

# Bioanalytical Method Development and Validation for the Determination of Levocetirizine in Pharmaceutical Dosage Form and Human Plasma by RP-HPLC

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## ABSTRACT

This study describes the development of a rapid, selective, precise and sensitive reverse phase high-performance liquid chromatography method for the quantitative determination of Levocetirizine Dihydrochloride (LCD) 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 ProntoSil C-18 column (4.6 x 250mm, 5 $\mu$  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 retention time was found to be 8 min for LCD. The calibration curve obtained was linear ( $r^2=0.9998$ ) over the concentration range of 2-10  $\mu$ g/mL. Method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.0057 and 0.174  $\mu$ g/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 LCD in tablet dosage forms and human plasma.

## INTRODUCTION

Levocetirizine dihydrochloride (LCD), chemically is (2-[4-[(R)-(4-chlorophenyl)henylmethyl]-1-piperazinyl]ethoxy]acetic acid dihydrochloride) is a third generation non sedative antihistamine (Neil, 2001) and developed from the second generation antihistamine cetirizine. In many cases, the two racemic 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 (Patel and Kothekar, 2006; Testa and Trager, 1990). It works by blocking histamine receptors and it is polar compound in nature.

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LCD 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 LCD (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 (Devalia *et al.*, 2001; Grant *et al.*, 2002).

LCD is official in Indian Pharmacopoeia (IP, 2007) and European Pharmacopoeia (EP, 2008) as well, in both the method describes for analysis of LCD is acid-base titration with 0.1 M NaOH in acetone/water medium. Literature survey revealed that LCD has been reported to be determined by UV Spectrophotometry based on charge transfer reaction (Raghu and Basavaiah, 2012; Basavaiah *et al.*, 2012), LC-MS-MS (Gunasakaran *et al.*, 2010; Morita *et al.*, 2008), RP-HPLC (Chaitanya Prasad *et al.*, 2011) and by HPTLC (Bhusari and Dhaneshwar, 2010) in a variety of samples.

LCD in combination with a number of other drugs in tablet dosage form has been assayed by UV- spectrophotometry (Prabhu *et al.*, 2008; Merukar *et al.*,2009), ratio derivative spectrophotometry (Choudhari *et al.*, 2010),TLC-densitometry (Smita *et al.*, 2010; Khedkar *et al.*,2014), Stability indicating (Venkateswari *et al.*, 2012) and RP-HPLC (Ashok Kumar *et al.*, 2009; Ambadas and Vaishali, 2010; Kamarapu *et al.*, 2010; Shaikh and Patil, 2010; Ishaq *et al.*, 2015; Rao *et al.*, 2015). However there is no method available for estimation of Levocetirizine in human plasma and pharmaceutical dosage form By RP-HPLC Therefore, an attempt was made to develop a new, rapid and sensitive method for the determination of LCD 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 (Code Q2A, 1994; Code Q2B, 1994).The chemical structures were represented in Figure 1.

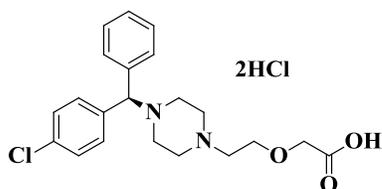


Fig. 1: Chemical structure of levocetirizine dihydrochloride.

## EXPERIMENTAL

### Materials

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

### Equipments

A high performance liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector (YL 9160 detector) connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used.

### 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 0.45 $\mu$  membrane filter and degassed by sonication before use.

The mobile phase was pumped isocratically at a flow rate of 1.0 mL/min during analysis at ambient temperature. The run time was set at 10 min and the volume of injection was 20 $\mu$ l and

eluent was detected at 232 nm on a Prontosil C-18 column (4.6 x 250mm, 5 $\mu$  particle size) Figure 2.

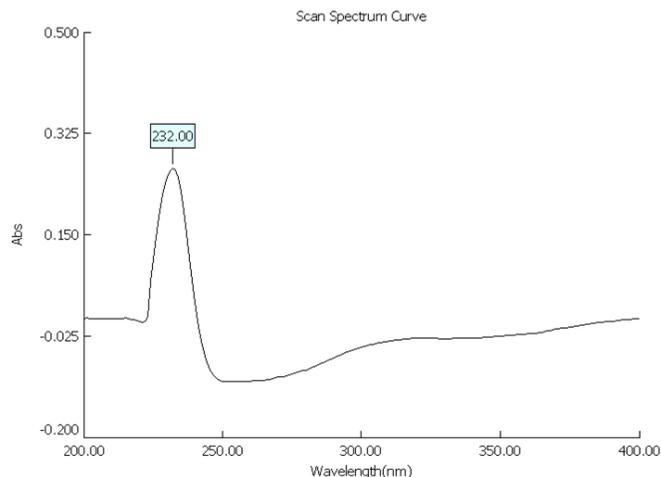


Fig. 2: UV spectra of LCD at 232.0 nm.

### Selection of mobile phase

Initially to estimate Levocetirizine 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 0.45 $\mu$  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 LCD 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 2-10 $\mu$ g/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  $\mu$  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 2-10 µg/mL for LCD. 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 LCD 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 for validation of analytical procedures. The method was validated for the parameters like linearity, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and robustness. The linearity of this method was proved using linear correlation of the peak-area values and appropriate concentrations.

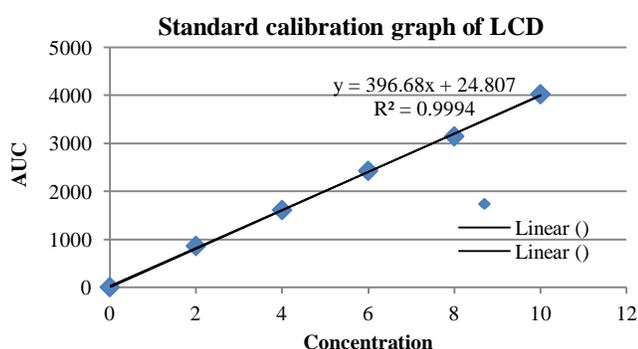
### Linearity, limit of detection and quantification

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.

**Table 1:** Regression statistics and LOD and LOQ.

Drug	Regression equation	*R <sup>2</sup>	*LOD (µg/mL)	*LOQ (µg/mL)
Levocetirizine	$y = 396.6x + 24.80$	0.999	0.0057	0.174

\*Average of five determination



**Fig. 3:** Standard calibration graph of LCD.

The LOD and LOQ at concentrations where the signal-to-noise ratios were equal to 3 and 10 respectively were determined to be 0.0057 µg/mL and 0.174 µg/mL for levocetirizine dihydrochloride respectively. The correlation coefficient of this dependence was calculated to be 0.999 for levocetirizine dihydrochloride Figure 3.

### 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  $\pm$  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	Percentage
	Mean $\pm$ S.D*. (n=15)	RSD*
Repeatability	99.23 $\pm$ 0.09	0.097
Reproducibility	97.40 $\pm$ 0.07	0.078
Intermediate precision		
Day to Day	97.24 $\pm$ 0.17	0.179
Analyst to Analyst	97.11 $\pm$ 0.13	0.144
<b>Robustness*</b>	96.87 $\pm$ 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 99.883 for LCD. Total amount of drug found and percentage recovery was calculated. Result of recovery studies are reported in Table 3.

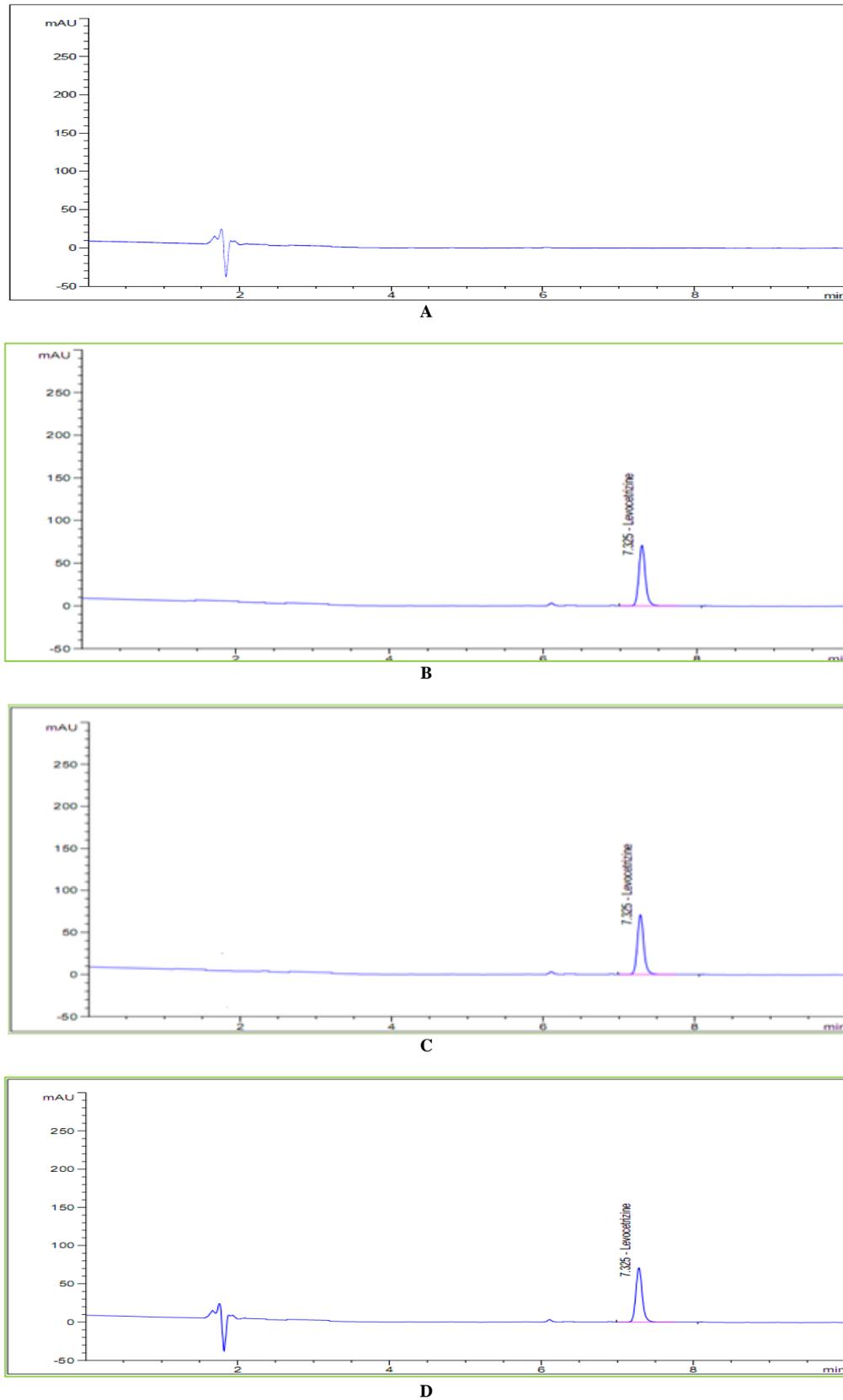
**Table 3:** Results from recovery studies of levocetirizine.

Recovery Level %	Levocetirizine	
	% MEAN $\pm$ SD*	% RSD*
80	99.88 $\pm$ 0.106	0.106
100	97.76 $\pm$ 1.317	1.347
120	99.35 $\pm$ 0.160	0.161

\*Average of five determination.

### Specificity

A representative chromatogram (Figure 4) 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.



**Fig. 4:** Representative chromatogram of blank plasma (A) and representative chromatogram of levocetirizine pure drug (B), representative chromatogram of levocetirizine in formulation (C), in human plasma (D).

### 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 (Figure 4).

**Table 4:** Accuracy and precision in human plasma.

Analyte	Spiked conc. ( $\mu\text{g/mL}$ )	Precision (% RSD)	Accuracy (%)
Levocetirizine	2	0.92	99.60
	4	1.02	98.56
	6	1.50	98.45

\*Average of five determination.

### CONCLUSION

Based on 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.

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**Conflict of Interests:** There are no conflicts of interest.

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Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.

Code Q2B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.

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