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Original Research Article

Development and Validation of Nevirapine- An Anti-Retro Viral Drug by UV-Visible Spectrophotometric Method and Its Degradtion Study under Various Stress Conditions

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Abstract

The aim of present work is to develop simple, economic, precise and cost-effective UV spectrophotometric method for determination of Nevirapine, an anti-retro viral drug, in bulk and pharmaceutical dosage form. Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. Forced degradation studies show the chemical behaviour of the molecule which in turn helps in the development of formulation and package. The method for Nevirapine was developed using methanol and water and absorbance maxima was found to be at 282nm with a correlation coefficient of 0.9978. Forced degradation studies of Nevirapine like Acid degradation, Base degradation, Thermal, Photolytic, and Peroxide was conducted in UV Spectrophotometer using methanol and water and percentage degradation was calculated.

Keywords: Nevirapine, Forced degradation, UV spectrophotometer, Photolytic.

INTRODUCTION [1-3]

Nevirapine is structurally a member of the dipyridodiazepinone 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). Its Official in Indian Pharmacopoeia [4] and United Pharmacopoeia [5] and British Pharmacopoeia [6]. Anti-HIV drugs such as nevirapine slow down damage to the immune system and prevent the occurrence of AIDS-defining illnesses. The enzyme reverse transcriptase converts single-stranded viral RNA into DNA. Drugs in the NNRTI class stop HIV from replicating within cells by binding near reverse transcriptase's active site and inhibiting polymerase activity. Nevirapine diffuses into the cell and binds to reverse transcriptase adjacent to the catalytic site. This induces conformational changes that inactivate the enzyme.

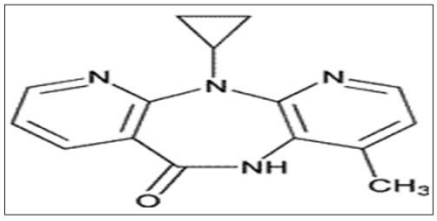

Fig-1: Structure of Nevirapine

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 dipyridodiazepinone chemical class of compounds. Viramune Tablets are for oral administration. Each tablet contains 200 mg of nevirapine and the inactive ingredients microcrystalline cellulose, lactose monohydrate, povidone, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate. Viramune Oral Suspension is for oral administration. Each 5 mL of viramune suspension contains 50 mg of nevirapine (as nevirapine hemihydrate). The suspension also contains the following excipients: carbomer 934P, methylparaben, propylparaben, sorbitol, sucrose, polysorbate 80, sodium hydroxide and purified water.

Nevirapine has official monograph in IP (Indian Pharmacopoeia 2018), which describes liquid chromatographic method for the assay of Nevirapine. Literature survey reveals that few analytical methods

have been published for the analysis of Nevirapine in bulk drug and formulation using HPLC Ch. Venkata Reddiah *et al*., Phani R. S. CH *et al*., Minaketan Sahoo *et al*., S. Vijayaraj *et al*., P. Ravisankar *et al*.,[9-15], and simultaneous estimation using UV spectrophotometry Dr. N. N Rajendran *et al*., [16]

Only one UV spectrophotometric method was reported for Nevirapine when present alone in a tablet dosage form. The method is based on absorbance measurement of the drug in ethanol at 282. 8nm Dharmaraju *et al*., [17] and obeys Beer' law in 1-5µg/ml concentration range.

MATERIALS AND METHODS

Nevirapine pure drug was obtained from Mylan Pharmaceuticals, Hyderabad, India. Nevimune brand is used for formulation assay. A UV- Visible spectrophotometer (Elico SL 210), Methanol (HPLC GRADE), Double distilled warer, with a pair of matched 1cm quartz cells and Spectral treats software was used for absorbance measurements.

Preparation of stock solution (1000µg/ml or PPM)

An accurately weighed amount of 10mg of nevirapine pure drug was taken in 10ml of volumetric flask and is dissolved with small portion of methanol and made up to the volume with water to form 1000 PPM.

Preparation of working standard (100µg/ml or PPM)

1ml from the stock solution was pipetted out into a 10 ml volumetric flask and volume was made up to the mark with water to form 100PPM.

Preparation of Serial Aliquots

Pipette out 0.5, 1, 1.5, 2, 2.5,3,3.5,4mlfrom working standard and transfer to separate 10ml volumetric flasks and make up the final volume to 10ml with water to yield 5,10,15,20,25,30,35,40µg/mL solutions respectively.

Selection of Detection Wavelength

Standard solution of Nevirapine was scanned in the UV range (200-400) and from the overlain spectrum, 282nm was selected as λ (lambda) max (Fig-3).

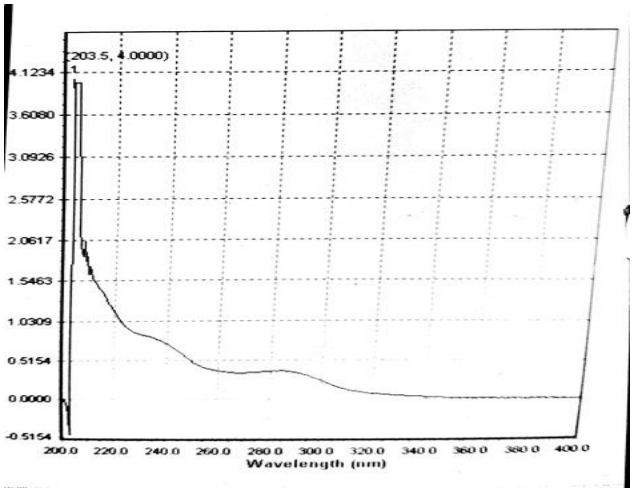

Fig-2: Showing maximum absorption wavelength

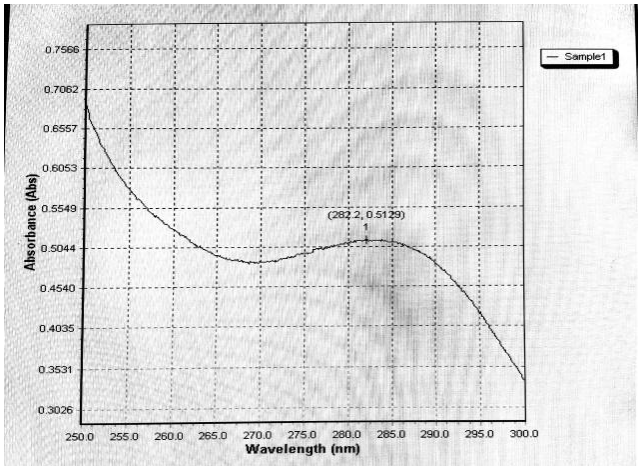

Fig-3: Absorption Spectrum

Analysis of Tablets

10 tablets from brand Nevimune-200mg were weighed and crushed into powder using a Pestle and Mortar. An amount equivalent to 10mg of Nevirapine was transferred into a 10ml volumetric flask. The content was shaken well with small volume of methanol and was made upto mark with water. Subsequent portion was diluted to get a concentration of 10ppm and %assay was calculated (refer Table-1).

$$\%Assay=\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}} \times 100$$

FORCED DEGRADATION STUDY[18,19,20]:

Forced degradation is a process whereby the natural degradation rate of a product or material is increased by the application of an additional stress.

Acid Degradation

1ml of standard 100ppm soultion is taken in a 10ml volumetric flask and add 1ml of 1N HCl and kept aside for 24hrs. Then it is neutralised with 1ml of 1N NaOH, made upto the volume with water and absorbance was noted using methanol and water as blank (Table-2).

Base Degradation

1ml of standard 100ppm soultion is taken in a 10ml volumetric flask and add 1ml of 1N NaOH and kept aside for 24hrs. Then it is neutralised with 1ml of 1N HCl, made upto the volume with water and absorbance was noted using methanol and water as blank (Table-2).

Peroxide Degradation

1ml of standard 100ppm soultion is taken in a 10ml volumetric flask and add 1ml of 10% hydrogen peroxide and kept aside for 24hrs. Then it is made upto the volume with water and absorbance was noted using methanol and water as blank.

Thermal Degradation

The standard drug powder was placed in incubator for 24hrs at 60°C and 10ppm solution was prepared using methanol and water and absorbance was noted.

Photolytic Degradation

The standard drug powder was placed in UV chamber for 24hrs and 10ppm solution was prepared using methanol and water and absorbance was noted.

VALIDATION

Method validation was performed in terms of specificity,selectivity, precision, accuracy, linearity as per International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity

Into a series of 10ml volumetric flasks, aliquots of standard drug solution(0.5-4ml from 100µg/ml) of Nevirapine accurately transferred and the volume was made with small portion of methanol and made up to mark with water. The absorbance of each solution was then measured at282nm against the blank (refer Table-1).

Calibration curve was prepared by plotting the absorbance versus concentration of drug.

Precision

The inter-day and intra-day precision of the method were determined. A repeatability study (intra-day precision) was performed by analysing Nevirapine standard solution (10µg/ml) within a day. An inter-day precision was performed using Nevirapine standard solution (10µg/ml) repeatedly on different days. Also method precision was performed using Nevirapine formulation (10µg/ml). %RSD was calculated (refer Table-1).

Accuracy and %recovery

The accuracy of the method can be known through recovery study. A constant known amount of pre-analysed tablet formulation was spiked to pure nevirapine solutions. Analysis of nevirapine was carried out at concentrations of 50%, 100% and 150% with concentrations of 15µg/ml, 20µg/ml and 25µg/ml respectively. %recovery of the method was calculated (refer Table-1).

$$\% \text{ recovery} = E/T+P^* 100$$

Where,

E= total obtained absorbance

T= Absorbance from pre-analysed nevirapine tablet

P= absorbance of pure drug.

Robustness and Ruggedness

The robustness of the method was performed by changing the wavelength maxima (282± 2) nm and the absorbance was noted for Nevirapine pure drug 10µg/ml solution. %RSD was calculated whereas ruggedness was calculated by performing with two different analysts. %RSD was calculated (refer Table-1).

Limit of detection and Limit of Quantification

The LOD and LOQ values were calculated (refer Table-1) using the formula:

$$LOD = (3.3 \times \sigma)/S$$

LOQ = (10 x σ)/S

Where,

σ is standard deviation of the response;

S is slope of calibration curve.

RESULTS AND DISCUSSIONS

Table-1:

PARAMETER	RESULTS
Λ max ,nm	282
Beer's law limit	5-40µg/ml)
Correlation coefficient	0.997
Slope	0.0248
Intercept	0.0207
Precision:	%RSD Interday-0.44 Intraday- 0.36 methodprecision-0.43
Robustness	280nm- 0.39 284nm-0.19
Ruggedness	1-Analyst-0.33 2-Analyst-0.35
% Recovery	50% - 99.2 99.5 99.2 100% - 99.2 98.9 99.1 150% - 99.2 99.5 100
LOD(µg/ml)	0.1374
LOQ(µg/ml)	0.4165
% Assay	100.7

Degradation Results

Table-2:

Type of degradation	Absorbance	% Degraded after 24hrs
Acid degradation	0.250	10.51
Base degradation	0.256	8.42
Peroxide degradation	0.257	7.94
Thermal degradation	0.258	7.85
Photolytic degradation	0.277	0.82

CONCLUSION

In this study, UV spectrophotometric method was developed for Nevirapine and the developed method was validated for parameters like linearity, precision, accuracy, ruggedness and rubustness. The degradation behaviour of nevirapine was studied by subjecting the drug to various stress conditions recommended by ICH. This method is advantageous over the reported method in terms of cost effectiveness and donot involve any tedious procedures.

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