METHOD DEVELOPMENT AND VALIDATION OF DARUNAVIR AND COBICISTAT IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Dr. M. David Raju[*1], R. Yesupadamu[2], B. Eswar Sai[3], T. Mounika[4], R. Uma Maheswar Reddy[5]

*[*1] Department of Chemistry, PB Siddhartha College of Arts and Science, Vijayawada, AP.*

[2] Department of Chemistry, Sri ABR Government Degree College, Repalle, Bapatla District, AP.

[3,4,5] M. Sc Final Year Students, PB Siddhartha College of Arts and Science, Vijayawada, AP.

*Corresponding author: davidm201926@gmail.com

Abstract

A stability indicating RP-UPLC method was developed and validated for simultaneous estimation Darunavir and Cobicistat in tablet dosage form. The separation was achieved under optimized chromatographic condition on aAgilient1290InfinityII LC system UPLC Equipped with PDA detector,Symmetry C18 (50x4.6mm, 2.1µ column) with mobile phase consist of Acetonitrile: Buffer 0.1%OPA in the ratio of 50:50 v/v. An isocratic elution at a flow rate of 1 ml/min at ambient oven temperature was carried out with PDA detection at 245 nm. The retention time for Darunavir and Cobicistat was 2.07 min and 3.24 min respectively. The degradation was observed under acidic, alkali, oxidative, photolytic and thermal conditions. The linearity was found to be in the concentration range of 100-600µg/ml for Darunavir and 18.75-112.5µg/ml for Cobicistat. The % recoveries at 50% were found to be 100.27% & 100.15% for Darunavir &Cobicistat respectively. The % recoveries at 100% were found to be 100.02% & 99.95% for Darunavir &Cobicistat respectively. The % recoveries at 150% were found to be 99.84% & 100.57% for Darunavir &Cobicistat respectively. The method was validated as per ICH guideline and the values were found to be within the limits. So, the proposed method was found to be simple, linear, accurate, precise, stability indicating robust and specific.

Keywords: *Darunavir, Cobicistat, UPLC, Method validation.*

1. Introduction

Darunavir (DRV), sold under the brand name Prezista among others, is an antiretroviral medication[1,2]. It is used to treat and prevent HIV/AIDS[3,4]. It is generally recommended for use with other antiretrovirals. It is often used with low doses of ritonavir[5,6] or cobicistat[7-9] to increase darunavir levels. It may be used for prevention after a needlestick injury[10] or other potential exposure. Common side effects include diarrhea[11], nausea, abdominal pain, headache, rash and vomiting. Severe side effects include allergic reactions, liver problems, and skin rashes such as toxic epidermal

necrolysis. While poorly studied in pregnancy it appears to be safe for the baby. It is of the protease inhibitor[12] (PI) class and works by blocking HIV protease[13].

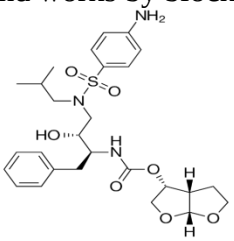

Fig no .1 structure of Darunavir

It is developed by pharmaceutical company Tibotec, darunavir is named after Arun K. Ghosh, the chemistry professor who discovered the molecule at the University of Illinois at Chicago. It was approved by the Food and Drug Administration (FDA) on June 23, 2006. It is on the World Health Organization's List of Essential Medicines. The fixed-dose combination medication darunavir/cobicistat (Rezolsta) is available as a single pill.

Cobicistat, sold under the brand name Tybost[14-15] is a medication for use in the treatment of human immunodeficiency virus infection (HIV/AIDS). Its major mechanism of action is through the inhibition of human CYP3A proteins. Like ritonavir (Norvir), cobicistat is of interest for its ability to inhibit liver enzymes that metabolize other medications used to treat HIV, notably elvitegravir, an HIV integrase inhibitor. By combining cobicistat with elvitegravir, higher concentrations of the latter are achieved in the body with lower dosing, theoretically enhancing elvitegravir's[16-17] viral suppression while diminishing its adverse side-effects. In contrast with ritonavir, the only other booster approved for use as a part of HAART, cobicistat has no anti-HIV activity of its own. Cobicistat is a potent inhibitor of cytochrome[18-19] P450 3A enzymes, including the important CYP3A4 subtype. It also inhibits intestinal transport proteins, increasing the overall absorption of several HIV medications, including atazanavir, darunavir, and tenofovir alafenamide [20].

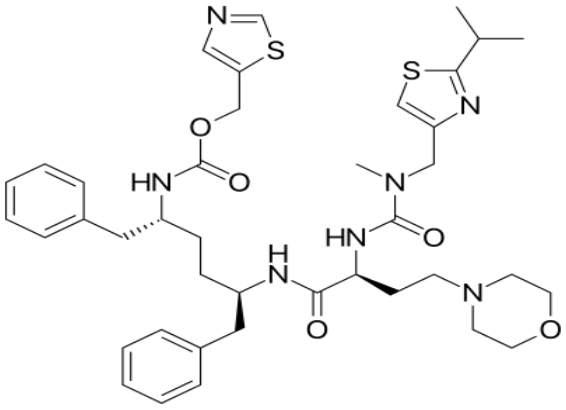

Fig no.2 Structure of Cobicistat.

Cobicistat is a drug analogue of ritonavir, in which the valine moiety is exchanged for a 2-morpholinoethyl group, and the backbone hydroxyl group is removed. These changes effectively eliminate the anti-HIV activity of ritonavir while preserving its inhibitory effects on the CYP3A isozyme family of proteins. Cobicistat is therefore able to increase plasma concentration of other coadministered anti-HIV drugs without the risk of causing cobicistat-resistant mutations in the HIV virus.

2. MATERIALS AND METHODS

Instrument

Agilient1290 Infinity II LC system UPLC with PDA detector equipped with Empower 2 software was used to perform the analysis of the tablet formulation.

Materials

Standard gift sample of Darunavir and Cobicistat were provided by Glenmark Pharmaceuticals, Mumbai (India). Tablet formulation was purchased from market, manufactured by Merck Pharmaceuticals.

Preparation of Standard Solution

Accurately weigh and transfer 400 mg of Darunavir, 75mg of Cobicistat working standard intoa 100 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and makevolume up to the mark with the same solvent. (Stock solution) .Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up tothe mark with diluent. (400ppm of Darunavir, 75ppm of Cobicistat).

Chromatographic Condition

The optimised Chromatographic Condition as given in table

Sample Preparation

Accurately weighed and transfer 1024mg of Darunavir and Cobicistat sample into a 100mLclean dry volumetric flask add Diluent and sonicate it up to 30 mins to dissolve, and centrifugefor 30min. to dissolve it completely and make volume up to the mark with the same solvent.Then it is filtered through 0.45 micron Injection filter. (Stock solution). Further pipette 5 ml ofthe above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent.(800ppm of Darunavir, 150ppm of Cobicistat).

Method Validation

The method was validated for linearity, accuracy, precision, repeatability and specificity. Accuracy was assessed by measuring recovery at three different levels, 50, 100 and 150% of the amount expected from analysis of the formulation, in accordance with ICH guidelines.RP-UPLC method was developed and validated for simultaneous estimation of Darunavir and Cobicistat in tablet dosage form. All system suitability parameters were passed in acceptable range. % Degradations of both drugs in different conditions was achieved as per ICH guidelines. Linearity of the developed method was near to 1, range was found 100–600µg/ml for Darunavir and 18.75–112.5µg/ml for Cobicistat. %RSD was found to be less than 2 for repeatability, intraday precision and intermediate precision. %Recoveries were found to be 99.67–99.75% and 99.83–100.98% for Darunavir and Cobicistat respectively. These results indicate that the developed method is accurate, precise, specific, robust and simple and less time consuming. It can be used in the routine quality control of marketed dosage form.

Forced Degradation Study

Standards of Darunavir, Cobicistat and formulation were subjected to forced degradation in acidic medium in presence of 0.1 N HCl at 80°C for 1 hour. Standards of Darunavir, Cobicistat and formulation were subjected to forced degradation in basic medium in presence of 0.05NNaOH at 50°C for 10 min. Standards of Darunavir, Cobicistat and Formulation were subjected to forced degradation in3% v/v solution of hydrogen peroxide (oxidizing medium) at room temperature for 24 hours. Thermal degradation study of standards of Darunavir, Cobicistat and formulation was carried out in a dry stability chamber at 105°C for 24 hours by exposing formulation in tablet form. Photo

degradation study of standards of Darunavir, Cobicistat and formulation was carried out in a photostability chamber by exposing to UV light in a Petridish for 1 ICH cycle.

System suitability

As per the test method, the standard solutions were prepared and injected into UPLC system from which the evaluated system suitability parameters are found to be within the limits

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyst in samples within the limits.

Precision

The degree of the closeness of agreement among individual test results when a method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.

Accuracy

The closeness of results was obtained by a method to the true value. It is a measure of the exactness of the method.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for each analyte were determined based on a signal-to-noise concept, as the lowest concentration at whichsignal-to-noise ratio between 3 or 2:1 and 10:1, respectively, with defined precision and accuracy under the given experimental conditions.

Stability

Standard and the sample solutions were subjected to 24 h stability studies at room temperature and 2–8°C. The stability of these solutions was studied and observed for changes in the area and retention time of thepeaks which were then compared with thepatternof the chromatogram of the freshly prepared solution.

Robustness

Robustness of the method was studied by slightly changes in experimental conditions such a flow rate and organic composition. Robustness on performed same instrument different chromatographic conditions.

.Ruggedness

Ruggedness of the method was studied using different source of analyst, instruments, and columns with same experimental conditions.

3. Results and Discussion

The aim of this study is to establish a single isocratic UPLC method for the simultaneous quantification of Darunavir, Cobicistat in bulk and pharmaceutical dosage forms that is

reliable, precise, and cost effective. According to the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

Optimization of the method

Using buffers (0.1% Trifluoro acetic acid, 0.1% formic acid, 0.1% triethylamine) and acetonitrile as mobile phase different trials were conducted in isocratic and gradient modes. Various stationary phases including phenyl, biphenyl, amino, C4, and C8, were used to test the system. The resolution and retention times were improved by changing the mobile step composition at each trial. In the end, the separation was achieved using a Symmetry C18 (50x4.6mm, 2.1µ column) and a mobile phase of 0.1% OPA: acetonitrile (50:50 v/v) with a flow rate of 0.5 ml/min and UV detection at a wavelength of 245 nm. The entire performance lasted three minutes. Conditions for optimized chromatography are provided in table 1.

System Suitability

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution.

Table 1. Method suitability conditions

Parameter	Suitable conditions
Column	Symmetry C18 (50x4.6mm, 2.1µcolumn
Moving Phase	0.1% Ortho Phosphoric acid: Acetonitrile (50:50 v/v)
Volume of injection	5 µl
Stream rate	0.5 mL/min
Temperature of column	Ambient
Wavelength	245 nm
Time duration	5 minutes
Retention time of Darunavir	2.07 min
Retention time of Cobicistat	3.24 min

Table 2. Results of system suitability

S.no	Parameter	Darunavir	Cobicistat
1	Retention time	2.127	3.248
2	Plate count	9865	12546
3	Tailing factor	0.75	1.02
4	Resolution	--	6.27
5	%RSD	0.49	1.34

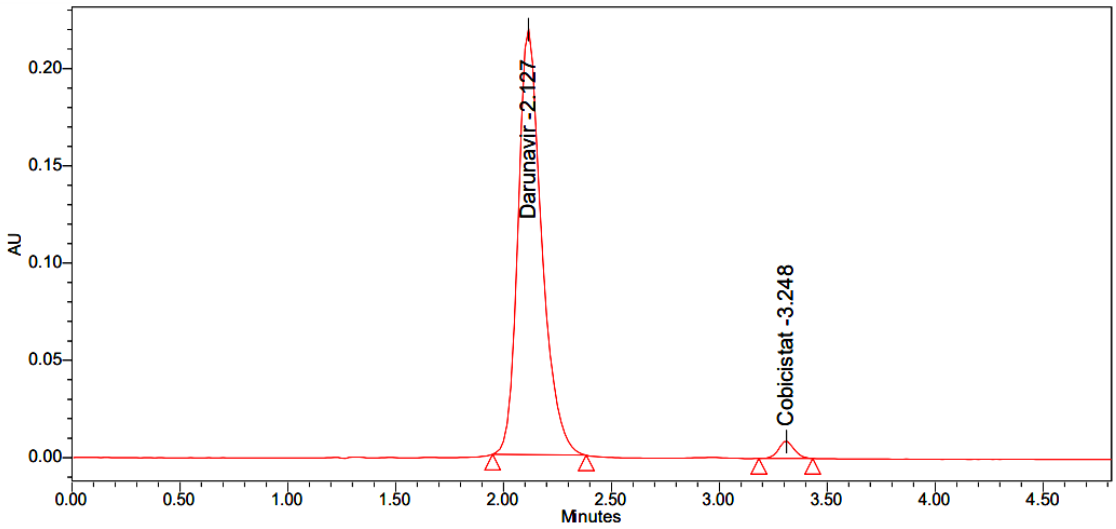

Figure 1. Chromatogram of standard

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Specificity

There was no participation from Darunavir and Cobicistat at the elution time. As seen in Figure 2, the blank chromatogram is present.

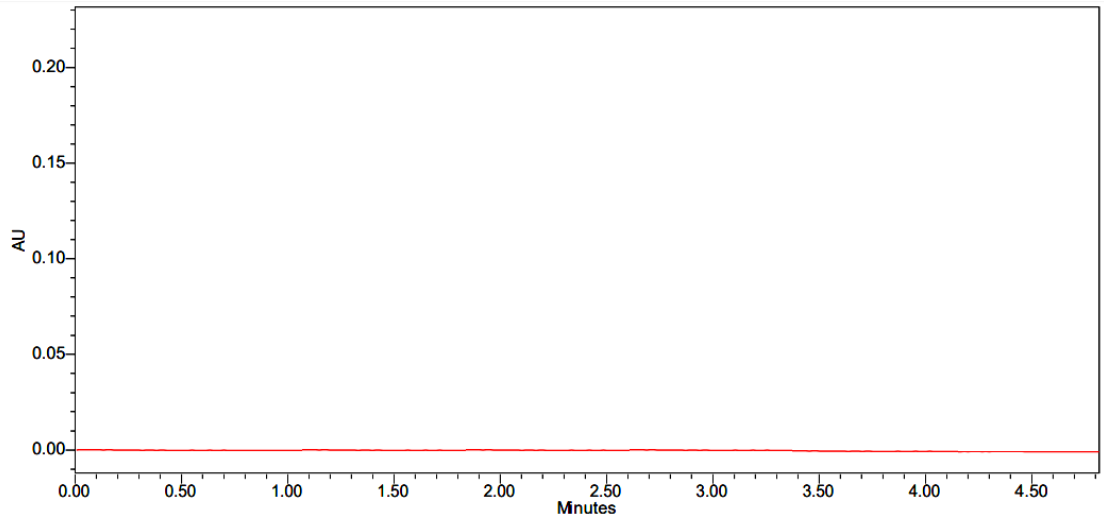

Figure 2. Chromatogram of blank

Linearity

By using a calibration curve to determine the linearity of the area of peak, its corresponding concentration was discovered. From this graph, it appears that the range of 100-500 µg/mL of Darunavir and 18.75-112.5 µg/mL of **C**obicistat had a straight line. Linearity results were demonstrated in table 3.

Table 3. Results of linearity

S. No	Darunavir	Cobicistat

	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	100	785496	18.75	310165
2	200	1542305	37.50	655947
3	300	2263512	56.25	953245
4	400	3125478	75.00	1256348
5	500	3896524	93.75	1502369
6	600	4602157	112.50	1845796

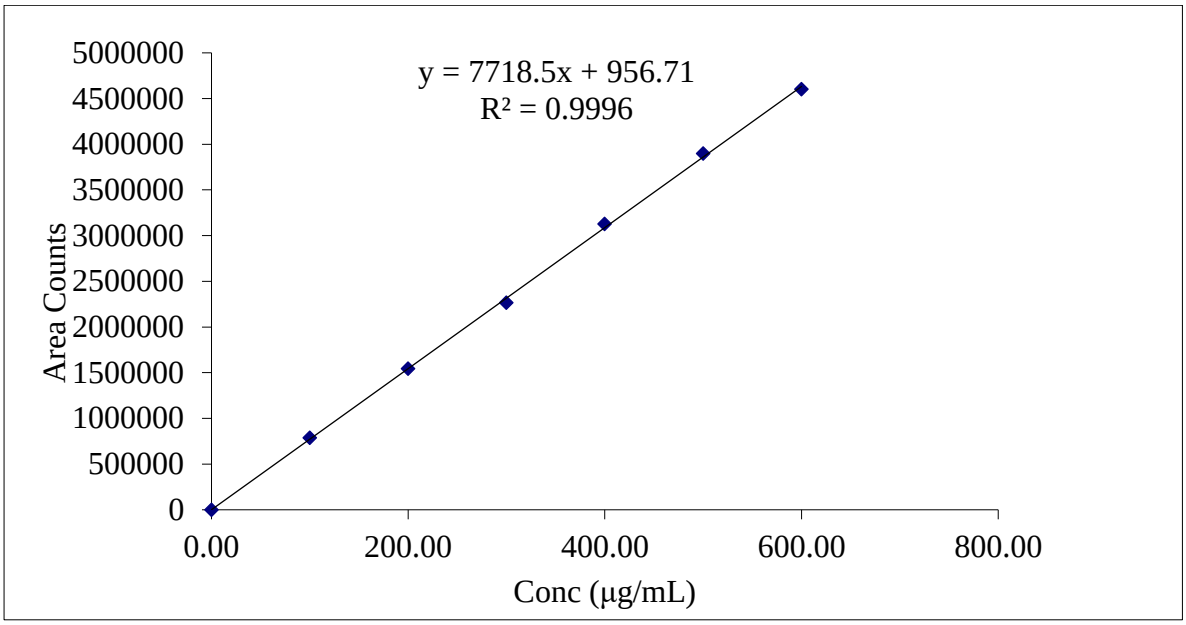

Figure 3. Calibration plot of Darunavir

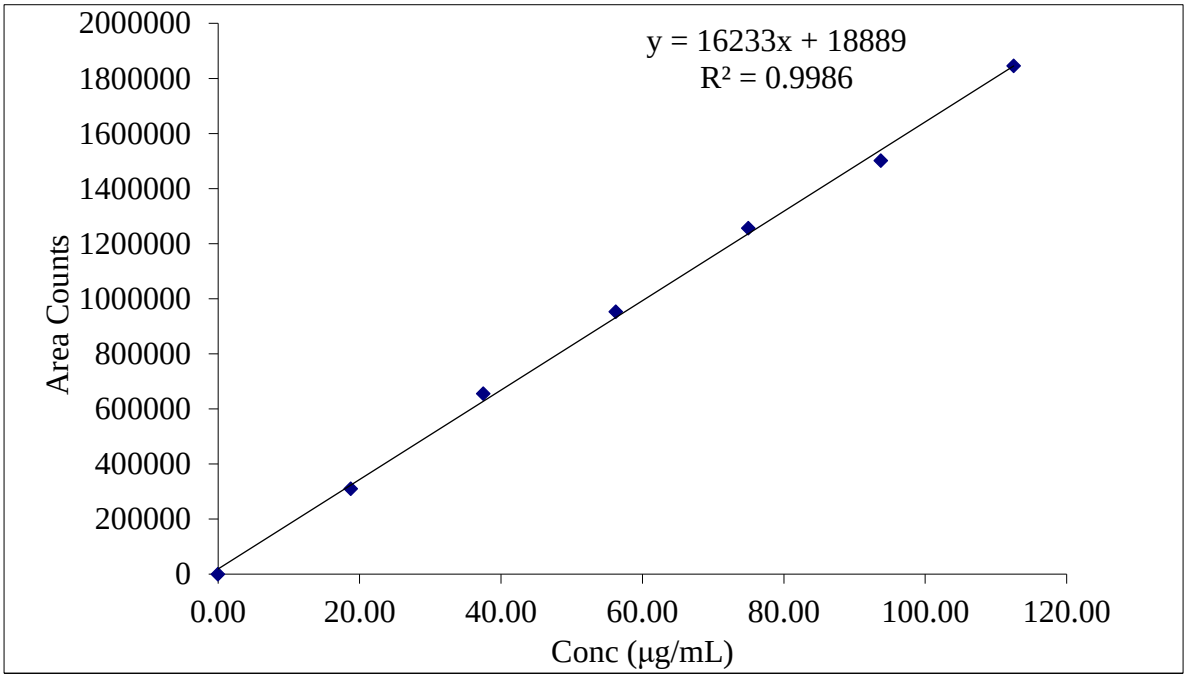

Figure 4. Calibration plot of Cobicistat

Precision

Intraday and intermediate precision variances were assessed in relation to the procedure's accuracy. The Standards were examined six times on the same day to obtain intraday results for Darunavir and Cobicistat. The system's intermediate precision was explored by analyzing data in the same laboratory using a variety of examiners and tools. It is very accurate, with an RSD percentage of less than 2%. The process was precise, yielding the best drug recoveries at each additional concentration. Table 4 shows the method precision results.

Table 4. Outcomes of method precision

S. No.	Darunavir		Cobicistat	
	Area	% Assay	Area	% Assay
1	3152468	99.55	854785	101.1
2	3142517	99.24	832654	99.34
3	3162543	99.88	828958	99.56
4	3185642	100.57	846523	100.42
5	3132568	98.81	822857	98.58
6	3165294	99.98	862539	101.72
Mean	3156839	99.65	841386	100.53
Std. dev	18691.94	0.591	15667.79	1.265
% RSD	0.592	0.59	1.262	1.27

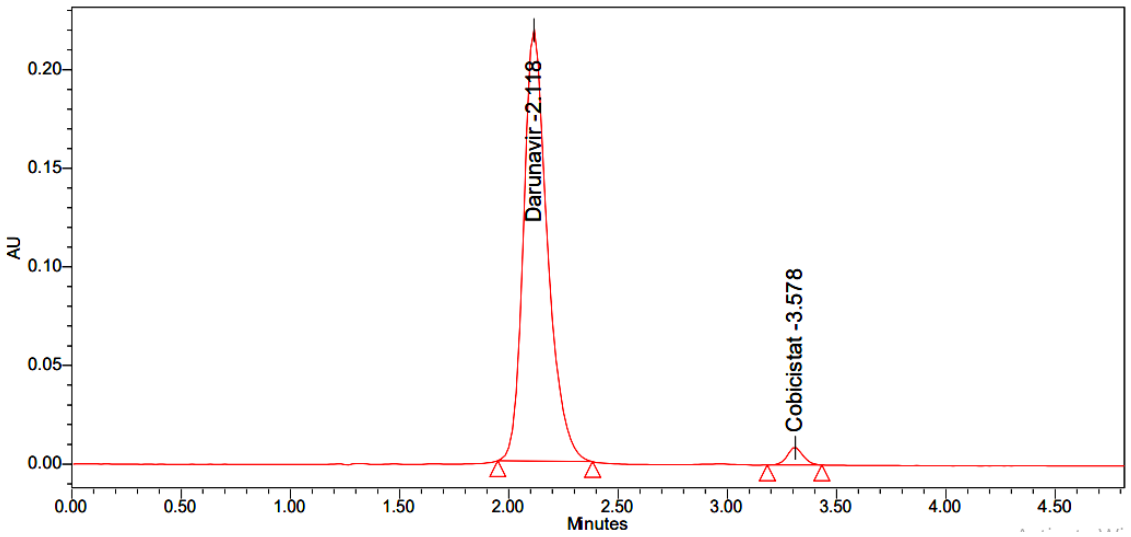

Figure 5. Chromatogram of method precision

Intermediate Precision (Ruggedness)

Intermediate precision results were shown in table 5.

Table 5. Results of intermediate precision

S.No.	Darunavir		Cobicistat	
	Area	% Assay	Area	% Assay
1	3147598	99.3	1265845	101.8
2	3131256	98.8	1248758	100.5
3	3145268	99.2	1239568	99.7
4	3168594	100.0	1222547	98.4
5	3145785	99.3	1258475	101.2
6	3195684	100.8	1235683	99.4

Mean	3155698	99.6	1245146	100.2
Std dev	22957.62	0.717	15814.77	1.247
% RSD	0.727	0.72	1.27	1.24

Accuracy

By measuring the recovery experiments at three stages, the method's precision was reached (50 percent, 100 percent, and 150 percent). APIs were made with concentrations of Darunavir of 50, 100, and 150 micrograms/mL and Cobicistat of 17.5, 35 and 52.5 micrograms/mL. For each stage of the spike, the test solution was injected three times, and the assay was performed in accordance with the test process. In addition to being able to determine the percentage of recovered data, the mean and relative standard deviations have also been found. The strategy was effective because the recovery values fell within the target range. Table 6 presents the accuracy results.

Table 6.Results of accuracy

Accuracy	Amount of Darunavir	% Recovery	Amount of Cobicistat	% Recovery
50*****	50	100.5	37.5	100.9
100*	100	100.5	75	101.3
150*	150	99.4	112.5	99.9

* Results are mean recovery of three sample preparations

LOD and LOQ

The concentration level at which the analytes are reliably detected and quantified is the limit of detection and quantification. Darunavir and Cobicistat had a LOD concentrations of 0.3 µg/ml, 0.11 µg/ml and their S/N values of 3, 10. The LOQ concentrations of Darunavir and Cobicistat were 1 µg/ml, 0.35 µg/ml, and their S/N values were 25, 22. (S/N is the ratio of signal to noise).

Robustness

To ensure the robustness of the chromatographic technique, the researchers evaluated flow rate and the composition of the mobile phase. By changing the flow rate and mobile phase ratio, the area of drugs changes. So, the percentage of relative standard deviation changes. Here in Table 7 (robustness results) the %RSD values are in within the acceptable limit.

Table 7. Outcomes of robustness

Parameter	% RSD of Darunavir	% RSD of Cobicistat
Flow Minus	0.53	0.61
Flow Plus	0.49	0.89
Organic phase (66:34)	0.74	1.27
Organic phase (54:46)	0.67	0.49

Forced Degradation

The proposed approach can be used for successful evaluations of release and stability tests, and it can be called a stability preferable technique. Acid, Alkali, oxidation, reduction, photo, and thermal degradation are all included in the ICH-required forced degradation analysis. The chromatograms show that the selected drugs remained stable under the stress conditions, despite the presence of degraded peaks. Results of forced degradation were given in table 8 and 9, forced degradation chromatograms were shown in figure 4.

Acid degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into UPLC system.

Alkali degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 ml of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the required concentration. Use a syringe filter to filter the solution, which will then be injected into the UPLC system.

Peroxide degradation

A volume of 1 ml sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into UPLC system.

Reduction degradation

Using a volumetric flask with a capacity of 10 ml, transfer 1 ml of sample stock solution and add 1 ml of 30% hydrogen peroxide solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the UPLC system.

Thermal degradation

During the 6-hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into an ultra-performance liquid chromatography system.

Photolytic degradation

A weight of 100mg sample was exposed to sunlight for 6 hrs. and the exposed Standard was analyzed. Prepare the Standard solution by using this Standard and inject into UPLC system.

Table 8. FD results

Stress Parameter (24 hrs)	% Degradation	
	Darunavir	Cobicistat
Acid degradation (1N HCl)	12.7	12.9
Alkali degradation (1N NaOH)	12.5	13.7
Peroxide degradation (30% Peroxide)	14.6	15.7
Reduction degradation (30% sodium bi sulphate)	9.4	10.4
Thermal (sample, 70°C, 6 Hrs)	5.2	2.8
Photo (UV-Vis light- (200 W h/m^2) and fluorescent light (1.2 milliion lux-h)	3.6	4.2

4. Conclusion

In this study, a novel, quick, sensitive, and easy-to-use UPLC method was developed for the simultaneous estimation of Darunavir and Cobicistat in API and pharmaceutical dosage types. Because there are no UPLC or HPLC methods published, this approach is the most practical option. Shorter run time, low cost, and all the other characteristics are benefits. Identifying many Standards necessitates considering these qualities. All the parameters were verified and were found to be within the acceptable range, including linearity, accuracy, specificity, robustness, and process precision. According to our research, the RSD values for all the parameters came in at less than 2%, showing that the procedure is accurate and that the results we found are consistent.

Therefore, it's possible to use the current approach in QC laboratories for routine study and manufacturing Darunavir and Cobicistat pharmaceuticals without having to separate the substances first.

Acknowledgement

Authors are thankful to my research guide for supporting this research.

Conflicts of Interest

None

Funding Support

None

5. References

[1] Burra P.Hepatitis C. Semin. Liver Dis.2009; 29 (1): 53–65..

[2] Nokes JD, Cane PA. New strategies for control of respiratory syncytial virus infection. Curr. Opin. Infect. Dis.December 2008; 21 (6): 639–43.

[3] Sepkowitz KA. AIDS – the first 20 years. The New England Journal of Medicine. June 2001 ;344 (23): 1764–72. .

[4] Montessori V, Press N, Harris M, Akagi L, Montaner JS. Adverse effects of antiretroviral therapy for HIV infection. Canadian Medical Association Journal. January 2004:170 (2): 229–38.

[5] World Health Organisation . World Health Organization model list of essential medicines: 21st list 2019. Geneva: World Health Organization.2009

[6] Nieminen TH, Hagelberg NM, Saari TI, Neuvonen M, Neuvonen PJ, Laine K, et al. Oxycodone concentrations are greatly increased by the concomitant use of ritonavir or lopinavir/ritonavir"(PDF). EuropeanJournal of Clinical Pharmacology.October2010 ;66 (10): 977–985.

[7] Lepist EI, Phan TK, Roy A, Tong L, Maclennan K, Murray B, Ray AS. Cobicistat boosts the intestinal absorption of transport substrates, including HIV protease inhibitors and GS-7340, in vitro". Antimicrobial Agents and Chemotherapy. October 2010; 56 (10): 5409–13.

[8] Xu L, Liu H, Murray BP, Callebaut C, Lee MS, Hong A, et al. Cobicistat (GS-9350): A Potent and Selective Inhibitor of Human CYP3A as a Novel Pharmacoenhancer ACS MedicinalChemistryLetters. 1 (5):August2010;20913.

[9] Sevrioukova IF, Poulos TL. Dissecting cytochrome P450 3A4-ligand interactions using ritonavir analogues. Biochemistry.July 2013; 52 (26): 447-81.

[10] Tarigan, Lukman H.; Cifuentes, Manuel; Quinn, Margaret; Kriebel, David. Prevention of needle-stick injuries in healthcare facilities: a meta-analysis. Infection Control and Hospital Epidemiology. July 2015;36 (7): 823–29.

[11] British National Formulary 69 (69 ed.). Pharmaceutical Pr. March 31, 2015

[12] Majorin F, Torondel B, Ka Seen Chan G, Clasen T, et al. (Cochrane Infectious Diseases Group) (September 2019). Interventions to improve disposal of child faeces for preventing diarrhoea and soil-transmitted helminth infection". The Cochrane Database of Systematic Reviews.

[13] Brik A, Wong CH. "HIV-1 protease: mechanism and drug discovery". Organic & Biomolecular Chemistry. January 2003.

[14] Mathias AA, German P, Murray BP, Wei L, Jain A, West S, et al. Pharmacokinetics andpharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIVactivity. Clinical Pharmacology and Therapeutics. March 2010: 87 (3): 3229.

[15] R Elion, J Gathe, B Rashbaum, and others. The Single-Tablet Regimen ofElvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (EVG/COBI/FTC/TDF; Quad) Maintains a High Rate of Virologic Suppression, and Cobicistat (COBI) is an Effective Pharmacoenhancer Through 48 Weeks. 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2010).Boston,Septmber12-15,2010.

[16] Horn, T. et al. Tenefovir Alafenamide Fumarate(TAF) sign-on letter to Gilead Archived 19 November 2019 at the Wayback Machine. 13 June 2013. Treatment Action Group.

[17] Eisenberg EJ, He GX, Lee WA. Metabolism of GS-7340, a novel phenyl monophosphoramidate intracellular prodrug of PMPA, in blood. Nucleosides NucleotidesNucleicAcids. 2001:20 (47):1098.

[18] Kumar, Sudhir . Molecular clocks: four decades of evolution. Nature Reviews.Genetics.2005: 6 (8):654662.

[19] Puustinen, A.; Wikström, M. The heme groups of cytochrome o from Escherichia coli.Proceedings of the National Academy of Sciences.1991-07-15: 88 (14): 6122–6126. Bibcode:1991PNAS...88.6122P.

[20] Gilead Press Release Gilead and Japan Tobacco Sign Licensing Agreement for Novel HIV Integrase Inhibitor March 22: 2008.

[21] Shimura K, Kodama E, Sakagami Y, et al.Broad Anti-Retroviral Activity and Resistance Profile of a Novel Human Immunodeficiency Virus Integrase Inhibitor, Elvitegravir (JTK-303/GS-9137) J virol. 2007:82 (2): 764–74.

[22] Rayudu S, Manoranjani M. Analytical method development and validation of Dexmethylphenidate and serdexmethylphenidate by using RP-HPLC in bulk and Pharmaceutical dosage forms, International journal of applied pharmaceutics .2022:14(2):110-115.

[23] Manoranjani M, Anitha D.A new stability indicating RP-HPLC method for the essay of Olmesartan in market formulations ,American journal of pharmatech research.2016:6(4):290-301.

[24] Manoranjani M. Assay method development and validation of Calnidipine and Ramipril, characterisation of its degradents by using LC-MS/MS, International journal of applied pharmaceutics.2022:

[25] Satya Dev T N V S S,Ramakrishna Ch..A New Related Substances Method Development And Validation Of Two Anticancer Drugs By Using Effective Liquid Chromatographic Method,International Journal of Applied Pharmaceutics,Vol 14, Issue 2, 2022.

[26] Satya Dev T N V S S. A new selective method development and validation of Cabozantinib and Nivolumab using HPLC. JPSR. 2021; 13 (3): 188-192.

[27] Satya Dev T N V S S. Stability indicating assay method development and validation of Aclidinium Bromide and Formoterol Fumarate by RP-HPLC. JETIR. 2019; 6 (6): 625-629.

[28] Manoranjani M. A study of method development, validation and forced degradation for simultaneous quantification of Cisplatin and Fluorouracil in bulk and pharmaceutical dosage form by RP-HPLC. JPSR. 2021; 13 (3): 155-161.

[29] Manoranjani M. Stability indicating assay method development and validation of Netarsudil and Latanoprost by RP-HPLC and its degradation. JETIR. 2019; 6 (6): 825-832.