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Development and Validation of a RP-HPLC Method for the Estimation Nascopine and Chlorpheniramine in Bulk and Dosage Form

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ABSTRACT

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Nascopine in active pharmaceutical ingredients and in its pharmaceutical dosage form by using Phenomenex LunaC18 (4.6mm x 150mm, 5µm) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with Ortho phosphoric acid in the ratio of 25:75%v/v. at a flow rate 1ml per minute. The retention time of Noscapine and Chlorpheniramine was found to be 2.427 mins and 4.432 mins respectively. The percentage recovery of the was Noscapine and Chlorpheniramine found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Noscapine and Chlorpheniramine in bulk drug and in its pharmaceutical dosage form. The proposed method were applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: Nascopine, Chlorpheniramine, RP-HPLC, Accuracy, Validation, ICH Guidelines.

INTRODUCTION

Noscapine ,(3S)-6,7-dimethoxy-3-[(5R)-4-methoxy-6-methyl-2H,5H,6H,7H,8H-[1,3]dioxolo[4,5-g] isoquinolin-5-yl]-1,3-dihydro-2-benzofuran-1-one is a non-sedating isoquinoline alkaloid used primarily for its antitussive properties. Its molecular formula is $C_{22}H_{23}N0_7$ **and molecular weight** 413.42. It is soluble in alcohol and water. It is primarily metabolized in liver with half life 1-1.5hr and exhibits high protein binding.

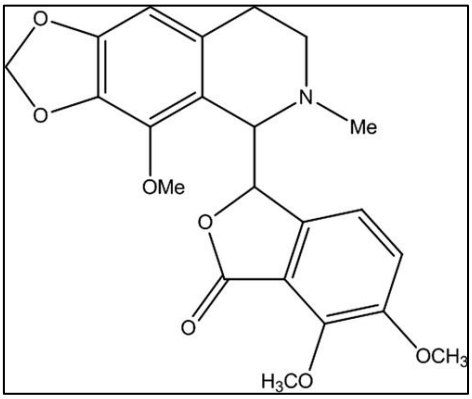

Figure no 1: Structure of Nascopine

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Chlorpheniramine: [3-(4-chlorophenyl)-3-(pyridin-2-yl) propyl] dimethylamine is a histamine-H1 receptor antagonist indicated for the management of symptoms associated with upper respiratory allergies. Its molecular formula is $_{C16}H_{19}ClN_2$ and molecular weight274.788. It is soluble in water and metabolized in liver and exhibits very low protein binding.

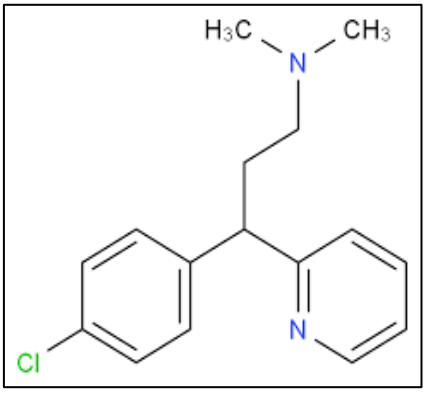

Figure no 2: Structure of Chlorpheniramine

MATERIALS AND METHODS

Instruments used:

The liquid chromatographic system used was WATERS, software: Empower 2, Alliance 2695 separation module 996 PDA detectors, Phenomenex LunaC18 (4.6mm x 150 mm, 5 µ) column, P^H meter Lab India, Ultrasonicator-Denver.

Chemicals used:

Gift samples of Noscapine and chlorpheniramine were procured from Sure Labs, Hyderabad, HPLC grade water, methanol, acetonitrile, and Potassium dihydrogen phosphate were purchased from MERCK laboratories, Mumbai.

Method Development

Preparation of Standard Solution:

Precisely weighed and transferred 15 milligram of Noscapine and 4 milligrams of Chlorpheniramine taken into a clean 10 milliliter flask as working standards. Following the addition of the standards, introduce approximately 7 milliliters of the diluent, and employ sonication to ensure complete dissolution. Finally, sufficient solvent was added to reach the desired final volume in the volumetric flask. 0.3 milliliter previously prepared stock solution was transferred into a 10-milliliter volumetric flask, & then diluted it with the appropriate diluent until the desired concentration is achieved.

Preparation of Sample Solution:

Precisely weighed and transferred 15 milligrams of Noscapine and 4 milligrams of Chlorpheniramine taken into a clean 10 milliliter flask as working standards. Following the addition of the standards, introduce approximately 7 milliliters of the diluent, and employ sonication to ensure complete dissolution. Finally, sufficient solvent was added to reach the desired final volume in the volumetric flask. 0.3 milliliter previously prepared stock solution was transferred into a 10-milliliter volumetric flask, & then dilute it with the appropriate diluent until the desired concentration is achieved.

Mobile Phase Optimization:

300ml of buffer is added to 700ml of methanol HPLC grade and ultrasonicate to remove dissolved gases and the solution was filtered through vacuum filtration (0.45µ).

Optimization of Column:

Columns of various makes were utilized and finally Dikma Spursil C18 ODS (4.6×150mm)5µ was identified as ideal based on its performance.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Water with auto sampler.
Column	:	Dikma Spursil C18 ODS (4.6×150mm)5µ
Mobile phase	:	30:70 (OPA buffer: Methanol)
Flow rate	:	1 ml/min
Wavelength	:	239 nm
Injection volume	:	20 µl
Run time	:	10 min.

Chromatogram is shown in the **fig 4.**

Method Validation:

System suitability: It was determined by six replicate injection of sample solution as per the procedure. The parameters like theoretical plates, tailing factor and resolution was recorded and tabulated and reported in table 1.

Specificity:

It was determined to rule out the interferences by any impurities and mobile phase. Standard, sample and blank solutions were injected into HPLC system and the chromatograms were recorded and reported in fig 5 to 7.

Linearity:

It was assessed by utilizing five different concentrations 40-200 µg/ml and 10-50 µg/ml for nascopine and chlorpheniramine respectively. Each level was injected in replicates and peak areas were measured. Plot a graph showing the area of the peak versus concentration (with concentration on the X-axis and peak area on the Y-axis), and then determine the correlation coefficient. The results were tabulated in table 2 and plots are shown in figures 8 and 9.

Accuracy:

It was determined by recovery studies. Three different levels with respect to target concentration ie 50%, 100% and 150% were prepared and each level was injected in triplicates. The amount recovered at each level was observed and mean recovery for both the analytes was observed and reported in Table 3 & 4.

Precision:

The standard solution was injected for six times and the peak area for all six injections was measured. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were given in table 5 to 8.

Limit of Detection and Limit of Quantification:

Limit of detection and Limit of Quantification of the analytes were calculated as follows:

$$LOD = 3.3\ \sigma / S$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = 10\ \sigma/S.$$

The chromatograms of the results of LOD and LOQ are shown in respectively

Robustness:

A study was conducted to determine the effect of variation in flow rate, change in mobile phase composition. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The same studies were also performed by varying mobile phase composition by 10%. The system suitability parameters were evaluated and reported in table 9 to 10.

RESULTS AND DISCUSSION

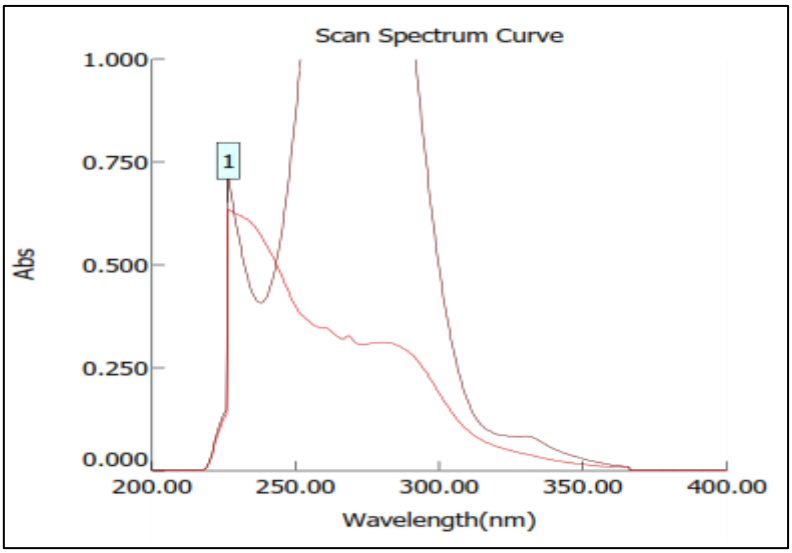

Fig: 3 Overlapping spectra in the spectrum of Noscapine & Chlorpheniramine

Optimized chromatographic conditions:

Instrument used	:	Water with auto sampler.
Column	:	Dikma Spursil C18 ODS (4.6×150mm)5µ
Mobile phase	:	30:70 (OPA buffer: Methanol)
Flow rate	:	1 ml/min
Wavelength	:	239 nm
Injection volume	:	20 µl
Run time	:	10 min.

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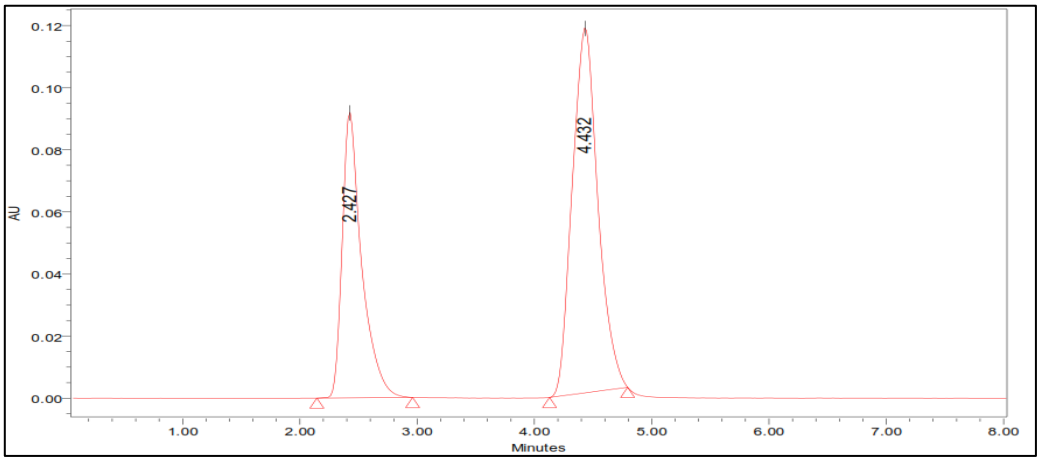

Fig.:4 Optimized Chromatogram

The retention time of Noscopine and Chlorpheniramine was found to be 2.427 mins and 4.432 mins respectively. And theoretical plates and tailing factor were found to be 2733, 1.6 and 3500, 1.4. Resolution was 4.6. The % purity for Noscopine and Chlorpheniramine is found to be 99.84 and 100.14% respectively.

System suitability: It was determined by six replicate injection of sample solution as per the procedure. The parameters like theoretical plates, tailing factor and resolution was recorded and tabulated.

Table : 1 System suitability studies of Noscapine and Chlorpheniramine

Nascopine			Chlorpheniramine		
Retention time	**Theoretical plates**	**Tailing Factor**	**Retention time**	**Theoretical plates**	**Tailing Factor**
2.356	3266.2	1.2	4.372	2251.1	1.3
2.352	3325.1	1.3	4.376	2656.7	1.2
2.387	3250.2	1.3	4.443	2127.2	1.2
2.383	3246.7	1.2	4.445	2267.6	1.2
2.337	3244.8	1.21	4.319	2345.1	1.2
2.367	3446.1	1.3	4.365	2356.2	1.2

Specificity

Specificity was carried out to rate out interference of any impurities and mobile phase.

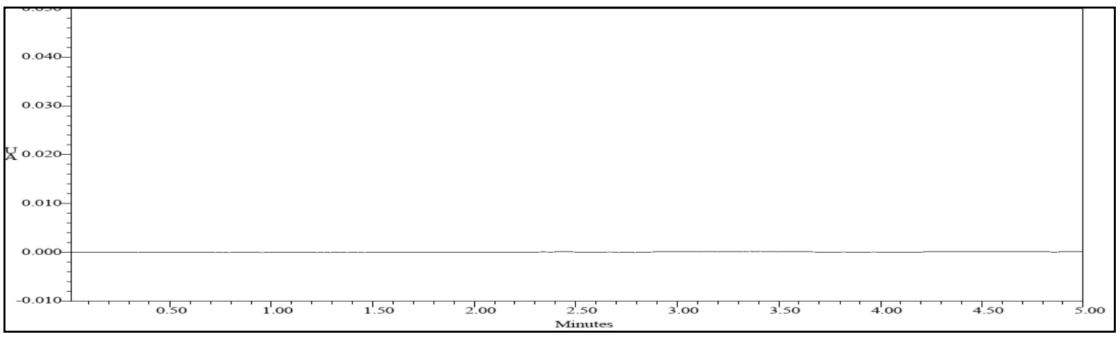

Fig :5 Chromatogram of Blank

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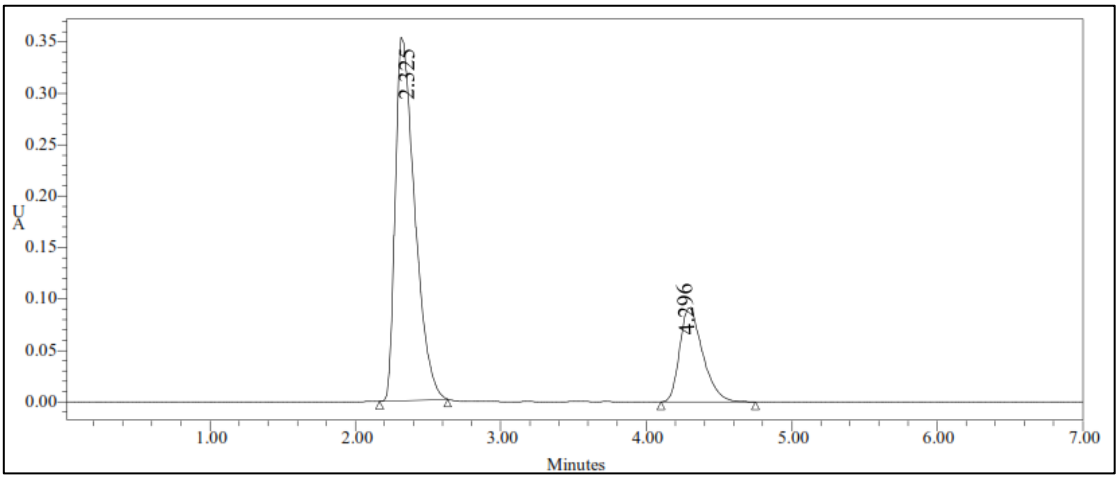

FIG:6 Chromatogram of Standard

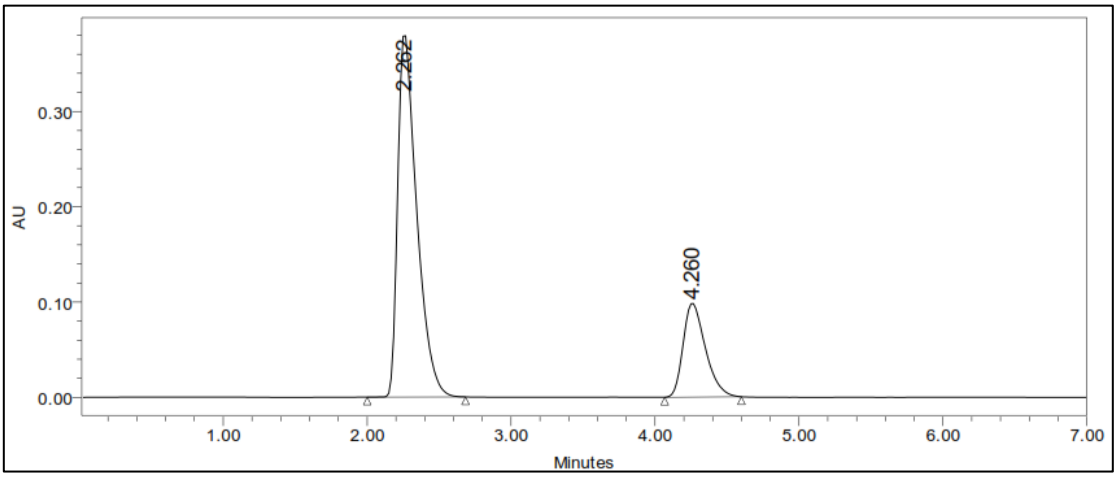

FIG:7 Chromatogram of Sample

Linearity : The linearity analysis was carried out for the levels of 40 ppm to 200 ppm chlorpheniramine and 20 ppm to 100 ppm Noscapine.

Table : 2 Linearity results of Noscapine and chlorpheniramine

Linearity level	Concentration	Area	Concentration	Area
I	40	363256	10	125624
II	80	752514	20	2256249
III	120	1063625	30	3536321
IV	160	1523652	40	4596526
V	200	1823625	50	5652413
Correlation coefficient		0.999	Correlation coefficient	0.999

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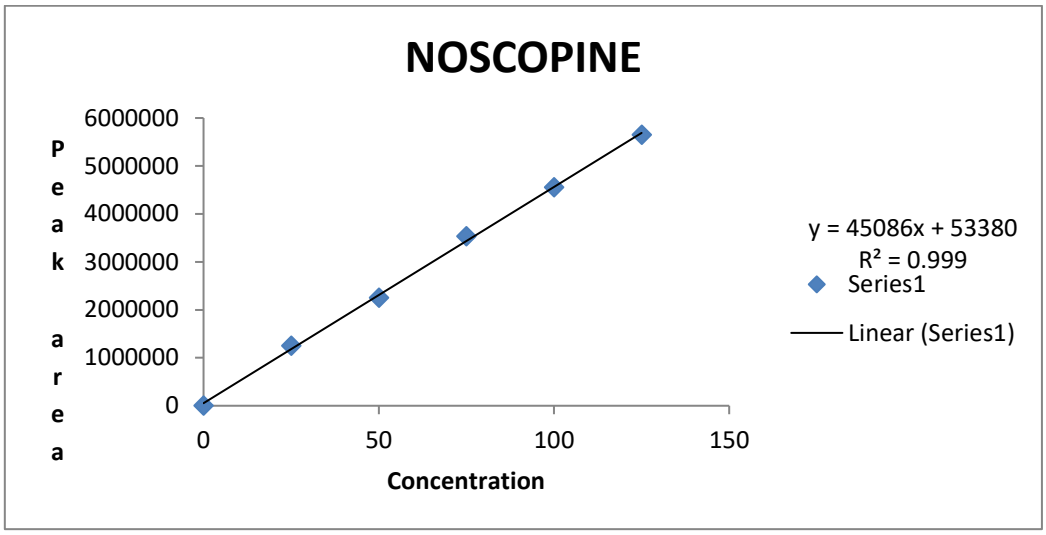

Fig:8 Linearity plot of Nascopine

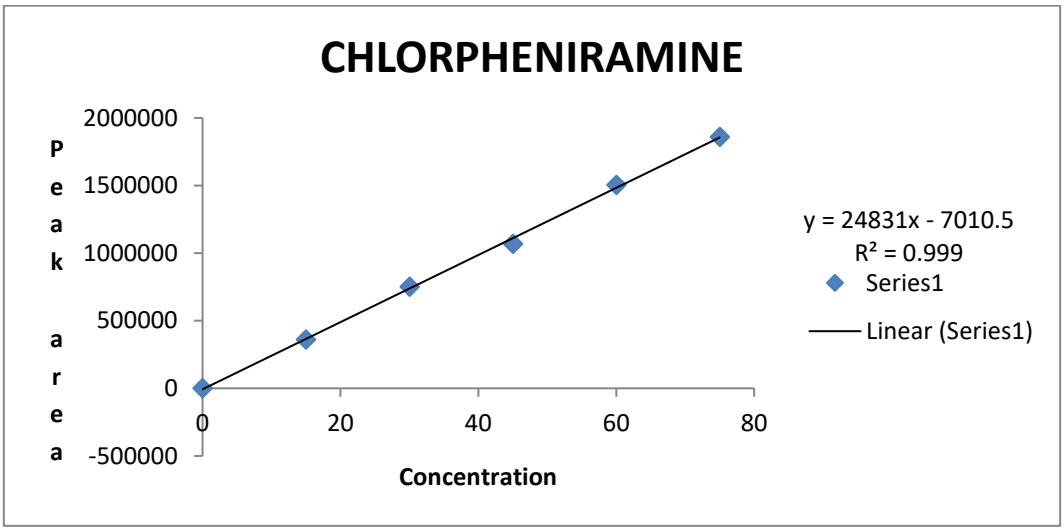

Fig :9 Linearity Plot of Chlorpheniramine

The linearity analysis was carried out over the concentration range of 10µg - 50µg chlorpheniramine and 40µg - 200µg noscapine, and the correlation coefficients were determined to be 0.999 and 0.999.

Accuracy:

Accuracy was determined at three levels i.e. 50%, 100% and 150%

Table : 3 Accuracy results of nascopine

level	Amount spiked (mg)	Amount achieved (mg)	% Recovery	Mean recovery
50%	5	5.0	100.0%	99.40%
100%	10	9.94	99.70%	
150%	15	14.6	98.50%	

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Table : 4 Accuracy results of Chlorpheniramine

level	Amount Spiked (mg)	Amount achieved (mg)	% Recovery	Mean recovery
50%	5	5.0	101.1%	100.30%
100%	10	9.98	99.98%	
150%	15	14.99	99.99%	

The percentage recoveries of noscapine and chlorpheniramine were found to be 99.40 and 100.30% respectively which were within the acceptance criteria**.**

Precision:

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

Table : 5 Precision results of noscapine

S.NO	Name of peak	Retention Time	Area
1	Noscapine	2.356	3365246
2	Noscapine	2.352	3366523
3	Noscapine	2.387	3365896
4	Noscapine	2.383	3362567
5	Noscapine	2.337	3366523
Mean			3363983
Standard deviation			30124.8
%Relative standard deviation			**1.0**

Table:6 Precision for Chlorpheniramine

S.NO	Name of peak	Retention Time	Area
1	Chlorpheniramine	4.372	1025868
2	Chlorpheniramine	4.376	1025846
3	Chlorpheniramine	4.443	102586
4	Chlorpheniramine	4.445	1025213
5	Chlorpheniramine	4.319	1025633
Mean			1025476
Standard. deviation			2268.3
%Relative standard deviation			**0.6**

To assess the intermediate precision the same procedure was repeated on different days.

Table:7 Intermediate Precision for Nascopine

S.NO	Name of peak	Retension Time	Area
1	Noscapine	2.331	3663527
2	Noscapine	2.323	3606588
3	Noscapine	2.316	3643255
4	Noscapine	2.277	3652654
5	Noscapine	2.289	3632456
Mean			3543255
Standard.deviation			98254.8
%Relative standard deviation			**1.5**

Table:8 Intermediate precision for Chlorpheniramine

S.NO	Name of peak	Retension Time	Area
1	Chlorpheniramine	4.448	1059563
2	Chlorpheniramine	4.456	1058546
3	Chlorpheniramine	4.304	1055697
4	Chlorpheniramine	4.267	1054523
5	Chlorpheniramine	4.211	1055625
Mean			1055675
Standard deviation			1646
%Relative standard deviation			**0.25**

The percentage RSD of peak areas of chlorpheniramine and noscapine were 0.26 and 1.6, respectively which were in agreement with acceptance criteria.

Limit of detection and Limit of Quantification:

The LOD for noscapine and chlorpheniramine was calculated to be 2.17 and 2.372, respectively.

The LOQ for noscapine and chlorpheniramine was determined to be 7.60 and 8.132, respectively.

Robustness:

Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min and altering the organic composition of the mobile phase.

Table:9 Variations in flow rate

Flow rate (ml/min)	**Chlorpheniramine**		**Noscapine**	
	Theoretical Plates	**Tailing Factor**	**Theoretical Plates**	**Tailing Factor**
0.8	2965.6	1.3	3286.2	1.2
1	2951.8	1.3	3425.1	1.2
1.2	2845.6	1.3	3350.2	1.3

Table: 10 Variations in mobile phase composition

Change in organic composition in the MP	Noscapine		Chlorpheniramine	
	Theoretical Plates	**Tailing Factor**	**Theoretical Plates**	**Tailing Factor**
5 % less	3266.2	1.2	2251.1	1.3
*Actual	3325.1	1.3	2656	1.4
5 % more	3250.2	1.4	2127.2	1.5

After deliberate changes in flowrate and organic phase composition there was no significant changes were observed in selected parameters.

CONCLUSION

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Noscapine in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Phenomenex LunaC18 (4.6mm x 150mm, 5µm) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with Ortho phosphoric acid in the ratio of 25:75%v/v. The retention time of Noscapine and Chlorpheniramine was found to be 2.427 mins and 4.432 mins respectively.

The method was found to be linear with a correlation coefficient of 0.999 and 0.999. The method was precise with % RSD values 1 and 0.6 for noscapine and chlorpheniramine respectively .Recovery studies were performed to determine the accuracy and thee mean recovery values were found to be 99.04 and 100.30 for noscapine and chlorpheniramine respectively. The LOD found to be 2.17,2.372 µg/ml and LOQ was found to be 7.60 and 8.132 µg/ml respectively. The method was established to be robust as there were no significant changes in system suitability parameters on deliberate changes in flow rate and mobile phase composition.

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Conflict of Interest Statement: All authors have nothing else to disclose.