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Development and validation of hplc method for analysis of chlorpheniramine maleate & levocetirizine in syrup formulation

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Abstract
A simple, rapid, accurate, precise and validated High-performance Liquid Chromatographic method for the simultaneous estimation of Chlorpheniramine Maleate and Levocetirizine HCl in bulk and Multicomponant formulation. The Reversed-phase liquid chromatographic analysis was performed on a Phenomenex -C18 column (250×4.6 mm i.d., 5 µm particle size) column with mobile Phase 0.01 M Disodium Hydrogen Phosphate Buffer PH 3.3 and Acetonitrile (98:02 v/v) and column temperature at 40°C. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 20 µl. Detection was performed at 230 nm. The retention time for CPM and LCET were 9.569 min and 14.096 min respectively. The method was validated and shown to be linear for CPM and LCET in 12.5-37.5 µg/mL (r2=0.998) and 25-75 µg/mL (r2=0.998) respectively. The proposed methods were successfully applied to the determination of Chlorpheniramine Maleate and Levocetirizine HCl in tablets, with high percentage of recovery, good accuracy and acceptable precision. Different analytical performance parameters such as Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantitation and Robustness were determined according to International Conference on Harmonization ICH Q2B guidelines. The developed RP- HPLC method is suitable for estimation of Chlorpheniramine Maleate and Levocetirizine HCl in tablet formulation. Hence this method can be used in quality control for routine analysis of the finish drug product.

Keywords: Chlorpheniramine maleate, levocetirizine hcl, rp-hplc, simultaneous estimation

Introduction

Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and assurance. Multicomponent formulations in market are increasing therefore it is very essential that two or more number of drugs should be estimated simultaneously. Chlorpheniramine Maleate is chemically 1-(N, N, Dimethylamine)-3-(p-chlorophenyl)-3-(alphapyridyl) propane maleate (Fig.1). CPM is a first-generation alkyl amine antihistamine. Mechanism of Chlorpheniramine binds to the histamine H1 receptor. It blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine [5]. Uses of Chlorpheniramine maleate is effectively used to treat common cold, conjunctivitis and acute allergic symptoms like rhinitis and urticaria, sneezing, rhinorrhea and itching of eyes, nose, throat and pruritus, atopic dermatitis, contact dermatitis and insect bites.

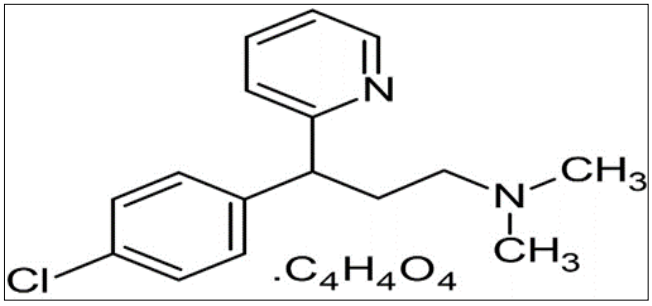

Fig 1: Structure ofChlorpheniramine Maleate

Levocetrizine, chemically 2-[2-[4-[(R)-(4- chlorophenyl)-phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid1. It is a third-generation nonsedative antihistamine and used in the form of levocetrizine dihydrochloride for the treatment of allergic rhinitis and chronic idiopathic urticaria. It is an active R-enantiomer of cetirizine, orally active, potent, and selective and long acting H1- histamine receptor antagonist with no anticholinergic activity.

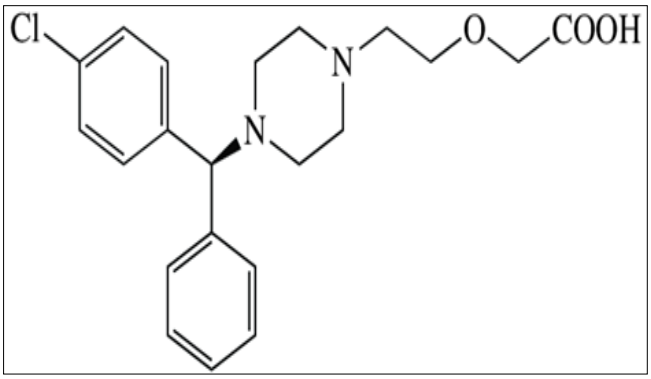

Fig 2: Structure of Levocetrizine

Materials and Methods

Pharmaceutical grade Chlorpheniramine Maleate and Levocetirizine HCl were pursued as a gift sample from Leben Pharma Private Limited, Akola (MH) India. All chemicals and solvents of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India.HPLC Instruement which has Perkin Elmer series 200, with PDA detector, Phenomenex C18 (250mm×4.6mm), 5µm). Marketed formulation Lyzevac Plus® Syrup containing CPM 10 mg and LCT 5 mg was used as sample purchased from local pharmacy. Calibrated glassware was used throughout the work.

Method Development

Different mobile phases containing methanol, water, Acetonitrile, and different buffers in different proportion were tried and finally of these 0.01M Disodium hydrogen Phosphate buffer pH 3.3 and acetonitrile (98:02) was selected as mobile phase which gave good resolution and acceptable peak parameters for both Chlorpheniramine Maleate an d Levocetirizine HCl.

Apparatus and chromatographic Conditions

Chromatographic separation was performed on a PerkinElmer series 200, with PDA detector, Phenomenex C18 (250mm×4.6mm), 5µm) was used for the separation. The mobile phase containing mixture of these 0.01M Disodium hydrogen Phosphate buffer pH 3.3 and acetonitrile (98:02) was delivered at a flow rate of 1.0 mL/min with detection at 230 nm. The mobile phase was filtered through a 0.2 and degassed. The injection volume was 20 µl; analysis was performed at temperature 25 ºC.

Preparation of Standard Solutions

10 mg of CPM & 10 mg of LCET was weighed accurately and transferred to 50 ml volumetric flask and dissolved in 25 ml of Acetonitrile and then the volume was made up to the mark with Acetonitrile to get 100 µg/ml and 100 µg/ml of stock solution of CPM and LCET respectively.

Preparation of Sample Solutions

For the test solution 5 ml (Each 5 ml marketed preparation contains CPM 2.5 mg and LCET 5mg) of syrup was added into a 100 ml volumetric flask the content were mixed with diluent & sonicated for 15 min & same content were filtered through 0.45 µ membrane filter. 1 ml of resultant was taken in a 10 ml of volumetric flask & volume was made up to the mark with diluent.

.A 20 µl volume of each sample solution was injected into the HPLC system under the chromatographic condition as stated above. The peak area of each peak was measured at 230 nm.With the optimized chromatographic conditions, as teady baseline was recorded, the mixed standard solution was injected and the chromatogram wasrecorded. The retention time of CPM and LCTZ were found to be 9.569 and 14.096 min respectively. This procedure was repeated for the sample solution obtained from the formulation. The proposed method was found to be specific and no interference from common excipients was observed.

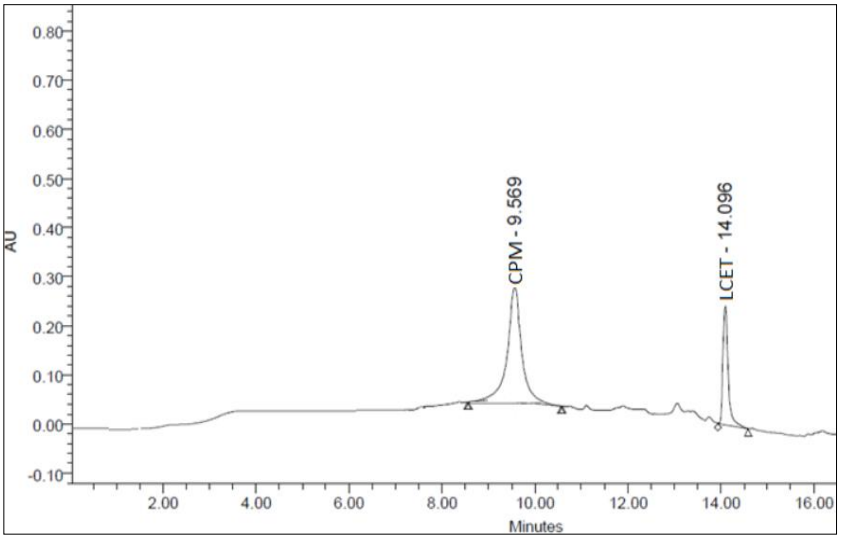

Fig 3: HPLC separation of the CPM and LCET drugs in selected mobile phase showing CPM at 9.569 min and LCET at 14.096 min.

Validation

The proposed method was validated by studying several parameters such as Linearity, Precision, Accuracy, Limit of Detection (LOD), Limit of Quantization, Robustness & Ruggedness.

Linearity and Range

Mixture of linearity dilutions were prepared ranging from 50% to 150% of labeled claim and injected into the HPLC system under developed chromatographic conditions, three times each the chromatograms were recorded and the peak responses for CPM and LCET were recorded. The concentrations for CPM were 12.5, 20, 25, 30 and 37.5 µg/ml. For LCET the concentrations were 25, 40, 50, 60 and 75 µg/ml. The average area response for each were recorded and the graph of concentration verses average area response for CPM and LCET.

Table 1: Linearity and range studies

Sr. No	Conc. range % (CPM)	Conc. (µg/ml) (CPM)	Mean Peak area (CPM) (n=3)	Conc. range % (LCET)	Conc. (µg/ml) (LCET)	Mean Peak area (LCET) (n=3)
1	50	12.5	2606740	50	25	96737
2	80	20	4188776	80	40	155179
3	100	25	5173470	100	50	191474

4	120	30	6258164	120	60	228768
5	150	37.5	7760205	150	75	288225

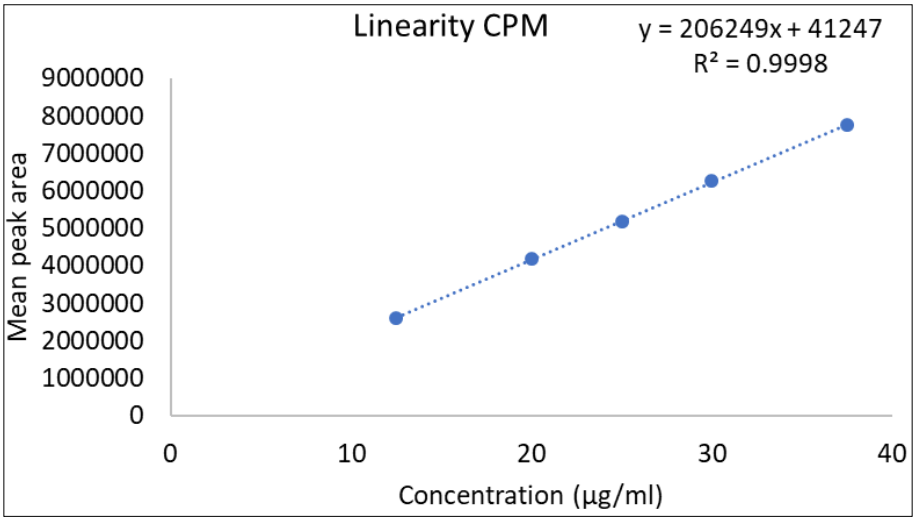

Fig 4: Graph of linearity of CPM.

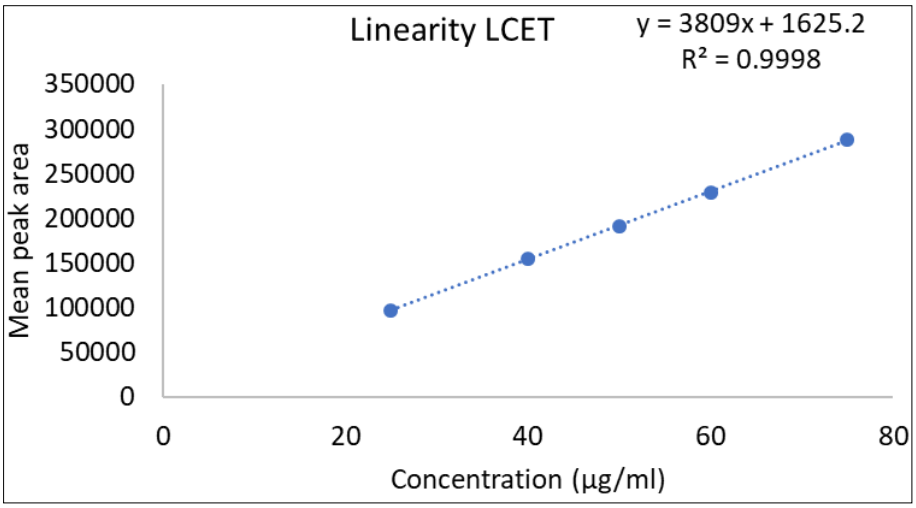

Fig 5: Graph of linearity of LCET.

Precision

Mixture of standard preparation was injected into the HPLC system chromatograms recorded and the peak responses were measured for the CPM and LCET. The assay was calculated in and % label claim for CPM and LCET for each of the injected system suitability solution. The average of % assay of the three preparations and % RSD for the three observations was recorded.

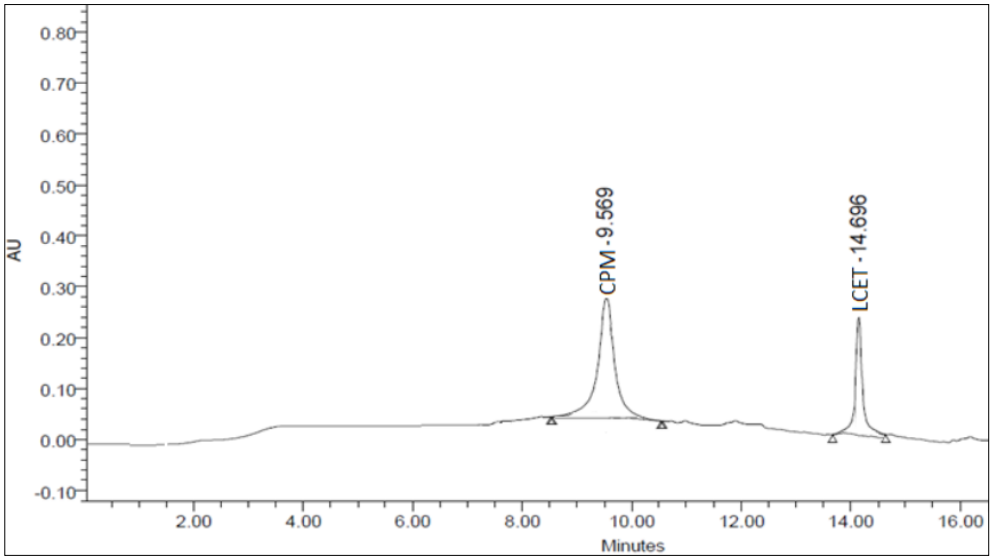

Fig 6: Chromatogram system precision showing repeatability

Table 2: System precision showing repeatability

Injection No.	Peak area Response	
	CPM	LCET
1	518443	1925629
2	517257	1904717
3	516340	1913900
Average	517347	1914749
SD	1054	10481.8
% RSD	0.20	0.54

Intermediate precision (Ruggedness)

As per the intermediate precision studies three mix standard solutions were prepared as per the method. Same were injected into different HPLC system (preferably with different manufacturer or same manufacturer with different configuration) by using the different column and by the different analyst at different date.

Table 3: Intermediate precision Studies

Sr.no.	CPM		LCET	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay% of LC
1	2.5	100.04	5.0	100.20
2	2.5	100.02	5.0	100.14
3	2.5	100.03	5.0	100.12
Average	2.5	100.03	5.0	100.15
SD	0.07	0.01	0.04	0.04
% RSD	0.02	0.009	0.8	0.04

Table 4: Results of method precision using marketed sample

Sr.No.	CPM		LCET	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay % of LC
1	2.5	100.05	5	100.25
2	2.5	99.96	5	100.18
3	2.5	99.95	5	100.10
Average	2.5	99.98	5	100.17
SD	----	0.05	----	0.08
% RSD	----	0.05	----	0.07

Accuracy

Accuracy studies were done by standard addition method. Known amount of standard was spiked into placebo at different concentration (80,100 & 120 %) of the labelled claim and resultant solution was subjected to chromatographic evaluation. Peak areas were reported & amount of standard added was reported for recovery studies. The results are depicted in table 19

Table 5: Accuracy studies by standard addition method

	CPM			LCET		
	Levels			Levels		
	80%	100%	120%	80%	100%	120%
Amt added (µg/ml)	20	25	30	40	50	60
	20	25	30	40	50	60
	20	25	30	40	50	60
Amt taken (µg/ml)	20	25	30	40	50	60
	20	25	30	40	50	60
	20	25	30	40	50	60
Amt recovered (µg/ml)	19.9	25.1	30.1	40.1	50.1	60
	19.95	25.2	30.1	40.1	50.1	60.1
	19.90	25.0	30	40	50.1	60.1
% Recovery	99.5	100.4	100.33	100.2	100.2	100
	99.75	100.8	100.33	100.2	100.2	100.16
	99.5	100	100	100	100.2	100.16
Mean recovery	99.58	100.4	100.22	100.13	100.2	100.1
% RSD	0.02	0.05	0.07	0.09	0.07	0.27

6.3.5. Robustness

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system

method parameters, the standard solution and test solutions were injected for each of the changes made to access the robustness of proposed analytical method.

Table 6: Results of robustness

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	CPM	0.20	0.12	0.33	NMT 2.0
		LCET	0.54	0.43	0.78	
2	Theoretical plates	CPM	1531592	1173880	1976612	NLT 2000
		LCET	102295	92604	12435	
3	Tailing factor	CPM	1.6	1.92	1.4	NMT 2.0
		LCET	1.02	0.99	1.14	
4	Retention Time (Min)	CPM	9.81	5.98	8.56	
		LCET	14.17	14.87	13.43	

Result and Discussion

A Syrup formulation containing Chlorphenamine maleate and Levocetirizine HCl is available in market (Lyzevac Plus® Syrup) for the treatment of cold. Due to this rise in the multicomponent formulations, the challenges faced by the analytical chemist are on the rise. Estimation of drugs from a multicomponent formulation requires a method capable of discriminating the two or more components. Approaches to multicomponent analysis can be broadly categorized into those which rely on physical separation of components prior to analysis (e.g. chromatographic methods) and those that do not actually separate the components (e.g. simultaneous equations method in spectroscopy).

The present work involved the development of accurate, precise, simple and suitable RP-HPLC method for estimation of the drugs in multicomponent tablet formulations.

Literature survey revealed few spectrophotometric methods like simultaneous equation method and FTIR method for simultaneous estimation of these drugs in pharmaceutical formulations.

Simple and reliable spectroscopic methods for estimation of Chlorphenamine maleate and Levocetirizine HCl in combined dosage form have been attempted.

In RP-HPLC method, the analyte was resolved using buffer (10mM): acetonitrile (98:02), pH 3.3 at a flow rate of 1.0ml/min, on HPLC auto-sampler system containing Photo diode array (PDA) detector with empower software and phenomenex C18 column (4.6 x 150 mm). The detection was carried out at 230 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range.

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of Chlorphenamine maleate and Levocetirizine HCl in their combined syrup dosage formulations. The RP-HPLC method is accurate, precise, specific, reproducible and sensitive.

No interference of additives, matrix etc. is encountered in RP-HPLC methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

The methods were found to be reliable, reproducible, rapid and economic also**.**

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