Validation of UV spectroscopy for simultaneous estimation of stavudine, lamivudine and nevirapine in tablet formulations

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Abstract

Mostly antiretroviral drugs are estimated using methods which require expensive equipment and trained technicians. Spectrophotometric method is relatively simple and accurate for the estimation of antiretroviral drugs in pharmaceutical formulations which is also cost effective when compared to other specialized methods. In the present study, simultaneous quantification of stavudine (STV), lamivudine (LMV) and nevirapine (NVP) by UV spectroscopy method was developed. The drugs STV, LMV and NVP were quantified at 266, 271 and 315 nm, respectively. Recovery values of 98.92 - 100.12 %, 99.40 – 100.13%, 99.01-100.32% for STV, LMV and NVP respectively with percentage relative standard deviation of <1 shows that the developed method was accurate and precise. This method can be employed for the routine analysis of tablets containing STV, LMV and NVP as comparable to other methods requiring higher facilities.

Keywords: Stavudine; Lamivudine; Nevirapine; UV spectrophotometry; Simultaneous

Introduction

Stavudine (STV), lamivudine (LMV) and nevirapine (NVP) are the first line drugs of the highly active antiretroviral therapy utilized for HIV. This triple drug combination rules out the several disadvantages of the single drug therapy by improving adherence to therapy and enhancing better patient compliance(1). STV (2–6), LMV (7–17) and NVP (18–29) have been reported to be estimated individually or in combination with other drugs by several methods. LMV along with zidovudine (30) has been reported to be estimated by RP-HPLC method. LMV and STV (31) have been reported to be simultaneously quantified in tablets by visible spectroscopy. Literature reviews reveal that there very few works are reported for the simultaneous estimation of STV, LMV and NVP in tablets. In the present study, simultaneous quantification of STV, LMV and NVP in tablets by uv-spectrophotometry method was developed and validated.

Materials and Methods

Materials

Hetero Drugs Ltd., India, provided the gift samples of pure STV, LMV and NVP. All the other chemicals used were of analytical grade.

Development of analytical method

Selection of solvent

Phosphate buffer pH – 6.8 was selected as the dissolution media because it proved to be most suitable for development of osmotic pressure by dissolving the osmogen and also has good permeability through the semi permeable membrane coating on the prepared tablets.

Solubility of Stavudine, Lamivudine and Nevirapine in Phosphate buffer

Solubility of the drugs in phosphate buffer pH – 6.8 was determined by dissolving the known quantity of the drugs in cumulative manner with the aid of sonication till the drug remains insoluble and the values noted.

Determination of Absorbance maximum

The λ_{max} of the drugs were determined by running the spectrum of drug solutions in double beam ultraviolet spectrophotometer. UV spectra of the drugs were carried out in phosphate buffer pH – 6.8. The drugs were (10 mg) accurately weighed and dissolved in the suitable medium. The solution was then diluted using the same medium. An 80 µg/m1 solution was kept in a fused silica cell and UV spectrum was recorded in the wavelength range of 200-400 in U.V. Spectrophotometer (Perkin Elmer Lamda-25).

Validation of analytical method (32-38)

Linearity and Range

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which is directly proportional to the concentration (amount) of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the active substance (by dilution of a standard stock solution) and/or on separate weighing of the mixture of the product components, using the proposed procedure.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The drugs (10mg) were accurately weighed and dissolved in 100 ml of phosphate buffer pH – 6.8 to give a stock solution of concentration 100 µg/ml. From this stock aliquots of solutions were transferred into nine 10 ml volumetric flasks and the final volume was adjusted with the appropriate medium to give concentrations of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 µg/ml. The absorbance was measured at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively against a blank medium. This experiment was performed in replicate of three and the average of absorbance was calculated by plotting the curve of absorbance verses concentration.

Specificity

Specificity is the ability to assess the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity test is done by measuring the absorbance of placebo solution, standard solution and mixture of placebo and standard solution.

Standard solutions were prepared by dissolving 10 mg of accurately weighed drugs in 500 ml of phosphate buffer pH – 6.8 with the aid of sonication till the solution is formed. From this stock solution, 30 ml of solution was withdrawn and diluted up to 100 ml with same medium in 100 ml volumetric flask.

Placebo solution was prepared by the addition and mixing of excipients required for the formulation along with the other two drugs in 500 ml of phosphate buffer pH – 6.8. From this solution 30 ml was withdrawn and diluted up to 100 ml with the same medium in a 100 ml volumetric flask.

Mixture of drug and placebo were prepared by dissolving accurately weighed drug (10 mg) and equivalent quantity of mixed excipients required in formulation in 500 ml of phosphate buffer pH – 6.8 in volumetric flask with aid of sonication for 20 minutes. From this solution 30 ml of solution was withdrawn and was diluted up to 100 ml with same medium in 100 ml volumetric flask. All these three solutions were filtered through 0.45µm filter and estimated by UV Spectrophotometry.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

A. Method precision

Method precision test was performed by measuring the absorbance of the target solution of target concentration at its λ max in six replicates.

Stock solutions of the drugs were prepared according to the earlier procedure and suitable dilution was made to prepare 25 µg/ml solutions. The absorbance of these solutions was measured at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively. This experiment was performed in six replicates. Standard deviation and % relative standard deviation (R.S.D) between six absorbance values were calculated.

B. System Precision

System precision test was done by measuring the absorbance of solution at small time interval in replicates of nine. This test is also known as repeatability.

The stock solutions of the three drugs were prepared as described previously and suitable dilution was made to prepare 25 µg/ml solutions. The absorbance of these solutions was read at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively at small time interval with nine replicates.

C. Interday and Intraday Precision

Interday precision was done by measuring the absorbance of solutions of the drugs of 30 µg/ml concentrations at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively at 2 hours interval within a day.

Intraday precision test was done by measuring the measuring the absorbance of the 25 µg/ml solution of the drugs in phosphate buffer pH – 6.8 at 266 nm, 271nm and 314nm for three days. The average, standard deviation and percentage RSD of the absorbance was calculated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy should be established across the specified range of the analytical procedure.

The blend of placebo and the drugs were prepared by mixing the active drug in to the placebo powder at 25%, 50%, 75%, 100%, 125%, 150% level of the target concentration each in triplicate. The recovery of sample was analyzed as per proposed test method and the amount of drug recovered was calculated at each spike level and 5 recovery at each spike level.

Robustness test

The robustness of an analytical method is a measure of capacity to be unaffected by small, deliberate variation in method parameters and provides an indication of its reliability during normal usage. Robustness test was done by measuring the solution stability for 8 hours.

The main stock solution was prepared as described previously. From this stock aliquots of solutions were transferred into 10 ml volumetric flasks and the final volume was adjusted with the appropriate medium to give concentrations of 10, 15, 20, 25 µg/ml solution. The absorbance of these solutions was measured at interval of 1 hour for 8 hours.

Estimation of drugs in tablets

About 20 tablets were weighed and powdered. A powder equivalent of 25 mg of the drug was weighed accurately and transferred to a 100 ml volumetric flask. The tablet powder was dissolved in 0.01M hydrochloric acid and filtered through a Whatmann filter paper. The solution was further diluted and absorbance was recorded.

Recovery studies

Recovery studies were carried out by adding known quantities of standards at different levels to the pre-analyzed sample to study the linearity, accuracy and precision of the proposed methods. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in the dosage forms.

Standard curve of Stavudine, Lamivudine and Nevirapine

The drugs (10mg) were accurately weighed and dissolved in 100 ml of medium, i.e. Phosphate buffer pH – 6.8 to give a stock solution of concentration 100 µg/ml. From this stock aliquots of solutions were transferred into 10 ml volumetric flasks and the final volume was adjusted with the appropriate medium to give concentrations of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80µg/ml. The absorbance was measured at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively against a blank medium in U.V. spectrophotometer (Perkin Elmer Lamda-25) based on the validated analytical method.

Results and discussion

Systematic quality control analysis of antiretroviral drugs is required for a successful AIDS therapy. The proposed spectrophotometric method is relatively simple and accurate for the estimation of antiretroviral drugs in pharmaceutical formulations

The absorption maxima of stavudine, lamivudine and nevirapine were found to be 266nm, 271nm and 314 nm in phosphate buffer pH – 6.8. The absorbance of the solutions was linear with the concentration of the drug in the range of 10 to 30 µg / ml. This analytical method was validated for accuracy, precisions, and specificity according to ICH guidelines. The analytical method described above is specific for the three drugs as there is no drug- drug or drug - placebo interference. The method is also precise because the % relative standard deviation of method precision was found to be less than 2% and that of the system precision was less than 1%. The results of the intraday and interday precisions show that the % RSD of the absorbance of the drugs is less than 1%. The results of the analytical methods are shown in tables 1-10. All the three drugs obeyed the Beer's law in the concentration range of 5 - 30µg / ml as shown in tables 11-13 and figures 1-3.

The method is linear in the concentration range reported. The developed method is free from interference due to the excipients present in various brands of tablets and can be used for routine simultaneous quantitative estimation of STV, LMV and NVP in pharmaceutical formulations. Previous reports suggest that the contents of stavudine, lamivudine and nevirapine in tablets estimated by HPLC and spectrophotometric methods were similar, and the variation in the amount of these drugs estimated by HPLC and spectrophotometric methods was below 10%. This suggests that the proposed spectrophotometric method is as accurate as the HPLC method for estimation of stavudine, lamivudine and nevirapine in pharmaceutical formulations (39).

Conclusions

The proposed uv-spectrophotometry method is precise, rugged, robust, simple and rapid. Hence the present method is suitable for the simultaneous estimation of stavudine, lamivudine and nevirapine in pharmaceutical formulations. Hence laboratories that do not have specialized equipments may also undertake these drug estimations using spectrophotometer.

References

[1] Simon, H. Everitt, J. Birtwistle, B. Stevenson, Oxford Hand Book of General Practice, Infective Disease. Human Immunodeficiency Virus. Oxford University Press, New Delhi, India, 2002.

[2] D.M. Burger, H. Rosing, R.V. Gijn, P.L. Meenhorst, O.V. Tellingen, J.H. Beijnen. Determination of stavudine, a new antiretroviral agent, in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection. Journal of Chromatography B: Biomedical Sciences and Applications, 1992, 584: 239–247.

[3] J.S. Janiszewski, D.E. Mulvana, S. Kaul, R.H.D. Barbhaiya. High-performance liquid chromatographic determination of 2′,3′-didehydro-3′-deoxythymidine, a new anti-human immunodeficiency virus agent, in human plasma and urine. Journal of Chromatography B: Biomedical Sciences and Applications, 1992, 577: 151–156.

[4] M. Sarasa, N. Riba, L. Zamora, X. Carne. Determination of stavudine in human plasma and urine by high-performance liquid chromatography using a reduced sample. J. Chromatogr. B Biomed. Sci. Appl., 2000, 746:183–189.

[5] G. Aymard, M. Legrand, N. Trichereau, D. Diquet. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl., 2000, 744: 227–240.

[6] A.J. Harker, G.L. Evans, A.E. Hawley, D.M. Mossis. High-performance liquid chromatographic assay for 2′-deoxy-3′-thiacytidine in human serum. J. Chromatogr. B Biomed. Sci. Appl., 1994, 657: 227–232.

[7] P.H. Hsyu, T.L. Lloyd. Automated high-performance liquid chromatographic analysis of (−)-2′-deoxy-3′-thiacytidine in biological fluids using the automated sequential trace enrichment of dialysate systems. J. Chromatogr. B Biomed. Sci. Appl., 1994, 655: 253–259.

[8] X.J. Zhou, J.P. Sommadossi. Rapid quantitation of (−)-2′-deoxy-3′-thiacytidine in human serum by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl., 1997, 691: 417–424.

[9] R.M. Hoetelmans, M. Profijit, P.L. Mennhorst, J.W. Mulder, J.H. Beijnen. Quantitative determination of (−)-2′-deoxy-3′-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl., 1998, 713: 387–394.

[10] J.D. Moore, G. Valette, A. Darque, X.J. Zhou, J.P. Sommadossi. Simultaneous quantitation of the 5'-triphosphate metabolites of zidovudine, lamivudine, and stavudine in peripheral mononuclear blood cells of HIV infected patients by high-performance liquid chromatography tandem mass spectrometry. J. Am. Soc. Mass Spectrom., 2000, 11: 1134–1143.

[11] S. Pereira, K.B. Kenny, M.S. Cohen, J.E. Hall, J.J. Eron, R.R. Tidwell, J.A. Donn. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. J. Chromatogr. B Biomed. Sci. Appl., 2000, 742: 173–183.

[12] Volosov, C. Alexander, L. Ting, S.J. Soldin. Simple rapid method for quantification of antiretrovirals by liquid chromatography-tandem mass-spectrometry. Clin. Biochem., 2000, 35: 199–203.

[13] J.J. Zheng, S.T. Wu, T.A. Emm. High-performance liquid chromatographic assay for the determination of 2′-deoxy-3′-thiacytidine (lamivudine) in human plasma. J. Chromatogr. B Biomed. Sci. Appl., 2001, 761: 195–201.

[14] V.A. Simon, M.D. Thiam, L.C. Lipford. Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. J. Chromatogr. A., 2001, 913: 447–453

[15] Fan, J.T. Stewart. Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC. J. Pharm. Biomed. Anal., 2002, 28: 903– 908.

[16] Fan, M.G. Bartlett, J.T. Stewart. Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch. Biomed. Chromatogr., 2002, 16: 383–389.

[17] Becher, A. Prurost, C. Goujard, C. Guerreiro, J.F. Delfraissy, J. Grassi, H. Benech. Improved method for the simultaneous determination of d4T, lamivudine and ddl intracellular phosphorylated anabolites in human peripheral-blood mononuclear cells with high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom., 2002, 16: 555–565.
[18] P.V. Heeswijk, R.M. Hoetelmans, P.L. Meenhorst, J.W. Mulder, J.H. Beijnen. Rapid determination of nevirapine in human plasma by ion-pair reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl., 1998, 713: 395–399.
[19] J.W. Par, L.S. Rowland, D.J. Korpalski. HPLC-UV method for the quantitation of nevirapine in biological matrices following solid phase extraction. J. Pharm. Biomed. Anal., 1999, 20: 91–98.
[20] T.P. Moyer, Z. Temesgen, R. Enger, L. Estes, J. Charlson, L. Oliver, A. Wright. Drug monitoring of antiretroviral therapy for HIV-1 infection: method validation and results of a pilot study. Clin. Chem., 1999, 45: 1465–1476.
[21] R.M. Hollanders, E.W.V. Ewijk, D.M. Burger, E.W. Wuis, P.P. Koopmans,Y.A. Hekster. Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl., 2000, 744: 65–71.
[22] R.M. Lopez, L. Pou, M.R. Gomez, I. Ruiz, J. Monterde. Simple and rapid determination of nevirapine in human serum by reversed-phase high-performance liquid chromatography. J. Chromatogr.B Biomed. Sci. Appl., 2001, 751: 371–376.
[23] P. Villani, M. Feroggio, L. Gilaneli, A. Bartoli, M. Montagna, R. Maserali, M.B. Regazzi. Antiretroviral: Simultaneous Determination of Five Protease Inhibitors and Three Nonnucleoside Transcriptase Inhibitors in Human Plasma by a Rapid High-Performance Liquid Chromatography–Mass Spectrometry Assay, Ther. Drug Monit., 2001, 23: 380–388.
[24] Dailly, L. Thomas, M.F. Kergueris, P. Jolliet, M. Bourin. High-performance liquid chromatographic assay to determine the plasma levels of HIV-protease inhibitors (amprenavir, indinavir, nelfinavir, ritonavir and saquinavir) and the non-nucleoside reverse transcriptase inhibitor (nevirapine) after liquid–liquid extraction. J. Chromatogr. B Biomed. Sci. Appl., 2001, 758: 129–135.
[25] Marzolini, A. Beguin, A. Telenti, A. Schreyer, T. Buclin, J. Biollaz, L.A. Decosterd. Determination of lopinavir and Nevirapine by high-performance liquid chromatography after solid-phase extraction: Application for the assessment of their transplacental passage at delivery. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 2002, 774: 127–140.
[26] K. Titier, F. Lagrange, F. Pehoureq, L. Edno-Mcheik, N. Moore, M. Molimard. High-performance liquid chromatographic method for the simultaneous determination of the six HIV-protease inhibitors and two non-nucleoside reverse transcriptase inhibitors in human plasma. Therapie J., 2002, 57: 169–174.
[27] N.L. Rezk, R.R. Tidwell, A.D. Kashuba. Simple and rapid quantification of the non-nucleoside reverse transcriptase inhibitors nevirapine, delavirdine, and efavirenz in human blood plasma using high-performance liquid chromatography with ultraviolet absorbance detection. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 2002, 774: 79–88.
[28] K. Marchei, L. Valvo, R. Pacifici, M. Pellergrini, G. Tossini, P. Zuccaro. Simultaneous determination of zidovudine and nevirapine in human plasma by RP-LC. Pharm. Biomed. Anal., 2002, 29: 1084–1088.
[29] T.L. Laurito, V. Santagoda, G. Caliendo, C.H. Oliveira, R.E. Barrientos, G. Denucci. Nevirapine quantification in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry. Application to bioequivalence study. J. Mass Spectrom., 2002, 37: 434–444.
[30] P.J. Wagh, N. Pai. Devlopment and validation of HPLC method for simultaneous determination of lavnivudine and zidovudine from pharmaceutical preparation. Indian drugs, 2002, 13: 654–657.
[31] D.G. Sankar, M.V.V.N. Reddy, J.M. Rajendrakumar, T.K. Murthy. Spectrophotometric determination of lamivudine and stavudine. Indian J. Pharm. Sci., 2002, 64 : 504–506.
[32] ICH guide lines for Validation of analytical method. Note for guidance on validation of analytical procedures: text and methodology (CPMP/ICH/381/95). Eropience Medicines Agency.
[33] United State Pharmacopoeias U.S.P. The official Compendia of Standard. Asian Edition. 2004.
[34] J.M.Green. A Practical Guide to Analytical Method Validation. Retrieved from http://pubs.acs.org/hotaartcl/ac/96/may/may.html on 28/04/2010.
[35] P.A.D.Edwardson, G.Bhaskar, J.E.Fairbrother. Method validation in pharmaceutical analysis. Journal of Pharmaceutical and Biomedical Analysis. 1990, 8(8-12): 929-933.
[36] G.C. Hokanson. A life Cycle Approach to the Validation of Analytical Methods during pharmaceutical product development, Part I: The initial method validation process. Pharmaceutical Technology. 1994, 18(9): 118-130.
[37] J.R.Lang, S.Bolton. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry – 1. Experimental considerations. Journal of Pharmaceutics and Biomedical Analysis. 1991, 9(5): 357-361.
[38] M.E Swatz and I.S.Krull. Analytical Method Development and Validation. Marcell Dekker Inc, 1997, pp 17-83.
[39] A.K. Hemanth Kumar, V. Sudha, Soumya Swaminathan ,Geetha Ramachandran. Comparison of HPLC & spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products. Indian J Med Res, 2010, 132: 390-394.

Table 1. Specificity test for analytical method development for the drugs

Test solution	Absorbance		
	Stavudine (266 nm)	Lamivudine (271 nm)	Nevirapine (314 nm)
Placebo	0.001	0.004	0.001
Standard	0.912	0.818	0.537
Standard + Placebo	0.913	0.817	0.538

Table 2. Method Precision study

Sample Number	Absorbance		
	Stavudine (266 nm)	Lamivudine (271 nm)	Nevirapine (314 nm)
1	0.912	0.818	0.538
2	0.913	0.817	0.538
3	0.912	0.818	0.537
4	0.914	0.816	0.539
5	0.912	0.818	0.538
6	0.914	0.816	0.537
Average	0.913	0.817	0.538
S.D.	0.00098	0.00098	0.00075
% RSD	0.1077	0.1203	0.1399

Table 3. System Precision study

Sample Number	Absorbance		
	Stavudine (266 nm)	Lamivudine (271 nm)	Nevirapine (314 nm)
1	0.912	0.818	0.538
2	0.913	0.819	0.538
3	0.912	0.818	0.536
4	0.911	0.816	0.539
5	0.912	0.818	0.538
6	0.912	0.816	0.537
7	0.912	0.818	0.538
8	0.911	0.818	0.538
9	0.912	0.818	0.538
Average	0.9116667	0.817667	0.538
S.D.	0.000600925	0.001	0.000833333
% RSD	0.065915012	0.122299176	0.154894672

Table 4. Inter day precision study

Time	Absorbance		
	Stavudine (266 nm)	Lamivudine (271 nm)	Nevirapine (314 nm)
9.00 a.m	0.912	0.818	0.538
12.00 pm	0.913	0.819	0.538
5.00 pm	0.912	0.818	0.537
Average	0.912	0.818	0.538
S.D.	0.0005	0.00047	0.00047
%RSD	0.0548	0.0576	0.0877

Table 5. Intraday precision study

Time	Absorbance		
	Stavudine (266 nm)	Lamivudine (271 nm)	Nevirapine (314 nm)
9.00 am	0.912	0.817	0.536
12.00 pm	0.913	0.817	0.537
5.00 pm	0.914	0.816	0.537
Average	0.913	0.816667	0.536667
S.D.	0.00082	0.00047	0.00047
%RSD	0.089	0.0577	0.0878

Table 6. Recovery studies of Stavudine

Recovery Level	Amount of drug recovered (mg)	Amount of drug added (mg)	% Recovery	Average	S.D.	% RSD
25%	19.93	20	99.67	100.04	0.377	0.377
	19.97	20	99.87			
	20.12	20	100.4			
50%	39.46	40	98.67	99.69	0.884	0.887
	40.12	40	100.23			
	40.10	40	100.17			
75%	58.93	60	98.22	99.48	1.135	1.141
	59.86	60	99.74			
	60.26	60	100.44			
100%	78.66	80	98.33	98.94	0.786	0.795
	79.86	80	99.83			
	78.93	80	98.67			
125%	99.33	100	98.93	100.13	1.141	1.140
	101.20	100	101.20			
	100.26	100	100.27			
150%	121.20	120	101	100.07	0.946	0.945
	118.93	120	99.11			
	120.13	120	100.11			

Table 7. Recovery studies of Lamivudine

Recovery Level	Amount of drug recovered (mg)	Amount of drug added (mg)	% Recovery	Average	S.D.	% RSD
25%	74.9	75	98.98	100.13	0.7596271	0.758640868
	74.2	75	98.93			
	75.2	75	100.27			
50%	149.10	150	99.40	100.09	0.6712178	0.670614246
	150.22	150	100.14			
	151.11	150	100.74			
75%	148.47	225	98.98	99.40	0.53724606	0.540488998
	148.84	225	99.23			
	150.01	225	100.01			
100%	298.95	300	99.65	99.72	0.7579138	0.760041921
	297.03	300	99.01			
	301.56	300	100.52			
125%	370.87	375	98.90	100.10	1.37263736	1.371266098
	381.00	375	101.60			
	374.32	375	99.82			
150%	449.22	450	99.83	99.94	0.27221315	0.272376578
	448.83	450	99.74			
	451.15	450	100.25			

Table 8. Recovery studies of Nevirapine

Recovery Level	Amount of Drug Recovered (mg)	Amount of Drug added (mg)	% Recovery	Average	S.D.	% RSD
25%	99.86	100	99.86	99.63	0.63129497	0.633639431
	100.12	100	100.12			
	98.92	100	98.92			
50%	200.16	200	100.08	99.66	0.61024585	0.612327767
	197.92	200	98.96			
	199.88	200	99.94			
75%	294.36	300	98.12	99.01	0.91045776	0.919561418
	296.94	300	98.98			
	299.82	300	99.94			
100%	400.40	400	100.10	99.32	0.67002488	0.674612238
	395.68	400	98.92			
	395.84	400	98.96			
125%	495.60	500	99.12	99.69	0.50921508	0.510798558
	499.25	500	99.85			
	500.50	500	100.10			
150%	607.38	600	101.23	100.32	0.80002083	0.797468932
	599.88	600	99.98			
	598.44	600	99.74			

Table 9. Solution Stability study for drugs

Time (hrs)	ABSORBANCE											
	10 µg / ml			15 µg / ml			20 µg / ml			25 µg / ml		
	STV	LMV	NVP	STV	LMV	NVP	STV	LMV	NVP	STV	LMV	NVP
Initial	0.365	0.327	0.216	0.57	0.488	0.327	0.726	0.667	0.421	0.912	0.818	0.538
1	0.364	0.326	0.217	0.572	0.49	0.328	0.725	0.665	0.422	0.913	0.819	0.538
2	0.366	0.328	0.216	0.571	0.488	0.329	0.727	0.666	0.423	0.912	0.818	0.539
3	0.364	0.327	0.215	0.572	0.488	0.328	0.727	0.665	0.422	0.911	0.816	0.538
4	0.365	0.328	0.216	0.571	0.489	0.328	0.727	0.667	0.422	0.912	0.818	0.537
5	0.364	0.328	0.216	0.57	0.489	0.327	0.727	0.666	0.422	0.912	0.816	0.538
6	0.364	0.328	0.217	0.571	0.491	0.328	0.726	0.666	0.422	0.912	0.818	0.538
7	0.364	0.326	0.216	0.571	0.489	0.328	0.728	0.668	0.423	0.911	0.818	0.538
8	0.363	0.329	0.217	0.572	0.489	0.328	0.727	0.665	0.421	0.912	0.818	0.538
10	0.365	0.328	0.216	0.572	0.49	0.328	0.728	0.666	0.423	0.913	0.818	0.539
12	0.364	0.328	0.217	0.572	0.489	0.327	0.727	0.666	0.422	0.912	0.817	0.538
16	0.363	0.327	0.216	0.571	0.488	0.326	0.726	0.665	0.421	0.912	0.817	0.538
20	0.363	0.326	0.216	0.57	0.487	0.326	0.725	0.665	0.421	0.911	0.816	0.537
24	0.363	0.326	0.215	0.57	0.487	0.325	0.725	0.664	0.42	0.911	0.816	0.537
Average	0.364	0.327	0.216	0.571	0.489	0.327	0.727	0.666	0.422	0.912	0.817	0.538
S.D.	0.0009	0.00099	0.0007	0.00083	0.00114	0.00108	0.00102	0.00105	0.00089	0.000663	0.00101	0.00062
% RSD	0.2518	0.30386	0.3067	0.14512	0.23301	0.33047	0.14027	0.15784	0.21162	0.072708	0.12335	0.11446

Table 10. Results of analytical studies

Parameters	Results	Acceptance criteria
Specificity	Specific ; No drug / placebo interference	Must be specific
Method Precision	% RSD ≤ 2	% RSD ≤ 2
System Precision	% RSD ≤ 1	% RSD ≤ 1
Interday Precision	% RSD ≤ 1	% RSD ≤ 1
Intraday Precision	% RSD ≤ 1	% RSD ≤ 1
Accuracy	99.26 %	98 – 102 %
Solution stability	Stable more than 24 hours	Solution should be stable

Table 11. Absorbance of Stavudine at 266 nm

Concentration (µg/ml)	Absorbance			Average
	1	2	3	
0	0	0	0	0
5	0.189	0.188	0.189	0.189
10	0.364	0.365	0.364	0.3649
15	0.571	0.571	0.572	0.5719
20	0.727	0.726	0.728	0.727
25	0.912	0.912	0.913	0.9129
30	0.998	0.997	0.998	0.998

Table 12. Absorbance of Lamivudine at 271 nm

Concentration (µg/ml)	Absorbance			Average
	1	2	3	
0	0	0	0	0
5	0.158	0.157	0.158	0.158
10	0.328	0.327	0.328	0.328
15	0.489	0.488	0.49	0.489
20	0.666	0.665	0.667	0.666
25	0.818	0.817	0.819	0.818
30	1.024	1.021	1.023	1.023

Table 13. Absorbance of Nevirapine at 314 nm.

Concentration (µg/ml)	Absorbance			Average
	1	2	3	
0	0	0	0	0
5	0.119	0.118	0.12	0.119
10	0.216	0.215	0.216	0.216
15	0.328	0.327	0.328	0.328
20	0.422	0.421	0.421	0.4218
25	0.538	0.538	0.538	0.538
30	0.645	0.644	0.646	0.645

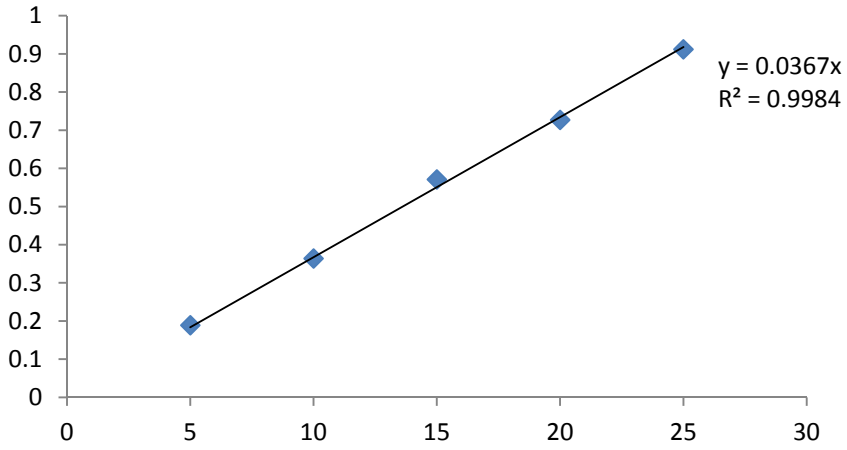

Fig. 1. Standard curve of Stavudine at 266 nm in Phosphate buffer pH 6.8

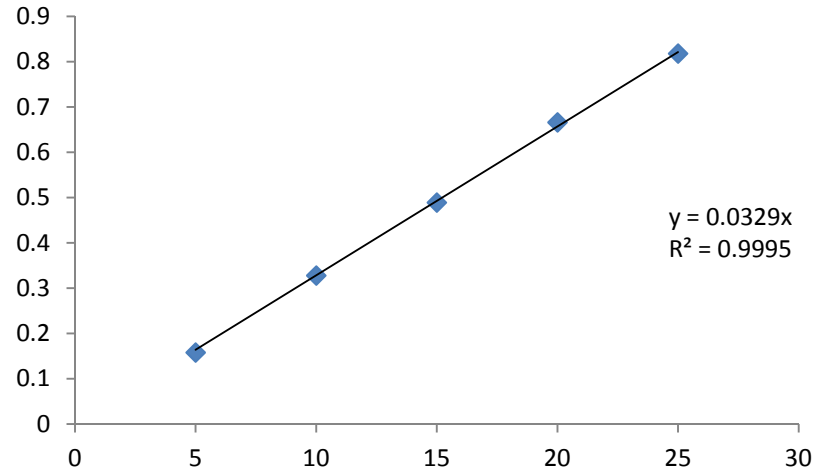

Fig. 2. Standard curve of Lamivudine at 271 nm in Phosphate buffer pH 6.8

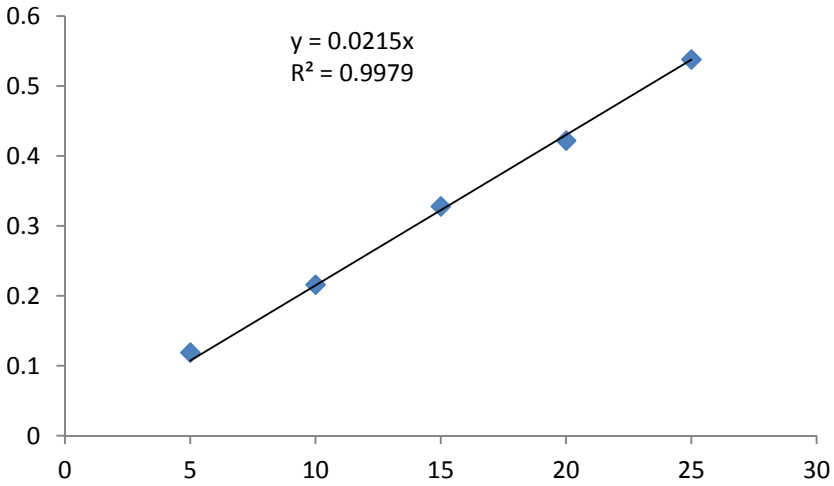

Fig.3. Standard curve of Nevirapine at 314 nm in Phosphate buffer pH 6.8