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Development and validation of dissolution method for levocetirizine dihydrochloride by isocratic reverse phase HPLC

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ABSTRACT

Levocetirizine hydrochloride is an orally active, non-sedative antihistamine used in the symptomatic relief of seasonal and perennial allergic rhinitis. The main objective of the proposed study is to develop and validate a dissolution method of Levocetirizine hydrochloride dosage form by reverse phase HPLC isocratic method. The method is optimized by various filteration methods such as centrifuge and whatman 42. The filteration methods are evaluated by reverse phase isocratic HPLC method using column- Phenomenex Luna - C 18 (250 mm x 4.6 mm; 5 µ), mobile phase – mixture of buffer and acetonitrile (580:420), adjusted pH to 6.0 with 10 % sodium hydroxide. buffer - 4.08 g Potassium dihydrogen orthophosphate in 600 mL of water. The flow rate is 1.0 mL/min and the elutant is monitored at 230 nm with UV detector. The retention time of levocetirizine hydrochloride is 5.5. Precision shows that % relative standard deviation is about 1.1%. The percentage recovery of the levocetirizine from the dosage formulation is 99.6%. The results obtained for robustness and ruggedness of levocetirizine hydrochloride are well within the acceptance criteria. The proposed method is found to be simple, rapid, accurate and precise. The proposed methods are validated as per ICH guidelines and successfully applied for the determination of Levocetirizine tablet.

***Keywords*:** Levocetirizine hydrochloride; Reverse Phase HPLC; Buffer; pH; Precision; Accuracy; Ruggedness

1. INTRODUCTION

Recently, fast-dissolving drug delivery systems have started gaining popularity and acceptance as new drug delivery systems, which aim to enhance safety and efficacy of a drug molecule by formulating it into a convenient dosage form for administration and to achieve better patient compliance. Some companies introduced more robust forms of fast-dissolving drug delivery, for example cipla labs, Swiss garnier and Lavipharm Laboratories Inc. (Lavipharm), invented an ideal fast-dissolving drug delivery system, which satisfied the unmet needs of the market.

This novel intra oral drug delivery system, trademarked as Quick-Dis is Lavipharm's proprietary, patented technology, flexible and quick-dissolving film. The film is placed on the top or the floor of the tongue [1]. When put on the tongue, this film disintegrates instantaneously, releasing the drug which dissolves in the saliva [2,3]. Some drugs absorbed from the mouth, pharynx, and esophagus as the saliva passes down into the stomach. In such cases, the bio availability of the drug is significantly greater than that observed for conventional tablets [4,5].

Levocetirizine dihydrochloride is an orally active, third generation non-sedative antihistamine used in the symptomatic relief of seasonal and perennial allergic rhinitis, hay fever and for the treatment of chronic idiopathic urticaria. It has twice the affinity for H1 histamine receptors when compared to cetirizine hydrochloride [6]. Levocetirizine dihydrochloride is rapidly and extensively absorbed following oral administration, with the peak plasma concentration usually attained in 0.9 h. Antihistaminic effects occur within 1 h. Symptomatic improvement is observed as early as 1 day after the initiation of therapy for allergic rhinitis orchronic idiopathic urticaria.

The duration of antihistaminic effect persist for at least 24 h [7-11]. Hence, for an antihistamine drug like levocetirizine dihydrochloride, a quick-disintegrating dosage form is suitable, since the disintegration and dissolution of the dosage form [5,12,13] occurs rapidly, thus providing a rapid onset of action. It was thought worth to formulate oro-dispersible formulations of the drug, so that the patient can ingest the dosage form anywhere and at anytime, without the aid of water which would be helpful especially in cases of unavailability of water, motion sickness, sudden episodes of allergic attacks and deglutition problems. Mouth-dissolving tablets of levocetirizine dihydrochloride (Fig. 1) were prepared by a direct compression method using different concentrations of spray-dried mannitol (Perlitol SD 200), menthol and camphor.

The sublimation technique was used to increase the porosity of the tablets in which menthol and camphor were used as subliming agents which in turn forms the porous structure on the surface of tablets after sublimation. The tablets were also evaluated for drug release for 30 min in 0.1 N HCl using the USP Type II dissolution apparatus. The in vitro drug release study revealed that menthol and camphor (1:1) at a concentration of 20% (Batch – LMD-6) of the total weight of the tablet offer a fast release of levocetirizine dihydrochloride within 5 min. These tablets also dissolved within 15-20 seconds in saliva with pleasant taste and smooth mouth feel [14,15].

The present investigation was aimed at the formulation of fast dissolving films of levocetirizine dihydrochloride and evaluates its physio-chemical properties [16], in vitro dissolution profile [17] and in vivo antihistamine efficacy performance in rats.

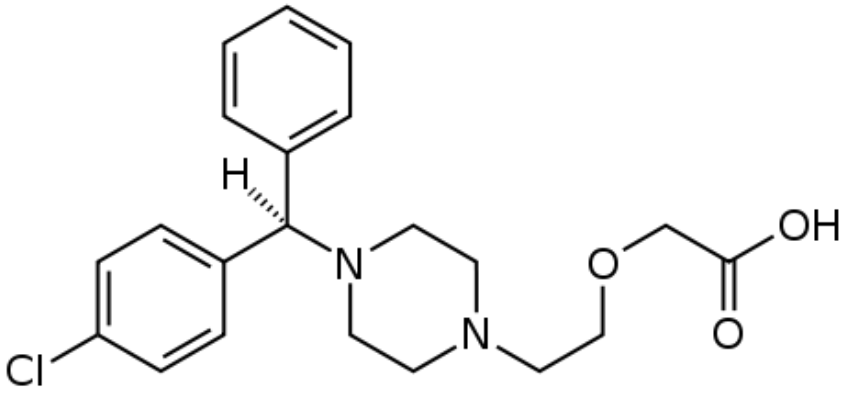

Fig. 1. 2-(2-{4-[(*R*)-(4-chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)acetic acid.

2. MATERIALS AND METHODS

2. 1. Instrumentation

Dissolution apparatus make Lab India and high performance liquid chromatography system consisting of Waters 2695 Separation (Alliance) Module with UV detector was used with data handling system Empower. Chemicals were weighed using Analytical balance, Sartorius and LC-GC all pH measurements were done on pH meter. Reagents, Chemicals, HPLC grade solvents, orthophosphoric acid and acetonitrile were obtained from Merck Specialities Pvt Ltd, India. Water was deionised and further purified by means of Milli-Q water purification system, Millipore Ltd (U.S.A). Levocetirizine hydrochloride were obtained as pure standards and samples [tablets of levocetiricine hydrochloride] from Cipla, India.

2. 2. Common Chromatographic conditions and measurement procedure

Column: Phenominex luna (250 x 4.6 mm), 5 µ
Column oven temperature : Ambiant
Flow Rate: 1.0 mL/min
Wave Length: 230 nm
Injection volume: 20 µL

2. 3. Dissolution Parameters

Apparatus	Paddle
Medium	Phosphate buffer 6.8
Volume	900 mL
Time	45 minutes
Speed	75 RPM
Temperature	37 ±0.5 °C

2. 4. Sample Preparation

Put the one Tablet in six dissolution vessels containing 900 mL of medium that has been equilibrated to 37 ±0.5 °C. Withdraw about 30 mL of dissolution medium after completion of the specified time. Filtered and discard first few mL of the filtrate and collect the filtrate.

2. 4. 1. Standard Preparation

Weighed accurately about 25 mg Levocetirizine dihydrochloride working standard, dissolve in mobile phase and diluted up to 100 mL with the mobile phase. Further diluted 1 mL of this solution to 50 mL with dissolution medium.

2. 4. 2. Evaluation of system suitability

Inject the standard solution into the chromatograph and record the chromatograms. The column efficiency determined from the Levocetirizine Dihydrochloride peak is not less than 5000 theoretical plates. The tailing factor of Levocetirizine Dihydrochloride peak is not more than 2.0. The relative standard deviation for five replicate injections is not more than 2 %.

2. 4. 3. Procedure

Separately inject 20 micro liters of the dissolution medium. Standard and sample Preparation into the chromatograph and record the chromatograms calculate the release of Levocetirizine dihydrochloride per tablet in percentage with respect to label claim from the following expression

$$\frac{AA \times WS \times 1 \times 900 \times P \times 100}{AB \times 100 \times 50 \times 1 \times 100 \times LC}$$

Where;
AA = Average area of Levocetirizine Dihydrochloride for sample preparation.
AB = Average area of for Levocetirizine Dihydrochloride Standard preparation
WS = Weight of Levocetirizine Dihydrochloride working standard taken in mg
P = Percent purity of Levocetirizine Dihydrochloride working standard on as such basis
LC = Label claim

2. 5. Procedure

The prepared sample was injected into chromatograph and chromatograms were recorded by using the chromatographic conditions, various trials were made for the determination of assay. Each trial mixture of known components were injected and observed for resolution and tailing factor of the peaks. Different flow rates of the mobile phase were tried for good resolution. Levocetricine hydrochloride was found to be soluble and stable in a mixture of buffer pH 6.86. Finally the chromatographic conditions were optimized at flow rate 1.00 mL/min, injection volume of 20 µL, run time of 15 minutes, a Phenomenex Luna (250 x 4.6 mm), 5 µ column. The %RSD for levocetrizine hydrochloride was found to be 0.8 and tailing factor was less than 1.5. Absorption maximum was found to be 230 nm.

Shapes of peaks were good. The work was divided into two parts. The first part describes the system suitability and the second part describes the validation data

3. RESULTS AND DISCUSSION

3. 1. System suitability

The method was validated under the chromatographic conditions; the parameters were given in Table 1.

3. 2. Validation parameters

3. 2. 1. Specificity [18-23] and system suitability

Specificity of the analytical method is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. The specificity results were tabulated in Table 1.

Table 1. Specificity results.

<table>
<tr><th>Validation Parameter</th><th colspan="2">Results</th><th colspan="2">Acceptance Criteria</th></tr>
<tr><td colspan="5">Specificity and system suitability:</td></tr>
<tr><td rowspan="2">RT of Levocitirizine hydrochloride peak</td><td>Standard</td><td>Sample</td><td colspan="2" rowspan="2">Standard and sample were comparable with respect to RT.</td></tr>
<tr><td>5.5 min</td><td>5.5 min.</td></tr>
<tr><td colspan="5">Blank Interference:</td></tr>
<tr><td>At RT of Levocitracine HCl peak</td><td colspan="2">No peak observed at the RT of Olmesartan</td><td colspan="2">Blank not showed no interference at the RT of Olmesartan peak.</td></tr>
<tr><td colspan="5">System Suitability:</td></tr>
<tr><td rowspan="3">Levocetirazine HCl</td><td colspan="2">Theoretical Plates</td><td>8981</td><td>NLT 5000</td></tr>
<tr><td colspan="2">Tailing factor</td><td>1.2</td><td>Between 0.8 and 1.5</td></tr>
<tr><td colspan="2">The relative standard deviation for five replicate injections</td><td>0.3</td><td>The relative standard deviation for five replicate injections is not more than 2.0 %.</td></tr>
</table>

3. 2. 2. Precision [18-23]

Precision of the method was studied the results and acceptance criteria were given in Table 2.

Table 2. Precision measurements.

Parmeter	Results		Acceptance Criteria
System Precision: The system precision is the closeness of agreement between the responses of detector. It is usually expressed as the standard deviation or relative standard deviation.			
Levocetirizine HCl	Theoretical Plates	8912	NLT 5000
	Tailing factor	1.1	Between 0.8 and 1.2
	The relative standard deviation for five replicate injections	0.3	The relative standard deviation for five replicate injections is not more than 2.0 %.
Method Precision: The precision of the analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of homogenious sample. It is usually expressed as the standard deviation or relative standard deviation [8-11].			
Olmesartan	The %RSD of Assay from six dissolution preparation	0.5%	The %RSD of Assay from six sample preparation should be NMT 2.0

3. 2. 3. Intermediate precision [18-23]

Table 3. Results and acceptance criteria for intermediate precision.

Validation Parameter	Results		Acceptance Criteria
System Precision:			
Levocitirazine HCl	Theoretical Plates	7912	NLT 5000
	Tailing factor	1.2	Between 0.8 and 1.2
	The relative standard deviation for five replicate injections	0.5	The relative standard deviation for five replicate injections is not more than 2.0 %.

Intermediate precision expresses within laboratory variation with different analyst or equipment within the same laboratory using same drug product as specified under precision. Intermediate precision of the method was studied the results and acceptance criteria were given in Table 3

3. 2. 4. Accuracy [18-23]

The accuracy of analytical procedure express the closeness of agreement between the value which is accepted either as a conversional true value or an accepted reference value and the volume found. The accuracy shall be established across the specified range of the analytical procedure.

3. 2. 5. Solution stability [18-23]

It is essential when validating an analytical method to confirm that the analyte has adequate stability in both the standard and sample solution during analytical measurement stages of the testing.

Solution stability of the method was studied the results and acceptance criteria were given in Table 4.

Table 4. Results and acceptance criteria of solution stability.

Validation Parameter	**Acceptance Criteria**			
Levocitirazine hydrochloride	Parameter	Initial	24^{th} H	
	Theoretical Plates	7982	8167	NLT 5000
	Tailing factor	1.2	1.3	Between 0.8 and 1.2
	The relative standard replicate injections for five replicate injections	0.2	0.1	The relative standard deviation for five replicate injections and bracketing together was not more than 2.0 %

3. 3. Robustness (Filter validation) [18-23]

The method shall show readability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions and mention the same in test method.

Robustness of the method was studied the results and acceptance criteria were given in Table 5.

Table 5. Results and acceptance criteria of robustness.

Validation Parameter	Acceptance Criteria			
Levocitirazine hydrochloride	Parameter	Initial		
	Theoretical Plates	8021		NLT 5000
	Tailing factor	1.2		Between 0.8 and 1.2
	The relative standard replicate injections for five replicate injections	0.2	0.4	The relative standard deviation for five replicate injections and bracketing together was not more than 2.0 %

3. 4. Validation data

Validation of an analytical method is the process by which is established that the Reference characteristics meet the requirements for the intended analytical applications. Reference characteristics are expressed in terms of analytical parameters the following analytical parameters are applied in the present validation process Specificity, Precision, Accuracy, Ruggedness, Solution stability, Filter validation and Effect of de aeration.

3. 4. 1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix.

3. 4. 2. Determination

Specificity is expressed as the degree of bias of the test results obtained by analysis of samples containing placebo ingredients when compared.

3. 4. 3. Demonstration

Table 6. The specificity results.

Test solution	Mean Area	% RSD
Placebo Interference	Nil	Nil
Standard (Five replicates)	191781	0.1
Placebo + Standard(duplicate)	196543	0.2
Interference of Montelukast Sodium	Nil	Nil

Specificity is demonstrated by measuring responses of placebo ingredients, analyte containing the placebo ingredients and the analyte. The results are tabulated in Table 6.

3. 4. 4. Acceptance criteria

Analysis of the non-active formulation components (placebo), should not interference with the quantitative determination of the active substance.

3. 4. 5. Inference

The area values demonstrate that there is no interference to the area of the analyte in the presence of placebo ingredient.

3. 5. Precision

3. 5. 1. Definition

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samples of a homogeneous sample.

3. 5. 2. Determination

The precision of an analytical method is determined by Dissolution sufficient number of aliquots of a homogeneous sample. Precision is determined in terms of Method precision in the table given below.

3. 5. 3. Demonstration

Determine the precision of test method by analyzing six samples prepared by spiking Levocetirizine HCl working standard with the equivalent amount of placebo present in one tablet. Also performed dissolution test with 6 tablets as per STP. Summarized the results in the Table 7.

Table 7. Obtained precision results.

Number	**Placebo + Levocetirizine HCl WS** (%)	**Actual % of dissolution (Determined by 6 Tablets) (%)**
1	100.3	93.5
2	100.9	96.9
3	98.6	94.1
4	99.3	94.7
5	99.7	93.6
6	99.5	93.9
Mean	99.7	94.4
RSD	0.8	1.3

3. 5. 4. Acceptance criteria

The % relative standard deviation of individual % Dissolution from the six samples preparations should be not more than 5.0 %

3. 5. 5. Demonstration

Determine the precision of test method by analyzing six samples prepared by spiking Levocetirizine HCl working standard with the equivalent amount of placebo present in one tablet. Also performed dissolution test with 6 tablets as per STP. Summarize the results in the table given below. The results are tabulated in Table 8.

Table 8. Results of demonstrations.

Number	**Placebo + Levocetirizine HCl WS** (%)	**Actual % of dissolution (Determined by 6 Tablets) (%)**
1	100.1	94.5
2	100.2	96.8
3	98.9	94.6
4	99.4	94.5
5	99.6	94.6
6	99.5	93.9
Mean	99.6	94.8
RSD	0.5	1.1

3. 5. 6. Acceptance criteria

The % relative standard deviation of individual % Dissolution from the six samples preparations should be not more than 5.0 %

3. 5. 7. Inference

The RSD of the replicate injection (in %) = 1.1%

3. 6. Accuracy

3. 6. 1. Definition

The accuracy of an analytical procedure is the closeness of the test results obtained by that procedure to that of the true value. The accuracy of an analytical procedure should be established across its range.

3. 6. 2. Determination

The accuracy of an analytical method is determined by applying that method to mixtures of excipients to which known amounts of analyte have been added both above and below the normal levels expected in the sample. Calculate the percentage of the recovery and RSD.

3. 6. 3. Demonstration

To validate, the test method can accurately quantify Levocetirizine HCl within the test tablet recipients, inject three samples each at higher and lower levels and three samples each at other levels prepared by spiking Levocetirizine HCl raw material with the equivalent amount of placebo or by weighing the sample proportionately to the concentrations at 100% and 150% of the dissolution target concentration. The results are tabulated in Table 9.

Table 9. Dissolution demonstration results.

S. No.	Concentration			Recovery		
	Spike level (%)	Qty (in mg) Added	Qty (in mg) Found	%	Mean %	RSD %
1	100	9.78	98.6	100.7	100.0	0.75
2	100	10.04	10.04	100.0		
3	100	9.92	9.84	99.25		
1	150	15.56	15.52	100.33	100.2	0.22
2	150	15.58	15.18	100.05		
3	150	15.54	15.52	100.47		
MIN				99.2		
MAX				100.7		

3. 6. 3. 1. Acceptance criteria

The mean recovery of Levocetirizine HCl at each level should be not less than 95.0% and not more than 105.0%. The relative standard deviation of % Dissolution of Levocetirizine HCl from the three sample preparations at higher and lower levels should be not more than 5.0.

3. 6. 3. 2. Inference

The percentage of recovery is within the limits 99.25 % to 100.74%

3. 6. 4. Ruggedness

The Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed Dissolution times, different Dissolution temperatures, different days.

3. 6. 5. Determination

The Ruggedness of an analytical method is determined as degree of reproducibility of test results by analysis of aliquots from homogeneous lots by different analysts using operational and environmental conditions that may differ but are still within specified parameters of the dissolution.

3. 6. 6. Demonstration

The Ruggedness of the method is demonstrated from the following parameters.

1. Bench top stability of standard preparation.
2. Bench top stability of test preparation.
3. Filter validation.
4. Effect of De aeration of dissolution media

3. 6. 7. Solution stability of standard preparation:

The Bench top stability was presented in Table 10.

Table 10. Scale of bench top stability.

Initial (area)	**After 24 h** (area)	**Difference** (%)
192931	200921	0.14

3. 6. 8. Solution stability of test preparation

Bench top stability in solution results were shown in Table 11.

Table 11. Solution stability.

Sample No.	**Initial dissolution in %**	**After 24 h dissolution in %**
01	93.4	94.6
02	96.8	96.9
03	94.2	97.3
04	94.5	94.4

05	93.6	97.3
06	93.2	97.0
Mean	94.3	96.3
%RSD	1.4	1.4
Difference between the mean values		1.9

3. 6. 9. Acceptance criteria

The difference between % dissolution for Levocetirizine HCl of bench top stability samples should be not more than 3.0.

3. 6. 10. Filter validation

The Filter validation results were tabulated in Table 12.

Table 12. Filter validation data.

Sample No.	Initial	Centrifuge	Whatman filter 42
01	93.5	93.8	93.3
02	96.8	91.9	93.5
03	94.2	90.2	91.1
04	94.5	95.4	92.4
05	93.6	92.6	92.9
06	93.2	93.5	93.7
Mean	94.30	92.9	92.8
%RSD	1.4	1.9	1.1
Difference between the mean values		1.4	1.5

3. 6. 10. 1. Acceptance criteria

The difference between % dissolution for Levocetirizine HCl of centrifuged samples and filtered samples should be not more than 3.0.

3. 6. 11. Effect of de aeration dissolution media

Effect of aeration in solution media results were tabulated in Table 13.

Table 13. Effect of de aeration dissolution media.

Sample No.	% Dissolution of Levocetirizine HCl from aerated media	% Dissolution of Levocetirizine HCl from deaerated media
01	93.5	97.7
02	96.8	93.5
03	94.2	92.4
04	94.5	98.4
05	93.6	96.1
06	93.2	96.2
Mean	94.3	95.7
%RSD	1.4	2.4
Difference between the mean values		1.41

3. 6. 11. 1. Acceptance criteria

The difference between Mean % dissolution for Levocetirizine HCl of aerated media samples and de aerated media samples should be not more than 5.0. The % RSD of % dissolution of Levocetirizine HCl from the six units should be not more than 5.0.

4. CONCLUSIONS

The dissolution method in different media such as aerated media and deaerated media were studied. Deaerated media gave better results. The stability of the solution, filtration by centrifuge and whatmann 42 were evaluated by HPLC. Whatmann 42 filtration gave better results.

The HPLC method is tested for specificity, precision, accuracy and robustness and found to meet the pre-determined acceptance criteria. Hence the dissolution method of Levocetirizine hydrochloride tablets also specific, accurate, robust and rugged.

Thus fast-dissolving of levocetirizine dihydrochloride can be considered suitable for clinical use in the treatment of allergic rhinitis and other conditions of allergies, where a quicker onset of action for a dosage form is desirable along with the convenience of administration. The conclusions of the results were tabulated in Table 14.

Table 14. Conclusions.

S. No	PARAMETERS	RESULT	ACCEPTANCE CRITERIA
1	System suitability	1.2 8981 0.3	Tailing factor NMT 2.0 Theoretical plates NLT 5000 Relative standard deviation NMT 2.0 %
2	Specificity	Complies 0.2%	The response of area of analyte should not interfere with blank as well as with Placebo The RSD of standard and Placebo + standard should not be more than 2.0
3	Precision	1.3 %	The % relative standard deviation of individual % dissolution from the six sample preparations should be not more than 5.0 %
4	Accuracy	99.2 % to 100.7% 100 % level of RSD = 0.75 % 150 % level of RSD = 0.22 %	The mean recovery of Levocetirizine HCl at each level should be not less than 95.0% and not more than 105.0%. The relative standard deviation of % dissolution of Levocetirizine HCl from the three sample preparations at higher and lower levels should be not more than 5.0.
5	Ruggedness 1. Bench top stability of standard preparation 2. Bench top stability of test preparation. 3. Filter validation Centrifuge Whatman ≠ 42 Deaeration RSD	0.1 % 1.9% 1.4 % 1.4 % 1.4 % 2.5 %	The difference between % dissolution for Levocetirizine HCl of bench top stability, centrifuged samples and filtered samples should be not more than 3.0. The difference between Mean % dissolution for Levocetirizine HCl of aerated media samples and de aerated media samples should be not more than 5.0.The % RSD of % dissolution of Levocetirizine HCl from the six units should be not more than 5.0.

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