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Method Development and Validation of Neverapine by Rp-Hplc

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A simple and accurate RP-HPLC method has been developed for the estimation of Neverapine in bulk and pharmaceutical dosage forms, using C-18 column 150 x 4.6 mm i.d, 5µm particle size in isocratic mode, with mobile phase comprising of phosphate buffer (pH3) and Acetonitrile in the ratio of 40:60 v/v. The flow rate was 0.8ml/min and the detection was monitored out by UV detector at 270nm. The retention time for Neverapine was found to be 3.308min.The proposed method has permitted the quantification of Neverapine over linearity in the range of 20-60 µg/ml and its percentage recovery was found to be 101.7%.The intraday and inter day precision were found 0.06% and 0.13%, respectively.

Keyword: Neverapine, HPLC, Isocratic, Validation.

INTRODUCTION: Neverapine, chemically 11-cyclopropyl-4-methyl-5, 11-dihydro-6*H*-dipyrido [3, 2-*b*: 2', 3'-*e*] [1, 4] diazepin-6-one (Fig.1) 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. transcriptase inhibitors such as stavudine and lamivudine[1, 2].

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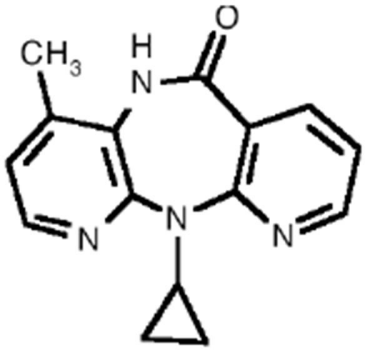
Neverapine is recommended for treating HIV infections in combination with other reverse
A literature survey revealed that the few analytical methods available for estimation of Neverapine from pharmaceutical formulations[3-8] and from human plasma[9-11]. The reported method for the estimation of Neverapine from pharmaceutical formulations includes HPLC[3-6], Spectrophotometry[7] and thin layer chromatography[8] method of analysis. The earlier reported methods were less sensitive and time consuming. Hence, the objective was to develop a new, simple, economical, selective, accurate and precise reverse phase high-performance liquid chromatographic method with good sensitivity for assay of Neverapine in tablet dosage form.

Fig.1 Structure of Neverapine



2.0 EXPERIMENTAL

2.1 Chemicals, Reagents and Apparatus

Neverapine (NAV) generous gift samples from Cipla Ltd. (Mumbai, India). A commercial NEVIMUNE (Cipla) tablets containing 200 mg of NAV were purchased from a local market and used within their shelf-life period. The HPLC grade acetonitrile, methanol and water were purchased from E.MERCK, India). All other chemicals used were of pharmaceutical or analytical grade from Qualigens Fine Chemicals Ltd., Mumbai, India). Waters Alliance HPLC system equipped with autosampler, binary Gradient pump and dual wavelength UV-visible detector were applied to perform the analysis. An analytical column, Symmetry C-18 (4.6 x 150mm, 5 µm, Make: XTerra) were used in the analysis. Chromatographic software Empower was used for data collection and processing.

2.2 Optimized chromatographic conditions

The chromatography elution was carried out in the isocratic mode using a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0, pH adjusted with ortho phosphoric acid) in a ratio of 60:40 v/v. The analysis performed at ambient temperature using a flow rate of 0.8 mL/min with a run time of 5 min. The eluent was monitored using DAD at a wavelength of 270 nm. The mobile phase was filtered through 0.45mm filters prior to use.

2.3 Preparation of mobile phase

Phosphate Buffer(Adjusted the pH to 3 with ortho phosphoric acid) and Acetonitrile in the ratio of 40:60 v/v were employed as a mobile phase and Buffer solution was prepared as directed by the procedure of Indian pharmacopoeia (1996).

2.4 Preparation of Standard Solution of Neverapine

Accurately weighed 10mg of Neverapine Working standard transferred into a 10 mL volumetric flask and about 7 mL of Diluent is added and sonicated to dissolve it completely and make the volume up to mark with the same solvent (Stock solution). Further 0.4 ml of the above stock solution is pipette into a 10ml volumetric flask and diluted up to the mark with diluent. Mix well and filtered through 0.45µm filter.

2.5 Preparation of Sample solution

Weigh five Neverapine Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Neverapine into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Inject 20 µL of the standard, sample into the chromatographic system and measure the area for the Neverapine peak and calculated the % assay.

2.6 Construction of linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.2 – 0.6 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 20-60 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times into the column. The calibration curve for Neverapine was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was

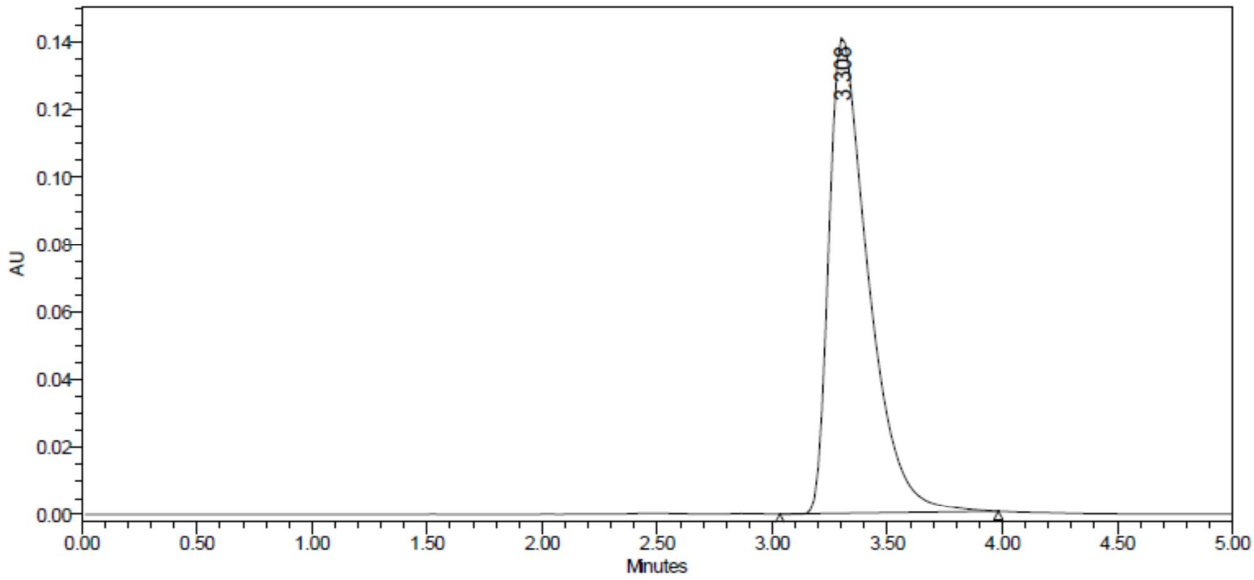
found to be linear in the concentration range 20-60 µg/ml with good correlation in between concentration and mean peak area.

3.0 RESULTS AND DISCUSSIONS

3.1 Method development

Phosphate Buffer pH 3 and Acetonitrile in the ratio of 40:60 v/v were employed as a mobile phase. The present RP – HPLC method for the quantification of Neverapine in bulk and pharmaceutical dosage forms, revealed as simple, accurate and precise method with significant shorter retention time of 3.308 min. The typical chromatogram of Neverapine was shown in Fig.2.

Fig.2 Typical chromatogram of Neverapine



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Neverapine	3.308	1675017	141771	1780.5	1.7

3.2 Method validation

3.2.1 Linearity The linearity for the detection of Nevirapine was 20-60µg/ml with (R^2=0.999). Results were shown in **table-1** and statistical data of calibration curves were shown in **table-2**. Correlation co-efficient – 0.999

Table-1 Linearity of Neverapine

	SampleName	Peak Name	RT	Area	Height (µV)
1	LINEARITY 20PPM	Neverapine	3.320	916934	76400
2	LINEARITY 30PPM	Neverapine	3.320	1294153	107530
3	LINEARITY 40PPM	Neverapine	3.322	1693798	142201
4	LINEARITY 50PPM	Neverapine	3.332	2150029	179505
5	LINEARITY 60PPM	Neverapine	3.323	2536197	214315

Fig.2 Calibration curve of Neverapine

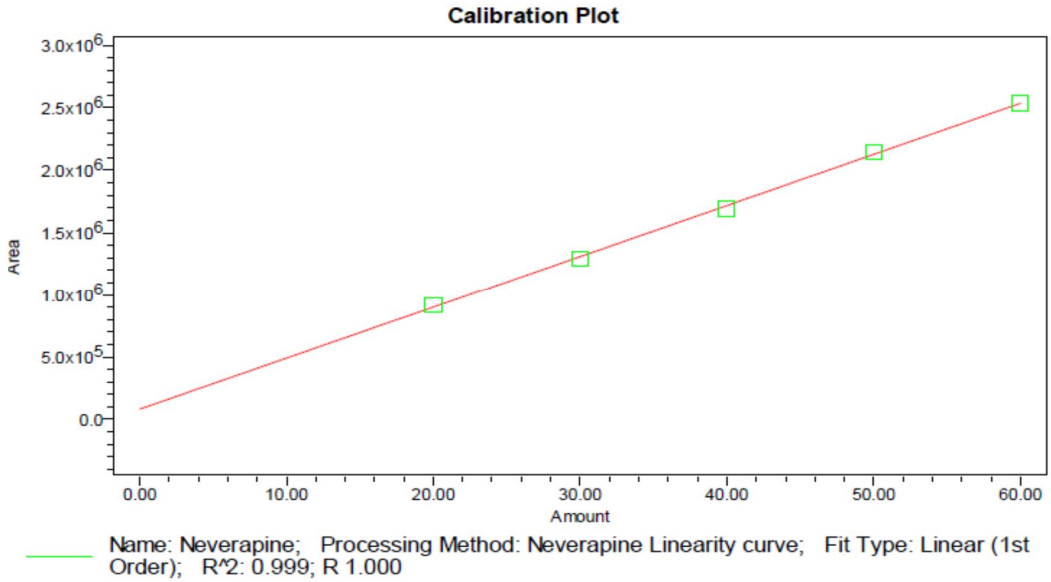


Table-2 Statistical data of calibration curve of Neverapine

Peak: Neverapine

	Conc. in µg/ml	Response
1	20	916934
2	30	1294153
3	40	1693798
4	50	2150029
5	60	2536197

3.2.2 Precision of the method

The intra day and inter day variations of the method were determined using five replicate injections of 40 µg/ml concentration and analysed on the same day and three different days over a period of two weeks. The result revealed the precision with %RSD (0.06% and 0.13%) respectively for intra day and inter day. Results were shown in Table–3 & 4.

3.2.3 Accuracy of the method To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with preanalysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting five times at three different concentrations equivalent to 50, 100, and 150% of the active ingredient, by adding a known amount of Nevirapine standard to a sample of known concentration and calculating the recovery of Nevirapine with RSD (%), and %recovery for each concentration. The mean % recoveries were in between 99.4-101.7% and were given in Table-5.

Table-3 Intra day Precision

Peak Name: Neverapine

	Peak Name	RT	Injection	Area
1	Neverapine	3.321	1	1695894
2	Neverapine	3.319	2	1696602
3	Neverapine	3.321	3	1696680
4	Neverapine	3.321	4	1698052
5	Neverapine	3.320	5	1695476
Mean				1696541
Std. Dev.				982.3
% RSD				0.06

Table-4 Inter day Precision

Peak Name: Neverapine

	Peak Name	RT	Injection	Area
1	Neverapine	3.322	1	1704111
2	Neverapine	3.322	2	1703528
3	Neverapine	3.322	3	1704391
4	Neverapine	3.322	4	1707805
5	Neverapine	3.321	5	1708012
Mean				1705569
Std. Dev.				2159.1
% RSD				0.13

Table-5 Accuracy Recovery

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	917495	5.28	5.37	101.7 %	100.0 %
100%	1697736	10.0	9.94	99.4%	
150%	2533074	15.0	14.8	98.8%	

3.2.4 Estimation of Neverapine in tablet formulation

The assay for the marketed tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 99.75% of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients.

Table-6 Amount of Neverapine in tablet dosage forms

Tablet Brand Name	Labeled Claim (mg)	Mean Amount ±SD	%Purity
NEVIMUNE	**200**	**200±1**	**99.75**

*Mean of five values

3.2.5 System suitability

To know reproducibility of the method ,system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values were shown in Table-7.

Table-7 System Suitability Parameters

Retention time	3.308
Theoretical Plates	2481
Tailing factor	1.3
Linearity Range (µg/ml)	20-60
Limit of Detection (LOD) (µg /ml)	3.04
Limit Of Quantitation (LOQ) (µg /ml)	10.08

3.2.6 Robustness Robustness of the proposed method was estimated by changing the organic composition in the mobile phase from 55% to 75%, where the actual mobile phase composition is buffer: acetonitrile 40:60v/v and, changing the flow rate from 0.6 ml to 1ml/min, and System suitability parameters were found to be within acceptable limits. Results were shown in Table-9 indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table-9 Robustness (with various flow rates)

FlowRate (ml/min)	System Suitability Results	
	USP Plate Count	SP Tailing
0.6	2367	1.2
0.8	2481	1.3
1.0	2432	1.3

Table-10 Robustness (change in organic composition in Mobile phase)

Sl. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2299	1.4
2	Actual	2481	1.3
3	10% more	332	1.3

3.2.7 Detection and quantification limits

Limits of Detection (LOD) and Quantification (LOQ), the limits of detection and quantitation were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

3.2.8 Specificity

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

4.0 CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of Neverapine from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures and low t_R. All these factors make this method suitable for quantification of Neverapine in tablet dosage forms. The method can be successfully used for routine analysis of Neverapine in bulk drugs and pharmaceutical dosage forms without interference.

REFERENCE:

1. Rey D. et al, Tolerance of a Short Course of Neverapine, Associated with 2 Nucleoside Analogues, in Postexposure Prophylaxis of HIV. J. Acquir. Immune. Defic. Syndr., 2004; 37: 1454-6.

2. USP-NF, The Official Compendia of Standards, published by The United States Pharmacopoeia Convention, City Press, Baltimore, US, 2009, Vol III, pp 3072-3.

3. Mohanraj P, Deb Kumar S, Choudhury T and Gauthaman K. A Simple and Rapid RP-HPLC Method for the Estimation of Neverapine in Bulk and Pharmaceutical Dosage Forms. E-J. Chem., 2008; 5 (S2): 1081-6.

4. Prasada Rao CH, Channabasavaraj KP, Lakshmi Aswini G. Development and validation of RP-HPLC method for the estimation of Neverapine in bulk and tablets. J. Pharm. Sci., 2009: 1(2): 78-82.

5. Ananth Kumar D, Naveen Babu MV, Seshagiri Rao JVNL and Jayathirtha Rao V. Simultaneous determination of Lamivudine, Zidovudine and Evirapine in Tablet Dosage Forms by RP-HPLC Method. Rasayana J. Chem., 2010; 3 (1): 94-9.

6. Vaishali PN and Kishor PB. A validated UV spectrophotometric method for the simultaneous estimation of Lamivudine, Neverapine and zidovudine in combined tablet dosage form. J. Pharm. Res., 2009; 2 (4): 666-9.

7. Venkatesh P and Rajesh Kumar R. Sepectrophotometric determination of Neverapine in pharmaceuticals after derivatization with 2,4-dinitro phenlyhydrazine. Asian J. Pharm. Health Sci., 2011; 1 (1): 33-4.

8. Purnima DH, Mitesh DP and Nitul S. Quantitative estimation of Neverapine by high performance thin layer chromatography. J. Pharm. Res. Health Care. 2009; 1 (2): 197-216.

9. Venkata Kumar Ch, Ananth Kumar D and Seshagiri Rao JVNL. A new validated RP-HPLC method for the determination of Neverapine in human plasma. E-J. Chem., 2010; 7 (3): 821-6.

10. Pav JW, Rowland LS and Korpalski DJ. HPLC-UV method for the quantitation of Neverapine in biological matrices following solid phase extraction. J. Pharm. Biomed. Anal., 1999; 20 (1-2): 91-8.

11. Heeswijk RPG, Hoetelmans RMW, Meenhorst PL, Mulder JW and Beijnen JH. Rapid determination of Neverapine in human plasma by ion-pair reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B, 1998; 713 (2): 395-9.