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Method Development and Validation of Nevirapine in Bulk Using Uv Spectroscopic Method

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ABSTRACT:
Nevirapine is a novel anti-HIV medication. There is currently no published easy UV spectrophotometric technique for the estimate, therefore a new, affordable, precise, linear, sensitive, accurate, and ultraviolet (UV) spectrophotometric approach is needed. Nevirapine has a wide range of potential formulations for use in HIV treatment, hence efforts were made to develop and validate the medication in accordance with ICH recommendations.This method was developed using Ethanol as a solvent. Nevirapine showed theabsorption maxima at 351nm. A UV-Visible spectrophotometer was used to carry out a spectral analysis.
The developed method was linear for a range of 10-60µg/ml and displayed a good correlation coefficient of 0.9944. The accuracy of the method was estimated using a recovery study. The amount of drug recovered was found to be in the range of 98.8-99.7%. The % relative standard deviation of intraday precision was found to be 0.972% and interday precision was found to be in the range of 0.26-0.45. The % relative standard deviation was found to be <2 which is indicative of the precision**.**The proposed UV Spectrophotometric technique's linearity, accuracy, precision, and Robustness were statistically verified; the findings demonstrated that the method may be used for regular nevirapine analysis.
Key words: Linearity, Precision, Calibration curve, LOD, LOQ

I. INTRODUCTION

Based on its molecular makeup, nevirapine belongs to the chemical class known as dipyridodiazepinones(11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[1,4diazepine-6-one]). is a non-nucleoside reverse transcriptase inhibitor (NNRTI), an anti-retroviral medication that possesses anti-human immunodeficiency virus type-1 (HIV-1) action. In the Indian Pharmacopoeia [4], United Pharmacopoeia [5], British Pharmacopoeia [6], it is officially recognized.

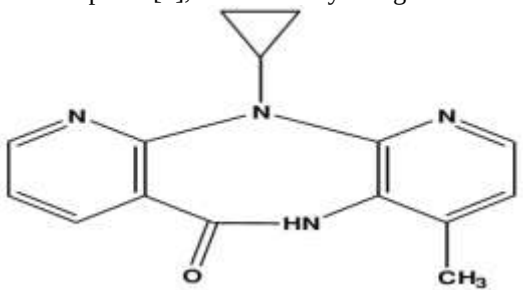

Fig-1Structure of Nevirapine

II. 2.DRUG PROFILE

MOLECULAR FORMULA: C15H14NO
NOMENCLATURE:2-cyclopropyl-7-methyl-2,4,9,15tetrazatricyclo[9.4.0.03,8]pentadeca 1(11),3,5,7,12,14-hexaen-10- one
MOLECULAR WEIGHT: 266.3040g/mol
CATEGORY: DNA polymerase inhibitor [anti viral]
SOLUBILITY: The maximum solubility is water at 25^0C 0.7046 mg/L
pka:2.8
PROTEIN BINDING: 60%
STORAGE: Stored at room temperature and protect from light and moisture
MELTING POINT:247-249^0C

MECHANISM OF ACTION:

Nevirapine is nonnucleoside reverse transcriptase inhibitor of HIV-1.Reverse transcriptasegets directly binded with nevirapine and block the RNA dependent and DNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of enzymes catalytic site. Activity of nevirapine does not compete with nucleoside triphosphates or template, eukaryoticDNA polymerases and HIV-2 RT are not inhibited by nevirapine.

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INSTRUMENTS:

- UV-Visible Spectrophotometer -ELsssICO SL-210 double beam with pair of 10mm matched quartz cells
- Weighing balance - Gold-300P
- Calibrated glassware

III. MATERIALS ANDREAGENTS:

All the chemicals and reagents used were of analytical grade which include

- Ethanol
- Distilled water

DRUG SAMPLES**:** includes
NEVIRAPINE active pharmaceutical ingredient was obtained from Aurobindo pharma LTD
FORMULATION USED:
Nevirapine bulk dosage form was used

IV. METHODOLOGY

Solubility studies: Nevirapine is soluble in organic or non-aqueous solvents such as ethanol, methanol, chloroform, etc., but it is poorly soluble in aqueous media like water. Absorbance was achieved using both types of solvents. By dissolving nevirapine in non-aqueous solvents such ethanol and adding distilled or demineralized water to reach volume, absorbance was achieved using this procedure. As a result, ethanol was utilized as a blank sample while estimating nevirapine.

Determination of absorption maxima of nevirapine: A volumetric flask of 100 ml was filled with 1 ml of the working standard solution, which was then diluted with ethanol to the mark to produce a solution containing 10 µg/ml. Utilizing ethanol as a reference, the spectra of this solution was examined in a UV spectrophotometer across a 200–400 nm range in order to determine the nevirapine's absorption maxima.

Preparation of standard stock of the nevirapine: Nevirapine, 100 mg of which had been carefully weighed, was put into a standard flask. A solution with a concentration of one milligram per milliliter (1 mg/ml) was created by adding five milliliters of ethanol and increasing the volume to 100 milliliters of water. We called this a conventional stock solution.

Preparation of working standard solution of nevirapine: A volumetric flask holding 100 ml was filled with 10 ml of the standard stock solution, which was then pipetted into it and diluted with distilled water to the desired level, producing a solution with a concentration of 100µg/ml. The term "working standard solution" was used to describe this.

V. METHOD VALIDATION

The ICH standards state that a technique must be verified in order to be reliable during routine usage. This procedure is sometimes known as method validation, and it involves supplying written proof that the method accomplishes its intended goals.

VALIDATION PARAMETERS OF PROPOSED UV SPECTROSCOPIC:

Validation parameters such as Linearity, Accuracy, Precision, Repeatability, and Robustness are validated statistically.

LINEARITY: After the working standard was diluted, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of series were taken and diluted with ethanol to create solutions that produced concentration ranges of 10-60 µg/ml in 10 ml volumetric flasks. Three replicates of each concentration's absorbance were measured at 351 nm, with ethanol serving as the blank solution. Plotting was done by taking concentration on the X-axis and absorbance was plotted on the Y-axis to construct the calibration curve.

ACCURACY: Using the conventional addition approach, the correctness of the suggested UV spectroscopic method was established. A solution with concentration ranges of 25, 50, and 75% was obtained by adding a known quantity of nevirapine to 30µg/ml of pre-analyzed sample solution. The standard deviation (S.D.) and percent RSD were computed after the absorbance was measured three times.

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PRECISION: The consistency of the findings gained by testing the same solution several times is measured by precision. The absorbance of six samples of the 30µg/ml nevirapine working standard solution was measured on the same day in order to assess repeatability, also referred to as intraday precision. The interday precision was computed at three distinct time intervals over the course of three days.

ROBUSTNESS: To evaluate the robustness of the procedure, the absorbance was measured and the analytical wavelength was changed. We examined the effect of sensing wavelength at ± 2nm.

VI. RESULTS

Absorption maxima of nevirapine:

The maximum of nevirapine was discovered to be 351nm (Figure-1). As a result, the maximum of the nevirapine was reported to be 351nm.

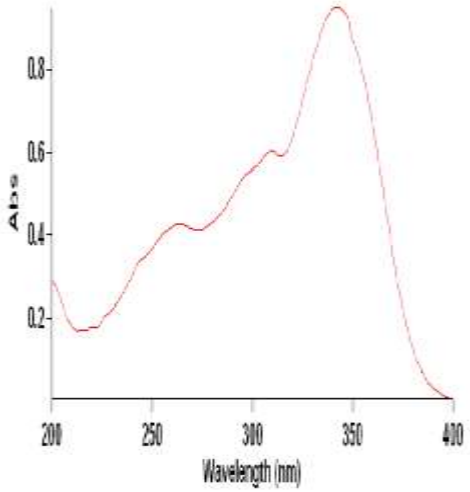

Figure-1 Absorption maxima of nevirapine (λmax) in ethanol at 351nm

LINEARITY:

The results for the linearity of nevirapine are shown in Table-1. Figure-2 depicts the nevirapine calibration curve ; it is clear that nevirapine obeys Beer's Lambert's law and has a linear equation at concentrations ranging from 0 to 10 µg/ml. **y= 0.0126x+0.0021** with a correlation coefficient **R^2=0.9944**, indicating a moderately positive correlation between nevirapine concentrations.

PRECISION:

Table-2 and 3 shows the intraday and interday precision results. The intraday RSD value was **0.972** and interday RSD value was between **0.20-0.42.** The RSD data obtained were within the acceptable limit, namely RSD<2%, indicating that the developed method was precise and acceptable.

ACCURACY:

Table-4 displays the acceptable accuracy results obtained by standard deviation and %Recovery, indicating that the established method was accurate.

Amount found (mg/ml) = Mean test Absorbance/Mean standard Absorbance×100

% Recovery=Amount found/Amount added×100

ROBUSTNESS:

For robustness, minor changes were made to the wavelength and temperature, resulting in percent RSD values that were within the acceptable range. That is <2%. The RSD values of robustness was discovered to be **1.39%** as shown in Table-5.

Table-1: Concentration vs Absorbance values for estimation of Nevirapine

S.No	CONCENTRATION (µg/ml)	ABSORBANCE	LINEAR REGRESSION EQUATION
1	10	0.128	y=0.0126x+0.0021 R^2=0.9944
2	20	0.246	
3	30	0.378	
4	40	0.512	
5	50	0.652	
6	60	0.734	

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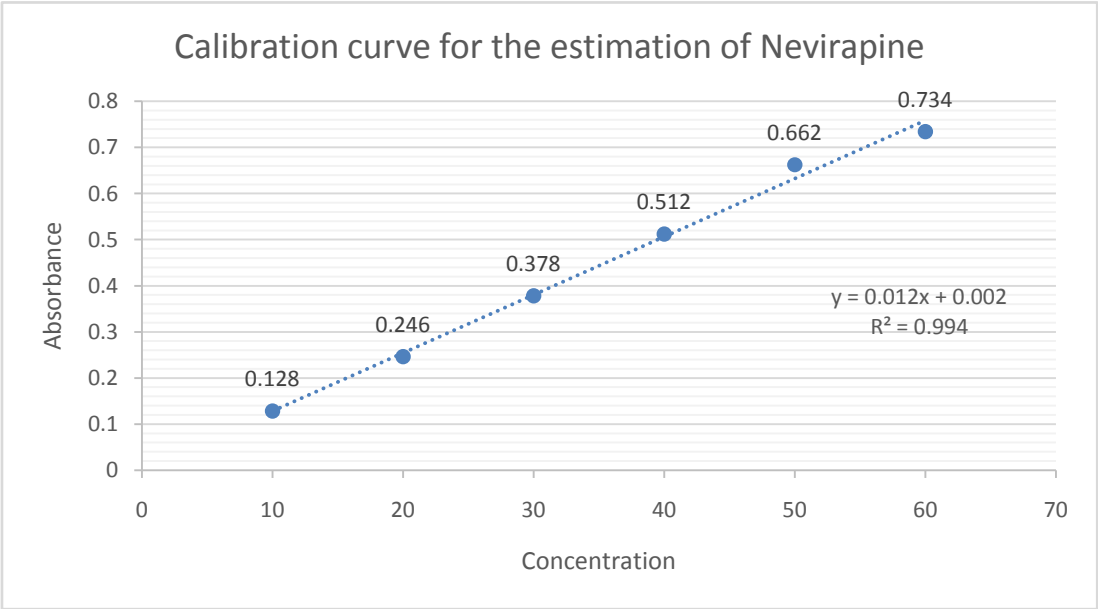

Figure-2: Calibration curve for the estimation of the nevirapine

Table-2: Intraday precision results for Nevirapine

S.NO	Amount of sample (concentration)	Absorbance	Mean	S.D	%RSD
1	30µg/ml	0.380	0.381	0.0017	0.972
2	30µg/ml	0.378			
3	30µg/ml	0.381			
4	30µg/ml	0.382			
5	30µg/ml	0.382			
6	30µg/ml	0.383			

SD: Standard Deviation
RSD: Relative Standard Deviation

Table-3: Interday precision results for Nevirapine

S.no	Day	Sample	Absorbance	Mean	S.D	%RSD
1	Day-1	30µg/ml	0.379	0.378	0.001	0.26
			0.378			
			0.377			
2	Day-2	30µg/ml	0.379	0.3781	0.0017	0.44
			0.380			
			0.377			
3	Day-3	30µg/ml	0.381	0.3793	0.0015	0.41
			0.379			
			0.378			

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Table-4: Accuracy results of Nevirapine

S.No	Recovery	Target in µg/ml	Spiked in µg/ml	Total in µg/ml	Absorbance	Amount found in µg/ml	% Recovery
1	25%	30	7.5	37.5	0.465	37.1	98.9
2	25%	30	7.5	37.5	0.466	37.2	99.2
3	25%	30	7.5	37.5	0.467	37.3	99.4
4	50%	30	15	45	0.563	44.6	99.1
5	50%	30	15	45	0.562	44.62	99.1
6	50%	30	15	45	0.564	44.7	99.3
7	75%	30	22.5	52.5	0.660	52.33	99.6
8	75%	30	22.5	52.5	0.662	52.4	99.8
9	75%	30	22.5	52.5	0.661	52.35	99.7

Table-5: Robustness results of Nevirapine

Sno	Sample	Wavelength	Absorbance	%RSD
1	**30**	**349**	**0.370**	**1.39**
2	**30**	**351(original)**	**0.378**	
3	**30**	**353**	**0.368**	

LIMIT OF DETECTION: Least amount of concentration to be detected
Formula: 3.3* standard deviation/mean
Value found-1.725µg/ml
LIMIT OF QUANTIFICATION: Least amount of concentration that is to be quantified
Formula: 10* standard deviation/mean
Value found- 5.227µg/ml

VII. CONCLUSION

In compliance with the ICH Guidelines, the linearity, precision, accuracy, robustness of this recently developed UV spectroscopic approach were confirmed. Every validation parameter was verified to be within the limit in accordance with ICH guidelines. A variety of commercial formulations' nevirapine content was successfully estimated using the developed approach.

Here, we conclude that the established UV Spectroscopic approach, which is straightforward, precise, accurate, inexpensive, and very sensitive, may be utilized for regular evaluation of nevirapine bulk.

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