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Reserch Article

VALIDATED UV-SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF DARUNAVIR BY ABSORPTION MAXIMA, FIRST ORDER DERIVATIVE AND AREA UNDER CURVE IN BULK AND ITS TABLET DOSAGE FORM

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ABSTRACT

Objective: Present study describes the Spectrophotometric method development and subsequent validation of Darunavir in its bulk and formulation with greater precision and accuracy.

Methods: Three simple methods were selected. Method [A] is absorption maximum. Method [B] which is First order derivative method and method [C] is Area under curve method. Spectrophotometric measurements were carried out using Schimadzu Double Beam (UV-1800 model) UltraViolet-Visible spectrophotometer with 10mm matched quartz cells and 70% methanol as solvent.

Results: Linearity for all three methods was found in the range of 2-24µg/ml (r^2 = 0.999). Tablet formulation was analyzed and the % assay for absorption max, first order derivative and area under curve methods were found to be 100.72%, 99.09% and 99.06% respectively.

Conclusion: Proposed methods were validated as per ICH guidelines. Validation studies demonstrated that proposed method is simple, precise, accurate, specific, rapid, reliable and reproducible.

Keywords: (DRN) Darunavir, First order derivative, (AUC) Area under curve, Spectrophotometry, Dosage form.

INTRODUCTION

Darunavir is chemically (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)(4-aminobenzene) sulfonamido]-1-phenylbutan-2-yl]carbamate. It is white to off-white powder that is very slightly soluble in water and soluble in methanol [1, 2]. DRN is generally co-administered along with Ritonavir (100mg) [3]. Darunavir is an inhibitor of Dimerisation and the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in the virus infected cells, thereby preventing the formation of infectious virus particles [4].

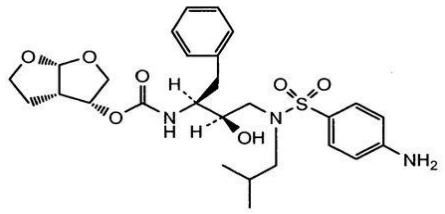


Fig. 1: Structure of Darunavir

Literature survey reveals that there are reports describing the determination of Darunavir in Plasma using liquid chromatography coupled with Tandem Mass Spectroscopy[5], HPTLC method for determination of drug in rat plasma [6], few HPLC methods [7,8] and infrared spectroscopy method for determination of Darunavir in tablets [9].The focus of present study is to develop and validate a rapid, stable, accurate, precise and economic Ultra Violet Spectrophotometric method for estimation of DRN in tablet dosage form.

MATERIALS AND METHODS

Apparatus

Spectrophotometric measurements were carried out using Schimadzu Double Beam (UV-1800 model) UltraViolet-Visible spectrophotometer with 10mm matched quartz cells. Analytical balance Keroy and pH meter Systronics 802 were used.

Chemicals and Materials

Working standard Darunavir (99.75%) was a gift sample obtained from Hetero Pharma Ltd. Hyderabad, India. Commercial tablet dosage form DRN tablets (Prezista 300mg) were purchased from local pharmacy. Methanol used was of AR grade and Double distilled water was used for entire study.

Preparation of standard solution

Working standard of DRN 10mg was accurately weighed and transferred into 10ml volumetric flask, containing 5ml of methanol and it was ultrasonicated for 10 min and diluted up to the mark with further quantity of methanol to get a concentration of 1000 µg/ml. From this 5ml was transferred into 50ml volumetric flask and made up the volume with 70% methanol (concentration 100µg/ml). From the above solution a series of aliquots 2-24µg/ml were prepared in 10ml volumetric flask using 70%methanol as diluents and readings were taken for these dilutions and the calibration curve was constructed by plotting absorbance of analyte versus their respective drug concentrations.

Preparation of Sample solution

An accurately weighed portion of powder equivalent to 10mg of Twenty tablets of PREZISTA (containing 300mg of DRN) were weighed and was transferred to 10ml standard volumetric flask containing 5ml of methanol. The solution was sonicated for 10min, and the final volume was made with remaining quantity of methanol to obtain solution of DRN (1000µg/ml). The mixture was then filtered through Whatman 41 filter paper. The above solution was suitably diluted with 70% methanol to obtain final concentration of DRN (10µg/ml).

Method [A] Absorption maxima[10].

Aliquots prepared from working standard in increasing order were scanned in the wavelength of 200-400. The λ_{max} was found at 262nm. The calibration curve was constructed and the regression equation was calculated and regression coefficient (r^2) was found 0.999. This equation was used for the estimation of Darunavir

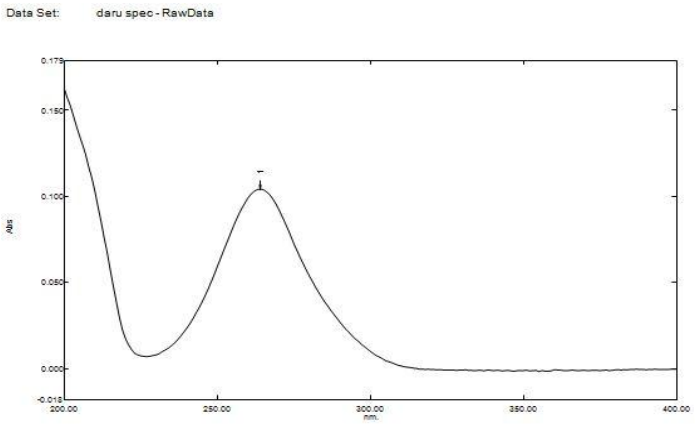
Method [B] first order derivative Spectroscopy

In this method, a standard concentration 10µg/ml was prepared and scanned and the spectra obtained was derivatized from zero order

to second order, where the first order derivative spectra were found to be suitable for analysis of the drug. Spectra showed λ_{max} at 248nm which was selected for its quantification purpose. The calibration curve was also found to be linear in the concentration range of 2-24µg/ml; graph was constructed by plotting concentration of analyte versus their respective dx/dy values [10]. Regression equation was calculated. This equation was used for estimation of Darunavir in tablet dosage form.

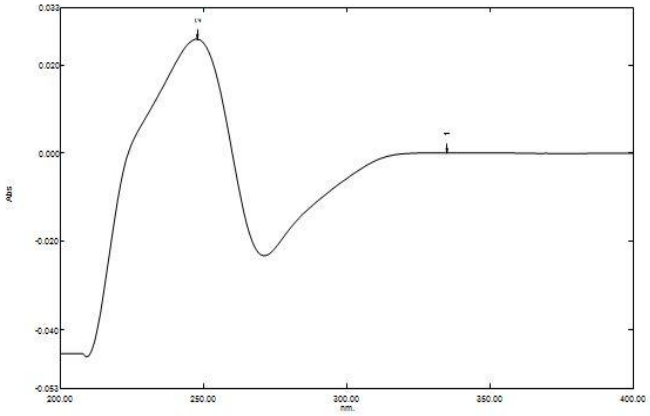
Method [c] Area under curve method

Spectra obtained after scanning standard solutions of Darunavir were subjected to area under curve method by selecting wavelength range from 257-267nm around the absorption maximum wavelength. The calibration curve was constructed by plotting the drug concentration versus their respective α+ β value [10]. Where, the linearity was found and regression equation was calculated and this equation utilized for estimation of Darunavir.



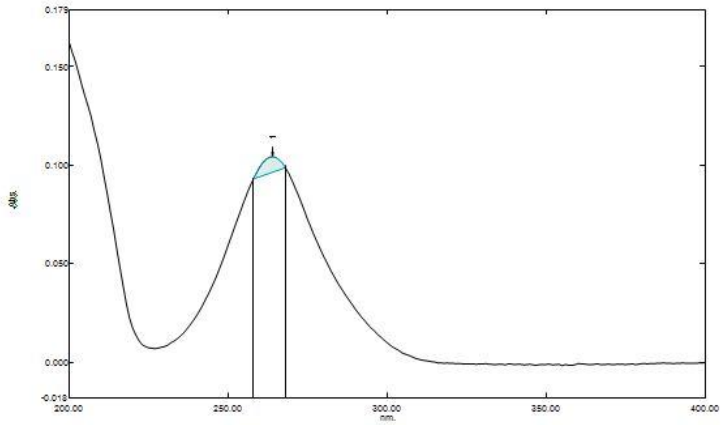
Wavelength

Fig. 2: Absorption maxima spectrum of Darunavir



Wavelength

Fig. 3: first order derivative spectrum of Darunavir



Wavelength

Fig. 4: Area under curve spectrum of Darunavir

Assay of Tablet formulation

Assay of marketed tablet formulation was determined by all three methods and the results were tabulated. Table.1

Validation of proposed methods

Validation was performed as per ICH guidelines[11]

Linearity

Linearity analyzed with different concentrations of standard solution prepared from standard stock of DRN and scanned at wavelength range from 200-400nm and the linearity for all three methods obeyed Beer's law in the concentration range from 2-24 µg/ml; and the correlation coefficient calculated was found to be r^2 = 0.999 for all three methods. Table 2

Accuracy

Accuracy of the method was checked by Recovery studies by adding known amount of standard to pre-analyzed sample. Studies were carried out at three different levels (80%, 100%, 120%). The proposed method affords recovery values within 98-102% shown in below table. Table 3

Precision

Precision is the measure of closeness of values between each concentration under same analytical conditions. It is determined by performing inter-day and intra-day studies. In intra-day studies three standard replicate injections of three different concentrations were injected on same day and same standard different concentrations were injected on three successive days in inter-day precision studies. Where, the %RSD was found to be within limits (<2). Table 4

Table 1: Assay results of tablet formulation

Formulation Name	Method	Labelled amount in mg	Assay concentration (µg/ml)	% Recovery Mean*	%RSD
Prezista	A	300	10	100.72	0.83
	B	300	10	99.09	0.36
	C	300	10	99.07	0.60

(*Average of six determinations)

Table 2: Linearity Parameters of Darunavir

Parameter	Result (n=6)		
	A	B	C
Linearity range	2-24 µg/ml	2-24 µg/ml	2-24 µg/ml
Slope	0.9992	0.9995	0.9993
Intercept	0.05	0.034	0.001
Correlation coefficient	0.041	0.407	0.001

Table 3: Accuracy results of darunavir

Test Concentration taken (µg/ml)	Recovery Level/ Spiked level(%)	Amount added (µg/ml)	Amount found (µg)mean*			%Recovery		
			A	B	C	A	B	C
10	80	8	18.1	19.95	18.05	101.25	99.33	100.6
10	100	10	20.19	20.02	19.92	101.98	100.02	99.2
10	120	12	21.8	21.95	22.04	98.4	99.6	100.4

(*average of Six determinations)

Table 4: Precision results of Darunavir

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	*mean±SD	%RSD	*mean±SD	%RSD
5	0.207±0.01	0.4	0.207666±0.001527	0.7
10	0.41566±0.00152752	0.36	0.416333±0.00251661	0.67
15	0.61666±0.004163	0.6	0.618666±0.005859	0.9

(*Average of three determinations (n=3))

Table 5: Statistical analysis of validated Parameters of Darunavir

Parameter	Method A	Method B	Method C
λ_{max}	262	248	257-267
Beer's range	2-24µg/ml	2-24 µg/ml	2-24 µg/ml
Correlation coefficient	0.9992	0.9995	0.9993
Regression Equation:	0.05	0.034	0.001
Intercept(c)	0.041	0.407	0.001
Slope(m)	0.02 µg/ml	0.625 µg/ml	0.002 µg/ml
Sandell's sensitivity	1.201 µg/ml	-	0.102 µg/ml
Confidence limit with 0.05 level	0.240 µg/ml	-	0.02 µg/ml
Confidence limit with 0.01 level	0.5%RSD	0.2%RSD	0.3%RSD
Precision	0.8%RSD	0.5%RSD	0.6%RSD
System Precision			
Method precision			

RESULTS AND DICUSSION

Three simple, precise, accurate and economic validated spectroscopic methods were developed and validated for their application in determination of Darunavir. Wavelength selected for first order derivative method is 248 which, is used for its quantification and linearity was found and regression equation was calculated (r^2=0.999), and for AUC method wavelength range selected for scanning was 257-267. Area was calculated and α+β values were used for quantification. %RSD was found to be within limits (<2). The proposed method is specific and gave no interference with placebo in estimation of drug. The mean %Assay was found to be 100.72 for method [A], 99.09 for method [B] and 99.06 for method [C] respectively. Table 5

CONCLUSION

A simple, precise, accurate, economic, sensitive, reliable and reproducible UV Spectrophotometric method for estimation of Darunavir in pharmaceutical dosage form has been developed and validated. Hence, these methods can be easily and conveniently used for routine analysis of DRN in pure and its pharmaceutical formulations.

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