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Validated RP - HPLC Method for the Estimation of Stavudine in Formulation and Serum

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Stavudine in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Methanol: 0.1 % O.P.A: Acetonitrile (40:50:10) was used. The flow rate was 1.2ml/min. and effluents were monitored at 267 nm. The retention time for Stavudine was 6.8 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.5ppm and 3.0ppm respectively and recovery of Stavudine from tablet formulation was found to be 97.2%. The proposed method was successfully applied for the quantitative determination of Stavudine in tablet formulation.

Key Words: Stavudine, HPLC, Linearity, Validation, 267 nm

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INTRODUCTION

Stavudine:

(2'-3'-didehydro-2'-3'-dideoxythymidine) is a nucleoside analog reverse transcriptase inhibitor (NARTI) active against HIV. It was first synthesized by Jerome Horwitz[1]. Stavudine is a crystalline solid with the molecular formula $C_{10}H_{12}N_2O_4$ and a molecular weight of 224.2. Stavudine, when used alone or in combination with other antiviral medications, may cause serious and possibly deadly damage to the liver and pancreas and a life-threatening condition called lactic acidosis. The I.U.P.AC name of stavudine is 1-((2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione

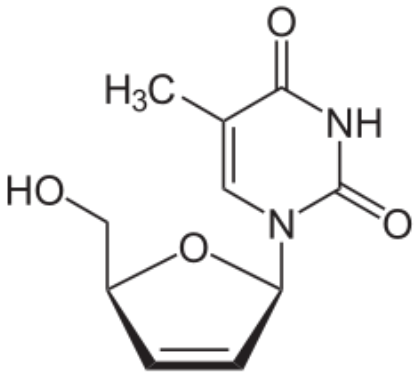

Figure 1: Molecular Structure of Stavudine / Deferasirox

Literature survey revealed that numerous methods have been reported for estimation of Stavudine [2-4] in pharmaceutical formulations has been reported.

Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of Stavudine in tablet dosage forms as well as subsequent validation of developed method according to I.C.H guide lines [5-8].

EXPERIMENTAL CONDITIONS

Instrument :

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

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Reagents and materials:

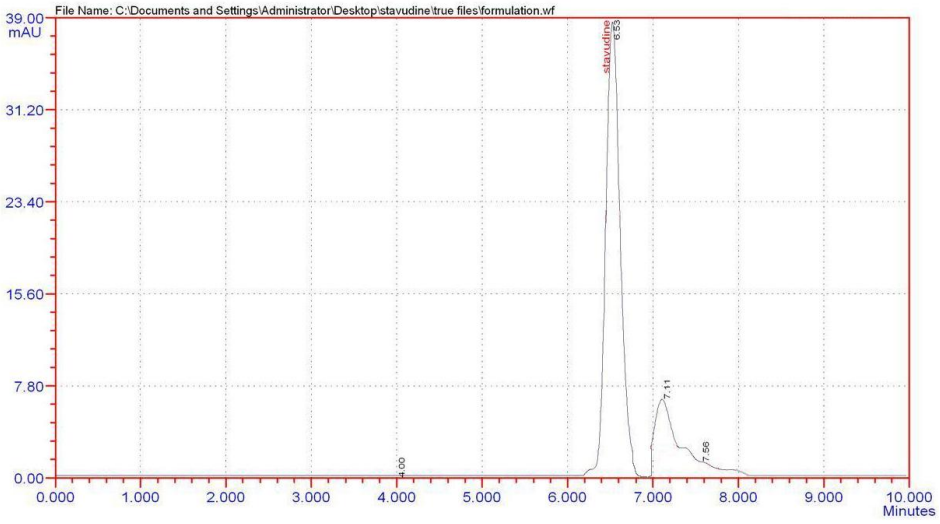
Methanol of HPLC grade was purchased from E.Merck, Mumbai, India. LC grader water was obtained by double distillation and purification through milli – Q water purification system. Ortho phosphoric acid of analytical grade was procured from Qualigens, Mumbai,India.

Preparation of Standard Stock Solution:

A stock solution of Stavudine was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 100ppm of Stavudine. Resultant solution was filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper.

Preparation of Sample Solution :

The formulation tablets of Stavudine were crushed to give finely powdered material. Powder equivalent to 1mg of Stavudine was taken in 100 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 10ppm.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		4.003	15	151.0	0.958	0.00	3153
2	stavudine	6.525	1653	10118.3	64.180	1.08	22647
3		7.107	469	5263.6	33.387	1.23	7992
4		7.563	19	232.5	1.475	25.00	7614
	Sum:		2156	15765.4	100.0000		

Figure 2: HPLC chromatogram of Stavudine formulation

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Chromatographic Conditions:

The mobile phase consisting of Methanol: Acetonitrile: OPA were filtered through 0.45µ Ultipor N_{66} Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 40:10:50,v/v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.2ml/min and detection wavelength was set at 267nm with a run time of 10min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Calibration Curve:

Appropriate aliquots of standard Stavudine stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 0.5,1.0,1.5,2.0,2.5 and 3ppm of Stavudine. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Stavudine was constructed by plotting peak area ratio versus applied concentration of Stavudine and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Stavudine in tablet sample was found out using regression equation.

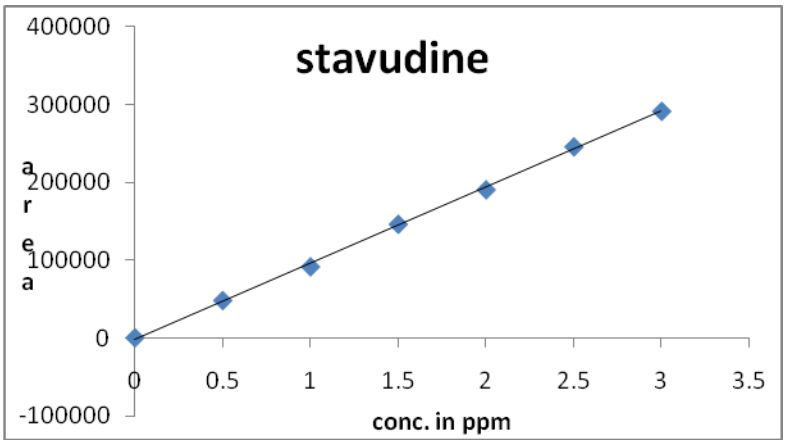

Figure-3

Method validation:

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy:

The accuracy of the method was determined by calculating recovery of Stavudine by the method of standard addition. Known amount of Stavudine (1.5ppm, 0.5ppm and 1.0ppm

.1.5ppm, 1.5ppm) was added to a pre quantified sample solution and the amount of Stavudine was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Stavudine was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision :

The intra-day precision study of Stavudine was carried out by estimating the correspondence responses six times on the same day with 2ppm concentration and inter-day precision study of Stavudine was carried out by estimating the correspondence responses six times next day with 2ppm concentration.

Linearity and range:

The linearity of the method was determined at six concentration levels ranging from 0.5-3.0ppm for Stavudine

Specificity:

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification:

Limit of detection = 15 ng/ml
Limit of quantification = 50 ng/ml

Stability:

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness:

Robustness of the method was studied by changing the composition of organic phase by ±5% and the P^H by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

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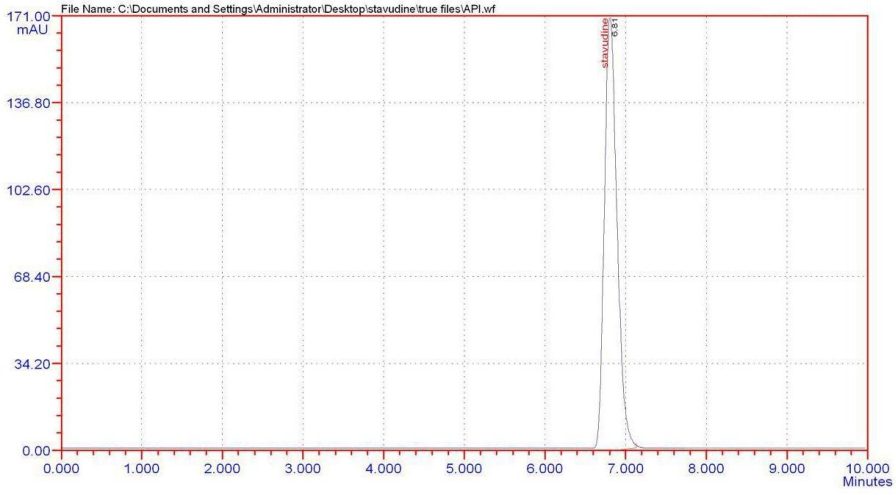
RESULTS AND DISCUSSION

The UV spectra of Deferasirox showed that the drug absorbs appreciably at 250nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol: Acetonitrile: 0.1 %OPA (40:50:10, v/v/v). The retention time of Stavudine was found to be 6.8 min, which indicates a good base line.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	stavudine	6.807	17120	187147.5	100.000	1.32	7728
	Sum:		17120	187147.5	100.0000		

Figure-4

HPLC chromatogram of Stavudine (Figure 3):

The number of theoretical plates was found to be 7727.66, which indicates efficient performance of the column, which indicates asymmetric nature of the peak. The calibration curve for Stavudine was obtained by plotting the peak area ratio versus the concentration of Stavudine over the range of 0.5-3.0 ppm, and it was found to be linear with r^2=0.999. The regression equation of Stavudine concentration over its peak area ratio was found to be y = (-3692.966 + 98516.62 x), where x is the concentration of Stavudine (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for Stavudine was found to be 0.5ppm and 3.0ppm, indicates the sensitivity of the method. The system suitability and validation parameters were given in table 2. The high percentage of recovery of Stavudine was found to be 97.2% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Stavudine in tablet formulation. The result for Stavudine was comparable with a corresponding

labeled amount (Table 3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	0.5-3.0
Slope	98516.6285
Intercept	3692.966
Correlation coefficient (r^2)	0.999

Table 2: System suitability and validation parameters

Parameters	Results
Theoretical plates (N)	7727.66
Retention time (min)	6.8
Asymmetric factor	1.8
LOD (ng/ml)	15
LOQ (ng/ml)	50
Accuracy (%)	97.2
R.S.D. (%)	0.198

Table 3: Assay results of tablet formulation

Formulation	Labelled claim (mg)	% of Deferasirox in Tablet
STADINE	500	18.4%

CONCLUSION

Proposed study describes new LC method for the estimation of Stavudine in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method is convenient and simple and can be used for routine analysis of estimation of Stavudine in its tablet formulation and serum.

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