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Short Communication

Development of Ultraviolet Spectrophotometric Methods for Analysis of Stavudine in Bulk and Pharmaceutical Dosage Forms

Sahoo Sunit Kumar*, Sahoo Gyanasis, Sahoo Sukanta Kumar, Das Dilip Kumar and Mishra Prachiprava

University Department of Pharmaceutical Sciences, Utkal University, VaniVihar, Orissa, India

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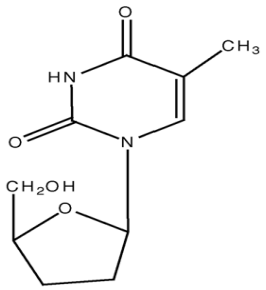
Abstract. A UV spectrophotometric method was developed and validated for the quantitative determination of stavudine, one of the first line regimens in antiretroviral therapy. The different analytical routine parameters such as linearity, precision, accuracy, limit of detection and limit of quantification were determined according to International Conference on Harmonization guidelines. Effect of various temperatures (25,50 and 60 °C) on stavudine solution in phosphate buffer pH 6.8 was also studied. Absorbance maximum in phosphate buffer pH 6.8 was found to be 266 nm. Beer's law is obeyed over concentration range of 3-24 μg/mL with correlation coefficient (r^2) value 0.999. The results were validated statistically and by recovery study. Degradation of stavudine was more at high temperature. The proposed method is highly sensitive, precise, cheap, reliable and less time consuming for estimation of stavudine in bulk as well as in pharmaceutical dosage forms.

Keywords: stavudine, spectrophotometry, phosphate buffer, correlation coefficient

HIV/AIDS requires enduring treatment with potent life saving drugs including nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Stavudine (d4T:2,3-didehydro-3-deoxythymidine), a non-nucleoside reverse transcriptase inhibitor is the first line therapy in treatment of AIDS (Dieterich *et al*., 2004) and is included officially in Indian Pharmacopoeia. Stavudine is chemically a thymidine nucleoside analogue which has complete and less unpredictable oral absorption as compared to other nucleoside analogues.



Chemical structure of stavudine

In the present study, a simple and precise spectrophotometric method has been developed using pH 6.8 phosphate buffer as a solvent; it can be considered a promising simple, faster, direct and relatively less expensive alternative with sufficient reliability for the determination of active drug content in pharmaceutical formulations, in bulk and in dosage forms.

*Author for correspondence; E-mail: sahoosunitkumar@rediffmail.com

Stavudine capsule, Avostav™ (30 mg stavudine), obtained as a gift from Ranbaxy Laboratories Ltd. (Paonta Sahib), was subjected to *in vitro* quality control tests like disintegration, weight variation and assay following the Indian pharmacopoeial procedures, whereas stavudine (analysed sample), received on gratis from Cipla Ltd. (Mumbai, India), was used as such without further purification. All other chemicals used were of analytical grade. All the solutions for analysis were prepared and analyzed afresh.

A solution of Stavudine was prepared by dissolving 100 mg of standard Stavudine in 100 mL pH 6.8 phosphate buffer and suitably diluted to get a working standard solution of 60 μg/mL. Aliquots (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 mL) of working standard solution were transferred to a series of 10 mL volumetric flasks to obtain final drug concentration of 3 to 24 μg/mL. Average weight of twenty capsules were determined, and then finely powdered. The powder (30 mg) was extracted with 100 mL pH 6.8 phosphate buffer for 45 min; the suspension was filtered using Whatman # 1 filter paper. 0.5 mL of this solution was diluted to 10 mL to obtain a concentration of 15 mg/mL of Stavudine (Borgmann *et al*., 2007). The absorbance of the solution was determined at 266 nm against a reagent blank. The repeatability of the method was established by carrying out (n = 8) the analysis of analyte (15 mg/mL) using the proposed method (Gandhi *et al*., 2008).

The accuracy of the method was evaluated by calculating the recovery of stavudine by standard addition method at concentration of 80%, 100% and 120% of the target level in

capsules (Saha *et al*., 2002). The results are presented in Table 1. The precision of the method was demonstrated by inter-day and intra-day variation studies using three different concentrations of the drug (in addition to calibration standards) covering the entire linearity range (Basavaiah and Kumar, 2006). In intra-day studies, drug was analyzed on the same day while inter-day precision was determined by analyzing drug for three days and results are presented as standard deviation and R.S.D. (%) in Table 2. Ruggedness of the proposed method was determined at 18 µg/mL on different instruments by different analyst under similar environment (El-Saharty *et al*., 2002). The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability (Argekar and Swant, 1999). The LOD and LOQ were calculated as $3.3\sigma / S$ and $10\sigma / S$, respectively, where σ is the standard deviation of the standard concentration and S is the slope of the standard curve. In order to determine the effect of solution temperature on stavudine solution, it was kept at 25, 50 and 60 °C for both 3 h and 6h and the drug degraded (%) was calculated.

Table 1. Results of precision studies

Amount taken (µg/mL)	Inter-day		Intra-day	
	Amount found (µg/mL)	RSD (%)	Amount found (µg/mL)	RSD (%)
12	11.94 ± 0.055	0.468	11.83 ± 0.224	1.95
15	14.91 ± 0.058	0.389	14.89 ± 0.080	0.54
18	17.89 ± 0.094	0.527	17.83 ± 0.124	0.704

Each value is the mean ± standard deviation of three observations.

Table 2. Recovery of stavudine capsule by the proposed method

Sample ID	Concentration of pure drug (µg/mL)	Concentration of capsule formulation (µg/mL)	Recovery of pure drug (%) (*n*=3)	RSD (%)
C : 80%	12	15	100.631 ± 0.414	0.411
C : 100%	15	15	99.012 ± 0.452	0.457
C : 120%	18	15	99.752 ± 0.280	0.281

Each value is the mean ± standard deviation of three observations.

The optical characteristics are: Beer's law limit (8-24 µg/mL), absorbance maxima (266 nm), molar absorptivity (472.079 L/M/cm), sandell's sensitivity (0.0212 µg/cm/0.001 absorbance unit), correlation coefficient ($r^2 > 0.999$), regression equation $y = 0.045X + 0.009$, (slope m = 0.045 and intercept c = 0.009, relative standard deviation (0.161%), range of error was found to be 0.101% and 0.077% at 0.05 and. 0.01 confidence limit, respectively. Molar absorptivity and sandell's sensitivity show that the method is perceptive. Limit of detection (LOD) and limit of quantification (LOQ) (Argekar and Sawant, 1999) were 0.0.194 and 0.578 µg/mL, respectively. The assay value for the marketed formulations was found to be 99.40% with R.S.D., - 0.149%.

The intra- and inter-day precision (RSD%) at different concentration levels (Basavaiah and Kumar, 2006) were found to be less than 2% (Table 1) indicating the meticulousness of the proposed method (Sarkar *et al*., 2006). Accuracy of the method was calculated by percentage mean recovery (*n* = 3). The recovery studies were carried out by the addition of standard analyte to the preanalyzed sample (Saha *et al*., 2002). The mean percentage recovery was found to be 99.75 for capsules (Table 2). Low values of percentage relative standard deviation, 0.111 and 0.148%, were observed in the ruggedness studies of the proposed method (El-Saharty *et al*., 2002). Higher temperatures (50 and 60 °C) had a deleterious effect on the drug stability (Table 3) as more than 70% drug degraded at the end of 6 h whereas at 25 °C it was below 3% (*n* = 3). Weight variation of the commercial capsules of stavudine was found to be 1-2% and all the capsules disintegrated within 2.5 min indicating the formulations were within the prescribed limits.

Table 3. Stavudine solution degradation (pH 6.8) at various temperatures

Temperature of solution (°C)	Degradation (%)	
	3 h	6 h
25	2.53	2.66
50	48.60	72.13
60	61.62	86.81

Each value is the mean of three observations.

The proposed UV method is simple and consistent providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover, the shorter duration of analysis of Stavudine makes this method suitable for routine quantitative analysis of the drug in bulk as well as in its pharmaceutical dosage formulation.

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