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Validated First Order Derivative Spectroscopic Method for the determination of Stavudine in Bulk and Pharmaceutical Dosage Forms

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Abstract :A simple, accurate, precise and sensitive First order derivative Spectrophotometric method was developed for the estimation of Stavudine in bulk and pharmaceutical dosage forms. The estimation of Stavudine was carried out at maximum absorbance of 250 nm. The method was found to be linear and obeys Beer's law in the concentration range of 2-20 mcg / ml. The developed method was validated according to ICH guidelines and was found to be accurate and precise. Thus the proposed method can be successfully applied for the estimation of Stavudine in bulk and pharmaceutical dosage forms.

Keywords: Stavudine, Validation, ICH guidelines, First order derivative spectroscopy.

INTRODUCTION AND EXPERIMENTAL

Stavudine is a synthetic nucleoside analogue with activity against HIV-1. The chemical name of Stavudine is 2^1, 3^1-didehydro-3^1-deoxythymidine. It has a molecular formula of $C_{10}H_{12}N_2O_4$ and a molecular weight of 224.22 g / mol and its structure was given in Fig: 1.

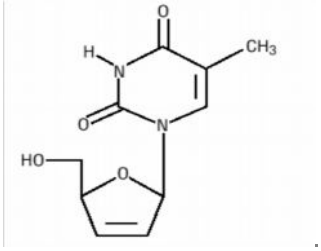

Fig. 1: Chemical Structure of Stavudine

Stavudine is a white or almost white powder. It is freely soluble in Ethanol (95 %) and sparingly soluble in Water.[1-2] The drug is officially listed in monograph of USP.[3] Several analytical methods that have been reported for the estimation of Stavudine in biological fluids or pharmaceutical formulations include High Performance Liquid Chromatography, Titrimetry and UV-Visible Spectrophotometry.[4-14] The objective of the work was to develop simple, accurate, precise and economic First order derivative Spectroscopic method to estimate the Stavudine in bulk and pharmaceutical dosage forms. The method is simple, reproducible and statistically valid.

UV-VIS Spectrophotometer Shimadzu UV-1800 with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement. Methanol, Distilled water and other reagents used were of analytical grade.

100 mg of standard Stavudine drug was weighed, transferred to a 100 ml volumetric flask and dissolved in Methanol. The flask was shaken and volume was made up to the mark with Methanol to give a solution

containing 1000 µg / ml. From this stock solution, 10 ml of solution was pippeted out and placed into 100ml volumetric flask. The volume was made up to mark with distilled water to give a solution containing 100 µg / ml.

From the standard stock solution of Stavudine, appropriate aliquots were pippeted out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 2 to 50 µg / ml. Absorbance for these solutions were measured at 250 nm and shown in Fig 2. For the standard solution analytical concentration range was found to be 2-20 µg / ml and those values were reported in Table: 1.

Appropriate volume of aliquots from standard Stavudine stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with distilled water to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg / ml. Absorbance spectra of each solution against distilled water as blank were measured at 250 nm and the graphs of absorbance against concentration was plotted and are shown in Fig 3. The regression equation and correlation coefficient was determined.

Twenty capsules of two brands were taken, removed the caps and the powder equivalent to 100 mg of Stavudine was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the Methanol and sonicated for 5 min. The flask was shaken and volume was made up to the mark with Methanol to give a solution of 1000 µg / ml. The above solution was centrifuged at 2000 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No. 41). From this solution, 10ml was taken and diluted to 100 ml with distilled water to give a solution of 100 µg / ml and used for the estimation of Stavudine. To examine the absence of either positive or negative interference of excipients used in formulation, recovery studies were carried out.

Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of Standard Stavudine drug to the sample solution of the capsules. Precision for assay were determined by repeatability, interday, intraday precision for drug (each in three replicate). Ruggedness studies were carried out by changing the analysts.

Table 1. Results of calibration curve at 250 nm for Stavudine by First order derivative UV Spectroscopy

Sl. No.	Concentration (mcg / ml)	Absorbance at 250 nm
1	2	0.003
2	4	0.006
3	6	0.009
4	8	0.012
5	10	0.015
6	12	0.018
7	14	0.021
8	16	0.024
9	18	0.027
10	20	0.031

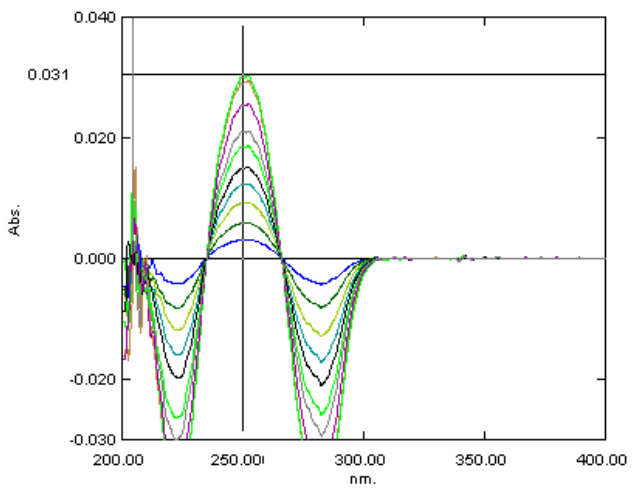

Fig. 2: First order spectra of Stavudine at 250 nm

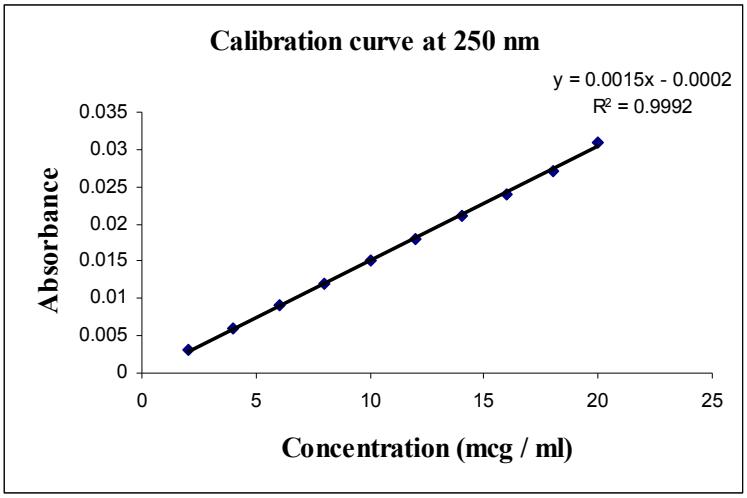

Fig. 3: Calibration curve of Stavudine by the proposed method.

Table 2: Shows Optical Characteristics of Stavudine:

PARAMETERS	RESULTS
Absorption maximum	250 nm
Beer's law limit (µg/ml)	2-20 µg / ml
Correlation coefficient (r^2)	0.9992
Sandell's sensitivity (µg/cm^2-0.001 absorbance units)	0.666
Regression equation (y=mx+c)	Y = 0.0015 X -0.0002
Slope (m)	0.0015
Intercept (c)	-0.0002
% RSD**	0.820
Limit of detection (µg/ml)	0.45
Limit of quantitation (µg/ml)	1.35

Table 3: Shows validation results of Stavudine:

PARAMETERS	RESULTS
Accuracy	
% Recovery ± SD**	101.22 ± 0.0687
Precision	
% RSD	0.13
Ruggedness	
% Recovery ± SD**	
Analyst I	101.14 ± 0.10
Analyst II	101.23 ± 0.06

** Average of six determinations, SD means standard deviation, RSD means relative standard deviation.*

RESULTS AND DISCUSSION

The absorption spectral analysis shows the λ max of Stavudine at 250 nm. The calibration curve was obtained for a series of concentration in the range of 2-20 mcg/ml. It was found to be linear and hence, suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics are summarized in Table 2. Regression analysis of Beer's law plot revealed a good correlation. The effects of various excipients generally present in the capsule dosage form of Stavudine were investigated. The results indicated that they did not interfere in the assay in amounts far in excess of their normal occurrence in it. The proposed method was validated as per the ICH guidelines[17]. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found to be with in the limits. This showed that the precision of the method is satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed method. For this, known quantities of the Stavudine solution was mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Stavudine was determined by using the proposed methods and the amount of added drug was calculated by the difference. The % RSD was less than ± 2.0. This showed that the recoveries of Stavudine by the proposed methods are satisfactory and the results are shown in Table 3. Ruggedness and Robustness were determined and the % RSD values were calculated from precision study was less than ± 2.0. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by the proposed method. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Stavudine in pharmaceutical dosage form.

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