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FORCED DEGRADATION STUDY OF DARUNAVIR ETHANOLATE AND RITONAVIR COMBINATION IN ACIDIC, BASIC AND OXIDATIVE CONDITIONS ESTABLISHING DEGRADATION PRODUCTS

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Keywords:

Darunavir ethanolate, Ritonavir, RP-HPLC, Validation, Stability and degradation products

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ABSTRACT: A simple, precise, and rapid stability indicating RP-HPLC method has been developed for the simultaneous estimation of darunavir ethanolate and Ritonavir in Pharmaceutical dosage form in the presence of degradation products. It involved the Zorbax bonus, 150 mm × 4.6 mm of 3.5 µm particles packing stationary phase. The separation was achieved using mobile phase a consists of ammonium acetate buffer: methanol; 50:50 (% v/v) mixture and mobile phase B consists of ammonium acetate buffer: acetonitrile; 45:55 (% v/v) mixture with the flow rate of 1.0 ml/min, gradient program was applied from 0.0 min to 35.0 min. The effluent is monitored at 240 nm. The retention time of darunavir ethanolate and ritonavir was found to be 7.39 ± 0.09 min and 16.18 ± 0.007 min, respectively, with a total run time of 35 min. Forced degradation study was carried out in acid, base, and oxidative conditions where 3 degradation products; DP1, DP2, and DP3 were observed in acidic condition at RT 1.765 min, 4.276 min, 8.261 min, and 4 degradation products; DP4, DP5, DP6, and DP7 were observed in basic condition at RT 1.789 min, 4.858 min, 7.518 min, and 13.179 min. However, no degradation products were visually observed in oxidative conditions. The method was found to be specific enough to separate degradation products from main analytes. The method was linear in the range of 240-560 µg/ml (R^2=0.999) and 30-70 µg/ml (R^2=0.999) for darunavir ethanolate and ritonavir, respectively. The described method was validated and result of each parameter was met with its acceptance criteria.

INTRODUCTION: Chemically, darunavir ethanolate is (3aS, 4R, 6aR)-2, 3, 3a, 4, 5, 6a-hexahydrofuro 2, 3-b furan-4-yl N-(2S, 3R)-4-(4-aminophenyl) sulfonyl- (2-methylpropyl) amino-3-hydroxy-1 phenylbutan-2-yl carbamate ethanol. It is an antiretroviral drug from the protease inhibitor class, which is used to treat infection of human immunodeficiency virus and also for AIDS.

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It selectively inhibits the cleavage of HIV encoded Gag-polyproteins in virus-infected cells, thereby preventing the formation of mature infectious virus particles. It can also change the shape of protease as it has molecular flexibility [1]. Chemically, ritonavir is 1, 3-thiazol-5-ylmethyl N-(2S, 3S, 5S) - 3-hydroxy-5- (2S)-3-methyl-2-methyl (2-(propan-2-yl)-1, 3-thiazol-4 yl methyl) carbamoyl amino butanamido-1, 6-diphenylhexan-2-yl carbamate.

It is an antiretroviral drug from the protease inhibitor class, which is used to treat infection of human immunodeficiency virus and also for AIDS. It ties to the protease dynamic site and hinders the action of the catalyst. This hindrance anticipates cleavage of the viral polyproteins bringing about

the arrangement of youthful non-irresistible viral particles [1]. RTV indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection.

DRV undergoes rapid absorption after oral administration, and its AUC is significantly increased by up to 14-fold in the presence of ritonavir. Dosage is twice daily when used with ritonavir, it is a potent CYP3A4 inhibitor, and thus the potential for significant drug interactions is high. Its elimination half-life is approximately 15 h when boosted with ritonavir [2]. Literature review revealed that there are many analytical methods like UV-Spectrophotometric, fluorescence, HPTLC, LC-MS, capillary electrophoresis, and Bio-analytical methods that have been developed for estimation of individual drugs with sample matrix like pure form, tablet, capsules, suspension and binary mixture and in combination with other drugs.

However, no method is reported describing the forced degradation study of DRV and RTV in combination using RP-HPLC [3]. So, a successful attempt was made to develop an accurate, precise, and simple method of analysis for the estimation of both the drugs in tablet dosage form by RP-HPLC.

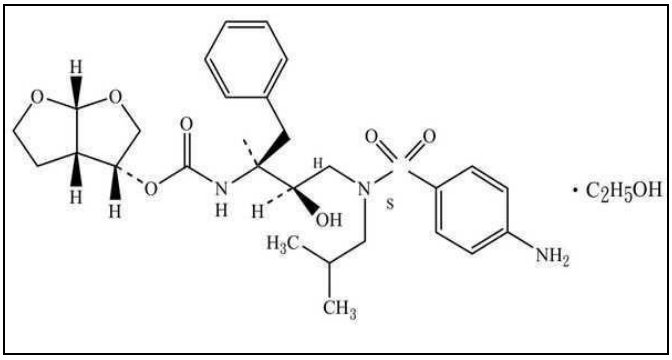

FIG. 1: CHEMICAL STRUCTURE OF DARUNAVIR ETHANOLATE

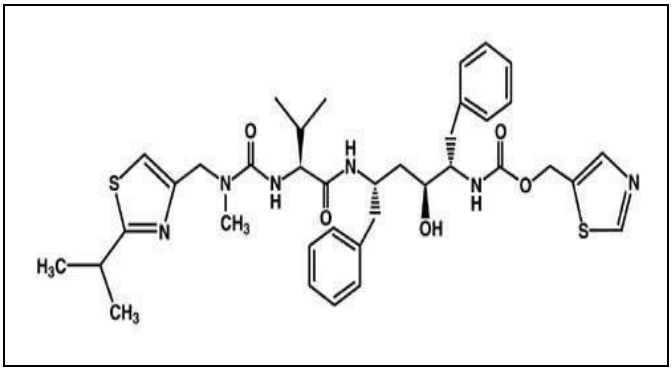

FIG. 2: CHEMICAL STRUCTURE OF RITONAVIR

MATERIALS AND METHODS:

Materials: Drug samples of Darunavir ethanolate (DRV) and Ritonavir (RTV) were procured from Emcure Pharmaceutical Ltd. R and D center, Gandhinagar, Gujarat.

TABLE 1: LIST OF CHEMICALS AND REAGENTS USED

Chemicals	Grade	Manufactured by
Acetonitrile	HPLC	Fisher scientific
Water	HPLC	Mili-Q
n-Butanol	HPLC	Merck
Tetrahydrofuran	HPLC	Merck
Methanol	HPLC	Fisher scientific
Ammonium acetate	AR	Merck
Potassium dihydrogen phosphate	AR	Merck
Hydrochloric acid	AR	Merck
Sodium hydroxide	AR	Merck
Hydrogen peroxide	AR	Merck

Chemicals and Reagents: Chemicals and reagents used in the experiment are of HPLC and AR grade quality procured from Merck and Fisher scientific.

Instruments: HPLC (Water alliance) equipped with PDA and UV Detector, Rheodyne injector, software: Empower 2.0 and Zorbax bonus column (150 mm × 4.6 mm of 3.5 µm particles packing). UV-Visible double beam spectrophotometer (UV-1800) with a matching pair of 1 cm quartz cuvettes and pH meter was of metrohm make.

Preparation of Darunavir Ethanolate Standard Stock Solution: Accurately weigh and transfer about 105 mg darunavir ethanolate (Mol wt. 593.73 gm/mol) working standard, which is 95.57 mg corrected weight of darunavir ethanolate into 50 ml clean and dry volumetric flask. Add about 30 ml of diluents and Sonicate to dissolve. Allow the solution to attain room temperature. Dilute to volume with diluents and mix well to obtain a stock solution of darunavir ethanolate (400 ppm).

Preparation of Ritonavir Standard Stock Solution: Accurately weigh and transfer about 20 mg ritonavir (Mol wt. 720.946 gm/mol) working standard into 100 ml clean and dry volumetric flask and add about 70 ml of diluent and sonicate to dissolve. Allow the solution to attain room temperature. Dilute to volume with diluent and mix well to obtain stock solution of ritonavir (50 ppm).

Preparation of Darunavir Ethanolate and Ritonavir Final Standard Solution: Transfer 5 ml of ritonavir and darunavir ethanolate standard stock

solution into 20 ml clean and dry volumetric flask. Dilute to volume with diluent and mix well.

Preparation of Sample Solution: Accurately weigh 20 tablets and determine the average weight of tablets and crush tablets weight equivalent to 200 mg ritonavir into 200 ml clean and dry volumetric flask.

Add about 160 ml of diluent and sonicate for 45 min at a temperature below 25 °C with intermittent shaking. Allow the solution to attain room temperature. Dilute to volume with diluents and mix well. Centrifuge the resulting solution at 3000 rpm for 10 min.

Dilute 5 ml of the supernatant into a 100 ml clean and dry volumetric flask. Dilute to volume with diluent and mix well. Filter this solution through a 0.45µ nylon filter by discarding the first 5 ml of filtrate.

Preparation of Optimized Mobile Phase:

Preparation of Buffer: Accurately weigh and dissolve about 1.54 g of ammonium acetate into 1000 ml water mix well (0.02 M). Filter through 0.45 µm nylon membrane filter paper.

- ✓ **Preparation of Mobile Phase A:** Prepare a mixture of ammonium acetate buffer and methanol in the ratio of 50:50 (% v/v). Sonicate to degas prior to use.
- ✓ **Preparation of Mobile Phase B:** Prepare a mixture of ammonium acetate buffer and ACN in the ratio of 45:55 (% v/v). Sonicate to degas prior to use.
- ✓ **Preparation of Diluents:** Prepare a mixture of water and ACN in the ratio of 50:50 (% v/v).
- ✓ **Preparation of Blank Solution:** Use diluents as a blank solution.

Selection of Detection Wavelength: Standard solutions of darunavir ethanolate 100 µg/ml and ritonavir 100 µg/ml were prepared for the selection of wavelength and scanned between 200-700 nm in UV-visible double beam spectro-photometer at a medium scanning speed.

It was found that darunavir ethanolate and ritonavir showed a reasonably good response at 240 nm. So 240 nm was selected as a detection wavelength for analysis.

Chromatographic Condition: Column used in the experiment was 'zorbax bonus, (150 mm × 4.6 mm of 3.5 µm particles) packing with detection wavelength at 240 nm and flow rate of 1.0 ml/min.

The injection volume selected was 50 µl with column oven temperature at 40 °C and sample cooler temperature at 25 °C with a total run time of 35 minutes using Water: ACN (10:90% v/v) as rinsing solvent.

Gradient Programme: The gradient program was used from 0.0 to 35.0 min.

TABLE 2: GRADIENT PROGRAMME

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	90	10
5.0	90	10
10.0	70	30
15.0	30	70
21.0	30	70
27.0	90	10
28.0	90	10
35.0	90	10

Method Validation:

System Suitability: System suitability was performed and calculated for each validation parameter at the start of the experiment.

The working standard solution was prepared as per the above-mentioned procedure and was injected 6 times as per optimized chromatographic conditions [4].

Specificity: Specificity of the method was obtained by comparing the chromatogram of blank, placebo, standard, and that of forced degradation studies.

Forced degradation studies were also performed to establish the stability-indicating property and specificity of the proposed method. Forced degradation studies were performed under acidic, basic, and oxidative conditions, as mentioned below [5, 6].

Acid Degradation: Weighed the fine powder equivalent accurately to 100 mg of darunavir ethanolate and transferred into 100 mL volumetric flask add about 50 mL of diluents and sonicate for 30 min with intermediate shaking.

Then add 5 mL of 0.72N HCl and kept at 98 ºC for 2 h and 23 min. Then 0.72 N NaOH was added to neutralize the solution and make up the volume up to the mark using diluents. Centrifuge this solution at 4000 rpm for 5 min. Transfer 4 ml of the filtrate to 10 mL volumetric flask and volume was made up with diluents. Filter solution through 0.45 µ nylon filter [7, 8].

Base Degradation: Weighed the fine powder equivalent accurately to 100 mg of darunavir ethanolate and transferred into 100 mL volumetric flask add about 50 ml of diluent sonicate for 30 min with intermediate shaking.

Then 1 ml 1.05 N NaOH was added and kept at 63 °C for 1 h 40 min. Then 1 ml 1.05 N HCl was added to neutralize the solution, and then the volume was made up with diluent. Centrifuge at 4000 RPM for 5 min. Transfer 4 mL of the filtrate to 10 mL volumetric flask and volume was made up with diluent. Filter solution through 0.45 µ nylon filter [7, 8].

Oxidative Degradation: Weighed the fine powder equivalent accurately to 100 mg of darunavir and transferred into 100 ml volumetric flask. Add about 50 ml of diluent sonicate for 30 min with intermediate shaking. Then 5 ml 2.15% H_2O_2 was added and heat on a water bath at 29 °C for 1 h 27 min. Then volume was made up with diluent. Centrifuge at 4000 RPM for 5 min.

Transfer 4 ml of the filtrate to 10 ml volumetric flask and volume was made up with diluent. Filter the solution through 0.45 µ nylon filter [7, 8.]

Linearity:

Preparation of Linearity Standard Stock Solution (LSSS): Accurately weigh 20 tablets and determine the average weight of tablets and crush tablets weight eq to 200 mg ritonavir into 200 ml clean and dry volumetric flask. Add about 160 ml of diluent and sonicate for 45 min at temperature. Below 25 °C with intermittent shaking allow the solution to attain room temperature. Dilute to volume with diluent and mix well. Centrifuge the resulting solution at 3000 rpm for 10 min. Dilute 5 ml of the supernatant into a 100 ml clean and dry volumetric flask. Dilute to volume with diluent and mix well. Filter this solution through a 0.45 µ nylon filter by discarding the first 5 ml of filtrate [9].

Preparation of Linearity Solution: Further, dilute above linearity standard stock solution (LSSS) as specified in table 3 and dilute to volume with diluent and mix. A graph of mean area versus concentration it was plotted, and the regression equation was determined.

TABLE 3: LINEARITY SOLUTION PREPARATION

% Linearity solution	LSSS (mL)	Diluted to (mL)	Concentration (µg/mL)	
			Darunavir ethanolate	Ritonavir
60	3.0	100	240	30
80	4.0	100	320	40
100	5.0	100	400	50
120	6.0	100	480	60
140	7.0	100	560	70

Precision: Precision was determined by repeatedly injecting (n=6) 400 µg/ml darunavir ethanolate and 50 µg/ml ritonavir and recording the responses as a peak area. The result was reported as % RSD [1].

Accuracy: It was carried out to determine the stability and reliability of the proposed method. Accuracy was determined by calculating the % recovery of darunavir ethanolate and ritonavir from the marketed formulation by the standard addition method in which known amounts of standard samples of darunavir ethanolate and ritonavir at 50%, 100%, and 150% levels are added to the pre-analyzed samples. The recovered amounts of darunavir ethanolate and ritonavir were calculated at each level, and % recovery was reported.

For performing accuracy study, known amounts of standard drug darunavir ethanolate (100, 200, 300 µg/mL) and ritonavir (12.5, 25, 37.5 µg/mL) were added to pre quantified sample solution of 200 µg/mL of darunavir ethanolate and 25 µg/mL of ritonavir. Each solution was injected in triplicate, and the % recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curve [11].

Robustness: Robustness of method was studied by a small but deliberate change in chromatographic condition conditions as listed below, and their effect was observed on system suitability [12].

Change in Flow Rate: 1.0 ml/min ± 0.1
Change in Temperature: 40 ºC ± 5
Change in Wavelength: 240 nm ± 2

Estimation of Darunavir Ethanolate and Ritonavir in Marketed Formulation by Proposed RP-HPLC Method: The tablet sample was prepared as per procedure mentioned above. The sample was then analyzed as per the chromatographic condition mention above in section.

A chromatogram was recorded, and area of darunavir ethanolate and ritonavir was measured. Con-centration was measured, and % assay was calculated for darunavir ethanolate and ritonavir present in the market formulation.

RESULTS AND DISCUSSION:

System Suitability: Inject the standard solution into the chromatographic system and record the chromatograms. The system is suitable for analysis if only if:

- The tailing factor for darunavir ethanolate and ritonavir peak is not more than 2.0 and resolution between two peaks is greater than 2.0.
- The number of theoretical plates determined from each component peak is not less than 2000.
- The relative standard deviation for the area from five replicate injection of standard preparation should NMT 2.0%.

SST results are given in **Table 4;** from the result table, it can be concluded that the system suitability parameter obtained from the analysis lies within limits; hence the system and chromatographic conditions are suitable for use.

Specificity: It was found that there is no interference from blank and no interference from excipients in the tablet from tablet formulation and also found a good correlation between retention time of standard and sample. Specificity results are shown in below **Table 5**.

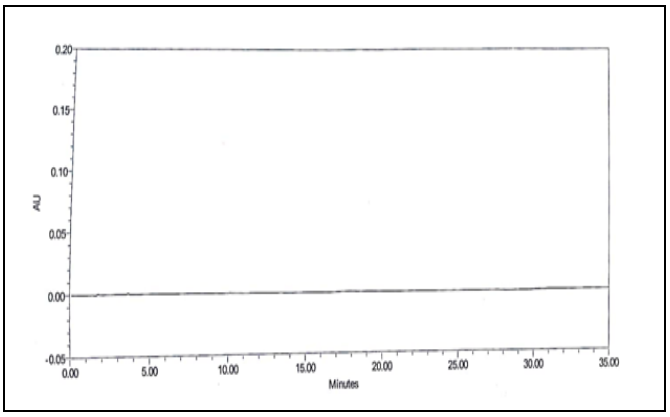

FIG. 3: CHROMATOGRAM OF BLANK AT 240 nm INJECTION VOLUME 50 µL

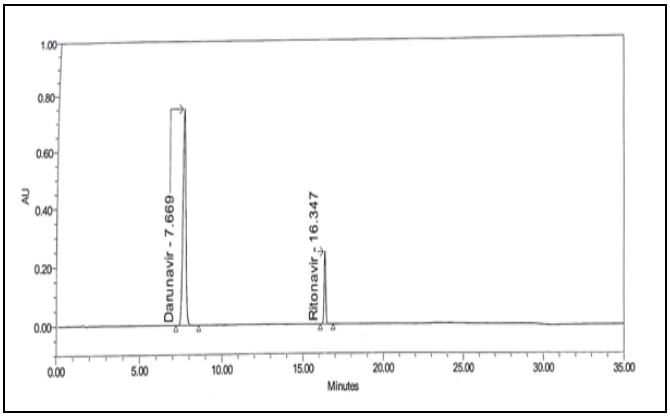

FIG. 4: CHROMATOGRAM OF STANDARD PREPARATION OF DARUNAVIR ETHANOLATE AND RITONAVIR AT 240 nm WITH RT OF 7.669 min (DRV) AND 16.347 min (RTV)

TABLE 4: SYSTEM SUITABILITY PARAMETER

No. of injections	Darunavir Ethanolate				Ritonavir				Resolution
	RT	Area	TF	TP	RT	Area	TF	TP	
1	7.38	946619	1.08	8424	16.17	172740	1.03	129824	34.20
2	7.38	937378	1.08	8433	16.17	173034	1.04	127915	34.28
3	7.39	937725	1.08	8415	16.18	172995	1.03	128781	34.23
4	7.40	947979	1.08	8466	16.18	172971	1.03	129777	34.26
5	7.40	937857	1.08	8480	16.18	173115	1.03	130356	34.23
Mean ±	7.39 ±	9375121 ±	1.081 ±	8443 ±	16.18 ±	1729714 ±	1.03 ±	974.698 ±	34.24 ±
SD	0.009	5470	0.001	28.032	0.007	1402	0.006	7.045	0.030
% RSD (n=5)	0.12	0.06	0.1	0.06	0.04	0.6	0.6	0.05	0.9

TABLE 5: SPECIFICITY DATA OF DARUNAVIR ETHANOLATE AND RITONAVIR

Name of the solution	Blank	Standard		Sample	
		DRV	RTV	DRV	RTV
Retention time (min)	No peak	7.669	16.347	7.393	16.174

TABLE 6: RESULTS OF FORCED DEGRADATION STUDIES OF DARUNAVIR ETHANOLATE AND RITONAVIR

S. no.	Injection	Darunavir ethanolate		Ritonavir	
		% Assay	% Degradation	% Assay	% Degradation
1	Acid degradation	84.4	14.36	74.9	26.4
2	Base degradation	89.4	8.9	69.8	26.94
3	Oxidative degradation	92.58	7.21	95.8	5.8

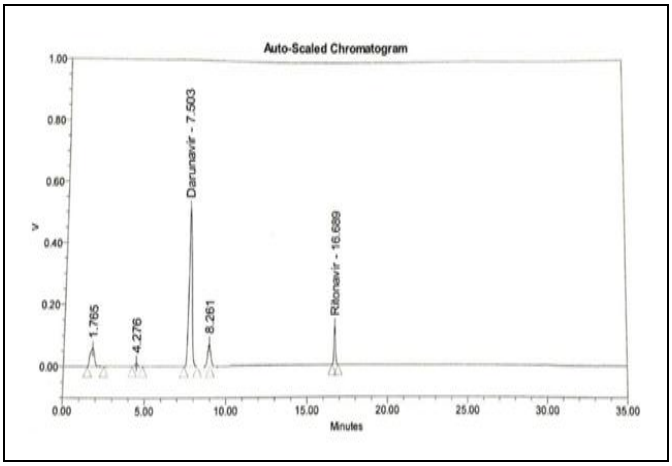

FIG. 5: CHROMATOGRAM OF DRV AND RTV IN ACIDIC CONDITION (0.72N HCL) SHOWS 5 PEAKS AT RT 7.503 min (DRV), 16.689 min (RTV), 1.765 min (DP1), 4.276 min (DP2) AND 8.261 MIN (DP3) WITH % DEGRADATION OF 14.36% (DRV) AND 26.4% (RTV)

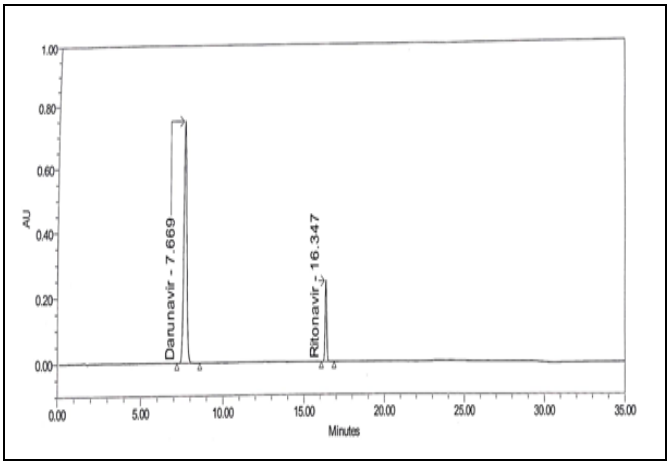

FIG. 6: CHROMATOGRAM OF DRV AND RTV IN BASIC CONDITION (1.05N NaOH) SHOWS 6 PEAKS AT RT 8.304 min (DRV), 16.664 min (RTV), 1.789 min (DP4), 4.858 min (DP5), 7.518 min (DP6) AND 13.179 min (DP7) WITH % DEGRADATION OF 8.9% (DRV) AND 26.94% (RTV)

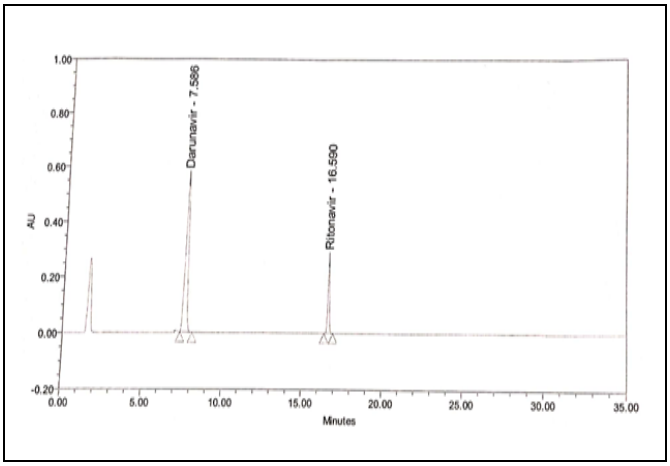

FIG. 7: CHROMATOGRAM OF DRV AND RTV IN OXIDATIVE CONDITION (2.15% H_2O_2) SHOWS 3 PEAKS AT RT 7.586 min (DRV), 16.590 min (RTV) AND 1.985 min (H_2O_2) HOWEVER NO DEGRADATION PRODUCT PEAKS ARE OBSERVED

Linearity: The calibration curve was plotted with the area obtained versus the concentration of both darunavir ethanolate and ritonavir **Fig. 9** and **10**. In the present study, six concentrations were chosen ranging between 240-560 µg/mL of darunavir ethanolate and 30-70 µg/mL of ritonavir.

The regression equation and correlation coefficient for darunavir ethanolate and ritonavir were found to be respectively, and results were given in **Table 7**.

$$Y = 19973_X\text{-}1E + 06 \text{ and } R^2 = 0.999$$

$$Y = 47153_X + 9095 \text{ and } R^2 = 0.999$$

TABLE 7: RESULT OF LINEARITY FOR DARUNAVIR ETHANOLATE AND RITONAVIR

% Linearity solution	Darunavir ethanolate		Ritonavir	
	Conc. (µg/ml)	Mean area ± SD	Conc. (µg/ml)	Mean area ± SD
60	240	3767428 ± 49	30	930980 ± 341
80	320	6867559 ± 1252.5	40	1455733 ± 36
100	400	8842808 ± 273	50	1798900 ± 146.3
120	480	10602857 ± 759.5	60	2180960 ± 22
140	560	13574125 ± 273.5	70	2743989 ± 1003

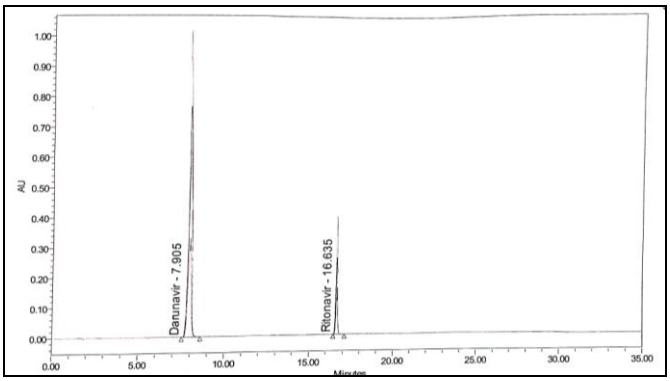

FIG. 8: OVERLAY CHROMATOGRAM OF DARUNAVIR ETHANOLATE AND RITONAVIR AT 240 nm WITH RT OF 7.905 min (DRV) AND 16.635 min (RTV)

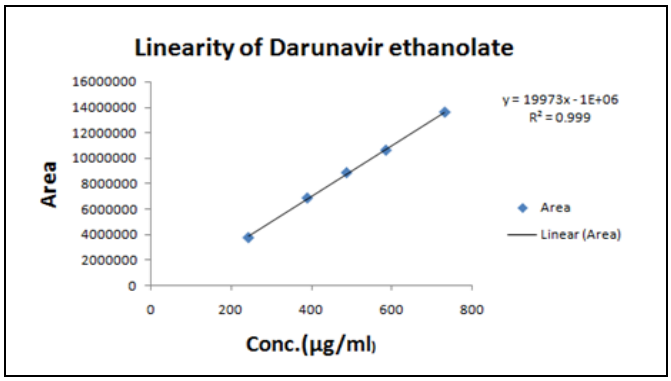

FIG. 9: CALIBRATION CURVE FOR DARUNAVIR ETHANOLATE (240-560 µg/ml)

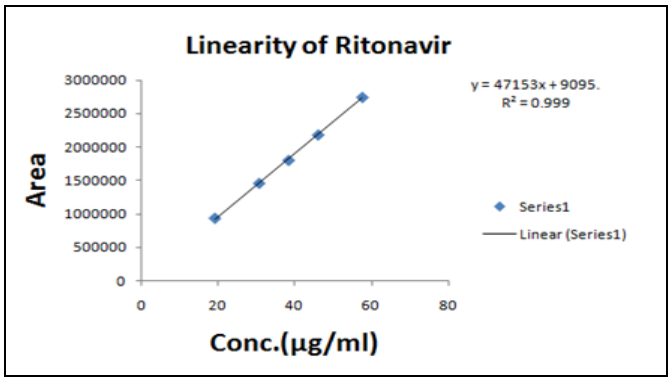

FIG. 10: CALIBRATION CURVE FOR RITONAVIR (30-70 µg/ml)

Precision: % RSD was calculated and found within limits. The low % RSD value was indicated that the method was precise and reproducible, and the results were shown in **Table 8**.

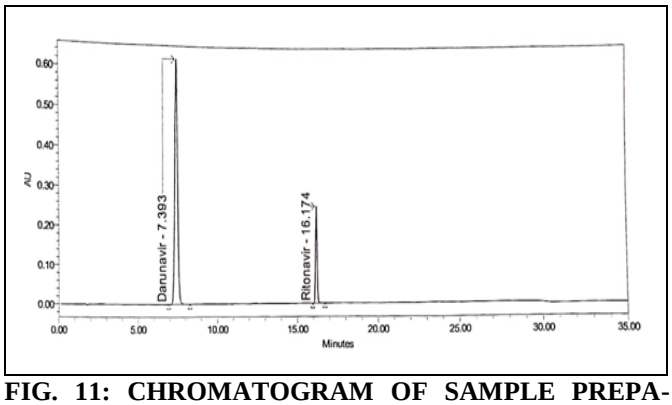

FIG. 11: CHROMATOGRAM OF SAMPLE PREPARATION FOR MARKETED FORMULATION SHOWS DARUNAVIR ETHANOLATE AT RT 7.393 min AND RITONAVIR AT 16.174 min

TABLE 8: PRECISION DATA FOR DARUNAVIR AND RITONAVIR

Darunavir ethanolate conc. (µg/mL)	Area	Ritonavir conc. (µg/mL)	Area
	7839869		1727152
	7852443		1709473
	7868558		1726422
400	7884895	50	1695123
	7895047		1713595
	7889508		1726237
Mean	7876536	Mean	1722243
%RSD	0.6	%RSD	0.6

Accuracy: The result shows that the percentage Recoveries for darunavir ethanolate and ritonavir were found to be in the range of 100.67-100.94% and 99.18-100.19%, respectively.

The result of accuracy study is shown in **Table 9** for darunavir ethanolate and ritonavir, respectively.

Robustness: From the robustness study, it was observed that deliberate change in above mention parameter has no significant effect on chromate-graphic behavior of the sample, and results are mention in **Table 10**.

TABLE 9: ACCURACY RESULTS FOR DARUNAVIR ETHANOLATE AND RITONAVIR

Level	Amount of DRV spiked (µg/ml)	Amount of DRV recovered (µg/ml)	Mean% recovery ± SD	% RSD	Amount of RTV spiked (µg/ml)	Amount of RTV recovered (µg/ml)	Mean % recovery ± SD	% RSD
		100.99				12.58		
50	100	101.31	100.67	0.85	12.5	12.46	100.19	0.43
		99.69	±0.86			12.52	±0.47	
		201.3				25.10		
100	200	202.60	100.94	0.33	25	24.88	99.89	0.46
		201.74	±0.33			24.93	±0.45	
		301.14				37.36		
150	300	304.17	100.88	0.50	37.5	36.99	99.18	0.52
		302.58	±0.51			37.21	±0.50	

TABLE 10: RESULTS OF ROBUSTNESS STUDY OF DARUNAVIR ETHANOLATE AND RITONAVIR

Parameters	Conditions	DRV		RTV	
		Mean area (n=5)	%RSD	Mean area (n=5)	% RSD
Flow rate (mL/min)	0.9 mL/min	10651049	0.64	2005863	0.14
	1.1 mL/min	8743819	0.08	1648415	0.17
Temperature (ºC)	35 ºC	9501491	0.01	1817207	0.37
	45 ºC	9452873	0.01	1837538	0.06
Wavelength (nm)	238 nm	7707829	0.03	1825049	0.06
	242 nm	11471671	0.23	1834658	0.19

TABLE 11: RESULT FOR ESTIMATION OF % ASSAY IN MARKET FORMULATION

Darunavir ethanolate			Ritonavir		
Label claim (mg)	Area	% Assay	Label claim (mg)	Area	% Assay
800	7913348	101.7	100	1726036	99.33
	7839869	100.8		1727152	99.4

DISCUSSION: A new stability-indicating RP-HPLC method has been developed for the estimation of darunavir ethanolate and ritonavir in the tablet dosage form. The column used in the method (zorbax bonus, 150 mm × 4.6 mm of 3.5 µm) particles packing stationary phase. The separation was achieved using mobile phase A which consists of ammonium acetate buffer: methanol; 50:50 (%v/v) mixture, and mobile phase B consists of ammonium acetate buffer: acetonitrile; 45:55 (%v/v) mixture with the flow rate of 1.0 ml/min, gradient program was applied from 0.0 min to 35.0 min.

The effluent is monitored at 240 nm. The retention time of darunavir ethanolate and ritonavir was found to be 7.39 ± 0.09 min and 16.18 ± 0.007 min, respectively, with a total run time of 35 min. Forced degradation study was carried out in acid, base, and oxidative conditions where 3 degradation products; DP1, DP2, and DP3 were observed in acidic conditions at RT 1.765 min, 4.276 min, 8.261 min with % degradation of 14.36% for DAR and 26.4% for RTV [4].

Degradation products; DP4, DP5, DP6, and DP7, were observed in basic condition at RT 1.789 min, 4.858 min, 7.518 min, and 13.179 min with % degradation of 8.9% for DAR and 26.94% for RTV; however, no degradation products were visually observed in oxidative condition.

Thus, from the above data, we can interpret that both drugs darunavir ethanolate (DRV) and ritonavir (RTV) are sensitive to acidic and basic condition and produce degradation products under such conditions; however, ritonavir was found to be more sensitive to both the conditions as it has undergone more % degradation compared to darunavir ethanolate. In the case of the oxidative condition, no degradation product was visually detected by UV detector, but as both the drugs have shown % degradation of 7.21% and 5.8% in the oxidative condition, we can predict that the impurities were generated but either they doesn't have a chromophoric group, or they were in a very negligible amount to be detected by the UV detector.

The method was found to be specific enough to separate degradation products from main analytes. The method was linear in the range of 240-560 µg/ml (R^2=0.999) and 30-70 µg/ml (R^2=0.999) for darunavir ethanolate and ritonavir, respectively. The developed method was validated, and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of darunavir and ritonavir in Tablet dosage forms. The forced degradation studies were carried out in accordance with ICH Q1(C) guideline, and the results revealed the suitability of the method to study the stability of darunavir and ritonavir under various degradation conditions like acid, base, and oxidative degradations.

CONCLUSION: Finally, it was concluded that the method is simple, sensitive, and has the ability to separate the drug from degradation products and recipients found in tablet the dosage form.

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