A simple, specific, accurate, precise and sensitive reverse phase high performance liquid chromatographic method has been developed for the quantitation of Stavudine in both pure and capsule dosage form. A Phenomenex Gemini C-18, 5 µm column having 250x4.6 mm i.d. in isocratic mode with mobile phase containing methanol: acetate buffer pH 6.0 (40:60). The flow rate was 1.0 ml/min and the effluents were monitored at 265 nm. The retention time was 3.58 min. The linearity was in the range of 25-75 mcg/ml. This method was validated for linearity, precision, specificity, limit of detection, limit of quantitation, accuracy, ruggedness and robustness. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug.

**Key words:** RP-HPLC, Stavudine, Validation.

**INTRODUCTION**

Stavudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV\(^1\). The chemical name of Stavudine is 2', 3'-didehydro-3'-deoxythymidine. It has a molecular formula of \(\text{C}_{12}\text{H}_{13}\text{N}_{2}\text{O}_{4}\) and a molecular weight of 224.2. It has the structural formula (Figure 1).

![Chemical Structure of Stavudine](image1.png)

**Fig. 1: Chemical Structure of Stavudine**

Stavudine is a white to off-white crystalline solid with a solubility of approximately 83 mg/ml in water at 23°C. 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\(^2\). The objective of the work is to develop simple, accurate, precise and economic RP-HPLC method with lesser run time to estimate the Stavudine in bulk and capsule dosage forms.

**MATERIALS AND METHODS**

A Shimadzu HPLC model containing LC-10 AT pump, variable wavelength programmable UV/VIS detector and Rheodyne injector was employed for the investigation. All the chemicals used in the investigation were of HPLC grade. The chromatographic analysis was performed on a Phenomenex Gemini C18 column. The mobile phase consisting of methanol and acetate buffer of pH 6.0 in the ratio of 40:60 v/v was selected. The optimized chromatographic conditions are summarized in table 1. The standard solution of Stavudine was prepared by dissolving 10 mg in 100 ml of mobile phase to give the concentration 100 µg/ml. The mobile phase and the solution were sonicated for 10 min. and filtered using whatman filter paper No.1 and used. The various dilutions of Stavudine in the concentration of 25, 37.5, 50, 62.5 and 75 µg/ml were prepared. The solutions were injected using a 20 µl fixed loop in to the chromatographic system at the flow rate of 1.0 ml/min and the effluents were monitored at 265 nm, chromatograms were recorded. The Stavudine was eluted at 3.58 min as shown in fig.2. The calibration curve was constructed by plotting the average peak area versus concentrations (fig. 3) and regression equation was computed. The method was extended for determination of Stavudine in capsule dosage form. The capsule containing 30 and 40 mg strength were taken.

**Table 1: Optimized Chromatographic conditions for the proposed method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Phenomenex Gemini C-18 (5µ)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Methanol: Acetate buffer (40:60)</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µl</td>
</tr>
<tr>
<td>Detection</td>
<td>265 nm in uv detector</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Retention time</td>
<td>3.58</td>
</tr>
<tr>
<td>Run time</td>
<td>6 min</td>
</tr>
</tbody>
</table>

**Fig. 2: Typical RP-HPLC Chromatogram of Stavudine by the proposed method.**

The content of twenty capsules were taken and weighed. The powder equivalent to 50 mg of Stavudine was transferred into 50 ml volumetric flask containing 50 ml of mobile phase and flask was kept for ultrasonication for 15 min, then it was diluted up to the mark with mobile phase and the solution was filtered through...
RESULTS AND DISCUSSION
A suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits (Table 2). Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 25-75 mcg/ml and it was found to be linear. The data of regression analysis of the calibration curves are shown in Table 3. Selectivity and specificity were studied for the examination of various excipients generally present in the dosage form of Stavudine. The results indicated that they did not interfere in the assay. The proposed method was validated as per the ICH guidelines.4-6. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying with in the range of ±2. This showed that the precision of the methods are satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Stavudine solution were mixed with definite amounts of pre-analyzed standards. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Stavudine solution were mixed with definite amounts of pre-analyzed standards. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Stavudine solution were mixed with definite amounts of pre-analyzed standards. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Stavudine solution were mixed with definite amounts of pre-analyzed standards. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. 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Table 5: Assay Results of Stavudine capsules using the proposed method

Thus it can be concluded that the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Stavudine in pure and capsule dosage forms.

REFERENCE


