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REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF EFAVIRENZ AS A DOSAGE FORM BY UV SPECTROPHOTOMETER

[1]Kajal Mane, [2]Anuj Kadam, [3]Mrunalini Patil, [4]Pallavi Sutar

Department of Pharmaceutical Chemistry,

Rajarambapu College of Pharmacy, Kasegaon, Sangli, Maharashtra, India

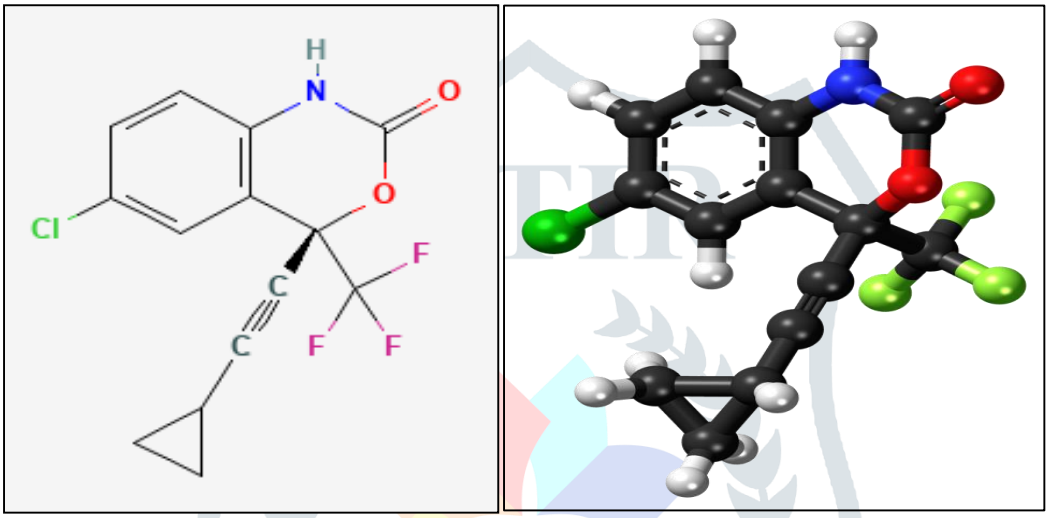
Abstract: Finding a straightforward, sensitive, accurate, precise, and quick ultraviolet (UV) spectrophotometric method for estimating Efavirenz in its pure form, as well as its formulations and stability samples, is the goal of this progress report. These techniques were validated in accordance with ICH requirements, and the accuracy, precision, and ruggedness results were within acceptable bounds. During the recovery study, excipients did not intervene. The approach was also effective for estimating and quantitatively evaluating Efavirenz from formulation. A few recoveries that did not significantly depart from 100% showed that the procedures were reliable and accurate, and that the common excipients utilised in tablet formulation did not cause any problems. Pharmacopeias have suggested several ways to regulate the quality of Efavirenz, an antiretroviral medication used to treat AIDS. These methods rely on costly equipment that is sometimes unavailable in African nations. A sensitive, dependable, easy-to-use, and quick method for determining the active ingredient Efavirenz in Efavirenz tablets has been validated for the purpose of controlling their quality by verifying their dosage. This problem has been resolved by employing UV-visible spectrophotometry, a low-cost and user-friendly instrument that is accessible in many laboratories across the African continent. Repeatability and intermediate precision were used to calculate the accuracy. Consistency over a brief period of time in the same operational environment is implied by repeatability. The intermediate precision analysis is expressed in terms of laboratory variance on different days and analyst-to-analyzer variation by different analysts.

Keywords: UV-visible spectrophotometry, Efavirenz, AIDS, Accuracy, Precision and Ruddedness.

1. INTRODUCTION

Efavirenz (EFV) is an antiviral drug used in HIV/AIDS treatment and prevention. It is marketed under several trade names, including Sustiva. It is typically advised to be used in conjunction with other antiretrovirals. It can be applied as a preventative measure following a needlestick wound or other possible exposure. You can buy Efavirenz, Emtricitabine and Tenofovir alone or in combination. It is taken orally.

STRUCTURE



Efavirenz (Monograph)

Brand name	Sustiva
Chemical name	(±)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4 (trifluoromethyl)-2H-3,1-benzoxazin-2-one
Molecular formula	C14H9ClF3NO2
Molar mass	315.68 g•mol−1
Partition coefficient	The [octanol /water] partition coefficient is determined to be P=5.4
Melting point	139-141 °C
Solubility	Almost insoluble in water (less than 10 mg/L)
CAS number	154635-17-3
VA class	AM800
Bioavailability	40–45% (under fasting conditions)

Protein binding	99.5–99.75%
Metabolism	Liver (CYP2A6 and CYP2B6-mediated)
Onset of action	3–5 hours
Elimination half-life	40–55 hours
Excretion	Kidney (14–34%) and feces (16–61%)
Routes of administration	By mouth [Capsule ,Tablet]

History

On September 21, 1998, the FDA approved efavirenz. The FDA authorised Mylan's generic tablet formulation on February 17, 2016. Thailand's Government Pharmaceutical Organisation (GPO) announced in late 2018 that it would start manufacturing Efavirenz after receiving WHO approval. Known by another name, DMP 266 (Efavirenz), Du Pont Pharma made the discovery. The production license for Efavirenz is scheduled to be granted to European nations in May 1999.

Chemical properties

(4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl) is the chemical formula for Efavirenz. 2-one-benzoxazin -1H-3,1-. $C_{14}H_9ClF_3NO_2$ is the empirical formula for it. Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.68 g/mol. It has a water solubility of less than 10 μg/mL.

Medical uses

The US Department of Health and Human Services Panel on Antiretroviral Guidelines recommends Efavirenz with Tenofovir/Emtricitabine (Truvada) as one of the NNRTI-based regimens for adults, adolescents and children with untreated HIV infection. Efavirenz is also used in combination with other antiretroviral drugs as part of a more thorough post-exposure preventive regimen to reduce the risk of HIV infection in individuals who have been exposed to a significant risk (e.g. needlestick injuries, certain types of unprotected sexual activity, etc.).

Efavirenz is safe to use in the first trimester of pregnancy. Efavirenz may be given to breastfed infants because it is a component of breast milk.

Contraindications

Those who have previously taken this drug and had an allergic response should refrain from taking additional dosages of Efavirenz. Toxic skin eruptions, erythema multiforme and Stevens-Johnson syndrome are examples of hypersensitivity reactions.

Adverse effects

Headaches, vertigo, dizziness, anxiety, and cognitive impairment (fatigue, disorientation, and problems with memory and concentration), depression, including suicidal thoughts, and disturbed sleep (nightmares, insomnia, and daytime fatigue) are among the most common

side effects. Euphoria is experienced by some people. There may be rashes and nausea. When Efavirenz is administered, some marijuana urine tests may produce a false-positive result. Because it may extend the QT interval, Efavirenz should not be administered to individuals who have torsades de pointes or are at risk for developing one. Using Efavirenz may cause convulsions in both adult and paediatric patients with a history of seizures.

Brands

As of 2016, Efavirenz is sold under several brand names including Adiva, Avifanz, Efamat, Efatec, Efavir, Efavirenz, Efcure, Eferven, Efrin, Erige, Estiva, Evirenz, Filginase, Stocrin, Sulfina V, Sustiva, Virorrever, and Zuletel. As of 2016, Efavirenz, Tenofovir, and Emtricitabine are marketed under the brands Atripla, Atroiza, Citenvir, Oditec, Teevir, Trustiva, Viraday, and Vonavir in several jurisdictions [1].

2. INSTRUMENTATION

2.1 The absorbance of the resultant solutions was to be measured using a Hitachi-U2000 spectrophotometer equipped with two identical quartz cells. Strides Pharma provided Efavirenz. Every other reagent that was utilized was analytical grade [2].

2.2A Misonixsonicator, a Mettler Toledo weighing scale and a Shimadzu double-beam spectrophotometer were utilized to detect absorbance [3].

2.3 All spectrum measurements were performed using a JASCO UV/Vis double beam spectrophotometer (model V-570) equipped with 1cm quartz cells [4].

2.4 With a spectral band width of 1 nm and a wavelength precision of 0.3 nm (automatically corrected for wavelength using a pair of 1 cm matched quartz cells), a Shimadzu model 1800 UV double beam spectrophotometer was used [5].

2.5 The Labindia model-3000+ series, which has 1cm quartz cells and a wavelength precision of ±1 nm, was utilized in the UV-spectrophotometric approach [6].

3. MATERIALS

Water, acetonitrile, methanol, sodium hydroxide, hydrochloric acid, lamivudine and Efavirenz.

Reagents:

The Efavirenz standard (United States Pharmacopoeia), the HPLC-grade ethanol required to dissolve the Efavirenz, and the Efavirenz tablets (EfavirenzMacleods® tablets) were supplied by the Senegalese National Medicines Control laboratory (LNCM, 39 Avenue Pasteur, Dakar).

A Milli-Q® water purification system (Millipore, Molsheim, France) provided the ultrapure water.

EFV, EMT, and TDF reference standards were a kind gift from the pharmaceutical company. We purchased methanol and HCl from Rankem, RFCL Limited, located in New

Delhi, India. The supplier of NaOH was Hi-Media Laboratory Pvt. Ltd. Every reagent and solvent was of analytical quality. On the day of preparation, all of the solutions were examined and shielded from the light. In-house triple-distilled water was produced. For the duration of the experiment, distilled water was obtained using Millipore's Mili Q apparatus (Milliford, USA) [7].

4. PROCEDURE

4.1Preparation of standard curve:

Ten milligrams of Efavirenz were dissolved in ten milliliters of methanol to create a 100 µg/mL stock solution, which was then topped up with 1%w/v SLS. The _max of Efavirenz was discovered by screening suitable dilutions with a high correlation coefficient. From the stock solution, a number of standard dilutions were created yielding solutions with 1, 2, 3, 4, and 5 µg/mL. Both their bandwidth and data pitch parameters were set at 0.5 nm and their corresponding absorbance values were measured at fixed _max. At each concentration the average absorbance values, standard deviation, and percentage coefficient of variation were computed. A one-way ANOVA test for linearity was performed using five randomly selected sets of calibration curves [8].

Sample preparation (Bulk drug)

50 mg of Efavirenz was carefully weighed and then added to a 100 mL volumetric flask that already had 10 mL of methanol in it. Next, a 1.5% w/v sodium lauryl sulphate solution was added to the flask until it was completely filled. To obtain 10, 20, 40, 50, and 100 µg/mL of this solution, it was serially diluted with 1.5% w/v sodium lauryl sulphate solution. The Efavirenz concentration was further determined by measuring the absorbance at 247 nm.

Sample preparation (Dosage forms)

Twenty tablets were weighed and ground into a powder, which was then precisely weighed to equal 50 mg of Efavirenz and added to a 100 mL volumetric flask that also contained 50 mL of methanol. After 30 minutes of vigorous shaking, the contents were mixed with methanol to reach a volume of 100 mL. After appropriately diluting this solution with 1.5% w/v sodium lauryl sulphate solution, the absorbance at 247 nm was measured to ascertain the Efavirenz concentration..

Recovery experiments

Analytical recovery tests were carried out by adding a known quantity of pure drug to the previously examined pharmaceutical preparation and analyzing it using the proposed method. This was done to investigate formulation additive interference and further validate the developed method's accuracy. The concentration level employed was 10µg/mL [9].

4.2Preparation of stock solution:

A 60:40 methanol and water mixture was used to dissolve 100 milligrammes of pure medicine Efavirenz following its transfer to a 100 millilitre volumetric flask and subsequent weighing. A solution of 1000µg/ml was obtained by shaking the flask and adding water and methanol (60:40) to get the volume up to the desired level. A 100 millilitre volumetric flask was filled with 10 millilitres of this solution after it was pipetted out. Methanol and water were added to bring the volume up to the required level, resulting in a solution containing 100µg/ml. To make the standard dilutions, the stock standard solution was suitably diluted using methanol and water to reach concentrations between 10 and 50 µg/ml [10].

Preparation of sample solution-

The EFAVIR-600 mg was employed in the analytical analysis. We were able to determine the average weight of the tablets by weighing twenty powdered tablets. A powdered pill containing 100 mg of Efavirenz was transferred to a volumetric flask following meticulous weighing. After adjusting the methanol and water solution's volume to the appropriate amount, scan it at 291 nm. A calibration curve covering 10–50 µg/ml was created. The Efavirenz calibration curve was made at a wavelength of 291 nm [11].

4.3 Preparation of solutions:

Tablets of EfavirenzMacleods® were ground into a powder. To get concentrations of the active ingredient Efavirenz ranging from 12 µg/mL to 0.625 µg/mL, the proper amounts were weighed and dissolved in ethanol. As stated earlier, two batches of EfavirenzMacleods® tablets were used to create two distinct solutions.. Ethanol was used to dissolve the required amount of Standard Efavirenz (USP), resulting in Efavirenz concentrations ranging from 12 µg/mL to 0.625 µg/mL.

Wavelength selection

Two 10 µg/mL solutions of Efavirenz tablets from two different batches were prepared, along with a 10 µg/mL solution of regular Efavirenz. The absorption spectra of the three solutions were recorded in the 200–800 nm region. Three absorption maxima at wavelengths of 207, 251 and 301 nm were found. The wavelength of 251 nm was chosen because it provides the best absorption (between 0.5 and 1) and is not too close to the visible spectrum (Fig. 1).

Validation of the method:

To verify the method, the following parameters were evaluated in compliance with the International Conference on Harmonisation protocol (ICH, 2005): linearity and range, accuracy, precision, selectivity, limit of detection (LOD) and limit of quantification (LOQ). The process has been validated using the accuracy profile, a fitness-to-purpose validation technique. It is based on a graphical decision-making tool called the accuracy profile. It makes it possible to use measurements made with repeatability or intermediate accuracy to calculate the interval where a specified percentage of subsequent measurements will be situated. One can easily determine the validity of a method by comparing this interval to an acceptability interval that the end-user of the results has set (Feinberg, 2007; Marlet and Lognay 2010; Smith et al., 2014; Mottier et al., 2016; Frampas et al., 2018; Ibrahim et al., 2020).

Linearity and range: Solutions containing 10, 7, 5, 3.5, 2.5, and 1.25 µg/mL of efavirenz were made. A spectrophotometer operating in the 200–400 nm UV range was used to scan the solutions. At 251 nm, the Efavirenz absorption was measured. The calibration curve was created using absorbance and concentration as parameters [12].

Intra-day and inter-day precision studies: Three replicates were used to perform intra-day precision (repeatability) at 5.6, 7.4 and 8.4 µg/mL. Three replicates and three consecutive days were used to accomplish inter-day precision, also known as intermediate precision, at the same doses. The relative standard deviation (RSD) values were calculated using the following formula: RSDIP is for Relative Standard Deviation of the Intermediate Precision, and RSDR is for Relative Standard Deviation of the Daily Repeatability.Additionally, the following formula was used to determine the RSDIP: By contrasting the actual concentrations with the theoretically anticipated concentrations (R), trueness (recovery) was ascertained. In order to do this, a calibration graph was created daily for the intra-day and inter-day precision investigations, and the experimental concentrations measured for each concentration and each repeat were computed using this curve.

- Limits of quantification (LOQ) and detection (LOD): The LOD was established by serially diluting working solutions until the lowest concentration was reached, at which point it was impossible to visually distinguish between the reported absorbance caused by the compound in the solution and the background noise from the spectrophotometer. The ICH rules, which specify that the LOQ should be equivalent to 3.3 times the LOD, were used to determine the LOQ [13].

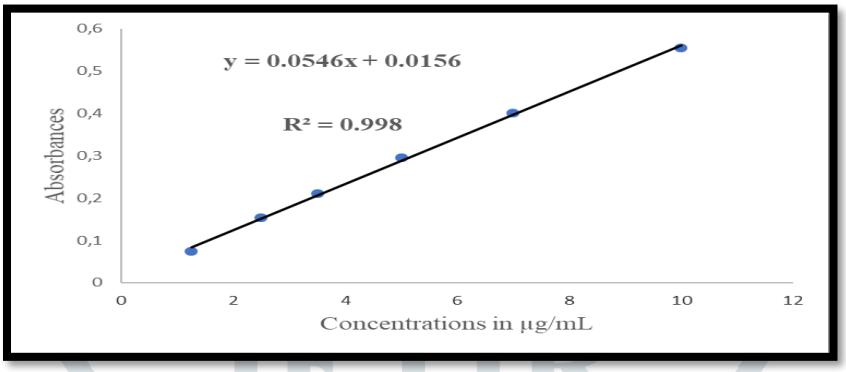


Figure 1: Calibration curve graph obtained with different concentrations of Efavirenz.

4.4 Method:

A range of solvents including methanol, water, acetonitrile, sodium hydroxide and hydrochloric acid, were used to record the UV spectra of Lamivudine and Efavirenz. These two drugs showed good absorbances when dissolved in acetonitrile. Acetonitrile was therefore chosen as the method's solvent. In order to obtain a solution of 100 µg/ml, Lamivudine and Efavirenz (10 mg each) were weighed separately, transferred to a 100 ml volumetric flask, and dissolved in acetonitrile. Working standard solutions of 50 µg/ml of each of the drugs were prepared and scanned in the range 400-200 nm to obtain the absorbance spectra and overlainspectra (Fig.2). Two wavelengths were chosen, 271 and 247 nm, which correspond to the λmax of the medications efavirenz and lamivudine, respectively. At each of the two chosen wavelengths, the absorbance of Lamivudine and Efavirenz was measured and the absorptivity values E (1%, 1 cm) were calculated [14].

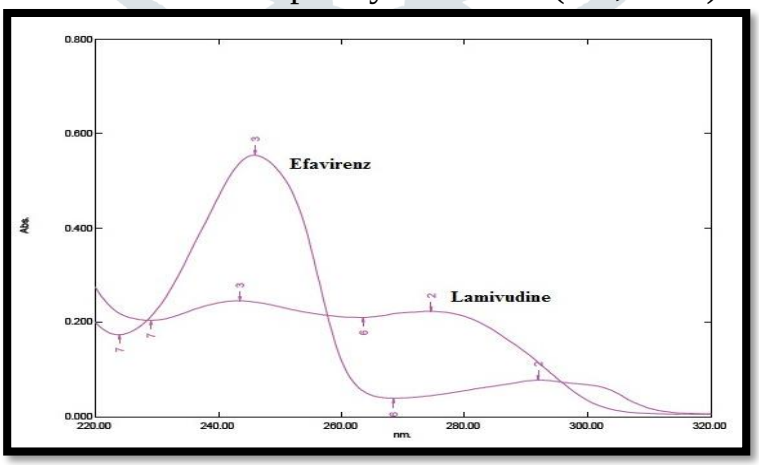


Figure. 2: Normal spectra of Efavirenz and Lamivudine overlaid in acetonitrile

4.5 Preparation of Solution

Preparation of Standard Stock Solution (Stock-A):

To make standard stock solutions, 100 mg of each drug were separately diluted in 50 mL of 0.1 N NaOH in a 100 ml volumetric flask. The flask was sonicated for around 10 minutes to dissolve the drug, and the volume was increased to 100 ml using 0.1 N NaOH in order to get a concentration of 1000 µg/ml (Stock-A) for both medications.

Preparation of Sub Stock Solution (Stock-B):

Using a pipette, 2.5 ml aliquots of EFV, EMT and TDF were taken out of standard stock solution A. They were then placed in a 25 ml volumetric flask and diluted with 0.1 N NaOH (Stock-B) at a concentration of 100 µg/ml.

Preparation of Standard Solution in Operation:

1) In a 10-ml volumetric flask 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml of the substock solution (Stock-B) were taken sequentially and the loudness was increased to 10 ml using 0.1 N NaOH. Accordingly, the EFV solutions were 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml.

2) A pipette was used to extract aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from the standard stock solution (Stock-B) and transfer them to a 10-ml volumetric flask. After that 0.1 N NaOH was added to raise the volume to 10 ml. Thus, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20µg/ml and 25µg/ml were the EMT solutions.

3) A pipette was used to extract aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from the standard stock solution (Stock-B) and transfer them to a 10-ml volumetric flask. After that 0.1 N NaOH was added to raise the volume to 10 ml. TDF solutions were 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml because of this.

Wavelength selection for linearity:

Separate solutions of 10 g/ml EFV, 10 g/ml EMT and 10 g/ml TDF were made. The spectrum mode was used to scan the solutions between 200 and 400 nm. The maximum absorbances were recorded by EFV, EMT and TDF at 240.0 nm, 256.0 nm and 316.0 nm, respectively (fig.3 and 4). Whereas EFV showed linearity in the concentration range of 10–50 g/ml, EMT and TDF showed linearity at their respective maxima of 5–25 g/ml. A plot of the calibration curve for absorbance vs concentration was made.

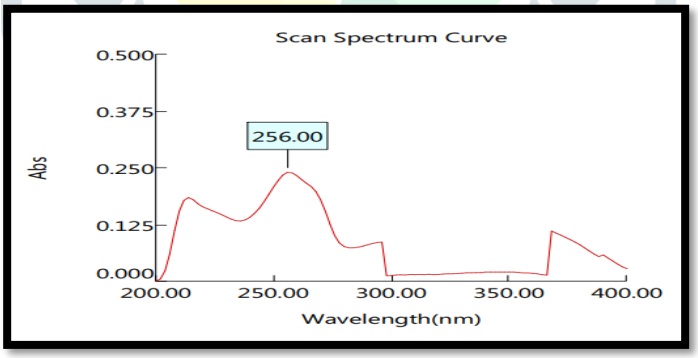


Figure.3: Determination of λmax of EMT

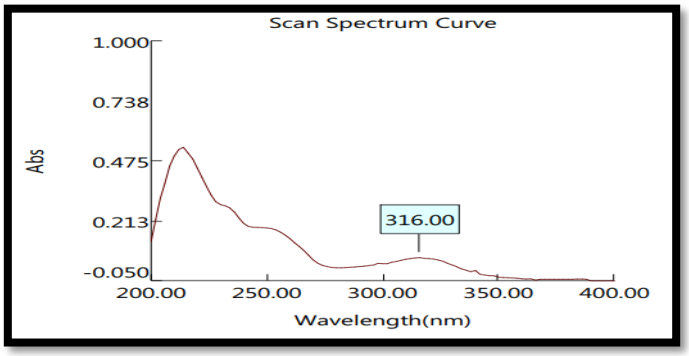


Figure.4: Determination of λmax of TDF

Examining Overlay Spectra

After preparing a standard stock solution with concentrations of 10µg/ml of EFV, 10µg/ml of EMT and 10µg/ml of TDF, the standard functioning solution was scanned in spectrum mode using 0.1 N NaOH as a blank. The two overlapped spectra were then recorded. While EMT and TDF displayed peaks at 256.0 and 316 nm, respectively, EFV displayed an absorbance peak at 240.0 nm. The overlain spectra also showed isoabsorptive

spots at 250.00 nm. The simultaneous equation technique can be applied to evaluate the two drugs concurrently because their absorbance maxima are distinct and do not conflict (fig.5) [15].

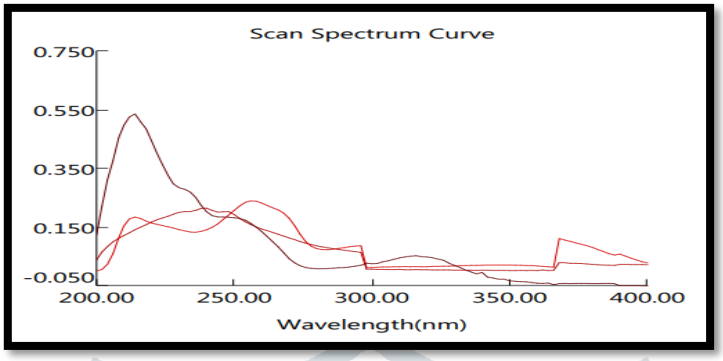


Figure.5: Overlay spectra of amox, ompr and rifb

5. RESULTS AND DISCUSSION

5.1Method development:

A variety of solvent systems, including water, methanol and others, were tested both separately and in tandem or with surfactants at varying proportions in order to create an accurate, precise and sensitive UV spectrophotometric technique for Efavirenz. Sensitivity, minimum interference, ease of preparation, suitability for drug content estimate, stability, analysis time and affordability were the factors that ultimately led to make use of 1% w/v SLS in water. Within the spectrum of concentrations of 1–5 µg/mL (Table 1&2), the _max for Efavirenz in 1% w/v SLS (Fig. 6) displayed a linear relationship (correlation coefficient of 0.9988).

Sample solution stability studies

Using the chosen solvent at controlled (25±2oC; 65±5%RH) and accelerated (40±2oC; 75±5%RH) conditions, overlay scans taken at zero time, 12, 24 and 48 hours showed no deterioration up to 48 hours. There may be a delay between sample collection and analysis since the drug remained stable for longer than 48 hours.

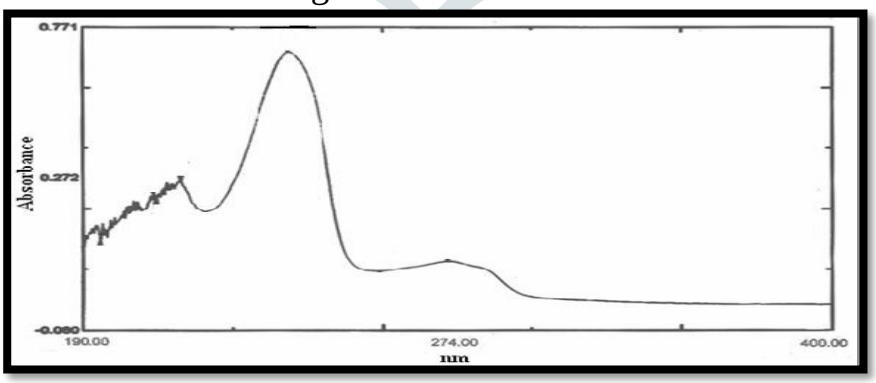


Figure.6:Spectra of 5µg/ml solution of Efavirenz in 1%w/v sodium lauryl sulphate.

Table 1. Calibration curve points of the suggested approach to Efavirenz estimation.

Parameters	Value
Absorption maxima, nm	247
Beer's law limit, µg/mL	1-20
Molar absorptivity, 1 mole-1/cm-1	2.21×10-4
Coefficient of correlation Regression equation*A	0.9998

Slope(b)	0.05306 ± 0.0004014
Intercept(a)	0.0005238 ± 0.001215
1/slope	-18.85
Correlation coefficient(f)	0.9999

Table 2. Results of least square regression analysis of uv methods for Efavirenz's estimate.

Concentration, µg/mL	Mean ±SD(n=6)	CV (%)
1	0.055 ±0.00634	1.20
2	0.107 ±0.00624	1.43
3	0.157 ±0.00610	1.58
4	0.214 ±0.00650	1.62
5	0.266 ±0.00818	1.71

Recovery studies

It was discovered that the technique created for estimating Efavirenz in bulk and in its dose forms was quick, easy, accurate and cost-effective. The drug content was uniform, ranging from 98 to 99.55%, as Table 3-5 makes evident and the SD and CV values were determined to be sufficiently low. Recovery studies were also carried out and the findings indicated that the recovery rates for both batches of pills were 98.89 and 98.89%. All that is needed to use the procedure is to measure the sample solution's absorbance at the selected wavelength and then perform basic calculations. It was therefore used further in our investigation.

Table 3. Efavirenz estimation in bulk by proposedmethod.

S.No	Efavirenz taken µg/mL	Efavirenz found	(% of efavirenz found(CV)
1	10	9.85(0.935)	98.5(0.76)
2	20	19.87(0.884)	99.4(0.85)
3	40	39.56(0.845)	98.9(1.12)
4	50	49.55(0.765)	99.1(0.99)
5	100	98.95(0.928)	98.9(0.10)

Table 4. Efavirenz estimate using a defined method in dose form.

Tablet sample	Label Claim, mg/tablet	Actual content found, mg±S.D	Percent Actual content found, ±S.D	CV
Sample-1 EfavirenzTab.(Generic)	100	98.89±0.153	98.89±0.153	0.959
Sample-2 Efavirenz Tab.(Generic)	600	590±2.04	98.33±0.74	0.769

Table 5.Efavirenz estimation in dosage form in recovery studies by proposed method.

Tablet sample	Concentration of added amount of drug in the final dilution, µg/mL ±S.D	Recovery, µg/mL ±S.D	Percent recovery,±S.D	CV
Sample-1Efavirenz Tab.(Generic)	10	9.85 ±0.0123	98.5 ±0.989	0.959
Sample-2Efavirenz Tab.(Generic)	10	9.89 ±0.0142	98.9 ±0.975	0.958

Method validation

The presented estimating approach demonstrated accuracy (ranging from 10.2-5.5%) and precision (with intra-day precisions below 4.5%). The technique has been verified for the 1–5 µg/mL range using a sodium lauryl sulphate solution that is 1%w/v. The F-test for lack of fit shows that the approach is linear over this concentration range. All locations on the standard curve showed analyte recovery of greater than 90%, intraday precision of greater than 5%CV, and accuracy ranging from 98 to 100% of nominal throughout this estimation range [16].

5.2METHOD OF VALIDATION:

Accuracy:

The standard solution for the Efavirenz test was added to the tablet formulation in order to evaluate the procedure's accuracy. This was done in triplicate and the average Efavirenz recovery was determined. Each level's recovery percentage (%) was determined to be significantly falling between 96.4% and 99.3%, showing that there was little to absence of excipient interference.

Table 6: Table displaying the conventional solution's accuracy

Concentration (µg/ml)	**Amount found**	**% RSD**
20	19.9 ±0.03	0.103
40	38.24 ±0.04	0.065
60	58.06± 0.19	1.025

Precision:

Typical samples for quality control were created in triplicate at various concentration levels throughout the whole linearity range in order to evaluate the precision. RSD% for repeated readings, repeatability (intra-day) and moderate level of accuracy (inter-day)were used to assess the assay's precision.

Ruggedness: Another analyst estimates ruggedness.

Table 7: The precision study's table

	Analyst1		**Analyst2**	
Drug	%Amount	%RSD	%Amount	%RSD
EFAVIR 600mg	95%	1.155	94.8%	1.911

Linearity:

The least-square regression approach is used to assess the linearity method.

Results and Discussions

The created technique for figuring out the dosage of Efavirenz tablets was discovered to be straightforward, sensitive, accurate, selective, quick and affordable. Efavirenz demonstrated peak absorption at 291 nm and adhered to Beer's law in the range of concentrations of 10–50 µg/ml. The suggested approach yielded linear regression with a correlation coefficient (r2) of 0.999, where y=0.181x+1.071 when calculating Efavirenz. Under typical operating settings, both intraday and interday investigations demonstrated a High level of reproducibility for an analytical procedure. Less than 2% is the precision percentage RSD. The formulation of the tablets remains unaffected [17].

5.3 Range and linearity; trueness and intra- and inter-day precision investigations; Limits of detection (LOD) and quantification (LOQ):

Linearity was attained at a concentration range of 1.25 µg/mL to 10 µg/mL with a correlation value (R2) of 0.9987 (Table 8, Figure 1). RSDs values for intra-day and inter-day precision investigations ranged from 0.7% to 3.5% and 2.9% to 3.6%, respectively, whereas recoveries ranged from 98.2% to 112.9% (Table 10). Finally, 0.15625 µg/mL and 0.515625 µg/ mL are the determined LOD and LOQ, respectively.

Selectivity

It was evident from recording the graphs produced by the three prepared solutions that they nearly fit one another (Figure 2). Thus, it could be said that the approach is selective. Additionally, the 251 nm maxima are smaller than 1 (the usual). Thus, the preferred wavelength for this technique is 251 nm.

Validation of the method

Given the numerous norms used to do the validation, the degree of freedom and risk were set at 6 and 5%, respectively. The result was 2.45 when the inverse Student Test (t) was calculated using those parameters. The confidence interval (CI), lower bound (LB), and upper limit (UB) of the accuracy profile of the procedure were calculated. The acceptance limit β was set at 15% in order to compute the accuracy profile's superior and inferior bounds. A overview of the values obtained for the several parameters computed to create the accuracy profile is given in Table 4 and the resulting graph is shown in Figure 7. According to the accuracy profile (Fig. 7), the method's validation could be set at 100% concentration of the analyte or tablet under inquiry (7 µg / mL in this study), with values not too far from this concentration. This concentration and its surrounding values actually lie exactly inside the boundaries of the graph [18].

Table 8: Absorbances recorded for different concentrations of efavirenz when establishing daily calibration graphs.

Concentration in µg/mL	Absorbance
10	0.55477679
7	0.40154345
5	0.29472345
3.5	0.21136679
2.5	0.15301679
1.25	0.07462345

Table 9: Summary of rsds examined for intra-day and inter-day precision studies
rsd : relative standard deviation. concentration : percentage (%) of 7 µg/ml.

Concentrations	80%	100%	120%
RSD inter-day precision	3.5%	0.7%	2.9%
RSD intra-day precision	3.6%	3.3%	2.9%

Table 10 : Recovery concentration: 7 µg/ml as a percentage (%).

Concentration (%)	Replicate 1	Replicate 2	Replicate 3
80	108.8	110.4	111.3
80	103.2	104.1	112.5
80	112.9	110.3	112.4
100	109.1	113.4	107.8
100	108.7	112.5	106.7
100	109.4	113.4	105.7
120	107.2	100.6	104.4
120	101.5	100.9	98.2

120	102.1	100.9	105.4

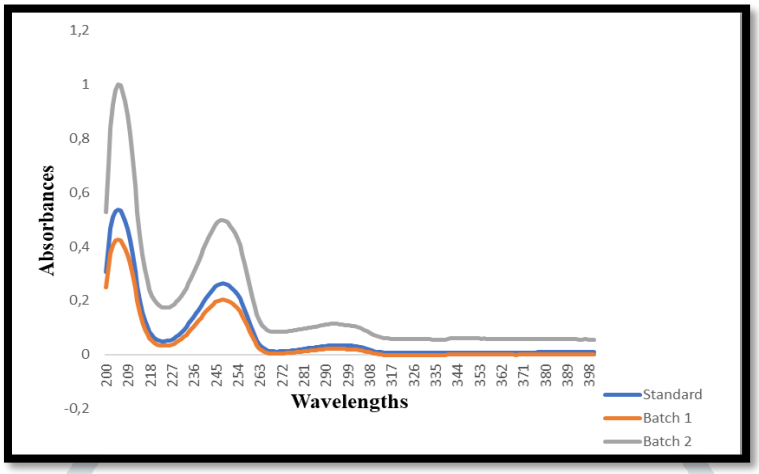


Figure 7: uv-visible absorption spectra of efavirenz at 10 µg/ml

Table 11: An overview of the accuracy profile.

conc	Accuracy	RSDR	RSDIP	CI	UB	LB	SL	IL
80%	104.9%	3.5%	3.6%	8.8%	113.7%	96.1%	115%	85%
100%	98.3%	0.7%	3.3%	8.0%	106.3%	90.3%	115%	85%
120%	93.2%	2.9%	2.9%	7.0%	100.2%	86.2%	115%	85%

Conc: concentration; SL: superior limit; IL: inferior limit; UB: upper bound; LB: lower bound; CI:confidence interval; RSDIP is the relative standard deviation for intermediate precision, and RSDR is the relative standard deviationforrepeatability. SL = 100 + β; IL = 100 - β; CI = RSDIP x t; UB; LB = Accuracy - CI = JustesseCI. SB stands for superior bond, IB for inferior bond, and SL for superior limit and IL for inferior limit.

DISCUSSION

A UV spectrophotometric approach was devised and validated by Pathade and associates. (2017) for the steadiness and determination research of tablets and bulk sitagliptin phosphate. In terms of linearity, the correlation coefficient in this study is nearly identical to that in that study (R2 = 0.999) and adheres to the norm (quite near to 1; R2 = 0.9987). Montgomery and associates (2001) developed and validated an HPLC technique in reverse phase for measuring Efavirenz and associated chemicals both in the drug's active ingredient and in a pill form and the results are comparable to theirs (better than 0.9999). The results from the current investigation utilising a UV-spectrophotometric technique and those from earlier research using an HPLC are similar, indicating that the current technology is appropriate for figuring out how much Efavirenz is in tablets. Furthermore, the value is comparable to that of a prior study conducted in the same facility by Djiambeu (2015), which validated. A spectrophotometric technique in the UV-visible range for assessing reformulated chlorpheniramin maleate (0.9993). Similar to Djiambeu (2015), the intra-day precision study's RSD values ranged from 1.5% to 3.1%, whereas the inter-day precision research's RSD values ranged from 2.1% to 3.1%. This approach, which is sensitive and effective for the antiretroviral's extraction, pre-concentration and concurrent determination medications Nelfinavir, Efavirenz and Nevirapine in biological samples and pharmacological formulations, is also comparable to the findings of (et al., 2019) Safari on the combination of solid-phase extraction and liquid-liquid dispersive microextraction using HPLCUV. As to the authors' findings, the intra-day precision (RSD in percentage) varied

between 2.2 and 4.2%, whereas the precision between days (RSD in percentage) varied between 3.1 and 5.2%. In contrast to the current study, their research on Efavirenz in particular demonstrated inter-day and intra-day accuracy 3.8% and 4.6%, respectively. These results provide credence to the notion that UV-visible spectrophotometry can yield results comparable to or even better than those obtained using high-performance liquid chromatography. The first study had mean recoveries of 99%, whereas the second trial had mean recoveries of 95% to 100.5%. the recovery values discovered (98.2% to 112.9%) in this study were comparable to those discovered by Safari (et al., 2019) and Montgomery (et al., 2001).These numbers are high compared to another study by Sarr and associates (2016) that found findings 94.7% to 100% on the creation and verification of an electrophoresis technique for pharmaceutical quality assessment based on metronidazole. The high results discovered in this research could be because of the complicated ingredients in Efavirenz tablets. In fact, Efavirenz pills include about 20 other compounds besides the primary substance, Efavirenz. Furthermore, this study's LOD and LOQ, which areBoth 0.16 and 0.45 µg/mL, respectively, are comparable to those discovered according to Pathade et al. (2017). They are however less than those discovered in a Jain (et al., 2011) study on the development and verification using the UV technique for the measurement of bulk terbinafine hydrochloride and formulation, which produced readings of1.30 µg and 0.42 µg, in that order. The observed difference shows how sensitive the approach created for this study is. It was also discovered that the technique was selective at 251 nm. This wavelength is quite similar to the one used in the High Performance Liquid Chromatography technology in the United States. The 15% permissible level is well below the present limits, which limit drug use to 5% to 10%. The European Union Directive 5/318/EEC actually sets a maximum of 5% and the only way to support higher limits is by altering the production and analytical procedures, claims Pinguet (2015). The drug's intricacy under investigation explains the 15% rate. In fact, besides the active component "Efavirenz," EfavirenzMacleods® also comprises binders and excipients, totalling over twenty other compounds. Additionally, instead of using a lab-generated Efavirenz pill during the method's validation phase, manufactured tablets were used. A bias resulting from this composition was therefore unavoidable because the tablets' composition is uncontrolled and consistency in composition among tablets cannot be guaranteed [19].

5.4 Validation of the method:

Linearity:

The curve used for calibration was analysed using least squares linear regression in order to prove linearity. Lamivudine and Efavirenz had curves for linear calibration for concentration ranges of 10–100 µg/ml and 10–70 µg/ml, respectively. Plotting absorbances against corresponding concentrations was done and the resulting curves were subjected to linear regression analysis. Lamivudine and Efavirenz were shown to have correlation values of 0.996 and 0.999, respectively (Fig. 8-9). Table 12 presents the findings.

Table 12: The correlation coefficient and linearity

Parameters	Lamivudine	Efavirenz
Regression equation	y = 0.020x + 0.030	y = 0.057x - 0.004
Linearity µg/ml	10 – 100	10 - 70
Correlation coefficient	0.996	0.999

Precision:

Using the proper statistical analysis, the method's degree of repeatability was evaluated. For the intra-day study, the drug concentrations were measured on the same day three times

at one-hour intervals, and for the inter-day study, three times on separate days. Calculations were made to determine both the Relative Standard Deviation (RSD) and Standard Deviation (SD). The results are shown in Table 13.

Table 13: Precision studies

Drug	Concentration µg/ml	Intraday Precision (n=3) % RSD	Inter day Precision (n=3) % RSD
Efavirenz	50	0.172	0.356
Lamivudine	50	0.487	0.762

Accuracy:

Using a pharmaceutical sample enriched with known concentrations of standard Lamivudine and Efavirenz equal 50, 100, and 150 percent of the label claim, recovery tests were conducted. At every level of the sum, six decisions were made. Table 14 displays the findings.

Table 14: Accuracy

Drug	% Amount added	Amount taken (mg)	Amount recovered (mg)	% Recovery	% *RSD
Efavirenz	50 100 150	300 600 900	299 598.23 895.14	99.94	0.546
Lamivudine	50 100 150	150 300 450	149.42 300.16 448	99.94	0.546

LOD and LOQ:

The LOD and LOQ of Lamivudine were determined to be 3.5 µg/ml and 3.0 µg/ml, in that order. While Efavirenz's were 10 µg/ml and 10 µg/ml. Table 15 presents the findings.

Table 15: LOD and LOQ analyses

Validation parameters	Lamivudine	Efavirenz
Limit of Detection (LOD) µg/ml	3.5	3.0
Limit of Quantification (LOQ) µg/ml	10	10

Examination of the marketed formulation:

Twenty Odivir kits with 600 mg of Efavirenz each and Lamivudine 300 mg, were weighed on average and ground into a powder. After being weighed, Lamivudine 300 mg and Efavirenz 600 mg were added into a 100 millilitre volumetric flask. Acetonitrile is used to extract it. To ensure that the drugs were completely dissolved sonicated the volumetric flask. for 20 minutes. Acetonitrile was then added to the solution after it had been filtered. To determine the focus between the two medications within the linearity range, suitable aliquots of the formulation were made and scanned. The simultaneous equation was used to calculate each analyte's concentration (Fig. 10) (Table 16) [20].

Table 16:A nalysis of formulation

Drug	Labelled amount (mg/tablet)	Amount found (mg/tablet)	% Label claim	% *RSD
Efavirenz	600	598.67	99.77	0.715
Lamivudine	300	298.92	99.64	0.095

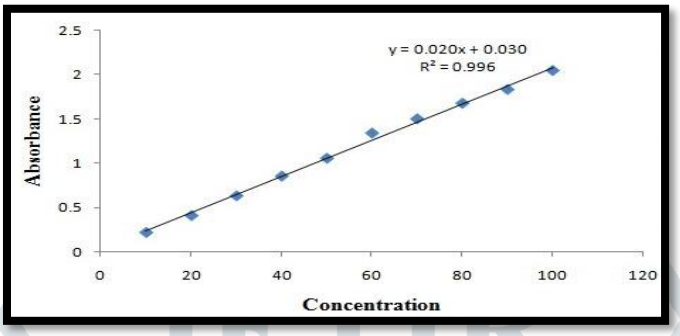


Figure. 8: Lamivudine calibration curve at 271 nm

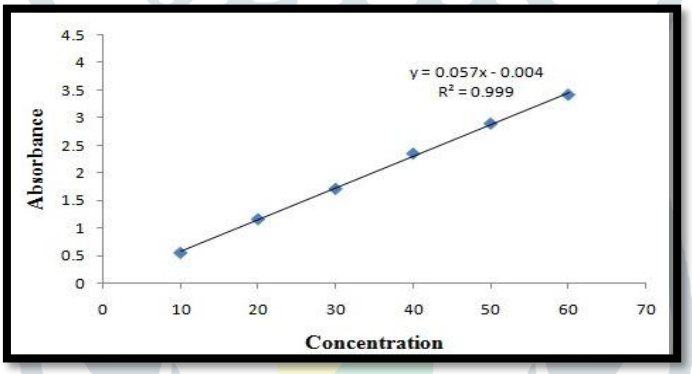


Figure. 9: Efavirenz calibration curve at 247 nm

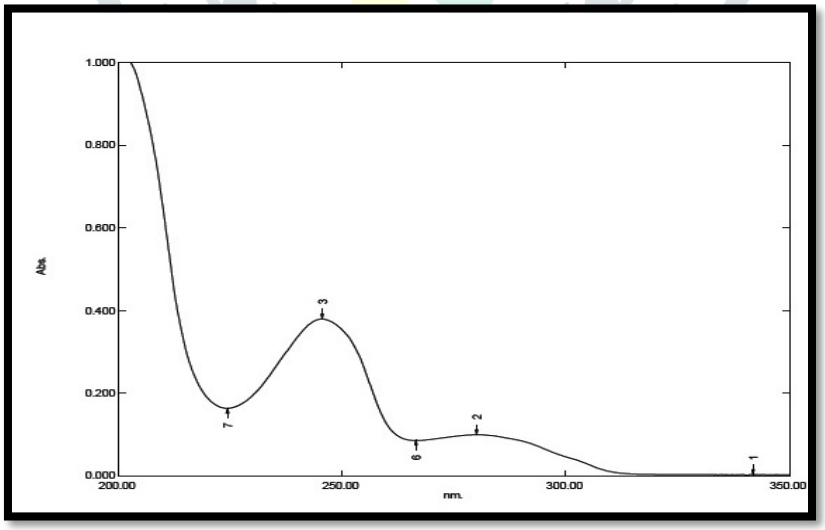


Figure. 10: Lamivudine with Efavirenz formulation spectrum

5.5Validation of simultaneous equation method:

Linearity : Drug response ratios were used to determine the linearity of both medications. The absorbance divided by the corresponding concentration yields the drug's response ratio. Table 17 was then used to draw a graph between concentration and response ratio.

Accuracy: Recovery studies assessed the accuracy of the proposed methods at three 80%, 100% and 120% are the levels. In order to conduct the recovery investigations, pre-analyzed

capsule solutions were mixed with a known quantity of standard EFV, EMT and TDF solutions. The suggested techniques were then used to re-analyze the final results. To determine the recovery of the additional drug sample, the entire analysis process was performed. Three replicates of five concentration levels were used for this recovery analysis (table 18).

Precision: Three levels of method precision were examined: reproducibility, intermediate precision (day-to-day and analyst-to-analyst) and repeatability. By analyzing the same drug concentration five times, repeatability was achieved. Analyzing five distinct drug concentrations over three days in a week was done on a daily basis (table 19).

Analysis of tablet Formulation: After weighing twenty pills and grinding them into a fine powder, 60 mg of EFV (20 mg EMT and 24 mg TDF) was taken in a 10-milliliter volumetric flask. To dissolve the medication in the capsule powder, 5 ml of 0.1 N NaOH was then added and the flask was sonicated for roughly 10 minutes. Next, 0.1 N NaOH was used to correct the volume. Following sonication, Whatman filter paper No. 41 was used for filtration. The final concentrations of all three medications within the working range were obtained by collecting the filtrate and further diluting it with 0.1 N NaOH. The simultaneous equation method was utilised to ascertain the concentrations after the absorbance of the final dilutions was measured at specific wavelengths. The procedure was run five times, as Table No. 17 demonstrates.

Table-17: Results of linearity of Efavirenz (EFV), Emtricitabine (EMT) and Tenofovir disoproxil fumarate (TDF)

PARAMETER	EFV	EMT	TDF
Concentration (µg/ml)	10-50	5-25	5-25
Correlation Coefficient (r2)*	0.999	0.999	0.999
Slope (m)*	0.011	0.023	0.016
Intercept (c)*	0.005	0.003	0.002

*Value of three replicate

Table-18: Results of recovery study

% Level	% MEAN±SD*		
	EFV	EMT	TDF
80%	99.54±0.314	99.02±0.723	99.22±0.555
100%	99.38±0.203	99.07±0.431	99.30±0.565
120%	99.35±0.543	99.22±0.549	99.35±0.455

* Value of three replicate and five concentrations.

Table-19: Results of precision

% MEAN±SD*			
Parameter	**EFV**	**EMT**	**TDF**
Repeatability	99.617±0.076	99.250±0.084	99.462±0.63
Intermediate precision			
Day to day precision	99.441±0.124	99.604±0.056	99.234±0.065

Analyst-to-Analyst	99.702±0.079	99.873±0.107	98.977±0.076
Reproducibility	99.637± 0.086	99.263±0.074	99.412±0.060

* Value of five replicate and five concentrations

Table-20: Assay of tablets formulation

% Conc. Found			
	EFV	**EMT**	**TDF**
Replicate 1	99.79	99.5	99.48
Replicate 2	99.65	99.27	99.49
Average	99.72	99.38	99.48
S. D.	0.099	0.163	0.007
% RSD	0.099	0.164	0.007

DISCUSSION:

To determine whether the analytical method could yield test results that were proportionate to the analyte concentration in the sample within a specific range, its linearity was examined. Standard solutions at varying concentrations were made, estimated in the UV and the outcomes were noted. Recovery studies evaluated the suggested approaches' validity and dependability. The recovery of the additional standards (80, 100 and 120 percent) was found at three concentration levels and three replicates. % means that are very close to 100, SD and % RSD that are less than 2 reflect the procedure's accuracy. Repeatability and the drug's intermediate precision were used to calculate precision. In the same operational circumstances, the repeatability result shows the precision over a brief period of time. Variability between laboratories on different days and between analysts is reflected in the intermediate precision study. The method's precision is indicated when the SD and RSD values are less than 2. The findings of the formulation analysis of the pills were published. Drug test results were near 100 and SD and % RSD less than 2 show that nothing was additional interference in drug evaluation [21].

6. CONCLUSION

According to the survey's findings, the well-established UV spectrophotometric approach for determining Efavirenz was simple, useful and had good repeatability, accuracy and precision. The sample recoveries in all formulations utilising the previously indicated approaches show that the procedure is valid and that there isn't any interference from formulation excipients in the estimation process, as they are in good agreement with their respective label claims or theoretical drug content.

Drugs remained stable in the chosen solvent system for over 48 hours, indicating that calculations don't always have to be done right away after sample collection. The method of UV spectrophotometry is rapid, simple, sensitive, specific and accurate. Since it has been verified to be in compliance with ICH requirements and no interference has been discovered, it is considered appropriate and helpful for industry for quality control. Fake or counterfeit pharmaceuticals are a major public health concern in developing countries when access to medications is limited for all populations.

Therefore, it's critical to make sure the medications on the market are of the highest calibre. Despite being crucial for quality control, many African countries' quality control labs typically lack analytical techniques like liquid chromatography with high performance and other highly costly equipment.

Some techniques have successfully used the simultaneous equation approach (Vierordt's method) to determine EFV, EMT and TDF in a combined sample solution at the same time. It has been discovered that these methods are exact, fast, easy to understand and accurate.

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