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DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND LEVOCETIRIZINE DIHYDROCHLORIDE

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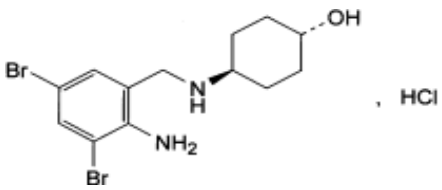
Abstract
A simple, precise and accurate method has been developed for simultaneous estimation of Ambroxol hydrochloride and Levocetirizine dihydrochloride. The proposed RP-HPLC method utilises Enable C18 G column (250 x 4.6mm, 5µm), mobile phase consisting of Phosphate buffer pH 3.0: Methanol in the ratio of 20:80 (v/v) and UV detection at 236nm using a photodiode array detector.The stability indicating capability of the method was proven by subjecting the drugs to stress conditions such as alkaline and acid hydrolysis, oxidation, photolysis, thermal degradation as per ich guidelines and resolution of the degradation products formed therein.The described method was linear over a range of 15 – 45 µg/mL for ambroxol hydrochloride and 1 – 3 µg/mL for levocetirizine dihydrochloride respectively. The method validation data showed excellent results for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness. The present method can be successfully used for routine quality control and stability studies.
***Keywords*:** Ambroxol hydrochloride, Levocetirizine dihydrochloride, RP-HPLC, Stability studies

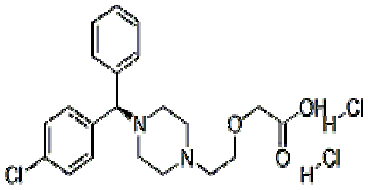
1. Introduction

Ambroxol hydrochloride (ABH) [1] chemically known as trans-4-[(2-Amino-3,5-dibromo benzyl) amino] cyclo hexanol hydrochloride is an active N-desmethyl metabolite of the mucolytic, bromhexine. Its mucolytic activity by which it facilitates breakdown of acid mucopolysaccharide fibres in the mucous making it thinner and less viscous and, therefore, easy for expectoration. It stimulates production of pulmonary surfactant, a substance found to play a major role in the lung host defence mechanism, thereby further protecting against lung inflammation and infection.

Levocetirizine dihydrochloride (LEVC) [2] chemically known as (2-(4-[(R)-(4-Chlorophenyl) (phenyl) methyl] piperazin-1-yl)ethoxy) acetic acid dihydrochloride, the (R) enantiomer of cetirizine, is a potent and selective antagonist of peripheral H_1-receptors. It has high affinity for human H1- receptors.



Ambroxol hydrochloride



Levocetirizine dihydrochloride

From literature survey it was found that many methods had been reported for determination of ambroxol hydrochloride [3-8] and levocetirizine dihydrochloride [9-12] individually and in combination [13] with other drugs. In this present research work, it was proposed to develop and validate a stability indicating HPLC method for simultaneous estimation of Ambroxol hydrochloride and Levocetirizine dihydrochloride in marketed dosage formulations.

2. Materials and methods

2.1 Instrumentation: The high pressure liquid chromatographic (HPLC) system used was of model SHIMADZU UFLC-2000 Prominance LC-20AD SPDM 20A Binary Gradient System equipped with Rheodyne injector and PDA detector controlled by LC solutions (version 1.25) software. A column Enable C-18 G Column

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(250mmx 4.6mm, 5µm) was used as a stationary phase.

2.2 Materials: Ambroxol hydrochloride was obtained as a gift sample from Hetero pharma, Hyd, India, Levocetirizine dihydrochloride was obtained as a gift sample from Metro Chem API Pvt. Ltd, Hyd, Sodium dihydrogen phosphate, HPLC grade methanol manufactured by Merck was procured from commercial source. High purity deionised water was obtained from [Millipore, Milli-Q] purification system. Relent-OD capsules (ambroxol hydrochloride 75mg and levocetirizine dihydrochloride 5mg) manufactured by Dr. Reddy's Laboratories, Hyd were purchased from local market.

2.3 Methods

2.3.1 Selection of Wavelength: The wavelength of maximum absorption for ambroxol hydrochloride 248nm and levocetirizine dihydrochloride at 230nm. A single wavelength has selected for estimation of ambroxol hydrochloride and levocetirizine dihydrochloride as 236nm as both the peaks have the significant response. Overlay spectrum of ambroxol hydrochloride and levocetirizine dihydrochloride given in fig 1.

2.3.2 Preparation of mobile phase: Sodium dihydrogen orthophosphate buffer (NaH2PO4) pH 3.0 was prepared by dissolving 15.61 gm of Sodium dihydrogen orthophosphate (NaH2PO4) in 1000 mL of water to get a concentration of 0.1M. Then it was adjusted to pH 3 with ortho phosphoric acid. The mobile phase was prepared in the ratio of 20:80 v/v (Sodium dihydrogen orthophosphate buffer pH 3.0: Methanol) filtered, degassed and sonicated for 10 min.

2.3.3. Preparation of Standard solutions: Standard stock solutions were prepared by dissolving 75 mg of ambroxol hydrochloride and 5 mg of levocetirizine dihydrochloride in 100 mL of mobile phase (stock I) and then 4 mL of the above solution was further diluted to 100 mL with the same mobile phase, to get a concentration of 30µg/mL for ambroxol hydrochloride and 2µg/mL for levocetirizine dihydrochloride respectively (stock II). A typical chromatogram is given in Figure 2.

2.3.4. Procedure for calibration curve: Five standard calibration solutions of ambroxol hydrochloride and levocetirizine dihydrochloride having concentration in the range of 15-45µg/mL and 1-3µg/mL respectively were prepared by diluting the stock I solution with mobile phase. (Table 5 & Fig 3 & 4)

2.3.5. Estimation of ambroxol hydrochloride and levocetirizine dihydrochloride in capsule formulation: Contents from 20 capsules were emptied; the powder equivalent to 75 mg of ambroxol hydrochloride and 5mg of levocetirizine dihydrochloride was accurately weighed and transfered into clean, dry 100mL volumetric flask. The powder was first dissolved in few mL of mobile phase by sonication, the volume was made up to 100mL and then filtered through a Whatmann filter to obtain the concentration 750µg/mL and 50µg/mL for ambroxol hydrochloride and levocetirizine dihydrochloride respectively. From the above stock, 10mL were transferred into a 100mL volumetric flask and volume made up to 100mL with the mobile phase to get the concentration of 75µg/mL for ambroxol hydrochloride and 5µg/mL for levocetirizine dihydrochloride respectively. 20 µL solution of the working sample solutions were injected repeatedly into the chromatograph, at a flow rate of 1 ml/min and detection at 236 nm and the % assay was calculated. (Table 1)

2.3.6. System Suitability Tests: System suitability was verified by injecting working standard solution of 30µg/mL of ambroxol hydrochloride and 2µg/mL of levocetirizine dihydrochloride. Various parameters such as HETP, number of theoretical plates, tailing factor and resolution between the peaks of ambroxol hydrochloride and levocetirizine dihydrochloride were obtained. (Table 2)

2.4. Validation parameters [14]: The HPLC method was validated in terms of accuracy, precision, LOD, LOQ, linearity, range and robustness as per ICH guidelines.

2.4.1. Accuracy: This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50%, 100% and 150%) and the % recovery of ambroxol hydrochloride and levocetirizine dihydrochloride was calculated. (Table 3)

2.4.2. Precision: The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of test sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analyzing the sample in different days. (Table 4)

2.4.3. Specificity: Specificity of the method was evaluated by injecting the blank, working standard and stressed samples into the chromatograph to check the co-elution, if any, at the retention time of ambroxol hydrochloride peak

and levocetirizine dihydrochloride peak. (Fig 5 & 6)

2.4.4. Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and the limit of quantitation (LOQ) for ambroxol hydrochloride and levocetirizine dihydrochloride were determined from standard deviation of the response and the slope. (Table 3)

$$LOD = \sigma/S \times 3.3$$

$$LOQ = \sigma/S \times 10$$

2.4.5. Robustness: The robustness of the method was determined as a measure of the analytical method capability to be unaffected by small variations in method parameters. The different variations such as variation in flow rate by ± 0.2 ml/minute, variation in wavelength by ± 2 nm. At these changed conditions, the standard solutions were injected. The amounts of ambroxol hydrochloride and levocetirizine dihydrochloride were calculated (% assay) in each varied condition. (Table 6)

2.4.6. Forced degradation study: To confirm the stability indicating nature of the analytical method, forced degradation of ambroxol hydrochloride and levocetirizine dihydrochloride was carried out under acid/base hydrolytic, oxidative, photolytic and thermal stress conditions as per ICH recommended test conditions [15].

The drugs were subjected to acid hydrolysis by using 0.1N hydrochloric acid for 6 hrs; base hydrolysis by using 0.1N sodium hydroxide solution for 6hrs; oxidation by using 3%v/v solution of hydrogen peroxide for 6hrs; thermal stress in a controlled- temperature oven at 60^{o}C for 48hrs and photolytic stress using UV lamp for 48hrs. (Fig. 7 & 8)

3. Results and Discussion

3.1 Method development: Several mobile phase compositions were tried to resolve the peaks of ambroxol hydrochloride, levocetirizine dihydrochloride and degraded components. The optimum mobile phase containing methanol–sodium dihydrogen phosphate buffer 80:20 (v/v) was selected because it could resolve the peaks of ambroxol hydrochloride (RT = 3.4 ± 0.02) and levocetirizine dihydrochloride (RT = 4.7 ± 0.03) with a resolution factor of 6.6. The pH was adjusted to 3.0 ± 0.02 with orthophosphoric acid. Quantification was achieved with UV detection at 236 nm on the basis of peak area. A typical HPLC chromatogram obtained during simultaneous estimation of ambroxol hydrochloride and levocetirizine dihydrochloride. (Fig. 2)

3.2 Method validation: The proposed method was found to be accurate as the percentage recoveries at three levels 103.93%, 102.14%, 103.84 for ABH and 104%, 99.19%, 102.2% for LEVC respectively which were found to be within the limits of acceptance criteria (90-110%). For precision and intermediate precision % RSD of ABH and LEVC were within 2.0% thus confirm good precision of the analytical method development. The method found to be specific as there is no interference of the degraded components with the standard drugs. The LOD and LOQ of ABH and LEVC were found to be 3ng/mL, 9ng/mL and 0.05ng/mL, 0.15ng/mL respectively. The calibration plot for the method was linear over the concentration range of 15-45µg/mL for ABH and 1-3µg/mL for LEVC respectively. The correlation coefficients (r^2) were 0.998 for ABH and LEVC respectively. Robustness of the method was performed by making deliberate changes in flow rate and wave length and it was by calculating established % RSD values and was within acceptance criteria range of 2.0%.

3.3 Forced Degradation: Forced degradation studies were carried out for the simultaneous estimation of ambroxol hydrochloride and levocetirizine dihydrochloride in acidic/alkaline hydrolysis, oxidation, thermal and photolytic stress. The peaks of the degradation components were well resolved from the peaks of main component and the drug components passed the purity test. Results of forced degradation study depicit that degradents appeared during oxidation stress study at retention time of 2.8min for ambroxol hydrochloride and 4.4min for levocetirizine dihydrochloride respectively where as during the alkali hydrolysis LEVC showed degradation at retention time of 4.4min.

4. Conclusion

A simple, precise and accurate method was developed for the quantitative estimation of ambroxol hydrochloride and levocetirizine dihydrochloride in bulk drug and marketed formulation without any interference from the excipients. The method is very simple and specific as both the peaks were well separated from their impurities and degraded components. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase and lack of extraction procedures which makes the method especially suitable for routine quality control analysis work.

Acknowledgement
The author thanks Hetero Pharma Ltd and Metrochem API Pvt. Ltd, Hyderabad for providing the gift samples of ambroxol hydrochloride and levocetirizine dihydrochloride. And thank to the EWCP management for supporting this work.

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Table 1: Analysis of formulation

Drug	Labelled amount(mg)	Amount found	%Label claim
ABH	75	75.55	100.93
LEVC	5	4.97	99.38

Table 2: System Suitability Data of ABH and LEVC

Compound	Retention time	Tailing factor	Asymmetry	Theoretical plate	Resolution
ABH	3.46	1.49	0.75	6412.44	
LEVC	4.75	1.38	1	7833.83	6.61

Table 3: Recovery data of ABH and LEVC

Drugs	Levels	Mean recovery	±SD	% RSD
ABH	**L_1**	103.42%	0.54	0.52
	L_2	101.78%	0.46	0.45
	L_3	103.27%	0.51	0.49
LEVC	**L_1**	103.71%	0.48	0.46
	L_2	99.75%	0.50	0.50
	L_3	101.8%	0.42	0.42

(N=3)

Table 4: Intermediate precision data of ABH and LEVC

Compound	Intra-day precision		Inter-day precision	
	% of Label	% RSD	% of Label	% RSD
ABH	100.15	0.55	99.48	0.65
LEVC	102.5	0.58	101.8	0.7

(N=6)

Table 5: Linearity data for ABH and LEVC

Linearity (n=5)	ABH	LEVC
Range	15-45µg/mL	1-3µg/Ml
Mean 'r^2' value	0.998	0.998
Regression equation	Y=13152X – 40621	Y= 52277 X- 1274.5

Table 6: Robustness data of ABH and LEVC

Changing Factor	Level	ABH (n=3), Mean % assay (% RSD)	LEVC (n=3), Mean % assay (% RSD)
Flow rate	0.9 Ml	98.66% (0.92%)	99.18% (0.83%)
	1.1 mL	99.09% (0.95%)	99.78% (0.94%)
Wavelength	231	99.2% (1.11%)	99.02% (0.92%)
	241	98.94% (0.95%)	99.34% (0.91%)

Fig 1: Overlay Spectrum of Ambroxol hydrochloride and Levocetirizine dihydrochloride

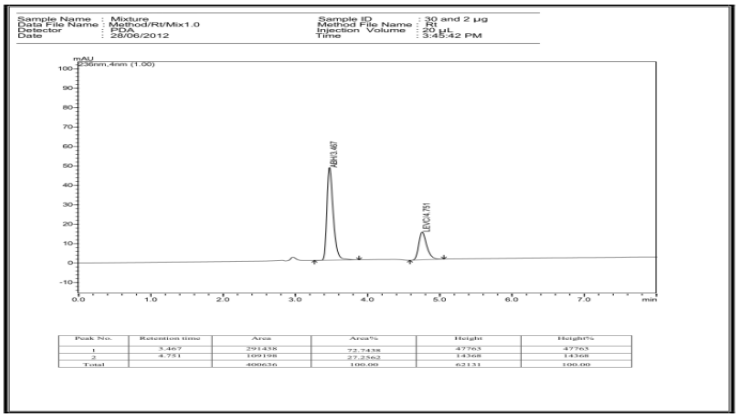

Fig 2: Chromatogram for retention time of Ambroxol hydrochloride anLevocetirizine dihydrochloride in combination

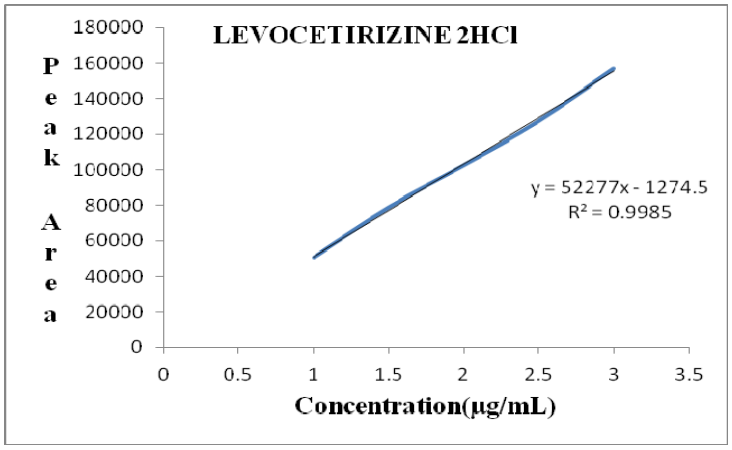

Fig 3: Calibration curve of ABH

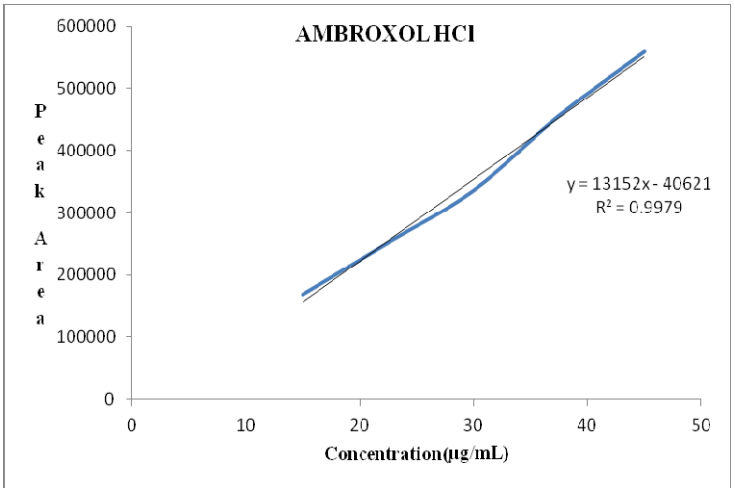

Fig 4: Calibration curve of LEVC

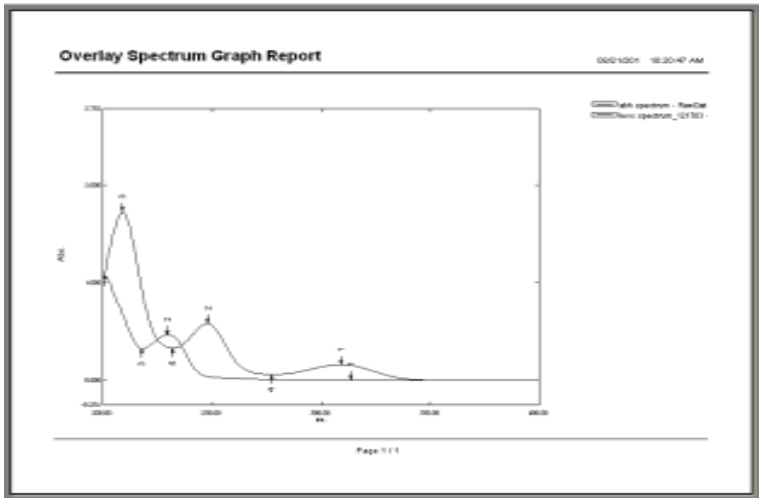

Fig No.5: Chromatogram for specificity (Peak purity profile of ABH)

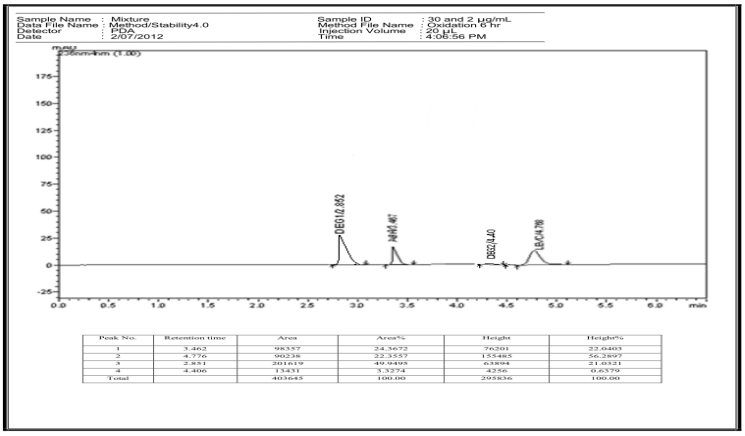

Fig No.6: Chromatogram for specificity (peak purity profile of LEVC)

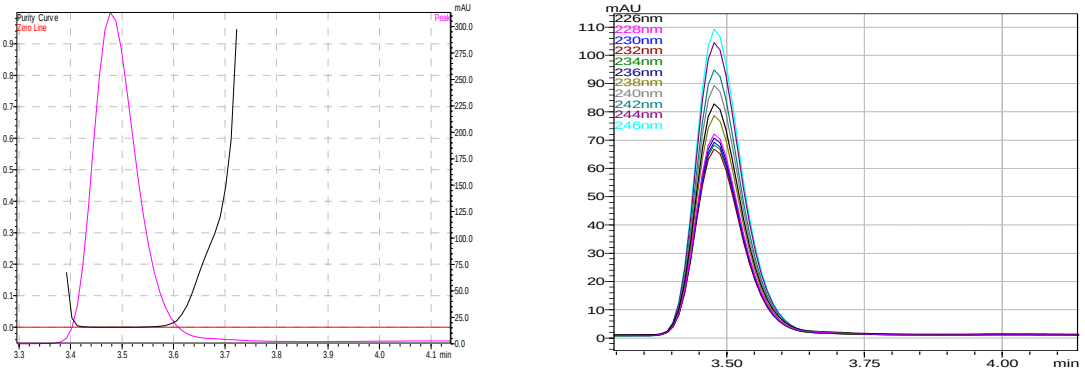

Fig 7: Chromatogram of ABH and LEVC in alkaline condition at 6th hr

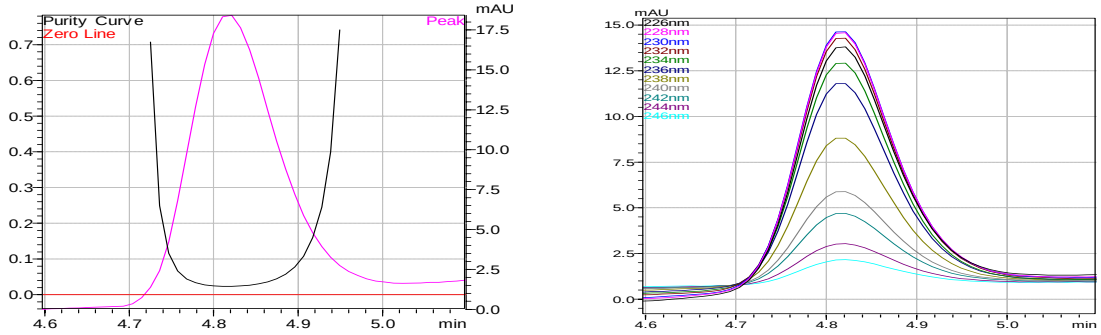

Fig 8: Chromatogram of ABH and LEVC in oxidation condition at 6th hr