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Research Article

Development and Validation of New Spectrophotometric Method for the Estimation of Stavudine in Bulk and Pharmaceutical Dosage Form

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Abstract: A simple, accurate, precise and sensitive 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 270 nm. The method was found to be linear and obeys Beer's law in the concentration range of 1-14 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, UV Spectrophotometry.

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INTRODUCTION

For the treatment of Acquired Immuno Deficiency Syndrome (AIDS): Five classes of anti retroviral drugs (ARV) have been developed such as nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry or fusion inhibitors and integrase inhibitors. Human immunodeficiency virus (HIV) gradually progressed to AIDs that lead to morbidity and mortality. To minimize the risk of this HIV, a combination of these ARV drugs are vital. For resource limited countries, a combination of two NRTIs with an NNRTI is the recommended for first line regimen. The commonly used NRTIs are Zidovudine, Stavudine and Lamivudine, while Nevirapine and Efavirenz are frequently used as NNRTIs.

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.

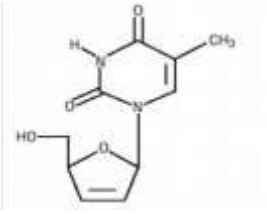

Fig. 1: Chemical Structure of Stavudine

Stavudine is a white or almost white powder. It is freely soluble in ethanol (95 %) and 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 Spectroscopic method to estimate the Stavudine in bulk and pharmaceutical dosage forms. The method is simple, reproducible and statistically valid.

MATERIALS AND METHODS

Authentic drug sample of Stavudine was given as a gift sample by Hetero drugs Ltd., Hyderabad. Tablets of Stavudine were procured from local market. Distilled water was used for the preparation of solutions.

Instrument

Labindia – 3000+ UV / Vis double beam Spectrophotometer with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

Preparation of stock solution

100 mg of standard Stavudine drug was weighed, transferred to a 100 ml volumetric flask and dissolved in distilled water. The flask was shaken and volume was made up to the mark with distilled water to give a solution containing 1000 µg / ml. From this stock solution, 10 ml of solution was pipetted 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.

Selection of Analytical concentration ranges

From the standard stock solution of Stavudine, appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with distilled

water to obtain working standard solutions of concentrations from 1 – 14 µg / ml. Absorbance for these solutions were measured at 270 nm and shown in Fig 2. For the standard solution analytical concentration range was found to be 1- 14 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 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 µg / ml. Absorbance spectra of each solution against distilled water as blank were measured at 270 nm and the graphs of absorbance against concentration was plotted and are shown in Fig 3. The regression equation and correlation coefficient was determined.

Preparation of sample solution

Twenty tablets of Stavudine were taken 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 distilled water and sonicated for 5 min. The flask was shaken and volume was made up to the mark with water 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.

Limits of detection (LOD) and limits of quantitation (LOQ)

The LOD and LOQ of Stavudine are determined by using calibration standards. Value of LOD is determined by using the formula: 3.3σ/S And the value of LOQ is determined by: 10σ/S , where 'σ ' is the standard deviation of the y intercept of the regression equation and **'S'** is the slope of calibration curve.

RESULTS AND DISCUSSION

Table 1: Results of calibration curve at 270 nm for Stavudine by UV Spectroscopy

Sl. No.	Conc. (mcg / ml)	Absorbance at 270 nm
1	1	0.048
2	2	0.086
3	3	0.133
4	4	0.175
5	5	0.197
6	6	0.242
7	8	0.308
8	10	0.372
9	12	0.452
10	14	0.528

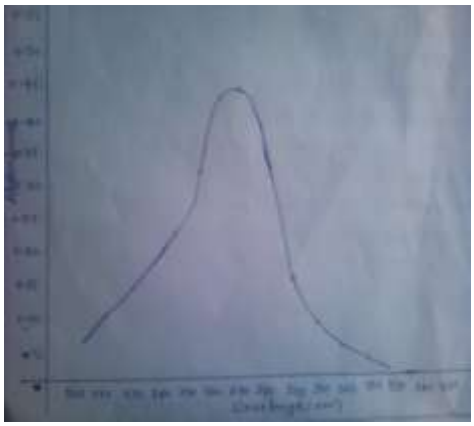

Fig: 2 Spectra of Stavudine at 270 nm

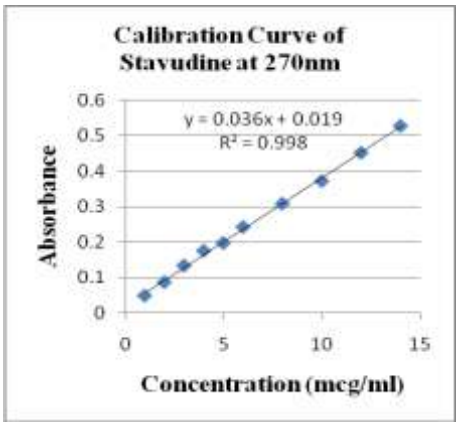

Fig. 3: Calibration curve of Stavudine

Table 2: Shows Optical Characteristics of Stavudine

Parameter	UV method
λ_{max} (nm)	270
Beer's law limits (mcg/ml)	1-14
Sandell's sensitivity (mcg/cm^2-0.001 absorbance units)	0.0271
Regression equation (Y*)	Y = 0.0036X + 0.019
Slope (b)	0.0036
Intercept (a)	+ 0.019
Correlation coefficient(r^2)	0.998
% RSD**	< 2%
Limit of detection (mcg/ml)	0.129
Limit of quantitation (mcg/ml)	0.392

*Y=bX + a where X is the concentration of Stavudine in mcg/ml and Y is the absorbance at the respective $\lambda_{max.}$

**Average of six determinations.

Table 3: Assay of Stavudine formulation

Sl. No.	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
1	Virostav	30	30.003±0.055	97.51%	1.088

Table: 4 Determination of Accuracy results for Stavudine at 270 nm

Brand name	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD**
Virostav	10	8	17.96	99.77±0.14
Virostav	10	10	19.96	99.83 ± 0.54
Virostav	10	12	21.80	99.11±0.46

**Average of six determinations.

Table: 5 Determination of Precision results for Stavudine at 270 nm

Conc. (mcg / ml)	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
LQC (2mcg/ml)	0.247 ± 0.0035	1.41	0.085 ± 0.001414	1.656
MQC (6mcg/ml)	0.532 ± 0.00189	0.35	0.248 ± 0.00208	0.39
HQC (14mcg/ml)	0.822 ± 0.00216	0.26	0.823 ± 0.00217	0.26

**Average of six determinations.

The absorption spectral analysis shows the λ_{max} of Stavudine at 270 nm. The calibration curve was obtained for a series of concentration in the range of 1-14 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 tablet 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 [15]. 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 within 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 & 4. 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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