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Research Article

A Validated Stability-Indicating HPLC Assay Method for Cetrizine HCl in Bulk Drug

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ABSTRACT

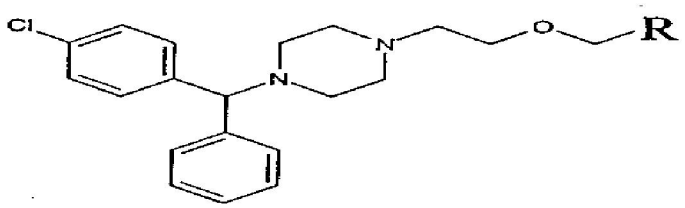
An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Cetrizine hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Thermo Hypersil C18 (250 x 4.6)mm,5µ column and the mobile phase containing 900 ml water and 200 ml 0.01M H_2SO_4 water filter and mixed. Prepare a homogenous mixture of buffer, and acetonitrile (80:20, v/v/v). The detection was carried out at wavelength 230 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Keywords: Cetrizine hydrochloride, Isocratic, Reversed phase HPLC, Linearity.

INTRODUCTION

Cetirizine HCl or 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-piperazin-1-yl]ethoxy]acetic acid 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, $C_{21}H_{27}C_{l3}N_2O_3$.[1-3] .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.[4-8]. Cetirizine is rapidly absorbed from the gastro-intestinal tract after oral administration, peak plasma concentration being attained in about one hour. It is highly bounded to plasma proteins and has an elimination half-life of about 11 hours. Cetirizine has been detected in breast milk and excreted primarily in the urine mainly as unchanged drug .[9-10].

Chemical structure of Cetrizine



R_1	COOH	Cetirizine
R_2	CH_2OH	Hydroxyzine

Literature Survey

Literature Survey reveals that there are number of methods reported in the literature for the determination of drug .[11] in tablets.[12-13] , serum [14-15] , urine[16-17] , plasma [18] based on HPLC, while an HP-TLC method for the determination of drug in human plasma is also available.[14] However, the limit of detection in all these methods does not exceed 3 µg/ ml. The goal of this study was to develop a rapid, more accurate, precise reliable, less expensive and least time consuming HPLC method for the analysis of Cetrizine HCl, in the form of raw materials, bulk drug samples and dosage formulations, using the most commonly employed C-18 column with UV detection and extremely low LOQ & LOD values. . In the present work reversed phase HPLC method was developed for the separation of Cetirizine in bulk drug and the impurities formed from its forced degradation under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat as per ICH standards[19-21].

Experimental

Material and reagents

Cetrizine hydrochloride bulk drug was made available from Merck Ltd. India (purity 99.8). Sulphuric acid were obtained from Qualigens fine chemicals, India Limited. Acetonitrile, were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades; Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system (Systronic) consisting of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and UV detector**.** The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Thermo Hypersil C18 stationary phase with particle size 5 micron and pore size 100A□ was used. The instrumental settings were a flow of 1 ml/min, the injection volume was 20 µl. and wavelength 230 nm.

Mobile Phase

The mobile phase containing 900 ml water and 200 ml 0.01M H_2SO_4 water filter and mixed. Prepare a homogenous mixture of buffer, and acetonitrile (80:20, v/v/v).

Preparation of Standard stock solutions

Standard stock solutions of 1000 ppm of Cetrizine hydrochloride in acetonitrile and water (70:30) were prepared in volumetric flasks.

Sample solution

1000 ppm of Cetrizine hydrochloride in 100ml calibrated flask containing acetonitrile and water mixture (70:30.The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure [22-23].

Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for Cetrizine hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Cetrizine hydrochloride bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5 N Hydrochloric acid), alkali (0.025N NaOH) hydrogen peroxide (30%), heat (60 ^oC) to evaluate the ability of the proposed method to separate Cetrizine hydrochloride from its degraded products. For heat study, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Cetrizine hydrochloride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of Cetrizine hydrochloride from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard Cetrizine hydrochloride from bulk. For this purpose, we have used Water nova pack C18(150X4.6)mm,5µ, Kromasil C18(150X4.6)mm,5µ, Inertsil ODS 3V C18(250X4.6)mm,5µ and Kromasil C18(250X4.6)mm,5µ,Star ODS-II C18 (250X4.6)mm,5µ and Grace Alpha C18 (250mm x 4.6)mm,5u Out of these used HPLC column, Thermo Hypersil C18 (250 x 4.6)mm,5u found to comparatively better and gave the graph with better Gaussian shape at retention time 9.27 min. To improve the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH_2PO_4 and Acetonitrile (60:40,v/v) in these trials peak shape is not good, another trials 0.01M Ammonium acetate P^H-5.9 and acetonitrile(20:80,v/v) peak shape not found well, trials Acetonitrile and water (80:20, v/v) column temperature 35 ^oC peak shape not found good, trials K_2HPO_4,Methanol and water (10:70:20,v/v/v)column temperature 35 ^oC, trials 1.0gm KH_2PO_4 and 0.45gm 1-Hexa sulphonic acid sodium salt make P^H-3.5 Ortho phosphoric acid and

methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for mobile phase containing 900 ml water and 200 ml 0.01M H_2SO_4 water filter and mixed. Prepare a homogenous mixture of buffer, and acetonitrile (80:20, v/v/v).

Result of forced degradation experiments

Considerable degradation was not observed in Cetrizine hydrochloride bulk samples, under stress conditions such acid , thermal stress .Considerable degradation of Cetrizine hydrochloride was observed under stress condition such as base ,and oxidative hydrolysis leads to the formation of some unknown degradation peaks. The mass balance of Cetrizine hydrochloride in stress samples was close to 100% and moreover, the unaffected assay of Cetrizine hydrochloride in the Tablets confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table 1.

Table 1: Summary of Forced degradation results

Stress condition	Time	Assay of active Substance%	Remarks
Acid Hydrolysis (0.5 N HCl)	48 Hrs	99.00	No Degradation
Base Hydrolysis (0.025 N NaOH)	2 Hrs	84.17	Degradation
Oxidation (30% H_2O_2)	48 Hrs	98.16	No Degradation
Thermal (80°C)	7 days	99.34	No Degradation
Photolytic degradation	1.2Lux million Hrs	98.59	negligible degradation

Method Validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table 2.

Table 2: System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Cetrizine HCl	9.27	1.33	1.13	5544

Precision

The precision of the method was studied by determining the concentrations of the drug Cetrizine hydrochloride in the tablet for six times [24] . The results of the precision study (Table 4) indicate the reliability of the method (RSD %< 2).

Table 4: Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Cetrizine HCl	0.67	80-120	2145.26	0.99911

*Standard deviation shown in parentheses

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Cetrizine hydrochloride ranged from 99.12% to 100.01% (Table 5). The average recoveries of three levels nine determinations for Cetrizine hydrochloride were 100.31- 100.51%.

Table 5: Results of the Recovery Tests for the Cetrizine HCl

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	50	98.11	98.22
100	100	99.14	99.04
120	150	100.01	100.33

* RSD shown in parenthesis.
^ Average recovery = the average of three levels, nine determinations

Calibration and linearity

Linearity test solutions for the method were prepared from Cetrizine hydrochloride stock solutions at six concentrations levels from tested from 80% to 120% of the targeted level of the assay concentration Cetrizine hydrochloride. Standard solutions containing 80-120 µg/ml of Cetrizine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations the slopes and correlation coefficients are shown in Table –3**.**

Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Cetrizine hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35^{o}C instead of 30^{o}C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in Table-6

Table 6: Results of robustness study

S. No.	Parameters	Variations	Resolutions between Cetrizine hydrochloride and base degraded product
1	Temperature	25 ^{o}C 35 ^{o}C	8.21 7.68
2	Flow rate	0.8 ml/min 1.2 ml/min	8.02 8.94
3	Mobile phase	40.5 ml 49.5 ml	3.7 3.3

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.15–0.27% of the assay concentration (40 µg mL−1) were prepared by dilution of the standard solutions. Each solution (20 µL) were injected five times, the areas were measured for the drug peak, and the standard deviation for the five injections was calculated for each concentration. On the basis of data obtained, the standard deviation was calculated and this value used for calculation of the LOD and LOQ. The results are shown in Table-3.

Table 3: Results of the LOD and LOQ

Name	%LOD	%LOQ
Cetrizine HCl	0.26	0.41

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Cetrizine hydrochloride was 0.35 %. The assay values were within $\pm$ 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSION

The method developed for quantitative determination of Cetrizine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be

used for assessing the stability of Cetrizine hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Cetrizine hydrochloride in bulk drugs and pharmaceutical dosage form.

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CHROMATOGRAMS

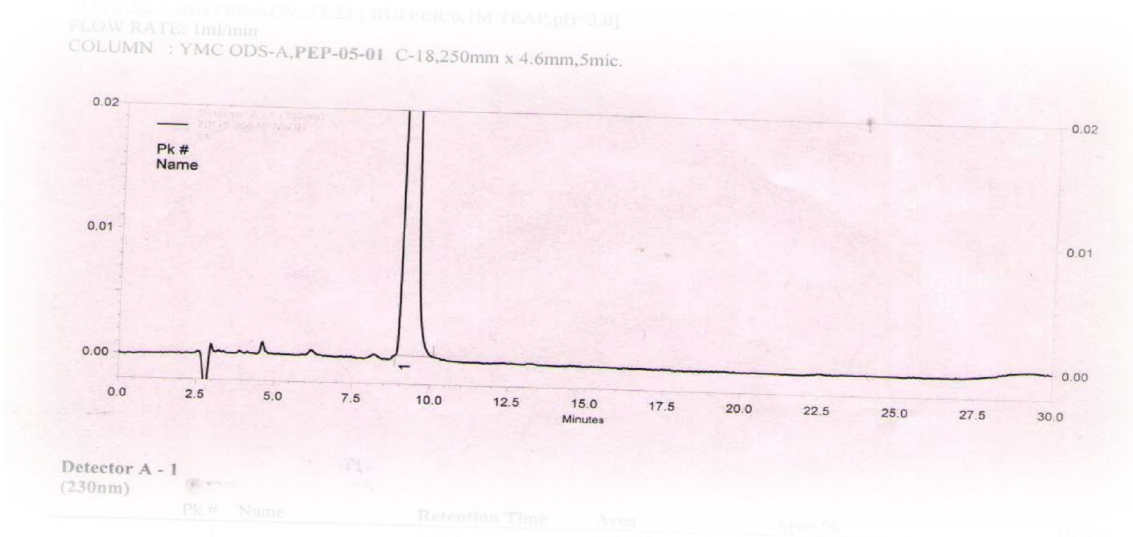

Fig. 1A: Typical Chromatogram of Cetrizine HCl Standard Preparation

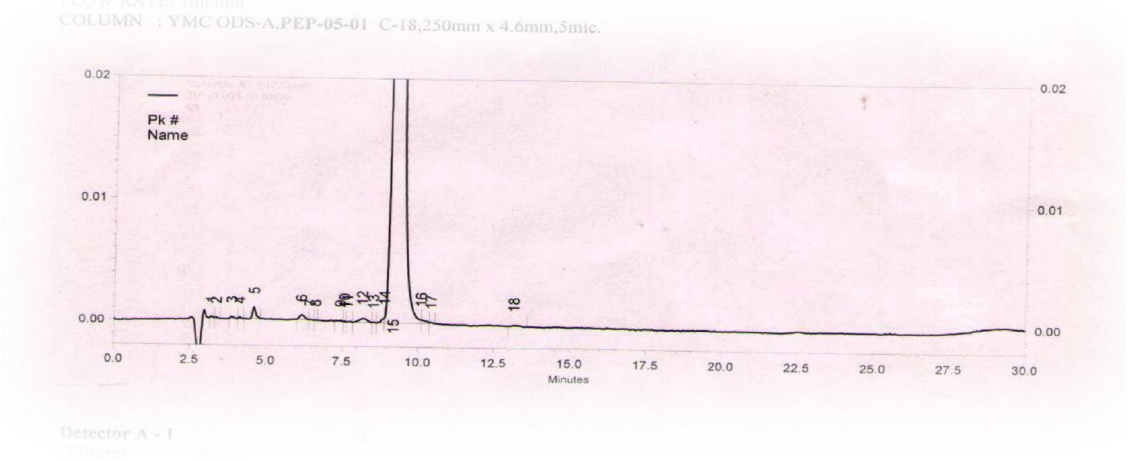

Fig. 2A: Typical Chromatogram of Cetrizine HCl Alkali Degradation

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