



PREFORMULATION STUDIES AND PREPERATION OF RIFABUTIN LOADED SOLID LIPID NANOPARTICLES

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Article Received on 21/03/2018

Article Revised on 12/04/2018

Article Accepted on 02/05/2018

ABSTRACT

Tuberculosis is a ubiquitous, highly contagious chronic granulomatous communicable bacterial infectious disease caused by *Mycobacterium tuberculosis* and other species of same genera. "Rifabutin" which is useful in the management of tuberculosis. Clinical management of tuberculosis poses serious problem because the efficacy of chemotherapy has been reduced which may be attributed to the degradation of drugs before reaching the target, the low level of cell permeability to drugs, or primary drug resistance. Other reason for the failure of chemotherapy may be the difficulty in achieving adequately high concentration at the infection site, inadequate penetration of drug into macrophages and low level in cells. These problems, which arise with conventional dosage forms of antitubercular drugs, may be overcome by designing and developing a site specific delivery of antitubercular drug using surface modified solid lipid nanoparticles. Hence in the present study, it was attempted to formulate Rifabutin in the form of solid lipid nanoparticle. Solid lipid nanoparticles of Rifabutin were obtained by adaption of lipid dispersion method. Preformulation studies were performed to check the compatibility of drug and excipient for the preparation of formulation by DSC and no interaction was found. Solubility study, partition coefficient determination, UV analysis, HPLC study, FTIR study were also performed. After the preformulation studies Rifabutin loaded solid lipid nanoparticles was also prepared. Hence it was concluded that solid lipid nanoparticle of Rifabutin could be formulated.

KEYWORDS: Rifabutin, Tuberculosis, solid lipid nanoparticle, preformulation studies.

INTRODUCTION

Tuberculosis is a ubiquitous, highly contagious chronic granulomatous communicable bacterial infectious disease caused by *Mycobacterium tuberculosis* and other species of same genera. Tuberculosis, which is easily transmitted through the air, already infects 1.9 billion people, and takes the lives of about two million people each year. The situation has been exacerbated because of the presence of numerous other complicating factors like multi drug resistant tuberculosis and HIV-coinfection. Tuberculosis is a leading cause of death amongst infectious diseases. Furthermore, this re-emerging disease has become one of the most important infections affecting human immunodeficiency virus (HIV)-positive patients worldwide. TB also is becoming increasingly resistant to existing drugs. It is estimated by the World Health Organization (WHO) that more than 2 billion people in the world are infected with *Mycobacterium tuberculosis*.^[1]

Mycobacterial infection is a challenging health problem that requires particular attention worldwide. The

characters of *Mycobacterium tuberculosis* are distinctly contrast to many other common bacteria.^[2]

The mycobacterium cell wall, lipid (e.g. mycolic acid) are linked to the underlying arabinogalactan and peptidoglycan. The structure is responsible for very low permeability of cell wall and thus for ineffectiveness of most of antibiotic against the organism. Clinical management of tuberculosis poses serious problem because the efficacy of chemotherapy has been reduced which may be attributed to the degradation of drugs before reaching the target, the low level of cell permeability to drugs, or primary drug resistance. Other reason for the failure of chemotherapy may be the difficulty in achieving adequately high concentration at the infection site, inadequate penetration of drug into macrophages and low level in cells. These problems, which arises with conventional dosage forms of antitubercular drugs, may be overcome by designing and developing a site specific delivery of antitubercular drug using surface modified solid lipid nanoparticles.^[3]

Advantages of SLNs^[4-6]

- Possibility of controlled drug release and drug targeting.
- Increased drug stability.
- High drug payload.
- Incorporation of lipophilic and hydrophilic drugs feasible.
- No biotoxicity of the carrier.
- Avoidance of organic solvents.
- Large-scale production and sterilization is easy.
- Good tolerability.
- High bioavailability.
- Better protection to drug against chemical degradation.
- No or little access of water to drug in the inner core of lipid particles.

Rifabutin is a first line drug for the treatment of tuberculosis and it is a derivative of Rifamycin S. It contains not less than 950 μg and not more than 1020 μg of $\text{C}_{46}\text{H}_{62}\text{N}_4\text{O}_{11}$ per mg, calculated on the anhydrous basis.

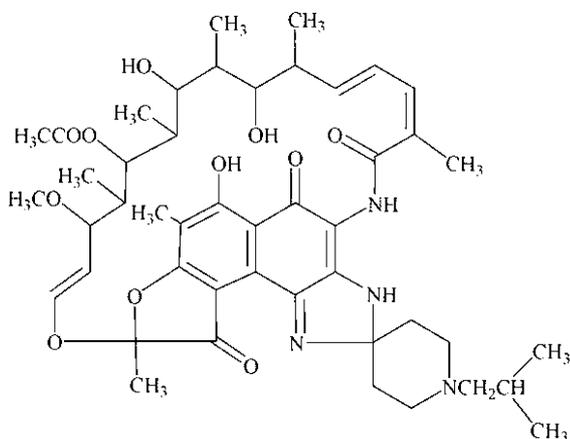


Table 1: Solubility profile of rifabutin.

S. No.	Solvent	Solubility
1.	Water	Insoluble
2.	PBS (pH 7.4)	Slightly soluble
3.	Sodium acetate buffer (pH 4.0)	Slightly soluble
4.	Methanol	Freely soluble
5.	Ethanol	Sparingly soluble
6.	Ether	Insoluble
7.	Chloroform	Freely soluble

+++ = Freely soluble 1-10 parts,

+++ = Sparingly soluble 30-100

++ = Slightly soluble 100-1000 Parts

- = Practically insoluble >10000 Parts

Determination of λ_{max}

Accurately weighed 10 mg of rifabutin was dissolved in 100 ml of methanol in a 100 ml volumetric flask. Then, 1 ml of this stock solution was pipetted into a 10 ml volumetric flask and volume made up to the mark with distilled water. The resulting solution was scanned between 200-400 nm using Cintra 10 GB UV-visible spectrophotometer. The λ_{max} was found to be 275 nm (Fig. 2.1). The same procedure was followed

Chemical Structure of Rifabutin

Preformulation studies are needed to ensure the development of a stable as well as therapeutically effective and safe dosage form. The preformulation studies, which were performed in this project, include identification of drug, solubility analysis, partition coefficient and drug compatibility with the lipids.^[7]

MATERIAL AND METHODS**1. Identification Tests****Physical Appearance**

The drug (Rifabutin) was obtained as a gift sample from Lupin Pharma Pvt.Ltd, Pune. The supplied sample of rifabutin was red-voilet, crystalline, odorless, hygroscopic powder.

Melting point

Melting point of rifabutin was determined by melting point apparatus (Tempo, Mumbai) and found to be 153 ± 2 °C.^[8]

Solubility

The sample was qualitatively tested for its solubility in various solvents⁹. It was determined by shaking 10 mg of drug sample in 10 ml of solvent (i.e., water, methanol, ethanol, ether, chloroform, benzene etc.) in small test tubes and noted down the time require to disappear the sample completely. Solubility profile of rifabutin is recorded in Table 1.

for determining the λ_{max} in PBS (pH7.4) and sodium acetate buffer of pH 4.0 except methanol was replaced with the respective solutions. The resulting solution was scanned between 200-400 nm using Cintra 10 GB UV-visible spectrophotometer. The λ_{max} was found to be 275 nm in these buffers also (Fig.1 to 3).

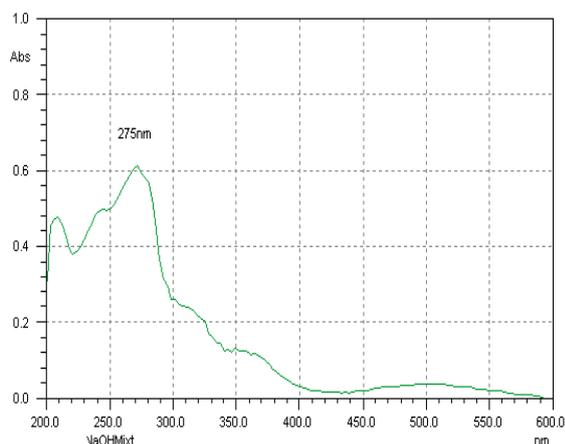


Fig 1: UV scan of rifabutin in Methanol.

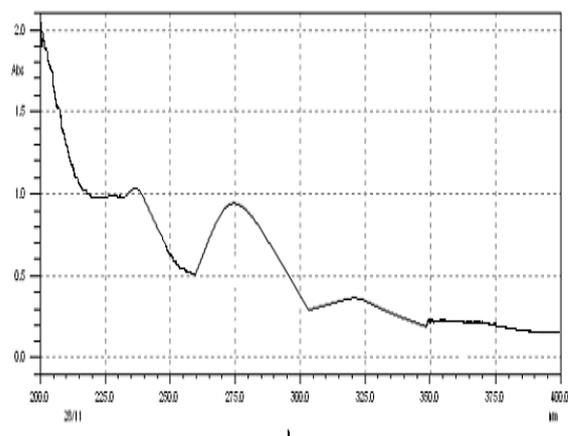
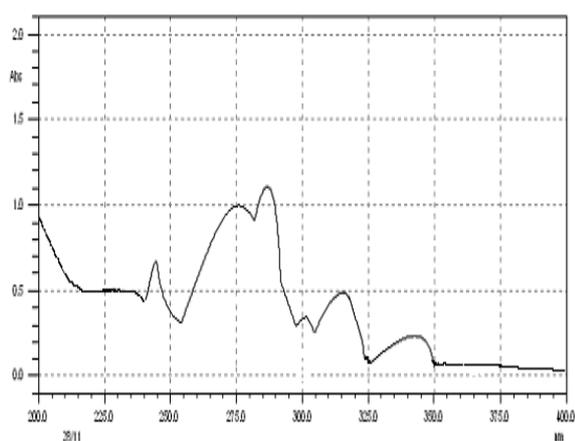


Fig 3: UV Scan of rifabutin in sodium acetate buffer (pH 4.0).



Figs 2: UV scan of rifabutin in PBS (pH7.4)

Infrared Spectroscopy

Drug sample was vacuum dried for 12 hours before IR studies. Drug (5mg) was mixed with potassium bromide (100mg) and compressed into pellets. The IR spectrum was taken in Department of Sophisticated analytical instrument facility in Panjab University, Chandigarh. The observed peaks were reported for functional groups (Fig4 and Table 2).

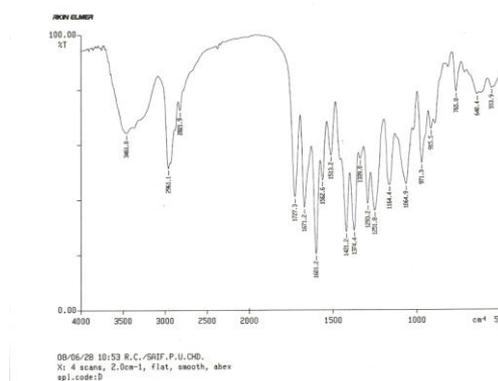


Fig. 4: IR spectrum of rifabutin sample.

Table 2: Important band frequencies in IR spectrum of rifabutin.

S.No.	IR Absorption band cm^{-1}	Assignments
1.	3461.0	O-H stretching (Alcohol, ROH)
2.	2961.1	Aliphatic C-H stretch
3.	2821.9	C-H stretch (Carboxylic acid)
4.	1727.3	C=O stretch of aldehyde
5.	1671.2	C=O stretch (cyclic amide)
6.	1601.2	C=C stretch Benzene (aromatic)
7.	1526.6	Asymmetric (ArNO_2) stretch
8.	1421.2	O-H bending (Carboxylic acid)
9.	915.5	C-H out of plane bend (alkenes)
10.	970	C-H out of plane bend (trans RCH=CHR)
11.	1064.9	C-N stretch (Amine)
12.	1164	C-O stretch (3 ROH)
13.	765.8	C-H out of plane bend (Ortho disubstituted benzene)

Partition coefficient

The partition behavior of drug was examined in n-octanol: water, n-octanol: PBS (7.4) system. It was determined by taking 5 mg of drug in two separating funnels one containing 10 ml portions of n-octanol and 10 ml water and the other containing, 10 ml of n-octanol and 10 ml of PBS (pH 7.4) respectively. The separating funnels were shaken for 2 hr in a wrist action shaker for equilibration. Two phases were separated and the amount

of the drug in aqueous phase was analyzed spectrophotometrically at 275 nm after appropriate dilution (Table 3). The partition coefficient of the drug was calculated by using the following formula.^[10]

The partition coefficient, $K =$

$$K = \frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}}$$

Table 3: Partition coefficient values of rifabutin

S. No.	Medium	Partition coefficient (n-octanol/aq. Phase)
1.	n-Octanol : Water	3.1
2.	n-Octanol : PBS pH (7.4)	2.9

Preparation of Standard Curve of rifabutin in different Solutions

Preparation of standard curve of rifabutin in Methanol

Accurately weighed 10 mg of rifabutin was dissolved in methanol and volume was made up the mark in a 100 ml volumetric flask. This resulted in 100 µg/ml stock solution. The aliquots of 0.2 ml, 0.4 ml, up to 2 ml of stock solution were transferred into a series of 10 ml volumetric flasks and volume was made up to the mark with double distilled water. The solutions were filtered through whatmann filter paper and filtrate analyzed at λ_{\max} 275 nm using Cintra 10 UV Visible Spectrophotometer. The standard curve was plotted between absorbance and concentration. (Fig. 6).

Standard curve of Rifabutin in phosphate buffer solution (pH 7.4)

Preparation of PBS pH 7.4

Disodium hydrogen orthophosphate (1.38g), potassium dihydrogen orthophosphate (0.19 g) and sodium chloride (8.0g) were added to about 100 ml of distilled water and the volume was made up to 1000 ml with distilled water. The pH of solution was adjusted to 7.4 immediately before use with 0.1 N hydrochloric acid or 0.1 N NaOH as required.

Preparation of standard curve in PBS pH 7.4

For the preparation of standard curve in PBS pH 7.4, all dilutions and measurements were made same as discussed above except the methanol was replaced with phosphate buffer saline pH 7.4. The absorbance of different drug solutions was taken at λ_{\max} 275 nm against a reagent blank. The standard curve was plotted between absorbance and concentration (Fig. 7).

Standard curve of Rifabutin in sodium acetate buffer solution (pH 4.0)

Preparation of sodium acetate buffer solution (pH 4.0)

Glacial acetic acid (2.78 ml) and 50% w/v of sodium hydroxide (1ml) were mixed and the volume was made up 100 ml with distilled water, the pH of solution was adjusted to 4.0 immediately before use with acetic acid.

Preparation of standard curve in sodium acetate buffer solution (pH 4.0)

For the preparation of standard curve in sodium acetate buffer solution (pH 4.0) all dilutions and measurements were made same as discussed above except the methanol was replaced with sodium acetate buffer solution (pH 4.0). The absorbance of different drug solutions was taken at λ_{\max} 275 nm against a reagent blank. The standard curve was plotted between absorbance and concentration (Fig. 8).

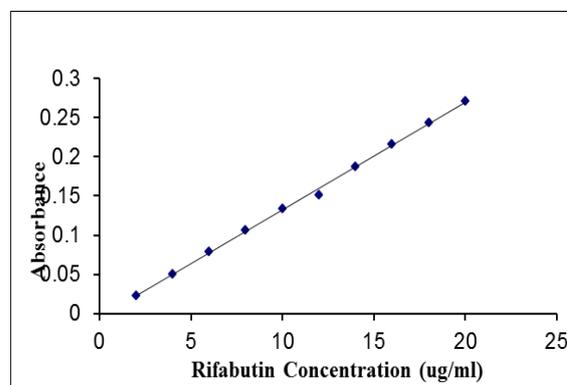


Fig. 6: Standard curve of rifabutin in methanol (λ_{\max} = 275 nm).

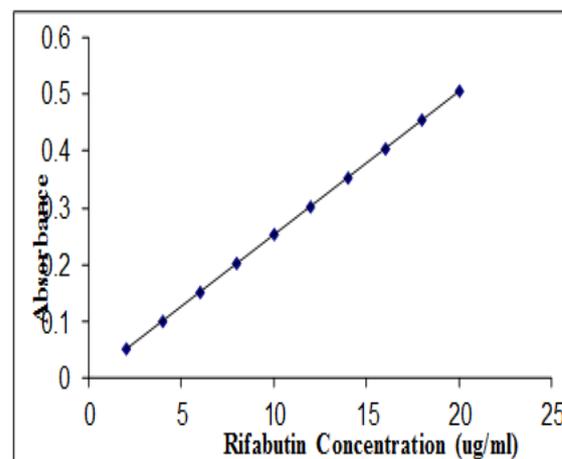


Fig. 7: Standard curve of rifabutin in PBS (pH 7.4) λ_{\max} 275 nm.

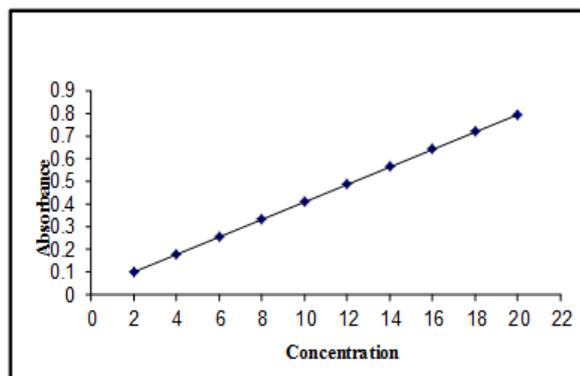


Fig. 8: Standard curve of rifabutin in buffer solution (pH 4.0) at λ_{\max} 275 nm.

Drug Compatibility Studies with Selected Lipids

Drug compatibility with tristearin, phosphatidyl choline (PC) and stearylamine was studied. Solution of rifabutin (8 μ g/ml) was prepared in methanol. Then, accurately weighed lipid (10 mg) was transferred separately into 10 ml volumetric flasks containing drug solution. The flasks were shaken for 3 hours and absorbance was measured for each solution using Cintra-10 UV Spectrophotometer against respective blank solution. The absorbance data are recorded in Table 4.

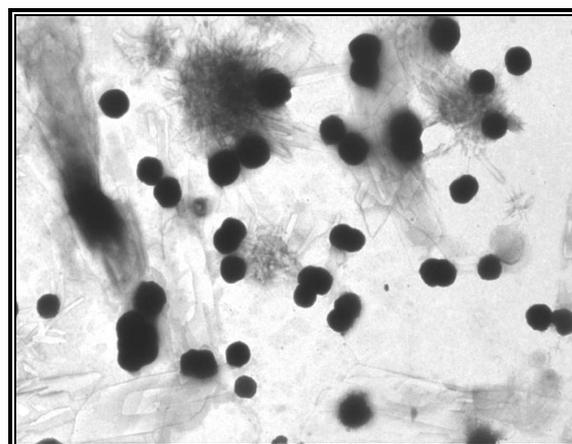
Table 4: Drug compatibilities studies with selected lipids.

S. No.	Composition	Absorption maxima (λ_{\max}) nm	Absorbance
1.	Rifabutin (8 μ g/ml)	275	0.2990
2.	Rifabutin+ tristearin	275	0.2950
3.	Rifabutin + Lecithin	275	0.2705
4.	Rifabutin+ Stearylamine	275	0.2497

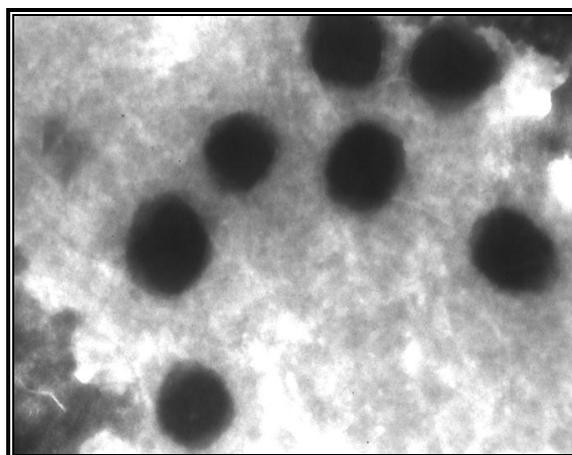
PREPARATION OF SOLID LIPID NANOPARTICLES

Solvent injection method reported by Schubert *et al.*, (2003) was used in the present work for the preparation of solid lipid nanoparticles. In this method, solid lipid nanoparticles (SLNs) can be prepared by rapidly injecting a solution of solid lipids in water-miscible organic solvents or a water-miscible organic solvent mixture into water. This method offers clear advantages over the existing methods such as the use of pharmaceutically acceptable organic solvents, no need for high-pressure homogenization, easy handling and a fast production process without use of technically sophisticated equipments. It is based on precipitation of lipid from dissolved solution. For this purpose, solution of the lipid in a water-miscible solvent or a water-miscible solvent mixture is rapidly injected into an aqueous phase with or without surfactant.^[11-13]

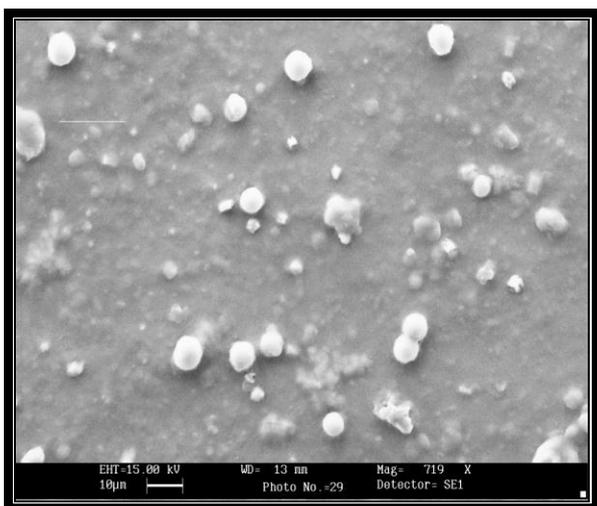
Briefly, tristearin, soya lecithin, stearylamine and drug (Rifabutin) were dissolved in a ethanol at 70°C and was then rapidly injected through an injection needle into a stirred aqueous phase maintained at same temperature containing the surfactant, Tween 80 which was previously dissolved in the aqueous phase. The resulting dispersion was then filtered with a paper filter in order to remove any excess lipid^[11]. Plain and drug loaded SLNs formulations were characterized by Scanning and Transmission electron microscopy (SEM and TEM) at AIIMS, New Delhi.



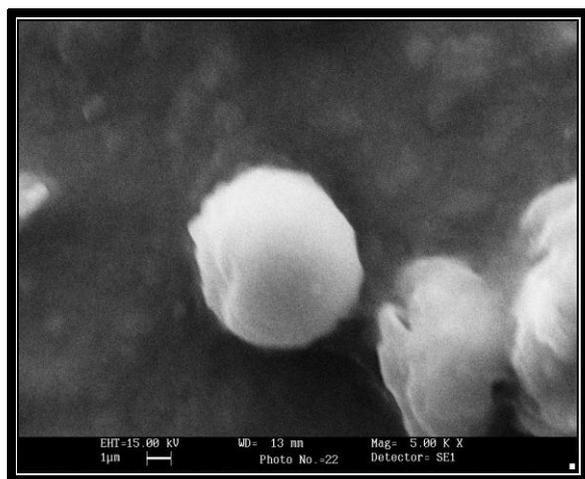
TEM of rifabutin loaded SLNs



TEM of rifabutin-loaded mannosylated SLNs.



SEM of rifabutin loaded SLNs.



SEM of rifabutin-loaded mannosylated SLNs.

RESULTS AND DISCUSSION

Rifabutin was obtained as a gift sample from Lupin Pharma pvt. Ltd. Pune, which was identified and tested for purity as per tests given in I.P.1996. The infrared spectrum of drug was satisfactory. Solubility studies in different solvents at room temperature suggested that it is soluble in methanol and chloroform, insoluble in distilled water.

Partition coefficient value of rifabutin also confirmed its lipophilic nature as it was found to be 3.1 in n-octanol/water system and 2.9 in n-octanol/PBS pH 7.4. Spectrophotometric method of analysis of rifabutin showed λ_{\max} at 275 nm in methanol, PBS (pH 7.4) and sodium acetate buffer (pH 4.0). The standard curves of rifabutin were prepared in methanol, PBS 7.4 and sodium acetate buffer pH 4.0 at λ_{\max} 275 nm and the absorbance data obtained subjected to linear regression. The correlation coefficients were found to be 0.9978, 0.9991 and 0.9953, in methanol, PBS (pH 7.4) and sodium acetate buffer solution (pH 4.0) respectively. The drug-lipid compatibility study was done spectrophotometrically and it was found that drug is

compatible with the lipids as no significant change in absorption maxima was observed in any case.

FUTURE PROSPECTS

Loading on to solid lipid nanoparticles can reduce the hemolytic toxicity of rifabutin. The solid lipid nanoparticles also hold a promising potential for treating the single or multiple ailments to improve patient compliance and reduce toxicity. However rigorous attempts need to be drawn to attain any unambiguous generalization.

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