ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Novel RP-UPLC Method Development and Validation for Simultaneous Quantification of Emtricitabine, Tenofovir and Efavirenz in Bulk and Tablet Dosage Forms

Sathish Kumar Konidala*, Ramu Samineni, Yamarthi Venkateswara Rao, Jithendra Chimakurthy, Chaitanya Sucharitha Kolakaluri, Vishali Korrapati

Faculty of Pharmacy, Department of Pharmaceutical Sciences, Vignan's Foundation for Science, Technology and Research, Vadlamudi, Guntur, Andhra Pradesh-522213.

*Corresponding Author E-mail: **sathishkonidala@gmail.com**

ABSTRACT:

Objective: The objective of this work is to develop a precise, accurate and validated reverse phase ultra-performance liquid chromatographic technique for effective simultaneous determination of Emtricitabine, Tenofovir and Efavirenz in bulk and tablet formulation. **Method:** Separation of the selected drugs was optimized after several trials including changing mobile phase and its composition, stationary phase, flow rate, column temperature, etc. Finally the separation of drugs was achieved on BEH C_{18} column using a mixture of methanol and phosphate buffer having pH 3.5 in the ratio of 65:35 v/v as mobile phase with flow rate of 0.3 ml/min and the analytes were detected at a wavelength of 260 nm. **Results:** The developed method was validated by determining the parameters like linearity, system suitability, recovery, precision, specificity, robustness, ruggedness, LOD, and LOQ as per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The system suitability parameters were within the limits, retention times (Rt) for Emtricitabine, Tenofovir and Efavirenz were found to be 0.432, 0.671, and 2.772 min respectively. The method showed linearity between the concentration range of 10-50 µg/ml for Emtricitabine (r^2= 0.9987), 15-75 µg/ml for Tenofovir (r^2= 0.9983) and 30-150 µg/ml for Efavirenz (r^2= 0.9982). The percentage recovery results at 50%, 100% and 150% of Emtricitabine, Tenofovir and Efavirenz were found to be in the range of 99.45 % - 100.15 %. Since there was no interference due to excipients and mobile phase, the method was found to be specific. The assay results of the combined tablet dosage form by the developed method were identified as in good agreement with the acceptance limit. **Conclusions:** The current method was proved to be effective for routine simultaneous determination of Emtricitabine, Tenofovir and Efavirenz in bulk and tablet formulation.

KEYWORDS: Reverse Phase Ultra-Performance Liquid chromatography (RP-UPLC), Validation, Emtricitabine, Tenofovir, Efavirenz, and Dosage Form.

INTRODUCTION:

Emtricitabine[1](EMT) (4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl) -1,3-oxathiolane-5-yl] -1,2-dihydropyrimidin-2-one; MF: $C_8H_{10}FN_3O_3S$; MW: 247.248 g/mol) is a reverse transcriptase inhibitor, highly soluble in water (112mg/ml), slightly soluble ethanol (3.5mg/ml), practically insoluble in dichloromethane, has the log P value 0.43 and pKa 2.65.

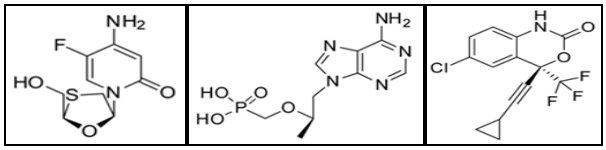
Tenofovir[2,3] (TEN) [(2*R*)-1-(6-aminopurin-9-yl)propan-2-yl]oxy methyl phosphonic acid; M.F.-$C_{19}H_{30}N_5O_{10}P$; M.Wt.-287.21 g/mol], is active against HIV-1 reverse transcriptase, it has solubility about 13.4 mg/ml in wateer, and <1 mg/ml in ethanol. It has the log P value 1.25 and pKa 2.65.

Efavirenz[4](EFA)[(4*S*)-6-chloro-4-(2-cyclopropylethanol) -4-(trifluoro-methyl) -2,4-dihydro-1*H*-3,1-benzoxazin -2-one; MF.-$C_{14}H_9ClF_3NO_2$; MW.-315.675 g/mol], it is a potent inhibitor of non-nucleoside reverse transcriptase, effectively used in human immunodeficiency virus (HIV) type 1 infections treatment, slightly soluble in

Received on 31.07.2020 Modified on 13.05.2021
Accepted on 11.09.2021
Research J. Pharm. and Tech. 2022; 15(7):3141-3146.
DOI: 10.52711/0974-360X.2022.00525

ethanol (20 mg/ml) and practically insoluble in water (<10 µg/ml), has the log P value 2.07 and pKa value 10.1.[5] The Chemical structures of EMT, TEN and EFA were shown in figure 1.

The selected drugs were currently used in combination as highly active antiretroviral therapy (HAAT) for effective treatment of HIV-1 infections[6].Few methods were reported in past for individual and simultaneous estimation of EMT, TEN and EFA through ultra-violet (UV) spectrophotoscopy[7-12], high performance liquid chromatography (HPLC)[12-28], ultra-performance liquid chromatography (UPLC)[6,29], high performance thin layer chromatography (HPTLC)[8,9] and liquid chromatography-mass spectrometry (LC-MS)[30-31] techniques, since the reported methods were time consuming, expensive in nature, so there is a need to develop a simple, rapid, accurate, selective, and validated chromatographic method for simultaneous estimation of EMT, TEN and EFA in pharmaceutical dosage form. The current research work elucidate the development and validation of simple, rapid, economical, accurate and selective RP-UPLC technique for simultaneous determination of EMT, TEN and EFA in bulk and tablet formulation, according to the International council for Harmonization of technical requirements for pharmaceuticals for human use (ICH) guidelines[32].



A B C

Fig. 1: Chemical structures of A-Emtricitabine (EMT), B-Tenofovir (TEN), C-Efavirenz (EFA)

MATERIALS AND METHODS:

Instrumentation:

The method was developed on Waters 2695 series (Empower software) UPLC System, (Milford, MA, USA), Semi micro balance (Sartorius ME235P, Germany) digital pH Meter (Thermo electron corporation orion 2 star, USA), Sonicator (Ultrasonic cleaner power sonic 420, Korea), Vacuum oven (Wadegati, Mumbai, India), Constant temperature water bath (Thermolab GMP, Mumbai, India) and 0.45 µm Nylon filter (Axivia, Haryana, India), 0.45µm PVDF filter (Rankem, Mumbai, India).

Chemicals and Reagents:

All the chemicals, reagents and solvents (HPLC grade) were procured from Merck Pvt. Ltd. (Mumbai), milliQ water (<20ppm) was produced in house by milliQ IQ7003 water plant (Merck, Mumbai, India), EMT, TEN and EFA active pharmaceutical ingredients were procured from Hetero drugs (Hyderabad, India) as gift samples, and the marketed formulation, Atripla (Gilead Sciences, US) (Labeled claim, EMT-200 mg, TEN-300 mg and EFA-600 mg) was purchased from the licensed pharmacy store.

Method Development:

The RP-UPLC method was developed through conducting number of trails in which the values chromatographic parameters like wavelength, mobile phase composition and ratio, flow rate, stationary phase, etc were altered to determine the effect of them on separation and identification of selected drugs, finally the chromatographic parameters were optimized as follows.

Selection of wavelength:

The wavelength was optimized by scanning the 10 µg/ml concentrated solutions prepared by using diluent of selected drugs through PDA detector of UPLC system and UV-Visible spectrophotometer in UV region. The optimum identification was achieved at 260 nm, hence the wavelength 260 nm was used throughout the method development and validation.

Selection of chromatographic conditions:

Preliminary trials were conducted by injecting the diluted standard solution of analyte to get the optimized chromatographic conditions which favor the optimum ionization of selected drugs in suitable mobile phase by changing the various solvents and solvent compositions, The effective separation and symmetrical peak shapes were achieved by changing chemical nature of columns (Octyl, Octadecyl) with different types, and manufacturers, the flow rate was adjusted to get the proper peak resolution and shape.

Optimized Chromatographic conditions:

Chromatographic separations of the selected drugs were performed on Waters corporation Acquity UPLC BEH C_{18} (2.1 x 50mm, 1.7µm) column with a mobile phase consisting methanol: 0.018 M phosphate buffer having pH 3.5 in the ratio of 65:35 v/v, pumped at a flow rate of 0.3 ml/min and detection wavelength of 260nm was set at room temperature, the injection volume of 6 µl and 5 min of run time for effective simultaneous separation of selected drugs were followed.

Preparation of mobile phase:

1.25 gm of potassium dihydrogen orthophosphate was accurately weighed, transferred to 500ml volumetric flask, dissolved with milliQ water, made up the final volume with milliQ water, and adjusted pH to 3.5 with ortho phosphoric acid. Mix 350 ml of the above prepared buffer with HPLC grade methanol, degassed in the mixture by ultrasonication, and was filtered through 0.45 µm Membrane filter using vacuum filtration assembly.

Preparation of standard stock solution:

10 mg of EMT, 15 mg of TEN and 30 mg of EFA were accurately weighted and transferred to 10ml of clean and dry flask, dissolved the contents with 7 ml of diluentby

the aid of sonication and the final volume was made up to the mark with diluent.

Preparation of sample solution:
The weight of 10 tablets (Atripla) containing selected drugs was determined and powdered in a glass mortar. The quantity of powder equivalent to 10 mg of EMT, 15 mg of TEN and 30 mg of EFA was transferred into 10 ml capacity clean and dry volumetric flask, add 7 ml diluent, sonicated for about 15 minutes in bath sonicator by shaking at intervals of five minutes each and was diluted up to the mark with diluent. Filter the sample solution through 0.45µm nylon membrane filter paper.

Assay of formulation:
0.2 ml of standard stock solution and sample stock solutions were transferred separately into 10 ml volumetric flasks, diluted and made up to the final volume with diluent, the resulting solutions were sonicated for about 15 min, and filtered through 0.45 mm filter. Standard and sample solutions were injected separately into the chromatographic system in triplicate.

Method Validation:
Validation of the optimized method parameters includes linearity, system suitability, accuracy, precision, ruggedness, robustness, limit of detection and limit of quantification was done according to ICH guidelines.[32]

System suitability:
The test was carried out by six replicate injections of working stock solution of formulation having 20µg/ml of EMT, 30µg/ml TEN and 60µg/ml of EFA into the UPLC system as per test procedure. The system suitability parameters were evaluated from chromatograms obtained, calculated the % RSD of retention times, tailing factor, theoretical plates and peak areas. Acceptance criteria: the % RSD for the retention times of principal peak from 3 replicate injections of each standard solution should be not more than 2.0 %, the number of theoretical plates (N) for the EMT, TEN and EFA peaks should be not less than 2000, the Tailing factor (T) for the EMT, TEN and EFA peaks should be not more than 2.0.

Linearity:
The linearity of the developed method was determined by preparing the aliquots of standard drug solutions with concentrations of 10-50 µg/ml of EMT 15-75 µg/ml of TEN and 30-150 µg/ml of and EFA from the standard stock solution, the linearity parameters like regression value (r^2), slope, y-intercept, were evaluated by plotting the calibration curve taking concentration in µg/ml on x-axis, and peak area on y-axis for linearity concentrations. The correlation coefficient (r^2) should be not less than 0.9990, % RSD of peak areas for replicated individual linearity concentrations should be not more than 2.0 % to accept the linearity of the method.

Precision:
Repeatability:
The intermediate concentration in linearity range of selected drug solution was loaded into the chromatographic system in five replications, the % RSD of peak area and Rt obtained from chromatograms was determined which should not be more than 2%.

Intermediate Precision/Ruggedness:
The intermediate precision was determined by injecting five replicate injections of intermediated concentrations of selected drugs into chromatographic system by different analyst on different days, The intermediate concentration in linearity range of selected drug solution was loaded into the chromatographic system in five replications, the % SD of peak area and Rt obtained from chromatograms was determined which should not be more than 2%.

Accuracy (Standard addition method):
Accuracy and specificity studies were performed by triplicate injection of the aliquots (50, 100, and 150 %). The aliquots (50, 100, and 150 %) were prepared by adding 0.1 ml, 0.2 ml, and 0.3 ml of standard stock solution respectively into three 10 ml volumetric flasks, add 0.2 ml of of formulation stock solution to each (fixed concentrations: 20µg/ml of EMT, 30 µg/ml of TEN and 60 µg/m of EFA) and make up the final volume with diluent. The %RSD of the amount recovered was determined. Acceptance criteria: the mean % recovery of EMT, TEN and EFA each spike level should be not less than 98.0 % and not more than 102.0 %.

Robustness:
The robustness of the proposed method was determined by triplicate injection of working stock solution from homogenous lots by differing physical parameters like flow rate and mobile phase composition, temperature variations which may differ but the responses were still within the specified limits of the assay. Acceptance criteria: % Relative standard deviation of peak areas and Rt should not be more than 2.0 %.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
The LOD and LOQ concentrations were determined to ascertain the sensitivity of the developed method by using the following formulae.

$$\text{Limit of detection} = (3.3 \times SD)/S$$

$$\text{Limit of Quantification} = (10 \times SD)/S$$

Where,
SD = standard deviation of y-intercepts
S = slope

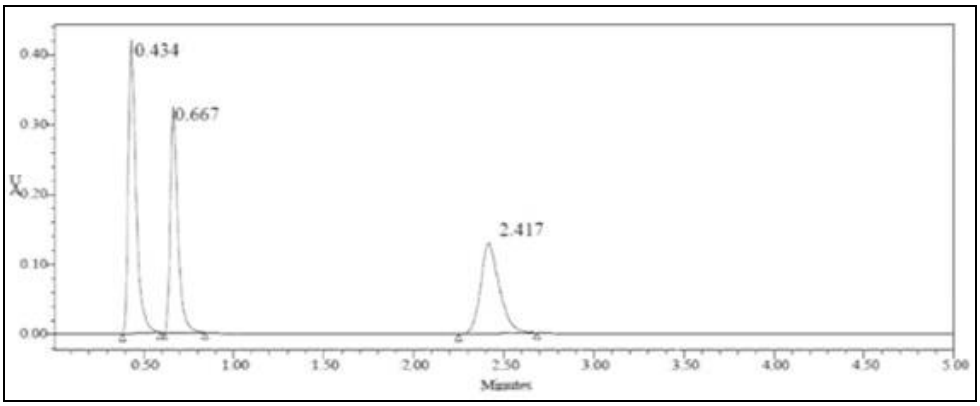


Fig. 2: UPLC Chromatogram of simultaneous separation of EMT, TEN and EFA sample (ATRIPLA)

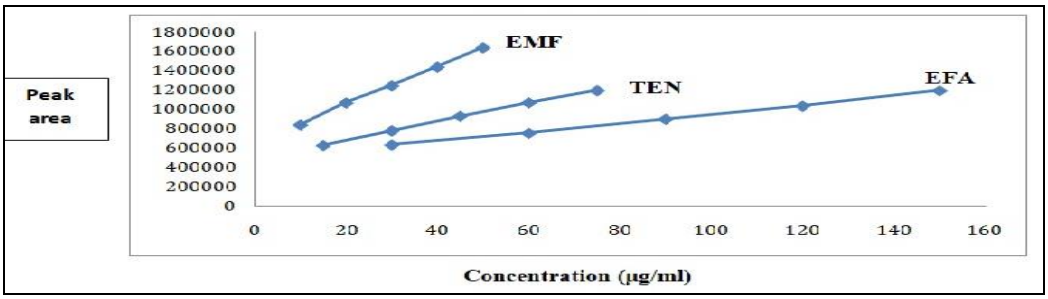


Fig. 3: Calibration curves of EMF, TEN and EFA

Acceptance Criteria: S/N Ratio of LOD concentration should be equal to 3 times of S/N ratio of blank solution, S/N Ratio of LOQ concentration should be equal to 10 times of S/N ratio of blank solution.

RESULTS AND DISCUSSION:

Method development and optimization:

The current research work enumerates the validated RP-UPLC method development for simultaneous determination of EMT, TEN and EFA in tablet formulation. The effective separation and good peak symmetry was achieved by using mobile phase methanol: 0.018M phosphate having buffer pH 3.5 [65:35, v/v] as mobile phase, on Acquity BEH C18 (4.6 x 50mm), analytical column under isocratic conditions.

System suitability:

The retention time (Rt) for EMT, TEN and EFA were found as 0.434 min, 0.667 min, and 2.417 min respectively with consistent reproducibility represented by %RSD shown in figure 2. The chromatographic parameters like USP Plate count, tailing factor, Resolution were found to be within the limits and given in table 1 which indicates the system suitability of the developed method.

Linearity:

The developed method showed the proportional relationship between peak area and concentration at different levels of standard drugs in the range of 10-50 µg/ml, 15-75 µg/ml, and 30-150 µg/ml with regression coefficients (r^2) 0.9987, 0.9983, and 0.9982 for EMT, TEN and EFA respectively. The linearity results were given in the table 2 and the linearity plots were shown in figure 3

Precision:

The developed method was provedas precise since the %RSD values for replicate (n=6) estimations of a same homology solution were within the limits. The intermediate precision which indicates the effect of deliberate changes in laboratory conditions or personal bias on replicate results (%RSD) were found to be less than 2%**.** The precision results were given in table 3.

Accuracy:

The accuracy study was performed (to know the degree of agreement or closeness between the estimated value and true value) by standard drug addition method, which the known concentration of standard drug was spiked to the pre-analyzed sample solution at different levels (50%, 100% and 150 %) within the linearity concentrations. The quantities recovered of EMT, TEN and EFA were within the limits (98 to 102 %) indicating good accuracy of the method. The RSD values of recovery for EMT, TNF and EFZ are 0.351, 0.306, and 0.200 respectively were found to be less than 2%.

Robustness:

Robustness of the method was determined by injecting the sample with deliberate changes in one or more chromatographic method conditions.

Table 1: System suitability Parameters

S. No.	Parameter	EMT		TEN		EFA	
		Mean±SD	**%RSD**	**Mean±SD**	**%RSD**	**Mean±SD**	**%RSD**
1	Rt (min)	0.432±0.002	0.462	0.666±0.005	0.307	2.405±0.044	1.856
2	Peak area	1251360±3850.68	0.836	937364.7±5374.93	0.573	929167.7±7598.933	0.817
3	USP Plate count	2487.333±1.527	0.061	2262±18.520	0.818	2556.333±43.981	43.981
4	Tailing Factor	1.613±0.030	1.893	1.483±0.023	1.556	1.243±0.011	0.928
5	Resolution	**3.060**			**13.113**		

Table 2: Linearity results for EMT, TEN and EFA

Linearity level	EMT		TEN		EFA	
	Con. (µg/ml)	**Peak area**	**Con. (µg/ml)**	**Area**	**Con.(µg/ml)**	**Area**
I	10	839286	15	626221	30	631737
II	20	1067774	30	778750	60	753615
III	30	1246474	45	931447	90	899796
IV	40	1439994	60	1070162	120	1035191
V	50	1639065	75	1196060	150	1194356
Correlation coefficient		0.9987		0.9983		0.9982

Table 3: Repeatability values for EMT, TEN and EFA

Precision Type		Peak area					
		EMT		TEN		EFA	
		(Mean±SD)	**%RSD**	**(Mean±SD)**	**%RSD**	**(Mean±SD)**	**%RSD**
Repeatability		1246389± 2965.62	0.23793	929858.6±4865.16	0.5232	945423.4±7200.575	0.761
Intermediate	Analyst 1	1233318±3061.06	0.2481	913200.4±2621.886	0.287	911687.4±2432.859	0.2668
	Analyst 2	1246389±2965.62	0.23793	929858.6±4865.16	0.5232	945423.4± 7200.575	0.761

Table 4: Robustness Results

Parameter	Change in flow rate (ml/min)	EMT		TEN		EFA	
		(Mean±SD)	**%RSD**	**(Mean±SD)**	**%RSD**	**(Mean±SD)**	**%RSD**
Rt	0.2	0.513 ± 0.002	0.435	0.791±0.004	0.546	2.848±0.050	1.761
	0.3	0.431±0.002	0.599	0.658±0.002	0.346	2.311±0.016	0.703
	0.4	0.368 ±0.003	1.016	0.562±0.002	0.446	1.938±0.004	0.210
USP Plate count	0.2	2511±1.015	0.392	2279±0.093	1.687	2346±1.18	0.482
	0.3	2490±0.037	0.078	2268±0.738	0.937	2556±0.925	0.037
	0.4	2484±1.051	1.028	2185±0.345	0.884	2096±0.738	0.633
USP tailing	0.2	1.16±0.058	0.726	1.27±0.188	0.285	1.28±0.523	0.428
	0.3	1.26±1.072	1.127	1.28±0.047	0.736	1.24±1.147	1.025
	0.4	1.32±0.829	0.698	1.28±1.027	1.004	1.27±0.742	0.578

The deliberate changes in the mobile phase flow rates (±0.2, ±0.3, ±0.4 ml/min) and in composition of organic phase (±0.5) in mobile phase, the results indicated that there is no significant effect on the results in terms of %RSD values (were found to be less than 2%) represented in table 4.

LOD and LOQ:

LOD & LOQ were calculated by substituting the standard deviation of y-intercepts of replicated calibration curves and slop of the calibration curve into respective formula. LOD and LOQ of EMT, TNF and EFZ were found to be 2.95, 3.02, 2.93µg/ml and 9.97, 10.13, 9.95µg/ml respectively.

DISCUSSION:

The aim in developing the UPLC method was to achieve simultaneous separation and estimation of three drugs in tablet dosage under common conditions that are applicable for routine quality control, research and development of these drugs in ordinary laboratories. Recently the RP-UPLC method development for the determination of drugs has received more attention because of their speed, sensitivity, resolution, less solvent consumption, cost effectiveness and more productivity which is important in the quality control of drugs and drug products. This work was intended to develop a precise, less time consuming, economical and a rapid method in reverse-phase UPLC separation combined with PDA detection for simultaneous estimation in bulk samples and in dosage formulations. The developed method was rapid for simultaneous estimation of EMT, TEN and EFA as it elutes the drugs with retention time (Rt) values 0.432, 0.666, and 2.405 min respectively than the previously existed work for simultaneous estimation of EMT, TEN and EFA[33] indicated that the long Rt values of EMT, TEN and EFA was 0.6, 1.88 and 3.23 minute respectively. Moreover, the LOD and LOQ values indicated that the method was more sensitive, %RSD values indicated that the method was précised, Accurate, and robust in nature.

CONCLUSION:

Till date most RP-UPLC reported methods were simultaneously estimated as the EMT, TEN and EFA with high retention times. Hence attempts were made towards the development of a method which should separate the compounds with good resolution and less

retention times. Different logical modifications were tried to get good separated symmetric peaks with less retention times this is achieved by changing the mobile phase composition. Results indicated that the developed and optimized method for simultaneous estimation of EMT, TEN and EFA in tablet dosage from is rapid, simple, accurate, precise, and robust in nature, this method can be utilized routine simultaneous estimation of EMT, TEN and EFA drugs in bulk and pharmaceutical dosage forms.

REFERENCES:

1. https://www.rxlist.com/emtriva-drug.htm#description(Accessed on 28thjune 2020)
2. https://pubchem.ncbi.nlm.nih.gov/compound/Tenofovir(Accessed on 28thjune 2020)
3. https://www.rxlist.com/viread-drug.htm(Accessed on 30thjune 2020)
4. Cristofoletti R, Nair A, Abrahamsson B, Groot DW, Kopp S, Langguth P, Polli JE, Shah VP, Dressman JB. Biowaiver monographs for immediate release solid oral dosage forms,efavirenz, Journal of pharmaceutical sciences. (2013)102(2)318-29.
5. Jacob J, Nadig S. A UPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and efavirenz in pharmaceutical dosage forms. Research Journal of Pharmacy and Technology. (2017) 10(12)4463-6.
6. El Barkil M, Gagnieu MC, Guitton J. Relevance of a combined UV and single mass spectrometry detection for the determination of tenofovir in human plasma by HPLC in therapeutic drug monitoring. Journal of Chromatography B, (2007)854(1-2)192-7.
7. Anbazhagan S, Indumathy N, Shanmugapandiyan 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) 39(3-4)801-4.
8. Shewiyo DH, Kaale E, Ugullum C, Sigonda MN, Risha PG, Dejaegher B, Smeyers–Verbeke J, Vander Heyden 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) 54(3)445-50.
9. Shelke A, Shinde M, Mogal R, Sable R, Jadhav A. Application of UV Spectrophotometric methods for simultaneous estimation of Emtricitabine and Tenofoviralafenamide fumarate in bulk. Asian Journal of Pharmacy and Technology. 2018;8(2):103-7.
10. Rao DS, Balaji M, Reddy SH, Ramu S, Rao KK, Krishna GR. Development and UV-spectrophotometric Methods for Quantitative Estimation of Tenofovirdisproxilfumarate in Bulk and Pharmaceutical Dosage Form. Asian Journal of Research in Chemistry. 2012;5(3):422-4.
11. Vidyadhara S, Sasidhar RL, Rao BV, Kumari PR. Simultaneous UV Spectrophotometric Method for the Determination of Tenofovir, Efavirenz and Lamivudine in Bulk and Combined Dosage Form. Asian Journal of Pharmaceutical Analysis. 2016;6(4):253-8.
12. Montgomery ER, Edmanson AL, Cook SC, Hovsepian PK. Development and validation of a reverse-phase HPLC method for analysis of efavirenz and its related substances in the drug substance and in a capsule formulation, Journal of pharmaceutical and biomedical analysis. 200125(2)267-84.
13. Kappelhoff BS, Rosing H, Huitema AD, Beijnen JH. Simple and rapid method for the simultaneous determination of the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine in human plasma using liquid chromatography, Journal of Chromatography B. 2003 Jul 25;792(2):353-62.
14. Jayaseelan S, Ganesh S, Rajasekar M, Sekar V, Perumal P. A new analytical method development and validation for the simultaneous estimation of lamivudine and stavudine in tablet dosage form by RP-HPLC method. Int J Pharm Tech Res. ((2010) 2(2)1539-42.
15. Jayakar B, Kumar M, Saravanan C, Kumudhavalli MV. Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine. J Chem Pharm Res. (2010)2(1)478-81.
16. Sharma R, Gupta P. Simultaneous quantification and validation of emtricitabine and tenofovirdisoproxil fumarate in a tablet dosage form. Eurasian journal of analytical chemistry. (2009) 4(3)276-84.
17. Jayaseelan S, Suresh S, Sathishkumar G, Sekar V, Perumal P. Bioanalytical method development and validation of Lamivudine by RP-HPLC method. Int J Chem Tech Res. (2010) 2163-7.
18. Rao BU, Nikalje AP. Stability-indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. African journal of Pharmacy and Pharmacology. (2009) Dec 313(12)643-50.
19. Malipatil SM, Nandedkar MA. Determination of TenofovirDisoproxilFumarate by a Sensitive Simple Isocratic RP-HPLC Method, Journal of Indian Council of Chemists. (2009) 26(1):67-9.
20. Mandloi DK, Tyagi PK, Rai VK, Dey S, Ashada RK, Mohanraj P. Method development and validation of RP-HPLC in the application of in vitro dissolution study of lamivudine in bulk drug and tablet formulation, J Chem Pharm Res. (2009) 1286-96.
21. Sarat M, Krishna PM, Rambabu C. Development and Validation of RP-UPLC Method for Simultaneous Estimation of AbacavirSulphate and Lamivudine in Combined Tablet Dosage Form, International Journal of ChemTech Research. 2012 Jul3(4)939-44.
22. Patel BN, Suhagia BN, Patel CN. Development and Validation of HPLC Method for the Estimation of Emtricitabine in Capsule Dosage Form. Asian Journal of Research in Chemistry. 2010;3(4):869-71.
23. Rao BS, Nagaraju S, Kiran BV. Development, Validation and Stress Degradation Studies of Emtricitabine and TenofovirDisoproxilFumerate by High Performance Liquid Chromatography. Asian Journal of Research in Chemistry. 2013;6(10):936-44.
24. Sreelatha P, Devi BR. Development and Validation of Stability-indicating HPLC method for simultaneous estimation of Tenofovir, Emtricitabine and Efavirenz in fixed dose combination drug product. Asian Journal of Research in Chemistry. 2018;11(1):23-31.
25. Shaheen R, Rizwan SH. A Stability Indicating Method for estimating Emtricitabine and Tenofovirdisoproxil fumarate simultaneously in Bulk and Combined dosage form by RP-HPLC. Asian Journal of Research in Chemistry. 2020;13(3):203-8.
26. Sundar BS, Balamuralikrishna K. Development and Validation of New Reversed Phase High Performance Liquid Chromatography Method for the Estimation of Efavirenz in Bulk and in Pharmaceutical Dosage Forms. Asian Journal of Research in Chemistry. 2011;4(4):555-7.
27. Raju NA, Begum S. Simultaneous RP-HPLC method for the estimation of the emtricitabine, tenofovirdisoproxilfumerate and efavirenz in tablet dosage forms. Research Journal of Pharmacy and Technology. 2008;1(4):522-5.
28. Jacob J, Nadig S. A UPLC method for simultaneous estimation of emtricitabine, tenofovirdisoproxilfumarate and efavirenz in pharmaceutical dosage forms. Research Journal of Pharmacy and Technology. 2017;10(12):4463-6.
29. Gomes NA, Vaidya VV, Pudage A, Joshi SS, Parekh SA. 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) Nov 448(3)918-26.
30. Prathipati PK, Mandal S, Destache CJ. LC–MS/MS method for the simultaneous determination of tenofovir, emtricitabine, elvitegravir and rilpivirine in dried blood spots. Biomedical Chromatography. (2018) Sep32(9) e4270.
31. US Food and Drug Administration, "ICH Guidelines on Validation of Analytical Procedures, Text and Methodology Q2 (R1)," Federal Register, FDA, Silver Spring, **(2005)**.
32. Jacob J, Nadig S. A UPLC method for simultaneous estimation of emtricitabine, tenofovirdisoproxilfumarate and efavirenz in pharmaceutical dosage forms. Research Journal of Pharmacy and Technology. (2017)10(12)4463-6.