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UV Spectrophotometric Method Development and Validation for Determination of Levocetirizine Dihydrochloride

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ABSTRACT

Levocetirizine Dihydrochloride is an orally active, non-sedative antihistamine drug. To determine the assay content of Levocetirizine Dihydrochloride drug substance, a very simple, accurate, specific and precise UV- spectrophotometric method has been built up as well as evaluated. The suggested method comprises dissolving Levocetirizine Dihydrochloride in 0.1M Hydrochloric acid solution and subjecting the consequential solution to UV Spectroscopic measurement. An Absorption maximum was found to lie at about 231nm and the measurements were carried out at this wavelength. Beer's law was followed in the concentration range of 7.5 to 22.5 µg/mL. The linearity showed on the calibration curve between concentration and absorbance by the line equation of $y = 0.0377x - 0.0043$ ($R^2 = 0.9992$). Reproducibility by repeating methods as %RSD was found to be less than 2%. The results of the accuracy and precision were found very satisfactory and here the suggested method was statically validated as per the ICH guidelines in terms of the specificity, linearity, accuracy, precision and robustness. Validation studies have discovered that the method is simple, specific, rapid, reproducible, precise, accurate and economical which is useful for the routine analysis of Levocetirizine Dihydrochloride

Keywords: Spectrophotometry, Levocetirizine Dihydrochloride, Assay, Validation.

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INTRODUCTION

Levocetirizine Dihydrochloride is the third-generation antihistamine[1] that is non-sedative and it is derived from cetirizine that is a second-generation antihistamine. This is actually a racemic mixture and R-enantiomer of the Cetirizine hydrochloride which has antihistaminic properties. Its chemical name[2] is Acetic acid, [2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-,dihydrochloride;(2-{4-[(*R*)-(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetic acid dihydrochloride. and the chemical formula is $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$. Its molecular weight is about 461.82.

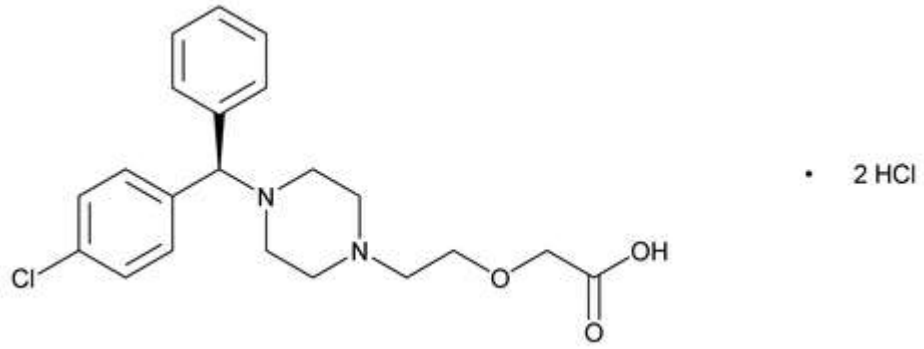

Figure 1: Structural formula of Levocetirizine Dihydrochloride

Levocetirizine Dihydrochloride is usually prescribed[3-4] to treat a runny nose, sneezing and watering eyes, itching as well as rashes. This antihistamine can also help to treat allergy by inhibiting the activity of the histamine in the human body and it has a quick onset of action. Levocetirizine Dihydrochloride helps to inhibit the histamine to bind with its receptors but doesn't prevent the release of histamine .This helps to prevent the release of the other allergy like chemicals and also increased the blood supply to this area. Literature survey reveals that very few methods have been developed for analysis of Levocetirizine Dihydrochloride drug substance. The present investigation was aimed to develop an accurate, rapid and reproducible method.

MATERIALS AND METHOD

Materials

Hydrochloric acid, Water, Levocetirizine Dihydrochloride Standard and Test Sample

Instruments

Weights were taken by an METTLER TOLEDO MS-TS Balance. An ultrasonic bath used to prepare the solution. Absorption was measured in 1cm quartz cell using a Double Beam Shimadzu UV-1800 UV-VIS spectrophotometer with a Spectral bandwidth of 1 nm Shimadzu UV-1800 UV-VIS spectrophotometer was equipped with UVProbe software .

METHOD DEVELOPMENT

Quantification of medicinal substance using spectrophotometer[5-6] may carry out by preparing a solution in transparent solvent and measuring it's absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λmax), where a small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal.

Preparation of Diluent (0.1M Hydrochloric acid)

8.6 mL of concentrated Hydrochloric acid(37%) was diluted in 1000 mL of water

Preparation of Analytical Standard Solution

In a 100-mL volumetric flask, about 30 mg of Levocetirizine Dihydrochloride standard was taken and the standard was dissolved by adding 30 mL of 0.1 M Hydrochloric acid and was added to sonication for 2 to 3 minutes. After sample temperature came to room temperature, volume the sample up to mark with 0.1 M Hydrochloric acid.Then,5 mL of this solution was taken in a 100-mL volumetric flask and was diluted to 100 mL with 0.1 M Hydrochloric acid.

Preparation of Sample Solution

In a 100-mL volumetric flask, about 30 mg of test sample was taken and the sample was dissolved by adding 30 mL of 0.1 M Hydrochloric acid and was added to sonication for 2 to 3 minutes. After sample temperature came to room temperature, volume the sample up to mark with 0.1 M Hydrochloric acid.Then,5 mL of this solution was taken in a 100-mL volumetric flask and was diluted to 100 mL with 0.1 M Hydrochloric acid.

Preparation of Linearity Solution

15.0 mg, 18.0 mg, 24.0 mg, 30.0 mg, 36.0 mg, 42.0 mg, and 45.0 mg of Levocetirizine Dihydrochloride sample were taken into seven different 100-mL volumetric flasks. Then 30 mL of 0.1 M Hydrochloric acid was added to each volumetric flask and mixed for 2 to 3 minutes in an ultrasonic bath. The samples were cooled to room temperature. Finally, 0.1 M Hydrochloric acid was added up to to 100 mL. 5 mL of this solution was taken from each volumetric solution into seven different 100-mL volumetric flasks and was diluted to 100 mL with 0.1 M Hydrochloric acid to achieve 50%, 60%, 80%, 100%, 120%, 140% and 150% of nominal concentration respectively.

Preparation of Blank

0.1M Hydrochloric acid was used as blank.

Spectrophotometric Measurements

The absorbance of Sample and Standard solutions were recorded in the range of 200-400 nm at 231 nm in1-cm cells by a calibrated ultraviolet spectrophotometer using 0.1 M Hydrochloric acid as blank.

VALIDATION OF THE PROPOSED UV METHOD

Method validation[7-8] is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

This method was validated per ICH guidelines. The following parameters were evaluated:

a) Specificity

b) Accuracy

c) Precision

- Repeatability
- Intermediate Precision

d) Linearity

e) Robustness

Specificity

Specificity study was resolved by comparison of the chromatograms of the blank solution, standard solution and sample solution.

Accuracy

Accuracy was determined by the preparation of 3 (three) different concentrations. Three replicate measurements were made for each concentration level. The % Recovery should be between 98.0% and 102.0%.

Precision

- **Repeatability (intra-assay precision)**

Repeatability was determined from six test samples against a standard solution to determine relative standard deviation (RSD of six test samples).The RSD of Repeatability of the method should not be more than 2.0%.

- **Intermediate precision**

A second analyst performed the same experiment as a repeatability experiment on different days and different equipment. For determination of intermediate precision, calculated the %RSD of two analyst's results. The RSD of intermediate precision of the method should not be more than 2.0%.

Linearity

Linearity was determined from concentration 50-150% of nominal concentration for a total of seven different concentrations. Data was evaluated by plotting the absorbance of Levocetirizine Dihydrochloride (Y-axis) against its respective concentration (X-axis). The linearity regression coefficient should not be less than 0.995.

Robustness

The robustness of this method was conducted by changing one parameter (maximum wavelength) of the method by using test sample of the same concentration of repeatability sample. Concentration value was calculated from the corresponding absorbance for the concentration. The absorbance of the test sample was measured maintaining same spectrophotometric conditions mentioned for Levocetirizine Dihydrochloride. Change the spectrophotometric condition by shifting the maximum wavelength ±2 nm from the required wavelength of analysis and measured the absorbance of the test sample. The %RSD should be less than 2.0% of Levocetirizine Dihydrochloride .

RESULTS AND DISCUSSION

Specificity

Specificity study was resolved by comparison of the chromatograms of the blank solution, standard solution and sample solution.

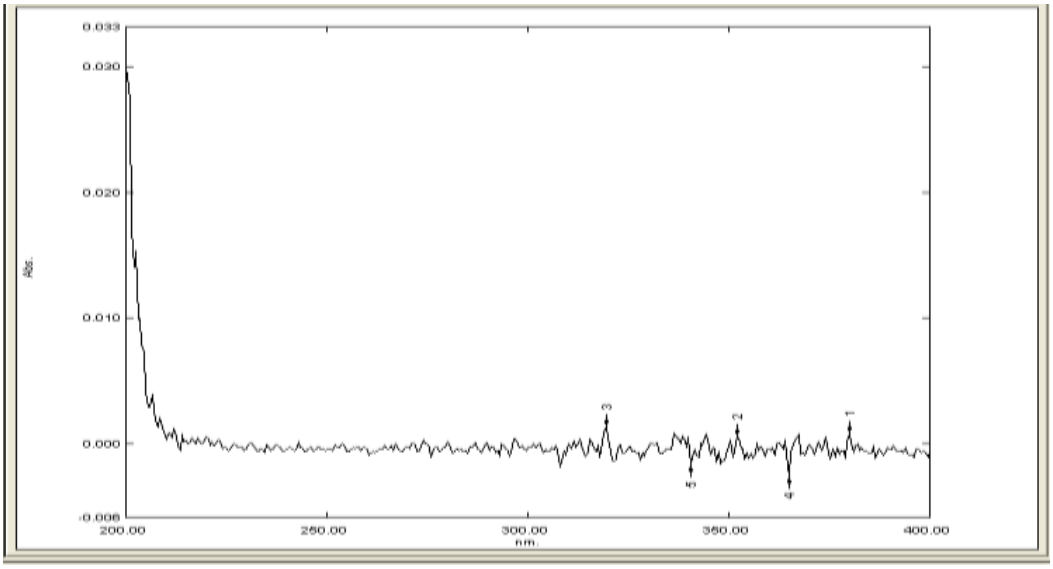

Figure 2: Spectrum of blank

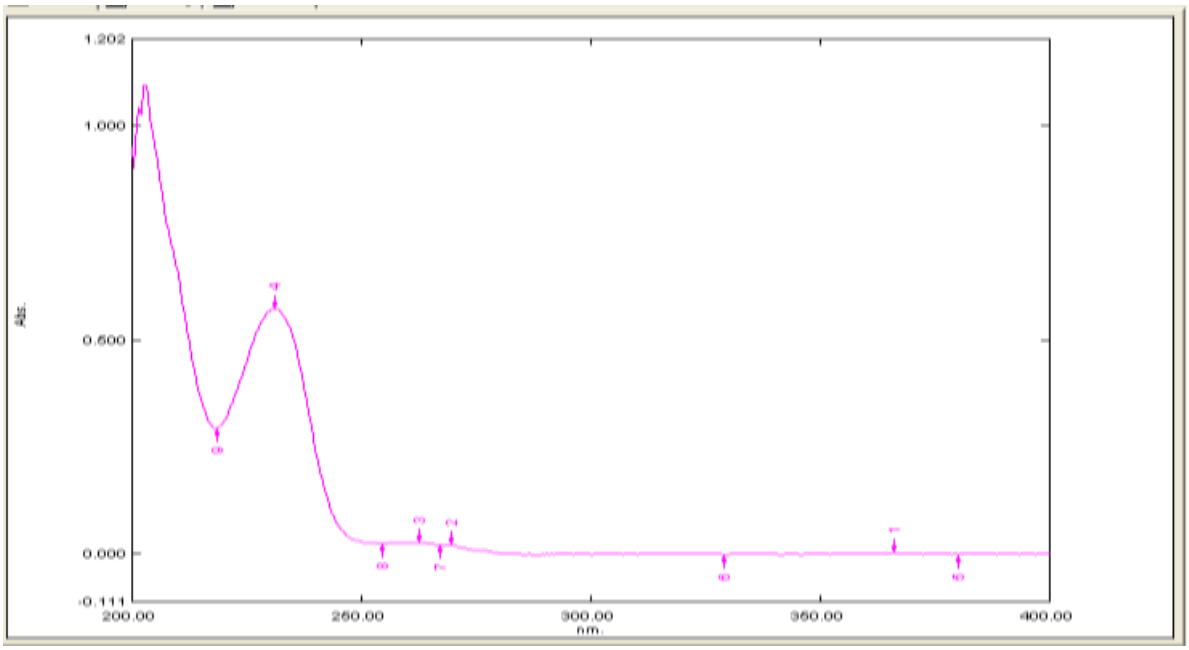

Figure 3: Spectrum of standard solution

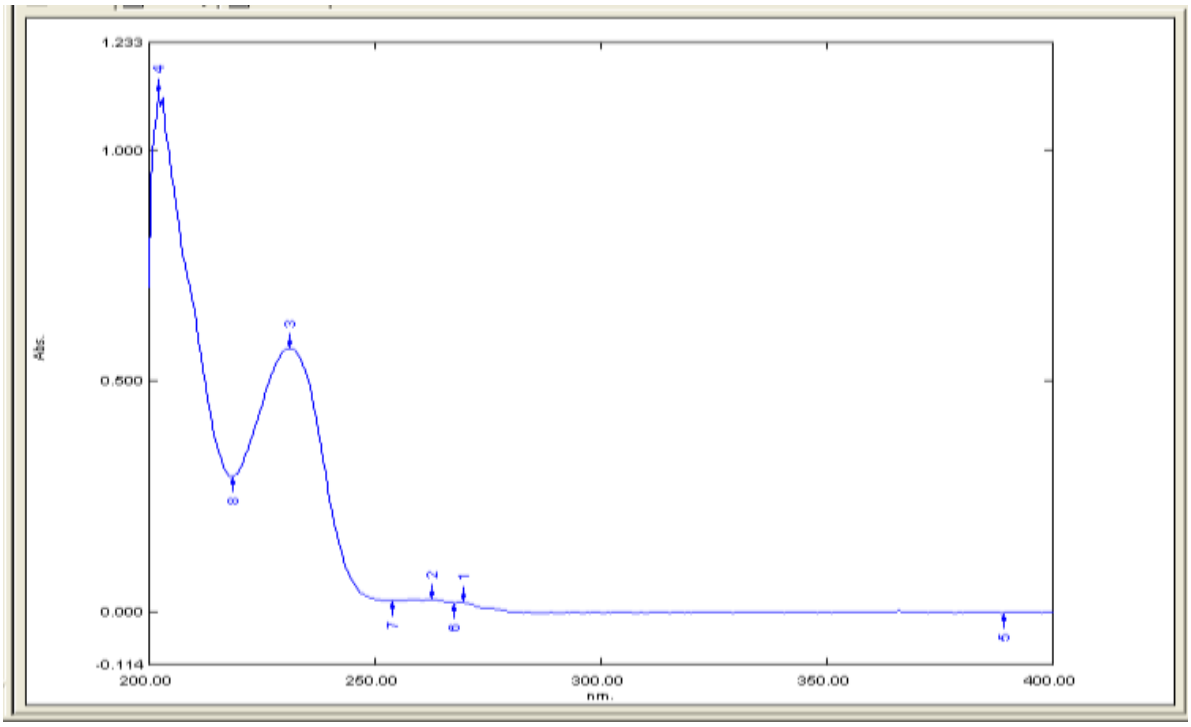

Figure 4: Spectrum of sample solution

Accuracy

Accuracy was determined by the preparation of 3 (three) different concentrations. Three replicate measurements were made for each concentration level. As shown from the data in Table 1, satisfactory % recoveries with small relative standard deviations (% RSD) were obtained .

Table 1: Accuracy study

Nominal Value (%)	Wt. of sample (mg)	Abs of Sample	Wt. of standard (mg)	Abs of standard	Recovery (%) Acceptance limit	Result	%RSD Acceptance limit	Result
80%	23.9	0.446	30.3	0.571	**98.0% to 102.0%.**	98.9	**NMT 2.0%**	0.8%
		0.445				98.7		
		0.446				98.9		
100%	30.3	0.575				100.5		
		0.573				100.2		
		0.574				100.4		
120%	35.3	0.657				98.6		
		0.658				98.8		
		0.658				98.8		
Average (n=9)						99.3		

Precision

- **Repeatability (intra-assay precision)**

Repeatability was determined from six test samples against a standard solution to determine relative standard deviation (RSD of six test samples). As shown from the data in **Table 2** the repeatability of the method was found to be 99.45%.

Table 2: Result of Repeatability

Serial No.	Wt. of sample (mg)	Abs of Sample	Wt. of standard (mg)	Abs of standard	Recovery (%) Acceptance limit	Result	RSD (%) Acceptance limit	Result
1	30.2	0.586	29.8	0.584	**98.0% to 102.0%.**	98.7	**NMT 2.0**	0.5
2	30.6	0.601				100.1		
3	30.6	0.599				99.7		
4	30.3	0.591				99.4		
5	30.3	0.591				99.4		
6	30.4	0.593				99.4		
Average (n=6)						99.45		

- **Intermediate precision**

A second analyst performed the same experiment as a repeatability experiment on different days and different equipment. For determination of intermediate precision, calculated the %RSD of two analyst's results. As shown from the data in Table 3 the %RSD of intermediate precision of the method was found to be 0.4%.

Table 3: Result of Repeatability

Serial No.		Wt. of sample (mg)	Abs of Sample	Wt. of standard (mg)	Abs of standard	Recovery (%) Acceptance limit	Result	RSD (%) Acceptance limit	Result
Analyst 1	1	30.2	0.586	29.8	0.584	**98.0% to 102.0%.**	98.7	**NMT 2.0**	0.5
	2	30.6	0.601				100.1		
	3	30.6	0.599				99.7		
	4	30.3	0.591				99.4		
	5	30.3	0.591				99.4		
	6	30.4	0.593				99.4		
Analyst 2	1	29.8	0.578	29.7	0.576		99.9		0.3
	2	29.7	0.575				99.7		
	3	29.6	0.573				99.7		
	4	29.7	0.576				99.9		
	5	30.2	0.589				100.4		
	6	30.1	0.585				100.1		
Average %RSD (n=12)									0.4

Linearity

Linearity was determined from concentration 50-150% of nominal concentration for a total of seven different concentrations. Data was evaluated by plotting the absorbance of Levocetirizine Dihydrochloride (Y-axis) against its respective concentration (X-axis). The result of the linearity experiment is shown in Table 4 and it was 0.9992.

Table 4: Linearity study

No.	Nominal value (%)	Conc. of Std (µg/mL)	Absorbance of Std	Regression coefficient(R^2) Acceptance limit	Result
1	50	7.45	0.279	**0.9950**	0.9992
2	60	8.90	0.327		
3	80	11.95	0.446		
4	100	15.15	0.575		
5	120	17.85	0.657		
6	140	21.05	0.791		
7	150	22.55	0.847		

Concentration (ug/mL)	Absorbance
7.45	0.279
8.90	0.327
11.95	0.446
15.15	0.575
17.85	0.657
21.05	0.791
22.55	0.847

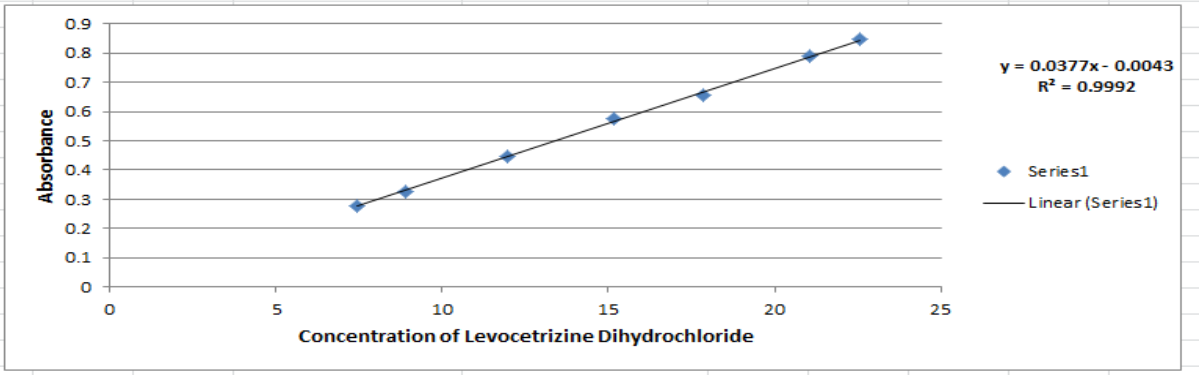

Figure 5: Calibration curve of Linearity

Robustness

The robustness of this method was conducted by changing one parameter (maximum wavelength) of the method by using the test sample of the same concentration of repeatability sample. Concentration value was calculated from the corresponding absorbance for the concentration. The absorbance of the test samples were measured maintaining the same spectrophotometric conditions mentioned for Levocetirizine Dihydrochloride. Change the spectrophotometric condition by shifting the maximum wavelength ±2 nm from the required wavelength of analysis and measured the absorbance of test sample. The %RSD results were found less than 2.0% of Levocetirizine Dihydrochloride which was shown in Table 5.

Table 5: Evaluation data of robustness study.

Serial No.	229 nm	231nm	233 nm
1	0.552	0.554	0.553
2	0.549	0.552	0.550
3	0.551	0.554	0.550
4	0.552	0.559	0.555
5	0.559	0.558	0.560
6	0.559	0.560	0.555
Mean	0.554	0.556	0.554
%RSD	0.8	0.6	0.7

CONCLUSION

From the above test parameters, it is proved that the linearity range, accuracy, precision (Repeatability, Intermediate), specificity were found okay and within the required range. The

proposed method provides simple, accurate and reproducible methods of quantitative analysis for determination of Levocetirizine Dihydrochloride. It was established that the settled method offered several advantages such as rapid, cost effective, simple and easy sample preparation.

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