A New Reverse Phase HPLC Method for Analysis of Levocetirizine Dihydrochloride in Raw Materials and Tablets

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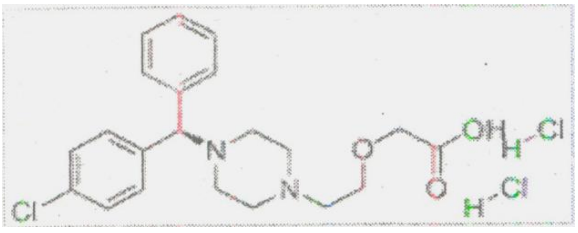
Abstract: A precise, simple and rapid high performance liquid chromatographic (HPLC) method for the determination and quantification of Levocetrizine has been developed. The chromatographic system consisted of a shimadzu LC-10ATVP pump, SPD-10AVP UV / Visible detector and autosampler (SIL 20A). Separation was achieved on the µ Bondapak C18 125A^0 10µm column at room temperature. The sample was introduced through an injector valve of autosampler with a 10µl sample loop, Buffer: Acetonitril (58 : 42 v/v) was used as mobile phase, with flow rate of 1.2ml / minute. PH was adjusted 2.4 with 1N sulfuric acid. UV detection was performed at 230nm. The obtained result showed a good agreement with declared content. Recovery values of Levocetrizine dihydrochloride in tablet (In Xyazl 05mg tablets) were from 99.88 % to 100.08%. The puroposed method is rapid, accurate and selective; it may be used for the quantitative analysis of Levocetirizine dihydrochloride from raw material, in bulk drugs and other dosage formulation.

Key Words: Levocetirizine, Sodium Heptane Sulfonate, HPLC determination.

INTRODUCTION

Levocetirizine dihydrochloride is [2-[4-[(R)-(4-chloro-phenyl) Phenylmethy l0]-1-piperazinyl] ethoxy]-acetic acid dihydrochloride having molecular formula $C_{21}H_{25}ClN_2O_3$. 2HCl, molecular weight 461.81 and melting point 215 – 220^{0}C. It is white or almost white, powder, freely soluble in water, very slightly soluble in chloroform and acetone.



Levocetirizine Dihydrochloride

Levocetirizine dihydrochloride, is an anti-histamine. It is an orally active and selective H1 receptor antagonist. Histamines act on H1 receptors, causign the symptoms commonly seen in allergic reaction. Levocetirizine dihydrochloride de inhibits these H_1 receptors. It is used in condition such as the allergic rhinitis and uncomplicated skin manifestation of chronic idiopathic urticaria [Journal of the British society for allergy and clinical immunology 2006]. Levocetirizine (as levocetirizine dihydrochloride) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine is the active enantiomer of cetirizine.It is the L-enantiomer of the cetirizine racemate. Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever.

Levocetirizine is called a non-sedating antihistamine as it does not enter the brain in significant amounts, and is therefore unlikely to cause drowsiness and it reduces asthma attacks by 70% in children

There have been no analytical testing method of Levocetrizine dihydrochloride available in USP, BP and EP.

EXPERIMENTAL

MATERIAL AND REAGENTS

Levocetirizine dihydrochloride reference standard were a kind gift from AGP (Private) Limited. Xyzal tablet were purchased from market. HPLC grade Acetonitirile, Sodium heptane sulfonate and sulphuric acid were obtained from Merck. The mobile phase and solution were prepared in double distilled deionized water. Stock solution of the compounds were prepared in deinozed water stored at room temperature. Fresh working solutions were prepared daily. All solutions were filtered (0.45µm) and degassed by sonicator.

APPARATUS

The HPLC system was LC-10ATVP Shimadzu pump, SIL 20A autosampler, SPD-10AV VP shimadzu UV visible detector and a µ bondapak 125A^0 C18 column (Particle size 10µm) was used for separation. The chromatographic and

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integrated data were recorded using GC software on an IBM PC.

CHROMATOGRAPHIC CONDITIONS

The mobile phase was buffer-acetonitirile (58:42 v/v); the pH of this mobile phase was adjusted to 2.4 with sulfuric acid (1N). Before delivering in to the system it was filtered through 0.45µm filter and degassed using a vacuum. The analysis was carried out under isocratic conditions using a flow rate 1.2ml / minute at room temperature. Chromatograms were recorded at 230nm using SPD-10AV VP Shimadzu UV visible detector; Autosampler with 10µl sample loop introduced the samples.

ANALYTICAL PROCEDURE

25mg of Levocetirizine dihydrochloride reference standard were dissolved in dilution solvent (Purified water : Acetonitril 60:40 v/v) in 100ml volumetric flask separately and made up volume with its solvent. With the help of stock solution aliquots of desired concentration of Levocetirizine dihydrochloride were prepared by dilution. Twenty tablets of Xyzal tablet were weight to obtain the average tablet weight (103mg) and were them powdered; 515mg of the powdered tablets (equivalent to 25mg of active substance) was with 100ml dilution solvent (purified water : Acetonitril 60:40 v/v). This mixture was allowed to stand for 30 minutes with intermittent sonication to ensure complete solubility of the drug. This stock solution was filtered to obtain clear filtrate; from this clear filtrate working solution were prepared of desire concentration. 10µl volume of each sample was injected and chromatographed under above condition.

RESULT AND DISCUSSION

The determination and quantification by HPLC in the quality control of drug products has received considerable attention and is advantageous due number of reason. The goal of this study was to develop a rapid, accurate, precise, reliable, less expensive and least time consuming HPLC method for the analysis of Levocetirizine dihydrochloride. The drug can be estimated efficiently in the form of raw materials, bulk drug samples and its tablets formulation using the most commonly employed C-18 column with UV detection. Present HPLC method was developed on the basis of chemical structure and other physical properties which are most important facts that predict chromatographic behavior.

Retention time = Levocetrizine Dihydrochloride = 3.7 minutes.

In the present investigation the best separation was achieved using a µ Bondapak 125A^0 C18 (10µm) columns. Using other types of column under the same experimental condition, peak tailing and peak broadening was observed. For the separation of Levocetirizine dihydrochloride and quantification of Levocetirizine dihydrochloride the best results were obtained using mobile phase buffer : Acetonitril (58%:42% v/v). The lower percentage of acetonitirile in mobile phase resulted also in peak tailing of component and long analysis duration, while higher percentage of Acetonitril in mobile phase resulted in very little analysis duration.

Optimal retention time (Levocetirizine dihydrochloride) 3.7 minutes were achieved when the pH of mobile phase was adjusted to 2.4 with 1N sulphuric acid.

Small changes in pH of the mobile phase had a great influence to the chromatographic behavior of these substances. At the higher pH of the mobile phase peak tailing was observed, while at lower pH values retention time of Levocetrizine dihydrochloride was extremely long. A typical chromatogram separation of Levocetirizine dihydrochloride is shown in figure 2.

ACCURACY AND PRECISION

The accuracy of the method was evaluated by analyzing independently prepared solution of Levocetirizine dihydro-

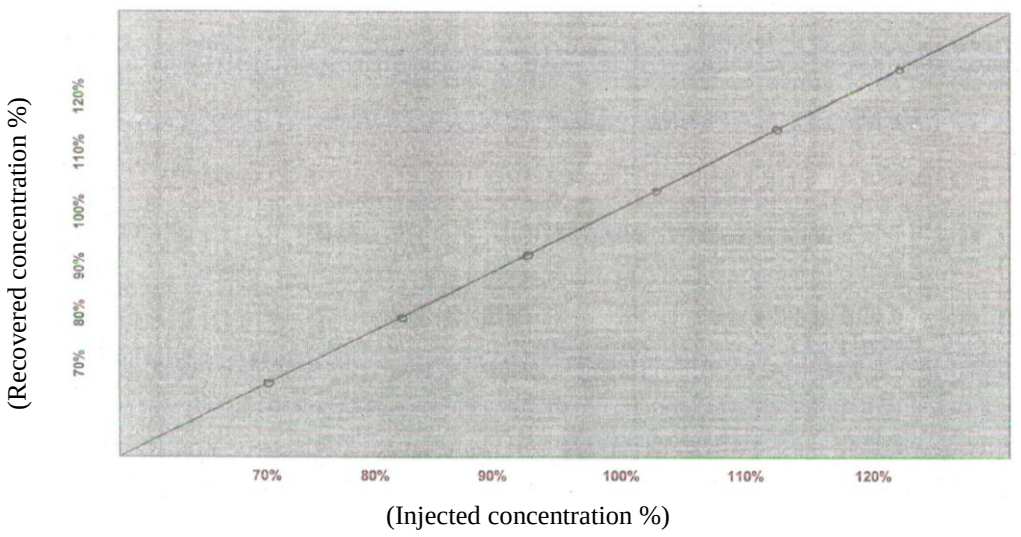


Table 1. Recovery of Levocetirizine Dihydrochloride in Reference Drug

Conc. Injected %	Reference Drug			Dosage Form		
	Found	Recovered %	Mean (Area)	Found	Recovered %	Mean (AUC)
70%	69.9%	99.85%	32966	69.8%	99.71%	33001
80%	79.8%	99.75%	37046	79.9%	99.87%	42526
90%	89.7%	99.66%	42502	89.8%	99.77%	47229
100%	99.9%	99.9%	47229	99.8%	99.8%	47212
110%	109.09%	99.9%	52059	109.8%	99.81%	52067
120%	119.9%	99.9%	56058	119.8%	99.83%	56346

Table 2. Inter Day Accuracy and Precision of Proposed Method

Conc. Injected %	Day 1		Day 2		Day 3		Day 4	
	Found	Recovery	Found	Recovery	Found	Recovery	Found	Recovery
70%	69.85%	99.78	69.72%	99.6%	69.70%	99.5%	69.70%	99.5%
80%	79.9%	99.87%	79.8%	99.7%	79.8%	99.7%	79.7%	99.6%
90%	89.9%	99.88%	89.9%	99.88%	89.7%	99.6%	89.6%	99.5%
100%	99.7%	99.7%	99.6%	99.6%	99.6%	99.6%	99.6%	99.6%
110%	109.9%	99.9%	109.8%	99.8%	109.7%	99.7%	109.7%	99.72%
120%	119.8%	99.8%	120.1%	100.08%	119.7%	99.75%	119.6%	99.6%
	%R.S.D = 0.07		%R.S.D = 0.18		%R.S.D = 0.09		%R.S.D = 0.08	

Table 3. Recovery and Regression Characteristics of Proposed Method

Concentration Injected (%)	Recovered Concentration			
	Day 1	Day 2	Day 3	Day 4
70%	69.85%	69.72%	69.70%	69.70%
80%	79.9%	79.8%	79.8%	79.7%
90%	89.9%	89.9%	89.7%	89.6%
100%	99.7%	99.6%	99.6%	99.6%
110%	109.9%	109.8%	109.7%	109.7%
120%	119.8%	119.7%	119.7%	119.6%
Correlation Coefficient (r)	0.999	0.999	0.999	0.999
Intercept	0.102	0.102	0.023	0.023
Slope	0.999	0.999	1.000	1.000

chloride. The recovery data is expressed in Table **4**. These tables show that the method is accurate for determination of Levocetirizine dihydrochloride. Data of regression characteristic is expressed in Table **3**. All calibration curves have a correlation coefficient vaule of at least 0.999 to 1.000. The accuracy was calculated as a percentage of the nominal concentration:

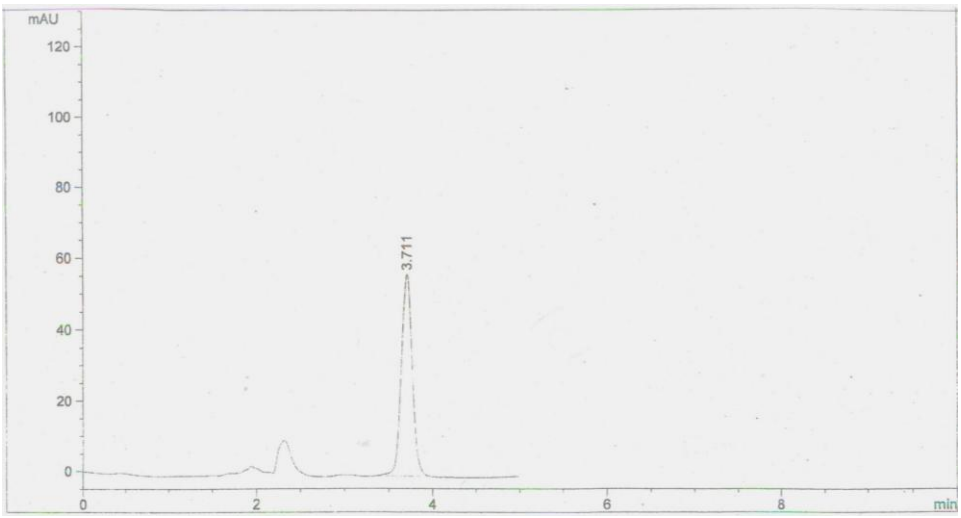
Accuracy = (concentration observed / nominal concentration) x 100.

The precision of the method was investigated with respect to repeatability. For intra-day precision, six concentration of each compound were analyzed on the same day. Each concentration of sample was injected 4 times.

Table 4. Recovery of Levocetirizine Dihydrochloride from Tablet (Xyzal tablet) by Proposed Method

Recovered (mg)	Error (%)	Recovery (%)	Recovered (mg)	Error (%)	Recovery (%)
4.995	0.005%	99.9%	4.991	0.009%	99.82%
5.010	0.01%	100.2%	5.020	0.02%	100.4%
4.975	0.02%	99.5%	4.961	0.039%	99.22%

Label claim of Xyzal Tablet = 05mg



A typical chromatogram showing Levocetirizine Dihydrochloride.

Table **2** summarizes the percent relative standard deviation (%RSD).

Generally acceptable repeatability of the results with in one day and day-to-day were observed. Data of the relative retention times obtained in a series of four consecutive injections also showed acceptable repeatability when analyzed not only on the same day but also on three consecutive days.

SYSTEM SUITABILITY AND SPECIFICITY

System suitability of the method was evaluated by analyzing the symmetry of the standard (Levocetirizine dihydrochloride) peak and theoretical plates of the column.

The specification of the method was evaluated to ensure separation of Levocetirizine dihydrochloride. The specificity of the method was demonstrated by assaying a sample of Levocetrizine dihydrochloride.

RUGGEDNESS

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab No. 1 was in the department of chemistry, university of Karachi while Lab No. 2 was the Bosch Pharmaceuticals lab Karachi.

CONCLUSION

The paper describes a new method for determination of Levocetirizine dihydrochloride by RP-HPLC.

The proposed RP-HPLC method enables determination and quantification of Levocetirizine dihydrochloride in raw materials as well as dosage formulations because of good separation and resolution of the chromatographic peaks. The proposed method is rapid, precise and the obtained results are in a good agreement with the declared contents. The accuracy and precision of the method has been confirmed by the statistical parameters.

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