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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR LEVODROPROPIZIN

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Keywords:

RP-HPLC, Force Degradation study for Levodropropizine

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ABSTRACT

The emphasis of present research work is to improve solubility behavior of NSAIDs by different amino acid conjugation. Present conjugate approach stands for modification to overcome pharmaceutical barriers like solubility behavior. The prodrugs designed by classical approach increase lipophilicity of the drug, which decreases the water solubility thus decreasing the concentration gradient, which controls drug absorption. To overcome the limitations of traditional prodrug approach, water soluble conjugates are designed by adding selected amino acid to the drug moiety that are the substrates for the enzyme located at the intestinal brushborder. Amino acid conjugate of ketoprofen was synthesized by conventional coupling method and it was characterized by Melting Point, TLC, UV, IR, NMR and Mass Spectroscopy. Serine - Ketoprofen conjugate has maximum water solubility. Present research work indicates that conjugates synthesized with amino acid possess more water solubility. From the pH rate profile it was found that all the synthesized compounds, are stable at low pH values (pH 1.2), while undergo hydrolysis as the pH is increased (pH 7.4). This indicates that the synthesized compounds will be hydrolyzed and subsequently absorbed through intestine. In future this approach can be applied to other NSAIDs having free carboxyl functional group as well as in vivo bioavailability study can be undertaken in animals and can be correlated in humans.

INTRODUCTION

The general pharmacological profile of Leodropropizine (S (-) 3-(4-phenyl-piperizine-1-yl)-propane-1, 2-diol)' a new antitussive drug.

Fig No.1 Leodropropizine

The mechanism of action of Leodropropizine exerts peripheral action and suppress cough in patients with non-productive cough. Leodropropizine is a peripherally acting agent inhibiting the afferent pathways that mediate the generation of the cough reflux. Compared with the racemic drug. Leodropropizine maintains the antitussive activity but considerably lower central nervous system depressant actions. Leodropropizine is activated in the broncopulmonary system as the inhibitor of brancospasm induced by histamine, serotononin and bradiquinine. When given intravenously or intraperitoneally, Leodropropizine did not exert any significant effects on the cardiovascular and respiratory systems. Receptor binding data excluded interaction with beta adrenergic, muscarinic and opiate receptors. On the contrary, Leodropropizine has affinity for H1-histaminic and alpha adrenergic receptors. Literature survey revealed that, there are few analytical methods such as spectrophotometry, HPLC, HPTLC, Electroanalytical characterization and LC/MS has been reported for estimation of levodropropizine[1-3] but there is no stability indicating method by RP-HPLC was reported for Levodropropizine According to the stability test guidelines issued by ICH, in the present study the stress induced stability studies were carried out for Levodropropizine to establish its stability characteristics and also an analytical method develop by RP-HPLC and developed method was validated.

EXPERIMENTAL

Material and Reagents:

Acetonitrile (HPLC grade), Methanol (HPLC grade), Concentrated Hydrochloric acid (AR grade), Sodium Hydroxide (AR grade), Hydrogen Peroxide (AR grade), Potassium dihydrogen o-phoshate(AR grade), Potassium phosphate dibasic(AR grade) All the chemicals were purchased

from Thomas Baker (Chemicals), Mumbai (India).Membrane filter paper was used of 0.45 µ purchased from Milipore Pvt. Ltd., Peenya, Banglore, India.

Instrumentation and Chromatographic Condition

JASCO, Binary high pressure gradient RP-HPLC Equipped with UV -2075 detector was employed in this method development, force degradation study, and method validation. Pump PU-2080 plus, Rheodyne manul loop injector (20 µL) , Analytical column –Phenomenex C18 column (250 mm× 4.6 mm, 5 µm), Seperation was perform by using Methanol:Phosphate buffer (60:40) (pH 6.8). Mobile Phase filtered through 0.45 µm membrane filter (0.45 µ, Millipore) and degassed in ultrasonic bath. Injection volume 20 µL, Flow rate 1 ml/min. uv detection carried out at 248 nm.

Preparation of standard solution

An accurately weighed quantity of 10 mg Levodropropizine was transferred to 10 mL volumetric flask, dissolved with sufficient quantity of methanol and volume was then made up to the mark with same solvent and sonicated for 15 min. From the resulting solution 0.1mL was transferred to 10 mL volumetric flask and the volume was made up to the mark with same solvent. The resulting 10 µg/mL of solution was subjected to chromatographic analyses using mobile phases of different strengths.

Sample Solution Preparation:

To determine the content of Levodropropizine in marketed tablets (label claim 60 mg per tablet), twenty tablets were weighed, and average weight was calculated. Tablets were triturated and powder equivalent to 10 mg of Leodropropizine was weighed. The drug was extracted from the tablet powder with 100 mL methanol. To ensure complete extraction it was sonicated for 15 min. 0.1mL of supernatant was then diluted up to 10 mL with mobile phase.

Method validation[4-6]:

The developed stability indicating method is then validated according to ICH guideline for linearity, accuracy, precision, specificity, limit of quantification, limit of detection, ruggedness, robustness of the method.

Force Degradation Studies:

Stress studies are performing according to ICH Guidelines Drug was subjected to variety of stress conditions to effect degradation up to about 5-20 %. The stress testing was performed using heating mantle with temperature controller. Drug was stressed under variety of stress conditions like acid, alkali, wet heat, effect by oxidation, light and dry heat. Further, the stressed samples

were subjected to chromatographic separation using mobile phase to resolve the drug from potential degradation products.

Acid degradation study:

Stressed sample- In a round-bottom flask (RBF), 10 mg of Levodropropizine was taken, to it 10 mL of x N HCl was added, it was then heated under reflux on a heating mantle at y °C for z hrs. diluted up to 50 mL with methanol and sonicated for 15 min.

Alkali degradation study:

In a round-bottom flask (RBF) 10 mg of Levodropropizine was taken, to it 10 ml of x N NaOH was added, it was then heated under reflux on a heating mantle at y °C for z hrs diluted up to 50 ml with methanol and sonicated for 15 min..

Wet heat degradation:

In a round bottom flask (RBF) 10 mg of Levodropropizine was taken, to it 10 ml of water was added, it was then heated under reflux on a heating mantle at y °C for z hrs. diluted up to 50 ml with methanol and sonicated for 15 min.

Dry heat degradation:

Levodropropizine drug was spread as thin film layer in Petri plate kept in a hot air oven at x °C for y hrs. From the above stressed sample, 10 mg was weighed accurately and diluted with methanol to make up the volume to 10 ml and sonicated for 15 min.

Photolytic Condition

Levodropropizine drug was taken in petri plate, spread as thin layer and exposed to sunlight for x hrs. From the above stressed sample, 10 mg was weighed accurately and diluted with methanol to make up the volume to 50 ml and sonicated for 15 min.

Stress testing under oxidative condition

10 mg of Levodropropizine was taken in a 10 mL stoppered volumetric flask, to it 10 mL x % v/v solution of hydrogen peroxide was added, it was sonicated for 10 min to ensure even mixing of drug in solution and then kept in dark at room temperature for y hrs. After y hrs the solution was heated to boiling temperature for 5 min to remove the excess of hydrogen peroxide.

RESULT AND DISCUSSION

Optimized Chromatographic condition:

The main aim of development of RP-HPLC method was to get reliable method for the quantification of levodropropizine from bulk and pharmaceutical dosage form and which will be applicable for degradation product also different chromatographic condition where employed for the analysis of levodropropizine. Finally the analysis was perform by using Methanol: Phosphate

Buffer (Ph6.8) 20 Mm (60:40v/V). Sample wher analyse at 248 nm. at an Injection volume 20 µL, Flow rate 1 ml/min. the proposed method was optimized to give a sharp peak with minimum tailing for levodropropizine.

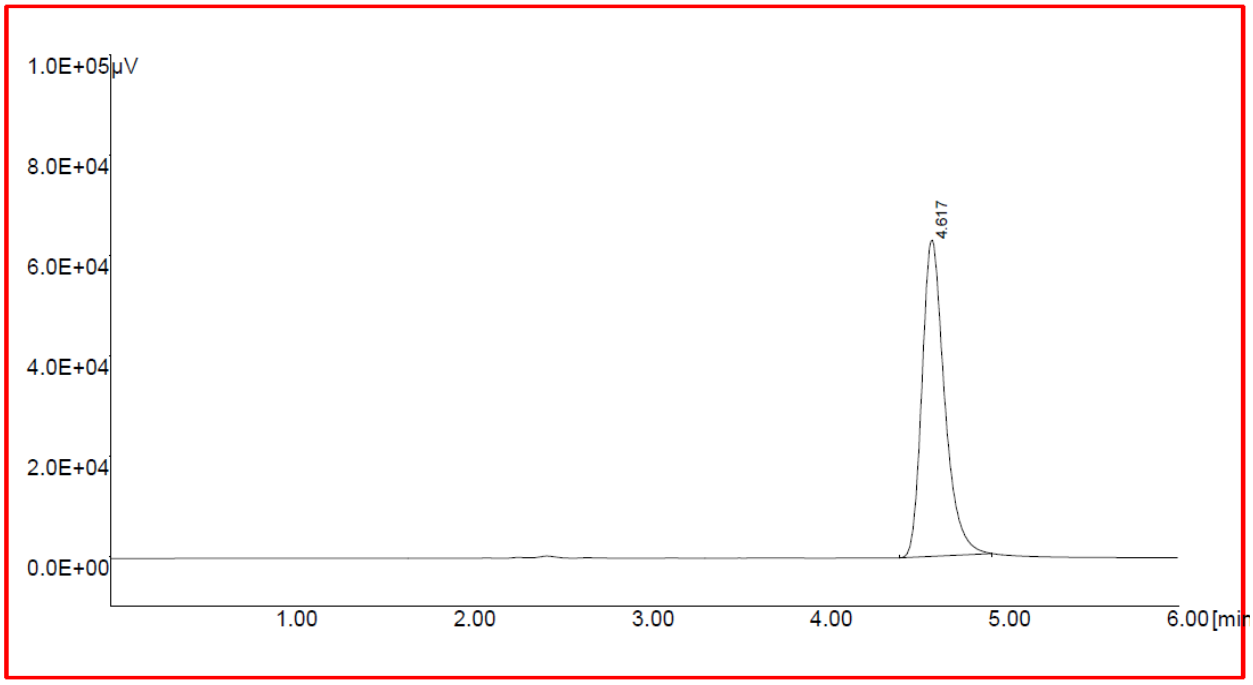

Fig No. 2: Representative Chromatogram of Levodropropazine[4-6]

Assay of levodropropizine: assay of in house levodropropizine formulation was carried out by using developed method sample solution where prepared and injected into HPLC System. The sample solution scanned at 248 nm the % drug was found to be 98.11% a single peak where observed with retaintion time 2.6

Accuracy and precision:

The result of intra and interday variation of Levodropropizine at three different concentration levels (80%, 100% and 120%) are depicted in **TABLE:1**

The mean values of amount estimated of the drug was found to be very close to the amount added and the % RSD values of intra-day were found to be very low indicating acceptable accuracy and precision of the method. The intra and inter-day results at each level were subjected to one way ANOVA and F values at each level were obtained as a ratio of Between Mean Square to the Within Mean Square (F = BMS/WMS). The obtained values were found to be less than the tabulated F (2, 6) at $\alpha = 0.05$ (Tabulated F value = 5.14). These indicated that there was no significant difference between intra and inter-day variability, suggested good intermediate precision of the method.

Table No.1Accuracy and precision[4-6]

Amount Added	Amount Found (mg)			Within mean square	Between mean square	F Value
	Day1	Day 2	Day 3			
48 mg 80%	46.44	46.63	45.29	0.2702	2.139	7.91
	46.66	45.62	44.41			
	45.72	45.75	44.38			
Mean	46.27333	46	44.69333			
S.D	0.491664	0.549454	0.516946			
%RSD	1.062521	1.194466	1.156652			
60 mg 100%	59.39	58.47	58.58	0.2587	0.5	2.09
	58.5	58.37	58.56			
	58.62	57.5	59.45			
Mean	58.83667	58.11333	58.86333			
S.D	0.482942	0.53351	0.539255			
%RSD	0.918052	0.918052	0.918052			
72 mg 120%	70.93	70.9		0.5716	0.0304	0.0531
	69.5	69.62	70.19			
	70.81	70.97	70.21			
Mean	70.41333	70.49333	70.61333			
S.D	0.793242	0.756924	0.715984			
%RSD	1.126551	1.073752	1.01395			

Linearity:

Linearity of proposed method was evaluated according to ICH guidelines. The data obtained in the calibration experiments when subjected to linear – regression analysis showed a linear relationship between peak areas and concentrations in the range 2-14 µg/mL. **Table No.2** depicts the calibration data of Levodropropizine. The respective linear equation was y =50015x - 5850 where x is the concentration and y is area of peak. The correlation coefficient was 0.998. The calibration curve of Levodropropizine is depicted in **Fig** No.3

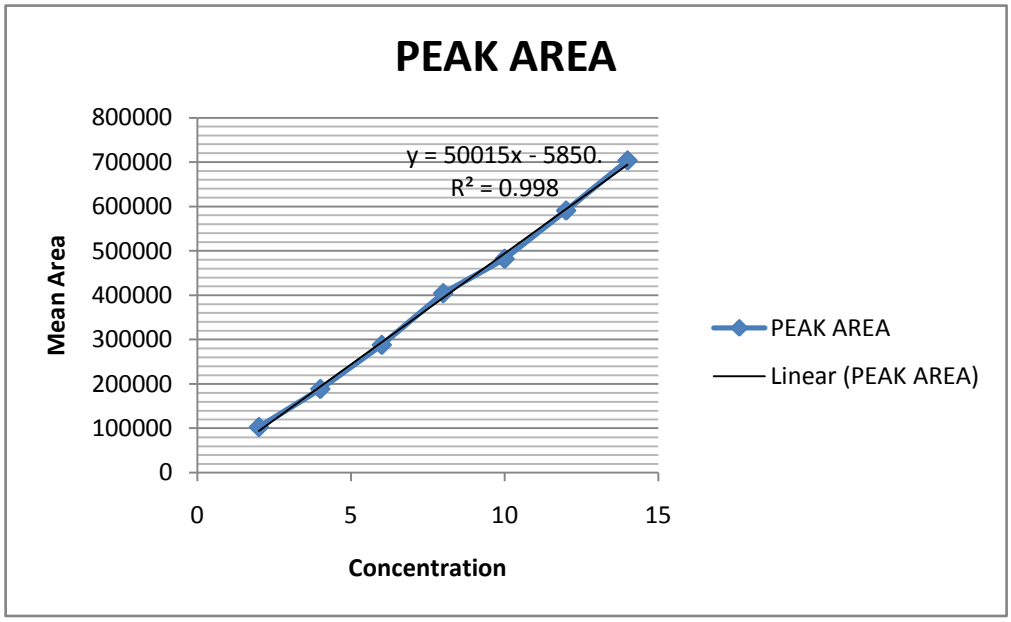

Fig No.3 Calibration curve of Levodropropizine[4-6]

Table No.2 Linearity[4-6]

Sr. No.	Conc µg/mL	PEAK AREA			MEAN PEAK AREA	STANDARD DEVIATION	% RSD
1	2	102367	103654	102158	102726.3333	810.150809	0.78865
2	4	188791	187698	189274	188587.6667	807.435653	0.428149
3	6	288707	287602	287404	287904.3333	702.144097	0.243881
4	8	409160	395229	408770	404386.3333	7932.88033	1.961708
5	10	478008	487907	479981	481965.3333	5239.34484	1.087079
6	12	591109	589876	591418	590801	815.836381	0.13809
7	14	707749	706949	695824	703507.3333	6665.97392	0.947534
Equation		y = 50015x - 5850					
R^2 Value		0.998					

Robustness:

The robustness was evaluated by making small changes in validation parameters such as mobile phase flow rate, detection wavelength and mobile phase concentration, the result where given in table No.3,4& 5

Table No.3 Validation Parameter mobile phase flow rate

Sr.no	Flow rate	Retention Time	Area	Plates	Asymmetry
1	0.8	5.5	969925	7659	1.36
2	1	4.6	554950	7015	1.2
3	1.2	3.6	4708045	6273	1.26

Table No.4 Validation Parameter Detection of wavelength

Sr.no	Detection Wavelength	Retention Time	Area	Plates	Asymmetry
1	243	5.3	796579	7475	1.34
2	248	4.4	577237	7191	1.3
3	253	4.425	448718	6833	1.35

Table No.5 Validation Parameter mobile phase concentration

Sr.no	Mobile Phase concentration	Retention Time	Area	Plates	Asymmetry
1	55:45	5.3	693143	7675	1.33
2	60:40	4.6	554950	7015	1.2
3	65:35	3.8	711381	7320	1.37

Specificity

The HPLC chromatograms recorded for the blank solution and blank solution exposed to the degradation conditions showed no peaks at the retention time of Levodropropizine and also the representative chromatograms of stressed samples under various stress conditions showed that Levodropropizine was well resolved from its degradation products, indicating the specificity of the method. The HPLC chromatograms recorded for blank, placebo, standard and sample solution showed that Levodropropizine peak was not affected by diluents and placebo.

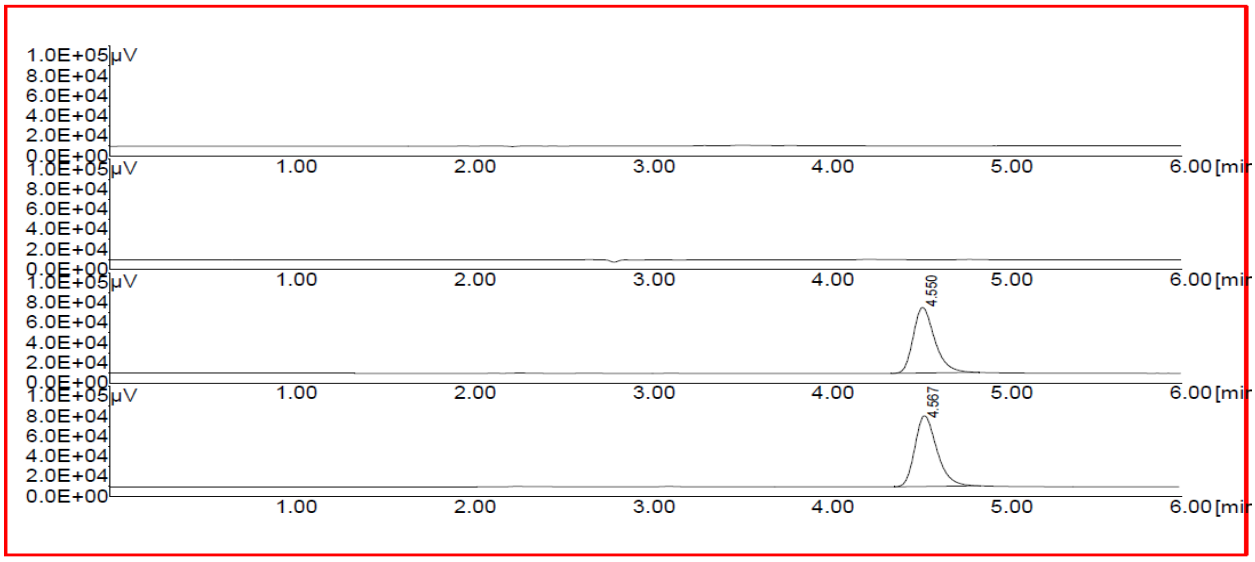

Fig No.4 Specificity

System Suitability[4-6]

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time ($t_{R),}$ number of theoretical plates (N), tailing factor(T) and peak asymmetry(A_f) were evaluated for five replicate injections of the drug at a concentration of 10µg/mL. The result are given in table were within acceptable limits. Atypical chromatogram of Levodropropizine is presented in table No.6

Table No.6 Validation Parameter System suitability

Sr. No.	Parameter	Value
1	Theoretical plates	5994.432
2	Retention time (min)	4.40
3	Asymmetry	1.43

Analysis of the marketed formulation

The chromatograms of the drug samples extracted did not show any change in the retention time. There was no interference from excipients, which are commonly present in the tablets. The drug content was found to be 101.21% with a % RSD of 0.0069% as shown in **TABLE:** Therefore it was concluded that, degradation of Leodropropizine had not occurred in marketed formulations. The % RSD value indicated the suitability of the method for the routine analysis of Leodropropizine in marketed formulation.

Table No.7 Analysis of the marketed formulation

Amount per tablet(mg)	Amount Found (mg/mL)	% Found	Average	±SD	%RSD
60	58.58666559	97.64444	98.11446	0.84563	0.008619
60	58.56495371	97.60826			
60	59.45441313	99.09069			

Force degradation study of levodropropizine:

Stress testing under acidic condition[4-6]

when the drug was refluxed with 0.01 N HCl for 1 hr at 65°C. When stressed sample was analyzed, there was one additional peak at the retention time 3.4 min. There was no additional peak at the same retention time when zero time, stressed blank and blank sample were analyzed confirming the formation of one degradation product. Comparison of the peak area of Levodropropizine in stressed condition with that of the zero time sample give 15.26% degradation.

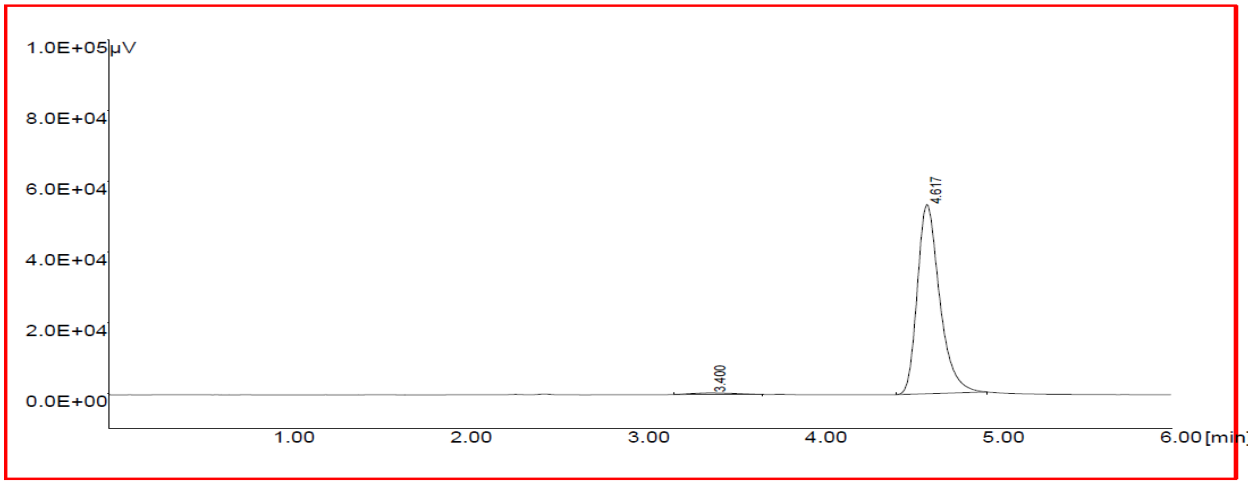

Fig No. 5 Representative Chromatogram of acid treated Levodropropazine (0.01N HCL for 1 hr reflux at 65°C).Alkali degradation

There were two degradants found when the drug was refluxed with 0.01N NaOH for 1 hr. at 80°C. When this stressed sample was analyzed, there was one additional peak at the retention of 2.4. There was no additional peak at the same retention time when blank, zero and stressed blank samples were analyzed confirming the formation of one degradation product. Comparison of the peak area of Levodropropizine in stressed condition with that of the zero time samples gave 18.16% degradation.

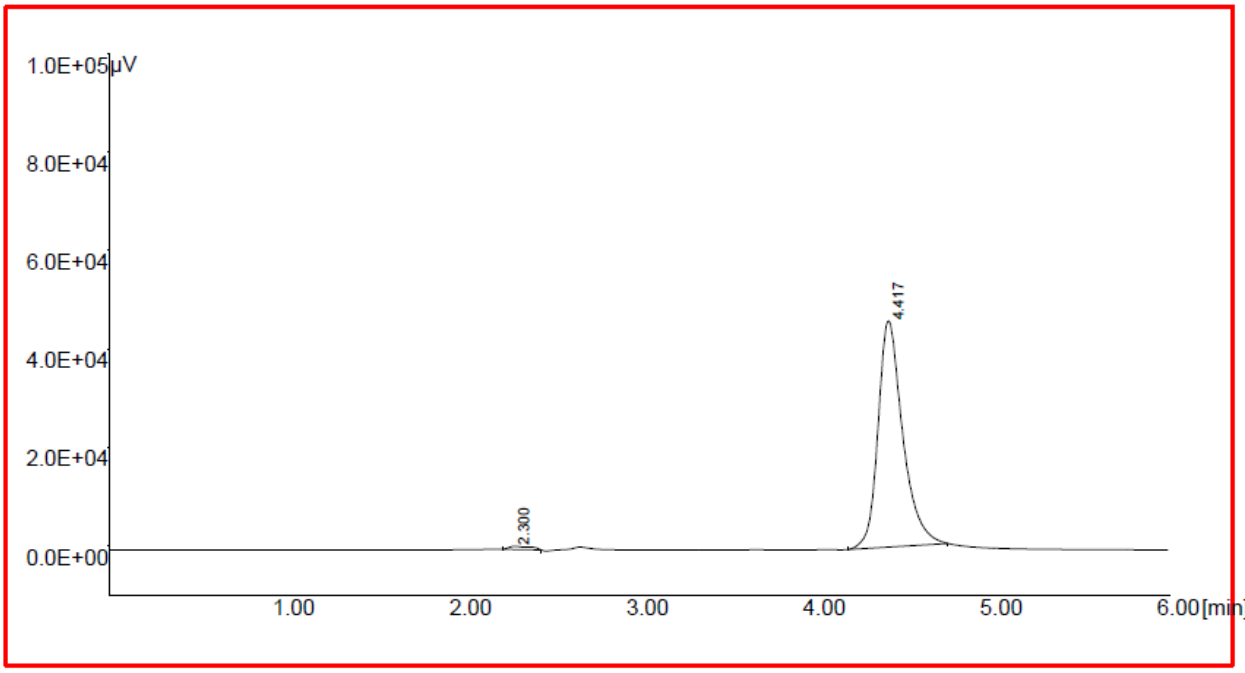

Fig No.6 Representative Chromatogram of base treated Levodropropazine (0.01N NaOH for 1 hr reflux at 80°C).

Wet heat degradation

There were two degradation products found when the drug was refluxed with Water for 45 minutes at 80°C. When stressed sample was analyzed, there were two additional peak at the retention time 2.3 min and 2.6 min. There was no additional peak at the same retention time when zero time, stressed blank and blank sample were analyzed confirming the formation of one degradation product. Comparison of the peak area of Levodropropizine in stressed condition with that of the zero time samples gave 21.12 %degradation.

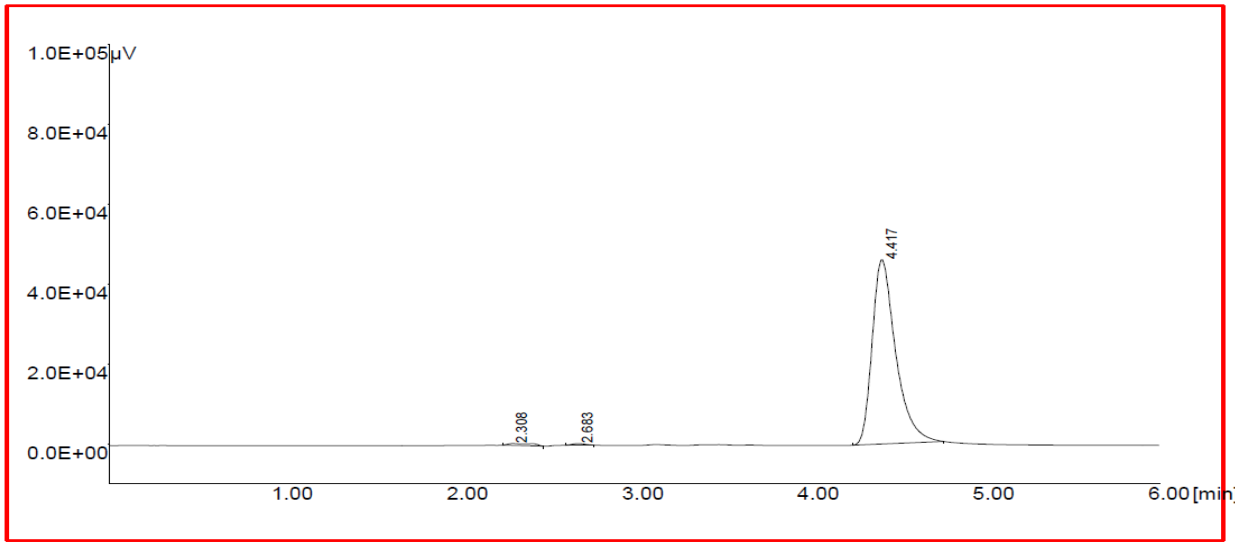

Fig No.7 Representative chromatogram of wet heat degradation for 45 min 80°C reflux

Dry heat degradation

Stability of Levodropropizine in dry heat was studied by keeping it for 1 hr at 60 °C. When the stressed sample was analyzed, no degradation was found and hence it was decided to extended the heating time for 2hrs, 3hrs, 4hrs and 5hrs with increased temperature of 60°C.When the stressed sample was analyzed, there was no additional peak found. Also the comparison between the peak areas of stressed sample of Levodropropizine with that of zero time samples showed no difference, indicating that there was no degradation. Hence it was concluded that the drug was stable under the conditions tested.

Photolytic degradation

There were one degradation products found when the powdered drug was exposed to sunlight for 36hrs. When stressed sample was analyzed, there was one additional peak at the retention time 3.4 min. There was no additional peak at the same retention time when zero time, stressed blank and blank sample were analyzed confirming the formation of one degradation product. Comparison of the peak area of Levodropropizine in stressed condition with that of the zero time sample gave 18.04 %degradation

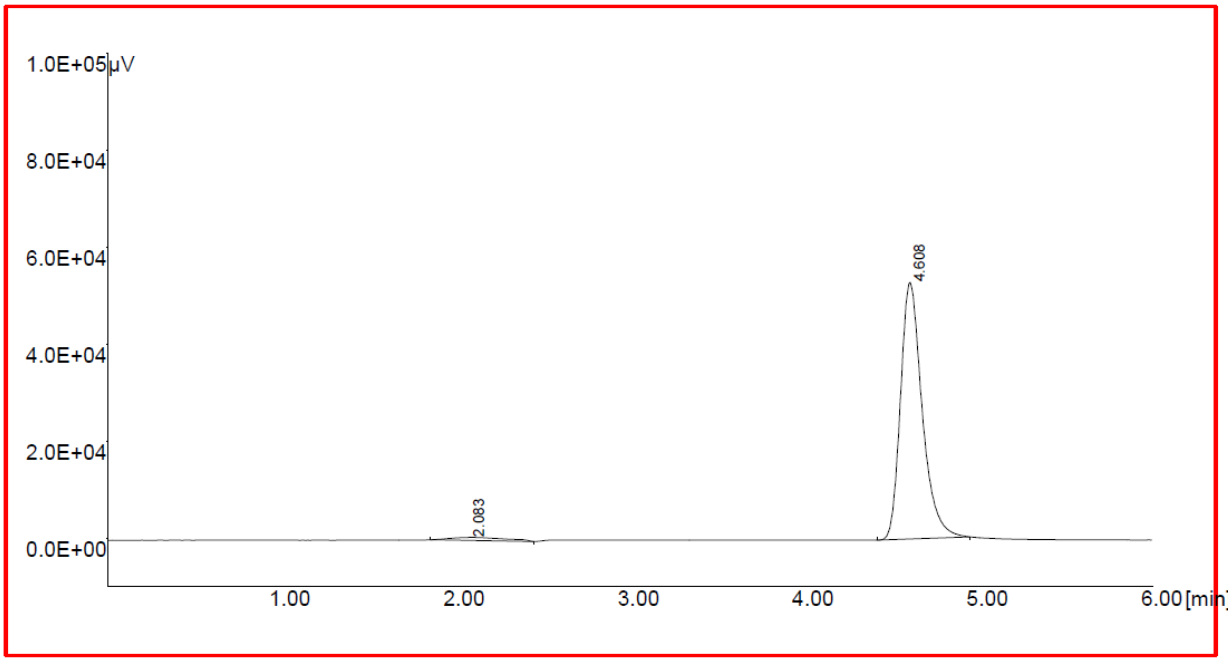

Fig No.8 Representative Chromatogram of Photolytic treated Levodropropazine for 36 hrs

Oxidative degradation

Oxidative degradation of levodropropazine was studied using 0.3 % H_2O_2 for 24 hrs when the samples were analyzed 70% drug degradation found. Hence it was concluded that drug was oxidative under the conditions tested.

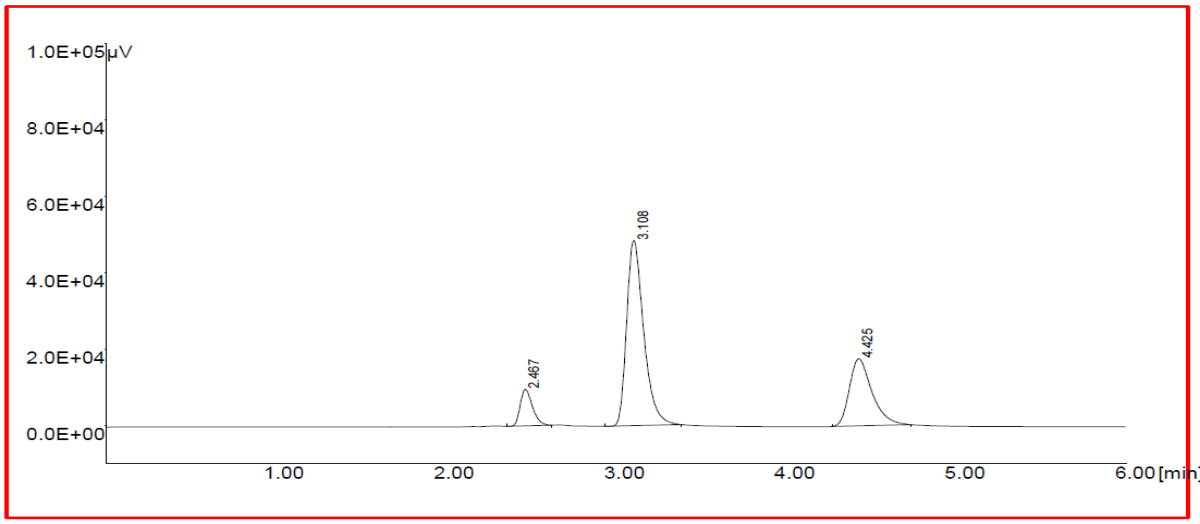

Fig No.9 Representative Chromatogram of oxidative degradation studies of levodropropizine (0.3% H_2O_2 for 24 hrs)[4-6].

Table No.8 Summary of Forced degradation study[4-6]

Sr.no	Stress Condition	Drug peak area at zero time sample	Drug peak area of stressed sample (mc.V.sec)	Retention time(s) of degradation products (min)	%Degradation
1	Acid 0.01N HCL (Refluxed for 1hrs)(65^{0}C)	554950	470247	3.4	15.26%
2	Alkali 0.01N NaOH (1 hrs) (80^{0}C)	554950	446955	2.3	19.46%
3	Wet heat 80°Cfor 45min	554950	430771	2.3	22.37
4	Oxidative 30%v/v H_2O_2(in direct room temperature	554950	161884	3.1	No degradation
5	Dry heat 70°C(kept in oven for 48hrs)	554950	-	No degradation	No degradation
6	Photolytic (exposed to sunlight for 48 hrs)	554950	456630	2.08	18.04 %

SUMMARY AND CONCLUSION:

- Stress testing of Levodropropizine was carried out under acidic, alkaline, neutral, oxidative, photolytic and dry heat conditions.
- The stressed samples and standard drug were subjected to chromatographic separations. The HPLC analysis was carried out using Phenomenex column (250×4.6 mm, 5 µm).
- The degradation products and in house tablet excipients in levodropropizine assay were well resolved from the drug using the mobile phase Methanol:Phosphate buffer (60:40 % v/v) (pH 6.8).
- The mobile phase flow rate was maintained at 1 mL/min and the detection was performed at wavelength 248 nm.
- The average retention time for Levodropropizine was 4.6 min. Levodropropizine was found to be degraded under acidic, alkaline and wet heat photolytic conditions, while it was stable under dry heat, and oxidative conditions. **Table No.8** summarizes the degradation behaviour of Levodropropizine and the retention times of degradation

products under tested conditions. **Fig.5-9** gives the representative chromatograms of stressed samples of Levodropropizine showing well resolved peaks of degradation products under various stress conditions.

- The developed method was validated as per ICH Guidelines Q2 (R1). The method was found to be economic, accurate, precise, specific, robust and linear in the range of 2-14 µg/mL. The Detection limit was found to be 0.16 µg/mL. The Quantitation limit was found to be 0.46µg/mL. It can be concluded that the HPLC method developed for Levodropropizine is capable of discriminating between the drug and the degradation products.
- Validation of the method is suitable for the analysis of Levodropropizine in tablet formulation without any interference from common excipients or potential degradation products of Levodropropizine and excipients.
- The developed method can be used for routine analysis of Levodropropizine in marketed formulation.

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