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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Chlorpheniramine maleate (CPM) and Phenylephrine hydrochloride (PEH) combination is used to reduce symptoms of cold. Extensive literature survey revealed the existence of few chromatographic methods for the simultaneous estimation of the mentioned drugs. Hence, it was aimed to develop a simple, accurate and precise RP-HPLC method to estimate CPM and PEH in pharmaceutical dosage forms. The chromatography system employed was Shimadzu Prominence 2695 series. Chromatography was performed on an Inertsil C-18 column as the stationary phase and an isocratic mobile phase constituting of mixed phosphate buffer pH 5.5: Acetonitrile (55:45). The flow rate of 0.8 ml/min was employed for the separation at ambient temperature conditions. The detection of the separated components was done with UV detector at 266 nm. The retention time for PEH was at 2.54 min and for CPM at 5.10 min. The developed method was validated as per Q2 (R1) of ICH guidelines. The detector response was found to be linear for CPM in the range 24-56 µg/ml and for PEH in the range 18-42 µg/ml. Precision results were decorous & within limits (% RSD<2). The method was found to be specific for the detection of CPM and PEH. The accuracy values were 101% for CPM and 100.31% for PEH, which are indicative of good recovery values. The assay results were 100.05% for PEH and 99.88% for CPM. The method was also found to be robust for change in flow rate and change in detection wavelength. All the results obtained were indicative of a simple, precise, specific and accurate RP-HPLC method, which can be applied for the analysis of CPM and PEH in bulk and dosage forms.

INTRODUCTION:

The emergence of multi drug dosage forms is hypothecated to the increase in health care needs of the people. These multi drug combinations pose a daunting challenge to the pharmaceutical industry in various aspects like formulation, manufacturing and analysis. Among them, the assessment of the quality of these combinations is of major concern. Thus, simultaneous estimation of the multi drug dosage forms by various analytical methods in gaining importance. The developed analytical methods should be simple without complex steps, economical

and offer good accuracy, precision and robustness for the analysis of the multi drugs in the dosage forms. Chlorpheniramine Maleate[1-2](CPM), is a histamine H1 antagonist (or more correctly, an inverse histamine agonist) of the alkyl amine class. It competes with histamine for the normal H1-receptor sites on effector cells of the gastrointestinal tract, blood vessels and respiratory tract. It provides effective, temporary relief of sneezing, watery and itchy eyes, and runny nose due to hay fever and other upper respiratory allergies. Phenylephrine Hydrochloride[3-4](PEH) acts predominantly by a direct effect on alpha-adrenergic receptors. Phenylephrine Hydrochloride also has an indirect effect by releasing norepinephrine from its storage sites. These two drugs in combination were predominantly used to reduce symptoms of cold. The chemical structures of the two drugs were shown in figure 1 and 2. Extensive literature[5-10] survey revealed that various analytical methods like potentiometric titrations, UV spectroscopic methods and HPLC methods were reported for the analysis of these two drugs individually and in their combination with other drugs. However, only few methods[11-15] were reported for the simultaneous analysis of these two drugs simultaneously. Thus, there is a need to develop an analytical method, which is simple, accurate, precise and specific for the estimation of both the drugs. The aim of the current research work is to develop a RP-HPLC method for the simultaneous estimation of Chlorpheniramine Maleate and Phenylephrine Hydrochloride in their pharmaceutical dosage forms.

MATERIALS AND METHODS

Instruments used: Analytical Balance (Shimadzu, AY-220), Shimadzu Prominence HPLC system (Model-2695) with LC solutions, Ultra sonicator (PCI Analytics Ltd.-6.5L) and pH meter (Elico) were used in present study.

Materials used:

The working standards of Chlorpheniramine maleate and Phenylephrine hydrochloride were procured from Lupin Pharmaceuticals Pvt.Ltd. Commercial formulation of the drugs were purchased from local market. Methanol HPLC-grade, Acetonitrile-HPLC grade, HPLC water, Potassium dihydrogen ortho phosphate-AR grade, Di potassium hydrogen phosphate-AR grade, Orthophosporic acid-AR grade were procured from E. Merck (India) Ltd., Mumbai. Double distilled water was obtained from in-house distillation unit.

Methods:

Preparation of Standard Solutions:

Weigh accurately 10 mg of Phenylephrine and 10 mg of Chlorpheniramine maleate in 10 ml of volumetric flask separately and dissolve in 10ml of mobile phase to obtain final concentration of 1mg/ml.

Preparation of working solutions: Standard solutions of Phenylephrine Hydrochloride (1ml) and Chlorpheniramine Maleate (1ml) from the stock was transferred to a 10ml volumetric flask and diluted to the mark to obtain 100µg/ml.

Preparation of Mixed Phosphate Buffer pH 5.5: Solution I - Dissolve 13.61 g of potassium phosphate in sufficient water to produce 1000 ml. Solution II- Dissolve 35.81 g of disodium hydrogen phosphate in sufficient water to produce 1000 ml. Mix 96.4 ml of solution I with 3.6 ml of solution II.

Preparation of Mobile Phase:

A mixture of 55 volumes of Mixed Phosphate buffer pH 5.5 and 45 volumes of Acetonitrile was used as mobile phase. The mobile phase was sonicated for 10 min to remove gases. Using a 100µl syringe, 20µl volumes of each solution were injected into the liquid Chromatograph under the chromatographic conditions mentioned in table 1.

Analysis of the marketed formulation

Twenty tablets taken and weighed. The quantity of the powder equivalent to 4 mg of Chlorpheniramine maleate and 10 mg of Phenylephrine Hydrochloride was weighed accurately and then transferred to 100 ml volumetric flask containing 70 ml of mobile phase. It was then sonicated for 15 min. The solution was filtered through a 0.45 µ filter and volume was made up to the mark with mobile phase. The dilution was made by taking 0.8 ml of the above solution into 10ml of volumetric flask and made up to the mark with mobile phase. The final dilution contained about 4 µg/ml of Chlorpheniramine Maleate and 10 µg/ml of Phenylephrine Hydrochloride respectively. Using a 100µl syringe, 20µl volumes of standard solution and sample solution were injected, each 5 times into the liquid chromatography under the previously mentioned chromatographic conditions.

Calculations: The amount of Phenylephrine Hydrochloride and Chlorpheniramine Maleate present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation.
AT: Peak area due to assay preparation.
WS: Weight of Phenylephrine Hydrochloride /Chlorpheniramine maleate in mg.
WT: Weight of sample in assay preparation.
DT: Dilution of assay preparation.
LC: Label claim
P : Purity.
DS: Standard dilution.

RESULTS AND DISCUSSION:

Method development:

A series of trials were conducted using phosphate and citrate buffers having different pH to obtain the required separations. After reviewing the results, mixed phosphate buffer of pH 5.5 was selected as the buffer and Acetonitrile was selected as organic modifier. The detection wavelength was selected from the UV spectra of the two drugs, which was an isobestic point. The developed method produced symmetric peaks 2.547 minutes for CPM and 5.103 minutes for PEH and satisfied all the peak properties as per USP guidelines[16]. The results were shown in Table 2 and figure 2.

Method validation: The developed RP-HPLC method was validated as per ICH guidelines[17]. And the results were mentioned below.

System suitability parameters: System Suitability was performed on five individual injections of CPM and PEH and the results were shown in table 3 & 4.

Stability of the solution: The stability of the prepared standard solutions were tested by the proposed method and all the solutions were found to be stable up to 24 hours after their preparation.

Specificity: The developed method was tested for the specificity of the drugs in presence of mobile phase and other matrices and was found to be specific. The chromatograms were shown in figures 3 & 4.

Linearity and Range: The detector linear response was tested in the range 24-56 µg/ml for CPM and for PEH in the range 18-42 µg/ml for PEH. The results were shown in table 5 and figure 6 & 7.

Precision: The repeatability of the method was tested by using intra and inter day precision. All the results were found to be < 2% of RSD and hence the method was precise.

Accuracy: Accuracy of the method was established by % recovery studies using standard addition method. All the result were found to be in acceptable limits as shown in table 6 & 7.

Table 1: Optimized Parameters for RP-HPLC

Column	Inertsil (250×4.6× 5µ)
Mobile Phase	Mixed phosphate buffer, pH 5.5 : Acetonitrile (55:45)
Solvent/diluent	Mixed phosphate buffer, pH 5.5: Acetonitrile (55:45)
Flow Rate	0.8 ml/min
Injection Volume	20µl
Pump Mode	Isocratic
Column Temperature	Ambient
UV detection	266 nm

Table 2: Chromatogram Optimized parameters

Peak	Ret. Time	Theoretical plates	Name	Resolution (USP)
1	2.547	7501	Phenylephrine Hydrochloride	--
2	5.103	8091	Chlorpheniramine Maleate	9.027

Table 3: Summary of System Suitability Parameters for Phenylephrine Hydrochloride

Inj. No	RT	Peak Area	Theoretical Plates	USP Tailing Factor
1	2.540	1067890	7501	0.733
2	2.539	1067899	7481	0.733
3	2.540	1067893	7471	0.734
4	2.543	1067791	7463	0.737
5	2.539	1066792	7499	0.737
Mean		1067653		
SD		483.3865		
% RSD		0.045276		

Table 4: Summary of System Suitability Parameters for Chlorpheniramine Maleate

Inj. No	RT	Peak Area	Theoretical Plates	USP Tailing Factor
1	5.101	1529049	8091	0.912
2	5.99	1529038	8093	0.919
3	5.102	1529100	8088	0.901
4	5.101	1529050	8079	0.913
5	5.102	1529041	8076	0.917
Mean		1529056		
SD		25.34364		
% RSD		0.001657		

Table 5: Linearity of Phenylephrine Hydrochloride & Chlorpheniramine Maleate by RP-HPLC

Phenylephrine Hydrochloride			Chlorpheniramine Maleate	
S. No	Con.mcg	Area	Con.mcg	Area
1	18	840397	24	1336678
2	24	1055899	32	1533549
3	30	1268990	40	1728050
4	36	1483905	48	1945117
5	42	1728715	56	2161966

Table 6: Recovery of Chlorpheniramine Maleate from Formulation

%addition of label claimed	Label claimed µg/ml	Spiked Conc.	Obtained Amount µg/ml	%Recovery
50%	4	2	6.12	102%
100%	4	4	7.98	99.75 %
150%	4	6	10.14	101.4 %

Table 7: Recovery of Phenylephrine from Formulation

%addition of label claimed	Label claimed µg/ml	Spiked Conc. µg/ml	Obtained Amount µg/ml	%Recovery
50%	10	5	14.86	99.06 %
100%	10	10	20.12	100.6 %
150%	10	15	25.32	101.28 %

*Average of 3 experiments

Table 8: Summary of Robustness Data for Phenylephrine Hydrochloride

Parameter	Condition	System suitability parameters	
		Theoretical plates	USP Tailing factor
Change in flow rate(± 0.2 ml/ min)	0.8 ml/ min	3820909	0.629
	1.2 ml/ min	2433649	0.891
Change in detector wavelength	258 nm	3338952	0.819
	262nm	3220914	0.653

Table 9: Summary of Robustness Data for Chlorpheniramine Maleate

Parameter	Condition	System suitability parameters	
		Theoretical plates	USP Tailing factor
Change in flow rate(± 0.2 ml/ min)	0.8 ml/ min	3161869	1.011
	1.2 ml/ min	3571961	1.744
Change in detector wavelength	258 nm	2869890	1.155
	262nm	3131899	0.419

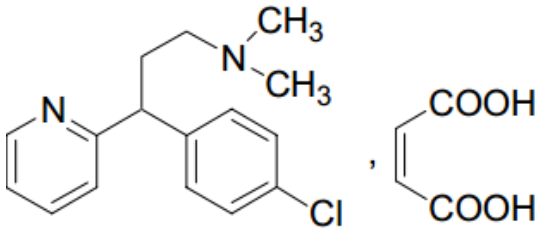

Figure 1: Chemical structure of Chlorpheniramine Maleate

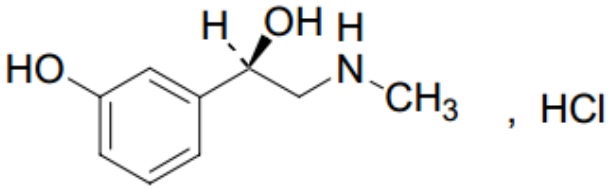

Figure 2: Chemical structure of Phenylephrine Hydrochloride

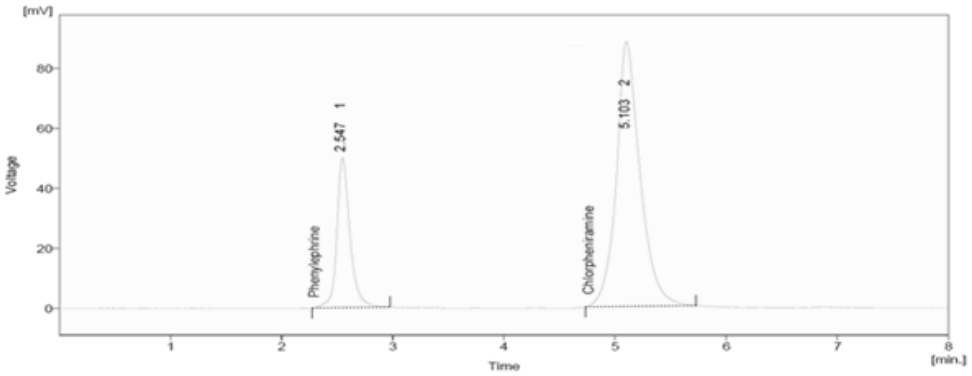

Figure 3: Chromatogram for Optimized Method

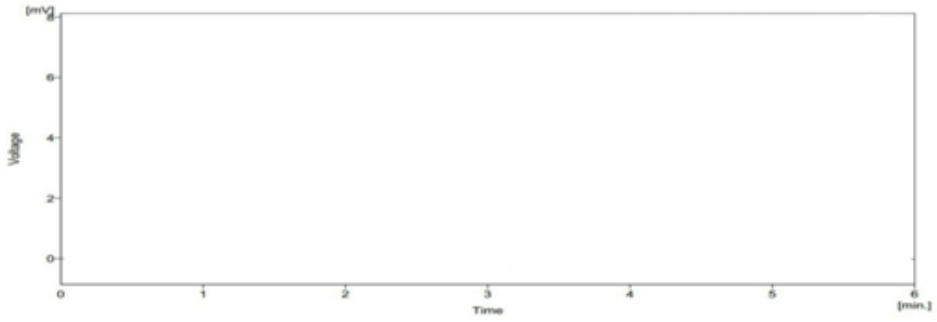

Figure 4 : Chromatogram of Blank

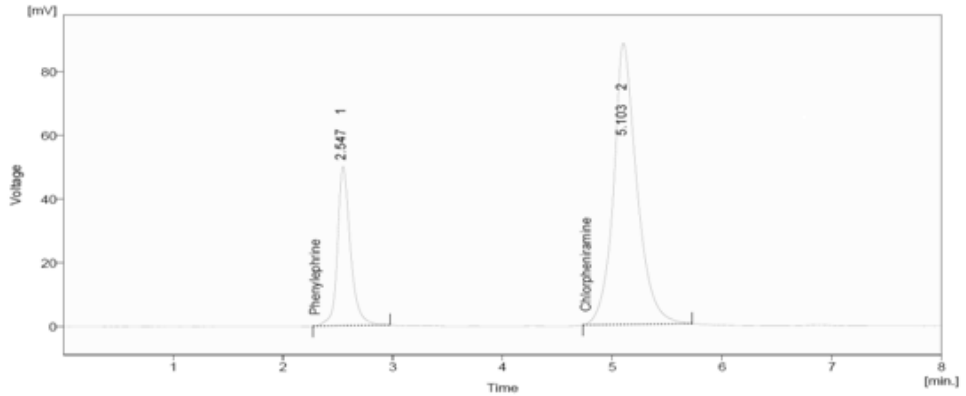

Figure 5: Chromatogram of Phenylephrine Hydrochloride and Chlorpheniramine Maleate

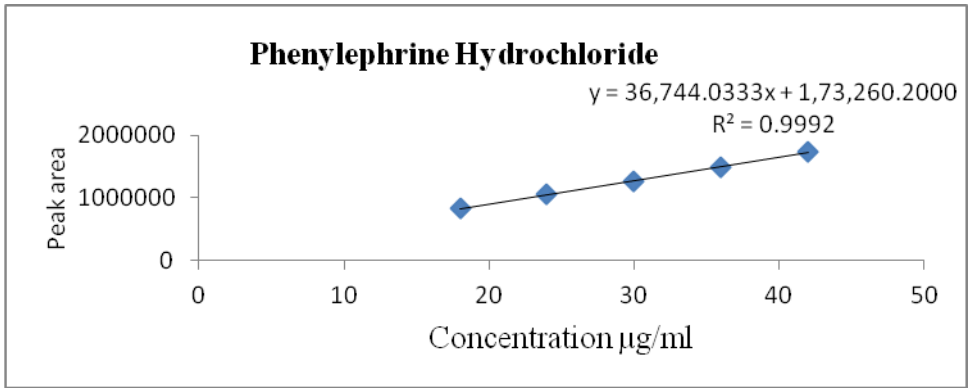

Figure 6: Calibration Curves for the Linearity Set of Phenylephrine Hydrochloride

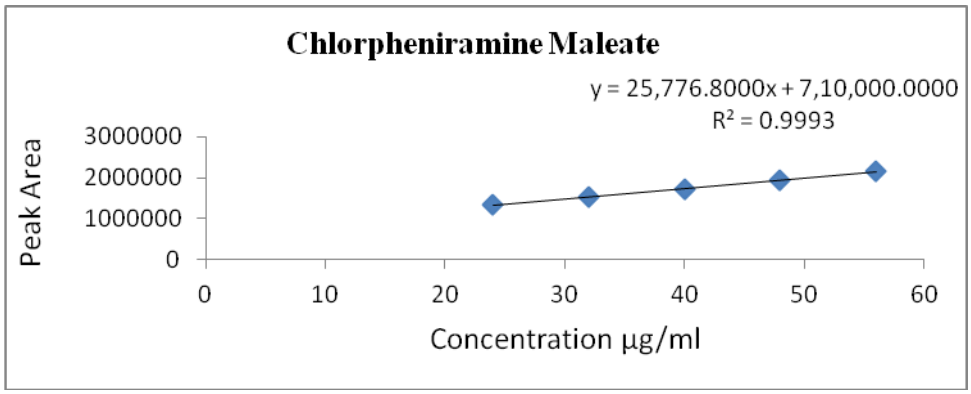

Figure 7: Calibration Curves for the Linearity Set of Chlorpheniramine Maleate

Assay: The assay of the tablet formulation containing PEH and CPM was carried out by the developed method and was found to be 100.05% for PEH and 99.88% for CPM.

Robustness: The robustness of proposed method was tested for change in flow rate (± 0.2 ml/ min) and change in detector wavelength. All the system suitability parameters for the robustness data was found to be within limits. The results were shown in table 8 & 9.

CONCLUSION:

All the results obtained for the developed method were indicative of a simple, precise, specific and accurate RP-HPLC method, which can be applied for the analysis of CPM and PEH in bulk and dosage forms.

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