



Study on Quality Control of Tenofovir Disoproxil Fumarate Enantiomers by High-Performance Liquid Chromatography–Mass Spectrometry

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Abstract

This study aims at developing a high-performance liquid chromatography–mass spectrometry (LC–MS) method to analyze tenofovir disoproxil fumarate (TDF) and its pharmaceutical preparations. Several cyclodextrin mobile-phase additives were applied to reversed-phase and normal-phase chromatography, and the effects of three chiral stationary phases on the TDF separation were investigated in this study. The R-type and S-type of TDF tablets were quantitatively analyzed in the single ion monitoring (SIM) scanning mode with a Unichiral CMD column. This method has been successfully applied to the separation and quantification of TDF and its isomers. The linear ranges of (R)-TDF and (S)-enantiomer were 1–20 and 0.2–16 µg/mL, respectively. The limit of detection for (R)-TDF and (S)-enantiomer was 0.0015 and 0.0012 µg/mL, respectively. (S)-enantiomer was not detected in the formulas from all the seven manufacturers, and the drug content of each took more than 98.5% of the labeled amount, which complies with the regulations. The method shows its advantages on high sensitivity, low detection limit, good practicability, and repeatability. The proposed method may provide a novel platform for separation of TDF enantiomers and quality control of TDF raw materials and preparations.

Keywords Tenofovir disoproxil fumarate · Enantiomers · Chiral resolution · Determination · High-performance liquid chromatography–mass spectrometry

Abbreviations

HPLC–MS	High-performance liquid chromatography–mass spectrometry
TDF	Tenofovir disoproxil fumarate
US FDA	The United States Food and Drug Administration
GC	Gas chromatography
CE	Capillary electrophoresis
GC–MS	GC–mass spectrometry
LC–MS	Liquid chromatography–MS
CE–MS	Capillary electrophoresis–MS
CCC	Counter current chromatography
CSP	Chiral stationary phase
CPMA	Chiral mobile-phase additive

t_R	Retention time
SIM	Select ion monitoring
CD	Cyclodextrin
β -CD	β -Cyclodextrin
CM- β -CD	Carboxymethyl- β -cyclodextrin
HP- β -CD	Hydroxypropyl- β -cyclodextrin
HP- γ -CD	Hydroxypropyl- γ -cyclodextrin
Me- β -CD	Methyl- β -cyclodextrin
SBE- β -CD	Sulfobutylether- β -cyclodextrin
LOD	Limit of detection
LOQ	Limit of quantitation

Introduction

Tenofovir disoproxil fumarate (TDF), a water-soluble tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$, M:519.44), is a prodrug of nucleotide reverse transcriptase inhibitor. It can be rapidly absorbed and hydrolyzed into active tenofovir after oral administration, and then phosphorylated and converted into active tenofovir diphosphate in cells. Tenofovir diphosphate competitively binds itself to the natural substrate

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TDF enantiomers. The effects of chromatographic column filled with cellulose and amylose and the composition of different mobile phases on the separation of TDF enantiomers were investigated. The method for the determination of TDF tablets was established as well. Combined with the high separation ability of HPLC and the high detection ability of mass spectrometry, SIM mode was selected for quantitative analysis of samples, aiming at enhancing the sensitivity and selectivity. The proposed method may provide a novel platform for separation of (R) and (S) TDF enantiomers and chiral quality control of preparation products.

Experimental

Reagents and Materials

Chromatographic-grade acetonitrile and methanol (MeOH) were obtained from Sigma Aldrich (St., Louis, MO, USA). Anhydrous ethanol and n-hexane were obtained from Aladdin Biochemical Technology (Shanghai, China). Isopropanol was obtained from McLean Biochemical Technology (Shanghai, China). β -cyclodextrin (β -CD) was obtained from Sinopharm Chemical Reagent (Shanghai, China). Hydroxypropyl- β -cyclodextrin (HP-CD, $\geq 98\%$), hydroxypropyl- γ -cyclodextrin (HP- γ -CD, $\geq 98\%$), methyl- β -cyclodextrin (Me- β -CD, $\geq 98\%$), and sulfobutylether- β -cyclodextrin (SBE- β -CD, $\geq 98\%$) were obtained from Yuanye Bio-Technology (Shanghai, China). Carboxymethyl- β -cyclodextrin (CM- β -CD, $\geq 99\%$) was obtained from Titan Scientific (Shanghai, China). Redistilled water was used for the experiment.

The drug information of TDF tablets from seven manufacturers is summarized in the Supplemental Materials (Table 1). These tablets were randomly obtained from pharmacies.

Instrumentation

LC-20AD HPLC coupled with MS-2020 single quadrupole mass spectrometer was obtained from Shimadzu (Kyoto, Japan). Unichiral CMD column (4.6×250 mm, $5 \mu\text{m}$) and ChromCore™ CN column (4.6×250 mm, $5 \mu\text{m}$) were obtained from Nano Spectrum Analysis (Suzhou, China). Chiralpak IB N-5 column (4.6×250 mm, $5 \mu\text{m}$) and Chiralcel OJ-RH column (4.6×250 mm, $5 \mu\text{m}$) were obtained from Daicel Chiral Technologies (Tokyo, Japan). C18 column (4.6×250 mm, $5 \mu\text{m}$) was obtained from Zhongpu Technology (Fuzhou, China).

Methods

Chromatographic Conditions

Unichiral CMD (4.6×250 mm, $5 \mu\text{m}$) column was selected for chromatographic separation. The mobile phase was anhydrous ethanol, eluted with isocratic elution. The mobile phase was anhydrous ethanol, eluted with isocratic elution. The flow rate was controlled at 1 mL/min, the detection wavelength was 260 nm, the column temperature was 30°C , and the injection volume was 20 μL .

MS Conditions

Mass spectrometry was conducted using a Shimadzu LC-MS/MS-2020 single quadrupole mass spectrometer equipped with an electrospray ionization interface. SIM in the positive mode was adopted to detect TDF enantiomers. The molecular ion peak $[\text{M} + \text{Na}]^+$ of tenofovir plus sodium was selected as the monitoring ion for (R)-TDF or (S)-enantiomer with m/z of 542. Other parameters were as follows: dry gas flow rate of 3 L/min, atomizer flow of 1.5 L/min, interface temperature of 350°C , DL temperature of 250°C , and heating block temperature of 200°C . The analyses were performed in scan mode within the range of 100–600 m/z .

Table 1 Drug weighing quality and determined results of tenofovir disoproxil fumarate tablets

Manufacturer	Weighing (g)	Percentage of drug content (%) ($n=3$)
Aspen Port Elizabeth	0.2381	100.4
Haisco Pharmaceutical (Meishan)	0.2321	104.7
Chengdu Brilliant Pharmaceutical	0.2324	101.5
Anhui Anke Hengyi Pharmaceutical	0.2267	101.9
Jiangsu Chia Tai-Tianqing Pharmaceutical	0.2180	98.5
Hangzhou Heze Medical Technology	0.2430	101.8
Qilu Pharmaceutical	0.2336	102.3

Standard Solution Preparation

7.50 mg enantiomeric standard of (R)-TDF and 5.00 mg enantiomeric standard of (S)-enantiomer were precisely weighed and dissolved separately in methanol and fixed into 25 mL volumetric flask to prepare (R)-TDF and (S)-enantiomer reserve solution. (R)-TDF with concentrations of 0.100, 1.00, 5.00, 10.0, 20.0, 50.0 and 80.0 $\mu\text{g/mL}$ and (S)-enantiomer with concentrations of 0.020, 0.200, 1.00, 2.00, 4.00, 10.0 and 16.0 $\mu\text{g/mL}$ were obtained through continuous dilution.

Precisely, 1.330 mL of 0.300 mg/mL (R)-TDF solution and 2000 mL of 0.200 mg/mL (S)-enantiomer solution were separately pipetted into a 10 mL volumetric flask. The volume was fixed to the scale with methanol and evenly mixed to prepare a diluted standard solution of (R)-TDF and (S)-enantiomer at the concentration of 40 $\mu\text{g/mL}$. All standard solutions were stored in refrigerator at $-20\text{ }^\circ\text{C}$. The standard solution was filtered with 0.22 μm organic microporous membrane before 20 μL was taken and injected into LC-MS.

Sample Preparation

Ten drug tablets were taken from each of the different manufacturers and weighed accurately. The tablets were ground, and then a certain amount of powder was weighed and dissolved with methanol in the 100 mL capacity bottle. After filtered, 1 mL of the filtrate was pipetted into a 100 mL volumetric flask and diluted to the scale with methanol. Then, the 10 $\mu\text{g/mL}$ sample was prepared and ready to be tested. The exact weighing of each tablet is shown in Table 1.

Results and Discussion

Chiral Additive Method

CD Chiral Additive Method

Among all chiral separation techniques, HPLC is the most simple, reproducible, selective, sensitive, and widely used method. CMPA was employed at the beginning of the experiment because of its simplicity and flexibility [15].

CDs usually contain 6–12 D-glucopyranose units. Each glucose unit forms a slightly conical ring with 1,4-glycoside bond, has a hydrophilic shell and a hydrophobic cavity structure [12, 13]. CDs can form an inclusion complex with the enantiomer for the separation and recognition of chiral compounds [13]. The cyclodextrin containing 7 glucose units called β -CD is the focus of the study [13, 14]. In the experiment, six kinds of CDs, including 15 mmol/mL β -CD, 10 mmol/mL CM- β -CD, 25 mmol/mL HP- β -CD, 20 mmol/

mL HP- γ -CD, 20 mmol/mL Me- β -CD, and 5% SBE- β -CD were dissolved in aqueous phase as chiral additives. On C18, these chiral additives and methanol or acetonitrile formed a chiral mobile phase to separate TDF enantiomers. However, the results showed that no separation occurred within 60 min. Then, the TDF enantiomers were separated on a normal-phase ChromCore™ CN column. First, 5 mmol/mL Me- β -CD was used as chiral additive. The mobile phases were 5 mmol/mL and 0.5 mmol/mL Me- β -CD with ethanol:n-hexane (35:65), respectively. The composition of the mobile phase is 5 mmol/mL Me- β -CD with ethanol:n-hexane (35:65) was too sticky for the experiment. The TDF isomer was injected at the concentration of 0.5 mmol/mL Me- β -CD with absolute ethanol:n-hexane (35:65) as the mobile phase, and the separation was not realized.

Crown Ether Chiral Additive

Crown ethers are macrocyclic polyether compounds that can bind to some cations, such as basic ions or protonated primary amines, and are potential chiral additives with high selectivity and affinity [18]. Using 18-crown ether-6 as a chiral additive in ethanol and n-hexane as mobile phase, resolution experiments were carried out on a normal-phase ChromCore™ CN column. The results showed that the enantiomers of TDF were not separated.

In conclusion, the CMPA using CDs and its derivatives or crown ethers as additives cannot achieve the separation of TDF enantiomers.

Analysis of (R)-Tenofovir and (S)-Enantiomer by CSP and LC-MS

Optimization of Chromatographic Conditions

CSPs were a common approach to chiral chromatographic separation, and polysaccharide-based CSPs derived from cellulose and amylose are the most commonly used CSP in HPLC [19–22]. To separate the two enantiomers of TDF, we investigated the effects of three chromatographic columns and different mobile phases on the separation of (R)-tenofovir and (S)-enantiomer. The chromatographic columns included CHIRALPAK IB N-5 [cellulose-tris(3,5-dimethylphenylcarbamate) immobilized to silica gel], CHIRALCEL OJ-RH [Cellulose tris(4-methylbenzoate) coated on silica gel], and Unichiral CMD [Amylose-tris(3,5-dimethylphenylcarbamate)]. The results showed that the separation effects differed from different surface functional groups and polysaccharides.

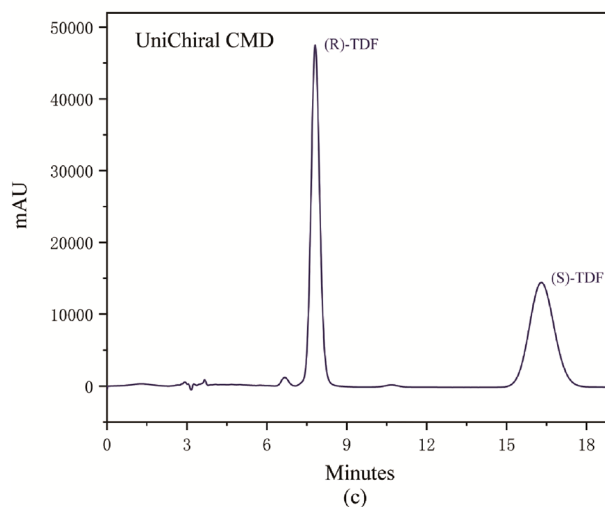
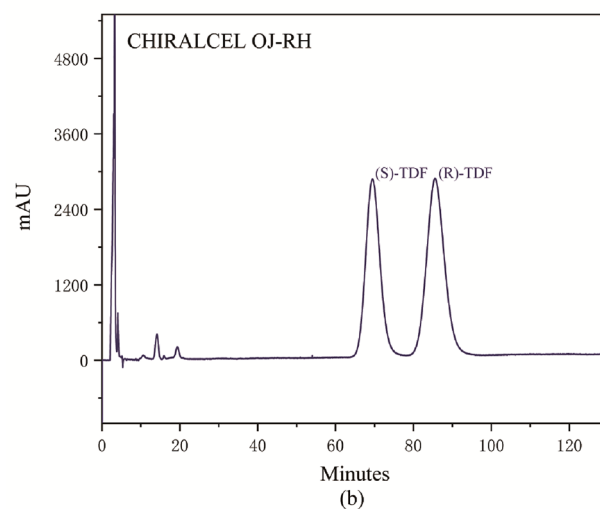
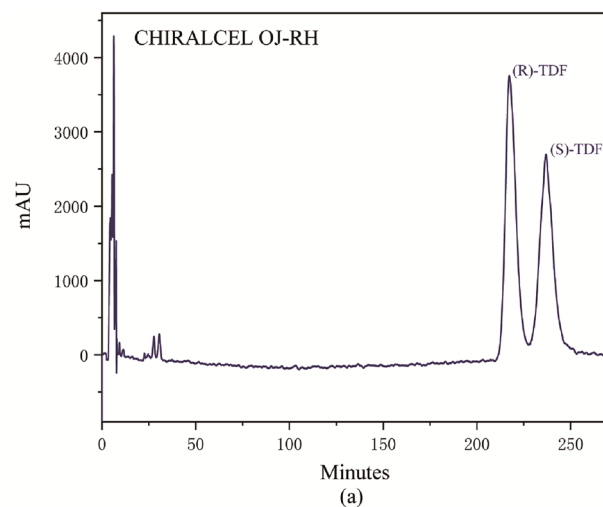
CHIRALPAK IB N-5 is a normal-phase column. N-hexane and ethanol were chosen to compose the mobile phase, and the separation effect of different proportions of n-hexane and ethanol on (R)-TDF and (S)-enantiomer was determined.

Fig. 2 Typical chromatograms of (R)-tenofovir disoproxil fumarate and its enantiomer by CHIRALCEL OJ-RH and Unichiral CMD column **a** CHIRALCEL OJ-RH, water: acetonitrile (87:13), 0.5 mL/min; **b** CHIRALCEL OJ-RH, water: methanol (50:50), 0.8 ml/min; **c** Unichiral CMD, ethanol (100%), 1.0 mL/min

When the mobile phase was n-hexane:anhydrous ethanol (80:20) at the flow rate of 0.6 ml/min, both (R)-tenofovir and (S)-enantiomer were eluted simultaneously at 33.88 min, which means the separation was not achieved under this condition. At ethanol ratios of 100%, 95%, 90%, and 85%, the retention time of mixed analytes was shortened to 8.53–8.89 min, and no peak separation was observed. Under above conditions, no resolution was observed with CHIRALPAK IB N-5 as stationary phase. Therefore, the column is considered as not suitable for the separation of (R)-TDF and (S)-enantiomer.

Then, CHIRALCEL OJ-RH column and Unichiral CMD column were chosen for the study forward. The CHIRALCEL OJ-RH chiral reverse phase column was coated with cellulose-tris(4-methylbenzoate) on the surface of silica gel. The results showed that when the mobile phase was water:acetonitrile (87:13) at the flow rate of 0.5 mL/min, (R)-tenofovir and (S)-enantiomer could be separated. The t_R of (R)-TDF and (S)-enantiomer were 217.29 min and 236.81 min, the peak width at half height were 5.95 min and 7.37 min, and the numbers of theoretical plates were 7355 and 6804. The degree of separation was 1.81, but the analysis was time-consuming. As shown in Fig. 2a, the separation effect was not ideal. To shorten the separation time and improve the separation degree, 0.1% tetrahydrofuran was added to the mobile phase, but the results showed that the separation effect was not good. When the mobile phase was water:methanol (50:50) at the flow rate of 0.8 ml/min, the results showed that the separation effect of (R)-tenofovir and (S)-enantiomer was the best, and the separation degree was 2.17. As shown in Fig. 2b, the retention times of (R)-tenofovir and (S)-enantiomer were 85.51 min and 69.48 min, shorter than the previous values, with half-peak widths of 3.99 min and 4.92 min, respectively.

(R)-tenofovir and (S)-enantiomer can be well separated on a Unichiral CMD column with amylose-tris(3,5-dimethylphenylcarbamate) as stationary phase when the mobile phase was ethanol, and the flow rate was 1.0 mL/min. The retention times of (R)-tenofovir and (S)-enantiomer were 7.787 min and 16.164 min, and the half-peak widths were 0.36 min and 1.02 min, respectively. The degree of resolution was 7.153. When Unichiral CMD was chosen as the separation column, the retention time of (R)-tenofovir and (S)-enantiomer was greatly shortened, and the separation effect was effectively improved, as shown in Fig. 2c. The results indicated that amylose-tris(3, 5-dimethylphenylcarbamate) as stationary phase was more suitable for the separation of



(R)-TDF and (S)-enantiomer than cellulose-tris(3, 5-dimethylphenylcarbamate) and cellulose-tris(4-methylbenzoate) in CSP method.

Amylose-tris(3, 5-dimethyl-phenylcarbamate) is a polysaccharide-based chiral selector. The separation is thought to be achieved by the formation of hydrogen bonds between the –NH or –CO groups on the carbamate structure and the analyte, as well as by π – π and van der Waals interactions with the benzene ring. The carbamate bond is around the cavity of the main chain of polysaccharide, providing more flexibility to the benzene ring, thus the cavity can be adjusted for different analytes, and the π – π interaction can be maximized [23]. The (R)-TDF and (S)-enantiomer contain C=O and –NH functional groups, which can form hydrogen bonds with carbamates in amylose-tris(3,5-dimethylphenylcarbamate). (R)-tenofovir and (S)-enantiomer have different structures, and the hydrogen bond forces between them and stationary phase are different. Therefore, a high degree of separation can be obtained on UniChiral CMD column.

Optimization of MS Conditions

The mixed solution of TDF was analyzed by mass spectrometry, and Fig. 3 shows the mass spectra of 50 $\mu\text{g/mL}$ (R)-TDF and 10 $\mu\text{g/mL}$ (S)-enantiomer with retention times of 7.787 min and 16.164 min, respectively. The ion peak of m/z 520.2 was tenofovir dipivoxil quasi-molecular ion $[M + H]^+$ and that of m/z 542.2 was $[M + Na]^+$, the two major fragment ion peaks were m/z 300.2 and 270.1. Figure 4 shows cleavage diagram of tenofovir disoproxil.

In comparison with the full scanning mode, SIM enables the mass spectrometer to spend more time in detecting the ion current of the selected ions, thereby improving the sensitivity for quantitative analysis. Based on the results of full scan (Fig. 3), the signal intensity of ion peak $[M + Na]^+$ was the highest. Therefore, $[M + Na]^+$ was selected as the quantitative measuring ion of (R)-TDF and (S)-enantiomer. Figure 5 shows the selective ion monitoring chromatograms of (R)-TDF and (S)-enantiomer standard and sample solutions with $[M + Na]^+$ selected as quantitative monitoring ions.

According to the retention time of chromatographic peak, mass spectrum data, and literature [8], the two peaks at 7.633 min and 16.100 min were attributed to (R)-tenofovir and (S)-enantiomer, respectively.

Method Validation and Performance

Calibration Curve, Precision, Repeatability, and Stability Test

The calibration curve was obtained by triple injection of a series of working solutions and constructed by plotting the peak areas (A) versus reference concentration (C , $\mu\text{g/}$

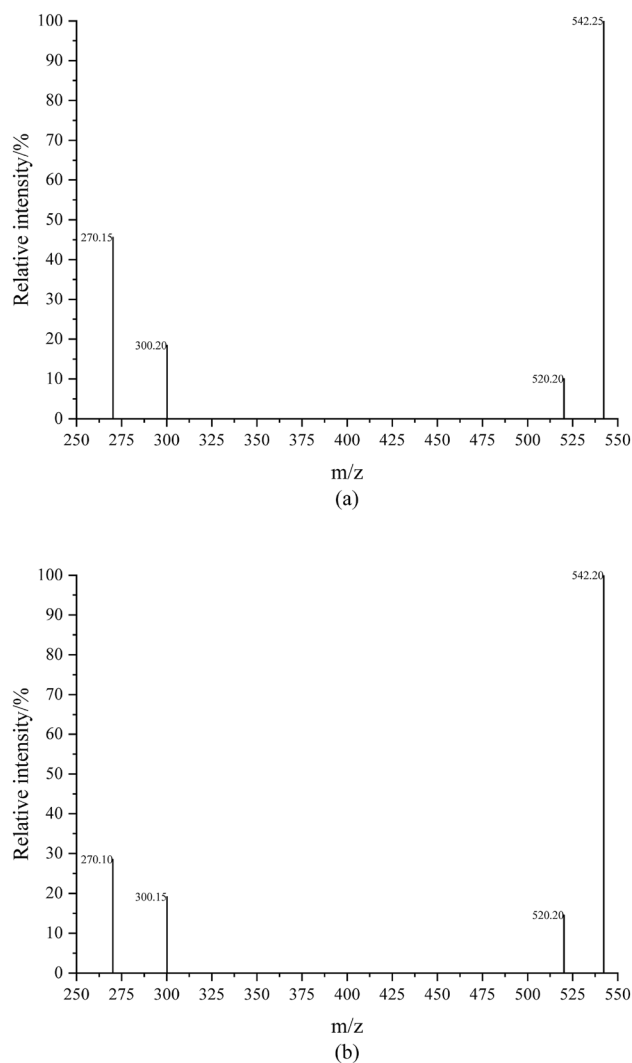
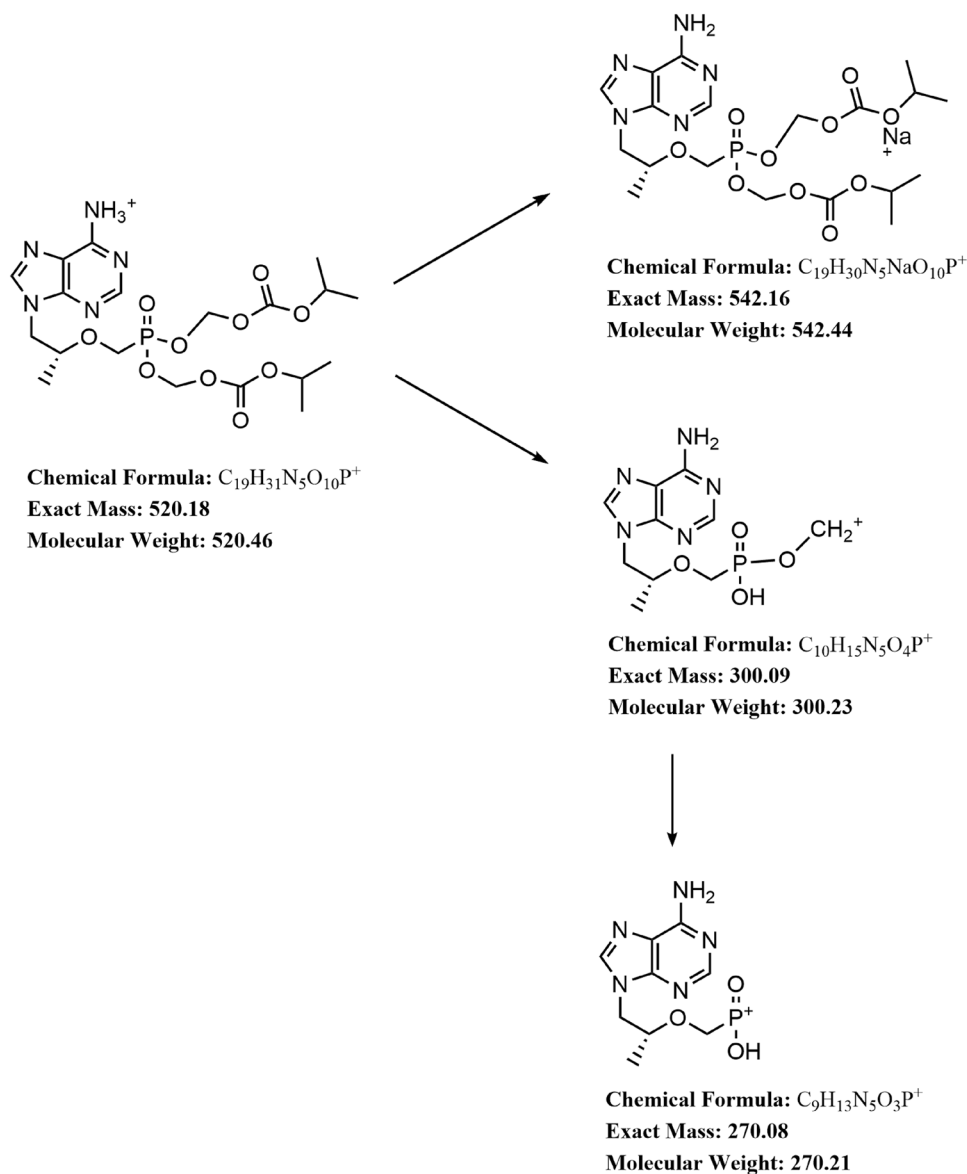


Fig. 3 Mass spectra of mixed standards of 50 $\mu\text{g/mL}$ (R)-TDF and 10 $\mu\text{g/mL}$ (S)-TDF with retention time of 7.787 and 16.164 min **a** Mixed solution of 50 $\mu\text{g/mL}$ (R)-TDF and 10 $\mu\text{g/mL}$ (S)-TDF Standard; **b** 20 $\mu\text{g/mL}$ TDF solution (Aspen Port Elizabeth (Pty) Ltd, Batch number: A867401)

mL). Table 2 shows that the compounds have a good linear relationship in the corresponding linear range, with the corresponding correlation coefficient (r^2), linear range ($\mu\text{g/mL}$), LOD, and LOQ. The limit of detection (LOD) and limit of quantitation (LOQ) of (R)-TDF and (S)-enantiomer were calculated at S/N values of 3 and 10.

Mixed standard solutions of (R)-TDF and (S)-TDF at concentrations of 5 $\mu\text{g/mL}$, respectively, were prepared as QC samples. And during the LC–MS experiment, a QC sample was injected after every 10 injections of sample analysis to verify the stability of the instrument. The results showed that the retention times of all QC samples were stable and the RSD of signal response was less than 5.0%, indicating good instrument stability.

Fig. 4 Shows cleavage diagram of tenofovir disoproxil



For precision, the mixed reference solution of 5 µg/ml (R)-tenofovir and 1 µg/ml (S)-enantiomer was selected. The chromatographic peak areas of (R)-tenofovir and (S)-enantiomer were determined ($n=6$). The RSD values of (R)-tenofovir and (S)-enantiomer were 1.14% and 1.42%, respectively, indicating that the precision of the method was good.

For repeatability, the mixed reference solution of 5 µg/ml (R)-tenofovir and 1 µg/ml (S)-enantiomer was selected, and its response for 3 continuous days was measured by MS. The RSD values of (R)-tenofovir and (S)-enantiomers were determined to be 1.34% and 1.68% ($n=6$), respectively, indicating that the method had good repeatability.

For stability, TDF solution (production batch no: a867401, Aspen Port Elizabeth Pty. Ltd.) was prepared by the method under item ("Sample Preparation" section)

and placed under room temperature for 0, 1, 2, 4, 8, and 12 h. Then, 20 µL of the solution was injected for LC-MS analysis at each time point. The RSD values of chromatographic peak area of (R)-tenofovir and (S)-enantiomers were determined to be 1.83% and 2.26% ($n=6$), respectively, indicating that the stability of the test sample was good within 12 h.

Percentage of Labeled Amount Test

The sample solution prepared under the condition of item ("Sample Preparation" section) was filtered with 0.22 µm organic microporous membrane, and the content was determined by LC-MS. The percentage of labeled amount of TDF tablets produced by seven manufacturers was 98.5%–104.7%

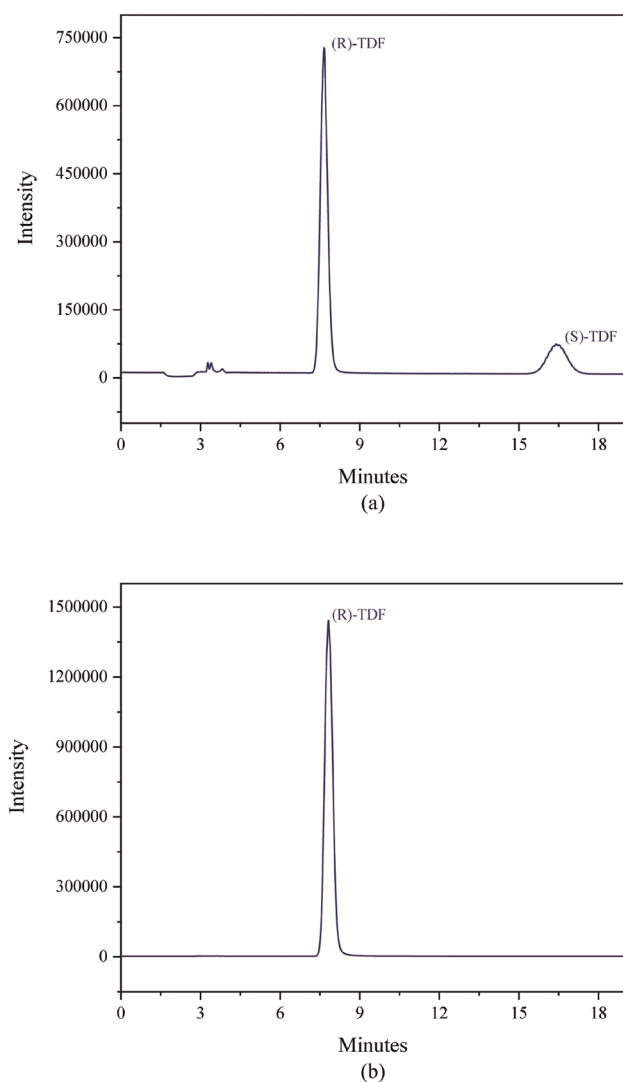


Fig. 5 Typical selective ion monitoring chromatograms of (R)-tenofovir disoproxil and its enantiomer **a** Mixed solution of 10 µg/mL (R)-TDF and 2 µg/mL (S)-TDF Standard **b** 20 µg/mL tenofovir fumarate disofuroximate solution (Brilliant Pharmaceutical)

of the labeled amount (Table 1), and the (S)-enantiomer was not detected. At present, TDF is not included in the 2020 edition of Chinese Pharmacopoeia. In 2020, the Chinese Pharmacopoeia Commission released the draft National Drug Standard Table of tenofovir disoproxil fumarate, which stipulated that the percentage of labeled amount of drug should be at least 98.5% of the labeled amount [24]. The area of each impurity peak shall not be larger than that of the main peak of the

Table 3 The result of recovery test

Components to be tested	Standard addition level	Original content (µg)	Addition amount (µg)	Average recovery (%)
(R)-TDF	80%	5.00	3.00	105.2
	80%	5.00	3.00	
	80%	5.00	3.00	
	100%	5.00	5.00	98.8
	100%	5.00	5.00	
	100%	5.00	5.00	
	120%	5.00	7.00	99.0
	120%	5.00	7.00	
(S)-enantiomer	80%	0	0.60	95.7
	80%	0	0.60	
	80%	0	0.60	
	100%	0	1.00	95.1
	100%	0	1.00	
	100%	0	1.00	
	120%	0	1.40	96.7
	120%	0	1.40	

control solution, if there is any (impurity limit < 1.0%). If the peak area is smaller than that of the main peak of the sensitivity solution, the peak area in the chromatogram of the test solution is negligible, and the percentage of labeled amount of TDF tablets produced by seven manufacturers meets this standard. According to relevant literature, the percentage of labeled amount of TDF in Chinese National Medical Products Administration import drug registration standard (No: jx20080009) is 90%–105% [25], and that of TDF tablets produced by seven manufacturers also meets this standard. The USP Medicines Compendium requires that the percentage of labeled amount of TDF be between 98.0% and 102.0% [26]. The percentage of labeled amount of TDF tablets produced by five manufacturers meets this standard. The Indian Pharmacopoeia requires that the percentage of labeled amount of TDF be between 90% and 110.0% [27], and the percentage of labeled amount of TDF tablets produced by seven manufacturers meets the standard.

In summary, the drug content of TDF tablets produced by seven manufacturers met the standard of the Draft National Drug Standard for TDF issued by the Chinese

Table 2 Regression equations, correlations coefficients (r^2) linear ranges, LOD, LOQ

Compound	Linear equation	r^2	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
(R)-TDF	$A = 1989590c + 12351.2$	0.9928	0.1–20	0.0015	0.005
(S)-enantiomer	$A = 2958970c + 8019.2$	0.9993	0.2–16	0.0012	0.004

Table 4 Comparison of the present method with other published methods for the enantiomeric separation of TDF

Method	Analyte	LOD	LOQ	Recovery rate (%)	Reference
HPLC–MS	(R)-TDF	0.0015	0.005	98.8–105.2	The method of this paper
	(S)-TDF	0.0012	0.004	95.1–96.7	
HPLC	(S)-TDF	0.005	0.015	98.7–109.2	Gao L. Y. et al. [11]
HPLC	(S)-TDF	0.02	0.07	98.67–101.0	Seshachalam U et al. [16]
HPLC	(S)-TDF	0.06	0.2	99.2–105.0	Heydari R. et al. [8]
HPLC	(S)-TDF	0.15	0.45	99.34–101.59	Nguyen B. T. et al. [5]

Pharmacopoeia Commission, and standard for TDF of Indian Pharmacopoeia; five manufacturers met the standard of American Pharmacopoeia.

Sample Recovery Rate

TDF solution (production batch no: 210104, Beite pharmaceutical) was prepared by the method under item ("Sample Preparation" section). The solution was added with appropriate mixed reference solution at low, medium, and high levels, and the volume was fixed to 2 mL. Precisely, 20 μ L of the solution was injected into LC–MS after filtered through a 0.45 μ m microporous membrane. The content of each component was calculated according to the standard curve. The experimental results are shown in Table 3. The recovery of this method in the sample ranged from 95.1% to 105.7%, indicating good recovery. The recovery rate is calculated using the following formula:

$$\text{Recovery rate (\%)} = 100 \times \frac{A}{B + C},$$

where: A: measured quantity of samples added with reference materials B: "true value" of the substance in the sample C: standard substance quality added

Comparison Between LC–MS and Other Methods

Limited studies have investigated the enantiomeric separation and quantification of TDF, and the quantitative determination of TDF by SIM has not been reported. In the present paper, the LOD of LC–MS method was lower than that of general method, and the result of qualitative analysis was reliable. The results are summarized in Table 4.

Conclusion

In this paper, the LC–MS method was used for the quantitative analysis of TDF, and the sensitivity was greatly improved in the SIM mode. The values of LOD and LOQ were less than 1/2 of the literature data. The (S)-enantiomers

were not detected in the TDF tablets produced by seven manufacturers, and the drug content were all above 98.5% of labeled amount. In addition, under the conditions of reversed- and normal-phase chromatography, several CD chiral additives were excluded for they are not feasible in CMPA method for TDF resolution. Finally, the results confirm that the Unichiral CMD column with amylose-tris(3,5-dimethylphenyl carbamate) as CSP was suitable for the chiral separation of TDF. The method established in this study showed its advantages on high sensitivity, low detection limit, good practicability, and repeatability. It was successfully applied to the separation and quantification of TDF and its isomers. The method provides a sensitive, simple and rapid approach for the quality control of TDF.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10337-023-04240-9>.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Li Lin and Yanbing Ke. The first draft of the manuscript was written by Li Lin. Research program planning, writing-review & editing were performed by Lingyi Huang. Methodology and formal analysis were done by Xiaowen Xu. Conceptualization, writing-review & editing, supervision, project administration, funding acquisition were performed by Liying Huang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Availability of data is available upon request from the corresponding author.

Declarations

Conflict of Interest The authors have declared no conflict of interest.

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