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Original Research Article

Validated Spectrophotometric and HPLC Method for the Estimation of Stavudine in Synthetic Mixture

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ABSTRACT

Stavudine is an antiretroviral medication used to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretroviral. It may be used for prevention after a needle stick injury or other potential exposure. However, it is not a first-line treatment. In present study a simple, sensitive and accurate isocratic reverse phase high performance liquid chromatography (RP-HPLC) and UV spectrophotometric method was developed for determination of stavudine in synthetic mixture. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Thermo C_{18} (250 × 4.6 mm, 5µm) column with mobile phase consisting of methanol: acetonitrile (50:50v/v) at flow rate of 1.0ml/min. The retention time for stavudine was 2.715±0.3min. The UV spectrophotometric determinations were performed at 266 nm using water as a solvent. The linearity range for stavudine was 5-25µg/ml for both HPLC and UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good (r2 >0.999) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. Moreover, the accuracy and precision obtained with HPLC co-related well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis.

Key words: HPLC, UV Spectrophotometry, Stavudine, Synthetic mixture, Method validation, Quantitative analysis.

INTRODUCTION

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), develops resistance rapidly (Valle-Bahena et al., 2006; Richman, 2006) Therapeutic strategy regimens require the combination of antiretroviral drugs to inhibit HIV (Claes et al., 2004; De Clercq, 2004). For the treatment of 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 (Van et al., 2004; Laurent et al., 2004). Stavudine is a thymidine analogue with activity against HIV I. The chemical name of stavudine is 2'3'deoxythymidine. Its molecular formula is $C_{10}H_{12}N_2O_4$ and molecular weight is 224.22g /mol. The structure of stavudine is given in Figure 1. Stavudine is a powder, white or almost white in colour. It is freely soluble in water. In USP Monograph it is officially listed (Rockville,1985; I.P., 1996). By HPLC, Titrimetry & UV visible spectrophotometry, stavudine can be estimated in biological fluid or pharmaceutical formulation alone or combination with other drugs (Verma et al., 2010; Ghosal, 2012; Panditi and Vinukonda. 2011; Mohamed and Mikre, 2009; Sarkar et al., 2006; Notari et al., 2006). However, there is no method available for the determination of stavudine by UV and HPLC. Therefore, an attempt was made to develop a new, rapid and sensitive method for the estimation of stavudine. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is mandatory also (Code Q2A, 1994; Code Q2B, 1994).

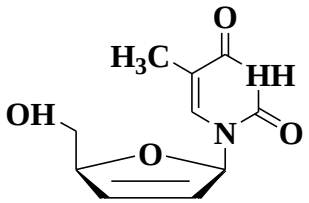

Figure1: Chemical structure of Stavudine

Experimental

Reagents and chemicals

An analytically pure sample of stavudine was procured as gift sample from Ranbaxy Lab, Dewas, India. HPLC grade methanol, ACN and water were procured from Sigma Aldrich and Millipore (India) Ltd., Bangalore. Tablet formulations of stavudine were prepared in lab by using common excipients.

Instrument

In UV-spectrophotometric method, Labindia model- 3000 + series were used, which is a

wavelength accuracy ±1 nm, with 1cm quartz cells.

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

UV spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) of stavudine

Wavelength of maximum absorption was determined by scanning 15µg/ml solution of stavudine using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 266 nm (Figure 2).

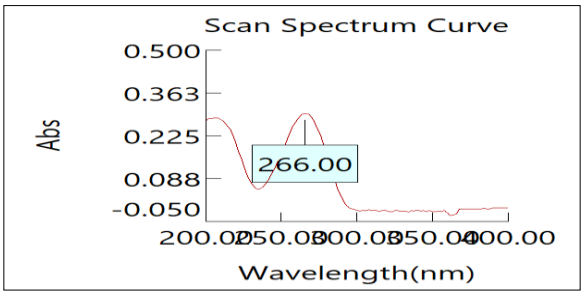

Figure 2 Selection of λ_{max} of stavudine

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving 100 mg of drug in 50 ml of HPLC grade water and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with water to get a concentration of 1000 µg/ml (Stock-A) for drug.

Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of stavudine and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with water that gave concentration of 100 µg/ml (Stock-B).

Preparation of working standard solution

0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for stavudine.

Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25µg/ml at 266 nm for stavudine. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n =5 determinations was plotted as the standard curve (Figure 3).

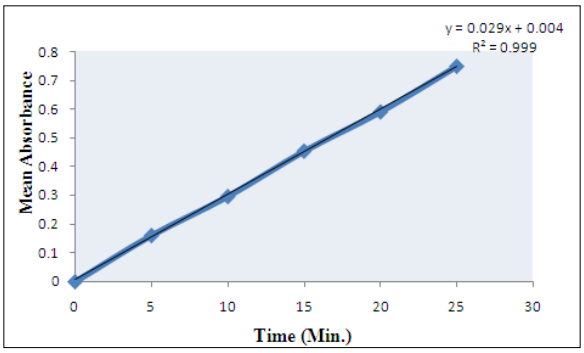

Figure 3 Calibration Curve of Standard Stavudine

RP-HPLC method

Chromatographic condition

The isocratic mobile phase consisted of methanol: ACN in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 266 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

10mg of stavudine was weighed accurately and transferred to separate 10 ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000 µg/ml.

Working standard solution

From stock solutions of stavudine 1 ml was taken and diluted up to 10 ml with methanol. From this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25µg/ml for stavudine solutions manually in triplicate to the HPLC system at detection wavelength of 266 nm. Mean of n =5 determinations was plotted as the standard curve (Figure 4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements.

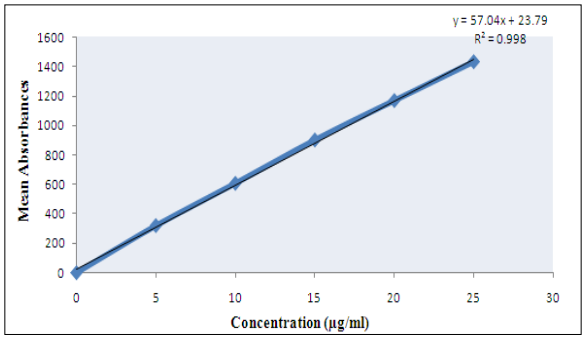

Figure 4 Calibration Graph of Stavudine

System suitability

The system suitability parameter was carried out to verify that the analytical system was

working properly and could give accurate and precise result. The six replicates of reference standard, 10µg/ml of stavudine were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in Table1.

Table 1 Results of system suitability parameters

Parameters	Stavudine
AUC	613.716
No. of Theoretical Plates	2594.00
Tailing Factor	1.800
Retention time	2.715

Validation Parameters

Linearity

Linearity was studied by analyzing five standard solutions (n=5) in the range of 5-25µg/ml of stavudine in both UV spectrophotometric and HPLC method. Calibration curves with concentration verses absorbance or peak area was plotted for each method and the obtained data were subjected to regression analysis using the least squares method. Linearity of stavudine was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance or peak area with respective concentration (Table 2).

Table 2 Response ratios of stavudine

Concentration (µg/ml)	HPLC Method		UV Method	
Stavudine	AUC	RR	ABS	RR
5	325.431	65.08	0.250	0.050
10	613.716	61.37	0.501	0.051
15	891.458	59.43	0.751	0.050
20	1154.152	57.70	1.012	0.051
25	1420.200	56.80	1.214	0.049

Accuracy

The validity and reliability of the proposed methods was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies were carried out by adding known amount of standard solution of stavudine to pre-analyzed tablet solutions. The resulting solutions were then re-analyzed by proposed methods. In UV Spectrophotometric method, the value of mean recoveries was found to be in the range of 99.63% to 99.79% for stavudine. The value of SD and %RSD less than 2 indicate the accuracy of the method. In RP-HPLC method, the value of mean recoveries was found in the range of 100.00% to 100.66 % for stavudine. Total amount of drug found and percentage recovery was calculated. Results of recovery studies are reported in Table 3.

Table 3 Results of recovery study

Recovery Level%	% Mean±SD*	
	U.V Method	RP-HPLC Method
80%	99.79±0.439	100.66±1.607
100%	99.63±0.153	100.66±1.989
120%	99.77±0.173	100.00±1.667

***** Value of three replicate and three concentrations

Precision

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD is less than 2 indicate the precision of method. Result of precision shown in Table 4.

As per ICH norms, small, but deliberate variations in concentration of the mobile phase, flow rate and temperature were made to check the method's capacity to remain unaffected. Results of robustness are reported in Table 5.

Table 4 Results of precision

Parameters	UV Method		RP-HPLC Method	
Precision (Mean± SD)*	Stavudine	%RSD	Stavudine	%RSD
Repeatability	99.93±0.125	0.126	99.96±0.586	0.598
Intra-day Precision	99.17±0.254	0.256	99.13±0.546	0.551
Inter-day Precision	98.39±0.484	0.492	97.50±0.500	0.512
Analyst to Analyst (1)	99.83±0.025	0.065	99.60±0.110	0.158
Analyst to Analyst (2)	99.26±0.023	0.049	99.80±0.225	0.159

*Average of 5 replicate and 5 concentration

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Table 5 Result of robustness of formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 ºC	+ 5 ºC
Stavudine	0.34	0.67	0.58
Flow rate		(-10%)	(+10%)
Stavudine	0.49	0.79	0.99
Mobile phase ratio		- 2 %	+ 2 %
Stavudine	0.34	0.85	0.90

LOD and LOQ

LOD and LOQ of described method were observed as 0.15µg/ml and 0.45µg/ml for stavudine in UV spectrophotometric method and 0.570 µg/ml and 0.500 µg/ml for stavudine in RP-HPLC method, based on the SD of response and slope, which meet the requirement of new method.

Analysis of marketed formulation

20 tablets (In house preparation) were weighed and ground to a fine powder. An equivalent amount to 30 mg of stavudine was taken in 50 ml volumetric flask. This was dissolve in 25 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method and RP-HPLC method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The statistical evaluation of tablet analysis by both methods is reported in Table 6.

Table 6 Results and statistical parameters for tablet analysis

S. No	Drug	Label claim	Amount found	% Label claimed	SD*	%RSD*
UV Method	Stavudine	30	29.82	99.40	0.145	0.235
RP-HPLC	Stavudine	30	29.99	99.67	0.586	0.598

*Average of five determination

Result and discussion

RP-HPLC and UV-Spectrophotometric methods were developed for Stavudine which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of stavudine was 2.715±0.3min. The chromatograms have been shown in Figure 5. A five-point calibration curve was constructed with working standards and was found linear (r2 = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed HPLC method was accurate, precise, reproducible and very sensitive.

For UV Method: Y = 0.029 x +0.004 (r2= 0.999)

For RP-HPLC: Y = 57.04 x +23.79 (r2= 0.998)

All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. Table 3 lists the percent recovery (content uniformity) of stavudine in the synthetic mixture by HPLC and UV methods. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 4). Moreover, the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying stavudine in synthetic mixture (Table 6).

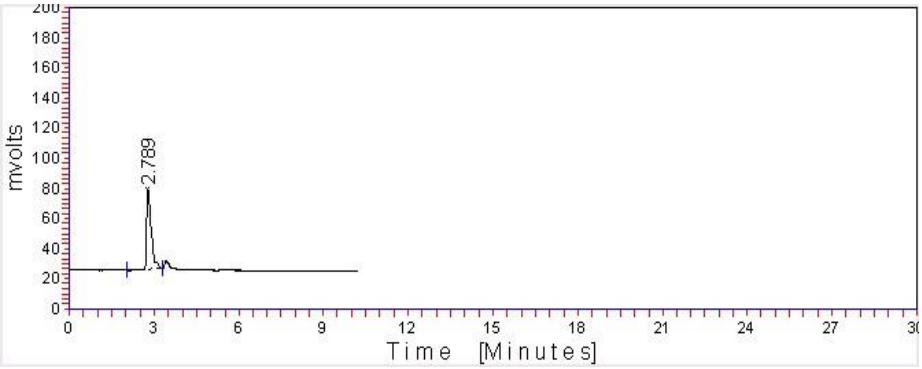

Figure 5 Chromatogram of stavudine

Conclusion

The HPLC method and the UV spectrophotometric method for the determination of stavudine in synthetic mixture were found to be simple, rapid, precise, accurate and sensitive. Moreover, the UV method offers a cost effective and time saving alternative to HPLC method of analysis for stavudine from mixture. The HPLC method enables faster quantification of stavudine within run time of five minutes without interference of excipients. In summary, the proposed methods can be used for routine quality control of pharmaceutical formulation containing stavudine.

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References

1. Valle-Bahena, O.M., Jimenez, J.R., Ortiz-Lopez, R., *et al.* (2006). Frequency of protease and reverse transcriptase drug resistance mutations in naïve HIV-infected patients. Arch Med Res., 37: 1022- 1027.
2. Richman, D.D. (2006). Antiviral drug resistance. Antiviral Res. 71:117-121.
3. Claes, P., Wintzen, M., Allard, S., Simons, P., De Coninck, A., Lacor, P. (2004). Nevirapine-induced toxic epidermal necrolysis and toxic hepatitis treated successfully with a combination of intravenous immunoglobulins and N-acetylcysteine. Euro J Intern Med. 15: 255-258.
4. De Clercq, E. (2004) Antiviral drugs in current clinical use. J Clin Virol. 30:115-133.
5. Van, L.F., Phanuphak, P., Ruxrungtham, K., *et al.* (2004). Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. Lancet, 363:1253-1263.
6. Laurent, C., Kouanfack, C., Koulla-Shiro, S., *et al.* (2004). Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial. Lancet, 364:29-34.
7. http://www.rxlist.com/zerit-drug/patient-images-side-effects.htm
8. Rockville, M.D. (1985) United States Pharmacopoeia (USP-NF XXIV), United States Pharmacopoeia Convention Inc. 3218.
9. Indian pharmacopoeia (1996) Addendum, 30-31.
10. Verma, J., Jain, D., Jain, N., Pandey, S.P., Jain, D.K. (2010). Simultaneous Estimation of Lamivudine, Stavudine and Nevirapine by RP-HPLC in Tablet Formulation. Research J. Pharm. and Tech. 3(2): 490-493
11. Ghosal K. (2012). UV-Spectroscopic methods for estimation of stavudine: finding the best order. Open Access Scientific Reports, 1(7): 1-3.
12. Panditi, V.R., Vinukonda, A. (2011). Validated First-order derivative spectroscopic method for the determination of stavudine in bulk and pharmaceutical dosage forms. International journal of Chem Tech Research, 3: 18-22.
13. Mohamed, E.I., Mikre, W. (2009). Determination of lamivudine and stavudine in pharmaceutical preparations using chemometrics-assisted

spectrophotometry Saudi Pharmaceutical Journal, 17: 275-281.

14. Sarkar, M., Khandavilli, S., Panchanula, R. (2006). Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. J Chromatogr B. 830: 349-354.
15. Notari, S., Bocedi, A., Ippolito, G., *et al.* (2006) Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. J Chromatogr B., 831:258-266.
16. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline, (1994). ICH Harmonised Tripartite Guideline.
17. Code Q2B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline, (1994). ICH Harmonised Tripartite Guideline.