



SPECTROPHOTOMETRIC ESTIMATION OF DARUNAVIR IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple spectrophotometric method for the determination of Darunavir was described. The method was based on bromination of the darunavir with excess brominating mixture in acidic medium. The yellow colour developed was measured at 350 nm against distilled water blank. Beer's law was obeyed in the concentration range of 40-200 µg/mL. The proposed methods were simple, rapid, and validated and can be used successfully for routine analysis of darunavir in a pure and tablet dosage form.

Key words: Darunavir, Spectrophotometry, Potassium bromate and Potassium bromide mixture, Formulations.

INTRODUCTION

Darunavir (DRV), [(1*S*,2*R*)-3-[[[4-aminophenyl] sulfonyl](2-methylpropyl) amino]-2-hydroxy-1-(phenylmethyl) propyl]-carbamic acid (3*R*,3*aS*,6*aR*)-hexa hydro furo [2,3-*b*] furan-3-yl ester, a new protease inhibitor (PI), is used to treat human immunodeficiency virus (HIV) type-1. According to *in vitro* experiments, DRV was active against HIV-1 with PI resistance mutations and against PI resistant clinical isolates. DRV is expected to be effective in antiretroviral treatment-experienced patients, such as those possessing HIV-1 strains, which are resistant to more than one protease inhibitor.

Literature survey revealed that different analytical methods for the determination of darunavir have been reported, which include high-performance liquid chromatography with UV detection to determine DRV in human plasma¹, HPLC-MS method for the simultaneous determination of DRV and 11 other antiretroviral agents in plasma of HIV infected patients², validation of plasma DRV concentrations by the HPLC method³, electrophoretic method for the separation of darunavir⁴, RP-HPLC method development and validation for estimation of

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darunavir ethanolate in tablet dosage form⁵, simultaneous determination and validation of Darunavir ethanolate and Ritonavir in binary mixture by Liquid chromatography⁶, LC-tandem MS assay for the simultaneous measurement of new antiretroviral agents⁷ and RP-HPLC method for the estimation of Darunavir in tablet dosage form⁸.

The aim of the present study was to develop a simple, precise and accurate spectrophotometric method for the estimation of darunavir in bulk drug and pharmaceutical dosage form.

EXPERIMENTAL

Materials and methods

All measurements were done on Milton Roy 1001 plus spectrophotometer by using 10 mm matched quartz cuvettes. All analytical grade chemicals were used and all the solutions were freshly prepared with double distilled water. Hydrochloric acid (4 N) was prepared and standardized using standard procedure. Darunavir tablets (Prezista 300 mg, Tibotec Company)) were procured from local market.

Chemicals and reagents

Potassium iodide 0.1 N: Potassium iodide 0.1 was prepared by dissolving 0.166 g in 100 mL distilled water. Brominating mixture solution 0.1 N: Brominating mixture solution was prepared by dissolving 0.695 g of potassium bromate and 1.75 g of potassium bromide in distilled water and diluted to 100 mL with distilled water. Further dilution was done to obtain working concentration of 0.02 N brominating mixture solution.

Preparation of sample solution

One hundred milligrams of pure darunavir was dissolved in methanol and diluted to 100 mL with methanol. This stock solution was further diluted to get the desirable working concentration of 200 µg/mL.

Calibration curve procedure

The following procedure has been adopted for obtaining the standard curve. An aliquot each of 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the darunavir solution was transferred into a series of 25 mL standard flasks. To each flask, 1 mL of 4 N hydrochloric acid and 1 mL of 0.02 N brominating mixture were added. The flask were shaken well and kept aside for 5 min for complete bromination. Then, 1.0 mL of 0.1 N potassium iodide was added to each flask and diluted to 25 mL with distilled water. The yellow colour solution formed was

measured at 350 nm against distilled water. The calibration curve was obtained by plotting absorbance values against amount of standard drug in $\mu\text{g/mL}$. The amount of drug present in the sample was computed from calibration curve. The calibration curve was found to be linear over the concentration range of 40-200 $\mu\text{g/mL}$.

Pharmaceutical formulations

Twenty tablets contents were accurately weighed, their mean weight was determined and they were mixed and finely powdered. A portion equivalent to 100 mg of darunavir was accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was filtered through Whatmann filter paper no 42. The original stock solution was further diluted to get sample solution of drug concentration of 200 $\mu\text{g/mL}$ and analyzed as given under the assay procedures for bulk samples. The results are represented in Table 1.

Table 1: Assay and recovery of darunavir in pharmaceutical formulations

Tablet formulations	Labeled amount	*Amount found		% Recovery
		Proposed Method	Official Method	
Tablet 1	300	300.04	299.96	100.02
Tablet 2	600	600.3	600.06	99.98

*Average five determinations based on the label claim

RESULTS AND DISCUSSION

The present study was carried out to develop a simple rapid, precise and reproducible spectrophotometric method for the estimation of darunavir in pharmaceutical dosage forms. The drug in slightly acidic conditions undergoes bromination with brominating mixture. After bromination was completed, the excess brominating mixture was treated with potassium iodide to form yellow coloured complex. The coloured complex was measured at 350 nm against distilled water blank. The experimental conditions were optimized by studying the effect of brominating mixture, hydrochloric acid, potassium iodide by the sequence of addition. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The percent relative standard deviation, standard deviation and student's 't' test values calculated from the five measurements of darunavir are presented in Table 2.

Table 2: Results of statistical analysis of the proposed method

Tablet formulation	*Standard deviation	% Relative standard deviation*	*t value
Tablet 1	0.3646	0.1215	0.2152
Tablet 2	0.3469	0.0577	1.934

*Average five determinations based on the label claim

Relative standard deviation values and standard deviation were low that indicates the reproducibility of the proposed methods. In the student's 't' tests, no significant differences were found between the calculated and theoretical values of both the proposed methods at 95% confidence level. This indicated similar precision and accuracy in the analysis of darunavir in its tablets.

CONCLUSION

The developed spectrophotometric method was simple, sensitive, precise and accurate, hence can be used in routine for the determination of darunavir in bulk and pharmaceutical dosage form.

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