*ejpmr, 2021,8(8), 573-586.*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND STAVUDINE BY RP-HPLC

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**Article Received on 12/06/2021** **Article Revised on 02/07/2021** **Article Accepted on 22/07/2021**

## ABSTRACT

Pharmaceutical analysis comprises those procedures necessary to determine the "*identity, strength, quality and purity*" of the drug. Again it may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. It also deals the analysis of raw materials and intermediates in the manufacture of drugs. The pharmaceutical analyst must therefore, have a firm background in basic organic analysis and in addition he should have special skills in the quality evaluation of drug products. Pharmaceutical analysis include both qualitative and quantitative analysis of drugs and pharmaceutical substances which starts from bulk drugs (starting materials to the finished dosage forms) which is applied for identifying or quantifying constituents in a sample. During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.The mean percentage recovery above 95% indicates the reproducibility and accuracy of new developed method compared. The result of study include the proposed method is highly accurate, simple, precise and specific. The simple recoveries in all formulations were in good agreement with their respective label claims. And they suggest non-interference of formulation excipients in the estimation.

**KEYWORDS:** Lamivudine, Stavudine, RP-HPLC, Optimization, Pharmaceutical analysis, Accuracy etc.

## INTRODUCTION

Pharmaceutical chemistry deals with the chemistry of substances used as a therapeutic agent in medicine. It embraces the main branches of chemistry, radiochemistry analytical, physical, and organic chemistry. Its scope expands with development in medicinal and allied studies, and the emphasis shifts as knowledge advances.

Pharmaceutical analysis include both qualitative and quantitative analysis of drugs and pharmaceutical substances which starts from bulk drugs (starting materials to the finished dosage forms) which is applied for identifying or quantifying constituents in a sample.

There are various analytical techniques used for quantitative analysis and qualitative analysis of mixtures. Various pharmaceutical analytical techniques, which are being used can be categorized as follows:

**A) Spectral methods:** Where we use light absorption or emission characteristics of drugs.
Eg: U.V spectroscopy, I.R spectroscopy, NMR spectroscopy, Flourimetry.

**B) Chromatographic methods**: Where we use affinity or partition coefficient difference between drugs.
Eg: Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), Paper chromatography, i.e.,

**C) Electro analytical techniques**: Based on the electrochemical property of drugs.
Eg: Potentiometry, Conductometry, Polarography, Amperometry, Paper electro-phoresis.

**D) Biological and microbiological methods**: Where we used either animals or microorganisms for analysis
Eg: biological assay of some vitamins, microbiological assay of antibiotics and vitamins.

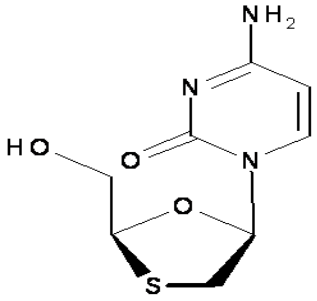
**E) Radioactive methods**: like radioimmunoassay and related techniques.

**F) Physical methods**: where we measure some physical characteristics of drugs
Eg: Differential Thermal Analysis (DTA), Differential Scanning Caloimetry (DSC)
Thermo Mechanical Analysis (TMA), Thermo Gravimetric Analysis (TGA), e.t.c

**G) Miscellaneous techniques**: like conventional titrimetric methods, polarimetric methods e.t.c

**Drug profile**
**Lamivudine**
**Chemical Name**
(-) 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one.

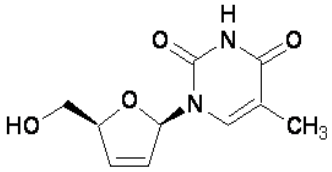
**Molecular Structure**



**Molecular Formula**: $C_8H_{11}N_3O_3S$
**Molecular Weight**: 229.3
**Category**: Antiretroviral (nucleoside reverse transcriptase inhibitor)
**Appearance**: A white to off-white crystalline solid
**Solubility**: Soluble in water and methanol; insoluble in acetone.

**Table 1: Stavudine**
**Chemical Name**
1-[(2R, 5S)-5-(hydroxymethyl)-2, 5-dihydrofuran-2-yl]-5-methyl pyrimidine- 2, 4(1H, 3H)- dione

**Molecular Structure**



**Molecular Formula**: $C_{10}H_{12}N_2O_4$
**Molecular Weight**: 224.2
**Category**: Antiretroviral (nucleoside reverse transcriptase inhibitor)
**Appearance**: A white to almost white powder
**Solubility**: Soluble in water and methanol.

## MATERIALS AND METHODS

**Materials Required**

**Table 1: Name of the Instruments and its companies used.**

| Instruments | Name of the company |
|---|---|
| HPLC | Water's model 2695 |
| U.V detector | Dual λ absorbance detector model 2487 |
| Analytical balance | Ascoset |
| Ultrasonicate water bath | Ultrasonicate SE-COUS |
| PH meter | ADWA Model AD1020 |

**Drugs and Chemicals Required**

**Table 2: Name of the chemicals and its companies.**

| CHEMICALS | NAME OF THE COMPANY |
|---|---|
| Lamivudine | Pharmatrain Research centre,Hyderabad |
| Stavudine | Pharmatrain Research centre,Hyderabad |
| Potassium dihydrogen phosphate | E.Merck |
| Methanol | E.Merck |
| Orthophosphoric acid | E.Merck |
| Water | E.Merck |

**Chromatographic Parameters**

**Table 3: Optimized Chromatographic Parameters.**

| Optimized chromatographic conditions | |
|---|---|
| Mode of separation | Isocratic elution |
| Mobile phase | Phosphate buffer pH 2.5**:**Methanol (80:20) |
| Column | C-18, Thermo (100×4.6, 3.5µm) |
| Flow rate | 0.8 mL/ min |
| Detection Wavelength | 266 nm |
| Injection volume | 20 µl |
| Column oven | Ambient |
| Run time | 6 min |

**Preparation of Phosphate buffer pH 2.5**

Weigh accurately 3.5 grams of $KH_2PO_4$ into a 500ml beaker, dissolved and diluted to 500ml with HPLC water. Adjusted the pH to 2.5 with Orthophosporic acid and degassed in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

**Preparation of mobile phase**

The mobile phase is prepared by mixing a mixture of above buffer 80ml of pH 2.5 phosphate buffer and 20ml of Methanol (HPLC grade) in 100ml of volumetric flask.

**Preparation of Standard Solution**

Accurately weigh and transfer 25 mg of Lamivudine & 25mg of Stavudine working standard into a 100 mL volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**Preparation of Sample Solution**

Weigh 4 tablets of Lamivudine & Stavudine and calculate the average weight. Weight accurately and transfer the sample equivalent to 25 mg of Lamivudine and 25mg of Stavudine into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipette 2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**Selection of wavelength**

From the UV-visible spectrophotometric results, a detection wavelength of 262nm for Stavudine and 271nm for Lamivudine was selected. Because at this wavelength they shows maximum absorbance and then 266nm was selected as common wavelength for simultaneous estimation of both the drugs, as these are eluting in the same mobile phase with good absorbance. The maximum absorbance with good peak intensity, good peak shape and height was observed at 266n.

**Overlaid Spectra of Lamivudine and Stavudine**

**Maximum absorbance occur at 266nm in the Overlaid spectra of Lamivudine and Stavudine**

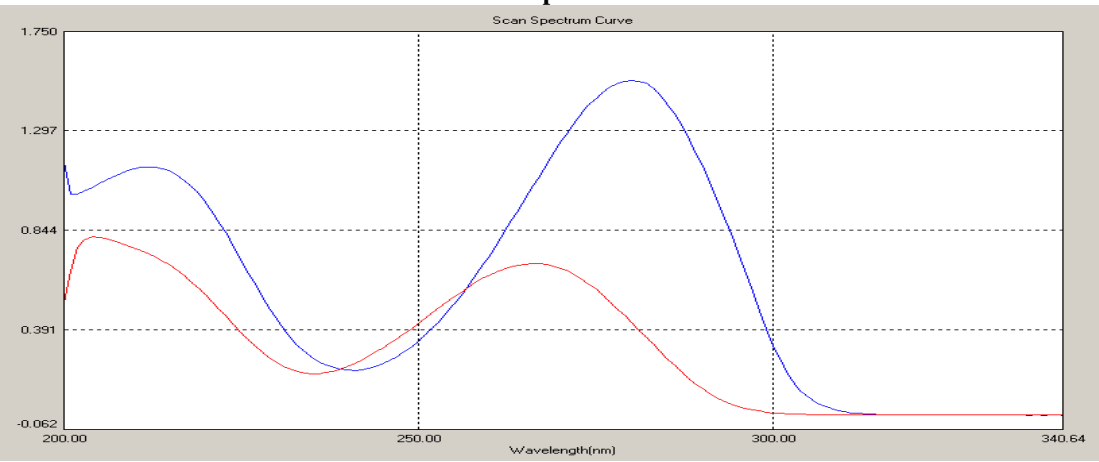



**Figure 1: UV maximum absorption spectrum of Lamivudine and Stavudine.**

## RESULTS

**Accuracy 50%**

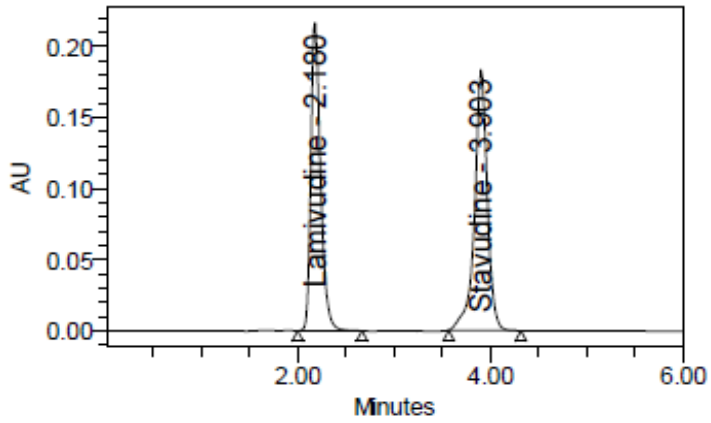



**Figure 2: Chromatogram of accuracy 50% solution.**

**Accuracy 100%**

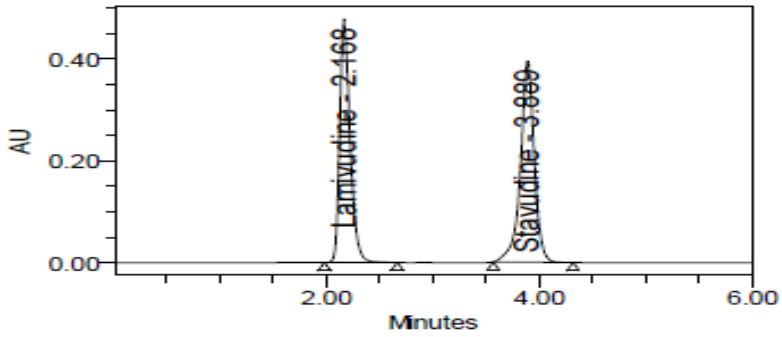



**Figure 3: Chromatogram of accuracy 100% solution.**

**Accuracy 150%**

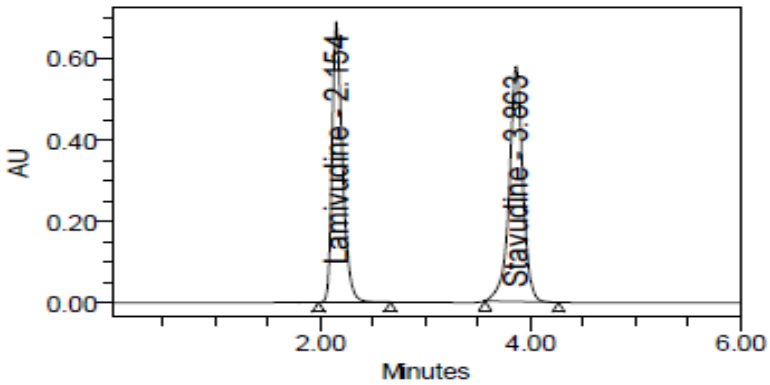



**Figure 4: Chromatogram of accuracy 150% solution**

**Table 4: Recovery studies for Lamivudine and Stavudine.**

| Inj.sample | Spike level | Mean area | Amount present | Amount recovered | % recovered | Mean recovery |
|---|---|---|---|---|---|---|
| **LAMIVUDINE** | 50 % | 1499303 | 11.8mg | 11.6mg | 98.3% | 98.7% |
| | 100 % | 3207970 | 25.12mg | 24.83mg | 98.8% | |
| | 150 % | 4706055 | 36.85mg | 36.43mg | 98.8% | |
| **STAVUDINE** | 50 % | 1672538 | 11.9mg | 11.71mg | 98.41% | 99.1% |
| | 100 % | 3566497 | 25.15mg | 24.97mg | 99.3% | |
| | 150 % | 5196283 | 36.53mg | 36.38mg | 99.5% | |

The mean recoveries of both the drugs were found to be 98.7 and 99.1%. The acceptance criteria limit is should be within 98.0% to 120.0%.

❖ **Precision**

**Intraday Precision**

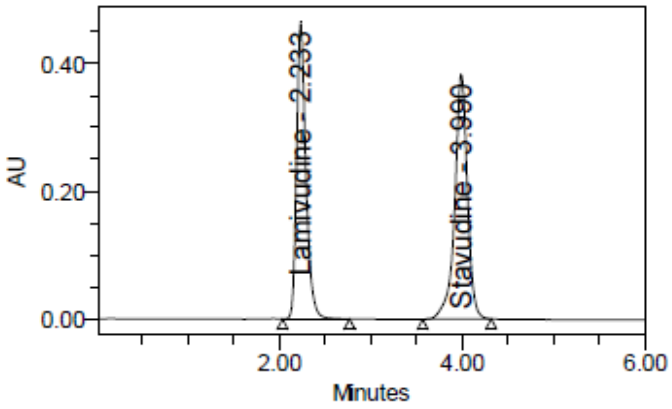



**Figure 5: Chromatogram for Precision.**

**ID Precision**

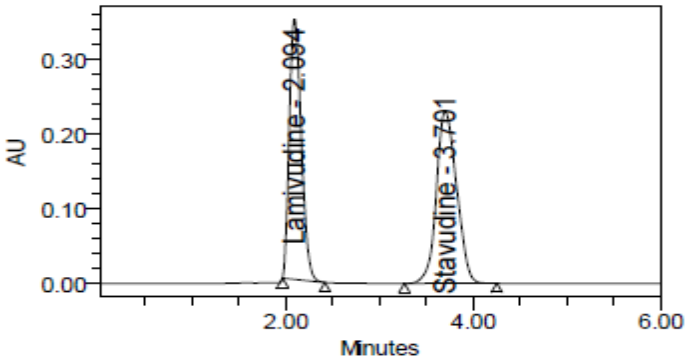



**Figure 6: Chromatogram for Interday Precision.**

**Table 5**: **Chromatographic parameters for Intraday and Interday Precision.**

| Parameter | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|
| Average area | Lamivudine | Stavudine | Lamivudine | Stavudine |
| | 3284765 | 3675650 | 3103171 | 3458791 |
| SD | 13312.8 | 3724.3 | 34255.5 | 15284.9 |
| %RSD | 0.41 | 0.10 | 1.09 | 0.44 |

n = 5; n = no. of injections

The %RSD values for five injection samples of Lamivudine and Stavudine were found to be 0.41 and 0.10 which are well within the acceptance criteria limit of less than 2%

**Linearity**

**[1] For Lamivudine**

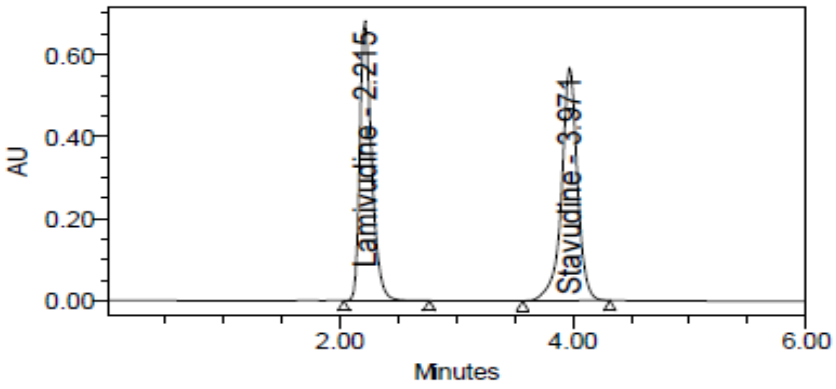



**Figure 7: Chromatogram of linearity curve.**

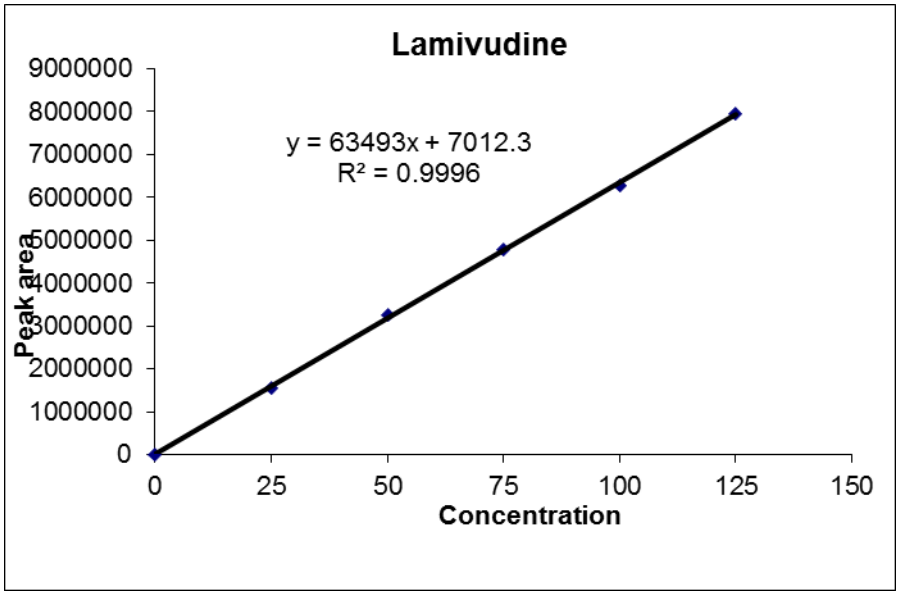



**Figure 8: Chromatogram for linearity curve of Lamivudine.**

**Linearity Results: Lamivudine**

**Table 6: Linearity of Lamivudine.**

| S. No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 25 µg/ml | 1544662 |
| 2 | II | 50µg/ml | 3270619 |
| 3 | III | 75µg/ml | 4790107 |
| 4 | IV | 100 µg/ml | 6281099 |
| 5 | V | 125 µg/ml | 7965562 |
| Correlation Coefficient | | | 0.999 |

The acceptance criteria for correlation coefficient should be not less than 0.999.

**[2] Stavudine**

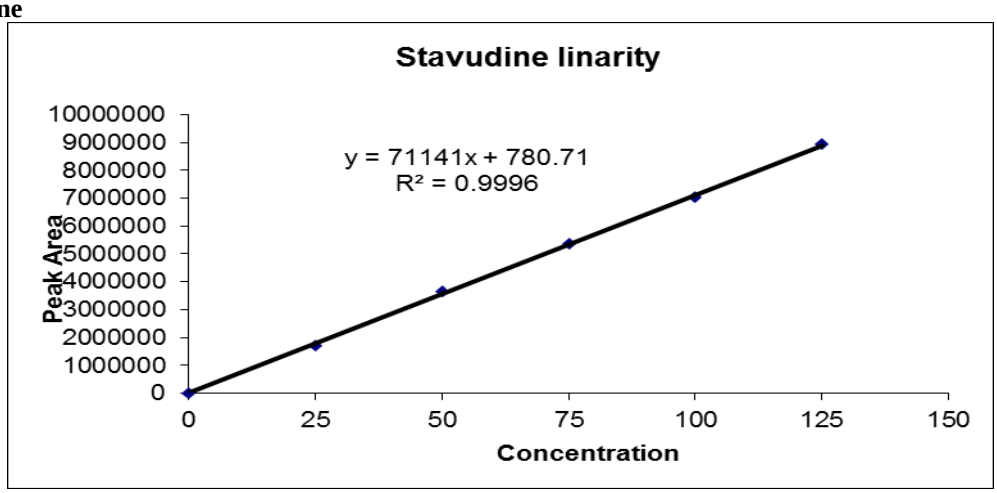



**Figure 9: Chromatogram for linearity curve of Stavudine Linearity Results: Stavudine.**

**Table 7: Linearity of Stavudine**.

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 25 µg/ml | 1721699 |
| 2 | II | 50µg/ml | 3656699 |
| 3 | III | 75 µg/ml | 5346468 |
| 4 | IV | 100 µg/ml | 7032616 |
| 5 | V | 125 µg/ml | 8925253 |
| Correlation Coefficient | | | 0.999 |

The acceptance criteria for correlation coefficient should be not less than 0.99.The correlation coefficient values were found to be within the acceptance criteria for both the drugs.

**System suitability**

**Table 8: System suitability parameters for Linearity curve.**

| Parameters | Lamivudine | Stavudine |
|---|---|---|
| Tailing factor (T) | 1.24 | 0.97 |
| Number of theoretical plate (N)(per meter) | 2772 | 2687 |
| Retention time ($R_t$)(min) | 2.16 | 4.32 |
| %RSD | 0.41 | 0.10 |
| Correlation coefficient | 0.999 | 0.999 |

The tailing factors were found to be 1.24 and 0.97 which were found to be in the acceptance criteria of not more than 2%. The number of theoretical plates were found to be 2772, 2687 which were found to be in the acceptance criteria of not less than 2000. The %RSD values were found to be 0.41 and 0.10 which were found to be in the acceptance criteria limit of less than 2%. The correlation coefficient was found to be 0.99 which is found to be in the acceptance criteria limit of less than 1%.

❖ **LOD (Limit Of Detection)**

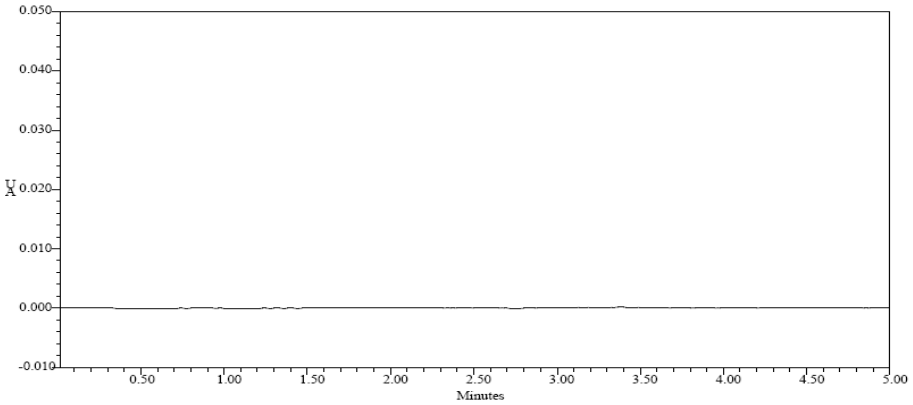



**Figure 10: Chromatogram for checking baseline noise of LOD solution.**

| | Baseline Noise (mV) |
|---|---|
| 1 | 0.052 |

**[3] Lamivudine**

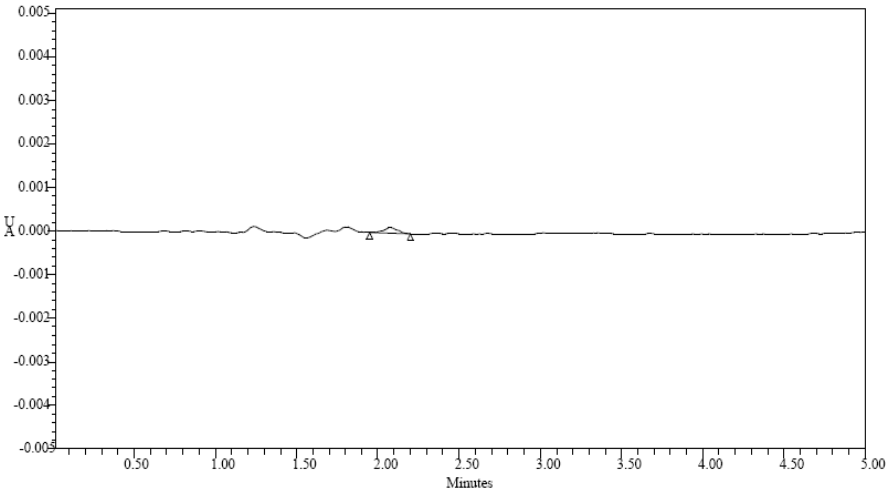



**Figure 11: Chromatogram of LOD for Lamivudine.**

| Peak Name | RT | Height (µV) |
|---|---|---|
| Lamivudine | 2.166 | 152 |

**Calculation of S/N Ratio** S/N = 152/52 = 2.9

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.04%oftarget assay concentration) : 152µV

**Acceptance Criteria**

- S/N Ratio value shall be 3 for LOD solution.

**[4] Stavudine**

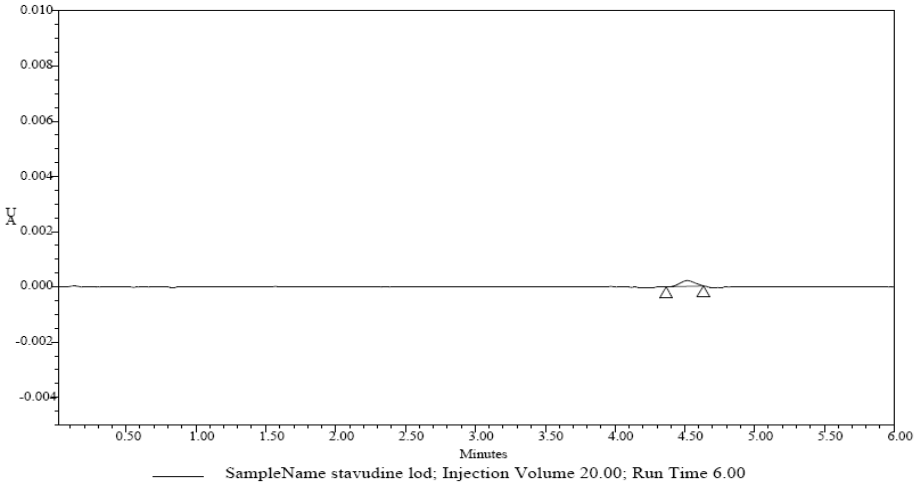



**Figure 12: Chromatogram of LOD for Stavudine.**

| Peak Name | RT | Area | Height |
|---|---|---|---|
| stavudine | 4.321 | 1492 | 168 |

**Calculation of S/N Ratio** S/N = 168/52 = 3.2

Average Baseline Noise obtained from Blank

: 52 µV

Signal Obtained from LOD solution (0.04%oftarget assay concentration) : 168µV

**Acceptance Criteria**

S/N Ratio value shall be 3 for LOD solution.

❖ **LOQ (Loss of Quantification)**

**[5] Lamivudine**

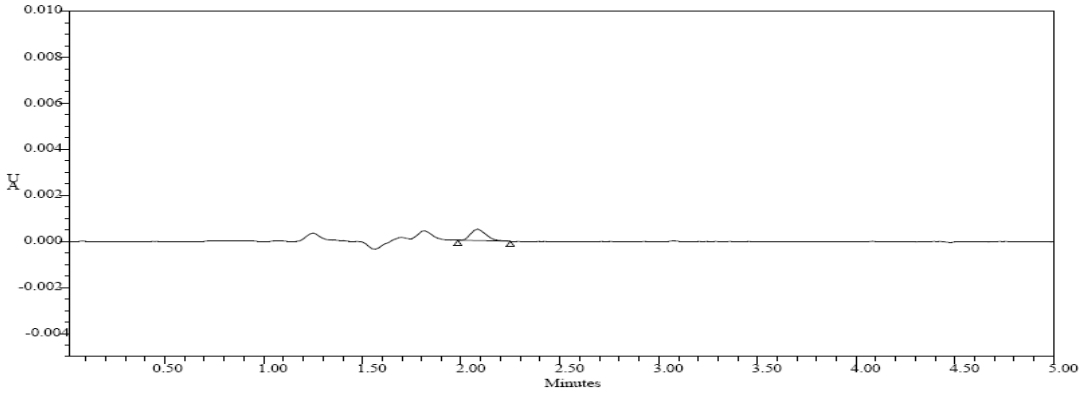



**Figure 13: Chromatogram of LOQ for Lamivudine.**

| Peak Name | RT | Height (µV) |
|---|---|---|
| Lamivudine | 2.094 | 497 |

**Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.6% of target assay concentration) 497µV

S/N = 497/52 = 9.5

**Acceptance Criteria**

S/N Ratio value shall be 10 for LOQ solution.

**5) Stavudine**

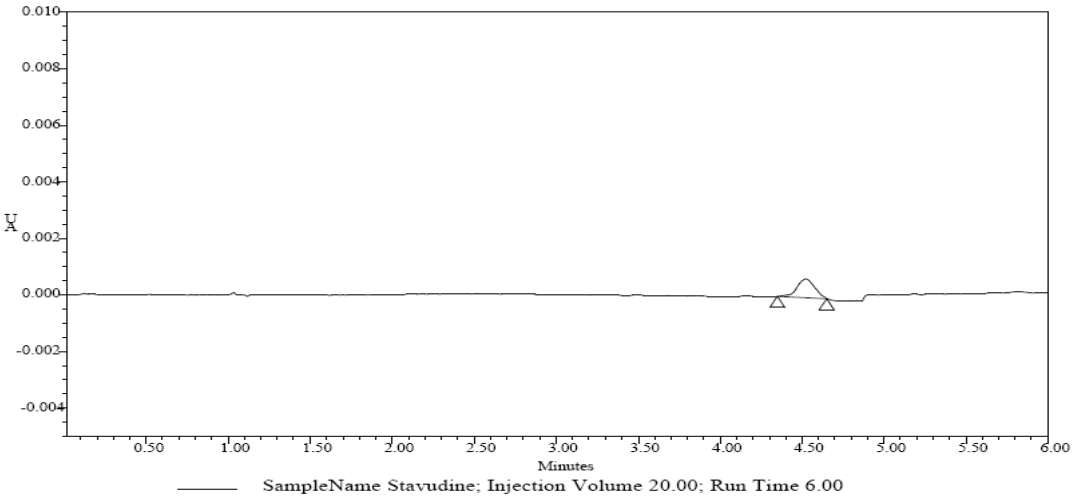



**Figure 14: Chromatogram of LOQ for Stavudine.**

| Peak Name | RT | Area | Height |
|---|---|---|---|
| Stavudine | 4.321 | 4885 | 540 |

**Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.7% of target assay concentration): 540µV

S/N = 540/52 = 10.3

**Acceptance Criteria**

S/N Ratio value shall be 10 for LOQ solution.

The S/N ratio of LOD and LOQ values were found to be with in the acceptance criteria limit of 3 and 10.

❖ **Robustness**

**[6] More Flow**

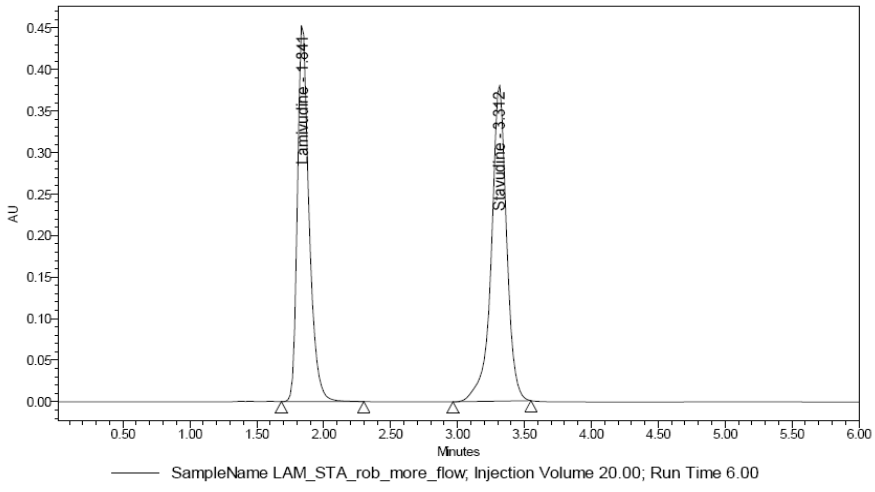



**Figure 15: Chromatogram of more flow.**

**[7] Less Flow**

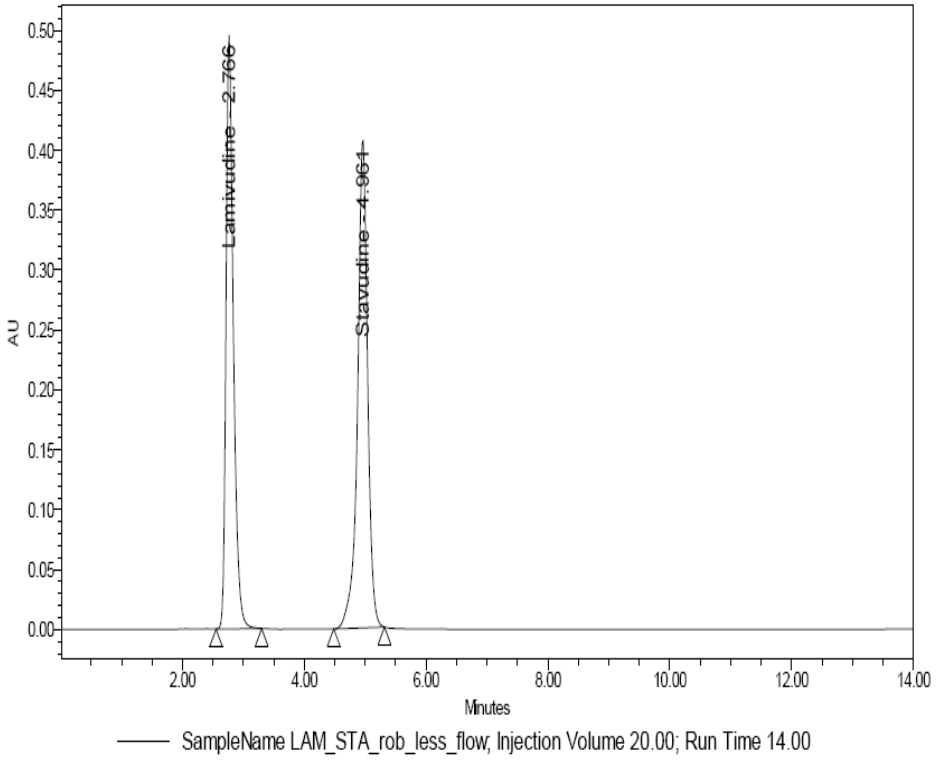



**Figure 16: Chromatogram of Less flow.**

**[8] More Organic**

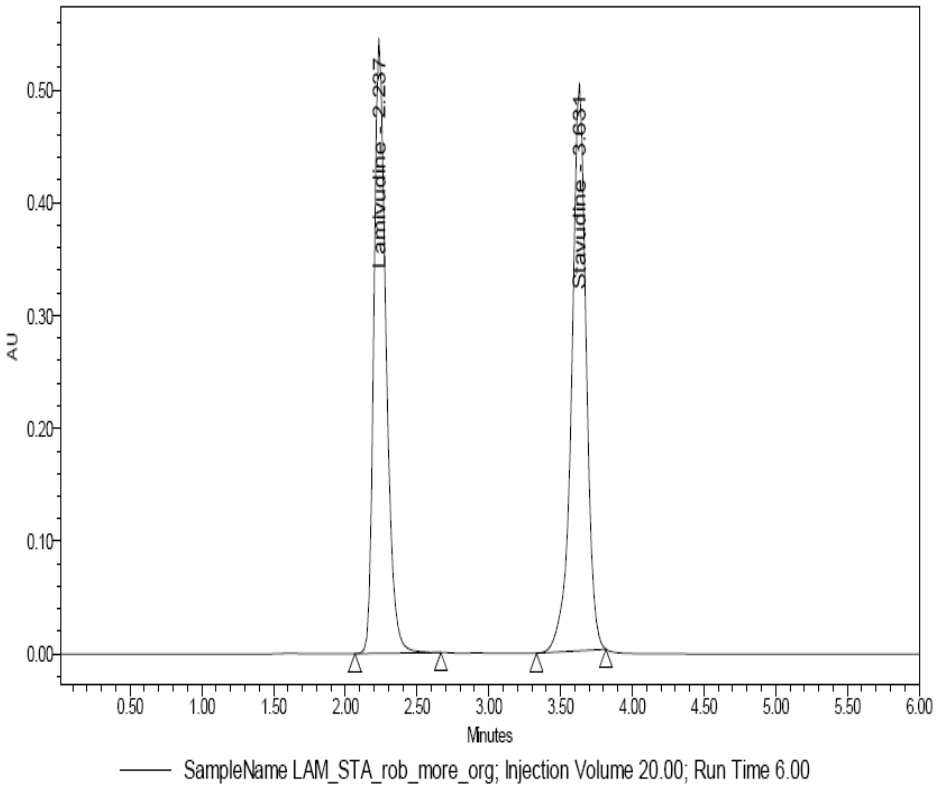



**Figure 17: Chromatogram of more organic.**

**[9] Less Organic**

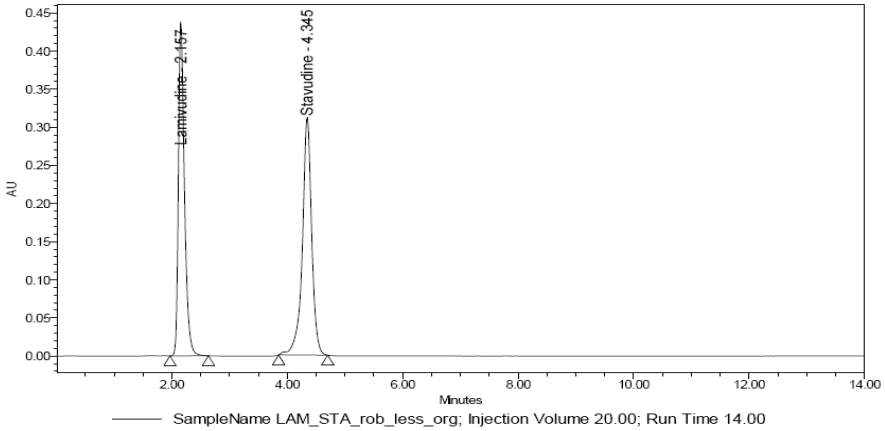



**Figure 18: Chromatogram of less organic.**

***** Results for actual flow (0.8ml/min) have been considered from Assay standard

***** Results for actual Mobile phase composition (80:20 Buffer: Methanol) have been consider

**Table 9: System suitability parameters for Lamivudine and Stavudine.**

| Parameter | | Plate count | Tailing | RT | Plate count | Tailing | RT |
|---|---|---|---|---|---|---|---|
| | Lamivudine | | | | Stavudine | | |
| Flow Rate (ml/min) | 0.6 | 2547.26 | 1.37 | 2.7 | 4939.24 | 0.89 | 4.9 |
| | 0.8* | 2771.79 | 1.24 | 2.0 | 2687 | 0.97 | 3.6 |
| | 1.0 | 2318.19 | 1.36 | 1.8 | 4513.50 | 0.90 | 3.3 |
| pH | 10%less | 2085.10 | 1.41 | 2.1 | 3983.39 | 1.27 | 4.3 |
| | 0 actual | 2771.79 | 1.24 | 2.0 | 2687 | 0.97 | 3.3 |
| | 10%more | 3347.46 | 1.27 | 2.2 | 6777.54 | 0.89 | 3.6 |

**[10] Lamivudine Assay**

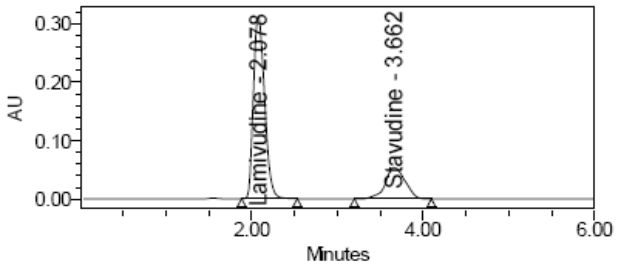



**Figure 19: Chromatogram for Lamivudine assay.**

| Peak name | Rt | Area |
|---|---|---|
| Lamivudine | 2.018 | 2861263 |
| Stavudine | 3.662 | 863654 |

**[11] Stavudine And Lamivudine Standard Graph**

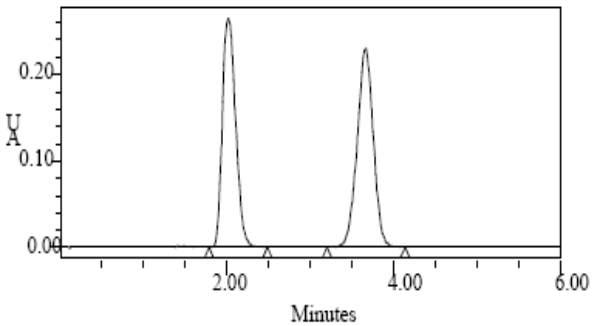



**Figure 20: Chromatogram of Standard graph.**

| Peak Name | RT | Area | USP Plate Count | USP Tailing |
|---|---|---|---|---|
| Lamivudine | 2.017 | 2917920 | 2771.79 | 1.24 |
| Stavudine | 3.660 | 3095838 | 2687.36 | 0.97 |

1). Tailing factor Obtained from the standard injection is 1.24
2). Theoretical Plates Obtained from the standard injection is 2772

$$\frac{2838202}{2917920} \times \frac{25.5}{100} \times \frac{2}{10} \times \frac{100}{83} \times \frac{10}{2} \times \frac{99.7}{100} \times 100 \times \frac{500}{150}$$

% Recovery of Lamivudine formulation = 99.31%

**Table 10**: **Percentage Label claim details for Lamivudine and Stavudine.**

| DRUG | Label Claim mg/tablet | Amount Estimated | Percentage Label Claim |
|---|---|---|---|
| Lamivudine | 150 | 147.08 | 98.7 |
| Stavudine | 40 | 38.74 | 98.1 |

The percentage of label claim was found as 98.7 and 98.1 are within the limits of acceptance.

**[12] Stavudine Assay**

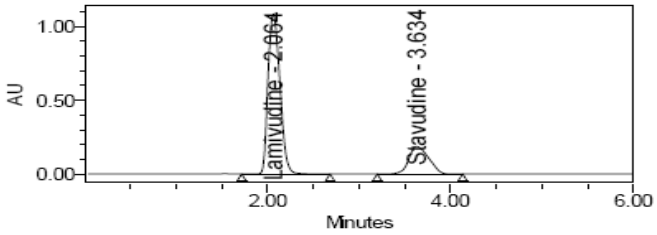



**Figure 21: Chromatogram of Stavudine assay.**

| Peak name | Rt | Area |
|---|---|---|
| Lamivudine | 2.064 | 9836282 |
| Stavudine | 3.634 | 2938089 |

1). Tailing factor Obtained from the standard injection is 0.97
2). Theoretical Plates Obtained from the standard injection is 2687

**[13] Assay Results**

Weight of 10 tablets : 2.000 grams
Average Weight : 0.500 grams

$$\frac{2838089}{3095838} \times \frac{25.05}{100} \times \frac{2}{10} \times \frac{100}{290} \times \frac{10}{2} \times \frac{99.7}{100} \times 100 \times \frac{500}{40}$$

**% Recovery of Stavudine formulation = 98.68%**

**DISCUSSION**

An effort has been made to identify a simple, precise, specific and accurate method for the estimation of Lamivudine and Stavudine in formulations by using RP-HPLC method.

During the selection of mobile phase several solvents were tried at various levels and finally selected mobile phase system was methanol and phosphate buffer $p^H$ 2.5 at ratio 80:20.

The solution of 10µg/ml of Lamivudine and Stavudine in mobile phase (methanol: phosphate buffer $p^H$2.5) was prepared and the solution was scanned in the range of 200-400nm. At 266nm the drugs shows maximum absorbance overlapping spectrum. Hence this was selected as a detection wavelength of U.V spectrum shown in figure 2. After considering all the system suitability parameters methanol and phosphate buffer pH 2.5 (80:20) was selected for analysis at optimized flow rate of 0.8ml/min. The retention time of Lamivudine and Stavudine was found to be 2.017min and 3.66 min. The system suitability parameters are caliculated are shown in Table 8.

The calibration was done with the optimized chromatographic conditions, stock solution of Lamivudine and Stavudine using mobile phase and various concentration ranges of 25 to 125µg/ml were prepared. From this 20µl of each solution were injected individually and the chromatogram was recorded at 266nm. The linearity graph was plotted using concentration against peak area. The correlation coefficient for both drugs was found to be 0.995 and 0.996 indicates that the concentration of Lamivudine and Stavudine had given good linearity as shown in Figures 9 & 10 and Tables 6 & 7.

Accuracy was confirmed by recovery studies by proposed method and their chromatograms were recorded as shown in the Figures 3, 5 & 6. The percentage recovery of Lamivudine and Stavudine was found to be 98.7% and 99.1% which are within the limits as shown in Table 4. The high percentage of recovery indicates that there are no interfere will be produced. Hence the developed method was found to be accurate.

The precision has done in two ways i.e. Intraday and Inte-rday precision. 20µl of 5 injection samples are injected for each precision. For Intraday precision the %RSD values were found to be 0.41 and 0.10 for Lamivudine and Stavudine respectively. For the Inter day precision the %RSD values were found to be 1.09 and 0.44 for Lamivudine and Stavudine respectively as shown in table 5.

The limit of detection and the limit of quantification were determined from the Signal to Noise ratio as shown in the Figure11. The limit of detection was found to be 2.9 and 3.0 for Lamivudine and Stavudine respectively as shown in Figure 12 & 13. The limit of quantification was found to be 9.5 and 10.3 for Lamivudine and Stavudine respectively as shown in Figure 14 &15.

The robustness of the method developed was validated by changing the flow rate and mobile phase composition has shown in the figure 16, 17, 18 and 19. & table 9. The selected flow rate and mobile phase composition gives good separation of drugs.

The tablet formulation was selected for analysis. The nominal concentration (50µg/ml) considered and 20µl of formulation was injected. The percentage of Lamivudine and Stavudine present in the sample was found to be 99.0% and 98.0% respectively as shown in fig 20 and 22.

All the above parameters combined with the simplicity and ease of operation ensures that the RP-HPLC method can be applied for the Simultaneous estimation of Lamivudine and Stavudine in tablet dosage form.

## CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the determination of Lamivudine and Stavudine in combined tablet formulation. The developed method was checked for the performance characteristics and has also been validated.

The method was found to be linear(r>0.999) precise (RSD: 0.41 for Lamivudine, 0.10 for Stavudine) and accuracy (mean percentage recovery fields 98.7% for Lamivudine, 99.1% for Stavudine) the proposed HPLC method was simple, precise because of commonly used buffer and shorter run time.

The mean percentage recovery above 95% indicates the reproducibility and accuracy of new developed method compared. The result of study include the proposed method is highly accurate, simple, precise and specific. The simple recoveries in all formulations were in good agreement with their respective label claims. And they suggest non-interference of formulation excipients in the estimation. After validating proposed method as per ICH guidelines and correlating obtained values with the standard values, satisfactory results were obtained.

Hence the method can easily and conveniently adopted for the estimation of combined dosage form of Lamivudine and Stavudine.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in publication of this paper.

## REFERENCE


1. Bently and Drivers, "Text book of Pharmaceutical Chemistry," 8th Edition, O'Brein, Oxford University Press, 1985; 1 – 3.
2. Napoleon A.A. "Pharmaceutical Titrimetric Analysis theory and practical," Kalaimani Publishers and Distributors, Kanchipuram, 1.1 – 1.4.
3. Ewing, G.W, "Instrumental Methods of Chemical Analysis", 2nd Edition, MC Graw Hill Book Company, 1960; 1-3.
4. GurdeepR.Chatwal and Sham K.Anand, "Instrumental Methods of Chemical Analysis 5th Edition, Himalaya Publishing House, 1.2 – 1.5.
5. U.S.P. Asian Edition, United Pharmacopoeial Convention Inc, Rockville, 2005; 2386-2389.
6. James.W Munson, "Modern Methods of Pharmaceutical Analysis, Medical Book Distributors, Mumbai, 2001; 17-54.
7. Sethi P.D. HPLC 'High Performance Liquid Chromatography and Quantitative analysis Pharmaceutical Formulations", 1st Edition, CBS Publishers and Distributors, 2001; 3-5.
8. Erwing, G. W., "Instrumental Methods of Chemical Analysis,' 2nd Edn., McGraw Hill Book Company, 1960; 3-5.
9. Jenkins GL., Knevel, A.M and Digangi, F. E., "Quantitative Pharmaceutical Chemistry". 7th Edn. McGraw Hill Book Co.,New York, Edition, 1977; 297-299.
10. Beckett, A. H and Stenlake, J. B., "Practical Pharmaceutical Chemistry"4th Edition. Part II, CBS

Publisher and Distributor, New Delhi, 1997; 277-278
11. Sethi P.D, "*HPLC* Quantitative Analysis of Pharmaceutical formulations"CBS Publisher and Distributor, New Delhi, 1996; 5.
12. L. R. Snyder, J.J. Glajch, J.J "Practical HPLC Method Development'. Kirland, 2nd Edition A Wiley- Inter-science Publication, 234-265.
13. A.H. Beckett and J.B. Stanlake "Text Book of Practical Pharmaceutical Chemistry" 4th Edition Part II, C.B.S., Publishers and Distributors, New Delhi, 158-164.
14. Loyd. K. Snyder, Joseph. J. Kirkland and joseph. L. Glajch, "Practical HPLC Method Development 2nd Edition, Wiley – Interscience Publication, USA, 705-710.
15. Loyd R. Snyder. Practical HPLC Method Development. 4th ed. A-Wiley- inter Science publication, 1997; 234-265.
16. Sharma B.K., "Instrumental Method of Chemical Analysis", 23rd Edn. Goal publication house, Merrut, 1991; 7-10.
17. RamaRao, G.Murthy, S.S.N and Khadgapathi P., "High Performance Liquid Chromatograpy and its role in pharmaceutical analysis", Eastern Pharmacist, 1986; 20(346): 53-59.
18. Sastry C.S.P., Prasad T.N.V. and Rao E.V., "Recent application of High Performance Liquid Chromatography in Pharmaceutical Analysis", Indian. Pharm. Education, 1987; 21: 37-40.
19. Loyd. K. Snyder, Joseph. J. Kirkland and joseph. L. Glajch, "Practical HPLC Method Development 2nd Edition, Wiley – Interscience Publication, USA, 1991; 742-747.
20. Willard, H.Y. Merritt L.L., Dean J.A. and Settle FA "Instrumental Methods of Analysis", 7th Edition CBS Publishers and Distributors, New Delhi, 1991; 436-439.
21. Kapoor N, Khandavilli S, Panchagnula R. "Simultaneous determination of Lamivudine, Stavudine and Nevirapine in Anti-Retroviral fixed dose combinations by High Performance Liquid Chromatography". Journal of pharmaceutical and biomedical analysis. Edition, 2006; 41: 761-765.
22. Socklingam Anbazhagan S, Indumathy N, Shanmiga Pandiyan P, Sridhar S K, " Simultaneous quantification of Lamivudine , Stavudine and Nevirapine by HPLC and HPTLC in tabletsJ Pharm Biomed AnalEd, 2005; 39: 801-804.
23. S.K Patro, S.R.Swain, V.J.Patro and N.S.K. Choudhury: "Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine And Stavudine".E-journal of chemistry Edition, 2010; 7(1): 117-122.
24. Weerasaksamee, ParonSrilamai, Sasithorn Ongort, Chayanid Sornchaithawatwong and Suwanna Vorarat: "Simultaneous Determination of Lamivudine, Stavudine and Nevirapine in the Presence of Their Acid-Induced Degradation Products by HPLC";The pharmaceutical and health science journal, Edition, 2007; 2(1): 39-45.
25. D.K.Mandloi, P.K.Tyagi, V.K.Rai, S. Dey, R.K Ashada, P.Mohanraj, "Method development and validation of RP- HPLC in the application of *invitro*Dissolution study of Lamivudine in bulk drug and tablet formulation"Journal of Chemical and Pharmaceutical Research, *2009*; 1: 286-296.
26. Ch.Balasekarreddy , BahlulZ.Awen, ch.Baburao, N.Sreekanth and P.Ramalingam, "Validated HPLC method for Determination of Lamivudine and Stavudine in their formulations" An International Journal of Advances In Pharmaceutical Sciences, Editionep, 2010; 1(1): 22-28.
27. J. Nijamdeen, B. Jayalakshmi, N. Senthilkumar, Vijayamirtharaj and C. Saravanan, **"**Method development and validation of RP-HPLC Method for Simultaneous Determination of Lamivudine and Zidovudine",Journal of Chemical and Pharmaceutical Research,Edition, 2010; 2(3): 92-96.
28. S.Jayaseelan,S.Ganesh, M.Rajasekar, V.Sekar, P.Perumal,"A New Analytical Method Development and Validation for the Simultaneous Estimation of Lamivudine and Stavudine in Tablet Dosage Form by RP-HPLC Method", International Journal of PharmTech Research, April-June2010; 2(2): 1539-1542.
29. D.Anantha Kumar, G. Srinivasa Rao and J.V.L.N. Seshagiri Rao, **"**Simultaneous Determination of Lamivudine, Zidovudine and Abacavir in Tablet Dosage Forms by RP HPLC Method", E-Journal of Chemistry, 2010; 7(1): 180-184.
30. SurajpalVerma, Pooja Mullic, M.Dsleem Butt, Nadeem Siddiqui, OzairAlam, InduBala and Suroor A. Khan, "Bio analytical Method Development and Validation for the Simultaneous Estimation of Lamivudine and Stavudine in Human Plasma by HPLC", ActaPoloniae Pharmaceutical Drug Research, Edition, 2010; 67(4): 429-437.