WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 12, 798-820. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DARUNAVIR IN PHARMACEUTICAL DOSAGE FORM

Prathamesh Dhiwar*, Rahul Dumbre and Narendra Gowekar

[1]Department of Quality Assurance and Techniques Siddhant College of Pharmacy, Sudumbare, Pune-412109.

Article Received on
05 August 2020,

Revised on 25 August 2020,
Accepted on 15 Sept. 2020

DOI: 10.20959/wjpr202012-18662

***Corresponding Author**
Prathamesh Dhiwar
Department of Quality Assurance and Techniques Siddhant College of Pharmacy, Sudumbare, Pune-412109.

ABSTRACT

High-performance liquid chromatography is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase (s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent (s) used. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. For the characterization of the drug some identification tests were done such as identification of drug, melting point of the drug and solubility test etc. methods were perform during entire process are as follows Analysis of tablet formulation of DAR in which determination of Recovery study of marketed formulation of DAR and Validation of RP-HPLC method for analysis of DAR along with Specificity, linearity, accuracy, precision, reproducibility, LOD and LOQ To access the stability of drug it is subjected to various stress conditions like acid, alkali, neutral oxidative and photo degredation these test were performed. For the assessment of different test dilution were prepared in a different concentration to obtain the accurate results dilutions were in range 0f 80-240µg/ml.Developed and estimated stability indicating RP-HPLC method for darunavir

which complies with ICH guidlines. Seperation was accomplished on agilent C18 colums (150 mm × 4.6mm, 3µm) using acetonitrile and phosphate buffer (40:60) as mobile phase. Flow rate of 1.5ml/min and optimised wavelength was 266nm. The calibration graph showed linear relationship with R=0.999 in range 0f 80-240µg/ml. To access the stability of drug it is subjected to various stress conditions like photo degradation, acid degradation, alkali degradation and oxidative degradation. With the conclusion from above information that the proposed HPLC method can be used successfully for estimation of Darunavir.

KEYWORDS: Darunavir, ICH, Reverse phase High performance liquid chromatography, Forced Degradation, Development, validation.

INTRODUCTION

High-performance liquid chromatography is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase (s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent (s) used. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Darunavir (formerly TMC114) is a second-generation, sulfonamide-based, peptidomimetic protease inhibitor (PI) with a modified 3-dimensional structure enabling more efficient binding to HIV protease. It has become an important drug Daruanvir is chemically (3R, 3As, 6aR)-Hexahydrofuro 2, 3-b furan-3-yl N-[(2s, 3R) - 3-hydroxy-4-[N-(2-methylpropyl) 4 aminobenzenesulfonamido]-1-phenylbutan-2-yl] Carbamate Literature survey observe that HPLC, LC-MS, and UV methods methods were reported for estimation of Darunavir.

Method Development of Reverse Phase Chromatography were performed with the mobile phase is more polar than the stationary phase in revers-phase chromatography. In these system mobile phase used is mixtures of two or more solvents with or without any additives or any organic solvent modifiers. The selection is completely depend on the, what appears on the most appropriate column, and then mobile phase is design, that will optimize the

selectivity and retention of the system. pH selection of buffer based on pKa value i.e. acid dissociation/ionization constant for a drug means the pH at which equal concentrations of the acidic and basic forms of the molecule are present in aqueous solutions. In mobile phase at pKa value of any drug shows broad or tailing peaks. It is very important that the pH value must be at least 1 to get proper and sharp peak of analyte. Among all the sample 99% of the sample is in one form because it help to shifts the equilibrium so result sustain that chromatography peak. Mobile phase selection was based on choosing the optimum mobile phase compositions of organic & aqueous portions. During the selection of column the following points were considered Packing Material of column The particle size & shape, length of the column and its diameter, carbon % load, Pore Volume, End capping. There are various types of detectors are used in the HPLC .Selection of detectors will be depend on the nature of API, degraded products & relates substances.

MATERIAL AND METHODS

Material and reagents

Hydrochloric acid (AR grade), Acetonitrile (HPLC grade), HPLC grade water, 3% Hydrogen peroxide (H_2O_2) (AR grade), sodium hydroxide (AR grade), Potassium dihydrogen phosphate, Orthophosphoric acid were purchased from MERCK Pvt. Ltd., Mumbai.

Standard stock solution of DAR (1600 µg/mL)

Weighed accurately 80 mg of DAR and transferred to 50 mL volumetric flask, add about 30 mL of methanol and sonicated for 10 min. and volume was made up to the mark by using double distilled water.

Selection of analytical wavelength

More dilutions were made using methanol from the standard stock solution and the concentration 10 µg/mL was tested over the range of 400-200 nm and the spectrum was obtained. The observed spectra showed maximum absorbance at 265.75 nm.

Table no. 1: Chromatographic conditions.

Parameters	Conditions
Stationary phase	ACE C18, 3µ (150 × 4.6 mm)
Mobile phase	ACN : Phosphate buffer pH 4.0 (40:60v/v)
Flow rate	1.5 ml/min
Detection wavelength	266 nm
Pump mode	Isocratic
Injection volume	20 µl

Column temperature	35-37°C
Sample temperature	15°C

Identification of the drug

The drug was identified by the use of Infrared absorption spectroscopy. The required quantity of drug was taken and mixed with potassium bromide and packed into the compact disk and spectrum was recorded shown in graph no 1.

Table no 2: IR peak assignment value of darunavir.

Assignment	Frequency (Cm^{-1})	
	Reported Value	Observed value
O-H/N-H stretch	3462, 3447, 3366	3450
C=O stretch	2961, 2933, 2872	2932
C=C stretch (skeleton of benzene)	1636,1597,1522, 1456	1595, 1541, 1502
$-SO_2-$	1315,1150	1307,1146
C-H bend (out of plane of benzene	833.702	840.767

Determination of melting point

Open capillary tube method was used for determining the melting point of the drug. In this process, single end of the capillary tube was fused and little quantity of the drug was inserted from another open end by spatula. The filled capillary was inserted into melting point apparatus and reading was recorded. This procedure was performed in triplicate and mean of three observations is considered as a melting point. The melting point of respective drug is summarized in table no 12.

Assessment of solubility of drug

The solubility of the drug was performed by placing of a drug in the conical flask by use of different solvents, which was then placed in a rotary shaker and was continuously shaken for 24 hours. Solvents used for solubility determination that was distilled water, methanol, acetonitrile, phosphate buffer pH 3, 4.6 and 6.8. This practice was performed thrice. For analysis of drug, solvents were finalized as shown in Table no 13.

Estimation of maximum absorbance wavelength of drug

Working standard solution of drugs was prepared for estimation of maximum absorbance wavelength as shown in Table no 14. UV spectrum of drug solutions was recorded in the wavelength range 400-200nm and maximum absorbance was observed at the particular wavelength as shown in graph 2 and Table no 14**.** This wavelength was further used as a detection wavelength ($\lambda_{max)}$ for analysis of drug.

Standardization of drugs

This drug is not official in any of the pharmacopeias, the purity of the drug sample was considered as stated in the certificate of analysis supplied by the supplier and summarized in Table no 3.

Table no. 3: determination of % Purity.

Sr. no	Drug	%purity
1.	Darunavir	≥100.8

Table no. 4: Common HPLC Chromatographic conditions for analysis of darunavir.

Parameters	Standards
HPLC Pump	Agilent 1120 compact LC gradient pump.
Injection	Manual Rheodyne injector
Column	Agilent TC C18 (250mm X 4.6 mm i.d., 5µm)
Detector	Variable Wavelength Detector
Software	EZChrom Elite Compact software
Injection Volume	20µL
Flow rate	1.5µL/min

Trials for finalising mobile phase

Mobile phase was finalized after taken several trials for the finalization of the mobile phase there are 5 trials have been done to determination of the mobile phase along with the different concentrations. The different trials as shown below with the observed records.

Trial-1

In trial 1 the minimum flow rate were performed for the determination of the mobile phase the flow rate was 1.5 mL/min with the injection volume was 20 µL the temperature of the column was 35°C.

Table no. 5: Chromatographic Conditions for trial 1.

Chromatographic Conditions	
Column	Zorbax, C8, 250 x 4.6 mm, 5.0 µm
Flow rate	1.5 mL/min
Injection volume	20 µL
Wavelength	265 nm
Column temperature	35°C

Trial 2

In comparison with trial 1, trial 2 had different flow rate were performed for the determination of the mobile phase the flow rate was 2.0 mL/min with the injection volume was 20 µL the temperature of the column was 35°C.

Table no. 6: Chromatographic conditions for trial 2.

Chromatographic Conditions	
Column	Zorbax, C8, 250 x 4.6 mm, 5.0 µm
Flow rate	2.0 mL/min
Injection volume	20 µL
Wavelength	265 nm
Column temperature	35°C

Trial 3-

In comparison with trial 1and trial 2, trial 3 had different column were introduced to determination of the mobile phase. Flow rate were performed for the determination of the mobile phase the flow rate was 1.0 mL/min with the injection volume was 20 µL the temperature of the column was 35°C.

Table no. 7: Chromatographic conditions for trial 3.

Chromatographic Conditions	
Column	Inertsil, C8, 150 x 4.6 mm, 3.0 µm
Flow rate	1.0 mL/min
Injection volume	20 µL
Wavelength	265 nm
Column temperature	35°C

Trial 4-

In comparison with trial 3 had different flow rate to determination of the mobile phase. flow rate were changed from 1.0 to 1.5 mL/min for the determination of the mobile phase with the same column which was used in trial 3 i.e Inertsil, C8, 150 x 4.6 mm, 3.0 µm with the injection volume was 20 µL the temperature of the column was 35°C. Prepared a mixture of buffer solution pH 4.0 and acetonitrile in the ratio 40:60 v/v, respectively. Mixed and sonicated to degas.

Table no. 8: Chromatographic conditions for trial 4.

Chromatographic Conditions	
Column	Inertsil, C8, 150 x 4.6 mm, 3.0 µm
Flow rate	1.5ml/min
Injection volume	20 µL
Wavelength	265 nm
Column temperature	35°C

Trial 5

The column was changed during the tial 5 to determination of the mobile phase. flow rate were 1.5 mL/min for the determination of the mobile phase with the column were changed

from Inertsil, C8, 150 x 4.6 mm, 3.0 µm to ACE C18, 150 x 4.6 mm, 3.0 µm with the injection volume was 20 µL the temperature of the column was 35°C.

Table no. 9: Chromatographic conditions for trial 5.

Chromatographic Conditions	
Column	ACE C18, 150 x 4.6 mm, 3.0 µm
Flow rate	1.5 mL/min
Injection volume	20 µL
Wavelength	265 nm
Column temperature	35°C

Preparation of working standards

Analytical standard solutions of DAR containing 80, 120, 160, 200 and 240 µg/mL were prepared by dilutions of aliquots portion of the standard stock solution of DAR and volume was adjusted to 10mL with double distilled water as per the procedure described in Table no 21. Aliquots of 20µL were injected into the HPLC system and separated with the mobile phase under the optimized chromatographic conditions. The analytical range was finalized 80-240µg/mL and their calibration data is summarized in Table no 21. A graph of concentration (µg/mL) versus average peak area was plotted is shown in Graph 8.

Analysis of tablet formulation of darunavir

Twenty tablets each of DAR were weighed. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent 80mg of Darunavir was weighed and transferred to a 100mL volumetric flask, dissolve in small portion of methanol sonicated for about 15 min and volume was made up to mark with finalised diluent and filtered into a 100mL volumetric flask through 0.22 µm membrane filter. 10 mL of the filtrate was collected and diluted to 50 mL with diluent in order to obtain a final concentration of 1600 µg/mL of DAR. The final concentration solution was injected into HPLC system and quantitate under standard chromatographic condition. Standard calibration curve of DAR was used to determine an amount of drug present in the sample solutions of the tablet. The result of analysis of tablet dosage form is shown in Table no 22.

Recovery study of marketed formulation of darunavir

Recovery study was carried out to the determined accuracy of the method. Known amount of pure Darunavir was added to get three levels at 80%, 100% and 120% (For 80 mg of reanalysed tablet 640 mg, 800 mg and 960 mg pure DAR was incorporated) The sample

solution was prepared and quantitate under standard chromatographic conditions. The results are shown in Table no 23.

- **Validation of RP-HPLC method for analysis of DAR**

Validation of stability indicating method was carried out as per ICH guidelines in terms of linearity, sensitivity, accuracy, precision, LOD, LOQ and robustness. Study the validation parameters were carried out at three concentrations 80µg/mL, 160µg/ml and 240µg/ml. These analytical standard solutions were prepared as described in Table No 21.

Specificity

The specificity of method was determined by complete separation of DAR tablet dosage form by applying parameters like Theoretical plate, capacity factor and the tailing factor is shown in Table no 10.

Table no. 10: Observed specificity value.

Parameter	Obtained value*
Capacity factor (k')	3.075
Theoretical plates (USP)	20692
Tailing factor (T_f)	1.01

Linearity

The linearity of the developed method was determined by diluting standard stock solution at different concentration levels. The plot between concentration data versus peak area was constructed for least-squares linear regression study. The result is shown in Table no 24.

Accuracy

The accuracy of an analytical method expresses the closeness between the reference value and found value. Accuracy was determined by the standard addition method. Previously analyzed samples of DAR (80µg/mL) were spiked with 0, 80, 100, and 120% extra DAR standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. % Recovery, RSD, bias and SEM were calculated for each concentration is used for validation study. The result is shown in Table no 25.

Precision

In accordance with ICH recommendations, precision was determined as both intermediate precision and repeatability. Intermediate precision was determined by measurement of inter-day variation and repeatability was estimated as an intra-day variation. For both intra-day and

inter-day variation, solutions of drug at three different (80µg/mL, 160µg/mL and 240µg/mL) concentrations were used. The result is given in Table no 26.

Reproducibility

The reproducibility of the method was checked by determining precision on a different instrument, the analysis being performed by another person in a different laboratory. For both intra-day and inter-day variation, solutions of drug at three different concentrations were determined and details are shown in Table no 27.

Limits of Quantification (LOQ) and Detection (LOD)

The LOQ and LOD were achieved by signal to noise ratio method observations is given in Table no 11.

LOD = 3.3 x σ/s

LOQ = 10 x σ/s

Where, σ = Standard deviation of response

S = Calibration curve slope

Table no. 11: determined LOD and LOQ values.

Limit of detection (LOD µg/mL)	1.23
Limit of quantitation (LOQ µg/mL)	3.74

Robustness

To determine the robustness of the developed method, various experimental conditions were purposefully changed. The mobile phase composition (actual±10%), the flow rate of the mobile phase (1.5 ± 0.1mL/min), column oven temperature (37 ± 5°C), and detection wavelength (actual ± 5nm), pH of (4 ± 0.2) were the varied parameters. In each case, the % RSD were calculated for the obtained retention time and peak area. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. The studies were carried out on the same day at different time intervals and on three different days for one concentration (80µg/mL) of drug by three different analysts (triplicate injections). The area obtained from each concentration was matched with that of the optimized one. The Results are shown in Table no 28.

Forced degradation studies

DAR Stock solution was prepared separately by weighing accurately 80mg of DAR and transferred the powder to 50mL volumetric flask, add about 30mL of methanol and sonicated for 10 min and volume was made up to mark by using double distilled water.

The drug was exposed to forced degradation studies. The experimental study was carried out separately for acid, alkali, neutral, thermal, oxidative and photolytic degradation.

Acid degradation

To 10mL of working standard solution (80µg/mL), 10mL of 0.1N HCl was added. This mixture was reflux at 80^0C for 2 hours, after that neutralized this solution with 0.1 M NaOH. 1mL of this solution was diluted to 10mL with the mobile phase and resultant solution injected into the HPLC system and the recorded chromatogram (graph 9) was used to evaluate the stability of the sample.

Alkali degradation

To 10 mL of working standard solution (80µg/mL), 10mL of 0.1N NaOH was added. This mixture was reflux at 80^0C for 2 hours, after that neutralized this solution with 0.1 M HCl. 1 mL of this solution was diluted to 10mL with the mobile phase and resultant solution injected into the HPLC system and the recorded chromatogram (graph 10) were used to evaluate the stability of the sample.

Neutral degradation

To 10mL of working standard solution (80µg/mL) was mixed with 10mL water and reflux for 60 min at 60^0C. Then 1mL solution was diluted to 10mL with the mobile phase and resultant solution injected into the HPLC system and the recorded chromatogram (graph 11) was used to evaluate the stability of the sample.

Photo degradation

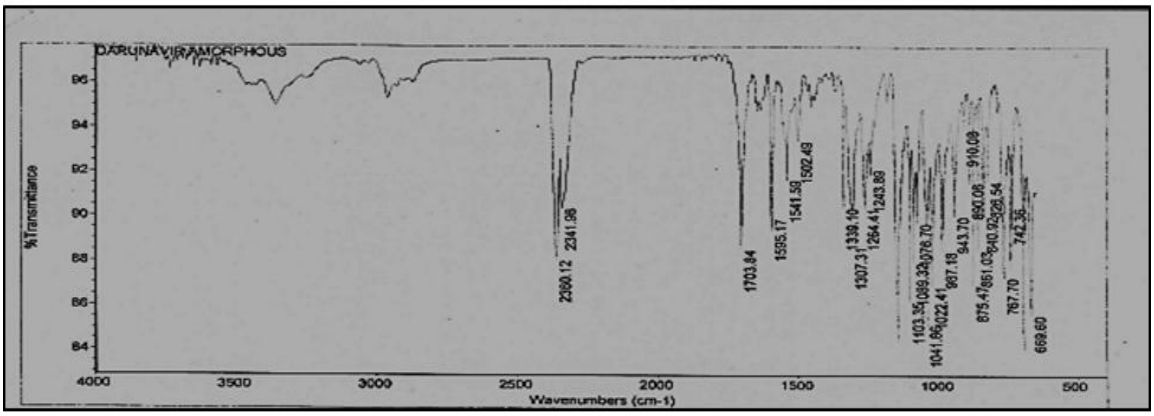
For photolytic degradation, a drug in the solid state kept in photo stability chamber was exposed to 1.2 million lux for 1hr. 8mg sample was dissolved in 10mL HPLC grade methanol and further diluted with mobile phase to obtained concentration 80µg/mL, a 20µL solution was injected into the HPLC system and the recorded chromatogram (graph 12) were used to evaluate the stability of the sample.

Oxidative degradation

To 10mL of working standard solution (80µg/mL), 10mL of 3 % H_2O_2 (v/v) was added. This solution was heated for 10 min in boiling water bath to remove the excess of H_2O_2 completely and reflux at 50^0C for 20 min. 1mL of the solution was diluted to 10mL with optimised mobile phase and resultant solution injected into the HPLC system and the recorded chromatogram (graph 13) were used to evaluate the stability of the sample.

RESULTS AND DISCUSSION

Identification of the Drug



Graph 1: Infrared spectra for Darunavir

Determination of melting point

Table no. 12: Determined value of darunavir by melting point method.

Sr. no	Drug	Melting Point (^{0}C) ± S.D.
1.	Darunavir (DAR)	74 ± 2.35

Assessment of Solubility of Darunavir Drug

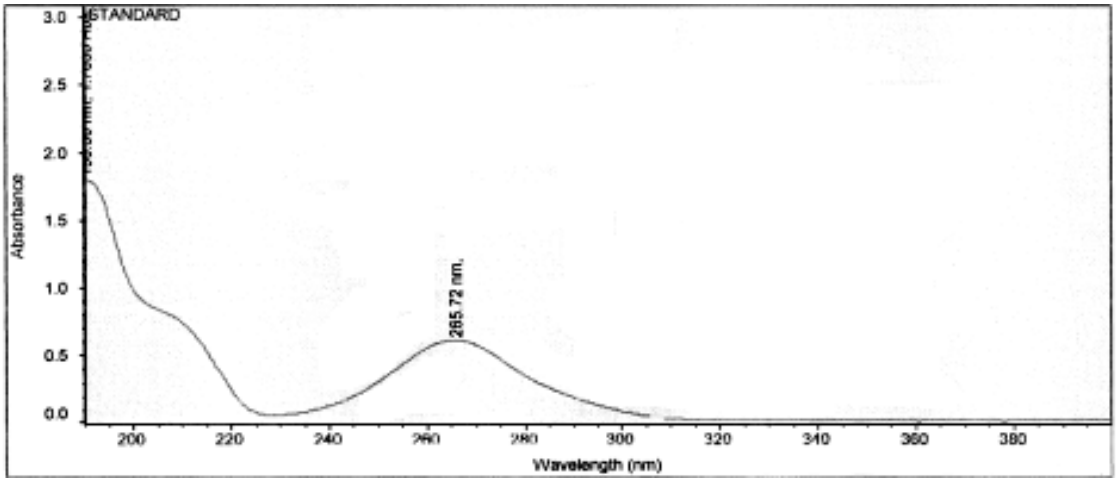
Table no. 13: Determined value of darunavir by solubility method.

Sr. No.	Drug	Solvent
1	Darunavir (DAR)	Methanol

Estimation of maximum absorbance wavelength of drug

Table no. 14: Determined maximum absorbance wavelength of Darunavir $\lambda_{max.}$

Sr. no	Drug	Stock Concentration	Working Std.	Reported Value (λmax)	Observed Value
1.	Darunavir	1 mg of drug dissolved in 10 mL Methanol (100µg/mL)	1 mL of stock solution diluted to 10 mL with Methanol (100µg/mL)	266 nm	265.75nm



Graph 2: UV spectrum of darunavir standard solution.

Standardization of drugs

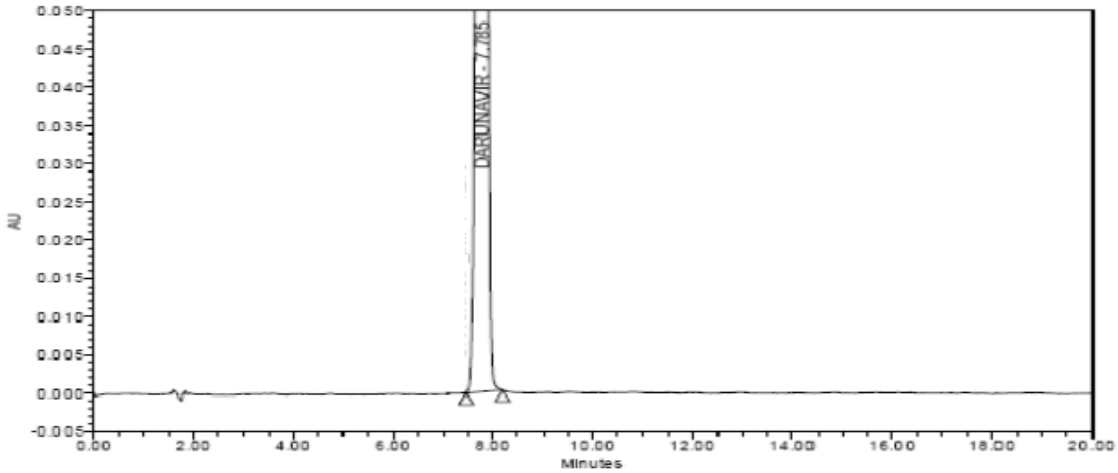
Table no. 15: determined % purity of darunavir.

Sr. no	Drug	%purity
1.	Darunavir	≥100.8

Trial-1

Table no. 16: Observed RT, area theoretical plate and symmetry values during trial 1.

Component	R.T (retention time)	Area(mAU)	Theoretical plate	Symmetry
Darunavir	7.785	4411294	12248	0.93

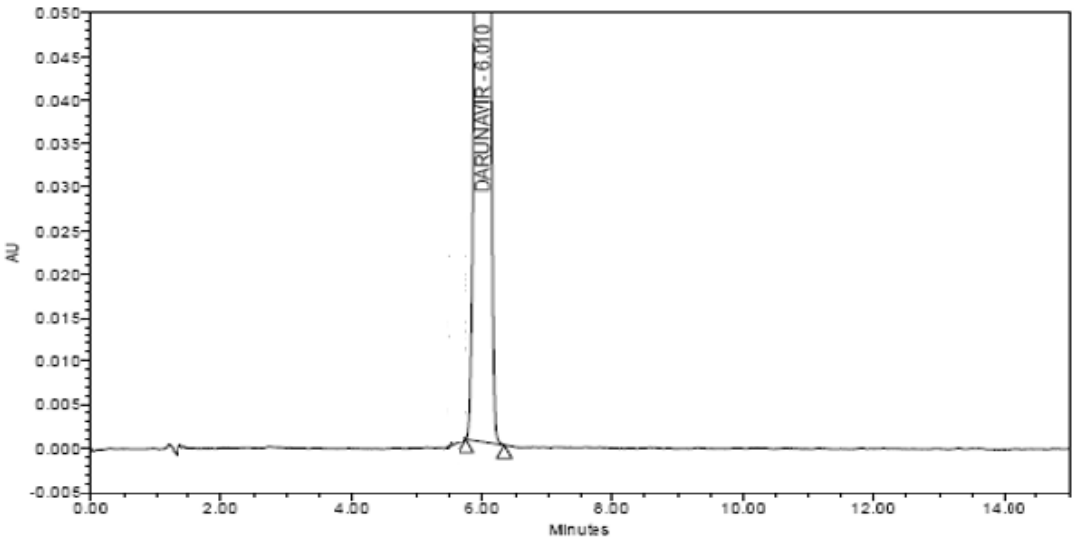


Chromatogram Graph no 3: Observed graph shown during trial 1 to determination of mobile phase of standard Darunavir

Trial 2-

Table no 17: Observed RT, area theoretical plate and symmetry values during trial 2.

Component	R.T (retention time)	Area(mAU)	Theoretical plate	Symmetry
Darunavir	6.010	3274607	7132	0.95

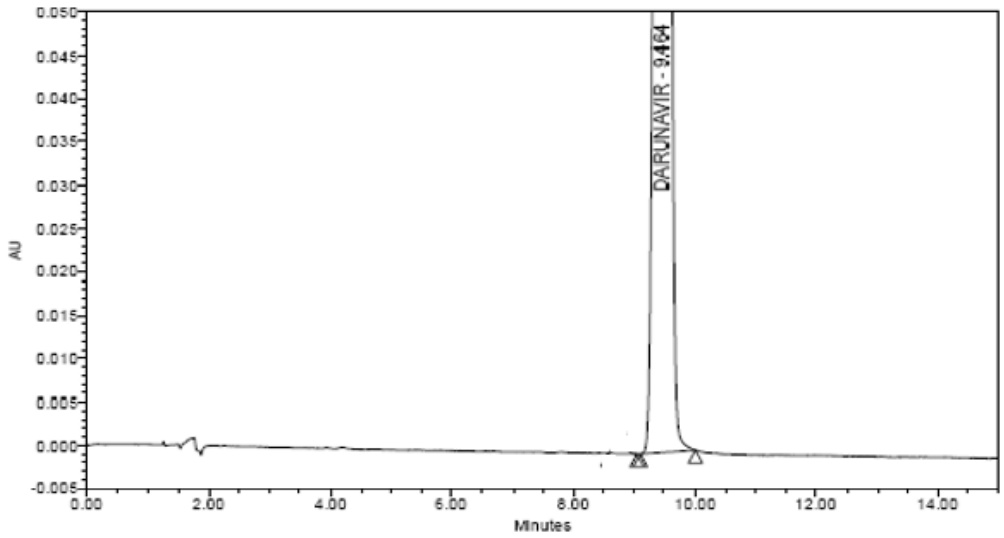


Chromatogram Graph no 4: Observed graph shown during trial 2 to determination of mobile phase of standard Darunavir

Trial 3-

Table no. 18: Observed RT, area theoretical plate and symmetry values during trial 3.

Component	R.T (retention time)	Area(mAU)	Theoretical plate	Symmetry
Darunavir	9.464	6647698	19725	0.98

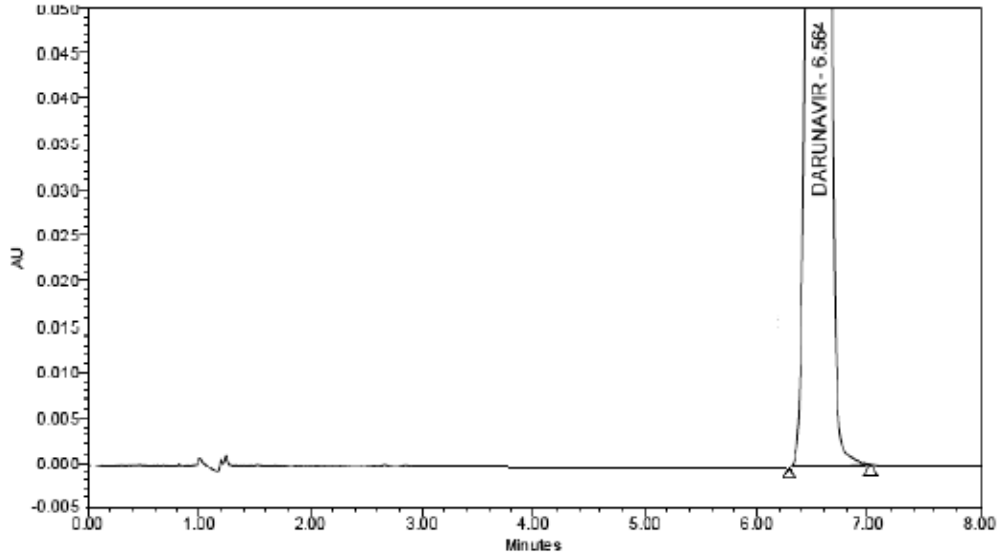


Chromatogram Graph no 5: Observed graph shown during trial 3 to determination of mobile phase of standard darunavir.

Trial 4-

Table no. 19: Observed RT, area theoretical plate and symmetry values during trial 4.

Component	R.T (retention time)	Area(mAU)	Theoretical plate	Symmetry
Darunavir	6.564	4390180	14039	0.94

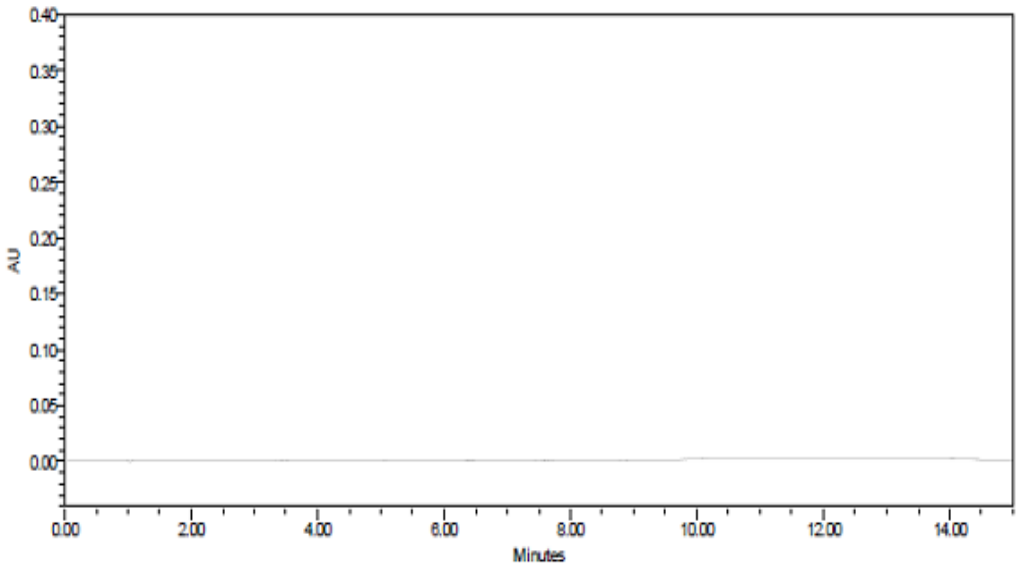


Chromatogram Graph no. 6: Observed graph shown during trial 4 to determination of mobile phase of standard darunavir.

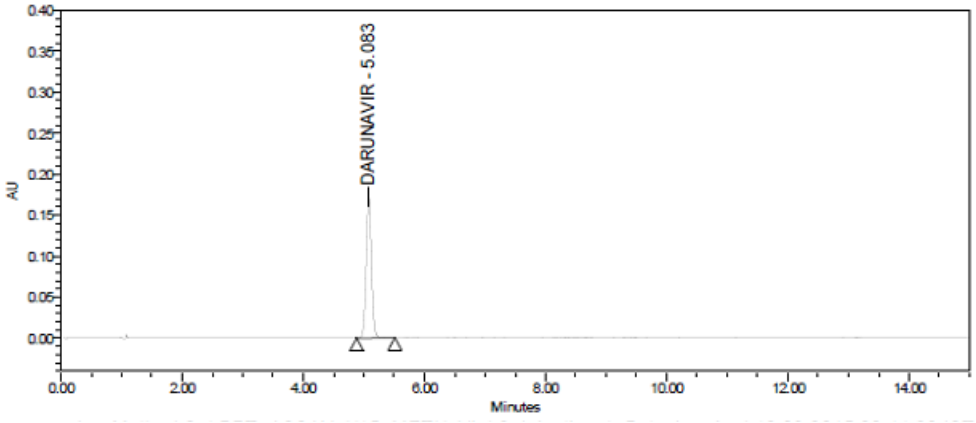
Trial 5-

Table no. 20: Observed RT, area theoretical plate and symmetry values during trial 5.

Component	R.T (retention time)	Area(mAU)	Theoretical plate	Symmetry
Darunavir	5.083	4407637	20692	1.01



Chromatogram Graph no. 7(A): Observed graph shown during trial 5 to Determination of mobile phase of blank darunavir.



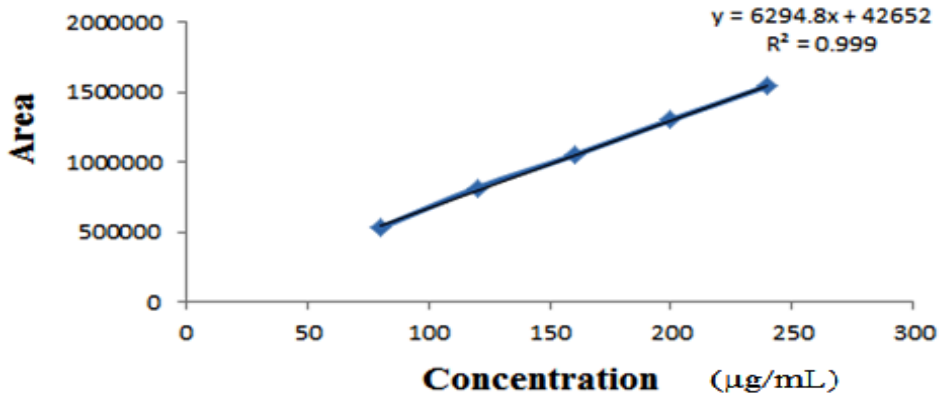
Chromatogram Graph no 7(B): Observed graph shown during trial 5 to determination of mobile phase of standard darunavir.

Table no. 21: Trials of mobile phase used for analysis of DAR.

Sr. no	Trial taken	Flow rate	Observation	RT of Darunavir in min	Remarks
1	MP: ACN: P.Buffer pH 4.0 (40:60)	1.5 mL/min	Tailing factor more than 2	7.785	Not Satisfactory
2	MP: ACN: P.Buffer pH 4.0 (40:60)	2.0 mL/min	Broadening and Tailing of peak	6.010	Not Satisfactory
3	Column changed to Inertsil C8 MP: ACN: P.Buffer pH 4.0 (40:60)	1.5 mL/min	Broadening and Tailing of peak	9.464	Not Satisfactory
4	Column changed to Inertsil C8 MP: ACN: P.Buffer pH 4.0 (60:40)	1.5 mL/min	Broadening and Tailing of peak	6.564	Not Satisfactory
5	Column changed to ACE C18, MP: ACN: P.Buffer pH 4.0 (40:60)	1.5 mL/min	Sharp peak with tailing factor less than 2	5.083	Satisfactory

Table no. 22: Calibration data of darunavir.

Stock volume	Diluent volume	Final volume	Final concentration spiking sol^{n} (µg/ml)	Mean area* ± *SD*	RSD %	SEM
50	9950	10	80	5310490 ± 3593.4	0.007	2074.6
75	9925	10	120	816837 ± 2356.0	0.003	1360.2
100	9900	10	160	1050765 ± 8312.8	0.008	4799.4
125	9875	10	200	1304088 ± 11335	0.009	6544.3
150	9850	10	240	1546391 ± 5112	0.0033	2893.2



Graph no. 8: Calibration curve of DAR.

Analysis of tablet formulation of darunavir.

Table no. 23: Analysis of marketed tablet formulations of DAR.

Drug	Marketed form	Amount Present (mg)	Amount Found (mg)	Amount Found* (%)	S.D.	SEM	% RSD
Darunavir	Daruvir 800mg (Cipla Ltd)	800	795.04	99.38	0.0017	0.0007	0.57

Recovery study of marketed formulation of darunavir.

Table no. 24: Recovery study of marketed tablet formulations of DAR.

Marketed Form	Level of standard addition (%)	Amount of tablet powder (mg)	Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery*	% RSD
Daruvir 800mg (Cipla Ltd)	80	800	640	1432.62	99.16	1.05
	100	800	800	1602.64	100.33	1.69
	120	800	960	1765.28	100.55	0.86

Linearity

Table no. 25: Calibration data for darunavir.

Conc^{n} (µg/mL)	Mean area* ± *SD*	RSD (%)	SEM
80	5310490 ± 3593.4	0.007	2074.6
120	816837 ± 2356.0	0.003	1360.2
160	1050765 ± 8312.8	0.008	4799.4
200	1304088 ± 11335	0.009	6544.3
240	1546391 ± 5112	0.0033	28932

Accuracy

Table no. 26: Determined of accuracy by observed value darunavir.

Amount (%) of drug added to analyte	Theoretical content (µg/mL)	Conc^n found (µg/mL) ± SD	Recovery (%)	RSD (%)	SEM
0	80	80.16±0.0226	100.20	0.282	0.15
80	144	144.40±0.401	100.28	0.278	0.76
100	160	159.84±0.179	99.90	0.112	0.57
120	176	177.36±0.100	100.77	0.056	0.43

Precision

Table no. 27: Determined of precision by observed value darunavir.

Conc^n (µg/mL)	Repeatability (intra-day precision)			Intermediate precision (inter-day)		
	Mean area* ± SD	SEM	RSD (%)	Mean area* ± SD	SEM	RSD (%)
80	5310680 ±183.63	106.02	0.003	5290671 ± 198.14	114.39	0.003
160	1050660 ±354.37	204.59	0.034	1051623 ± 356.91	206.06	0.034
240	1546680 ±294.64	170.11	0.02	1543894 ± 290.40	167.66	0.019

* Mean of three determinations

Reproducibility

Table no. 28: Determined of reproducibility by observed value darunavir.

Concn (µg/mL)	Repeatability (intra-day)			Intermediate precision (inter-day)		
	Mean area* ± SD	SEM	RSD (%)	Mean area* ± SD	SEM	RSD (%)
80	5289772 ± 216.52	125.01	0.004	5283339 ± 249.13	143.83	0.04
160	1051463 ± 334.54	193.14	0.03	1051126 ± 414.32	239.21	0.039
240	1543622 ± 360.26	208.00	0.024	15427730 ± 366.84	211.79	0.024

Robustness

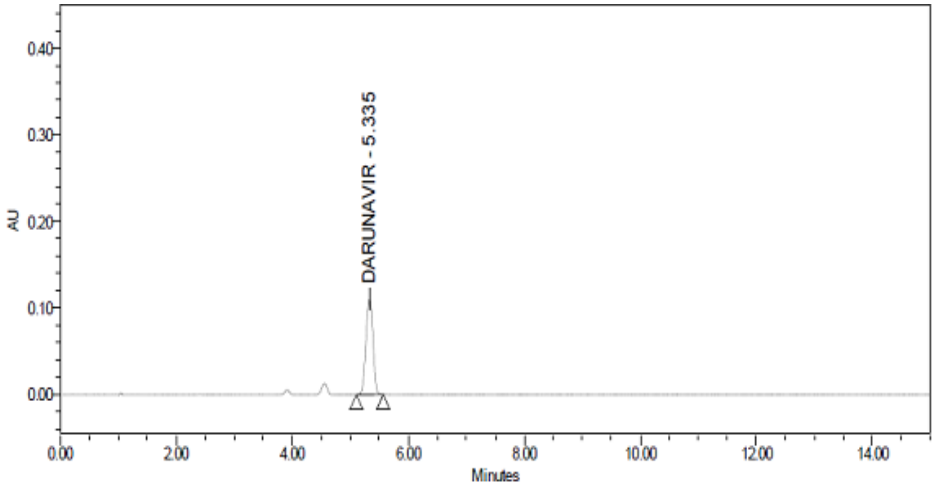
Table no. 29: Results from testing of the robustness of the method for DAR.

Original	Used	Level	Mean area* ± SD	SEM	% RSD
Flow rate (mL/min.)					
1.5	1.4	-1	5312631 ±235.26	135.83	0.004
	1.5	0	5310799 ±119.79	69.16	0.0023
	1.6	+1	5319749 ±209.32	120.85	0.004
Temperature (^{0}C)					
37	30	-5	5313736±261.09	150.74	0.005
	35	0	5310694±294.52	170.04	0.0056
	40	+5	5319750±245.81	141.92	0.0046
Wavelength (nm)					
265	260	- 5	5311829±205.61	118.71	0.0041
	265	0	5310606±362.97	209.56	0.0068
	270	+5	5318643±356.77	205.98	0.0067
pH					

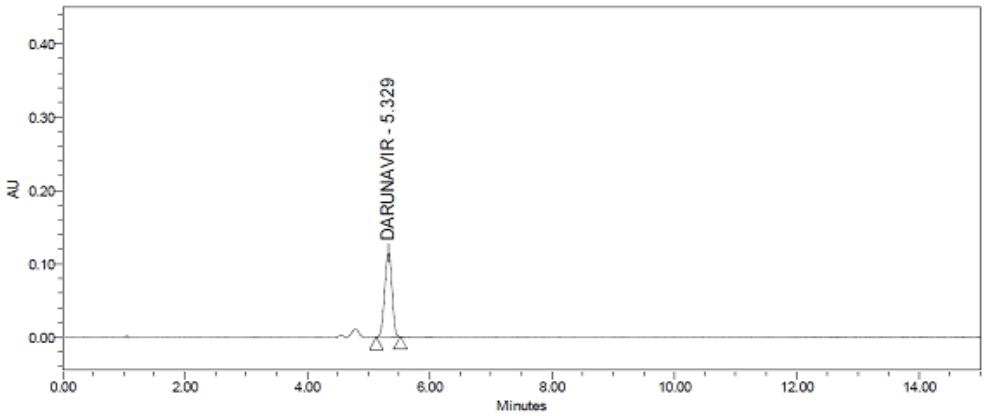
4	3.8	- 0.2	5312806 ±176.17	101.71	0.003
	4	0	5310844 ±153.7	88.74	0.0029
	4.2	+0.2	5319550 ±279.92	161.61	0.0052

* Mean of three determinations

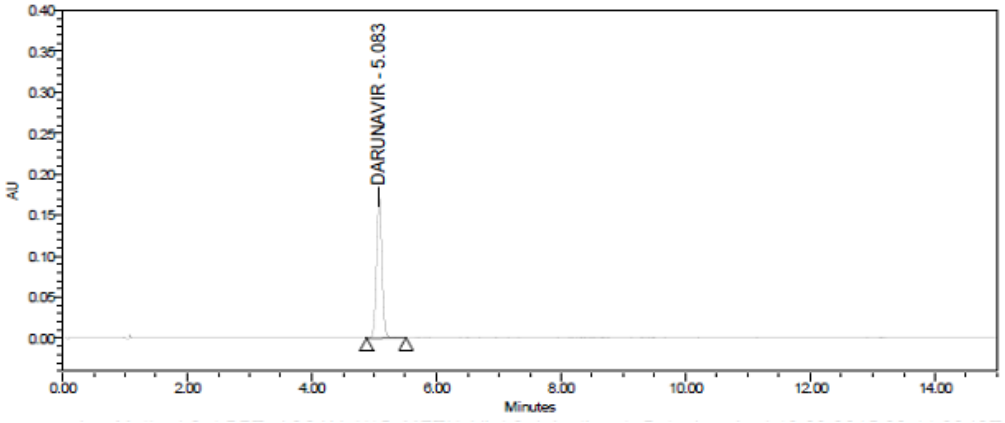
Forced degradation studies



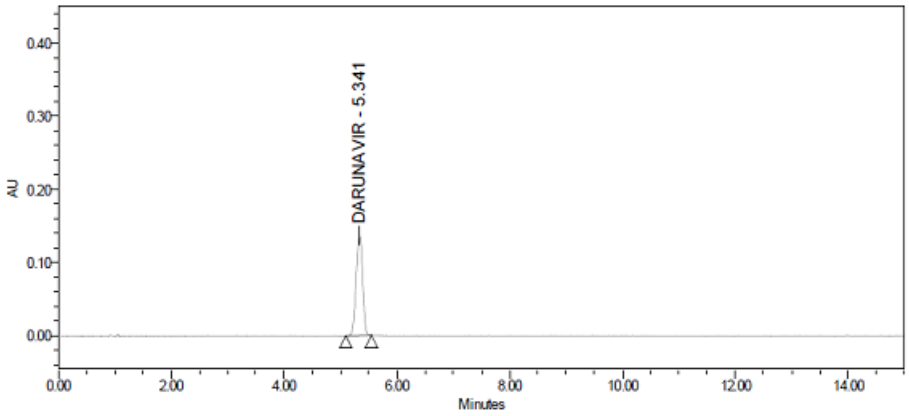
Graph 9: Acid degradation chromatogram of darunavir sample in 1M HCl.



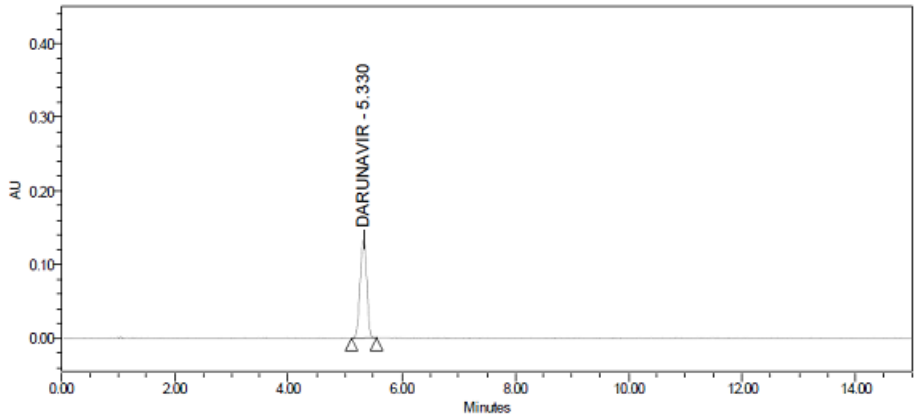
Graph no. 10: Alkali degradation chromatogram of darunavir sample in 1M NaOH.



Graph no 11: Neutral degradation Chromatogram of Darunavir sample in water.



Graph no. 12: Chromatogram of darunavir sample in photolytic degradation.



Graph no. 13: Chromatogram of darunavir sample in oxidative degradation.

Table no. 30: Results from forced degradation of DAR solution.

Condition	RT (min)	Area(mAU)	Ungraded Darunavir %	% degradation
Control	5.083	5312055	100.6	-
Acid degradation (1 N HCL)	5.335	4562242	86.4	14.2
Base degradation (1 N NaOH)	5.329	4667850	88.4	12.2
Peroxide degradation (30% H_2O_2) 70°C 5 hrs.	5.330	4752335	90	10.6
Photolytic study	5.341	4699532	89	11.6

RESULT AND DISCUSSION

The performed study value is shown that the Darunavir drug is performed under the different methods shown the significant values and this method is significant. The methods performed with Darunavir was under different columns which shown in the different trials.

There was five different trails taken to determine the mobile phase with the different flow rates but with the one column it was not accurate to determine the mobile phase during the study it was seen that for the determination of the mobile phase which not only obtained by

changing only one parameter it was the work done on both change in flow rate and change in column. General factors were also determined like accuracy precision, robustness etc. with the help of under the HPLC those values were strongly stated that the performed method under HPLC were confirmed the Darunavir drug was appropriated for the given method and the method is developed.

CONCLUSION

From the above values it is stated that the study performed on Darunavir drug was, we can conclude that the proposed HPLC method can be used successfully for estimation of Darunavir. Further this method may be applied to preparative HPLC for qualification of unknown impurities which might be generated during forced degradation studies. The results obtained by HPLC method for determination of Darunavir are reliable, accurate and precise. The method can be employed for routine quality control analysis of Darunavir in formulation.

AKNOWLEDGMENT

The authors are grateful to the management of CAYMET's Siddhant college of Pharmacy, Pune for providing the necessary support and guidance.

REFERENCES

1. Martin M., Guiochon, G. Effects of high pressures in liquid chromatography.J. Chromatogr. A, 2005; (1-2)7: 16-38.
2. Liu Y., Lee M.L. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography, 2006; 1104(1-2): 198–202.
3. Abidi, S.L. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J. Chromatogr, 1991; 587: 193-203.
4. Watson GD, Pharmaceutical Analysis, Elsevier Churchill Livingstone, 2005; 5: 280-294.
5. Gray DC, Analytical Chemistry, Jhon wiley & sons, INC, 2004; 6: 604-619.
6. Skoog DA, Holler FJ, Crouch SR, Principle of Instrumental Analysis, 6th ed; Eastern press, Banglore, 2007; 1-13, 372-442, 816-825.
7. Riter LS, Vitek O, Gooding KM, Hodge BD, Julian RK, *Journal of High Performance Liquid Chromatography*, 2005; 40: 565.
8. Medhan J, Denney RC, Barnes D, Thomas MJM, Vogel's Textbook of Quantitative Chemical Analysis, Pearson education, 6: 29-39, 103-110, 261-288.
9. Chatwal GR, Anand SK, Instrumental Methods of Chemical Analysis, Himalaya Publishing house, 5(2): 624-2.639.

10. Willard HH, Merrit LL, Dean JA, Settle FA, Instrumental methods of analysis. CBS Publisher and Distributors Pvt. Ltd, 7: 614-628.
11. Kasture AV, Wadodkar SG, Mahadik KR, More HM, Pharmaceutical Analysis, Instrumental methods, Nirali Prakashan, 18: 48-57.
12. Beckett AH, Stenlake JB, Practical pharmaceutical chemistry, CBS Publishers and Distributors, 4(2): 85-157, 255-325.
13. Satinder A, Michael DW, Handbook of Pharmaceutical Analysis by HPLC, Separation science and technology, 6: 1-216.
14. Sethi PD, High Performance Liquid Chromatography: Quantitative analysis of pharmaceutical formulation, CBS Publishers and Distributors, 1: 141-145.
15. www.hiv.gov/hivbasic/aids overview/what-are-hiv-and-aids.
16. http://medchrome.com/basic-science/pharmacology/antiviral-drug-classification-and-anti-herpes-virus-drugs/.
17. https://pubchem.ncbi.nlm.nih.gov/compound/Darunavir.
18. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, 1996; 1-5: 886-892.
19. ICH, Q2B, Validation of Analytical Procedure: Methodology, International Conference on Harmonization, Geneva, March, 1996; 1-8: 645-655.
20. http://medchrome.com/basic-science/pharmacology/antiviral-drugs-classification-and-anti-herpes-virus-drugs/.
21. http://www.aidsmap.com/types-of-antiretroviral-drugs/page/1412436.
22. Tripathi KD, Essential of medical pharmacology, Jaypee Brothers Publishers ltd.; 7: 798-801, 811-816.
23. M. M. Eswarudu*, P. Siva Krishna, P. Srinivasa Babu, N. Punna Reddy, Darunavir: A Review on its Analytical Methods, 2018; 2(12): 120-135.
24. B. Prathap*, V. Haribaskar, B. Kumar, S. Harika, “Stability indicating RP-HPLC method for simultaneous estimation of Ritonavir and darunavir in bulk and its synthetic mixture”, 2018; 9(2): 5549-5560.
25. P. Siva Krishna, G. Sneha Latha “method development and validation of uv spectrophotometric methods for the estimation of darunavir in bulk and its pharmaceutical dosage forms”, 2018; 5(4): 2230-2237.
26. Kamlesh Valuba Bhuse “RP-HPLC method development and validation for determination of darunavir in bilk and dosage form”, 2017; 6(7): 1752-1766.

27. Mathews B, Rao RN, Mukkanti K, Sarbani P, Development and validation of a simple, sensitive, selective and stability-indicating method for the determination of Darunavir and Cobicistat in pharmaceutical combined dosage forms, IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 2017; 12: 69-78.
28. J. Sathish Kumar Reddy KR, Prasad S and Suresh K, A stability indicating RP-HPLC method for simultaneous estimation of Darunavir and Cobicistat in bulk and tablet dosage form, Scholars Research Library Der Pharmacia Lettre, 2016; 8(12): 89-96.
29. Manisha BM, Pranali JG, Anuja VP, Ashish SM, RP-HPLC method for determination of darunavir in bulk and pharmaceutical preparations, Int. J. Pharm. Sci. Rev. Res., 2013; 21(2): 20-23.
30. Sigamala SK, Donthireddy SP and Paul RM, RP-HPLC method development and validation for simultaneous estimation of Cobicistat and Darunavir in tablet dosage form, World Journal of Pharmacy and Pharmaceutical Sciences, 2016; 5(6): 490-499.
31. Sindu PD, Sankar DG and Jaya CD, Stability indicating RP-HPLC method for the simultaneous estimation of Darunavir ethanolate and Cobicistat in bulk and tablet dosage form, Scholars Research Library, 2016; 8(14): 1-11.
32. Hemant K. Jain "Develpoment and validation of rp-hplc method for estimation of darunavir ethanolate in bulk and tablet", 2015; 7(10): 386-389.
33. Rizwan SH, Sastry VG, Shaik GQI, Khatija MB, A new and validated stability indicating RP-HPLC analysis of Darunavir and Cobicistat in bulk drug and tablet dosage form, International Journal of Pharmaceutical Sciences Review and Research, 2016; 36(1): 180-185.
34. Raveendra G, Lakshmana AR and Venkateswara JR, Development and validation of novel HPLC method for estimation of Darunavir in pharmaceutical formulations, International Journal of Research in Pharmacy and Chemistry, 2013; 3(2): 438-445.
35. Josilene CRC, Cristina Helena DRS and Hérida RN, Salgado, Stability study of Darunavir ethanolate tablets applying a new stability-indicating HPLC method, Hindawi Publishing Corporation Chromatography Research International, 2013; 20: 1-7.
36. Reddy BV, Jyothi G, Reddy BS, Raman BK, Reddy SC and Rambabu C, Stability-indicating HPLC method for the determination of Darunavir ethanolate, Journal of Chromatographic Science, 2013; 55: 471–476.
37. https://drugsdetails.com/darunavir/.
38. https://www.drugbank.ca/drugs/db01264.

39. https://pubchem.ncbi.nlm.nih.gov/compound/darunavir/sectionpharmacolophand-biochemistry.
40. http://www.irjponline.com/admin/php/uploads/1723_pdf.pdf.
41. http://www.irjponline.com/admin/php/uploads/1723_pdf.pdf.
42. http://www.mims.com/malaysia/drug/info/darunavir?mtype=generic.
43. http://www.aidsmap.com/drug-interactions/page/1730943/.