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Development and Validation of RP-HPLC Method for the Simultaneous Determination of Levocetirizine Dihydrochloride and Ambroxol Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

Levocetirizine dihydrochloride (LCD) and Ambroxol Hydrochloride (ABH) are two chemicals used for the treatment of upper respiratory tract diseases and elevation of allergy symptoms. Few HPLC methods were reported for the estimation of LCD and ABH in bulk and in tablet dosage form without extraction. The present work describes a simple, precise and accurate isocratic reversed-phase HPLC method that was developed and validated for the estimation of Levocetirizine dihydrochloride and Ambroxol hydrochloride in bulk and in tablet dosage form. The proposed RP-HPLC method was carried out using Intersil C8 column (5 μm, 25 cm, 4.6 mm i.d.). The mobile phase of water: acetonitrile mixture (50:50 v/v) was adjusted to pH 3.3 using ortho-phosphoric acid and applied at a flow rate of 1mL/min and 20 μL injection volume. The detection was achieved with UV at 225 nm. The retention time of ABH and LCD was 1.80 ± 0.01 min and 3.21 ± 0.07 min, respectively. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration plot was linear over the concentration range of 5-400 μg/mL for ABH and 1-35 μg/mL for LCD. The mean absolute recoveries for ABH and LCD were about 98.97 % and 100.8 %, respectively. From the validation study, it was found that the method was specific, rapid, accurate, sensitive, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine pharmaceutical quality control of both these drugs separately and in their combined dosage form.

***Keywords**:* Levocetirizine dihydrochloride; Ambroxol hydrochloride; HPLC, commercial formulations and bulk.

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INTRODUCTION

Levocetirizine dihydrochloride (**Figure 1**) is the chemical name for [2-[4-[(R)-(4-chloro- phenyl) Phenylmethy l0]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride that has the molecular formula of $C_{21}H_{25}ClN_2O_3.2HCl$ and a molecular weight 461.8. LCD is a third-generation non-sedative antihistamine developed from the second generation antihistamine cetirizine[1]. It is the *R*-enantiomer of the cetirizine which functions to block histamine receptors. More specfically, LCD does not prevent the actual release of histamine from mast cells but prevents its binding to its receptors. This, in turn, prevents the release of other allergy chemicals and increases the blood supply to the area providing relief from the typical symptoms of hay fever[2].

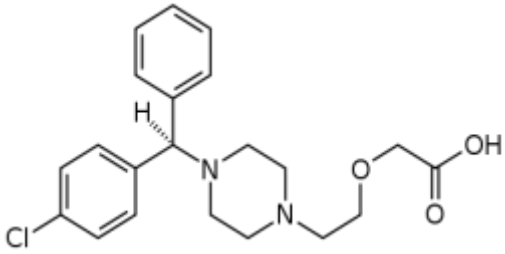

Figure 1. Chemical Structure of Levocetirizine dihydrochloride (LCD)

Ambroxol hydrochloride, trans-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol hydrochloride (**Figure 2**), is an active N-desmethyl metabolite of the mucolytic, bromhexine[3]. It is used in the treatment of the upper respiratory tract diseases. With its mucolytic activity, ABH facilitates the breakdown of acid mucopolysaccharide fibres in the mucous thus making it thinner and less viscous for expectoration[3]. As well, it stimulates the production of pulmonary surfactant, a substance found to play a major role in the lung defense mechanism and thereby further protect it against inflammation and infection.

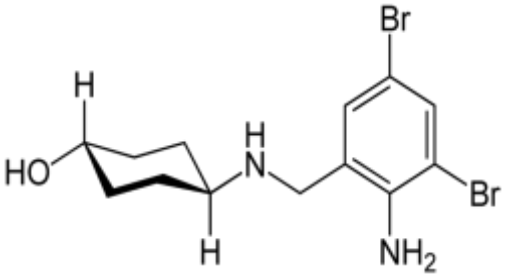

Figure 2. Chemical Structure of Ambroxol hydrochloride (ABH)

A literature survey reveals that few HPLC methods were reported for the estimation of LCD and ABH combined in bulk and in tablet dosage form without prior extraction. However, there have been some analytical methods reported for the individual determination of LCD[4-8], LCD in combination with other drugs in human plasma[9,10], in pharmaceutical dosage form by HPLC[11-16],

using TLC[17] and applying charge transfer method[18,19]. As well, a number of methods were reported for the individual determination of ABH[20-22], in combination with other drugs in pharmaceutical dosage form by HPLC[23-28], in human plasma[29,30], by TLC[31], UV spectrophotometry[32] and HPTLC[33].

In the current research work, a stability indicating method was developed and validated for the simultaneous HPLC determination of Levocetirizine dihydrochloride and Ambroxol hydrochloride in bulk and marketed dosage formulations.

2. Materials and Methods:

Equipment:

High performance liquid chromatography HPLC system used was Agilent 1100 with Degasser (G1322A), Quat. Pump (G1311A), Auto Sampler (Als G1313A), UV Absorbance Detector (G1314A) and an Intersil C8 (5 µm, 25 cm, 4.6 mm i.d.) column was used as the stationary phase. Other equipment used: analytical weighing balance (Radwag XA60/220/X), pH meter (HANNA HI 253) and vacuum filter pump (model XI 5522050, Millipore).

Materials:

Pure sample of LCD was obtained from Dr. Reddy's Laboratories Ltd. Pharmaceutical Company, India, and ABH was obtained from Pharco Pharmaceutical Company, Egypt. Samples of tablets Baret-la each containing 5 mg LCD and 60 mg ABH were purchased from A Five Pharmaceutical Company, India. HPLC grade acetonitrile (Scharlau, Spain) was used for preparing the mobile phase. Analytical grade Orthophosphoric acid (85%) (ADWIC, Egypt) was used to adjust mobile phase pH. High purity deionised water was obtained using Veolia-Water, Pure Lab Flex purification system.

Methods:

Selection of Wavelength: The wavelength of maximum absorption for ABH was obtained at 248 nm and that for LCD at 230 nm. A single wavelength selected for estimation of the mixture of ABH and LCD was found to be at 225 nm where both chemical showed a significant response. The overlay spectrum for the individual solutions of ABH, LCD and their mixture is provided in **Figure 3**.

Preparation of mobile phase:

The mobile phase was prepared using water: acetonitrile mixture (50:50 v/v) and the final pH was adjusted 3.3 using orthophosphoric acid (85%). The prepared mobile phase was filtered (0.45 µm) under vacuum, degassed and sonicated for 5 min. This mobile phase was also used as diluent.

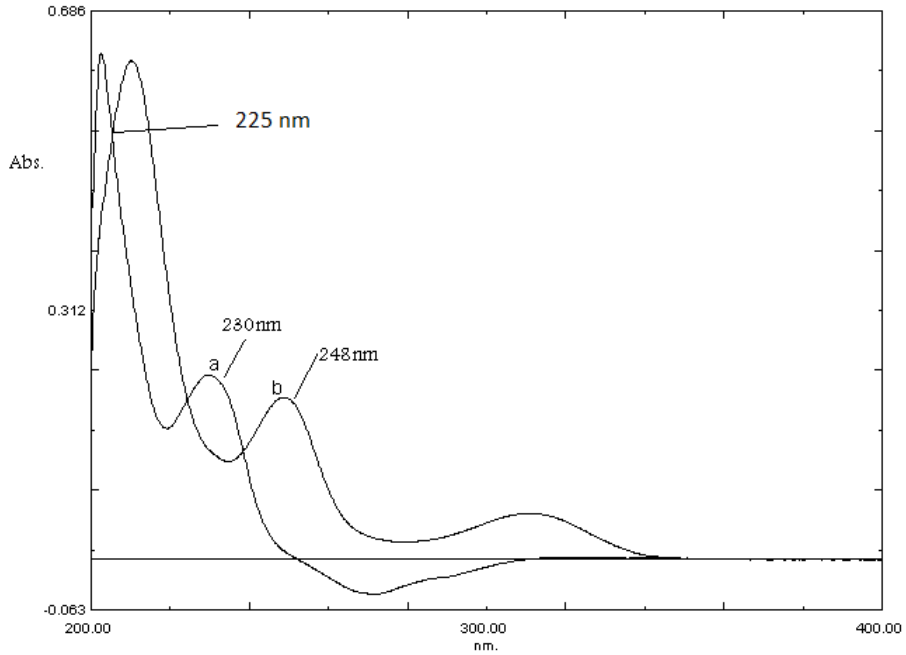

Figure 3: Overlay spectra for (a) 5.0 µg mL^{-1} of LCD and (b) 6 µg mL^{-1} of ABH which indicates λ_{max} for the mixture of LCD and ABH at 225 nm.

Preparation of Standard solutions:

Levocetirizine Dihydrochloride Standard Stock Solution:

25 mg of LCD were accurately weighed and transferred into 50 mL clean dry volumetric flask and were dissolved in and completed to the mark using mobile phase. 5 mL of this stock solution were transferred into 50 mL volumetric flask and diluted up to the mark to obtain a stock standard solution of 0.05 mg/mL LCD.

Ambroxol Hydrochloride Standard Stock Solutions:

60 mg of ABH were accurately weighed and transferred into 100 mL clean dry volumetric flask and dissolved in the mobile phase to the mark to obtain a stock standard solution of 0.6 mg/mL ABH.

Mixed Standard Solution:

5 mL of standard stock solution of LCD (0.05 mg/mL) and 5 mL of standard stock solution of ABH (0.6 mg/mL) were transferred into 50 mL clean dry volumetric flask and the mobile phase was added to the mark. This solution has a final concentration of 5 µg/mL and 60 µg/mL for LCD and ABH, respectively. **Figure 4** shows the typical chromatogram obtained for the prepared mixed standard solution.

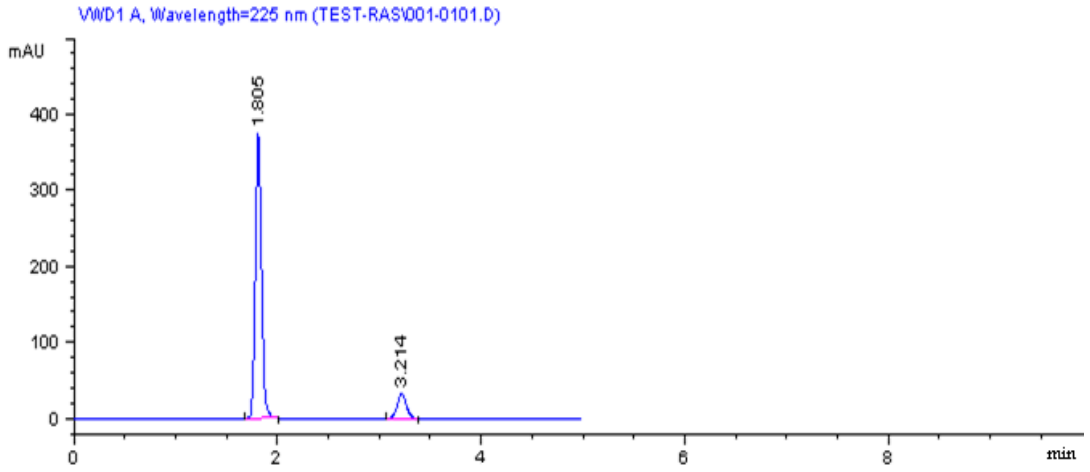

Figure 4: Chromatogram of 5 µg/mL LCD and 60 µg/mL ambroxol hydrochloride (standard mixture solution).

Estimation of ABH and LCD in capsule formulation:

Twenty tablets of **BARET-LA** were weighed and finely grounded to powder. An equivalent weight of one tablet in the powder form knowing to contain 60 mg ABH and 5 mg LCD was transferred into a 100 mL clean dry volumetric flask. 70 mL of diluent was added and the mixture was sonicated to dissolve the powder completely. The final solution volume was made up to the mark with the same solvent. 5 mL of this sample stock solution was pipetted into a 50 mL volumetric flask and diluted to the mark with the mobile phase to get a final sample solution with the concentration 5 µg/mL and 60 µg/mL of LCD and ABH, respectively (**Figure 5**).

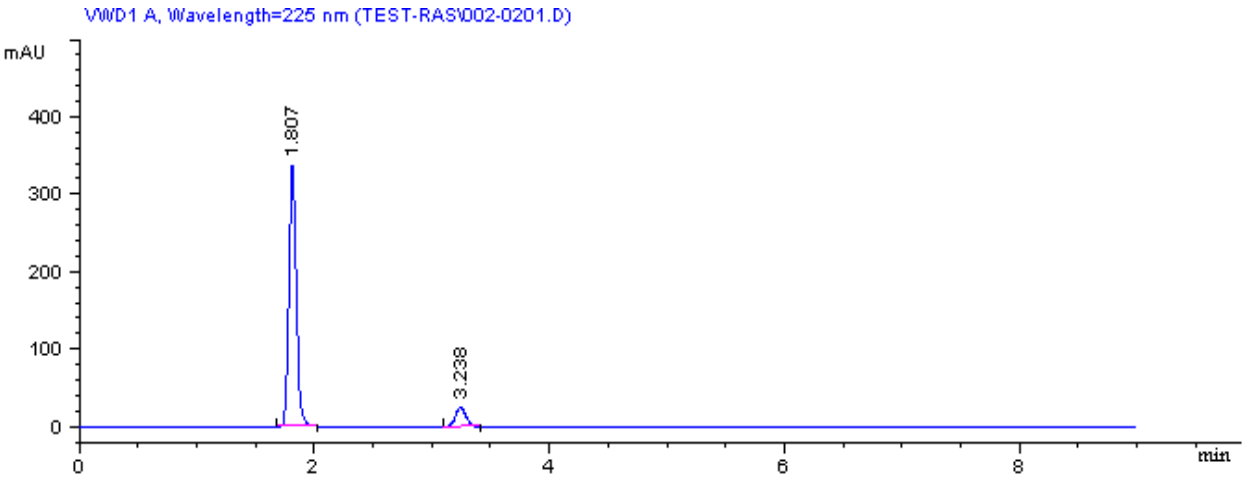

Figure 5: Typical chromatogram of LCD and ABH in sample containing 5 µg/mL and 60 µg/mL, respectively.

RESULTS AND DISCUSSION

Method validation:

The HPLC method developed was validated in terms of accuracy, precision, LOD, LOQ,

linearity, range and robustness as per ICH guidelines. System suitability was verified by injecting ten replicate samples from the working standard solution of LCD and ABH mixture containing the respective amount of 5 µg/mL and 60 µg/mL. Various parameters for acceptability of the method includes the fact that % RSD of peak areas that did not exceed 2%, the theoretical plates numbers (N) was at least 2000 for each peak and tailing factors did not exceed 2.0 for both LCD and ABH. The data obtained for these experimental trials are provided in **Table 1**.

Table 1: System Suitability parameters of ABH and LCD for the proposed method

Parameter	Levocetirizine Dihydrochloride	Ambroxol Hydrochloride
Theoretical plates	6770	4676
symmetry factor	0.92	0.88
% RSD (Peak area)	0.75	0.78
Resolution	10.24	------
Tailing factor	1.059	1.153

Table 2: Linearity parameters of the proposed method

Parameters	LCD	ABH
Slope	39.851	26.286
Intercept	10.176	18.993
Correlation coefficient (R^2)	0.9999	1.0000
LOD (µg/mL)	0.04	0.07
LOQ (µg/mL)	0.12	0.23

Linearity:

The calibration curves for linearity of this method were obtained by plotting the peak area against concentrations of LCD and ABH ranging between of 1 - 35 µg/mL and 5 - 400 µg/mL, respectively. The results obtained for these calibration plots of LCD and ABH are provided in **Table 2** and depicted in **Figures 6 and 7**.

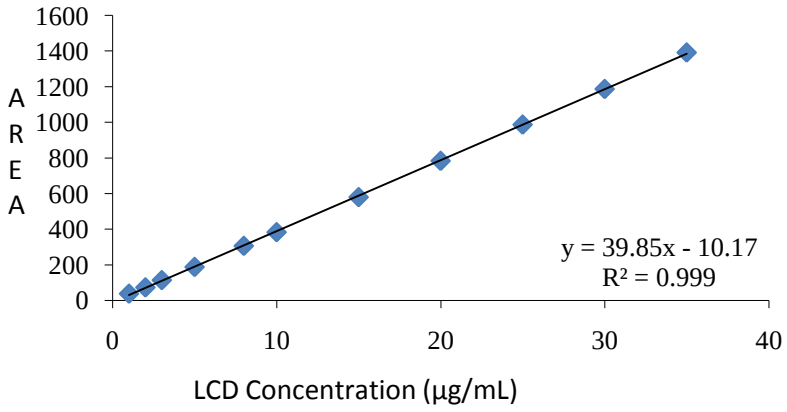

Figure 6: Calibration curve for LCD (concentration range :1.0-35.0 µg/mL)

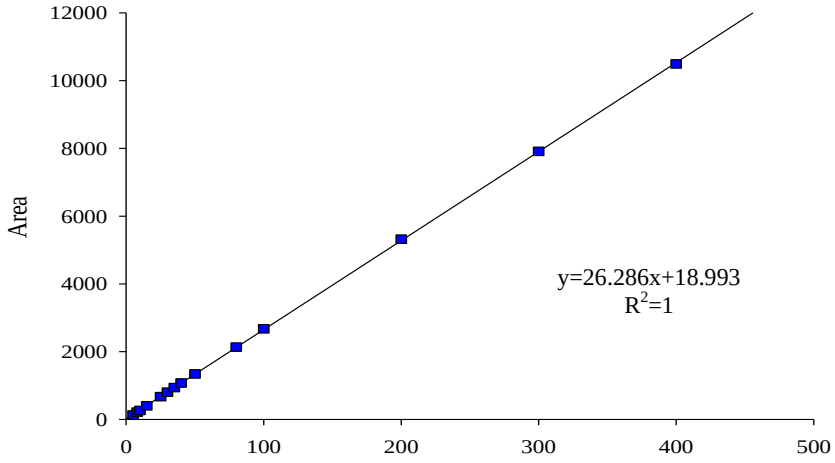

Figure 7: Calibration plot for ABH (concentration range: 5.0-400.0 µg/mL)

Precision:

The repeatability of the analytical method was evaluated by assaying three sample solutions of LCD and ABH within the same day and under the same experimental conditions (Intra-Day). Intermediate precision was evaluated by assaying similar solutions on three consecutive days. The peak areas were determined and compared and the precision was expressed as % Relative Standard Deviation (RSD% <2). From the data obtained in **Table 3**, the developed RP-HPLC method was found to be precise.

Table 3: Precision data for LCD and ABH using the proposed method

Drug	Concentration (µg/mL)	Intra-day precision		%Mean Recovery ± SD	% Mean RSD
		Amount Found[(a)]	% Recovery[(a)]		
LCD	5	5.03	100.7	100.8±0.45	0.45
	10	10.04	100.4		
	15	15.19	101.3		
ABH	60	59.74	99.57	98.97±0.57	0.58
	120	118.10	98.42		
	180	178.06	98.92		
Drug	**Concentration (µg/mL)**	**Inter-day precision**		**%Mean Recovery ± SD**	**% Mean RSD**
		Amount Found[(a)]	**% Recovery[(a)]**		
LCD	5	4.99	99.83	99.46±0.49	0.49
	10	9.89	98.90		
	15	14.95	99.67		
ABH	60	59.83	99.72	99.38±0.42	0.42
	120	118.69	98.91		
	180	179.16	99.53		

[(a)] mean of three different samples for each concentration**Accuracy:**

The accuracy of the method was determined by calculating the % recovery of LCD and ABH using the standard addition method. Known amounts of LCD and ABH were added to a pre-quantified sample solutions and the amount of LCD and ABH were estimated by measuring the peak areas and fitting the values to the straight-line equation obtained for the respective calibration curve (**Table 4**).

Table 4: Accuracy data for the determination of LCD and ABH using proposed method

Drugs	Label claim /tablet (µg/mL)	Amount of standard added (µg/mL)	Amount Found (µg/mL)	% Recovery[(a)]	%Mean Recovery ±SD	Mean RSD
LCD	5	5	5.01	100.2	98.87±0.94	0.95%
		10	9.85	98.5		
		15	14.70	98.0		
		20	19.76	98.8		
ABH	60	60	59.00	98.3	99.43±1.25	1.26%
		120	121.13	100.9		
		180	180.00	100.0		
		240	236.42	98.5		

[(a)] mean of three different samples for each concentration

Robustness:

The robustness of a method refers to it's the ability to remain unaffected even as small changes are being introduced to the main parameters. To determine the robustness of the developed method, experimental conditions were purposely altered and the chromatographic resolution between LCD and ABH were evaluated using a constant flow rate of 1.0 mL/min. To study the effect of flow rate upon the peak resolution of LCD and ABH, the rate was changed from 0.8 to 1.2 mL/min using 0.2 increments. The effect of pH variation of the mobile phase upon the resolution was studied at pH 3.1, 3.3 and 3.5. The cumulative results are provided in **Table 5**. Form these results; no significant change in the obtained chromatogram was observed which demonstrates that the HPLC method developed was robust.

Table 5: System suitability parameters and robustness in normal and changed condition

System suitability Parameter	No	Robustness	Levocetirizine dihydrochloride	Ambroxol Hydrochloride	Mean ± S.D	
Resolution	1.	Flow rate			LCD	ABH
		0.8 mL	11.19	11.19	10.54±0.56	
		1.0 mL	10.24	10.24		
		1.2 mL	10.20	10.20		
	2.	pH of Buffer			10.07±0.77	
		3.1	9.22	9.22		

		3.3	10.73	10.73		
		3.5	10.26	10.26		
Peak symmetry	1.	Flow rate			LCD	ABH
		0.8 mL	0.92	0.87	0.93±0.01	0.88±0.01
		1.0 mL	0.93	0.88		
		1.2 mL	0.95	0.90		
	2.	pH of Buffer			0.94±0.01	0.85±0.03
		3.1	0.96	0.81		
		3.3	0.93	0.88		
		3.5	0.94	0.87		
Retention time	1.	Flow rate			LCD	ABH
		0.8 mL	4.16	2.26	3.39±0.69	1.85±0.37
		1.0 mL	3.21	1.80		
		1.2 mL	2.81	1.51		
	2.	pH of Buffer			LCD	ABH
		3.1	3.71	2.16	3.58±0.32	2.01±0.18
		3.3	3.21	1.80		
		3.5	3.82	2.08		
Theoretical plates	1.	Flow rate				
		0.8 mL	7388	3896	--------	--------
		1.0 mL	6770	4676	--------	--------
		1.2 mL	6008	3119	--------	--------
	2.	pH of Buffer				
		3.1	6716	3117	--------	--------
		3.3	6770	4676	--------	--------
		3.5	6812	2960	--------	--------

Assay:

The assay was performed on the marketed formulation of LCD and ABH under the brand name of (BARET-LA). This was done by taking the equivalent weight of one tablet, dissolving it in the mobile phase, diluting it and injecting in HPLC. The mean recovery using the proposed method in tablets of LCD and ABH was 101.0 ± 0.17 and 98.81 ± 0.05, respectively. The obtained results are provided in **Table 6**. The *Student t-* and *F- tests* (at 95 % confidence level) were applied[34,35] and the results showed that the calculated t- and F- values did not exceed the theoretical values (**Table 7**).

Table 6: Analysis of marketed formulation BARET-LA tablets using the proposed method

Brand name		Label claim (mg)	Amount Found (mg) [(a)]	% Label claim[(a)]
Baret-la	LCD	5	5.05	101.0±0.17
	ABH	60	59.29	98.81±0.05

[(a)] mean of six different determinations

Table 7: Statistical analysis of data obtained for the determination of LCD and ABH in BARET-LA tablets

Parameter	Proposed method	Reference[34] method	Proposed method	Reference[35] method
	LCD		ABH	
Std. Dev	0.17	0.49	0.052	0.64
Std. Error	0.07	0.20	0.02	0.26
F-Value	0.34	----------	0.08	----------
t-Value	0.85	----------	0.30	----------

F-tabulated = 9.28 at 95% confidence limit.

t-tabulated = 2.447 at 95% confidence limit under n= 6 degrees of freedom .

CONCLUSION

A simple, precise and accurate method was developed for the quantitative estimation of ambroxol hydrochloride and levocetirizine dihydrochloride in bulk drug and marketed pharmaceutical formulations without any interference from the excipients. The method is very simple and specific as both the peaks were well separated. The developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps, improved sensitivity and comparative short run time. The lack of extraction procedures makes the method especially suitable for routine quality control analysis work particularly when large numbers of samples are encountered.

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