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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2008, 5(S2), 1081-1086

A Simple and Rapid RP-HPLC Method for the Estimation of Nevirapine in Bulk and Pharmaceutical Dosage Forms

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Received 9 March 2008; Accepted 22 April 2008

Abstract: A reverse phase HPLC method is described for the determination of nevirapine in tablet dosage forms. Chromatography was carried on an ODS column using a mixture of methanol and water (89:11 v/v) as the mobile phase at a flow rate of 1 mL/min with detection at 284 nm. The retention time of the drug was 3.22 min. The detector response was linear in the concentration of 25-200 mcg/mL. The limit of detection and limit of quantification was 0.004 and 0.0121 mcg/mL respectively. The percentage assay of nevirapine was 99.52%. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, economical, fast, accurate and precise and hence can be applied for routine quality control of nevirapine in bulk and tablet dosage forms.

Keywords: Nevirapine, RP-HPLC, Estimation, Tablet dosage forms.

Introduction

Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2',3'-*e*][1,4] diazepin 6-one¹ is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). Treatment with nevirapine mono-therapy is notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI binding site². However, in association with two other antiretroviral products, nucleoside reverse transcriptase inhibitors (NRTIs) and/or protease inhibitors (PIs), nevirapine significantly reduces the viral load and increases CD4 cell count, particularly in treatment-naïve patients³.

Nevirapine is metabolized by cytochrome P450 (CYP3A4) and is a relatively potent inductor of the enzyme; consequently, it has the ability to reduce plasma concentrations of other drugs that are also biotransformed by CYP3A4 as PIs⁴. Therapeutic drug monitoring of nevirapine may be warranted to prevent or delay the occurrence of viral resistance, and to ensure optimal therapy for HIV-infected patients⁵. Literature survey reveals that chromatographic methods for the determination of nevirapine in tablet dosage forms by simultaneous quantification with other drugs HPTLC⁶, several HPLC⁷⁻¹³ methods in combination with other antiretrovirals by plasma levels. Marzolini C¹⁴ *et al.* describe method in transplacental passage at delivery in pregnant woman, but due to ethical concern, pregnant women are generally excluded from clinical trials¹⁵ and LC/MS/MS¹⁶ method in human plasma with other antiretroviral drugs, Few individual HPLC methods developed for nevirapine like Hollanders R M F¹⁷ *et al.* developed a method in phosphate buffer and acetonitrile with flow rate 1.5 mL/min and retention time was 6.5 min, Joesph W P¹⁸ *et al.* developed method LC-8 analytical column with mobile phase phosphate buffer, 1-butane sulfonic acid as anion pair reagent, methanol and acetonitrile and peak area was detected with retention time 10 min at a flow rate of 1.0 mL/min. Lopaz R M¹⁹ *et al.* showed chromatogram with phosphate buffer and acetonitrile as mobile phase at a flow rate of 1 mL/min with retention time was 2 min. Our aim is to report an isocratic RP-HPLC method for the estimation of nevirapine with mobile phase methanol and water used as mobile phase in the ratio of (89:11), and flow rate 1 mL/min, retention time was 3.22 min. This paper reports simple, economical, accurate, reliable and reproducible analytical method for the estimation of nevirapine in formulation. This procedure is applied successfully for the analysis of the commercial tablets, purchased from market and the results are compared statistically.

Experimental

Nevirapine was obtained as a gift sample from Cipla Ltd, Methanol HPLC grade (S.d. fine-Chem Ltd.), Water HPLC grade (Universal Laboratories Pvt. Ltd.), were used.

Instrument

RP-HPLC was performed in Shimadzu (Japan) HPLC VP Series, Pump LC 20AT & UV Detector model SPD-20A with Rheodyne injector and 20 μ L loop. The column used was C18-ODS-Hypersil column (250 mm $\times$ 4.6 mm, 5 μ). Spinchrome (Mumbai, India) computer based data station was used.

Chromatographic conditions

Chromatographic separations were achieved using a Luna 5u C18 Hypersil column (250 $\times$ 4.6 mm, 5 μ). The mobile phase consisting of methanol and water (89:11 v/v) was passed through 0.45 μ m membrane filter and degassed by ultrasonication. The flow rate was maintained at 1 mL/min and the measurements were made at 284 nm. The column and the RP-HPLC system were kept in ambient temperature.

Preparation of standard stock solution

Accurately weighed 100 mg of nevirapine standard was taken in 100 mL volumetric flask. This was dissolved in 70 mL of methanol and sonicated for 30 min and then diluted to 30 mL with the mobile phase to get 1 mg/mL standard stock solution.

Preparation of sample solution

Each twenty tablets (Nevimune, Nevivir, Cipla Ltd.) were weighed accurately and finely powdered. The powder equivalent to 100 mg was taken in 100 mL volumetric flask.

This was dissolved in 70 mL methanol and sonicated for 30 min with internal shaking. Then the volume was finally made to 100 mL with mobile phase to get a clear solution of 1 mg/mL. The above solution was centrifuged at 3000 rpm for 5 min.

Linearity

Several aliquots of standard stock solutions 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mL of nevirapine were taken in different 10 mL volumetric flask and diluted up to the mark with methanol. Evaluation was performed with Ultra-Violet Dual alpha absorbance detector at 284 nm. Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area *versus* concentration of nevirapine (Figure 1). The plot of peak area of each sample against respective concentration of nevirapine was found to be linear in the range of 25.0-200.0 µg/mL, using regression analysis the linear equation $Y = 15.71x - 2.116$ with correlation coefficient of $r^2 = 0.9999$. Linear regression least square fit data obtained from the measurements are given in Table 1.

Figure 1. Calibration curve of nevirapine by RP- HPLC

Table 1. Calibration curve points of the proposed method

Concentration of nevirapine µg/mL	Peak area* mV.s
25	396.208
50	770.874
75	1179.528
100	1574.426
125	1969.221
150	2351.261
175	2745.28
200	3142.885

*Mean of five determinations.

Results and Discussion

The system suitability tests were carried out on freshly prepared standard stock solution of nevirapine. Parameters that were studied to evaluate the suitability of the system are given in Table 2.

Table 2. System suitability test parameters

Parameters	Values
HETP, mm	0.009
Tailing factor	1.113
Theoretical plates/m	110099.73
Resolution	2.38
LOD, µg/mL	0.004
LOQ, µg/mL	0.0121

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for nevirapine were found to be 0.004 and 0.0121 µg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of nevirapine as shown in Figure 2, it was found that the retention time was 3.22 min. A mixture of methanol and water ratio of 89:11 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship ($r^2=0.9999$) was observed between the concentration range of 25.0-200.0 mcg/mL. The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as CV (%) of a series of measurement Table 3. The result obtained shows CV (%) less than 1% indicating good intra-day precision.

Figure 2. A typical chromatogram of nevirapine by RP- HPLC**Table 3.** Intra and inter - day precision for nevirapine assay

Conc. of nevirapine µg/mL	Concentration of nevirapine found			
	Intra-day		Inter-day	
	Mean, n= 5	CV, %	Mean, n=5	CV, %
50	50.05	0.61	50.51	0.99
100	99.63	1.21	99.47	0.28
200	200.14	0.93	200.16	1.03

The assay of nevirapine tablets was found to be 99.52%. The results are given in Table 4. From the recovery studies it was found that about 99.56% of nevirapine was recovered which indicates high accuracy of the method. The results are given in Table 5. The absence of additional peaks in the chromatogram indicates non interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of nevirapine within a short analysis time.

Table 4. Results of assay of formulation by the proposed method

Sample	Amount claim mg/tab	Amount found mg/tab	% Recovery
1	200	199.44	99.72
2	200	199.79	99.33

**Mean of five determinations.*

Table 5. The recovery studies for nevirapine by proposed method

Labeled Claim (200 mg)	Amount of drug added, mg	Mean amount found, mg	Mean % Recovery*
1	10	10.24	100.39
2	20	19.07	98.96
3	30	29.88	99.33

**Mean of five determinations.*

Assay

20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3.22 min. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 4.

Recovery studies

Accuracy was determined by recovery studies of nevirapine, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 5. The study was done at three different concentration levels.

Conclusion

Developed high performance liquid chromatographic method is simple, reliable, reproducible and economical for the analysis of nevirapine in pharmaceutical formulations. The reported method can be used successfully for the effective qualitative and quantitative analysis of nevirapine in Tablet or other pharmaceutical formulations.

Acknowledgement

The authors are very grateful to, Dr. H. P.Chhetri, The Director, Himalayan Pharmacy Institute, E. Sikkim for providing facility to perform this work.

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