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Development and validation of a sensitive reversed phase HPLC method to determine total, free drug and encapsulation efficiency of cytotoxic Nano pharmaceutical

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Abstract : The intracellular accumulation of anti-cancer agents strongly influences the efficiency of chemotherapy for cancer. In the present research work, a simple rapid, sensitive reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed and validated to determine free, encapsulated and total drug in Doxorubicin liposome a cytotoxic Nano pharmaceutical formulation. The free drug and total drug were measured by RP-HPLC with a C18 column after extraction with waters Oasis SPE cartridge using solid phase extraction assembly with methanol and mobile phase as diluent. The mobile phase contained 0.1% trifluro acetic acid and solvent mixture of acetonitrile and methanol in the ratio of 80:20. UV/PDA detector with wavelength 254 nm was used for determination of free and encapsulated drug. The calibration curve was linear from 5 to 100 µg/ml with correlation coefficient of 0.999 while the recovery of total assay was between 98% and 101%. The intra and inter day coefficient of variation (RSD) were less than 1%. Furthermore, the validated method was used to determine the free, encapsulated and total drug for the developmental liposome as Nano pharmaceutical formulation.

Keywords:HPLC,Doxorubicin Liposomes, validation, freedrug, Encapsulation efficiency.

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