Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Method Development and Validation for Tenofovir an Antiretroviral Drug in Plasma by LC-MS/MS Technique

Sarang SALUNKE [1], Sagar WANKHEDE [1] Sharad MEDHE [2*] Hemlata NIMJE [1] Samir RANJAN [2] Vikas KENDRE [1] Payal BHASKAR [1]

1 Department of Pharmaceutical Quality Assurance, Jayawant Shikshan Prasarak Mandal's Charak College of Pharmacy and Research, Wagholi, Pune - 412 207, Maharashtra, India.
2 10, B.A.T House, Plot No 985, 720, Lal Bahadur Shastri Road, Pune, Maharashtra 411030.
* Corresponding Author. E-mail: sharadmedhe@gmail.com (S.M.); Tel. +91-020-67335103.

Received: 02 August 2022 / Revised: 13 October 2022 / Accepted: 13 October 2022

ABSTRACT: Bioanalytical method development for Tenofovir (TFR) as an antiretroviral drug by LCMS Technique. A developed Bioanalytical analysis method for TFR can be used routinely in a commercial laboratory. All the solvents used were of HPLC grade. 4000 QTrap along with the Shimadzu LC 20AD LC System used to develop and validate the method. The LLOQ and LOQ for Tenofovir was found were 5ng/mL and 15ng/mL. The method was accurate (within ±15% of control) and precise (coefficient of variation ≤15%). Analytes were stable for five freeze/thaw cycles and up to 6 days at room temperature, whereas long-term at −20°C or at −80°C. For Precision study using QCs of the drug- 85%, 100% and 115% concentration of drug chosen and the levels M1QC (75ng/mL), MQC (300ng/mL) and HQC (600ng/mL) where the % CV were observed of ≤ 15%. In a Precision and Accuracy study (inter day and intraday), the % CV obtained for Tenofovir was observed ≤ 15%. Recovery studies for extracted samples with LQC (15ng/mL), MQC (300ng/mL) and HQC (600ng/mL) were 94.51%, 91.83% and 90.91% respectively. Stability was within 15% deviation. The results of System Suitability Test for TFR and Acyclovir (ACR) are an internal standard with observed %CV ≤ 2.0%. The aim of the study was to develop a method that could be used as an alternative to the existing Tenofovir indirect method. The existing method observes separating the parent drug from the metabolite in LCMS/MS. This method is a good alternative to the indirect methods currently in use.

KEYWORDS: Tenofovir; LCMS; Method Development; Validation.

1. INTRODUCTION

An estimated 2.1 million Indians were living with HIV in 2015, of which 0.9 million were on antiretroviral therapy [1]. Now, with the test and treat policy coming into existence [2] the number of people living with HIV and AIDS (PLHIV) coming under an ART regimen is expected to increase as every person confirmed, having an HIV-positive report is immediately started on antiretroviral therapy. As per the NACO guidelines, the first line recommended regimen for ART initiation is a tenofovir based regimen [Tenofovir 300mg, Lamivudine 300 mg and Efavirenz 600mg][1]. Tenofovir Disoproxil Fumarate along with Emtricitabine is not only indicated for treatment but also has proven its efficacy in pre-exposure prophylaxis where the drugs given to high-risk groups like FSW's, MSM along with barrier contraceptives to prevent transmission of infection [3].

Tenofovir Disoproxil Fumarate (TDF), ((2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methyl) phosphonic acid) belongs to a class of antiretroviral drugs known as nucleoside analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, a crucial virus enzyme in human immunodeficiency virus 1 (HIV-1). In this version of the drug, the two negative charges of the tenofovir phosphonic acid group are masked, thus enhancing oral absorption. It differs from the rest of the group in not only having a high polarity but also a phosphate group bonded to the alkyl side chain. Tenofovir disoproxil fumarate is a prodrug which undergoes hydrolysis in the intestine, hence tenofovir is absorbed into blood. Tenofovir gets phosphorylated intra-cellularly to form the active intracellular diphosphate [4]. This drug has a comparatively longer half-life of 17 hours, and the elimination of its active metabolite is 52 hours half-life, hence estimating the tenofovir levels in plasma could give us a better idea about the drug intake of the patient [5].

HIV is now a chronic manageable disease with the use of antiretroviral drugs and India is trying to achieve the 90:90:90 goal by 2020. These goals envisage that 90% of the HIV infected individuals should know

How to cite this article: Salunke S, Wankhede S, Medhe S, Nimje H, Ranjan S, Kendre V, Bhaskar P . Method Development and Validation for Tenofovir an Antiretroviral Drug in Plasma by LC-MS/MS Technique. J Res Pharm. 2023; 27(5): 1790-1798.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.463

their status. 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of these will achieve viral suppression [6]. To achieve the last 90-it is important to have adherence of more than 95%. Efficacy of pre-exposure prophylaxis also depends on adherence. Drug intake cannot be confirmed by pill counts and qualitative scales. The best method to confirm adherence is to measure the levels of tenofovir in plasma. Hence it was necessary to develop and validate a low-cost method which has a shorter run time.

2. RESULTS

2.1. System suitability

The system suitability was determined by six replicated injections of MQC samples. The precision of the system suitability test (% CV) was within 5% for retention time of analyte and 15% for the area ratio of analyte/IS. No carryover was observed when a blank plasma sample was run after each highest concentration sample during validation.

2.2. Specificity and Selectivity

No endogenous substances were found to interfere with the retention time of analyte(s) or internal standard in blank plasma extracts.

2.3. Lower limit of quantification

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with acceptable accuracy and precision. Lower limit of quantification of 5ng /ml with 2.5-fold linearity (r^2 =0.9944) at four QC points could be achieved.

2.4. Lower limit of Detection (LOD)

1ng/mL, The LOD is the lowest amount of analyte in a sample that can be detected with probability, although not quantified as an exact value. The lower limit for detection of our method was 1ng/mL.

2.5. Linearity, Precision & Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the actual value (concentration) of the analyte. Accuracy is determined by replicated analysis of samples containing known amounts of the analyte (i.e., QCs). The deviation of the mean from the nominal value serves as the measure of accuracy. The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of a biological matrix. Precision was measured using six determinations (replicate injections) per concentration. We used four concentrations (LQC: 15ng/ml, M1QC: 75ng/ml, MQC: 300 and HQC: 600 ng/ml) in the range of expected study sample concentrations as recommended. The precision determined at each concentration level did not exceed 15% of the coefficient of variation (CV).

The method was validated under the above criteria and found to be linear from concentrations of 1 to 1000 ng/mL. Intra-day and inter-day accuracy and precision were determined by assaying extracted samples (n=6) at four different concentrations (QCs) for drugs for 5 analytical batches. The regression coefficient (r) of all calibration curves was ≥ 0.9944 for tenofovir.

The correlation coefficient (r) from inter-day analysis was >0.99 in all cases. The calibration model was selected based on the analysis of the data by linear regression with or without weighing factors (none, $1/\times$ and $1/\times^2$). The best linear fit and least-square residuals for the calibration curve were achieved with a $1/\times^2$ weighing factor, giving a representative mean linear regression equation for the calibration curve of Tenofovir: Y=0.706(±0.0003) X + 0.00213 (±0.00100). Y represents the peak area ratio of the analyte to the internal standard and x is the concentration of the analyte. The mean correlation coefficient of the weighted calibration curve for tenofovir using various weights is as shown below. Weighing (none): r=0.9990 (±0.0010); Weighing ($1/\times$): r=0.9981 (±0.0005) and Weighing ($1/\times^2$): r=0.9974 (±0.0003). Based on these determinations, we chose $1/\times^2$ as the weighing factor for linear regression. The sensitivity at LLOQ (lower limit of quantification) of 5ng /ml was with 2.5-fold linearity (r^2 =0.9974) at four QC points, demonstrating a% CV of less than 4.23% (precision) and an accuracy in the range of 80–105%, with a signal-to-noise (S/N) ratio of >7. The intra-batch and inter-batch precision for all four QC levels is shown in Table 1.

Table 1. Intra-day and Inter-day precision and Accuracy

Level	Nominal Concentration (ng/mL)	Intra-day					Inter-day				
	Mean back calculated concentration (ng/mL)a	n	Mean back calculated concentration (ng/mL)a	%RE	%CV	Accuracy	n	Mean back calculated concentration (ng/mL) b	%RE	%CV	Accuracy
LQC	15	6	14.9705	-0.1966	7.3425	99.73	30	15.0407	104.8420	7.1083	101.86
M1QC	75	6	68.0786	-9.2284	9.6235	90.65	30	67.2334	598.6327	9.4958	92.50
MQC	300	6	263.7561	-12.0813	8.0176	89.21	30	266.6313	3225.5370	10.8791	95.18
HQC	600	6	534.2416	-10.9597	3.4116	89.05	30	557.7090	16247.3600	6.4124	93.40

a; mean back calculated concentration of 6 replicate injections at each concentration
b; mean of six replicates of 5 batches at each concentration

Table 2: Summary of calibration curve with back calculated concentrations

Tenofovir P&A Samples										
		Calibration Concentration								
Sr.No	BATCH	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	CS-9
	Lower Concentration	4.250	8.500	17.000	42.5	85	170.000	340.000	680.000	885.000
	Nominal Concentration	5.000	10.000	20.000	50.000	100.000	200.000	400.000	800.000	1000.000
	Higher Concentration	5.750	11.500	23.000	57.500	115.000	230.000	460.000	920.000	1115.000
1	22022017	4.976	10.033	19.920	51.621	97.420	221.904	395.719	785.547	917.980
2	03032017	4.500	11.482	22.472	46.763	110.581	173.456	370.765	784.043	938.007
3	06032017	5.084	11.181	18.246	50.174	109.417	202.717	399.819	742.786	1031.174
4	07032017	4.840	10.410	21.611	45.824	98.172	197.061	449.664	742.102	975.127
5	09032017	4.919	11.019	20.940	54.301	95.215	189.049	392.455	706.090	1000.052
6	09032017	5.207	8.549	21.556	55.692	104.199	195.664	366.224	763.195	1022.210
7	15032017	5.031	9.391	20.975	53.632	102.492	204.413	359.315	682.144	1035.322
8	16032017	4.976	9.338	22.894	50.975	102.183	212.245	355.745	720.500	1055.614
	AVERAGE	4.941	9.603	21.076	51.122	102.084	199.563	380.088	740.800	996.935
	Std Dev	0.209	1.162	0.738	3.495	3.310	14.672	18.059	36.7167	29.155
	%CV	4.237	12.100	3.503	6.837	3.242	7.3524	4.751	4.956	2.924

Representative chromatogram of blank, LLOQ, internal standard is shown in Figure 1 and a representative calibration curve is shown in Figure 2.

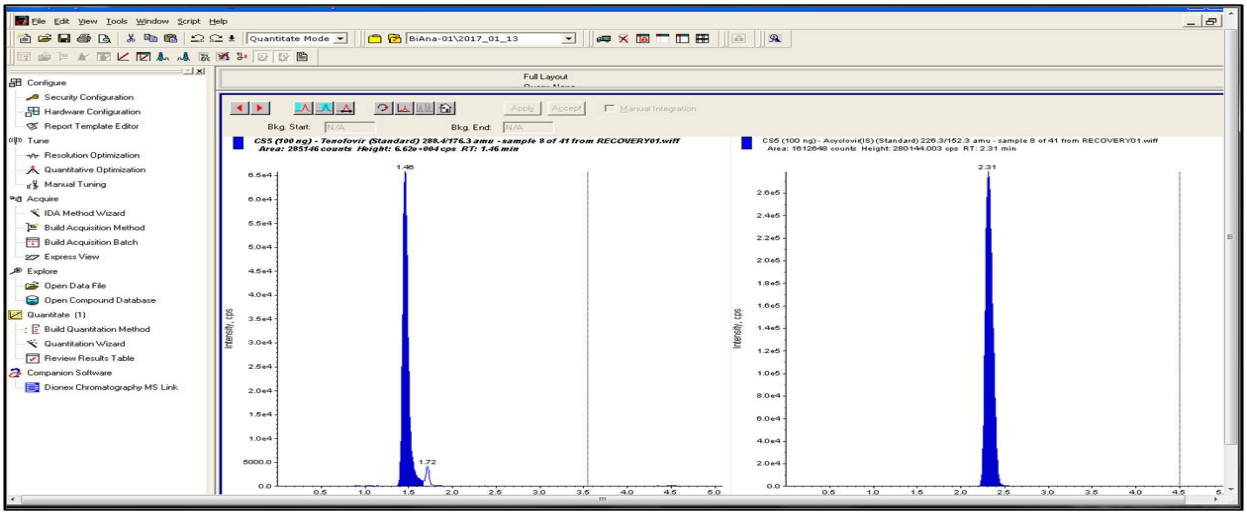

Figure 1. Chromatogram of Tenofovir and Internal Standard (Acyclovir) with retention time of 1.46 min and 2.31 min respectively

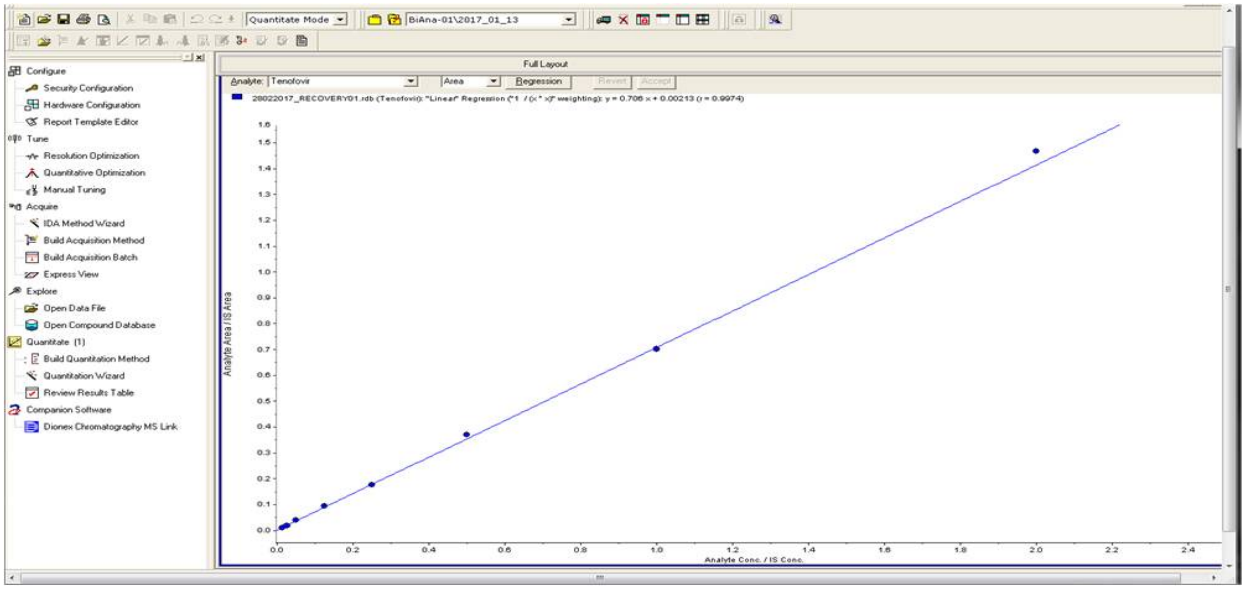

Figure 2. Linearity, peak area of the analyte and IS ratios for the calibration standards were proportional to the concentration of the analyte in plasma over the range (5ng to 1000ng) tested.

2.6. Recovery

The % extraction efficiency (recoveries) of an analytical process, reported as a percentage of the known amount of an analyte at low (15.0 ng/ml) and HQC (600 ng/ml) carried through the sample extraction against the Unextracted aqueous samples were 55.15%, 59.84% for tenofovir and 57.95% and 62.35% for internal standard Acyclovir.

2.7. Matrix effect

The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample. The matrix effect was performed using six different blank plasma lots at LQC and HQC concentration in triplicate by calculating the % accuracy, precision (% CV), which was 108%, (1.2%) and 95%, (1.5%) for Tenofovir and 89.44% (1.02%) and 88.47% (1.03%) for Acyclovir.

2.8. Ruggedness

Ruggedness is a measure of reproducibility of test results under the variations in conditions normally expected from analyst to analyst. Ruggedness of the method was evaluated using re-injection of one precision and accuracy batch samples using different columns of the same make and also with different analysts. The precision (% CV) and % accuracy values for different columns ranged from 3.9% to 7.5% and 89 to 107.1% accuracy for tenofovir at all LQC, MQC and HQC levels.

2.9. Dilution Integrity

The precision (% CV) and % accuracy values for different column were ranging from 2.1% to 5.1% and 99.6% to 107.1% for tenofovir and 3.5% to 4.1% and 99.2% to 106.1% for Emtricitabine respectively at all four QC levels. One of the samples should not affect accuracy and precision. If applicable, dilution integrity should be demonstrated by spiking the matrix with an analyte concentration above the ULOQ and diluting this sample with a blank matrix, e.g., 1/2 and 1/4 dilution factors (at least five determinations per dilution factor). Accuracy and precision should be within the set criteria, i.e., within ±15%. Dilution integrity should cover the dilution applied to the study samples.

2.10. Reinjection reproducibility

It should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument interruptions.

2.11. Stability Experiments

2.11.1. Bench-Top Stability

Bench top stability experiments were designed and conducted to cover the laboratory handling conditions that are expected for study samples. The tenofovir in samples was stable for 16 hours.

Table 3. Bench top stability of Tenofovir 16 hours.

Bench Top Stability				
	Fresh		BT-16Hr	
Concentrations /Sr. No	LQC	HQC	LQC	HQC
Lower Concentration	12.750	510.000	12.750	510.000
Nominal Concentration	15.000	600.000	15.000	600.000
Higher Concentration	17.250	690.000	17.250	690.000
1	14.562	565.646	16.549	569.130
2	16.480	527.225	15.267	540.293
3	15.079	538.122	14.030	559.414
4	15.597	566.169	15.978	549.992
5	16.044	562.028	14.467	582.741
6	15.664	551.644	14.914	553.196
AVERAGE	15.571	551.805	15.200	559.127
Std Dev	0.621	16.086	0.940	15.040
%CV	3.993	2.915	6.183	2.690
% Nominal			100.8614	101.3269
% Change			-98.9913	-98.9867

2.11.2. Freeze and Thaw Stability

During freeze/thaw stability evaluations, the freezing and thawing of stability samples mimicked the intended sample handling conditions to be used during sample analysis. The Tenofovir in samples was stable for 5 freeze thaw cycles at -70 ° C.

Table 4. Freeze thaw stability after 5 freeze thaw cycles.

Freeze & Thaw Stability				
Concentrations /Sr. No	**Fresh**		**FT-5 Cycles**	
	LQC	**HQC**	**LQC**	**HQC**
Lower Concentration	14.750	590.000	14.750	570.000
Nominal Concentration	15.000	600.000	15.000	600.000
Higher Concentration	17.250	610.000	15.010	602.000
1	17.046	592.904	13.900	550.177
2	15.423	578.484	13.131	560.301
3	14.863	575.408	14.142	550.812
4	14.887	608.056	13.881	529.405
5	15.147	587.112	13.417	514.919
6	15.340	582.697	15.063	539.947
AVERAGE	15.523	591.629	14.255	557.507
Std Dev	0.8943	11.6322	0.6885	27.8079
%CV	5.7612	1.9661	4.8304	4.9879
% Nominal			88.5329	88.3317
% Change			-99.1146	-99.1166

2.11.3. Auto Sampler Stability

Six replicates of Lower and Higher quality control samples were kept in Autosampler prior to check for 48 hours. The stability of the analyte was assessed by running against fresh calibration curve standards and quality control samples. The tenofovir was found to be stable after 48 hours when kept at 5 °C.

Table 5. Auto sampler stability at 48 hours

Auto sampler stability				
	Fresh		**AT-48Hrs**	
Concentration /Sr.No	**LQC**	**HQC**	**LQC**	**HQC**
Lower Concentration	12.750	510.000	12.750	510.000
Nominal Concentration	15.000	600.000	15.000	600.000
Higher Concentration	17.250	690.000	17.250	690.000
1	14.562	565.646	15.331	585.802
2	16.480	527.225	16.594	548.739
3	15.079	538.122	11.641	537.175
4	16.597	566.169	14.050	572.261
5	16.044	562.028	17.293	555.196
6	15.664	551.644	14.493	581.904
AVERAGE	15.0710	551.8057	14.9003	563.5128
Std Dev	1.3902	16.0862	2.0145	19.4561
%CV	9.2244	2.9152	13.5198	3.4526
% Nominal			98.8675	102.1216
% Change			-99.0113	-98.9787

3. DISCUSSION

The method development and validation study for Tenofovir (TFR) as a drug substance with its internal standard Acyclovir (ACR) has been carried out in human blood plasma by LCMS/MS technique. The drug Tenofovir belongs to the class of acyclic nucleoside phosphonate (nucleotide) analogs of adenosine 5′-monophosphate. This class of compounds has significant potency directed at HIV. Tenofovir has been used in combination with antiretroviral therapy of HIV-1 infection since 2001 and also to treat hepatitis B virus. Once the drug is intracellular, it is converted into the active form Tenofovir Diphosphate, which undergoes Nucleoside reverse transcriptase inhibitors (NRTIs) blocking an enzyme called reverse transcriptase. This prevents HIV from copying itself, which reduces the amount of HIV in the body.

In this study, the method developed (for TFR) by keeping in mind to use less solvent (by reducing the total run time), well resolved peak (of TFR and ACR), increases extraction efficacy (by using SPE) in a method. After method development, the validation study was carried out as per the ICH guidelines [8]. Where the linearity range of 5–1000 ng/mL for TFR was fixed as per the response of the drug. As the study was carried out with human plasma, the interference of plasma was checked in a specificity study which showed there was not any interference observed in it.

The lower limit of quantification for Tenofovir was 15ng/mL. The LLOQ found was 5ng/mL. The method was accurate (within ±15% of control) and precise (coefficient of variation ≤15%). Analytes were stable for five freeze/thaw cycles and up to 6 days at room temperature, whereas long-term required storage was maintained at −20°C or at −80°C.

For Precision study using QCs of the drug- 85%, 100% and 115% concentration of drug chosen and the levels considered LLQC (5ng/mL), LQC (15ng/mL), M1QC (75ng/mL), MQC (300ng/mL) and HQC (600ng/mL) where the % CV of 6 replicate of each level were observed≤ 15% as per the acceptance criteria of Bioanalytical study. In a Precision and Accuracy study (inter day and intraday), % CV obtained for Tenofovir was observed ≤ 15%. Recovery studies for extracted samples with LQC (15ng/mL), MQC (300ng/mL) and HQC (600ng/mL) were 94.51%, 91.83% and 90.91% respectively. The stability of TFR matrix-based samples was evaluated at LQC and HQC concentrations under various conditions. Stability was assessed by comparing samples undergoing a stability test to the QC nominal concentration and considered stable if the percent difference was within 15% deviation. The internal standard substance (ACR) can be used for calibration by plotting the ratio of the analyte (TFR) signal to the internal standard (ACR) signal as a function of the analyte standard concentration. This is done to correct analyte losses during sample preparation. The results of System Suitability Test for TFR and ACR are an internal standard with observed %CV ≤ 2.0%.

4. CONCLUSION

The cost of the sample analysis could be reduced by using the internal standard which resembles the analyte during extraction, elution and detection. This chromatographic resemblance not only compensates for the inconsistent response due to the matrix effect but also reduces the interferences. Ideally, a stable isotope should have been used but use of acyclovir, the nucleoside analogue, was used as an internal standard since it does not have drug interaction during extraction separation and analysis and also has similar chromatographic characteristics [7]. It was competent to minimize any analytical variation due to solvent evaporation, integrity of the column, and ionization efficiency as reported earlier [8]. Cost is less and easily available. Many investigators have used other drugs as internal standards rather than stable isotopes, for example, Adefovir, Lamivudine, voriconazole and Atenolol [9, 10, 11 and 12]. The results from liquid-liquid extraction were not satisfactory, hence, we used solid phase extraction which concentrates and purifies the samples. One disadvantage of SPE is the time required for processing the samples and also the increase in cost. The retention time in our method is around 1.46 and 2.31 for tenofovir and acyclovir respectively, which is quite lower than the methods described, resulting in a shorter run time of 5 minutes only [10, 11 and 12]. When developing a method for an analyte on an HPLC system, the analyst can maximize sample throughput in a quality control lab by reducing analysis time by considering precautionary things like better resolution, higher sensitivity, pump pressure, mobile phase flow rate with column selection, and less solvent consumption.

The back calculated results showed good day-to-day accuracy and precision. Hence the tenofovir standard curve produced by this method could be used reliably to determine plasma drug concentrations in a consistent fashion. Our method had recovery of less than 65% but it was consistent and reproducible.

5. MATERIALS AND METHODS

5.1. Chemicals and Reagents

The Tenofovir reference standard and Acyclovir reference standard was procured from Clearsynth and plasma was made available from a blood bank after getting permission. Water, Acetonitrile, Isopropyl alcohol, Methanol, Ammonia Solution 30% and formic acid of HPLC grades were used.

5.2. LCMS Instrumentation

The 4000 QTrap along with the Shimadzu LC 20AD LC System, controlled by Analyst 1.4.2 Software was used for analysis.

5.3. Solid phase extraction

Thermo Scientific- SOLA SCX 10mg/1mL was used for Solid phase extraction. The cartridges were preconditioned with 1ml methanol and 1ml 1% formic acid in water. Then the cartridges were washed with

0.5mL of 1%v/v formic acid in water followed by 0.5 mL of 1% formic acid in methanol and dried completely by applying full nitrogen pressure. Analytes and IS were eluted with 0.500ml twice with elution solution (0.5% Ammonia in water: Methanol; 60:40; v/v, adjust pH 8.00) and transferred into HPLC recovery vials and 2 µL of the sample was injected into the column.

5.4. HPLC conditions/ Chromatographic separation and MS parameters

Chromatographic separation was performed at 40°C using a column oven, on Thermo C18 column (5µm, 4.6 X 150mm) Chromatographic run was performed with mobile phase A (0.1%Formic acid in methanol) and mobile phase B (0.1% Ammonia in water) with a initial ratio of (85:15 v/v then 0:100 after first minute and 85:15 at fourth and fifth minute) &1.0ml/minute flow rate. Throughout the analysis, the samples were maintained in an auto-sampler at a 5°C temperature. Nitrogen was generated by peak scientific (NM20Z) from compressed air. The analyte(s) and IS were eluted within a run time of 2.8 minutes.

For MS/MS, ESI with positive MRM was selected for ionization. Tenofovir & Internal Standard Acyclovir were monitored by MRM transitions, Tenofovir-288.4>176.3, and Internal standard, Acyclovir-226.3>152.3 were monitored by MRM transitions. De-clustering Potential (DP) & Entrance Potential (EP) for Tenofovir and Acyclovir were 55.00V &10.0V respectively. Collision Energy (CE) for Tenofovir and Acyclovir was 35.0V respectively. Collision Cell Exit Potential (CXP) for Tenofovir, and Acyclovir were 15.0V. Curtain Gas, Capillary voltage, Temperature, Collision Gas for all the MRM transitions were maintained as 20psi, 5500V, 550°C and 6psi. Dwell time for all the MRM transitions was 200msec**.**

5.5. Stock solutions

Stock solutions of TFV and the IS (Acyclovir) were prepared at 1 mg/ml in methanol. A series of standard working solutions for TFV was prepared by diluting stock solutions with methanol to concentrations in the range of 5ng/ml to 1000 ng/ml. The IS solution was brought to a final concentration of 2 mg/ml in water. All working solutions were stored at 4 °C and brought to room temperature before use. Prepared (5, 10, 20, 50, 100, 200, 400, 800 & 1000 ng/mL as calibration standard) and (15, 75, 300 & 600 ng/mL) set as an Internal QC) with proper labeling. All four QC samples applied n=6 times. A vial containing matrix (as a blank) is also kept with the above set to check any interference in it.2µl of stock IS (1000 µg/mL) was added to 9.998 ml of Diluent to get 200 ng/mL of intermediate solution. The validation was carried out as per the USFDA guidelines for bioanalytical experiments [13].

Acknowledgements: Author Sarang Salunke Like to acknowledge the College, Jayawant Shikshan Prasarak Mandal's Charak College of Pharmacy and Research, Wagholi, Pune where he persuing his M. Pharm. and Bioanalytical Technologies India Pvt Ltd Pune.

Author contributions: Concept – S.S.; Design – S.S.; Supervision – S.W.; Resources – B.P.; Materials – C.S.; Data Collection and/or Processing – S.S.; Analysis and/or Interpretation – S.W.; Literature Search – S.S.; Writing – S.S.; Critical Reviews – H. N.; Other– S.M., S.R., V.K., P.B.

Conflict of interest: None

REFERENCES

[1] Pandey A, Dhingra N, Kumar P, Sahu D, Reddy DC, Narayan P, Raj Y, Sangal B, Chandra N, Nair S, Singh J. Sustained progress, but no room for complacency: Results of 2015 HIV estimations in India. Indian J Med Res. 2017; 146(1): 83-96. [CrossRef]

[2] Nah K, Nishiura H, Tsuchiya N. Test-and-treat approach to HIV/AIDS: a primer for mathematical modeling. Theor Biol Med Model. 2017; 14(1): 1-11. [CrossRef]

[3] Interim Guidance for Clinicians Considering the Use of Preexposure Prophylaxis for the Prevention of HIV Infection in Heterosexually Active Adults. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6131a2.htm (accessed on 13 Oct 2022).

[4] VIREAD, https://www.rxlist.com/viread-drug.htm (accessed on 13 Oct 2022).

[5] Donnell D, Baeten JM, Bumpus NN, Brantley J, Bangsberg DR, Haberer JE, Mujugira A, Mugo N, Ndase P, Hendrix C, Celum C. HIV protective efficacy and correlates of tenofovir blood concentrations in a clinical trial of PrEP for HIV prevention. J Acq Immun Def Synd. 2014; 66(3): 340-348. [CrossRef]

[6] An ambitious treatment target to help end the AIDS epidemic. https://www.unaids.org/en/resources/documents/2017/90-90-90 (accessed on 13 Oct 2022).

[7] Rani R, Medhe S, Srivastava M. HPTLC–MS based method development and validation for the detection of adulterants in spices. J Food Meas Charact. 2015; 9(2): 186-194. [CrossRef]

[8] Yadav M, Singhal P, Goswami S, Pande UC, Sanyal M, Shrivastav PS. 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 Chromatogra Sci. 2010; 48(9): 704-13. [CrossRef]

[9] Delahunty T, Bushman L, Fletcher CV. Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS. J Chromatogr B. 2006; 830(1): 6-12. [CrossRef]

[10] 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. J Pharmaceut Biomed. 2008; 48(3): 918-26. [CrossRef]

[11] Kim DH, Cho JY, Chae SI, Kang BK, An TG, Shim WS, Noh YS, Hwang SJ, Chung EK, Lee KT. Development of a simple and sensitive HPLC-MS/MS method for determination of diazepam in human plasma and its application to a bioequivalence study. Transl Clin Pharmacol. 2017; 25(4): 173-178. [CrossRef]

[12] Takahashi M, Kudaka Y, Okumura N, Hirano A, Banno K, Kaneda T. Determination of plasma tenofovir concentrations using a conventional LC-MS method. Biol Pharm Bull. 2007; 30(9): 1784-1786. [CrossRef]

[13] Bioanalytical Method Validation Guidance for Industry May 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry (accessed on 13 Oct 2022).