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Darunavir: A Review on its Analytical Methods

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ABSTRACT

Darunavir is a second-generation protease inhibitor this drug is pharmaceutically known as TMC114, i.e., to treat human immune deficiency virus. It works by drastically reduces viral load and increases CD4 cell counts. Darunavir is recommended as treatment option for human immunodeficiency virus (HIV) infection in antiretroviral treatment-experienced adult patients i.e. patients infected with more than one protease inhibitor-resistant HIV-1 strains. This medicine is also used along with other protease inhibitors drugs for the treatment of multiple HIV protease inhibitor-resistant strain of HIV-1. For the determination of Darunavir in the pharmaceutical dosage form and bulk form, several analytical methods including UV, HPLC, LC-MS and HPTLC, GC and Electrophoresis techniques have been developed. Methods indicating human plasma stability and impurity profiling are also described for Darunavir. For qualitative and quantitative estimation of Darunavir, these analytical methods can be used and it can also be used for its related degradants in bulk formulations and biological fluids. The following study depicts the review on analytical methods which includes estimating the antiretroviral drugs.

INTRODUCTION

Darunavir (Fig.1) is an antiviral drug and inhibitor of the human immunodeficiency virus (HIV) protease in adults and children 6 years of age and older [1]. It was approved by the Food and Drug Administration on June 23, 2006. DRV, a second-generation protease inhibitor, is discovered to overcome the problems with early protease inhibitor (PIs) like severe side effects and drug toxicities, require a high therapeutic dose, are costly to manufacture, and show a disturbing susceptibility to drug-resistant mutations. DRV is used with ritonavir and other medications to treat HIV. It works by slowing the spread of HIV in the body.DRV selectively inhibits the cleavage of HIV-1 encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles[2]. DRV was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from treatment-experienced patients with multiple resistance mutations to PIs. It blocks HIV protease, an enzyme which is needed for HIV to multiply. HIV infection destroys CD4 (T) cells, which are important to the immune system. The immune system helps fight infection. Reducing the amount of HIV and increasing the CD4 (T) cell count may improve your immune system and, thus, reduce the risk of death or infections that can happen when your immune system is weak (opportunistic infections). Darunavir is co-administered with ritonavir and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV-1) infection [3].

DRUG PROFILE:

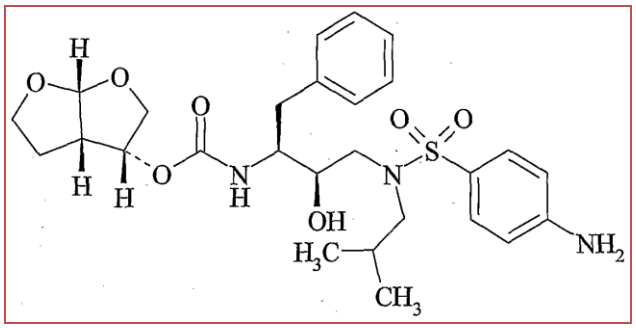

Figure 1: Chemical structure of Darunavir

Chemical name: Chemically it is [(3aS,4R,6aR)-2,3,3a,4,5,6a hexahydrofuro[2,3-b]furan-4-yl]N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2yl]carbamate.

Chemical formula: $C_{27}H_{37}N_3O_7S$

Molecular weight: 593.73 g/mol

Solubility: Methanol, slightly soluble in water, Sodium hydroxide, Hydrochloric acid.

Category: Protease inhibitor.

Mechanism of Action:

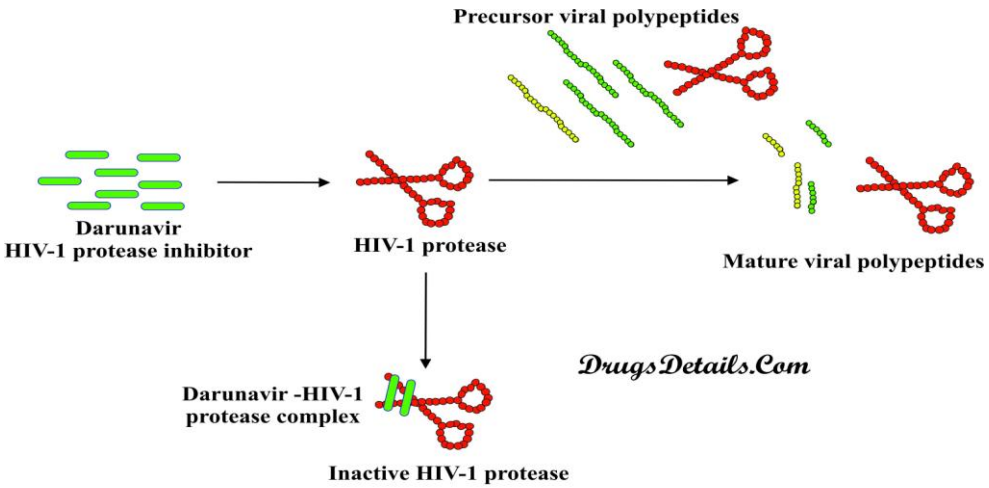

Figure 2: Mechanism of Action of darunavir

Literature survey revealed that few Spectrophotometric [4-12], HPLC [13-29], LC-MS [30-34], HPTLC [35] and electrophoresis [36] methods were reported earlier for the determination of Darunavir in bulk and pharmaceutical dosage forms.

Reported methods are categorized depending on the following considerations:

1. Analysis of Darunavir Single and combination with other class of drugs components by UV-Visible Spectrophotometric methods.

2. Analysis of Darunavir Single and combination with other class drugs by Chromatographic methods, biological methods, and Electrophoretic methods.

UV-Visible Spectrophotometric methods.

M.M. Eswarudu et al., [4] has been developed two simple, precise and economical UV Spectrophotometric methods for the estimation of Darunavir ethanolate (DRV) in bulk and its

pharmaceutical dosage form. Two methods were developed based on measurement of absorption at maximum wavelengths, for Method Ⅰ 272.1 nm and Method Ⅰ Ⅰ 272.4 nm. Linearity for detector response was observed in the concentration range of 2-10 µg/ml for both methods. The developed methods were validated with respect to linearity, accuracy (recovery), precision and specificity. The accuracy of the methods was assessed by recovery studies and was found to be 98.3% and 99% for Method Ⅰ and Method Ⅰ Ⅰ respectively. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory. The proposed methods were successfully applied for the determination of DRV in the pharmaceutical dosage form.

Parameswara Rao K [5] has developed new, selective, accurate and economical Spectrophotometric methods for the determination of Darunavir in pure and dosage forms. He has 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. The 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 a 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.

Vijayalakshmi. R et al., [6] has developed simple, precise and accurate colorimetric methods for the estimation of Darunavir ethanolate (DAR) using PDAB (M1) and vanillin (M2) reagents. DAR is an antiretroviral protease inhibitor. The methods are based mainly on the reaction of the free amino group in the drug with the reagents undergoing the condensation reaction to form colored 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.

Mrinalini Damle et al., [7] has developed two simple and rapid UV Spectrophotometric methods have been developed for the estimation of Darunavir ethanolate and Ritonavir in combination. Method A is Absorbance Ratio Method which is based on measurement of absorption at a maximum wavelength of Ritonavir i.e. 239 nm and isosbestic point i.e. 251 nm. Method B is Absorbance correction method, at wavelengths 251nm and 267 nm. Linearity for detector response was observed in the concentration range of 10-50 µg/ml for both drugs and both methods. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The accuracy of the methods was assessed by recovery studies of Darunavir ethanolate was found to be 100.83% and 98.25% for Method A and Method B respectively and Ritonavir was found to be 100.52% and 99.25% for Method A and Method B respectively.

Minal R et al., [8] has developed two simple, precise and economical UV methods have been developed for the estimation of Darunavir ethanolate (DRV) in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at a maximum wavelength, 266nm. Method B is an area under the curve (AUC), in the wavelength range of 255-275 nm. Linearity for detector response was observed in the concentration range of 3-18 µg/ml for both methods. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The accuracy of the methods was assessed by recovery studies and was found to be 100.07% and 99.58% for Method A and Method B respectively. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory. The proposed methods were successfully applied for the determination of DRV in tablet dosage form.

Say Sirisha et al., [9] has developed three simple methods were developed and subsequent validation of Darunavir in its bulk and formulation with greater precision and accuracy. 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 Shimadzu Double Beam (UV-1800 model) Ultraviolet-Visible spectrophotometer with 10 mm matched quartz cells and 70% methanol as solvent. Linearity for all three methods was found in the range of 2-24 µg/ml (r^2 = 0.999). Tablet

formulation was analyzed and the % assay for absorption max, first-order derivative, and area under curve methods was found to be 100.72%, 99.09%, and 99.06% respectively.

Shinde VR et al., [10] has been developed the simple accurate and sensitive UV Spectrophotometric method for the quantitative estimation of Darunavir in bulk and its pharmaceutical formulation. In this method, 70% methanol was used as a solvent. Darunavir shows maximum absorbance at 262.5 nm and obeys Beer-Lamberts law in the concentration range of 2-25 µg/ml. The linearity was observed in concentration range of 2-25 µg/ml. The method was validated statistically for accuracy, precision, and sensitivity.

Ana Carolina Kogawa et al., [11] has developed and validation of an infrared spectroscopy method for the determination of darunavir in tablets. The method was completely validated according to the International Conference on Harmonization guidelines, showing accuracy, precision, selectivity, robustness, and linearity. It was linear over the concentration range of 1.5-3.5 mg with correlation coefficients greater than 0.9991 and limits of detection and quantification of 0.12 and 0.36 mg, respectively. The validated method is very useful to the routine quality control of darunavir since it does not use polluting reagents, it is simple and has low-cost.

Josilene Chaves Ruela Corrêa et al., [12] has developed a selective, easy and fast method was achieved employing simple and cheap instrumentation by using first-order derivative spectrophotometry. Results: The first-derivation of a spectrum of the drug measured between 200 and 400 nm allowed identification of the analyte and showed absence of placebo interference. The assay was based on the absorbance at 276 nm. The linear concentration range was established from 11 to 21 µg/ml. The intra-day and inter-day precision expressed as RSD was 0.06% and 3.75% respectively with a mean recovery of 99.84%.Conclusion: The proposed analytical method is able to quantify darunavir as raw material and tablets and can be used routinely by any laboratory applying a spectrophotometer with a derivative accessory. The great difference of the method proposed here is that it proves to be free of placebo interferences as well as simple, fast and low cost.

Chromatographic methods

Anantha Siva Nageswararao G et al., [13] the objective of the present research work is to develop and validated analytical method for the determination of Darunavir in pure and tablet

dosage form. A simple, rapid, precise, selective and accurate novel RP-HPLC method was developed and validated for separation and determination for the Darunavir in pure and tablet dosage form. Darunavir was analyzed by Zodiac C18, (250×4.6mm, 5µ), Shimadzu LC-20AT Prominence Liquid Chromatograph and mobile phase constituted of Triethylamine buffer: Acetonitrile (60:40 v/v). The pH of the buffer was adjusted to 4.5 with diluted ortho-phosphoric acid. The flow rate of mobile phase was 1.0 mL/min and the analysis was performed using UV-Visible detector at 260nm. The Darunavir was eluted within 6 min and retention time was showed 2.753 min. The developed assay method was validated by the guidelines of ICH Q2R1. The method was found to be linear in the drug concentration range of 20 µg/ml -100 µg/ml. The value of correlation coefficient was found to be 0.999. The method was found good percentage recovery it indicates the method is highly accurate. The method has been successfully applied for the determination of Darunavir in the pharmaceutical dosage form in regular quality control analysis.

Mallikarjuna Rao. N et al., [14] has developed a new simple, efficient, and sensitive reverse phase high performance liquid chromatographic method has been developed for simultaneous extraction and determination of the concentrations of lamivudine, tenofovir, darunavir, and ritonavir in bulk and in their tablets. Four compounds were separated on a Reversed-phase C18 column at 30±2.0° using a gradient mobile phase combination containing potassium dihydrogen phosphate, Acetonitrile, and methanol. The pH was adjusted to 3.5±0.05 by the addition of Orthophosphoric acid. The samples were detected using a UV detector, 260 nm for lamivudine, tenofovir and darunavir and 240 nm for ritonavir. The procedure separated analyte and its potential degradation products such as lamivudine, tenofovir, darunavir and ritonavir eluting at about 2.385, 4.055, 11.353 and14.010 mins, respectively. The linear range of lamivudine, tenofovir, darunavir and ritonavir was 58.32-174.96 µg/ml, 58.32-174.96 µg/ml, 72.00-216.00 µg/ml and 112.50-337.5 µg/ml, respectively. The relative standard deviation for precision was less than 2.0%. The drug was subjected to acid, alkaline peroxide and photolytic stress conditions and the performance of the method were validated according to the International Conference on Harmonization guidelines for specificity, linearity, accuracy, precision, and robustness.

Venkata Siva Sri Nalini .M et al., [15] has developed a simple, sensitive, precise stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of Darunavir and Cobicistat simultaneously in the

combined dosage form. The stationary phase used was Phenomenex C18 (150 x 4.6 mm,5 µm particle size). The mobile phase used was a mixture of0.1 M NaH_2PO4 and methanol (70:30 v/v). Quantification was done with photodiode array detection at 260 nm over the concentration range of 80-240 µg/mL and 15-45 µg/mL for darunavir and cobicistat, respectively. The method had accuracy in the range of 100.11-100.31% for darunavir and 99.87-99.89%for cobicistat. Darunavir and cobicistat were also subjected to acid, base, oxidation, heat, photo and UV degradation. The degradation products obtained were well resolved from the darunavir and cobicistat with different retention times. Since the method can effectively separate the darunavir and cobicistat from its degradation products, it can be used as the stability indicating method.

Chenthilnathan A et al., [16] has been developed a simple, selective, linear, precise and accurate RP-HPLC method for quantification of Darunavir ethanolate in bulk and its tablet dosage form. The separation was carried out on Shiseido C8 (250 × 4.6mm; 5µm) column at ambient temperature using phosphate buffer pH 3: Acetonitrile (40:60) as eluent. The flow rate was 1.0 ml/min and effluent was detected at 270 nm. The retention time of Darunavir ethanolate was 4.30 min. The percentage recovery was within the range between 99.46% and 100.17% for Darunavir ethanolate. The linear ranges were found to be 5-15µg/ml (r2 = 0.9997) for Darunavir ethanolate. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the method could be successfully applied for routine analysis of Darunavir ethanolate in pharmaceutical formulations.

A. Charbe. N et al., [17] have developed a new method using high-performance liquid chromatography coupled with ultraviolet detection (HPLC–UV) were developed and validated for the simultaneous quantification of Atazanavir, Dolutegravir, Darunavir, Efavirenz, Etravirine Lopinavir, Raltegravir, Rilpivirine, and Tipranavir in human plasma. For the first time we reported here the development and validation of an HPLC–UV assay to quantify the frequently administered 9 antiretroviral compounds including dolutegravir and rilpivirine. A simple solid phase extraction procedure was applied to 500 µL aliquots of plasma. The chromatographic separation of the drugs and internal standard (Quinoxaline) was achieved with a gradient of Acetonitrile and sodium acetate buffer on a C18 reverse-phase analytical column with a 25 min analytical run time. Calibration curves were optimized according to the therapeutic range of drug concentrations in patients, and the coefficient of determination (r2) was higher than 0.99 for all analyte. Mean intraday and interday precisions

(RSD) for all compounds were less than 15.0%, and the mean accuracy (% deviation from nominal concentration) was also found to be less than 15.0%. Extraction recovery range was between 80% and 120% for all drugs analyzed. The solid phase extraction and HPLC–UV method enable a specific, sensitive, and reliable simultaneous determination of nine antiretroviral agents in plasma. Good extraction efficiency and low limit of HPLC–UV quantification make this method suitable for use in clinical trials and therapeutic drug monitoring.

Hemant K. Jain et al.,[18] has been developed a new precise, accurate, sensitive and robust RP-HPLC method for estimation of darunavir ethanolate in bulk and tablets. Methods**:** The chromatographic separation was achieved on Enable C_{18} column (250 × 4.6 mm, 5 µm) at an ambient temperature. The mobile phase consists of Acetonitrile and 0.01M potassium acetate buffer, pH 5.1 (75:25 v/v) was at the flow rate 1 ml/min and UV detection was done at 268 nm. Results**:** The method was linear over the concentration range of 40-90 µg/ml (r^2= 0.998) of the drug. The percentage content was found in darunavir ethanolate 99.19±0.58 in tablets. The low value of the drug %RSD (0.11) indicates that reproducibility of this method. A low value of LOD and LOQ suggests the sensitivity of the method. Conclusion**:** It can be concluded from the results that the proposed RP-HPLC method was found to be rapid, simple, accurate, robust and precise for the analysis of darunavir ethanolate in bulk and tablet dosage form. The developed method can be applied in the routine analysis of this drug in the pharmaceutical industry.

Nagendrakumar AVD et al., [19] has been developed a simple, selective, linear, precise and accurate RP-HPLC method was for rapid assay of Darunavir in bulk and pharmaceutical formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on symmetry C18 (250 mm x 4.6 mm, 5µm) column at ambient temperature. The mobile phase consisted of Acetonitrile: Methanol in the ratio of 90:10 v/v. The UV detection wavelength was 271nm and 20µl sample was injected. The retention time for Darunavir was 2.59 min. The percentage RSD for precision and accuracy of the method was found to be 0.48%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Darunavir in the rapid and reliable determination of Darunavir in a pharmaceutical formulation.

Chaudhary PG et al., [20] has been developed High performance liquid chromatographic method for the determination of Darunavir Ethanolate in tablet dosage form. The method was

carried out on a Phenomenex Luna C18 Column (150 × 4.6 mm id, 5µ) maintained at 30°C. The mobile phase consisted of water-Acetonitrile (60 + 40, v/v) pumped at a flow rate of 1.0 mL/min. Photodiode array detection was at 265 nm. The chromatographic separation was obtained with a retention time of 11.8 min, and the method was linear in the range of 1-30 µg/mL (r2 = 0.9997). The specificity and stability indicating a capability of the method were proven through forced degradation studies, which also showed that there was no interference of the excipients. The method was validated for linearity, precision, accuracy, robustness, and specificity, limit of detection and limit of quantitation. The developed method, after being validated was successively applied to the analysis of tablet formulations. The drug could be effectively separated from different degradation products and hence the method can be used for stability analysis.

Raveendra Babu. G et al., [21] has developed a simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Darunavir in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase Hypersil, BDS C18 Column (250 mm x 4.6 mm, 5µm). The method showed a linear response for concentration in the range of 5-25 µg/mL using phosphate buffer pH 3: Acetonitrile as the mobile phase in the ratio of 40:60, v/v with detection at 270 nm with a flow rate of 1 ml/min and retention time was 2.711 min. The method was statistically validated for linearity, accuracy, precision, and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the %RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity, and accuracy of the method, we believe that the method will be used for routine quality control analysis of Darunavir in pharmaceutical formulations.

Manisha B et al., [22] has developed a Simple, economic, sensitive and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for Darunavir in tablets. Isocratic chromatography was performed on a C18 column with methanol-Acetonitrile 95:5(v/v) as a mobile phase at a flow rate of 0.7ml/min. The effluent was monitored by UV- detector at 264 nm and total run time was 10 min. A calibration curve was linear over the concentration range of 3-27 µg/ml. The method was validated with respect to accuracy, linearity, precision, selectivity, and robustness; these parameters examined met the current recommendations of U.S.P for analytical method validation. The developed RP-HPLC method was successfully applied for the quantitative determination of Darunavir in pharmaceutical dosage forms.

Josilenechavesruelacorrêa et al., [23] has developed Chemical and physical degradation of drugs may result in altered therapeutic efficacy and even toxic effects. Therefore, the aim of this work was to study the stability of darunavir and to develop and validate a liquid chromatography (LC) method to determine darunavir in raw material and tablets in the presence of degradation products. The novel method showed to be linear from 6.0to 21.0 μg/mL, with high precision (CV < 2%) and accuracy (recuperation of 99.64%). It is simple and reliable, free of placebo interferences. The robustness of the method was evaluated by a factorial design using seven different parameters. Forced degradation study was done under alkaline, acidic, and oxidative stress at ambient temperature and by heating. The LC method was able to quantify and separate darunavir and its degradation products. Darunavir showed to be unstable under alkaline, acid, and oxidative conditions. The novelty of this study understands the factors that affect darunavir ethanolate stability in tablets, which is the first step to unravel the path to know the degradation products. The novel stability-indicating method can be used to monitor the drug and the main degradation products in low concentrations in which there is linearity.

B.V. Rami Reddy et al., [24] A novel stability-indicating reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the quantitative determination of darunavir ethanolate, an HIV-1 protease inhibitor. The chromatographic separation was achieved using an X-Bridge C18 (150 3 4.6 mm 33.5 mm) HPLC column in isocratic mode employing 0.01M ammonium format (pH.3.0) buffer and Acetonitrile in the ratio of 55:45 (v/v) with a flow rate of 1.0 ml /min. The detector wavelength was monitored at 265 nm and the column temperature was maintained at 308C. Darunavir ethanolate was exposed to thermal, photolytic, acid, base and oxidative stress conditions. Considerable degradation of the drug substance was found to occur under acid, base and oxidative stress conditions. The peak homogeneity data of Darunavir ethanolate obtained by photodiode array detection demonstrated the specificity of the method in the presence of degradants. The degradation products were well resolved from a primary peak of Darunavir, indicating that the method is specific and stability-indicating. The HPLC method was validated as per International Conference on Harmonization guidelines with respect to specificity, precision, linearity, accuracy, and robustness. Regression analysis showed a correlation coefficient value greater than 0.999. The accuracy of the method was established based on the recovery obtained for Darunavir ethanolate.

Bhavini N. Patel et al., [25] A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Darunavir ethanolate in tablet dosage form. A Phenomenex Luna C18 column (250 mm i.d., 4.6mm, 5µm) with the mobile phase consisting of water-Acetonitrile (40: 60, v/v; pH adjusted to 3.2 ± 0.02 with formic acid) was used. The flow rate was 1.0 ml/min and the effluents were monitored at 267 nm. The retention time was 5.02 min. The detector response was linear in the concentration of 2-20 µg/ml. The limit of detection and limit of quantification was found to be 0.085 µg/ml and 0.38 µg/ml respectively. This method was simple, precise, and sensitive, and they are applicable for the determination of Darunavir ethanolate in tablet dosage form.

L. Satyanarayana et al., [26] has developed a simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Darunavir in tablet dosage form. An RP Inertsil ODS-3V C-18, 250x4.6 mm, 5_m particle size, with a mobile phase consisting of 0.02M dipotassium hydrogen orthophosphate +0.02M Potassium Dihydrogen orthophosphate in water and Acetonitrile in the ratio of 40:60 v/v was used. The flow rate was 1.0 ml/min and the effluents were monitored at 265nm. The retention time was 5.859 min. The detector response was linear in the concentration of 80 240 µg/ml. The respective linear regression equation being Y= 44076.08X +726603. The limit of detection and limit of quantification was 0.1µg and 0.3µg/ml respectively. The percentage assay of Darunavir was 98.58%. The method was validated by determining its accuracy, precision and system suitability.

Raveendra. B. Ganduri et al.,[27] To develop a simple, rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic method for the determination of Darunavir in tablet dosage form. Waters, Symmetry shield RP18 (250X4.6mm, 5µm) column, 0.1% Orthophosphoric acid and Acetonitrile (50:50 % v/v) as mobile phase, the detection wavelength of 265 nm, the flow rate of 1.0 mL min-1. The method is linear from 25µg mL-1 to 100µg mL-1, accuracy was found to be 99.54%, mean inter and intraday assay relative standard deviation (RSD) were less than 1.0%. The method is simple, accurate, specific and precise, can be used for the determination of Darunavir.

Bhavini N et al., [28] has developed a simple and accurate method to determine Darunavir ethanolate (DRV) and ritonavir (RTV), in the binary mixture, was developed and validated using liquid chromatography (LC). The LC separation was achieved on a Phenomenex Luna

C18 column (250 mm, 4.6mm i.d., 5µm), in the isocratic mode using Acetonitrile: water (60: 40, v/v), pH adjusted to 3.2 ± 0.02 with formic acid at a flow rate of 1 mL/min. The retention times were about 5.02 and 7.60 min for DRV and RTV, respectively. Quantification was achieved with a UV-visible detector at 245 nm over the concentration range of 6-60 and 2-20 µg/mL for DRV and RTV respectively, with mean recoveries of 99.54-100.18 % and 99.16-100.20 % for DRV and RTV, respectively. The method was validated and was found to be simple, specific, accurate, precise and robust. The method was successfully applied for the determination of DRV and RTV in the binary mixture without any interference from common excipients.

Masaaki Takahashi et al., [29] has developed Darunavir (DRV) is a new protease inhibitor used to treat human immune deficiency virus (HIV) type-1. The aim of this study was to validate the determination of plasma DRV concentrations using the HPLC method, a simple procedure for the simultaneous determination of seven HIV protease inhibitors and efavirenz. The calibration curve was linear (range of 0.13 to 10.36 mg/ml). The average accuracy ranged from 100.7 to 105.6%. Both the interday and intraday coefficients of variation were less than 6.7%, which was similar to or much lower than previously reported values by the LC/MS/MS method. It is concluded that HPLC can be used to determine plasma DRV concentrations and routinely in the clinical setting; thus, this HPLC method enables further study of DRV pharmacokinetics in conventional hospital laboratories.

Ajay Gupta et al., [30] has been developed a simple, precise and rapid ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) method for the quantification of darunavir, a protease inhibitor, using darunavir-d9 as internal standard (IS). The method involved liquid-liquid extraction of darunavir and IS in the methyl-*tert*-butyl ether from 50 µL human plasma. The chromatographic separation was achieved on an Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm particle size) analytical column under gradient conditions, in a run time of 1.6 min. The precursor → production transitions for darunavir (*m*/*z* 548.1 → 392.0) and IS (*m*/*z*557.1 → 401.0) were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) and positive ion mode. The method was extensively validated for its selectivity, sensitivity, carryover check, linearity, precision and accuracy, reinjection reproducibility, recovery, matrix effect, ion suppression/enhancement, stability and dilution integrity. The linearity of the method was established in the concentration range of 1.0–5000 ng/mL. The mean relative

recovery for darunavir (100.8%) and IS (89.8%) from spiked plasma samples was consistent and reproducible. The application of this method for routine measurement of plasma darunavir concentration was demonstrated by a bioequivalence study conducted in 40 healthy Indian subjects for a 600 mg tablet formulation along with 100 mg ritonavir as the booster under fast and fed conditions. To demonstrate the reproducibility in the measurement of study data, an incurred sample reanalysis was done with 400 subject samples and the % change in concentration was within ±12%.

Ravi Kanneti et al., [31] has developed a simple, rapid, sensitive, selective and high-performance liquid chromatography method with MS/MS was developed and validated for the determination of Darunavir (DRV) in human plasma. Extraction from the plasma was by solid phase extraction (SPE) extraction procedure. Carbamazepine was used as an internal standard (IS). The chromatographic separation was performed by Thermo Hypurity, advance column (50 X 4.6mm, 5µ) with a mobile phase comprising of 5mM ammonium acetate: Acetonitrile (15:85% v/v). Quantification was performed using multiple reactions monitoring (MRM) of the transition *m/z* 548.20 (parent ion) → *m/z* 392.30 (production); *m/z* 273.20 (parent ion) → *m/z* 194.10 (production) for DRV and IS respectively. The assay linearity ranged 50.14 to 2007.43 ng/mL and the lower limit of quantitation is 50.14 ng/mL. Frequently co-administered drugs did not interfere with the described methodology. The validated method is suitable to support a wide range of therapeutic drug monitoring and pharmacokinetic studies.

Fayet A, et al., [32] describes a sensitive and accurate liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of plasma drug levels. Raltegravir (RAL), Maraviroc (MVC), Darunavir (DRV), and Etravirine (ETV) are new antiretroviral agents with significant potential for drug interactions. Single-step extraction of RAL, MVC, DRV, ETV and RTV from plasma (100µl) is performed by protein precipitation using 600 µl of Acetonitrile, after the addition of 100 µl darunavir-d(9) (DRV-d(9)) at 1000 ng/ml in MeOH/H(2)O 50/50 as internal standard (I.S.). The mixture is vortexed, sonicated for 10 min, vortex-mixed again and centrifuged. An aliquot of supernatant (150 µl) is diluted 1:1 with a mixture of 20 mM ammonium acetate/MeOH 40/60 and 10 µl is injected onto a 2.1 x 50 mm Waters Atlantis-d C18 3 µm analytical column. Chromatographic separations are performed using a gradient program with 2 mM ammonium acetate containing 0.1% formic acid and Acetonitrile with 0.1% formic acid. Analytes quantification is performed by electrospray

ionization-triple quadrupole mass spectrometry using the selected reaction monitoring detection in the positive mode. The method has been validated over the clinically relevant concentrations ranging from 12.5 to 5000 ng/ml, 2.5 to 1000 ng/ml, 25 to 10,000 ng/ml, 10 to 4000 ng/ml, and 5 to 2000 ng/ml for RAL, MRV, DRV, ETV, and RTV, respectively. The extraction recovery for all antiretroviral drugs is always above 91%. The method is precise, with mean inter-day CV% within 5.1-9.8%, and accurate (range of inter-day deviation from nominal values -3.3 to +5.1%). In addition, our method enables the simultaneous assessment of raltegravir-glucuronide. This is the first analytical method allowing the simultaneous assay of antiretroviral agents targeted to four different steps of HIV replication. The proposed method is suitable for the Therapeutic Drug Monitoring Service of these new regimen combinations administered as salvage therapy to patients having experienced treatment failure, and for whom exposure, tolerance and adherence assessments are critical.

Heine RT et al.,[33] For the quantification of all currently approved non-nucleoside reverse transcriptase inhibitors and protease inhibitors, including the new protease inhibitor darunavir and the active Nelfinavir metabolite M8, an assay was developed, using liquid chromatography coupled with tandem mass spectrometry. The sample pre-treatment consisted of a protein precipitation with a mixture of methanol and Acetonitrile using only 100 µL plasma. Chromatographic separation was performed on a reversed-phase C18 column (150 × 2.0 mm, particle size 5 µm) with a quick stepwise gradient using an acetate buffer (pH 5) and methanol, at a flow rate of 0.25 mL/min. The analytical run time was only 10 min. The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over a range of 0.1 to 20 µg/mL for Amprenavir, Atazanavir, efavirenz, Indinavir, Lopinavir, Nelfinavir, the active Nelfinavir metabolite M8, Nevirapine and ritonavir, a range of 0.05 to 10 µg/mL for Saquinavir and darunavir and a range of 0.5 to 100 µg/mL for Tipranavir, based on observed concentration ranges in patients treated with these drugs. D5-saquinavir, D6-indinavir, 13C6-efavirenz, and dibenzepin were used as internal standards. The method was proven to be specific, accurate, precise and robust. Accuracies ranged from 88.5% to 102.2% and all precisions were less than 9.5%. Furthermore, the assay demonstrates a high sensitivity for all analytes and the stepwise gradient allows the addition of new analytes into the same method. The method is now successfully applied for routine therapeutic drug monitoring and pharmacokinetic studies in HIV-infected patients.

Patel BN et al., [34] has been developed a quantitative high-performance thin-layer chromatography (HPTLC) method for determination of darunavir ethanolate (DRV) in tablets has been established and validated. DRV from the formulations was separated and identified on silica gel 60 F254 HPTLC plates with toluene-ethyl acetate-methanol 7.0:2.0:1.0 (v/v) as a mobile phase. The plates were developed to a distance of 8 cm. Quantification was performed at λ = 267 nm. Well-resolved bands were obtained for DRV. The method was validated for specificity, precision, robustness, and accuracy. The calibration plot for DRV standard was linear in the range 250–1750 ng per band with r = 0.9994, slope = 0.4253, and intercept = 44.81. The limits of detection and quantification were 15.28 and 45.84 ng per band, respectively. The method is selective, sensitive, and specific, with potential application in pharmaceutical analysis.

Leonard S et al., [35] has been developed a capillary Electrophoretic (CE) method was for the separation of diastereoisomers of a new human immunodeficiency virus (HIV) protease inhibitor TMC114. In total 16 isomers of this drug have been synthesized (eight pairs of enantiomers). We succeeded in the separation of the eight diastereoisomers, but no enantiomers could be separated. Because of the high similarity and water-insolubility of these isomers, the separation is a real challenge. Different CE modes were tried out: capillary zone electrophoresis (CZE), nonaqueous capillary electrophoresis (NACE), micellar electrokinetic capillary chromatography (MEKC), and microemulsion electrokinetic capillary chromatography (MEEKC). Only MEEKC offered a resolution of these compounds.

CONCLUSION

This review depicts the reported Spectrophotometric and Chromatographic methods; developed and validated for the estimation of protease inhibitor. According to this review, it was concluded that for protease inhibitor (Darunavir) different Spectroscopic & Chromatographic methods are available for a Single component as well as for combination and also it was found that the Mobile phase Containing Phosphate buffer, Methanol and Acetonitrile was common for most of the chromatographic method to provide more resolution. It was observed that most common combination of Darunavir with Ritonavir. For chromatographic method flow rate is observed in the range of 0.8-1.5 ml/min to get good retention time. For most of the Spectroscopic methods, a common solvent is Methanol. Hence this all methods found to be simple, accurate, economical, precise, and reproducible in nature.

Most of the Methods were of RP-HPLC and UV absorbance detection because of these methods provided with best available reliability, repeatability, analysis time and Sensitivity.

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