Available online on 15.10.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Open Access Research Article

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LEVOCETIRIZINE IN BLOOD PLASMA BY USING RP-HPLC

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ABSTRACT

A rapid, selective, precise and sensitive reverse phase high-performance liquid chromatography method was developed for the quantitative estimation of Levocetirizine Dihydrochloride (LD) in human plasma and pharmaceutical dosage form. Extraction of drug from plasma was done by employing optimized liquid-liquid extraction procedure. The sample was analyzed using acetonitrile: methanol: 20mM ammonium acetate buffer pH-5 (25:55:20 % v/v/v) as mobile phase. Chromatographic separation was achieved on Thermo C-18 column (4.6 x 250mm, 5µ particle size) as stationary phase using isocratic elution (at a flow rate of 1 ml/min). The peak was detected using UV-PDA detector set at 232 nm and the total time for a chromatographic separation was 8 min. The calibration curve obtained was linear (r^2= 0.9998) over the concentration range of 2-10 µg/ml. Method was validated for precision, robustness and recovery. The limit of detection and limit of quantitation was 0.0057 and 0.174 µg/ml respectively. There was no significant difference between the amount of drug spiked in plasma and the amount recovered and plasma did not interfere in estimation. Thus, the proposed method is suitable for the analysis of LD in tablet dosage forms and human plasma.

Keywords: RP-HPLC, Levocetirizine Dihydrochloride, Human plasma, Liquid-liquid extraction

Article Info: Received 08 Sep, 2018; Review Completed 07 Oct 2018; Accepted 08 Oct 2018; Available online 15 Oct 2018

Cite this article as:

Khatri S, Bioanalytical method development and validation for the estimation of levocetirizine in blood plasma by using RP-HPLC , Journal of Drug Delivery and Therapeutics. 2018; 8(5-s):288-292

DOI: http://dx.doi.org/10.22270/jddt.v8i5-s.1977

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INTRODUCTION

Levocetirizine dihydrochloride (LD, Fig.1), chemically is (2-[4-[(R)-(4-chlorophenyl) henylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride) is a third generation non sedative antihistamine[1] and developed from the second generation antihistamine cetirizine. In many cases, the two racemic enantiomers differ in their pharmacokinetic and pharmacodynamic properties. Replacing existing recemates with single isomers has resulted in improved safety and/or efficacy profile of various recemates[2,3]. It works by blocking histamine receptors and polar in nature. LD has the advantages of higher efficacy, less side effects and longer duration over other antihistamines and has begun to replace cetirizine in clinical therapy stepwise. It has been chemically proved that the half dosage form of LD (2.5 mg) has comparable antihistaminic activity to normal amount (5.0 mg) of cetirizine in the treatment of allergic rhinitis and chronic idiopathic urticaria[4, 5]. LD is official in Indian Pharmacopoeia [6] and European Pharmacopoeia[7] this describes acid-base titration with 0.1 M NaOH in acetone/water medium. Literature survey revealed that LD has been reported to be determined by UV Spectrophotometry based on charge transfer reaction[8, 9], LC-MS-MS[10,11], RP-HPLC[12] and by HPTLC[13] in a variety of samples. LD in combination with a number of other drugs in tablet dosage form has been assayed by UV- spectrophotometry [14,15], ratio derivative spectrophotometry[16], TLC-densitometry [17,18], Stability indicating[19] and RP-HPLC [20-25]. However there is no method available for estimation of LD in human plasma By RP-HPLC Therefore, an attempt was made to develop a new, rapid and sensitive method for the determination of LD in human plasma. To access the reproducibility and wide applicability of the developed

method, it was validated as per ICH norm, which is mandatory also[26, 27].

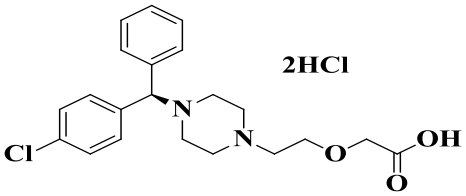


Figure 1: Chemical structure of levocetirizine dihydrochloride

EXPERIMENTAL

Materials

The pure drug sample of LD (99.56%) was obtained as gift sample from Reddy's Laboratory, Hyderabad. HPLC grade acetonitrile; methanol was purchased from Merck Ltd (Mumbai, India). All other chemicals and reagents used were of analytical grade. The human plasma was received from Peoples Hospital, Bhopal, MP, India. Triple distilled water was used throughout the process and prepared in-house; Liancet-L (5mg) Tablet was purchased from the local market.

Equipments

A high performance liquid chromatographic system from Waters comprising of manual injector, quaternary pump for constant flow and constant pressure delivery and Photodiode array detector connected to Ace software for controlling the instrumentation as well as processing the data generated was used. A thermo spectronic model of Lab India 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λmax.

Chromatographic conditions

The chromatographic analysis was performed by using a mobile phase of 20mM ammonium acetate buffer (pH 5.0), methanol and acetonitrile (20:55:25 v/v). These were filtered through 0.45µ membrane filter and degassed by sonication before use. The mobile phase was pumped isocratically at a flow rate of 1.0ml/min during analysis at ambient temperature. The run time was set at 10 min and the volume of injection was 20µl and eluent was detected at 232 nm on a Thermo C-18 column (4.6 x 250mm, 5µ particle size) Fig. 2.

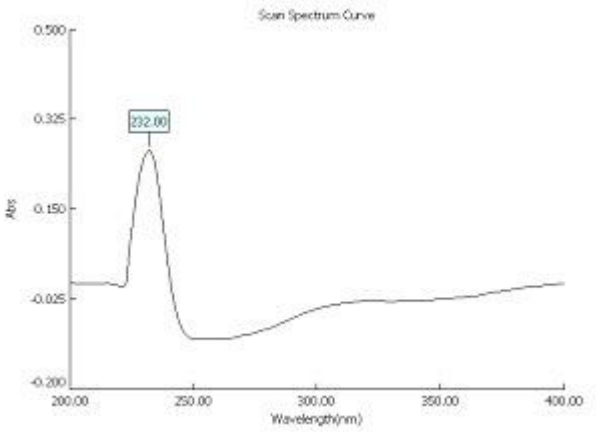


Figure 2: UV spectra of LD at 232 nm

Selection of mobile phase

Initially to estimate LD number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was acetonitrile: methanol: 20 mM ammonium acetate buffer (pH 5.0) in the ratio of 25:55:20 v/v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of standard stock solution

Accurately weigh and transfer 10mg of LD of working standard into 50ml clean dry volumetric flask add about 40ml of diluents (Mobile Phase) and sonicate to dissolve it completely and make volume up to the mark with same solvent (Stock Solution). Further pipette 5ml of above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.2 to 1.0 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. This gives the solutions of 2-10µg/ml for drug.

Preparation of blank plasma

To 1 ml of plasma, 4 ml of acetonitrile was added; the solution was mixed thoroughly and left to stand for 5 min at room temperature. After 5 min the solution was centrifuged at 10000 rpm for 12 min at 4°C. The clear supernatant liquid was removed, filtered through 0.22 µ syringe filter and injected directly into HPLC system.

Preparation of standard plasma stock solution

To prepare calibration standards and quality control samples, appropriate quantities of the various diluted standard solutions added to blank plasma to obtain drugs in the concentrations range of 2-10µg/ml for LD. These were stored at –20°C and 20 µL volume of each sample was injected and chromatographed.

Assay in formulations

To determine the content of the drug in the formulations, twenty marketed tablets of Liancet-L (Azillian Healthcare Pvt. Ltd., Ahmedabad) were weighed and ground to a fine powder; amount equal to 5 mg of LD was taken in 50-ml volumetric flask before 50 ml diluents was added. The content of the flask was shaken for about 60 min. This solution was filtered through Whatman filter paper to separate out the insoluble excipients, and further dilutions were carried out to obtain the desired concentration. Final solutions were filtered through a 0.45-µm Millipore filter before injection into the HPLC.

RESULT AND DISCUSSION

Method validation

The method was validated according to ICH guidelines such as linearity, precision, specificity, LOD, LOQ, accuracy and robustness. The linearity of this method was proved using linear correlation of the peak-area values and appropriate concentrations.

Linearity, limit of detection and quantification

Under the previously described experimental conditions, linear correlation between the peak area and applied concentration was found in the concentration range 2-10 µg/ml. The regression statistics are shown in Table 1. The LOD and LOQ at concentrations where the signal-to-noise ratios were equal to 3 and 10 respectively were determined to be 0.0057µg/ml and 0.174µg/ml for LD respectively. The correlation coefficient of this dependence was calculated to be 0.999 for LD.

Table 1 Regression statistics and LOD and LOQ

Drug	Regression equation	*r^2	*LOD (µg/ml)	*LOQ (µg/ml)
Levocetirizine	y = 396.6x +24.80	0.999	0.0057	0.174

*Average of five determination

Precision and robustness

Precision of the methods was studied at three levels as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility in synthetic samples using placebo mixtures. Mean ± SD and % relative standard deviation (RSD) values were used to express precision. As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, ACN: methanol: ammonium acetate buffer pH-5 (25:55:20 % V/V/V), to (25:54:21 % V/V/V).Results of precision and robustness are reported in Table 2.

Table 2: Result of precision and robustness

Validation Parameter	Percentage Mean ± S.D*. (n=15)	Percentage RSD*
Repeatability	99.23±0.09	0.097
Reproducibility	97.40±0.07	0.078
Intermediate precision		
Day to Day	97.24 ±0.17	0.179
Analyst to Analyst	97.11±0.13	0.144
Robustness*	96.87±0.10	0.110

* Mean of fifteen determinations (3 replicates at 5 concentration level)

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. The recovery studies were carried out by adding a definite concentration of standard drug (80%, 100%, and 120%) to preanalyzed sample solutions. The resulting solutions were then re-analyzed by proposed methods. The value of mean recoveries was found to be in ranging from 97.76 to 99.88 for LD. Total amount of drug found and percentage recovery was calculated. Result of recovery studies are reported in Table 3**.**

Table 3: Results from recovery studies of levocetirizine

Recovery Level %	Levocetirizine	
	% Mean±SD*	% RSD*
80	99.88±0.106	0.106
100	97.76±1.317	1.347
120	99.35±0.160	0.161

*Average of five determination

Specificity

A representative chromatogram (Fig. 3) was generated to show that other components, which could be present in the sample matrix, are resolved from the parent analyte. No significant changes in retention times of the drugs in the presence and the absence of excipients clearly indicated the specificity of the method**.**

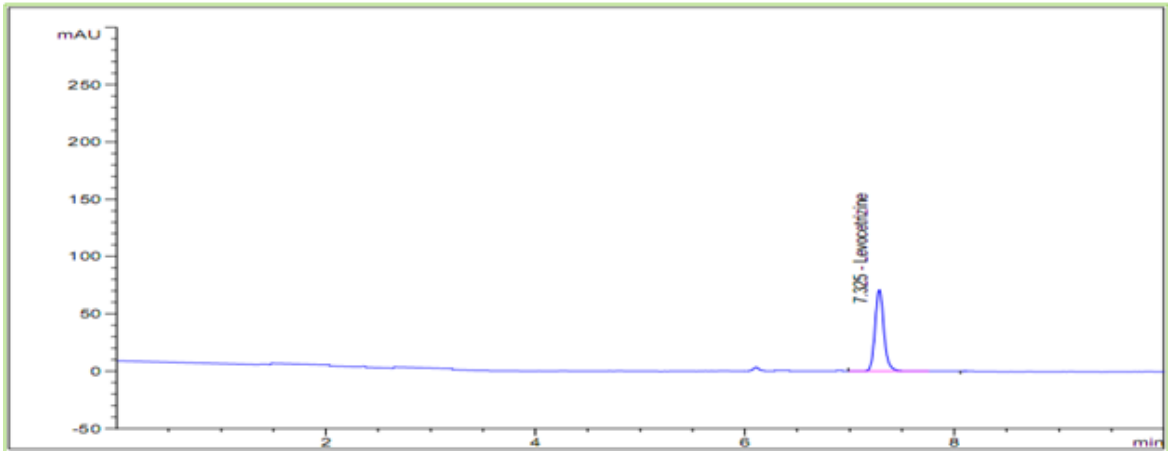
Application in human Plasma

It was observed after spiking the analyte in the plasma sample that there was no significant difference between the amount of drug spiked in plasma and the amount recovered. The recovery values (Table 4) in human plasma clearly indicate the applicability of the present method for the required purpose (Fig. 3).

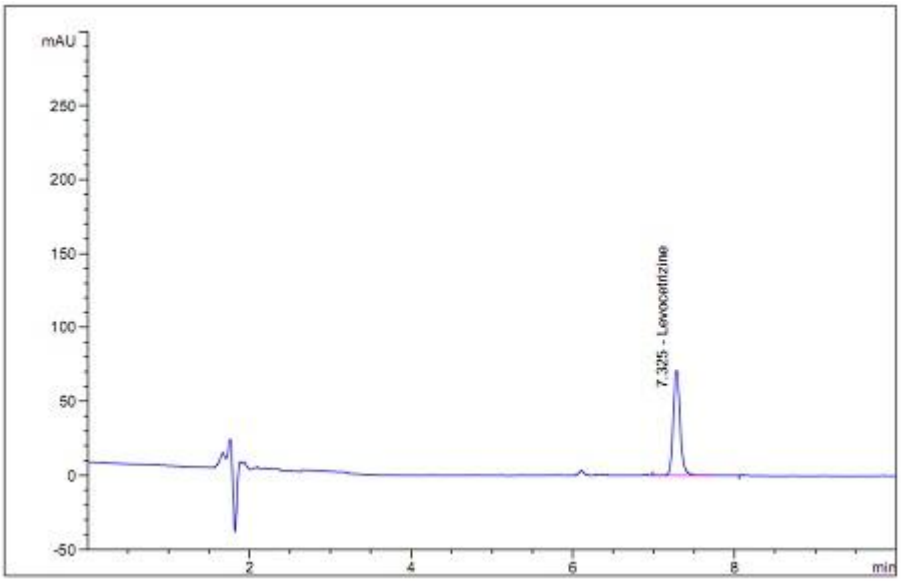
Table 4: Accuracy and precision in human plasma

Analyte	Spiked conc. (µg/ml)	Precision (% RSD)	Accuracy (%)
Levocetirizine	**2**	0.92	99.60
	4	1.02	98.56
	6	1.50	98.45

*Average of five determination



(A)



(B)

Figure 3: Representative chromatogram of LD in formulation (A), in human plasma (B).

CONCLUSION

All the results obtained by HPLC studies, it was concluded that the present method was fast and easy to perform. The linearity range, precision, accuracy, robustness, LOD, LOQ and specificity were processed to establish the suitability of the method and the confirmed results were obtained. HPLC has several superiorities compared with UV spectrophotometry, such as smaller detection and quantification limits, small sample volumes and specificity. Thus, the developed HPLC method is rapid, reliable, cost-effective and can be proposed for routine analysis laboratories and quality control purposes and are very beneficial for pharmaceutical companies, clinicians and physicians and also can be beneficial for the studies of drug interaction with other combinations.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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