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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTIFICATION OF LEVODROPROPIZINE AND CHLORPHENIRAMINE MALEATE IN BULK AND PHARMACEUTICAL FORMULATION BY RP-HPLC

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ABSTRACT

A high performance liquid chromatographic assay method was developed for the simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in syrup dosage form. Chromatogram was run through Discovery C18 (4.6 x 150mm, 5µm) column. Mobile phase containing 0.01N KH_2PO_4 buffer (p^H 2.8): acetonitrile (50:50) pumped through the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C and the detector was monitored at a wavelength of 215 nm. The injection volume was 10 µl with a total run time of 7 min. Retention time of Levodropropizine and Chlorpheniramine maleate were found to be 2.451 min and 3.595 min. The calibration curves were linear in the concentration range of 15-90 µg/ml and 1-6 µg/ml of Levodropropizine and Chlorpheniramine maleate respectively (r^2 = 0.999). The percentage recoveries were found to be 99.26 % for Levodropropizine and 99.43 % for Chlorpheniramine maleate. The limit of detection was found to be 0.33 µg/ml & 0.03 µg/ml and limit of quantitation was found to be 1.00 µg/ml & 0.09 µg/ml for Levodropropizine and Chlorpheniramine maleate respectively. The most effective RP-HPLC method was developed for the estimation of syrup dosage form containing Levodropropizine and Chlorpheniramine maleate. The developed method was validated for system suitability, specificity, accuracy, precision, linearity, limit of detection, limit of quantitation and robustness according to International Conference on Harmonization (ICH) guidelines.

Keywords: Levodropropizine, Chlorpheniramine maleate, RP-HPLC, Method development, Method validation.

INTRODUCTION

Levodropropizine is chemically (-)-(S)-3-(4-Phenyl-1-piperazinyl)-1,2-propanediol. It is the levo-rotatory (S)-enantiomer of dropropizine. It is a non-opioid agent whose peripheral antitussive action may result from its modulation of sensory neuropeptide levels within the respiratory tract. Chlorpheniramine maleate is chemically [3-(4-chlorophenyl)-3-(pyridin-2-yl)propyl] dimethylamine is a antihistaminic used in the treatment of allergy. It acts by competing with histamine for H1-receptor sites on effector cells. A combination of Levodropropizine and Chlorpheniramine maleate is used as cough suppressant and also for allergy, itchy throat, common cold, hay fever, watery eyes, and runny nose. Extensive literature survey revealed that there were liquid chromatographic methods for the estimation Levodropropizine, Chlorpheniramine maleate alone[1,2] and with other combinations.[3-9] But no HPLC method has been reported for the simultaneous estimation of proposed drugs. Hence a validated RP HPLC method has been developed for the simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in bulk and syrup formulation.

MATERIALS AND METHODS

Apparatus

HPLC (Waters 2695) equipped with quaternary pumps, photo diode array detector and auto sampler. The output signal was monitored and processed using Empower software. Ultra sonicator (BVK enterprises), p^H meter (BVK enterprises) and analytical balance (Denver) were used.

Reagents and chemicals

Levodropropizine and Chlorpheniramine maleate syrup containing 30 mg Levodropropizine and 2 mg of Chlorpheniramine maleate, manufactured by Dr. Reddy's Laboratories was purchased from local pharmacy. Orthophosphoric acid of analytical reagent grade was purchased from Rankem, New Delhi, India. Acetonitrile, Milli-Q water and methanol of HPLC grade was purchased from Rankem, Maharashtra, India.

Chromatographic conditions

The chromatographic column used was Discovery C18 (4.6 x 150mm, 5µm). The separation was achieved on isocratic mode. Mobile phase containing 0.01N KH_2PO_4 buffer (p^H 2.8): acetonitrile (50:50) pumped through column at a flow rate of 1.0 ml/min. The column temperature was maintained at 30 °C and the detector was monitored at a wavelength of 215 nm. The injection volume was 10 µl with a total run time 7 min.

Preparation of diluent

The diluent used was water and acetonitrile in the ratio 50:50 %v/v.

Preparation of standard solution

A standard solution of Levodropropizine and Chlorpheniramine maleate was prepared by dissolving 30 mg of Levodropropizine, 2 mg of Chlorpheniramine maleate standards in 25 ml of diluent, sonicated for 10 min, filtered and final volume was made up to 50 ml with diluent. From this 1 ml has been taken and diluted to 10 ml so as to get final concentration of 60 µg/ml of Levodropropizine and 4 µg/ml of Chlorpheniramine maleate.

Preparation of sample solution
The quantity of syrup equivalent to 30 mg Levodropropizine and 2 mg of Chlorpheniramine maleate was transferred into a 50 ml volumetric flask, 25 ml of diluent was added and sonicated for 25 min. Then it was filtered and further the volume was made up with diluent. From this 1 ml has been taken and diluted to 10 ml so as to get final concentration of 60 µg/ml of Levodropropizine and 4 µg/ml of Chlorpheniramine maleate.

RESULTS AND DISCUSSION

Method development
Several chromatographic conditions were tried for better separation and resolution. A number of trials were performed with different solvents in different ratios over a wide p^H range, with different flow rates and column temperatures to get good, sharp peaks with better retention times for efficient resolution between two peaks. Satisfactory results were achieved in terms of retention time, resolution, symmetry and sensitivity on isocratic trial Chromatogram was run through Discovery C18 (4.6 x 150mm, 5µm) column. Mobile phase containing 0.01N KH_2PO_4 buffer (p^H 2.8): acetonitrile (50:50) pumped at a flow rate of 1.0 ml/min. The column temperature was maintained at 30 °C and the detector was monitored at a wavelength of 215 nm. The injection volume was 10 µl with a total run time of 7 min. The optimized standard chromatogram was presented in Figure 1.

Method validation
The developed method was validated for parameters such as system suitability, specificity, linearity, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines for analytical procedures Q2 [R1].[10]

System suitability
To verify the system performance, six replicate samples containing 600 µg/ml of Levodropropizine and 40 µg/ml of Chlorpheniramine maleate were analyzed using the developed method. The factors such as theoretical plate count, tailing factor and resolution between the peaks were taken into consideration for testing system suitability. The results were presented in Table 1. From the results, it was found that the resolution between two peaks was 45.4 and the tailing factor for both drugs was 1.30 and 1.25. The theoretical plate count was 5646 and 7770 for Levodropropizine and Chlorpheniramine maleate respectively. All the parameters were found to be within the limits.

Specificity
To check the specificity of the method the blank and placebo were prepared as per test method and injected into the chromatographic column and checked for the interfering peaks at the retention times of analyte peaks and thereby found no interfering peaks at the retention times of anlayte peaks. Hence the results prove that the developed method was specific for the estimation of Levodropropizine and Chlorpheniramine maleate.

Precision
Precision of method was verified by repeatability. Repeatability was checked by injecting six individual homogenous preparations of standard solution under the same operating conditions over a short interval of time. Relative standard deviation (RSD) was calculated. The results of precision studies were tabulated in Table 2. From the data obtained from precision studies, it was found that the RSD values were less than 2 and hence assure the precision of the developed method.

Accuracy
Accuracy was determined in triplicate at three concentration levels 50%, 100% and 150% of target assay concentration. Known quantities of drug substances corresponding to the specified level of the label claim were added to the pre-analyzed sample. Each set of addition were repeated three times. The results were expressed as the percentage of analytes recovered by the assay. The recoveries of the drugs from a series of spiked concentrations were presented in Table 3. According to statistical data, the recoveries of drugs were found to be within the specified range of 98 to 102%. Hence it can be concluded that the method was highly accurate for the determination of Levodropropizine and Chlorpheniramine maleate.

Linearity
A linear relationship was evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance by diluting standard stock solution of Levodropropizine and Chlorpheniramine maleate. The calibration curves were plotted for Levodropropizine and Chlorpheniramine maleate (Figure 2 & 3). The data was subjected to statistical analysis using a linear-regression model, the regression equation and correlation coefficient. The results were tabulated in Table 4. They were found linear over the concentration range of 15-90 µg/ml of Levodropropizine and 1-6 µg/ml of Chlorpheniramine maleate). The correlation coefficient (r^2) was found to be 0.999 which show a good linearity between concentration and peak area.

Limit of detection and limit of quantitation
The detection limit was determined based on the standard deviation of y-intercepts and the slope from set of three calibration plots using the formula method. The LOD and LOQ of Levodropropizine and Chlorpheniramine maleate were found to be 0.33 µg/ml & 1.00 µg/ml and 0.03 µg/ml & 0.09 µg/ml respectively. From the results of LOD and LOQ it was concluded that the developed method has good sensitivity.

Robustness
The robustness of the developed method was established to make sure that the developed analytical method was unaffected by small, but deliberate changes in the method parameters. For this, the experimental conditions like flow rate, column temperature and mobile phase ratio were deliberately altered and the system suitability parameters were evaluated. The solutions were prepared as per the test method and injected at different variable conditions like flow rate (±0.1 ml/min), column temperature (± 2 ºC) and mobile phase ratio (± 2 % organic phase). The results were presented in Table 5. In all the varied chromatographic conditions no significant differences have been observed in system suitability parameters and were found to be within the limits. The result indicates that the method was unaffected and found to be robust.

Applicability of the developed method
The assay of Levodropropizine and Chlorpheniramine maleate in syrup dosage form was performed to check the applicability of the developed method. The standard preparations and sample preparations were made from the pure drugs and formulation respectively. The prepared solutions were injected six times into the chromatographic system. The drugs present in the formulation were estimated by comparing with the reference standards. The average percentage of drugs was calculated and they were found to be 99.43 % and 99.91 % for Levodropropizine and Chlorpheniramine maleate respectively. Hence the developed method was successfully applied for the determination of drug content in the formulation.

TABLE 1: RESULTS OF SYSTEM SUITABILITY

Analytes	Resolution*	Tailing factor*	Theoretical plates*
Levodropropizine	-	1.30	5646
Chlorpheniramine maleate	45.4	1.23	7770

*Average of six determinations

TABLE 2: RESULTS OF REPEATABILITY

N	Levodropropizine		Chlorpheniramine maleate	
	Rt	Peak area	Rt	Peak area
Injection 1	2.440	1235415	3.595	131106
Injection 2	2.441	1229557	3.595	132663
Injection 3	2.441	1218046	3.599	132169
Injection 4	2.445	1221573	3.599	131819
Injection 5	2.448	1217562	3.600	130047
Injection 6	2.452	1239817	3.604	132602
Mean	1226995		131734	
SD	9384.3		1005.3	
RSD (%)	0.8		0.8	

RSD = relative standard deviation; SD = standard deviation; Rt=retention time

TABLE 3: RESULTS OF ACCURACY (RECOVERY STUDIES)

Analyte	Pre analysed sample concentration (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	*Mean recovery
Levodropropizine	60	30	29.83	99.43	100.36
	60	30	29.55	98.51	
	60	30	30.04	100.14	
	60	60	60.58	100.97	
	60	60	60.80	101.33	
	60	60	60.70	101.16	
	60	90	90.97	101.08	
	60	90	90.89	100.99	
	60	90	89.65	99.61	
Chlorpheniramine maleate	4	2	1.98	98.86	99.55
	4	2	1.96	98.14	
	4	2	1.99	99.61	
	4	4	3.97	99.28	
	4	4	3.98	99.45	
	4	4	4.01	100.24	
	4	6	6.04	100.71	
	4	6	6.02	100.30	
	4	6	5.96	99.35	

*Average of triplicate determinations

TABLE 4: RESULTS OF LINEARITY

% Level	Levodropropizine		Chlorpheniramine maleate	
	Concentration (µg/ml)	Peak areas	Concentration (µg/ml)	Peak areas
25	15	355792	1	40254
50	30	652918	2	70785
75	45	957368	3	106409
100	60	1283348	4	137995
125	75	1564438	5	171769
150	90	1859495	6	205755
Correlation coefficient		0.999	0.999	
Regression equation		y = 20539x + 29101	y = 33839x + 3191	

TABLE 5: RESULTS OF ROBUSTNESS

Parameter	Levodropropizine		Chlorpheniramine maleate	
	Tailing	Plate count	Tailing	Plate count
Less flow rate (0.9 ml/min)	1.31	5285	1.27	7532
;.More flow rate (1.1 ml/min)	1.29	5882	1.28	7941
Less column temperature (25ºC)	1.28	6022	1.28	7893
More column temperature (35ºC)	1.28	5625	1.28	8089
Less organic phase (45:55)	1.31	5579	1.22	7834
More organic phase (55:45)	1.27	6319	1.16	7800

*Average of six determinations

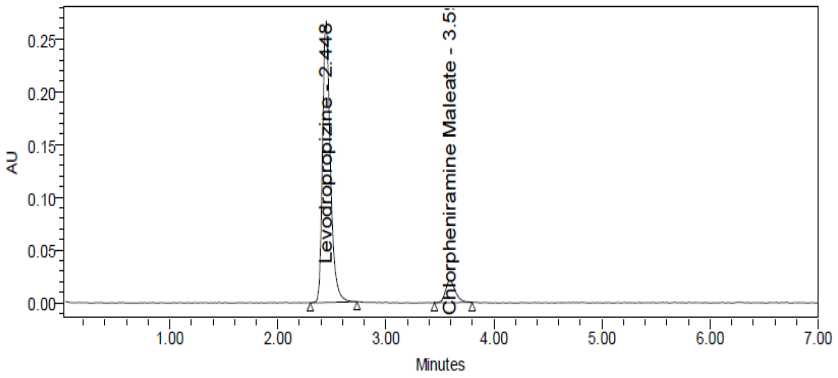

Figure 1: Chromatogram of Levodropropizine and Chlorpheniramine maleate standard preparation

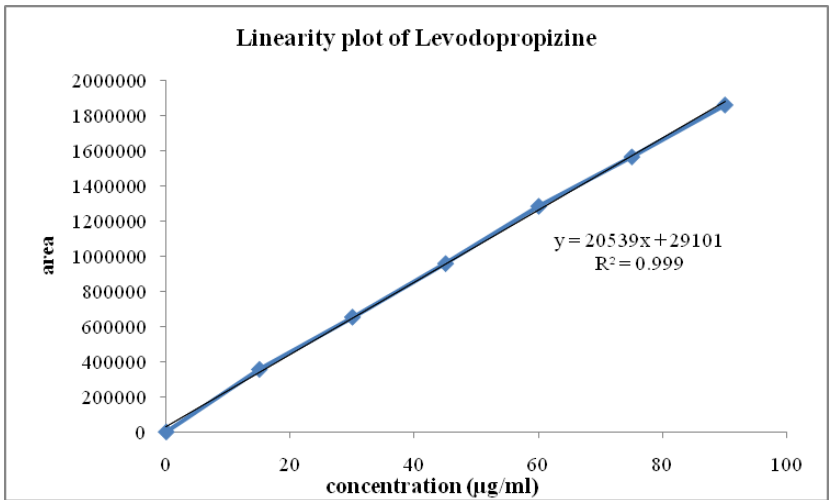

Figure 2: Linearity chart of Levodropropizine

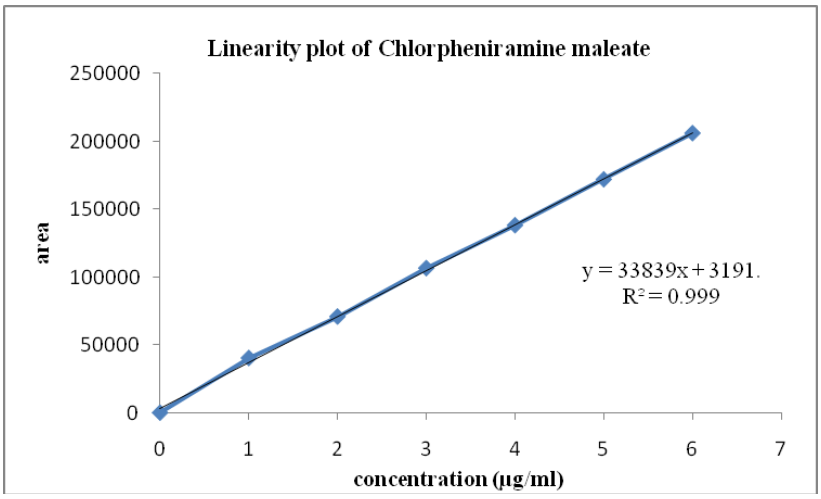

Figure 3: Linearity chart of Chlorpheniramine maleate

CONCLUSION

The study was undertaken to develop and validate a simple, sensitive and rapid analytical RP-HPLC method for simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in syrup dosage form. The developed method was validated by means of system suitability, specificity, accuracy, precision, linearity, LOD, LOQ and robustness as per ICH guidelines. The results of the study indicate that the proposed HPLC method of analysis can be successfully used in routine analysis of formulation containing Levodropropizine and Chlorpheniramine maleate.

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