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SPECTROPHOTOMETRIC DETERMINATION OF AN ANTIRETROVIRAL DRUG STAVUDINE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Two simple, sensitive and accurate spectrophotometric methods have been developed for the determination of stavudine in pure and dosage forms. Method-A is based on the reaction of stavudine(STD) with 1,2-napthaquinone-4-sulphonic acid (NQS) to form N-alkylamono naphthaquinone by replacement of the sulphonate group of the naphthaquinone sulphonic acid by an amino group. The colored chromogen shows absorption maximum at 464 nm and linear within the limits 2.0-12.0 µg/mL. Method-B is based on oxidation of the drug with 1, 10-phenanthroline producing red colored chromogen which is measured at 480nm. Beer's law is obeyed in the concentration range of 5.0-30.0 µg/mL for the developed method. Different experimental parameters affecting the color development and stability of colored product are carefully studied and optimized. The developed methods could be successfully applied to pharmaceutical formulations. The results obtained are in good agreement with those obtained using official methods.

Keywords: Stavudine, Spectrophotometry, dosage forms 1, 2-napthaquinone-4-sulphonic acid (NQS), 1, 10-phenanthroline (O-PHEN).

INTRODUCTION

Stavudine is a white to off-white crystalline solid with the molecular formula $C_{10}H_{12}N_2O_4$ and a molecular weight of 224.2 grams. Its solubility at 23°C is approximately 83 mg/mL in water and 30 mg/mL in propylene glycol. It is chemically known as 2'-3'-didehydro-2'-3'-dideoxythymidine, d4T. It is a nucleoside analog reverse transcriptase inhibitor (NARTI) active against HIV. d4T. It is approved by the U.S. Food and Drug Administration (FDA) for pediatric use. FDA granted approval for three generic formulations of stavudine. The approved generic formulations are stavudine capsules (15 mg, 20 mg, 30 mg and 40 mg), and stavudine for oral solution (1 mg/mL), both manufactured by Aurobindo Pharma; and stavudine capsules (15 mg, 20 mg, 30 mg and 40 mg) manufactured by Hetero Drugs Limited, both of Hyberdad, India. Each capsule also contains inactive ingredients microcrystalline cellulose, sodium starch glycolate, lactose, and magnesium stearate. The hard gelatin shell consists of gelatin, titanium dioxide, and iron oxides. Zerit for Oral Solution is supplied as a dye-free, fruit-flavored powder in bottles with child-resistant closures providing 200 mL of a 1 mg/mL stavudine solution upon constitution with water per label instructions. The powder for oral solution contains the following inactive ingredients: methylparaben, propylparaben, sodium carboxymethylcellulose, sucrose, and antifoaming and flavoring agents.

The literature survey indicates that several analytical methods such as reverse phase HPLC, spectrophotometric methods are reported for the determination of stavudine in pure and formulations and mostly in biological fluids. Mohamed et al reported a chemometric assisted spectrophotometric method[1] for the determination of stavudine in combination with lamivudine in pharmaceutical formulations.UV spectrophotometric methods[2] is reported for the estimation of stavudine. Basavaiah et.al developed a

titrametric and visible spectrophotometric method[3] using bromate and bromide using dyes for the estimation of stavudine in tablets. Several methods are reported for the determination of stavudine in combination with several other HIV drugs in biological fluids by liquid chromatography- tandem mass spectrometry[4-6]. A number of methods are reported based on high performance liquid chromatography for the determination of the stavudine in biological fluids[7-12] in combination with other HIV drugs. Determination of stavudine/ didanosine/saquinavir and stavudine/didanosine/efavirenz in human serum by micellar electrokinetic chromatography[13], several HPLC methods[14-21] are reported for the assay of stavudine in formulations.

EXPERIMENTAL

Instrumentation

An ELICO SL-159 model, 2nm high resolution, double beam, 1cm length quartz coated optics; Wavelength range190-1100nm; High stability, linearity, precision instrument is used for all the spectral measurements. All chemicals and reagents used in the analysis are of analytical grade and doubly distilled water is used for the preparation of all the solutions.

Materials and Methods

All the chemicals and reagents used are of analytical reagent grade. Stavudine reference standard is kindly gifted by Hetero Drugs, Hyderabad. Pharmaceutical preparations containing stavudine capsules named as zerit contain 15mg, 20mg, 30mg and 40mg per tablet are purchased locally.

Preparation of drug standard solution

100mg of the Stavudine is accurately weighed and transferred into a 100mL standard flask and dissolved with doubled distilled water and made up to the mark with constant shaking and then 10mL and 20.0mL of this solution is accurately transferred into two100mL standard flasks by means of a burette and made up to the mark with doubled distilled water to obtain 100µg/mL and 200µg/mL working standard solutions.

Preparation of Reagents

0.5% (w/v) solution of NQS is prepared by dissolving 500mg of the NQS in 100mL of double distilled water, 20% (w/v) solution of NaOH is prepared by dissolving 20.0g of sodium hydroxide pellets in double distilled water and made up to 100mL, 0.054% (w/v) Fe (III) solution is prepared by dissolving 54mg of anhydrous ferric chloride in 100mL of double distilled water, 0.2% (w/v) o-phenanthroline is prepared by dissolving 200mg of the reagent in 100mL of double distilled water with warming and 1.27% (v/v) O-phosphoric acid solution is prepared by diluting 1.27 mL of laboratory reagent (AR Grade) of o-phosphoric acid to 100mL with distilled water.

Experimental Procedure

Method –A

Aliquots of working standard solution of STD (0.2mL-1.2mL, 100µg/mL) are transferred into a series of 10mL calibrated test tubes. Then 2mL of NaOH and 0.5mL of NQS reagent solutions are added to each tube and the contents are kept aside for 2min. at room temperature. The solutions are made up to the mark with double distilled water. The absorbance is measured from 350 to 600nm against a reagent blank prepared similarly and maximum absorbance is found at 464 nm(Fig.1). The amount of the STD is calculated from its calibration graph (Fig.3)

Method-B

Different portions (0.5- 3.0mL, 200µg/mL) of standard STD solution is delivered into a series of 20mL calibrated tubes and then 1.5mL of 3.32×10^{-3}M of Fe (III) solution, 2.0mL of 1.10×10^{-2}M o-phenanthroline are added successively. The total volume in each tube is brought to 10mL with distilled water. The tubes are kept on a boiling water bath for 30min. The tubes are removed and cooled to room temperature.2.0mL of 2.0×10^{-2}M of o-phosphoric acid is added and volume in each tube is made up to the mark with distilled water. The absorbance of the colored complex solution is measured after 5min against a reagent blank prepared similarly except drug from 400 to 600nm and maximum absorbance is found to be at 480nm(Fig.2). The amount of the drug is computed from the appropriate calibration graph (Fig.4).

Optimum conditions established in developing Methods

The optimum conditions for the developed methods are fixed based on the study of the effects of various parameters such as concentration of the drug standard, type of acid, concentration of the acid, concentration of the reagents such as NQS and Fe(III) and o-phenanthroline and choice of the base. Control experiments are carried out by measuring absorbance at 464 nm and 480 nm of series of the solutions varying one and fixing the other parameter for method A&B respectively.

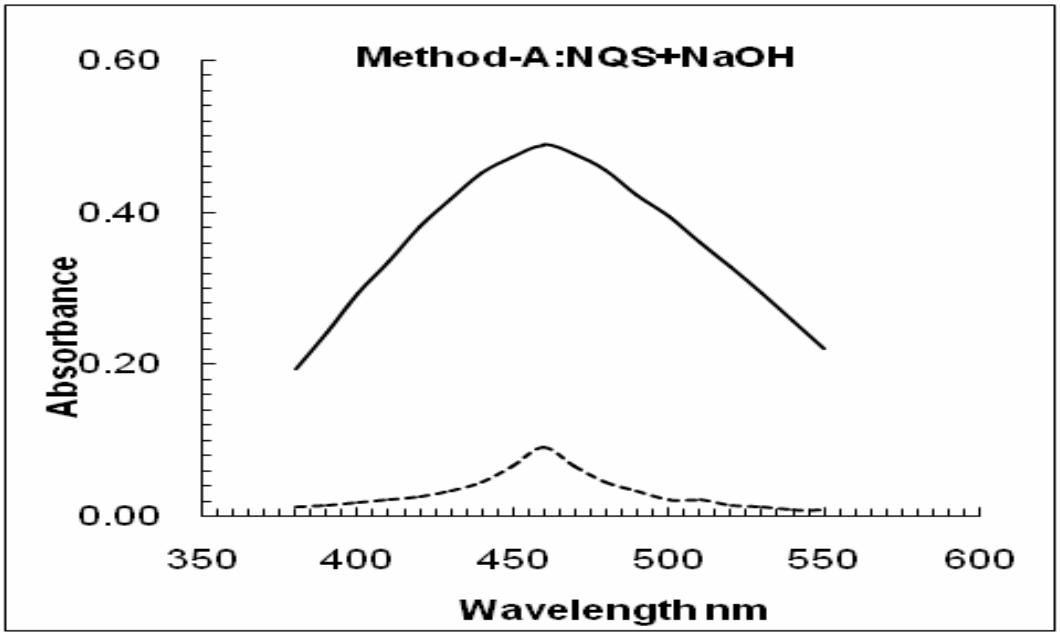


Fig.-1: Absorption spectra of Stavudine with NQS/NaOH

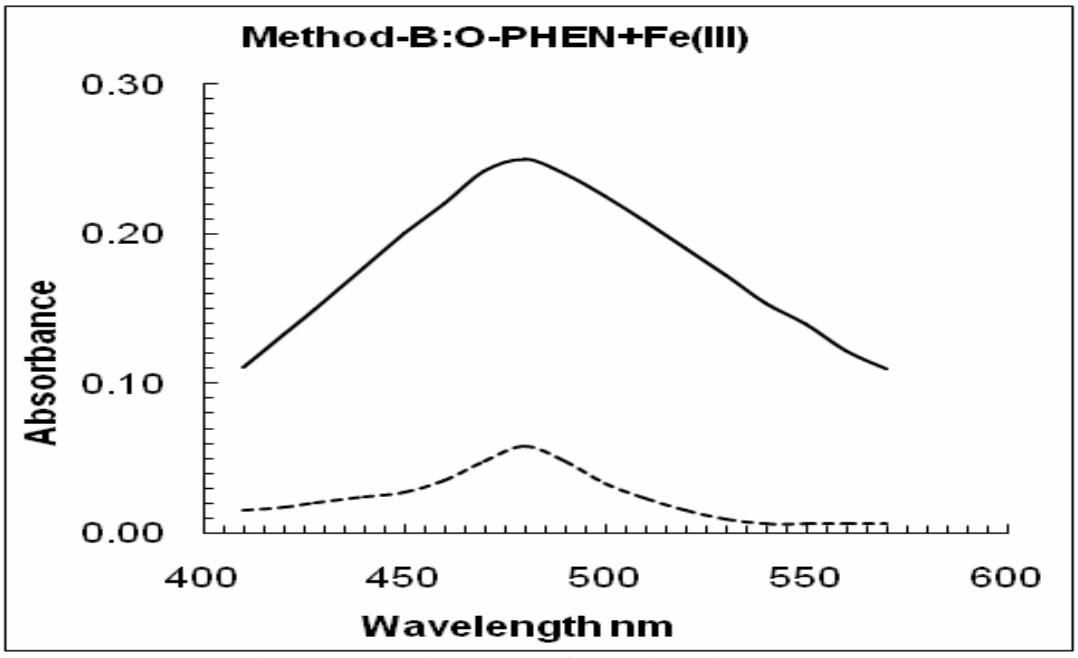


Fig.-2L: Absorption spectra of Stavudine with Fe (III)/O-PHEN

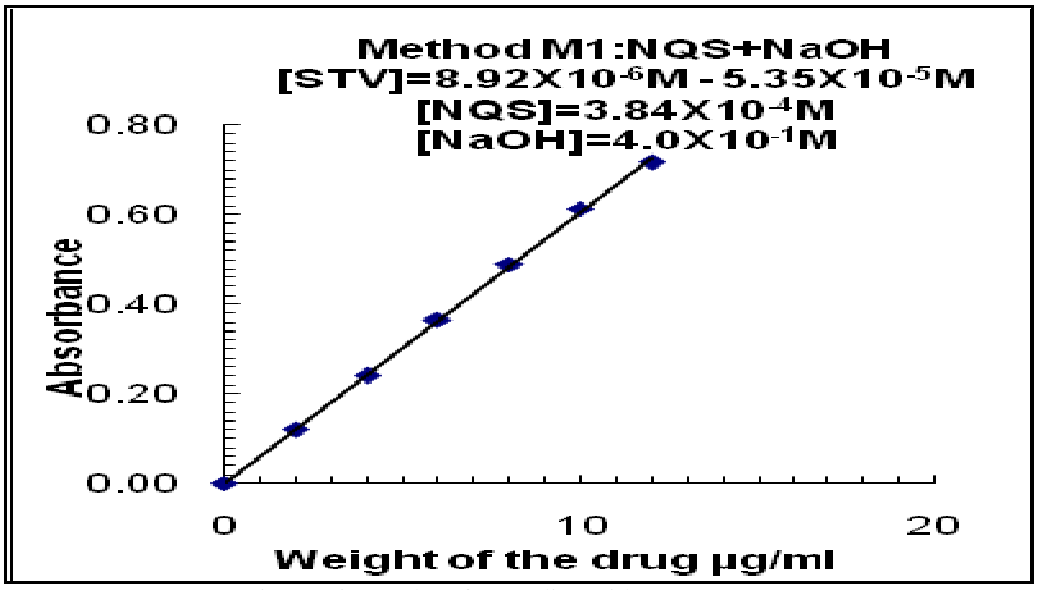


Fig.-3: Linear plot of Stavudine with NQS

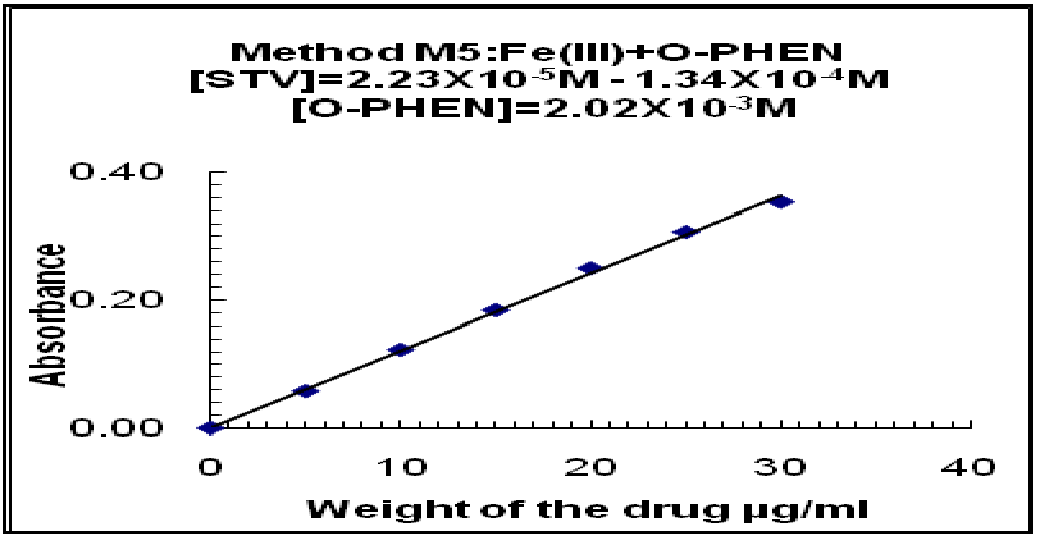


Fig.-4: Linear plot of Stavudine with Fe (III)/O-PHEN

RESULTS AND DISCUSSION

In order to test whether the colored products formed in these methods adhere to Beer's law, the absorbences at maximum wavelength of a series of six concentrations are plotted against concentration of the drug in µg/mL (Fig.3 and Fig.4). Beer's law is obeyed within the limits 2.0-12.0 µg/mL and 5.0-30.0 µg/mL , molar absorptivity is found to be $1.35x10^4$ and $2.68x10^3$ for method-A and Method-B respectively. Regression analysis of the Beer's law plots at λ_{max} reveals a good correlation. The graphs

show negligible intercept and are described by the regression equation, Y= *bC* + *a* (where *Y* is the absorbance of 1 cm layer, *b* is the slope, *a* is the intercept and *C* is the concentration of the measured solution in µg/mL). The high molar absorptivites of the resulting colored complexes indicate the high sensitivity of the methods.

Precision of the developed methods is ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of the test in total solution. The percent of relative standard deviation and percent range of error are calculated for the developed methods. To determine the accuracy of these methods, three different amounts of bulk samples within the linearity limits are prepared and analyzed by the developed methods. The percent recoveries of the drug by these methods are found to be within the range which indicates that the developed methods are accurate.

Optical characteristics, linear regression parameters, precision and accuracy of the proposed methods are presented in Table-1. These methods have been successfully applied for the determination of stavudine in pharmaceutical preparations. Zerit 30.0mg and 40.0mg are taken for the analysis. The percent of recovery of the drug is calculated and is compared with a reference method statistically by means of t-test and F-test at 95% confidence level and found the developed methods are not significantly different. The results obtained by the developed methods are shown in Table-2.

Table-1; Optical characteristics, Regression parameters, Precision and Accuracy of the proposed methods

Parameter	Method-A	Method-B
Maximum Wavelength λ_{max}	464 nm	480 nm
Beer's Law Limits µg/mL	2.0-12.0	5.0-30.0
Optimum photometric Range µg/mL	4.0-10.0	10.0-30.0
Sandell's Sensitivity ($\mu g/cm^2$/0.0001 Absorbance)	1.67E-02	8.62E-02
Molar Absorptivity Lt/mole/cm	1.35E+04	2.68E+03
Slope (b)[a]	6.03E-02	1.20E-02
Intercept(a)[a]	1.27E-03	3.20E-03
Standard Deviation on slope(S_b)	6.53E-04	2.59E-04
Standard Deviation on slope(S_a)	5.09E-03	5.04E-03
Standard Error on Estimation(S_e)	7.13E-03	7.05E-03
Correlation Coefficient (r)	0.9996	0.9985
Standard Deviation (S)	0.1686	0.2197
%Relative Standard Deviation[b]	2.1213	1.1025
0.05 level confidence limit	0.2774	0.3615
Limit of Detection (LOD)µg/mL	0.2534	1.2634
Limit of Quantification (LOQ)µg/mL	0.8445	4.2112

[a]Regression equation Y=a+bC , Where Y stands for absorbance and C is concentration in µg/mL
[b]%Relative standard deviation is calculated for six determination

Table-2: Analysis of Pharmaceutical Formulations of Stavudine

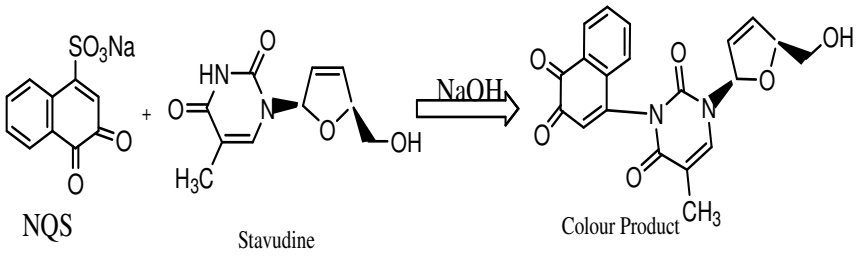
Method	Formulation	Mean[c]	SD	Proposed Method		Reference Method		F-test	t-test
				%REC	% RSD	%REC	%RSD		
Method-A	Zerit-Tablet, 30mg	30.15	0.450	100.50	1.493	101.1	1.42	1.095	0.691
	Zerit-Tablet, 40mg	40.12	0.801	100.30	1.994	99.7	1.98	1.025	0.418
Method-B	Zerit-Tablet, 30mg	30.45	0.400	101.50	1.314	101.1	1.42	1.075	0.460
	Zerit-Tablet, 40mg	39.58	0.770	98.95	1.945	99.7	1.98	1.026	0.710

[c] Mean of six determinations, %REC means percent of recovery of the formulation, %RSD means percent of relative standard deviation, SD means standard deviation

Scheme of the colored products

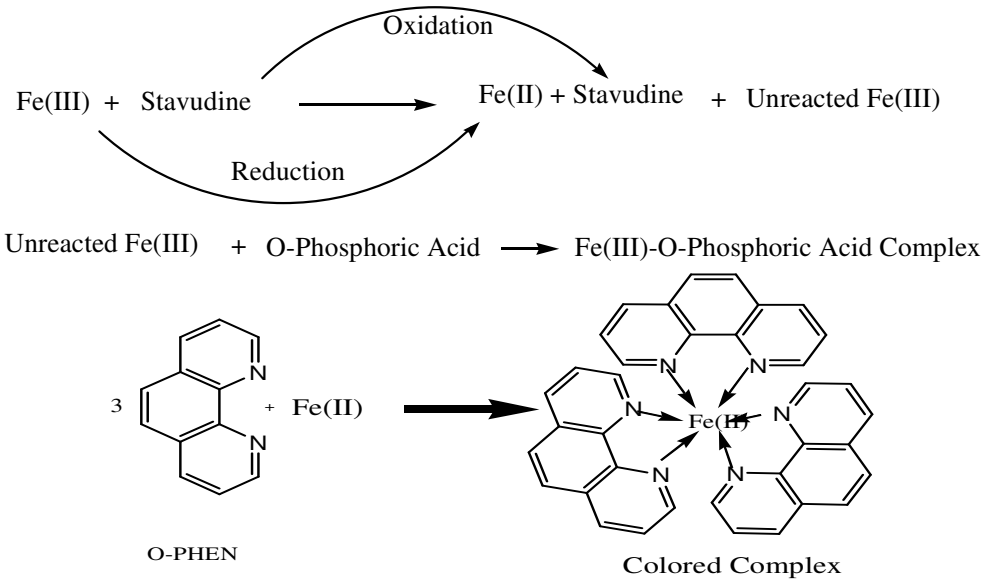
Method-A

In this method, the presence of secondary amino group of stavudine permits the development of new spectrophotometric method for its determination through the nucleophilic substitution reaction with NQS. The colored product of the reaction is given below.



Method-B

Ferric salt converts into a ferrous salt upon oxidation and can be easily detected by the usual reagent o-phenanthroline. The reduction product is tris complex of Fe (II), well known as ferroin. The colored product of the reaction is given below.



CONCLUSIONS

The developed methods are simple, sensitive, accurate and economic. These methods can be successfully applied for the analysis of pharmaceutical formulations in any laboratory.

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