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Formulation of Rifabutin Liquisolid system using mixed solvency concept and their evaluation

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Abstract
Rifabutin is broad spectrum antimicrobial drug, used for the treatment of infection caused by *M. tuberculosis*, *M. avium* and *M. Leprae* and also used in the treatment of multidrug resistant TB. Rifabutin is poorly water soluble drug (0.19mg/ml) with high permeability. In the present study, Rifabutin Liquisolid system were prepared using mixed solvency concept for the improvement of solubility and dissolution rate that improve drug release and oral bioavailability. Different blends were prepared using various solid solubilizers like sodium benzoate, sodium caprylate and niacinamide in propylene glycol (co-solvent). In this blend K (35%SC+5%NM+5%SB) gives highest solubility and selected for further study. Liquisolid system was prepared using blend K, microcrystalline cellulose as carrier material and aerosil as coating material. In all the formulations, formulation LSS-01 was selected as best formulation as it released 98.20% of drug in 1 hrs. The prepared Liquisolid system (LSS-01) using mixed solvency concept was evaluated for Drug content, X-RD analysis, dissolution study and stability study. Drug content in LSS-01 was found to be 100.24± 0.04% & in Physical mixture was found to be 98.9± 0.03%. X-ray diffraction study showed that the crystallinity of drug is not much reduced. Drug release profile of optimized batch LSS-01 showed enhanced dissolution behavior (98% in 60min.) as compare to pure drug (46.26±0.011), physical mixture (51.42±0.006) and marketed formulation (48.92±0.009).

Keywords: mixed solvency, liquisolid system, rifabutin, solubility enhancement, solubilizers, synergistic

1. Introduction
Solubility in quantitative terms defined as the concentration of the solute in a saturated solution at a certain temperature and in the qualitative terms, defined as the spontaneous interaction of two or more substances to form a homogenous molecular dispersion [1].

There are varieties of new drug and their derivatives are available. But less than 40% of lipophillic drug candidates fail to reach market due to poor bioavailability, even though these drugs might exhibit potential pharmaco-dynamics activities. Therefore it is necessary to enhance the solubility of poorly water soluble drug. Rifabutin is a first line drug use in the treatment of TB with high permeability and low solubility [2]. Mixed solvency concept proposed by R.K. Maheshwari. He is one of the opinion that all substances whether liquid, gases or solids has got solublizing property. As per his statement, each substance is solubilizer. A concentrated aqueous solution containing various water soluble substances makes it strong solvent for poorly water soluble drugs. Such concentrated solutions may show synergistic or additive solubilizing actions of solubilizers present in the solution for a particular solute [3].

Liquisolid system is novel technique which involves the water insoluble drugs dissolved in non-volatile solvent and this liquid medication can be converted into free flowing, non-adherent and compressible powder with the use of carrier and coating material. When drug is dissolved in non volatile solvent and mixed with carrier materials which have a porous surface as a result of absorption and adsorption take place. After this process of adsorption of liquid onto the external and internal surface of the porous carrier particle occurs then coating material added [4].

2. Materials and Methods
Materials
Rifabutin was obtained as gift sample from Lupin Ltd. Aurangabad. Sodium caprylate and Niacinamide were obtained from Ambika lab. Indore. Other ingredients and excipients used were laboratory analytical grade

Methods
Preformulation Study of Drug
Preformulation studies are needed to ensure the development of stable as well as therapeutically effective and safe dosage form [5]. The preformulation studies, which were performed in this project include identification of drug, partition coefficient, solubility analysis, development of solvent system and drug solubilizer interference study.

Identification of Drug
The melting point of drug was determined by Thiele tube. The λ_{max} of Rifabutin was determined in methanol. For this 10mg of rifabutin was accurately weighed and dissolved in 100ml of methanol in 100ml of volumetric flask. Then, 1ml of this solution was pipette into a 10ml volumetric flask and volume was made upto 10ml with distilled water. The resulting solution was scanned between 200-400nm using double- beam UV- visible spectrophotometer (Shimadzu 1800) [5].

Small quantity of rifabutin was placed on holder and the FTIR spectrum of drug was recorded in the wave number region of 400-4000cm-1 on FTIR spectrophotometer (Shimadzu Affinity 1) [6].

Calibration Curve by U.V. Spectroscopy Method
a) Preparation of Stock Solution of Rifabutin in Presence of Sodium Caprylate
Rifabutin was accurately weighed (20mg) and transferred to 50ml volumetric flask. To this 20ml of 25% w/v sodium caprylate solution was added to dissolve the drug and the volume was made up to 50ml with distilled water to prepare 400µg/ml solution. The solution thus produced was

sufficiently diluted with distilled water to obtain 40µg/ml solution. It was scanned on double- beam UV- visible spectrophotometer (Shimadzu 1800) between wavelength 200-400 nm against reagent blank. The λ_{max} was found to be at 322nm.

b) Preparation of Calibration Curve in Presence of Sodium Caprylate

Rifabutin was accurately weighed (20mg) and transferred to 50ml volumetric flask. To this 20ml of 25% w/v sodium caprylate solution was added to dissolve the drug and the volume was made up to 50ml with distilled water to prepare 400µg/ml solution. Appropriate dilutions from the stock solution were made in concentration range of 20-80 µg/ml. The absorbances were noted at 322nm against respective reagent blank [7].

Partition Coefficient

The partition coefficient of rifabutin was examined in n-octanol: water system. It was determined by taking 10mg of drug in separating funnel, containing 10ml of n- octanol and 10ml of water. The separating funnel was shaken for 1 hour. The drug was completely dissolved in two phases, i.e. there were no undissolved drug particles. Two phases were separated and the amount of drug in aqueous phase was analyzed spectrometrically at 322nm after appropriated dilution. Partition coefficient was calculated using formula [5]

Partition coefficient=

$$\frac{\text{concentration of drug in organic phase}}{\text{concentration of drug in aqueous phase}} \text{ ----- (1)}$$

Solubility of Rifabutin in Different Solvent

The solubility of Rifabutin was determined in propylene glycol, PEG 400 & water. Excess quantity of Rifabutin was added to each vial containing 5ml of solvent. As the saturation point was reached a pinch of drug was added to it and the flask was shaken for 15min and placed in the flask shaker for 24 hrs. After 24 hrs it was removed and observed. Since un-dissolved drug was found it was kept for 24hrs undisturbed. After 24 hrs, the solution was filtered and diluted suitably with reagent blank and absorbance was taken against reagent blank and recorded.

Development of Solvent Systems

Since the determined solubility in Propylene glycol and in PEG 400 is less than the desired solubility, so the mixed solvency approach was used to create a solvent system in which various solid solubilizers were dissolved as per their respective safe concentrations in the propylene glycol and making propylene glycol a strong solvent which can be use for preparation of dosage form. Various solid solubilizers were used individually; blends of solubilizers and drug solubility (approximate) studies were performed by weight difference method [7].

Table 1: Approximate Solubility of Rifabutin in Solution of Different Solubilizers and their Blends

S. No.	Blend	Solubilizers	Approximate solubility (mg/ml)	Solubility enhancement ratio
1	A	10% Sodium Benzoate (SB)	75	1:394
2	B	10% Sodium Caprylate (SC)	100	1:526
3	C	10% Niacinamide (NM)	70	1:368
4	D	20%SC+5% SB	200	1:1052
5	E	20% SB+ 5% NM	200	1:1052
5	F	10%SC+10%NM+10%SB	400	1:2105
6	G	20%SC+10%NM+10%SB	360	1:1894
7	H	25%SC+10%NM+5%SB	150	1:789
8	I	25%SC+5%NM+5%SB	225	1:1184
9	J	30%SC+5%SB+5%NM	370	1:1947
10	K	35%SC+5%NM+5%SB	500	1:2631

Determination of Equilibrium Solubility in Selected Solubilizers

On the basis of result of approximate solubility in different blends; F, G, J and K blends were selected for equilibrium solubility. For determining solubility, precisely measured 5ml of a particular blend was taken in 10 ml volumetric flask and excess amount of drug was added and shaken until the saturated solution was formed. The volumetric flask was shaken on mechanical shaker for 12h so that equilibrium solubility can be achieved and solution was allowed to equilibrate for 24h. Then solution was centrifuged at 2000rpm for 5min in ultracentrifuge and then solution was filtered through Whatsman filter paper. Aliquot was suitably diluted with distilled water and analyzed using UV spectrophotometer at 322nm against respective reagent blanks.

Drug- Solubilizers Interference Study

Thin Layer Chromatography

In order to examine the possibility of interaction between drug and excipients, thin layer chromatography studies were performed. A plate of silica gel was activated at 110° C for 1 hour and used. The methanolic solution of drug alone and the methanolic solution drug plus various excipients (Rifabutin+ sodium benzoate, Rifabutin+ sodium caprylate, Rifabutin+ Niacinamide, Rifabutin+ MCC and Liquisolid system of rifabutin prepared using blend K) were spotted on the base line with the help of capillary. Then the plate was left in air for 10min to dry and transferred to a solvent jar, saturated with solvent containing acetone. The solvent system was allowed to run. Then plate was transferred to an oven maintained at 80° C for 5 min. and then observed [8].

Formulation

i) Selection of Carrier

Determination of Amount of Carrier Required to Prepare Liquisolid System

Drug solution equivalent to 150 mg of drug was transferred to cleaned dried pestle mortar and triturate. Trituration yielding a reddish colored clear solution. Into the solution gross amount of carrier was added and allowed to adsorb the drug. The mixture was then triturated to allow and check the uniform mixing and adhesiveness of the powder and remaining amount of carrier was again added to reduce adhesiveness.

Different carriers and combination in various proportions of

the same were used to adsorb drug solution and batches were developed [7].

Dissolution Profile of Different Liquisolid System

Dissolution profile of each batch was studied to select the most suitable batch for scale up. For dissolution, distilled water was taken as media and the paddle rotation speed was kept at 50rpm at 37±0.5°C in 900 ml of distilled water.

ii) Preparation of Fast Release Capsule

Blend k was taken (0.3ml) and accurately weighed 150mg drug was dissolved in it by trituration. Trituration yielding a reddish coloured clear solution. Into the solution gross amount of carrier was added and allowed to adsorb the drug. Coating material (Aerosil) in 1% was added in it. Then prepared Liquisolid mass filled manually in size "00" capsule.

Table 2: Final Formula for the Preparation of Liquisolid System

S. No.	Ingredients	Quantity	Function
1.	Rifabutin	150 mg	Drug
2.	Sodium Caprylate	3.5g	Solubilizer
3.	Sodium Benzoate	500mg	Solubilizer
4.	Niacinamide	500mg	Solubilizer
5.	MCC	650mg	Carrier material

Evaluation

The prepared Liquisolid capsule were evaluated for the following properties.

Drug Content of Formulated PM and LSS-01

For the determination of drug content, Liquisolid system LSS-01 and physical mixture (PM) was taken separately and equivalent to 150mg of drug powder was transferred in 1000 ml volumetric flask, 800ml of distilled water was added and it was briskly shaken for 15 minutes and the volume was made up to 1000ml. after filtration, the filtrate was approximately diluted with distilled water and the absorbance was then noted at 322nm against blank.

Powder X-ray Diffraction Studies of Formulated PM and LSS-01

The powder X-ray diffraction spectra of the prepared physical mixtures and LSS-01 formulation were obtained using Bruker D8 advance x-rays diffractometer with tube copper anode over the interval 5 to 60 ^{0} of 2Ɵ. The x-ray were produced using a sealed tube and the wavelength of x-ray was 0.154nm (Cu-K-alpha). The operation data were as follows: generator current 30mA and scanning speed 0.02^0/min. The x-ray were detected using a fast counting detector based on silicon strip technology (Bruker LynxEye detector) [9].

Comparative Dissolution Study

The dissolution of the prepared batch of Liquisolid system (LSS-01), Bulk drug sample of Rifabutin (PD), Physical mixture (PM) and marketed formulation was performed. The in-vitro dissolution study was determined by using USP type 2 (Paddle type) dissolution apparatus. For dissolution, distilled water was taken as media and the rotation speed was kept at 50rpm at 37±0.5°C in 900ml of distilled water.

Stability Study

Physical mixture of drug and LSS-01 formulation were subjected to chemical stability testing. Physical mixture and LSS-01 formulation were kept in 10ml amber colored glass vials and vials are plugged and sealed. Vials are kept at 40˚C±2°C and 75± 5%RH. The samples were withdrawn at different time intervals and drug contents were determined by UV spectrophotometric analysis. The initial drug content for each formulation was considered as 100.00%.

3. Result and Discussion

Melting Point

Table 3: Melting Point of Drug

S. No.	Drug	Melting Pont	
		Standard	Experimental*
1	Rifabutin	169-171°C	168-171°C

*average of 3 determinants

U.V. Spectrum

Rifabutin shown absorbance at 238nm in methanol (Fig.1). While reported λ max of drug is also 238nm [10].

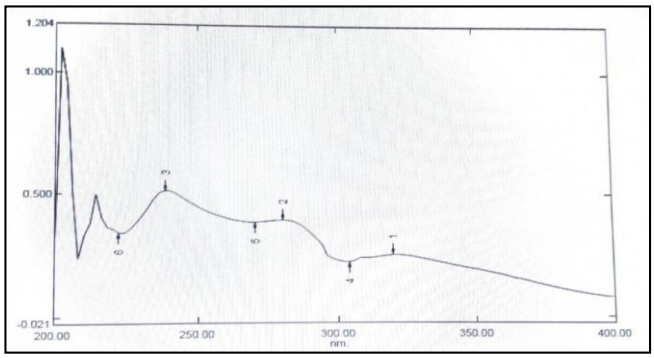

Fig 1: UV Absorbance Spectra of Rifabutin

FTIR Spectrum

For characterization of pure Rifabutin FTIR studies were carried out. The observed and reported characteristic peaks of functional group have been shown in table 4 and FTIR spectrum is shown in (Fig.2).

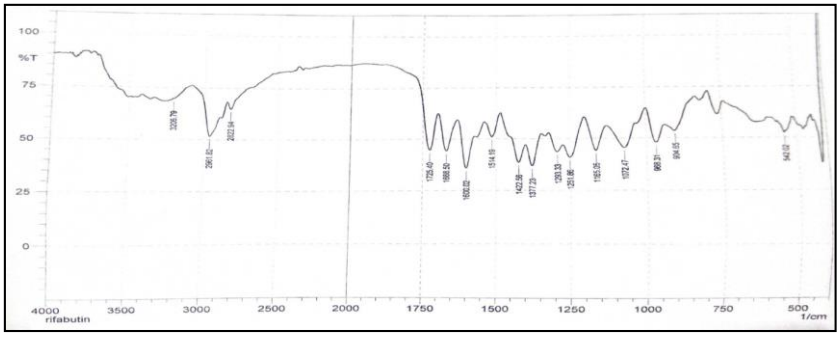

Fig 2: FTIR Spectra of Rifabutin

Table 4: Reported and Observed Principal Peaks of Rifabutin in FTIR

S. No.	Interpretation	Observed Wave no.	Reported Wave no.[9]
1	Secondary alcohol	1072.47	1067.97
2	C=O stretching	1251.86	1247.86
3	N-H deformation of 2° amine	1647.10	1647.10
4	N-H stretching	3206.79	3215.09
5	Stretching vibration of C=O group	1668.5, 1725.4	1670.24, 1730.03
6.	C-H deformation of CH_2	1422.46, 1377.23	1458.08, 1373.22
7.	Stretching vibration of C=C	1600.02	1600.81

Pure Rifabutin showed principal absorption peaks at 1251.86 cm^{-1} (C=O stretching), 1647.10 cm^{-1} (N-H deformation of 2° amine), 3206.79 cm^{-1} (N-H stretching), 1668.5, 1725.4 cm^{-1} (Stretching vibration of C=O group) 1072.47 cm^{-1} (Secondary alcohol) which resembles with groups present in drug molecule. This indicates that the drug has maintained its identity without losing its characteristic properties.

Calibration Curve by U.V. Spectroscopy Method

a) Preparation of Stock Solution of Rifabutin in Presence of Sodium Caprylate

Rifabutin shown absorbance at 322nm in presence of sodium caprylate (Fig. 3).

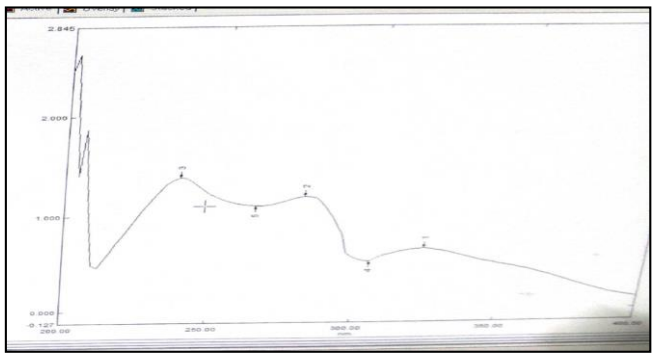

Fig 3: UV Absorbance Spectra of Rifabutin in Presence of Sodium Caprylate

b) Preparation of Calibration Curve of Rifabutin in Presence of Sodium Caprylate

A linear relationship was obtained in Beer-Lamberts plot of Rifabutin (y =0.011x+0.013, R^2=0.998). The calibration data is given in Table 5 and graph obtained after plotting absorbance (y) vs. concentration (x) is shown in Fig.4.

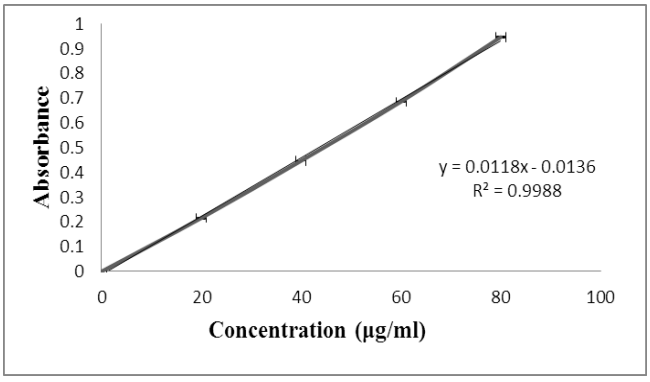

Fig 4: Calibration Curve of Rifabutin in Presence of Sodium Caprylate

Partition coefficient

Partition coefficient was found by shake flask method. And the value was found to be

Table 5: Partition Coefficient value of Rifabutin

S. No.	Medium	Partition Coefficient (n-octanol/aq. Phase)	
		Experimental*	Standard
1.	n-octanol : water	3.2	3.1

*Average of 3 determinants

Solubility in different solvent

Equilibrium solubility of rifabutin in different solvents (PG, PEG 400 and water) were determined by excess solute method.

Table 6: Solubility Profile of Rifabutin in Different Solvent System

S. No.	Solvent	Solubility* mg/ml (At RT)
1.	Water	0.19±0.021
2.	Propylene Glycol	13.99±0.010
3.	PEG 400	8.78±0.019

*****Average of 3 determinants

Equilibrium Solubility in Selected Solubilizers

Different blends were prepared in propylene glycol and equilibrium solubility was determined for selected solubilizers. From the result of solubility data it was concluded that aqueous solubility of rifabutin was increased more than 2500 times in blend K containing 35 % Sodium Caprylate, 5% Sodium Benzoate and 5% Niacinamide.

Table 7: Equilibrium solubility of Rifabutin in solution of selected solubilizers

S. No.	Blend	Solubilizers	Equilibrium solubility*(mg/ml)	Solubility enhancement ratio
1	F	10%SC+10%NM+10%SB	375± 0.016	1:1973
2	G	20%SC+10%NM+10%SB	350±0.011	1:1842
3	J	30%SC+5%SB+5%NM	364±0.019	1:1915
4	K	35%SC+5%NM+5%SB	492±0.012	1:2589

*Average of 3 determinants

Drug- Solubilizers Interference Studies (TLC study)

TLC method was also employed for drug solublizer's interference study. The result of TLC study revealed that there are no considerable changes in R_F values and no changes in number of spots of pure drug, drug with excipients and Liquisolid system of drug.

Table 8: R_F Values of Rifabutin Alone and with Excipients

System	R_F value	No. of spot
Rifabutin in methanol	0.84	01
Rifabutin+ SB in methanol	0.83	01
Rifabutin+ SC in methanol	0.83	01
Rifabutin+ NM in methanol	0.84	01
Rifabutin+ MCC in methanol	0.84	01
Physical mixture in methanol	0.83	01

Formulation

i. Selection of Carrier

Determination of Amount of Carrier Required to Prepare Liquisolid System

The fast release capsule of rifabutin was prepared by Liquisolid technique using propylene glycol as non- volatile solvent, blend K (35% SC, 5% SB, 5% NM) and different carriers in various proportions.

Table 9: Carrier and Their Amount Used

Batch no.	Carrier	Amt. of carrier used (mg)	Blend used	Volume of blend used	Net weight (mg)
LSS-01	MCC	650	K	0.3	850
LSS-02	$MgCO_3$	600	K	0.3	800
LSS-03	Ethyl cellulose	500	K	0.3	752
LSS-04	Mannitol	800	K	0.3	1069
LSS-05	Ethyl cellulose+ Mannitol	800	K	0.3	1000

1.2 Dissolution Profile of Different Liquisolid System

Dissolution profile of Liquisolid system of rifabutin prepared using different carriers, and it is observed that LSS-01 gives better drug release. So, LSS-01 selected for further study.

Table 10: Dissolution Profile of Different Batches Using Different Carriers

Time (min.)	% Cumulative drug release				
	LSS-01 (MCC)	LSS-02 ($MgCO_3$)	LSS-03 (Ethyl cellulose)	LSS-04 (Mannitol)	LSS-05 (Ethyl cellulose+ Mannitol)
00	00	00	00	00	00
02	51.74	39.21	31.04	21.60	23.7
05	60.86	44.12	37.34	36.97	35.81
10	69.93	49.23	43.88	40.52	41.09
15	78.92	51.3	55.69	47.8	48.86
30	83.14	54.67	64.79	53.09	57.1
45	89.92	60.92	71.12	63.90	69.50
60	98.20	71.16	77.78	72.48	77.91

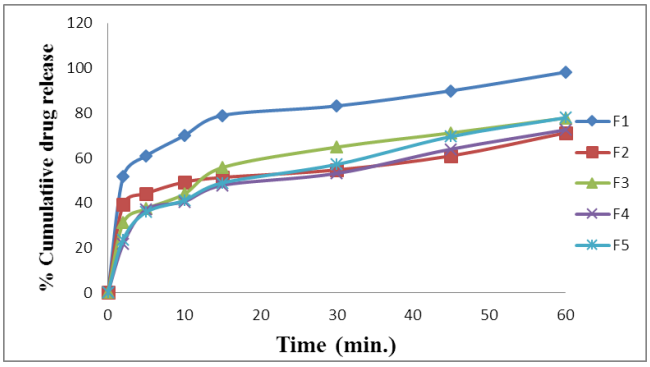

Fig 5: Dissolution Profile of Various Batches of Liquisolid System

4. Evaluation

Drug Content of Formulated PM and LSS-01

Drug content of LSS-01 and Physical mixture was determined. The result showed that drug content in LSS-01 was 100.24± 0.04% and Physical mixture of that gives 98.9± 0.03% drug content.

Table 11: Drug Content in LSS-01 and PM

Batch No.	Percent drug content (mean ± S.D.)
LSS-01	100.24± 0.04
Physical Mixture	98.9± 0.03

Powder X-ray Diffraction Studies of Formulated PM and LSS-01

The physical mixture showed intense peaks due to crystallinity. The peaks in the Liquisolid system (LSS-01) sample showed less intense peaks at same 2Ɵ values which shows that the crystallinity of drug is not much reduced.

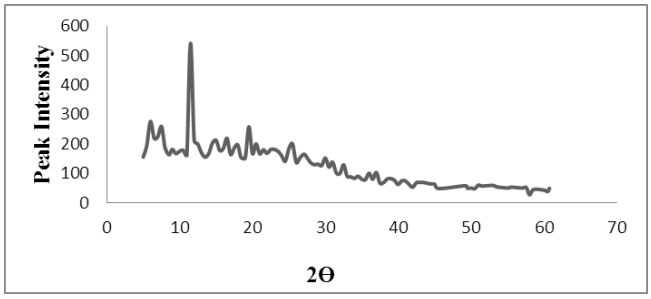

Fig 6: X-RD of Physical Mixture

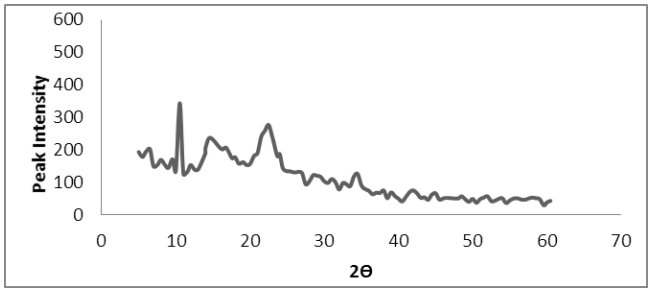

Fig 7: X-RD of LSS-01

Comparative Dissolution Study

The proposed formulation batch LSS-01 released the 98.20±0.014 of drug in 60 min in comparison to marketed formulation (MF) 48.92±0.009 with reference to the pure bulk drug (PD) 46.26±0.011 and physical mixture (PM) 51.42±0.006.

Table 12: Comparative Dissolution Study of Batch LSS-01, PD, PM, MF

Time (min)	% Cumulative drug release, Mean ±SD			
	Pure Drug (PD)	Physical Mixture (PM)	Marketed Formulation (MF)	LSS-01
00	00±000	00±000	00±000	00±000
02	12.56±0.011	33.56±0.003	14.46±0.011	51.74±0.001
05	21.73±0.012	37.94±0.001	23.45±0.014	60.86±0.005
10	29.76±0.006	39.3±0.012	34.02±0.002	69.93±0.002
15	35.30±0.004	40.54±0.013	37.01±0.011	78.92±0.013
30	40.08±0.011	43.54±0.011	41.45±0.010	83.14±0.011
45	43.24±0.009	47.66±0.002	44.52±0.011	89.92±0.012
60	46.26±0.011	51.42±0.006	48.92±0.009	98.20±0.014

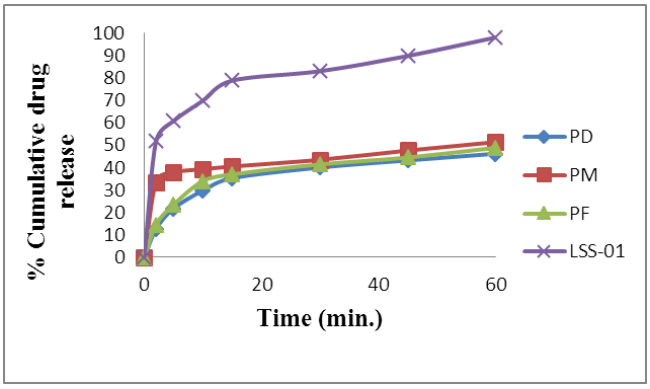

Fig 8: Comparative Dissolution study of Batch LSS-01, PD, PM, MF in distilled water

Stability Study

Stability study of developed formulation (LSS-01 and PM) was performed. The result showed that the % residual drug content after storage for 1 month was above 99% at 40˚C±2°C and 75± 5%RH, showing good stability.

Table 14: Stability Study Data of LSS-01 and Physical Mixture (PM)

Condition	Time (days)	% residual drug in formulation (mean ±SD)	
		LSS-01	PM
40˚C±2°C and 75± 5%RH	0	100.00	100.00
	15	99.98±0.012	99.99±0.009
	30	99.97±0.011	99.99±0.014

5. Conclusion

The research findings showed that, a stable liquid solid system containing Rifabutin was successfully developed using mixed solvency concept showing dissolution rate enhancement which may further enhance the bioavailability of rifbutin. The proposed techniques would be economical, convenient and safe. Thus the study open the chances of preparing such other formulations of poorly water soluble drugs. If chemical stability of the drug remains unaffected, to open a new era of more stable economic and safe products in the market.

6. Acknowledgement

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7. References

1. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Lippincott Williams & Wilkins Philadelphia, Third Edition, 2004.
2. Seth SD, Seth V. Textbook of Pharmacology. Elsevier New Delhi, Third Edition, 2009.
3. Gayakwad PS, Maheshwari RK. Formulation development and evaluation of an aqueous injection of gatifloxacin by novel mixed solvency technique. World Journal of Pharmaceutical Research. 2014; 3:1642-1644.
4. Panda S, Varaprasad R, Priyanka K, Swain RP. Liquisolid technique: a novel approach for dosage form design. International Journal of Applied Pharmaceutics. 2017; 9:8-14.
5. Kumari M, Agrawal A, Kumari M. Development of novel nanoparticulate drug delivery system of rifabutin. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 6:1404-1417.
6. Verma A, Pandit J. Rifabutin loaded floating gellan gum beads: invitro and invivo evaluation. African Journal of Pharmacy and Pharmacology. 2011; 5:589-595.
7. Joshi A, Maheshwari RK. Novel application of mixed solvency concept in the development of oral Liquisolid system of a poorly soluble drug Indomethacin and its evaluation. S.G.S.I.T.S, 2016, 76.
8. Maheshwari RK, Indurkhya A. Formulation and evaluation of aceclofenac injection made by mixed hydrotropic solubilization technique. International Journal of Pharmaceutical Research, 2010, 233-242.
9. Nighute AB, Bhise SB. Enhancement of dissolution rate of rifabutin by preparation of microcrystals using solvent change method. International Journal of Pharm Technology Research. 2009; 1:142-148.
10. Rifabutin absorbance. https://pubchem. ncbi. nlm. nih. gov/ compound/ rifabutin#section=Spectral-Properties, 2018.