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Development and Validation of a UV Spectroscopic Method for Quantitative Analysis of Nevirapine in Bulk Drug Substances

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Abstract

Nevirapine is an innovative anti-HIV drug. Currently, there is no straightforward UV spectrophotometric method for its estimation. Therefore, a new, cost-effective, precise, linear, sensitive, and accurate UV spectrophotometric method is necessary. Given Nevirapine's broad potential formulations for HIV treatment, we aimed to develop and validate this method in line with ICH guidelines. This method utilized Ethanol as the solvent, with Nevirapine showing an absorption maximum at 351 nm. Spectral analysis was performed using a UV-Visible spectrophotometer. The developed method demonstrated linearity within a range of 10-60 µg/ml, with an excellent correlation coefficient of 0.9944. Method accuracy was confirmed through a recovery study, showing drug recovery between 98.8% and 99.7%. The intraday precision relative standard deviation (RSD) was 0.972%, and interday precision RSD ranged from 0.26% to 0.45%. The RSD was consistently below 2%, indicating high precision. The linearity, accuracy, precision, and robustness of the proposed UV spectrophotometric method were statistically validated, confirming its suitability for routine Nevirapine analysis.

Key words: Linearity, Precision, Calibration curve, LOD, LOQ.

INTRODUCTION

Based on its molecular structure, nevirapine belongs to the chemical class known as dipyridodiazepinones (11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[1,4-diazepine-6-one]). It is a non-nucleoside reverse transcriptase inhibitor (NNRTI), an antiretroviral medication with activity against human immunodeficiency virus type-1 (HIV-1). Nevirapine is officially recognized in the Indian Pharmacopoeia, United States Pharmacopoeia, and British Pharmacopoeia[1].

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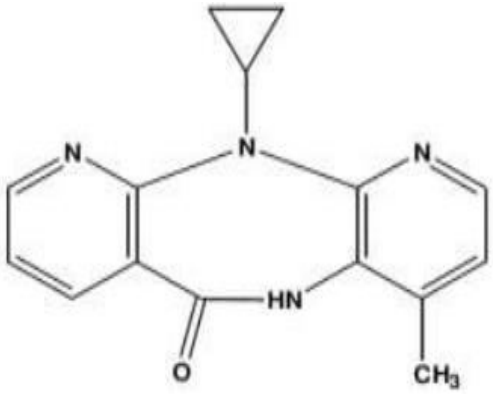

Fig-1Structure of Nevirapine

2. DRUG PROFILE

MECHANISM OF ACTION:

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. It binds directly to the reverse transcriptase enzyme, disrupting its catalytic site and blocking RNA-dependent and DNA-dependent DNA polymerase activities. Nevirapine's action does not compete with nucleoside triphosphates or templates, and it does not inhibit eukaryotic DNA polymerases or HIV-2 reverse transcriptase[2].

MATERIALS ANDREAGENTS

All chemicals and reagents used were of analytical grade, including:

- Ethanol
- Distilled water

Drug Samples: Nevirapine active pharmaceutical ingredient (API) was obtained from Aurobindo Pharma Ltd.

Formulation Used: Nevirapine bulk dosage form was used.

METHODOLOGY

Solubility Studies: Nevirapine is soluble in organic or non-aqueous solvents such as ethanol, methanol, and chloroform, but it is poorly soluble in aqueous media like water. Absorbance was measured using both types of solvents. To achieve this, nevirapine was dissolved in non-aqueous solvents like ethanol, and distilled or demineralized water was added to reach the desired volume. Consequently, ethanol was used as the blank sample during the estimation of nevirapine[3].

Figure 2: Solubility Studies

Determination of Absorption Maxima of Nevirapine: A 100 ml volumetric flask was filled with 1 ml of the working standard solution and then diluted to the mark with ethanol to produce a solution containing 10 µg/ml. Using ethanol as a reference, the spectrum of this solution was analysed with a UV spectrophotometer across a 200–400 nm range to determine the absorption maxima of nevirapine[4].

METHOD VALIDATION

The ICH standards mandate that a technique must be verified to ensure its reliability during routine usage. This process, known as method validation, involves providing documented evidence that the method achieves its intended goals.

Validation Parameters of the Proposed UV Spectroscopic Method:

Linearity: The working standard was diluted, and 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of the series were taken and further diluted with ethanol to create solutions with concentrations ranging from 10-60 µg/ml in 10 ml volumetric flasks. The absorbance of each concentration was measured in triplicate at 351 nm, using ethanol as the blank solution. A calibration curve was constructed by plotting concentration on the X-axis and absorbance on the Y-axis[5].

Accuracy: The accuracy of the proposed UV spectroscopic method was established using the conventional addition approach. A solution with concentration ranges of 25%, 50%, and 75% was obtained by adding a known quantity of nevirapine to a 30 µg/ml pre-analyzed sample solution[6]. Absorbance was measured three times for each concentration. The standard deviation (S.D.) and percent relative standard deviation (RSD) were calculated to determine the method's accuracy.

Precision, Repeatability, and Robustness: These validation parameters were statistically validated to ensure the reliability of the proposed method[7].

Precision: Precision measures the consistency of results obtained by repeatedly testing the same solution. The absorbance of six samples of the 30 µg/ml nevirapine working standard solution was measured on the same day to assess repeatability, also known as intraday precision. Interday precision was calculated by measuring the absorbance at three different time intervals over three consecutive days[8].

Robustness: To evaluate the robustness of the procedure, the absorbance was measured while altering the analytical wavelength. The effect of sensing wavelength was examined at ± 2 nm to assess the method's robustness.

RESULTS

Absorption Maxima of Nevirapine: The absorption maximum of nevirapine was found to be at 351 nm (Figure-1). Therefore, the absorption maximum of nevirapine is reported to be 351 nm[9].

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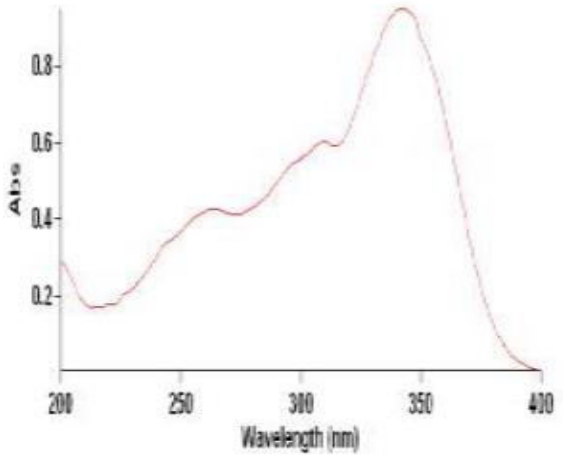

Figure ;3 Absorption maxima of Nevirapine (ƛ max) in ethanol at 351nm

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	

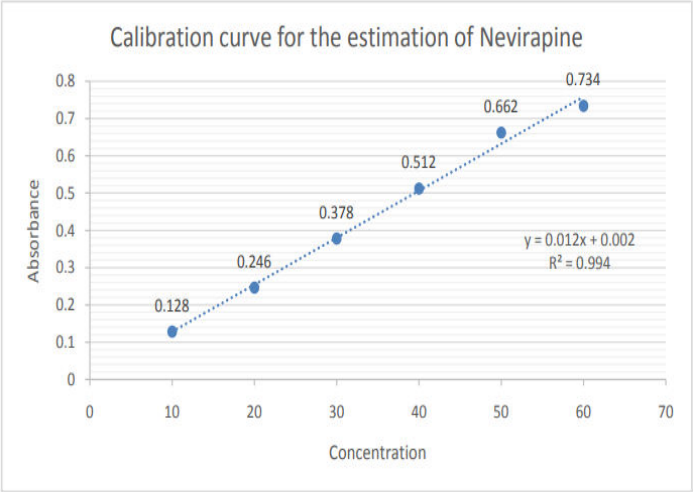

Figure 4: Calibration curve for the estimation of the Nevirapine

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

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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	0.378	0.001	0.26
			0.377			
2	Day-2	30µg/ml	0.379			
			0.380	0.3781	0.0017	0.44
			0.377			
3	Day-3	30µg/ml	0.381			
			0.379	0.3793	0.0015	0.41
			0.378			

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	
2	30	351(original)	0.378	1.39
3	30	353	0.368	

CONCLUSION

In accordance with ICH guidelines, the linearity, precision, accuracy, and robustness of this newly developed UV spectroscopic method were confirmed. All validation parameters were found to be within the acceptable limits specified by the ICH guidelines. The developed method successfully estimated the nevirapine content in various commercial formulations.In conclusion, this established UV spectroscopic method is simple, precise, accurate, cost-effective, and highly sensitive, making it suitable for the routine evaluation of nevirapine bulk.

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