation 0.175% for Levocetirizine hydrochloride in Levocetirizine hydrochloride tablets, there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision:
(Within laboratory variations such as different days, analyst & equipments):

Analyst: (II)
Chromatographic Condition:-

- A stainless steel column 25 cm X 4.6 mm, packed with octadecylesilane bonded to porous silica (5um).
- Flow rate 1.0ml per minute,
- spectrophotometer set at 230 nm,
- Injection volume. 20 µl.

Buffer: A solution containing 0.05M of potassium dihydrogen phosphate.

Mobile Phase: A mixture of 60 volumes of buffer and 40 volumes of Acetonitrile, mix well, adjust pH to 6.0 with 10 per cent w/v of sodium hydroxide. Then degas and filter through with 0.45 µm filter paper.

Standard preparation:
Weigh accurately 25.6mg of Levocetirizine hydrochloride in 50 ml of mobile phase, shake and makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Sample Preparation:
Weigh accurately as required quantity of the sample powdered tablets eq. to 25mg of Levocetirizine hydrochloride add 50 ml of mobile phase and sonicate to dissolve, makeup 100 ml with mobile phase, filter and dilute 5 ml of filtrate to 25 ml of mobile phase.

Chromatographic system:
Inject the standard and sample solutions. If the tailing factor more than 2.0 and column efficiency is less than 2000 theoretical plates the test is not valid. The relative standard deviation for replicate injection is not more than 2.0 %. Inject the test solution and reference solution.

Procedure:
Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Levocetirizine hydrochloride by the formula given below:-

$$= \frac{\text{Sample area X Std.dilution X Potency X 100 X Avg. Wt.}}{\text{Standard area X SPL dilution X 100 X Claim}} \ \%$$

Sample Dilutions:

(A) Take 824.6 mg of the sample and proceed as per above.

(B) Take 821.3 mg of the sample and proceed as per above.

(C) Take 820.8 mg of the sample and proceed as per above.

(D) Take 829.4 mg of the sample and proceed as per above.

(E) Take 831.2 mg of the sample and proceed as per above.

(F) Take 827.7 mg of the sample and proceed as per above.

Test Data Collection:-

Standards	Area of Levocetirizine
Standard 1	3575796
Standard 2	3575771
Standard 3	3575381
Standard 4	3575092
Standard 5	3575058
Standard 6	3575897
Mean	3575499
%RSD	0.010%

Samples		Sample Area Levocetirizine	Mean
Sample A	T1	3522216	3522360
	T2	3522503	
Sample B	T1	3514361	3514467
	T2	3514574	
Sample C	T1	3508235	3509718
	T2	3511202	
Sample D	T1	3549977	3552060
	T2	3554143	
Sample E	T1	3562377	3563322
	T2	3564266	
Sample F	T1	3543786	3542625
	T2	3541464	

Estimated Amount of Levocetirizine:-

- Assay on % of Theory for sample A---- 99.24%
- Assay on % of Theory for sample B---- 99.41%
- Assay on % of Theory for sample C-----99.34%
- Assay on % of Theory for sample D----- 99.49%
- Assay on % of Theory for sample E----- 99.59%
- Assay on % of Theory for sample F----- 99.43%

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Table for Six Replicate Assays:

Sample Number	Estimated % Amount of Levocetirizine	Mean	% RSD
Sample A	99.24	99.42%	0.124%
Sample B	99.41		
Sample C	99.34		
Sample D	99.49		
Sample E	99.59		
Sample F	99.43		

Table for Six Replicate Assays analyst by two different Analysts & days:

Test Data analyst by **……..:-**

Table for Six Replicate Assays:

Sample Number	Estimated % Amount of Levocetirizine	Mean	% RSD
Sample A	99.24	99.39%	0.175%
Sample B	99.33		
Sample C	99.19		
Sample D	99.64		
Sample E	99.54		
Sample F	99.40		

Test Data analyst by **……..:**

Table for Six Replicate Assays

Sample Number	Estimated % Amount of Levocetirizine	Mean	% RSD
Sample A	99.24	99.42%	0.124%
Sample B	99.41		
Sample C	99.34		
Sample D	99.49		
Sample E	99.59		
Sample F	99.43		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.175% & 0.124% of Levocetirizine hydrochloride there is no significant difference between two analysts Within laboratory variations such as different days , analyst & equipments.

Therefore reproducibility of the method considered to be acceptable.

Robustness:

To demonstrate the analytical method is capable to yield reproducibility results under; small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.

- Change in Flow rate of mobile phase. Record the observation in below observation table.

Observation Table:-

Change ratio in the mobile phase at 230nm

Mobile phase		Flow rate ml/min	System suitability		
Buffer	**Acetonitrile**		**Retention Time**	**Theoretical Plates**	**Tailing Factor**
610ml	390ml	1.0ml/min	4.031	3636.930	1.460
600ml	400ml	1.0ml/min	3.768	3507.463	1.484
590ml	410ml	1.0ml/min	3.591	3594.325	1.448
Change in flow rate at 230 nm					
Mobile phase		**Change in Flow rate**	**System Suitability**		
Buffer	**Acetonitrile**		**Retention time**	**Theoretical Plates**	**Tailing Factor**
600ml	400ml	0.8ml/min	4.675	3754.003	1.537
600ml	400ml	1.0ml/min	3.759	3482.034	1.493
600ml	400ml	1.2ml/min	3.169	3267.962	1.458

Acceptance criteria:

Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 2000 & tailing factor is not more than 2.0).

Conclusion for Robustness:

There is no significant difference for Levocetirizine hydrochloride in Levocetirizine hydrochloride tablets for different conditions, such as composition & Flow rate of mobile phase. Therefore Robustness of the method considered acceptable.

Conclusion:

All the analytical parameter are checked as per the approved validation process and found well within specified acceptance criteria. Hence, it is concluded that, this method is suitable for accurate & precise results for routine analysis.