Pavani and Susithra
Future Journal of Pharmaceutical Sciences (2022) 8:2
https://doi.org/10.1186/s43094-021-00390-5

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

A novel simultaneous high performance liquid chromatography-PDA method for the determination of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat in bulk and its application to marketed formulation

Challamalla Pavani and E. Susithra*

Abstract

Background: The present research article involves the simultaneous determination of Tenofovir alafenamide, Darunavir, Emtricitabine and Cobicistat in bulk as well as in tablet dosage form using high performance liquid chromatography.

Result: The separation was performed using DIKMA Spursil, C_{18}, ODS, (4.6 × 150 mm × 5 µm) analytical column using the mobile phase acetonitrile and 0.1% Orthophosphoric acid in the volume ratio of 70:30 at pH 3. The eluents were detected using PDA detector at 254.0 nm. After optimization subsequent validation study of different parameters was performed by utilizing the optimised condition as per the ICH guidelines. Under this optimised conditions Tenofovir alafenamide, Darunavir, Emtricitabine and Cobicistat were eluted at 2.287 min, 2.507 min, 4.062 min, 6.011 min respectively. Percentage assay was found 99.21% for Tenofovir alafenamide, 99.80% for Darunavir, 99.80% for Emtricitabine and 99.84% for Cobicistat. Tenofovir alafenamide was found linear in the range of 2.0–10.0 µg/mL, Darunavir (160.0–800.0 µg/mL), Emtricitabine (40.0–200.0 µg/mL) and for cobicistat (30.0–150.0 µg/mL). The corelation coefficient was found 0.999 for all the APIs. The detection limit was found 0.14 µg/mL for Tenofovir alafenamide, 2.14 µg/mL for Darunavir, 0.6 µg/mL for Emtricitabine and 7.32 µg/mL for cobicistat. In the LOQ study the quantitation limit was found 0.47 µg/mL for Tenofovir alafenamide, 7.12 µg/mL for Darunavir, 2.10 µg/mL, for Emtricitabine and 24.42 µg/mL for cobicistat.

Conclusion: All the studied API's has been highly resolute utilizing the optimised condition and found extremely suitable for the determination of all of them simultaneously in marketed dosage form as well as in the bulk form.

Keywords: Tenofovir alafenamide, Darunavir, Emtricitabine, Cobicistat, HPLC

Background

Antiretroviral therapy (ART) for HIV-1 infection has vastly improved over time [1], and HIV-1 is currently regarded as a chronic but manageable condition [2, 3]. The first protease inhibitor (PI)-based single-tablet regimen for treatment-naive or some treatment-experienced persons living with HIV is the fixed-dose combination tablet darunavir-cobicistat-tenofovir alafenamide-emtricitabine [4]. Symtuza is a prescription medicine approved by the United States Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children weighing at least 88 pounds (40 kg) who

*Correspondence: susithra123e@gmail.com
Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamilnadu 600117, India

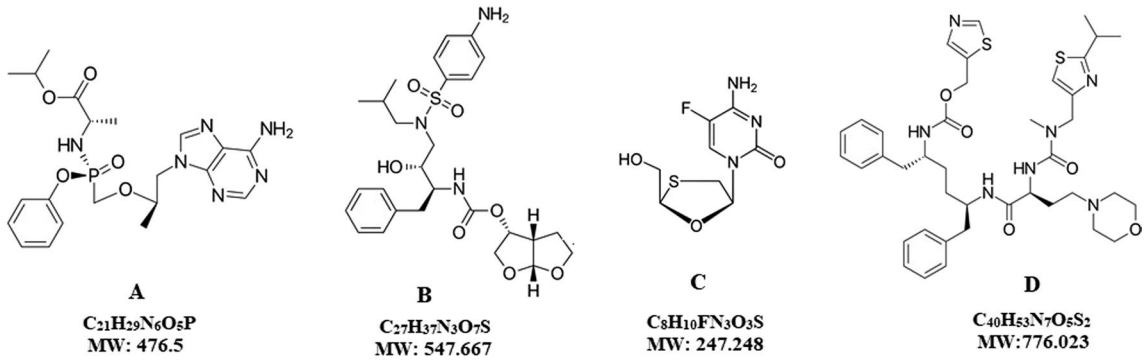

Fig. 1 Chemical structures of Tenofovir AF (**A**), Darunavir (**B**) Emtricitabine (**C**), Cobicistat (**D**)

have never taken HIV medications before or who meet certain criteria as determined by a health care providers [5]. Symtuza decreases the amount of HIV-1 in the blood and keeps it at a low level by blocking protease. Cobicistat works as a 'booster,' extending the antiviral effects of darunavir by slowing down its breakdown and therefore prolonging its antiviral activity in the body. Tenofovir alafenamide is a tenofovir 'prodrug,' meaning that it is transformed in the body into the active component tenofovir is a reverse transcriptase inhibitor. Emtricitabine is a reverse transcriptase inhibitor that functions similarly to tenofovir [6, 7]. The chemical structure of all the four compounds was shown in Fig. 1. The extensive literature review has been performed for the simultaneous determination of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat and it was observed that no such methods was available in any pharmacopeial official monograph. There is an availability of few reported method for the determination each of the studied drug with other antiviral agents, which is not related to the present simultaneous dosage form. Only two reported published method was available for the combination of present four APIs for which we developed the method for. In one of the UPLC method [8] reported by Gandi et al., in 2021, found huge baseline noise in the LOD chromatogram. The reported lower level of the linearity was very high, which questioned about the sensitivity. In the reported degradation chromatograms, no degradation peaks was observed but degradation values up to 5% has been cited in the table, which is quite surprising. In another HPLC method [9], reported by Parameswari et.al, shows very narrow resolution between the eluted compounds, which can be improve. The lower level of the linearity reported is high in almost all of the APIs, which can be possible to minimize. The authors utilised the in house formulation for the assay instead of marketed formulation and even not provide specified information about the content amount and type of doses form prepared. This is not justifiable as per the ICH guidelines. After considering the aforementioned drawbacks in the previously published methods, and to understand the importance of wide applicability of this combined dosage form of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat, there is a need to develop and report a reliable, simple and validated method for the simultaneous quantification of Tenofovir AF, Darunavir, Emtricitabine and cobicistat in bulk and in marketed formulations. Therefore in this present method we had taken an effort to eradicate the mentioned drawbacks and to develop a fast, easy reliable HPLC method for the Tenofovir AF, Darunavir, Emtricitabine and Cobicistat in bulk and marketed tablet dosage form and to validate as per the ICH Q2B guidelines of analytical method[10] development and validation.

Methods

Pure samples

Pure Tenofovir AF, Darunavir, Emtricitabine and Cobicistat active pharmaceutical ingredients with 99.19%, 99.56% and 99.13% purity were procured from Honour labs, R&D division, Bonthapally, Hyderabad, India.

Formulation

Marketed tablets were purchased from the local pharmacy shop in Hyderabad, India. The water and methanol used was HPLC grade and obtained from Lichrosolv, Merck, Mumbai).

Chemicals reagents and instruments

HPLC grade acetonitrile which are used were obtained from Molychem, Mumbai, India. Ortho phosphoric acid was obtained from Finer chemical limited, Mumbai, India. The Adwa AD 1020 pH meter was used and Afcoset ER 200A weighing balance was used. Chromatographic separation was achieved on Waters 2695

separation module, equipped with empower 3 software, with PDA detector. Detector wavelength was set at 254.0 nm and 1 mL/min flow rate was maintained for the mobile phase. The separation was performed using DIKMA Spursil, C_{18}, ODS, (4.6 × 150 mm × 3 µm) analytical column. The used mobile phase was the mixture of acetonitrile and 0.1% Orthophosphoric acid in the volume ratio of 70:30 at pH 3.

Preparation of 0.1% OPA buffer

Accurately taken 1 mL of orthophosphoric acid (HPLC Grade) dissolve in 1000 mL of HPLC grade water. And adjust the pH with diluted Sodium hydroxide up to 3.0, finally the solution was filtered by using 0.45 Micron membrane filter, and sonicate it for 10 min.

Preparation of mobile phase

Accurately measured 300 mL (30%) of above buffer and 700 mL (70%) of Acetonitrile for HPLC were mixed and degassed in an ultrasonic water bath for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

Standard solution preparation

Accurately weigh and transfer 10 mg of Tenofovir AF, 800 mg of Darunavir, 200 mg of Emtricitabine and 150 mg of Cobicistat working standard into a 100 mL clean dry volumetric flask add about 70 mL of diluent (mobile phase composition) and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.6 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent to achieve 6.0 µg/mL of Tenofovir AF, 480.0 µg/mL of Darunavir, 120.0 µg/mL of Emtricitabine, and 90.0 µg/mL of Cobicistat.

Preparation of sample solution for the assay of combined tablet

Accurately weigh 10 tablets and crush in the mortar and pestle. Accurately transfer the tablet powder (1420 mg) equivalent to 10.0 mg of Tenofovir AF, 800 mg of Darunavir, 200 mg of Emtricitabine and 150.0 mg of Cobicistat into a 100 mL clean dry volumetric flask. 10 mL of acetonitrile as added to dissolve, and 50 mL of diluent was also added and sonicate to dissolve the content completely. Filter the entire solution using membrane filter. The volume was made up to the mark with the diluent and further pipette 0.6 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent and injected in to the chromatographic system.

Validation study of the developed method

Precision

For the precision study of the present method, 6.0 µg/mL of Tenofovir AF, 480.0 µg/mL of Darunavir, 120.0 µg/mL of Emtricitabine, and 90.0 µg/mL of Cobicistat spiked solution was prepared from the primary standard stock solution. The prepared solution was injected for six times and measured the area for all six injections using the HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The % RSD for the area of six standard injections results should not be more than 2%.

Intermediate precision

For this study the spiked solution of all four APIs was prepared like precision study and was injected on the other day for six times and measured the area for all six injections in HPLC. The % RSD for the area of six standard injections results should not be more than 2%.

Accuracy

Accuracy of the present developed method was studied by recovery study. The fixed dose combination tablet powder was kept constant and standard solutions of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat were spiked at the levels of 50%, 100% and 150%. The total amount obtain was calculated for each concentration level. Percentage recovery was calculated for each level and overall recovery were calculated for the all studied drugs. The % Recovery for each level should be between 98.0 and 102.0%.

System suitability

It was performed to validate whether an analytical system working properly. It was evaluated by injecting the working standard drugs of Tenofovir AF (6.0 µg/mL), Darunavir (480.0 µg/mL) and Emtricitabine (120.0 µg/mL) and cobicistat (90.0 µg/mL) six times. Several parameters like theoretical plate, retention time, asymmetric factor has been considered for the calculation of percentage relative standard.

Linearity

For the study of linearity of the present developed method, different levels of the Tenofovir AF, Darunavir, Emtricitabine and Cobicistat has been prepared from the standard solution with suitable dilution. For Tenofovir AF 2.0–10.0 µg/mL, for Darunavir 160.0–180.0 µg/mL, for Emtricitabine 40.0–200.0 µg/mL, and 30.0–150.0 µg/mL solutions were prepared at different levels and injected for the linearity study. Calibration curve was plotted for

all the drugs at different levels. Concentration and peak area were considered to construct the linearity graph and obtained data was subjected to regression analysis.

Limit of detection

For the study of detection limit of the individual component of the spiked solution, the specified sample has been prepared from the standard stock solution which contains Tenofovir AF (6 µg/mL), Darunavir (480 µg/mL) and Emtricitabine (120 µg/mL) and cobicistat (90 µg/mL). For the Tenofovir AF 2 mL was pipetted in to the 10 mL flask, filled with diluent and further 1.19 mL was transferred to 10 mL volumetric flask and volume was filled with the diluent to achieve 0.14 µg/mL of tenofovir. Similarly, for darunavir 2 mL of standard solution was diluted to 10 mL, and further 0.45 mL was diluted to 10 mL with diluent to obtained 2.14 µg/mL. For Emtricitabine, 1 mL of the standard solution was diluted to 10 mL and further 0.5 mL was diluted to 10 mL to achieve 0.6 µg/mL. Cobicistat LOD solution was prepared by diluting 6 mL of the standard solution to 10 mL, then 1.36 mL from the prepared solution was transferred and diluted to 10 mL with the diluent to obtain 7.32 µg/mL of Cobicistat. Signal to noise ratio was calculated from the baseline and signal noise and should be 3.

Limit of quantitation

For the study of quantitation limit, sample solutions were prepared from the working standard.

Solution. 0.47 µg/mL of Tenofovir AF, 7.12 µg/mL of Darunavir, 2.10 µg/mL of Emtricitabine, 24.42 µg/mL of Cobicistat diluted solutions were prepared from the standard solution by diluting suitable volumes with the diluent in 10 mL of volumetric flask. The prepared solutions were injected into the chromatographic system for the analysis. Signal to noise ratio was calculated from the baseline noise and signal and quantitation limit ratio should be 10.

Robustness

In the robustness study of the present developed method, several optimised parameters like, flow rate of the mobile phase, Organic composition of the mobile phase, detection wavelength of the optimised conditions were deliberately changes in minor level. The flow rate was changes at the level of ± 0.1, The organic composition was changes to $\pm 10\%$, and the detection wavelength was changes to ± 2 nm. The peak areas in all such conditions for all the studied APIs were considered to observed the robustness. The percentage relative standard deviation was calculated for the parameters and should not be more than 2.

Force degradation study

Force degradation study of the present sample solution [11] was conducted using ICH prescribed stress condition such as acidic, alkaline, oxidative, thermal and

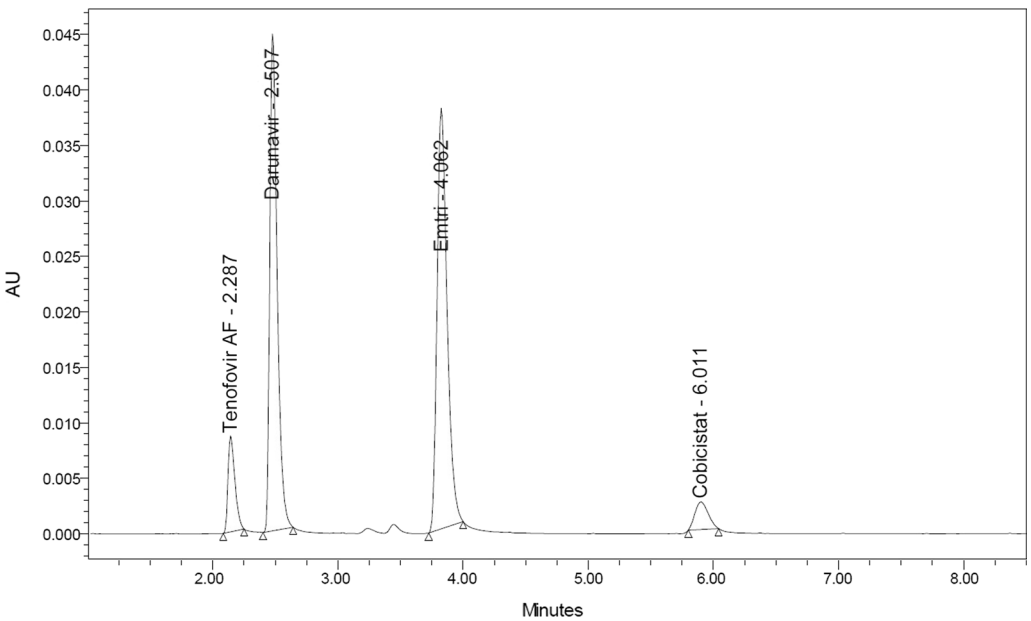

Fig. 2 Optimised chromatograms of Tenofovir AF, Darunavir, Emtricitabine, Cobicistat using optimised chromatographic conditions

Table 1 Assay of marketed formulations

Formulation	Tenofovir AF			Darunavir			Emtricitabine			Cobicistat		
	Amount taken (mg)	Amount obtained (mg)	% Assay*	Amount taken (mg)	Amount obtained (mg)	% Assay*	Amount taken (mg)	Amount obtained	% Assay*	Amount taken	Amount obtained	% Assay*
Symtuza tablets (Tenofovir AF + Darunavir + Emtricitabine + Cobicistat)	10	9.921	99.21	800	798.42	99.80	200	199.62	99.81	150	149.76	99.84

* Average of three replicates

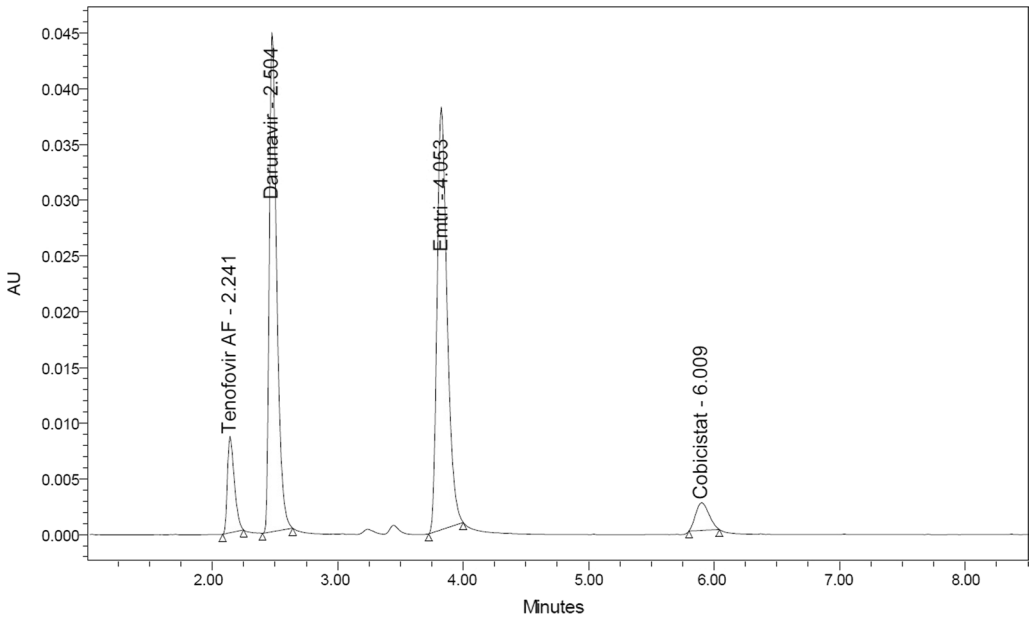

Fig. 3 Assay chromatograms of Tenofovir AF, Darunavir, Emtricitabine, Cobicistat marketed formulation

Table 2 Results of the validation parameters

Parameters	Tenofovir	Darunavir	Emtricitabine	Cobicistat
Precision (%RSD)	1.0	0.6	0.8	0.6
Intermediate precision (%RSD)	0.2	0.8	0.2	0.9
Accuracy (Mean recovery)	100.11	100.26	100.64	100.08
Linearity (µg/mL)	2–10	160–800	40–200	30–150
Regression coefficient	0.999	0.999	0.999	0.999
LOD (µg/mL) (signal to noise ratio method)	0.14	2.14	0.6	7.32
LOQ(µg/mL) (signal to noise ratio method)	0.47	7.12	2.10	24.42

Table 3 Robustness study

Parameters	Changes done	Tenofovir	Darunavir	Emtricitabine	Cobicistat
Change in flow rate		Area*	Area*	Area*	Area*
	0.9 mL	59,662	345,373	428,300	36,922
	1 mL	62,377	343,268	410,983	37,868
	1.1 mL	61,702	330,567	403,890	36,036
Organic composition in mobile phase	10%less	60,835	334,251	418,302	36,554
	Actual	62,377	343,268	410,983	37,868
	10% more	61,473	319,463	420,831	37,831
Detection wavelength	252 nm	59,870	325,549	408,822	37,402
	254 nm	62,377	343,268	410,983	37,868
	256 nm	61,352	338,546	412,297	36,451
% RSD	–	1.64	1.85	1.89	1.32

* Average of three readings

Table 4 Result of the forced degradation study

Stressed conditions	Tenofovir			Darunavir			Emtricitabine			Cobicistat		
	% Degradation	Purity Angle	Purity Threshold	% Degradation	Purity Angle	Purity Threshold	% Degradation	Purity Angle	Purity Threshold	% Degradation	Purity Angle	Purity Threshold
Acid	3.64	0.169	1.009	3.31	0.137	1.013	4.67	0.223	1.005	5.08	0.144	1.012
Alkaline	3.10	0.308	1.672	3.70	0.654	1.857	3.13	0.126	1.152	4.57	0.193	1.642
Peroxided	3.46	0.206	1.034	7.51	0.423	1.797	3.76	0.735	1.310	6.24	0.190	1.856
Photolytic	3.92	0.289	1.673	4.89	0.486	1.538	7.74	0.187	1.563	5.49	0.397	1.675
Thermal	3.76	0.217	1.429	5.02	0.265	1.497	4.59	0.543	1.241	5.64	0.315	1.211

photolytic stress conditions. All the types of degradation studies have been performed in triplicate and mean peak area has been considered for the calculation.

Degradation under acidic condition

Pipette 2 mL of above solution into a 10 mL volumetric flask and 3 mL of 0.1N HCl was added. Then, the volumetric flask was kept at 60°C for 6 h and then neutralized with 0.1N NaOH and make up to 10 mL with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Degradation under alkaline condition

Pipette 2 mL of above solution into a 10 mL volumetric flask and 3 mL of 0.1N NaOH was added in 10 mL of volumetric flask. Then, the volumetric flask was kept at 60°C for 6 h and then neutralized with 0.1N HCl and make up to 10 mL with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation

Tenofovir, Darunavir, Emtricitabine and Cobicistat sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 h. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation

Pipette 2 mL of above stock solution into a 10 mL volumetric flask and 1 mL of 3% w/v of hydrogen peroxide added in 10 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photolytic degradation

The photolytic degradation was done by exposing of drug content under the UV light for 15 min to 7 days. The drug degradation observed in the above specific photolytic degradation condition.

Results

Method optimization

For the optimization of the developed method, preliminary trials has been conducted sufficiently by varying the several parameters. Methanol, acetonitrile and buffers with different pH has been used in different volume ratio. Flow rate of the mobile phase, with different column temperature has been varied to obtain ideal peak shape, resolution, retention time and other chromatographic parameters. Finally, the mobile phase consisting of acetonitrile and 0.1%OPA buffer at pH-3, satisfactorily eluted all the four APIs with high resolution with proper shape of all the peaks. DIKMA Spursil, C_{18}, ODS, (4.6×150 mm $\times 5$ µm) analytical column was found suitable for the elution with greater resolution at 1 mL/min flow rate and the detection wavelength at 254 nm using PDA detector. Under the above optimised conditions Tenofovir AF, Darunavir, Emtricitabine and Cobicistat were eluted at 2.287 min, 2.507 min, 4.062 min, 6.011 min respectively. The optimized chromatogram for all the studied APIs has been shown in Fig. 2. Now this optimized condition has been maintained throughout the study of validation parameters.

Method validation

The marketed dosage form of all the four drugs has been assayed using the developed method and percentage assay was found 99.21% for Tenofovir AF, 99.80% for Darunavir, 99.80% for Emtricitabine and 99.84% for Cobicistat as shown in the Table 1 and chromatogram was depicted in the Fig. 3. In the precision study of the present method the %relative standard deviation was found 1%, 0.6%, 0.8% and 0.6% for the Tenofovir AF, Darunavir, Emtricitabine and Cobicistat respectively. In the intermediate precision, the %RSD values for all the studied parameters for all APIs were found less than 2. In accuracy study, the percentage recovery was studied and were obtained 100.11% for Tenofovir AF, 100.26% for Darunavir, 100.64% for Emtricitabine and 100.08% for Cobicistat respectively, cited in the Table 2. In the linearity study it was observed that Tenofovir AF was found linear in the range of 2–10 µg/mL, Darunavir was found linear in the range of 160–800 µg/mL, for Emtricitabine it was found 40–200 µg/mL and for cobicistat it was 30–150 µg/mL. From the linearity graph of all the active ingredient the corelation coefficient was found 0.999. The detection limit was found 0.14 µg/mL for Tenofovir AF, 2.14 µg/mL for Darunavir, 0.6 µg/mL for Emtricitabine and 7.32 µg/mL for cobicistat. In the LOQ study the quantitation limit was found 0.47 µg/mL for Tenofovir AF, 7.12 µg/mL for Darunavir, 2.10 µg/mL for Emtricitabine and 24.42 µg/mL for cobicistat. In the specificity study no excipients peaks were found at the analyte the retention time. System Suitability study was conducted to confirm the proper operational of the equipment used for analytical measurements. Several parameters like tailing factor and theoretical plates were taken into consideration. The % relative standard deviation of peak area, theoretical plates, tailing factors and retention time were 0.31%, 0.28%, 1.19% and 1.03% respectively for Tenofovir AF, The percentage RSD of 0.12%, 1.93%, 1.01% and 0.43% were obtained for Darunavir. Similarly, for the Emtricitabine and cobicistat the %RSD of all the studied parameters were also found less than 2. In the robustness

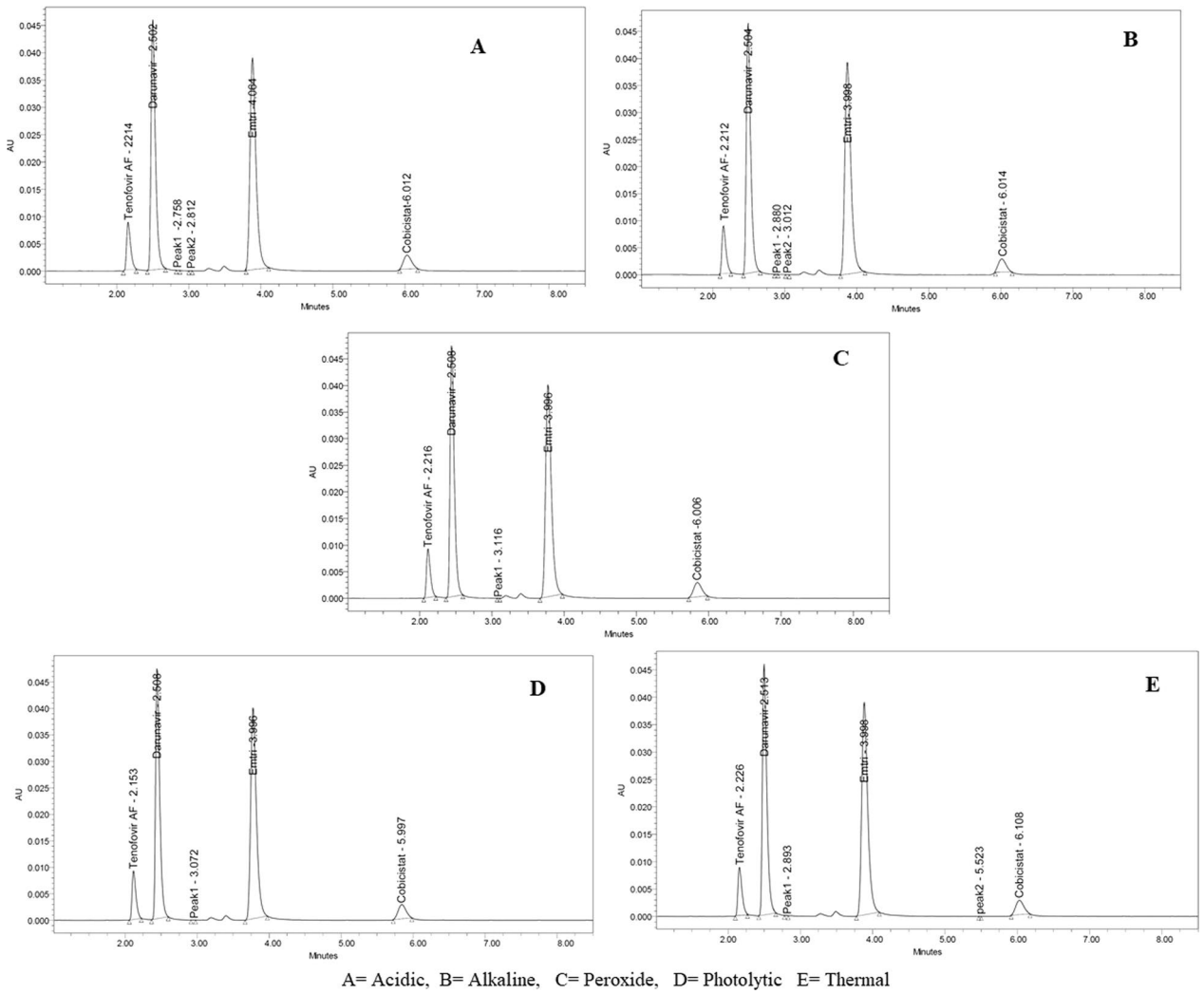

Fig. 4 Chromatograms of various stressed degradation conditions

study where the changes in the flow rate, changes in the organic composition and changes in the detection wavelength were done and the % RSD of peak area has been considered and found 1.64% and 1.85% for Tenofovir AF, 1.89% and 1.32% was obtained for Darunavir, 0.82% and 1.06% was for Emtricitabine, for cobicistat it was 1.02% and 0.85%. the details of the robustness study results was given in the Table 3. From the result of force degradation study it was observed that in the acidic stressed condition 3.64% degradation was found for Tenofovir AF, 3.31% was found for Darunavir, 4.67% was found for Emtricitabine and for cobicistat it was 5.08%. In the same way in alkaline stressed condition 3.10% degradation was found for Tenofovir AF, 3.70% was found for Darunavir, 3.13% was found for Emtricitabine and for cobicistat it was 4.57%. The details of all the % degradation for all the APIs at peroxide, photolytic and thermal stressed conditions have been cited in the Table 4, with purity angle and purity threshold. The chromatograms of all the stressed condition were depicted in the Fig. 4. The HPLC chromatograms of purity angle and purity threshold at all the stressed conditions also depicted in the Fig. 5.

Discussion

In this present research work, we optimised the chromatographic parameters using HPLC instrument for the four APIs, viz: Tenofovir AF, Darunavir, Emtricitabine and Cobicistat. Initially we found a biggest challenge to elute the four drugs with satisfactory resolution. In most of the preliminary trials all four drugs has not been eluted. Even eluted, it was found very hard to resolute them as per the ICH guidelines. Finally, all of them has been eluted with satisfactory resolution using acetonitrile

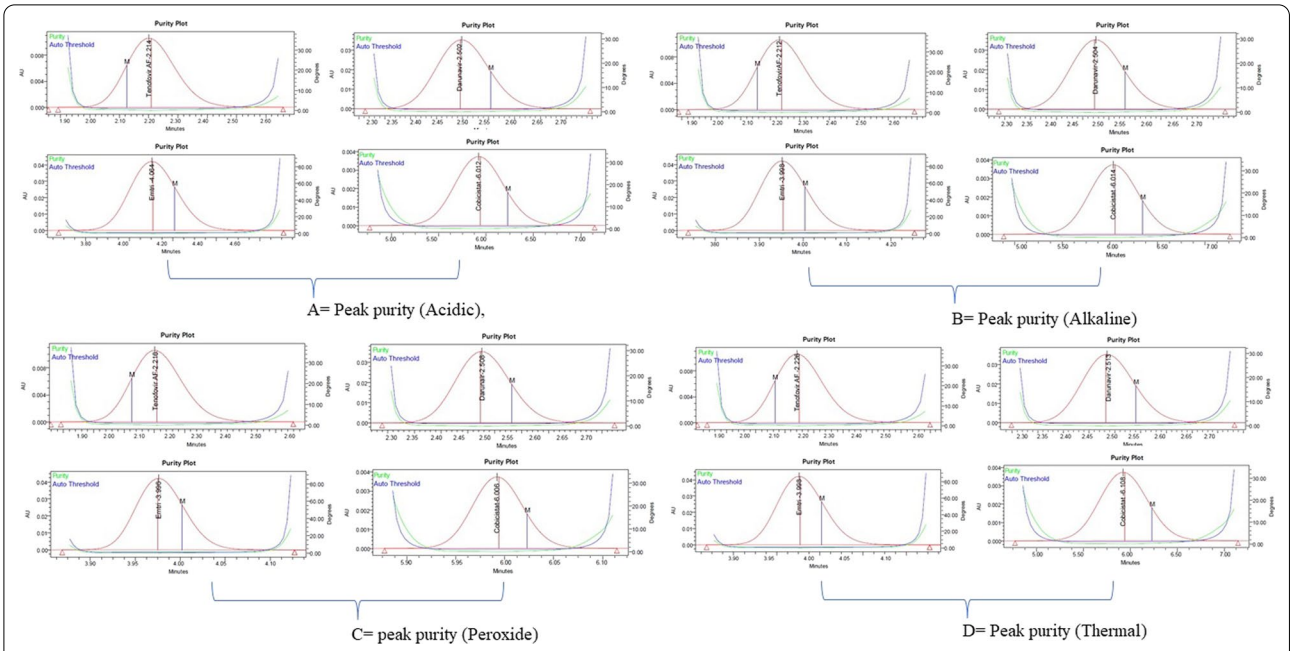

Fig. 5 Peak purity chromatograms of all APIs at various degradation conditions

and 0.1% OPA buffer at the volume ratio of 70:30, using the HPLC instrument at 254.0 nm PDA detection wavelength. The elution for all the APIs has been completed within 6 min. The percentage assay results of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat in the marketed dosage form was found very close to the label claim and found within the limits, which indicates the aptness of the developed method to analyse all the four APIs simultaneously in the marketed dosage form. The result of system suitability indicates that the utilized HPLC system with optimized conditions was found suitable for the determination of all the studied APIs. Accuracy and precision of the present method were determined as per the guidelines and the % recovery for Tenofovir AF, Darunavir, Emtricitabine and Cobicistat were found within the acceptable limit i.e. within 98–102% as revealed in the result and it confirms the accuracy of the present developed method. In the precision study, and intermediate precision the amount of all the four-ingredient found was calculated and %RSD was found satisfactory and within the limit. Therefore, the results of precision study specified the precision of the developed method for the simultaneous determination of all the APIs and in their marketed dosage form. In the linearity study of the present simultaneous method the correlation coefficient was obtained close to 1 for all the four APIs, which is within the prescribed limit and indicates the linearity. The least squares method was used to establish the regression line and the curves were found linear for all of the APIs. The limit of detection and limit of quantitation results for all the studied APIs, proved the sensitivity of the developed method and indicated that the noise levels are also within the level. The results of the robustness study exposed that the present method was found highly robust for all of the APIs, upon changing the minor chromatographic parameters and eluted with their specified retention time with greater resolution. From the stability study results it can be discussed that, all the stressed condition degrades the all four APIs, but percentage degradations were at very minor level and it was found within the acceptable criteria as per the guidelines. Peak purity results concludes that the obtained purity angle and purity threshold are within the limit and justified the stability of the developed method. The desired chromatograms of all the four APIs has been eluted in their specific positions with proper resolution along with the degraded peaks.

Conclusions

The described developed method based on the empirical evidences for the determination of Tenofovir AF, Darunavir, Emtricitabine and Cobicistat simultaneously using high performance liquid chromatography was found very specific. In comparison to the reported methods the present method was found advantageous in all the aspects. All the studied APIs has been highly resolute utilizing the

optimised condition and found extremely suitable for the determination of all of them simultaneously in marketed dosage form as well as in the bulk form.

Abbreviations
Tenofovir AF: Tenofovir alafenamide; ODS: Octadecyl silica; ICH: International Conference on Harmonization; ART: Antiretroviral therapy; NRTIs: Nucleotide reverse transcriptase inhibitors; HIV: Human immunodeficiency virus; OPA: Ortho phosphoric acid; PDA: Photo diode array.

Acknowledgements
The authors are Thankful to Management Vels Institute of Science, Technology and Advance Studies (VISTAS), Pallavaram, Chennai for providing necessary facilities to conduct this research work.

Authors' contributions
Dr. ES and Mrs. CP designed the entire project work. Mrs. CP has carried out the entire method development and validation study. Dr. ES and Mrs. CP carried out the stability study. Both authors revised and approved the final manuscript and the research work was performed in collaboration between the authors.

Funding
This research did not receive any specific grant from any funding agencies.

Availability of data and materials
All data and material is available upon request.

Declarations

Ethics approval and consent to participate
Non applicable.

Consent for publication
Non applicable.

Competing interests
The authors declares that they have no competing interest.

Received: 1 November 2021 Accepted: 17 December 2021
Published online: 04 January 2022

References

1. British HIV Association (2016) BHIVA guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy (2016 interim update). http://www.bhiva.org. Accessed 3 June 2021
2. Baker D, Pell C, Donovan B (2014) HIV as a chronic disease. Optimizing outcomes. Med Today 15(2):16–26
3. European AIDS Clinical Society. Guidelines Version 9.0. (2017). http://eacsociety.org/. Accessed 16 June 2021
4. Shah BM, Schafer JJ, Priano J (2013) Cobicistat: a new boost for the treatment of human immunodefciency virus infection. Pharmacotherapy 33(10):1107–1116
5. Deeks ED (2018) Darunavir/Cobicistat/Emtricitabine/Tenofovir Alafenamide: a review in HIV-1 infection. Drugs 78(10):1013–1024
6. Cossu MV, Astuti N, Capetti A, Rizzardini G (2015) Impact and diferential clinical utility of cobicistat-boosted darunavir in HIV/AIDS. Virus Adapt Treat 7:47–56
7. Europian Medicine Agency (2017) An overview of Symtuza. https://www.ema.europa.eu/en/medicines/human/EPAR/symtuza. Accessed 12 June 2021
8. Suresh G, Manikandan A, Rao SV (2021) A simple reverse phase UPLC validated method for concurrent estimation of Emtricitabine, Darunavir, Tenofovir and Cobicistat in drug substances and drug product. Res J Chem Environ 25(9):79–88
9. Parameswari SA, Ankinapalli AKR, Tsegaye T, Alagusundaram M (2021) Stability indicating RP-HPLC method development and validation for the simultaneous estimation of darunavir, cobicistat, emtricitabine and tenofovir alafenamide in bulk and pharmaceutical formulation. Int J Pharm Sci Res 12(6):3216–3224
10. Mondal P, Mahender K, Padmaja B (2018) A novel UPLC-PDA method for the simultaneous determination of Lamivudine, Zidovudine and Nevirapine in bulk and tablet dosage form. Anal Chem Lett 8(1):131–138

Publisher's Note