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# High Performance Liquid-Chromatographic Determination Of Cetirizine In Pharmaceuticals

Corresponding Author

**K.Basavaiah**
**Department of Chemistry,**
**University of Mysore, Manasagangotri,**
**Mysore 570 006, (INDIA)**
**E-mail : basavaiahk@yahoo.co.in**

Co-Author

**B.C.Somashekar**
Department of Chemistry,
University of Mysore, Manasagangotri,
Mysore 570 006, (INDIA)

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## ABSTRACT

One rapid assay procedure based on high performance liquid chromatography (HPLC) has been developed for cetirizine in bulk drug and in tablets. The determination was performed on a reversed phase column (ODS $C_{18}$; 250x4.6 mm i.d) using a mobile phase(1.5 ml/min) consisting of potassium dihydrogen phosphate, acetonitrile and methanol (pH = 5.5)-58:33:9(v/v) with UV-detection at 210 nm. A reactilinear relationship between mean peak area and concentration of CTH was observed in the range 2-30 μg/ml with a detection limit of 0.2 μg/ml and a quantitation limit of 0.66 μg/ml. Intra-day and inter-day precision, and accuracy of the methods have been established according to the current ICH guidelines. The methods have been successfully applied to the assay of CTH in tablet preparations with recoveries varying from 99.8 to 101.9 % with standard deviation in the range of 0.68-1.12 %. The results were statistically compared with those of the reference method by applying Student's t-test and F-test. Accuracy, evaluated by means of the spike recovery method was in the range 98.2 to 104.1 % with precision (RSD) better than 3%. 

## KEYWORDS

Cetirizine;
Determination;
HPLC;
Pharmaceuticals

## INTRODUCTION

Cetirizine hydrochloride (CTH) is chemically designated as (RS)–2[2-{(4-chlorophenyl phenyl methyl)-1-piperazinyl} ethoxy] acetic acid dihydrochloride (Figure 1). It is a new antihistaminic drug used for the treatment of perennial and seasonal allergic rhinitis and also for chronic urticaria[1]. The drug is official in European Pharmacopoea[2], which describes a titrimetric method with 0.1N sodium hy-

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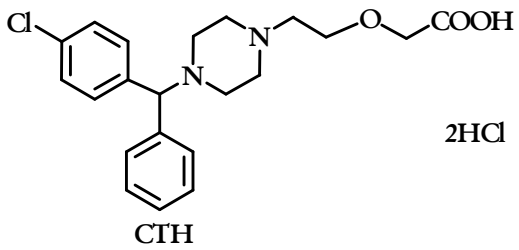


CTH

**Figure 1: Structure of drug**

droxide for bulk drug. There are several reports available in the literature for the determination of CTH by techniques such as titrimetry[3], UV-spectrophotometry[3-5], and visible spectrophotometry[6-10].

The drug in biological fluids has been determined by high performance liquid chromatography (HPLC)[11,12]. and gas chromatography(GC)[13]. The four HPLC techniques have been applied to determine CTH in tablet. The method presented by El-Walily et al[10] consisted of analysis on a 10 micro m Bondapak C18 column (30 cm × 3.9 m m i.d.) with acetonitrile/0.1 M-ammonium dihydrogen phosphate solution (8:17) of pH 3 containing 1 g/l tetrabutyl ammonium hydrogen sulphate as mobile phase (2 ml/min) and detection at 230 nm. Salicylic acid was used as the internal standard. Suryanarayana- et al[14] have reported a method in which the separation was caused on a column (30 cm × 3.9 mm) of micro Bondapak C18 (10 micro m) with a mobile phase (1ml/min) of acetonitrile-0.01 M-$KH_2PO_4$ (7:3) and detection at 230 nm. Chlorobenzophenone was used as internal standard. The method reported by Jaber et al[15] used Hypersil BDS C18, 5µm column (4.6 × 250 mm) and detector was set at 230 nm in conjunction with a mobile phase of 0.05 M dihydrogen phosphate:acetonitrile:methanol:tetrahydrofuran (12:5:2:1, v/v/) at a pH of 5.5 and flow rate of 1 ml/min. A reverse phase HPLC determination of CTH presented by Zarapkar et al[16] consisted of determination of CTH on a C18 BDS Hypersil column (5 micron, 25 cm X 3.9 mm), using water : acetonitrile : triethylamine (63 : 37 : 0.2 v/v) as the mobile phase and uv detection at 254 nm.

This paper describes the development and validation of an HPLC method without incorporating the use of internal standard for the determination of CTH in pharmaceutical formulations. The method has been demonstrated to be both accurate and precise in addition to being more sensitive than the reported HPLC procedures(TABLE 1)

## EXPERIMENTAL

### Apparatus

An HPLC Shimadzu 1100 series, Schimadzu Technologies, Japan, equipped with an inbuilt solvent degasser, quaternary pump, photodiode array detector with variable injector and an auto sampler, and a reverse phase ODS $C_{18}$ (250 × 4.6 mm) 3V.

### Reagents and standards

All chemicals used were of analytical reagent

**TABLE 1: Comparison of the existing HPLC methods with the proposed methods**

| Sl No. | Chromatographic conditions | $\lambda_{max}$, nm | Linear range µg/ml | Remarks | Ref |
|---|---|---|---|---|---|
| 1. | 10 micro m Bondapak C18(30 x 3.9 mm i. d.);acetonitril/0.1 M-ammonium dihydrogen phosphate solution(8:17) of pH 3 containing tetrabutyl ammonium hydrogen sulphate as mobile phase(2 ml/min) and Salicylic acid is used as internal standard | 230 | --- | Uses internal standard | 10 |
| 2. | 10 micro m Bondapak C18(30 x 3.9 mm i. d.) with mobile phase of acetonitrile: 0.01M $KH_2PO_4$(7:3) at 1 ml/min. | 230 | 25-200 | Less sensitive | 14 |
| 3. | Hypersil BDS C18(5µm) (4.6 x 250 mm);0.005 M dihydrogen phosphate: acetonitrile:methanol:tetrahydrofuran(12:5:2:1, v/v) at pH 5.5 and flow rate of 1.0 ml/mn. | 230 | - | - | 15 |
| 4. | Hypersil BDS C18(5µm) (25 cm x 3.9 mm)with a mobile phase consisting of water:acetonitrile:triethylamine(63:37:0.2 v/v) | 254 | 200-700 | Least sensitive | 16 |
| 5. | Reverse phase ODS $C_{18}$ (250 x 4.6 mm); $KH_2PO_4$:acetonitrile: methanol (58:33:9) at 1.5 ml/min | 210 | 2-30 | Sensitive | Present method |

grade. HPLC grade acetonitrile(Rankem, India), methanol(E. Merck Ltd., Mumbai. India) distilled water were filtered through a 0. 45 μ filter (Millipore) before use.

### Mobile phase

A 0.06 M of $KH_2PO_4$ was first prepared by dissolving 0.82 g of chemical (Thomas Baker, England) in 1 liter of water and filtered through 0.45 μ filter and degassed by sanication. The mobile phase was prepared by mixing $KH_2PO_4$, acetonitrile, methanol in the ratio 58:33:9 (pH = 5.5)

Pharmaceutical grade CTH, was received from UCB, Pharmaceuticals, India Ltd, Mumbai, as gift and was used as received. A stock standard solution equivalent to 100 μg/ ml CTH was prepared by dissolving accurately weighed amount of pure drug in mobile phase and diluting to the mark in a 100 ml calibrated flask with the same solvent,

### Procedures

### Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reversed phase ODS $C_{18}$ (250 × 4.6 mm) 3V column using a mobile phase consisting of solution of potassium dihydrogen phosphate, acetonitrile and methanol (58+33+9) (pH =5.5) at a flow rate of 1.5 ml/min The detector wavelength was set at 210 nm with 0.2 a.u.f.s.

### Preparation of calibration graph

Working standard solutions equivalent to 2-30 μg/ml CTH were prepared by appropriate dilution of the stock standard solution with mobile phase. A 20 μl volume was injected automatically into the chromatograph in duplicate and chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area against CTH concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

### Procedure for tablets

Tablets of different brands containing CTH were purchased from local commercial sources. Twenty tablets were weighed accurately and ground into a fine powder with agate pestle and mortar. An amount of the powder equivalent to 10 mg of CTH was extracted with three 20 ml portions of mobile phase, and the extracts were filtered, combined in a 100 ml calibrated flask and diluted to the volume with the mobile phase. A small portion of the filtrate (~ 10 ml) was withdrawn and filtered through a 0.2 μm filter to ensure the absence of particulate matter. This solution (100 μg/ml with respect to cetirizine) was appropriately diluted with the mobile phase to get the final concentration, and then subjected to analysis.

## RESULTS AND DISCUSSION

### Method development

An HPLC method was developed to provide a specific procedure suitable for the rapid quality control of CTH formulations. The conditions used gave a well-defined peak (Figure 2). A mobile phase consisting of potassium dihydrogen phosphate, acetonitrile and methanol (58+33+9) was chosen after several trials with acetonitrile-water, methanol-water, acetonitrile-potassium dihydrogen phosphate and methanol-potassium dihydrogen phosphate mobile phase systems. The described chromatographic conditions gave the peak in a reasonable time of ~10.85 min(Figure 2). For quantitative determinations, a linear calibraton graph (Y = -977.80 + 38099.84X; n=5 where Y and X are mean peak area and concentration in μg/ml, respectively) with a regression coefficient of 0.9994 (n=7) was obtained over the working concentration range of 2-30 μg/ml (Figure 3). The limits of detection and quantification were 0.2 and 0.66 μg/ml, respectively.

### Method validation

### Accuracy and precision

The accuracy and precision of the method were established by performing seven replicate analyses on pure drug solution at three different concentration levels (within the linear range). The relative error (%), an indicator of accuracy was less than 1%, and the intra-day precision expressed as relative standard deviation, RSD (%) was also around 1.5% indicating high accuracy and precision of the methods.

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TABLE 2: Evaluation of accuracy and precision

| CTH taken, µg/ ml | CTH* found, µg /ml | Range, µg/ml | Relative error, % | Standard deviation, µg/ml | RSD, % | ROE, % |
|---|---|---|---|---|---|---|
| 10.0 | 9.964 | 0.14 | 0.36 | 0.04 | 0.43 | ± 0.43 |
| 20.0 | 19.745 | 0.65 | 1.28 | 0.24 | 1.22 | ± 1.21 |
| 30.0 | 29.790 | 0.35 | 0.70 | 0.12 | 0.41 | ± 0.40 |

*Mean value of seven determinations; RSD - Relative standard deviation; ROE - Range of error at 95% confidence level for six degrees of freedom.

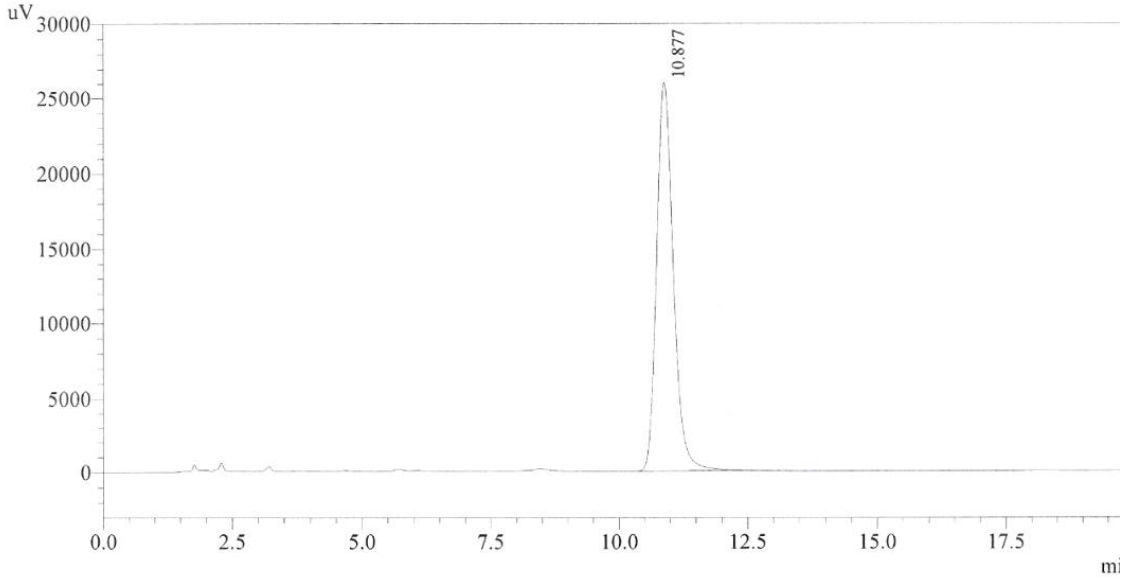


Figure 2: Typical chromatogram

The results of this study are given in TABLE 2. The day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three concentration levels over a period of five days by preparing all solutions a fresh each day. The day-to-day RSD values were less than 2% reflecting the usefullness of the method in routine analysis.

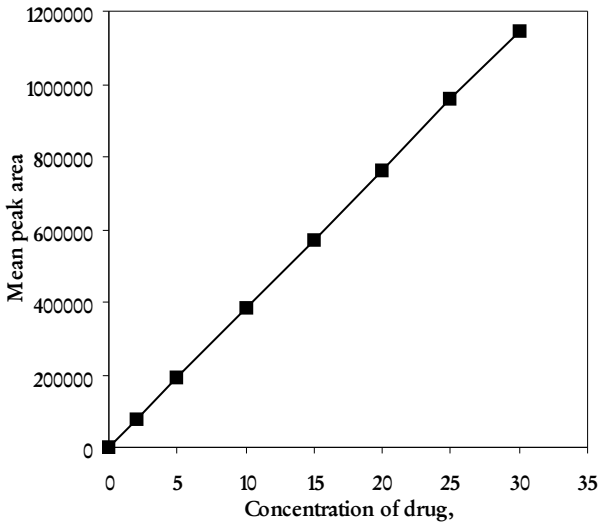


Figure 3: Linearity curve

## Application to tablet analysis

Commercially available cetirizine tablets were analysed by the described HPLC method. The results are summarized in TABLE 3. For the purpose of comparision, the proposed methods were compared with the reference method[3]. which consist of extraction of drug from tablet with 0.1 M HCl and

TABLE 3: Results of assay of tablets containing cetirizine by the proposed methods

| Tablet brand name** | Nominal amount (mg) | Found** (% of label claim ± SD) | | Student's t-value | F-value |
|---|---|---|---|---|---|
| | | HPLC | Reference method | | |
| Cetiriz[a] | 10 | 99.80 ±0.68 | 98.86 ±1.26 | 1.53 | 3.43 |
| Cetmac[b] | 10 | 101.96 ±1.12 | 100.65 ±1.32 | 1.70 | 1.39 |

*Marketed by: a. Alkem laboratories ltd; b. Ikon remedies pvt ltd;
**Mean value of five determinations;
Tabulated t-value at 95% confidence level is 2.77;
Tabulated F-value at 95% confidence level is 6.39.

**TABLE 4: Results of recovery study by standard-addition method**

| Tablet studied | Drug in tablet, µg/ml | Pure drug added, µg/ml | Total found, µg/ml | Recovery* of pure drug, % |
|---|---|---|---|---|
| Cetmac 10 mg | 5.1 | 5 | 10.31 | 104.1 |
| | 5.1 | 10 | 15.36 | 102.6 |
| | 5.1 | 15 | 19.83 | 98.2 |

*Mean value of three determinations.

measurement of absorbance at 232 nm, these results are shown in TABLE 3.

The performance of the methods was further judged through the calculation of student's t-value and F-value. At 95 % confidence level, the calculated values did not exceed the tabulated values, indicating that the proposed methods are as accurate and precise as the official method.

### Recovery experiment

In order to demonstrate the validity and applicability the methods, recovery studies were performed via standard-addition technique. Tablets were spiked with pure CTH at three different levels and the total was found by the proposed methods. The experiment at each level was repeated three times. The percent recoveries of the pure drug added which are compiled in TABLE 4 reveal that commonly added excipients such as sucrose, talc, starch, gumacacia, sodium alignate and magnesium stearate did not interfere in the assay methods.

## CONCLUSION

The present HPLC method is superior to many reported previously interms of linear range of response and sensitivity. It does not require an internal standard for quantitation. Though the method is intended to determine CTH in single component tablets, it can be conveniently applied to combined dosage forms. The method with a relative error less than 1.5% and RSD less than 0.5% is found to be more accurate and precise, also it is highly specific to CTH. However, as shown by the results of assay, commonly added tablet excipients did not interfere.

## ACKNOWLEDGEMENT

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