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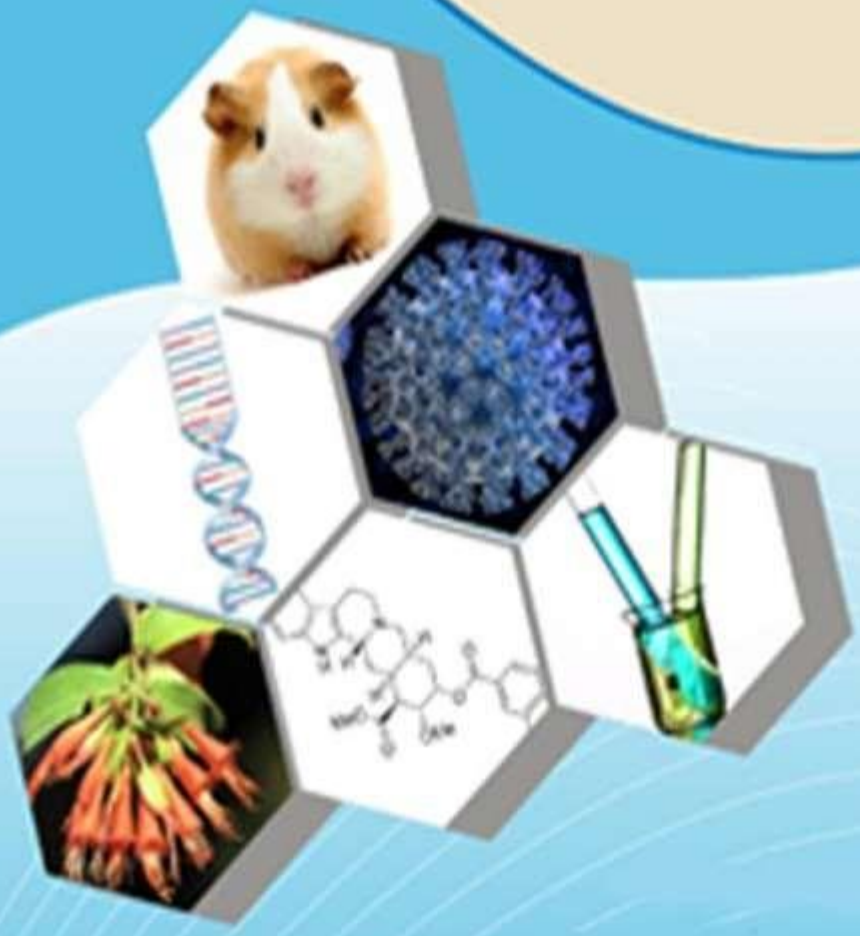

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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF COBICISTAT AND DARUNAVIR BY USING RP-HPLC

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ABSTRACT

The purpose of this research was to create an RP-HPLC technique that could detect cobicistat and darunavir simultaneously while being quick, easy, and accurate. To provide sufficient separation of all three medicines with internal standard, a variety of solvents, buffer-solvent ratios, and flow rates were investigated throughout the development of the procedure. The results of validating the proposed method showed that it meets ICH standards Q2 R1.A Xterra C18 5m (4.6*250mm) column was used to separate Cobicistat and Darunavir at a flow rate of 1 ml/min, a mobile phase ratio of Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), and a detection wave length of 255 nm. The instruments utilized in this analysis were a WATERS HPLC Auto Sampler, a separation module 2695, a PDA detector 996, and Empower-software version 2. You will recall both 2.39 and 3.907 minutes, according to the computer. Laboratory analysis confirmed that Cobicistat and Darunavir were 99.9 percent and 101.4 percent pure, respectively. Combining Cobicistat and Darunavir resulted in a resolution of 8.0, theoretical plates of 1.3, and a tailing factor of 1.4. The validation of the analytical technique was carried out in accordance with ICH standards (ICH, Q2 (R1)). Cobicistat and Darunavir were found to have linear concentration-response curves from 1 to 5 grams and 100 to 500 grams, respectively, with mean recoveries of 100 and 100.5, repeatability standard deviations (RSDs) of 2 and 4, and intermediate RSDs of 5 and 16 percent, respectively.

Keywords: Parameters for validation of reversed-phase HPLC for determination of cobicistat and darunavir.

INTRODUCTION

A drug is any chemical that may have medicinal, intoxicating, performance-enhancing, or other effects when consumed orally or injected into a live body. Medicines are defined as "chemical substances used in the treatment, cure, prevention, or diagnosis of disease or used in other ways to enhance physical or mental well-being" in the field of pharmacology. Drug therapy cycles may be unnecessary or excessive for certain illnesses. The building blocks of large pharmacological compounds are typically hydrogen, carbon, and sometimes oxygen and nitrogen. Hydrate is only one kind of drug that may have zero or one nitrogen atoms. Substances with a psychoactive effect on the central nervous system, such as opioids and hallucinogens, are often used Recreationally. They may have a positive effect on one's thoughts, feelings, personality, and conduct, making them a viable therapeutic option. Addiction and/or habituation may occur with a number of drugs. Analytical chemistry techniques are utilized extensively throughout many fields, including business, health, and the sciences. Doctors utilize the results of gas analysis on millions of blood samples every day to make diagnoses and decisions about patient care.

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The efficiency of smog-control equipment may be determined by analyzing the concentrations of hydrocarbons, nitrogen oxides, and carbon monoxide in vehicle exhaust. Parathyroid illness in humans may be diagnosed using quantitative measures of ionized calcium in blood serum. A food's protein content and, by extension, its nutritional value, may be quantified by measuring its nitrogen concentration. In-process analysis has made it possible to adjust the carbon, nickel, and ductility ratios in steel. To warn residents of potential gas leaks, the mercaptan content of their gas supply is frequently checked. Farmers need to be adaptable in terms of when they water and fertilize their crops, since plants have varying requirements throughout the growing season. Using measurements of plants and soil, we may make educated guesses about what is required.

FOR USE IN LABORATORY EXPERIMENTS:

We have used the following apparatus in our experimental work:

Table 1: Equipment Listing

S.No	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance PDA Detector	Waters 2695 Waters 996	Empower	Waters
2	UV double beam spectrophotometer	UV 3000	UV Win 5	Lab India
3	Digital weighing balance	BSA224SCW	-	Sartorius
4	pH meter	AD102U	-	Lab India
5	Ultra sonicator	UE600S	-	-
6	Suction pump	VE115N	-	-

Several substances are used in the experimental inquiry.

The following are some commonly used compounds:

Material Criteria Table 2

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R
5	Cobicistat and Darunavir	-	-

METHODDEVELOPMENTS:

The following procedures constitute the development of a method for the simultaneous estimation of Cobicistat and darunavir in pharmaceutical dose forms.

- Selection of detection wavelength (λ-max)
- Selection of column
- Selection of mobile phase
- Selection of flow rate
- Preparations and procedures

Selection of detection wavelength:

Mobile phase was used to dissolve cobicistat and darunavir (10 mg). These spectra were collected by scanning the solution at various wavelengths between 200 and 400 nm. The optimal wavelength for each therapy was determined by comparing their shared y-spectra. The isosbestic point was used to determine the detecting wavelength.

Selected Cell in Table

The analytes are separated using a [Column: Inertial C18 (4.6 x 250mm, 5m, Make: Waters)].

Picking a Portable Playground:

Phosphate buffer, pH 4.6 (0.05M), and acetonitrile (ACN) will be mixed in a concentration range of 30% (v/v) to 70% (v/v) to make the mobile phase. A buffer should have a pH value between 2 and 8, at most. In a pH2 buffer, simazine linkages are broken. Buffers with a pH greater than 8 may dissolve silica. The elution quality is controlled by the ionization

properties, which in turn are controlled by pH. It's conceivable that irregularity may rise as regularity declines. There should be sufficient RF, AR, TF, and RF clarity.

Selection of flow rate:

- Flow rate is selected based on
- Retention time
- Column back pressure
- Peak symmetry
- Separation of impurities

Preventive Measures:

Here's how you whip up some pH-4.6 phosphate buffer: a.

In a 1000 mL beaker, the pH of HPLC water was corrected to 4.6 using ortho phosphoric acid. After then, 6.8 grams of KH2PO4 were dissolved in the water.

Getting ready for the "mobile" stage

After degassing for 5 minutes in an ultrasonic water bath, 30 mL of phosphate buffer at pH 4.6 is mixed with 70 mL of acetone. After that, a 0.45-micron filter is used in a vacuum to filter the solution.

The Preparation of the Diluent (b)

The "Diluent" in this context is the "Mobile Phase."

Preparing a Cobicistat follows the usual steps listed in (d).

About 2 ml of DMF was poured to a dry, clean 10 ml volumetric flask containing 10 mg of Cobicistat to use as a standard. The diluent is added after the mixture has been sonicated to achieve complete dissolve and get the desired volume. Common Reply. A volumetric flask holding 100 ml was diluted with 10.0 ml of the stock solution to reach the desired tolerance level.

Darunavir Dosage Consistency for Individuals

In a dry, clean 10 ml volumetric flask, add 2 ml of dry DMF, followed by 10 mg of Darunavir standard. To help dissolve the contents, sonicating the mixture is done first; then the diluents are added to get the desired volume. Common Reply. A volumetric flask holding 100 ml was diluted with 10.0 ml of the stock solution to reach the desired tolerance level.

Tabulating, or compressing, the test solution

In a clean, dry 10 mL volumetric flask, crush 10 tablets containing commercially available Darunavir and Cobicistat into a fine powder using a crusher and pestle. In order to get the appropriate concentration after dissolving 7 mL of diluents by sanitation, additional of the same solvent must be added to the final amount. Getting ready for action a further 3 mL of the stock solution was pipette into the 10 mL volumetric flask after the necessary amount of solvent was added.

Procedure:

Adding 20 liters of the standard sample to the chromatographic apparatus and calculating the peak areas for Darunavir and Cobicistat yields the% Assay.

Whether or not the System is Appropriate:

Darunavir and Cobicistat peaks in standard solutions should have a tailing factor of no more than 2.0.

Darunavir and Cobicistat peaks in Standard solution should have theoretical plate values of at least 2000.

Assay calculation:

$$Assay\,\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

P = % Purity of Reference Material

Lc = Label Claim Units of Drug in Mg/mL.

ANALYTICALMETHODVALIDATION

Accuracy:

Rational dosing of cobicistat and darunavir:

In a 10 mL volumetric flask and a 100 mL volumetric flask, respectively, we put the 10 mg doses of Darunavir and Cobicistat. Add extra solvent and 7 –70 mL of diluents to get the desired volume, and then sonicate the mixture to ensure thorough dissolve. Gathering for Preparation The necessary concentration of 3 ml was achieved by pipetting the aforementioned stock solution into a 10 ml volumetric flask and then further diluting it using diluents.

Preparing and Scrubbing Down the Test Solution:

If you follow these instructions, you'll end up with a solution that's half as concentrated (with respect to the desired Assay value).

To a 10 mL and a 100 mL clean, dry volumetric flask, respectively, 5 mg of Darunavir and 5 mg of Cobicistat working standard were weighed and transferred; 7 mL of Diluents were added, sonicated to dissolve it completely, and the volume was brought up to the mark using the same solvent. Standardization of the Respondents). A 10 ml volumetric flask was equipped with a 3 ml dilution of Darunavir stock solution and a 0.3 ml dilution of Cobicistat stock solution.

Assay concentration-specific solutions may be obtained by:

Sanitation was used to dissolve 10 mg of Darunavir and 10 mg of Cobicistat in 7 mL of Diluents; the concentrations of both drugs were then adjusted using additional of the same solvent. Standardization of the Respondents). A 10 ml volumetric flask was equipped with a 3 ml dilution of Darunavir stock solution and a 0.3 ml dilution of Cobicistat stock solution.

To make an Assay solution with a concentration 150% higher than needed, you would:

To dissolve 15 mg of Darunavir and 15 mg of Cobicistat, respectively, the quantities of diluents used were little over 7 mL and 15 mL Standardization of the

Respondents). Fill a 10-milliliter volumetric flask with 3 milliliters.

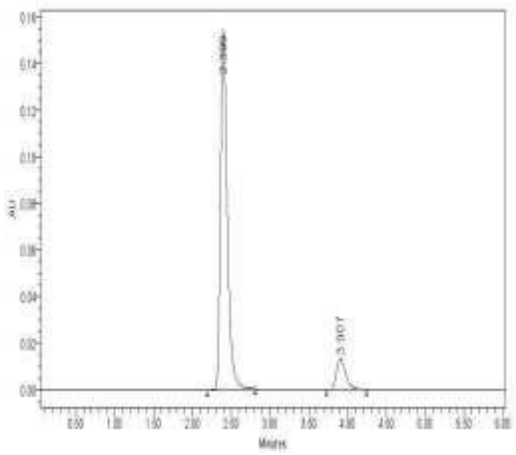

Fig.1 Trail Chromatogram

Table3 Details of Trail

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Cobicistat	2.399	946124	155429	5105	1.3	8.1
2	Darunavir	3.907	111541	13239	3788	1.4	

Observation:

All of the chromatogram's components may be seen to be separated from one another. The parameters and maximal system symmetry both represent ideal cases. That's why we've decided to go with this strategy.

VALIDATION RESULTS

1. Accuracy:

Table 4Accuracy results of Darunavir

Acceptance criteria:

Each tier's % Recovery needs to be between 98.0 and 102.0%.

Table 5Accuracy results of Cobicistat

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4725008	10	9.94	99.4%	
150%	5911798	15	14.8	98.2%	

Acceptance criteria:

Each tier's % Recovery needs to be between 98.0 and 102.0%.

2. Precision

i) Repeatability

ii) Intermediate precision (Ruggedness)

Repeatability

Table 6 Repeatability results of Cobicistat & Darunavir.

Name: Darunavir

	Name	RT	Area	Height (µV)
1	Darunavir	4.304	1501417	100275
2	Darunavir	4.300	1486940	100079
3	Darunavir	4.308	1490656	98257
4	Darunavir	4.310	1487329	98165
5	Darunavir	4.314	1490384	98153
Mean			1491345	
Std. Dev.			5881.4	
% RSD			0.39	

Name: Cobicistat

	Name	RT	Area	Height (µV)
1	Cobicistat	2.321	2235319	196999
2	Cobicistat	2.317	2240678	198254
3	Cobicistat	2.323	2249490	195128
4	Cobicistat	2.322	2245822	196164
5	Cobicistat	2.324	2251694	195887
Mean			2244601	
Std. Dev.			6656.8	
% RSD			0.30	

Acceptance criteria:

There should be no more than a 2% RSD difference between the regions of five standard injections. Method precision research revealed that for both cobicistat and diazepam, the % RSD was 0.3 (NMT2).

Intermediate precision/Ruggedness

Table7Ruggedness results of Darunavir & Cobicistat

Name: Cobocistat

	Name	RT	Area	Height (µV)
1	Cobocistat	2.328	2194758	189693
2	Cobocistat	2.326	2195700	190025
3	Cobocistat	2.327	2196191	189862
4	Cobocistat	2.326	2195326	190700
5	Cobocistat	2.331	2200951	189426
Mean			2196585	
Std. Dev.			2496.0	
% RSD			0.11	

Name: Darunavir

	Name	RT	Area	Height (µV)
1	Darunavir	4.335	1456296	95623
2	Darunavir	4.336	1457422	95150
3	Darunavir	4.334	1456513	95165
4	Darunavir	4.337	1454579	95298
5	Darunavir	4.340	1451483	95251
Mean			1455259	
Std. Dev.			2347.6	
% RSD			0.16	

Acceptance criteria:

The relative standard deviation (RSD) between the means of five separate injections shouldn't exceed 2%.

The NMT2 trial found that the RSD for both Cobicistat and Darunavir was less than 0.1%.

3. Specificity:

Table8Standard results of Darunavir & Cobicistat

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Cobicistat	2.237	7913799	394185	2632	1.8	
2	Darunavir	4.342	1855381	162758	2614	1.6	5.23

Linearity:

Both low concentrations (from 1 ppm to 5 ppm) and high concentrations (from 100 ppm to 500 ppm) were used to evaluate the linearity. The chromatographic machine needed injections at each new stage. It is possible to get a correlation coefficient by comparing the regions of the various levels. Comparison of Darunavir with Cobicistat in Terms of Their Linearity.

Table10 Linearity results of Cobicistat and Darunavir

	SampleName	Name	RT	Area	Height (µV)
1	Linearty 1	Cobicistat	2.309	1810101	145957
2	Linearty 1	Darunavir	4.307	1164173	75128
3	Linearty 2	Cobicistat	2.322	2044287	176935
4	Linearty 2	Darunavir	4.317	1342535	87703
5	Linearty 3	Cobicistat	2.324	2367133	206622
6	Linearty 3	Darunavir	4.323	1555931	101999
7	Linearty 4	Cobicistat	2.336	2602279	228576
8	Linearty 4	Darunavir	4.340	1777973	117084
9	Linearty 5	Cobicistat	2.345	2869778	259346
10	Linearty 5	Darunavir	4.340	1942319	129409

Criteria for acceptance:

The minimum value for the correlation coefficient is 0.999.

Calibration graphs are created by comparing the areas of linearity peaks to the concentrations at which the calibration was performed.

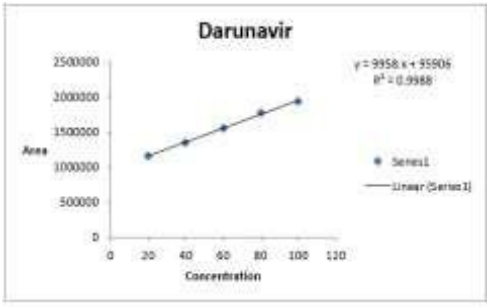

Fig.2Calibration curve of Darunavir

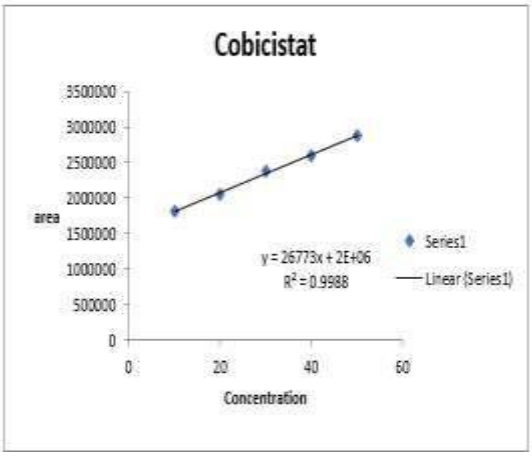

Fig.3Calibration curve of Cobicistat

Range

Both Cobicistat and Darunavir were shown to be linear throughout a concentration range of 10–50 mg, with a correlation value of 0.9988, and 20–100 mg, with a correlation coefficient of 0.999(NLT0.9988).

Robustness

We investigated the method's resilience by changing the flow rate, mobile phase composition, and temperature.

A) Flow Rate:

Darunavir flow rate system suitability findings tabulated.

S.No	Flow Rate (ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

*Assay standard results for real flow (1.0ml/min) were used.

Cobicistat (Flow rate) System Suitability Data Table

S.No	Flow Rate (ml/min)	System suitability results	
		USP Plate count	Upscaling
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

The assay standard data were analyzed at a flow rate of 1.0 ml/min.

Transitioning to Stage

The organic percentage of the mobile phase, which was formerly 70%, is now just 60%. To do this, we created and evaluated a range of mobile phase compositions while testing a standard solution of 300 g/ml Darunavir and 3 g/ml Cobicistat.

Final Thoughts on the Darunavir System's Safety:

Darunavir system suitability findings (Mobile phase) are shown in Table.13.

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10%More	1948.0	1.2

From the results of the accuracy standard, we inferred that the actual mobile phase composition is 45:55 Buffers: ACN.

Compliance testing of the Cobicistat system:

Table 14: Cobicistat system suitability findings (mobile phase).

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	883.3	1.56
2	Actual	1234.0	1.1
3	10%More	969.2	1.6

According to the Accuracy requirements, we considered the real 55:45 buffer: ACN mobile phase composition.

Conclusion

With some tweaks to the RP-HPLC method, we were able to measure both cobicistat and darunavir at the same time. With a flow rate of 1 ml/min, a mobile phase ratio of Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), and a detection wave length of 255 nm, Cobicistat and Darunavir were separated using an Xterra C18 5m (4.6*250mm) column. WATERS HPLC Auto Sampler, separation module 2695, PDA detector 996, and Empower-software version 2 were used in this investigation. The machine says you'll remember 2.39 hours and 3.907 hours. The purity levels of Cobicistat (99.9%) and Darunavir (101.4%) were verified in a laboratory setting. The resolution was 8.0, the theoretical plates were 1.3, and the tailing factor was 1.4 when Cobicistat and Darunavir were used together. The analytical method was validated in compliance with ICH (ICH, Q2 (R1)) guidelines. Concentration response curves for 1–5 grams of Cobicistat and 100–500 grams of Darunavir were found to be linear, with mean recoveries of 100 and 100.5, repeatability standard deviations (RSDs) of 2 and 4, and intermediate RSDs of 5 and 16 percent. The findings of the precision investigation were confirmed to be accurate. The LOQ and LOD values were both in the low 3s and the upper 9s, respectively. The suggested RP-HPLC method may be used to regularly evaluate Cobicistat and Darunavir in API and Pharmaceutical dose form.

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