DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RITONAVIR AND ALPHA TOCOPHEROL IN NANOFORMULATION

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Purpose: Acquired immunodeficiency syndrome (AIDS), a life-threatening chronic condition in which the immune system of the body is affected mainly. Human immunodeficiency virus (HIV) is a lentivirus belongs to the family *Retroviridae* is a causative organism and attacks the lymphocytes with CD4 glycoprotein. Ritonavir is a drug used for the reatment of HIV, but having severe side effects and hepatotoxicity. The major side effect of this drug is hepatotoxicity. Ritonavir-induced hepatotoxicity is unknown, but increase in the oxidative stress levels is one of the typical indications seen. Alpha-tocopherol, a well known vitamin with a very good anti-oxidant properties. Hence, a nanoformulation that can encapsulate ritonavir and alpha-tocopherol would help in reducing the oxidative stress which helps to reduce the hepatotoxicity also. But there is no specific analytical method for the estimation of both the drugs in nanoformulation. Hence, in the present study an analytical HPLC method was developed and validated as per the ICH guidelines for the estimation of ritonavir and alpha-tocopherol in nanoformulations.

Methods: The method was developed by using Inertsil ODS-3V C18 column (250 mm × 4.6 mm, 5 µm, 100 Å). The mobile phase used was acetonitrile, mathanol and orthophosphoric acid (with pH 3.0). The samples were detected at a wavelength of 242 and 290 nm for the quantification of ritonavir and alpha tocopherol, respectively. The developed method was validated for parameters such as system suitability, specificity and selectivity, linearity, precison, recovery and robusteness of the method.

Results: The responses were found to be linear over a range of 300 ng/mL to 30 µg/mL with a correlation coefficient value of 1 and 0.9999 for ritonavir and alpha tocopherol, respectively. The method was found to be precise with intraday and interday precision values less than 1% and less than 2%, respectively for both ritonavir and alpha-tocopherol. The limit of detection and limit of quantification of ritonavir were found to be 37.92 and 114.91 ng/mL, respectively for ritonavir and

129.94 and 393.76 for alpha-tocopherol, which revealed the sensitivity of developed method. The mean recovery values for ritnovir were 105.04, 102.28 and 101.48% and for alpha-tocopherol 101.69, 97.23 and 97.29 for concentration levels 75, 100, and 125%, respectively. The method showed good robustness deliberate changes in various chromatographic conditions such as pH of the buffer, column oven temperature, wavelength, and injection volume. The validated method was successfully applied for the quantification of ritonavir and alpha tocopherol in prepared nanoformulations. The assay value was found to be more than 92.86 and 106.89% for ritonavir and alpha-tocopherol, respectively.

Conclusions: The proposed method was sensitive, precise, and accurate hence can be used for the simultaneous quantitative analysis of ritonavir and alphatocopherol in the nanoformulations.