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ANALYTICAL AAPROACH FOR DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF NOSCAPINE AND CHLORPHENIRAMINE MALEATE IN SYNTHETIC MIXTURE

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ABSTRACT: Simple, rapid, economical, precise and accurate Stability indicating RP- HPLC method for the estimation of Noscapine and Chlorpheniramine Maleate in synthetic mixture has been developed. A reverse phase high performance liquid chromatographic method was developed for the estimation of Noscapine and Chlorpheniramine Maleate in synthetic mixture has been developed. The separation was achieved Column Kromasil C18 (250 x 4.6) 5 µm ID, Isocratic program0.1%TFA: Acetonitrile, as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out at 254 nm retention time of Noscapine and Chlorpheniramine Maleate was found to be 5.035 and 2.111 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Noscapine and Chlorpheniramine Maleate 74.90-224.70 µg/ml. Developed method was found to be accurate, precise and rapid for estimation of Noscapine and Chlorpheniramine Maleate in synthetic mixture. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, under same chromatographic condition. The stress samples were assayed on RP-HPLC system.

KEYWORDS:

Noscapine, Chlorpheniramine Maleate, Stability indicating RP- HPLC Method, Validation.

I . INTRODUCTION:

Allergy occurs when a person reacts to substance in the environment that are harmless to most people. These substances are known as allergens and are found in the dust mites, pets, pollen, insects, ticks, moulds, food and some of medications. The symptoms of allergy are caused by your immune system reacting or over-reacting to these otherwise harmless substances. Allergy can produce many and varied symptoms. [1]. Allergies occurs when your immune system reacts to a foreign substance. When an allergic reaction occurs, allergens bind to antibodies that the body produced called immunoglobin E(igE). [2] Allergy treatment is based on your medical history, the results of your allergy tests and how severe your symptoms are. It can include three treatment types: avoiding allergens, medicine options and immunotherapy. [3]. Structure of Noscapine and Chlorpheniramine Maleate is shown in Figure. [4-5] Noscapine's antitussive effects appear to be primarily mediated by its sigma receptor agonist activity. Chlorpheniramine binds to the histamine H1 receptor. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine. [6-8] By the literature survey it was found that analytical methods are available for estimation of Noscapine and Chlorpheniramine Maleate alone and with other combination. [9-15]. So, there is thought to perform Stability indicating RP-HPLC method development and validation for simultaneous estimation of synthetic mixture. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products. [16-17] Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of Noscapine and Chlorpheniramine Maleate.

II .MATERIALS AND METHODS

Shimadzu HPLC, LC 2010 CHT model and LC Solution software was used. Acetonitrile, methanol, Diammonium hydrogen phosphate, Mili-Q water and ortho phosphoric acid of AR grade from Merck Life Science Pvt. Ltd, was used.

IR identification and wavelength selection

The individual standard drugs, Noscapine and Chlorpheniramine Maleate were mixed with KBr and KBr pallets were prepared. These KBr pallets of drugs were used for FTIR analysis. And then FTIR spectra were interpreted and results were co-related with M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

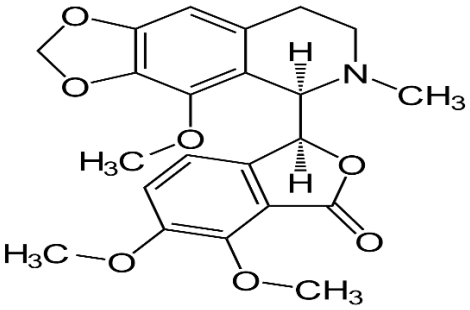

Figure 1. Structure of Noscapine

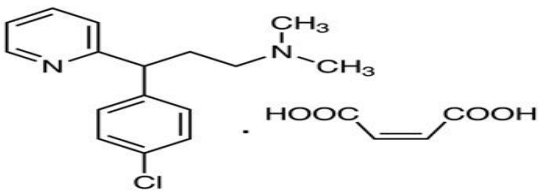

Figure 2. Structure of Chlorpheniramine Maleate

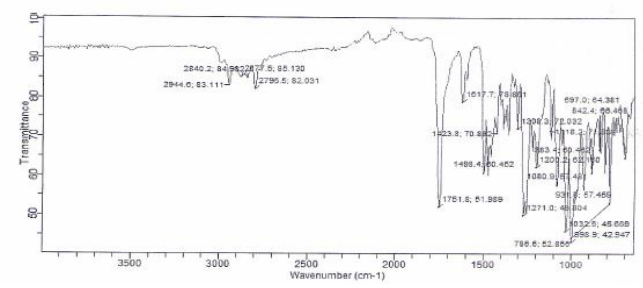

Figure 3: IR spectrum of Noscapine (API)

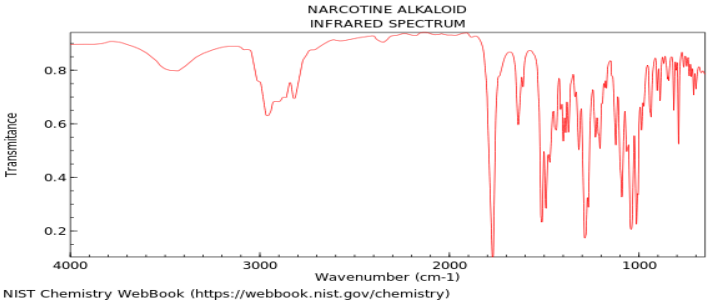

Figure 4: IR Spectrum of Noscapine (Std.)

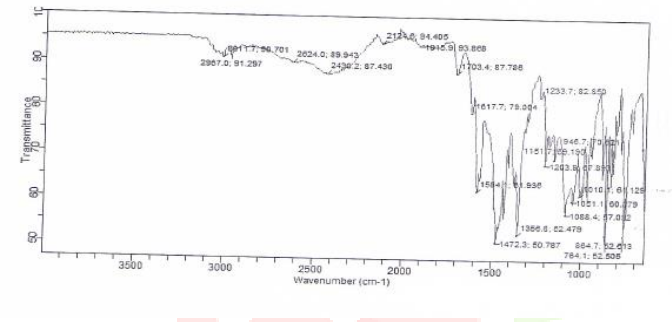

Figure 5: IR spectrum of Chlorpheniramine (API)

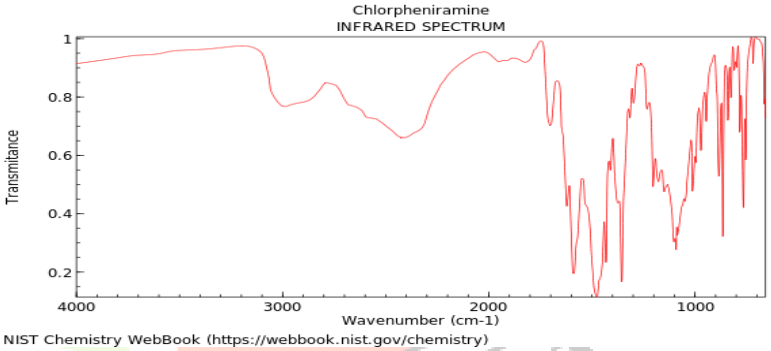

Figure 6: IR Spectrum of Chlorpheniramine (Std.)

Table 1: IR spectrum of Noscapine

Sr. No.	Functional group	Observed value
1	N-H stretching	2944.0
2	C-H stretching	2795
3	C=O stretching	1751.8
4	C-H bending	1617.7
5	C-O stretching	1271.1

Table 2: IR spectrum of Chlorpheniramine Maleate

Sr. No.	Functional group	Observed value
1	C=O stretching	1703.4
2	C-H bending	1358.5
3	C-N stretching(aromatic)	1203.5
4	C-N stretching	1088.4
5	C-Cl stretching	764.1

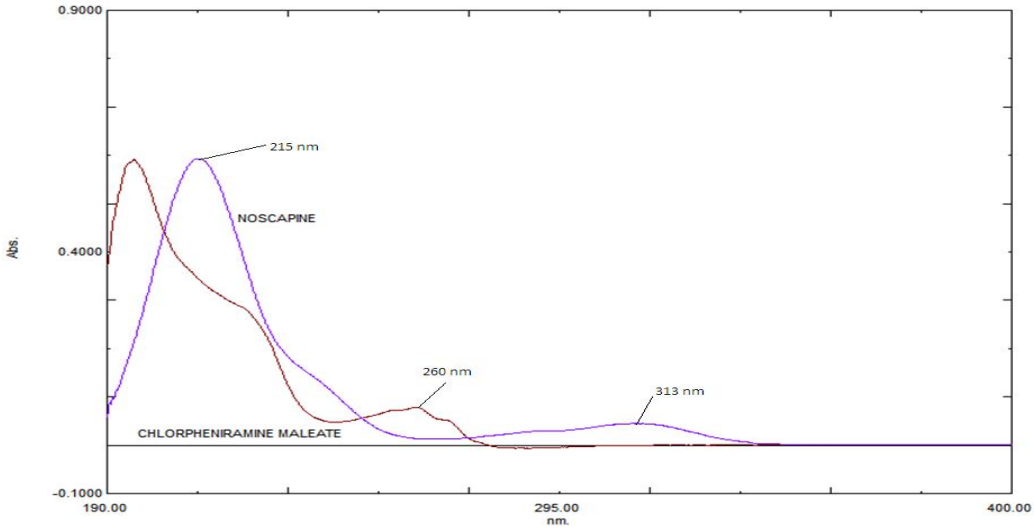

Figure 7: Determination of wavelength maximum (250 nm)

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, methanol, Sodium dihydrogen phosphate, sodium hydroxide, ortho phosphoric acid, Trifluoroacetic acid in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Standard stock solution of Noscapine and Chlorpheniramine Maleate were further diluted with Water into 10 ml volumetric flask which contain 50,100,150,200,250 µg/ml for both drugs and volume was made up mark with mobile phase. Scan in HPLC and plot the graph of peak area vs concentration. Calibration curve were plotted over a concentration range of 50-250 µg/ml for both drugs.

Preparation of sample solution

Pipette out 5 ml of synthetic mixture into 100 ml volumetric flask. Add 20 ml of diluent and sonicated for 15 minutes to dissolve the content. Make the volume with diluent. Sample solution was filtered through Whatman 0.45 µm PVDF filter and used as a sample solution.

METHOD DEVELOPMENT

Trial-1

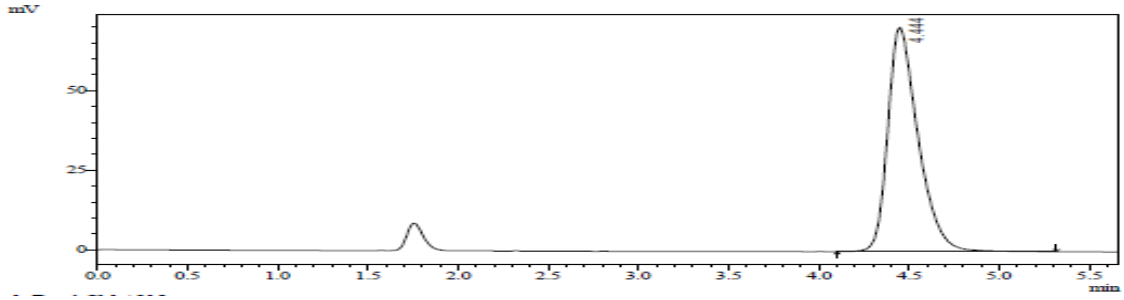

Figure 8: Chromatogram of Chlorpheniramine Maleate 40 ppm 0.1%TFA:Acetonitrile (50:50)

Trial-2

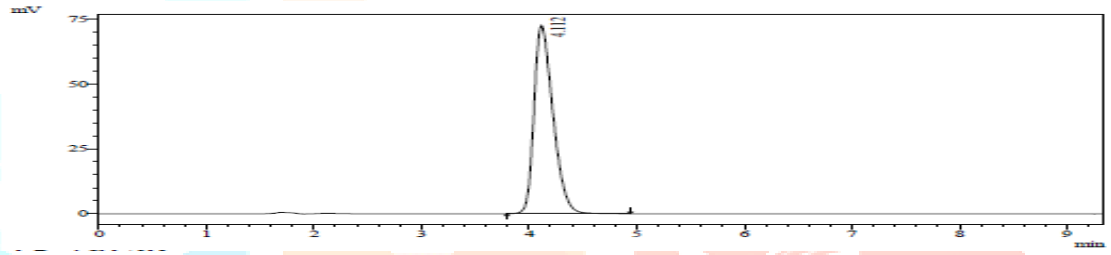

Figure 9: Chromatogram of Noscapine 150 ppm 0.1%TFA: Acetonitrile (50:50)

Trial-3

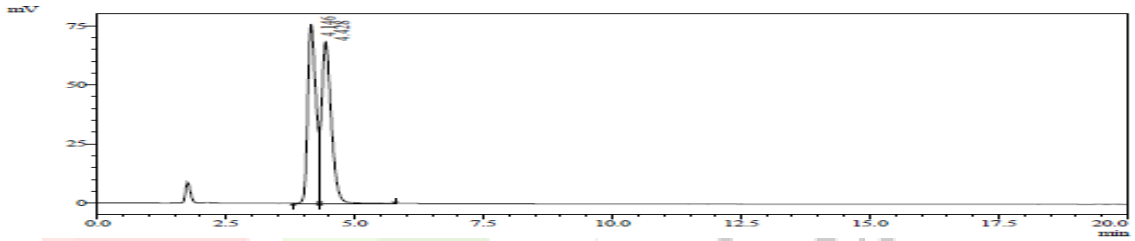

Figure 10: Chromatogram of Chlorpheniramine Maleate 40 ppm+ Noscapine 150 ppm 0.1%TFA: Acetonitrile (50:50)

Trial-4

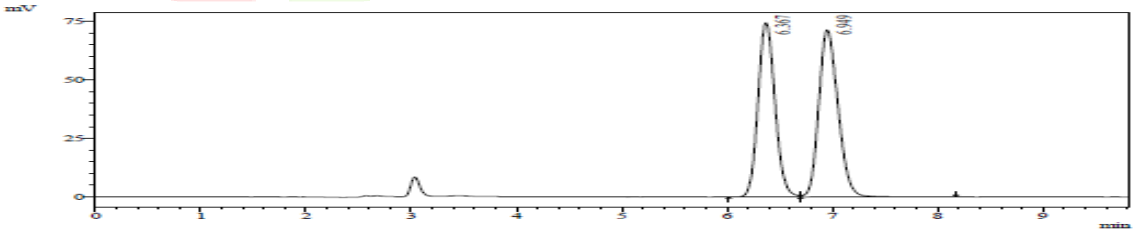

Figure 11: Chromatogram of Chlorpheniramine Maleate 40 ppm+ Noscapine 150 ppm 0.1%TFA: Acetonitrile (50:50)

Trial-5

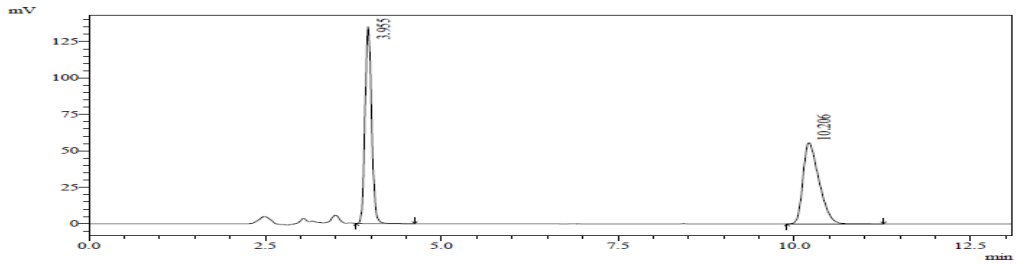

Figure 12: Chromatogram of Chlorpheniramine Maleate 40 ppm+ Noscapine 150 ppm 0.1%TFA: Acetonitrile (70:30)

Trial-6

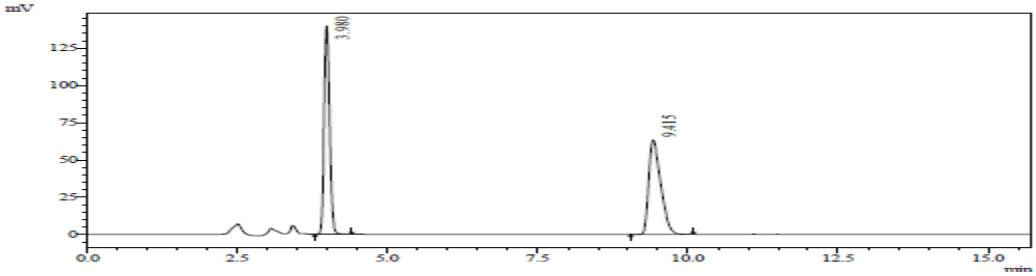

Figure 13 Chromatogram of Chlorpheniramine Maleate 40 ppm+ Noscapine 150 ppm 0.1%TFA: Acetonitrile (70:30)

Trial-7

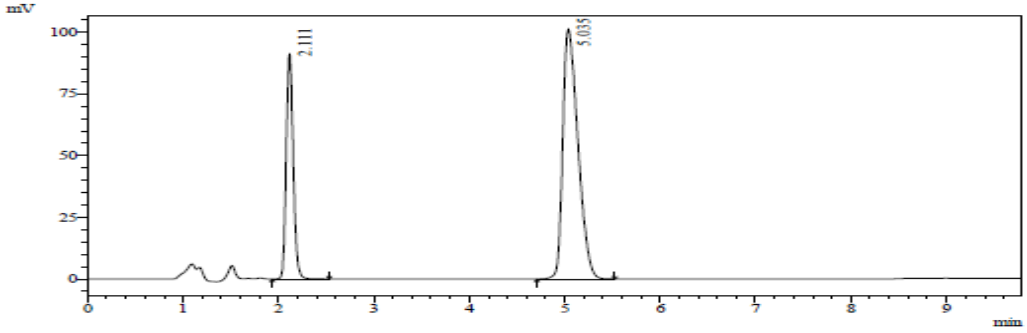

Figure 14 Chromatogram of Chlorpheniramine Maleate 40 ppm+ Noscapine 150 ppm 0.1%TFA: Acetonitrile (70:30)

Table: 3 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	0.1%TFA: Acetonitrile (50:50)	Proper peak elutes
2	0.1%TFA: Acetonitrile (50:50)	Proper peak elutes
3	0.1%TFA: Acetonitrile (50:50)	Both peaks merged
4	0.1%TFA: Acetonitrile (50:50)	Alter select column (pear are not merged)
5	0.1%TFA: Acetonitrile (70:30)	peaks eluted (Further trials taken to shorten and optimize method.)
6	0.1%TFA: Acetonitrile (70:30)	Peak observed both drugs (Further trials taken to shorten and optimize method.)
7	0.1%TFA: Acetonitrile (70:30)	Peak observed both drugs with proper shape trial finalised

IV. METHOD VALIDATION

Specificity

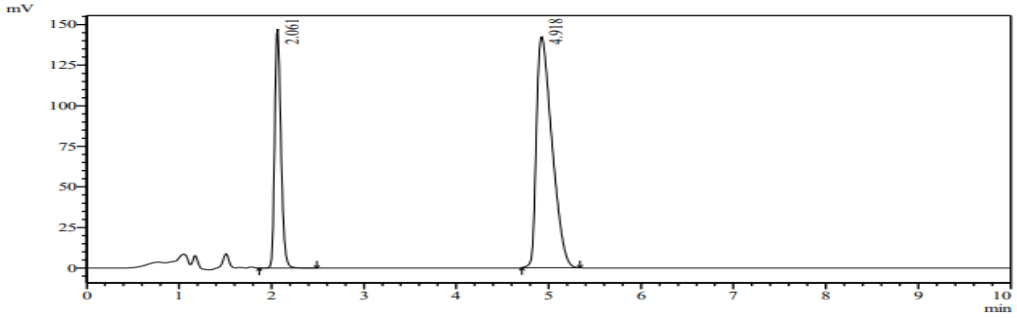

Figure 15: Chromatogram of Standard Noscapine and Chlorpheniramine Maleate

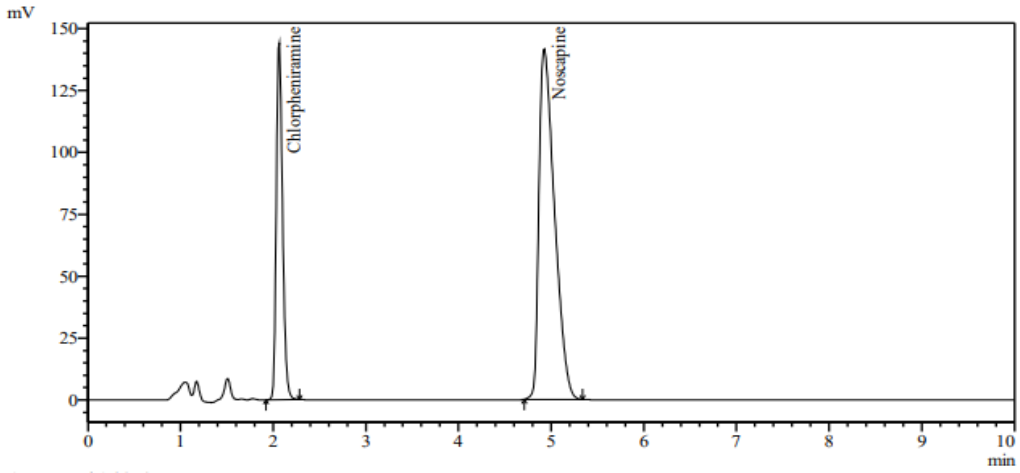

Figure 16: Chromatogram of Sample Noscapine and Chlorpheniramine Maleate

Linearity

For the linearity study 5,10,15,20,25,30 ml of Noscapine, 5,10,15,20,25,30 ml of Chlorpheniramine Maleate was mixed in six 10ml volumetric flask and volume was made up to mark by water. Calibration curve Noscapine and Chlorpheniramine Maleate are shown in figure.

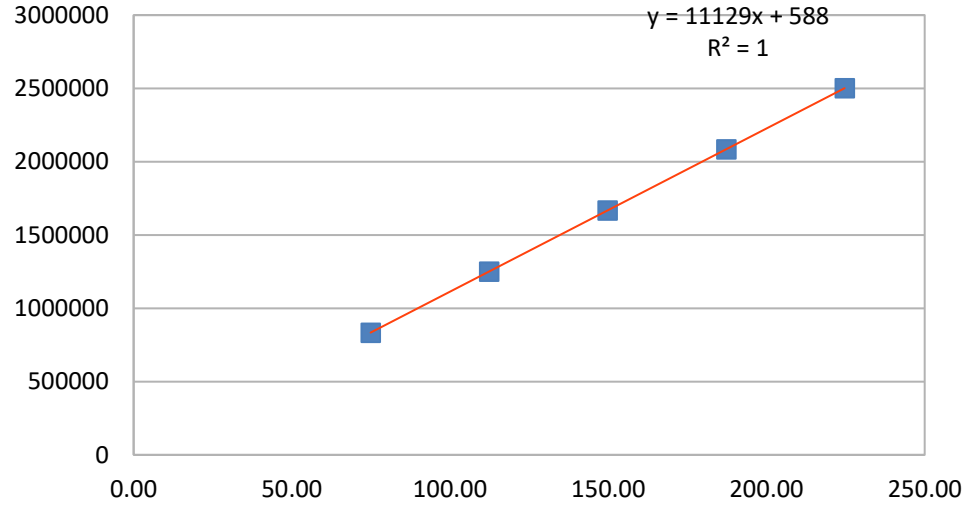

Figure 17: Calibration Curve of Noscapine

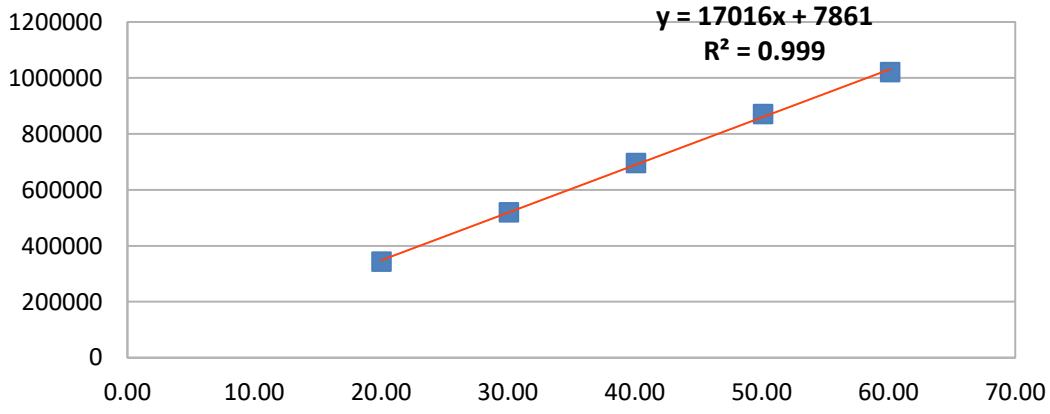

Figure 18: Calibration curve of Chlorpheniramine Maleate

Table 5: Linearity study of Noscapine and Chlorpheniramine Maleate

Noscapine		Chlorpheniramine Maleate	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
74.90	834257	20.05	343226
112.35	1251248	30.07	519839
149.80	1666978	40.10	696305
187.25	2084789	50.12	871024
224.70	2501426	60.15	1020541

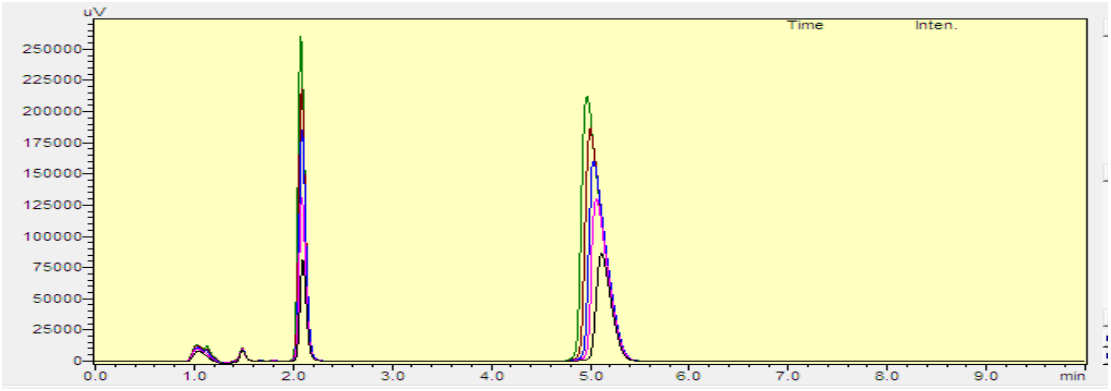

Figure 19: Overlaying linearity chromatogram of Noscapine and Chlorpheniramine Maleate

Repeatability

The data for repeatability of peak area measurement for Noscapine and Chlorpheniramine Maleate based on six measurements of same solution. The % RSD for Noscapine and Chlorpheniramine Maleate are shown in table.

Intra-day and inter-day Precision

In the intra-day studies, three repeated injections of standard solution were made and response factor of drug peaks and % RSD were calculated. In inter-day variation studies, three injections of standard solution were made for three consecutive days and response of drug peaks and % RSD were calculated.

Table 6: Repeatability study

Concentrationof Noscapine(µg/ml)	Noscapine		Concentration of Chlorpheniramine Maleate(µg/ml)	Chlorpheniramine Maleate	
	Mean ± SD (n=6)	% RSD		Mean ± SD (n=6)	% RSD
150	1669600.800 ±439.20	0.0	40	708952.000±796.38	0.1

Table 7: Intraday & Interday precision study of Noscapine

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD(n=3)	% RSD	Mean ± SD (n=3)	% RSD
Noscapine	150	1614925 ±398.0188438	0.0	1613937± 2408.785794	0.1

Table 8: Intraday & Interday precision study of Chlorpheniramine Maleate

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
Chlorpheniramine Maleate	40	700151±1051.085312	0.2	701078±930.5487628	0.1

Accuracy:

Table 9: Recovery study for Noscapine and Chlorpheniramine Maleate

Drug	% Of Level	Amount (mg/ml)	AmountAdded (mg/ml)	Total Amount Found (mg/ml)	% Recovery ± SD (n=3)
Noscapine	50 %	1	0.76	0.77	101.0± 0.1
	100 %	1	1.52	1.53	100.4 ± 0.6
	150 %	1	2.29	2.30	100.3 ± 1.1
Chlorpheniramine Maleate	50 %	1	0.20	0.20	101.4 ± 0.2
	100 %	1	0.39	0.39	99.3 ±0.4
	150 %	1	0.59	0.58	98.9 ±0.1

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 10 %).
2. Temp of Mobile phase was changed (± 5°C).
3. Ratio of Mobile phase was changed (± 2 %). The results were shown in table.

Table:10 Robustness data for Noscapine and Chlorpheniramine Maleate

Drug	Area at Temp. (-5°C)	Area at Temp. (+5°C)	Area at Flow (-10% ml/min)	Area at Flow (+10% ml/min)	Area at Mobile Phase (-2%)	Area at Mobile Phase (+2%)
Noscapine	1669108	1669108	1669108	1669108	1669108	1669108
	1705928	1709182	1887069	1548255	1736672	1702705
	1706482	1712309	1887037	1548696	1739352	1703541
% RSD	1.3	1.4	6.9	4.4	2.3	1.2
Chlorpheniramine Maleate	698488	702975	773159	635118	711264	697253
	699679	702104	774457	634969	711306	699138
	700053	702845	774957	635684	712899	699718
% RSD	0.1	0.1	0.1	0.1	0.1	0.2

LOD and LOQ

Calibration curve was repeated for Three times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts. The results were shown in table.

Table 12: Limit of Detection and Limit of Quantitation Data of Noscapine and Chlorpheniramine Maleate

Noscapine	Chlorpheniramine Maleate
LOD = 3.3 x (SD / Slope) = 3.3 x (658999.6762/ 11129.30178) = 195.393 mg/ml = 0.195 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (269848.8431 /17016.24749) = 52.3324 mg/ml = 0.0523 µg/ml
LOQ = 10 x (SD / Slope) = 10 x (658999.6762/ 11129.30178) =592.130 mg/ml = 0.592 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (269848.8431 /17016.24749) = 158.5830mg/ml = 0.1585 µg/ml

V. Forced Degradation Condition

1. Acid Degradation

- **Acid degradation Standard:** Accurately measured 1 ml of Noscapine standard stock and 1 ml Chlorpheniramine Maleate standard stock solutions were taken into 10 mL volumetric flask. 1 ml 5 N HCl was added into the flask. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 1 ml 5 N NaOH Volume was made up to the mark with water and injected in to HPLC system.
- **Acid degradation Sample:** 1 ml of sample stock solution was taken into 20 ml volumetric flask. To this, 1 ml 5 N HCl was added. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 1 ml 5 N NaOH. Volume was made up to the mark with water and injected in to HPLC system.

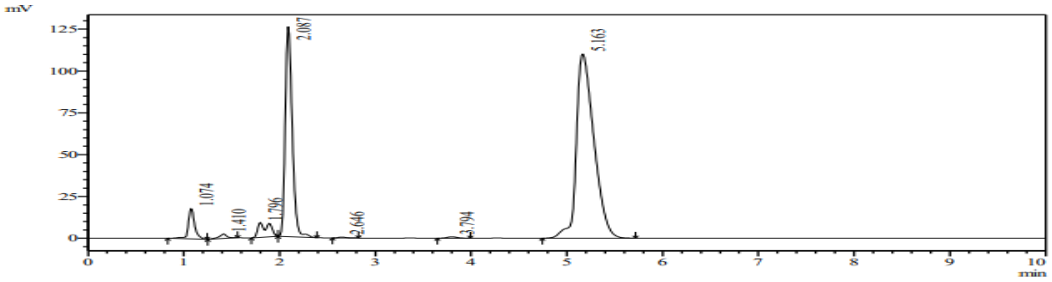

Figure 20: Chromatogram of Noscapine and Chlorpheniramine Maleate under acid Degradation Standard

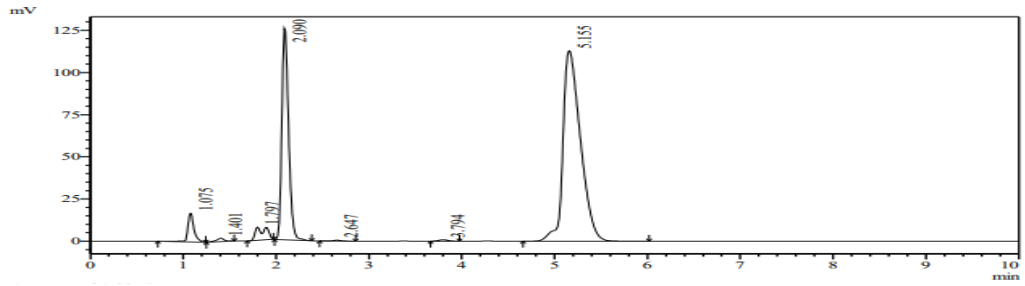

Figure 21: Chromatogram of Noscapine and Chlorpheniramine Maleate under acid Degradation Sample

2. Base Degradation

- **Base degradation Standard:** Accurately measured 1 ml of Noscapine standard stock and 1 ml Chlorpheniramine Maleate standard stock solutions were taken into 10 mL volumetric flask. 1 ml 5 N NaOH was added into the flask. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 1 ml 5 N HCL. Volume was made up to the mark with water and injected in to HPLC system
- **Base degradation Sample**1 ml of sample stock solution was taken into 20 ml volumetric flask. To this, 1 ml 5 N NaOH was added. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 1 ml 5 N HCL. Volume was made up to the mark with water and injected in to HPLC system.

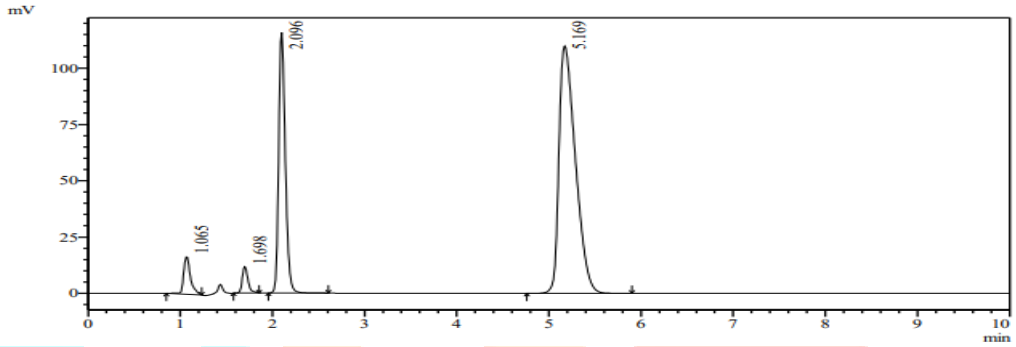

Figure 22: Chromatogram of Noscapine and Chlorpheniramine Maleate under base Degradation Standard

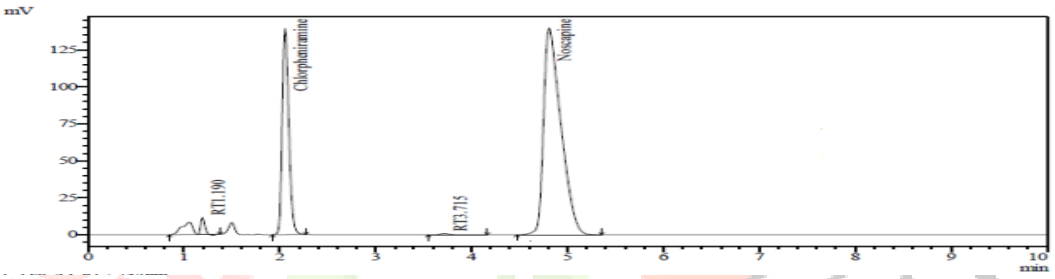

Figure 23: Chromatogram of Noscapine and Chlorpheniramine Maleate under base Degradation Sample

3. Oxidative Degradation

- **Peroxide degradation Standard:** Accurately measured 1 ml of Noscapine standard stock and 1 ml Chlorpheniramine Maleate standard stock solutions were taken into 10 mL volumetric flask and 1 ml 3 % H_2O_2 was added and solution was kept at room temperature for 6 hours for an Oxidative hydrolysis and made volume up to mark with diluent and injected in to HPLC system.

- **Peroxide degradation Sample:** 1ml stock solution into 20 ml volumetric flask and 1 ml 3% H_2O_2. Set for 6 hours at room temperature. Volume made with diluents and injected into HPLC.

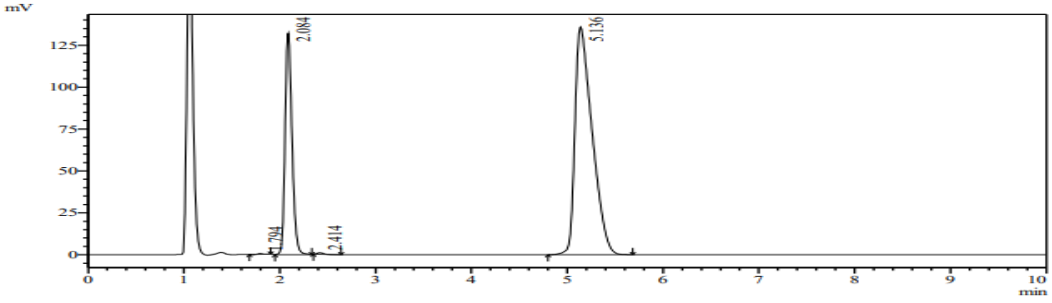

Figure 24: Chromatogram of Noscapine and Chlorpheniramine Maleate under oxidation Degradation Standard

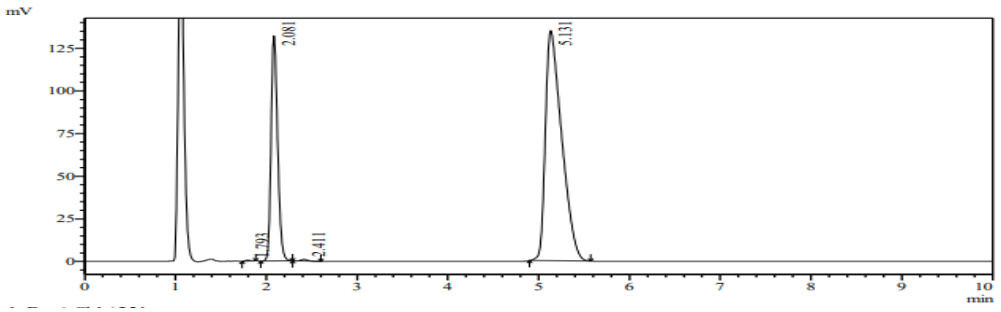

Figure 25: Chromatogram of Noscapine and Chlorpheniramine Maleate under oxidation Degradation Sample

4. Photo Degradation

- Synthetic mixture and APIs were kept into sunlight for 2 days. Solutions were made as per method preparation and injected into HPLC.

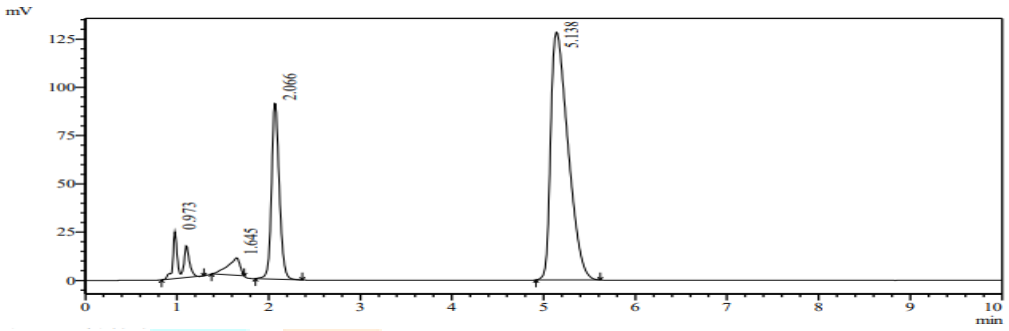

Figure 26: Chromatogram of Noscapine and Chlorpheniramine Maleate under photo Degradation Standard

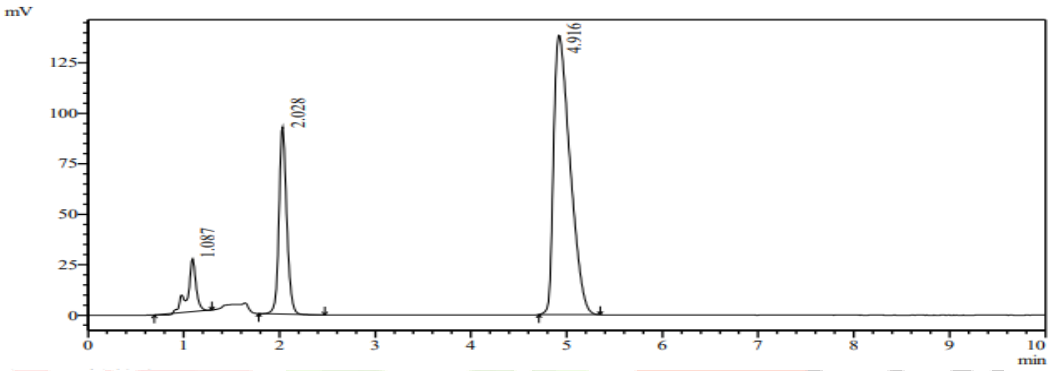

Figure 27: Chromatogram of Noscapine and Chlorpheniramine Maleate under photo Degradation Sample

5. Thermal Degradation

- Solution were exposed to wet heat at 70℃ and then solutions were made as per test sample preparation and injected into HPLC.

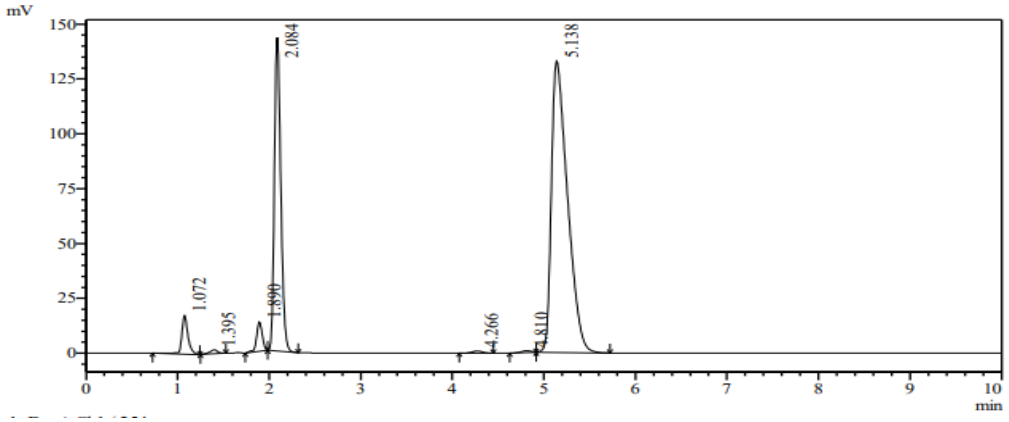

Figure 28: Chromatogram of Noscapine and Chlorpheniramine Maleate under thermal Degradation Standard

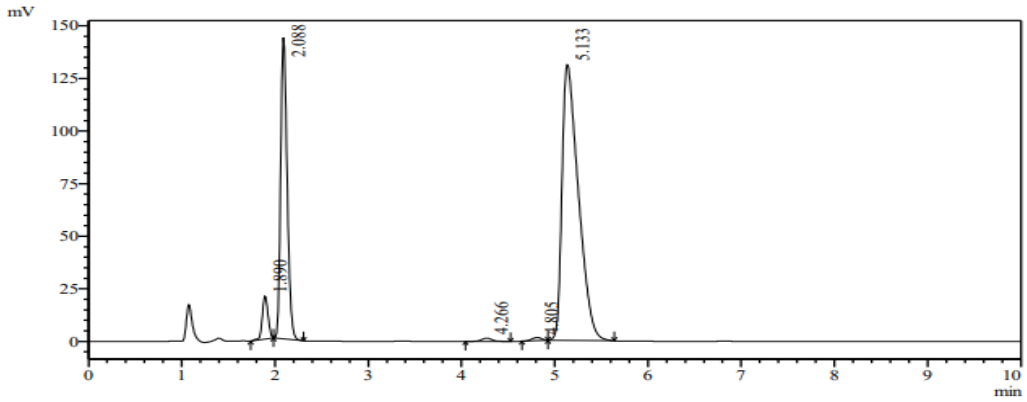

Figure 29: Chromatogram of Noscapine and Chlorpheniramine Maleate under thermal Degradation Sample

Table 15: Result of stability study of Noscapine and Chlorpheniramine Maleate

Condition	% Degradation Noscapine		% Degradation Chlorpheniramine Maleate	
	Sample	Standard	Sample	Standard
Acid	11.37	14.98	6.6	9.09
Base	13.5	17.58	14.12	18.53
Oxidation	2.72	2.88	2.48	5.81
Thermal	4.0	2.14	0.53	1.75
Photo	0.54	0.54	8.18	15.67

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of Noscapine and Chlorpheniramine Maleate. The melting point of Noscapine (173-177 °C) and Chlorpheniramine Maleate (132-135 °C) was found in the range. Method was developed in mobile phase containing Isocratic program0.1%TFA: Acetonitrile. Detection was carried out at 254 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % Recovery was within the range (99% - 102%). Results were shown in table. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day and inter-day precision. Results were shown in table. So, the developed method was found to be precise. LOD and LOQ values were shown in table. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. So, the developed method was found to be robust. Various forced degradation conditions were performed in proposed method and it can efficiently separate all the degradation products from the drugs. % degradation values are 1% to 31% degradation of the drug substance, have been considered as reasonable and acceptable for validation of chromatographic assays. So, the developed method is stability indicating.

CONCLUSION

Noscapine's antitussive effects appear to be primarily mediated by its sigma receptor agonist activity. Agents that suppress cough. They act centrally on the medullary cough centre. Chlorpheniramine Maleate binds to the histamine H1 receptor. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine.

RP-HPLC method was developed for simultaneous estimation Noscapine and Chlorpheniramine Maleate. In RP-HPLC method, good resolution and separation of two drugs was achieved. Isocratic program0.1%TFA: Acetonitrile, mobile phase. Retention time of Noscapine and Chlorpheniramine Maleate were found to be 5.035 and 2.11 min respectively with a flow rate of 1.5 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Noscapine and Chlorpheniramine Maleate in Synthetic mixture.

Forced degradation study of Noscapine and Chlorpheniramine Maleate was performed by RP-HPLC method which includes Acid, Base, Oxidative and Thermal degradation. Results of degradation were found within limit.

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