



DETERMINATION OF LEVOCETIRIZINE CONFIGURATIONAL STABILITY IN TABLETS USING CHIRAL HPLC METHOD

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ABSTRACT

Levocetirizine dihydrochloride is the active enantiomer of cetirizine a second generation H₁-Antagonist. A chiral HPLC method has been validated and applied for determination the configurational stability of levocetirizine dihydrochloride in tablets. Enantiomeric resolution was achieved using a chiralcel column and a mobile phase consists of buffer and acetonitrile. Detection was performed at 230 nm. The method was validated and all the validation parameters were within the accepted limits. Stress conditions including acid, alkali hydrolysis, oxidation, photolysis, and heat were applied. The degradation products did not interfere with the detection of levocetirizine, thus the method can be considered as stability indicating method. The accelerated stability study of the tablets was performed according to ICH guidelines. There was no significant change from the initial value and levocetirizine was not subjected to racemization.

Keywords: Levocetirizine; enantiomers; chiral switch; configurational stability; HPLC; stability indicating method.

INTRODUCTION

Several drugs currently used are mixtures of enantiomers (racemates). In many cases the two enantiomers differ in their pharmacokinetic and pharmacodynamic properties. Replacing existing racemates with single isomers has resulted in improved safety and/or efficacy profile of various racemates^{1,2}.

Many optically active enantiomers of a variety of drugs racemize in vivo, resulting into various side effects, therefore, it is very important to study the racemization of optically active drugs³.

Levocetirizine is a second generation H₁ antihistamines marketed for the treatment of perennial and seasonal allergic rhinitis and chronic idiopathic urticaria. It is the most active enantiomer of cetirizine and has a favorable pharmacokinetic profile. Levocetirizine is rapidly and extensively absorbed, minimally metabolized and has a volume of distribution (V_d) which is lower than other compounds from the same group^{4,5}.

To provide exact, safe and correct dose of an optically active pharmaceutical, it is important to study their racemization.

Since it is difficult to study racemization of drugs in vivo therefore, studying it in vitro is simpler method to deal with this problem. Many factors such as drug concentration, pH, temperature, ionic concentration, etc. are controlling racemization process^{1,3}.

Configurational stability and instability are relative phenomena, no stereoisomer is configurationally stable⁶. On the other hand, there are two concern, the pharmaceutical time scale and the pharmacological time scale. The pharmaceutical time scale which concern with conditions required to keep the drugs configurationally stable during manufacturing processes and for the whole shelf life. While pharmacological time scale is concerned with the stability under physiological condition (37 °C, pH 7.4) and for the drug residence time in the body⁶.

In case of pharmacological time scale it appears that following the oral administration of [¹⁴C] levocetirizine dihydrochloride to four subjects, there was no appearance of dextrocetirizine in human plasma or urine samples. This is an indication that levocetirizine dose not racemize in the body^{7,8}.

Several bioanalytical methods have been described for the analysis of levocetirizine in plasma or urine using stereoselective analytical method⁹⁻¹³. On the other hand, methods intended to determine levocetirizine in dosage forms were not stereoselective¹⁴⁻¹⁶. This means that these methods could not differentiate between the two

enantiomer. Therefore results collected from these methods were inaccurate and highly misleading, particularly when attempting to relate concentration to a pharmacological effect or therapeutic benefit. Actually, the previous methods do not differentiate between levocetirizine and dextrocetirizine and the AUC obtained is a sum of levocetirizine and dextrocetirizine peaks. Even if we started with levocetirizine dihydrochloride of 99.9% enantiomeric purity, it might be difficult to maintain the percentage during processing and storage. A deep literature search indicates that there is no enantioselective stability indicating HPLC method published for determination of levocetirizine in pharmaceutical formulation.

The present work focused on the ability of a modified HPLC method to separate and determine levocetirizine in pharmaceutical formulations and serving as stability indicating method in order to estimate levocetirizine chiral inversion and stability.

MATERIALS AND METHODS

Samples of levocetirizine dihydrochloride, levocetirizine dihydrochloride working standard and dextrocetirizine dihydrochloride working standard were obtained from Enaltic Labs (Pvt.Ltd, India).

RS-1-[(4-chlorophenyl) phenylmethyl]piperazine, cetirizine impurity A according to BP (imp A BP), obtained from Enaltic Labs (Pvt.Ltd, India).

RS-2-[2-[4-(4-chlorophenyl) phenylmethyl]piperazine-1-yl]ethoxy] acetic acid, ethyl ester) or cetirizine ethyl ester, cetirizine impurity A according to USP (imp A USP), obtained from USP reference materials supplier.

Xyzal® tablet reference product batch NO. 08H28C, (UCB-Pharma AG, Zurich, Switzerland). All other chemicals were of HPLC or analytical grade and obtained from (Merck, Germany).

Methods

Chiral separations were performed with a HPLC (LA Chrom ELITE, VWR Hitachi, Germany, equipped with a L-2130 pump, L-2300 thermostatted column compartment, and programmable detector module UV Photo diod array),

using a 250 X 4.6 mm 10 μm Chiralcel column, and a mobile phase consisting a mixture of 0.5 mol L⁻¹ NaClO₄ buffer and Acetonitrile (60:40 v/v), the pH was adjust at 2. Flow rate was kept at 0.4 mL min⁻¹, column temperature was maintained at 15 °C, and the injection volume was 10 μL while the elution was monitored at 230 nm.

Resolution solution

About 10 mg of each of levocetirizine dihydrochloride and dextrocetirizine dihydrochloride standard were accurately weighed, transferred into a 100 mL volumetric flask, and dissolved with purified water in order to get an individual concentration of 0.1 mg mL⁻¹. This solution was used for enantiomeric separation and resolution estimation.

Standard solution

About 10 mg of levocetirizine dihydrochloride was accurately weighed, transferred into a 100 mL volumetric flask and dissolved in 50 mL purified water, sonicated and then dilute to volume with purified water to obtain 0.1 mg mL⁻¹ concentration of levocetirizine dihydrochloride.

Sample preparation

Five tablets were weighed and transferred into a 250 mL volumetric flask. About 100 mL purified water were added and sonicated for 10 min with intermittent shaking, then the solution was brought back to room temperature and diluted to volume with purified water. The sample was filtered using a 0.45 µm nylon syringe filter. The concentration obtained was 0.1 mg mL⁻¹ levocetirizine.

Method validation

The validation was performed according to the ICH guideline

Specificity

The specificity was carried out in the presence of Imp-A USP and Imp-A Bp and tablet excipients.

Linearity

The calibration curve was drawn between the peak areas of levocetirizine versus its concentration in a range 70 – 130% of the standard concentration.

Accuracy

Three samples of levocetirizine dihydrochloride covering the concentration range (70 – 130 %) were prepared and each sample was read three times. The Assay value was calculated of each sample and the RSD % was calculated.

In addition accuracy of the method was studied by recovery experiments. The recovery experiments were performed by spiking solution of known amounts of the drugs in the tablet matrix.

Precision**Repeatability**

Repeatability was evaluated by calculating the RSD % of nine determinations by injection nine freshly prepare solutions containing levocetirizine dihydrochloride at three concentration levels on the same day.

Intermediate Precision

Samples at the above three concentration levels were injected on different days and the RSD % was calculated.

Detection Limit

The limit of detection (LOD) was determined as 3 times the baseline noise.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the buffer pH (± 0.2 units), and HPLC column temperature (± 0.2 units).

Mobile Phase Stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4 – 8 °C and for 15 days. The aged mobile phase was compared using a freshly prepared one.

Procedure for forced degradation study

Since there is no suggestion of conducting stress studies directly on formulations¹⁷, therefore forced degradation studies was applied on standard solution of the drug.

Standard solution for degradation study were prepared by transferring 250 mg levocetirizine hydrochloride, into a 100 mL volumetric flask. The prepared samples were exposed to acidic, alkaline, oxidizing and thermal and incubated in the dark in order to exclude any potential photolytic effects, then photolytic conditions were studied under UV and sun light.

Forced degradation studies were summarized in Table 1.

After the degradation treatments were completed, the stress content solutions were diluted with distilled water to attain a 0.1 mg mL⁻¹ concentration. The above validated method was used with same parameter instead of injection volume which become 50µL (to detect small concentration of degradation product).

Table 1: Summary of levocetirizine forced degradation conditions

Stress condition	Medium	Temperature	Duration
Acidic	1 N HCl	60 °C	24 hours
Alkaline	2 N NaOH	60 °C	24 hours
Oxidative	10 % H ₂ O ₂	60 °C	24 hours
Thermal	Standard solution /60 °C	60 °C	15 days
Photo	Standard solution /Sun light	Ambient	15 days
	Standard solution / UV254 nm	Ambient	48 hours

Accelerated Stability Study:

Xyzal® tablets, were studied for the accelerated stability study as per the ICH guidelines. Levocetirizine content and its configurational stability were investigated using the above validated analytical method.

RESULTS

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of the resolution solution were made, and asymmetry, theoretical plate

number, resolution and RSD% of the peak areas were determined (Fig. 1). For all system suitability injections, asymmetry was < 1.5, theoretical plate number was > 3000, resolution > 2.0 and RSD% of the peak areas was < 2.0%.

Photodiode array detection was used as an evidence of the specificity of the method to evaluate were complete separation of levocetirizine, imp A USP, and imp A BP was noticed.

Well-resolved peaks for levocetirizine, imp A BP, imp A USP were noted (Fig. 2,3). In addition there was no interference at the retention time of levocetirizine in the chromatogram of placebo.

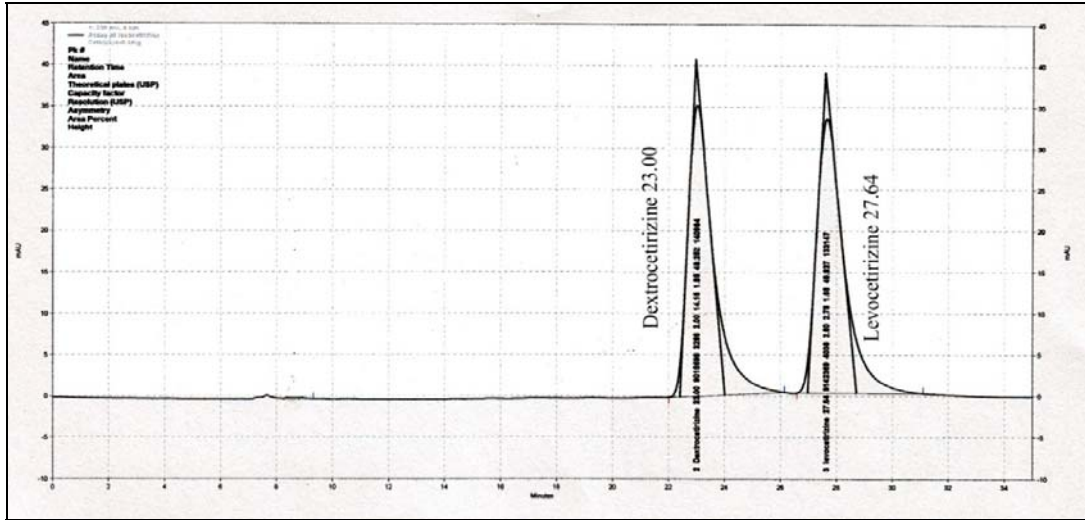


Fig. 1: HPLC chromatogram for Levocetirizine and Dextrocetirizine

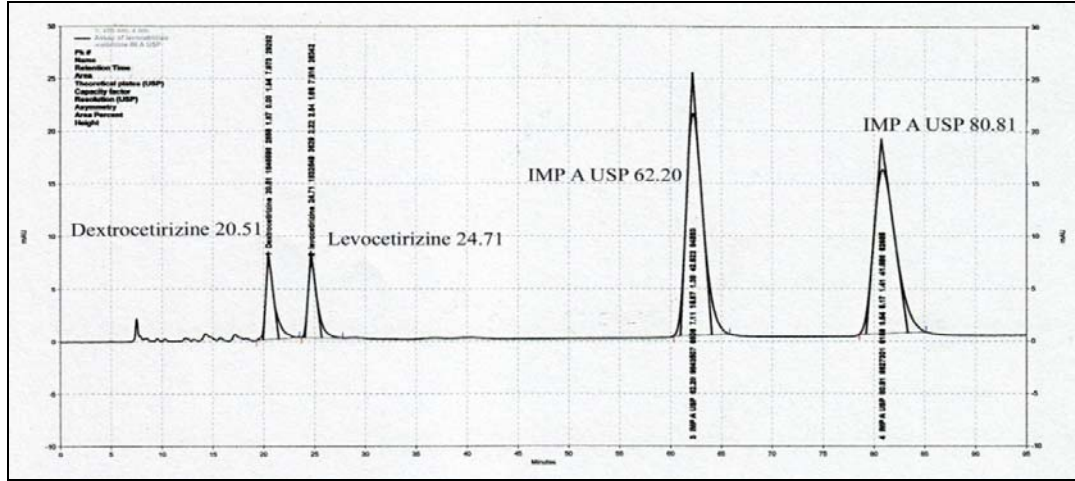


Fig. 2: HPLC chromatogram for Dextrocetirizine, Levocetirizine and imp A USP

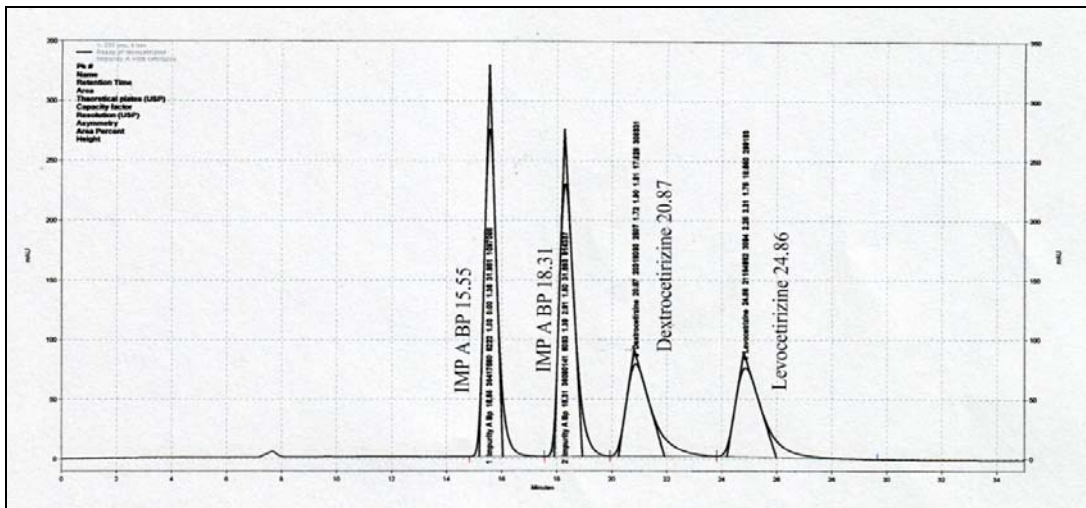


Fig. 3: HPLC chromatogram for Dextrocetirizine, Levocetirizine and imp A BP

During all variation conditions, the assay value of the test preparation solution was not affected and was in accordance with the actual value and the mobile phase was stable up to 15 days at 4⁰ C. Other validation results were summarized in Table 2.

When stress conditions applied to levocetirizine, the HPLC results showed that the method is stability indicating since there was no interference between the tested drug and the degradation products (Fig. 4).

Table 2: Validation studies results

S. No.	Parameters	Results
1	Linearity range ($\mu\text{g mL}^{-1}$)	0.7 – 1.3
2	Correlation coefficient	0.998
3	Accuracy (%)	0.65
4	Recovery (%)	99.68
5	Repeatability (RSD%)	< 1.0
6	Intermediate precision (RSD%)	< 1.5
7	LOD ($\mu\text{g/mL}$)	0.01

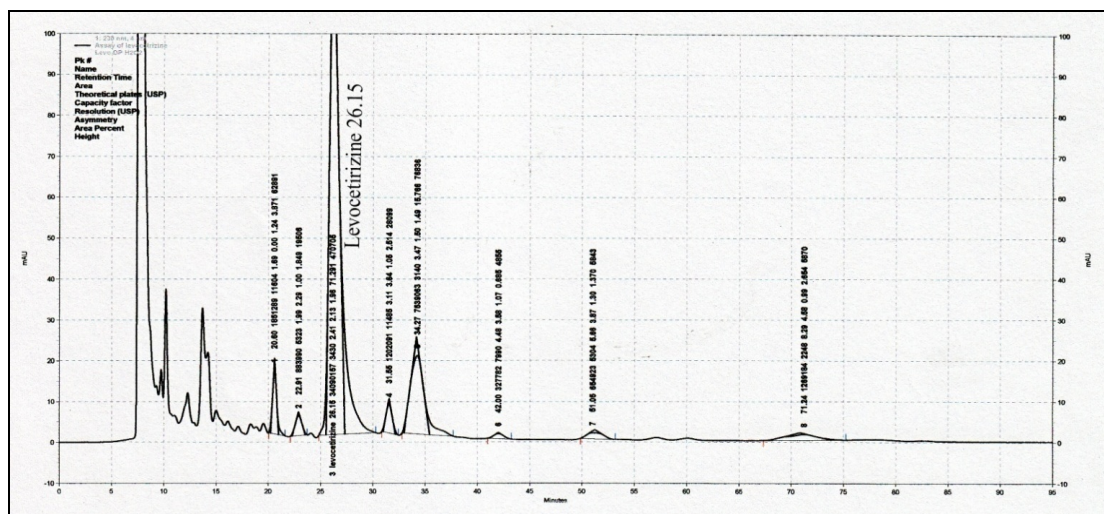


Fig. 4: HPLC chromatogram for levocetirizine degradation in 10 % H₂O₂

Levocetirizine was stable in alkaline and wet heat, but was less stable in acidic and UV light and susceptible to oxidation and sun light.

The concentrations of levocetirizine in the stored tablets were calculated and compared with the concentration of levocetirizine in the tablets before storing. There was no significant difference between the two concentrations. In addition there is no chiral inversion of levocetirizine to dextrocetirizine.

CONCLUSION

Dhaneshwar S. et al. analyze levocetirizine and study tablets stability using non selective analytical method. An HPLC method was modified, validate and approved as stability indicating one to utilize it as inspector for levocetirizine stability and chiral inversion in the tablets. The method was accurate, precise and selective for levocetirizine determination in the presence of other impurity and tablet matrix without interferences.

Degradation studies concluded that the levocetirizine degraded under oxidation and photolytic condition while it is more stable in an acidic and alkaline media.

Previous studies on levocetirizine and cetirizine stability gave different results with acidic and alkaline degradation, but all gave a parallel result with oxidation degradation^{14,15,18-21}.

Levocetirizine was stable during the accelerated stability study period with no chiral inversion to dextrocetirizine. Even under acidic, alkaline, thermal or other condition it was noticed a decrease in levocetirizine content without conversion to dextrocetirizine,

which indicate strong configurational stability of levocetirizine, and show a good correlation between in vivo and in vitro studies^{7,8}.

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