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Research

DESIGN CHROMATOGRAPHIC METHOD FOR NEVIRAPINE ASSAY BY HPLC METHOD USING ISOCRATIC ELUTION

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	Abstract
Published on: 17 Nov 2024	<p>Design chromatographic method for Nevirapine assay by HPLC method using isocratic elution. Scope of the study is to designed Isocratic chromatographic method for Nevirapine assay by HPLC method and validate assay parameter by linearity, Precision, accuracy and solution stability parameter by HPLC method The aim of present work is to develop simple, economic, precise and cost-effective HPLC method for determination of Nevirapine, an anti-retro viral drug, in bulk and pharmaceutical dosage form Nevirapine has official monograph in IP (Indian Pharmacopoeia 2018), which describes liquid chromatographic method for the assay of Nevirapine.</p>
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 Creative Commons Attribution 4.0 International License.	Keywords: Nevirapine, HPLC, Isocratic elution

INTRODUCTION

Drug Profile: Nevirapine is structurally a member of the dipyrido diazepinone chemical class of compounds (11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2- b:20,30e][1,4diazepine-6-one]) is an anti-retroviral drug which is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against human immunodeficiency virus type-1 (HIV-1). It's Official in Indian Pharmacopoeia and United Pharmacopoeia [5] and British Pharmacopoeia Anti-HIV drugs such Nevirapine is a white to off-white crystalline powder with the molecular weight of 266.30 and the molecular formula C₁₅H₁₄N₄O. VIRAMUNE is the brand name for Nevirapine (NVP) Nevirapine is structurally a member of the dipyrido diazepinone chemical class of compounds.

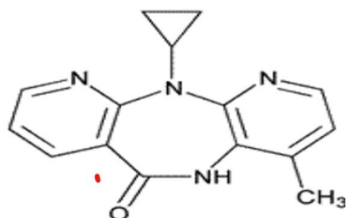


Fig: 1 structure of Nevirapine

Nevirapine 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. [Pub Chem] Structurally, Nevirapine belongs to a member of the dipyrido diazepinone chemical class of compounds (11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2- b:20,30e][1,4diazepine-6-one]) is an anti-retroviral drug .It's Official in Indian Pharmacopoeia [4].Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of nevirapine does not compete with template or nucleoside triphosphates. Anti-HIV drugs such as nevirapine slow down damage to the immune system and prevent the occurrence of AIDS-defining illnesses. Nevirapine is, in general, only prescribed after the immune system has declined and 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.

MATERIALS AND METHODS

Equipment and Instrument: HPLC Model: Shimadzu,

pH meter Model: Mettler Instrument number : QC/pH/0158

Analytical Balance: Model: Mettler Instrument number: QC/BAL/029

Standards: Reference standards of Nevirapine which has purity 99.99 %

Solvent and Chemicals Used during analysis: Acetonitrile, HPLC grade, Water, HPLC grade, Dehydrate alcohol

DESIGN ANALYTICAL METHOD VALIDATION

Specificity and System Suitability, Linearity and Range Precision, System Solution, Stability System, Stability Solution, Robustness

Chemical and Equipment: Acetonitrile HPLC grade, Water HPLC grade, Dehydrate alcohol

Method Parameter for the Validation: The main objective of method validation process is to prove that an analytical method is acceptable for its intended purpose. The necessity for laboratories to use fully validated methods is now universally accepted as a way to obtain reliable results. There are diverse documents for method validation including information about different performance parameters. The classical performance characteristics are accuracy limit of detection, precision, recovery, robustness, ruggedness, selectivity, specificity and trueness. Unfortunately, contradictory information is normally present among the method validation documents used by laboratories.

Specificity and System Suitability: This test is performed for the identification of analyte and placebo interference and peak purity of Analyte For reagent, diluent preparation, buffer preparation, chromatographic condition, resolution solution, standard solution and sample proceed as per Methodology defined

Preparation of the placebo solution: Weight an accurately portion of the placebo powder equivalent to 200mg of Nevirapine into a 200 ml of volumetric flask, add about 150 ml diluent.

Sonicate the solution for about 20 minute and then shake for about 20 minute Allow the solution cool to room temperature, dilute to volume with diluents and mix. Centrifuge a portion of the resulting solution at about 1500 rpm for about 5 minutes Dilute 5 ml of the supernatant into 200 ml volumetric flask and dilute to volume with diluents and mix.

Preparation of test solution: Weight powder not less than 20 tablets. Transfer an accurately weighed portion of the tablets powder equivalent to 200mg of Nevirapine into a 200 ml of volumetric flask, add about 150 ml diluents .Sonicate the solution for about 20 minute and then shake for about 20 minute allow the solution cool to room temperature , dilute to volume with diluent and mix. Centrifuge a portion of the resulting solution at about 1500 rpm for about 5 minutes, Dilute 5 ml of the supernatant into 200 ml volumetric flask and dilute to volume with diluent and mix. Inject 20 µl of sample solution into the chromatographic system

Instrumental Procedure: Inject 20 µl of resolution solution into the chromatographic system

Inject 20 µl of diluent as a blank into the chromatographic system, Inject 20 µl of placebo solution into the chromatographic system, Inject 20 µl of five times standard into the chromatographic system, Inject 20 µl of sample solution into the chromatographic system.

Acceptance Criteria

Identification: The retention time of standard solution and sample solution should be comparable with respect to retention time,

Placebo and Blank Interference: There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard.

System Suitability: The resolution between Nevirapine related compound A is NLT 3.0. The relative standard deviation for Nevirapine peak areas obtained from five replica injection of standard solution is not more than 2.0%

Identification

Results of identification	
Solution	Retention T
Standard solution- Nevirapine	4.819 min
Test solution- Nevirapine	4.819 min
Conclusion: RT of Nevirapine obtained with standard and test sample are comparable. Hence method is specific	
Acceptance Criteria: The retention time of standard solution and sample solution should be comparable with respect to retention time	

Placebo Interference

Results of Placebo Interference

There is no interference peak observed due to placebo at the retention time of Nevirapine peak. Hence method is specific

Acceptance Criteria: There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard

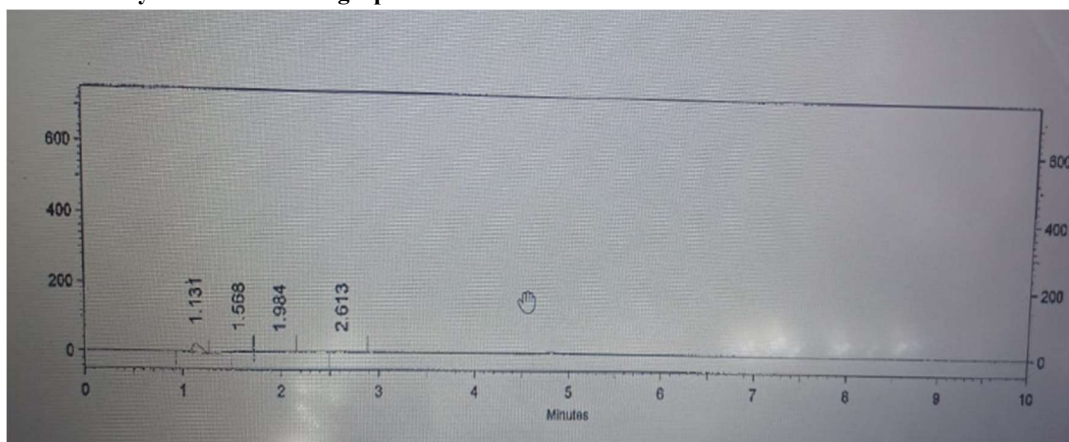
System Suitability- Nevirapine

Results of System Suitability	
Injection	Peak Area
1	2778665
2	2790412
3	2801567
4	2779371
5	2788130
Mean	2787629
% RSD	0.336

The resolution between Nevirapine related compound A is 11.3 Hence method is specific

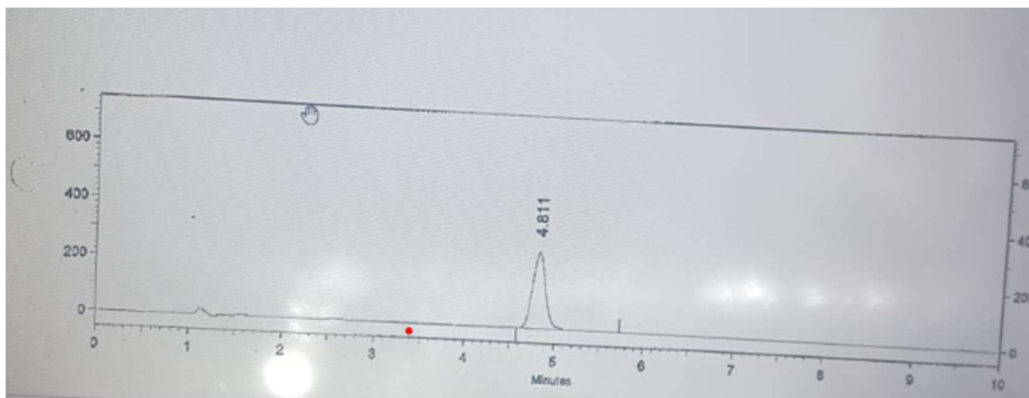
Acceptance Criteria: Resolution: The resolution between Nevirapine related compound A is NLT 3.0.

System Suitability Blank Chromatographs



System Suitability Placebo Chromatographs

System Suitability Standard System Suitability Chromatographs



System Suitability Sample System Suitability

Linearity:

To demonstrate the linearity of analyst response from 50 % to 150 % of target concentration. Evaluate the range of method using the data from linearity, precision and accuracy studies. For reagent, diluent preparation, buffer preparation, chromatographic condition, resolution solution, standard solution and sample proceed as per Methodology defined. Accurately weight and transfer about 25 mg of Nevirapine anhydrous standard into a 250 ml volumetric flask, dissolved to volume diluent.

Linearity level	Take ___ ml stock solution	Dilute up to ___ ml diluent	Concentration in ppm
L1 (50%)	2.5	20	12.50
L2 (80%)	4.0	20	20.00
L3 (100%)	5.0	20	25.00
L4 (120%)	6.0	20	30.00
L5 (150%)	7.5	20	37.50

Inject 20 μ l of diluent as a blank into the chromatographic system, Inject 20 μ l of 50 % and 150 % concentration in 6 times and other levels in duplicate.

Instrumental Procedure: Into the chromatographic system Plot a graph of concentration against the peak Responses and calculate the linearity regression coefficient, % Y – intercept and % RSD peak area response for 50 % level and 150 % level

Acceptance criteria: The co-relation is not less than 0.999, The % Y intercept is between -2 % to +2 %, % RSD of peak Responses of 50 % level and 150% level should be NMT 2.0.

Linearity level	Concentration in ppm	Area-Average	% of RSD	Statistic Analysis	
L1 (50%)	12.50	1342892	0.04	Y Intercept	2255.7
L2 (80%)	20.00	2113529	--		
L3 (100%)	25.00	2642630	--	% Y Intercept	0.86
L4 (120%)	30.00	3159909	--	Correlation coefficient	0.99999
L5 (150%)	37.50	3961036	0.22	R ²	0.9998

Conclusion: Response of Nevirapine is linear over the concentration range 50% to 150% target concentration

A. Precision:**Results of System Precision**

Results of System Precision	
Injection	Peak Area
1	2669770
2	2671632
3	2672925
4	2673531
5	2671711
Mean	2671914
% RSD	0.05

The resolution between Nevirapine related compound A is 10.9 Hence method is specific

Acceptance Criteria: Resolution: The resolution between Nevirapine related compound A is NLT 3.0.

Method Precision:

Weight powder not less than 20 tablets. Transfer an accurately weighed portion of the tablets powder equivalent to 200mg of Nevirapine into a 200 ml of volumetric flask, add about 150 ml diluent. Sonicate the solution for about 20 minute and then shake for about 20 minute Allow the solution cool to room temperature, dilute to volume with diluent and mix. Centrifuge a portion of the resulting solution at about 1500 rpm for about 5 minutes Dilute 5 ml of the supernatant into 200 ml volumetric flask and dilute to volume with diluent and mix.

Instrumental Procedure: Inject 20 µl of sample solution into the chromatographic system Calculate the % of dissolution and determine the % RSD for dissolution solution from the data of six preparations.

Calculation: Nevirapine (% labeled amount)

$$\frac{A1 \times Ws \times 5 \times 200 \times 200 \times P \times T \times 100}{A2 \times 250 \times 20 \times wt \times 5 \times 100 \times L}$$

A1= Area of Nevirapine peak in sample solution, A2= Average area of Nevirapine peak in standard solution, A3= Weight of Nevirapine working standard taken in mg, Wt= Weight of sample taken in mg, P= Purity of standard, T = Average weight of tablet in mg, L = Label claim of Nevirapine.

Acceptance criteria: The % RSD and % Assay from six sampled should be NMT 2.0

Method Precision:

Results of Method Precision			
Sample	% Assay	Statistical Analysis	
1	100.2	Mean	100.0
2	99.9		
3	99.8	SD	0.16
4	100.1		
5	99.8	% RSD	0.16
6	99.9		

Test results are showing that the test method is precise.

B. Accuracy:

The recovery of Nevirapine in table is asses by adding a series of known amount of drug substance to placebo from 50 % to 150 % of test concentration in separate volumetric flask add about 150 ml diluent. Sonicate the solution for about 20 minute and then shake for about 20 minutes, Allow the solution cool to room temperature , dilute to volume with diluent and mix. Centrifuge a portion of the resulting solution at about 1500 rpm for about 5 minutes, Dilute 5 ml of the supernatant into 200 ml volumetric flask and dilute to volume with diluent and mix.

Preparation of the accuracy solution

Accuracy level	Weight of drug added in mg	Placebo of added in mg	Dilute upto ___ with diluent solution	Further ___ dilute ___ ml	Dilute up to ___ ml with diluent solution
L1 (50 %)	100 mg	160 mg	200 ml	5.0 ml	200 ml
	100 mg	160 mg	200 ml	5.0 ml	200 ml
	100 mg	160 mg	200 ml	5.0 ml	200 ml
L2 (100 %)	200 mg	160 mg	200 ml	5.0 ml	200 ml
	200 mg	160 mg	200 ml	5.0 ml	200 ml
	200 mg	160 mg	200 ml	5.0 ml	200 ml
L3 (150 %)	300 mg	160 mg	200 ml	5.0 ml	200 ml
	L1 (50 %)	160 mg	200 ml	5.0 ml	200 ml
	300 mg	160 mg	200 ml	5.0 ml	200 ml

Instrumental Procedure: Inject 20 µl of blank, accuracy level samples into the chromatographic system and check the % recovery.

Calculation: Nevirapine (% labeled amount)

$$\frac{\text{Sample area} \times \text{standard dilution} \times \text{purity} \times \text{weight of drug added (mg)}}{\text{Mean area of standard} \times \text{sample dilution} \times 100}$$

% Recovery = $\frac{\text{Amount found in mg} \times 100}{\text{Amount added (mg)}}$

Acceptance criteria

System suitability: The resolution between Nevirapine and Nevirapine related compound A is NLT 3.0. The relative standard deviation for Nevirapine peak area obtained from five replica injection of standards solution is not more than 2.0 %

% Recovery: The % recovery of accuracy levels should be not less than 98.0 and not more than 102.0

Results of System Precision	
Injection	Peak Area
1	2669770
2	2671632
3	2672925
4	2673531
5	2671711
Mean	2671914
% RSD	0.05

The resolution between Nevirapine related compound A is 10.9 Hence method is specific

Acceptance Criteria: Resolution : The resolution between Nevirapine related compound A is NLT 3.0.

Result of Linearity					
Linearity level	Amount added (mg)	Amount found (mg)	% Recovery	Statistic Analysis	
L1 (50%) Sample-1	100.05	101.07	101.0	Mean	100.8
L1 (50%) Sample-2	100.03	100.80	100.8	SD	0.25
L1 (50%) Sample-3	100.09	100.64	100.8	% RSD	0.25
L3 (100%) Sample-1	200.01	197.80	98.9	Mean	98.8
L3 (100%) Sample-2	200.05	197.82	98.9	SD	.12
L3 (100%) Sample-3	200.07	197.56	98.7	% RSD	.12
L5 (150%) Sample-1	300.11	297.81	99.2	Mean	99.2
L5 (150%) Sample-2	300.16	297.83	99.2	SD	0.00
L5 (150%) Sample-3	300.17	297.81	99.2	% RSD	0.00
Over all Statistical Analysis					
Mean	99.6	SD	0.90	%RSD	0.90

The recovery results indicating that the test methods has an acceptable level of accuracy

Solution Stability:

Preparation of sample solution: The recovery of Nevirapine in table is asses by adding a series of known amount of drug substance to placebo from 50 % to 150 % of test concentration in separate volumetric flask add about 150 ml diluent. Sonicate the solution for about 20 minute and then shake for about 20 minute. Allow the solution cool to room temperature, dilute to volume with diluent and mix. Centrifuge a portion of the resulting solution at about 1500 rpm for about 5 minutes. Dilute 5 ml of the supernatant into 200 ml volumetric flask and dilute to volume with diluent and mix

Instrumental Procedure: Inject 20 µl of resolution into the chromatographic system. Inject 20 µl of standard solution 5 replicate and sample solution at regular interval for initial, 6,12,24 hours along with freshly prepared standard, into chromatographic system. Calculate the % assay of standard and sample, Monitored pattern of chromatogram of standard and sample for chromatographic system.

Calculation: Nevirapine (% labeled amount) $A_1 =$ Area of Nevirapine peak in sample solution, $A_2 =$ Average area of Nevirapine peak in standard solution, $A_3 =$ Weight of Nevirapine working standard taken in mg, $W_t =$ Weight of sample taken in mg, $P =$ Purity of standard, $T =$ Average weight of tablet in mg, $L =$ Label claim of Nevirapine.

Acceptance criteria: The % RSD and % Assay from six sampled should be NMT 2.0

Calculation: Nevirapine Stanadr % Assay

$$A_1 \times W_s \times X_5 \times 250 \times 20 \times 100$$

$$A_2 \times 250 \times 20 \times w_t \times X_5$$

$A_1 =$ Area of Nevirapine peak in sample solution, $A_2 =$ Average area of Nevirapine peak in standard solution, $A_3 =$ Weight of Nevirapine working standard taken in mg, $W_t =$ Weight of Standard taken in mg, $W_s =$ Weight of Standard taken in mg

Acceptance criteria: System suitability: The resolution between Nevirapine and Nevirapine related compound A is NLT 3.0, The relative standard deviation for Nevirapine peak area obtained from five replica injection of standards solution is not more than 2.0 %

Solution Stability: There should be no extraneous peak or degradants are observed in chromatogram of sample and standard for chromatographic method. For standard the % difference of Assay of initial standard to standard at regular interval should be NMT 2.0, The % difference of assay of the sample solution at initial to that at regular interval should be more than 2.0

Results: Standard Solution: The % difference for the peak area response from 5 replica injection standard solution .From precision study and one from solution stability should be not more than 2.0

Time in Hours	% Assay	% Difference
Initial	99.9	-
Standard about 6 hours	99.8	0.1
Standard about 12 hours	99.8	0.1
Standard about 24 hours	99.2	0.7

Sample Solution:

The % difference of % assay of sample solution from initial to the % assay at regular intervals should be not more than 2.0

Time in Hours	% Assay	% Difference
Initial	99.9	-
Standard about 6 hours	99.8	0.1
Standard about 12 hours	99.8	0.1
Standard about 24 hours	99.3	0.6

Conclusion: Standard and sample are stable for 24 hours

CONCLUSION:

This study, isocratic chromatography by HPLC method was developed for Nevirapine and the developed method was validated for parameters like linearity, precision, accuracy, ruggedness and robustness. This method is advantageous over the reported method in terms of cost effectiveness and do not involve any tedious procedures.

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