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STRESS DEGRADATION STUDIES ON DARUNAVIR ETHANOLATE USING VALIDATED STABILITY-INDICATING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Palak G. Chaudhary and Prof. Dr. Dhrubo Jyoti Sen

Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

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***Corresponding Author**
Palak G. Chaudhary
Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

ABSTRACT

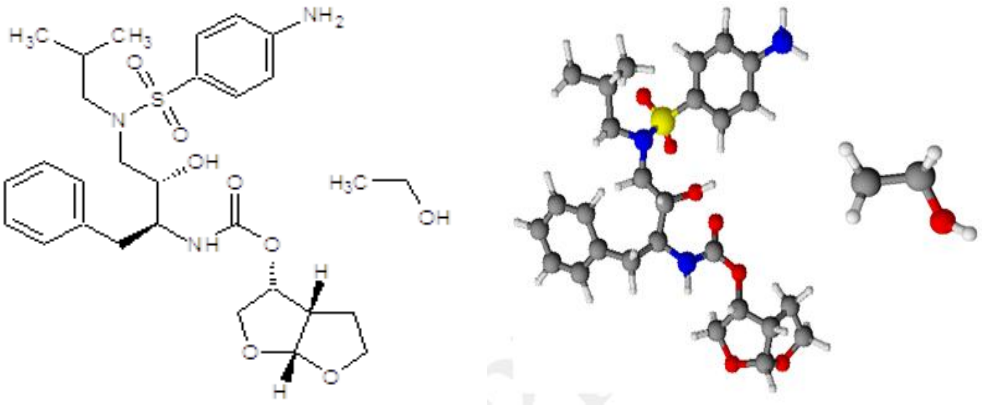
Darunavir Ethanolate is a novel nonpeptidic human immunodeficiency virus type 1 (HIV-1) protease inhibitor (PI). A sensitive, selective, accurate, precise and stability-indicating high-performance thin-layer chromatographic method for analysis of Darunavir Ethanolate has been developed and validated. The method uses aluminum-backed silica gel $60F_{254}$ plates with toluene – ethyl acetate – methanol 6.5:2.0:1.5 (v/v) as mobile phase. The system gave compact bands for Darunavir Ethanolate (R_F 0.37). Densitometric analysis of Darunavir Ethanolate was performed in absorbance mode at 270 nm. The method was linear in the range of 500-3500 ng/band (R^2 = 0.9995). The limits of detection and quantification were 130 and 390 ng per band, respectively. Darunavir Ethanolate was subjected to different stress conditions – acid and alkaline hydrolysis, oxidation, photo degradation and thermal degradation. The degradation products were well resolved from the pure drug with significantly different R_F values. Since the method could effectively separate the drug from its degradation products, it could be used as a stability-indicating method for analysis of Darunavir Ethanolate.

KEYWORDS: Darunavir Ethanolate (DRV), HPTLC, Stress degradation, Validation, Limit of Detection (LOD), Limit of Quantitation (LOQ), RSD, ICH.

INTRODUCTION

A novel nonpeptidic human immunodeficiency virus type 1 (HIV-1) protease inhibitor (PI) Darunavir Ethanolate (Figure-1) is [(1S,2R)-3-[[(4-aminophenyl)sulfonyl](2-

methylpropyl)amino]-2-hydroxy-1-(phenyl methyl) propyl]-carbamic acid (3R,3aS,6aR)-hexahydrofuro[2,3-b] furan-3-yl ester monoethanolate. The molecular weight of Darunavir base and Darunavir Ethanolate are 547.73 and 593.73 g/mol, respectively. DRV was active against HIV-1 with PI-resistance mutations and against PI-resistant clinical isolates. This drug is expected to be effective in patients experienced in antiretroviral treatment, such as those carrying HIV-1 strains which are resistant to more than one PI.[1-5]



Formula Weight= 593.736 gm/mole

Figure-1: Darunavir Ethanolate

From the literature it is revealed that various analytical methods for the determination of DRV have been reported, which include high-performance liquid chromatography (HPLC) with UV detection (HPLC-UV) to determine DRV* in human plasma; RP-HPLC method; HPTLC method; A novel LC–ESI-MS method; RP HPLC–MS method for the simultaneous determination of DRV and 11 other antiretroviral agents in plasma of HIV infected patients; LC-tandem MS assay; and validation of plasma DRV concentrations by the HPLC for PIs.[6-14]

Currently HPTLC is becoming a routine technique for analysis of drugs. It has proved to be a very useful technique because of its low operating cost, high sample-throughput and need for minimum sample clean-up. The major advantage of HPTLC lies in reducing analysis time and cost per analysis. Unlike HPLC, wherein substantial amounts of mobile phase and time are required for analysis of multiple samples, HPTLC has the advantage that several samples can be analyzed simultaneously using a small quantity of mobile phase.

The development of stability indicating assays, using the approach of stress testing as determined by the ICH guidelines, is highly recommended for the QC of pharmaceutical formulations. DRV is commercially available, but not much work was reported with HPTLC

for quantitative analysis of DRV in the presence of its degradation products. Therefore, the aim of the present research was to develop and validate a simple, accurate and precise stability-indicating HPTLC method. This paper describes an accurate, specific, repeatable and stability-indicating method for analysis of DRV in the presence of its degradation products for assessment of the purity of the bulk drug and the stability of its dosage forms.

HPTLC CHEMISTRY

[(1R,5S,6R)-2,8-dioxabicyclo[3.3.0]oct-6-yl]N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl) amino]-3-hydroxy-1-phenyl-butan-2-yl]carbamate. It is a chiral compound having **five asymmetric carbons** so 1R,5S,6R and 2S,3R are the **five** points to generate chirality.

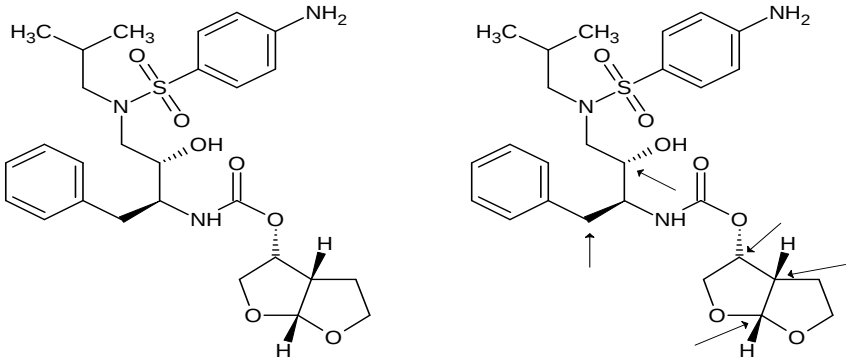


Figure-2: Structure and chirality (arrows) of DRV

It is the **amino ester** of **sulfonamide** moiety having logP=3.94 (nonpolar), pKb=13.59 (strong basic), pKa=2.39 (moderate acidic). Basicity generates from free amino (-NH_2) group (pKb=13.59) and acidity generates from carbamate linkage (-NH-COO-) after keto-enol tautomerism (pKa=2.39).

Tautomerism

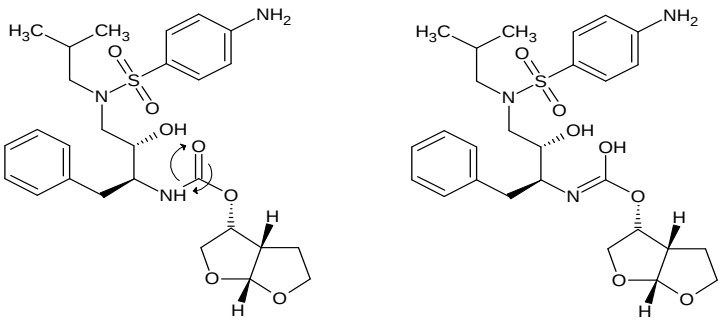


Figure-3: Tautomerism

It has biotransformation property by acidic and basic hydrolysis to breakdown of ester/amide part of carbamate linkage and sulfonamide part. Molecular Formula: $C_{27}H_{37}N_3O_7S$, Formula Weight: 547.66358.

Ester and amide or sulfonamides under hydrolysis because in ester (-COO-) four lone pairs of electrons **(2+2=4)** are present each of one oxygen and electronegativity of oxygen is 3.44 so total electronegativity is **6.88 (ester)**. Whereas in amide (-CONH-) total lone pairs of electrons are **2+1=3**; 2 for oxygen and 1 for nitrogen and electronegativity of oxygen is 3.44 and electronegativity of nitrogen is 3.04 so total electronegativity is **6.48 (amide)**. In sulfonamide ($-SO_2NH-$) total lone pairs of electrons are **2+2+2+1=7**; 2 for sulfur, 2+2 for two oxygen and 1 for nitrogen and electronegativity is 2.58 for sulfur and 3.04 for nitrogen so total electronegativity is **2.58+3.44+3.44+3.04=12.5 (sulfonamide)**.

Correlation approach

sulfonamide>ester>amide because 12.5>6.88>6.48 (total electronegativity approach)

sulfonamide>ester>amide because 7>4>3 (total electron pairs).

This proves that sulfonamide is much more susceptible to hydrolysis than ester rather than amide.

Acid hydrolysis

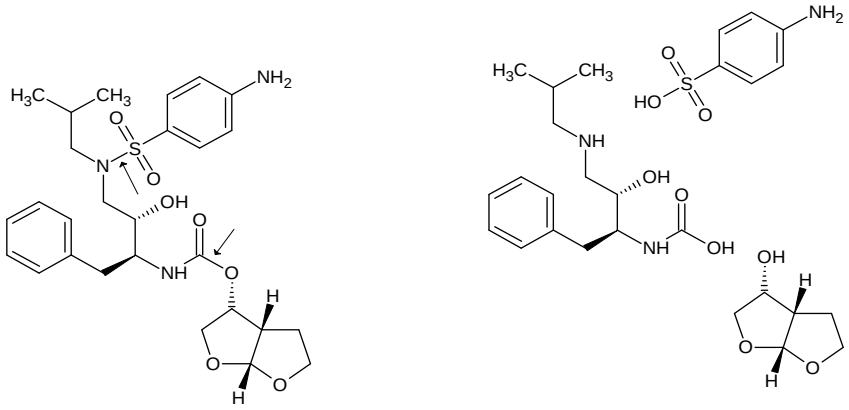


Figure-4: Sulfonamide bond acid hydrolysis (produces amino, alcohol and sulfonic acid)

Three HPTLC peaks are obtained.

Alkali hydrolysis

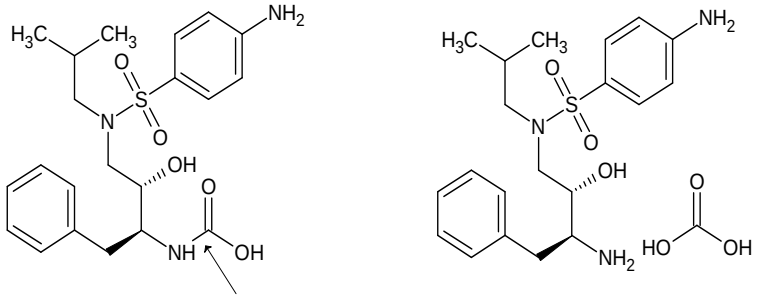


Figure-5: Amide bond alkali hydrolysis (produces amino & carboxylic acid)

One HPTLC peak is obtained because here H_2CO_3 is generated which is carbonic acid and highly unstable product which decomposes into CO_2 and H_2O. So one peak is obtained. Total peaks obtained are 3+1=4 for hydrolysis.

Oxidation

It is oxidized at two points; amino (-NH_2) is converted into hydroxyl amine (-NHOH) and secondary alcohol (-CHOH) is converted into ketone (>C=O) and isobutyl (-CH<) is oxidized into tertiary alcohol, so three peaks are obtained for oxidation.

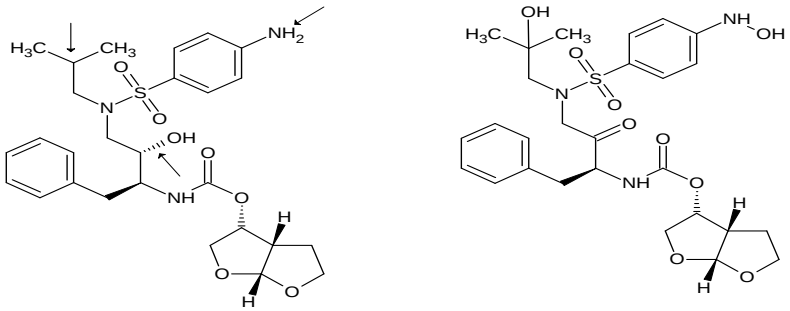
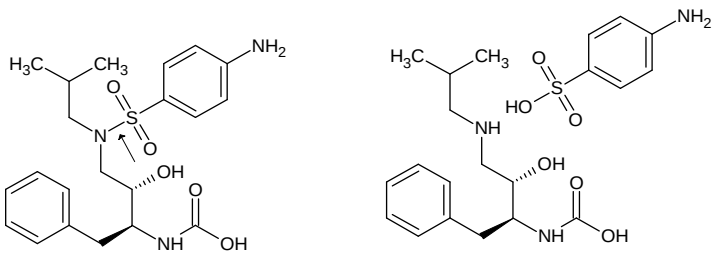


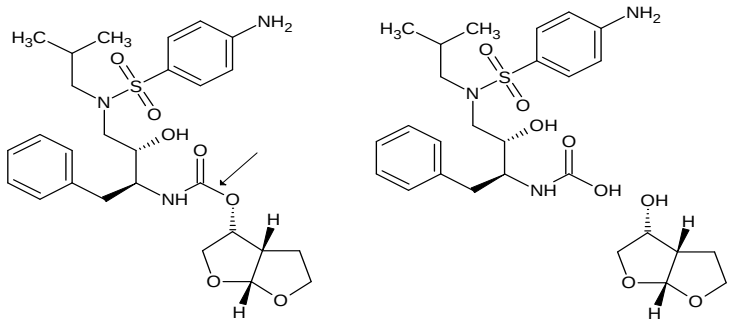
Figure-6: Oxidation

Thermal degradation and **photolytic degradation** are not possible because the drug structure has no such functional groups.

Sulfonamide bond hydrolysis and **Ester bond hydrolysis**



Sulfonamide bond hydrolysis (produces amino & sulfonic acid)



Ester bond hydrolysis (produces carboxylic acid & alcohol)

Figure-7: Sulfonamide bond hydrolysis and Ester bond hydrolysis

Correlation between acid and alkali hydrolysis

Two HPTLC peaks are obtained in sulfonamide and two in ester bond hydrolysis, so 2+2=4 peaks are obtained in acid hydrolysis which matches with 2+2=4 peaks of alkali hydrolysis. Sulfonamide group is first hydrolyzed then ester and finally amide is hydrolyzed.

MATERIALS AND METHODS

Experimental

Reagents and Materials: DRV standard was procured from Cipla Pharmaceutical Ltd., Mumbai, India and tablets of Darunavir ethanolate, "Daruvir®-300mg" tablets (300 mg, Cipla Pharma, Mumbai, India) were procured from a local pharmacy. AR grade methanol, toluene and ethylacetate (Finar Chemicals Pvt. Ltd, Ahmedabad, India), Silica gel 60 GF_{254} plates (EMerck, Mumbai, India).

HPTLC Chromatographic Conditions

Thin layer chromatography was performed on 20 cm × 10 cm aluminum-backed HPTLC plates coated with 250-µm layers of silica gel $60F_{254}$ (E. Merck, supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 105–110°C for 15 min before use for chromatography. The samples in methanol were applied as bands 5 mm wide, 5 mm apart and 20 mm from the bottom under continuous flow of nitrogen, by means of a DESAGA AS 30 sample applicator fitted with a 100-µL syringe. The plates were then conditioned for 20 min in a pre-saturated twin-trough chamber with the mobile phase, toluene–ethyl acetate–methanol 6.5:2.0:1.5 (v/v). The plate was then placed in the mobile phase and ascending development was performed to a distance of 80 mm from the point of application at ambient temperature. After development, plates were air dried and densitometric scanning was performed at 270 nm with a DESAGA CD 60 Densitometer

operated in the reflectance–absorbance mode and controlled by proquont software. The slit dimensions were 1 mm×0.1 mm. Evaluation was done by linear regression of peak area against amount of DRV per band.

Forced degradation study and development of stability indicating assay method

For development of stability indicating assay method, DRV was subjected for forced degradation study. DRV (10mg) was subjected to forced degradation study like acid hydrolysis, alkaline hydrolysis, thermal decomposition, photolytic degradation and oxidative degradation. The stress conditions were adjusted to achieve minimum 10% degradation. From the literature survey and based on different trial, following conditions were optimized for stress testing and finalized for further experiment.

Table-1: Forced Degradation conditions for DRV

Sr.No.	Stress Type	Stress Conditions
1	Acid hydrolysis	1 N HCl, 24 hr at R.T.
2	Alkali hydrolysis	1 N NaOH, 12 hr at R.T.
3	Oxidative Degradation	30% H_2O_2, 24 hr at R.T.
4	Thermal Degradation	Thermal 85^{o}C for 24 hours
5	Photolytic Degradation	U.V light, 72 hours

Preparation of solutions

(a) 1(N) HCl: 0.85 ml of HCl (36.5%) analytical grade was taken in 100ml volumetric flask and diluted up to the mark with triple distilled water to get 1 N HCl.

(b) 1(N) NaOH: Accurately weighed 0.4 gm of NaOH was taken in 100ml volumetric flask and dissolved in triple distilled water, sonicated and made up to the mark with triple distilled water to get 1(N) NaOH then filtered thorough Whatman no 41 filter.

Solution preparation for analytical method validation

Preparation of Standard solution

Accurately weighed DRV (10 mg) was transferred to a 10 mL volumetric flask, dissolved in and diluted to the mark with methanol to obtain a standard stock solution (1000 µg/ml). An aliquot (1 mL) was transferred to a 10 mL volumetric flask and diluted to the mark with methanol to obtain a working standard solution 100 µg/mL of DRV.

Preparation of Test solution

Twenty tablets were weighed and average weight was calculated. The tablets were powdered, a quantity of powder equivalent to 10 mg DRV was weighed and transferred to a

10 mL of volumetric flask containing 5 mL methanol and sonicated for 10 minutes. The flask was allowed to stand at room temperature for 5 min and the volume was made up to the mark with methanol to obtain the sample stock solution (1000 µg/mL). The solution was filtered through 0.45µm-47mm membrane filter. An aliquot (1 mL) was transferred to a 10 mL volumetric flask and diluted to the mark with methanol to obtain a sample solution (100 µg/mL).

Preparation of samples for Stress Degradation study of DRV

A stock solution containing 1000 µg/mL of DRV was used for forced degradation in accordance with ICH guidelines.

Acid Hydrolysis

Accurately weighed DRV (10 mg) was transferred to 10 mL volumetric flask and dissolved in 3 mL methanol and add 1 N hydrochloric acid solution (7 mL) for acid hydrolysis at room temperature for 24 hr. Take 1 mL from this and the sample was neutralized with base as appropriate and diluted to the mark with diluent to obtain solution (100 µg/mL).

Alkaline Hydrolysis

Accurately weighed DRV (10 mg) was transferred to 10 mL volumetric flask and dissolved in 3 mL methanol and add 1 N sodium hydroxide solution (7 mL) for alkaline hydrolysis at room temperature for 12 hr. Take 1 mL from this and the sample was neutralized with acid as appropriate and diluted to the mark with diluent to obtain the solution (100 µg/mL).

Oxidative Degradation

Accurately weighed DRV (10 mg) was transferred to 10 mL volumetric flask and dissolved in 3 mL methanol and add 30% Hydrogen peroxide solution (7 mL) for oxidative degradation at room temperature for 24 hr. Take 1 mL from this and the sample was diluted to the mark with diluent to obtain the solution (100 µg/mL).

METHOD VALIDATION

The developed method was validated as per ICH guidelines.

Stability in Sample Solution

Solutions of analytical concentration (1000 µg/mL) were prepared from the sample solution and stored for 12 and 24 hr at room temperature on a laboratory bench in tightly capped volumetric flasks protected from light. They were then applied on the TLC plate and

analyzed by the developed method for the presence of any additional band other than the drug peak.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The band for DRV in sample was confirmed by comparing the R_F and spectra of the band with those obtained from standard. The peak purity was assessed in spectrum mode of densitometer. The peak purity of DRV was assessed by comparing spectra acquired at three different positions on the band, i.e. peak start (S), peak apex (M) and peak end (E).

Linearity

The linearity was determined by analyzing seven independent levels of calibration curve in the range of 500-3500 ng/band of DRV. From the working standard solution (100µg/ml DRV, aliquots of 5, 10, 15, 20, 25, 30 and 35µL were spotted on the TLC plate under nitrogen stream using AS-30 sample applicator. The plate was dried in air and developed in mobile phase in twin trough developing chamber (20×10cm) with stainless steel lid. The twin trough developing chamber was previously saturated with the mobile phase for 30 min. Plate was removed from the chamber after 80mm solvent run and dried in air. Then the plate was scanned and quantified at 270 nm in absorption mode with CD60 TLC scanner using Pro Quant software. The calibration curve of peak area vs. concentration was plotted. Correlation co-efficient and regression line equation for DRV was determined from the calibration curve.

Precision

For Repeatability, DRV test solution was analyzed six times and % RSD was calculated. Application and measurement of peak area were measured using six replicates of the same band (1000 ng DRV per band). Intra-day and inter-day precision for analysis of DRV was measured at three different concentrations–1000, 2000 and 3000 ng per band for three times on the same day and on three different days over a period of one week. The results were reported in terms of % RSD.

Accuracy

Accuracy of the method was determined by standard addition method. To check the accuracy of the method, recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150%.

Limit of Detection and Limit of Quantitation

The LOD and LOQ were calculated, as defined by ICH, using the mean values of six independent analytical curves, determined by a linear-regression model, where the factors 3.3 and 10 for the detection and quantitation limits, respectively were multiplied by the ratio from the SD of the intercept and the slope.

LOD = 3.3 × N/S

LOQ = 10 × N/S

Where,

N is standard deviation of y-intercept of the calibration curves

S is mean slope of calibration curves.

Analysis of Tablet Formulation

To prepare the sample solution, twenty tablets were accurately weighed and crushed to a fine powder. The accurately weighed powder equivalent to 10 mg DRV was transferred to 10 mL volumetric flask and methanol (5.0 mL) was added. The solution was sonicated for 15 min. The flask was allowed to stand at room temperature for 5 min and the volume was diluted to the mark with methanol to obtain the sample stock solution (1000 µg/mL). The solution was filtered and suitably diluted with methanol to obtain sample solution of DRV (100 µg/mL) and then analyzed for assay determination. From this 3500 ng/band was applied to TLC plate followed by development and scanning as described above.

RESULT AND DISCUSSION

Optimization of mobile phase for HPTLC method

Various solvents like toluene, methanol and ethyl acetate were tried in different proportions to obtain good spot resolution of the DRV and its degradation products. The TLC procedure was optimized to develop a stability-indicating assay method to quantify DRV in marketed formulations. Both the pure drug and the degraded solutions were applied to the TLC plates and chromatographed with different mobile phases. Initially toluene–methanol in different ratios was tried, but separation of the degradation products was not achieved. Ethyl acetate was added to this mobile phase in different ratios to achieve good resolution of the drug peak

and the degradation peaks. The mobile phase toluene–ethyl acetate–methanol 6.5:2.0:1.5 (v/v) gave a sharp and symmetrical peak for DRV at an R_F value of 0.37 (Figure-11) with a good resolution of the DRV peak from those of degradation products. Well defined bands were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Selection of scanning wavelength

Wavelength was selected by scanning 1000ng/band of DRV under the multi-wavelength mode. A band was scanned between 200-400nm. At 270 nm, peak shape and baseline were found to be good and degradation spots were also detected at this wavelength. So, 270 nm was finalized for TLC densitometer scanning.

Table-2: Optimization of mobile phase

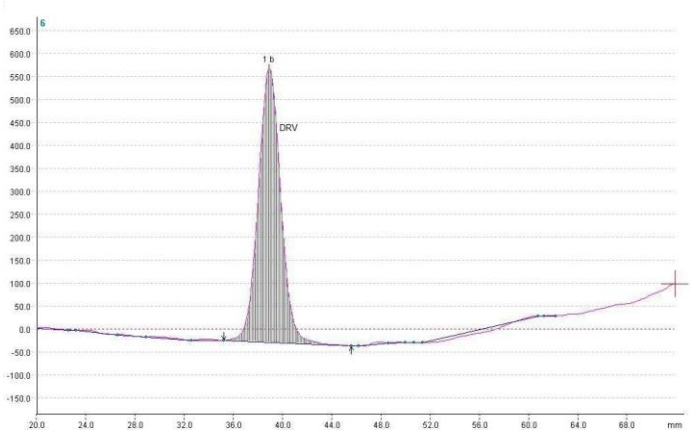
Sr.No.	Mobile Phase	Ratio	Remarks
1	Toluene	1	Separation was not observed, component stay at bottom.
2	Methanol	1	Component reached to solvent front.
3	Toluene : Methanol	5:5	Component reached to solvent front and required to decrease the ratio of methanol and addition of less
4	Toluene : Methanol : Ethyl acetate	6:3:1	Spot was not sharp enough and reached near to solvent front also.
5	Toluene : Methanol : Ethyl acetate	6:2:2	Spot was good but spots of degradation products were not sharp enough.
6	**Toluene : Methanol : Ethyl acetate**	**6.5:1.5:2**	**Spot was Sharp with the good separation of the degradation products.**

Stress Degradation study of DRV

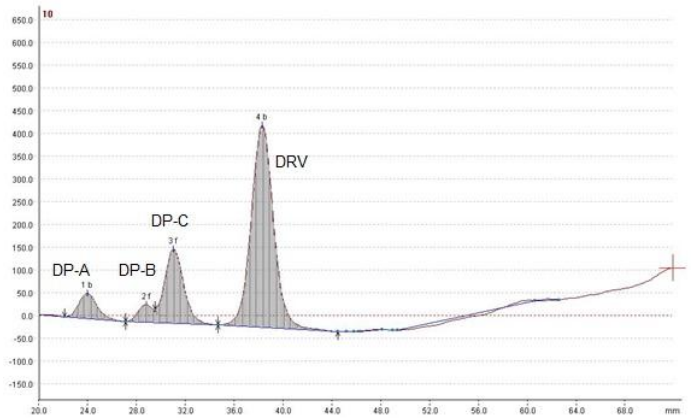
The chromatograms obtained from samples treated with acid, base, hydrogen peroxide, dry heat depicted well separated bands of pure DRV and some additional peaks at different R_F values. The number of degradation products with their R_F values is listed in Table-3.

Stress degradations are performed to provide indications of the stability-indicating properties of an analytical method. The forced degradation studies in thermal as well as photolytic conditions resulted in a negligible decrease of the DRV peak area without any detectable degradation products. The basic hydrolysis caused significant decrease of DRV peak area, with one additional peak detected. Under the acidic condition, a significant decrease of DRV peak area, with three additional peaks detected. In oxidative degradation, a significant

decrease of DRV peak area was also observed, with three additional peaks detected.[15] (Figure: 8-10).

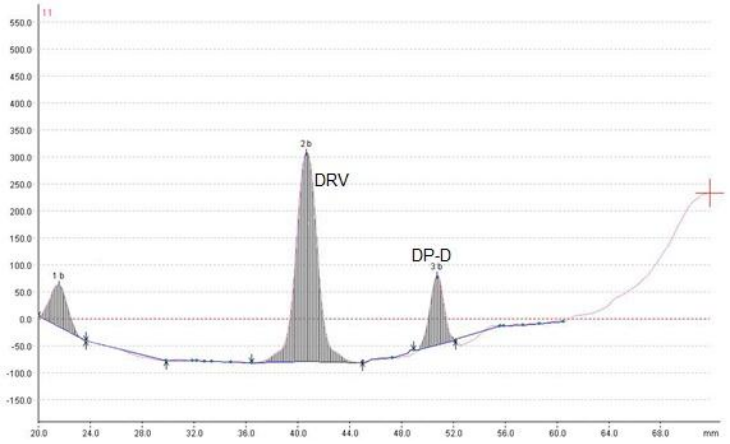


(a)

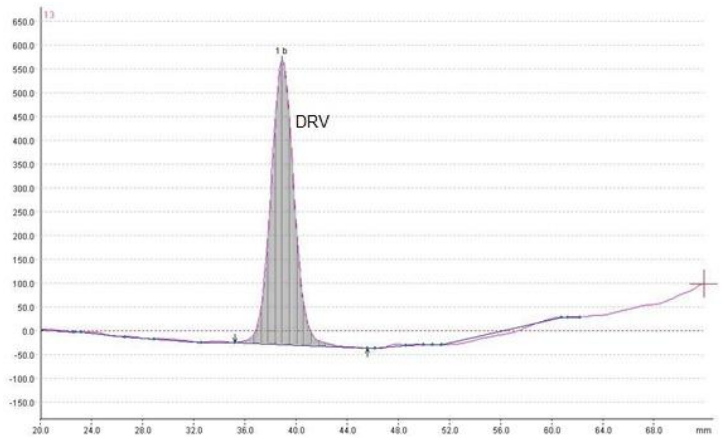


(b)

Figure-8: (a) HPTLC Chromatogram of standard DRV and (b) HPTLC Chromatogram of acid hydrolysis

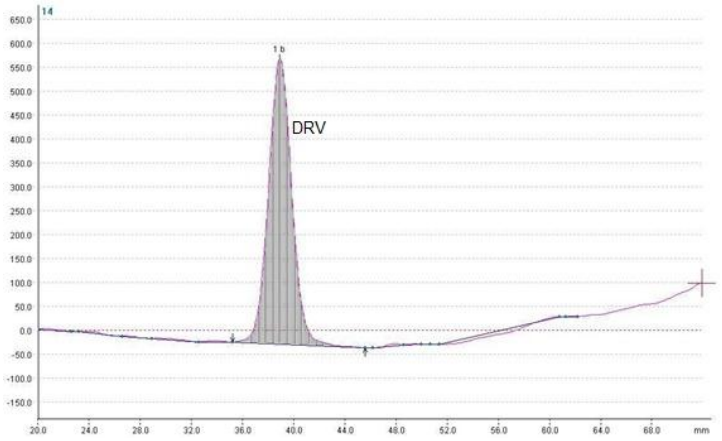


(a)

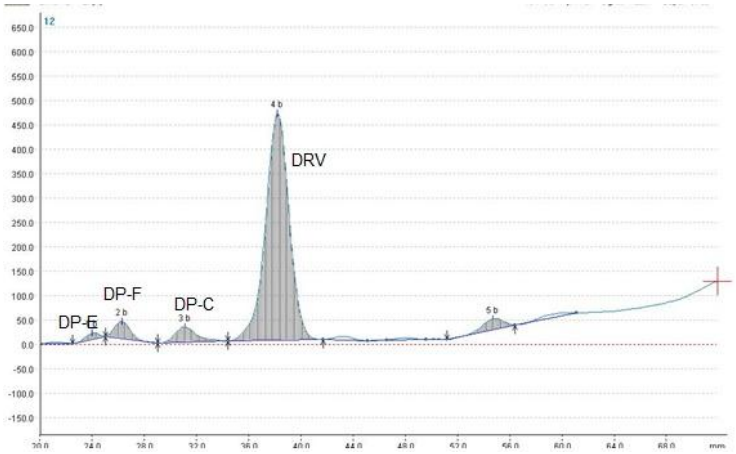


(b)

Figure-9: (a) HPTLC Chromatogram of alkali hydrolysis and (b) HPTLC Chromatogram of thermal degradation



(a)



(b)

Figure-10: (a) HPTLC Chromatogram of photolytic degradation and (b) HPTLC Chromatogram of oxidative degradation

Table-3: Result from Stress Degradation Study

Stress conditions	% Degradation	R_f value	
1 N HCl, 24 hr at R.T.	14.99%	DP-A	0.09
		DP-B	0.21
		DP-C	0.25
1 N NaOH, 12 hr at R.T.	23.15%	DP-D	0.49
30% H_2O_2, 24 hr at R.T.	18.44%	DP-E	0.14
		DP-F	0.17
		DP-C	0.28
Thermal 85°C for 24 hours	negligible	-	
U.V light, 72 hours	negligible	-	

Method Validation

Specificity

Peak purity for DRV was assessed by comparing spectra acquired at the peak start, apex and end positions of the band, r^2(S,M) = 0.9999 and r^2(M,E) = 0.9996. Good correlation (r^2 = 0.9995) was also obtained between standard and sample spectra of DRV. These correlation values indicate the ability of the method to separate and specifically detect DRV from sample solutions.

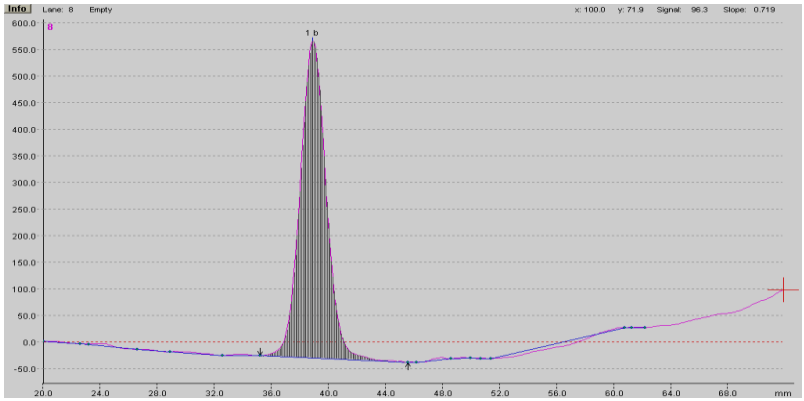


Figure-11: Chromatogram of DRV from tablet with R_f value of 0.37

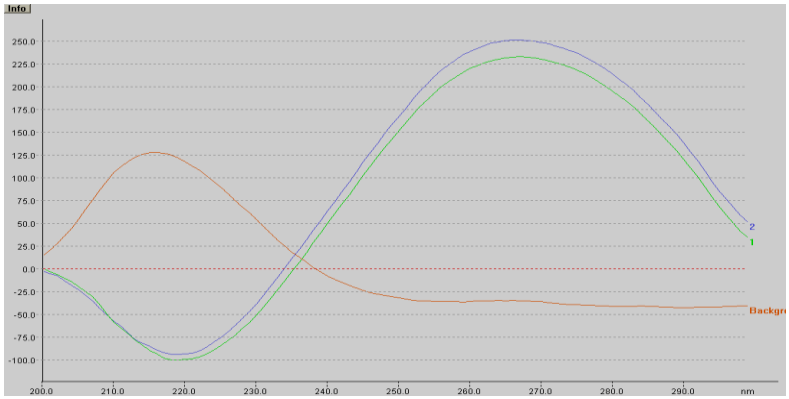


Figure-12: Peak purity spectra of DRV

Linearity

A standard calibration plot for the drug was constructed based on standard drug peak area versus concentration of standard solutions. The plot showed a good linearity over a concentration range of 500-3500 ng/band. Table 04 shows the linearity data. The linearity of the calibration plots and adherence of the system to Beer's law was validated by the high value of correlation coefficient $R^2 = 0.9995$.

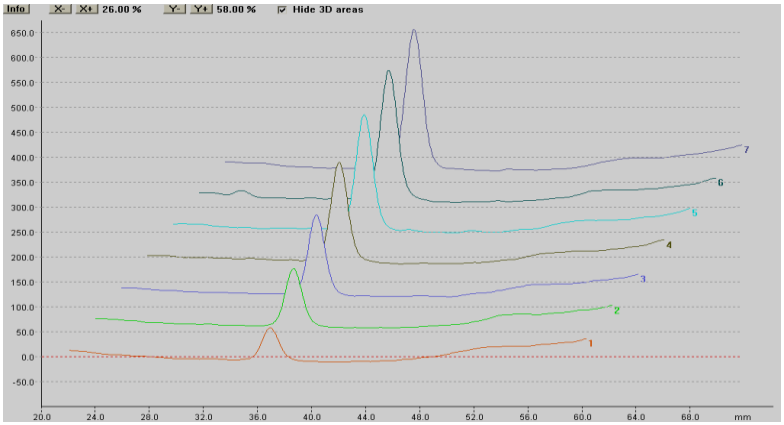


Figure-13: Overlain Chromatogram showing Linearity of DRV

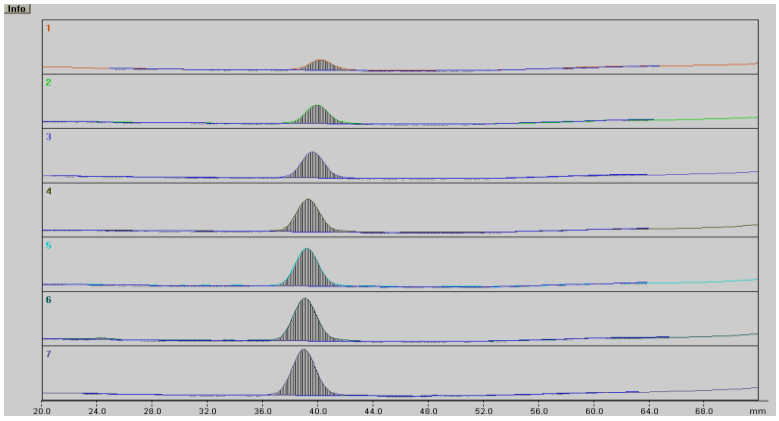


Figure-14: Vertical Chromatogram showing Linearity of DRV

Table-4: Linearity data of DRV

Sr. No.	Conc. of DRV (ng/band)	Mean Peak Area±SD (n=6)	%RSD
1	500	398.32±1.4	0.35
2	1000	567.55±2.8	0.49
3	1500	741.82±2.0	0.26
4	2000	934.45±2.2	0.24
5	2500	1128.99±5.8	0.51
6	3000	1292.16±6.7	0.52
7	3500	1472.7±6.9	0.47

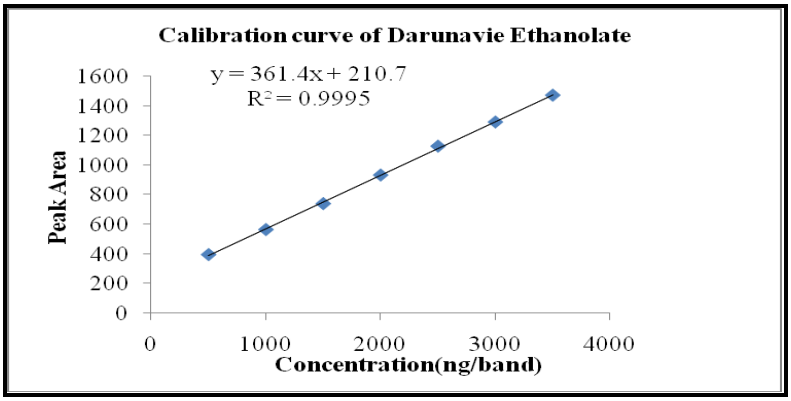


Figure-15: Calibration curve of Darunavir Ethanolate

Table-5: Linear Regression Data (n=7)

Parameters	**HPTLC method**
Linearity	500-3500 ng/band
Linear regression equation [a]	
Intercept (c)	210.7
Slope (m)	361.4
Correlation coefficient (R^2)	0.9995
LOD	150 ng/band
LOQ	390 ng/band

[a] y=mx+c.

Precision

The repeatability of application, expressed as RSD was 0.91%. Results from measurement of intra-day and inter-day variation at three different concentrations - 1000, 2000 and 3000 ng per band are listed in Table 06. RSD for within day and day-to-day analysis was found to be less than 2%. These low RSD values are indicative of good repeatability and precision of the method.[16]

Table-6: Results of repeatability (n=6)

Drug	**DRV(1000ng/band)**
1	529.05
2	527.99
3	524.79
4	520.98
5	529.88
6	517.99
Mean	525.11
SD	5.01
%RSD	0.91

Table-7: Results of Intra-day and Inter-day precision (n=3)

DRV(ng/band)	Intra-day precision		Inter-day precision	
	Mean±SD	% RSD	Mean±SD	% RSD
1000	569.14±6.01	1.06	577.22±9.93	1.72
2000	930.99±9.14	0.98	934.07±12.35	1.32
3000	1276.04±12.25	0.96	1263.36±18.18	1.44

Accuracy

The accuracy study was carried out by the standard addition method. The percent recovery was found in the range of 99.54-100.33%, which indicates accuracy of the method.

Table-8: Accuracy data (recovery studies) (n=3)

Amount taken from test (ng/band)	Amount added from std (ng/band)	Amount recovered (ng/band)	%Recovery±SD
1000	500	499.8	99.96±0.83
1000	1000	1015.5	99.54±0.59
1000	1500	1504.95	100.33±0.74

Limit of Detection and Limit of Quantitation

The LOD and LOQ values of DRV were found to be 150 ng/band and 390 ng/band, respectively which indicate the sensitivity of the method. (Table-5).

Stability in Sample Solution

The analyte was stable in solution, because no significant deviation in peak area (RSD < 2%) was observed on analysis up to 24hr. There was no indication of drug instability in the sample solution.

Table-9: Results of Standard and Sample Solution Stability

Time	Assay of DRV (%)		% Difference	
	Standard	Sample	Standard	Sample
Initial	100.52	100.24	---	---
After 12 hours	99.84	99.79	0.68	0.45
After 24 hours	100.01	99.53	0.51	0.71

Analysis of Tablet Formulation

Drug bands at R_F 0.37 corresponding to DRV were observed in chromatograms obtained from tablet extracts. There was no interference from excipients present in the tablets. The DRV content of marketed formulation was 99.56%, RSD 0.43%. The good performance of

the method indicated the suitability of this method for routine analysis of DRV in pharmaceutical dosage form.

Table-10: Analysis results of tablet dosage form (n=3)

Formulation	Labeled amount (mg)	Amount found (mg)	%Assay±SD
Daruvir®-300mg	300	298.68	99.56±0.43

The validation data are summarized in Table 11.

Table-11: Summary of Validation Parameters

Parameters	HPTLC method
Linearity	500-3500 ng/band
Intercept (c)	210.7
Slope (m)	361.4
Correlation coefficient (R^2)	0.9995
LOD	150 ng/band
LOQ	390 ng/band
Precision (%RSD)	
Intraday (n=3), %	1.0
Interday (n=3), %	1.49
Repeatability of injection (n=6), %	0.91
% Recovery (at 100% level)	99.54 ± 0.59

CONCLUSION

Two HPTLC peaks are obtained in sulfonamide and two in ester bond hydrolysis, so 2+2=4 peaks are obtained in acid hydrolysis which matches with 2+2=4 peaks of alkali hydrolysis. Sulfonamide group is first hydrolyzed then ester and finally amide is hydrolyzed. In summary, the method described is rapid, sensitive, specific, accurate and repeatable. It possesses significant linearity (R^2 = 0.9995), precision within acceptable range of RSD and no interference from the excipients or degradation products. It was successfully used to study the stability of DRV under different stress degradation conditions. As the method separates the drug from its degradation products, it can be used as a stability-indicating method. The proposed method gives idea about the degradation products of DRV and used for the routine QC analysis of DRV in pharmaceutical dosage form.

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