Research Article

Available Online at: www.ijphr.com

International Journal of Pharmaceuticals and Health care Research

ISSN: - 2306 – 6091

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE, NEVIRAPINE AND ZIDOVUDINE IN BULK AND TABLET FORMULATION BY HPTLC METHOD

[*]Ramesh Jayaprakash, Senthil Kumar Natesan, Vijay Amirtharaj Ramasamy, Rajasekhar Kommi, Kiran Gandhi R

Department of Pharmaceutical Analysis

JKK Munirajah Medical Research Foundation's- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal -DT, Tamilnadu, India.

Abstract

A high performance thin layer chromatography (HPTLC) method was developed and validated for determination of three anti-retro viral drugs, Lamivudine, Nevirapine and Zidovudine in bulk and tablet formulation. Study was performed on pre-coated silica gel HPTLC plates using Ethyl acetate: Toluene: Methanol: 25%Ammonia (4: 5: 1: 0.1 v/v/v/v) as the mobile phase. A TLC scanner set at 254nm was used for direct evaluation of the chromatograms in the reflectance/ absorbance mode. Method was validated according to ICH guidelines. The correlation co-efficient of calibration curves were found to be 0.998, 0.998 and 0.998 in the concentration range of 90-540ng/spot, 150-900ng/spot and 180-1080ng/spot for Lamivudine, Nevirapine and Zidovudine, respectively. The method had an accuracy of 98.68% for Lamivudine, 98.56% for Nevirapine and 98.65% for Zidovudine. The method had the potential to determine these drugs simultaneously from dosage forms without any interference of the tablet excipients.

Keywords: High performance thin layer chromatography, Lamivudine, Nevirapine, Zidovudine.

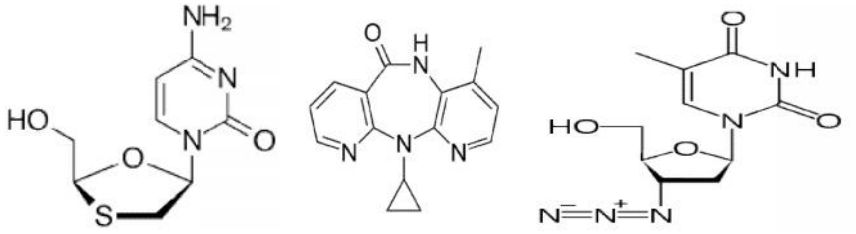
Introduction

Lamivudine(Fig.1a), Nevirapine(Fig. 1b) and zidovudine(Fig.1c) were active against human immuno deficiency virus (HIV) which is a retro virus.[1] They are useful in prolonging and improving the quality of life and postponing complications of acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC), but do not cure the infection. The clinical efficiency of antiretro virus drugs is monitored primarily by plasma HIV-RNA assays and CD4 lymphocyte count carried out at regular intervals.[2]

Validated assays have been reported for each drug individually and combinations with other drugs. For analysis of Lamivudine (LVD), Stavudine (STV) and Nevirapine (NVP) in fixed dose combination table,[3] HPLC and HPTLC determination ,[1] HPTLC determination,[4] An interlaboratory investigation on the use of High – performance thin layer chromatography,[5]Spectrophotometric determination,[6] Simultaneous determination of Lamivudine, Lopinavir, Ritonavir, and Zidovudine concentration in plasma of HIV- infected patients by HPLC – MS / MS,[7] Determination of Zidovudine, Lamivudine,

Author for Correspondence:
Ramesh Jayaprakash,
Department of Pharmaceutical Analysis,
JKK Munirajah Medical Research Foundation College of Pharmacy,
Komarapalayam-638 183, Namakkal (DT), Tamilnadu, India.
Email: rameshj1974@gmail.com

Nevirapine in human plasma using ion-pair HPLC,[8] Development and validation of UV spectrophoto - metric method for simultaneous estimation of Lamivudine and Efavirenz in the pharmaceutical dosage form,[9] Development and validation of a normal – phase HPTLC method for the simultaneous analysis of Lamivudine and Zidovudine in fixed-dose combination tablets.[10]



(a) (b) (c)

Fig. No. 01: Chemical structure of (a) Lamivudine (b) Nevirapine and (c) Zidovudine

Material and Methods

Materials

Analytical pure samples of lamivudine, nevirapine and zidovudine (Mylan labs, puducherry, India) were used in the study. The pharmaceutical dosage form used in this study was procured from the same company and labelled to contain 30mg of lamivudine, 50mg of nevirapine and 60mg of zidovudine per tablet. The solvents and chemicals used in the study were AR grade (Thomas baker, Mumbai, Maharastra, India).

Instrumentation

Microsyringe (Linomat 659.0014, camag, Switzerland), pre-coated silica gel 60 F_{254} aluminium plates (10×10, 10×6, 6×4cm, 250µm thickness; Merck, Germany), Linomat 5 applicator (Camag , Switzerland), twin trough chamber (20×10, 10×10cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner III (Camag, Switzerland), win CATS version 1.4.3, SNR 1502W010 software (Camag, Switzerland), Dryer (Orpat) were used in this study. Microsoft excel was also used to treat data statistically.

Preparation of standard solution

Weigh accurately about 3, 5 and 6 mg of standard lamivudine (LAM), nevirapine (NEV) and zidovudine (ZDV) standard drugs in to a three individual 100 ml volumetric flask, add 10 ml of methanol to dissolve and finally make up the volume to 100 ml with distilled water to get the concentration of 30, 50 and 60µg/ml or 30, 50 and 60ng/µL respectively.

Preparation of sample solution

Weigh 20 tablets and calculate the average weight of tablets, crush the tablets and take the powder weight equivalent to 3,5 and 6mg of Lamivudine, Nevirapine and Zidovudine, and transferred in to a 100ml volumetric flask, add 10ml of methanol to dissolve and make up the volume to 100ml with distilled water, sonicate the solution for 15 minutes to complete dissolve and filter the solution through whattman filter paper (No.14), and the resulting solution was used for the analysis.

Optimized chromatographic conditions

Suitable volumes of standard and sample solutions (µL) were applied to the HPTLC plates, 10mm from the bottom and 10mm from the side edges in the form of bands or streaks with the band length of 8mm. The mobile phase consisting of ethyl acetate: toluene: methanol: 25% ammonia (4: 5: 1: 0.1 v/v/v/v) was used in each chromatographic run. Ascending development technique was carried out in twin trough chamber. The optimized chamber saturation time for the mobile phase was 30 min at room temperature that was assisted by saturation pads. The distance covered by the solvent front was 6cm which took about 10 min. The spots were scanned using the TLC scanner III in the reflectance/ absorbance mode at 254nm and all measurements were operated by winCATS software. Concentrations of the separated compounds were determined from the intensity of reflected light and peak areas were used for evaluation.

Analysis of Marketed formulation

Tablet sample solutions were prepared as discussed above. Suitable working sample solution (12µL) containing concentration of 360ng/spot of lamivudine, 600ng/spot of nevirapine and 720ng of

zidovudine were prepared, applied on HPTLC plates and analysed under the optimized chromatographic conditions.

Method validation

The method was in compliance with ICH guidelines (ICH, Q2 (R1), 2005). The following parameters were used for validation of the developed method.

Linearity

Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng/spot by making six replicate measurements in the concentration range of 90-540ng/spot of lamivudine, 150-900ng/spot of nevirapine and 180-1080ng/spot of zidovudine, respectively.

Precision

Precision of the developed method was studied by performing system precision, repeatability and intermediate precision studies. The sample application and measurement of peak area was determined by performing six replicate measurements of the same band using a standard solution for system precision and sample solution for the intermediate and repeatability studies, these solutions containing 360ng/spot of lamivudine, 600ng/spot of nevirapine and 720ng/spot of zidovudine, respectively.

Recovery studies

Recovery studies were carried out by spiking three different known amounts of the standard substances to the drug product (standard addition method). Hence, 288, 360 and 432ng/spot of lamivudine, 480, 600 and 720ng/spot of nevirapine and 576, 720 and 864ng/spot of zidovudine were spiked to the dosage form that contained 360, 600 and 720 ng/spot of lamivudine, nevirapine and zidovudine, respectively, after sample dilution.

Specificity

Peak purity of lamivudine, nevirapine and zidovudine was assessed to evaluate the specificity of the method. The sample and standard bands were scanned at three different levels, i.e. peak start (S), peak apex (M), and peak end (E) positions.

Results and discussion

HPTLC method optimization

For the selection of appropriate mobile phase for the effective separation of lamivudine, nevirapine and zidovudine, several runs were made by using mobile phase containing solvents of varying polarity at different concentration levels. Different mobile phases systems like ethyl acetate: methanol: chloroform, ethyl acetate: toluene: chloroform, acetone: toluene: methanol etc, at different concentrations ratios tried. Among the different mobile phase combinations employed, the mobile phase consisting of ethyl acetate: toluene: methanol: 25%ammonia (4: 5: 1: 0.1 v/v/v/v) gave the best resolution with sharp well defined peaks with R_f values of 0.08±0.02, 0.31±0.22 and 0.22±0.02 for lamivudine, nevirapine and zidovudine, respectively. For the selection of analytical wavelength for the quantification of the drugs, the standard solutions were scanned in the UV-Visible spectroscopy at 200-800nm and their overlay spectra were obtained. From the overlay spectra (Fig.2), it was observed that the three drugs exhibited strong absorbance at about 254nm which was selected as the analytical wavelength for further analysis. The densitogram of the blank mobile phase (Fig.3) also showed no peak confirming the purity of the standard peaks obtained using the proposed mobile phase.

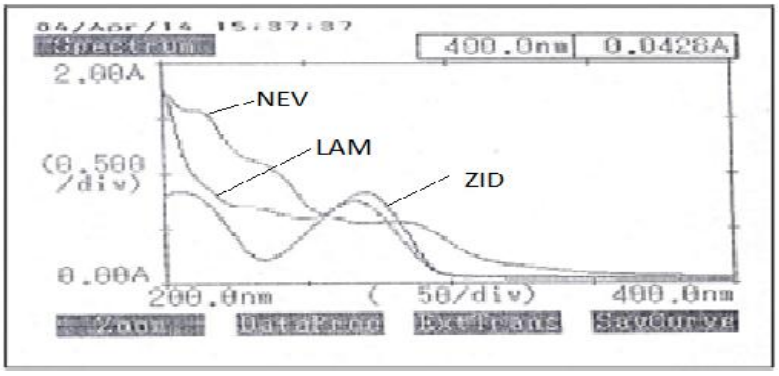


Fig. No. 02: Overlay UV spectrum of lamivudine, nevirapine and zidovudine

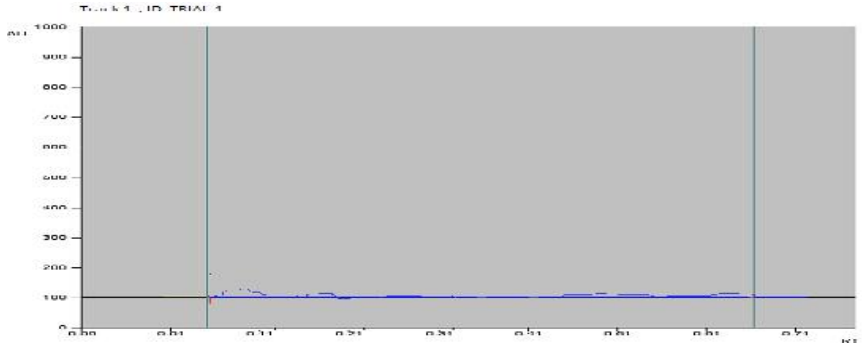


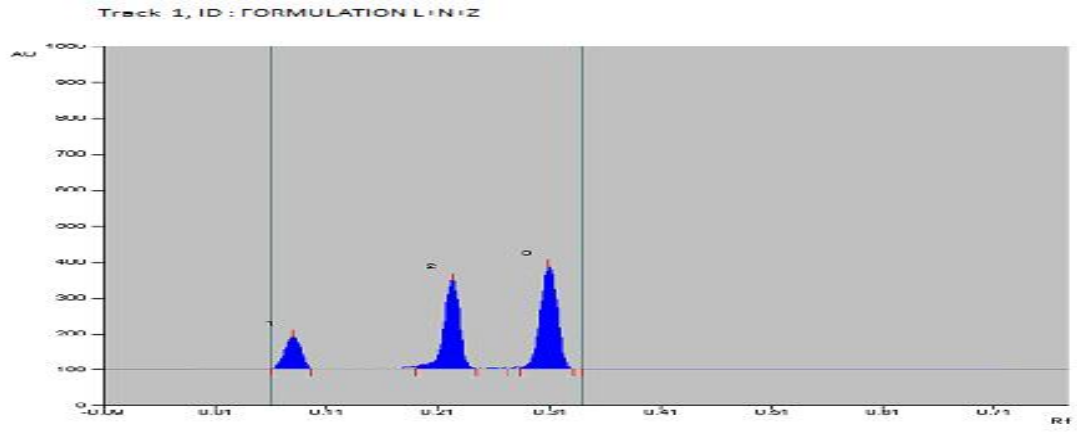
Fig. No. 03: Typical chromatogram of blank mobile phase

Analysis of marketed formulation

The marketed formulation was analysed using the developed method. The chromatogram of tablet sample showed only three peaks at 0.08, 0.31 and 0.22 for lamivudine, nevirapine and zidovudine, respectively, indicating that there is no interference of the excipients presents in the tablet formulation. The content of lamivudine, nevirapine and zidovudine was calculated by comparing peak areas of sample with that of the standard (Table.1). The chromatogram of tablet formulation is shown in Fig.4

Table No. 01: Assay results of the pharmaceutical dosage form

Drug	Amount (mg/tablet)		%Drug content	SD	% RSD
	Lable	Estimated			
Lamivudine	30	29.71	99.05	0.457282	0.461637
Nevirapine	50	49.82	99.64	0.099331	0.099683
Zidovudine	60	59.87	99.78	0.03559	0.03566



(1) Lamivudine =R_f (0.08) (2) Nevirapine = R_f(0.31) (3) Zidovudine= R_f(0.22)

Fig. No. 04: Typical chromatogram of pharmaceutical dosage form

Method validation

Linearity

Peak areas were found to have better linear relationship with the concentration than the peak heights. For lamivudine, the r^2 value was found to be 0.998, for nevirapine the r^2 value was 0.998 and for zidovudine the r^2 value was found to 0.998.

Calibration graphs were constructed in the concentration range of 90-540 ng/spot for lamivudine, 150-900ng/spot for nevirapine and 180-1080ng/spot for zidovudine. The correlation coefficients, y-intercepts and slope of the regression lines of the two drugs were calculated and are shown in (Table.2), and the calibration curves were shown in Fig.5.1-5.3

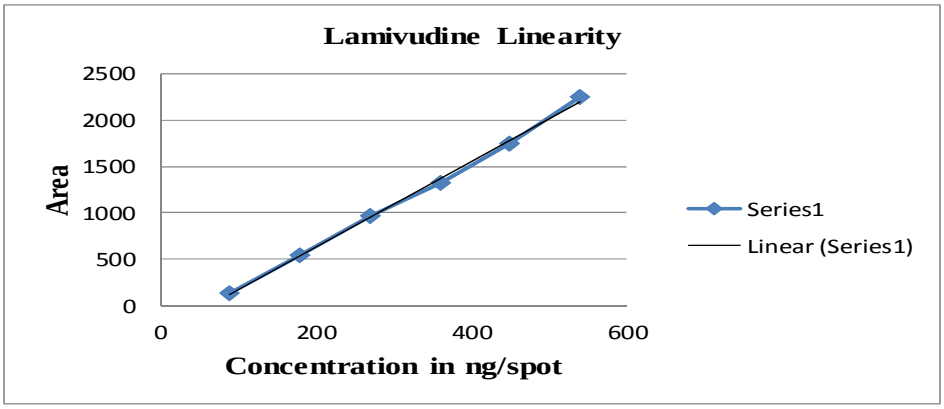


Fig. No. 5.1

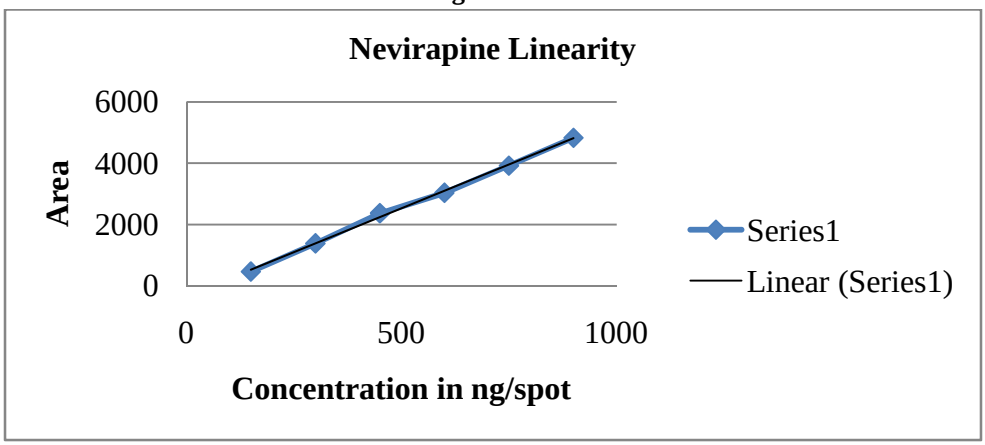


Fig. No. 5.2

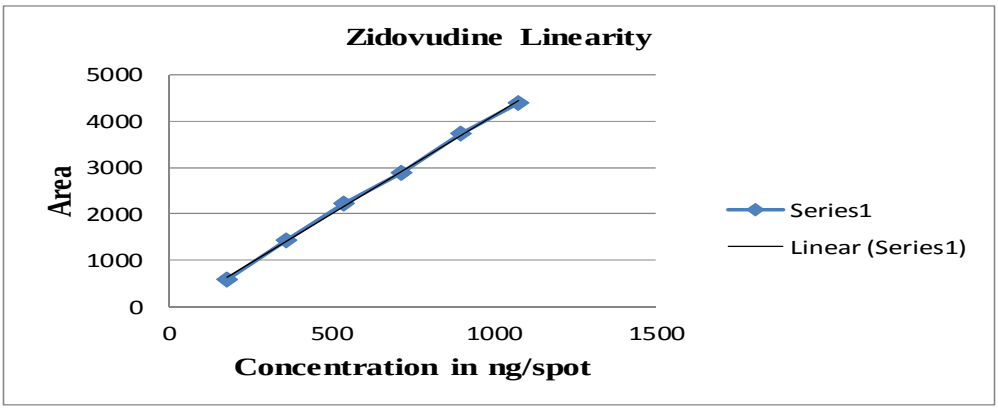


Fig. No. 5.3

Table No. 02: Summery of linear regression and validation data

Parameters	Lamivudine	Nevirapine	Zidovudine
Linearity range*	90-540 ng/spot	150-900 ng/spot	180-1080 ng/spot
Slope	256.1099206	329.2352381	4.162850529
Intercept	-157.5703571	-181.0928571	-73.63214286
Correlation Coefficient(r^2)	0.998	0.998	0.998
System precision* (%RSD)	0.040752	0.961922	1.410114
Repeatability* (%RSD)	0.297642	0.113596	1.013182
Intra-day (%RSD)	0.039796	0.087966	0.5697
Inter-day (%RSD)	0.037631	0.038014	0.362700

*Denotes average of six estimations

Precision

System precision, repeatability and intermediate precision of the developed method were expressed in the terms of relative standard deviation (RSD) of the peak area. The results showed that the system precision, repeatability, intra- and inter day variation of the results at concentration of 360ng/spot for lamivudine, 600ng/spot for nevirapine and 720ng/spot for zidovudine were within the acceptance range. The % RSD values were found to be less than 2% for the three drugs. (Table.2)

Accuracy/recovery studies

The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The percentage recovery of lamivudine, nevirapine and zidovudine at all the three levels was found to be satisfactory (Table.3). For the lamivudine % recovery was found between 98.60% and 98.73%, for nevirapine between 98.56% and 98.65% and for zidovudine between 98.59% and 98.73%, respectively.

Specificity

The peak purity test of lamivudine, nevirapine and zidovudine spots were assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot and their spectra were overlaid to assess spectral matching.

Table No. 03: Recovery study of the method

Drugs	% solutions	Amount Added (ng)	Amount Recovered (ng)	% of amount recovered	SD	%RSD
Lamivudine	80%	288	284.066	98.73	0.075056	0.076054
	100%	360	354.966	98.60		
	120%	432	426.533	98.73		
Nevirapine	80%	480	473.133	98.56	0.085049	0.086289
	100%	600	590.906	98.48		
	120%	720	710.283	98.65		
Zidovudine	80%	576	567.93	98.59	0.070238	0.071194
	100%	720	710.87	98.73		
	120%	864	852.42	98.65		

*Number of three replicate applications

Conclusion

A combination of lamivudine, nevirapine and zidovudine is currently available for the treatment of HIV. As there are no reported methods for their simultaneous estimation, a High performance thin layer chromatography (HPTLC) method was developed and validated for the determination of lamivudine, nevirapine and zidovudine on pre-coated silica gel TLC plates using ethyl acetate: toluene: methanol: 25% ammonia (4: 5: 1: 0.1 v/v/v/v) as the mobile phase with densitometry detection at 254nm. The developed method was found to be simple, rapid, selective, sensitive and suitable for simultaneous determination of lamivudine, nevirapine and zidovudine. The HPTLC method offers several advantages over liquid chromatographic methods such as the possibility of simultaneous analysis of sample and standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, higher mobile phase pH, large sample capacity, short run time, minimum solution consumption and no prior treatment for solvents like filtration and degassing.

Acknowledgements

The author would like to thank Mr.Sundar raj (Mylan laboratories , pudhucherry), for providing the drugs to perform this study.

References

1. Anbazhagan S, Indumathy N, Shanmuga pandivan P, Sridhar SK. Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets, *Journal of pharmaceutical and biomedical Analysis*, 2005 Sep 15;39 (3-4):801-4.
2. Broder.S, "The development of antiretroviral therapy and its impact on the HIV-1/

AIDSpandemic.*AntiviralResearch*,(2009),85(1): 12.dio:10.1016/j.antiviral.2009.10.002.

3. Shewivo DH, Kaale E, Ugullum C, Sigonda MN, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Hevden Y. Development and validation of a normal phase HPTLC method for the simultaneous analysis of lamivudine, stavudine and nevirapine in fixed dose combination tablets, *Journal of pharmaceutical and biomedical Analysis*, 2011 Feb 20;54 (3), 2010 Sep 17,445-50.
4. GirumHabte, AriyanaHymete& Abdel-Maaboud Ismail Mohamed. Determination of lamivudine and zidovudine in pharmaceutical formulations using the HPTLC method, *published online*, 26 Jun 2009.
5. Kaale E, Risha P, Reich E, Layloff TP. An inter laboratory investigation on the use of HPTLC to perform assays of lamivudine, zidovudine, metronidazole, nevirapine and quinine composite samples, *Association Of Analytical Comunities, Int*, 2010 Nov-Dec;93(6):1836-43.
6. Sohrabi MR, TayefehZarkesh M. Spectra resolution for simultaneous spectrophotometric determination of lamivudine and zidovudinecomponants in pharmaceutical formulation of HIV drug based on using continuous wavelet transform and derivative transform technique, *Talanta*, 2014 May ; Epub 2014 Feb 4, 122:223.
7. Notari S, Sergi M, Montesano C, Ivanovic J, Narciso P, Pucillo LP, Ascenzi P. simultaneous determination of lamivudine, lopinavir, ritonavir and zidovudine concentration in plasma of HIV-infected patients by HPLC-MS/MS, *IUBMB(International Union of Biochemistry and Molecular biology) Life*.2012 May; 64(5),Epub 2012 Apr 4,443-9.
8. Fan B, Stewart JT. Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC, *Journal of pharmaceutical and biomedical Analysis*. 2002 Jun 1; 28(5):903-8.
9. Manikanta Kumar A, B. Naga Sandhya, Mahesh Nasare, V.V.L.N Prasad, Prakash.V.Diwan. Development and validation of UV spectro - photometric method for simultaneous estimation of lamivudine and efavirenz in the pharmaceutical dosage form.
10. PalaniVenkatesh, Mahesh Daggumati, development and validation of a normal-phase HPTLC method for the simultaneous analysis of lamivudine and zidovudine in fixed dose combination tablets, *Journal of Pharmaceutical Analysis*, volume 2, Issue 2 , April 2012, 152-155.
11. Basic equipment for modern thin layer chromatography,*CAMAG*, 2010-11, Switzerland.
12. Dr.Sethi P.D, the text book of HPTLC *Quantitative Analysis of Pharmaceutical Formulations*, first edition, vol-3, 2013,1217-31.
13. ICH, Q2 (R1), Validation of Analytical procedures: Methodology, *International Conference of Harmonization*, November 2005,1-12.
14. Kaul N, Agarwal H, Paradkar AR, Mahadik KR. HPTLC method for determination of nevirapine in pharmaceutical dosage form, *Journal of Talanta* , 2004 Mar 10;62(4):, 843-52.
15. Sudha T, V.R.Ravikumar 2 and P.V.Hemalatha 2. Validated HPTLC method for simultaneous determination of lamivudine and abacavir sulphate in tablet dosage form, International *Journal of Pharmaceutical sciences and Research*(IJPSR), 2010; vol. 1 (11), 26 October, 2010, 107-111.