Method development, validation and rapid determination of Cetirizine Hydrochloride drug by chromatographic technique.

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***Abstract*- A simple, sensitive, precise and accurate reverse phase liquid chromatographic method for the analysis of Cetirizine hydrochloride has been developed and validated. This method is used for the determination of compounds in commercial pharmaceutical products. The compounds were well separated on a C_{18} column [Inertsil C_{18}, 5µm, 150 x 4.6 mm] utilizing a mobile phase consisting of acetonitrile: phosphate buffer (45:55 (v/v), pH7.0) at a flow rate of 1.0 ml/min with UV detection at 230 nm. The retention time of Cetirizine hydrochloride was found to be 3.11minute.With this method linearity was observed (Correlation coefficient=0.999). According to the validation results, the proposed method was found to be specific, accurate, precise and rapid. Hence the same can be applied to the quantitative analysis of tablets containing Cetirizine hydrochloride.**

***Keywords*- Cetirizine hydrochloride, RP-HPLC, C_{18} column, Method validation.**

I. INTRODUCTION:

Cetirizine HCLor2-[2-[4-[(4-chlorophenyl) phenylmethyl]-piperazin-1-yl] ethoxy] aceticacid dihydrochloride, is white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride, molecular weight 461.8, molecular formula, $C_{21}H_{27}Cl_3N_2O_3$[1-5] (figure 1).

Cetirizine is a piperazine derivative and metabolite of hydroxyzine, is an antihistamine, reported to be a long acting and with some mast-cell stabilizing activity. It is used for the symptomatic relief of hypersensitivity reactions including rhinitis and chronic urticaria. [6-10]. Analytical methods for estimation of cetirizine hydrochloride in different combinations by reverse phase chromatography have been reported [11-17]. Spectrophotometry [18] and TLC [19]. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [20-22].

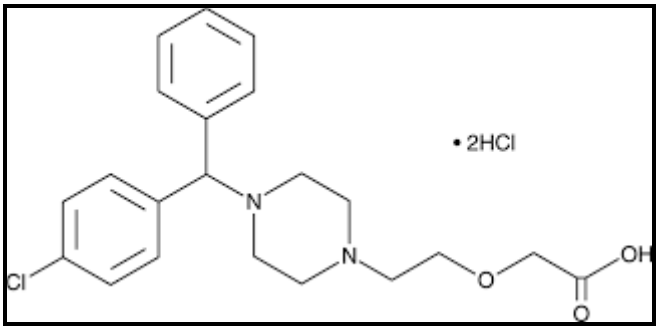


Fig.1.Cetirizine hydrochloride

II. MATERIALS AND METHODS:

- **Chemicals and reagents:**
  Active pharmaceutical ingredient of Cetirizine Hydrochloride was procured from GSK Ltd. Cetzine tablet dosage form was procured from the pharmacy. HPLC grade Acetonitrile Rankem Ltd., milli -Q water used, Phospate (AR grade) buffer was purchased from loba Chem.

- **Instrumentation and chromatographic conditions:**
  The chromatographic separation was performed on the Agilent Technologies1200 series liquid chromatographic system equipped with G1311A quaternary pump and G1315DDAD detector. An Inertsil C_{18} column (150 mm x 4.6mm, 5µmparticle size) was used for the separation. A rheodyne syringe loading auto injector with a 20µl sample loop was used for the injection of analyte. The system was controlled; data was collected and processed by EZ Chrom Eliet software.

- **Preparation of buffer:**

10mM phosphate buffer was prepared in water and pH was adjusted to 7.0 with NaOH solution.

- **Preparation of mobile phase:**
  Mix buffer and acetonitrile in the ratio of 45:55 v/v, mobile phase is degassed before use.

- **Preparation of Standard stock solution:**
  The stock solution of Cetirizine hydrochloride (100µg/mL) was prepared by dissolving 10 mg of Cetirizine Hydrochloride (99.9 %) in a 100mL volumetric flask (stock solution) make up with diluent up to the mark.

  Transferred (10,15,10,5, 15mL) of each stock solution to a diferrent volumetric flask and diluted up to the mark with diluents. This is working standard solution containing (10,15,20,25 and 30µg/ml) of Cetirizine hydrochloride.Refer-Table no.2 Linearity of Citrizine HCL

- **Preparation of test solution:**
  Accurately 20 Tablets were weighed to determine average weight of tablets. Then tablets were finely crushed and powder equivalent to 10 mg Cetirizine Hydrochloride was transferred into 100 ml volumetric flask. Added 70 ml diluent and sonicated for 5 minute with intermittent shaking. Make up volume up to the mark to100ml. From that solution take 10ml of Sample Solution was used and make up volume up to the mark 50 ml using Diluent. This solution was filtered through 0.45µm nylon syringe filter and the final concentration of test sample solution had concentration of Cetirizine 20µg/ml respectively.

Method development:

1. Optimization of the chromatographic conditions:

Mobile phase containing Acetonitrile and phosphate buffer was initially used. In this view Acetonitrile with phosphate buffer of different ratios was tried as a mobile phase at a different pH and ratios were tried along with change in column temperatures. All the times peak shape was not proper and that for Cetirizine retention time was also too long. Mobile phase containing (45:55 v/v) Acetonitrile and phosphate buffer was tried. In this mobile phase drug showed the peak shape with reduced tailing. [Inertsil C_{18}, 5µm, 150 mm x 4.6 mm] the column was the most suitable one since it produced symmetrical peak shape, improved the quality of peak and earlier retention time for Cetirizine. Flow rate was set to1ml/min and UV detection was carried out at 230nm. Retention time of Cetirizine was 3.11 min. The mobile phase was filtered through 0.45µm nylon membrane filter paper and sample solutions were filtered using 0.45µm nylon syringe filter prior to use

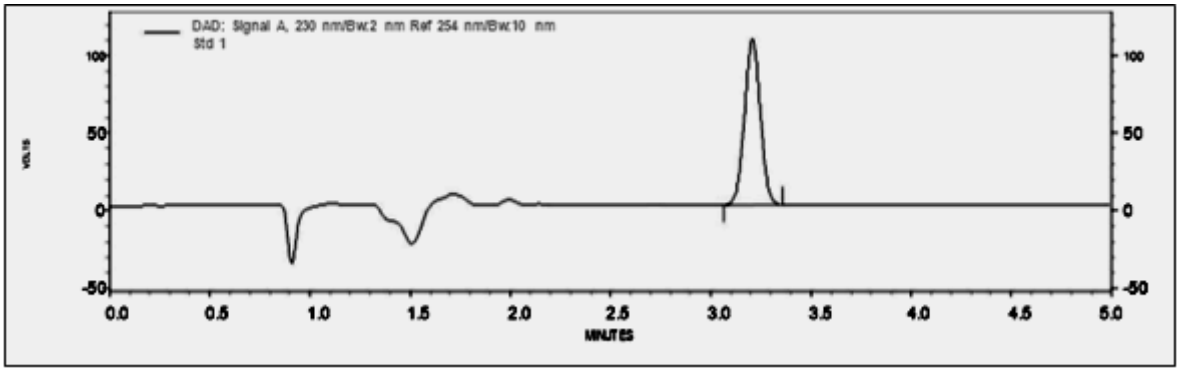
System Suitability:

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. It indicates good performance.

Table no.1 system suitability parameters

Parameter	Cetirizine HCL
Retention time	3.11
Tailing factor	1.06
%RSD	0.04

Figure no.2 representative chromatogram of test solution



III. METHOD VALIDATION:

The objective of the method 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 requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Specificity:

Spectral purities of Cetirizine HCL peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurity as per the methodology. In the work, a solution of the tablet excipients were prepared using the sample preparation to evaluate possible interfering peaks.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtained test result which are directly proportional to the concentration of the analyte in the sample. Linearity was evaluated by visual inspection of a calibration graph. The calibration curves were plotted over a concentration range of 10-30 µg/ml for Cetirizine. Accurately measured standard stock solutions of Cetirizine was prepared by diluting the (1, 1.5, 2, 2.5, and 3) mL of the stock solution in five different 20 mL volumetric flasks and diluted up to the mark with the mobile phase to the give the following concentrations. The absorbance of solution was then measured at 230nm curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Table .2 linearity concentration level of Cetirizine HCL

Standard weight	Diluted to volume	ml taken	Diluted to	PPM	% Level
10	100	10	100	10	50
10	100	15	100	15	75
10	100	10	50	20	100
10	100	5	20	25	125
10	100	15	50	30	150

- **Accuracy:**

  The accuracy of the method was determined by recovery experiments known concentration 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. recovery was performed at 50%, 100%, 150% level and percentage 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 injecting replicate injection of 20µg/ml of solution for 6 times. The mean area and % relative standard deviation (RSD) was calculated. %RSD should not be more than (NMT) 2%.

- **Intermediate Precision:**

  The intermediate precision of the assay method is established by comparison of two independent repeatability experiment on 2 different days .The data of the 1st day is taken from the analysis of "repeatability''. The second set of experiment is performed by a different analyst and HPLC system as well. The standard deviation, relative standard deviation and mean value difference is calculated from the results obtained on each day.

- **Robustness:**

  The robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. It was observed that the variation like flow rate & mobile phase composition, variation in wavelength by ± 2nm. Etc.

IV. Result and Discussion:

Cetirizine hydrochloride showed maximum absorbance at 230 nm. The proposed method for estimation of Cetirizine HCL drug was validated as per the ICH guidelines.

Specificity:

By comparing the chromatogram of blank, placebo solution, reference solution & test solution is it observed that there

is no interference of any peaks at the retention time of Cetirizine HCL. The retention time of the main peaks in the chromatogram obtained with the reference solution & test solution are matching .This confirmed the specificity of the method.

Fig no.3 chromatogram of blank solution

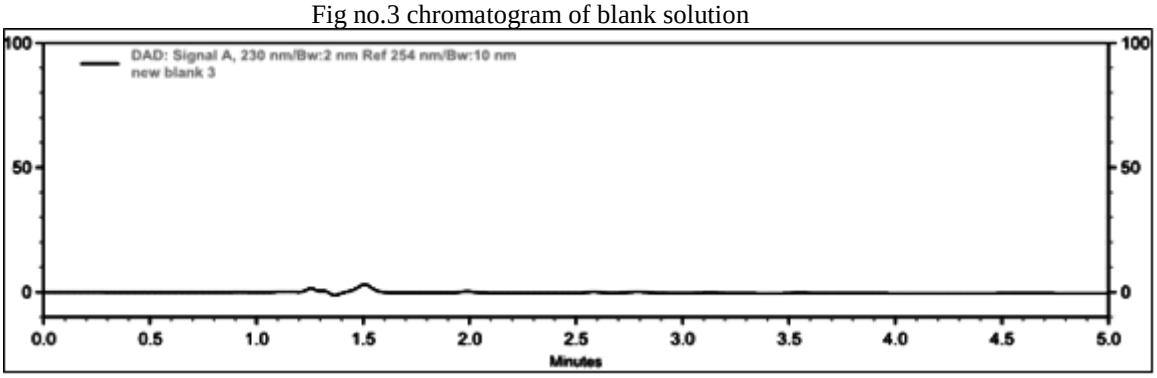


Fig no.4 chromatogram of standard solution

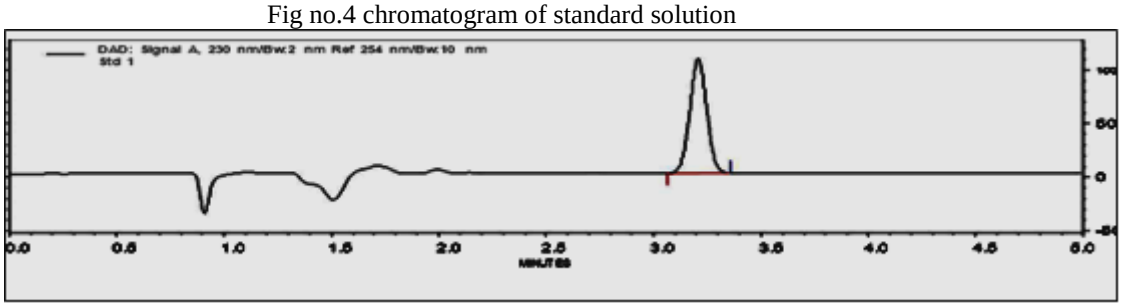


Fig no.5 chromatogram of sample solution

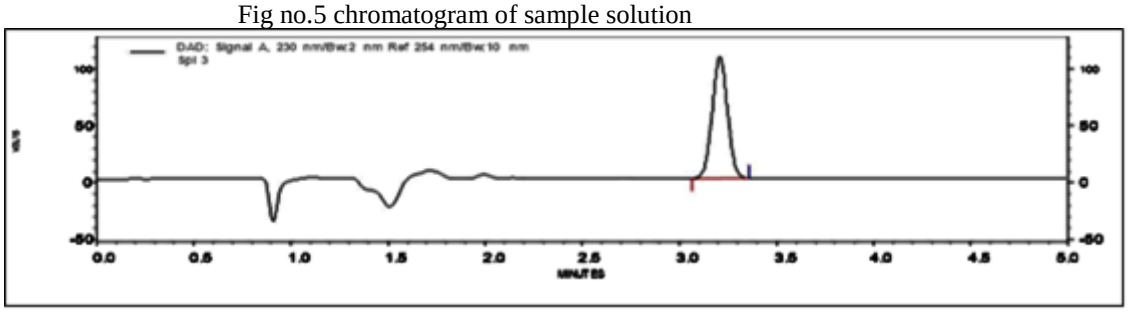
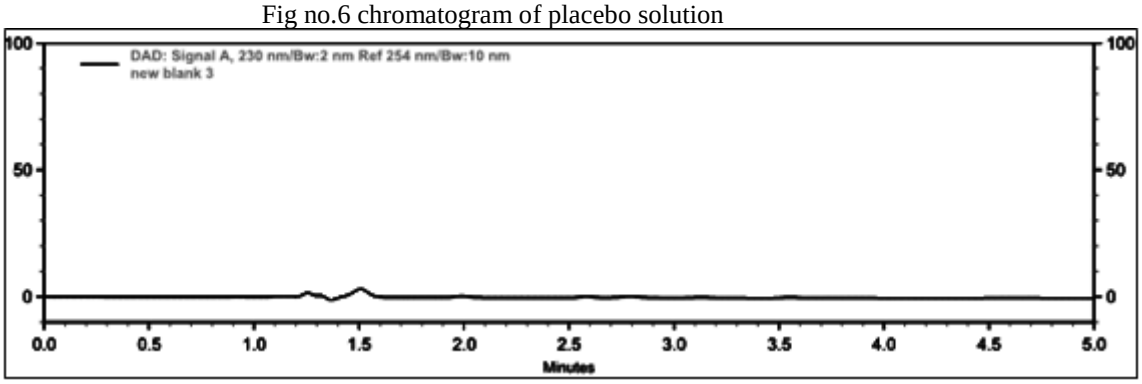


Fig no.6 chromatogram of placebo solution



Linearity:

Five concentration such as 10, 15. 20, 25, 30 µg/ml for Cetirizine HCL and linearity graph was plotted using concentration verses peak area and shown in figure no.(7). A linear relationship was obtained between peak area and quantity analyzed in the range of 50 to 150 %.

Fig no. 7 Linearity plot of Cetirizine HCL

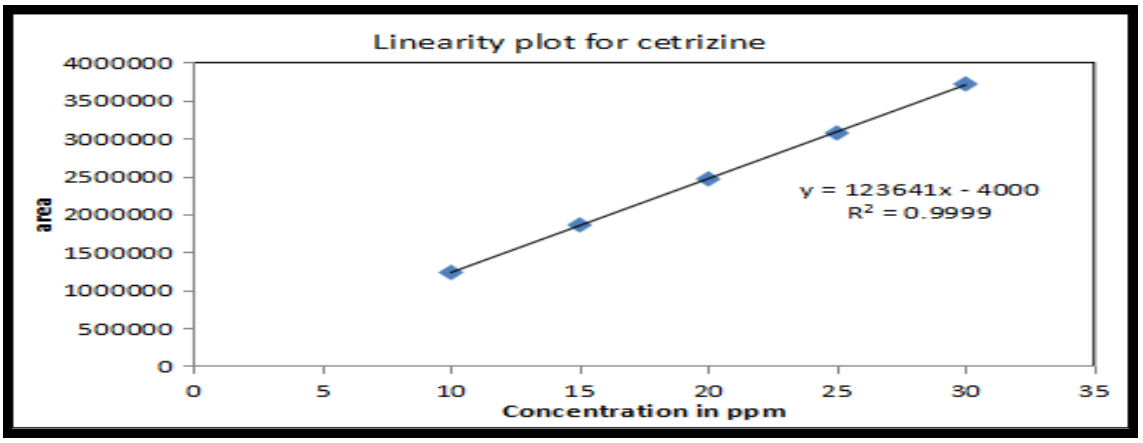


Table no .3 linearity of solution

Parameter of linearity	Value	Acceptance criteria
Correlation coefficient R	0.9999	≥ 0.999

The method is considered to be linear in the range on 10-30 µg/ml for Cetirizine Hcl and correlation coefficient & y-axis intercept should be within the limit.

Accuracy:

The percentage recovery of Cetirizine HCL is 99.83 %. Which shows the accuracy of the method. Refer table no. (4). For recovery at different concentration levels. The recovery values between prescribed limit of 98-102 % shown that method is free from interference of excipients present in formulation.

Table no. 4 accuracy of method

Accuracy level	% Recovery of Cetirizine Hcl
50%	99.5
100%	100.5
150%	99.5
Mean recovery	99.83

Precision:

The exactness of the method as defined by precision and method is considered to be précised as since the relative standard from 6 determinations is well within the acceptance limit. Refer table (5)

Table no .5

Sample no.	% assay of Cetirizine HCL
Sample 01	100.1
Sample 02	99.9
Sample 03	99.8
Sample 04	99.9
Sample 05	100.1
Sample 06	99.6
Mean	99.9
Standrd deviation	0.19

%RSD	0.19

Intermediate precision:

The intermediate precision of the assay method is established by comparison of two independent repeatability experiments on 2 different days. Refer table no.6

Table no.6

Sample no.	% assay of Cetirizine HCL
Sample 01	100.1
Sample 02	99.8
Sample 03	99.9
Sample 04	100.2
Sample 05	99.7
Sample 06	99.8
Mean	99.9
Standard deviation	0.19
% RSD	0.19

Robustness:

Robustness result of Cetirizine HCL:

Method is found to be robust as system suitability criteria is achieved for all the robustness parameter tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is Robust. The results were shown in table no.7

Table no.7 Robustness result for Cetirizine HCL

Sr. No.	**Robustness parameter (flow)**	**% RSD**	**Tailing factor**	**Assay**
1	**0.8 ml/min**	**0.10**	**1.02**	**100**
2	**1.2 ml/min**	**0.10**	**1.02**	**99.9**

Sr. No.	**Robustness parameter (Column oven temperature)**	**% RSD**	**Tailing factor**	**Assay**
1	30 ^{0}C	**0.10**	**1.02**	**100**
2	40 ^{0}C	**0.10**	**1.02**	**99.9**

V.CONCLUSION:

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for estimation of Cetirizine HCL in pharmaceutical tablet dosage form in accordance with the ICH guidelines. This method gives short analysis time, reproducible and showed data for all the method validation parameter. Thus, the method is very simple and all peaks were well separated and there is no interference by excipient speaks with total run time, which makes it especially suitable for routine quality control analysis work. The method can be used for individual analysis of the titled drugs or

their binary combinations.

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