Determination of Darunavir in Pharmaceutical Dosage Form

K. Parameswara Rao

Department of Chemistry, Andhra Loyola College, Vijayawada-520008, India

ABSTRACT

An accurate and economical spectrophotometric method for the determination of Darunavir in pure and dosage forms has been described in the present work. Stock solution of Darunavir was prepared by initially dissolving 100 mg of Darunavir in 10 mL of methanol and made up to 100 mL with distilled water. 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. This developed method has been extended to pharmaceutical formulations as they are simple, economical and sensitive. The present method involves 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, M11 Method, Optical Characteristics and Nature of the Colored Species.

INTRODUCTION

Formulations containing various drugs and combinations of drugs for potentiating or complementing one another in therapy are available in market. Pharmaceutical equivalents containing identical amounts of the same active ingredient(s) in the same dosage form and targeted to give in the same route of administration are called as generics drugs. For a generic drug to be approved it must be shown to be pharmaceutically equivalent and bioequivalent to the Reference Listed Drug (RLD). They must also meet all relevant standards of strength, quality, purity and identity. In view of the foregoing discussion the assaying and stability testing in pharmaceutical analysis [1] occupies an important role to meet the requirement of statutory certification of drugs and their formulations by the industry. The analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. The best way to characterize the quality of a bulk drug is to determine its purity. There are two possible approaches to reach this goal.

Darunavir (Figure 1) is a protease inhibitor class used to treat human immune deficiency virus (HIV) 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[2]. Darunavir selectively inhibits the cleavage of HIV-1 encoded Gag- Pol polypeptides in infected cells, thereby preventing the formation of mature virus particles. Several analytical methods have been reported for the determination of Darunavir in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry liquid chromatography, electro kinetic chromatography high performance thin layer chromatography either in single or in combined forms.
The principle of UV-Vis spectrophotometry[3] is based on the ability of the molecule to absorb ultraviolet and visible light. The absorption of light corresponds to the excitation of outer electrons in the molecule. When a molecule absorbs energy and the outer electrons in the molecule excited from the Highest Occupied Molecular Orbital (HOMO) to Lowest Unoccupied Molecule Orbital (LUMO). The occupied molecular orbitals with lowest energy are known the σ orbitals, at slightly higher energy are called π orbitals and at still higher energy are known non-bonding orbitals (unshared pair electrons). So far only two spectrophotometric methods[4] 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 [5-12]. Rao et al. have presented the results on different oxide materials, polymers, nanopowders, glasses and drug materials in their earlier studies [13-65]. This paper describes the development and validation of UV-Visible spectrophotometric method 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-M11, PCA solution (Loba 0.1 % w/v): Prepared by dissolving 100 mg of parachloranilic acid in 100 mL of 1,4-dioxane (Merck, Mumbai, India) was prepared and kept in dark when not in use.

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 200 µg/mL for the Method-M11.

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 procedure 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 procedure.
Proposed Procedures

Method-M_11, PCA: Aliquots of standard Darunavir solutions (0.5-2.5 mL; 200 µg/mL) were accurately transferred into a series of 10.0 mL calibrated tubes and the total volume was adjusted to 3.0 mL by adding adequate quantity of acetonitrile. To each tube 2.0 mL of 0.1 % p-chloranilic acid was added, and the contents were mixed well and kept aside for 10 min. The mixture was diluted to the volume with acetonitrile and the absorbance of the colored complex developed in each tube was measured at 528 nm against a reagent blank prepared similarly. The concentration of the Darunavir was read from the standard graph using the Beer’s law data (Figure 2(b)).

Method Validation: The proposed method was 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 maximum absorption (λ_max) of the colored species formed in each of spectrophotometric methods, specified amounts of Darunavir were taken and colors were developed separately by following the above mentioned procedure individually. The absorption spectrum was scanned on a spectrophotometer in the wavelength region of 340 to 900 nm against similar reagent blank. The reagent blank absorption spectrum of the method was also recorded against distilled water. The results were graphically represented in Figure 2(a&b) for M_11 respectively. The absorption curves of the colored species in this method show characteristics absorption maxima whereas the blank in each this has low or no absorption in this region.

Optical Characteristics (Linearity & Range): Under the abovementioned experimental conditions, calibration graph was constructed for this proposed method after the analysis of five different concentrations of Darunavir with each concentration was measured in triplicate that are represented in Figure 2(a).

Precision: The precision of proposed method 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 method.

Accuracy: To ensure the reliability and accuracy of the 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 method and the result (percent error) are recorded.

Analysis of Formulations: Commercial formulations (tablets) containing Darunavir were successfully analyzed by the proposed method. The value obtained by the proposed and reference method for formulations was compared statistically with F and t tests and found not to different significantly.
Nature of the Colored Species: It is difficult to predict the exact nature of colored species formed in the proposed method. An attempt have been made by the author to describe the nature of colored species in the proposed method for Darunavir the basis of reactive functional moiety (tertiary amine group) in drug and the reagents used and their respective scheme was given below (Scheme 1).

Scheme 1: Reaction schemes of proposed methods for Darunavir

CONCLUSION

In this paper a simple, reliable and economical visible spectrophotometric method was 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 method was found to be accurate and precise that enabled the use of the proposed method 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 method 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.

REFERENCES