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Full Length Research Paper

Development and validation of stability indicating assay method of cetirizine hydrochloride by HPLC

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A new simple and rapid high performance liquid chromatography (HPLC) method was developed for the determination of cetirizine hydrochloride (CTZ) in tablets using CLC-ODS reverse phase column (4.6 × 250 mm, 5 µm). Salicylic acid was used as internal standard. A mixture of methanol and water of 70:30 with pH 4 (adjusted with o-phosphoric acid) was used as mobile phase. The eluents were detected at 231 nm. The coefficient of determination of calibration curve for CTZ and salicylic acid in mobile phase were 0.9898 and 0.9925, respectively. The limit of detection for CTZ was 4 µg ml^{-1}. The proposed method was successfully applied for the stability study of CTZ. The CTZ was found to be stable at accelerated condition of temperature and relative humidity after storage of six months. This method can be used for the routine quality control and dosage form assay of CTZ in pharmaceutical preparations.

Key words: High performance liquid chromatography, cetirizine hydrochloride, pharmaceutical preparations.

INTRODUCTION

Cetirizine hydrochloride (CTZ) is a major metabolite of hydroxyzine and a racemic selective histamine receptor (H_1) antagonist used in the treatment of urticaria, angioedema, allergies and hay fever (Anderson and Knoben, 2002). It is a white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride (British Pharmacopoeia, 2007). The CTZ is rapidly absorbed within 1 h to maximum concentration following oral administration of tablet or syrup. Food has no effect on the extent of CTZ absorption. The mean plasma protein binding of CTZ is 93%. Its apparent volume of distribution is substantially smaller than other H_1-selective antihistamines. The CTZ is not extensively metabolized by the liver. The studies indicate that 70% of the administered CTZ was recovered in the urine and 10% in the faeces. Approximately 60% of oral dose is excreted unchanged in the urine within 24 h. The mean elimination half-life of CTZ is 8.3 h and the apparent total body clearance for CTZ is approximately 53 ml min^{-1} (Fireman et al., 1993).

Numerous authors have reported CTZ detection methods in biological fluids and pharmaceutical formulations (Arayne et al., 2005; Maa et al., 2007; Kuchekar et al., 2003; Azhagvuela and Sekar, 2007; Maithani et al.,

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2010). Arayne et al. (2005) developed a sensitive and rapid HPLC method for the analysis of CTZ using hyoscine butyl bromide as internal standard. Beer's law was obeyed in a concentration range of 5 to 30 ng.ml^{-1}. Maa et al. (2007) developed a simple, selective and sensitive liquid chromatography tandem mass spectrometry method to determine the plasma concentrations of pseudoephedrine and cetirizine in humans. Kuchekar et al. (2003) presented an HPLC method for the determination of dual mixtures of pseudoephedrine hydrochloride with CTZ. The chromatographic separation of PSE, FEX and CET was obtained on a Zorbax C8 column using UV detection at 218 and 222 nm.

The mobile phase was consisted of triethanolamine solution (0.5%, pH 4.5), methanol, acetonitrile (50:20:30) and Sekar, et al. (2007) proposed a capillary zone electrophoresis method for the separation and determination of cetirizine dihydrochloride, phenylpropanolamine hydrochloride and paracetamol in tablets. The LOQ of the cetirizine, paracetamol, and phenylpropanolamine hydrochloride was found to be 2.0, 2.0, and 4.0 µg ml^{-1}, respectively. Maithani et al. (2010) reported a highly selective method for simultaneous determination of hydroxyzine and cetirizine in human serum. Haloperidol was used as an internal standard. The linearity range was 0.025 to 2.00 mg ml^{-1} for hydroxyzine and cetirizine.

However, few of these methods are simple and rapid as these methods involve costly and time consuming procedures such as the application of solid phase extraction, use of mobile phase containing buffer etc. In this presentation, we report a simple yet rapid assay with sufficient sensitivity for the quantification of CTZ in terms of short run time and the use of liquid phase extraction and mobile phase containing no buffer.

The objective of the present study was to develop, optimize and validate a simple and rapid isocratic HPLC method with diode array detection for the evaluation of CTZ in tablets. In addition, experiments were carried out to evaluate the stability of CTZ in tablets.

EXPERIMENTAL

Materials

Methanol HPLC grade (Lab Scan), ortho-phosphoric acid (Merck), salicylic acid (Merck), acetonitrile (Lab Scan), benzoic acid (Merck), cetirizine hydrochloride (Siza Pharmaceuticals). These chemicals were used without further purification.

Calibration of HPLC system

The HPLC analysis were carried out using the system consisting of G1311A quaternary pump, G1315B DAD detector, auto-sampler of Agilent Technolgies (1200 series). A quaternary pump (G 1311A) of Agilent Technologies 1200 series was used. The flow rate of quaternary pump was checked by pumping filtered distilled water at temperature (25 ± 2°C) at a flow rate of 1 ml min^{-1}. The effluent water was collected in a pre-calibrated 50 ml of volumetric flask. The time required for collection of 50 ml water was recorded by using pre-calibrated stop watch. The precision of flow rate was ± 0.3%.

Wavelength accuracy of diod array detector

The detector was disconnected from the other equipment and a spectral scan holmium per chlorate solution in a stopped flow mode was obtained. A 4% m/v solution of holmium oxide in a 1.4 M perchloric acid was used for this purpose. Having first flushed the flow cell with water, the instrument baseline was adjusted to zero between 200 and 400 nm while a solution of holmium perchlorate was circulating through the system. A manual scan of the spectrum afforded absorbance maximum at 241.0, 361.4 and 536.0 nm. These maxima were with in the allowed tolerances. The tolerance is with in 3 nm of 240.1, 361.4 and 536.0 nm. The wavelength reproducibility was with in ± 0.1 nm.

Method development

Standard solution

Standards of different concentrations of CTZ (powder) were prepared in the mobile phase consisting of methanol and water in 70:30 ratio. A 100 mg of standard CTZ was dissolved in 100 ml of mobile phase to prepare 1 mg ml^{-1} solution. This standard solution was further diluted to prepare solutions of different concentrations. The prepared standard solutions were scanned over a UV/Visible range of 200 to 800 nm. The maximum absorption was set at 231 nm. Standard solutions of concentration 10 to 200 µg ml^{-1} were prepared. The absorbance of these solutions was measured and a graph of concentration versus absorbance was plotted. Beers-Lambert Law was verified according to this proportionality:

$A \propto C$

Sample solution

Sample solutions of CTZ (tablets) were prepared by grinding 20 tablets with a smooth pestle and mortar. Then, a specific amount of tablets powder was weighed to prepare 1 mg ml^{-1} solution. Then absorbance of these samples was noted by UV/Visible spectrophotometer.

Internal standard solution

Accurately weighed quantity (100 mg) of salicylic acid was dissolved in mobile phase (100 ml), and then it was diluted to known concentration of about 0.1 mg ml^{-1}, filtered through 0.45 µm filter paper and degassed for 5 to 10 minn using ultrasonic bath.

Optimized chromatographic conditions

The optimized conditions were: The column used for the present study was a stainless steel shim pack CLC-ODS(M) 25 cm C18 having stationary phase: Octa-decyl group; particle diameter: 5 µm: column dimensions: 4.6 × 250 mm; separation mode: Reversed phase. Mobile phase: Methanol:water (70:30).

The pH of mobile phase was adjusted at 4 with ortho-phosphoric acid and then filtered through 0.45 µm pore size filter paper and degassed for 5 to 10 min using ultrasonic bath. Flow rate: 1.5 ml min^{-1}; injection volume: 10 µl; detection wavelength: 231 nm, and injection sequence: Standard, samples, samples, standard.

Equal volumes of about 10 µl of samples were injected separately and chromatograms were recorded. The responses of major peaks were measured. The retention times (t_R) for standards and samples were noted. The major peaks of the chromatogram obtained with standard and sample were compared. Spiking with the known drug substance was done to identify the peaks of the mixture of standard preparations and sample preparation.

Method validation

Noise

The baseline was recorded under the following conditions using optimized chromatographic conditions. Wavelength: 260 nm (D2 lamp), 600 nm (tungsten W lamp); attenuation: 0; flow rate: 1.5 ml min^{-1}. After stabilization of the baseline the noise was checked. The noise amplitude is expressed in volts, amperes, or absorbance unit of the envelope of the baseline, which includes all random variations of the detector signal, the frequency of which is of the order of 1 or more cycles per minute. The baseline was within ± 0.5 × 10^{-5} AU maximum (250 nm, air in the cell).

Column efficiency

The numbers of theoretical plates N were calculated and the column efficiency determined by the equation:

$$N = 16(t_R/W)^2$$

$$N = 5.5(t_R/W_h)^2$$

where t_R is the retention time of a substance and W is the width of the peak at the base obtained by the extrapolating relatively straight sides of the peak to its baseline and W_h is the peak width at half-height.

Resolution

The resolution is defined as the ability of chromatograph to separate the peaks. The resolution was determined by the following equation:

$$R = 2(t_{R2}-t_{R1})/(W_2+W_1)$$

where R is the resolution, t_{R2} and t_{R1} are retention times of two components, and W_2 and W_1 are their corresponding widths at bases of the peaks obtained by extrapolating relatively straight sides of the peak to respective baseline.

Precision

The precision of new HPLC method was determined by injecting the replicates of 10 µl sample size.

Accuracy

The accuracy of new HPLC method was determined by measuring the response of solution of analyte concentration in replicate. The concentrations of analyte were calculated.

Linearity

The linearity of new HPLC method was determined for CTZ. Seven solutions of different concentrations of CTZ were prepared in mobile phase. Then the sample size of 10 µl of eight concentrations of each analyte was injected into the HPLC. The detector response was measured at 231 nm for the seven solutions of each analyte. The calibration plots (concentration versus peak area) were obtained using the linear regression method.

Limit of detection (LOD)

It is the concentration or mass flow of a sample component in the mobile phase that gives a signal equal to twice of the noise level. It was calculated from measured sensitivity(S) and noise (N). That is: LOD = 2S/N. Both the sensitivity and limit of detection were determined for the same substance.

Limit of quantification (LOQ)

The LOQ can be determined by the following formula: LOQ = 2 × LOD

Specificity

Specificity is the ability to find and quantify the compound of interest also in the presence of other compounds. This means for chromatographic methods, that the analyte can be separated with sufficient resolution and that it can be directed with suitable instrument. The specificity of method was checked by analyzing the peaks of CTZ in the stability sample kept at accelerated conditions of temperature and moisture.

Repeatability

The ability to re-run as an analysis with low standard deviation is known as repeatability. This newly developed stability indicating method was found to be repeatable.

Quality control

The quality control samples were used to accept or reject the run. The replicate measurements were made at three concentrations, one at lower limit of quantification, one in the mid range and one approaching the high end of the range.

Stability study in mobile phase

Stability study of sample in the mobile phase can also be carried out to evaluate the time period that encompasses the duration of typical sample preparation, sample handling and analytical run time. The stability study can be extended to 72 h in this work.

Stability study of commercially available tablets of cetirizine hydrochloride

A sample of CTZ tablets was placed at accelerated conditions of

Table 1. Stability protocol.

1st month	2nd month	3rd month	4th month	5th month	6th month
Daily	Weekly	Weekly	Fortnightly	Fortnightly	One month

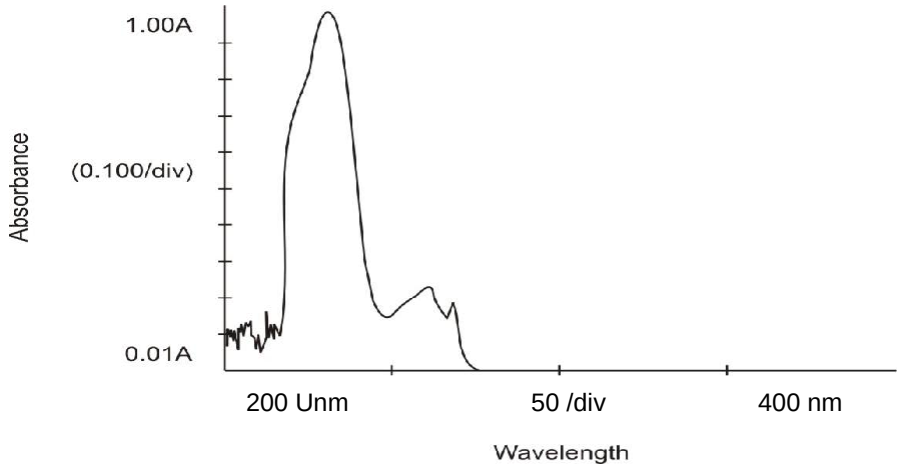

Figure 1. Spectrum of cetirizine hydrochloride by UV/visible spectrophotometer.

temperature that is at 40 and 50°C with 75% relative humidity in environmental chamber for six months. The stability protocol in Table 1 was followed and assays were made as mentioned in method development.

RESULTS AND DISCUSSION

Calibration of HPLC system

All the components of HPLC system (Agilent Technologies 1200 series) were calibrated according to the instructions provided in the manual of the equipment. The calibrations were checked on quarterly basis or as and when there was a need after service/repairs. The criterion used for qualification was a specified in the service manual of the equipment. During these studies, no major breakdown of any of the equipment occurred. Every time the results of the calibration were found to be satisfactory. System suitability was determined in methods for the individual substances under study.

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Method development

Determination of λ_{max} for cetirizine hydrochloride

The solutions of different concentrations of CTZ were scanned. The maximum absorbance was at 231 nm. The spectrum obtained is shown in Figure 1.

Optimization of chromatographic conditions

By varying the wavelength of detector, sample extracting agents, composition of mobile phase and flow rate of the mobile phase, optimization of conditions was carried out. Then methanol and water in 70:30 ratio with pH 4 at a flow rate of 1.5 ml min^{-1} and detection wavelength of 231 nm were found to be the most suitable because it afforded better resolution and shorter run time. These conditions were used for the subsequent study.

Analysis of mixture of cetirizine hydrochloride and salicylic acid as internal standard

By using the mobile phase and chromatographic conditions as described in experimental, the analysis of mixture of CTZ and salicylic acid was performed. A typical chromatogram is shown in Figure 2. By spiking with salicylic acid, the identification of CTZ and salicylic acid was carried out. The retention time of CTZ and salicylic acid was found to be at 3.4 and 1.4 min, respectively. The total run time was set at 6 min.

Method validation

Accuracy and precision

The accuracy (%) was in terms of recovery and its value

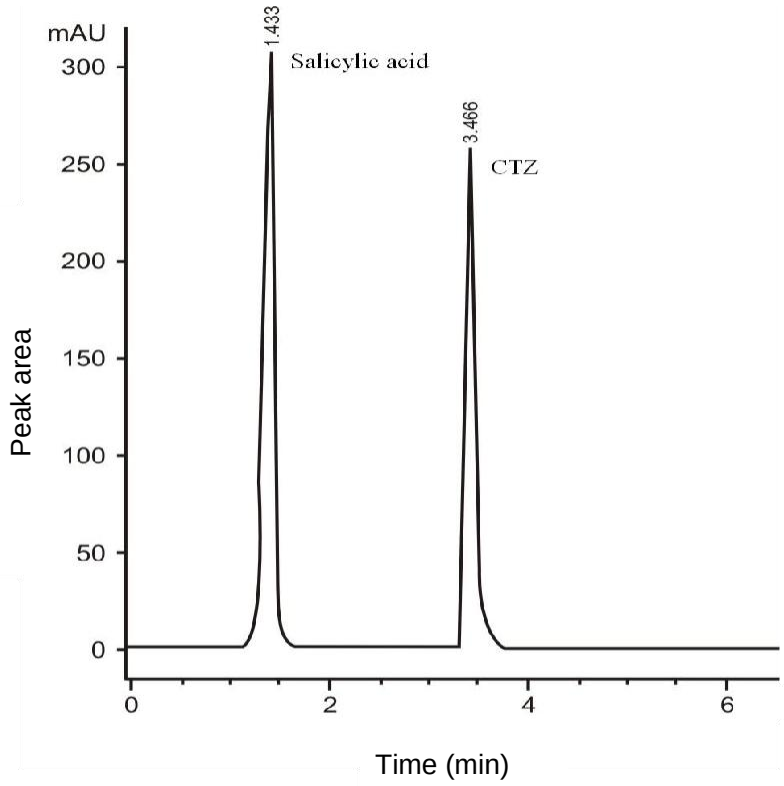

Figure 2. A typical chromatogram of mixture of cetirizine hydrochloride and salicylic acid.

Table 2. Validation parameters of analyte in the mobile phase at different concentration levels.

Matrix	Parameter	Cetirizine hydrochloride
In mobile phase	LOD (µg/ml)	4.0
	LOQ (µg/ml)	8.0
	Concentration range (µg/ml)	10-200
	Tailing factor	1
	No. of theoretical plates (N)	15572

was 98.59%. Within day and between days precisions were determined which were less than 4%. The analyses were performed at three different concentration levels covering the entire linear range. The results are given in Table 2.

Linearity

The concentration and peak area were found to be linearly related for CTZ concentration ranges under study. The linearity data showed linearity over a concentration rage of 10-200 µg/ml and the coefficient of determination was equal to 1. The calibration curve in mobile phase is shown in Figure 3.

Limit of detection (LOD) and limit of quantification (LOQ)

The values of LOD and LOQ are given in Table 2. The low levels of LOD and LOQ indicate that the method is quite sensitive for the determination of CTZ and salicylic acid in the presence of each other.

Specificity and reproducibility

The method was found to be specific for the determination of particular analyte in dosage forms as the precision for CTZ was less than 4% and the reproducibility of measurement of five different concentration samples

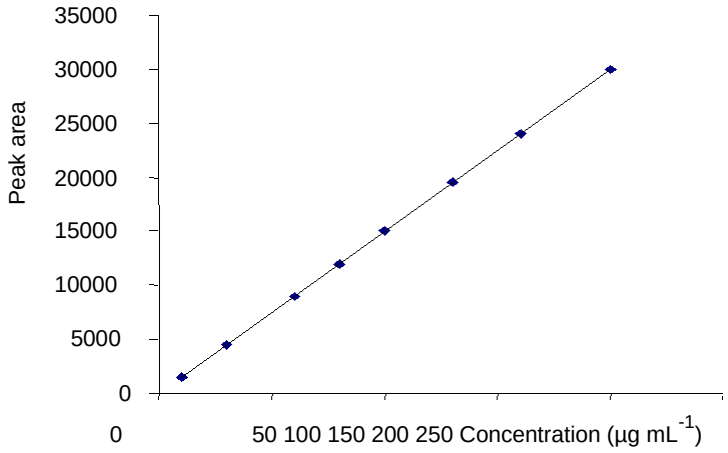

Figure 3. Concentration versus peak area showing linear response of cetirizine hydrochloride.

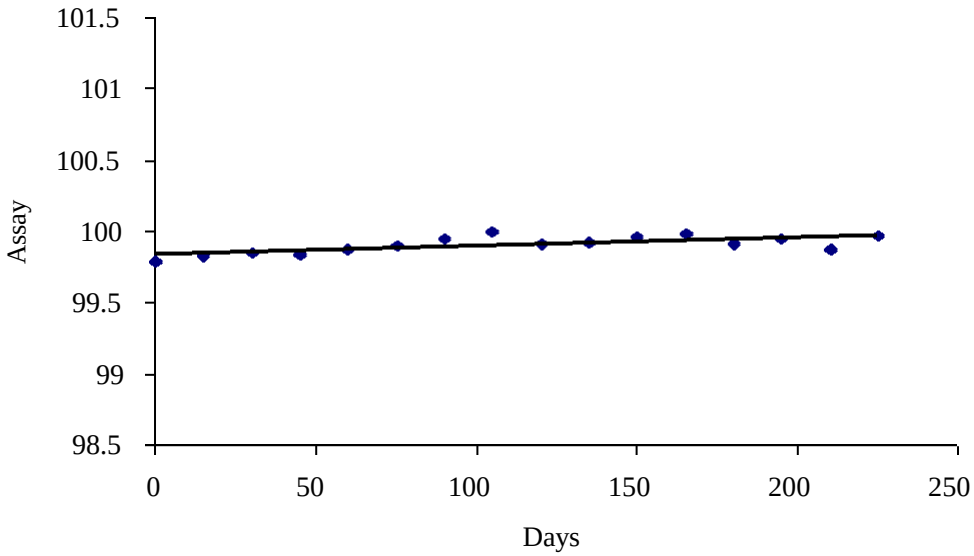

Figure 4. The assay (% contents) vs number of days showing stability study of cetirizine hydrochloride at 40°C with 75% relative humidity.

spiked with standard was also very high.

Stability study of cetirizine hydrochloride at 40°C with 75% relative humidity

The proposed assay method was applied to stability study of commercially available CTZ tablets. The sample was placed at 40°C with relative humidity of 75% relative humidity (Figure 4). Stability study was performed according to stability protocol as described in previous section. Sample was analyzed and percentage of contents was measured.

According to the results obtained CTZ was found to be stable at accelerated conditions of temperature and relative humidity.

Stability study of cetirizine hydrochloride at 50°C with 75% relative humidity

The proposed assay method was applied to stability study of commercially available CTZ. The sample was placed at 50°C with relative humidity of 75% (Figure 5).

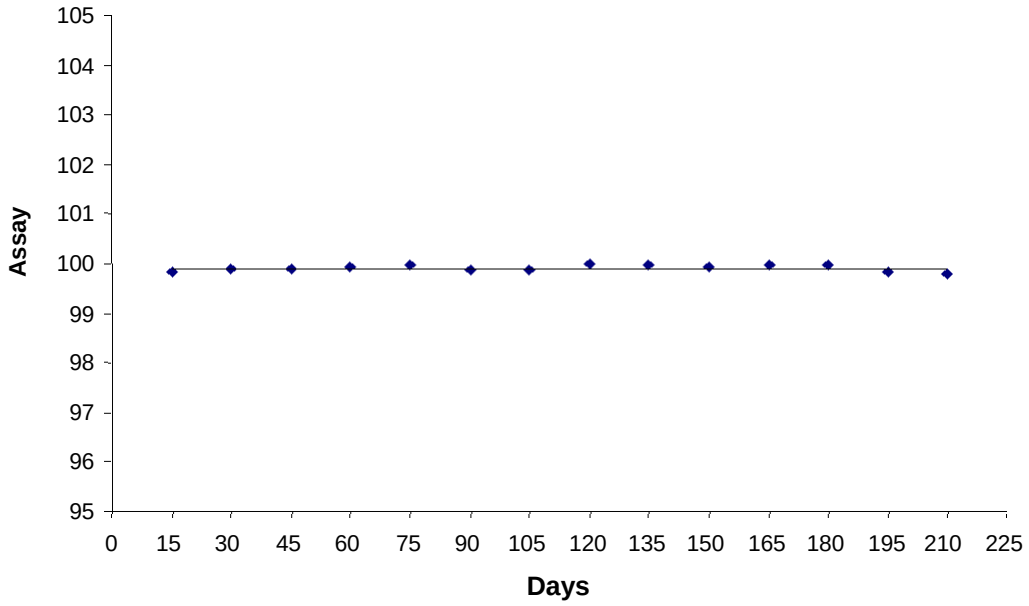

Figure 5. The assay (% contents) versus number of days showing the stability of cetirizine hydrochloride at 50°C with 75% relative humidity.

Stability study was performed according to stability protocol as described in previous section. Sample was analyzed and percentage of contents was measured. According to the results obtained CTZ was found to be stable at accelerated conditions of temperature and relative humidity.

Conclusion

The newly developed stability indicating method for cetirizine hydrochloride on HPLC was validated by employing it in the stability study of cetirizine hydrochloride at accelerated conditions of temperature and relative humidity. The method appears to be efficient, convenient, employing simple solvent system and better in terms of high resolution and short analysis time.

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