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ESTIMATION OF ETRAVIRINE BY UV-VIS SPECTROSCOPIC METHOD IN TABLET DOSAGE FORMS AND ITS INVITRO DISSOLUTION ASSESSMENT.

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ABSTRACT
A simple, sensitive, rapid and reproducible UV-VIS Spectroscopic Method has been developed and validated for estimation of Etravirine simultaneously and also the comparative study of invitro data in tablet formulation. The solvent used was Acetonitrile : Water (50:50) %v/v and the λmax or the absorption maxima of the drug was found to be 310 nm. A linear response was observed in the range of 5.0-37.5µg/ml with a regression coefficient of 0.99. The invitro release of various test units was compared for their similarity using the f2 test which limits were found with in the acceptance criteria. All the validation parameters were with in the acceptance range according to ICH norms. The described method was successfully employed for quality control assay of the component simultaneously and dissolution data helpful in generating the further information regarding invivo absorption rate in tablet dosage form.

Keywords Etravirine; Invitro dissolution study; UV-VIS Spectroscopic

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INTRODUCTION

Ultra performance liquid chromatography is a new category of analytical separation science that retains the practicality and principles of HPLC, while increasing the overall interlaced attributes of speed, sensitivity and resolution, which utilizes sub-2-µm particles for the stationary phase. Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Hence this technique has gained considerable attention in recent days for pharmaceutical analysis. In this preset work the sub-2-µm particle technology has been applied for the method development and method validation of related compounds and assay

determination of etravirine API and its dosage forms. Etravirine is a diarylpyrimidine (DAPY), a type of organic molecule with some conformational isomerism that can bind the enzyme reverse transcriptase in multiple conformations, allowing for a more robust interaction between etravirine and the enzyme, even in the presence of mutations [1], etravirine can be used by patients infected with HIV that is resistant to other NNRTIs [2]. When HIV infects a CD4 cell in a person's body, it copies its own genetic code into the cell's DNA. In this way, the cell is then "programmed" to create new copies of HIV. HIV's genetic material is in the form of RNA. In order for it to infect CD4 cells, it must first convert its RNA into DNA. HIV's reverse transcriptase enzyme is needed to perform this process. NNRTIs, also known as "non-nucleosides" or "non-nukes" for short, attach themselves to reverse transcriptase and prevent the enzyme from converting RNA to DNA [3, 4]. In turn; HIV's genetic material cannot be incorporated into the healthy genetic material of the cell, and prevents the cell from producing new virus [5]. The objective of the work is to develop an economic, time-efficient, RP-UPLC method and demonstrate its stability-indicating capabilities by forced degradation followed with method validation. In this study, the kinetics of degradation of etravirine in solution and in solid state has been studied since it is a part of developmental strategy under the ICH requirements and is carried out under more severe conditions. These studies provides valuable information on stability of drug, the degradation pathways and storage of drug, and also helps in the validation of analytical methods to be used in stability studies [6-8]. Currently there are limited number of LC methods were reported for the determination of etravirine in pharmaceutical preparations. Mass spectrometry and LC have been reported for the determination of assays in human and rat plasma and its application in pharmacokinetic studies [9, 10] and some more methods for simultaneous determination of etravirine, and other antiretroviral drugs [11-13]. However there are no methods reported in the literature for the quantification etravirine and its related compounds. Hence a reproducible stability – indicating RP UPLC method was developed for the quantitative determination of etravirine and its seven impurities. This paper mainly deals with the forced degradation of etravirine drug product under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat, humidity and light. This paper also deals with validation of the developed method for the accurate quantification of impurities and assay of etravirine in bulk drug samples.

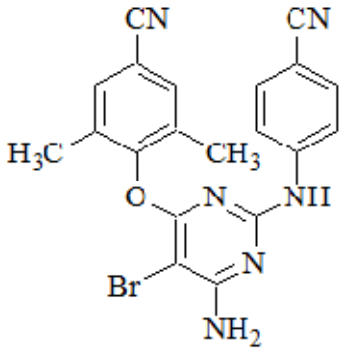


Fig. 1 Structure of Etravirine

MATERIAL AND METHODS

Chemicals and Materials:

Hetero Labs Ltd supplied Etravirine respectively. Sodium Lauryl Sulfate (Merck,AR grade), (methanol (HPLC grade) ,Acetonitrile (HPLC grade) and Hydrochloric acid (AR grade) respectively. In-house purified water (USP grade) was used throughout the study.

Dissolution parameters:

Phase –I
Medium : Degassed 0.01M HCl
Volume : 500 ml
Apparatus : Paddle (USP-II)
Speed : 50 rpm
Temperature : 37.0± 0.5°c
Time : 10 min
Phase -II
Medium : 2.25 % Sodium lauryl sulphate in 0.01M HCl (Finally we get 1% SLS in 0.01M HCl)
Volume : 400 ml
Apparatus : Paddles
Speed : 50 rpm
Temperature : 37.0°c± 0.5°c

For Single point : 60 minutes
For profile : 5 ,10 ,20,30,45,60 & 90 minutes
Note : Sampling is not required for phase -I
Instrumentation**:**
The chromatographic separations were performed using Shimadzu UV 1800 series with UV probe software -VIS Spectroscopic. Electrolab TDT-08L auto sampler dissolution apparatus were used for comparative dissolution study.

Buffer preparation:

Preparation of 2.25 % Sodium lauryl sulphate in 0.01M HCl :

Weigh and transfer about 225 gr of sodium lauryl sulphate into a beaker contgaining 10000 mL of 0.01M HCl and dissolve with the aid of sonication .

Prepartion of 0.01M hydrochloric acid solution:

Dilute 8.5 ml of hydrochloric acid to 10,000 mLwith water and mix.

For Dissolution:

Standard preparation:

Accurately weigh and transfer about 50 mg of Etravirine working standard into a 100 ml volumetric flask, add about 30 ml of methanol and sonicate to dissolve. Dilute to volume with methanol. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask. Dilute to volume with dissolution medium and mix.

Sample preparation:

Place 1 tablet each in six different vessels and operate the instrument as mentioned above. Withdraw about 10 mL of the sample solution, filter and dilute 5.0 mL of this to 25ml volumetric flask. Dilute to volume with dissolution medium and mix.

The samples withdrawn above were analyzed on UV-VIS Spectroscopic about λmax 310nm using 0.2 cm^2 cell.

Applied method to compare dissolution profiles:

The description of the in vitro dissolution profiles was calculated by using model-independent method. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f2 (similarity factor).

The specification of dissolution method is set by considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparison in the FDA's guides for industry.

For Assay:

Diluent: Prepared a mixture of (acetonitrile: Water) 50:50%v/v

Standard preparation:

Accurately weigh and transfer about 50 mg of Etravirine working standard into a 100 ml volumetric flask, add about 30 ml of methanol and sonicate to dissolve. Dilute to volume with methanol. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask. Dilute to volume with diluent and mix.

Sample preparation:

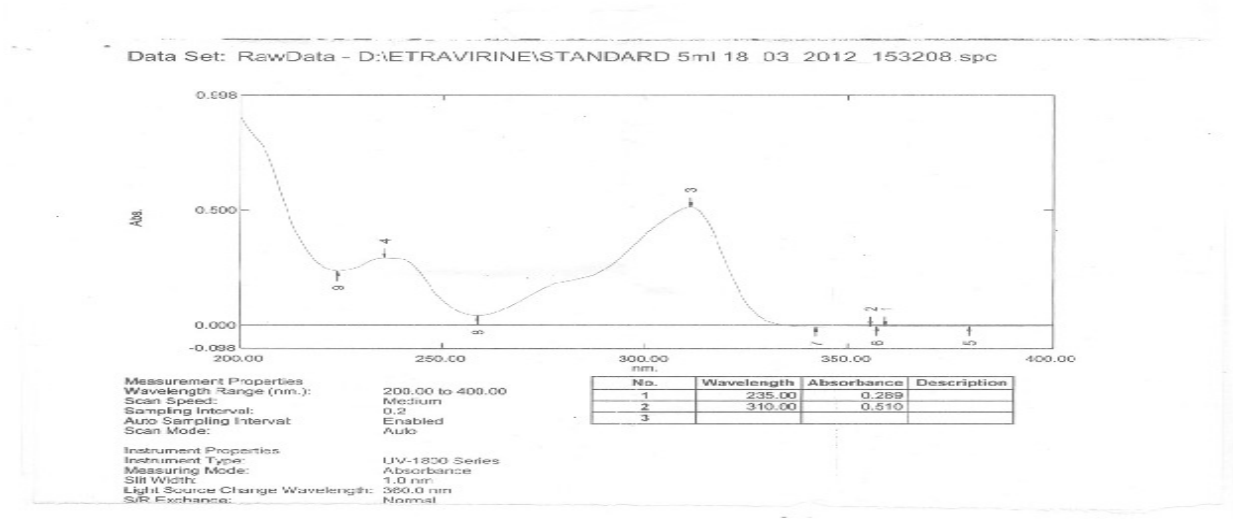
Weigh accurately tablets powdered equivalent to about 100 mg of Etravirine in to 200-mL volumetric flask. Add about 100-mL methanol and sonicate it for 30 minute to dissolve. Dilute to volume with water. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask. Dilute to volume with diluent and mix. Filtered it through 0.45 µ HVLP nylon filter.

RESULTS

The UV-VIS Spectroscopic wavelength of 310 nm was chosen in order to achieve a good sensitivity for quantitative determination of Etravirine in tablet dosage.

Table 1. Method Precision				
Compound	**Concentration (µg/mL) (n=6)**		**% Assay Mean (n=6)**	**%RSD of Assay**
Etravirine	25		99.85	1.0

Standard Solution(fig.1)



Test Solution(fig.2)

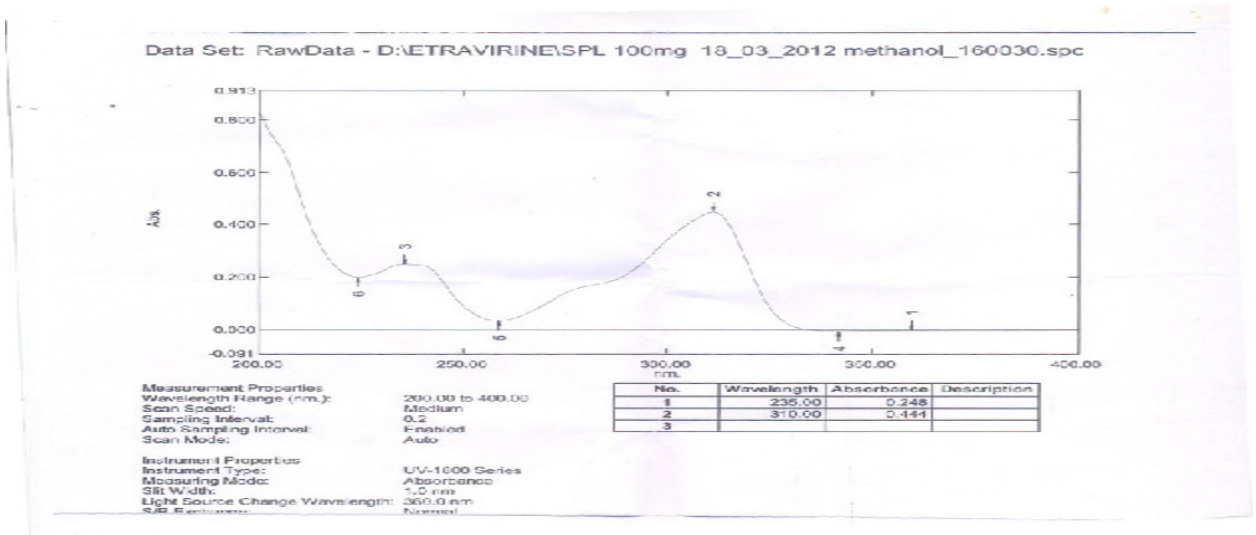
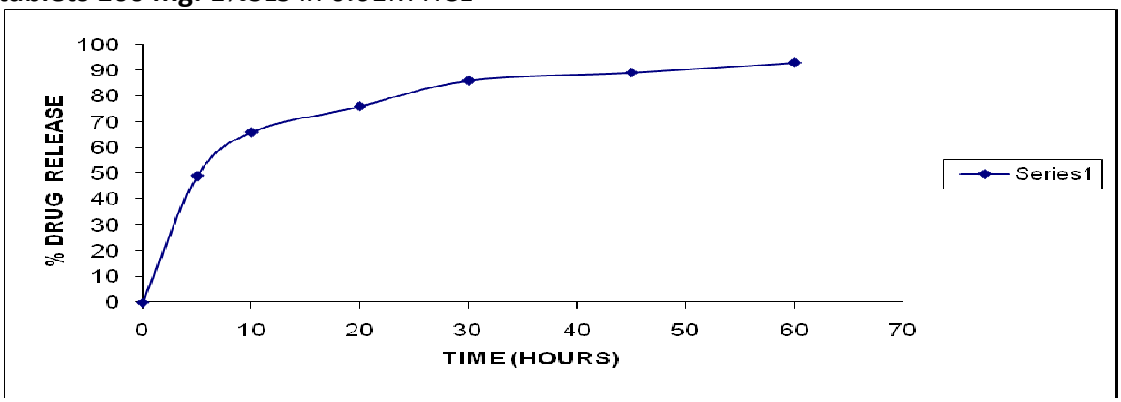


Table 2. Method Accuracy

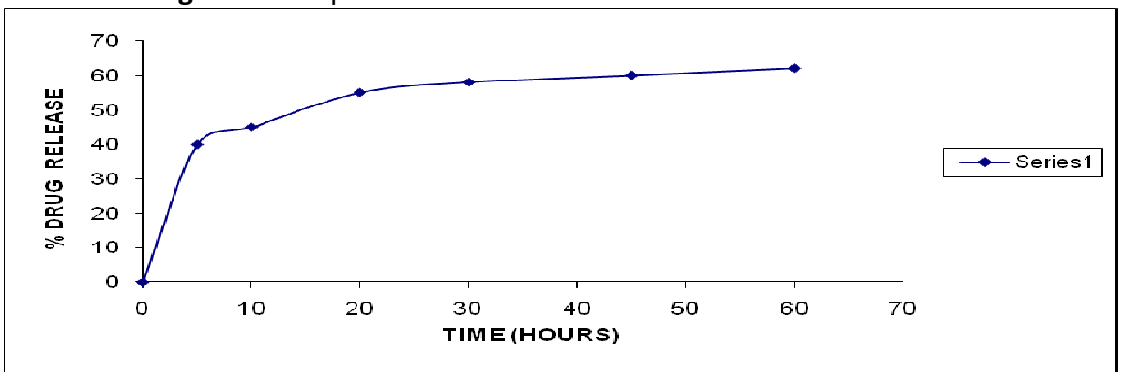
Drug	Level	Drug Added	Drug recovered	%Assay	% Assay(n=3)
Etravirine	50%	25.62	25.89	102.14	0.5
	100%	50.56	50.10	98.20	0.7
	150%	75.23	75.92	101.80	0.5

Comparative Dissolution Data:

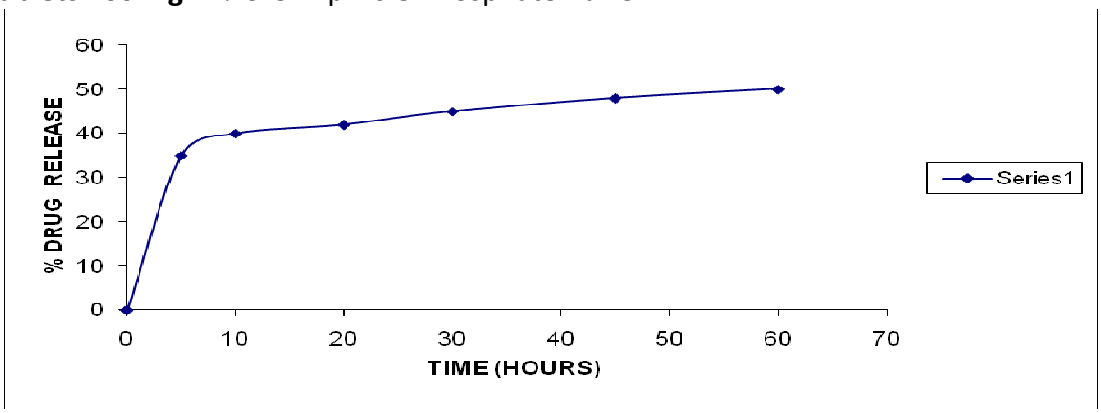
INTELENCE tablets 100 mg: 1%SLS in 0.01M HCL



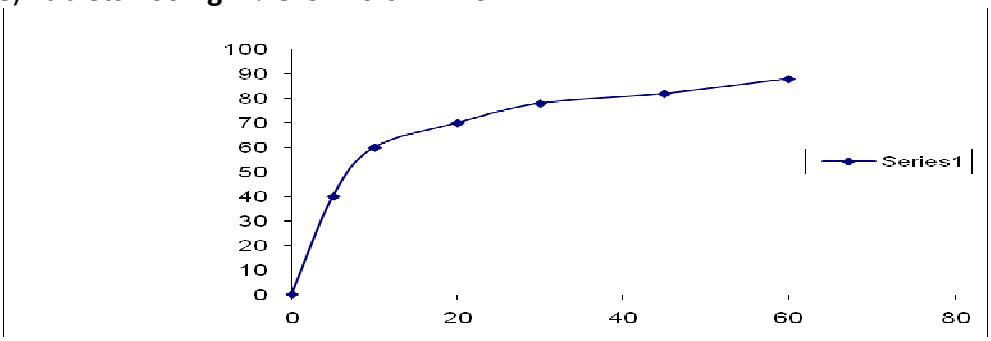
INTELENCE tablets 100 mg: 1%SLS in pH 4.5 Acetate Buffer



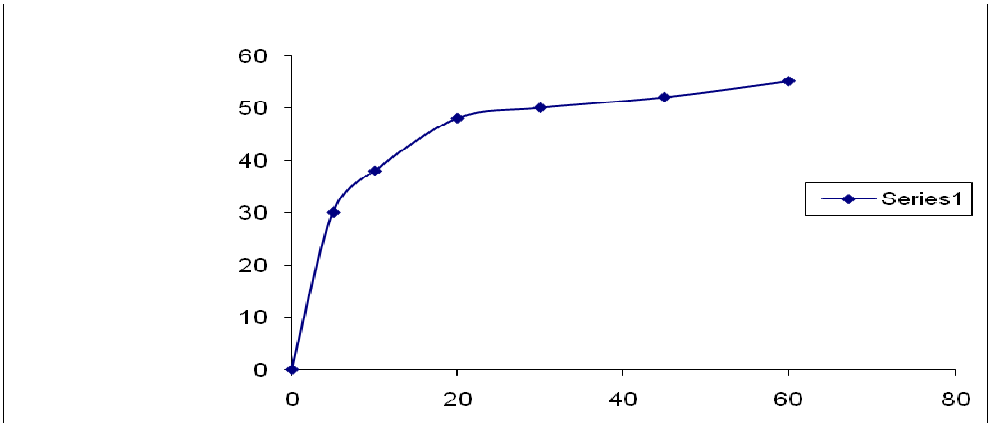
INTELENCE tablets 100 mg : 1%SLS in pH 6.8 Phosphate Buffer



Hetero (Etravirine) Tablets 100mg: 1%SLS in 0.01M HCL



Hetero (Etravirine) Tablets 100mg: 1%SLS in pH 4.5 Acetate Buffer



Hetero (Etravirine) Tablets 100mg: 1%SLS in pH 6.8 Phosphate Buffer

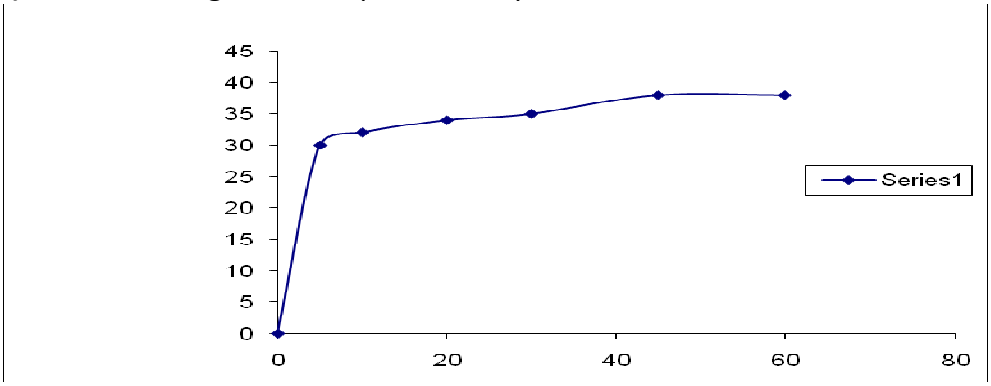


Table 3: Method Ruggedness

Day	Compound	% Assay Mean	%RSD
Day 1	**Etravirine**	99.56	0.6
Day 2	**Etravirine**	98.45	0.5

Table 4: Linearity

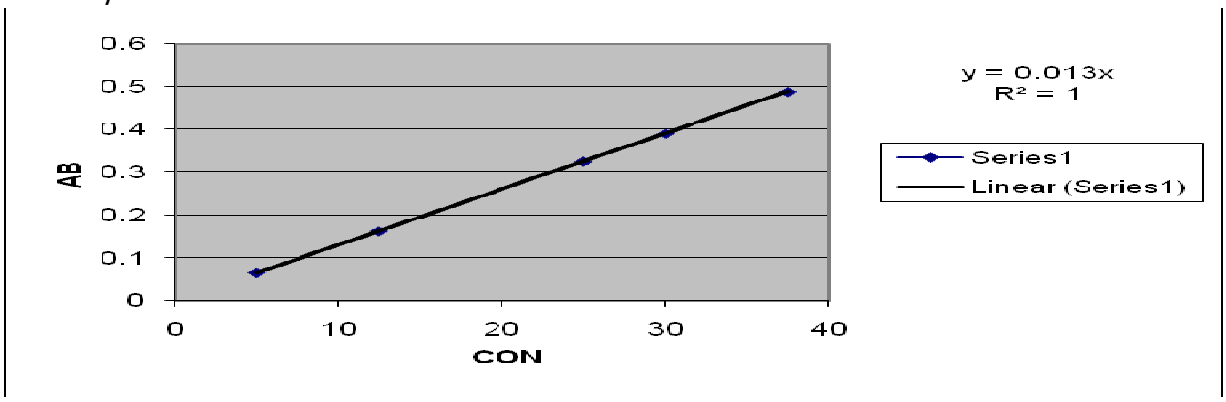


Table 5. Comparative Dissolution Profile for Etravirine tablets

	Reference (**INTELENCE)**	Test
Manufactured by	**Janassen Cilag S.P.A., Latina, Italy**,	Hetero
Apparatus	USP-II Paddle	
RPM	50	
Dissolution Media	900 ml, 1%SLS in 0.01M HCL	
% of Drug release for Etravirine		
Time in minutes	Reference	test
5	49	40
10	66	60
20	76	70
30	86	78
45	89	82
60	93	88
90	95	94
F2(similarity factor)	59.24	
Dissolution Media	900 ml, 1%SLS in pH 4.5 Acetate Buffer	
% of Drug release for Etravirine		
Time in minutes	Reference	test
5	40	30
10	45	38
20	55	48
30	58	50
45	60	52
60	62	55
90	65	58
F2(similarity factor)	55.27	
Dissolution Media	900 ml, 1%SLS in pH 6.8 Phosphate Buffer	
% of Drug release for Etravirine		
Time in minutes	Reference	test
5	35	30
10	40	32
20	42	34
30	45	35
45	48	38
60	50	38
90	52	40
F2(similarity factor)	50.84	

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 48 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Specificity:

There was no interference from Standard, sample, placebo and The values of f2 were calculated for

the dissolution in three different medias. As can be seen in Table 5 data obtained for f2 were found to be with in the acceptable criteria. In three different media phosphate buffer shows the better result and water was not found to be suitable as dissolution media.

Method precision:

The relative standard deviation for six replicate injections was less than 1.0 %, which met the acceptance criteria established for the method. The results obtained were presented in Table 1.

Accuracy/recovery:

The data presented in Table 2 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50,100, and 150% levels were with in the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method is suitably accurate for potency assay of Etravirine simultaneously in the drug substances.

Linearity:

The plot of peak area responses against concentration. It can be seen that plot is linear over the concentration range of 5.0 to 37.5 µg/mL of Etravirine respectively with a correlation coefficient (r2) 0.999. The results of linearity, limit of detection and limit of quantification were presented in Table 4.

Method Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 3

DISCUSSION

Considering the efficiency of UV-VIS Spectroscopic, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Etravirine in a tablet dosage form. Thus method described enables to the quantification of Etravirine. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. Dissolution testing is very important invitro test to evaluate drug product. This data form the part of the pharmaceutical development report, but can also be included in the bioequivalance study report. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this UV-VIS Spectroscopic method can be used for analysis of commercial formulation and dissolution data provides useful information for invivo studies.

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