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SIMPLE AND SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NEVIRAPINE IN BULK AND FORMULATIONS

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ABSTRACT

A sensitive and direct spectrophotometric method is developed, which is free from extraction, derivatization, evaporation and complexation for the determination of nevirapine (NEV) in bulk drug and pharmaceutical formulation. The optimum conditions for the analysis of the drug are established. The method permits the determination of NEV over a concentration range of 1μg/mL to 10 μg/mL. Detection and quantification limit was found to be that 1.63 μg/mL and 3.12 μg/mL, respectively. The attained results shows good recoveries of 100.21%, with relative standard deviation of 0.28. All the calibration curve show a linear relation between the absorbance and concentration with correlation coefficient higher than 0.999. Precision and accuracy of the developed method is used for recovery study. The proposed method is applicable for the assay of NEV in dosage form and the results are in good agreement.

Key words *:* Nevirapine, Spectrophotometry, Determination

INTRODUCTION

Nevirapine (NEV) is 1-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido [3, 2-b : 2', 3'-e][1, 4] diazepin-6-one is a non-nucleoside reverse transcriptase inhibitor, which inhibits the replication of retroviruses, including HIV. Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme, which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs bind within a pocket termed the NNRTI pocket. Resistance to NEV develops rapidly if viral replication is not completely suppressed. The most common mutations observed after NEV treatment are Y181C and K103N, which are also

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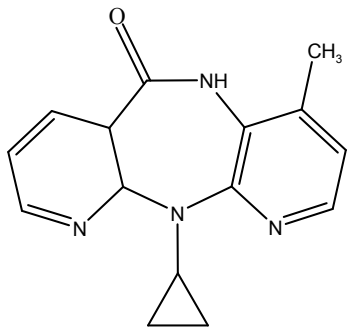
observed with other NNRTIs. As all NNRTIs bind within the same pocket, viral strains which are resistant to NEV are usually also resistant to the other NNRTIs, Efavirenz and delavirdine[1].

The structural formula of nevirapine (NEV) by AM 1 method is illustrated below :



NEV absorbed, through oral, is more than 90%, bioavailability is 93% (tablets) and 91% (oral solution), t_{max} is 4 h and C_{max} is approximately 2 µg/mL. It crosses the placenta and is found in breast milk. Protein binding is approximately 60% and is highly lipophilic and widely distributed. NEV Vd is 1.21 L/kg (IV) and cerebrospinal fluid approximates 45% of concentration in plasma. NEV is eliminated in urine (81.3%) and feces (10.1%). Less than 3% of the parent compound is excreted in urine. Autoinduction results in a decrease of the $t_{1/2}$ from 45 h (single dose) to approximately 25 to 30 h (multiple dose). It is administered at dosages of PO 200 mg daily for 14 days. Total daily dose not to exceed 400 mg, for maintanence dose PO 200 mg twice daily in combination with other antiretroviral agents. For children (2 months to 8 years of age) is PO 4 mg/kg daily for 14 days followed by 7 mg/kg twice daily. Total daily dose not to exceed 400 mg and for children (at least 8 yr of age) is PO 4 mg/kg daily for 14 days followed by 4 mg/kg twice daily. Total daily dose not to exceed 400 mg[2].

The adverse effect of NEV fatigue (5%); headache (4%); somnolence, paresthesia, malaise (postmarketing) in CNS, Rash (all grades 24% [life-threatening 2%]); Stevens-Johnson syndrome; toxic epidermal necrolysis; angioedema, bullous eruptions, urticaria, blistering (postmarketing); facial edema in dermatologic, in GIT it leads to Nausea (9%); abdominal pain, diarrhea (2%); vomiting, ulcerative stomatitis, oral lesions (postmarketing) and in hepatic is Hepatitis (including fatal fulminant hepatitis); hepatic failure; jaundice, cholestatic hepatitis, hepatic necrosis (postmarketing). It shows hypersensitivity, including severe rash or rash accompanied by fever, general malaise, fatigue, muscle or joint aches, blisters, oral lesions, conjunctivitis, facial edema;

anaphylaxis (post marketing).

The literature reveals various methods for the determination of NEV in biological fluids and pharmaceutical formulations. They are HPLC with UV detector[3-16], HPTLC[11], TLC[18], UV spectroscopy[11], Electrospray tandem mass spectroscopy[19, 20], capillary electrophoresis[21], solid phase extraction[22-23], GC/MS[25], LC-MS/MS[26], GC[27] and ion pair HPLC[28] used to determine the component in formulation and biological fluids.

The objective of the present study is to develop a simple, precise, accurate and economic analytical method with a better detection range for the estimation of NEV in bulk drugs and in pharmaceutical formulations. No extraction, derivatization, or evaporation step, no complexation agent and no baleful chemicals are involved in the proposed method; thereby, decreasing the time and the error in the quantization. This paper describes a simple, reliable method for assaying NEV by spectrophotometrically, which has been used to analyze the bulk drugs and formulation of NEV.

EXPERIMENTAL

Apparatus

A double-beam spectrophotometer Shimadzu UV 1601 PC Model was used.

Chemicals and reagents

NEV pure sample was supplied by M/s Hetero Drugs Pvt. Ltd., India as gift sample and used as such. Pharmaceutical dosage forms were procured from local pharmacies : Viramune (200 mg NEV/Tablet) was manufactured from M/s Hetero Drugs Pvt. Ltd, Hyderabad, India. Methanol used was spectro grade from S. D. fine chemicals Ltd., India.

Standard solutions

Stock solution

A primary stock solution of NEV (200 mg) was prepared in methanol and stored in dark in a refrigerator. All the measurements were performed at room temperature. The standard solutions were prepared by the proper dilution of the primary stock solution with methanol to obtain working standard. For linearity study, serial dilutions were made for nevirapine (NEV) in the range of 1 to 10 µg/mL concentrations were prepared by diluting the stock solution with methanol. The absorbances of these solutions were fitted in the calibration curve to calculate the accuracy and precision of the method.

For formulation

The average weight of the tablets were determined by weighing 20 tablets and these were powdered. Tablet powder equivalent to 50 mg of NEV was weighed and transferred to a 100 mL volumetric flask. About 60 mL of methanol was added and sonicated for 15 minutes for complete dissolution of drugs, the volume was made up with methanol and filtered through filter paper. Dilutions were made with methanol to attain a concentration of 5 µg/mL and spectra was recorded. Six replicates of analysis were carried out with sample weighed individually. The average weight of the tablet was found to be 0.8957 g.

Method validation

Linearity

The method was validated according to ICH Q2B guidelines[29] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte[30-34]. For NEV, five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. The linearity was evaluated by the least-square regression method using unweighted data.

Precision and accuracy

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements[29]. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD and LOQ

The limit of detection (LOD) is defied as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations –

LOD = 3 s/m; LOQ = 10 s/m

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability[29-32]. The values of LOD and LOQ are given in Table 1.

Stability

The stability of NEV in methanolic solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4 and 25°C for 12, 24, 26, 48, 60 and 72 h. The stability of these solutions was studied by performing the experiment and looking for the change in the spectrophotometric pattern compared with freshly prepared solutions.

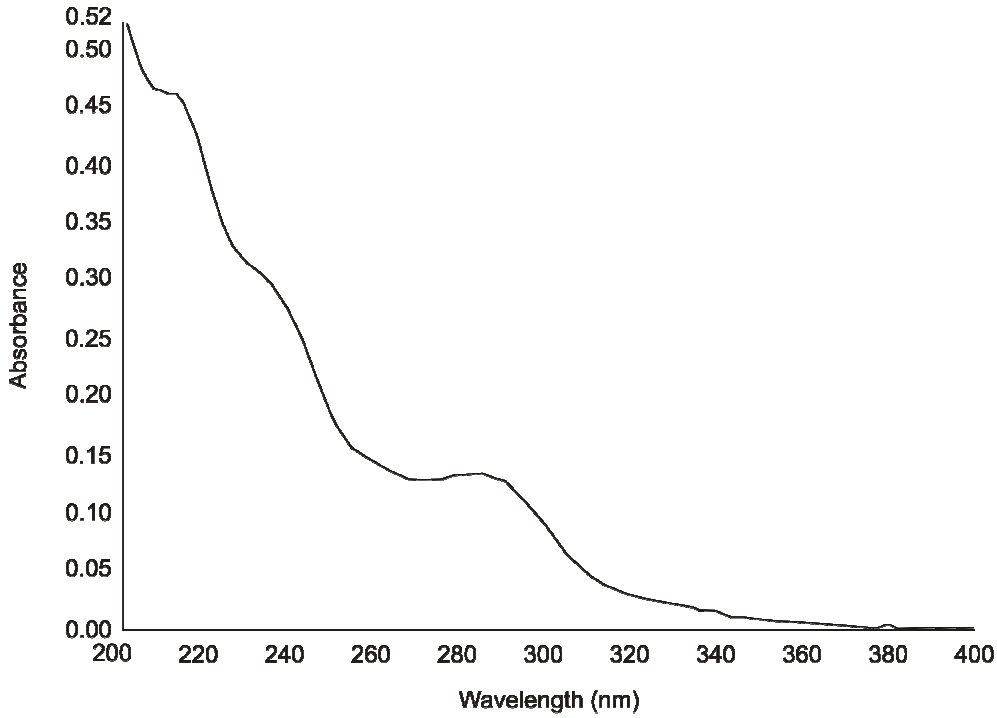


Fig. 1 : Absorption spectrum of NEV

Recovery study

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations, as employed in the linearity studies, was used. To study the accuracy, precision and reproducibility of the proposed method and dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by the addition of known amounts of pure NEV to the pre-analyzed tablet formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. NEV is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of NEV in methanolic solution is shown in Fig. 1.

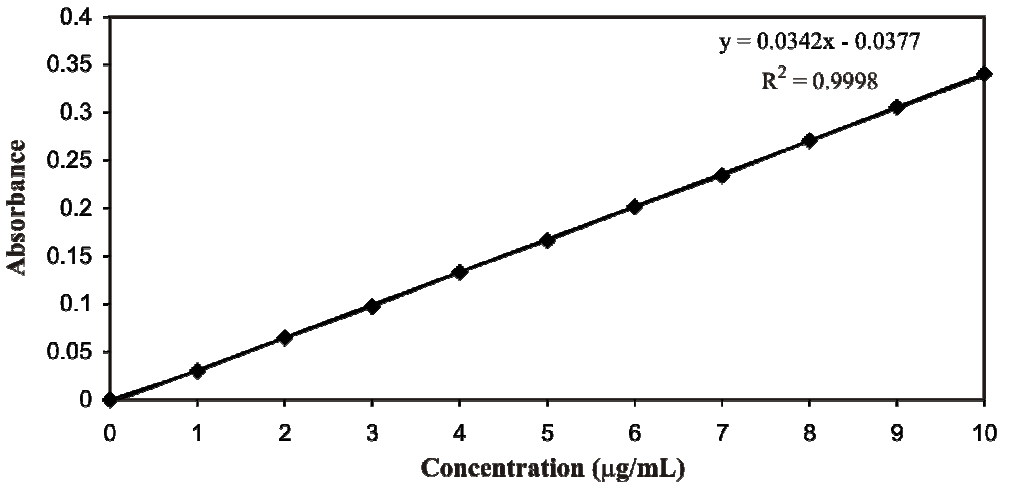


Fig. 2: Regression analysis of the calibration curve for NEV showed a linear relationship between the intensity of absorbance and the concentration, with correlation coefficients higher than 0.999 in all the curves assayed

Calibration curves

Calibration curve data were constructed in the range of the expected concentrations of 1 µg/mL to 10 µg/mL. Beer's law was obeyed over this concentration range. The regression equation was found to be y = 0.0342x – 0.0377. The correlation coefficient (r) of the standard curve was found to be greater than 0.999.The stock solutions and working standards were made in methanol. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region.

The characteristic of the calibration plot is presented in Fig. 2 and the analytical characteristics and necessary validation parameters for the UV techniques for NEV are presented in Table 1.

Table 1: Regression data of the calibration lines for quantitative determination of NEV by UV method

Parameters	Nevirapine (NEV)
Measured wavelength (λ_{max})	281
Linearity range, µg/mL	1-10
Slope	0.0342
Intercept	-0.0377
Correlation coefficient (r)	0.999
SE of slope	9.21×10^{-2}
SE of intercept	4.9×10^{-4}
LOD, µg/mL	1.63
LOQ, µg/mL	3.12
Repeatability of absorbance, RSD %	0.13
Repeatability of wavelength, RSD %	0.17
Reproducibility of absorbance, RSD %	0.30
Reproducibility of wavelength, RSD %	0.03

Performing replicate analyses of the standard solutions was used to assess the accuracy, precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in methanol and analyzed with the

relevant calibration curves to determine the intra- and interday variability. The intra- and interday precision were determined as the RSD %. The precision, accuracy and reproducibility of the results are given in Tables 1 and 2, which demonstrate a good precision, accuracy and reproducibility.

The proposed methods can be successfully applied for NEV assay in tablet dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim (Table 2). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table 2). In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets (Table 2). These results reveal that the developed method have an adequate precision and accuracy and consequently, can be applied to the determination of NEV tablet in pharmaceuticals without any interference from the excipients.

Table 2 : Assay results from NEV tablets and mean recoveries in spiked tablets

Parameters	Nevirapine (NEV)
Labelled claim, mg	200
Amount found, mg*	201.01
RSD %	1.92
Added, %	50, 75, 100
Found, %**	101.04, 99.73, 99.86
Recovery, %	100.21
RSD, % of recovery	0.28

*Mean of six determinations,
** three determinations

The stability of NEV in methanolic solution was evaluated to verify whether any spontaneous degradation occurs, when the samples were prepared. Fig. 3 shows the stability profile at 4 and 25^{o}C for 12, 24, 48, 60 and 72 h. The results were expressed as a percentage of the drugs remaining. The obtained data showed that the sample solutions

were stable during 48 h when stored at 4 and 25°C with a degradation of less than 5%. NEV was less stable at 25°C with degradation of 4.8% after 72 h.

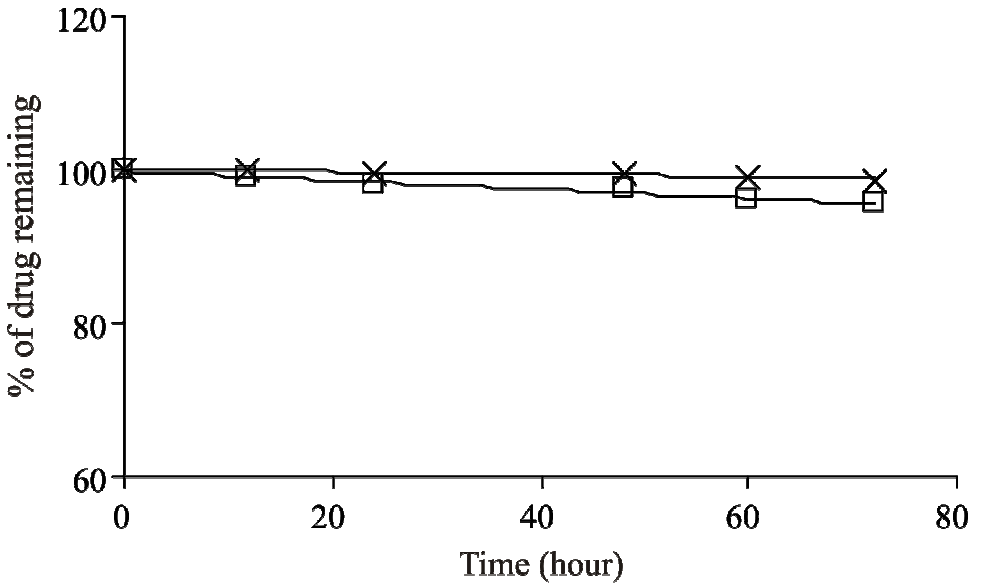


Fig. 3: Curve of the NEV in methanolic solution stability stored at 4 and 25°C during 12, 24, 48, 60 and 72 h

CONCLUSIONS

A spectrophotometric method for quantifying NEV in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. NEV can be estimated as low as 3.12 µg/mL in formulation. It could be precisely quantified and LOD was approximately 1.63 µg/mL in formulation. In summary, the proposed method can be used for the drug analysis in routine quality control.

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