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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR ANALYSIS OF POTENCIAL GENOTOXIC IMPURITIES OF CETIRIZINE DIHYDROCHLORIDE

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ABSTRACT

The present paper describes a simple gradient reverse phase HPLC Method for separation and determination of two potencial genotoxic impurities i.e. 1-chloro-2-(chloro(phenyl)methyl) benzene (2-CCPB) and (chloromethylene)dibenzene (CPB) from the other 18 impurities in Cetirizine Dihydrochloride. Good resolution between two impurities was achieved with Inertsil ODS-3 HP (150 x 4.6 x 3µm) column using gradient of 0.2% Orthophosphoric acid and Acetonitrile. The flow rate was 0.8ml/min and elution was monitored at 225nm. The factors involved in method development are discussed. This method is validated as per International Conference on Harmonization (ICH) guidelines.

KEYWORDS: Cetirizine Dihydrochloride, Genotoxic, RP-HPLC, Gradient, Internation Conference on Harmonization (ICH) guidelines.

1.INTRODUCTION

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurtities may have unwanted toxicities, including genotoxicity and carcinogenicity (5-7) and are to be controlled based on the maximum daily dose.

Regulatory issues related to the presence of genotoxic impurities have arisen with a greater frequency due to enhanced technological capability in identifying impurities and increased

focus on their potential impact on human health. As per the guideline from the European Medicines Agencies (8) on the limits of genotoxic impurities, a threshold of toxilogical convern (TTC) value of 1.5 µg/day. Intake of a genotoxic impurity in ppm is the ratio of TTC in microgram/day and dose in gram/day.This method is validated as per ICH guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and specificity.

Cetirizine dihydrochloride, is a non-selective antagonist of H_1-receptor. Cetirizine dihydrochloride is a potent and non-sedating antihistamine belongs to the piperazine class of second generation of antihistamines. Cetirizine dihydrochloride is used for symptomatic treatment of allergic conditions including seasonal rhinitis and chronic urticaria (9). Cetirizine dihydrochloride is a potent second-generation antihistamine used in the treatment of hay fever, allergies, angioedema, and urticaria.

2. OBJECTIVES OF THE STUDY

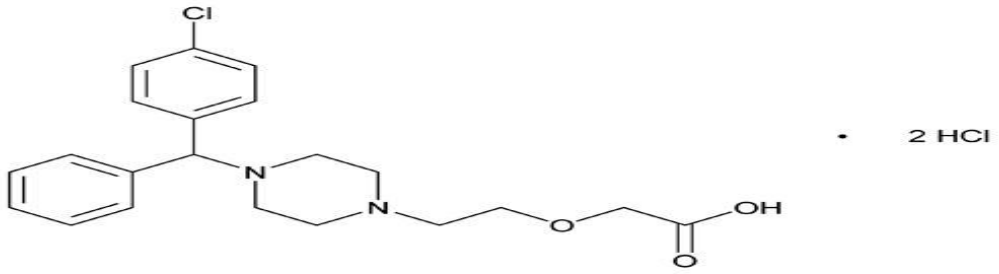
Although it is official in IP(1), BP(2), USP(3), the literature survey reveals that few analytical methods have been reported for genotoxic impurities of Cetirizine Dihydrochloride. Our objective is to develop and validate new RP-HPLC method for determination of Cetirizine Dihydrochloride and its related compounds. The proposed RP-HPLC method utilizes economical solvent system having advantage like better rentention time, sharp and symmetric peak shape. The method is validated according to ICH Guidelines(4)(10).

3. Instrumentation

Waters, Alliance 2695 series HPLC system comprising a quaternary pump, an autosampler, a thermostatted column compartment, a solvent cabinet with degasser along with photodiode array (PDA) 2998 and ultraviolet (UV) 2487 detectors were used for separation and detection. Data acquisition and calculations were carried out using Waters Empower3 software (Milford). Sartorius (Germany) analytical balance was used for weighing material.

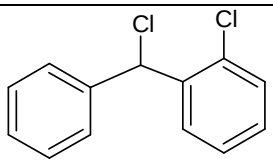
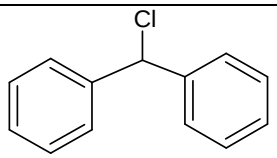
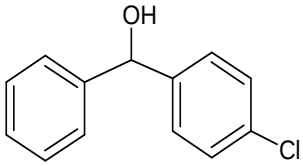
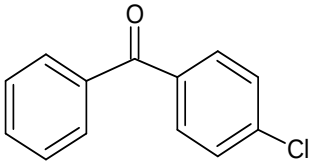
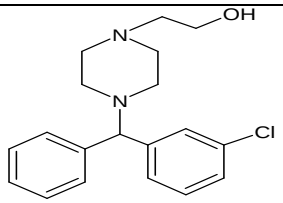
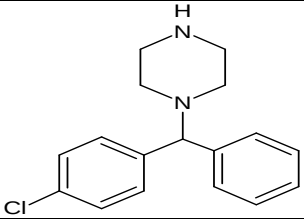
4. Materials and Reagent

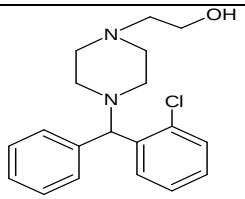
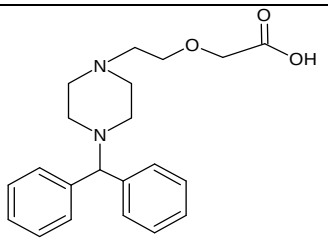
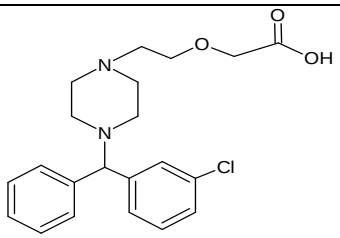
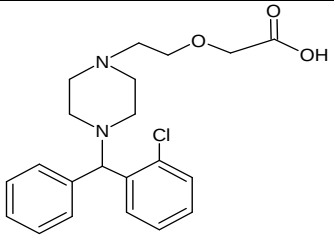
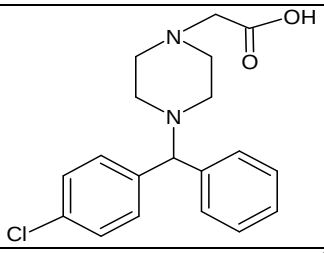
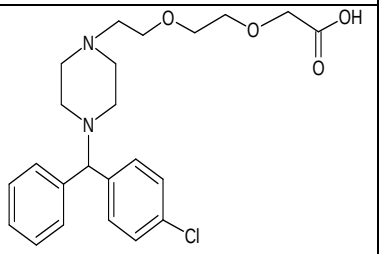
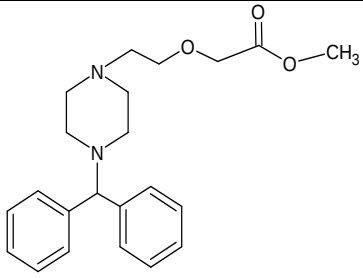
Cetirizine Dihydrochloride HCl sample, woking standard and its related substances working standard were received from Analytical Research and Development department of Indoco Research Centre (Navi Mumbai). HPLC grade Orthophosphoric Acid, Acetonitrile and HPLC grade water were purchased from Merck (India).

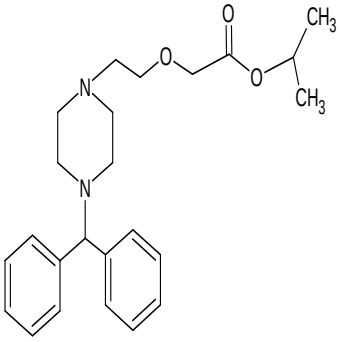
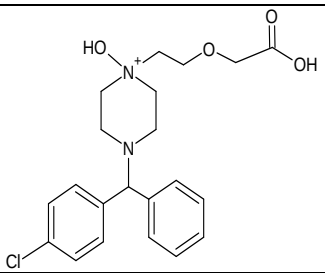
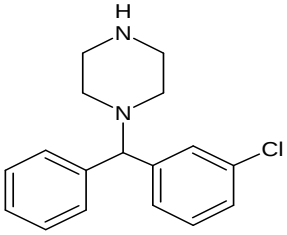
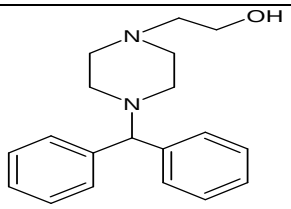
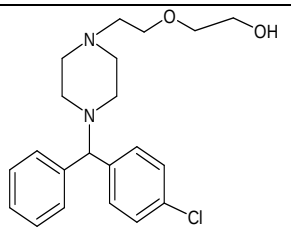
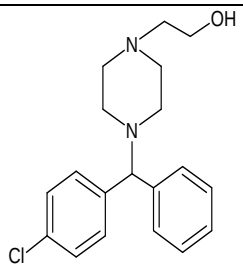
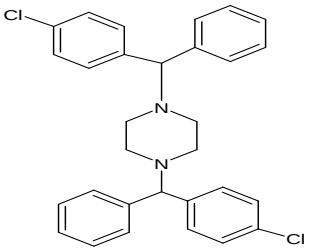


Chemical/IUPAC Name: 2-(2-(4-((4-chlorophenyl)(phenyl)methyl)piperazine-1-yl)ethoxy acetic acid dihydrochloride **Component Name: CTZ**

Table 1: Chemical name of Cetirize dihydrochloride and its related substances.

Sr No.	Component Name	Chemical Name	Structure
1	2-CCPB	1-chloro-2-(chloro(phenyl)methyl) benzene	
2	CPB	(chloromethylene)dibenzene	
3	4-CBH	(4-chlorophenyl)(phenyl)methanol	
4	4-CBPO	(4-chlorophenyl)(phenyl)methanone	
5	3-CPPE	2-(4-((3-chlorophenyl)(phenyl)methyl) piperazin-1-yl)ethan-1-ol	
6	CBHP	1-((4-chlorophenyl)(phenyl)methyl) piperazine	

7	2-CPPE	2-(4-((2-chlorophenyl)(phenyl)methyl) piperazin-1-yl)ethan-1-ol	
8	IMP-F	2-(2-(4-benzhydrylpiperazin-1-yl)ethoxy) acetic acid	
9	3-Chloro CTZ	2-(2-(4-((3-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethoxy)acetic acid	
10	IMP-C	2-(2-(4-((2-chlorophenyl)(phenyl) methyl) piperazin-1-yl)ethoxy)acetic acid	
11	IMP-B	2-(4-((4-chlorophenyl)(phenyl)methyl) piperazin-1-yl)acetic acid	
12	IMP-E	2-(2-(2-(4((4-chlorophenyl)(phenyl) methyl)piperazin-1-yl)ethoxy) ethoxy) acetic acid	
13	CTZ Methyl Ester	Methyl 2-(2-(4-((4-chlorophenyl)(phenyl) methyl)piperazin-1-yl)ethoxy)acetate di hydrochloride	

14	CTZ Isopropyl Ester	Isopropyl 2-(2-(4-((4-chlorophenyl) (phenyl) methyl)piperazin-1-yl) ethoxy)acetate di hydrochloride	
15	N-Oxide	1-(2-(carboxymethoxy)ethyl)-4-((4-chlorophenyl) (phenyl)methyl) piperazine 1,4-dioxide di hydrochloride	
16	3-CBHP	1-((3-chlorophenyl)(phenyl)methyl) piperazine	
17	PPE	2-(4-benzhydrylpiperazin-1-yl)ethan-1-ol	
18	4-PPEE	2-(2-(4-((4-chlorophenyl)(phenyl) methyl)piperazin-1-yl) ethoxy)ethan-1-ol	
19	IMP-G	2-(4-((4-chlorophenyl)(phenyl) Methyl)piperazin-1-yl)ethan-1-ol	
20	IMP-D	1,4-bis((4-chlorophenyl)(phenyl) methyl)piperazine	

5. Chromatographic Condition And Measurement Procedure

5.1 Preparation of Mobile Phase

5.1.1 Mobile phase-A

Transfer 2ml of Orthophosphoric Acid into 1L bottle, containing 1000 mL of water, dissolve and shake well. Filter the solution through a 0.45µm membrane filter, and degas by sonication for 2 minutes.

5.1.1 Mobile phase-B

Acetonitrile

5.2 Diluent

Mix equal volumes of acetonitrile and water and degas by sonication for 2 minutes.

5.3 Preparation of Blank

Use diluent as a blank

Table 2: Chromatographic Conditions.

Column	**Inertsil ODS-3 HP(150 mm x 4.6 mm, 3µm)**		
Column Temperature	40°C±5°C		
Flow Rate	0.8 mL/min		
Gradient Program	Time (min)	MP-A	MP-B
	0 min	75%	25%
	15 min	75%	25%
	35 min	20%	80%
	40 min	20%	80%
	45 min	75%	25%
	50 min	75%	25%
Injection Volume	50 µL		
Detector Wavelength	225 nm		
Run Time	50 minutes		
Retention Time	Cetirize Dihydrochloride , about 18.9 minutes.		
Needle Wash	ACN:Water (50:50)		

5.4 Preparation of solutions

5.4.1 Reference solution (a)

Transfer about 20 mg of CPB and 2-CCPB into 100 mL volumetric flask, dissolve in about 10 mL of diluent and make upto the mark with diluent.

Transfer 1.0 mL of above solution to 100 mL volumetric flask and make upto mark with diluent.

Further transfer 1.0 mL of above solution to 10 mL volumetric flask and make upto mark with diluent.

5.4.3 Test solution

Transfer about 80 mg of Cetirizine Dihydrochloride sample into 10 mL volumetric flask, dissolve in about 5 mL of diluent and make upto the mark with diluent.

Table 3: Injection sequence.

SI#	Description	No. of Injections
1	Blank	1
2	Reference solution (a)	5
3	Test solution	2

5.5 Procedure

Equilibrate the HPLC system with the initial composition until a steady baseline is obtained. Inject Blank and reference solution (a). Ensure that all the system suitability parameters meet the requirements. Inject blank, reference solution (b) and test solution as per injection sequence and record the chromatograms. Make blank correction if necessary.

Table 4: Peak name with Retention Time.

Sr. No.	Peak Name	Retention Time
1	CTZ	18.9
2	CPB	27.6
3	2-CCPB	30.5
4	3-Chloro CTZ	2.0
5	IMP-C	2.1
6	IMP-F	2.2
7	PPE	4.8
8	2-CPPE	9.3
9	CBHP	11.0
10	3 - CBHP	11.4
11	CTZ1	12.5
12	4 – PPEE	13.7
13	IMP-E	16.8
14	3 – CPPE	17.5
15	N-Oxide	21.2
16	IMP-B	21.2
17	Methyl Ester	21.3
18	Isopropyl Ester	23.7
19	IMP-D	29.9
20	4-CBH	31.1
21	4 - CBPO	34.9

6. System suitability

6.1 Acceptance criteria

6.1.1 Tailing Factor*:* The tailing factor of peaks due to CPB and 2-CCPB in reference solution (b) should be not more than 2.0.

6.1.2 %RSD*:* The percent relative standard deviation of five replicates for the peak due to Cetirizine Dihydrochloride in the chromatogram obtained with reference solution (b) should not be more than 5.0.

7. Calculation

Calculate impurity content by formula given below:

$$\text{Content of Genotoxic Impurities in ppm} = \frac{AI \times WS \times 1 \times 1 \times 10 \times 10^6}{AS \times 100 \times 100 \times 10 \times WT \times 100} \times P$$

Where,

AI = Average peak area for respective impurity in test solution.

AS = Average peak area of impurities in reference solution (b).

WS = Weight in mg of each CPB and 2-CCPB working standard taken for reference Solution (b) preparation.

WT = Weight in mg of Cetirizine Dihydrochloride sample taken for test solution preparation.

P = Potency of CPB and 2-CCPB working standard.

8. RESULT AND DISCUSSION

To develop a selective and sensitive method, the primary concern was to separate the genotoxoic impurities from rest of eighteen known impurities and Cetirizine Dihydrochloride. To begin with method from USP was refered. It was observed that many known impurities were co-eluting and merging into one another. Hence method was deviated. Different types of buffer for example phosphate buffer of pH 3-6, citrate buffer of pH 3-5 and ammonium acetate buffer of pH 4-6 were studies in combination with acetonitrile and methanol. It was observed that pH of buffer did not play important role in separation of impurities. To improve separation, modifier was added at lower pH, at limit level all impurities were found to be separate but when spiked at test concentration, one of genotoxic impurity was found to be merging with principal peak. Hence pH of solution was eliminated and only 0.2% Orthophosphoric Acid was used to bring about the separation. The polarity did

not seem to play a role as results both Methanol and Acetonitrile gave similar results, but with Acetonitrile the peak shape of principal peak was observed to be good.

Since most of the impurities are non-polar in nature, various C-18 columns were screened. The parameters like tailing factor and theoretical plates were recorded during the study. From obtained data Inertsil ODS-3 HP (150 x 4.6 x 3µm) was found suitable for analysis.

All impurities were prepared at 100 ppm and their UV-visible spectra was acquired. The Cetirizine Dihydrochloride and all its impurities has good and satisfactory response at 225nm.

9. Analytical Method Validation

The developed method is subjected to analytical method validation, which is conducted according to International Council for harmonisation (ICH) guidelines. The parameters with which analytical method is validated are specificity, limit of detection and limit of quantitation, linearity, accuracy and precision.

9.1 Specificity

Specificity is capability of the method to measure the analyte response in presence of impurities. The typical chromatograms of Blank solution and Impurities spike solution are given from figure 1 to figure 3 respectively. The results indicate that potencial genotoxic impurities are well separated under the current chromatographic conditions from remaining other impurities and as well as from product peak. There was no interference of peak from blank solution. Peak purity for both the genotoxic impurities were passing. The signal to noise ratio was also found to be satisfactory at limit level. For retention time of each impurity and its peak purity refer table no.5

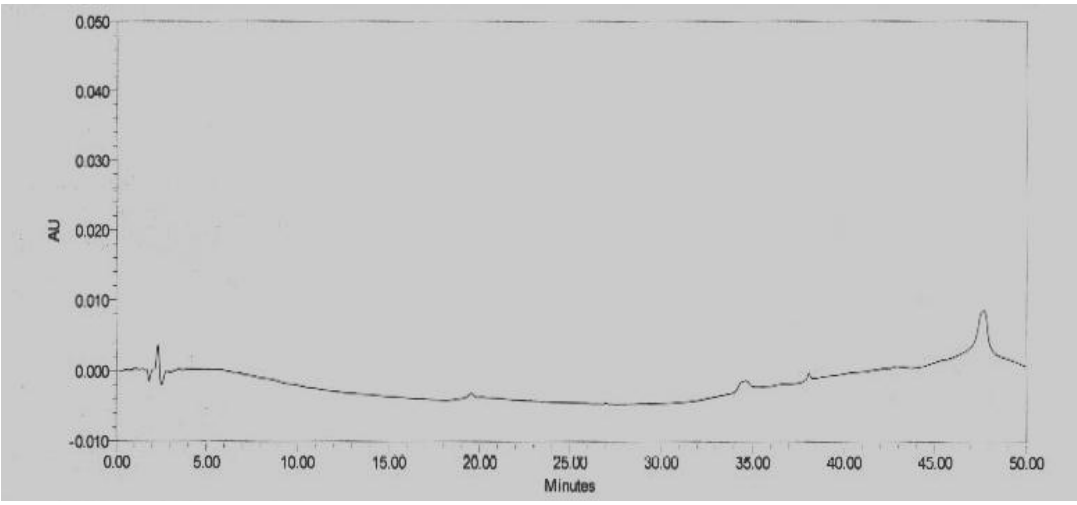


Figure 1: Blank.

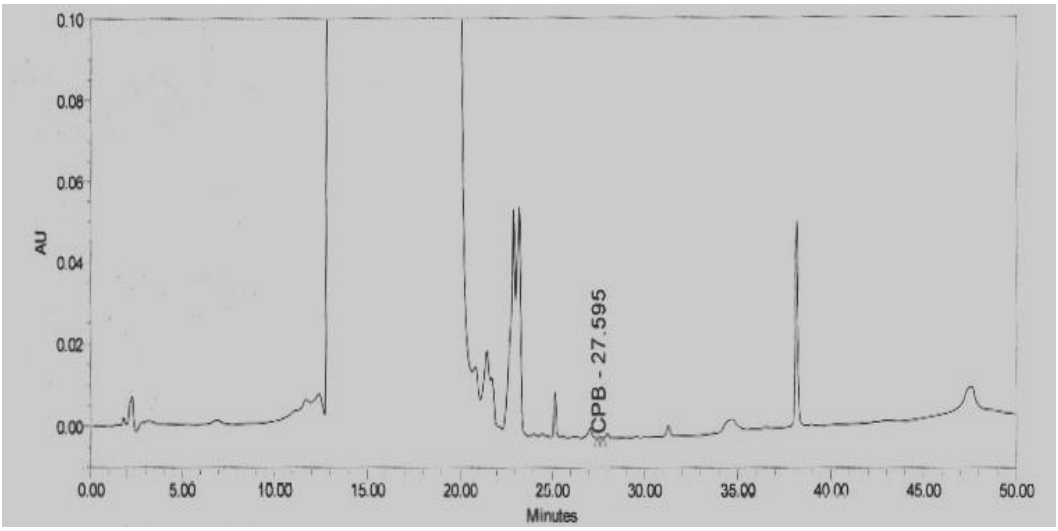


Figure 2: Spike Soltuion containing impurity CCB.

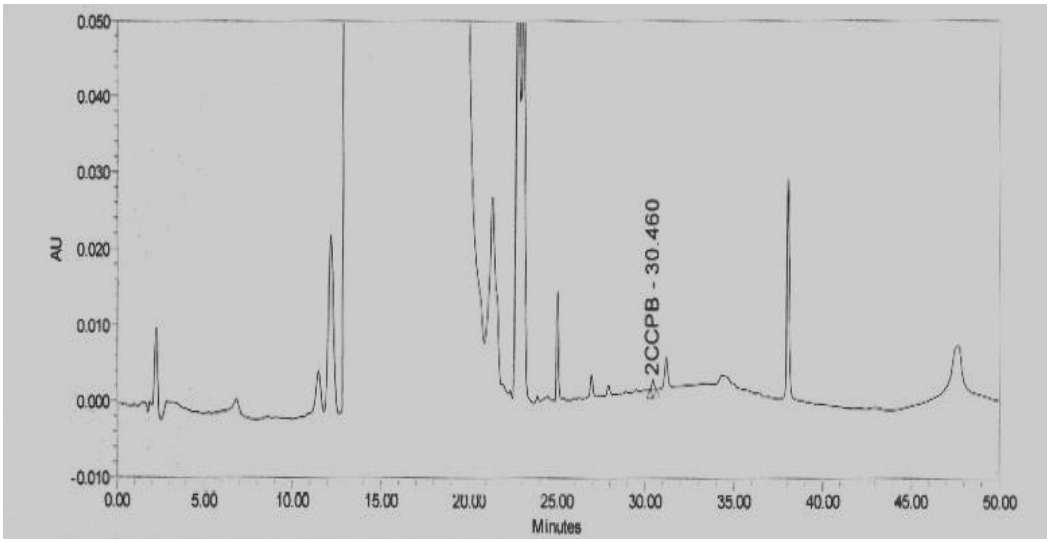


Figure 3: Spike Soltuion containing impurity 2-CCPB.

Table 5: Peak purity and RT Ratio for Cetirizine Dihydrochloride and its impurities.

Sr. no.	Peak name	RT	s/n Ratio	Purity Angle	Purity Threshold
1	CPB	27.5	94.7	1.902	2.055
2	2-CCPB	30.3	144.0	1.714	1.870

9.2 Limit of Detection and Limit of Quantification

Series of standard solution of Cetirizine Dihydrochloride and its genotoxic impurities were prepared and injected in concentration ranging from 50% to 1000%. Limit of detection (LOD) and Limit of Quantitation (LOQ) was calculated based on residual standard deviation of regression line and slope. Both calculated LOD and LOQ were well within limit. For complete details of LOQ and LOD refer Table 6.

Table 6: LOD and LOQ.

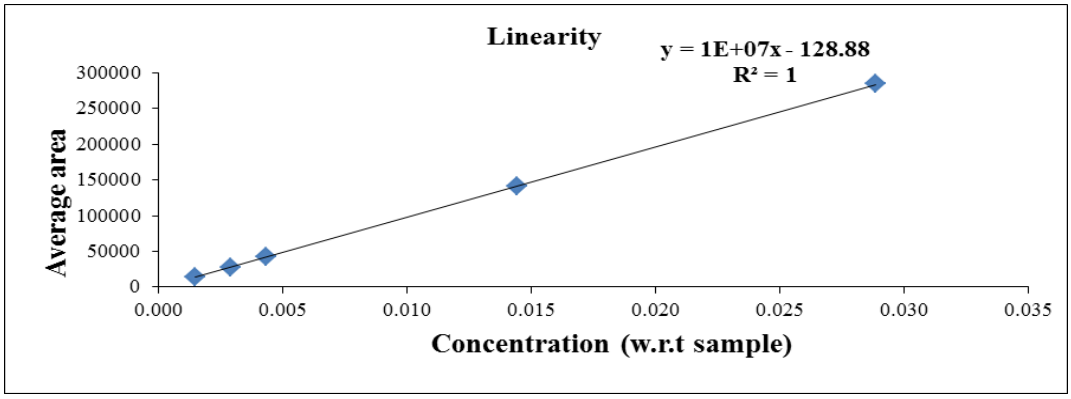
Sr. No.	Name of Impurity	LOD (ppm)	LOQ (ppm)
1	CPB	2.0	7.0
2	2-CCPB	3.0	9.0

9.3 Linearity

Series of linearity solution of Cetirizine Dihydrochloride and its genotoxic impurities were prepared from 50% to 1000% of test concentration. Lineariy curves were drawn by plotting the peak areas of Cetirizine Dihydrochloride and its genotoxic impurities against its corresponding concentration of linearity solution. Observed regression coefficient was greater than 0.998 and % y-intercept was less than 5.0%

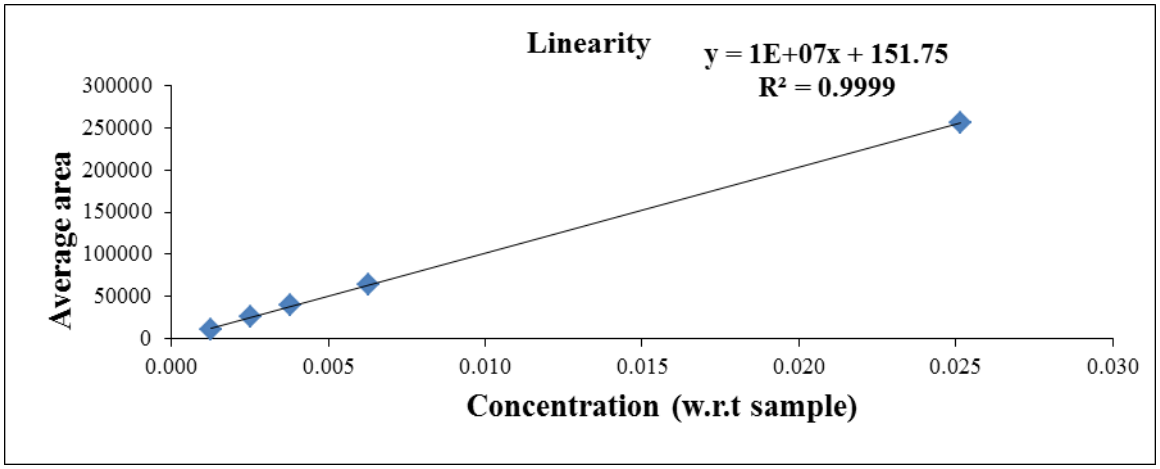
Table 7: Linearity table and its R^2 values CPB

Slope	**9836435.27**
Intercept	**-128.88**
Corelation Coefficent (R^2)	**1.0000**
% Y-Intercept	**-0.30**
STD	**710.68**



2-CCPB

Slope	**10170032.00**
Intercept	**151.75**
Corelation Coefficent (R^2)	**0.9999**
% Y-Intercept	**0.39**
STD	**875.34**



9.4 Accuracy

Accuracy of method is calculated and established by carrying out recovery studies of Impurities. The test solution was spiked with imputrities solution at specific limit level concentration 50%, 100%, 150%. Each spiked test solution was analyzed for recovery study of impurities. Recovery established is between 85% to 110%.

Table 8: Recovery of impurities at 50%.

Sr.No.	IMP Name	As Such Test Area	Area of 0.05% Level	RRF	Thereotical imp Added in	IMP Observed in ppm	% of Recovery
1	2CCPB	0	12033	**1.00**	11.44	10.83	**94.70**

Sr.No.	IMP Name	As Such Test Area	Area of 0.05% Level	RRF	Thereotical imp Added in	IMP Observed in ppm	% of Recovery
1	CPB	897	15178	**1.00**	13.61	14.28	**104.93**

Table 9: Recovery of impurities at 100%.

Sr.No.	IMP Name	As Such Test Area	Area of 0.10% Level	RRF	Thereotical imp Added in	% of IMP Observed	% of Recovery
1	2CCPB	0	25043	**1.00**	22.85	22.52	**98.55**

Sr.No.	IMP Name	As Such Test Area	Area of 0.10% Level	RRF	Thereotical imp Added in	IMP Observed in ppm	% of Recovery
1	CPB	897	28346	**1.00**	27.22	27.45	**100.84**

Table 10: Recovery of impurities at 150%.

Sr.No.	IMP Name	As Such Test Area	Area of 0.15% Level	RRF	Thereotical imp Added in	% of IMP Observed	% of Recovery
1	2CCPB	0	42685	**1.00**	34.37	38.49	**111.98**

Sr.No.	IMP Name	As Such Test Area	Area of 0.15% Level	RRF	Thereotical imp Added in	% of IMP Observed	% of Recovery
1	CPB	897	42563	**1.00**	40.85	41.69	**102.05**

9.5 Precision

System precision was carried out by analyzing five injections of reference solution (b) of Cetirizine Dihydrochloride API at limit level concentration. Relative standard deviation for peak area of Cetirizine Dihydrochloride was calculated and found to be 0.61%.

10. CONCLUSION

The analytical method validation for Cetirizine Dihydrochloride by Reverse Phase HPLC was carried out by performing the parameters such as specificity, limit of detection and limit of quantitation, linearity, accuracy, precision. All the data has been compiled and found to be satisfactory. Hence, method developed for Reverse Phase HPLC can be suitably used for analysis genotoxic impurities of Cetirizine Dihydrochloride.

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