International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.7, pp 240-249, 2016

Stability Indicating RP-HPLC Method Development and Validation for the Quantification of Tenofovir Disoproxil Fumarate in Bulk and its Dosage form

K. Nandini*, Shaik Muneer, K.B Chandra Sekhar, B. Siva Sai Kiran

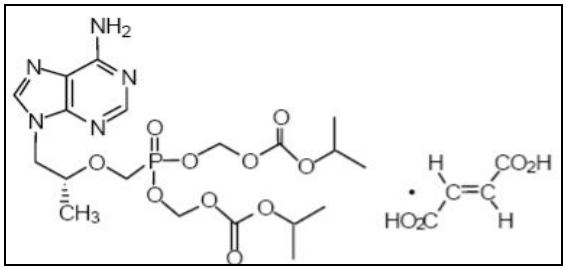
Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur- Oil Technological & Pharmaceutical Research Institute, Anantapur, A.P. India.

Abstract : A simple, selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been proposed for the estimation of Tenofovir Disoproxil Fumarate in pure form as well as in its pharmaceutical formulation. The chromatography was carried on Phenomenex Luna C18 (250 x 4.6 mm x 5 µm) column, with mobile phase Orthophosphoric Acid: Acetonitrile: Methanol in the ratio of (40:50:10% v/v) and pH adjusted to 3.0. The flow rate was 0.9 ml/min with detection at 254 nm. The retention time was found to be 2.21 min. The proposed method was validated in accordance with ICH guidelines. The linearity was found in the range of 10-60 µg/mL respectively. All validation parameters were within the acceptable range. From the recovery studies, non interference of excipients with the drug was identified and % recovery was found to be 100.19. The drug was subjected to stress studies by subjecting to various conditions like acid, base, oxidative, thermal and photolytic from which sensitivity of drug can be determined. The developed method was successfully applied to estimate the amount of drug in tablet dosage form and to study the stability of the product in various stress conditions as per ICH guidelines.
Keywords: Tenofovir disoproxil fumarate, RP-HPLC Method development, Validation, Stress studies.

1. Introduction

Tenofovir disoproxil fumarate (TDF), chemically is 9-[(*R*)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy] -phosphinyl]-methoxy]propyl] adenine fumarate (1:1). It inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and after incorporation into DNA, by 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. It is used in the treatment of HIV infection and chronic hepatitis B infection. [1]The chemical structure of Tenofovir disoproxil Fumarate was shown in Fig. 1.

Structure of Tenofovir Disoproxil Fumarate



Extensive literature survey very few methods were reported in stability indicating assay methods for estimation of TDF using HPLC in bulk and its pharmaceutical formulation individually[2-4] and in its combination[5-13] with other drugs. The present study is an attempt made to develop a novel, simple, rapid, accurate, efficient & reproducible stability indicating[13-18] analytical method for the estimation of TDF using RP-HPLC & to validate[19-25] the analytical procedures Q2 (R1) and Stability Testing Q1A (R2) as per ICH guidelines[26-29].

Experimental:

Instruments:

The chromatographic technique was performed on a Shimadzu HPLC separation module LC 20 AD with Photo diode array detector and a Rheodyne manual injector model 7725i with 20µL sample loop connected to LC solutions chemstation. Chromatographic separation was carried using Phenomenex Luna C18 (250 x 4.6 mm, 5 µm) column. Mobile phase was filtered through a 0.45µ membrane filter (Millipore Pvt. Ltd., Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai, India).

Materials:

Tenofovir disoproxil Fumarate was obtained as gift sample from Ranbaxy laboratories Pvt. ltd, Ahmadabad, India. The drug was used without further purification.

HPLC-grade solvents like Acetonitrile, Methanol and Water and was obtained from Spectrochem Pvt. Ltd., Mumbai, India. Analytical reagent grade solvents orthophosphoric acid, hydrogen peroxide (H_2O_2) 30% w/v, sodium hydroxide pellets and hydrochloric acid were procured from SD Fine–Chem Limited, Mumbai, India.

A tablet formulation of Tenofovir disoproxil Fumarate (300 mg) (TAVIN, Emcure Pharmaceuticals) was procured from local market.

Preparation of mobile phase:

The mobile phase consists of orthophosphoric acid (0.1 % v/v) adjusted to pH-3, acetonitrile, methanol in the ratio of 40:50:10% v/v. Before proceeding for analysis the mobile phase was degassed by use of ultra sonication and filtered through a 0.45 µm HPLC filtration assembly. The system was equilibrated before each injection. Isocratic elution system was performed.

Stock and working standard preparations:

Weigh about 10 mg of TDF and transfer into 10 ml volumetric flask, sufficient amount of Methanol was added to dissolve it and final volume was made up to 10 ml using mobile phase (Stock A; 1000 µg/ml). Various aliquots from stock A were prepared to give concentrations between 10-60 µg/ml, which were further diluted using mobile phase.

Sample preparation (Assay):

To carry out the sample solution, 20 tablets were taken and weighed individually, finally grounded them to fine powder. An appropriate portion of this powder, equivalent to 50 mg of TDF was weighed and

placed in a 50 ml volumetric flask, dissolving it in the mobile phase. This solution was sonicated for 10 min to dissolve and remove the entire active from the tablet and the solution was filtered if necessary. From the above 0.3 ml of aliquot was taken and transferred to volumetric flask of 10 ml capacity and volume was made up to the mark with the diluents to give final concentration 30 µg/ml.

Results and Discussion

Preliminary trials have been done to develop cost effective simple accurate and precise method. The method has been tried with various mobile phase mixtures of methanol with water, methanol with buffer, and acetonitrile with water and buffer which doesn't show good elution of peak and broad peaks were eluted. The mobile phase composition of orthophosphoric acid: acetonitrile: methanol in the ratio of 40:50:10% v/v with 0.9 mL/min flow rate was found to be satisfactory and gave good symmetric peak for TDF. The column used for this method was Phenomenex Luna C18 (250 x 4.6 mm, 5 µm). The detection was carried at 254 nm using PDA detector which shows good response factor and linearity with retention time as 2.21 min. The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can originate, by injecting blank preparation (single injection) and standard preparation (six replicates), record the chromatograms to evaluate the system suitability parameters like retention time, tailing factor, theoretical Plates and % RSD for peak area of all six replicates. The system suitability data is reported in Table No. 1 and blank chromatogram was shown in Fig. 2. The proposed method was validated for analytical procedures Q2 (R1) and for Stability Testing Q1A (R2). The total summary of all validation parameters was reported in Table No. 10.

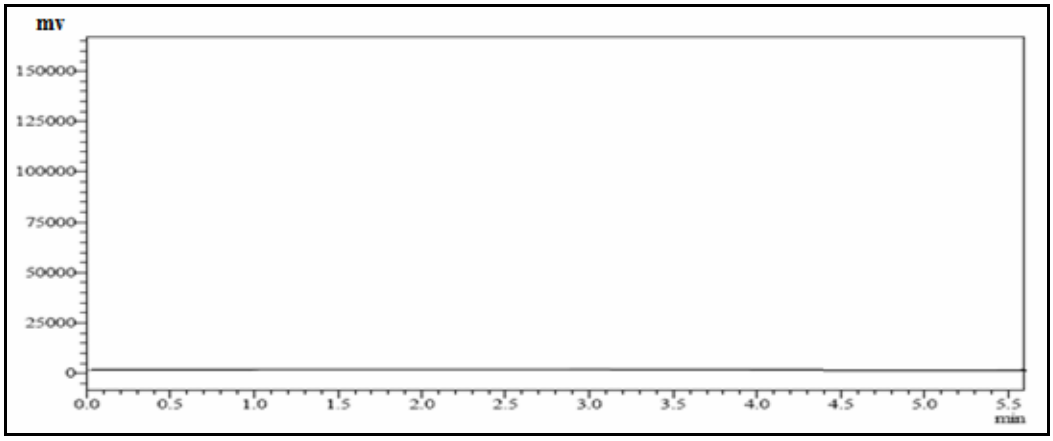


Figure 2: Blank chromatogram

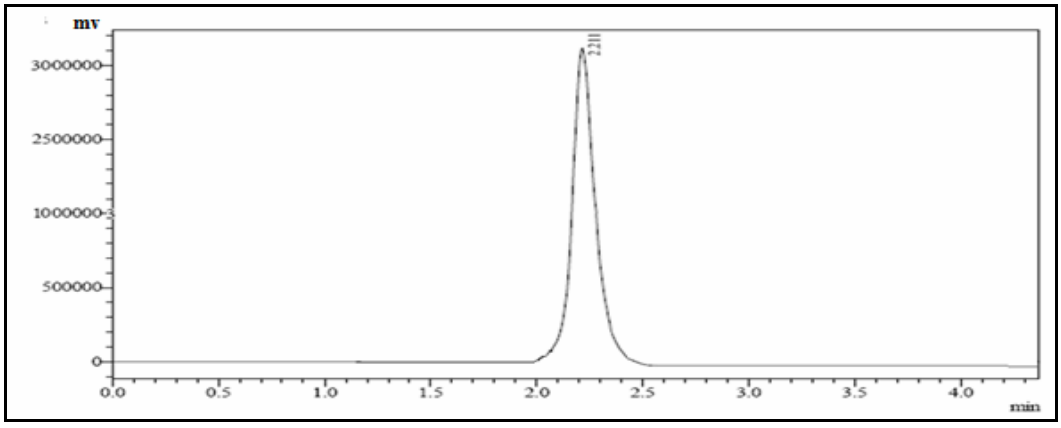


Figure 3 : Typical Chromatogram of Tenofovir Disoproxil Fumarate (Pure)

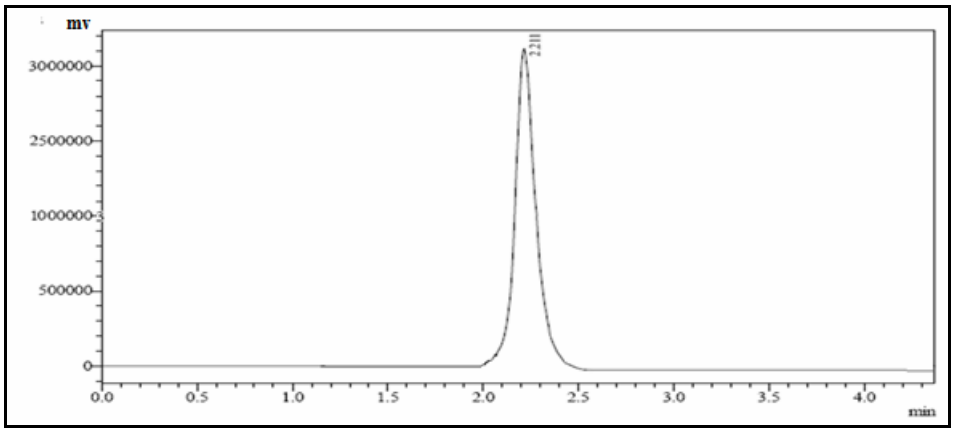


Figure 4: Typical Chromatogram of Tablet formulation

Table No: 1 – System Suitability Parameters

S.No	Parameter	Chromatographic Condition
1.	Instrument	Shimazdu LC 20 AD
2.	Column	C18 phenomenex Luna (250 x 4.6 mm x 5 µm)
3.	Mobile Phase	Orthophosphoric Acid: Acetonitrile: Methanol (40:50:10% v/v) (pH Adjusted to 3.0)
4.	Detector	PDA
5.	Detection Wavelength	254 nm
6.	Flow rate	0.9 ml/min
7.	Injection Mode & Volume	Manual & 20 µl
8.	Column Temperature	Ambient
9.	Retention time	2.211 min
10.	Theoretical Plates	3685
11.	Tailing Factor	1.21

Method validation:

Linearity:

From the tablet sample solution various aliquots were pipetted out to prepare six standard solutions covering the concentration range of 10-60 µg/ml. Calibration curve showing concentration versus peak area was plotted and the data obtained was subjected to regression analysis using the least square method. The calibration data is presented Table No. 2 and calibration curve is shown in Fig. 5.

Table No: 2 – Calibration Data for Tenofovir disoproxil Fumarate

S.No	Concentration (µg/ml)	Peak Area
1	10	775141
2	20	1569320
3	30	2389310
4	40	3101096
5	50	3914394
6	60	4603658

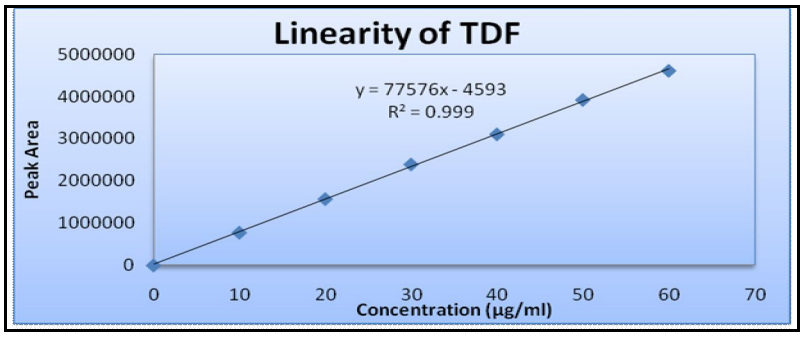


Fig. 5 Calibration curve of Tenofovir Disoproxil Fumarate

Precision (Repeatability) & Intermediate precision (Reproducibility):

The precision of the instrument was checked by repeated injections of a concentration and measurement of peak areas and retention times of solutions (n = 6) for TDF without changing the parameter of the proposed chromatographic method. The method was also validated for both Intraday and Interday precision, by injecting replicate injections six times with similar concentrations on the same day and on six different days. The result of repeatability and reproducibility was reported in terms of % RSD shown in Table -3 & 4.

Table No: 3 Method Precision (Repeatability)

S.No.	Peak Area	% Assay
Injection 1	2395179	100.04
Injection 2	2386138	99.66
Injection 3	2378205	99.33
Injection 4	2389987	99.82
Injection 5	2395182	100.04
Injection 6	2391169	99.87
Mean	2389310	99.80
Std Dev	5862.626	0.244
% RSD	0.245	0.245

Table No: 4 Intraday Precision and Interday Precision

S.No	Precision studies	
	Intraday precision (Peak area)	Interday precision (Peak area)
Sample (n = 6)	2389310	2372965.5
Std Dev	5862.626	12256.460
% RSD	0.245	0.516

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the formula

LOD = 3.3 σ /S

LOQ =10 σ /S

The slope S may be estimated from the calibration curve of the analyte and standard deviation from peak responses. Results are shown in Table – 5.

Table No: 5 LOD and LOQ values

S.No	Parameter	LOD & LOQ (µg/ml)
1	Limit of Detection (LOD)	0.249
2	Limit of Quantitation (LOQ)	0.755

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of TDF by the standard addition method. Known amounts of standard solutions of TDF were added at 50%, 100% and 150% concentration to pre quantified sample solutions of TDF (15, 30, 45 µg/ml) and the amount of drug recovered was estimated. Results are shown in Table –6.

Table No: 6 Recovery Data

S.No	Spike level (%)	Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% Mean Recovery
1	50	1189668	7.5	7.468	99.58	100.19
2	100	2379961	30.0	29.882	99.60	
3	150	3634528	67.5	68.452	101.41	

Specificity:

In an assay, expression of specificity requires that it can be shown that the procedure is unaffected by the presence of excipients. In fact, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and representing that the assay results are unaffected by the presence of these discrete materials. There should be no interference of the diluents, placebo at retention time of drug substances. The assay results were depicted in table no. 7.

Table No: 7 Assay results

S.No.	Peak Area	% Assay
Injection 1	2395179	100.04
Injection 2	2386138	99.66
Injection 3	2378205	99.33
Injection 4	2389987	99.82
Injection 5	2395182	100.04
Injection 6	2391169	99.87
Mean		99.80
Std Dev		0.244
% RSD		0.245

Robustness:

In this method, wavelength (± 2 nm) and flow rate (± 0.1 mL/min) were slightly changed to lower and higher sides of the actual values to find if the change the peak area and retention time were within limits. Evaluate the system suitability values as required by the test method for both the altered parameters. Results were shown in Table – 8.

Table No: 8 Robustness study

S. No	Robust condition	Parameter change	Peak Area	Retention Time (min)
1	Wave length ± 2 nm	252 nm	2321648	2.120
2		*254 nm	2389310	2.211
3		256 nm	2298957	2.114
4	Flow rate ± 0.1 mL/min	0.8 mL/min	2198321	2.374
5		*0.9 mL/min	2389310	2.211
6		1.0 mL/min	2208694	1.914

Forced Degradation Study:

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/ml of TDF and refluxed for 30 min at 70 ºC. All samples were then diluted in mobile phase to give a final concentration of 30 µg/ml and filtered if necessary before injection.

It was performed by treating the drug solution of TDF (1 mg/mL) with 1 N hydrochloric acid (HCl), 1 N Sodium hydroxide, 30 % v/v Hydrogen peroxide and to thermal studies individually for 24 hrs in a thermostat maintained at 70 ºC, cooled and then the stressed sample was neutralized and diluted with mobile phase as per the requirement before injecting in to the HPLC system. The drug solution (1 mg/mL) for photo stability testing was exposed to UV light chamber for 24 hours and then analyzed.

Table No: 9 Forced Degradation Data

S. No	Type of degradation	TDF		
		Peak Area	% Purity	% Degradation
1	Acid (1M HCl)	1502404	62.75474	37.245
2	Base (1M NaOH)	1872764	78.2245	21.7755
3	Peroxide (30 % v/v H_2O_2)	1827124	76.3181	23.6819
4	Thermal	2293498	95.79838	4.201625
5	Photolytic	2315395	96.713	3.286997

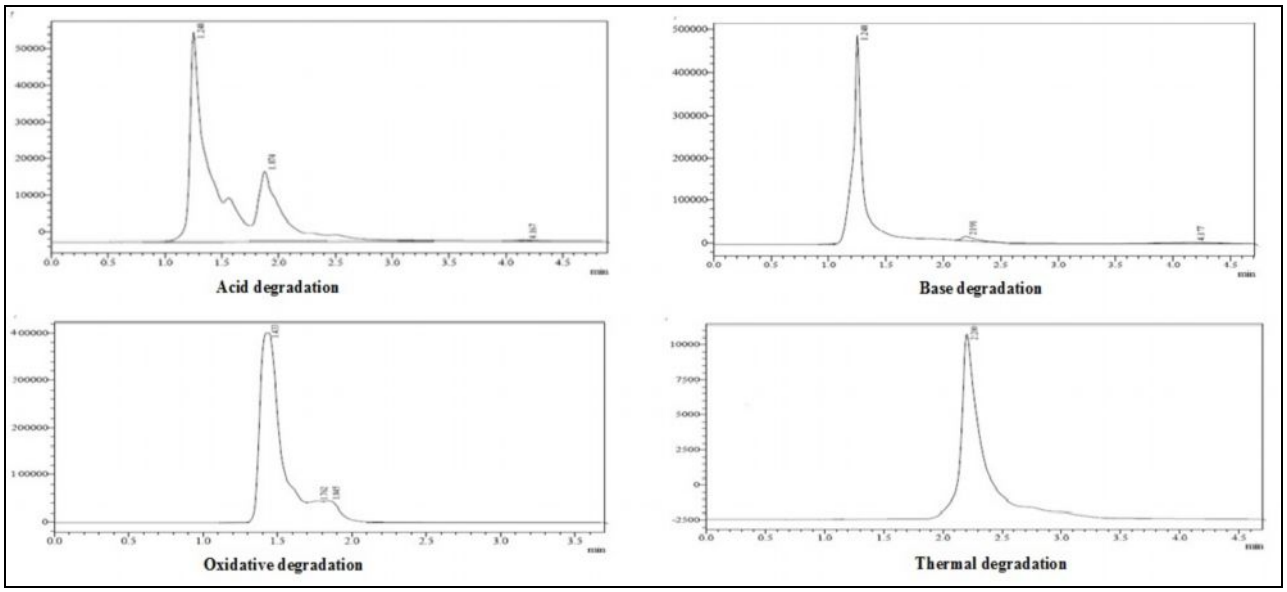


Figure 6: Degradation studies (Acid, Base, Oxidative and Thermal)

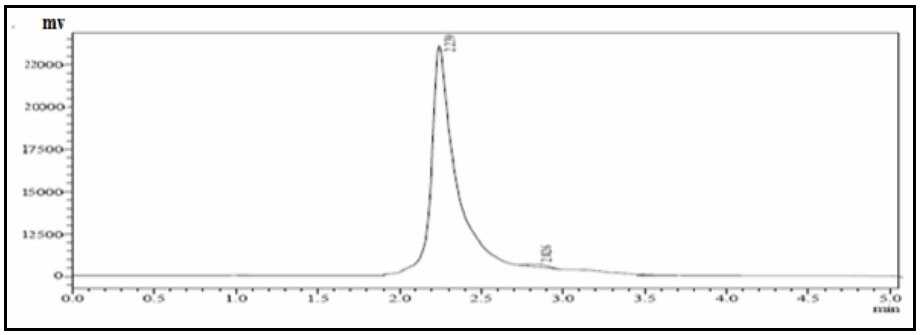


Figure 7: Photolytic degradation

Table No: 10 Summary of validation parameters

S.No	Validation parameter	Results
1	Linearity	10-60 (µg/ml)
2	Regression equation (y)	Y=77576x – 4593
3	Regression coefficient (r^2)	0.999
4	Limit of detection (µg/ml)	0.249
5	Limit of Quantitation (µg/ml)	0.755
6	Accuracy (Mean % recovery)	100.19
7	Precision	
	Intra-day precision (%RSD)	0.245
	Inter-day precision (%RSD)	0.516
8	Mean Assay (% Purity)	99.80
9	Tailing factor	1.21
10	Number of theoretical plates	3685
11	Retention time	2.21 min

5. Conclusion:

The proposed study describes a novel RP-HPLC method for the estimation of Tenofovir Disoproxil Fumarate (TDF) in bulk using simple mobile phase. The method gives good resolution for the compound with a short analysis time (<3 min). The method was validated and found to be simple, rapid, selective, accurate and precise when compared to the reported methods. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. The method is also cost effective with respect to solvent consumption. Therefore, the proposed method can be used for routine analysis of TDF in its dosage form.

Acknowledgement

I thank Ranbaxy laboratories Pvt. Ltd, Ahemedabad for providing gift sample of Tenofovir. I also feel privilege to thank Director, Jawaharlal Nehru Technological University Anantapur- Oil Technological & Pharmaceutical Research Institute for support and providing necessary facilities to carry out this work.

References

1. www.drugbank.ca/drugs/DB00300 (Tenofovir Disproxil Fumarate)

2. Syed sajjad hussen, Premnath Shenoy, Udupa, Muddu Krishna. Development and validation of stability indicating RP-HPLC method for Tenofovir nanoparticle formulation. Int J Pharm Pharm Sci. 2013, 5(2):245-248.
3. Sivaram Manavarthi, Gurmeet Singh Chhabra. Stability indicating RP-HPLC method development and validation of Tenofovir in Bulk and Pharmaceutical formulation, Der Pharma Chemica. 2014, 6 (2):401-409.
4. Narendra Devanaboyina, Satyanarayana T and Ganga Rao B. HPLC Method Development And Validation For Simultaneous Estimation of Tenofovir And Emtricitabine in Combined Pharmaceutical Dosage Form. Int. J Res. Pharm Biomed. Sci. 2012, 3(1):362-367.
5. Gurupadayya BM, Trinath MN, Shiva Prasad, Shilpa Kache. Development and validation of stability indicating method for simultaneous estimation of Emtricitabine, Tenofovir. Am. J. pharm tech res. 2013, 3(6):280-293.
6. Anusha N, Vanaja P, Giri Prasad V S. Development and validation of a RP-HPLC method for simultaneous estimation of Lamivudine, Tenofovir Disoproxil Fumarate And Efavirenz In a combined tablet dosage form, Int J Pharm Pharm Sci. 2013, 5(3):116-121.
7. Deepthi Komaroju , Nagarjuna Reddy G, Dhanalakshmi K. Method Development and Validation for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Pure and Tablet Dosage Form by using RP-HPLC. Int. J Pharm Res. Review. 2013, 2(10):1-11.
8. Arun ramaswamy, Anton Smith Arul Gnana Dhas. Development and Validation of Analytical Method for Quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC, Arab J Chem. 2014,1-7.
9. Dara s bhavsar, Bhavini N. Patel, and Chhaganbhai N. Patel. RP-HPLC method for simultaneous estimation of Tenofovir Disoproxil Fumarate, Lamivudine, and Efavirenz in combined tablet dosage form. Pharm Methods. 2012, 3(2):73-78.
10. Honey Diana B, Shaik Khader Bibi and Sita Kumari K. New validated RP-HPLC method for simultaneous estimation of Lamivudine and Tenofovir Disoproxil Fumarate in tablets. Int J of Adv in pharmacy Ana. 2015, 5(1):10-13.
11. Anjaneyulu N. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Tenofovir Disproxil Fumerate in Combined Dosage Form. Asian J. biomed. Pharm. Sci. 2013, 3(23):7-11.
12. Budagam lavanya, Perumalla Hariprasad, Allumellu Venkatapraveen,Dudipala Prasanna lakshmi, Maram Ramireddy**.** Simultaneous estimation of Emtricitabine and Tenofovir Disproxil Fumerate by HPLC method. Der Pharmacia Lettre. 2012, 4 (6):1855-1862.
13. Atul P. Sherje, Vaishali Londhe, Stability Indicating HPLC method for Determination of Paliperidone in Bulk, International Journal of PharmTech Research, 2015, 8(8), 157-163.
14. Rizwan S.H, Girija Sastry. V, Imad. Q, Stability Indicating Method Development and Validation of Bosentan in Bulk Drug and Formulation by Rp-Hplc Method, International Journal of PharmTech Research, 2015, 8(4), 569-579
15. Somsubhra Ghosh, Venkatesh S, Ravikumar B. V. V, Development of Stability Indicating RP-HPLC Method and Validation for the Estimation of Vilazodone Hydrochloride, International Journal of PharmTech Research, 2014-2015, 7(1), 204-211.
16. Prateek Kumar Mishra, Savita Upadhyay, Avinash C. Tripathi, Shailendra K. Saraf, Stability Indicating HPLC-UV Method for Simultaneous Estimation of Pantoprazole, Domperidone and Drotaverine, International Journal of PharmTech Research, 2015, 8 (5), 912-923.
17. Muralee Krishna, Meghana Nadre, Aniruddha V. Sherikar, Ranjith Reddy, Stability Indicating Analytical Method Validation for determination of Related Substances by Rphplc for Phenytoin Sodium Inphenytoin Sodium Capsules, International Journal of PharmTech Research, 2015, 8(6),78-87.
18. Ashish. P. Gorle, Jayashri S. Shinde, Development and Validation of Stability Indicating Assay Method of Ofloxacin in Bulk and Pharmaceutical Dosage Form by RP-HPLC, International Journal of PharmTech Research, 2016, 9(4), 289-298.
19. Nerella M.Madhu, Sreeram.V, Nagendrakumar A.V.D, Reddy T.V, A Newer RP - HPLC Method for the Estimation of Isosorbide Dinitrate in Tablet Formulation, International Journal of Pharm Tech Research, 2014-2015, 7(1), 01-09.
20. Sridhar Goud, Garlapati Achaiah, Sivaramakrishna.V, Mayuri. P, Development and Validation of RP-LC Method for Lisinopril Dihydrate in Bulk and its Pharmaceutical Formulations, International Journal of PharmTech Research, 2015, 8(3), 448-452.
21. Uttam Prasad Panigrahy, Sunil Kumar Reddy.A, A novel validated RP-HPLC-DAD method for the

simultaneous estimation of Netupitant and Palonosetron in bulk and pharmaceutical dosage form with forced degradation studies, International Journal of ChemTech Research, 2015, 8(10), 317-337.
22. Pankaj Dangre, Vilas Sawale, Satish Meshram, Mahendra Gunde, Development and validation of RP-HPLC method for the Simultaneous Estimation of Eprosartan mesylate and chlorthalidone in Tablet Dosage Form, International Journal of PharmTech Research, 2015, 8(2), 163-168.
23. Sunil kumar. Y.V, Usenni Reddy Mallu, Kasi Viswanath I V, RP-HPLC Method Development and Cleaning Method Validation for the Analysis of Triclabendazole in Veterinary Pharmaceutical Dosage Forms, International Journal of ChemTech Research, 2016, 9(5), 507-520.
24. Pranesh Dwivedi, Savita Yadav, Janhavi Rao, Validated RP HPLC Method for the Determination of Related Substance of Oxcarbazepine an Antiepileptic Drug, International Journal of PharmTech Research, 2016, 9(3), 444-451.
25. Kalpana J, Himaja M, Validated LC Method for the Estimation of Hydralazine, Hydrochloride in Pharmaceutical Dosage Forms, International Journal of PharmTech Research, 2016, 9(1), 142-146.
26. International Conference on Harmonization (ICH) Guidelines. Q1A (R2), ICH Guidelines: Stability Testing of New Drug Substances and Products, Geneva, 2003.
27. International Conference on Harmonization (ICH) Guidelines.Q2 (R1), ICH Guidelines: Validation of analytical procedures: Text and methodology Geneva, 2005.
28. International Conference on Harmonization (ICH) GuidelinesQ2A, ICH Guidelines: Text on validation of analytical procedures: 1995.
29. LR Snyder, JJ Kirkland, JI Glajch; Practical HPLC Method Development. A Wiley-Inter science Publication, 1997, 2, 21-173,685-713.

*****