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Development and Validation of RP-HPLC Method For quantitative Estimation of Cetirizine HCL in Pharmaceutical Formulation as Per ICH Guideline.

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Abstract:
Background: A simple, specific, linear, precise and accurate reverse phase liquid chromatographic (RP-HPLC) method was developed to analysis speed by minimizing run time and retention timeof Cetirizine Hydrochloride in tablet dosage forms. The chromatographic separation was performed using Phenomenex Luna 5µ C18 100A (250 x 4.6 mm). Mobile phase composed of Acetonitrile and water (60:40 v/v) was selected and a flow rate of 1.000 ml/minute is monitored with injection volume of 20µl. Detection was carried out at 229 nm. The method was validated as per ICH guidelines. The retention time for CetirizineHydrochloride is observed as 2.3 minutes. Linearity range was observed in concentration of 50 - 150 µg/ml for Cetirizine Hydrochloride. The percentage recovery of Cetirizine Hydrochlorideis 100%. The correlation coefficients for both the components are close to 1. The proposed method was validated and successfully applied to the estimation of CetirizineHydrochloride in tablet dosage forms.
Key Word: Cetirizine Hydrochloride, method development, Validation.

I. INTRODUCTION

Cetirizine (CEZ) (Figure 1-A) is the carboxylated metabolite of hydroxyzine, and it has a high specific affinity for histamine H1 receptors[1]. CetirizineHydrochloride is a relatively new second-generation antihistamine in the market. It has, however, been found that cetirizine hydrochloride has both sedative and anti-cholinergic effects, though to a smaller extent than that seen in the first generation antihistamines[2]. Cetirizine (CTZ) is an orally active and selective H1-receptor antagonist. It is piperazine derivative and metabolite of hydroxyzine.

Cetirizine Hydrochloridehas three ionizable moieties resulting in pKa values of 2.2, 2.9 and 8.0. At physiological pH, it predominantly exists as a zwitterion or an anion. CetirizineHydrochloride is a whiteor almost white powder that is freely soluble in water, practically insoluble in acetone and in methylene chloride. Its melting point is 110°C to 115°C.

A literature survey reveals that so many RP-HPLC and spectroscopic methods have been reported for the estimation of cetirizineHydrochloride. The main object of present work is to develop a new, cost effective, Solvent saving RP-HPLC method for estimation of Cetirizine Hydrochloride in Tablet form. The present work describes Simple, Gradient RP-HPLC method for the determination of cetirizine Hydrochloride tablet form as for ICH guidelines [6-12].

The aim of this study is practical aspect of analyzing drug using minimum consumption of solvents and to increase speed by minimize run time and retention time so that solvent consumption is less and it will be cost effective and its application to commercial product and develop a simple, fast, precise and accurate reverse-phase HPLC (RP-HPLC) method for the estimation of cetirizine Hydrochloridein pharmaceutical dosage forms as per ICH guidelines.

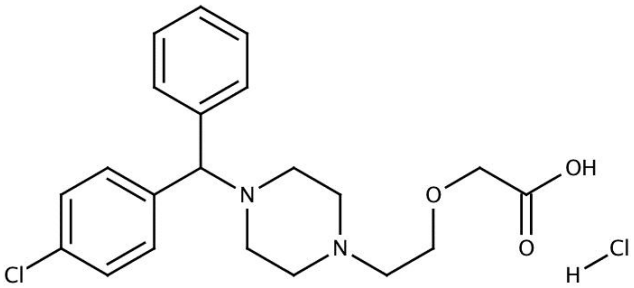

Figure 1:*Cetirizine Hydrochloride*[1]

II. MATERIAL AND METHOD DEVELOPMENT

Instrumental

The HPLC analysis was carried with Waters 2695 with software version Empower 2- PDA detector and Shimadzu LC-2010C HT HPLC system with UV detector and auto sampler integrated with software LCsolution Version 1.25. The column used is Phenomenex Luna 5µ C18 100A (250 x 4.6 mm) and detection was performed at 229 nm. The injection volume of sample was 20µl and the run time was 5minutes. An isocratic mobile phase consisted of Acetonitrile and water (60:40 v/v). The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed before use.

Reagents and chemicals

Cetirizine Hydrochloridewas taken from commercial source and tablets were obtained from local market of Mumbai. HPLC grade Acetonitrile was obtained from Finar Ltd. All other chemicals used were AR grade.

Preparation of mobile phase

Mix acetonitrile and water in the ratio of (60:40 v/v). Mobile phase is degassed before use.

Diluent

Water

Preparation of standard solution

Weigh & transfer accurately about 10 mg of cetirizine Hydrochlorideworking standard in to 10 ml volumetric flask. Add to it 7 ml of diluent and sonicate until it dissolve completely. Dilute up to the mark with diluent& mix well.

Further dilute 2mL of above standard stock to 20mL with diluent.

Preparation of sample solution

Take 10 tablets and crush. Mix uniformly and take equivalent to 10mg of cetirizine Hydrochloride powder and transfer it into 10 ml volumetric flask. Add to it 7 mL diluent, sonicate for 10 minutes, cool to room temperature. Dilute up to the mark with diluent. Mix well and inject.

Further dilute 2mL of above standard stock to 20mL with diluent.

METHOD DEVELOPMENT

Various mobile phase combination were tried to develop new method of cetirizine Hydrochlorideon C18 column. In order to achieve acceptable peak shapes and suitable run time various combinations were tried systematically. Mobile phase composed of acetonitrile and water (60:40 v/v) indicated that peak shape was proper with lesser run time. Therefore acetonitrile and water (60:40 v/v) at a flow rate of 1 ml/min was selected as optimized mobile phase. Phenomenex Luna 5µ C18 100A (250 x 4.6 mm) was used as the stationary phase to reduce the run time. To analyze drugs, detection was tried at various wavelengths but 229 nm was selected as the detection wavelength as drug showed maximum absorption. The retention time was found to be 2.333 minutes. The chromatogram obtained was shown in figure (2). The system suitability parameters were shown in Table (1)

Table I: System Suitability Parameters

Parameter	Cetirizine Hydrochloride
Retention Time	2.33
Tailing factor	1.1
% RSD	0.3

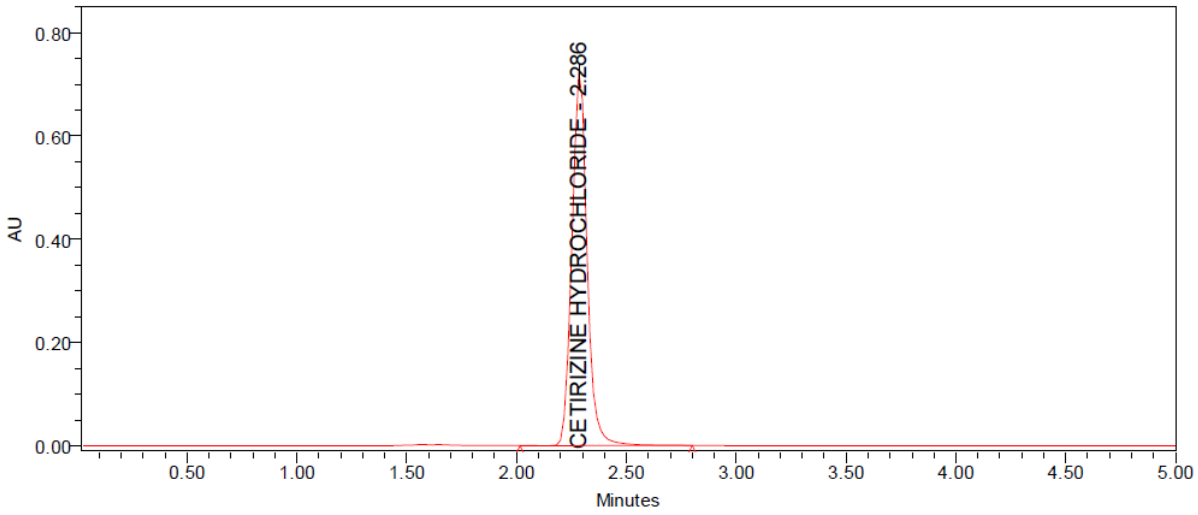

Figure 2: Representive chromatogram of test solution

III. MATERIAL VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristic of a method (expressed in terms of analytical parameters) to meet the requirement for the intended application of the method.
They were tested using the optimize chromatographic conditions and instruments.

Specificity
Spectral purities of cetirizine Hydrochloridepeakwere evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks.

Linearity
The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of for cetirizine Hydrochloridewas established by analyzing serial dilutions of a stock solution of the working standard.Five concentrations such as 50, 75, 100, 125&150 µg/ml for cetirizine Hydrochloridewere prepared as per table (1) and analyzed. Correlation coefficient & %Y-axis should be within the limit.

Table 2: Linearity Concentration Levels of cetirizine Hydrochloride

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	1 ml	20	50
75%	1.5 ml	20	75
100%	2 ml	20	100
125%	2.5 ml	20	125
150%	3 ml	20	150

Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with pre-analyzed sample. Three different solutions of cetirizine Hydrochloride were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (50, 100, 150 µg/mL)and the percentage mean and individual recovery was calculated. Data from the linearity was considered for accuracy.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of cetirizine Hydrochlorideat100 µg/ml concentration. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Intermediate precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst or on different instrument. The standard deviation, relative standard deviation and mean value difference was calculated from the results obtained on each day.

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 was observed that the variations like sonication time&change in wavelength etc.

IV. RESULT AND DISCUSSION

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines.The above method was validated to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.cetirizine Hydrochlorideshowed maximum absorbance at 229 nm.

Specificity

By comparing the chromatogramsof blank solution, placebo solution, reference solution & test solution itis observed that there is no interference of any peaks at the retention time of cetirizine Hydrochloride. The retention time of the main peak in the chromatogram obtained with the reference solution & test solution are matching. This confirmed the specificity of the method.

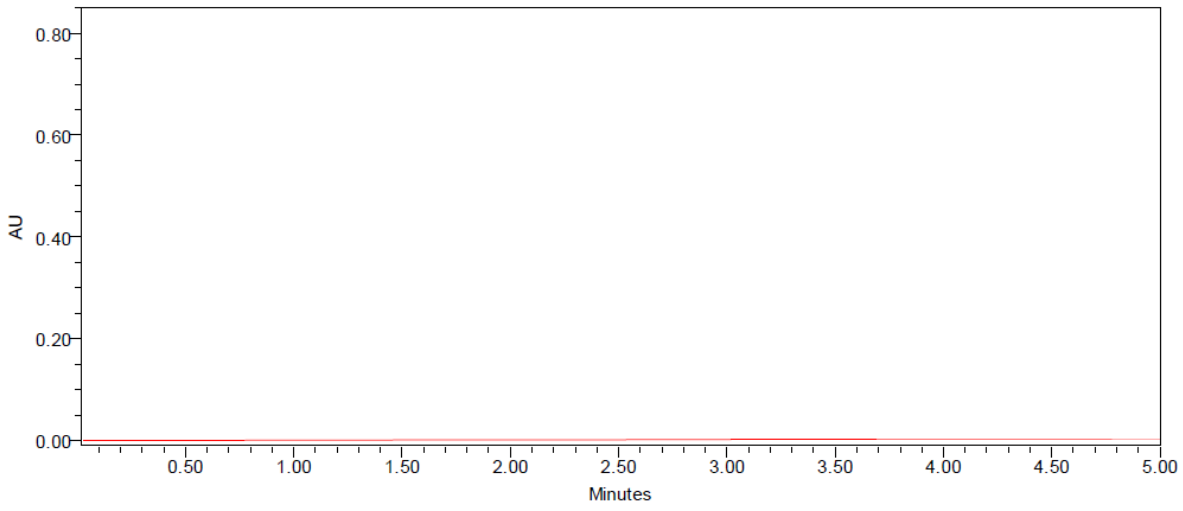

Figure 3: Chromatogram of blank solution

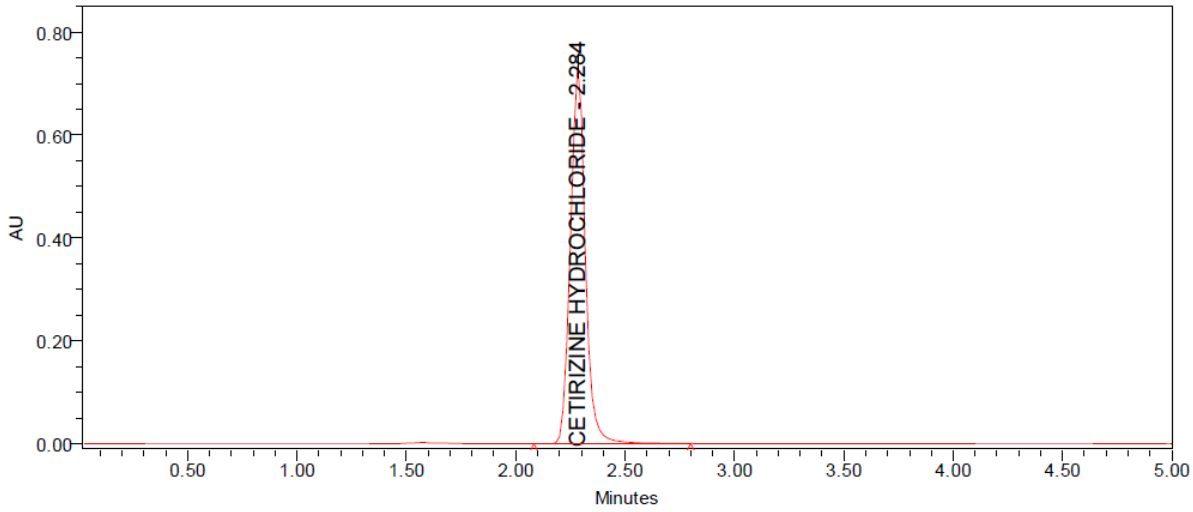

Figure 4: Chromatogram of standard solution

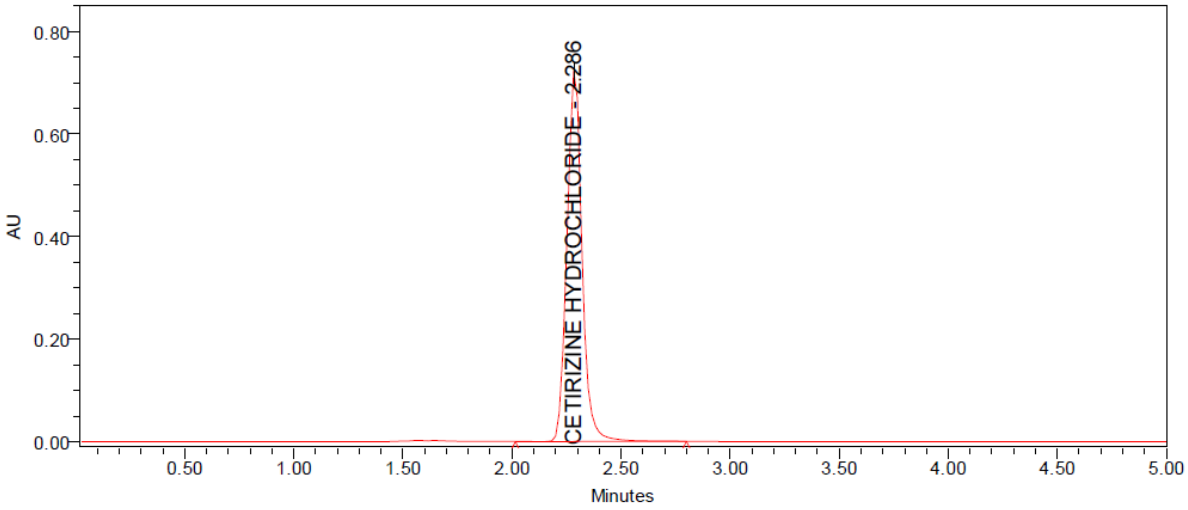

Figure 5: Chromatogram of sample solution

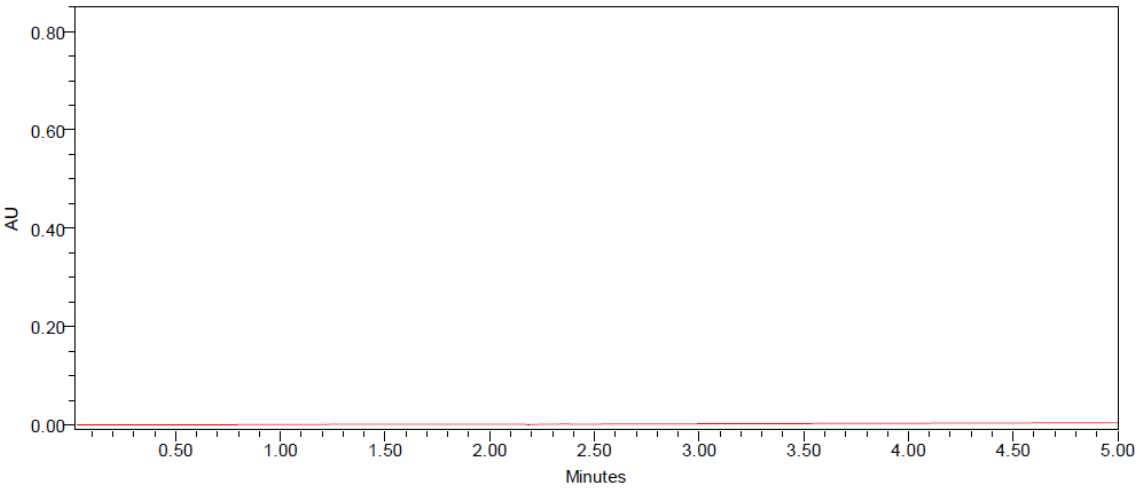

Figure 6: Chromatogram of placebo solution

Linearity

Five concentrations such as 50, 75, 100, 125 & 150µg/ml for Cetirizine Hydrochloridewere prepared and the linearity graph was plotted using concentration verses peak areaas shown in Figure (6).Graph of Residuals against concentration was also plotted as per shown in Figure (7).A linear relationship was obtained between peak areas and quantity analyzed in the range of 50% to 150% (25 -75µg/ml)

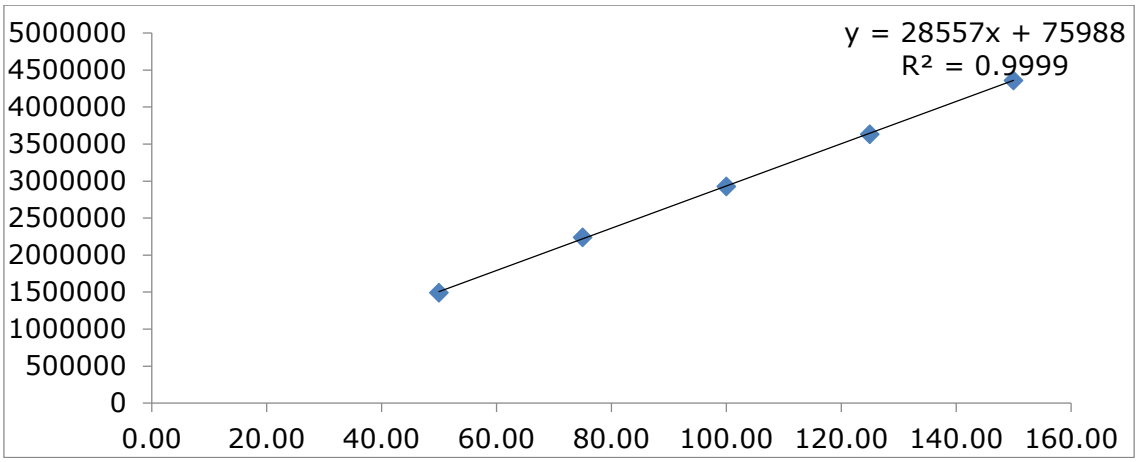

Figure 7: Linearity plot for cetirizine Hydrochloride

Table 3: Observation table for linearity of cetirizine Hydrochloride

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	0.99993	$\geq$ 0.999
%Y – axis intercept	2.59	$\leq \pm$ 5 %
Slope of regression line	28556.50	To be reported
Y Intercept	75988.20	To be reported

The method was considered to be linear in the range on 50 – 150 µg/ml for cetirizine Hydrochloride as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy

The percentage recovery of cetirizine Hydrochloridewas tabulated in table (4). The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 4: Recovery at Different Concentration Levels

Accuracy level	% recovery of cetirizine Hydrochloride
50%	100.6
	101.0
	101.3
100%	99.7
	101.9
	99.9
150%	98.7
	98.5
	98.7
Means recovery	100.4

Minimum recovery	98.7
Maximum recovery	101.0

Precision

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table (5).

Table 5: Method Precision

Sample No.	% Assay of cetirizine Hydrochloride
Sample 01	100.2
Sample 02	100.4
Sample 03	98.4
Sample 04	99.2
Sample 05	100.0
Sample 06	100.8
Mean	99.8
STD Dev	0.89
% RSD	0.89

Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table (6) for % Assay of cetirizine Hydrochlorideand table (6) for comparison of two independent repeatability

Table 6: Intermediate Precision

Sample No.	% Assay of cetirizine Hydrochloride
Sample 01	100.9
Sample 02	100.8
Sample 03	101.3
Sample 04	101.1
Sample 05	100.4
Sample 05	101.4
Mean	101.0
STD Dev	0.36
% RSD	0.35

Table 7: Comparison of two independent repeatability

Parameter	1st day Repeatability	2nd day Repeatability
Number of determinations	6	6
Mean (%) assay	99.8	101.0
RSD (%)	0.89	0.35
Mean value difference (%) Acceptance Criteria: < 2.0 % absolute	0.62	

Robustness

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed HPLC method was robust. The results were shown in Table (8).

Table 8: Robustness Result forcetirizineHydrochloride

Parameter	System suitability	% Assay
	% RSD	
As per method		
--	0.3	100.4
pH		
3.2	0.38	99.7
2.8	0.31	99.5
Flow rate		
0.8 mL/Minutes	0.22	100.1
1.2 mL/minutes	0.06	100.1

Stability of Analytical solution

The sample and standard preparation were tested against freshly prepared standard preparation for 24 hrs. Results found within the acceptance limit of ± 2%.Refer table (9)

Table 9: Stability of Analytical solution

Solution Stability	% Assay of Cetirizine Hydrochloride
0 Hr	100.4
24 Hr	101.0

Application

The same method is applied for topical dosage form i.e. Syrup, Same Results Found as tablet dosage form and Found within the acceptance limit of ± 2%. Refer table (10)

Table 10: Assay of Syrup

Sample No.	%Assay of cetirizine Hydrochloride
Sample 1	99.3
Sample 2	99.3
Mean	99.3
STD Dev	10541.26
%RSD	0.30

V. CONCLUSION

In this present work a new simple, selective, precise, accurate and robust, linear, precise,HPLCmethod was developed and validated for the estimation of cetirizine Hydrochloridein pharmaceutical dosage form in accordance with the ICH guidelines.

The present work is having short run time and retention time hence reduced time and solvent. This method is cost effective and easy to determine.

The current work is worthwhile as developed HPLC spectroscopic method is selective, simple and rapid whichcan be very beneficial for the routine analysis of cetirizine Hydrochloridein pharmaceutical tablet dosage form.

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