IJBPAS, July, 2022, 11(7): 3080-3089

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF A NEW UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DARUNAVIR ETHANOLATE BOTH IN BULK AND MARKETED DOSAGE FORM

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Received 15th June 2020; Revised 20th July 2020; Accepted 23rd Oct. 2021; Available online 1st July 2022

https://doi.org/10.31032/IJBPAS/2022/11.7.6209

ABSTRACT

Objective: The main aim of this study was to develop a spectrophotometric method for the determination of Darunavir Ethanloate in bulk.

Method: Standard and working solutions of darunavir ethanolate were prepared then aliquots of working solutions of different concentrations were prepared then linearity, precision, accuracy, robustness, ruggedness, quantitation limit and detection limit were determined.

Result: The linearity for this method was found to be within the range of 2-24µg/ml. Correlation coefficient (R^2) was found to be 0.998. Regression equation was found to be y = 0.034x + 0.063.

Conclusion: A simple, specific and sensitive UV spectroscopy method was developed for the evaluation of darunavir ethanolate in bulk. The projected method may be duly applied for the analysis of darunavir ethanolate in bulk for routine analysis.

Keywords: Darunavir Ethanolate; UV Spectrometry; Validation; Assay

INTRODUCTION

Darunavir Ethanolate (DRV) is an inhibitor of the human immunodeficiency virus (HIV-1) protease. It selectively inhibits the cleavage of HIV-1 encoded GagPol polyproteins in infected cells, thereby preventing the formation of mature virus

particles. Development of a spectrophotometric method is based on the knowledge of the chromatographic process. A good method development strategy requires only as many experimental runs as are necessary to achieve the desired final result. The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise the main objective for that is to improve the condition and parameter, which should be followed in the development and validation.

In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method using diluent acetonitrile for the determination of darunavir ethanolate in the raw materials as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization and demonstrated excellent specificity, linearity, precision and accuracy for DRV. The chemical structure of DRV is shown in **Figure 1 [1-14]**.

MATERIALS AND METHODS:

Instruments and methods: A gift sample of DARUNAVIR ETHANOLATE with purity of 100.1was obtained. LAB INDIA (T60) double beam UV/Visible spectrophotometer and ELITE analytical balance were the instruments used. Chemicals and reagents are of analytical grade. DARUNAVIR ETHANOLATE of 300mg with a brand name DANAVIR was purchased from the local market.

Preparation of standard stock solution (1000µg/ml): A standard drug solution of DARUNAVIR was prepared by adding 100mg of the drug into a 100 mL volumetric flask and made up to the mark with Acetonitrile to get a concentration of 1000µg/ml.

Preparation of working standard solution (100µg/ml): From the above standard stock solution 10 ml of the sample was transferred to a 100mL volumetric flask and made up to mark with acetonitrile to get a concentration of 100µg/mL. It was then scanned by a UV Spectrophotometer in the range of 200-400nm using acetonitrile as a blank. The absorbance was found to be maximum at 263nm.

CONSTRUCTION OF CALIBRATION CURVE: Aliquots ranging from 2-24µg/mL solutions were prepared by using acetonitrile as solvent. The samples were then analyzed at a λmax of 263nm to get respective absorbance. The values are then plotted to get a calibration curve.

PREPARATION OF THE ASSAY SOLUTION: The proposed method was applied to analyze the commercially available Darunavir Ethanolate tablets

(300mg).10 tablets are weighed and powdered, the amount of powder is equivalent to 100mg of darunavir was weighed accurately and transferred into a 100 mL volumetric flask containing acetonitrile which was further sonicated for 15min with vigorous shaking and the volume was brought up to 100ml with acetonitrile. The solution was subjected to filtration to Whatman filter paper #44. The filtrate was diluted suitably with acetonitrile to get a final solution of 300µg/ml concentration. This was subsequently analyzed using a Double beam UV-VIS spectrophotometer and taking acetonitrile as blank in the UV range 200-400nm. The spectrum was recorded as 263nm.The concentrations of the drug were calculated from the linear regression equation.

METHOD VALIDATION: Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity will consistently produce the desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, Range and Sensitivity **[15-17]**.

Linearity: The ability of an analytical procedure is to produce test results that are directly proportional to the concentration of an analyte. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. For estimation of linearity at least 5 concentrations are required.

Accuracy: Accuracy means the expression of closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference true value and the value found. Accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations.

Precision: The closeness of agreement between the obtained values by analyzing the same sample multiple times under prescribed conditions. There are 3 levels of repeatability, intermediate precision and reproducibility. Repeatability is a measure of the exactness under the same working conditions more than a short in term of time, that is, under ordinary working states of the scientific technique with the same hardware it is also known as intraday precision. Reproducibility is also known as intra day precision. Precision is expressed in terms of % Relative Standard Deviation.

% RSD = (Standard deviation)/Mean x100

Standard deviation(SD)

$SD = \sqrt{[\sum(x-x)^2/(n-1)]}$

Where n = no of entries

Ruggedness: The ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing the same sample under a variety of conditions like laboratories, instruments, analysis, reagents etc.

Robustness: Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

LOD = (3.3xσ)/S

Where S = standard deviation

Limit of Quantification: It is an amount of analyte that can be quantified with a specified limit of accuracy and precision,

LOQ = (10xσ)/S

Linearity: Different aliquots of darunavir ethanolate were prepared from the working standard solution (100µg/mL) in the range of 2-24µg/mL. The solutions were scanned on a Double beam UV-VIS spectrophotometer in the range of 200-400nm using acetonitrile as the blank. The spectrum was recorded at 263 nm. The calibration plot was constructed as concentration Vs absorbance and can be shown.

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of the same concentration 12µg/mL were prepared and analyzed six times, for three consecutive days and the absorbance was recorded **(Table 4)**. In the intra-day variation study ,six different solutions of the same concentration 12µg/mL were prepared and analyzed thrice a day(Morning , Afternoon and Evening) and the %RSD was calculated and reported **(Table 3)**.

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations i.e.,80,100, and 120%, in which the amount of marketed formulation darunavir ethanolate was kept constant(12µg/ml) and the amount drug was varied, that is 96µg,120µg,144µg for 80,100, and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery was calculated and reported in the **(Table 5)**.

Robustness: The Robustness of the method was carried out by analyzing the sample using three different wavelengths (±1 of lambda max) that were and respective absorbance were recorded. The results are indicated in **(Table 6)**.

Ruggedness: The ruggedness of the method was carried out by analyzing the sample using two different analysts and two different cuvettes and respective absorbance were recorded. The results are indicated in **(Table 7 & 8)**.

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines. They are calculated by checking absorbance using solvent and calculated using formulae and the results are shown in

(Table 9).

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. Beer's law obeyed over the concentration range of 2-24µg/mL, using regression analysis the linear equation y=0.034x+0.063 with a correlation coefficient if R^2 O. 998. The precision results show %RSD less than 2 at each level which indicates clearly that the method is precise enough for the analysis of Darunavir. The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed method. The robustness and ruggedness studies reveal that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicating that the method is specific. Determination of Darunavir in tablet formulation showed the content of Darunavir was very close to the label amount. The percentage RSD values in all the parameters were within the acceptable limit (<2%) all the characteristics of the method are represented in the **(Table 10)**.

Table 1: Linearity of working standard solutions

Concentration(µg/ml)	Absorbance
2	0.1337
4	0.1960
6	0.2651
8	0.3455
10	0.4161
12	0.4840
14	0.5594
16	0.6269
18	0.6705
20	0.7506
22	0.8144
24	0.9105

Table 2: Repeatability data

Concentration(µg/ml)	Absorbance	Statistics analysis
12	0.4630	
12	0.4593	
12	0.4589	Mean:0.4641
12	0.4681	%RSD:1.23
12	0.4653	
12	0.4733	

Table 3: Intra-day Study

Concentration (µg/ml)	%RSD			Average %RSD
	1	2	3	
12	1.37%	1.49%	1.60%	1.48%

Table 4:Inter-day study

Concentration (µg/ml)	%RSD			Average %RSD
	Day 1	Day 2	Day 3	
12	1.75%	0.59%	0.79%	1.04%

Table 5: Accuracy data

Levels of addition (%)	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	%Mean Recovery
80	96	95.5	99.47	99.62%
100	120	119	99.44	
120	144	143	99.95	

Table 6: Robustness data

Concentration (µg/ml)	Absorbance		
	262nm	263nm	264nm
12	0.4546	0.4616	0.4522
12	0.4380	0.4480	0.4614
12	0.4468	0.4549	0.4566
12	0.4586	0.4465	0.4550
12	0.4430	0.4481	0.4594
12	0.4418	0.4494	0.4578

Table 7: Ruggedness data

Concentration (µg/ml)	Absorbance	
	Analyst	Analyst
12	0.4497	0.4558
12	0.4606	0.4562
12	0.4571	0.4541
12	0.4510	0.4605
12	0.4576	0.4606
12	0.4550	0.4551

Table 8: Ruggedness data

Concentration (µg/mL)	Absorbance	
	Cuvette 1	Cuvette 2
12	0.4669	0.4850
12	0.4687	0.4852
12	0.4682	0.4824
12	0.4653	0.4809
12	0.4694	0.4839
12	0.4714	0.4962

Table 9: LOD & LOQ

Limit of Detection	Limit of Qualification
0.0693µg/ml	0.21µg/ml

Table 10: Results of validation parameters

Parameters	Results
Absorption maxima (nm)	263
Linearity range (µg/ml)	2-24
Regression equation	Y=0.034x+0.063
Correlation coefficient ($_R^2$)	0.998
Molar extinction coefficient LOD(µg/ml) LOQ(µg/ml)	25431.91 0.0693 0.21
Accuracy (%Recovery± SD)	99.62%
Precision Intraday Precision (%RSD) Interday Precision (%RSD)	1.48% 1.04%
Sand ell's sensitivity (µg/cm^2 /0.001 absorbance units)	0.0294

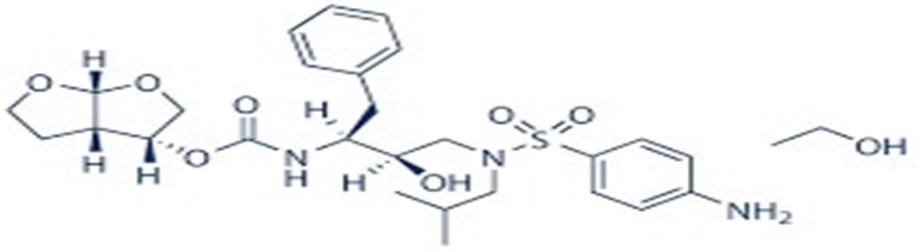

Figure 1: Structure of Darunavir Ethanolate

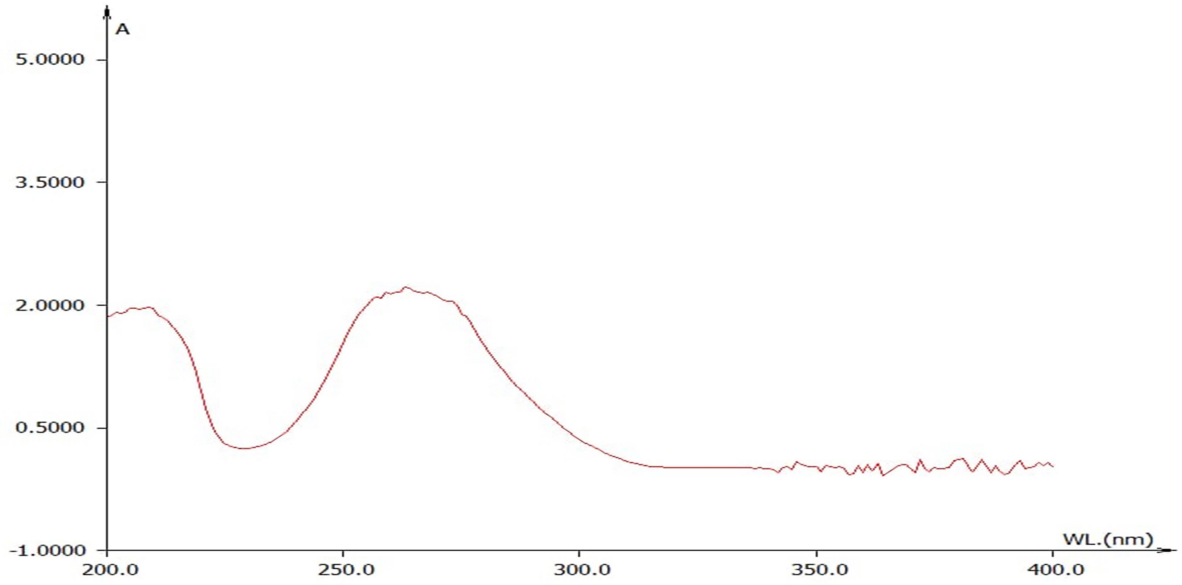

Figure 2: Absorbance of Darunavir Enthanolate at 263

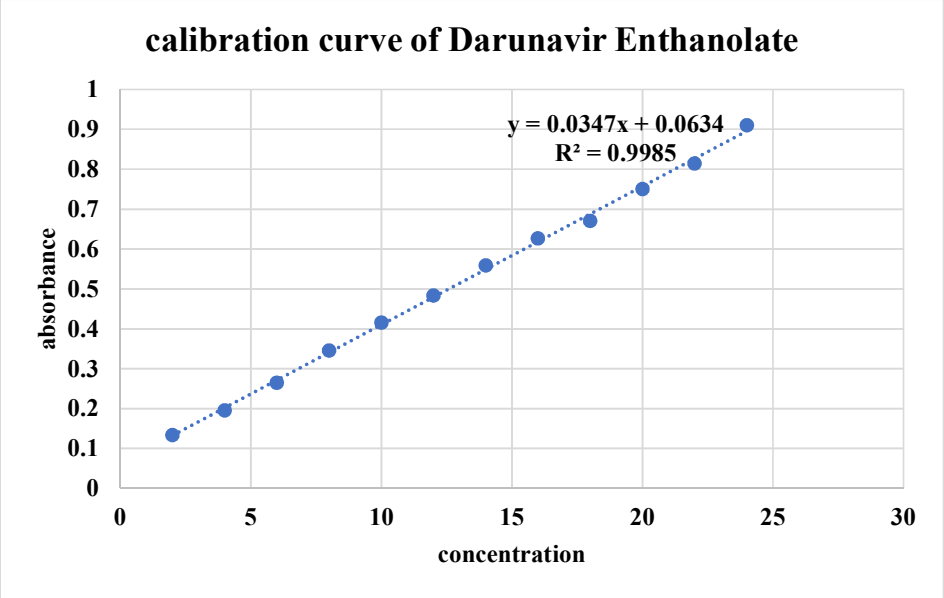

Figure 3: Calibration curve of Darunavir Enthanolate

CONCLUSION

A UV spectrophotometric method has been validated for the estimation of Darunavir in bulk as well as the pharmaceutical dosage form. The developed method was found to be simple, accurate, precise, specific, reproducible and linear over the concentration range studied. The proposed method can be used for the routine analysis of Darunavir in bulk as well as pharmaceutical formulations.

ACKNOWLEDGEMENT

The authors are thankful to the principal and the management, Vignan Institute of Pharmaceutical Technology, Visakhapatnam for providing necessary facilities to carry out my research work.

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