INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL.4 ISSUE 1, 2016; 24 – 29 ; http://ijpda.com; ISSN: 2348-8948

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF TENOFOVIR AND EMTRICITABINE IN PHARMACEUTICAL DOSAGE FORMS BY HPLC

Z. M. Sayyed[*1], Aijaz A. Sheikh[*2], Zakirhussain A. Shaikh[3], S. A. Shinde[4], V. J. Chaware[5], K. R. Biyani[6]

[1,2,4-6] Department of Quality Assurance, Anuradha College of Pharmacy, Chikhli, Dist.-Buldana, Pin - 443201, Maharashtra (India).

[3] B.Sci. (PCM), Siddharth Sci. & Jr. College, Jafrabad, Dist.- Jalna, Pin- 431206, Maharashtra (India).

Date Received: 24th December 2015; Date Accepted: 11th January 2016 Date published: 15th January 2016

Email: sayyedzuber11@gmail.com

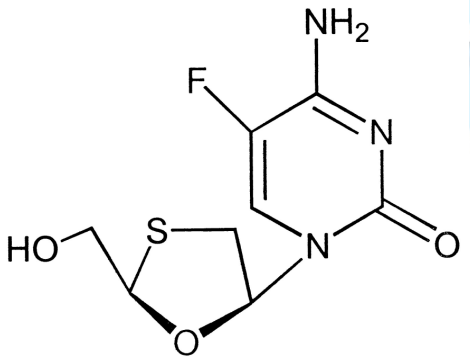
Abstract: A simple, accurate, rapid and precise High performance Liquid Chromatography (HPLC) method was developed and validated for the simultaneous estimation of Tenofovir (TFR) and Emtricitabine (ECB) in combined tablet dosage form. Chromatogram was run through column of Hi Q C18 W (150 mm: 4.6 mm, 5 µ). Mobile phase containing Buffer, Methanol and Acetonitrile (40: 50: 10) was pumped through column at a flow rate of 1.3 ml/ min. 0.01 N KH_2PO_4 was used as Buffer. UV detection was carried out at 265 nm and 278 nm for Tenofovir and Emtricitabine respectively. Retention times of Tenofovir and Emtricitabine were found to be 4.38 min and 3.22 min respectively. % RSD of the Tenofovir and Emtricitabine was found to be less than 2 %. The Calibration curves were plotted in the concentration range of 6-36 µg/ ml for Tenofovir and 5-30 µg/ml for Emtricitabine with R^2= 0.99. % Recovery was found between 97-100 % for both the drugs.

Key words: Tenofovir, Emtricitabine, Simultaneous Estimation, Validation, HPLC.

INTRODUCTION:

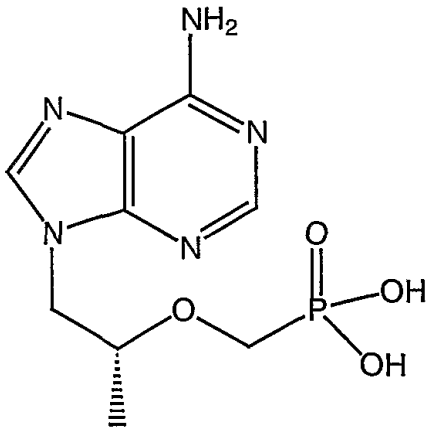
Emtricitabine and Tenofovir belongs to a class of Anti-Retrovirus (Anti-HIV) drugs. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI). Its IUPAC name is 4-amino-5-fluro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxanthion-5-yl]-1,2-dihydropyrimidine-2-one. Emtricitabine act by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, It helps to lower the amount of HIV or viral load in patient's body and can indirectly increase the number of immune system (called T cells / Cd4+ cells). Molecular weight of Emtricitabine is 247.248 g/mol. Its chemical formula is $C_8H_{10}FN_3O_3S$ [1-4] **(Figure No. 1)**.

Tenofovir is a, Bis{[(isopropoxycarbonyl)oxy]methyl} ({[(2R)-1-(6-amino-9H-purine-9-yl)-2-propanyl]oxy} methyl) phosphonate. It having molecular weight 519.443 g/mol and chemical formula is $C_{19}H_{30}N_5O_{10}P$. Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine-5'-triphosphate and after incorporation into DNA, by DNA chain termination, It gets converted into diphosphate intracellularly, which inhibits the DNA synthesis of HIV by competitive inhibition of reverse transcriptase and incorporation into viral DNA. It also inhibits hepatitis B virus polymerase, resulting in inhibition of viral replication [5-8] **(Figure No. 2)**.



Emtricitabine

Literature survey reveals that few analytical methods were reported for Tenofovir and Emtricitabine in individual and in combination with other drugs.[9-11] But no simple, precise and accurate method has been reported so far by using RP-HPLC for simultaneous estimation of Tenofovir and Emtricitabine.



Tenofovir

Materials and Methods

Instrument:

HPLC separation module: HPLC JASCO PU- 2080 Plus Intelligent HPLC Pump, JASCO PU- 2075 Plus Intelligent HPLC Detector, with column of Hi Q C18 W (150 mm × 4.6 mm: 5µ) is used. Mobile phase containing Buffer, Methanol and Acetonitrile (40: 50: 10) was pumped through column at a flow rate of 1.3 ml/ min. 0.01 N KH_2PO_4 was used as Buffer. UV detection was carried out at 265 nm and 278 nm for Tenofovir and Emtricitabine respectively.

Reagents and Chemicals:

Tenofovir and Emtricitabine pure drug samples were procured as a gift samples. The tablet dosage form Tavin-EM (contains Tenofovir: 300 mg and Emtricitabine: 200 mg) was procured from local market. All chemicals and reagents used were of analytical reagent grade.

Preparation of standard stock solution:

Stock solutions were prepared by dissolving 10 mg of Tenofovir and 10 mg of Emtricitabine in 100 ml of mobile phase separately. Aliquots of the standard stock solutions of Tenofovir and Emtricitabine were transferred into 10 ml volumetric flasks and solution was made up to the volume to yield required concentrations of both drugs within the linearity range. Standard stock solution of 300 µg/ml of TFR and 200 µg/ml of ECB was also prepared.

Selection of Detection Wavelengths:

UV- Detector was selected, as it is reliable and easy to set at constant wavelengths. UV detection was carried out at 265 nm and 278 nm for Tenofovir and Emtricitabine respectively, **Figure No. 3**.

Analysis of Marketed Formulation:

20 tablets were accurately weighed and the average weight was calculated. The tablets were crushed to fine powder. Then the weight of powder equivalent to an average weight of tablet was transferred into a 25 ml volumetric flask, 15 ml of mobile phase was added and sonicated for 20 min and made up the final volume with mobile phase and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC. Results of analysis of Marketed Combined Tablet Formulation are shown in **Table No. 3**.

Recording of Chromatograms:

A steady baseline was recorded with the fixed chromatographic conditions and standard drug solutions were injected and chromatograms were recorded. Retention times of TFR and ECB were found to be 4.38 and 3.22 minutes, **Figure No. 4**. This was followed by injection of sample solution obtained from the formulation. Calibration curves were plotted using peak areas of standard drug vs. concentration of corresponding standard solutions. Calibration curves are shown in **Figure No. 5-6** and data for Calibration curves are given in **Table No. 1-2**. Peak areas of the sample chromatograms were compared and the amount of TFR and ECB were calculated.

Validation of Proposed Method

(1) Linearity: The linearity of the method was determined by constructing Calibration curves. The Calibration curves were linear over the concentration range of 6-36 µg/ml for Tenofovir and 5-30 µg/ml for Emtricitabine. Peak areas were plotted versus respective concentrations. The correlation coefficient was found to be 0.99 (R^2 = 0.99) for both the drugs. Calibration curves are shown in **Figure No. 5-6** and data for Calibration curves are given in **Table No. 1-2**.

(2) Accuracy: Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual results obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the mixture of the sample to be analysed. For this prepare three different concentrations of solution like 80%, 100%, 120%. For each concentration was injected and the % recovery was calculated. Results of Recovery study are shown in **Table No. 4**.

(3) Precision: Method precision was determined by injecting six replicates of the drug sample solution. The retention times and peak areas of six replicates were recorded. The precision is expresses as the % RSD of peak areas and it should be NMT 2 %. Results of Precision study are given in **Table No. 5**.

(4) Limit of Detection (LOD) and Limit of Quantitation (LOQ): Limit of detection and Limit of quantitation represents the concentration of analyte that would yield

signal to noise ratio of 3 for LOD and 10 for LOQ respectively. LOD and LOQ was calculated from linear curve using formulae.

$$LOD = 3.3 \times \sigma / S$$
$$LOQ = 10 \times \sigma / S$$

Where, σ = Standard deviation of the response and S = Slope of calibration curve. Results of LOD/ LOQ are given in **Table No. 6**.

(5) Repeatability: It was performed by injecting samples of Tenofovir and Emtricitabine into the system and measuring the peak area. It was repeated for three times. Results of Repeatability study are shown in **Table No. 7**.

(6) Ruggedness: Ruggedness of the method was studied by two different analyst using same operational and environmental conditions. Results of Ruggedness study are shown in **Table No. 8**.

Results and Discussion

Mobile phase of different compositions were used to resolve the peaks of Tenofovir and Emtricitabine. The mobile phase was found to be suitable was Buffer: Methanol: Acetonitrile in the ratio of 40: 50: 10. UV detection was carried out at 265 nm and 278 nm for Tenofovir and Emtricitabine respectively, **Figure No. 3**. Retention times of Tenofovir and Emtricitabine were found to be 4.38 min and 3.22 min respectively, **Figure No. 4**. Calibration graphs were plotted using standard peak areas vs. concentration of standard solutions. This drugs showed linearity in the concentration range of 6-36 µg/ ml for Tenofovir and 5-30 µg/ml for Emtricitabine. Correlation coefficient values were found to be R^2= 0.99 for both the drugs. Calibration data for TFR and ECB were shown in **Table No. 1-2** and Calibration curves were shown in **Figure No. 5-6**. Results of analysis of Marketed Combined Tablet Formulation are shown in **Table No. 3**. Accuracy of the developed method was determined on the basis of Recovery study and was found to be between 97-100 %. Results of Recovery study are shown in **Table No. 4**. Precision study was conducted by Intraday and Interday precision. % RSD was found to be NMT 2 %. Results of Precision study are given in **Table No. 5**. Results of LOD/ LOQ, Repeatability and Ruggedness are shown in **Table No. 6-8**. The developed method was found to be robust. System suitability parameters like number of theoretical plates (N), Retention time (Rt), and Tailing factor were also studied. The validated liquid chromatographic method was applied for simultaneous determination of Tenofovir and Emtricitabine in bulk and pharmaceutical dosage form.

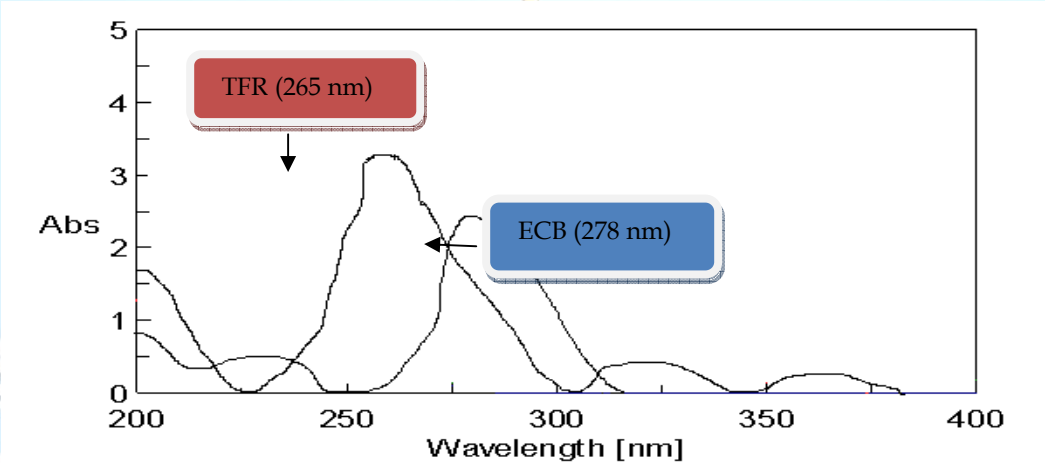


Figure No. 3: UV Spectra of Tenofovir and Emtricitabine

Table No. 1: Calibration data for Tenofovir

Sr. No.	Concentrations of TFR (µg/ml)	Peak Area	% RSD
1	06	424981	0.42
2	12	536212	0.73
3	18	639468	1.23
4	24	746795	0.58
5	30	852387	0.33
6	36	963640	0.85

Table No. 2: Calibration data for Emtricitabine

Sr. No.	Concentrations of ECB (µg/ml)	Peak Area	% RSD
1	05	3231404	1.61
2	10	4378281	0.43
3	15	5486263	0.71
4	20	6525922	0.22
5	25	7612848	1.18
6	30	8741376	0.92

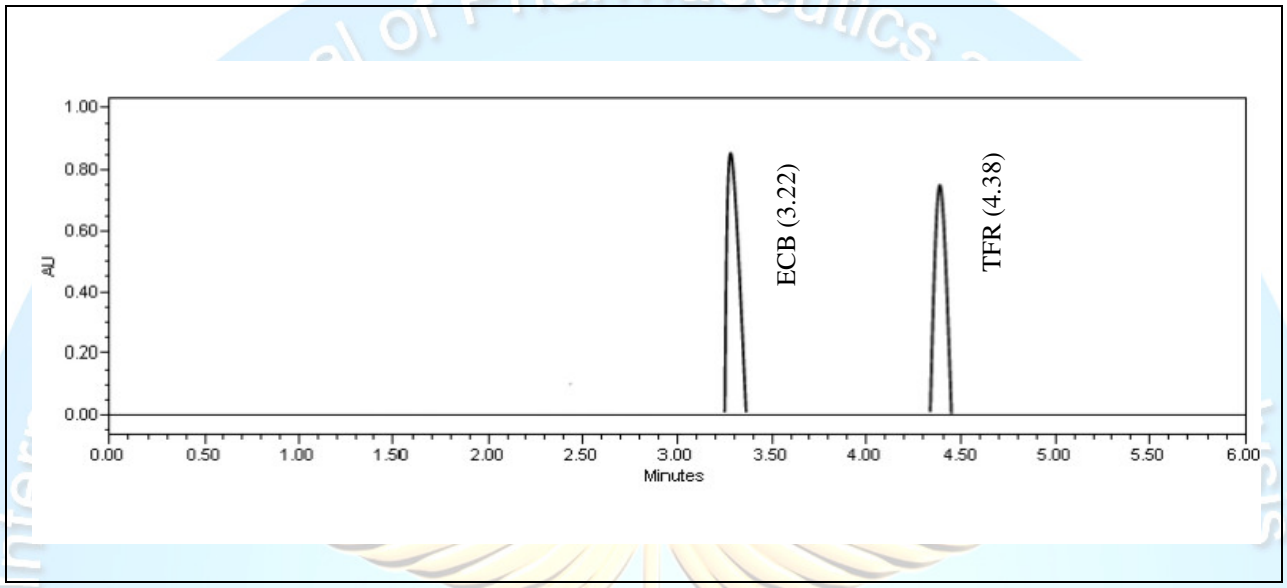


Figure No. 4: Chromatogram of Tenofovir and Emtricitabine

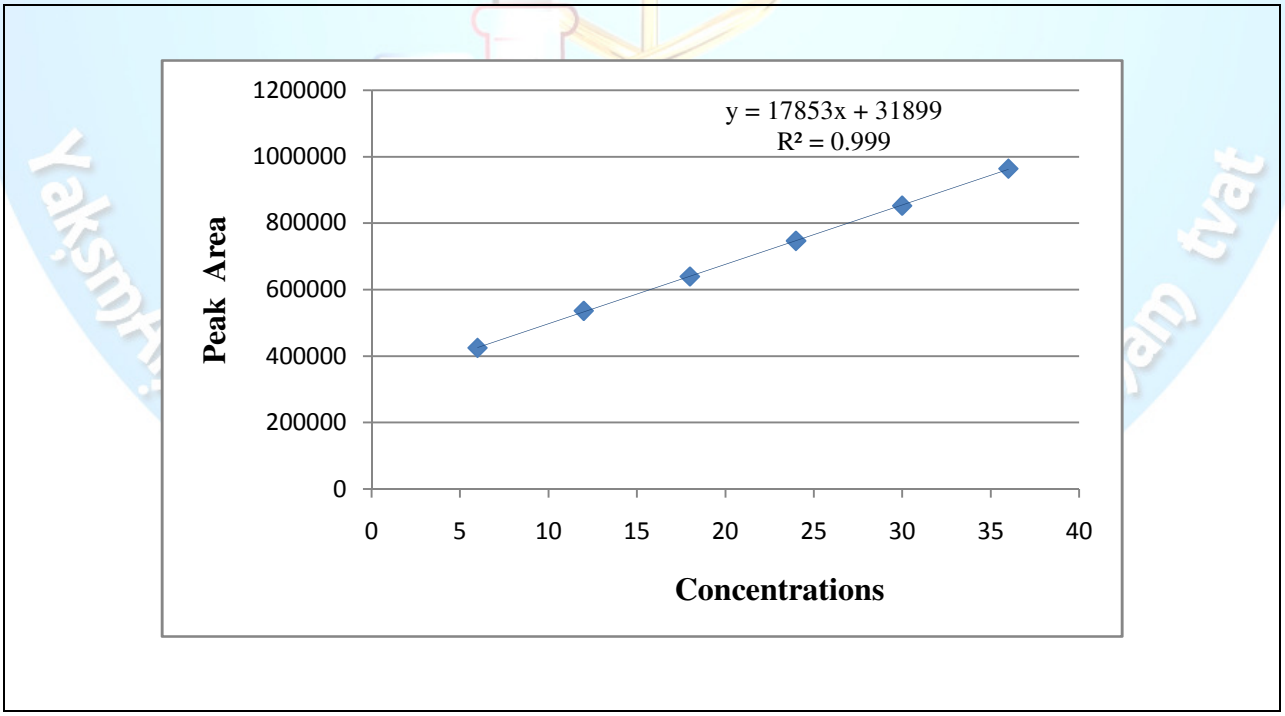


Figure No. 5: Calibration Curve of Tenofovir

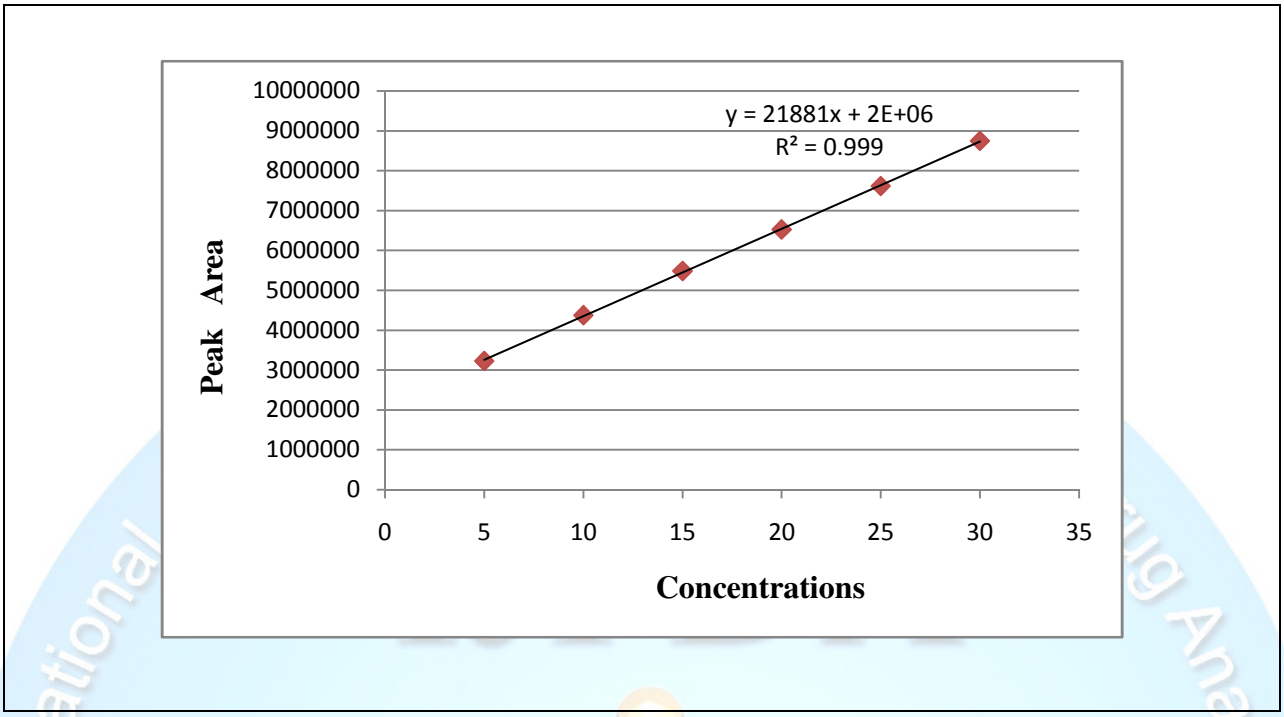


Figure No. 6: Calibration Curve of Emtricitabine

Table No. 3: Results of Analysis of Marketed Combined Tablet Formulation

Sr. No.	Drugs	Labeled amount (mg/tab)	Amount found (mg/tab)	% Amount found	% RSD
1	TFR	300	297.06	99.02	0.87
2	ECB	200	198.1	99.05	0.48

Table No. 4: Results of Recovery study

Sr. No.	Drugs		Recovery Levels		% Recovery		% RSD (n= 5)	
			TFR	ECB	TFR	ECB	TFR	ECB
1	TFR	ECB	80 %	80 %	99.51	97.98	0.36	0.86
2			100 %	100 %	99.80	99.96	0.13	0.44
3			120%	120 %	98.71	98.89	0.76	0.79

Table No. 5: Results of Precision study (Intra-day and Inter-day)

Sr. No.	Drugs	Conc. taken (µg/ml)	Intra-day amount found		Inter-day amount found	
			Mean	% RSD	Mean	% RSD
1	TFR	24	23.11	1.01	23.96	0.15
		30	28.97	0.29	29.01	0.23
		36	34.92	0.64	35.16	0.77
2	ECB	16	15.74	0.83	15.81	1.20
		20	19.66	0.72	19.22	0.40
		24	23.25	0.48	23.38	0.59

Table No. 6: Results of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Sr. No.	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1	TFR	1.56	2.75
2	ECB	1.25	2.31

Table No. 7: Results of Repeatability study

Sr. No.	Drugs	Conc. (µg/ml)	Mean Peak Area	% RSD (n= 3)
1	TFR	300	5547832	0.94
2	ECB	200	401287	1.08

Table No. 8: Results of Ruggedness study

Sr. No.	Analysts	Mean		% RSD	
		TFR	ECB	TFR	ECB
1	Analyst-I	524986	4125765	1.25	0.48
2	Analyst- II	535489	4248973	0.81	0.19

Conclusion

The new RP-HPLC method was developed and found to be simple, accurate, rapid and precise. The developed RP-HPLC method was used to estimate Tenofovir and Emtricitabine quantitatively in bulk and pharmaceutical dosage form. The HPLC method was validated and showed good linearity, precision, accuracy. The method was validated as per international conference on harmonization (ICH) guidelines.

Acknowledgement

The authors wish to thank Emcure Pharmaceuticals Ltd. for providing gift samples of pure Tenofovir and Emtricitabine.

References

1. Sujata K, Sheelarani T, Anand Babu K, et al. RP-HPLC method development and validation of Emtricitabine in synthetic mixture. World Journal of Pharmaceutical Research. 2014; 36: 499-505.
2. Arjun G, Sathis Kumar D, Yogeswaran P, et al. A simple HPLC method for the quantification of Emtricitabine in capsule dosage form. Der pharma Chemica. 2010; 22: 281-285.
3. Nagaraju P, Channabasavaraj K, Shantha Kumar P, Development and validation of spectrophotometric method for estimation of Emtricitabine in tablet dosage form. Int. J. Chem Tech Res. 2011; 31: 23-28.
4. Ali Mohammed., A Text Book of Pharmaceutical Organic Chemistry, 1st Edn, CBS Publication New Delhi: 285-289, (2008).
5. Manavarthi S, Chhabra S. Stability indicating RP-HPLC method development and validation of Tenofovir in bulk and pharmaceutical formulation. Der pharma Chemica. 2014; 62: 401-409.
6. Pratap R, Chakravarthy E. New spectrophotometric determination of tenofovir disoproxil fumarate in bulk and pharmaceutical dosage form. IOSR Journal of Applied Chemistry. 2012; 12: 29–33.
7. Kandagal P, Manjunatha D, Seetharamappa J, et al. RP-HPLC method for the determination of tenofovir in pharmaceutical formulations and spiked human plasma. Analytical Letters. 2008; 414: 561–570.
8. Mahadik K. R., Kuchekar B. S. and Deshmukh K. R., Concise Organic Pharmaceutical Chemistry, 13th Edn, Nirali Publication Pune: 189-199, (2009).
9. Patel S, Baghel S, Rajesh P, et al. Spectrophotometric method development and validation of Tenofovir disoproxil fumarate and Emtricitabine in bulk drug and tablet dosage form. International Journal of Pharmaceutical and Clinical Research. 2009; 11: 28–30.
10. Joshi M, Nikalje AP, Shahed M, et al. HPTLC method for the simultaneous estimation of Emtricitabine and Tenofovir in tablet dosage from. Indian J. Pharm. Sci. 2009; 71: 95-97.
11. Gomes NA, Vaidya VV, Pudage A, et al. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of Tenofovir and Emtricitabine in human plasma and its application to a bioequivalence study. Journal of Pharmaceutical and Biomedical Analysis. 2008; 483: 918–926.