Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com

Development of validated liquid chromatographic method for estimation of levocetirizine from pharmaceutical dosage forms

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ABSTRACT

A simple and rapid high-performance liquid chromatographic (HPLC) method for the determination levocetirizine has been developed. The chromatographic system consisted of a Water 2695 binary gradient pump, Water 2487 dual wavelength absorbance detector, and Empower 2 software. Separation was achieved on the XTerra symmetry C18 column at room temperature. The sample was introduced through an injector valve with a 20 μL sample loop. The results obtained showed a good agreement with the declared content. Recovery values of levocetirizine in tablets were from 99.57-100.48 %. The proposed method is rapid, accurate and selective; it may be used for the quantitative analysis of levocetirizine from raw materials, in bulk drugs and other dosage formulations.

Keywords: Levocetirizine, Tablets, Reverse phase, HPLC and Quality control.

INTRODUCTION

Levocetirizine, chemically is [2-[4- [(r)-(4-chlorophenyl) phenylmethyl]-1- piperazinyl] ethoxy] acetic acid is a third generation non-sedative antihistamine, developed from the second generation antihistamine 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 (Grant *et al.,* 2002). Literature review reveals that some analytical methods have been reported for Levocetirizine (Morita et al., 2008; Arayne et al., 2008; Selvan et al., 2006; Birajdar et al., 2008; Ashokkumar et al., 2009; Sharmaa et al., 2010) individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. The aim of the present work was to develop a simple, sensitive, accurate, and precise HPLC method for routine analysis. The proposed method was validated according to ICH guidelines (ICH, 2005).

EXPERIMENTAL

Materials and Instrumentation

The pure drug sample of LTZ was obtained as gift sample from Reddy's Laboratory, Hyderabad. Acetonitrile (HPLC-grade) was purchased from Merck, India. Millipore purification

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system was used for high purity water. All other chemicals and reagents employed were of analytical grade and were purchased from S.D. Fine Chemicals, India. The chromatograph system comprised of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower-2 software. The column used was XTerra symmetry C_{18} (150×4.6 mm, 3.5μm). The mobile phase consisted of a mixture of phosphate buffer with pH adjusted at 3.0 and acetonitrile (35:65% v/v). The flow rate was set at 0.7 mL/min and a 20μL aliquot was injected into the HPLC column. The eluent was monitored at 230 nm and these conditions the retention time observed for LTZ was 2.552 min.

Preparation of standard solution

The LTZ was weighed and dissolved in mobile phase at room temperature to obtain a stock solution of 1000μg/mL. Serial dilutions of the stock solution were made for spiking the calibration standards. The calibration curve for LTZ was prepared at five concentrations range from 20-60 µg/mL.
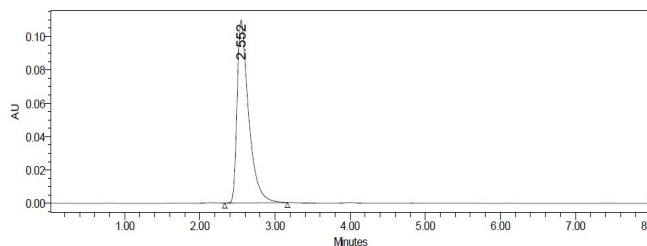
Quantification of levocetirizine in formulation

Twenty tablets were accurately weighed and finely powdered. The powdered tablet equivalent to 5 mg of LTZ was weighed and transferred into a 50 ml volumetric flask, added sufficient quantity of acetonitrile and was sonicated for few minutes and made up to the mark with acetonitrile. The solution was filtered through Whatmann filter paper No.41. From this clear solution, further dilutions were made with acetonitrile to get 30 µg/mL solutions. The peak area measurements were done by injecting the sample six times and the amount of LTZ was calculated from the respective calibration curve.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drug by RP-HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The mobile phase containing phosphate buffer (pH 3.0 with ortho phosphoric acid) and acetonitrile in the proportion 35:63 v/v was selected because it was found to give a peak for LTZ with minimal tailing. With the above mentioned composition of mobile phase, sharp peak was achieved with reasonable short run time of 2.552 min. The criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparatory steps involved in the use of same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. A typical chromatogram of test solution is shown in Figure 1.

The peak shape was symmetrical and asymmetry factor was less than 2. When the concentrations of LTZ and its respective peak areas were subjected to regression analysis by least square method, a good linear relationship (r^2=0.9996) was observed between the concentration of LTZ and the respective peak areas in

Fig 1: A typical chromatogram of Levocetirizine.

Table1. Results from regression analysis and system suitability of LTZ.

Parameters	Results
Retention time (min)	2.552
Linear range (µg/mL)	20-60
Limit of detection (µg/mL)	0.25
Limit of quantification (µg/mL)	0.76
Regression line	Y=54609X+79190
Correlation coefficient (r)	0.9996
Theoretical plates	2865
Tailing factor	1.26

Table 2. Results of recovery studies and precision.

Actual Conc. (µg/mL)	% Recovery		Precision			
			Intra-day		Inter-day	
	Mean±SD	%RSD	*Mean±SD	%RSD	*Mean±SD	%RSD
30	100.22±1.3153	1.31	100.45±1.3741	1.37	99.5±0.9765	0.98
40	100.48±0.7136	0.71	100.82±0.6713	0.67	100.14±1.1736	1.17
50	99.57±1.4488	1.46	100.11±1.3493	1.35	100.52±0.4028	0.4

*Concentration (µg/mL)

the range of 20-60 µg/ mL. The regression of LTZ was found to be Y= 54609X+79190 where Y is the peak area and X is the concentration of LTZ. The regression equation was used to estimate the amount of LTZ either in tablet formulations or in validation study. The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y- intercepts of regression lines and slope of the calibration curves were used to calculate LOD and LOQ. The LOD and LOQ values are 0.25 and 0.76 µg/mL respectively. Results from the linear regression analysis with system suitability data were listed in Table 1. To examine the accuracy of the method, recovery studies were carried out by standard addition method. The results were shown in Table 2. The average percent recoveries obtained as 99.57-100.48%, indicating that the method was accurate. High percentage recovery and low % RSD showed that the method was free from interference of the commonly used excipients in the formulation. The results of intra-day and inter-day precision studies were shown in Table 2. They revealed that % RSD values for intra-day studies ranged between 0.67-1.37% and for inter-day precision between 0.4-1.17percent, which are within the permissible limits of 2.0%.

The RP-HPLC method developed in the present study has been used to quantify LTZ in tablet dosage forms. LTZ tablets

were analyzed as per procedure described above and the average drug content was found to be 99.47% of the labeled amount (Table 3). No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

Table 3: Analysis of LTZ in tablets.

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
ALERCY	5	99.47±0.6946	0.6983	0.2836

CONCLUSION

The procedure described here is simple, rapid, sensitive, selective and cost effective. It is evident from the results that the recommended procedure is well suited for assay and evaluation of drug, in dosage forms. It can be applied for direct determination of LTZ in quality control laboratories.

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