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RP-HPLC method development and stability studies of nevirapine in pharmaceutical dosage form

Dr.V.Uma Maheshwar Rao[1], B.Swapna[*1], P.Manisha[1], Palawar Dinesh Kumar[1], Puri Dinesh[1], Pola Madhuri[1], Priyanka Sharma[1], Priya Sharma[1]

[1]Teegala Ram Reddy College of Pharmacy, #4-202, Meerpet, Saroornagar (M), Rangareddy District, Hyderabad-500079, Telangana, India.

***Corresponding Author: B.Swapna**

Email: swapna.klsss@gmail.com

ABSTRACT

A selective, specific and sensitive stability-indicating Reverse phase high-performance liquid chromatographic method (RP-HPLC) was developed and validated for the estimation of Nevirapine in pharmaceutical dosage forms. The method was performed on HPLC WATERS equipped with Isocratic with UV-Visible Detector using Develosil ODS HG-5 RP C18 column (15cm × 4.6 mm, 5µm) and Mobile phase used is a mixture of Acetate Buffer (pH=3.9): Acetonitrile (45:55) with a flow rate of 1.0 ml/min. Detection wavelength was 246nm and temperature was Ambient. The percentage relative standard deviation in precision and accuracy studies was found to be less than 2%. The proposed method was validated according to ICH guidelines. Nevirapine was subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation, photolysis and thermal degradation and it was found that the drug is highly resistant towards all degradations. The developed method was validated with regard to linearity, accuracy, precision, selectivity and robustness and the method was found to be precise, accurate, linear and specific. As the proposed HPLC method achieved satisfactory resolution between Nevirapine and its degradation products.

Keywords: Nevirapine, RP-HPLC, Accuracy, Linearity, Precision, Method Validation.

INTRODUCTION

It is a potent; 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. Structurally Nevirapine belongs to the dipyridodiazepinone chemical class [1] [2] [3]. For use in combination with other antiretroviral drugs in the ongoing treatment of HIV-1 infection.

In general Nevirapine only prescribed after the immune system has declined and [4] 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 binds directly to reverse transcriptase enzyme (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase [5][6] activities by causing a disruption of the enzyme's catalytic site. The activity of Nevirapine does not compete with template or nucleoside triphosphates.

Chemically Nevirapine is a 2-cyclopropyl-7-methyl-2,4,9,15-tetraazatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3,5,7,12,14-hexaen-10-one, the molecular formula of Nevirapine is $C_{15}H_{14}N_4O$ and the molecular weight is 266.2979 g/mol[7]. The Chemical structure of Nevirapine is follows

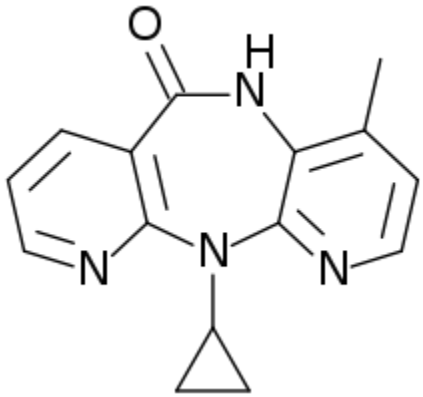

Fig-1: Chemical Structure of Nevirapine

MATERIALS AND METHODS

Table-1:- List of equipments used

Sl. No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	LABINDIA T-60 UV – Vis spectrophotometer
3.	Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Develosil ODS HG-5 RP C_{18}, 5µm, 15cm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit

Table-2:- List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	HPLC grade water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Potassium dihydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Ortho phosphoric acid	99.9	L.R.	Sd fine-Chem ltd; Mumbai

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm [8-10]. This has been performed to know the maxima of Nevirapine, so that the same wave number can be utilized in HPLC UV detector for estimating the Nevirapine. While scanning the Nevirapine solution we observed the maxima at 246nm. The UV spectrum has been recorded on LABINDIA make UV – Vis spectrophotometer model T-60. The scanned UV spectrum is attached in the following page

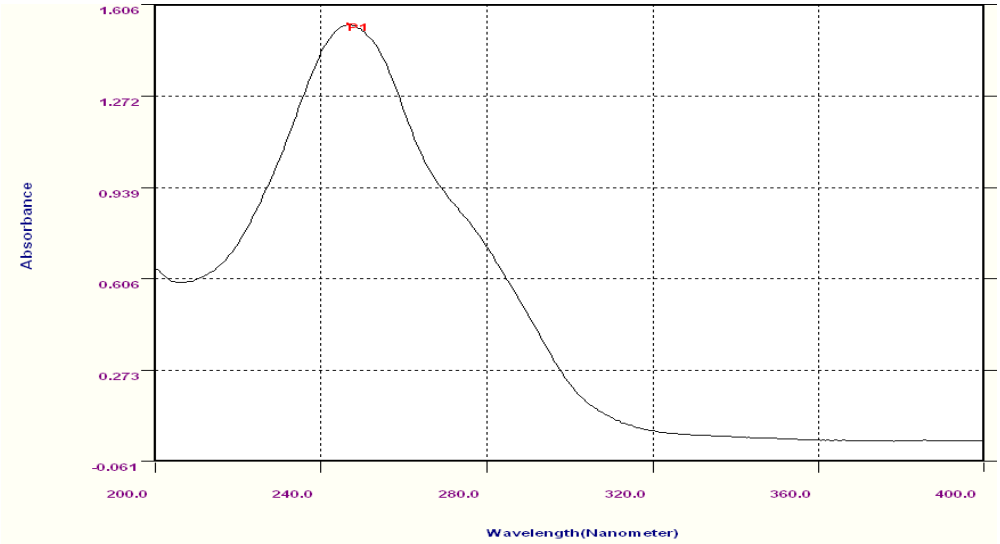

Fig-2:- UV-Spectrum for Nevirapine

Mobile phase preparation

The mobile phase used in this analysis consists of a mixture of acetate buffer (pH adjusted to 3.9 with orthophosphoric acid) and Acetonitrile in a ratio of 45:55.

450 ml of this buffer solution was added and properly mixed with 550 ml of Acetonitrile and a homogenous solution is achieved [10-14]. This mobile phase was filled and sonicated for 15 minutes before using in the experiment

Sample & standard preparation for the analysis

25 mg of Rilpivirine standard was transferred into 25 ml volumetric flask, make up to volume with mobile phase.

Further dilution was done by transferring 4 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

RESULTS AND DISCUSSION

Method development

Table-3: Trials for Method development and optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol : Phosphate Buffer = 20 : 80	1.0 ml/min	246 nm	Did nt get any Peaks	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol : Phosphate Buffer = 60 : 40	1.0 ml/min	246 nm	Pseudo peaks interfering main peak	Method rejected

Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol : Acetate Buffer = 50 : 50	1.0 ml/ min	246 nm	Low response & Broad Peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : Acetate Buffer (pH=2.2) = 40:60	1.0 ml/min	246 nm	Peak broadening	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : Acetate buffer (pH=3.9) = 55:45	1.0 ml/min	246 nm	Nice peak	Method accepted

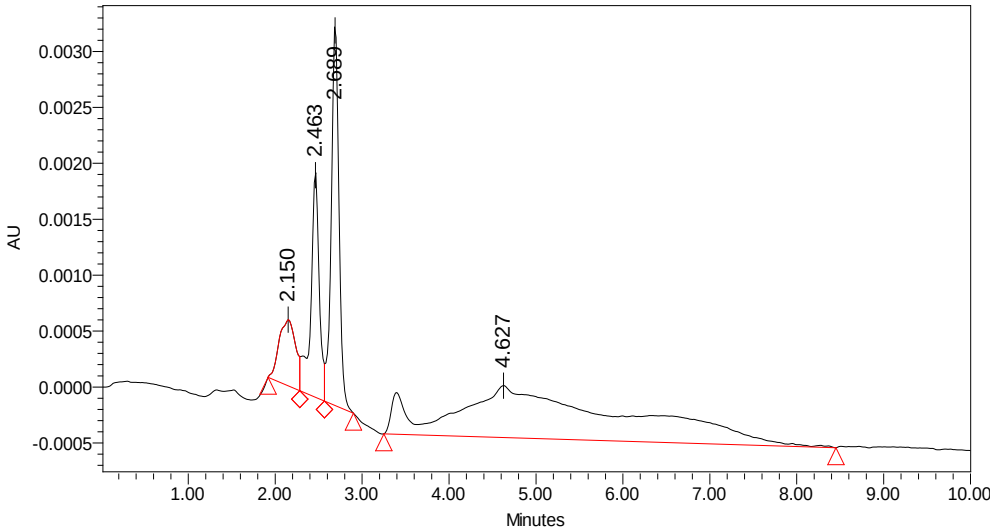

Fig-3: Chromatogram for Trial-1

Table-4: Results of Trial-1

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	2.689	10325	1.26	2985

Conclusion

Here tailing peak was observed, so method was rejected.

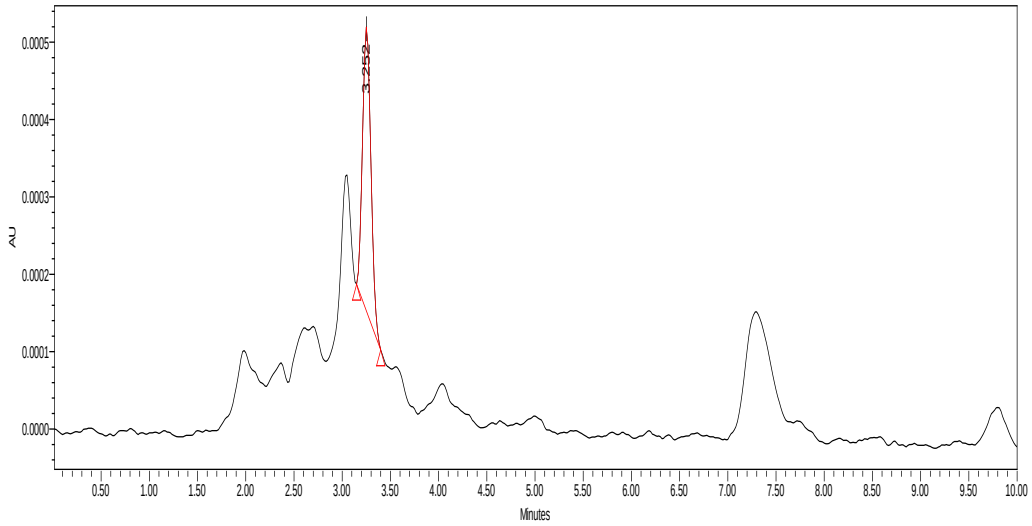

Fig-4:- Chromatogram for Trial-2

Table-5: Results of Trial-2

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	3.252	102131	1.35	3152

Conclusion

Here also tailing peaks was observed, so method was rejected.

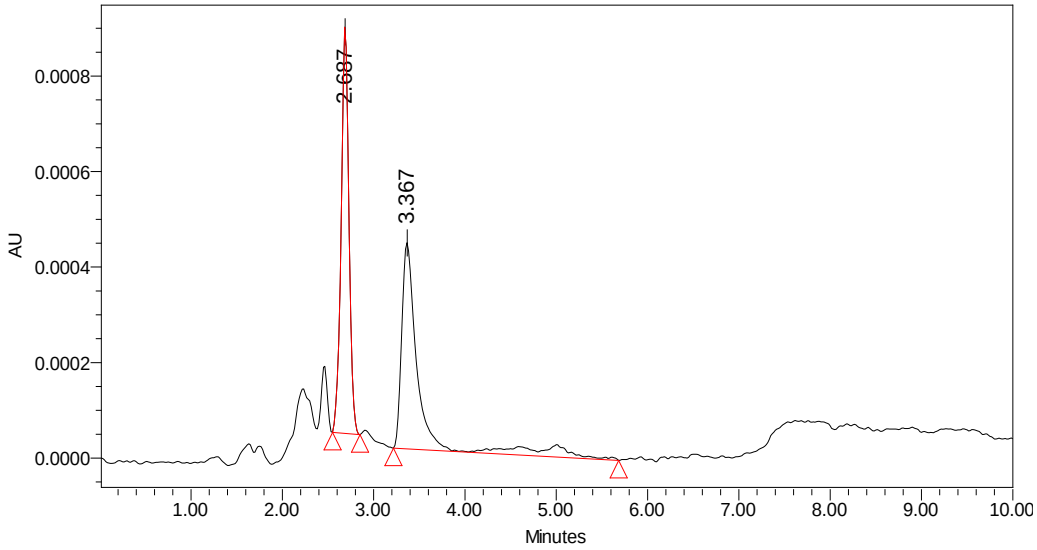

Fig-5: Chromatogram for Trial-3

Table-6: Results of Trial-3

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	2.687	10782	1.32	3214

Conclusion

Here broad peak and impurities peaks were observed, so method was rejected.

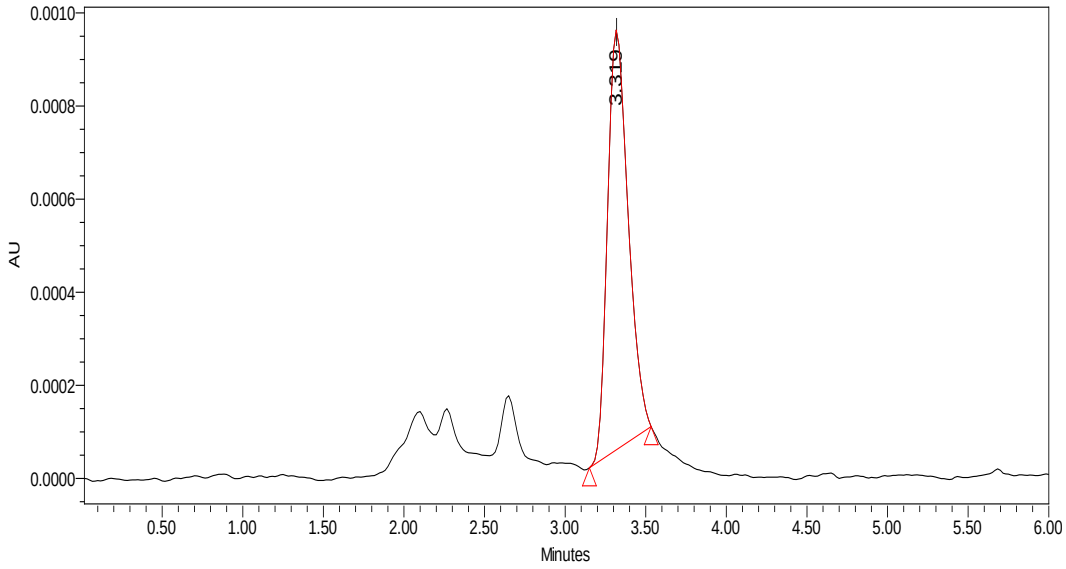

Fig-6: Chromatogram for Trial-4

Table-7: Results of Trial-4

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	3.319	102354	1.48	3524

Conclusion

Here peak was good but tailings were observed. So method was rejected.

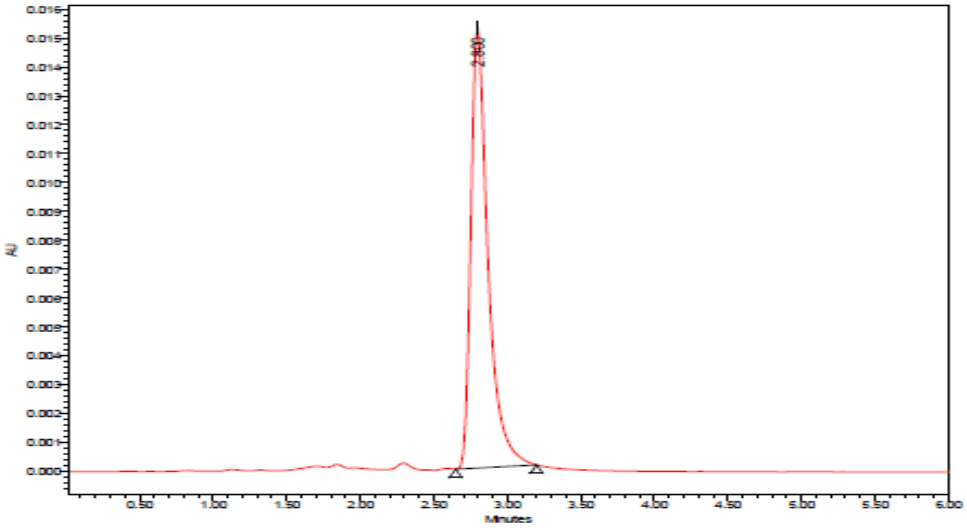

Fig-7: Chromatogram for Trial-5

Table-8: Results of Trial-5

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	2.800	1023541	1.15	3568

Conclusion

Here Sharp peak was observed. So method was accepted.

Optimized chromatographic conditions

Column : Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Mobile Phase : Acetate Buffer (pH=3.9): Acetonitrile (45:55)
Flow Rate : 1.0ml/minute
Wave length : 246 nm
Injection volume : 20 µl
Run time : 06 minutes
Column temperature : Ambient
Sampler cooler : Ambient

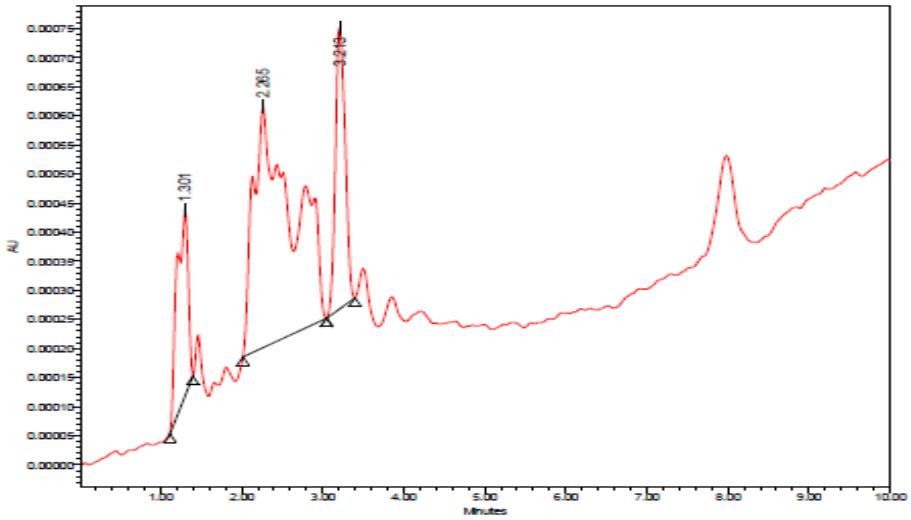

Fig-8: Chromatogram for Blank

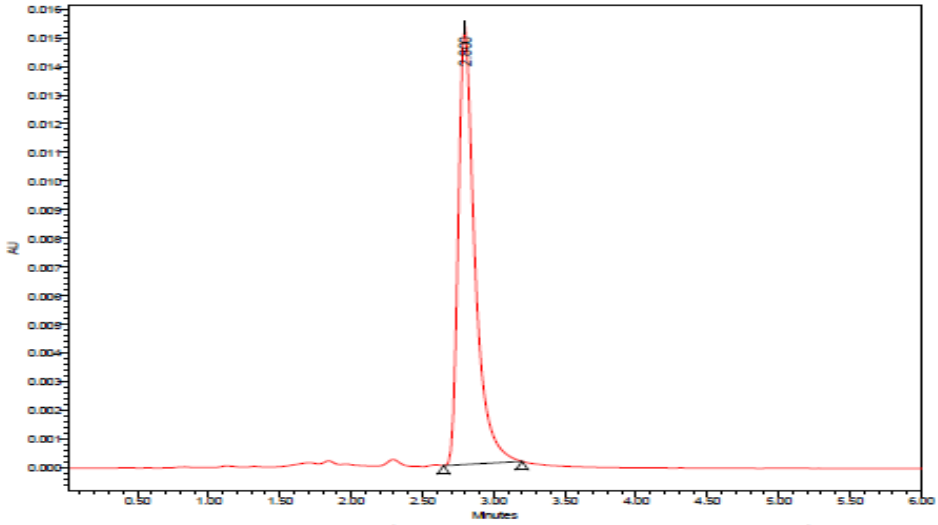

Fig-9: Optimized Chromatogram for Nevirapine (Rt. 2.800min)

Table-9: Results of Nevirapine in optimized condition

Name	Rt	Peak Area	Tailing Factor	Plate Count
Nevirapine	2.800	1023541	1.15	3568

FINAL RESULT & DISCUSSION

The selected and optimized mobile phase was Acetate Buffer (pH=3.9): Acetonitrile (45:55) and conditions optimized were: flow rate (1.0 ml/minute), wavelength (246nm), Run time was 06 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

STABILITY STUDIES

Acid hydrolysis

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs [14][15]. from that 0.5 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

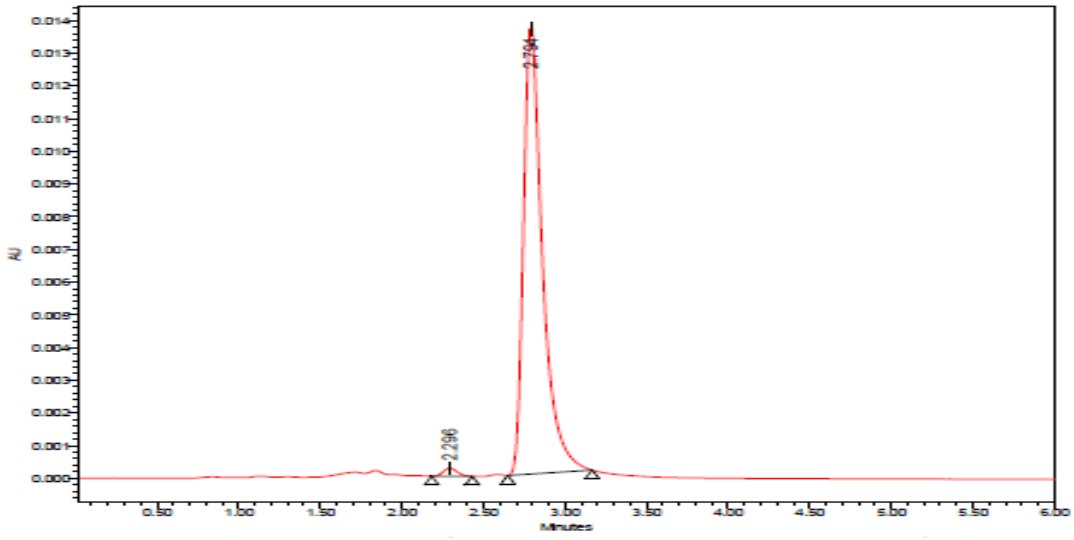

Fig-10: Chromatogram showing degradation for Nevirapine in 0.1 N HCl

Table-10: Results of acid hydrolysis of Nevirapine

Name	Rt	Peak Area	Theoretical Plates	Plate Count
Nevirapine	2.794	921106.99	1.29	3215

Basic hydrolysis

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.5N Sodium hydroxide [16][17] was added & make up to the mark & kept for 24 hrs. from that 0.5 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

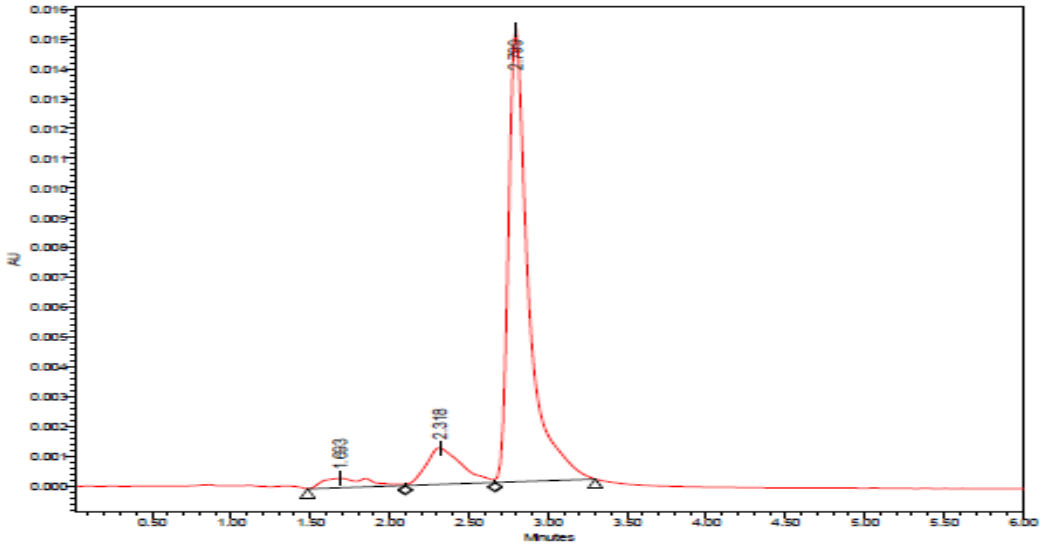

Fig-11: Chromatogram showing degradation related impurity in 0.1 N NaOH

Table-11: Results of basic hydrolysis of Nevirapine

Name	Rt	Peak Area	Theoretical Plates	Plate Count
Nevirapine	2.799	1023541	1.46	3168

Dry heat degradation

An accurately weighed 1 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 ^{0}C. for 24 hrs. Then injected into[18][19] the HPLC system against a blank of mobile phase (after all optimized conditions).

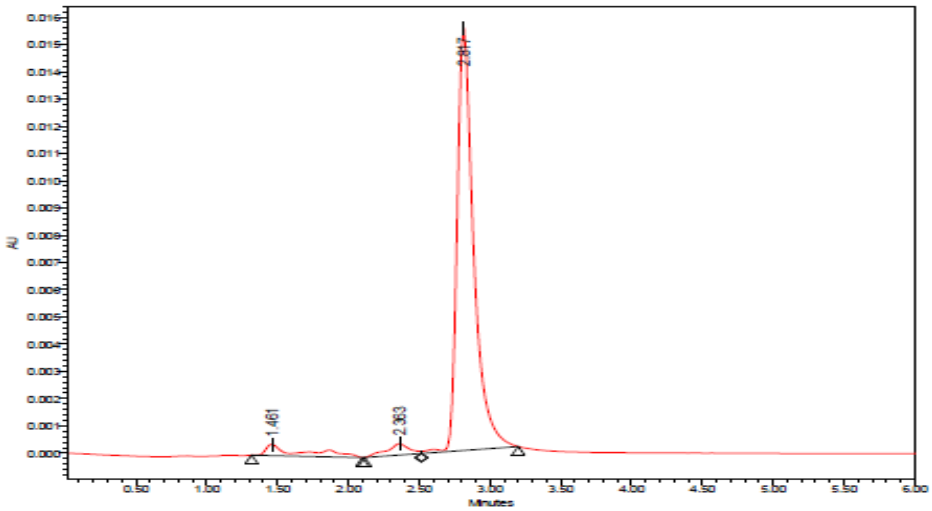

Fig-12: Chromatogram showing thermal degradation studies

Table-12: Results of thermal degradation of Nevirapine

Name	Rt	Peak Area	Theoretical Plates	Plate Count
Nevirapine	2.817	1177072.15	1.62	3652

Photolytic degradation

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 100 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark [20][21].Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

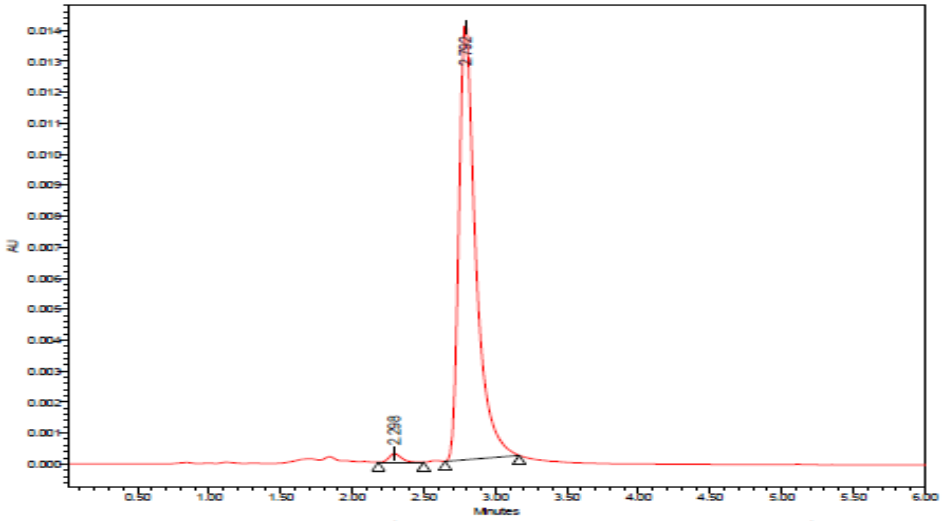

Fig-13:- Chromatogram is showing photolytic degradation.

Table-13: Results of Photolytic degradation of Nevirapine

Name	Rt	Peak Area	Theoretical Plates	Plate Count
Nevirapine	2.792	839303.62	1.37	3426

Oxidation with (3%) H_2O_2

Accurately weighed 1 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 50ppm solution [22-24]. The above sample was injected into the HPLC system.

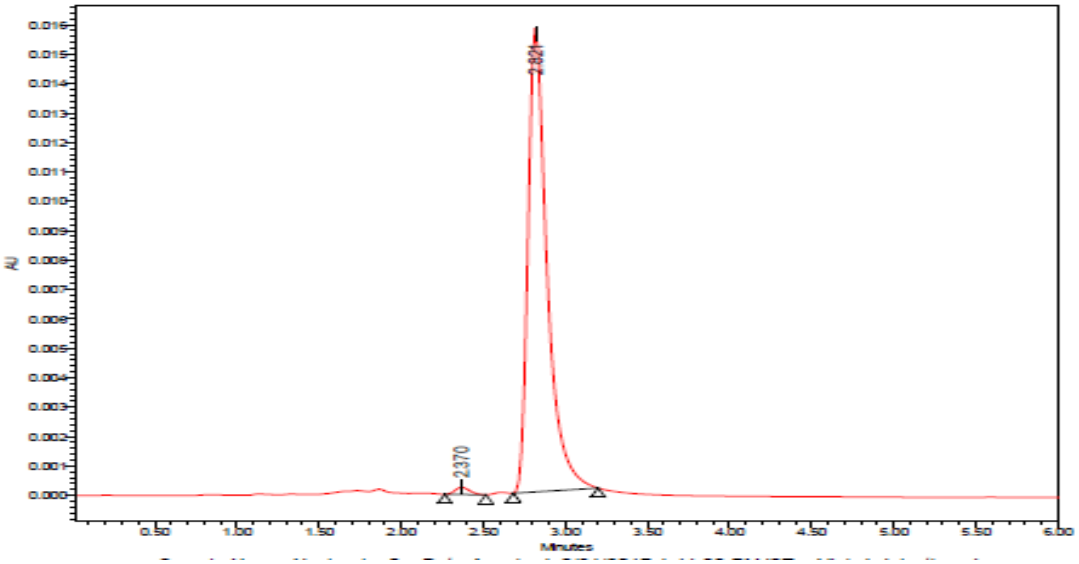

Fig-14:- Chromatogram shows oxidative degradation.

Table-14: Results of oxidative degradation of Nevirapine

Name	Rt	Peak Area	Theoretical Plates	Plate Count
Nevirapine	2.821	1084953.46	1.60	3314

Results of forced degradation studies

The results of the stress studies indicated the **specificity** of the method that has been developed. Nevirapine was stable in basic, photolytic stress conditions. The result of forced degradation studies are given in the following Table-15.

Table -15: Results of forced degradation studies of Nevirapine API

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	89.99	9.71	99.70
Basic Hydrolysis (0.I M NaOH)	24Hrs.	99.54	---------	99.54
Thermal Degradation (50 ^{0}C)	24Hrs.	98.03	0.61	98.64
UV (254nm)	24Hrs.	99.51	----------	99.51
3 % Hydrogen peroxide	24Hrs.	95.23	3.81	99.04

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Nevirapine, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Waters C18, 5µm, 25cm x 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, DMSO, ethanol, propylene glycol).

The drug was found to be highly soluble in Acetonitrile & DMSO and slightly soluble in methanol, ethanol and propylene glycol, dichloromethane. Drug was practically insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Nevirapine it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Nevirapine in different formulations.

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Nevirapine API. Further the developed RP-HPLC method has excellent sensitivity, accuracy, precision and reproducibility. The results show the proposed method is yet another suitable method for assay, purity which can help in the analysis of Nevirapine in different formulations. The method development and stability studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Nevirapine under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations.

Finally it was concluded that the developed method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the pharmaceutical dosage form.

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