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Development and Validation of Stability Indicating RP-HPLC Method for Niacin in its Pharmaceutical Formulations

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Niacin in tablet formulations. The separation was achieved by using column Primsep A 100, 150 × 4.6 mm, 5 µm, in mobile phase pH 2.5 Phosphate Buffer and Acetonitrile in the ratio of 600:400 v/v. The flow rate was 1.0 mL.min^{-1} and the separated Niacin was detected using UV detector at the wavelength of 262 nm. The retention time of Niacin, was noted to be 3.68 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography, Niacin, Validation.

INTRODUCTION

Niacin is official drug in I.P, B.P, U.S.P, E.P, Japanese and International Pharmacopoeia [1-6]. IUPAC name is Pyridine-3-Carboxylic acid. Category: Anti-lipidemic Agent, Vasodilator, Vitamin B Complex for the treatment of type IV and V hyper lipidemic. It is indicated as adjunctive therapy. Molecular Formula is $C_6H_5NO_2$, Molecular mass is 123.1096 g/mol, soluble in water and ethanol (96%) dissolves in dilute solutions of alkali hydroxides and carbonates and practically insoluble in ether. Niacin was indicated for prevention and treatment of vitamin B_3 deficiency states. Vitamin B_3 also acts to reduce LDL cholesterol, triglycerides and HDL cholesterol. The magnitude of individual lipid and lipoprotein responses may be influenced by the severity and type of HDL sub fractions (as defined by Ultra centrifugation) with an increase in the HDL2: HDL3 ratio and an increase in apolipoprotein A1 content. Vitamin B3 (Niacin) treatment also decreases the serum levels of apolipoprotein B100 (apo B), the major protein component of the VLDL lipo protein and LDL fractions, and of lipoprotein-a, A variant form of LDL independently associated with coronary risk.

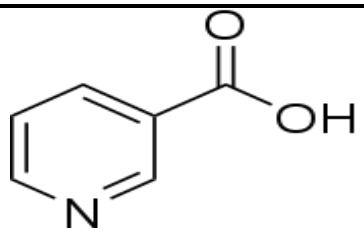

Figure-1 The structure of Niacin

Literature survey revealed a few HPLC methods for the estimation of Niacin [7-22]. In the present study, an attempt has been made to develop RP-HPLC method for the estimation of Niacin.

MATERIALS AND METHODS

Chemicals and Reagents: Milli-Q Water, Acetonitrile and Methanol (HPLC Grade), Orthophosphoric acid (AR Grade), Potassium dihydrogen phosphate (AR Grade) were obtained from Merck, Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation and Chromatographic Conditions

Instrumentation: The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Primsep A 100, 150×4.6 mm, 5 µm was used. The output of signal was monitored and integrated using waters Empower 2 software.

Preparation of Buffer: Weighed and dissolved 1.36 g of potassium dihydrogen orthophosphate in 1000 mL of water and adjusted the pH to 2.50 with dilute orthophosphoric acid. Filter the solution through 0.45µm membrane filter.

Preparation of Mobile Phase: Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 600:400 v/v respectively.

Preparation of Diluent: The diluent was prepared by methanol and Milli-Q water in ratio of 1:1.

Preparation of Standard: About 50mg niacin was accurately weighed and transferred into a 100mL volumetric flask. The sample was dissolved in small volume of diluent and then the volume was made up with more diluent to prepare niacin standard stock solution. From this 5mL of this solution was accurately measured by means of a pipette and delivered to a 25mL standard volumetric flask. This diluted solution was referred as niacin standard solution that contained 100µg of niacin per mL.

Preparation of Sample: 5 tablets were transferred into 1000 mL volumetric flask. 100 mL of tetra hydro furan (THF) was added and sonicated for 10 min. Then 300 mL of methanol was added and sonicated for 60 min. Then 300 mL of milli-Q-water was added and mixed well with the help of magnetic stirrer till the small granular particles completely disappear and the volume was made up with 10 mL of the above milli-Q water solution was centrifuged at 8000 RPM for 10 min. 5mL of above clear sample solution was pipetted into 200mL volumetric flask and diluted with diluent.

Chromatographic conditions: A Primsep A 100 (150 mmx4.6 mm I.D; particle size 5µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 262nm for the detection and Chromatographic runtime was 6 min.

RESULTS AND DISCUSSION

Method Development: Spectroscopic analysis of compound Niacin showed that maximum UV absorbance (λmax) at 262 nm respectively. To develop a suitable and robust LC method for the determination of Niacin, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Primsep A 100, 150mm x4.6mm, 5µ with the following different pH Phosphate buffers like that pH 6.8, 4.6 and 2.5. It was observed that when Niacin was injected, Peak Tailing, not satisfactory. In the next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer pH 2.5 and Acetonitrile in the ratio of 600:400 v/v as eluent at flow rate 1.0 mL/min. UV detection was performed at 262nm. The retention time of Niacin is 3.68 min (**Fig.2**) and the peak shape was good. The chromatogram of Niacin standard using the proposed method is shown in **(Fig.2)** System suitability results of the method are presented in **table-1.**

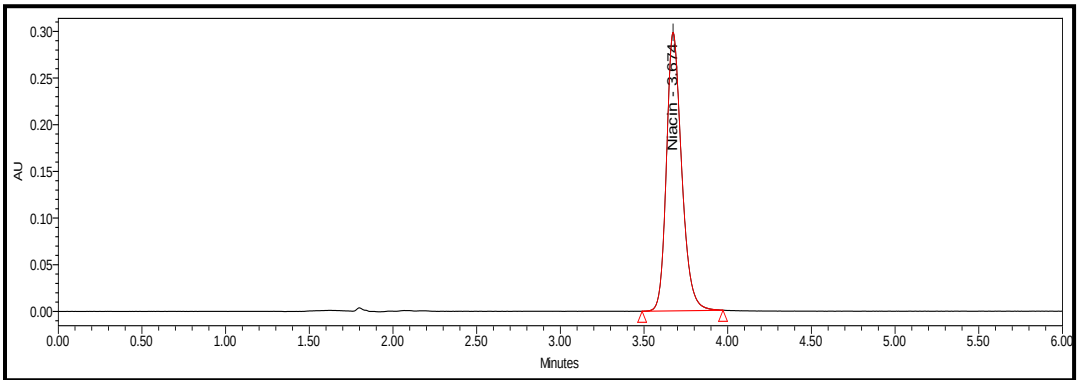

Fig. 2 HPLC Chromatogram showing the peak of Niacin

Method validation: The developed RP-LC method extensively validated for assay of Niacin using the following Parameters.

Specificity: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Fig.3)** showed no peak at the retention time of Niacin peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Niacin in Niacin tablets. Similarly Chromatogram of Placebo solution **(Fig.4)** showed no peaks at the retention time of Niacin peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of Niacin in Niacin tablets.

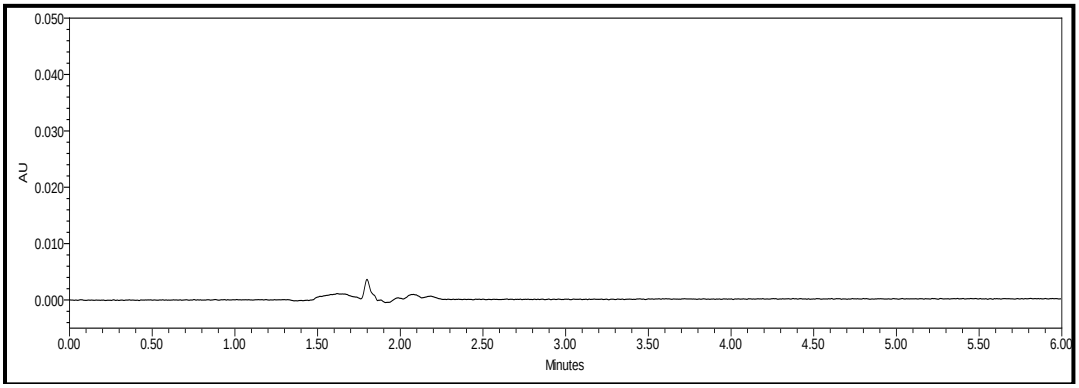

Fig.3 HPLC Chromatogram showing the no interference of diluent of Niacin

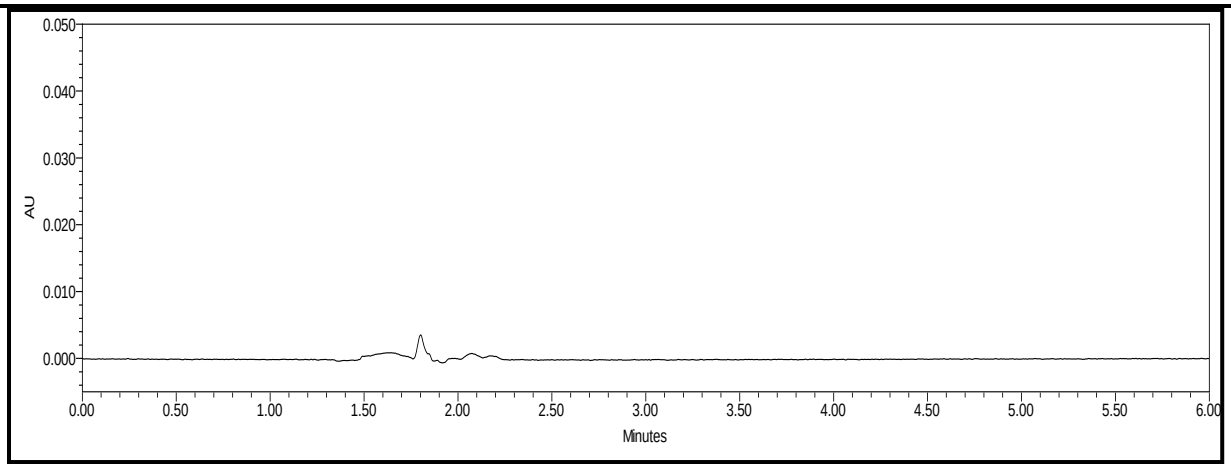

Fig.4 HPLC Chromatogram showing the no interference of placebo of Niacin

Table-1 System suitability parameters of Niacin for proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Niacin	3.679	8149	1.24

Forced Degradation:

Acid Degradation Sample: Sample of 750 mg strength and its placebo were stressed using 5N hydrochloric acid by heating at 60°C for 60 min. Solutions were then neutralized with dilute sodium hydroxide and analysed (**Fig.5**).

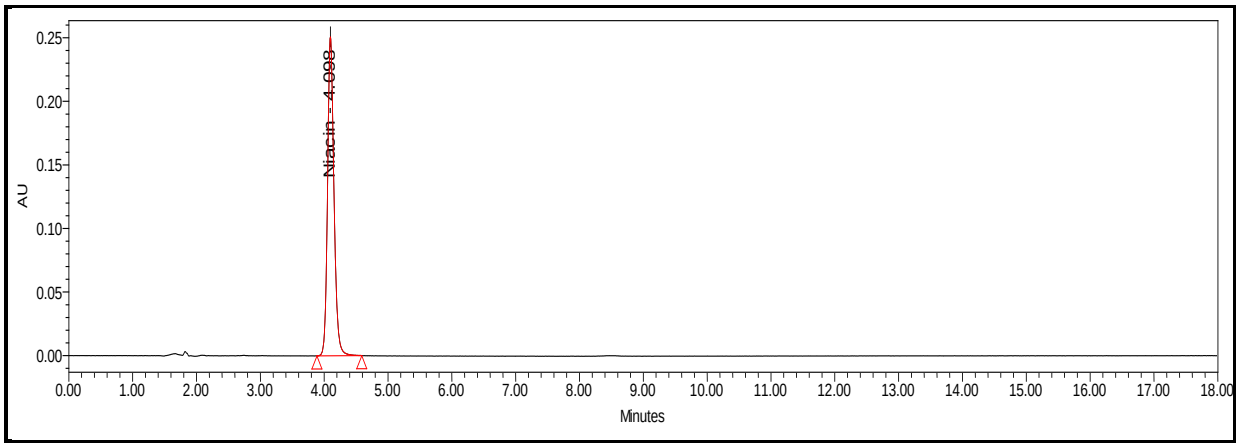

Fig.5 HPLC Chromatogram acid stressed sample of Niacin

Base Degradation Sample: Sample of 750 mg strength and its placebo were stressed using 5N sodium hydroxide by heating at 60°C for 60 min. Solutions were then neutralized with dilute hydrochloric acid and analyzed **(Fig.6)**.

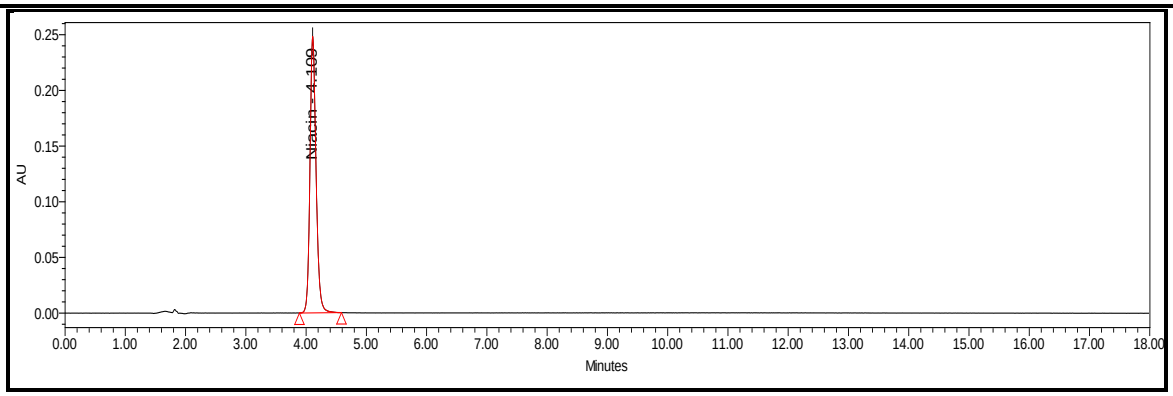

Fig.6 HPLC Chromatogram of base stressed sample of Niacin

Peroxide Degradation Sample: Sample of 750 mg strength and its placebo were stressed using 10% H_2O_2 by heating at 60°C for 60 min and solutions were analyzed **(Fig.7)**.

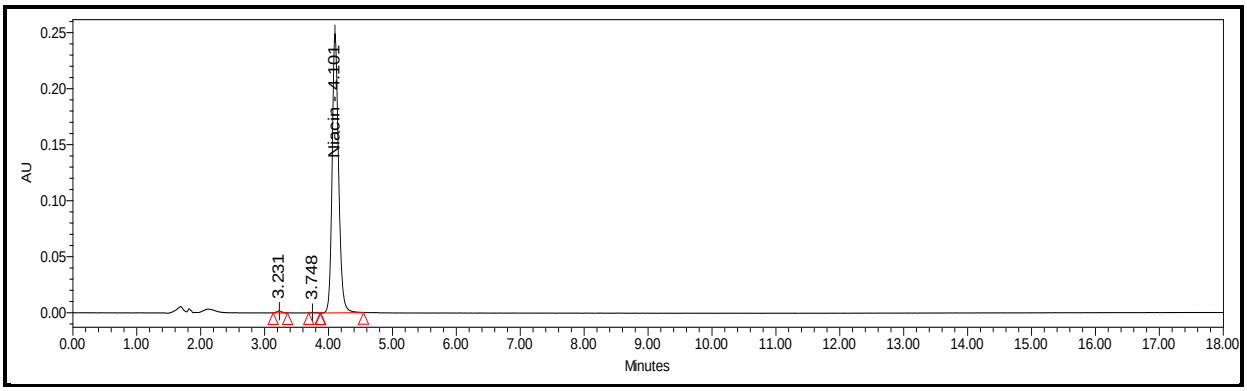

Fig.7 HPLC Chromatogram of peroxide stressed sample of Niacin

Thermal Degradation Sample: Sample of 750 mg strength and its placebo were placed in a glass petridish and stressed at 105°C for 12 h in an oven and analyzed **(Fig.8)**.

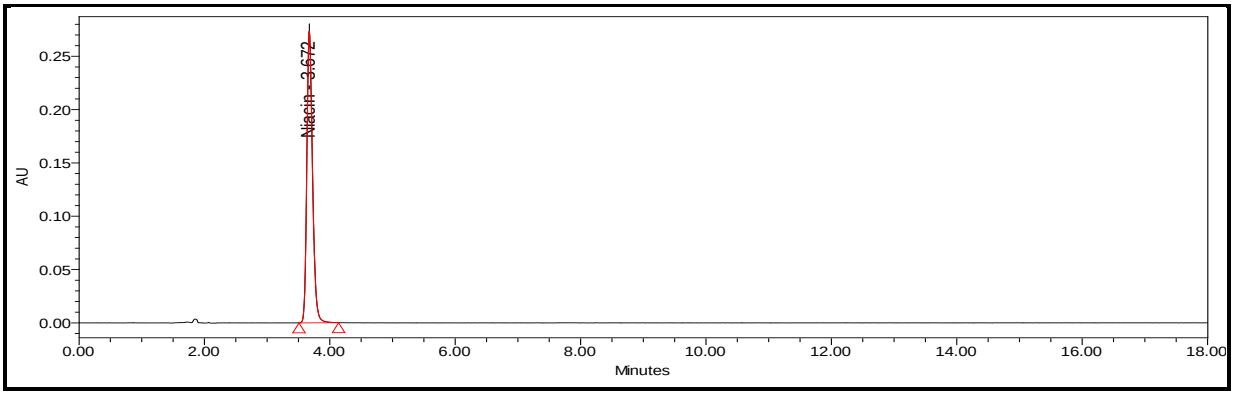

Fig.8 HPLC Chromatogram Thermal degradation sample of Niacin

Humidity Degradation Sample: Sample of 750 mg strength and its placebo were stressed at 90% RH (using a saturated solution of potassium nitrate) at room temperature for 7 days and solutions were analyzed **(Fig.9)**.

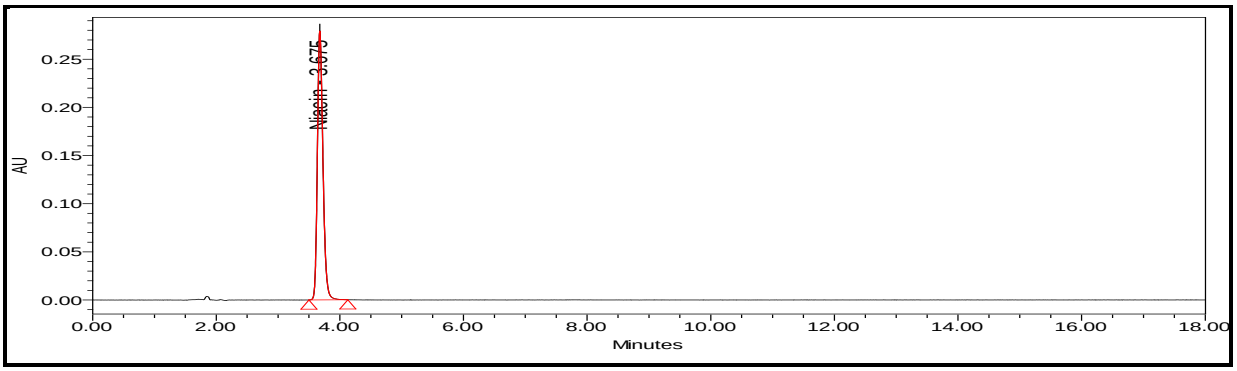

Fig.9 HPLC Chromatogram of humidity stressed sample of Niacin

UV-Light Degradation Sample: Sample of 750 mg strength and its placebo were stressed in a light chamber equipped with light sources as per ICH guideline on photo stability and exposed to 200 Watt hours/min of UV light. Solutions were prepared and analyzed **(Fig.10)**.

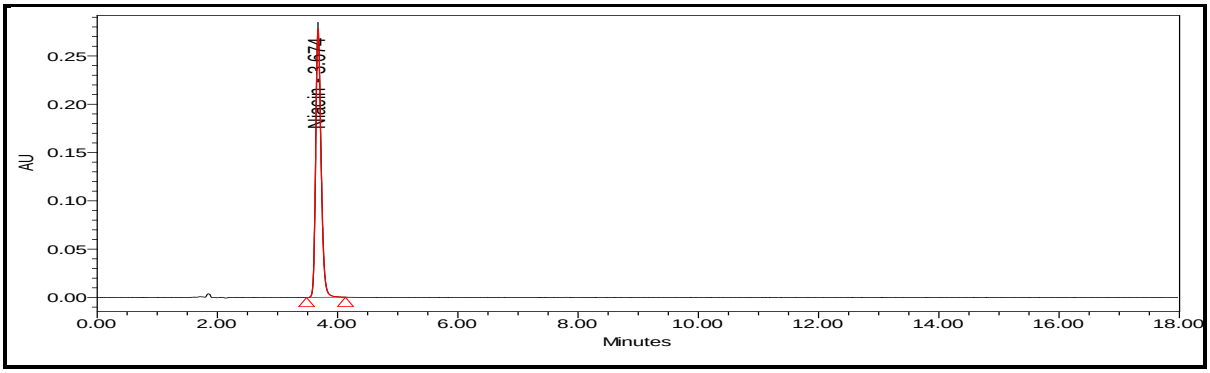

Fig.10 HPLC Chromatogram of UV-Light stressed sample of Niacin

Visible Light Degradation Sample: Sample of 750 mg strength and its placebo were stressed in a light chamber equipped with light sources as per ICH guideline on photo stability and exposed to 1.2 million lux hours of visible light. Solutions were prepared and analyzed **(Fig.11)**.

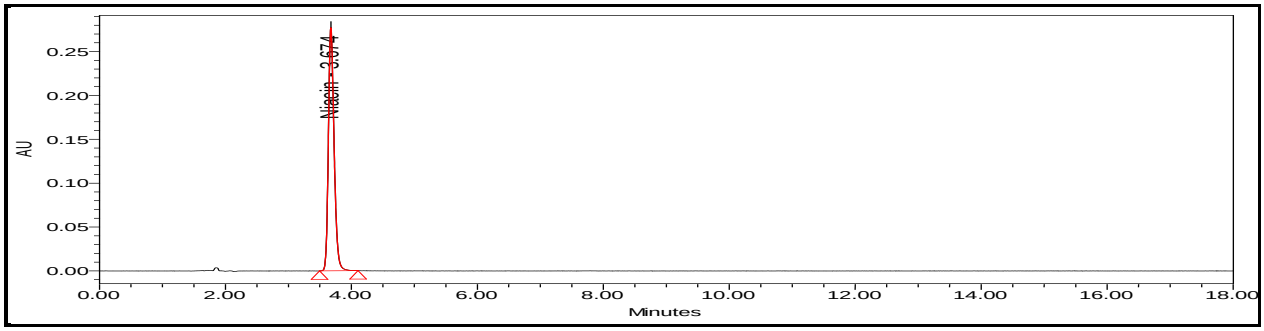

Fig.11 HPLC Chromatogram of Visible-Light stressed sample of Niacin

Neutral Hydrolysis Degradation Sample: Sample of 750mg strength and its placebo were stressed using water by heating at 60°C for 60 min and solutions were analyzed **(Fig.12)**.

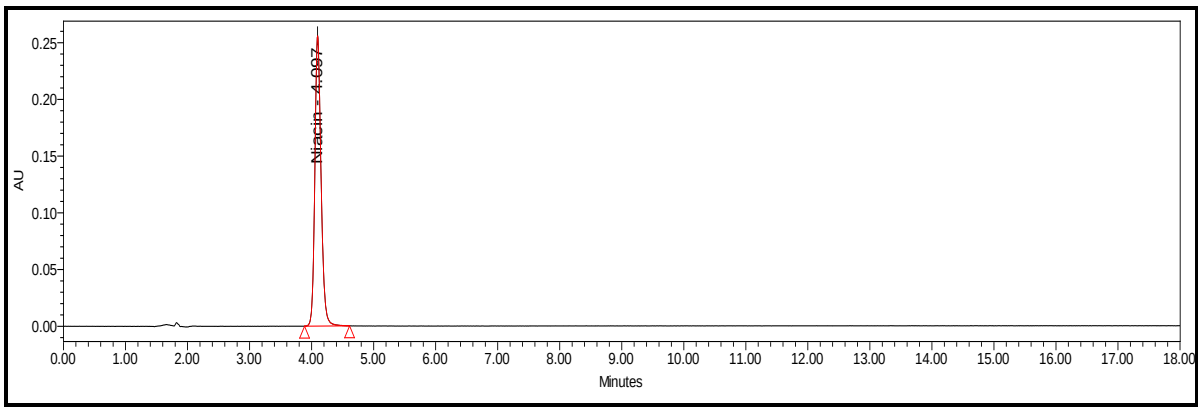

Fig.12 HPLC Chromatogram Neutral Hydrolysis stressed sample of Niacin

The results of drug and placebo stressed under different conditions were depicted in the following tables **table-2** and **3.**

Table-2 Stress Conditions and Percent Degradation of Niacin

Stress Type	Stress Conditions	Percent Degradation
Acid Hydrolysis	Heated at 60°C with 5N HCl for 60 minutes	0.0
Base Hydrolysis	Heated at 60°C with 5N NaOH for 60 minutes	0.0
Neutral Hydrolysis	Heated at 60°C with water	0.0
Oxidation	Heated at 60°C with 10% H_2O_2 for 60 minutes	0.0
High Humidity	90% RH at RT for 7 days	0.0
Photolysis – Visible Light	1.2 Million Lux Hours	0.0
Photolysis – UV Light	200 Watts per m^2	0.0
Thermal	105°C for 12 Hours	0.0

Table-3 Percent Assay of Stressed Samples

Stress Condition	Percent Assay of Niacin
Control Sample	98.1
Acid Hydrolysis	99.0
Base Hydrolysis	98.6
Neutral Hydrolysis	100.8
Oxidation	98.8
Degradation under high humidity	100.9
Photolysis – Visible Light	100.9
Photolysis – UV Light	100.7
Thermal	99.9

Accuracy: The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected

from 5 tablets of Niacin, analyzed as per the proposed method. The percentage recoveries with found in the range of 99.3 to 101.2 for Niacin. From the data obtained which given in **table-4** the method was found to be accurate.

Table-4 Recovery studies of Niacin for proposed method

% Level	Recovery Range
50	100.3-101.1
100	99.3-100.3
150	99.3-101.2

Linearity: The standard curve was obtained in the concentration range of 50 -150µg/mL for Niacin. The linearity of this method was evaluated by linear regression analysis the results were shown in **table-5.** Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **fig.13** to demonstrate the linearity of the proposed method from the data obtained which was given in **table-6** the method was found to be linear within the proposed range.

Table-5 Peak results of Niacin for Linearity

S. No.	Sample name	Name	RT	Area	USP tailing	USP plate count	Height
1	Assay-Linearity-50%	Niacin	4.041	950751	1.07	6372	122921
2	Assay-Linearity-75%	Niacin	4.037	1422128	1.09	6243	183382
3	Assay-Linearity-100%	Niacin	4.033	1903148	1.10	6047	244017
4	Assay-Linearity-125%	Niacin	4.028	2378543	1.12	6099	302130
5	Assay-Linearity-150%	Niacin	4.020	2864639	1.14	5965	362662

Table-6 Linearity studies of Niacin for proposed method

S. No.	Nominal concentration	Concentration (mg/mL)	Area
1	50%	0.0504	950751
2	75%	0.0756	1422128
3	100%	0.1009	1903148
4	125%	0.1261	2378543
5	150%	0.1513	2864639
Slope			$2x10^7$
y-intercept			-3107
R			1.0000
Bias at 100% level			-0.517

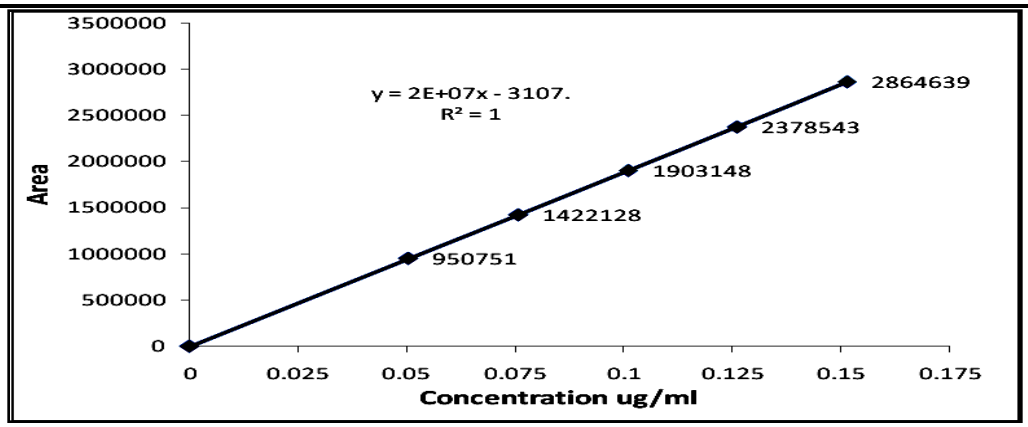

Fig.16 Linearity plot of Niacin

APPLICATIONS

We have developed a fast, simple and reliable analytical method for determination of Niacin in pharmaceutical preparation using RP-LC. There is no interference of blank and placebo at the retention time of Niacin. It is very fast, with good reproducibility and good response short time analysis of RP-HPLC method of Niacin comparing reported literature survey methods of RP-HPLC.

CONCLUSIONS

An RP-HPLC method for estimation of Niacin was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 50-150µg mL^{-1}. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the drug or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and linearity. It allows reliably the analysis of Niacin in its different pharmaceutical dosage forms.

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