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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF DARUNAVIR IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Darunavir in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point which is done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Darunavir in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, very slightly soluble in water and Insoluble in Ethanol. So, the analytical grade methanol is used as a diluent in method. The melting point of Darunavir was found to be 98-99˚C (uncorrected). It showed absorption maxima 266 nm in analytical grade methanol. On the basis of absorption spectrum, the working concentration was set on 50µg/ml (PPM). The linearity was observed between 10-90 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.81% and for Ruggedness is 0.30%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Darunavir in tablet dosage form. The method could be considered for the determination of Darunavir in quality control laboratories.

KEYWORDS: Darunavir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Darunavir ethanolate (DRV) [Fig.1] is an antiviral drug and inhibitor of the human immunodeficiency virus (HIV) protease in adults and children 6 years of age and older. It was approved by the Food and Drug Administration on June 23, 2006.[1] Chemically it 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. DRV, a second-generation protease inhibitor, is discovered to overcome the problems with early protease inhibitor (PIs) like severe side effects and drug toxicities, require a high therapeutic dose, are costly to manufacture, and show a disturbing susceptibility to drug resistant mutations.[2] DRV is used with ritonavir and other medications to treat HIV. It works by slowing the spread of HIV in the body. It was initially approved by the FDA in 2006.[3] Darunavir is being studied as a possible treatment for SARS-CoV-2, the coronavirus responsible for COVID-19, due to in vitro evidence supporting its ability to combat this infection. Clinical trials are underway and are expected to conclude in August 2020.[4]

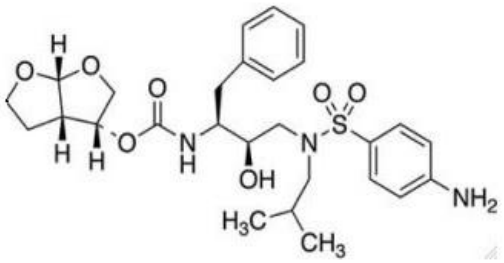

Fig. 1: Chemical structure of darunavir.

Literature survey revealed that a few Spectrophotometric,[5-7] HPLC,[8-12] LC-MS,[13-14] HPTLC,[15] and electrophoresis[16] methods were reported earlier for the determination of Darunavir in bulk and

pharmaceutical dosage forms. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity, and low cost. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Darunavir alone in tablet dosage form.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair.

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.
Melting point apparatus of Equiptronics were used.

- **Materials**

Darunavir was obtained as a gift sample. Darunavir tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (15 PPM)[17,18,19]

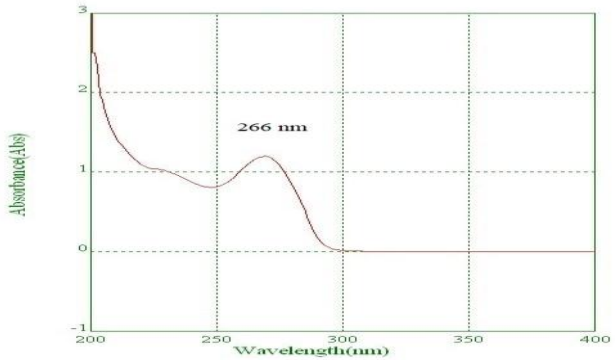

Fig. 2: Calibration curve.

50 mg weighed amount of Darunavir was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 10 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of working concentration

Preparation of standard stock solution

Standard stock was prepared by dissolving 50 mg of Darunavir in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

Preparation of standard solution

Pipette out 1 ml from standard stock solution and diluted up to 10 ml with analytical grade methanol to get concentration of 50 µg/ml (PPM).

C. Procedure for UV reading

Blank solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[17,18,19]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Darunavir was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1 ml of the filtered solution and diluted up to 10 ml with analytical grade methanol. The absorbance was measured at 266 nm. The absorbance was recorded.

Table 1: Absorbance of dosage form.

Cipla Pharma Pvt. Ltd. (Darunavir 300 mg Tablets)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.5234
3	Sample	0.5141

Table 2: Dosage form specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Daruvir ® - 300 Cipla Pharma Pvt LTD (300mg)	12/2022	8/2025	MNB/01/104	0.4854	98.22

E. Method of validation[20,21,22,23]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 90 PPM at 266 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity studies.

Sr. no.	Sample Concentration	Absorbance
1	10 PPM	0.1163
2	30 PPM	0.3208
3	50 PPM	0.5314
4	70 PPM	0.7640
5	90 PPM	0.9565
Correlation coefficient		0.9993

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade methanol and measuring the absorbance at 266nm.

Table 4: Accuracy studies.

Spectrophotometric method			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	0.98	98.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5321
2	Sample Solution 2	0.5305
3	Sample Solution 3	0.5216
4	Sample Solution 4	0.5254
5	Sample Solution 5	0.5251
MEAN		0.5269
SD		0.0043
% RSD		0.8139

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5219	0.5187	99.09	0.3006
		0.5154			
2	Analyst 2	0.5145	0.5165	98.67	
		0.5184			

RESULTS

1. Solubility of darunavir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	DMSO, Methanol	Freely Soluble
2	Water	Very Slightly soluble
3	Ethanol	Insoluble

2. Melting point of darunavir

The melting point of Darunavir was found to be 98-99˚C (uncorrected).

3. Results for linearity for assay method of darunavir

The linearity of method was determined at concentration level ranging from 10 to 90 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9993**

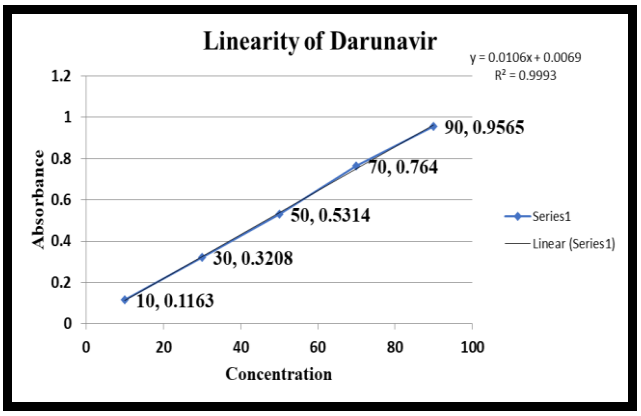

Fig. 3: Darunavir standard curve.

4. Results for accuracy for assay method of darunavir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of darunavir

The % RSD for different sample of precision was found to be 0.8139 ~ 0.81 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of darunavir

The %RSD for different sample of ruggedness was found to be 0.3006 ~ 0.30 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Darunavir in tablet form has been developed. From the spectrum of Darunavir, it was found that the maximum absorbance was 266 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 10-90 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Darunavir in solid dosage forms. Hence, the method

could be considered for the determination of Darunavir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. MS - Mass Spectroscopy
6. LC - Liquid Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml - Microgram per milliliter
15. Avg - Average
16. Wt - Weight
17. g - gm
18. DRV - Darunavir
19. DMF - Dimethylformamide
20. HIV - Human Immunodeficiency Virus

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