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Research Article

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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE DETERMINATION OF CHLORPHENIRAMINE MALEATE AND PHENYLEPHRINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

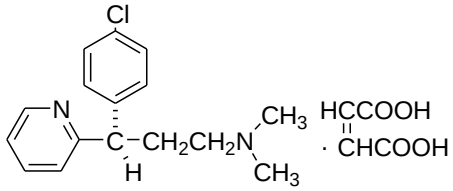
A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of chlorpheniramine maleate and phenylephrine in tablet dosage forms. A reversed-phase C-18 column (250 mm × 8 mm i.d., particle size 10 µm) column with mobile phase consisting of acetonitrile and phosphate buffer 55:45 (v/v) (pH 5.6 ± 0.02, adjusted with triethylamine) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 255 nm. The retention times of chlorpheniramine maleate and phenylephrine were found to be 3.13 min and 4.58 min, respectively. The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for both the drugs was found in the range of 10-70 µg/ml. The % recoveries of chlorpheniramine maleate and phenylephrine were found to be between 101.09 and 98.99. The proposed method was successfully applied to the estimation of chlorpheniramine maleate and phenylephrine in combined tablet dosage forms.

Keywords: Chlorpheniramine maleate, phenylephrine, simultaneous estimation, RP-HPLC, tablet dosage forms.

INTRODUCTION

Chlorpheniramine maleate (CM) chemically, 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1-amine is an antihistamine drug that is widely used in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases. Phenylephrine (PE) chemically, (1R)-1-(3hydroxy-phenyl)-2-(methylamino) ethanol hydrochloride is used as a sympathomimetic.[1-4] The structures of CM and PE are shown in (Figure **1**). Numerous UV, HPLC and HPTLC based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms.[5-14] But no method had yet been reported for simultaneous estimation of these two drugs using HPLC in bulk drug and pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of CM and PE in pharmaceutical dosage forms.

Figure 1. The structures of chlorpheniramine maleate (CM) and phenylephrine (PE)



Chlorpheniramine maleate (CM)

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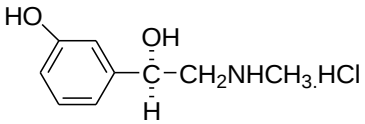


Phenylephrine (PE)

MATERIALS AND METHODS

Chemicals and Reagents

Reference standards of CM and PE were procured as gift samples from Torrent Pharmaceutical (Gandhinagar, India). HPLC grade acetonitrile, water and triethylamine were obtained from Rankem, RFCL Limited, New Delhi, India. Potassium dihydrogen orthophosphate AR and ortho phosphoric acid AR grade were procured from Central Drug House (P) Limited, New Delhi, India.

Instrumentation

The HPLC (PerkinElmer series 200) instrument equipped with a model series 200 pump, vacuum degasser, rheodyne injector with a 20µl loop, UV-Visible detector and C-18 column was used.

Chromatographic Conditions

The isocratic mobile phase was consisted of acetonitrile and phosphate buffer 55:45 (v/v) (pH 5.6 ± 0.02, adjusted with triethylamine). The mobile phase was sonicated for 15 min and filtered through a 0.45 µ membrane filter paper. Flow rate of mobile phase was 1.0 ml/min. The variable wavelength UV–visible detector was set at 255 nm. All analyses were performed at ambient temperature.

Preparation of Standard Stock Solution

25 mg CM and 25 mg PE were accurately weighed and transferred to 100 ml volumetric flasks separately and

dissolved in the mobile phase to give stock solutions of 250 µg/ml each of CM and PE.

Preparation of Sample Solution

Twenty tablets (T-MINIC Tab, Novartis) were weighed and powdered finely. Tablet powder equivalent to 2 mg of CM and 2.5 mg of PE was transferred to a 100 ml volumetric flask and dissolved in 50 ml of mobile phase. The solution was ultrasonicated for 15 min and filtered through 0.45 micron membrane filter. The solutions were further diluted with mobile phase to obtain concentration of 10 µg/ml of CM and 12.5 µg/ml of PE and were subjected to HPLC analysis as described earlier. From the peak area of CM and PE, the amount of drugs in samples was computed.

Method Validation[15-18]

Specificity: Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of CM and PE in sample solution.

Linearity: Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at seven different concentrations and analyses were performed in triplicate. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

Precision: The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability, or intra-day precision, was determined by performing nine analyses at three concentrations on the same day. Intermediate precision was determined by analyzing the same sample in the same way on different days. Results from determination of repeatability and intermediate precision were expressed as SD and RSD.

Accuracy: The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 80, 100, and 120% of the standard and analyzed. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

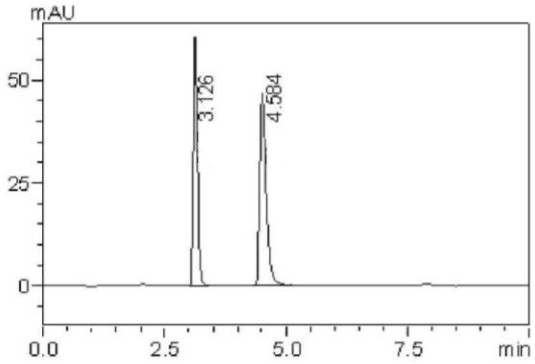
Limits of Detection and Limit of Quantitation: The LOD and LOQ were separately determined on the basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Method Development

Several mobile phase compositions were tried to resolve the peaks of CM and PE. The optimum mobile phase containing Acetonitrile and phosphate buffer 55:45 (v/v) (pH 5.6 ± 0.02, adjusted with triethylamine) was selected because it could resolve the peaks of CM (RT = 3.13±0.03 min) and PE (RT = 4.58±0.05 min) with a resolution factor of 9.0. Quantification was achieved with UV detection at 255 nm on the basis of peak area at 1.0 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of CM and PE is given in (Figure **2**).

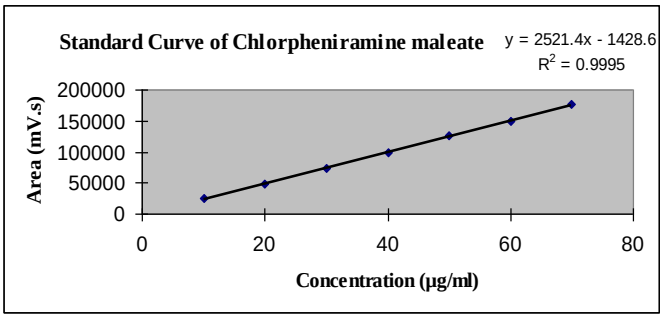
Figure 2. HPLC chromatogram obtained during simultaneous determination of CM and PE



Method Validation

Linearity and Range: Seven different concentrations (10, 20, 30, 40, 50, 60 and 70 µg/ml) of the mixture of two drugs were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 10-70 µg/ml for both the drugs. The linear regression equations for CM and PE were found to be y = 2521.4x - 1428.6 and y = 2000x - 428.57 respectively. The regression coefficient values (r^2) were found to be 0.9995 and 0.9993 respectively indicating a high degree of linearity. Calibration curves of CM and PE are shown in (Figure **3**). Regression characteristics of the proposed HPLC method are given in (Table **1**).

Figure 3. Calibration curves of CM and PE



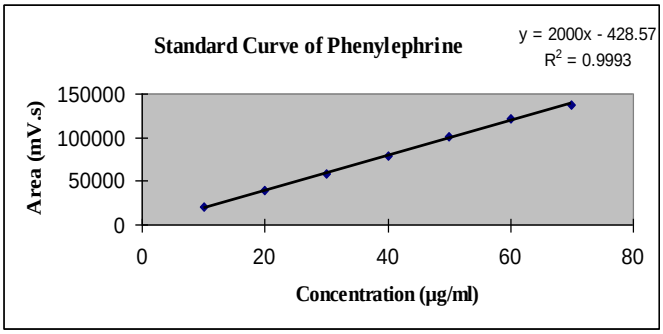


Table 1. Regression characteristics of the proposed HPLC method

Linearity experiment	CM	PE
Range (µg/ml)	10 – 70	10 – 70
Regression coefficient (r^2)	0.9995	0.9993
Slope	2521.4	2000
Intercept	1428.6	428.57

Specificity: The specificity studies proved the absence of interference, since none of the peaks appeared at the retention time of CM and PE. The interaction study in standard solution was also carried out by comparing peak of each drug individually and in drug mixture.

Precision: From the standard stock solutions, mixed

standards containing CM and PE were prepared. Standard solutions (n=3) were injected using a universal rheodyne injector with injection volume of 20 µl. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The % RSD was found to be between 0.78 and 0.60 for both the drugs. The lower values of % RSD indicate that the method is precise.

Accuracy: Recovery studies were carried out by applying the standard addition method. Known amounts of standard CM and PE corresponding to 80%, 100%, and 120% of the label claim were added to sample of tablet dosage form separately. The average % recoveries for CM and PE in marketed formulation were found to be between 101.09 and 98.99. The results revealed that there was no interference of excipients. The results of accuracy are shown in (Table **2**).

Table 2. Percent recovery data

Drug	% simulated dosage nominal	% Mean (n=3)	±SD	RSD (%)
CM	80	100.65	0.35	0.61
PE	80	101.06	0.67	0.76
CM	100	99.51	0.11	0.82
PE	100	98.99	0.59	0.33
CM	120	100.34	0.95	0.54
PE	120	101.09	0.28	0.17

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The limit of detection and limit of quantification were found to be 0.23 and 0.40 µg/ml for CM and 0.15 and 0.32 µg/ml for PE. The values indicate that the method is sensitive.

Analysis of Marketed Formulation

Analysis of marketed tablets ((T-MINIC Tab, Novartis) was carried out using optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for CM and PE was found to be 100.5 and 101.1, respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 105%. The results are given in Table **3**.

Table 3. Analysis of marketed tablets

Drug	Label claim (mg/tablet)	Quantity found (mg/tablet) (n = 3)	RSD (%)	% Assay
Chlorpheniramine maleate	2	2.01	0.40	100.5
Phenylephrine	2.5	2.53	0.51	101.1

System Suitability Parameters

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the resolution, capacity factor, tailing factor, theoretical plate, retention volume and asymmetry factor of the peaks were calculated. The results are shown in Table **4**.

Table 4. System suitability data

Parameters	CM	PE
Resolution	-	6.0
Capacity factor	0.12	0.57
Tailing factor	1.03	1.40
Theoretical plates	13450	15342
Asymmetry factor	1.15	1.39

CONCLUSION

A novel RP- HPLC method has been developed for the simultaneous estimation of CM and PE in marketed formulations. The method gave good resolution for both the drugs with a short analysis time below 6 minutes. The developed method was validated. It was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of CM and PE in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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