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Reverse Phase - High-performance liquid chromatography method for simultaneous estimation of levocetirizine and pseudoephedrine in raw and tablet formulation

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ABSTRACT

A simple, reproducible method was developed for the determination of levocetirizine and pseudoephedrine in tablet formulation using reverse phase HPLC method. HPLC separation was carried over on a Thermo hypersil – keystone C18 (250 x 4.6mm, 0.5µ) isocratic mode column using a mobile phase comprising a 50:50 (% v/v) of acetonitrile and 0.5mM phosphate buffer. The detection was proceeded out by UV detector at 257 nm. The range taken for linearity for levocetirizine and pseudoephedrine were 5 – 25 µg/ml and 120 – 600 µg/ml. The percentage recovery was 100.11 % and 110.04 % for levocetirizine and pseudoephedrine respectively. The amount of levocetirizine and pseudoephedrine found per tablet was 5.05 mg and 119.84 mg respectively.

Keywords: levocetirizine, pseudoephedrine and RP-HPLC.

INTRODUCTION

Levocetirizine is chemically known as 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid dihydrochloride(1-3). It is a non sedating anti-histamine drug and works by preventing the actions of histamine(4-6). Pseudoephedrine HCl is chemically knows as (1S, 2S)-2-methylamino-1-phenylpropan-1-ol hydrochloride (7). Pseudoephedrine comes under sympathomimetic decongestant, which acts through the alpha-adrenergic receptors. Analytical methods such as HPLC and UV spectrophotometry have been reported for these drugs (8). No much analytical method has been reported or developed for these two drugs in pharmaceutical formulations (9-11). In this proposed method we present a simple, precise, reproducible and specific method for determination of tablet form levocetirizine and pseudoephedrine simultaneous.

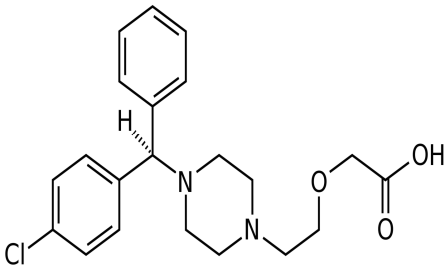

Figure 1: Levocetirizine Chemical structure

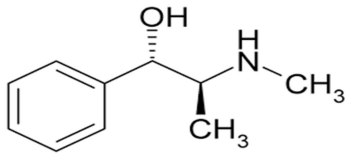

Figure 2: Pseudoephedrine Chemical structure

MATERIALS AND METHODS

Reagents: 99.27% of levocetirizine working standard and 98.92% pseudoephedrine were obtained purchased from Sigma, UK. Combined tablet formulation Levocetirizine HCl 5 mg and Pseudoephedrine HCl 120 brand Verizet D, Manufactured by Cipla pharmaceuticals. Acetonitrile and water (HPLC grade) were obtained from Merck, Darmstadt, Germany. Analytical grade Phosphoric acid, potassium dihydrogen phosphate and ortho phosphate used were from Merck, Darmstadt, Germany.

Preparation of Mobile phase:
The phosphate buffer was prepared using 0.51g of KH_2PO_4 in 1000 ml of HPLC grade water, the pH adjusted to a 5.0 (±0.5) by using phosphoric acid. The resulting solution was filtered with 0.45µ membrane filters and degassed in an ultrasonic bath for 10 mins. The ratio of Acetonitrile: phosphate buffer was (50:50) v/v

HPLC and chromatographic conditions: A Shimadzu class LC-10A HPLC system equipped with LC – 10ATvp pump, SPD – 10A UV detector, and Rheodyne injector was used. Compounds were separated on a C18 hypersil (250 x 4.5mm, 0.5µ) column. The flow rate of 1.3 ml/min was set with 0.5mM phosphate buffer and acetonitrile 50:50 v/v used as a mobile phase. The wavelength of 257nm was set in detector. The peak responses area integrated using Shimadzu chromatographic software.

STANDARD SOLUTIONS:
Preparation of stock solution (standard): 5mg of levocetirizine RS and 120 mg of pseudoephedrine was taken separately in volumetric flask 100ml and with mobile phase diluted to the mark. The mixture kept stand for 15 min, for complete solubility by intermittent sonication, and filtered through a 0.45 µm membrane filter.

Working standard solution: 4ml of each stock solution (standard) from each were taken in a 10ml volumetric flask and diluted to 10ml with mobile phase to get a concentration of 5 µg / ml of levocetirizine and 120 µg/ml of pseudoephedrine.

Sample solution: Twenty tablets of commercially available brand tablets were taken and weighed and grinded into fine powder. 15mg of powder equivalent was weighed and transferred into 25ml volumetric flask then extracted with 25ml of mobile phase. The resulting solution then filtered by membrane filter 0.45 µm and 1ml of the aliquot was diluted again with 25ml of mobile phase to get a concentration of 25µg/ml levocetirizine and 600 µg/ml pseudoephedrine respectively.

Assay: 20µl of sample and standard solutions were injected individually at different time point, into an HPLC injector, from the obtained HPLC peak area of levocetirizine and pseudoephedrine amount of drug in sample were computed. The values are given in Table -2.

Method validation: The present method was conducted to obtain a new, sensitive and convenient method for simultaneous estimation by HPLC. The experimental method was validated according to the ICH guidelines recommendations and USP-30 for parameters such as, system suitability, accuracy, precision, repeatability and specificity.

System suitability: Suitability parameters like resolution, retention time, column theoretical plates and tailing factor was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

Linearity: The linearity of chromatographic method was determined by plotting a graph to concentration vs peak area of levocetirizine and pseudoephedrine standard and determining the correlation coefficients (R2) of the two compounds. For the linearity studies of levocetirizine and pseudoephedrine the specific range was determined at 5 - 25 µg/ml and 120-600 µg/ml for levocetirizine and pseudoephedrine respectively were injected into the HPLC system. For 60 minutes column was equilibrated with the mobile phase before injection of the solutions.

Accuracy: The method accuracy was determined by recovery experiments. The experiment was performed by adding levocetirizine and pseudoephedrine working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %,) and expressed as percent (%) recovered. Three sets were prepared for each level recovery. The recovery statistical results are under the acceptance range (S.D. < 2.0) value for levocetirizine and pseudoephedrine

Precision: The precision of intraday and interday of the analyzed method was determined by 4 repeats of the sample responses on the same day and 4 different days of a week for 4 different concentrations of standard solutions of levocetirizine and pseudoephedrine. 5 - 25 µg/ml and 120-600 µg/ml for levocetirizine and pseudoephedrine respectively, and results are represented in terms of % RSD.

Specificity: The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The RP-HPLC of standard mixture and formulation shows specificity of method. The RP-HPLC method is able to access the analyte in presence of excipients.

Statistical Parameters: The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

RESULTS AND DISCUSSION

According to USP-XXIII the system suitability test were tested on freshly prepared standard stock solution of levocetirizine and pseudoephedrine. The optimum mobile phase of 0.5mM phosphate buffer and acetonitrile of 50:50 % v/v ratios was selected. The ratios was found to resolve peaks ideally of pseudoephedrine (2.2), levocetirizine (5.2) as the retention shown in figure 3 and 4 (chromatograph of test sample). By scanning wide range of wavelength 200-400nm wavelengths, the 257 nm was selected, levocetirizine and pseudoephedrine showed a good response at 257 nm. The Linearity was evaluated by plotting peak area as a functional of analyte concentration for both levocetirizine and pseudoephedrine. The graphical representation was given in figure 5 and data presented in table 1 and 2.

The specific range was determined from linearity studies, for both drugs which is 5-25 µg/ml for levocetirizine and 120-600 µg/ml for pseudoephedrine. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data are presented in table 3. The suitability parameters of the system like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method are presented in figure 6; the data are presented in table 4. The chromatographic retention time of pseudoephedrine and levocetirizine was found to be 2.2 and 5.2 minutes respectively. This is well within the specific limits of 10 minutes.

The tailing factor was found to be 1.41 and 1.29 for levocetirizine and pseudoephedrine respectively. The peaks are symmetrical and theoretical plates for levocetirizine and pseudoephedrine were 9296 and 7456 respectively which shows the column efficient performance. The LOD and LOQ for levocetirizine and pseudoephedrine are presented in table 5. The quantitative estimation of the tablet formulation is presented in table 6 and graphically presented in figure 7. The recovery study for spiked concentration of drugs to the pre analyzes form is represented in table 7.

The tablet formulation assay shows percentage purity ranging from 98.09 to 100.92% for levocetirizine and 101.21 to 99.01% for pseudoephedrine. The percentage deviation was found to be -1.1 to +1.1% and – 1.8 to +0.8 for levocetirizine and pseudoephedrine respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

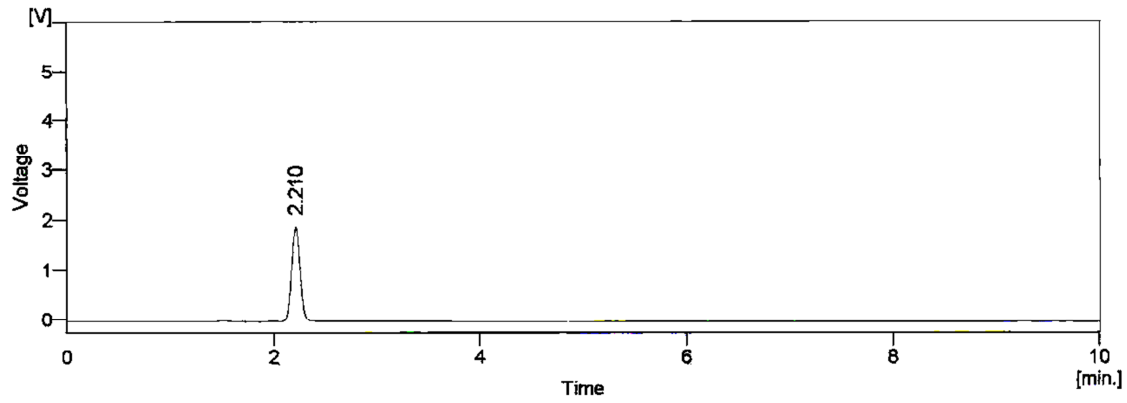

Figure 3: Chromatogram of Pseudoephedrine

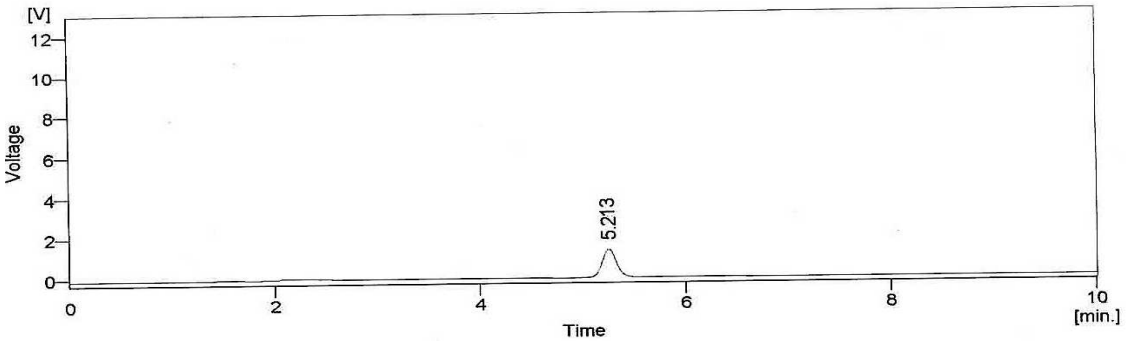

Figure 4: Chromatogram of Levocetirizine

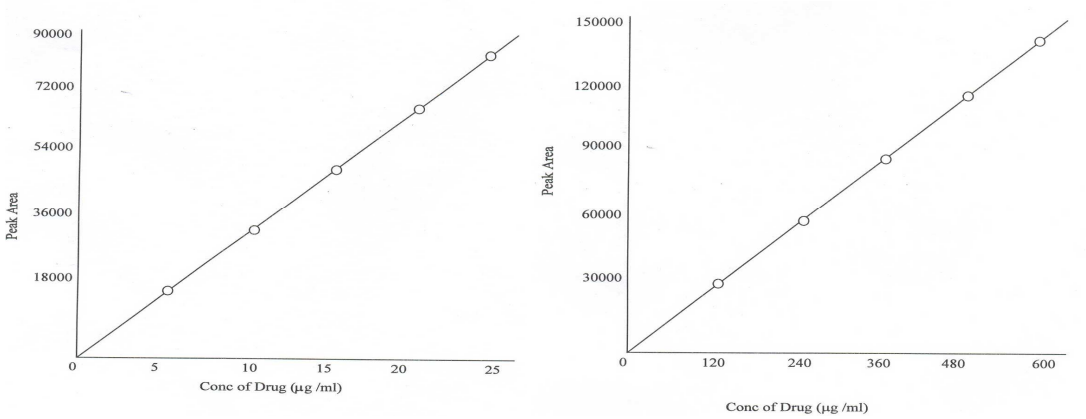

Figure 5: Calibration curve of Levocetirizine and Pseudoephedrine

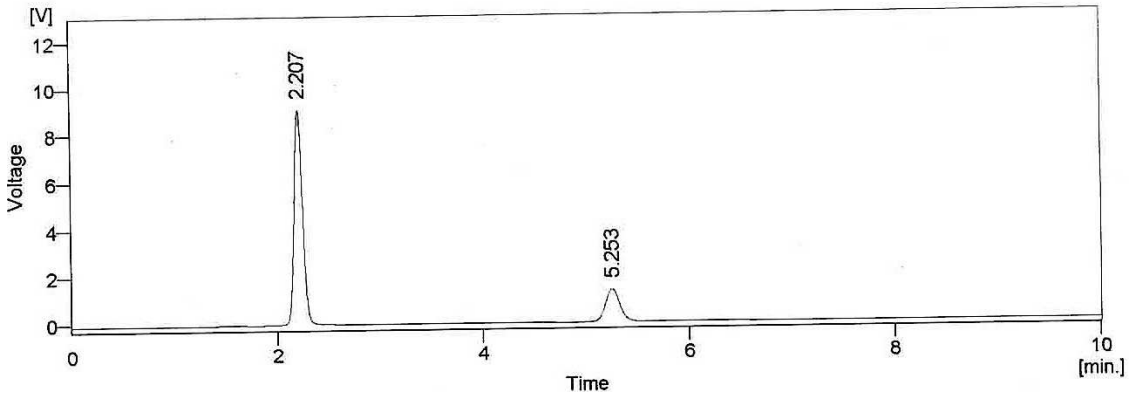

Figure 6: Chromatogram of Pseudoephedrine and Levocetirizine

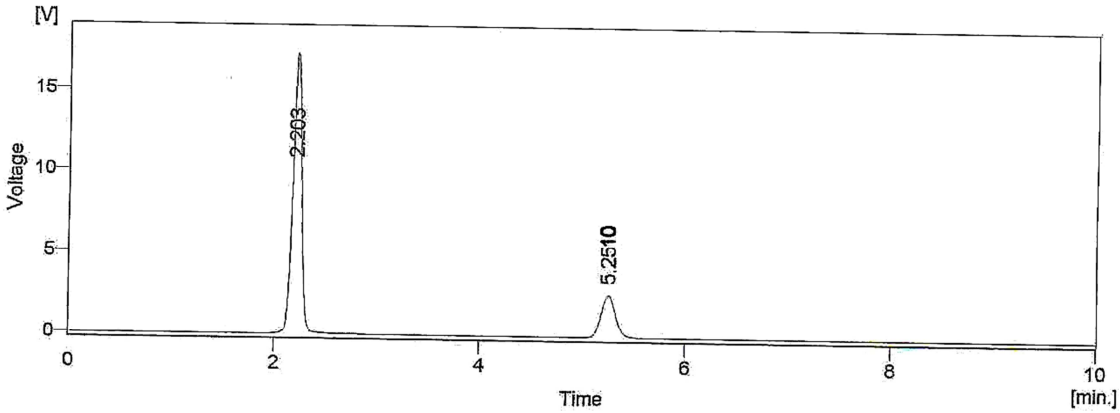

Figure 7: Quantitative estimation (Assay) of levocetirizine and pseudoephedrine in tablet formulation

Table 1: HPLC linearity data for Levocetirizine

SNo	Concentration (µg/ml)	Peak area
1	5	17233
2	10	34194
3	15	50814
4	20	66559
5	25	81069

Table 2: HPLC linearity data for Pseudoephedrine

SNo	Concentration (µg/ml)	Peak area
1	120	27449
2	240	54220
3	360	82789
4	480	129253
5	600	137768

Table 3: Results of statistical parameters

SNo	Parameters	Levocetirizine	Pseudoephedrine
1	Standard deviation (SD)	6.849	8.883
2	Relative standard deviation (RSD)	0.01332	0.01059
3	% RSD	1.332	1.059
4	Standard error (SE)	0.0195	0.0175
5	Correlation Coefficient (r)	0.9992	0.9991
6	Slope (a)	32.0075	24.6393
7	Intercept (b)	1962.70	-2404.08
8	Regression equation Y = (a X + b)	Y = 33.076 X + 1962.7	Y = 24.6393X + -2404.08

Table 4: System suitability parameters

Parameter	Levocetirizine	Pseudoephedrine
R T	5.2	2.2
Theoretical plates	9296	7456
Tailing factor	1.41	1.29
Resolution factor	13.7	13.7
Calibration range (or) Linear dynamic range (LDR)	5 – 25	120 - 600

Table 5: Results of Limit of detection (LOD) & limit of quantification LOQ

Parameters	Levocetirizine	Pseudoephedrine
LOD (µg/ml)	3.01×10^{-4}	2.13×10^{-4}
LOQ (µg/ml)	9.2×10^{-4}	7.2×10^{-4}

Table 6: Quantitative estimation (Assay) of data of Levocetirizine and Pseudoephedrine

Levocetirizine		Pseudoephedrine	
Amount claimed mg/tablet	Amount found mg/tablet	Amount claimed mg/tablet	Amount found mg/tablet
5	4.98	120	118.96
	5.01		119.68
	5.04		119.67
	4.92		119.52
	5.08		121.40
Mean	5.05	Mean	119.84

Table 7: Recovery study for spiked concentration of drugs to the pre analyzes form.

Levocetirizine			Pseudoephedrine		
Amount added (mg)	Amount found (mg)	Amount Recovered (% mg)	Amount added (mg)	Amount found (mg)	Amount Recovered (% mg)
5	4.90	98.09	120	127.52	101.21
10	10.09	100.92	240	237.53	99.01

CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations. The method shows good resolution time between levocetirizine and pseudoephedrine with short time (< 10min). The proposed method is repeatable, very simple, rapid and involves no complicated sample preparation. High percentage of recovery result shows the method is free from interference of excipients in the formulations.

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