Novel approaches for determination of antiretroviral reverse transcriptase inhibitor agent in commercial dosage forms by using spectrofluorimetric, first derivative spectrophotometric, and HPLC methods

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Abstract

Background: Rapid, simple, and sensitive spectrofluorimetric, first derivative spectrophotometric, and high-performance liquid chromatographic (HPLC) methods have been developed and validated for determination of tenofovir in pharmaceutical preparations. Spectrofluorimetric method is based on measuring the native fluorescence intensity of tenofovir at 375.0 nm after excitation at 275.0 nm. Calibration graphics were plotted and were found linear over 4.72–15.75 μg/mL concentration range (r² = 0.9994). The second method developed was the first derivative spectrophotometric method for the analysis of tenofovir performed by measuring the amplitude at 251.7 and 272.6 nm. Linearity was observed in the concentration range 10.0–28.0 μg/mL (r² = 0.9998). On the other hand, HPLC with a diode array detector (DAD). Ritonavir was used as internal standard (IS). HPLC analysis was carried out on a C₁₈ column (Wakosil-II 5 C₁₈ AR, 4.6 × 250 mm) using a mobile phase consisting of acetonitrile: 0.5% formic acid (99.5:0.5; v/v) at a flow rate of 1.0 mL/min. Injection volume was 5.0 μL. DAD signals at 260.0 nm were used. HPLC method was found to be linear over the concentration range of 10.0–100.0 μg/mL (r² = 0.9990).

Result: Intra- and inter-day analysis and recovery studies were carried out to investigate precision and accuracy of the proposed spectrofluorimetric, first derivative spectrophotometry and HPLC methods.

Conclusion: We successfully applied the developed methods for determination of tenofovir in tablet formulation. Finally, the proposed methods were compared statistically.

Keywords: Tenofovir, Spectrofluorimetry, HPLC, First derivative spectrophotometry, Quantitative analysis

Background

Tenofovir disoproxil fumarate (TDF) is a pro-drug of tenofovir which is a phosphorylated adenosine analogue [1]. Tenofovir disoproxil fumarate is an antiretroviral reverse transcriptase inhibitor [2]. Chemical structure of tenofovir disoproxil fumarate is shown in Fig. 1.

Several methods have been published for the determination of tenofovir in bulk or pharmaceutical formulations and in biological fluids and materials. In the literature, tenofovir has been determined as the only active ingredient and in combination with other active ingredients. These methods include HPLC [3–8], HPLC-MS [9], HPLC-MS/MS [10, 11], LC-MS [12–14], LC-MS/MS [15–18], UPLC [19–25], UPLC-MS and UPLC-MS/MS [22–25], TLC [26], HPTLC [27–29], and spectrophotometric [7, 30–34] methods.
This paper describes new, simple, sensitive, and rapid spectrofluorimetric method for the determination of tenofovir in pharmaceutical dosage form. To the best of our knowledge, no spectrofluorimetric method was proposed for the quantitative determination of tenofovir in pharmaceutical preparations. A new HPLC method was also validated for the analysis of tenofovir in pharmaceutical dosage form, and this HPLC method was used as the reference method, and we compared the results obtained from the proposed methods statistically.

Methods
Apparatus
An Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer equipped with Agilent Technologies 10.0 mm quartz fluorescence cells was used for the spectrofluorimetric method. Absorbance measurements were measured on a double beam UV–VIS spectrophotometer model UV-1800 PC by using quartz cells with 10.0-mm path length. For the HPLC analysis, an Agilent 1100 Series HPLC with DAD detector was used.

Software
Agilent Cary Eclipse™, UV-Probe 2.52 software, and Agilent ChemStation™ products were used for spectrofluorimetric, spectrophotometric methods, and HPLC, respectively. Origin Pro8™ software was used for plotting the calibration spectrums and chromatograms shown in this paper.

Reagents and materials
Acetonitrile and boric acid were purchased from Riedel-de Haën®, Germany, and methanol was purchased from LiChrosolv®, Germany. Dimethylformamide, diethylamine, phosphoric acid, acetic acid, formic acid, sodium hydroxide, sodium dodecyl sulphate (SDS), tween® 80, cetrimonium bromide (CTAB), tenofovir, caffeine, hydrochlorothiazide, zanamivir, oseltamivir, lopinavir, and ritonavir were all purchased from Sigma-Aldrich®, USA.

Pharmaceutical compounds and dosage forms
TDF and its dosage form “Ternavir® 245.0 mg film coated tablet” was kindly provided by ATABAY Pharmaceuticals and Fine Chemicals (Turkey). Tablet formulation contains 300.0 mg TDF which is equal to 245.0 mg tenofovir disoproxil per tablet. Ritonavir which was used as the internal standard in HPLC analysis was kindly provided by AbbVie Medical Drugs Industry and Trade Ltd. Co. (Turkey).

Preparation of standard solutions
16.0 mg TDF was weighed and transferred to 50.0-mL volumetric flask and dissolved with distilled water and completed to the volume with the distilled water for the spectrofluorimetric and spectrophotometric methods. Surfactant stock solutions were prepared with distilled water to contain 2.0%, v/v for Tween® 80, and 2.0%, w/v for SDS and CTAB.

For the HPLC method, 22.5 mg TDF standard was transferred to a 10.0-mL volumetric flask and completed to the volume with methanol. 33.6 mg ritonavir standard was transferred to a 25.0-mL volumetric flask, dissolved and completed to the volume with methanol. Further dilutions from these two stock solutions were made with methanol. All solutions were filtered through 0.45-μm filter before injection to the HPLC system.

Preparation of pharmaceutical dosage form
Ten tablets were weighted accurately and finely powdered and mixed. Powder amount that is equal to the average tablet weight was transferred to a 500.0-mL volumetric flask. Some distilled water was added, and the solution was sonicated for about 10.0 min for the spectrofluorimetric and spectrophotometric methods. Then, completed to the volume with the same solvent and mixed on a magnetic mixer for about 45.0 min. Further dilutions were made with water.

Powder amount that is equal to average tablet weight was transferred to a 250.0-mL volumetric flask; some methanol was added and sonicated for about 10.0 min and then completed with methanol to the volume for HPLC analysis. This solution was mixed on a magnetic mixer for about 30.0 min. Further dilutions were made with methanol.

All tablet solutions were filtered through 0.45-μm filter before analysis for both methods.
General analytical procedures

Spectrofluorimetric measurements were recorded at emission mode after excitation at 275.0 nm. Detector voltage was set at 660.0 volts. Excitation and emission slit widths were 10.0 nm. Native fluorescence intensities at 375.0 nm were used for the calculations.

HPLC analysis was carried out through Wakosil-II 5 C₁₈ AR, 4.6 × 250 mm analytical column. Acetonitrile 0.5% formic acid (99.5: 0.5; v/v) was used as mobile phase at a flow rate of 1.0 mL/min. Injection volume was 5.0 μL. DAD detector was set at 250.0, 254.0, 260.0, 265.0, and 270.0 nm. Chromatograms obtained at 260.0 nm were used for the analysis.

The developed analytical method was validated according to international guidelines with respect to certain parameters such as accuracy, precision, and linearity [35].

Results

Optimization of the spectrofluorimetric method

Effect of the diluting solvent

Firstly, the effect of the diluting solvent on the native fluorescence intensity was investigated. Distilled water, methanol (MeOH), acetonitrile (ACN), and dimethylformamide (DMF) was used as the diluting solvent. Sample concentration was 14.86 μg/mL in each different solution. Spectrums of the sample and blank solutions were recorded. Results obtained are shown in Fig. 2. Distilled water was chosen as the diluting solvent because of the higher native fluorescence intensity obtained.

Effect of pH

After choosing the diluting solvent, effect of pH on the fluorescence intensity was investigated. A stock solution of Britton-Rabinson solution was prepared. Buffer solutions at different pH values (pH 2.26–10.11) were prepared from this solution by using 5.0 N NaOH solution. Appropriate volumes of the stock TDF solution were transferred to 10-mL volumetric flasks and completed to the volume with the buffer solutions prepared. Final tenofovir concentration was 14.86 μg/mL. Spectrums of the blank and sample solutions were recorded. Native fluorescence intensities of each sample solution with different pH values are shown in Fig. 3.

As it is seen on the graph, the highest native fluorescence intensity was obtained with the pH 2.26 buffer solution; that is why this pH was chosen for the spectrofluorimetric analysis.

Effect of volume of the buffer solution

After choosing the pH value, effect of the buffer volume used in the sample preparation process was investigated. Dilutions were made from the TDF stock solution to 10.0-mL volumetric flasks to end up with a 14.86 μg/mL tenofovir concentration. Different volumes of the pH 2.26 buffer solution were added to the flasks and completed to the volume with distilled water or buffer. Native fluorescence intensities were measured, and the results are shown in Fig. 4.

As it is seen in Fig. 4, pH 2.26 buffer solution volume does not affect the native fluorescence intensity significantly. That is why pH 2.26 volume added to the sample solutions was decided to be 1.0 mL to minimize the use of buffer solution.

Effect of surfactant

The effect of the surfactant was also investigated. Three different kind of surfactants were chosen: Tween® 80...
(non-ionic), SDS (anionic), and CTAB (cationic). Solutions were prepared at the rate of 2.0% v/v Tween® 80, w/v SDS, and w/v CTAB. One milliliter stock TDF solution, 1.0 mL pH 2.26 buffer solution, and 1.0 mL surfactant (0.5 mL for Tween® 80) solution were transferred to 10.0-mL volumetric flasks and completed to the volume with water. Results that are obtained are shown in Fig. 5.

Using SDS as the surfactant was found to be the best choice since it provides the highest native fluorescence intensity.

**Effect of volume of the surfactant**

Different volumes of the SDS (2.0%, w/v) solution were used to prepare sample solutions. In each solution, 1.0 mL pH 2.26 buffer solution was also added and the final tenofovir concentration was 14.86 μg/mL.

As shown in Fig. 6, there is no significant difference between fluorescence intensities of solutions which were prepared with different volumes of SDS solution. Therefore, SDS solution volume to be added to sample solutions was decided to be 1.0 mL for the analysis.

**Effect of temperature**

The effect of temperature on the native fluorescence intensity was also investigated. Working solutions using 1.0 mL pH 2.26 buffer and 1.0 mL 2.0% SDS (w/v) solutions and appropriate volume of TDF stock solution and distilled water were prepared. Solutions prepared were kept for 10.0 min in a thermostatically controlled water bath which was set at different temperatures. Native fluorescence intensities of these solutions are shown in Fig. 7.
There is a decrease in native fluorescence intensity as the temperature increases. Especially after 50.0 °C, this decrease is much more significant. Therefore, it was decided to perform the analysis at room temperature.

Optimization of the first derivative spectrophotometric method

The direct UV spectrum and the first derivative spectrum of tenofovir in methanol solutions are shown in Figs. 8 and 9, respectively. Derivative spectra of different orders were studied for tenofovir, and experimental results showed that the first derivative spectra gave well-defined peaks around $\lambda_{251.0}$ and $\lambda_{272.0}$ with suitable precision.

Optimization of the HPLC method

Different column and mobile phase systems and different internal standards were used to achieve the best resolution between tenofovir and the internal standard and to gain the best peak shape and performance.
parameters. Stationary and mobile phase systems and different internal standards used for the optimization of the HPLC method are summarized in Table 1. DAD detector was set at 250.0, 254.0, 260.0, 265.0, and 270.0 nm for all conditions.

Best separation and system performance was achieved on a Wakosil-II 5 C_{18} AR, 4.6 × 250 mm analytical column using acetonitrile: 0.5% formic acid (99.5:0.5; v/v) as mobile phase. The best resolution is obtained tenofovir and ritonavir. Flow rate was 1.0 mL/min. Injection volume was 5.0 μL. Analytical data of 260.0 nm was used for the analysis. System performance data is summarized in Table 2.

Validation parameters

Purposed methods were validated for their linearity, accuracy, and precision according to ICH guidelines [35].

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**Fig. 7** Effect of temperature on native fluorescence intensity

**Fig. 8** UV-spectrums of the tenofovir calibration solutions

- a- blank
- b- 4.72 μg/mL
- c- 6.30 μg/mL
- d- 7.87 μg/mL
- e- 9.45 μg/mL
- f- 11.02 μg/mL
- g- 12.60 μg/mL
- h- 14.17 μg/mL
- i- 15.75 μg/mL
Linearity

Appropriate volumes of TDF stock solution were transferred to 10.0-mL volumetric flasks; 1.0 mL of pH 2.26 buffer and 2.0% SDS solutions were added and completed to the volume with distilled water. Tenofovir concentration was between 4.72 and 15.75 μg/mL range for the spectrofluorimetric method (Method I). Spectrums of these solutions are shown in Fig. 10. Regression line was calculated by the least squares method [35]. Coefficient of determination (\( r^2 \)) was found to be 0.9994 which indicates good linearity.

In the first derivative spectrophotometric method (Method II), Beer’s law range, linear regression equations, and correlation coefficients determined for each method are given in Table 3.

For HPLC method (Method III), appropriate dilutions were made from the stock solutions of ritonavir and TDF. Tenofovir concentrations were between 10.0 and 100.0 μg/mL, and the ritonavir concentration was 125.4 μg/mL in each solution. Calibration graph was plotted via least square method (\( r^2 = 0.9990 \)) [35]. Chromatograms of these solutions are shown in Fig. 11. Calculations were made by the formula below for the HPLC method.

\[
\text{Relative tenofovir peak area} = \frac{\text{Tenofovir peak area}}{\text{Ritonavir peak area}}
\]

For Method I, eight sets of solutions were prepared as three series. For Method III, ten sets of solutions were prepared in three series and 3 injections were made for each serial of each concentration. Statistical data of the calibration graphs of the proposed methods are shown in Table 3. Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated as described at ICH guidelines by the formulas given below, where \( S_o \) is the standard deviation of the intercept of the regression line and \( a \) is the slope of the calibration curve [35].

\[
\text{LOD} = \frac{3.3 S_o}{a}
\]

\[
\text{LOQ} = \frac{10 S_o}{a}
\]

![Fig. 9 First derivative spectrums of the tenofovir calibration solutions](image)

| Table 1 | Column, mobile phase systems, and internal standards used for the optimization of the HPLC method |
| --- | --- | --- |
| Column | Mobile phase* | Internal standard |
| Thermo Hypersil Phenyl 250 × 4.6 mm 5 μ | ACN:MeOH | Caffeine |
| | ACN:MeOH:Diethylamine | Hydrochlorothiazide |
| | ACN:0.5% Formic acid | Zanamivir |
| Waters Xterra RP8 5 μ 250 × 4.6 mm | ACN:MeOH | Oseltamivir |
| | ACN:0.5% Formic acid | Lopinavir |
| Wakosil-II S C18 AR, 4.6 × 250 mm | ACN:0.5% Formic acid | Ritonavir |

*Mobile phase components were at different rates
Accuracy and precision
Accuracy of the purposed methods was investigated via standard addition method. Known amount of tenofovir was added to a known concentration of tablet formulation. Five solutions at different concentrations in three series were prepared for the recovery studies. The statistical data of the recovery studies of Methods I, II, and III are given in Table 4. The results given in Table 3 indicate good accuracy and show that there is no interference with tablet excipients that are commonly used in formulations.

Precision of the purposed methods was investigated via intra- and inter-day analysis by preparing working solutions from the stock solutions mentioned before at three different concentrations as three series and by analyzing these samples at different times and days for intra- and inter-day analysis, respectively. Samples were kept at room, dark, and refrigerator conditions for the inter-day analysis. For each condition, solutions at three different concentrations were prepared and analyzed.

For Method I, samples were analyzed through 5 h and 5 days’ timeline for the intra- and inter-day analysis, respectively. Samples were analyzed for 7.5 h and 5 days for intra- and inter-day analysis, respectively, for Method III. The results of the intra- and inter-day analysis are given in Table 5.

Assay
Methods I, II, and III were successfully applied to the finished product Ternavir® tablets. Results that were obtained are shown in Table 6.

\[ t \text{ and } f \text{ values were calculated by the formulas given below. As it is seen on Table 6, calculated } t \text{ and } f \text{ values are smaller than the theoretical table } t \text{ and } f \text{ values.} \]

Table 2 System performance parameters obtained by HPLC for tenofovir

| Parameters                              | HPLC | Reference value [36, 37] |
|-----------------------------------------|------|-------------------------|
| Retention time, \( t_r \)              | 2.64 | —                       |
| Capacity factor, \( K' \)              | 3.19 | \( K' > 2.0 \)          |
| Resolution, \( R_s \)                  | 3.806| \( R_s > 2.0 \)         |
| Selectivity, \( \alpha \)              | 4.437| \( \alpha > 1.0 \)      |
| Theoretical plates, \( N \) (plates/column) | 11207| \( N > 2000 \)         |
| Tailing factor, \( T \)                | 1.191| \( T \leq 2.0 \)       |
| RSD(%)                                  | 0.12 | RSD \leq 1%             |

LOD = 3.3 \( S_b/a \); LOQ = 10 \( S_b/a \)

Fig. 10 Spectrums of the blank solution and the solutions containing increasing concentrations of tenofovir
which are 2.78 and 6.39, respectively (for \( n = 5 \) and \( p = 0.05 \)). This result indicates that there is no significant difference between the purposed methods' means and standard deviation values.

\[
f \text{value} = \frac{(SD_1)^2}{(SD_2)^2}
\]

where \( SD_1 \) and \( SD_2 \) are standard deviations of five quantitative determinations of Method I and Method II, respectively (\( SD_1 > SD_2 \)).

\[
t \text{value} = \frac{|C_{\text{mean}} - C_{\text{real}}|}{SD/\sqrt{n}}
\]

where \( C_{\text{mean}} \) is the mean of five determinations (mg/tablet), \( C_{\text{real}} \) is the label value (135.58 mg tenofovir/tablet), SD is standard deviation of five determinations, and \( n \) is the number of determinations (\( n = 5 \)).

**Discussion**

In this study, spectofluorometric, chromatographic, and spectrophotometric methods were developed for determination of tenofovir disoproxil fumarate active substance in Ternavir® preparation.
Various experiments have been conducted to optimize the spectrofluorimetric method. Dilutions were made with different solvents such as distilled water, methanol, and acetonitrile, and distilled water giving the highest fluorescence intensity was selected.

After the dilution solution was selected, buffer solutions with different pH values were prepared and the best solution was selected as pH 2.26. In order to investigate the effect of the volume of the buffer solution, measurements were made by adding an increasing amount of buffer solution to the 10.0-ml flask. Since there was not much difference in fluorescence intensity and to use the buffer solution at least, it was decided to add 1.0 ml of the buffer solution.

Different surfactants were tried to optimize the fluorescence intensity, and sodium dodecyl sulfate solution (2.0%, w/v) was selected. 1.0 ml of sodium dodecyl sulphate solution was decided to be added, because the

| C_{drug} (μg/mL) | C_{add} (μg/mL) | C_{det}* (μg/mL) | %Recovery ±SD | %RSD |
|------------------|-----------------|------------------|--------------|------|
| 5.42             | 1.57            | 7.01             | 101.31 ± 2.74 | 2.70 |
| 3.18             | 8.64            | 101.24 ± 0.53    | 0.52          | 4.90 |
| 4.76             | 10.16           | 99.61 ± 0.73     | 0.74          | 6.79 |
| 6.30             | 11.98           | 104.13 ± 1.25    | 1.20          | 8.20 |
| 7.87             | 13.22           | 99.04 ± 2.25     | 2.27          | 10.00|

| C_{drug} (μg/mL) | C_{add} (μg/mL) | C_{det}* (μg/mL) | %Recovery ±SD | %RSD |
|------------------|-----------------|------------------|--------------|------|
| 100.06 ± 1.11    | 14.02           | 27.09            | 30.00        | 57.64|
| 99.25 ± 0.93     | 15.96           | 20.10            | 80.00        | 65.16|
| 97.82 ± 1.08     | 17.99           | 20.10            | 80.00        | 65.16|
| 98.91 ± 0.88     | 20.10           | 22.08            | 70.00        | 95.91|
| 99.04 ± 0.99     | 22.08           | 57.64            | 70.00        | 95.91|

| C_{drug} (μg/mL) | C_{add} (μg/mL) | C_{det}* (μg/mL) | %Recovery ±SD | %RSD |
|------------------|-----------------|------------------|--------------|------|
| 101.85 ± 0.71    | 14.02           | 27.09            | 30.00        | 57.64|
| 95.18 ± 2.43     | 15.96           | 20.10            | 80.00        | 65.16|
| 96.98 ± 1.38     | 17.99           | 20.10            | 80.00        | 65.16|
| 98.32 ± 1.39     | 22.08           | 57.64            | 70.00        | 95.91|

C_{drug} drug concentration, C_{add} added concentration, C_{det} detected concentration, SD standard deviation, RSD relative standard deviation

*Mean concentration of three determinations

Table 4 Results of the recovery studies using standard addition method

| Method I | Method II | Method III |
|----------|-----------|------------|
| C_{drug} (μg/mL) | C_{add} (μg/mL) | C_{det}* (μg/mL) | %Recovery ±SD | %RSD |
| 5.42 | 1.57 | 7.01 | 101.31 ± 2.74 | 2.70 |
| 3.18 | 8.64 | 101.24 ± 0.53 | 0.52 | 4.90 |
| 4.76 | 10.16 | 99.61 ± 0.73 | 0.74 | 6.79 |
| 6.30 | 11.98 | 104.13 ± 1.25 | 1.20 | 8.20 |
| 7.87 | 13.22 | 99.04 ± 2.25 | 2.27 | 10.00|

Table 5 Precision data of tenofovir by proposed methods

| Method I | Method II | Method III |
|----------|-----------|------------|
| C_{real} (μg/mL) | Conc.* Found (μg/mL) | %Recovery*±SD | %RSD |
| Intra-day analysis | | | |
| Room | 6.26 | 6.43 | 102.64 ± 3.17 | 3.09 |
| 9.40 | 9.95 | 105.87 ± 1.29 | 1.22 |
| 12.53 | 12.86 | 102.61 ± 0.57 | 0.56 |
| Inter-day analysis | | | |
| Room | 30.00 | 30.33 | 101.09 ± 0.79 | 0.79 |
| 9.40 | 50.00 | 48.64 | 97.28 ± 0.50 | 0.51 |
| 12.53 | 70.00 | 68.82 | 98.31 ± 0.74 | 0.75 |
| Dark | 6.26 | 6.28 | 100.38 ± 0.81 | 0.80 |
| 9.40 | 106.3 | 113.09 ± 5.52 | 4.88 |
| 12.53 | 13.72 | 109.52 ± 5.14 | 4.69 |
| Refrigerator | 70.00 | 68.05 | 97.21 ± 2.17 | 2.23 |

| C_{real} (μg/mL) | Conc.* Found (μg/mL) | %Recovery*±SD | %RSD |
| Intra-day analysis | | | |
| Room | 30.00 | 30.33 | 101.09 ± 0.79 | 0.79 |
| 9.40 | 50.00 | 48.64 | 97.28 ± 0.50 | 0.51 |
| 12.53 | 70.00 | 68.82 | 98.31 ± 0.74 | 0.75 |
| Inter-day analysis | | | |
| Room | 30.00 | 29.78 | 99.25 ± 2.56 | 2.58 |
| 9.40 | 50.00 | 48.13 | 96.27 ± 1.80 | 1.87 |
| 12.53 | 70.00 | 68.05 | 97.21 ± 2.17 | 2.23 |
| Dark | 6.26 | 7.02 | 112.15 ± 6.61 | 5.89 |
| 9.40 | 10.56 | 112.34 ± 5.36 | 4.77 |
| 12.53 | 13.68 | 109.21 ± 4.59 | 4.21 |
| Refrigerator | 70.00 | 68.48 | 97.83 ± 2.30 | 2.36 |

C_{real} real concentration of the solution, Conc. concentration, Recov. recovery, SD standard deviation, RSD relative standard deviation, RE relative error

*Mean concentration of three determinations

**Mean % recovery of three determinations
amount added in different volumes did not change the fluorescence intensity.

The solutions prepared to examine the effect of temperature were kept in a water bath, and different temperatures were tested. As the spectrofluorimetric density decreased at 50.0 °C, the experiments were decided to be carried out at room temperature.

HPLC and spectrophotometric methods were optimized. For HPLC experiments, the column and mobile phase were chosen which gave the best results and are shown in Table 1. In HPLC study, the system suitability parameters for tenofovir were found to be more sensitive compared to the studies in the literature and the analysis was completed faster [6].

Although the study with spectrofluorimetric detector is more sensitive in the literature, it is an expensive method. Our study is simple to be used in routine laboratorries and its analysis time is short [8]. Our study is suitable for green chemistry compared to the studies in the literature [15, 30, 32].

In order to prove the validity of the developed methods, the parameters reported in the sources were selected for the method validity tests and the relevant validity criteria were accepted [35]. For this purpose, validation studies’ linearity, range, sensitivity, precision, recovery, repeatability, etc., parameters were investigated and statistical evaluations were made.

### Conclusion

Spectrofluorimetric, first derivative spectrophotometric, and HPLC methods were developed for the determination of Tenofovir in pharmaceutical preparations. LOD and LOQ values indicate that both methods are sensitive. Correlation coefficients of the methods are close to 1.0 which supports the sensitivity. Intra- and inter-day analyses were performed, and the results that are obtained showed that the purposes methods are precise. Recovery studies point out good accuracy, and they indicate that there is no interference with the commonly used excipients in pharmaceutical preparations. Purposed methods were applied to the tablet formulation successfully. Proposed methods were also compared statistically via calculating $t$ and $f$ values, and no difference was found between methods. Proposed methods are rapid, simple, sensitive, accurate, and precise and can be used in many control laboratories for the determination of tenofovir in pharmaceutical preparations.

### Abbreviations

- HPLC: High-performance liquid chromatography
- DAD: Diode array detector
- UV: Ultraviolet
- TDF: Tenofovir disoproxil fumarate
- SDS: Sodium dodecyl sulfate
- CTAB: Cetrimonium bromide
- ACN: Acetonitrile
- MeOH: Methanol
- DMF: Dimethyl formamide
- TLC: Thin layer chromatography
- LC-MS/MS: Liquid chromatography mass spectrometry/mass spectrometry
- HPLC: High-performance thin layer chromatography
- ICH: International Council for Harmonization
- SD: Standard deviation
- RSD: Relative standard deviation
- LOD: Limit of detection
- LOQ: Limit of quantification

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### Authors' contributions

ME designed and supervised the experiments, analyzed the data, and wrote the paper. EC performed the experiments. GT helped to draft the finally manuscript. All authors read and approved the final manuscript.

### Availability of data and materials

All data and materials are available upon request.

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

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