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Research Article

Zero order and area under curve spectrophotometric methods for determination of Levocetirizine in pharmaceutical formulation

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Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Levocetirizine in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the zero order derivative values measured at 230 nm and the area under the curve method values measured at 227-234 nm (n=2). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Levocetirizine using 5-25µg/ml (r^2=0.998 and r^2=0.999) for zero order and area under the curve spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Levocetirizine in tablets.

Keywords: Levocetirizine, UV visible spectrophotometry, Accuracy, Precission, AUC, Method Validation.

1. 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. [1,2] 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. [3-5] 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**.** [6,7] Literature survey revealed several analytical methods UV spectrophotometry [8,9] and HPLC [10-12] have been reported in bulk, pharmaceutical dosage form for determination of Levocetirizine. To our notice, so far no UV- spectrophotometric method using Zero Order and Area under Curve (AUC) has been reported for the determination of Levocetirizine in bulk and tablets. Hence an attempt has been made to develop new Zero Order and Area under Curve Spectrophotometric methodsmethod for estimation of Levocetirizine in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Molecular formula: $C_{21}H_{25}ClN_2O$
Molecular weight: 388.888 g/mol

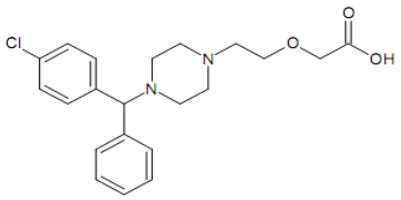

Fig. 1: Chemical Structure of Levocetirizine

2. Materials and Methods

2.1 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan

Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India).Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.2 Materials

Reference standard of Levocetirizine API was supplied as gift sample by Lupin Pharmaceutical Limited Aurangabad. Tablet sample with label claim 5 mg per tablet were purchased from local market Pune.

2.3 Method development

2.3.1 Preparation of Standard and Sample Solutions:-

Stock solution of 10µg/ml of Levocetirizine was prepared in methanol, for zero order and area under the curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with methanol in a concentration range of 5, 10, 15, 20, and 25µg/ml with methanol for zero order and area under the curve spectrophotometric methods. Methanol was used as a blank solution.

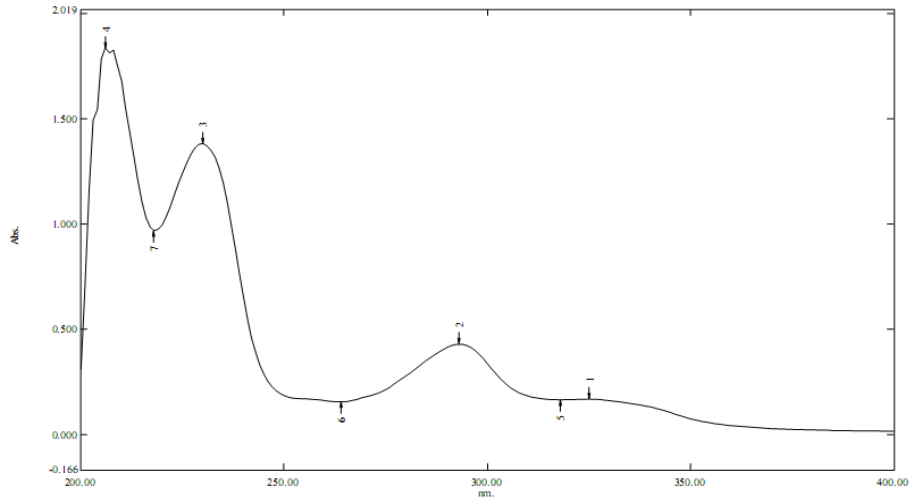

Fig. 2: Zero order derivative spectrum of Levocetirizine in Methanol (20µg/ml)

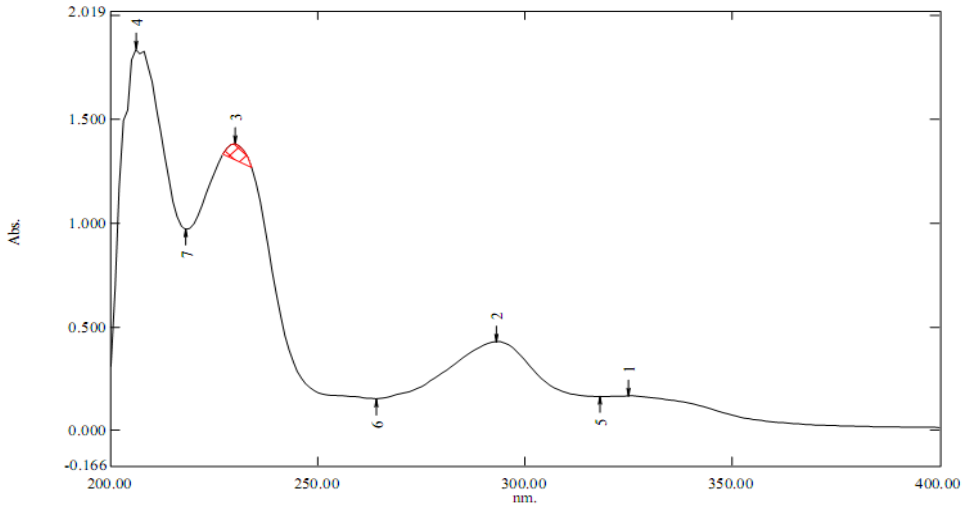

Fig. 3: UV AUC spectrum of Levocetirizine in Methanol (20µg/ml)

2.3.2 Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 227 to 234 nm.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelength range start and end point of curve region**.** [13,14]

2.3.3 Assay Procedure

Twenty tablets each containing 5 mg of Levocetirizine were weighed crushed to powder and average weight was calculated. Powder equivalent to

10 mg of Levocetirizine was transferred in 100 ml of volumetric flask. A 50 ml of methanol was added and sonicated for 15minutes. Then solution was further diluted up to the mark with methanol. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 15µg/mL solution with methanol subjected for UV analysis using methanol as blank. Appropriate dilutions were made with methanol from stock solution for both zero order and area under the curve spectrophotometric methods.

Table 1: Assay of tablet dosage form

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)* Zero derivative	Amount found (%)* AUC	Mean % Found zero derivative	Mean % Found AUC	% RSD zero derivative	% RSD AUC
1	15	102.25	101.19				
2	15	100.11	98.12	101.10	100.52	0.7258	0.7413
3	15	100.96	102.26				

*n=3, % RSD = % Relative Standard Deviation.

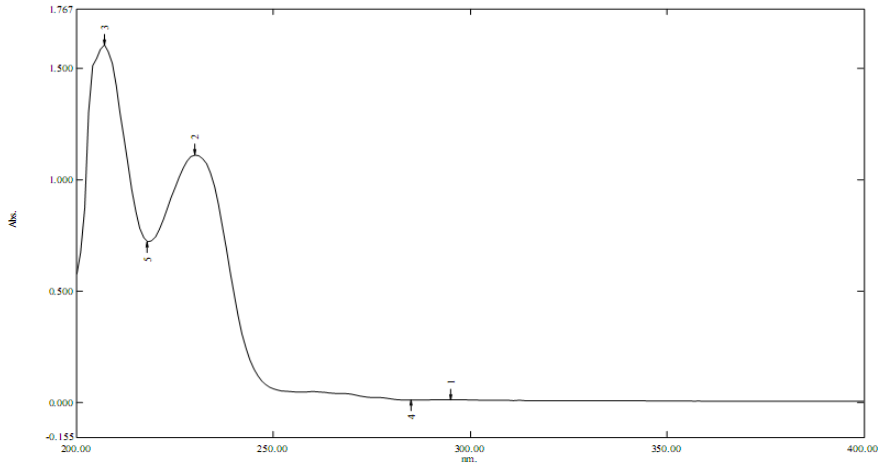

Fig. 4 Zero order derivative spectrum of Levocetirizine dosage form (25µg/ml).

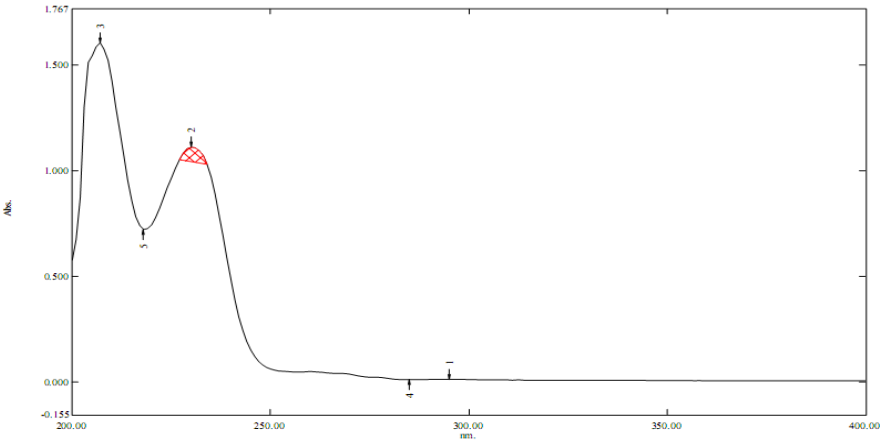

Fig. 5 Zero order UV AUC spectrum of Levocetirizine dosage form (25µg/ml).

3. Results and Discussion

The zero order and area under the curve spectra for Levocetirizine were recorded at the wavelength of 230 nm and 227-234 nm respectively.

3.1 Linearity and Range

Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y=0.022x+0.012 (r^2=0.998) at 230 nm for zero order derivative spectrophotometry and y=0.021x+0.004 (r^2=0.999) at 227-234 nm for area under the curve spectrophotometry. The range was found to be 5-25µg/ml for both zero order and area under the curve spectrophotometric methods.

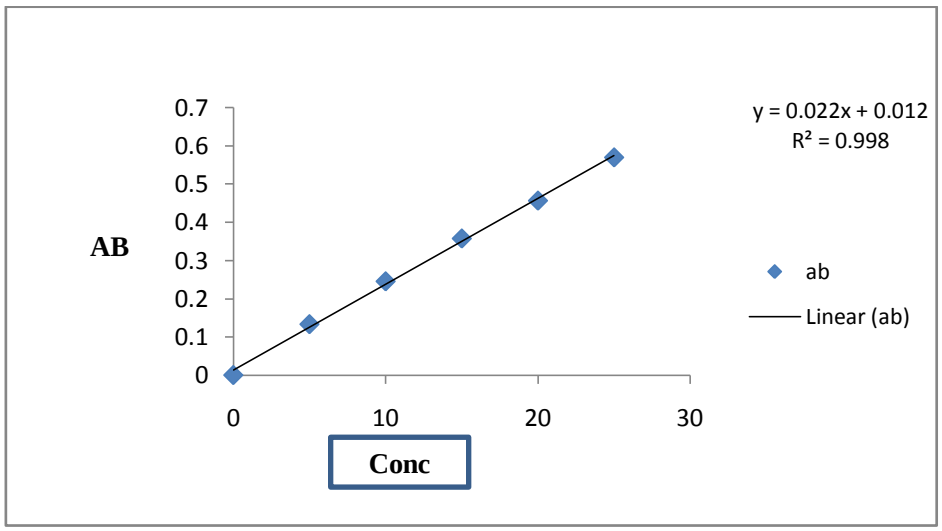

Fig.5: Linearity of Levocetirizine by Absorbance

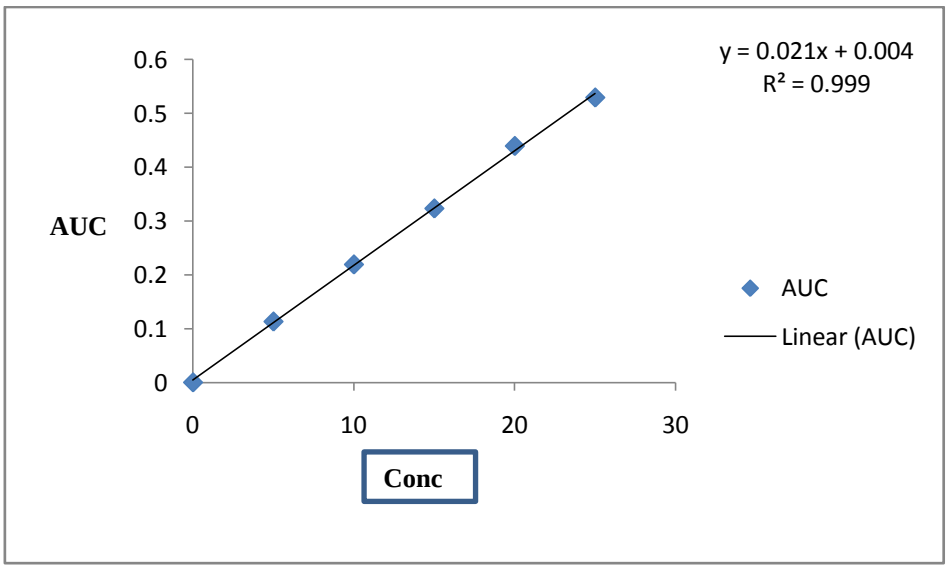

Fig.6: Linearity of Levocetirizine by AUC.

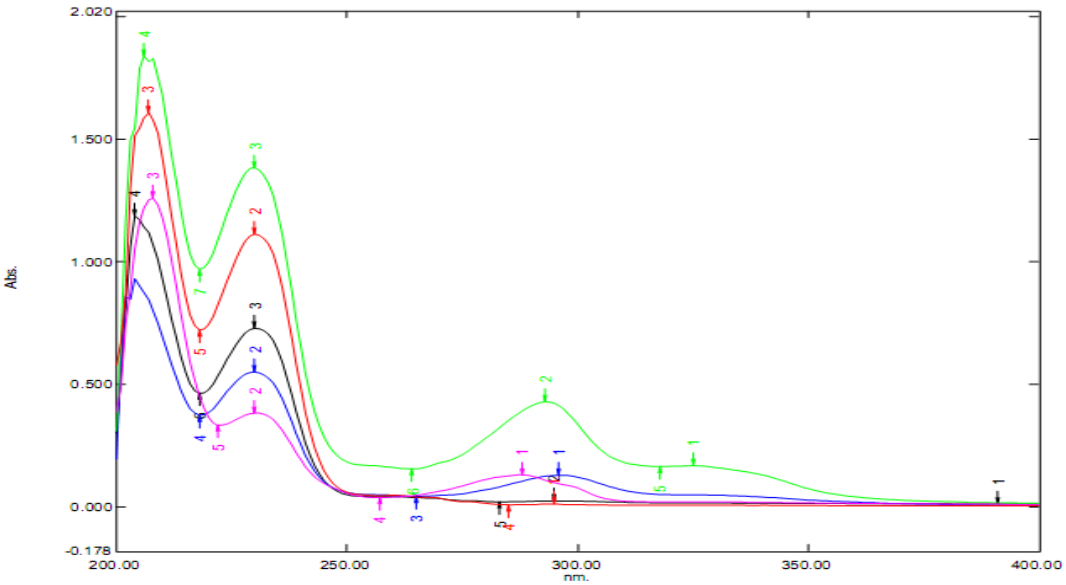

Fig. 7: Zero order derivative overlay of Levocetirizine at 5,10,15,20 and 25 µg/ml Concentrations.

Table 2: Statistical data for the calibration graphs for determination of Levocetirizine by Proposed methods.

Parameters	Zero order derivative	Area Under the Curve
Linearity range (µg/ml)*	5-25	5-25
r^2± S.D*	0.998	0.999

3.2 Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml standard solution. For Area under curve (AUC) was measured in wavelength range 227-234 nm and For Zero order derivative at 230 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 3: Accuracy results for Levocetirizine

Accuracy level	Sample conc (µg/)	Std. conc	Total amnt. Added (µg/m)	%Recovery zero derivatie	% Recovery Auc*	Mean of Zero derivative*	Mean of Auc	% RSD Zero derivative	% RSD Auc
80	15	12	27	98.46	101.01				
100	15	15	30	98.24	102.09	98.36	101.46	0.658	0.673
120	15	18	33	98.39	101.28				

*n=3, % RSD = % Relative Standard Deviation.

3.3 Precision

To determine the precision of the method, Levocetirizine solutions at a concentration of 10 µg/ml were analysed each three times for both zero order and area under the curve spectrophotometric methods. Solutions for the standard curves were prepared fresh every day.

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.0064	0.6617	0.0033	0.4114
Area under the curve	0.8374	0.5421	0.8612	1.6109

3.4 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.5960 µg/ml and 1.782µg/ml respectively for zero order derivative and The LOD and LOQ were found to be 0.5826 µg/ml & 1.7472 µg/ml for area under the curve methods respectively.

3.5 Analysis of the Marketed Formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.47 % and 100.93 % zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Levocetirizine had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Levocetirizine in pharmaceutical dosage form.

Table 5: Summary of validation parameters

Parameter	Zero derivative	AUC
λ range	200-400 nm	200-400 nm
Regression Equation (y=mx+c)	Y=0.022x+ 0.012	Y=0.053x+ 0.011
Measured wavelength	230 nm	227-234 nm
Linearity range	5-25µg/ml	5-25µg/ml
Slope	0.022	0.053
Intercept	0.012	0.011
Correlation coefficient (R^2)	0.998	0.999
Limit of Detection (LOD) µg/ml	0.5960	0.5826
Limit of Quantitation (LOQ) µg/ml	1.782	1.7472
Accuracy (Mean % Recovery)	98.36	101.46
Precission (%RSD)	0.658	0.673

4. Conclusion

No UV or Area under Curve spectrophotometric methods have been described for the determination of Levocetirizine. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Levocetirizine. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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