



## **SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF STAVUDINE RESIDUES IN SWAB SAMPLES**

**ROHIT DUTT\*, VANDANA DUTT, KUMAR GAURAV, D. P. PATHAK<sup>a</sup>  
and GAJENDRA SINGH<sup>a</sup>**

Guru Gobind Singh College of Pharmacy, YAMUNANAGAR – 135001 (Haryana) INDIA

<sup>a</sup>Department of Pharmacy, Post Graduate Institute of Medical Sciences,  
ROHTAK (Haryana) INDIA.

### **ABSTRACT**

The objective of present investigation was to develop a simple, cheap, fast, accurate, sensitive and precise UV spectrophotometric method that can be used for the determination of stavudine residues in swab samples. The drug standard solution was scanned over entire UV range and a wavelength of 266 nm was selected for analysis. The swabbing procedure was optimized in order to obtain a suitable recovery from stainless steel surface using Tex Wipe Polyurethane swab stick. The drug solution showed sufficient absorption at 266 nm and low quantities of stavudine may be detected correctly. A mean recovery of 99 % was obtained, when swabs dipped in water were used. Beer's law is valid in concentration range of 1-20 mcg/mL. The results were found to be satisfactory with good stability up to 48 hours at ambient temperature.

**Key words:** Spectrophotometric, Stavudine, Cleaning validation, Swab sample.

### **INTRODUCTION**

Cleaning validation is gaining importance as various regulatory authorities are putting stress for the need of establishment of cleaning validation program in pharmaceutical industries<sup>1-3</sup>. The main purpose of cleaning validation is to prevent cross contamination<sup>4</sup>.

The FDA has not published specific guidelines on the issue of setting acceptance specification because of multipurpose use of equipment<sup>4</sup>. One set of acceptance criterion is unlikely to apply all products and type of equipments. Therefore, pharmaceutical

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\* Author for correspondence; Rohit Dutt, 35- Indira Colony, Jagadhari, District- Yamunanagar -135001, Haryana; Phone No. – 9896732222; E-mail- rohitdatt23@rediffmail.com

companies are expected to establish acceptance criteria based on scientific and logical rational. Several acceptance criteria have been reported in literature <sup>5</sup>.

Chemically, stavudine is designated as 2', 3'-didehydro-3'-deoxythymidine. Stavudine is an analog of thymidine. It is phosphorylated by cellular kinases into active triphosphate. Stavudine triphosphate inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA synthesis by incorporating into it<sup>6,7</sup>. Very few methods appeared in the literature for the spectrophotometric determination of stavudine <sup>8, 9</sup>. No spectrophotometric study has been described for the residual determination of stavudine in swab samples. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for swab sample analysis of stavudine. The analytical method has been validated in terms of linearity, precision, accuracy, LOD, LOQ and selectivity.

## EXPERIMENTAL

### Reagents and chemicals

The stavudine working standard was an in house qualified (Ranbaxy Lab.Ltd.) standard having potency of 99.5 % (w/w). All reagents used, were of analytical grade, supplied by Rankem, Ranbaxy Fine Chemicals Ltd. The water used in the preparation of sample and standard solution was purified through a Milli-Q-System (Millipore USA). The sample and standard solution was filtered through a 0.45  $\mu$  membrane filter (Type BD, Millipore, USA). Swab sampling was achieved by using clean Tip Tx <sup>Tm</sup> 714 A swabs from Tex Wipe Company (Upper Saddle River, NJ).

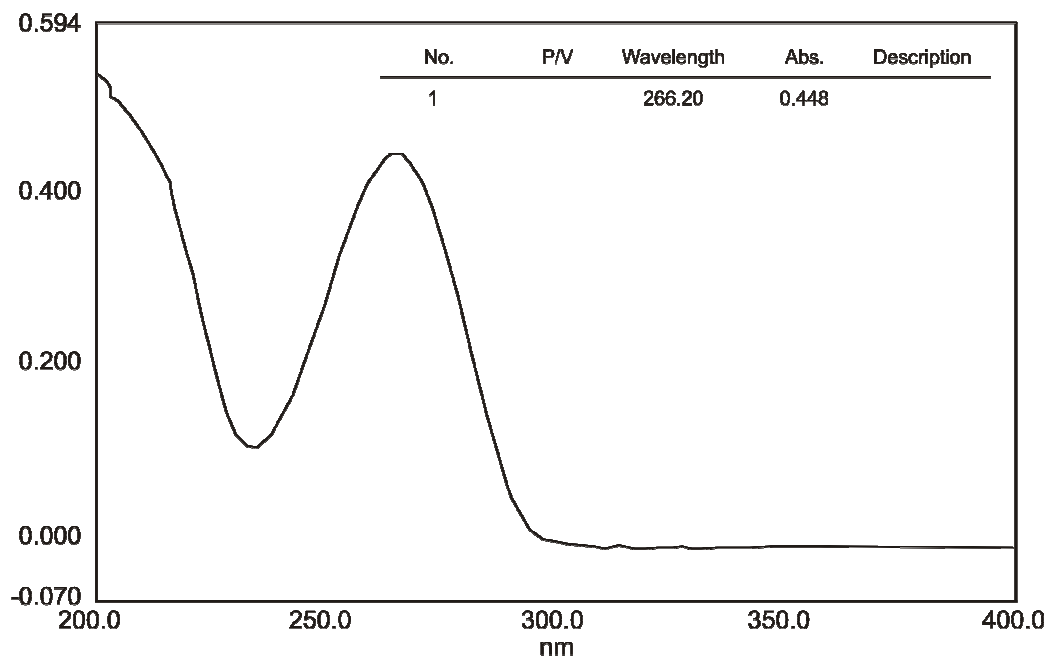
### Equipments

The instrument used in the experiment was UV-Visible spectrophotometer (UV 2450, Shimadzu, Japan) controlled by a UV probe (Version 1.10) software. An AG-285 analytical balance with LC-P45 printer was used (Mettler Toledo, USA). An ultrasonic bath from Toshniwal Instruments Pvt. Ltd. (Toschon) was used.

### Preparation of standard solution

A stavudine stock solution was prepared by accurately weighing about 25 mg of stavudine working standard and transferring it into a 100 mL volumetric flask. 10 mL of methanol was added to it and sonicated to dissolve. Volume was made up with a mixture of methanol-water (50 : 50 v/v). 4 mL of this solution was further diluted to 100 mL with a mixture of methanol-water (50 : 50 v/v) to obtain standard solution of 10 mcg/mL

concentration.



**Fig. 1: UV Scan of standard**

### **Determination of absorbance maxima**

The standard solution (10 mcg/mL) was scanned against a solvent blank between 200–400 nm. A spectrum was recorded and the suitable absorption maximum was selected as 266 nm.

### **Preparation of calibration standard**

A stavudine stock solution was prepared by accurately weighing about 25 mg of stavudine working standard and transferring it into a 100 mL volumetric flask. 10 mL of methanol was added to it and sonicated to dissolve. Volume was made up with a mixture of methanol -water (50 : 50 v/v). This solution was further diluted with a mixture of methanol-water (50 : 50 v/v) in order to obtain the calibration solutions at various concentration levels i.e. 1, 2, 5, 10, 15, and 20 mcg/mL.

### **Sample preparation**

**For recovery studies of stavudine from clean tip swabs and stainless steel plate**

The surface tested was stainless steel (2 x 2 sq.inch.) plate prepared in-house. A spiking solution was prepared by dissolving 25 mg of stavudine into 100 mL of methanol. The sample preparation for controlling the cleaning step of manufacturing process was performed as followed:

Heads of fine T<sup>x</sup> T<sup>m</sup> 714A swab sticks were rinsed with water. Using appropriate glass syringe spiking solutions (7.5, 10 and 12.5 mcg/mL) were transferred on to three sets of five plates each. The solution on the test surfaces were allowed to dry. Swabs sticks previously placed in 20 mL glass test tubes containing 5 mL water were used for swabbing the stainless steel plate. Swabbing was done first in horizontal and then in vertical direction. Finally, swab sticks were put again in 20 mL test tubes and 5mL of extraction solvent (methanol) was added to each test tube and sonicated for 5 minutes at ambient temperature. This resulted in spiked sample solution at three-concentration level i.e. 7.5, 10, 12.5 mcg/mL, respectively. Finally, absorbance of these sample solutions was measured at detection wavelength of 266 nm.

### **Procedure for actual samples collected from the equipment train**

5 mL aliquot of the extraction solvent (methanol) was pipetted directly on top of swab sample contained in glass test tubes having 5 mL of water. Each sample test tube was wrapped and sonicated it for 5 min. The extracted stavudine sample solution was filtered through 0.45  $\mu$  membrane filter before measuring the absorbance.

## **Method**

### **Spectrophotometric method**

The absorbance of sample and calibration solutions was measured against a solvent blank at detection wavelength of 266 nm. The amount of residual stavudine was determined by comparing the stavudine absorbance obtained from the sample to linear calibration curve.

## **RESULTS AND DISCUSSION**

### **Limit acceptance level**

The following criteria were used to establish acceptance criterion:

#### **Visual inspection**

Visual limit is estimated to be 100 mcg/ 4 sq. inch for each equipment.

## 10 ppm criteria

Generally, this criteria is preferred along with visual inspection. Calculation formula under this criteria is –

$$\text{SRAL} = \frac{\text{R} \times \text{SBS} \times 1000 \times 4}{\text{U}}$$

SRAL = Specific residual acceptance limit / 4 sq. inch.

R = 10 mg ingredient of product stavudine / kg of next product.

SBS = No. of kg per batch of next product

U = Total contact surface area of all equipments used for processing expressed as sq. inch

## Development of spectrophotometric method

The main objective in the present study has been to develop a sensitive, accurate, precise, linear and selective spectrophotometric method for the analysis of trace level of stavudine in swab samples. Detection wavelength of 266 nm was selected for analysis because the drug has sufficient absorption and low quantities of stavudine residues may be detected correctly. Furthermore, the calibration curve obtained at 266 nm showed good linearity.

## Validation of spectrophotometric method

The validation of spectrophotometric method was carried out by determining the selectivity, linearity, precision, accuracy, quantitation and detection limit. Stability of analytical solution was also investigated.

## Stability of stavudine in cleaning swab

Stability of stavudine in cleaning swab was studied at three concentration levels (5, 10, and 15 mcg/mL) up to 48 hours. The cumulative RSD values were determined by making three replicate measurements at each concentration level investigated. The results of stability studies at various time intervals (0, 4, 8, 12, 24 and 48 hours) are presented in the Table 1, which suggest that standard solutions are stable up to 48 hours, as cumulative RSD values were less than 10%.

**Table 1: Stability studies of stavudine**

Concentration (mcg/mL)	Time interval (hours)	Cumulative % RSD
(n = 18)	0-48	2.45
(n = 18)	0-48	1.89
15 (n = 18)	0-48	1.74

**Linearity**

Linearity was studied over the drug concentration range from 1-20 mcg/mL (n =12). The correlation coefficient ( $R^2 = 0.99998$ ) obtained for regression line showed excellent linear relationship between absorbance and concentration of stavudine. Results of linearity studies are shown in Table 2.

**Table 2: Validation of the spectroscopic method for determination of stavudine**

Validation criterion	Concentration (mcg/mL)	Results
<i>Linearity of response:</i>		
<i>Absorption maximum</i>	1-20	266 nm
Beer's law limit	1,2,5,10,15,20	1-20
Slope	-	0.045
Intercept	-	0.001
Regression equation	-	$Y = 0.001 + 0.045 X$
LOD	0.75	RSD = 19.10%
LOQ	2.3	RSD = 5.25%

**Precision:**

(a) Repeatability

% RSD (n = 6) 10 0.74%

(b) Intermediate precision

% RSD (n = 6) 10 0.14%

Cont...

Validation criterion	Concentration (mcg/mL)	Results
<b>(c) Reproducibility</b>		
% RSD (n = 6)	10	1.95%
<b>Accuracy:</b> (Mean % Recovery)		
(n = 5)	7.5	95.89%
(n = 5)	10	95.80%
(n = 5)	12.5	99.75%

### Precision

The precision of spectrophotometric method reported, as % RSD, was estimated by measuring repeatability, time dependent intermediate precision and reproducibility by measuring absorbance of six individual preparation of standard solution of stavudine. The % RSD value represented in Table 2 was less than 5%, that illustrates the good precision of this analytical method.

The precision of swabbing procedure was evaluated by considering the repeatability and reproducibility by performing recovery studies at three concentrations level of stavudine (7.5, 10 and 12.5 mcg/mL). The results expressed, as % RSD, are included in Table 3. The obtained values were found to be less than 5 % that revealed the good precision of swabbing procedure.

### Accuracy

The accuracy of the procedure was assessed by comparing the analytical amount determined vs known amount (Standard 10 mcg/mL) spiked at three concentration level (7.5, 10 and 12.5 mcg/mL) with five replicates (n = 5) for each concentration level investigated. The accuracy defined as, mean %age recovery, of 92 % indicated that spectrophotometric method developed for the stavudine could be considered as accurate with in the concentration range investigated as clearly indicated in Table 2.

### Limit of detection and quantitation

The LOD and LOQ of stavudine estimated from the intercept (a) of the regression line and corresponding standard deviation was found to be 0.75 mcg/mL and 2.30 mcg/mL respectively and results are clearly indicated in Table 2. Further, the estimated values of LOD and LOQ were confirmed by measuring RSD values (precision) of six measurements

of respective concentration for LOD (RSD = 19.10%) and LOQ (RSD = 5.25%).

**Table 3: Precision of swabbing procedure**

Amount of stavudine (mcg/mL)	Repeatability % RSD (n = 5)	Reproducibility % RSD (n = 5)
7.5	1.58	1.14
10	1.92	1.15
12.5	1.63	1.78

### Selectivity

During sample preparation, some potential contaminant substance may extract from swab tip, which would interfere with quantitation of stavudine. The selectivity was studied by comparing absorbance of swab blank solution and standard solution (10 mcg/mL) of stavudine at 266 nm. No swab interference was found in terms of absorbance of analyte.

### Swab sample analysis

The results obtained for the determination of stavudine in actual swab samples collected from critical sites on major manufacturing equipments are presented in Table 4.

**Table 4: Actual swab sample analysis (UV method)**

Equipment	Area swabbed (Sq. inch)	Absorbance	Drug content (µg/mL)
Cage blender (CBL01)	Total = 3930	0.235	5.21
Coating machine (GAC01)	Total = 3100	0.145	3.21
Coating machine (GAC01)	Total = 1365	0.168	3.72
Rotary compression machine (RCM01)	Total = 1365	0.210	4.65
Vibro sifter (VBS01)	Total = 1250	0.075	1.66
Double cone blender (DCB01)	Total = 1120	0.125	2.77

## CONCLUSION

The proposed method is simple, rapid and sensitive and hence, it can be used for



the routine determination of swab samples in quality control laboratories. The method requires inexpensive chemicals and can give rapid results as compared to other analytical techniques.

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