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Development and validation of new analytical method for the simultaneous estimation of levodropropizine and chlorpheniramine in pharmaceutical dosage form

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Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Levodropropizine and Chlorpheniramine in Tablet dosage form. The chromatogram was run through Ascentis C18 150 x 4.6 mm, 5m. Mobile phase containing Buffer Kh2po4: Acetonitrile was taken in the ratio 40:60was pumped through the column at a flow rate of 1.0ml/min. The buffer used in this method was Kh2po4. The temperature was maintained at 30°C. The optimized wavelength selected was260nm. The retention time of Levodropropizine and Chlorpheniramine was found to be 2.276min and 2.848. %RSD of the Levodropropizine and Chlorpheniramine was and found to be 0.7 and 0.7 respectively. %Recovery was obtained as 100.73% and 99.03% for Levodropropizine and Chlorpheniramine respectively. LOD, LOQ values obtained from regression equations of Levodropropizine and Chlorpheniramine were 0.14, 0.02, and 0.43, 0.06 respectively. Regression equation of Levodropropizine is y = 67089x + 5956.8 and y = 226526x + 13941 of Chlorpheniramine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control tests in Industries.

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Introduction

Levodropropizine is chemically (-)-(S)-3-(4-Phenyl-1-piperazinyl)-1,2-propanediol. It is the levo-rotatory (S)-enantiomer of dropropizine. It is a non-opioid agent whose peripheral antitussive action may result from its modulation of sensory neuropeptide levels within the respiratory tract. Levodropropizine is a peripherally acting agent inhibiting the afferent pathways that mediate the generation of the cough reflex. Compared with the racemic drug, levodropropizine maintains the antitussive activity but considerably lower central nervous system depressant actions. Levodropropizine is activated in the bronchopulmonary system as the inhibitor of bronchospasm induced by histamine, serotonin and bradiquinina.

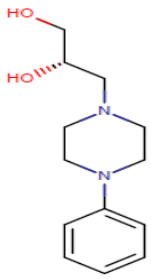

Fig 01: Structure of levodropropazine

Chlorpheniramine maleate is chemically [3-(4-chlorophenyl)-3- (pyridin-2-yl)propyl] dimethylamine is a antihistaminic used in the treatment of allergy. It acts by competing with histamine for H1-receptor sites on effector cells.

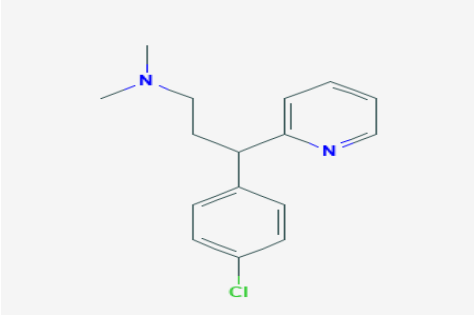

Fig 02: Structure of Chlorphenamine

A combination of Levodropropizine and Chlorpheniramine maleate is used as cough suppressant and also for allergy, itchy throat, common cold, hay fever, watery eyes, and runny nose. Extensive literature survey revealed that there were liquid chromatographic methods for the estimation Levodropropizine, Chlorpheniramine maleate alone[1,2] and with other combinations [3-9]. But no HPLC method has been reported for the simultaneous estimation of proposed drugs. Hence a validated RP HPLC method has been developed for the simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in bulk and syrup formulation.

Materials and Methods

Materials

Chlorpheniramine and Levodropropizine pure drugs (API), Combination Chlorpheniramine and Levodropropizine(**Reswas**) Syrup, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals andsolvents are from Rankem

Instruments

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps,Photo Diode Array detector and Auto sampler integrated with Empower 2 Software , UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Chlorpheniramine and Levodropropizine solutions.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions

Accurately weighed 30mg of Levodropropazine, 2mg of Chloropheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chloropheniramine)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml Levodropropazine of and 4µg/ml of Chloropheniramine)

Preparation of Sample stock solutions

Syrup equivalent to 30mg Levodropropazine and 2mg of Chloropheniramine was transferred into a 100 ml volumetric flask, 20ml of diluents as added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (300µg/ml of Levodropropazine and 20µg/ml of Chloropheniramine)

Preparation of Sample working solutions (100% solution)

2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(60µg/ml of Levodropropazine and 4µg/ml of Chloropheniramine)

Preparation of buffer

0.1% Formic acidBuffer:1ml of Conc Formic acid was diluted to 1000ml with water.

Validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Levodropropazine (60ppm) and Chloropheniramine (4ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Method Validation [10-11]

Specificity Checking of the interference in the optimized method.We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions Accurately weighed 30mg of Levodropropazine, 2mg of Chloropheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chloropheniramine)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml Levodropropazine of and 4µg/ml of Chloropheniramine)

Preparation of Sample stock solutions

Syrup equivalent to 30mg Levodropropazine and 2mg of Chloropheniramine was transferred into a 100 ml volumetric flask, 20ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (300µg/ml of Levodropropazine and 20µg/ml of Chloropheniramine)

Preparation of Sample working solutions (100% solution)

2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(60µg/ml of Levodropropazine and 4µg/ml of Chloropheniramine)

Linearity

Preparation of Standard stock solutions

Accurately weighed 30mg of Levodropropazine, 2mg of Chloropheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chloropheniramine)

25% Standard solution

0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Levodropropazine and 1µg/ml of Chloropheniramine)

50% Standard solution

0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Levodropropazine and 2µg/ml of Chloropheniramine)

75% Standard solution

0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (45µg/ml of Levodropropazine and 3µg/ml of Chloropheniramine)

100% Standard solution

1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (60µg/ml of Levodropropazine and 4µg/ml of Chloropheniramine)

125% Standard solution

1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Levodropropazine and 5µg/ml of Chloropheniramine)

150% Standard solution

1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (90µg/ml of Levodropropazine and 6µg/ml of Chloropheniramine)

Accuracy

Preparation of Standard stock solutions

Accurately weighed 30mg of Levodropropazine, 2mg of Chloropheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chloropheniramine)

Preparation of 50% Spiked Solution

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness

Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature

minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Levodropropazine, Chloropheniramine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Levodropropazine, Chloropheniramine, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies [12-13]

Oxidation

To 1 ml of stock solution of Chlorpheniramine and Levodropropizine, 1 ml of 20% hydrogen peroxide (H2O2) wasa dded separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 60µg/ml & 4µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stocks solution Chlorpheniramine and Levodropropizine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 60 µg/ml & 4µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Chlorpheniramine and Levodropropizine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 60 µg/ml & 4µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution w a s placed in oven at105°C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 60 µg/ml & 4µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 600µg/ml& 40µg/ml to UV Light by keeping the beaker in UV Chamber for 1days or 4000 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 60µg/ml& 4µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for1h r s atatemperature of $60^º$. For HPLC study, the resultant solution was diluted to 60µg/ml& 4µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Results and Discussion

Optimized conditions

Chromatographic conditions:

Mobile phase :0.01N kh2po4:Acetonitrile (60:40)

Flow rate : 1.0ml/min

Column : AscentisC18 (4.6 x 150mm, 5µm)

Detector wave length : 260nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 10 min

Diluent : Water and Acetonitrile in the ratio 50:50

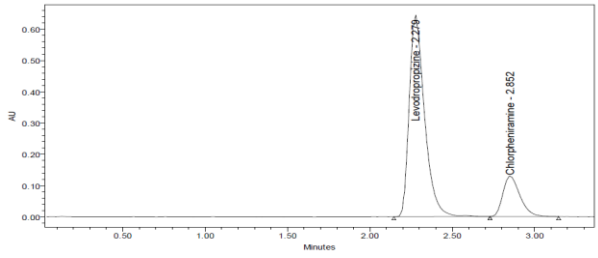

Fig 03: typical chromatogram

Observation

Levodropropizine and Chlorpheniramine were eluted at 2.279 min and 2.852 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table 01: Systemsuitability parameters for Levodropropizine and Chlorpheniramine

S no	Levodropropizine			Chlorpheniramine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.276	2943	1.40	2.848	3796	1.34	3.2
2	2.280	3057	1.38	2.855	3765	1.35	3.2
3	2.280	2954	1.39	2.855	3824	1.34	3.2
4	2.280	2902	1.40	2.856	3819	1.36	3.2
5	2.280	3017	1.37	2.856	3731	1.34	3.2
6	2.281	2968	1.39	2.857	3835	1.31	3.2

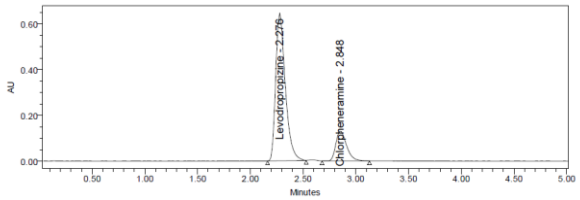

Fig 04: System suitability Chromatogram

Discussion

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation

Specificity

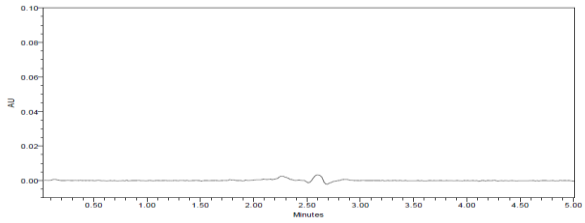

Fig 05: Chromatogram of blank

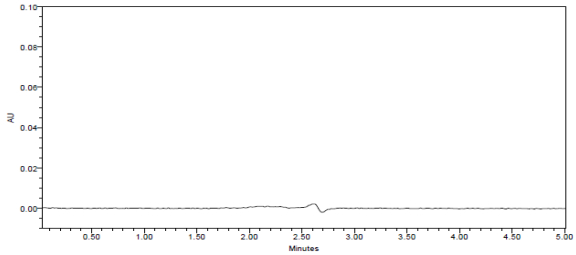

Fig 06: Chromatogram of placebo

Linearity

Table 02: Linearity table forLevodropropizine and Chlorpheniramine.

Levodropropizine		Chlorpheniramine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
15	1061194	1	242381
30	1994923	2	492293
45	2956215	3	685116
60	4087434	4	920941
75	5017766	5	1142880
90	6057273	6	1371032

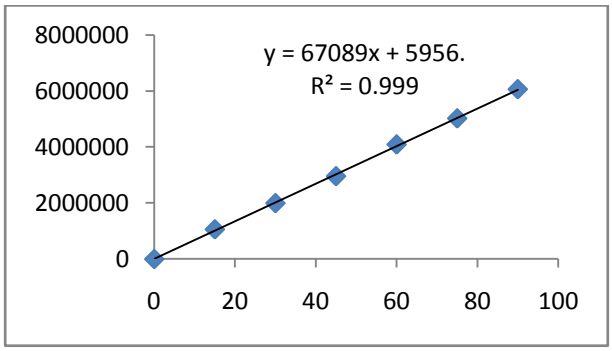

Fig 07: Calibrationcurveof Levodropropizine

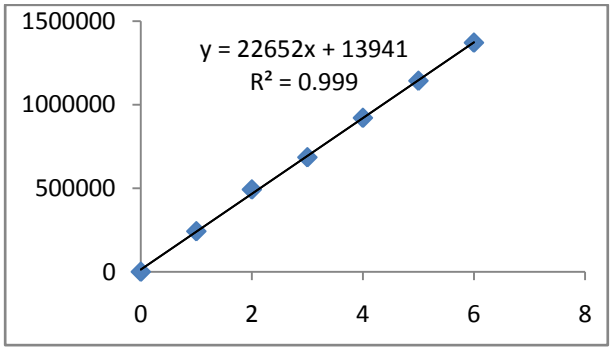

Fig 07: Calibrationcurveof Chlorpheniramine

Discussion

Six linear concentrations of Levodropropizine (15-90µg/ml) and Chlorpheniramine (1-6µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Levodropropizine was y = 67089x + 5956.8 and of Chlorpheniramine was y = 226526 x + 13941 Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System Precision

Table 03: System precision table of Levodropropizine and Chlorpheniramine

S. No	Area of Levodropropizine	Area of Chlorpheniramine
1.	4043221	918686
2.	4087891	918284
3.	4058297	916869
4.	4029594	924941
5.	4061933	909503
6.	4087812	919243
Mean	4061458	917921
S.D	23457.9	4974.2
%RSD	0.6	0.5

Repeatability

Table 04: Repeatability table of Levodropropizine and Chlorpheniramine

S. No	Area of Levodropropizine	Area of Chlorpheniramine
1.	4063209	911247
2.	4053333	908454
3.	4098738	915791
4.	4017469	921275
5.	4088506	903916
6.	4040395	917554
Mean	4060275	913040
S.D	30201.9	6375.8
%RSD	0.7	0.7

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.7% respectively for Levodropropizine and Chlorpheniramine. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_Day Precision)

Table 05: Intermediate precision table of Levodropropizine and Chlorpheniramine

S. No	Area of Levodropropizine	Area of Chlorpheniramine
1.	4043221	918686
2.	4087891	918284
3.	4058297	916869
4.	4029594	924941
5.	4061933	909503
6.	4087812	919243
Mean	4061458	917921
S.D	23457.9	4974.2
%RSD	0.6	0.5

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.6% and 0.5% respectively for Levodropropizine and Chlorpheniramine. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Table 06: Accuracy table of Levodropropizine

% Level	Amount Spiked(µg/mL)	Amountrecovered(µg/mL)	% Recovery	%
50 %	30	30.562924	101.88	100.73%
	30	30.222525	100.74	
	30	29.525749	98.42	
100 %	60	59.644159	99.41	
	60	60.428729	100.71	
	60	60.843357	101.41	
150 %	90	91.780456	101.98	
	90	90.684389	100.76	
	90	91.113059	101.24	

Table 07: Accuracy table of Chlorpheniramine

% Le	AmountSpiked(µg/mL)	Amount recovere	% Rec	Mean% Recover
50 %	2	1.972	98.59	99.03%
	2	1.994	99.72	
	2	1.969	98.44	
10 0%	4	4.005	100.14	
	4	3.993	99.82	
	4	3.932	98.31	
15 0%	6	5.932	98.87	
	6	5.957	99.29	
	6	5.885	98.08	

Discussion

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.73% and 99.03% for Levodropropizine and Chlorpheniramine respectively.

Sensitivity

Table 08: Sensitivity table of Levodropropizine and Chlorpheniramine

Molecule	LOD	LOQ
Levodropropizine	0.13	0.43
Chlorpheniramine	0.02	0.06

Robustness

Table 09: Robustness data for Levodropropizine and Chlorpheniramine

S.no	Condition	%RSD of Levodropropizine	%RSD of Chlorpheniramine
1	Flow rate (-) 0.9ml/min	0.6	0.4
2	Flow rate (+) 1.1ml/min	0.2	0.4
3	Mobile phase (-) 65B:35A	0.8	1.3
4	Mobile phase (+) 55B:45A	0.9	0.7
5	Temperature (-) 25°C	0.9	0.8
6	Temperature (+) 35°C	0.9	0.7

Discussion

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay

Reswas bearing the label claim Levodropropizine 30mg, Chlorpheniramine 2mg. Assay was performed with the above formulation. Average % Assay for Levodropropizine 99.57% and Chlorpheniramine 99.27% obtained was and respectively.

Table 10: Assay Data of Levodropropizine

S.no	Standard Area	Sample area	% Assay
1	4043221	4063209	99.64
2	4087891	4053333	99.40
3	4058297	4098738	100.51
4	4029594	4017469	98.52
5	4061933	4088506	100.26
6	4087812	4040395	99.08
Avg	4061458	4060275	99.57
Stdev	23457.9	30201.9	0.74
%RSD	0.6	0.7	0.7

Table 11: Assay Data of Chlorpheniramine

S.no	Standard Area	Sample area	% Assay
1	918686	911247	99.07
2	918284	908454	98.77
3	916869	915791	99.57
4	924941	921275	100.16
5	909503	903916	98.28
6	919243	917554	99.76
Avg	917921	913040	99.27
Stdev	4974.2	6375.8	0.7
%RSD	0.5	0.7	0.7

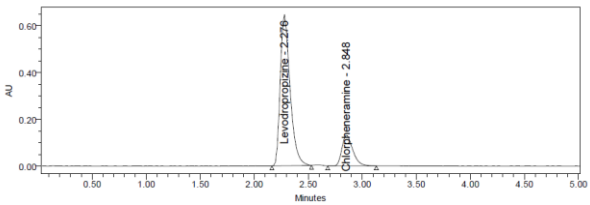

Fig 08: Chromatogram of working standard solution

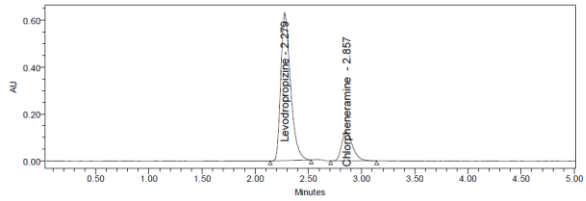

Fig 09: Chromatogram of working sample solution

Degradation data

Table 12: Degradation data for Levodropropizine and Chlorpheniramine

Type of degradation	Levodropropizine			Chlorpheniramine		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	3913602	95.97	4.03	886513	96.39	3.61
Base	3941275	96.65	3.35	865599	94.11	5.89
Peroxide	3822375	93.74	6.26	884893	96.21	3.79
Thermal	3990183	97.85	2.15	893907	97.19	2.81
Uv	4020395	99.55	0.45	895791	97.39	2.61
Water	4059294	98.59	1.41	912503	99.21	0.79

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Levodropropizine and Chlorpheniramine in in pharmaceutical dosage form. Retention time of Levodropropizine and Chlorpheniramine were found to be 2.276min and 2.848. %RSD of the Levodropropizine and Chlorpheniramine were and found to be 0.7 and 0.7 respectively.

%Recovery was obtained as 100.73% and 99.03% for Levodropropizine and Chlorpheniramine respectively. LOD, LOQ values obtained from regression equations of Levodropropizine and Chlorpheniramine were 0.14, 0.02 and 0.43, 0.06 respectively. Regression equation of Levodropropizine is y = 67089x + 5956.8 and y = 226526x + 13941 of Chlorpheniramine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Author Contribution

All authors are Contributed Equally

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