

RESEARCH

Open Access



Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR

Anuradha Vejendla^{1*}, Subrahmanyam Talari^{2,4}, Raju Moturu³, S. N. Murthy Boddapati⁴ and A. Emmanuel Kola⁴

Abstract

Background: Using a Symmetry C18 (4.6 × 150 mm, 3.5) column, a high-performance liquid chromatographic method for quantification of Rilpivirine and Cabotegravir in active pharmaceutical ingredients was developed and validated. The mobile phase is made up of buffer, acetonitrile, and 0.1 percent formic acid in a 20:80v/v ratio. The flow rate was kept constant at 1.0 ml/min, and detection was accomplished through absorption at 231 nm with a photodiode array detector.

Results: The calibration curve was linear, with a regression coefficient (R²) value of 0.999 and concentrations ranging from 30 to 450 g/ml of Rilpivirine and 20–300 g/ml of Cabotegravir. The method's LOD and LOQ were 0.375 g/ml, 1.238 g/ml, and 0.25 g/ml, 0.825 g/ml for Rilpivirine and Cabotegravir, respectively.

Conclusions: In the forced degradation studies, the degradants were characterized by using LCMS and FTIR. The current application was found to be simple, economical, and suitable, and validated according to ICH guidelines.

Keywords: Rilpivirine, Cabotegravir, LCMS, FTIR development, Characterization

Background

Cabotegravir is a medicine utilized for the treatment of acquired immunodeficiency syndrome [1, 2]. It is available in tablet form and as an intramuscular injection [3, 4], as well as an injectable blend with Rilpivirine under the brand name Cabenuva. The injection forms are used once every month or every two months. Cabotegravir in combination with Rilpivirine has been shown to treat human immunodeficiency virus type 1 in adults (Human immune virus-1). And, if the virus has not developed resistance to the inhibitors, the combination injection is planned for the treatment of adults who do not have detectable human immune virus levels in their blood

despite their current antiretroviral treatment [5, 6] and integrase strand transfer inhibitors [7]. Before beginning injection therapy, the tablets are used to see if a person responds to the medication. The two drugs are the first antiretroviral drugs to be available in an injectable formulation with a long half-life. This implies that rather than a day by day pills, individuals get intramuscular injections month to month.

Rilpivirine, also known as Edurant and Rekambys, is a Tibotec prescription medication which is used to treat human immune virus/acquired immune deficiency syndrome [8]. It is a second-generation non-nucleoside reverse transcriptase inhibitor with a lower side-effect profile, higher strength, and a longer half-life than older non-nucleoside reverse transcriptase inhibitors like efavirenz [9]. The well-known side effects of the injectable formulation include reactions at the injection site (in

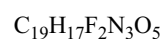
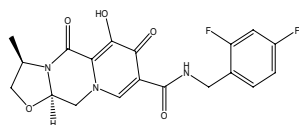
*Correspondence: vanuradha372@gmail.com

¹ Department of Chemistry, Vignan Degree College, Guntur, AP 522009, India

Full list of author information is available at the end of the article

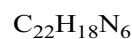
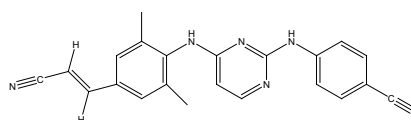
up to 84 percent of patients), such as pain and swelling, just cerebral pain [10, 11], and fever [12, 13] or feeling hot. Depressive disorders, insomnia [14, 15], and rashes are less common (less than 10%). Depressive disorders [16], headache, rashes, and insomnia are some of the less common side effects of the tablets. These side effects occurred when Rilpivirine was combined with

one or more other anti-human immune virus drugs. Heart rhythm prolongation [17, 18] has been observed at extremely high doses of the drug, but it is not clinically relevant at standard doses. Figure 1 shows structures of Cabotegravir, Rilpivirine, and its degradation products.



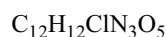
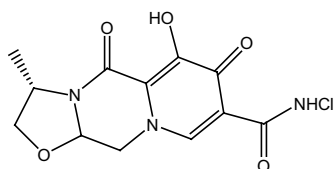
Mol. Wt.: 405.35

AP1



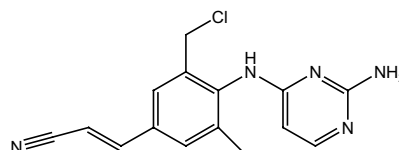
Mol. Wt.: 366.42

AP2



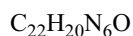
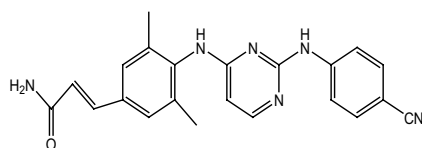
Mol. Wt.: 313.69

DP1



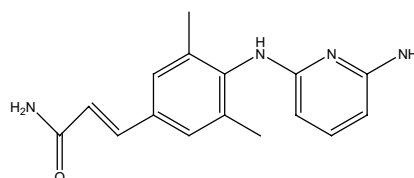
Mol. Wt.: 299.76

DP2



Mol. Wt.: 384.43

DP3



Mol. Wt.: 282.34

DP4

Fig. 1 Structures of Cabotegravir (AP1), Rilpivirine (AP2) and DPs (DP1, DP2, DP3, and DP4)

Methods

Chemicals

Merck Pvt. Ltd. Worli, Mumbai, India, provided water, formic acid, and acetonitrile (High-performance liquid chromatography grade). Spectrum Pharma research solutions Pvt. Ltd., Hyderabad, provided the reference standards and APIs for Rilpivirine and Cabotegravir.

Instrumentation

HPLC

Waters Alliance LC (model 2695) monitored with empowering 2 data handling systems and fitted with asymmetry C₁₈ (150 × 4.6 mm, 3.5μ) and a detector of photodiode array (model 2998) was used for this study.

LC-MS/MS conditions

In the stress degradation study, HPLC was linked to a mass spectrophotometer with the splitter placed before the ESI source, allowing only 35% of eluent to enter. The following were the standard operating source conditions for Cabotegravir and Rilpivirine MS scans on positive ESI mode: the fragmented voltage was set at 80 V, the capillary at 3000 V, the skimmer at 60 V, nitrogen was used as a drying and nebulizing gas (45 psi), and highly filtered nitrogen gas was used as collision gas.

FTIR conditions

FTIR was utilized to get insights concerning the presence of different functional groups like keto, Aldehydes, cyano alcohol, and amides present in the degradation samples. Perkin Elmer range 100 models were utilized with KBr as a dispersion medium to make the specimen pellets. Approximately 1.5–3.5 mg of specimens were mixed with 4.5 mg of KBr for a complete examination.

Buffer preparation

1 mL formic acid is dissolved in 1L high-performance liquid chromatography-grade water and filtered through 0.45 filter paper.

Mobile phase preparation

After thoroughly mixing buffer and acetonitrile in 20:80 ratios, the mixture was sonicated for 5 min and filtered through a 0.22 μm membrane filter. The HPLC analysis was carried out on a reversed phase-HPLC system with isocratic elution mode using a mobile phase of 0.1 percent formic acid and acetonitrile (80:20) on a Symmetry C18 column (150 × 4.6 mm, 3.5) with a flow rate of 1 mL/min and a photodiode array detector at 231 nm. As a diluent, the mobile phase was used.

Standard solution preparation (Rilpivirine 300 g/ml and Cabotegravir 200 g/ml)

Weigh out 300 mg of Rilpivirine and 200 mg of Cabotegravir working standards into a 100 mL volumetric flask, add 70 mL of diluent, sonicated for 30 min to dissolve it, and makeup to the mark with diluent. Transfer 5 mL of this stock solution to a 50 mL volumetric flask and dilute to the desired concentration with diluent.

Chromatographic condition optimization

Different mobile phase combinations were tested to determine theoretical plate count, resolution, tailing, and other system suitability parameters. Finally, the separation was accomplished using a freshly prepared mobile phase composed of buffer: acetonitrile in a 20:80 ratio with a flow rate of 1.0 mL/min. The injection volume of 10 μL and the ambient temperature were kept constant throughout the process to obtain the symmetric peak of Rilpivirine and Cabotegravir at 231 nm.

Results

To achieve the best chromatographic conditions, various columns such as C18, C8, and CN-propyl, as well as mobile phases, were tested. The best chromatographic separation occurred on a Symmetry C18 column with acetonitrile and 0.1 percent formic acid in (80:20) mobile phase at a flow rate of 1 mL/min and PDA detection at 231 nm (Fig. 2). Table 1 depicts the optimized chromatographic conditions.

System suitability

Six replicates of a standard solution containing 300 g/mL of Rilpivirine and 200 g/mL of Cabotegravir were used to assess system suitability. The results show that the system's suitability is within the acceptable range. Table 2 and Fig. 3 show the results.

Linearity

The current application's linearity was determined by plotting a graph between concentration and corresponding peak area for Rilpivirine and Cabotegravir over concentration ranges of 30–450 g/mL and 20–300 g/mL, respectively (Fig. 4). For both drugs, the correlation coefficient was found to be 0.999. Table 3 contains a summary of the linearity results.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for Rilpivirine and Cabotegravir were 0.375 g/mL, 1.238 g/mL, and 0.25 g/mL, 0.825 g/mL, respectively. Table 4 summarizes the findings.

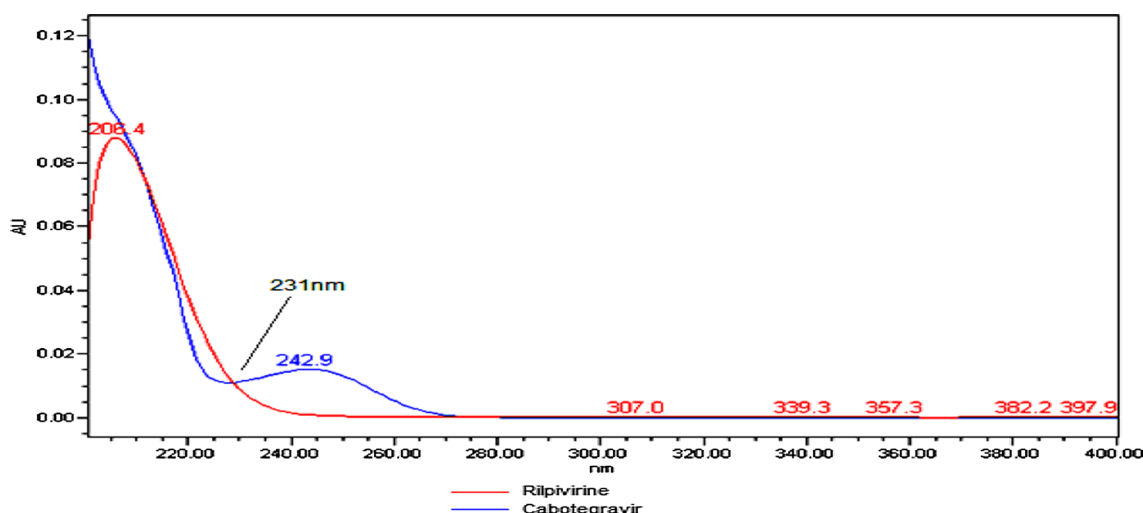


Fig. 2 Photodiode array detector spectrum of Cabotegravir and Rilpivirine

Table 1 Optimized chromatographic conditions

Stationary phase	Symmetry C ₁₈ (150 × 4.6 mm, 3.5μ)
Carrying phase	Acetonitrile: 0.1% formic acid (80:20)
Injection volume	10 μL
Flow rate	1.0 ml/min
Column temperature	25 °C
Wavelength	231 nm
Run time	5 min
RT of Rilpivirine	2.050 min
RT of Cabotegravir	3.942 min

Table 2 System suitability results

Parameter	Rilpivirine	Cabotegravir
USP plate count	2079	3852
Tailing factor	1.25	1.18
Resolution	–	7.73
RT	2.050	3.942

Precision

Repeatability (or) method precision

The percentage of RSD value for six replicate injections of known concentrations of Rilpivirine and Cabotegravir performed on the same day was discovered to be 2%, indicating that the method precision is within the limit. Table 5 summarizes the findings.

Accuracy

Rilpivirine and Cabotegravir were prepared in three concentration levels: 50%, 100%, and 150%. The

recovery percentage was found to be within the acceptable range of 98–102 percent. Based on these findings, it was determined that the developed method is precise and accurate. Table 6 summarizes the findings.

Robustness

The current method's robustness was tested by varying the mobile phase composition and flow rate. The percentage of RSD was discovered to be within an acceptable range. Table 7 contains a summary of the robustness results.

Degradation effects and its characterization

Rilpivirine and Cabotegravir samples were subjected to a variety of stress degradation conditions to observe the drugs partial degradation. The stress studies revealed the conditions under which the drug becomes unstable; these measures can be implemented during formulation to avoid potential instabilities. LC-MS and FTIR are used to characterize these degradation products.

Acid degradation

Initially, no degradant peaks were formed when Rilpivirine and Cabotegravir were studied in 0.1 N HCl. When the acid concentration was increased to 1 N HCl and heated at 60 °C for 30 min, 16.5 percent of Rilpivirine and 15.3 percent of Cabotegravir were degraded, and two degradations were observed. (DP1 and DP2) products were formed on acid hydrolysis.

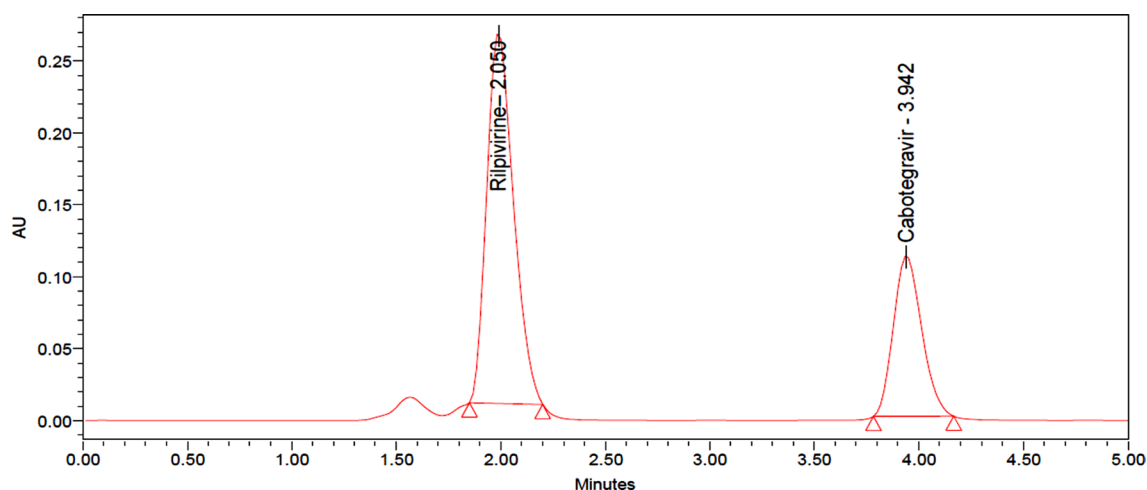


Fig. 3 Chromatogram of system precision

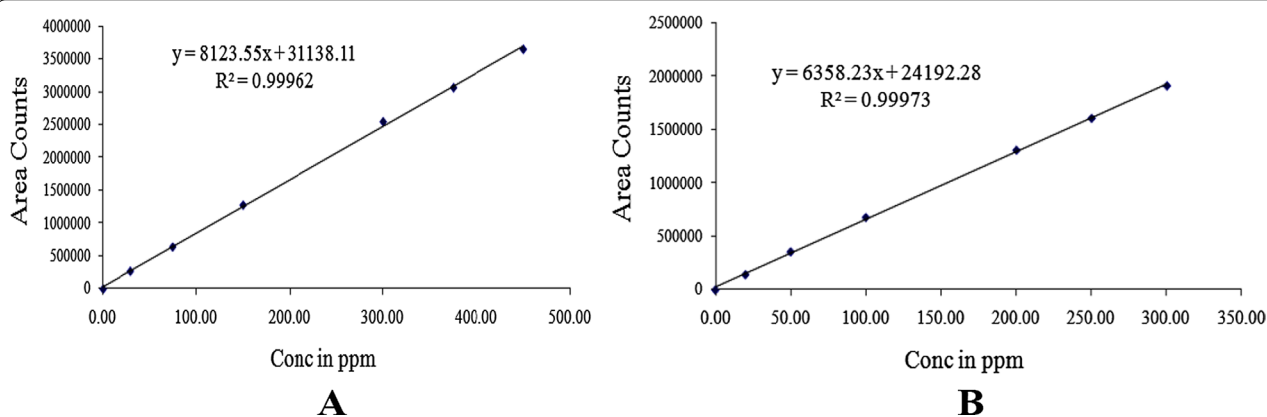


Fig. 4 Calibration curve of **A** Rilpivirine, **B** Cabotegravir

Table 3 Results of linearity

S. no	Rilpivirine		Cabotegravir	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	30.00	270,078	20.00	143,686
2	75.00	637,392	50.00	358,414
3	150.00	1,273,025	100.00	679,383
4	300.00	2,544,502	200.00	1,311,917
5	375.00	3,060,044	250.00	1,610,677
6	450.00	3,643,429	300.00	1,914,838

Alkali degradation

Rilpivirine and Cabotegravir were stressed under in 0.1 N NaOH, and no degradant products are formed. When the strength of alkali was increase to 1 N NaOH and

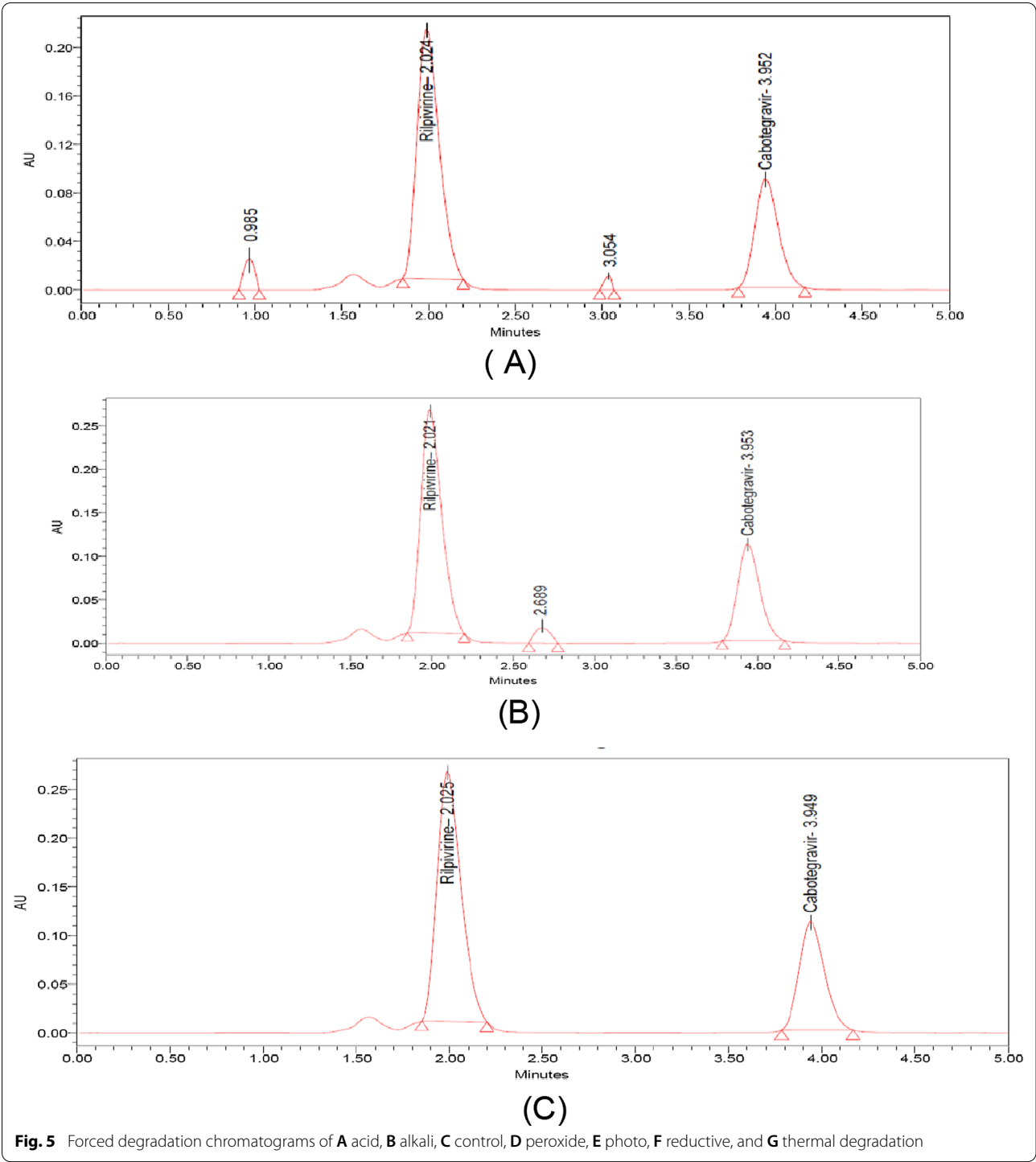
Table 4 Results of LOD AND LOQ

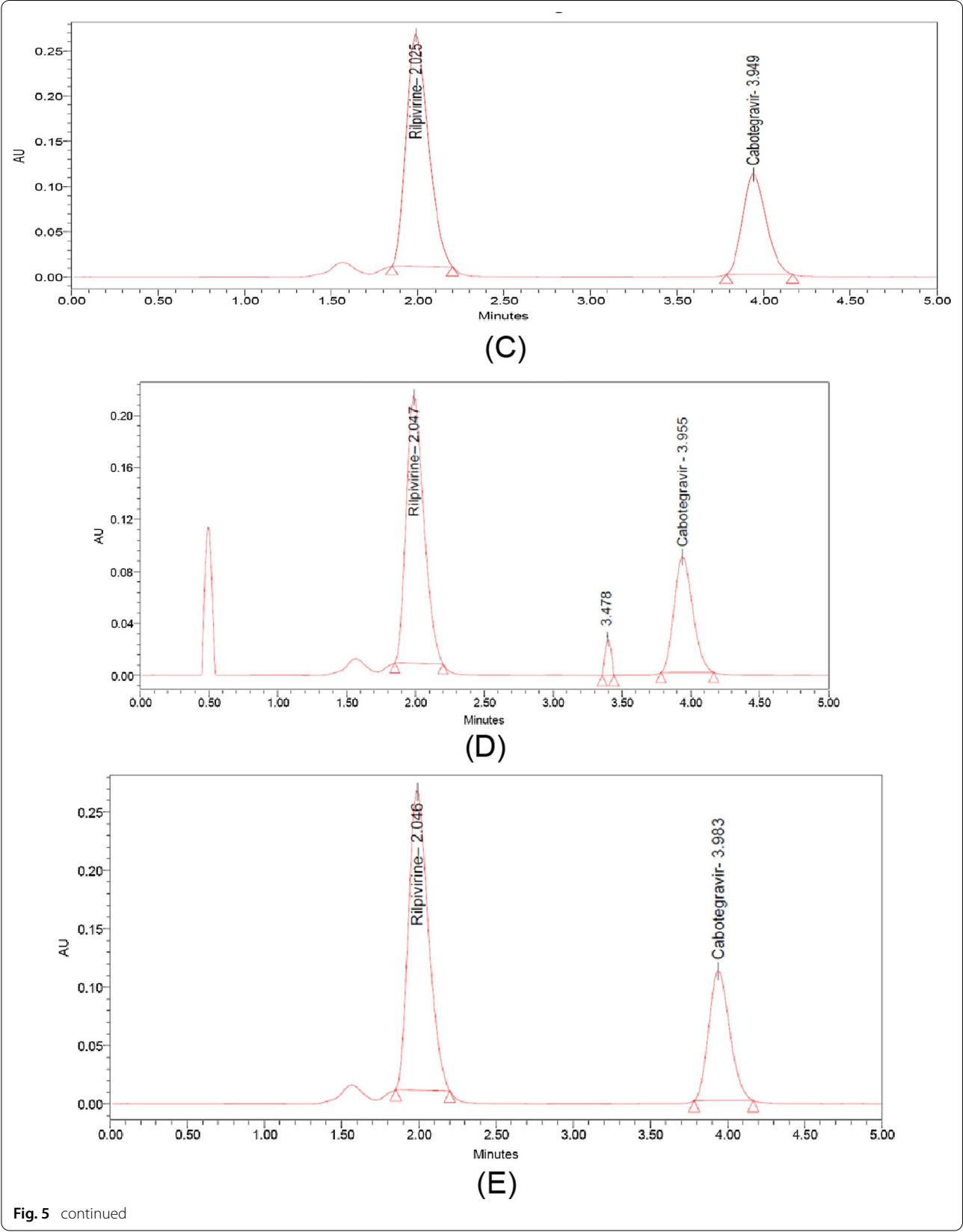
Drug	LOD	LOQ
Rilpivirine	0.375	1.238
Cabotegravir	0.25	0.825

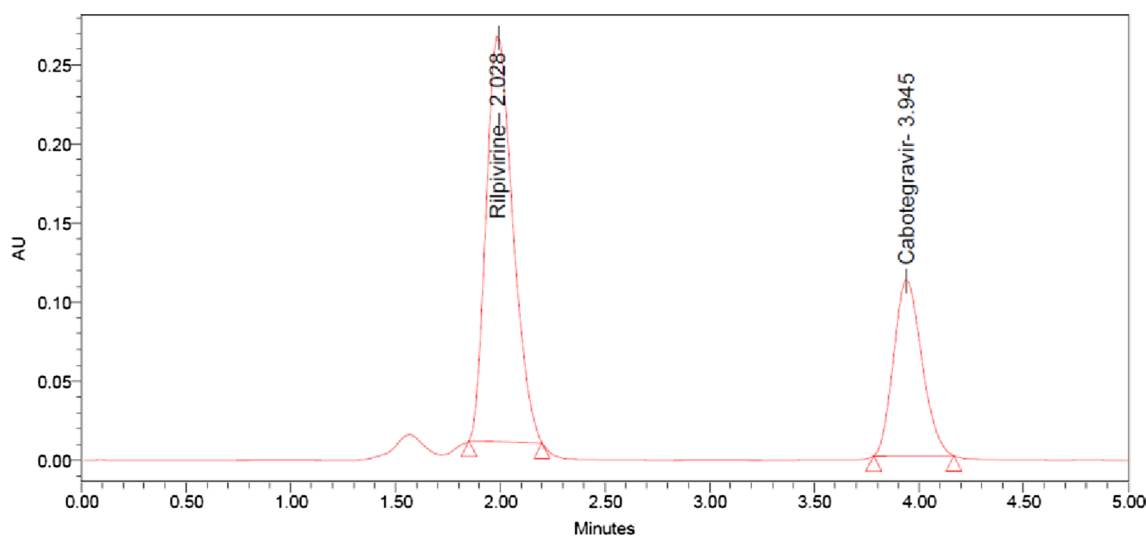
heated at 60 °C for 30 min, 12.5% of Rilpivirine and 12.1% of Cabotegravir degradation were observed and one degradation (DP3) product was formed on alkali hydrolysis.

Oxidative degradation

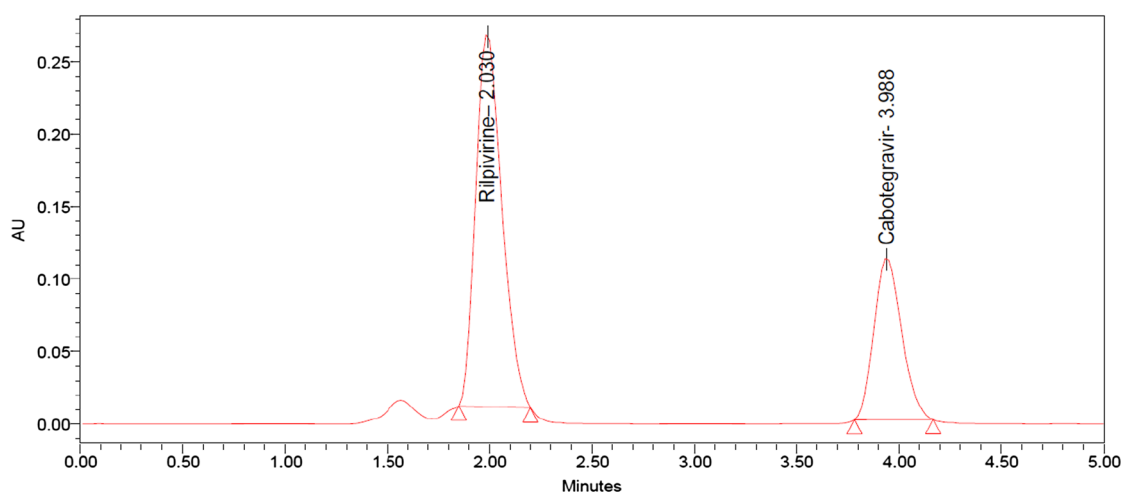
Rilpivirine and Cabotegravir were studied in 10% peroxide condition, and no degradant peaks are formed. Then, the strength of peroxide was increased to 30% and refluxed for 3 h, one degradant (DP4) was formed.







(F)



(G)

Fig. 5 continued

Reductive degradation

The first trial of Rilpivirine and Cabotegravir were studied in 10% sodium bisulfate solution, for reduction degradation process, and no degradant was formed. After that, the above solution refluxed for 3 h, and no degradants were formed.

Thermal degradation

For thermal degradation, the sample was exposed at 105 °C for 3 h, and no degradant products are formed. After that, the above solution was refluxed for 3 h, and no degradation peaks were formed.

Photolytic degradation

For the first trial of photolytic degradation, the sample was exposed to UV light for 6Hrs and the exposed sample was analyzed, and no degradants peaks are formed. After that, the exposed sample was refluxed for 3 h, and no degradants peaks were formed.

The details of degradation products and chromatograms are represented in Table 8 and Fig. 5. FTIR spectra show the frequencies (Fig. 6) of all different functional groups like acid, amide, cyano, and keto. The details of FTIR spectra for all degradation products are provided in Table 9.

Table 5 Results of method precision

S. no	Area of Rilpivirine	Area of Cabotegravir
1	2,542,689	1,344,264
2	2,553,130	1,371,927
3	2,505,117	1,323,328
4	2,577,854	1,384,891
5	2,591,109	1,355,421
6	2,514,689	1,391,485
Mean	2,547,431	1,361,886
SD	33,914.023	25,865.819
%RSD	1.33	1.90

Table 6 Accuracy results

Accuracy (%)	Amount of Rilpivirine	% Recovery	Amount of Cabotegravir	% Recovery
50	150	100.6	100	100.9
100	300	99.8	200	98.6
150	450	101.0	300	100.4

Table 7 Results of robustness

Parameter	% RSD of Rilpivirine	% RSD of Cabotegravir
FP (1.2 ml/min)	0.36	0.52
FM (0.8 ml/min)	0.79	0.33
OP (88:12)	0.64	0.84
OM (72:28)	1.39	1.11

Collision-induced dissociation and FTIR details of Cabotegravir and Rilpivirine:

The degradation products of **DP1**, **DP2**, **DP3**, and **DP4**, which were observed under acid, alkali, and peroxide conditions. The ESI spectrum showed most intense $[M+H]^+$ ion of m/z -313.69, 299.76, 384.43, and m/z -282.34. The MS/MS spectrums of DP1, DP2, DP3,

and DP4 are displayed in Fig. 7, and fragmentation pathway mentioned in Fig. 8

Acid degradation product (DP1) characterization

The **DP1**, which was observed under acid degradation conditions. The ESI spectrum showed the most intense $[M+H]^+$ ion of m/z -313.69. The MS/MS spectrum of DP1 displayed abundant product ions at m/z -241.23 (loss of $C_7H_4N_2O_3$), m/z -201.23 (loss of $C_{10}H_1F_2NO_2$), m/z -143.13 (loss of $C_{12}H_{10}N_2O_5$). IR spectrum of DP1 showed a peak at 3406.8 cm^{-1} indicates the presence of hydroxyl group, 3318.2 cm^{-1} correspondings to N–H stretching, 3162.3 cm^{-1} indicates the presence of C–H alkene stretching, 1725.3 cm^{-1} belongs to C=O stretching present in amide and, 1288.6 cm^{-1} , 1051.7 cm^{-1} indicates the presence of the C–N stretching.

Acid degradation product (DP2) characterization

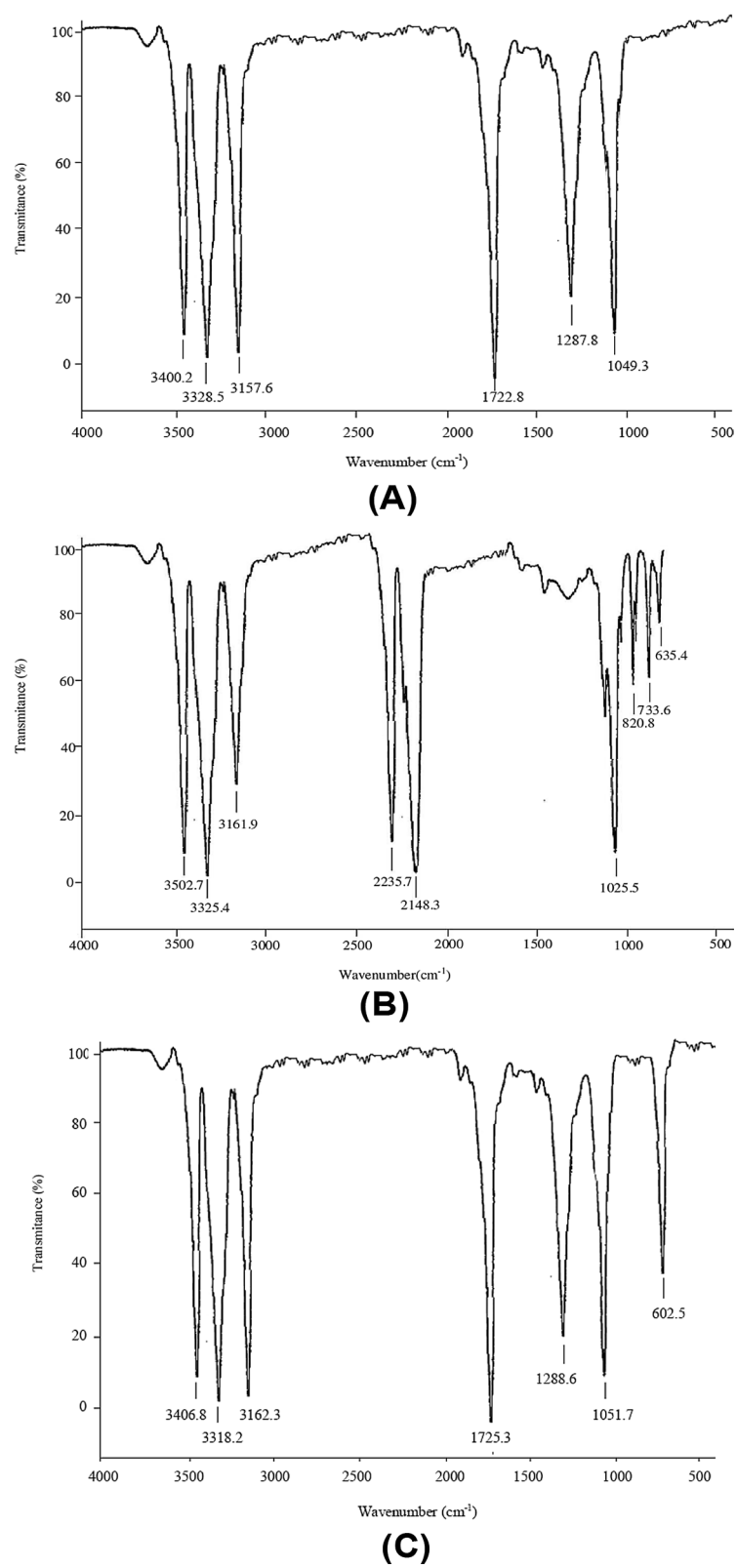
The fragmentation mechanism of **DP2** and the MS/MS spectrum showed more intense protonated molecular ion of m/z -299.76 which was noticed under acid conditions. The spectrum displayed abundant product ions at m/z -248.71 (loss of C_3H_3N from m/z -299.76), m/z -155.62 (loss of $C_5H_5N_3$ from m/z 248.71). IR spectrum of DP2 showed at 3115.3 cm^{-1} , 3327.8 cm^{-1} indicates the presence of N–H stretching in primary amines, 2236.2 cm^{-1} , 2152.5 belongs to $C\equiv N$ stretching, 1487.8 cm^{-1} belongs to $C\equiv C$ stretching and 1028.7 cm^{-1} corresponding to C–N stretching. Finally, the DP2 was characterized as (E)-3-(4-(2-amino pyrimidine-4-ylamino)-3-(chloromethyl)-5-methyl phenyl) acrylonitrile.

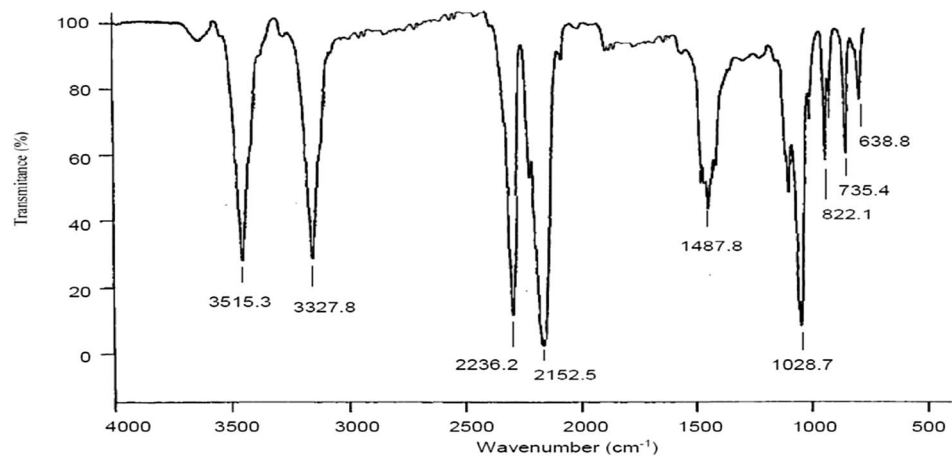
Alkali degradation product (DP3) characterization

The molecular ion of m/z 384.43 with molecular formula $C_{22}H_{20}N_6O$ which was noticed under alkali condition and fragmented into product ions at m/z -315.27 (loss of C_3H_5NO), m/z -211.22 (loss of m/z C_8H_{10} from m/z 315.27), and m/z -96.05 (loss of $C_7H_6N_2$ from m/z 211.22).

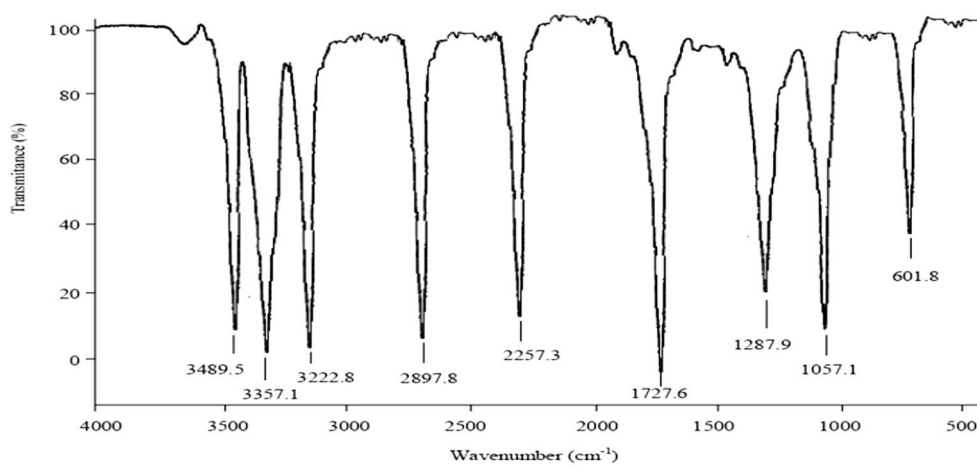
Table 8 Stress degradation results

Results: % degradation results	Rilpivirine		Cabotegravir		Number of DPs formed
	Area	% degradation	Area	% degradation	
Control	2,535,823	0	1,335,136	0.1	–
Reduction	2,277,678	12.2	1,190,347	13.9	–
Base	2,244,102	12.5	1,173,639	12.1	DP3
Acid	2,208,598	16.5	1,161,321	15.3	DP1 and DP2
Thermal	2,197,980	11.4	1,150,247	10.9	–
Photo	2,176,871	10.2	1,138,264	12.8	–
Peroxide	2,114,687	13.5	1,335,136	13	DP4

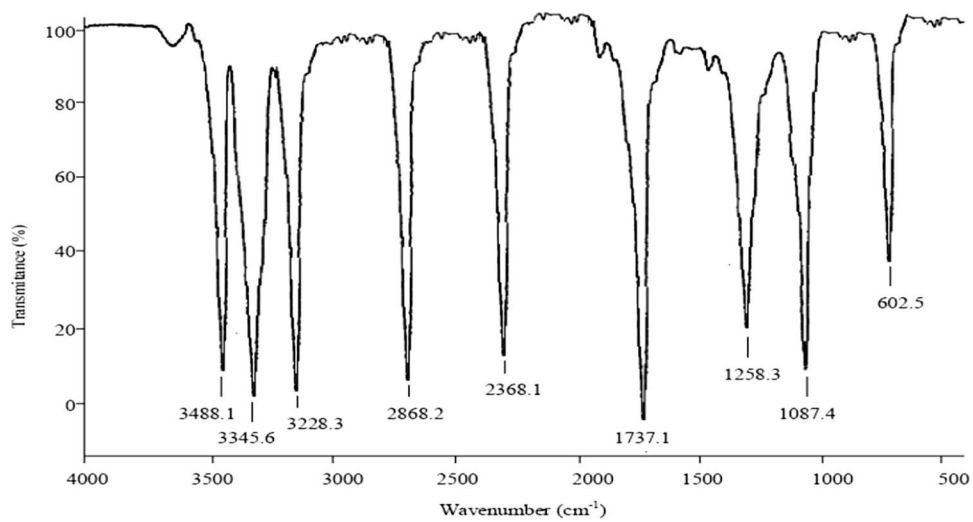
**Fig. 6** FTIR Spectrums of **A** Cabotegravir, **B** Rilpivirine, **C** DP1, **D** DP2, **E** DP3, **F** DP4



(D)

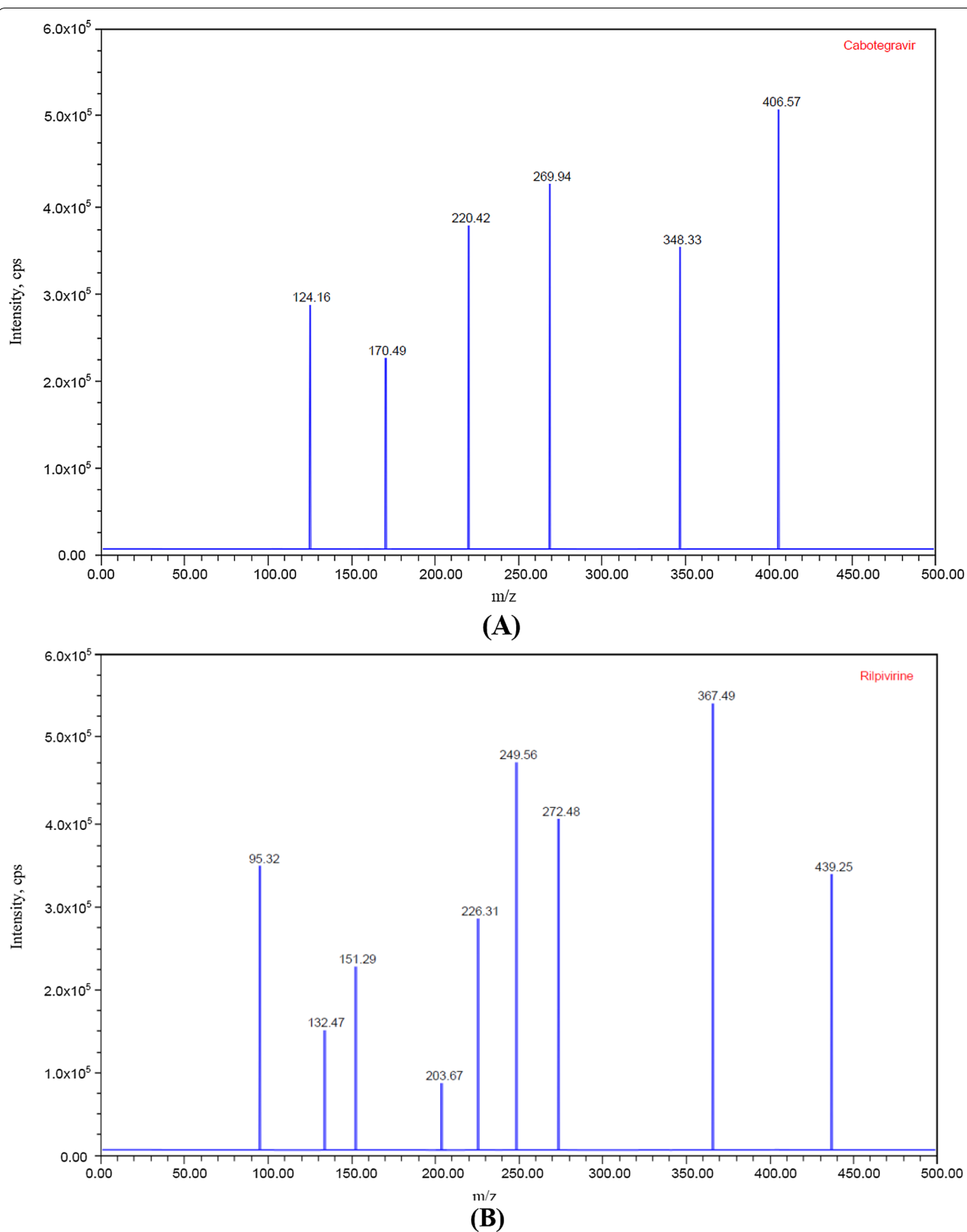


(E)



(F)

Fig. 6 continued

**Fig. 7** Mass spectrums of **A** Cabotegravir, **B** Rilpivirine, **C** DP1, **D** DP2, **E** DP3, **F** DP4

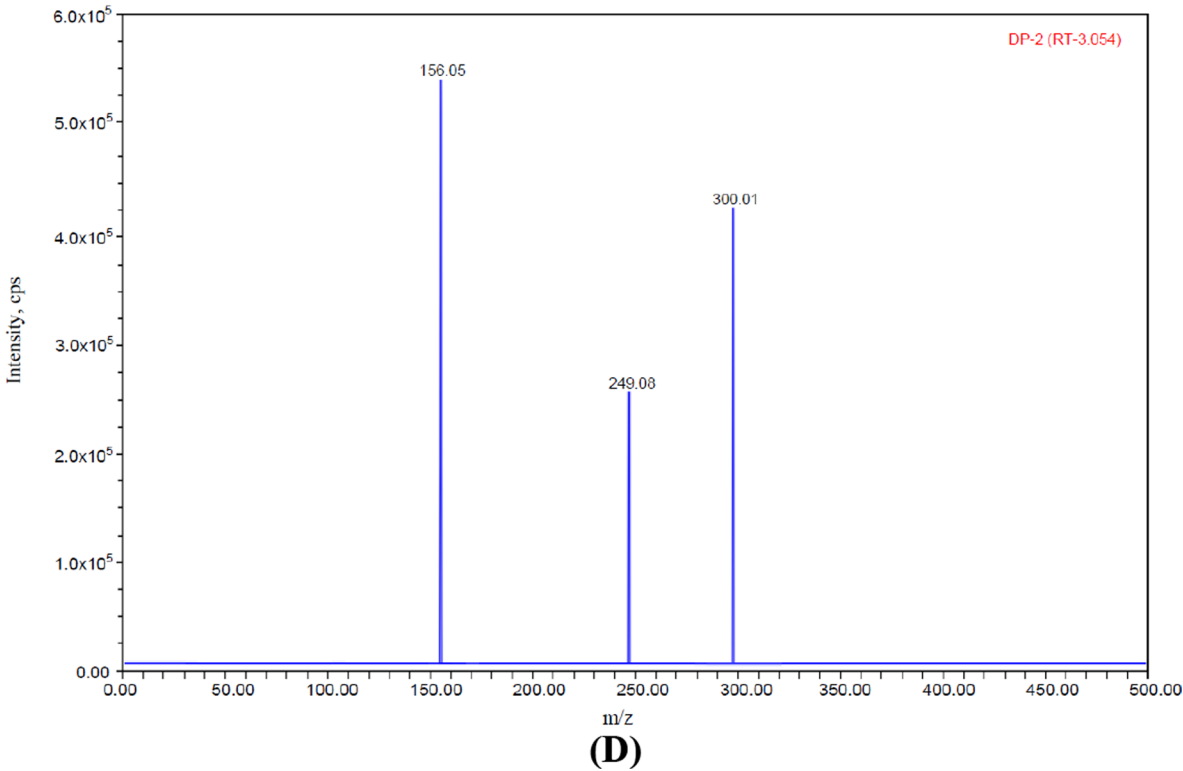
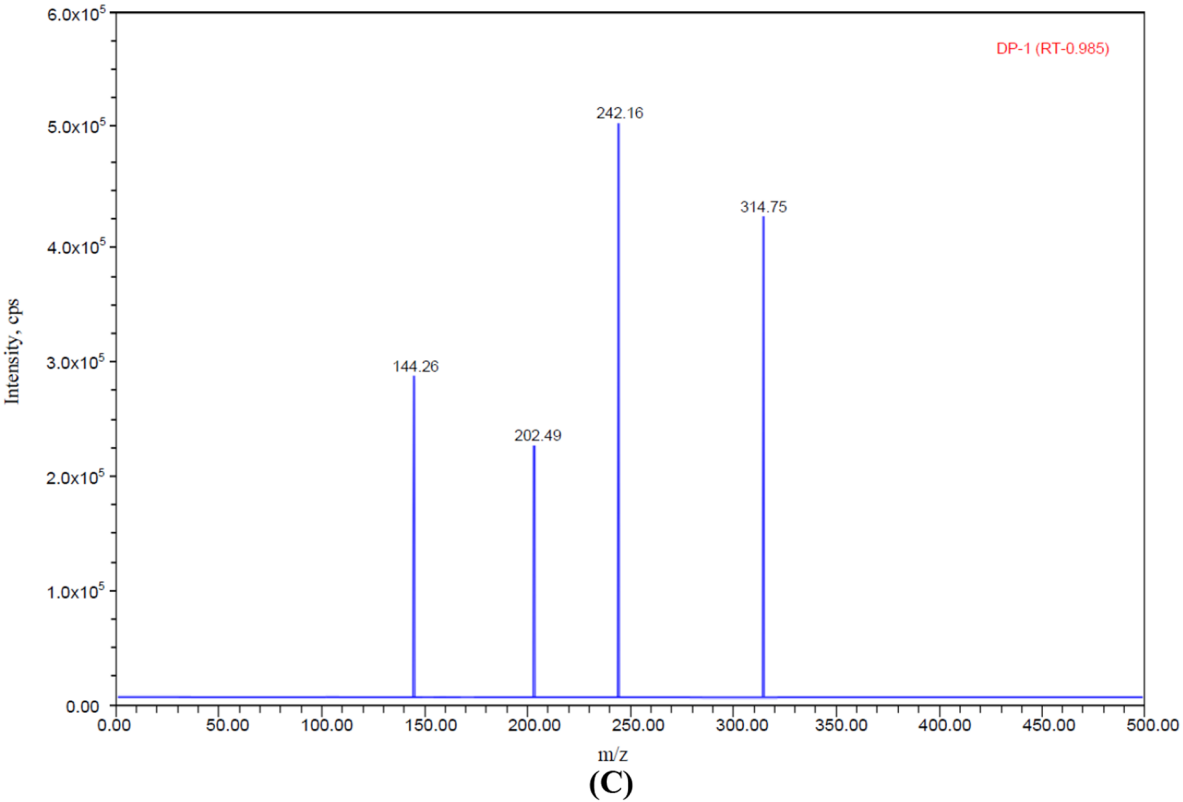


Fig. 7 continued

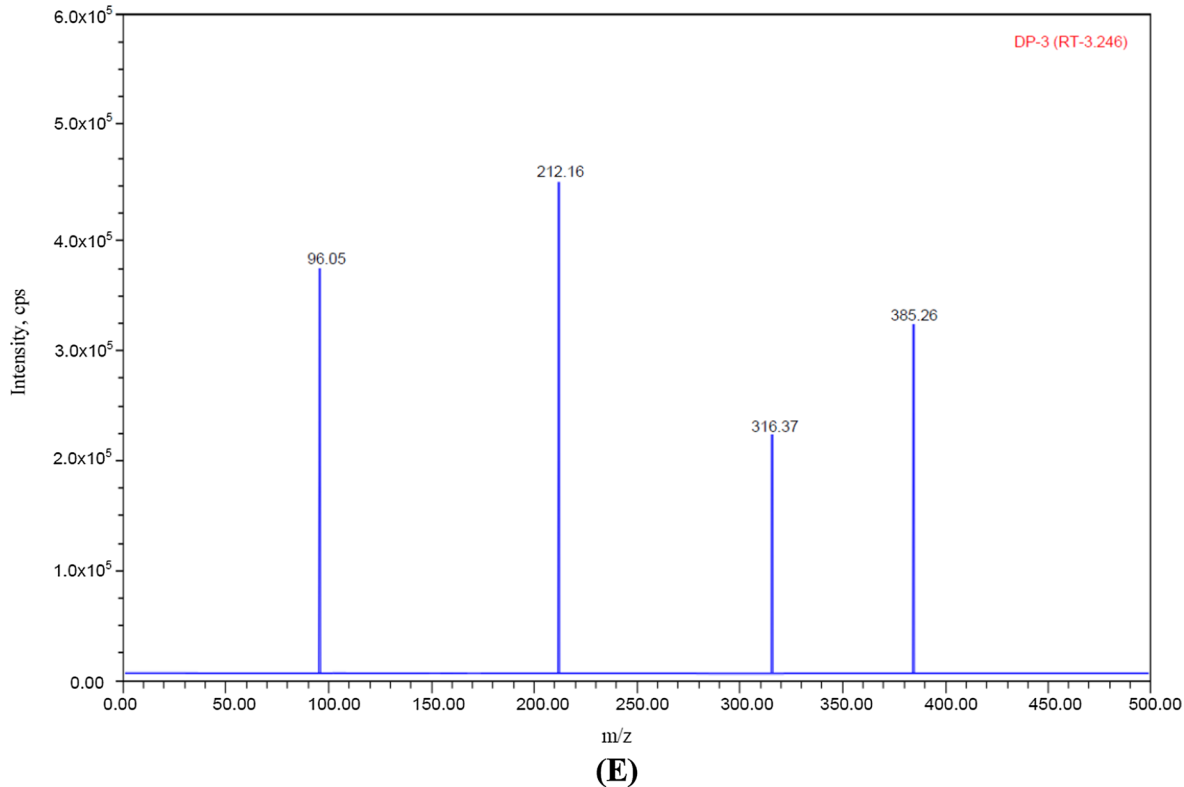
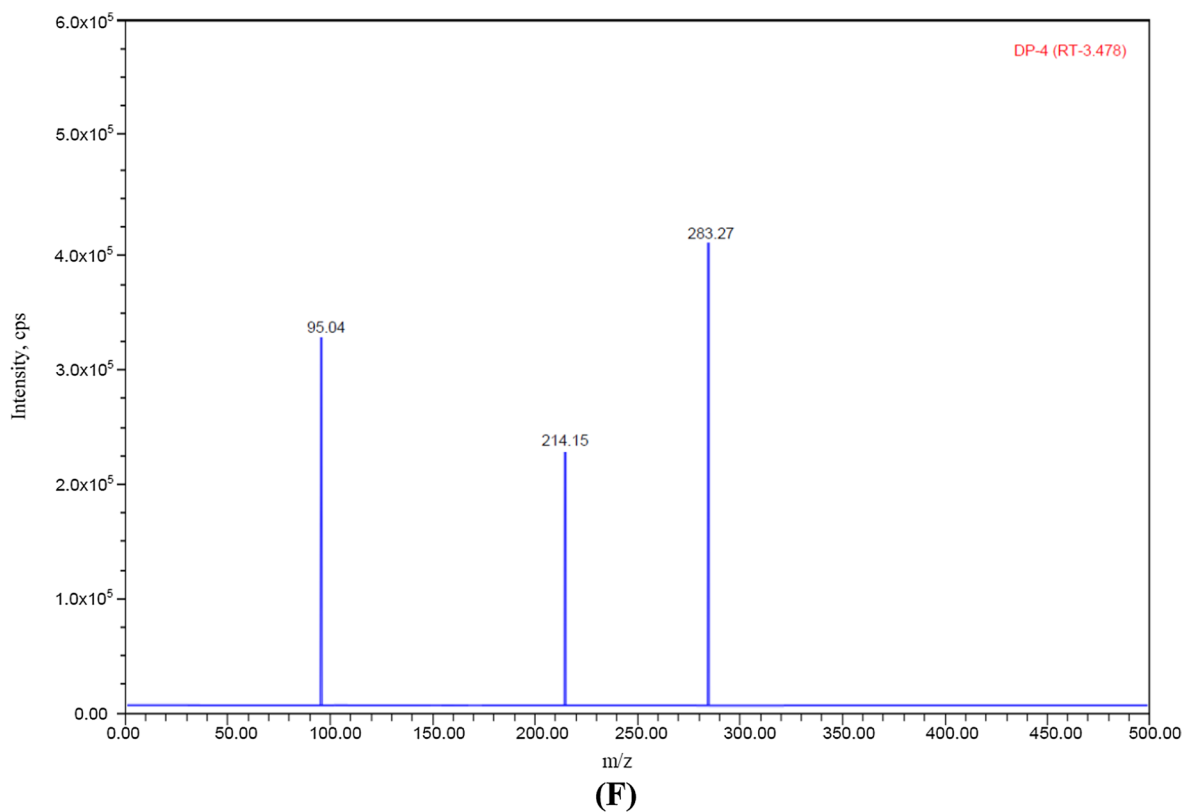


Fig. 7 continued

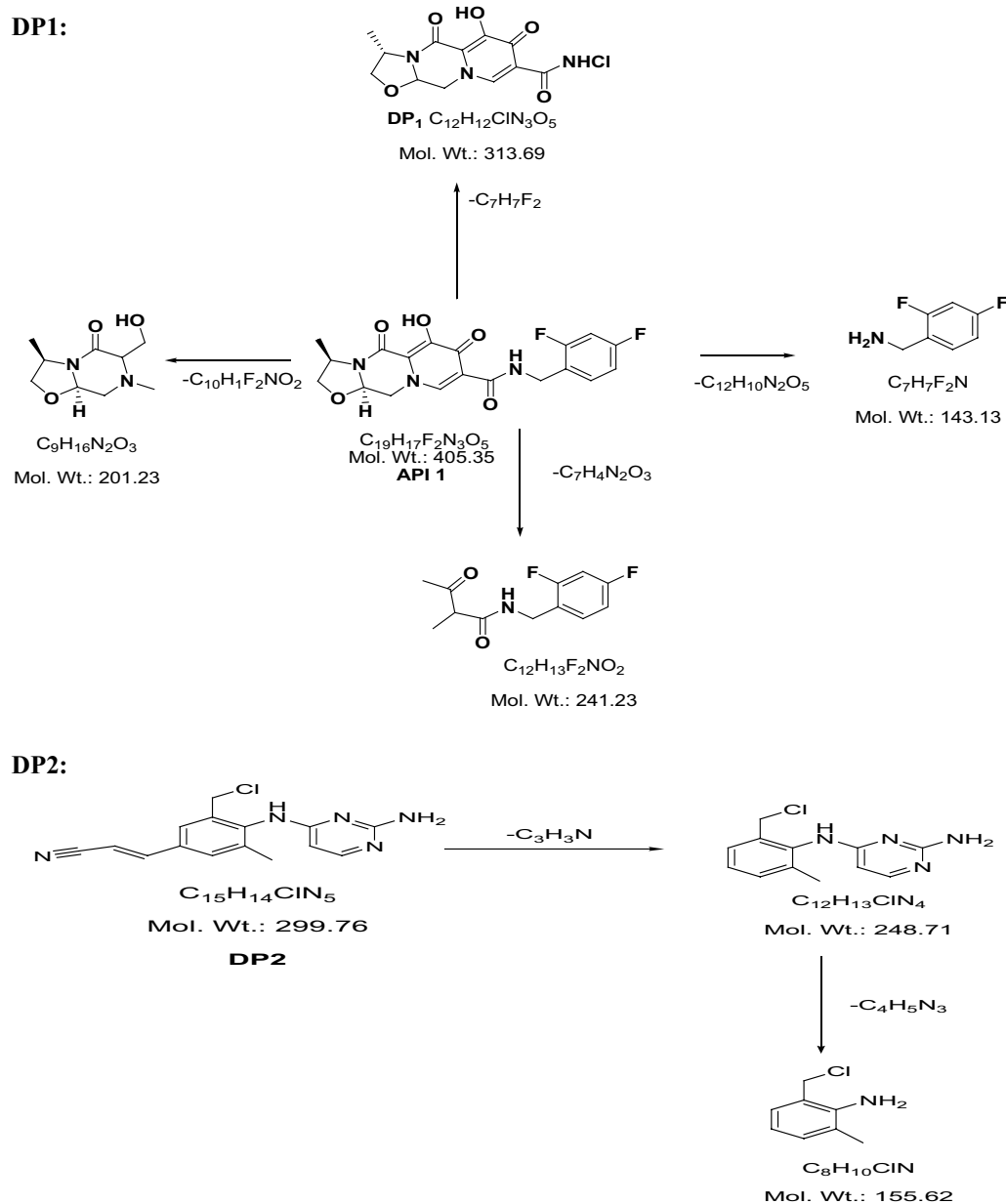


Fig. 8 Collision-induced dissociation mechanisms of DP1, DP2, DP3, and DP4

IR spectrum of DP3 showed at 3489.5 cm^{-1} , 3357.1 cm^{-1} indicates the presence of N–H stretching, 2257.3 cm^{-1} belongs to $\text{C}\equiv\text{N}$ stretching, 1727.6 cm^{-1} belongs to $\text{C}=\text{O}$ stretching in amides, 3222.8 cm^{-1} C–H alkene stretching and 2897.8 indicates C–H aliphatic stretching.

Peroxide degradation product (DP4) characterization

DP4 degradation product for m/z -282.34 which was noticed under peroxide degradation condition. The

product ions formed at m/z -213.28 (loss of $\text{C}_3\text{H}_5\text{NO}$), m/z -94.05 (loss of $\text{C}_8\text{H}_{11}\text{N}$). IR spectrum of DP3 showed at 3488.1 cm^{-1} , 3345.6 cm^{-1} indicates the presence of N–H stretching, 2368.1 cm^{-1} belongs to $\text{C}\equiv\text{N}$ stretching, 1737.1 cm^{-1} belongs to $\text{C}=\text{O}$ stretching in amides, 3228.3 cm^{-1} C–H alkene stretching, 2868.2 indicates C–H aliphatic stretching, and 1258.3 cm^{-1} , 1087.4 related to C–N stretching. It was characterized as (E)-3-(4-(6-aminopyridine-2-ylamino)-3,5-dimethyl phenyl)acrylamide. Finally, FTIR data clears that the

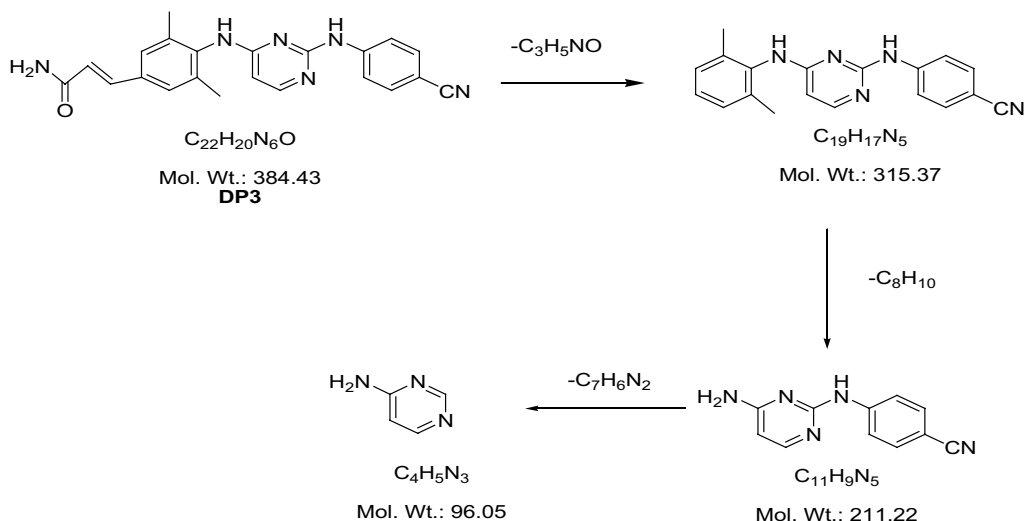
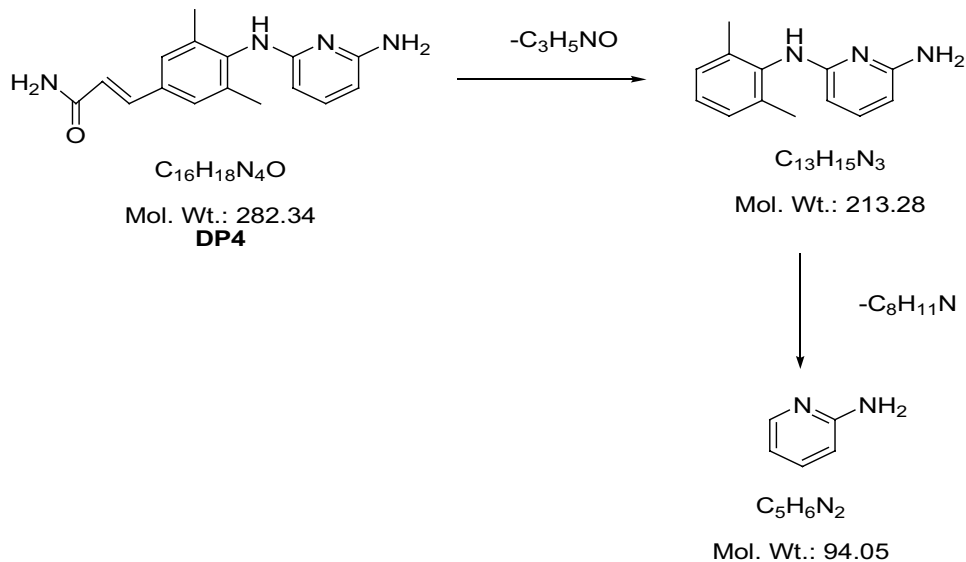
DP3:**DP4:**

Fig. 8 continued

presence of cyano, primary amide, amine, hydroxyl group, and keto functional groups in DP4.

Discussion

We developed a high-performance liquid chromatography method for indicating stability, and forced degradation products are characterized using LC-MS and FTIR under ICH guidelines [19]. Very few articles were reported in the last few decades for determining the Cabotegravir and Rilpivirine by using HPLC [20–22]. In

the present study, we intended to explore a specific, sensitive, and new HPLC method towards the analysis of Cabotegravir, Rilpivirine, and characterization of its degradation products by LC-MS and FTIR.

Conclusion

Till today there is no HPLC method to estimate the combination of Rilpivirine and Cabotegravir. A single HPLC method was validated and developed under ICH

Table 9 FTIR data of Cabotegravir, Rilpivirine, and its degradation products

S. no	API 1	API 2	DP1	DP2	DP3	DP4	Region (cm ⁻¹)	Assignment
1	3400	3502	3406.8	3515.3	3489.5	3488.1	3500–3300	N–H stretching
	3328.5	3325.4	3318.2	3327.8	3357.1	3345.6		
2	3157.6	3161.9	3162.3	–	3222.8	3228.3	3200–3000	C–H Alkene stretching
3	–	–	–	–	2897.8	2868.2	2840–3000	C–H Aliphatic stretching
4	–	2235.7, 2148.3	–	2236.2, 2152.5	2257.3	2368.1	2354	C≡N Stretching
5	1722.8	–	1725.3	–	1727.6	1737.1	1680–1630	C=O Stretching (Amides)
6	–	–	–	1487.8	–	–	1600–1450	C≡C Stretching
7	1287.8	1025.5	1288.6	1028.7	1287.9	1258.3	1350–1000	C–N Stretching
	1049.3		1051.7		1057.1	1087.4		

guidelines for the estimation of these two drugs. All validation parameters are within the limits, including system suitability, method precision, accuracy, LOD, LOQ, and robustness. This method has several advantages, including a shorter run time, a lower cost, accessibility, sensitivity, reliability, and reproducibility. The drugs' degradation actions were investigated under acid, base, oxidation, reduction, photolytic, and thermal stress conditions. The drugs were discovered to be stable under reduction, thermal, and photolytic conditions, but unstable under acid, alkali, and oxidative conditions. LCMS and FTIR experiments were used to characterize the degradation products.

Abbreviations

HPLC: High-performance liquid chromatography; LOQ: Limit of quantization; LOD: Limit of detection; ICH: International Conference on Harmonization; FTIR: Fourier Transform Infrared spectroscopy; MS: Mass spectrometry.

Acknowledgements

The authors are grateful to thank for the Acharya Nagarjuna University and management committee of Sir C R Reddy College, Eluru, for providing facilities to complete this research work.

Authors' contributions

Author 'SMT AND AV' designed the study, performed the method development and validation, wrote the protocol, and wrote the first draft of the manuscript. 'Author MR' and 'BSNM' helped in the analyses of the study. 'Author KAE' managed the literature searches. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data materials

The data for verification are provided with a Supplementary file and the rest. Of the data, if required, will be available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Chemistry, Vignan Degree College, Guntur, AP 522009, India. ²Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, AP 522510, India. ³Department of Physics, Sir CR Reddy College, Eluru, AP 534007, India. ⁴Department of Chemistry, Sir CR Reddy College, Eluru, AP 534007, India.

Received: 24 July 2021 Accepted: 3 October 2021

Published online: 06 November 2021

References

- De Cock KM, Jaffe HW, Curran JW (2012) The evolving epidemiology of HIV/AIDS. *AIDS* 26(10):1205–1213
- Blankson JN (2010) Control of HIV-1 replication in elite suppressors. *Discov Med* 9(46):261–266
- Sisson H (2015) Aspiring during the intramuscular injection procedure: a systematic literature review. *J Clin Nurs* 24(18):2368–2375
- Şanlıalp Zeyrek A, Takmak Ş, Kurban NK, Arslan S (2019) Systematic review and meta-analysis: physical-procedural interventions used to reduce pain during intramuscular injections in adults. *J Adv Nurs* 75(12):3346–3361
- De Clercq E (1998) The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antivir Res* 38(3):153–179
- Das K, Sarafianos SG, Clark AD Jr, Boyer PL, Hughes SH, Arnold E (2007) Crystal structures of clinically relevant Lys103Asn/Tyr181Cys double mutant HIV-1 reverse transcriptase in complexes with ATP and non-nucleoside inhibitor HBY 097. *J Mol Biol* 365(1):77–89
- Savarino A (2006) A historical sketch of the discovery and development of HIV-1 integrase inhibitors. *Expert Opin Investig Drugs* 15(12):1507–1522
- Stellbrink HJ (2007) Antiviral drugs in the treatment of AIDS: what is in the pipeline? *Eur J Med Res* 12(9):483–495
- Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoeltelmanns R, de Béthune MP, Peeters M, Woodfall B (2006) Short-term antiviral activity of TMC278—a novel NNRTI—in treatment-naïve HIV-1-infected subjects. *AIDS* 20(13):1721–6
- Cámara-Lemarroy CR, Rodríguez-Gutiérrez R, Monreal-Robles R, Marfil-Rivera A (2016) Gastrointestinal disorders associated with migraine: a comprehensive review. *World J Gastroenterol (Review)* 22(36):8149–8160
- Stovner L, Hagen K, Jensen R, Katsarava Z, RB Lipton, Scher A, Steiner T, Zwart JA (2007) The global burden of headache: a documentation of headache prevalence and disability worldwide. *Cephalalgia Int J Headache* 27(3):193–210
- Dayal R, Agarwal D (2016) Fever in children and fever of unknown origin. *Indian J Pediatr* 83(1):38–43
- White L, Ybarra M (2017) Neutropenic fever. *Hematol Oncol Clin N Am* 31(6):981–993

14. Wilson JF (2008) In the clinic. Insomnia. *Ann Intern Med* 148(9):ITC13-1-ITC13-16
15. Drake CL, Roehrs T, Roth T (2003) Insomnia causes, consequences, and therapeutics: an overview. *Depress Anxiety* 18(4):163–176
16. Schuckit MA, Tipp JE, Bergman M, Reich W, Hesselbrock VM, Smith TL (1997) Comparison of induced and independent major depressive disorders in 2,945 alcoholics. *Am J Psychiatry* 154(7):948–957
17. Gillis AM (2013) Heart Rhythm Journal celebrates 10 years. *Heart Rhythm* 10(5):619
18. Poole JE (2020) Heart Rhythm O2: bringing up-to-date heart rhythm science and information to anyone, anywhere. *Heart Rhythm* 1(1):1–2
19. ICH (2003) Q1A (R2) Stability testing of new drug substances and products. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2_Guideline.pdf.
20. Courlet P, Alves Saldanha S, Cavassini M, Marzolini C, Choong E, Csajka C, Günthard HF, André P, Buclin T, Desfontaine V, Decosterd LA (2020) Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HIV. *J Mass Spectrom* 55:4506
21. Kumar BMS, Rajkamal B, Chandramowli B (2019) Development and validation of Rilpivirine in pharmaceutical formulation by RP-HPLC. *Am J PharmTech Res* 9(03):345–353
22. Veeraswami B, Naveen VMK (2019) Development and validation of RP-HPLC method for the estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. *Asian J Pharm Clin Res* 12(2):267–271

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)