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Pharmaceutical Analysis

Simultaneous Method Development, Validation and Stress Studies of Darunavir and Ritonavir in Bulk and Combined Dosage Form Using UV Spectroscopy

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Abstract Original Research Article

A simple, specific, accurate and precise UV Spectrophotometric method has been developed for the simultaneous estimation of Darunavir and Ritonavir in pharmaceutical dosage form. The absorption maxima of the Darunavir and Ritonavir were found to be 267 nm by using methanol and 240nm using methanol: water as solvent and isosbestic point found at 254nm. This method obeys beers law in the employed concentration range of 2-18µg/ml and 5-100µg/ml for Darunavir and Ritonavir respectively. Different analytical validation parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines. The accuracy of the method was confirmed by recovery studies of tablet dosage form and was found to be 100% and 99% for Darunavir and Ritonavir respectively. The LOD of Darunavir and Ritonavir was found to be 0.043µg/ml and 0.024µg/ml respectively and LOQ of Darunavir and Ritonavir was found to be 0.132µg/ml and 00.078µg/ml respectively. The developed method was free from interferences due to excipients present in formulation and it can be used for routine quality control analysis.

Keywords: Absorption maxima, analytical, validation parameters, Darunavir, Ritonavir, ICH guidelines.

INTRODUCTION

Darunavir ethanolate (DRV) is an antiviral drug used in the treatment of human immunodeficiency virus (HIV) protease. It was approved in the year 2006 by the Food and Drug Administration(FDA),it isschemically[(1*S*,2*R*)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl) propyl]- carbamic acid (3*R*,3a*S*,6a*R*) hexahydrofuro [2,3-b] furan-3-yl ester monoethanolate.

DRV is a second-generation protease inhibitor, it will selectively inhibit the cleavage of HIV-1 encoded Gag- Pol polyproteins in the infected cells, and thereby it prevents the formation of mature virus cells [1-6].

DRV is used with Ritonavir and other medications to treat HIV. It works by slowing the spread of HIV in the body. Ritonavir is an antiretroviral drug from the protease inhibitor class it is used to treat HIV infection and AIDS. The chemical it is (5S,8S, 10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but it will inhibit the same host enzyme that metabolizes other protease inhibitors. This leads to increases in plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy.

From the literature survey it was found that the analytical methods have been reported for the determination of the darunavir in spectrophotometric method and individually in human plasma by liquid chromatography/tandem mass spectrometry, and from the literature survey it was found that Ritonavir estimated by analytical methods such as spectrophotometric methods, reversed phase high performance liquid chromatographic (RP-HPLC) method7-13, LC-MS14 and HPTLC15 method. Apart from the above, no other methods are also present such as zero and first order derivative spectrophotometric

method was reported for the quantitative determination of (RIT)in pharmaceutical dosage forms. The present work describes the simple precise, accurate and specific for the simultaneous determination of drugs by UV spectrophotometric method [7-12].

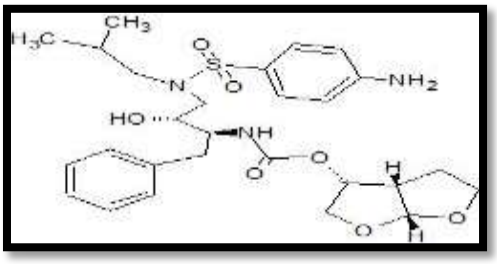

Fig-1: Structure of Darunavir

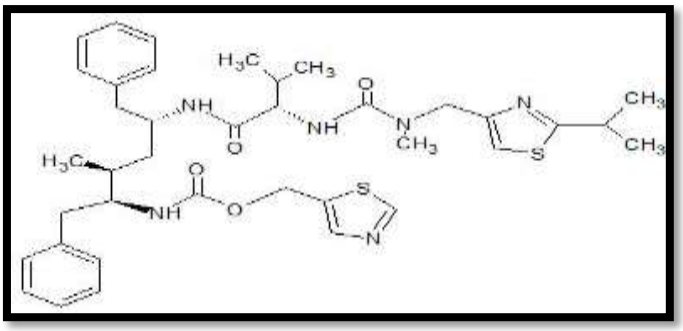

Fig-2: Structure of Ritonavir

Introduction to UV spectroscopy

UV-Visible Spectroscopy is type of absorption Spectroscopy in which light of ultra-violet region (200-400 nm) is absorbed by the molecule.

Principle: UV-Visible Spectroscopy obeys the beer-lamberts law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

This law is expressed as

$A = \log (I_0/I) = \varepsilon cl$

A=Absorbance.

I_0= Intensity of light upon a sample cell.

I = Intensity of light departing the sample cell.

C= Concentration of the solute.

L=Length of the sample cell and

E=Molar absorptivity [13-18].

MATERIALS AND METHODS

Chemical and reagents

Darunavir and Ritonavir were obtained as a gift sample from Pharmaceuticals, Hyderabad, telangana, Methanol and other chemicals were analytical grade from Rankem chemicals limited.

Instrumentation

Spectroscopic analysis was carried out using Elico SL-210UV/Vis-Double beam spectrophotometer with Spectral treaties software. Spectrophotometer with spectral width2nm, wavelength accuracy of 0.5nm and a pair of 10mmmatching quartz cells was used to measure absorbance of the resulting solutions.

Selection of common solvent

After assessing the solubility of drugs in different solvents Methanol has been selected as common solvent for developing spectral characteristics.

Selection of wavelength

Weigh accurately about 10mg of Darunavir in a 10ml volumetric flask and add methanol until the substance completely dissolves and make the volume up to10ml with methanol in 10 ml volumetric flask to obtain the 1000µg/ml of the component and 10mg of Ritonavir in a 10ml volumetric flask and add methanol: water (6:4) and make the volume up to10ml with methanol: water in 10 ml volumetric flask to obtain the 1000µg/ml of the Ritonavir and these both the drugs are further diluted to get 10µg/ml of Darunavir (standard stock solution A) and 10µg/ml of Ritonavir (standard stock solution B) in a separate volumetric flask. These dilutions were scanned from 200-400 nm respectively. Wavelengthsof darunavir 267 nm and 240nm for the Ritonavir respectively and according to the label claim 1; 8 dilutions of DRV and RIT were scanned between 200-400nm isosbestic points found at 254nm.

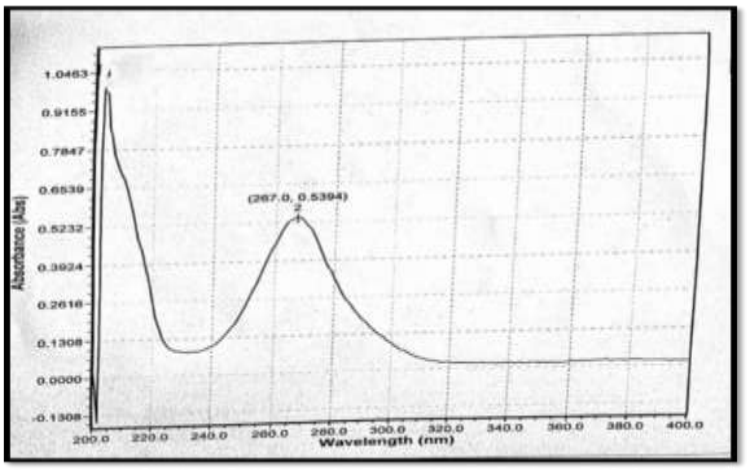

Fig-3: Spectra of Darunavir

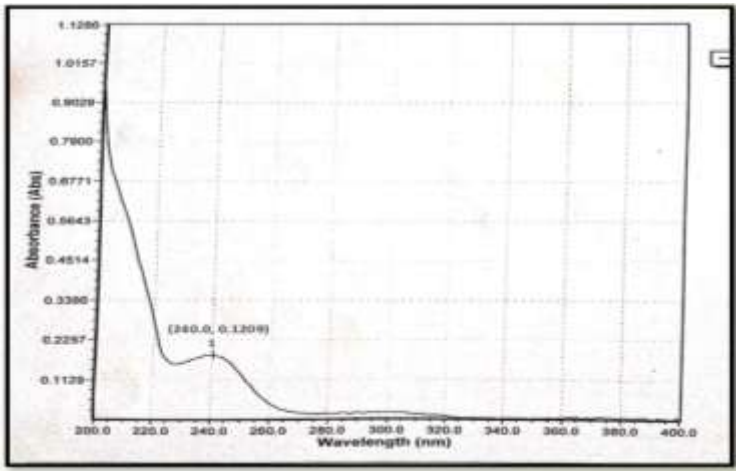

Fig 4: Spectra of Ritonavir

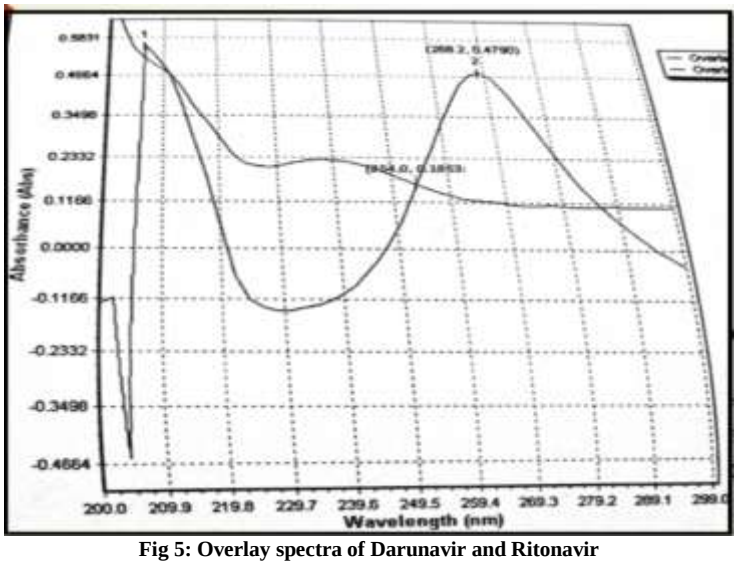

Fig 5: Overlay spectra of Darunavir and Ritonavir

Preparation of standard stock solution

10mg of DRV and RIT were separately weighed in 10 ml volumetric flask and dissolve with methanol for DRV and methanol; water (6; 4) for RIT to get concentration of 1000ppm from this 1 in 10ml was prepared to get the concentration of 100ppm.

Validation parameters

Validation: It is defined as establishing documented evidence which provides a high degree of assurance that a specific process will produce a product meeting its predetermined specifications and quality characteristics.

Linearity

various standard solutions of Darunavir were prepared by pipetting 0.2 to 2 ml of stock solutions (100 µg/ml) of DAR in 10 ml of volumetric flasks and volume made up to the mark by methanol to obtain the concentrations of 2-20 µg/ml.then,thestandard solutions of Ritonavir were prepared by pipetting 0.5-10 ml from(stock solutions 100 µg/ml) of RIT in 10 ml of volumetric flasks and the volumetric flasks and volume was made up to the mark by methanol: water(6:4) to obtain the final concentrations of 5-100 µg/ml.

Precision

Darunavir (10ppm) using methanol and RTV (50ppm) using methanol: water (6:4) as diluents the standard solutions were prepared separately and precision study was performed This procedure is repeated 6 times and absorbance of all were measured at 267nmand 240nm for DAR and RTV, and its %RSD was calculated by using the formula:%RSD = (standard deviation of the measurement / mean value of measurement)*100 repeated for 6 times and its %RSD was calculated.

Accuracy

The Accuracy was determined by spiking the sample matrix of interest with a known concentration of analyte, the recovery studies were carried at three different levels (50, 100 and 150%) on the basis of the label claim. At each level, three determinations were performed simultaneously and percentage recovery was calculated.

Robustness

Small Deliberate change in the method are made such as wavelength **t**he determination of the Robustness was performed at +1 nm and -1nm from the fixed wave length, %RSD was calculated.

Ruggedness

The Ruggedness of both the methods was performed by changing analysts and instruments, %RSD were calculated.

LOD & LOQ

LOD is defined as the lowest concentration of an analyte in sample that can be detected, not quantified. LOD = 3.3 σ/S

σ = standard deviation of the response,
S = slope of calibration curve.

LOQ Is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

LOQ = 10 σ/s

σ = standard deviation of the response,
S = slope of calibration curve.

Analysis of formulation: Simultaneous Equation Method

For the analysis of formulation take the Marketed tablet formulation of DURART-R 450mg (400 mg of Darunavir + 50 mg of Ritonavir).

Weigh the 5 Tablets and powder them, Weigh accurately 10mg equivalent tablet powder in 10ml of volumetric flask dissolve it in methanol filtered and sonicated for 5min which gives the stock solution of (1000ppm), from this take 0.1ml of the solution in to 10 ml volumetric flask and make up the volume up to 10ml with methanol (10ppm), this solution scanned.

Then, the Reported Amount of Darunavir and Ritonavir was calculated using simultaneous equation method given below:

$C_X = A_2ay_1 - A_1ay_2 / ax_2\ ay_1 - ax_1ay_2$
$C_Y = A_1ax_2 - A_2ax1 / ax_2ay_1 - ax_1ay_2$

A_1= Absorbance of formulation at 267 nm.
A_2= Absorbance of formulation at 240nm.
ax_1=Absorptivity of DRV at 267 nm.
ax_2=Absorptivity of DRV at 240 nm.
ay_1=Absorptivity of RIT at 267 nm.
ay_2 =Absorptivity of RIT at 240 nm.
Cx = Concentration of Darunavir.
Cy =Concentration of Ritonavir.

Forced Degradation Studies

Forced degradation studies are also known as stress testing. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule.

Objective for forced Degradation Studies

- To recognize the chemical properties of drug molecules.
- To elucidate the structure of degradation products.
- To resolve stability-related problems.
- To establish the stability of a drug substance in the formulation.
- To reveal the degradation mechanisms of the drug substance and drug product.
- To distinguish degradation products that is related to drug products.

ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, Q1B exemplify the forced degradation studies

ICH Q1A: stability testing of new drug substances and products

ICH Q1B: photo stability testing of new drug substances and products [19-21].

Procedure

Acid Degradation

From 10ppm of drug solution, taken 1 ml of the 10ppm solution into 10ml volumetric flask +1 ml of 0.1 N HCl kept for 24 hours. After 24 hours neutralize with 1 ml of 0.1N NaOH Measured its absorbance at 251nm.

Alkali Degradation

From 10ppm of drug solution, taken 1 ml of 10ppm solution into 10 ml volumetric flask +1 ml of 0.1N NaOH Was kept for 24 hours. After 24 hours neutralize with 1 ml of 0.1 N HCl Measured its absorbance at 251nm.

Photolytic Degradation

10mg of drug was exposed to UV light in UV chamber for 3hrs by placing the drug in Petri dish. After 3hrs Sample was diluted to get concentration of 10 µg/ml and absorbance was measured at 251nm.

Thermal Degradation

Drug was exposed to dry heat 40 C in oven at for 3hrs by placing the drugs in Petri dish. Weighed 10mg of drug and diluted to get a final concentration of 10 µg/ml Measure the absorbance at 251nm and calculate the percentage of Degradation.

RESULTS AND DISCUSSION

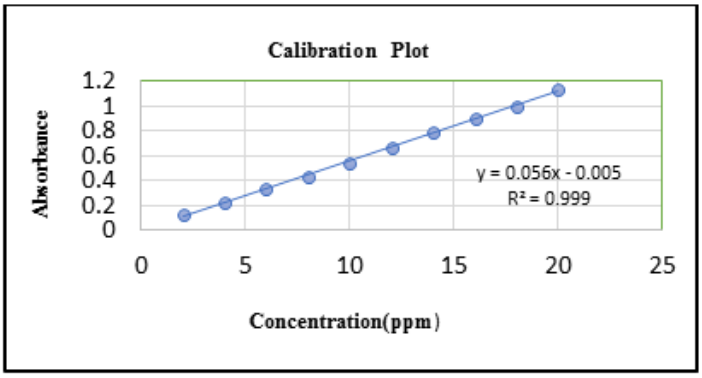

Fig-6: Calibration curve of Darunavir

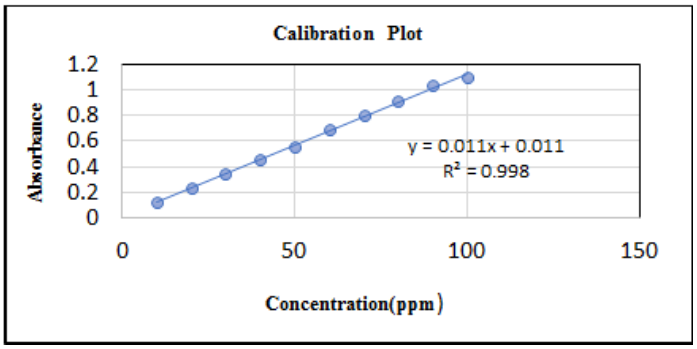

Fig-7: Calibration curve of Ritonavir

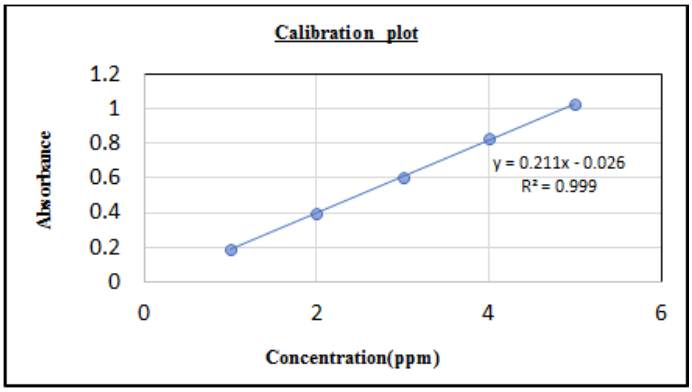

Fig-8: calibration plot of combined drugs

Table-1: precision data

S. No	Darunavir (10ppm) 267nm	Ritonavir (50ppm) 240nm	Combined Drugs 254nm (1:8)
1	0.5147	0.5780	0.6076
2	0.5145	0.5781	0.6075
3	0.5140	0.5782	0.6074
4	0.5135	0.5780	0.6075
5	0.5145	0.5781	0.6074
6	0.5157	0.5782	0.6073
Mean	0.5144	0.5781	0.6074
SD	0.0007386	0.0000894	0.000104
%RSD	0.143%	0.0321%	0.0172%

Table-2: Accuracy data of darunavir

% level	Combined concentration	DRV concentration	%Recovery	Mean% Recovery
50%	1:8	2ppm	99.1% 99.5% 99.6%	99.3%
100%	1:8	4ppm	99.2% 99.1% 99.3%	99.2%
150%	1:8	6ppm	100.1% 100.1% 100%	100%

Table-3: Accuracy data of Ritonavir

% level	Combined concentration	RIT Concentration	%Recovery	%Mean Recovery
50%	1:8	5ppm	99.1% 99.2% 99.3%	99.2%
100%	1:8	10ppm	99.1% 99.5% 99.3%	99.3%
150%	1:8	15ppm	99% 100% 100%	99.6%

Table-4: Robustness data of Darunavir

S. No	266nm	268nm
1	0.5152	0.5189
2	0.5152	0.5188
3	0.5150	0.5188
Mean	0.5151	0.5188
SD	0.000154	0.000057
%RSD	0.0298%	0.0111%

Table-5: Robustness data of Ritonavir

S.NO	239nm	241nm
1	0.5691	0.5792
2	0.5653	0.5796
3	0.5670	0.5802
Mean	0.5671	0.5796
SD	0.00190	0.000503
%RSD	0.711%	0.1800%

Table-6: Robustness data of combined drugs

S. No	253nm	255nm
1	0.5932	0.6154
2	0.5936	0.6156
3	0.5935	0.6154
Mean	0.5934	0.6156
SD	0.000208	0.0002
%RSD	0.0350%	0.0324%

Table-7: Ruggedness data of darunavir

S. No	Absorbance (Abs)	
	Analyst-1	Analyst-2
1	0.5086	0.5069
2	0.5089	0.5073
3	0.5080	0.5078
Mean	0.5085	0.5073
SD	0.000458	0.000450
%RSD	0.09%	0.0887%

Table-8: Ruggedness data of Ritonavir

S. No	Absorbance (Abs)	
	Analyst-1	Analyst-2
1	0.5691	0.5792
2	0.5653	0.5796
3	0.5673	0.5802
Mean	0.5672	0.5796
SD	0.000190	0.0005033
%RSD	0.0334%	0.0868%

Table-9: Ruggedness data of combined drugs

S. No	Absorbance (Abs)	
	Analyst-1	Analyst-2
1	0.6132	0.6025
2	0.6131	0.6025
3	0.6132	0.6024
4	0.6133	0.6023
5	0.6132	0.6024
6	0.6131	0.6024
Mean	0.6131	0.6025
SD	0.0000752	0.0000816
%RSD	0.0122%	0.0135%

Table-10: LOD&LOQ data of Darunavir and Ritonavir

	LOD	LOQ
DRV	0.043µg/ml	0.132µg/ml
RIT	0.024µg/ml	0.078µg/ml

Table-10: Analysis of formulation by simultaneous method

Drugs name	Concentrations
Darunavir C_x	8.8µg/ml
Ritonavir C_y	1.1µg/ml

Table-11: stress studies

Type of Degradation	Darunavir	Ritonavir
Acid Degradation (0.1N HCL)	19.1%	15%
Alkali Degradation (0.1N NaOH)	19%	17%
Photolytic Degradation (UV Chamber)	17%	18.5%
Peroxide Degradation ($3\%H_2O_2$)	19.6%	17.8%
Thermal Degradation (Hot air Oven-40°c)	15%	16.4%

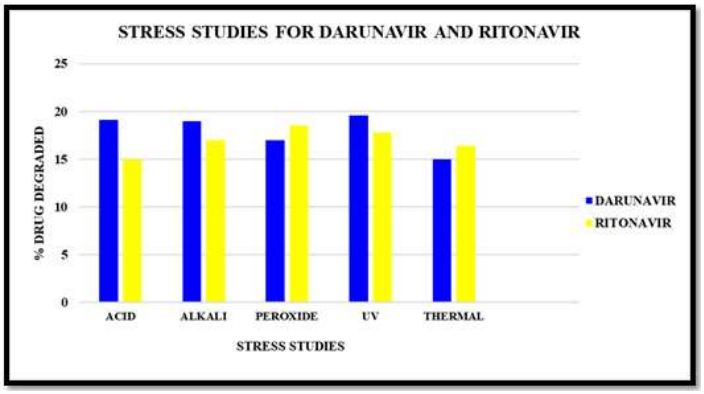

Fig-9: Stress studies of Darunavir and Ritonavir

CONCLUSION

The developed UV spectrophotometric method is simple, precise, accurate, linear, and reproducible for the estimation of Darunavir and Ritonavir in combined dosage form without any interference from the excipients. It can be successfully applied for the routine analysis of both the drugs in pharmaceutical dosage form.

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REFERENCES

1. Mrinalini damle and ajinkya deosthali Uv-spectrophotometric absorbance correction method and absorbance ratio method for the simultaneous estimation of ritonavir and darunavir ethanolate world journal of pharmacy and pharmaceutical sciences. 2016; 5(8): 1179-1187
2. Gholve Sachin B, Asware Baburao S, Kadam Shrihari C, Bhusnure Omprakash G, Thonte Sanjay S. Development and validation of a simple UV spectrophotometric method for the determination of Darunavir ethanolate both in bulk and marketed dosage formulations.
3. Seetaramaiah K, Smith AA, Ramyateja K, Alagumanivasagam G, Manavalan R. Spectrophotometric determination of ritonavir in bulk and pharmaceutical formulation. Scientific Reviews & Chemical Communications. 2012;2:1-6.
4. Redasani VK, Patel PR, Marathe DY, Chaudhari SR, Shirkhedkar AA, Surana SJ. A review on derivative Uv-spectrophotometry analysis of drugs in pharmaceutical formulations and biological samples review. Journal of the Chilean Chemical Society. 2018;63(3):4126-34.
5. Ghante MR, Shelar RS, Sawant SD, Kadam MM. Development and validation of UV spectrophotometric method for estimation of darunavir ethanolate in bulk and tablet dosage form. Int. J. Pharm. Pharm. Sci. 2014;6:240-.
6. Corrêa JC, Serra CH, Salgado HR. Stability study of darunavir ethanolate tablets applying a new stability-indicating HPLC method. Chromatography Research International. 2013;2013.
7. Satyanarayana L, Naidu SV, Rao MN, Kumar A, Suresh K. The Estimation of Darunavir in Tablet dosage form by RP-HPLC. Asian Journal of Research in Pharmaceutical Science. 2011;1(3):74-6.
8. Patel BN, Suhagia BN. Simultaneous determination and validation of Darunavir ethanolate and Ritonavir in binary mixture by liquid chromatography. Int. J Pharm Tech Res. 2012;4(4):1450-6.
9. Karthika A, Rama K, Chenthilnathan A. RP-HPLC method development and validation for quantification of darunavir ethanolate in tablet dosage form. Der Pharmacia Lettre. 2016;8(5):291-6.
10. Curran A, Gutirerrez M, Deig E, Mateo G, Lopez RM, Imaz A, Crespo M, Ocana I, Domingo P, Ribera E. Efficacy, safety and pharmacokinetics of

900/100 mg of darunavir/ritonavir once daily in treatment-experienced patients. Journal of antimicrobial chemotherapy. 2010 Oct 1;65(10):2195-203.

11. Garcia SP, Tunica DG, Serra MB. Development and assessment of a method for the determination of darunavir in plasma by lC-MS/MS. Desarrollo y validación de un método para la determinación de darunavir en plasma mediante lC-MS/MS.) Rev lab Clin. 2011;4:127-33.
12. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. Journal of Chromatography B. 2010 Jun 1;878(19):1455-65.
13. Atole DM, RAJPUT HH. Ultraviolet spectroscopy and its pharmaceutical applications-a brief review. Asian J Pharm Clin Res. 2018;11(2):59-66.
14. Shah RS, Shah RR, Pawar RB, Gayakar PP. International Journal of Institutional Pharmacy and Life Sciences. International Journal of Institutional pharmacy and life sciences.;5:490-505.
15. Choudhary A. The principle of Ultra Violet (UV) Spectrophotometer. Medium. com. 2017.
16. Curran A, Gutirerrez M, Deig E, Mateo G, Lopez RM, Imaz A, Crespo M, Ocana I, Domingo P, Ribera E. Efficacy, safety and pharmacokinetics of

900/100 mg of darunavir/ritonavir once daily in treatment-experienced patients. Journal of antimicrobial chemotherapy. 2010 Oct 1;65(10):2195-203.

17. Garcia SP, Tunica DG, Serra MB. Development and assessment of a method for the determination of darunavir in plasma by lC-MS/MS. Desarrollo y validación de un método para la determinación de darunavir en plasma mediante lC-MS/MS.) Rev lab Clin. 2011;4:127-33.
18. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. Journal of Chromatography B. 2010 Jun 1;878(19):1455-65.

19. Blessy MR, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. Journal of pharmaceutical analysis. 2014 Jun 1;4(3):159-65.
20. Chan CP. Forced degradation studies: current trends and future perspectives for protein-based therapeutics. Expert Review of Proteomics. 2016 Jul 2;13(7):651-8.
21. Iram F, Iram H, Iqbal AZ, Husain A. Forced degradation studies. J Anal Pharm Res. 2016;3(6):00073.