Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 suppl 2, 2014

Research Article

# DEVELOPMENT AND VALIDATION OF A ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY OF CETIRIZINE DIHYDROCHLORIDE

**P. RAVI KUMAR REDDY*, DR. V. KRISHNA REDDY, E. SASIKIRAN GOUD[2], Y. RAMACHANDRA REDDY[3]**

**Sri Krishnadevaraya University, Department of Chemistry, Anantapur, A.P, India.**

*Received: 28 Nov 2013, Revised and Accepted: 03 Feb 2014*

**ABSTRACT**

Objective: The main objective of current study is to develop and validate UPLC method, simple, precise, accurate and specific chromatographic method for the determination of cetirizine dihydrochloride in tablets.

Methods: An ultra performance liquid chromatography instrument and silica, 33 x 4.6 mm, 3 µ were used for determination of cetirizine dihydrochloride. The flow rate of 1.0 mL/min was set with isocratic, the temperature of column compartment maintained at 25°C and Ultra violet detection done at 230nm wavelength. The injection volume was 5 µL. The cetirizine dihydrochloride peaks eluted at 1.202 minute and run time was set as about 2 minutes.

Results: The correlation coefficient (≥ 0.999) shows the linearity of response against concentration over the range of 10 to 300%. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated an ultra performance liquid chromatographic method was suitable for determination of cetirizine dihydrochloride in pharmaceutical formulations which is more useful with respect to regular laboratory analysis. This method can be conveniently used in a quality control laboratory for routine analysis of cetirizine dihydrochloride.

**Keywords:** Cetirizine dihydrochloride, Method development, UPLC, Validation

## INTRODUCTION

Cetirizine hydrochloride (Fig. 1) is chemically 2-{2-{4-[(4-Chlorophenyl) (phenyl)-methyl] piperazino} ethoxy) acetic acid hydrochloride. It is a second-generation antihistamine, is a major metabolite of hydroxyzine, and a racemic selective H1 receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. Cetirizine crosses the blood-brain barrier only slightly, reducing the sedative side effect common with older antihistamines. It has also been shown to inhibit eosinophil chemotaxis and LTB4 release. At a dosage of 20 mg it was found that it inhibited the expression of VCAM-1 in patients with atopic dermatitis. The levorotary enantiomer of cetirizine, known as levocetirizine, is the more active form. [1].The literature survey reveals that a so many RP-HPLC and spectroscopic methods have been reported for the estimation of cetirizine dihydrochloride [2-11]. The main object of present work is to develop a new UPLC method for estimation of Cetirizine Dihydrochloride in tablet form. The present work describes a simple, gradient UPLC method for the determination of Cetirizine Dihydrochloride tablet form as for ICH guidelines [12-14].

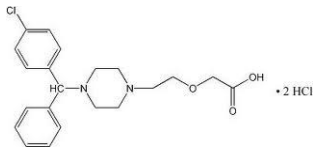


**Fig. 1: Structure of Cetirizine Dihydrochloride**

## MATERIALS AND METHODS

### Chemicals

Qualified standards and samples were obtained from local laboratories and were used without any further purification. The chemicals like sulfuric acid, acetonitrile and methanol were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was 33 X 4.6 mm, 3 µ silica.

### Instruments

An Acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of cetirizine dihydrochloride. UPLC instrument was controlled by Waters Empower chromatographic software. Silica, 33 x 4.6 mm, column with particle size of 3 µm was used as stationary phase for chromatographic separation and determination of Cetirizine Dihydrochloride. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment, and Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

### Standard preparation

Weighed and transferred 50.0mg of cetirizine dihydrochloride standard in to 100 mL volumetric flask dissolve and make up the volume with mobile phase and mix well. Further dilute 5.0mL of above solution in 25mL volumetric flask, make up the volume with mobile phase and mix well.

### Sample preparation

Weighed and transferred 50.0mg of cetirizine dihydrochloride sample in to 100 mL volumetric flask dissolve and make up the volume with mobile phase and mix well. Centrifuge a portion of this preparation at 3000 RPM for 10 minutes. Pipette out 5 mL of the clear supernatant solution into a 25 mL volumetric flask, dilute to volume with the mobile phase and mix and filter through 0.45µm filter.

### Chromatographic conditions

The chromatographic column used was Silica column with dimensions of 33 mm X 4.6 mm with 3 µm particle size. The isocratic method was employed with the mobile phase dilute sulfuric acid, water and Acetonitrile in the ratio of 4:66:930 (v/v/v).

The column temperature was maintained at 25.0°C and detection was monitored at a wavelength of 230 nm. Injection volume was 5 µl and the mobile phase flow was set at 1.0 mL/min. The mobile phase used as diluents for preparation of solutions.

## METHOD VALIDATION

The developed method for determination of cetirizine dihydrochloride was validated for system suitability along with

method selectivity, specificity, linearity, range, precision (Repeatability and Intermediate precision), accuracy, limits of detection and Limit of quantification according to the ICH guidelines.

**System suitability**

The system suitability was conducted using diluted standard preparation and evaluated by injecting five replicate injections.

**Specificity**

Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte.

**Linearity**

Performed the linearity with cetirizine dihydrochloride in the range of 10 to 300% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system

**Precision and Accuracy**

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample.

The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements. The system precision was conducted using cetirizine dihydrochloride and evaluated by making six replicate injections. The accuracy of the method by recoveries of cetirizine dihydrochloride sample solutions at different concentration levels ranging from 10 to 300%

**Robustness**

The method robustness was studied by deliberately changing the percentage of organic modifier, flow rate and column temperature.

**RESULTS AND DISCUSSION**

**Optimization of chromatographic conditions:**

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 230 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Cetirizine Dihydrochloride. Preliminary development trials were performed with various silica columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Silica,33 X 4.6 mm, 3µm, column there was a substantial increase in the theoretical plates(2693) with a significant improvement in the peak shapes with 1.5 tailing factor.

**System suitability**

The RSD from five replicate injections of standard preparation was 0.1 %. Theoretical plates for cetirizine dihydrochloride peak 2693 with tailing factor 1.5.

**Selectivity**

Performed the specificity parameter of the method by injecting diluent, placebo, standard preparation and sample preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of cetirizine dihydrochloride. Specificity results of cetirizine dihydrochloride given in the below Table-1.The selectivity Chromatograms Shown in the Figure-2

**Table 1: Selectivity results of cetirizine dihydrochloride**

| Solution Retention Time in (min) |
|---|
| Diluent - |
| Placebo - |
| Standard preparation- 1.201 |
| Sample as such- 1.210 |

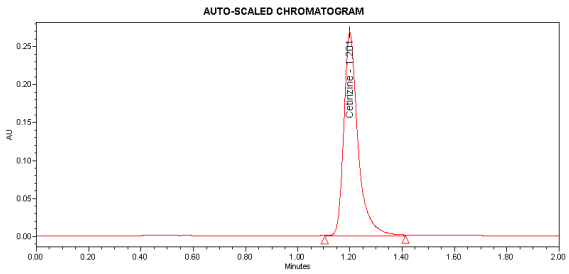


**Fig. 2: Chromatogram of cetirizine dihydrochloride**

**Linearity**

To demonstrate the linearity with cetirizine dihydrochloride standard in the range of 10 to 300% of specification limit**.** Correlation coefficient of cetirizine dihydrochloride was 1.000.The linearity results of cetirizine dihydrochloride shown in the Table-2.

**Table 2: Linearity results of cetirizine dihydrochloride**

| S. No. | Level in % | Area Response |
|---|---|---|
| 1 | 10 | 106273 |
| 2 | 50 | 511098 |
| 3 | 100 | 1012506 |
| 4 | 150 | 1501533 |
| 5 | 200 | 2027603 |
| 6 | 300 | 3085002 |

**Accuracy**

Accuracy study found that the mean % of recovery was more than 97.0% and less than 103.0% at each level from 10 to 300% of concentration levels, hence method was accurate. The accuracy results are given in the Table-3

**Table 3: Accuracy results**

| S. No | Level in % | % Mean Recovery |
|---|---|---|
| 1 | 10 | 101.6 |
| 2 | 50 | 100.6 |
| 3 | 100 | 99.8 |
| 4 | 150 | 100.6 |
| 5 | 200 | 100.1 |
| 6 | 300 | 99.8 |

**Precision**

The precision of test method was validated by assaying six samples prepared on cetirizine dihydrochloride and calculate relative standard deviation of assay results. The precision results are given in the Table-4

**Table 4: Precision results**

| S. No. | Preparation No. | % Assay of cetirizine |
|---|---|---|
| 1 | Preparation No - 1 | 99.31 |
| 2 | Preparation No - 2 | 99.52 |
| 3 | Preparation No - 3 | 99.13 |
| 4 | Preparation No - 4 | 97.69 |
| 5 | Preparation No - 5 | 99.42 |
| 6 | Preparation No - 6 | 99.33 |
| Average % Assay of Cetirizine | 99.07 | |
| % Relative Standard Deviation | 0.69 | |

**Robustness**

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier, flow rate, and column temperature. The results were obtained as shown in the below Table-5.

**Table 5: Robustness results**

| Condition | Tailing factor | Theoritical plates | . % RSD |
|---|---|---|---|
| Limits | NLT 2.0 | NLT 1500 | NMT 2.0 |
| Normal Condition | 1.5 | 2693 | |
| Flow rate 1.2ml/min | 1.5 | 2675 | 0.1 |
| Flow rate 0.8/min | 1.4 | 2985 | 0.1 |
| Column Temperature 30°C | 1.4 | 2918 | 0.1 |
| Column Temperature 20°C | 1.4 | 2849 | 0.1 |
| Organic phase +10.0% | 1.4 | 2853 | 0.1 |
| Organic phase - 10.0% | 1.5 | 2818 | 0.1 |

**ACKNOWLEDGEMENT**

The author thankful to Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India for encouragement

**CONCLUSIONS**

A simple isocratic UPLC method has been developed and validated for the determination of cetirizine dihydrochloride in tablets. The developed method has been found to selective, sensitive, precise, robust, and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to quantification of cetirizine assay and also for the analysis of stability samples.

**REFERENCES**

**1.** Azhagvuel S, Sekar R. Method development and validation for the simultaneous determ-ination of Cetirizine dihydrochloride, Paracetamol and Phenylpropanolamine hydrochloride in tablets by capillary zone electrophoresis, Journal of Pharmaceutical and Biomedical Analysis 2007; 43: 873–878.

2. L. Suryan, V. K. Bhusari, K. S. Rasal, S. R. Dhaneshwar*, Simultaneous Quantitation and Validation of Paracetamol, Phenylpropanolamine Hydrochloride and Cetirizine Hydrochloride by RP-HPLC in Bulk Drug and Formulation, International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(4): 303-308.
3. Rosseel MT and Lefebvre RA, "Determination of cetirizine In human urine by high performance liquid chromatography" Journal of Chromatography,1991;565(1-2):504-510.
4. Jaber AMY, Al Sherife HA, Al Omari MM, Badwan AA, "Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC" Journal of Pharmaceutical and Biomedical Analysis, 2004; 36(2): 341-350.
5. El Walily AFM, Korany MA, El Gindy A, Bedair MF, "Spectrophotometric and hig performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets" Journal of Pharmaceutical and Biomedical Analysis, 1998; 17(3): 435-442.
6. Ramesh KC, Melwanki MB, Gowda BG, Seetharamappa J, Keshavayya J"New spectrophotometric method for the determination of cetirizine hydrochloride inpharmaceutical preparations and biological samples" Indian journal of Pharmaceutical science, 2002; 64(5): 455-458.
7. S. B. Wankhede*, K. A. Lad, S. S. Chitlange, Development and Validation of UV-spectrophotometric methods for simultaneous estimation of eetirizine hydrochloride and phenylephrine hydrochloride in Tablets. IJPSDR, 2012; Vol 4, Issue 3,222-226.
8. Patel BA, Patel GS, Patel PD, Patel HB, Patel MM. Stability indicating HPTLC method development and validation for estimation of Ambroxol hydrochloride and Cetirizine dihydrochloride in combine tablet dosage form, International Research Journal of Pharmacy 2011; 2(3): 95-99.
9. Makhija NS, Vavia RP. Stability indicating HPTLC method for the simultaneous determination of Pseudoephedrine and Cetirizine in pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis, 2001; 25: 663–667.
10. Dhongle SP, Sahare JS, Dhongle SS, Mundhey SA, Wate PS. Development and validation of HPTLC method for simultaneous estimation of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in tablet dosage form, Der Pharmacia Lettre.2011; 3 (4): 111-119.
11. Nitin d. rawool1,*, a. venkatchalam1 and k.h. singh2, development and validation of a rapid RR-HPLC method for the simultaneous estimation of cetirizine and pseudoephedrine in pharmaceutical dosage forms, Int. J. Curr. Pharm. Res,2005; Vol 5, Issue 1, 54-60.
12. Validation of Analytical Procedures: Methodology (Q2B), ICH Harmonized Tripartite Guidelines, Geneva, 1996
13. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization,2005.
14. ICH Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization, 2003.