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METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF DARUNAVIR IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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Abstract:

Two simple, precise and economical UV Spectrophotometric methods have been developed for the estimation of Darunavir ethanolate (DRV) in bulk and its pharmaceutical dosage form. Two methods were developed based on measurement of absorption at maximum wavelengths, for Method I 272.1 nm and Method II 272.4 nm. Linearity for detector response was observed in the concentration range of 2-10 µg/ml for the both methods. The developed methods were validated with respect to linearity, accuracy (recovery), precision and specificity. The accuracy of the methods was assessed by recovery studies and was found to be 98.3% and 99% for Method I and Method II respectively. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory. The proposed methods were successfully applied for the determination of DRV in pharmaceutical dosage form.

Key words: *Darunavir, UV Spectrophotometry, Absorbance maxima methods and Validation.*

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INTRODUCTION:

Darunavir (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 [1]. It was approved by the Food and Drug Administration (FDA) on June 23, 2006. Chemically it is [(3aS,4R,6aR)-2,3,3a,4,5,6a-hexahydrofuro[2,3 b]furan-4-yl]N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2-yl]carbamate. 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. DRV is used with ritonavir and other medications to treat HIV. It works by slowing the spread of HIV in the body.DRV selectively inhibits the cleavage of HIV-1 encoded Gag- Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles[2]. DRV was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from treatment-experienced patients with multiple resistance mutations to PIs. It blocks HIV protease, an enzyme which is needed for HIV to multiply. HIV infection destroys CD4 (T) cells, which are important to the immune system. The immune system helps fight infection. Reducing the amount of HIV and increasing the CD4 (T) cell count may improve your immune system and, thus, reduce the risk of death or infections that can happen when your immune system is weak (opportunistic infections). Darunavir is co-administered with ritonavir and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV-1) infection [3].

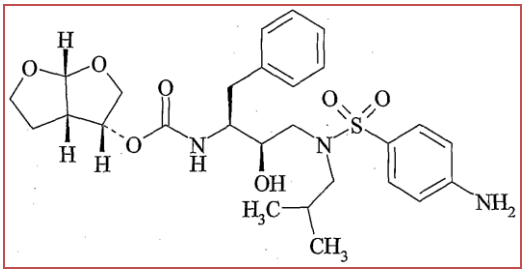

Figure 1: Chemical structure of Darunavir

Literature survey revealed that a few Spectrophotometric [4-11], HPLC [12-28], LC-MS [29-32], HPTLC [33] and electrophoresis [34] methods were reported earlier for the determination of Darunavir in bulk and pharmaceutical dosage forms. The objective of the present study is to develop two simple, accurate, precise, economic methods for the estimation of Darunavir in bulk and pharmaceutical dosage form. Validate the developed methods according to ICH guidelines [35].

MATERIALS AND METHODS:

Chemicals: Darunavir was generous gift samples from MARS Therapeutics and Chemicals Ltd. Hyderabad, India. Commercial tablets with brand name, Prezista (Janssen Pharmaceutical Limited) containing 300 mg was purchased from local Pharmacy store and used within their shelf-life period. All chemicals and solvents are of analytical grade reagents were used in this study.

Equipments: Double beam UV-Visible spectrophotometer (SYSTRONICS- 2203) with 1cm matched quartz cells was used for the measurement of absorbance. Electronic balance (Essay- AJ-220 L) 1mg Sensitivity was used for weighing the samples. Ultrasonicator (Citizen) and Class 'A' volumetric glassware's were used for this work

Selection of Solvent for Analysis: The drug was insoluble in chloroform but soluble in Methanol and 0.1N sodium hydroxide& distilled water. The stability of Darunavir was found to be 24 hours. In methanol the stability of Darunavir is less. At the end of these studies, Distilled water and 0.1N Sodium hydroxide were chosen as solvents for this study, because of the time gain while preparing solutions and cost saving by eliminating the purchase and disposal of organic solvents.

Preparation of standard stock solution:

Method I: 10 mg of Darunavir was weighed and transferred to 100 ml of clean and dry volumetric flask and dissolved in distilled water and the solution was made up to volume100 ml with distilled water to get 100 µg/ml of Darunavir. Method II: 10 mg of Darunavir was weighed and transferred to 100 ml of clean and dry volumetric flask and dissolved in 0.1N NaoH and the solution was made up to volume100 ml with 0.1N sodium hydroxide solution to get 100 µg/ml of Darunavir.

Determination of Absorption Maxima (λmax): Standard solutions of Darunavir 10 µg /ml were prepared in two 10 ml volumetric flasks and each one diluted by Distilled water and 0.1N Sodium hydroxide. The above solutions were scanned over the range of 200 nm to 400 nm against reagent blank. The Maximum Absorbance of Darunavir at 272.1nm and 272.4 nm for method I and method II. UV Spectra of Method I and Method II were shown in Figure 3 & Figure 4.

Calibration curve: Aliquots (0.2, 0.4, 0.6, 0.8, 1.0 ml) of prepared standard stock solution were transferred into series of 10 mL volumetric flasks and diluted by distilled water and 0.1N Sodium hydroxide to give the concentration range of 2-10 µg/ml. The above solutions were taken the absorbance values at 272.1nm and 272.4 nm. Calibration curves were prepared by plotting absorbance vs. concentration.

Estimation of Darunavir in Tablets: For the analysis of tablet dosage form, twenty tablets of Prezista (300 mg) were ground to fine powdered and mixed thoroughly. A quantity of tablet powder equivalent to 10 mg of the drug was transferred in to Two 100 ml clean and dry volumetric flasks and dissolved in about 40 ml of distilled water and 40 ml of 0.1N Sodium hydroxide and sonicated to dissolve completely and volume was made with the same solvents. The above sample solution was filtered and suitably diluted to get concentrations of10 µg/ml. Absorbance was recorded against the blank at 272.1 nm and 272.4 nm.

Method Validation:
The proposed method was validated according to the ICH guidelines which include linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. Under the validation study, the following parameters were studied.

Linearity: Linearity is the ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by method of least squares. Calibration curves were constructed by plotting absorbance vs. concentration of Darunavir for method I and Method II and the regression equations were calculated. The calibration curves of Darunavir for Method I and Method II were plotted over 6 different concentrations.

Accuracy: Accuracy of analytical method is 'measure of how close the experimental value to the true value' accuracy of the method was determined by standard addition method. A known amount of standard drug is added to the fixed amount of pre-analysed injection solution. Percent recovery is calculated by comparing the area before and after addition of the standard drug. The standard addition method is performed at 50%, 100% and 150% level. The solutions are analysed in triplicate at each level as per the proposed method.

Precision: Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate solutions of a known concentration of Darunavir 6 µg/ml for method I and Method II have been analyzed by taking the absorbances of them by UV Spectrophotometer on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The absorbance's ratios of all readings were taken and standard deviation, % relative standard deviation (RSD), was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection and limit of quantification of Darunavir were determined by calibration curve method. Solutions of method I and method II were prepared in linearity range and taken the absorbance's values in triplicate. Average absorbance's values of three analyses were plotted against concentration.

LOD and LOQ were calculated by using the following equations:

$$LOD = 3.3 \times N / B$$
$$LOQ = 10 \times N / B$$

Where
N is residual variance due to regression; B is the slope.

Robustness: The standard and samples of Darunavir were taken absorbance's values by changing the conditions like wavelength, pH of the solvent and composition of the solvent. There was no significant change in the parameters like detection wavelength of drug substance, absorbance values etc.

RESULTS AND DISCUSSION:

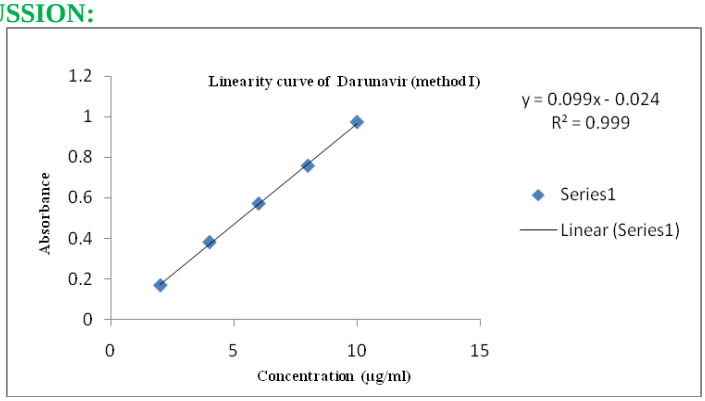

Fig. 2: Linearity curve of Darunavir in Method-I

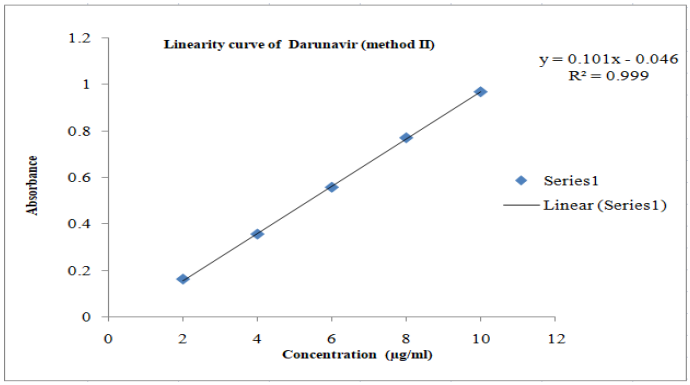

Fig. 3: Linearity curve of Darunavir in Method-II

Table 1: Linearity results of Darunavir in proposed methods

S.No.	Concentration (µg/ml)	Absorbance	
		Method I	Method II
1	2	0.169	0.161
2	4	0.381	0.355
3	6	0.571	0.557
4	8	0.757	0.77
5	10	0.972	0.968

Table 2: Recovery studies results of proposed methods

S.No.	Spike Level	µg/ml Added	Method I			Method II		
			µg/ml Found	% recovery	Mean% recovery	µg/ml Found	% recovery	Mean% recovery
1	20	2	1.974	98.71	98.49	1.975	98.75	98.96
2	20	2	1.961	98.07		1.987	99.38	
3	20	2	1.974	98.71		1.975	98.95	
1	60	6	5.955	99.25	99.44	5.978	99.64	99.40
2	60	6	5.977	99.63		5.946	99.11	
3	60	6	5.966	99.45		5.967	99.46	
1	100	10	9.969	99.69	100.05	9.979	99.79	99.79
2	100	10	9.979	99.79		9.969	99.69	
3	100	10	10.04	100.4		9.986	99.89	

Table 3: Intra-day precision (Repeatability) data of proposed methods

S.No.	Concentration (µg/ml)	Method I Absorbance at 272.1 nm		Method II Absorbance at 272.4 nm	
		Morning	Evening	Morning	Evening
1	6	0.571	0.572	0.557	0.559
2	6	0.572	0.571	0.557	0.558
3	6	0.569	0.569	0.559	0.557
4	6	0.570	0.569	0.558	0.558
5	6	0.570	0.570	0.556	0.559
6	6	0.571	0.572	0.558	0.556
7	Avg	0.978	0.978	0.557	0.557
8	SD	0.0010	0.0013	0.0010	0.0011
9	%RSD	0.107	0.133	0.188	0.209

Table 4: Inter-day precision (Reproducibility) data of proposed methods

S.No.	Concentration (µg/ml)	Method I Absorbance at 272.1 nm			Method II Absorbance at 272.4 nm		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	6	0.571	0.574	0.569	0.557	0.560	0.565
2	6	0.569	0.572	0.568	0.554	0.561	0.568
3	6	0.568	0.571	0.569	0.556	0.562	0.569
4	6	0.570	0.573	0.572	0.555	0.559	0.567
5	6	0.571	0.574	0.571	0.557	0.560	0.569
6	6	0.569	0.571	0.567	0.554	0.562	0.564
7	Avg	0.569	0.9814	0.569	0.555	0.506	0.567
8	SD	0.001	0.0013	0.0018	0.0013	0.0012	0.002
9	%RSD	0.2108	0.139	0.316	0.234	0.231	0.352

Table 5: Robustness studies results of proposed methods

S.No.	Concentration (µg/ml)	Absorbance					
		Method I			Method II		
		270.1nm	272.1nm	274.1nm	270.4nm	272.4nm	274.4nm
1	8	0.755	0.756	0.755	0.77	0.77	0.767
2	8	0.756	0.757	0.753	0.776	0.772	0.766
3	8	0.754	0.754	0.754	0.777	0.772	0.77
4	8	0.753	0.757	0.754	0.778	0.771	0.768
5	8	0.754	0.751	0.755	0.776	0.77	0.769
6	8	0.756	0.755	0.756	0.776	0.771	0.77
7	Avg	0.754	0.755	0.754	0.754	0.771	0.768
8	SD	0.0007	0.0007	0.0007	0.0007	0.0010	0.0016
9	%RSD	0.0929	0.093	0.093	0.093	0.134	0.212

Table-6: Optical characteristics and Validation summary

S.No.	Optical characteristics	Observed values	
		Method I	Method II
1	Beer's law limit	2-10 µg/ml	2-10 µg/ml
2	Correlation coefficient (r2)	0.999	0.999
3	Regression equation	y = 0.0951x+0.001	Y=0.0957x+0.001
4	Slope (m)	0.099	0.101
5	Intercept (c)	0.024	0.046
6	Intra Day Precision	0.1072	0.1881
7	Inter Day precision	0.455	0.272
8	Mean % recovery	98.49-100.06	98.96-99.79
9	LOD	0.8 µg/ml	1.50 µg/ml
10	LOQ	2.42 µg/ml	4.55 µg/ml

Table 7: Assay results of Darunavir in Tablets

Formulation	Label Claim	Method 1	% Assay	Method II	% Assay
PREZISTA	300 mg/ tablet	297 mg/ tablet	99	295 mg/ tablet	98.3

Optimized methods were linear in the range of 2-10 µg/ml for Darunavir with correlation coefficient 0.999 for methods. Linear regression data for method I and method II were given in Table 1, the linearity curves of darunavir for method I and method II were shown in Fig.2 and Fig.3. The mean % recoveries of developed methods for Darunavir were found to be 98.49 % to 100.15 % and 98.96 % to 99.97 % for method I and method II respectively. From the recovery studies it was clear that the methods were accurate for quantitative estimation of darunavir in tablet dosage form, as the statistical results were within the acceptance range. The accuracy results were shown in Table 2. In intraday study, concentration of replicates of drug was calculated on the same day for two times. In inter-day study the concentration of drug were calculated on three successive days which expresses the laboratory variation in different days. In both intra and inter day precision study for the proposed methods % RSD was calculated and results were shown in Table 3 and Table 4. The limit of detection and limit of quantification of Darunavir by proposed methods were determined using calibration curves. The LOD and LOQ of Darunavir were found to be 0.8 µg/ml and 2.42. µg /ml and 1.5 µg/ml and 4.55 µg/ml for method I and method II respectively, which indicate the sensitivity of the methods. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough was shown in Table 5.

Optical characteristics such as Beer's law limit (µg/ml)**,** correlation coefficient, Regression equation, Slope (m), and Intercept (c) were calculated and shown in Table-6. Validated methods were applied for the determination of Darunavir in commercial formulations. The % assay was found to be 99 % and 98.3 % for method I and method II respectively. And assay results were shown in Table 7. These data show that the proposed methods are simple, economic, precise and sensitive for the determination of Darunavir in bulk and its pharmaceutical dosage forms.

CONCLUSION:

The proposed methods were simple, sensitive, and cost-effective. It was validated in terms of linearity accuracy, precision LOD and LOQ. The results are reproducible, and can be used successfully for the estimation of Darunavir in bulk and its pharmaceutical formulations.

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