RP-HPLC Method Development, Validation including Stability indicating and Forced Degradation Studies for the Estimation of Pemetrexed in API and Pharmaceutical Dosage form

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**Abstract**. Stability indicating analytical RP-HPLC method was developed for the analysis of the drug in the presence of its degradation products and subsequent validation of Pemetrexed was conducted in API (Active pharmaceutical ingredient) and pharmaceutical dosage form. Pemetrexed was subjected to different stress conditions as per International Conference on Harmonization (ICH) guidelines, stress conditions applied were including the effect of acid, base, oxidative, hydrolysis, thermal and photolytic degradation conditions. Chromatographic separation of API was achieved on Water’s HPLC instrument and ODS C18, 250×4.6 mm,5µm particle size column with gradient elution of 0.1% ortho phosphoric acid buffer: Acetonitrile 80:20 ratio was taken as the eluent (mobile phase). The buffer pH was maintained at 3, the flow rate was 1.0 ml/min and wavelength were detected at 225 nm. The RT (Retention time) of Pemetrexed was Found at 2.9 minutes, column temperature was kept at ambient and the runtime was 6 min. System suitability parameters like theoretical plates, tailing factor, and Asymmetry values were showed at 3391, 1.41, and 1.101 respectively. The percentage drug purity was found to be 99.99%. Validation of the method was proved that linear relationship over the range of 25-150 µg/mL, with linear regression curve (correlation coefficient) r2 value was noticed at 0.999 and limit of detection, limit of quantification values was established at 0.33µg/mL, 0.99µg/mL respectively. Recovery studies carried out at the concentrations of 50%, 100% and 150% with percentage recovery values of 100.06, 99.8 and 99.87 respectively. The precision of intraday was 0.64% and inter-day was 0.95%. Robustness of flow rate and mobile phase was changed even also method was Robust. Forced degradation studies were also conducted to check the stability and suitability of this method to resolve the degradation products. Method validation was proved to be accurate, precise, linear, repeatable and robust. Thus, the accepted method can be proceeded for the estimation of Pemetrexed in pharmaceutical dosage form in a daily basis, due to their easy, suitable, accuracy, robustness and reproducibility.

**Key words:** Pemetrexed, method validation, stability indicating, degradation, pharmaceutical dosage form, RP-HPLC.

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