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## Original Research Paper

### STABILITY INDICATING HPLC METHOD FOR IMPURITIES ESTIMATION OF NEVIRAPINE IN EXTENDED RELEASE TABLET DOSE

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#### ABSTRACT

A novel, sensitive and selective reverse phase high performance liquid chromatographic method was developed for quantitative determination of nevirapine in its extended tablet dosage forms. The synthetic non-nucleoside reverse transcriptase inhibitor analogues (NNRTI) nevirapine form one of the extended dosage in HIV. It belongs to a group of anti-HIV medicines called non-nucleoside reverse transcriptase inhibitors (NNRTIs). The method is applicable to the quantification of related compounds of Nevirapine form one of the fixed extended dosage. Chromatographic separation of nevirapine from the possible impurities and the degradation products was achieved on a Supelcosil LC-ABZ, 150 x 4.6 mm, and 5.0 µm column. Ammonium orthophosphate pH 5.0 as mobile phase A and acetonitrile taken as mobile phase B in the ratio (85 : 15). The flow rate was 1.0 mL/min, and the detection was done at 220 nm. The above developed HPLC method was further subjected to hydrolytic, oxidative, photolytic and thermal stress conditions. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, and ruggedness.

**Keywords:** Nevirapine XR, Force degradation studies, Validation.

#### INTRODUCTION

Nevirapine (NEV) is an antiretroviral drug, The chemical name of nevirapine is 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2',3'-*e*][1,4]diazepin-6-one .Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against human immunodeficiency virus type 1 (HIV-1) that is already marketed for the treatment of HIV-1 infected adults. Nevirapine is recommended for treating HIV infections in combination with other reverse transcriptase inhibitors such as stavudine, zidovudine, and lamivudine.<sup>1</sup> The method has been reported for individual nevirapine in United

States Pharmacopeia<sup>2</sup> and European Pharmacopoeia.<sup>3</sup> High-performance thin-layer chromatography (HPTLC) method is reported for determination of nevirapine in pharmaceutical dosage form.<sup>4</sup> Some methods are reported for estimation of nevirapine with other antiviral drugs.<sup>5-7</sup> Methods are reported in biological fluids.<sup>8-11</sup> Survey of literature revealed that the nevirapine was determined by stripping voltammetry<sup>12</sup> and by spectrophotometry.<sup>13</sup> Viramune was recently featured in Health Magazine<sup>19</sup> as one of the top ten medical advances of 1999 and on CNN's list (December

29, 1999) of the top 10 health improvement stories of 1999. Viramune was lauded as the ninth greatest health advance in 1999 based on its ability to reduce the transmission of HIV from mother to infant. In addition, nevirapine is more affordable and practical than any other drug examined to date. The Health Magazine<sup>19</sup> article reports, "The new drug [nevirapine] is as cheap as it is effective, costing about one-seventieth as much as a short course of AZT. The Ugandan government is working with the manufacturer to make the drug widely available in that country and other African nations may follow. If so, researchers estimate 400,000 infants could be spared from HIV each year." Three papers have been published on chromatographic methods that study different aspects of nevirapine. Dinallo *et al.*<sup>20</sup> reported the characterization of synthetic

byproducts by B/E linked scanning and high-resolution thermo spray mass spectrometry. Cohen *et al.*<sup>21</sup> and Palladino *et al.*<sup>22</sup> separately reported the studies of the binding environment of nevirapine to reverse transcriptase of HIV-1 by high-performance liquid chromatography (HPLC) and photo affinity cross-linking. The present paper is the first to describe the validation of a specific, sensitive, and stability-indicating HPLC method for the assay and determination of related organic impurities in nevirapine drug substance. The validation generally complies with the International Conference on Harmonization (ICH) guidelines on the impurities in new drug substances<sup>23</sup> and the validation of analytical methods: definition and terminology<sup>24</sup> and methodology.<sup>25</sup>

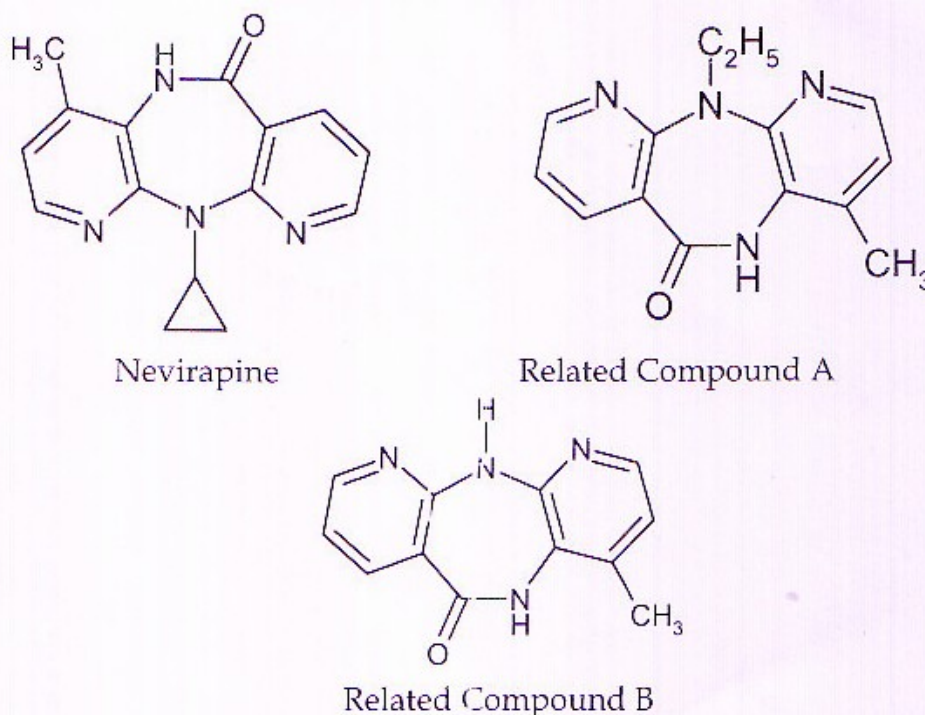


Fig. 1. Chemical structures of nevirapine, related compound A, and related compound B

### Figure 1: Nevirapine and its related compounds structures.

## CHEMICALS AND MATERIALS

Acetonitrile (HPLC Grade) and Ammonium orthophosphate & Sodium hydroxide (AR Grade), were purchased from Spectrochem and E-Merck Limited respectively. In-house purified water (USP Grade) was used throughout the study. Active pharmaceutical ingredients and its related

impurities (Figure 1) were procured from Hetero, India, commercially available.

## Equipments

The High performance liquid chromatography (Waters) used was equipped with Photo diode array detector with gradient elution capacity and an auto sampler with data handling system (Empower software) on Lenovo computer.

## Chromatographic Conditions

The chromatographic separation was achieved using a isocratic method on an Supelcosil LC-ABZ, 150 x 4.6 mm, 5- $\mu$ m column; Selection of Liquid chromatographic degassed Buffer solution A and acetonitrile in the ratio (85 : 15 ) as mobile phase. The solution A contains 2.88 g of Ammonium ortho phosphate into a beaker containing 1000 ml of water and mix. Adjust pH of the solution to 5.0  $\pm$ 0.05 with dilute 1N sodium hydroxide. Filter the solution through 0.45 $\mu$ m membrane filter. The flow rate was 1.0 mL/min is used. The column temperature was maintained at 35 °C and the detection wavelength was 220 nm for identified and unidentified impurities. The injection volume of 20 $\mu$ L was used.

## Diluent

Use mobile phase as a diluents.

## Standard Solutions

### Preparation of Nevirapine Standard Solution

Accurately weighed and transferred about 20 mg of the Nevirapine anhydrous into a 100 ml volumetric flask, added 20 ml acetonitrile and sonicate to dissolve and further dilute to volume with diluent and mix.

### Preparation of Impurity Standard Stock Solution

Accurately weighed and transferred about 2.5 mg each of Nevirapine RC-A and Nevirapine RC-B into a 50 mL VF. And add 10 mL Acetonitrile and sonicate to dissolve. Dilute to volume with diluent and mix.

### Preparation of Resolution Solution

Accurately weighed and transferred about 50 mg of Nevirapine anhydrous working standard into a 50 ml volumetric flask. Added about 10 ml of acetonitrile and sonicate to dissolve. Add 2.0 mL of impurity stock solution and make up with diluent and mix.

## Preparation of Sample Solutions

Accurately weighed and transferred tablets powder equivalent to about 100 mg of Nevirapine into a 100 ml volumetric flask, added about 20ml of acetonitrile and rotate for not less than 20 minutes on rotary shaker. Add about 50 mL diluents and sonicate not less than 20 minutes (maintain the sonicator temperature between 20 to 25°C). Dilute to volume with diluent and mix. Filter a portion of the solution through 0.45 $\mu$ m membrane filter and discard first few ml of the filtrate.

## Force Degradation Studies

Specificity is the ability of method to measure the analyte response in the presence of its potential impurities and degradation products. The specificity of the developed RP-HPLC method of Nevirapine was carried out in presence of its two potential impurities, namely Nevirapine RC-A, & Nevirapine RC-B. Forced degradation studies were performed on for Nevirapine bulk drugs (Figure 2). Intentional degradation was attempted with stress conditions of UV light (254 nm), heat & humidity (105°C at 90 % RH), acid (5N HCl), base (5N NaOH) and oxidation (30 % H<sub>2</sub>O<sub>2</sub>) to determine the ability of the proposed method to separate Nevirapine from its impurities and degradation products generated during forced decomposition studies. For heat and light studies, study period was 7 days where as for acid, base and oxidation it was 24 hrs. Peak purity test was carried out on the stressed samples by using PDA. Related compounds studies were carried out for stress samples against qualified reference standard. Related compounds were also calculated for bulk sample by spiking with its impurities at its specification level (0.1%).

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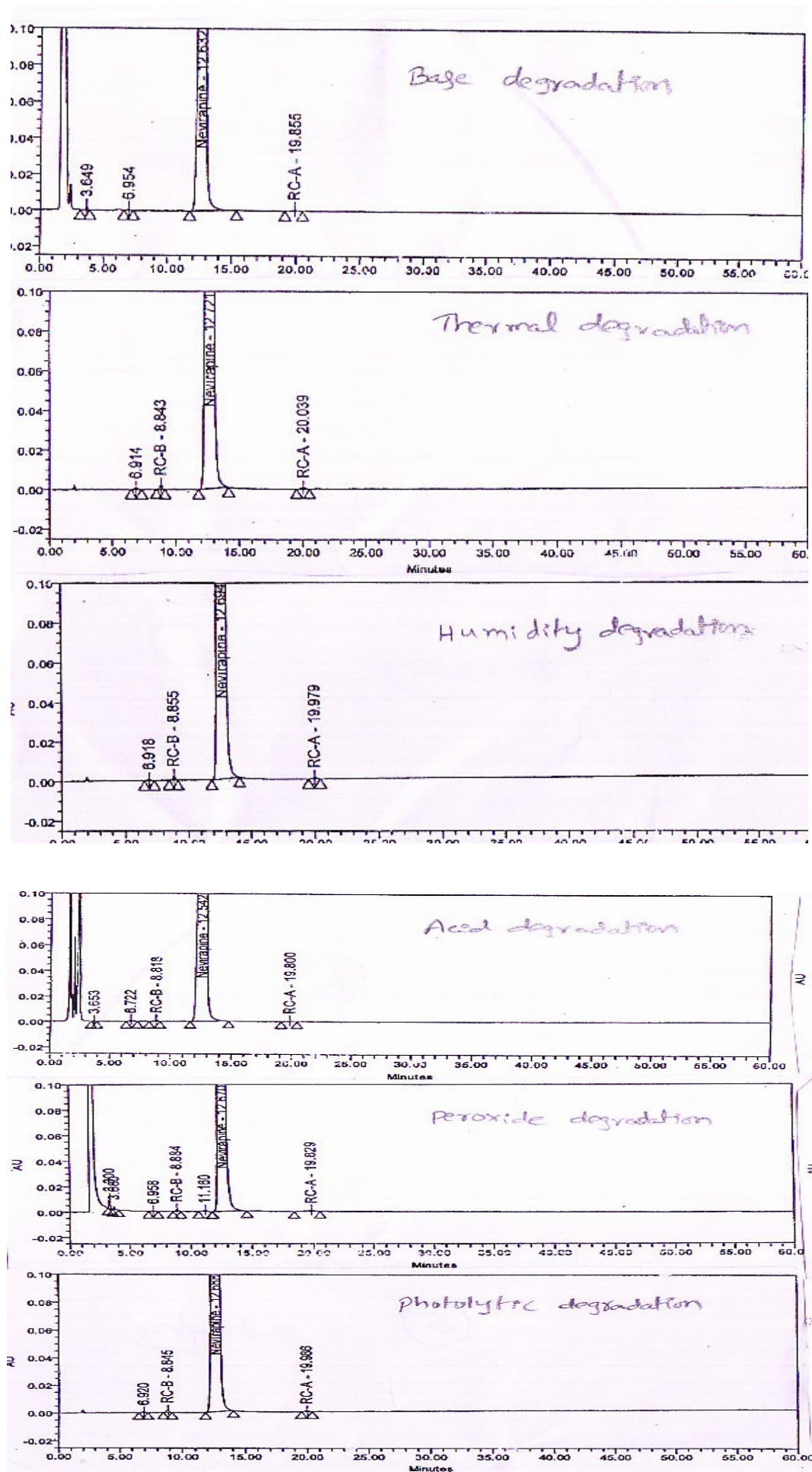


Figure 2: Chromatograms of forced degradation studies

## Method Validation

### System & Method Precision

The system precision is indicated by the repeatability of multiple injections and indicates the performance of the HPLC instrument under the prescribed chromatographic conditions. The variance of the values obtained is represented as the percent relative standard deviation (% RSD). A working standard solution of Nevirapine and its related compounds was consecutively injected six times under the same analytical conditions. The % RSD of peak areas, difference of retention times, tailing factor (T) column efficiency (N) and resolution (R) are calculated. The intermediate precision of the method was also evaluated using one unspiked sample and 6 independent sample preparations spiked with a 100 % of the target concentrations as defined by the method. The samples were injected using a different instrument and column.

### Linearity

The linearity is determined by the ability of the method to obtain test results, which are directly proportional to the concentration of the compounds of interest in the sample. Stock solutions were serially diluted to produce solutions containing concentration levels from QL to 150% with respect to impurity specification limits of 0.1 %. The calibration curve was drawn by plotting the peak areas of Nevirapine RC-A and RC-B versus its corresponding concentrations. The % RSD value of the slope and Y intercept of the calibration curve was calculated.

### Quantification limit (QL) and Detection Limit (DL)

The lower end of the linear range was considered to be the QL for the method. The QL concentrations were determined by injecting diluted standard solution to a level such that % RSD was not more than 10 %, precision study was also carried at the QL level by injecting six individual preparations of Nevirapine , Nevirapine RC-A and Nevirapine RC-B.

### Accuracy

Nevirapine ; sample solution was spiked with impurity standard solutions containing Nevirapine RC-A and Nevirapine RC-B at three concentration levels corresponding to QL , 100 % and 150 % of analyte concentration. The % recovery is the amount of the compound of interest analyzed as a percentage of the theoretical amount present in the medium was calculated from the slope and the Y-intercept of the calibration curve.

### Robustness

Deliberate variations in critical method parameters were done to assess the robustness of the related compounds method to evaluate method reliability. The flow rate of the mobile phase was 1.0mL/min, to study the effect of flow rate on the resolution; it was changed by 0.1 unit from 0.9 to 1.1mL/min. The effect of column temperature on resolution was studied at 30 and 40 °C instead of 35 °C. The pH of Buffer Mobile phase 4.8 and 5.2 instead of 5.0. The mobile phase ratio changes (87:13) and (83:17) instead of (85:15).

### Solution Stability

The stability of the analyte was established for standard and sample solutions under conditions as prescribed in the method. The purpose of this procedure was to determine the time during which the standard and sample solutions remain stable. In this validation three solutions were studied: Stock standard solution, Working standard solution and Sample solution.

## RESULTS AND DISCUSSIONS

### Method Development and Optimization

The main aim of the chromatographic method is to achieve the separation of precursors, intermediates and the main components Nevirapine. From the UV profiling it was found that the suitable wavelength for the Nevirapine drug and its related impurities is 220 nm. Hence it was concluded anticipating the possible base line interferences at lower wavelength 220 nm was selected as the detection wavelength for the quantification of Nevirapine its identified and unidentified impurities. When developing a reversed phase method for acidic compounds, like

Nevirapine you can expect a more robust method when using basic mobile phases. Based on the experimental data & the opted wavelength it was found ammonium ortho-phosphate is suitable. The chromatographic separation was achieved on a Supelcosil LC-ABZ 150 x 4.6 mm, 5  $\mu$ m column. The isocratic liquid chromatographic method employs solution A and Solution B as mobile phase. Solution A contains 2.88 g of Ammonium orthophosphate a beaker containing 1000 ml of water and mix, Adjust pH of the solution to  $5.0 \pm 0.05$  with 1N sodium hydroxide. Selection of mobile phase is mixture of HPLC grade Solution A : Solution B is Acetonitrile (85:15). The flow rate was 1.0 mL/min. The column temperature was maintained at 35  $^{\circ}$ C,

sample compartment temperature is maintained at 5 $^{\circ}$ C and the detection wavelength was 220 nm for identified and unidentified impurities. The injection volume 20  $\mu$ L. The peak shape of Nevirapine was found to be symmetric and well separated by its potential process impurities and degradants. In the optimized conditions, Nevirapine, Nevirapine RC-A and Nevirapine RC-B were well separated with a resolution greater than 1.5 and the typical retention times for Nevirapine, Nevirapine RC-A and Nevirapine RC-B were about 12.6, 19.9, and 8.8 respectively. The system suitability results were tabulated and the developed method for Nevirapine and its impurities was found to be specific (Table 1).

**Table 1: Forced degradation results**

Impurities (%)	Stress Condition					
	HCl	NaOH	H <sub>2</sub> O <sub>2</sub>	UV	Thermal	Humidity
Nevirapine Related Compound -A	0.025	0.024	0.053	0.020	0.023	0.021
Nevirapine Related Compound-B	0.020	NA	0.026	0.025	0.026	0.026

### Results of Forced Degradation

Forced degradation samples were analyzed with a sample concentration of 1000 mg/mL of Nevirapine equivalent with above mentioned chromatographic conditions using a PDA detector to monitor the homogeneity and purity of the Nevirapine peak. Degradation was not observed under stress condition like, heat & humidity (105  $^{\circ}$ C & 90 % RH for 7 days) oxidative (30 % H<sub>2</sub>O<sub>2</sub> at RT for 24 hours) and light exposure in solid state and liquid state. Very mild degradation of drug material was observed during acid hydrolysis (5 N HCl 24 hours at 80 $^{\circ}$ C) however the drug is more susceptible to base hydrolysis (5 N NaOH 24 hours at 60  $^{\circ}$ C) (Figure 2). The RS studies were carried out for the stress samples against a Nevirapine qualified reference standard. The mass balance (%assay + % sum of all related compounds + % sum of all degradants) were calculated for all of the stressed samples and were found to be more than 95 %. Peak purity test results obtained from PDA confirm that the Nevirapine peak was homogeneous and pure in all analyzed stress samples, which confirms the

stability indicating power of the developed method.

### Results of Method Validation

#### Precision

The injection (system) precision was evaluated by performing six replicate injections for its related compounds at 100 % working standard concentration. The % relative standard deviation of 6 injections was calculated, the % RSD Nevirapine, Nevirapine RC-A and Nevirapine RC-B were found to be 0.6, 0.35 and 0.77% respectively. The RSDs of the % recovery values meet the requirement of not more than 10% for all impurities. (Table 2)

#### Linearity

For all two impurities, a linear calibration curve was obtained ranging from QL to 0.15 %. The analytical data and linearity results for Nevirapine RC-A, Nevirapine RC-B were tabulated in (Table 2). The coefficient of determination ( $r^2$ ) is 0.99999, and 0.99997 respectively, which meets the specification for the  $r^2$  value of not more than 0.99, confirming the linearity of the method.

#### Accuracy

The related compounds of Nevirapine also be determined accurately over a concentration range varying from QL to 150 % of their respective target analyte concentrations when in Nevirapine sample solution. The percentage recovery for the related compounds Nevirapine RC-A and Nevirapine RC-B were ranged from 99.7 to 102.2 (Table 2).

### Robustness

In all the deliberate varied conditions (flow rate and column compartment temperature) the resolution between Nevirapine Related compound B and , Nevirapine and its impurities was greater than 5.0, and the resolution between Nevirapine and Nevirapine Related compound A is not less than 3.0.

**Table 2:** Summary of method validation

Validation Parameter	Nevirapine	Nevirapine Related Compound A	Nevirapine Related Compound B
<b>System Precision</b>			
% RSD of peak area	0.60	0.35	0.77
Tailing Factor	0.97		
Column efficiency	10483		
<b>Linearity</b>			
Slope	91208.27	97668.47	119722.79
Intercept	154.89	607.65	670.21
r <sup>2</sup>	0.99998	0.99999	0.99997
RRF	--	1.07	1.31
Accuracy Mean % Recovery at			
QL		99.8%	102.2%
50%		100.9%	102.1 %
100 %		100.5%	100.9 %
150 % of target		100.0%	99.7 %
<b>Intermediate Method Precision</b>			
% RSD	0.68	1.38	1.11
<b>Quantitation limit(µg/mL)</b>	0.012	0.014	0.006
<b>Detection limit(µg/mL)</b>	0.004	0.005	0.002
<b>Stability of Solutions</b>			
Working Standard Stock , Std Solutions (Room temp) and Sample Solution (5±3°C) (Room temp) .	0 hour to 48 hours stable	0 hours to 48 hours stable	0 hours to 48 hours stable

### Solution Stability

The Stock standard solution, Working standard solution and Sample solution were prepared as per the method, after dispensing an amount for the testing of initial time , the solutions were stored in volumetric flasks and kept in refrigerator (5 ±3 °C) prior to the testing at each time interval of 1st week, 2nd week, 3rd week & 4th week for Stock standard solution and 24 hours & 48 hours, for Working standard solution and Sample solution, the flasks were taken out of the refrigerator,

allowed to equilibrate to room temperature before use. The % recovery of each analyte meets the requirement of 90 to 110 % after 2<sup>nd</sup> day for Stock standard solution; however working standard is stable up to 2<sup>nd</sup> day. No extra peaks detected, no peaks disappeared and no peak areas are increased or decreased by more than the respective QL level after 48 hours in case of sample solution. Therefore sample solution was found to be stable for 48 hours. However working standard and

sample stored at room temperature showed a stability of 24 hours.

## CONCLUSION

A stability indicating HPLC related compounds method was developed for the quantification of, Nevirapine and its potential impurities in active pharmaceutical ingredients and its dosage forms. The developed method is specific, precise, accurate, linear and robust for, Nevirapine and its impurities. Degradation products formed during forced decomposition studies were very well separated from analyte peak, which demonstrates that the developed method was specific and stability indicating. This method can be used to carry out the analysis of Nevirapine drug product in regular quality check and stability samples.

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