

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CHLOROPHENIRAMINE MALAETE AND LEVODROPROPazine BY USING RP-HPLC

Gella Sasi Kala*, Gadi Vijaya Lakshmi, Yandamuri Narayudu and Kanchumarti Namratha

Department of Pharmaceutical Analysis & Quality Assurance VJ'S College of Pharmacy, Diwancheruvu, Rajamahendravaram, Andhra Pradesh-533296.

***Corresponding Author: Gella Sasi Kala**

Department of Pharmaceutical Analysis & Quality Assurance VJ'S College of Pharmacy, Diwancheruvu, Rajamahendravaram, Andhra Pradesh-533296.

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Levodropropazine and Chlorpheniramine Maleate in syrup dosage form. Chromatogram was run through Denali C18 150 x 4.6 mm, 5 μ . Mobile phase containing Buffer 0.02N KH₂PO₄ (3.47pH): Acetonitrile taken in the ratio 65:35 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was 0.02N KH₂PO₄. Temperature was maintained at 30°C. Optimized wavelength selected was 252 nm. Retention time of Levodropropazine and Chlorpheniramine Maleate were found to be 2.300 min and 3.187 min. %RSD of the Levodropropazine and Chlorpheniramine Maleate were and found to be 0.6 and 0.6 respectively. %Recovery was obtained as 100.12% and 100.40% for Levodropropazine and Chlorpheniramine Maleate respectively. LOD, LOQ values obtained from regression equations of Levodropropazine and Chlorpheniramine Maleate were 0.63, 1.90 and 0.09, 0.27 respectively. Regression equation of Levodropropazine is $y = 16729x + 5154$. And $y = 52826x + 2622$. of Chlorpheniramine Maleate. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Levodropropazine, Chlorpheniramine Maleate, RP-HPLC.**INTRODUCTION**

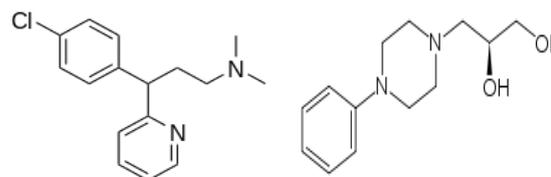
Chemically Chlorpheniramine (CLP) was an [3-(4-chlorophenyl)-3-(pyridin-2-yl)propyl]dimethylamine] Molecular weight and molecular formula of CLP were 274.788 g/mole and C₁₆H₁₉ClN₂ respectively. Chlorpheniramine binds to the histamine H₁ receptor. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine. Structure of the CLP was shown in figure 1 (A)1.

Chemically Levodropropazine (LEV) was an (2S)-3-(4-phenylpiperazin-1-yl) propane-1,2-diol. Molecular weight and molecular formula of LEV were 236.315 g/mole and C₁₃H₂₀N₂O₂ respectively. Levodropropazine is the levo-rotatory (S)-enantiome of dropropazine, a racemic non-opiate antitussive agent. Levodropropazine acts through a mainly peripheral tracheobronchial antitussive effect by inhibition of vagal C-fiber and its sensor neuropeptide.

Compared with dropropazine, levodropropazine exhibits in animal models similar antitussive activity but considerably lower central nervous system (CNS)

depressant effects. It is also less likely to cause sedation in treated patients. Structure of the MET was shown in figure 1 (B)2.

Literature survey reveals there are several methods to estimated two drugs in single or in combination of two drugs.^[5-9] but there is only very few HPLC methods are available for simultaneous estimation of CLP and LEV, so the scope of developing and alidating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

**Figure 1: Structure of (A) Chlorpheniramine (B) Levodropropazine.**

MATERIALS AND METHODS

Reagents and Chemicals: The active pharmaceutical ingredient samples of Chlorpheniramine and Levodropropazine were obtained from Spectra Pharma Pvt. Ltd., Hyderabad. All the chemicals and solvents used were HPLC grade. The tablet pharmaceutical dosage of combination of these drugs was purchased from local pharmacy.

Instrumentation: Waters HPLC(2695 series) with quaternary pumps, Photo Diode array detector and auto sampler integrated with Empower software-2 was used for separation of these drugs.

Chromatographic conditions: Discovery C₁₈ (4.6 x 150mm, 5µm) Column was used for analytical separation. Potassium dihydrogen ortho phosphate and Acetonitrile was taken in the ratio of (65:35% v/v) mobile phase for the investigation with a flow rate of a 0.8ml/min. The temperature was maintained at 30°C. The injection volume was 10µl and the UV detection was achieved at 252nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.47): Accurately weighed 2.72gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.47 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Mixture of 600 ml of 0.01N KH₂PO₄ buffer (pH-3.47) and 400 ml of Acetonitrile in the ration of 60:35 v/v were

mixed and degased in ultrasonic water bath for 15 minutes and filtered through 0.45 µ filter paper. Mobile phase was used as a diluent.

Preparation of mixture Standard stock solution (CLP 600µg/ml and LEV 40µg/ml): Accurately weighed 30mg of Levodropropazine, 2mg of Chlorpheniramine Maleate and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. The concentrations of 600µg/ml of LEV and 40µg/ml of CLP were achieved respectively.

Preparation of Sample (Tablet) stock solutions: Syrup equivalent to 30mg Levodropropazine and 2mg of Chlorpheniramine Maleate was transferred into a 50 ml volumetric flask, 20ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (600µg/ml of Levodropropazine and 40µg/ml of Chlorpheniramine Maleate).

Optimized chromatographic conditions

Column : Discovery C₁₈ (4.6 x 150mm, 5µm)
Mobile phase : 0.02N KH₂PO₄ (3.47pH): Acetonitrile (35:65)
Flow rate : 0.8 ml/min
Wavelength : 252.0nm
Temperature : 30°C
Injection Volume: 10µL

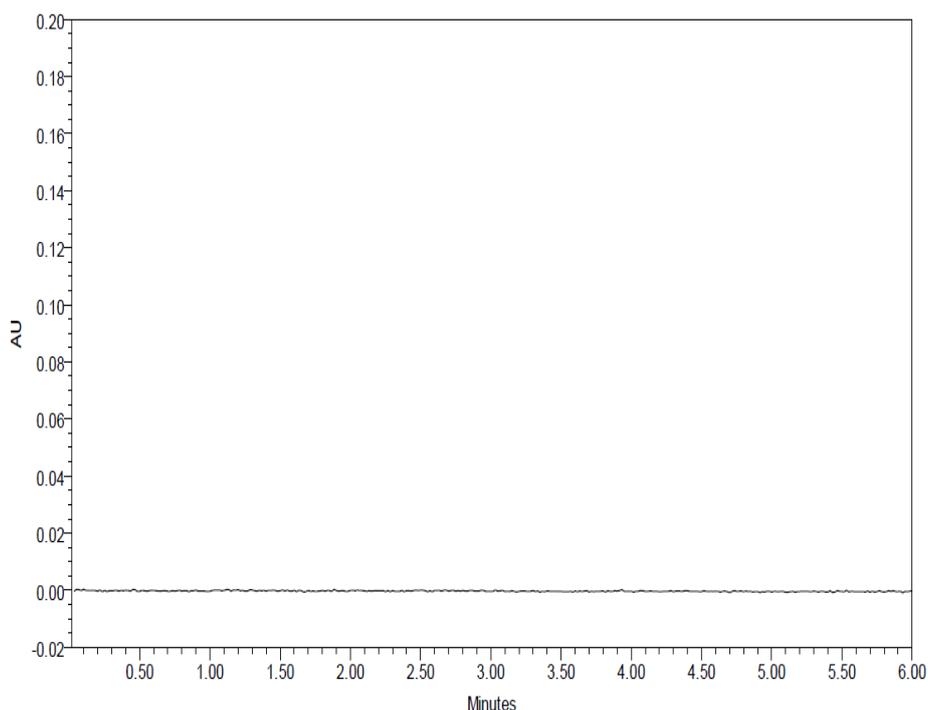
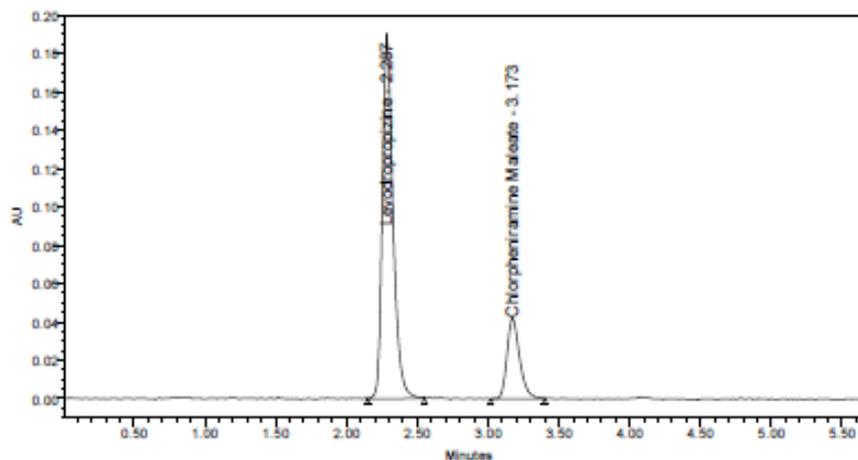


Figure 2: Blank Chromatogram.



	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Levodropropizine	2.267	993700	4475.2		1.3
2	Chlorpheniramine Maleate	3.173	261681	6110.0	5.8	1.3

Figure 3: Chromatogram of standard mixture of CLP and LEV.

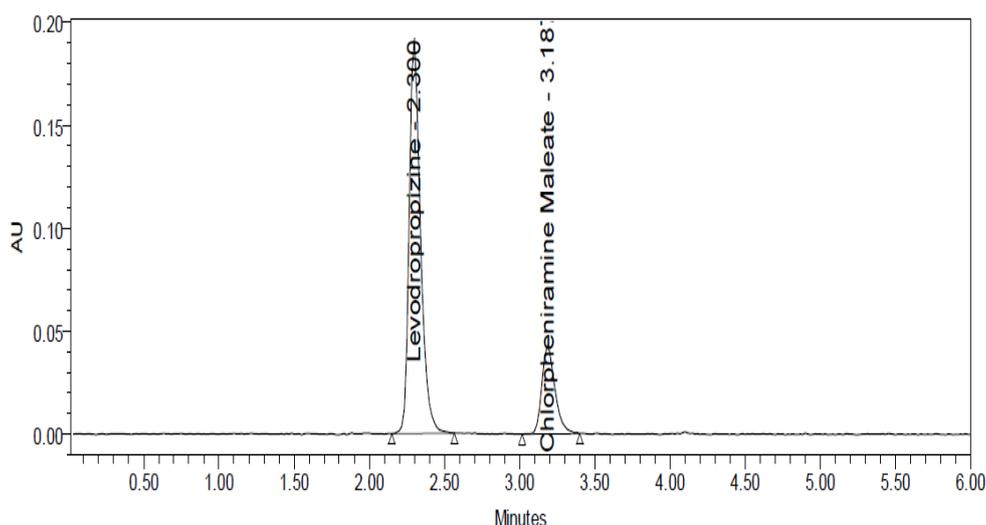


Figure 4: Chromatogram of sample mixture of CLP and LEV.

Validation

The above optimized chromatographic method has been validated for the assay of CLP and LEV using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of CLP and LEV drug mixtures (600µg/ml of CLP, 40µg/ml of LEV respectively). Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of CLP and LEV 600µg/ml, 40µg/ml respectively. The precision of each method was

ascertained separately from the peak area by actual determination of five replicates of a fixed amount of drug (of CLP and LEV 600µg/ml, 40µg/ml respectively). The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 50,100 and 150% of CLP and LEV. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae $LOD = 3.3 \times \text{standard deviation} / \text{slope}$; $LOQ = 10 \times \text{standard deviation} / \text{slope}$. Robustness was performed by following the same method with different flow rate.

RESULT AND DISCUSSION

The regression equation for LEV was found to be $y = 16729x + 5154$ (slope, intercept and correlation coefficient were found to be 16729, 5154 and 0.999 respectively) and linear over beer's range of 15-90 $\mu\text{g/ml}$. The regression equation for CLP was found to be $y = 52826x + 2622$ (slope, intercept and correlation coefficient were found to be 52826, 2622 and 0.999 respectively) and linear over beer's range of 1-6 $\mu\text{g/ml}$. Linearity graph of CLP and LEV were shown in Figure 5 & 6 respectively. Linearity data was shown in table 1. The percentage of content of CLP and LEV in tablet dosage form was $99.50 \pm 0.64\%$ & $99.67 \pm 0.6\%$ respectively. The precision and ruggedness were determined using the %RSD of the peak area for six replicate preparations of the drug. The %RSD of precision and ruggedness of LEV were found to be 0.9 and 0.3 respectively and for CLP were 0.7 and 0.4 respectively. The calculated RSD values were less than

2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug CLP and LEV and along with 5 $\mu\text{g/mL}$ of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 100.33 ± 0.5 , 99.53 ± 0.9 and $100.48 \pm 0.1\%$ w/w for 50%, 100% and 150% respectively for LEV. The mean percentage recoveries were found to be 99.98 ± 1.0 , 99.59 ± 0.4 and $100.28 \pm 0.7\%$ w/w for 50%, 100% and 150% respectively for CLP. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for CLP and LEV was found to be $0.63\mu\text{g}$ and $0.09\mu\text{g}$ respectively. LOQ for CLP and LEV was found to be $1.90\mu\text{g}$ and $0.27\mu\text{g}$ respectively. Summary of all the validation parameter shown in table 4.

Table 1: Linearity data of standard mixture of CLP and LEV.

Levodropropazine		Chlorpheniramine Maleate	
Conc($\mu\text{g/mL}$)	Peak area	Conc($\mu\text{g/mL}$)	Peak area
0	0	0	0
15	257630	1	56358
30	511973	2	110504
45	755905	3	160200
60	1016621	4	217011
75	1253737	5	265544
90	1509712	6	318082

Table 2: System precision data of CLP and LEV.

S. No	Area of Levodropropazine	Area of Chlorpheniramine Maleate
1.	1027653	211151
2.	1025971	212504
3.	1006238	215172
4.	1028483	211373
5.	1034558	212405
6.	1027260	213333
Mean	1025027	212656
S.D	9680.7	1468.7
%RSD	0.9	0.7

Table 3: Degradation data of CLP and LEV.

Type of degradation	Levodropropazine			Chlorpheniramine Maleate		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	952480	92.83	7.17	195429	91.81	8.19
Base	955576	93.13	6.87	198693	93.34	6.66
Peroxide	994010	96.88	3.12	202607	95.18	4.82
Thermal	1002292	97.68	2.32	207932	97.68	2.32
Uv	1011829	98.61	1.39	209425	98.38	1.62
Water	1021093	98.61	1.39	210767	99.01	0.99

Table 4: Summary of validation data of CLP and LEV.

Validation	Parameters	Ivabradine	Metoprolol
Linearity	Range ($\mu\text{g/ml}$)	15-90 $\mu\text{g/ml}$	1-6 $\mu\text{g/ml}$
	Regression coefficient	0.999	0.999
	Slope(m)	16729	52826
	Intercept(c)	5154	2622
Assay	Mean % content	99.50%	99.67%
Specificity		Specific	Specific
System precision	%RSD	0.9	0.7
Method precision	%RSD	0.6	0.6
Accuracy % recovery	% recovery	100.12%	100.40%
LOD		0.63	0.09
LOQ		1.90	0.27

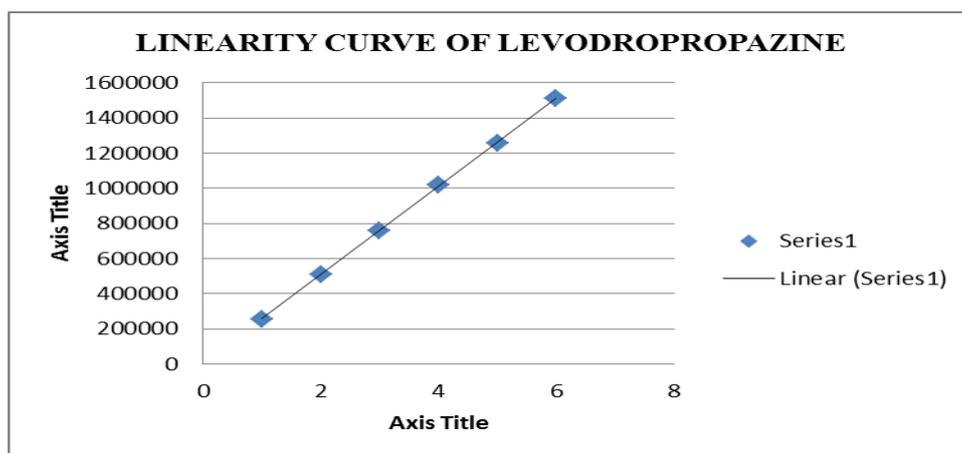


Figure 5: Linearity curve of Levodropropazine

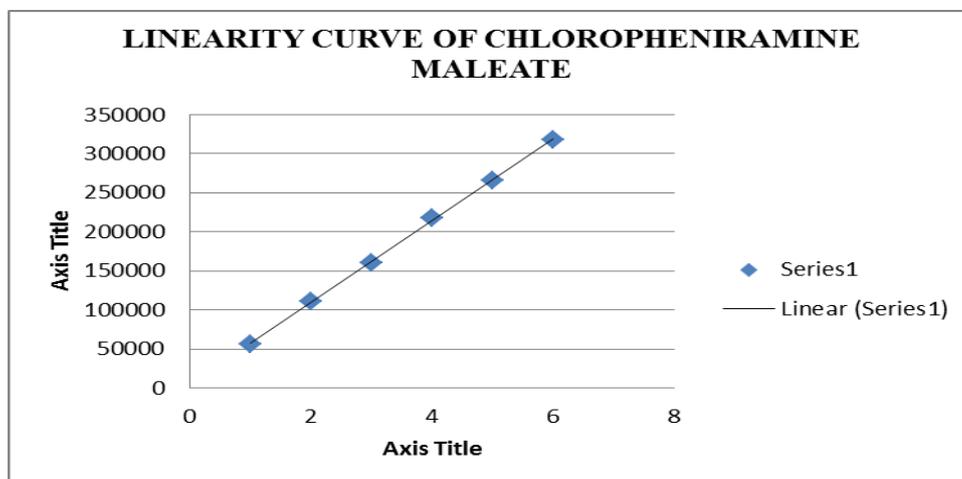


Figure 6: Linearity curve of Chlorpheniramine Maleate.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Degradation data shown in table 3.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Levodropropazine and

Chlorpheniramine in Syrup dosage form was developed and the proposed method as suitable for routine analysis of CLP and LEV.

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