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Spectrophotometric Determination of Darunavir Ethanolate by Condensation Technique

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Abstract: The objective of the present work is to develop simple, precise and accurate colorimetric methods for the estimation of Darunavir ethanolate(DAR) using PDAB (M1) and vanillin (M2) reagents. DAR is antiretroviral protease inhibitor. The methods are based mainly on the reaction of the free amino group in the drug with the reagents undergoing condensation reaction to form coloured condensation products (schiff's bases). The products were quantified at 452 nm by PDAB and 406 nm by vanillin. The linearity of the methods was assessed in the range of 50-350 µg/ml and 50-300µg/ml, respectively. The LOD and LOQ are 6.24 and 18.93; 4.30 and 13.04 for both the methods, respectively. The colorimetric methods were extensively validated as per ICH guidelines and all the parameters were within the acceptance criteria with a correlation of 0.9998 and 0.9999 and the % RSD less than 2. The results of the accuracy studies were nearer to 100%. The methods were proven to be more accurate, simple, precise and rapid by statistical validation.

Keywords : Darunavir ethanolate (DAR), Para dimethyl amino benzaldehyde (PDAB), Vanillin.

Introduction:

Darunavir ethanolate is chemically, [(1R,5S,6R)-2,8-dioxabicyclo[3.3.0]oct-6-yl] N-[(2S,3R)-4- [(4-aminophenyl)sulfonyl- (2-methylpropyl)amino]-3-hydroxy-1-phenyl- butan-2-yl] carbamate, which is a HIV protease inhibitor[1-2]. Its molecular formula is $C_{27}H_{37}N_3O_7S$. C_2H_5OH and its molecular weight is 593.73 g/mol[3]. It prevents HIV replication by binding to the enzyme's active site, there by preventing the dimerization and the catalytic activity of the HIV-1 protease. It also selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus-infected cells, which prevents the formation of mature infectious virus particles[4].

A literature survey of the drug revealed that there are very few spectrophotometric[5-7,12-15] and HPLC[8-11,16-25] methods for the determination of DAR. The purpose of this work was to develop a novel, simple, economical and efficient colorimetric method for quantitative analysis of the drugs and to validate the methods according to the ICH guidelines.

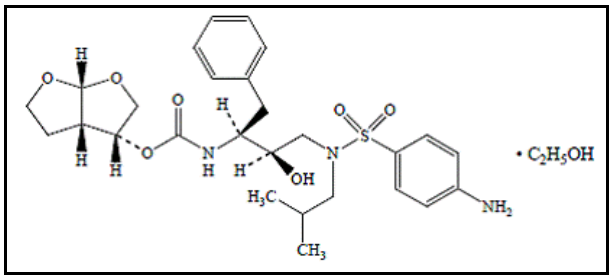

Fig.1: Structure of Darunavir ethanolate

Materials and Methods:

Instrumentation

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer was used for spectral measurements.

Chemicals

DAR is obtained as gift sample from Aurobindo Pharma Ltd, Hyd., methanol, sulphuric acid, vanillin and PDAB of AR grade was used for the experimental work.

Preparation of Stock Solutions:

Stock Solution for Method M1

Accurately weighed 25 mg of DAR was transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with methanol. The resulting solution has a concentration of 1mg/ml.

Stock Solution for Method M2

Accurately weighed 125 mg of DAR was transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with methanol. The resulting solution has a concentration of 5 mg/ml.

Preparation of (0.25%W/V) PDAB

0.5 g of PDAB was weighed and dissolved in a mixture of 12.5 ml sulphuric acid and distilled water made up to 50 ml.

Preparation of (0.25%W/V) Vanillin

0.5 g of vanillin was weighed and dissolved in a mixture of 25 ml sulphuric acid and 25 ml distilled water.

Preparation of Calibration Standards for M1 And M2:

As per the linearity range given in the table, all the calibration standards were prepared using the specified diluent and followed as per order of reagent addition given in the table1. The absorbances were measured at the λ_{max} of the reaction product given in the table 2.

Assay Procedure for M1 and M2:

Twenty tablets of commercial samples (Daruvir 300 mg) of DAR were accurately weighed and powdered. Tablet powder equivalent to 25 mg was weighed and dissolved in 25 ml methanol and powder equivalent to 125 mg was weighed and dissolved in 25 ml methanol, filtered and the procedure was carried out as mentioned.

Results and Discussion

Optimization of the Method

The spectral characteristics of all the methods using PDAB and vanillin reagents were performed by optimizing the methods for several optimization parameters as given below.

Reagent Concentration: In order to obtain the optimum conditions for determination of drugs ,the absorbance were measured for a series of solutions by varying the concentration of one with respect to other against the corresponding reagent blank in each case. The optimum conditions were presented in table1.

Effect of Temperature: Effect of temperature on reaction conditions was studied by lowering/increasing the temperature for the reaction products. The complex formation was complete in 2-3 min time interval at room temperature for both the methods.

Stability of Colour: The influence of the time on the maximum color development and stability of the colored species were studied by measuring the absorbance at gradual increase in time interval, standing time above 5 min doesn't increase the color intensity of the complex.

Table 1: Order of addition and concentration of reagents

M1:DAR+ 1 ml PDAB (0.25%w/v) + ethanol
M2:DAR + 2 ml vanillin (0.25%w/v) + 5N H_2SO_4

Method Validation

DAR is validated for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory. Regression parameters were presented in table 2.

Table 2: Optical and regression parameters

Parameters	M1	M2
λ_{max} nm	452	406
Beer's law range (µg/ml)	50-350	50-300
Molar extinction coefficient ($L.mole^{-1}.cm^{-1}$)	6.2×10^5	7.5×10^5
Sandell'sensitivity ($\mu g/cm^2$)/0.001 absorbance unit	2×10^5	2×10^5
LOD, µg/ml	6.24	4.30
LOQ, µg/ml	18.93	13.04
Slope(m)	0.0063	0.007
Intercept(b)	-0.020	0.013
Correlation coefficient(r)	0.9998	0.999

Linearity and Range

Linearity was assessed by performing single measurement at several analyte concentrations of DAR showed good correlation between the concentration range of 50-350 µg/ml for PDAB and 50-300µg/ml for vanillin, respectively. The results were reported in table 3 and shown in fig. 2and 3.

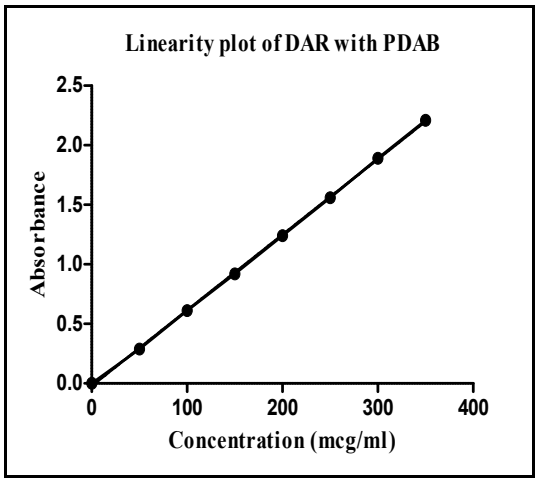

Fig.2: Linearity plot of DAR with PDAB

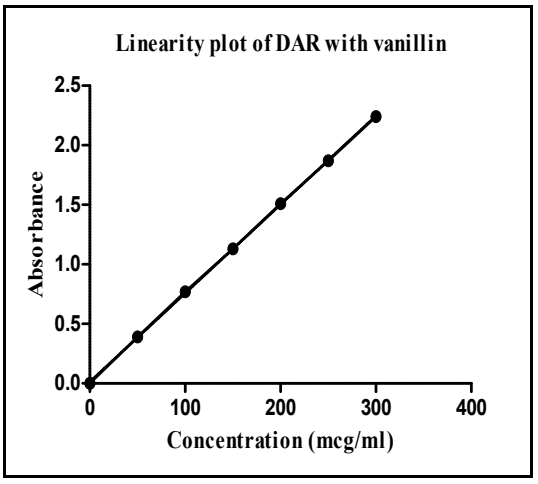

Fig.3: Linearity plot of DAR with vanillin

Table 3: Linearity of DAR with PDAB and vanillin

M1		M2	
Conc.(µg/ml)	**Absorbance**	**Conc.(µg/ml)**	**Absorbance**
50	0.289	50	0.39
100	0.611	100	0.77
150	0.92	150	1.13
200	1.24	200	1.51
250	1.56	250	1.87
300	1.89	300	2.24
350	2.21	-	-

Precision

The precision of the proposed methods were assessed by determining the relative standard deviation (RSD) of six replicate analyses on the same solution containing fixed concentration of DAR (within Beer's law limit). The low % RSD of the intraday and interday repeatability studies corroborates precision of the method. Table 4 represents the results of precision studies.

Table 4: Results showing Precision studies

Parameter	**M1**		**M2**	
	Inter day*	**Intra day***	**Inter day***	**Intra day***
Conc, (µg/ml)	200		200	
Mean abs	1.235	1.233	1.51	1.506
SD	0.0083	0.0081	0.0063	0.0081
% RSD	0.677	0.66	0.418	0.544

N=6 mean of six determinations

Robustness

Robustness was checked by altering the optimized parameters and the % RSD was found to be within the acceptable limit and reported in table 5.

Table 5: Results showing robustness for methods M1 and M2

Methods	**Parameters**			
	λ_{max}	**%RSD N=3**	**PDAB/vanillin**	**%RSD N=3**
M1	452 ± 2 nm	0.67	1 ml± 0.1 ml	0.52
M2	406 ± 2 nm	0.418	2 ml± 0.1 ml	0.68

N=3 *mean of three determinations

Ruggedness

System to system/ analyst to analyst/ variability study was conducted on different colorimeters and the results were satisfactory and reported in table 6.

Table 6: Results showing ruggedness for the methods

parameters	**M1**	**M2**
Analyst-1	1.21	1.50
Analyst-2	1.24	1.51
Mean abs	1.233	1.506
SD	0.0081	0.0081
%RSD	0.66	0.544

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by analyzing progressively lower concentrations of standard solution using optimized conditions and the results were found to be satisfactory and presented in table 2.

Accuracy

In order to determine the accuracy of the proposed methods, pure drug solution at three different concentration levels (within the working range) were prepared and analyzed, the results were presented in table 7. The percentage relative error indicates that the accuracy of the methods was found to be satisfactory.

Table 7: Results of accuracy studies by the proposed methods

S. NO.	M1		M2	
	% Recovery	% RSD N=3	% Recovery	% RSD N=3
80%	99.75	0.199	99.80	0.136
100%	99.69	0.163	99.67	0.120
120%	99.76	0.138	99.84	0.153

Application of the Proposed Methods to Formulations

To evaluate the proposed methods, they were applied to the determination of DAR in commercial formulations. The recoveries are close to 100%, indicating that there is no serious interference in samples. The good agreement between these results and known values indicate the successful applicability of the proposed methods for the determination of DAR in formulations. The results are given in table 8.

Table 8: Assay results for methods M1 and M2

Methods	Formulation	Label claim (mg)	Amount found(mg)	%Recovery N=3
M1	Daruvir (Cipla Ltd)	300	298.75	99.58
M2		300	298.63	99.54

Conclusion:

Two new, cost effective, simple and sensitive visible spectrophotometric methods, using PDAB and vanillin as reagents, were developed for the determination of DAR in bulk and in pharmaceutical formulations. The developed methods were also validated. From the statistical data, it was found that the proposed methods were accurate, precise and reproducible and can be successfully applied to the analysis of the same and could make a better alternative to the existing methods.

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