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RP-HPLC METHOD FOR THE SIMULTANEOUS ANALYSIS OF CODEINE AND CHLORPHENIRAMINE MALEATE IN ITS TABLET DOSAGE FORM

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Codeine, Chlorpheniramine, Chromatography, RP-HPLC, Validation etc.

ABSTRACT

The present research focuses on simultaneous estimation of Codeine and Chlorpheniramine maleate in its tablet dosage form by using Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). Literature review reveals that HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence present work is aimed to develop a new, simple, fast, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Codeine and Chlorpheniramine maleate and validated according to ICH guidelines. Both the drugs are simultaneously assayed and validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The chromatographic conditions were success fully developed for the separation of Chlorpheniramine and Codeine by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoric acid) with detection wavelength 252nm. The linearity study of Chlorpheniramine and Codeine was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999. % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19, LOD with 3.17 and 5.68, LOQ with 0.0172 and 0.2125 respectively. Hence the above RP-HPLC method can be used for routine analysis of Codeine and Chlorpheniramine in API and Pharmaceutical dosage form.

CONTENTS

Citation: **G.Kumari*, D.Saravanan et al. RP-HPLC Method for the Simultaneous Analysis of Codeine and Chlorpheniramine Maleate in its Tablet Dosage Form. International Journal of Pharmacometrics and Integrated Biosciences. 2016: Volume1 Issue 4: p 87-95.**

INTRODUCTION

Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.[1] Codeine is an opioid analgesic related to morphine but with less potent analgesic properties and mild sedative effects where as chlorpheniramine is a histamine H1 antagonist used in allergic reactions, hay fever, rhinitis etc. In the present research work the above drugs are analyzed simultaneously using RP-HPLC. [2]

Literature review reveals that both the drugs are simultaneously analysed using RP-HPLC in its liquid dosage form by Geetha Lakshmi.E et al [2] and syrup by Vijai Anand P. et al [3] etc. So this research focused on simultaneous estimation of codeine and chlorpheniramine maleate using RP-HPLC in its tablet dosage form.

RP-HPLC consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily.[4] RP-HPLC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. [5] By using the above technique a simple, accurate and efficient method has been developed for the simultaneous estimation of Chlorpheniramine and codeine in its tablet dosage form.

MATERIALS AND METHODS

Materials:
Water, Methanol, Acetonitrile, Orthophosphoric acid, Potassium dihydrogen phosphate were procured from Merck. Codeine and Chlorpheniramine were purchased from In- House laboratories. All the solvents and filters used were of HPLC grade.

Instrumentation:
Instrument used was WATERS HPLC auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2.

Method Development [7]
The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of codeine and chlorpheniramine was obtained and the isobestic point of codeine and chlorpheniramine showed absorbance maxima at 252 nm. The spectrum is shown in Fig no – 1

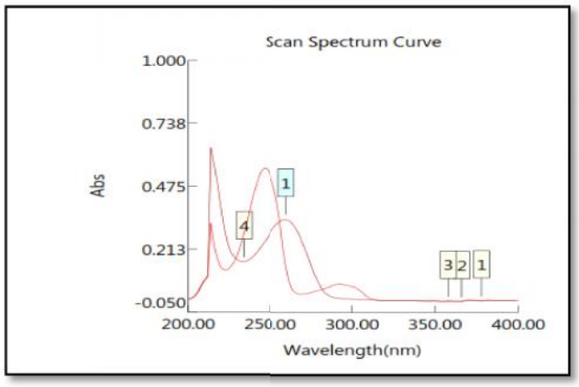


Fig.No.01. Spectrum showing overlapping spectrum of Codeine and Chlorpheniramine

Optimized Method development:
After performing various trails using various columns, buffers with flow rates. Optimized Chromatographic conditions are shown below.

Chromatographic conditions

Column	:	Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase ratio	:	Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength	:	252 nm
Flow rate	:	0.7 ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	8min
Retention time	:	2.449 & 3.191 min

By using the above conditions, separation was found to be good, peak shape was good and the chromatogram was shown in fig no 2, 3.

Preparation of solutions:

Preparation of phosphate buffer
6.8 grams of sodium acetate was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase
Mix a mixture of above buffer 30 ml (30%) and 70 ml of Methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation
Mobile phase was used as the diluent.

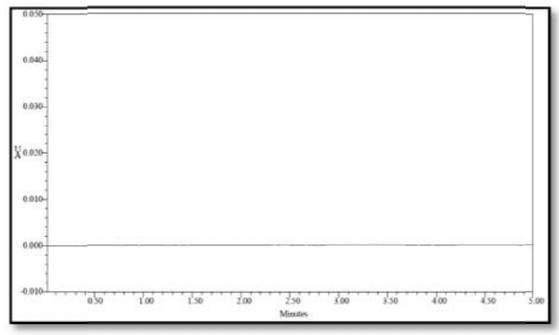


Fig.No.2. Chromatogram showing blank preparation (mobile phase)

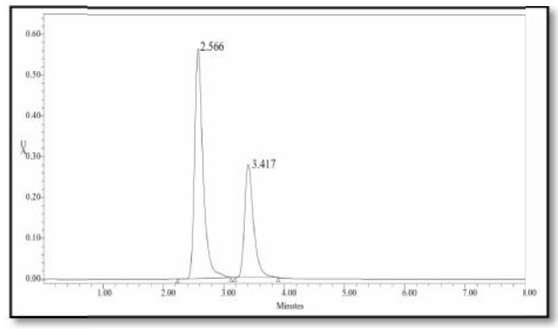


Fig.No.03. Chromatogram showing trial-5 injection

Preparation of solutions for peak identification:

Chlorpheniramine standard preparation
10 mg of chlorpheniramine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Codeine standard preparation
10 mg of Codeine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of Codeine and Chlorpheniramine standard and sample solution

Sample preparation:
1mg of chlorophinaramine and 10 mg codeine tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard preparation

1mg chlorpheniramine and 10 mg codeine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

10µL of the blank, standard and sample were injected into the chromatographic system and peak areas for the Codeine and chlorpheniramine were used for calculating the % assay by using the formulae.

Assay calculation

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where: Avg.wt = average weight of tablets, P= Percentage purity of working standard, LC= Label Claim of chlorpheniramine mg/ml.[6]

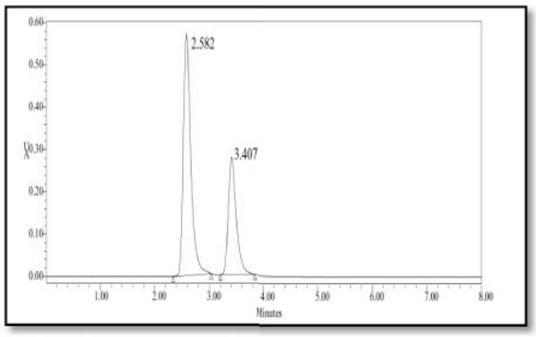


Fig.No.04. Chromatogram showing assay of sample injection

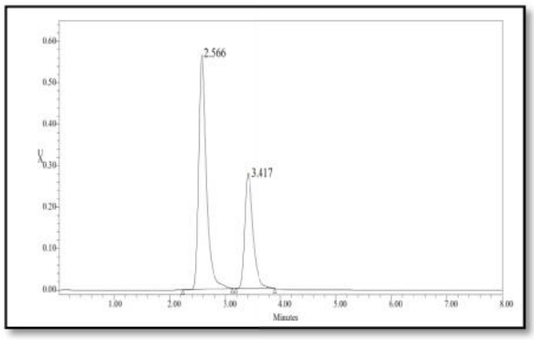


Fig.No.05. Chromatogram showing assay of standard injection

Table.No.01. Showing assay results

S.No	Name of compound	Amount taken	%purity
1	Codeine	754.7	99.24
2	Chlorpheniramine	735.6	101.04

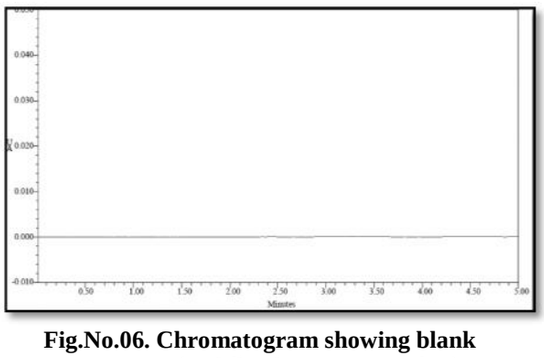


Fig.No.06. Chromatogram showing blank (mobile phase preparation)

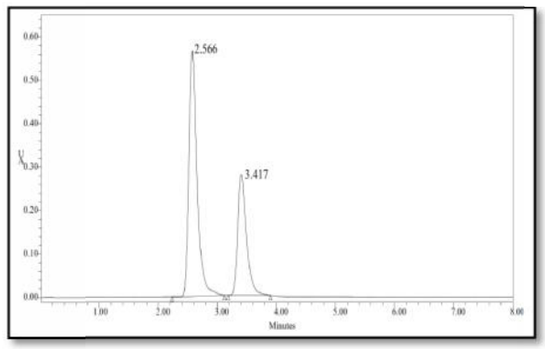


Fig.No.07. Chromatogram showing standard injection

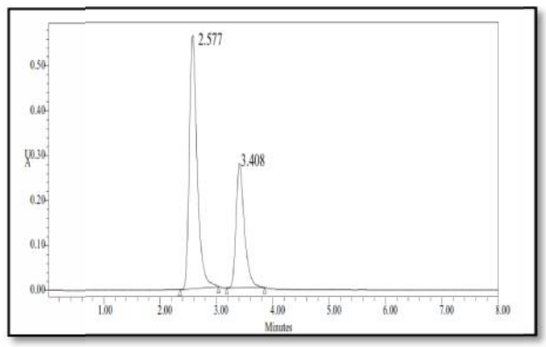


Fig.No.08. Chromatogram showing sample injection

METHOD VALIDATION [8-12]

1. Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

2. Linearity;

Preparation of stock solution

1 mg of chlorpheniramine and 10 mg of codeine working standard were accurately weighed and was transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (5 ppm of Chlorpheniramine and 50 ppm of Codeine)

0.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluents

.

Preparation of Level – II (10 ppm of Chlorpheniramine and 100ppm of Codeine)

1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – III (15 ppm of Chlorpheniramine and 150ppm of Codeine)

1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – IV (20 ppm of Chlorpheniramine and 200ppm of Codeine)

2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – V (25 ppm of Chlorpheniramine and 250ppm of Codeine)

2.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

Chromatograms showing linearity level-1 to level 5 (5ppm-25 ppm of Codeine and 50ppm -250ppm of Chlorpheniramine) injections.

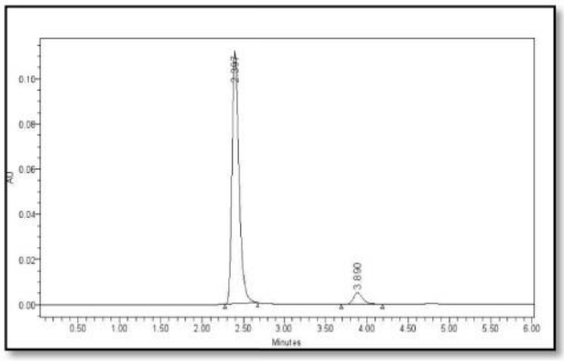


Fig.No.09. Level – 1

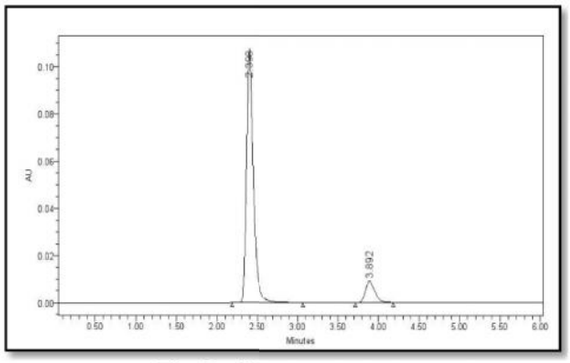


Fig.No.10. Level – 2

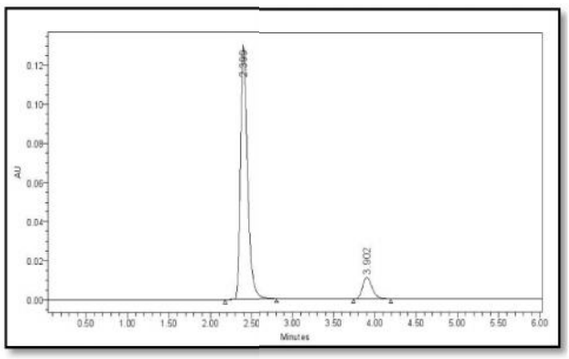


Fig.No.11. Level - 3

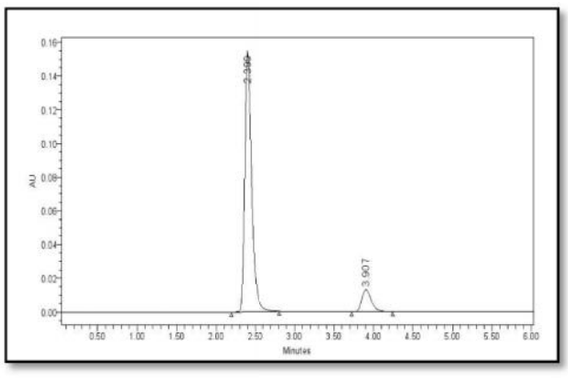


Fig.No.12. Level - 4

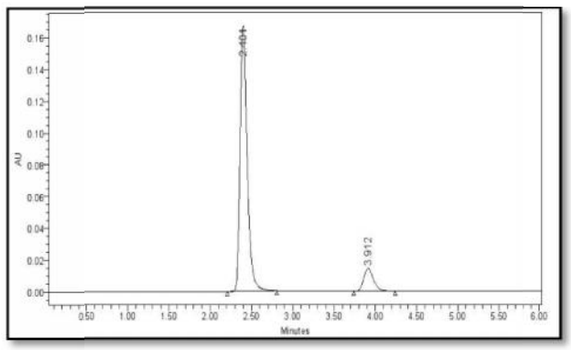
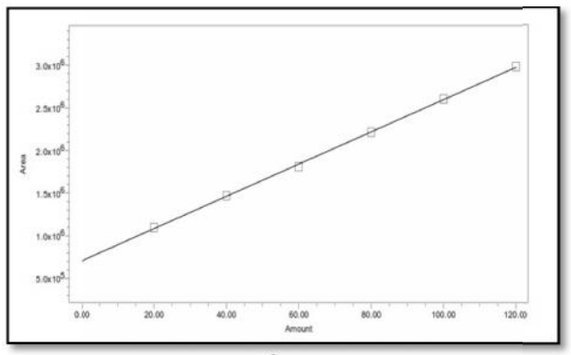


Fig.No.13. Level - 5

Table.No.02. Linearity Results for Codeine:

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277
3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient	0.999		

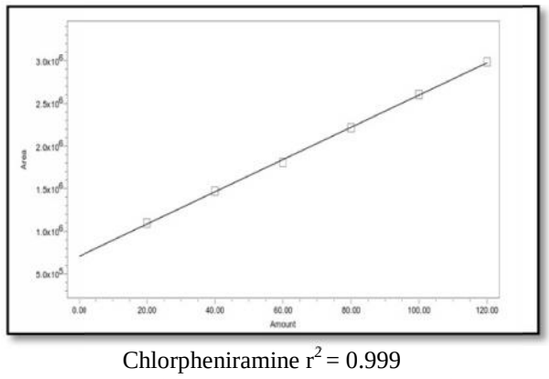


Codeine $r^2 = 0.999$

Fig.No.14. Showing calibration graph for codeine

Table.No. 03.
Linearity Results for Chlorpheniramine:

S.No	Linearity Level	Concentration	Area
1	I	50ppm	56472
2	II	100 ppm	73841
3	III	150ppm	92655
4	IV	200ppm	111541
5	V	250ppm	130567
Correlation Coefficient			0.999



Chlorpheniramine $r^2 = 0.999$

Fig.No.15. Showing calibration graph for Chlorpheniramine

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 5µg/ml-25µg/ml and

50µg/ml-250µg/ml of codeine and chlorpheniramine respectively.

4. Accuracy:

Preparation of standard stock solution

1mg of chlorpheniramine and 10mg of codeine working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipetted out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

0.5mg of chlorpheniramine and 5 mg of codeine working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipetted out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)

1 mg of chlorpheniramine and 10 mg of codeine working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipetted out 1ml of above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 150% solution (with respect to target assay concentration)

2 mg of chlorpheniramine and 15 mg of Codeine working standards into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solutions of accuracy 50%, 100% and 150% were injected into chromatographic system. Calculate the amount found and amount added for codeine and chlorpheniramine and calculate the individual % recovery and mean % recovery values.

Accuracy -50%

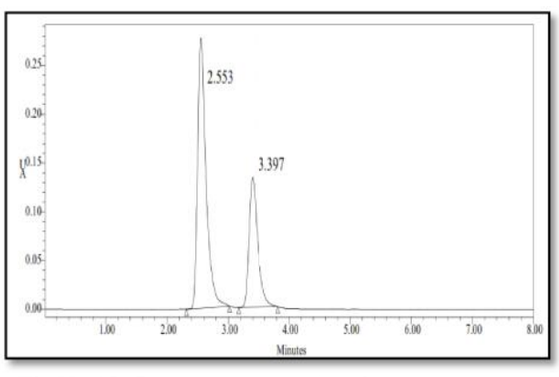


Fig.No.16. Chromatograms showing accuracy-50% injection

Accuracy -100%

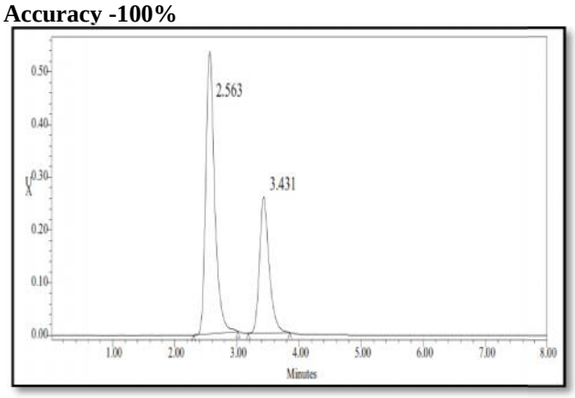


Fig.No.17. Chromatogram showing accuracy -100% injection.

Accuracy 150%

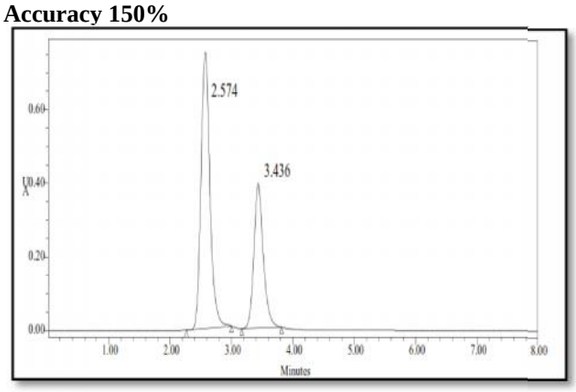


Fig.No.18. Chromatogram showing accuracy -150 % injection.

5. Precision

5.1 Repeatability

Preparation of stock solution

1mg of chlorpheniramine and 10 mg of codeine working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

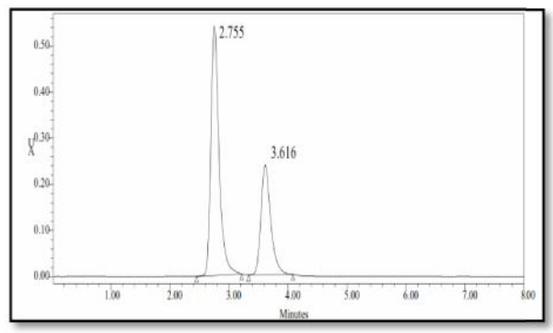


Fig.No.19. Chromatograms showing precision injections

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution

1 mg of Chlorpheniramine and 10 mg of Codeine

Table.No.04. Showing accuracy results for Codeine

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table.No.05. Showing accuracy results for Chlorpheniramine

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table.No.06. Showing % RSD results for Codeine

Sl.No	Peak Name	RT	Area	Height (µV)
1	Codeine	3.616	2742453	238643.4
2	Codeine	3.634	2762750	271543.5
3	Codeine	3.460	2797670	281711.6
4	Codeine	3.446	2793578	274499.8
5	Codeine	3.437	2778483	276713.0
Mean			2774987	
Std Dev			22806.9	
%RSD			0.82	

Table.No.07. Showing %RSD results for Chlorpheniramine

Sl.No	Peak Name	RT	Area	Height (µV)
1	Chlorpheniramine	2.755	5223559	541538.3
2	Chlorpheniramine	2.687	5208511	485548.5
3	Chlorpheniramine	2.632	5323569	574440.4
4	Chlorpheniramine	2.612	5259147	557413.5
5	Chlorpheniramine	2.616	5273463	565020.1
Mean			5257650	
Std Dev			45206.4	
%RSD			0.86	

Table.No.08. Showing results for intermediate precision of Codiene

Sl.No	Peak Name	RT	Area	Height (µV)
1	Codeine	3.617	2624315	231325.6
2	Codeine	3.635	2623598	231315.4
3	Codeine	3.461	2623541	231250.1
4	Codeine	3.447	2624987	231342.6
5	Codeine	3.438	2635698	231765.2
Mean			2626428	
Std Dev			5215.78	
%RSD			0.19	

Table.No.09. Showing results for intermediate precision of Chlorpheniramine

Sl.No	Peak Name	RT	Area	Height (µV)
1	Chlorpheniramine	2.756	5698542	539568.1
2	Chlorpheniramine	2.688	5682534	536985.4
3	Chlorpheniramine	2.633	5695846	539584.1
4	Chlorpheniramine	2.613	5689452	534569.8
5	Chlorpheniramine	2.617	5636591	534985.5
Mean			5600593	
Std Dev			203577.3	
%RSD			0.44	

working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The

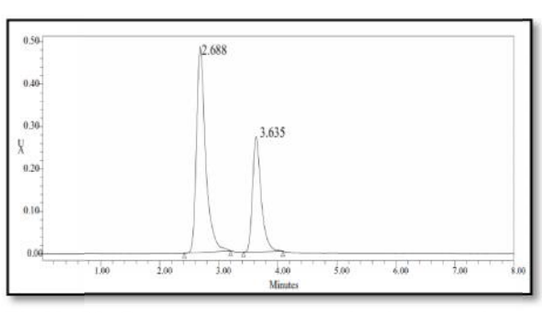


Fig.No.20. Chromatograms showing intermediate precision injections

%RSD for the area of five replicate injections was found to be within the specified limits.

6. Limit of detection (LOD):

LOD can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

$$\mathbf{LOD = 3.3\ X\ \frac{\sigma}{S}}$$

Where σ - Standard deviation (SD), S - Slope

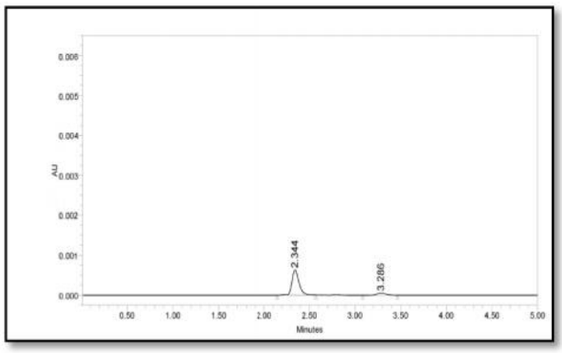


Fig .No.21 Showing results for Limit of Detection

Table.No.10 Showing results for Limit of Detection

Drug name	Standard deviation (σ)	Slope (s)	LOD (µg)
Codeine	373625.50	581075863	3.17
Chlorpheniramine	5772.40	476579210	0.0172

7. Limit of quantification:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula: $\mathbf{LOQ = 10\ X\ \frac{\sigma}{S}}$

Where σ - Standard deviation, S - Slope

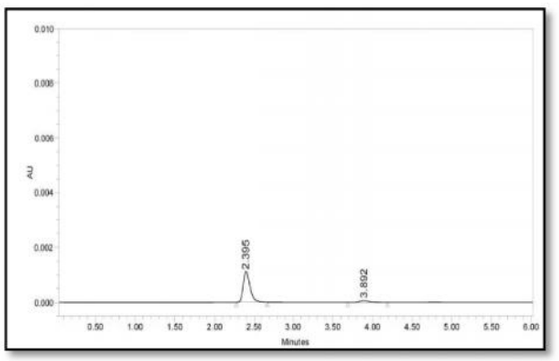


Fig.No.22. Showing results for Limit of Quantification

Table.No.11. Showing results for Limit of Quantification

Drug name	Standard deviation (σ)	Slope(s)	LOQ(µg)
Codiene	372727.80	574265980	5.80
Chlorophinaramine	5761.30	478828490	0.212

8. Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 15ppm of chlorpheniramine and 150 ppm of codeine was prepared and analysed using the varied flow rates along with method flow rate.
b) The organic composition in the mobile phase was varied from 65% to75 % standard solution 15 µg/ml of chlorpheniramine and 150 µg/ml of codeine was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

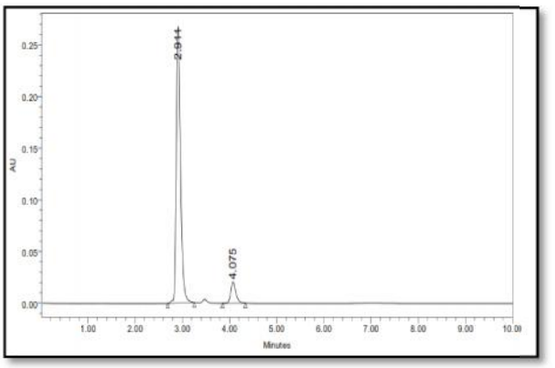


Fig.No.23. Chromatogram showing less flow rate 0.8ml/min

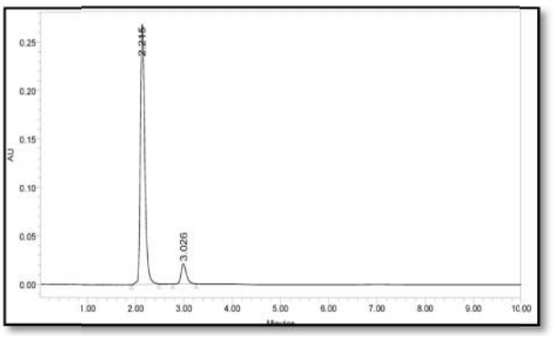


Fig.No.24. Chromatogram showing less flow rate 1.2 ml/min

9. System suitability:

1mg of chlorpheniramine and 10 mg of codeine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of codeine and chlorpheniramine from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

RESULTS AND DISCUSSION

A new analytical method was developed for the simultaneous estimation of codeine and chlorpheniramine in its tablet dosage form.

Assay:

The retention time of codeine and chlorpheniramine was found to be 2.566 min and 3.417 min respectively. The system suitability parameters for Codeine and Chlorpheniramine such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089, 1.2.

Resolution was 6.0 the % purity Codeine and Chlorpheniramine in Pharmaceutical dosage form was found to be 99.24 and 101.2% respectively. Results were shown in table no-1 and fig no – 4, 5.

Specificity:
Specificity was performed for codeine and chlorpheniramine. It was found that there was no interference of impurities in retention time of analytical peak. Results were shown in fig no:6-8

Linearity:
The linearity study was performed for concentration range of 5µg-25µg and 50µg-250µg of codeine and chlorpheniramine and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999). Results were shown in table no-2,3 and fig no: 9-15

Accuracy:
The accuracy study was performed for % recovery of codeine and chlorpheniramine. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%). Results were shown in table no-4,5 and fig no:16-18

Precision:
Method precision was performed for the %RSD of codeine and chlorpheniramine and found to be 0.82 and 0.86 (NMT 2). Results were shown in table no-6,7 and fig no – 19.
Intermediate precision was performed for %RSD of codeine and chlorpheniramine was found to be 0.19 and 0.44 respectively (NMT 2). Results were shown in table no-8,9 and fig no – 20.

LOD:
LOD was performed for codeine and chlorpheniramine was found to be 3.17and 0.0172 respectively. Results were shown in table no-10 and fig no – 21.

LOQ:
LOQ was performed for codeine and chlorpheniramine was found to be 5.80 and 0.212 respectively. Results were shown in table no-11 and fig no – 22.

Robustness:
The results are summarized on evaluation of the above results, it was concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition. On evaluation of the above results, it was concluded that the variation in ±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%. Results were shown in table no:12-14 and fig no: 23-26.

Table.No.12. Showing system suitability results for Codeine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table.No.13. Showing system suitability results for Chlorpheniramine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

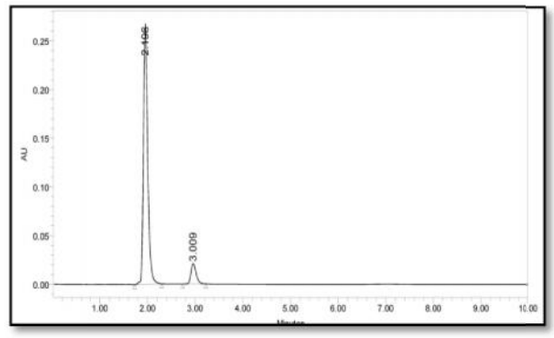


Fig.No.25 Chromatogram showing more organic phase ratio

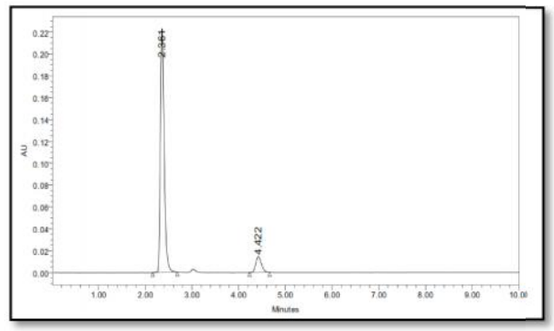


Fig.No.26. Chromatogram showing less organic phase ratio

Table.No.13. Showing system suitability results for Codeine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	*Actual	4668	1.3
3	5 % more	6387	1.4

Table.No.14. Showing system suitability results for Chlorpheniramine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

CONCLUSION
A new method was established for simultaneous estimation of Chlorpheniramine and Codeine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Chlorpheniramine and Codeine. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). Hence the suggested RP-HPLC method can be used for routine analysis of Chlorpheniramine and Codeine in API and Pharmaceutical dosage form.

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