3. Plan of Work

3.1 Proposed Hypothesis

The proposed study is aimed to prepare the Nano Lipid Carrier System for a drug carrier in the treatment of Tuberculosis. Which is present in macrophage cell of different body organ these particulate system loaded with active therapeutic compound are aimed to enhanced phagocytosis and penetrate the barriers and show good penetration through the cellular compartment and increase the drug concentration. It is assumed that the system will increase the concentration of drug in the cellular compartment. To obtain the targeted and extended release of drug intracellularly to macrophage, this could be a beneficial therapeutic strategy for better management for targeting body organ by reduction of dosing frequency and reduce resistance.

3.2 Objectives

- HPLC method development of rifabutin as per ICH guidelines.
- Screening and optimisation of Important factors used in the preparation method of Nano Lipid Carrier
- To prepare and characterised Nano Lipid Carrier (NLC) with capmul EP of rifabutin.
- To prepare and characterised Nano Lipid Carrier (NLC) with capmul C-8 of rifabutin.
- To perform a pharmacokinetic study to assess the bioavailability of the formulations.
- Target the drug at the site of action (macrophage cell line J7441.A)

3.3 Detailed Experimental plan

3.3.1 Aqueous Solubility and Stability at Different Solvents

3.3.2 Analytical Study

- Estimation and Evaluation of λ max of Rifabutin by UV Visible Spectrophotometric.
- HPLC analytical Method Development for estimation of Rifabutin.

3.3.3 Development of Nano Lipid Carrier (NLC) with capmul EP of Rifabutin

1. **Selection of Ingredient**
   i) Selection of Lipid
   ii) Selection of Stabilizer and other ingredients.
2. **Preparation of NLC**
   i. Screening of significant factors in process through Design of Experiment.

ii. Optimisation of significant factors in process through Design of Experiment.

3.3.4 Characterisation of NLC

1. Particle Size, Polydispersity Index (PDI)
2. Zeta potential
3. Entrapment Efficiency
4. *In-vitro* release

Solid State Characterisation of NLC

a) Differential Scanning Chromatography
b) X-Ray Diffraction

Morphological Study of NLC

1. Scanning Electron Microscopy (SEM)
2. Transmission Electron Microscopy

Release Kinetic Study

Stability Studies of NLC as ICH Guidelines

3.3.5 Pharmacokinetic Studies

3.3.6 *ex-vivo* profile Studies

- In vitro cytotoxicity study

3.3.7 *In-vivo* studies

3.3.8 Rationale of the study

Drug targeting to a specific tissue or organ has been a subject of creative and innovative research in pharmaceutical field since the beginning of 20th century. In many diseases (e.g. tuberculosis) a considerable therapeutic advantage could be gained if drugs were delivering in selective and controlled manner to their target site.

Thus, NLCs reduce the systemic toxicity improving the therapeutic index by restricting the drug effect on large cells. Biodegradable surfactant from the verity of aggregates from NLCs, which can be used as vesicles for drug delivery. This approach with reduction of Rifabutin treatment with effective targeting to cell with increase concentration of drug with in macrophages, the host cell for mycobacterium tubercular bacteria. The mode of cellular delivery thus evades the lysosomal degradation. This may lead to sustained however targeted cytosolic delivery of drug with preparation of suitable size for penetration in to the third degree cell targeted. Finally it includes reduction of drug dose, dose frequency and toxicity. Improvement of patient compliances and most importantly targeting macrophage that harbor the tuberculosis bacteria.