

**Stability indicating HPLC method for impurities estimation of nevirapine in extended release tablet dose**

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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 forming one of the fixed Extended dosage. Chromatographic separation of Nevirapine from the possible impurities and the degradation products was achieved on an Supelcosil LC-ABZ 150 x 4.6 mm, 5.0µm 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

## 1. 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 [1]. The method has been reported for individual nevirapine in United States Pharmacopeia [2] and European Pharmacopoeia [3].

High-performance thin-layer chromatography (HPTLC) method is reported for determination of nevirapine in pharmaceutical dosage form [4]. Some methods are reported for estimation of nevirapine with other antiviral drugs [5–7]. Methods are reported in biological fluids [8–11]. Survey of literature revealed that nevirapine was determined by stripping voltammetry [12] and by spectrophotometry [13]. Viramune was recently featured in *Health Magazine* [19] 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* [19] 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. [20] reported the characterization of synthetic byproducts by B/E linked scanning and high-resolution thermospray mass spectrometry. Cohen et al. [21] and Palladino et al. [22] separately reported the studies of the binding environment of nevirapine to reverse transcriptase of HIV-1 by high-performance liquid chromatography (HPLC) and photoaffinity crosslinking.

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 [23] and the validation of analytical methods: definition and terminology [24] and methodology [25].

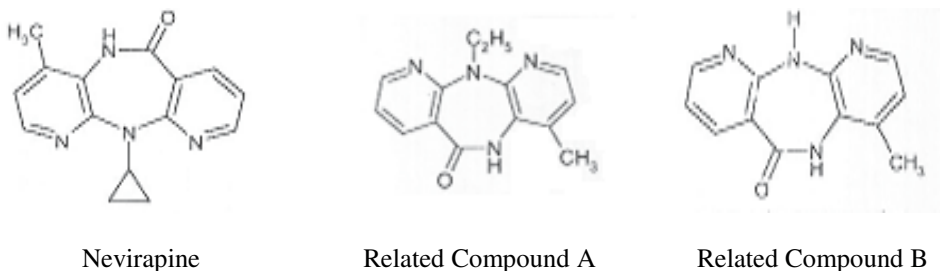


Fig. 1 Nevirapine and its related compounds A and B.

## 2. EXPERIMENTAL

### 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 (Fig. 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 an 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 ( 85:5 ) as mobile phase. The solution A contains 2.88 g of

Ammonium orthophosphate in a beaker with 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  $0.45 \mu\text{m}$  membrane filter. The flow rate was  $1.0 \text{ cm}^3/\text{min}$  is used. The column temperature was maintained at  $35^\circ\text{C}$  and the detection wavelength was 220 nm for identified and unidentified impurities. The injection volume of  $20 \text{ mm}^3$  was used.

### **Diluent**

Use mobile phase as a diluent.

### **Standard Solutions**

#### Preparation of Nevirapine Standard solution

Accurately weigh and transfer about 20 mg of the Nevirapine anhydrous to a  $100 \text{ cm}^3$  volumetric flask, add  $20 \text{ cm}^3$  acetonitrile and sonicate to dissolve and further dilute to volume with diluent and mix.

#### Preparation of Impurity standard stock solution

Accurately weigh and transfer about 2.5 mg each of Nevirapine RC-A and Nevirapine RC-B to a  $50 \text{ cm}^3$  VF. Add  $10 \text{ cm}^3$  Acetonitrile and sonicate to dissolve. Dilute to volume with diluent and mix.

#### Preparation of Resolution solution

Accurately weigh and transfer about 50 mg of Nevirapine anhydrous working standard to a  $50 \text{ cm}^3$  volumetric flask. Add about  $10 \text{ cm}^3$  of acetonitrile and sonicate to dissolve. Add  $2.0 \text{ cm}^3$  of impurity stock solution and make up with diluent and mix.

#### Preparation of Sample Solutions:

Accurately weigh and transfer tablets powder equivalent to about 100 mg of Nevirapine to a  $100 \text{ cm}^3$  volumetric flask, add about 20ml of acetonitrile and rotate for not less than 20 minutes on rotary shaker. Add about  $50 \text{ cm}^3$  diluents and sonicate for not less than 20 minutes (maintain the sonicator temperature between  $20$  to  $25^\circ\text{C}$ ). Dilute to volume with diluent and mix. Filter a portion of the solution through  $0.45 \mu\text{m}$  membrane filter and discard first few ml of the filtrate.

### 3. 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 of 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 that anticipating the possible base line interferences at lower wavelength 220 nm was to be 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 and the opted wavelength, it was found that ammonium orthophosphate is suitable. The chromatographic separation was achieved on a Supelcosil LC-ABZ  $150 \times 4.6$  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 in a beaker containing 1000  $\text{cm}^3$  of water and mix. Adjust pH of the solution to  $5.0 \pm 0.05$  with 1N sodium hydroxide. Selection of mobile phase is a mixture of HPLC grade Solution A: Solution B is Acetonitrile (85 : 15). The flow rate was 1.0  $\text{cm}^3/\text{min}$ . The column temperature was maintained at  $35^\circ\text{C}$ , sample compartment temperature was maintained at  $5^\circ\text{C}$  and the detection wavelength was 220 nm for identified and unidentified impurities. The injection volume was 20  $\text{mm}^3$ .

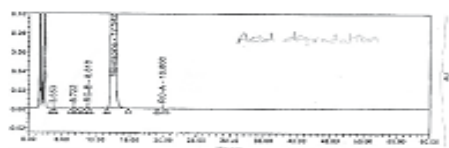
The peak shape of Nevirapine was found to be symmetric and well separated by its potential process impurities and degradants. In the optimized conditions, Nevirapine, Nevirapine RC-A and Nevirapine RC-B were well separated with a resolution greater than 1.5 and the typical retention times for Nevirapine, 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.

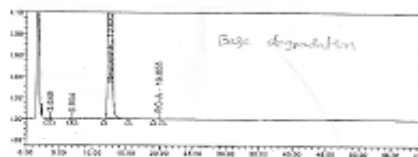
Impurities [%]	Stress Condition					
	HCl	NaOH	H <sub>2</sub> O <sub>2</sub>	UV	Thermal	Humidity
Nevirapine Related Compound-A	0.025	0.024	0.053	0.020	0.023	0.021
Nevirapine Related Compound-B	0.020	NA	0.026	0.025	0.026	0.026

### Results of Forced Degradation

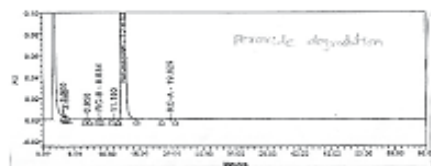
Forced degradation samples were analyzed with a sample concentration of 1000 mg/cm<sup>3</sup> of Nevirapine equivalent with above mentioned chromatographic conditions using a PDA detector to monitor the homogeneity and purity of the Nevirapine peak.



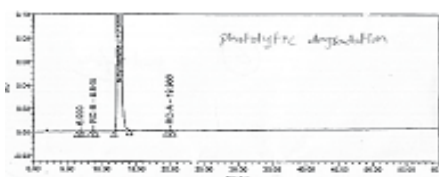
Chromatogram of Acid Degradation



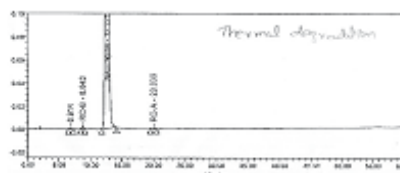
Chromatogram of Base Degradation



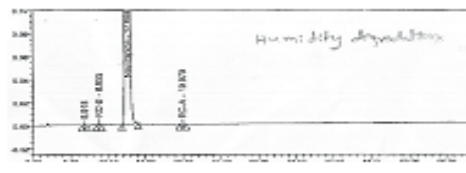
Chromatogram of peroxide degradation



Chromatogram of Phtolytic degradation



Chromatogram of Thermal degradation



Chromatogram of Humidity degradation

Fig. 2. Chromatograms of forced degradation studies.

Degradation was not observed under stress conditions, like heat and humidity (105°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°C) however the drug is more susceptible to base hydrolysis (5 N NaOH 24 hours at 60°C) (Fig. 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 the 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 entered 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 were also 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 ranged from 99.7 to 102.2 (Table 2).

Table 2. Summary of method validation.

Validation Parameter	Nevirapine	Nevirapine Related Compound A	Nevirapine Related Compound B
<u>System Precision</u>			
(%) RSD of peak area	0.60	0.35	0.77
Tailing Factor	0.97		
Column efficiency	10483		
<u>Linearity</u>			
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
<u>Accuracy Mean [%] Recovery at</u>			
QL		99.8%	102.2%
50%		100.9%	102.1%
100%		100.5%	100.9%
150% of target		100.0%	99.7%
<u>Intermediate Method Precision</u>			
(%) RSD	0.68	1.38	1.11
<u>Quantification limit [<math>\mu\text{g}/\text{cm}^3</math>]</u>	0.012	0.014	0.006
<u>Detection limit [<math>\mu\text{g}/\text{cm}^3</math>]</u>	0.004	0.005	0.002
<u>Stability of Solutions</u>			
Working Standard Stock , Std Solutions (Room temp) and Sample Solution ( $5 \pm 3^\circ\text{C}$ ) (Room temp)	0 hour to 48 hours stable	0 hours to 48 hours stable	0 hours to 48 hours stable



### 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 was not less than 3.0.

## 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 degradation 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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