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Validation of Visible Spectrophotometric Methods of Darunavir in Pure and Dosage Forms

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ABSTRACT

Some new selective accurate and economical spectrophotometric methods for the determination of Darunavir in pure and dosage forms have been described in the present work. An Elico, UV-Visible digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements. Stock solution of Darunavir was prepared by initially by dissolving 100 mg of Darunavir in 10 mL of methanol and made up to 100 mL with distilled water. The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Darunavir under optimum conditions were investigated. The values obtained by the proposed and reference method for formulations were compared statistically with F and t tests and found not to be different significantly. These developed methods have been extended to pharmaceutical formulations as they are simple, economical and sensitive. The present methods involve the formation of highly stable colored species which makes it easier for the determination of Darunavir in pharmaceutical dosage at the given optimum conditions.

Keywords: Darunavir, UV spectrophotometric Methods, Optical Characteristics, Precision, Accuracy and Nature of the Colored Species.

INTRODUCTION

Darunavir is a protease inhibitor class used to treat human immune deficiency virus (HIV) 1*R*, 5*S*, 6*R*)-2, 8-dioxabicyclo [3.3.0] oct-6-yl] *N* [(2*S*, 3*R*)-4-[(4-aminophenyl) sulfonyl- (2 methylpropyl) amino]-3-hydroxy-1-phenyl- butan-2yl] carbamate [1-11**].** Darunavir selectively inhibits the cleavage of HIV-1 encoded Gag- Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles. When used with other anti-HIV medicines it may reduce the amount of HIV in our blood (called "viral load") and increase our CD4 (T) cell count. HIV infection destroys CD4 (T) cells. Several analytical methods have been reported for the determination of Darunavir in pure drug, pharmaceutical dosage forms and in biological samples using spcetrophotometry liquid chromatography, electro kinetic chromatography high performance thin layer chromatography either in single or in combined forms. So far only two spectrophotometric methods [12, 13] have been reported in the literature for its quantitative estimation in pharmaceutical formulations and this made the author an attempt to develop and validate few simple economical visible spectrophotometric methods for the above said drug. In this accord six new economical visible spectrophotometric methods have been developed and validated by the author basing on the reactivity of various functional groups of the Darunavir with various organic reagents. Rao et al. have presented the results on different oxide materials, polymers, nanopowders, glasses and drug materials in their earlier studies [14-66]. This paper describes the development and validation of some new UV-Visible spectrophotometric methods for the assay of Darunavir in pure and dosage forms.

MATERIALS AND METHODS

Instrumentation: An Elico, UV-Visible digital spectrophotometer (SL-160) with 1 cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements and officially calibrated Pyrex glassware [Borosil] was used throughout this study.

Preparation of Reagents*:* All the chemicals and reagents used are of analytical grade and their corresponding solutions were prepared using double distilled water.

Method-M_1, Resorcinol (Loba, 0.2 % w/v): Prepared by dissolving 200mg of resorcinol in 100 mL distilled water. HCl solution (Qualigens, 0.25 M): Prepared by dissolving 2.15 mL of conc. HCl to 100 mL distilled water and standardized. $NaNO_2$ solution (Loba, 0.1 % w/v): Prepared by dissolving 100 mg of $NaNO_2$ in 100 mL distilled water. NaOH solution (Loba, 4 % w/v): Prepared by dissolving 4 gms of NaOH to 100 mL distilled water and standardized.

Method-M_2, Phloroglucinol (Loba, 0.1 %w/v): Prepared by dissolving 100 mg of phloroglucinol in 100 mL distilled water. HCl solution (Qualigens, 0.25 M): Prepared by dissolving 2.15 mL of Conc. HCl to 100 mL distilled water and standardized. $NaNO_2$ solution (Loba, 0.1 % w/v): Prepared by dissolving 100 mg of $NaNO_2$ in 100 mL distilled water. NaOH solution (Loba, 4 % w/v): Prepared by dissolving 4 gms of NaOH to 100 mL distilled water and standardized.

Method-M_3, PDAB solution (Loba 0.1 % w/v)]: Prepared by dissolving 100 mg of para dimethyl amino Benzaldehyde in 100 mL of methanol (Merck, Mumbai, India).

Method-M_4, PDAC solution (Loba 0.1 % w/v)]: Prepared by dissolving 100 mg of para dimethyl amino cinnamaldehyde in 100 mL of methanol (Merck, Mumbai, India).

Method-M_5, Vanillin solution (Loba 0.2 % w/v)]: Prepared by dissolving 200 mg of vanillin in 100 mL of methanol.

Preparation of Stock and Working Standard Solutions: Stock solution (1.0 mg/mL) of Darunavir was prepared by initially by dissolving 100 mg of Darunavir (99.98 % pure) in 10 mL of methanol and made up to 100 mL with distilled water. From this stock appropriate volumes were diluted step wise with distilled water in separate volumetric flasks to get the working standard solutions of concentrations of 160 µg/mL for the Method-M_3 & M_4; 200 µg/mL for the Methods-M_1, M_5 and 250 µg/mL for the Method-M_2 respectively.

Procedure for Market Formulations: About ten tablets of Darunavir PREZISTA [Each tablet containing 600 mg of Darunavir] purchased from local pharmacy were pulverized to fine powder. Then powder equivalent to 100 mg of Darunavir was accurately weighed and transferred into a 100 mL calibrated flask containing 10 mL of methanol was added and the content shaken thoroughly for 15-20 min and later the volume was finally diluted to the mark with double distilled water and filtered through Whatman filter paper No 41. Aliquots of this filtrate were accurately diluted with distilled water as per the working standard solutions and these solutions were used for the determination of Darunavir in formulations as per the proposed procedures described below respectively.

RESULTS AND DISCUSSION

Method Development: In development of the proposed methods for Darunavir various reaction conditions were optimized by varying one parameter, keeping the others at a time fixed and observing the effect produced on the absorbance of the colored species. In designing this, the regroups experiments were conducted by the author and the conditions so obtained were incorporated in proposed procedures.

Proposed Procedures

Method – M_1, Resorcinol: Into a series of 25 mL volumetric flasks (0.5-2.5 mL, 200 µg/mL) Darunavir was transferred. Then 1.0 mL of hydrochloric acid (dilute) and 1.0 mL cold aqueous solution of sodium nitrite were added and set aside for 10 min. at 0 – 5 ^oC temperature. Later 1.0 mL of 0.2 % resorcinol and 1.5 mL of 1.0 M aqueous sodium hydroxide were added successively and then the volume in each flask was made up to 25 mL with distilled water. The absorbance was measured at 600 nm against reagent blank and the amount of Darunavir was calculated from the calibration graph plotted (Figure 1(a)).

Method–M₂, Phloroglucinol: Aliquots of (0.5-2.5 mL, 250 µg/mL) Darunavir were transferred into a series of 25 mL calibrated flasks. To each of the above aliquots, 1.0 mL of hydrochloric acid (dilute) and 1.0 mL cold aqueous solution of 0.1 % sodium nitrite were added and set aside for 10 min. at 0 – 5 ^{o}C temperatures Later 1.0 mL of 0.2 % phloroglucinol and 1.5 mL of 1.0 M aqueous sodium hydroxide were added successively and then the volume in each flask was made up to the mark with distilled water. The absorbance was measured at 510 nm against reagent blank and the amount of Darunavir was deduced from its calibration graph (Figure 2(a)).

Method – M₃, PDAB: To a series of volumetric flasks, Darunavir solutions ranging from 0.5-2.5 mL (160 µg/mL) and 2.0 mL of PDAB was added and the solution was kept aside for 5 min. Later the solutions in each flask were finally made up to the mark with methanol. The absorbance of the yellow colored solution was measured at 482 nm against the corresponding reagent blank. The amount of Darunavir was computed from the corresponding Beer-Lambert's plot (Figure 3(a)).

Method–M₄, PDAC: To a series of volumetric flasks, Darunavir solutions ranging from 0.5-2.5 mL (160 µg/mL) and 2.0 mL of PDAC was added and the solution was kept aside for 5 min. Later the solutions in each flask were finally made up to the mark with methanol. The absorbance of the yellow colored solution was measured at 530 nm against the corresponding reagent blank. The amount of Darunavir was computed from the corresponding Beer-Lambert's plot (Figure 4(a)).

Method – M₅, Vanillin: To each of 10 mL calibrated tubes, aliquots (0.5-2.5 mL, 200 µg/mL) of standard Darunavir solution, 2.0 mL of vanillin and 3.0 mL of con sulphuric acid were added successively and the total volume in each flask was brought to 10 mL by the addition of methanol and placed in heating water bath (maintained at 50 ^{O}C) for 10 min. Then the colored developed in each tube is diluted up to the mark with methanol and the absorbance was measured at 440 nm against a reagent blank prepared in a similar way. The concentration of drug in a sample was computed from Beer-Lambert plot (Figure 5(a)).

Method Validation: The proposed methods were validated in terms of linearity, accuracy, precision and specificity of the sample applications as per the ICH guidelines.

Spectral Characteristics: In order to ascertain the optimum wavelength of maxim absorption (λ_{max}) of the colored species formed in each of nine spectrophotometric methods, specified amounts of Darunavir were taken and colors were developed separately by following the above mentioned procedures individually. The absorption spectra were scanned on a spectrophotometer in the wavelength region of 340 to 900 nm against similar reagent blank. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in Figure 1(b) for M_1, Figure 2(b) for M_2, Figure 3(b) for M_3, Figure 4(b) for M_4 Figure 5(b) for M_5 respectively. The absorption curves of the colored species in each method show characteristics absorption maxima whereas the blank in each method has low or no absorption in this region.

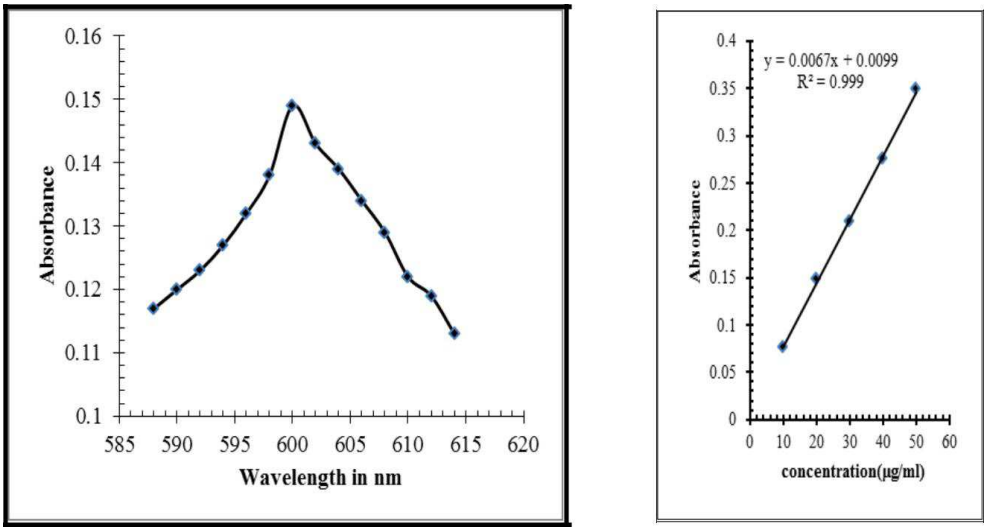

Figure 1(a&b): Absorption spectra and Beer's law plot of Duranavir for Method-M_1

Optical Characteristics (Linearity & Range): Under the above mentioned experimental conditions, calibration graphs were constructed for each proposed method after the analysis of five different concentrations of Darunavir

with each concentration was measured in triplicate that are represented in Figures 1(a) to Figure 5(a) respectively. The regression equations (with standard error of intercept and slope) and correlation of each proposed methods were given in Table (1&2) and these results showed that there is an excellent correlation between the absorbance recorded and the concentrations of Darunavir in the range tested. The values of LOD revealed that the developed methods more sensitive and are suitable for the analysis of Darunavir in different brands of dosage forms.

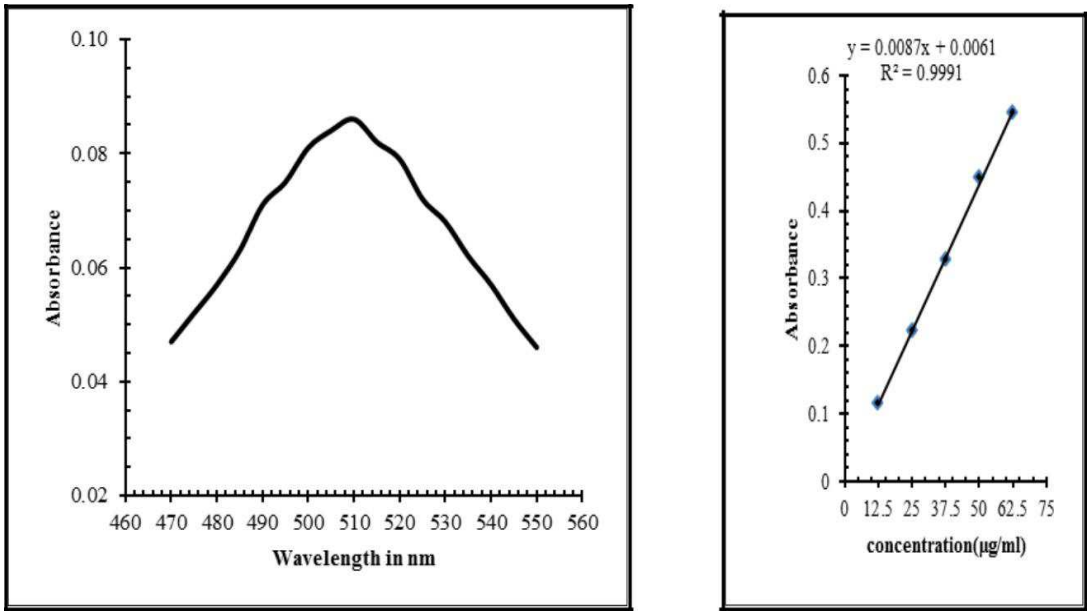

Figure 2(a&b): Absorption spectra and Beer's law plot of Duranavir for Method-M_2

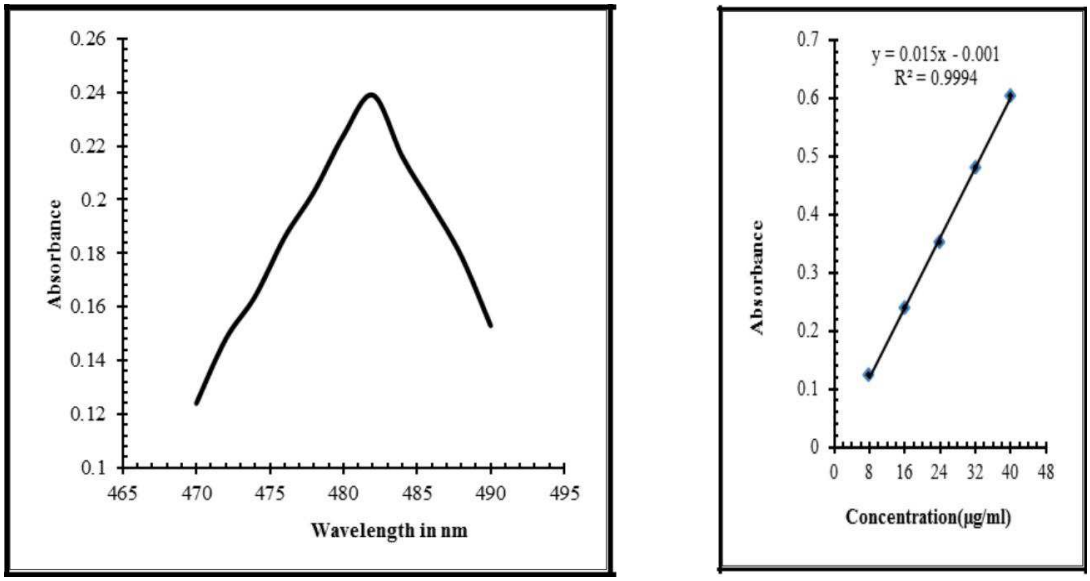

Figure 3(a&b): Absorption spectra and Beer's law plot of Duranavir for Method-M_3

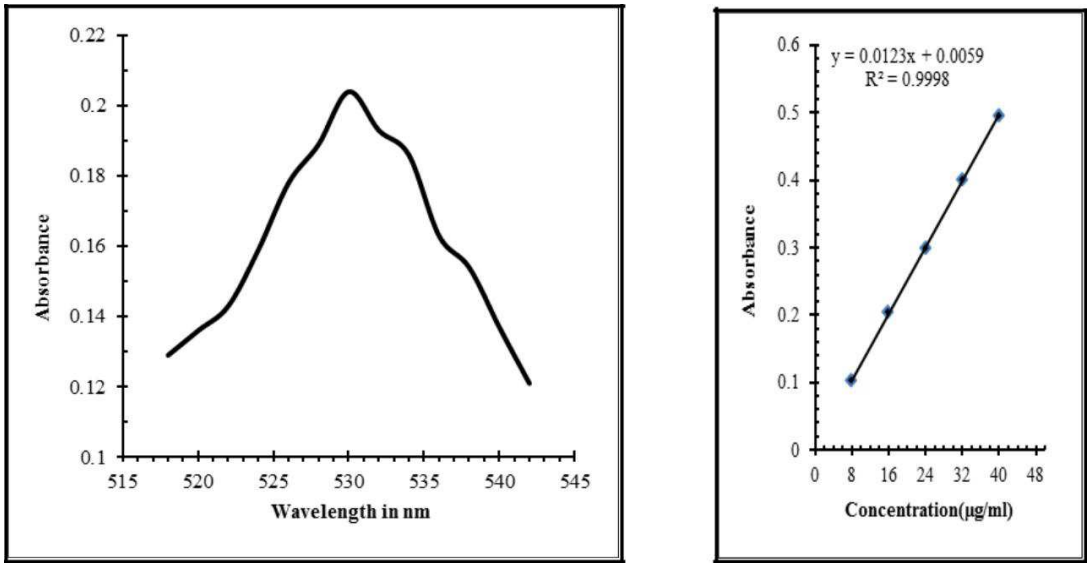

Figure 4(a&b): Absorption spectra and Beer's law plot of Duranavir for Method-M_4

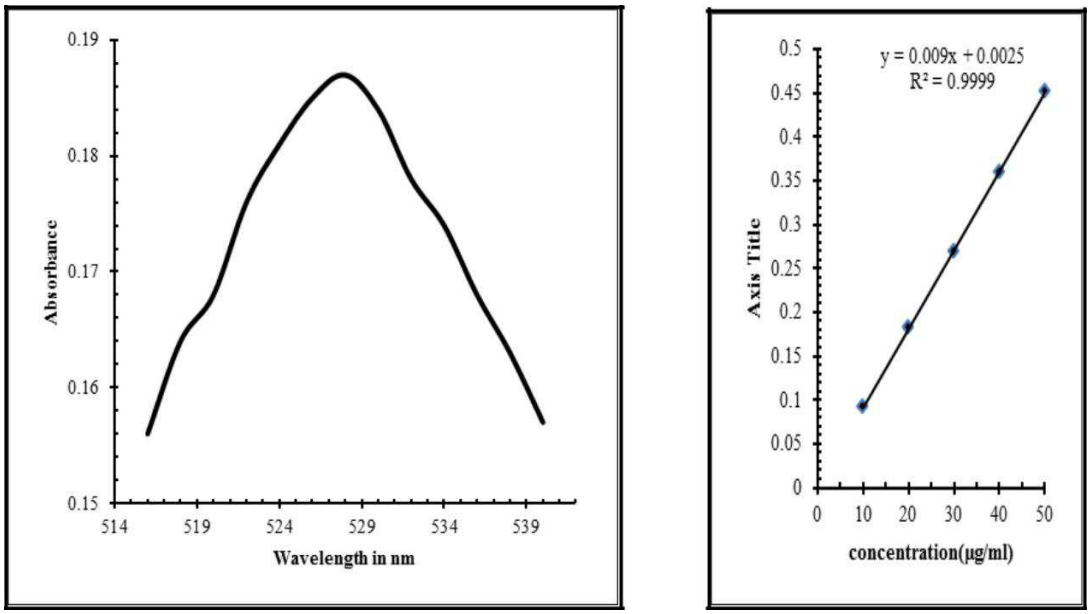

Figure 5(a&b): Absorption spectra and Beer's law plot of Duranavir for Method-M_5

Precision: The precision of each proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of Darunavir in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table (1&2)).

Accuracy: To ensure the reliability and accuracy of each proposed method Recovery experiment was performed by the standard addition method. For this different amount of bulk samples of Darunavir within the Beer's law limits were taken and analyzed by the proposed methods and the results (percent error) are recorded in Table 3 The % recovery of Darunavir for the proposed methods was almost found to be 100 % (Table 3) were within the acceptable limits revealing the high accuracy of the proposed methods.

Table -1: Results of Method Validation Obtained by Applying the Proposed Methods for the Determination of Darunavir

Parameter	M1	M2	M3
λ_{max} (nm)	600	510	482
Beer's law limits (µg/mL)	10.0-50.0	12.5-62.5	8.0-40.0
Molar absorptivity (1 mol^{-1}. cm^{-1})	$1.911x10^3$	$1.790x10^3$	$2.634x10^3$
Sandell's sensitivity (µg.cm^{-2}/0.001 A.U)	0.0573	0.0764	0.0332
Regression equation (Y=a+bc); Slope (b)	0.0067	0.0061	-0.001
Intercept (a)	0.0099	$4.999x10^{-4}$	$4.999x10^{-1}$
Correlation coefficient (r)	0.9999	0.9991	0.9994
Relative standard deviation (%)*	1.379	0.7646	0.5034
% Range of error (confidence limits)			
0.05 level	1.153	0.6394	0.4204
0.01 level	1.706	0.9458	0.6225
LOD	0.0318	0.0299	0.0239

** Average of six determinations*

Table-2: Results of Method Validation Obtained by Applying the Proposed Methods for the Determination of Darunavir

Parameter	M_4	M_5
λ_{max} (nm)	530	440
Beer's law limits (µg/mL)	8.0-40.0	10.0-50.0
Molar absorptivity (1 mol^{-1}. cm^{-1})	$1.632x10^3$	$2.402x10^3$
Sandell's sensitivity (µg. cm^{-2}/0.001 A.U)	0.0536	0.0452
Regression equation (Y=a+bc); Slope (b)	0.0123	0.0099
Intercept (a)	0.0059	0.0047
Correlation coefficient (r)	0.9998	0.9998
Relative standard deviation (%)*	0.0835	0.7035
% Range of error (confidence limits)		
0.05 level	0.6903	0.5883
0.01 level	1.0211	0.8703
LOD	0.0139	0.0132

**Average of six determinations*

Table-3: Results of (Accuracy Studies) of Darunavir by the Proposed Visible Spectrophotometric Methods

Proposed methods	DRV in tablet µg. mL^{-1}	Pure DRV added µg. mL^{-1}	Total found µg. mL^{-1*}	Pure DRV recovered %
Method M_1	20.0	5.0	24.93	99.72
Method M_2	25.0	5.0	29.94	99.80
Method M_3	40.0	5.0	44.99	99.97
Method M_4	40.0	5.0	44.94	99.86
Method M_5	20.0	5.0	24.96	99.84

** Average of six determinations considered*

Analysis of Formulations: Commercial formulations (tablets) containing Darunavir were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method [13] for formulations were

compared statistically with F and t tests and found not to different significantly. The results F and t tests and recovery experiments for the proposed methods are summarized in Table 3.

Nature of the Colored Species: It is difficult to predict the exact nature of colored species formed in the proposed methods. An attempt have been made by the author to describe the nature of colored species in each of the proposed methods for Darunavir the basis of reactive functional moiety (tertiary amine group) in drug and the reagents used.

CONCLUSION

In this paper six simple, reliable and economical visible spectrophotometric methods were developed and validated for the determination of Darunavir in pure and in pharmaceutical formulations. The results of statistical analysis depicted that the developed visible spectrophotometric methods were found to be accurate and precise that enabled the use of the proposed methods for the quantitative and qualitative estimation of Darunavir in different brands of Darunavir tablets without any excipient interference. Therefore, it can be concluded that the proposed visible spectrophotometric methods could find practical implementations as an economical quality control tool for the analysis of active pharmaceutical ingredients from their final dosage forms on industrial as well as laboratory scale.

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