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Research

Design of Experimental approach for the quantification of Levocetirizine dihydrochloride dissolution in Tablets formulation using RL HPLC method

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Abstract

The development of an analytical technique for the determination of drug by RP-HPLC. A simple, sensitive and reproducible method was established and validated for the concurrent estimation of Levocetirizine in its tablet preparation by reverse phase high performance liquid chromatography. The mobile phase containing phosphate buffer (pH 4.0 with ortho phosphoric acid) and acetonitrile in the proportion 65:35 v/v was selected because it was found to give a peak for Levocetirizine with minimum tailing. With the mentioned composition of mobile phase, sharp peak was achieved with reasonable short run time of 4.552 min. The criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparatory steps involved in the use of same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. Hence this method can be applied for quantification of different formulations containing Levocetirizine simultaneously.

Keywords: RP-HPLC, Levocetirizine dihydrochloride, Tablet, Dissolution, Formulation.

INTRODUCTION

Design of Experimental approach for the quantification of Levocetirizine dihydrochloride dissolution in Tablets formulation using RL HPLC method. The present scope is to: Development and Validation of HPLC method for the estimation of dissolution Levocetirizine dihydrochloride in Levocetirizine dihydrochloride Tablets USP 5 mg

- The proposed method shall be used for the quantification of active material Levocetirizine dihydrochloride.
- The proposed method shall be validated for Specificity, System suitability, Linearity, Accuracy, Range, Precision, and Repeatability and Robustness as per ICH guideline.

Levocetirizine, chemically is [2-[4- [(r)-(4-chlorophenyl) phenyl methyl]-1- piperazinyl] ethyl] acetic acid is a third generation non-sedative antihistamine, developed from the second generation antihistamine cetirizine.

It is the L-enantiomer of the cetirizine racemate. Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever (Grant et al., 2002).

Literature review reveals that some analytical methods have been reported for Levocetirizine (Morita et al., 2008; Arayne et al., 2008; Selvan et al., 2006; Birajdar et al., 2008; Ashokkumar et al., 2009; Sharmaa et al., 2010) individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. The aim of the present work was to develop a simple, sensitive, accurate, and precise HPLC method for routine analysis. The proposed method was validated according to ICH guidelines (ICH, 2005*).*

Characteristics: Levocetirizine is in a class of medications called antihistamines. It works by blocking the action of histamine, a substance in the body that causes allergic symptoms

Description: white powder, Boiling point: 542.1 °C, Other names: Acetic acid, 2-(2-(4-((R)-(4-chlorophenyl)phenylmethyl)-1-piperazinyl)ethoxy)-, hydrochloride (1:1), Solubility : Levocetirizine is a highly soluble (94.6 g/100 mL) and a moderately permeable, Molecular Formula :$C_{21}H_{26}Cl_2N2O3$, Molar mass: 388.888 g/mole.

Materials and methods: Sodium Di hydrogen phosphate, Ortho Phosphoric acid, Acetonitrile, Levocetirizine dihydrochloride Requirement: Chemical, Reagent, Placebo and Standards, HPLC, Dissolution Apparatus, Analytical Balance, pH Meter, Column, Detector, 0.45 nylon membrane filter, Glassware.

DESIGN OF EXPERIMENT BY DIFFERENT TRAILS BY RP-HPLC METHOD

Selection of Chromatographic System: Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1222A), binary pump (G1212A), auto injector (G1267C), column oven (G1210B), DAD detector (G1215C) and Empower (software). were used for method development trials to optimize the method as a stability indicating method for determination of Levocetirizine dihydrochloride.

Selection of Buffer in Mobile Phase: Dilute orthophosphoric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (65:35) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of Mobile Phase: Different ratios of Acetonitrile and Buffer were used to optimize the retention time from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Acetonitrile) (65:35) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of HPLC Column: For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Xterra RP, 150 mm X 4.6, µm or equivalent column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

Selection of Diluent / Solvent for extraction: Different solvents were tried including single solvent and combination of solvents like ACN: Water, Buffer in different concentrations, But Levocetirizine dihydrochloride.

Tablet gets dissolved in Acetonitrile. Hence first stock was prepared in methanol and followed by second dilution done in diluents as (Buffer: Acetonitrile) (65:35) %v/v same as that of mobile phase to reduce the peak shape related problems.

The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria.

Methodology to be adopted for design

A. Specificity and System Suitability: Identification, Blank interference of the Experiment, System Suitability,

Linearity and Range: Precision: System Precision, Method Precision

Accuracy, Solution, Stability, System Stability, Solution stability

B. Robustness: Change in wave length, Filter variability

- Linearity will be performed from about 50% to 150 % of target concentration
- Accuracy will be performed from about 50% to 150 % of stock solution.
- The following experimental design is drawn in order to prove test method is capable to yield consistent, reproduction results within the pre-determine acceptance limits.

- Acceptance criteria design is drawn validation parameters are specified in individual experimental design.
- Observations and results are recorded individual method validation data sheet

Preparation of dilute orthophosphoric acid: Dilute 10 ml of orthophosphoric acid to 100 ml with water and mix.
Preparation of Buffer solution: Weight and transfer about 2.76 of Sodium Di hydrogen phosphate dehydrate into a beaker containing 1000ml of water. Adjust pH of the solution to 4.0± 0.05 with Orthophosphoric acid and filter through 0.45µ nylon membrane filter.
Preparation of mobile phase: Thoroughly mix buffer and acetonitrile solution in the ratio of 65: 35 % v/v.
Dissolution

Medium	:	Purified Water
Volume	:	900ml
Apparatus	:	USP type ll
Speed	:	50rpm
Temperature	:	37±0.5ºC
Sampling point	:	30 minutes

Chromatographic conditions
Column: Xterra RP, 150 mm X 4.6, µm or equivalent column or Equivalent Wavelength: UV 230 n m,
Flow rate Injection : 1.0ml/minute
Volume : 20µL,
Column oven,
Temperature : 30°C
Run time : 10 minute
Preparation of the standard solution: Accurately weigh and transfer about 55 mg of Levocetirine dihydrochloride standard into a 200 ml volumetric flask add about 120 0ml of dissolution media t and sonicate to dissolve and dilute to volume with diluent. Transfer 2.0 ml of this solution to 100 ml volumetric flask and dilute the volume with dissolution media and mix.
Preparation of Test Sample solution: Set the parameters of dissolution apparatus as mentioned above. Place one tablet into each of the dissolution jar. At the end of the specified time point withdraw 10ml of the sample solution through 10 µm full flow filters from each dissolution vessel .Filter the solution through 0.45 membrane filter by discarding first 5ml of filtrate
Procedure: Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 1.0ml/minute. Inject 20 µL of blank solution into the Chromatographic system, record the Chromatogram. Program the data processor to inhibit the integration of peaks due to blank. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of test sample ample solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard).Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Evaluation of System Suitability parameters

Sr.l	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	**Identification**	Prepared standard and sample solution as per the test method and inject into the chromatographic system	
2.	**Blank and Pla Interference**	Prepared blank and placebo solution as per the test method and inject into the chromatographic system	
3.	**System Suitabilit**	Prepared standard as per the test method and inject five times into the chromatographic system	
4.	**System Suitability**	**Acceptance Criteria**	
5.	**Identification and RT Confirmation:**The retention time of standard solution and sample solution should be comparable with respect to retention time.The retention time of analyte peak obtained from sample solution should be within ±0.5 minutes of the retention time of analytic peak obtained from the standard solution		

Sr.l	Validation Parameter	Results		Acceptance Criteria
	Blank and Placebo Interference: There should not be any interfering peak in the chromatogram obtained f rom blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard **System Suitability:** The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 32000 theoretical plates. Tailing factor for the same peak is not more than 2, The relative standard deviation for Levocetirine dihydrochloride peak area obtained from five replicate injections of standard solution is not more 2.			
Observed Value				
6.	Identification and RT Confirmation	Name	Retention time in minute	The retention time of standard solution and sample solution should be comparable with respect to retention time The retention time of analyte peak obtained from sample solution should be within ±0.5 minutes of the retention time of analytic peak obtained from the standard solution
		Standard	4.769	
		Sample 5 mg	4.767	
Conclusion: RT of Levocetirine dihydrochloride obtained with standard and test sample are comparable. Hence method is specific				
7.	**Blank and Placebo Interference**	There are no interference peak observed due to place to at the retention time of Levocetirine dihydrochloride peak. Hence method is specific		There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analytic peak in the chromatogram obtained with the standard
8.	System Suitability	Theoretical plate	6422	The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 3000 theoretical plates
		Tailing Factor	1.04	Tailing factor for the same peak is not more than 2.
		Peak Area	0.09	The relative standard deviation for Levocetirine dihydrochloride peak area obtained from five replicate injections of standard solution is not more 2.0

System Suitability

Sr.No	Retention Time	Peak Area	Theoretical Plates	Asymmetry
1.	4.811	205760	6511	1.04
2.	4.832	205807	6475	1.03
3.	4.832	205933	6503	1.04
4.	4.832	206178	6440	1.05
5.	4.856	206104	6422	1.04
Mean	4.832	205956	6422	1.04
SD	0.25	181.89		
% RSD	0.12	0.09		

Conclusion :

- The retention time of standard solution and sample solution is comparable with respect to retention time
- There is no any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard
- The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 2000 theoretical plates
- Tailing factor for the same peak is not more than 2.
- The relative standard deviation for Levocetirine dihydrochloride peak area obtained from five replicate injections of standard solution is not more 2

A. Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	**Method**	Five linearity solutions were prepared by using Levocetirine dihydrochloride standard at concentration levels ranging from 50% to 150 % of target concentration of Levocetirine dihydrochloride. Measured the peak area response of solution at Level 1 and Level 5 six times and other levels		
2.	**Acceptance crite**	• **Linearity :** • The co-relation is not less than 0.999 • The % Y intercept is between +5 % • % RSD of peak Reponses of 02 % level and 120% level should be NMT 2.0		
3.	Observed results	Correlation Coefficient	0.99996	Correlation coefficient should be not less than 0.999
		%y-intercept	-0.36	%y-intercept should be ±2.0
		Residual were within ±5 % of the 100% concentration response		Residual should be within ±5 % of the 100% concentration response
		% RSD at lower level	0.11	% RSD of peak area response of 6 replicates at lower and higher levels should be more than 2.0
		% RSD at higher level	0.04	

Linearity level	Concentration in ppm	Area-Average	% of RSD	Statistic Analysis	
Level -1	1.114	40638	0.11	R^2	0.99996
Level -2	2.786	102380	---	Slope	37215.08
Level -3	4.458	16111	---	Y Intercept	-743.08
Level -4	5.572	207057	--	% Y Intercept	-0.36
Level 5	6.687	247362	0.04	Correlation coefficient	0.999999

Conclusion :

Response of Levocetirine dihydrochloride is linear overt the concentration range 20% to 120% target concentration

Linerity level	Concentraion in ppm	Y –Practical responses i.e mean peak areas are obtaines	Therotical repbse	Residaul	Residual Squares
Level-1	1.114	40638	40175	-77	5929
Level-2	2.786	102380	102938	-558	311364
Level-3	4.458	166111	165162	949	900601
Level-4	5.572	207057	206619	438	191844
Level5	6.687	247362	248114	-752	565504
Reqsidual sum of square					1975242

- Trend line equation,: Y=MX + (C),Y= Response,X= Concentration, M(Slope)=37215.08, C (Y-Intercept) = -743.08, Response of 1000% Concentration = 207057, 5 % of 100% Concentration= 10353

B. Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Sr.No	Validation Parameter	Results		Acceptance Criteria
A. System Suitability : Method and Procedure				
1.	**System Suitability**	Prepared standard solution as per the test methods and inject six times into the chromatographic system		
	Acceptanc criteria	• The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 3200 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for Levocetirine dihydrochloride peak area obtained from five replicate injections of standard solution is not more 2.0		
2. Observed Values				
System Precision		Theoretical Plates	5794	The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 2000 theoretical plates.
		Tailing Factors	1.07	Tailing factor for the same peak is not more than 2.0
		% RSD	0.31	The % RSD of % assay from Five samples should be more than 2.0
3. **Results :**				
System Suitability and System Precision	**Sr.No**	**Peak Area**	**Theoretic factor**	**Tailing Factor**
	1	202989	5794	10.7
	2	202380	5883	1.06
	3	20317	5875	1.06
	4	202694	5875	1.06
	5	201622	5847	1.07
	6	201722	5926	1.07
	Mean	202438		
	SD	635.48		
	% RSD	0.31		

Observed Results:

- The observed theoretical plates obtained for the Levocetirine dihydrochloride from standard solution is r than 3000 theoretical plates.
- The Observed Tailing factor obtained for the Levocetirine dihydrochloride peak from the standard soluti less than 2.0.
- The % RSD of the peak area of Levocetirine dihydrochloride obtained from five replica injections o standard solution is 0.73

Conclusion :

The above data shows that the system is precise.

B. **Method Precision : Method and Procedure**				
1.	**Methods Precision**	Prepared six sample solution of Levocetirine dihydrochloride tablets 5 mg as per the test methods and inject into the chromatographic system		
	Acceptanc criteria	The % RSD of % assay from six samples should be more than 2.0		
2. Observed Value				
Method Precision		% RSD	1.78	The % RSD of % assay from six samples should be more than 2.0
3. **Results:**				

Sr.No	Dissolution % labeled amount
Injection-1	102.0
Injection-2	100.3
Injection-3	100.4
Injection-4	102.9
Injection-5	102.9
Injection-6	98.3
Mean	**101.1**

Sr.No	Validation Parameter	Results	Acceptance Criteria
SD		**1.80**	
% RSD		**1.78**	
95% confidence interval of mean		**99.7 to 102.5**	

Conclusion :
The above results show that the methods is precise

Intermediate Precision

Intermediate precision expresses within laboratories variation: different days, different analyst, different equipment's, etc. It is the term synonymous with the term 'ruggedness'. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used.

Sr.No	Validation Parameter	Results	Acceptance Criteria
A. System Suitability : Method and Procedure			
Intermediate precision was performed on Levocetirine dihydrochloride by different analyst on different day by using different column of same make and different instruments			
4.	**System Suitability**	Prepared standard solution as per the test methods and inject five times into the chromatographic system	
	Acceptance criteria	• The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 2000 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for Levocetirine dihydrochloride peak area obtained from five replicate injections of standard solution is not more 2.0	
5. Observed Values			
System Precision		Theoretical Plates	4059	The column efficiency as determined for the Levocetirine dihydrochloride from standard solution is not less than 3000 theoretical plates.
		Tailing Factors	1.07	Tailing factor for the same peak is not more than 2.0
		% RSD	0.1	The % RSD of % assay from Five samples should be more than 2.0

6. **Results :**

	Sr.No	Peak Area	Theoretical factor	Tailing Factor
System Suitability and System Precision	1	204794	4061	1.06
	2	205274	4059	1.07
	3	204912	4072	1.08
	4	204875	4060	1.06
	5	205288	4075	1.06
	Mean	205029		
	SD	234.38		
	% RSD	0.11		

Observed Results:

- The observed theoretical plates obtained for the Levocetirine dihydrochloride from standard solution is r than 2000 theoretical plates.
- The Observed Tailing factor obtained for the Levocetirine dihydrochloride peak from the standard solutic less than 2.0.
- The % RSD of the peak area of Levocetirine dihydrochloride obtained from five replica injections of the standard solution is 0.07

Sr.No	Validation Parameter	Results		Acceptance Criteria
Conclusion :				
The above data shows that the system is precise.				

B. **Intermediate Precision : Method and Procedure**			
4.	**Acceptance criteria**	• The % RSD of % dissolution results six samples should be in specified test criteria as per test method. • The % RSD of % dissolution from six samples should be not more than 5.0 • Report the 95 % confidence interval of mean • The overall % RSD of % release of drug from 12 units of precision and intermediate precision together should not be more than 5% • The difference of mean % dissolution from method precision study and intermediate precision study should be more than 5.0%	
5. Observed Value			
Intermediate Precision	% RSD	0.98	The % RSD of % assay from six samples should be more than 2.0
	Over all RSD	0.81	The overall % RSD assay from method precision study and intermediate precision study together should not be more than 2.0
	Difference of mean % dissolution	0.5	The difference of mean % dissolution from method precision study and intermediate precision study , should not be more than 5.0%

6. **Results:**

Sr.No	% Dissolution
Injection-1	100.8
Injection-2	100.5
Injection-3	99.5
Injection-4	101.1
Injection-5	101.7
Injection-6	99.7
Mean	**100.6**
SD	**0.84**
% RSD	**0.83**
95% confidence interval of mean	99.9 to 101.3

Conclusion :
The above results show that the methods is precise

Results :

Overall % RSD

Dissolution (% Labeled amount)

Sr.No	% Dissolution Precision Set -1	Intermediate Dissolution Set ll
1	102.0	100.8
2	100.3	100.5
3	100.4	99.5
4	102.9	101.1
5	1002.9	101.7
6	98.3	99.7

Sr.No	Validation Parameter	Results	Acceptance Criteria
Mean	**101.1**	100.6	
SD	**1.80**	0.84	
% RSD	**1.78**	0.83	
Overall Mean	100.8		
Overall SD	1.37		
Overall % RSD	1.36		
Difference of Mean $ dissolution	0.5		
Analyst	**Mr. Dayakar Raju**	**Mr. Meera**	
HPLC	**QC-HPLC-002**	**QC-HPLC-003**	
Column	**231**	**239**	
Day	**13-02-2024**	**17-02-2024**	

Conclusion:
The above results shows that the method is rugged

ACCURACY
The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	Accuracy was performed by spiking the Levocetirine dihydrochloride drugs substance to the placebo at 20% to 120 % of target concentration of Levocetirine dihydrochloride in triplicate at each level and analyzed as per the test method			
	Acceptance Criteria	The % recovery of accuracy levels should be not less than 95.0 and not more than 105% Report the 95 % confidence interval of mean		
2. Observed Values				
Accuracy		Mean % recovery	99.1	The % recovery of accuracy levels should be not less than 95.0 and not more than 105.0

3. Results

Accuracy level		Amount added in mg	Amount found in mg	% Recov	Statistical Analysis		
					Mean	**SD**	**%RSD**
Level 1	Sample -1	1.002	0.993	99.1	99.2	0.12	0.12
	Sample -2	1.002	0.993	99.1			
	Sample -3	1.002	0.995	99.3			
Level 2	Sample -1	2.505	2.481	99.0	98.7	0.23	0.23
	Sample -2	2.505	2.469	98.6			
	Sample -3	2.505	2.469	98.6			
Level 3	Sample -1	5.010	4.956	98.7	98.8	0.15	`0.15
	Sample -2	5.010	4.940	98.9			
	Sample -3	5.010	4.948	98.7			
Level 4	Sample -1	6.012	6.013	99.9	99.9	0.15	0.15
	Sample -2	6..012	5.995	99.8			
	Sample -3	6.012	6.007	10.00			

Overall Statistical Analysis					
Mean	99.1	**SD**	0.50	**% RSD**	0.50

Conclusion : The Form the above results , it is concluded that the test method is accurate from 20 % to 120% of test stock concentration

RANGE
Range of an analytical method is the interval between the upper and lower concentration 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. The range is normally derived from the linearity studies and depends on the intended application of the procedure.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	Range of analytical method can be obtained from linearity, Precision and accuracy data. Report range in % with respect to sample concentration.		
Observed Values			
2.	Range	The analytical method is linear, Precise and accurate from 20% to 120% of target concentration	----
Conclusion : It was concluded from the linearity, Precision and accuracy data that the analytical method is linear , Precise and accurate from 20% to 120% of target concentration			

SOLUTION STABILITY

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs. 24 hrs., 48 hrs. etc.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	Standard Solution and Sample Solution was prepared as per test methods and stored at refrigerator condition. Solution Stability was evaluated at initial, 24 hours and 48 hours.			
A.	**Acceptance Criteria**	- The overall % RSA from initial replicate standard peaks and bracketing standards peak should be more than 2.0. - The % assay difference from initial and corresponding time intervals should be more than 2		
2. Observed Values				
	Standard Solution	Standard Solution is stable up to 48 hou refrigerator condition		The 5 assay difference from initial and at regular interval more than 2.0
	Sample Solution	Sample solution is table up to 48 hours at refrigerator condition		The % dissolution difference from initial and corresponding time intervals should be more than 2.0
3. Results :				
	Standard Solution :: Over % RSD	Time Interval	% RSD	Difference
		Initial	100.4	---
		24 hours	100.2	0.2
		48 Hours	99.5	0.9
	Sample Solution :: Over % RSD	**Time Interval**	**% Assay**	**Difference of % Assay**
		Initial	102.6	--
		24 hours	101.3	1.3
		48 Hours	101.03	1.3
Conclusion : From the above results it is concluded that standard and sample solutions are stable up to 48 Hrs. at Refrigerator				

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages.

CONCLUSION

In the current study the effort has been undertaken to improve most simple, economical, sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives resolution with a short analysis time (< 10 min). The method parameter was validated and establishes to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of Levocetirine dihydrochloride in medical dosage form. The procedure designated now is simple, rapid, sensitive, selective and cost effective. It is apparent from the results that the suggested procedure is well suited for dissolution and assay and evaluation of drug, in dosage forms. It can be applied for direct determination of Levocetirizine dihydrochloride in laboratories.

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