tions), which has meant that it is a particularly successful anti-HIV treatment for young children.

Several analytical techniques have been reported for the determination of nevirapine [15-25]. The method in the United States Pharmacopoeia (USP)—monograph for determining nevirapine and its related compounds, A and B—uses a reversed-phase separation with UV detection [26]. The method calls for a 4.6 ×150 mm column packed with L60 (spherical, porous silica gel, 10 øm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped). Due to the strong retention of impurity C, the separation requires about 30 minutes. In the present work we discuss a rapid spectrophotometric method for the routine determination of nevirapine using tetrathiocyanatocobalt(II) ion as a reagent in pure and dosage forms.

Experimental

Apparatus

A Shimadzu UV-2550 UV-VIS Spectrophotometer with 1cm matched quartz cells was used for absorbance measurements.

Reagents and Solutions

All chemicals used were of analytical reagent grade. NVP drug was obtained as gift sample from SeQuent Scientific Ltd, Mangalore. Commercial tablets containing 200 mg were used for the study of dosage forms.

A 1000 µg mL^{-1} standard drug solution was prepared by dissolving 0.1g of NVP in alcohol diluting to the mark in a 100 mL standard flask. For the calibration samples, a working solution was prepared by appropriate dilution of the stock concentration in ethanol. Buffer of *p*H 4 was prepared by transferring one buffer tablet of *p*H 4 in 100 mL. Tetrathiocyanatocobalt(II) ion (TTC) was prepared by mixing 4g cobalt(II) chloride with 20g KSCN and made up to 100 mL with distilled water in a standard flask.

Procedure

Determination of nevirapine using tetrathiocyanatocobalt(II) ion

Different aliquots containing 0.2 - 2.0 µg mL^{-1} of NVP were transferred into a series of 10 mL standard flasks using a micro burette. To this 4 mL of tetrathiocyanatocobalt(II) ion solution was added followed by 2 mL of *p*H 4 buffer solution. The contents were shaken well and set aside for 10 minutes and diluted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 624.5 nm against the corresponding reagent blank.

Assay of formulations

To determine the content of nevirapine in conventional tablets (label claim: 200 mg/tablet), the tablets were powdered and powder equivalent to 100 mg of nevirapine was weighed. The drug from the powder was extracted with ethanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 mL. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

Results and Discussion

The method involves the reaction of NVP with TTC in *p*H 4 to form a complex [Scheme 1], which has an absorption maximum at 624.5 nm [Fig. 1].

Analytical Data

The adherence of Beer's law was studied by measuring the absorbance values of the solutions varying analyte concentration [Fig. 2]. A linear relation was found between absorbance at λ_{max} and concentration ranges given in table 1. Regression analysis of Beer's law data using the method of least squares were made to evaluate the slope (a), intercept (b) and correlation coefficient (R), for each system of NVP and are also presented in table 1. Sensitivity parameters such as molar absorptivity, Sandell's sensitivity, detection limit and quantification limit are also compiled in table 1. The limit of detection and quantitation are calculated according to ICH guidelines.

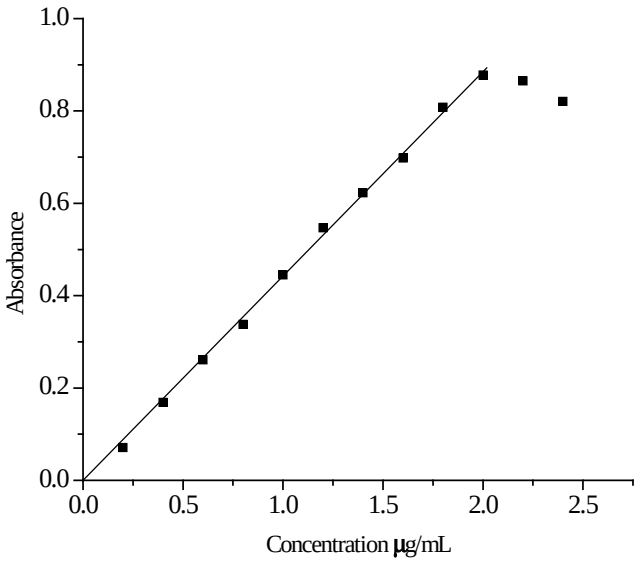

Figure 2. Adherence of Beer's law

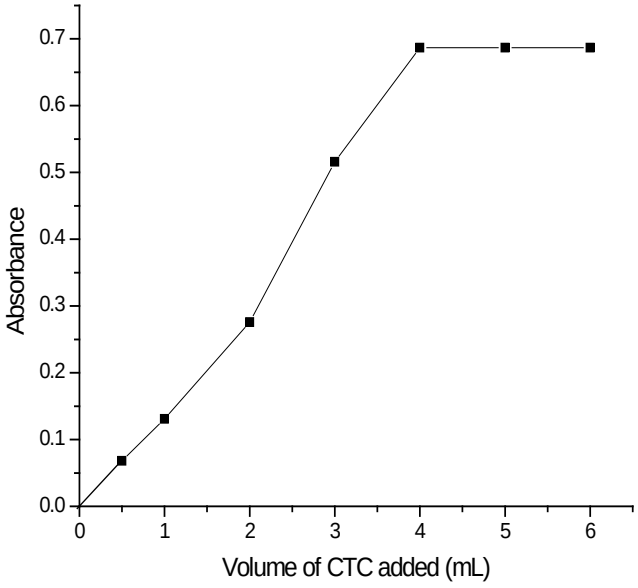

Figure 3. Optimum concentration of reagent TTC

Optimum Volume of Reagent

An aliquot containing 2 μg mL^{-1} (2 mL) of NVP was pipetted out from the stock solution of NVP (10 μg mL^{-1}) into a 10 mL calibrated flask along with 2 mL of buffer solution of *p*H 4. Then it was mixed with TTC in the order of 0.5 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 6 mL. The complexes were made up to 10 mL and absorbance was measured at 624.5 nm. It was found that the volume between 4 and 6 mL is optimum volume of the reagent to get the maximum absorbance and 4 mL was chosen for the experiment [Fig. 4].

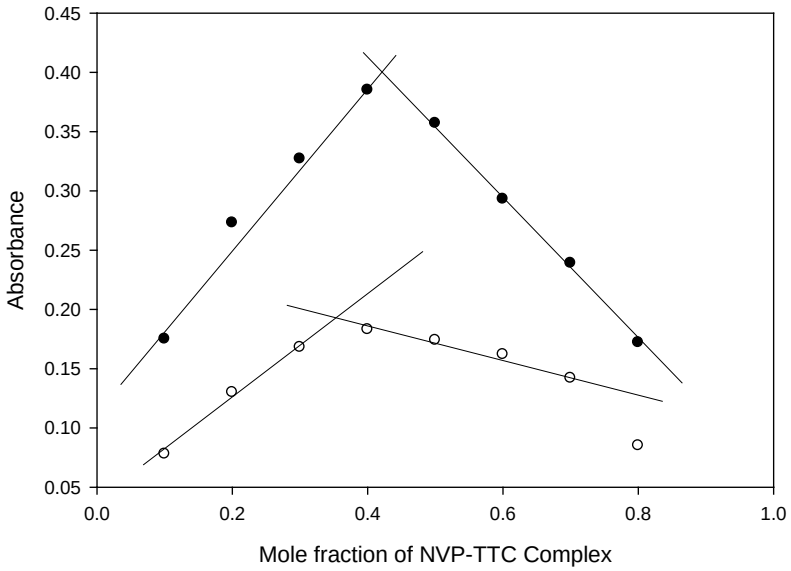

Figure 5. Continuous variation graph for TTC-NVP Complex

Method Validation

The proposed method is applied to the assay of NVP in three commercially available dietary supplements. An aliquot containing 1.2 μg mL^{-1} drug solution is taken and assayed according to the proposed methods. The content of the tablet formulation is calculated by applying suitable dilution factor. The proposed methods are checked by a thorough analysis of each spiked sample and the results are compiled in table 3 .The accuracy and reliability of the proposed method are further established by performing recovery studies. The relative error and relative standard deviation indicate the high accuracy and precision for the method and are compiled in table 2. For a better picture of reproducibility on a day- to-day basis, a series of experiments are performed in which standard drug solution at three levels is determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values represent the best appraisal of the method in routine use.

Conclusions

Simple spectrophotometric method for the determination of NVP have been developed and validated according to ICH guidelines. The method is simple and easy to perform compared to other existing methods and do not entail any rigorous experimental variables which affect the reliability of the results. The ingredients usually present in the pharmaceutical formulations of these drugs seldom interfere in the proposed methods. The proposed method is simple, accurate and easy to perform and can be used for the routine determination of NVP in bulk and in dosage forms.

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