BAOJ Pharmaceutical Sciences

Suneetha A, et al, BAOJ Pharm Sci 2017, 3: 3
3: 041

Research

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Efavirenz, Lamivudine, and Stavudine in Pharmaceutical Dosage forms

Suneetha A[1]*, Bhavana G[2] and Ayesha Siddikha Sk[2]

[1]Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur-522 002, A. P, India

[2]Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur-522 002, A. P, India

Abstract

RP-HPLC method is developed for simultaneous estimation of Efavirenz, Lamivudine and Stavudine in Pharmaceutical Dosage Forms. The mobile phase used was a combination of Potassium Dihydrogen Ortho Phosphate p^H 4 Buffer: Methanol 30:70 v/v. The reversed phase column used was Inertsil ODS C18(4.6 X 250 mm, i.d., 5.0µm) at ambient temperature. The detection was carried out at 254 nm and a flow rate employed was 1.2 ml/min. The retention time for Efavirenz, Lamivudine and Stavudine was found to be 3.105, 4.810and 7.164 min, respectively. The method was validated in terms of linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for Efavirenz, Lamivudine and Stavudine was found in the concentration range of 15- 75ppm, 4-20ppm and 60-300ppm, respectively. The mean percentage recovery of Efavirenz, Lamivudine and Stavudine was found to be101.53%, 99.65% and 98.61%, respectively. The percentage relative standard deviation (% RSD) of Efavirenz, Lamivudine and Stavudine for intraday precision was found to be 0.95, 1.25 and 1.37, respectively. The LOD values for Efavirenz, Lamivudine and Stavudine was found to be 2.98, 2.94 and 2.92 µg/ml, respectively. The LOQ values for Efavirenz, Lamivudine and Stavudine was found to be 9.96, 9.98 and 9.94 µg/ml, respectively. Thus the proposed method was successfully applied for simultaneous estimation of Efavirenz, Lamivudine and Stavudine for routine analysis.

Keywords: Efavirenz; Lamivudine; Stavudine; RP-HPLC

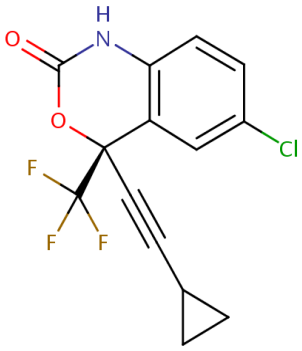
Introduction

Efavirenz IUPAC name (4S)-6-Chlor-4-(cyclopropylethinyl)-4-(trifluormethyl)-1, 4-dihydro-2H-3, 1-benzoxazin-2-on. It falls in the NNRTI class of anti retrovirals. Non-Nucleoside Reverse Transecriptase Inhibitors inhibit the same target, the reverse transcriptase enzyme. Efavirenz is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class. Lamivudine its IUPAC name was 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and polymerase, resulting in DNA chain termination.StavudineIUPAC name is 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methyl-1,2,3,4tetra-hydropyrimidine-2,4-dione.Stavudine is an analog of thymidine. It is phosphorylated by cellular kinases into active triphosphate. Stavudine triphosphate inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA replication by incorporating into the DNA strand. The review of literature revealed that various analytical methods involving UV-Spectrophotometry [1,2,3] HPLC [2,3-12] and LC MS [13] have been reported for Efavirenz, Lamivudine and Stavudine [5] individually or with other combinations. But there is no HPLC method was reported for this combination of drugs. Hence the necessity of developing simple and cost effective RP-HPLC method always a continuing interest.

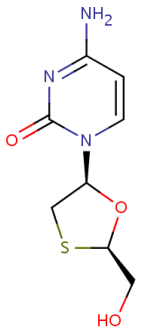
***Corresponding author:** Suneetha A, Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur-522 002, A. P, India, E-mail: drasuneetha@gmail.com

Sub Date: June 15, 2017, **Acc Date:** July 14, 2017, **Pub Date:** July 14, 2017.

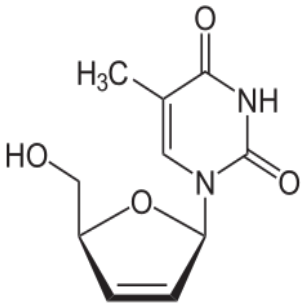
Citation: Suneetha A, Bhavana G and Ayesha Siddikha Sk (2017) Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Efavirenz, Lamivudine, and Stavudine in Pharmaceutical Dosage forms. BAOJ Pharm Sci 3: 041.



EFAVIRENZ



LAMIVUDINE



STAVUDINE

Materials and Methods

Instrumentation [14]

Waters HPLC System is equipped with Empower software-2.Waters HPLC 2695 series consisting four pumps and an auto sampler with five racks, each having twenty four vials holding capacity with a temperature controller. This is equipped with an auto injector having a capacity of injecting 5µL to 500µL and a UV-V is DAD Detector which is connected to thermostat column compartment having a capacity to maintain 5°C to 60°C column temperature.

Experimental Conditions

The HPLC system was operated iso-cratically at flow rate of 1.2 ml/min at 30°C ±0.5°Cfor 10min. The mobile phase found to be most suitable for analysis was buffer: Methanol in 30:70%v/v, and detection was carried out at 254 nm.

Preparation of Standard Stock Solution

Accurately weigh and transfer 150 mg of Lamivudine, 40 mg of Stavudine and 600 mg of Efavirenzin a 100ml volumetric flask, and dissolve in water, make up to the mark with the same solvent. Further pipette out 1.0 ml from the above stock solution into 10ml volumetric flask and dilute up to the mark with diluent.

From the above solution pipette out 3ml of sample into a 10ml clean dry volumetric flask and make up with diluents.

Sample Solution

Accurately weighed and transferred 150mg of Lamivudine, 600 mg of Efavirenzand 40mg of Stavudine into a 100ml clean dry volumetric flask and is sonicatedto dissolve it completely and made up to the mark with the same solvent.

Further pipette out 1.0 ml of the above sample solution into a 10ml volumetric flask and diluted to the mark with diluent and it was further diluted to get the concentration within the calibration range.

Results

Method Development and Optimization

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution were tested for a good chromatographic separation. Trials showed that an acidic mobile phase with reverse phase C18 column gives symmetric and sharp peaks. Mobile phase composition of 30:70 (v/v) at a flow rate of 1.2 mL/min showed good resolution. When Potassium Di hydrogen Ortho Phosphate was used as modifier, resolution between Efavirenz, Lamivudine and Stavudine was much better at pH 4.0. For the quantitative analytical purposes the wavelength was set at 254 nm. For the quantitative determination of Efavirenz, Lamivudine and Stavudine in formulations, initially mixed standard solution was injected into the column five times and the retention times of Efavirenz. Lamivudine and Stavudine were found to be3.105, 4.810, and 7.164 min, respectively (Figure 1).

Validation

The described method has been validated for the simultaneous estimation of Efavirenz, Lamivudine and Stavudine using following parameters.

Linearity

To establish linearity [15] of the proposed method, five different sets of drug solutions were prepared and analyzed. Standard curves were constructed in the concentration range of 15-75µg/ml for Lamivudine, 4-20 µg/ml for Stavudine and 60-300µg/ml for Efavirenz lope, intercept and the correlation coefficient were determined from the calibration curves shown in (Figure 2-4) and sample chromatogram was shown in (Figure 5).

Accuracy

The accuracy [16] of the method was demonstrated at three different concentration levels (50, 100,150%) by spiking a known quantity of standard drugs into a previously analyzed sample in

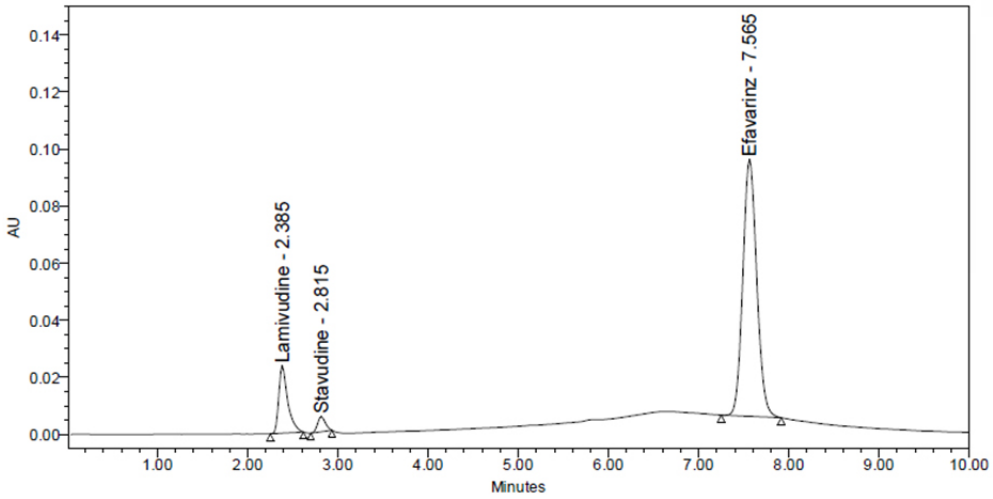


Figure 1. Standard chromatogram of Lamivudine, Stavudine and Efavirenz

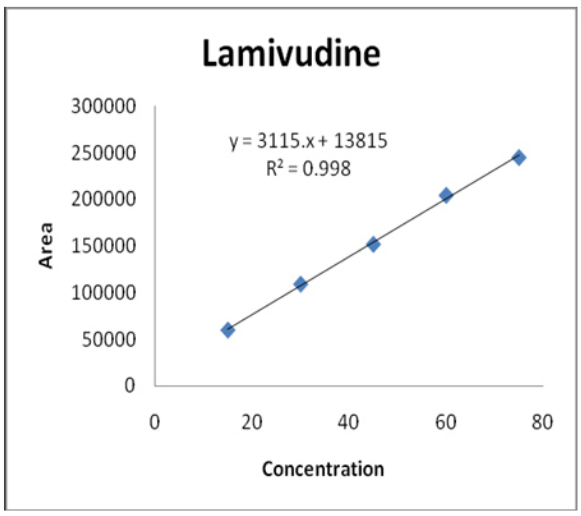


Figure 2. Calibration curve of Lamivudine

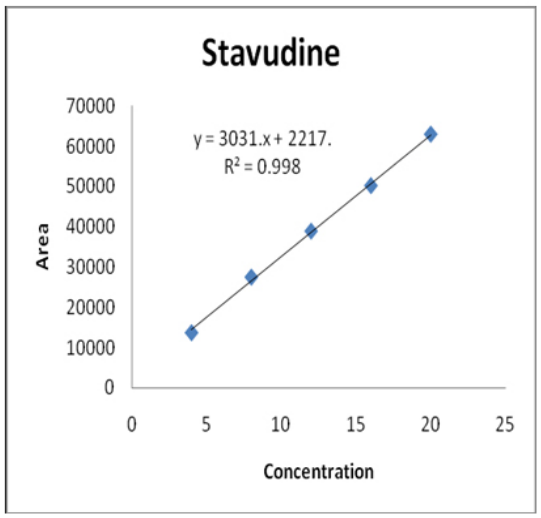


Figure 3. Calibration curve of Stavudine

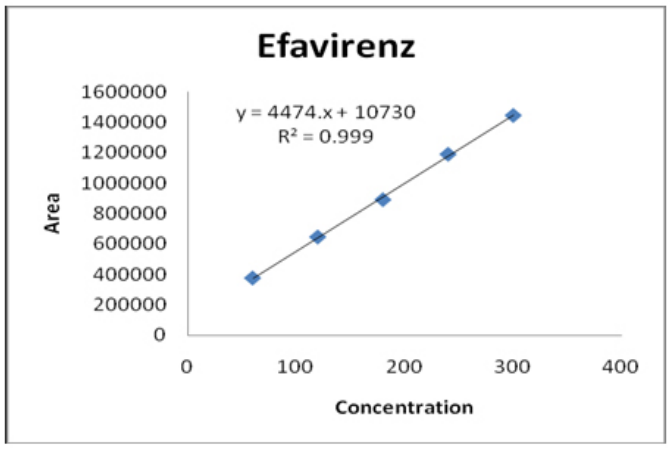


Figure 4. Calibration curve of Efavirenz

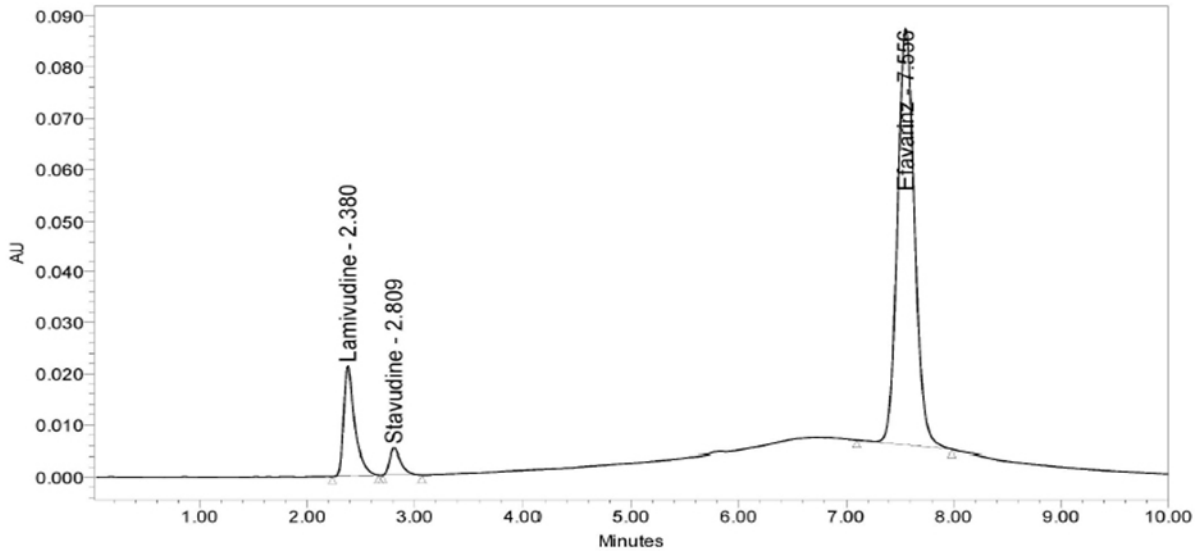


Figure 5. Sample Chromatogram of Lamivudine, Stavudine and Efavirenzin formulation

triplicate. The results of accuracy revealed that the method is more accurate. Results of accuracy were given in (Table 1).

Precision

The precision [17] of the method was verified by repeatability and intermediate precision studies, three replicates were injected into the system on two different non-consecutive days, in each case %RSD was calculated. Results of precision were given in (Table 2).

Limit of detection (LOD) and Limit of quantization (LOQ)

The limit of detection and limit of quantization for Efavirenz, Lamivudine and Stavudine were calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. The limit of detection of a compound is defined as the lowest concentration of analyte that can be detected. LOD value of Efavirenz, Lamivudine and Stavudine were found to be 2.98, 2.94 µg/ml and 2.92 µg/ml, respectively. The limit of quantization is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy.

LOQ values for Efavirenz, Lamivudine and Stavudine were found to be 9.96, 9.98 µg/ml and 9.94 µg/ml, respectively.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making out intentional

Injection	Spike Level	Amount present(mg)	Amount recovered(mg)	% recovery	Mean % recovery
Lamivudine	50%	76	77.03	101.35%	
	100%	150	149.45	99.64%	100.93%
	150%	226	230.05	101.79%	
Stavudine	50%	20	20.27	101.33%	
	100%	40	40.27	100.67%	101.31%
	150%	60	61.15	101.92%	
Efavirenz	50%	300	297.92	99.31%	
	100%	600	595.64	99.27%	99.46%
	150%	900	898.13	99.97%	

Table 1. Recovery study of Lamivudine, Stavudine and Efavirenz.

Mean peak area*	Intraday presicion		
	Lamivudine	**Stavudine**	**Efavirenz**
SD	154132	38601.8	897935.8
	2526.33	611.52	4411.57
%RSD	1.6390	1.5841	0.4913

*Average of six determinations

Table 2. Method precision for Lamivudine, Stavudine and Efavirenz in combined dosage form.

method variations like mobile phase, flow changes, pH, mobile phase compositions and column oven temperature variations etc.

The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

To ascertain the system suitability for the proposed method a number of statistical values such as theoretical plates, peak symmetry, resolution have been calculated with the observed readings and the results are tabulated in (Table 3).

Discussion

The objective of the proposed work was to develop novel analytical method for the simultaneous estimation of Efavirenz [18], Lamivudine and Stavudine to validate the method according to ICH guidelines and applying the same for its estimation in pharmaceutical formulations. A few methods appeared in the literature, for individual drugs both in UV spectrophotometric method and RP- HPLC method were reported and for the simultaneous estimation of Efavirenz, Lamivudine and Stavudine only HPLC method has been reported. In view of the above fact, a simple RP- HPLC method was planned to develop with high sensitivity, accuracy, precision and economical.

A rapid and sensitive HPLC method was developed for the analysis of Efavirenz, Lamivudine and Stavudine in bulk drug and in its Pharmaceutical dosage forms using the thermo Inertsil ODS C-18 column. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates) runtime, resolution.

The system with Potassium Di hydrogen Ortho Phosphate buffer and Methanol (30: 70) v/v of $P^{H}4$ and 1.2 ml/min flow rate was quite robust.

The optimum wavelength for detection was 254 nm at which the better detector response for the drug was obtained. The run time was set at 10 min and the retention time for Efavirenz, Lamivudine and Stavudine was found to be 3 .105, 4.810, and 7.164 min, shown in Fig. 4

Each sample was injected 6 times and the retention time was recorded. A good linear relationship was observed between the concentration of Efavirenz, Lamivudine and Stavudine. The proposed HPLC method was also validated for precision studies and results were within the range.

The precision [19] of the method was verified by repeatability and intermediate precision studies, and %RSD was calculated. Results of precision were within the range. Summary of precision results were shown in table No. 2.

The accuracy limit and the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 101.53%, 101.31% and 99.46% for Lamivudine, Stavudine and Efavirenz. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is

S.No	Parameters	Lamivudine	Stavudine	Efavirenz
1	Retention time(min)	3.105	4.810	7.164
2	Theoretical plates	2774	4343	11307
3	Tailing factor	1.55	1.11	1.11
4	Resolution	-	2.44	20.86
5	Range (mcg/ml)	15-75	4-20	60-300
6	%RSD	0.8008	1.25093	1.094656
7	Correlation coefficient	0.999	0.999	0.999
8	LOD(mcg/ml)	2.94	2.92	2.98
9	LOQ(mcg/ml)	9.98	9.94	9.96

Table 3. System Suitability Parameters

capable of showing good accuracy and reproducibility. Summary of Accuracy results were shown in table No. 1.

The change in flow rates such as 1.0 ml, 1.2 ml, and 1.4ml /min. At the flow rate of 1.2 ml/min, the peaks were sharp with good resolution, rest of the flow rates was found to be not satisfactory. So 1.2 ml/min flow rate was kept constant throughout the analysis. The temperature changes are observed at 25º, 30º and 35ºC respectively. At 30ºC temperature the peaks were sharp with good resolution, but with other temperatures the peaks were not satisfactory. So 30ºC Temperature is maintained constant for the analysis.

The LOD and LOQ were performed and percentage assay were calculated and reported. The values were found to be within the range. Summary of validation results were shown in table No. 3.

System suitability Parameters were calculated which includes Efficiency, Resolution and tailing factor.

The method was validated for linearity, accuracy, precision, robustness.

Conclusion

The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination of Efavirenz, Lamivudine and Stavudine in bulk and its pharmaceutical dosage form. The developed method gave good resolution between Efavirenz, Lamivudine and Stavudine with short analysis time (9 min).

Reference

1. Kajal G (2012) UV-spectroscopic methods for estimation of stavudine: finding the best order. Open Access Scientific Reports 1(7): 13.
2. Mohanreddy TR, Srikanth G (2016) Development and validation of HPLC-UV method for the estimation of stavudine in human plasma. Indo American Journal of Pharmaceutical Research 6(1): 3996-4001.
3. Srinath BA, Sneha, Akhila A, Rayeesa A, Kulkarni RG (2015) Method development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV-spectroscopic method. International journal of pharmaceutical sciences and research 1(11) : 5491-5497.
4. Dhara SB, Bhavini Patel N, Chhaganbhai Patel N (2012) RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined dosage form. Pharmaceutical Methods 3(2): 73-78.
5. Karthikeya D, Srinivas M, Santhosh Kumar C (2013) Formulation and Evaluation of Stavudine Nanoparticles. International Journal of Novel Trends in Pharmaceutical Sciences 3: 24-32.
6. Rajkumar B, Bhavya T, Kulsum S, Ashok kumar A (2014) RP-HPLC method development and validation for the simultaneous quantitative estimation of efavirenz, lamivudine and zidovudine in tablets. International journal of pharmacy and pharmaceutical sciences 6(2).
7. Honey Diana B, Bibi Khader SK, Sita Kumari K (2015) New validated RP-HPLC method for simultaneous estimation of lamivudine and tenofovirdisproxilfumerate in tablets. International journal of advanced pharmaceutical analysis 5(1).
8. Srinivasa Rao A, Naveen Kumar G, Srilekha K, Aruna Kumari N (2016) Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC. International journal of advanced research in science and engineering 5(5): 2319-8354.
9. PSRCHNP Varma D and Lakshmana Rao (2014) Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Efavirenz, Tenofovir and Emtricitabine in Pharmaceutical Formulations. Indian Journal of Pharmacy and Pharmacology 1(1): 1-17.
10. Mallikarjuna Rao N, Gowri Sankar D (2015) Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage forms. Future journal of pharmaceutical sciences 1(2): 73-77.
11. Santoshkumar M, VenkateshwarRao J (2014) A stability indicating RP-HPLC method development and validation for simultaneous determination of lamivudine and zidovudine in combined dosage form. Indo American Journal of Pharmaceutical Research 4(3): 1289-1297.
12. Umesh Patel M, Nageswara Rao R (2011) Development and validation of a stability indicating RP-HPLC method for simultaneous determination of Lamivudine and Stavudine in combined dosage forms. Journal of Chemical and Pharmaceutical Research 3(6): 200-211.
13. Fan B, Bartlett GB, Stewart JT (2002) Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch. Biomed Chormatogr 16(6): 383-389.
14. Suneetha A, Syama Sundar B (2010) A Validated RP-HPLC method for estimation of Almotriptan malate in pharmaceutical dosage form. The Journal of Chinese Chemical Society 57(5A): 1067-1070.
15. Balaji S, Suneetha A (2010) Development and validation of spectrophotometeric method for simultaneous determination of simvastatin and ezetimibe in tablet formulations. Pak J Pharm Science 23(4): 375-8.
16. Sundar BS, Suneetha A (2010) Validation of HPTLC method for estimation of Rizatriptan Benzoate in tablet dosage form. Indian J Pharm Sci 72(6): 798-801.
17. Suneetha A, Chandana Priya T (2014) RP- HPLC Method for Simultaneous Estimation of Paracetmol and Ondonsetron in Bulk & Oral suspension. Journal of Pharmaceutical Research 13(4): 106-110.
18. Djerada Z, Feliu C, Tournois C, Vautier D, Binet L, et al. (2013) Validation of a fast method for quantitative analysis of elvitegravir and 10 other antiretroviral agents in human plasma samples with a new UPLC-MS/MS technology. J Pharm Biomed Anal 86: 100-11. doi: 10.1016/j.jpba.2013.08.002
19. Carmen R, Antonio C, Simon S (1983) Acceptable Limits for Precision and Accuracy. Clinical Chemistry 29(3).