



NOVEL, GRADIENT RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE IN A MARKETED FORMULATION

¹Varsha. S. Dhanawade, ^{*}Dr. Nutan Rao

¹Research Student, Oriental College of Pharmacy, Sector 2, Behind Sanpada Railway Station, Sanpada West, Navi Mumbai, Maharashtra 400705, Email: varshad11297@gmail.com.

^{*}Associate Professor, Dept of Pharmaceutical Chemistry, Vivekanand Education Society's college of Pharmacy, Chembur, Mumbai, 400074, Email: nutan.rao@ves.ac.in

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ABSTRACT

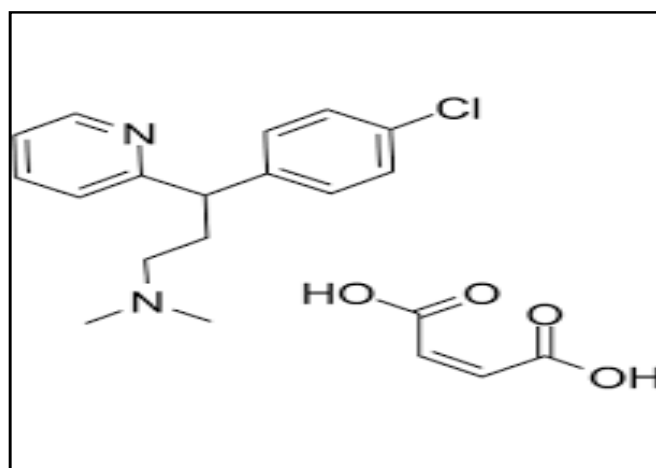
A novel, simple and cost-efficient gradient RP-HPLC method was developed for the simultaneous analysis of phenylephrine hydrochloride (PE) and Chlorpheniramine maleate (CM) within the marketed formulation. A reverse-phase C-18 column (Intersil ODS-3V; particle size 5 μ m 4.6*250mm) with mobile phase A consisting of potassium dihydrogen phosphate (pH 3.5 adjusted with ortho phosphoric acid), and mobile phase B consisting 100% Acetonitrile was used. The flow rate was 1.0 ml/min and effluents were monitored at 225 nm. The retention times of Phenylephrine hydrochloride and Chlorpheniramine maleate were found to be 3.75 min and 17.47 min respectively. The method was validated in terms of linearity, specificity, accuracy, precision, robustness and forced degradation. The percentage recoveries of Phenylephrine hydrochloride and Chlorpheniramine maleate were found to be 99.8 and 99.3. The developed method was successfully applied for the simultaneous analysis of adrenergic drug and chlorpheniramine in a tablet dosage form.

KEYWORDS: *Phenylephrine hydrochloride, Chlorpheniramine maleate, simultaneous analysis, RP-HPLC, tablet dosage form.*

INTRODUCTION:

Phenylephrine hydrochloride (PE)(1R)-1-(3hydroxy-phenyl)-2-(methylamino) ethanol hydrochloride, is a direct-acting adrenergic aminoalkane with chemical structure associated with adrenaline and ephedrine with potent vasoconstrictive property. Phenylephrine is employed to alleviate nasal discomfort caused by colds, allergies and pollinosis. It belongs to category of medicines known as nasal decongestants, which works by reducing swelling of the blood vessels within the nasal passage. ⁽¹⁾ Chlorpheniramine Maleate (CM) 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1- amine, is 1st generation histamine H₁ antagonist, which is used to relieve the symptoms of hay fever, rhinitis, hives and asthma. It

has a role as an H₁ receptor antagonist, antipruritic drug, histamine antagonist, serotonin reuptake inhibitor, antidepressant and antiallergic agent. ⁽²⁾ The structures of Phenylephrine hydrochloride and Chlorpheniramine maleate are shown in Figure 1.



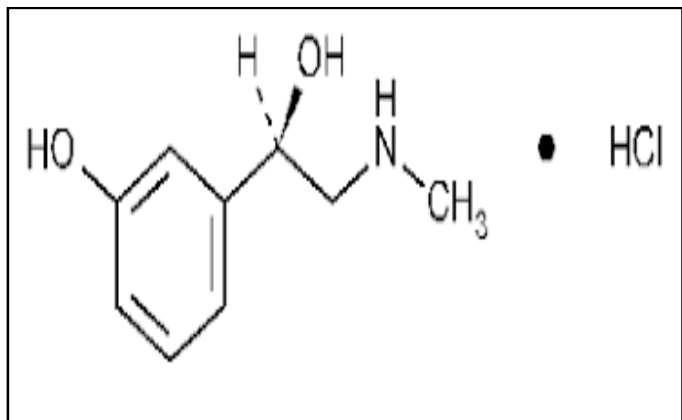


Figure 1: Structure of (a) Chlorpheniramine maleate and (b) Phenylephrine hydrochloride

MATERIALS AND METHODS:

Chemicals and Reagents:

Reference standards of Phenylephrine and Chlorpheniramine were procured as gift samples from Alembic Pharmaceuticals (Gorwa, Baroda-Gujarat, India). HPLC grade acetonitrile (HPLC grade), milli Q water and ortho phosphoric acid (AR grade) were obtained from Piramal Lab Solution, Rabale, India. Potassium dihydrogen phosphate (LiChropur grade) and ortho phosphoric acid (AR grade) were obtained from Sigma Aldrich.

Instrumentation:

The HPLC (Shimadzu) instrument equipped with model series SPD-M20A-LC-20AD pump, vacuum degasser, rheodyne injector with 10 µl, Photodiode Array detector and Intersil C-18 column was used

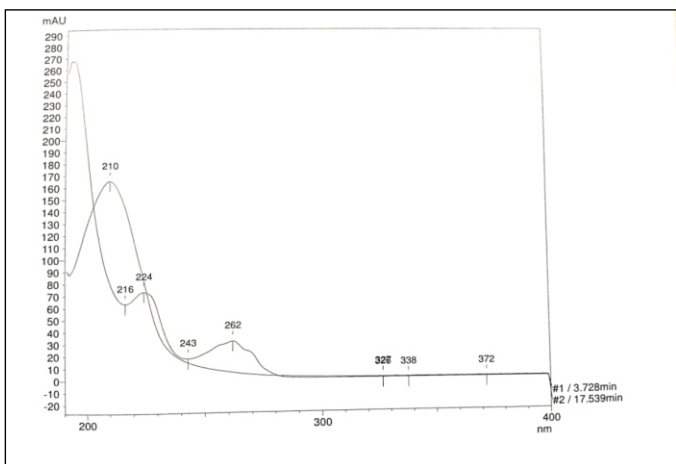


Figure 2: UV graph of Phenylephrine hydrochloride and chlorpheniramine Maleate..

Chromatographic Conditions: –

For the gradient elution, mobile phase A consisting of potassium dihydrogen phosphate (pH 3.5 adjusted with ortho phosphoric acid), and mobile phase B consisting of 100% Acetonitrile was used as per the gradient program given in Table 1. The mobile phase was sonicated for 10 min and filtered through a 0.45µ membrane filter paper. Diluent was prepared as Buffer: Acetonitrile (90:10). Flow rate of mobile phase was 1.0 ml/min. The specified wavelength of Photo diode detector was set at 225 nm. All analyses were performed at ambient temperature.

Table 1: Gradient program

Time	Mobile phase- A	Mobile phase-B
0.01	80	20
10	80	20
20	45	55
25	30	70
30	30	70
35	80	20
45	80	20

Preparation of Standard Solution:

Phenylephrine (10 mg) and Chlorpheniramine (4 mg) were weighed and transferred to 100 ml volumetric flasks separately. Diluent (30 ml) was added, solution obtained was sonicated for 5 mins and then volume was made up with the diluent to give stock solution of 100 µg/ml and 40 µg /ml each of Phenylephrine and Chlorpheniramine respectively.

Preparation of Sample Solution:

Two tablets (Maxtra Tablet, Zuventus Heathcare Ltd.) were weighed and powdered finely. Tablet powder equivalent to 4 mg of Chlorpheniramine and 10 mg of Phenylephrine was transferred to a 100 ml volumetric flask and dissolved in 50 ml of diluent. The solution was sonicated for 25 min and volume made up with the diluent and then filtered through 0.45-micron membrane filter. The mixture obtained with concentration of 40µg /ml of Chlorpheniramine and 100µg/ml of Phenylephrine

were subjected to HPLC analysis as described earlier. Based on the peak area of Phenylephrine and Chlorpheniramine, the amount of drugs in samples was computed.

Method Validation:

Specificity: The Specificity test was performed against the reference standard and against possible interference in the occupancy of placebo. No involvement was detected in the retention time of Chlorpheniramine and Phenylephrine in the sample in the solution. No peak was observed at retention time of peak in the blank solution.⁽⁵⁾

Linearity: Linearity is studied to see the range over that analyte response. This study was performed by preparing standard solutions at seven different concentrations and analyses were performed in triplicate. Responses were measured as peak space. Responses were measured as peak area. The calibration curves were obtained by plotting the peak area versus the concentration.⁽⁶⁾

Precision: The precision of an analytical technique is the closeness of replicate results obtained from analysis of a similar homogenous sample. Precision was considered at 2 levels, i.e., repeatability and intermediate precision (ruggedness), in accordance with ICH recommendations. Repeatability was decided by performing 9 analyses at 3 concentrations on a similar day. Intermediate precision was decided by analyzing a similar sample within the same approach by different analyst. Results from determination of repeatability and intermediate precision were expressed as standard deviation and RSD.⁽⁷⁾

Accuracy: The accuracy of an analytical technique is the closeness of results obtained by that technique to true value for the sample. It is expressed as recovery (%), which is set by the standard addition technique. Samples were spiked with 80, 100 and 120 µg/ml of the standard and analyzed. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.⁽⁸⁾

Robustness: The robustness of a method is its ability to remain unaffected by tiny changes in parameters

like percent organic content and pH of the mobile phase, buffer concentration, temperature, and injection volume. These methodology parameters could also be evaluated one factor at a time. Obtaining data on the results of those parameters could permit a variety of acceptable values to be enclosed within the final methodology procedure.⁽⁹⁾

System suitability: System suitability tests are performed to confirm instrumentation, electronics, analytical operations, associated samples to be analyzed represent an integral system which will be evaluated intrinsically. System suitability check parameters to be established for a specific procedure depend upon the sort of procedure being evaluated. System suitability was evaluated in every condition and the results were compared with technique preciseness results.⁽¹⁰⁾

RESULTS AND DISCUSSION

Method Development

Several mobile phase compositions were tried to resolve the peaks of Chlorpheniramine and Phenylephrine. The optimum mobile phase containing Acetonitrile and phosphate buffer 10:90 (v/v) (pH 3.5 ± 0.02 , adjusted with ortho phosphoric acid) was selected because it could resolve the peaks of Phenylephrine (RT = 3.72 ± 0.03 min) and Chlorpheniramine (RT = 17.54 ± 0.05 min). Quantification was achieved with UV detection at 225 nm on the basis of peak area at 1.0 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of PE and CM is given in (Figure 3).

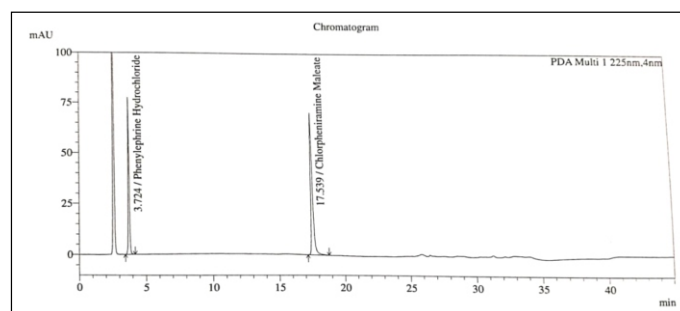


Figure 3. HPLC chromatogram obtained during simultaneous determination of Phenylephrine and Chlorpheniramine

Method Validation

Linearity and Range: Five different concentrations (80, 90, 100, 110 and 120 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 80-120 µg/ml for both the drugs. The linear regression equations for Phenylephrine and Chlorpheniramine were found to be $y = 4600x - 8563$ and $y = 17990x - 31887$ respectively. The regression coefficient values (r^2) were found to be 0.9998 and 0.9997 respectively indicating a high degree of linearity. Calibration curves of Phenylephrine and Chlorpheniramine are shown in (Figure 4a and 4b). Regression characteristics of the proposed HPLC method are given in (Table 2).

appeared at the retention time of Phenylephrine and Chlorpheniramine. The interaction study in standard solution was also carried out by comparing peak of each drug individually and in drug mixture.

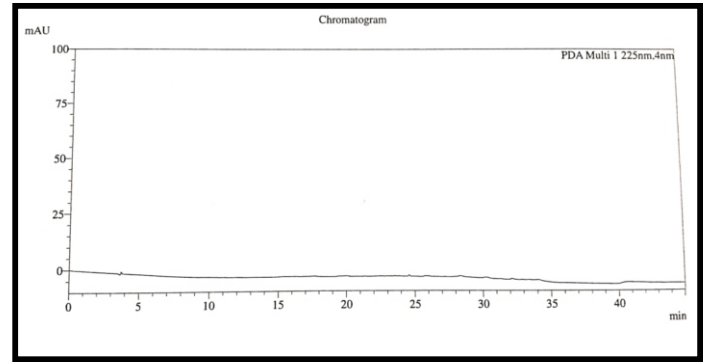


Figure 5: Chromatogram of blank solution

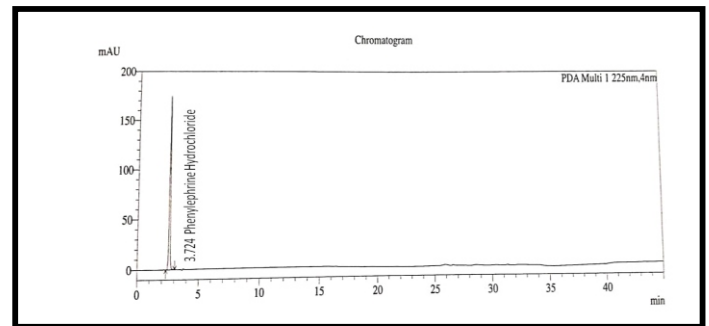


Figure 6: Chromatogram of individual standard of Phenylephrine hydrochloride

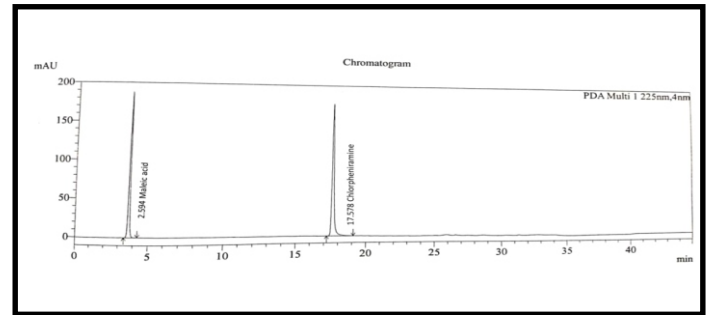


Figure 7: Chromatogram of individual standard of Chlorpheniramine maleate

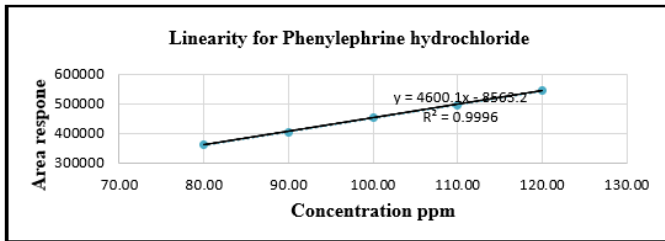


Figure 4a: Linearity graph of Phenylephrine hydrochloride

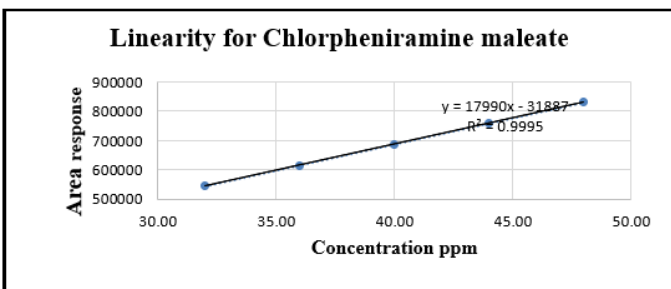


Figure 4b: Linearity graph of Chlorpheniramine maleate

Table 2. Regression characteristics of the proposed HPLC method

Linearity experiment	Chlorpheniramine	Phenylephrine
Range (µg/ml)	80-120	80-120
Regression coefficient (r ²)	0.9998	0.9997
Slope	4600	17900
Intercept	8563	31887

Specificity: The specificity studies proved the absence of interference, since none of the peaks

Precision: From the standard stock solutions, mixed standards containing Phenylephrine and Chlorpheniramine were prepared. Standard solutions (n=3) were injected using a universal rheodyne injector with injection volume of 10 µl. The system, method (repeatability) and intermediate precisions were assessed by analyzing standard solutions. The % RSD of Phenylephrine for system precision was 0.08, for method precision was 0.18 and for intermediate precision was found to be 0.14.

The % RSD of Chlorpheniramine for system precision was 0.17, for method precision was 0.38 and for intermediate precision was found to be 0.08. 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 Phenylephrine and Chlorpheniramine corresponding to 80%, 100%, and 120% of the label claim were added to sample of tablet dosage form separately. The average % recoveries for Phenylephrine and Chlorpheniramine in marketed formulation were found to be between 99.3 and 101.53. The results revealed that there was no interference of excipients. The results of accuracy are shown in (Table 3).

Table 3. Percent recovery data

Drug	% Simulated dosage nominal	% Mean (n=3)	±SD	RSD (%)
PE	80	99.93	0.09	0.09
CM	80	101.1	0.35	0.35
PE	100	100.23	0.09	0.09
CM	100	100.7	0.57	0.57
PE	120	101.53	0.09	0.09
CM	120	100.8	0.28	0.28

Robustness: Robustness of the method was verified by deliberately varying the following instrumental conditions.

By changing the flow rate by plus and minus 1 ml/min.

By changing the temperature by 2°C.

System suitability was evaluated in each condition and the results were compared with method precision results. The results of robustness for Phenylephrine and Chlorpheniramine are shown in (Table 5).

Table 4: Robustness Parameters with respect to the number shown in next table

Sr. No.	Experimental (Actual Value)
1	Method precision mean
2	Flow rate (plus) (1.1 ml/min)
3	Flow rate (minus) (0.9ml/min)

4	Column oven temperature (plus) (27°C)
5	Column oven temperature (minus) (23°C)

Table 5: Result of robustness for Phenylephrine hydrochloride and Chlorpheniramine maleate

Sr. No	Phenylephrine hydrochloride	Chlorpheniramine maleate
1	101.2	100.9
2	100.8	100.6
3	100.9	100.7
4	100.8	100.6
5	101	100.7

Analysis of Marketed Formulation

Analysis of marketed tablets (Maxtra, Zuventus) was carried out using optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for PE and CM was found to be 99.8 and 99.3, 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 7.

Table 7: Assay of marketed formulation

Tablet	Drug	%Assay
Maxtra tablet (10 mg Phenylephrine hydrochloride and 4 mg Chlorpheniramine maleate)	Phenylephrine hydrochloride	99.8
	Chlorpheniramine maleate	99.3

System Suitability Parameters

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the retention time, theoretical plates and tailing factors were calculated. The results are shown in Table 8.

Table 8: System Suitability Data

Parameters	Phenylephrine	Chlorpheniramine
Retention time (min)	3.742	17.53
Tailing factor	1.23	1.33
Theoretical plates	52009	688064

The results showed that the method is specific, linear, precise and exact. Therefore, the method can be used for routine test analysis of selected drugs. ⁽¹¹⁾ All

forced degradation samples were compared to control sample solution. When subjected to various forced degradation conditions, no other impurity peaks were found to interfere with the standard peaks. Thus, the peaks were found to be pure.⁽¹²⁾

CONCLUSION

A novel, gradient RP-HPLC method was developed and validated for simultaneous estimation of Phenylephrine hydrochloride and Chlorpheniramine maleate in bulk and pharmaceutical dosage form. The method assured the satisfactory results of validation parameters and it was found to be novel, simple, accurate, economical and rapid, hence it can be applied for routine analysis. The developed gradient method can be thus successfully applied for analysis of various formulations containing Phenylephrine hydrochloride and Chlorpheniramine maleate.

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