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Stability indicating RP-HPLC method for the simultaneous estimation of darunavir ethanolate and cobicistat in bulk and tablet dosage form

D. Sindu Priya*, D. Gowri Sankar and D. Jaya Chandrika

Department of Pharmaceutical Analysis and Quality Assurance, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam,530003 Andhra Pradesh, India.

ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Darunavirethanolate and Cobicistatin bulk and pharmaceutical dosage forms. Chromatography was carried out on Enable C_{18} (250mm x 4.6 mm, 5 µ particlesize) column using a mobile phase of water (adjusted to pH 3 with 0.1% orthophosphoric acid): Acetonitrile (55:45 % v/v) at a flow rate of 1ml/min. The analytes were monitored using PDA detector at 245 nm. The retention time was found to be 3.074min and 6.081min for Darunavirethanolate and Cobicistat respectively. The proposed method was found to be having linearity in the concentration range of 30-120 µg/ml for Darunavirethanolate and 6-50µg/ml for Cobicistat. The mean % recoveries obtained were found to be 99.99-100.00% for Darunavirethanolate and 99.85-100.08 % for Cobicistat. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guidelines and found to be simple, specific, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the stability indicating simultaneous estimation of Darunavirethanolate and Cobicistatin bulk and Pharmaceutical formulations and in routine quality control analysis.

Keywords: Darunavirethanolate, Cobicistat, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Darunavir(DRV), in the form of darunavirethanolate, has the following chemical name: [(1*S*,2*R*)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-phenylmethyl)propyl]-carbamic acid(3*R*,3a*S*,6a*R*)-hexa hydrofuro [2,3-*b*]furan-3-yl ester monoethanolate. Its molecular formula is$C_{27}H_{37}N_3O_7S \cdot C_2H_5OH$ and its molecular weight is 593.73.

Cobicistat(COBI) is adsorbed onto silicon dioxide. The chemical name for cobicistat is 1,3-thiazol-5-ylmethyl[(2*R*,5*R*)-5-{[(2*S*)2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4yl) butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate.

It has a molecular formula of $C_{40}H_{53}N_7O_5S_2$ and a molecular weight of 776.0.

Literature survey reveals that a few analytical methods are reported like Spectrophotometric methods[2,3] and RP-HPLC methods[4-6] in alone or in combination with other drugs in pharmaceutical dosage forms but no simple stability indicating RP-HPLC method for the simultaneous estimation of DRV and COBI in Pharmaceutical dosage forms have been reported so far. Hence author has planned to develop a simple, accurate, precise and sensitive Stability Indicating RP-HPLC method for the simultaneous estimation of DRV and COBI in bulk and its pharmaceutical dosage forms suitable for routine quality control analysis.

MATERIALS AND METHODS

Chemicals and solutions
HPLC grade Acetonitrile(ACN) (Merck), HPLC grade milli-Q water, and Orthophosphoricacid(OPA) was used for the analysis.

Instrumentation
Quantitative HPLC was performed on a Shimadzu LC20 –AD, SPD M20A prominence PDA Detector, Rheodyne universal injector 7725 port and Hamilton 50 µl manual injector. Data processing was performed with shimadzu LC Solutions software version 1.25 for LC peak integration.

Mobile phase Preparation
A 55:45 v/v mixture of water (pH 3) and ACN was prepared by mixing 550 ml of water (pH adjusted to 3with 0.1%OPA) and 450 ml of ACN in a 1000 ml volumetric flask. The mixture was filtered through 0.45 µ membrane filter and sonicated before use. The same mixture was used as diluents for preparing working standard solutions of the drugs.

Preparation of standard stock solution
Accurately weighed and transferred 80 mg & 15 mg of DRV and COBI working standards into a 100 ml clean and dry volumetric flask, 30 ml of diluent was added, sonicated for 30 minutes and then made up to the final volume with diluent. From the above stock solution, 1.0 ml was pipetted out in to a 10 ml volumetric flask and then make up to the final volume with diluent.

Preparation of sample stock solution
20 tablets were weighed and average weight of each tablet was taken and then powder equivalent to 80 mg & 15 mg of DRV and COBI was transferred into a 100 ml volumetric flask, 30 ml of diluent was added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution, 1.0 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

Chromatographic conditions
Preliminary studies were conducted and trails were made for the method development. Separation and analysis was carried out on Enable C_{18} (250mm x 4.6mm, 5µ particle size) column. The optimized mobile phase consisting of water (pH adjusted to 3 with 0.1% OPA) and ACN and in the ratio of 55:45 % v/v. Flow rate was maintained at 1 ml/min and run time for 8 min. Prior to sample injection, column was saturated with mobile phase for 40 min and injection volume of 10 µl injected by manual Injector. The detection response was measured at 245 nm and maintained at ambient temperature.

Method Validation[7]
The method was validated for its linearity, accuracy, precision, sensitivity and specificity. Method validation is carried out as per ICH guidelines.

System suitability
System suitability was carried out by injecting standard solutions five times into the chromatographic system. The system suitability parameters were then evaluated using mean peak area and % RSD of the individual drugs of standard chromatograms.

Specificity
Specificity is the ability to assess unequivocally the analytes in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from standard, sample, blank and placebo solutions.

Linearity
The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatograms were recorded.

System Precision
The system precision was carried out by injecting standard solution preparations six times into the chromatographic system and calculate %RSD of retention time and peak area for both DRV and COBI in standard preparations.

Method precision
In method precision, a homogenous sample of a single batch should be analyzed six times by injecting sample solution preparations six times into the chromatographic system and calculate %RSD of retention time and peak area for both DRV and COBI in sample preparations.

Accuracy
The accuracy was performed by making three different standard concentrations at 50%, 100% and 150% levels of known amounts of studied drugs. The accuracy of an analytical method should be established across its range. The mixture was then analyzed by the proposed HPLC method at 245 nm.

Robustness and Ruggedness
The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and wavelength. The standard and sample solution were injected into the chromatograph at varied conditions of flow ± 0.2 ml/min, mobile phase composition with altered organic phase of ±2 % and wavelength ±2 nm.

Forced Degradation[8]
Stress testing of the drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule.

Acid degradation studies
To 1.0 ml of stock solution of DRV and COBI, 1ml of 2N HCl was added and refluxed for 30mins.The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Alkali degradation studies
To 1.0 ml of stock solution of DRV and COBI, 1 ml of 2N NaOH was added and refluxed for 30mins. The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Hydrolytic studies
Stress testing under neutral conditions was studied by refluxing 1.0 ml of stock solution of DRV and COBI on water bath for 6 hrs. The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Peroxide studies
To 1.0 ml of stock solution of DRV and COBI,1 ml of 20 % H_2O_2 was added. The solution was kept for 30 min. The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Photolytic studies

It is studied by exposing the 1.0 ml stock solution of DRV and COBI to UV light by keeping in the UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Thermal studies

It is carried out by keeping 1.0 ml stock solution of DRV and COBI in an oven at 105°C for 6 hrs to study dry heat degradation. The resultant solution was further diluted to required concentration and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

Bench top Stability

Standard and sample solutions of DRV and COBI were prepared as per the test method and injected into the chromatographic system at initial and 24 hours by keeping solutions at room temperature.

RESULTS AND DISCUSSION

From this study, it was found that a Simple, precise, accurate, sensitive and efficient Stability Indicating RP-HPLC method has been developed and validated for the estimation of DRV and COBI in pharmaceutical dosage form. Separation was done by using mobile phase composed of Water (adjusted to pH 3 with 0.1% OPA) and ACN in the ratio (55:45% v/v). Chromatographic separation was carried out on Enable C_{18} column (250 mm x 4.6mm, 5µ particle size) at a flow rate 1 ml/min using PDA detection at 245 nm. The retention times of DRV and COBI were found to be 3.074 and 6.081 min respectively. There was no interference from the excipients commonly present in the formulation and from the mobile phase. It may therefore be inferred that no degradation of DRV and COBI in the pharmaceutical formulation was detected by using this method. And in the validation of the assay, formulation, placebo samples and blank, yielded clean chromatograms [Fig. 2. a-d]; with no interference from the excipients and mobile phase; The peaks of the degradation products were well resolved from that of DRV and COBI [Fig. 2. e–j] this is indicative of the specificity of the method. System suitability results are shown in Table 2. Linearity was evaluated in the concentration range of 30-120µg/ml for DRV and 6-50µg/ml for COBI and results are shown in Table3. The calibration curves of DRV and COBI were described by the equation y = 44817x - 19056 and y = 16363x + 2868 respectively with correlation coefficient 0.999 as shown in [Fig 3 a, b)]. The %RSD in system and method precisionas shown in Table 4, were found to be less than 2.0%, indicating that the method is precise. Accuracy data was shown in Table 5, where the drug recoveries are found to be between 99.9 to 100 % for DRV where 99.85 to 100.08% for COBI which explains that the method is accurate. The proposed method was found to be robust as the deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. Robustness values are given in table no. 6. Standard solutions of DRV and COBI were found to be stable in the mobile phase for a period of 24hours, because no peaks corresponding to degradation products were observed and there was no significant change in the peak area of the drug (RSD <1%). The forced degradation data is given in Table7.

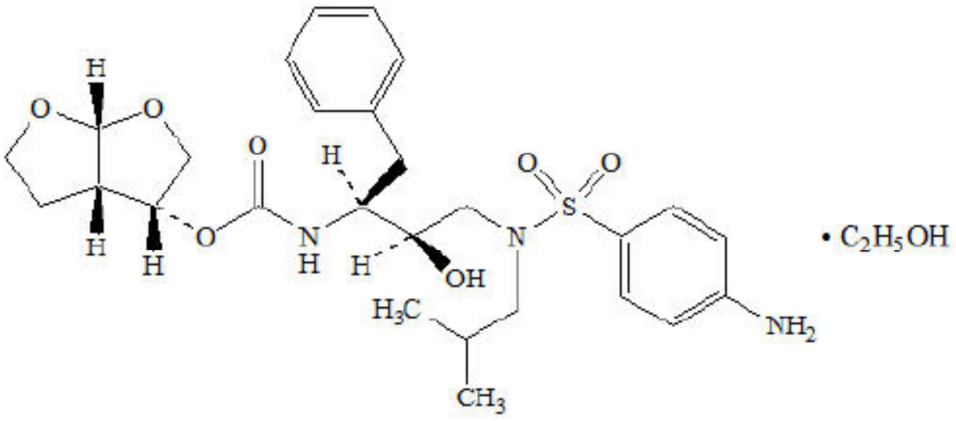

Figure 1 a. DarunavirEthanolate

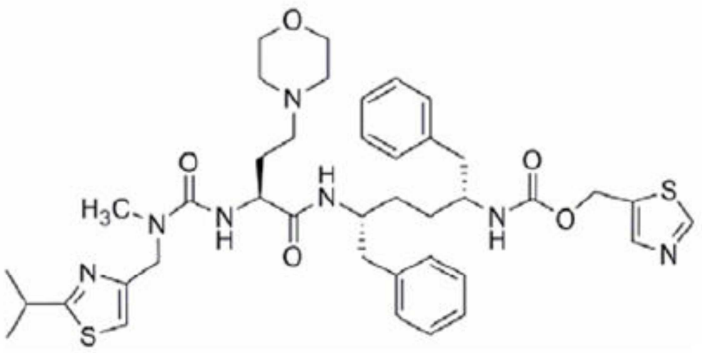

Figure 1 b. Cobicistat

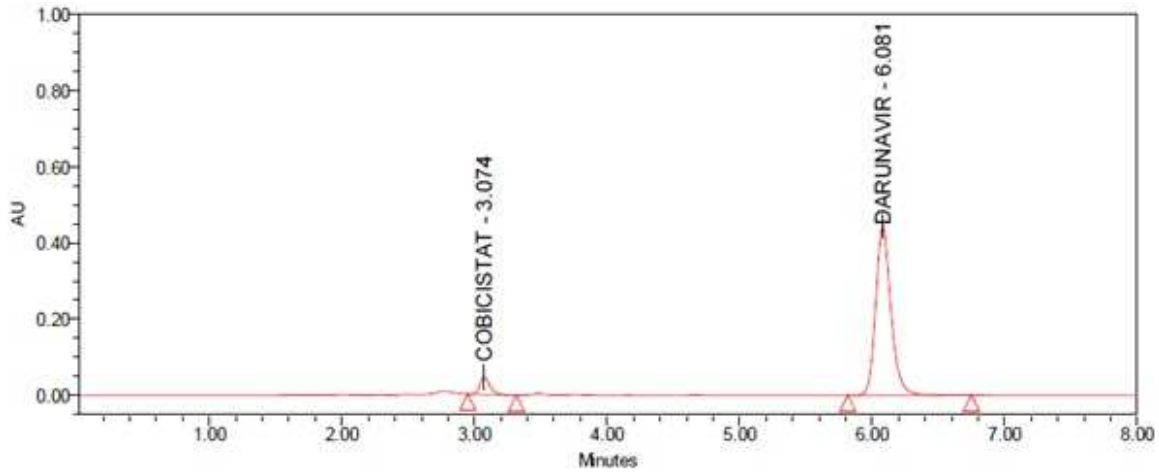

Figure 2 a. Chromatogram of Standard

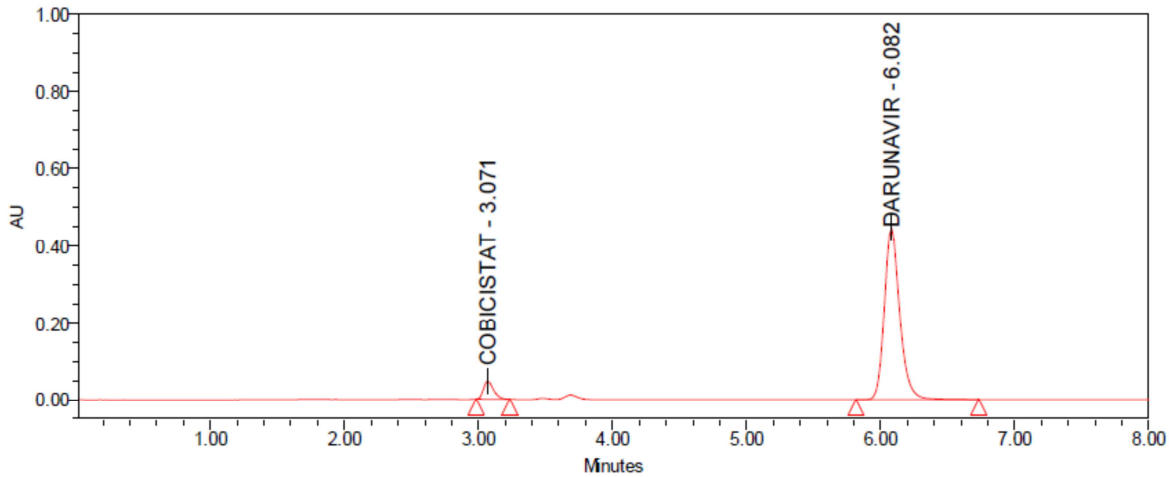

Figure 2 b. Chromatogram of Formulation

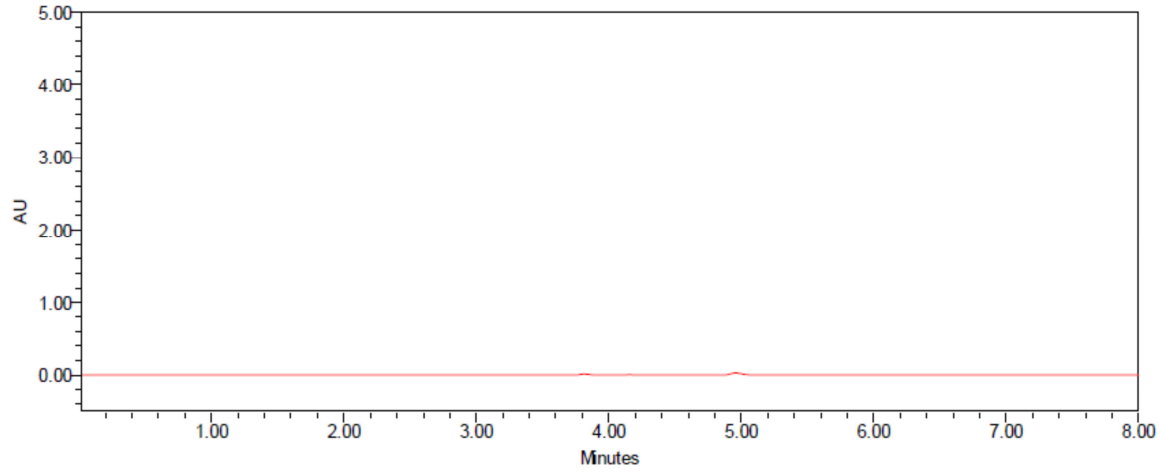

Figure 2 c. Chromatogram of Placebo

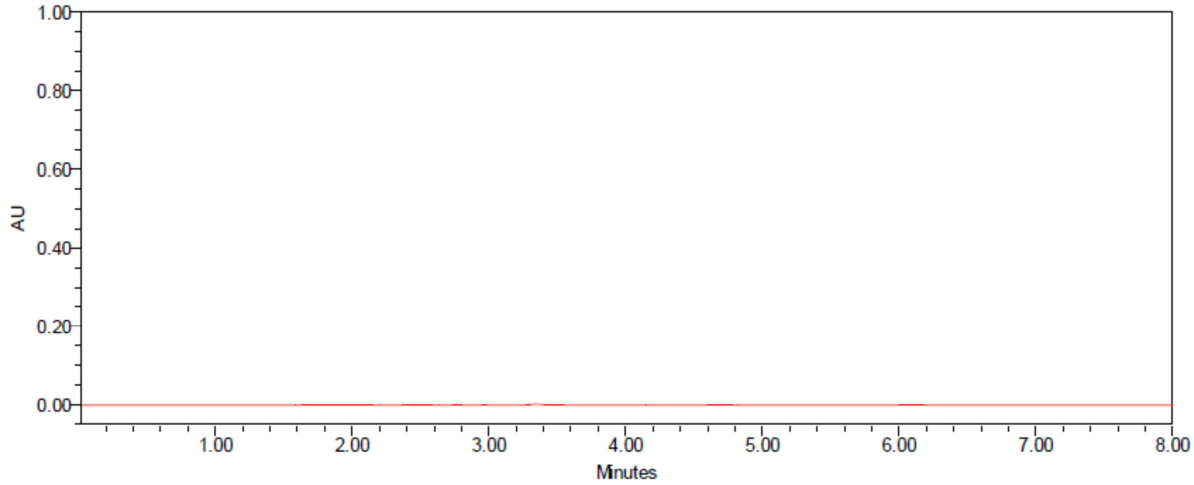

Figure 2 d. Chromatogram of Blank

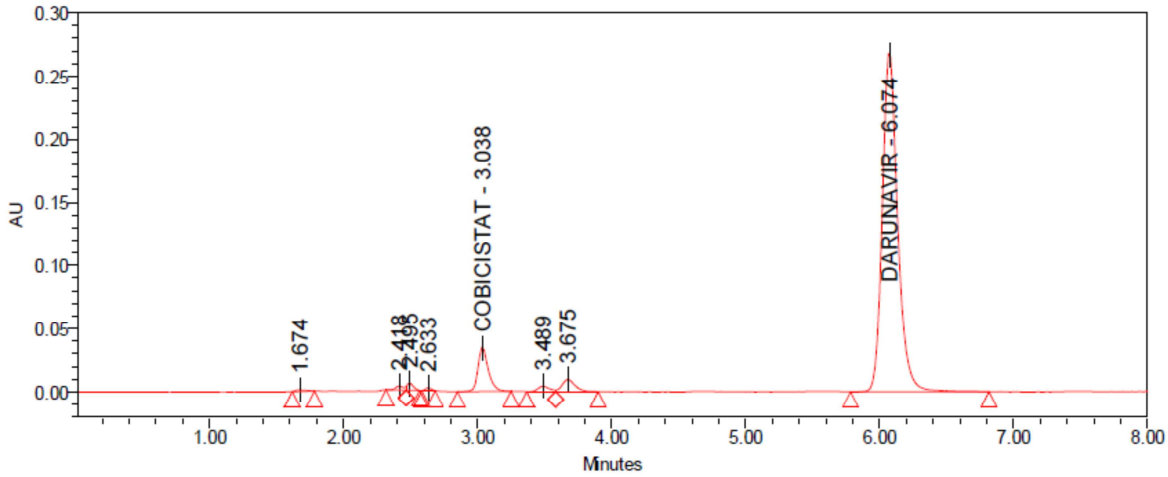

Figure 2 e. Chromatogram of Acid Degradation

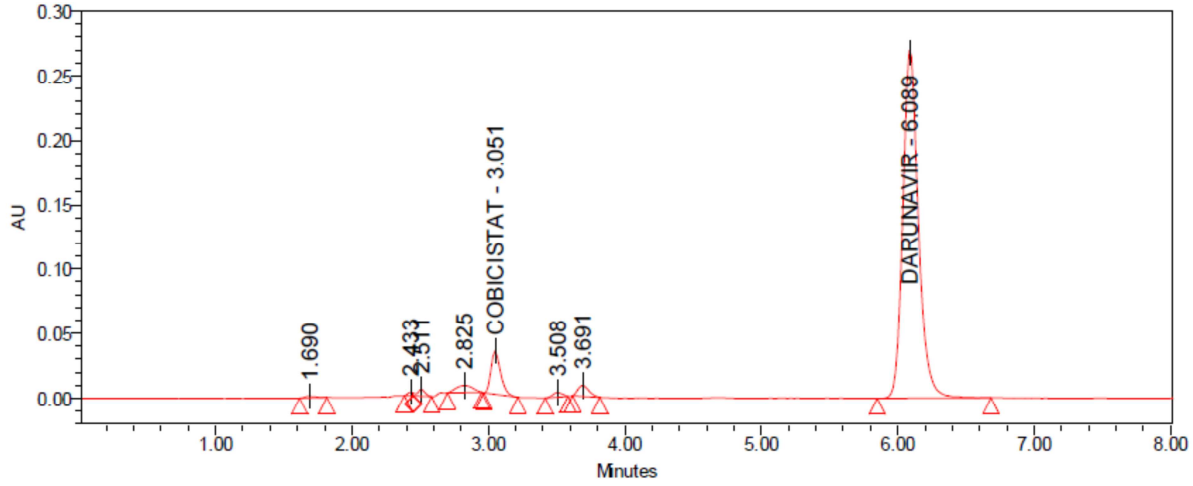

Figure 2 f. Chromatogram of Alkali degradation

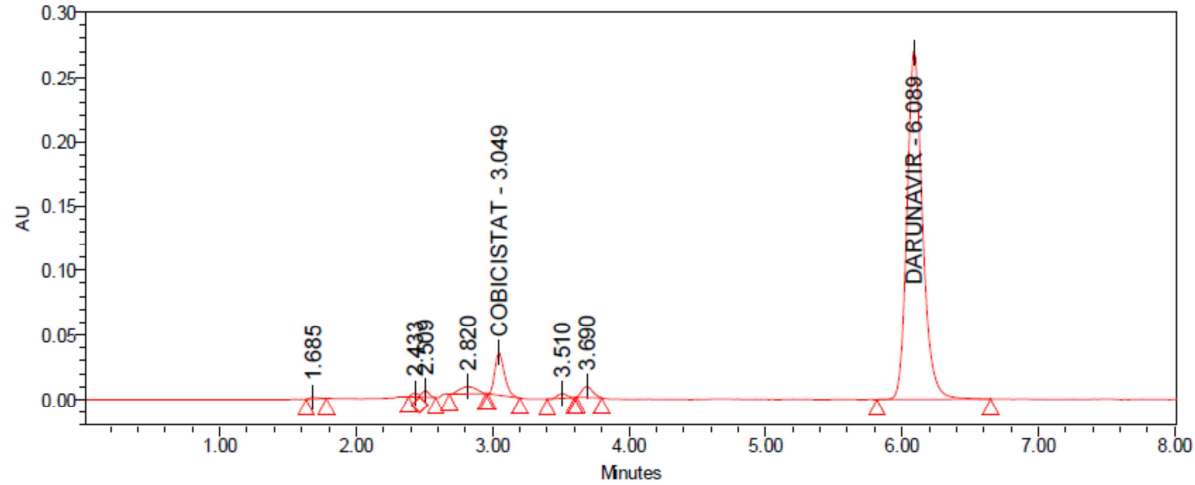

Figure 2 g. Chromatogram of Peroxide Degradation

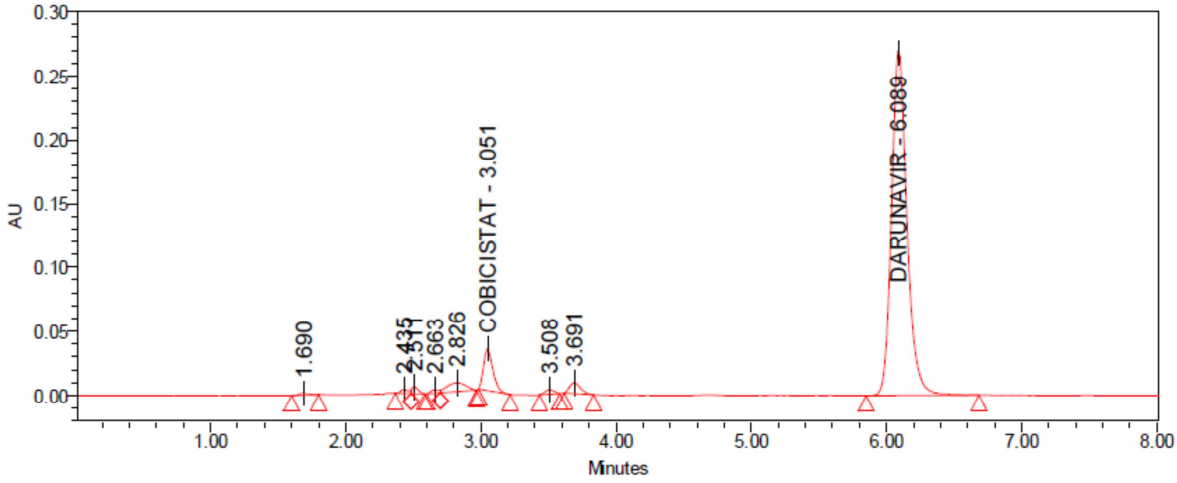

Figure 2 h. Chromatogram of Photolytic degradation

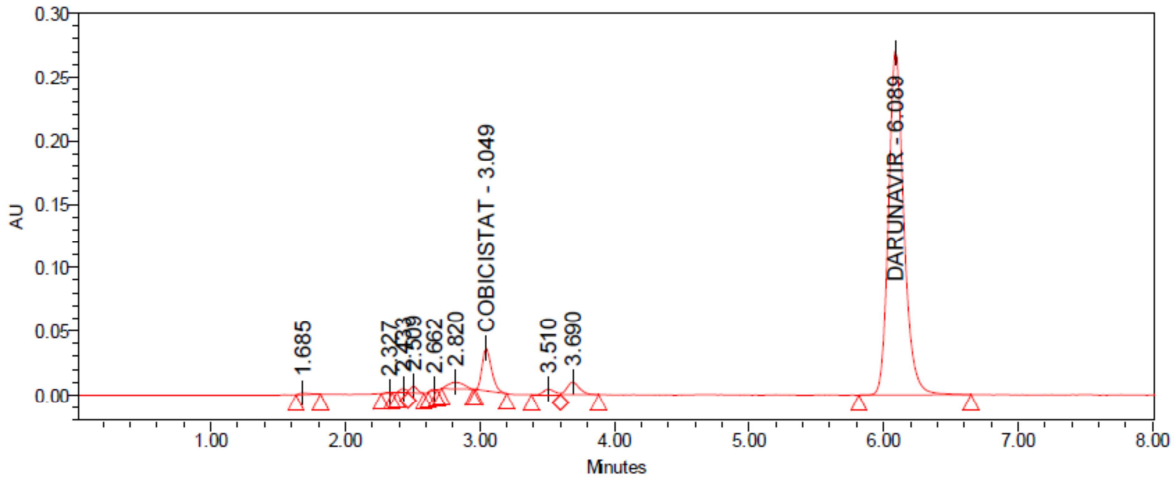

Figure 2 i. Chromatogram of Hydrolysis degradation

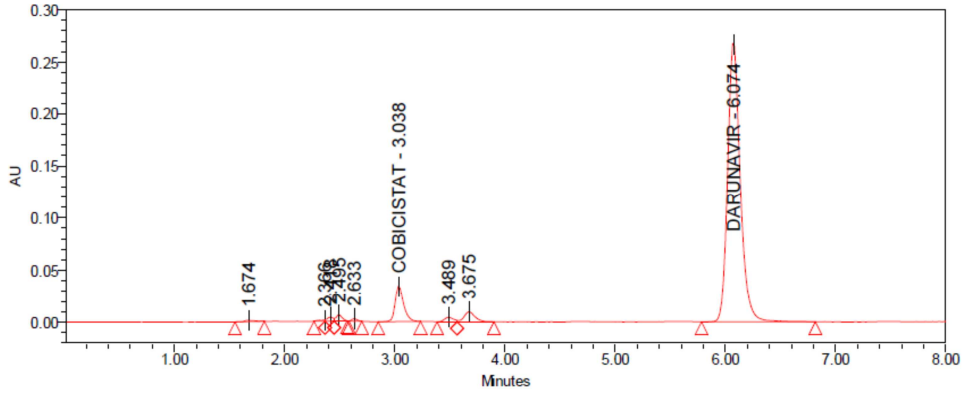

Figure 2 j. Chromatogram of Thermal Degradation

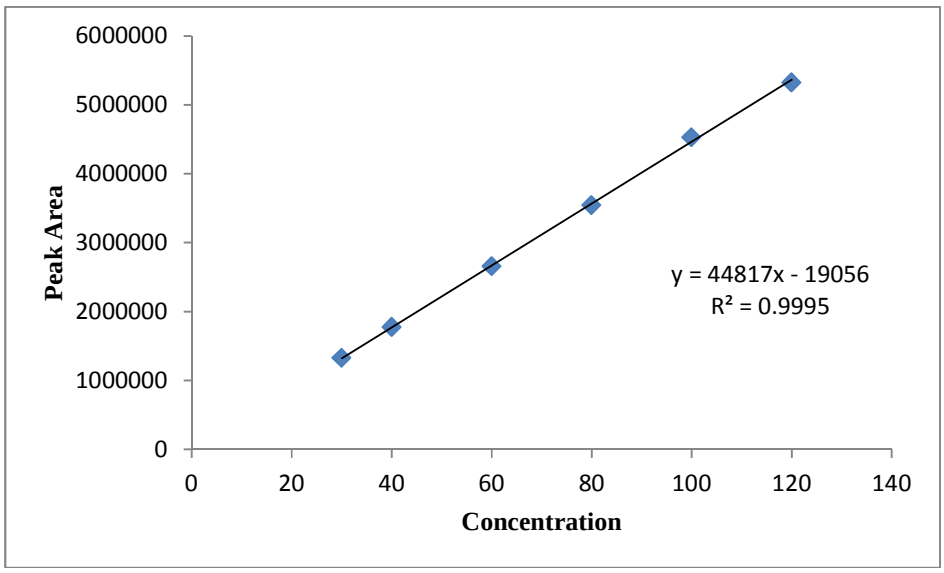

Figure 3 a. Calibration curve of DRV

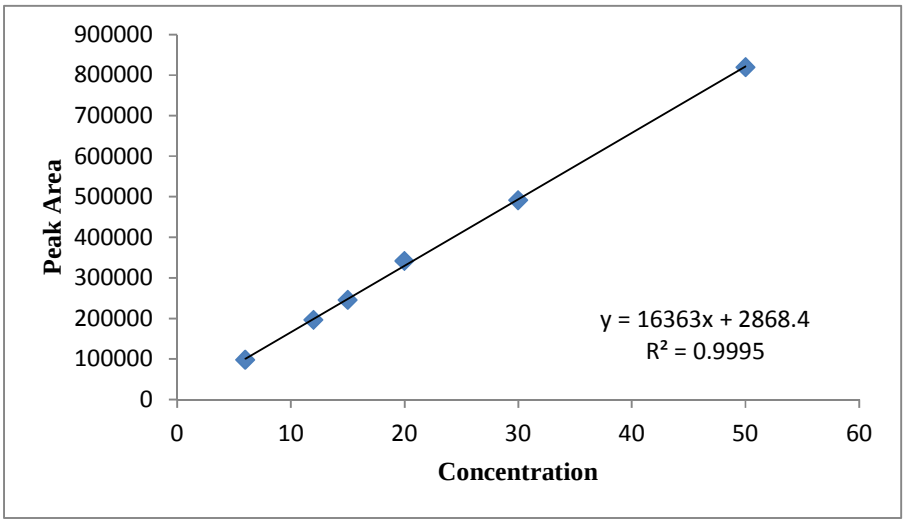

Figure 3 b. Calibration curve of COBI

Table 1. Assay Result of DRV and COBI

S.No.	Drug	Label Claim	% Amount Found*	% RSD
1	DRV	800	99.98	0.26
2	COBI	150	99.99	0.04

** Mean of Three Determinations*

Table 2. System suitability

S.No.	Drug	Peak Area*	SD	% RSD
1	DRV	3542109	1166.66	0.03
2	COBI	250913	1383.21	0.55

** Mean of Five Determinations*

Table 3. Linearity of the proposed method

DRV		COBI	
Concentration	**Peak Area***	**Concentration**	**Peak Area***
30	1328754	6	98320
40	1774512	12	196640
60	2657268	15	245800
80	3543024	20	341733
100	4528771	30	491600
120	5324576	50	819333
Y- Intercept	-19056	Y- Intercept	2868
Slope	44817	Slope	16363
R^2	0.999	R^2	0.999

** Mean of Three Determinations*

Table 4. Precision data of the proposed method

Injection	DRV		COBI	
	Method Precision	**System Precision**	**Method Precision**	**System Precision**
	Peak Area	**Peak Area**	**Peak Area**	**Peak Area**
1	3511001	3482452	240746	249326
2	3532975	3529521	243815	248531
3	3514135	3498715	241918	249027
4	3501317	3510627	239862	250174
5	3518362	3495403	240023	248945
6	3493926	3486031	241298	247942
Mean	3511952.66	3500458.17	241277.0	248990.83
SD	13614.43	17372.46	1463.52	751.94
RSD	0.39%	0.50%	0.61%	0.30%

Table 5. Accuracy data (Triplicate values at 50, 100 and 150 percent levels) of DRV and COBI

Concentration of spiked level	Amount added	Amount found	% Recovery	Mean Recovery %	%RSD
DRV					
50%	40.2	40.18	99.95	100.00	0.05
	40.09	40.11	100.04		
	40.15	40.16	100.02		
100%	80.5	80.45	99.93	99.99	0.05
	80.02	80.04	100.02		
	80.14	80.16	100.02		
150%	120.24	120.31	100.05	100.00	0.04
	120.07	120.04	99.97		
	120.18	120.19	100.00		
COBI					
50%	7.63	7.70	100.91	99.85	0.95
	7.45	7.38	99.06		
	7.50	7.47	99.6		
100%	15.14	15.15	100.06	100.01	0.08
	15.21	15.20	99.93		
	15.18	15.19	100.06		
150%	22.51	22.61	100.44	100.08	0.41
	22.64	22.56	99.64		
	22.58	22.62	100.17		

Table 6. Robustness data of DAR and COBI

Variations	DAR			COBI		
	%Assay*	Theoretical plates*	Tailing factor*	%Assay*	Theoretical plates*	Tailing factor*
43% of ACN in mobile phase	100.02	14793	1.20	99.99	8221	1.28
47% of ACN in mobile phase	100.3	14410	1.21	100.3	8474	1.25
Flow rate at 0.9ml/min	100.5	15753	1.20	100.3	7794	1.18
Flow rate at 1.1ml/min	100.8	15341	1.19	100.2	7812	1.95
Wave length at 243nm	100.6	14427	1.18	100.5	8127	1.27
Wave length at 247nm	100.3	14245	1.20	100.3	7730	1.33

** Mean of Three Determinations*

Table 7. Forced Degradation Data

Treatment	% Label Claim	% Degradation	Peak Purity		Pass/Fail
			Purity angle	Purity Threshold	
DRV					
Acid	83.8	16.2	1.278	4.051	Pass
Alkali	84.2	15.8	1.780	5.327	Pass
Peroxide	84.7	15.3	1.381	4.792	Pass
Thermal	84.5	15.5	1.291	4.328	Pass
Photolytic	85.1	14.9	1.487	4.823	Pass
Hydrolysis	85.8	14.2	1.295	4.412	Pass
COBI					
Acid	80.2	19.8	1.325	4.189	Pass
Alkali	80.7	19.3	1.734	4.227	Pass
Peroxide	81.4	18.6	1.284	4.856	Pass
Thermal	80.6	19.4	1.343	4.338	Pass
Photolytic	81.3	18.7	1.285	4.423	Pass
Hydrolysis	81.6	18.4	1.235	4.129	Pass

CONCLUSION

From the above discussion it can be concluded that the proposed method is precise, accurate and stability indicating. Therefore the proposed method can be used for routine quality control and analysis of DRV and COBI during stability studies in bulk samples and in tablet dosage forms.

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