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Spectrophotometric Methods for Determination of Nevirapine Using *Indigo carmine*

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Abstract: UV, simultaneous estimation method and Area under curve (AUC) Spectrophotometric methods for the determination of Nevirapine in pharmaceutical formulations have been developed. For the first method, UV-Spectrophotometry, standard solutions were measured at 341 nm. The linearity ranges were found to be 1–10 μg/ml in Indigo Carmine and the regression equation was (r=0.9892). The second method was based on calculation of Area under Curve (AUC) for analysis of Nevirapine in the wavelength range of 308–314 nm. Calibration curve was constructed by plotting AUC values against concentrations, 1–10 μg ml^{-1} of Nevirapine standards in Indigo Carmine. Regression equation of linear calibration graph was calculated as AUC (r=0.9896). Hence attempts were made to develop simultaneous estimation AUC of Nevirapine in pharmaceutical dosage form by UV spectroscopy.

Key words: Nevirapine · UV-Spectrophotometry · AUC-spectrophotometry

INTRODUCTION

Nevirapine is chemically, 1-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido [3,2-b: 21,31-e] [1, 4] diazepin-6-one. It is a non-nucleoside reverse transcriptase inhibitor and antiretroviral used in the treatment of AIDS [1]. Nevirapine is official in Indian Pharmacopoeia. Literature survey reveals that few HPLC [2-8] methods were reported earlier for the estimation of Nevirapine in human plasma and in its formulation. The proposed method was validated as per ICH guidelines According to International Conference on Harmonization (ICH), the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [9, 10] and its updated international convention. The present groups of authors have already reported UV Method development different pharmaceutical dosage form [11-25] and Indigo Carmine, Methyl orange [26].Therefore the objective of the present study was to develop four simple, precise, accurate, validated, economic analytical methods, in accordance with International Conference on Harmonization for quantification of Nevirapine in bulk and pharmaceutical formulations.

MATERIAL AND METHODS

UV Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Single component tablet formulations of Nevirapine (200 mg) were purchased from local market. All chemicals and reagents used were of AR/HPLC grade, Chloroform, ammonia and methanol were used for mobile phase preparation and as solvent. All chemicals used in this study were analytical grade and used without further purification. Chloroform, Indigo Carmine (s.d. Finechem, Bombay, India).

Preparation of Standard Stock Solution: The standard stock solution of Nevirapine was prepared by dissolving accurately weighed 10 mg of the drug in 50mg Indigo Carmine and diluted to 100 ml with 50mg Indigo Carmine to obtain a final concentration of 100 μg/ml. This stock solution was used to prepare further dilutions of standard solutions.

Reagent Preparation: 50mg of Indigo Carmine was weighed and transferred into a 100 ml standard flask and

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the volume was made up to the mark to get the required concentration (0.1%w/v).

Method I: Simultaneous Estimation [27]: Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 6 and 10 ml stock solution into separate 10 ml volumetric flasks and diluting to volume with 50mg Indigo Carmine to produce the concentrations ranging from 1:10 μg ml^{-1}. The above solutions were scanned over the range of 400 nm to 200nm against blank. The λ max was found to be at 341 nm. The calibration curve was constructed by plotting concentration (1-10 μg ml^{-1}) versus absorbance at 341 nm.

Method II: Area Under Curve [28]: It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrums obtained in method I was used to calculate AUC. The calibration curve was constructed by plotting concentration (1:10 μg ml^{-1}) versus AUC.

Estimation of Nevirapine in Tablets: For the analysis of the pharmaceutical dosage form, a total of twenty tablets were weighed and finely powdered. A portion of the powder, equivalent to about 10 mg Nevirapine was weighed accurately and transferred into 100 ml volumetric flask and 50 ml Indigo Carmine was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with Indigo Carmine and filtered through Whatmann filter paper (No. 41). Appropriate dilution was made into 20 μg ml^{-1} with Indigo Carmine from the stock solution for all the methods and the amounts of Nevirapine were determined.

Validation: The proposed Spectrophotometric methods are indirect and are based on the determination of the residual Nevirapine. Linearity all the methods, six point calibration curves were prepared on three different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method. Precision of intraday and interday precisions of the proposed Spectrophotometric methods were determined by estimating the corresponding response three times on the same day and on three different days over a period three different concentrations of Nevirapine (5, 10 and 20 μg ml^{-1}), the results are reported in terms relative standard deviation. Accuracy of parameter was evaluated by the percent recovery studies at concentration levels of 80, 100 and 120%, which consisted of adding known amounts of Nevirapine reference materials to a prequantified sample solution. Aliquots of sample solutions containing Nevirapine at 5 μg ml^{-1} were transferred to three 10 ml volumetric flasks. The recoveries were verified by estimation of drugs in triplicate preparations at each specified concentration level. The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery. Specificity of results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific. The robustness of the proposed methods was tested by changing parameters such as wavelength range and slit width. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust. Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot in different laboratories by different analyst using similar operational and environmental conditions.

RESULTS AND DISCUSSION

In present research work a UV Spectrometric method has been developed for determination of Nevirapine from its tablet formulations. This method utilizes the active analogue principle that lies at the spectroscopic method [11-25]. The developed method was based on formation of absolute ethanol extractable complex of drug with Indigo Carmine in double distilled water. Wavelength maxima of Nevirapine were found to be at 341 nm Indigo Carmine. Linearity was observed in concentration range of 1-10 μg/ml. Percentage label claim estimated for tablet formulation was found to be in the range of 99.79-100.11 % and respective values of standard deviation were found in the range of 0.9892 for different colouring agents tablet formulations of Nevirapine. To fix the linearity a calibration curve was constructed by plotting the absorbance as a function of the corresponding concentrations. The regression equation for the results was:

$$A=0.6588 * -0.1746 (r = 0.\ 0.9892)$$

Table 1: Analytical and regression parameters of the proposed methods

Parameter	Indigo Carmine
Beer's law limits	1-10
□max, nm	341 nm
Molar absorptivity	2.3×10^4
Sandell sensitivity	0.421
Limit of detection	0.751
Limit of quantification	0.663
Regression equation *	
(a) Intercept	0.375
(b) Slope	0.659
Correlation coefficient, (r)	0.9892

Table 2: Accuracy of the proposed methods

Sample	Label Claim	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added	Amount of Drug recovered	% Recovery	RSD (% n=6)
Method							
I	200	200.08	50	20	200.05	100.90	0.23
			100	40	199.99	99.98	0.54
			150	60	199.92	99.96	0.65
Method							
II	200	200.12	50	20	199.91	100.05	0.28
			100	40	198.99	100.16	0.33
			150	60	199.87	99.88	0.30

Table. 3: Intraday, Interdays, data of tablet formulation

		Interday precision % COV		
Sample	Intra day precision % COV (n=6)	Day 1[a]	Day 2[a]	Day 3[a]
Method				
I	1.527	1.104	0.986	0.769
Method				
II	1.973	1.234	0.870	0.598

COV: Coefficient of variance

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κ S.D. *a*/*b*, where k= 1 for LOD and 10 for LOQ, S.D.*a* is the standard deviation of the intercept and *b* is the slope. The LOD and LOQ were 0.751 and 0.663 μg ml^{-1}, respectively. The detection and quantitation limits determined were 0.387 and 0.475 μg ml^{-1}, respectively. These low values indicated the high sensitivity of the purposed method. Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. The results of analysis and recovery studies are given in Table.1. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are listed in Table 3. The low relative standard deviation (RSD 0.163 0.275, 0.398) at three different levels (intra-day precision), 0.749, 0.328, 0.872 at three different levels (inter-day precision) showed the good precision of the method. The analysis of formulation was carried out for three times in the same day and on three successive days. The % RSD value for interday and intraday analysis of formulation was found to be less than 2%.The accuracy of method was confirmed by recovery studies. A known amount of standard drug material was added with pre-analysed formulation in different levels. The amount of drug recovered was calculated and the percentage recovery was found to be in the range of 99.96% - 101.24% for method. The procedure was repeated for six times for each concentration and the % RSD values were calculated in Table 2.

CONCLUSION

The developed UV Spectrophotometric method for the estimation of Nevirapine was found to be simple and useful with high accuracy, precision, repeatability. Sample recoveries in all formulations using the above method was

in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non inference of formulation excipients in the estimation. The good precision ensures the reliability of the method. From the recovery studies it was found that there are no interference due to excipients used in formulation. Hence these two methods can be effectively used for the routine analysis of Nevirapine in bulk and formulation.

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