World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
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Available online at: http://www.wjpsonline.org/
Original Article

Development and validation of a HPTLC method for simultaneous estimation of efavirenz and lamivudine in pharmaceutical formulations

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Received: 07-11-2015 / Revised: 15-01-2016 / Accepted: 28-01-2016 / Published: 30-01-2016

ABSTRACT

A simple, sensitive and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of efavirenz and lamivudine in pharmaceutical formulations. The chromatographic development was carried out on HPTLC plates pre-coated with silica gel 60G F_{254} using a mixture of ethyl acetate: methanol: formic acid in the ratio of 7.0:2.5:0.5 (v/v) as mobile phase. The calibration curve was found to be linear over the concentration range of 50-200 ng/spot for 3TC and 100-400 ng/spot for EFV with a regression coefficient for both analytes were greater than 0.999. The %RSD values for intra-day and inter-day variation were not more than 2.0. The method has demonstrated high sensitivity and specificity. The method is new, simple and economic for routine estimation of efavirenz and lamivudine in bulk, pre-formulation studies and pharmaceutical formulation to help the industries as well as researchers for their sensitive determination of efavirenz and lamivudine rapidly at low cost in routine analysis.

Keywords: Efavirenz, Lamivudine, HPTLC, Method Development

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs that were introduced as antiretroviral agents for the treatment of infection with human immune deficiency virus (HIV). Additional drug classes were developed. They are protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), fusion inhibitors[1]. Efavirenz, chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor. It is used in the treatment of HIV infection (Figure 1). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme and therefore, interferes with viral RNA to DNA directed polymerase activities[2]. Lamivudine, chemically 4-amino-1-{(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl}-1, 2-dihydropyrimidin-2-one, is a nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus (Figure 2)[3,4].

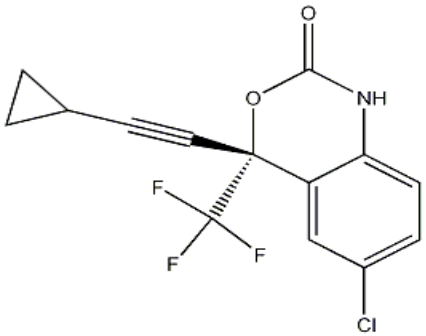

Figure 1: Chemical Structure of Efavirenz

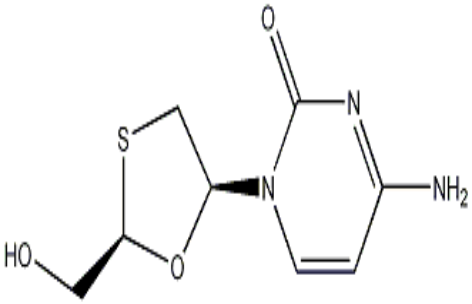

Figure 2: Chemical Structure of Lamivudine

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The literature survey reveals that several analytical methods have been reported for the quantification of these drugs individually or in combination with other drugs in pharmaceutical dosage forms or in human plasma by high performance liquid chromatography[5-8], Liquid chromatography/tandem mass spectrometry[9-12], UPLC[13,14] and high performance thin layer chromatography[15].

Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs high sample throughput, and need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus reducing the analysis time and cost per analysis. Accordingly, the aim of the present study involves development and validation of HPTLC method for the simultaneous estimation of efavirenz and lamivudine in combined tablet dosage form, which is fast, sensitive with better resolution and peak symmetry. Finally, the developed method was validated to assess the validity of research data means determining whether the method used during the study can be trusted to provide a genuine, account of the intervention being evaluated.

MATERIALS AND METHODS

Materials: Pure efavirenz (EFV) and lamivudine (3TC) used as working standards, were gifts from Hetero Drugs Pvt. Ltd., Hyderabad, India. All chemicals and reagents employed were of analytical grade, and purchased from Rankem, India. A commercial odivir tablets containing 300 mg of lamivudine and 600 mg of efavirenz were obtained from local pharmacies and used within their shelf life period.

Instrumentation and chromatographic conditions: Instruments used in the study were Camag HPTLC: A conventional CAMAG 20 × 10 cm twin-trough chamber and ultra sonicator were used for the development of chromatogram. Automatic Linomat 5 sampler was used as sample applicator with Hamilton syringe. Experiment performed on aluminium foil pre-coated with silica gel 60G F_{254} plates (10 × 10 cm, layer thickness 0.2 mm) (E. Merck, Darmstadt, Germany). Before use, the plates were prewashed with methanol and water mixture then dried in the current of dry air and activated at 120 °C for 5 min. Samples were sprayed at a speed of 150 nL/second to the plates with band length of 6 mm bands and distance of 7.8 mm between each track. The plates were developed by the ascending technique, to a total distance of 8 cm, at 25 ± 5°C, relative humidity 50-60%, in a CAMAG twin-trough glass chamber with a stainless steel lid, using a mobile phase of ethyl acetate:methanol: 0.1% formic acid in the ratio of 7.0:2.5:0.5 v/v and the chamber saturation time of 30 minutes. After development of plates, it was dried in an oven. Densitometric scanning was performed at 254 nm with a CAMAG TLC Scanner III in reflectance–absorbance mode controlled by winCATS software (version 1.4.8.2012; CAMAG) resident in the system.

Preparation of standard solutions: A mixed standard stock solution of EFV (1000 µg/mL) and 3TC (500 µg/mL) was prepared by accurately weighed EFV (100 mg) and 3TC (50 mg), and dissolved in 100 mL volumetric flask containing 30 mL methanol and the flask was sonicated to dissolve the contents and made up to the mark with methanol. Aliquots of these solution was transferred into 100 mL volumetric flask containing 30 mL methanol, sonicated for 2 min and the remaining volume was made up to mark with methanol to get final concentration of 100 µg/mL for EFV and 50 µg/mL for 3TC.

Method Validation: The method was validated in accordance with ICH guidelines[16].

***Specificity and sensitivity*:** The specificity of the developed method was established analyzing the sample solutions containing EFV and 3TC standards, and marketed tablets in relation to interferences from formulation ingredients. The spot for EFV and 3TC in the sample was confirmed by comparing Rf values of the spot with that of the standard. The sensitivity of measurement was estimated in terms of the limit of quantification (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated by the use of equations LOD = 3 × N/B and LOQ = 10 × N/B where N is the standard deviation of the peak area of the drug (n = 3), taken as a measure of noise and B is the slope of the corresponding calibration plot.

Linearity: Calibration curves were constructed by plotting peak areas versus concentrations of EFV and 3TC, and the regression equations were calculated. From the mixed standard stock solution containing 100 µg/mL of efavirenz and 50 µg/mL lamivudine, aliquots of standard solution (1, 1.6, 2.2, 2.8, 3.4, and 4.0 µL/spot) were spotted on TLC plate to obtain final concentration of 100-400 ng/spot and 50-200 ng/spot for efavirenz and lamivudine, respectively. Each concentration was applied three times to the TLC plate.

Accuracy: The accuracy was carried out by adding known amounts of each standard drug corresponding to three concentration levels - 50,

100 and 150 % - of the labeled claim to the analytes. At each level, three determinations were performed and the results were recorded. The accuracy was expressed as percent analyte recovered by the proposed method.

Precision: The precision of the method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for ten determinations of peak areas of EFV (280 ng/spot) and 3TC (140 ng/spot), performed on the same day. For both intra- and inter-assay variation, sample solutions of EFV (160, 220 and 280 ng/spot) and 3TC (80, 110 and 140 ng/spot) were injected in triplicate.

Robustness: The robustness of the proposed method was determined by carrying out the analysis, during which mobile phase composition and duration of saturation time (varied by 5 min) were altered.

Stability studies: To test the stability of the drugs on the TLC plates, the freshly prepared solutions of the analyte were applied to the plates and developed plates were scanned at different intervals of 2, 6, 24, 48 and 72 h.

RESULTS AND DISCUSSIONS

The HPTLC method, as described, was validated and successfully employed for the simultaneous quantification of EFV and 3TC in tablets. There is need to consider the successive steps for the development of HPTLC method. In particular, the problems relating to the standardization of sample preparations and selection of mobile phase needs to be emphasized. The mobile phase [i.e., ethyl acetate:methanol: 0.1 % formic acid in the ratio of 7.0:2.5:0.5 v/v] was found to give a sharp and well-defined peak at Rf of 0.57±0.02 and 0.72±0.01 for 3TC and EFV, respectively (Figure 3). Better resolution was obtained when the chamber was saturated for 30 minutes with the mobile phase at a room temperature. Thus, this system and aforementioned conditions were selected for the analysis.

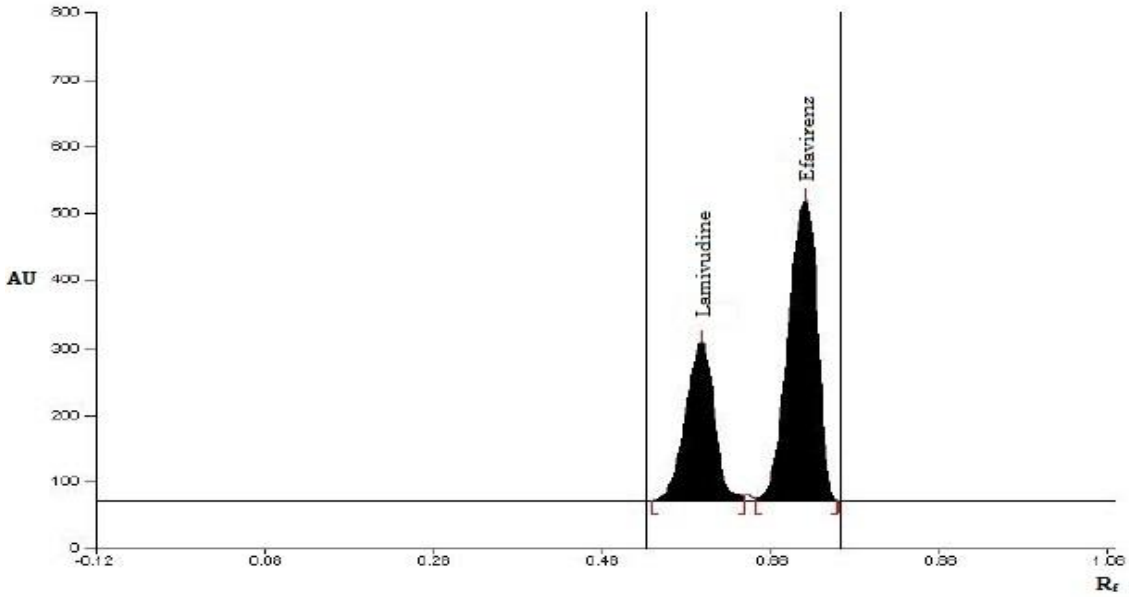

Figure 3: HPTLC chromatogram of standard EFV and 3TC

A calibration curve was constructed by plotting peak area against concentration (ng/spot). The results of regression analysis are shown in Table 1. They confirm the linearity of the standard curves over the concentration range of 50-200 ng/spot for 3TC and 100-400 ng/spot for EFV. The regression coefficients of 3TC (r^2 = 0.9991) and EFV (r^2 = 0.9992) signify that a decent linear relationship exhibited between peak area versus concentration over a wide range. The 3-D chromatographs of all calibration concentrations are shown in Figure 4.

The peak purity of EFV and 3TC was assessed by comparing the spectra at three different levels, that is, peak start (*S*), peak apex (*M*), and peak end (*E*) positions of the spot and the results obtained as r(S, M) = 0.9999 and r(M, E) = 0.9998 for 3TC; r(S, M) = 0.9998 and r(M, E) = 0.9998 for EFV. Good

correlation was obtained between standard and sample spectra of EFV and 3TC.Limit of Detection for EFV and 3TC was 8.45 ng/spot and 5.68 ng/spot, respectively, whereas Limit of quantification was 25.42 ng/spot and 17.05 ng/spot, respectively. The developed method showed high and consistent recoveries at all studied levels. The results obtained from recovery studies are presented in Table 2. The mean % recovery ranged from 98 to 102. Additionally, the obtained recoveries were found to be normally distributed with low %RSD (⩽2) at all concentration levels. The recovery study results signifying that the developed method was accurate.

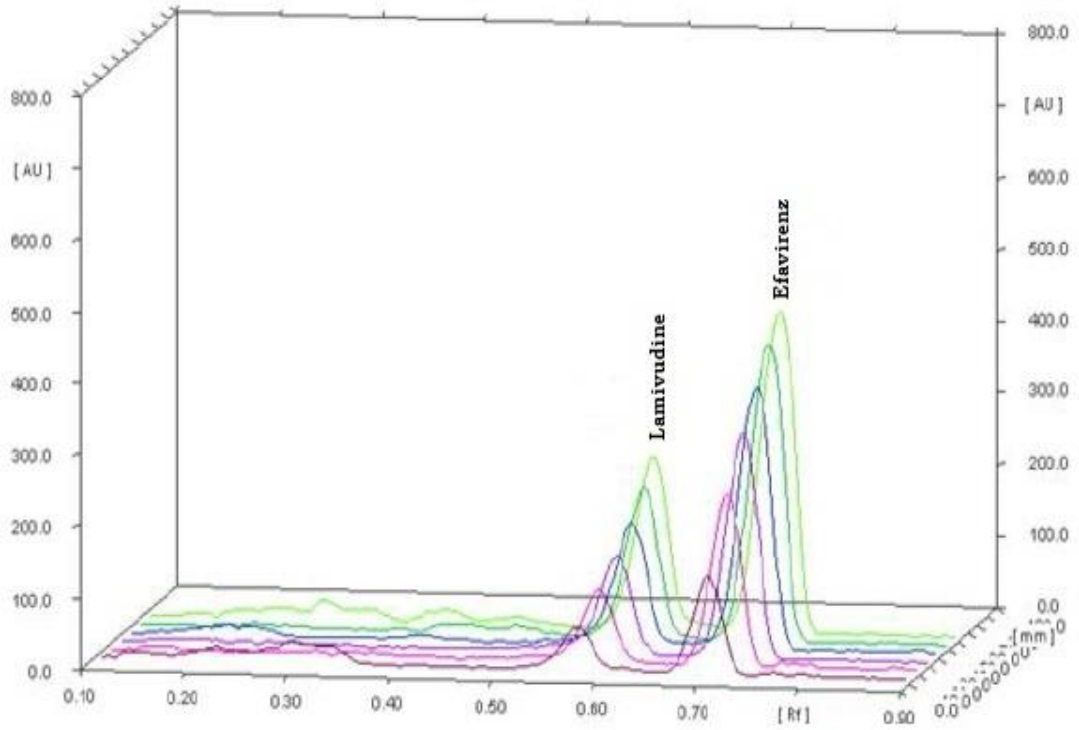

Figure 4: 3D chromatogram showing peaks of 3TC and EFV at different concentrations

Table 1: Linearity data of the proposed method

Analyte	Conc. (ng/spot)	Peak Area (Mean ± SD)*	RSD (%)	Linear regression equation
3TC	50	1278± 21	1.649	y= 49.075x – 1258.9 R^2= 0.9991
	80	2686± 50	1.877	
	110	4024± 49	1.218	
	140	5518± 27	0.481	
	170	7126± 109	1.532	
	200	8621± 70	0.809	
EFV	100	3662± 47	1.293	y= 39.102x – 196.76 R^2= 0.9992
	160	5976± 66	1.111	
	220	8472± 50	0.594	
	280	10981± 84	0.769	
	340	13033± 74	0.564	
	400	15349± 64	0.415	

No. of Replicates (N=3); SD: Standard Deviation; RSD: Relative Standard Deviation

Table 2: Results of recovery studies by standard addition method

Analyte	Amount of standard drug spiked		Amount of sample taken (mg)	% Recovery (Mean ± SD) {three replicates}	RSD (%)	SEM
	% Spiked	Quantity (mg)				
EFV	50	300	600	99.59± 1.404	1.410	0.8105
	100	600	600	100.41± 0.844	0.841	0.4874
	150	900	600	99.74± 1.563	1.567	0.9025
3TC	50	150	300	100.41± 0.920	0.917	0.5315
	100	300	300	98.81± 0.399	0.404	0.2302
	150	450	300	100.82±0.910	0.902	0.5252

Injection repeatability values (%RSD) of EFV and 3TC were found to be 1.853 and 0.938, respectively. The intra- and inter-assay precision results were expressed as %RSD values and were shown in Table 3. The low %RSD values proved that the method was precise. There was no significant difference between %RSD values, which indicates that the optimized method was reproducible. The results obtained in the new conditions were in accordance with the original results as shown in Table 4, though the R_f varied very slightly and the %RSD values for peak area was less than 2.0 indicating the highly robust nature of the developed method.

There was no significant deviation in peak area (RSD < 1.5%) observed on analysis up to 72 h. No decomposition of the drug was observed during chromatogram development. These observations suggest that the drug is stable under the typical processing and storage conditions of the analytical procedure. The results of the assay yielded 99.58% for EFV and 99.46 % for 3TC, of label claim of the tablets. The assay results show that the method was selective for the simultaneous determination of EFV and 3TC without interference from the excipients used in the tablet dosage form and the results were shown in the Table 5.

Table 3: Precision data of the proposed method

Analyte	Analyte Conc. (ng/spot)	Intra-assay precision*	Inter-assay precision*	Reproducibility*	
				Analyst one	Analyst two
EFV	160	1.051	0.911	1.382	1.085
	220	0.605	1.442	1.048	0.838
	280	1.341	1.241	0.763	0.637
3TC	80	0.515	1.042	0.584	0.779
	110	1.061	0.619	0.616	0.605
	140	0.805	0.651	1.246	0.298

**%RSD Values*

Table 4: Results for robustness of the proposed method

Parameter	Original	Used	Analyte	R_f Values	
				Mean± SD	RSD (%)
Mobile phase composition (ethyl acetate: methanol: formic acid)	(7:2.5:0.5)	6.8: 2.7:0.5	EFV	0.73±0.01	0.787
		7:2.5:0.5		0.72±0.01	0.806
		7.2:2.3:0.5		0.75±0.01	0.773
		6.8: 2.7:0.5	3TC	0.57±0.01	1.019
		7:2.5:0.5		0.58±0.01	0.990
		7.2:2.3:0.5		0.60±0.01	1.667
Duration of saturation time	30 min	25	EFV	0.70±0.01	0.821
		30		0.72±0.01	0.798
		35		0.75±0.01	0.773
		25	3TC	0.61±0.01	0.952
		30		0.58±0.01	0.990
		35		0.56±0.01	1.025

Table 5: Assay results for efavirenz and lamivudine in tablets

Product	Analyte	Label claim per tablet (mg)	% analyte estimated (Mean ±SD)*	RSD (%)	SEM
Odivir	EFV	600	99.58± 1.417	1.423	0.818
	3TC	300	99.46± 0.792	0.797	0.457

** n = 3; SEM = standard error of mean*

CONCLUSION

A convenient, rapid, accurate and precise HPTLC method was developed for the simultaneous determination of efavirenz and lamivudine in pharmaceutical formulations. The assay provides a linear response across a wide range of concentrations. This method can be said to be more economical as compared to other methods reported in literature. The method suitable for the determination of these drugs in tablets, and hence can be used for routine quality control of efavirenz and lamivudine in this dosage form.

REFERENCES

1. Farmer Paul, Léandre Fernet, Mukherjee Joia, Gupta Rajesh, Tarter Laura, Kim Jim Yong. Community-based treatment of advanced HIV disease: introducing DOT-HAART (directly observed therapy with highly active antiretroviral therapy). Bull World Health Organ 2001; 79(12): 1145-51.
2. Adkins J.C., Noble S. Efavirenz. Drugs 1998, 56 (6), 1055.
3. Zhou Z., Rodman J.H., Flynn P.M., Robbins B.L., Wilcox C.K., D'Argenio D.Z. Model for intracellular Lamivudine metabolism in peripheral blood mononuclear cells ex vivo and in human immunodeficiency virus type 1-infected adolescents. Antimicrob Agents Chemother 2006; 50(8): 2686-94.
4. Van Rompay A.R., Johansson M., Karlsson A. Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. Pharmacol Ther 2000; 87(2-3): 189-98.
5. Manikanta Kumar A, B. Naga Sandhya, Mahesh Nasare, V. V. L. N Prasad, Prakash V. Diwan. Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical Dosage Form. J Adv Pharm Edu Res 2012; 2(4): 232-8.
6. K. Anandakumar, G. Abirami, S. Murugan, B. Ashok. RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation. J Anal Chem 2013; 68(9): 815-21.
7. Akula Srinath, B. Sneha, Akhila Alladi, Rayees Ahmed, R.G. Kulkarni. Method development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV-spectroscopic method. Int J Pharm Sci Res 2014; 5(12): 5491-7.
8. B. Naga Sandhya, A. Manikanta Kumar, Mahesh K Nasare, P. Vijay Kumar, Jetta Satish, Prakash. V. Diwan. RP-HPLC method for simultaneous estimation of lamivudine, didanosine and efavirenz in pharmaceutical dosage forms. Der Pharmacia Lettre 2013; 5(3): 148-55.
9. Manish Yadav, Puran Singhal, Sailendra Goswami, Umesh C. Pande, Mallika Sanyal, Pranav S. Shrivastav. Selective Determination of Antiretroviral Agents Tenofovir, Emtricitabine, and Lamivudine in Human Plasma by a LC–MS–MS Method for a Bioequivalence Study in Healthy Indian Subjects. J Chromatogr Sci 2010; 48: 704-13.
10. Rower J.E., Klein B., Bushman L.R., Anderson P.L. Validation of a sensitive LC/MS/MS method for the determination of zidovudine and lamivudine in human plasma. Biomed Chromatogr 2012; 26(1): 12-20.
11. Li Z., Ding C., Ge Q., Zhou Z., Zhi X., Liu X. Simultaneous determination of lamivudine, stavudine and nevirapine in human plasma by LC-MS/MS and its application to pharmacokinetic study in clinic. Biomed Chromatogr 2010; 24(9): 926-34.
12. Kumar V.R., Reddy B.P., Kumar B.R., Sreekanth K., Babu K.N. High throughput LC-MS/MS method for simultaneous determination of zidovudine, lamivudine and nevirapine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2013; 15(921-922): 9-14.
13. Sk. Madeesh, Y. Ismail and V. Gunasekaran. Development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz by UPLC. Int J Pharm 2012; 2(3): 656-60.
14. Marina V. Antunes, Julia Poeta, Jorge P. Ribeiro, Eduardo Sprinz, Rafael Linden. Ultra-performance liquid chromatographic method for simultaneous quantification of HIV non-nucleoside reverse transcriptase inhibitors and protease inhibitors in human plasma. J Braz Chem Soc 2011; 22(1): 134-41.
15. Hamrapurkar P., Phale M., Shah N. Quantitative Estimation of Efavirenz by High Performance Thin Layer Chromatography. J Young Pharm 2009; 1(4): 359-63.
16. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure: Text and Methodology {ICH – Q2(R1)} November 2005: 1-13.