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Development, Validation of Nevirapine- An Anti-Retro Viral Drug by RP-HPLC Method and Its Degradation Study under Various Stress Conditions

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Abstract:
A simple, Precise, Accurate method was developed for the estimation of Nevirapine by RP-HPLC technique. Chromatographic conditions used are stationary phase Kromosil C18 250mm x 4.6 mm, 5m , Mobile phase Acetonitrile: Water in the ratio of 60:40 Then pH adjusted to 3.5 with orthophosphoric acid. And flow rate was maintained at 1ml/min, detection wave length was 282nm, column temperature was set to 30 o C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R 2 value was found to be as 0.999. Precision was found to be 0.3 for repeatability and 0.3 for intermediate precision. LOD and LOQ are 0.07µg/ml and 0.22µg/ml respectively. By using above method assay of marketed formulation was carried out 99.78% was present. Degradation studies of Nevirapine were done, in all conditions purity threshold was more than purity angle and within the acceptable range.
Key words: HPLC, ICH guideline, Method development, Nevirapine

1. INTRODUCTION [1,2,3]:

Nevirapine is apotent, non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with nucleoside analogues for treatment of Human Immunodeficiency Virus Type 1 (HIV-1) infection and AIDS. [Pub Chem] Structurally, nevirapine belongs to a member of the dipyrido diazepinone chemical class of compounds (11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:20,30e][1,4diazepine-6-one]) is an anti-retroviral drug .It's Official in Indian Pharmacopoeia [4].Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of nevirapine does not compete with template or nucleoside triphosphates. Anti-HIV drugs such as nevirapine slow down damage to the immune system and prevent the occurrence of AIDS-defining illnesses.

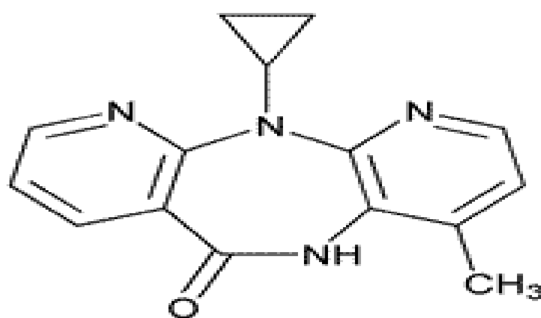


Fig: 1 structure of Nevirapine

Nevirapine is, in general, only prescribed after the immune system has declined and infections have become evident. It is always taken with at least one other HIV medication such as Retrovir or Videx. The virus can develop resistance to nevirapine if the drug is taken alone, although even if used properly, nevirapine is effective for only a limited time

Nevirapine is a white to off-white crystalline powder with the molecular weight of 266.30 and the molecular formula C15H14N4O. VIRAMUNE is the brand name for nevirapine (NVP) Nevirapine is structurally a member of the dipyrido diazepinone chemical class of compounds. Viramune Tablets are for oral administration. Each tablet contains 200 mg of nevirapine and the inactive ingredients microcrystalline cellulose, lactose monohydrate, povidone, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate. Viramune Oral Suspension is for oral administration. Each 5 mL of viramune suspension contains 50 mg of nevirapine (as nevirapine hemihydrate). The suspension also contains the following excipients: carbomer 934P, methylparaben, propylparaben, sorbitol, sucrose, polysorbate 80, sodium hydroxide and purified water.

Nevirapine has official monograph in IP (Indian Pharmacopoeia 2018), which describes liquid chromatographic method for the assay of Nevirapine. Literature survey reveals that few analytical methods have been published for the analysis of Nevirapine in bulk drug and formulation using HPLC, [9-15, 16]

2. MATERIALS AND METHODS:

Equipment and apparatus used:

Analysis was performed using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Nevirapine solutions.

1. Sonicator (Ultrasonic sonicator)
2. P^H meter (Thermo scientific)
3. Micro balance (Sartorius)
4. Vacuum filter pump

Reagents used:

1. Methanol HPLC Grade (RANKEM)
2. Acetonitrile HPLC Grade (RANKEM)
3. HPLC grade Water (RANKEM)
4. Phosphoric acid

Methods:

Diluent:

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

Preparation of Standard stock solutions:

Accurately weighed 10mg of Nevirapine transferred 25ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution (400µg/ml of Nevirapine)

Preparation of Standard working solutions (100% solution):

2.5ml of Nevirapine from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Nevirapine)

Preparation of Sample stock solutions:

10 tablets, each containing 200mg of nevirapine were weighed and average weight was calculated. Quantity equivalent to 10mg of tablet powder was taken in 10ml volumetric flask diluted with diluent gives 1000ppm and filtered through Whatman filter paper and suitable aliquots of formulation solutions were prepared to obtain concentration in the linearity range.(1000 µg/ml of Nevirapine)

Preparation of Sample working solutions (100% solution):

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Nevirapine)

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std. Dil. Fac}}{\text{Spl. Dil. Fac}} \times \frac{\text{Avg. Wt}}{\text{L.C}} \times \text{Potency}$$

Spl area – Sample Peak area
STD area – Standard Peak area
Std. Dil. Fac- standard dilution factor
Spl. Dil. Fac- sample dilution factor
Avg. Wt of Tab- average weight of tablet
L.C – label claim
Potency of Std

Optimization of chromatographic conditions: For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. Symmetrical peaks with good separation (retention time) was obtained with Kromosil C18 column and the mobile phase was Acetonitrile: Water (60:40). The pH of water was adjusted at a flow rate of 1ml/min at 282nm wavelength. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in figure.2

Preparation of Calibration curve:

Stock solution was diluted with mobile phase get a series of concentration ranging from 25%-150% were analysed. The peaks obtained were integrated, peak areas were noted and calibration graph was plotted using peak area ratios to internal standard peak areas vs. concentration of standard solutions.

3. METHOD VALIDATION:

The developed method was validated according to ICH guidelines for validation of analytical procedures.

Precision:

Precision studies were done in terms of repeatability (intra-day) and intermediate (inter-day) for a series of measurements and expressed as relative standard deviation. Intraday and Interday precision were calculated from six replicate readings and results were shown in Table 1.

Accuracy:

To study the accuracy of the method, recovery studies were carried out by spiking standard drug solution to pre analysed samples at 3 different levels i.e., 50, 100 and 150%. The resultant solutions were then analysed by the developed method. The recovery studies range from listed in Table 2.

Robustness: Small Deliberate changes in the method are made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated.

4. FORCED DEGRADATION IN UV

Acid degradation:

1ml of standard 100ppm solution is taken in a 10ml volumetric flask and add 1ml of 1N HCl and kept aside for 24hrs. Then it is neutralised with 1ml of 1N NaOH, made upto the volume with methanol and absorbance was noted using methanol as blank.

Base degradation:

1ml of standard 100ppm solution is taken in a 10ml volumetric flask and add 1ml of 1N NaOH and kept aside for 24hrs. Then it is neutralised with 1ml of 1N HCl, made upto the volume with methanol and absorbance was noted using methanol as blank.

Peroxide degradation:

1ml of standard 100ppm solution is taken in a 10ml volumetric flask and add 1ml of 10% hydrogen peroxide and kept aside for 24hrs. Then it is made upto the volume with methanol and absorbance was noted using methanol as blank.

Thermal degradation:

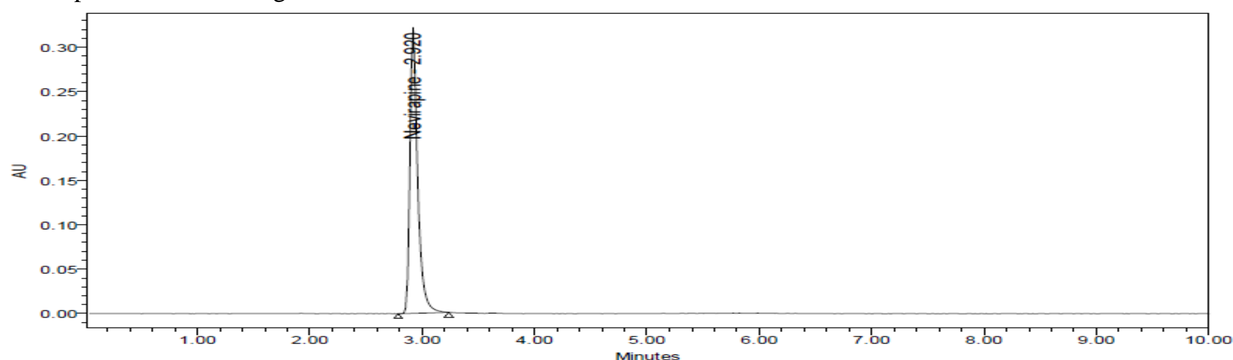
The standard drug powder was placed in incubator for 24hrs at 60°C and 10ppm solution was prepared using methanol and absorbance was noted.

Photolytic degradation:

The standard drug powder was placed in UV chamber for 24hrs and 10ppm solution was prepared using methanol and absorbance was noted.

5. RESULTS AND DISCUSSIONS:

Optimised chromatogram



Optimized Chromatographic Conditions

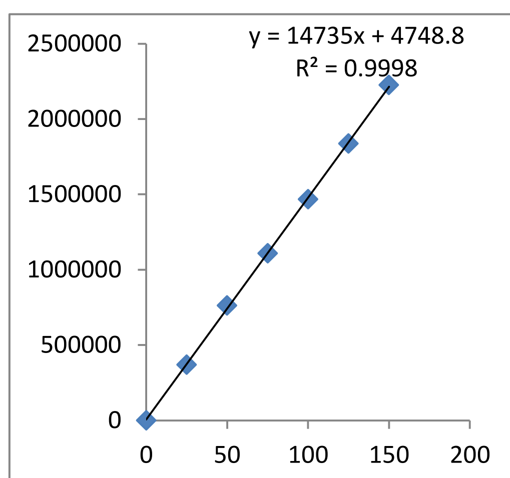
Column	Kromosil C18 250mm x 4.6 mm, 5m
Mobile phase	Water: Acetonitrile (60:40)
Flow rate	1ml/min
Detector	PDA 282nm
Injection Volume	20µl
Pump mode	Isocratic

Accuracy data

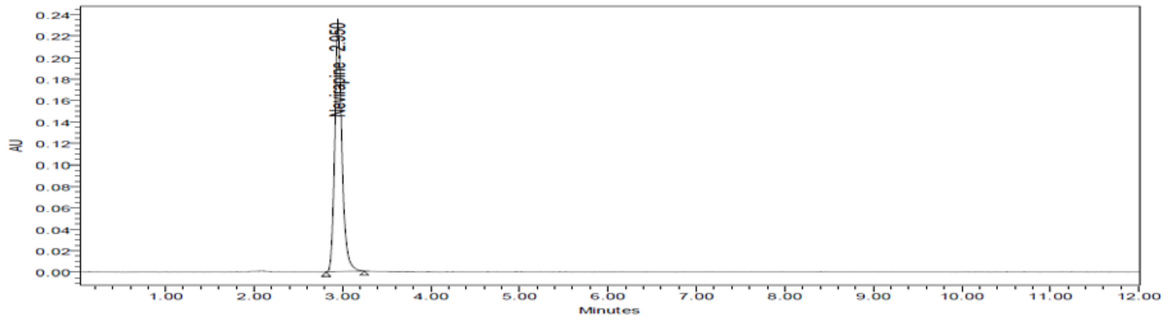
Parameters	Nevirapine
Calibration range(µg/ml)	25-150ppm
Optimized wavelength	282nm
Retention time	2.954min
Regression equation(Y)	y = 14375x + 4748
Correlation coefficient(r^2)	0.999
Precision (%RSD*)	0.3
% Recovery	100.04%
Limit of Detection(µg/ml)	0.07µg/ml
Limit of Quantitation(µg/ml)	0.22µg/ml

Assay Chromatogram of Sample

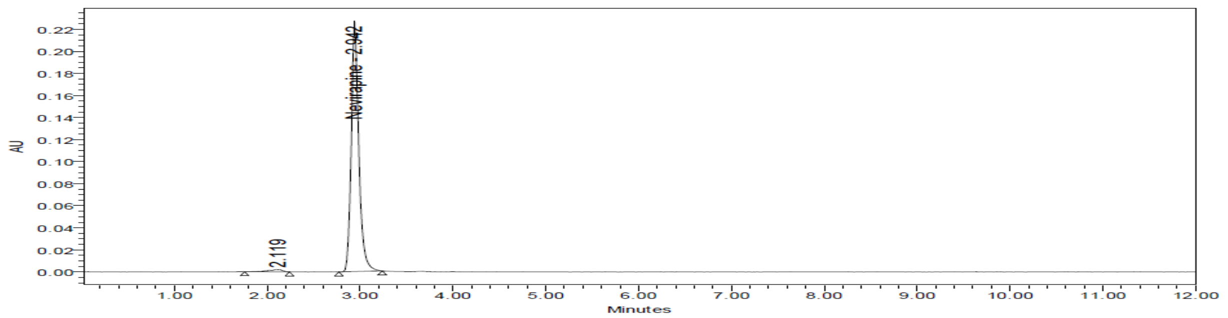
% Level	Amount Spiked	Amount	% Recovery	Mean%
50%	50	50.05	100.11	100.04%
	50	49.87	99.73	
	50	49.97	99.95	
100%	100	99.94	99.94	
	100	100.15	100.15	
	100	100.98	100.98	
150%	150	149.51	99.67	
	150	149.81	99.88	
	150	149.98	99.99	



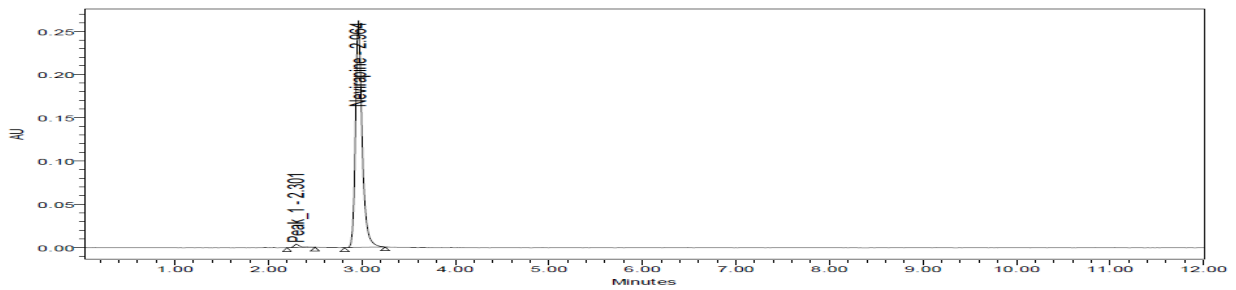
Forced degradations:



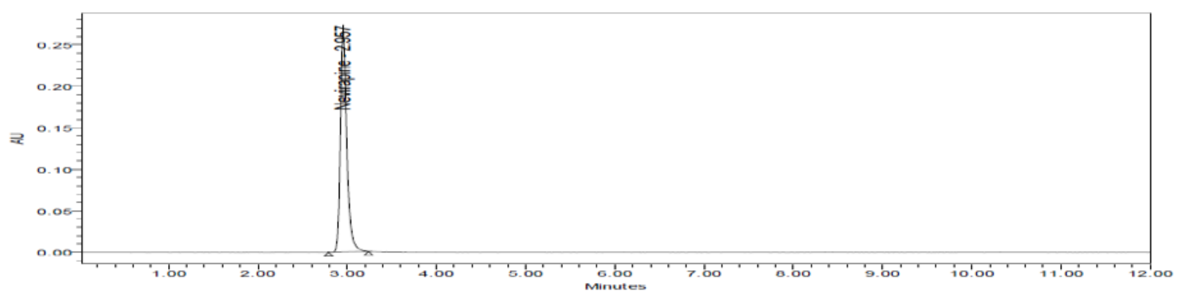
Base degradation chromatogram



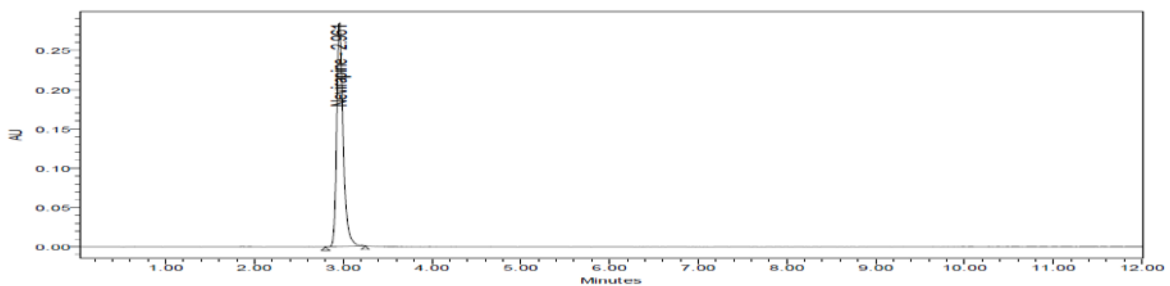
Acid degradation chromatogram



Peroxide degradation chromatogram



Thermal degradation chromatogram



UV degradation chromatogram

6. CONCLUSION

Chromatographic conditions used are stationary phase Kromosil (250mm*4.6mm 5µ.)Mobile phase Acetonitrile: Water (60:40), flow rate was maintained at 1ml/min, detection wavelength was 282 nm, Conditions were finalized as optimized method. Linearity study was carried out between25% to150 % levels, R^2 value was 0.999.Precision was found to be 0.3 for repeatability and intermediate precision. LOD and LOQ are 0.07µg/ml and 0.22µg/ml respectively. Assay of marketed formulation was carried out 99.78% was present. Degradation studies of Nevirapine were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

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