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Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Nortriptyline Hydrochloride in Tablet Dosage Form

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ABSTRACT

Stability indicating RP-HPLC method was developed for Nortriptyline Hydrochloride in the tablet dosage form. The chromatographic separation was performed on Hypersil ODS C18 (250 mm x 4.6 mm, 5µm) column, with a mobile phase, in a mixture of Methanol and Phosphate buffer [pH 6.3] with OPA in the ratio of 95:5 v/v at the flow rate of 1.0 ml/min. The detection was carried out at 239 nm using PDA detector. The retention time of NH was found to be 8.459 min with a run time of 10 min. As per ICH Q2R1 guideline, the method was validated for linearity, accuracy, precision, LOD, LOQ, and robustness. The linearity of NH was found in the range of 2-12 µg/ml. The correlation coefficient for NH was found 0.999. The LOD value was found to be 0.1049µg/ml and the LOQ value was found to be 0.3180µg/ml. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for the estimation of NH in tablet dosage form. The stability indicating method was developed using acid-base hydrolysis, Oxidative degradation, Photolytic degradation and Thermal degradation. The method was validated and was found to be stability indicating and can be successfully utilized for the quantitative analysis of pharmaceutical tablet dosage formulations containing Nortriptyline hydrochloride.

INTRODUCTION

Nortriptyline Hydrochloride (NH) is an antidepressant drug and chemically it is 3-(10, 11-dihydro-5H-dibenzo, [a, d] cyclohept-5-ylidene) propyl (methyl) amine hydrochloride (Mol Wt-299.842g/mol)[1]. It inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors it is used as an Antidepressant [2].

From the literature review, it is found that only a few analytical methods UV, HPLC, GC, and TLC are available for the estimation of NH in the combination with the other drugs [7-23] but there is no any official stability indicating RP-HPLC method available till date for the estimation of Nortriptyline Hydrochloride in tablet dosage form [3-6]. So in this article affords are put to develop and validate a stability indicating the RP-HPLC method for the estimation of NH in Tablet dosage form.

MATERIALS AND METHODS

Equipment

Chromatographic separation was performed on HPLC system-Agilent Technologies, 1220 infinity LC, PDA Detector 2695 series, equipped with a solvent delivery pump, auto-sampler, and column thermostats. Chem station software was applied for data collecting and processing.

Chemical and reagents

Methanol, Water, Potassium Dihydrogen Phosphate of HPLC grade was purchased from SD Fine Chem Ltd. Reference standards Nortriptyline Hydrochloride was obtained from R L Fine Chem Pvt. Ltd., Karnataka. PRIMOX tablets of Nortriptyline Hydrochloride (25 mg), manufactured by sun pharma laboratories Limited, were procured from local market.

Preparation of standard solution

Accurately weighed 10mg of Nortriptyline Hydrochloride is transferred into a 10ml volumetric flask, add about 5ml of diluents and shake it until drug dissolved. Make up the volume with diluent and mix well to get a solution of 1000µg/ml. Filter a portion of the solution through the 0.45µm membrane filter and discard first few ml of the filtrate. Transfer

1 ml of the filtered solution into a 10ml volumetric flask, dilute up to volume with diluents and mix well to get a solution of 100µg/ml.

Preparation of sample solution

Commercially available 20 tablets are weighed and powdered. Powder equivalent to the 10mg of Nortriptyline Hydrochloride is transferred into a 10ml volumetric flask, add about 5ml of diluents and sonicate for 15min with an occasional shaking. Make up the volume with diluent and mix well to get a solution of 1000µg/ml. Filter a portion of the solution through the 0.45µm membrane filter and discard first few ml of the filtrate. Transfer 1 ml of the filtered solution into a 10ml volumetric flask, dilute to volume with diluent and mix well to get a solution of 100µg/ml. 20µl of the standard and sample solutions of NH are injected into the chromatographic system and from the values of obtained peak areas, the assessment of % assay was done.

Preparation of 0.02 M Phosphate buffer pH 6.3

Accurately weigh and transfer of 2.72g phosphate buffer into a beaker containing 1000ml of water and sonicate to dissolve. Filter the solution through the 0.45µm membrane filter. To the buffer solution, add OPA to adjust pH 6.3 using PH Meter.

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RESULT AND DISCUSSION

Optimized chromatographic conditions

- **Diluent:** Methanol
- **Mobile phase:** Methanol: Phosphate buffer pH 6.3 adjusted with OPA
- **Flow rate:** 1.0 ml/min
- **Column:** Hypersil ODS (C18), (250 X 4.6mm, 5µm)
- **Detector wavelength:** 239nm
- **Injection volume:** 20µL
- **Runtime:** 10 min

- **Mode of Pump:** Isocratic

METHOD VALIDATION

The validation parameters for the proposed analytical method are elucidated as per the ICH guideline Q2R1.

Validation Parameters are as given below

Linearity

Different solutions were prepared with concentrations, 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml of Nortriptyline Hydrochloride. Each solution was injected and linearity was evaluated by linear regression analysis.

Table 1: Linearity data

Conc. (µg/ml)	Area(mV) Mean ± SD (n=6)	%RSD
2	91.4676±1.641111	1.794199
4	195.2593±3.102227	1.588773
6	295.8117±4.236164	1.432047
8	413.9355±7.7396083	1.869762
10	515.9475±7.996923	1.549949
12	609.1394±8.609366	1.413365

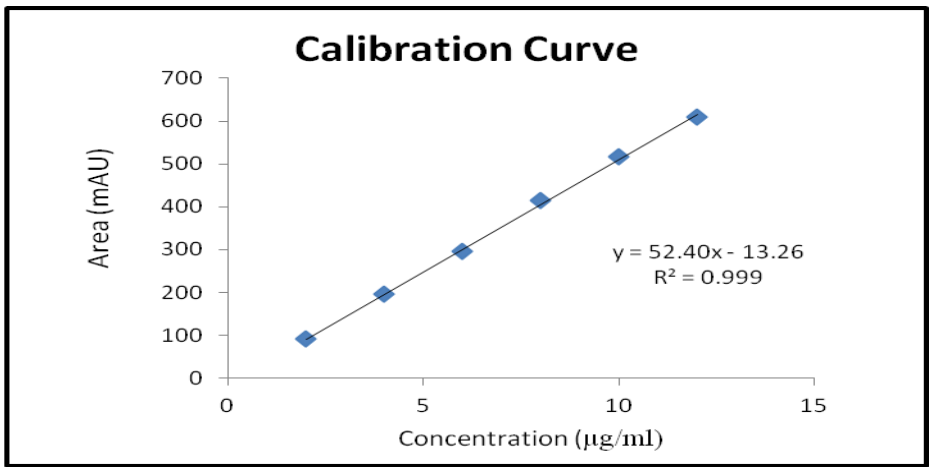

Figure 1: Calibration curve

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of the standard to pre-analyzed sample preparation. For each concentration, three sets were prepared and injected.

Table 2: Recovery data of NH

Drug	Spike Level	Amount present (µg/ml)	Amount added (µg/ml)	Amount recovered (mean* ± SD) (µg/ml) (n=3)	% recovery (Mean ± SD)
4 µg/ml	-	4 µg/ml	4 µg/ml	3.87±1.10	97.74±1.12
NH	50%	4	2	5.81±0.015	96.90±0.28
	100%	4	4	7.76±0.03	97.08±0.40
	150%	4	6	10.04±0.05	100.49±0.54

Precision

Repeatability: variations were determined using six replicate injections of one concentration and analyzed on the same day.

Table 3: Repeatability data

Replicates	NH (8µg /ml) Area (mAU)
1	414.4587
2	401.5894
3	419.7521
4	412.7534
5	421.1241
6	411.2106
Mean ± SD (n=6)	413.4814±7.011331
%RSD	1.695682

Intraday: variations were determined using 3 different concentrations and analyzed on the same day.

Table 4: Intraday data

Conc. (µg /ml)	Area(mAU) Mean ± SD(n=3)	%RSD
2	90.87466±1.200207	1.320728
8	409.3907±7.093052	1.732587
12	606.9007±7.323639	1.206728

Inter-day: variations were determined using 3 different concentrations and analyzed on the three consecutive days.

Table 5: Inter-day data

Conc. (µg /ml)	Area(mAU) Mean ± SD(n=3)	%RSD
2	90.6281±1.531974	1.690395
10	416.445±5.154196	1.237666
18	607.2138±7.695773	1.267391

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min) and the mobile phase ratio (±1 ml).

Table 6: Robustness data

Factors	Levels	Retention time (min) Mean ±SD (n=3)	% RSD
Flow rate (ml/min)	0.9	8.923±0.015275	0.171183
	1.0	8.533±0.025166	0.294915
	1.1	8.146±0.020817	0.255524
Mobile phase Ratio: Methanol: Buffer	94:6	8.49±0.01	0.117786
	95:5	8.52±0.01	0.117371
	96:4	8.61±0.01	0.116144

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated from linear curve using formulae

LOD=3.3*σ /slope,

LOQ=10*σ /slope

(Where σ = the standard deviation of the response and, S= Slope of calibration curve).

Table 7: LOD and LOQ data

Parameter	NH
LOD (µg /ml)	0.1049 µg /ml
LOQ (µg /ml)	0.3180 µg /ml

System Suitability Parameters

The data for system suitability parameters of developed HPLC method are presented in Table.

Table 8: System suitability parameters

System Suitability Parameters	Proposed method
Retention Time (Rt)	8.456
Theoretical Plate Number (N)	6934
Tailing Factor (T)	0.53

Specificity and Analysis of formulation in HPLC (% assay)

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate the separation of Nortriptyline Hydrochloride from its impurities

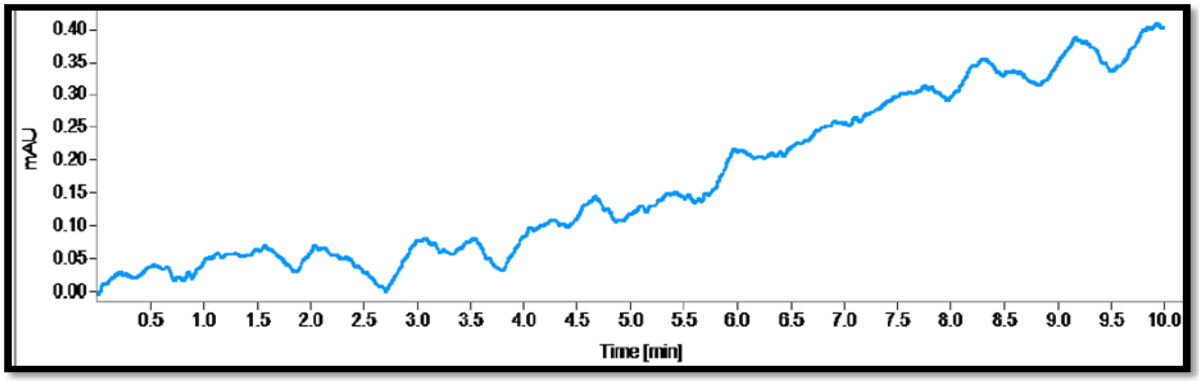

Figure 2: Representative chromatogram of the blank solution

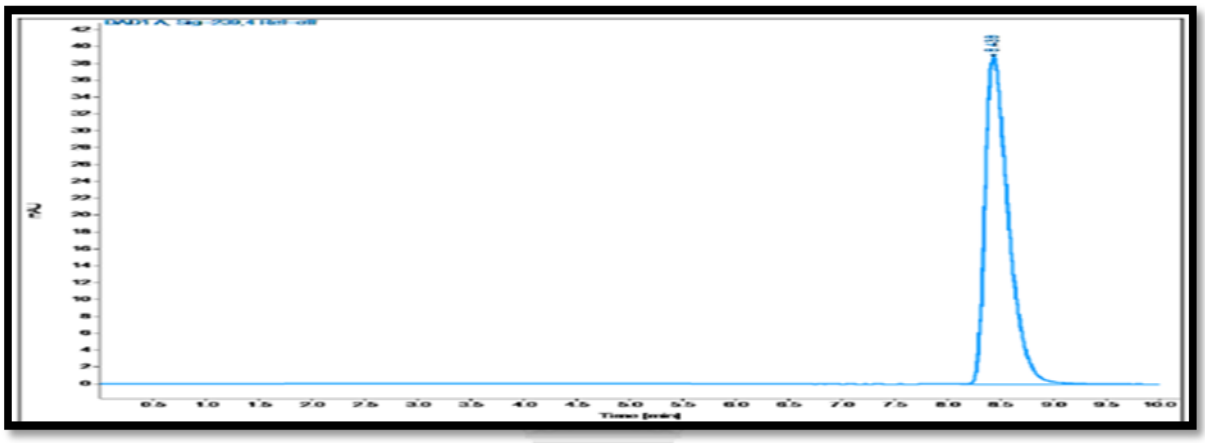

Figure 3: Representative chromatogram of standard NH (10µg/ml)

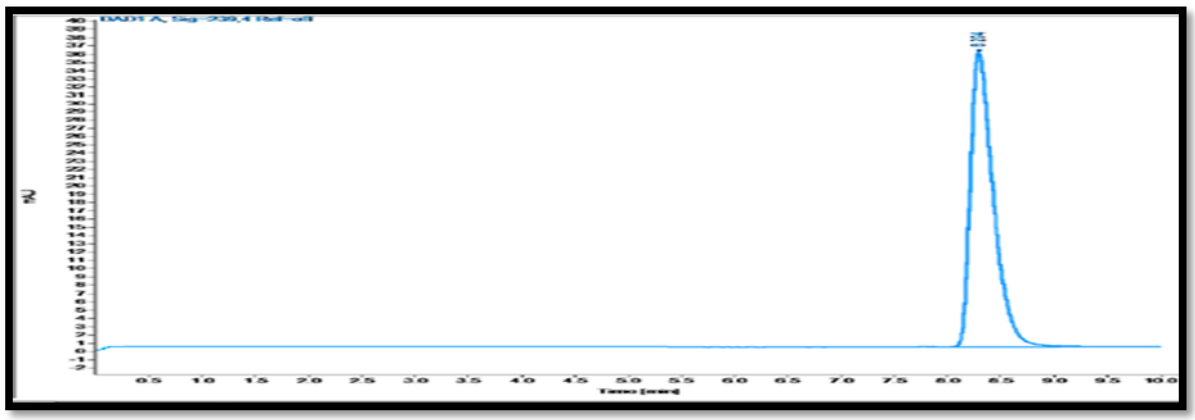

Figure 4: Representative chromatogram of the formulation of NH (10µg/ml)

The % amount of drug was found to be 100.02±1.05987 for NH (n=3)

Table 9: Analysis of formulation in HPLC

Amount of drug taken (µg/ml)	Amount of drug found (µg/ml)	%Amount of drug mean ±S.D (n=3)
10µg/ml	10.02	100.02±1.05987

Stability indicating RP-HPLC method [25]

For the purpose of indicating the stability of both standard and sample solutions during the analysis period, both solutions are tested for a time period of 24 hr at room temperature. The analysis revealed that no considerable degradation has occurred as the retention time and peak area of NH remained almost undisturbed (as % R.S.D. is less than 2.0), that indicates stability for a period of 24 hr for both the solutions, which was adequate to complete the entire analytical procedure.

Control Sample

Twenty tablets of which the labeled claim is NH 25 mg/tablet are taken and powdered very finely. Accurately weighed equivalent sample containing NH 10 mg was transferred into a 10 ml clean and dry volumetric flask and about 5 ml of diluent was added and sonicated to dissolve it completely and made up to the mark using the diluents. This freshly prepared solution was filtered using the HPLC filters and was labeled as the control sample. Then 1.0 ml of filtered control sample solution was transferred to a 10ml volumetric flask and made up to the mark with diluents to obtain 10µg/ ml of NH solution. A measured quantity (20 µL) of this solution was injected to obtain the chromatogram.

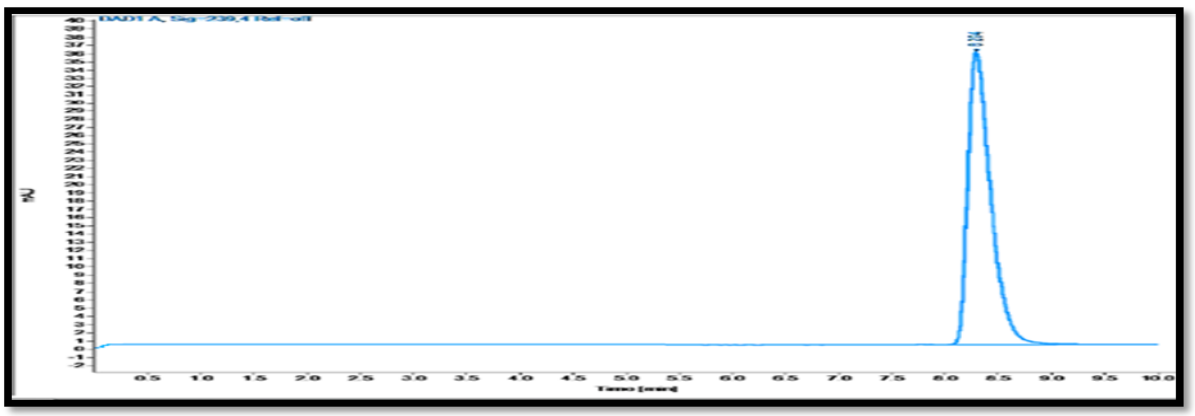

Figure 5: Chromatogram of Control sample

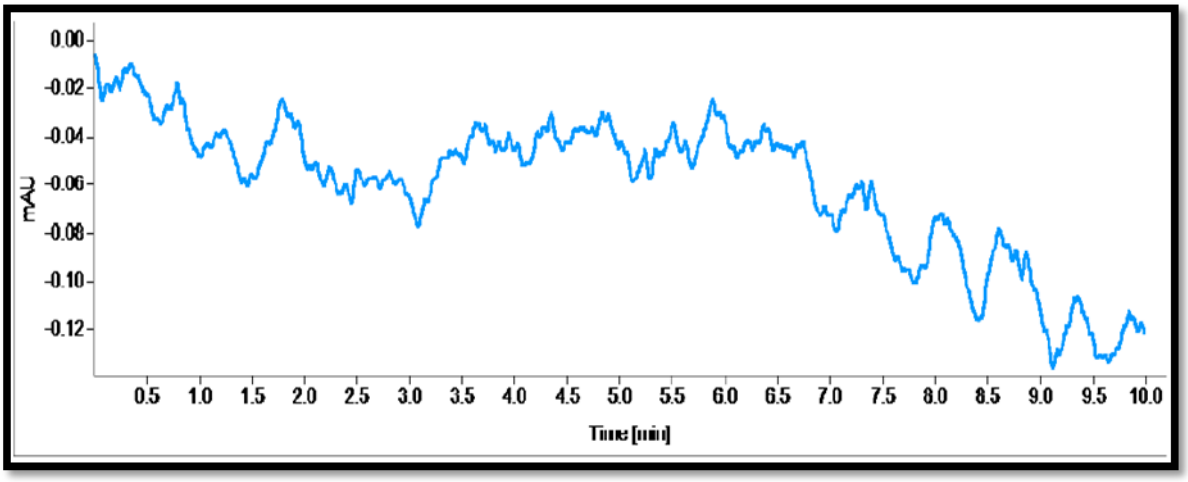

Figure 6: Blank Chromatogram of acid and base mobile phase

Acid Degradation

Aliquots of 1.0 ml containing standard working solutions of NH was transferred into 10 ml volumetric flask, 1.0 ml 0.1 N HCl was added to the sample drug solutions and kept for 3 hrs. at 25±2°C. The solutions were neutralized by adding 1 ml of 0.1 NaOH and diluted up to 10 ml with mobile phase to get the final concentration of 10 µg/ml of NH.

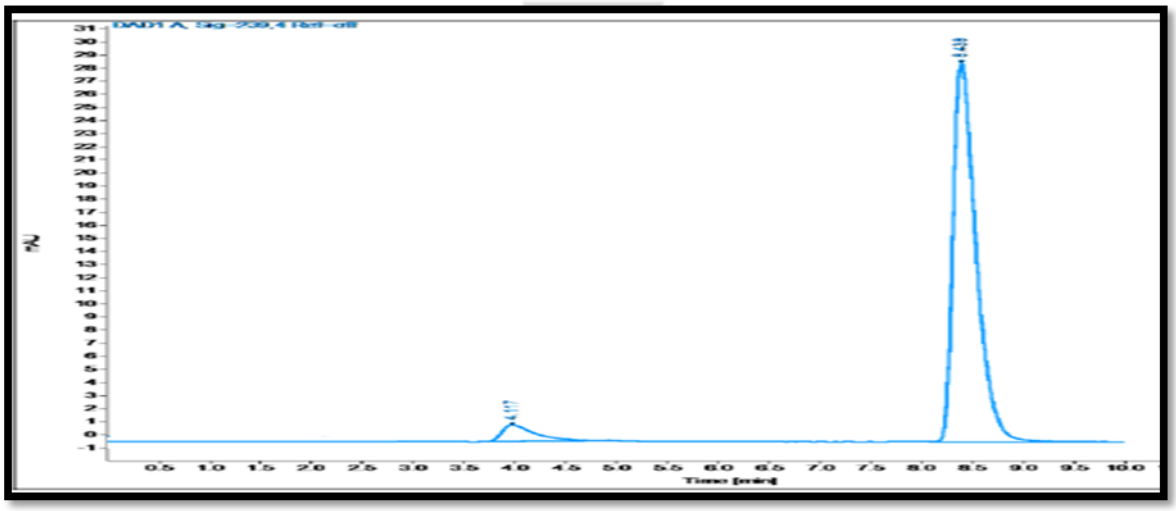

Figure 7: Acid degradation (0.1 N HCl, 3hrs)

Base Degradation

Aliquots of 1.0 ml containing standard working solution of NH was transferred into 10 ml volumetric flask, 1.0 ml 0.1 N NaOH was added to the sample drug solution and kept for 3

hrs. at 25±2°C. The solution was neutralized by adding 1.0 ml 0.1N HCl and diluted with mobile phase to get the final concentration of 10µg/ml of NH**.**

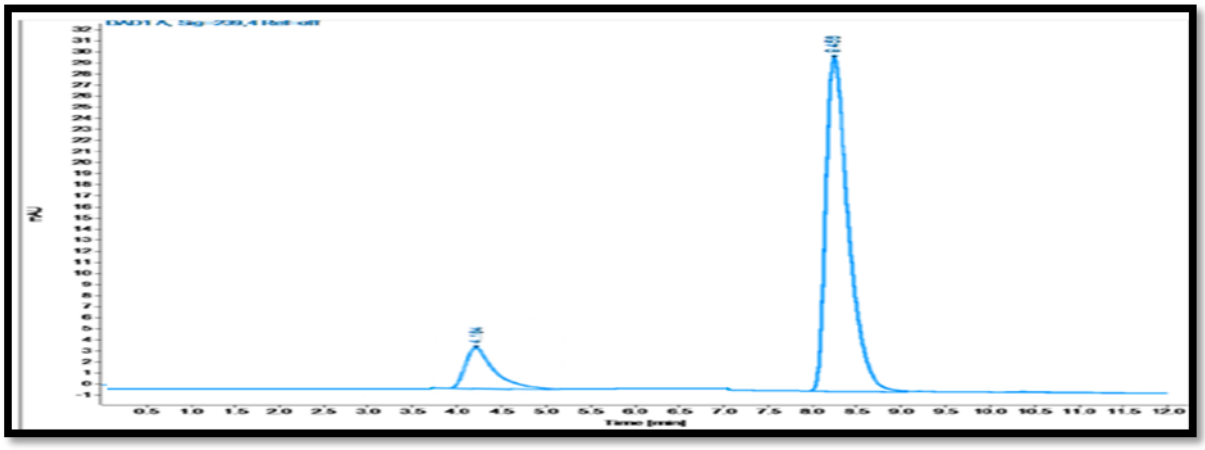

Figure 8: Base degradation (0.1 N NaOH, 3 hrs)

Photodegradation:

The drug sample of NH was exposed to UV light (254 nm) for 1 hr. in UV chamber. The powder was taken after 1 hr. and the solution was made with final concentration of 10µg/ml of NH.

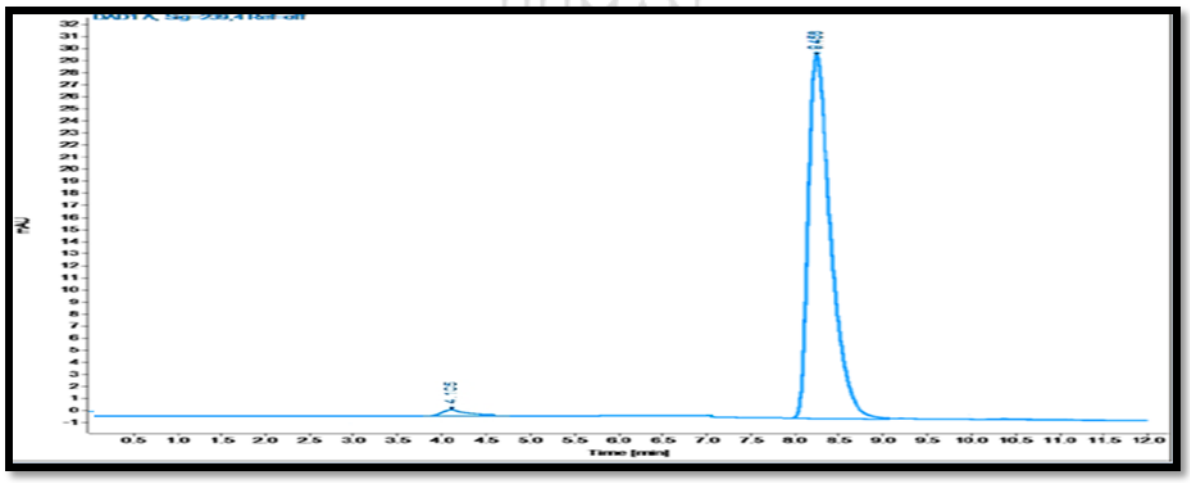

Figure 9: Photolytic degradation (UV light, 1 hr)

Oxidative Degradation:

Aliquots of 1.0 ml containing standard working solution of drug were transferred into 10 ml volumetric flask, 1 ml 3% Hydrogen peroxide (H_2O_2) was added and kept for 3 hrs. at

25±2°C. The solutions diluted up to 10 ml with mobile phase to get final concentration 10µg/ml of NH.

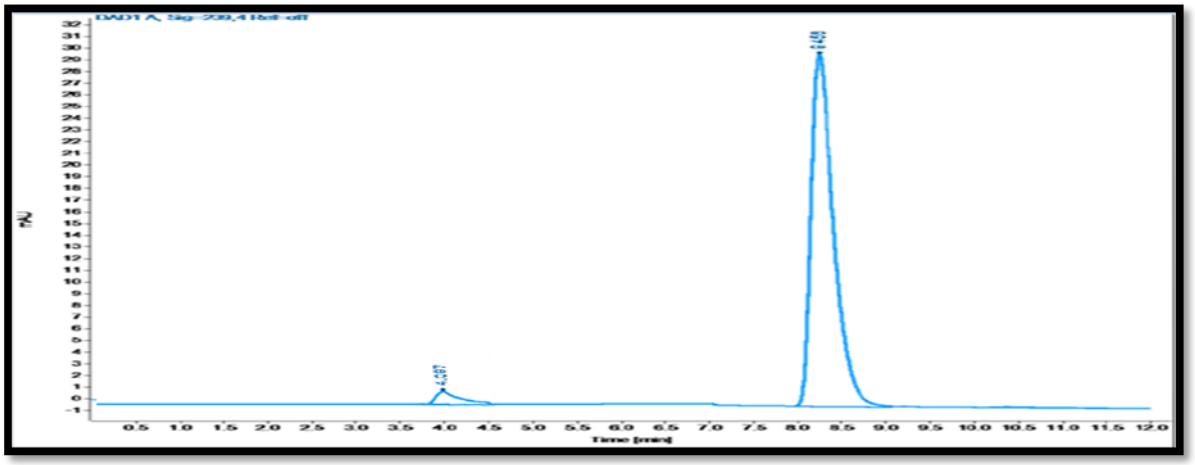

Figure 10: Oxidative degradation (3% H_2O_2, 3 hrs)

Thermal degradation:

The drug sample of NH was exposed to 60ºC in Hot air oven for 5 hrs. The powder was taken after 5 hrs. The solution was made to get the final concentration of 10µg/ml of NH.

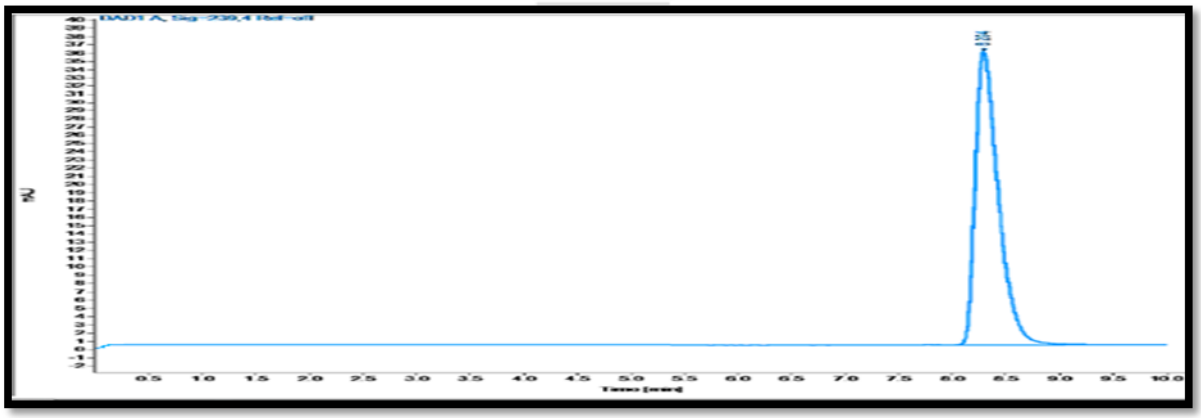

Figure 11: Thermal degradation (70ºC, 5 hrs)

Table 10: Forced degradation studies of NH

Stress Type (Degradation)	Stress conditions	Rt	Theo. Plates	Resolution	Area (mAU)	% NH assay	% NH Degradation
Control	-	8.456	6934	1.86	537.11108	104.03	-
Acid	0.1N HCl, 3 hrs	8.438	8059	13.50	512.39241	95.397	4.603
Base	0.1N NaOH, 3 hrs	8.438	7815	20.71	498.84392	92.875	7.125
Oxidative	3% H_2O_2, 3 hrs	8.458	8184	10.23	467.29403	87.001	12.999
Thermal	70ºC, 5 hrs	8.254	8421	1.25	535.15836	99.636	0.364
Photo	UV light, 1 hr	8.456	7525	15.71	456.90924	85.067	14.933

CONCLUSION

The research investigation presents a simple and validated HPLC stability indicating the method for analysis of NH in the presence of degradation products. The method developed was evaluated as specific, precise, accurate, sensitive, and robust. Very accurate and precise linear response was given by this method in the said range. The degradation products that are formed during the exposure of drug to stress conditions gave peaks that are well separated from that of the analyte peaks which establishes the fact that the developed method was specific and stability indicating**.** The developed method hence can be used for successful determination of marketed formulations containing NH.

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